Journal Pre-proof

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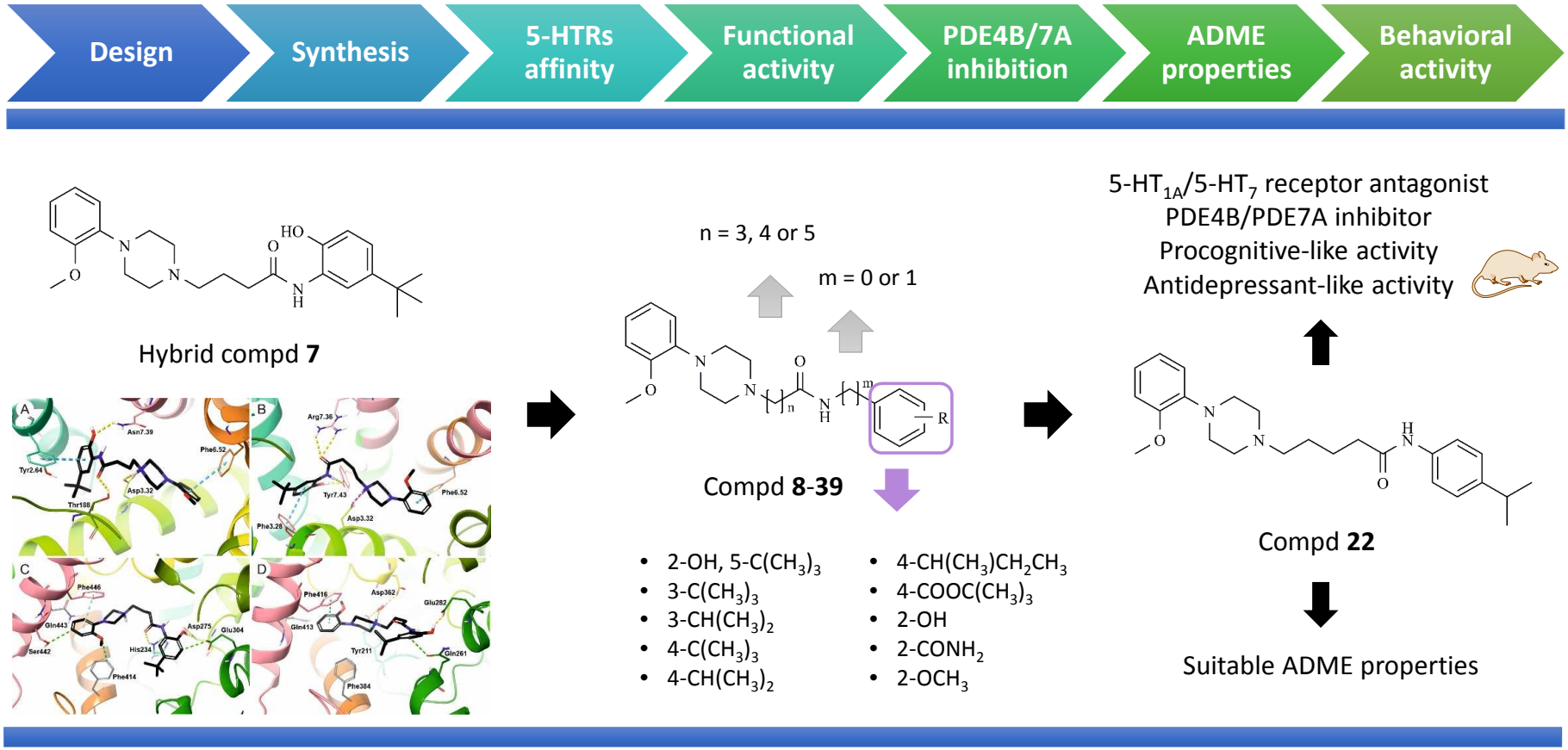
Design
Synthesis
5-HTRs affinity
Functional activity
PDE4B/7A inhibition
ADME properties
Behavioral activity
Hybrid compd 7
A
B
C
D
n = 3, 4 or 5
m = 0 or 1
Compd 8-39
2-OH, 5-C(CH3)3
3-C(CH3)3
3-CH(CH3)2
4-C(CH3)3
4-CH(CH3)2
4-CH(CH3)CH2CH3
4-COOC(CH3)3
2-OH
2-CONH2
2-OCH3
5-HT1A/5-HT7 receptor antagonist
PDE4B/PDE7A inhibitor
Procognitive-like activity
Antidepressant-like activity
Compd 22
Suitable ADME properties

Novel Anilide and Benzylamide Derivatives of Arylpiperazinylalkanoic Acids as 5-HT$_{1A}$/5-HT$_7$ Receptor Antagonists and Phosphodiesterase 4/7 Inhibitors with Procognitive and Antidepressant Activity

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Abstract: A library of novel anilide and benzylamide derivatives of ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoic acids as combined 5-HT_{1A}/5-HT_7 receptor ligands and phosphodiesterase PDE4B/PDE7A inhibitors was designed using a structure-based drug design approach. The *in vitro* studies of 33 newly synthesized compounds (**7**-**39**) allowed us to identify **22** as the most promising multifunctional 5-HT_{1A}/5-HT_7 receptor antagonist (5-HT_{1A} K_i = 8 nM, K_b = 0.04 nM; 5-HT_7 K_i = 451 nM, K_b = 460 nM) with PDE4B/PDE7A inhibitory activity (PDE4B IC_{50} = 80.4 μM; PDE7A IC_{50} = 151.3 μM). Compound **22** exerted a very good ability to passively penetrate through biological membranes and a high metabolic stability *in vitro*. Moreover, the pharmacological evaluation of **22** showed its procognitive and antidepressant properties in rat behavioral tests. Compound **22** at a dose of 3 mg/kg (*i.p.*) significantly reversed MK-801-induced episodic memory deficits in the novel object recognition test, while at a dose of 10 mg/kg (*i.p.*) reduced the immobility time of animals (by about 34 %) in the forced swimming test. The antidepressant-like effect produced by compound **22** was stronger than that of escitalopram used as a reference drug. This study opens a new perspective in the search for efficacious drugs for the treatment of cognitive and depressive disorders.

Keywords: anilide; benzylamide; 1-(2-methoxyphenyl)piperazine derivative; PDE4/PDE7 inhibitor; 5-HT_{1A}/5-HT_7 receptor antagonist; procognitive and antidepressant activity.

Abbreviations: PDE, phosphodiesterase; 5-HT receptor, serotonin receptor; cAMP, cyclic-3′,5′-adenosine monophosphate; LCAP, long-chain arylpiperazine; BBB, blood-brain barrier; RLMs, rat liver microsomes; IBMX, 3-isobutyl-1-methylxanthine; NOR test, novel object recognition test; FST, forced swimming test.

1. Introduction

Due to the growing number of patients suffering from memory and depressive disorders [1,2], the search for new drugs with procognitive and antidepressant properties is one of the leading research directions around the world [3–5]. Cognitive functioning is moderately to severely impaired in patients with depression [6], schizophrenia [7], and various forms of dementia including this caused by Alzheimer's disease [8]. The impairment of cognition is the prime driver of the significant disabilities in occupational, social, and economic functioning in patients. Unfortunately, we have limited treatment options for cognitive disorders, which are often accompanied by deterioration of mood, a factor that worsens patients' quality of life and leads to their premature death [9,10].

According to latest literature data, drugs with multidirectional activity combining procognitive and antidepressant effects may be achieved by interaction with serotonin 5-HT_{1A} and 5-HT_7 receptors [10] and inhibition of cyclic-3′,5′-adenosine monophosphate (cAMP)-specific phosphodiesterase (PDE) type 4 and 7 [11–13]. Therapeutic paradox that both agonists and antagonists of 5-HT_{1A} and 5-HT_7 receptors may exert procognitive activity is still unclear [14]. In turn, the action of PDE4/PDE7 inhibitors probably results from the regulation of cAMP signaling pathway and their anti-inflammatory [15,16] and neuroprotective properties [17–19].

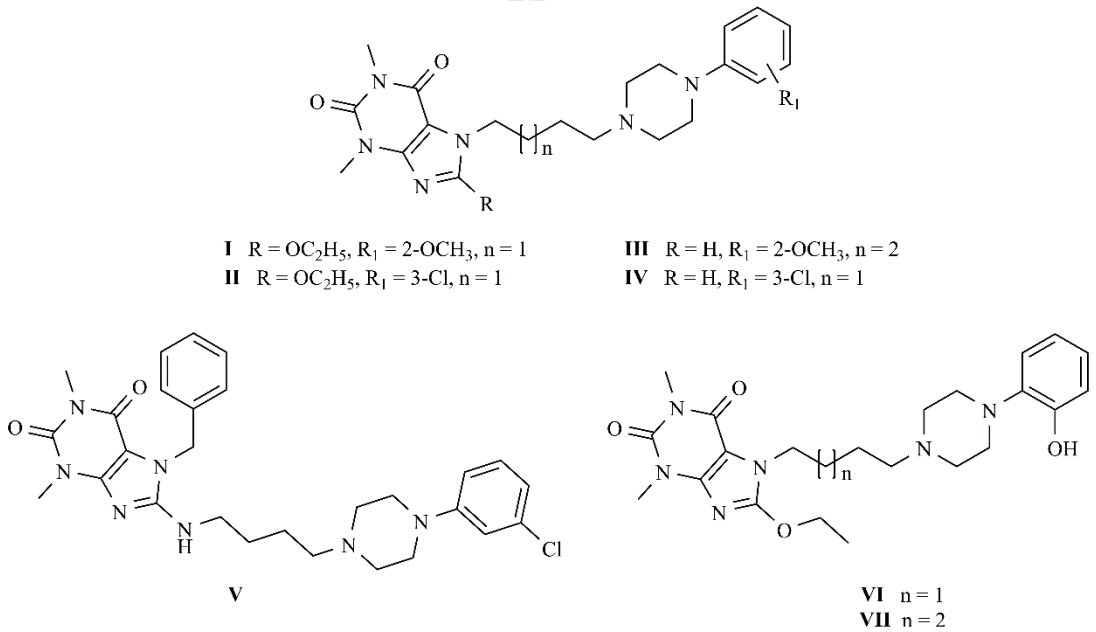


Fig. 1. Representative 5-HT_{1A}/5-HT_7 receptor ligands with antidepressant activity from previously described studies [20–23].

For several years, we have been developing 5-HT_{1A}/5-HT_7 receptor ligands with a diverse functional ago/antagonist profile in a long-chain arylpiperazines (LCAPs) class containing purine-2,6-dione scaffold as the terminal fragment. Some compounds from the evaluated series showed antidepressant activity in an animal behavioral test, *e.g.*, **I-V** (Fig. **1**) [20–23]. Recently, novel LCAP

derivatives of 8-alkoxypurine-2,6-dione and imidazo[2,1-*f*]purine-2,4-dione combining 5-HT_{1A}/5-HT_7 receptor affinity and PDE4B/PDE10A inhibitory activity have been designed, synthesized, and evaluated as potential antidepressant drugs, *e.g.*, **VI** and **VII** (Fig. **1**) [24–27]. However, the pharmacological studies aimed to assess the effects of above mentioned LCAP derivatives of purine-2,4-dione on cognitive functions have not been performed yet.

In this work we made an attempt to obtain innovative multifunctional ligands with ability to improve cognition and mood. Our research hypothesis is that simultaneous interaction with 5-HT_{1A}/5-HT_7 receptors and inhibition of PDE4B/PDE7A isoenzymes may provide both procognitive and antidepressant activities by their synergistic effect on the intracellular cAMP level.

The aim of this study was to design, synthesize, and evaluate procognitive and antidepressant activity of novel anilide and benzylamide derivatives of ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoic acids as 5-HT_{1A}/5-HT_7 receptor ligands and PDE4B/PDE7A inhibitors. The innovative group of hybrid compounds that linked the 1-(2-methoxyphenyl)piperazine scaffold with a differently substituted anilide or benzylamide moiety *via* 3, 4, or 5-carbon aliphatic chain was designed using a structure-based drug design approach. For the newly synthesized compounds, their 5-HT_{1A}/5-HT_7 receptor affinity and PDE4B/PDE7A inhibitory activity were determined *in vitro*. For the most potent ligands, the evaluation of functional activity toward targeted receptors as well as membrane permeability and metabolic stability was performed. Finally, for the most promising compound its procognitive and antidepressant-like activity was tested *in vivo* using well-established experimental paradigms.

2. Results and discussion

2.1. Computer-aided molecule design

In order to obtain innovative multifunctional ligands that combine 5-HT_{1A} and 5-HT_7 receptor affinity and ability to inhibit PDE4B and PDE7A isoforms, a ligand-based drug design approach was applied that relies on the knowledge of molecules that bind to the biological targets of interest. As a result of combining LCAP moiety being one of the most universal templates used for designing 5-HT_{1A} and 5-HT_7 receptors ligands (Fig. **2**) [28–31] and substituted anilide group found to be crucial for inhibitory activity of the 1,3-dimethylpurine-2,6-diones toward PDE4B and PDE7A (Fig. **3**) [32,33], a new hybrid compound **7**, anilide derivative of arylpiperazinylalkanoic acid as a potential multi-target-directed ligand was designed (Fig. **4**).

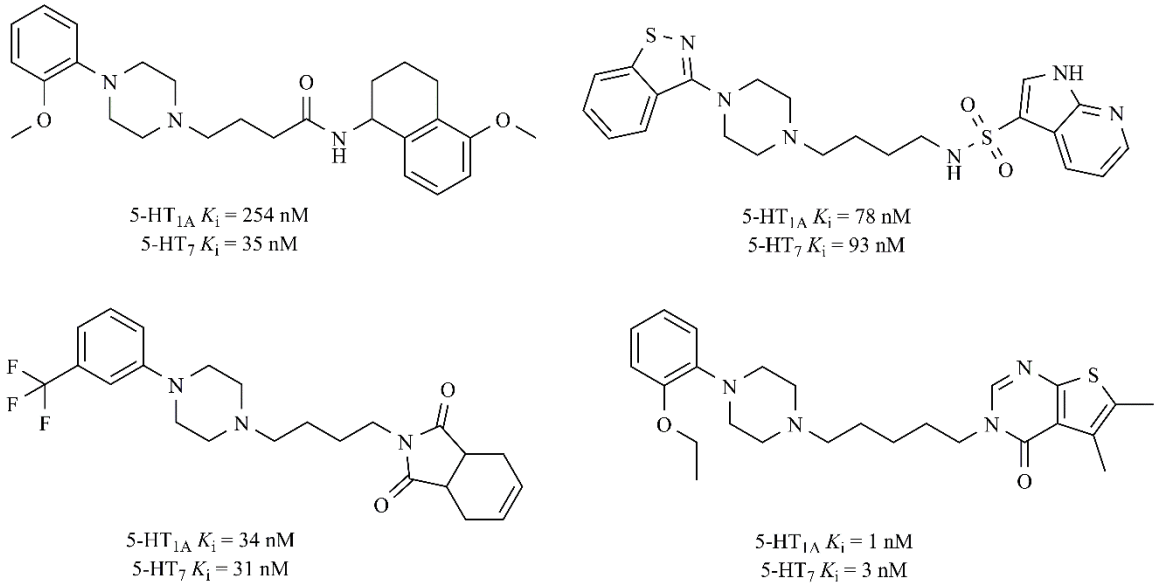


Fig. 2. The structures of representative 5-HT_{1A}/5-HT_7 receptor ligands based on the LCAP moiety [28–31].

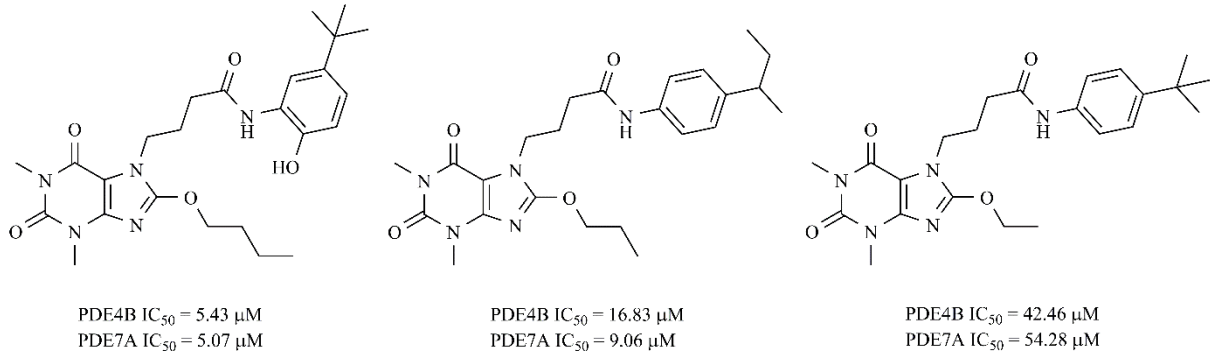


Fig. 3. The structures of representative PDE4B/PDE7A inhibitors based on the 1,3-dimethylpurine-2,6-dione scaffold [32,33].

To confirm the advisability of the proposed hybrid hypothesis, molecular modeling studies were performed. The results showed that compound **7** was able to interact as both a 5-HT_{1A} and 5-HT_7 receptor ligand as well as a PDE4B and PDE7A inhibitor.

In 5-HT_{1A} and 5-HT_7 receptors, hybrid compound **7** was docked in the orthosteric binding sites (the phenylpiperazine moiety), whereas the (5-*tert*-butyl-2-hydroxyphenyl)carbamoylpropyl fragment bound in the additional (allosteric) binding sites. The key anchoring interactions of phenylpiperazine fragment, common for both the receptors, are salt bridges (charge reinforced hydrogen bonds) to Asp3.32, and aromatic π-π stackings with Phe6.52. In the 5-HT_{1A} receptor, additional stabilizing interactions are as follows: π-π stacking with Tyr2.64, hydrogen bonds to Asn7.39 and Thr188 (Fig. **5A**). In the case of the 5-HT_7 receptor, the additional interactions comprise π-π stacking with Phe3.28 and hydrogen bonds to Arg7.36 and Tyr7.43 (Fig. **5B**). Favorable

complementarity of ligand-receptor interactions suggests a high affinity for the target proteins and justifies further research within this chemotype.

