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Original article

Novel 4-aryl-pyrido[1,2-*c*]pyrimidines with dual SSRI and 5-HT$_{1A}$ activity: Part 2☆

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ABSTRACT

Derivatives of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine were synthesized. These compounds contain the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy or 2-methyl derivative. In vitro binding tests were performed to determine the affinity of the compounds for the 5-HT$_{1A}$ receptor and serotonin transporter (SERT) proteins in the rat brain cortex. In vivo studies, particularly the inducible hypothermia test and forced swimming test, were conducted to determine agonistic/antagonistic activity with pre- and postsynaptic 5-HT$_{1A}$ receptors. Molecular modeling techniques were used to determine the binding modes of the selected compounds at the 5-HT$_{1A}$ receptor and SERT. The SAR analysis showed that the presence of the 3-(4-piperidyl)-1*H*-indole group or its 5-methoxy derivative, as well as a *para* substitution with –OCH$_3$ or –F in the aryl ring of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine, results in an increased affinity for both the 5-HT$_{1A}$ receptors and SERT. In contrast, the presence of the 2-methyl-3-(4-piperidyl)-1*H*-indole group resulted in a considerable decrease in binding affinity.

1. Introduction

The introduction of selective serotonin reuptake inhibitors (SSRIs) as second generation antidepressants was a breakthrough in the treatment of depression. The SSRIs are characterized by significantly reduced disadvantageous properties; firstly, these drugs produce notably fewer adverse drug reactions (ADRs) compared with older drugs such as tricyclic antidepressants (TCAs) or nonselective monoaminooxidase inhibitors (MAOIs). However, SSRIs also have several drawbacks, including lower clinical effectiveness, some ADRs, and a relatively long onset of action, which led to the idea to develop newer, more efficacious, and safer antidepressants [1–3].

The long latency of SSRIs is caused by their self-limiting action on extracellular serotonin in the forebrain. The administration of an SSRI leads to an increased serotonin concentration in the synaptic clefts of the raphe nucleus, which in turn activates somatodendritic serotoninergic autoreceptors (5-HT$_{1A}$) [4,5]. The activation of 5-HT$_{1A}$ causes, due to negative feedback, the inhibition of serotonin release from presynaptic terminals [6–8]. A renewed, permanent increase in 5-HT concentrations in various brain synapses is achieved after a 2–4 week period, when the desensitization of 5-HT$_{1A}$ autoreceptors occurs [9,10].

The latency can be shortened by the co-administration of an SSRI with a 5-HT$_{1A}$ antagonist [11,12]. A pre-clinical in vivo microdialysis study confirmed that the simultaneous administration of the 5-HT$_{1A}$ antagonist WAY 100635 with the SSRI fluoxetine leads to an immediate increase in the serotonin concentration of the rat brain cortex, whereas this effect was not observed after the administration of fluoxetine alone [13]. Also, in clinical studies, the

Abbreviations: SERT, serotonin transporter; SAR, structure–activity relationship; 5-HT, serotonin; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetraline; K_i, Inhibitor constant; TMS, tetramethylsilane.

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co-administration of a 5-HT_{1A} antagonist (pindolol, WAY 100135, or robalzotan) with an SSRI considerably shortened the latency period to 3–7 days [14–16]. Hence, the idea of incorporating two parts into one derivative molecule with a double mechanism of action may lead to the development of a new generation of antidepressant drugs [17–20].

In our previous study, we described a series of new pyrido[1,2-*c*]pyrimidine derivatives with a double mechanism of action: the inhibition of 5-HT reuptake and agonistic activity towards both pre- and postsynaptic 5-HT_{1A} receptors [21]. These compounds can be considered a good entry point for novel drug candidates. Presynaptic agonistic effects can lead to faster desensitization of the autoreceptors, which in turn could shorten the latency period. It has also been demonstrated that postsynaptic agonistic activity increases neurotransmission in the serotoninergic system [22].

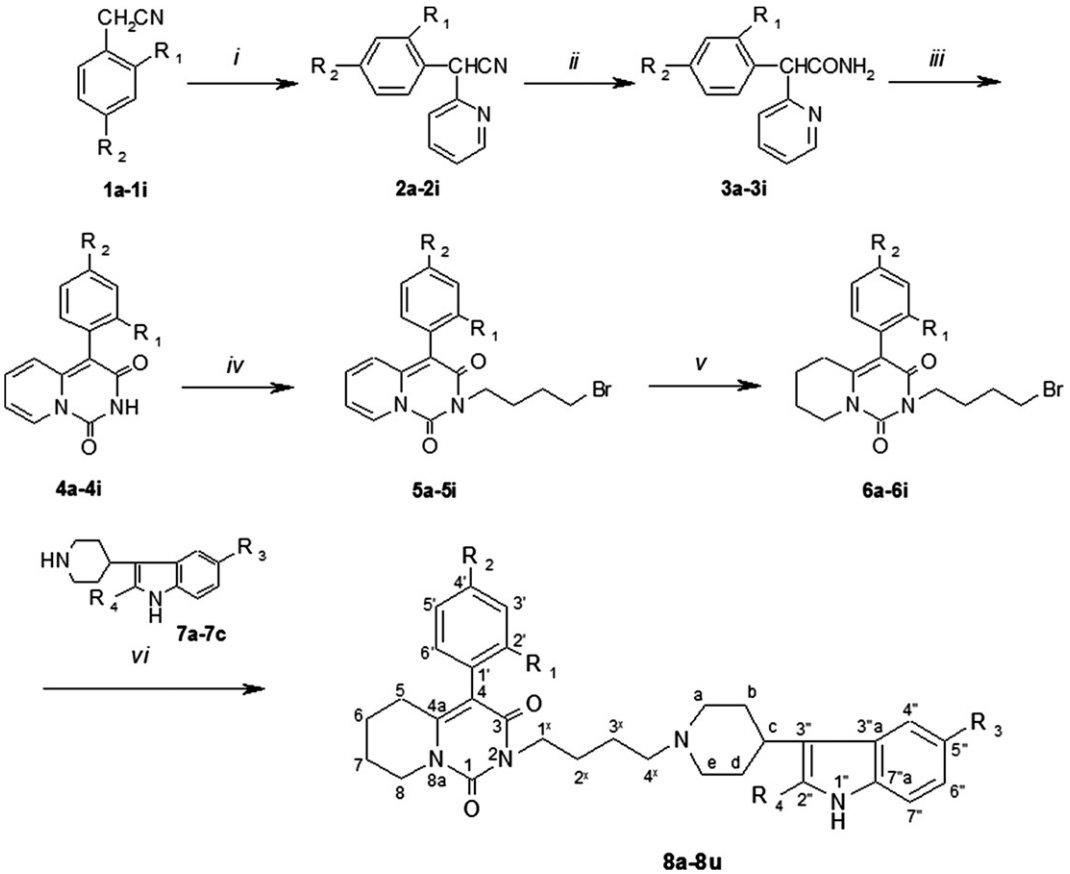
The aim of our present study was to synthesize and assess the biological properties of new tetrahydro-pyrido[1,2-*c*]pyrimidine derivatives with dual activity. New ligands were obtained after the modification of previously synthesized pyrido[1,2-*c*]pyrimidine derivatives belonging to the long chain arylpiperazine (LCAP) group with a high affinity for 5-HT_{1A} receptors [23]. Modifications were performed in the pharmacophore portion where the 3-(4-piperidyl)-1*H*-indole group or its 5-methoxy or 2-methyl derivative was inserted. These groups reveal the molecule's potential to inhibit the serotonin transporter (SERT) protein, as well as bind 5-HT_{1A} receptors [24–26]. The pharmacophore portion of the molecule was bound to the terminal tetrahydro-pyrido[1,2-*c*]pyrimidine moiety by an *n*-butyl chain. Biological investigations of the newly synthesized compounds (**8c**–**8u**) included determination of the binding affinity for the 5-HT_{1A} receptor and serotonin transporter SERT. Compounds **8c**, **8d**, **8e**, **8f**, **8h**, and **8i** were also investigated in vivo using a hypothermic test in mice, whereas compounds **8c**, **8f**, **8h**, and **8i** were investigated using a forced swimming test in mice (behavioral study). Compounds **8d**, **8e**, **8h**, **8l**, **8n**, **8q**, and **8u** were docked to the 5-HT_{1A} receptor model, whereas ligands **8d**, **8e**, **8h**, and **8l** were also docked to the SERT model.

2. Chemistry

The respective nitriles (**2a**–**2i**) were synthesized by C-arylation of the appropriate arylacetonitriles with 2-bromopyridine in an aprotic polar solvent (Scheme 1) [27,28]. Next, the nitriles (**2a**–**2i**) were hydrolyzed using a mixture of sulfuric acid and acetic acid to produce amides (**3a**–**3i**) in good yield. Compounds **4a**–**4i** were formed by the intermolecular cyclization of **3a**–**3i** with diethyl carbonate. The nitrogen in the imide group of compounds **4a**–**4i** was alkylated with 1,4-dibromobutane to form bromobutyl derivatives **5a**–**5i**, which were subsequently hydrogenated to form 5,6,7,8-tetrahydro derivatives (**6a**–**6i**). The final compounds (**8a**–**8u**) were obtained by the condensation of bromobutyl derivatives **6a**–**6i** with the appropriate piperidyl-indole (**7a**–**7c**) synthesized according to the method previously described [25].

3. Structural investigations

The new compounds (**8a**–**8u**) were characterized by physical constants, elemental analysis, IR, and ^{1}H, and ^{13}C NMR spectroscopy (see section 8.2). The NMR spectra mutually correlated and were in agreement with the literature for similar systems [23,27] as well as



Scheme 1. [a]Reagents: (*i*) 2-bromopyridine, KOH, DMSO, Δ; (*ii*) H_2SO_4, CH_3COOH, Δ; (*iii*) diethyl carbonate, EtONa, EtOH, Δ; (*iv*) 1,4-dibromobutane, K_2CO_3, acetone, Δ; (*v*) H_2, 10% Pt/C, 60 atm., 50 °C; (*vi*) **7a**–**7c**, acetonitrile, K_2CO_3, KI, Δ. Numbering system of **8** for NMR analysis.

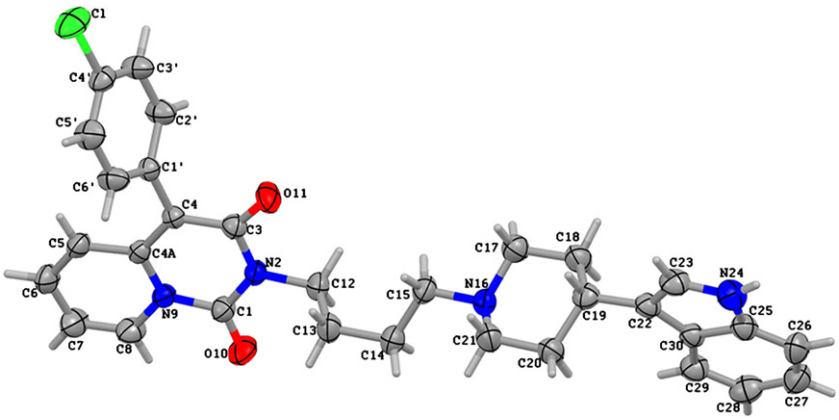


Fig. 1. A view of the 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21] molecule and its numbering scheme.

the theoretical spectra calculated according to the ACD/NMR Predictor v. 8.09 program.

The XRD experiments carried out for compound 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) were previously described in part 1 of this work [21]. The ORTEP view of the molecule, with drawings created by the Mercury program [29], is shown in Fig. 1. Selected bond lengths, bond angles, and torsion angles are listed in Table 2.

The piperidine ring adopts a chair conformation with N16 and C19, 0.672(2) and 0.666(2) Å above and below the plane formed by the piperidine ring carbons, respectively. The planarity of the indole substituent at C19 with respect to the piperidine ring is indicated by the value of the torsion angle C18–C19–C22–C23, 109.2(2)°. The butyl chain at N16 occupies the equatorial position and adopts the trans/gauche/trans conformation (see the torsion angles in Table 2).

The pyrido[1,2-*c*]pyrimidine fragment is essentially planar with no atomic deviation greater than 0.044(1) Å (for C1 atom) from its least-squares plane. The planar substituent at C4 creates an angle of 68.77(4)° with the best plane of the pyridopyrimidine system, and the orientation of this substituent with respect to the mentioned moiety can be described by the torsion angle C3–C4–C1′–C2′, which is 68.1(2)°.

The crystal packing of the molecules is determined by different N–H···O, C–H···Cl, and C–H···π intermolecular contacts (Fig. 2). The geometric parameters of all these bonds are listed in Table 3.

Table 1
Crystal data, data collection, and structure refinement for 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21].

Compound	4-(4-Chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*] pyrimidine-1,3-dione
Empirical formula	$C_{31}H_{31}N_4O_2Cl$
Formula weight	527.05
T (K)	293(2)
Wavelength (Å)	0.71073
Crystal system, space group	Triclinic, *P*-1
Unit cell dimensions	
a (Å)	10.4857(5)
b (Å)	10.9071(6)
c (Å)	12.4227(6)
α (°)	77.821(5)
β (°)	79.883(4)
γ (°)	76.435(5)
Volume (Å^3)	1338.1(1)
Z, D_x (Mg/m^3)	2, 1.308
μ (mm^{-1})	0.179
F(000)	556
Θ range for data collection (°)	3.08–30.04
hkl Range	$-13 < h < 14$ $-13 < k < 14$ $-16 < l < 17$
Reflections	
Collected	18056
Unique (R_{int})	6707 (0.024)
Observed ($I > 2((I))$	4480
Data/restraints/parameters	6707/0/343
Goodness-of-fit on F^2	1.049
$R(F)$ ($I > 2\sigma(I)$)	0.0461
$wR(F^2)$ (all data)	0.1287
Max/min. (e/Å^3)	0.307/−0.259

4. Pharmacology

The in vitro affinity of target compounds **8c–8u** for 5-HT$_{1A}$ receptors and SERT in rat brain was assessed using radioligand binding assays ([^{3}H]8-OH-DPAT and [^{3}H]citalopram, respectively). Data were analyzed using iterative curve-fitting routines to obtain IC_{50} values (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA),

Table 2
Selected bond lengths and angles and selected torsional angles for 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21].

