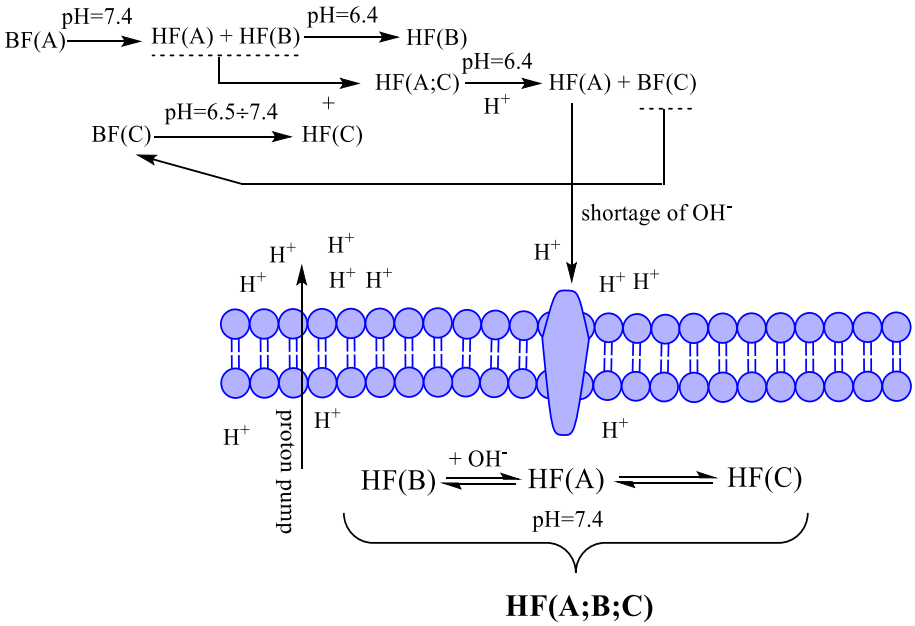
Theoretical study of anticancer activity of glycoside amides



Sofia, 2021

THEORETICAL STUDY OF ANTICANCER ACTIVITY OF GLYCOSIDE AMIDES

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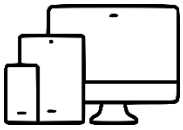
Genesis 1:29

"And God said, Behold, I have given you every herb bearing seed, which is upon the face of all the earth, and every tree, in the which is the fruit of a tree yielding seed; to you it shall be for meat"[i]



This book is a long-term study and analysis presented in a more scientifically popular form. It should not be cited in scientific publications. The matter is presented in a freer way in order to explain the matter. If you are interested, please quote the following three articles - they are entirely scientific and have passed all the rigor of the publisher:

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This book is structured in a structure aimed at limiting animal testing in laboratory and preclinical settings.

Only refurbished computers, tablets and smart devices were used in the creation of this book. Good practices have also been applied to reduce energy consumption. The goal is to prevent additional carbon load in the world around us.

INTRODUCTION

The apricot is a fruit known to people for millennia (WHAT MAKES ARMENIA SPECIAL?, 2016) (Harutyunyan, 2014). Archaeological excavations in the ancient Armenian city of Shenchovit near Yerevan revealed overlaid apricot excavations dating back to 6,000 years BC. The first written mention of apricot was 4,000 years ago in a letter from a Chinese resident. The well-known apricot comes from a variety of the high-mountainous region of Hindu Kush - Central Asia, where the borders of China, Tajikistan, Afghanistan and Pakistan meet today. Natural forest and very old apricot trees can still be found in northeast China and the Caucasus.

It is a well-known fact by derontologists that the Hunzi people (Ahmed, 2016) that inhabited the highlands of northern Pakistan, not far from where the apricot originates, are the healthiest and longest-lived people in the world. According to researchers and medical scientists who studied the life of the Huns in their natural environment in the 1950s and 1960s, one hundred percent of them had perfect vision, and cancer, heart attack, high blood pressure, high cholesterol and even appendicitis and gout were unknown states for them.

Throughout the year, their diet was rich in dried fruits and nuts, with apricots and apricot kernels predominating, and their main source of fat was apricot seeds. Apricots were an important part of the Hunzi life.

The apricot kernels contain (Femenia, Rossello, Mulet, & Canellas, 1995) an average of 21% protein and 52% vegetable oil and are widely used as a substitute for almonds in the food, cosmetic and pharmaceutical industries. Due to its high content of amygdalin, apricot seeds are a source of *Vitamin B17* and are used in alternative medicine for cancer therapy.

The American Cancer Society notes that apricots, as well as other carotene-rich fruits, reduce the risk of cancer of the larynx, esophagus and lungs.

This scientific research concentrates on the processes occurring in the medium around the cancer cell and the transfer of glycoside amides through their cell membrane. They are obtained by modification of *natural glycoside-nitriles* (*cyano-glycosides*). Hydrolysis of starting materials in the blood medium and associated volume around physiologically active healthy and cancer cells, based on quantum-chemical semi-empirical methods, are considered.

Based on the fact that the cancer cell feeds primarily on carbohydrates, it is likely that organisms have adapted to take food containing nitrile glycosides and/or modified forms to counteract "external" bioactive activity. For their part, cancers have evolved to create conditions around their cells that eliminate their active apoptotic forms. This is far more appropriate for them than changing their entire enzyme regulation to counteract it. In this way, it protects itself and the gene sets and develops accordingly.

Derived pedestal that closely defines the processes of hydrolysis in the blood, the transfer of a specific molecular hydrolytic form to the cancer cell membrane and with the help of time-dependent density-functional quantum-chemical methods, its passage and the processes of re-hydrolysis within the cell itself, to bioactive forms causing chemical apoptosis of the cell - independent of its non-genetic set, which seeks to counteract the process.

Used in oncology it could turn a cancer from a lethal to a chronic disease (such as diabetes). The causative agent and conditions for the development of the disease are not eliminated, but the amount of cancer cells could be kept low for a long time (even a lifetime).

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ABBREVIATIONS USED IN THE TEXT

AACF	Active Anticancer Cell molecules Forms
AAF	Active apoptotic forms
ACFSC	Atom Centered Fragments similarity check
APSM	Accuracy of prediction for similar molecules
COSMO	COnductor-like Screening Model
CPCM	conductor-like polarizable continuum model
CSM	Concordance for similar molecules
GADI	Global AD Index
MDRC	Model's Descriptors Range Check
MEPASM	Maximum Error of Prediction Among Similar Molecules
MM2	Molecular Mechanics force field were developed by Merck and are sometimes called the Merck
MMFF94	Molecular Force Field
NMNC	Neural map neurons concordance
QSAR	Quantitative structure–activity relationship
SMKEV	Similar molecules with known experimental value

[0.]	the value is rounded to an integer
[0.0]	the value is rounded to the nearest decimal place
[0.00]	the value is rounded to the nearest hundred place
[0.000]	the value is rounded to the nearest thousandth place

I. GOALS AND OBJECTIVES OF THE STUDY

1. First goal: *A study of what exactly is the reason for the long-term use of toxic amygdalin in the social group*

Annotation to the realization of the objective: By making a precise socio-anthropological analysis (Bernard, 1998) of the life of the ancient people of Botra (Hunza people, Burusho / Brusho people), we come to the hypothesis, which is confirmed by two proofs, through a number of modern quantum-mechanical, molecular-topological and bio-analytical checks. A convenient, harmless, form of amygdalin derivative is available that has the same biological and chemical activity and could be used in conservative clinical oncology. The article also presents a theoretical comparative analysis of biochemical reactivity in *in vivo* and *in vitro* media, by which we also determine the recommended dosage for patient administration. Based on a comparative analysis of the data, obtained in published clinical studies of amygdalin, is presented and summarized a scheme of the anti-tumor activity

Presentation and scientific popularization of the results: some of the conducted researches are published in the format of an article **- *Theoretical Analysis for the Safe Form and Dosage of Amygdalin Product*** (Tsanov & Tsanov, 2020).

2. Second goal: *Analysis of molecules forming activity in the environment around the cancer cell and their ability to cross the cell membrane*

Annotation to the realization of the objective: This scientific analysis is a continuation of the first goal (***§I.1.***). The hypothesis that hydrolyzed to amine/carboxylic acid cyano/nitrile glycosides are a potential anticancer drug has been proposed and theoretically confirmed there. Their biological activity remains unchanged directly from the natural compounds of this group, but their toxicity is many times lower than unmodified native molecules. After defining the chemical formula and determining the pharmaceutical form and dosage, most active groups are also identified, which directly determines their biological activity.

Presentation and scientific popularization of the results some of the conducted researches are published in the format of an article **- *Theoretical Study of the Process of Passage of Glycoside Amides through the Cell Membrane of Cancer Cell*** (Tsanov, H. & Tsanov, 2021)

3. Third goal: *Analysis of models for evaluation of the offered pharmaceutical forms*

Annotation to the realization of the objective: The pharmaceutical form allows deviation from the chemically pure substance. This is a convenient and at the same time affordable (from a financial and / or technological point of view) form of admission by patients. It is not necessary to use an "ideal" pure active substance (including a specific isomeric form). Due to the wide variety of natural glucosamide nitriles (starting material for the production of amide / carboxylic acid), modern pharmacology allows their combined use by chemical nature and concentration of the active form passing through the cell membrane.

Methodology: A comparative analysis is performed based on stoichiometric calculations for the concentration of the active form and the prediction of bioactivity. For this purpose, the following methodology is used: Analysis of data on the active molecular form of anticancer cells and determination of the drug dose.

Presentation and scientific popularization of the results: some of the conducted researches are published in the format of an article - ***Theoretical analysis of anticancer cellular effects of glycoside amides*** (Tsanov & Tsanov, Theoretical analysis of anticancer cellular effects of glycoside amides, 2022).

- ◊ -

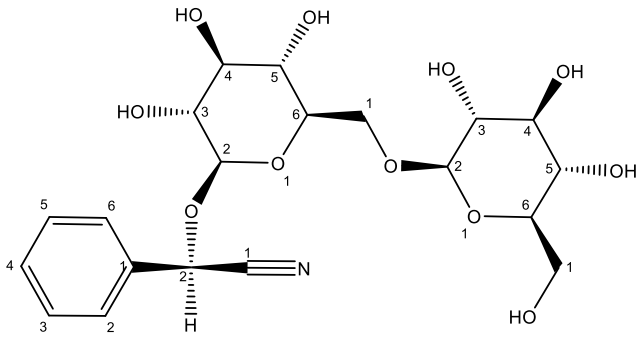
II. BRIEF OVERVIEW OF THE PROBLEM STATUS

1. On the first goal:

1.1. Pharmacological activity of amygdalin.

Amygdalin is a nitrile containing a diglycoside compound of the general formula $C_{20}H_{27}NO_{11}$, molecular weight 457.42, with the structure *D-mandelonitrile-β-D-glucoside-6-β-glucoside* (Vetter, 2000) and the structural formula ***Fig.II.1.1***.

(R)-2-phenyl-2-(((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-((((2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)acetonitrile



Amygdalin

Fig.II.1. 1 *Structural formula and full chemical name of Amygdalin*

Amygdalin is non-toxic, but under the action of digestive juices and enzymes in the blood it releases HCN, which, even at relatively low concentrations, is even deadly.

Numerous studies have been performed to prove its antitussive (Chang, et al., 2005) and antiasthmatic (Do , Hwang, Seo, Woo, & Nam, 2008) effects, analgesic (Holland, 1982) (Zhu, Su, & Li, 1994) (Hwang, et al., 2008) (Hwang, Lee, Kim, Shim, & Hahm, 2008) (Yang, et al., 2013) (Paoletti, et al., 2013), gastro-enterologic (Wei, Xie, & Ito, 2009) (Shim & Kwon, 2010), promoting apoptosis of human renal fibroblast (Guo, Wu, Shen, Yang, & Tan, 2013), boosting immunity synthesis (Jiagang, et al., 2011) (Baroni, et al., 2005) (Perez, 2013) (including to increase polyhydroxyalkanoates in induced human peripheral blood T-lymphocyte proliferation), anti-diabetic properties (Mirmiranpour, et al., 2012) (Heikkila & Cabbat, 1980) (including inhibiting alloxan in hyperglycemia), potential in the treatment of Hansen's disease, atherosclerosis, immune suppression and most of all antitumor effect (Kwon, Hong, Hahn, & Kim, 2003) (Fukuda, et al., 2003) (Barwina, Wiergowski , & Anand, 2013) (Howard-Ruben & Miller, 1984) (Yang, et al., 2012) (Milazzo, Ernst, Lejeune, Boehm, & Horneber, 2011) (Fenselau, et al., 1977) (Davignon, Trissel, & Kleinman, 1978) (Karabulutlu, 2014) (Ellison, Byar, & Newell, 1978) (Bolarinwa, Orfila, & Morgan, 2014) (Newmark, et al., 1981) (Syrigos, Rowlinson-Busza, & Epenetos, 1998) (Chen, et al., 2013) (Bitting, 1978) (Moertel C. G., et al., 1982) (Miller, Anderson, & Stoewsand, 1981) (Park, et al., 2005) (ACS, 1991) (Shishkovsky, 1980) (Liao, Ling, Zhong, & Ping, 2005) (Shils & Hermann, 1982) (Chang, et al., 2006) (Greenberg, 1980) (Herbert, 1979) (Milazzo, Lejeune, & Ernst, Laetrile for cancer: a systematic review of the clinical evidence, 2007) (Curran, 1980) (Zhou, et al., 2012) (Carter, McLafferty, & Goldman, 1980) (Kousparou, Epenetos, & Deonarain, 2002) (Biaglow & Durand, 1978) and etc.

1.2. Clinical trial of amygdalin in the treatment of human cancer

1.2.1. Conducting research

In summary: A group of authors (Moertel C. G., et al., 1982) published a detailed clinical trial of *amygdalin* (and its *Laetrile* derivative). For the purposes of our research, the exact methodology of clinical trial must be studied in great detail.

After fully informing the patients about the type and manner of the study, the test begins (***Tabl.II.1.1***), constantly monitoring the concentration of total cyanide in the blood. The first analysis was performed 2 hours after the start of oral drug administration. If the cyanide level is higher than 2µg/ml but less than 3µg/ml, *amygdalin* is discontinued for up to 48 hours or until all symptoms suggesting toxicity are discontinued.

Therapy was continued in all patients at least until they had irrefutable evidence of progressive malignancy or until severe clinical deterioration allowed further treatment and follow-up.

Tabl.II.1. 1 *Amygdalin and "Metabolic Therapy" Regimens for 21 days*

AGENT	STANDARD DOSE	HIGH DOSE
Amygdalin		
intravenous course	4.5 g/m^2 of body-surface	7 g/m^2/day X 21 days
oral maintenance	area/day x 21 days 0.5 g 3 times daily	0.5 g 4 times daily
Vitamins		
A	25,000 U/day	100,000 U/day
C	2 g/day	10 g/day
E	400 U/day	1200 U/day
B complex and minerals	1 capsule/day	1 capsule/day
Pancreatic enzymes (Viokase)	12 tablets/day	12 tablets/day
© Charles G. Moertel, M.D., Thomas R. Fleming, Ph.D., Joseph Rubin, M.D., Larry K. Kvols, M.D., Gregory Sarna, M.D., Robert Koch, M.D., Violante E. Currie, M.D., Charles W. Young, M.D., Stephen E. Jones, M.D., And J. Paul Davignon, Ph.D.		

In short, the author's research team points out that the methods of this study are completely comparable to those used in the studies of every new agent being developed and tested for cancer treatment through more traditional channels. They are designed to maximize the ability of amygdalin to exhibit therapeutic activity, if such potential exists.

1.2.2. Clinical results

The characteristics of all patients undergoing experimental treatment are listed in ***Tabl.II.1.2.*** Types of tumors include predominance of colorectal, lung, and breast cancer with a standard dose regimen.

Tabl.II.1. 2 *Characteristics of eligible patients subjected to clinical testing with amygdalin*

Characteristic	Standard-Dose Regimen	High-Dose Regimen No. Of pal lent s	All Patients
Sex			
Male	92	8	100
Female	72	6	78
Age (yr.)			
Median	57	60	57
Range	18-84	39-73	18-84
Primary tumor			
Colorectal *	44	14	58
Lung §	30	—	30
Breast *	21	—	21
Melanoma	15	—	15
Sarcoma ,	10	—	10
Pancreas *	8	—	8
Stomach *	7	—	7
Kidney *	6	—	6
Lymphoma	5	—	5
Ovary *	4	—	4
Other (1 or 2 each)	14	—	14
Prior radiation therapy ֍			
Yes	72	0	72
No	92	14	106(60)
Prior chemotherapy ֍			
Yes	109	9	118
No	55	5	60(34)
Performance status ֍ ○			
0-1	116	11	127 (71)
2-3	48	3	51
Institution			
University of Arizona	19	—	19
UCLA	40	—	40
Mayo Clinic	82	14	96
New York Memorial	23	—	23
* Adenocarcinoma § Non-small-cell carcinoma ֍ Figures in parentheses denote percentages. ○ Eastern Cooperative Oncology Group score: 0, fully active, to 4, totally disabled.			
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All patients selected for the high-dose regimen had colorectal cancer. Particularly remarkable is that over one third of all patients did not receive prior chemotherapy. In addition, 71% of the patients had a good working condition - that is, they were able to work full-time or part-time.

1.2.3. Toxic reactions and symptoms

Data on the possible toxicity of intravenous and oral amygdalin are shown in ***Tabl.II.1.3***.

Tabl.II.1. 3 *Toxicity of amygdalin therapy tested in a clinical setting*

Toxic REACTION	ROUTE	
	INTRAVENOUS	ORAL
	% of 178 patients	% of 132 patients
Nausea	31	30
Vomiting	25	17
Headache	7	8
Dizziness	7	10
Mental obtundation	4	5
Dermatitis	2	2
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The authors point out that adverse reactions are rare and also inherent in the oncologic diseases involved. The side effects of increased cyanide concentration (greater than 3 μg/ml) in the blood disappear after the amygdalin discontinuation.

Higher concentrations of amygdalin intake are also investigated, but then adverse reactions increase significantly

This is precisely the purpose of this section of the study: **To provide a dosage form that provides the necessary concentration of active molecules in vivo to patients in need of anti-tumor therapy**.

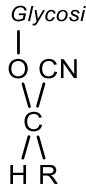
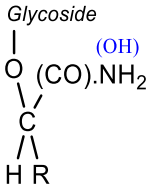
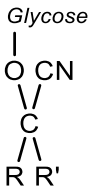
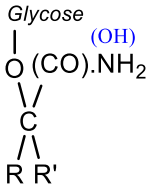
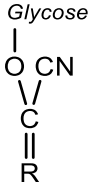
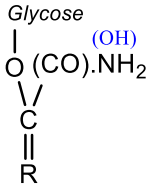
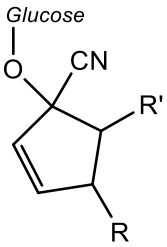
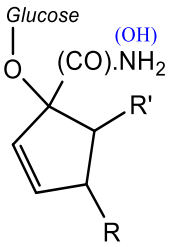
2. On the second goal

Here it focuses on the processes occurring in close proximity to the tumor cell and will present a model of the mechanism of passage through the cell membrane - to obtain an active molecular form for apoptosis.

Aromatically substituted glycosides: *Prunus spp.* (Karakas, et al., 2019), *Lucuma spp.* (Prabhu, Selvam, & Rajeswari, 2018), *Vicia spp.* (Salehi, et al., 2021), *Sambucus spp.* (Thole, et al., 2006), *Sorghum spp.* (Smolensky, et al., 2018), *Taxus spp.* (Durak, Büber, Devrim, Kocaoğlu, & Durak, 2014), *Zieria spp.* (Spalding, 1991), *Macadamia spp.* (Desegaulx, Sirdaarta, Rayan, Cock, & McDonnell, 2015). Glycosides with a free α-hydroxynitrile: *Nandina spp.* (Taha, Khalil, & Abubakr, 2020). Glycosides with aliphatic substituents: *Linum spp.* (Szewczyk, et al., 2014), *Trifolium spp.* (Sabudak & Guler, 2009), *Lotus spp.* (Tong, et al., 2020), *Maniholt spp.* (Veerapagu, Latha, Ramanathan, & Jeya, 2020), *Acacia spp.* (Sakthivel, Kannan, Angeline, & Guruvayoorappan, 2012), *Triglochin spp.* (Lellau & Liebezeit, 2003), *Deidamia spp.* и *Tetrapathaea spp.* (Yulvianti & Zidorn, 2021), *Gynocardia spp.* (Kalita, et al., 2018), *Pangium spp.* (Chye & Sim, 2009) and others.

The proposed pharmaceutical form of the amide/carboxyl derivative of amygdalin represents only one of the dozens of glycoside nitriles that have been analyzed and by which this claim is made. Some of them are listed (Vetter, 2000) (Barceloux, 2008) in ***Tabl.II.2.1***.

Tabl.II.2. 1 *Natural nitrile glycosides and their modified amide and carboxylic acid forms*

Base Structure	Substituent R	Glycoside	Sugar	Occurrence	Modified Structure
Glycosides with aromatic substituents					
	Phenyl	Prunasin	D-Glucose	*Prunus spp.*	
	Phenyl	Amygdalin	Gentiobiose	*Prunus spp.*	
	Phenyl	Lucumin	Primeverose	*Lucuma spp.*	
	Phenyl	Vicianin	Vicianose	*Vicia spp.*	
	Phenyl	Sambunigrin	D-Glucose	*Sambucus spp.*	
	p - Hydroxyphenyl	Dhurrin	D-Glucose	*Sorghum spp.*	
	p - Hydroxyphenyl	Taxiphyllin	D-Glucose	*Taxus spp.*	
	m - Hydroxyphenyl	Zierin	D-Glucose	*Zieria spp.*	
	p - Glucosyloxyphenyl	Proteacin	D-Glucose	*Macadamia spp.*	
Glycosides with a free α - hydroxynitrile					
		p-Glucosyloxymandelonitrile		*Nandina spp.*	
Glycosides with Aliphatic substituents					
	R=R'=CH_3	Linamarin	D-Glucose	*Linum spp.* *Trifolium spp.*	
	R=CH_3 R'=$CH_2.CH_3$	Lotaustralin	D-Glucose	*Loyus spp.* *Maniholt spp.*	
	R=$C(CH_3)_2$	Acacipetalin	D-Glucose	*Acacia spp.*	
	R=$C(CH_2.COOH).CH=CH-COOH$	Triglochinin	D-Glucose	*Triglochin spp.*	
	R=R'=H	Deidaclin	D-Glucose	*Deidamia spp.*	
		Tetraphyllin A	D-Glucose	*Tetrapathaea spp.*	
	R=OH; R'=H	Tetraphyllin B	D-Glucose	*Tetrapathaea spp.*	
	R=R'=OH	Gynocardin	D-Glucose	*Gynocardia spp.* *Pangium spp.*	

It is important to mention that the by-product of the modification process also produces their carboxyl derivatives in a ratio, the ratio for amygdalin being: -amide: -carboxyl = 4.87:1, and for other homologs and/or similar structural of compounds is in the order of 2.61÷5.13:1. Chemical bond of the type: -N(H)-OC(O) - between the two derivatives of amygdalin is possible, but it is within statistical error.

3. On the third goal

The physiologically active cancer cell itself is quite inert to external influences. It is far more stable than any physiologically active structural and/or functional organismal cell. Its defenses

are discussed in detail in the article (Tsanov, H. & Tsanov, 2021), and its main weakness was defined, namely: the cancer cell feeds mainly on carbohydrates and/ or carbohydrate complexes. In an effort to preserve its gene set, it has evolved to counteract biologically active substances by maximally preventing its passage through its cell membrane.

It is this property that could be used to minimize its effect on the whole body. In the same article, based on theoretical calculations and literature references, a hypothesis is stated: cancers could turn from severe infectious to controlled chronic ones (similar to diabetes, chronic hepatitis, etc.)

Regardless of whether the cancer cell is active and/or already has suppressed physiological functions, it also has its corresponding cellular effect: proliferation (De Berardinis , Lum, Hatzivassiliou, & Thompson, 2008) /including in combination with Wartburg's effects (Sun, Chen, Cao, Liang, & Xu, 2019) /, invasion (Krakhmal, Zavyalova, Denisov, Vtorushin, & Perelmuter, 2015), migration (Yamaguchi, Wyckoff, & Condeelis, 2005), metastasis (Seyfried & Huysentruyt, 2013), adhesion (Janiszewska, Primi, & Izard , 2020), interruption of cell cycle (Otto & Sicinski, 2017), cytotoxicity (Ribeiro, et al., 2017) and apoptosis (Hu, Xu, Meng, Huang, & Sun, 2018) or a combination of two or more simultaneous actions.

Tabl.II.3. 1 Specific antitumor mechanisms of amygdalin in different tumors

Types	Cell lines	Dosage of amygdalin [mg/mL]	Treatment time	Cellular Effects
Lung cancer	H1299 PA	2.5 ÷ 5	48 hours	proliferation, invasion, migration
$integrin\beta1\downarrow, integrin\beta4\downarrow \{ILK\downarrow\ p-FAK\downarrow \Longrightarrow\ \beta-catenin\downarrow, E-cadherin\uparrow\} \Longrightarrow AKT\downarrow, RICTOR\downarrow \frac{AKT}{mTOR\downarrow}$				
Bladder cancer	UMUC-3 RT112 TCCSUP	10	24 hours or 2 weeks	proliferation, adhesion, invasion, migration, cell cycle, cytotoxicity
$\{integrin\beta1\downarrow, integrin\beta4\downarrow \{ILK\downarrow\ p-FAK\downarrow \Longrightarrow\ \beta-catenin\downarrow, E-cadherin\uparrow\ \ cdk1\downarrow, cdk2\downarrow, cdk4\downarrow\ cyclin\ A\downarrow, cyclin\ B\downarrow, cyclin\ D1\downarrow\} \Rightarrow \frac{G0}{G1} phase\uparrow\ p-AKT\downarrow, p-RICTOR\downarrow \Rightarrow \frac{AKT}{mTOR\downarrow}$				
Renal cell carcinoma	Caki-1 KTC-26 A498	10	24 hours or 2 weeks	proliferation, apoptosis, adhesion, cell cycle

$\{cdk1\downarrow, cdk2\downarrow, cdk4\downarrow, cyclin\ A\downarrow, cyclin\ B\downarrow \Longrightarrow \frac{G0}{1} phase\uparrow, \frac{G2}{M} phase\ \downarrow, S\ phase\uparrow\ E-cadherin \downarrow, N-cadherin\downarrow$				
Prostate cancer	LNCaP DU-145 PC3	0.1 ÷ 20	24 hours	proliferation, apoptosis, cell cycle
$\{cdk1\downarrow, cdk2\downarrow, cdk4\downarrow\ cyclin\ A\downarrow, cyclin\ B\downarrow\ cyclin\ D\downarrow, p19\downarrow, p27\downarrow\} \frac{G0}{G1} phase\uparrow, \frac{G2}{M} pase\downarrow, S\ phase \downarrow\ p-AKT\downarrow, p-RICTOR\downarrow, p-Raptor\downarrow \Longrightarrow \frac{AKT}{mTOR\downarrow} Bcl-2\downarrow, Bax\uparrow, caspase-3\uparrow$				
Cervical cancer	Hela cell	10 ÷ 20	24 hours	proliferation, apoptosis
$Bcl-2\downarrow, Bax\uparrow, caspase-3\uparrow$				
Colon cancer	SNU-C4	5	24 hours	proliferation, cell cycle, cytotoxicity
cell cycle-related gene:		$EXO1\downarrow, ABCF2\downarrow, MRE11A, TOP\ I.\downarrow, FRAP1\downarrow\ ATP-binding\ cassette\downarrow, sub-family\ F\downarrow$		
Promyelocytic leukemia	HL-60	1 ÷ 20	48 hours	proliferation, apoptosis
combinate with β-glucosidase		$Bcl-2\downarrow, Bax\uparrow$		
Breast Cancer	Hs578T MDA-MB-231 ER-positive MCF7	10 ÷ 40	24 hours	cytotoxicity, apoptosis, adhesion
The information is provided by (Shi, et al., 2019). They are summarized by studies of: (Qian, Xie, Wang, & Qian, 2015), (Makarević, et al., 2014), (Makarevic, et al., 2014), (Makarević, et al., 2016), (Syrigos, Rowlinson-Busza, & Epenetos, 1998), (Juengel, et al., 2016), (Chang, et al., 2006), (Chen, et al., 2013), (Park, et al., 2005), (Lee & Moon, 2016) & (Young, Pyo, Hoon, & Hee, 2003).				

Data in ***Tabl.II.3.1*** are applicable to the use of "pure" unmodified Amygdalin [§1.2 of article (Tsanov & Tsanov, Theoretical Analysis for the Safe Form and Dosage of Amygdalin Product, 2020)] and concentrations consistent with its nitrile chemical nature.

Therefore, the possible chemical apoptosis (or other type of cellular reaction) will occur independently of all enzymes synthesized according to instructions from cancer DNA (for example, as - *linamarase gene* to *linamarase*).

- ◊ -

III. METHODOLOGY

1. Information support

The computer configurations used have an operating system installed *MS*[ii] *Windows 10 Pro* and *MS Office 2016*.

Calculations are performed with computer programs that are provided for free use by academic institutions[iii] and/or those with non-commercial use, incl. and those covered by the GNU License[iv].

Virtualizations from other operating systems (*openSUSE*[v] и *MacOS*[vi]) are performed with *Oracle VM VirtualBox*[vii].

Input and output data is stored and synchronized in *Google Drive*[viii] with synchronization repositories[ix].

1.1. Software products

Graphical representation of chemical formulas was done with *MarvinSketch*[x] and *ACD/ChemSketch*[xi]. The main standard for working in *2D* is: file extension - .*CDX*[xii] and media type: *chemical/x-cdx*.

1.1.1. Mathematical software

SnapPy[xiii] – is a topological software that investigates linear connections between points, PL arcs and PL circles.

Maxima[xiv] – this program is used for algebraic and stoichiometric calculations of chemical reactions.

GAP[xv] – the software product is used for cross-checks and extrapolations of graph data.

1.1.2. Quantum Chemical Software

A. Chemical Informatics

Open Babel[xvi] – through it the conversions from and to the various file standards required for the respective software product have been performed.

B. Chemical kinetics

Cantera[xvii] - it was used to analyze data regarding the relationship between molecular dynamics and enthalpy change.

KPP[xviii] – it was used to cross-check the calculations received from Cantera (Damian, Sandu, Damian, Potra, & Carmichael, 2002).

C. Molecular modeling and visualization

Avogadro[xix] – this molecular editor and used to generate source code for quantum chemical software.

Jmol[xx] – through it are applied data entering from different databases and/or manually entered from performed calculations, etc. package type.

D. Molecular docking

AutoDock Suite[xxi] – used to study the passage of substances across the cell membrane and the enzymatic effects of products.

E. Molecular dynamics

GROMACS[xxii] – the resulting protein complexes are described and simulated by a molecular dynamics package.

F. Quantum calculations

MOPAC[xxiii] – it is used for molecular optimizations and calculations using semi-empirical quantum methods.

GAMESS US[xxiv] - This program is used for optimizations of geometries-minimums and transients in the main one. It determines most thermodynamic quantities: *entropies, enthalpies, energies, dipole moments, Gibbs free energy* and others.

1.1.3. Drug design

DruLiTo[xxv] – is an open source virtual screening software tool. It contains a significant set of algorithms based on highly cited scientific articles for calculating drug similarity. It is built on the basis of CDK[xxvi] (Chemistry Development Kit) in Java[xxvii].

T.E.S.T.[xxviii] – A toxicity assessment software tool has been developed to allow users to easily assess the toxicity of chemical compounds using quantitative structural linkages (QSARs) methodologies.

Molinspiration[xxix] – large set of algorithms for chemical informatics for generation, manipulation and processing of molecules, such as: normalization of molecules, generation of tautomers, fragmentation of molecules, calculation of various molecular properties required in QSAR, molecular modeling and drug design, high quality imaging of molecules, molecular database tools supporting substructure search and similarity.

OSIRIS Property Explorer[xxx] – software package allowing evaluation of the drug/toxic properties of molecules, emphasizing those with high risk and/or difficult absorption in the intestine.

VEGA[xxxi] – Java based set of models investigating toxicity, mutagenicity, carcinogenicity and more.[1]

SwissADME[xxxii] – web based platform to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

1.1.4. Processing of statistical information

SciDAVis[xxxiii] – open source software optimized for scientific information analysis and data virtualization.

GnuPlot[xxxiv] – open source software using command-line data entry. It has a good set of statistical and mathematical algorithms optimized for scientific work.

1.2. Databases with empirical information

PubChem[xxxv] - database with information on chemical molecules, emphasizing their biological activity. Administered by NCBI[xxxvi] and NIH[xxxvii].

ChemSpider[xxxviii] - database of chemical compounds. Administered by RSC[xxxix].

ChEBI[xl] - database of so-called "Small molecules". Administered by EBI[xli], through OBO[xlii].

DrugBank[xliii] - open access and editing database. It focuses on information about drugs and drug therapy.

[1] Istituto di ricerche farmacologiche Mario Negri – IRCS, emilio.benfenati@marionegri.it (Benfenati & Marzo, 2020)

FoodData Central[xliv] - database with information on nutrients in foods. It also contains information arrays with statistical analyzes of food and can be modeled according to various criteria.

2. General foundations of the proposed methodological scheme

1) Semi-empirical methods are used in conducting comparative analyzes in terms of physicochemical parameters. Their genesis does not "claim" accuracy and/or maximum approximation to the real structural, and hence reactive properties of substances. Semi-empirical methods have three key advantages (apart from the speed of implementation) over other methods in computational chemistry:

   - The set of parameters included in their algorithms is constantly consistent with experimental data. This significantly reduces the moments of "abstractness" and dualistic interpretation;
   - They have good repeatability of the results, and hence the statistical processing of the data is relatively reliable;
   - Based on the latter, the results of the semi-empirical methods are amenable to comparison in absolute value, or in the present case we use the approximation that they are equally NOT accurate, i.e. the change of any physicochemical and/or structural indicator will reflect their properties in a way that coincides with this dependence in another compound.

2) Emphasis on pre-laboratory research in order to limit animal testing.

3. Methodological schemes for achieving the defined goals

3.1. On the first goal

The first goal is defined in detail in ***§I.1*** and ***§II.1.***

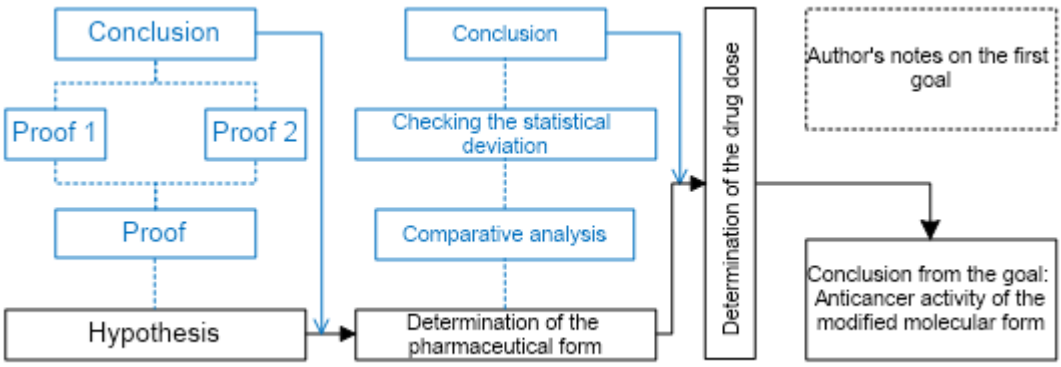


Fig. III.3. 1 *Methodological scheme for achieving the first goal - A study of what exactly is the reason for the long-term use of toxic amygdalin in the social group*

The present study is conducted on the basis of a hypothesis, which is subjected to theoretical and logical analysis in order to confirm and/or reject it. The methodological scheme (***Fig. III.3. 1***) is:

1) Determining the optimal chemical formula - after definition, the hypothesis is subjected to two logical proofs depending on the environment:
   - *in vivo* – this evidence is based on a unique chemical property of the nitrile group to catalytically convert to an amide in a hydrochloric acid medium. The conditions are close to those of the digestive juices in the stomach, and the catalyst is an ion that is available under certain conditions;
   - *in vitro* – undergoes an enzymatic reaction analysis, which achieves the same result, i.e. modification of the amygdalin molecule to a form overlapping with the input hypothesis.

It comes out with a conclusion from the part.

2) Determination of the pharmaceutical molecular form

After confirming the defined hypothesis, it is necessary to compare the physical and chemical ratios of the modified molecule with those of the parent amygdalin. With the help of mathematical chemical methods a number of comparative methodologies are carried out, which aim to compare each individual studied parameter between the obtained molecular forms. For this purpose, the indicators are analyzed:

- molecular topology - *Balaban index* (Babić, Klein, Lukovits, Nikolić, & Trinajstić, 2001), (Balaban, 1982), (Devillers & Balaban, 1999), (Mercader, Castro, & Toropov, 2001) & (Randic, 1975), *Cluster Count* (Mingos & Wales, 1990), *Molecular Topological Index*[2] (Mueller, Szymanski, Knop, & Trinajstic, 1990), *Num. Rotatable Bonds* (Khanna & Ranganathan, 2009), *Polar Surface Area* [0.] (Palm, Stenberg, & Luthman, 1997), *Shape Coefficient*[3] (Langmuir, 1917), *Topological Diameter* (Petitjean, 1992), *Total Connectivity* [0.00] (Kier & Hall, 2002), *Total Valence Connectivity* [0.00] (Unger, 1987), *Wiener Index* (Rouvray, 2002) (Todeschini R. , Consonni, Mannhold, Kubinyi, & Timmerman, 2008), *Shape Attribute* [0.00], *Sum Of Degrees* & *Valence Degrees* (Mezey, 1993);

- molecular networks[xlv] - distribution coefficients: *LogP* [0.0] (Leo, Hansch, & Elkins, 1971), *LogS* [0.00] (Wang & Hou, 2011), Dissociation Constant: *PKa* [0.00] (Perrin, Dempsey, & Serjeant, 1981);

- electronic treatment in atomic-molecular system (Baue, Schneider, & Göller, 2019), (Clayden, 2001), (Bodor, Buchwald, & Huang, 1999) – *Number of HBond Donors*, *Number of HBond Acceptors*, *Formal Charge*[4] (Welsh & Allison, 2019), *Principal*

[2] MTI – defined in the form: $MTI = \sum_{i=1}^{n} E_i$, where E_i are the components of a vector: $E = (A + D)d$, and A is the neighborhood matrix, D is the distance matrix of the column and d is the vector of degrees of the graph. Only adjacent non-hydrogen atoms are taken.

[3] The coefficient of the form I is given as: $= \frac{D-R}{R}$, where the diameter D represents the distance between the two farthest atoms in the molecule, and the radius R is the distance between the central atom(s) and the farthest.

[4] Formal charge is the charge assigned to an atom in a molecule, assuming that the electrons in the chemical bond are equally divided between the atoms, regardless of the relative electronegativity.

Moment [0.] (Sims, Abbott, Cowling, Goodby, & Moore, 2017), *Henry's Law Constant* [0.00] (Sander, 2015), *Mol Refraction* [0.00] (Born & Wolf, 1999);

- molecular properties (at 310.15 K and 1.02 bar) obtained by semi-empirical methods (Wagnière, 1976) (Kříž & Řezáč, 2019), as: *Core-Core Repulsion* [0.]; *COSMO Area* [0.0]; *COSMO Volume* [0.0]; *Dipole* [0.00], *Ionization Potential* [0.00], *Total Energy* [0.]; *Heat Capacity* [0.0], *Thermodynamic Energy* [0.0] on CPCM (Takano & Houk, 2005).

Immediately after determining the average deviation between the parameters of the starting and modified molecules, a check is performed by comparison with an already well-studied, close to the experimental, modification obtained by the same methods and conditions.

It comes out with a conclusion from the part.

3) Determination of the drug dose;

In order to determine the drug dose, it is necessary to consider the most probable active forms of the amide already present *in vivo* medium. Knowing the conditions (pH, temperature, etc.) in the human body and comparing them with concentrations of similar molecular structure and possible chemical transfer of already passed clinical trials of medicinal products. Stoichiometric calculations are applied and a relatively accurate dose is obtained for administration of the new pharmaceutical form in order to achieve their maximum anti-cancer action.

4) The antitumor activity of the modified molecular form should be inferred. A collection scheme with clearly defined and interpreted molecular transitions is prepared;

5) Author's notes on the set goal.

3.2. On the second goal

The present study is based on the scientific fact that the relatively conditioned associated volume around the tumor cell (Swietach, Vaughan-Jones, Harris, & Hulikova, 2014) has reduced acidity (Raghunand, et al., 1999) (pH = 6.5, at normal for a physiologically healthy cell pH = 7.4). This circumstance from the point of view of quantum chemical and molecular topological methods (Brown, 2009) allows to conduct a sufficiently reliable comparative analysis (Martone, Fulginei, & Salvini, 2007) of substances that are in close proximity to cells and to differentiate their chemical behavior in the respective media.

Semi-empirical methods (Stewart J. J., Optimization of parameters for semiempirical methods I. Method, 1989) (Stewart J. J., Optimization of parameters for semiempirical methods, 2013) are extremely suitable for comparing individual parameters directly related to the chemical and bio-active properties of individual molecules. They do not claim to be accurate, but give a fairly clear picture when comparing individual calculated and/or measured values, and are sufficiently reliable, especially the identification in the individual structural relationships.

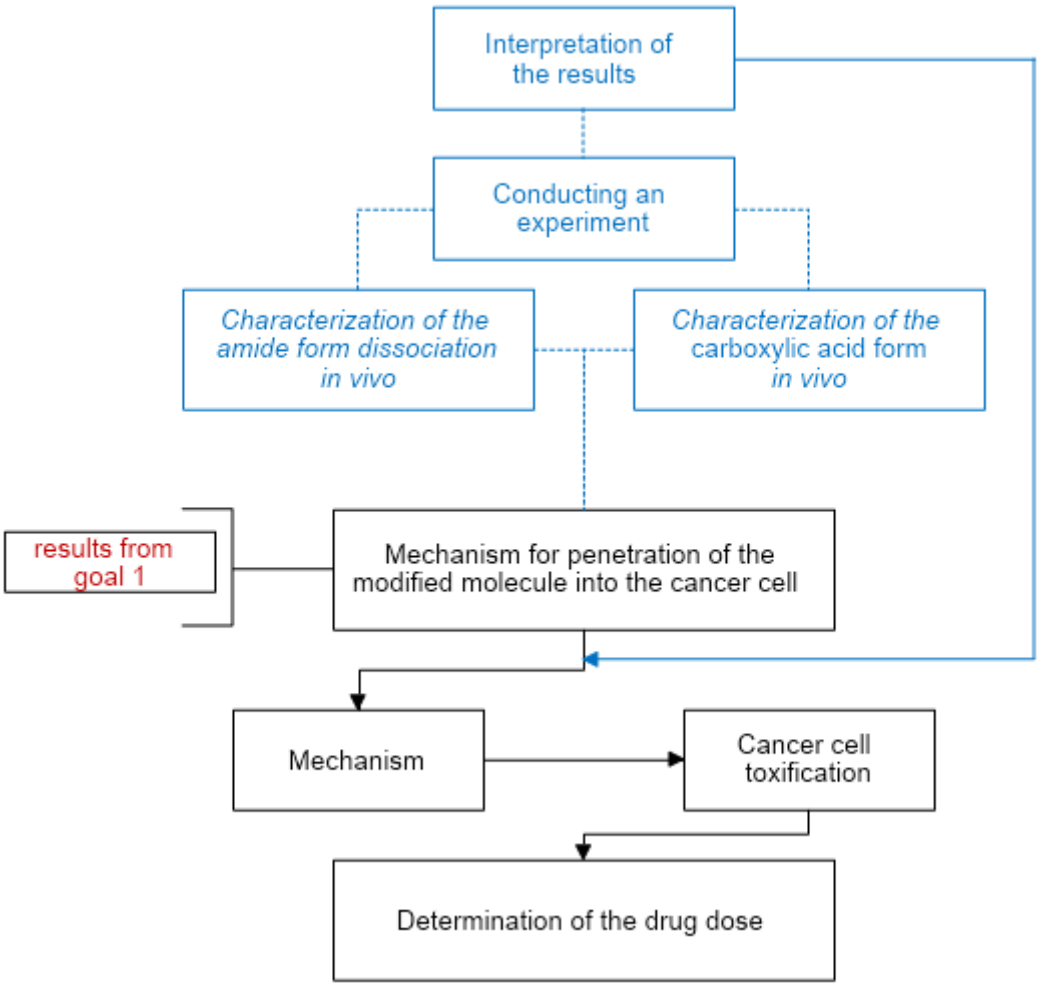


Fig. III.3. 2 *Methodological scheme for realization of the second goal - Analysis of molecules forming activity in the environment around the cancer cell and their ability to cross the cell membrane*

The conditions selected to be maximally covered by *in vivo*: temperature 308K, in a blood environment (reported by the dielectric constant, failure to introduce this indicator in the equations leads to a deviation of 5 to 8% - which is not physiologically justified).

The dialectic constant of human blood has a different value in different blood groups (Salahuddin, Farrugia, Sammut, O'Halloran, & Porter, 2017) (Rauf, 2013), so we take its average arithmetic as a starting point – 68.15 x10^3 at 308K and at frequency of 1KHz.

The electrostatic potential, created in the space around a molecule (Ren, et al., 2012) of its nuclei and electrons (treated as static charge distributions), is a well-applied property for analyzing and predicting molecularly reactive behavior. Hence the approximation that the molecular electrostatic potential is the potential energy of a proton at a specific location near a molecule. In this case, it is particularly useful as an indicator of the sites or zones of a molecule, initially attracted by an approaching electrophile, and has also been successfully applied to investigate interactions, that involve a certain optimal relative orientation of the reagents, in our case as an active pharmaceutical form cell to pass a specific cell membrane (cancer). This, however, prevents the recording of charges originating from the local electron density (charges density), which could be achieved with *Mulliken Changes*. The use of both variables from different methodologies more objectively illustrates the charges in the molecule and facilitate

subsequent interpretation. When comparing the outputs, it is necessary to use the same functional and basic set to make an accurate comparison, since the electron density is sensitive precisely to the likelihood of the process (whether at the electrostatic level).

With the introduction of *Ionization potential*, as a corrective for the interpretation of individual factors in the construction of the overall picture, more dualistic interpretation values (including variables) are eliminated.

All other investigated indicators are introduced into the analysis in order to better characterize the processes and their approximation to the actual *patho & sanus* physiological environment in the body.

The whole presentation is based on the amide and carboxyl acid form of *amygdalin* as the best studied, but they are only one representative of the homologous order. The conclusions are absolutely comparable to all its representatives (in their respective relation – amine/amide to carboxylic acid ***§I.1*** & ***§II.1)***.

Each source molecule is pre-exposed to *MM2 minimized energy* (Allinger, Conformational analysis. 130. MM2. A hydrocarbon force field utilizing V1 and V2 torsional terms, 1977), followed by molecular dynamics simulation at 308K (with 100 iterations) in order to generate an input to the subsequent *Z-matrix* calculations (Gidopoulos & Wilson, 2003). All values obtained by semi-empirical methods were performed in MOPAC (Maia, et al., 2012) environment with type *PM7* (Dral, Wu, Spörkel, Koslowski, & Thiel, 2016) and those of time-dependent density-functional theory (TD-DFT) in a GAMESS US (Gordon & Schmidt, 2005) environment. Each value is calculated five times with five different computer configurations and OS (Linux - Ubuntu™ & OpenSUSE™; Windows™ - 7 Pro & 10 Workstation). The results were statistically analyzed by the mean squared error (Hughes & Hase, 2010) and the hypotheses with the *Student's T-test* (Spiegel, 1992) for independent samples. The final numeric value is the one closest to its neighbor (also represented).

3.2.1. Characterization of amide dissociation *in vivo*

Analyzes the non-hydrolyzed amide / Basic Form A - BF(A) / both hydrolytic forms / conditionally accepted on Hydrolytic Form A - HF(A) and Hydrolytic Form B - HF(B) / in steps according to the indicators:

- *Electrostatic Potential* (Politzer & Laurence, 1985) (of reactive atoms), *Mulliken Changes* (Ohlinger, Klunzinger, Deppmeier, & Hehre, 2009), *Core-Core Repulsion* (Stewart J. , 2021), *COSMO* (Klamt A. , 2018) (Schürmann & Klamt, 1993) *Area* (Klamt, 2005) & *Volume* (Moya, Klamt, & Palomar, 2015), *Dipoles* (Tro, 2008) (vector Debye), *Electronic* & *Total Energy* and *Ionization Potential* (Lang & Smith, 2003);
- *Polar Surface Area* (Ertl, Rohde, & Selzer, 2000), *Radius* (Oxtoby, Gillis, & Campion, 2007) and *Topological Diameter* (Petitjean, 1992);
- *LogP*, *LogS* and *Partition Coefficient* (Kwon Y. , 2002) (Sangster, 1997);
- calculation and structural representation of *pKa* (Rossotti & Rossotti, 1961) (BHAGAVAN, 2002) of each atom and/or group of the whole molecule.

To carry out the check is applied and *Principal Moment* (Foote & Raman, 2000) and *Lipinski Rule* (Lipinski, Feeney, Lombardo, & Dominy, 2001) /etc. *Lipinski's rule of five*/.

3.2.2. Characterization of the carboxylic acid obtained as a by-product of nitrile hydrolysis

It shall be performed identically according to ***§III.3.2.1***.

3.2.3. Schematic presentation of the data from ***§III.3.2.1*** and ***§III.3.2.2*** with the necessary interpretations and conclusions - with the title: ***Mechanism of penetration of the modified molecule into the cancer cell*.**

TD-DFT is applied with respect to potential energy (Paul & Guchhait, 2011) and average enthalpy (Fifen, Nsangou, Dhaouadi, Motapon, & Jaidane, 2011).

3.2.4. Toxication of the cancer cell

Compounds that could be obtained immediately after the penetration of the active molecules into the cancer cell are considered. Their possible natural precursors are also presented. The results are interpreted.

3.2.5. Determination of the drug dose

3.3. On the third goal

It is mandatory to follow the tree structure of presentation, because it is also a function of the subsequent interpretation of the results.

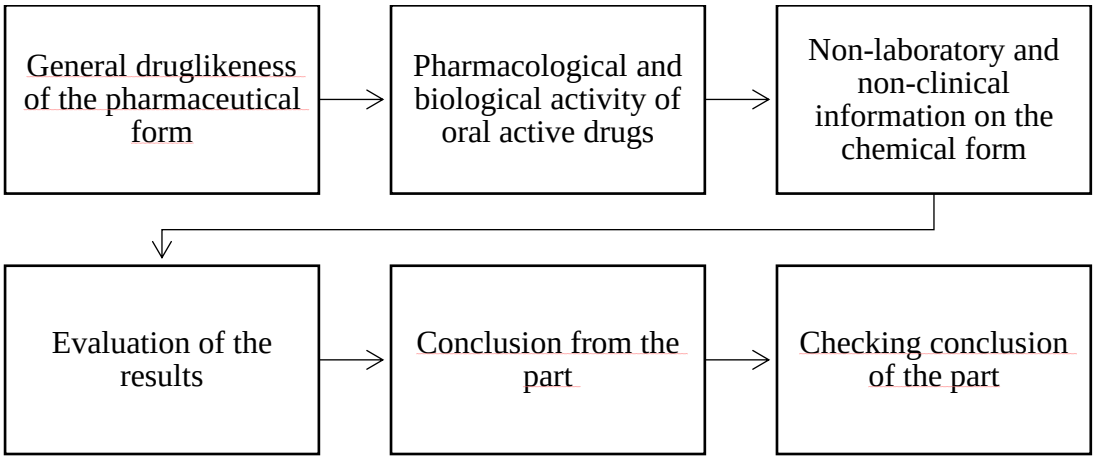


Fig. III.3. 3 *Methodological scheme for realization of the third goal - Analysis of models for evaluation of the offered pharmaceutical forms*

Amide/carboxylic acid hydrolyzed natural nitrile glycosides can be conditionally divided into 16 groups according (***Tabl.IV.2. 6*** **-** prepared on the basis ***§III.3.2.3***, ***-4***) to the *Active Anticancer Cell molecules Forms* (AACF) secreted inside the cancer cell. Some of the groups have more than one homologous representative and it is necessary to find the optimal one for

application (in terms of toxicity, working concentration, time of administration, etc.). Methods for non-laboratory and non-clinical assessment are applied, which are as close as possible to the real conditions and minimize as much as possible the errors in the theoretical research.

A comparative analysis is performed on the basis of stoichiometric calculations for the concentration of the active form and the prediction of the bioactivity. For this purpose, the following methodology is applied:

3.3.1. Data analysis for active anticancer cell molecular form

The derived chemicals obtained immediately after the passage of glucosamide across the cancer cell membrane [***§6*** of article (Tsanov & Tsanov, Theoretical Analysis for the Safe Form and Dosage of Amygdalin Product, 2020) are: *(R)-2-hydroxy-2-phenylacetamide, (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide, (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide, 2-hydroxy-2-methylpropanamide, (S)-2-hydroxy-2-methylbutanamide, 2-hydroxy-3-methylbut-2-enamide, (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid, (S)-1-hydroxycyclopent-2-ene-1-carboxamide, (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide, (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide, (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide, (R)-2-hydroxy-3-methylbutanamide, (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide, (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide, (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* и *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

The analysis is conducted in several phases:

3.3.2. Determining the exact molecular shape

Here is considered the geometry of the molecule (Ouyang, et al., 2013) on the basis of literature data (National Center for Biotechnology Information, 2020) and *MM2* (National Center for Biotechnology Information, 2020), and as a corrective is taken into account and *MMFF94* (Halgren T. A., 1996).

3.3.3. Druglikeness of the pharmaceutical form

Amides/carboxylic acids obtained by hydrolysis of natural nitrile glycosides are analyzed. Their concentrations and quantitative ratios are not considered here, but only the nature of the substances. Chemical ratios affecting biological activity were compared by *Molinspiration Drug Property* (Molinspiration:, 2020): *GPCR ligand* (Kristiansen, 2004), *Ion channel modulator* (Kaczorowski, McManus, Priest, & Garcia, 2008), *Kinase inhibitor* (Bhullar, et al., 2018), *Nuclear receptor ligand* (Zhao, Zhou, & Gustafsson, 2019), *Protease inhibitor* (Srikanth & Chen, 2016), (Eatemadi, et al., 2017) and *Enzyme inhibitor* (Aoyagi, shizuka, Takeuchi, & Umezawa, 1977), (Scatena, Bottoni, Pontoglio, Mastrototaro, & Giardina, 2008), (Song, Wu, & Wu, 2016) in order to characterize the overall *Druglikeness* (Li, et al., 2019).

