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Original article

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

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ABSTRACT

A series of new derivatives of 4-aryl-pyrido[1,2-*c*]pyrimidine containing the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy derivative were synthesized. They were characterized (*i*) in vitro by binding to 5-HT$_{1A}$ receptors and 5-HT transporter proteins in rat brain cortex membranes and (*ii*) in vivo in the mouse by induced hypothermia and forced swimming models for antagonist/agonist activity against the 5-HT$_{1A}$ autoreceptors and postsynaptic 5-HT$_{1A}$ receptors, respectively. Structure activity relationship evaluation indicated that the presence of the 3-(4-piperidyl)-1*H*-indole residue and *ortho*- or *para*-substituents with –F or –CH_3 groups in the aryl ring as well as an unsubstituted aryl in the 4-aryl-pyrido[1,2-*c*]pyrimidine moiety promoted low K_i values for both receptors. In contrast, the presence of a 5-methoxy-3-(4-piperidyl)-1*H*-indole residue as well as –Cl or –OCH_3 substituents at the *para* position markedly reduced the receptor affinity.

1. Introduction

Approximately 20% of the adult population suffers from depression, a widespread affective psychiatric disease. According to the monoamine theory, the pathomechanism of this disease is connected with disturbances in serotoninergic transmission. Correction of these disturbances has led to the design of new compounds with antidepressant activity [1].

First generation drugs, tricyclic antidepressants (TCA) and nonselective inhibitors of monoaminooxidase (MAO-I), exerted several adverse drug reactions (ADR) because of low receptor selectivity. Second generation antidepressants, including mainly selective serotonin reuptake inhibitors (SSRI), produced markedly less ADR than TCA or nonselective MAO-I. However, newer antidepressants exhibit relatively long onset of action (2–6 weeks) as well as lower clinical effectiveness (on average only 70% of patients respond positively to these drugs) [2,3].

Long latency of antidepressive drugs is related to the desensitization of somatodendritic serotoninergic autoreceptors. An initial increase in serotonin concentration, after administration of the drug, leads to attenuation of 5-HT neurotransmission due to recurrent inhibition (negative feedback). After desensitization of autoreceptors (2–6 weeks after administration), release of 5-HT from presynaptic terminals normalizes [4,5].

Artigas et al. found that latency can be shortened after coadministration of SSRI with antagonist of 5-HT$_{1A}$ autoreceptor, e.g. fluoxetine, paroxetine or fluvoxamine and pindolol, citalopram, fluoxetine or fluvoxamine and WAY 100635 [6–8]. Results of these investigations led to attempts to synthesize a molecule consisting of two parts: one inhibiting 5-HT reuptake, and the second blocking presynaptic 5-HT$_{1A}$ receptor. This innovation may result in obtaining new antidepressant drugs with a double mechanism of action, characterized by better clinical parameters than drugs previously used.

Several compounds with double pharmacological properties have been described [9–11]. Introduction of a moiety with 5-HT$_{1A}$ antagonistic properties into the basic structure of the SSRI seemed justified, although a component with unselective antagonistic action on both pre- and postsynaptic receptors may abolish the advantageous effects of presynaptic inhibition [12].

Another possibility is the synthesis of compounds with dual activity consisting of a component with agonistic action toward the 5-HT$_{1A}$ receptor and a residue inhibiting the reuptake of 5-HT. Presynaptic agonistic effects led to faster desensitization of the autoreceptor which, in turn, shortened the latency period, whereas postsynaptic agonistic activity may increase neurotransmission in

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serotoninergic systems [13]. Several compounds possessing such properties have been described (vilazodone, VN2222) (Fig. 1) [12,14,15]. Promising results were obtained with vilazodone which recently entered phase III of clinical trials.

The aim of this study was to synthesize and evaluate the biological properties of new compounds with double activity: agonism to 5-HT_{1A} receptor and inhibition of 5-HT reuptake. New ligands were obtained after modification of the design of the previously acquired pyrido[1,2-*c*]pyrimidine derivatives belonging to the long chain piperazine (LCP) group with high affinity to 5-HT_{1A} receptors [16]. Modifications were performed in the pharmacophore part of the molecule by replacing the arylpiperazine residue with 3-(4-piperidyl)-1*H*-indole group or its 5-methoxy derivative, resulting in the formation of agents inhibiting the serotonin transporter protein (5-HT-T) and possessing high affinity to 5-HT_{1A} receptors [17–19]. Biological investigations of the newly synthesized compounds (**7a**,**b**–**12a**,**b**) included determination of the binding affinity to 5-HT_{1A} receptor as well as to serotonin transporter (5-HT-T). Compounds **7a**–**12a** were also investigated in vivo using a hypothermic test in mice, while compounds **8a**, **10a**, **11a** and **12a** were investigated by means of a forced swimming test in mice.

2. Chemistry

The respective nitriles (**2a**–**2f**) were synthesized by the reaction of *C*-arylation of appropriate arylacetonitriles (**1a**–**1f**) with 2-bromopyridine in an aprotic polar solvent [20,21] (Fig. 2). Next, the nitriles (**2a**–**2f**) were hydrolyzed using a mixture of sulfuric acid and acetic acid to obtain the amides (**3a**–**3f**) in good yields. Compounds **4a**–**4f** were formed by the intermolecular cyclization of **3a**–**3f** with diethyl carbonate. The imide group of compounds **4a**–**4f** was then *N*-alkylated by 1,4-dibromobutane to yield bromobutyl derivatives **5a**–**5f**. The final new target compounds (**7a**,**b**–**12a**,**b**) were obtained by the condensation of bromobutyl derivatives **5a**–**5f** with the appropriate piperidyl-indole (**6a**, **6b**) synthesized according to the method previously described [19].

3. Structural investigation

All new compounds (**7a**,**b**–**12a**,**b**) were characterized by physical constants, elemental analysis, IR, ^{1}H, and ^{13}C NMR spectroscopy (see Section 7). The structures of these compounds were elucidated from their analytical and spectroscopic data. The NMR spectra were mutually correlated and were in agreement with the literature data for similar systems [16,20], as well as with the theoretical spectra calculated according to ACD/NMR Predictor v. 8.09 program.

4. Pharmacology

Target compounds **7a**,**b**–**12a**,**b** were assessed for in vitro affinity for 5-HT_{1A} and 5-HT-T receptors by radioligand binding assay, using [^{3}H]8-OH-DPAT and [^{3}H]citalopram, respectively, in rat brain tissues. Data were analyzed using iterative curve-fitting routines to obtain IC_{50} values (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA), which in turn were used to calculate inhibition constant K_i according to the Cheng–Prusoff formula (see Table 1) [22].

The final compounds were further evaluated in mice for their agonist/antagonist properties toward presynaptic and postsynaptic 5-HT_{1A} receptors, using the inducible hypothermia and forced swimming tests, respectively.

In the inducible hypothermia test, the effects of administration of 6 compounds (**7a**–**12a**) were recorded as well as the effect of WAY 100635 (5-HT_{1A} receptor antagonist) on the hypothermia induced by the tested compounds. The results were expressed as a change in body temperature (Δt) with respect to 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. [23]. The effect of selected compounds on spontaneous locomotor activity in mice was also recorded. The obtained data were presented as the mean ± SEM. Comparisons between groups were carried out by a one-way analysis of variance (ANOVA) followed by intergroup comparisons using the Dunnett's or Newman–Keuls test.

5. Results and discussion

Several new derivatives of 4-aryl-pyrido[1,2-*c*]pyrimidine containing the 3-(4-piperidyl)-1*H*-indole residue or its 5-methoxy counterpart were obtained. In the 4-aryl-pyrido[1,2-*c*]pyrimidine system, aryl substituent in compounds **12a** and **12b** consisted of a phenyl ring, whereas compounds **11a** and **11b** also possessed –F at *ortho* position and the remaining compounds had $–OCH_3$ (**7a**, **7b**), $–CH_3$ (**8a**, **8b**), –Cl (**9a**, **9b**) or –F (**10a**, **10b**) located at the *para* position.

Binding values of compounds **7a**,**b**–**12a**,**b** toward the 5-HT_{1A} receptor as well as to the 5-HT transporter were analyzed by investigating the influence of substituents bound to the 3-(4-piperidyl)-1*H*-indole moiety (–H or $–OCH_3$ substituents at position 5) as well as substitutions in the aryl ring of the pyrido[1,2-*c*]pyrimidine at the *ortho* and *para* positions (Table 1).