Bond	Length (Å)
C4′–Cl	1.741(1)
C4–C4A	1.374(2)
C4–C1′	1.493(2)
C3–O11	1.231(2)
C1–O10	1.212(2)
C12–N2	1.484(2)
C15–N16	1.468(2)
C23–N24	1.360(2)
C25–N24	1.370(2)
Bonds	**Angle (deg)**
C1–N2–C3	125.4(1)
C3–C4–C1′	118.0(1)
C15–N16–C21	111.7(1)
C15–N16–C17	109.8(1)
C23–N24–C25	108.9(1)
Bonds	**Torsional angles (deg)**
C2′–C1′–C4–C4A	−111.8(2)
N2–C12–C13–C14	−175.7(1)
C12–C13–C14–C15	71.5(2)
C13–C14–C15–N16	174.6(1)
C18–C19–C22–C23	109.2(2)

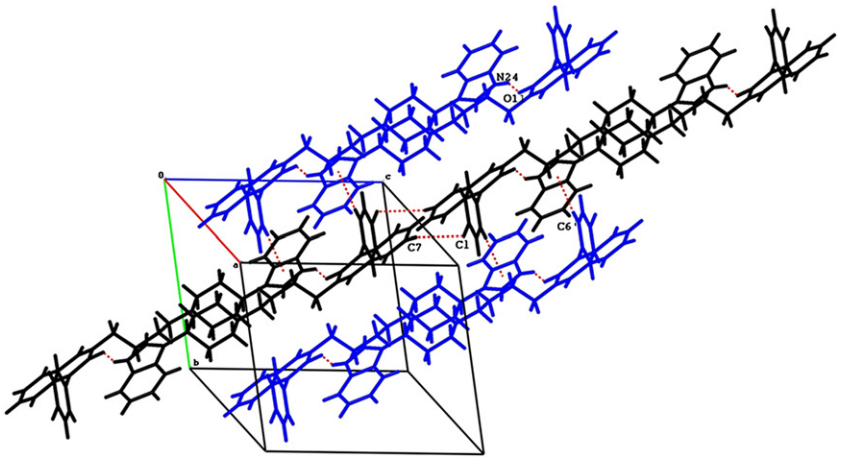


Fig. 2. The packing arrangement of 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21] with intermolecular interactions.

which in turn were used to calculate inhibition constant K_i according to the Cheng–Prusoff formula (Table 4) [30].

The final compounds were further evaluated in mice for their agonist/antagonist properties towards pre- and postsynaptic 5-HT_{1A} receptors using the inducible hypothermia and forced swimming tests, respectively. In the inducible hypothermia test, the effects of the administration of six compounds (**8c**, **8d**, **8e**, **8f**, **8h**, and **8i**) on body temperature, as well as the effects of WAY 100635 (5-HT_{1A} receptor antagonist) on the hypothermia induced by the tested compounds were investigated. The results were expressed as changes in body temperature (Δt) compared with basal body temperature as measured at the beginning of the experiment. The forced swimming test was carried out according to the method of Porsolt et al. for **8c**, **8f**, **8h**, and **8i** [31]. The effect of these compounds on the spontaneous locomotor activity in mice was also recorded. The obtained data were presented as the mean ± S.E.M. The significance of differences between groups was evaluated by a one-way analysis of variance (ANOVA) followed by intergroup comparisons using Dunnett's or Newman–Keuls test; $p \leq 0.01$ was accepted as significant.

5. Molecular modeling

Ligand binding modes were studied by the fully flexible molecular docking of seven ligands (**8d**, **8e**, **8h**, **8l**, **8n**, **8r**, and **8v**) to the model of the transmembrane portion of the 5-HT_{1A} receptor. Additionally, three compounds described in Part 1 of this article (**8a**, **10a**, and **11a**) were also docked [21]. One hundred 5-HT_{1A} receptor models were used that were previously "tuned" for aryl-piperazine/arylpiperidine ligands [32]. Two pharmacophore constraints were used to force the interactions crucial for the recognition of arylpiperidine serotoninergic ligands: the ionic interaction between Asp3.32 and the basic moiety of the ligand, and the aromatic contact between the ligand and Phe6.52.

Table 3
Hydrogen-bonding geometry for 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21].

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	<(D–H···A) (deg)
N24–H24A···O11^{i}	0.86	2.16	2.845(2)	136
C7–HA···Cl^{ii}	0.93	2.95	3.798(2)	152
C6′–H6′A···Cg^{iii}	0.93	2.64	3.535(2)	161

Cg represents the centroid of the five-membered ring of the indole moiety.
Symmetry codes: (i) $1-x, 1-y, -z$; (ii) $1-x, -y, 2-z$; (iii) $x, -1+y, 1+z$.

Four ligands (**8d**, **8e**, **8h**, and **8l**; Table 4) were also docked to the SERT model using a docking procedure as implemented in the ICM software [33]. The SERT model was based on the X-ray crystal structure of the leucine transporter from *Aquifex aeolicus* ($LeuT_{Aa}$) [34,35]. This SERT model corresponds to the substrate-bound conformation of the transporter model, closed on both sides of the membrane. All compounds studied have a nitrogen atom attached to the protonated butyl fragment. The functionally important residue for ligand interaction with SERT, $Asp98_{1.45}$ in TMH1 [36], was used as an anchoring point for docking fully flexible ligands to the serotonin transporter.

6. Results and discussion

Several new derivatives of 4-aryl-tetrahydro-pyrido[1,2-*c*]pyrimidine containing 3-(4-piperidyl)-1*H*-indole or its 5-methoxy or 2-methyl derivative in the pharmacophore portion of the molecule were obtained (Fig. 1). In the 4-aryl-tetrahydro-pyrido[1,2-*c*]pyrimidine system, an aryl substitute in compounds **8b** and **8o** consisted of a non-substituted phenyl ring, whereas other derivatives possessed additional –F (**8f**, **8i**, **8n**), –Cl (**8m**, **8t**), $-OCH_3$ (**8p**), or $-CH_3$ (**8j**, **8k**) groups at the *ortho* position of the phenyl ring, as well as –F (**8l**, **8u**), –Cl (**8a**, **8g**, **8s**), $-OCH_3$ (**8d**, **8e**, **8r**), or $-CH_3$ (**8c**, **8h**, **8q**) groups at the *para* position.

Table 4
Inhibition constant (K_i) values of the investigated compounds for 5-HT_{1A} receptors and SERT.

Derivative	R_1	R_2	R_3	R_4	$K_{i\ 5\text{-}HT1A}$ (nM)	$K_{i\ SERT}$ (nM)
8c	H	CH_3	OCH_3	H	23.0 ± 1.4	135.5 ± 4.5
8d	H	OCH_3	H	H	20.5 ± 2.8	71.6 ± 0.9
8e	H	OCH_3	OCH_3	H	10.4 ± 2.6	83.5 ± 7.1
8f	F	H	OCH_3	H	45.6 ± 3.9	184.4 ± 5.0
8g	H	Cl	OCH_3	H	14.6 ± 0.8	222.0 ± 8.0
8h	H	CH_3	H	H	48.3 ± 4.3	76.7 ± 1.3
8i	F	H	H	H	27.8 ± 3.6	101.9 ± 9.3
8j	CH_3	H	H	H	53.8 ± 1.9	123.6 ± 8.8
8k	CH_3	H	OCH_3	H	51.7 ± 4.1	123.7 ± 5.2
8l	H	F	OCH_3	H	13.9 ± 0.5	76.1 ± 6.1
8m	Cl	H	H	H	36.2 ± 1.9	101.3 ± 5.8
8n	F	H	H	CH_3	2.2 ± 0.2 (μM)	944 ± 22
8o	H	H	H	CH_3	1.4 ± 0.1 (μM)	273.6 ± 4.5
8p	OCH_3	H	H	CH_3	1.6 ± 0.1 (μM)	719 ± 48
8q	H	CH_3	H	CH_3	1.5 ± 0.1 (μM)	115.1 ± 6.9
8r	H	OCH_3	H	CH_3	835.6 ± 64.4	203 ± 4
8s	H	Cl	H	CH_3	1.5 ± 0.3 (μM)	460 ± 25
8t	Cl	H	H	CH_3	2.1 ± 0.1 (μM)	480 ± 43
8u	H	F	H	CH_3	3.2 ± 0.2 (μM)	116.1 ± 9.9

6.1. SAR analysis

The binding values obtained for compounds **8c**–**8u** for the 5-HT_{1A} receptor and SERT provided the possibility of analyzing the influence of substituents bound to the 3-(4-piperidyl)-1*H*-indole moiety (–H or $–OCH_3$ at 5 position, $–CH_3$ or –H at 2 position), the influence of various substitutes at the *ortho*/*para* position of the aryl ring, and the impact of the degree of saturation of the tetrahydro-pyrido[1,2-*c*]pyrimidine system (compare to Ref. [21]). The binding tests for **8c**–**8u** revealed a very high affinity of derivatives **8e** ($K_i = 10.4$ nM), **8g** ($K_i = 14.6$ nM), and **8l** ($K_i = 13.9$ nM) for 5-HT_{1A} receptors. The remaining compounds possessed high to weak binding activity with K_i ranging from 20.5 nM to 3.2 μM (compounds **8d**, **8c**, **8i**, **8m**, **8f**, **8h**, **8k**, **8j**, **8r**, **8o**, **8q**, **8p**, **8s**, **8t**, **8n**, and **8u** according to increasing K_i values; see Table 4).

Analyzing the effect of substituents at the indole moiety on binding activity found that compounds bearing a non-substituted 3-(4-piperidyl)-1*H*-indole group or analogous 3-(4-piperidyl)-5-methoxy-1*H*-indole derivatives possessed very high or high affinity for 5-HT_{1A} receptors. The presence of the 2-methyl-3-(4-piperidyl)-1*H*-indole group resulted in a considerable loss in affinity. An analysis of the effect of the substitution of the aryl ring in tetrahydro-pyrido[1,2-*c*]pyrimidine found that, on the condition that there is no $–CH_3$ substituent at the 2 position of the 3-(4-piperidyl)-1*H*-indole group, substitution with $–OCH_3$ (**8d**, **8e**), –F (**8l**), or –Cl (**8g**) at the *para* position results in a higher affinity for 5–HT_{1A} receptors than ligands with –F (**8f**, **8i**), $–CH_3$ (**8j**, **8k**), or –Cl (**8m**) at the *ortho* position and compounds with $–CH_3$ (**8c**, **8h**) at the *para* position. In addition, we found that the degree of saturation of the tetrahydro-pyrido[1,2-*c*]pyrimidine system has no major influence on 5-HT_{1A} receptor affinity compared to unsaturated pyrido[1,2-*c*]pyrimidine derivatives previously described in Part 1 of this study [21]. The SERT binding studies demonstrated a moderate to poor affinity for the newly synthesized compounds with K_i values ranging from 71.6 to 943.7 nM (derivatives **8d**, **8l**, **8h**, **8e**, **8m**, **8i**, **8q**, **8u**, **8j**, **8k**, **8c**, **8f**, **8r**, **8g**, **8o**, **8s**, **8t**, **8p**, and **8n** according to increasing K_i values).

When the influence of the indole moiety substitution on SERT binding was evaluated, we observed that the non-substituted 3-(4-piperidyl)-1*H*-indole derivatives and 3-(4-piperidyl)-5-methoxy-1*H*-indole analogues possessed relatively similar and substantial affinities ($K_i = 71.6$–222.0 nM), whereas the 2-methylindole derivatives were mostly characterized by high K_i values. Analyzing the influence of the aryl ring substitution on tetrahydro-pyrido[1,2-*c*]pyrimidine revealed that substitution with $–OCH_3$ (**8d**, **8e**, **8r**), $–CH_3$ (**8c**, **8h**, **8q**), or –F (**8l**, **8u**) at the *para* position results in relatively high affinity. The remaining derivatives exhibited a lower affinity. Saturation of the tetrahydro-pyrido[1,2-*c*]pyrimidine system decreased SERT inhibition compared to the unsaturated pyrido[1,2-*c*]pyrimidine derivatives previously described in Part 1 [21].

6.2. Behavioral studies

Compounds with the most promising affinity for both 5–HT_{1A} receptor and SERT were selected for in vivo experiments (**8c**, **8d**, **8e**, **8f**, **8h**, and **8i**) in models commonly used for evaluating 5-HT_{1A} receptor function. The 5-HT_{1A} receptor agonist 8-OH-DPAT induces hypothermia in mice, an effect mediated through the 5-HT_{1A} somatodendritic receptor [37,38]. This hypothermia is abolished by the 5-HT_{1A} receptor antagonist WAY 100635 [39]. Compounds **8c**, **8e**, **8f**, **8h**, and **8i** induced hypothermia in mice similar to 8-OH-DPAT, whereas **8d**, analogous to WAY 100635, did not alter the body temperature in mice (Table 5). The hypothermia induced by compounds **8c** (20 mg/kg), **8f** (20 mg/kg), **8h** (10 mg/kg), and **8i** (10 mg/kg) was attenuated by WAY 100635 (0.1 mg/kg) (Table 6).

Table 5
The effect of the tested compounds on the body temperature of mice.