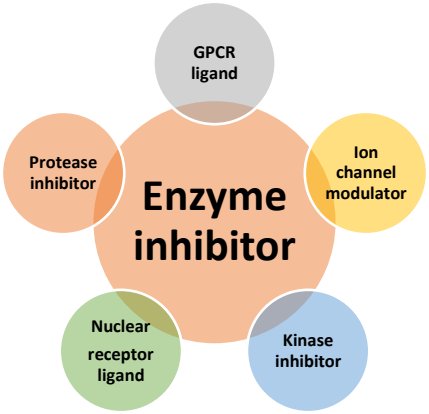


Fig. III.3. 4 *Methodological scheme for conducting analysis of General Druglikeness of the pharmaceutical form*

The data are presented in tabular form and the values that cover the minimum requirements for the respective indicator are marked in light green, and in more saturated green - those covering the optimal requirements. In order to consider that a substance has the bio-activity of a medicinal product, it needs to have at least two of the sets of minimum values.

The results are extremely insufficient and too dualistic. They are relatively comparative and not give information about possible deviations in the direction of toxicity. Other (mutually exclusive) methodologies for drug evaluation are also considered.

3.3.3.1. Pharmacological and biological activity of oral active drugs

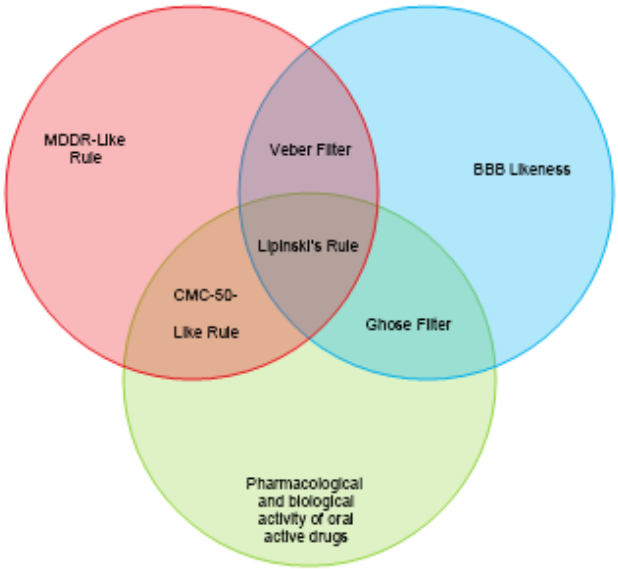


Fig. III.3. 5 *Methodological scheme for research on the indicators Lipinski's Rule, Ghose Filter and CMC-50-Like Rule and their interrelations*

The proposed pharmaceutical molecular form is recommended to be administered in the body in a solid state by mouth [***§5*** of (Tsanov & Tsanov, Theoretical Analysis for the Safe Form and Dosage of Amygdalin Product, 2020)].

The main goal here is not to precisely define the drug "strength", but to compare, empirically, indicators for the evaluation of molecules and the interchangeability of the starting precursors. Therefore, it is not the absolute accuracy that matters, but only the extremely strict repeatability of the assessment methodologies. No deviation in rounding of values affecting the precise statistical processing of the results is allowed. Calculations: (Lipinski, Lombardo, Dominy, & Feeney, 1997), (Ghose, Viswanadhan, & Wendoloski, 1999), (Oprea, 2000), (Veber, et al., 2002), (Steinbeck, et al., 2003), (Brenk, et al., 2008), (Di & Kerns, 2008), (Bickerton, Paolini, Besnard, Muresan, & Hopkins, 2012), (Yusof & Segall, 2013), are performed with the drug-likeness tool *DruLiTo 2018* (DruLiTo, 2020) /NIPER S.A.S., Nagar, India/.

The analysis is divided into three main groups of methods:

3.3.3.1.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

Lipinski's rule of five (Doak, Over, Giordanetto, & Kihlberg, 2014) (also known as *Pfizer's rule*) as a basic rule for the evaluation of chemical compounds having pharmacological and/or biological activity for use as a drug for oral use. This rule is conditional because there are over time drugs that do not cover some of its postulates.

Data from *Lipinski's rule* are compared with those of the adapted *Ghose Filter* (Azad, Nasibullah, Khan, Hassan, & Akhter, 2018) and *CMC-50-Like Rule* (Kadam & Roy, 2007). The data are presented in tabular form, with the corresponding color identification, showing covering and / or exceeding requirements for each individual evaluation parameter: *molecular weight* (MW), partition coefficient (*LogP*), *H-bond acceptor* (HBA), *H-bond donor* (HBD), *Atom Molar Refractivity* (AMR) and *number of atoms in the molecule* (nAtom). This is necessary to more precisely clarify the genesis of the deviations, for each indicator.

It is expected that *LogP* values in *Ghose Filter* and *CMC-50-Like Rule* and the molecular weight in *CMC-50-Like Rule* directly reflect more on the time of penetration through the stomach wall. In cancer patients, there is often a deviation from the normal physiology of the stomach, so it is assumed that the deviations are not drastic and reflect more on the individual anamnesis of the patient.

3.3.3.1.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Regardless of the analysis in ***§3.3.3.1.1*** an analytically differentiable is conducted with regard to methodologies for evaluation of medicinal products (Ani, Anand, Sreenath, & Deepa, 2020), based on *total polar surface area* (TPSA), *number of rotatable bonds* (nRB), *rotatable bond count* (RC), *number of rigid bonds* (nRingidB), *MW, nAcidGroup* and *number of hydrogen bonds* (nHB), via *Weber Filter, MDDR-Like Rule* and *BBB Likeness*.

Here, the goal is to evaluate chemical molecules by a set of variables not affected by the partition coefficient (*LogP*), but at the same time taking into account the influence of *molecular weight* (part of *BBB Likeness*).

The color identification of the results specified in ***§3.3.3.1.1***, shall be applied again in a tabular form.

The information obtained from this set of empirical rules cannot be interpreted unambiguously. These are molecules have a larger *associated volume* and a *total number of bonds* (and hence axes and points of rotation). Much of the molecule is occupied by a carbohydrate residue, which in most cases does not slow down the action of the drug in the blood, simply prolongs the time spent in the stomach. Suffice it to say that no drastic differences (in orders of magnitude) of deviations are expected.

3.3.3.1.3. QED

The methods used in ***§3.3.3.1.1*** and ***§3.3.3.1.2*** are based on physicochemical parameters which are considered separately. Using the *Quantitative Estimate of Druglikeness* (QED) methodology, an approximation of the interchangeability of individual indicators is applied.

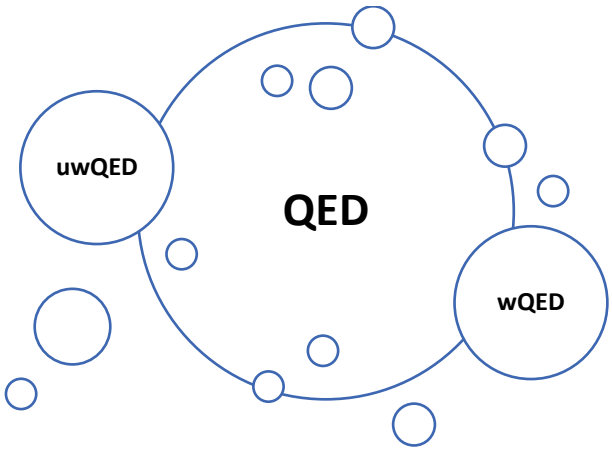


Fig. III.3. 6 *Dependence between QED with uwQED and wQED*

They enter into a general linear relationship (***Fig. III.3. 6***) and give a solution in the form of a scalar coefficient. This greatly facilitates the comparison of druglikeness properties of individual molecules of a homologous order. For this purpose, other empirical and/or calculated variables are added: *octanol-water* partition coefficient (AlogP), *numbers of Structural alerts* (sAlerts) and *numbers of Aromatic bond count* (nAromaRing).

They are two separate forms of evaluation that appear next (Jablonsky, Haz, Burcova, Kreps, & Jablonsky, 2019):

A. Unweighted Quantitative Estimate of Druglikeness Unweighted Quantitative Estimate of Druglikeness (UwQED):

UwQED is defined as (***Equat.III. 1***):

Equat.III. 1

$$UwQED = exp(\frac{1}{n}\sum_{i=1}^{n} ln\ ln\ d_i\ )$$

where: d is the individual desirability function, and *n* is the number of descriptors.

B. Weighted Quantitative Estimate of Druglikeness

Weighted Quantitative Estimate of Druglikeness (wQED) represents the functional dependence (***Equat.III. 2***):

Equat.III. 2

$$wQED = exp(\frac{\sum_{i=1}^{n} w_i ln\, d_i}{\sum_{i=1}^{n} w_i})$$

where: *d* is the individual desirability function, *w* is the weight applied to each function and *n* is the number of descriptors.

3.3.4. Non-laboratory and non-clinical information on the chemical form

Activities as a factor in conducting analysis, we could divide it into two main groups (***Fig.III.3. 7***): those that require time for the process (carcinogenicity and mutagenicity) and those that must be considered in real-time (drug absorption, toxicity and receptor activity).

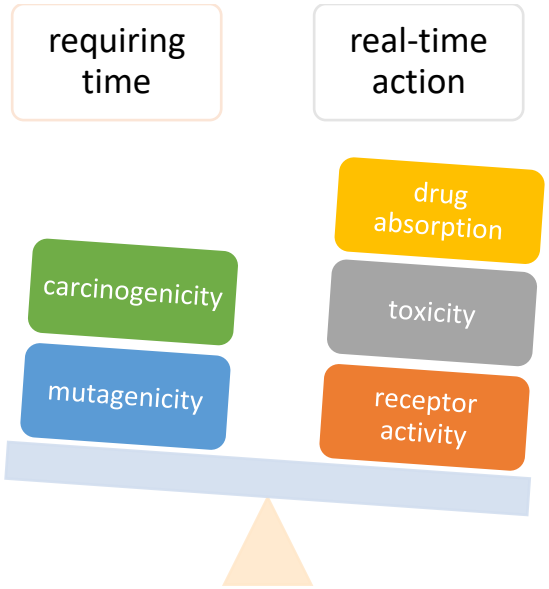


Fig.III.3. 7 *Factors influencing the overall assessment when conducting analysis for data obtained in non-laboratory and non-clinical methods*

This is an important circumstance, which is also valid in the case of real conservative treatment of a patient with the studied pharmaceutical forms.

3.3.4.1. Receptor activity

To achieve this goal, a model of qualitative prognosis of 12 classical endocrine abnormalities associated with the so-called receptor-mediated endocrine disruptions. They include: receptor-mediated endocrine disruptions. They include: *Androgen Receptor* (AR) (Davey & Grossmann, 2016), *Estrogen Receptor a* (ERa) & *Estrogen Receptor b* (ERb) (Paterni, Granchi, Katzenellenbogen, & Minutolo, 2014), *Glucocorticoid Receptor* (GR) (Nicolaides, Chrousos, &

Kino, updated 2020 Nov 21), *Mineralocorticoid Receptor* (MR)[5] (Gomez-Sanchez & Gomez-Sanchez, 2014), *Progesterone Receptor* (PR) (Scarpin, Graham, Mote, & Clarke, 2009) (Jacobsen & Horwitz, 2012), *Retinoic Acid Receptor a* (RARa) (Wang, et al., 2017), *Retinoic Acid Receptor b* (RARb) (Busby & Burris, 2012) (Doan, et al., 2020), *Retinoic Acid Receptor r* (RARr) (le Maire, et al., 2012) (le Maire, Teyssier, Balaguer, Bourguet, & Germain, 2019), *Thyroid Hormone Receptor a* (TRa) (Moran & Chatterjee, 2015) (Keijzer, et al., 2007), *Thyroid Hormone Receptor b* (TRb) (Cömert, et al., 2010) and *Vitamin D Receptor* (VDR) (Kato, 2000) (Pike & Meyer, 2010).

Each of them is directly related to the normal physiology of the body. The change of any hormonal regulation can lead to complications during treatment, and in case of larger deviations to permanent damage to the organism (including death).

After the analysis of literature data from scientific articles and reports (Hall & Greco, 2019) (Evans & Mangelsdorf, 2014), for the needs of the analysis the so-called *Nuclear Receptor-mediated Endocrine Activity* (NRMEA) Model[xlvi], part of the VEGA software package (***§III.1.1.3***).

The data are presented in tabular form, applying a color estimate of the result obtained. Exact values are not displayed due to incompatibility of dimensions and minimization of mathematization. The color scheme is: green - meets the requirements for inertia to the respective receptor, orange - is active and colorless - there is not enough data and/or the data cannot be unambiguously interpreted.

3.3.4.2. Mutagenicity

The study for possible mutagenic activity includes QSAR methodologies based on empirical information on already reported molecules and/or their fragments and some topological, physical and physicochemical molecular dependencies.

They are conditionally divided (***Fig.III.3. 8***) into two groups: Stand-alone models (CAESAR, SarPy / IRFMN, ISS and KNN / Read-Across) and the unifying Consensus model.

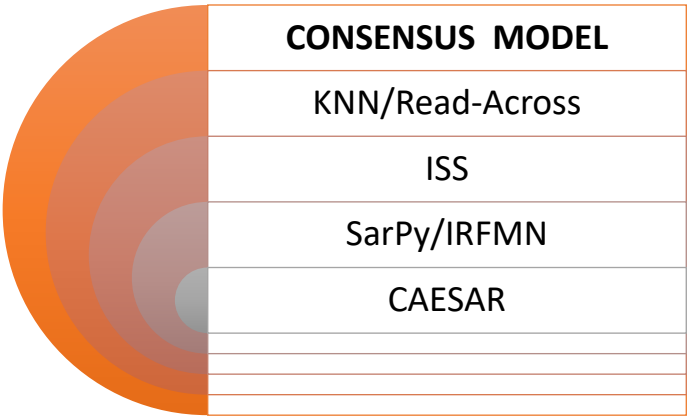


Fig. III.3. 8 *Relationship between Stand-alone models and Consensus model in the assessment of a molecular form for mutagenicity*

[5] Not every compound can be subjected to this analysis. The training set is not adapted to glycosidic derivatives.

A. Stand-alone models

It is also important to eliminate the possibility that the tested pharmaceutical forms, especially in the case of a continuous example, may lead to mutagenic abnormalities. For the purposes of the present study, *Ames*[xlvii] mutagenicity (Ames, Durston, Yamasaki, & Lee, 1973) (Mortelmans & Zeiger, 2000) gives a sufficiently reliable result, in a form convenient for interpretation. Due to the relative freedom of application of the method, sub-methods have been created, which insert into the conclusions and additional knowledge that we have accumulated after its creation.

Data on the structural signals (for the respective sub-methods) for mutagenicity and carcinogenicity are derived from the article: (Benigni, Bossa, Jeliazkova, Netzeva, & Worth, 2008).

a) CAESAR

The model (Ferrari & Gini, An open source multistep model to predict mutagenicity from statistical analysis and relevant structural alerts, 2010) provides a qualitative prediction[6] for mutagenicity. The area of applicability of the forecasts is assessed using *a domain applicability index* (ADI), which gives values from 0 (for an indicator that is as close as possible to a negative answer) and to 1 (for a maximum approach to a positive one). The ADI is calculated by grouping several other indices, each of which takes into account a specific "applicability problem". Most of the indices are based on the calculation of the most similar compounds found in the database to the respective model, i.e. relies on the available set of empirical data in it.

For each index, including the final ADI, three intervals are defined for its values, so that the first interval corresponds to a positive assessment, the second to a presumed assessment, and the last to a negative one.

The subsequent process in the method algorithm takes into account all components of the applicability domain, along with their values and intervals between them. For the purposes of estimating the accuracy and compliance index, the prediction of a 'presumed mutagenic' is considered a 'mutagenic'.

The individual indices are:

- Similar molecules with known experimental value (SMKEV): this index takes into account how similar the first three most similar compounds found are. Values close to 1 mean that the predicted compound is well represented in the data set used to build the model, otherwise the prediction may be extrapolation. The defined intervals are: at $1 \geq$ index > 0.85 - strongly similar compounds with known experimental value were found in the database set; $0.85 \geq$ index > 0.7 - "moderately similar" compounds with known experimental value were found and at index ≤ 0.7 - no similar compounds with known experimental value were found in the set of molecules;

[6] **CAESAR model statistics:** The following statistics are obtained, applying the model to its initial data set: ● Training set: n = 3253; Accuracy = 0.92; Specificity = 0.86; Sensitivity = 0.97 ● Test set: n = 798; Accuracy = 0.83; Specificity = 0.74; Sensitivity = 0.90. Compounds intended as a suspicious mutagen have been omitted from this statistic. Structural signals for putative mutagenicity were identified as follows: ● In the training set: n = 114 (18 mutagenic compounds, 96 non-mutagenic compounds) ● In the test set: n = 39 (19 mutagenic compounds, 20 non-mutagenic compounds)

- Accuracy of prediction for similar molecules (APSM) (Zubatyuk, Smith, Leszczynski, & Isayev, 2019): This index takes into account the accuracy of classification in predicting the three most similar compounds found. Values close to 1 mean that the analyzed compounds fall into an area of the model space where reliable predictions are given (i.e. without erroneous classifications), otherwise the lower the value, the greater the deviation from the model. The defined intervals are: 1 ≥ index > 0.9 - the accuracy in predicting such molecules is good; 0.9 ≥ index > 0.5 - the accuracy in predicting such molecules is average and index ≤ 0.5 - here it is low;

- Concordance for similar molecules (CSM): This index takes into account the difference between the predicted and experimental values of the three most similar compounds. Values close to 0 mean that the forecast made does not correlate with the values found in the model space, which is why the forecast is considered unreliable. The defined intervals are: 1 ≥ index > 0.9 - experimental data of similar molecules are available, which agree with the calculated value; 0.9 ≥ Index > 0.5 - similar molecules were found whose experimental data partially covered the calculated values and index ≤ 0.5 - similar molecules were found whose experimental data did not overlap with the calculated value;

- Atom Centered Fragments similarity check (ACFSC) (Kühne, Eber, & Schürmann, 2009) (Batista, Tan, & Bajorath, 2010): This index detects the presence of one or more fragments that are not found in the training kit and/or are rare. Fragments from the center of the active atom of the first order of all molecules in the set of experimental data are calculated, after which similar fragments in the analyzed molecule are calculated; then the index is calculated as follows: the first index (conditionally written as "RARE") takes into account rare fragments (those that occur less than three times in the set of molecules). It acquires a value of 1 if no such fragments are found, 0.85 if up to two fragments are found, 0.7 if more than two fragments are found; the second index (conditionally written as "NOTFOUND") takes into account fragments not found. Respectively, the value is 1 if no such fragments are found, 0.6 if such a fragment is found and 0.4 if more than one fragment is found. The operation ends with the generation of the final index, with answers: "rare" and "non-core". The definition intervals are: index = 1 - all atomically centered fragments are found in the database; 1 > index ≥ 0.7 - any of the atom-centered fragments of the compound were not found in the available set of molecules and index = 0.7 - no atom-centered fragments were found in the test molecule and / or rare fragments;

- Model's descriptors range check (MDRC) (Votano, et al., 2004): This index checks whether the descriptors calculated for the predicted compound are in the range of the descriptors of the molecules in the database (training set). The index takes a value of 1 - if all descriptors are within range and 0 - if at least one descriptor is out of range. The defined intervals are respectively: index = true and index = false;

- Global AD Index (GADI) (Votano, et al., 2004): The final index takes into account all the previous ones in order to summarize the assessment of the applicability of the analyzed compound. The determination intervals are: 1 ≥ index > 0.9 - the analyzed molecule is in the applicability of the model; 0.9 ≥ index> 0.7 - the result is not eloquent and index ≤ 0.7 - the analyzed molecule is outside the applicable area of the model. Since GADI is not related to the standard set and goes a little out of the model, it will be presented first in the scoreboard.

Data are presented in tabular form with the corresponding color indexation of the values: green - the compound is classified as non-mutagenic, red - the compound is classified as mutagenic and yellow - impossibility of unambiguous interpretation.

b) SarPy/IRFMN

The model is built with a set of instructions from CAESAR (see ***§a***) (Ferrari, et al., 2013). The initial model has been further developed, leading to a set of instructions for mutagenicity (up to 112) and non-mutagenicity (up to 93). If at least one mutagenicity rule matches the test compound, a "mutagen" prediction is given; if only one or more non-mutagenicity rules match, a "non-mutagenic" prediction is given; "Possible non-mutagenic".

The definition of the indices is identical to that for CAESAR, but with new intervals:

- SMKEV: $1 \geq$ index > 0.8 - the accuracy in predicting such molecules is good; $0.8 \geq$ index > 0.6 - the accuracy in predicting such molecules is average and index ≤ 0.6 - here it is low;
- APSM, CSM, ACFFSC: are identical to those of CAESAR;
- GADI: is identical to that of CAESAR, only the lower limit is reduced from 0.7 to 0.65.

IMPORTANT: MDRC - absent from the method!

The data are presented in the form of the main method.

c) ISS

The ISS model was developed as a set of rules similar to those of SarPy / IRFMN (Benigni & Bossa, Mechanisms of chemical carcinogenicity and mutagenicity: a review with implications for, 2011) (Benigni, Bossa, & Tcheremenskaia, In vitro cell transformation assays for an integrated, alternative assessment of carcinogenicity: a databased, 2013).

ISS applies all SarPy / IRFMN mutagenicity rules. If at least one rule coincides with a test compound, a prognosis is "mutagenic", otherwise "non-mutagenic".

All indexes and color identifications are identical to those of SarPy / IRFMN.

d) KNN/Read-Across

The model re-examines a data set[7] (Hansen, et al., 2009). It is very close to ISS and SarPy/IRFMN. Uses identical definition of intervals and their color representation.

[7] **KNN/Read-Across model statistics**: Statistics obtained then, applying the reading forecast through the initial data set, with an initial approach (re-reading for each compound was performed on the whole data set without the compound itself) ● n = 5764; Accuracy = 0.80; Specificity = 0.76; Sensitivity = 0.83 ● Unpredictable compounds: n = 6

Then obtained statistics applying the reading forecast through the initial data set, with a baseline approach (re-reading for each compound was performed on the whole data set without the compound itself).

B. Consensus model

For the performance of a complex assessment (according to Ames) of the data from the available methods (CAESAR, SarPy, ISS and KNN) the so-called consensus model of mutagenicity (Votano, et al., 2004). The algorithms in this model compare the solutions of each model, find their repetitive results and the severity of the final answer is determined not so much by the number of matches as by the "accumulated" in mathematical expression deviations from the general provisions of each method.

The assessment of the applicability of the result is converted into a numerical value in the range [0, .., 1], in the following scheme: experimental value - [1]; high reliability - [0.9]; moderate reliability - [0.6] and low reliability - [0.2]. For each prediction class (i.e. mutagenic or non-mutagenic), the result is calculated as the sum of the values for each model that created this prediction. For the purposes of the consensus approach, "putative mutagenic/non-mutagenic" predictions are considered as "mutagenic/non-mutagenic" predictions. The calculated result is normalized to the number of models used so that there is a theoretical range [0,..,1], followed by the class of predictions with the highest score is considered consensus forecasting. However, if a model has found an experimental value, it is considered final. In each case, the model always generates a response with a lower value, i.e. closer to mutagenicity

Data are presented in tabular form with the corresponding color indexation of the values: *green* - the compound is classified as non-mutagenic, *red* - the compound is classified as mutagenic and *yellow* - impossibility of unambiguous interpretation.

3.3.4.3. Carcinogenicity

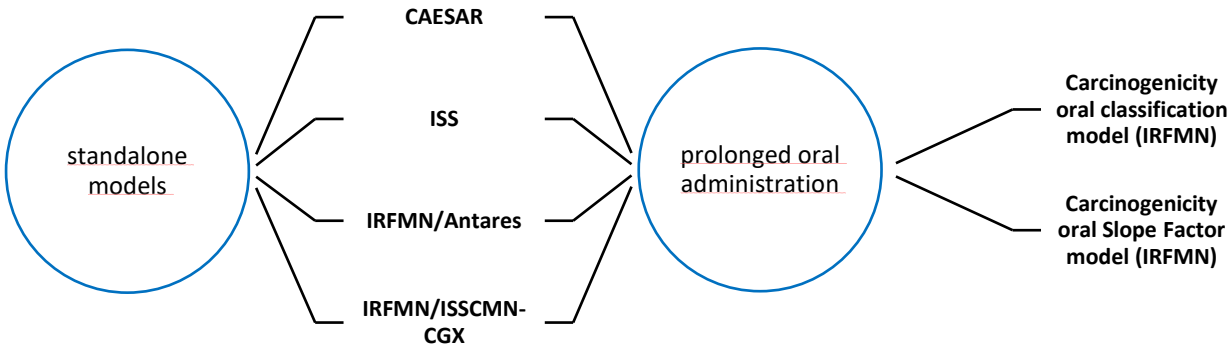


Fig. III.3. 9 Relationship between Stand-alone models and Prolonged oral administration in the assessment of a molecular form for carcinogenicity

The study for the manifestation of possible carcinogenicity of the studied molecules in non-laboratory and non-clinical conditions (***Fig.III.3. 9***) is carried out in two directions: Stand-alone models (CAESAR, ISS, IRFMN/Antares and IRFMN/ISSCMN-CGX) and prolonged oral

administration (Carcinogenicity oral classification model (IRFMN) and Carcinogenicity oral Slope Factor model (IRFMN)).

A. stand-alone models

a) CAESAR

The model (Fjodorova, Vračko, Novič, Roncaglioni, & Benfenati, 2010) (Fjodorova & Novič, Comparison of criteria used to access carcinogenicity in CPANN QSAR models versus the knowledge-based expert system Toxtree , 2014) is built as an artificial neural network for counteraction of distribution (CP ANN)[8]. The output of the neural network consists of two values, denoted as "positive" and "non-positive", which are in the range [0,..., 1] and their sum is equal to one. They represent the extent to which the neuron into which the test compound belongs to the class of "carcinogenic" or "non-carcinogenic" compounds. The higher between the two values leads to the forecast. Descriptors are calculated from values presented in (Todeschini & Consonni, 2009).

The definition of the individual indices is identical to that of CAESAR for mutagenicity, but at certain intervals:

- SMKEV: at $1 \geq$ index > 0.8 - strongly similar compounds with known experimental value were found in the database set; $0.8 \geq$ index > 0.6 - "moderately similar" compounds with known experimental value were found and at index ≤ 0.6 - no similar compounds with known experimental value were found in the set of molecules;

- APSM: $1 \geq$ index > 0.9 - the accuracy in predicting such molecules is good; $0.9 \geq$ index > 0.5 - the accuracy in predicting such molecules is average and index ≤ 0.5 - here it is low;

- CSM: $1 \geq$ index > 0.9 - experimental data of similar molecules are available, which agree with the calculated value; $0.9 \geq$ index > 0.5 - similar molecules were found, whose experimental data partially overlap with the calculated values, and index ≤ 0.5 - similar molecules were found, whose experimental data did not overlap with the calculated value;

- ACFSC: $1 >$ index ≥ 0.7 - any of the atom-centered fragments of the compound, not found in the available set of molecules and index = 0.7 - no atom-centered fragments found in the test molecule and/or rare fragments;

- MDRC: 1 - if all descriptors are within range and 0 - if at least one descriptor is out of range. The defined intervals are respectively: index = true and index = false;

[8] ***CAESAR Ccarcinogenicity Model statistics:*** The following statistics are obtained, applying the model to its initial data set: ● Training set: n = 645; Accuracy = 0.87; Specificity = 0.86; Sensitivity = 0.89 ● Test set: n = 161; Accuracy = 0.67; Specificity = 0.61; Sensitivity = 0.72

- GADI: 1 ≥ index > 0.8 - the analyzed molecule is in the applicability of the model; 0.8 ≥ index > 0.6 - the result is not eloquent and index≤0.6 - the analyzed molecule is outside the applicable area of the model.

Two new indices are also introduced here:

- MCAR: This index checks whether the two neural network outputs (positive and non-positive) lead to an unreliable prediction; when the difference between these two values is less than 0.1, the neuron where the test compound falls cannot provide a good classification, so the index is set to 0. Otherwise, the index is set to 1, i.e. index = 1 - the task of the model class is well defined and for index = 0 - here it is uncertain;

- Neural map neurons concordance (NMNC): This index checks the compliance of the analyzed compound with the experimental values of the compounds from the database that fall into the same neuron. The index is constructed taking into account two sub-indices: population and concordance. Low values mean that the predicted compound falls within a neural network area that has no experimental compounds and / or that has those but with heterogeneous ones, leading to low reliability. The defined intervals are: index = 1 - the predicted value agrees with the experimental ones of the compounds from the database, applied in the same neuron; index = 0.75 - the predicted value does not agree with the experimental ones from the database placed in the same neuron and index = 0.50 - the analyzed substance falls into a neuron in which there are no similar compounds in the database.

Data are presented in tabular form with the corresponding color indexation of the values: green - the compound is classified as non-carcinogenic, red - the compound is classified as carcinogenic and yellow - impossibility of unambiguous interpretation.

b) ISS

In essence, the model is identical to that of the ISS in mutagenicity (Benigni, Bossa, & Tcheremenskaia, Nongenotoxic carcinogenicity of chemicals: mechanisms of action and early recognition through a new set of structural alerts, 2013).

The model applies[9] all rules related to carcinogenicity, but not the full decision tree. If at least one carcinogenic rule coincides with the test compound, a prognosis for 'carcinogenic' is given, otherwise the expression 'non-carcinogenic' is generated.

The SMKEV, APSM, CSM and ACFFSC indices assume the same defined intervals and color identifications as for CAESAR for carcinogenicity. In GADI alone, the internal response limits are 0.90 and 0.65.

c) IRFMN/Antares

The model is made up of a set of rules[10] and a database from the Antares[xlviii] project and its like. It includes data from the chemical carcinogenesis of rats with 127 structural warnings. If at

[9] ***ISS Carcinogenicity Training set:*** n = 797; Accuracy = 0.76; Specificity = 0.58; Sensitivity = 0.81
[10] ***IRFMN/AntaresTraining carcinogenicity set:*** n = 1543; Accuracy = 0.66; Specificity = 0.48; Sensitivity = 0.82

least one rule coincides with the studied molecule, a "carcinogenic" prognosis is given. Otherwise, the prognosis is "possible non-carcinogenic".

The SMKEV, ACFFSC and GADI indices assume the same defining intervals and color identifications as for CAESAR for carcinogenicity. Only the internal limits for APSM and CSM are 0.80 and 0.60.

d) IRFMN/ISSCMN-CGX

The model[11] is built as a set of rules obtained from the union of the ISS database and the CGX[xlix] data set. A cross-validated procedure is applied, ending with the extraction of a set of 43 rules (structural warnings) related to carcinogenic activity, representing molecular fragments.

If at least one rule matches the test compound, a "carcinogenic" prediction is given, otherwise a "possible non-carcinogenic" prediction is given.

The SMKEV, ACFSC, APSM, CSM and GADI indices assume the same definition intervals and color identifications as for IRFMN / Antares for carcinogenicity.

B. Prolonged oral administration

a) Carcinogenicity oral classification model (IRFMN)

The model[12] was developed using the RAIS risk information system toxicity database. The data cover different categories of different categories of substances, including organic and inorganic.

An internal tool (Manganaro, 2020), developed in the statistical platform R, was used to select the best set of descriptors and size to be used for the final model. The approach is based on pre-selection technology: starting from the descriptor most correlated with the experimental data, a descriptor leading to the best model (among all available descriptors) is added to each iteration, up to the size of 25 descriptors. The models were constructed using linear discriminant analysis (LDA) modeling and were applied with a starting band cross-checking approach (n = 100 iterations). The fitness function was calculated for each model as a linear combination of the average values of accuracy, sensitivity and specificity obtained from the models built into each iteration of the bootstrap. This function is used to select the best descriptor to add to move to the next iteration.

The GADI, SMKEV, APSM, and CSM indices assume the same definition intervals and color identifications as for IRFMN / Antares for carcinogenicity, and the MDRC and ACFSC as for CAESAR.

[11] ***IRFMN/ISSCMN-CGX carcinogenicity Model statistics:*** The following statistics are obtained, applying the model to its initial data set: ● Training set: n = 986; Accuracy = 0.73; Specificity = 0.60; Sensitivity = 0.785
[12] ***IRFMN statistics:*** training kit (593 chemicals) Accuracy = 0.81 Sensitivity Specificity = 0.82 = 0.79

b) Carcinogenicity oral Slope Factor model (IRFMN)

The model[13] is close to the Carcinogenicity oral classification model (IRFMN) and is described in detail (Benfenati, Roncaglioni, Lombardo, & Manganaro, 2019).

The selection of characteristics was performed with the package "gaselect" R, which implements a partial algorithm with the least square (PLS-GA) and re-double cross-checking for statistical evaluation of a subset of descriptors. The following settings were applied to PLS-GA: initial population 2000; number of iterations 5000; minimum number of variable 5; maximum number of variables 12. Optimal subsets of descriptors returned by the final iteration of the execution were used to derive a model using r^2 as a fitness function.

Here the forecast assumes a numerical value.

3.3.4.4. Toxicity

Assessing the toxicity of the studied molecules and their fragments requires a comprehensive examination of models that are as far apart as possible when conducting methodologies. In ***Fig.III.3. 10*** shows the methodological scheme followed in the analysis.

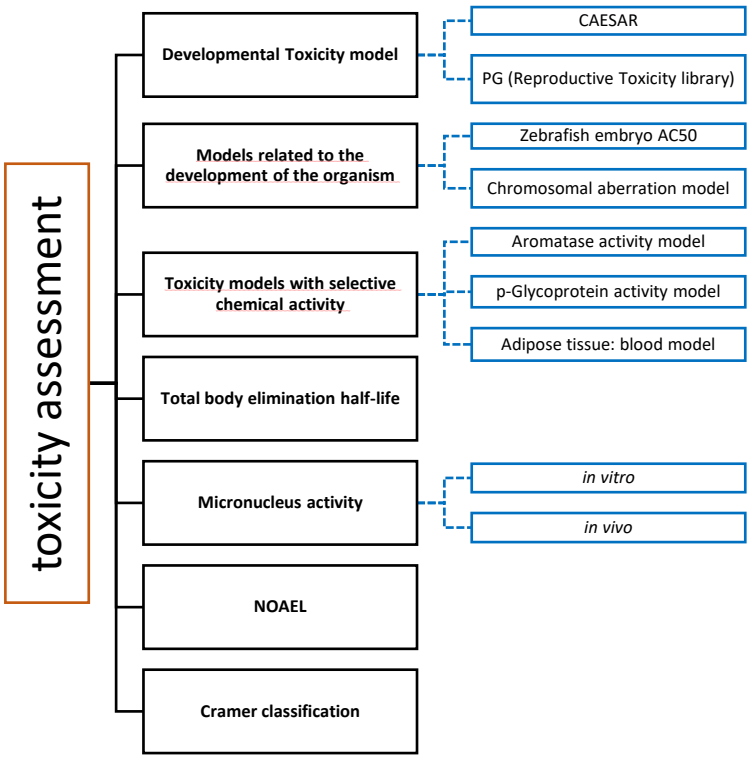


Fig. III.3. 10 Methodological scheme for assessing the toxicity of the studied molecular form

[13] ***Predictability Carcinogenicity oral Slope Factor model***- Statistics: obtained by external validation: Test set (32 chemicals) R2 = 0.573; RMSE = 1.28

A. Developmental Toxicity model

a) CAESAR

The model (Cassano, et al., 2010) is a QSAR classification model based on a Random Forest method, implemented using WEKA open-source libraries.

The analyzed variables[14] are classified in the order:

- SMKEV: Values close to or equal to one indicate that the test compound is well represented in the data set used in the construction of the model. When moving away from one - the resulting slenderness is extrapolated on the basis of overlapping molecular fragments. The defined intervals are: 1≥index> 0.8 - molecules and / or their fragments with strong similarity to the test substance were found; 0.8≥index> 0.7 - moderately similar molecular similarities were found; index≤0.7 - no similarities with the molecules from the training set;
- APSM: this index takes into account the classification accuracy when predicting two most similar compounds found in a training set. Values close to 1 mean that the predicted compounds fall within an area of the model space. The determination intervals are similar to those of SMKEV but are defined as: 1≥index> 0.8 | 0.8≥index> 0.6 | 0.6≥index;
- CSM: this index is a function between the predicted and experimental value of the two closest compounds in the training set data set. The closer the value is to zero, the more unreliable it is. The determination intervals are the same as for APSM;
- ACFSC: This index takes into account the presence of one or more fragments that are not found in the training set or are reported as very rare. Higher values obtained show better predictive overlap and more accurate end result. The algorithm is mathematical and is well described in the cited article. The defining intervals for it are: 1 = index | 1> index≥0.7 | index <0.7;
- MDRC: This expression checks whether the descriptors calculated for the test compound are in the range of the descriptors of the training set and test set. The index takes the value 1 (displayed as: true) if the descriptors are in the range and 0 (displayed as: false) if at least one descriptor is out of range;
- GADI: This index summarizes all the information from each index. Values closer to one indicate that the test substance is within the scope of the model. The determination intervals are: 1≥index> 0.8 | 0.8> index≥0.7 | index <0.7.

b) PG (Reproductive Toxicity library)

The attached Developmental / Reproductive Toxicity library (PG) model is part of the Vega QSAR package (§III.1.1.3). It is based on the study of Shengde Wu's team (Wu, et al., 2013).

The model is close to CAESAR toxicity and uses the same indices (GADI, SMKEV, APSM, CSM & ACFSC). The defining intervals for it are described in (Benfenati & Marzo, QMRF Title:Developmental/Reproductive Toxicity library (PG) (version 1.1.0), 2020).

[14] Training set: n = 234; Accuracy = 1.00; Specificity = 1.00; Sensitivity = 1.00 | Test set: n = 58; Accuracy = 0.84; Specificity = 0.59; Sensitivity = 0.95

B. Models related to the development of the organism

a) Zebrafish embryo AC50

This model was developed by a team of scientists (Toropova, et al., 2017), based on (Padilla, et al., 2012) and implemented by the QSAR package VEGA (§III.1.1.3.)

Due to the much more complex evaluation of the intermediate / working results (well described in the already presented articles), a color scheme for evaluation of the individual indicators is applied: GADI, SMKEV, APSM, CSM, MEPASM, MDRC and ACFSC. The final result is a baseline numerical value indicating at what concentration of the test substance there is a real risk of harm to the fetus. The higher concentration, in mg/l, illustrates higher inertness relative to the embryo.

b) Chromosomal aberration model (CORAL)

The Chromosomal aberration model analyzes the high level of chromosomal aberrations in peripheral blood lymphocytes, which is also an early marker of cancer risk, but data on the risk of specific cancers and types of chromosomal aberrations are limited. This study was conducted by a group of scientists (Toropov, Toropova, Ritano, & Benfenati, 2019), and the analysis of the putative anti-cancer molecular forms was performed in VEGA medium.

Each indicator (GADI, SMKEV, APSM, CSM, MEPASM, MDRC and ACFSC) is analyzed, a color scheme is used for evaluation and a conclusion is drawn about the activity.

C. Toxity models with selective chemical activity

a) Aromatase activity model

Aromatase (Shoombuatong, Schaduangrat, & Nantasenamat, 2018) is a rate-limiting enzyme for estrogen biosynthesis that is overproduced in breast cancer tissue. The VEGA software package (§III.1.1.3) is used to analyze active anti-cancer molecular forms.

When presenting the results in tabular form, a color evaluation of the values for each individual indicator (GADI, SMKEV, APSM, CSM and ACFSC) is applied, followed by an evaluation of the activity (Active Agonist, Active Antagonist and Inactive). The study ends with a prediction of the activity (according to the numerical values of the individual assessments).

b) P-Glycoprotein activity model

P-Glycoprotein (Lagares, Minovski, & Novič, 2019) is a transmembrane protein that actively transports a wide variety of chemically different compounds from the cell. It is strongly associated with ADMET (absorption, distribution, metabolism, excretion and toxicity) properties

of drugs / drug candidates and contributes to the reduction of toxicity by eliminating compounds from cells, thus preventing intracellular accumulation.

The model applied (Lagares L. , et al., 2020) in a VegaHub environment (***§III.1.1.3***) includes evaluation by indicators: GADI, SMKEV, APSM, CSM, MDRC and ACFSC. It is taken into account, etc. Euclidean Distance from the central neuron (Prajapati, et al., 2013), data[15] in training and test sets and is assumed.

c) Adipose tissue: blood model (INERIS)

Adipose tissue: blood model (INERIS): blood distribution is a key endpoint for predicting the pharmacokinetics of chemicals in humans and animals, as the affinities of other organs: blood can be assessed as a function of this parameter (Cappelli, Manganelli, Toma, Benfenati, & Mombelli, 2021).

The analysis (Benfenati E. , Adipose tissue:blood model (INERIS) - v. 1.0.0, 2020) is performed by calculation of parameter departments (GADI, SMKEV, APSM, CSM, MEPASM, MDRC and ACFSC), using color evaluation. LogK (in log units) is calculated, and hence K (in numerical units).

D. Total body elimination half-life (QSARINS)

This study was performed with the VEGA software package on the indicators: GADI, SMKEV, APSM, CSM, MEPASM, MDRC and ACFSC. LogHLt (in log units) is also predicted, and hence the Total half-life indicator (in min).

E. Micronucleus activity

Genotoxicity is the ability of an agent to cause DNA damage as an alteration in the structure or information content of genetic material in cells, including those that are permanently transmissible.

Micronucleus activity (IRFMN/VERMEER) QSAR test is based on (Ferrari, et al., 2013) and is performed through the VEGA software package. You study the indicators GADI, SMKEV, APSM, CSM and ACFS and come up with Prediction in terms of activity.

The assay is performed *in vitro* and *in vivo*.

[15] Considering only the “Inhibitor” class vs the remaining “Substrate” and “Non active”: Training set: n=1777 (+ 8 non predicted compounds); Accuracy=0.95; Specificity=0.95; Sensitivity=0.95, Test set: n=717 (+ 9 non predicted compounds); Accuracy=0.85; Specificity=0.86; Sensitivity = 0.84 | Considering only the “Substrate” class vs the remaining “Inhibitor” and “Non active”: Training set: n=1777 (+ 8 non predicted compounds); Accuracy=0.97; Specificity=0.98; Sensitivity=0.92, Test set: n=717 (+ 9 non predicted compounds); Accuracy=0.88; Specificity=0.93; Sensitivity=0.69

a) In vitro (Baderna & Benfenati, 2019)

b) In vivo

F. NOAEL

This method (Toropov, Toropova, & Benfenati, NOAEL (IRFMN/CORAL) - v. 1.0.0, 2020) involves testing drugs for oral administration to rats for 90 days. The 28-day tests are also methodologically compliant. The indicators GADI, SMKEV, APSM, CSM, MEPASM, MDRC and ACFSC are analyzed. The final result for Prediction is in -log(mg/kg), and from there in mg/kg.

G. Cramer classification (Jeliazkova & Benfenati, 2020)

3.3.5. Evaluation of the results

In the case of more than one homologous and/or isomeric representative of the test dosage form, it is necessary to differentiate, by correlation analysis (Mirkin, 2019) of the data used all the tested methods, the most optimal molecules.

3.3.6. Conclusion from the part

The selected pharmaceutical form is analyzed according to the indicators: Oral rat LD50 and Bioaccumulation factor. The aim is that the methods used to evaluate the molecules do not differ from the statistical processing. It concludes with lethal and cumulative doses.

Using statistical methods (Божанов & Вучков, 1973), (Devore, 2011) for optimization i.e. statement of the task is created. It involves finding such factor values (in this case, they overlap with the empirical results of each set of rules) in which to isolate the most optimal dosage form suitable for conservative treatment. For this purpose, we assume that the optimum of the objective function coincides with its extremum - minimum or maximum (by definition of the respective rule). Here we will deliberately eliminate the statistical disturbances of the analysis - it is not necessary to maintain chemical purity close to p.a. or higher. To avoid this difficulty, we will apply a "step-by-step" procedure: they will be analyzed in packages according to three rules (corresponding only in terms of their molecular weight, i.e. whether they swell or not) of the two molecular structures: amide or carboxyl. Here we do not take into account factors such as concentration, share relations, etc. quantitative variables.

In order to isolate one or at most two molecular forms, the components of the gradient are also determined by *Box & Wilson method* (Box & Wilson, 1951).

These results are obtained for the optimal molecular package (amide/carboxylic acid). A conclusion should be made about druglikeness for the proposed pharmaceutical forms. It is based on a comparison of each individual group of assays and optimally applicable molecules are derived. The final molecules were also compared for toxicity by *Hierarchical clustering method*

(Böcker, Derksen, Schmidt, Teckentrup, & Schneider, 2005) (HCM) in a T.E.S.T. (Martin, Harten, Venkatapathy, & Young, 2008), (EPA, 2020) for *Oral rat LD50* in the form (*equations III.3 & 4*).

Model ellipsoid:

Equat.III. 3

$$h_{00} = X_0^T (X^T X)^{-1} X_0$$

Test molecule must be within ellipsoid of descriptor values for model chemicals. The model ellipsoid constaint is satisfied if the leverage of the test compound (h_{00}) is less than the maximum leverage value for compounds.

Rmax:

Equat.III. 4

$$distance_i = \sum_{j=1}^{d} \left(x_{ij} - C_j\right)^2$$

Distance to the centroid of the cluster must be less than the maximum distance for any cluster molecule

where: Fragment constraints - Compounds in the cluster must have at least one example of each of the fragments contained in the test molecule. Not used for binary endpoints (i.e. mutagenicity)!

T.pyriformis IGC50 (48hr), Daphnia magna LC50 (48hr) and *Fathead minnow LC50 (96hr)* (Martin & Young, Prediction of the Acute Toxicity (96-h LC50) of Organic Compounds in the Fathead Minnow (Pimephales Promelas) Using a Group Contribution Method, 2001) are relatively more accurate in terms of *Coefficient of determination (R2)*. *Oral rat LD50* and *Bioaccumulation factor* are chosen because they give more general (not so profiled) results for clinical reactions, taking into account: Predicted value and Prediction interval. *Nearest neighbor (Nn)* in the form: is considered as a control sample confirming the dependence:

Three most similar molecules must exceed a minimum cosine (***equat.III.5***) similarity coefficient of 0.5:

Equat.III. 5

$$SC_{i,k} = \frac{\sum_{j=1}^{\#descriptors} . x_{ij} x_{kj}}{\sqrt{\sum_{j=1}^{\#descriptors} . x_{ij}{}^2 . \sum_{j=1}^{\#descriptors} . x_{kj}{}^2}}$$

The neighbors are those with highest similarity coefficient. All neighbors must exceed a minimum cosine similarity coefficient.

All coefficients, constants and other variables using a database (Martin, Lilavois, & Barron, Prediction of pesticide acute toxicity using two dimensional chemical descriptors and target species classification, 2017) of U.S. Pat. Environmental Protection Agency. HCM are accepted as the final result for analysis and subsequent interpretation of the results (Young, Martin, Venkatapathy, & Harten, 2008) (Benfenati, et al., 2009), (Zhu, Martin, Young, & Tropsha, 2009), (Sushko, et al., 2010), (Cassano, et al., 2010).

All values in the part are rounded to: integer for values over 10; up to a decimal value for results of 10÷1; up to hundreds of answers under 1.

Once we have determined the working concentrations in the event of treatment with the studied chemical molecules, it is necessary to more fully evaluate the active substances in order

to analyze and relate to other pathological influences. They can be studied through treatment models in the so-called no laboratory and no clinical testing.

3.3.7. Checking conclusion of the part

This part of the scientific evidence is based (Daina, Michielin, & Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, 2017) (Daina, Michielin, & Zoete, iLOGP: A Simple, Robust, and Efficient Description of n-Octanol/Water Partition Coefficient for Drug Design Using the GB/SA Approach, 2014) on the calculation of physico-chemical descriptors, as well as to predict the ADME parameters: Lipophilicity, water solubility, pharmacokinetics, drugliness and medical chemistry indicators.

In ***Tabl.III.3. 11*** shows the research structure of the conducted analysis.

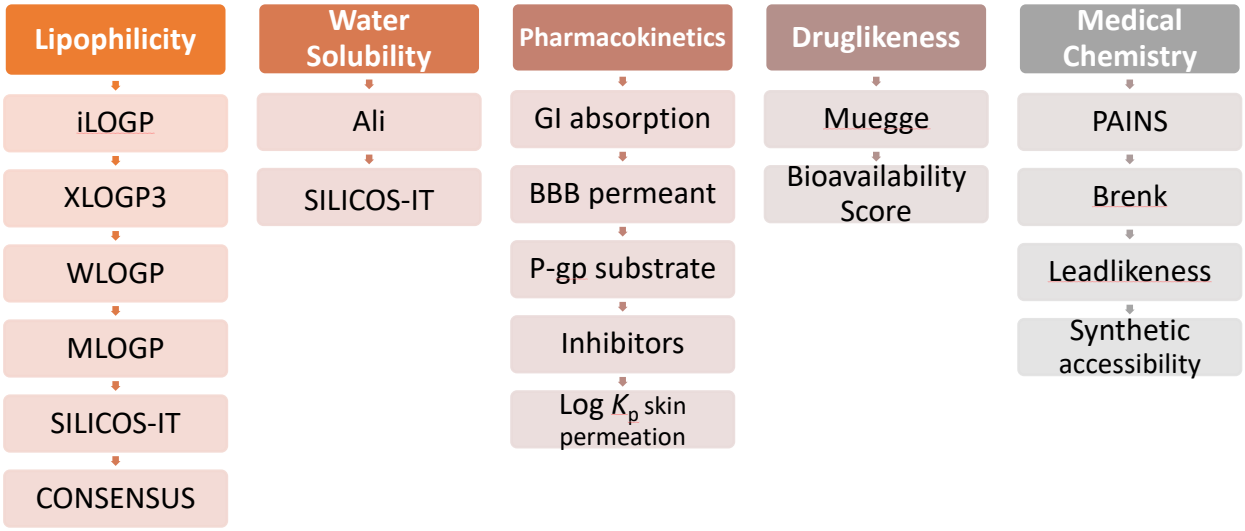


Fig. III.3. 11 *Structure for conducting an analysis to verify the conclusion of the part in the evaluation of modified molecular forms*

The ultimate goal of this operation is, through methods not applied in the methodology introduced so far, to check for significant deviations and/or to confirm the working conclusions. As a final answer, we get the exact molecular modified form, which already needs to be determined its dosage.

3.4. Determination of the drug dose

To determine the drug dose, we need to consider all possible substances obtained by the final hydrolysis of the glycosidic bond inside the cancer cell. For this purpose, it is necessary to calculate the concentration of active antitumor cell forms [§6 of Article 2]. Stoichiometric calculations based on data from [§5 of Article 1] shall be applied, using also the data on the mass

ratios of amide and carboxylic acid in the preparation of a natural precursor (in this case nitrile glycoside).

- ◊ -

IV. RESULTS AND INTERPRETATION

1. On the first goal

1.1. Hypothesis:

The bioactive form of amygdalin is it's hydrolyzed to amide nitrile group.

1.1.1. Evidences

1.1.1.1. Proof 1

Based on the chemical deposition of nitriles (***Fig.IV.1.1***) (Петров, 1996/2019):

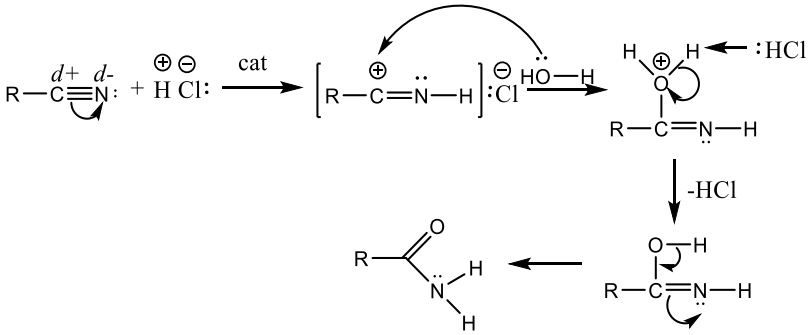


Fig.IV.1. 1 *Catalytic chemical hydrolysis of nitrile to amide in an acidic environment*

The catalyst for this reaction can only be non-coordinatively bonded *Co*. Cobalt in the human body is in the order of 22-59 nmol/l (in the age group 30-56 years for men and women) (Бошев, и др., 1986). Its daily requirement is 0.08 mg/day (for 1-2 year olds) and reaches 0.30 mg/day (for over 60 year olds). Let us clarify that cobalamin (known as *vitamin B12*) is made up of a corin ring that associates cobalt in its complexing form - therefore cobalt in cobalamin may NOT be a catalyst for the reaction.

Based on the fact that the total content of cobalt in urine is from 3.9 to 30 nmol/d, and that of cobalamin is 23.3 to 44.5 pmol/d, therefore there is a constant exchange of non-coordinated cobalt in the body.

After analyzing the foods consumed by *Hunza people*, those containing cobalt were clearly identified, but there were no traces of *vitamin B12*: spinach (0.07-1.20 mg/kg); beet leaves (0.39-0.41 mg/kg); onions (0.13 mg/kg); carrots (0.02 mg/kg) (FoodData, 1984/2004), etc.

Entered into the stomach uncoordinated cobalt (regardless of its form) will quickly dissolve under the action of hydrochloric acid and thus enter the blood, therefore, the times of admission of amygdalin and cobalt will not coincide. The amygdalin will enter the blood with its nitrile group from which it will release cyanide ion.

The spinach also contains significant amounts of oxalic acid (320 mg/kg). Oxalic acid forms insoluble cobalt oxalate (3.15×10^{-9} gr/100ml at 308K) with cobalt ions and therefore will not pass through the gastric walls to the blood.

After analysis and simulations (based on average enthalpy and potential energy of the molecule) by TD-DFT (Petersilka, Gossmann, & Gross, 1996), an extremely stable retention cycle of the non-coordinated cobalt in the stomach is shown in GAMESS US (Young D. C., 2001), shown in ***Fig.IV.1.2***.

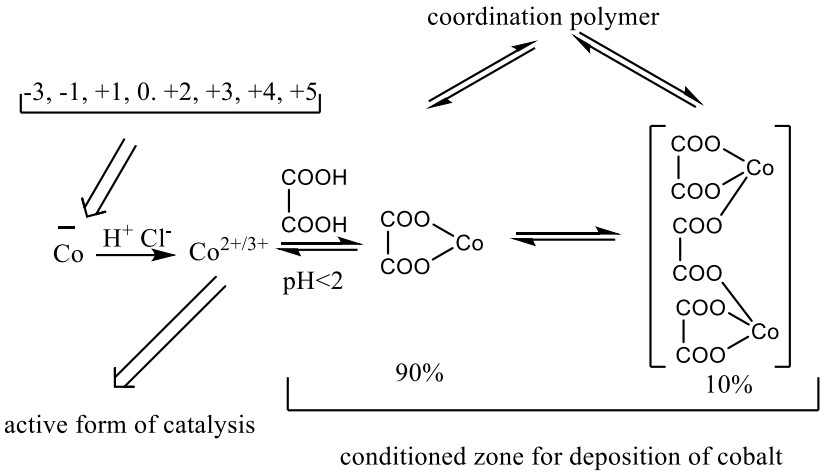


Fig.IV.1. 2 *Schematic depicting the physiological retention of cobalt in the stomach*

However, at pH <2, the oxalic salts are partially hydrolyzed and metal anionic (in this case cobalt) separated. The reaction is reversible and displaced in the direction of the starting material (cobalt oxalate). Thus, the residence time of cobalt in the stomach increases significantly. In addition to spinach, other cultivated plants typical of the area contain oxalic acid: tea (370 mg/kg), sorrel (360 mg/kg), rhubarb (240 mg/kg), fig (100 mg/kg), beet (40 mg/kg), plums (12 mg/kg), grapes (7 mg/kg), etc.

