Accepted Manuscript

Synthesis and *in vitro* characterization of novel fluorinated derivatives of the TSPO 18 kDa ligand SSR180575

Annelaure Damont, Frank Marguet, Frédéric Puech, Frédéric Dollé

PII: S0223-5234(15)30165-3

DOI: 10.1016/j.ejmech.2015.07.033

Reference: EJMECH 8017

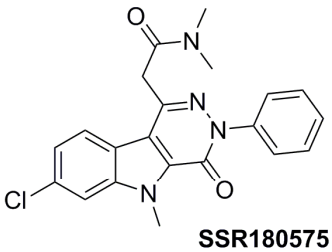
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 20 March 2015

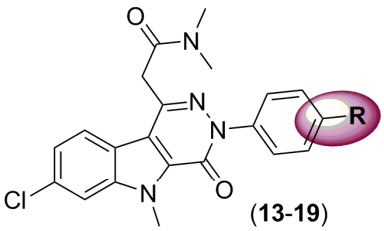
Revised Date: 15 July 2015

Accepted Date: 16 July 2015

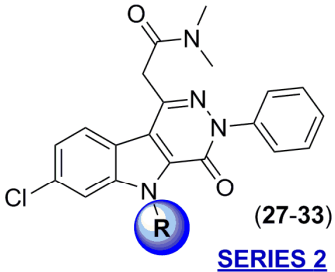
Please cite this article as: A. Damont, F. Marguet, F. Puech, F. Dollé, Synthesis and *in vitro* characterization of novel fluorinated derivatives of the TSPO 18 kDa ligand SSR180575, *European Journal of Medicinal Chemistry* (2015), doi: 10.1016/j.ejmech.2015.07.033.



SSR180575 (1)
Ki TSPO = 0.83 nM
Ki CBR > 1.0 μM
$\log D_{7.4}$ = 2.97



(**13-19**)
SERIES 1



(**27-33**)
SERIES 2

New fluorinated analogues

Synthesis and *in vitro* characterization of novel fluorinated derivatives of the TSPO 18 kDa ligand SSR180575

Annelaure Damont [a,b], Frank Marguet [c], Frédéric Puech [c], Frédéric Dollé [a,b]

[a] *CEA, I2BM, Service Hospitalier Frédéric Joliot, Orsay, France.*

[b] *Inserm / CEA / Université Paris Sud, UMR 1023 – ERL 9218 CNRS, IMIV, Orsay, France.*

[c] *Exploratory Unit, Sanofi, Chilly-Mazarin, France.*

Corresponding Author.

Annelaure Damont. CEA, I2BM, Service Hospitalier Frédéric Joliot, 4 Place du Général Leclerc, 91400 Orsay, France.
E-mail address: annelaure.damont@cea.fr

Abstract

SSR180575 (**1**) is a high-affinity (0.83 nM) TSPO 18 kDa ligand belonging to the pyridazino[4,5-*b*]indole-1-acetamides family. Herein we describe the synthesis and *in vitro* characterization of two series of new fluorinated analogues. Eleven compounds (out of fifteen) displayed nanomolar to subnamolar affinities (0.30 – 8.1 nM) and high selectivities (Ki(CBR) / Ki(TSPO) > 10^3). Two derivatives stand out as promising candidates for drug development or use as PET probes for *in vivo* neuroinflammation imaging, once fluorine-18-labeled.

Keywords:

TSPO 18 kDa

pyridazino[4,5-*b*]indole-1-acetamides

SSR180575

Positron emission tomography

Neuroinflammation

1. Introduction

As the population ages, diseases associated with central nervous system (CNS) damages constitute an increasing public health concern. Diseases such as Alzheimer's (AD) or related afflictions, Parkinson's and Huntington's or multiple sclerosis and ischemic brain injury are examples of CNS pathologies that suffer from the absence of efficient treatments to prevent or reverse the progression of the diseases. An important aspect is also the development of innovative diagnostic tools and methodologies permitting an early and accurate detection of the related biochemical and physiological events associated with these diseases, and as such, treatment monitoring is greatly considered. Neuroinflammatory processes are today recognized as an early response to these CNS afflictions and notably involve activation of the resident immune cells, microglia [1]. Indeed, microglial cells provide a first defence against brain damage and disease progression and contribute to an environment that supports neuronal viability and regeneration, and myelin sheath formation [2]. However, chronic activation of microglia may also become deleterious for neuronal cells and constitutes an important factor in neurodegenerative processes [2]. Microglia activation is associated with the overexpression of a highly lipophilic tryptophan-rich protein of 169 amino acids, the

TSPO 18 kDa, formerly known as the peripheral benzodiazepine receptor (PBR), and located primarily in the outer mitochondrial membrane [3]. TSPO is involved in the transport of cholesterol from the outer to the inner mitochondrial membrane, which is the rate-limiting step in the synthesis of steroid hormones and neurosteroids and is one of the best characterized functions of this protein [4] that contributes to neuroprotection and nerve regeneration [2]. As a matter of fact, TSPO is a highly investigated target for the development of neuroprotective drugs [2]. It is also a major focus for the development of novel imaging probes allowing to quantify *in vivo* the level of brain inflammation, in a non-invasive way. The first reported ligands of TSPO were PK11195 and Ro5-4864 (Figure 1), that both have proven their efficacy to reduce the level of microglial activation and the production of pro-inflammatory cytokines [2]. PK11195 labelled with the short-lived positron-emitter carbon-11 ($T_{1/2}$: 20.4 min) is also the first reported and still widely used TSPO PET radioligand [5,6], even though novel and more potent structures have now emerged [7-12], such as [^{18}F]DPA-714 labelled with the longer-lived radioisotope fluorine-18 ($T_{1/2}$: 109.8 min) [13-15]. SSR180575 (**1**), a compound belonging to the chemical class of the pyridazino[4,5-*b*]indole-1-acetamides [16], binds to the TSPO with a high affinity and represents thus an attractive lead for the development of TSPO ligands as therapeutic drugs. SSR180575 has also been labelled with carbon-11 and displayed encouraging *in vivo* PET imaging properties in both rodents and non-human-primates [17-19].

In this context, we aimed at designing derivatives of **1** and investigating their affinity and selectivity for the TSPO, both for therapeutic and diagnostic purposes. All new compounds were designed with a fluorine atom in their structure at a position compatible with further isotopic labelling with fluorine-18. A first series of compounds modified at the para-position of the freely rotating phenyl group was developed to investigate the steric tolerance of the TSPO binding pocket toward this part of the molecule. Beside this first series of alkoxyaryl SSR180575 analogues, a second series of derivatives, where the methyl substituent at the *N*-indole position was replaced with fluoroalkyl chains, were also prepared.

Fig. 1. Structures of PK11195, Ro5-4864, DPA-714 and the herein investigated series 1 and 2 derived from SSR180575 (**1**).

We report herein the synthesis and initial *in vitro* biological evaluation of fifteen new SSR181575 analogues in an aim to explore the pharmacophoric model within the pyridazino[4,5-*b*]indole-1-acetamides class for optimal binding to TSPO and guide the choice, within this class of compounds, of new radioligands as potential *in vivo* PET imaging probes to visualize neuroinflammatory processes.

2. Chemistry

Two series of new pyridazino[4,5-*b*]indole-1-acetamides, structurally closely related to SSR180575 (**1**), were synthesized. In series 1 (scheme 1), derivatization at the *para*-position of the phenyl group of SSR180575 led to the generation of eight alkyl ethers: the methoxy derivative **11** and the fluorinated analogues **13-19.**

Scheme 1. Synthesis of methoxy derivative **11** and fluorinated analogues **13-19** of SSR180575 modified at the *para*-phenyl position (series 1). **Reagents and conditions**: (a) KOEt, diethyl oxalate, EtOH, Et_2O, r.t., 15 h; (b) Fe, AcOH, EtOH, reflux, 4 h; (c) 1) NaH, DMF, r.t., 1 h, 2) MeI, r.t., 4 h; (d) $TiCl_4$, ethyl oxalyl chloride, $(CH_2Cl)_2$, r.t., 4 h; (e) *p*-MeO-phenylhydrazine.HCl, AcOH, 30 min, then reflux, 3 days; (f) $NaBH_4$, THF, MeOH, reflux, 6 h; (g) TsCl, TEA, CH_2Cl_2, 30 °C, 3 h; (h) KCN, DMF, 70 °C, 1 h, then r.t., 8 h; (i) $HCl_{(g)}$/MeOH, 60 °C, 2 h; (j) $AlMe_3$, $NH(Me)_2$.HCl, toluene, r.t., 30

min, then reflux, 1 h; (k) BBr_3, CH_2Cl_2, -60 °C, 2 h, then warming to -10 °C, 1 h; (l) alkylating agent, K_2CO_3, DMF, 70 °C, 2 h.

As depicted in Scheme 1, the indole ring is first generated in two steps from 4-chloro-2-nitrotoluene according to Reissert synthesis [20]. In this reaction, the acidity of the methyl group *ortho* to nitro on the benzene ring is the means for condensation with oxalate. Then, the nitro group is reduced to amino that spontaneously undergoes a cyclisation-dehydration sequence leading to the indole ring formation in 94 % yield. The resulting indole **3** is subsequently methylated at the *N*-indole position using methyl iodide and sodium hydride in *N*,*N*-dimethylformamide to generate compound **4** quantitatively. Glyoxylic acid ethyl ester substituent was introduced at the 3-position of indole **4** using ethyl oxalyl chloride and titanium (IV) chloride to provide **5** in excellent yield (95 %) that was subsequently condensed with *p*-methoxyphenyl hydrazine to give **6** featuring the pyridazino[4,5-*b*]indole backbone (31 % yield). Reduction of the ester group of **6** with sodium borohydride gave in high yield alcohol **7** (93 %) which was converted to nitrile **9** via chloro- compound **8** by displacement with potassium cyanide in hot *N*,*N*-dimethylformamide (53 % yield over two steps). The methyl ester **10** was then cleanly obtained in excellent yield (96 %) by reacting nitrile **9** with a freshly prepared methanolic solution of hydrochloric acid. Conversion of methyl ester **10** to *N*,*N*-dimethylamide **11** was achieved in refluxing toluene (75 % yield) using an *in situ* generated aluminium-amine complex by reaction of trimethylaluminium with dimethylamine hydrochloride. Methyl phenyl ether **11** was converted in high yield (93 %) to its corresponding phenol **12** by treatment with boron tribromide in dichloromethane at low temperature. Compound **12**, as a common intermediate, was then alkylated by heating with the appropriate, commercially available or previously prepared, alkylating agent at 70°C in *N*,*N*-dimethylformamide in the presence of potassium carbonate as also depicted in scheme 1. Thus, seven fluorinated analogues (**13-19**) were generated from **12** with yields ranging from 35 to 70 %.

Non-commercially available fluorinated alkylating agents **22**, **23**, **24** and **25** were prepared as presented in Scheme 2.

(*E*)-1-Fluoro-4-tosyloxybut-2-ene (**22**) was obtained in 21 % yield from dimethyl fumarate following the three-step sequence already described by Dollé *et al.* [21] 4-Fluorobut-2-yn-1-yl 4-methylbenzenesulfonate (**23**) was obtained from 1,4-butynediol via ditosylation followed by monofluorination with tetrabutylammonium fluoride in hot tetrahydrofuran (11 % overall yield). Compounds **24** and **25** were obtained by monofluorination of di- and tri(ethyleneglycol)di-p-tosylate, respectively (37-43 %).

Scheme 2. Synthesis of fluorinated alkylating agents **22-25**. **Reagents and conditions**: (a) DIBAL-H, toluene, -10 °C, 2 h, then r.t., 16 h; (b) 1) KOH_{aq}, $BnMe_3NOH$, dioxane, r.t., 10 min, 2) Ts_2O, r.t., 5 min; (c) TBAF, THF, reflux, 1-2 h.

In series 2, derivatisation of SSR180575 was investigated at the *N*-indole position with replacement of the methyl substituent with various fluoroalkyl groups, leading to the generation of seven new analogues **27-33** as depicted in Scheme 3. All compounds were obtained from free indole **26** by reaction with the appropriate alkylating agent and in the presence of potassium carbonate in *N*,*N*-dimethylformamide at 60 °C. Yields ranged from 42 % to 72 %.

Scheme 3. Synthesis of fluorinated analogues **27-33** modified at the *N*-indole position (series 2). **Reagents and conditions**: (a) alkylating agent, K_2CO_3, DMF, 60 °C, 2-16 h.

3. Results and discussion

3.1. Binding studies

Table 1. *In vitro* TSPO binding affinity and selectivity of SSR180575 (**1**), its methoxy derivative **11** and the fluorinated analogues **13-19** and **27-33**.

In vitro TSPO binding affinities (Ki) of all target compounds, the methoxy derivative **11** as well as the fluorinated analogues **13-19** (series 1) and **27-33** (series 2), were determined using membrane homogenates from rat heart and screening against [^{3}H]PK11195 (Table 1) for comparison with the lead compound SSR180575 (**1**). As shown, most of the synthesized compounds show similar or even higher binding affinity for TSPO than the parent molecule. As a general rule, alkoxy substitution at the 4-position of the phenyl ring (series 1) did not significantly affect the TSPO binding (Ki ranging from 0.40 to 4.2 nM) when compared to the parent molecule **1** (0.83 nM). Introduction of a methoxy function (**11**) or a fluoroalkoxy chain (**13-19**) was well tolerated, the most beneficial effect among this series being observed for the fluoropropoxy- and fluorobutoxy- analogues (**14**, **15**). More constrained side chains such as fluorobutenoxy (**16**) or fluorobutynoxy (**17**) did not improve the binding for TSPO. Interestingly, elongation of the fluorinated side chain with a polyethylene glycol motif (**18**: n = 2 and **19**: n = 3), to further explore the steric tolerance of the TSPO binding pocket while retaining acceptable lipophilicity, resulted in a slight loss of affinity. These results demonstrate the rather good tolerance to alkoxy substitution at the 4-position of the phenyl ring of SSR180575 (**1**). In the second series of compounds, modification at the *N*-indole position (series 2) resulted in a marked decrease of TSPO affinity compared to the parent molecule SSR180575 (**1**). Among them, only three compounds (**27**, **30** and **31**) exhibited binding affinities in the nanomolar range, while the four other compounds (**28**, **29**, **32**, **33**) demonstrated a significantly less pronounced TSPO binding efficiency with Ki values above 60 nM and even close to the micromolar range for compound **33**. Noteworthy, the loss of affinity correlated well with the lengthening of the saturated fluorinated side chain (**27**, **28**, **29**, **32** and **33**) and interestingly, nanomolar affinity was restored when an unsaturation is present in the alkyl chain (**30**, **31**).

This second set of results proved that replacement of the *N*-methylindole group of **1** with an *N*-fluoroalkyl moiety is not appropriate to generate compounds with improved affinity for the TSPO. Indeed, such a modification caused between five to thousand-fold losses in affinity, demonstrating possible unfavourable steric interactions between *N*-indole substituents and the binding domain of the TSPO.

The TSPO versus CBR (central benzodiazepine receptor) selectivity of compounds **11**, **13-19** and **27-33**, was also assessed, using membrane homogenates from rat cerebral cortex and screening against [^{3}H]flunitrazepam, and compared to SSR180575 (**1**). All compounds previously reported for having nanomolar to subnanomolar affinities to the TSPO, showed no inhibition of [^{3}H]flunitrazepam binding to the CBR at 1 μM, attesting the excellent selectivity over the CBR for these compounds.

Evaluation of the lipophilic properties of all target compounds was investigated by determination of their log$D_{7.4}$ (*n*-octanol/buffer pH 7.4 partition coefficients) values, using a validated and standardized HPLC method. For compounds targeting the CNS, these data give a first indication of potential brain penetration and possible *in vivo* non-specific binding. Measured log$D_{7.4}$ values ranged from 3.01 to 3.75. These values are all slightly higher than the one of the parent molecule (2.97) but are still in the range for acceptable *in vivo* biodistribution characteristics and compatible with brain entry.

3.2. Microsomal stability

Table 2. In vitro microsomal stability of **1**, **11**, **13-19**, **27** and solubility of all novel analogues.

The synthesized analogues, and more particularly compounds displaying good binding affinity for the TSPO (**11**, **13-19**, **27, 30** and **31**) with Ki values below 10 nM, were also evaluated for their *in vitro* microsomal metabolic stability as presented in Table 2. Human, rat and mouse microsomes were used to obtain a preliminary indication of the *in vivo* metabolic clearance of the most promising ligands. Solubility in 50 mM phosphate buffer (pH 7.4) of all novel compounds was also assessed. SSR180575 (**1**) as well as compounds with slight modifications (e.g. **13**, **14**, **27**) compared to the parent molecule (**1**), showed good metabolic stability across the three species. As a general trend, the lengthening of the fluoroalkoxy substitution at the 4-position of the phenyl ring (series 1) resulted in an increase of the percentage of biotransformation at 20 min (compare **13**, **15** and **19** for examples). Insertion of unsaturation into the fluoroalkyl side chain also led to a marked decrease in the microsomal stability of the compounds as illustrated by comparing analogues **15** to **16** and **17** but also **27** to **30** and **31**.

4. Conclusion

Chemical syntheses of fifteen new fluorinated derivatives of SSR180575 (**1**) have been achieved. Of these new analogues, being structurally modified either at the para-positon of the phenyl ring or at the *N*-indole substitution, compounds **14** and **15** stand out as promising candidates for drug development. These new structures also provide unique opportunities for fluorine-18-labeling and *in vivo* PET-imaging of neuroinflammation.

5. Experimental section

5.1. Chemistry

5.1.1. General

Chemicals were purchased from Aldrich France and were used without further purification. Flash chromatographies were conducted on silica gel (0.63-0.200 mm, VWR France) columns. TLCs were run on aluminum pre-coated plates of silica gel $60F_{254}$ (VWR France). The compounds were localized at 254 nm using a UV-lamp and by dipping the TLC-plates in a basic potassium permanganate aqueous solution and heating on a hot plate. Melting points (Mp) were measured on a 9200 Electrothermal (Villepinte - Roissy Charles de Gaulle, France) instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker (Wissembourg, France) Avance 400 MHz apparatus and chemical shifts were referenced to the hydrogenated residue of the deuterated solvent ($\delta[CDHCl_2]$ = 5.32 ppm and $\delta[CHCl_3]$ = 7.23 ppm) for ^{1}H NMR and to the deuterated solvent (($\delta[CD_2Cl_2]$ = 53.5 ppm and $\delta[CDCl_3]$ = 77.0 ppm) for ^{13}C NMR experiments. The standard concentration of the analysed samples was 20 mg/mL. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, m and b for singlet, doublet, triplet, quadruplet, multiplet and broad, respectively). High resolution mass spectrometry analyses (HRMS) were performed by the Small Molecule Mass Spectrometry platform of IMAGIF, (Gif-sur-Yvette, France, www.imagif.cnrs.fr) by electrospray with positive (ESI+) or negative (ESI-) ionization mode. Purity of the synthesized compounds was determined using analytical HPLC (HPLC A): UPLC/SQD Acquity Waters, Acquity BEH C18 (2.1 x 50 mm) column, 1.7 µm, mobile phase: H_2O (A), CH_3CN + 0.1 % formic acid (B), gradient: 2 to 100 % (B) in 3 min, 1.0 mL/min. $LogD_{7.4}$ values were determined based on a validated and standardized HPLC method (HPLC B): Alliance 2695 - PDA Waters, X-Terra MS C18 (4.6 x 20 mm, 3.5 µm) column, mobile phase: 5 mM MOPS/$(CH_3)_4NOH$ pH 7.4 (A), 5 % MOPS/$(CH_3)_4NOH$ (100 mM, pH 7.4) / 95 % CH_3CN (B), gradient (A / B): 98:2 (0.5 min), 0:100 (4.8 min), 98:2 (1.6 min),

1.2 mL/min, 25 °C, detection at 254 nm. Purity of all synthesized compounds was determined using HPLC A and was found to be more than 95%.

5.1.1. Procedures for the preparation of compounds **1-33**

SSR180575 (1). Compound **1** (as reference) was prepared according to patent WO00/44384 and obtained as white fluffy needles. Mp: 232-233 °C. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 1.6 Hz, 1H), 7.49 (t, J = 8.0 Hz, 2H), 7.39 (t, J = 8.0 Hz, 1H), 7.34 (dd, J = 8.8, 1.6 Hz, 1H), 4.32 (s, 3H), 4.19 (s, 2H), 3.22 (s, 3H), 3.00 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.4 [C], 155.3 [C], 141.6 [C], 141.4 [C], 140.4 [C], 133.3 [C], 131.3 [C], 128.7 [2CH], 127.8 [CH], 126.1 [2CH], 123.3 [CH], 123.0 [CH], 118.9 [C], 117.4 [C], 110.6 [CH], 39.6 [CH_2], 37.6 [CH_3], 35.7 [CH_3], 31.6 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{21}H_{20}N_4O_2Cl$: 395.1275 $[M+H]^+$, found 395.1268.

3-(4-Chloro-2-nitro-phenyl)-2-oxo-propionic acid ethyl ester (2). To a suspension of potassium ethoxide (9.8 g, 117 mmol) in dry diethyl ether (96 mL) were added, at room temperature under argon, ethanol (29 mL), diethyl oxalate (20 mL, 146 mmol), and 4-chloro-2-nitrotoluene (5 g, 29 mmol) in solution in diethyl ether (50 mL). The reaction mixture turned red and stirring was continued for 15 h. The reaction mixture was poured onto cold 1.0 M aqueous hydrochloric acid solution (400 mL) and the aqueous layer was extracted with ethyl acetate twice (2 x 250 mL). The combined organic layers were washed with brine and dried (Na_2SO_4). After evaporation, crude compound **2** was directly used in the next step without further purification. R_f (heptane/ethyl acetate, 8/2): 0.23.

Ethyl 6-chloro-1*H*-indole-2-carboxylate (3). To crude **2** (29 mmol) in a mixture of ethanol and glacial acetic acid (1:1, v:v, 108 mL) at room temperature was added iron powder (14.4 g, 257 mmol) and the reaction mixture was stirred at reflux for 4 h. The mixture was filtered and evaporated to dryness to give a residue which was redistributed in dichloromethane (300 mL) / 1.0 M aqueous hydrochloric acid solution (400 mL). The organic layer was separated and successively washed with a saturated aqueous sodium bicarbonate solution (300 mL), brine (300 mL) and dried (Na_2SO_4). Evaporation followed by crystallization (hexane-dichloromethane) gave **3** as pale off-white needles (6.12 g, 94 % over 2 steps). R_f (heptane/ethyl acetate, 8/2): 0.48. Mp: 176-177 °C. ^{1}H NMR (CD_2Cl_2) δ 9.03 (bs, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.48 (s, 1H), 7.20 (d, J = 1.2 Hz, 1H), 7.14 (dd, J = 8.6, 1.6 Hz, 1H), 4.41 (q^4, J = 7.1 Hz, 2H), 1.42 (t, J = 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 161.7 [C], 136.9 [C], 131.2 [C], 128.1 [C], 125.9 [C], 123.4 [CH], 121.8 [CH], 111.6 [CH], 108.5 [CH], 61.2 [CH_2], 14.3 [CH_3].

Ethyl 6-chloro-1-methyl-1*H*-indole-2-carboxylate (4). To a suspension of sodium hydride (0.73 g, 29 mmol) in dry *N*,*N*-dimethylformamide (58 mL) was added **3** (6.1 g, 27.4 mmol) at room temperature and the reaction mixture was stirred under argon for 1 h before addition of methyl iodide (1.82 mL, 29 mmol). The reaction mixture was stirred at room temperature for 4 h and evaporated to give a residue which was redistributed in dichloromethane (200 mL) and water (300 mL). The organic layer was separated and washed twice with water (2 x 200 mL) before being dried (Na_2SO_4). Evaporation followed by crystallization in ethanol gave **4** as beige crystals (6.5 g, quantitative). R_f (heptane/ethyl acetate, 7/3): 0.52. Mp: 75-76 °C. ^{1}H NMR ($CDCl_3$) δ 7.56 (d, J = 8.4 Hz, 1H), 7.35 (d, J = 1.6 Hz, 1H), 7.24 (s, 1H), 7.09 (d, J = 8.4 Hz, 1H), 4.36 (q^4, J = 6.8 Hz, 2H), 4.01 (s, 3H), 1.41 (t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 161.8 [C], 139.8 [C], 130.9 [C], 128.7 [C], 124.2 [C], 123.4 [CH], 121.4 [CH], 110.1 [CH], 110.0 [CH], 60.6 [CH_2], 31.7 [CH_3], 14.3 [CH_3].

Ethyl 6-chloro-3-(2-ethoxy-2-oxoacetyl)-1-methyl-1*H*-indole-2-carboxylate (5). To a solution of ethyl chlorooxoacetate (1.51 mL, 13.5 mmol) in dichloroethane (25 mL) was added titanium chloride IV (13.6 mL of a 1.0 M solution in dichloromethane). The reaction mixture was stirred at room temperature for 30 min and **4** (2.92 g, 12.3 mmol) dissolved in dichloroethane (12 mL) was added. Stirring was continued for 4 h at room temperature before evaporation. The residue was redistributed in dichloromethane (100 mL) and water (150 mL). The organic layer was separated and dried (Na_2SO_4). Evapo-

ration followed by crystallization from heptane gave **5** as pale yellow crystals (3.88 g, 95 %). R_f (heptane/ethyl acetate, 7/3): 0.24. Mp: 94-95 °C. ^{1}H NMR (CD_2Cl_2) δ 8.10 (d, J = 8.4 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 7.33 (dd, J = 8.4, 1.6 Hz, 1H), 4.38 (q^4, J = 7.2 Hz, 2H), 4.33 (q^4, J = 7.2 Hz, 2H), 3.99 (s, 3H), 1.40 (t, J = 7.2 Hz, 3H), 1.38 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 181.5 [C], 162.9 [C], 160.7 [C], 137.9 [C], 134.3 [C], 131.8 [C], 124.7 [CH], 124.2 [C], 123.5 [CH], 114.8 [C], 110.5 [CH], 62.4 [CH_2], 62.3 [CH_2], 32.2 [CH_3], 13.9 [CH_3], 13.8 [CH_3].