Treatment	Dose (mg/kg)	Δ*t* ± SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	–	0.0 ± 0.0	−0.0 ± 0.1	−0.1 ± 0.0	0.0 ± 0.1
8c	5	−0.3 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.0
	10	−0.6 ± 0.1[b]	−0.4 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1
	20	−1.3 ± 0.1[c]	−1.3 ± 0.2[c]	−0.8 ± 0.1[c]	−0.7 ± 0.1[b]
Vehicle	–	−0.1 ± 0.0	−0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
8d	5	−0.5 ± 0.1[a]	−0.2 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1
	10	−0.7 ± 0.1[b]	−0.5 ± 0.1[a]	−0.4 ± 0.1	−0.3 ± 0.1
	20	−0.6 ± 0.1[b]	−0.7 ± 0.1[b]	−0.4 ± 0.1	−0.3 ± 0.1
Vehicle	–	0.0 ± 0.0	−0.0 ± 0.0	−0.1 ± 0.1	0.0 ± 0.0
8e	1.25	−0.3 ± 0.1	−0.4 ± 0.1	−0.4 ± 0.1	−0.2 ± 0.1
	2.5	−0.8 ± 0.1[c]	−0.6 ± 0.1[b]	−0.6 ± 0.1[b]	−0.5 ± 0.0[a]
	5	−1.1 ± 0.2[c]	−0.5 ± 0.1[a]	−0.3 ± 0.1	−0.1 ± 0.0
Vehicle	–	−0.0 ± 0.0	−0.0 ± 0.1	−0.1 ± 0.0	−0.0 ± 0.1
8f	5	−0.7 ± 0.1[b]	−0.6 ± 0.1[b]	−0.4 ± 0.1	−0.3 ± 0.1
	10	−0.7 ± 0.1[b]	−0.8 ± 0.1[c]	−0.6 ± 0.2[b]	−0.4 ± 0.1
	20	−1.2 ± 0.1[c]	−1.0 ± 0.1[c]	−0.8 ± 0.2[c]	−0.7 ± 0.2[b]
Vehicle	–	−0.1 ± 0.1	−0.1 ± 0.0	−0.1 ± 0.1	−0.1 ± 0.1
8h	5	−0.6 ± 0.1[b]	−0.6 ± 0.1[b]	−0.3 ± 0.1	−0.2 ± 0.1
	10	−1.0 ± 0.1[c]	−0.5 ± 0.1[a]	−0.3 ± 0.1	−0.2 ± 0.1
Vehicle	–	−0.0 ± 0.0	−0.1 ± 0.1	−0.1 ± 0.1	−0.0 ± 0.1
8i	5	−0.3 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1
	10	−1.2 ± 0.2[c]	−1.0 ± 0.1[c]	−0.8 ± 0.1[c]	−0.5 ± 0.1[a]
Vehicle	–	0.0 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.1
8-OH-DPAT	5	−1.5 ± 0.1[c]	−1.1 ± 0.1[c]	−0.7 ± 0.1[b]	−0.2 ± 0.1
WAY 100635	0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.2

The tested compounds were administered by ip injection 30 min before the test. 8-OH-DPAT and WAY 100635 were injected subcutaneously (sc). The absolute mean body temperatures were 36 ± 0.5 °C; $n = 7$–8 mice per group.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$ vs. respective vehicle.

Table 6
The effect of WAY 100635 on the hypothermia induced in mice by the tested compounds.

Compound and dose (mg/kg)	Δ*t* ± SEM (°C)			
	30 min		60 min	
Vehicle + vehicle	−0.1	±0.1	−0.0	±0.0
Vehicle + **8c** (20)	−1.3	±0.2[b]	−0.8	±0.1[a]
WAY 100635 (0.1) + **8c** (20)	−0.4	±0.1[d]	−0.4	±0.1
Vehicle + vehicle	−0.1	±0.1	−0.1	±0.1
Vehicle + **8e** (5)	−1.1	±0.1[b]	−0.6	±0.1[a]
WAY 100635 (0.1) + **8e** (5)	−1.2	±0.2[b]	−0.9	±0.2[b]
Vehicle + vehicle	−0.0	±0.1	−0.1	±0.1
Vehicle + **8f** (20)	−1.3	±0.2[b]	−1.0	±0.2[b]
WAY 100635 (0.1) + **8f** (20)	−0.5	±0.1[d]	−0.4	±0.1[d]
Vehicle + vehicle	−0.1	±0.0	−0.1	±0.1
Vehicle + **8h** (10)	−1.0	±0.1[b]	−0.5	±0.2
WAY 100635 (0.1) + **8h** (10)	−0.6	±0.1[b,c]	−0.6	±0.1[b]
Vehicle + vehicle	−0.0	±0.1	−0.1	±0.1
Vehicle + **8i** (10)	−1.2	±0.2[b]	−1.0	±0.2[b]
WAY 100635 (0.1) + **8i** (10)	−0.6	±0.1[a,d]	−0.4	±0.1[d]
Vehicle + vehicle	0.0	±0.1	−0.1	±0.1
Vehicle + 8-OH-DPAT (5)	−1.5	±0.1[b]	−1.1	±0.1[b]
WAY 100635 (0.1) + 8-OH-DPAT(5)	−0.1	±0.1[d]	−0.2	±0.1[d]

WAY 100635 was administered subcutaneously 15 min before the investigated compounds, $n = 7$–8 mice per group. The test was performed 30 min after the injection of the tested compounds (ip). The absolute mean body temperatures were 36.0 ± 0.4 °C.
[a] $p < 0.01$.
[b] $p < 0.001$ vs. respective vehicle + vehicle group.
[c] $p < 0.01$.
[d] $p < 0.001$ vs. respective vehicle + compound group.

Table 7
The effect of compound **8d** and WAY 100635 on 5 mg/kg 8-OH-DPAT-induced hypothermia in mice.

Treatment and dose (mg/kg)	$\Delta t \pm$ SEM (°C)			
	15 min	30 min	45 min	60 min
Vehicle + vehicle	-0.0 ± 0.0	-0.1 ± 0.1	-0.1 ± 0.1	-0.1 ± 0.1
Vehicle + 8-OH-DPAT	-1.2 ± 0.2[b]	-1.6 ± 0.1[b]	-0.8 ± 0.1[a]	-0.2 ± 0.1
8d (20) + 8-OH-DPAT	0.6 ± 0.1[a,d]	-0.5 ± 0.1[a,c]	-0.3 ± 0.1	-0.2 ± 0.1
Vehicle + vehicle	-0.1 ± 0.0	-0.1 ± 0.0	-0.1 ± 0.0	0.0 ± 0.1
Vehicle + 8-OH-DPAT (5)	-1.2 ± 0.1[b]	-1.5 ± 0.1[b]	-0.7 ± 0.1[a]	-0.3 ± 0.1
WAY100635(0.1) + 8-OH-DPAT	-0.1 ± 0.1[d]	-0.1 ± 0.1[d]	-0.1 ± 0.1[d]	-0.2 ± 0.1[d]

Compound **8d** was administered (ip) 45 min prior to 8-OH-DPAT, WAY 100635 (sc) 15 min prior to 8-OH-DPAT, $n = 8$ mice per group. The absolute mean initial body temperatures were 36.0 ± 0.5 °C.
[a] $p < 0.01$.
[b] $p < 0.001$ vs. respective vehicle + vehicle group.
[c] $p < 0.01$.
[d] $p < 0.001$ vs. respective vehicle + 8-OH-DPAT.

At the same time, the decrease in body temperature induced by 8-OH-DPAT (5 mg/kg) was completely blocked by WAY 100635 (0.1 mg/kg) (Table 6). Therefore, the decrease in body temperature produced in mice by **8c**, **8f**, **8h**, and **8i** can be regarded as a measure of its presynaptic 5-HT_{1A} receptor agonist activity. Compound **8d** (20 mg/kg) decreased the hypothermia induced by 8-OH-DPAT (5 mg/kg) in mice (Table 7). Therefore, the decrease in body temperature produced in mice by 8-OH-DPAT can be regarded as a measure of its presynaptic 5-HT_{1A} receptor antagonist activity. WAY 100635 did not change the hypothermia induced by **8e** (5 mg/kg) in mice; therefore, it seems that the action of **8e** on those receptors is of no importance to its hypothermic effect.

Subsequently, to establish the agonist/antagonistic actions on the postsynaptic 5-HT_{1A} receptor, compounds **8c**, **8f**, **8h**, and **8i** were investigated using the forced swimming test in mice, and their effect on mouse locomotor activity was studied. Compound **8f** reduced the immobility time of the mice by 26% [$F(2,27) = 6.864$, $p < 0.01$] at a dose of 20 mg/kg but not 10 mg/kg. At the same time, compounds **8h** and **8i** reduced the immobility time by 34% and 27%, respectively [**8h**: $F(2,27) = 21.95$, $p < 0.001$; **8i**: $F(2,27) = 17.04$, $p < 0.001$] at a dose of 10 mg/kg but not 5 mg/kg. Compound **8c** was ineffective at doses of 10 mg/kg and 20 mg/kg. We found that 8-OH-DPAT reduced the immobility time of the mice by 45% and 57% ($F(2,26) = 53.92$, $p < 0.0001$) at doses of 1 mg/kg and 2 mg/kg, respectively (Fig. 3). All of the compounds tested (**8c**, **8f**, **8h**, and **8i**) reduced the spontaneous locomotor activity in mice during a 6-min (i.e. for the time equivalent to the observation period in the forced swimming test) or a 30-min observation period (Table 8).

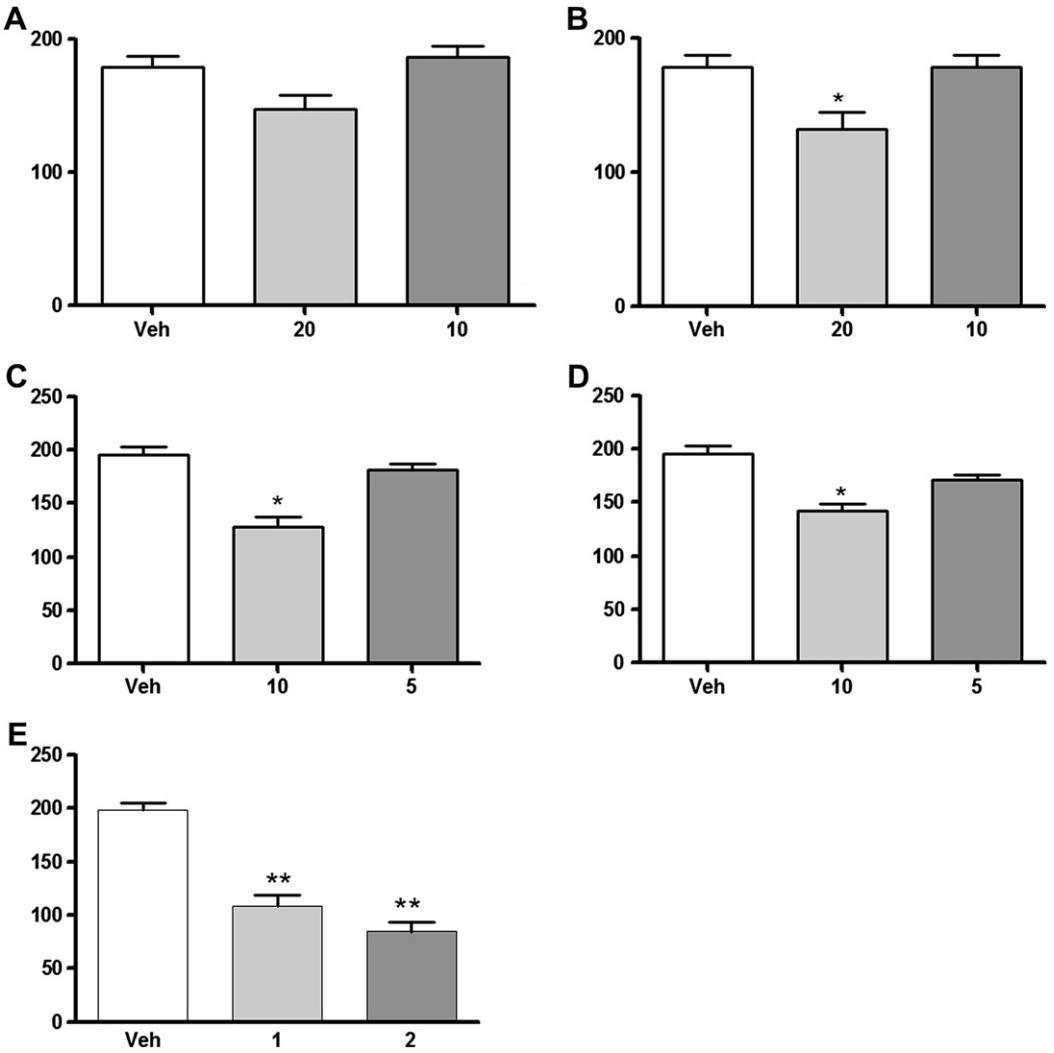


Fig. 3. Effects of compounds **8c** (A), **8f** (B), **8h** (C), **8i** (D), and 8-OH-DPAT (E) on Albino Swiss mice in the forced swimming test. Each bar represents the mean ± SEM of 9–10 mice. All compounds were injected 30 min before the test. *$p < 0.01$, **$p < 0.001$ vs. respective vehicle group (Dunnett's test). *X*-Axis: dose [mg/kg]; *Y*-axis: immobility time [s].

Table 8
Effect of compounds **8f**, **8h**, and **8i** on locomotor activity in mice.

Treatment (mg/kg)	Locomotor activity	
	Number of crossings ± S.E.M.	
	6 min	30 min
Vehicle	414 ± 34	1010 ± 60
8f (20)	272 ± 42[a]	520 ± 80[a]
8h (10)	114 ± 21[a]	221 ± 42[a]
8i (10)	217 ± 22[a]	333 ± 60[a]
	$F(3,36) = 16.57$, $p < 0.0001$	$F(3,36) = 31.35$, $p < 0.0001$

All compounds were injected (ip) 30 min before the test.
[a] $p < 0.01$ vs. vehicle group, $n = 10$.

6.3. Molecular modeling

The overall conformation of the alkyl spacer of the docked ligands is extended and resembles the X-ray structure of 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**; Fig. 1) described in Part 1 of this article [21]. The majority of the docking results do not explicitly explain the influence of the methyl substituent at position 2 of the indole ring that decreases the affinity to 5-HT_{1A} to the micromolar level. In approximately 80% of modeled complexes, the methyl group was exposed towards the exterior of the binding site, whereas the benzene portion of the indole ring penetrated the binding pocket. In some of these models the position of the indole moiety is additionally stabilized by H-bonding between the indole NH group and Ser5.43 (Fig. 4C, H, I). However, in some poses the alternative orientation of the indole moiety is visible in the non-methylated ligands, with the NH group of the indole ring pointing towards the interior of the binding pocket. This can be seen in the top-scored pose of **8l** (Fig. 4G) and in selected poses for **10a** (Fig. 5). In such a conformation, the addition of the methyl substituent will cause a steric hindrance with the surface of the binding pocket.