Under these conditions, the constantly consuming amygdalin has the conditions (concentration of reagents, catalyst, pH, temperature, etc.) to react to catalytic hydrolysis to its corresponding amide.

1.1.1.2. Proof 2

In addition to cobalt ions, the catalyst may also be cobalt linked to a covalent bond. For example, *nitrile hydratases* (*NHases; EC 4.2.1.84*). They are metal-containing enzymes that convert the nitrile and / or cyano group to amides, including *amygdalin*.

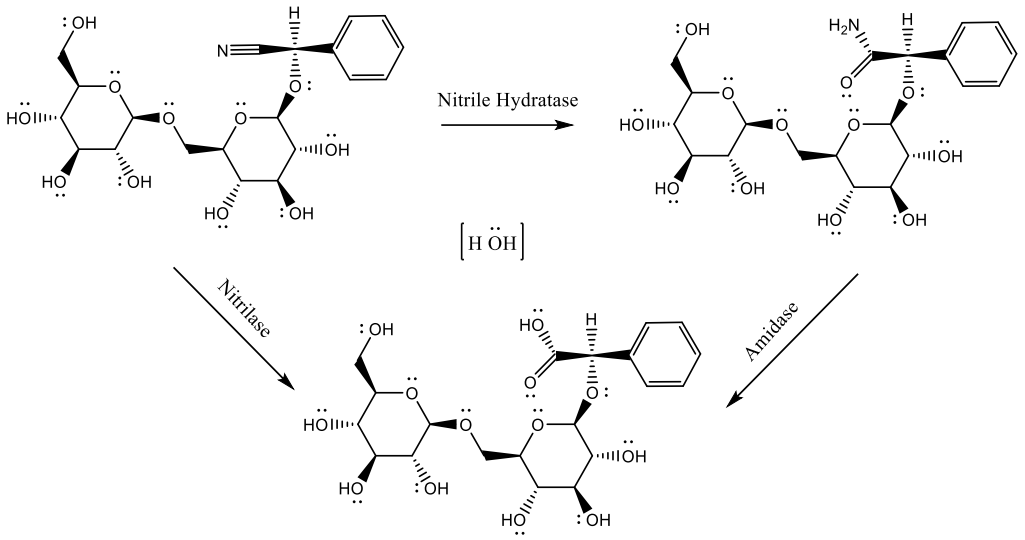


Fig.IV.1. 3 *Schematic diagram of the enzymatic hydrolysis of the amygdalin-nitrile group to its amides and carboxylic acid*

The optimum action of nitrile hydratase, for example from *Rhodococcus rhodochrous*, is from 5.5 to 8.5 pH . Their activity is highest in the range of 10-80 degrees, and their effect is from 20 minutes to 8 hours. The acidity in the stomach is much higher and from there these enzymes will be inhibited and even agglutinated.

The peoples studied have consumed large quantities of apricot and other fruit oils. Much of the prokaryotes and eukaryotes that produce nitrile hydratases live in lakes or in the moist environment around them. There is a possibility, due to technological processes, to enter and live for a long time in these oils. Thus, these insecurities may have lived in unprotected vessels and catalytically hydrolyze the amygdalin nitrile group to amide and/or carboxylic acid.

1.1.2. Conclusion of the part

Regardless of the manner of preparation of the hydrolyzed nitrile group of amygdalin to amide and / or carboxylic acid, its already modified form has a much safer chemical structure to the body. The absence of the nitrile group, which readily converts to the cyano ion, makes it possible to increase its constant concentration many times in the *in vivo* environment.

1.2. Determination of pharmaceutical form by mathematical chemical indicators

All further calculations were made after optimization with respect to the minimum energy of the molecules via *MM2* (Leach, 2001) and *MMFF94* (Halgren T. A., 1996) and followed by molecular dynamics responsible for 310 K, 1.02 bar in a medium of 0.9% solution of NaCl in water using CPCM (Liton, Ali, & Hossain, 2012) methodology.

1.2.1. Comparative analysis

For a more complete characterization of the hypothetical molecules, they were compared with the starting one (*amygdalin*). Comparisons were made with respect to: *Molecular Topology* (***Tabl.IV.1. 1.***), *Molecular Networks* (***Tabl.IV.1. 2***), electronic reference in the atomic-molecular system (***Tabl.IV.1. 3***) and physical and thermodynamic parameters (***Tabl.IV.1. 4***).

Tabl.IV.1. 1 *Comparative analysis of the molecular topology of pure amygdalin, its amide and carboxyl acid derivatives, resulting from the hydrolysis of its nitrile group*

indicator	-CN	-C(O)NH_2	-COOH
Balaban Index	1660564	1893740	1893740
Cluster Count	32	33	33
Molecular Topological Index	20391	21571	21342
Num Rotatable Bonds, [No. of bonds]	8	8	8
Polar Surface Area, [A^2]	202	221	216
Radius, atoms	8	8	8
Shape Attribute	30.03	31.03	31.03
Shape Coefficient	0	0	0
Sum Of Degrees	68	70	70
Sum Of Valence Degrees	124	128	130
Topological Diameter	15	15	15
Total Connectivity	2.14E-05	1.75E-05	1.75E-05
Total Valence Connectivity	8.47E-10	4.46E-10	3.46E-10
Wiener Index	3080	3308	3308

Tabl.IV.1. 2 *Partition coefficients of amygdalin and its hydrolysates of the nitrile group to amide and carboxylic acid in the 0.9% NaCl phase in water*

indicator	-CN	-C(O)NH_2	-COOH
LogP	-2.8	-3.7	-2.9
LogS	-0.05	0.22	-0.04
PKa			
O-1	16.64	16.74	18.80
O-2	17.41	17.41	17.59
O-3	12.89	12.96	13.07
O-4	13.03	13.03	13.03
O-5	18.99	19.01	16.98
O-6	20.67	20.67	20.67
O-7	14.94	14.94	14.94
OH(COOH)			3.03
Molar Refractivity	10.53	10.92	10.70

Tabl.IV.1. 3 *Electron Assignment in the Atomolecular System of amygdalin and its hydrolysates of the nitrile group to amide and carboxylic acid*

indicator	-CN	-C(O)NH_2	-COOH
Formal Charge	0	0	0
Number of HBond Acceptors	12	12	12
Number of HBond Donors	7	8	8
Principal Moment	1902 6153 6969	2042 6108 7033	2101 6066 7046
Henry's Law Constant	24.51	27.95	26.26

Tabl.IV.1. 4 *Molecular and electron-configuration properties of amygdalin and its hydrolysates of the nitrile group to amide and carboxylic acid obtained by semi-empirical methods*

indicator	method*	-CN	-C(O)NH_2	-COOH
Core-Core Repulsion [eV]	PM7	51868	56375	56412
COSMO Area [$Å^2$]		378.8	385.9	387.5
COSMO Volume [$Å^3$]		489.3	508.8	507.2
Dipole [Debye]		4.99	5.84	5.71
Ionization Potential [eV]		9.93	9.87	9.90
Total Energy [eV]		-6255	-6578	-6674
Heat Capacity [Cal/Mol-Kelvin]	PM6	96.3	100.2	97.0
Thermodynamic Energy [Kcal/Mol]		283.9	297.8	289.4
*- by 310.15K , 1.02 bar				

Data in ***Tabl.IV.1. 1÷4*** show that values (including their equated statistical error) over 23% of the indicators are the same. No drastic topological and molecular network deviations and electron-configuration anomalies are observed. The differences represent up to 12% mean deviation of the amygdalin index with its hydrolyzed derivatives.

1.2.2. Checking the statistical deviation

To test the tolerance of 12%$^{-th}$ still not jeopardize the normal physiology of the body, let us consider a well-studied enzyme hydrolytic industrial process (***Fig.IV.1. 4***) of a passage of *3-Cyanopyridine* to *Nicotinamide* (Nagasawa, Mathew, Mauger, & Yamada, 1988) (*vitamin B3*) by means of nitrile hydratase. The thermodynamic quantities are compared: *total energy, thermal capacity, thermodynamic energy*, since these physical functions are the consequence of most morphological, and electron-configuration relationships of each molecule in ***Tabl.IV.1. 4.***

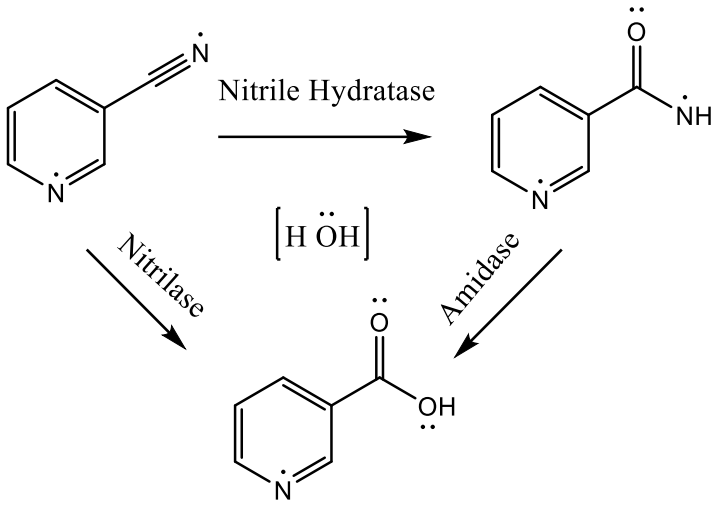


Fig.IV.1. 4 *Enzyme hydrolysis of 3-Cyanopyridine to Nicotinamide and nicotinic acid*

It is important to note that all three enzymes are almost always together, whether of micro-bacterial or bio-physicochemical origin.

Tabl.IV.1. 5 *Total Energy, Heat Capacity and Thermodynamic Energy on 3-Cyanopyridine, Nicotinamide and Nicotinic Acid by 310K, 1.02 bar in medium of 0.9% NaCl solution in water using CPCM methodology*

indicator	3-Cyanopyridine	Nicotinamide	Nicotinic acid
Total Energy PM7 [eV]	-1162	-1486	-1581
Heat Capacity [Cal/Mol-Kelvin]	16.7	19.8	20.4
Thermodynamic Energy [Kcal/Mol]	55.8	70.8	63.6

By comparing the data in ***Tabl.IV.1. 5*** with the conclusions of ***Tabl.IV.1.1÷4***, the average standard deviation is confirmed when comparing the molecular nature of the nitriles, the amide and their corresponding carboxylic acid.

1.2.3. Conclusion of the part

Due to the theoretical molecular modification, namely the transition from condensed and / or polymerized nitrile to a carbohydrate in its amide and as a by-product and its acid, it does not change the total activity of the starting compound and its active groups not involved in hydrolysis. The chemical stability and predictability of hydrolysis products are the basis for obtaining a pharmaceutical form covering all international requirements for a conservative medicinal product.

1.3. Determine drug dose

In order to determine the dosage, it is necessary to consider the most likely active forms of amide already *in vivo*.

In oral use (which is also recommended), the connection between the two glycosidic nuclei will be attacked by the salivary gland (mainly from *α-amylase*). Due to the relatively short time for complete contact between the substance and the enzyme, the probability of a reaction being below 3% and the probability of reading the reaction itself being less than 1%, it is assumed that amygdalin is unchanged in the stomach.

The amino derivative of *amygdalin* is relatively stable in a highly acidic environment and so over 87% will pass into the blood. Due to my poorly studied biochemical activity, let's look at the possible chemical relations of its active forms in those already described *in vitro* (Fig.IV.1. 5).

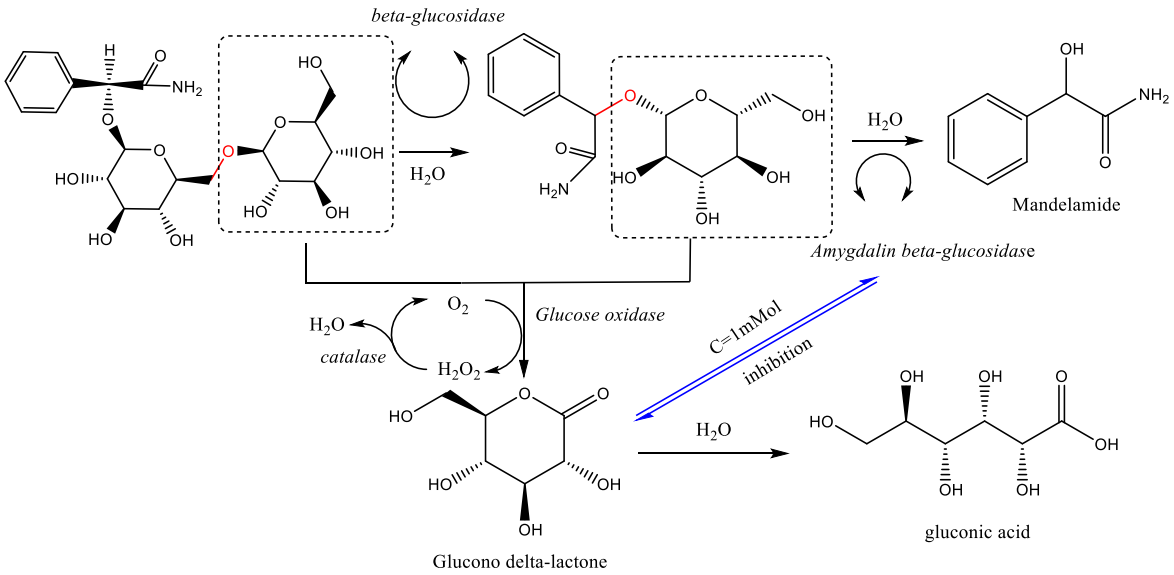


Fig.IV.1. 5 *Enzyme hydrolysis of hydrolyzed amygdalin to amide*

Each of the enzyme reactions has an analogue also in the *in vivo* medium, and thus the following hypothetical active forms stand out (***Fig.IV.1. 6***).

Chemical bond of type: -N(H)-OC(O)- between the two derivatives of amygdalin is possible, but it's a statistical error. Under standard physiological conditions in the body, the presence of the carboxyl derivative is not a hindrance factor, but on the contrary, it would stabilize the reactions of the amine derivative of amygdalin to some extent due to its well-expressed proton activity.

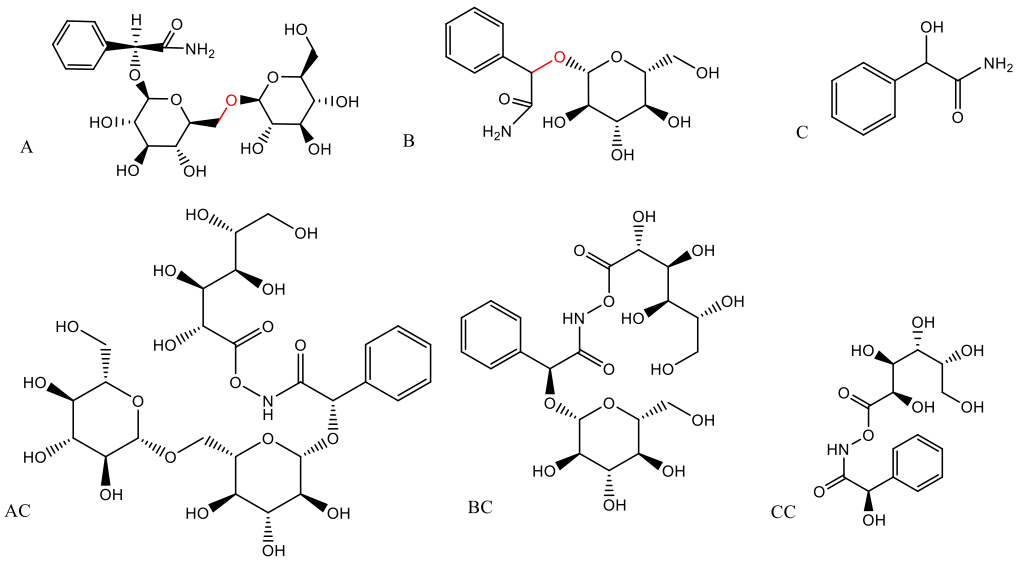


Fig.IV.1. 6 *Calculated active forms of hydrolyzed to the amine amygdalin by TD-DFT in an environment of GAMESS US*

As a by-product, their carboxyl derivatives are also obtained in the ratio:

-amide: -carboxyl = 4.87: 1.

After exposure of the above and adopting the approximation that the amide group will identify all of the activity of the active forms of the substance, it can be referred concentrations for the treatment of *pellagra* with nicotinamide to dose ranging and for the test substance. It is important to note that the approximation for nicotinamide is only made in terms of the mass of its amide group to the mass of the whole molecule (not evaluating the reactions of the nitrogen atom in the pyridine ring, as in the case of *Nicotinamide riboside*, etc.).

Stoichiometric calculations with respect to the fat of the active groups to the total mass of the molecule should indicate that the dosages should be:

150-375 mg PO q6-8hr; not to exceed 1.8 g/day.

1.4. Conclusion from the goal: Antitumor activity of the modified molecular form

The hydrolyzed to amide / carboxylic acid cyano / nitrile glycosides are potential drugs. Their biological activity remains unchanged, but their toxicity is many times lower than unmodified native molecules. The amygdalin/dhurrin-derived amide is only one of the dozens of studies we have conducted and we make this claim.

In Fig.IV.1. 7 depicts a summary scheme of theoretical derivation of reactions.

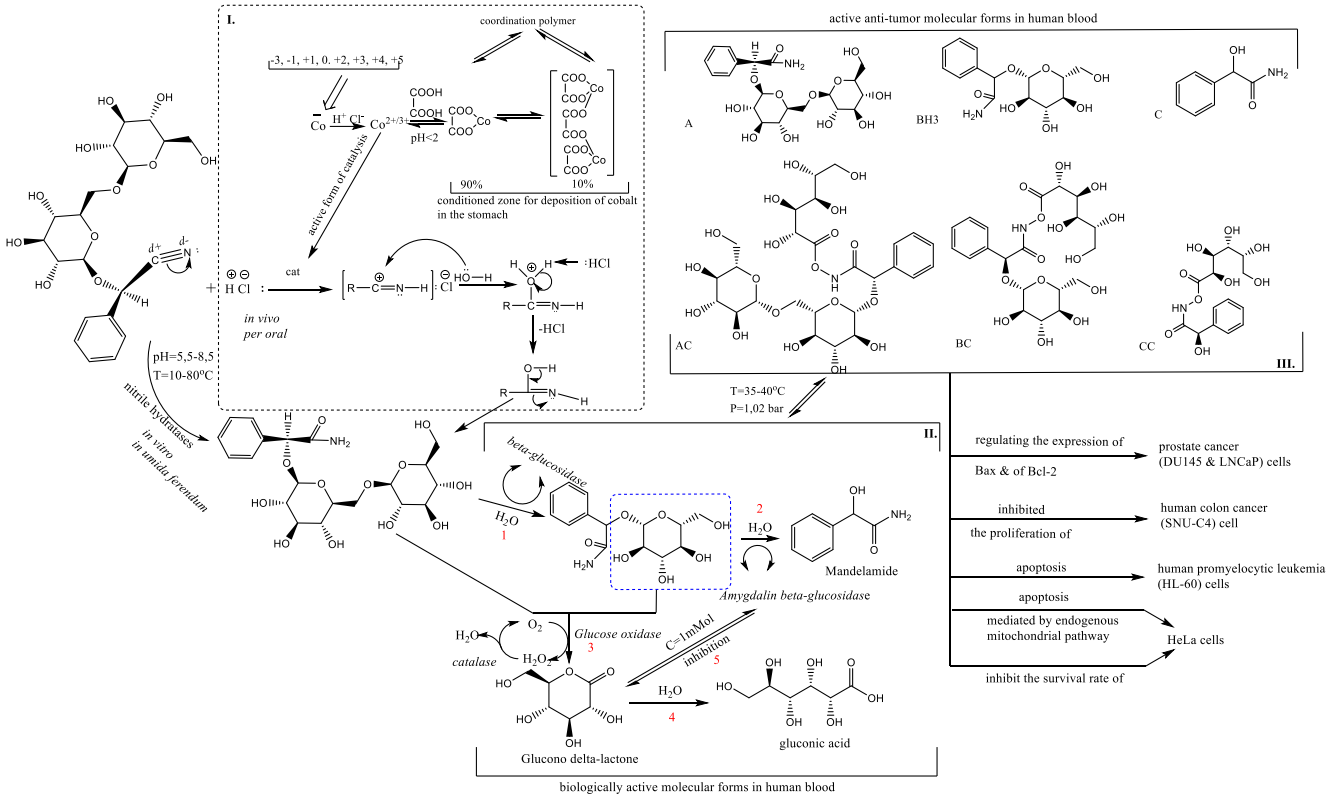


Fig.IV.1. 7 *Summary scheme of the theoretically calculated antitumor activity of the biologically modified amygdalin and the site of the dosage form throughout the biochemical cycle*

We conditionally divide the bio-activity into three conditional sub-schemes:

Molecular Transition I: In the stomach under the action of hydrochloric acid and coordination-unrelated cobalt (I.), i.e. in the form of an ion, hydrolysis occurs by acid catalysis. For the retention of cobalt ions in the stomach long enough and with the necessary concentration during the diet, we have theoretically derived the most probable and physiologically justified reaction, namely in the form of cobalt oxalates. The corresponding amide is obtained. The same end product can also be obtained in an *in-vitro* environment using enzymes. Regardless of the method and place of production, an already modified molecule of amygdalin enters the bloodstream;

Molecular Transition II: In the blood under the action of *beta-glucosidases*[16] and water (reaction #1), begins to break down glycosidic bonds and secrete free glycosides and linamarine amide derivative. It undergoes amygdalin *beta-glucosidase* in *mandelamide* (reaction #2). The remaining free glycosides and a small fraction of the amide derivative of amygdalin are also attacked by Glucose oxidases (reaction #3). Glaucoma delta-lactone is obtained which under the action of water is converted to gluconic acid (reaction #4). It is important to note that with an increase in the concentration of g*lucono delta-lactone* above 1mMol / ml, inhibition of the activity of amygdalin beta-glucosidase also occurs (reaction #5). The presence of such active molecules at this stage also results in increased biological activity and by-products of the reaction.

[16] To take into account the possible presence of a concomitant disease of the patient - such as diabetes, impaired renal function, etc., which would decrease the concentration in the human blood.

Molecular Transition III: Based on an author's study (Song & Xu, 2014) on the achievements in the anti-tumor mechanism of amygdalin and when standard living organisms are set, six molecular forms (III. A, BH3, C, AC, BC, CC) are clearly distinguished, and they are thought to exhibit anti-tumor activity. Based on the paragraph four that it will be at least the same as in the clinical trials of pure *amygdalin*.

1.5. Author's notes on first goal

Our legacy of the *Hunza people* and the contribution from tens-of-thousands of scientists who created modern synthesis and biochemistry make the production of nitrile amide into a routine (especially with nitrile hydratase). Thus, humanity holds in its hands a huge medicinal resource that can provide treatment for diseases of all parts of conservative medicine.

The hydrolyzed to amide / carboxylic acid nitrile / cyanide carbohydrates will occupy one of the fundamental steps of countless future clinical practices. This is the purpose of our modest research!

Other substances in these groups with pronounced biological activity (including anti-tumor) are the hydrolyzed nitrile groups of ***Linamarin***, ***(R) -Lotaustralin***, ***S-Sambunigrin***, etc., to their amide / carboxylic acid.

2. On second goal

2.1. Characterization of amide dissociation *in vivo*

2.1.1. Conducting the experiment

For the reaction-determining atoms, the following were calculated: *Mulliken Changes*, *Electrostatical Potential*, *Core-Core Repulsion*, *COSMO Area* & *Volume*, *Dipoles* (vector Debye), *Electronic* & *Total Energy* and *Ionization Potential*.

Mulliken Changes and *Electrostatical Potential* of the reactive atoms in amide derivative of amygdalin and its two hydrolytic forms in vivo are shown in (**Fig.IV.2. 1**).

The negative electrostatic potential corresponds to the attraction of the proton from the electron density concentrated in the geometric space in the molecules (from single pairs, π-bonds, etc.). The positive electrostatic potential corresponds to the repulsion of the proton from the atomic nuclei in regions with low electron density and the nuclear charge is incompletely shielded.

The data of ***Fig.IV.2. 1*** clearly illustrates that during hydrolysis of glycoside amide, obtained by hydrolytic modification of nitrile glycoside (in this case *amygdalin*) *in vivo*, the electrostatic equilibrium shifts to the ammonia-saturated form (assumed conditionally for **Hydrolytic Form A)**. This is confirmed by *Mulliken Changes* (Murray & Sen, 1996) of the three molecular forms.

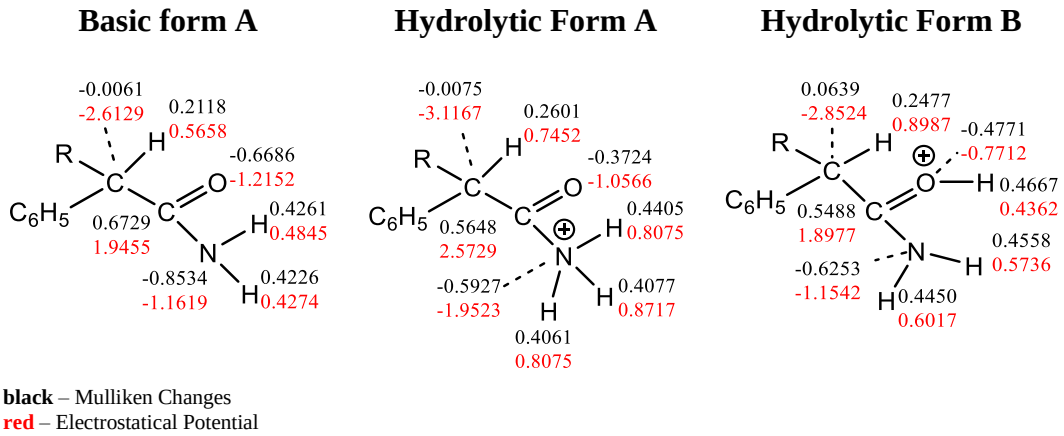


Fig.IV.2. 1 *Mulliken Changes and Electrostatical Potential of the amide derivative of amygdalin and its two hydrolytic forms in vivo*

Therefore, in terms of Electrostatical Potential and Mulliken Changes, the medium around the cancer cell tends to shift the hydrolytic equilibrium to HF(A). This is provided that the molecule is relatively static and already reoriented in a closed volume around the cancer cell.

The *Core-Core Repulsion*, *COSMO Area & Volume*, *Electronic Energy*, *Ionization Potential* and *Total Energy* of the reactive atoms in amide derivative of amygdalin and its two hydrolytic forms in vivo are depicted in ***Fig.IV.2. 2*** .

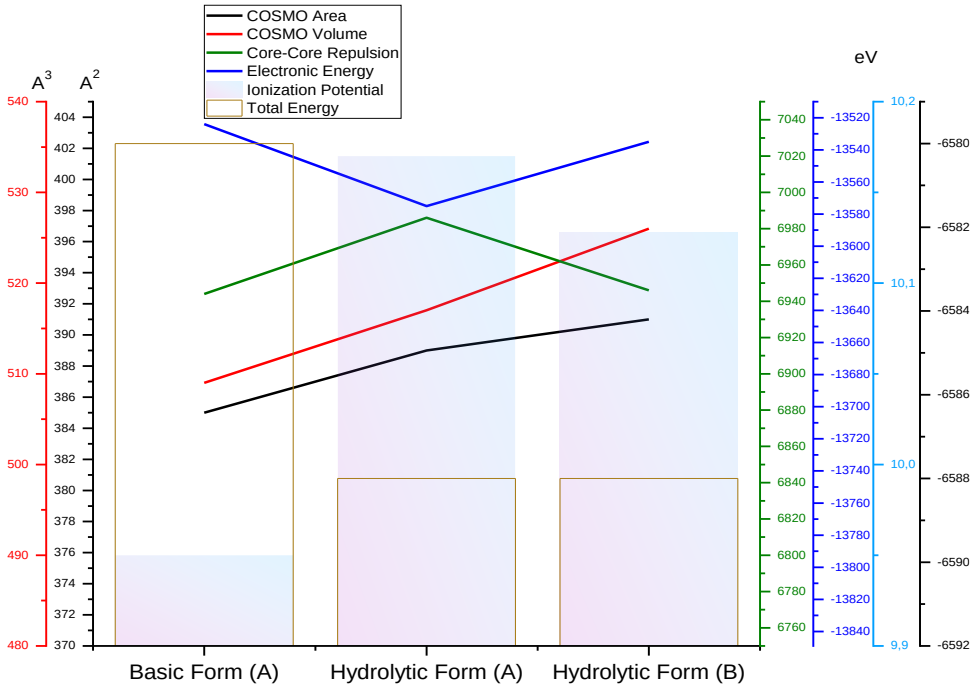


Fig.IV.2. 2 *Core-Core Repulsion, COSMO Area and Volume, Electronic Energy, Ionization Potential and Total Energy of amygdalin amide derivative and its two hydrolytic forms in vivo*

Core-Core Repulsion has the highest value at **HF(A)**. This is an indicator of a slight reorientation of the proton centers, which is confirmed by the slight geometric deformation observed in this form. *COSMO Area* & *Volume* grow in the direction from the ammonia-saturated

to the hydroxy-saturated hydrolytic molecular form. These circumstances indicate that **HF(B)** occupies a larger geometric volume (along with associated blood / water / intercellular fluid / CSF), which minimizes the shielding of externally charged molecules (including dipoles, ions, domains, etc.). This is confirmed by the lower ionization energy compared to **HF(A)**. This effect is not decisive in the expression of total energy (i.e. total energy affects *ionization potential*, not the other way around). It is apparently lower than **BF(A)**, and is relatively the same in **HF(A)** and **HF(B)**.

Therefore, in terms of Core-Core Repulsion, COSMO Area and Volume, Electronic Energy, Ionization Potential and Total Energy, the environment around the cancer cell tends to support hydrolysis to HF(B).

There is a certain dualism in this summary: Provided that the reorientation in space is due to the elimination of energy and/or partial charges, and they in turn increase the polar hydration volume (an integral part of the total volume of the molecule), non-uniform polarization inside the basic structure of the molecule under relatively constant conditions. The latter is an indisputable fact, since the in vivo medium is buffered to both pH and temperature.

The polarizations within the molecular forms can be compared by so-called *Debye vectors* on the molecular spatial axes along the X, Y, Z and total dipole moment of the amide derivative of amygdalin and its two hydrolytic forms in vivo are shown in ***Fig.IV.2. 3***.

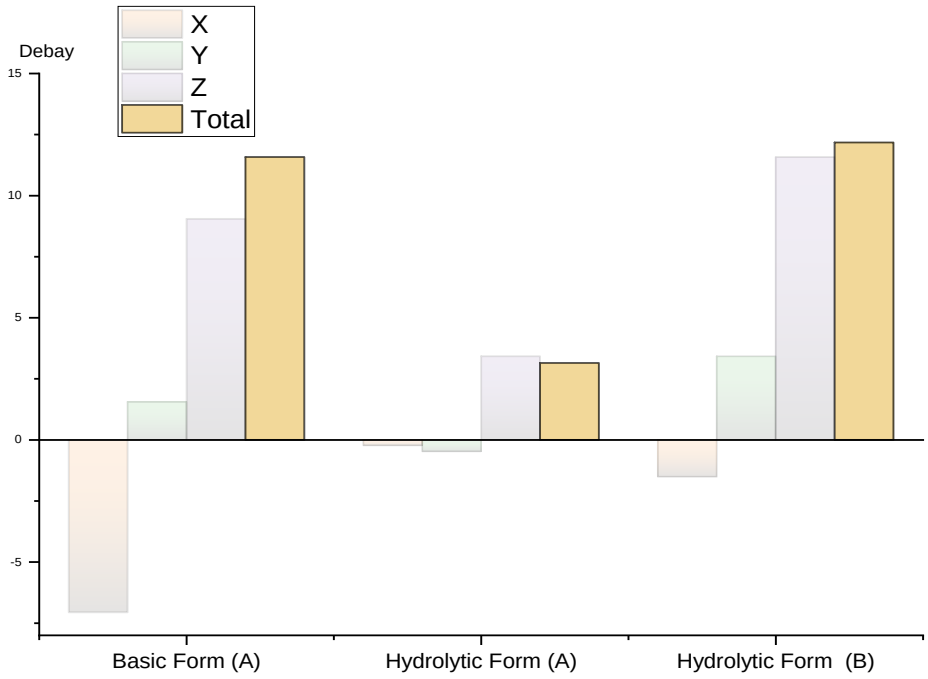


Fig.IV.2. 3 *Dipole moment of amygdalin amide derivative molecule and its two hydrolytic forms in vivo*

HF(A) has a lower dipole moment, with the same molecular radius, topological diameter, and polar surface area (***Tabl.IV.2. 1***). The analysis thus performed does not take into account the change of the dipole moment under the dynamic action of the active molecules.

Tabl.IV.2. 1 *Polar Surface Area, Molecular Radius and Topological Diameter of amygdalin amide derivative molecule and its two hydrolytic forms in vivo*

indicators		**Basic Form A**	**Hydrolytic Form A**	**Hydrolytic Form B**
Molecular Topology				
Polar Surface Area	Å	221±4	223±4	226±4
Radius	Atom(s)	8	8	8
Topological Diameter	Bond(s)	15	15	15

This circumstance does not allow us to assume the common belief that the lower dipole moment has lower molecular activity in a polar environment. Therefore, we need to analyze the partition coefficients *pKa* (***Fig.IV.2. 4***) *LogP*, *LogS* and *Partition Coefficient* (***Tabl.IV.2. 2)*** in the starting conditions and conditions (***§III.1.1.2.***).

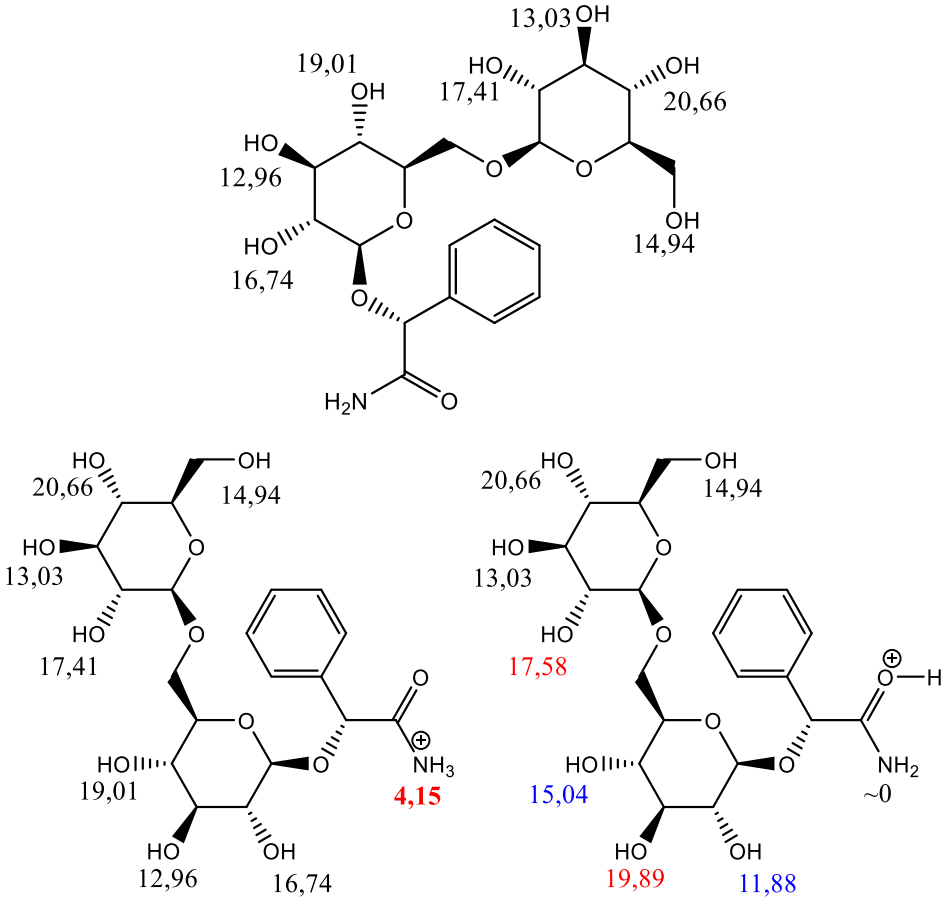


Fig.IV.2. 4 *pKa per atom and/or group of the whole molecule in amide of both its hydrolytic forms of amygdalin*

The calculation and structural representation of *pKa* of each atom and/or group of the entire molecule in all three forms is presented in ***Fig.IV.2. 4***. Black values are unchanged during hydrolysis, red those that increase and blue are those that decrease.

Based on the definition of *pKa*, namely that it is inversely proportional to the "strength" of the acid (or acid residue) and the degree of more complete dissociation, it follows that **HF(A**) is more alkaline than **HF(B)**, relative to the major hydrolytically active groups (-[C=O]-NH_3^+ и –[C=O^+H]-NH_2).

HF(B) has a change in the hydrolytically active hydroxyl groups of the glucosidal nuclei. From thence, the internal polarization of the molecule also grows, which is also confirmed by the higher dipole moment (***Fig.IV.2. 3***) with respect to **HF(A)**. It is important to note that this does not compare the dipole moments in the molecule itself with that of the molecule and the solvated volume around it. These are two different dimensions in which external factors are crucial. In our case, even with constant temperature and acidity, the molecule is also affected by other ions and molecules, and in some cases by mechanical effects (including movement of blood, cerebrospinal fluid, lymph, tissue and intercellular fluids).

In terms of distribution coefficients, both hydration forms are equally likely to occur. On the one hand, the more acidic medium will shift the equilibrium to ammonia-saturated **HF(A)**, and on the other hand, the speed and stability of hydrolysis will be faster on **HF(B)** - probably due to incomplete protonation by the more acidic medium around the cancer cell. The data from *Principal Moment* (***Tabl.IV.2. 2)*** cannot be interpreted uniquely.

Tabl.IV.2. 2 *Principal Moment, Lipinski's rule of five, LogP, LogS, Partition Coefficient of amygdalin amide derivative*

indicators	**dimension**	**Basic Form A**	**Hydrolytic Form A**	**Hydrolytic Form B**
Principal Moment				
		2743 4688 5994	2711 4606 5876	2824 4484 5910
Lipinski's rule of five				
molecular weight		475	476	476
number of HBond acceptors	units	12	12	11
number of HBond donors	units	8	8	9
number of rotatable bonds	units	8	8	8
LogP	Log Units	-2.98	-2.67	-2.67
LogS	Log Units	0.22	0.20	0.93
Partition Coefficient	conditional units	-2.98	-2.67	-2.67

There is a deviation from the *Lipinski's rule of five* application indicator. This is due to the molecular topology of the three forms - **BF(A)** and **HF(A; B)**. The molecules are divided into four major parts – two substituent (or H), sugar[17] (***Fig.IV.1.11***) and an amide group. They have different electron density (and/or protons) transported in different directions, with different

[17] During the enzymatic reaction, a carbohydrate residue and/or condensate between two or more carbohydrates may also be observed. Precision is required when changing the concentration, medium or introducing other reagents into the reaction.

capacities and intensities. The very algorithm for deriving the *Lipinski's rule of five* is quite subjective and with a few variables that we eliminate through analysis. Behind each statement is at least three counter-analyzes and the necessary statistical processing (according to the methodology – ***§III.***).

2.1.2. Conclusion from the part

Based on the aforementioned theoretical-analytical considerations, it can be concluded that the cancer cell seeks to maintain hydrolysis to **HF(B)**. Following the same analysis using a completely identical methodology, but at pH=7.4, it is concluded that a physiologically sound cell tends to undergo more alkaline hydrolysis, namely **HF(A)**.

As mentioned in ***§IV.1***, in addition to the amide derivative, there are invariably significant amounts of the corresponding carboxylic acid / **BF(C)** / with it. This compound (even at low concentrations) plays a significant role in the ionic activity of the medium and hence the chemical activity of the hydrolyzed amide / **BF(A)** /.

2.2. Characterization of carboxylic acid dissociation in vivo

2.2.1. Conducting the experiment

For the reaction-determining atoms, the following were calculated: *Mulliken Changes*, *Electrostatical Potential*, *Core-Core Repulsion*, *COSMO Area & Volume*, *Dipoles* (vector Debye), *Electronic & Total Energy* and *Ionization Potential*.

Mulliken Changes and *Electrostatical Potential* of the reactive atom's amide derivative of *amygdalin* and its two hydrolytic forms *in vivo* are shown in ***Fig.IV.2. 5***.

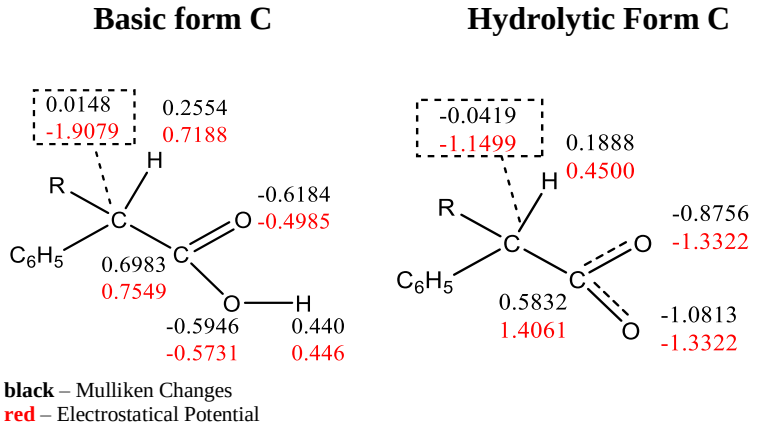


Fig.IV.2. 5 *Mulliken Charges and Electrostatic Potential of the carboxyl derivative of amygdalin and its hydrolytic forms in vivo*

According to the analytical wording in methodology concludes that the hydrolytic equilibrium of the reaction is shifted from **BF(C)** to **HF(C)**. The difference in ionic activity at

pH=6.5 and 7.4 is within less than 1% deviation (due to the presence of only one hydrolytic form), it is evident that the values are in the statistical error of the methods.

Therefore, from the perspective of Electrostatical Potential and Mulliken Changes, the medium around the cancer cell tends to shift the hydrolytic equilibrium to HF(C). The influence of the dynamic relationships of the molecule is not essential for hydrolysis around healthy and cancer cells.

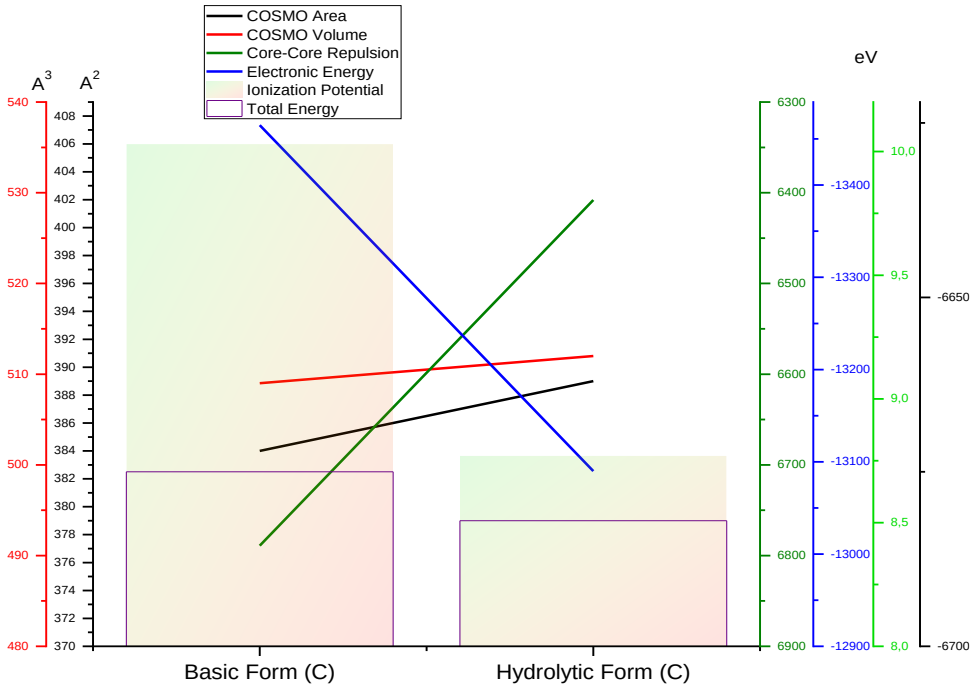


Fig.IV.2. 6 *Core-Core Repulsion, COSMO Area and Volume, Electronic Energy, Ionization Potential and Total Energy of amygdalin carboxyl derivative and its hydrolytic forms in vivo*

Tabl.IV.2. 3 *Polar Surface Area, Molecular Radius and Topological Diameter of carboxyl derivative of amygdalin and its hydrolytic forms in vivo*

indicators		**Basic Form C [pH=7.4]**	**Hydrolytic Form C [pH=6.5]**
Molecular Topology			
Polar Surface Area	Å	216±4	225±4
Radius	Atom(s)	8	8
Topological Diameter	Bond(s)	15	15

Data of ***Fig.IV.2. 6***, ***Fig.IV.2. 7*** and ***Tabl.IV.2. 4*** shall be interpreted in proportion to what the data of ***§ III. 2.1***.

The pKa of the starting material **BF(C)** and the reaction product **HF(C)** in this case are not significant when considering the reaction.

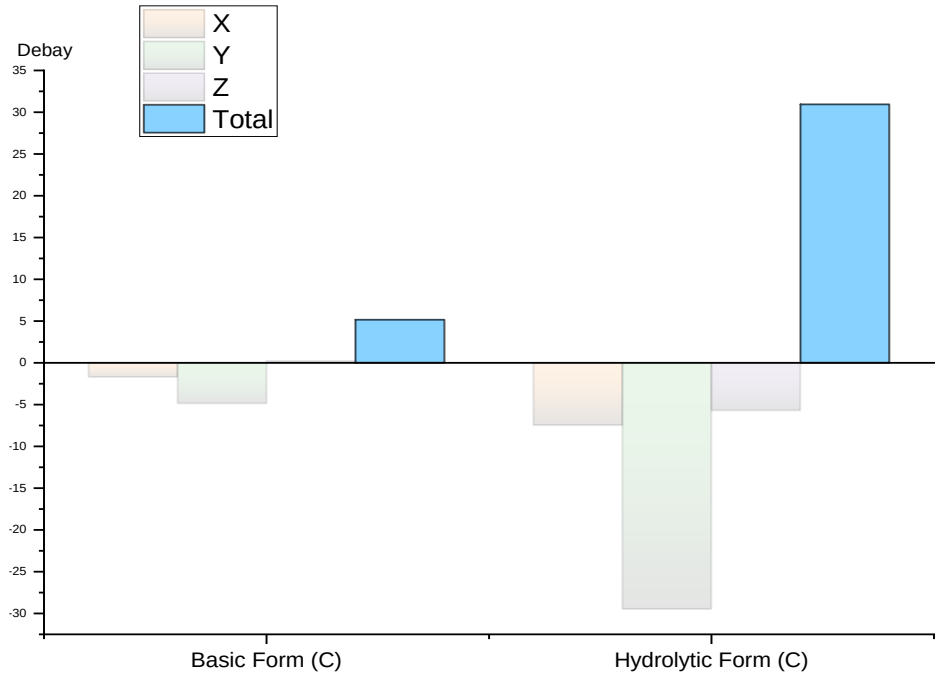


Fig.IV.2. 7 *Dipole moment of the carboxyl derivative of amygdalin and its hydrolytic forms in vivo*

This is confirmed by the same *Polar Surface Area* (***Tabl.IV.2. 3)*** for the molecular forms in the two acidic states. The activity of the hydroxyl groups in the non-structural position will assume a value close to that of the natural nitrile. In order not to get an active chemical form that is completely different in bio-reactivity, the starting carboxylic molecule is also compared with respect to *Principal Moment*, *Lipinski's rule of five* and the *partition coefficients* (***Tabl.IV.2. 5)***. The deviation from them is comparable to that of the amide form (***§III. 2.1***).

Tabl.IV.2. 5 *Principal Moment, Lipinski's rule of five, LogP, LogS, Partition Coefficient of amygdalin carboxyl derivative*

indicators		**Basic Form (C)**	**Hydrolytic Form (C)**
Principal Moment			
		2833 4587 5953	2881 4321 5827
Lipinski's rule of five			
molecular weight	g/mol	476	476
number of HBond acceptors	numbers	12	13
number of HBond donors	numbers	8	7
number of rotatable bonds	numbers	8	8
LogP	Log Units	-2.93	-3.11
LogS	Log Units	-0.06	0.812
Partition Coefficient	conditional units	-2.08	-10.55

2.2.2. Conclusion of the part

Based on the analysis, we conclude that the acidity around the tumor cell is not a significant factor influencing the hydrolysis. It is again shifted in the direction of the product, i.e. **HF(C)**.

2.3. Mechanism of penetration of the modified molecule into the cancer cell

2.3.1. Mechanism

The biological activity of the hydrolyzed to amide/carboxylic acid nitrile / cyano glycosides (***Tabl.II.2. 1***) is approximately the same as that of the native (basic) molecules. This allows high doses (***§IV.1.3***) to be administered, which in turn significantly increases their anticancer activity (in terms of concentration).

In ***Fig.IV.2. 8*** is a general schematic view of the data of ***Tabl.II.2. 1*** and their hydrolysis (Петров, 1996/2019).

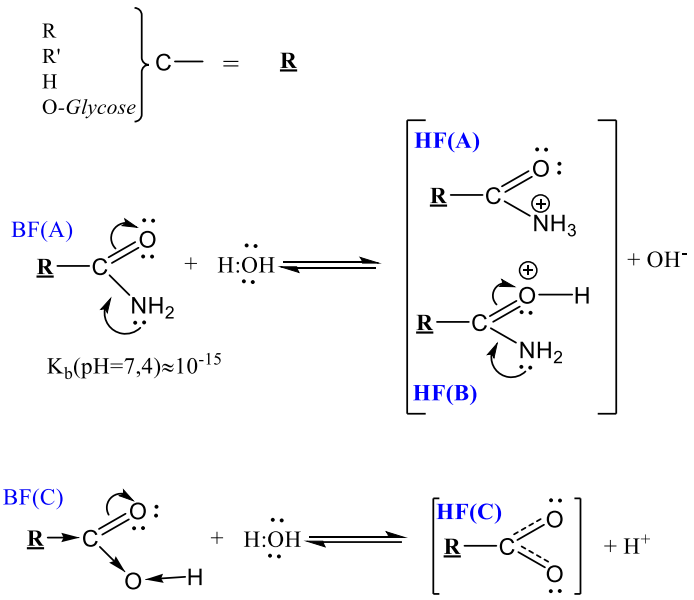


Fig.IV.2. 8 *Hydrolysis of amide and carboxylic acid derivatives of nitrile (cyano) glycosides*

Fig.IV.2. 9 presents two physiologically active cells: healthy (III.) with pH=7.2 and cancer (V.) with pH=7.4. They are found in a volume of blood (I.) with pH=7.4. Around each cell has an associated volume of liquid (VI.) With specific ionic activity and hence different acidity. For a healthy cell, pH=7.4 and for cancer pH=6.5. Based on the data of ***§IV.2.1-2*** it follows that in a healthy cell, all three hydration forms **HF(A;B;C)** will be in significant concentration both in the blood (I.) and in the closed volume (II.) around it. In the volume around the cancer cell, the hydrolytic equilibrium is shifted in the **HF** direction **(B)**. In this form, the molecule loses its activity in an environment of excess protons, i.e. behaves like a "regular carbohydrate".

Based on the fact that the cancer cell feeds primarily on carbohydrates, it is likely that the organisms have adapted to receive food containing nitrile glycosides and/or their modified forms to counteract "external" biological effects. Cancers, for their part, have evolved to the extent that they create conditions around their cells that eliminate the active apoptotic forms. This is far more appropriate for them than changing their entire enzyme regulation to counteract it. In this way, it protects itself and the gene set and develops according to its instructions.

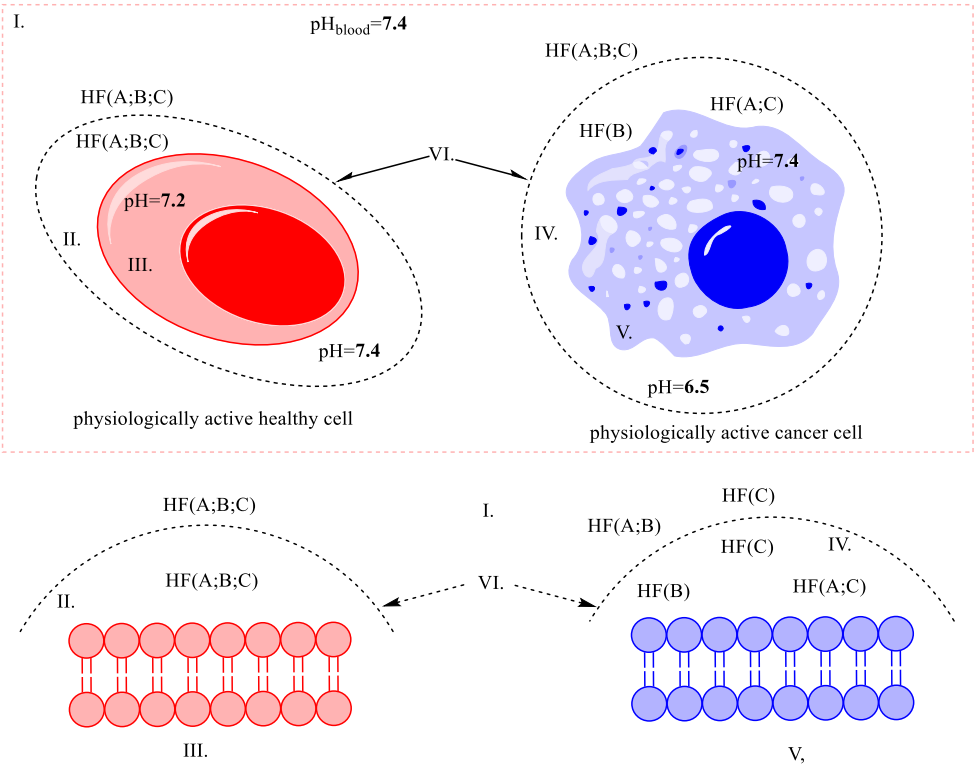


Fig.IV.2. 9 *Schematic representation of the type of hydrolysis of amide and carboxylic acid derivatives of nitrile (cyano) glycosides around a physiologically active cancer and healthy cell*

Therefore, the hydration balance of **HF(A,B)** in the blood, the medium around the cancer cell shifts it in the direction of **HF(B)**. Parallel to this hydrolysis, the carboxylic acid, i.e. from **BF(C)** to **HF(C)**. It is not sensitive to this change in acidity and the equilibrium is shifted towards the product. This concludes that the concentration of **HF(C)** is approximately the same in (I.) and (IV.) in healthy and cancer cells. This form also could hardly pass through the cell membrane in considerable concentration.

The presence of both types of hydrolysates in one volume in the blood (I.) changes the whole picture. The equilibrium at the amide derivative is shifted (***Fig.IV.2. 10***) in direction **HF(A)**.