Compounds **11a**, **8a**, **10a**, and **12a** exhibited high affinity toward the 5-HT_{1A} receptors, with K_i values varying from 4.8 to 10.9 nM. The remaining compounds possessed high to moderate binding activity with K_i ranging from 16.7 to 78.1 nM (compounds **11b**, **10b**, **9a**, **9b**, **7a**, **12b**, **8b** and **7b** according to the increasing K_i values) (Table 1).

An analysis of the effect of substituents at the indole moiety on K_i values revealed that compounds with the 3-(4-piperidyl)-1*H*-indole group had higher affinity for the 5-HT_{1A} receptor than the analogous 5-methoxy-3-(4-piperidyl)-1*H*-indole derivatives. Substitution on the aryl ring in 4-aryl-pyrido[1,2-*c*]pyrimidine with fluorine at both the *para* (**10a**, **10b**) and *ortho* positions (**11a**, **11b**) as well as $–CH_3$ at the *para* position (**8a**, **8b**) or a lack of substitution (**12a**, **12b**) resulted in markedly higher binding values than with the remaining ligands, i.e. derivatives with –Cl (**9a**, **9b**) and $–OCH_3$ (**7a**, **7b**) at the *para* position.

Compounds **8a** and **11a** showed the best binding affinity for both the serotonin protein transporter (K_i values less than 1.0 nM, Table 1) and for the 5-HT_{1A} receptor (K_i values ~ 5 nM). Compound

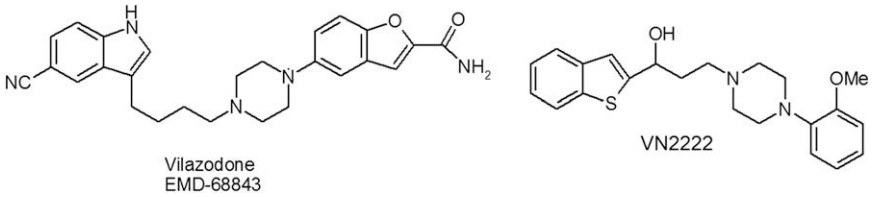

Fig. 1. Compounds with double action (SSRI plus 5-HT_{1A} agonist).

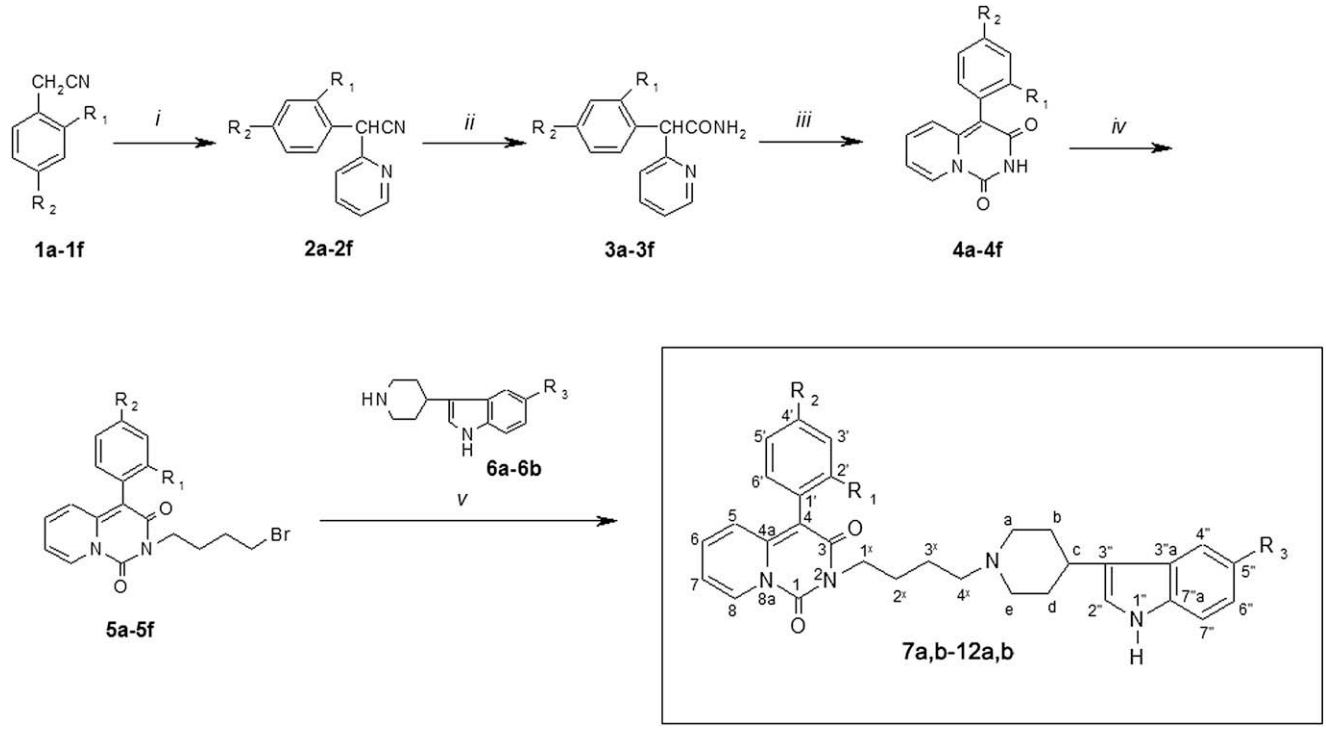

Fig. 2. Synthetic scheme for novel compounds **7a**–**12a** and **7b**–**12b**. Reagents: (*i*) 2-bromopyridine, KOH, DMSO, Δ; (*ii*) H_2SO_4, CH_3COOH, Δ; (*iii*) diethyl carbonate, EtONa, EtOH, Δ; (*iv*) 1,4-dibromobutane, K_2CO_3, acetone, Δ; (*v*) **6a**, **6b**, acetonitrile, K_2CO_3, KI, Δ. Numbering system of **7**–**12** for NMR analysis. R_1, R_2, R_3: **7a** H, OCH_3, H; **8a** H, CH_3, H; **9a** H, Cl, H; **10a** H, F, H; **11a** F, H, H; **12a** H, H, H; **7b** H, OCH_3, OCH_3; **8b** H, CH_3, OCH_3; **9b** H, Cl, OCH_3; **10b** H, F, OCH_3; **11b** F, H, OCH_3; **12b** H, H, OCH_3.

10a also showed high levels of binding level whereas the binding affinity of the remaining compounds to 5-HT transporter was moderate to low. For the remaining compounds **12a**, **10b**, **11b**, **12b**, **7b**, **7a**, **9b** and **9a**, values of K_i lay between 54 and 280 nM (Table 1).

Evaluation of substitution in the indole moiety indicated that some non-substituted 3-(4-piperidyl)-1*H*-indole derivatives possessed significantly lower K_i values toward the serotonin transporter protein than toward the 5-methoxy-3-(4-piperidyl)-1*H*-indole analogues (compounds: **8a** vs. **8b**, **10a** vs. **10b**, **11a** vs. **11b**, **12a** vs. **12b**), while other compounds showed somewhat higher K_i values (i.e. lower affinity) than the 5-methoxy analogues (compounds **7a** vs. **7b**, **9a** vs. **9b**). A comparison of the substituents on the aryl ring of the pyrido[1,2-*c*]pyrimidine moiety indicated that compounds with the highest affinity for the 5-HT transporter had a fluorine atom located at either the *ortho* (**11a**, **11b**) or *para* (**10a**, **10b**) positions, $-OCH_3$ or $-CH_3$ substituent at *para* (**7a**, **7b**, **8a**) position, or carried no substituents on the phenyl ring (**12a**, **12b**).

Since radioligand binding technique does not distinguish agonist/antagonist properties, we employed behavioral tests to resolve this issue. Based on these results obtained in vitro, compounds **7a**–**12a** were selected for further in vivo experiments because they had the most promising binding affinity for both the 5-HT_{1A} receptor and 5-HT transporter.