Another explanation for the influence of the methyl substitution at the indole moiety could be a possible interaction with extracellular loop 2 (EL2). Extracellular loops were not modeled due to the unsatisfactory quality of loop modeling in Modeler [40]. The superimposition of our receptor models on recent crystal structures for the adrenergic receptors (data not shown) suggests that the area where the methyl substituent is situated is not occupied by any atoms of the EL2, suggesting that the steric hindrance of this loop is not the reason for the loss of 5-HT_{1A} affinity for ligands with a methylated indole moiety.

The position of the 4-aryl-pyrido[1,2-*c*]pyrimidine or 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine moiety was similar in most of the top-scored poses for all ligands; it occupies the smaller binding subpocket outlined by transmembrane helices (TMHs) 1–3 and 7. One of the carbonylic oxygen atoms of that moiety forms bifurcated hydrogen bonds with Tyr7.43 and/or Asn7.39. The phenyl ring substituted with a uracil moiety is, in most cases (Fig. 4C–J), stacked between Phe3.28 and Tyr2.64 and forms aromatic contacts with these residues.

The docking studies indicated that favorable interactions between ligands (**8d**, **8e**, **8h**, **8l**) and the SERT model in closed conformation occurred in the pore formed between TMHs 1, 3, 6, and 8 (Fig. 6). The residues involved in ligand binding were (the generic number of the residue [41] is shown as lower index): $Tyr95_{1.42}$, $Asp98_{1.45}$, $Gly100_{1.48}$, $Trp103_{1.50}$, and $Arg104_{1.51}$ (TMH1); $Ala169_{3.43}$, $Ile172_{3.46}$, $Tyr175_{3.49}$, $Tyr176_{3.50}$, and $Ile179_{3.53}$ (TMH3); $Phe335_{6.53}$, $Ser336_{6.54}$, $Gly338_{6.56}$,

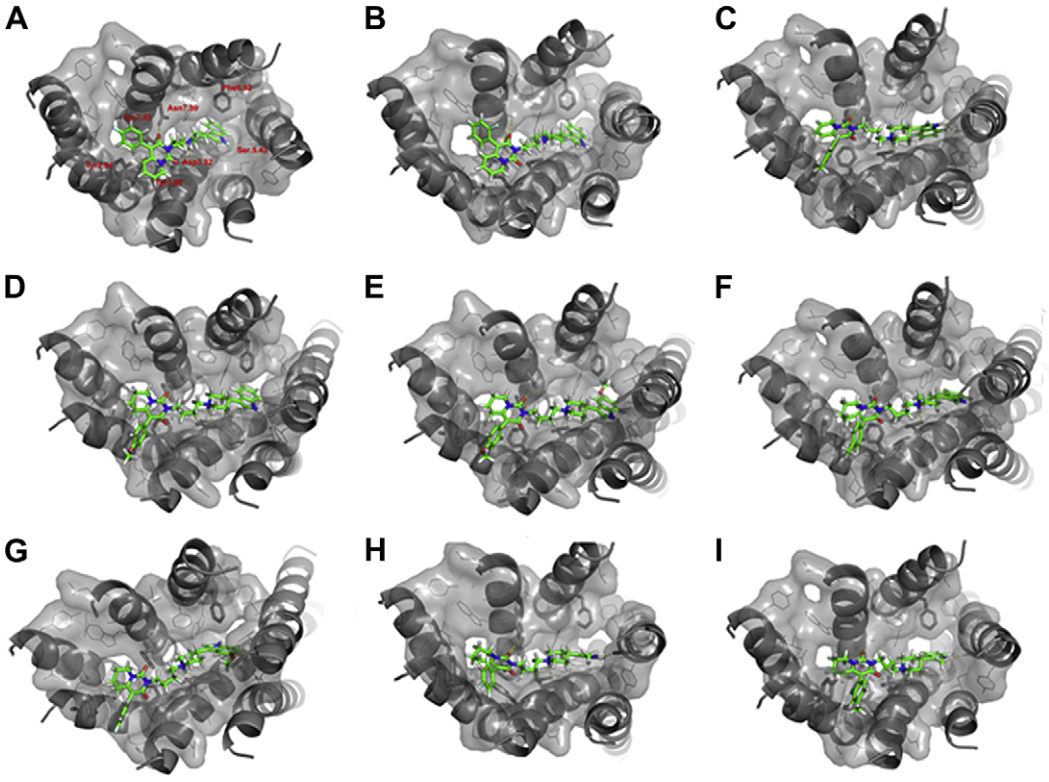


Fig. 4. Ligand binding modes predicted by flexible docking. The transmembrane portion of the receptor is represented as ribbons. Side chains and the molecular surface are visualized only for amino acids defined as being in the active site. Residues forming specific contacts with the ligand are shown as sticks. H-bonds are represented by yellow dotted lines. (A) **10a** [21], (B) **11a** [21], (C) **8a** [21], (D) **8d**, (E) **8e**, (F) **8h**, (G) **8l**, (H) **8n**, (I) **8q**.

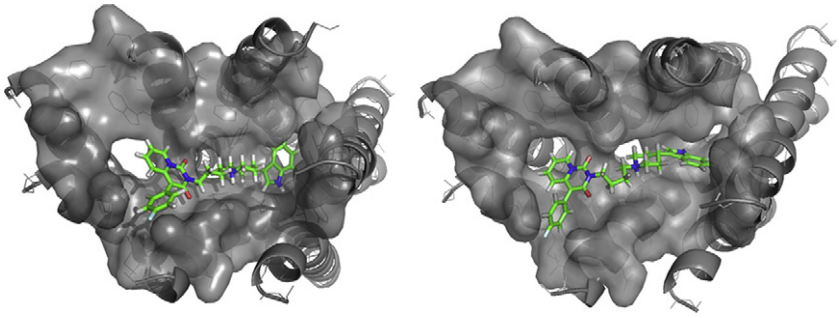


Fig. 5. Alternative binding modes present in approximately 20% of docking solutions, with a possible steric hindrance around position 2 in the indole ring.

Phe341$_{6.59}$, and Val343$_{6.62}$ (TMH6); Ser438$_{8.60}$, Thr439$_{8.61}$, and Gly442$_{8.64}$ (TMH8). All docked compounds are placed in the binding site of SERT in a similar position. The ligand indole moiety was located in a position corresponding to the SERT substrate binding site, while the tetrahydro-pyrido[1,2-*c*]pyrimidine moiety was close to the putative low affinity binding site [34,42]. For all docked ligands, close interactions with Asp98$_{1.45}$ in TMH1 were observed. However, the steric hindrance of Phe355$_{6.53}$ in TMH6 with the ligands' butyl chains was observed. In addition, steric interactions between the tetrahydro-pyrido[1,2-*c*]pyrimidine moiety and Arg104$_{1.51}$ in TMH1 can affect the ligand binding affinity for SERT.

7. Conclusions

Several new 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine derivatives possessing a 3-(4-piperidyl)-1*H*-indole group or its 5-methoxy or 2-methyl derivatives were synthesized. These compounds are considered to have a double mechanism of action. In vitro receptor binding studies of newly synthesized ligands showed a very high to minimal binding affinity for 5-HT$_{1A}$ receptors, as well as a moderate to weak binding affinity for SERT. The K_i values for both 5-HT$_{1A}$ receptor and SERT showed that compounds **8e**, **8d**, **8i**, and **8l** had the highest affinity among new derivatives. The remaining compounds either revealed a relatively higher affinity for one of the proteins (**8g** and **8c** for 5-HT$_{1A}$ receptors; **8h** and **8m** for SERT) or bound both 5-HT$_{1A}$ receptor and SERT with moderate or slight strength and were characterized by K_i values ranging from intermediate to very high (majority of compounds).

It was established that the presence of the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy derivative, as well as the fluorine atom at the *ortho*/*para* position or –OCH$_3$/–CH$_3$ group at the *para* position of the phenyl group in the aryl ring of the 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine system, are at an advantage with regard to binding affinity.

We also found that the degree of saturation of the 5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine system has no influence on the binding activity for both 5-HT$_{1A}$ receptor and SERT compared to unsaturated pyrido[1,2-*c*]pyrimidine derivatives described previously [21]. In contrast, the presence of the 2-methyl-3-(4-

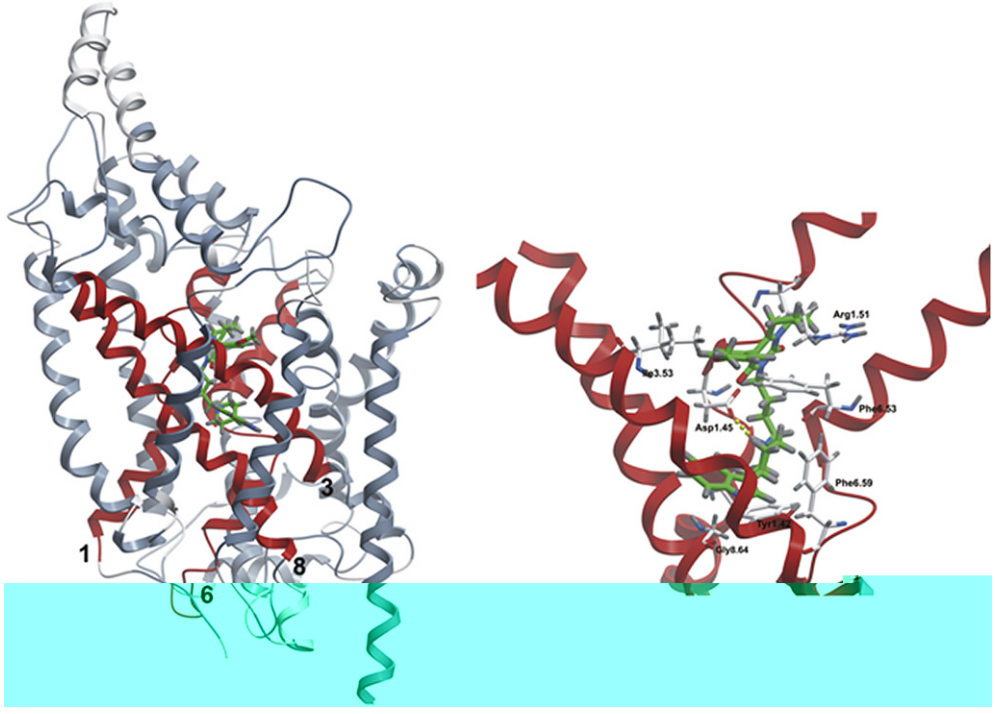


Fig. 6. Ligand **8d** binding to the SERT model.

piperidyl)-1*H*-indole group results in a drastic affinity decrease with regard to 5-HT_{1A} receptors and a significant reduction in the SERT-inhibiting action of relevant compounds.

In an induced hypothermia test in mice, compounds **8c**, **8f**, **8h**, and **8i** exhibited agonistic properties towards presynaptic 5-HT_{1A} receptors when administered at doses of 20 mg/kg, 20 mg/kg, 10 mg/kg, and 10 mg/kg, respectively. In contrast, compound **8d** exhibited antagonistic properties when administered at a dose of 20 mg/kg. The agonistic effects of compounds **8f**, **8h**, and **8i** on postsynaptic 5-HT_{1A} receptors were also demonstrated in forced swimming tests in mice. Compounds **8h** and **8i** have an effect at a 10 mg/kg dose, whereas compound **8f** has an effect a dose of 20 mg/kg.

8. Experimental protocols

8.1. Chemistry

8.1.1. General remarks

Catalytic hydrogenation reactions were performed using a Roth Model IV apparatus. Melting points were determined on an Electrothermal 9100 apparatus with open capillary tubes and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400 analyzer and were within ±0.4% of the theoretical values. Infrared spectra (KBr) were recorded on a Shimadzu FTIR-8300 spectrometer. The ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AVANCE DMX 400WB instrument in $CDCl_3$ with TMS as an internal reference. Chemical shifts are expressed in δ units and coupling constants (J) are expressed in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), m (multiplet), p (pseudo-), and b (broad-). For the two-dimensional experiments, the pulse sequences, acquisition, and processing parameters were taken from the standard Bruker software library.

Flash column chromatography was carried out on Merck Silica gel 60 (230–400 mesh ASTM) using methylene chloride/methanol (99:1, 97:3, 95:5 *v*/*v*) as the eluent. Thin layer chromatography was run on Merck Silica gel 60 F_{254} plates with a mobile phase consisting of dioxane, toluene, ethanol, and 25% NH_4OH (6.0:3.2:0.5:0.2, *v*/*v*). Compounds were visualized by UV light (254 nm).

Crystals of 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21] suitable for X-ray analysis were grown by the slow evaporation of an EtOH solution. All details of the measurements, crystal data, and structure refinement are given in Table 1. The data were collected on an Oxford Diffraction KM4CCD diffractometer [43] at 293 K using graphite-monochromated MoK_α radiation. The unit cell parameters were determined by the least-squares treatment of setting angles at the highest-intensity reflections chosen from the whole experiment. Intensity data were corrected for the Lorentz and polarization effects [44]. The structure was solved by direct methods using the SHELXS97 program [45] and refined by the full-matrix least-squares method with the SHELXL97 program [46]. The function $\Sigma w(|F_o|^2 - |F_c|^2)^2$ was minimized with $w^{-1} = [\sigma^2(F_o)^2 + (0.0691P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$.

All non-hydrogen atoms were refined with anisotropic thermal parameters. The coordinates of the hydrogen atoms were calculated in idealized positions and refined as a riding model with their thermal parameters calculated as 1.2 times U_{eq} of the respective carrier carbon atom.

The deposition number CCDC 686643 for 4-(4-chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-*c*]pyrimidine-1,3-dione (**9a**) [21] contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

8.1.2. Preparation of 2-(4-bromobutyl)-4-aryl-pyrido[1,2-c] pyrimidine-1,3-diones (**5a**–**f**)

The starting compounds **2a**–**i**, **3a**–**i**, **4a**–**i**, and **5a**–**i** were obtained according to previously described procedures [27,28].