The compound thus obtained exhibits hydrolytic inertness at pH=6.4. Thus, the resulting **HF(A;C)** form reaches unaltered to the cell membrane of the cancer cell.

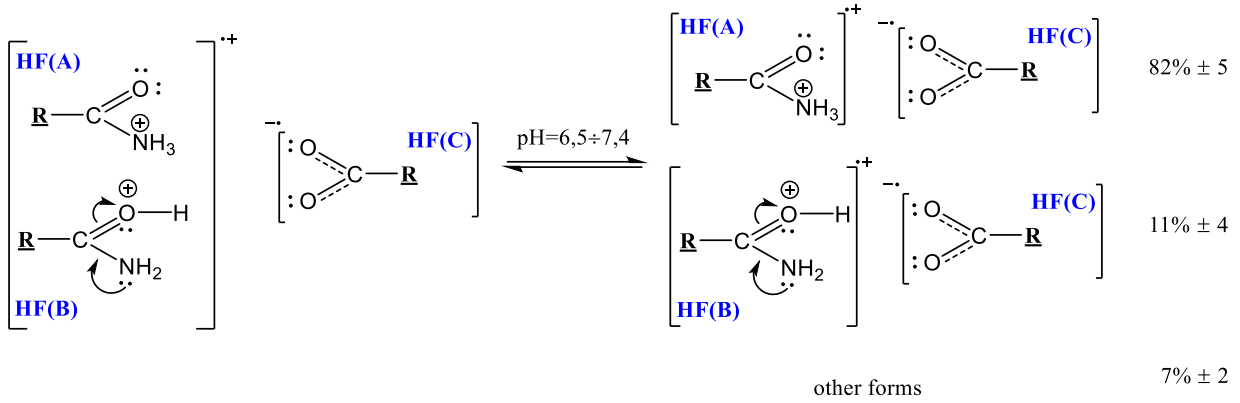


Fig.IV.2. 10 *Formation of a complex with incomplete counter-charge between hydrolysis of amide and carboxylic acid derivatives of nitrile (cyano) glycosides*

These compounds are due to the sharing of incomplete electron charges and are stabilized by a solvate shell of water. The shortage of -OH groups further stabilizes the process, due to the slightly acidic medium and free protons oriented directly above the cell membrane. In parallel with this process, reverse hydrolysis takes place (***Fig.IV.2. 11***) from **HF(C)** to **BF(C)** - which retains activity and leaves the associated volume around the cancer cell, re-hydrolyzes and binds a new amount of **HF(A)** and enters into the closed volume.

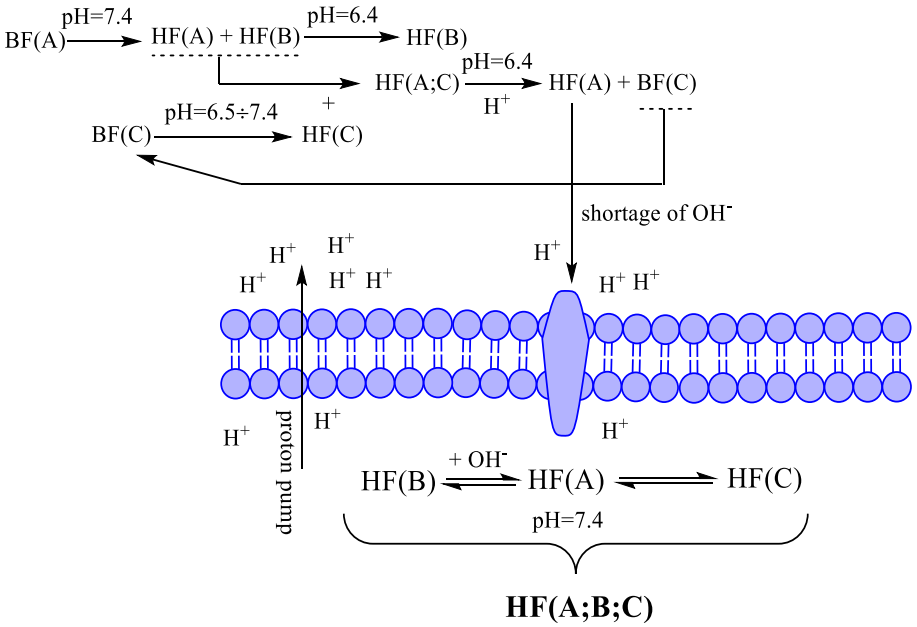


Fig.IV.2. 11 *Scheme of chemical bonding between basic and hydrolyzed forms of amide and carboxyl forms of nitrile glycosides under various conditions in vivo around and in cancer cell*

HF(A) molecule contains at least one glycosidic group. The compound is saturated with ammonia (-NH_3^+) and readily binds with protein to glycoprotein. The hydrolysis-modified form is passed through a protein carrier through the cell membrane. Here, however, pH is equal to 7.4 and saturation of -OH groups. Thus, **HF(B)** is obtained, i.e. there is a partial shift of the hydrolytic equilibrium from **HF(A)** through **BF(A)** to **HF(B)**. An enzyme amidase (Valiña, Mazumder-Shivakumar, & Bruice, 2004) is also synthesized in the cell, which converts -(CO).NH_2 to -

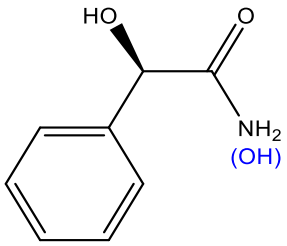
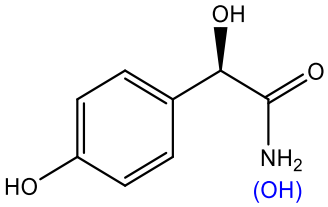
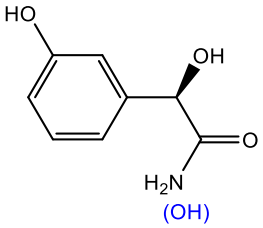
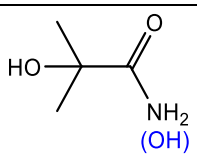
(COOH). As a final product under these conditions we have all three hydrolytic forms - **HF(A;B;C)**.

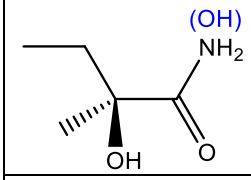
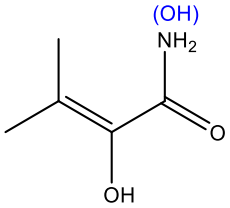
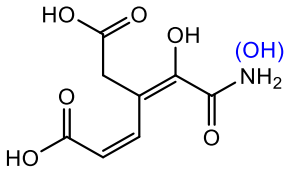
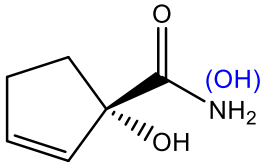
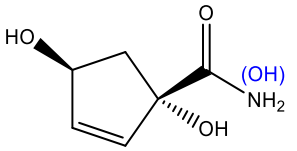
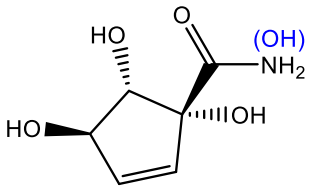
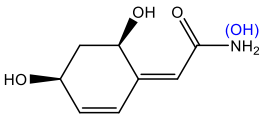
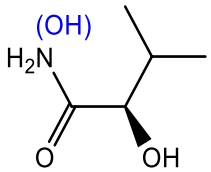
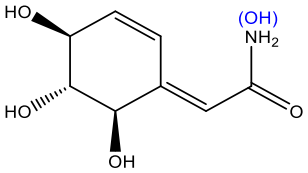
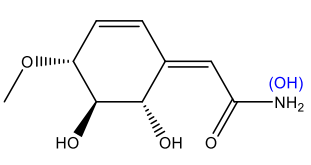
Therefore, the eventual chemical apoptosis will proceed independently of all enzymes synthesized according to instructions from cancer DNA (for example, the *linamarase gene* to *linamarase*).

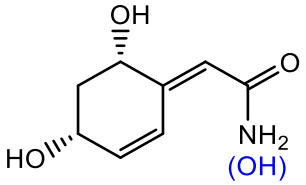
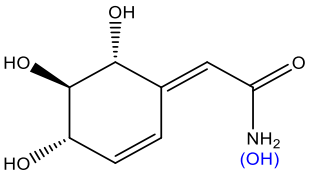
2.3.2. Toxication of the cancer cell

Active apoptotic form (AAF) with manifested anticancer activity are formed according to their molecular structure. For diglycoside compounds (*Amygdalin* / *Gentiobiose* / *Lucumin* / *Primeverose* / *Vicianin* / *Vicianose* , etc.) primary enzymatic hydrolysis (gluconases - which are abundant in tissue fluids) of the glycosidic bonds between the individual sugars takes place. The relationship between the secondary carbohydrate and the reaction-determining group is stronger and requires a longer reaction time and/or a specific enzyme such as amygdalin beta-gluconase. The latter is synthesized mainly inside the cell itself. This leads us to conclude that the passage through the cell membrane of the cancer cell (**§IV.2.1-2**) occurs with only one carbohydrate molecule. Once inside the cell, the only glycosidic bond is broken. This is how the **AAF**s themselves are created. Some of them are listed in ***Tabl.IV.2. 6***.

Tabl.IV.2. 6 *Active apoptotic amide/carboxyl acid molecular forms*

chemical formula	name	natural precursor
	(R)-2-hydroxy-2-phenylacetamide	Prunasin Amygdalin Lucumin Vicianin Sambunigrin
	(R)-2-hydroxy-2-phenylacetic acid	
	(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide	Dhurrin Taxiphyllein Proteacin p-Glucosyloxymendelonitrile
	(R)-2-hydroxy-2-(4-hydroxyphenyl)acetic acid	
	(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide	Zierin
	(R)-2-hydroxy-2-(3-hydroxyphenyl)acetic acid	
	2-hydroxy-2-methylpropanamide	Linamarin
	2-hydroxy-2-methylpropanoic acid	

	(S)-2-hydroxy-2-methylbutanamide	Lotaustralin
	(S)-2-hydroxy-2-methylbutanoic acid	
	2-hydroxy-3-methylbut-2-enamide	Acacipetalin
	2-hydroxy-3-methylbut-2-enoic acid	
	(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid	Triglochinin
	(2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid	
	(S)-1-hydroxycyclopent-2-ene-1-carboxamide	Deidaclin Tetraphyllin A
	(S)-1-hydroxycyclopent-2-ene-1-carboxylic acid	
	(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide	Tetraphyllin B Volkenin Taraktophyllin
	(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxylic acid	
	(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide	Gynocardin
	(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxylic acid	
	(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide	Menisdaurin
	(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid	
	(R)-2-hydroxy-3-methylbutanamide	Volkenin
	(R)-2-hydroxy-3-methylbutanoic acid	
	(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide	Griffonin
	(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid	
	(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide	Bauhinin
	(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid	
	(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide	Purshianin

	(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid	
	(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid	Lithospermoside

Each of these molecules alone would not cross the cell membrane of the cancer cell. Only those related to carbohydrate and fulfilling the conditions of (***§*IV.2.1-2**). they will block and/or permanently damage her normal physiology. The use of **AAF** (***Tabl.IV.2. 6***) directly for treatment will lead to severe toxic reactions and allergic responses of the body.

By themselves, these compounds or their homologues are still used in conservative chemotherapy (Chabner & Longo, 2018) (Airley, 2009) (Priestman, 2012). Glycosides such as *Rehmapicroside*, *Loganic acid*, *HMBOA D-glucoside*, *Glucose beta-1,3-isofagamine*, *Vanillyl beta-D-glucopyranoside* and others. Although they contain **AAF** of the proposed type, they would not cross the cell membrane of the cancer cell. They do not fulfill the condition of ***§IV.2.2.***, in the part of the amide derivative which is to be hydrolyzed by a transitional complex with a carboxylic acid.

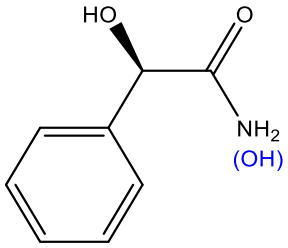
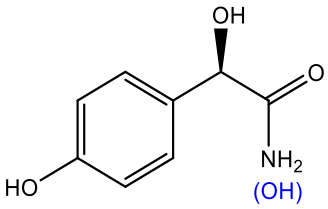
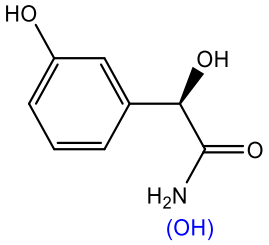
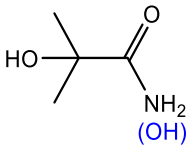
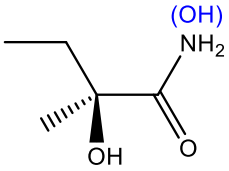
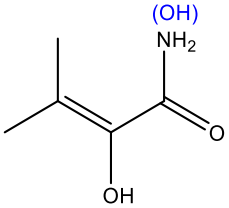
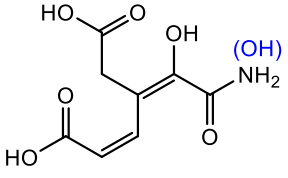
The relative inertness of the glycosidic bond (*in vivo*) also allows the use of different amide-carboxyl glycosides simultaneously. This is also observed in nature with regard to the distribution of nitrile glycosides - they are often more than one representative in one plant. Thus, different AAFs can be injected simultaneously, at different concentrations and at different times, in order to closely differentiate the different types of cancers, through the synergistic action of the controlled toxicity itself inside the "attacked" cell.

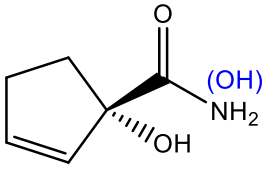
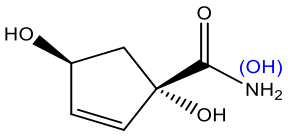
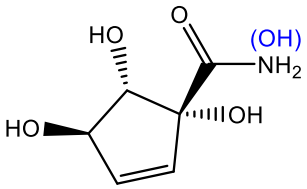
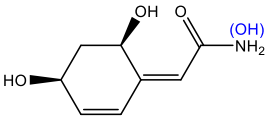
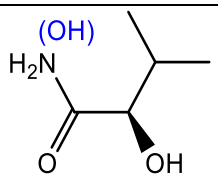
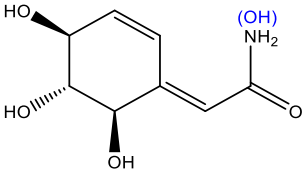
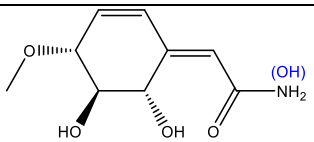
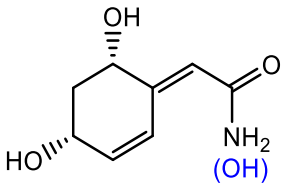
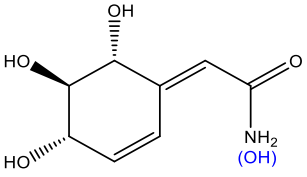
Natural nitrile glycosides would not cross the cancer cell membrane. They decompose to HCN-acid, phenyl methanol and carbohydrate. They do NOT have anticancer activity due to their inability to reach the target unchanged. These compounds, in their natural form, are extremely toxic to the human body. Applying them is not a cure, even at a higher concentration, they do more than they can help. We have theoretically derived dozens of their modified forms, but their amides and their carboxylic acids are the most promising for their introduction into conservative oncology. The fact is that the cancer cell itself tries to counteract it in a fairly certain way.

2.4. Determination of the drug dose

The drug dose is determined by considering all possible substances obtained by the final hydrolysis of the glycosidic bond inside the cancer cell (***Tabl. IV.2. 6***, ***-7***).

Tabl.IV.2. 7 *Nature and concentration of active anticancer cell molecules obtained after crossing the cell membrane by their natural precursors*

AACF chemical formula obtained after crossing the cell membrane	**natural precursor enzymatically modified to amide and carboxylic acid**	**AACF concentration derived from 1 mg/ml pharmacological form [mg/ml]**
	Prunasin 4.87:1 Amygdalin 4.87:1 Lucumin 4.87:1 Vicianin 4.87:1 Sambunigrin 4.87:1	0.40 0.27 0.27 0.27 0.40
	Dhurrin 4.87:1 Taxiphyllin 4.87:1 Proteacin 4.87:1 p-Glucosyloxymandelonitrile 4.87:1	0.42 0.42 0.31 0.42
	Zierin 4.87:1	0.42
	Linamarin 4.87:1	0.32
	Lotaustralin 4.87:1	0.35
	Acacipetalin 4.87:1	0.34
	Triglochinin 4.87:1	0.47

	Deidaclin 4.87:1 Tetraphyllin A 4.87:1	0.36 0.36
	Tetraphyllin B 4.87:1 Volkenin 4.87:1 Taraktophyllin 4.87:1	0.39 0.39 0.39
	Gynocardin 4.87:1	0.41
	Menisdaurin 4.87:1	0.42
	Epiheterodendrin 4.87:1	0.35
	Griffonin 4.87:1	0.44
	Bauhinin 4.87:1	0.46
	Purshianin 4.87:1	0.42
	Lithospermoside 4.87:1	0.44

The use of two or more pharmaceutical forms would not prevent their penetration subject to the mass ratios between the active antitumor amide and the active carboxyl transfer form.

The chemical compounds listed in ***Tabl. IV.2. 6, -7*** and are currently used as: anti-migrane, anti-atherosclerotic, anticoagulant, treatment of HIV, anti-cancer, anti-asthmatic, anti-hypertensive, anti-epileptic, analgesic, ocular anti-inflammatory, anti-hypertensive, hypnotic, anesthetic, anti-allergic, aromatase inhibitor, anti-ulcerative, anti-neoplastic, antibacterial, anticoccidial, contraceptive, tyrosine-kinase inhibitor treatment of mast cell tumors, etc.. The difference is that with the proposed technology they are formed inside the cell itself and thus minimize their overall toxicity in the body.

The known cellular reactions, which in this case are a function of one of the fundamental principles of medical chemistry - "structure-activity", define conclusions that give some of the scientific answers on the topic "Theoretical analysis of anticancer cellular effects of glycosamidamides":

3. On the third goal

It is mandatory to follow the tree structure of presentation, because it is also a function of the subsequent interpretation of the results.

Natural nitrile glycosides hydrolyzed to amide/carboxylic acid are still unexplored, but with great theoretical potential. As biologically active substances, these compounds also have significant toxicity. Lack of sufficient information (including mathematical and/or statistical models) for their accumulation, assimilation, decomposition, separation, etc. in the body raises many questions about the chemical and pharmacological molecular form, the concentration used for treatment, the time to maintain the necessary activity in the body. One of the purposes of this article is to limit laboratory testing to animals.

3.1. Analysis of data for active anticancer cell form

To produce an active anticancer molecule, two molecular forms must be present inside the cancer cell: amide and carboxylic acid. The amide molecule crosses the cell membrane and the carboxyl molecule minimizes the protection of the cancer cell [see §5 of (Tsanov, H. & Tsanov, 2021)]. They are easily obtained from natural nitrile glycosides [§3.2.2. of Article (Tsanov & Tsanov, Theoretical Analysis for the Safe Form and Dosage of Amygdalin Product, 2020)].

- ◊ -

3.1.1. (R)-2-hydroxy-2-phenylacetamide

Subjected to analysis potential pharmaceutical forms for release within the cancer cell of *(R)-2-hydroxy-2-phenylacetamide*, comprising an amides and carboxylic acids obtained by hydrolysis of the nitrile groups of *Prunasin, Amygdalin, Lucumin, Vicianin* and *Sambunigrin*. The process proceeds according to ***§IV.2.3.***

3.1.1.1. General Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.1. 1*** are listed the values of *GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-phenylacetamide.*

Tabl.IV.3.1. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-phenylacetamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Prunasin / Sambunigrin						
amide	0.23	-0.07	-0.05	-0.20	0.27	0.42
acid	0.39	0.13	0.02	0.19	0.32	0.60
Amygdalin						
amide	0.20	-0.05	0	-0.21	0.21	0.33
acid	0.31	0.09	0.04	0.05	0.24	0.44
Lucumin / Vicianin						
amide	0.15	-0.07	-0.11	-0.29	0.19	0.32
acid	0.26	0.07	-0.06	-0.01	0.22	0.44

Data in ***Tabl.IV.3.1. 1*** show that the amides and carboxylic acids of *Prunasin* and *Sambunigrin* have more pronounced overall drug activity *in vivo*.

3.1.1.2. Pharmacological and biological activity of oral active drugs

3.1.1.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.1. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule, Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-phenylacetamide.*

Tabl.IV.3.1. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-phenylacetamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Prunasin / Sambunigrin												
amide	313	-2.0	8	5	313	-2.0	75	41	313	-2.0	75	41
acid	314	-1.3	8	5	314	-1.3	75	40	314	-1.3	75	40
Amygdalin												
amide	475	-3.5	13	8	475	-3.5	108	62	475	-3.5	108	62
acid	476	-2.8	13	8	476	-2.8	108	61	476	-2.8	108	61
Lucumin / Vicianin												
amide	445	-3.3	12	7	445	-3.3	102	58	445	-3.3	102	58
acid	446	-2.6	12	7	446	-2.6	102	57	446	-2.6	102	57

Two molecular forms stand out here (the corresponding amides and carboxylic acid of *Prunasin* and *Sambunigrin*, which cover most of the requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.1.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-phenylacetamide* are listed in ***Tabl.IV.3.1. 3***.

Tabl.IV.3.1. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-phenylacetamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcid Group	nHB
Prunasin / Sambunigrin								
amide	142	5	5	2	18	313	0	13
acid	137	5	5	2	18	314	1	13
Amygdalin								
amide	222	8	8	3	27	475	0	21
acid	215	8	8	3	27	476	1	21
Lucumin / Vicianin								
amide	201	7	7	3	26	445	0	19
acid	196	7	7	3	26	446	1	19

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1.2***.

3.1.1.2.3. QED

The analysis is performed according to ***§III.3.3.3.1.3***.

A. uwQED rules

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.1. 4***.

Tabl.IV.3.1. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-phenylacetamide*

								uwQED
	MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Prunasin / Sambunigrin									
amide	313	-1.8	8	5	142	5	0	1	0.45
acid	314	-1.4	8	5	137	5	0	1	0.48
Amygdalin									
amide	475	-3.5	13	8	222	8	0	1	0.12
acid	476	-3.1	13	8	216	8	0	1	0.13
Lucumin / Vicianin									
amide	445	-3.0	12	7	201	7	0	1	0.17
acid	446	-2.6	12	7	196	7	0	1	0,18

The information obtained from the calculations indicates that the amides and carboxylic acids obtained by hydrolysis of the nitrile groups of *Prunasin* and *Sambunigrin* are more applicable in a treatment.

B. wQED

In ***Tabl.IV.3.1. 5*** *Unweighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-phenylacetamide.*

Tabl.IV.3.1. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-phenylacetamide*

									wQED
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Prunasin / Sambunigrin									
amide	313	-1.8	8	5	142	5	0	1	0.52
acid	314	-1.4	8	5	137	5	0	1	0.55
Amygdalin									
amide	475	-3.5	13	8	222	8	0	1	0.20
acid	476	-3.1	13	8	216	8	0	1	0.22
Lucumin / Vicianin									
amide	445	-3.0	12	7	201	7	0	1	0.26
acid	446	-2.6	12	7	196	7	0	1	0.28

The information obtained from the calculations indicates the amides and carboxylic acids of *Prunasin* and *Sambunigrin* as covering the requirements for *Weighted Quantitative Estimate of Druglikeness*.

3.1.1.3. Non-laboratory and no clinical information on the chemical form

3.1.1.3.1. Receptor activity

In ***Tabl.IV.3.1. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Prunasin, Sambunigrin, Amygdalin, Lucumin* and *Vicianin* to receptors (according to ***§III.3.3.4.1***).

Tabl.IV.3.1. 6 *Receptor activity of amide and carboxyl derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

indicator	Prunasin / Sambunigrin / Amygdalin / Lucumin / Vicianin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR		
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

From the presented it is unambiguously concluded that the studied molecules show inertness to the studied receptor set.

3.1.1.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.1. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Prunasin* and *Sambunigrin*.

Tabl.IV.3.1. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

CAESAR indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0	0.83	0.74	0.74
SMKEV	0.80	0.80	0.82	0.83
APSM	0.67	1	0.66	0.66
CSM	0	0.68	0.67	0.67
MDRC	true	true	true	true
ACFFSC	1	1	1	1
Prediction	M	NM	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; M- mutagenicity; NM- non mutagenicity				

However, molecular fragments of the amide of *Prunasin* and *Sambunigrin* coincide with those of other molecules with mutagenicity already studied[18].

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* did not show activity (***Tabl.IV.3.1. 8***).

[18] Similarity: 0.77-9 by CAS: 51-34-3, CAS: 7195-43-9 and CAS: 3544-94-3

Tabl.IV.3.1. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

SarPy/IRFMN indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.81	0.81	0.74	0.74
SMKEV	0.80	0.80	0.82	0.83
APSM	0.67	1	0.66	0.66
CSM	1	0.68	0.67	0.67
ACFFSC	1	1	1	1
Prediction	NM	NM	NM	NM
NM- non mutagenicity				

c) ISS

Amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* are non-mutagenic according to *ISS* methodology (***Tabl.IV.3.1. 9***).

Tabl.IV.3.1. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

ISS indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.75	0.75	0.76	0.76
SMKEV	0.79	0.79	0.80	0.81
APSM	0.50	0.52	1	1
CSM	1	1	0.51	0.52
ACFFSC	1	1	1	1
Prediction	NM	NM	NM	NM
NM- non mutagenicity				

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Amygdalin*, *Lucumin* and *Vicianin* show some deviation from *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.1. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

KNN/Read-Across indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.71	0.78	0.60	0.60
SMKEV	0.82	0.81	0.82	0.83
APSM	0.50	0.75	0.25	0.26
CSM	0.75	0.75	0.75	0.76
ACFFSC	1	1	1	1
Prediction	NM	NM	NM	NM
NM- non mutagenicity				

B. Consensus model

Data from ***Tabl.IV.3.1. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Prunasin, Sambunigrin, Amygdalin, Lucumin* and *Vicianin* to mutagenicity.

Tabl.IV.3.1. 11 *Consensus model for mutagenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

Consensus model mutagenicity indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
numerical value	0.45	0.60	0.50	0.50

3.1.1.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

CAESAR carcinogenicity method recognizes that amide and carboxyl acid derivatives of amygdalin are the most non-carcinogenic of all the model forms studied (***Tab.IV.3.1. 12***). We attribute this to two facts: the molecules of *Amygdalin, Lucumin* and *Vicianin* have two glycosidic nuclei, therefore the electron density is more evenly distributed in the direction of the functional group (***§IV.2.1***), and hence the activity decreases - especially in dilute solutions; *amygdalin* is the best studied molecule of all the compounds studied, and hence the training set is more detailed.

Tabl.IV.3.1. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

CAESAR indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.74	0.50	0.86	0.86	0.60	0.60
SMKEV	0.77	0.76	0.71	0.75	0.73	0.73
APSM	0.50	0.50	1	1	0.50	0.59
CSM	1	0.50	1	1	0.50	0.50
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
MCAR	0.39	0.39	0.35	0.35	0.35	0.35
NMNC	1	0.50	1	1	1	1
Carcinogen	0.31	**0.59**	0.33	0.33	0.33	0.33
NON-Carcinogen	**0.69**	0.41	**0.67**	**0.67**	**0.67**	**0.67**
Prediction	NC	C	NC	NC	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen; C- carcinogen						

Molecular sites of carboxyl acid derivatives of *Prunasin* and *Sambunigrin* coincide with fragments of molecules with proven carcinogenicity[19].

b) ISS

ISS for amides and carboxyl acids derivatives of *Prunasin*, S*ambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* do not give an unambiguous answer about carcinogenicity (***Tabl.IV.3.1. 13***).

Tabl.IV.3.1. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

ISS indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.75	0.75	0.76	0.76	0.76	0.76
SMKEV	0.79	0.79	0.80	0.80	0.80	0.80
APSM	0.50	0.52	1	1	1	1
CSM	1	1	0.51	0.82	0.52	0.52
ACFSC	1	1	1	1	1	1
Prediction	PNC	PNC	PNC	PNC	PNC	PNC
PNC- possible non-carcinogenic						

[19] Similarity: 0.76 by CAS: 51-55-8 | Similarity: 0.74 by CAS: 18883-66-4 and CAS: 54749-90-5

c) IRFMN/Antares

The carcinogenicity data (***Table IV.3.1. 14***) of amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* are comparable to those of *ISS* (***§b***).

Tabl.IV.3.1. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN/Antares indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.61	0.62	0.62	0.62	0.62	0.62
SMKEV	0.79	0.81	0.82	0.83	0.82	0.82
APSM	0.67	0.34	0.34	0.33	0.34	0.34
CSM	0.34	0.67	0.66	0.66	0.65	0.65
ACFSC	1	1	1	1	1	1
Prediction	PNC	PNC	PNC	PNC	PNC	PNC
PNC- possible non-carcinogenic						

d) IRFMN/ISSCMN-CGX

The acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* coincides with part of a molecule that is listed in the training set as alert[20] for carcinogenicity (***Tabl.IV.3.1. 15***).

Tabl.IV.3.1. 15 *IRFMN/ISSCMN-CGX carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN/ISSCAN-CGX indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.72	0.60	0.68	0.81
SMKEV	0.78	0.77	0.80	0.80
APSM	0.67	0.68	1	1
CSM	0.67	0.32	0.34	0.66
ACFSC	1	1	1	1
Prediction	PNC	C	PNC	C
PNC- possible non-carcinogenic; C- carcinogen				

[20] Similarity: 0.74-6 by CAS: 51-55-8, CAS: 9000-07-1, CAS: 69644-85-5, CAS: 17924-92-4, CAS: 18883-66-4 which have been shown to be carcinogenic

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The method did not report information (***Tabl.IV.3.1. 16***) on carcinogenicity for the amide and carboxyl derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin*.

Tabl.IV.3.1. 16 *Carcinogenicity oral classification model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.73	0.88	0.70	0.84	0.71	0.60
SMKEV	0.77	0.77	0.70	0.71	0.71	0.71
APSM	1	1	1	1	1	0.51
CSM	0.49	1	0.50	1	0.50	0.51
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
Prediction	NC	NC	NC	NC	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen						

b) Carcinogenicity oral Slope Factor model

It is noteworthy (***Tabl.IV.3.1. 17***) that the molecules with two glycosidic nuclei (*Amygdalin*, *Lucumin* and *Vicianin*) allow higher allowable working concentrations. However, when checking the molecular weight ratio of the compound to the functional group mass, the results were comparable to those with a single glycoside nucleus (*Prunasin* and *Sambunigrin*).

Tabl.IV.3.1. 17 *Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.64	0.63	0	0
SMKEV	0.76	0.74	0.70	0.70
APSM	0.18	0.18	0.57	0.57
CSM	1.95	2.01	5.03	5.04
MEPASM	0.28	0,28	1.07	1.07
MDRC	true	true	n-true	n-true
ACFSC	0.85	0.85	0.85	0.85

Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$			
	14.4	16.6	42.2	42.2
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$			
	5.8		**11.4**	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; n-true - does not cover.				

3.1.1.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

Data in ***Tabl.IV.3.1. 18*** visualize the lack of toxicity in amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin*. Given that amygdalin is the best studied nitrile and with the most clinical information, toxins[21] have been reported in the training set.

Tabl.IV.3.1. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

CAESAR indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.64	0.75	0.69	0.91
SMKEV	0.76	0.79	0.70	0.83
APSM	0.18	0.51	0.57	1
CSM	1.96	1	5.03	1
MEPASM	0.28	?	1.07	?
MDRC	true	true	N-true	true
ACFSC	0.85	1	0.85	1
Prediction	NT	NT	T	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; N-true - does not cover; T- toxic; NT- non-toxic				

[21] Similarity: 0.78-84 by CAS: 59-01-8, CAS: 32986-56-4, CAS: 33419-42-0 and CAS: 23214-92-8

b) PG (Reproductive Toxicity library)

The results of the comparative analysis of amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* in *PG* (Reproductive Toxicity library) did not show toxicity (***Tab.IV.3.1. 19***).

Tabl.IV.3.1. 19 *PG toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

PG indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0	0	0	0
SMKEV	0.78	0.77	0.83	0.81
APSM	1	1	1	1
CSM	0	0	0	0
ACFSC	1	1	1	1
Prediction	NT	NT	NT	NT
NT- non-toxic				

B. Models related to the development of the organism

a) Zebrafish embryo AC50

The toxicity assessment of *Zebrafish embryo AC50* did not detect potentially dangerous for the development of the organism fragments in the molecules of amide and carboxyl acid derivatives of *Amygdalin*, *Lucumin* and *Vicianin* (***Tab.IV.3.1. 20***).

Tabl.IV.3.1. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN/CORAL indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.30	0.46	0.27	0.41	0.28	0.42
SMKEV	0.76	0.76	0.69	0.68	0.69	0.69
APSM	0.73	0.73	0.12	0.12	0.14	0.12
CSM	1.20	1.60	1,44	1.84	1.39	1.79
MEPASM	1.04	1.01	0.18	0.18	0.18	0.18
MDRC	true	true	true	true	true	true
ACFSC	0.40	0.60	0.40	0.60	0.40	0.60
Prediction	[mg/L]					
	4.50	**11.4**	**74.8**	**189.5**	**63.1**	**159.8**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

The amide and carboxylic acid derivatives of *Prunasin* and *Sambunigrin* would show some activity, but it is mainly due to the weaker connection of the glycoside residue with the functionally determining structure.

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.1. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.1. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

CORAL indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.30	0.46	0.27	0.41	0.28	0.42
SMKEV	0.76	0.76	0.69	0.68	0.69	0.69
APSM	0.73	0.73	0.12	0.12	0.12	0.12
CSM	1.20	1.60	1.44	1.84	1.39	1.79
MEPASM	1.01	1.01	0.18	0.18	0.18	0.17
MDRC	true	true	true	true	true	true
ACFSC	0.40	0.60	0.40	0.60	0.40	0.60
Prediction	**A**	**A**	**A**	**A**	**A**	**A**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* (***Tab.IV.3.1. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.1. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.63	0.64	0.74	0.75	0.64	0.74
SMKEV	0.77	0.78	0.77	0.77	0.76	0.76
APSM	1	1	1	1	1	1
CSM	0.52	0.52	1	1	0.55	1

ACFSC	0.85	0.85	0.85	0.85	0.85	0.85
Active Agonist	0.10	0.10	0.14	0.14	0.14	0.13
Active Antagonist:	0.04	0.03	0.08	0.07	0.06	0.06
Inactive:	**0.86**	**0.87**	**0.78**	**0.79**	**0.80**	**0.81**
Prediction	inA	inA	inA	inA	inA	inA
inA- inactive						

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* did not report any deviations (***Tabl.IV.3.1. 23***) affecting the studied process.

Tabl.IV.3.1. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

NIC indicator	Prunasin / Sambunigrin		Amygdalin / Lucumin / Vicianin	
	amide	acid	amide	acid
GADI	0.76	0.91	0.78	0.77
SMKEV	0.82	0.82	0.86	0.86
APSM	1	1	1	1
CSM	0.50	1	0.48	0.48
MDRC	true	true	true	true
ACFSC	1	1	1	1
Euclidean Distance from the central neuron:	1.39	2.44	2.69	4.04
Prediction	NA	NA	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NA- Non active				

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* we understand (***Tabl.IV.3.1. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.1. 24 *Adipose tissue: blood model for toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

INERIS indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0	0	0	0	0	0
SMKEV	0.77	0.77	0.71	0.70	0.72	0.71
APSM	0.31	0.31	0.14	0.33	0.14	0.31
CSM	0.43	0.35	0.34	0.24	0.34	0.31
MEPASM	0.50	0.50	0.15	0.50	0.15	0.50
MDRC	N-true	N-true	N-true	N-true	N-true	N-true
ACFSC	0.51	0.51	0.51	0.51	0.51	0.51
Prediction						
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]					
	0.183	0.264	0.195	0.299	0.238	0.306
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]					
	1.525	1.837	1.583	1.991	1.718	2.023
N-true - does not cover						

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.1. 25***).

Tabl.IV.3.1. 25 *Total body elimination half-life toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

QSARINS indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.85	0.85	0.85	0.85	0.85	0.85
SMKEV	0.79	0.79	0.82	0.82	0.82	0.82
APSM	0.09	0.09	0.33	0.13	0.33	0.33
CSM	0.20	0.07	0.52	0.34	0.46	0.42
MEPASM	0.15	0.15	0.62	0.23	0.62	0.62
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
Prediction						
LogHLt	[log units]					
	0.30	0.33	0.13	0.17	0.20	0.23
Total half-life	[min]					
	120	**130**	**80**	**90**	**95**	**105**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.1. 26***).

Tabl.IV.3.1. 26 *Micronucleus activity - In vitro for toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN/VERMEER indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.86	0.86	0.89	0.89	0.89	0.89
SMKEV	0.73	0.74	0.79	0.79	0.78	0.78
APSM	1	1	1	1	1	1
CSM	1	1	1	1	1	1
ACFSC	1	1	1	1	1	1
Prediction	A	A	A	A	A	A
A- active						

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Prunasin*, *Sambunigrin*, *Amygdalin*, *Lucumin* and *Vicianin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.1. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.1. 27 *NOAEL toxicity of amide and carboxyl acid derivatives of Prunasin, Sambunigrin, Amygdalin, Lucumin and Vicianin*

IRFMN/CORAL indicator	Prunasin / Sambunigrin		Amygdalin		Lucumin / Vicianin	
	amide	acid	amide	acid	amide	acid
GADI	0.69	0.70	0.69	0.85	0.85	0.85
SMKEV	0.82	0.82	0.82	0.83	0.83	0.84
APSM	0.25	0.25	0.25	0.25	0.25	0.25
CSM	1.42	1.26	0.69	0.55	0.93	0.79
MEPASM	0.38	0.38	0.38	0.3u	0.38	0.38
MDRC	true	true	true	true	true	true
ACFSC	0.85	0.85	0.85	0.85	0.85	0.85

Prediction	[-log(mg/kg)]					
	-2.25	-2.39	-2.96	-3.10	-2.72	-2.86
Prediction	[mg/kg]					
	178	**248**	**918**	**1276**	**528**	**734**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

Prunasin/*Sambunigrin* derivatives are expected to be more active and therefore have lower toxicity limits.

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.1.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.1.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Prunasin* and *Sambunigrin* would be optimal for drugs taken orally to poison the cancer cell with *(R)-2-hydroxy-2-phenylacetamide* as performed in ***§IV.2*** second objective of the study.

Alternatively, amygdalin derivatives are available.

These two conclusions do not preclude the use of *Lucumin* and *Vicianin* derivatives in clinical need.

3.1.1.5. Conclusion from the part

The application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***) proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for Prunasin amide and Sambunigrin amide $2229 \leq 5588 \leq 14008$, Prunasin acid and Sambunigrin acid $1904 \leq 5178 \leq 14079$ and Bioaccumulation factor [conditional units] Prunasin amide and Sambunigrin amide $1.03 \leq 16.3 \leq 255.9$, Prunasin acid and Sambunigrin acid are $0.00 \leq 0.30 \leq 617$; $0.05 \leq 0.39 \leq 3.2$. This is understandable because both compounds are in isomeric form.

3.1.1.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.1.6.1. Lipophilicity

Data from ***Tabl.IV.3.1. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.1. 28 *Lipophilicity of amide and carboxylic acid derivatives of Prunasin and Sambunigrin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Prunasin / Sambunigrin						
amide	0.97	-1.55	-2.29	-1.96	-1.47	-1.29
acid	1.01	-0.90	-1.70	-1.55	-1.23	-0.87

3.1.1.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.1. 29***).

Tabl.IV.3.1. 29 *Water solubility of amide and carboxylic acid derivatives of Prunasin and Sambunigrin*

studied indicator	Prunasin / Sambunigrin	
	amide	acid
ESOL		
Log S	-0.68	-1.09
Solubility, [mg/ml]	6.58e+01	2.53e+01
Class	vs	vs
Ali		
Log S	-0.93	-1.49
Solubility, [mg/ml]	3.64e+01	1.02e+01
Class	vs	vs
SILICOS-IT		
Log S	0.16	0.38
Solubility, [mg/ml]	4.53e+02	7.49e+02
Class	s	s
vs - very soluble; s - soluble		

3.1.1.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Prunasin* and *Sambunigrin* meets the pharmacokinetic requirements (***Table IV.3.1. 30***).

Tabl.IV.3.1. 30 *Pharmacokinetic indicators of amide and derivatives of Prunasin and Sambunigrin*

studied indicator	Prunasin / Sambunigrin

	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	no	no
inhibitors		
CYP1A2	low	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	low	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.31	-8.86

3.1.1.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.1. 31***) containing amide and derivatives of *Prunasin* and *Sambunigrin*.

Tabl.IV.3.1. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Prunasin and Sambunigrin*

studied indicator	Prunasin / Sambunigrin	
	amide	acid
Muegge	Yes	Yes
Bioavailability Score	0.55	0.56

3.1.1.6.5. Medical Chemistry

Data from ***Tabl.IV.3.1. 32*** confirm the drug safety of amide and derivatives of *Prunasin* and *Sambunigrin*.

Tabl.IV.3.1. 32 *Medical chemistry indicators for amide and derivatives of Prunasin and Sambunigrin*

studied indicator	Prunasin / Sambunigrin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	Yes	Yes
Synthetic accessibility	4.35	4.35

- ◊ -

3.1.2. (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide

Subjected to analysis are potential pharmaceutical forms for release within the cancer cell of *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*, comprising an amides and carboxylic acids obtained by hydrolysis of the nitrile groups of *Dhurrin*, *Taxiphyllin*, *Proteacin* and *p-Glucosyloxymandelin*. The process proceeds according to ***§IV.2.3.***

3.1.2.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.2. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*.

Tabl.IV.3.2. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Dhurrin / Taxiphyllin							
	amide	0.27	-0.04	0.01	-0.05	0.28	0.44
	acid	0.42	0.16	0.07	0.33	0.32	0.61
Proteacin							
	amide	0.14	-0.08	-0.08	-0.11	0.17	0.29
	acid	0.24	0.05	-0.04	0.14	0.20	0.41
p-Glucosyloxymandelo-							
	amide	0.13	-0.11	-0.08	-0.06	0.14	0.39
	acid	0.29	0.02	-0.05	0.32	0.17	0.45

The data in ***Tabl.IV.3.2. 1*** show that the amides and carboxylic acids of *Dhurrin* and Taxiphyllin have more pronounced general drug activity *in vivo*.

3.1.2.2. Pharmacological and biological activity of oral active drugs

3.1.2.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.2. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide.*

Tabl.IV.3.2. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Dhurrin / Taxiphyllin / p-Glucosyloxymandelo-												
amide	329	-2.7	9	6	329	-2.7	77	42	329	-2.7	77	42
acid	330	-2.0	9	6	330	-2.0	77	41	330	-2.0	77	41
Proteacin												
amide	491	-4.0	14	9	491	-4.0	110	63	491	-4.0	110	63
acid	492	-3.3	14	9	492	-3.3	110	62	492	-3.3	110	62

Here there are two molecular forms that stand out (the respective amides and carboxylic acid of *Dhurrin* and *Taxiphyllin* which cover most of the requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.2.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide* are listed in ***Tabl.IV.3.2. 3***.

Tabl.IV.3.2. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Dhurrin								
amide	167	5	5	2	19	329	0	15
acid	157	5	5	2	19	330	1	15
Taxiphyllin / p-Glucosyloxymandelo-								
amide	163	5	5	2	19	329	0	15
acid	157	5	5	2	19	330	1	15
Proteacin								
amide	242	8	8	3	28	491	0	23
acid	236	8	8	3	28	492	1	23

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1.***

3.1.2.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.2. 4***.

Tabl.IV.3.2. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAromaRing	uwQED
Dhurrin / Taxiphyllin										
	amide	329	-2.4	9	6	163	5	0	1	0.32
	acid	330	-2.0	9	6	157	5	0	1	0.35
Proteacin										
	amide	491	-4.4	14	9	242	8	0	1	0.10
	acid	492	-4.0	14	9	236	8	0	1	0.15
p-Glucosyloxymandelo-										
	amide	329	-2.7	9	6	163	5	0	1	0.30
	acid	330	-2.3	9	6	157	5	0	1	0.33

The information obtained from the calculations indicates that the amides and carboxylic acids of the studied molecules to the same extent deviate from the defined rules. In the absolute approximation, *Dhurrin* and *Taxiphyllin* derivatives would be more applicable for treatment.

B. wQED

In ***Tabl.IV.3.2. 5*** *Unweighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide* .

Tabl.IV.3.2. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide*

									wQED
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Dhurrin / Taxiphyllin										
	amide	329	-2.4	9	6	163	5	0	1	0.41
	acid	330	-2.0	9	6	157	5	0	1	0.44
Proteacin										
	amide	491	-4.4	14	9	242	8	0	1	0.17
	acid	492	-4.0	14	9	236	8	0	1	0.18
p-Glucosyloxymandelo-										
	amide	329	-2.7	9	6	163	5	0	1	0.39
	acid	330	-2.3	9	6	157	5	0	1	0.42

uwQED (***Tabl.IV.3.2. 4***) and *wQED* (***Tabl.11***) of potential pharmaceutical forms including amides and carboxylic acids obtained by hydrolysis of the nitrile group of *Dhurrin*, *Taxiphyllin*, *Proteacin* and *p-Glucosyloxymandelin* meet the requirements for conservative treatment.

3.1.2.3. Non-laboratory and no clinical information on the chemical

3.1.2.3.1. Receptor activity

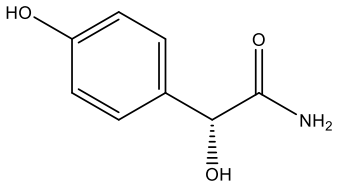
In ***Tabl.IV.3.2. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Dhurrin*, *Taxiphyllin*, *Proteacin* and *p-Glucosyloxymandelo-* to receptors (according to ***§III.3.3.4.1***).

Tabl.IV.3.2. 6 *Receptor activity of amide and carboxyl derivatives of Dhurrin, Taxiphyllin, Proteacin and p-Glucosyloxymandelo-*

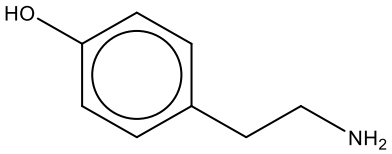
indicator	Dhurrin / Taxiphyllin / Proteacin / p-Glucosyloxymandelo-	
	amide	acid
AR		
ERa	active*	
ERb		
GR		
MR		
PR		
RARa		
RARb		
RARr		
TRa		

TRb		
VDR		
*- antagonist		

The amide form exhibits biological activity as an antagonist of *Estrogen Receptor a* (ERa). This is due to the close chemical nature (Scott, et al., 2016) (Lipfert, et al., 2006) with an already proven molecule: *4-(2-aminoethyl)phenol* (***Fig.IV.3. 1***).



(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide



4-(2-aminoethyl)phenol

Fig.IV.3. 1 *Structural formulas of (R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide u 4-(2-aminoethyl)phenol*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.2.3.2. Mutagenicity

A. Stand -alone models

It is held respectively with ***§III.3.3.4.2***

a) CAESAR

Data from ***Tabl.IV.3.2. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *p-Glucosyloxymandeloamide/acid.*

Tabl.IV.3.2. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Dhurrin, Taxiphyllin, Proteacin and p-Glucosyloxymandelo-*

CAESAR indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.81	0.82	0.75	0.75	0.82	0.69
SMKEV	0.80	0.81	0.84	0.85	0.81	0.82
APSM	0.68	1	0.67	0.67	1	1
CSM	1	0.68	0.67	0.67	0.67	0.33
MDRC	true	true	true	true	true	true

ACFFSC	1	1	1	1	1	1
prediction	NM	NM	NM	NM	M	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; M- mutagenicity; NM- non mutagenicity						

However, molecular fragments of *p-Glucosyloxymandeloamide* coincide with those of other molecules with mutagenicity already studied[22].

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* did not show activity (***Table IV.3.2. 8***).

Tabl.IV.3.2. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

SarPy/IRFMN indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.81	0.82	0.75	0.75	0.68	0.69
SMKEV	0.80	0.81	0.84	0.85	0.81	0.82
APSM	0.68	1	0.67	0.67	1	1
CSM	1	0.68	0.67	0.67	0.32	0.33
ACFFSC	1	1	1	1	1	1
prediction	NM	NM	NM	NM	NM	NM
NM- non mutagenicity						

c) ISS

Amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* are non-mutagenic according to *ISS* methodology (***Table IV.3.2. 9***).

Tabl.IV.3.2. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

ISS indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.75	0.75	0.74	0.75	0.75	0.88
SMKEV	0.78	0.79	0.78	0.78	0.77	0.78

[22] Similarity: 0.82 by CAS: 54954-12-0 and CAS: 23445-00-3; Similarity: 0.78 by CAS: 152-84-1

APSM	0.51	0.52	1	1	1	1
CSM	1	1	0.50	0.50	0.52	1
ACFFSC	1	1	1	1	1	1
Prediction	NM	NM	NM	NM	NM	NM
NM- non mutagenicity						

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Proteacin*, *Dhurrin* and *Taxiphyllin* show some deviation from *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.2. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

KNN/Read-Across indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.71	0.65	0.72	0.72	0.72	0.79
SMKEV	0.81	0.83	0.83	0.85	0.85	0.85
APSM	0.50	0.25	0.50	0.50	0.74	0.74
CSM	0.75	1	0.75	0.75	0.52	1
ACFFSC	1	1	1	1	1	1
prediction	NM	NM	NM	NM	NM	M
M- mutagenicity; NM- non mutagenicity						

However, molecular fragments of *p-Glucosyloxymandeloacid* coincide with those of other molecules with mutagenicity already studied[23].

B. Consensus model

Data from ***Tabl.IV.3.2. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* to mutagenicity.

[23] Similarity: 0.88 by CAS: 531-75-9; Similarity: 0.84 by CAS: 39115-11-2, CAS: 53797-18-5 and CAS: 69686-05-1; Similarity: 0.82 by CAS: 60262-82-0

Tabl.IV.3.2. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

Consensus model mutagenicity indicator	Dhurrin / Taxiphyllin / Proteacin / p-Glucosyloxymandelo	
	amide	acid
numerical value	0.60	0.50

3.1.2.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data from ***Tabl.IV.3.2. 12*** show that despite some deviations in the individual indicators, all studied representatives are non-carcinogenic.

Tabl.IV.3.2. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

CAESAR indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.73	0	0.85	0.85	0	0
SMKEV	0.76	0.75	0.73	0.72	0.76	0.75
APSM	0.50	1	1	1	1	1
CSM	1	0.51	1	1	0	0
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
MCAR	0.39	0.02	0.35	0.35	0.02	0.02
NMNC	1	0.50	1	1	0.50	0.50
Carcinogen	0.31	0.49	0.33	0.33	0.49	0.49
NON-Carcinogen	**0.69**	**0.51**	**0.67**	**0.67**	**0.51**	**0.51**
Prediction	NC	NC	NC	NC	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen						

b) ISS

The non-carcinogenicity of amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* was confirmed (***Tab.IV.3.2. 13***) and using *ISS* methodology.

Tabl.IV.3.2. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

ISS indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.75	0.75	0.74	0.75	0.63	0.75
SMKEV	0.78	0.78	0.78	0.78	0.78	0.78
APSM	0.51	0.52	1	1	0.51	0.52
CSM	1	1	0.50	0.50	0.51	1
ACFSC	1	1	1	1	1	1
Prediction	NC	NC	NC	NC	NC	NC
NC- NON-Carcinogen						

c) IRFMN/Antares

The carcinogenicity data (***Table IV.3.2. 14***) of amide and carboxyl acid derivatives of *Proteacin, p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* by *IRFMN/Antares* method could not be interpreted unambiguously.

Tabl.IV.3.2. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN/Antares indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.62	0.62	0.73	0.81	0.52	0.52
SMKEV	0.80	0.81	0.79	0.80	0.80	0.80
APSM	0.67	0.34	0.67	0.67	0.33	0.34
CSM	0.34	0.66	0.67	1	0.33	0.34
ACFSC	1	1	1	1	1	1
Prediction	PNC	PNC	PNC	PNC	PNC	PNC
PNC- possible non-carcinogenic						

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX method reports increased carcinogenicity (***Tab.IV.3.2. 15***). This is due to compounds already studied similar to them[24].

[24] Similarity: 0.76-8 by CAS: 23214-92-8, CAS: 51-55-8, CAS: 17924-92-4, CAS: 69644-85-5, CAS: 9000-07-1 and CAS: 53973-98-1; Similarity: 0.71-2 by CAS: 643-22-1, CAS: 12663-46-6, CAS: 18883-66-4 and CAS: 116355-83-0 which have been shown to be carcinogenic

Tabl.IV.3.2. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN/ISSCAN-CGX indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.68	0.79	0.79	0.80	0.79	0.79
SMKEV	0.77	0.77	0.77	0.78	0.77	0.77
APSM	1	1	1	1	1	1
CSM	0.35	0.64	0.67	0.66	0.65	0.65
ACFSC	1	1	1	1	1	1
Prediction	PNC	C	C	C	C	C
PNC- possible non-carcinogenic; C- carcinogen						

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The method did not report information (***Tabl.IV.3.2. 16***) on carcinogenicity for the amide and carboxyl derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin*.

Tabl.IV.3.2. 16 *Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN/ISSCAN-CGX indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.74	0.87	0.71	0.71	0.74	0.73
SMKEV	0.76	0.76	0.71	0.70	0.76	0.76
APSM	1	1	1	1	1	1
CSM	0.51	1	0.50	0.50	0.51	0.51
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
Prediction	NC	NC	NC	NC	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; PNC- possible non-carcinogenic						

b) Carcinogenicity oral Slope Factor model

It is noteworthy (***Tabl.IV.3.2. 17***) that the molecules with two glycosidic nuclei (*Proteacin* and *p-Glucosyloxymandelo-*) allow higher allowable working concentrations. However, when checking the molecular weight ratio of the compound to the functional group mass, the results were comparable to those with a single glycoside nucleus (*Dhurrin* and *Taxiphyllin*).

Tabl.IV.3.2. 17 *Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.64	0.63	0	0	0	0
SMKEV	0.75	0.74	0.70	0.69	0.76	0.75
APSM	0.18	0.18	0.57	0.57	0.18	0.18
CSM	1.94	2.02	5.05	5.06	2.23	2.39
MEPASM	0.28	0.28	1.07	1.07	0.28	0.28
MDRC	true	true	n-true	n-true	n-true	n-true
ACFSC	0.85	0.85	0.85	0.85	0.85	0.85
Predicted						
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$					
	14.1	16.6	44.7	45.7	27.5	39.8
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$					
	5.9		**13.9**		**11.6**	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; n-true - does not cover.						

3.1.2.3.4. Toxity

A. Developmental Toxicity model

a) CAESAR

The application of the *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Proteacin, p-Glucosyloxymandelo*, *Dhurrin* and *Taxiphyllin* highlights the lack of toxicity (***Tabl. IV.3.2. 18***).