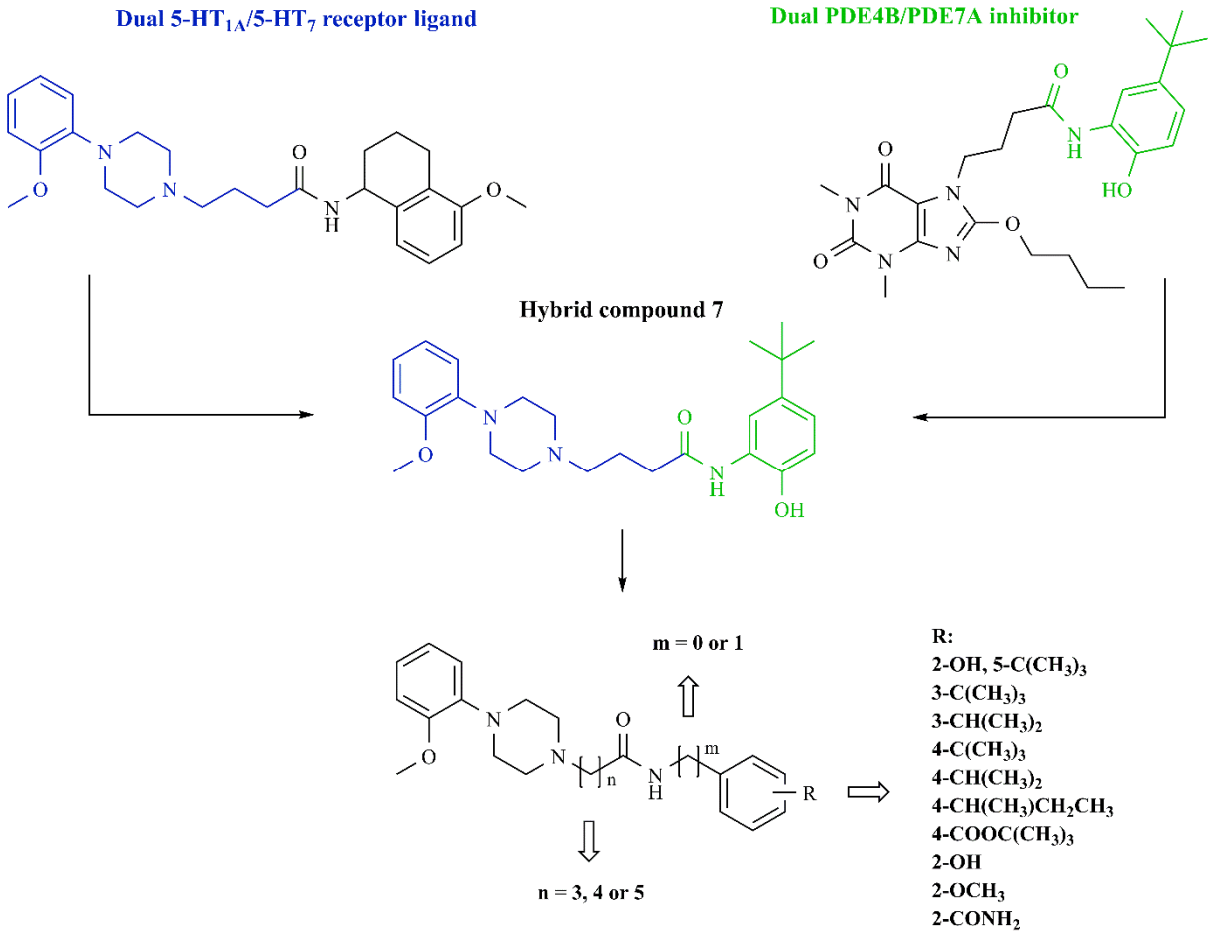


Fig. 4. Design of anilide and benzylamide derivatives of arylpiperazinylalkanoic acids as potential multifunctional ligands combining 5-HT_{1A}/5-HT_7 receptor affinity and PDE4B/PDE7A inhibitory activity.

In the case of PDE4B, compound **7** was docked in the center of the catalytic site, placing the phenylpiperazine moiety (responsible for dual 5-HT_{1A} and 5-HT_7 receptor activity) in the hydrophobic pocket (H pocket) between Phe446 and Phe414 residues. Its phenyl ring formed π-π stacking interactions with Phe446. Another fragment of the structure - (5-*tert*-butyl-2-hydroxyphenyl)carbamoylpropyl was situated in the area of the metal-binding site (M site) and formed two hydrogen bonds with His234 (carbonyl group) and Asp275 (hydroxy group) amino acid residues. Additionally, there were several weak interactions found – aromatic hydrogen bonds with Ser442, Phe414 and Glu304 (Fig. **5C**).

In the active site of PDE7A, compound **7** was placed analogously. The phenyl ring of the phenylpiperazine moiety formed π-π stacking interactions with Phe416. The protonated nitrogen atom of piperazine ring formed an H-bond network with a water molecule, which in turn interacted

simultaneously with Tyr211 (hydroxy group) and Asp362 (carbonyl group). (5-*tert*-butyl-2-hydroxyphenyl)carbamoylpropyl fragment also stabilized the proper compound conformation through a hydrogen bond with Glu282 (a hydroxy group at position 2 of phenyl ring) and aromatic hydrogen bond with Gln261 (Fig. **5D**).

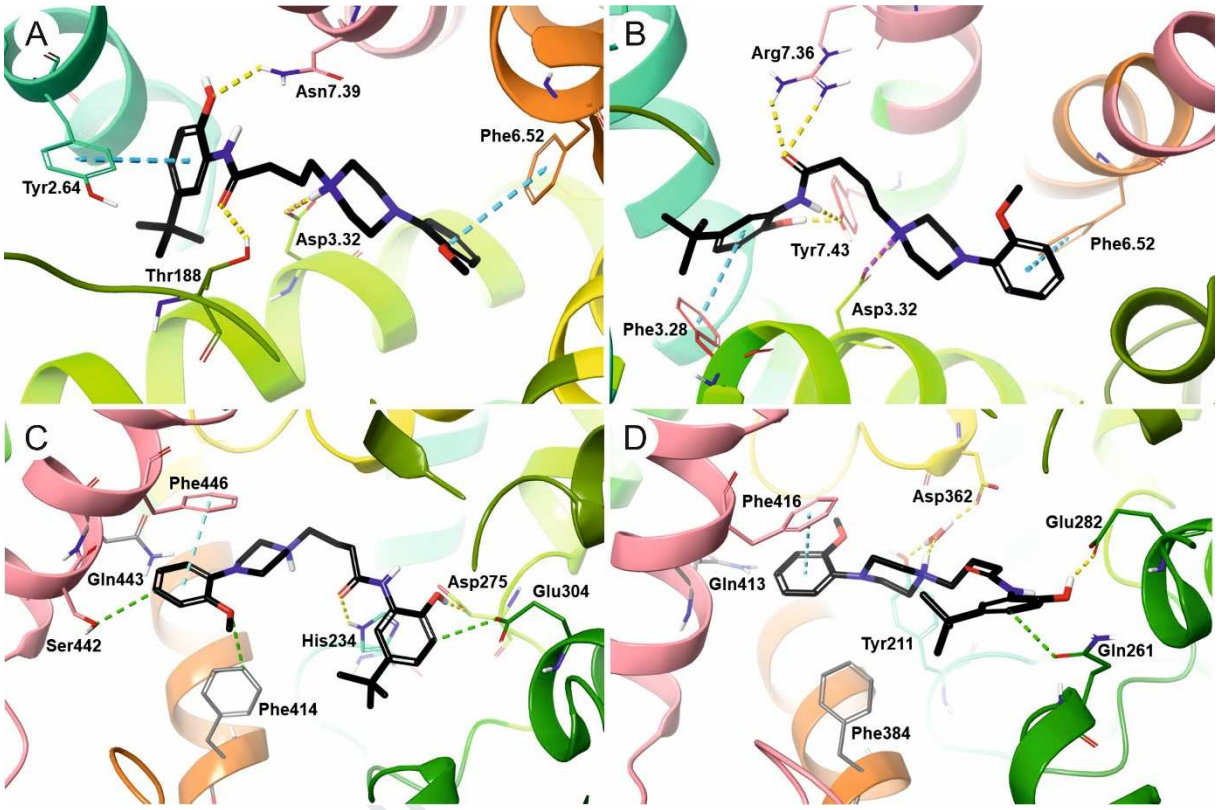


Fig. 5. The predicted binding modes of hybrid compound **7** in the models of 5-HT_{1A} (A) and 5-HT_7 (B) receptors, as well as PDE4B (C) and PDE7A (D) catalytic sites. Interactions with the key amino acids are displayed as follows: hydrogen bonds (yellow), aromatic hydrogen bonds (green), and aromatic π-π stacking (blue).

It was proved that PDE4B and PDE7A inhibitory activity depends on the occurrence of 3 interactions: hydrogen bond with Gln443/Gln413, and π-π stacking interactions with Phe446/Phe416, and Phe414/Phe384 [34]. The lack of these important hydrogen bonds and one of the aromatic interactions could pose an explanation of compound **7** weak PDE4B and PDE7A inhibitory activity.

For the hybrid compound **7** its molecular parameters, physicochemical, pharmacological, and toxicological properties were determined using appropriate software (Table **1**). Based on the Lipinski's rule of five [35], it was estimated using the Instant JChem software that compound **7** has physical and chemical properties that would make it a likely orally active drug in humans. Using the Van de Watembeerd [36] and Kelder [37] rules, and the brain or intestinal estimated permeation (BOILED-Egg) method [38], it was found that compound **7** is likely to cross the blood-brain barrier (BBB) and act within CNS. The risk of mutagenicity, tumorigenicity, irritating, and reproductive

effects of compound **7** were assessed as low using the OSIRIS Property Explorer software [39]. Finally, using the SwissADME program [40], no structural fragments known as PAINS (Pan-assay interference compounds) were found in the designed hybrid compound **7** that could give false positive results in *in vitro* pharmacological tests.

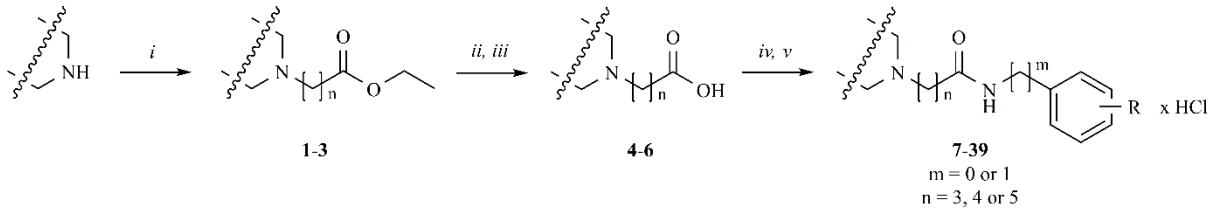
Table 1. Prediction of ability to cross the BBB and toxicity of compound **7** using Instant JChem[a], SwissADME[b], or OSIRIS Property Explorer[c].

Molecular and physicochemical properties[a]		**CNS penetration[a,b]**		**Risk of toxicity[c]**	
MW	425.56	Lipinski's rule	Yes	Mutagenicity	No
HBD	2	Van de Waterbeemd's rule	Yes	Tumorigenicity	No
HBA	5	Kelder's rule	Yes	Irritating effect	No
CLogP	3.53	BOILED-Egg	Yes	Reproductive effect	Low
TPSA	65.04 Å^2				

Taking into account the results of *in silico* studies, a library of new hybrid compounds was designed as potential 5-HT_{1A}/5-HT_7 receptor ligands and PDE4B/PDE7A inhibitors based on the structure of the pilot compound **7** (Fig. **4**). 2-Methoxyphenylpiperazine was left as a terminal amine fragment. The benzene ring at the anilide group was substituted with a branched alkyl, ester, hydroxy, methoxy, or carboxamide group. Moreover, instead of the anilide group, the benzylamide fragment was introduced. Both terminal fragments were connected by a 3, 4, or 5-carbon aliphatic chain. The modifications were aimed at checking how the length of aliphatic linker and changing the substitution mode of the benzene ring at the amide fragment affect the receptor and enzymatic affinities of the obtained compounds.

2.2. Synthesis

Synthesis of the designed compounds **7-39** was performed according to the multistep procedure presented in Scheme **1**. In the first step, commercially available 1-(2-methoxyphenyl)piperazine was alkylated using an appropriate ethyl bromoalkanoate in the presence of anhydrous K_2CO_3 in refluxing acetonitrile, yielding ethyl esters **1-3**. Intermediate esters were subsequently hydrolyzed with KOH solution in a water-acetone medium, and the resulting acids **4-6** were isolated after acidification of the reaction mixture. In the next step, the obtained acids were coupled with amine (aniline or benzylamine derivative) in the presence of di(1*H*-imidazol-1-yl)methanone (CDI) in DMF. The synthesized amides were treated with 1 N HCl in methanol to give the final products **7-39** as hydrochloride salts.



Scheme 1. The synthesis of the anilide and benzylamide derivatives of ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoic acids **7-39**. Reagents and conditions: (*i*) ethyl 4-bromobutanoate, ethyl 5-bromopentanoate, or ethyl 6-bromohexanoate, K_2CO_3, acetonitrile, reflux, 15 h; (*ii*) KOH, acetone/water, reflux, 8 h; (*iii*) conc. HCl; (*iv*) aniline or benzylamine derivative, CDI, DMF, room temp., 72 h; (*v*) HCl in methanol.

2.3. Preliminary in vitro pharmacology

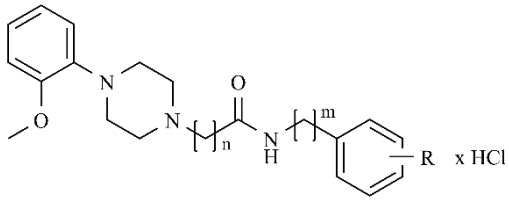
2.3.1. Radioligand binding assays

All the newly synthesized compounds **7-39** were tested for their 5-HT_{1A} and 5-HT_7 receptor affinity in the radioligand binding assays according to the previously described protocols [41]. The inhibition constants (K_i) were calculated from the Cheng-Prusoff equation and presented in Table **2**. The experiments were carried out using [^{3}H]-8-OH-DPAT (135.2 Ci/ mmol) and [^{3}H]-5-CT (39.2 Ci/mmol) for the human 5-HT_{1A} and 5-HT_7 receptors, respectively, which were all stably expressed in human embryonic kidney 293 (HEK293) cells. Buspirone and clozapine were used as reference 5-HT_{1A} and 5-HT_7 receptor ligands, respectively.

In general, the synthesized 2-methoxyphenylpiperazine derivatives **7-39** containing 3, 4, or 5-carbon aliphatic linker and differently substituted benzene ring at the amide fragment exerted higher affinity for the 5-HT_{1A} receptor than for the 5-HT_7 receptor (Table **2**). The compounds showed 5-HT_{1A} receptor affinity in the range of 8-590 nM. The most potent 5-HT_{1A} receptor ligands were compounds **12**, **18-28**, **30-35**, and **37-39** exerting K_i < 100 nM. Among them, compounds **19-24** provided a similar or higher affinity (K_i = 8-29 nM) comparing with buspirone (K_i = 20 nM). In turn, compounds **8**, **9**, **11**, **13**, **14**, **16**, **17**, **29**, and **36** behaved as moderate 5-HT_{1A} receptor ligands (100 nM < K_i < 500 nM). The lowest 5-HT_{1A} receptor affinity (K_i > 500 nM) was found for compounds **7**, **10**, and **15**.

As presented, all the structural modifications of the prototype hybrid molecule (pilot compound **7**) resulted in an improved affinity for the 5-HT_{1A} receptor. Considering the aliphatic linker length between 2-methoxyphenylpiperazine and amide fragments it can be concluded that the 4-carbon linker was more preferred for the enhanced 5-HT_{1A} receptor affinity than 3- or 5-carbon ones (compounds **18-29** *vs.* **7-17** and **30-39**) what is in line with the previously reported SAR analysis results in the group of purine-based 5-HT_{1A} receptor ligands [20,23,24]. Moreover, the analysis of the benzene ring substitution at the amide group revealed that branched alkyl groups were more favorable than hydroxy, ester, and amide groups (*e.g.*, compounds **19-23** *vs.* **18** and **24-26**).

Table 2. Structures and 5-HT_{1A} and 5-HT_7 receptor affinities of compounds **7**-**39**.