7-Chloro-5-methyl-4-oxo-3-(4-methoxyphenyl)-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indole-1-carboxylic acid ethyl ester (6). To a suspension of **5** (5.6 g, 16.6 mmol) in acetic acid (166 mL) was added 4-methoxyphenylhydrazine hydrochloride (3.47 g, 19.9 mmol) and the reaction mixture was stirred for 30 min at room temperature and then refluxed for 3 days. The mixture was poured on water (200 mL) and extracted with dichloromethane (150 mL). The organic layer was successively washed with a saturated aqueous sodium bicarbonate solution (200 mL) and water (200 mL) before being dried (Na_2SO_4). After evaporation, the residue was purified by silica gel column chromatography (toluene/acetone 98/2) to afford **6** (2.12 g, 31 %) as a white powder and unreacted material (2.7 g, 48 %). R_f (toluene/acetone, 19/1): 0.38. Mp: 209-210 °C. ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 9.2 Hz, 2H), 7.49 (d, J = 1.2 Hz, 1H), 7.33 (dd, J = 8.8, 1.2 Hz, 1H), 7.02 (d, J = 9.2 Hz, 2H), 4.53 (q^4, J = 7.2 Hz, 2H), 4.32 (s, 3H), 3.87 (s, 3H), 1.47 (t, J = 7.2 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ 164.0 [C], 159.4 [C], 155.3 [C], 141.7 [C], 134.6 [C], 134.1 [C], 134.0 [C], 131.5 [C], 127.3 [2CH], 126.7 [CH], 123.1 [CH], 118.2 [C], 115.7 [C], 114.0 [2CH], 110.1 [CH], 62.2 [CH_2], 55.5 [CH_3], 31.7 [CH_3], 14.2 [CH_3].

7-Chloro-1-(hydroxymethyl)-5-methyl-3-(4-methoxyphenyl)-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-4-one (7). To a solution of **6** (0.3 g, 0.73 mmol) and sodium borohydride (166 mg, 4.38 mmol) in dry tetrahydrofuran (11 mL) was added methanol (153 µL) while stirring at room temperature. The reaction mixture was refluxed for 6 h and poured onto cold 1.0 M aqueous hydrochloric acid solution (20 mL). The resulting precipitate was separated by filtration and washed successively with water (20 mL) and diethyl ether (20 mL). Heat drying of the cake gave **7** (250 mg, 93 %) as a white powder. R_f (toluene/acetone, 19/1): 0.12. Mp: 233-234 °C. ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 8.8 Hz, 1H), 7.50 (s, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.32 (dd, J = 8.8 Hz, 1.6 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 4.98 (s, 2H), 4.26 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (DMF-d_7) δ 159.9 [C], 156.0 [C], 145.5 [C], 142.4 [C], 136.2 [C], 133.4 [C], 132.4 [C], 128.6 [2CH], 126.2 [CH], 123.4 [CH], 119.9 [C], 117.2 [C], 114.6 [2CH], 112.1 [CH], 64.0 [CH_2], 56.3 [CH_3], 32.4 [CH_3].

7-Chloro-1-(2-chloromethyl)-5-methyl-3-(4-methoxyphenyl)-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-4-one (8). To a solution of **7** (600 mg, 1.63 mmol) in dichloromethane (6 mL) was added, at room temperature, toluenesulfonyl chloride (371 mg, 1.95 mmol) and triethylamine (0.45 mL, 3.25 mmol). The reaction mixture was stirred for 3 h at 30 °C before addition of a 1.0 M aqueous hydrochloric acid solution (150 mL) and extraction with dichloromethane (100 mL). The organic layer was washed with water (100 mL), dried (Na_2SO_4) and evaporated to dryness. The residue was purified by silica gel column chromatography (dichloromethane/ethyl acetate 99/1) to afford **8** (410 mg, 65 %) as a white solid. R_f (toluene/acetone, 19/1): 0.38. Mp: 236-237 °C. ^{1}H NMR ($CDCl_3$) δ 8.11 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 1.6 Hz, 1H), 7.53 (d, J = 8.8 Hz, 2H), 7.42 (dd, J = 8.8, 1.6 Hz, 1H), 7.02 (d, J = 8.8 Hz, 2H), 4.96 (s, 2H), 4.35 (s, 3H), 3.87 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 159.2 [C], 155.2 [C], 141.4 [C], 140.3 [C], 134.2 [C], 133.7 [C], 131.5 [C], 127.3 [2CH], 123.9 [CH], 123.3 [CH], 118.0 [C], 115.6 [C], 114.0 [2CH], 110.8 [CH], 55.5 [CH_3], 43.7 [CH_2], 31.7 [CH_3].

2-(7-Chloro-3-(4-methoxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)acetonitrile (9). To a solution of **8** (1.0 g, 2.58 mmol) in *N*,*N*-dimethylformamide (30 mL) was added under argon potassium cyanide (318 mg, 4.90 mmol). The reaction mixture was stirred for 1 h at 70 °C and then allowed to cool down to room temperature for 8 h. A 1.0 M aqueous sodium hydroxide solution (250 mL) was added and the resulting mixture was extracted with ethyl acetate (150 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by silica gel column

chromatography (dichloromethane/ethyl acetate 98/2) and the collected fraction was crystallized in a 9:1 mixture of diethyl ether and ethanol to afford **9** (789 mg, 81 %) as white crystals. R_f (toluene/acetone, 19/1): 0.24. ^{1}H NMR ($CDCl_3$) δ 7.91 (d, J = 8.8 Hz, 1H), 7.55 (d, J = 1.6 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 7.37 (dd, J = 8.8, 1.6 Hz, 1H), 6.96 (d, J = 8.8 Hz, 2H), 4.27 (s, 3H), 4.17 (s, 2H), 3.80 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 159.2 [C], 155.1 [C], 141.4 [C], 134.7 [C], 134.0 [C], 133.9 [C], 131.3 [C], 127.1 [2CH], 123.6 [CH], 122.6 [CH], 117.7 [C], 115.5 [C], 115.2 [C], 114.0 [2CH], 111.1 [CH], 55.4 [CH_3], 31.7 [CH_3], 23.3 [CH_2].

Methyl 2-(7-chloro-3-(4-methoxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)acetate (10). To a suspension of **9** (180 mg, 0.47 mmol) in methanol (10 mL) was added dropwise acetyl chloride (5 mL) under argon. The reaction mixture was stirred for 2 h at 60 °C, then cooled down to room temperature and a 10% aqueous solution of sodium bicarbonate (50 mL) was added followed by extraction with ethyl acetate (30 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness. Triturating the residue in diethyl ether afforded **10** (188 mg, 96 %) as a white solid and no further purification was required. R_f (dichloromethane/methanol, 49/1): 0.60. ^{1}H NMR (CD_2Cl_2) δ 7.88 (d, J = 8.6 Hz, 1H), 7.61 (d, J = 1.6 Hz, 1H), 7.53 (d, J = 8.9 Hz, 2H), 7.37 (dd, J = 8.6, 1.5 Hz, 1H), 7.01 (d, J = 8.9 Hz, 2H), 4.33 (s, 3H), 4.14 (s, 2H), 3.87 (s, 3H), 3.70 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 169.9 [C], 159.1 [C], 155.3 [C], 141.3 [C], 138.5 [C], 134.5 [C], 133.4 [C], 131.4 [C], 127.3 [2CH], 123.2 [CH], 122.7 [CH], 118.7 [C], 116.8 [C], 114.0 [2CH], 110.9 [CH], 55.5 [CH_3], 52.5 [CH_3], 40.0 [CH_2], 31.7 [CH_3].

2-(7-Chloro-3-(4-methoxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (11). To a suspension of dimethylamine hydrochloride (333 mg, 4.09 mmol) in dry toluene (15 mL) at 5 °C was slowly added a 2 M solution of trimethylaluminium in toluene (2.05 mL, 4.09 mmol). After the addition was complete, the reaction mixture was allowed to warm to room temperature and was stirred for 30 min until gas evolution has ceased. A solution of **10** (840 mg, 2.05 mmol) in toluene (10 mL) was added to the former solution. The resulting mixture was refluxed under argon until no starting ester was observed on TLC (approximately 1 h). The reaction mixture was cooled to room temperature and was carefully quenched with a 5 % hydrochloric acid aqueous solution (30 mL). The organic layer was separated and the aqueous layer was extracted three times with ethyl acetate (3 x 20 mL). The organic extracts were combined, dried (Na_2SO_4) and evaporated to dryness. Triturating the residue with diethyl ether-methanol afforded **11** (650 mg, 75 %) as a ligh yellow solid. R_f (dichloromethane/methanol, 49/1): 0.30. t_R (HPLC A) = 1.07 min. Mp: 233-234 °C. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 1H), 7.53 (m, 3H), 7.33 (dd, J = 8.4, 1.6 Hz, 1H), 7.00 (d, J = 8.8 Hz, 2H), 4.32 (s, 3H), 4.18 (s, 2H), 3.86 (s, 3H), 3.22 (s, 3H), 3.00 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.4 [C], 158.9 [C], 155.3 [C], 141.6 [C], 140.1 [C], 134.6 [C], 133.2 [C], 131.3 [C], 127.3 [2CH], 123.3 [CH], 123.0 [CH], 119.0 [C], 117.4 [C], 113.9 [2CH], 110.6 [CH], 55.5 [CH_3], 39.6 [CH_2], 37.6 [CH_3], 35.7 [CH_3], 31.6 [CH_3]. HR-ESI(+)-MS m/z calcd for $C_{22}H_{22}N_4O_3Cl$: 425.1380 $[M+H]^+$, found 425.1395.

2-(7-Chloro-3-(4-hydroxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (12). To a solution of **11** (2.27 g, 5.37 mmol) in dichloromethane (50 mL), was added dropwise at -60°C a 1.0 M solution of boron tribromide in dichloromethane (26.8 mL, 26.8 mmol). The reaction mixture was stirred for 2 h at -60 °C, then warmed to -10 °C for 1 h before quenching with methanol (10 mL). The resulting mixture was then neutralized with concentrated aqueous ammonia and filtered through a pad of celite®. The filtrate was evaporated to half its volume under reduced pressure, diluted with dichloromethane (50 mL) and extracted three times with an aqueous 2.0 M sodium hydroxide solution (3 x 50 mL). The combined aqueous extracts were combined and acidified with concentrated hydrochloric acid until generation of a white precipitate which was collected by filtration to give pure **12** (2.03 g, 93 %) as white crystals. R_f (dichloromethane/methanol, 19/1): 0.16. t_R (analytical HPLC) = 1.07 min. Mp: 298-300 °C. ^{1}H NMR (DMSO-d_6) δ

9.71 (s, 1H), 7.94 (s, 1H), 7.86 (d, J = 8.4 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 8.8 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 4.27 (s, 3H), 4.20 (s, 2H), 3.16 (s, 3H), 2.84 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 168.6 [C], 157.1 [C], 154.8 [C], 141.2 [C], 140.9 [C], 133.6 [C], 132.1 [C], 130.9 [C], 127.8 [2CH], 124.0 [CH], 122.6 [CH], 118.9 [C], 117.3 [C], 115.3 [2CH], 111.6 [CH], 40.0 [CH_2], 37.4 [CH_3], 35.4 [CH_3], 32.0 [CH_3].

General procedure A - Preparation of *O*-fluoroalkylated compounds 13-19 (series 1). To a mixture of phenol **12** (100-150 mg) and potassium carbonate (2 eq.) in *N*,*N*-dimethylformamide (10-12 mL) was gradually added the alkylating agent (2 eq.) in solution in *N*,*N*-dimethylformamide (2 mL). The whole mixture was stirred for 2 h at 70 °C, cooled to room temperature, quenched by the addition of a saturated aqueous ammonium chloride solution (20-30 mL) and extracted with dichloromethane (15-20 mL). The organic extract was washed with brine, dried (Na_2SO_4) and evaporated to dryness to afford the crude product. Purification by silica gel column chromatography (dichloromethane/methanol 98/2 to 95/5) gave the expected compounds **13** to **19** (35-70 %).

2-(7-Chloro-3-(4-(2-fluoroethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (13). Using general procedure A and 2-fluoroethyl 4-methylbenzenesulfonate as alkylating agent, **13** (105 mg, 63 %) was obtained as a white powder. R_f (dichloromethane/methanol, 19/1): 0.38. t_R (HPLC A) = 1.08 min. Mp: 234-236 °C. ^{1}H NMR (CD_2Cl_2) δ 7.89 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 9.2 Hz, 2H), 7.34 (dd, J = 8.8, 1.6 Hz, 1H), 7.03 (d, J = 9.2 Hz, 2H), 4.78 (dt, $^2J_{H-F}$ = 47.6 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 4.32 (s, 3H), 4.27 (dt, $^3J_{H-F}$ = 28.4 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 4.16 (s, 2H), 3.19 (s, 3H), 2.96 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 157.6 [C], 155.1 [C], 141.3 [C], 140.7 [C], 140.3 [C], 135.4 [C], 132.8 [C], 127.4 [2CH], 123.2 [CH], 122.6 [CH], 118.9 [C], 117.2 [C], 114.3 [2CH], 110.7 [CH], 82.0 [d, $^1J_{C-F}$ = 169.0 Hz, CH_2], 67.5 [d, $^2J_{C-F}$ = 20 Hz, CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3], 31.6 [CH_3]. HR-ESI(+)-MS m/z calcd for $C_{23}H_{23}N_4O_3FCl$: 457.1442 $[M+H]^+$, found 457.1443.

2-(7-Chloro-3-(4-(3-fluoropropoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (14). Using general procedure A and 3-fluoropropyl 4-methylbenzenesulfonate as alkylating agent, **14** (100 mg, 58 %) was obtained as a white powder. R_f (dichloromethane/acetone, 7/3): 0.71. t_R (HPLC A) = 1.15 min. Mp: 199-200 °C. ^{1}H NMR (CD_2Cl_2) δ 7.89 (d, J = 8.4 Hz, 1H), 7.58 (d, J = 1.6 Hz, 1H), 7.52 (d, J = 9.2 Hz, 2H), 7.34 (dd, J = 8.4, 1.6 Hz, 1H), 7.01 (d, J = 9.2 Hz, 2H), 4.67 (dt, $^2J_{H-F}$ = 46.8 Hz, $^3J_{H-H}$ = 6.0 Hz, 2H), 4.31 (s, 3H), 4.16 (m, 4H), 3.19 (s, 3H), 2.96 (s, 3H), 2.20 (dq^5, $^3J_{H-F}$ = 26.0 Hz, $^3J_{H-H}$ = 6.0 Hz, 2H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 158.0 [C], 155.1 [C], 141.3 [C], 140.3 [C], 135.0 [C], 132.8 [C], 131.3 [C], 127.3 [2CH], 123.2 [CH], 122.5 [CH], 119.0 [C], 117.2 [C], 114.2 [2CH], 110.7 [CH], 80.8 [d, $^1J_{C-F}$ = 163.0 Hz, CH_2], 63.9 [d, $^3J_{C-F}$ = 6.0 Hz, CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3], 31.5 [CH_3], 30.3 [CH_2, $^2J_{C-F}$ = 20.0 Hz]. HR-ESI(+)-MS m/z calcd for $C_{24}H_{25}N_4O_3FCl$: 471.1599 $[M+H]^+$, found 471.1613.

2-(7-Chloro-3-(4-(4-fluorobutoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (15). Using general procedure A and 1-bromo-4-fluorobutane as alkylating agent, **15** (99 mg, 70 %) was obtained as a white powder. R_f (dichloromethane/methanol, 19/1): 0.68. t_R (HPLC A) = 1.19 min. Mp: 219-220 °C. ^{1}H NMR (CD_2Cl_2) δ 7.90 (d, J = 8.8 Hz, 1H), 7.58 (d, J = 1.6 Hz, 1H), 7.51 (d, J = 9.2 Hz, 2H), 7.34 (dd, J = 8.8, 1.6 Hz, 1H), 6.99 (d, J = 9.2 Hz, 2H), 4.54 (dt, $^2J_{H-F}$ = 47.2 Hz, $^3J_{H-H}$ = 5.6 Hz, 2H), 4.33 (s, 3H), 4.16 (s, 2H), 4.08 (t, J = 5.6 Hz, 2H), 3.19 (s, 3H), 2.96 (s, 3H), 1.97-1.85 (m, 4H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 158.2 [C], 155.1 [C], 141.3 [C], 140.2 [C], 134.9 [C], 132.8 [C], 131.4 [C], 127.3 [2CH], 123.2 [CH], 122.5 [CH], 119.0 [C], 117.2 [C], 114.2 [2CH], 110.7 [CH], 83.8 [d, $^1J_{C-F}$ = 163.0 Hz, CH_2], 67.6 [CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3], 31.5 [CH_3], 27.1 [CH_2, $^2J_{C-F}$ = 20.0 Hz], 25.1 [CH_2, $^3J_{C-F}$ = 5.0 Hz]. HR-ESI(+)-MS m/z calcd for $C_{25}H_{27}N_4O_3FCl$: 485.1755 $[M+H]^+$, found 485.1738.

(*E*)-2-(7-Chloro-3-(4-((4-fluorobut-2-en-1-yl)oxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (16). Using general procedure A and (*E*)-4-fluorobut-2-en-1-yl 4-methylbenzenesulfonate (**22**)

as alkylating agent, **16** (70 mg, 50 %) was obtained as white crystals. R_f (dichloromethane/methanol, 19/1): 0.46. t_R (HPLC A) = 1.17 min. Mp: 247-248 °C. ^{1}H NMR (CD_2Cl_2) δ 7.86 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 1.6 Hz, 1H), 7.50 (d, J = 8.8 Hz, 2H), 7.36 (dd, J = 8.8, 1.6 Hz, 1H), 7.03 (d, J = 8.8 Hz, 2H), 6.08 (m, 2H), 4.92 (dd, $^2J_{H-F}$ = 46.8 Hz, $^3J_{H-H}$ = 3.6 Hz, 2H), 4.65 (d, J = 4.4 Hz, 2H), 4.31 (s, 3H), 4.20 (s, 2H), 3.20 (s, 3H), 2.97 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.9 [C], 157.8 [C], 155.3 [C], 141.4 [C], 140.4 [C], 134.9 [C], 133.1 [C], 131.2 [C], 129.1 [d, $^3J_{C-F}$: 12 Hz, CH], 127.6 [d, $^2J_{C-F}$ = 17 Hz, CH], 127.4 [2CH], 123.0 [CH], 122.7 [CH], 118.8 [C], 117.4 [C], 114.5 [2CH], 110.8 [CH], 82.5 [d, $^1J_{C-F}$ = 162 Hz, CH_2], 67.5 [CH_2], 39.3 [CH_2], 37.5 [CH_3], 35.3 [CH_3], 31.5 [CH_3]. HR-ESI(+)-MS m/z calcd for $C_{25}H_{25}N_4O_3FCl$: 483.1599 $[M+H]^+$, found 483.1591.

2-(7-Chloro-3-(4-((4-fluorobut-2-yn-1-yl)oxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (17). Using general procedure A and 4-fluorobut-2-yn-1-yl 4-methylbenzenesulfonate (**23**) as alkylating agent, **17** (57 mg, 35 %) was obtained as a white solid. R_f (dichloromethane/acetone, 8/2): 0.60. t_R (HPLC A) = 1.35 min. Mp: 259-261 °C. ^{1}H NMR ($CDCl_3$) δ 7.94 (d, J = 8.8 Hz, 1H), 7.56 (d, J = 8.8 Hz, 2H), 7.53 (d, J = 1.6 Hz, 1H), 7.35 (dd, J = 8.8, 1.6 Hz, 1H), 7.06 (d, J = 8.8 Hz, 2H), 5.01 (d, $^2J_{H-F}$ = 47.2 Hz, 2H), 4.81 (d, $^5J_{H-F}$ = 7.2 Hz, 2H), 4.33 (s, 3H), 4.19 (s, 2H), 3.23 (s, 3H), 3.00 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.8 [C], 157.0 [C], 155.5 [C], 141.5 [C], 140.5 [C], 135.4 [C], 133.5 [C], 131.3 [C], 127.5 [2CH], 123.2 [2CH], 118.9 [C], 117.6 [C], 115.0 [2CH], 110.8 [CH], 84.6 [d, $^3J_{C-F}$: 11 Hz, C], 81,4 [d, $^2J_{C-F}$ = 22 Hz, C], 70.5 [d, $^1J_{C-F}$ = 165 Hz, CH_2], 56.1 [d, $^4J_{C-F}$ = 3 Hz, CH_2], 39.5 [CH_2], 37.8 [CH_3], 35.8 [CH_3], 31.7 [CH_2]. HR-ESI(+)-MS m/z calcd for $C_{25}H_{23}N_4O_3FCl$: 481.1442 $[M+H]^+$, found 481.1437.

2-(7-Chloro-3-(4-(2-(2-fluoroethoxy)ethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (18). Using general procedure A and 2-(2-fluoroethoxy)ethyl 4-methylbenzenesulfonate (**24**) as alkylating agent, **18** (100 mg, 69 %) was obtained as a white solid. R_f (dichloromethane/acetone, 8/2): 0.44. t_R (HPLC A) = 1.08 min. Mp: 230-231 °C. ^{1}H NMR (CD_2Cl_2) δ 7.91 (d, J = 8.4 Hz, 1H), 7.60 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 8.8 Hz, 2H), 7.36 (dd, J = 8.4, 1.6 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 4.61 (dt, $^2J_{H-F}$ = 48.0 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 4.34 (s, 3H), 4.22 (t, J = 4.8 Hz, 2H), 4.17 (s, 2H), 3.91 (t, J = 4.8 Hz, 2H), 3.83 (dt, $^3J_{H-F}$ = 30.0 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 3.21 (s, 3H), 2.98 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 157.9 [C], 155.1 [C], 141.3 [C], 140.3 [C], 135.1 [C], 132.8 [C], 131.4 [C], 127.3 [2CH], 123.2 [CH], 122.5 [CH], 119.0 [C], 117.2 [C], 114.3 [2CH], 110.7 [CH], 83.2 [d, $^1J_{C-F}$ = 167.0 Hz, CH_2], 70.4 [d, $^2J_{C-F}$ = 19.0 Hz, CH_2], 69.7 [CH_2], 67.8 [CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3], 31.6 [CH_3]. HR-ESI(+)-MS m/z calcd for $C_{25}H_{27}N_4O_4FCl$: 501.1705 $[M+H]^+$, found 501.1711.

2-(7-Chloro-3-(4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (19). Using general procedure A and 2-(2-(2-fluoroethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**25**) as alkylating agent, **19** (110 mg, 63 %) was obtained as a white solid. R_f (dichloromethane/acetone, 8/2): 0.34. t_R (HPLC A) = 1.08 min. Mp: 136-138 °C. ^{1}H NMR (CD_2Cl_2) δ 7.91 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 8.8 Hz, 2H), 7.36 (dd, J = 8.8, 1.6 Hz, 1H), 7.04 (d, J = 8.8 Hz, 2H), 4.57 (dt, $^2J_{H-F}$ = 47.6 Hz, $^3J_{H-H}$ = 4.4 Hz, 2H), 4.33 (s, 3H), 4.21 (t, J = 4.4 Hz, 2H), 4.17 (s, 2H), 3.88 (t, J = 4.8 Hz, 2H), 3.80-3.65 (m, 6H), 3.21 (s, 3H), 2.98 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 158.0 [C], 155.1 [C], 141.3 [C], 140.3 [C], 135.1 [C], 132.8 [C], 131.4 [C], 127.3 [2CH], 123.2 [CH], 122.5 [CH], 119.0 [C], 117.2 [C], 114.3 [2CH], 110.7 [CH], 83.2 [d, $^1J_{C-F}$ = 167.0 Hz, CH_2], 70.7 [CH_2], 70.6 [CH_2], 70.3 [d, $^2J_{C-F}$ = 19.0 Hz, CH_2], 69.6 [CH_2], 67.8 [CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3], 31.6 [CH_3]. HR-ESI(+)-MS m/z calcd for $C_{27}H_{31}N_4O_5FCl$: 545.1967 $[M+H]^+$, found 545.1979.

(*E*)-But-2-ene-1,4-diyl bis(4-methylbenzenesulfonate) (20). Compound **20** was prepared in 2 steps from dimethyl fumarate (2.08 g, 14.4 mmol) according to the procedure described by Reddy *et al*. [22] and was obtained as a white solid (1.7 g, 30 %). R_f (heptane/ethyl acetate, 2/1): 0.19. Mp: 90-91 °C. ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 8.0 Hz, 4H), 7.36 (d, J =

8.0 Hz, 4H), 5.75-5.73 (m, 2H), 4.47 (dd, J = 4.0, 1.6 Hz, 4H), 2.45 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 146.0 [2C], 132.5 [2C], 130.4 [4C], 128.3 [4C], 128.1 [2C], 68.7 [2C], 22.7 [2C].

