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Short communication

New 9- or 10-arylpiperazinoalkyl substituted pyrimido- or diazepino[2,1-*f*]purines with partial or full 5-HT$_{1A}$ agonistic activity

Maciej Pawlowski[a]*, Jacek Katlabi[a], Anna Drabczynska[a], Beata Duszynska[b], Sijka Charakchieva-Minol[b], Anna Deren-Wesolek[b], Ewa Tatarczynska[b], Ewa Chojnacka-Wójcik[b], Maria J. Mokrosz[b], Andrzej J. Bojarski[b]

[a]*Department of Pharmaceutical Chemistry, Collegium Medicum of Jagiellonian University, 9 Medyczna St., 30-688 Kraków, Poland*
[b]*Institute of Pharmacology, Polish Academy of Sciences, 12 Smetna St., 31-343 Kraków, Poland*

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Abstract – 5HT$_{1A}$ and 5HT$_{2A}$ receptor affinities of several γ-(3'-chloro- or 2'-methoxyphenyl)-piperazinopropyl derivatives of pyrimido[2,1-*f*]purines or 1,3-diazepino[2,1-*f*]purines are reported. Some behavioral models demonstrated that 1,3-dimethyl-9-{3-[4-(2'-methoxyphenyl)-1-piperazinyl]propyl}-2,4-dioxo-1,3,6,7,8,9-hexahydropyrimido[2,1-*f*]purine **5b** and 1,3-dimethyl-9-{3-[4-(2'-methoxyphenyl)-1-piperazinyl]propyl}-2,4,8-trioxo-1,3-dihydro-9H-pyrimido[2,1-*f*]purine **8b**, may be classified as partial agonist and a full agonist, respectively of pre- and post-synaptic 5HT$_{1A}$ receptors. © Elsevier, Paris

1-arylpiperazine / 5-HT$_{1A}$ agonists / pyrimidopurines / diazepinopurines

1. Introduction

The majority of 1-arylpiperazines is classified as partial agonists of 5-HT$_{1A}$ receptors [1–3], although the postsynaptic antagonism may also be regarded as their common pharmacological property [1, 2, 3]. There are, however, only few examples of either full (pre- and post-synaptic) agonists [4, 5] **1a,b** or antagonists [6–8] **2** and **3**. *(figure 1)*.

In the course of searching for the ligands possessing one of the above pharmacological properties, we tested 1-phenyl and 1-(2-pyrimidyl)piperazines **4** which contained a complex heterocyclic ring system. *(figure 2)*. These derivatives have been found to be the potent 5-HT$_{1A}$ receptor ligands and behaved either like antagonists or partial agonist of the postsynaptic 5-HT$_{1A}$ receptors [9]. In the present study we further examined the influence of structural modifications pyrimido[2,1-*f*]purine and diazepino[2,1-*f*]purines together with 1-(3-chlorophenyl)- and 1-(3-methoxyphenyl)piperazine on 5-HT$_{1A}$ affinity and 5-HT$_{1A}$/5-HT$_{2A}$ selectivity of the

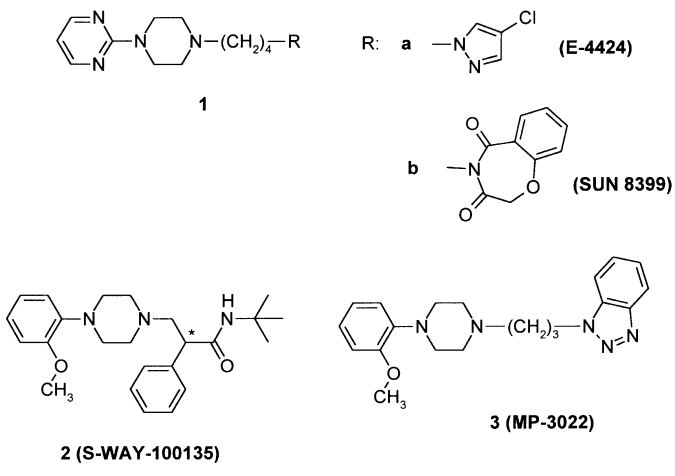

Figure 1. Structure of full (pre- and post-synaptic) 5-HT$_{1A}$ receptor agonists **1a,b** and antagonists **2, 3**.

