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A new putative 5-HT_{1A} receptor antagonist of the 1-arylpiperazine class of ligands

E Chojnacka-Wójcik[1], A Kłodzińska[1], A Drabczyńska[2], M Pawłowski[3], S Charakchieva-Minol[1], G Chłoń[3], M Gorczyca[3]

[1]*Institute of Pharmacology, Polish Academy of Sciences, 12, Smętna St, 31-343 Krakow;*
[2]*Department of Chemical Technology of Drugs, Collegium Medicum, Jagiellonian University;*
[3]*Department of Pharmaceutical Chemistry, Collegium Medicum, Jagiellonian University, 9, Medyczna St, 30-688 Krakow, Poland*

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Summary — 5-HT_{1A} and 5-HT_{2A} receptor affinities of several ω-alkyl-1-arylpiperazines containing a terminal pyrimidopurine or 1,3-diazepinopurine ring system are reported. Several behavioral models demonstrated that 1,3-dimethyl-9-[3-(4-phenyl-1-piperazinyl)propyl]-2,4,8-trioxo-1,3,9-trihydropyrimidino[2,1-*f*]purine **8** and its analogs **5** and **6** may be classified as 5-HT_{1A} postsynaptic antagonists, whereas **7**, **9** and **10** are partial agonists of 5-HT_{1A} receptors.

1-arylpiperazine / 5-HT_{1A}-antagonist / 5-HT_{1A}-partial agonist

Introduction

1-Arylpiperazines constitute one of the most important classes of the 5-HT_{1A} receptor ligands. The majority are classified as partial agonists of 5-HT_{1A} receptors [1, 2]. Only two examples of full (presynaptic and postsynaptic) agonists of 5-HT_{1A} receptors of this class of ligands have been reported in the literature: E-4424 **1** [3] and SUN-8399 **2** [4]. Although the postsynaptic antagonism of 1-arylpiperazines at 5-HT_{1A} receptors may be regarded as their common pharmacological property [1, 2, 5, 6], only two derivatives of 1-(*o*-methoxyphenyl)piperazine are classified as full, pre- and postsynaptic antagonists of these receptors; they are (S)-WAY-100135 **3** [7, 8] and MP-3022 **4** [9].

As a continuation of our research program to develop compounds which show antagonism towards 5-HT_{1A} receptors, in the present paper we describe some structure–affinity relationships and pharmacological properties of several 1-arylpiperazines **5–11** which contain a complex heterocyclic ring system in their structure.

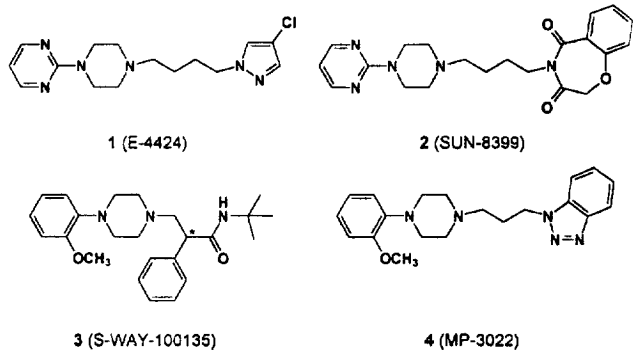


Chart 1. Structure of full (presynaptic and postsynaptic) 5-HT_{1A} receptor agonists (**1**, **2**) and antagonists (**3**, **4**).

Chemistry

The following compounds are known and were synthesized according to the published procedures: **5**, **7** [10], **6** [11], **8** [12] and **9** [13]. Two additional derivatives **10** and **11** were prepared by condensation of the chloropropyl substrate **12** or **13** with 1-(2-pyrimidinyl)piperazine in the boiling 2-methoxyethanol, with yields of 63 and 74%, respectively (scheme 1).

Abbreviations: MM-77: 1-(2-methoxyphenyl)-4-[(4-succinimido)butyl]piperazine; NAN-190: 1-(2-methoxyphenyl)-4-[(*N*-phthalimido)butyl]piperazine.