But-2-yne-1,4-diyl bis(4-methylbenzenesulfonate) (21). To a solution of but-2-yne-1,4-diol (600 mg, 6.97 mmol) in dioxane (10 mL) were successively added benzyltrimethylammonium hydroxide (58 mg, 0.35 mmol) and a 50% aqueous potassium hydroxide solution (10 mL). The resulting mixture was stirred for 10 min at ambient temperature and a suspension of 4-methylbenzenesulfonic anhydride (5.0 g, 15.33 mmol) in dioxane (20 mL) was added portionwise while a white precipitate appeared. Stirring was continued for an additional 5 min and the mixture was partitioned between ethyl acetate (50 mL) and a 1.0 M aqueous hydrochloric acid solution (80 mL). The organic layer was separated, washed with brine, dried (Na_2SO_4) and evaporated under vacuum. The residue was retaken and triturated in ethanol to give, after filtration, expected compound **21** (1.53 g, 56 %) as a white solid that was used without further purification. R_f (heptane/ethyl acetate, 2/1): 0.22. Mp: 94-95 °C. ^{1}H NMR ($CDCl_3$) δ 7.77 (d, J = 8.0 Hz, 4H), 7.35 (d, J = 8.0 Hz, 4H), 4.58 (s, 4H), 2.46 (s, 6H). ^{13}C NMR ($CDCl_3$) δ 145.6 [2C], 133.0 [2C], 130.1 [4CH], 128.3 [4CH], 81.2 [2C], 57.3 [$2CH_2$], 21.9 [$2CH_3$].

(*E*)-4-Fluorobut-2-en-1-yl 4-methylbenzenesulfonate (22). Compound **22** was prepared from **20** (600 mg, 1.52 mmol) according to the procedure described by Dollé *et al.* [21] and was obtained as a colourless oil (145 mg, 39 %). R_f (heptane/ethyl acetate, 2/1): 0.37. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 5.98-5.85 (m, 1H), 5.84-5.75 (m, 1H), 4.83 (ddd, $^2J_{H-F}$ = 47.2 Hz, $^3J_{H-H}$ = 4.8 Hz, $^4J_{H-H}$ = 0.8 Hz, 2H), 4.57 (m, 2H), 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 144.9 [C], 133.1 [C], 130.2 [CH], 129.8 [2CH], 127.9 [2CH], 125.4 [CH], 81.7 [d, $^1J_{C-F}$ = 165.0 Hz, CH_2], 69.1 [CH_2], 21.6 [CH_3].

4-Fluorobut-2-yn-1-yl 4-methylbenzenesulfonate (23). To a solution of **21** (1.5 g, 3.81 mmol) in tetrahydrofuran (70 mL) at ambient temperature was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (4.2 mL, 4.19 mmol). The resulting mixture was refluxed for 1 h and evaporated to dryness. The residue was purified by silica gel column chromatography (heptane/ethyl acetate 4/1) to afford **23** (240 mg, 20 %) as a colourless oil. R_f (heptane/ethyl acetate, 2/1): 0.34. ^{1}H NMR ($CDCl_3$) δ 7.82 (d, J = 8.0 Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 4.84 (d, $^2J_{H-F}$ = 52.8 Hz), 4.78 (s, 2H), 2.46 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 145.2 [C], 132.8 [C], 129.8 [2CH], 128.1 [2CH], 82.9 [d, $^2J_{C-F}$ = 22.1 Hz, C], 81.5 [d, $^3J_{C-F}$ = 11.6 Hz, C], 69.9 [d, $^1J_{C-F}$ = 166.3 Hz, CH_2], 57.2 [d, $^4J_{C-F}$ = 3.1 Hz, CH_2], 21.6 [CH_3].

2-(2-Fluoroethoxy)ethyl 4-methylbenzenesulfonate (24). To a solution of commercially available di(ethyleneglycol)di-p-tosylate (2.49 g, 6.0 mmol) in tetrahydrofuran (20 mL) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (8 mL, 8.0 mmol). The resulting mixture was refluxed for 2 h and evaporated to dryness. The residue was purified by silica gel column chromatography (heptane/ethyl acetate 4/1 to 2/1) to afford pure **24** (578 mg, 37 %) as a colourless oil. R_f (petroleum ether/ethyl acetate, 2/1): 0.31. ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 4.49 (dt, $^2J_{H-F}$ = 47.6 Hz, $^3J_{H-H}$ = 4.4 Hz, 2H), 4.18 (m, 2H), 3.72 (m, 2H), 3.67 (dt, $^3J_{H-F}$ = 29.6 Hz, $^3J_{H-H}$ = 4.4 Hz, 2H), 2.44 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 144.8 [C], 132.9 [C], 129.8 [2CH], 127.9 [2CH], 82.9 [d, $^1J_{C-F}$ = 169.0 Hz, CH_2], 70.3 [d, $^2J_{C-F}$ = 20.0 Hz, CH_2], 69.1 [CH_2], 68.8 [CH_2], 21.6 [CH_3].

2-(2-(2-Fluoroethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (25). To a solution of commercially available tri(ethyleneglycol)di-p-tosylate (2.61 g , 5.7 mmol) in tetrahydrofuran (20 mL) was added dropwise a 1.0 M solution of tetrabutylammonium fluoride in tetrahydrofuran (7.5 mL, 7.5 mmol). The resulting mixture was refluxed for 2 h and evaporated to dryness. The residue was purified by silica gel column chromatography (heptane/ethyl acetate 4/1 to 2/1) to give pure **25** (775 mg, 43 %) as a colourless oil. R_f (petroleum ether/ethyl acetate, 2/1): 0.23. ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.54 (dt, $^2J_{H-F}$ = 48.0 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 4.17 (t, J = 4.8 Hz, 2H), 3.71 (dt, $^3J_{H-F}$ = 29.6 Hz, $^3J_{H-H}$ = 4.0 Hz, 2H), 3.69 (t, J = 4.8 Hz, 2H), 3.60 (m, 4H), 2.45 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 144.7 [C], 132.9 [C], 129.8 [2CH],

127.9 [2CH], 83.0 [d, $^1J_{C-F}$ = 168.0 Hz, CH_2], 70.7 [CH_2], 70.6 [CH_2], 70.4 [d, $^2J_{C-F}$ = 19.0 Hz, CH_2], 69.2 [CH_2], 68.7 [CH_2], 21.6 [CH_3].

2-(7-Chloro-4-oxo-3-phenyl-4,5-dihydro-3H-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (26). Compound **26** (as key-intermediate for the preparation of series 2) was prepared according to patent WO00/44384 and obtained as a white solid. Mp: 310-312 °C. ^{1}H NMR (DMSO-d_6) δ 7.86 (d, J = 8.8 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.61 (d, J = 7.6 Hz, 2H), 7.52 (t, J = 7.6 Hz, 2H), 7.42 (t, J = 7.6 Hz, 1H), 7.35 (dd, J = 8.8, 1.6 Hz, 1H), 4.22 (s, 2H), 3.17 (s, 3H), 2.86 (s, 3H). ^{13}C NMR (DMSO-d_6) δ 168.7 [C], 154.3 [C], 141.9 [C], 141.6 [C], 140.1 [C], 132.6 [C], 131.8 [C], 129.0 [2CH], 128.0 [CH], 126.5 [2CH], 124.1 [CH], 122.5 [CH], 120.1 [C], 117.6 [C], 112.9 [CH], 39.1 [CH_2], 37.5 [CH_3], 35.4 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{20}H_{17}N_4O_2Cl$: 381.1113 $[M+H]^+$, found 381.1150.

General procedure B - Preparation of *N*-fluoroalkylated compounds 27-33 (series 2). To a mixture of indole **26** (100-150 mg) and potassium carbonate (2-5 eq.) in *N*,*N*-dimethylformamide (10-12 mL) was gradually added the alkylating agent (1.3 eq.) in solution in *N*,*N*-dimethylformamide (2 mL). The whole mixture was stirred for 2 to 16 h at 60 °C (depending on the starting material conversion rate), cooled to room temperature, quenched by the addition of a saturated aqueous ammonium chloride solution (50 mL) and extracted with dichloromethane (50 mL). The organic extract was washed with brine (50 mL), dried (Na_2SO_4) and evaporated to dryness to afford the crude product. Purification by silica gel column chromatography (dichloromethane/methanol 99/1 to 97/3) gave the expected compounds **27** to **33** (42-72%).

2-(7-Chloro-5-(2-fluoroethyl)-4-oxo-3-phenyl-4,5-dihydro-3H-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (27). Using general procedure B and 2-fluoroethyl 4-methylbenzenesulfonate as alkylating agent, **27** (133 mg, 59 %) was obtained as white crystals. R_f (dichloromethane/methanol, 19/1): 0.48. t_R (HPLC A) = 1.08 min. Mp: 242-244 °C. ^{1}H NMR (CD_2Cl_2) δ 7.91 (d, J = 8.8 Hz, 1H), 7.68 (d, J = 2.0 Hz, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.52 (t, J = 8.0 Hz, 2H), 7.43 (t, J = 8.0 Hz, 1H), 7.37 (dd, J = 8.8, 2.0 Hz, 1H), 5.09 (dt, $^3J_{H-F}$ = 26.4 Hz, $^3J_{H-H}$ = 4.8 Hz, 2H), 4.90 (dt, $^2J_{H-F}$ = 47.2 Hz, $^3J_{H-H}$ = 4.8 Hz, 2H), 4.20 (s, 2H), 3.22 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.1 [C], 154.9 [C], 141.7 [C], 141.5 [C], 140.6 [C], 133.2 [C], 130.8 [C], 128.5 [2CH], 127.7 [CH], 126.1 [2CH], 123.2 [CH], 122.9 [CH], 119.0 [C], 118.1 [C], 111.2 [d, J = 3.0 Hz, CH], 83.9 [d, $^1J_{C-F}$ = 169.0 Hz, CH_2], 45.5 [d, $^2J_{C-F}$ = 20.0 Hz, CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{22}H_{21}N_4O_2FCl$: 427.1337 $[M+H]^+$, found 427.1344.

2-(7-Chloro-5-(3-fluoropropyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (28). Using general procedure B and 3-fluoropropyl 4-methylbenzenesulfonate as alkylating agent, **28** (67 mg, 58 %) was obtained as white crystals. R_f (dichloromethane/methanol, 19/1): 0.54. t_R (HPLC A) = 1.13 min. Mp: 236-237 °C. ^{1}H NMR (CD_2Cl_2) δ 7.90 (d, J = 8.8 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.63 (d, J = 7.6 Hz, 2H), 7.50 (t, J = 7.6 Hz, 2H), 7.41 (t, J = 7.6 Hz, 1H), 7.35 (dd, J = 8.8, 1.6 Hz, 1H), 4.90 (t, J = 7.2 Hz, 2H), 4.50 (dt, $^2J_{H-F}$ = 47.2 Hz, $^3J_{H-H}$ = 6.0 Hz, 2H), 4.18 (s, 2H), 3.20 (s, 3H), 2.97 (s, 3H), 2.32 (dq^5, $^3J_{H-F}$ = 27.6 Hz, $^3J_{H-H}$ = 6.0 Hz, 2H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 154.7 [C], 141.8 [C], 140.7 [C], 140.5 [C], 133.1 [C], 131.0 [C], 128.5 [2CH], 127.6 [CH], 126.1 [2CH], 123.3 [CH], 122.7 [CH], 119.0 [C], 117.8 [C], 110.7 [CH], 81.3 [d, $^1J_{C-F}$ = 164.0 Hz, CH_2], 41.5 [d, $^3J_{C-F}$ = 5.0 Hz, CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.3 [CH_3], 31.7 [d, $^2J_{C-F}$ = 20.0 Hz, CH_2]. HR-(ESI+)-MS *m/z* calcd for $C_{23}H_{23}N_4O_2FCl$: 441.1493 $[M+H]^+$, found 441.1505.

2-(7-Chloro-5-(4-fluorobutyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide (29). Using general procedure B and 1-bromo-4-fluorobutane as alkylating agent, **29** (125 mg, 72 %) was obtained as white crystals. R_f (dichloromethane/methanol, 19/1): 0.58. t_R (HPLC A) = 1.18 min. Mp: 229-231 °C. ^{1}H NMR (CD_2Cl_2) δ 7.91 (d, J = 8.8 Hz, 1H), 7.63 (m, 3H), 7.52 (t, J = 8.0 Hz, 2H), 7.42 (t, J = 7.2 Hz, 1H), 7.36 (dd, J = 8.8, 1.6 Hz, 1H), 4.87 (t, J = 7.2 Hz, 2H), 4.48 (dt, $^2J_{H-F}$ = 47.2 Hz, $^3J_{H-H}$ = 6.0 Hz, 2H), 4.19 (s, 2H), 3.22 (s, 3H), 2.98 (s, 3H), 2.03 (q^5, J = 7.6 Hz, 2H), 1.80

(dtt, $^3J_{H-F}$ = 25.6 Hz, $^3J_{H-H}$ = 7.6, 6.0 Hz, 2H). ^{13}C NMR (CD_2Cl_2) δ 168.1 [C], 154.7 [C], 141.9 [C], 140.5 [C], 140.4 [C], 132.9 [C], 131.8 [C], 128.5 [2CH], 127.6 [CH], 126.2 [2CH], 123.4 [CH], 122.6 [CH], 119.1 [C], 117.5 [C], 110.8 [CH], 83.7 [d, $^1J_{C-F}$ = 163.0 Hz, CH_2], 44.6 [CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.3 [CH_3], 27.5 [d, $^2J_{C-F}$ = 20.0 Hz, CH_2], 26.6 [d, $^3J_{C-F}$ = 5.0 Hz, CH_2]. HR-(ESI+)-MS *m/z* calcd for $C_{24}H_{25}N_4O_2FCl$: 455.1650 $[M+H]^+$, found 455.1660.

(*E*)-2-(7-Chloro-5-(4-fluorobut-2-en-1-yl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-N,N-dimethylacetamide (30). Using general procedure B and 4-fluorobut-2-yn-1-yl 4-methylbenzenesulfonate (**22**) as alkylating agent, **30** (94 mg, 57 %) was obtained as a white powder. R_f (dichloromethane/acetone, 37/3): 0.40. t_R (HPLC A) = 1.34 min. Mp: 192-193 °C. ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 7.6 Hz, 2H), 7.50 (m, 3H), 7.40 (d, J = 7.6 Hz, 1H), 7.36 (dd, J = 8.8, 1.6 Hz, 1H), 6.06 (m, 1H), 5.72 (m, 1H), 5.53 (bs, 2H), 4.80 (dd, $^2J_{H-F}$ = 46.8 Hz, $^3J_{H-H}$ = 4.4 Hz, 2H), 4.22 (s, 2H), 3.24 (s, 3H), 3.01 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.3 [C], 154.9 [C], 141.5 [C], 140.5 [C], 140.3 [C], 133.5 [C], 130.7 [C], 128.7 [d, $^3J_{C-F}$: 12 Hz, CH], 128.6 [2CH], 127.8 [CH], 127.7 [d, $^2J_{C-F}$ = 17 Hz, CH], 126.1 [2CH], 123.5 [CH], 123.3 [CH], 119.2 [C], 117.9 [C], 110.9 [CH], 82.2 [d, $^1J_{C-F}$ = 164.0 Hz, CH_2], 45.8 [CH_2], 39.6 [CH_2], 37.7 [CH_3], 35.7 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{24}H_{23}N_4O_2FCl$: 453.1493 $[M+H]^+$, found 453.1498.

2-(7-Chloro-5-(4-fluorobut-2-yn-1-yl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (31). Using general procedure B and 4-fluorobut-2-yn-1-yl 4-methylbenzenesulfonate (**23**) as alkylating agent, **31** (110 mg, 66 %) was obtained as a white solid. R_f (dichloromethane/acetone, 19/1): 0.62. t_R (HPLC A) = 1.33 min. Mp: 203-204 °C. ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 8.8 Hz, 1H), 7.68 (s, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.50 (t, J = 8.0 Hz, 2H), 7.40 (t, J = 8.0 Hz, 2H), 5.87 (s, 1H), 5.85 (s, 1H), 4.92 (d, $^2J_{H-F}$ = 47.2 Hz), 4.21 (s, 2H), 3.24 (s, 3H), 3.01 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 168.2 [C], 154.9 [C], 141.3 [C], 140.4 [C], 140.3 [C], 133.7 [C], 130.1 [C], 128.7 [2CH], 127.9 [CH], 126.1 [2CH], 123.6 [CH], 123.5 [CH], 119.4 [C], 118.1 [C], 111.2 [CH], 83.5 [d, $^3J_{C-F}$: 11 Hz, C], 79,2 [d, $^2J_{C-F}$ = 21 Hz, C], 70.4 [d, $^1J_{C-F}$ = 165.0 Hz, CH_2], 39.6 [CH_2], 37.6 [CH_3], 35.7 [CH_3], 34.3 [CH_2]. HR-(ESI+)-MS *m/z* calcd for $C_{24}H_{21}N_4O_2FCl$: 451.1337 $[M+H]^+$, found 451.1342.

2-(7-Chloro-5-(2-(2-fluoroethoxy)ethyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (32). Using general procedure B and 2-(2-fluoroethoxy)ethyl 4-methylbenzenesulfonate (**24**) as alkylating agent, **32** (78 mg, 42 %) was obtained as a white solid. R_f (dichloromethane/methanol, 19/1): 0.45. t_R (HPLC A) = 1.10 min. Mp: 149-150 °C. ^{1}H NMR (CD_2Cl_2) δ 7.90 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 1.6 Hz, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.52 (t, J = 8.0 Hz, 2H), 7.42 (t, J = 8.0 Hz, 1H), 7.35 (dd, J = 8.8, 1.6 Hz, 1H), 5.00 (t, J = 5.6 Hz, 2H), 4.44 (dt, $^2J_{H-F}$ = 47.6 Hz, $^2J_{H-H}$ = 4.0 Hz, 2H), 4.19 (s, 2H), 3.98 (t, J = 5.6 Hz, 2H), 3.66 (dt, $^3J_{H-F}$ = 29.6 Hz, $^2J_{H-H}$ = 4.0 Hz, 2H), 3.22 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (DMF-d_7) δ 168.8 [C], 155.0 [C], 142.7 [C], 141.8 [C], 141.6 [C], 132.5 [C], 131.3 [C], 128.8 [2CH], 127.8 [CH], 126.6 [2CH], 124.1 [CH], 122.8 [CH], 119.5 [C], 118.2 [C], 112.4 [CH], 83.5 [d, $^1J_{C-F}$ = 166.0 Hz, CH_2], 70.4 [d, $^2J_{C-F}$ = 19.1 Hz, CH_2], 45.1 [CH_2], 39.4 [CH_2], 37.2 [CH_3], 34.9 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{24}H_{25}N_4O_3FCl$: 471.1599 $[M+H]^+$, found 471.1594.

2-(7-Chloro-5-(2-(2-(2-fluoroethoxy)ethoxy)ethyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N,N*-dimethylacetamide (33). Using general procedure B and 2-(2-(2-fluoroethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (**25**) as alkylating agent, **33** (99 mg, 49 %) was obtained as a white solid. R_f (dichloromethane/methanol, 19/1): 0.39. t_R (HPLC A) = 1.10 min. Mp: 110-112 °C. ^{1}H NMR (CD_2Cl_2) δ 7.89 (d, J = 8.8 Hz, 1H), 7.78 (d, J = 1.6 Hz, 1H), 7.64 (d, J = 8.0 Hz, 2H), 7.52 (t, J = 8.0 Hz, 2H), 7.42 (t, J = 8.0 Hz, 1H), 7.34 (dd, J = 8.8, 1.6 Hz, 1H), 4.99 (t, J = 5.6 Hz, 2H), 4.42 (dt, $^2J_{H-F}$ = 48.0 Hz, $^2J_{H-H}$ = 4.0 Hz, 2H), 4.19 (s, 2H), 3.94 (t, J = 5.6 Hz, 2H), 3.61-3.51 (m, 6H), 3.22 (s, 3H), 2.99 (s, 3H). ^{13}C NMR (CD_2Cl_2) δ 168.2 [C], 154.8 [C], 141.9 [C], 141.5 [C], 140.5 [C], 132.7 [C], 130.9 [C], 128.4 [2CH], 127.6 [CH], 126.1 [2CH], 123.0 [CH], 122.6 [CH], 118.9 [C], 117.7 [C], 112.1 [CH], 83.2 [d, $^1J_{C-F}$ = 166.0 Hz, CH_2], 71.1 [CH_2], 70.8

[CH_2], 70.5 [CH_2], 70.3 [d, $^2J_{C-F}$ = 19.2 Hz, CH_2], 45.3 [CH_2], 39.5 [CH_2], 37.4 [CH_3], 35.2 [CH_3]. HR-(ESI+)-MS *m/z* calcd for $C_{26}H_{29}N_4O_4FCl$: 515.1861 $[M+H]^+$, found 515.1850.

5.2. Binding studies

Binding affinities for the TSPO 18 kDa (Ki) were determined using membrane homogenates from rat heart and screened against [^{3}H]PK11195 (K_d = 1.8 nM, C = 0.2 nM). Affinities for the CBR were determined at a unique concentration (1 µM), using membrane homogenates from rat cerebral cortex and screened against [^{3}H]flunitrazepam (K_d = 2.1 nM, C = 0.4 nM). This work was done by CEREP (http://www.cerep.fr).

5.3. $LogD_{7.4}$ determination

$LogD_{7.4}$ (*n*-octanol/buffer pH 7.4 partition coefficient) values were determined based on a validated and standardized HPLC method, by conversion of the recorded retention time for each compound (correlation between retention times and known logD values of similar compounds). HPLC conditions: Alliance 2695 - PDA Waters, X-Terra MS C18 (4.6 x 20 mm, 3.5 µm) column ; mobile phase 5 mM MOPS / $(CH_3)_4NOH$ pH 7.4 (A), 5 % MOPS / $(CH_3)_4NOH$ (100 mM, pH 7.4) / 95 % CH_3CN (B) ; gradient (A / B): 98:2 (0.5 min), 0:100 (4.8 min), 98:2 (1.6 min) ; 1.2 mL/min ; 25 °C ; detection at 254 nm.

5.4. Microsomal metabolic stability evaluation

Compounds were incubated with hepatic microsomal fractions (male CD1 mouse, male Sprague-Dawley rat or humans (BD pool)) using the following experimental conditions throughout the study: microsomal proteins concentration = 1 mg/mL; bovine serum albumin (BSA) concentration = 1 mg/mL; substrate concentration = 5 µM; cofactor (if used, see below): 1 mM aq. NADPH. For each compound to be tested, 2 samples were prepared: Sample A: microsomal incubation, 0 min, without cofactor; Sample B: microsomal incubation, 20 min, with cofactor. Enzyme activity was stopped with 1 volume of ACN, and proteins removed by centrifugation. The supernatant fluids were then analysed by HPLC/ESI-MS/MS with the mass spectrometer set in selected ion recording (SIR) in positive mode. The data were collected and processed using MassLynx 4.0 software from Waters-Micromass, leading to quantification of the unchanged tested compound. Analytical HPLC conditions: C18 (125 x 2.1 mm, 3 µm) column; mobile phase: (A) H_2O containing NH_4OAc (0.25 g/L) and HCO_2H (2 mL/L), (B) ACN / MeOH (80 / 20) containing NH_4OAc (0.15 g/L), HCO_2H (2 mL/L) and H_2O (10 mL/L) ; gradient (A/B): 90:10 (0.75 min), 0:100 (3.25 min), 90:10 (2.0 min) ; 0.3 mL/min; injection volume: 10 µL.

The percentages of biotransformation, consisting in oxidative reactions as well as non-co-factor-dependent reactions such as ester bond hydrolysis, were calculated using the following formula and are reported in Table 2:

$$[\%\ \text{biotransformation}] = [1 - (\frac{\text{Peak Area corresponding to unchanged compound in Sample B}}{\text{Peak Area corresponding to unchanged compound in Sample A}})] \times 100$$

5.5. Solubility determination

The solubility in 50 mM phosphate buffer (pH 7.4) was determined as follows: 50 mM phosphate buffer (pH 7.4) [prepared from 40.5 mL aq. 0.1 M Na_2HPO_4 (40.5 mL), aq. 0.1 M NaH_2PO_4 (9.5 mL) and water (volume adjustment to 100 mL)] was added to a known weight of the compound to be tested in order to reach a 2 mg/mL concentration. The mixture was then mixed for 20 h (Rock and Roll shaker), then filtered under vacuum using a Solvinert Millipore plate (0.45 µm, hydrophilic PTFE). The concentration of the filtrate was then determined by HPLC-dosage using a 0.2 mg / mL sonicated (10 min, r.t.)

solution in DMSO of the compound. HPLC conditions: Acquity BEH C18 (50 x 2.1 mm, 1.7 µm) column ; mobile phase: H_2O + 0.05% TFA (A) / CH_3CN + 0.035% TFA (B) ; gradient (A / B): 98:2 (3.5 min), 0:100 (4 min) ; 1 mL/min ; 40 °C.

Acknowledgements

This work was supported by CEA-I^{2}BM intramural programs, as well as the European Union's Seventh Framework Programme [FP7/2007-2013] INMiND (Grant agreement n° HEALTH-F2-2011-278850). The authors wish to thank LGCR Analytical Sciences (Sanofi, Chilly Mazarin, France) for $logD_{7.4}$ measurements and DMPK (Sanofi, Montpellier, France) for preliminary metabolism studies. HRMS determination has benefited from the facilities and expertise of the Small Molecule Mass Spectrometry platform of IMAGIF (www.imagif.cnrs.fr) (Gif-sur-Yvette, France).

References

[1] Venneti, S.; Lopresti, B. J.; Wiley, C. A. The peripheral benzodiazepine receptor (Translocator protein 18kDa) in microglia: from pathology to imaging. *Prog. Neurobiol.* **2006**, *80*, 308-322.

[2] Rupprecht, R.; Papadopoulos, V.; Rammes, G.; Baghai, T.C.; Fan, J.; Akula, N.; Groyer, G.; Adams, D.; Schumacher, M. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat. Rev. Drug Discovery* **2010**, *9*, 971-988.