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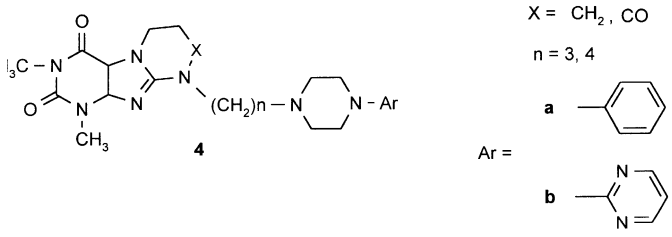


Figure 2. Structure of tricyclic theophylline derivatives, antagonists and partial agonists of postsynaptic 5-HT_{1A} receptors.

ligands and the consequences of these modifications on in vivo pharmacological properties of the most active derivatives.

2. Chemistry

The following new compounds **5**–**9** were synthesized by condensation of the bromo(chloro)propyl substrate with corresponding substituted piperazines *(figure 3)*.

The starting bromo(chloro)propyl derivatives of tricyclic pyrimido[2,1-*f*]theophylline or diazepino[2,1-*f*]theophylline were obtained in the several-step synthesis reported previously [10–13].

Melting points, yields and results from elemental and TLC analyses are given in *table I*. The final products were characterized by their ^{1}H-NMR and MS spectral data, which were in accordance with the proposed structures *(table II* and *III)*.

3. Pharmacology

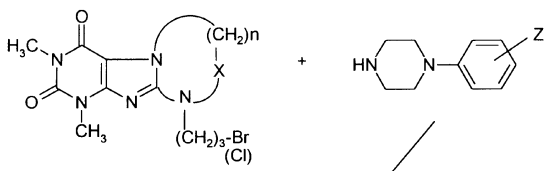
The receptor affinities of the investigated compounds **5**–**9** (hydrochlorides) were determined in competition experiments using 8-hydroxy-2-di-n-propylaminotetraline, [^{3}H]-8-OH-DPAT (hippocampus) for 5-HT_{1A} and [^{3}H] ketanserin (cortex) for 5-HT_{2A} receptors. The results are presented in *table IV*.

The functional profile of **5b** and **8b** at pre- and post-synaptic 5-HT_{1A} receptors was demonstrated in several in vivo models. The 8-OH-DPAT-induced lower lip retraction (LLR) in rats [14] and behavioral syndrome

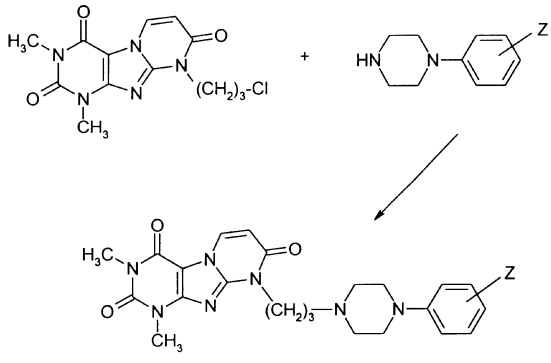
Table I. Yields, melting points and analytical data of new compounds.