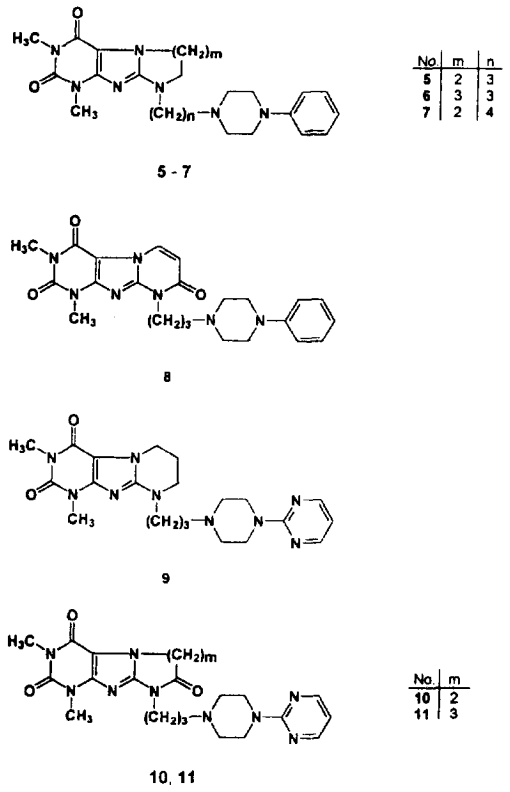
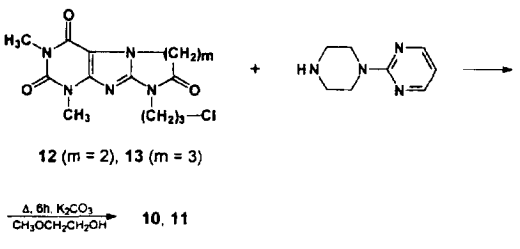


Chart 2. Structures of the investigated compounds.

Pharmacology

All the target compounds **5–11** were evaluated for their affinities for 5-HT_{1A} and 5-HT_{2A} receptors by determining their ability to displace [^{3}H]-8-OH-DPAT and [^{3}H]-ketanserin, respectively, from the rat hippocampus or cortex membranes. The affinity of **8** to α_1 receptors was determined using [^{3}H]prazosin and the rat cortex. The results are shown in table I.

The functional activity of the investigated compounds **5–11** at presynaptic and postsynaptic



Scheme 1.

Table I. 5-HT_{1A} and 5-HT_{2A} receptor affinities of compounds **5–11**.

Compound	*K_i ± SEM (nM)*[a]		*Selectivity*
	5-HT_{1A}	*5-HT_{2A}*	*5-HT_{2A}/5-HT_{1A}*
5	16 ± 2	422 ± 51	26
6	31 ± 2	258 ± 14	8.3
7	2.1 ± 0.2	243 ± 46	116
8[b]	25 ± 2	4680 ± 50	187
9	36 ± 4	8140 ± 540	226
10	42 ± 4	28 000 ± 2000	667
11	474 ± 58	38 800 ± 990	82

[a]Mean values from at least three independent experiments; [b]K_i = 44 ± 6 nM for α_1 adrenoreceptors.

5-HT_{1A} receptors was demonstrated in several commonly used behavioral models. The 8-OH-DPAT-induced behavioral syndrome (flat body posture and forepaw treading) in reserpinized rats, and the lower lip retraction (LLR) in rats were mediated by postsynaptic receptors [14–16]. However, 8-OH-DPAT-induced hypothermia in mice was mediated by presynaptic 5-HT_{1A} receptors [17, 18]. The results of *in vivo* studies are reported in tables II–IV.

Results and discussion

All the investigated compounds showed a high to moderate 5-HT_{1A} receptor affinity, which ranged from 10^{-9} to 10^{-7} M. On the other hand, compounds **5–7** had an only moderate affinity for 5-HT_{1A} receptors, whereas **8–11** could bind to 5-HT_{2A} receptors in the micromolar range (10^{-6}–10^{-5} M). Hence derivatives **5–10** are highly potent 5-HT_{1A} receptor ligands of different selectivity towards 5-HT_{2A} receptors as shown in table I. It should be stressed, however, that the 5-HT_{2A} affinity of **7** (K_i = 243 nM) should not be neglected. A few tentative conclusions on the structure–affinity relationships may also be drawn on the basis of the 5-HT_{1A} binding data presented in table I. The enlargement of the ring built into the terminal heterocyclic fragment significantly decreased the observed 5-HT_{1A} affinity, if **5** was compared with **6**, or **10** with **11**. The elongation of the alkenyl chain from three (**6**) to four (**7**) carbon atoms resulted in a potent enhancement of the 5-HT_{1A} affinity, often observed for this class of ligands [1, 2]. However, the replacement of the methylene group with the carbonyl one (*cf* **5** and **8**, or **9** and **10**) did not contribute to the 5-HT_{1A} receptor affinity (table I).