[3] Papadopoulos, V.; Baraldi, M.; Guilarte, T. R.; Knudsen, T. B.; Lacapere, J. J.; Lindemann, P.; Norenberg, M. D.; Nutt, D.; Weizman, A.; Zhang, M. R.; Gavish, M. Translocator protein (18 kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol. Sci.* **2006**, *27*, 402-409.

[4] Papadopoulos, V.; Amri, H.; Boujrad, N.; Cascio, C.; Culty, M.; Garnier, M.; Hardwick, M.; Li, H.; Vidic, B.; Brown, A.S.; Reversa, J.L.; Bernassau, J.M.; Drieu, K. Peripheral benzodiazepine receptor in cholesterol transport and steroidogenesis. *Steroids* **1997**, *62*, 21-28.

[5] Camsonne, R.; Crouzel, C.; Comar, D.; Mazière, M.; Prenant, C.; Sastre, J.; Moulin, M.A.; Syrota, A. Synthesis of N-(^{11}C) methyl, N-(methyl-1 propyl), (chloro-2 phenyl)-1 isoquinoleine carboxamide-3 (PK 11195): A new ligand for peripheral benzodiazepine receptors. *J. Label. Compds Radiopharm*. **1984**, *21*, 985-991.

[6] Banati, R.B.; Newcombe, J.; Gunn, R.N.; Cagnin, A.; Turkheimer, F.; Heppner, F.; Price, G.; Wegner, F.; Giovannoni, G.; Miller, D.H.; Perkin, D.G.; Smith, T.; Hewson, A.K.; Bydder, G.; Kreutzberg, G.W.; Jones, T.; Cuzner, M.L.; Myers, R. The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative in vivo imaging of microglia as a measure of disease activity. *Brain* **2000**, *123*, 2321-2337.

[7] Damont, A.; Roeda, D.; Dollé, F. The potential of carbon-11 and fluorine-18 chemistry: illustration through the development of positron emission tomography radioligands targeting the translocator protein 18 kDa. *J. Label. Compds Radiopharm.* **2013**, *56*, 96-104.

[8] Roeda, D.; Kuhnast, B.; Damont, A.; Dollé, F. Synthesis of fluorine-18-labelled TSPO ligands for imaging neuroinflammation with Positron Emission Tomography. *J. Fluor. Chem.* **2012**, *134*, 107-114.

[9] Scarf, A. M.; Kassiou, M. The translocator protein. *J. Nucl. Med.* **2011**, *52*, 677-680.

[10] Winkeler, A.; Boisgard, R.; Martin, A.; Tavitian, B. Radioisotopic imaging of neuroinflammation. *J. Nucl. Med.* 2010, *51*, 1-4. (11) Dollé, F.; Luus, C.; Reynolds, A.; Kassiou, M. Radiolabelled molecules for imaging the translocator protein (18 kDa) using positron emission tomography. *Curr. Med. Chem.* **2009**, *16*, 2899-2923.

[12] Chauveau, F.; Boutin, H.; Van Camp, N.; Dollé, F.; Tavitian, B. Nuclear imaging of neuroinflammation: a comprehensive review of [^{11}C]PK11195 challengers. *Eur. J. Nucl. Med. Mol. Imaging.* **2008**, *35*, 2304-2319.

[13] Kuhnast, B.; Damont, A.; Hinnen, F.; Catarina, T.; Demphel, S.; Le Helleix, S.; Coulon, C.; Goutal, S.; Gervais, P.; Dollé, F. [^{18}F]DPA-714, [^{18}F]PBR111 and [^{18}F]FEDAA1106–Selective radioligands for imaging TSPO 18 kDa with PET: Automated radiosynthesis on a TRACERLAb FX-FN synthesizer and quality controls. *Appl. Rad. Isot.* **2012**, *70*, 489-497.

[14] Damont, A.; Hinnen, F.; Kuhnast, B.; Schöllhorn-Peyronneau, M. A.; James, M. L.; Luus, C.; Tavitian, B.; Kassiou, M.; Dollé, F. Radiosynthesis of [^{18}F]DPA-714, a selective radioligand for imaging the translocator protein (18 kDa) with PET. *J Label. Compds Radiopharm.* **2008**, *51*, 286-292.

[15] James, M. L.; Fulton, R. R.; Vercouillie, J.; Henderson, D. J.; Garreau, L.; Chalon, S.; Dollé, F. Costa, B.; Guilloteau, D.; Kassiou, M. DPA-714, a new translocator protein-specific ligand: synthesis, radiofluorination, and pharmacologic characterization. *J. Nucl. Med*. **2008**, *49*, 814-822.

[16] Ferzaz, B.; Brault, E.; Bourliaud, G.; Robert, J. P.; Poughon, G.; Claustre, Y.; Marguet, F.; Liere, P.; Schumacher, M.; Nowicki, J. P.; Fournier, J.; Marabout, B.; Sevrin, M.; George, P.; Soubrie, P.; Benavides, J.; Scatton, B. SSR180575 (7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-*b*]indole-1-acetamide), a peripheral benzodiazepine receptor ligand, promotes neuronal survival and repair. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1067-1078.

[17] Van Camp, N.; Lavisse, S.; Rbah, L.; Aron Badin, R.; Jan, C.; Dollé, F.; Rooney, T.; Brouillet, E.; Hantraye, P. ^{11}C-SSR180575 evaluated under normal and pathological conditions in nonhuman primate brain. *J. Nucl. Med.* **2012**, *53*, 356.

[18] Chauveau, F.; Boutin, H.; Van Camp, N.; Thominiaux, C.; Hantraye, P.; Rivron, L.; Marguet, F.; Castel, M. N.; Rooney, T.; Benavides, J.; Dollé, F.; Tavitian, B. In vivo imaging of neuroinflammation in the rodent brain with [^{11}C]SSR180575, a novel indoleacetamide radioligand of the translocator protein (18 kDa). *Eur. J. Nucl. Med. Mol. Imaging* **2011**, *38*, 509-514.

[19] Thominiaux, C.; Damont, A.; Kuhnast, B.; Demphel, S.; Le Helleix, S.; Boisnard, S.; Rivron, L.; Chauveau, F.; Boutin, H.; Van Camp, N.; Boisgard, R.; Roy, S.; Allen, J.; Rooney, T.; Benavides, J.; Hantraye, P.; Tavitian, B.; Dollé, F. Radiosynthesis of 7-chloro-*N*,*N*-dimethyl-5-[^{11}C]methyl-4-oxo-3-phenyl-3,5-dihydro-4*H*-pyridazino[4,5-*b*]indole-1-acetamide, [^{11}C]SSR180575, a novel radioligand for imaging the TSPO (peripheral benzodiazepine receptor) with PET. *J. Label. Compds Radiopharm*. **2010**, *53*, 767-773.

[20] Noland, W. E.; Baude, F. J. Ethyl indole-2-carboxylate. *Org. Synth.* **1963**, *43*, 40.

[21] Dollé, F.; Hinnen, F.; Emond, P.; Mavel, S.; Mincheva, Z.; Saba, W.; Schöllhorn-Peyronneau, M. A.; Valette, H.; Garreau, L.; Chalon, S.; Halldin, C.; Helfenbein, J.; Legaillard, J.; Madelmont, J. C.; Deloye, J. B.; Bottlaender, M.; Guilloteau, D. Radiosynthesis of [^{18}F]LBT-999, a selective radioligand for the visualization of the dopamine transporter with PET. *J. Label. Compds Radiopharm*. **2006**, *49***,** 687-698.

[22] Reddy, V. K.; Valasinas, A.; Sarkar, A.; Basu, H. S.; Marton, L. J.; Frydman, B. Conformationally restricted analogues of 1N,12N-bisethylspermine: synthesis and growth inhibitory effects on human tumor cell lines. *J. Med. Chem.* **1998**, *41*, 4723-4732.

Table 1. *In vitro* TSPO binding affinity and selectivity of SSR180575 (**1**), its methoxy derivative **11** and the fluorinated analogues **13-19** and **27-33**.

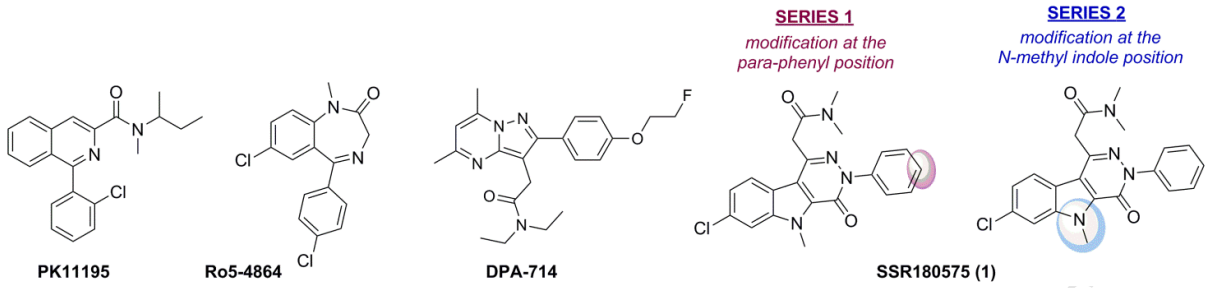
Ligand	TSPO [a] K_i (*nM*)	TSPO/CBR [b] *selectivity*	Log$D_{7.4}$
1	0.83 ± 0.10	> 10^3	2.97
11	0.68 ± 0.09	> 10^3	3.01
13	1.4 ± 0.3	> 10^3	3.12
14	0.40 ± 0.05	> 10^3	3.48
15	0.42 ± 0.08	> 10^3	3.75
16	0.96 ± 0.13	> 10^3	3.62
17	0.30 ± 0.05	> 10^3	3.54
18	4.2 ± 0.2	> 10^3	3.11
19	1.5 ± 0.2	> 10^3	3.08
27	4.1 ± 0.3	> 10^3	3.09
28	61 ± 6	> 10^3	3.38
29	120 ± 13	> 10^2	3.66
30	8.1 ± 1.2	> 10^3	3.48
31	4.2 ± 0.5	> 10^3	3.44
32	130 ± 25	> 10^2	3.20
33	920 ± 53	> 10	3.19

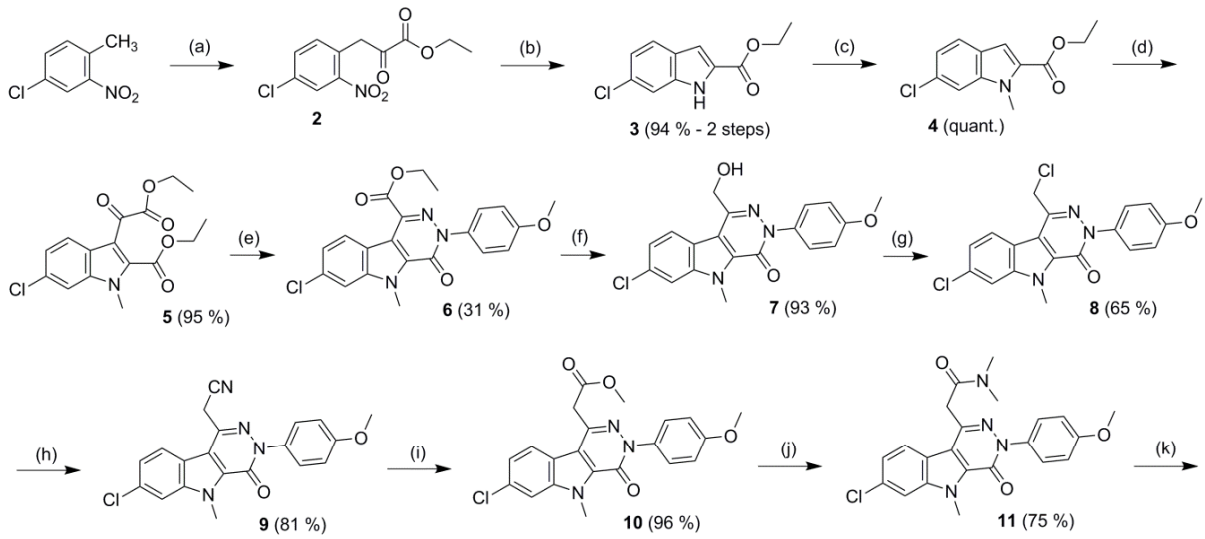
[a] TSPO Ki values (mean ± SD, duplicate) were determined using membrane homogenates of rat heart and screened against [^{3}H]PK11195 (Kd = 1.8 nM, C = 0.2 nM).
[b] CBR binding affinity was evaluated using homogenates of rat cerebral cortex against [^{3}H]flunitrazepam (Kd = 2.1 nM, C = 0.4 nM).
[c] Log$D_{7.4}$ were determined by converting the retention time recorded for the tested compounds using a validated, standardized HPLC method.

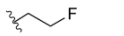
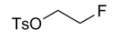
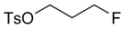
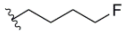
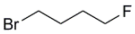
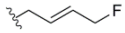
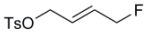
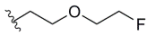
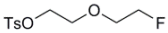
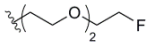
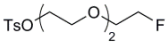
Table 2. In vitro microsomal stability of **1**, **11**, **13-19**, **27** and solubility of all novel analogues.

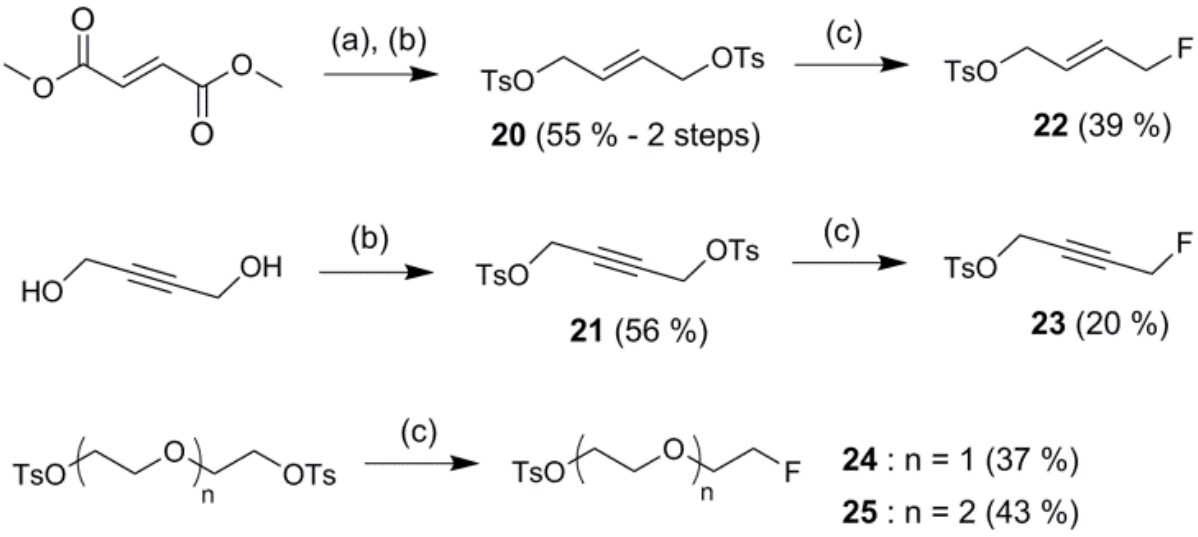
Compound	% biotransformation at 20 min[a]			Solubility[b] (mg/mL)
	Human	Rat	Mouse	
SSR180575 **(1)**	10 %	14 %	21 %	$< 10^{-3}$
11	22 %	4 %	22 %	$1.\ 10^{-3}$
13	0 %	2 %	0 %	$< 10^{-3}$
14	4 %	3 %	4 %	$< 10^{-3}$
15	16 %	13 %	1 %	$< 10^{-3}$
16	76 %	41 %	53 %	$< 10^{-3}$
17	47 %	25 %	42 %	$6.\ 10^{-3}$
18	17 %	11 %	17 %	$< 10^{-3}$
19	81 %	52 %	69 %	$< 10^{-3}$
27	19 %	14 %	5 %	$1.\ 10^{-3}$
28	n.d.	n.d.	n.d.	$1.\ 10^{-3}$
29	n.d.	n.d.	n.d.	$< 10^{-3}$
30	38 %	44 %	31 %	$< 10^{-3}$
31	30 %	26 %	39 %	$< 10^{-3}$
32	n.d.	n.d.	n.d.	$11.\ 10^{-3}$
33	n.d.	n.d.	n.d.	$27.\ 10^{-3}$

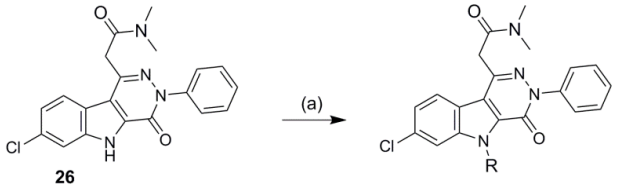
[a] hepatic microsomal (male CD1 mouse, male Sprague-Dawley rat or humans (BD pool)) incubation, followed by analysis of the supernatant using HPLC/ESI-MS/MS; [b] Solubility in 50 mM phosphate buffer (pH 7.4).

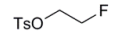
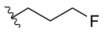
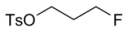
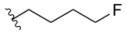
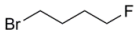
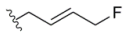
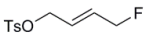
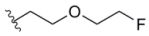
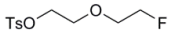
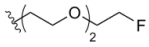
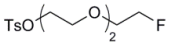




Compound	R	Alkylating agent	Yield
13			63 %
14			58 %
15			70 %
16		 (**22**)	50 %
17		 (**23**)	35 %
18		 (**24**)	69 %
19		 (**25**)	63 %





Compound	R	Alkylating agent		Yield
27				59 %
28				58 %
29				72 %
30			(**22**)	57 %
31			(**23**)	66 %
32			(**24**)	42 %
33			(**25**)	49 %

Highlights

- Fifteen novel pyridazino[4,5-b]indole-1-acetamides, closely related to SSR180575, were synthesized as TSPO ligands.
- Four compounds exhibited higher affinity for the TSPO than the parent molecule.
- Two candidates stand out as promising candidates for drug development and also provide unique opportunities for fluorine-18-labeling and *in vivo* PET-imaging of neuroinflammation.

SUPPLEMENTARY DATA

Synthesis and *in vitro* characterization of novel fluorinated derivatives of the TSPO 18 kDa ligand SSR180575

Annelaure Damont [a,b], Frank Marguet [c], Frédéric Puech [c], Frédéric Dollé [a,b]

[a] *CEA, I2BM, Service Hospitalier Frédéric Joliot, Orsay, France.*

[b] *Inserm / CEA / Université Paris Sud, UMR 1023 – ERL 9218 CNRS, IMIV, Orsay, France.*

[c] *Exploratory Unit, Sanofi, Chilly-Mazarin, France.*

Corresponding Author.

Annelaure Damont. CEA, I2BM, Service Hospitalier Frédéric Joliot, 4 Place du Général Leclerc, 91400 Orsay, France.

E-mail address: annelaure.damont@cea.fr

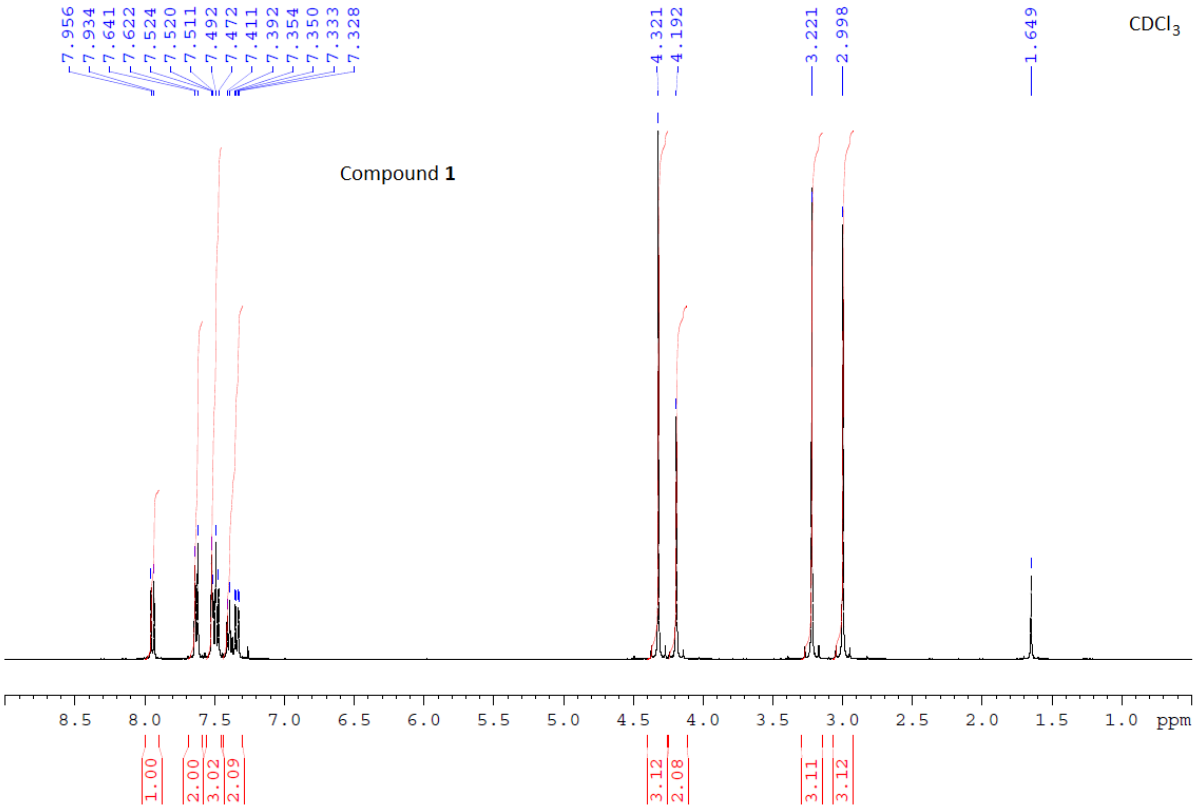
Table of contents

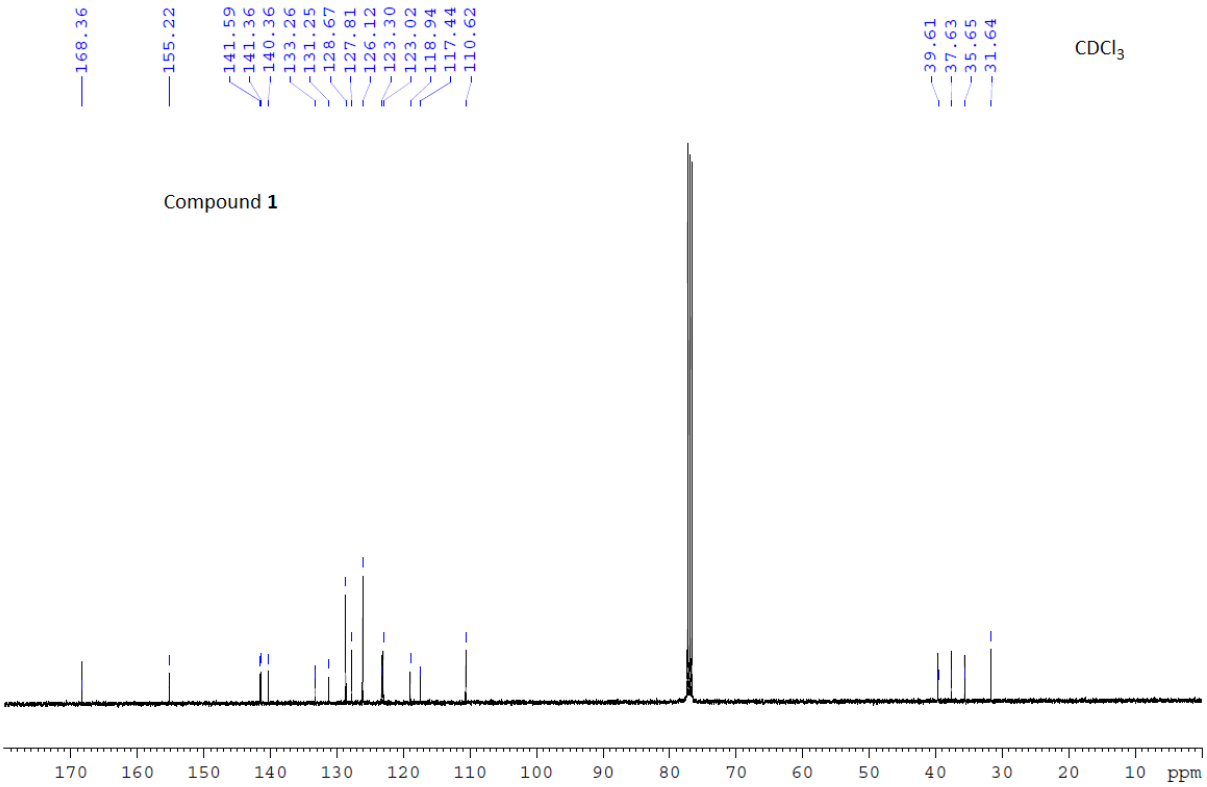
*^{1}H and ^{13}C NMR spectra for compounds **1**, **11-19** and **26-33**:*

Compound **1** (SSR180575):

2-(7-Chloro-3-phenyl-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$)