Compd	n	m	R	K_i [nM]		Ratio K_i
				5-HT_{1A}	**5-HT_7**	**5-HT_7/5-HT_{1A}**
7	3	-	2-OH, 5-$C(CH_3)_3$	539	328	<1
8	3	-	3-$C(CH_3)_3$	224	130	<1
9	3	-	3-$CH(CH_3)_2$	187	157	<1
10	3	-	4-$C(CH_3)_3$	590	1196	2
11	3	-	4-$CH(CH_3)_2$	150	954	6
12	3	-	4-$CH(CH_3)CH_2CH_3$	37	354	10
13	3	-	4-$COOC(CH_3)_3$	287	3282	11
14	3	-	2-OH	405	471	1
15	3	-	2-$CONH_2$	526	1143	2
16	3	1	2-OH	339	319	1
17	3	1	2-OCH_3	292	869	3
18	4	-	2-OH, 5-$C(CH_3)_3$	61	159	3
19	4	-	3-$C(CH_3)_3$	16	75	5
20	4	-	3-$CH(CH_3)_2$	21	151	7
21	4	-	4-$C(CH_3)_3$	21	496	24
22	4	-	4-$CH(CH_3)_2$	8	451	56
23	4	-	4-$CH(CH_3)CH_2CH_3$	14	323	23
24	4	-	4-$COOC(CH_3)_3$	29	927	32
25	4	-	2-OH	31	300	10
26	4	-	2-$CONH_2$	82	1874	23
27	4	1	2-OH	43	343	8
28	4	1	2-OCH_3	82	366	4
29	4	1	4-$C(CH_3)_3$	138	391	3
30	5	-	2-OH, 5-$C(CH_3)_3$	82	154	2
31	5	-	3-$C(CH_3)_3$	53	190	4
32	5	-	3-$CH(CH_3)_2$	34	141	4
33	5	-	4-$C(CH_3)_3$	40	514	13
34	5	-	4-$CH(CH_3)_2$	43	434	10
35	5	-	4-$CH(CH_3)CH_2CH_3$	69	260	4
36	5	-	4-$COOC(CH_3)_3$	125	799	6
37	5	-	2-OH	95	254	3
38	5	1	2-OH	70	289	4
39	5	1	2-OCH_3	64	515	8
Buspirone				20	-	
Clozapine				-	18	

The replacement of the anilide fragment by benzylamide moiety resulted in a slight increase in 5-HT_{1A} receptor affinity in the case of 2-hydroxy phenyl derivatives (**14** *vs.* **16** and **37** *vs.* **38**). On the other hand, in the 4-*tert*-butyl analogs this modification decreased (about 7-fold) 5-HT_{1A} receptor affinity (**21** vs. **29**).

The evaluated compounds **7**-**39** exerted 5-HT$_7$ receptor affinity in the range of 75-3282 nM. Compound **19** showed the highest 5-HT$_7$ receptor affinity (K_i = 75 nM) in this group of derivatives but its activity was 4-fold lower than that for clozapine (K_i = 18 nM). Compounds **7**-**9**, **12**, **14**, **16**, **18**, **20**-**23**, **25**, **27**-**32**, **34**, **35**, **37**, and **38** were characterized as moderate 5-HT$_7$ receptor ligands (100 nM < K_i < 500 nM), while other studied compounds possessed a low affinity toward the 5-HT$_7$ receptor (K_i > 500 nM).

Similarly to the 5-HT$_{1A}$ receptor, the 5-HT$_7$ receptor affinity of the tested compounds was highest for the derivatives with 4-carbon chain than for the compounds with 3- and 5-carbon linker. Considering the benzene ring substitution at the amide moiety it can also be concluded that branched alkyl groups were preferred, especially, at the *meta* position (*e.g.*, compounds **8**, **9**, **19**, **20**, **31**, and **32**).

It was also found that only the combination of 2-methoxyphenylpiperazine, a 4-carbon aliphatic linker, and a 3-(isopropyl)phenyl group provided a potent and dual 5-HT$_{1A}$ and 5-HT$_7$ receptor ligand (compound **19**) with K_i value of 16 and 75 nM, respectively. The shortening of the alkyl linker from 4-carbon to 3-carbon one maintained the dual nature of interaction of compound **8** but its 5-HT receptor affinity was reduced.

Finally, the SAR study around amide group showed that the replacement of the anilide by benzylamide (compounds **14** *vs.* **16**, **21** *vs.* **29**, **25** *vs.* **27**, **38** *vs.* **39**) did not significantly change the pharmacological activity of the tested compounds toward the 5-HT$_7$ receptor.

2.3.2. Functional activity evaluation

On the basis of the binding affinity results, a series of the high affinity 5-HT$_{1A}$ and 5-HT$_7$ receptor ligands was selected for functional profile analysis according to previously described protocols using *in vitro* cellular assays [42]. For the 5-HT$_{1A}$ receptor ligands, a cellular aequorin-based functional assay was performed with recombinant Chinese hamster ovary (CHO)-K1 cells expressing mitochondrially targeted aequorin, the human 5-HT$_{1A}$ receptor, and the promiscuous G protein α_{16} for the 5-HT$_{1A}$ receptor. For the 5-HT$_7$ receptor ligands, adenylyl cyclase activity was monitored using cryopreserved CHO-K1 cells with expression of the human 5-HT$_7$ receptor. NAN-190 and SB-269970 were used as reference 5-HT$_{1A}$ and 5-HT$_7$ receptor antagonists, respectively, and serotonin as a 5-HT$_{1A}$/5-HT$_7$ receptor agonist.

All the tested compounds showed antagonistic properties toward 5-HT$_{1A}$ and 5-HT$_7$ receptors (Table **3**), while no activation of 5-HT$_{1A}$ and 5-HT$_7$ receptors was observed in the cell-based assays. The evaluated compounds were characterized as very potent 5-HT$_{1A}$ receptor antagonists and weak to moderate 5-HT$_7$ receptor antagonists. The most potent 5-HT$_{1A}$ receptor antagonists (compounds **19**, **20**, **22**, and **24**) affected the receptor with a similar or higher potency than that of NAN-190 (K_b = 0.07 nM) used as a reference. Other 5-HT$_{1A}$ receptor antagonists (compounds **7**, **10**, **16**, **27**, **30**, and **33**) showed the K_b value in the range of 0.89 to 4.20 nM. The antagonistic activity of all tested compounds toward 5-HT$_7$ receptor was much more diverse and provided the K_b value in the range of 54 nM

(compound **19**) to 9900 nM (compound **24**) compared to K_b value of 0.2 nM for the reference 5-HT_7 receptor antagonist SB-269970. However, most of the tested 5-HT_7 receptor antagonists **16**, **20**, **22**, **27**, **30**, and **33** gave the K_b value at submicromolar level in the range of 120-870 nM.

Table 3. Antagonistic activity of the selected 5-HT_{1A} and 5-HT_7 receptor ligands.

Compd	K_b [nM]	
	5-HT_{1A}	**5-HT_7**
7	3.40	2300
10	4.20	N.C.
16	4.20	120
19	0.21	54
20	0.13	100
22	0.04	460
24	0.11	9900
27	0.89	240
30	1.10	480
33	1.40	870
NAN-190	0.07	-
SB-269970	-	0.2

N.C. – not calculable

The results obtained in this selected set of compounds showed the importance of the aliphatic linker length on the functional profile toward 5-HT_{1A} receptor as the potency of the antagonistic activity was observed in the following order: 4-carbon (compounds **19**, **20**, **22**, **24**, and **27**) > 5-carbon (compounds **30** and **33**) > 3-carbon aliphatic chain (compounds **7**, **10**, and **16**). No similar relationship was found for the 5-HT_7 receptor.

2.3.3. PDE4B and PDE7A inhibitory activity

All the synthesized compounds **7-39** were tested *in vitro* for their PDE4B and PDE7A inhibitory activity using PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE4B and PDE7A expressed in *Spodoptera frugiperda* 9 (Sf9) cells according to previously described protocols [43]. A non-selective PDE inhibitor, 3-isobutyl-1-methylxanthine (IBMX) was used as a reference compound in this study. The data obtained were analyzed using nonlinear regression (ADAPT 5 (BMSR, Los Angeles, CA, USA). PDE4B and PDE7A inhibitory activities were expressed as IC_{50} values.

Compounds **7-39** exerted various PDE4B and PDE7A inhibitory activity *in vitro*. The IC_{50} values for active compounds **7**, **10**, and **18-23** were summarized in Table **4**. Other tested compounds **8**, **9**, **11-17**, and **24-39** showed PDE4B and PDE7A IC_{50} values > 200 μM.

Table 4. The PDE4B and PDE7A inhibitory activities of compounds **7**, **10**, and **18**-**23**.

Compd	IC_{50} [μM]	
	PDE4B	**PDE7A**
7	69.0	57.0
10	96.0	27.0
18	36.5	> 200
19	130.0	> 200
20	88.1	> 200
21	138.5	> 200
22	80.4	151.3
23	168.9	125.5
IBMX	28.2	85.6

The hybrid compound **7** showed double-digit micromolar IC_{50} values of 69.0 and 57.0 μM for PDE4B and PDE7A, respectively, and its inhibitory activity was similar to that obtained for IBMX (Table **4**). The structural modification of compound **7** which comprised the elongation of the aliphatic linker between 2-methoxyphenylpiperazine and amide fragments from 3- to 4-carbon chain gave compound **18** with increased activity toward PDE4B (IC_{50} = 36.5 μM). However, this structural change decreased PDE7A inhibitory activity (IC_{50} > 200 μM). In turn, the removal of the hydroxy group from the amide fragment and introduction of the *tert*-butyl group at *para* position of the benzene ring provided compound **10** with increased activity toward PDE7A (IC_{50} = 27.0 μM). On the other hand, this modification decreased activity toward PDE4B (IC_{50} = 96.0 μM). For several other compounds **19**-**23**, their PDE4B IC_{50} values were determined in the range of 80.4-168.9 μM. For compounds **22** and **23**, their PDE7A inhibitory activity was estimated as 151.3 and 125.5 μM, respectively. Compounds **7**, **10**, **22**, and **23** were found to be weak dual PDE4B/PDE7A inhibitors.

The results obtained in this set of compounds confirmed the influence of the aliphatic linker length and the mode of the benzene ring substitution at the amide fragment on PDE4B and PDE7A inhibition. Some compounds (**7**, **10**, **22**, and **23**) with 3- or 4-carbon aliphatic chain were characterized as dual PDE4B/PDE7A inhibitors, while the 5-carbon homologs were inactive. Furthermore, the replacement of the anilide by benzylamide moiety (compound **21** *vs*. **29**) decreased the inhibitory activity toward both PDE4B and PDE7A. Finally, analysis of the benzene ring substitution at the amide group revealed that branched alkyl groups were more favorable than hydroxy, ester, and amide groups for the activity toward PDE4B/PDE7A (*e.g.*, compounds **18**-**23** *vs*. **24**-**26**). For the alkyl substituents, the activity was established in the following order: isopropyl > *tert*-butyl > *sec*-butyl group. It is worth noting that according to the results of molecular modeling studies, the combination of 2-hydroxy and 5-*tert*-butyl substituents at the benzene ring leads to the strongest inhibitory activity toward PDE4B (compounds **7** and **18**) but has a different effect on PDE7A.

2.4. In vitro ADME studies

2.4.1. Parallel artificial membrane permeability assay

The penetration through biological membranes is the crucial aspect determining *in vivo* activity of CNS-targeted compounds. Thus, compounds **19**, **20**, and **22** characterized as potent 5-HT_{1A} receptor antagonists, weak or moderate 5-HT_7 receptor antagonists with additional inhibitory activity toward PDE4B or PDE7A were selected to extended *in vitro* ADME studies in order to determine their passive transport and predict oral absorption.

The membrane permeability of compounds **19**, **20**, and **22** was estimated by the commercially available Pre-coated PAMPA Plate System Gentest™ according to the previously described protocols and formulas [44,45]. Norfloxacin and caffeine were used as a low- and high-permeable reference drug, respectively. The probes were analyzed by LC/MS Waters ACQUITY™ TQD system with the TQ Detector.

Table 5. Membrane permeability of the selected compounds **19**, **20**, **22**, and reference substances.

Compd	P_e [a] [10^{-6} cm/s] ± SD
19	4.8 ± 1.0
20	8.6 ± 1.0
22	10.5 ± 3.1
Norfloxacin	0.6 ± 0.1
Caffeine	15.1 ± 0.4

[a]PAMPA plate's manufacturer breakpoint for permeable compounds: $P_e \geq 1.5 \times 10^{-6}$ cm/s [46]

The calculated permeability coefficients (P_e) for compounds **19**, **20**, and **22** were much higher than that for a low-permeable reference drug, norfloxacin and lower than that for a high-permeable compound, caffeine (Table **5**). Moreover, the obtained P_e values for compounds **19**, **20**, and **22** were 3-, 6-, or 7-fold higher, respectively, than that indicated as the breakpoint for permeable compounds ($P_e \geq 1.5 \times 10^{-6}$ cm/s) [46].

In this study, the passive transport of compounds **19**, **20**, and **22** across the cell membranes as well as an opportunity for their oral absorption were estimated as high. The highest ability to passively penetrate through biological membranes was found for the 4-isopropylanilide derivative **22**.

2.4.2. Metabolic stability study

Determination of metabolic stability and metabolites of new chemical entities is a key step in the process of drug discovery, since it influences pharmacological, pharmacokinetic, and toxicological properties of the tested compounds. The preliminary metabolic stability study of compounds **19**, **20**, and **22** was performed *in vitro* using rat liver microsomes (RLMs) according to the previously described protocols [47]. The supernatants were analyzed by UPLC/MS using LC/MS Waters ACQUITY™ TQD system with the TQ Detector. The results of biotransformation study and characteristics of metabolites were presented in Table **6**. Additionally, the *in silico* study was

performed by MetaSite 6.0.1 and the sites of biotransformation of compound **22** were predicted (Fig. **6**).

UPLC/MS analyses of the reaction mixtures after 2 hours of incubation with RLMs indicated the rapid and almost complete decomposition of compounds **19** and **20**, possibly due to the hydrolysis of anilide (amide) bond to alkylcarboxylic acid (**19-M1**, **20-M1**) and an appropriate aniline derivative (**19-M2**, **20-M2**) (Table **6**).

Table 6. Biotransformation study results of compounds **19**, **20**, and **22** and their metabolite characteristics.

Parent compounds and their metabolites	$[M+H]^+$	Metabolic pathway	% content after incubation with RLMs
19	424.34	hydrolysis	23.8
19-M1	293.21	-	66.2
19-M2	150.14	-	10.0
20	410.38	hydrolysis	6.5
20-M1	293.21	-	80.9
20-M2	136.11	-	12.6
22	410.38	hydroxylation	90.1
22-M1	426.33	-	5.9
22-M2	426.33	-	4.0

According to the UPLC/MS spectra, compound **22** was characterized by a high metabolic stability using RLMs. After 2 hours of incubation with RLMs, only around 10 % of compound **22** (t_R = 5.19 min, $[M+H]^+$ = 410.38) was metabolized into two metabolites: **22-M1** (t_R = 4.64 min, $[M+H]^+$ = 426.33) and **22-M2** (t_R = 3.72 min, $[M+H]^+$ = 426.33) (Supp. Fig. **S1-4**). The MetaSite *in silico* prediction (Fig. **6**), the ion fragmentation analyses performed for compound **22** and its metabolites as well as increased $[M+H]^+$ values of both metabolites **22-M1** and **22-M2** by 16 mass units relative to the initial $[M+H]^+$ value of compound **22** indicated the hydroxylation at the benzene ring of the phenylpiperazine scaffold (**22-M1**) or the hydroxylation at the isopropyl group of the anilide fragment (**22-M2**) as the most probable biotransformation sites of compound **22**.

Analysis of the structure-metabolic stability relationship in the studied group of anilide derivatives enabled the statement that branched alkyl substituent at the *para* position of the benzene ring at the amide fragment increased the metabolic stability (compound **22**), while substitution at the *meta* position decreased the metabolic stability in the RLMs (compound **19** and **20**). The results obtained make compound **22** a good candidate for further studies, including behavioral tests in animals.

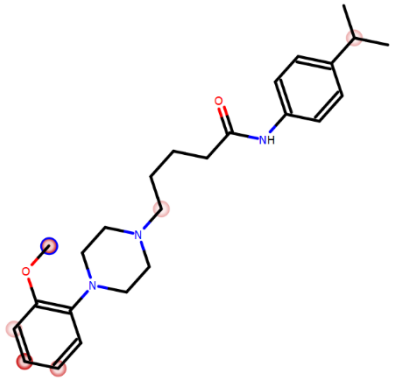


Fig. 6. The plot of MetaSite 6.0.1 prediction for sites of biotransformation of compound **22**. The darker red color of the marked functional group indicates its higher probability to be involved in the metabolism pathway. The blue circle marked the site of compound **22** which may be involved in metabolism with the highest probability.

2.5. In vivo behavioral evaluation

The *in vitro* pharmacological profile and suitable ADME properties of the presented compounds prompted us to evaluate their procognitive and antidepressant activity *in vivo*. We selected for behavioral studies compound **22** which was characterized as a potent 5-HT_{1A} receptor antagonist with weak 5-HT_7 receptor antagonistic properties and an inhibitory activity toward both PDE4B and PDE7A isoenzymes. The choice was dictated by the results of functional studies suggesting the insignificance of antagonistic properties of compound **22** toward the 5-HT_7 receptor and its negligible effect on the reduction of cAMP level. Moreover, several studies provided evidence that 5-HT_7 receptor blockade may be also useful in treating cognitive and antidepressant disorders [5,48].

The procognitive and antidepressant activity of compound **22** was tested in Wistar rats at doses of 1, 3, and 10 mg/kg (*i.p.*) using well-established experimental paradigms, *i.e.*, novel object recognition (NOR) and forced swimming (FST) tests, respectively. Moreover, in order to exclude the possibility of competing behaviors, such as general locomotor activity, the open field (OF) test was carried out and the influence of effective doses of **22** was studied.

*2.5.1. Ability of compound **22** to reverse memory impairment*

Following a single administration, compound **22** at doses of 1 and 3 mg/kg reversed MK-801-induced memory impairment in the NOR test in rats (Fig. **7**) but the effect was statistically significant only at the dose of 3 mg/kg. Moreover, effective dose of 3 mg/kg obtained in the NOR test had no influence on the spontaneous locomotor activity of the animals in the OF test (Table **7**).