Compounds **7a**–**12a** were tested in in vivo models commonly used for evaluating functional 5-HT_{1A} receptor activity. Compound 8-OH-DPAT, a 5-HT_{1A} receptor agonist, induces hypothermia in mice; it is an effect mediated through 5-HT_{1A} somatodendritic receptor [24,25]. This hypothermia is abolished by 5-HT_{1A} receptor's antagonist WAY 100635 [26]. Compounds **8a**–**12a**, like 8-OH-DPAT, induced hypothermia, whereas **7a**, like WAY 100635, did not change body temperature in mice (Table 2). The hypothermia induced by compounds **8a** (20 mg/kg), **9a** (20 mg/kg), **10a** (10 mg/kg), **11a** (20 mg/kg) and **12a** (20 mg/kg) was attenuated by WAY 100635 (0.1 mg/kg) (Table 3). 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 3). Therefore, the decrease in mouse body temperature, produced by **8a**–**12a**, can be regarded as a measure of their presynaptic 5-HT_{1A} receptor agonistic activity. However, since the hypothermia induced by the tested compounds (especially by **9a**, **10a** and **12a**) was not completely antagonized by WAY 100635, receptors other than presynaptic 5-HT_{1A} receptors may participate in this effect. Compound **7a** (20 mg/kg) did not change the hypothermia induced by 8-OH-DPAT (5 mg/kg) in mice; therefore, it seems that the functional activity of **7a** at those receptors was negligible in that experimental paradigm.

Subsequently, to establish agonist/antagonistic action on the postsynaptic 5-HT_{1A} receptor, compounds **8a**, **10a**, **11a** and **12a** were investigated in the forced swimming test in mice. Compounds **11a** and **12a** at doses of 20 mg/kg (but not 10 mg/kg) reduced immobility time by 40% and 29%, respectively, while compound **10a** at 10 mg/kg reduced the immobility time by 34% in mice in the forced swimming test (Fig. 3A–C). ANOVA revealed for the following compounds: **12a**: $F(2,27) = 14.57$, $p < 0.0001$, **11a**: $F(2,27) = 15.92$, $p < 0.0001$, **10a**: $F(2,26) = 21.12$, $p < 0.0001$. Compound **8a** was

Table 1
5-HT_{1A} and 5-HT-T binding affinities of compounds **7a**–**12a** and **7b**–**12b**.

Compound	R_1	R_2	R_3	K_i for 5-HT_{1A} (nM)	K_i for 5-HT-T (nM)
7a	H	OCH_3	H	42.1 ± 3.5	145.0 ± 3.9
8a	H	CH_3	H	5.8 ± 1.4	0.3 ± 0.1
9a	H	Cl	H	37.1 ± 2.2	280.0 ± 1.6
10a	H	F	H	9.2 ± 1.5	37.6 ± 5.7
11a	F	H	H	4.8 ± 1.3	0.7 ± 0.2
12a	H	H	H	10.9 ± 4.2	58.1 ± 3.6
7b	H	OCH_3	OCH_3	78 ± 10	129.0 ± 1.7
8b	H	CH_3	OCH_3	59 ± 20	222.9 ± 2.9
9b	H	Cl	OCH_3	41.1 ± 1.6	251 ± 13
10b	H	F	OCH_3	34.6 ± 2.1	54.6 ± 6.2
11b	F	H	OCH_3	16.7 ± 4.9	59.3 ± 4.7
12b	H	H	OCH_3	52.3 ± 2.9	128.7 ± 0.6

Table 2
The effect of the tested compounds on the body temperature in mice.

Treatment	Dose (mg/kg)	$\Delta t \pm$ SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	K_i	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	0.0 ± 0.1
7a	5	−0.3 ± 0.1	−0.6 ± 0.1[a]	−0.6 ± 0.1[a]	−0.4 ± 0.1
	10	−0.3 ± 0.1	−0.3 ± 0.1	−0.1 ± 0.0	−0.2 ± 0.0
	20	−0.3 ± 0.1	−0.2 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1
Vehicle	–	0.0 ± 0.1	0.0 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
8a	5	−0.4 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.0
	10	−0.5 ± 0.1[a]	−0.3 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
	20	−1.0 ± 0.1[c]	−0.8 ± 0.1[c]	−0.7 ± 0.1[b]	−0.8 ± 0.1[c]
Vehicle	–	−0.1 ± 0.1	0.0 ± 0.1	−0.2 ± 0.1	−0.1 ± 0.0
9a	5	−0.5 ± 0.1[a]	−0.4 ± 0.1	−0.4 ± 0.1	−0.2 ± 0.1
	10	−0.4 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.0
	20	−1.2 ± 0.1[c]	−1.0 ± 0.1[c]	−1.1 ± 0.1[c]	−0.7 ± 0.1[b]
Vehicle	–	0.0 ± 0.1	−0.1 ± 0.0	0.0 ± 0.1	0.0 ± 0.1
10a	5	−0.4 ± 0.1	−0.3 ± 0.1	−0.4 ± 0.1	−0.3 ± 0.1
	10	−1.2 ± 0.2[c]	−1.2 ± 0.2[c]	−1.0 ± 0.2[c]	−0.8 ± 0.2[c]
	20	−1.2 ± 0.2[c]	−1.0 ± 0.1[c]	−0.8 ± 0.1[c]	−0.6 ± 0.1[b]
Vehicle	–	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1
11a	5	−0.5 ± 0.1[a]	−0.3 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.0
	10	−0.8 ± 0.1[c]	−0.2 ± 0.2	−0.3 ± 0.1	−0.1 ± 0.0
	20	−1.1 ± 0.1[c]	−0.8 ± 0.2[b]	−0.7 ± 0.1[b]	−0.7 ± 0.1[b]
Vehicle	–	−0.1 ± 0.1	−0.1 ± 0.1	−0.1 ± 0.1	0.0 ± 0.1
12a	5	−0.4 ± 0.1	−0.5 ± 0.2[a]	−0.4 ± 0.1	−0.1 ± 0.0
	10	−0.8 ± 0.1[c]	−0.4 ± 0.1	−0.3 ± 0.1	−0.1 ± 0.0
	20	−1.2 ± 0.2[c]	−1.3 ± 0.2[c]	−1.1 ± 0.3[c]	−1.9 ± 0.8[c]
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 (*ip*) 30 min before the test. The absolute mean body temperatures were within a range of 36 ± 0.5 °C; $n = 7$–8 mice per group.
[a] $p < 0.05$ vs. respective vehicle.
[b] $p < 0.01$ vs. respective vehicle.
[c] $p < 0.001$ vs. respective vehicle.

ineffective at doses of 10 mg/kg and 20 mg/kg in that test (data not shown). Compound 8-OH-DPAT, at doses of 1 mg/kg and 2 mg/kg, reduced the immobility time by 45% and 57%, respectively (Fig. 3D; ANOVA: $F(2,26) = 53.92$, $p < 0.0001$).

Compounds **10a** and **12a** at a dose of 20 mg/kg did not change the spontaneous locomotor activity during a 6-min (i.e. the time equivalent to the observation period in the forced swimming test) or a 30-min observation period in mice (Table 4). In contrast, compound **11a** reduced the spontaneous locomotor activity of mice during both the 6-min and 30-min observation period in mice (Table 4).

6. Conclusions

Several new derivatives of 4-aryl-pyrido[1,2-*c*]pyrimidine possessing either 3-(4-piperidyl)-1*H*-indole group or its 5-methoxy derivative were synthesized (**7a**,**b**–**12a**,**b**). These compounds are considered to possess double biological activity. In in vitro experiments newly synthesized compounds possessed high to moderate binding activity to the 5-HT_{1A} receptor as well as to the 5-HT transporter.

Based on K_i values in the low nM range, we determined that compounds **8a** and **11a** possessed the highest binding affinity for both the 5-HT_{1A} receptor and the 5-HT transporter. The remaining compounds also bind to both the 5-HT_{1A} receptor and 5-HT transporter with moderate to relatively low affinity.

Table 3
The effect of WAY 100635 on the hypothermia induced by the tested compounds in mice.