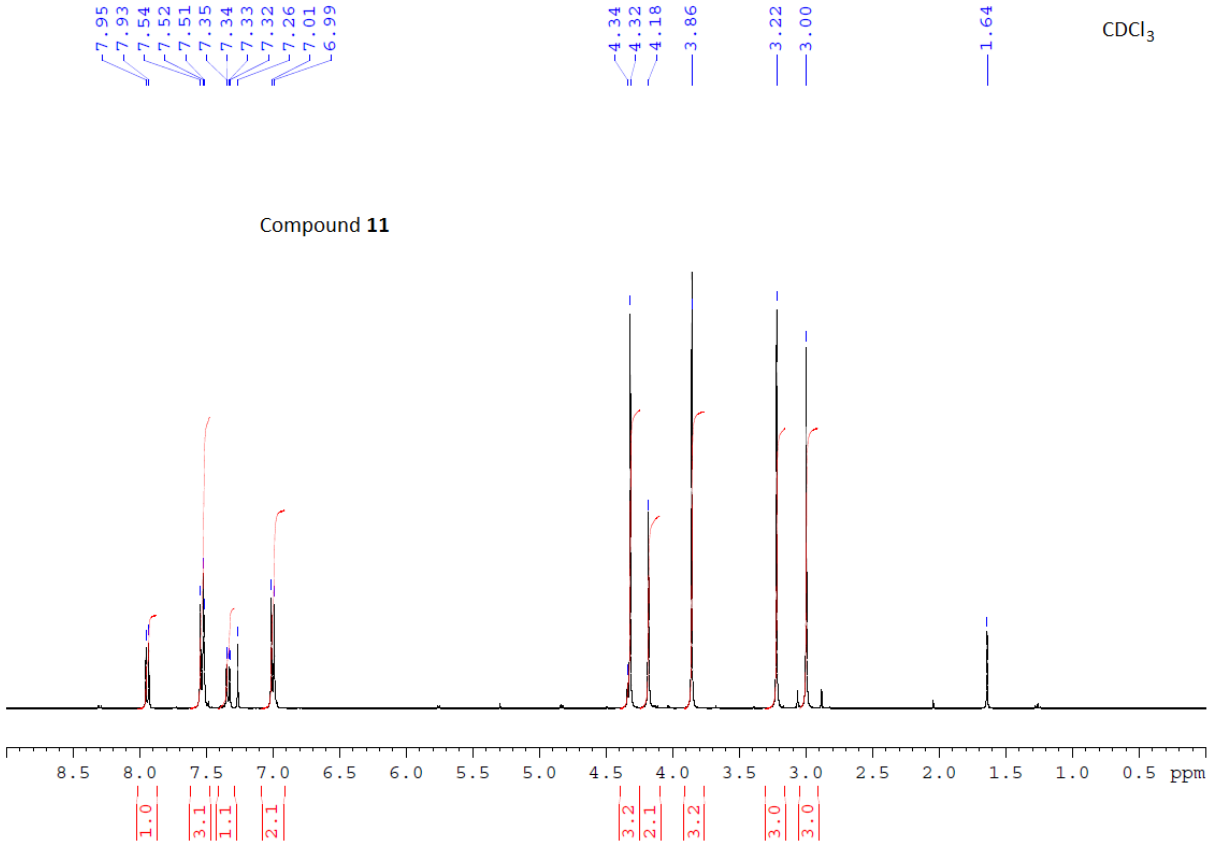


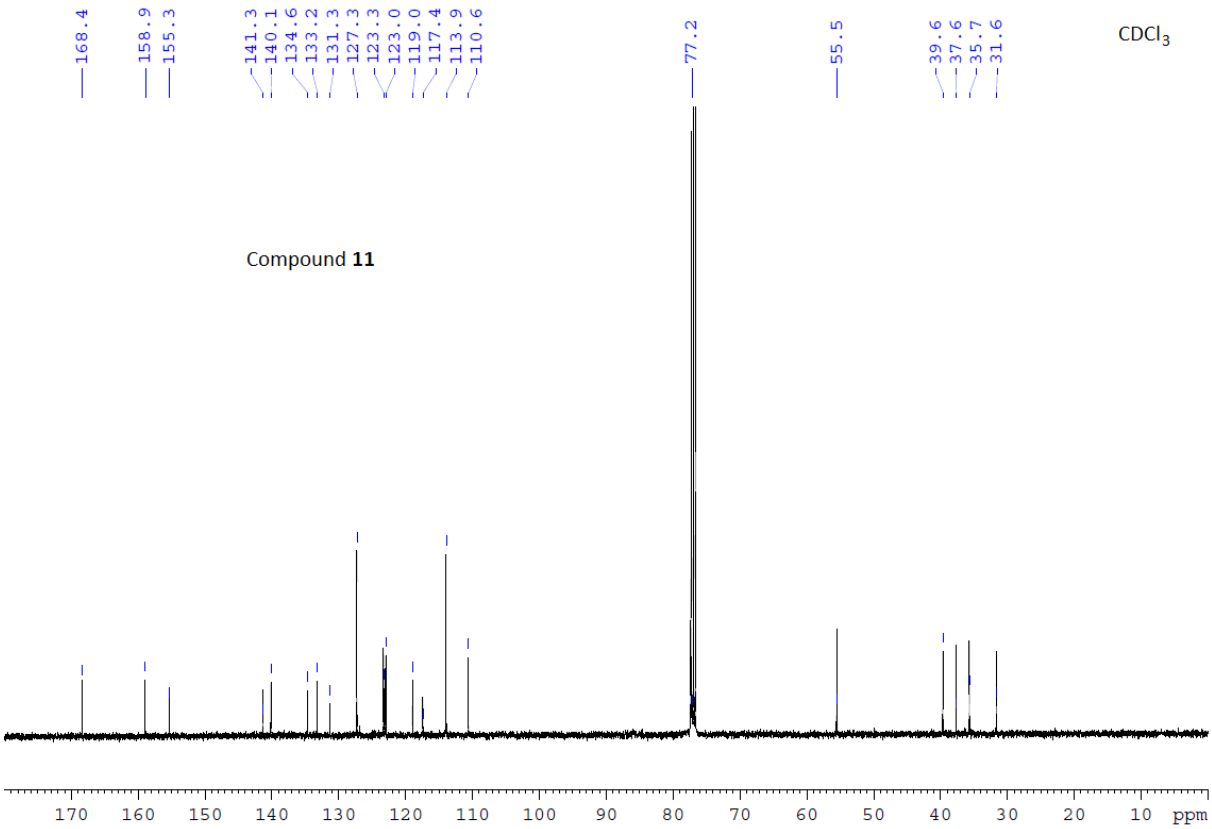


Compound **11**:

2-(7-Chloro-3-(4-methoxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$)

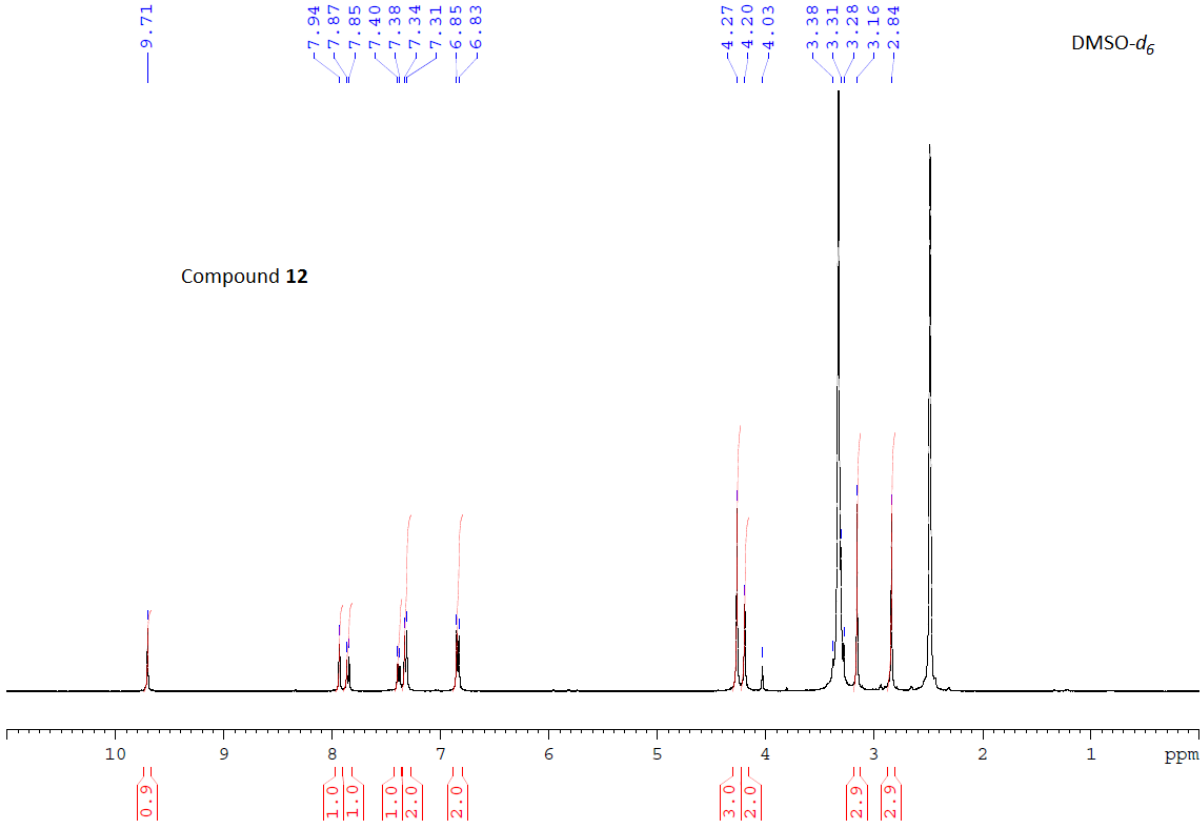


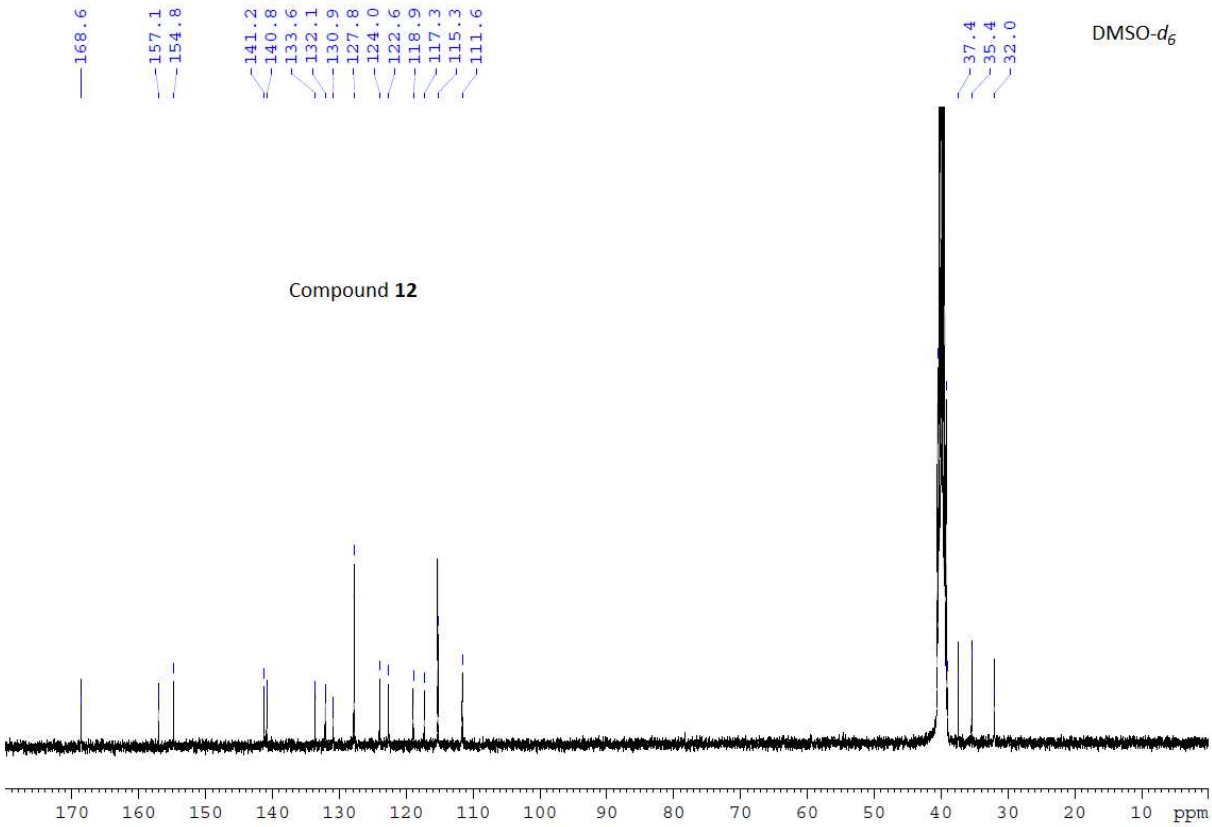


Compound **12**:

2-(7-Chloro-3-(4-hydroxyphenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6)

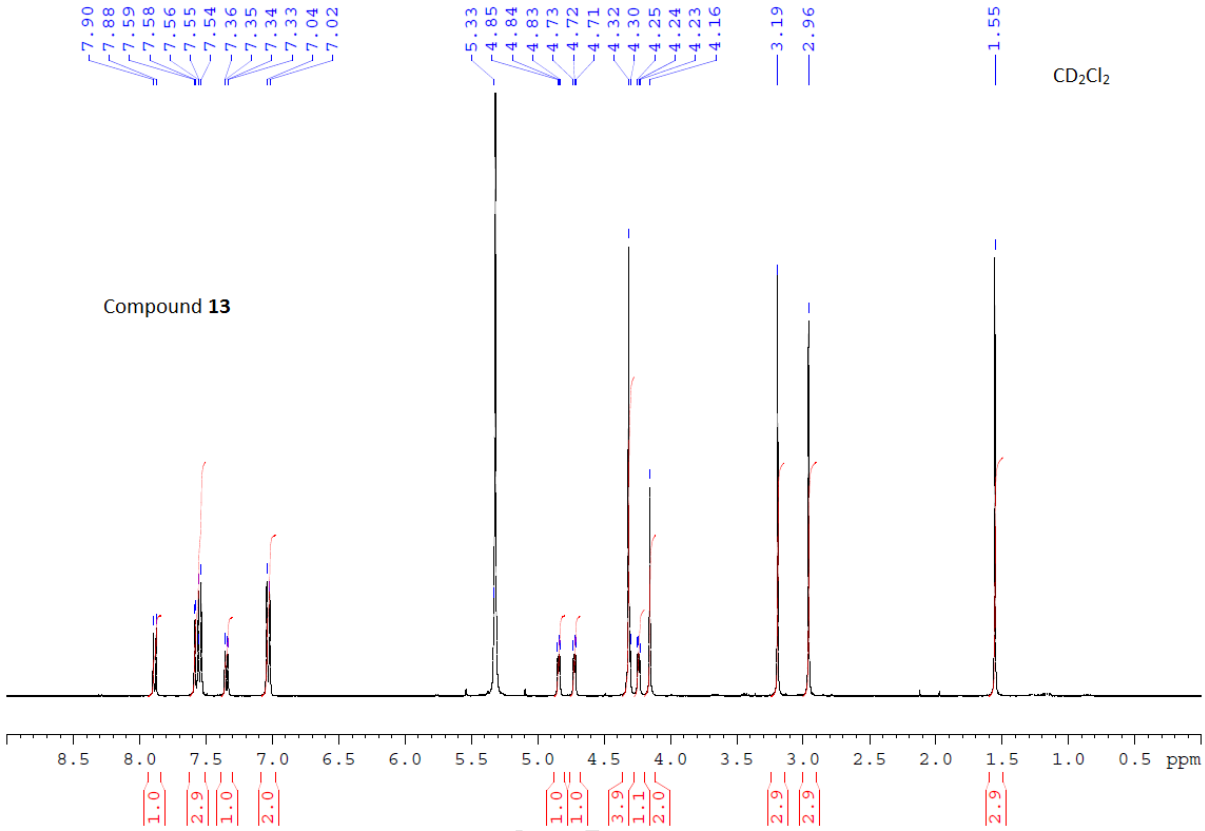


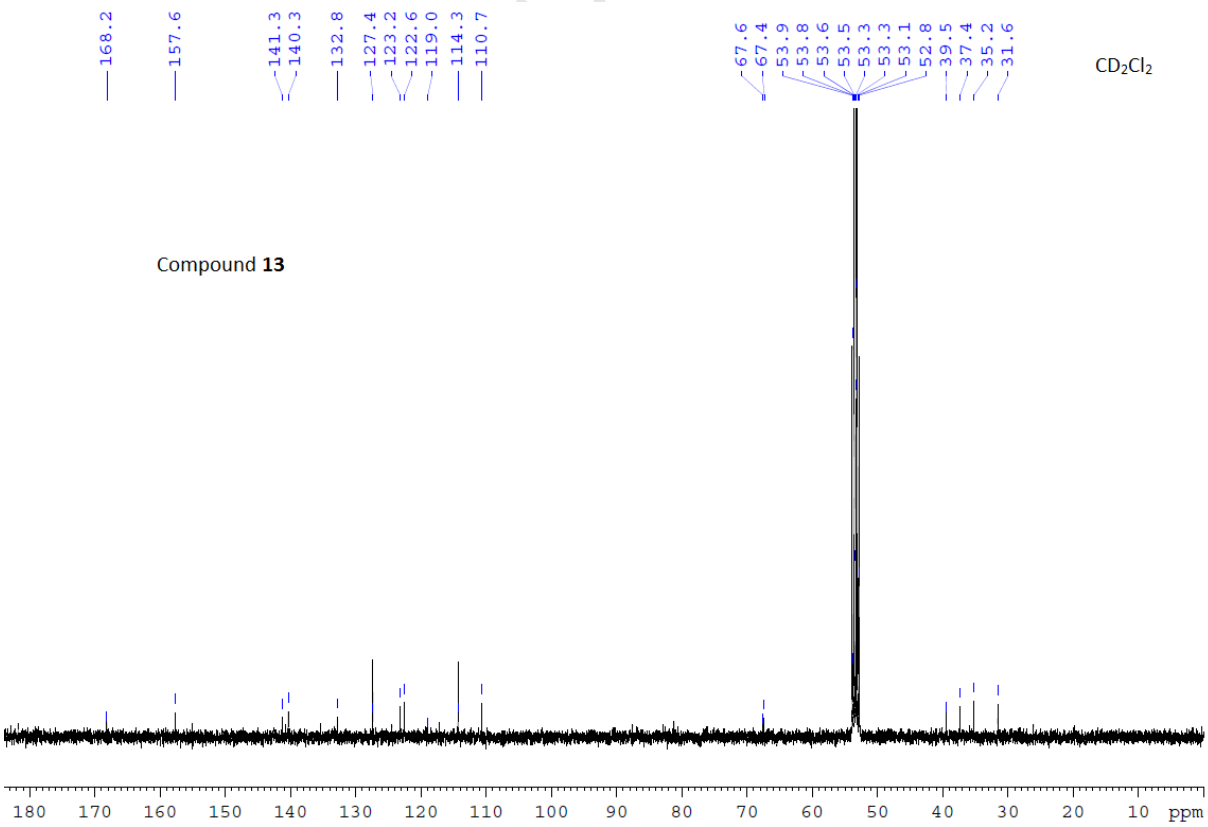


Compound **13**:

2-(7-Chloro-3-(4-(2-fluoroethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

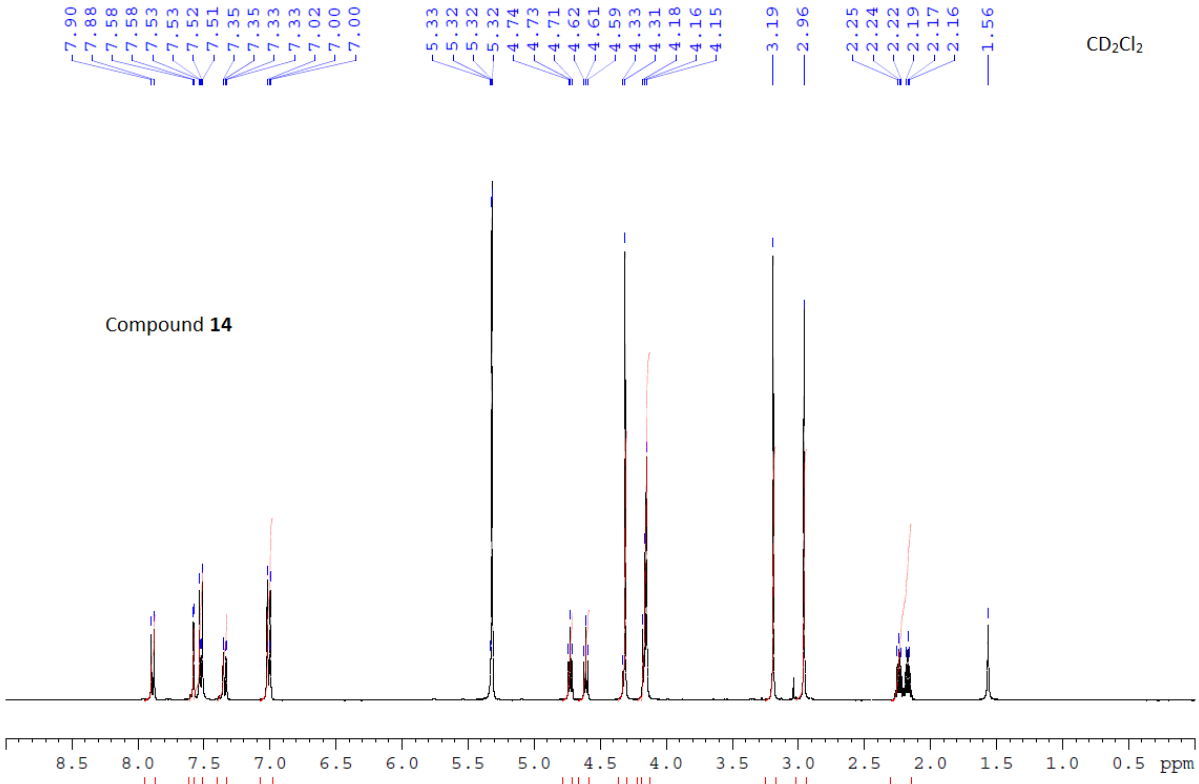


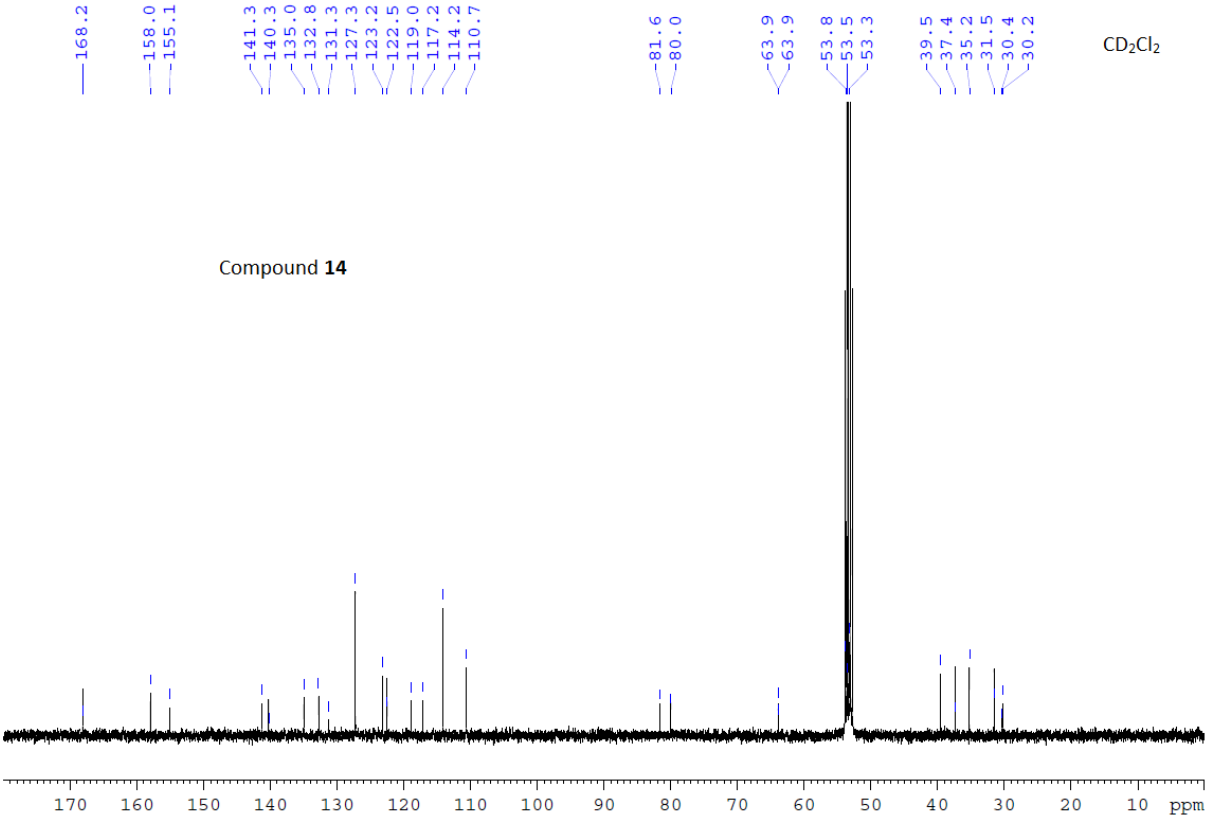


Compound **14**:

2-(7-Chloro-3-(4-(3-fluoropropoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