8.1.3. Preparation of 2-(4-bromobutyl)-4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-diones (**6a**–**i**)

The derivatives **5a**–**5i** (0.01 mol) were hydrogenated in 100 ml acetic acid with 0.1 g 10% Pt/C, and the catalytic reduction was carried out under 60 atmospheres of hydrogen pressure at 50 °C for 10 h. The catalyst was removed by filtration and the solvent by evaporation in vacuo. Compounds **6a**–**6i** were purified by column chromatography (chloroform:methanol, 97:3 *v*/*v*).

8.1.4. General procedure for the synthesis of 3-piperidin-4-yl-1H-indoles (**7a**, **7b**)

The starting compounds **7a** and **7b** were obtained according to previously described procedures [25].

8.1.5. General procedure for the synthesis of 2-methyl-3-piperidin-4-yl-1H-indole (**6c**)

The starting compound **7c** was obtained according to previously described procedures [47].

8.1.6. General procedure for the synthesis of 2-{4-[4-(1H-indol-3-yl)-piperidin-1-yl]-butyl}-4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-c] pyrimidine-1,3-diones (**8a**–**8v**)

Compounds **6a**–**6i** (0.0026 mol) and **7a**–**7c** (0.0026 mol) and K_2CO_3 (0.005 mol), 70 ml acetonitrile, and a catalytic amount of KI were stirred and refluxed for 4–5 h. Reaction time was monitored using TLC. After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The crude residue was purified by flash chromatography using CH_2Cl_2/MeOH (96:4 *v*/*v*) as an eluent. Proper fractions were identified by TLC and evaporated to dryness giving analytically pure compounds **8a**–**8v**.

8.2. ^{1}H NMR, ^{13}C NMR, IR and elementary analysis data

8.2.1. 2-(4-Bromobutyl)-4-(4-chloro-phenyl)-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**6a**)

The title compound was isolated as white powder (64%); mp 91–92 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: mp: 91–92 °C, 1.71 (q, $^3J = 6.8$, C7H$_2$), 1.83 (q, C2 × H$_2$), 1.93 (m, C6H$_2$, C3 × H$_2$), 2.52 (t, $^3J = 6.6$, C5H$_2$), 3.44 (t, $^3J = 6.6$, C4 × H$_2$), 3.94 (t, $^3J = 6.4$, C1 × H$_2$), 4.03 (t, $^3J = 7.0$, C8H$_2$), 7.14 (d, $^3J = 8.4$, C2′H, C6′H), 7.37 (d, C3′H, C5′H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.7 (C7), 21.9 (C6), 26.6 (C2×), 27.0 (C5), 30.4 (C3×), 33.4 (C4×), 41.0 (C8), 42.9 (C1×), 111.5 (C4), 128.9 (C3′, C5′), 131.9 (C1′), 132.4 (C2′, C6′), 134.0 (C4′), 150.3 (C4a), 151.8 (C1), 162.0 (C3).

8.2.2. 2-(4-Bromobutyl)-4-(4-fluoro-phenyl)-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**6b**)

The title compound was isolated as white powder (85%); mp 64.0–66.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71 (q, $^3J = 6.4$ Hz, 2H, C–6H$_2$), 1.84 (q, 2H, C2 × H$_2$), 1.93 (m, 4H, C–7H$_2$, C–3 × H$_2$), 2.52 (t, $^3J = 6.6$ Hz, 2H, C–5H$_2$), 3.44 (t, $^3J = 6.6$ Hz, 2H, C–4 × H$_2$), 3.94 (t, $^3J = 6.4$ Hz, 2H, C–8H$_2$), 4.03 (t, $^3J = 6.8$ Hz, 2H, C–1 × H$_2$), 7.09 (m, 2H, C–3′H, C–5′H), 7.17 (m, 2H, C–2′H, C–6′H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.7 (C-6), 21.9 (C-7), 26.6 (C-2×), 26.9 (C-5), 30.4 (C-3×), 33.4 (C-4×), 40.9 (C-1×), 42.9 (C-8), 111.5 (C-4), 115.7

(d*, ^2J = 21.4 Hz, C-3′, C-5′), 129.3 (d*, ^4J = 3.5 Hz, C-1′), 132.7 (d*, ^3J = 8.1 Hz, C-2′, C-6′), 150.2 (C4a), 151.8 (C-1), 162.1 (C-3), 162.4 (d*, ^1J = 246.9 Hz, C-4′). IR (KBr) *ν*: 1635 (C═O), 1695 (C═O). Anal. Calc. for $C_{18}H_{20}FN_2O_2Br$: C, 54.7; H 5.1; N 7.1. Found: C, 54.5; H, 5.1; N, 7.1.

*8.2.3. 4-(4-Chloro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c] pyrimidine-1,3-dione (**8a**)*

The title compound was isolated as white powder (67.5%); mp 131–132 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.71 (m, CbH_{ax}, CdH_{ax}), 1.93 (m, $C7H_2$, $C2 \times H_2$), 2.06 (pd, CbH_{eq}, CdH_{eq}), 2.22 (pt, CaH_{ax}, CeH_{ax}), 2.51 (m, $C5H_2$, $C4 \times H_2$), 2.86 (m, CcH), 3.13 (pd, CaH_{eq}, CeH_{eq}), 3.94 (t, ^3J = 6.4, $C8H_2$), 4.04 (t, ^3J = 6.8, $C1 \times H_2$), 6.96 (bs, C2″H), 7.10 (t, ^3J = 7.2, C5″H), 7.14 (d, ^3J = 8.4, C2′H, C6′H), 7.17 (t, ^3J = 7.2, C6″H), 7.37 (d, C3′H, C5′H), 7.63 (d, ^3J = 7.6, C4″H), 8.15 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.7 (C6), 21.9 (C7), 24.1 (C2×), 25.8 (C3×), 27.0 (C5), 32.6 (Cb, Cd), 33.4 (Cc), 41.6 (C1×), 42.9 (C8), 54.4 (Ca, Ce), 58.7 (C4×), 111.4 (C4, C7″), 119.2 (C5″), 119.3 (C4″), 120.0 (C2″), 121.1 (C3″), 126.8 (C3″a), 129.0 (C3′, C5′), 132.0 (C1′), 132.4 (C2′, C6′), 133.9 (C4′), 136.6 (C7″a), 150.2 (C4a), 151.8 (C1), 162.1 (C3). IR *ν*: 1635 (C═O), 1697 (C═O), Anal. Calcd. for $C_{31}H_{35}ClN_4O_2$: C, 70.1; H, 6.6; N, 10.6. Found: C, 69.8; H, 6.7; N, 10.6.

*8.2.4. 2-{4-[4-(1*H*-Indol-3-yl)-piperidin-1-yl]-butyl}-4-phenyl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8b**)*

The title compound was isolated as white powder (70%); mp 189–190 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.70 (m, CbH_{ax}, CdH_{ax}), 1.83 (q, $C2 \times H_2$), 1.91 (q, $C7H_2$), 2.03 (pd, CbH_{eq}, CdH_{eq}), 2.11 (pt, CaH_{ax}, CeH_{ax}), 2.44 (t, ^3J = 7.4, $C4 \times H_2$), 2.52 (t, ^3J = 6.4, $C5H_2$), 2.82 (m, CcH), 3.05 (pd, CaH_{eq}, CeH_{eq}), 3.93 (t, ^3J = 6.2, $C8H_2$), 4.05 (t, ^3J = 7.2, $C1 \times H_2$), 6.91 (bs, C2″H), 7.07 (t, ^3J = 7.6, C5″H), 7.15 (t, ^3J = 7.6, C6″H), 7.20 (d, ^3J = 7.2, C2′H, C6′H), 7.33 (pd, C4′H, C7″H), 7.39 (t, ^3J = 7.0, C3′H, C5′H), 7.63 (d, ^3J = 7.6, C4″H), 8.28 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.8 (C6), 22.0 (C7), 24.7 (C2×), 26.0 (C3×), 26.9 (C5), 33.2 (Cb, Cd), 33.7 (Cc), 41.8 (C1×), 42.9 (C8), 54.7 (Ca, Ce), 59.1 (C4×), 111.5 (C7″), 112.7 (C4), 119.1 (C5″), 119.3 (C4″), 120.0 (C2″), 121.5 (C3″), 121.9 (C6″), 126.9 (C3″a), 127.9 (C4′), 128.7 (C3′, C5′), 131.0 (C2′, C6′), 133.6 (C1′), 136.6 (C7″a), 149.9 (C4a), 152.0 (C1), 162.2 (C3). IR *ν*: 1663 (C═O), 1690 (C═O), Anal. Calcd. for $C_{31}H_{36}N_4O_2$: C, 75.0; H, 7.3; N, 11.3. Found: C, 74.6; H, 7.2; N, 11.1.

*8.2.5. 2-{4-[4-(5-Methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-*p*-tolyl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8c**)*

The title compound was isolated as white powder (74%); mp 205–207 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.68 (m, $C6H_2$, $C2 \times H_2$, $C3 \times H_2$), 1.87 (pt, CbH_{ax}, CdH_{ax}), 1.91 (q, $C7H_2$), 2.02 (pd, CbH_{eq}, CdH_{eq}), 2.15 (pt, CaH_{ax}, CeH_{ax}), 2.36 (s, CH_3), 2.47 (t, ^3J = 6.8, $C4 \times H_2$), 2.53 (t, ^3J = 6.6, $C5H_2$), 2.77 (m, CcH), 3.08 (pd, CaH_{eq}, CeH_{eq}), 3.86 (s, OCH_3), 3.93 (t, ^3J = 6.4, $C8H_2$), 4.05 (t, ^3J = 6.8, $C1 \times H_2$), 6.83 (dd, ^3J = 8.4, ^4J = 1.2, C6″H), 6.90 (s, C2″H), 7.07 (m, C3′H, C5′H, C4″H), 7.21 (m, C2′H, C6′H, C7″H), 8.20 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.8 (C6), 21.4 (CH_3), 21.9 (C7), 24.3 (C2×), 25.9 (C3×), 26.9 (C5), 32.8 (Cb, Cd), 33.5 (Cc), 41.6 (C1×), 42.8 (C8), 54.5 (Ca, Ce), 56.3 (OCH_3), 58.8 (C4×), 101.2 (C4″), 112.1 (C6″, C7″), 112.5 (C4), 120.9 (C2″, C3″), 127.1 (C3″a), 129.4 (C2′, C6′), 130.5 (C1′), 130.7 (C3′, C5′), 137.6 (C4′), 149.8 (C4a), 151.9 (C1), 153.9 (C5″), 162.3 (C3). IR *ν*: 16433 (C═O), 1693 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_3$: C, 73.3; H, 7.5; N, 10.4. Found: C, 73.0; H, 7.3; N, 10.4.

*8.2.6. 2-{4-[4-(1*H*-Indol-3-yl)-piperidin-1-yl]-butyl}-4-(4-methoxy-phenyl)-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8d**)*

The title compound was isolated as white powder (79.2%); mp 218–219 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.69 (m, $C6H_2$, $C2 \times H_2$, $C3 \times H_2$), 1.91 (m, $C7H_2$, CbH_{ax}, CdH_{ax}), 2.04 (pd, CbH_{eq}, CdH_{eq}), 2.15 (pt, CaH_{ax}, CeH_{ax}), 2.48 (t, $C4 \times H_2$), 2.54 (t, ^3J = 6.6, $C5H_2$), 2.83 (pt, CcH), 3.09 (pd, CaH_{eq}, CeH_{eq}), 3.81 (s, OCH_3), 3.93 (t, ^3J = 6.2, $C8H_2$), 4.05 (t, ^3J = 6.8, $C1 \times H_2$), 6.93 (d, C3′H, C5′H), 6.94 (s, C2″H), 7.06 (t, ^3J = 8.0, C6″H), 7.09 (t, ^3J = 8.0, C5″H), 7.12 (d, ^3J = 8.4, C2′H, C6′H), 7.34 (d, ^3J = 8.0, C7″H), 7.63 (d, ^3J = 8.0, C4″H), 8.19 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.8 (C6), 22.0 (C7), 24.4 (C2×), 25.9 (C3×), 26.9 (C5), 32.9 (Cb, Cd), 33.5 (Cc), 41.6 (C1×), 42.8 (C8), 54.5 (Ca, Ce), 58.8 (C4×), 111.4 (C7″), 112.2 (C4), 114.2 (C3′, C5′), 119.2 (C5″), 119.2 (C4″), 120.0 (C2″), 122.0 (C6″), 123.5 (C3″), 125.7 (C1′), 126.8 (C3″a), 132.0 (C2′, C6′), 136.5 (C7″a), 149.9 (C4a), 151.9 (C1), 159.2 (C4′), 162.4 (C3). IR *ν*: 1636 (C═O), 1693 (C═O), Anal. Calcd. for $C_{32}H_{38}N_4O_3$: C, 73.0; H, 7.3; N, 10.7. Found: C, 72.7; H, 7.3; N, 10.7.

*8.2.7. 2-{4-[4-(5-Methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-(4-methoxy-phenyl)-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8e**)*

The title compound was isolated as white powder (81.1%); mp 179–181 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.68 (m, $C6H_2$, $C2 \times H_2$, $C3 \times H_2$), 1.81 (pt, CbH_{ax}, CdH_{ax}), 1.90 (q, ^3J = 6.8, $C7H_2$), 2.02 (pd, CbH_{eq}, CdH_{eq}), 2.12 (pt, CaH_{ax}, CeH_{ax}), 2.45 (t, ^3J = 7.6, $C4 \times H_2$), 2.53 (t, ^3J = 6.8, $C5H_2$), 2.76 (tt, $^3J_{ax-ax}$ = 12.0, $^3J_{ax-eq}$ = 3.6, CcH), 3.06 (pd, CaH_{eq}, CeH_{eq}), 3.80 (s, OCH_3), 3.85 (s, $OC11H_3$), 3.92 (t, ^3J = 6.4, $C8H_2$), 4.05 (t, ^3J = 7.4, $C1 \times H_2$), 6.82 (dd, ^3J = 8.8, ^4J = 2.0, C6″H), 6.90 (d, ^3J = 2.0, C2″H), 6.92 (d, C3′H, C5′H), 7.05 (d, ^4J = 2.0, C4″H), 7.11 (d, ^3J = 8.8, C2′H, C6′H), 7.20 (d, ^3J = 8.8, C7″H), 8.3 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.8 (C6), 22.0 (C7), 24.6 (C2×), 26.0 (C3×), 27.0 (C5), 33.0 (Cb, Cd), 33.6 (Cc), 41.7 (C1×), 42.8 (C8), 54.6 (Ca, Ce), 55.5(C10), 56.3 (C11), 59.0 (C4×), 101.2 (C4″), 112.1 (C6″, C7″), 112.2 (C4), 114.2 (C3′, C5′), 121.0 (C2″), 121.1 (C3″), 125.7 (C3″a), 127.2 (C1′), 131.8 (C7″a), 132.1 (C2′, C6′), 149.9 (C4a), 151.9 (C1), 153.8 (C5″), 159.2 (C4′), 162.5 (C3). IR *ν*: 1636 (C═O), 1690 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_4$: C, 71.2; H, 7.2; N, 10.1. Found: C, 70.8; H, 7.2; N, 10.1.