Compound and dose (mg/kg)	$\Delta t \pm$ SEM (°C)	
	30 min	60 min
Vehicle + vehicle	0.0 ± 0.1	0.0 ± 0.1
Vehicle + **8a** (20)	−1.0 ± 0.1[b]	−0.8 ± 0.1[b]
WAY 100635 (0.1) + **8a** (20)	−0.3 ± 0.1[d]	−0.2 ± 0.1[d]
Vehicle + vehicle	−0.1 ± 0.1	0.0 ± 0.1
Vehicle + **9a** (20)	−1.2 ± 0.1[b]	−1.0 ± 0.1[b]
WAY 100635 (0.1) + **9a** (20)	−0.4 ± 0.1[d]	−0.2 ± 0.1[d]
Vehicle + vehicle	0.0 ± 0.1	−0.1 ± 0.0
Vehicle + **10a** (10)	−1.0 ± 0.1[b]	−1.0 ± 0.1[b]
WAY 100635 (0.1) + **10a** (10)	−0.8 ± 0.1[b,c]	−0.8 ± 0.1[b,c]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **11a** (20)	−1.1 ± 0.1[b]	−0.8 ± 0.2[a]
WAY 100635 (0.1) + **11a** (20)	−0.3 ± 0.1[d]	−0.1 ± 0.1[c]
Vehicle + vehicle	−0.1 ± 0.1	−0.1 ± 0.1
Vehicle + **12a** (20)	−1.2 ± 0.2[b]	−1.3 ± 0.2[b]
WAY 100635 (0.1) + **12a** (20)	−0.5 ± 0.1[a,d]	−0.3 ± 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 (*sc*) 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 within a range of 36.0 ± 0.4 °C.
[a] $p < 0.01$ vs. respective vehicle + vehicle group.
[b] $p < 0.001$ vs. respective vehicle + vehicle group.
[c] $p < 0.01$ vs. respective vehicle + compound group.
[d] $p < 0.001$ vs. respective vehicle + compound group.

The presence of the 3-(4-piperidyl)-1*H*-indole residue, as well as a fluorine atom or methyl group at *ortho*/*para* positions of the phenyl group (or unsubstituted phenyl) in the aryl ring of 4-aryl-pyrido[1,2-*c*]pyrimidine system is of special advantage with regard to binding affinity.

In the induced hypothermia model in mice compounds **8a**–**12a** showed agonistic properties to presynaptic 5-HT_{1A} receptor. Compound **10a** decreased body temperature when administered at 10 mg/kg dose, whereas for the remaining compounds (**8a**, **9a**, **11a** and **12a**) doses of 20 mg/kg were needed.

Agonistic effects on postsynaptic 5-HT_{1A} receptor of compounds **10a**, **11a** and **12a** were also demonstrated in the forced swimming test in mice. Compound **10a** has an effect at 10 mg/kg dose, while compounds **11a** and **12a** required higher doses of 20 mg/kg to be effective.

Compounds **8a**, **10a** and **11a** are currently being tested for multi-receptor binding profile (D_1, D_2, α_1 and β_1 receptors) as well as for chronic toxicity. These results will be required for further neurobiological evaluation.

7. Experimental protocols

7.1. Chemistry

7.1.1. General remarks

Melting points were determined on an Electrothermal 9100 apparatus with open capillary tubes and are uncorrected. Elementar analyses were performed on a Perkin–Elmer 2400 analyzer and were within ±0.4% of the theoretical values. Infrared spectra were recorded on Shimadzu FTIR-8300 spectrometer. ^1H and ^{13}C NMR spectra were obtained on Bruker AVANCE DMX 400WB instrument in $CDCl_3$ (chemical shifts are reported in δ units).

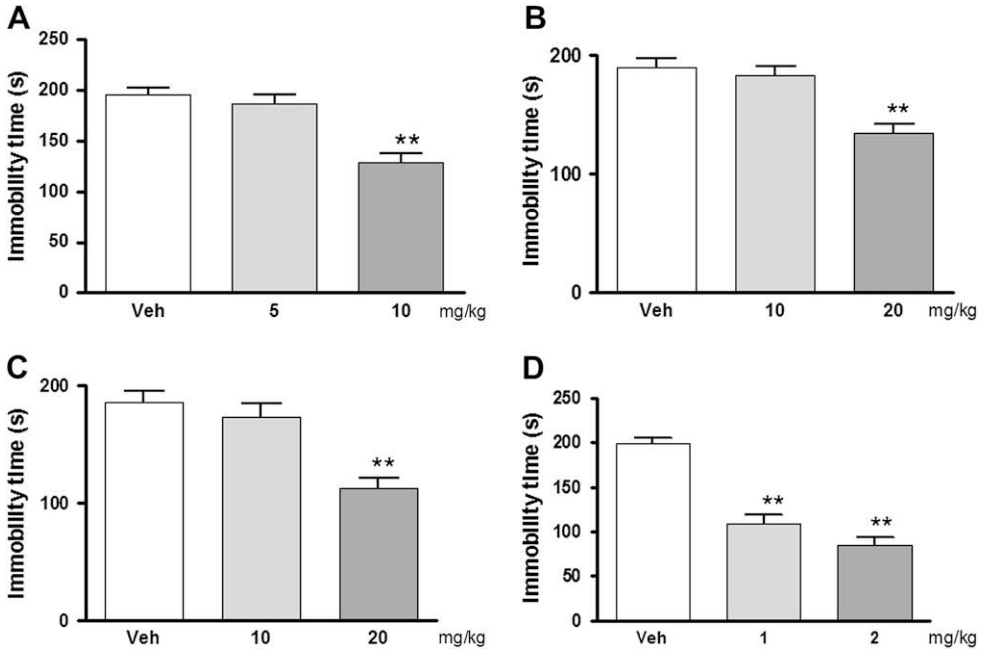

Fig. 3. Effects of compounds **12a** (A), **11a** (B), **10a** (C) and 8-OH-DPAT (D) on immobility time in the forced swimming test in Albino Swiss mice. Each bar represents the mean ± SEM of 9–10 mice. All compounds were injected 30 min before the test. $^{**}p < 0.001$ vs. respective vehicle group (Dunnett's test).

Coupling constants (*J*) are in hertz (Hz), the internal reference was TMS. 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-), 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 the solvent methylene chloride/methanol (99:1, 97:3, 95:5, v/v). Thin layer chromatography was run on Merck Silica gel 60 F_{254} plates using mobile phase 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).

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

The starting compounds **2a**–**2f**, **3a**–**3f**, **4a**–**4f** and **5a**–**5f** were obtained according to procedures described in Refs. [20,21].

*7.1.3. General procedure for the synthesis of 3-piperidin-4-yl-1H-indoles (**6a**, **6b**)*

A mixture of 0.1 mol of appropriate indole, 0.2 mol of *N*-benzyl-4-piperidone and 150 ml of 2 N KOH/MeOH was refluxed while stirring under an atmosphere of nitrogen for 8 h. The mixture was further stirred for 8 h at room temperature and poured onto ice/water. The crude product was extracted with $CHCl_3$. Organic layers were combined and dried with $MgSO_4$. The mixture was then filtered and the filtrate was evaporated to dryness. The crude residue was purified with diethylether. The obtained yellow solid was dissolved in 300 ml of methanol, hydrogenated for 8 h in 45 °C and 1 atm on 10% Pd/C as catalyst. The catalyst was then filtered off and the filtrate was evaporated to dryness. The crude product was purified by crystallization from ethyl acetate.

Table 4
Effect of compounds **10a**, **11a** and **12a** on the locomotor activity in mice.

Treatment (mg/kg)	Locomotor activity	
	Number of crossings ± SEM during	
	6 min	30 min
Vehicle	343 ± 41	889 ± 111
10a (10)	376 ± 46	965 ± 125
11a (20)	179 ± 50	399 ± 131
12a (20)	409 ± 23**	926 ± 70**
	$F(3,36) = 6.184$; $P < 0.001$	$F(3,36) = 5.656$; $P < 0.001$

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

*7.1.4. General procedure for the synthesis of 2-{4-[4-(1H-indol-3-yl)-piperidin-1-yl]-butyl}-4-aryl-pyrido[1,2-c]pyrimidine-1,3-diones (**7a**,**b**–**12a**,**b**)*

Compounds **5a**–**5f** (0.0026 mol), **6a**, **6b** (0.0026 mol), K_2CO_3 (0.005 mol), 70 ml of 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) mixture. Proper fractions were identified by TLC and evaporated to dryness giving analytically pure compounds **7a**,**b**–**12a**,**b**.

