New Arylpiperazine 5-HT$_{1A}$ Receptor Ligands Containing the Pyrimido[2,1-*f*]purine Fragment: Synthesis, in Vitro, and in Vivo Pharmacological Evaluation

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Received July 4, 2003

New 1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione derivatives of arylpiperazine (**11**−**22**) were prepared and evaluated in vitro for their affinity for 5-HT$_{1A}$, 5-HT$_{2A}$, α_1, and D$_2$ receptors. The tested compounds showed high affinity for 5-HT$_{1A}$ and α_1 receptors (K_i = 1.1−87 and 10−62 nM, respectively) and moderate to low affinity for 5-HT$_{2A}$ (K_i = 56−881 nM) and D$_2$ receptors (K_i = 94−1245 nM). Compounds **14**, **15**, **18**, **19**, and **21**, mostly 3′-chlorophenylpiperazine derivatives, can be classified as mixed 5-HT$_{1A}$/5-HT$_{2A}$/α_1 ligands. Compound **13**, which showed the highest 5-HT$_{1A}$ receptor affinity (K_i = 1.1 nM), was 50-fold selective in relation to α_1 adrenoceptors and at least 250-fold over 5-HT$_{2A}$ and D$_2$ sites. On the basis of in vivo functional tests, 8-phenylpiperazinoethylamino (**11**), 8-(2′-methoxyphenylpiperazino)ethylamino (**13**), and 8-phenylpiperazinopropylamino (**14**) derivatives of 1,3-dimethyl-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione were identified as potent pre- and postsynaptic 5-HT$_{1A}$ receptor antagonists. 1,3-Dimethyl-7-bromo-8-(phenylpiperazinopropylamino)-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (**20**) behaved like an agonist of presynaptic and as a partial agonist of postsynaptic 5-HT$_{1A}$ receptors and resembled ipsapirone in terms of functional intrinsic activity. It revealed marked anxiolytic-like activity in the Vogel test in rats, comparable to that of the reference drug diazepam, and exhibited antidepressant-like activity in the Porsolt test in rats. The sedative effect of **20**, evaluated in the open field test in rats, appeared at doses twice as high as those inducing a minimal anxiolytic-like effect and was similar to the effects of diazepam.

Introduction

Psychiatric disorders such as depression, psychosis, or anxiety are closely connected with disturbances in the function of different neuromediator systems. In a systematic attempt to look for more effective treatments of these disorders, it is essential to search for either new drugs or new tool substances useful in exploring complicated neurochemical mechanisms.

Arylpiperazine is a core fragment of many bioactive compounds exhibiting a variety of pharmacological effects. It has been shown that their action can be mediated by different subpopulations of serotonin (5-hydroxytryptamine, 5-HT), dopamine, and adrenergic receptors.[1] Such a multireceptor potential implicates their frequent use as a source of new agents with different therapeutic properties.

The most thoroughly studied group of arylpiperazine derivatives, called long-chain arylpiperazines (LCAPs), have been found as serotonin receptor ligands, in particular 5-HT$_{1A}$ and 5-HT$_{2A}$ ones.[2,3] Their general chemical structure contains an alkyl chain (two to four methylene units) attached to the N4 atom of the piperazine moiety and a terminal amide or an imide fragment. The significance of the respective parts of LCAPs for 5-HT$_{1A}$ receptor affinity, intrinsic activity, and selectivity has been the subject of many structure−activity relationship studies.[1] In particular, much effort has been devoted to the role of the terminal part in a ligand−receptor interaction; in consequence, a great many different fragments were used.[4]

In our earlier attempt to find new 5-HT$_{1A}$ and/or 5-HT$_{2A}$ receptor ligands, a series of arylpiperazinealkyl derivatives with a complex terminal part based on the purine moiety had been synthesized (Figure 1).[5,6] The majority of the obtained compounds showed high (K_i < 50 nM) or very high (K_i < 10 nM) 5-HT$_{1A}$ receptor affinity and a diversified pharmacological profile.[6,7] In such a relatively uniform group of 5-HT$_{1A}$ receptor ligands, full (**1**) and partial (**2**) agonists or antagonists (**3**) were found.[6]

On the basis of the above data, we synthesized 12 new compounds (**11**−**22**) with a novel pyrimido[2,1-*f*]purine fragment as a terminal part of LCAPs (Table 1). To explore its influence on the serotonergic activity, the original, tricyclic terminus was combined with the most thoroughly studied phenylpiperazines (i.e., unsubstituted, *m*-Cl or *o*-OCH$_3$) via a three- or a four-unit spacer

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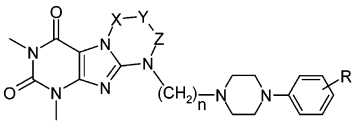
10.1021/jm030946u CCC: $27.50
Published on Web 04/10/2004



compd	X-Y	Z	n	R	K_i [nM] 5-HT_{1A}	K_i [nM] 5-HT_{2A}
1	CH=CH	CO	3	*o*-OCH_3	10.4	3882
2	CH_2–CH_2	CH_2	3	*o*-OCH_3	17.2	562
3	CH=CH	CO	3	H	25.0	4680
4	CH_2–CH_2	CH_2	4	H	2.1	243

Figure 1. Examples of previously synthesized arylpiperazinealkylpurine derivatives.

chain. All the new compounds were tested for their affinity for 5-HT_{1A} and 5-HT_{2A} receptors; moreover, with respect to the potential multireceptor profile of such derivatives, binding affinities for dopaminergic D_2 and α_1 adrenoceptors were also evaluated.

On the basis of binding studies, a functional 5-HT_{1A} receptor profile was determined for all the compounds. Additionally, 5-HT_{2A} receptor antagonistic activity in vivo was assessed for the most in vitro active derivatives (**14**, **15**, **18**, and **21**). Compound **20**, which showed the most interesting 5-HT_{1A} receptor functional profile, was examined in animal models of anxiety and depression.