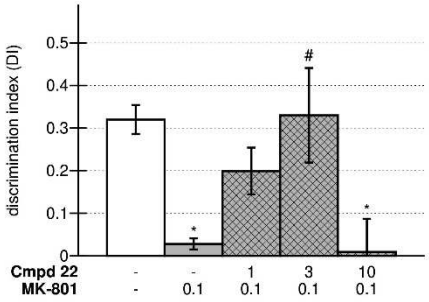


Fig. 7. Effect of compound **22** on MK-801-induced memory deficits in rat NOR test. MK-801 was administered *i.p.* 30 min and the tested compound 60 min before the T1 phase. Data represent mean ± SEM. Rats per group N = 6–8. *p < 0.05 *vs.* vehicle-treated group, #p < 0.05 *vs.* MK-801-treated group (one-way ANOVA followed by Bonferroni's posthoc test).

Table 7. Effect of compound **22** and escitalopram on the rat locomotor activity measured in the OF test.

Treatment	Dose (mg/kg)	Total distance (cm)	Rearings	Ambulations X	Ambulations Y
Vehicle	0	2334.7 ± 201.4	48.0 ± 6.5	223.5 ± 23.5	203.3 ± 31.1
Cmpd 22	3	2257.2 ± 229.6	44.0 ± 4.1	116.8 ± 32.1	219.8 ± 35.4
	10	1156.8 ± 105.0**	18.5 ± 1.3***	103.0 ± 14.6*	82.7 ± 6.7**
Vehicle	0	1897.3 ± 98.6	42.6 ± 4.8	194.5 ± 21.4	201.1 ± 26.3
S-cit	20	2058.5 ± 113.1	39.9 ± 6.3	205.1 ± 24.0	192.5 ± 15.2

The compound **22** was injected *i.p.* 60 min, while escitalopram (S-cit) was given *i.p.* 30 min before the test. Values represent the mean ± SEM during 5-min test session compared to the respective vehicle-treated group. Rats per group N = 4–6. *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA followed by the Bonferroni's post hoc test).

*2.5.1. Antidepressant-like effect of compound **22***

Following a single administration, compound **22** reduced the immobility time of animals in the FST by about 34 % at dose of 10 mg/kg, while the reference drug, escitalopram, revealed antidepressant-like effect at 2-fold higher dose of 20 mg/kg, shortening the immobility time by about 49 % (Fig. **8**). The antidepressant-like effect produced by compound **22** as well as escitalopram seems to be specific, since both compounds given at their effective doses did not increase the spontaneous locomotor activity of rats in the OF test (Table **7**). However, compound **22** significantly reduced the mobility of animals which indicates its sedative properties.

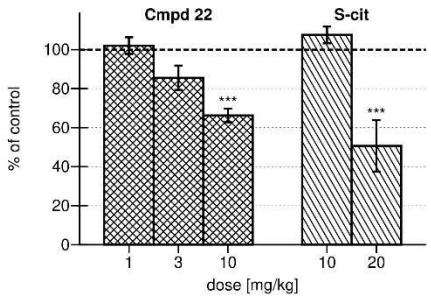


Fig. 8. Effect of compound **22** and escitalopram in the FST in rats. The compound **22** was injected *i.p.* 60 min, while escitalopram 30 min before the test. Values represent mean ± SEM and are expressed as percentage of control. Rats per group N = 6–8. ***p < 0.001 *vs.* vehicle-treated group (one-way ANOVA followed by the Bonferroni's post hoc test).

Animal tests showed both procognitive and antidepressant effects of compound **22** being a representative of a new class of compounds with unique mechanism of action. However, the *in vivo* activity of compound **22** seems to be related to its antagonistic properties toward the 5-HT_{1A} receptor. Further *in vivo* studies, including pharmacokinetic evaluation, are needed to provide evidence for the participation of individual targets in the *in vivo* activity. In addition, the *in vitro* studies to specify the affinity of obtained compounds for other receptors which are recognized therapeutic targets in the treatment of CNS disorders or are associated with adverse effects should be performed. The obtained compound **22** represents the new lead structure for further optimization to find more active and metabolically stable derivatives showing this desired multi-target mechanism of action. An improved multifunctional ligand with a balanced activity profile toward 5-HT_{1A}/5-HT_7 receptors and PDE4B/PDE7A will be eventually tested for its efficacy in an animal model of Alzheimer's disease.

3. Conclusions

In this paper, the computer-aided design and synthesis of 33 innovative anilide and benzylamide derivatives of ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoic acids were described. The synthesized compounds were found to be potent to moderate 5-HT_{1A} receptor antagonists with moderate to weak 5-HT_7 receptor antagonistic properties and additional PDE4B and PDE7A inhibitory activities. Binding mode predictions shed light on structural requirements for multifunctional 5-HT_{1A}/5-HT_7 receptor ligands and PDE4B/PDE7A inhibitors. SAR analysis showed that the 4-carbon aliphatic chain between cyclic amine and amide fragments was the most favorable for obtaining multi-target-directed ligands of 5-HT_{1A}/5-HT_7 receptors and PDE4B/PDE7A inhibitors. Moreover, the benzene ring substitution site at the amide fragment was found as a possible modification site of the metabolic stability of the tested compounds. The selected compound **22** characterized as a potent 5-HT_{1A} receptor antagonist, a weak 5-HT_7 receptor antagonist with an additional PDE4B/PDE7A inhibitory activity and suitable *in vitro* ADME properties showed *in vivo* procognitive-like and antidepressant-

like activity in well-established experimental tests in rats. The *in vitro* studies suggested that the behavioral effects of compound **22** were most likely related to its antagonistic properties toward 5-HT_{1A} receptor; however, the detailed selectivity and pharmacokinetic studies are needed to establish the participation of individual targets in *in vivo* activity. The obtained compound **22** represents a new lead structure for further optimization to find more active and metabolically stable derivatives with procognitive and antidepressant activity.

4. Materials and methods

4.1. In silico studies

The design of compounds was supported by calculation of molecular properties and prediction of physicochemical properties, BBB permeation, and risk of toxicity using appropriate software. The calculation of molecular properties and prediction of physicochemical characteristics were performed using Instant JChem software [49]. PAINS alerts and the ability of compound to cross the BBB was checked using SwissADME server [50]. OSIRIS Property Explorer was used to estimate the risk of toxicity [39]. The prediction of metabolic biotransformation was performed using MetaSite 6.0.1 (Molecular Discovery Ltd, UK) [51].

The studies on the binding mode were performed using Small-Molecule Drug Discovery Suite (Schrödinger Ltd.) [52]. Ligand structures were optimized using LigPrep tool and OPLS-3 force field. The structural models of serotonin receptors 5-HT_{1A} and 5-HT_7 and PDEs 4 and 7 have been described previously [4,43]. The docking procedure was carried out by Glide XP tool using default settings. Complexes were additionally optimized by Prime tool (Refine Protein-Ligand Complex) in order to minimize their energy and correct ligand arrangement in the active site. The results were analyzed for both Glide gscore and the presence of proper interactions.

4.2. Synthesis

All the reagents and solvents were purchased from Sigma-Aldrich, TCI Europe, or Fluorochem. Thin layer chromatography was performed on Merck silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany) with the following solvent: (A) dichloromethane/methanol (9:1). Spots were detected by their absorption under UV light (λ = 254 nm). Column chromatography was performed on Merck silica gel 60 (63-200 mm) with the solvent A. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Mercury 300 at 300 MHz and 75 MHz, respectively, using TMS (0.00 ppm) as an internal standard and DMSO-d_6 as solvent. Chemical shifts were expressed in δ (ppm) and the coupling constants *J* in Hertz (Hz) and splitting patterns were designated as follows: s (singlet), b.s. (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). LC/MS analyses were performed on Waters Acquity TQD apparatus with eλ DAD detector. For mass spectrometry, ESI^+ (electrospray positive) ionization mode was used. UV spectra were taken in the range of 200-700 nm. For establishing the purity of compounds, UV chromatograms were used. The UPLC/MS purity of all the

synthesized compounds was determined to be over 98 %. Melting points (mp) were determined with a Büchi apparatus and are uncorrected. Elemental analyses were taken with Elementar Vario EL III apparatus. Analyses indicated by the symbols of the elements were within ± 0.4 % of the theoretical values.

*4.2.1. General procedure for the synthesis of ethyl ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoates (**1**-**3**) and ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoic acids (**4**-**6**)*

The synthesis of the intermediates **1**-**3** from 1-(2-methoxyphenyl)piperazine and **4**-**6** from ethyl ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanoates **1**-**3** has been reported previously [53]:

- Ethyl 4-(4-(2-methoxyphenyl)piperazin-1-yl)butanoate (**1**),
- Ethyl 5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanoate (**2**),
- Ethyl 6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanoate (**3**),
- 4-(4-(2-Methoxyphenyl)piperazin-1-yl)butanoic acid (**4**),
- 5-(4-(2-Methoxyphenyl)piperazin-1-yl)pentanoic acid (**5**),
- 6-(4-(2-Methoxyphenyl)piperazin-1-yl)hexanoic acid (**6**).

*4.2.2. General procedure for the synthesis of hydrochlorides of the ω-(4-(2-methoxyphenyl)piperazin-1-yl)alkanamides (**7**-**39**)*

A mixture of 1 mmol of alkanoic acid **4**, **5**, or **6** with 1.5 mmol of CDI in DMF (5 mL) was stirred at room temperature for 30 minutes. Then, 1 mmol of appropriate aniline or benzylamine was added and stirring was continued for 72 hours. Afterwards, 2-3 drops of water were added and the mixture was concentrated under reduced pressure. The obtained crude product was purified by silica gel chromatography with solvent A as eluent. The hydrochlorides of compounds **7**-**39** were prepared by dissolving the compounds in a minimum quantity of methanol. Then, the solution was treated with 1 N solution of HCl(g) in methanol, evaporated under reduced pressure, washed, and dried.

*4.2.2.1. N-(5-(tert-butyl)-2-hydroxyphenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**7**)*

The title compound was obtained from **4** and 2-amino-4-(*tert*-butyl)phenol as a white solid; yield 59 %; mp 176-178 °C; R_f = 0.42 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.21 (s, 9H, C(C*H*$_3$)$_3$), 1.89 - 2.13 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.53 (t, *J* = 7.00 Hz, 2H, CH$_2$CH$_2$C*H*$_2$), 2.94 - 3.23 (m, 6H, N(CH$_2$C*H*$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$), 3.42 - 3.60 (m, 4H, N(C*H*$_2$CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.78 (d, *J* = 8.79 Hz, 1H, 3-Ph'), 6.84 - 7.07 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.74 (d, *J* = 2.34 Hz, 1H, 6-Ph'), 9.44 (s, 1H, CON*H*), 9.55 (s, 1H, O*H*), 10.65 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.8, 31.8, 33.2, 34.2, 47.3, 51.6, 55.6, 55.8, 112.3, 116.0, 118.6, 120.0, 121.3, 121.9, 123.9, 126.0, 139.9,

141.6, 146.1, 152.2, 171.0; LC/MS: *m/z* calc. 426.28, found 426.27; MW 462.02; Anal. $C_{25}H_{36}ClN_3O_3$ (C, H, N).

*4.2.2.2. N-(3-(tert-butyl)phenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**8**)*

The title compound was obtained from **4** and 3-(*tert*-butyl)aniline as a white solid; yield 69 %; mp 201-203 °C; R_f = 0.39 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.24 (s, 9H, C(C*H*$_3$)$_3$), 1.97 - 2.11 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.43 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$C*H*$_2$), 3.00 - 3.22 (m, 6H, N(CH$_2$C*H*$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$), 3.40 - 3.61 (m, 4H, N(C*H*$_2$CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.85 - 7.08 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.19 (t, *J* = 7.91 Hz, 1H, 5-Ph'), 7.48 (d, *J* = 7.60 Hz, 1H, 6-Ph'), 7.61 (s, 1H, 2-Ph'), 10.11 (s, 1H, CON*H*), 10.92 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.7, 31.6, 33.6, 34.8, 47.3, 51.6, 55.5, 55.8, 112.3, 116.6, 116.8, 118.6, 120.5, 121.3, 123.8, 128.7, 139.4, 139.9, 151.6, 152.2, 170.3; LC/MS: *m/z* calc. 410.28, found 410.25; MW 446.03; Anal. $C_{25}H_{36}ClN_3O_2$ (C, H, N).

*4.2.2.3. N-(3-isopropylphenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**9**)*

The title compound was obtained from **4** and 3-isopropylaniline as a white solid; yield 57 %; mp 197-199 °C; R_f = 0.41 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, *J* = 7.03 Hz, 6H, CH(C*H*$_3$)$_2$), 1.95 - 2.11 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.43 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$C*H*$_2$), 2.72 - 2.89 (m, 1H, C*H*(CH$_3$)$_2$), 2.99 - 3.19 (m, 6H, N(C*H*$_2$CH$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$), 3.42 - 3.61 (m, 4H, N(CH$_2$C*H*$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.84 - 7.05 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.18 (t, *J* = 7.91 Hz, 1H, 5-Ph'), 7.43 (d, *J* = 8.21 Hz, 1H, 6-Ph'), 7.48 (s, 1H, 2-Ph'), 10.07 (s, 1H, CON*H*), 10.75 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.7, 24.3, 33.6, 33.9, 47.2, 51.5, 55.5, 55.8, 112.3, 117.2, 117.5, 118.6, 121.3, 121.6, 123.8, 129.0, 139.7, 139.9, 149.3, 152.2, 170.3; LC/MS: *m/z* calc. 396.26, found 396.29; MW 432.00; Anal. $C_{24}H_{34}ClN_3O_2$ (C, H, N).

*4.2.2.4. N-(4-(tert-butyl)phenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**10**)*

The title compound was obtained from **4** and 4-(*tert*-butyl)aniline as a white solid; yield 58 %; mp 196-198 °C; R_f = 0.44 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (s, 9H, C(C*H*$_3$)$_3$), 1.76 (m, *J* = 7.03 Hz, 2H, CH$_2$C*H*$_2$CH$_2$), 2.21 - 2.42 (m, *J* = 7.03 Hz, 4H, C*H*$_2$CH$_2$C*H*$_2$), 2.51 (m, 2H, N(C*H*$_2$CH$_2$)$_2$NH$^+$), 2.93 (m, 4H, N(CH$_2$C*H*$_2$)$_2$NH$^+$), 3.74 (s, 3H, OC*H*$_3$), 6.76 - 6.99 (m, *J* = 2.34 Hz, 4H, 3,4,5,6-Ph), 7.27 (m, *J* = 8.79 Hz, 2H, 3,5-Ph'), 7.49 (m, *J* = 8.79 Hz, 2H, 2,6-Ph'), 9.77 (s, 1H, CON*H*), 10.78 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.7, 31.6, 33.6, 34.4, 47.3, 51.6, 55.5, 55.8, 112.4, 118.6, 119.4, 121.3, 123.9, 125.7, 137.1, 139.9, 145.8, 152.2, 170.2; LC/MS: *m/z* calc. 410.28, found 410.32; MW 446.03; Anal. $C_{25}H_{36}ClN_3O_2$ (C, H, N).

*4.2.2.5. N-(4-isopropylphenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**11**)*

The title compound was obtained from **4** and 4-isopropylaniline as a white solid; yield 59 %; mp 200-202 °C; R_f = 0.40 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.15 (d, *J* = 7.03 Hz, 6H, CH(C*H*$_3$)$_2$),

1.83 - 2.05 (m, 2H, $CH_2CH_2CH_2$), 2.39 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2$), 2.72 - 2.85 (m, 1H, $CH(CH_3)_2$), 2.85 - 3.47 (m, 10H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.77 (s, 3H, OCH_3), 6.84 - 7.03 (m, 4H, 3,4,5,6-Ph), 7.14 (d, *J* = 8.79 Hz, 2H, 3,5-Ph'), 7.50 (d, *J* = 8.21 Hz, 2H, 2,6-Ph'), 9.97 (s, 1H, CONH), 10.73 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 20.3, 24.4, 33.3, 33.8, 47.9, 52.0, 55.8, 56.0, 112.3, 118.6, 119.7, 121.3, 123.6, 126.8, 137.5, 140.2, 143.5, 152.3, 170.4; LC/MS: *m/z* calc. 396.26, found 396.22; MW 432.00; Anal. $C_{24}H_{34}ClN_3O_2$ (C, H, N).

*4.2.2.6. N-(4-(sec-butyl)phenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**12**)*

The title compound was obtained from **4** and 4-(*sec*-butyl)aniline as a white solid; yield 55 %; mp 168-170 °C; R_f = 0.42 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.73 (t, *J* = 7.60 Hz, 3H, $CH(CH_3)CH_2CH_3$), 1.14 (d, *J* = 6.45 Hz, 3H, $CH(CH_3)CH_2CH_3$), 1.38 - 1.60 (m, 2H, $CH(CH_3)CH_2CH_3$), 1.93 - 2.12 (m, 2H, $CH_2CH_2CH_2$), 2.34 - 2.46 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2$), 2.93 - 3.24 (m, 7H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$, $CH(CH_3)CH_2CH_3$), 3.38 - 3.61 (m, 4H, $N(CH_2CH_2)_2NH^+$,), 3.77 (s, 3H, OCH_3), 6.81 - 7.04 (m, 4H, 3,4,5,6-Ph), 7.10 (d, *J* = 8.80 Hz, 2H, 3,5-Ph'), 7.50 (d, *J* = 8.20 Hz, 2H, 2,6-Ph'), 10.05 (s, 1H, CONH), 10.74 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.5, 19.7, 22.3, 31.0, 33.6, 40.8, 47.2, 51.6, 55.5, 55.8, 112.4, 118.6, 119.7, 121.3, 123.8, 127.4, 137.5, 139.9, 142.3, 152.2, 170.2; LC/MS: *m/z* calc. 410.28, found 410.25; MW 445.25; Anal. $C_{25}H_{36}ClN_3O_2$ (C, H, N).