Tabl.IV.3.2. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

CAESAR indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.75	0.74	0.90	0.90	0.74	0.73
SMKEV	0.78	0.77	0.80	0.79	0.77	0.76
APSM	0.51	0.51	1	1	1	1
CSM	1	1	1	1	0.49	0.49
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
Prediction	NT	NT	NT	NT	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic						

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* did not report values for GADI and CSM. Molecular fragments close to *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.2. 19*** cannot be considered reliable.

Tabl.IV.3.2. 19 *PG toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

PG indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0	0	0	0	0	0
SMKEV	0.78	0.77	0.80	0.79	0.79	0.77
APSM	1	1	1	1	1	1
CSM	0	0	0	0	0	0
ACFSC	1	1	1	1	1	1
Prediction	NT	NT	NT	NT	NT	NT
NT- non-toxic						

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to IRFMN/CORAL toxicity test on amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.2. 20***).

Tabl.IV.3.2. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN/CORAL indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.30	0.45	0.28	0.42	0.30	0.44
SMKEV	0.75	0.75	0.69	0.69	0.74	0.74
APSM	0.59	0.59	0.12	0.12	0.25	0.25
CSM	1.34	1.74	1.39	1.80	0.98	1.38
MEPASM	1.01	1.01	0.18	0.18	0.32	0.32
MDRC	true	true	true	true	true	true
ACFSC	0.40	0.60	0.40	0.60	0.40	0.60
Prediction	[mg/L]					

	18.0	**45.7**	**69.8**	**176.8**	**18.0**	**45.8**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.2. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.2. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

CORAL indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.64	0.64	0.73	0.73	0.63	0.64
SMKEV	0.78	0.79	0.74	0.75	0.77	0.78
APSM	0.52	0.53	1	1	1	1
CSM	1	1	1	1	0.52	0.52
ACFSC	0.85	0.85	0.85	0.85	0.85	0.85
Prediction	A	A	A	A	A	A
A- active						

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* (***Tab.IV.3.2. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.2. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

CORAL indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.92	0.93	0.92	0.92	0.94	0.95
SMKEV	0.85	0.87	0.84	0.85	0.88	0.89
APSM	1	1	1	1	1	1
CSM	1	1	1	1	1	1
ACFSC	1	1	1	1	1	1

Active Agonist	0.14	0.13	0.15	0.14	0.14	0.14
Active Antagonist:	0.03	0.03	0.07	0.07	0.04	0.04
Inactive:	**0.83**	**0.84**	**0.78**	**0.79**	**0.82**	**0.82**
Prediction	inA	inA	inA	inA	inA	inA
inA- inactive						

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Proteacin, p-Glucosyloxymandelo-, Dhurrin* and *Taxiphyllin* did not report any deviations (***Tabl.IV.3.2. 23***) affecting the studied process.

Tabl.IV.3.2. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

CORAL indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.91	0.92	0.92	0.92	0.93	0.93
SMKEV	0.83	0.85	0.84	0.85	0.86	0.87
APSM	1	1	1	1	1	1
CSM	1	1	1	1	1	1
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
Euclidean Distance from the central neuron:	1.47	2.52	2.62	4.04	1.58	3.04
Prediction	nonA	nonA	nonA	nonA	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active						

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Proteacin, p-Glucosyloxymandelo-, Dhurrin* and *Taxiphyllin* we understand (***Tabl.IV.3.2. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.2. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

INERIS indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0	0	0	0	0	0
SMKEV	0.76	0.76	0.72	0.71	0.78	0.77
APSM	0.31	0.31	0.11	0.11	0.08	0.07
CSM	0.42	0.33	0.24	0.34	0.17	0.26
MEPASM	0.50	0.50	0.15	0.15	0.10	0.10
MDRC	N-true	N-true	N-true	N-true	N-true	N-true

ACFSC	0.51	0.51	0.51	0.51	0.51	0.51
Prediction						
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]					
	0.192	0.291	0.197	0.301	0.193	0.247
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]					
	1.556	**1.954**	**1.574**	**2.000**	**1.560**	**1.766**
N-true - does not cover						

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.2. 25***).

Tabl.IV.3.2. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

QSARINS indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.85	0.85	0.85	0.85	0.85	0.85
SMKEV	0.79	0.80	0.77	0.78	0.79	0.79
APSM	0.09	0.09	0.13	0.13	0.15	0.15
CSM	0.02	0.02	0.37	0.34	0.26	0.26
MEPASM	0.15	0.15	0.23	0.23	0.26	0.25
MDRC	true	true	true	true	true	true
ACFSC	1	1	1	1	1	1
LogHLt	[log units]					
	0.279	0.312	0.138	0.173	0.335	0.379
Total half-life	[min]					
	115	**125**	**85**	**90**	**130**	**145**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Proteacin*, *p-Glucosyloxymandelo-*, *Dhurrin* and *Taxiphyllin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.2. 26***).

Tabl.IV.3.2. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN/VERMEER indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.86	0.86	0.89	0.89	0.72	0.72
SMKEV	0.74	0.73	0.78	0.78	0.73	0.74
APSM	1	1	1	1	1	1
CSM	1	1	1	1	0.50	0.50
ACFSC	1	1	1	1	1	1
Prediction	A	A	A	A	A	A
A- active						

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.2.3.2***), carcinogenicity (***§IV.3.1.2.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.2.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Proteacin, p-Glucosyloxymandelo-, Dhurrin* and *Taxiphyllin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.2. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.2. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Proteacin, p-Glucosyloxymandelo-, Dhurrin and Taxiphyllin*

IRFMN indicator	Dhurrin / Taxiphyllin		Proteacin		p-Glucosyloxymandelo	
	amide	acid	amide	acid	amide	acid
GADI	0.69	0.85	0.66	0.67	0.68	0.69
SMKEV	0.81	0.83	0.78	0.79	0.80	0.81
APSM	0.25	0.25	0.25	0.25	0.25	0.25
CSM	1.05	0.91	0.34	0.19	1.05	0.91
MEPASM	0.38	0.38	0.38	0.38	0.38	0.38
MDRC	true	true	true	true	true	true
ACFSC	0.85	0.85	0.85	0.85	0.85	0.85
Prediction	[-log(mg/kg)]					
	-2.605	-2.748	-3.317	-3.46	-2.605	-2.748

Prediction	[mg/kg]					
	403	**560**	**2075**	**2884**	**403**	**560**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set						

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.2.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.2.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Prunasin* and *Sambunigrin* would be optimal for drugs taken orally to poison the cancer cell with *(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide* as performed in ***§IV.2*** second objective of the study.

Alternatively, *Proteacin* derivatives are available.

These two conclusions do not preclude the use of *p-Glucosyloxymandeloamide/acid* derivatives in clinical need.

3.1.2.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values are respectively: *Oral rat LD50* [mg/kg] for Dhurrin amide and Taxiphyllin amide 2541 ≤ 7077 ≤ 19709, Dhurrin acid and Taxiphyllin acid 1159 ≤ 2970 ≤ 7612 and *Bioaccumulation factor* [conditional units] Dhurrin amide and Taxiphyllin amide 1.1 ≤ 17 ≤ 272, Dhurrin acid and Taxiphyllin acid are 0.01 ≤ 0.28 ≤ 6.1. This is understandable because both compounds are in isomeric form.

3.1.2.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.2.6.1. Lipophilicity

Data from ***Tabl.IV.3.2. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.2. 28 *Lipophilicity of amide and carboxylic acid derivatives of Dhurrin and Taxiphyllin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Dhurrin / Taxiphyllin						
amide	0.22	-1.77	-2.59	-2.47	-1.93	-1.71
acid	0.64	-1.12	-1.99	-2.06	-1.69	-1.24

3.1.2.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.2. 29***).

Tabl.IV.3.2. 29 *Water solubility of amide and carboxylic acid derivatives of Dhurrin and Taxiphyllin*

studied indicator	Dhurrin / Taxiphyllin	
	amide	acid
ESOL		
Log S	-0.63	-1.05
Solubility, [mg/ml]	7.73e+01	2.98e+01
Class	vs	vs
Ali		
Log S	-1.13	-1.68
Solubility, [mg/ml]	2.43e+01	6.84e+00
Class	vs	vs
SILICOS-IT		
Log S	0,74	0.96
Solubility, [mg/ml]	1.83e+03	3.01e+03
Class	s	s
vs - very soluble; s - soluble		

3.1.2.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Dhurrin* and *Taxiphyllin* meets the pharmacokinetic requirements (***Tabl.IV.3.2. 30***).

Tabl.IV.3.2. 30 *Pharmacokinetic indicators of amide and derivatives of Dhurrin and Taxiphyllin*

studied indicator	Dhurrin / Taxiphyllin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	no	no
inhibitors		

CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.57	-9.11

3.1.2.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.1. 31***) containing amide and derivatives of *Dhurrin* and *Taxiphyllin*.

Tabl.IV.3.2. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Dhurrin and Taxiphyllin*

studied indicator	Dhurrin / Taxiphyllin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* 2 violations: TPSA>150, H-don>5		

3.1.2.6.5. Medical Chemistry

Data from ***Tabl.IV.3.2. 32*** confirm the drug safety of amide and derivatives of *Dhurrin* and *Taxiphyllin*.

Tabl.IV.3.2. 32 *Medical chemistry indicators for amide and derivatives of Dhurrin and Taxiphyllin*

studied indicator	Dhurrin / Taxiphyllin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	yes	yes
Synthetic accessibility	4.31	4.30

- ◊ -

3.1.3. (R)-2-hydroxy-2-(3-hydroxypheny3l)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell *of (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Zierin*. The process proceeds according to ***§IV.2.3***.

3.1.3.1.Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.3. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*.

Tabl.IV.3.3. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Zierin						
amide	0.26	-0.05	-0.01	-0.04	0.27	0.44
acid	0.41	0.15	0.05	0.33	0.31	0.61

Data in ***Tabl.IV.3.3. 1*** show that the amides and carboxylic acids of *Zierin* have more pronounced overall drug activity *in vivo*.

3.1.3.2. Pharmacological and biological activity of oral active drugs

3.1.3.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.3. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*.

Tabl.IV.3.3. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Zierin												
amide	329	-2.7	9	6	329	-2.7	77	42	329	-2.7	77	42
acid	330	-2.0	9	6	330	-2.0	77	41	330	-2.0	77	41

The two molecular modified forms of *Zierin* cover most of the requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.3.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide* are listed in ***Tabl.IV.3.3. 3***.

Tabl.IV.3.3. 3 *Tabl.14 Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Zierin								
amide	163	5	5	2	19	329	0	15
acid	157	5	5	2	19	330	1	15

There are no significant fluctuations in the individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.3.2.3. QED

The analysis is performed according to ***§3.3.3.1.3.***

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.3. 4***.

Tabl.IV.3.3. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

	uwQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Zierin									
amide	329	-2.4	9	6	163	5	0	1	0.32
acid	330	-2.0	9	6	157	5	0	1	0.35

B. wQED

In ***Tabl.IV.3.3. 5*** *Unweighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide.*

Tabl.IV.3.3. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

		wQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Zierin										
	amide	329	-2.4	9	6	163	5	0	1	0.41
	acid	330	-2.0	9	6	157	5	0	1	0.44

uwQED (***Tabl.IV.3.3. 4***) and *wQED* (***Tabl.IV.3.3. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Zierin* meets the requirements for conservative treatment.

3.1.3.3. Non-laboratory and no clinical information on the chemical form

3.1.3.3.1. Receptor activity

In ***Tabl.IV.3.3. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Zierin* (according to ***§III.3.3.4.1***).

Tabl.IV.3.3. 6 *Receptor activity of amide and carboxyl derivatives of Zierin*

indicator	Zierin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR		
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

From the presented it is unambiguously concluded that the studied molecules show inertness to the studied receptor set.

3.1.3.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.3. 7*** illustrate the mutagenic activity of amide and carboxylic acid derivatives of *Zierin*.

Tabl.IV.3.3. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Zierin*

CAESAR indicator	Zierin	
	amide	acid
GADI	0.81	0.82
SMKEV	0.80	0.81
APSM	0.68	1
CSM	1	0.68
MDRC	true	true
ACFFSC	1	1
prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Zierin* did not show activity (***Tabl.IV.3.3. 8***).

Tabl.IV.3.3. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Zierin*

SarPy/IRFMN indicator	Zierin	
	amide	acid
GADI	0.81	0.82
SMKEV	0.80	0.81
APSM	0.68	1
CSM	1	0.68
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Zierin* are non-mutagenic according to *ISS* methodology (***Tabl.IV.3.3. 9***).

Tabl.IV.3.3. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Zierin*

ISS indicator	Zierin	
	amide	acid
GADI	0.75	0.88
SMKEV	0.78	0.78
APSM	0.51	1
CSM	1	1
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Zierin* show some deviation from *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.3. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Zierin*

KNN/Read-Across indicator	Zierin	
	amide	acid
GADI	0.71	0.64
SMKEV	0.81	0.81
APSM	0.50	0.25
CSM	0.75	1
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.3. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Zierin* to mutagenicity.

Tabl.IV.3.3. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Zierin*

Consensus model mutagenicity indicator	Zierin	
	amide	acid
numerical value	0.60	0.50

3.1.3.3.3. Carcinogenicity

Stand-alone models

a) CAESAR

Data on carcinogenicity in methodology *CAESAR* (***Tabl.IV.3.3. 12***) rejects any carcinogenicity of amide and carboxyl acid derivatives of *Zierin*.

Tabl.IV.3.3. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Zierin*

CAESAR indicator	Zierin	
	amide	acid
GADI	0.73	0.73
SMKEV	0.75	0.75
APSM	0.50	1
CSM	1	0.50
MDRC	true	true
ACFSC	1	1
MCAR	0.39	0.39
NMNC	1	1
Carcinogen	0.31	0.31
NON-Carcinogen	**0.69**	**0.69**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

Like *CAESAR* (***§a***) *ISS* methodology (***Tabl.IV.3.3. 13***), it also did not detect carcinogenicity in amide and carboxyl acid derivatives of *Zierin*.

Tabl.IV.3.3. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Zierin*

ISS indicator	Zierin	
	amide	acid
GADI	0.75	0.64
SMKEV	0.78	0.78
APSM	0.51	0.53
CSM	1	0.53
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares methodology (***Tabl.IV.3.3. 14***) provides quite dualistic information regarding the carcinogenicity of amide and carboxyl acid derivatives of *Zierin*.

Tabl.IV.3.3. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Zierin*

IRFMN/Antares indicator	Zierin	
	amide	acid
GADI	0.61	0.62
SMKEV	0.79	0.81
APSM	0.34	0.34
CSM	0.67	0.67
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

Carboxylic acid form of *Zierin* derivative (***Tabl.IV.3.3. 15***) coincides with some molecules that have already been reported to be carcinogenic[25].

[25] Similarity: 0.74-5 by CAS: 17924-92-4; CAS: 69644-85-5; CAS: 303-47-9; CAS: 23214-92-8 and CAS: 53973-98-1 which have been shown to be carcinogenic

Tabl.IV.3.3. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Zierin*

IRFMN/ISSCAN-CGX indicator	Zierin	
	amide	acid
GADI	0.67	0.78
SMKEV	0.77	0.77
APSM	1	1
CSM	0.35	0.64
ACFSC	1	1
Prediction	PNC	C
PNC- possible non-carcinogenic; C- carcinogen		

A. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The method did not report information (***Tabl.IV.3.3. 16***) on carcinogenicity for the amide and carboxyl derivatives of *Zierin*.

Tabl.IV.3.3. 16 *Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Zierin*

IRFMN indicator	Zierin	
	amide	acid
GADI	0.74	0.87
SMKEV	0.76	0.76
APSM	1	1
CSM	0.51	1
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.3. 17*** shows the data on oral concentration limits of amide and carboxyl acid derivatives of *Zierin*.

Tabl.IV.3.3. 17 *Carcinogenicity oral Slope Factor model (IRFMN) of amide and carboxyl acid derivatives of Zierin*

IRFMN indicator	Zierin	
	amide	acid
GADI	0.64	0.63
SMKEV	0.75	0.73
APSM	0.18	0.18
CSM	1.90	1.96
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	12.9	14.8
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	5.4	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.3.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Zierin* highlights the lack of toxicity (***Table IV.3.3. 18***).

Tabl.IV.3.3. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Zierin*

CAESAR indicator	Zierin	
	amide	acid
GADI	0.75	0.74
SMKEV	0.78	0.77
APSM	0.51	0.51
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) method reports zeros in response to most evaluation indicators. This is due to the lack of clinical and QSAR data for *(R)-2-hydroxy-2-(3-hydroxypheny3l)acetamide*.

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Zierin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.3. 20***).

Tabl.IV.3.3. 19 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Zierin*

IRFMN/CORAL indicator	Zierin	
	amide	acid
GADI	0.29	0.45
SMKEV	0.75	0.75
APSM	0.59	0.59
CSM	1.17	1.57
MEPASM	1.01	1.01
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	12.2	**31.0**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Zierin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.3. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.3. 20 *Chromosomal aberration model of amide and carboxyl acid derivatives of Zierin*

CORAL indicator	Zierin	
	amide	acid
GADI	0.64	0.64
SMKEV	0.77	0.78
APSM	0.52	0.53
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Zierin* (***Tab.IV.3.3. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.3. 21 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Zierin*

IRFMN indicator	Zierin	
	amide	acid
GADI	0.92	0.93
SMKEV	0.85	0.87
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.14	0.14
Active Antagonist:	0.02	0.02
Inactive:	**0.84**	**0.84**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Zierin* did not report any deviations (***Tabl.IV.3.3. 23***) affecting the studied process.

Tabl.IV.3.3. 22 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Zierin*

NIC indicator	Zierin	
	amide	acid
GADI	0.91	0.92
SMKEV	0.83	0.84
APSM	1	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	1.66	2.78
Prediction	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NA- Non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Zierin* we understand (***Tabl.IV.3.3. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.3. 23 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Zierin*

INERIS indicator	Zierin	
	amide	acid
GADI	0	0
SMKEV	0.76	0.75
APSM	0.31	0.31
CSM	0.42	0.33
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.192	0.291
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.556	**1.954**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Zierin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.3. 25***).

Tabl.IV.3.3. 24 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Zierin*

QSARINS indicator	Zierin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.79	0.79
APSM	0.09	0.09
CSM	0.02	0.05
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.281	0.313
Total half-life	[min]	
	115	**125**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Zierin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.3. 26***).

Tabl.IV.3.3. 25 *Micronucleus toxicity activity – in vitro for amide and carboxyl acid derivatives of Zierin*

IRFMN/VERMEER indicator	Zierin	
	amide	acid
GADI	0.86	0.86
SMKEV	0.74	0.74
APSM	1	1
CSM	1	1
ACFSC	1	1
Prediction	A	A
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.3.3.2***), carcinogenicity (***§IV.3.1.3.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.3.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives *Zierin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.3. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.3. 26 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Zierin*

IRFMN/VERMEER indicator	Zierin	
	amide	acid
GADI	0.69	0.85
SMKEV	0.82	0.83
APSM	0.25	0.25
CSM	1.05	0.91
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-2.605	-2.748
Prediction	[mg/kg]	
	403	**560**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.3.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.3.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Zierin* would be optimal for drugs taken orally to poison the cancer cell with *(R)-2-hydroxy-2-(3-hydroxypheny3l)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.3.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide 2646 ≤ 7387 ≤ 20620, acid 2283 ≤ 5725 ≤ 14356 and *Bioaccumulation factor* [conditional units] amide 1.13 ≤ 17.7 ≤ 279, acid are 0.03 ≤ 0.64 ≤ 14.31.

3.1.3.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7.***

3.1.3.6.1. Lipophilicity

Data from ***Tabl.IV.3.3. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.3. 27 *Lipophilicity of amide and carboxylic acid derivatives of Zierin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Zierin						
amide	0.67	-1.77	-2.59	-2.47	-1.93	-1.62
acid	1.11	-1.12	-1.99	-2.06	-1.63	-1.15

3.1.3.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.3. 29***).

Tabl.IV.3.3. 28 *Water solubility of amide and carboxylic acid derivatives of Zierin*

studied indicator	Zierin	
	amide	acid
ESOL		
Log S	-0.63	-1.05
Solubility, [mg/ml]	7.73e+01	2.98e+01
Class	vs	vs
Ali		
Log S	-1.13	-1.68
Solubility, [mg/ml]	2.43e+01	6.84e+00
Class	vs	vs
SILICOS-IT		
Log S	0,74	0.96

Solubility, [mg/ml]	1.83e+03	3.01e+03
Class	s	s
vs - very soluble; s - soluble		

3.1.3.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Zierin* meets the pharmacokinetic requirements (***Table IV.3.3. 30***).

Tabl.IV.3.3. 29 *Pharmacokinetic indicators of amide and derivatives of Zierin*

studied indicator	Zierin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	no	no
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.57	-9.11

3.1.3.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.3. 31***) containing amide and derivatives of *Zierin*

Tabl.IV.3.3. 30 *Muegge activity and Bioavailability Score of amide and derivatives of Zierin*

studied indicator	Zierin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* 2 violations: TPSA>150, H-don>5		

3.1.3.6.5. Medical Chemistry

Data from ***Tabl.IV.3.3. 32*** confirm the drug safety of amide and derivatives *Zierin*.

Tabl.IV.3.3. 31 *Medical chemistry indicators for amide and derivatives of Zierin*

studied indicator	Zierin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	yes	yes
Synthetic accessibility	4.38	4.37

- ◊ -

3.1.4. 2-hydroxy-2-methylpropanamide

Subject to analysis is a potential pharmaceutical form for release within the cancer cell of *2-hydroxy-2-methylpropanamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Linamarin*. The process proceeds according to ***§IV.2.3.***

3.1.4.1.Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.4. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-2-methylpropanamide*.

Tabl.IV.3.4. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-2-methylpropanamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Linamarin							
	amide	0.15	0.18	0	-0,20	0.19	0.57
	acid	0.22	0.20	-0.13	0.28	0.09	0.75

Data in ***Tabl.IV.3.4. 1*** show that the amides and carboxylic acids of *Linamarin* have more pronounced overall drug activity *in vivo*.

3.1.4.2. Pharmacological and biological activity of oral active drug

3.1.4.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.4. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-2-methylpropanamide*.

Tabl.IV.3.4. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-2-methylpropanamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Linamarin													
	amide	265	-2.3	8	5	265	-2.3	56	37	265	-2.3	56	37
	acid	266	-1.6	8	5	266	-1.6	56	36	266	-1.6	56	36

The two molecular modified forms of *Linamarin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.4.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-2-methylpropanamide* are listed in ***Tabl.IV.3.4. 3***.

Tabl.IV.3.4. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-2-methylpropanamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Linamarin								
amide	142	4	4	1	14	265	0	13
acid	137	4	4	1	14	266	1	13

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.4.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.4. 4.***

Tabl.IV.3.4. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-2-methylpropanamide*

	uwQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAromaRing	uwQED
Linamarin									
amide	265	-2.2	8	5	142	4	0	0	0.38
acid	266	-1.8	8	5	137	4	0	0	0.41

B. wQED

In ***Tabl.IV.3.4. 5*** *Unweighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-2-methylpropanamide*.

Tabl.IV.3.4. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-2-methylpropanamide*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	Salerts	nAtomRing	wQED
Linamarin										
	amide	265	-2.2	8	5	142	4	0	0	0.45
	acid	266	-1.8	8	5	137	4	0	0	0.47

uwQED (***Tabl.IV.3.4. 4)*** and *wQED* (***Tabl.IV.3.4. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Linamarin* meets the requirements for conservative treatment.

3.1.4.3. Non -laboratory and no clinical information on the chemical form

3.1.4.3.1. Receptor activity

In ***Tabl.IV.3.4. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Linamarin* (according to ***§III.3.3.4.1***).

Tabl.IV.3.4. 6 *Receptor activity of amide and carboxyl derivatives of Linamarin*

indicator	Linamarin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

With the exception of *Mineralocorticoid Receptor* (MR), the studied molecules show inertness to the studied receptor set.

3.1.4.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.4. 7*** illustrate explicitly the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied.

Tabl.IV.3.4. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Linamarin*

CAESAR indicator	Linamarin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.83	0.82
APSM	0.67	0.67
CSM	0.67	0.68
MDRC	true	true
ACFFSC	1	1
prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Linamarin* did not show activity (***Table IV.3.4. 8***).

Tabl.IV.3.4. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Linamarin*

SarPy/IRFMN indicator	Linamarin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.83	0.82
APSM	0.67	0.67
CSM	0.67	0.68

ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Linamarin* are non-mutagenic according to *ISS* methodology (***Table IV.3.4. 9***).

Tabl.IV.3.4. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Linamarin*

ISS indicator	Linamarin	
	amide	acid
GADI	0.77	0.77
SMKEV	0.83	0.83
APSM	1	1
CSM	0.51	0.52
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Linamarin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.4. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Linamarin*

KNN/Read-Across indicator	Linamarin	
	amide	acid
GADI	0.54	0.54
SMKEV	0.83	0.83
APSM	0.24	0.24
CSM	0.51	0.52
ACFFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.4. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Linamarin* to mutagenicity.

Tabl.IV.3.4. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Linamarin*

Consensus model mutagenicity indicator	Linamarin	
	amide	acid
numerical value	0.50	0.50

3.1.4.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using the *CAESAR* methodology (***Tab.IV.3.4. 12***), for amide and carboxyl acid derivatives of *Linamarin* did not indicate the presence of carcinogenicity (by training set[26]).

Tabl.IV.3.4. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Linamarin*

CAESAR indicator	Linamarin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.82	0.80
APSM	1	1
CSM	0.50	0.50
MDRC	true	true
ACFSC	1	1
MCAR	0.61	0.25
NMNC	1	1
Carcinogen	0.19	**0.62**
NON-Carcinogen	**0.81**	0.38
Prediction	NC	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

[26] Similarity: 0.80 by CAS: 18883-66-4 and CAS: 54749-90-5

b) ISS

Like *CAESAR* (***§a***) and *ISS* methodology (***Tab.IV.3.4. 13***), it identifies amide and carboxyl acid derivatives of *Linamarin* as non-carcinogenic.

Tabl.IV.3.4. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Linamarin*

ISS indicator	Linamarin	
	amide	acid
GADI	0.77	0.78
SMKEV	0.83	0.83
APSM	1	1
CSM	0.51	0.52
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

c) IRFMN/Antares

Carboxyl acid form of *Linamarin* is prone to carcinogenicity (***Table IV.3.4. 14***) according to *IRFMN/Antares* methodology. In the training set there are molecules with close to analyzed fragments.

Tabl.IV.3.4. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN/Antares indicator	Linamarin	
	amide	acid
GADI	0.63	0.76
SMKEV	0.84	0.86
APSM	0.67	0.67
CSM	0.33	0.66
ACFSC	1	1
Prediction	PNC	C
PNC- possible non-carcinogenic; C- carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.4. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Linamarin*.

Tabl.IV.3.4. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN/ISSCAN-CGX indicator	Linamarin	
	amide	acid
GADI	0.69	0.70
SMKEV	0.82	0.81
APSM	1	1
CSM	0.34	0.35
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Linamarin* was confirmed (***Table IV.3.4. 16***) by *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.4. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Linamarin*

IRFMN indicator	Linamarin	
	amide	acid
GADI	0	0
SMKEV	0.82	0.80
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.4. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Linamarin* should not be administered orally.

Tabl.IV.3.4. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN indicator	Linamarin	
	amide	acid
GADI	0.69	0.68
SMKEV	0.82	0.80
APSM	0.18	0.18
CSM	2.17	2.17
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	23.4	24.0
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	7.5	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.4.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Linamarin* highlights the lack of toxicity (***Table IV.3.3. 18***).

Tabl.IV.3.4. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Linamarin*

CAESAR indicator	Linamarin	
	amide	acid
GADI	0.89	0.89
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) method reports zeros in response to most evaluation indicators. This is due to the lack of clinical and QSAR data for *2-hydroxy-2-methylpropanamide*.

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Linamarin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.4. 19***).

Tabl.IV.3.4. 19 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN/CORAL indicator	Linamarin	
	amide	acid
GADI	0.27	0.41
SMKEV	0.68	0.68
APSM	0.58	0.58
CSM	0.81	1.04
MEPASM	1.01	1.01
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	4.2	**10.6**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Linamarin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.4. 20***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.4. 20 *Chromosomal aberration model of amide and carboxyl acid derivatives of Linamarin*

CORAL indicator	Linamarin	
	amide	acid
GADI	0.65	0.64
SMKEV	0.80	0.82
APSM	1	1
CSM	0.52	0.48
ACFSC	0.85	0.85

Prediction	A	inA
A- active; inA- inactive		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Linamarin* (***Tab.IV.3.4. 21***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.4. 21 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN indicator	Linamarin	
	amide	acid
GADI	0.93	0.94
SMKEV	0.87	0.88
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.04	0.02
Active Antagonist:	0.01	0.08
Inactive:	0.95	0.90
Prediction	**inA**	**inA**
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Linamarin* did not report any deviations (***Tabl.IV.3.4. 22***) affecting the studied process.

Tabl.IV.3.4. 22 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Linamarin*

NIC indicator	Linamarin	
	amide	acid
GADI	0.78	0.77
SMKEV	0.85	0.83
APSM	0.51	0.52
CSM	1	1
MDRC	true	true
ACFSC	1	1

Euclidean Distance from the central neuron:	2.62	2.43
Prediction	NA	NA

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Linamarin* we understand (***Tabl.IV.3.4. 23***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.4. 23 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Linamarin*

INERIS indicator	Linamarin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.66
APSM	0.05	0.29
CSM	0.16	0.30
MEPASM	0.08	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.158	-0.041
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.439	**1.099**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Linamarin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.4. 24***).

Tabl.IV.3.4. 24 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Linamarin*

QSARINS indicator	Linamarin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.82	0.83
APSM	0.09	0.03
CSM	0.02	0.12

MEPASM	0.15	0.03
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.281	0.301
Total half-life	[min]	
	115	**120**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Linamarin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.4. 25***).

Tabl.IV.3.4. 25 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Linamarin*

IRFMN/VERMEER indicator	Linamarin	
	amide	acid
GADI	0.73	0.72
SMKEV	0.76	0.75
APSM	1	1
CSM	0.49	0.48
ACFSC	1	1
Prediction	A	A
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.4.3.2***), carcinogenicity (***§IV.3.1.4.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.4.3.4***).

b) in vivo

Linamarin is a well-studied natural product, and hence *2-hydroxy-2-methylpropanamide* is well known. It is used in a clinical setting. *Micronucleus* activity - *in vivo* does not report genotoxic loading of the tested substances (***Tabl.IV.3.4. 26***).

Tabl.IV.3.4. 26 *Micronucleus toxicity activity model – in vivo of amide and carboxyl acid derivatives of Linamarin*

IRFMN indicator	Linamarin	
	amide	acid
GADI	0.91	0.93
SMKEV	0.83	0.86
APSM	1	1
CSM	1	1
ACFSC	1	1
Prediction	NON-genotoxic	NON-genotoxic

F. NOAEL

The amide and carboxylic acid derivatives of *Linamarin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.4. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.4. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Linamarin*

IRFMN/VERMEER indicator	Linamarin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.85	0.87
APSM	0.25	0.25
CSM	0.97	0.82
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-2.691	-2.834
Prediction	[mg/kg]	
	491	**682**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.4.4.Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.10.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Linamarin* would be optimal for drugs taken orally to poison the cancer cell with *2-hydroxy-2-methylpropanamide* as performed in ***§IV.2*** second objective of the study.

3.1.4.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide $1092 \leq 2218 \leq 4506$, acid $1218 \leq 2280 \leq 4266$ and *Bioaccumulation factor* [conditional units] amide $0.02 \leq 18.3 \leq 13516$, acid form are $0.24 \leq 1.22 \leq 6.21$.

3.1.4.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.4.6.1. Lipophilicity

Data from ***Tabl.IV.3.4. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.4. 28 *Lipophilicity of amide and carboxylic acid derivatives of Linamarin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Linamarin						
amide	0.74	-3.11	-2.93	-2.77	-2.37	-2.09
acid	0.14	-2.46	-2.33	-2.37	-2.13	-1.83

3.1.4.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.4. 29***).

Tabl.IV.3.4. 29 *Water solubility of amide and carboxylic acid derivatives of Linamarin*

studied indicator	Linamarin	
	amide	acid
ESOL		
Log S	0.74	0.31
Solubility, [mg/ml]	1.45e+03	5.60e+02
Class	hs	hs
Ali		
Log S	0.68	0.13
Solubility, [mg/ml]	1.28e+03	3.60e+02
Class	hs	hs
SILICOS-IT		
Log S	1.88	2.10
Solubility, [mg/ml]	2.03e+04	3.34e+04
Class	s	s
hs - highly soluble; s - soluble		

3.1.4.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Dhurrin* and *Taxiphyllin* meets the pharmacokinetic requirements (***Table IV.3.4. 30***).

Tabl.IV.3.4. 30 *Pharmacokinetic indicators of amide and derivatives of Linamarin*

studied indicator	Linamarin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	no	no
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-10.13	-9.67

3.1.4.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.4. 31***) containing amide and derivatives of *Linamarin*.

Tabl.IV.3.4. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Linamarin*

studied indicator	Linamarin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.56
* 1 violations: XLOGP3<-2		

3.1.4.6.5. Medical Chemistry

Data from ***Tabl.IV.3.4. 32*** confirm the drug safety of amide and derivatives of *Linamarin*.

Tabl.IV.3.4. 32 *Medical chemistry indicators for amide and derivatives of Linamarin*

studied indicator	Linamarin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	yes	yes
Synthetic accessibility	4.43	4.46

- ◊ -

3.1.5. (S)-2-hydroxy-2-methylbutanamide

Subject to analysis is a potential pharmaceutical form for release within the cancer cell of *(S)-2-hydroxy-2-methylbutanamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Lotaustralin*. The process proceeds according to ***§IV.2.3.***

3.1.5.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.5. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*.

Tabl.IV.3.5. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-2-(3-hydroxyphenyl)acetamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Lotaustralin							
	amide	0.14	0.14	0	-0.60	0.24	0.58
	acid	0.20	0.16	-0.12	0.31	0.15	0.75

Data in ***Tabl.IV.3.5. 1*** show that the amides and carboxylic acids of *Lotaustralin* have more pronounced overall drug activity *in vivo*.

3.1.5.2. Pharmacological and biological activity of oral active drugs

3.1.5.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.5. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-2-hydroxy-2-methylbutanamide*.

Tabl.IV.3.5. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-2-hydroxy-2-methylbutanamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Lotaustralin													
	amide	279	-2.0	8	5	279	-2.0	59	40	279	-2.0	59	40

acid	280	-1.2	8	5	280	-1.2	59	39	280	-1.2	59	39

The two molecular modified forms of *Lotaustralin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.5.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-2-hydroxy-2-methylbutanamide* are listed in ***Tabl.IV.3.5. 3.***

Tabl.IV.3.5. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-2-hydroxy-2-methylbutanamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Lotaustralin								
amide	142	5	5	1	14	279	0	13
acid	137	5	5	1	14	280	1	13

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.5.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.5. 4***.

Tabl.IV.3.5. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-2-hydroxy-2-methylbutanamide*

	uwQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Lotaustralin									
amide	279	-2.9	8	5	142	5	0	0	0.34
acid	280	-2.5	8	5	137	5	0	0	0.37

B. wQED

In ***Tabl.IV.3.5. 5*** shows *Unweighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-2-hydroxy-2-methylbutanamide.*

Tabl.IV.3.5. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially able to overcome the cancer cell and release (S)-2-hydroxy-2-methylbutanamide in it*

		wQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Lotaustralin										
	amide	279	-2.9	8	5	142	5	0	0	0.40
	acid	280	-2.5	8	5	137	5	0	0	0.43

uwQED (***Tabl.IV.3.5. 4***) and *wQED* (***Tabl.IV.3.5. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Lotaustralin* meets the requirements for conservative treatment.

3.1.5.3. Non -laboratory and no clinical information on the chemical form

3.1.5.3.1. Receptor activity

In ***Tabl.IV.3.5. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Lotaustralin* to receptors (according to ***§III.3.3.4.1***).

Tabl.IV.3.5. 6 *Receptor activity of amide and carboxyl derivatives of Lotaustralin*

indicator	Lotaustralin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

With the exception of *Mineralocorticoid Receptor* (MR), the studied molecules show inertness to the studied receptor set.

3.1.5.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.5. 7*** explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of *Lotaustralin*.

Tabl.IV.3.5. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Lotaustralin*

CAESAR indicator	Lotaustralin	
	amide	acid
GADI	0.83	0.75
SMKEV	0.83	0.83
APSM	1	0.68
CSM	0.67	0.68
MDRC	true	true
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Lotaustralin* did not show activity (***Table IV.3.5. 8***).

Tabl.IV.3.5. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Lotaustralin*

SarPy/IRFMN indicator	Lotaustralin	
	amide	acid
GADI	0.83	0.64
SMKEV	0.83	0.83
APSM	1	0.36
CSM	0.68	0.68
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Lotaustralin* are non-mutagenic according to *ISS* methodology (***Table IV.3.5. 9***).

Tabl.IV.3.5. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Lotaustralin*

ISS indicator	Lotaustralin	
	amide	acid
GADI	0.77	0.78
SMKEV	0.83	0.82
APSM	1	1
CSM	0.52	0.53
ACFFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Lotaustralin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.5. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Lotaustralin*

KNN/Read-Across indicator	Lotaustralin	
	amide	acid
GADI	0.60	0.55
SMKEV	0.85	0.84
APSM	0.24	0.24
CSM	0.75	0.52
ACFFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.5. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Lotaustralin* to mutagenicity.

Tabl.IV.3.5. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Lotaustralin*

Consensus model mutagenicity indicator	Lotaustralin	
	amide	acid
numerical value	0.50	0.40

3.1.5.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data from ***Tabl.IV.3.5. 12*** show us that *CAESAR* carcinogenicity assessment methodology (with training set[27]) does not detect a deviation for of amide and carboxyl acid derivatives of *Lotaustralin*.

Tabl.IV.3.5. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Lotaustralin*

CAESAR indicator	Lotaustralin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.81	0.79
APSM	1	1
CSM	0.50	0.50
MDRC	true	true
ACFSC	1	1
MCAR	0.61	0.25
NMNC	1	1
Carcinogen	0.19	**0.62**
NON-Carcinogen	**0.81**	0.38
Prediction	NC	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

Carboxylic acid form of *Lotaustralin* is carcinogenic[28], according to *ISS* evaluation methodology (***Tab.IV.3.5. 13***).

[27] Similarity: 0.79 by CAS: 18883-66-4 and CAS: 54749-90-5
[28] Similarity: 0.74-9 by CAS: 18883-66-4, CAS: 51333-22-3, CAS: 60102-37-6 and CAS: 315-22-0

Tabl.IV.3.5. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Lotaustralin*

ISS indicator	Lotaustralin	
	amide	acid
GADI	0.77	0.75
SMKEV	0.83	0.82
APSM	1	1
CSM	0.52	0.47
ACFSC	1	1
Prediction	NC	C
NC- NON-Carcinogen; C- carcinogen		

c) IRFMN/Antares

Carboxylic acid form of *Lotaustralin* is carcinogenic, according to *IRFMN/Antares* evaluation methodology (***Tab.IV.3.5. 14***).

Tabl.IV.3.5. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/Antares indicator	Lotaustralin	
	amide	acid
GADI	0.65	0.54
SMKEV	0.85	0.87
APSM	0.67	0.34
CSM	0.34	0.33
ACFSC	1	1
Prediction	PNC	C
PNC- possible non-carcinogenic; C- carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX (***Table IV.3.5. 15***) provides quite dualistic information regarding the carcinogenicity of amide and carboxyl acid derivatives of *Lotaustralin*.

Tabl.IV.3.5. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/ISSCAN-CGX indicator	Lotaustralin	
	amide	acid
GADI	0.70	0.70
SMKEV	0.82	0.81
APSM	1	1
CSM	0.35	0.36
ACFSC	1	1

Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The method did not report information (***Tabl.IV.3.5. 16***) on carcinogenicity for the amide and carboxyl derivatives of *Lotaustralin*.

Tabl.IV.3.5. 16 *Data of Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN indicator	Lotaustralin	
	amide	acid
GADI	0	0
SMKEV	0.80	0.79
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.5. 17*** shows the data on oral concentration limits of amide and carboxyl acid derivatives of *Lotaustralin*.

Tabl.IV.3.5. 17 *Data of Carcinogenicity oral Slope Factor model (IRFMN) of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN indicator	Lotaustralin	
	amide	acid
GADI	0.68	0.67
SMKEV	0.80	0.79
APSM	0.18	0.18
CSM	2.14	2.13
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85

Predicted Oral Carcinogenicity SF for molecular forms	(g/kg-day)$^{-1}$	
	22.4	**21.9**
Presumed concentration of the active form inside the cancer cell	(g/kg-day)$^{-1}$	
	7.8	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.5.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Lotaustralin* highlights the lack of toxicity (***Table IV.3.5. 18***).

Tabl.IV.3.5. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Lotaustralin*

CAESAR indicator	Lotaustralin	
	amide	acid
GADI	0.76	0.89
SMKEV	0.80	0.80
APSM	1	1
CSM	0.53	1
MEPASM	true	true
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Lotaustralin* did not report values for GADI and CSM. Molecular fragments close to *(S)-2-hydroxy-2-methylbutanamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.5. 19*** cannot be considered reliable.

Tabl.IV.3.5. 19 *PG toxicity of amide and carboxyl acid derivatives of Lotaustralin*

PG indicator	Lotaustralin	
	amide	acid
GADI	0	0
SMKEV	0.77	0.76
APSM	1	1
CSM	0	0

ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Lotaustralin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.5. 20***).

Tabl.IV.3.5. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/CORAL indicator	Lotaustralin	
	amide	acid
GADI	0.27	0.41
SMKEV	0.68	0.68
APSM	0.35	0.58
CSM	0.18	1.05
MEPASM	0.54	1.01
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	4.46	**11.32**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide derivative of *Lotaustralin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.5. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.5. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Lotaustralin*

CORAL indicator	Lotaustralin	
	amide	acid
GADI	0.65	0.64
SMKEV	0.80	0.82
APSM	1	1
CSM	0.53	0.47
ACFSC	0.85	0.85

Prediction	A	inA
A- active; inA- inactive		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Lotaustralin* (***Tab.IV.3.5. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.5. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN indicator	Lotaustralin	
	amide	acid
GADI	0.93	0.94
SMKEV	0.87	0.88
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.03	0.01
Active Antagonist:	0.01	0.01
Inactive:	0.96	0.98
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Lotaustralin* did not report any deviations (***Tabl.IV.3.5. 23***) affecting the studied process.

Tabl.IV.3.5. 23 *p-Glycoprotein activity toxicity model of amide and carboxyl acid derivatives of Lotaustralin*

NIC indicator	Lotaustralin	
	amide	acid
GADI	0.78	0.78
SMKEV	0.87	0.84
APSM	0.51	0.51
CSM	1	1
MDRC	true	true
ACFSC	1	1

Euclidean Distance from the central neuron:	2.27	2.24
Prediction	NA	NA

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Lotaustralin* we understand (***Tabl.IV.3.5. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.5. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Lotaustralin*

INERIS indicator	Lotaustralin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.67
APSM	0.16	0.31
CSM	0.57	0.34
MDRC	0.19	0.50
ACFSC	N-true	N-true
GADI	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.181	0.278
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.517	**1.897**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Lotaustralin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.5. 25***).

Tabl.IV.3.5. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Lotaustralin*

QSARINS indicator	Lotaustralin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.84	0.85
APSM	0.09	0.02
CSM	0.05	0.12
MEPASM	0.15	0.03
MDRC	true	true
ACFSC	1	1

Prediction		
LogHLt	[log units]	
	0.310	0.333
Total half-life	[min]	
	125	**130**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Lotaustralin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.5. 26***).

Tabl.IV.3.5. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/VERMEER indicator	Lotaustralin	
	amide	acid
GADI	0.73	0.73
SMKEV	0.77	0.76
APSM	1	1
CSM	0.49	0.48
ACFSC	1	1
Prediction	A	A
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.5.3.2***), carcinogenicity (***§IV.3.1.5.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.5.3.4***).

b) in vivo

Linamarin is a well-studied natural product, and hence *(S)-2-hydroxy-2-methylbutanamide* is well known. It is used in a clinical setting. *Micronucleus* activity - *in vivo* does not report genotoxic loading of the tested substances (***Tabl.IV.3.5. 27***).

Tabl.IV.3.5. 27 *Micronucleus toxicity activity model – in vivo of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/VERMEER indicator	Lotaustralin	
	amide	acid
GADI	0.92	0.93
SMKEV	0.84	0.86
APSM	1	1
CSM	1	1
ACFSC	1	1
Prediction	**NON-genotoxic**	**NON-genotoxic**

F. NOAEL

The amide and carboxylic acid derivatives of *Lotaustralin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.5. 28***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.5. 28 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Lotaustralin*

IRFMN/VERMEER indicator	Lotaustralin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.87	0.89
APSM	0.25	0.25
CSM	0.94	0.80
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-2.714	-2.857
Prediction	[mg/kg]	
	518	**719**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.5.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.5.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Lotaustralin* would be optimal for drugs taken orally to poison the cancer cell with *(S)-2-hydroxy-2-methylbutanamide* as performed in ***§IV.2*** second goal of the study.

3.1.5.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. No toxicity deviations were found and the values were respectively: *Oral rat LD50* [mg/kg] for amide $3000 \leq 8420 \leq 23633$, acid $5279 \leq 13466 \leq 34351$ and *Bioaccumulation factor* [conditional units] amide $4.01 \leq 64.6 \leq 1043$, acid are $0.04 \leq 0.34 \leq 2.98$.

3.1.5.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.5.6.1. Lipophilicity

Data from ***Tabl.IV.3.5. 29*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.5. 29 *Lipophilicity of amide and carboxylic acid derivatives of Lotaustralin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Lotaustralin						
amide	1.16	-1.99	-2.54	-2.48	-2.00	-1.57
acid	1.00	-1.34	-1.94	-2.08	-1.76	-1.22

3.1.5.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.5. 30***).

Tabl.IV.3.5. 30 *Water solubility of amide and carboxylic acid derivatives of Lotaustralin*

studied indicator	Lotaustralin	
	amide	acid
ESOL		
Log S	0.01	-0.40
Solubility, [mg/ml]	2.87e+02	1.11e+02
Class	hs	vs
Ali		
Log S	-0.48	-1.03
Solubility, [mg/ml]	9.29e+01	2.61e+01
Class	s	vs
SILICOS-IT		
Log S	1.48	1.70
Solubility, [mg/ml]	8.49e+03	1.40e+04
Class	s	s
vs - very soluble; hs - highly soluble; s - soluble		

3.1.5.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Lotaustralin* meets the pharmacokinetic requirements (***Table IV.3.5. 31***).

Tabl.IV.3.5. 31 *Pharmacokinetic indicators of amide and derivatives of Lotaustralin*

studied indicator	Lotaustralin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	no	no
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.42	-8.96

3.1.5.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.5. 32***) containing amide and derivatives of *Lotaustralin*.

Tabl.IV.3.5. 32 *Muegge activity and Bioavailability Score of amide and derivatives of Lotaustralin*

studied indicator	Lotaustralin	
	amide	acid
Muegge	Yes	Yes
Bioavailability Score	0.55	0.56

3.1.5.6.5. Medical Chemistry

Data from ***Tabl.IV.3.5. 33*** confirm the drug safety of amide and derivatives of *Lotaustralin*.

Tabl.IV.3.5. 33 *Medical chemistry indicators for amide and derivatives of Lotaustralin*

studied indicator	Lotaustralin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	yes	yes
Synthetic accessibility	4.60	4.67

- ◊ -

3.1.6. 2-hydroxy-3-methylbut-2-enamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *2-hydroxy-3-methylbut-2-enamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Acacipetalin*. The process proceeds according to ***§IV.2.3.***

3.1.6.1.Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.6. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-3-methylbut-2-enamide*.

Tabl.IV.3.6. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-3-methylbut-2-enamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Acacipetalin						
amide	0.11	0.08	-0.17	-0.21	0.08	0.58
acid	0.25	0.14	-0.25	-0.02	0.03	0.75

Data in ***Tabl.IV.3.6. 1*** show that the amides and carboxylic acids of *Acacipetalin* have more pronounced overall drug activity *in vivo*.

3.1.6.2. Pharmacological and biological activity of oral active drugs

3.1.6.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.6. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-3-methylbut-2-enamide*.

Tabl.IV.3.6. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-3-methylbut-2-enamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Acacipetalin												
amide	277	-1.7	8	5	277	-1.7	62	38	277	-1.7	62	38
acid	278	-1.0	8	5	278	-1.0	62	37	278	-1.0	62	37

The two molecular modified forms of Acacipetalin meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.6.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-3-methylbut-2-enamide* are listed in ***Tabl.IV.3.6. 3***.

Tabl.IV.3.6. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-3-methylbut-2-enamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Acacipetalin									
	amide	142	4	4	1	15	277	0	13
	acid	137	4	4	1	15	278	1	13

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.6.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.6. 4***.

Tabl.IV.3.6. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-3-methylbut-2-enamide*

										uwQED
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Acacipetalin										
	amide	277	-1.7	8	5	142	4	2	0	0.39
	acid	278	-1.3	8	5	137	4	2	0	0.41

B. wQED

In ***Tabl.IV.3.6. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *2-hydroxy-3-methylbut-2-enamide*.

Tabl.IV.3.6. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release 2-hydroxy-3-methylbut-2-enamide*

		wQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Acacipetalin										
	amide	277	-1.7	8	5	142	4	2	0	0.42
	acid	278	-1.3	8	5	137	4	2	0	0.44

uwQED (***Tabl.IV.3.6. 4***) and *wQED* (***Tabl.IV.3.6. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Acacipetalin* meets the requirements for conservative treatment.

3.1.6.3. Non -laboratory and no clinical information on the chemical form

3.1.6.3.1. Receptor activity

In ***Tabl.IV.3.6. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Acacipetalin* to receptors (according to ***§III.3.3.4.1***).

Tabl.IV.3.6. 6 *Receptor activity of amide and carboxyl derivatives of Acacipetalin*

indicator	Acacipetalin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

With the exception of *Mineralocorticoid Receptor* (MR), the studied molecules show inertness to the studied receptor set.

3.1.6.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.6. 7*** explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of *Acacipetalin*.

Tabl.IV.3.6. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Acacipetalin*

CAESAR indicator	Acacipetalin	
	amide	acid
GADI	0.69	0.69
SMKEV	0.80	0.81
APSM	0.34	0.35
CSM	1	1
MDRC	true	true
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Acacipetalin* did not show activity (***Table IV.3.6. 8***).

Tabl.IV.3.6. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Acacipetalin*

SarPy/IRFMN indicator	Acacipetalin	
	amide	acid
GADI	0.69	0.69
SMKEV	0.80	0.81
APSM	0.34	0.35
CSM	1	1
ACFSC	1	1

prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Acacipetalin* are non-mutagenic according to the *ISS* methodology (***Table IV.3.6. 9***).

Tabl.IV.3.6. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Acacipetalin*

ISS indicator	Acacipetalin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.50	0.52
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Acacipetalin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.6. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Acacipetalin*

KNN/Read-Across indicator	Acacipetalin	
	amide	acid
GADI	0.53	0.75
SMKEV	0.81	0.81
APSM	0.24	0.48
CSM	0.51	1
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.6. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Acacipetalin* to mutagenicity.

Tabl.IV.3.6. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Acacipetalin*

Consensus model mutagenicity indicator	Acacipetalin	
	amide	acid
numerical value	0.25	0.50

3.1.6.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

The carcinogenicity data of the studied derivatives of *Acacipetalin* (***Tabl. IV.3.6. 12***) according to *CAESAR* methodology (with training set[29]) could not be interpreted unambiguously. Due to some inaccuracies in the algorithm, the data is too dualistic. *Example:* if there are individual indicators for which there is no information - the algorithm writes zero (while not accepting them as loading the value of the output), and at the same time if the training set has a close molecule with carcinogenicity - an empirical value is written (then the value is determining the final result).

Tabl.IV.3.6. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Acacipetalin*

CAESAR indicator	Acacipetalin	
	amide	acid
GADI	0	0.74
SMKEV	0.79	0.78
APSM	1	1
CSM	0	0.50
MDRC	true	true
ACFSC	1	1
MCAR	0.61	0.25
NMNC	1	1
Carcinogen	0.19	**0.63**
NON-Carcinogen	**0.81**	0.37
Prediction	NC	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen; C- Carcinogen		

[29] Similarity: 0.77 by CAS: 18883-66-4 and CAS: 54749-90-5

b) ISS

Like *CAESAR* (§a) and *ISS*, the carcinogenicity assessment methodology does not provide amide and carboxyl acid derivatives of *Acacipetalin*. In this case we get identical (and/or those in the statistical error of the method) results for CSM, but neither can be accepted – i.e. is both below and above 0.50.

Tabl.IV.3.6. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Acacipetalin*

ISS indicator	Acacipetalin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.49	0.52
ACFSC	1	1
Prediction	C	NC
NC- NON-Carcinogen; C- Carcinogen		

However, the training set reports structurally similar molecules and/or fragments thereof for amide[30] and carboxylic acid[31] forms.

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.6. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Acacipetalin*.

Tabl.IV.3.6. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN/Antares indicator	Acacipetalin	
	amide	acid
GADI	0.62	0.63
SMKEV	0.81	0.83
APSM	0.67	0.67
CSM	0.33	0.34
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

[30] Similarity: 0.79 by CAS: 18883-66-4; Similarity: 0.75 by CAS: 23246-96-0 and CAS: 60102-37-6 which have been shown to be carcinogenic.
[31] Similarity: 0.74 by CAS: 51333-22-3 plus some of the amide form

d) IRFMN/ISSCAN-CGX

The amide derivative of *Acacipetalin* (***Tabl. IV.3.6. 15***) contains molecular fragments close to those more reported in the training set of *IRFMN/ISSCAN-CGX* methodology for carcinogenicity assessment[32].

Tabl.IV.3.6. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN/ISSCAN-CGX indicator	Acacipetalin	
	amide	acid
GADI	0.81	0.67
SMKEV	0.80	0.79
APSM	1	1
CSM	0.66	0.35
ACFSC	1	1
Prediction	C	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Acacipetalin* was confirmed (***Table IV.3.6. 16***) by *Carcinogenicity oral classification model* (IRFMN) model.

Tabl.IV.3.6. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN indicator	Acacipetalin	
	amide	acid
GADI	0	0
SMKEV	0.79	0.77
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	0.85	0.79
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

[32] Similarity: 0.79 by CAS: 18883-66-4 and CAS: 54749-90-5; Similarity: 0.75 by CAS: 3131-60-0 and CAS: 23246-96-0

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.6. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Acacipetalin* should not be administered orally.

Tabl.IV.3.6. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN indicator	Acacipetalin	
	amide	acid
GADI	0.67	0.66
SMKEV	0.79	0.77
APSM	0.18	0.18
CSM	2.13	2.10
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	21.9	20.4
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	7.4	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.6.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Acacipetalin* highlights the lack of toxicity (***Table IV.3.6. 18***).