*7.1.4.1. 2-{4-[4-(1H-Indol-3-yl)-piperidin-1-yl]-butyl}-4-(4-methoxyphenyl)-pyrido[1,2-c]pyrimidine-1,3-dione (**7a**).* Yield: 90%; yellow crystals, m.p. 235–236 °C; ^{1}H NMR (400 MHz) δ: 1.67 (m, C-3 x H_2), 1.79 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.07 (m, 4H, Ca,eH_{ax}, Cb,dH_{eq}), 2.44 (t, $^3J = 7.6$ Hz, C-4 x H_2), 2.82 (pt, CcH), 3.06 (pd, 2H, Ca,e$_{eq}$), 3.84 (s, OCH_3), 4.19 (t, $^3J = 7.2$ Hz, C-1 x H_2), 6.36 (t, $^3J = 7.2$ Hz, C-7H), 6.90 (m, C-5H, C-6H), 6.96 (bs, C-2″H), 6.98 (d, C-3′H, C-5′H), 7.09 (t, $^3J = 7.6$ Hz, C-5″H), 7.17 (t, $^3J = 7.6$ Hz, C-6″H), 7.24 (d, $^3J = 8.4$ Hz, C-2′H, C-6′H), 7.34 (d, $^3J = 8.0$ Hz, C-7″H), 7.65 (d, $^3J = 7.6$ Hz, C-4″H), 8.0 (bs, NH), 8.34 (d, $^3J = 7.6$ Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.8 (C-3x), 25.9 (C-2x), 33.3 (Cb, Cd), 33.7 (Cc), 42.7 (C1x), 54.7 (Ca, Ce), 55.6 (OCH_3), 59.0 (C-4x), 104.8 (C-4), 110.8 (C-7), 111.4 (C-7″), 114.5 (C-3′, C-5′), 119.2 (C-5″), 119.3 (C-4″), 119.7 (C-3″), 119.9 (C-2″), 121.8 (C-5), 122.0 (C-6″), 125.1 (C-1′), 126.9 (C-3″a), 128.1 (C-8), 132.3 (C-6), 132.6 (C-2′, C-6′), 136.5 (C-7″a), 143.7 (C-4a), 149.2 (C-1), 159.3 (C-4′), 160.6 (C-3); IR *v*: 1635 (C═O), 1705 (C═O). Anal. Calcd for $C_{32}H_{34}N_4O_3$: C, 73.5; H, 6.6; N, 10.7. Found: C, 72.9; H, 6.3; N, 10.2.

*7.1.4.2. 2-{4-[4-(5-Methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-(4-methoxyphenyl)-pyrido[1,2-c]pyrimidine-1,3-dione (**7b**).* Yield: 58.8%; yellow crystals, m.p. 162–164 °C; ^{1}H NMR (400 MHz) δ: 1.64 (q, C-3 x H_2), 2.03 (m, C-2 x H_2, Cb,dH_{ax}), 2.03 (pd, Cb,dH_{eq}), 2.44 (t, ^3J = 7.6 Hz, C-4 x H_2), 2.77 (tt, CcH), 3.83 (s, OCH_3), 3.86 (s, OC-10H_3), 4.19 (t, ^3J = 7.4 Hz, C-1 x H_2), 6.35 (t, ^3J = 7.2 Hz, C-7H), 6.83 (dd, ^3J = 8.8 Hz, ^4J = 2.0 Hz, C-6″H), 6.90 (m, C-5H, C-6H), 6.92 (s, C-2″H), 7.05 (d, ^4J = 1.6 Hz, C-4″H), 7.05 (d, C-3′H, C-5′H), 7.21 (d, ^3J = 8.4 Hz, C-7″H), 7.26 (d, ^3J = 8.4 Hz, C-2′H, C-6′H), 8.04 (bs, NH), 8.31 (d, ^3J = 7.2 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.8 (C-3x), 25.9 (C-2x), 33.2 (Cb, Cd), 33.7 (Cc), 42.7 (C-1x), 54.7 (Ca, Ce), 55.6 (OCH_3), 56.3 (OC-10H_3), 59.1 (C-4x), 101.3 (C-4″), 104.8 (C-4), 110.8 (C-7), 112.1 (C-7″), 112.2 (C-6″), 114.5 (C-3′, C-5′), 121.8 (C-5), 125.1 (C-1′), 127.3 (C-3″a), 128.1 (C-8), 131.8 (C-7″a), 132.6 (C-2′, C-6′), 143.7 (C-4a), 149.2 (C-5″), 153.9 (C-1), 159.4 (C-4′), 160.6 (C-3); IR *ν*: 1647 (C═O), 1720 (C═O). Anal. Calcd for $C_{33}H_{36}N_4O_4$: C, 71.7; H, 6.6; N, 10.1. Found: C, 71.1; H, 6.4; N, 10.0.

*7.1.4.3. 2-{4-[4-(1*H*-Indol-3-yl)-piperidin-1-yl]-butyl}-4-*p*-tolyl-pyrido[1,2-c]pyrimidine-1,3-dione (**8a**).* Yield: 50%; yellow crystals, m.p. 178–179 °C; ^{1}H NMR (400 MHz) δ: 1.64 (q, ^3J = 7.6 Hz, C3 x H_2), 1.78 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.07 (m, 4H, Ca,eH_{ax}, Cb,dH_{eq}), 2.38 (s, CH_3), 2.43 (t, ^3J = 7.6 Hz, C-4 x H_2), 2.82 (pt, CcH), 4.20 (t, ^3J = 7.4 Hz, C-1 x H_2), 6.35 (td, ^3J = 6.0 Hz, ^4J = 0.8 Hz, C-7H), 6.88 (m, C-5H, C-6H), 6.93 (bs, C-2″H), 7.08 (t, ^3J = 7.4 Hz, C-5″H), 7.16 (t, ^3J = 7.6 Hz, C-6″H), 7.20 (d, C-3′H, C-5′H), 7.25 (d, ^3J = 7.2 Hz, C-2′H, C-6′H), 7.33 (d, ^3J = 8.0 Hz, C-7″H), 7.63 (d, ^3J = 7.6 Hz, C-4″H), 8.09 (bs, NH), 8.31 (d, ^3J = 7.2 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 21.5 (CH_3), 24.8 (C-3x), 25.9 (C-2x), 33.3 (Cb, Cd), 33.8 (Cc), 42.7 (C-1x), 54.7 (Ca, Ce), 59.1 (C-4x), 105.2 (C-4), 110.8 (C-7), 111.4 (C-7″), 119.2 (C-5″), 119.3 (C-4″), 119.7 (C-3″), 119.9 (C-2″), 121.8 (C-5), 122.0 (C-6″), 126.9 (C-3″a), 128.1 (C-8), 129.7 (C-2′, C-6′), 129.9 (C-1′), 131.3 (C-3′, C-5′), 132.3 (C-6), 136.6 (C-7″a), 137.8 (C-4′), 143.6 (C-4a), 149.2 (C-1), 160.5 (C-3); IR *ν*: 1635 (C═O), 1709 (C═O). Anal. Calcd for $C_{32}H_{34}N_4O_2$: C, 75.8; H, 6.7; N, 11.0. Found: C, 75.3; H, 6.7; N, 11.1.

*7.1.4.4. 2-{4-[4-(5-Methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-*p*-tolyl-pyrido[1,2-c]pyrimidine-1,3-dione (**8b**).* Yield: 78%; yellow crystals, m.p. 110–112 °C; ^{1}H NMR (400 MHz) δ: 1.67 (q, C3 x H_2), 1.79 (m, C-2 x H_2, Cb,dH_{ax}), 2.03 (pd, Cb,dH_{eq}), 2.15 (pt, Ca,eH_{ax}), 2.39 (s, CH_3), 2.46 (t, ^3J = 7.8 Hz, C-4 x H_2), 2.78 (pt, CcH), 3.06 (pd, Ca,e$_{eq}$), 3.87 (s, OCH_3), 4.19 (t, ^3J = 7.2 Hz, C-1 x H_2), 6.39 (m, C-7H), 6.83 (dd, ^3J = 8.8 Hz, ^4J = 2.4 Hz, C-6″H), 6.91 (bps, C-5H, C-6H), 7.07 (t, ^4J = 2.0 Hz, C-4″H), 7.20 (d, ^3J = 8.0 Hz, C-3′H, C-5′H), 7.22–7.31 (m, C-2′H, C-6′H, C-7″H), 7.33 (d, ^3J = 8.0 Hz, C-7″H), 8.32 (d, ^3J = 7.2 Hz, C-8H), 8.40 (bs, NH).

^{13}C NMR (100 MHz) δ: 21.4 (CH_3), 24.3 (C-3x), 25.8 (C-2x), 32.8 (Cb, Cd), 33.6 (Cc), 42.6 (C-1x), 54.5 (Ca, Ce), 56.2 (OCH_3), 58.9 (C-4x), 101.2 (C-4″), 105.1 (C-4), 110.9 (C-7), 112.0 (C-6″, C-7″), 120.8 (C-2″), 120.9 (C-3″), 121.8 (C-5), 127.0 (C-3″a), 128.0 (C-8), 129.6 (C-2′, C-6″), 129.7 (C-1′), 131.2 (C-7″a), 132.5 (C-6), 137.8 (C-4′), 143.8 (C-4a), 149.1 (C-5″), 153.7 (C-1), 160.6 (C-3); IR *ν*: 1620 (C═O), 1709 (C═O). Anal. Calcd for $C_{33}H_{36}N_4O_3$: C, 73.9; H, 6.8; N, 10.4. Found: C, 73.3; H, 6.8; N, 10.3.