*4.2.2.7. Tert-butyl 4-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamido)benzoate hydrochloride (**13**)*

The title compound was obtained from **4** and *tert*-butyl 4-aminobenzoate as a white solid; yield 79 %; mp 180-182 °C; R_f = 0.42 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.51 (s, 9H, $C(CH_3)_3$), 1.96 - 2.13 (m, 2H, $CH_2CH_2CH_2$), 2.53 (t, *J* = 7.00 Hz, 2H, $CH_2CH_2CH_2$), 3.01 - 3.22 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.38 - 3.53 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 6.75 - 7.09 (m, 4H, 3,4,5,6-Ph), 7.73 (d, *J* = 8.80 Hz, 2H, 3,5-Ph'), 7.83 (d, *J* = 8.80 Hz, 2H, 2,6-Ph'), 10.55 (s, 1H, CONH), 10.90 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.5, 28.3, 33.7, 47.3, 51.6, 55.5, 55.8, 80.7, 112.3, 118.6, 118.7, 121.3, 123.9, 126.0, 130.5, 139.9, 143.7, 152.2, 165.0, 171.0; LC/MS: *m/z* calc. 454.27, found 454.18; MW 490.03; Anal. $C_{26}H_{36}ClN_3O_4$ (C, H, N).

*4.2.2.8. N-(2-hydroxyphenyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**14**)*

The title compound was obtained from **4** and 2-aminophenol as a white solid; yield 55 %; mp 229-231 °C; R_f = 0.38 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.91 - 2.13 (m, 2H, $CH_2CH_2CH_2$), 2.52 - 2.56 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2$), 2.93 - 3.25 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.37 - 3.61 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 6.67 - 6.79 (m, 1H, 3-Ph'), 6.82 - 7.06 (m, 6H, 3,4,5,6-Ph, 4,5-Ph'), 7.74 (d, *J* = 7.03 Hz, 1H, 6-Ph'), 9.38 (s, 1H, CONH), 9.80 (s, 1H, OH), 10.67 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.8, 33.3, 47.3, 51.6, 55.6, 55.8, 112.4,

116.3, 118.6, 119.3, 121.3, 123.0, 123.9, 125.1, 126.6, 139.9, 148.5, 152.2, 170.8; LC/MS: *m/z* calc. 370.21, found 370.17; MW 405.92; Anal. $C_{21}H_{28}ClN_3O_3$ (C, H, N).

*4.2.2.9. 2-(4-(4-(2-Methoxyphenyl)piperazin-1-yl)butanamido)benzamide hydrochloride (**15**)*

The title compound was obtained from **4** and 2-aminobenzamide as a white solid; yield 60 %; mp 209-211 °C; R_f = 0.33 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.99 - 2.12 (m, 2H, $CH_2CH_2CH_2$), 2.41 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2$), 2.96 - 3.24 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.39 - 3.61 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 6.83 - 7.04 (m, 4H, 3,4,5,6-Ph), 7.10 (t, *J* = 7.00 Hz, 1H, 5-Ph'), 7.47 (t, *J* = 7.60 Hz, 1H, 4-Ph'), 7.79 (dd, *J* = 7.91, 1.47 Hz, 1H, 3-Ph'), 8.28 (br.s., 2H, $CONH_2$), 8.42 (d, *J* = 7.62 Hz, 1H, 6-Ph'), 10.70 (br.s., 1H, CONH), 11.68 (s, 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 18.9, 34.0, 46.7, 51.1, 54.9, 55.3, 111.8, 118.1, 119.9, 120.3, 120.8, 122.4, 123.3, 128.6, 132.0, 139.3, 139.4, 151.7, 169.7, 170.6; LC/MS: *m/z* calc. 397.22, found 397.22; MW 432.94; Anal. $C_{22}H_{29}ClN_4O_3$ (C, H, N).

*4.2.2.10. N-(2-hydroxybenzyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**16**)*

The title compound was obtained from **4** and 2-(aminomethyl)phenol as a white solid; yield 63 %; mp 144-146 °C; R_f = 0.40 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.86 - 2.04 (m, 2, $CH_2CH_2CH_2$), 2.20 - 2.32 (m, 2H, $CH_2CH_2CH_2$), 2.93 - 3.20 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.43 - 3.56 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 4.19 (d, *J* = 5.86 Hz, 2H, $CONHCH_2$), 6.70 - 7.11 (m, 8H, 3,4,5,6-Ph, 3,4,5,6-Ph'), 8.39 (t, *J* = 5.86 Hz, 1H, CONH), 9.59 (s, 1H, OH), 10.65 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.9, 32.5, 38.0, 47.3, 51.5, 55.6, 55.8, 112.4, 115.5, 118.6, 119.2, 121.3, 123.9, 125.5, 128.3, 129.0, 139.8, 152.2, 155.4, 171.8; LC/MS: *m/z* calc. 384.23, found 384.26; MW 419.94; Anal. $C_{22}H_{30}ClN_3O_3$ (C, H, N).

*4.2.2.11. N-(2-methoxybenzyl)-4-(4-(2-methoxyphenyl)piperazin-1-yl)butanamide hydrochloride (**17**)*

The title compound was obtained from **4** and (2-methoxyphenyl)methanamine as a white solid; yield 82 %; mp 97-99 °C; R_f = 0.40 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.90 - 2.05 (m, 2H, $CH_2CH_2CH_2$), 2.23 - 2.32 (m, 2H, $CH_2CH_2CH_2$), 2.93 - 3.21 (m, 6H, $(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2$), 3.39 - 3.57 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, CH_3OPh), 3.78 (s, 2H, CH_3OPh'), 4.22 (d, *J* = 5.86 Hz, 2H, $CONHCH_2$), 6.79 - 7.07 (m, 6H, 3,4,5,6-Ph, 3,5-Ph'), 7.08 - 7.28 (m, 2H, 4,6-Ph'), 8.33 (t, *J* = 5.86 Hz, 1H, CONH), 10.83 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 19.9, 32.6, 37.6, 47.2, 51.5, 55.6, 55.7, 55.8, 110.9, 112.3, 118.6, 120.6, 121.3, 123.9, 127.1, 128.2, 128.5, 139.9, 152.2, 157.0, 171.5; LC/MS: *m/z* calc. 398.24, found 398.22; MW 433.97; Anal. $C_{23}H_{32}ClN_3O_3$ (C, H, N).

*4.2.2.12. N-(5-(tert-butyl)-2-hydroxyphenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**18**)*

The title compound was obtained from **5** and 2-amino-4-(*tert*-butyl)phenol as a white solid; yield 63 %; mp 86-88 °C; R_f = 0.49 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20 (s, 9H, C(CH_3)$_3$), 1.54 - 1.69 (m, 2H, CH$_2$CH_2CH$_2$CH$_2$), 1.69 - 1.85 (m, 2H, CH$_2$CH$_2$CH_2CH$_2$), 2.91 - 3.23 (m, 6H, N(CH$_2$CH_2)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$), 3.47 - 3.59 (m, 4H, N(CH_2CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.77 (d, *J* = 8.21 Hz, 1H, 3-Ph'), 6.83 - 7.07 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.69 (d, *J* = 2.34 Hz, 1H, 6-Ph'), 9.45 (s, 1H, CON*H*), 9.57 (s, 1H, O*H*), 10.55 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.9, 23.1, 31.8, 34.2, 35.6, 47.3, 51.5, 55.6, 55.8, 112.3, 116.3, 118.6, 119.9, 121.3, 122.0, 123.9, 126.1, 139.8, 141.6, 146.1, 152.2, 171.9; LC/MS: *m/z* calc. 440.29, found 440.16; MW 476.05; Anal. $C_{26}H_{38}ClN_3O_3$ (C, H, N).

*4.2.2.13. N-(3-(tert-butyl)phenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**19**)*

The title compound was obtained from **5** and 3-(*tert*-butyl)aniline as a white solid; yield 78 %; mp 216-218 °C; R_f = 0.45 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (s, 9H, C(CH_3)$_3$), 1.55 - 1.67 (m, 2H, CH$_2$CH_2CH$_2$CH$_2$), 1.69 - 1.84 (m, 2H, CH$_2$CH$_2$CH_2CH$_2$), 2.36 (t, *J* = 7.33 Hz, 2H, CH$_2$CH$_2$CH$_2$CH_2), 3.03 - 3.21 (m, 6H, N(CH_2CH$_2$)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$), 3.38 - 3.57 (m, 4H, N(CH$_2$CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.85 - 7.07 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.18 (t, *J* = 7.91 Hz, 1H, 5-Ph'), 7.45 - 7.53 (m, 1H, 6-Ph'), 7.61 (m, 1H, 2-Ph'), 10.04 (s, 1H, CON*H*), 10.95 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.7, 23.2, 31.6, 34.8, 36.1, 47.3, 51.4, 55.6, 55.8, 112.4, 116.5, 116.8, 118.7, 120.4, 121.3, 124.1, 128.7, 139.5, 139.6, 151.6, 152.2, 171.1; LC/MS: *m/z* calc. 424.30, found 424.34; MW 460.05; Anal. $C_{26}H_{38}ClN_3O_2$ (C, H, N).

*4.2.2.14. N-(3-isopropylphenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**20**)*

The title compound was obtained from **5** and 3-isopropylaniline as a white solid; yield 64 %; mp 227-229 °C; R_f = 0.47 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, *J* = 7.03 Hz, 6H, CH(CH_3)$_2$), 1.53 - 1.68 (m, 2H, CH$_2$CH_2CH$_2$CH$_2$), 1.69 - 1.84 (m, 2H, CH$_2$CH$_2$CH_2CH$_2$), 2.36 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$CH$_2$CH_2), 2.80 (m, 1H, C*H*(CH$_3$)$_2$), 2.98 - 3.18 (m, 6H, N(CH_2CH$_2$)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$), 3.39 - 3.58 (m, 4H, N(CH$_2$CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.79 - 7.04 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.17 (t, *J* = 7.62 Hz, 1H, 5-Ph'), 7.37 - 7.52 (m, 2H, 2,6-Ph'), 10.00 (s, 1H, CON*H*), 10.69 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.8, 23.1, 24.3, 33.9, 36.1, 47.2, 51.5, 55.6, 55.8, 112.3, 117.1, 117.4, 118.6, 121.3, 121.5, 123.9, 128.9, 139.8, 139.9, 149.3, 152.2, 171.1; LC/MS: *m/z* calc. 410.28, found 410.32; MW 446.03; Anal. $C_{25}H_{36}ClN_3O_2$ (C, H, N).

*4.2.2.15. N-(4-(tert-butyl)phenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**21**)*

The title compound was obtained from **5** and 4-(*tert*-butyl)aniline as a white solid; yield 62 %; mp 222-224 °C; R_f = 0.45 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (s, 9H, C(C*H*$_3$)$_3$), 1.53 - 1.84 (m, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 2.35 (t, *J* = 6.74 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.94 - 3.22 (m, 6H, N(C*H*$_2$CH$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$), 3.46 (t, *J* = 14.07 Hz, 4H, N(CH$_2$C*H*$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.83 - 7.05 (m, 4H, 3,4,5,6-Ph), 7.28 (d, *J* = 8.21 Hz, 2H, 3,5-Ph'), 7.51 (d, *J* = 8.79 Hz, 2H, 2,6-Ph'), 9.97 (s, 1H, CON*H*), 10.65 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.8, 23.1, 31.7, 34.4, 36.0, 47.2, 51.5, 55.6, 55.8, 112.3, 118.6, 119.3, 121.3, 123.9, 125.6, 137.2, 139.8, 145.7, 152.2, 171.0; LC/MS: *m/z* calc. 424.30, found 424.21; MW 460.05; Anal. $C_{26}H_{38}ClN_3O_2$ (C, H, N).

*4.2.2.16. N-(4-isopropylphenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**22**)*

The title compound was obtained from **5** and 4-isopropylaniline as a white solid; yield 64 %; mp 204-206 °C; R_f = 0.45 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.15 (d, *J* = 6.45 Hz, 6H, CH(C*H*$_3$)$_2$), 1.53 - 1.84 (m, 4H, CH$_2$C*H*$_2$C*H*$_2$CH$_2$), 2.35 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.80 (m, 1H, C*H*(CH$_3$)$_2$), 2.94 - 3.20 (m, 6H, N(C*H*$_2$CH$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$), 3.48 (t, *J* = 11.00 Hz, 4H, N(CH$_2$C*H*$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.83 - 7.05 (m, 4H, 3,4,5,6-Ph), 7.13 (d, *J* = 8.20 Hz, 2H, 3,5-Ph'), 7.50 (d, *J* = 8.20 Hz, 2H, 2,6-Ph'), 9.98 (s, 1H, CON*H*), 10.70 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.8, 23.1, 24.4, 33.3, 36.0, 47.2, 51.4, 55.6, 55.8, 112.3, 118.6, 119.6, 121.3, 123.9, 126.7, 137.6, 139.8, 143.4, 152.2, 170.9; LC/MS: *m/z* calc. 410.28, found 410.25; MW 446.03; Anal. $C_{25}H_{36}ClN_3O_2$ (C, H, N).

*4.2.2.17. N-(4-(sec-butyl)phenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**23**)*

The title compound was obtained from **5** and 4-(*sec*-butyl)aniline as a white solid; yield 67 %; mp 198-200 °C; R_f = 0.47 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.73 (t, *J* = 7.33 Hz, 3H, CH(CH$_3$)CH$_2$C*H*$_3$), 1.13 (d, *J* = 7.03 Hz, 3H, CH(C*H*$_3$)CH$_2$CH$_3$), 1.42 - 1.55 (m, 2H, CH(CH$_3$)C*H*$_2$CH$_3$), 1.56 - 1.68 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$), 1.69 - 1.84 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_2$), 2.35 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.94 - 3.21 (m, 7H, N(CH$_2$C*H*$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$, C*H*(CH$_3$)CH$_2$CH$_3$), 3.38 - 3.56 (m, 4H, N(C*H*$_2$CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.83 - 7.04 (m, 4H, 3,4,5,6-Ph), 7.08 (d, *J* = 8.80 Hz, 2H, 3,5-Ph'), 7.51 (d, *J* = 8.20 Hz, 2H, 2,6-Ph'), 9.97 (s, 1H, CON*H*), 10.69 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.5, 22.3, 22.9, 23.1, 31.1, 36.1, 40.8, 47.2, 51.5, 55.6, 55.8, 112.3, 118.6, 119.6, 121.3, 123.8, 127.3, 137.6, 139.9, 142.1, 152.2, 170.9; LC/MS: *m/z* calc. 424.30, found 424.27; MW 460.05; Anal. $C_{26}H_{38}ClN_3O_2$ (C, H, N).

*4.2.2.18. Tert-butyl 4-(5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamido)benzoate hydrochloride (**24**)*

The title compound was obtained from **5** and *tert*-butyl 4-aminobenzoate as a white solid; yield 57 %; mp 242-244 °C; R_f = 0.47 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.50 (s, 9H, C(C*H*$_3$)$_3$), 1.55 - 1.68 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$), 1.68 - 1.79 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_2$), 2.42 (t, *J* = 6.74 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.93 - 3.37 (m, 10H, N(C*H*$_2$C*H*$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$), 3.76 (s, 3H, OC*H*$_3$), 6.84 - 7.02 (m, 4H, 3,4,5,6-Ph), 7.73 (d, *J* = 9.40 Hz, 2H, 3,5-Ph'), 7.82 (d, *J* = 8.80 Hz, 2H, 2,6-Ph'), 10.44 (s, 1H, CON*H*), 10.80 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.6, 23.3, 28.3, 36.2, 47.5, 51.7, 55.8, 80.6, 110.0, 112.3, 118.6, 118.7, 121.3, 123.8, 125.9, 130.4, 140.0, 143.8, 152.2, 165.1, 171.9; LC/MS: *m/z* calc. 468.34, found 468.29; MW 504.06; Anal. $C_{27}H_{38}ClN_3O_4$ (C, H, N).

*4.2.2.19. N-(2-hydroxyphenyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**25**)*

The title compound was obtained from **5** and 2-aminophenol as a white solid; yield 52 %; mp 218-220 °C; R_f = 0.43 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.57 - 1.70 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$), 1.70 - 1.79 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_2$), 2.43 (t, *J* = 6.50 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.96 - 3.18 (m, 6H, N(CH$_2$C*H*$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$), 3.46 (t, *J* = 12.30 Hz, 4H, N(C*H*$_2$CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.67 - 6.80 (m, 1H, 3-Ph'), 6.84 - 7.04 (m, 6H, 3,4,5,6-Ph, 4,5-Ph'), 7.71 (d, *J* = 7.03 Hz, 1H, 6-Ph'), 9.34 (s, 1H, CON*H*), 9.79 (s, 1H, O*H*), 10.50 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.9, 23.1, 35.6, 47.2, 51.5, 55.6, 55.8, 112.3, 116.4, 118.6, 119.3, 121.3, 122.9, 123.9, 125.1, 126.8, 139.9, 148.4, 152.2, 171.7; LC/MS: *m/z* calc. 384.23, found 384.13; MW 419.94; Anal. $C_{22}H_{30}ClN_3O_3$ (C, H, N).

*4.2.2.20. 2-(5-(4-(2-Methoxyphenyl)piperazin-1-yl)pentanamido)benzamide hydrochloride (**26**)*

The title compound was obtained from **5** and 2-aminobenzamide as a white solid; yield 58 %; mp 199-201 °C; R_f = 0.48 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.54 - 1.70 (m, 2H, CH$_2$C*H*$_2$CH$_2$CH$_2$), 1.70 - 1.91 (m, 2H, CH$_2$CH$_2$C*H*$_2$CH$_2$), 2.41 (t, *J* = 7.03 Hz, 2H, CH$_2$CH$_2$CH$_2$C*H*$_2$), 2.93 - 3.09 (m, 6H, N(C*H*$_2$CH$_2$)$_2$NH$^+$, C*H*$_2$CH$_2$CH$_2$CH$_2$), 3.37 - 3.61 (m, 4H, N(CH$_2$C*H*$_2$)$_2$NH$^+$), 3.77 (s, 3H, OC*H*$_3$), 6.81 - 7.05 (m, 4H, 3,4,5,6-Ph), 7.10 (t, *J* = 7.60 Hz, 1H, 5-Ph'), 7.47 (t, *J* = 7.00 Hz, 1H, 4-Ph'), 7.79 (dd, *J* = 7.91, 1.47 Hz, 1H, 3-Ph'), 8.28 (br.s., 2H, CONH$_2$), 8.44 (d, *J* = 7.60 Hz, 1H, 6-Ph'), 10.61 (br.s., 1H, CON*H*), 11.67 (s, 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.5, 23.0, 37.1, 47.2, 51.5, 55.6, 55.8, 112.3, 118.6, 120.3, 120.7, 121.3, 122.8, 123.9, 129.1, 132.6, 139.9, 140.0, 152.2, 170.9, 171.2; LC/MS: *m/z* calc. 411.24, found 411.38; MW 446.97; Anal. $C_{23}H_{31}ClN_4O_3$ (C, H, N).