Tabl.IV.3.6. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Acacipetalin*

CAESAR indicator	Acacipetalin	
	amide	acid
GADI	0.75	0.88
SMKEV	0.78	0.77
APSM	1	1
CSM	0.52	1
MDRC	true	true
ACFSC	1	1

Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of carboxyl acid derivative of *Acacipetalin* did not report values for GADI and CSM. Molecular fragments close to *2-hydroxy-3-methylbut-2-enamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.6. 19*** cannot be considered reliable.

Tabl.IV.3.6. 19 *PG toxicity of amide and carboxyl acid derivatives of Acacipetalin*

PG indicator	Acacipetalin	
	amide	acid
GADI	0.62	0
SMKEV	0.76	0.74
APSM	0.51	1
CSM	0.49	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Acacipetalin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.6. 20***).

Tabl.IV.3.6. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN/CORAL indicator	Acacipetalin	
	amide	acid
GADI	0.27	0.41
SMKEV	0.68	0,68
APSM	0.35	0.32
CSM	0.18	0.37
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60

Prediction	[mg/L]	
	4.6	**11.7**

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Acacipetalin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.6. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.6. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Acacipetalin*

CORAL indicator	Acacipetalin	
	amide	acid
GADI	0.75	0.62
SMKEV	0.78	0.79
APSM	1	1
CSM	1	0.47
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Acacipetalin* (***Tab.IV.3.6. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.6. 22 *Aromatase activity toxicity model for amide and carboxyl acid derivatives of Acacipetalin*

IRFMN indicator	Acacipetalin	
	amide	acid
GADI	0.91	0.92
SMKEV	0.83	0.84
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.05	0.03
Active Antagonist:	0.02	0.01
Inactive:	**0.93**	**0.97**
Prediction	inA	inA
inA- in active		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Acacipetalin* did not report any deviations (***Tabl.IV.3.6. 23***) affecting the studied process.

Tabl.IV.3.6. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Acacipetalin*

NIC indicator	Acacipetalin	
	amide	acid
GADI	0.77	0.76
SMKEV	0.83	0.81
APSM	0.51	0.52
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.26	1.99
Prediction	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Acacipetalin* we understand (***Tabl.IV.3.6. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.6. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Acacipetalin*

INERIS indicator	Acacipetalin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.69
APSM	0.13	0.32
CSM	0.23	0.43
MEPASM	0.14	0.50
MDRC	N-true	N-true
ACFSC	0.34	0.40
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	-0.162	-0.062
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.452	**1.153**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Acacipetalin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.6. 25***).

Tabl.IV.3.6. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Acacipetalin*

QSARINS indicator	Acacipetalin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.80	0.81
APSM	0.09	0.03
CSM	0.05	0.12
MEPASM	0.15	0.03
MDRC	true	true
ACFSC	1	1
LogHLt	[log units]	
	0.31	0.34
Total half-life	[min]	
	125	**135**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) In vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Acacipetalin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.6. 26***).

Tabl.IV.3.6. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN/VERMEER indicator	Acacipetalin	
	amide	acid
GADI	0.73	0.86
SMKEV	0.76	0.74
APSM	1	1
CSM	0.50	1
ACFSC	1	1
Prediction	A	A
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.7.3.2***), carcinogenicity (***§IV.3.1.7.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.7.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Acacipetalin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.6. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.6. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Acacipetalin*

IRFMN/VERMEER indicator	Acacipetalin	
	amide	acid
GADI	0.42	0.43
SMKEV	0.82	0.84
APSM	0.25	0.25
CSM	0.83	0.69
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.51	0.51
Prediction	[-log(mg/kg)]	
	-2.824	-2.967
Prediction	[mg/kg]	
	667	**927**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.6.4.Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.6.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Acacipetalin* would be optimal for drugs taken orally to poison the cancer cell with *2-hydroxy-3-methylbut-2-enamide* as performed in ***§IV.2*** second objective of the study.

3.1.6.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide 4475 ≤ 11298 ≤ 28524, acid 5245 ≤ 13299 ≤ 33714 and *Bioaccumulation factor* [conditional units] amide 2.45 ≤ 39.49 ≤ 637.29, acid form are 0.02 ≤ 0.17 ≤ 1.49.

3.1.6.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.6.6.1. Lipophilicity

Data from ***Tabl.IV.3.6. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.6. 28 *Lipophilicity of amide and carboxylic acid derivatives of Acacipetalin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Acacipetalin						
amide	0.82	-1.36	-2.42	-2.59	-2.16	-1.54
acid	0.38	-0.71	-1.82	-2.18	-1.92	-1.25

3.1.6.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.6. 29***).

Tabl.IV.3.6. 29 *Water solubility of amide and carboxylic acid derivatives of Acacipetalin*

studied indicator	Acacipetalin	
	amide	acid
ESOL		
Log S	-0.44	-0.85
Solubility, [mg/ml]	1.01e+02	3.90e+01
Class	vs	vs
Ali		
Log S	-1.13	-1.68
Solubility, [mg/ml]	2.05e+01	5.75e+00
Class	vs	vs
SILICOS-IT		
Log S	1.84	2.06
Solubility, [mg/ml]	1.94e+04	3.21e+04
Class	s	s
vs - very soluble; s - soluble		

3.1.6.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Acacipetalin* meets the pharmacokinetic requirements (***Table IV.3.6. 30***).

Tabl.IV.3.6. 30 *Pharmacokinetic indicators of amide and derivatives of Acacipetalin*

studied indicator	Acacipetalin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-8.50	-8.86

3.1.6.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.6. 31***) containing amide and derivatives of *Acacipetalin*.

Tabl.IV.3.6. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Acacipetalin*

studied indicator	Acacipetalin	
	amide	acid
Muegge	Yes	Yes
Bioavailability Score	0.56	0.51

3.1.6.6.5. Medical Chemistry

Data from ***Tabl.IV.3.6. 32*** confirm the drug safety of amide and derivatives of *Acacipetalin*.

Tabl.IV.3.6. 32 *Medical chemistry indicators for amide and derivatives of Acacipetalin*

studied indicator	Acacipetalin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	Yes*	Yes*
Leadlikeness	Yes	Yes
Synthetic accessibility	4.82	4.82
* 2 alerts: acyclic_C=C-O, michael_acceptor_1		

- ◊ -

3.1.7. (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Triglochinin*. The process proceeds according to ***§IV.2.3.***

3.1.7.1.Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.7. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*.

Tabl.IV.3.7. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Triglochinin							
	amide	0.37	0.10	-0.10	0.22	0.27	0.56
	acid	0.40	0.12	-0.12	0.28	0.18	0.64

Data in ***Tabl.IV.3.7. 1*** show that the amides and carboxylic acids of *Triglochinin* have more pronounced overall drug activity *in vivo*.

3.1.7.2. Pharmacological and biological activity of oral active drugs

3.1.7.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.7. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*.

Tabl.IV.3.7. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Triglochinin													
	amide	377	-2.9	12	7	377	-2.9	80	45	377	-2.9	80	45
	acid	378	-2.1	12	7	378	-2.1	80	44	378	-2.1	80	44

The two molecular modified forms of *Triglochinin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.7.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid* are listed in ***Tabl.IV.3.7. 3***.

Tabl.IV.3.7. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Triglochinin									
	amide	217	8	8	1	18	377	2	19
	acid	211	8	8	1	18	378	3	19

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.7.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.7. 4***.

Tabl.IV.3.7. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Triglochinin										
	amide	377	-3.1	12	7	217	8	3	0	0.13
	acid	378	-2.7	12	7	211	8	3	0	0.14

B. wQED

In ***Tabl.IV.3.7. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*.

Tabl.IV.3.7. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Triglochinin										
	amide	277	-3.1	12	7	217	8	3	0	0.18
	acid	378	-2.7	12	7	211	8	3	0	0.19

uwQED (***Tabl.IV.3.7. 4***) and *wQED* (***Tabl.IV.3.7. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Triglochinin* meets the requirements for conservative treatment.

3.1.7.3. Non -laboratory and no clinical information on the chemical form

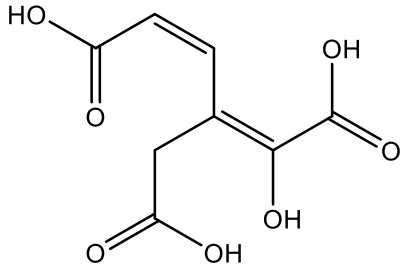
3.1.7.3.1. Receptor activity

In ***Tabl.IV.3.7. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Triglochinin* to receptors (according to ***§III.3.3.4.1***).

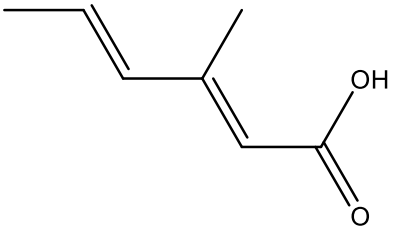
Tabl.IV.3.7. 6 *Receptor activity of amide and carboxyl derivatives of Triglochinin*

indicator	Triglochinin	
	amide	acid
AR		
ERa		
ERb	active*	active *
GR		
MR	-	-
PR		
RARa		active *
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from ***Tabl.IV.3.7. 6*** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to an overlap of a fragment of *(2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid* с *(2E,4E)-3-methylhexa-2,4-dienoic acid (**Fig.IV.3. 2**).*



(2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid

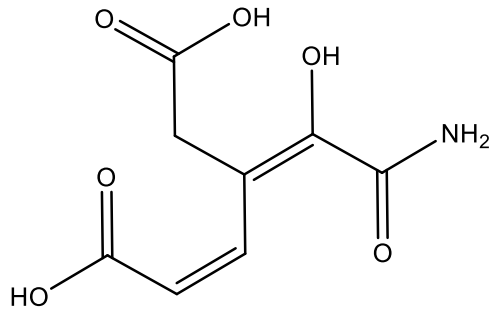


(2E,4E)-3-methylhexa-2,4-dienoic acid

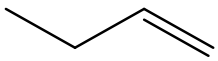
***Fig.IV.3. 2** Structural formulas of (2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid и (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***). On the other hand, the amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(2E,4Z)-3-(carboxymethyl)-2-hydroxyhexa-2,4-dienedioic acid* (***Fig.IV.3. 2***), *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid* with *but-1-ene* chain (***Fig.IV.3. 3***).



(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid



but-1-ene

***Fig.IV.3. 3** Structural formulas of (2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid и but-1-ene*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.7.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.7. 7*** explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of *Triglochinin*.

Tabl.IV.3.7. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Triglochinin*

CAESAR indicator	Triglochinin	
	amide	acid
GADI	0.80	0.80
SMKEV	0.77	0.78
APSM	0.68	0.68
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Triglochinin* show activity (***Table IV.3.7. 8***).

Tabl.IV.3.7. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Triglochinin*

SarPy/IRFMN indicator	Triglochinin	
	amide	acid
GADI	0	0
SMKEV	0.77	0.78
APSM	0.68	0.68
CSM	0	0
ACFSC	1	1
prediction	M	M
M- mutagenicity		

This is due to already reported mutagenic molecules with a similar structure in the training set[33].

c) ISS

Carboxyl acid derivative of *Triglochinin* is non-mutagenic according to *ISS* methodology (***Table IV.3.7. 9***). However, the amide form coincides with already reported molecules leading to mutagenicity[34].

Tabl.IV.3.7. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Triglochinin*

ISS indicator	Triglochinin	
	amide	acid
GADI	0.72	0.75
SMKEV	0.76	0.76
APSM	1	1
CSM	0.47	0.54
ACFSC	1	1
prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Triglochinin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.7. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Triglochinin*

KNN/Read-Across indicator	Triglochinin	
	amide	acid
GADI	0.58	0.74
SMKEV	0.79	0.79
APSM	0.24	0.48
CSM	0.75	1
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

[33] Similarity: 0.76 by CAS: 23282-20-4 and CAS: 23255-69-8
[34] Similarity: 0.70-3 by CAS: 23246-96-0, CAS: 18883-66-4, CAS: 2058-46-0, CAS: 303-34-4 and CAS: 64-75-5

B. Consensus model

Data from ***Tabl.IV.3.7. 11*** show that the amide derivative has a mutagenic effect on the organism, and the acid form does not.

Tabl.IV.3.7. 11 *Consensus mutagenicity model of amide and carboxyl acid derivatives of Triglochinin*

Consensus model mutagenicity indicator	Triglochinin	
	amide	acid
numerical value	0.20	0.45

3.1.7.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Like ***§IV.3.1.6.3.3.A.a***, CAESAR methodology (with training set[35]) cannot accurately assess the carcinogenicity of amide and carboxyl acid derivatives of *Triglochinin* (***Table IV.3.7. 12***).

Tabl.IV.3.7. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Triglochinin*

CAESAR indicator	Triglochinin	
	amide	acid
GADI	0	0
SMKEV	0.75	0.76
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
MCAR	0.01	0.01
NMNC	0.50	0.50
Carcinogen	**0.51**	**0.51**
NON-Carcinogen	0.49	0.49
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- Carcinogen		

[35] Similarity: 0.73 by CAS: 54749-90-5, CAS: 15503-86-3, CAS: 18883-66-4 and CAS: 480-54-6

b) ISS

Similar to ***§IV.3.1.6.3.3.A.b*** *ISS* data for the assessment of the carcinogenicity of amide and carboxyl acid derivatives of *Triglochinin* cannot be interpreted unambiguously (***Table IV.3.7. 13***).

Tabl.IV.3.7. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Triglochinin*

ISS indicator	Triglochinin	
	amide	acid
GADI	0.72	0.75
SMKEV	0.76	0.76
APSM	1	1
CSM	0.47	0.54
ACFSC	1	1
Prediction	C	NC
NC- NON-Carcinogen; C- Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.7. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Triglochinin*.

Tabl.IV.3.7. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Triglochinin*

IRFMN/Antares indicator	Triglochinin	
	amide	acid
GADI	0.61	0.62
SMKEV	0.79	0.80
APSM	0.34	0.35
CSM	0.66	0.66
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.7. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Triglochinin*.

Tabl.IV.3.7. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Triglochinin*

IRFMN/ISSCAN-CGX indicator	Triglochinin	
	amide	acid
GADI	0.78	0.77
SMKEV	0.75	0.75
APSM	1	1
CSM	0.64	0.63
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Triglochinin* was confirmed (***Table IV.3.7. 16***) by *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.7. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Triglochinin*

IRFMN indicator	Triglochinin	
	amide	acid
GADI	0	0
SMKEV	0.73	0.72
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	0.85	0.85
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.7. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Triglochinin* should not be administered orally.

Tabl.IV.3.7. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Triglochinin*

IRFMN indicator	Triglochinin	
	amide	acid
GADI	0.62	0.61
SMKEV	0.73	0.72
APSM	0.18	0.18
CSM	2.13	2.11
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	21.9	20.9
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	10.3	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set;		

3.1.7.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Triglochinin* highlights the lack of toxicity (***Table IV.3.7. 18***).

Tabl.IV.3.7. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Triglochinin*

CAESAR indicator	Triglochinin	
	amide	acid
GADI	0.73	0.86
SMKEV	0.75	0.75
APSM	1	1
CSM	0.51	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Triglochinin* did not report values for GADI and CSM. Molecular fragments close to *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.7. 19*** cannot be considered reliable.

Tabl.IV.3.7. 19 *PG toxicity of amide and carboxyl acid derivatives of Triglochinin*

PG indicator	Triglochinin	
	amide	acid
GADI	0.62	0.61
SMKEV	0.76	0.74
APSM	0.49	0.49
CSM	0.51	0.51
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Triglochinin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.7. 20***).

Tabl.IV.3.7. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Triglochinin*

IRFMN/CORAL indicator	Triglochinin	
	amide	acid
GADI	0.27	0.39
SMKEV	0.67	0.65
APSM	0.31	0.31
CSM	0.54	0.60
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	7.2	**8.2**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Triglochinin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.7. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.7. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Triglochinin*

CORAL indicator	Triglochinin	
	amide	acid
GADI	0.74	0.74
SMKEV	0.76	0.76
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic procesis is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Triglochinin* (***Tab.IV.3.7. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.7. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Triglochinin*

IRFMN indicator	Triglochinin	
	amide	acid
GADI	0.87	0.87
SMKEV	0.75	0.76
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.13	0.12
Active Antagonist:	0.02	0.02
Inactive:	**0.85**	**0.86**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Triglochinin* did not report any deviations (***Tabl.IV.3.7. 23***) affecting the studied process.

Tabl.IV.3.7. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Triglochinin*

NIC indicator	Triglochinin	
	amide	acid
GADI	0.74	0.87
SMKEV	0.77	0.76
APSM	0.50	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron	**4.05**	**4.10**
Prediction	NA	NA

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Triglochinin* we understand (***Tabl.IV.3.7. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.7. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Triglochinin*

INERIS indicator	Triglochinin	
	amide	acid
GADI	0	0
SMKEV	0.66	0.65
APSM	0.33	0.52
CSM	0.91	0.66
MEPASM	0.54	0.54
MDRC	N-true	N-true
ACFSC	0.34	0.40
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.139	0.234
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.377	1.714
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Triglochinin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.7. 25***).

Tabl.IV.3.7. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Triglochinin*

QSARINS indicator	Triglochinin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.79	0.79
APSM	0.07	0.07
CSM	0.11	0.08
MEPASM	0.11	0.11
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.135	0.168
Total half-life	[min]	
	80	**90**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Triglochinin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.7. 26***).

Tabl.IV.3.7. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Triglochinin*

IRFMN/VERMEER indicator	Triglochinin	
	amide	acid
GADI	0.84	0.83
SMKEV	0.70	0.69
APSM	1	1
CSM	1	1
ACFSC	1	1
Prediction	A	A
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.7.3.2***), carcinogenicity (***§IV.3.1.7.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.7.3.4***).

b) in vivo

The *in vivo* toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of Triglochinin can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.7. 27***). They are relatively safe in terms of NOAEL toxicity model.

Tabl.IV.3.7. 27 *Toxicity of NOAEL of amide and carboxyl acid derivatives of Triglochinin*

IRFMN/VERMEER indicator	Triglochinin	
	amide	acid
GADI	0.42	0.42
SMKEV	0.81	0.83
APSM	0.25	0.25
CSM	0.19	0.33
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.51	0.51
Prediction	[-log(mg/kg)]	
	-3.84	-3.99
Prediction	[mg/kg]	
	6918	**9772**

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.7.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.7.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Triglochinin* would be optimal for drugs taken orally to poison

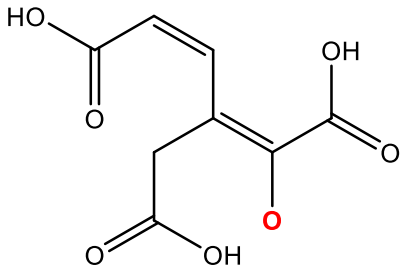
the cancer cell with *(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid* as performed in ***§IV.2*** second objective of the study.

3.1.7.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. No toxicity deviations were found and the values were respectively: *Oral rat LD50* [mg/kg] for amide 4809 ≤ 13902 ≤ 40189, acid 3803 ≤ 9794 ≤ 25226 and *Bioaccumulation factor* [conditional units] amide 0.00 ≤ 2.28E-02 ≤ 0.57, acid are values close to 0.

3.1.7.6. Checking conclusion of the part

Due to the triple symmetry in the functional group (***Fig.IV.3. 4***) of the condensate with the carbohydrate, the methodological scheme (***§III.3.3.7***) cannot be applied.



(2E,4Z)-3-(carboxymethyl)-2-(λ^1-oxidaneyl)hexa-2,4-dienedioic acid

Fig.IV.3. 4 *Structural formula of (2E,4Z)-3-(carboxymethyl)-2-(λ^1-oxidaneyl)hexa-2,4-dienedioic acid*

- ◊ -

3.1.8. (S)-1-hydroxycyclopent-2-ene-1-carboxamide

Subject to analysis are potential pharmaceutical forms for release within the cancer cell of *(S)-1-hydroxycyclopent-2-ene-1-carboxamide*, comprising an amides and carboxylic acids obtained by hydrolysis of the nitrile groups of *Deidaclin* and *Tetraphyllin A*. The process proceeds according to ***§IV.2.3.***

3.1.8.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.8. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-1-hydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.8. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-1-hydroxycyclopent-2-ene-1-carboxamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Deidaclin / Tetraphyllin A						
amide	0.15	0.09	-0.05	-0.13	0.23	0.53
acid	0.21	0.11	-0.16	0.22	0.14	0.69

Data in ***Tabl.IV.3.8. 1*** show that the amides and carboxylic acids of *Deidaclin* и *Tetraphyllin A* have more pronounced overall drug activity *in vivo*.

3.1.8.2. Pharmacological and biological activity of oral active drugs

3.1.8.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.8. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-1-hydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.8. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-1-hydroxycyclopent-2-ene-1-carboxamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Deidaclin / Tetraphyllin A												
amide	289	-2.1	8	5	289	-2.1	63	39	289	-2.1	64	39
acid	290	-1.4	8	5	290	-1.4	67	38	290	-1.4	67	38

They distinguish the three molecular forms (the corresponding amides and carboxylic acids of *Deidaclin* and *Tetraphyllin A*) that meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.8.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter, MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-1-hydroxycyclopent-2-ene-1-carboxamide* are listed in ***Tabl.IV.3.8. 3***.

Tabl.IV.3.8. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-1-hydroxycyclopent-2-ene-1-carboxamideca*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Deidaclin / Tetraphyllin A								
amide	142	4	4	2	17	289	0	13
acid	137	4	4	2	17	290	1	13

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.8.2.3. QED

The analysis is performed according to **§3.3.3.1.3**.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.8. 4***.

Tabl.IV.3.8. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-1-hydroxycyclopent-2-ene-1-carboxamide*

	uwQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAromaRing	uwQED
Deidaclin / Tetraphyllin A									
amide	289	-2.1	8	5	142	4	1	0	0.39
acid	290	-1.8	8	5	137	4	1	0	0.42

B. wQED

In ***Tabl.IV.3.8. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(S)-1-hydroxycyclopent-2-ene-1-carboxamide*

Tabl.IV.3.8. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (S)-1-hydroxycyclopent-2-ene-1-carboxamide*

	wQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Deidaclin / Tetraphyllin A									
amide	289	-2.1	8	5	142	4	1	0	0.45
acid	290	-1.8	8	5	137	4	1	0	0.48

uwQED (***Tabl.IV.3.8. 4)*** and *wQED* (***Tabl.IV.3.8. 5)*** of potential pharmaceutical forms including amides and carboxylic acids obtained by hydrolysis of the nitrile group of *Deidaclin* and *Tetraphyllin A* meets the requirements for conservative treatment.

3.1.8.3. Non -laboratory and no clinical information on the chemical form

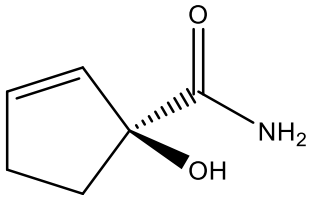
3.1.8.3.1. Receptor activity

In ***Tabl.IV.3.8. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Deidaclin* and *Tetraphyllin A* to receptors (according to ***§III.3.3.4.1***).

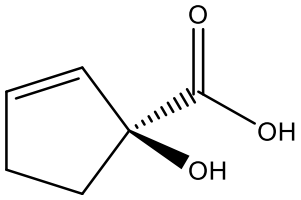
Tabl.IV.3.8. 6 *Receptor activity of amide and carboxyl derivatives of Deidaclin and Tetraphyllin A*

indicator	Deidaclin / Tetraphyllin A	
	amide	acid
AR		
ERa		
ERb	active*	active *
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

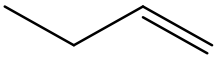
The amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb). This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(S)-1-hydroxycyclopent-2-ene-1-carboxamide* and *(S)-1-hydroxycyclopent-2-ene-1-carboxylic acid* with *but-1-ene* chain (***Fig.IV.3. 5***).



(S)-1-hydroxycyclopent-2-ene-1-carboxamide



(S)-1-hydroxycyclopent-2-ene-1-carboxylic acid



but-1-ene

Fig.IV.3. 5 *Structural formulas of (S)-1-hydroxycyclopent-2-ene-1-carboxamide, (S)-1-hydroxycyclopent-2-ene-1-carboxylic acid and but-1-ene*

It is important to note that *(S)-1-hydroxycyclopent-2-ene-1-carboxylic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***).

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.8.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.8. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Deidaclin* and *Tetraphyllin A*.

Tabl.IV.3.8. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

CAESAR indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.85
APSM	0.67	0.67
CSM	0.67	0.66
MDRC	true	true
ACFSC	1	1
prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* did not show activity (***Table IV.3.8. 8***).

Tabl.IV.3.8. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

SarPy/IRFMN indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.63	0.63
SMKEV	0.83	0.85
APSM	0.33	0.33
CSM	0.67	0.66
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Deidaclin and Tetraphyllin A* are non-mutagenic according to *ISS* methodology (***Table IV.3.8. 9***).

Tabl.IV.3.8. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

ISS indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.76	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.52	0.53
ACFSC	1	1
prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Deidaclin and Tetraphyllin A* show some deviation from *KNN/Read-Across* method due to the incomplete training set. At the same time, the model results in mutagenic activity[36] of their amide form.

Tabl.IV.3.8. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

KNN/Read-Across indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.65	0.72
SMKEV	0.83	0.85
APSM	0.50	0.50
CSM	0.50	0.75
ACFSC	1	1
prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.8. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* to mutagenicity.

Tabl.IV.3.8. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

Consensus model mutagenicity indicator	Deidaclin / Tetraphyllin A	
	amide	acid
numerical value	0.35	0.50

[36] Similarity: 0.82-3 by CAS: 3947-65-7, CAS: 87625-62-5 and CAS: 585-86-4 and CAS: 57-50-1

3.1.8.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

CAESAR methodology (with training set[37]) for assessment of carcinogenicity of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A*, similar to ***§IV.3.1.6.3.3.A.a*** and ***§IV.3.1.7.3.3.A.a*** does not give an unambiguous prognosis for tabulation (***Tabl. IV.3.8. 12***).

Tabl.IV.3.8. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

CAESAR indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0	0
SMKEV	0.80	0.79
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
MCAR	0.24	0.24
NMNC	0.50	0.50
Carcinogen	**0.62**	**0.62**
NON-Carcinogen	0.38	0.38
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- Carcinogen		

b) ISS

ISS methodology (***Tab.IV.3.8. 13***) is identifies amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* as non-carcinogenic.

Tabl.IV.3.8. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

ISS indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.76	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.52	0.53

[37] Similarity: 0.76 by CAS: 15503-86-3 and CAS: 18883-66-4

ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

c) IRFMN/Antares

Training set of *IRFMN/Antares* carcinogenicity assessment methodology reported for alerts with close molecular fragments to carboxylic acid forms of *Deidaclin* and *Tetraphyllin A* (***Tabl.IV.3.8. 14***).

Tabl.IV.3.8. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN/Antares indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.63	0.75
SMKEV	0.83	0.85
APSM	0.67	0.67
CSM	0.33	0.67
ACFSC	1	1
Prediction	PNC	C
NC- NON-Carcinogen; C- Carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.8. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A*.

Tabl.IV.3.8. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN/ISSCAN-CGX indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.80	0.79
SMKEV	0.79	0.79
APSM	1	1
CSM	0.65	0.64
ACFSC	1	1
Prediction	PNC	PNC
NC- NON-Carcinogen		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* was confirmed (***Table IV.3.8. 16***) by *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.8. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.88	0.83
SMKEV	0.78	0.72
APSM	1	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.8. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* should not be administered orally.

Tabl.IV.3.8. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.66	0.65
SMKEV	0.78	0.76
APSM	0.18	0.18
CSM	1.85	1.85
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
	$(g/kg\text{-}day)^{-1}$	

Predicted Oral Carcinogenicity SF for molecular forms)	**11.5**	**11.5**
Presumed concentration of the active form inside the cancer cell	(g/kg-day)$^{-1}$	
	4.1	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.8.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* highlights the lack of toxicity (***Table IV.3.8. 18***).

Tabl.IV.3.8. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

CAESAR indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.76	0.88
SMKEV	0.78	0.78
APSM	1	1
CSM	0.53	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* did not report values for GADI and CSM. Molecular fragments close to *(S)-1-hydroxycyclopent-2-ene-1-carboxamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.8. 19*** cannot be considered reliable.

Tabl.IV.3.8. 19 *PG toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

PG indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.62	0
SMKEV	0.77	0.78
APSM	0.51	1
CSM	0.49	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.8. 20***).

Tabl.IV.3.8. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN/CORAL indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.28	0.41
SMKEV	0.69	0.68
APSM	0.33	0.33
CSM	1.02	1.42
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
	[mg/L]	
Prediction	**9.85**	**24.99**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.8. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.8. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

CORAL indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.76	0.65
SMKEV	0.79	0.79
APSM	1	1
CSM	1	0.53
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* (***Tab.IV.3.8. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.8. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.91	0.92
SMKEV	0.84	0.84
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.07	0.06
Active Antagonist:	0.04	0.04
Inactive:	**0.89**	**0.90**
Prediction	inA	inA

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* did not report any deviations (***Tabl.IV.3.8. 23***) affecting the studied process.

Tabl.IV.3.8. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

NIC indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.77	0.76
SMKEV	0.84	0.81
APSM	0.50	0.51
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.01	2.74
Prediction	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NA- Non active		

c) Adipose tissue: blood model

Applying Adipose tissue: blood model for toxicity of amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* we understand (***Tabl.IV.3.8. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.8. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

INERIS indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0	0
SMKEV	0.71	0.70
APSM	0.31	0.311
CSM	0.48	0.65
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.138	-0.029
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.374	**1.069**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.8. 25***).

Tabl.IV.3.8. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

QSARINS indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.85	0.85
SMKEV	0.82	0.82
APSM	0.09	0.09
CSM	0.06	0.08
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.32	0.34
Total half-life	[min]	
	125	**135**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Deidaclin* and *Tetraphyllin A* show activity for Micronucleus in *in vitro* was confirmed (***Tab.IV.3.8. 26***).

Tabl.IV.3.8. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN/VERMEER indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.74	0.73
SMKEV	0.76	0.75
APSM	1	1
CSM	0.51	0.50
ACFSC	1	1
Prediction	A	inA
A- active; inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.8.3.2***), carcinogenicity (***§IV.3.1.8.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.8.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Deidaclin* and *Tetraphyllin A* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.8. 27***). They are relatively safe in terms of *NOAEL* toxicity model.

Tabl.IV.3.8. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Deidaclin and Tetraphyllin A*

IRFMN/VERMEER indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.85
APSM	0.25	0.25
CSM	0.87	0.73
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-2.72	-2.93
Prediction	[mg/kg]	
	525	**851**

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.8.4. Evaluation of the result

After a comparative analysis of the results (***§IV.3.1.10.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Deidaclin / Tetraphyllin A* would be optimal for drugs taken

orally to poison the cancer cell with *(S)-1-hydroxycyclopent-2-ene-1-carboxamide* as performed in ***§IV.2*** second objective of the study.

3.1.8.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), demonstrates maximum coverage of the requirements for oral medicinal products. No toxicity deviations were found and the values were respectively: *Oral rat LD50* [mg/kg] for Deidaclin and Tetraphyllin A amide 1447 ≤ 3284 ≤ 7449, Deidaclin acid and Tetraphyllin A acid 1132 ≤ 2555 ≤ 5765 and *Bioaccumulation factor* [conditional units] Deidaclin amide and Tetraphyllin A 0.00 ≤ 1.50 ≤ 4230, Deidaclin acid and Tetraphyllin A acid are 0.02 ≤ 0.16 ≤ 1.45. This is understandable because both compounds are in isomeric form.

3.1.8.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.8.6.1. Lipophilicity

Data from ***Tabl.IV.3.8. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.8. 28 *Lipophilicity of amide and carboxylic acid derivatives of Deidaclin and Tetraphyllin A*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Deidaclin / Tetraphyllin A						
amide	1.06	-2.04	-2.62	-2.31	-2.10	-1.60
acid	-0.32	-1.38	-2.02	-1.90	-1.86	-1.50

3.1.8.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.8. 29***).

Tabl.IV.3.8. 29 *Water solubility of amide and carboxylic acid derivatives of Deidaclin and Tetraphyllin A*

studied indicator	Deidaclin / Tetraphyllin A	
	amide	acid
ESOL		
Log S	-0.08	-0.51
Solubility, [mg/ml]	2.38e+02	9.05e+01
Class	vs	vs
Ali		
Log S	-0.43	-0.99
Solubility, [mg/ml]	1.08e+02	2.97e+01
Class	vs	vs
SILICOS-IT		
Log S	1.81	2.02
Solubility, [mg/ml]	1.85e+04	3.05e+04
Class	s	s
vs - very soluble; s - soluble		

3.1.8.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Deidaclin* and *Tetraphyllin A* meets the pharmacokinetic requirements (***Table IV.3.8. 30***).

Tabl.IV.3.8. 30 *Pharmacokinetic indicators of amide and derivatives of Deidaclin and Tetraphyllin A*

studied indicator	Deidaclin / Tetraphyllin A	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.51	-9.05

3.1.8.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.8. 31***) containing amide and derivatives of *Deidaclin* and *Tetraphyllin A*.

Tabl.IV.3.8. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Deidaclin and Tetraphyllin A*

studied indicator	Deidaclin / Tetraphyllin A	
	amide	acid
Muegge	No*	Yes
Bioavailability Score	0.55	0.56
* 1 violation: XLOGP3<-2		

3.1.8.6.5. Medical Chemistry

Data from ***Tabl.IV.3.8. 32*** confirm the drug safety of amide and derivatives of *Deidaclin* and *Tetraphyllin A*.

Tabl.IV.3.8. 32 *Medical chemistry indicators for amide and derivatives of Deidaclin and Tetraphyllin A*

studied indicator	Deidaclin / Tetraphyllin A	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.00	5.05
* 1 alert: isolated_alkene		

- ◊ -

3.1.9. (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide

Subjected to analysis potential pharmaceutical forms for release within the cancer cell of *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*, comprising an amides and carboxylic acids obtained by hydrolysis of the nitrile groups of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin*. The process proceeds according to ***§IV.2.3.***

3.1.9.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.9. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.9. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Tetraphyllin B / Volkenin / Taraktophyllin						
amide	0.22	0.15	0.13	0.03	0.28	0.66
acid	0.28	0.17	0.01	0.36	0.19	0.81

Data in ***Tabl.IV.3.9. 1*** show that the amides and carboxylic acids of Tetraphyllin B, Volkenin and Taraktophyllin have more pronounced overall drug activity *in vivo*.

3.1.9.2. Pharmacological and biological activity of oral active drugs

3.1.9.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.9. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.9. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Tetraphyllin B / Volkenin / Taraktophyllin												
amide	308	-2.9	9	6	305	-2.9	65	40	305	-2.9	65	40
acid	306	-2.1	9	6	306	-2.1	65	39	306	-2.1	65	39

They distinguish the three molecular forms (the corresponding amides and carboxylic acids of *Tetraphyllin B, Volkenin* and *Taraktophyllin)* that meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.9.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide* are listed in ***Tabl.IV.3.9. 3***.

Tabl.IV.3.9. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

	Veber Filter		MDDR-Like Rule			BBB Likeness		
	TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Tetraphyllin B / Volkenin / Taraktophyllin								
amide	163	4	4	2	18	305	0	15
acid	157	4	4	2	18	306	1	15

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.9.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.9. 4***.

Tabl.IV.3.9. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

									uwQED
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAromaRing	uwQED
Tetraphyllin B / Volkenin / Taraktophyllin									
amide	305	-2.9	9	6	163	4	1	0	0.27
acid	306	-2.5	9	6	157	4	1	0	0.29

B. wQED

In ***Tabl.IV.3.9. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and *release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

Tabl.IV.3.9. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide*

	wQED								
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Tetraphyllin B / Volkenin / Taraktophyllin									
amide	305	-2.9	9	6	163	4	1	0	0.35
acid	306	-2.5	9	6	157	4	1	0	0.37

uwQED (***Tabl.IV.3.9. 4***) and *wQED* (***Tabl.IV.3.9. 5***) of potential pharmaceutical forms including amides and carboxylic acids obtained by hydrolysis of the nitrile group of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* meets the requirements for conservative treatment.

3.1.9.3. Non -laboratory and no clinical information on the chemical form

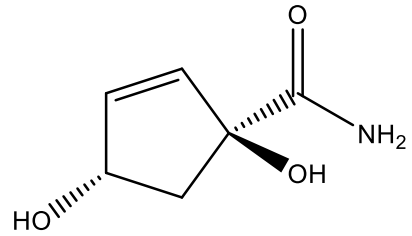
3.1.9.3.1. Receptor activity

In ***Tabl.IV.3.9. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* to receptors (according to ***§III.3.3.4.1***).

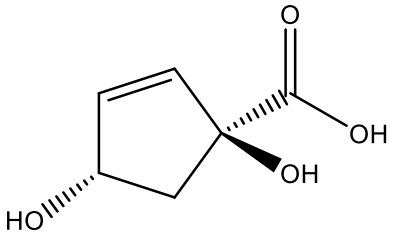
Tabl.IV.3.9. 6 *Receptor activity of amide and carboxyl derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
AR		
ERa		
ERb	active*	active
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

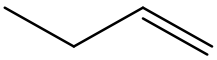
The amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb). This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide, (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxylic acid* with *but-1-ene* chain (***Fig.IV.3. 6***).



(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide



(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxylic acid



but-1-ene

Fig.IV.3. 6 *Structural formulas of (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide, (1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxylic acid and but-1-ene*

It is important to note that *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxylic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***).

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.9.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.9. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Tetraphyllin B, Volkenin and Taraktophyllin*.

Tabl.IV.3.9. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

CAESAR indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.83	0.84
APSM	0.67	0.67
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* did not show activity (***Table IV.3.9. 8***).

Tabl.IV.3.9. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

SarPy/IRFMN indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.63	0.63
SMKEV	0.83	0.84
APSM	0.34	0.34
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* are non-mutagenic according to *ISS* methodology (***Table IV.3.9. 9***).

Tabl.IV.3.9. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

ISS indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.76	0.77
SMKEV	0.81	0.80
APSM	1	1
CSM	0.52	0.54
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin and Taraktophyllin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.9. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

KNN/Read-Across indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.54	0.72
SMKEV	0.84	0.85
APSM	0.25	0.49
CSM	0.50	0.75
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.9. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* to mutagenicity.

Tabl.IV.3.9. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

Consensus model mutagenicity indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
numerical value	0.40	0.50

3.1.9.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

CAESAR methodology (with training set[38]) for assessment of carcinogenicity of amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin*, similar to ***§IV.3.1.6.3.3.A.a***, ***§IV.3.1.7.3.3.A.a*** and ***§IV.3.1.8.3.3.A.a*** does not give an unambiguous prognosis for tabulation (***Tabl. IV.3.9. 12***).

Tabl.IV.3.9. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

CAESAR indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0	0
SMKEV	0.79	0.78
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
MCAR	0.01	0.01
NMNC	0.50	0.50
Carcinogen	**0.51**	**0.51**
NON-Carcinogen	0.49	0.49
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- Carcinogen		

b) ISS

ISS methodology (***Tab.IV.3.9. 13***), it identifies amide and carboxylic acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* as non-carcinogenic.

[38] Similarity: 0.77 by CAS: 83480-29-9, CAS: 18883-66-4, CAS: 54749-90-5, CAS: 15503-86-3 and CAS: 480-54-6

Tabl.IV.3.9. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

ISS indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.76	0.77
SMKEV	0.81	0.80
APSM	1	1
CSM	0.52	0.54
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

c) IRFMN/Antares

Carboxyl acid form of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* is prone to carcinogenicity (***Table IV.3.9. 14***) according to *IRFMN/Antares* methodology. In the training set there are molecules with close to analyzed fragments.

Tabl.IV.3.9. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN/Antares indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.63	0.75
SMKEV	0.84	0.85
APSM	0.67	0.67
CSM	0.34	0.66
ACFSC	1	1
Prediction	PNC	C
PNC- possible non-carcinogenic; C- carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.9. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin*.

Tabl.IV.3.9. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN/ISSCAN-CGX indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.80	0.79
SMKEV	0.79	0.79
APSM	1	1
CSM	0.64	0.63
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* was confirmed (***Table IV.3.9. 16***) by *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.9. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0	0
SMKEV	0.78	0.75
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.9. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* should not be administered orally.

Tabl.IV.3.9. 17 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.66	0.64
SMKEV	0.78	0.75
APSM	0.18	0.18
CSM	1.83	1.80
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	11.0	10.2
Presumed concentration of the active form inside the cancer cell	(g/kg-day)−1	
	4.3	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.9.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of the *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* highlights the lack of toxicity (***Table IV.3.9. 18***).

Tabl.IV.3.9. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

CAESAR indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.76	0.88
SMKEV	0.79	0.77
APSM	1	1
CSM	0.53	1
MDRC	true	true
ACFSC	1	1

Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* did not report values for GADI and CSM. Molecular fragments close to *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.9. 19*** cannot be considered reliable.

Tabl.IV.3.9. 19 *PG toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

PG indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.62	0
SMKEV	0.77	0.77
APSM	0.50	1
CSM	0.49	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxylic acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.9. 20***).

Tabl.IV.3.9. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN/CORAL indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.27	0.40
SMKEV	0.67	0.67
APSM	0.33	0.33
CSM	1.32	1.72

MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	20.9	**53.0**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.2. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.9. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

CORAL indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.76	0.76
SMKEV	0,79	0.79
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

A. Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* (***Tab.IV.3.9. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.9. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.91	0.91
SMKEV	0.83	0.83
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.11	0.09
Active Antagonist:	0.03	0.03
Inactive:	**0.86**	**0.88**
Prediction	inA	inA
inA- inactive		

B. p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* did not report any deviations (***Tabl.IV.3.9. 23***) affecting the studied process.

Tabl.IV.3.9. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

NIC indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.77	0.76
SMKEV	0.84	0.81
APSM	0.49	0.50
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	1.89	3.11
Prediction	NA	NA

C. Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* we understand (***Tabl.IV.3.9. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.9. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

INERIS indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0	0
SMKEV	0.69	0.68
APSM	0.31	0.31
CSM	0.44	0.38
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.180	0.278
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.514	**1.897**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.9. 25***).

Tabl.IV.3.9. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

QSARINS indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.83
APSM	0.09	0.09
CSM	0.03	0.06
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.29	0.32
Total half-life	[min]	
	115	**125**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.9. 26***).

Tabl.IV.3.9. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN/VERMEER indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.73	0.72
SMKEV	0.74	0.74
APSM	1	1
CSM	0.52	0.49
ACFSC	1	1
Prediction	A	inA
A- active; inA - inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.6.3.2***), carcinogenicity (***§IV.3.1.6.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.6.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Tetraphyllin B*, *Volkenin* and *Taraktophyllin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.9. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.3.9. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

IRFMN/VERMEER indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.85	0.86
APSM	0.25	0.25
CSM	0.66	0.51
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0,85
Prediction	[-log(mg/kg)]	
	-3.00	-3.14
Prediction	[mg/kg]	
	1000	1380
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.9.4. Evaluation of the result

After a comparative analysis of the results (***§IV.3.1.10.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Tetraphyllin B, Volkenin and Taraktophyllin* would be optimal for drugs taken orally to poison the cancer cell with *(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide* as performed in ***§IV.2*** second objective of the study.

3.1.9.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. No toxicity deviations were found and the values were respectively: *Oral rat LD50* [mg/kg] for amide $1605 \leq 4502 \leq 12623$, acid $1719 \leq 4318 \leq 10842$ and *Bioaccumulation factor* [conditional units] amide $2.06 \leq 33.1 \leq 533$, acid are $0.00 \leq 0.32 \leq 678$. This is understandable because both compounds are in isomeric form.

3.1.9.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.9.6.1. Lipophilicity

Data from ***Tabl.IV.3.9. 29*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.9. 28 *Lipophilicity of amide and carboxylic acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Tetraphyllin B / Volkenin / Taraktophyllin						
amide	0.43	-3.15	-3.65	-3.09	-2.98	-2.49
acid	0.30	-2.50	-3.05	-2.68	-2.74	-2.13

3.1.9.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.9. 30***).

Tabl.IV.3.9. 29 *Water solubility of amide and carboxylic acid derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

studied indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
ESOL		
Log S	0.52	0.10
Solubility, [mg/ml]	1.00e+03	3.86e+02
Class	vs	vs
Ali		
Log S	0.30	-0.25
Solubility, [mg/ml]	6.10e+02	1.71e+02
Class	vs	vs
SILICOS-IT		
Log S	2.62	2.83
Solubility, [mg/ml]	1.27e+05	2.09e+05
Class	s	s
vs - very soluble; s - soluble		

3.1.9.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin* meets the pharmacokinetic requirements (***Table IV.3.9. 31***).

Tabl.IV.3.9. 30 *Pharmacokinetic indicators of amide and derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

studied indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-10.40	-9.94

3.1.9.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.9. 32***) containing amide and derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin*.

Tabl.IV.3.9. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

studied indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.17	0.11
* 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.9.6.5. Medical Chemistry

Data from ***Tabl.IV.3.9. 33*** confirm the drug safety of amide and derivatives of *Tetraphyllin B, Volkenin* and *Taraktophyllin*.

Tabl.IV.3.9. 32 *Medical chemistry indicators for amide and derivatives of Tetraphyllin B, Volkenin and Taraktophyllin*

studied indicator	Tetraphyllin B / Volkenin / Taraktophyllin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.18	5.22
* 1 alert: isolated_alkene		

- ◊ -

3.1.10. (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Gynocardin*. The process proceeds according to ***§IV.2.3***.

3.1.10.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.10. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.10. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Gynocardin							
	amide	0.23	0.14	0.15	0.05	0.29	0.69
	acid	0.29	0.15	0.04	0.37	0.21	0.83

Data in ***Tabl.IV.3.10. 1*** show that the amides and carboxylic acids of *Gynocardin* have more pronounced overall drug activity *in vivo*.

3.1.10.2. Pharmacological and biological activity of oral active drugs

3.1.10.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.10. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*.

Tabl.IV.3.10. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Gynocardin													
	amide	321	-2.6	10	7	321	-2.6	67	41	321	-2.6	67	41
	acid	322	-2.3	10	7	322	-2.3	67	40	322	-2.3	67	40

The two molecular modified forms of *Gynocardin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.10.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide* are listed in ***Tabl.IV.3.10. 3***.

Tabl.IV.3.10. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Gynocardin									
	amide	183	4	4	2	19	321	0	17
	acid	177	4	4	2	19	322	1	17

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.10.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.10. 4***.

Tabl.IV.3.10. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Gynocardin										
	amide	321	-3.7	10	7	183	4	1	0	0.19
	acid	322	-3.3	10	7	177	4	1	0	0.21

B. wQED

In ***Tabl.IV.3.10. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide* .

Tabl.IV.3.10. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Gynocardin										
	amide	321	-3.7	10	7	183	4	1	0	0.28
	acid	322	-3.3	10	7	177	4	1	0	0.29

uwQED (***Tabl.IV.3.10. 4***) and *wQED* (***Tabl.IV.3.10. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Gynocardin* meets the requirements for conservative treatment.

3.1.10.3. Non -laboratory and no clinical information on the chemical form

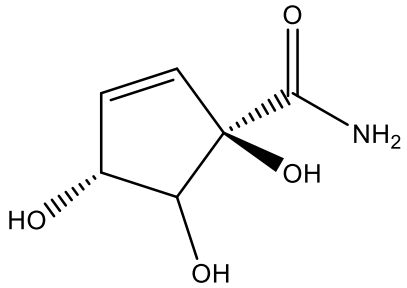
3.1.10.3.1. Receptor activity

In ***Tabl.IV.3.10. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Gynocardin* to receptors (according to ***§III.3.3.4.1***).

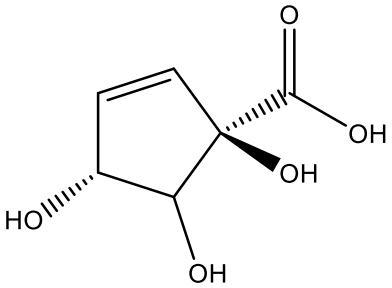
Tabl.IV.3.10. 6 *Receptor activity of amide and carboxyl derivatives of Gynocardin*

indicator	Gynocardin	
	amide	acid
AR		
ERa		
ERb	active *	active *
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

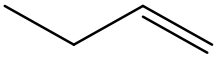
The amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb). This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide* and *(S)-1-hydroxycyclopent-2-ene-1-carboxylic acid* and *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxylic acid* with *but-1-ene* chain (***Fig.IV.3. 7***).



(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide



(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxylic acid



but-1-ene

Fig.IV.3. 7 *Structural formulas of (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide and (S)-1-hydroxycyclopent-2-ene-1-carboxylic acid, (1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxylic acid and but-1-ene*

It is important to note that *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxylic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane (***§IV.2.3 .1***).

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.10.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.10. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Gynocardin*.

Tabl.IV.3.10. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Gynocardin*

CAESAR indicator	Gynocardin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.83	0.84
APSM	0.67	0.68
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Gynocardin* did not show activity (***Table IV.3.10. 8***).

Tabl.IV.3.10. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Gynocardin*

SarPy/IRFMN indicator	Gynocardin	
	amide	acid
GADI	0.63	0.64
SMKEV	0.83	0.84
APSM	0.34	0.35
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Gynocardin* are non-mutagenic according to *ISS* methodology (***Table IV.3.10. 9***).

Tabl.IV.3.10. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Gynocardin*

ISS indicator	Gynocardin	
	amide	acid
GADI	0.77	0.77
SMKEV	0.81	0.80
APSM	1	1

CSM	0.53	0.55
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Gynocardin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.10. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Gynocardin*

KNN/Read-Across indicator	Gynocardin	
	amide	acid
GADI	0.55	0.72
SMKEV	0.84	0.85
APSM	0.24	0.49
CSM	0.51	0.75
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.10. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Gynocardin* to mutagenicity.

Tabl.IV.3.10. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Gynocardin*

Consensus model mutagenicity indicator	Gynocardin	
	amide	acid
numerical value	0.40	0.50

3.1.10.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

CAESAR methodology concludes that amide and carboxyl acid derivatives of *Gynocardin* are carcinogenic (***Tabl.IV.3.10. 12***). They have close molecular fragments with a series of alerts[39].

Tabl.IV.3.10. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Gynocardin*

CAESAR indicator	Gynocardin	
	amide	acid
GADI	0.37	0.37
SMKEV	0.78	0.77
APSM	1	1
CSM	0.49	0.48
MDRC	true	true
ACFSC	1	1
MCAR	0.61	0.61
NMNC	0.50	0.50
Carcinogen	**0.84**	**0.84**
NON-Carcinogen	0.16	0.16
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- carcinogen		

b) ISS

ISS methodology (***Tab.IV.3.10. 13***), it identifies amide and carboxyl acid derivatives of *Gynocardin* as non-carcinogenic.

Tabl.IV.3.10. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Gynocardin*

ISS indicator	Gynocardin	
	amide	acid
GADI	0.77	0.77
SMKEV	0.81	0.80
APSM	1	1
CSM	0.53	0.55
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

[39] Similarity: 0.77 by CAS: 18883-66-4, CAS: 54749-90-5, CAS: 15503-86-3 and CAS: 480-54-6

c) IRFMN/Antares

Carboxylic acid form of *Gynocardin* is prone to carcinogenicity (***Table IV.3.10. 14***) according to *IRFMN/Antares* methodology. In the training set there are molecules with close to analyzed fragments[40].

Tabl.IV.3.10. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN/Antares indicator	Gynocardin	
	amide	acid
GADI	0.63	0.75
SMKEV	0.84	0.86
APSM	0.66	0.66
CSM	0.34	0.66
ACFSC	1	1
Prediction	PNC	C
PNC- possible non-carcinogenic; C- carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.10. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Gynocardin*.

Tabl.IV.3.10. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN/ISSCAN-CGX indicator	Gynocardin	
	amide	acid
GADI	0.79	0.79
SMKEV	0.79	0.78
APSM	1	1
CSM	0.64	0.63
ACFSC	1	1
Prediction	PNC	PNC
C- carcinogen		

[40] Similarity: 0.77 by CAS: 18883-66-4, CAS: 54749-90-5, CAS: 15503-86-3 and CAS: 480-54-6

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Gynocardin* was confirmed (***Table IV.3.10. 16***) by the *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.10. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Gynocardin*

IRFMN indicator	Gynocardin	
	amide	acid
GADI	0	0
SMKEV	0.77	0.75
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.10. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Gynocardin* should not be administered orally.

Tabl.IV.3.10. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN indicator	Gynocardin	
	amide	acid
GADI	0	0
SMKEV	0.77	0.75
APSM	0.18	0.18
CSM	2.36	2.36
MEPASM	0.28	0.28
MDRC	N-true	N-true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	37.2	37.2
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	15.3	
n-true - does not cover		

3.1.10.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Gynocardin* highlights the lack of toxicity (***Table IV.3.10. 18***).

Tabl.IV.3.10. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Gynocardin*

CAESAR indicator	Gynocardin	
	amide	acid
GADI	0.76	0.88
SMKEV	0.79	0.78
APSM	1	1
CSM	0.52	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Gynocardin* did not report values for GADI and CSM. Molecular fragments close to *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.10. 19*** cannot be considered reliable.

Tabl.IV.3.10. 19 *PG toxicity of amide and carboxyl acid derivatives of Gynocardin*

PG indicator	Gynocardin	
	amide	acid
GADI	0	0
SMKEV	0.78	0.76
APSM	1	1
CSM	0	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Gynocardin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.10. 20***).

Tabl.IV.3.10. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN/CORAL indicator	Gynocardin	
	amide	acid
GADI	0.27	0.39
SMKEV	0.66	0.66
APSM	0.33	0.33
CSM	1.78	2.18
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	63.1	**160.0**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Gynocardin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.10. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.10. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Gynocardin*

CORAL indicator	Gynocardin	
	amide	acid
GADI	0.76	0.76
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
MDRC	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Gynocardin* (***Tab.IV.3.10. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.10. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN indicator	Gynocardin	
	amide	acid
GADI	0.90	0.90
SMKEV	0.81	0.15
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.13	0.12
Active Antagonist:	0.01	0.01
Inactive:	**0.86**	**0.87**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Gynocardin* did not report any deviations (***Tabl.IV.3.10. 23***) affecting the studied process.

Tabl.IV.3.10. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Gynocardin*

NIC indicator	Gynocardin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.83	0.81
APSM	0.49	0.49
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.20	3.86
Prediction	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxylic acid derivatives of *Gynocardin* we understand (***Tabl.IV.3.10. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.10. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Gynocardin*

INERIS indicator	Gynocardin	
	amide	acid
GADI	0	0
SMKEV	0.69	0.68
APSM	0.31	0.31
CSM	0.41	0.32
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.201	0.294
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.589	**1.968**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Gynocardin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.10. 25***).

Tabl.IV.3.10. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Gynocardin*

QSARINS indicator	Gynocardin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.83
APSM	0.09	0.09
CSM	0.05	0.03
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.21	0.23
Total half-life	[min]	

	100	105
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Gynocardin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.10. 26***).

Tabl.IV.3.10. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Gynocardin*

IRFMN/VERMEER indicator	Gynocardin	
	amide	acid
GADI	0.73	0.71
SMKEV	0.73	0.73
APSM	1	1
CSM	0.52	0.49
ACFSC	1	1
Prediction	A	inA
A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.10.3.2***), carcinogenicity (***§IV.3.1.10.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.10.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Gynocardin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.10. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.3.10. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Gynocardin*

IRFMN/VERMEER indicator	Gynocardin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.86	0.88
APSM	0.25	0.25
CSM	0.44	0.29
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.22	-3.36
	[mg/kg]	
	1660	**2291**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.10.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.10.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Gynocardin* would be optimal for drugs taken orally to poison the cancer cell with *(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide* as performed in ***§IV.2*** second objective of the study.

3.1.10.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide $2957 \leq 8312 \leq 23360$, acid $3059 \leq 7704 \leq 19402$ and *Bioaccumulation factor* [conditional units] amide $2.45 \leq 39 \leq 638$, acid forms are $0.00 \leq 0.31 \leq 707$.

3.1.10.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.10.6.1. Lipophilicity

Data from ***Tabl.IV.3.10. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.10. 28 *Lipophilicity of amide and carboxylic acid derivatives of Gynocardin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Gynocardin						
amide	0.12	-3.57	-4.68	-3.86	-3.85	-3.17
acid	0.11	-2.92	-4.08	-3.45	-3.61	-2.84

3.1.10.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.10. 29***).

Tabl.IV.3.10. 29 *Water solubility of amide and carboxylic acid derivatives of Gynocardin*

studied indicator	Gynocardin	
	amide	acid
ESOL		
Log S	0.68	0.27
Solubility, [mg/ml]	1.54e+03	5.94e+02
Class	vs	vs
Ali		
Log S	0.31	-0.24
Solubility, [mg/ml]	6.59e+02	1.85e+02
Class	vs	vs
SILICOS-IT		
Log S	3.43	3.65
Solubility, [mg/ml]	8.69e+05	1.43e+06
Class	s	s
vs - very soluble; s - soluble		

3.1.10.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Gynocardin* meets the pharmacokinetic requirements (***Table IV.3.10. 30***).

Tabl.IV.3.10. 30 *Pharmacokinetic indicators of amide and derivatives of Gynocardin*

studied indicator	Gynocardin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-10.79	-10.34

3.1.10.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.10. 31***) containing amide and derivatives of *Gynocardin*.

Tabl.IV.3.10. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Gynocardin*

studied indicator	Gynocardin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* No; 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.10.6.5. Medical Chemistry

Data from ***Tabl.IV.3.10. 32*** confirm the drug safety of amide and derivatives of *Gynocardin*.

Tabl.IV.3.10. 32 *Medical chemistry indicators for amide and derivatives of Gynocardin*

studied indicator	Gynocardin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.31	5.34
* 1 alert: isolated_alkene		

- ◊ -

3.1.11. (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell *of (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Menisdaurin*. The process proceeds according to ***§IV.2.3.***

3.1.11.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.11. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.3.11. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Menisdaurin						
amide	0.53	0.35	0.22	0.42	0.27	0.99
acid	0.57	0.47	0.21	0.68	0.34	1.01

Data in ***Tabl.IV.3.11. 1*** show that the amides and carboxylic acids of *Menisdaurin* have more pronounced overall drug activity *in vivo*.

3.1.11.2. Pharmacological and biological activity of oral active drugs

3.1.11.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.11. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.3.11. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Menisdaurin												
amide	331	-2.7	9	6	331	-2.6	74	44	331	-2.6	74	44
acid	332	-1.9	9	6	332	-1.9	74	43	332	-1.9	74	43

The two molecular modified forms of *Menisdaurin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.11.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* are listed in ***Tabl.IV.3.11. 3***.

Tabl.IV.3.11. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Menisdaurin									
	amide	163	4	4	2	20	331	0	15
	acid	157	4	4	2	20	332	1	15

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.11.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.11. 4***.

Tabl.IV.3.11. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Menisdaurin										
	amide	331	-3.1	9	6	163	4	1	0	0.26
	acid	332	-2.7	9	6	157	4	1	0	0.29

B. wQED

In ***Tabl.IV.3.11. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide.*

Tabl.IV.3.11. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		wQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Menisdaurin										
	amide	331	-3.1	9	6	163	4	1	0	0.34
	acid	332	-2.7	9	6	157	4	1	0	0.36

uwQED (***Tabl.IV.3.11. 4***) and *wQED* (***Tabl.IV.3.11. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of Menisdaurin meets the requirements for conservative treatment.

3.1.11.3. Non -laboratory and no clinical information on the chemical form

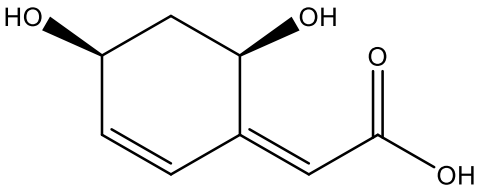
3.1.11.3.1. Receptor activity

In ***Tabl.IV.3.11. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Menisdaurin* to receptors (according to ***§III.3.3.4.1***).

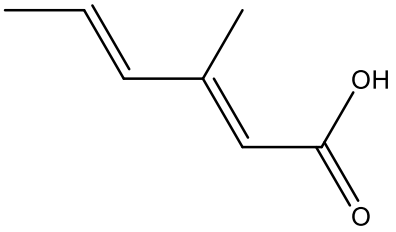
Tabl.IV.3.11. 6 *Receptor activity of amide and carboxyl derivatives of Menisdaurin*

indicator	Menisdaurin	
	amide	acid
AR		
ERa		
ERb	active *	active *
GR		
MR	-	-
PR		
RARa		active*
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from ***Tabl.IV.3.11. 6*** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to an overlap of a fragment of *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* с *(2E,4E)-3-methylhexa-2,4-dienoic acid* (***Fig.IV.3. 8***).



(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid

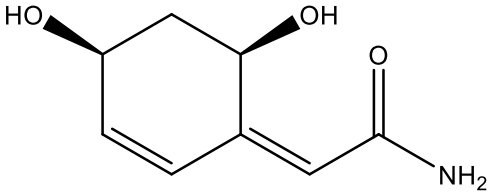


(2E,4E)-3-methylhexa-2,4-dienoic acid

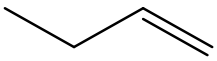
Fig.IV.3. 8 *Structural formulas of (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid u (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***). On the other hand, the amide and acid forms exhibit agonist activity to Estrogen Receptor b (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* (***Fig.IV.3. 2***), *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* with *but-1-ene* chain (***Fig.IV.3. 3***).



(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide



but-1-ene

Fig.IV.3. 9 *Structural formulas of (Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide u but-1-ene*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.11.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.11. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Menisdaurin*.

Tabl.IV.3.11. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Menisdaurin*

CAESAR indicator	Menisdaurin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.83
APSM	0.67	0.67
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Menisdaurin* did not show activity (***Table IV.3.11. 8***).

Tabl.IV.3.11. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Menisdaurin*

SarPy/IRFMN indicator	Menisdaurin	
	amide	acid
GADI	0.63	0.63
SMKEV	0.83	0.83
APSM	0.34	0.34
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Carboxyl acid derivatives of *Menisdaurin* is non-mutagenic according to *ISS* methodology (***Table IV.3.11. 9***). The implementation of the algorithm gives for the amide form – mutagenicity[41]. This is due to already reported mutagenic molecules with a similar structure in the training set.

Tabl.IV.3.11. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Menisdaurin*

ISS indicator	Menisdaurin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Menisdaurin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.11. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Menisdaurin*

KNN/Read-Across indicator	Menisdaurin	
	amide	acid
GADI	0.71	0.71
SMKEV	0.83	0.83
APSM	0.50	0.50
CSM	0.75	0.75
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

[41] Similarity: 0.71-8 by CAS: 23246-96-0, CAS: 303-34-4, CAS: 315-22-0, CAS: 18883-66-4 and CAS: 50-07-7

B. Consensus model

Data from ***Tabl.IV.3.11. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Menisdaurin* to mutagenicity.

Tabl.IV.3.11. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Menisdaurin*

Consensus model mutagenicity indicator	Menisdaurin	
	amide	acid
numerical value	0.35	0.50

3.1.11.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using the *CAESAR* methodology (***Tab.IV.3.11. 12***), for amide and carboxyl acid derivatives of *Menisdaurin* did not indicate the presence of carcinogenicity.

Tabl.IV.3.11. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Menisdaurin*

CAESAR indicator	Menisdaurin	
	amide	acid
GADI	0.75	0.64
SMKEV	0.79	0.79
APSM	1	0.52
CSM	0.51	0.52
MDRC	true	true
ACFSC	1	1
MCAR	0.39	0.39
NMNC	1	1
Carcinogen	0.31	0.31
NON-Carcinogen	**0.69**	**0.69**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) ISS

ISS carcinogenicity assessment methodology does not provide amide and carboxyl acid derivatives of *Menisdaurin* (***Tabl.IV.3.11. 13***). In this case we get identical (and/or those in the

statistical error of the method) results for CSM, but neither can be accepted – i.e. is both below and above 0.50.

Tabl.IV.3.11. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Menisdaurin*

ISS indicator	Menisdaurin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	C	NC
C- carcinogen; NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.11. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Menisdaurin*.

Tabl.IV.3.11. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN/Antares indicator	Menisdaurin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.84
APSM	0.67	0.67
CSM	0.67	0.67
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.11. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Menisdaurin*.

Tabl.IV.3.11. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN/ISSCAN-CGX indicator	Menisdaurin	
	amide	acid
GADI	0.80	0.79
SMKEV	0.79	0.78
APSM	1	1
CSM	0.65	0.64
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Menisdaurin* was confirmed (***Table IV.3.11. 16***) by the *Carcinogenicity oral classification model* (IRFMN).

Tabl.IV.3.11. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN indicator	Menisdaurin	
	amide	acid
GADI	0.73	0.72
SMKEV	0.76	0.74
APSM	0.50	0.50
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.11. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Menisdaurin* should not be administered orally.

Tabl.IV.3.11. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN indicator	Menisdaurin	
	amide	acid
GADI	0.64	0.63
SMKEV	0.76	0.74
APSM	0.07	0.07
CSM	2.65	2.60
MEPASM	0.11	0.11
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	[(g/kg-day)$^{-1}$	
	3.9	3.5
Presumed concentration of the active form inside the cancer cell	[(g/kg-day)$^{-1}$	
	1.6	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.11.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Menisdaurin* highlights the lack of toxicity (***Table IV.3.11. 18***).

Tabl.IV.3.11. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Menisdaurin*

CAESAR indicator	Menisdaurin	
	amide	acid
GADI	0.75	0.88
SMKEV	0.77	0.77
APSM	1	1
CSM	0.52	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Menisdaurin* did not report values for GADI and CSM. Molecular fragments close to *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.11. 19*** cannot be considered reliable.

Tabl.IV.3.11. 19 *PG toxicity of amide and carboxyl acid derivatives of Menisdaurin*

PG indicator	Menisdaurin	
	amide	acid
GADI	0	0
SMKEV	0.76	0.77
APSM	1	1
CSM	0	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Menisdaurin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.11. 20***).

Tabl.IV.3.11. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN/CORAL indicator	Menisdaurin	
	amide	acid
GADI	0.28	0.41
SMKEV	0.69	0.68
APSM	0.31	0.33
CSM	1.39	1.96
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	39.5	**100.2**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Menisdaurin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.11. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.11. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Menisdaurin*

CORAL indicator	Menisdaurin	
	amide	acid
GADI	0.76	0.76
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Menisdaurin* (***Tab.IV.3.11. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.11. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN indicator	Menisdaurin	
	amide	acid
GADI	0.90	0.90
SMKEV	0.81	0.81
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.09	0.08
Active Antagonist:	0.03	0.03
Inactive:	**0.88**	**0.88**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Menisdaurin* did not report any deviations (***Tabl.IV.3.11. 23***) affecting the studied process.

Tabl.IV.3.11. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Menisdaurin*

NIC indicator	Menisdaurin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.80	0.80
APSM	0.49	0.50
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.27	3.27
Prediction	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Menisdaurin* we understand (***Tabl.IV.3.11. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.11. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Menisdaurin*

INERIS indicator	Menisdaurin	
	amide	acid
GADI	0	0
SMKEV	0.69	0.68
APSM	0.31	0.31
CSM	0.63	0.63
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.190	0.231
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.549	**1.702**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Menisdaurin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.11. 25***).

Tabl.IV.3.11. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Menisdaurin*

QSARINS indicator	Menisdaurin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.81	0.81
APSM	0.09	0.09
CSM	0.07	0.10
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.33	0.36
Total half-life	[min]	
	130	**135**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Menisdaurin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.11. 26***).

Tabl.IV.3.11. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN/VERMEER indicator	Menisdaurin	
	amide	acid
GADI	0	0.86
SMKEV	0.75	0.75
APSM	1	1
CSM	0	1
ACFSC	1	1
Prediction	inA	inA
inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.11.3.2***), carcinogenicity (***§IV.3.1.11.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.11.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus* activity could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Menisdaurin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.11. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.3.11. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Menisdaurin*

IRFMN/CORAL indicator	Menisdaurin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.84
APSM	0.25	0.25
CSM	0.50	0.36
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.15	-3.30
Prediction	[mg/kg]	
	1413	**1995**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.11.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.11.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Menisdaurin* would be optimal for drugs taken orally to poison the cancer cell with *(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.11.5. Conclusion from the part

Applying of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide $3924 \leq 10845 \leq 29968$, acid $2977 \leq 73832.64 \leq 18307.03$ and Bioaccumulation factor [conditional units] amide $0.85 \leq 13.78 \leq 224.33$, acid are $0.00 \leq 0.30 \leq 617.14$

3.1.11.6. Evaluation of the results

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.11.6.1. Lipophilicity

Data from ***Tabl.IV.3.11. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.11. 28 *Lipophilicity of amide and carboxylic acid derivatives of Menisdaurin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Menisdaurin						
amide	1.82	-3.15	-3.10	-2.65	-2.66	-1.95
acid	0.19	-2.50	-2.50	-2.24	-2.42	-1.89

3.1.11.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.11. 29***).

Tabl.IV.3.11. 29 *Water solubility of amide and carboxylic acid derivatives of Menisdaurin*

studied indicator	Menisdaurin	
	amide	acid
ESOL		
Log S	0.35	-0.06
Solubility, [mg/ml]	7.49e+02	2.89e+02
Class	vs	vs
Ali		
Log S	0.30	-0.25
Solubility, [mg/ml]	6.62e+02	1.86e+02
Class	vs	vs
SILICOS-IT		
Log S	2.54	2.76
Solubility, [mg/ml]	1.15e+05	1.91e+05
Class	s	s
vs - very soluble; s - soluble		

3.1.11.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Menisdaurin* meets the pharmacokinetic requirements (***Table IV.3.11. 30***).

Tabl.IV.3.11. 30 *Pharmacokinetic indicators of amide and derivatives of Menisdaurin*

studied indicator	Menisdaurin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-10.56	-10.10

3.1.11.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.11. 31***) containing amide and derivatives of *Menisdaurin*.

Tabl.IV.3.11. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Menisdaurin*

studied indicator	Menisdaurin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* No; 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.11.6.5. Medical Chemistry

Data from ***Tabl.IV.3.11. 32*** confirm the drug safety of amide and derivatives of *Menisdaurin*.

Tabl.IV.3.11. 32 *Medical chemistry indicators for amide and derivatives of Menisdaurin*

studied indicator	Menisdaurin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.35	5.42
* 1 alert: michael_acceptor_1		

- ◊ -

3.1.12. (R)-2-hydroxy-3-methylbutanamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(R)-2-hydroxy-3-methylbutanamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Epiheterodendrin*. The process proceeds according to ***§IV.2.3.***

3.1.12.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.3.12. 1*** are listed values of *GPCR ligand, Ion channel modulator, Kinase inhibitor, Nuclear receptor ligand, Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-3-methylbutanamide*.

Tabl.IV.3.12. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-3-methylbutanamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Epiheterodendrin							
	amide	0.02	-0.08	-0.26	-0.44	0.15	0.31
	acid	0.17	0.17	-0.25	0.16	0.21	0.52

Data in ***Tabl.IV.3.12. 1*** show that the amides and carboxylic acids of *Epiheterodendrin* have more pronounced overall drug activity *in vivo*.

3.1.12.2. Pharmacological and biological activity of oral active drugs

3.1.12.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.3.12. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule, Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-3-methylbutanamide*.

Tabl.IV.3.12. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-3-methylbutanamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Epiheterodendrin													
	amide	279	-1.8	8	5	279	-1.8	60	40	279	-1.8	60	40
	acid	280	-1.0	8	5	280	-1.0	60	39	280	-1.0	60	39

The two molecular modified forms of *Epiheterodendrin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.12.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-3-methylbutanamide* are listed in ***Tabl.IV.3.12. 3.***

Tabl.IV.3.12. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-3-methylbutanamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Epiheterodendrin									
	amide	142	5	5	1	14	279	0	13
	acid	137	5	5	1	14	280	1	13

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.12.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.3.12. 4***.

Tabl.IV.3.12. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-3-methylbutanamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Epiheterodendrin										
	amide	279	-2.4	8	5	142	5	0	0	0.37
	acid	280	-2.0	8	5	137	5	0	0	0.40

B. wQED

In ***Tabl.IV.3.12. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(R)-2-hydroxy-3-methylbutanamide*.

Tabl.IV.3.12. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-2-hydroxy-3-methylbutanamide*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Epiheterodendrin										
	amide	279	-2.4	8	5	142	5	0	0	0.43
	acid	280	-2.0	8	5	137	5	0	0	0.46

uwQED (***Tabl.IV.3.12. 4***) and *wQED* (***Tabl.IV.3.12. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Epiheterodendrin* meets the requirements for conservative treatment.

3.1.12.3. Non -laboratory and no clinical information on the chemical form

3.1.12.3.1. Receptor activity

In ***Tabl.IV.3.12. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Epiheterodendrin* to receptors (according to ***§III.3.3.4.1***).

Tabl.IV.3.12. 6 *Receptor activity of amide and carboxyl derivatives of Epiheterodendrin*

indicator	Epiheterodendrin	
	amide	acid
AR		
ERa		
ERb		
GR		
MR	-	-
PR		
RARa		
RARb		
RARr		
TRa		
TRb		
VDR		

With the exception of *Mineralocorticoid Receptor* (MR), the studied molecules show inertness to the studied receptor set.

3.1.12.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.12. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Epiheterodendrin*.

Tabl.IV.3.12. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

CAESAR indicator	Epiheterodendrin	
	amide	acid
GADI	0.83	0.75
SMKEV	0.83	0.83
APSM	1	1
CSM	0.67	0.68
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Epiheterodendrin* did not show activity (***Table IV.3.1. 8***).

Tabl.IV.3.12. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

SarPy/IRFMN indicator	Epiheterodendrin	
	amide	acid
GADI	0.83	0.64
SMKEV	0.83	0.83
APSM	1	0.36
CSM	0.68	0.68
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Amide and carboxyl acid derivatives of *Epiheterodendrin* are non-mutagenic according to *ISS* methodology (***Table IV.3.12. 9***).

Tabl.IV.3.12. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

ISS indicator	Epiheterodendrin	
	amide	acid
GADI	0.77	0.78
SMKEV	0.83	0.82
APSM	1	1
CSM	0.52	0.53
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Epiheterodendrin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.3.12. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

KNN/Read-Across indicator	Epiheterodendrin	
	amide	acid
GADI	0.60	0.55
SMKEV	0.85	0.84
APSM	0.24	0.24
CSM	0.75	0.52
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.12. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Epiheterodendrin* to mutagenicity.

Tabl.IV.3.12. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

Consensus model mutagenicity indicator	Epiheterodendrin	
	amide	acid
numerical value	0.50	0.40

3.1.12.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using *CAESAR* methodology (***Tab.IV.3.12. 12***), for amide and carboxyl acid derivatives of *Epiheterodendrin* did not indicate the presence of carcinogenicity.

Tabl.IV.3.12. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

CAESAR indicator	Epiheterodendrin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.81	0.79
APSM	1	1
CSM	0.50	0.50
MDRC	true	true
ACFSC	1	1
MCAR	0.61	0.25
NMNC	1	1
Carcinogen	0.19	0.38
NON-Carcinogen	**0.81**	**0.62**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

Carboxyl acid form of *Epiheterodendrin* is prone to carcinogenicity (***Table IV.3.12. 13***) according to *ISS* methodology. In the training set there are molecules with close to analyzed fragments[42].

[42] Similarity: 0.81 by CAS: 18883-66-4; Similarity: 0.76 by CAS: 315-22-0, CAS: 51333-22-3 and CAS: 54749-90-5

Tabl.IV.3.12. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

ISS indicator	Epiheterodendrin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.83	0.82
APSM	1	1
CSM	0.48	0.47
ACFSC	1	1
Prediction	C	C
C- Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.12. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Epiheterodendrin*.

Tabl.IV.3.12. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN/Antares indicator	Epiheterodendrin	
	amide	acid
GADI	0.64	0.64
SMKEV	0.85	0.87
APSM	0.67	0.34
CSM	0.34	0.67
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.12. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxylic acid derivatives of *Epiheterodendrin*.

Tabl.IV.3.12. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxylic acid derivatives of Epiheterodendrin*

IRFMN/ISSCAN-CGX indicator	Epiheterodendrin	
	amide	acid
GADI	0.81	0.80
SMKEV	0.82	0.81
APSM	1	1
CSM	0.65	0.64

ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

Data from *Carcinogenicity oral classification model* (IRFMN) of amide and carboxylic acid derivatives of *Epiheterodendrin* (***Tabl.IV.3.12. 16***).

Tabl.IV.3.12. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN indicator	Epiheterodendrin	
	amide	acid
GADI	0	0
SMKEV	0.80	0.79
APSM	1	1
CSM	0	0
MDRC	true	true
ACFSC	1	1
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.12. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Epiheterodendrin* should not be administered orally.

Tabl.IV.3.12. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN indicator	Epiheterodendrin	
	amide	acid
GADI	0.68	0.67
SMKEV	0.80	0.79
APSM	0.18	0.18
CSM	2.20	2.17
MEPASM	0.28	0.28
MDRC	true	true
ACFSC	0.85	0.85

Predicted Oral Carcinogenicity SF for molecular forms	(g/kg-day)$^{-1}$	
	25.70	23.99
Presumed concentration of the active form inside the cancer cell	(g/kg-day)$^{-1}$	
	9.0	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.12.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Epiheterodendrin* highlights the lack of toxicity (***Table IV.3.12. 18***).

Tabl.IV.3.12. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

CAESAR indicator	Epiheterodendrin	
	amide	acid
GADI	0.76	0.89
SMKEV	0.80	0.80
APSM	1	1
CSM	0.53	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Epiheterodendrin* did not report values for GADI and CSM. Molecular fragments close to *(R)-2-hydroxy-3-methylbutanamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.12. 19*** cannot be considered reliable.

Tabl.IV.3.12. 19 *PG toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

PG indicator	Epiheterodendrin	
	amide	acid
GADI	0	0
SMKEV	0.77	0.76
APSM	1	1
CSM	0	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Epiheterodendrin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.12. 20***).

Tabl.IV.3.12. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN/CORAL indicator	Epiheterodendrin	
	amide	acid
GADI	0.27	0.41
SMKEV	0.68	0.68
APSM	0.35	0.58
CSM	0.18	1.11
MEPASM	0.54	1.01
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	5.2	**13.1**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Epiheterodendrin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.12. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.3.12. 21 *Chromosomal aberration toxicity model of amide and carboxyl acid derivatives of Epiheterodendrin*

CORAL indicator	Epiheterodendrin	
	amide	acid
GADI	0.65	0.64
SMKEV	0.80	0.82
APSM	1	1
CSM	0.53	0.47
ACFSC	0.85	0.85
Prediction	A	inA
A- active; inA- inactive		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Epiheterodendrin* (***Tab.IV.3.12. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.3.12. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN indicator	Epiheterodendrin	
	amide	acid
GADI	0.93	0.94
SMKEV	0.87	0.88
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.04	0.02
Active Antagonist:	0.01	0.01
Inactive:	**0.95**	**0.97**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Epiheterodendrin* did not report any deviations (***Tabl.IV.3.12. 23***) affecting the studied process.

Tabl.IV.3.12. 23 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Epiheterodendrin*

NIC indicator	Epiheterodendrin	
	amide	acid
GADI	0.78	0.78
SMKEV	0.87	0.84
APSM	0.51	0.51
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.22	1.78
Prediction	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Epiheterodendrin* we understand (***Tabl.IV.3.12. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.3.12. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Epiheterodendrin*

INERIS indicator	Epiheterodendrin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.67
APSM	0.16	0.31
CSM	0.59	0.47
MEPASM	0.19	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.60
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.167	0.144
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.469	**1.393**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Epiheterodendrin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.12. 25***).

Tabl.IV.3.12. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

QSARINS indicator	Epiheterodendrin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.84	0.85
APSM	0.09	0.03
CSM	0.01	0.12
MEPASM	0.15	0.03
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.28	0.30
Total half-life	[min]	
	115	**120**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Epiheterodendrin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.12. 26***).

Tabl.IV.3.12. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN/VERMEER indicator	Epiheterodendrin	
	amide	acid
GADI	0.74	0.74
SMKEV	0.77	0.76
APSM	1	1
CSM	0.49	0.52
ACFSC	1	1
Prediction	A	inA
A- active; inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.12.3.2***), carcinogenicity (***§IV.3.1.12.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.12.3.4***).

b) in vivo

The amides and carboxylic acid derivatives of *Epiheterodendrin*, as well as the *(R)-2-hydroxy-3-methylbutanamide* secreted after the passage of the cancer cell membrane, have been well studied in the clinical setting. Applying the *Micronucleus* toxicity activity model in vivo, the analysis showed the absence of genotoxic activity (***Tabl.IV.3.12. 27***).

Tabl.IV.3.12. 27 *Micronucleus toxicity activity model – in vivo of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN indicator	Epiheterodendrin	
	amide	acid
GADI	0.92	0.93
SMKEV	0.84	0.86
APSM	1	1
CSM	1	1
ACFSC	1	1
Prediction	**NON-genotoxic**	**NON-genotoxic**

F. NOAEL

The amide and carboxylic acid derivatives of *Epiheterodendrin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.12. 28***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.3.12. 28 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Epiheterodendrin*

IRFMN/VERMEER indicator	Epiheterodendrin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.87	0.89
APSM	0.25	0.25
CSM	0.83	0.69
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	

	-2.82	-2.97
Prediction	[mg/kg]	
	667	**933**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.12.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.12.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Epiheterodendrin* would be optimal for drugs taken orally to poison the cancer cell with *(R)-2-hydroxy-3-methylbutanamide* as performed in ***§IV.2*** second objective of the study.

3.1.12.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide $2991 \leq 8379 \leq 23479$, acid $5167 \leq 13130 \leq 33364$ and *Bioaccumulation factor* [conditional units] amide $5.5 \leq 89 \leq 1448$ and acid form are $0.03 \leq 0.61 \leq 13.7$.

3.1.12.6. Checking conclusion of the part

Conducted according to the methodological scheme §III.3.3.7.

3.1.12.6.1. Lipophilicity

Data from ***Tabl.IV.3.12. 29*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.3.12. 29 *Lipophilicity of amide and carboxylic acid derivatives of Epiheterodendrin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Epiheterodendrin						
amide	1.63	-2.34	-2.69	-2.48	-2.01	-1.58
acid	1.05	-1.69	-2.09	-2.08	-1.77	-1.32

3.1.12.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.12. 30***).

Tabl.IV.3.12. 30 *Water solubility of amide and carboxylic acid derivatives of Epiheterodendrin*

studied indicator	Epiheterodendrin	
	amide	acid
ESOL		
Log S	0.23	-0.18
Solubility, [mg/ml]	4.77e+02	1.84e+02
Class	vs	vs
Ali		
Log S	-0.11	-0.67
Solubility, [mg/ml]	2.14e+02	6.02e+01
Class	vs	vs
SILICOS-IT		
Log S	1.84	2.06
Solubility, [mg/ml]	1.93e+04	3.18e+04
Class	s	s
vs - very soluble; s - soluble		

3.1.12.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Epiheterodendrin* meets the pharmacokinetic requirements (***Table IV.3.12. 31***).

Tabl.IV.3.12. 31 *Pharmacokinetic indicators of amide and derivatives of Epiheterodendrin*

studied indicator	Epiheterodendrin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-9.67	-9.21

3.1.12.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.12. 31***) containing amide and derivatives of *Epiheterodendrin*.

Tabl.IV.3.12. 32 *Muegge activity and Bioavailability Score of amide and derivatives of Epiheterodendrin*

studied indicator	Epiheterodendrin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.56
1 violation: XLOGP3<-2		

3.1.12.6.5. Medical Chemistry

Data from ***Tabl.IV.3.12. 33*** confirm the drug safety of amide and derivatives of *Epiheterodendrin*.

Tabl.IV.3.12. 33 *Medical chemistry indicators for amide and derivatives of Epiheterodendrin*

studied indicator	Epiheterodendrin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	0	0
Leadlikeness	Yes	Yes
Synthetic accessibility	4.60	4.67

- ◊ -

3.1.13. (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Griffonin*. The process proceeds according to ***§IV.2.3.***

3.1.13.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.13. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.13. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Griffonin							
	amide	0.42	0.13	0.13	0.30	0.10	0.39
	acid	0.47	0.24	0.12	0.55	0.17	0.70

Data in ***Tabl.IV.13. 1*** show that the amides and carboxylic acids of *Griffonin* have pronounced overall drug activity *in vivo*.

3.1.13.2. Pharmacological and biological activity of oral active drugs

3.1.13.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.13. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.13. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Griffonin													
	amide	347	-3.2	10	7	347	-3.2	77	45	347	-3.2	77	45
	acid	348	-2.5	10	7	348	-2.5	77	44	348	-2.5	77	44

The two molecular modified forms of *Griffonin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.13.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* are listed in ***Tabl.IV.13. 3.***

Tabl.IV.13. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Griffonin									
	amide	183	4	4	2	21	347	0	17
	acid	177	4	4	2	21	348	1	17

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§3.1.1.2.1***.

3.1.13.2.3. QED

The analysis is performed according to ***§3.1.1.2.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.13. 4***.

Tabl.IV.13. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Griffonin										
	amide	347	-3.3	10	7	183	4	1	0	0.20
	acid	348	-2.9	10	7	177	4	1	0	0.22

B. wQED

In ***Tabl.IV.13. 5*** presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer

cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide.*

Tabl.IV.13. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		wQED							
	MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Griffonin									
amide	347	-3.3	10	7	183	4	1	0	0.29
acid	348	-2.9	10	7	177	4	1	0	0.31

uwQED (***Tabl.IV.13. 4***) and *wQED* (***Tabl.IV.13. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Griffonin* meets the requirements for conservative treatment.

3.1.13.3. Non -laboratory and no clinical information on the chemical form

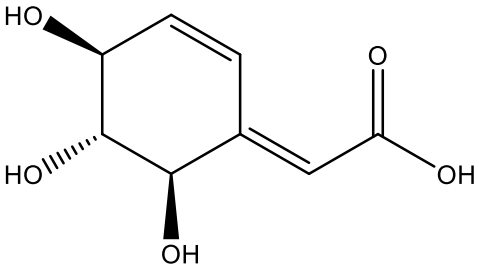
3.1.13.3.1. Receptor activity

In ***Tabl.IV.3.13. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Griffonin* to receptors (according to ***§III.3.3.4.1***).

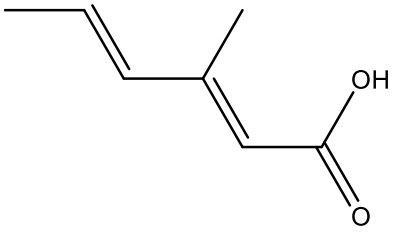
Tabl.IV.13. 6 *Receptor activity of amide and carboxyl derivatives of Griffonin*

indicator	Griffonin	
	amide	acid
AR		
ERa		
ERb	active *	active *
GR		
MR	-	-
PR		
RARa		active*
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from **Tabl.IV.3.13. 6** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to an overlap of a fragment of *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* c *(2E,4E)-3-methylhexa-2,4-dienoic acid* (***Fig.IV.3. 10***).



(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid

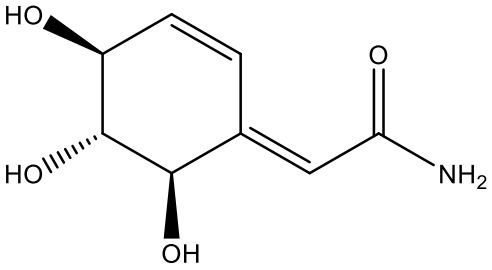


(2E,4E)-3-methylhexa-2,4-dienoic acid

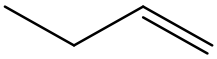
Fig.IV.3. 10 *Structural formulas of (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid и (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***). On the other hand, the amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* (***Fig.IV.3. 10***) and *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* with *but-1-ene* chain (***Fig.IV.3. 11***).



(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide



but-1-ene

Fig.IV.3. 11 *Structural formulas of (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide и but-1-ene*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.13.3.2. Mutagenicity

A. Stand-alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.13. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Griffonin*.

Tabl.IV.13. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Griffonin*

CAESAR indicator	Griffonin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.82	0.83
APSM	0.67	0.67
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Griffonin* did not show activity (***Table IV.3.13. 8***).

Tabl.IV.13. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Griffonin*

SarPy/IRFMN indicator	Griffonin	
	amide	acid
GADI	0.63	0.63
SMKEV	0.82	0.83
APSM	0.34	0.35
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Carboxyl acid derivative of *Griffonin* is non-mutagenic according to *ISS* methodology (***Table IV.3.13. 9***). However, the amide is mutagenic because there are similar molecules in the training set[43].

[43] Similarity: 0.72-7 by CAS: 23246-96-0, CAS: 303-34-4, CAS: 315-22-0, CAS: 18883-66-4 and CAS: 2058-46-0

Tabl.IV.13. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Griffonin*

ISS indicator	Griffonin	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.47	0.54
ACFSC	1	1
Prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Griffonin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.13. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Griffonin*

KNN/Read-Across indicator	Griffonin	
	amide	acid
GADI	0.54	0.71
SMKEV	0.83	0.83
APSM	0.24	0.49
CSM	0.51	0.76
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.13. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Griffonin* to mutagenicity.

Tabl.IV.13. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Griffonin*

Consensus model mutagenicity indicator	Griffonin	
	amide	acid
numerical value	0.25	0.50

3.1.13.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using *CAESAR* methodology (***Tab.IV.3.13. 12***), for amide and carboxyl acid derivatives of *Griffonin* did not indicate the presence of carcinogenicity.

Tabl.IV.13. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Griffonin*

CAESAR indicator	Griffonin	
	amide	acid
GADI	0.75	0.88
SMKEV	0.78	0.78
APSM	1	1
CSM	0.51	1
MDRC	true	true
ACFSC	1	1
MCAR	0.47	0.47
NMNC	1	1
Carcinogen	0.27	0.27
NON-Carcinogen	**0.73**	**0.73**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

ISS carcinogenicity assessment methodology does not provide amide and carboxyl acid derivatives of *Griffonin* (***Tabl.IV.3.13. 13***). In this case we get identical (and/or those in the statistical error of the method) results for CSM, but neither can be accepted – i.e. is both below and above 0.50.

Tabl.IV.13. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Griffonin*

ISS indicator	Griffonin	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.47	0.54
ACFSC	1	1
Prediction	C	NC
C- carcinogen; NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.13. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Griffonin*.

Tabl.IV.13. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN/Antares indicator	Griffonin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.84
APSM	0.66	0.66
CSM	0.66	0.66
ACFSC	1	1
Prediction	PNC	PNC
C- carcinogen		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.13. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Griffonin*.

Tabl.IV.13. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN/ISSCAN-CGX indicator	Griffonin	
	amide	acid
GADI	0.79	0.79
SMKEV	0.78	0.78
APSM	1	1
CSM	0.64	0.63
ACFSC	1	1
Prediction	PNC	PNC
C- carcinogen		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

The non-carcinogenicity of amide and carboxyl acid derivatives of *Griffonin* was confirmed (***Table IV.3.13. 16***) by the *Carcinogenicity oral classification model* (IRFMN) model.

Tabl.IV.13. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Griffonin*

IRFMN indicator	Griffonin	
	amide	acid
GADI	0.73	0.85
SMKEV	0.75	0.73
APSM	0.50	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	PNC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; PNC- possible non-carcinogenic; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.13. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Griffonin* should not be administered orally.

Tabl.IV.13. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN indicator	Griffonin	
	amide	acid
GADI	0	0
SMKEV	0.75	0.73
APSM	0.07	0.10
CSM	3.56	2.86
MEPASM	0.11	0.11
MDRC	N-true	N-true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	32.4	31.6
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	14.3	
n-true - does not cover		

3.1.13.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Griffonin* highlights the lack of toxicity (***Table IV.3.13. 18***).

Tabl.IV.13. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Griffonin*

CAESAR indicator	Griffonin	
	amide	acid
GADI	0.88	0.88
SMKEV	0.77	0.77
APSM	1	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives *Griffonin* did not report values for GADI and CSM. Molecular fragments close to *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.13. 19*** cannot be considered reliable.

Tabl.IV.13. 19 *PG toxicity of amide and carboxyl acid derivatives of Griffonin*

PG indicator	Griffonin	
	amide	acid
GADI	0.62	0
SMKEV	0.76	0.76
APSM	0.49	1
CSM	0.50	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Griffonin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.13. 20***).

Tabl.IV.13. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN/CORAL indicator	Griffonin	
	amide	acid
GADI	0.28	0.40
SMKEV	0.69	0.67
APSM	0.31	0.31
CSM	1.37	1.77
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	44.4	**11.3**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Griffonin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.13. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.13. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Griffonin*

CORAL indicator	Griffonin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.79	0.78
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Griffonin* (***Tab.IV.3.13. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.13. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN indicator	Griffonin	
	amide	acid
GADI	0.89	0.90
SMKEV	0.79	0.80
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.12	0.12
Active Antagonist:	0.04	0.04
Inactive:	**0.84**	**0.84**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Griffonin* did not report any deviations (***Tabl.IV.3.13. 23***) affecting the studied process.

Tabl.IV.13. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Griffonin*

NIC indicator	Griffonin	
	amide	acid
GADI	0.75	0.75
SMKEV	0.79	0.79
APSM	0.49	0.49
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.42	3.94
Prediction	nonA	nonA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying Adipose tissue: blood model for toxicity of amide and carboxyl acid derivatives of *Griffonin* we understand (***Tabl.IV.3.13. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.13. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Griffonin*

INERIS indicator	Griffonin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.68
APSM	0.31	0.31
CSM	0.63	0.63
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.60
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.192	0.237
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.556	**1.726**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Griffonin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.13. 25***).

Tabl.IV.13. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Griffonin*

QSARINS indicator	Griffonin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.81	0.81
APSM	0.09	0.41
CSM	0.02	0.29
MEPASM	0.15	0.79
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	

	0.24	0.28
Total half-life	[min]	
	105	**115**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodic part that amide and carboxyl acid derivatives of *Griffonin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.13. 26***).

Tabl.IV.13. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Griffonin*

IRFMN/VERMEER indicator	Griffonin	
	amide	acid
GADI	0	0.86
SMKEV	0.74	0.74
APSM	1	1
CSM	0	1
ACFSC	1	1
Prediction	inA	inA
inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.13.3.2***), carcinogenicity (***§IV.3.1.13.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.13.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus activity* could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Griffonin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.13. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.13. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Griffonin*

IRFMN/VERMEER indicator	Griffonin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.84	0.86
APSM	0.25	0.25
CSM	0.28	0.19
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.37	-3.52
Prediction	[mg/kg]	
	2344	**3311**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.13.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.13.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Griffonin* would be optimal for drugs taken orally to poison the cancer cell with *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.13.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values are respectively: *Oral rat LD50* [mg/kg] for amide $5016 \leq 13956 \leq 38832$, acid $4015 \leq 10006 \leq 24938$ and *Bioaccumulation factor* [conditional units] amide $0.86 \leq 13.9 \leq 228$, acid are $0.00 \leq 0.25 \leq 567$.

3.1.13.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.13.6.1. Lipophilicity

Data from ***Tabl.IV.3.13. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.13. 28 *Lipophilicity of amide and carboxylic acid derivatives of Griffonin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Griffonin						
amide	0.67	-4.13	-4.13	-3.42	-3.54	-2.91
acid	0.61	-3.48	-3.53	-3.01	-3.30	-2.54

3.1.13.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.313. 29***).

Tabl.IV.13. 29 *Water solubility of amide and carboxylic acid derivatives of Griffonin*

studied indicator	Griffonin	
	amide	acid
ESOL		
Log S	0.87	0.46
Solubility, [mg/ml]	2.59e+03	9.97e+02
Class	vs	hs
Ali		
Log S	0.89	0.34
Solubility, [mg/ml]	2.71e+03	7.62e+02
Class	vs	hs
SILICOS-IT		
Log S	3.36	3.58
Solubility, [mg/ml]	7.92e+05	1.31e+06
Class	s	s
vs - very soluble; hs - highly soluble; s - soluble		

3.1.13.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Griffonin* meets the pharmacokinetic requirements (***Table IV.3.13. 30***).

Tabl.IV.13. 30 *Pharmacokinetic indicators of amide and derivatives of Griffonin*

studied indicator	Griffonin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-11.35	-10.90

3.1.13.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.13. 31***) containing amide and derivatives of *Griffonin*.

Tabl.IV.13. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Griffonin*

studied indicator	Griffonin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.13.6.5. Medical Chemistry

Data from ***Tabl.IV.3.13. 32*** confirm the drug safety of amide and derivatives of *Griffonin*.

Tabl.IV.13. 32 *Medical chemistry indicators for amide and derivatives of Griffonin*

studied indicator	Griffonin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.49	5.55
* 1 alert: michael_acceptor_1		

- ◊ -

3.1.14. (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Bauhinin*. The process proceeds according to ***§IV.2.3.***

3.1.14.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.14. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.14. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Bauhinin							
	amide	0.42	0.18	0.19	0.30	0.13	0.64
	acid	0.46	0.29	0.18	0.54	0.19	0.65

Data in ***Tabl.IV.14. 1*** show that the amides and carboxylic acids of *Bauhinin* have pronounced overall drug activity *in vivo*.

3.1.14.2. Pharmacological and biological activity of oral active drugs

3.1.14.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.14. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.14. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Bauhinin													
	amide	361	-3.1	10	6	362	-3.1	82	48	361	-3.1	82	48
	acid	362	-2.4	10	6	362	-2.4	82	47	362	-2.4	82	47

The two molecular modified forms of Bauhinin meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.14.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and *release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide* are listed in ***Tabl.IV.14. 3.***

Tabl.IV.14. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Bauhinin									
	amide	172	5	5	2	21	361	0	16
	acid	166	5	5	2	21	362	1	16

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.14.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.14. 4***.

Tabl.IV.14. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Bauhinin										
	amide	361	-2.9	10	6	172	5	1	0	0.24
	acid	362	-2.5	10	6	166	5	1	0	0.26

B. wQED

Tabl.IV.14. 5 presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide.*

Tabl.IV.14. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Bauhinin										
	amide	361	-2.9	10	6	172	5	1	0	0.33
	acid	362	-2.5	10	6	166	5	1	0	0.36

uwQED (***Tabl.IV.14. 4***) and *wQED* (***Tabl.IV.14. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Lithospermoside* meets the requirements for conservative treatment.

3.1.14.3. Non -laboratory and no clinical information on the chemical form

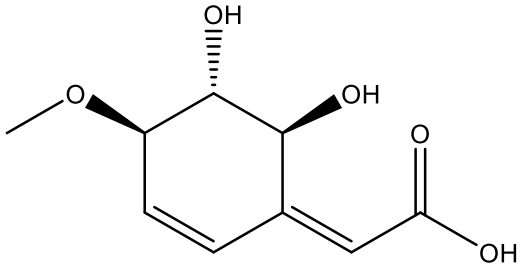
3.1.14.3.1. Receptor activity

In ***Tabl.IV.3.14. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Bauhinin* to receptors (according to ***§III.3.3.4.1***).

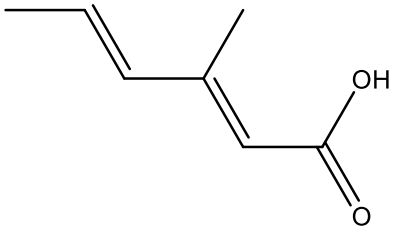
Tabl.IV.14. 6 *Receptor activity of amide and carboxyl derivatives of Bauhinin*

indicator	Bauhinin	
	amide	acid
AR		
ERa		
ERb	active *	active *
GR		
MR	-	-
PR		
RARa		active*
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from **Tabl.IV.3.14. 6** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to an overlap of a fragment of *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid* с *(2E,4E)-3-methylhexa-2,4-dienoic acid* (***Fig.IV.3. 12***).



(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid

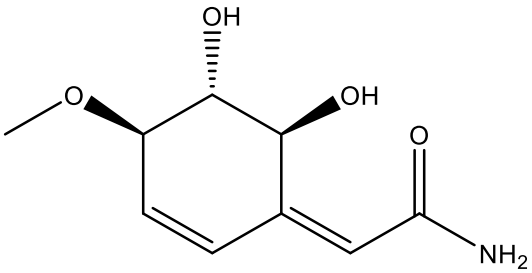


(2E,4E)-3-methylhexa-2,4-dienoic acid

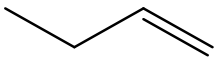
Fig.IV.3. 12 *Structural formulas of (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid u (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid* is formed as a by-product after the passage of **HF(A)** across the cancer cell membrane ***(§IV.2.3 .1***). On the other hand, the amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetic acid* (***Fig.IV.3. 12***), *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide* with *but-1-ene* chain (***Fig.IV.3. 13***).



(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide



but-1-ene

Fig.IV.3. 13 *Structural formulas of (Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide u but-1-ene*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.14.3.2. Mutagenicity

A. Stand -alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.14. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Bauhinin*.

Tabl.IV.14. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Bauhinin*

CAESAR indicator	Bauhinin	
	amide	acid
GADI	0.74	0.74
SMKEV	0.82	0.83
APSM	0.67	0.67
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Bauhinin* did not show activity (***Table IV.3.14. 8***).

Tabl.IV.14. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Bauhinin*

SarPy/IRFMN indicator	Bauhinin	
	amide	acid
GADI	0.62	0.63
SMKEV	0.82	0.83
APSM	0.34	0.34
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Carboxyl acid derivative of *Bauhinin* is non-mutagenic according to the *ISS* methodology (***Table IV.3.14. 9***). The amide derivative exhibits mutagenic activity[44] against the organism.

Tabl.IV.14. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Bauhinin*

ISS indicator	Bauhinin	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.79
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Bauhinin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.14. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Bauhinin*

KNN/Read-Across indicator	Bauhinin	
	amide	acid
GADI	0.71	0.71
SMKEV	0.82	0.83
APSM	0.50	0.49
CSM	0.75	0.75
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.14. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Bauhinin* to mutagenicity.

[44] Similarity: 0.74-7 by CAS: 23246-96-0 (SA37 Pyrrolizidine Alkaloid), CAS: 303-34-4 (SA37 Pyrrolizidine Alkaloid) and CAS: 315-22-0 (SA37 Pyrrolizidine Alkaloids); Similarity: 0.7203 by CAS: 18883-66-4 (SA21 Alkyl and aryl N-nitroso groups) and CAS: 64-75-5 (SA10 alfa, beta unsaturated carbonyls | SA38 Alkenylbenzenes)

Tabl.IV.14. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Bauhinin*

Consensus model mutagenicity indicator	Bauhinin	
	amide	acid
numerical value	0.35	0.50

3.1.14.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using *CAESAR* methodology (***Tab.IV.3.14. 12***), for amide and carboxyl acid derivatives of *Bauhinin* did not indicate the presence of carcinogenicity.

Tabl.IV.14. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Bauhinin*

CAESAR indicator	Bauhinin	
	amide	acid
GADI	0.75	0.89
SMKEV	0.79	0.79
APSM	1	1
CSM	0.52	1
MDRC	true	true
ACFSC	1	1
MCAR	0.47	0.47
NMNC	1	1
Carcinogen	0.26	0.26
NON-Carcinogen	**0.74**	**0.74**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

ISS carcinogenicity assessment methodology does not provide amide and carboxyl acid derivatives of *Bauhinin* (***Tabl.IV.3.14. 13***). In this case we get identical (and/or those in the statistical error of the method) results for CSM, but neither can be accepted – i.e. is both below and above 0.50.

Tabl.IV.14. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Bauhinin*

ISS indicator	Bauhinin	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.79
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	C	NC
C- carcinogen; NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.14. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Bauhinin*.

Tabl.IV.14. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN/Antares indicator	Bauhinin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.82	0.83
APSM	0.67	0.67
CSM	0.67	0.67
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.14. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Bauhinin*.

Tabl.IV.14. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN/ISSCAN-CGX indicator	Bauhinin	
	amide	acid
GADI	0.79	0.79
SMKEV	0.79	0.78
APSM	1	1

CSM	0.65	0.64
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

Carcinogenic activity (***Table IV.14. 16***) was observed with a single dose of amide and carboxylic acid derivatives of *Bauhinin* according to the *Carcinogenicity oral classification model* (IRFMN). The molecules reported[45] in APSM also determine the final prediction.

Tabl.IV.14. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Bauhinin*

IRFMN indicator	Bauhinin	
	amide	acid
GADI	0.73	0.72
SMKEV	0.75	0.73
APSM	0.51	0.51
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.14. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Bauhinin* should not be administered orally.

Tabl.IV.14. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN indicator	Bauhinin	
	amide	acid
GADI	0	0
SMKEV	0.75	0.73
APSM	0.07	0.07

[45] Similarity: 0.73-5 by CAS: 303-34-4, CAS: 315-22-0, CAS: 18883-66-4, CAS: 54749-90-5; Similarity: 0.69 by CAS: 50-07-7

CSM	3.43	3.41
MEPASM	0.11	0.11
MDRC	N-true	N-true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	24.0	22.9
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	11.0	
N-true – do not cover		

3.1.14.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of the *CAESAR* toxicity method on amide and carboxyl acid derivatives of *Bauhinin* highlights the lack of toxicity (***Table IV.3.14. 18***).

Tabl.IV.14. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Bauhinin*

CAESAR indicator	Bauhinin	
	amide	acid
GADI	0.75	0.88
SMKEV	0.78	0.78
APSM	1	1
CSM	0.51	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Bauhinin* did not report values for GADI and CSM. Molecular fragments close to *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.14. 19*** cannot be considered reliable.

Tabl.IV.14. 19 *PG toxicity of amide and carboxyl acid derivatives of Bauhinin*

PG indicator	Bauhinin	
	amide	acid
GADI	0.62	0
SMKEV	0.77	0.77
APSM	0.50	1
CSM	0.50	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Bauhinin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.14. 20***).

Tabl.IV.14. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN/CORAL indicator	Bauhinin	
	amide	acid
GADI	0.28	0.41
SMKEV	0.69	0.68
APSM	0.31	0.30
CSM	1.16	1.56
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	28.6	**72.6**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Bauhinin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.14. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.14. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Bauhinin*

CORAL indicator	Bauhinin	
	amide	acid
GADI	0.76	0.75
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and / or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Bauhinin* (***Tab.IV.3.14. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.14. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN indicator	Bauhinin	
	amide	acid
GADI	0.89	0.88
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.13	0.12
Active Antagonist:	0.03	0.03
Inactive:	**0.84**	**0.85**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Bauhinin* did not report any deviations (***Tabl.IV.3.14. 23***) affecting the studied process.

Tabl.IV.14. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Bauhinin*

NIC indicator	Bauhinin	
	amide	acid
GADI	0	0
SMKEV	0.79	0.79
APSM	0	0
CSM	0.51	0.50
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.18	3.70
Prediction	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NA- Non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Bauhinin* we understand (***Tabl.IV.3.14. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.14. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Bauhinin*

INERIS indicator	Bauhinin	
	amide	acid
GADI	0	0
SMKEV	0.68	0.68
APSM	0.31	0.31
CSM	0.63	0.63
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.51
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.181	0.223
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.517	1.671
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Bauhinin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.14. 25***).

Tabl.IV.14. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Bauhinin*

QSARINS indicator	Bauhinin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.81	0.82
APSM	0.09	0.41
CSM	0.06	0.37
MEPASM	0.15	0.79
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.33	0.36
Total half-life	[min]	
	130	**140**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Bauhinin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.14. 26***).

Tabl.IV.14. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Bauhinin*

IRFMN/VERMEER indicator	Bauhinin	
	amide	acid
GADI	0	0.73
SMKEV	0.75	0.75
APSM	1	1
CSM	0	0.52
ACFSC	1	1
Prediction	inA	A
A- active; inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.14.3.2***), carcinogenicity (***§IV.3.1.14.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.14.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus activity* could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives *Bauhinin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.14. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.14. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Bauhinin*

IRFMN/VERMEER indicator	Bauhinin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.84
APSM	0.25	0.25
CSM	0.54	0.39
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.12	-3.26
Prediction	[mg/kg]	
	1318	**1820**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.14.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.14.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Bauhinin* would be optimal for drugs taken orally to poison the cancer cell with *(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.14.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg / kg] for amide 3765 ≤ 10452 ≤ 29017, acid 3521 ≤ 9817 ≤ 27375 and *Bioaccumulation factor* [conditional units] amide 0.96 ≤ 15.6 ≤ 255, acid form are 0.00 ≤ 0.28 ≤ 560.

3.1.14.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.14.6.1. Lipophilicity

Data from ***Tabl.IV.3.14. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.14. 28 *Lipophilicity of amide and carboxylic acid derivatives of Bauhinin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Bauhinin						
amide	1.72	-3.59	-3.47	-3.16	-3.00	-2.30
acid	1.14	-2.94	-2.87	-2.75	-2.77	-2.04

3.1.14.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.14. 29***).

Tabl.IV.14. 29 *Water solubility of amide and carboxylic acid derivatives of Bauhinin*

studied indicator	Bauhinin	
	amide	acid
ESOL		
Log S	0.51	0.10
Solubility, [mg/ml]	1.17e+03	4.52e+02
Class	hs	hs
Ali		
Log S	0.56	0.01
Solubility, [mg/ml]	1.32e+03	3.71e+02
Class	hs	hs
SILICOS-IT		
Log S	2.67	2.88

Solubility, [mg/ml]	1.67e+05	2.76e+05
Class	s	s
vs - very soluble; hs - highly soluble; s - soluble		

3.1.14.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Bauhinin* meets the pharmacokinetic requirements (***Table IV.3.14. 30***).

Tabl.IV.14. 30 Pharmacokinetic indicators of amide and derivatives of Bauhinin

studied indicator	Bauhinin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-11.05	-10.60

3.1.14.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.14. 31***) containing amide and derivatives of *Bauhinin*.

Tabl.IV.14. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Bauhinin*

studied indicator	Bauhinin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.14.6.5. Medical Chemistry

Data from ***Tabl.IV.3.14. 32*** confirm the drug safety of amide and derivatives of *Bauhinin*.

Tabl.IV.14. 32 *Medical chemistry indicators for amide and derivatives of Bauhinin*

studied indicator	Bauhinin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	No**	No**
Synthetic accessibility	5.62	5.68
* 1 alert: michael_acceptor_1; ** 1 violation: MW>350		

- ◊ -

3.1.15. (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Purshianin*. The process proceeds according to ***§IV.2.3.***

3.1.15.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.15. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.15. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Purshianin							
	amide	0.53	0.35	0.22	0.42	0.27	0.99
	acid	0.57	0.47	0.21	0.68	0.34	1.01

Data in ***Tabl.IV.15. 1*** show that the amides and carboxylic acids of *Purshianin* have pronounced overall drug activity *in vivo*.

3.1.15.2. Pharmacological and biological activity of oral active drugs

3.1.15.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.15. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.15. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (R)-(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
		MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Purshianin													
	amide	331	-2.6	9	6	331	-2.6	74	44	331	-2.6	74	44
	acid	332	-1.9	9	6	332	-1.9	74	43	332	-1.9	74	43

The two molecular modified forms of *Purshianin* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.15.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on *Veber Filter*, *MDDR-Like Rule* and *BBB Likeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* are listed in ***Tabl.IV.15. 3***

Tabl.IV.15. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Purshianin									
	amide	163	4	4	2	20	331	0	15
	acid	157	4	4	2	20	332	1	15

There are no significant fluctuations in individual indicators. All "problem" values correlate with pre-entered deviations **§III.3.3.3.1**.

3.1.15.2.3. QED

The analysis is performed according to ***§3.3.3.1.3***.

A. uwQED

Data for *Unweighted Quantitative Estimate of Druglikeness* of the tested compounds are given in ***Tabl.IV.15. 4***.

Tabl.IV.15. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Purshianin										
	amide	331	-3.1	9	6	163	4	1	0	0.27
	acid	332	-2.7	9	6	157	4	1	0	0.29

B. wQED

Tabl.IV.15. 5 presents the data from the calculations for a *Weighted Quantitative Estimate of Druglikeness* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.15. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*

		wQED							
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Purshianin										
	amide	331	-3.1	9	6	163	4	1	0	0.34
	acid	322	-2.7	9	6	157	4	1	0	0.36

uwQED (***Tabl.IV.15. 4***) and *wQED* (***Tabl.IV.15. 5***) of a potential pharmaceutical form including amide and carboxylic acid obtained by hydrolysis of the nitrile group of *Lithospermoside* meets the requirements for conservative treatment.

3.1.15.3. Non -laboratory and no clinical information on the chemical form

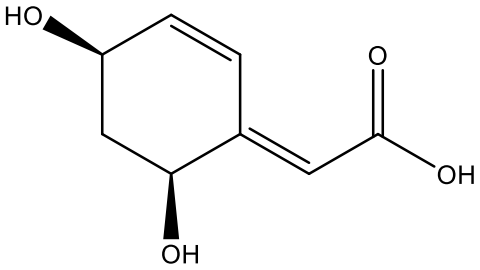
3.1.15.3.1. Receptor activity

In ***Tabl.IV.3.15. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Purshianin* to receptors (according to ***§III.3.3.4.1***).

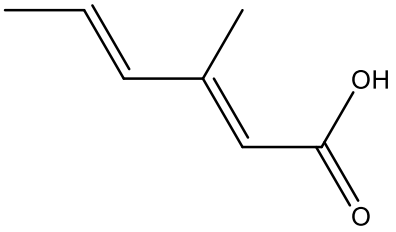
Tabl.IV.15. 6 *Receptor activity of amide and carboxyl derivatives of Purshianin*

indicator	Purshianin	
	amide	acid
AR		
ERa		
ERb	active *	active *
GR		
MR	-	-
PR		
RARa		active*
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from ***Tabl.IV.3.15. 6*** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to an overlap of a fragment of *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* c *(2E,4E)-3-methylhexa-2,4-dienoic acid* (***Fig.IV.3. 14***).



(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid

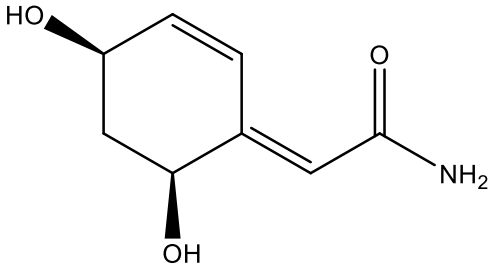


(2E,4E)-3-methylhexa-2,4-dienoic acid

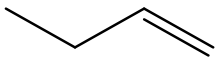
Fig.IV.3. 14 *Structural formulas of (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid u (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* is formed as a by-product after the passage of HF(A) across the cancer cell membrane ***(§IV.2.3 .1***). On the other hand, the amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetic acid* (***Fig.IV.3. 14***) and *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide*with *but-1-ene* chain (***Fig.IV.3. 15***).



(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide



but-1-ene

Fig.IV.3. 15 *Structural formulas of (E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide u but-1-ene*

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.15.3.2. Mutagenicity

A. Stand -alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.15. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Purshianin*.

Tabl.IV.15. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Purshianin*

CAESAR indicator	Purshianin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.83
APSM	0.67	0.67
CSM	0.70	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Purshianin* did not show activity (***Table IV.3.15. 8***).

Tabl.IV.15. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Purshianin*

SarPy/IRFMN indicator	Purshianin	
	amide	acid
GADI	0.63	0.63
SMKEV	0.83	0.83
APSM	0.34	0.34
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Carboxyl acid derivative of *Purshianin* is non-mutagenic according to the *ISS* methodology (***Table IV.3.15. 9***). The amide derivative exhibits mutagenic activity[46] against the organism.

[46] Similarity: 0.76-8 by CAS: 23246-96-0 (SA37 Pyrrolizidine Alkaloids), CAS: 303-34-4 (SA37 Pyrrolizidine Alkaloids) and CAS: 315-22-0 (SA37 Pyrrolizidine Alkaloids); Similarity: 0.71-4 by CAS: 18883-66-4 (SA21 Alkyl and aryl N-nitroso groups) and CAS: 50-07-7 (SA7 Epoxides and aziridines; SA12 Quinones; SA16 Alkyl carbamate and thiocarbamate)

Tabl.IV.15. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Purshianin*

ISS indicator	Purshianin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Purshianin* show some deviation from the *KNN/Read-Across* method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.15. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Purshianin*

KNN/Read-Across indicator	Purshianin	
	amide	acid
GADI	0.71	0.71
SMKEV	0.83	0.83
APSM	0.50	0.50
CSM	0.75	0.75
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

B. Consensus model

Data from ***Tabl.IV.3.15. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Purshianin* to mutagenicity

Tabl.IV.15. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Purshianin*

Consensus model mutagenicity indicator	Purshianin	
	amide	acid
numerical value	0.35	0.50

3.1.15.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using the CAESAR methodology (***Tab.IV.3.15. 12***), for amide and carboxyl acid derivatives of *Purshianin* did not indicate the presence of carcinogenicity.

Tabl.IV.15. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Purshianin*

CAESAR indicator	Purshianin	
	amide	acid
GADI	0.75	0.64
SMKEV	0.79	0.79
APSM	1	0.52
CSM	0.51	0.52
MDRC	true	true
ACFSC	1	1
MCAR	0.39	0.39
NMNC	1	1
Carcinogen	0.31	0.31
NON-Carcinogen	**0.69**	**0.69**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

ISS carcinogenicity assessment methodology does not provide amide and carboxyl acid derivatives of *Purshianin* (***Tabl.IV.3.15. 13***). In this case we get identical (and/or those in the statistical error of the method) results for CSM, but neither can be accepted – i.e. is both below and above 0.50.

Tabl.IV.15. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Purshianin*

ISS indicator	Purshianin	
	amide	acid
GADI	0.75	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.48	0.53
ACFSC	1	1
Prediction	C	NC
C- Carcinogen, NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.15. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Purshianin*.

Tabl.IV.15. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN/Antares indicator	Purshianin	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.83
APSM	0.67	0.67
CSM	0.67	0.67
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.15. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxyl acid derivatives of *Purshianin*.

Tabl.IV.15. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN/ISSCAN-CGX indicator	Purshianin	
	amide	acid
GADI	0.80	0.79
SMKEV	0.79	0.78
APSM	1	1
CSM	0.65	0.64
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

Carcinogenic activity (***Table IV.15. 16***) was observed with a single dose of amide and carboxylic acid derivatives of *Purshianin* according to the *Carcinogenicity oral classification model* (IRFMN). The molecules reported[47] in APSM also determine the final prediction.

[47] Similarity: 0.74-6 by CAS: 303-34-4, CAS: 315-22-0, CAS: 18883-66-4, CAS: 54749-90-5; Similarity: 0.71 by CAS: 50-07-7

Tabl.IV.15. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Purshianin*

IRFMN indicator	Purshianin	
	amide	acid
GADI	0.73	0.72
SMKEV	0.76	0.74
APSM	0.50	0.50
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	C	C
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; C- -Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.15. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Purshianin* should not be administered orally.

Tabl.IV.15. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN indicator	Purshianin	
	amide	acid
GADI	0.64	0.63
SMKEV	0.76	0.74
APSM	0.07	0.07
CSM	2.65	2.60
MEPASM	0.11	0.11
MDRC	true	true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF (log form)	$(g/kg\text{-}day)^{-1}$	
	3.89	3.47
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	1.6	
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

3.1.15.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of the CAESAR toxicity method on amide and carboxyl acid derivatives of *Purshianin* highlights the lack of toxicity (***Table IV.3.15. 18***).

Tabl.IV.15. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Purshianin*

CAESAR indicator	Purshianin	
	amide	acid
SMKEV	0.75	0.88
APSM	0.77	0.77
CSM	1	1
MDRC	0.52	1
.CFSC	true	true
SMKEV	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG (Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Purshianin* did not report values for GADI and CSM. Molecular fragments close to *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.15. 19*** cannot be considered reliable.

Tabl.IV.15. 19 *PG toxicity of amide and carboxyl acid derivatives of Purshianin*

PG indicator	Purshianin	
	amide	acid
GADI	0	0
SMKEV	0.76	0.77
APSM	1	1
CSM	0	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Purshianin*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.15. 20***).

Tabl.IV.15. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN/CORAL indicator	Purshianin	
	amide	acid
GADI	0.28	0.41
SMKEV	0.69	0.68
APSM	0.31	0.33
CSM	1.34	1.96
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
	[mg/L]	
Prediction	**39.5**	**100.2**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Purshianin* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.15. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.15. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Purshianin*

CORAL indicator	Purshianin	
	amide	acid
GADI	0.76	0.76
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Toxity models with selective chemical activity

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Purshianin* (***Tab.IV.3.15. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.15. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN indicator	Purshianin	
	amide	acid
GADI	0.89	0.90
SMKEV	0.81	0.81
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.09	0.08
Active Antagonist:	0.03	0.03
Inactive:	**0.88**	**0.89**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Purshianin* did not report any deviations (***Tabl.IV.3.15. 23***) affecting the studied process.

Tabl.IV.15. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Purshianin*

NIC indicator	Purshianin	
	amide	acid
GADI	0.75	0.65
SMKEV	0.79	0.98
APSM	0.49	0.50
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.27	3.27
Prediction	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; nonA- non active		

c) Adipose tissue: blood model

Applying *Adipose tissue*: blood model for toxicity of amide and carboxyl acid derivatives of *Purshianin* we understand (***Tabl.IV.3.15. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.15. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Purshianin*

INERIS indicator	Purshianin	
	amide	acid
GADI	0	0
SMKEV	0.69	0.68
APSM	0.31	0.30
CSM	0.63	0.63
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.60
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.19	0.23
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.549	**1.702**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Purshianin* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.15. 25***).

Tabl.IV.15. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Purshianin*

QSARINS indicator	Purshianin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.81	0.81
APSM	0.09	0.09
CSM	0.07	0.10
MEPASM	0.15	0.15
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.33	0.36

Total half-life	[min]	
	130	**135**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Purshianin* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.15. 26***).

Tabl.IV.15. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Purshianin*

IRFMN/VERMEER indicator	Purshianin	
	amide	acid
GADI	0	0.86
SMKEV	0.75	0.75
APSM	1	1
CSM	0	1
ACFSC	1	1
Prediction	inA	A
A- active; inA- inactive		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.15.3.2***), carcinogenicity (***§IV.3.1.15.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.15.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus activity* could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Purshianin* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.15. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.15. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Purshianin*

IRFMN/CORAL indicator	Purshianin	
	amide	acid
GADI	0.85	0.85
SMKEV	0.83	0.84
APSM	0.25	0.25
CSM	0.50	0.36
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.15	-3.30
Prediction	[mg/kg]	
	1413	**1995**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of ***Cramer*** activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.15.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.15.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Purshianin* would be optimal for drugs taken orally to poison the cancer cell with *(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.15.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide $3924 \leq 10845 \leq 29968$, acid $2977 \leq 7382 \leq 18307$ and *Bioaccumulation factor* [conditional units] amide $0.85 \leq 13.8 \leq 224$ and acid form is $0.00 \leq 0.30 \leq 617$.

3.1.15.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7.***

3.1.15.6.1. Lipophilicity

Data from ***Tabl.IV.3.15. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.15. 28 *Lipophilicity of amide and carboxylic acid derivatives of Purshianin*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Purshianin						
amide	1.13	-3.15	-3.10	-2.65	-2.66	-2.09
acid	0.64	-2.50	-2.50	-2.24	-2.42	-1.80

3.1.15.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - *ESOL*, *Ali* and *SILICOS-IT* (***Tab.IV.3.15. 29***).

Tabl.IV.15. 29 *Water solubility of amide and carboxylic acid derivatives of Purshianin*

studied indicator	Purshianin	
	amide	acid
ESOL		
Log S	0.35	-0.06
Solubility, [mg/ml]	7.49e+02	2.89e+02
Class	hs	vs
Ali		
Log S	0.30	-0.25
Solubility, [mg/ml]	6.62e+02	1.86e+02
Class	hs	vs
SILICOS-IT		
Log S	2.54	2.76
Solubility, [mg/ml]	1.15e+05	1.91e+05
Class	s	s
vs- very soluble; hs - highly soluble; s - soluble		

3.1.15.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of Purshianin meets the pharmacokinetic requirements (***Table IV.3.15. 30***).

Tabl.IV.15. 30 *Pharmacokinetic indicators of amide and derivatives of Purshianin*

studied indicator	Purshianin	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes

inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-10.56	-10.10

3.1.15.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.15. 31***) containing amide and derivatives of *Purshianin*.

Tabl.IV.15. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Purshianin*

studied indicator	Purshianin	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
* - 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.15.6.5. Medical Chemistry

Data from ***Tabl.IV.3.2. 32*** confirm the drug safety of amide and derivatives of *Purshianin*.

Tabl.IV.15. 32 *Medical chemistry indicators for amide and derivatives of Purshianin*

studied indicator	Purshianin	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.35	5.42
*- 1 alert: michael_acceptor_1		

- ◊ -

3.1.16. (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide

Subjected to analysis potential pharmaceutical form for release within the cancer cell of *(R)-2-hydroxy-2-phenylacetamide*, comprising an amide and a carboxylic acid obtained by hydrolysis of the nitrile group of *Lithospermoside*. The process proceeds according to ***§IV.2.3.***

3.1.16.1. Druglikeness of the pharmaceutical form

In ***Tabl.IV.16. 1*** are listed values of *GPCR ligand*, *Ion channel modulator*, *Kinase inhibitor*, *Nuclear receptor ligand*, *Protease inhibitor* and *Enzyme inhibitor* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.16. 1 *Data for total druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Lithospermoside						
amide	0.43	0.13	0.13	0.30	0.10	0.69
acid	0.47	0.24	0.12	0.55	0.17	0.70

Data in ***Tabl.IV.16. 1*** show that the amides and carboxylic acids of *Lithospermoside* have pronounced overall drug activity *in vivo*.

3.1.16.2. Pharmacological and biological activity of oral active drugs

3.1.16.2.1. Lipinski's Rule, Ghose Filter and CMC-50-Like Rule

In ***Tabl.IV.16. 2*** shows the empirical values (without their dimensions) for *Lipinski's Rule*, *Ghose Filter* and *CMC-50-Like Rule* of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.16. 2 *Lipinski's Rule, Ghose Filter and CMC-50-Like Rule of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

	Lipinski's Rule				Ghose Filter				CMC-50-Like Rule			
	MW	logP	HBA	HBD	MW	logP	AMR	nAtom	MW	logP	AMR	nAtom
Lithospermoside												
amide	347	-3.2	10	7	347	-3.2	77	45	347	-3.2	77	45
acid	348	-2.5	10	7	348	-2.5	77	44	348	-2.5	77	44

The two molecular modified forms of *Lithospermoside* meet most requirements. All deviations in individual indicators confirm the preliminary conditionally accepted approximations in the methodology.

3.1.16.2.2. Veber Filter, MDDR-Like Rule and BBB Likeness

Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* are listed in ***Tabl.IV.16. 3.***

Tabl.IV.16. 3 *Data on Veber Filter, MDDR-Like Rule and BBB Likeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		Veber Filter		MDDR-Like Rule			BBB Likeness		
		TPSA	nRB	nRB	RC	nRingidB	MW	nAcidGroup	nHB
Lithospermoside									
	amide	183	4	4	2	21	347	0	17
	acid	177	4	4	2	21	348	1	17

There are no significant fluctuations in individual indicators. All "problematic" values correlate with the pre-entered deviations ***§III.3.3.3.1***.

3.1.16.2.3. QED

The analysis is performed according to ***§3.1.1.2.3***.

A. uwQED

Data for Unweighted Quantitative Estimate of Druglikeness of the tested compounds are given in ***Tabl.IV.16. 4.***

Tabl.IV.16. 4 *Unweighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

		uwQED								
		MW	AlogP	HBA	HBD	TPSA	nRB	sAlerts	nAromaRing	uwQED
Lithospermoside										
	amide	347	-3.3	10	7	183	4	1	0	0.20
	acid	348	-2.9	10	7	177	4	1	0	0.22

B. wQED

Tabl.IV.16. 5 presents the data from the calculations for a Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*.

Tabl.IV.16. 5 *Weighted Quantitative Estimate of Druglikeness of chemical molecules potentially possible to pass through the cancer cell membrane and release (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide*

									wQED
		MW	AlogP	HBA	HBD	TPSA	nRB	SAlerts	nAtomRing	wQED
Lithospermoside										
	amide	347	-3.3	10	7	183	4	1	0	0.29
	acid	348	-2.9	10	7	177	4	1	0	0.31

uwQED (***Tabl.IV.16. 5***) and wQED (***Tabl.IV.16. 5***) of a potential pharmaceutical form including amide and carboxylic acid, obtained by hydrolysis of the nitrile group of *Lithospermoside* meets the requirements for conservative treatment.

3.1.16.3. Non -laboratory and no clinical information on the chemical form

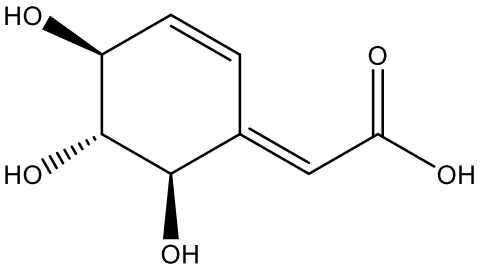
3.1.16.3.1. Receptor activity

In ***Tabl.IV.3.7. 6*** shows the bioactivity of amide and carboxylic acid derivatives of *Lithospermoside* to receptors (according to ***§III.3.3.4.1***).

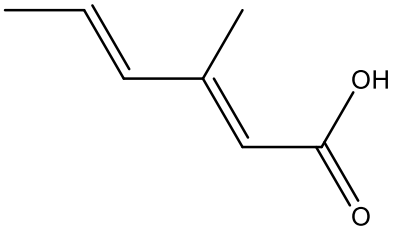
Tabl.IV.16. 6 *Receptor activity of amide and carboxyl derivatives of Lithospermoside*

indicator	Lithospermoside	
	amide	acid
AR		
ERa		
ERb	active *	active*
GR		
MR	-	-
PR		
RARa		active*
RARb		
RARr		
TRa		
TRb		
VDR		
*- agonist		

Data from ***Tabl.IV.3.7. 6*** show that the acid form exhibits *Retinoic Acid Receptor* (RARa) agonist activity. The active molecular form would inhibit the development of a number of tumors (oral cavity, stomach) and melanoma (Soprano & Soprano, 2002). This is probably due to the overlap of a fragment of *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* c *(2E,4E)-3-methylhexa-2,4-dienoic acid* (***Fig.IV.3. 16***).



(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid

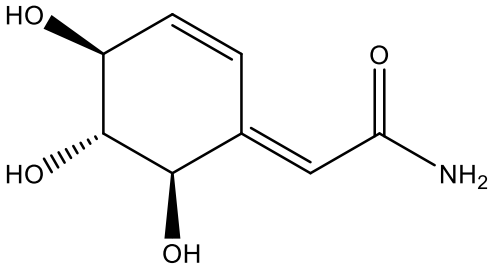


(2E,4E)-3-methylhexa-2,4-dienoic acid

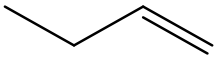
Fig.IV.3. 16 *Structural formulas of (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid u (2E,4E)-3-methylhexa-2,4-dienoic acid*

It is important to note that *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* is formed as a by-product after the passage of **HF(A)** across the cancer cell membrane ***(§IV.2.3. 1***). On the other hand, the amide and acid forms exhibit agonist activity to *Estrogen Receptor b* (ERb).

This property also leads to an increase in the proliferation of cancer cells in the breast (Zhou & Liu, 2020). We attribute it to the complexity in the construction of *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetic acid* (***Fig.IV.3. 16***) and *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* with *but-1-ene* chain (***Fig.IV.3. 17***).



(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide



but-1-ene

Fig.IV.3. 17 *Structural formulas of (E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide u* but-1-ene

Therefore, a synergistic effect will be manifested: as a primary, it could be an attack on cancer cells (Tsanov, H. & Tsanov, 2021) – further developed in goal No.2, and as a secondary - suppression of the development of solid tumors in the breast.

3.1.16.3.2. Mutagenicity

A. Stand -alone models

It is held respectively with ***§III.3.3.4.2***:

a) CAESAR

Data from ***Tabl.IV.3.16. 7*** do not explicitly illustrate the mutagenic activity of amide and carboxylic acid derivatives of the nitrile glycosides studied. We attribute it to the fact that the training set has insufficient data for *Lithospermoside*.

Tabl.IV.16. 7 *CAESAR mutagenicity of amide and carboxyl acid derivatives of Lithospermoside*

CAESAR indicator	Lithospermoside	
	amide	acid
GADI	0.74	0.75
SMKEV	0.82	0.83
APSM	0.67	0.67
CSM	0.67	0.67
MDRC	true	true
ACFSC	1	1
Prediction	NM	NM
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NM- non mutagenicity		

b) SarPy/IRFMN

In terms of mutagenicity, calculated using the *SarPy/IRFMN* methodology, amide and carboxyl acid derivatives of *Lithospermoside* did not show activity (***Table IV.3.16. 8***).

Tabl.IV.16. 8 *SarPy/IRFMN mutagenicity of amide and carboxyl acid derivatives of Lithospermoside*

SarPy/IRFMN indicator	Lithospermoside	
	amide	acid
GADI	0.63	0.63
SMKEV	0.82	0.83
APSM	0.34	0.35
CSM	0.67	0.67
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

c) ISS

Carboxyl acid derivative of *Lithospermoside* is non-mutagenic according to the ISS methodology (***Table IV.3.16. 9***). The amide derivative exhibits mutagenic activity[48] against the organism.

Tabl.IV.16. 9 *ISS mutagenicity of amide and carboxyl acid derivatives of Lithospermoside*

ISS indicator	Lithospermoside	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.47	0.54
ACFSC	1	1
Prediction	M	NM
M- mutagenicity; NM- non mutagenicity		

d) KNN/Read-Across

Amide and carboxyl acid derivatives of *Lithospermoside* show some deviation from the KNN/Read-Across method due to the incomplete training set. At the same time, the model is well adapted for such cases with algorithms that eliminate inaccuracies. Ultimately, all compounds tested were non-mutagenic.

Tabl.IV.16. 10 *KNN/Read-Across mutagenicity of amide and carboxyl acid derivatives of Lithospermoside*

KNN/Read-Across indicator	Lithospermoside	
	amide	acid
GADI	0.54	0.71
SMKEV	0.83	0.83
APSM	0.24	0.49
CSM	0.51	0.76
ACFSC	1	1
Prediction	NM	NM
NM- non mutagenicity		

[48] Similarity: 0.76-8 by CAS: 23246-96-0 (SA37 Pyrrolizidine Alkaloids), CAS: 303-34-4 (SA37 Pyrrolizidine Alkaloids) and CAS: 315-22-0 (SA37 Pyrrolizidine Alkaloids); Similarity: 0.71-4 by CAS: 18883-66-4 (SA21 Alkyl and aryl N-nitroso groups) and CAS: 2058-46-0 (SA10 alfa, beta unsaturated carbonyls | SA38 Alkenylbenzenes)

B. Consensus model

Data from ***Tabl.IV.3.16. 11*** confirmed the inertness of amide and carboxyl acid derivatives of *Lithospermoside*.

Tabl.IV.16. 11 *CONSENSUS model for mutagenicity of amide and carboxyl acid derivatives of Lithospermoside*

Consensus model mutagenicity indicator	Lithospermoside	
	amide	acid
numerical value	0.25	0.50

3.1.16.3.3. Carcinogenicity

A. Stand-alone models

a) CAESAR

Data on carcinogenicity activity, using the *CAESAR* methodology (***Tab.IV.3.16. 12***), for amide and carboxyl acid derivatives of *Lithospermoside* did not indicate the presence of carcinogenicity.

Tabl.IV.16. 12 *CAESAR carcinogenicity of amide and carboxyl acid derivatives of Lithospermoside*

CAESAR indicator	Lithospermoside	
	amide	acid
GADI	0.75	0.88
SMKEV	0.78	0.78
APSM	1	1
CSM	0.51	1
MDRC	true	true
ACFSC	1	1
MCAR	0.47	0.47
NMNC	1	1
Carcinogen	0.26	0.23
NON-Carcinogen	**0.74**	**0.77**
Prediction	NC	NC
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NC- NON-Carcinogen		

b) ISS

Data on carcinogenicity activity, using the *ISS* methodology (***Tab.IV.3.16. 13***), for amide and carboxylic acid derivatives of *Lithospermoside* did not indicate the presence of carcinogenicity.

Tabl.IV.16. 13 *ISS carcinogenicity of amide and carboxyl acid derivatives of Lithospermoside*

ISS indicator	Lithospermoside	
	amide	acid
GADI	0.74	0.76
SMKEV	0.80	0.80
APSM	1	1
CSM	0.74	0.54
ACFSC	1	1
Prediction	NC	NC
C- carcinogen; NC- NON-Carcinogen		

c) IRFMN/Antares

IRFMN/Antares does not give well-distinguishable results (***Tab.IV.3.16. 14***), susceptible to interpretation, for carcinogenicity of amide and carboxylic acid derivatives of *Lithospermoside*.

Tabl.IV.16. 14 *IRFMN/Antares carcinogenicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN/Antares indicator	Lithospermoside	
	amide	acid
GADI	0.74	0.75
SMKEV	0.83	0.84
APSM	0.66	0.66
CSM	0.66	0.66
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

d) IRFMN/ISSCAN-CGX

IRFMN/ISSCAN-CGX does not give well-distinguishable results (***Tab.IV.3.16. 15***), susceptible to interpretation, for carcinogenicity of amide and carboxylic acid derivatives of *Lithospermoside*.

Tabl.IV.16. 15 *IRFMN/ISSCAN-CGX carcinogenicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN/ISSCAN-CGX indicator	Lithospermoside	
	amide	acid
GADI	0.79	0.79
SMKEV	0.78	0.78
APSM	1	1
CSM	0.64	0.63
ACFSC	1	1
Prediction	PNC	PNC
PNC- possible non-carcinogenic		

B. Prolonged intake. Carcinogenicity oral Slope Factor model

a) Carcinogenicity oral classification model (IRFMN)

Fragments of the amide derivative of *Lithospermoside* coincide with those reported[49] in the training set in APSM, according to *Carcinogenicity oral classification model* (IRFMN) methodology (***Tabl.IV.16. 16***).

Tabl.IV.16. 16 *Data from Carcinogenicity oral classification model (IRFMN) of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN indicator	Lithospermoside	
	amide	acid
GADI	0.73	0.85
SMKEV	0.75	0.73
APSM	0.50	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	C	NC
C- carcinogen; NC- NON-Carcinogen		

b) Carcinogenicity oral Slope Factor model

In ***Tabl.IV.3.16. 17*** determines the concentrations above which oral amide and carboxyl acid derivatives of *Lithospermoside* should not be administered orally.

[49] Similarity: 0.75 by CAS: 303-34-4, CAS: 315-22-0, CAS: 18883-66-4 and CAS: 54749-90-5; Similarity: 0.70 by CAS: 50-07-7

Tabl.IV.16. 17 *Data for Carcinogenicity oral Slope Factor model (IRFMN) for carcinogenicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN indicator	Lithospermoside	
	amide	acid
GADI	0	0
SMKEV	0.75	0.73
APSM	0.07	0.10
CSM	3.56	2.86
MEPASM	0.11	0.11
MDRC	N-true	N-true
ACFSC	0.85	0.85
Predicted Oral Carcinogenicity SF for molecular forms	$(g/kg\text{-}day)^{-1}$	
	32.4	31.6
Presumed concentration of the active form inside the cancer cell	$(g/kg\text{-}day)^{-1}$	
	14.3	
n-true - does not cover		

3.1.16.3.4. Toxicity

A. Developmental Toxicity model

a) CAESAR

The application of the CAESAR toxicity method on amide and carboxyl acid derivatives of *Lithospermoside* highlights the lack of toxicity (***Tabl.IV.3.16. 18***).

Tabl.IV.16. 18 *CAESAR toxicity of amide and carboxyl acid derivatives of Lithospermoside*

CAESAR indicator	Lithospermoside	
	amide	acid
GADI	0.88	0.88
SMKEV	0.78	0.78
APSM	1	1
CSM	1	1
MDRC	true	true
ACFSC	1	1
Prediction	NT	NT
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NT- non-toxic		

b) PG(Reproductive Toxicity library)

PG (Reproductive Toxicity library) test for the toxicity of amide and carboxyl acid derivatives of *Lithospermoside* did not report values for GADI and CSM. Molecular fragments close to *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* have not been well studied and there are no clinical data on them. The data from ***Tabl.IV.3.16. 19*** cannot be considered reliable.

Tabl.IV.16. 19 *PG toxicity of amide and carboxyl acid derivatives of Lithospermoside*

PG indicator	Lithospermoside	
	amide	acid
GADI	0.61	0
SMKEV	0.76	0.76
APSM	0.49	1
CSM	0.50	0
ACFSC	1	1
Prediction	NT	NT
NT- non-toxic		

B. Models related to the development of the organism

a) Zebrafish embryo AC50

When *Zebrafish embryo AC50* was subjected to *IRFMN/CORAL* toxicity test on amide and carboxyl acid derivatives of *Lithospermoside*, no serious deviations from the generally accepted reference standards were observed (***Tab.IV.3.16. 20***).

Tabl.IV.16. 20 *Zebrafish embryo AC50 at IRFMN/CORAL toxicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN/CORAL indicator	Lithospermoside	
	amide	acid
GADI	0.27	0.40
SMKEV	0.69	0.67
APSM	0.31	0.31
CSM	1.37	1.77
MEPASM	0.54	0.54
MDRC	true	true
ACFSC	0.40	0.60
Prediction	[mg/L]	
	44.4	**112.6**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

b) Chromosomal aberration model

Like any biologically active substance, amide and carboxyl acid derivatives of *Lithospermoside* exhibit would lead to a burden on the chromosome set (***Tab.IV.3.16. 21***). Everything is determined by the concentration and time of treatment.

Tabl.IV.16. 21 *Chromosomal aberration model of amide and carboxyl acid derivatives of Lithospermoside*

CORAL indicator	Lithospermoside	
	amide	acid
GADI	0.76	0.75
SMKEV	0.79	0.79
APSM	1	1
CSM	1	1
ACFSC	0.85	0.85
Prediction	A	A
A- active		

C. Models related to the development of the organism

a) Aromatase activity model

The ability to inhibit or protonate hormones (whether as antagonists or agonists) and/or enzymatic processes is clearly expressed for biologically active substances, incl. and amide and carboxyl acid derivatives of *Lithospermoside* (***Tab.IV.3.16. 22***). Concentration and treatment time are crucial in accurately describing the process.

Tabl.IV.16. 22 *Aromatase activity model for toxicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN indicator	Lithospermoside	
	amide	acid
GADI	0.89	0.90
SMKEV	0.79	0.80
APSM	1	1
CSM	1	1
ACFSC	1	1
Active Agonist	0.12	0.12
Active Antagonist:	0.04	0.04
Inactive:	**0.84**	**0.84**
Prediction	inA	inA
inA- inactive		

b) p-Glycoprotein activity model

p-Glycoprotein activity model for analysis of amide and carboxyl acid derivatives of *Lithospermoside* did not report any deviations (***Tabl.IV.3.16. 23***) affecting the studied process.

Tabl.IV.16. 23 *p-Glycoprotein activity model for toxicity of amide and carboxyl acid derivatives of Lithospermoside*

NIC indicator	Lithospermoside	
	amide	acid
GADI	0.75	0.75
SMKEV	0.79	0.79
APSM	0.49	0.49
CSM	1	1
MDRC	true	true
ACFSC	1	1
Euclidean Distance from the central neuron:	2.42	3.94
Prediction	NA	NA
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set; NA- Non active		

c) Adipose tissue: blood model

Applying *Adipose tissue: blood model* for toxicity of amide and carboxyl acid derivatives of *Lithospermoside* we understand (***Tabl.IV.3.16. 24***) that the chemical equilibrium of possible reactions are in the direction of the product, i.e. when treated with the studied molecules, we will also have some accumulation in adipose tissue.

Tabl.IV.16. 24 *Toxicity at Adipose tissue: blood model amide and carboxyl acid derivatives of Lithospermoside*

INERIS indicator	Lithospermoside	
	amide	acid
GADI	0	0
SMKEV	0.68	0.68
APSM	0.32	0.31
CSM	0.63	0.63
MEPASM	0.50	0.50
MDRC	N-true	N-true
ACFSC	0.51	0.60
Prediction		
logK ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[log units]	
	0.192	0.237
K ($C_{HF(A,B)}$,$C_{adipose\ tissue}$)	[numerical units]	
	1.556	**1.726**
N-true - does not cover		

D. Total body elimination half-life

The studied amide and carboxyl acid derivatives of *Lithospermoside* have a retention time in the body, comparable to the main conservative drugs (***Tab.IV.3.16. 25***).

Tabl.IV.16. 25 *Total body elimination half-life model toxicity of amide and carboxyl acid derivatives of Lithospermoside*

QSARINS indicator	Lithospermoside	
	amide	acid
GADI	0.85	0.85
SMKEV	0.81	0.81
APSM	0.09	0.41
CSM	0.02	0.29
MEPASM	0.15	0.79
MDRC	true	true
ACFSC	1	1
Prediction		
LogHLt	[log units]	
	0.24	0.28
Total half-life	[min]	
	105	**115**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set;		

E. Micronucleus activity

a) in vitro

The hypothesis set in the methodical part that amide and carboxyl acid derivatives of *Lithospermoside* show activity for *Micronucleus* in *in vitro* was confirmed (***Tab.IV.3.16. 26***).

Tabl.IV.16. 26 *Micronucleus toxicity activity model – in vitro of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN/VERMEER indicator	Lithospermoside	
	amide	acid
GADI	0	0.86
SMKEV	0.74	0.74
APSM	1	1
CSM	0	1
ACFSC	1	1
Prediction	inA	A
inA- inactive, A- active		

The reported molecules from the training set coincide with those from those already analyzed: mutagenicity (***§IV.3.1.16.3.2***), carcinogenicity (***§IV.3.1.16.3.3***) and the previously analyzed toxicity methods (***§IV.3.1.16.3.4***).

b) in vivo

The in vivo toxicity analysis of *Micronucleus activity* could not be performed due to the lack of experimental empirical information on glycosidamides and glycosacids in the training set.

F. NOAEL

The amide and carboxylic acid derivatives of *Lithospermoside* can be treated as a drug even at doses above those generally accepted by good medical practice (***Table IV.3.16. 27***). They are relatively safe in terms of the *NOAEL* toxicity model.

Tabl.IV.16. 27 *NOAEL methodology for toxicity of amide and carboxyl acid derivatives of Lithospermoside*

IRFMN/VERMEER indicator	Lithospermoside	
	amide	acid
GADI	0.85	0.85
SMKEV	0.84	0.86
APSM	0.25	0.25
CSM	0.28	0.19
MEPASM	0.38	0.38
MDRC	true	true
ACFSC	0.85	0.85
Prediction	[-log(mg/kg)]	
	-3.37	-3.52
Prediction	[mg/kg]	
	2344	**3311**
true- descriptors for this compound have values inside the descriptor range of the compounds of the training set		

G. Cramer classification

The toxicity analysis of *Cramer* activity could not be performed due to the lack of experimental empirical information on gl*ycosidamides and gly*cosacids in the training set.

3.1.16.4. Evaluation of the results

After a comparative analysis of the results (***§IV.3.1.16.1, -2*** and ***-3***) we assume that amide and carboxyl acid derivatives of *Lithospermoside* would be optimal for drugs taken orally to poison the cancer cell with *(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide* as performed in ***§IV.2*** second objective of the study.

3.1.16.5. Conclusion from the part

Application of the methodological scheme of ***§III.3.3.6*** for a potential pharmacological form (***§IV.3.1.16***), proves maximum coverage of the requirements for oral medicinal products. Toxicity did not deviate from the rules and the values were respectively: *Oral rat LD50* [mg/kg] for amide 5016 ≤ 13956 ≤ 38832, acid 4015 ≤ 10006 ≤ 24938 and *Bioaccumulation factor* [conditional units] amide 0.86 ≤ 13.9 ≤ 228 and for acid are 0.00 ≤ 0.25 ≤ 567.

3.1.16.6. Checking conclusion of the part

Conducted according to the methodological scheme ***§III.3.3.7***.

3.1.16.6.1. Lipophilicity

Data from ***Tabl.IV.3.16. 28*** that the test compounds are rather hydrophilic and will be separated from the aqueous medium of the stomach in the blood and will not reach, in sufficient concentration, the intestine.

Tabl.IV.16. 28 *Lipophilicity of amide and carboxylic acid derivatives of Lithospermoside*

	Log $P_{o/w}$					
	iLOGP	XLOGP3	WLOGP	MLOGP	SILICOS-IT	Consensus
Lithospermoside						
amide	0.67	-4.13	-4.13	-3.42	-3.54	-2.91
acid	0.61	-3.48	-3.53	-3.01	-3.30	-2.54

3.1.16.6.2. Water Solubility

The tested compounds have very good solubility in water, confirmed by the three applied methodologies - ESOL, Ali and SILICOS-IT (***Tab.IV.3.16. 29***).

Tabl.IV.16. 29 *Water solubility of amide and carboxylic acid derivatives of Lithospermoside*

studied indicator	Lithospermoside	
	amide	acid
ESOL		
Log S	0.87	0.46
Solubility, [mg/ml]	2.59e+03	9.97e+02
Class	hs	hs
Ali		
Log S	0.89	0.34
Solubility, [mg/ml]	2.71e+03	7.62e+02
Class	hs	hs
SILICOS-IT		
Log S	3.36	3.58
Solubility, [mg/ml]	7.92e+05	1.31e+06
Class	s	s
hs – highly soluble; s - soluble		

3.1.16.6.3. Pharmacokinetics

A medicinal product based on amide and carboxylic acid derivatives of *Lithospermoside* meets the pharmacokinetic requirements (***Table IV.3.16. 30***).

Tabl.IV.16. 30 *Pharmacokinetic indicators of amide and derivatives of Lithospermoside*

studied indicator	Lithospermoside	
	amide	acid
GI absorption	low	low
BBB permeant	no	no
P-gp substrate	Yes	Yes
inhibitors		
CYP1A2	no	no
CYP2C19	no	no
CYP2C9	no	no
CYP2D6	no	no
CYP3A4	no	no
Log K_p		
skin permeation, [cm/s]	-11.35	-10.90

3.1.16.6.4. Druglikeness

Studies have shown that druglike determinants confirm the drug safety of a product (***Tabl.IV.3.16. 31***) containing amide and derivatives of *Lithospermoside*.

Tabl.IV.16. 31 *Muegge activity and Bioavailability Score of amide and derivatives of Lithospermoside*

studied indicator	Lithospermoside	
	amide	acid
Muegge	No*	No*
Bioavailability Score	0.55	0.11
*- 3 violations: XLOGP3<-2, TPSA>150, H-don>5		

3.1.16.6.5. Medical Chemistry

Data from ***Tabl.IV.3.16. 32*** confirm the drug safety of amide and derivatives of *Lithospermoside*.

Tabl.IV.16. 32 *Medical chemistry indicators for amide and derivatives of Lithospermoside*

studied indicator	Lithospermoside	
	amide	acid
PAINS, [number of alerts]	0	0
Brenk, [number of alerts]	1*	1*
Leadlikeness	Yes	Yes
Synthetic accessibility	5.49	5.55
*- 1 alert: michael_acceptor_1		

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V. SYNTHESIS OF RESULTS

The overall view of the hypothesis, the presented evidence, the methodology, the analysis, the interpretations and the tests represent the synthesis of the results.

In ***Tabl.VI. 1*** the possible applications of the proposed molecular forms in the treatment of oncological diseases are summarized.

Tabl.VI. 1 *Optimal natural precursors for obtaining an active anti-tumor molecular form released inside a cancer cell and causing its toxicity*

	active anti-cancer molecular form	**natural precursor**
treatment of cancer of the breast		
	(R)-2-hydroxy-2-(4-hydroxyphenyl)acetamide	Dhurrin, Taxiphyllin
	(S)-1-hydroxycyclopent-2-ene-1-carboxamide	Deidaclin, Tetraphyllin A
	(1S,4S)-1,4-dihydroxycyclopent-2-ene-1-carboxamide	Tetraphyllin B, Volkenin, Taraktophyllin
	(1R,4R)-1,4,5-trihydroxycyclopent-2-ene-1-carboxamide	Gynocardin
treatment of cancer of the breast, skin, oral cavity, melanoma, stomach		
	(2Z,4E)-4-(2-amino-1-hydroxy-2-oxoethylidene)hex-2-enedioic acid	Triglochinin
	(Z)-2-((4S,6R)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide	Menisdaurin
	(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide	Griffonin
	(Z)-2-((4R,5R,6S)-5,6-dihydroxy-4-methoxycyclohex-2-en-1-ylidene)acetamide	Bauhinin
	(E)-2-((4R,6S)-4,6-dihydroxycyclohex-2-en-1-ylidene)acetamide	Purshianin
	(E)-2-((4S,5R,6R)-4,5,6-trihydroxycyclohex-2-en-1-ylidene)acetamide	Lithospermoside
general anti-cancer		
	(R)-2-hydroxy-2-phenylacetamide	Prunasin, Sambunigrin
	(R)-2-hydroxy-2-(3-hydroxypheny3l)acetamide	Zierin
	2-hydroxy-2-methylpropanamide	Linamarin
	(S)-2-hydroxy-2-methylbutanamide	Lotaustralin
	2-hydroxy-3-methylbut-2-enamide	Acacipetalin
	(R)-2-hydroxy-3-methylbutanamide	Epiheterodendrin

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VI. CONCLUSIONS

1. On the first goal

Our legacy of the *Hunza people* and the knowledge from tens-of-thousands of scientists who created modern synthesis and biochemistry make the production of nitrile amide into a routine (especially with nitrile hydratase). Thus, humanity holds in its hands a huge medicinal resource that can provide treatment for diseases of all parts of conservative medicine (including all listed in ***Section 1.1***.).

The hydrolyzed to amide/carboxylic acid nitrile/cyanide carbohydrates will occupy one of the fundamental steps of countless future clinical practices. This is the purpose of our modest research!

Other substances in these groups with pronounced biological activity (including anti-tumor) are the hydrolyzed nitrile groups of ***Linamarin***, ***(R) -Lotaustralin***, ***S-Sambunigrin***, etc., to their amide/carboxylic acid.

2. The second goal

1) The amide derivatives of nitrile glycosides are potential chemical compounds with anti-cancer activity;

2) the cancer cell seeks to shift the hydrolysis of these derivatives in a direction that would not pass through its cell membrane;

3) the amide-carboxyl derivatives of nitrile glycosides can deliver extremely toxic compounds inside the tumor cell itself and thus block and / or permanently damage its normal physiology;

4) the use of these compounds in oncology could turn cancer from a lethal to a chronic disease (such as diabetes). The cause and conditions of the disease are not eliminated, but the number of cancer cells could be kept low for a long time (even a lifetime).

3. On the third goal

1) Amides resulting from the hydrolysis of nitrile glycosides would be able to cross the cell membrane of a cancer cell and thus cause its cellular response;

2) the pharmaceutical form must represent the exact amide/carboxylic acid ratio for the corresponding active anticancer cell form;

3) clinical concentrations are more than 7 times higher than those of nitrile glycosides due to their reduced toxicity;

4) no significant deviations are observed, on a theoretical level, in the complex use of several pharmaceutical forms together and/or sequentially.

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VII. CLINICAL CONTROL

1. Correlation of bio constants

The bio constants of the human organism should not be accepted as a dogma, should be abolished, but should be used as benchmarks of the norm. Deviation from them should not necessarily be considered pathology. The human body adapts extremely well to the environment and strives to respond adequately to each stimulus.

Especially when performing chemotherapy on cancer patients, it is necessary to monitor the overall reference picture of the patient. It is good to avoid interpreting individual deviations from the physiologically healthy organism and to direct the treatment of cancer in the direction of "suppression" of individual symptoms.

In Tabl.VII.1. the control forms that the clinician must comply with before and during chemotherapy are indicated. These are reference correlations that would directly affect the release of the active anticancer molecular form (***Tabl.IV.2. 6***) within the cancer cell.

A. **VOLUME OF BLOOD**: it is directly related to the fluid ratio, and secondarily to the water content in the body - hence the change in a number of physicochemical parameters

When the total blood volume increases, it is important for the treating physician to rule out diagnoses:

- chronic leucosis;
- uremia (due to the change in nitrogen balance, which will prevent the transport of amide derivatives in the body) - is often accompanied by hyperkalemia and hyperchloremia.

When the total blood volume decreases, it is necessary to exclude the diagnoses:

- acidosis - increased water content is also reported;
- tubular acidosis - IMPORTANT: do not rush with tenal tubular acidosis - we also have hypophosphatemia.

B. **HEMATOCRIT**:

In case of a decrease in the value of the hematocrit in the blood, it is obligatory for the attending physician to reject the diagnoses:

- spherocytic anemia - at the beginning of treatment with amide / carboxyl derivative of nitrile glycosides there is a **REAL POSSIBILITY FOR CRISIS** !;
- This condition is often an indicator of brain metastases.

C. NATREMIA:

Particular attention should be paid to cases of hyponatremia. Here the attending physician needs to comply with diagnoses such as:

- cystic fibrosis (CP) / mucoviscidose/- in these cases treatment should begin with a very low concentration of the dosage form;
- this condition is often an indicator of lung cancer.

D. KALEMIA:

To some extent, hyperkalemia has a synergistic effect on the action of the studied dosage forms. It is good for your doctor to maintain higher blood potassium reference values. In cases where this is difficult, two circumstances must be taken into account:

- to reject Cushing's syndrome, by control test and for hyperchloremia - gives both increase and decrease;
- is often observed together with hypophosphatemia. If necessary, to introduce phosphorus preparations into the body.

E. CHLORAEMIA:

Hypochloraemia alters the ionic and electrostatic activity of both amides and carboxylic acids - especially when they are in low concentrations in the blood. It is good to consider treatment with:

- presence of liver cirrhosis - the analysis should be done at least 4 hours after glucose infusion and diuretics taken. The results should be differentiated from Hepato-renal syndrome.

F. CALCEMIA:

Hypercalcemia can suppress the spread of the drug form. Treatment should be resected and any comorbidities considered:

- extensive metastases;
- osteorenal sarcoma;
- breast carcinoma with bone metastases during treatment with ANDROGENS and ESTRONES;
- sarcoidosis.

G. SIDERINEMIA:

Hyposiderinemia could delay the detection of a more acidic environment around the cancer cell and from there slow down the action of the drug under study. The clinician should be aware that this is often accompanied by:

- ballast leukemia;
- carcinomas;
- uterine fibroids;
- myelosis and lymphadenosis (in terminal stage);
- erythraemia vera (in terminal stage).

Correction of iron in the blood is one of the most important factors in treatment with these doses.

Tabl. VII. 1 *Correlation data of microcomponents in human blood that affect the digestibility and activity of amides and carboxylic acids derivatives of natural nitrile glycosides*

control form	raise		reduction	
	indicator	indicator control	indicator	indicator control
volume of blood	chronic leucosis		acidosis	increased water content is also reported
			tubular acidosis*	hypophosphatemia
	uremia⁂	hyperkalemia		
		hyperchloremia		
hematocrit			spherocytic anemia☼	
			indicator of brain metastases	
natremia▲			mucoviscidose⁂	
			indicator of lung cancer	
kalemia			to reject Cushing's syndrome◊	
			often seen with hypophosphatemia	
chloraemia			indicator and of liver cirrhosis§	
calcemia	extensive metastases			
	osteorenal sarcoma			
	breast carcinoma with bone metastases during treatment with ANDROGENS and ESTRONES			
	sarcoidosis			
siderinemia			ballast leukemia	
			carcinomas	
			uterine fibroids	
			myelosis and lymphadenosis (in terminal stage)	
			erythraemia vera (in terminal stage)	
total iron-binding capacity	bone marrow hypoplasia		hemosiderosis	
			malignant tumors	
cupremia	malignant tumors	increase in iodine in the blood		
	malignant melanoma			
	hemochromatosis			
	malignant hemopathy (T-leucosis)	increase of sulfates in the blood		

	meligenic lymphomas		
	treatment with estrogen		
	cirrhosis of the liver	reduction of iodine in the blood	
Zinc content in the blood	in the serum		
	erythraemia vera	bone marrow hypoplasia	
	atrophic cirrhosis of the liver	lymphadenosis	
	acute leucosis	myelosis	
	in erythrocytes		
	bone marrow hypoplasia	myelosis	
	erythraemia vera	lifadenosis	
*- IMPORTANT: do not rush with tenal tubular acidosis - we also have hypophosphatemia ⁑- due to a change in the nitrogen balance ☼- **REAL POSSIBILITY FOR CRISIS !** ⁂- MANDATORY- the treatment should begin with a very low concentration of the dosage form ▲- to rule out myxedema as a concomitant disease ◊- control test and for hyperchloremia - gives both increase and decrease §- the analysis should be performed at least 4 hours after glucose infusion and diuretics taken. The results should be differentiated from Hepato-renal syndrome			

H. TOTAL IRON-BINDING CAPACITY

In case of increased content of total iron-binding capacity, it is necessary for the clinician to take into account the possible presence of:

- bone marrow hypoplasia,

and at reduced content with:

- hemosiderosis;
- malignant tumors.

I. CUPREMIA:

Hypercupressia would significantly increase the need for a higher drug dose. She herself is also an instructor for:

- malignant tumors - as an control sample can be used and the increase in iodine in the blood; - malignant melanoma;
- hemochromatosis;
- malignant hemopathy (T-leukemia) - as an control sample can be used and the increase of sulfates in the blood;
- meligenic lymphomas;
- treatment with estrogen;
- cirrhosis of the liver - as a control sample can be used and the reduction of iodine in the blood.

J. ZINC CONTENT IN THE BLOOD

The content of zinc in the blood determines its extremely complex role in cancer. Its compounds are both inhibitors and promoters. It can displace a number of metals from organometallic biologically active substances, but at the same time its coordination compounds in an in vivo environment are volatile.

We recommend a mandatory blood test for zinc in the blood before starting chemotherapy according to the studied experimental methodology. The following reference deviations must be taken into account:

a. in the serum:

increased serum zinc concentration:

- erythraemia vera;
- atrophic cirrhosis of the liver;
- acute leukemia.

decreased serum zinc concentration:

- bone marrow hypoplasia;
- lymphadenosis;
- myelosis.

b. in erythrocytes:

increased concentration of zinc in erythrocytes:

- bone marrow hypoplasia;
- erythraemia vera.

decreased concentration of zinc in erythrocytes:

- myelosis;
- lifadenosis.

2. Chemoprevention and Homeopathy

The proposed methodological program for conservative treatment of oncological diseases does not contradict the good medical practices for chemotherapy. In order to improve the general condition of patients, chemoprevention (Lele, 2021) and/or homeopathy could be applied, but not at the expense of a varied diet, incl. table salt, water, culinary acidifiers and fats. Alternative medicine should only be used to treat individual symptoms, not syndromes.

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AUTHOR'S NOTES

With the present scientific work we have tried to present in a more generalized form our long-term theoretical research. We tried to draw every value, every dependence and every conclusion precisely, in a form that is not subject to any personal view and/or to be enslaved to a generally "accepted" opinion.

Natural nitrile glycosides would not cross the tumor cell membrane. They decompose to HCN-acid, phenyl methanol and carbohydrate. They do NOT have antitumor activity due to their inability to reach the target unchanged. These compounds, in their natural form, are extremely toxic to the human body. Applying them is not a cure, even at higher concentrations they do more harm than good. Theoretically, we have derived dozens of their modified forms, but their amides and carboxylic acids are the most promising for their introduction in conservative oncology. The fact is that the tumor cell itself is trying to counteract in a way that is quite safe for it.

The knowledge that humanity has gained from the millennial battle between it and tumors, combined with the development of mathematics, statistical and quantum molecular thermodynamics, molecular topology and geometry, clinical oncology, pathophysiology, etc., with the unequivocal contribution of thousands of scientists, we tried to we present this thesis as a sentence and the most modest way to try to confirm and prove it.

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[xli] European Bioinformatics Institute - https://www.ebi.ac.uk
[xlii] Open Biological and Biomedical Ontologies (OBO) Foundry - http://obofoundry.org
[xliii] DRUGBANK ONLINE: https://go.drugbank.com
[xliv] U.S. Department of Agriculture – FoodData: https://fdc.nal.usda.gov
[xlv] Ready-made algorithms from the software product are used: Molecular Networks: www.molecular-networks.com/moses
[xlvi] version 1.1.1 applies. - developers: Wei Shi (njushiwei@nju.edu.cn), Haoyue Tan, Qinchang Chen, Hongxia Yu
[xlvii] Bruce Ames (1928) was an American professor of biochemistry and molecular biology at the University of California, Berkeley, and supervisor at the Children's Hospital and Research Institute in Auckland (CHORI). He works in the field of mutagenicity and has developed authorial methodologies for its evaluation, based on his study of different strains of the bacterium Salmonella. Winner of dozens of awards.
[xlviii] EU-funded project ANTARES - https://antaresproject.eu/
[xlix] Carcinogenicity Genotoxicity eXperience CGX

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