*7.1.4.5. 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**).* Yield: 75.5%; yellow crystals, m.p. 173–174 °C; ^{1}H NMR (400 MHz) δ: 1.69 (q, C-3 x H_2), 1.78 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.01 (pd, Cb,dH_{eq}), 2.43 (t, ^3J = 7.6 Hz, C-4 x H_2), 2.82 (tt, CcH), 4.19 (t, ^3J = 7.4 Hz, C-1 x H_2), 6.39 (td, ^3J = 6.8 Hz, ^4J = 0.8 Hz, C-7H), 6.87 (d, ^3J = 9.2 Hz, C-5H), 6.93 (t, C-6H), 6.93 (s, C-2″H), 7.08 (t, ^3J = 7.2 Hz, C-5″H), 7.16 (t, ^3J = 7.2 Hz, C-6″H), 7.26 (d, C-3′H, C-5′H), 7.33 (d, ^3J = 8.0 Hz, C-7″H), 7.41 (d, ^3J = 8.4 Hz, C-2′H, C-6′H), 7.64 (d, ^3J = 8.0 Hz, C-4″H), 8.11 (bs, NH), 8.34 (d, ^3J = 7.2 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.8 (C-3x), 25.9 (C-2x), 33.3 (Cb, Cd), 33.8 (Cc), 42.8 (C-1x), 54.7 (Ca, Ce), 59.0 (C-4x), 103.8 (C-4), 111.0 (C-7), 111.4 (C-7″), 119.2 (C-5″), 119.9 (C-2″), 121.3 (C-5), 121.8 (C-3″), 122.0 (C-6″), 126.9 (C-3″a), 128.4 (C-8), 129.2 (C-2′, C-6′), 131.6 (C-1′), 132.9 (C-3′, C-5′), 133.1 (C-6), 133.9 (C-4′), 136.6 (C-7″a), 143.8 (C-4a), 149.1 (C-1), 160.2 (C-3); IR *ν*: 1635 (C═O), 1713 (C═O). Anal. Calcd for $C_{31}H_{31}N_4O_2Cl$: C, 70.6; H, 5.9; N, 10.6. Found: C, 70.0; H, 6.2; N, 10.6.

*7.1.4.6. 4-(4-Chloro-phenyl)-2-{4-[4-(5-methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-c]pyrimidine-1,3-dione (**9b**).* Yield: 53.8%; yellow crystals, m.p. 110–112 °C; ^{1}H NMR (400 MHz) δ: 1.64 (q, ^3J = 7.2 Hz, C-3 x H_2), 1.77 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.03 (pd, Cb,dH_{eq}), 2.11 (pt, Ca,eH_{ax}), 2.44 (t, ^3J = 7.8 Hz, C-4 x H_2), 2.77 (tt, $^3J_{A-A}$ = 12.0 Hz, $^3J_{A-E}$ = 3.6 Hz, CcH), 3.04 (pd, Ca,e$_{eq}$), 3.86 (s, OCH_3), 4.19 (t, ^3J = 7.4 Hz, C-1 x H_2), 6.40 (td, ^3J = 6.8 Hz, ^4J = 1.0 Hz, C-7H), 6.84 (dd, ^3J = 8.8 Hz, ^4J = 2.4 Hz, C-6″H), 6.88 (d, ^3J = 9.6 Hz, C-5H), 6.94 (s, C-2″H), 6.94 (td, ^3J = 7.8 Hz, ^4J = 0.8 Hz, C-6H), 7.06 (d, ^4J = 2.4 Hz, C-4″H), 7.23 (d, ^3J = 8.4 Hz, C-7″H), 7.27 (d, C-3′H, C-5′H), 7.42 (d, ^3J = 8.4 Hz, C-2′H, C-6′H), 7.94 (bs, NH), 8.35 (d, ^3J = 7.6 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.5 (C-3x), 25.6 (C-2x), 32.9 (Cb, Cd), 33.5 (Cc), 42.5 (C-1x), 54.5 (Ca, Ce), 56.0 (OCH_3), 58.8 (C-4x), 101.0 (C-4″), 103.5 (C-4), 110.8 (C-7), 111.8 (C-7″), 112.0 (C-6″), 121.0 (C-5), 121.3 (C-3″), 125.5 (C-3″a), 128.1 (C-8), 129.0 (C-2′, C-6′), 131.2 (C-1′), 131.5 (C-7″a), 132.7 (C-3′, C-5′), 132.9 (C-6), 133.7 (C-4′), 143.6 (C-4a), 148.8 (C-5″), 153.7 (C-1), 160.0 (C-3); IR *ν*: 1624 (C═O), 1709 (C═O). Anal. Calcd for $C_{32}H_{33}N_4O_3Cl$: C, 69.0; H, 6.0; N, 10.1. Found: C, 68.6; H, 6.1; N, 9.5.

*7.1.4.7. 4-(4-Fluoro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-c]pyrimidine-1,3-dione (**10a**).* Yield: 56.5%; yellow crystals, m.p. 182–183 °C; ^{1}H NMR (400 MHz) δ: 1.64 (q, C-3 x H_2), 1.79 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.02 (pd, Cb,dH_{eq}), 2.10 (pt, Ca,eH_{ax}), 2.44 (t, ^3J = 7.6 Hz, C-4 x H_2), 2.83 (tt, CcH), 3.05 (pd, Ca,eH_{eq}), 4.19 (t, ^3J = 7.4 Hz, C-1 x H_2), 6.39 (t, ^3J = 6.8 Hz, ^4J = 0.8 Hz, C-7H), 6.86 (d, ^3J = 9.2 Hz, C-5H), 6.92 (t, ^3J = 6.0 Hz, C-6H), 6.95 (s, C-2″H), 7.05–7.23 (m, C-5″H, C-6″H), 7.27–7.33 (m, C-2′H, C-6′H), 7.34 (d, ^3J = 8.4 Hz, C-7″H), 7.64 (d, ^3J = 7.6 Hz, C-4″H), 8.02 (bs, NH), 8.34 (d, ^3J = 7.6 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.8 (C-3x), 25.9 (C-2x), 33.3 (Cb, Cd), 33.7 (Cc), 42.8 (C-1x), 54.7 (Ca, Ce), 59.0 (C-4x), 104.0 (C-4), 110.9 (C-7), 111.3 (C-7″), 116.0 (d^, ^2J = 21.4 Hz, C-3′, C-5′), 119.2 (C-5″), 119.3 (C-4″), 119.7 (C-3″), 119.8 (C-2″), 121.4 (C-5), 122.1 (C-6″), 126.9 (C-3″a), 128.3 (C-8), 128.9 (d^, ^4J = 3.1 Hz, C-1′), 132.9 (C-6), 133.2 (d^, ^3J = 8.0 Hz, C-2′, C-6′), 136.5 (C-7″a), 143.9 (C-4a), 149.1 (C-1), 160.4 (C-3), 162.5 (d^, ^1J = 246.9 Hz, C-4′); IR *ν*: 1643 (C═O), 1720 (C═O). Anal. Calcd for $C_{31}H_{31}N_4O_2F$: C, 72.8; H, 6.1; N, 11.0. Found: C, 72.3; H, 6.1; N, 10.8.