*4.2.2.21. N-(2-hydroxybenzyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**27**)*

The title compound was obtained from **5** and 2-(aminomethyl)phenol as a white solid; yield 69 %; mp 207-209 °C; R_f = 0.39 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.52 - 1.63 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.63 - 1.74 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.20 (t, J = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2$), 2.98 - 3.14 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2$), 3.45 (t, J = 7.60 Hz, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 4.18 (d, J = 5.27 Hz, 2H, $CONHCH_2$), 6.70 - 7.11 (m, 8H, 3,4,5,6-Ph, 3,4,5,6-Ph'), 8.34 (t, J = 5.86 Hz, 1H, CONH), 9.61 (s, 1H, OH), 10.61 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 22.9, 23.1, 34.9, 37.9, 47.3, 51.5, 55.6, 55.8, 112.3, 115.6, 118.6, 119.2, 121.3, 123.9, 125.7, 128.3, 129.1, 139.8, 152.2, 155.4, 172.6; LC/MS: *m/z* calc. 398.24, found 398.29; MW 433.97; Anal. $C_{23}H_{32}ClN_3O_3$ (C, H, N).

*4.2.2.22. N-(2-methoxybenzyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**28**)*

The title compound was obtained from **5** and (2-methoxyphenyl)methanamine as a white solid; yield 57 %; mp 213-215 °C; R_f = 0.43 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.43 - 1.62 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.62 - 1.79 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.20 (t, J = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2$), 2.90 - 3.19 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2$), 3.36 - 3.57 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, CH_3OPh), 3.78 (s, 3H, CH_3OPh'), 4.21 (d, J = 5.86 Hz, 2H, $CONHCH_2$), 6.80 - 7.05 (m, 6H, 3,4,5,6-Ph, 3,5-Ph'), 7.10 - 7.27 (m, 2H, 4,6-Ph'), 8.22 (t, J = 5.57 Hz, 1H, CONH), 10.69 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.0, 23.2, 35.0, 37.4, 47.3, 51.5, 55.7, 55.7, 55.8, 110.9, 112.3, 118.6, 120.6, 121.3, 123.9, 127.3, 128.1, 128.4, 139.9, 152.2, 157.0, 172.2; LC/MS: *m/z* calc. 412.26, found 412.24; MW 448.00; Anal. $C_{24}H_{34}ClN_3O_3$ (C, H, N).

*4.2.2.23. N-(4-(tert-butyl)benzyl)-5-(4-(2-methoxyphenyl)piperazin-1-yl)pentanamide hydrochloride (**29**)*

The title compound was obtained from **5** and (4-(*tert*-butyl)phenyl)methanamine as a white solid; yield 78 %; mp 119-121 °C; R_f = 0.49 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.24 (s, 9H, $C(CH_3)_3$), 1.56 (d, J = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2$), 1.61 - 1.83 (m, 2H, $CH_2CH_2CH_2CH_2$), 2.18 (t, J = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2$), 3.00 - 3.16 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2$), 3.39 - 3.57 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 4.20 (d, J = 5.86 Hz, 2H, $CONHCH_2$), 6.84 - 7.05 (m, 4H, 3,4,5,6-Ph), 7.13 - 7.19 (m, J = 8.21 Hz, 2H, 3,5-Ph'), 7.28 - 7.35 (m, 2H, 2,6-Ph'), 8.36 (s, 1H, CONH), 10.64 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.0, 23.1, 31.6, 34.6, 35.0, 42.2, 47.2, 51.4, 55.6, 55.8, 112.3, 118.6, 121.3, 123.9, 125.4, 127.5, 137.1, 139.9, 149.5, 152.2, 172.0; LC/MS: *m/z* calc. 438.31, found 438.23; MW 474.08; Anal. $C_{27}H_{40}ClN_3O_2$ (C, H, N).

*4.2.2.24. N-(5-(tert-butyl)-2-hydroxyphenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**30**)*

The title compound was obtained from **6** and 2-amino-4-(*tert*-butyl)phenol as a white solid; yield 73 %; mp 63-65 °C; R_f = 0.48 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20 (s, 9H, C(CH_3)$_3$), 1.26 - 1.42 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.52 - 1.92 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.42 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.88 - 3.24 (m, 6H, N(CH_2CH_2)$_2$NH$^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.36 - 3.51 (m, 4H, N(CH_2CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.77 (d, *J* = 8.21 Hz, 1H, 3-Ph'), 6.82 - 7.11 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.67 (d, *J* = 2.34 Hz, 1H, 6-Ph'), 9.46 (s, 1H, CONH), 9.59 (br.s., 1H, OH), 10.72 (br.s., 1H, NH$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 25.2, 26.1, 31.8, 34.2, 35.9, 47.3, 51.4, 55.7, 55.8, 112.3, 116.4, 118.6, 119.8, 121.3, 122.0, 124.0, 126.2, 139.7, 141.7, 146.1, 152.2, 172.4; LC/MS: *m/z* calc. 453.62, found 454.31; MW 490.08; Anal. $C_{27}H_{40}ClN_3O_3$ (C, H, N).

*4.2.2.25. N-(3-(tert-butyl)phenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**31**)*

The title compound was obtained from **6** and 3-(*tert*-butyl)aniline as a white solid; yield 73 %; mp 143-145 °C; R_f = 0.46 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (s, 9H, C(CH_3)$_3$), 1.28 - 1.38 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.58 - 1.78 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.32 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.99 - 3.18 (m, 6H, N(CH_2CH_2)$_2$NH$^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.40 - 3.58 (m, 4H, N(CH_2CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.84 - 7.07 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.18 (t, *J* = 7.91 Hz, 1H, 5-Ph'), 7.48 (d, *J* = 8.79 Hz, 1H, 6-Ph'), 7.59 (s, 1H, 2-Ph'), 9.93 (s, 1H, CONH), 10.62 (br.s., 1H, NH$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 25.1, 26.2, 31.6, 34.8, 36.5, 47.2, 51.5, 55.7, 55.8, 112.3, 116.4, 116.7, 118.6, 120.3, 121.3, 123.9, 128.7, 139.6, 139.9, 151.5, 152.2, 171.5; LC/MS: *m/z* calc. 438.31, found 438.30; MW 474.08; Anal. $C_{27}H_{40}ClN_3O_2$ (C, H, N).

*4.2.2.26. N-(3-isopropylphenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**32**)*

The title compound was obtained from **6** and 3-isopropylaniline as a white solid; yield 72 %; mp 166-168 °C; R_f = 0.48 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, *J* = 6.45 Hz, 6H, CH(CH_3)$_2$), 1.24 - 1.38 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.54 - 1.82 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.32 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.71 - 2.85 (m, 1H, CH(CH$_3$)$_2$), 2.92 - 3.20 (m, 6H, N(CH_2CH_2)$_2$NH$^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.38 - 3.57 (m, 4H, N(CH_2CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.83 - 7.05 (m, 5H, 3,4,5,6-Ph, 4-Ph'), 7.17 (t, *J* = 7.91 Hz, 1H, 5-Ph'), 7.41 (d, *J* = 8.21 Hz, 1H, 6-Ph'), 7.48 (s, 1H, 2-Ph'), 9.92 (s, 1H, CONH), 10.62 (br.s., 1H, NH$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 24.3, 25.1, 26.1, 33.9, 36.5, 47.2, 51.4, 55.7, 55.8, 112.3, 117.1, 117.4, 118.6, 121.3, 121.4, 123.9, 128.9, 139.8, 139.9, 149.2, 152.2, 171.5; LC/MS: *m/z* calc. 424.30, found 424.21; MW 460.05; Anal. $C_{26}H_{38}ClN_3O_2$ (C, H, N).

*4.2.2.27. N-(4-(tert-butyl)phenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**33**)*

The title compound was obtained from **6** and 4-(*tert*-butyl)aniline as a white solid; yield 65 %; mp 109-111 °C; R_f = 0.46 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (s, 9H, C(CH_3)$_3$), 1.27 - 1.39 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.54 - 1.82 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.31 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.97 - 3.15 (m, 6H, N(CH_2CH$_2$)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$CH$_2$), 3.45 - 3.56 (m, 4H, N(CH$_2$CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.85 - 7.07 (m, 4H, 3,4,5,6-Ph), 7.27 (d, *J* = 8.79 Hz, 2H, 3,5-Ph'), 7.50 (d, *J* = 8.21 Hz, 2H, 2,6-Ph'), 9.91 (s, 1H, CON*H*), 10.69 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 25.6, 26.2, 26.9, 31.6, 34.4, 36.7, 50.2, 53.3, 55.7, 58.0, 112.3, 118.3, 119.3, 121.2, 122.9, 125.6, 137.2, 141.5, 145.6, 152.4, 171.4; LC/MS: *m/z* calc. 438.31, found 438.36; MW 474.08; Anal. $C_{27}H_{40}ClN_3O_2$ (C, H, N).

*4.2.2.28. N-(4-isopropylphenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**34**)*

The title compound was obtained from **6** and 4-isopropylaniline as a white solid; yield 58 %; mp 80-82 °C; R_f = 0.46 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.14 (d, *J* = 6.45 Hz, 6H, CH(CH_3)$_2$), 1.23 - 1.44 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.52 - 1.84 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.31 (t, *J* = 7.03 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.64 - 2.90 (m, 1H, C*H*(CH$_3$)$_2$), 2.90 - 3.22 (m, 6H, N(CH_2CH$_2$)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$CH$_2$), 3.37 - 3.59 (m, 4H, N(CH$_2$CH_2)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.80 - 7.05 (m, 4H, 3,4,5,6-Ph), 7.12 (d, *J* = 8.20 Hz, 2H, 3,5-Ph'), 7.50 (d, *J* = 8.20 Hz, 2H, 2,6-Ph'), 9.91 (s, 1H, CON*H*), 10.69 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 24.4, 25.1, 26.1, 33.3, 36.4, 47.3, 51.5, 55.7, 55.8, 112.3, 118.6, 119.6, 121.3, 123.8, 126.7, 137.6, 139.9, 143.4, 152.2, 171.3; LC/MS: *m/z* calc. 424.30, found 424.27; MW 460.05; Anal. $C_{26}H_{38}ClN_3O_2$ (C, H, N).

*4.2.2.29. N-(4-(sec-butyl)phenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**35**)*

The title compound was obtained from **6** and 4-(*sec*-butyl)aniline as a white solid; yield 69 %; mp 74-76 °C; R_f = 0.48 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.72 (t, *J* = 7.33 Hz, 3H, CH(CH$_3$)CH$_2$CH_3), 1.13 (d, *J* = 7.03 Hz, 3H, CH(CH_3)CH$_2$CH$_3$), 1.26 - 1.38 (m, 2H, CH(CH$_3$)CH_2CH$_3$), 1.43 - 1.54 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.56 - 1.65 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.67 - 1.82 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.31 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.80 - 3.20 (m, 7H, N(CH$_2$CH_2)$_2$NH$^+$, CH_2CH$_2$CH$_2$CH$_2$CH$_2$, C*H*(CH$_3$)CH$_2$CH$_3$), 3.37 - 3.60 (m, 4H, N(CH_2CH$_2$)$_2$NH$^+$), 3.77 (s, 3H, OCH_3), 6.87 - 7.02 (m, 4H, 3,4,5,6-Ph), 7.08 (d, *J* = 8.79 Hz, 2H, 3,5-Ph'), 7.49 (d, *J* = 8.79 Hz, 2H, 2,6-Ph'), 9.89 (s, 1H, CON*H*), 10.54 (br.s., 1H, N*H*$^+$); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 12.5, 22.3, 23.2, 25.1, 26.1, 31.1, 36.4, 40.8, 47.3, 51.5, 55.7, 55.8, 112.3, 118.6, 119.6, 121.3, 123.9, 127.3, 137.6, 139.9, 142.1, 152.2, 171.3; LC/MS: *m/z* calc. 438.31, found 438.30; MW 474.08; Anal. $C_{27}H_{40}ClN_3O_2$ (C, H, N).

*4.2.2.30. Tert-butyl 4-(6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamido)benzoate hydrochloride (**36**)*

The title compound was obtained from **6** and *tert*-butyl 4-aminobenzoate as a white solid; yield 61 %; mp 169-171 °C; R_f = 0.50 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.25 - 1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.51 (s, 9H, $C(CH_3)_3$), 1.59 - 1.78 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 2.38 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 3.00 - 3.14 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.44 - 3.55 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 6.78 - 7.07 (m, 4H, 3,4,5,6-Ph), 7.72 (d, *J* = 9.40 Hz, 2H, 3,5-Ph'), 7.82 (d, *J* = 8.80 Hz, 2H, 2,6-Ph'), 10.39 (s, 1H, CONH), 10.70 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 24.9, 26.1, 28.3, 36.5, 47.3, 51.5, 55.7, 55.8, 80.6, 112.3, 118.6, 121.3, 123.9, 125.8, 130.5, 139.8, 143.9, 152.2, 165.1, 172.2; LC/MS: *m/z* calc. 482.30, found 482.23; MW 518.09; Anal. $C_{28}H_{40}ClN_3O_4$ (C, H, N).

*4.2.2.31. N-(2-hydroxyphenyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**37**)*

The title compound was obtained from **6** and 2-aminophenol as a white solid; yield 49 %; mp 229-231 °C; R_f = 0.42 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.21 - 1.43 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.53 - 1.70 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.70 - 1.87 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.41 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.98 - 3.21 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.38 - 3.64 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 6.67 - 6.80 (m, 1H, 3-Ph'), 6.81 - 7.06 (m, 6H, 3,4,5,6-Ph, 4,5-Ph'), 7.69 (d, *J* = 7.62 Hz, 1H, 6-Ph'), 9.32 (s, 1H, CONH), 9.80 (s, 1H, OH), 10.58 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.3, 25.2, 26.1, 36.0, 47.4, 51.5, 55.8, 112.3, 116.5, 118.6, 119.3, 121.3, 122.8, 123.8, 125.0, 126.8, 139.9, 148.3, 152.2, 172.1; LC/MS: *m/z* calc. 397.51, found 398.29; MW 433.97; Anal. $C_{23}H_{32}ClN_3O_3$ (C, H, N).

*4.2.2.32. N-(2-hydroxybenzyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**38**)*

The title compound was obtained from **6** and 2-(aminomethyl)phenol as a white solid; yield 64 %; mp 180-182 °C; R_f = 0.40 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.19 - 1.38 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.55 (quin, *J* = 7.47 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.61 - 1.84 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.17 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.90 - 3.22 (m, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.47 (t, *J* = 11.72 Hz, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, OCH_3), 4.17 (d, *J* = 5.86 Hz, 2H, $CONHCH_2$), 6.69 - 7.10 (m, 8H, 3,4,5,6-Ph, 3,4,5,6-Ph'), 8.29 (t, *J* = 5.86 Hz, 1H, CONH), 9.60 (s, 1H, OH), 10.59 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 25.2, 26.1, 35.3, 37.9, 47.3, 51.5, 55.7, 55.8, 112.3, 115.6, 118.6, 119.2, 121.3, 123.9, 125.7, 128.3, 129.0, 139.9, 152.2, 155.4, 173.0; LC/MS: *m/z* calc. 412.26, found 412.18; MW 448.00; Anal. $C_{24}H_{34}ClN_3O_3$ (C, H, N).

*4.2.2.33. N-(2-methoxybenzyl)-6-(4-(2-methoxyphenyl)piperazin-1-yl)hexanamide hydrochloride (**39**)*

The title compound was obtained from **6** and (2-methoxyphenyl)methanamine as a white solid; yield 68 %; mp 200-202 °C; R_f = 0.46 (A); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.18 - 1.40 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.44 - 1.64 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 1.64 - 1.87 (m, 2H, $CH_2CH_2CH_2CH_2CH_2$), 2.17 (t, *J* = 7.33 Hz, 2H, $CH_2CH_2CH_2CH_2CH_2$), 3.06 (t, *J* = 7.91 Hz, 6H, $N(CH_2CH_2)_2NH^+$, $CH_2CH_2CH_2CH_2CH_2$), 3.36 - 3.59 (m, 4H, $N(CH_2CH_2)_2NH^+$), 3.77 (s, 3H, CH_3OPh), 3.78 (s, 3H, CH_3OPh'), 4.20 (d, *J* = 5.86 Hz, 2H, CONHCH_2), 6.81 - 7.06 (m, 6H, 3,4,5,6-Ph, 3,5-Ph'), 7.09 - 7.26 (m, 2H, 4,6-Ph'), 8.17 (t, *J* = 5.57 Hz, 1H, CONH), 10.72 (br.s., 1H, NH^+); ^{13}C NMR (75 MHz, DMSO-d_6) δ ppm 23.2, 25.2, 26.2, 31.2, 35.4, 37.4, 47.3, 51.4, 55.7, 55.8, 110.8, 112.3, 118.6, 120.5, 121.3, 123.9, 127.4, 128.0, 128.4, 139.9, 152.2, 157.0, 172.4; LC/MS: *m/z* calc. 426.28, found 426.20; MW 462.02; Anal. $C_{25}H_{36}ClN_3O_3$ (C, H, N).