Chemistry

We used 1,3-dimethyl-9-benzyl-1*H*,3*H*,9*H*-pyrimido-[2,1-*f*]purine-2,4,8-trione (**5**) and 1,3-dimethyl-7-bromo-9-benzyl-1*H*,3*H*,9*H*-pyrimido[2,1-*f*]purine-2,4,8-trione (**6**), prepared according to the methods described earlier, as starting tricyclic theophylline derivatives.[8] Those compounds were debenzylated in a 90% sulfuric acid environment yielding compounds **7** and **8**, which were subsequently treated with phosphorus oxychloride to obtain 1,3-dimethyl-8-chloro-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (**9**) and 1,3-dimethyl-7-bromo-8-chloro-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (**10**). Those compounds were converted into target substances by coupling them with the appropriate phenylpiperazinealkylamine derivatives (Scheme 1). The tricyclic 7-bromo-8-chloro derivative **10** reacted with amines only in position 8, yielding compounds with an unchanged 7-bromo substituent, which was confirmed by spectral (MS and ^{1}H NMR) analyses. The phenylpiperazinealkylamines used were prepared according to the Gabriel method.[9] For pharmacological studies, all the final compounds (**11**−**22**) were converted into their hydrochloride salts, better dissolved in water.

Pharmacology

The newly synthesized compounds were tested in vitro for their ability to bind to central serotonin 5-HT_{1A} and 5-HT_{2A}, D_2, and α_1 receptors.

All the compounds prepared in this study were tested in vivo to evaluate their functional activity at 5-HT_{1A} receptors; additionally, in the cases of **14**, **15**, **18**, and **21**, their potential central 5-HT_{2A} receptor antagonistic activity was assessed.

The functional activity of the investigated compounds at 5-HT_{1A} receptors was tested in several commonly used in vivo models. It was demonstrated previously that the hypothermia induced by the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) in mice was connected with the activation of presynaptic 5-HT_{1A} receptors[10,11] and was abolished by 5-HT_{1A} receptor antagonists such as *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-piridinyl)cyclohexanecarboxamide (WAY 100635)[12] or 4-[3-(benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine (MP 3022).[13] Thus, the hypothermia produced by the compounds tested in mice (and reduced by WAY 100635, a well-known 5-HT_{1A} receptor antagonist) was regarded as a measure of presynaptic 5-HT_{1A} receptor agonistic activity. Similarly, the ability of those compounds to inhibit the 8-OH-DPAT-induced hypothermia was taken as a measure of presynaptic antagonistic activity. To determine a postsynaptic 5-HT_{1A} receptor agonistic effect of the tested 5-HT_{1A} ligands, their ability to induce lower lip retraction (LLR) in rats and behavioral syndrome, i.e., flat body posture (FBP) and forepaw treading (FT), in reserpinized rats was tested. The 8-OH-DPAT-induced LLR and behavioral syndrome in rats depended on stimulation of postsynaptic 5-HT_{1A} receptors;[14−16] moreover, it was shown that those symptoms were sensitive to 5-HT_{1A} receptor antagonists.[12,13] Hence, the ability of the investigated compounds to inhibit those symptoms induced by 8-OH-DPAT was regarded as postsynaptic 5-HT_{1A} receptor antagonistic activity.

The ability of the tested compounds to antagonize head twitches in mice, observed after administration of (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)-DOI), a 5-HT_{2A} receptor agonist, was used to evaluate 5-HT_{2A} receptor antagonistic properties.[17]

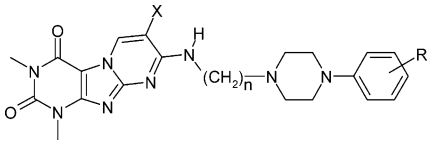
Potential anxiolytic and antidepressant activity of compound **20** was evaluated in the conflict drinking (Vogel) test in rats[18] and the forced swimming test in rats,[19] respectively. Its effect on the exploratory activity of rats was investigated in the open field test.

Results and Discussion

As mentioned in the Introduction, 12 new pyrimido-[2,1-*f*]purine analogues of three common phenylpiperazines were synthesized. Slight structural modifications concerned the spacer chain length (three to four units) and the introduction of a bromine atom into position 7 of the crucial heterocyclic system in order to increase the compound lipophilicity. As anticipated on the basis of our previous investigations, all the compounds tested were potent 5-HT_{1A} receptor ligands. Besides the outstanding 5-HT_{1A} receptor affinity of compound **13** (K_i = 1.1 nM), the K_i values of all other compounds were in a relatively narrow range, from 11 nM (**11** and **20**) to 87 nM (**17**). Generally, the introduction of a bromine atom into compounds with a shorter spacer has a negative impact on 5-HT_{1A} receptor affinity (which is most clearly visible in the case of methoxy derivatives **13** vs **19**; K_i = 1.1 and 62 nM, respectively), but it is practically without effect in the case of derivatives with a longer chain.

The 5-HT_{2A} receptor affinity of the tested compounds was generally lower than those observed for 5-HT_{1A} receptors and ranged from 56 to 880 nM for **15** and **22**, respectively. This observation is in line with our previous data on pyrimido[2,1-*f*]purines;[6,7] however, in the present study, five compounds showed affinity lower than 100 nM (**14**, **15**, **18**, **19**, and **21**). These five compounds can be regarded as mixed 5-HT_{1A}/5-HT_{2A} receptor ligands; furthermore, it is noteworthy that three of them contain an *m*-Cl-phenylpiperazine fragment.

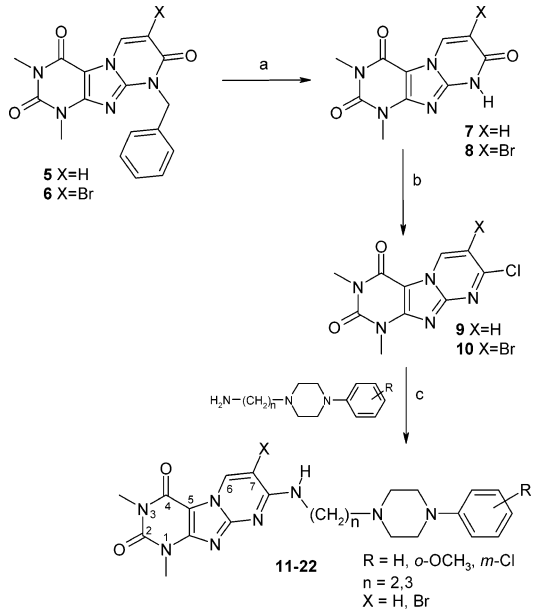
Table 1. Affinities of Compounds **11**−**22** for 5-HT_{1A}, 5-HT_{2A}, D_2, and α_1 Receptors and Their Pre- and Postsynaptic 5-HT_{1A} in Vivo Functional Profile[a]