Compounds **5–10** in the highest doses used antagonized the 8-OH-DPAT-induced behavioral syn-

Table II. The effect of **5–11** on the 8-OH-DPAT-induced (A) behavioral syndrome in reserpine-pretreated rats[a] and (B) lower lip retraction (LLR)[b], and (C) induction of LLR by the investigated compounds in rats[c].

Treatment	*Dose (mg/kg)*	*Mean ± SEM behavioral score*			
		(A)		*(B)*	*(C)*
		Flat body posture	*Forepaw treading*	*LLR*	
Vehicle	–	14.7 ± 0.3	13.5 ± 0.6	2.7 ± 0.1	0.3 ± 0.1
5	5	6.0 ± 1.2[d]	6.4 ± 1.7[d]	NT	NT
	10	3.7 ± 1.2[d]	2.6 ± 1.3[d]	2.8 ± 0.4	0.7 ± 0.3
	20	NT	NT	1.7 ± 0.2[d]	0.9 ± 0.3
Vehicle	–	14.7 ± 0.3	13.5 ± 0.6	2.7 ± 0.1	0.1 ± 0.1
6	5	5.5 ± 1.0[d]	6.1 ± 1.7[d]	NT	NT
	10	5.5 ± 1.3[d]	3.2 ± 1.2[d]	2.3 ± 0.2	0.1 ± 0.1
	20	NT	NT	0.7 ± 0.3[d]	0.2 ± 0.2
Vehicle	–	14.7 ± 0.3	13.5 ± 0.6	2.3 ± 0.3	0.2 ± 0.1
7	10	9.8 ± 1.7[e]	5.5 ± 1.7[d]	2.6 ± 0.2	1.0 ± 0.3
	20	2.8 ± 1.6[d]	1.5 ± 0.9[d]	NT	2.3 ± 0.3[d]
Vehicle	–	11.7 ± 0.3	11.2 ± 0.7	2.2 ± 0.2	0.3 ± 0.2
8	10	4.7 ± 1.2[d]	4.2 ± 0.3[d]	2.1 ± 0.3	0.4 ± 0.2
	20	6.5 ± 1.6[e]	3.5 ± 0.8[d]	1.0 ± 0.3[e]	0.5 ± 0.2
Vehicle	–	13.0 ± 0.4	12.5 ± 0.8	2.3 ± 0.2	0.3 ± 0.1
9	10	7.7 ± 0.5[d]	5.8 ± 0.7[d]	2.8 ± 0.1	1.3 ± 0.2[e]
	20	3.3 ± 1.2[d]	6.8 ± 1.0[d]	1.9 ± 0.4	1.1 ± 0.2[e]
Vehicle	–	13.0 ± 0.4	12.5 ± 0.8	2.3 ± 0.2	0.1 ± 0.1
10	10	11.0 ± 0.6	8.6 ± 0.9[d]	2.3 ± 0.2	0.6 ± 0.3
	20	2.7 ± 0.8[b]	3.0 ± 1.0[d]	NT	2.0 ± 0.2[d]
Vehicle	–	13.0 ± 0.4	12.5 ± 0.8	2.3 ± 0.2	0.1 ± 0.1
11	10	11.3 ± 0.9	10.7 ± 1.0	2.5 ± 0.3	0.3 ± 0.2
	20	10.8 ± 0.9	7.8 ± 1.2[d]	1.3 ± 0.2[d]	1.8 ± 0.3