Compound	M.p. (°C), recryst. solvent	Yield (%)	Formula, molecular weight	Analyses [a]	TLC, R_f solvent
5a, HCl	244–245, EtOH/H_2O	88	$C_{23}H_{30}N_7O_2Cl{\cdot}HCl$, 508.45	C, H, N	0.79 E
5b, HCl	176 decomp., EtOH/H_2O	74	$C_{24}H_{33}N_7O_3{\cdot}H_2O{\cdot}HCl$, 522.04	C, H, N	0.73 E
6a, HCl	245–247, EtOH/H_2O	65	$C_{24}H_{32}N_7O_2Cl{\cdot}HCl$, 522.47	C, H, N	0.87 E
6b, HCl	190 decomp., EtOH/H_2O	82	$C_{25}H_{32}N_7O_3{\cdot}H_2O{\cdot}HCl$, 536.01	C [b] H, N [c]	0.78 E
7a, base	190–191, 96° EtOH	64	$C_{23}H_{28}N_7O_3Cl$, 486.00	C [b], H, N	0.23 A, 0.53 B, 0.76 C
7a, HCl	272 decomp., 80° EtOH		$C_{23}H_{28}N_7O_3Cl{\cdot}HCl$, 522.44	C, H, N	
7b, base	230–231, 2-methoxyethanol	50	$C_{24}H_{31}N_7O_4$, 481.56	C, H, N	0.10 A, 0.30 B, 0.55 C
7b, HCl	258 decomp., 96° EtOH		$C_{24}H_{31}N_7O_4{\cdot}HCl$, 518.02	C [b], H, N	
8a, base	174–175, 96° EtOH	62	$C_{23}H_{26}N_7O_3Cl$, 483.96	C, H, N	0.31 A, 0.60 B, 0.75 C
8a, HCl	288–290 decomp., 10° EtOH	–	$C_{23}H_{26}N_7O_3Cl{\cdot}HCl$, 520.42	C [b], H, N	
8b, base	216–218, 96° EtOH	68	$C_{24}H_{29}N_7O_4$, 479.55	C, H, N	0.18 A, 0.39 B, 0.56 C
8b, HCl	273–275 decomp., 96° EtOH		$C_{24}H_{29}N_7O_4{\cdot}HCl$, 515.99	C [b], H, N	
9a, base	145–146, 96° EtOH	40	$C_{24}H_{30}N_7O_3Cl$, 500.00	C, H, N	0.30 A, 0.56 B, 0.75 C
9a, HCl	167–169 decomp., anh. EtOH	–	$C_{24}H_{30}N_7O_3Cl{\cdot}HCl$, 536.45	C, H, N [c]	
9b, base	145–147, 96° EtOH	41	$C_{25}H_{33}N_7O_4$, 495.59	C, H, N [c]	0.15 A, 0.36 B, 0.80 D
9b, HCl	242–244 decomp., 96° EtOH	–	$C_{25}H_{33}N_7O_4{\cdot}HCl$, 532.04	C [b], H, N	

[a] Elemental analysis is within ±0.4% for elements indicated unless otherwise noted; [b] C found (calc.): **6b** HCl 56.52 (56.02); **7a** base 56.40 (56.84); **7b** HCl 55.08 (55.64); **8a** HCl 52.62 (53.08); **8b** HCl 55.28 (55.86); **9b** HCl 55.90 (56.43); [c] N found (calc.) **6b** HCl 18.93 (18.29); **9a** HCl 18.70 (18.27); **9b** base 19.33 (19.78).



	n	X	Z
5a	2	CH_2	3'-Cl
5b	2	CH_2	2'-OCH_3
6a	3	CH_2	3'-Cl
6b	3	CH_2	2'-OCH_3
7a	2	CO	3'-Cl
7b	2	CO	2'-OCH_3
9a	3	CO	3'-Cl
9b	3	CO	2'-OCH_3



	Z
8a	3'-Cl
8b	2'-OCH_3

Figure 3. Synthesis of tricyclic chloro- and methoxy-arylpiperazinopropyl derivatives.

(flat body posture and forepaw treading) in reserpinized rats [15] were mediated by postsynaptic receptors.

However, the 8-OH-DPAT-induced hypothermia in mice was mediated by presynaptic 5-HT_{1A} receptors [16, 17]. The results of in vivo studies are given in *tables V, VI, VII* and *VIII*.

4. Results and discussion

The radioligand binding data on the 5-HT_{1A} receptor affinities of **5**–**9** show large differences and depend on both, 1-arylpiperazine moiety and the amide fragment. All the investigated compounds are potent 5-HT_{1A} receptor ligand with K_i within the region 2.8–43 nM for **5b** and **7a** respectively, except **9a** and **9b** of which the affinity is moderate.