	substituents			receptor binding, $K_i \pm$ SEM (nM)				functional profile		
								5-HT_{1A}		
compd	X	*n*	R	5-HT_{1A}	5-HT_{2A}	D_2	α_1	presynaptic	postsynaptic	5-HT_{2A}
11	H	2	H	11.5 ± 0.1	326 ± 30	nd	nd	antagonist	antagonist	nd
12	H	2	3′-Cl	23 ± 4	234 ± 11	182 ± 15	29 ± 4	agonist	antagonist	nd
13	H	2	2′-OCH_3	1.1 ± 0.5	246 ± 6	1330 ± 550	58 ± 12	antagonist	antagonist	nd
14	H	3	H	19 ± 3	72 ± 2	430 ± 4	25 ± 9	antagonist	antagonist	antagonist
15	H	3	3′-Cl	46 ± 3	56 ± 2	94 ± 13	21 ± 4	agonist	antagonist	antagonist
16	H	3	2′-OCH_3	34 ± 3	835 ± 7	1175 ± 410	33 ± 1		antagonist	nd
17	Br	2	H	87 ± 8	440 ± 40	1245 ± 730	nd		antagonist	nd
18	Br	2	3′-Cl	68 ± 7	64 ± 10	165 ± 20	nd		antagonist	antagonist
19	Br	2	2′-OCH_3	62 ± 5	86 ± 12	226 ± 3	62 ± 2	agonist	antagonist	nd
20	Br	3	H	11 ± 0.1	297 ± 20	414 ± 120	11 ± 2	agonist	partial agonist	nd
21	Br	3	3′-Cl	49 ± 6	85 ± 8	264 ± 12	46 ± 4	agonist	antagonist	antagonist
22	Br	3	2′-OCH_3	36 ± 2	880 ± 12	325 ± 39	38 ± 5	agonist	antagonist	nd

[a] nd: not determined.

Scheme 1[a]



[a] Conditions: (a) 90% H_2SO_4; (b) $POCl_3$, reflux; (c) K_2CO_3, EtOH, reflux.

The majority of the tested compounds show moderate (K_i = 160−430 nM; **12**, **14** and **18**−**22**) or low (K_i = 1180−1330 nM; **13**, **16**, and **17**) affinity for D_2 receptors. Since the most active derivative at D_2 sites, **15** (K_i = 94 nM), also significantly binds to 5-HT_{1A} and 5-HT_{2A} receptors, its profile resembles those of atypical antipsychotic agents. Regarding α_1 adrenoceptors, all the compounds tested were found to be active, and except for derivative **13**, their K_i values were similar to those obtained for 5-HT_{1A} receptors. The above-discussed results are not surprising, since there is great similarity between 5-HT_{1A} and α_1 receptors in the amino acid sequence,[20] especially in the ligand binding region.

In summary, our in vitro binding study has shown that the investigated compounds actually possess a multireceptor profile and that most of them are devoid of 5-HT_{1A}/α_1 selectivity. The only exception, compound **13** showing the highest 5-HT_{1A} receptor affinity, was 50-fold selective in relation to α_1 adrenoceptors and at least 250-fold selective over 5-HT_{2A} and D_2 sites. Further studies, based on the already known 5-HT_{1A}/α_1 selectivity, are necessary to reduce the α_1-adrenoreceptor affinity of pyrimido[2,1-*f*]purines.

As already mentioned in the Introduction, the previously described analogues of the tested compounds reveal different intrinsic activity at 5-HT_{1A} receptors.[6,7] For this reason, functional in vivo studies were also carried out with all the new derivatives (**11**−**22**). In addition, potential central 5-HT_{2A} receptor antagonistic activity was assessed for **14**, **15**, **18**, and **21**.

The tested compounds **12** and **15**−**22** administered alone (like 8-OH-DPAT, a 5-HT_{1A} receptor agonist) produced hypothermia in mice, whereas compounds **11**, **13**, and **14** (like WAY 100635, a 5-HT_{1A} receptor antagonist) did not change body temperature in mice (see Supporting Information Table 1). The hypothermias evoked by **12**, **15, 19**, **20**, **21**, or **22** were reduced or abolished (like that induced by 8-OH-DPAT) by WAY 100635 (see Supporting Information, Table 2). These results suggest that **12**, **15**, and **19**−**22** show features of presynaptic 5-HT_{1A} receptor agonists. At the same time, WAY 100635 did not change the hypothermic effects induced by **16**, **17**, or **18** (see Supporting Information Table 2), so it seems that the presynaptic 5-HT_{1A} receptor activity of these compounds is negligible in this experimental paradigm. On the other hand, compounds **11**, **13**, and **14** reduced the 8-OH-DPAT-induced hypothermia in mice, while WAY 100635 completely abolished the effect of 8-OH-DPAT in that test (see Supporting Information Table 3); hence, these derivatives may by classified as presynaptic 5-HT_{1A} receptor antagonists.

In behavioral experiments with rats, used to evaluate postsynaptic 5-HT_{1A} receptor activity, analogue **20** (10 mg/kg) given alone induced weak LLR in rats; on the other hand, the other compounds (5−20 mg/kg) like WAY 100635 did not mimic the effect of 8-OH-DPAT in that test (data not shown). The LLR induced by 8-OH-DPAT was significantly reduced by all the compounds

tested (including **20**) or by WAY 100635 (see Supporting Information Table 4). Therefore, in the LLR model in rats, compound **20** can be classified as a partial agonist, and compounds **11**−**19**, **21**, and **22** can be classified as antagonists of postsynaptic 5-HT_{1A} receptors. Successive results obtained in the behavioral syndrome (FBP and FT) model in reserpinized rats showed that none of the investigated compounds given alone (up to 20 mg/kg) mimicked the effect of 8-OH-DPAT (data not shown). On the other hand, **11**−**17**, **19**, **20**, and **22** effectively reduced the 8-OH-DPAT-induced symptoms. Compounds **18** and **21** only slightly reduced FT but failed to inhibit the FBP produced by 8-OH-DPAT (see Supporting Information Table 4). These results indicate that the tested compounds **11**−**22** exhibited features of postsynaptic 5-HT_{1A} receptor antagonists in reserpinized rats, their activity being diversified though.