[a]Reserpine (1 mg/kg, sc) and compounds **5–11** (ip) were administered 18 h and 60 min, respectively, before 8-OH-DPAT (5 mg/kg, sc); [b]**5–11** were administered ip 45 min before 8-OH-DPAT (1 mg/kg, sc); [c]**5–11** were administered ip 15 min before the test; [d]$p < 0.05$ *vs* vehicle; [e]$p < 0.01$ *vs* vehicle; NT: not tested.

drome in reserpine-pretreated rats by at least 50%. Moreover, used in the same doses, derivatives **5**, **7** and **10** antagonized the symptoms in a dose-dependent manner and produced an almost complete blockade of the flat body posture and forepaw treading (table II). In the other model used, 8-OH-DPAT-induced lower lip retraction (LLR) in rats, only derivatives **5**, **6** and **8** at doses of 20 mg/kg antagonized that effect. However, the same compounds given alone had no activity in that test. Although compounds **7**, **9** and **10** did not affect the LLR induced by 8-OH-DPAT, they produced that effect when given alone in doses of 10–20 mg/kg (table II). Compound **11** was practically inactive in both models.

Compound **8** in doses of 2.5–5 mg/kg reduced 8-OH-DPAT-induced hypothermia in mice, which was completely abolished after 45 and 60 min. However, derivatives **5–7** or **9–11** practically did not change the hypothermia induced by 8-OH-DPAT (table III). On the other hand, compounds **6** and **7** given alone produced hypothermia in mice throughout the whole experimental period. Compounds **5** and **8** given alone also evoked hypothermia in mice. That effect was only observed up to 60 min after administration of **5** and **8**, and their activity was lower than that observed for **6** and **7**. Derivatives **9–11** did not change the body temperature of mice (table IV).

Derivatives **5**, **6** and **8** behaved like typical postsynaptic 5-HT_{1A} antagonists, *eg*, NAN-190 or MM-77, in the *in vivo* tests used. However, the observed *in vivo* activity of **5** and **6** was somewhat weaker than that of NAN-190 and MM-77 [19–21]. Three other compounds, **7**, **9** and **10**, behaved like potential partial agonists of the postsynaptic 5-HT_{1A} receptors. They

Table III. The effect of **5–11** on the 8-OH-DPAT-induced hypothermia in mice[a].

Treatment	*Dose (mg/kg)*	*Δt ± SEM (°C)*[b]			
		15 min	*30 min*	*45 min*	*60 min*
Vehicle	–	–0.3 ± 0.1	–0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.1
8-OH-DPAT	–	–1.0 ± 0.2[c]	–1.1 ± 0.2[c]	–0.9 ± 0.2[c]	–0.6 ± 0.1[c]
5	2.5	–1.2 ± 0.4[c]	–0.6 ± 0.4	–0.8 ± 0.4	–0.6 ± 0.3
	5	–1.4 ± 0.4[d]	–1.4 ± 0.4[d]	–1.2 ± 0.3[d]	–0.9 ± 0.2[d]
Vehicle	–	–0.1 ± 0.2	–0.1 ± 0.2	0.2 ± 0.1	0.0 ± 0.2
8-OH-DPAT	–	–1.1 ± 0.1[d]	–1.4 ± 0.2[d]	–1.2 ± 0.1[d]	–0.9 ± 0.1[d]
6	2.5	–1.7 ± 0.3[d]	–1.5 ± 0.2[d]	–1.1 ± 0.2[d]	–0.8 ± 0.1[d]
	5	–1.6 ± 0.3[d]	–1.5 ± 0.3[d]	–1.1 ± 0.3[d]	–0.8 ± 0.3[d]
7	–	NT	NT	NT	NT
Vehicle	–	–0.2 ± 0.1	–0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.2
8-OH-DPAT	–	–1.1 ± 0.2[d]	–0.9 ± 0.2[c]	–1.0 ± 0.2[d]	–0.5 ± 0.2
8	2.5	–0.8 ± 0.2	–0.2 ± 0.2[e]	–0.1 ± 0.2[e]	0.1 ± 0.2
	5	–0.9 ± 0.2[c]	–0.3 ± 0.2	0.1 ± 0.2[f]	0.3 ± 0.2[e]
Vehicle	–	–0.3 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1	–0.1 ± 0.2
8-OH-DPAT	–	–0.9 ± 0.1[c]	–0.9 ± 0.1[c]	–0.9 ± 0.1[d]	–0.7 ± 0.2[c]
9	5	–1.2 ± 0.2[d]	–0.6 ± 0.2[c]	–0.7 ± 0.1[c]	–0.4 ± 0.2
	10	–1.2 ± 0.1[d]	–0.7 ± 0.1[c]	–0.4 ± 0.2	–0.5 ± 0.3
Vehicle	–	–0.4 ± 0.1	–0.3 ± 0.1	–0.2 ± 0.2	–0.2 ± 0.1
8-OH-DPAT	–	–1.1 ± 0.1[c]	–1.1 ± 0.2[e]	–1.1 ± 0.2[d]	–0.9 ± 0.1[c]
10	5	–1.5 ± 0.3[d]	–1.0 ± 0.3[c]	–0.9 ± 0.2[c]	–0.7 ± 0.2
	10	–1.6 ± 0.3[d]	–1.2 ± 0.1[d]	–1.0 ± 0.1[c]	–0.4 ± 0.2
Vehicle	–	–0.3 ± 0.1	–0.1 ± 0.1	0.0 ± 0.1	0.1 ± 0.2
8-OH-DPAT	–	–1.3 ± 0.1[d]	–1.1 ± 0.1[d]	–0.8 ± 0.1[c]	–0.6 ± 0.1[c]
11	5	–1.3 ± 0.2[d]	–1.1 ± 0.3[d]	–0.7 ± 0.2[c]	–0.7 ± 0.2[c]
	10	–1.6 ± 0.2[d]	–1.6 ± 0.2[d]	–1.6 ± 0.3[e]	–0.9 ± 0.2[d]