*8.2.8. 4-(2-Fluoro-phenyl)-2-{4-[4-(5-methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8f**)*

The title compound was isolated as white powder (71%); mp 115–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.74 (m, $C6H_2$, $C2 \times H_2$, $C3 \times H_2$), 1.94 (q, $C7H_2$), 1.94 (bs, CbH_{eq}, CdH_{eq}), 2.37 (bs, CaH_{ax}, CeH_{ax}), 2.48 (pt, $C5H_2$), 2.67 (pt, $C4 \times H_2$), 2.82 (m, CcH), 3.22 (pd, CaH_{eq}, CeH_{eq}), 3.87 (s, OCH_3), 3.94 (m, $C8H_2$), 4.00 (pt, $C1 \times H_2$), 6.83 (dd, ^3J = 8.8, ^4J = 4.8, C6″H), 6.93 (d, ^3J = 4.0, C2″H), 7.05 (d, ^4J = 4.8, C4″H), 7.13 (pt, C3′H), 7.20 (m, C5′H, C6′H), 7.24 (m, C4′H), 7.24 (d, ^3J = 8.8, C7″H), 8.24 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 18.6 (C6), 21.9 (C7), 23.2 (C3×), 25.5 (C2×), 26.7 (C5), 31.7 (bs, Cb, Cd), 32.9 (Cc), 41.1 (C1×), 43.2 (C8), 54.0, 54.2 (Ca, Ce), 56.4 (OCH_3), 58.2 (C4×), 101.1 (C4″), 106.2 (C4), 112.2 (C7″), 112.2 (C6″), 116.0 (d*, ^2J = 22.3, C3′), 119.9 (C2″), 121.0 (d*, ^2J = 16.0, C1′), 121.1 (C3″), 124.5 (d*, ^4J = 3.4, C5′), 126.9 (C3″a), 130.3 (d*, ^3J = 8.2, C4′), 131.7 (C7″a), 133.1 (d*, ^3J = 2.7, C6′), 151.4 (C4a), 151.8 (C1), 154.0 (C5″), 160.6 (d*, ^1J = 16.0, C1′), 161.8 (C3). IR *ν*: 1651 (C═O), 1701 (C═O), Anal. Calcd. for $C_{31}H_{35}FN_4O_2$: C, 70.6; H, 6.9; N, 10.3. Found: C, 70.2; H, 6.9; N, 10.1.

*8.2.9. 4-(4-Chloro-phenyl)-2-{4-[4-(5-methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8g**)*

The title compound was isolated as white powder (79%); mp 199.0–200.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.71 (m, $C6H_2$, $C2 \times H_2$, $C3 \times H_2$), 1.93 (q, ^3J = 6.6, $C7H_2$), 2.04 (pd, CbH_{eq}, CdH_{eq}), 2.28 (bps, CaH_{ax}, CeH_{ax}), 2.52 (t, ^3J = 6.6, $C5H_2$), 2.59 (bps, $C4 \times H_2$), 2.82 (bps, CcH), 3.19 (pd, CaH_{eq}, CeH_{eq}), 3.87 (s, OCH_3), 3.94 (t, ^3J = 6.4, $C8H_2$), 4.05 (t, ^3J = 6.4, $C1 \times H_2$), 6.85 (dd, ^3J = 8.6, ^4J = 2.2, C6″H), 6.95 (bs, C2″H), 7.06 (d, C4″H), 7.15 (d, ^3J = 8.4, C2′H, C6′H),

7.25 (d, C7″H), 7.37 (d, C3′H, C5′H), 8.03 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.7 (C6), 21.9 (C7), 23.8 (C2×), 25.7 (C3×), 27.0 (C5), 32.3 (Cb, Cd), 33.3 (Cc), 41.5 (C1×), 43.0 (C8), 54.4 (Ca, Ce), 56.3 (OCH_3), 58.6 (C4×), 101.2 (C4″), 111.5 (C4), 112.1 (C7″), 112.3 (C6″), 121.0 (C2″, C3″), 127.1 (C3″a), 129.0 (C3′, C5′), 132.0 (C1′), 132.4 (C2′, C6′), 133.4 (C4′), 150.3 (C4a), 151.8 (C1), 154.0 (C5″), 162.1 (C3). IR *v*: 1636 (C═O), 1697 (C═O), Anal. Calcd. for $C_{32}H_{37}ClN_4O_3$: C, 68.5; H, 6.7; N, 10.0. Found: C, 68.4; H, 6.6; N, 10.0.

8.2.10. 2-{4-[4-(1H-Indol-3-yl)-piperidin-1-yl]-butyl}-4-p-tolyl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8h**)

The title compound was isolated as white powder (80%); mp 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70 (q, $^3J = 6.4$, C6H$_2$), 1.75 (pd, 6H, CbH$_{ax}$, CdH$_{ax}$), 1.92 (q, $^3J = 6.4$, C7H$_2$), 2.05 (bps, CbH$_{eq}$, CdH$_{eq}$), 2.32 (bpt, CaH$_{ax}$, CeH$_{ax}$), 2.36 (s, CH$_3$), 2.54 (t, $^3J = 6.4$, C5H$_2$), 2.64 (bps, C4 × H$_2$), 2.87 (m, CcH), 3.20 (bpd, CaH$_{eq}$, CeH$_{eq}$), 3.94 (t, $^3J = 6.4$, C8H$_2$), 4.05 (t, $^3J = 6.4$, C1 × H$_2$), 6.96 (bs, C2″H), 7.08 (d, C3′H, C5′H), 7.09 (t, C5″H), 7.17 (t, $^3J = 7.6$, C6″H), 7.21 (d, $^3J = 8.0$, C2′H, C6′H), 7.36 (d, $^3J = 8.0$, C7″H), 7.61 (d, $^3J = 7.6$, C4″H), 8.27 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.8 (C6), 21.5 (CH_3), 22.0 (C7), 26.2 (C3×), 26.6 (C2×), 27.0 (C5), 31.8 (Cc), 32.1 (Cb, Cd), 41.2 (C1×), 42.9 (C8), 55.0 (Ca, Ce), 58.0 (C4×), 111.5 (C7″), 112.5 (C4), 119.1 (C5″), 119.3 (C4″), 120.2 (C2″), 121.8 (C3″), 122.1 (C6″), 126.7 (C3″a), 129.5 (C2′, C6′), 130.5 (C1′), 130.7 (C3′, C5′), 136.5 (C7″a), 137.7 (C4′), 148.0 (C4a), 152.0 (C1), 162.4 (C3). IR *v*: 1639 (C═O), 1701 (C═O), Anal. Calcd. for $C_{32}H_{38}N_4O_2$: C, 75.3; H, 7.5; N, 11.0. Found: C, 74.8; H, 7.4; N, 10.6.

8.2.11. 4-(2-Fluoro-phenyl)-2-{4-[4-(1H-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8i**)

The title compound was isolated as white powder (73%); mp 106–108 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72 (m, C6H$_2$, C3 × H$_2$), 1.92 (q, C7H$_2$), 2.01 (m, 6H, CbH$_{eq}$, CdH$_{eq}$ C2 × H$_2$), 2.48 (m, C5H$_2$), 2.65 (t, $^3J = 7.4$, C4 × H$_2$), 2.84 (tt, CcH), 3.18 (m, CaH$_{ax}$, CeH$_{ax}$), 3.21 (pd, CaH$_{eq}$, CeH$_{eq}$), 3.93 (m, C8H$_2$), 4.04 (t, $^3J = 7.2$, C1 × H$_2$), 6.90 (bs, C2″H), 7.12 (pt, C3′H), 7.15 (C6″H), 7.20 (m, C5′H, C6′H), 7.22 (bs, NH), 7.34 (m, C4′H, C7″H), 7.59 (d, $^3J = 8.0$, C4″H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.5 (C6), 21.8 (C7), 22.9 (C2×), 25.5 (C3×), 26.6 (C5), 31.6 (Cb, Cd), 32.8 (Cc), 41.2 (C1×), 43.1 (C8), 53.6, 53.7 (Ca, Ce), 57.8 (C4×), 106.2 (C4), 111.5 (C7″), 115.9 (d*, $^2J = 22.2$, C3′), 119.0 (C4″), 120.0 (C2″), 120.3(C3″), 120.9 (d*, $^2J = 16.2$, C1′), 124.3 (d*, $^4J = 3.4$, C5′), 126.6 (C3″a), 130.2 (d*, $^3J = 8.1$, C4′), 133.0 (d*, $^3J = 2.7$, C6′), 136.6 (C7″a), 151.4 (C4a), 151.8 (C1), 160.6 (d*, $^1J = 247.9$, C2′), 161.7 (C3). IR *v*: 1651 (C═O), 1701 (C═O), Anal. Calcd. for $C_{31}H_{35}FN_4O_2$: C, 72.4; H, 6.9; N, 10.9. Found: C, 71.9; H, 6.7; N, 11.0.

8.2.12. 2-{4-[4-(1H-Indol-3-yl)-piperidin-1-yl]-butyl}-4-o-tolyl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8j**)

The title compound was isolated as white powder (5.2%); mp 128.0–130.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.74 (m, C2 × H$_2$, C3 × H$_2$), 1.94 (q, C7H$_2$), 2.03 (m, CbH$_2$, CdH$_2$), 2.14 (s, CH$_3$), 2.25 (pk CaH$_{ax}$, CeH$_{ax}$), 2.28 (m, C5(″)H), 2.42 (C5(′)H), 2.84 (m, CcH), 3.17 (pd, CaH$_{eq}$, CeH$_{eq}$), 3.94 (m, C8H$_2$), 4.06 (t, $^3J = 6.0$, C1 × H$_2$), 6.92 (s, C2″H), 7.05 (d, $^3J = 7.2$, C3′H), 7.09 (t, $^3J = 7.6$, C5″H), 7.16 (t, $^3J = 7.6$, C6″H), 7.18–7.30 (m, C4′H–C6′H), 7.35 (d, $^3J = 8.0$, C7″H), 7.60 (d, $^3J = 7.6$, C4″H), 8.31 (d, NH). IR *v*: 1635 (C═O), 1697 (C═O), Anal. Calcd. for $C_{32}H_{38}N_4O_2$: C, 75.3; H, 7.5; N, 11.0. Found: C, 74.9; H, 7.5; N, 11.1.

8.2.13. 2-{4-[4-(5-Methoxy-1H-indol-3-yl)-piperidin-1-yl]-butyl}-4-o-tolyl-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8k**)

The title compound was isolated as white powder (55%); mp 101.5–103.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71 (m, C2 × H$_2$, C3 × H$_2$), 1.90 (m, CdH$_{ax}$), 2.01 (pd, CbH$_{eq}$, CdH$_{eq}$), 2.13 (s, CH$_3$), 2.20 (pt, CaH$_{ax}$, CeH$_{ax}$), 2.27 (m, C5″H), 2.43 (m, C5′H), 2.53 (t, C4 × H$_2$), 2.77 (pt, CcH), 3.06 (pd, CaH$_{eq}$, CeH$_{eq}$), 3.85 (s, OCH_3), 3.92 (m, C8H$_2$), 4.06 (t, C1 × H$_2$), 6.82 (d, $^3J = 8.8$, C6″H), 6.88 (bs, C2″H), 7.04 (bps, C3′H, C4″H), 7.21 (d, $^3J = 8.4$, C7″H), 7.25 (m, C4′H, C6′H), 8.40 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.7 (C6), 19.8 (CH_3), 22.0 (C7), 23.9 (C2×), 25.8 (C3×), 26.7 (C5), 32.4 (Cb, Cd), 33.2 (Cc), 41.3 (C1×), 43.1 (C8), 54.3 (Ca, Ce), 56.2 (OCH_3), 58.6 (C4×), 101.0 (C4″), 111.8 (C4), 112.1 (C7″), 112.2 (C6″), 120.4 (C3″), 121.0 (C2″), 126.4 (C5′), 127.0 (C3″a), 128.4 (C4′), 130.4 (C3′), 130.9 (C6′), 131.7 (C7″a), 133.1 (C1′), 137.8 (C2′), 152.0 (C4a), 153.8 (C1), 161.7 (C3). IR *v*: 1635 (C═O), 1693 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_3$: C, 73.3; H, 7.5; N, 10.4. Found: C, 73.0; H, 7.4; N, 10.3.