*7.1.4.8. 4-(4-Fluoro-phenyl)-2-{4-[4-(5-methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-c]pyrimidine-1,3-dione (**10b**).* Yield: 78.7%; yellow crystals, m.p. 151–152 °C; ^{1}H NMR (400 MHz) δ: 1.82 (ps, 4H, C-3 x H_2, Cb,H$_{ax}$, CdH$_{ax}$), 2.08 (ps, C2 x H_2, CbH$_{eq}$, CdH$_{eq}$), 2.41 (bps, C-4 x H_2), 2.72 (ps, CaH$_{ax}$, CeH$_{ax}$), 2.86 (ps, CcH), 3.26 (ps, Ca,H$_{eq}$, CeH$_{eq}$), 3.88 (s, OCH_3), 4.20 (t, C-1 x H_2), 6.41 (t, ^3J = 6.4 Hz, C-7H), 6.86 (m, C-5H, C-6″H), 6.94 (m, C-6H), 6.97 (s, C-2″H), 7.06 (d, ^4J = 1.6 Hz, C-4″H), 7,14 (t, C-3′H, C-5′H), 7.27 (m, C-2′H, C-6′H, C-7″H), 8.08 (bs, NH), 8.35 (t, ^3J = 7.6 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 25.4 (C-3x), 25.5 (C-2x), 36.0 (Cb, Cd), 54.2 (Ca, Ce), 56.4 (OCH_3), 101.14 (C-4″), 103.9 (C-4), 111.1 (C-7), 112.2 (C-7″), 112.4 (C-6″), 116.1 (d^, ^2J = 21.4 Hz, C-3′, C-5′), 119.4 (C-3″), 121.1 (C-2″), 121.4 (C-5), 127.0 (C-3″a), 128.3 (C-8), 128.9 (d^, ^4J = 3.0 Hz, C-1′), 132.1 (C-6), 133.2 (d^, ^3J = 8.2 Hz, C-2′, C-6′), 131.7 (C-7″a), 144.1 (C-4a), 154.1 (C-1), 160.5 (C-3); IR *ν*: 1635 (C═O),

1697 (C=O). Anal. Calcd for $C_{32}H_{33}N_4O_3F$: C, 71.1; H, 6.2; N, 10.4. Found: C, 67.3; H, 6.1; N, 9.8.

*7.1.4.9. 4-(2-Fluoro-phenyl)-2-{4-[4-(1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-c]pyrimidine-1,3-dione (**11a**).* Yield: 37.6%; yellow crystals, m.p. 191–192.6 °C; ^{1}H NMR (400 MHz) δ: 1.65 (q, C-3 x H_2), 1.79 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.01 (pd, Cb,dH_{eq}), 2.10 (pt, Ca,eH_{ax}), 2.44 (t, ^3J = 7.2 Hz, C-4 x H_2), 2.82 (tt, CcH), 3.05 (pd, Ca,eH_{eq}), 4.20 (t, ^3J = 7.2 Hz, C-1 x H_2), 6.41 (t, ^3J = 6.8 Hz, C-7H), 6.73 (d, ^3J = 9.2 Hz, C-5H), 6.96 (t, ^3J = 8.8 Hz, C-6H), 6.94 (s, C-2″H), 7.08 (t, ^3J = 7.2 Hz, C-5″H), 7.16 (m, C-3′H, C-6′H), 7.26 (t, ^3J = 7.2 Hz, C-5′H), 7.30–7.42 (m, C-4′H, C-6′H, C-7″H), 7.64 (d, ^3J = 8.0 Hz, C-4″H), 8.07 (bs, NH), 8.36 (d, ^3J = 7.2 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.7 (C-3x), 25.8 (C-2x), 33.3 (Cb, Cd), 33.7 (Cc), 42.8 (C-1x), 54.7 (Ca, Ce), 59.0 (C-4x), 98.6 (C-4), 111.0 (C-7), 111.3 (C-7″), 116.3 (d^, ^2J = 22.2 Hz, C-3′), 119.2 (C-5″), 119.3 (C-4″), 119.9 (C-2″), 120.5 (d^, ^2J = 16.0 Hz, C-1′), 121.4 (C-5), 121.8 (C-3″), 122.0 (C-6″), 124.6 (d^, ^4J = 3.5 Hz, C-5′), 126.9 (C-3″a), 128.4 (C-8), 130.3 (d^, ^3J = 9.3 Hz, C-4′), 133.3 (C-6), 133.7 (d^, ^3J = 3.0 Hz, C-6′), 136.5 (C-7″a), 144.2 (C-4a), 149.1 (C-1), 159.8 (C-3), 161.0 (d^, ^1J = 249.5 Hz, C-2′); IR *v*: 1647 (C=O), 1713 (C=O). Anal. Calcd for $C_{31}H_{31}N_4O_2F$: C, 72.9; H, 6.3; N, 11.0. Found: C, 73.0; H, 6.2; N, 10.7.

*7.1.4.10. 4-(2-Fluoro-phenyl)-2-{4-[4-(5-methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-pyrido[1,2-c]pyrimidine-1,3-dione (**11b**).* Yield: 65%; yellow crystals, m.p. 88–92 °C; ^{1}H NMR (400 MHz) δ: 1.73 (q, C-3 x H_2), 1.79 (q, C-2 x H_2), 1.94 (pt, CbH_{ax},CdH_{ax}), 2.04 (pd, CbH_{eq}, CdH_{eq}), 2.27 (pt, CaH_{ax}, CeH_{ax}), 2.59 (t, ^3J = 7.2 Hz, C-4 x H_2), 2.81 (m, CcH), 3.16 (pd, CaH_{eq}, CeH_{eq}), 3.87 (s, OCH_3), 4.20 (t, ^3J = 7.0 Hz, C-1 x H_2), 6.44 (t, ^3J = 6.8 Hz, C-7H), 6.74 (d, ^3J = 9.6 Hz, C-5H), 6.83 (dd, ^3J = 8.8 Hz, ^4J = 2.0 Hz, C-6″H), 6.94 (s, C-2″H), 6.99 (dd, C-6H), 7.05 (d, ^4J = 1.6 Hz, C-4″H), 7.16 (m, C-3′H), 7.26 (m, C-5′H, C-7′H), 7.33 (t, C-4′H), 7.37 (m, C-6′H), 8.18 (bs, NH), 8.37 (d, ^3J = 7.6 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.0 (C-3x), 25.5 (C-2x), 33.3 (Cb, Cd), 32.0 (Cb, Cd), 32.5 (Cc), 42.2 (C-1x), 54.2 (Ca, Ce), 56.3 (C-4x, OCH_3), 99.8 (C-4), 101.1 (C-4″), 111.2 (C-7), 112.2 (C-6″, C-7″), 116.3 (d^, ^2J = 22.3 Hz, C-3′), 119.6 (C-2″), 120.4 (C-3″), 121.0 (d^, ^2J = 16.0 Hz, C-1′), 121.4 (C-5), 124.7 (d^, ^4J = 3.5 Hz, C-5′), 127.0 (C-3″a), 128.4 (C-8), 130.4 (d^, ^3J = 7.5 Hz, C-4′), 131.7 (C-7″a), 133.5 (C-6), 133.6 (d^, ^3J = 3.0 Hz, C-6′), 144.4 (C-4a), 149.1 (C-1), 158.6 (d^, ^1J = 249.0 Hz, C-2′), 159.9 (C-3); IR *v*: 1643 (C=O), 1713 (C=O). Anal. Calcd for $C_{32}H_{33}N_4O_3F$: C, 71.1; H, 6.1; N, 10.4. Found: C, 70.8; H, 6.0; N, 10.1.

*7.1.4.11. 2-{4-[4-(1*H*-Indol-3-yl)-piperidin-1-yl]-butyl}-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-dione (**12a**).* Yield: 70.3%; yellow crystals, m.p. 219–220 °C; ^{1}H NMR (400 MHz) δ: 1.67 (q, C-3 x H_2), 1.80 (m, 4H, C-2 x H_2, Cb,dH_{ax}), 2.03 (pd, 2H, Cb,dH_{eq}), 2.15 (pt, 2H, Ca,eH_{ax}), 2.49 (t, ^3J = 7.6 Hz, C-4 x H_2), 2.83 (tt, ^3J = 11.6 Hz, ^4J = 7.6 Hz), 3.08 (pd, 2H, Ca,eH_{eq}), 4.20 (t, ^3J = 7.2 Hz, C-1 x H_2), 6.37 (q, ^3J = 7.6 Hz, ^4J = 3.6 Hz, C-7H), 6.89 (pd, C-5H, C-6H), 6.92 (bs, C-2″H), 7.08 (t, ^3J = 7.4 Hz, C-5″H), 7.16 (t, ^3J = 7.4 Hz, C-6″H), 7.32 (m, 4H, C-6′H, C-7″H), 7.62 (d, ^3J = 7.6 Hz, C-4′H), 8.18 (bs, NH), 8.33 (d, ^3J = 7.6 Hz, C-8H).