[a]The investigated compounds were administered ip 45 min before 8-OH-DPAT (5 mg/kg, ip); [b]the absolute mean initial body temperatures were within a range of 36.3 ± 0.5°C; [c]$p < 0.05$ *vs* vehicle; [d]$p < 0.01$ *vs* vehicle; [e]$p < 0.05$ *vs* 8-OH-DPAT; [f]$p < 0.01$ *vs* 8-OH-DPAT; NT: not tested.

induced LLR in rats, but also inhibited the behavioral syndrome induced by 8-OH-DPAT in reserpinized rats. Although compounds **7**, **9** and **10** mimic the pharmacological profile of some partial agonists, the observed *in vivo* effects (tables II and III) appeared at doses at least twice as high as those reported for ipsapirone or buspirone [12, 22, 23].

It may be concluded that the observed *in vivo* activity of compounds **5–10** stems from their high affinity for 5-HT_{1A} sites. By contrast, a fairly low 5-HT_{1A} affinity of derivative **11** may be the cause of its negligible *in vivo* activity, or even of the lack of activity in the tests used. Moreover, all the compounds active *in vivo* may be classified as postsynaptic antagonists (**5**, **6** and **8**), or partial agonists (**7**, **9** and **10**) of 5-HT_{1A} receptors, as all of them inhibited or even completely blocked the 8-OH-DPAT-induced behavioral syndrome in reserpinized rats. Consequently, each of them showed the same component of the functional profile, *ie* postsynaptic 5-HT_{1A} antagonism.

Experimental protocols

Chemistry

Melting points (mp) were determined on a Boetius apparatus and are uncorrected. ^{1}H-NMR spectra were obtained on a Varian EM-360L (60 MHz) spectrometer in $CDCl_3$ solution, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are reported in δ units, and coupling constants are reported in Hertz. Elemental analyses indicated by the symbols were within ±0.4% of the theoretical value.

*Synthesis of compounds **10** and **11** (general procedure)*

A solution of 1-(2-pyrimidinyl)piperazine dihydrochloride (7.2 g, 0.03 mol) in 30% KOH (60 ml) was extracted with

Table IV. The effect of **5–11** on the body temperature in mice[a].