8.2.14. 4-(4-Fluoro-phenyl)-2-{4-[4-(5-methoxy-1H-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8l**)

The title compound was isolated as white powder (54%); mp 162–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.63 (m, C2 × H$_2$, C3 × H$_2$), 1.70 (q, C6H$_2$), 1.80 (pt, CbH$_{ax}$, CdH$_{ax}$), 1.91 (q, $^3J = 6.4$, C7H$_2$), 2.02 (pd, CbH$_{eq}$, CdH$_{eq}$), 2.14 (pt, CaH$_{ax}$, CeH$_{ax}$), 2.46 (t, $^3J = 7.2$, C4 × H$_2$), 2.51 (t, $^3J = 6.8$, C5H$_2$), 2.78 (tt, $^3J_{ax-ax} = 11.6$, $^3J_{ax-eq} = 3.6$, CcH), 3.08 (pd, CaH$_{eq}$, CeH$_{eq}$), 3.86 (s, OCH_3), 3.93 (t, $^3J = 6.4$), 4.04 (t, $^3J = 7.2$, C1 × H$_2$), 6.84 (dd, $^3J = 8.6$, $^4J = 2.2$, C6″H), 7.05 (d, $^4J = 2.0$, C4″H), 7.16 (m, C2′H, C6′H), 7.22 (d, C7″H), 8.15 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18.7 (C6), 21.9 (C7), 24.5 (C2×), 25.9 (C3×), 26.9 (C5), 32.9 (Cb, Cd), 33.6 (Cc), 41.7 (C1×), 42.9 (C8), 54.6 (Ca, Ce), 56.3 (OCH_3), 58.9 (C4×), 101.2 (C4″), 111.6 (C4), 112.1 (C6″, C7″), 115.7 (d*, $^2J = 21.5$, C3′, C5′), 120.8 (C2″), 121.1 (C3″), 127.1 (C3″a), 129.4 (d*, $^4J = 3.4$, C1′), 131.7 (C7″a), 132.7 (d*, $^3J = 7.9$, C2′, C6′), 150.1 (C5″), 151.8 (C4a), 153.9 (C1), 162.2 (C3), 162.4 (d*, $^1J = 246.9$, C4′). IR *v*: 1645 (C═O), 1718 (C═O), Anal. Calcd. for $C_{32}H_{37}FN_4O_3$: C, 70.6; H, 6.9; N, 10.1. Found: C, 69.7; H, 6.8; N, 10.1.

8.2.15. 4-(2-Chloro-phenyl)-2-{4-[4-(1H-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8m**)

The title compound was isolated as white powder (60%); mp 92.4–93.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.72 (m, C6H$_2$, C2 × H$_2$, C3 × H$_2$), 1.91 (m, C7H$_2$, CbH$_{ax}$, CdH$_{ax}$), 2.04 (pd, CbH$_{eq}$, CdH$_{eq}$), 2.19 (pt, CaH$_{ax}$, CeH$_{ax}$), 2.40 (dt, C5H$_2$), 2.52 (t, $^3J = 6.8$, C4 × H$_2$), 2.83 (t, $^3J_{ax-ax} = 11.6$, $^3J_{ax-eq} = 3.6$, CcH), 3.1 (pd, CaH$_{eq}$, CeH$_{eq}$), 3.93 (m, $^2J = 13.8$, $^3J = 6.8$, C8H$_2$), 4.06 (t, $^3J = 7.0$, C1 × H$_2$), 6.94 (d, $^3J = 1.6$), 7.08 (t, $^3J = 7.2$, C5″H), 7.16 (t, $^3J = 7.4$, C6″H), 7.21 (m, C6′H), 7.30 9 (m, C4′H, C5′H), 7.34 (d, $^3J = 8.4$, C7″H), 7.45 (m, C3′H), 7.62 (d, $^3J = 8.0$, C4″H), 8.29 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 18,4 (C6), 21.8 (C7), 23.0 (C2×), 25.6 (C3×), 26.4 (C5), 33.1 (Cb, Cd), 33.2 (Cc), 40.0 (C1×), 55.0 (Ca, Ce), 58.3 (C4×), 110.0 (C7″), 111.2 (C4), 118.9 (C4″, C5″), 119.8 (C2″), 121.7 (C6″), 121.8 (C3″), 127.0 (C3″a), 127.1 (C5′), 129.5 (C4′), 129.7 (C4′), 132.4 (C1′), 132.5 (C6′), 135.0 (C2′), 136.3 (C7″a), 151.7 (C1), 161.2 (C3). IR *v*: 1632 (C═O), 1704 (C═O), Anal. Calcd. for $C_{31}H_{35}ClN_4O_2$: C, 70.1; H, 6.6; N, 10.6. Found: C, 69.4; H, 6.9; N, 10.5.

8.2.16. 4-(2-Fluoro-phenyl)-2-{4-[4-(2-methyl-1H-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-c]pyrimidine-1,3-dione (**8n**)

The title compound was isolated as white powder (48%); mp 192.2–194.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.74 (m, 8H, CbH$_{ax}$, CdH$_{ax}$), 1.94 (q, $^3J = 6.3$, C7H$_2$), 2.24 (bps, CbH$_{eq}$, CdH$_{eq}$), 2.33 (bps, CaH$_{ax}$, CeH$_{ax}$), 2.40 (m + s, C4 × H$_2$, CH$_3$), 2.49 (td, $^3J = 5.4$, $^4J = 2.1$), 2.77 (bps, CcH), 3.19 (bps, CaH$_{eq}$, CeH$_{eq}$), 3.93 (m, C8H$_2$), 4.05 (t, $^3J = 6.3$, C1 × H$_2$), 7.03 (t, C5″H), 7.10 (t, C5″H), 7.20 (m, C3′H), 7.23 (dd, $^3J = 6.0$, $^4J = 1.2$, C7″H), 7.35 (m, C4′H, C5′H, C6′H), 7.73 (d, $^3J = 7.5$, C4″H), 7.94 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 12.6 (CH_3), 18.6 (C6), 21.9 (C7), 25.8 (C2×), 26.7 (C3×, C5), 31.5 (Cb, Cd), 34.2 (Cc), 43.0 (C1×), 43.1 (C8), 54.8 (Ca, Ce), 58.8 (C4×), 106.3 (C4),

110.4 (C7″), 116.0 (d*, ^2J = 22.1, C3′), 119.1 (C3″), 119.4 (C4″, C5″), 120.9 (C6″), 121.0 (d*, ^2J = 17.7, C1′), 124.4 (d*, ^4J = 3.3, C5′), 127.6 (C3″a), 130.2 (d*, ^3J = 8.3, C4′), 130.3 (C7″a), 133.9 (d*, ^3J = 3.3, C6′), 151.4 (C4a), 151.8 (C1), 160.6 (d*, ^1J = 245.5, C2′), 161.7 (C3). IR *v*: 1634 (C═O), 1715 (C═O), Anal. Calcd. for $C_{32}H_{37}FN_4O_2$: C, 72.7; H, 7.1; N, 10.6. Found: C, 72.2; H, 7.0; N, 10.3.

*8.2.17. 2-{4-[4-(2-Methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-phenyl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8o**)*

The title compound was isolated as white powder (68%); mp 196.0–197.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.64 (pd, CbH_{ax}, CdH_{ax}), 1.72 (m, C2 × H_2, C6H_2, C3 × H_2), 1.93 (q, C7H_2), 2.04 (pt, CbH_{eq}, CdH_{eq}), 2.23 (pk, CaH_{ax}, CeH_{ax}), 2.40 (s, CH_3), 2.42 (t, C4 × H_2), 2.54 (t, ^3J = 6.6, C5H_2), 2.73 (m, CcH), 3.08 (pd, CaH_{eq}, CeH_{eq}), 3.95 (t, ^3J = 6.6, C8H_2), 4.05 (t, ^3J = 7.0, C1 × H_2), 7.02 (t, ^3J = 7.4, C5″H), 7.07 (t, ^3J = 7.2, C6″H), 7.21 (d, ^3J = 7.6, C2′H, C6′H), 7.24 (d, C7″H), 7.33 (t, ^3J = 7.2, C4′H), 7.40 (t, ^3J = 7.2, C3′H, C5′H), 7.71 (s, NH), 7.74 (d, C4″H). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 12.8 (CH_3), 18.9 (C6), 21.9 (C7), 25.7 (C2×), 26.9 (C3×, C5), 33.0 (Cb, Cd), 34.7 (Cc), 41.8 (C1×), 42.6 (C8), 54.3 (Ca, Ce), 57.1 (C4×), 110.5 (C7″), 111.2 (C4), 119.3 (C5″), 119.4 (C4″), 120.1 (C2″), 121.7 (C3″), 121.9 (C6″), 126.5 (C3″a), 127.9 (C4′), 128.7 (C3′, C5′), 131.1 (C2′, C6′), 133.9 (C1′), 136.6 (C7″a), 149.6 (C4a), 152.4 (C1), 162.9 (C3). IR *v*: 1648 (C═O), 1695 (C═O), Anal. Calcd. for $C_{32}H_{38}N_4O_2$: C, 75.3; H, 7.5; N, 11.0. Found: C, 74.8; H, 7.2; N, 10.7.

*8.2.18. 4-(2-Methoxy-phenyl)-2-{4-[4-(2-methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8p**)*

The title compound was isolated as white powder (41%); mp 189.7–191.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.76 (m, 8H, CbH_{ax}, CdH_{ax}), 1.92 (q, ^3J = 6.4, C7H_2), 2.00 (bps, CbH_{eq}, CdH_{eq}), 2.25 (pk, CaH_{ax}, CeH_{ax}), 2.39 (m + s, C4 × H_2, CH_3), 2.43 (t, ^3J = 6.8, C5H_2), 2.77 (bps, CcH), 3.20 (bps, CaH_{eq}, CeH_{eq}), 3.76 (s, OCH_3), 3.91 (t, C8H_2), 4.05 (t, ^3J = 6.0, C1 × H_2), 6.93 (d, ^3J = 8.4, C3′H), 6.99 (t, ^3J = 7.6, C5′H), 7.05 (td, ^3J = 7.2, ^4J = 0.9, C5″H), 7.10 (td, ^3J = 7.6, ^4J = 1.6, C6″H), 7.23 (dd, ^3J = 7.6, ^4J = 0.8, C6′H), 7.32 (m, C4′H, C7′H), 7.74 (d, ^3J = 6.8, C4″H), 7.99 (bs, NH). IR *v*: 1631 (C═O), 1693 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_3$: C, 73.3; H, 7.5; N, 10.4. Found: C, 73.0; H, 7.2; N, 10.2.

*8.2.19. 2-{4-[4-(2-Methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-p-tolyl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8q**)*

The title compound was isolated as white powder (41%); mp 163.5–166.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.61 (ps, CbH_{ax}, CdH_{ax}), 1.72 (m, C2 × H_2, C6H_2, C3 × H_2), 1.93 (q, ^3J = 6.4, C7H_2), 2.03 (pt, CbH_{eq}, CdH_{eq}), 2.22 (pk, CaH_{ax}, CeH_{ax}), 2.40 (t + s, C4 × H_2, CH_3), 2.55 (t, ^3J = 6.6, C5H_2), 2.73 (m, CcH), 3.08 (pd, CaH_{eq}, CeH_{eq}), 3.82 (s, OCH_3), 3.94 (t, ^3J = 6.4, C8H_2), 4.05 (t, ^3J = 7.2, C1 × H_2), 6.94 (d, ^3J = 8.8, C3′H, C5′H), 7.02 (t, ^3J = 7.2, C5″H), 7.07 (t, ^3J = 6.8, C6″H), 7.13 (d, C2′H, C6′H), 7.25 (d, C7″H), 7.72 (m, C4″H, NH). IR *v*: 1642 (C═O), 1697 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_2$: C, 75.5; H, 7.7; N, 10.7. Found: C, 75.3; H, 7.6; N, 10.8.

*8.2.20. 4-(4-Methoxy-phenyl)-2-{4-[4-(2-methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8r**)*

The title compound was isolated as white powder (72%); mp 180–182.3 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 1.58–1.78 (m, 8H, CbH_{ax}, CdH_{ax}), 1.89 (q, ^3J = 6.6, C7H_2), 2.02 (m, CaH_{eq}, CeH_{eq}), 2.21 (m, CaH_{ax}, CeH_{ax}), 2.34 (s, CH_3), 2.41 (t, ^3J = 7.8, C4 × H_2), 2.52 (t, ^3J = 6.8, C5H_2), 2.71 (tt, $^3J_{ax-ax}$ = 12.4, $^3J_{ax-eq}$ = 3.7, CcH), 3.79 (s, OCH_3), 3.92 (t, ^3J = 6.3, C8H_2), 4.06 (t, ^3J = 7.8, C4 × H_2), 6.91 (dt, C3′H, C5′H), 6.98 (m, C5″H), 7.03 (m, C6″H), 7.08 (d, ^3J = 8.1, C7″H), 7.11 (dt, ^3J = 8.8, ^4J = 1.9), 8.04 (bs, NH). ^{13}C NMR (500 MHz, $CDCl_3$) *δ*: 12.2 (CH_3), 18.6 (C6), 21.8 (C7), 24.3 (C2×), 25.6 (C3×), 26.7 (C5), 32.2 (Cb, Cd), 34.8 (Cc), 41.6 (C1×), 42.6 (C8), 55.0 (Ca, Ce), 55.3 (OCH_3), 59.0 (C4×), 110.3 (C4), 112.0 (C7″), 114.0 (C3′, C5′), 115.3 (C3″), 118.6 (C5″), 119.3 (C4″), 120.4 (C6″), 125.5 (C1′), 127.6 (C3″a), 130.0 (C7″a), 131.9 (C2′, C6′), 149.7 (C4a), 151.7 (C1), 159.0 (C4′), 162.2 (C3). IR *v*: 1636 (C═O), 1690 (C═O), Anal. Calcd. for $C_{33}H_{40}N_4O_3$: C, 73.3; H, 7.5; N, 10.4. Found: C, 72.7; H, 7.4; N, 10.3.