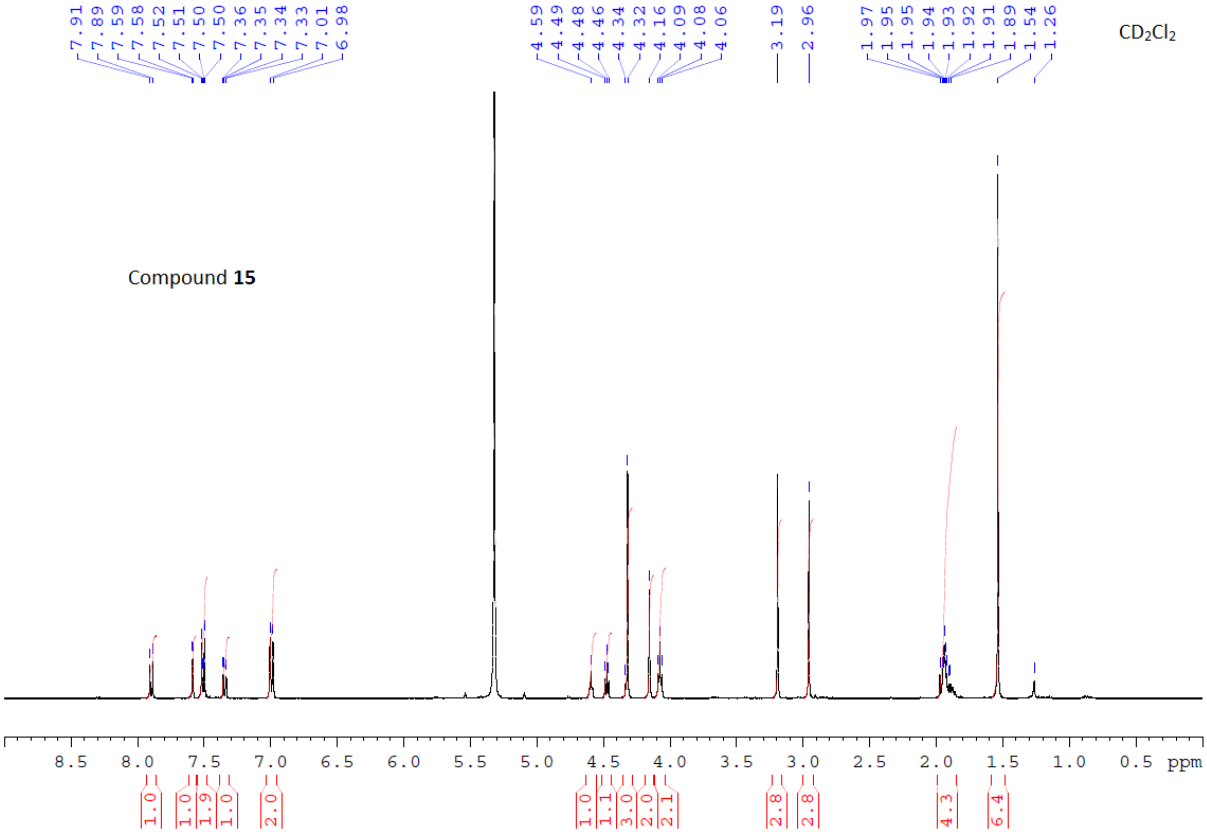


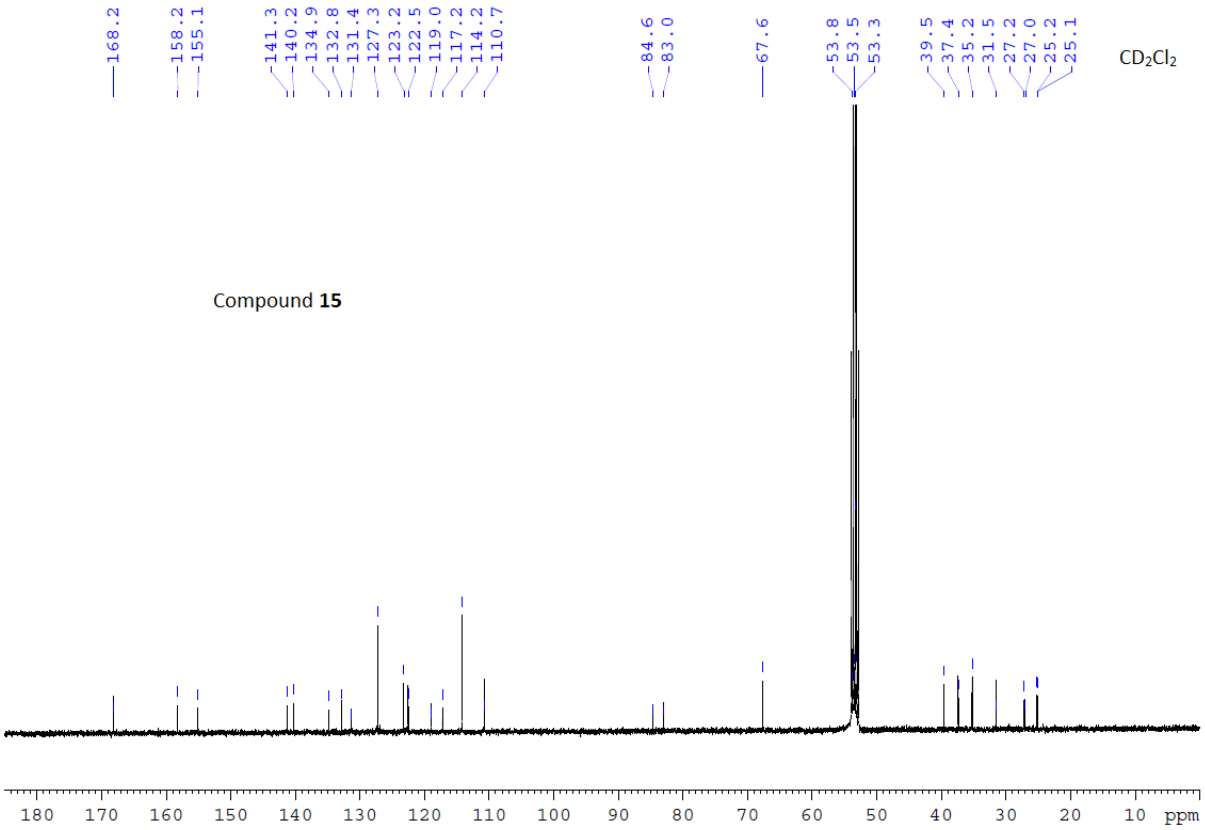


Compound **15**:

2-(7-Chloro-3-(4-(4-fluorobutoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

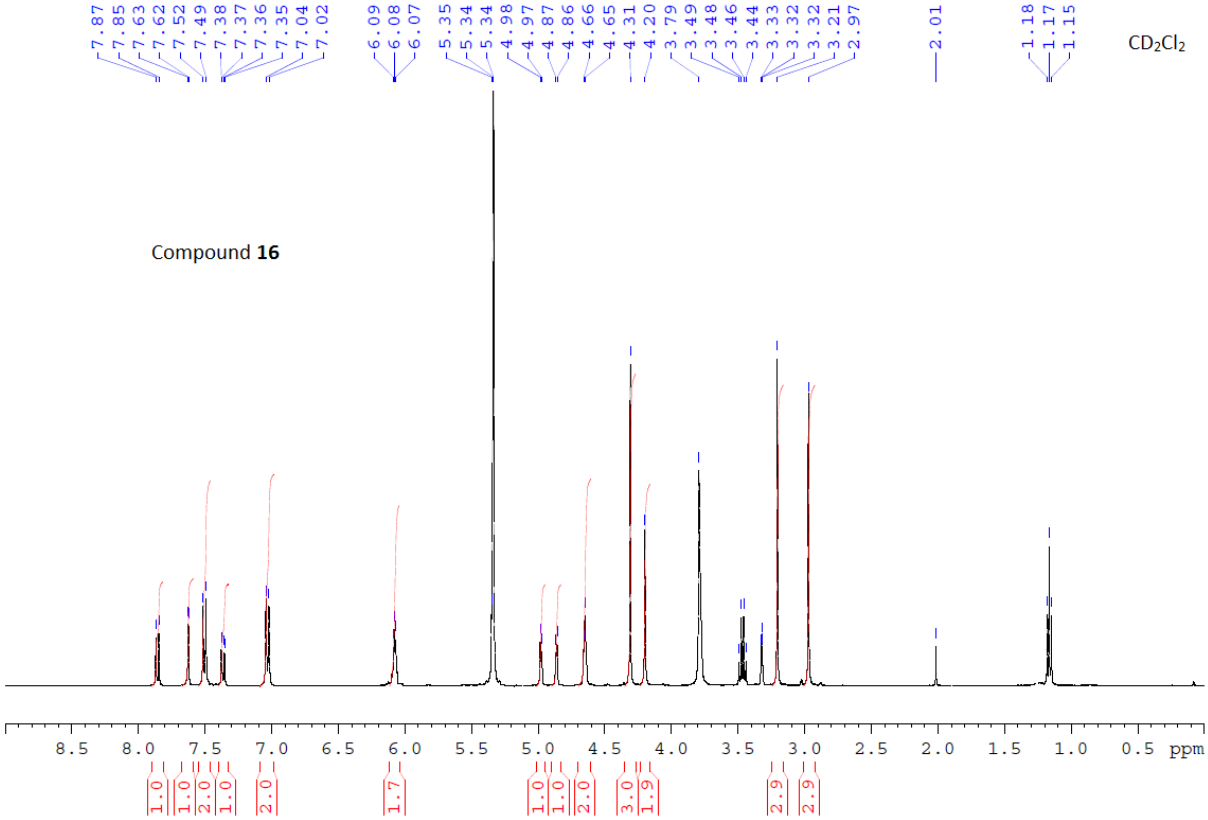


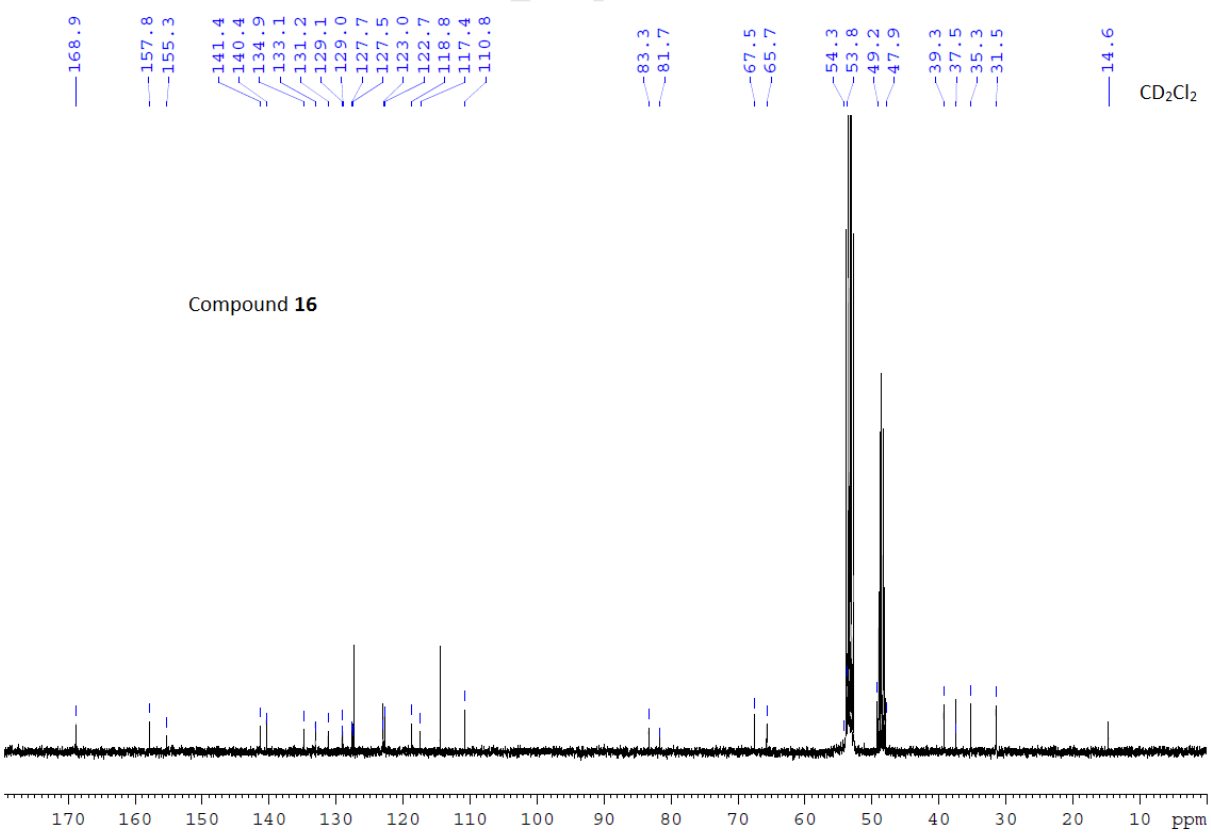


Compound **16**:

(*E*)-2-(7-Chloro-3-(4-((4-fluorobut-2-en-1-yl)oxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

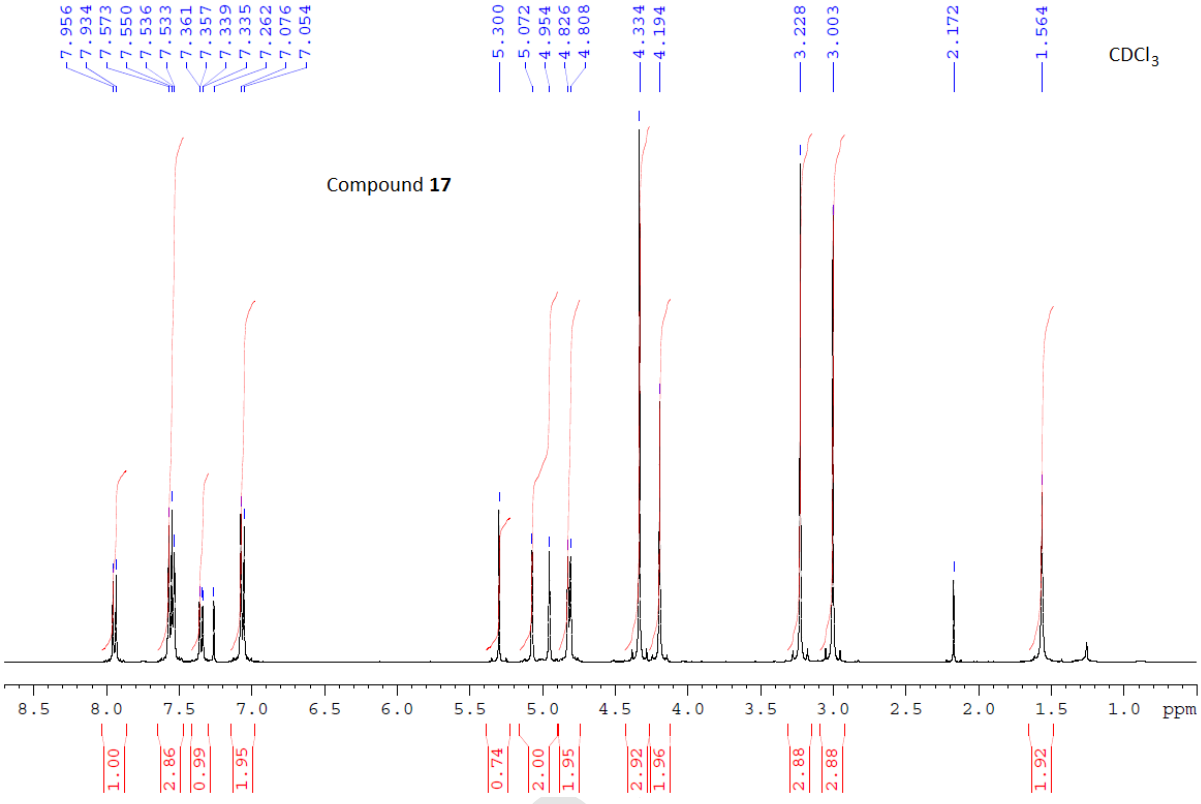


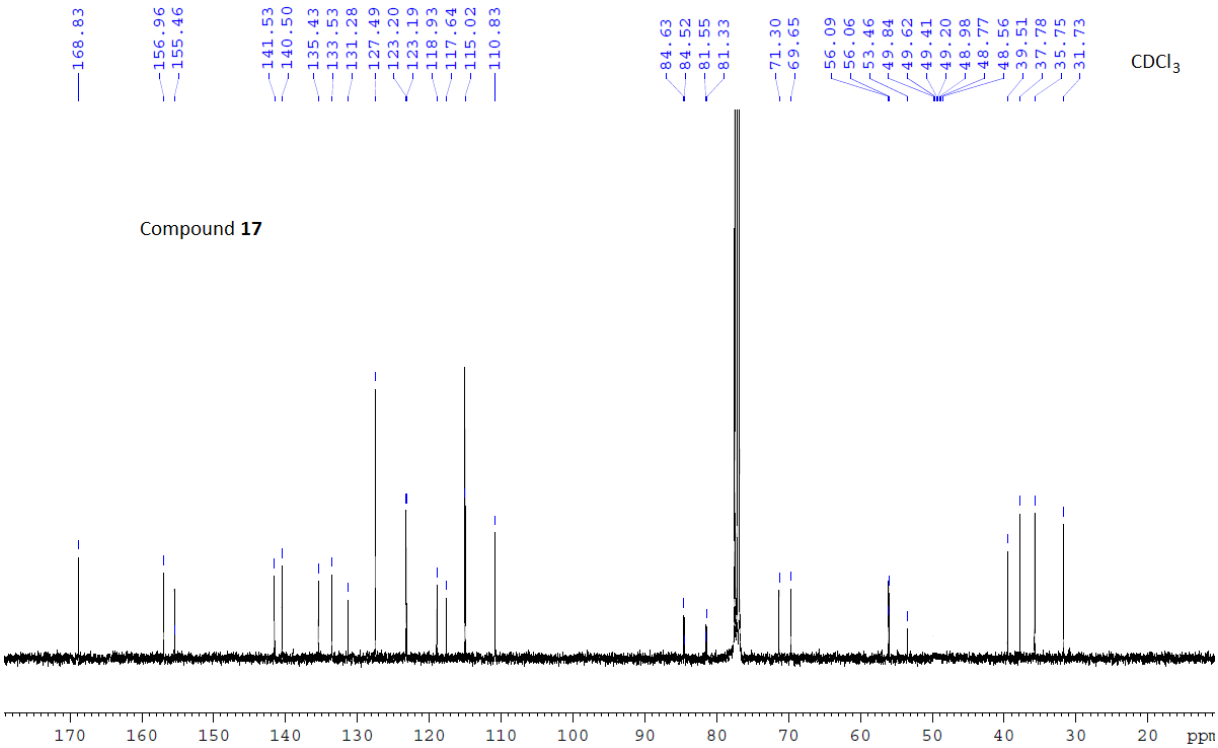


Compound **17**:

2-(7-Chloro-3-(4-((4-fluorobut-2-yn-1-yl)oxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$)

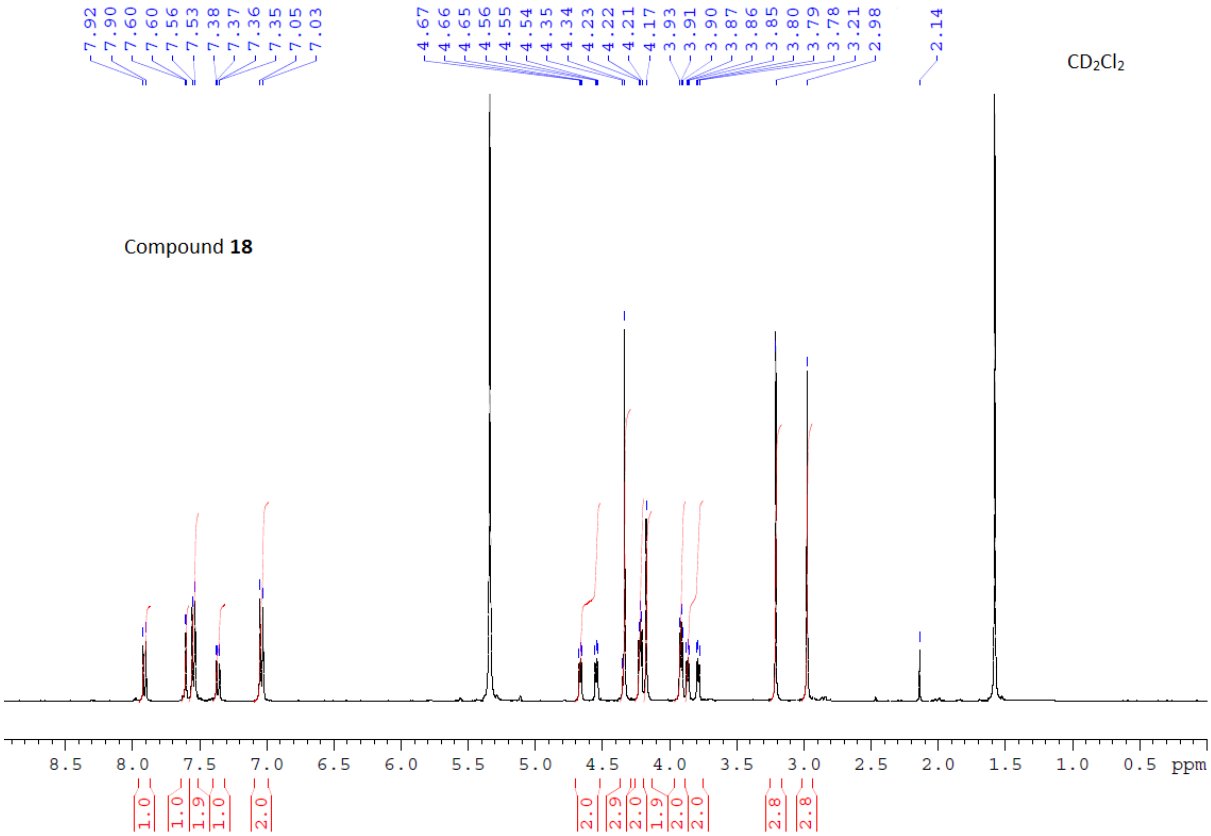


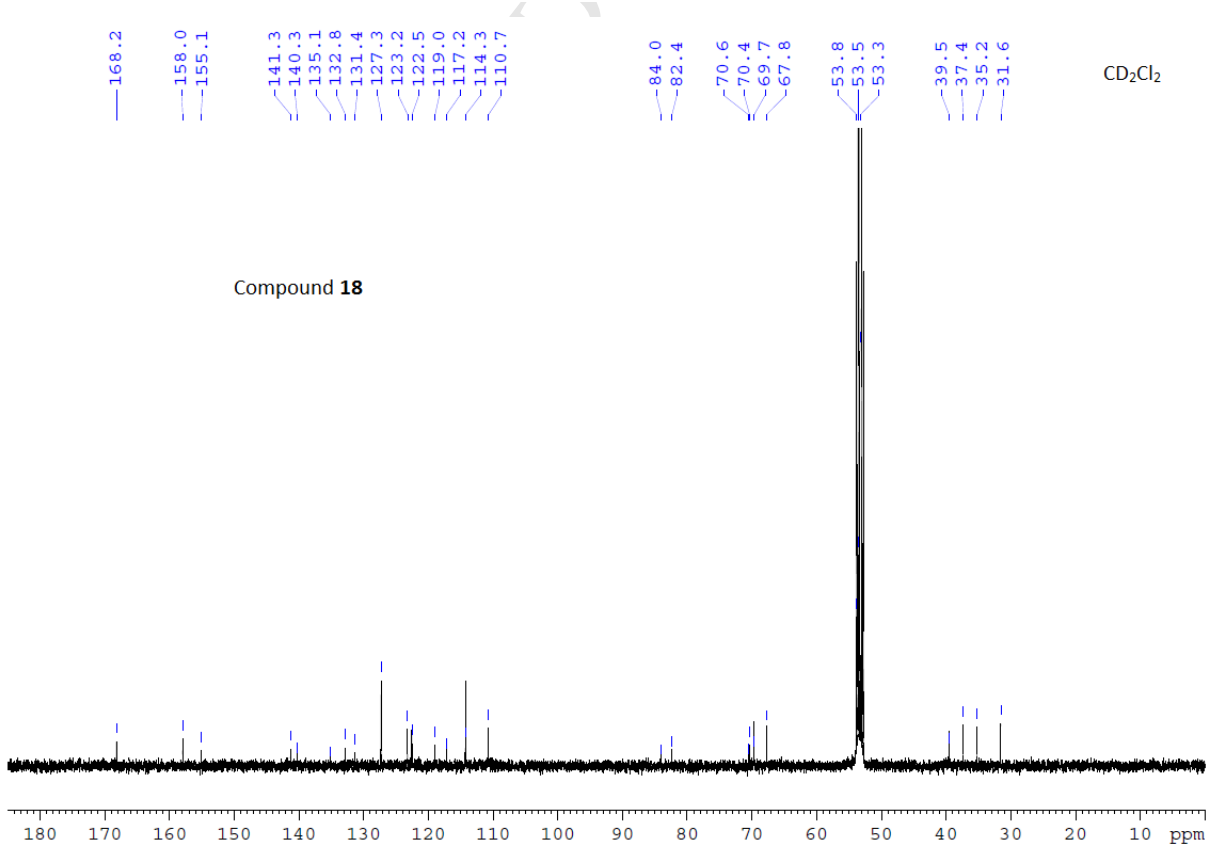


Compound **18**:

2-(7-Chloro-3-(4-(2-(2-fluoroethoxy)ethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