^{13}C NMR (100 MHz) δ: 24.4 (C-3x), 25.7 (C-2x), 32.9 (Cb, Cd), 33.5 (Cc), 42.6 (C1x), 54.5 (Ca, Ce), 58.8 (C-4x), 105.1 (C-4), 110.9 (C-7), 111.4 (C-7″), 119.2 (C-4″, C-5″), 120.0 (C-2″), 121.3 (C-3″), 121.6 (C-5), 122.0 (C-6″), 126.8 (C-3″a), 128.0 (C-4′), 128.1 (C-8), 129.0 (C-3′, C-5′), 131.4 (C-2′, C-6′), 132.6 (C-6), 133.0 (C-1′), 136.5 (C-7″a), 143.7 (C-4a), 149.1 (C-1), 160.4 (C-3); IR *v*: 1639 (C=O), 1709 (C=O). Anal. Calcd for $C_{31}H_{32}N_4O_2$: C, 75.6; H, 6.5; N, 11.4. Found: C, 75.6; H, 6.6; N, 11.2.

*7.1.4.12. 2-{4-[4-(5-Methoxy-1*H*-indol-3-yl)-piperidin-1-yl]-butyl}-4-phenyl-pyrido[1,2-c]pyrimidine-1,3-dione (**12b**).* Yield: 55%; yellow crystals, m.p. 158–160 °C; ^{1}H NMR (400 MHz) δ: 1.87 (q, ^3J = 6.8 Hz, C-3 x H_2), 1.98 (ps, C-2 x H_2,), 2.10 (bps, CbH_{ax}, CdH_{ax}), 2.47 (bps, CbH_{eq}, CdH_{eq}), 2.94 (bps, CcH), 3.05 (bps, CaH_{ax}, CeH_{ax}), 3.45 (bps, CaH_{eq}, CeH_{eq}), 3.89 (s, OCH_3), 4.21 (t, ^3J = 6.8 Hz, C-1 x H_2), 6.43 (td, ^3J = 6.8 Hz, ^4J = 1.2 Hz, C-7H), 6.85 (dd, ^3J = 8.8 Hz, ^4J = 2.0 Hz, C-6″H), 6.89 (ps, C-2″H), 6.94 (m, C-5H, C-6H), 7.07 (ps, C-4″H), 7.26 (d, ^3J = 8.8 Hz, C-7″H), 7.31 (d, ^3J = 7.6 Hz, C-2′H, C-6′H), 7.37 (t, C-4′H), 7.45 (t, ^3J = 7.2 Hz, C-3′H, C-5′H), 8.26 (bs, NH), 8.35 (d, ^3J = 7.6 Hz, C-8H); IR *v*: 1620 (C=O), 1709 (C=O). Anal. Calcd for $C_{32}H_{34}N_4O_3$: C, 73.5; H, 6.6; N, 10.7. Found: C, 73.2; H, 6.4; N, 10.6.

7.2. Pharmacology

7.2.1. In vitro experiments

7.2.1.1. 5-HT$_{1A}$ binding assay. [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)-tetralin ([^{3}H]-8-OH-DPAT, spec. act. 106 Ci/mmol, NEN Chemicals) was used for labeling 5-HT$_{1A}$ receptors. The membrane preparation and the assay procedure were carried out according to the published procedure [27] with slight modifications. Briefly, the hippocampus tissue was homogenized in 20 vol. of 50 mM Tris–HCl buffer (pH 7.7 at 25 °C) using Ultra-Turrax® T 25, and was then centrifuged at 32,000 x *g* for 10 min. The supernatant fraction was discarded, and the pellet was resuspended in the same volume of Tris–HCl buffer and was then centrifuged. 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. One milliliter of the tissue suspension (5 mg of wet weight), 100 μl of 10 μM serotonin (for unspecific 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 a rapid vacuum filtration through Whatman GF/B glass filters, and was then washed three times with 5 ml of a 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.

7.2.1.2. 5-HT-T binding assay. The assay was performed according to the method of Owens et al. [28] with slight modifications. Rat cerebral cortex was homogenized in 30 vol. of ice-cold 50 mM Tris–HCl containing 150 mM NaCl and 5 mM KCl, pH 7.7 at 25 °C and centrifuged at 20,000 x *g* for 20 min. The supernatant was decanted and the pellet was resuspended in 30 vol. of buffer and centrifuged again. The resulting pellet was resuspended in the same quantity of the buffer and centrifuged for the third time in the same conditions. [^{3}H]-citalopram (spec. act. 50 Ci/mmol, NEN Chemicals) was used for labeling 5-HT-transporter. Aliquots 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 analyzed compounds ranged from 10^{-10} to 10^{-4} M. Incubations were terminated by vacuum filtration over Whatman GF/B filters and washed two times with 100 μl of ice-cold buffer. Radioactivity was measured in a WALLAC 1409 DSA liquid scintillation counter. All assays were done in duplicates. Radioligand binding data were analyzed using iterative curve-fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). K_i values were calculated from the Cheng and Prusoff equation [21].

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

L_O – labeled ligand concentration
K_D – dissociation constant of labeled ligand

7.2.2. In vivo experiments

7.2.2.1. Induced hypothermia in mice

7.2.2.1.1. Animals and housing. The experiments were performed on male Albino Swiss mice (24–28 g; purchased from licenced dealer Staniszewska, Ilkowice, Poland). The animals were kept at room temperature (20 ± 1 °C) on a natural day-light cycle (January–February) and housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group consisted of 7–8 animals/dose, and all the animals were used only once. 8-Hydroxy-2-(di-*n*-propylamino)-tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.) and *N*-{2-[4[(2[methoxyphenyl)-1-piperazinyl]-ethyl}-*N*-(2-pyridinyl)-cyclohexane-carboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were used as aqueous solutions. Compounds **7a**–**12a** were suspended in a 1% aqueous solution of Tween 80. Compounds 8-OH-DPAT and WAY 100635 were injected subcutaneously (*sc*), **7a**–**12a** were given interperitoneally (*ip*) in a volume of 10 mg/kg (mice). The obtained data were analyzed by Dunnett's test (when only one drug was given) or by the Newman–Keuls test (when two drugs were administered).

7.2.2.1.2. Body temperature in mice. The effects of the tested compounds given alone on the rectal body temperature in mice (measured with Ellab thermometer) were recorded 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 **8a**–**12a** or 8-OH-DPAT was tested. WAY 100635 was administered 15 min before **8a**–**12a** or 8-OH-DPAT and the rectal body temperature was recorded 30 and 60 min after injection of the tested compounds. In another experiment, the effect of **7a** (which did not change body temperature in mice) on the 8-OH-DPAT (5 mg/kg)-induced hypothermia was tested. Compound **7a** was administered 45 min before 8-OH-DPAT, and the rectal body temperature was measured 15, 30, 45 and 60 min after injection of 8-OH-DPAT. The results were expressed as a change in body temperature (Δt) with respect to basal body temperature, as measured at the beginning of the experiment.

7.2.2.2. Forced swimming test in mice

7.2.2.2.1. Animals and housing. The experiments were performed on male Albino Swiss mice (24–28 g; purchased from licenced dealer Staniszewska, Ilkowice, Poland). The animals were kept in groups of 20 in a cage (60 x 38 x 20 cm) at a temperature of 20 ± 1 °C, and had free access to food (standard laboratory pallets) and tap water before experiment. All investigations were conducted in the light phase, on a natural light/dark cycle (January–February), between 9 AM and 2 PM. Each experimental group consisted of 9–10 animals per drug dose. Animals were tested in a counterbalanced order and were used only once in each test. The experiments were performed by an observer unaware of the treatment. All the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

7.2.2.2.2. Drug treatment. The following drugs were used 8-OH-DPAT (Research Biochemical Inc.), compounds **8a**, **10a**, **11a** and **12a**. All compounds were suspended in a 1% aqueous solution of Tween 80. Compound 8-OH-DPAT was injected subcutaneously (*sc*), **8a**, **10a**, **11a**, and **12a** were given interperitoneally (*ip*), 30 min before the test in a volume of 10 ml/kg (mice).

7.2.2.2.3. Forced swimming test in mice. The experiment was carried out according to the method of Porsolt et al. [23]. Briefly, the mice were individually placed in a glass cylinder (25 cm high, 10 cm in diameter) containing 6 cm of water maintained at 23–25 °C, and were left therein for 6 min. A mouse was regarded as immobile when it remained floating on water, making only small movements to keep its head above it. The total duration of immobility was measured by an experimenter running the final 4 min of 6-min test session, after a 2-min habituation period.

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

7.2.2.2.5. Statistical analysis. The obtained data were presented as the mean ± SEM. Comparisons between groups were carried out by a one-way analysis of variance (ANOVA) followed by intergroup comparisons using the Dunnett's test. A p value < 0.05 was considered significant.

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