*8.2.21. 4-(4-Chloro-phenyl)-2-{4-[4-(2-methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8s**)*

The title compound was isolated as white powder (43%); mp 173.5–174.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 1.62 (m, C3 × H_2), 1.68–1.77 (m, 6H, CbH_{ax}, CdH_{ax}), 1.92 (q, ^3J = 6.6, C7H_2), 2.03 (m, CbH_{eq}, CdH_{eq}), 2.22 (m, CaH_{ax}, CeH_{ax}), 2.38 (s, CH_3), 2.42 (t, ^3J = 7.8, C4 × H_2), 2.51 (t, ^3J = 6.6, C5H_2), 2.72 (tt, $^3J_{ax-ax}$ = 12.4, $^3J_{ax-eq}$ = 3.7, CcH), 3.07 (pd, CaH_{eq}, CeH_{eq}), 3.94 (t, C8H_2), 4.05 (t, ^3J = 7.1, C1 × H_2), 7.00 (m, C5″H), 7.06 (td, ^3J = 7.1, ^4J = 1.0, C6″H), 7.15 (dt, C3′H, C5′H), 7.23 (d, ^3J = 8.1, C7″H), 7.37 (dt, ^3J = 8.5, ^4J = 2.4, C2′H, C6′H), 7.70 (d, ^3J = 7.8, C4″H), 7.77 (bs, NH). ^{13}C NMR (500 MHz, $CDCl_3$) *δ*: 12.4 (CH_3), 18.6 (C6), 21.7 (C7), 24.6 (C2×), 25.9 (C3×), 26.7 (C5), 32.2 (Cb, Cd), 34.8 (Cc), 41.7 (C1×), 42.7 (C8), 55.0 (Ca, Ce), 59.0 (C4×), 110.2 (C4), 111.3 (C7″), 115.5 (C3″), 118.8 (C5″), 119.4 (C4″), 120.6 (C6″), 127.6 (C3″a), 128.7 (C3′, C5′), 129.85 (C7″a), 131.9 (C1′), 132.2 (C2′, C6′), 133.7 (C4′), 149.9 (C4a), 151.6 (C1), 161.8 (C3). IR *v*: 1643 (C═O), 1703 (C═O), Anal. Calcd. for $C_{32}H_{37}ClN_4O_2$: C, 72.7; H, 7.0; N, 10.6. Found: C, 72.0; H, 6.8; N, 10.7.

*8.2.22. 4-(2-Chloro-phenyl)-2-{4-[4-(2-methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8t**)*

The title compound was isolated as white powder (67%); mp 170.0–173.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.71 (m, 8H, CbH_{ax}, CdH_{ax}), 1.95 (q, ^3J = 6.3, C7H_2), 2.06 (bps, CbH_{eq}, CdH_{eq}), 2.25 (pk, CaH_{ax}, CeH_{ax}), 2.41 (m + s, C4 × H_2, CH_3), 2.43 (td, ^3J = 6.6, ^4J = 1.8, C5H_2), 2.75 (tt, CcH), 3.00 (pd, CaH_{eq}, CeH_{eq}), 3.96 (m, C8H_2), 4.08 (t, ^3J = 7.2, C1 × H_2), 7.03 (t, ^3J = 6.9, C5″H), 7.09 (t, ^3J = 6.3, C6″H), 7.22 (d, C6′H), 7.24 (d, C7″H), 7.32 (m, C4′H, C5′H),7.48 (dd, C3′H), 7.73 (d, ^3J = 7.5, C4″H), 7.80 (bs, NH). ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 12.6 (CH_3), 18.7 (C6), 22.0 (C7), 24.6 (C2×), 26.0 (C3×), 26.6 (C5), 32.3 (Cb, Cd), 35.0 (Cc), 41.7 (C1×), 43.2 (C8), 55.1 (Ca, Ce), 59.1 (C4×), 110.3 (C4), 110.4 (C7″), 115.7 (C3″), 118.9 (C5″), 119.6 (C4″), 120.8 (C6″), 127.3 (C5′), 127.8 (C3″a), 129.7 (C4′), 129.9 (C3′), 130.1 (C7″a), 132.7 (C6′), 132.8 (C1′), 135.3 (C2′), 135.4 (C2″), 150.6 (C4a), 152.0 (C1), 161.4 (C3). IR *v*: 1639 (C═O), 1695 (C═O), Anal. Calcd. for $C_{32}H_{37}ClN_4O_2$: C, 70.5; H, 6.8; N, 10.3. Found: C, 71.0; H, 6.5; N, 10.0.

*8.2.23. 4-(4-Fluoro-phenyl)-2-{4-[4-(2-methyl-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidine-1,3-dione (**8u**)*

The title compound was isolated as white powder (53%); mp 199.3–200.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.71 (m, 8H, CbH_{ax}, CdH_{ax}), 1.94 (q, ^3J = 6.3, C7H_2), 2.04 (pt, CbH_{eq}, CdH_{eq}), 2.23 (pk, CaH_{ax}, CeH_{ax}), 2.39 (s, CH_3), 2.43 (t, C4 × H_2), 2.52 (t, ^3J = 6.9, C5H_2), 2.73 (tt, CcH), 3.09 (pd, CaH_{eq}, CeH_{eq}), 3.95 (t, ^3J = 6.3, C8H_2), 4.06 (t, ^3J = 6.9, C1 × H_2), 7.02 (t, ^3J = 7.2, C5″H), 7.08 (t, C6″H), 7.09 (d, C3′H, C5′H), 7.18 (d, C2′H, C6′H), 7.24 (d, ^3J = 8.1, C7″H), 7.74 (d, ^3J = 7.5, C4″H), 7.79 (bs, NH), ^{13}C NMR (400 MHz, $CDCl_3$) *δ*: 12.6 (CH_3), 18.8 (C6), 21.9 (C7), 24.8 (C2×), 26.0 (C3×), 27.0 (C5), 32.3 (Cb, Cd), 35.0 (Cc), 41.9 (C1×), 42.9 (C8), 55.2 (Ca, Ce), 59.1 (C4×), 110.4 (C4), 111.6 (C7″), 115.7 (C3″), 115.7 (d*, ^2J = 21.6, C3′, C5′), 118.9 (C5″), 119.6 (C4″), 120.8 (C6″), 127.8 (C3″a), 129.4 (d*, ^4J = 3.9, C1′), 130.1 (C7″a), 132.7 (d*, ^3J = 8.3, C2′, C6′), 135.4 (C2″), 150.1 (C4a), 151.9 (C1), 162.2 (C3) 162.4 (d*, ^1J = 246.6, C4′). IR *v*: 1635 (C═O), 1692 (C═O), Anal.

Calcd. for $C_{32}H_{37}FN_4O_2$: C, 72.7; H, 7.0; N, 10.6. Found: C, 72.6; H, 6.8; N, 10.3.

8.3. Pharmacology

8.3.1. In vitro experiments

8.3.1.1. 5-HT_{1A} binding assay. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)tetraline ([^{3}H]-8-OH−DPAT, spec. act. 106 Ci/mmol, NEN Chemicals) was used for labeling 5-HT_{1A} receptors. The membrane preparation and assay procedure were carried out according to a previously described procedure [48] with slight modifications. In brief, the hippocampus tissue was homogenized in 20 volumes of 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) using Ultra-Turrax® T 25 and then centrifuged at 32 000*g* for 10 min. The supernatant fraction was discarded, the pellet resuspended in the same volume of Tris–HCl buffer, and the solution centrifuged again. Before the third centrifugation, the samples were incubated at 37 °C for 10 min. The final pellet was resuspended in Tris–HCl buffer containing 10 μM pargyline, 4 mM $CaCl_2$, and 0.1% ascorbic acid. Samples containing 1 ml of the tissue suspension (5 mg wet weight), 100 μl of 10 μM serotonin for non-specific binding, 100 μl of [^{3}H]8-OH-DPAT, and 100 μl of analyzed compound were incubated at 37 °C for 15 min. The incubation was followed by rapid vacuum filtration through Whatman GF/B glass filters, and the remainder was washed three times with 5 ml cold buffer (50 mM Tris–HCl, pH 7.7) using a Brandel cell harvester. The final [^{3}H]8-OH-DPAT concentration was 1 nM, and the concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-4} M.

8.3.1.2. SERT binding assay. The assay was performed according to the method of Owens et al. [49] with slight modifications. Rat cerebral cortex was homogenized in 30 volumes of ice-cold 50 mM Tris–HCl buffer containing 150 mM NaCl and 5 mM KCl (pH 7.7, 25 °C) and centrifuged at 20 000*g* for 20 min. The supernatant was decanted and the pellet resuspended in 30 volumes of buffer and centrifuged again. The resulting pellet was resuspended in the same volume of buffer and centrifuged a third time in the same way as above. [^{3}H]-citalopram (spec. act. 50 Ci/mmol, NEN Chemicals) was used for labeling the 5-HT-transporter. Samples consisting of the tissue suspension (240 μl), 30 μl of 1 μM imipramine (displacer), 30 μl of 1 nM [^{3}H]-citalopram, and 100 μl of the analyzed compound were incubated at 22 °C for 1 h. The concentrations of the analyzed compounds ranged from 10^{-10} to 10^{-4} M. Incubations were terminated by vacuum filtration through Whatman GF/B filters and washed two times with 100 μl ice-cold buffer. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter. All assays were performed in duplicates. Radioligand binding data were analyzed using iterative curve-fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). The K_i values were calculated from the Cheng and Prusoff equation [30].

$$K_i = \frac{IC_{50}}{1 + \frac{L_O}{K_D}}$$

L_O = labeled ligand concentration.
K_D = dissociation constant of labeled ligand.

8.3.2. In vivo experiments

8.3.2.1. Induced hypothermia in mice

8.3.2.1.1. Animals and housing. The experiments were performed in male Albino Swiss mice (24–28 g; purchased from licensed dealer Staniszewska, Ilkowice, Poland). The animals were housed under standard laboratory conditions in a room maintained at a temperature of 20 ± 1 °C with a natural (January to February) light/dark cycle. Animals had free access to food and water before the experiment. Each experimental group consisted of 7–8 animals/dose, and all the animals were used only once.

8.3.2.1.2. Drug treatment. 8-Hydroxy-2-(di-*n*-propylamino)-tetraline hydrobromide (8-OH-DPAT, Research Biochemical Inc.) and N-{2-[4-(2-methoxy-phenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Cracow, Poland) were used as aqueous solutions and injected subcutaneously (sc). Compounds **8c**, **8d**, **8e**, **8f**, **8h**, and **8i** were suspended in a 1% aqueous solution of Tween 80 and given intraperitoneally (ip) at a dose of 10 mg/kg. The data were analyzed by Dunnett's test when only one drug was given or by the Newman–Keuls test when two drugs were administered.

8.3.2.1.3. Effect of investigated compounds on body temperature in mice. The effects of the tested compounds on the rectal body temperature in mice when given alone were measured with an Ellab thermometer 30, 60, 90, and 120 min after their administration. In an independent experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **8c**, **8e**, **8f**, **8h**, and **8i** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before **8c**, **8e**, **8f**, **8h**, **8i**, or 8-OH-DPAT and the rectal body temperature recorded 30 min and 60 min after the injection of the investigated compounds. In another experiment, the effect of **8d,** which did not change the body temperature in mice, on the hypothermia induced by 8-OH-DPAT (5 mg/kg) was tested. Compound **8d** was administered 45 min before 8-OH-DPAT, and the rectal body temperature was measured 15, 30, 45, and 60 min after the injection of 8-OH-DPAT. The results were expressed as a change in body temperature (Δt) compared to basal body temperature measured at the beginning of the experiment.

8.3.2.2. Forced swimming test in mice

8.3.2.2.1. Animals and housing. The experiments were performed in male Albino Swiss mice (24–28 g; purchased from licensed dealer Staniszewska, Ilkowice, Poland). The animals were kept in groups of twenty per cage (dimensions: 60 × 38 × 20 cm) at a temperature of 20 ± 1 °C and had free access to food (standard laboratory pellets) and water before the experiments. All investigations were conducted in the light phase of a natural light/dark cycle (January to February) between 9 AM and 2 PM. Each experimental group consisted of 9–10 animals per drug dose. Animals were tested in a counter-balanced order and were used in only one experiment. The experiments were conducted by an investigator blinded to treatment. All of the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences, Cracow.

8.3.2.2.2. Drug treatment. The following drugs were used: 8-OH-DPAT (Research Biochemical Inc.) and compounds **8c**, **8f**, **8h**, and **8i**. All compounds were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT was injected subcutaneously (sc), and **8c**, **8f**, **8h**, and **8i** were given intraperitoneally (ip) 30 min before the test at a dose of 10 ml/kg.

8.3.2.2.3. Effect of the investigated compounds on forced swimming test results. The experiment was carried out according to the method of Porsolt et al. [31]. In brief, the mice were placed individually in a glass cylinder (25 cm high, 10 cm in diameter, filled with water to a height of 6 cm) maintained at 23–25 °C and were left inside for 6 min. A mouse was regarded as immobile when it remained floating on the water, making only small movements to keep its head above. The total immobility time was measured by an experimenter during the final 4 min of a 6-min test session after a 2-min habituation period.

8.3.2.2.4. Locomotor activity in mice. The spontaneous locomotor activity of mice was recorded for compounds **8c**, **8f**, **8h**, and

8i in photoresistor actometers (24 cm in diameter, illuminated by two light beams) connected to a counter for the recording of light beam interruptions. The mice were placed individually in the actometers, and the number of light beam crossings was counted twice: first during the first 6-min period, i.e. the time equal to the observation period in the forced swimming test, and during a 30-min experimental session.

8.3.2.2.5. Statistical analysis. The obtained data were presented as the mean ± S.E.M. The statistical significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) followed by comparisons using Dunnett's test. A p value <0.05 was accepted as significant.

8.3.2.2.6. Molecular docking. The population of one hundred 5-HT_{1A}R models described previously was reused in the present work [32]. The models were constructed on the basis of a slightly modified bovine rhodopsin template. All of the docking experiments were conducted using the FlexX software with the FlexX-Pharm extension. Pharmacophore constraints were applied: H-bond acceptor at the carboxylic oxygen of Asp3.32, CH-π edge-to-face interaction with Phe6.52, and H-bond donor at the hydroxylic group of Ser5.43. The results were rescored with four additional scoring functions and subjected to a consensus scoring procedure as implemented in SYBYL. The highest PMF-scored solutions out of those having a consensus score of 5 were considered representative. The PMF score was used for the final scoring of the docking solutions because it was proven to give the best enrichment factors in virtual screening experiments [50].

The ligands were docked to the SERT model using a flexible Monte Carlo docking procedure as implemented in the ICM program [35]. The functionally important residue for ligand anchoring was $Asp98_{1.45}$ in TMH1. The ligand molecules were fully flexible and the protein was represented by grid interaction potentials. The geometrically different and low-energy conformations of the ligand in the complex ligand-serotonin transporter accumulated in the conformational stack. For each ligand, the best pose was selected using a visual inspection of the interactions with amino acid residues inside the binding site and the scoring functions implemented in the Xscore program [51].

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