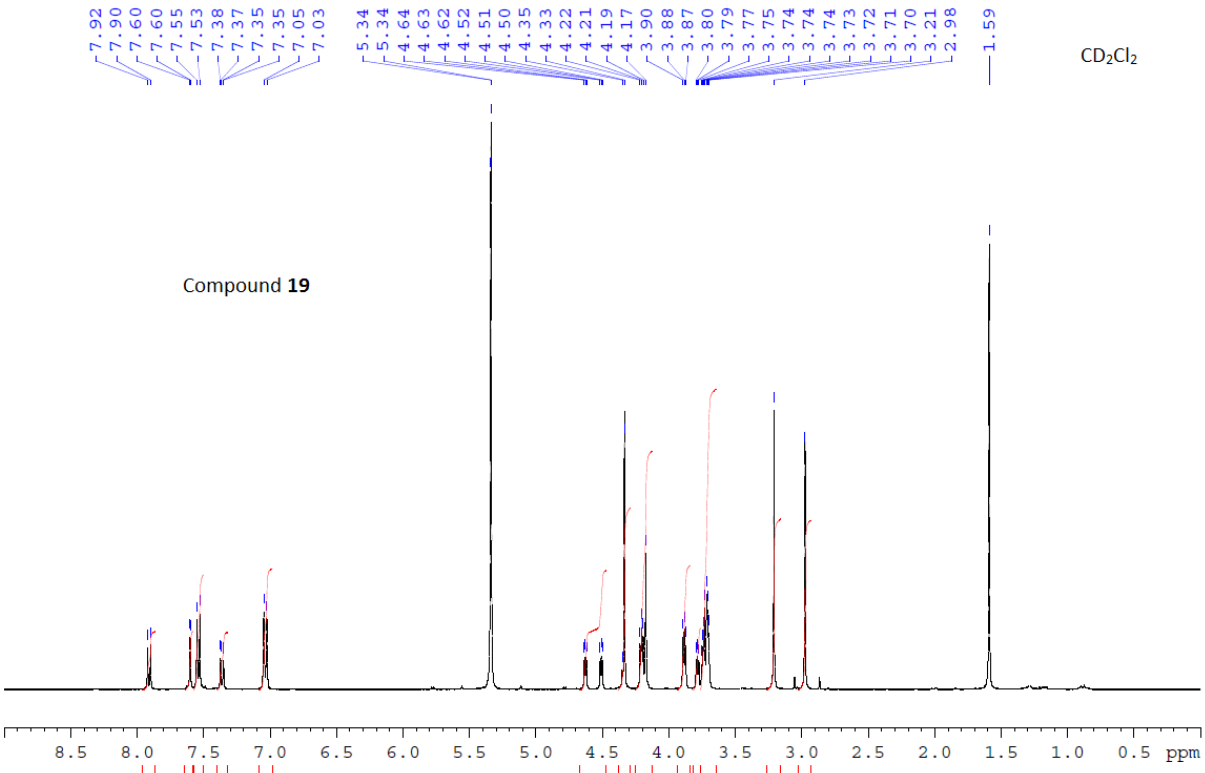


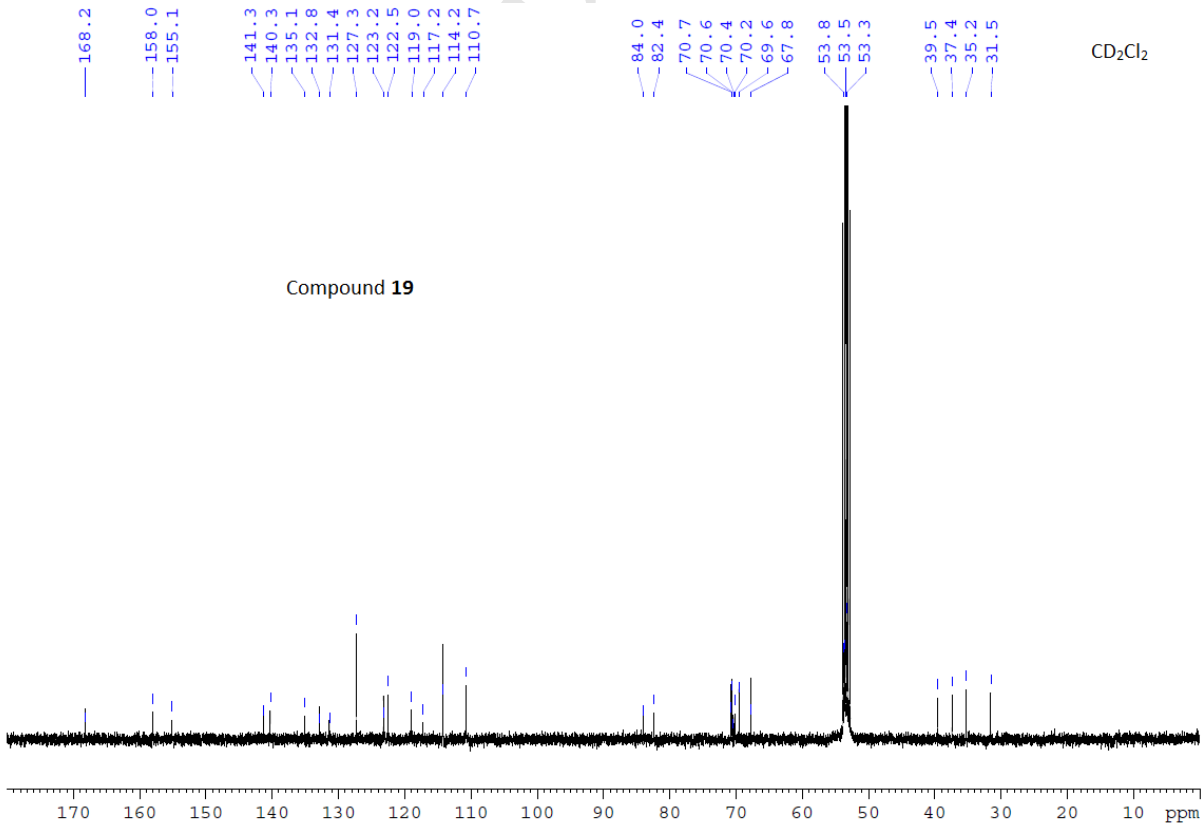


Compound **19**:

2-(7-Chloro-3-(4-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)phenyl)-5-methyl-4-oxo-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

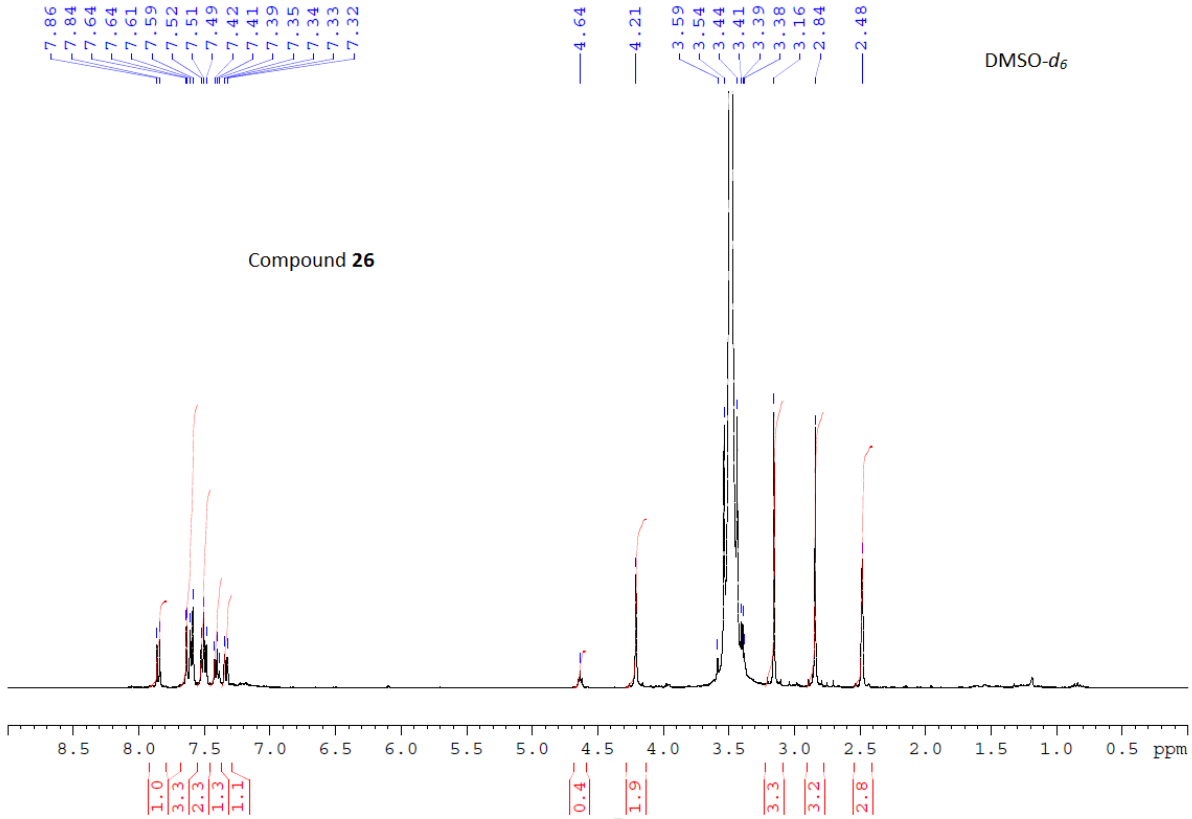


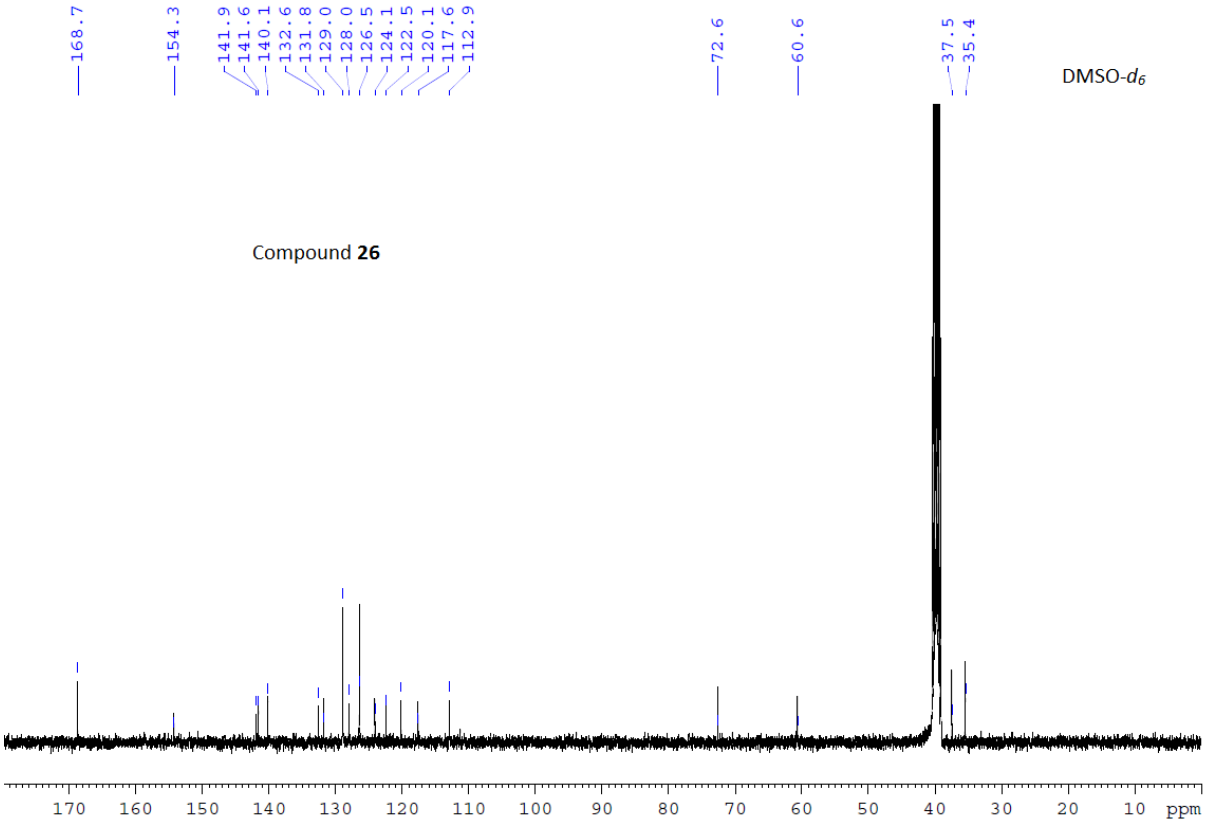


Compound **26**:

2-(7-Chloro-4-oxo-3-phenyl-4,5-dihydro-3H-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6)

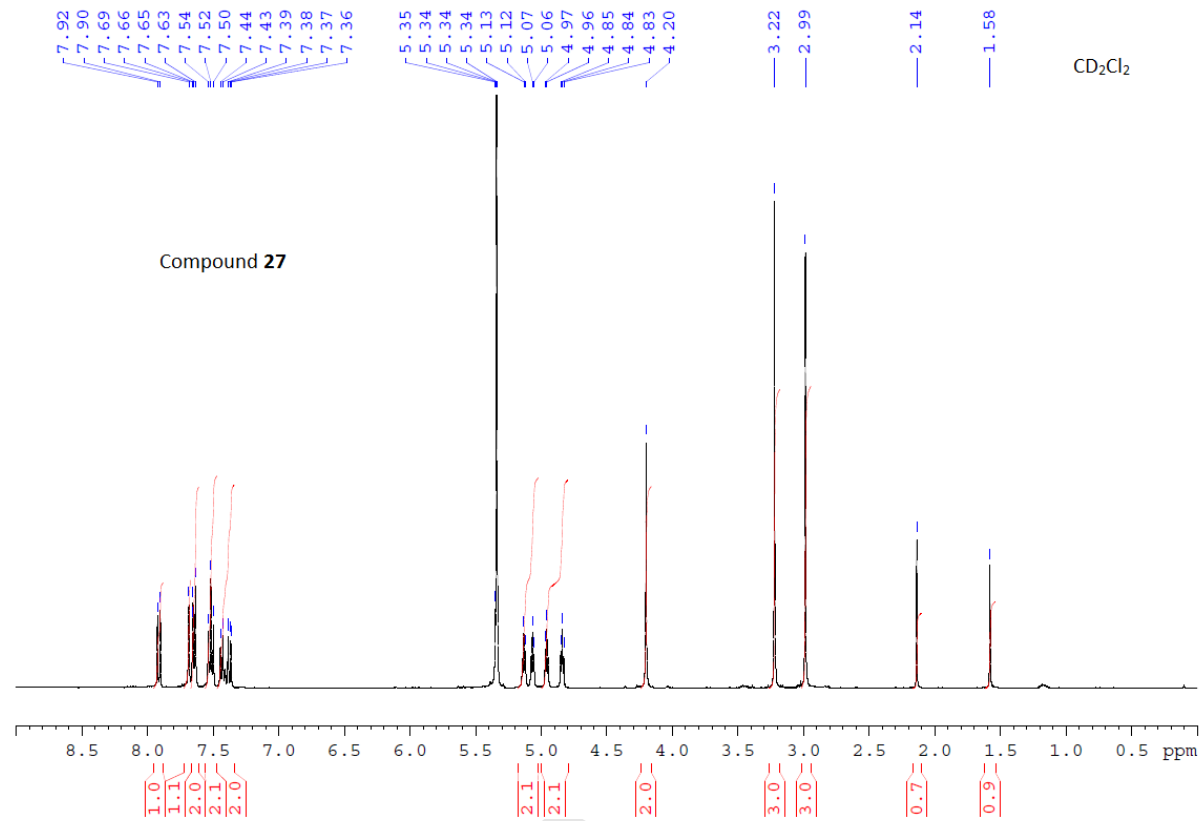


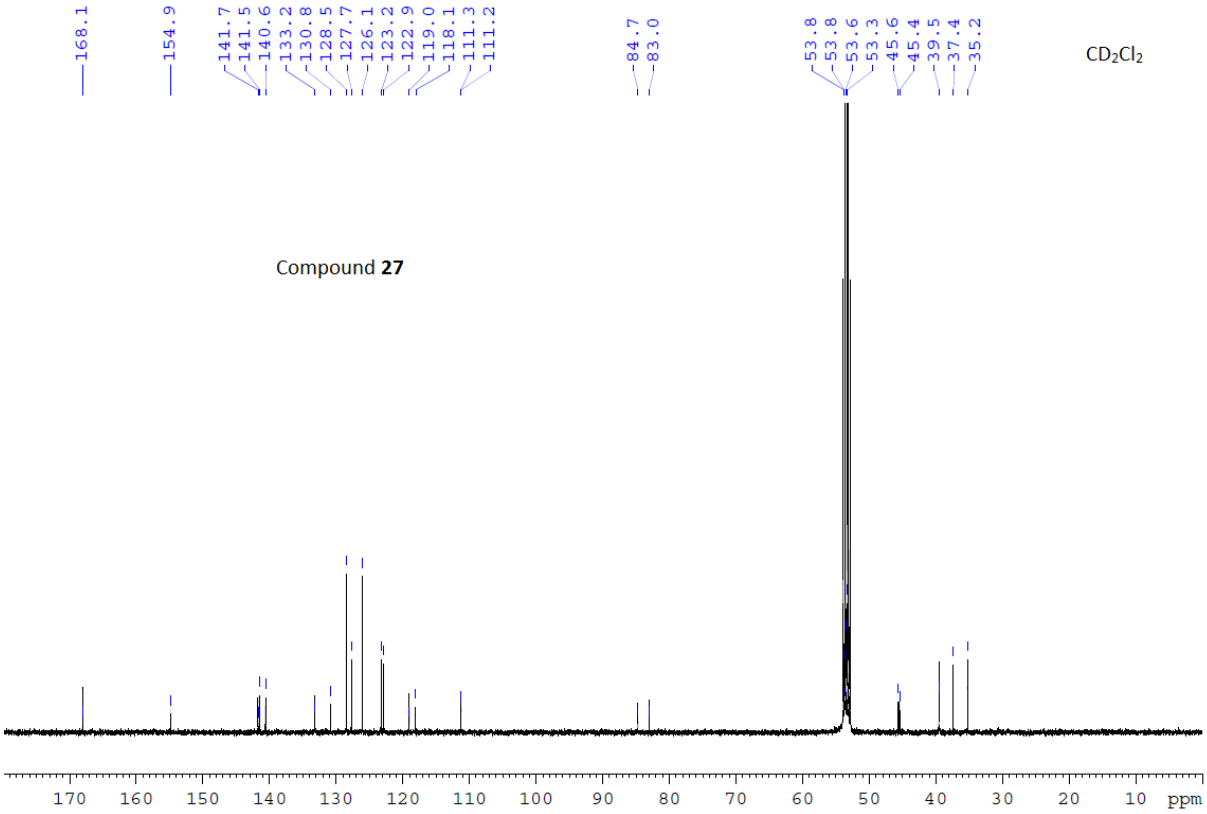


Compound **27**:

2-(7-Chloro-5-(2-fluoroethyl)-4-oxo-3-phenyl-4,5-dihydro-3H-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

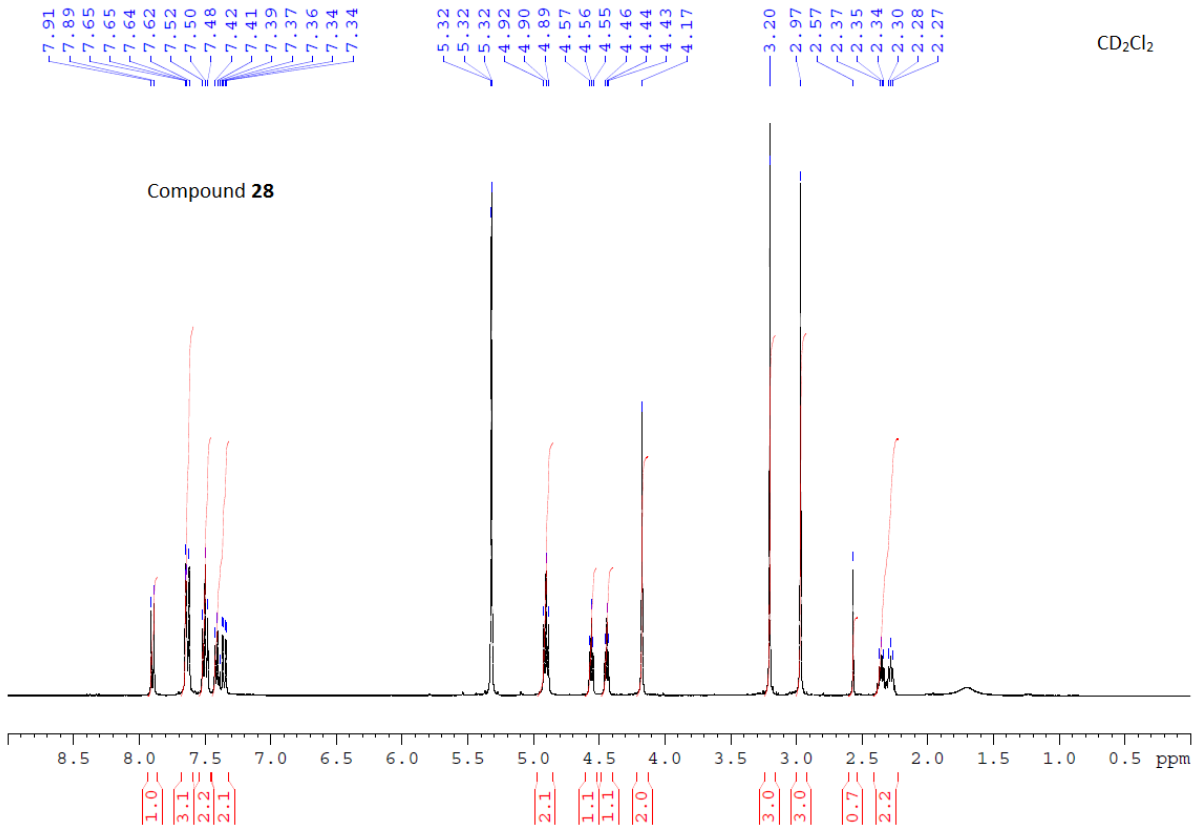


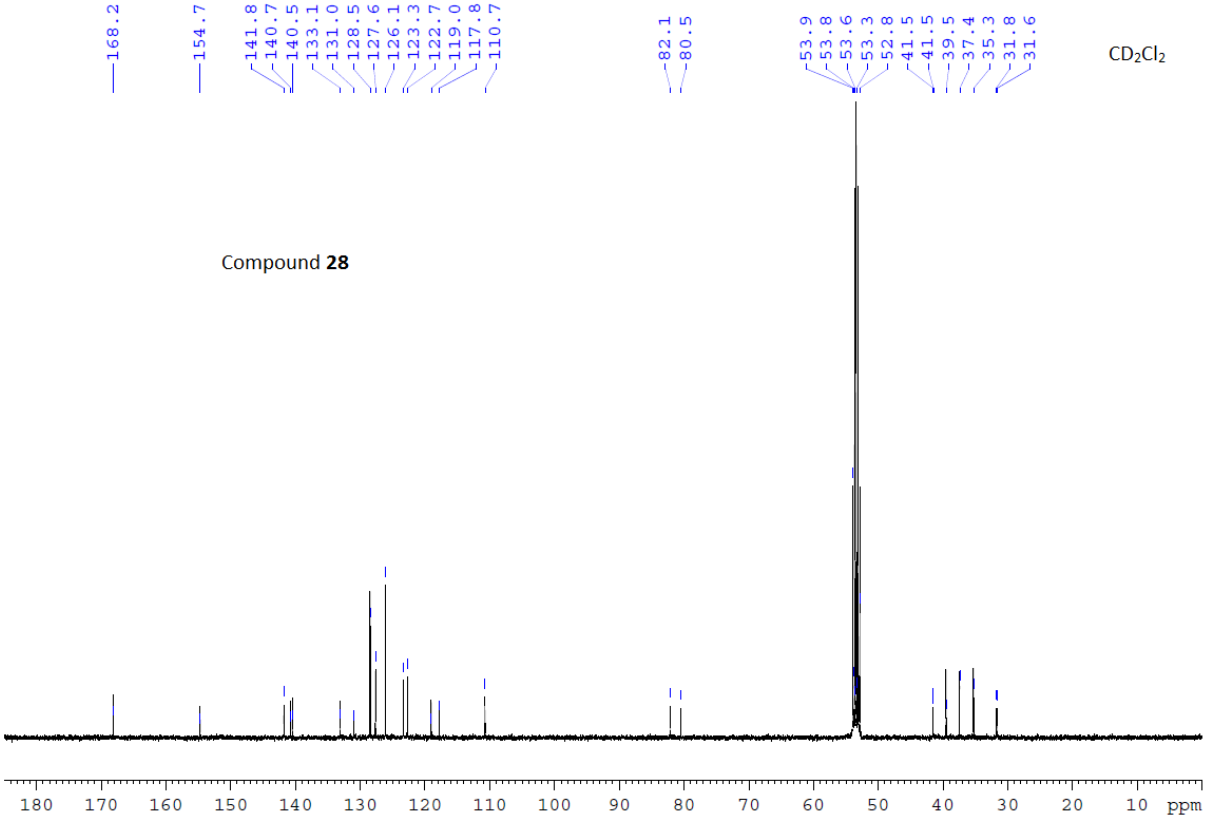


Compound **28**:

2-(7-Chloro-5-(3-fluoropropyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