The compounds containing o-methoxyphenylpiperazine (o-OMePhP, series **b**) are generally more active at 5-HT_{1A} receptor sites than their counterparts with m-chloro-phenylpiperazine (m-CPP, series **a**) if comparing **5b** vs. **5a** (K_i = 2.8 and 25.7 nM) or **8b** vs. **8a** (K_i = 10.4 and 32.8 nM). On the other hand their high affinity makes them more sensitive for any change in the amide fragment. Enlargement of the ring of the alkyl substitution causes 6-fold loss of activity for o-methoxy derivative **6b** vs. **5b** and retains activity of **6a** comparing to **5a**. Addition of the amide function makes both series of the compounds less afin but again o-methoxy derivatives are more affected, K_i ratios are **7b/5b** = 7.7 and **7a/5a** = 1.7. Both modifications, amide function along with enlargement of the ring, dramatically decrease activity of **9b**, 60 times in relation to the most active compound **5b**, while **9a** is only slightly, above 2 times less potent than **5a** *(table IV, figure 4)*.

The 5-HT_{2A} affinities of the derivatives studied extend from the moderate, K_i = 126 nM for **5a**, to very weak, 5014 nM for **9b**. The influence of the structure modifications on 5-HT_{2A} affinity is comparable in both series of the investigated compounds but not as strong as in case of the 5-HT_{1A} affinity however, the direction of these changes is similar *(table IV, figure 4)*.

The selectivity factor $S_{1,2}$, for the m-CPP series varies from 4.2 for **7a** to 33.9 for **8a** and these derivatives should be regarded rather non-selective ones. On the contrary, the o-OMePhP derivatives are highly selective 5-HT_{1A}/5-HT_{2A} receptor ligands as their selectivity factor is within 30 for **9b** up to 373 for **8b**, especially that their 5-HT_{2A} affinities are within the range which can not be expected to evoke any behavioral effects.

It has been shown that lipophilicity or dipole moment of the terminal amide or cycloamide fragment of 1-arylpiperazines significantly affects the 5-HT_{1A} and

Table II. ^{1}H-NMR data on selected compounds.