Moreover, compounds **14**, **15**, **18**, and **21**, which have almost identical 5-HT_{2A} receptor affinities, behaved like 5-HT_{2A} receptor antagonists; in fact, like ketanserin, a 5-HT_{2A} receptor antagonist, they effectively inhibited the (±)-DOI-induced head twitches in mice, but their ID_{50} values ranged between 4.5 and 22.1 mg/kg (see Supporting Information Table 5). The reason for such variety of doses is not clear, but it should be borne in mind that compounds **18** and **21** (with the lowest ID_{50} values) contain a bromine atom in position 7 of the pyrimido[2,1-*f*]purine moiety, which by increasing their lipophilicity may facilitate their brain penetration.

The in vivo obtained results indicate that, like the previously described derivatives **1**−**3**,[6,7] the tested 5-HT_{1A} ligands, pyrimido[2,1-*f*]purine derivatives, were characterized by their diverse in vivo activity at 5-HT_{1A} receptors. In fact, in the models used, those compounds showed features of antagonists of pre- and postsynaptic 5-HT_{1A} receptors (**11**, **13**, and **14**), of antagonists of postsynaptic sites (**16**−**19**), of agonists of presynaptic sites and antagonists of postsynaptic sites (**12**, **15**, **21**, and **22**), or of agonist of presynaptic receptors and partial agonist of postsynaptic 5-HT_{1A} receptors (**20**). Moreover, compounds **14**, **15**, **18**, and **21** can be classified as weak 5-HT_{2A} receptor antagonists (Table 1).

The functional profile of the investigated 5-HT_{1A} receptor ligands suggests that some of them may show anxiolytic- and/or antidepressant-like activity. In fact, several authors have reported that 5-HT_{1A} receptor antagonists (including WAY 100635) as well as agonists of presynaptic and antagonists of postsynaptic 5-HT_{1A} receptors exert anxiolytic-like activity in animals.[21−29] It should, however, be kept in mind that the possibility of using 5-HT_{1A} receptor antagonists (silent or with some presynaptic intrinsic activity) must be further validated.

Regarding mixed 5-HT_{1A}/5-HT_{2A} receptor antagonists (**14**, **15**, **18**, **19**, and **21**), it cannot be excluded that such a combined activity may be beneficial to anxiolytic properties. For example, Griebel et al.[30] demonstrated that the 5-HT_{1A}/5-HT_{2A} receptor antagonist S 21357 (3-(2-[4-(-4-fluorobenzoyl)piperidino]ethyl)-6-([4-(2-methoxyphenyl)-1-piperazinyl]-1-*n*-butyl)benzothiazolin-2-one) produced anxiolytic-like effects in animals. However, at present, more interest has focused on investigations into the potential clinical activity of compounds acting like 5-HT_{1A} receptor agonists and 5-HT_{2A} receptor antagonists.[31] It is noteworthy that in in vivo functional tests, compound **20** behaved like the well-known anxiolytic ipsapirone, a partial 5-HT_{1A} receptor agonist.[14,32,33] Taking into account the functional activity of **20**, that compound was selected as a potential anxiolytic and/or antidepressant for further in vivo preclinical studies. Our results show that **20** exerts anxiolytic-like activity in the conflict drinking test in rats (Table 2). Its effect seems to be specifically anxiolytic because when it is given in doses evoking anticonflict activity, **20** affects neither the shock threshold nor the nonpunished water consumption (data not shown). It should be noted that the anticonflict effect of **20** is comparable, in terms of its potency and active doses, with the effect produced by diazepam (used a reference drug) (Table 2) and ipsapirone.[34] Buspirone, another 5-HT_{1A} receptor partial agonist and an anxiolytic drug, induced anticonflict effects in rats at doses lower than those of **20** or ipsapirone, but its dose−response curve was bell-shaped.[35] The results of our successive experiments also show that **20** reduces immobility time in the despair test in rats, this effect not being dose-dependent though (Table 3). The typical antidepressant imipramine, used as a reference drug, exerts dose-dependent and distinct activity in this model (Table 3). In contrast, buspirone and ipsapirone do not affect the immobility time in rats, but they produce antidepressant-like activity in rats pretreated with an inhibitor of drug metabolism in this test.[36] It has also been shown that **20** inhibits the exploratory activity of rats at a dose at least twice as high as this, producing a minimal but statistically significant effect in the conflict drinking test or the forced swimming test. Diazepam also shows a sedative effect in rats at a dose twice as high as this, inducing a minimal anxiolytic-like effect, whereas imipramine at doses active in the forced swimming test does not change

Table 2. Effect of Compound **20** and Diazepam in the Conflict Drinking Test in Rats[a]

treatment	dose, mg/kg	no. of shocks accepted/ 5 min, mean ± SEM	%
vehicle		7.4 ± 0.8	100
20	2.5	15.8 ± 2.9	213.5
	5	36.4 ± 4.7[b]	491.9
	10	54.3 ± 3.3[b]	733.8
vehicle		7.7 ± 0.1	100
diazepam	2.5	23.6 ± 2.7[b]	306.5
	5	41.1 ± 6.7[b]	533.8
	10	52.8 ± 5.7[b]	685.7

[a] Compound **20** and diazepam were administered 60 min before the test. $n = 8$ rats per group. [b] $p < 0.01$ vs vehicle (Dunnett test).

Table 3. Effect of Compound **20** and Imipramine in the Forced Swimming Test in Rats[a]

treatment	dose, mg/kg	immobility time (s), mean ± SEM	%
vehicle		237.9 ± 7.6	100
20	5	173.9 ± 11.6[b]	73.1
	10	153.1 ± 13.3[b]	64.4
	20	165.6 ± 5.8[b]	69.6
vehicle		189.3 ± 13.1	100
imipramine	20	155.0 ± 14.9	81.9
	30	120.4 ± 7.4[a]	63.3
	40	99.7 ± 8.8[a]	52.7

[a] Compound **20** and imipramine were administered 60 min before the test. $n = 8$ rats per group. [b] $p < 0.01$ vs vehicle (Dunnett test).

the exploratory activity of rats (see Supporting Information Table 6).

Conclusions

We synthesized a series of arylpiperazine 5-HT_{1A} ligands containing the novel pyrimido[2,1-*f*]purine fragment. All the new compounds showed high in vitro activity at 5-HT_{1A} receptors; moreover, five of them could be regarded as mixed 5-HT_{1A}/5-HT_{2A} ligands. Compound **13**, the most potent 5-HT_{1A} receptor ligand ($K_i = 1.1$ nM), was also the most selective in relation to α_1 adrenoceptors, 5-HT_{2A}, and D_2 sites (50-, 250-, and 1200-fold, respectively). It was demonstrated that the structural modifications applied had limited influence on the binding profile observed in vitro; on the other hand, the intrinsic in vivo activity at 5-HT_{1A} receptors was diversified. For the first time, three potential pre- and postsynaptic 5-HT_{1A} receptor antagonists (**11**, **13**, and **14**) were identified in arylpiperazinealkylpurine derivatives. In addition, compound **20** (an agonist of presynaptic 5-HT_{1A} receptor and a partial agonist of postsynaptic ones) revealed marked anxiolytic-like activity in the Vogel test in rats and exhibited antidepresant-like activity in the Porsolt test in rats.