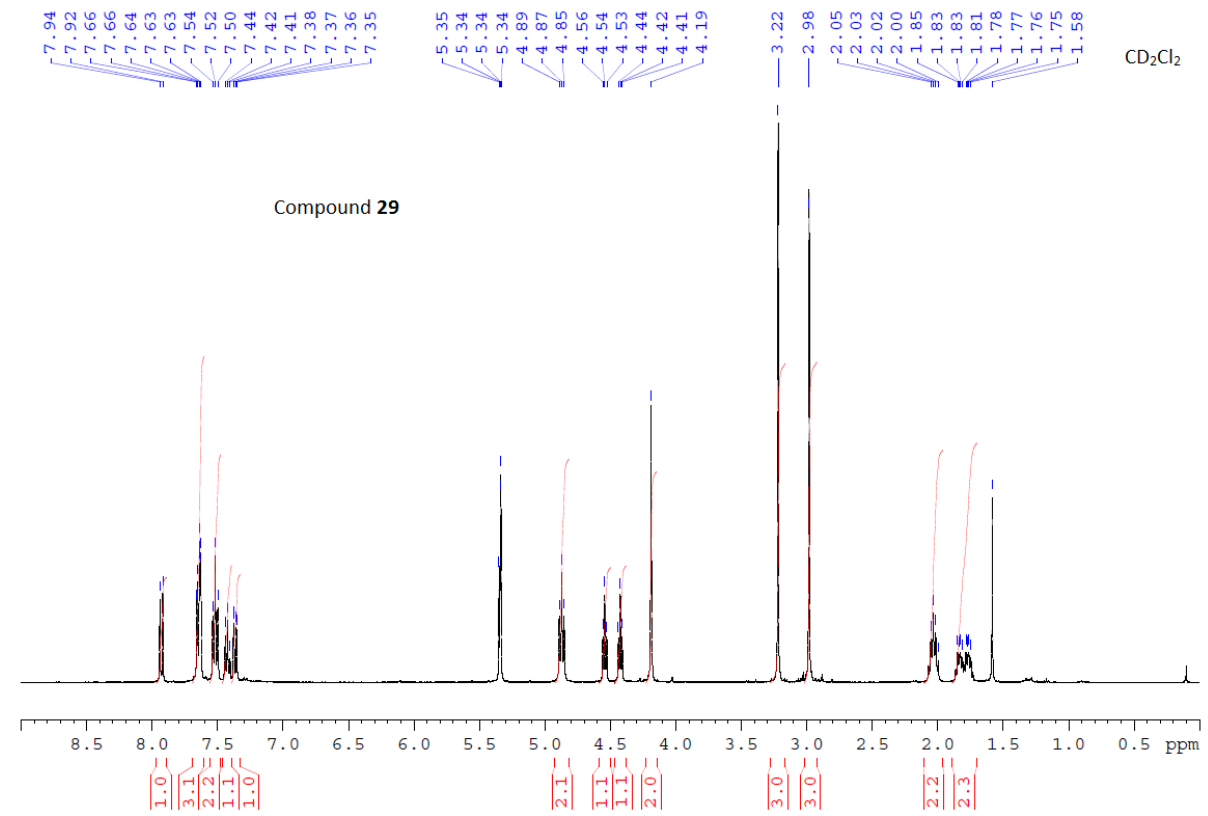


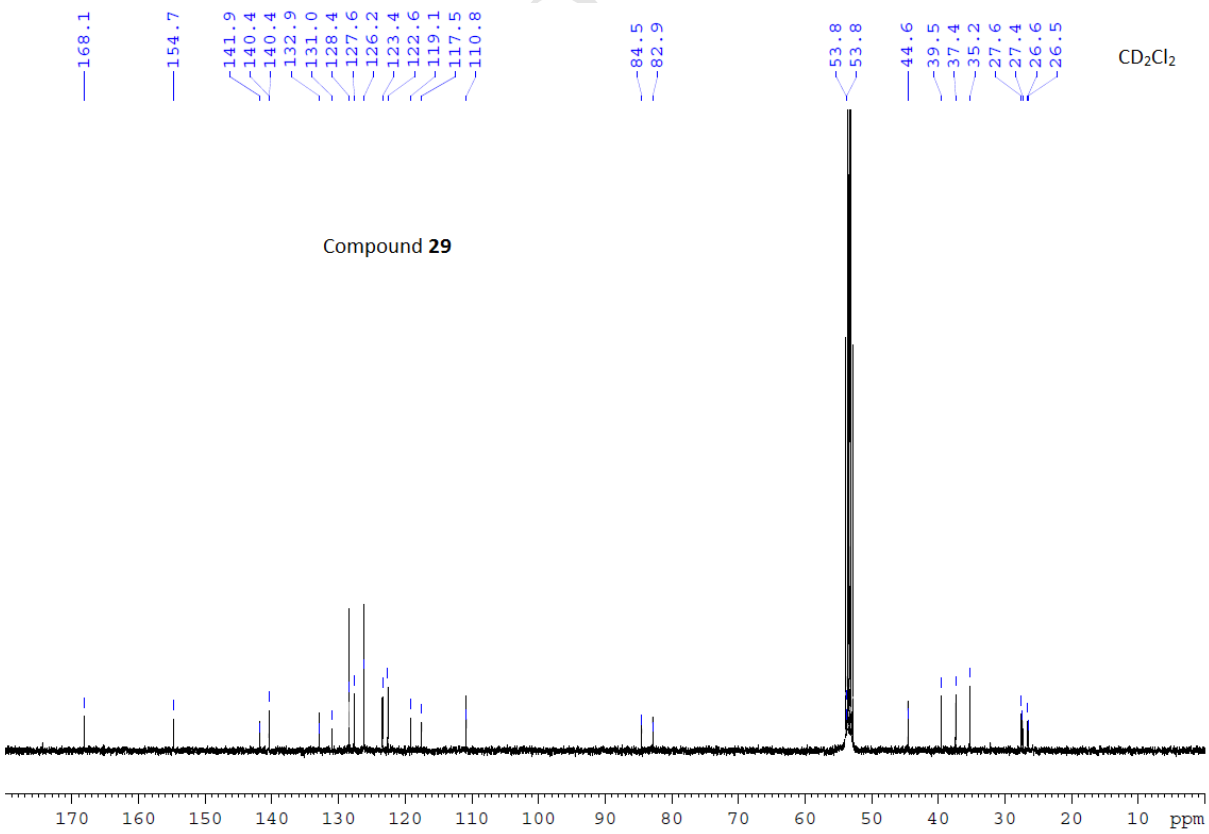


Compound **29**:

2-(7-Chloro-5-(4-fluorobutyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (CD_2Cl_2)

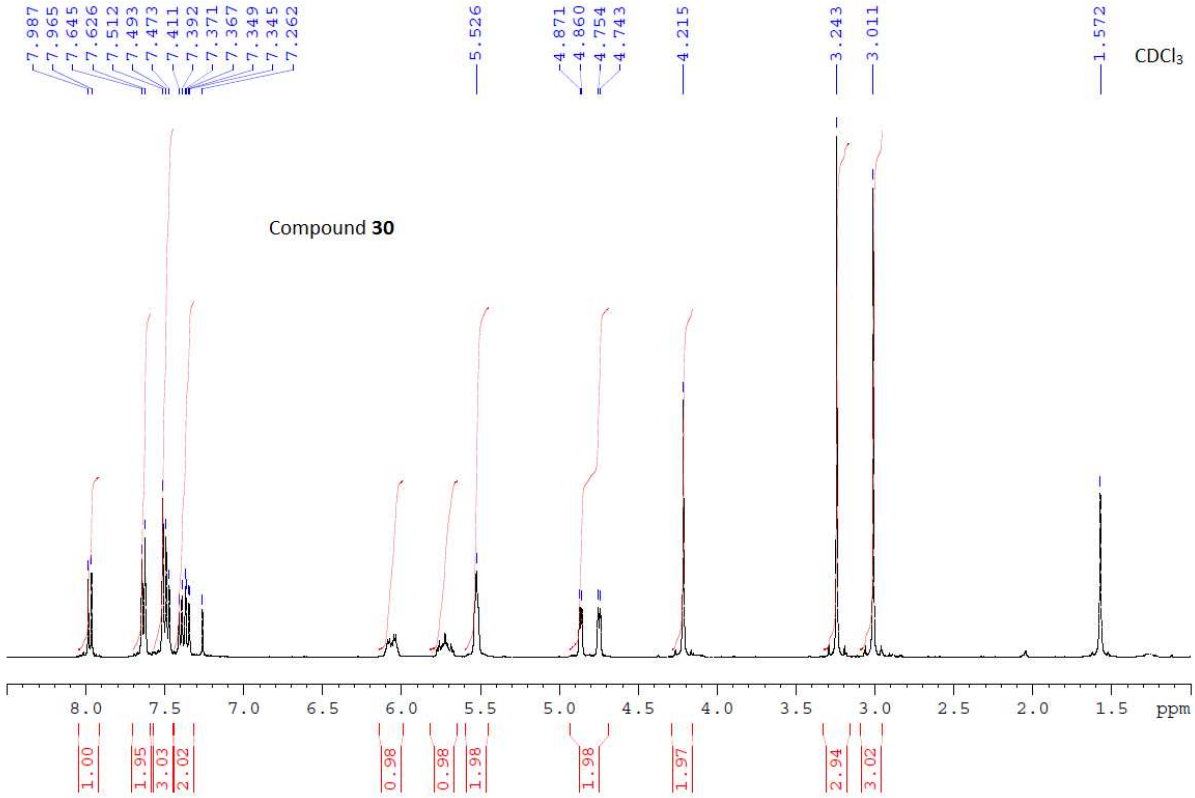


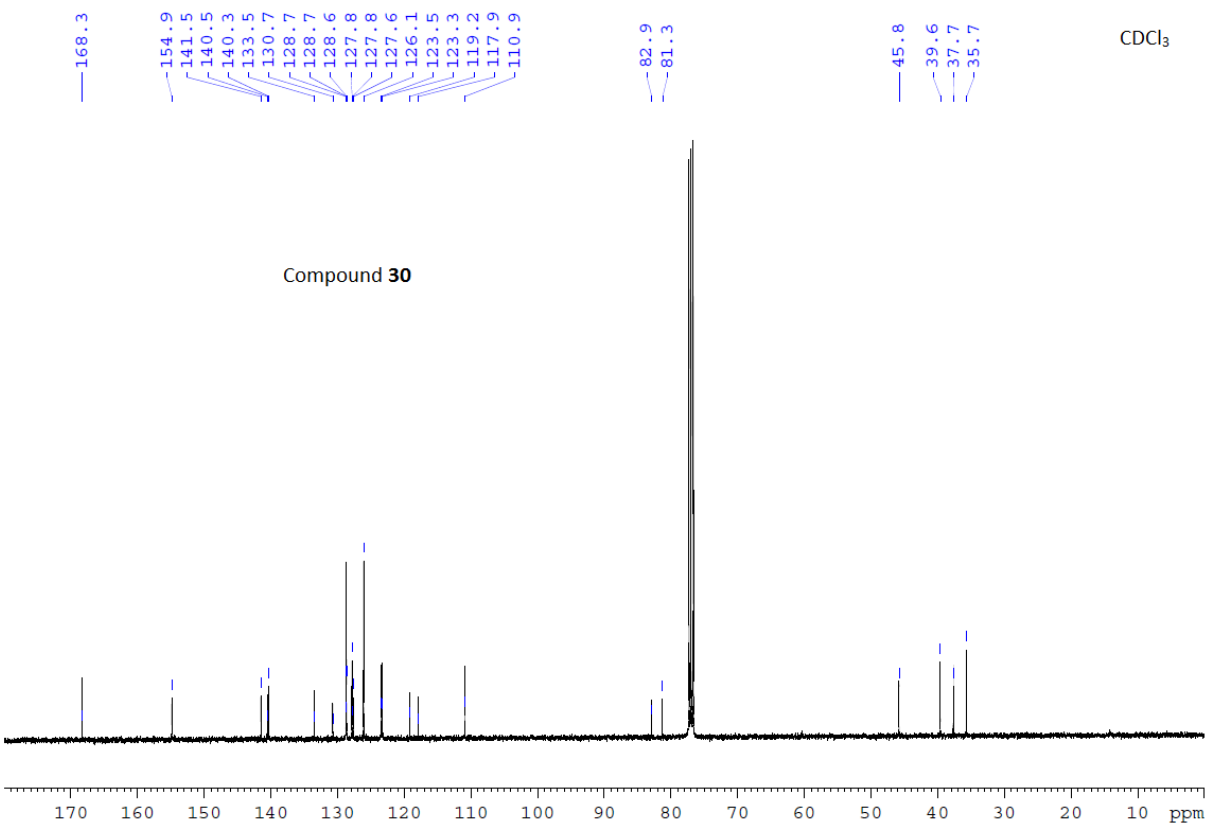


Compound **30**:

(*E*)-2-(7-Chloro-5-(4-fluorobut-2-en-1-yl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-N,N-dimethylacetamide

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$)

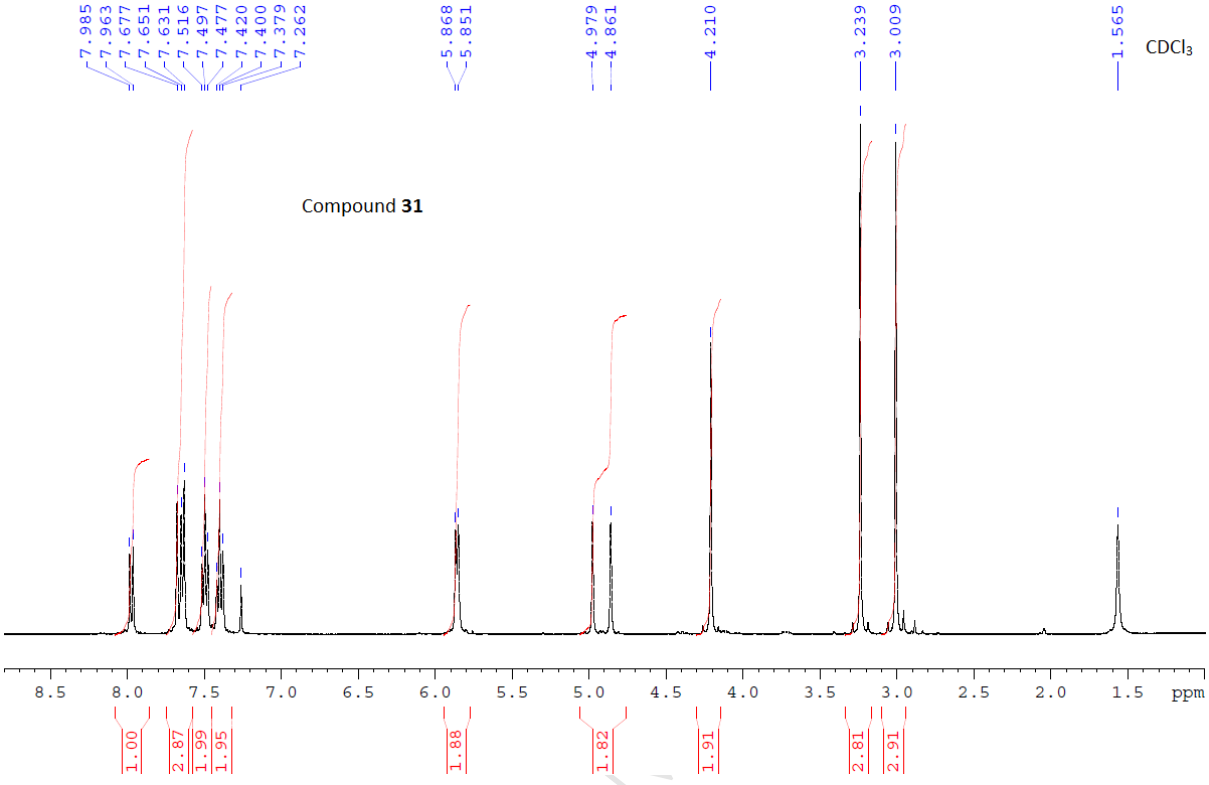


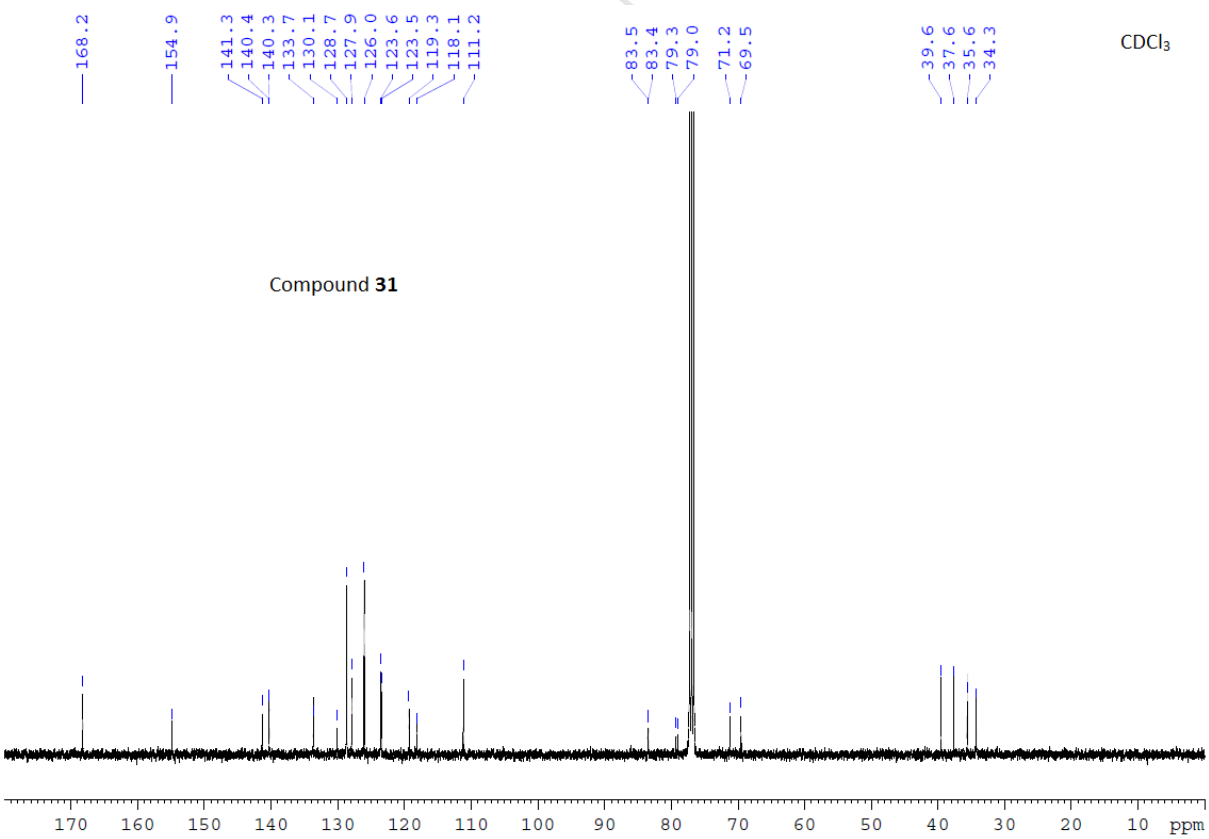


Compound **31**:

2-(7-Chloro-5-(4-fluorobut-2-yn-1-yl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$)

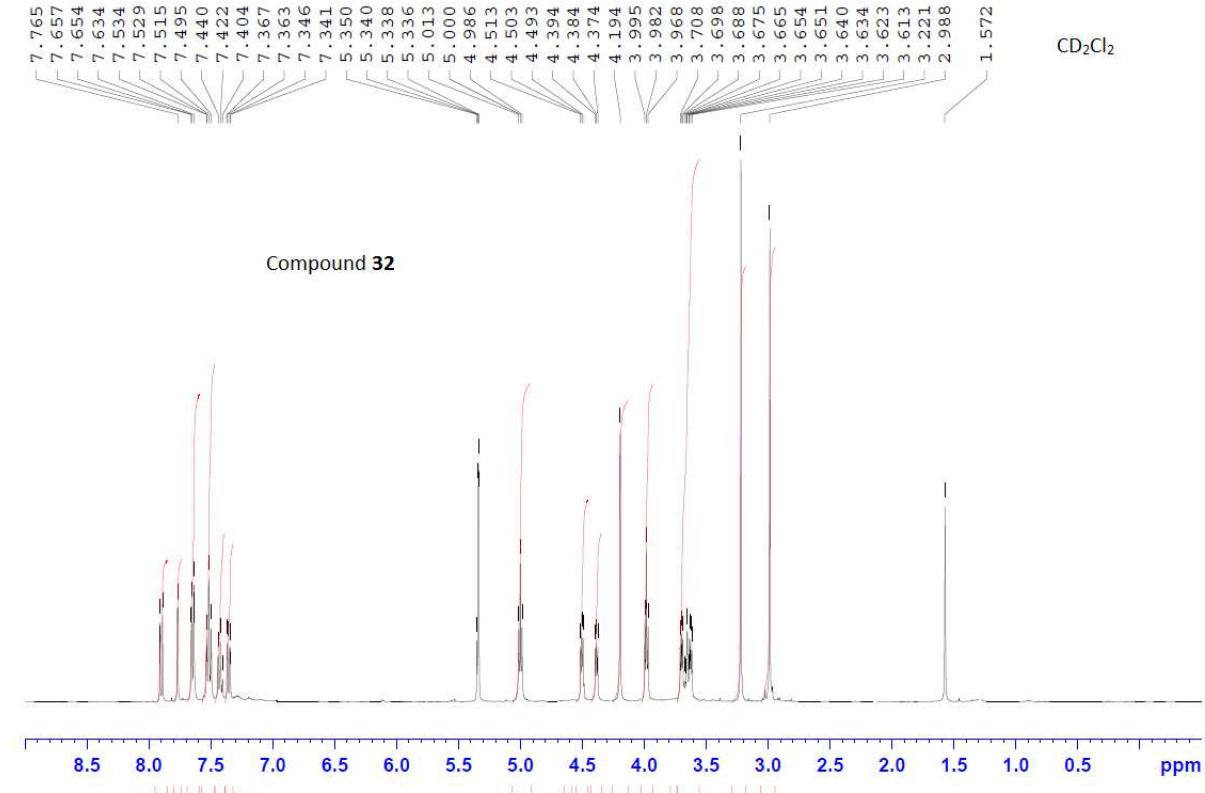


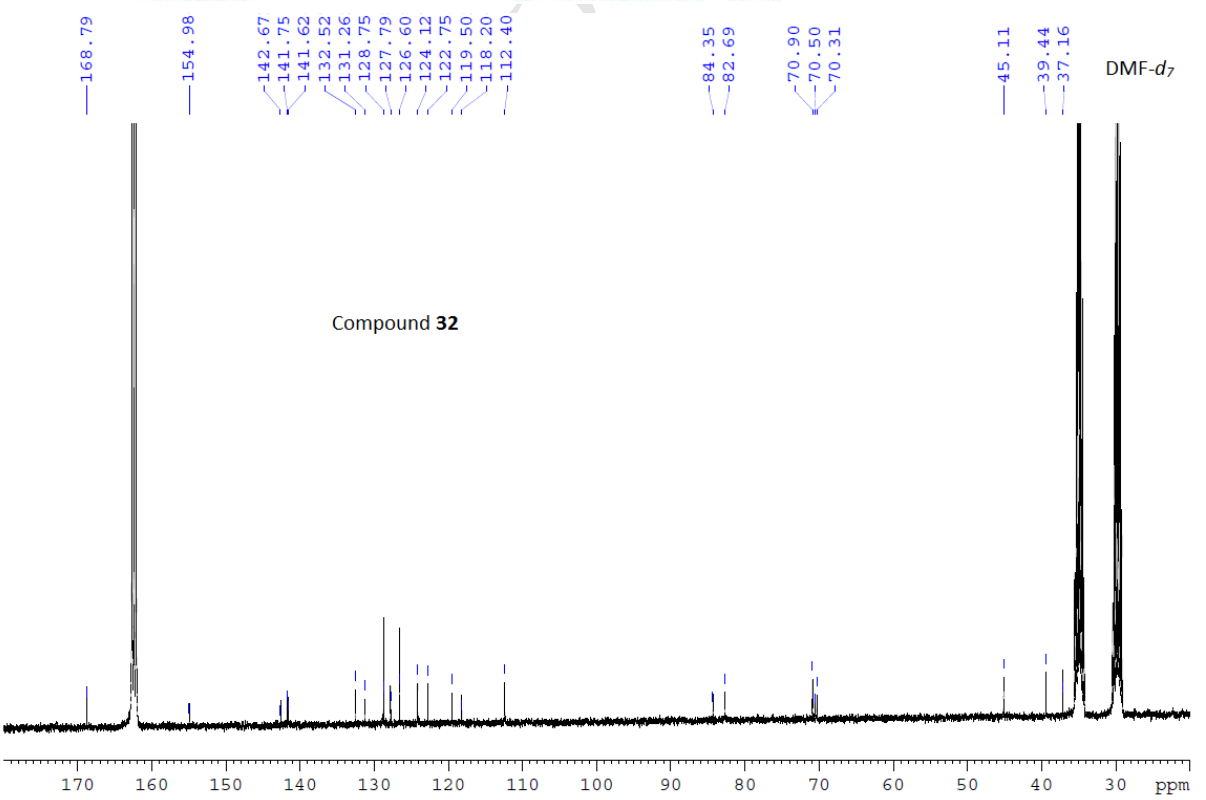


Compound **32**:

2-(7-Chloro-5-(2-(2-fluoroethoxy)ethyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (DMF-d_7)





Compound **33**:

2-(7-Chloro-5-(2-(2-(2-fluoroethoxy)ethoxy)ethyl)-4-oxo-3-phenyl-4,5-dihydro-3*H*-pyridazino[4,5-*b*]indol-1-yl)-*N*,*N*-dimethylacetamide

^{1}H NMR (CD_2Cl_2) and ^{13}C NMR (DMF-d_7)