Treatment	*Dose (mg/kg)*	*Δt ± SEM (°C)*[b]			
		30 min	*60 min*	*90 min*	*120 min*
Vehicle	–	–0.3 ± 0.1	–0.2 ± 0.1	–0.4 ± 0.1	–0.2 ± 0.2
5	2.5	–0.4 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1[c]	–0.4 ± 0.1[c]
	5	–1.2 ± 0.2[d]	–0.4 ± 0.2[d]	–0.1 ± 0.1	0.2 ± 0.1
	10	–1.8 ± 0.2[d]	–1.2 ± 0.3[d]	–0.6 ± 0.2	–0.2 ± 0.2
Vehicle	–	–0.2 ± 0.1	–0.3 ± 0.2	–0.1 ± 0.2	–0.2 ± 0.2
6	2.5	–0.7 ± 0.2[c]	–0.6 ± 0.1	–0.2 ± 0.1	–0.1 ± 0.1
	5	–1.3 ± 0.3[d]	–0.7 ± 0.3	–0.3 ± 0.3	–0.3 ± 0.2
	10	–2.2 ± 0.4[d]	–1.8 ± 0.4[d]	–1.2 ± 0.4[c]	–0.5 ± 0.3
Vehicle	–	–0.2 ± 0.1	–0.4 ± 0.1	–0.1 ± 0.2	–0.1 ± 0.3
7	5	–2.4 ± 0.3[d]	–1.8 ± 0.3[d]	–1.5 ± 0.2[d]	–1.0 ± 0.2[c]
	10	–2.5 ± 0.4[d]	–2.5 ± 0.4[d]	–2.1 ± 0.3[d]	–1.6 ± 0.3[d]
Vehicle	–	–0.2 ± 0.1	–0.1 ± 0.1	–0.3 ± 0.2	–0.1 ± 0.2
8	2.5	–0.3 ± 0.2	–0.3 ± 0.3	–0.2 ± 0.1	0.1 ± 0.1
	5	–0.5 ± 0.2	0.1 ± 0.3	0.1 ± 0.2	0.3 ± 0.3
	10	–1.6 ± 0.2[d]	–1.0 ± 0.2[d]	–0.6 ± 0.3	–0.3 ± 0.3
Vehicle	–	–0.3 ± 0.1	–0.2 ± 0.1	–0.3 ± 0.2	–0.1 ± 0.1
9	5	–0.3 ± 0.1	0.0 ± 0.1	–0.1 ± 0.1	0.0 ± 0.1
	10	–0.3 ± 0.3	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
Vehicle	–	–0.3 ± 0.1	–0.1 ± 0.2	–0.5 ± 0.2	–0.5 ± 0.1
10	5	0.2 ± 0.2	–0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
	10	0.2 ± 0.1	0.3 ± 0.2	–0.2 ± 0.2	–0.3 ± 0.2
Vehicle	–	–0.3 ± 0.1	–0.1 ± 0.2	–0.5 ± 0.2	–0.5 ± 0.1
11	5	–0.1 ± 0.2	–0.1 ± 0.2	0.1 ± 0.3	0.2 ± 0.3
	10	0.3 ± 0.2[c]	0.5 ± 0.3	0.1 ± 0.2	0.3 ± 0.3

[a]The investigated compounds were administered ip 30 min before the test; [b]the absolute mean initial body temperatures were within a range of 36.3 ± 0.5°C; [c]$p < 0.05$ *vs* vehicle; [d]$p < 0.01$ *vs* vehicle.

chloroform (3 x 30 ml). The combined organic layers were dried over anhydrous K_2CO_3, filtered off, and evaporated. The oily residue was treated with 2-methoxyethanol (30 ml), and the appropriate γ-chloropropyl derivative **12** or **13** (0.015 mol) was added in one portion. The reaction mixture was refluxed for 6 h. After cooling, the crystalline product was filtered off, washed with ethanol and water, and recrystallized.

Hydrochloride salts of **10** and **11** were prepared as follows: a suspension of free bases (1.0 g) in 99.8% ethanol (20 ml) was saturated with gaseous HCl until the precipitate dissolved and crystals of the salt appeared.