Experimental Section

Chemistry. The melting points are given uncorrected. ^{1}H NMR spectra were determined with a Varian BB 200 (300 MHz) instrument. Chemical shifts are expressed in ppm downfield from the internal TMS as a reference. Mass spectra (MS) were recorded using a Finnigan MAP 95-S mass spectrometer (EI mode, 70 eV). Elemental analyses were carried out with a Elementar Vario EL III and were within ±0.4% of the theoretical values. Preparative column chromatography was performed using silica gel 100 (Fluka, 70−230 mesh). The purity of the products were confirmed by the TLC on Merck plates (Kieselgel 60 F_{254}); the appropriate solvents were used, and spots were visualized under the UV light.

1,3-Dimethyl-1*H*,3*H*,9*H*-pyrimido[2,1-*f*]purine-2,4,8-trione (7) was prepared according to the previously described method.[8]

1,3-Dimethyl-7-bromo-1*H*,3*H*,9*H*-pyrimido[2,1-*f*]purine-2,4,8-trione (8). A solution of 20.8 g (0.05 mol) of compound **2** in 90% H_2SO_4 (75 mL) was allowed to react at room temperature for 20 h. Next, 75 mL of water was carefully added to the ice-cooled solution. The precipitated product was filtered off, and the solution was diluted with 350 mL of water. The separated white crystals were filtered off, washed with water, and dried. Yield 15.2 g (93%); mp >330 °C; ^{1}H NMR (DMSO-d_6) δ 3.20 (s, 3H, N3−CH_3), 3.38 (s, 3H, N1−CH_3), 8.64 (s, 1H, C6-*H*). Anal. ($C_{10}H_8N_5O_3Br$) C, H, N.

1,3-Dimethyl-8-chloro-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (9). A mixture of 10.0 g (0.0405 mol) 1,3-dimethyl-1*H*,3*H*,9*H*-pyrimido[2,1-*f*]purine-2,4,8-trione (**7**) with 100 mL of $POCl_3$ was refluxed for 6 h. After cooling, the obtained brown solution was poured on 500 g of ice and left at a room temperature until the reaction of the excess of phosphorus oxychloride was completed. The formed precipitate was filtered off, washed with water, and dried. Yield 10.2 g (95%); mp >330 °C; ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 3H, N3−CH_3), 3.70 (s, 3H, N1−CH_3), 7.18 (d, 1H, C7-*H*), 9.15 (d, 1H, C6-*H*). Anal. ($C_{10}H_8N_5O_2Cl$) C, H, N.

1,3-Dimethyl-7-bromo-8-chloro-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (10). The compound was prepared according to **9**. Yield 70%; mp >330 °C; ^{1}H NMR ($CDCl_3$) δ 3.47 (s, 3H, N3−CH_3), 3.70 (s, 3H, N1−CH_3), 9.42 (s, 1H, C6-*H*). Anal. ($C_{10}H_7N_5O_2BrCl$) C, H, N.

Phenylpiperazinealkylamines. The amines were obtained using a procedure similar to that described earlier by Glennon.[9] The arylpiperazines were alkylated with the appropriate *N*-(bromoalkyl)phthalimide. The obtained products were transformed via hydrazinolysis to the primary amines isolated as free bases by extraction to dichloromethane. After evaporation of the solvent, the final compounds were used without further purification. The obtained phenylpiperazinealkylamines were the following: 2-(4-phenylpiperazin-1-yl)ethylamine, 2-[4-(3-chlorophenyl)piperazin-1-yl]ethylamine, 2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamine, 3-(4-phenylpiperazin-1-yl)propylamine, 3-[4-(3-chlorophenyl)piperazin-1-yl]propylamine, and 3-[4-(2-methoxyphenyl)piperazin-1-yl]-propylamine.

General Procedure for the Preparation of Compounds 11−22. A mixture of 0.003 mol of compound **9** or **10** (for **11**−**16** and **17**−**22** approximately) and a 2-fold excess of the substituted phenylpiperazinalkylamines in 50 mL of 2-methoxyethanol were heated under reflux for 3 h. After evaporation of the solvent, the brown oil residue was dissolved in dichloromethane, dried over Na_2SO_4 and purified by flash column chromatography on silica gel (eluted with dichloromethane/methanol/acetic acid 30:2:1). The obtained products, in the form of acetic acid salts, were treated with an excess of triethylamine and recrystallized from ethanol to yield free bases.

General Procedure for the Preparation of Hydrochlorides. Free bases of compounds **11**−**22** were converted into their hydrochloride salts by treating with an excess of concentrated HCl, evaporating to dryness, and recrystallizating the obtained residue from ethanol/water.

1,3-Dimethyl-8-[2-(4-phenylpiperazin-1-yl)ethylamino]-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (11): obtained in 54% yield from **7** and 2-(4-phenylpiperazine)ethylamine; mp 245−248 °C; ^{1}H NMR ($CDCl_3$) δ 2.65−2.75 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.2−3.3 (m, 4H, $(CH_2)_2N$), 3.44 (s, 3H, N3−CH_3), 3.66 (s, 3H, N1−CH_3), 3.65−3.7 (m, 2H, NHCH_2), 5.99 (t, 1H, N*H*CH$_2$), 6.30 (d, 1H, C7−*H*), 6.8−7.0 (m, 3H, Ph), 7.2−7.35 (m, 2H, Ph), 8.65 (d, 1H, C6-*H*). Anal. ($C_{22}H_{26}N_8O_2$) C, H, N.