4.3. In vitro pharmacology

4.3.1. Radioligand binding assays

HEK293 cells with stable expression of human 5-HT_{1A} or 5-HT_{7b} receptors (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5 % CO_2 and grown in Dulbecco's Modifier Eagle Medium containing 10 % dialyzed fetal bovine serum and 500 µg/mL G418 sulfate. For membrane preparation, cells were subcultured in 150 cm^2 diameter dishes, grown to 90 % confluence, washed twice with prewarmed to 37 °C phosphate buffered saline (PBS) and pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol. Prior to membrane preparation, pellets were stored at –80 °C.

Cell pellets were thawed and homogenized in 10 volumes of assay buffer using an Ultra Turrax tissue homogenizer and centrifuged twice at 35,000 g for 15 minutes at 4 °C, with incubation for 15 minutes at 37 °C in between. The composition of the assay buffers were as follows: 50 mM Tris-HCl, 0.1 mM EDTA, 4 mM $MgCl_2$, 10 µM pargyline, and 0.1 % ascorbate (for the 5-HT_{1A} receptor); 50 mM Tris-HCl, 4 mM $MgCl_2$, 10 µM pargyline, and 0.1 % ascorbate (for the 5-HT_{7b} receptor).

All assays were incubated in a total volume of 200 µL in 96-well microtiter plates for 1 hour at room temperature for the 5-HT_{1A} receptor or at 37 °C for the 5-HT_{7b} receptor. The process of equilibration was terminated by a rapid filtration through Unifilter plates with a 96-well cell harvester and radioactivity retained on the filters was quantified on a Microbeta plate reader (PerkinElmer, USA).

For displacement studies, the assay samples contained as radioligands (PerkinElmer, USA): 2.5 nM [^{3}H]-8-OH-DPAT (135.2 Ci/ mmol) or 0.8 nM [^{3}H]-5-CT (39.2 Ci/mmol) for 5-HT_{1A} or 5-HT_7 receptor, respectively. Nonspecific binding was defined with 10 µM of serotonin in 5-HT_{1A} and 5-HT_7 receptor binding experiments. Each compound was tested in triplicate at 7 to 8 concentrations (10^{-10}–

10^{-4} M). K_i values were calculated from the Cheng-Prusoff equation [54]. For all binding assays, results were expressed as means of at least two separate experiments (SD ≤ 21 %).

4.3.2. Functional cell-based assays

The tested and reference compounds were dissolved in DMSO at a concentration of 1 mM. Serial dilutions were prepared in 96-well microplate in assay buffer and 8 to 10 concentrations were investigated in duplicate.

For the 5-HT_{1A} receptor ligands, a cellular aequorin-based functional assay was performed with recombinant CHO-K1 cells expressing mitochondrially targeted aequorin, the human 5-HT_{1A} receptor, and the promiscuous G protein α_{16} for 5-HT_{1A} receptor (PerkinElmer, USA). The assay was carried out according to the previously described protocol [42]. After thawing, cells were transferred to assay buffer (DMEM/HAM's F12 with 0.1 % protease-free BSA) and centrifuged. The cell pellet was resuspended in assay buffer and coelenterazine h was added at final concentrations of 5 μM. The cell suspension was incubated at 16 °C, protected from light with constant agitation for 16 hours and then diluted with assay buffer to a concentration of 100,000 cells/mL. After 1 hour of incubation, 50 μL of the cell suspension was dispensed using automatic injectors built into the radiometric and luminescence plate counter MicroBeta2 LumiJET (PerkinElmer, USA) into white opaque 96-well microplates preloaded with tested compounds. The immediate light emission generated following calcium mobilization was recorded for 30 seconds. In the antagonist mode, after 25 minutes of incubation, the reference agonist was added to the above assay mix and light emission was recorded again. The final concentration of the reference agonist was equal to EC_{80} (300 nM serotonin).

For the 5-HT_7 receptor ligands, adenylyl cyclase activity was monitored using cryopreserved CHO-K1 cells with expression of the human 5-HT_7 receptor. CHO-K1 cells were transfected with a beta lactamase reporter gene under control of the cAMP response element (Thermo Fisher Scientific, USA). Thawed cells were resuspended in stimulation buffer (HBSS, 5 mM HEPES, 0.5 mM IBMX, and 0.1 % BSA at pH 7.4) at concentration of 200 000 cells/mL. 10 μL of cell suspension was added to 10 μL of tested compounds loaded onto a white opaque half area 96-well microplate. The antagonist response experiment was performed with 10 nM serotonin as the reference agonist. The agonist and antagonist were added simultaneously. Cell stimulation was performed for 1 hour at room temperature. After incubation, cAMP measurements were performed with homogeneous TR-FRET immunoassay using the LANCE Ultra cAMP kit (PerkinElmer, USA). 10 μL of EucAMP Tracer Working Solution and 10 μL of ULight-anti-cAMP Tracer Working Solution were added, mixed, and incubated for 1 hour. The TR-FRET signal was read on an EnVision microplate reader (PerkinElmer, USA).

IC_{50} values were determined by nonlinear regression analysis using GraphPad Prism 6.0 software. The logIC_{50} was used to obtain the K_b by applying the Cheng-Prusoff equation. Results were expressed as means of at least two independent experiments.

4.3.3. PDE-Glo™ phosphodiesterase assays

The PDE inhibitory activity of the investigated compounds was evaluated using the PDE-Glo™ Phosphodiesterase Assay and human recombinant PDE4B and PDE7A expressed in Sf9 cells (Promega, USA). The optimal amount of both PDEs for screening and the optimal PDE reaction time were determined empirically from the PDE titration to achieve approximately 80 % of the maximum assay signal. All studied compounds were dissolved in DMSO and a serial dilution of these solutions was performed using the same solvent. In order to assess IC_{50} values, 1.5 µL of 1X PDE-Glo Reaction buffer containing an appropriate amount of purified human recombinant PDE4B or PDE7A (SignalChem, Canada) was added to each well of a 384-well plate (Thermo Fisher Scientific, USA) and 1 µL of diluted solutions of each compound in DMSO were added followed by 2.5 µL of cAMP (50 nM). The final concentrations of the compounds ranged from 0.01 to 200 µM and the concentration of DMSO in the reaction mixture was 2 %. After 10 minutes of incubation at 30 °C (Grant-bio Thermo-shaker PHMT, Grant Instruments, England), 2.5 µL of PDE-Glo™ Termination Buffer and 2.5 µL of PDE-Glo™ Detection Solution were added to each well. Following 20 min of incubation at room temperature, 10 µL of Kinase-Glo® Reagent was added and after 10 minutes luminescence was measured in each well using a microplate luminometer (POLARstar Omega, BMG LABTECH, Germany). Raw luminescence data were normalized to percentage activity of controls, where percent activity = (relative luminescence units [RLU] in the presence of test compound − mean RLU of the low controls)/(mean RLU of the high controls − mean RLU of the low controls) × 100. The high controls consisted of complete reaction mixtures with the addition of vehicle (2 % DMSO) and the low controls contained all components besides the enzyme.

All measurements were performed in triplicate for each inhibitor concentration using POLARstar Omega (BMG LABTECH, Germany). IC_{50} values were estimated using nonlinear regression with ADAPT 5 (BMSR, Los Angeles, CA, USA). The results were expressed as means of at least two independent experiments.

4.3.4. Parallel artificial membrane permeability assay

Evaluation of permeability through membranes was prepared using 96-wells Pre-coated PAMPA Plate System Gentest™ (Corning, Tewksbury, USA). The tested compounds and the references solutions (all at concentration of 200 µM) were prepared in PBS buffer (pH = 7.4) and added to the donor wells (300 µL/well). Then, 200 µL/well of PBS was added to the acceptor wells and the plates were incubated at room temperature for 5 hours without agitation. After incubation, the plates were separated and 100 µL of solution from each well of both: the acceptor plate and the donor plate was next diluted with 100 µL solution of an internal standard in PBS (pH = 7.4). The compounds' concentrations in acceptor and donor wells were determined by analyzed by UPLC/MS using LC/MS Waters ACQUITY™ TQD system with the TQ Detector (Waters, USA). All compounds were

analyzed in triplicate. P_e values were calculated based on the previously described formulas [44,45]. According to the PAMPA plate's manufacturer, compounds with $P_e \geq 1.5 \times 10^{-6}$ cm/s possess good human oral absorption capacity [46].

4.3.5. Liver microsomal stability assay

The reactions of biotransformation were conducted using commercial RLMs purchased from Sigma-Aldrich (St. Louis, USA) according to the previously described protocols [47]. The reaction mixtures consisted of 50 µM concentration of the tested compound in 0.1 mM Tris-HCl (pH = 7.4) with RLMs (1 mg/mL). All mixtures were preincubated first at 37 °C for 5 minutes. Then, the reaction was initiated by adding 50 µL of NADPH Regeneration System (Promega, USA). After 120 minutes of incubation at 37 °C, the cold methanol was used to terminate the reactions. The mixtures were centrifuged at 14 000 rpm for 15 minutes and the supernatants were analyzed by UPLC/MS using LC/MS Waters ACQUITY™ TQD system with the TQ Detector (Waters, USA).

4.4. In vivo pharmacology

4.4.1. Animals

Male Wistar rats weighing 230–260 g were used in experiments. The animals were housed in polycarbonate Makrolon type 3 cages (dimensions 26.5 × 15 × 42 cm) in an environmentally controlled room (ambient temperature 21 ± 2 °C; relative humidity 50–60 %; 12:12 light/dark cycle, lights on at 8:00), in groups of four rats, with free access to standard laboratory food (LSM-B) and filtered water. The animals were assigned randomly to treatment groups. All the experiments were performed by observers unaware of the type of treatment and were conducted between 9:00 and 14:00. All animals were used only once. The research protocol was approved by the Second Local Ethical Committee on Animal Testing at the Institute of Pharmacology, Polish Academy of Sciences (Kraków, Poland).

4.4.2. Novel object recognition test

The experiment was adapted from the method of Ennanceur and Delacour [55]. The test was conducted in opaque black boxes. After each animal, the boxes were wiped with alcohol. On the first day, rats were 2-day adapted to the test arena (without any objects) for 5 min. The test session comprising of two trials separated by an inter-trial interval (ITI) of 1 h was carried out 24 hours later. During the first trial (familiarization, T1) two identical objects (A1 and A2) were presented in the opposite corners of the box, approximately 10 cm from the walls. During the second trial (recognition, T2) one of the A objects was replaced by a novel object B, so that the animals were presented with the A = familiar and B = novel objects. Both trials lasted for 3 min and the animals were returned to their home cages after T1. The objects used were the glass jars filled with the gravel and the metal Coca-Cola cans. The heights of the objects were comparable (12 cm) and the objects were heavy enough not

to be displaced by the animals. The sequence of presentations and the location of the objects were randomly assigned to each rat. The animals explored the objects by looking, licking, sniffing or touching the object while sniffing, but not when leaning against, standing or sitting on the object. Any rat exploring the two objects for less than 5 s within 3 min of T1 or T2 was eliminated from the study. The experiments were video-recorded and the exploration time of the objects was measured by an observer blind to the drug treatment. Based on exploration time (E) of two objects during T2, discrimination index (DI) was calculated according to the formula: DI = (EB – EA)/(EA + AB). MK-801, used to attenuate learning, was administered at 30 min before T1.

4.4.3. Forced swimming test

The experiment was carried out according to the method of Porsolt *et al.* [56]. On the first day of experiment, the animals were individually placed in Plexiglas cylinders (40 cm high, 18 cm in diameter) filled with water at 25 °C to the height of 15 cm for 15 min. Upon removal from water, the rats were gently wiped and dried under a 60-W bulb in a Plexiglas box for 30 min. 24 hours after habituation session the rats were placed again in the cylinder and the total duration of immobility was recorded throughout a 5-min test period. Fresh water was used for each animal.

4.4.4. Open field test

The locomotor activity was recorded using Motor Monitor System (Campden Instruments Ltd., UK) consisted of two Smart Frame Open Field stations (40 × 40 × 38 cm) with 16 × 16 beams, located in sound attenuating chambers and connected to PC software. Rats were individually placed in the center of the station. Motor Monitor System recorded ambulations (in X and Y axes), the number of rearings, and total distance covered by a rat for 5 min. The cages were cleaned up with 70 % ethanol after each rat. The influence of effective doses only recorded in the FST and NOR tests was studied in the OF test in order to exclude the possibility of competing behaviors, such as general locomotor activity.

4.4.5. Statistical analysis

The data were evaluated by an one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. $p < 0.05$ was considered significant.

Author contributions

All authors have contributed and have given approval to the final version of the manuscript.

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References

[1] C. Gillis, F. Mirzaei, M. Potashman, M.A. Ikram, N. Maserejian, The incidence of mild cognitive impairment: A systematic review and data synthesis, Alzheimer's Dement. Diagnosis, Assess. Dis. Monit. 11 (2019) 248–256. https://doi.org/10.1016/j.dadm.2019.01.004.

[2] J. Wang, X. Wu, W. Lai, E. Long, X. Zhang, W. Li, Y. Zhu, C. Chen, X. Zhong, Z. Liu, D. Wang, H. Lin, Prevalence of depression and depressive symptoms among outpatients: a systematic review and meta-analysis, BMJ Open. 7 (2017) e017173. https://doi.org/10.1136/bmjopen-2017-017173.

[3] T.D. Prevot, G. Li, A. Vidojevic, K.A. Misquitta, C. Fee, A. Santrac, D.E. Knutson, M.R. Stephen, R. Kodali, N.M. Zahn, L.A. Arnold, P. Scholze, J.L. Fisher, B.D. Marković, M. Banasr, J.M. Cook, M. Savic, E. Sibille, Novel Benzodiazepine-Like Ligands with Various Anxiolytic, Antidepressant, or Pro-Cognitive Profiles, Mol. Neuropsychiatry. 5 (2019) 84–97. https://doi.org/10.1159/000496086.

[4] A. Bucki, M. Marcinkowska, J. Śniecikowska, K. Więckowski, M.H. Pawłowski, M. Głuch-Lutwin, A. Gryboś, A. Siwek, K. Pytka, M. Jastrzębska-Więsek, A. Partyka, A. Wesołowska, P. Mierzejewski, M. Kołaczkowski, Novel 3-(1,2,3,6-tetrahydropyridin-4-yl)-1H-indole-Based Multifunctional Ligands With Antipsychotic-like, Mood-Modulating And Procognitive Activity, J. Med. Chem. 60 (2017) 7483–7501. https://doi.org/10.1021/acs.jmedchem.7b00839.

[5] V. Canale, R. Kurczab, A. Partyka, G. Satała, K. Słoczyńska, T. Kos, M. Jastrzębska-Więsek, A. Siwek, E. Pękala, A.J. Bojarski, A. Wesołowska, P. Popik, P. Zajdel, N-Alkylated arylsulfonamides of (aryloxy)ethyl piperidines: 5-HT7 receptor selectivity versus multireceptor profile, Bioorganic Med. Chem. 24 (2016) 130–139. https://doi.org/10.1016/j.bmc.2015.11.041.

[6] G. Perini, M. Cotta Ramusino, E. Sinforiani, S. Bernini, R. Petrachi, A. Costa, Cognitive impairment in depression: recent advances and novel treatments, Neuropsychiatr. Dis. Treat. 15 (2019) 1249–1258. https://doi.org/10.2147/NDT.S199746.

[7] J. Dai, X. Du, G. Yin, Y. Zhang, H. Xia, X. Li, R. Cassidy, Q. Tong, D. Chen, A.L. Teixeira, Y. Zheng, Y. Ning, J.C. Soares, M.-X. He, X.Y. Zhang, Prevalence, demographic and clinical features of comorbid depressive symptoms in drug naïve patients with schizophrenia presenting with first episode psychosis, Schizophr. Res. 193 (2018) 182–187. https://doi.org/10.1016/j.schres.2017.06.029.

[8] J. Liu, E. Abdin, J.A. Vaingankar, S.B. Shafie, A. Jeyagurunathan, S. Shahwan, H. Magadi, L.L. Ng, S.A. Chong, M. Subramaniam, The relationship among unawareness of memory impairment, depression, and dementia in older adults with memory impairment in Singapore, Psychogeriatrics. 17 (2017) 430–438. https://doi.org/10.1111/psyg.12270.

[9] A. Jankowska, A. Wesołowska, M. Pawłowski, G. Chłoń-Rzepa, Diabetic Theory in Anti-Alzheimer's Drug Research and Development. Part 1: Therapeutic Potential of Antidiabetic Agents, Curr. Med. Chem. 26 (2019). https://doi.org/10.2174/0929867326666191011144818.

[10] A. Jankowska, A. Wesołowska, M. Pawłowski, G. Chłoń-Rzepa, Multi-Target-Directed Ligands Affecting Serotonergic Neurotransmission for Alzheimer's Disease Therapy: Advances in Chemical and Biological Research, Curr. Med. Chem. 25 (2018) 2045–2067. https://doi.org/10.2174/0929867324666170529122802.

[11] A. Jankowska, A. Świerczek, G. Chłoń-Rzepa, M. Pawłowski, E. Wyska, PDE7-Selective and Dual Inhibitors: Advances in Chemical and Biological Research, Curr. Med. Chem. 24 (2017) 673–700. https://doi.org/10.2174/0929867324666170116125159.

[12] A. Jankowska, A. Wesołowska, M. Pawłowski, G. Chłoń-Rzepa, Multifunctional Ligands Targeting Phosphodiesterase as the Future Strategy for the Symptomatic and Disease-Modifying Treatment of Alzheimer's Disease, Curr. Med. Chem. 26 (2019). https://doi.org/10.2174/0929867326666190620095623.