1,3-Dimethyl-9-{3-[4-(2-pyrimidinyl)-1-piperazinyl]propyl}-2,4,8-trioxo-1,3,6,7,9-pentahydropyrimidino[2,1-f]purine ***10***. Yield: 4.3 g (63%); mp 236–238°C (2-methoxyethanol). ^{1}H-NMR δ: 1.80–2.20 (m, 2H, $CH_2CH_2CH_2$); 2.35–2.75 (m, 4H, 2 x CH_2 piperazine); 2.85–4.30 (cluster, 16H, 5 x CH_2 and 2 x CH_3); 4.50 (t, J = 7 Hz, 2H, CH_2-piperazine); 6.50 (t, J = 6 Hz, 1H, pyrimidine-H_5); 8.30 (d, J = 6 Hz, 2H, pyrimidine-$H_{4,6}$). **10·HCl**: mp 287–289°C (99.8% ethanol). Anal $C_{21}H_{27}N_9O_3$·HCl (C, H, N).

1,3-Dimethyl-10-{3-[4-(2-pyrimidinyl)-1-piperazinyl]propyl}-2,4,9-trioxo-1,3,6,7,8,10-hexahydro-1,3-diazepino[2,1-f]purine ***11***. Yield: 5.2 g (74%); mp 134–136°C (ethanol). ^{1}H-NMR δ: 1.75–2.10 (m, 4H, $CH_2CH_2CH_2$ and 1,3-diazepine-5-CH_2); 2.30–2.75 (m, 10H, 5 x CH_2); 3.45 (s, 3H, 1-CH_3); 3.65 (s, 3H, 3-CH_3); 3.70–4.35 (m, 4H, 2 x CH_2); 4.50–4.75 (m, 2H, CH_2); 6.50 (t, J = 6 Hz, 1H, pyrimidine-H_5); 8.35 (d, J = 6 Hz, 2H, pyrimidine-$H_{4,6}$). **11·HCl**: mp 263–265°C (dilute ethanol). Anal $C_{22}H_{29}N_9O_3$·HCl (C, H, N).

Pharmacology

The experiments were performed on male Wistar rats (220–260 g) or male Albino Swiss mice (20–25 g). The animals were kept at ambient temperature (20 ± 1°C) on a natural day-night cycle (September–November), and were housed under standard laboratory conditions. They had free access to food (Bacutil pellets) and tap water before the experiment. Each experimental group consisted of six to eight animals per dose, and all the animals were used only once.

8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT·HBr, Research Biochemicals, Inc), reserpine (Ciba) and

the investigated hydrochloride salts of **5–11** were used in the form of freshly prepared aqueous solutions.

The obtained *in vivo* data were analyzed by Dunnett's test.

In vitro experiments

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 [24, 25]. 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 Chemicals) 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 binding experiments in which 10–14 drug concentrations (10^{-10}–10^{-3} M), run in triplicate, were used.

In vivo experiments

Behavioral syndrome induced by 8-OH-DPAT in reserpinized rats. The animals were individually placed in cages 5 min before injection of 8-OH-DPAT (5 mg/kg, ip). Observation sessions, lasting 45 s each, began 3 min after 8-OH-DPAT administration, 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, according to Tricklebank *et al* [14]. The maximum score, summed up over five observation periods, amounted to 15 for each symptom per animal. Reserpine (1 mg/kg, sc) and the investigated compounds were administered at 18 h and 60 min before 8-OH-DPAT injection, respectively.

Lower lip retraction (LLR) induced by 8-OH-DPAT in rats. The LLR was conducted according to the method described by Berendsen *et al* [15]. The animals were individually placed in cages, and were scored at 15, 30 and 45 min after 8-OH-DPAT administration as follows: 0 = lower incisors invisible, 0.5 = partly visible, 1 = clearly visible. The summed up, maximum score was up to 3 for each rat. The investigated compounds were administered ip 45 min before 8-OH-DPAT injection (1 mg/kg, sc).

The LLR induction by the investigated compounds given alone was tested in a separate experiment, and the animals were scored three times (at 15, 30 and 45 min) after the treatment.

Hypothermia induced by 8-OH-DPAT in mice. The rectal body temperature of mice (measured with an Ellab thermometer) was recorded at 15, 30, 45 and 60 min after injection of 8-OH-DPAT (5 mg/kg, ip). The investigated compounds were given 45 min before 8-OH-DPAT injection.

In a separate experiment, the effect of the investigated compounds given alone on the rectal body temperature was measured at 30, 60, 90 and 120 min after treatment.

The results are expressed as a change in the body temperature (Δt) in relation to the basal body temperature, as measured at the beginning of the experiments.

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