1,3-Dimethyl-8-{2-[4-(3-chlorophenyl)piperazin-1-yl]-ethylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (12): obtained in 62% yield from **7** and 2-(4−3′-chlorophenylpiperazine)ethylamine; mp 231−233 °C; ^{1}H NMR ($CDCl_3$) δ 2.62−2.75 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.18−3.23 (m, 4H, $(CH_2)_2N$), 3.46 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.65−3.7 (m, 2H, NHCH_2), 5.94 (t, 1H, N*H*CH$_2$), 6.30 (d, 1H, C7-*H*), 6.75−6.9 (m, 3H, Ph), 7.1−7.2 (m, 1H, Ph), 8.65 (d, 1H, C6-*H*). Anal. ($C_{22}H_{25}N_8O_2Cl$) C, H, N.

1,3-Dimethyl-8-{2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (13): obtained in 72% yield from **7** and 2-(4−2′-methoxyphenylpiperazine)ethylamine; mp 224−225 °C; ^{1}H NMR ($CDCl_3$) δ 2.65−2.77 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.1−3.2 (m, 4H, $(CH_2)_2N$), 3.43 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.65−3.7 (m, 2H, NHCH_2), 3.87 (s, 3H, OCH_3), 6.0 (t, 1H, N*H*CH$_2$), 6.30 (d, 1H, C7-*H*), 6.82−7.05 (m, 4H, Ph), 8.64 (d, 1H, C6-*H*); MS (EI) *m*/*z* (relative intensity) 464 ([M]$^+$, 6), 406 (2), 315 (3), 273 (3), 259 (7), 218 (11), 205 (100), 190 (12), 175 (7), 162 (27), 134 (6), 120 (6), 70 (6). Anal. ($C_{23}H_{28}N_8O_3$) C, H, N.

1,3-Dimethyl-8-[3-(4-phenyl-piperazin-1-yl)propylamino)-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (14): obtained in 70% yield from **7** and 2-(4-phenylpiperazine)propylamine; mp 204−206 °C; ^{1}H NMR ($CDCl_3$) δ 2.17 (m, 2H, $CH_2CH_2CH_2$), 3.0−3.2 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.43 (s, 3H, N3−CH_3), 3.5−3.6 (m, 4H, $(CH_2)_2N$), 3.53 (s, 3H, N1−CH_3), 3.77 (m, 2H, NHCH_2), 6.39 (d, 1H, C7-*H*), 6.7−6.95 (m, 3H, Ph), 7.15−7.25 (m, 2H, Ph), 7.77 (t, 1H, N*H*CH$_2$), 8.61 (d, 1H, C6-*H*). Anal. ($C_{23}H_{28}N_8O_2$) C, H, N.

1,3-Dimethyl-8-{3-[4-(3-chlorophenyl)piperazin-1-yl]-propylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (15): obtained in 63% yield from **7** and 2-(4−3′-chlorophenylpiperazine)propylamine; mp 206−208 °C; ^{1}H NMR ($CDCl_3$) δ 1.87 (q, 2H, $CH_2CH_2CH_2$), 2.6−2.7 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.2−3.25 (m, 4H, $(CH_2)_2N$), 3.43 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.7 (m, 2H, NHCH_2), 6.14 (d, 1H, C7-*H*), 6.78−6.95

(m, 3H, Ph), 7.17−7.21 (m, 1H, Ph), 7.44 (t, 1H, N*H*CH_2), 8.59 (d, 1H, C6-*H*). Anal. ($C_{23}H_{27}N_8O_2Cl$) C, H, N.

1,3-Dimethyl-8-{3-[4-(2-methoxyphenyl)piperazin-1-yl]-propylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (16): obtained in 75% yield from **7** and 2-(4−2′-methoxyphenylpiperazine)propylamine; mp 215−218 °C; ^{1}H NMR ($CDCl_3$) δ 1.86 (q, 2H, $CH_2CH_2CH_2$), 2.61−2.77 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.1−3.2 (m, 4H, $(CH_2)_2N$), 3.43 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.65−3.7 (m, 2H, NHCH_2), 3.86 (s, 3H, OCH_3), 6.15 (d, 1H, C7−*H*), 6.85−7.08 (m, 4H, Ph), 7.90 (t, 1H, N*H*CH_2), 8.57 (d, 1H, C6−*H*); MS (EI) *m*/*z* (relative intensity) 478 ([M]$^+$, 24), 463 (53), 343 (9), 329 (16), 316 (100), 287 (51), 273 (22), 260 (26), 246 (19), 231 (16), 205 (22), 190 (14), 162 (19), 149 (11), 136 (17), 120 (21), 97 (19), 70 (10). Anal. ($C_{24}H_{30}N_8O_3$) C, H, N.

1,3-Dimethyl-7-bromo-8-[2-(4-phenylpiperazin-1-yl)-ethylamino]-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (17): obtained in 51% yield from **8** and 2-(4-phenylpiperazine)-ethylamine; mp 224−225 °C; ^{1}H NMR ($CDCl_3$) δ 2.7−2.85 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.25−3.35 (m, 4H, $(CH_2)_2N$), 3.44 (s, 3H, N3−CH_3), 3.66 (s, 3H, N1−CH_3), 3.75 (m, 2H, NHCH_2), 6.9 (m, 1H, N*H*CH_2), 6.85−7.0 (m, 3H, Ph), 7.23−7.35 (m, 2H, Ph), 8.94 (s, 1H, C6−*H*). Anal. ($C_{22}H_{25}N_8O_2Br$) C, H, N.

1,3-Dimethyl-7-bromo-8-{2-[4-(3-chlorophenyl)piperazin-1-yl]ethylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (18): obtained in 62% yield from **8** and 2-(4−3′-chlorophenylpiperazine)ethylamine; mp 216−218 °C; ^{1}H NMR ($CDCl_3$) δ 3.0−3.2 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.3−3.4 (m, 4H, $(CH_2)_2N$), 3.45 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.65−3.7 (m, 2H, NHCH_2), 6.7−6.95 (m, 3H, Ph), 7.1−7.2 (m, 1H, Ph), 8.0 (t, 1H, N*H*CH_2), 9.0 (s, 1H, C6−*H*). Anal. ($C_{22}H_{24}N_8O_2BrCl$) C, H, N.

1,3-Dimethyl-7-bromo-8-{2-[4-(2-methoxyphenyl)piperazin-1-yl]ethylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (19): obtained in 51% yield from **8** and 2-(4−2′-methoxyphenylpiperazine)ethylamine; mp 217−219 °C; ^{1}H NMR ($CDCl_3$) δ 2.8−2.95 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.1−3.2 (m, 4H, $(CH_2)_2N$), 3.44 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.76 (m, 2H, NHCH_2), 3.87 (s, 3H, OCH_3), 6.82−7.05 (m, 5H, Ph, N*H*CH_2), 8.95 (s, 1H, C6-*H*); MS (EI) *m*/*z* (relative intensity) 544 ([M + 2]$^+$, 15), 542 ([M]$^+$, 14), 380 (10), 378 (11), 352 (5), 350 (8), 205 (100), 190 (14), 169 (22), 150 (13), 111 (18), 109 (15), 97 (38), 70 (20). Anal. ($C_{23}H_{27}N_8O_3Br$) C, H, N.

1,3-Dimethyl-7-bromo-8-[3-(4-phenylpiperazin-1-yl)-propylamino]-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (20): obtained in 64% yield from **8** and 2-(4-phenylpiperazine)-propylamine; mp 226−227 °C; ^{1}H NMR ($CDCl_3$) δ 1.91 (q, 2H, $CH_2CH_2CH_2$), 2.7−2.85 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.25−3.35 (m, 4H, $(CH_2)_2N$), 3.44 (s, 3H, N3−CH_3), 3.66 (s, 3H, N1−CH_3), 3.75 (m, 2H, NHCH_2), 6.9 (m, 1H, N*H*CH_2), 6.85−7.0 (m, 3H, Ph), 7.23−7.35 (m, 2H, Ph), 8.94 (s, 1H, C6-*H*). Anal. ($C_{23}H_{27}N_8O_2Br$) C, H, N.

1,3-Dimethyl-7-bromo-8-{3-[4-(3-chlorophenyl)piperazin-1-yl]propylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (21): obtained in 63% yield from **8** and 2-(4−3′-chlorophenylpiperazine)propylamine; mp 228−230 °C; ^{1}H NMR ($CDCl_3$) δ 1.91 (q, 2H, $CH_2CH_2CH_2$), 2.6−2.75 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.25−3.35 (m, 4H, $(CH_2)_2N$), 3.43 (s, 3H, N3−CH_3), 3.64 (s, 3H, N1−CH_3), 3.74 (m, 2H, NHCH_2), 6.75−6.9 (m, 3H, Ph), 7.15−7.22 (m, 1H, Ph), 7.72 (t, 1H, N*H*CH_2), 8.90 (s, 1H, C6−*H*). Anal. ($C_{23}H_{26}N_8O_2BrCl$) C, H, N.

1,3-Dimethyl-7-bromo-8-{3-[4-(2-methoxyphenyl)piperazin-1-yl]propylamino}-1*H*,3*H*-pyrimido[2,1-*f*]purine-2,4-dione (22): obtained in 71% yield from **8** and 2-(4−2′-methoxyphenylpiperazine)propylamine; mp 242−245 °C; ^{1}H NMR ($CDCl_3$) δ 1.9 (q, 2H, $CH_2CH_2CH_2$), 2.60−2.8 (m, 6H, $CH_2CH_2N(CH_2)_2$), 3.05−3.25 (m, 4H, $(CH_2)_2N$), 3.45 (s, 3H, N3−CH_3), 3.65 (s, 3H, N1−CH_3), 3.75 (m, 2H, NHCH_2), 3.85 (s, 3H, OCH_3), 6.8−7.2 (m, 4H, Ph), 7.85 (t, 1H, N*H*CH_2), 8.9 (s, 1H, C6-*H*); MS (EI) *m*/*z* (relative intensity) 558 ([M + 2]$^+$, 37), 556 ([M]$^+$, 44), 543 (100), 541 (97), 477 (10), 409 (11), 407 (10), 396 (86), 394 (90), 353 (26), 351 (36), 340 (26), 338 (29), 326 (6), 324 (25), 287 (19), 231 (24), 219 (28), 205 (73), 190 (44), 162 (51), 150 (59), 136 (56), 120 (56), 97 (88), 70 (41). Anal. ($C_{24}H_{29}N_8O_3Br$) C, H, N.

In Vitro Studies. Receptor Binding. 5-HT$_{1A}$, 5-HT$_{2A}$, and α_1 Receptor Binding Assays. Radioligand binding experiments were conducted in the hippocampus of the rat brain for 5-HT$_{1A}$ receptors and in the cortex for both 5-HT$_{2A}$ and α_1 receptors according to published procedures.[37,38] The following radioligands were used: [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham), [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals), and [^{3}H]-prazosin (26 Ci/mmol, NEN Chemical) for 5-HT$_{1A}$, 5-HT$_{2A}$, and α_1 receptors, respectively. K_i values were determined on the basis of at least three competition experiments in which 10−14 drug concentrations (10^{-10}−10^{-5} M), run in triplicate, were used.

D$_2$ Dopaminergic Receptor Binding Assay. Competition binding studies were performed in rat striatal membranes prepared according to the previously published procedure.[59] An assay was carried out in a 96-well filter plate (containing glass fiber type C, Millipore), presoaked with 100 μL of an ice-cold 50 mM potassium phosphate buffer (pH 7.4), and filtered using a Millipore vacuum manifold prior to sample addition. Samples of 150 μL aliquots of striatal membrane preparations, 50 μL of the radioligand ([^{3}H]-spiperone, 15.70 Ci/mmol, NEN Chemicals), and either 50 μL of the buffer (for total binding assay) or 50 μL of (±)-butaclamol (5 μM) to determine the unspecific binding, or 50 μL of the compounds to be tested, were added to each well. Additionally, to prevent [^{3}H]-spiperone binding to 5-HT$_{2A}$ receptors, ketanserin (50 nM) was included in the assay buffer. After incubation at 37 °C for 30 min, the binding reaction was terminated by vacuum filtration and the mixture was washed three times with 200 μL of buffer. Radioactivity was determined by liquid scintillation counting in a Beckman LS 6500 apparatus.

K_i values were determined on the basis of at least three competition binding experiments in which 10 drug concentrations, run in triplicate, were used. The Cheng and Prusoff equation was used for K_i calculations.[39]

In Vivo Experiments. The experiments were performed on male Wistar rats (250−300 g) or male Albino Swiss mice (24−28 g). The animals were kept at a room temperature of 20 ± 1 °C on a natural day−night cycle (September−March) and were housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group consisted of six to eight animals per dose, and each animal was used only once. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical, Inc.), reserpine (Ciba), and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridynyl)cyclohexane carboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were used in the form of aqueous solutions. Imipramine hydrochloride (Polfa) was dissolved in saline. Diazepam (Polfa) and the investigated compounds were suspended in a 1% aqueous solution of Tween 80. 8-OH-DPAT, reserpine, and WAY 100635 were injected subcutaneously (sc), and imipramine, diazepam, and all the compounds tested were given intraperitoneally (ip) in a volume of 2 mL/kg (rats) or 10 mL/kg (mice).

Body Temperature in Mice. Effects of the tested compounds given alone on the rectal body temperature of mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after administration. In an separate experiment, effects of **11**, **13**, and **14** (which did not change mouse body temperature) on the 8-OH-DPAT (5 mg/kg) induced hypothermia were assessed. Compounds **11**, **13**, and **14** were administered 45 min before 8-OH-DPAT, and rectal body temperature was measured 15, 30, 45, and 60 min after 8-OH-DPAT injection. In another experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **12** and **15**−**22** was tested. WAY 100635 was administered 15 min before the compounds were tested, and rectal body temperature was recorded 30 and 60 min after injection of 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.

Lower Lip Retraction (LLR) in Rats. LLR was assessed according to the method described by Berendsen et al.[14] The rats were individually placed in cages (30 cm × 25 cm × 25 cm) and were scored three times at 15, 30, and 45 min after administration of the tested compounds, as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The total maximum scores amounted to 3 for each rat. In a separate experiment, the effect of the tested compounds on the LLR induced by 8-OH-DPAT (1 mg/kg) was tested. The compounds were administered 45 min before 8-OH-DPAT, and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

Behavioral Syndrome in Reserpinized Rats. Reserpine (1 mg/kg) was administered 18 h before the test. The rats were individually placed in the experimental cages (30 cm × 25 cm × 25 cm) 5 min before injection of the tested compounds. Observation sessions, lasting 45 s each, began 3 min after injection and were repeated every 3 min. Reciprocal forepaw treading and flat body posture were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense. The total maximum score, of five observation periods, amounted to 15 for each symptom per rat.[16] The effect of the tested compounds on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg) in reserpinized rats was estimated in a independent experiment. The investigated compounds were administered 60 min before 8-OH-DPAT. Observations began 3 min after 8-OH-DPAT administration and were repeated every 3 min for a period of 15 min.

Head Twitches in Mice. To habituate mice to the experimental environment, each animal was randomly transferred to a 12 cm (diameter) × 20 cm (height) glass cage lined with sawdust 20 min before treatment. Head twitches in mice were induced by (±)-DOI (2.5 mg/kg). Immediately after treatment, the head twitches were counted throughout 20 min.[17] The investigated compounds were administered 60 min before (±)-DOI.

Conflict Drinking Test (Vogel Test) in Rats. A modification of the method by Vogel et al.[18] was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a Plexiglas box (27 cm × 27 cm × 50 cm) equipped with a grid floor made of stainless steel bars and a drinking bottle containing tap water. After an initial adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for another 10 min adaptation period during which they had free access to the drinking bottle. Afterward, they were allowed a 30 min free-drinking session in their home cage. After another 24 h period of water deprivation, the rats (which drank water the day before) were placed again in the test chamber and allowed to drink for 30 s. Immediately afterward, drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the preceding shock delivery) at 1 s periods between the grid floor and the spout of the drinking bottle. The shocks accepted throughout a 5 min experimental session were counted by an experimenter who observed the behavioral reaction (e.g., body jerks) of rats to an electric shock. The tested compound was administered 60 min before the test.

Shock Threshold and Free-Drinking Tests in Rats. To control the possibility of occurrence of drug-induced changes in the perception of a stimulus or in the thirst drive, which might contribute to the activity in the conflict drinking test, stimulus threshold measurements and a free-drinking experiment were also carried out. In both cases, the rats were treated before the experiment in the same manner as described in the conflict drinking test, including two 24 h water deprivation periods separated by 30 min of water availability. In the shock threshold test, the rats were placed individually in the box, and electric shocks were delivered through the grid floor. The shock threshold was determined stepwise at 15 s shock-free intervals by manually increasing the current (0.1, 0.2, 0.3, 0.4, and 0.5 mA). A shock lasted 1 s and was delivered through the grid floor until a rat showed an avoidance reaction (jump or jerk) to the electric stimulus. In the free-drinking test, each animal was allowed to drink from the water spout. Licking was not punished. The total amount of water (mL) consumed during 5 min was recorded for each rat. The tested compound was administered 60 min before the tests.

Forced Swimming Test in Rats. The studies were carried out on rats using the method of Porsolt et al.[19] Briefly, the animals were placed individually in Plexiglas cylinders (40 cm high, 18 cm in diameter) containing 15 cm of water maintained at 25 °C. After 15 min they were transferred to a drying room (30 °C) for 30 min. They were replaced in the cylinder 24 h later, and the total immobility time was measured during a 5 min test. The tested compound was administered 60 min before the test.

Open Field Test in Rats. The open field consisted of a circular arena, 1 m in diameter without walls, which was divided into six symmetrical sectors and illuminated with a 75 W electric bulb hung 75 cm directly above it. The laboratory was dark for all the experiments. Individual controls or drug-injected animals were placed gently at the center of the arena and were allowed to explore freely. The walking time, ambulation (the number of crossings of sector lines), and the number of rearing and peeping (looking over the edge of the arena) episodes were recorded for 3 min. The tested compound was administered 60 min before the test.

Statistics. The obtained data were analyzed by a one-way ANOVA, followed by Dunnett's test (when only one drug was given) or by the Newman−Keuls test (when two drugs were administered). ID_{50} values were calculated by the method of Litchfield and Wilcoxon.

Acknowledgment. The study was supported by Grant No. 4P05F 035 17 from the State Committee for Scientific Research (KBN), Warszawa, Poland.

Supporting Information Available: Six tables listing the effect of the investigated compounds on the body temperature in mice, the effect of WAY 100635 on the hypothermia induced by **12** and **15**−**22** in mice, the effect of **11**, **13**, and **14** on the 8-OH-DPAT-induced hypothermia in mice, the effects of the investigated compounds on 8-OH-DPAT-induced LLR in rats and 8-OH-DPAT-induced behavioral syndrome in reserpinized rats, the effects of **14**, **15**, **18**, **21**, and ketanserin on the (±)-DOI-induced head twitch response in mice, and the effect of **20**, imipramine, and Diazepam on exploratory activity in the open field test in rats. This material is available free of charge via the Internet at http://pubs.acs.org.

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