[13] R. Perez-Gonzalez, C. Pascual, D. Antequera, M. Bolos, M. Redondo, D.I. Perez, V. Pérez-Grijalba, A. Krzyzanowska, M. Sarasa, C. Gil, I. Ferrer, A. Martinez, E. Carro, Phosphodiesterase 7 inhibitor reduced cognitive impairment and pathological hallmarks in a mouse model of Alzheimer's disease, Neurobiol. Aging. 34 (2013) 2133–2145. https://doi.org/10.1016/j.neurobiolaging.2013.03.011.

[14] A. Meneses, W. Adriani, 5-HT7 receptor stimulation and blockade: a therapeutic paradox about memory formation and amnesia, Front. Behav. Neurosci. 8 (2014) 207. https://doi.org/10.3389/fnbeh.2014.00207.

[15] M. Goto, M. Murakawa, K. Kadoshima-Yamaoka, Y. Tanaka, H. Inoue, H. Murafuji, Y. Hayashi, K. Miura, T. Nakatsuka, K. Nagahira, K. Chamoto, Y. Fukuda, T. Nishimura, Phosphodiesterase 7A inhibitor ASB16165 suppresses proliferation and cytokine production of NKT cells, Cell. Immunol. 258 (2009) 147–151. https://doi.org/10.1016/j.cellimm.2009.04.005.

[16] L. Mestre, M. Redondo, F.J. Carrillo-Salinas, J.A. Morales-García, S. Alonso-Gil, A. Pérez-Castillo, C. Gil, A. Martínez, C. Guaza, PDE7 inhibitor TC3.6 ameliorates symptomatology in a model of primary progressive multiple sclerosis, Br. J. Pharmacol. 172 (2015) 4277–4290. https://doi.org/10.1111/bph.13192.

[17] J.A. Morales-Garcia, D. Aguilar-Morante, E. Hernandez-Encinas, S. Alonso-Gil, C. Gil, A. Martinez, A. Santos, A. Perez-Castillo, Silencing phosphodiesterase 7B gene by lentiviral-shRNA interference attenuates neurodegeneration and motor deficits in hemiparkinsonian mice, Neurobiol. Aging. 36 (2014) 1160–1173. https://doi.org/10.1016/j.neurobiolaging.2014.10.008.

[18] M. Redondo, J.G. Zarruk, P. Ceballos, D.I. Pérez, C. Pérez, A. Perez-Castillo, M.A. Moro, J.

Brea, C. Val, M.I. Cadavid, M.I. Loza, N.E. Campillo, A. Martínez, C. Gil, Neuroprotective efficacy of quinazoline type phosphodiesterase 7 inhibitors in cellular cultures and experimental stroke model, Eur. J. Med. Chem. 47 (2012) 175–185. https://doi.org/10.1016/j.ejmech.2011.10.040.

[19] E.M. Medina-Rodriguez, F.J. Arenzana, J. Pastor, M. Redondo, V. Palomo, R. Garcia De Sola, C. Gil, A. Martinez, A. Bribin, F. De Castro, Inhibition of endogenous phosphodiesterase 7 promotes oligodendrocyte precursor differentiation and survival, Cell. Mol. Life Sci. 70 (2013) 3449–3462. https://doi.org/10.1007/s00018-013-1340-2.

[20] G. Chłoń-Rzepa, P. Żmudzki, P. Zajdel, A.J. Bojarski, B. Duszyńska, A. Nikiforuk, E. Tatarczyńska, M. Pawłowski, 7-Arylpiperazinylalkyl and 7-tetrahydroisoquinolinylalkyl derivatives of 8-alkoxy-purine-2,6-dione and some of their purine-2,6,8-trione analogs as 5-HT1A, 5-HT2A, and 5-HT7 serotonin receptor ligands, Bioorg. Med. Chem. 15 (2007) 5239–5250. https://doi.org/10.1016/j.bmc.2007.05.017.

[21] M. Zygmunt, J. Sapa, G. Chłoń-Rzepa, A. Zagórska, A. Siwek, M. Pawłowski, G. Nowak, 7-3-Chlorophenypiperazinylalkyl derivatives of 8-alkoxy-purine-2,6-dione as a serotonin receptor ligands with potential antidepressant activity, Pharmacol. Reports. 66 (2014) 505–510. https://doi.org/10.1016/j.pharep.2013.12.014.

[22] A. Partyka, G. Chłoń-Rzepa, A. Wasik, M. Jastrzębska-Więsek, A. Bucki, M. Kołaczkowski, G. Satała, A.J. Bojarski, A. Wesołowska, Antidepressant- and anxiolytic-like activity of 7-phenylpiperazinylalkyl-1,3-dimethyl-purine-2,6-dione derivatives with diversified 5-HT1A receptor functional profile, Bioorg. Med. Chem. 23 (2015) 212–221. https://doi.org/10.1016/j.bmc.2014.11.008.

[23] G. Chłoń-Rzepa, P. Żmudzki, G. Satała, B. Duszyńska, A. Partyka, D. Wróbel, M. Jastrzębska-Więsek, A. Wesołowska, A.J. Bojarski, M. Pawłowski, P. Zajdel, New 8-aminoalkyl derivatives of purine-2,6-dione with arylalkyl, allyl or propynyl substituents in position 7, their 5-HT1A, 5-HT2A, and 5-HT7 receptor affinity and pharmacological evaluation, Pharmacol. Reports. 65 (2013) 15–29. https://doi.org/10.1016/S1734-1140(13)70960-5.

[24] A. Zagórska, A. Bucki, M. Kołaczkowski, A. Siwek, M. Głuch-Lutwin, G. Starowicz, G. Kazek, A. Partyka, A. Wesołowska, K. Słoczyńska, E. Pękala, M. Pawłowski, Synthesis and biological evaluation of 2-fluoro and 3-trifluoromethyl-phenyl-piperazinylalkyl derivatives of 1H-imidazo[2,1-f]purine-2,4(3H,8H)-dione as potential antidepressant agents., J. Enzyme Inhib. Med. Chem. 31 (2016) 10–24. https://doi.org/10.1080/14756366.2016.1198902.

[25] G. Chłoń-Rzepa, A. Zagórska, P. Żmudzki, A. Bucki, M. Kołaczkowski, A. Partyka, A. Wesołowska, G. Kazek, M. Głuch-Lutwin, A. Siwek, G. Starowicz, M. Pawłowski, Aminoalkyl Derivatives of 8-Alkoxypurine-2,6-diones: Multifunctional 5-HT1A/5-HT7 Receptor Ligands and PDE Inhibitors with Antidepressant Activity, Arch. Pharm. (Weinheim). 349 (2016) 889–903. https://doi.org/10.1002/ardp.201600260.

[26] A. Jankowska, A. Świerczek, E. Wyska, A. Gawalska, A. Bucki, M. Pawłowski, G. Chłoń-Rzepa, Advances in Discovery of PDE10A Inhibitors for CNS-Related Disorders. Part 1: Overview of the Chemical and Biological Research., Curr. Drug Targets. 20 (2019) 122–143. https://doi.org/10.2174/1389450119666180808105056.

[27] A. Świerczek, A. Jankowska, G. Chłoń-Rzepa, M. Pawłowski, E. Wyska, Advances in the Discovery of PDE10A Inhibitors for CNS-Related Disorders. Part 2: Focus on Schizophrenia, Curr. Drug Targets. 20 (2019) 1652–1669. https://doi.org/10.2174/1389450120666190801114210.

[28] M. Leopoldo, F. Berardi, N.A. Colabufo, M. Contino, E. Lacivita, M. Niso, R. Perrone, V. Tortorella, Structure-Affinity Relationship Study on N-(1,2,3,4-Tetrahydronaphthalen-1-yl)-4-Aryl-1-Piperazinealkylamides, a New Class of 5-Hydroxytryptamine 7 Receptor Agents, J Med Chem. 47 (2004) 6616–6624. https://doi.org/10.1021/jm049702f.

[29] M. Kołaczkowski, P. Kowalski, J. Jaskowska, M. Marcinkowska, K. Mitka, A. Bucki, A. Wesolowska, M. Pawlowski, Arylsulfonamides for the Treatment of CNS Diseases, PCT Int. Appl. WO 2012035123 (A1), Mar 22, 2012.

[30] M.H. Paluchowska, R. Bugno, B. Duszyńska, E. Tatarczyńska, A. Nikiforuk, T. Lenda, E. Chojnacka-Wójcik, The influence of modifications in imide fragment structure on 5-HT1A and 5-HT7 receptor affinity and in vivo pharmacological properties of some new 1-(m-trifluoromethylphenyl)piperazines, Bioorg. Med. Chem. 15 (2007) 7116–7125. https://doi.org/10.1016/j.bmc.2007.07.029.

[31] M.N. Modica, S. Intagliata, V. Pittalà, L. Salerno, M.A. Siracusa, A. Cagnotto, M. Salmona, G. Romeo, Synthesis and binding properties of new long-chain 4-substituted piperazine derivatives as 5-HT1A and 5-HT7 receptor ligands, Bioorg. Med. Chem. Lett. 25 (2015) 1427–1430. https://doi.org/10.1016/j.bmcl.2015.02.042.

[32] G. Chłoń-Rzepa, M. Ślusarczyk, A. Jankowska, A. Gawalska, A. Bucki, M. Kołaczkowski, A. Świerczek, K. Pociecha, E. Wyska, M. Zygmunt, G. Kazek, K. Sałat, M. Pawłowski, Novel amide derivatives of 1,3-dimethyl-2,6-dioxopurin-7-yl-alkylcarboxylic acids as multifunctional TRPA1 antagonists and PDE4/7 inhibitors: A new approach for the treatment of pain, Eur J Med Chem. 158 (2018) 517–533. https://doi.org/10.1016/j.ejmech.2018.09.021.

[33] K. Wójcik-Pszczoła, G. Chłoń-Rzepa, A. Jankowska, E. Ellen, A. Świerczek, K. Pociecha, P. Koczurkiewicz, K. Piska, A. Gawędzka, E. Wyska, M. Knapik-Czajka, E. Pękala, R. Gosens, Novel phosphodiesterases inhibitors from the group of purine-2,6-dione derivatives as potent modulators of airway smooth muscle cell remodelling, Eur. J. Pharmacol. 865 (2019) 172779. https://doi.org/10.1016/j.ejphar.2019.172779.

[34] R.X. Xu, W.J. Rocque, M.H. Lambert, D.E. Vanderwall, M.A. Luther, R.T. Nolte, Crystal Structures of the Catalytic Domain of Phosphodiesterase 4B Complexed with AMP, 8-Br-AMP, and Rolipram, J. Mol. Biol. 337 (2004) 355–365.

https://doi.org/10.1016/j.jmb.2004.01.040.

[35] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 46 (2001) 3–26. https://doi.org/10.1016/s0169-409x(00)00129-0.

[36] H. van de Waterbeemd, G. Camenisch, G. Folkers, J.R. Chretien, O.A. Raevsky, Estimation of Blood-Brain Barrier Crossing of Drugs Using Molecular Size and Shape, and H-Bonding Descriptors, J. Drug Target. 6 (1998) 151–165. https://doi.org/10.3109/10611869808997889.

[37] J. Kelder, P.D. Grootenhuis, D.M. Bayada, L.P. Delbressine, J.P. Ploemen, Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs., Pharm. Res. 16 (1999) 1514–9. https://doi.org/10.1023/a:1015040217741.

[38] A. Daina, V. Zoete, A BOILED-Egg To Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules, ChemMedChem. 11 (2016) 1117–1121. https://doi.org/10.1002/cmdc.201600182.

[39] OSIRIS Property Explorer Was Used for Prediction of Toxicity, 2018 (https://www.organic-chemistry.org/prog/peo/).

[40] A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 42717. https://doi.org/10.1038/srep42717.

[41] K. Kucwaj-Brysz, R. Kurczab, M. Jastrzębska-Więsek, E. Żesławska, G. Satała, W. Nitek, A. Partyka, A. Siwek, A. Jankowska, A. Wesołowska, K. Kieć-Kononowicz, J. Handzlik, Computer-aided insights into receptor-ligand interaction for novel 5-arylhydantoin derivatives as serotonin 5-HT7 receptor agents with antidepressant activity, Eur. J. Med. Chem. 147 (2018) 102–114. https://doi.org/10.1016/j.ejmech.2018.01.093.

[42] M. Kołaczkowski, M. Marcinkowska, A. Bucki, M. Pawłowski, K. Mitka, J. Jaśkowska, P. Kowalski, G. Kazek, A. Siwek, A. Wasik, A. Wesołowska, P. Mierzejewski, P. Bienkowski, Novel arylsulfonamide derivatives with 5-HT6/5-HT7 receptor antagonism targeting behavioral and psychological symptoms of dementia, J. Med. Chem. 57 (2014) 4543–4557. https://doi.org/10.1021/jm401895u.

[43] G. Chłoń-Rzepa, A. Jankowska, M. Ślusarczyk, A. Świerczek, K. Pociecha, E. Wyska, A. Bucki, A. Gawalska, M. Kołaczkowski, M. Pawłowski, Novel butanehydrazide derivatives of purine-2,6-dione as dual PDE4/7 inhibitors with potential anti-inflammatory activity: Design, synthesis and biological evaluation, Eur. J. Med. Chem. 146 (2018) 381–394. https://doi.org/10.1016/j.ejmech.2018.01.068.

[44] G. Latacz, A. Lubelska, M. Jastrzębska-Więsek, A. Partyka, A. Sobiło, A. Olejarz, K. Kucwaj-Brysz, G. Satała, A.J. Bojarski, A. Wesołowska, K. Kieć-Kononowicz, J. Handzlik, In the search for a lead structure among series of potent and selective hydantoin 5-HT7R agents: The drug-likeness in vitro study, Chem. Biol. Drug Des. 90 (2017) 1295–1306.

https://doi.org/10.1111/cbdd.13106.

[45] A. Lubelska, G. Latacz, M. Jastrzębska-Więsek, M. Kotańska, R. Kurczab, A. Partyka, M.A. Marć, D. Wilczyńska, A. Doroz-Płonka, D. Łażewska, A. Wesołowska, K. Kieć-Kononowicz, J. Handzlik, Are the Hydantoin-1,3,5-triazine 5-HT6R Ligands a Hope to a Find New Procognitive and Anti-Obesity Drug? Considerations Based on Primary In Vivo Assays and ADME-Tox Profile In Vitro, Molecules. 24 (2019) 4472. https://doi.org/10.3390/molecules24244472.

[46] X. Chen, A. Murawski, K. Patel, C.L. Crespi, P. V. Balimane, A Novel Design of Artificial Membrane for Improving the PAMPA Model, Pharm. Res. 25 (2008) 1511–1520. https://doi.org/10.1007/s11095-007-9517-8.

[47] G. Latacz, A. Lubelska, M. Jastrzębska-Więsek, A. Partyka, K. Kucwaj-Brysz, A. Wesołowska, K. Kieć-Kononowicz, J. Handzlik, MF-8, a novel promising arylpiperazine-hydantoin based 5-HT 7 receptor antagonist: In vitro drug-likeness studies and in vivo pharmacological evaluation, Bioorg. Med. Chem. Lett. 28 (2018) 878–883. https://doi.org/10.1016/j.bmcl.2018.02.003.

[48] T. Horisawa, T. Ishibashi, H. Nishikawa, T. Enomoto, S. Toma, T. Ishiyama, M. Taiji, The effects of selective antagonists of serotonin 5-HT7 and 5-HT1A receptors on MK-801-induced impairment of learning and memory in the passive avoidance and Morris water maze tests in rats: Mechanistic implications for the beneficial effects of the novel, Behav. Brain Res. 220 (2011) 83–90. https://doi.org/10.1016/j.bbr.2011.01.034.

[49] Instant JChem Was Used for Structure Property Prediction and Calculation, Instant JChem 18.4.0, 2018, ChemAxon (http://www.chemaxon.com).

[50] SwissADME Was Used for Property Prediction, 2018 (http://www.swissadme.ch/).

[51] G. Cruciani, E. Carosati, B. De Boeck, K. Ethirajulu, C. Mackie, T. Howe, R. Vianello, MetaSite: Understanding Metabolism in Human Cytochromes from the Perspective of the Chemist, J. Med. Chem. 48 (2005) 6970–6979. https://doi.org/10.1021/jm050529c.

[52] Schrödinger LLC, Small-Molecule Drug Discovery Suite 2017-4, (2017).

[53] Y. Kim, J. Tae, K. Lee, H. Rhim, I.H. Choo, H. Cho, W.K. Park, G. Keum, H. Choo, Novel N-biphenyl-2-ylmethyl 2-methoxyphenylpiperazinylalkanamides as 5-HT7R antagonists for the treatment of depression, Bioorganic Med. Chem. 22 (2014) 4587–4596. https://doi.org/10.1016/j.bmc.2014.07.026.

[54] C. Yung-Chi, W.H. Prusoff, Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (I50) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108. https://doi.org/10.1016/0006-2952(73)90196-2.

[55] A. Ennaceur, J. Delacour, A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data, Behav. Brain Res. 31 (1988) 47–59. https://doi.org/10.1016/0166-4328(88)90157-X.

[56] R.D. Porsolt, A. Bertin, M. Jalfre, Behavioral despair in mice: a primary screening test for antidepressants., Arch. Int. Pharmacodyn. Ther. 229 (1977) 327–36. http://www.ncbi.nlm.nih.gov/pubmed/596982.

Research highlights:

- A new series of anilide and benzylamide derivatives was designed and synthesized
- 5-HT_{1A}/5-HT_7R and PDE4B/7A binding modes were analyzed *via* molecular modeling study
- Compounds were characterized as 5-HT_{1A}/5-HT_7Rs antagonists and PDE4B/7A inhibitors
- Membrane permeability and metabolic stability studies were performed *in vitro*
- Selected ligand **22** showed procognitive and antidepressant activity *in vivo*

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: