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### RESEARCH ARTICLE

## SYNTHESIS, CHARACTERIZATION, ANTIBACTERIAL TEST AND MOLECULAR DOCKING OF SOME AMIDE DERIVATIVES

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### Abstract

The amide group is an organic group synthesized by reaction between an amine and carboxylic acid and is known to have many biological activities like antibacterial, antifungal, anti-inflammatory, antitumor and many other activities. The traditional and easy method to synthesize amides by first convert carboxylic acid to acyl chloride and then the acyl chloride react with amine to give the amide. The goal is to synthesize new amide derivatives by one pot reaction between two drugs one is amine (bromohexine which act as mucolytic) And the other drug is carboxylic acid like NSAIDs (diclofenac, naproxen, indomethacin and mefenamic acid) using thionyl chloride and triethylamine as catalytic agents and stirring for 1 hr. The result products will be characterized by physical examination, melting point and IR spectroscopy. As mentioned the amide derivatives have many biological activities so the antibacterial activity will be tested on some bacterial species 2 g+ve (staph.aureus and streptococcus) and 2 g-ve (E.coli and Klebsiella). Finally the docking study will be done on the synthesized derivatives on the proposed targets to see the inhibition score for each derivatives and compare it with the reference inhibitor and with antibacterial test.

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### Introduction:-

Amides are important chemical class present in many of biologically active compounds and they are wide spread structural units in nature because it found as the core linkage in natural proteins and peptides, also found in polymers, agrochemicals and pharmaceutical compounds. <sup>[1,2]</sup> Derivatives of amides where contain large spectrum of biological actions. These are used in treatment of convulsion, pain, tuberculosis, inflammation, tumor, insects, fungal and bacterial infections. <sup>[3]</sup> Amide group participate in the binding interaction of the drug with its target because amide group act as hydrogen bond donor and acceptor in hydrogen bonding interaction, so it is important for medicinal chemists. <sup>[4]</sup> The usual way for the preparation of amide products involve reaction between a carboxylic acid and an amine compound by first converting carboxyl group of acid into acyl chloride and then reacting the resulted product with the amine compound give the corresponding amide with the expulsion of water molecule. <sup>[5,6]</sup> The aim of this work is to synthesize new amide products by direct reaction between an amine drug (bromohexine) with carboxyl drugs like some of the non-steroidal anti-inflammatory drugs {NSAID} (indomethacin, diclofenac, mefenamic acid and naproxen). These drugs have activities like; anti-inflammatory, analgesic and antipyretic. <sup>[7,8]</sup> NSAID act by inhibition of enzyme called cyclooxygenase (COX), this enzyme have two isoform (COX 1 and 2) and it's responsible for the synthesis of a group of lipids with hormone-like actions

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called prostaglandins.<sup>[9,10]</sup> NSAID used in the treatment of diseases like rheumatoid arthritis, osteoarthritis, gouty arthritis and also headache.<sup>[11,12]</sup> Bromohexine is a weak base is a synthetic benzyl amine derivatives of vasicine.<sup>[13]</sup> Bromohexine is mucolytic act by reducing the viscosity of the septum and so will enhance lung ventilation and expectoration.<sup>[14]</sup> Nowadays used asco-therapy in the treatment of COVID19, COVID 19 virus mainly relies on the trans membrane protease serine 2(TMPS2) to enter the cell.<sup>[15,16]</sup> bromohexine was found to be interfere with this enzyme (TMPS2) so it has another benefit beside mucolytic and expectorant effect by interfering with the mechanism by which the virus enter the cell and cause cell injury.<sup>[17-19]</sup>

#### **Experimental:**

##### **Materials and Methods:-**

All the used solvents and solid were of pure type the suppliers were (alphchem, India.thomas baker, india). Bromohexine, mefenamic acid, diclofenac, indomethacin and naproxen was supplied by SDI company for drug industry in Samarra/ Iraq . Melting points were determined by capillary method on Bamstead / Electro-thermal 9100 an Electric melting point apparatus (England). The identification of compounds was done using a FTIR spectrum were recorded on a FTIR-spectrophotometer FTIR-6100 Type A as KBr disks.

##### **General procedure for the synthesis of amide derivatives (1a-d);**

To a solution of (1mmol) amine in (5-10ml) of dichloromethane add (3mmol) of triethylamine then add (1mmol) of the acid followed by (1mmol) of thionyl chloride (SOCl<sub>2</sub>) at room temperature, close the container tightly and stir the mixture at room temperature for one hour.To achieve the resulted product evaporate the solvent. The resulting material dissolved in dichloromethane and treated first with (1 N) HCl and then with (1N) NaOH. The organic phase was dried with (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to dryness to afford the corresponding carboxylic amide.<sup>[20]</sup>

##### **Antimicrobial activity assessment:**

The newly synthesized amide compounds (1a-d) were tested for their In vitro antimicrobial action against some Gram positive bacterial isolates (Staphylococcus aureus, streptococcus aeruginosa) and Gram negative bacteria (Escherichia coli, klebsiellapneumoniae).Dimethyl sulfoxide (DMSO) used to prepare the tested derivatives at(100mg/ml) concentrations. Disk diffusion method was utilized to define the initial activity of the microorganisms. The disks with a diameter of 6.25mm were perforated from Whatman no. 1 filter papers. The batch discs were distributed to each screw-covered bottle and made sterile by dry heat at (140°C) for one hour. Wells perforated in agar medium seeded with fresh and filled with 100µl of each concentration. The control that is used in this test was DMSO. The incubation was performed at (37°C) for one day. Erythromycin, gentamycin, sulfamethaxazole and trimethprim was used as a standard drugs in the current test.The diameter of the observed inhibition region were used to assess the antimicrobial activity of the tested derivatives.<sup>[21,22]</sup>

##### **Molecular docking:**

The molecular docking was made using autodock tool to check the binding of the derivatives with the proposed target and compare the result with the standard antibacterial and the in vitro antimicrobial test. The binding energy (Kcal/mol) for the tested compound was measured by autodock and the compound the lowest binding energy is the preferred compound.<sup>[23,24]</sup>

##### **Results and Discussion:-**

The synthetic pathway for the newly synthesized amide derivatives (1a-d) described in scheme 1.

**Scheme 1:-** synthesis of amide derivatives.  $\text{SOCl}_2$ (thionyl chloride),  $\text{Et}_3\text{N}$ (tri ethyl amine),  $\text{DCM}$ (di chloromethane).

**N-(2,4-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-(6-methoxynaphthalen-2-yl)propanamide (1a)** : brown powder ( 65% yield ), m.wt(588) , m.p (85-88) , IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 472 (C-Br), 1458 ( C-N ), 1558 (aromatic C=C), 1670 (amide C=O ), 3433 (amide N-H).<sup>[25]</sup>

**N-(2,4-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-((2,6-dichlorophenyl)amino)phenylacetamide (1b)** : reddish brown powder ( 85% yield ), m.wt (654) , m.p (110-113) , IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 453 (C-Br), 559 (C-Cl), 1452 ( C-N ), 1504 (aromatic C=C), 1575 (amide C=O ), 3261 ( amine N-H), 3386 (amide N-H).

**N-(2,4-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)-2-((2,3-dimethylphenyl)amino)benzamide (1c)**: sticky yellow material ( 40% yield ), m.wt (599) , IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 501 (C-Br), 1450 ( C-N ), 1508 (aromatic C=C), 1649 (amide C=O ), 3311 (amine N-H), 3350 (amide N-H).

**2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-(2,4-dibromo-6-((cyclohexyl(methyl)amino)methyl)phenyl)acetamide (1d)**: : dark brown powder (80% yield), m.wt (715.9) , m.p (89-92) , IR (KBr)  $\nu$  ( $\text{cm}^{-1}$ ): 511 (C-Br), 551 (C-Cl), 1454 ( C-N ), 1481 (aromatic C=C), 1552 ( indomethacin amide C=O), 1575 (amide C=O ), 3440 (amide N-H).

**Table 1:-** Antimicrobial test results.

Pathogenic Bacteria	Inhibition zone diameter (mm)																				
	1d				1b				1c				1a				CON +				
	1	2	3	4	1	2	3	4	1		3	4	1	2	3	4	1 S M T	2 T R M	3 E R T	4 A M P	5 G N T
Staph.	2	-	-	-	14	14	12	10	11	10	11	12	12	16	14	14	0	10	10		
Strep.	-	-	-	-	10	9	9	7	8	8	8	8	8	8	8	-	-	16	10	-	
E.coli	-	-	-	-	-	-	-	-	13	12	12	10	-	-	-	-	14	16	-	-	10
Klebs.	-	-	-	-	-	9	9	9	8	8	8	8	8	8	8	16	14	12	-	16	

**Note;** SMT (sulfamethaxazole), TRM (trimethoprim), ERT (erythromycin), AMP (ampicillin), GNT (gentamicin). (-) mean no inhibition zone. (1,2,3,4) refer to concentrations of 1000,500,250,125 Mcg/ml respectively.

**Table 2:-** Autodock results on dihydrofolatereductase target (PBD ID: 2w9g).

Compound	Binding score
trimethoprim	-7.9
1a	-9.5
1b	-9.5
1c	-9.9
1d	-8.2

**Table 3:-** Autodock results on dihydropteroate synthase target (PBD ID: 1ad4).

Compound	Binding score
sulfamethaxazole	-6
1a	-6.6
1b	-6
1c	-6.3
1d	-5.5

Chemically ; the use of catalytic agents  $\text{SOCl}_2$  and  $\text{Et}_3\text{N}$  making one pot synthesis of amide possible this achieved by first reaction of  $\text{SOCl}_2$  with carboxylic acid to produce acid chloride followed by reaction with amine to give the corresponding amide derivative.

The antimicrobial test (table 1) reveals that these derivatives have some antibacterial activity (1a,1b) have better activity on staph. Than all of the tested drugs. (1a,1b,1c) have better results on strept. Than trimethoprim, sulfamethaxazole and gentamicin. (1c) have some activity on E.coli better than ampicillin and erythromycin. (1a,1b,1c) have some activity on klebsila and better than ampicillin .

The proposed mechanism of antibacterial action according to the structure of the products is either inhibition of dihydrofolatereductase enzyme like trimethoprim or inhibition of dihydropteroate synthase like sulfamethaxazole . the results of docking (table2 and 3) show that all of the derivatives (except 1d on 1ad4 enzyme) have better inhibition scores than the corresponding drugs and in all the lowest activity between the derivatives was for (1d).the results is not completely fit with the antibacterial test because the proposed mechanism may not be that exact mechanism by with the derivatives act as antibacterial.

### Conclusion:-

1. The synthesis of the designed compounds has been successfully achieved.
2. Characterization and identification of the synthesized compounds were confirmed by determination of physical properties (melting point and description).
3. The antibacterial assessment of the final products indicates that the amide group add antibacterial activity on the reacted drug one is mucolytic the other is NSAID.
4. The docking study show that (according to the proposed mechanism of action) all of the derivatives (except 1d on 1ad4 enzyme) have better inhibition scores than the corresponding drugs and in all the lowest activity between the derivatives was for (1d).

### Further study:

For further work check the HNMR and CNMR spectrum on the prepared compounds because such measures are not found in Iraq. Also check other activities for amide compounds like ; anti-inflammatory, anti-tumor , anti-fungal and other activities.

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