No.	δ ppm in $CDCl_3$ (200 MHz)
5a, HCl	2.10–2.29 (m, 2H, $CH_2CH_2CH_2$); 2.32–2.45 (m, 10H, CH_2N-$(CH_2)_4$-N); 3.06–3.11 (m, 2H, $C7H_2$); 3.36 (s, 3H, $N3CH_3$); 3.47 (s, 3H, $N1CH_3$); 3.56–3.68 (t, 2H, *J* = 7 Hz, $N9CH_2$); 4.19–4.25 (t, 2H, *J* = 6 Hz, $N5CH_2$); 6.75–7.20 (m, 4H, aromat).
5b, HCl	2.17–2.30 (m, 2H, $CH_2CH_2CH_2$); 2.32–2.45 (m, 10H, CH_2N-$(CH_2)_4$-N); 3.06–3.15 (m, 2H, $C7H_2$); 3.36 (s, 3H, $N3CH_3$); 3.48 (s, 3H, $N1CH_3$); 3.65–3.72 (t, 2H, *J* = 7 Hz, $N9CH_2$); 3.86 (s, 3H, OCH_3); 4.15–4.25 (t, 2H, *J* = 6 Hz, $N5CH_2$); 6.80–7.10 (m, 4H, aromat).
6a, HCl	1.90–2.17 (m, 2H, $CH_2CH_2CH_2$); 2.32–2.41 (m, 10H, CH_2N-$(CH_2)_4$-N); 2.90–3.20 (m, 2H, $C7H_2$); 3.37 (s, 3H, $N3CH_3$); 3.48 (s, 3H, $N1CH_3$); 3.60–3.66 (t, 2H, *J* = 7 Hz, $N10CH_2$); 4.34–4.36 (d, 2H, *J* = 5 Hz, $N5CH_2$); 6.80–7.20 (m, 4H, aromat).
6b, base	1.92–2.00 (m, 2H, $CH_2CH_2CH_2$); 2.30–2.45 (m, 10H, CH_2N-$(CH_2)_4$-N); 3.10–3.20 (m, 2H, $C7H_2$); 3.36 (s, 3H, $N3CH_3$); 3.50 (s, 3H, $N1CH_3$); 3.90 (s, 3H, OCH_3); 4.30–4.40 (d, 2H, *J* = 5 Hz, $N5CH_2$); 6.90–7.20 (m, 4H, aromat).
7a, base	1.79–1.96 (m, 2H, $CH_2CH_2CH_2$); 2.37–2.59 (m, 6H, CH_2N-$(CH_2)_2$); 2.84–3.02 (t, 2H, $C7H_2$); 3.08–3.20 (m, 4H, $(CH_2)_2N$-C_6H_4Cl); 3.34 (s, 3H, $N3CH_3$); 3.50 (s, 3H, $N1CH_3$); 3.98–4.14 (t, 2H, $N9CH_2$); 4.50 (t, 2H, $N5CH_2$); 6.67–6.70 (m, 3H, aromat); 7.01–7.02 (d, 1H, 2'-C_6H_4Cl).
7b, base	1.80–2.02 (q, 2H, $CH_2CH_2CH_2$); 2.39–2.65 (m, 6H, CH_2N-$(CH_2)_2$); 2.84–3.10 (m, 6H, $(CH_2)_2N$-$C_6H_4OCH_3$ + $C7H_2$); 3.35 (s, 3H, $N3CH_3$); 3.50 (s, 3H, $N1CH_3$); 3.81 (s, 3H, OCH_3); 3.97–4.15 (t, 2H, $N9CH_2$); 4.33–4.51 (t, 2H, $N5CH_2$); 6.86–6.97 (m, 4H, aromat).
8a, base	1.81–1.98 (m, 2H, $CH_2CH_2CH_2$); 2.43–2.59 (m, 6H, CH_2N-$(CH_2)_2$); 3.04–3.17 (m, 4H, $(CH_2)_2N$-C_6H_4Cl); 3.38 (s, 3H, $N3CH_3$); 3.52 (s, 3H, $N1CH_3$); 4.24–4.42 (t, 2H, $N9CH_2$); 6.19–6.29 (d, 1H, 7CH); 6.68–6.78 (m, 3H, aromat); 7.00–7.02 (d, 1H, 2'-C_6H_4Cl); 8.41–8.51 (d, 1H, C6H).
8b, base	1.81–2.16 (m, 2H, $CH_2CH_2CH_2$); 2.45–2.64 (m, 6H, CH_2N-$(CH_2)_2$); 2.94–3.16 (m, 4H, $(CH_2)_2N$-$C_6H_4OCH_3$); 3.38 (s, 3H, $N3CH_3$); 3.56 (s, 3H, $N1CH_3$); 3.81 (s, 3H, OCH_3); 4.25–4.43 (t, 2H, $N9CH_2$); 6.20–6.29 (d, 1H, C7H); 6.75–7.02 (m, 4H, aromat); 8.42–8.51 (d, 1H, C6H).
9a, base	1.74–2.01 (q, 2H, $CH_2CH_2CH_2$); 2.32–2.57 (m, 10H, CH_2N-$(CH_2)_2$ + $C7H_2$ + $C8H_2$); 3.06–3.18 (m, 4H, $(CH_2)_2N$-C_6H_4Cl); 3.37 (s, 3H, $N3CH_3$); 3.52 (s, 3H, $N1CH_3$); 3.87–4.05 (t, 2H, $N9CH_2$); 4.35–4.52 (t, 2H, $N5CH_2$); 6.67–6.79 (m, 3H, aromat); 7.01–7.03 (d, 1H, 2'-C_6H_4Cl).
9b, base	1.65–2.00 (m, 2H, $CH_2CH_2CH_2$); 2.32–2.62 (m, 10H, CH_2N-$(CH_2)_2$ + $C7H_2$ + $C8H_2$); 2.84–3.07 (m, 4H, $(CH_2)_2N$-$C_6H_4OCH_3$); 3.37 (s, 3H, $N3CH_3$); 3.52 (s, 3H, $N1CH_3$); 3.81 (s, 3H, OCH_3); 3.88–4.06 (t, 2H, $N9CH_2$); 4.21–4.51 (t, 2H, $N5CH_2$); 6.75–7.02 (m, 4H, aromat).

5-HT_{2A} receptor affinity [2, 18, 19]. This is not however, to be such an obvious case. Although, in both series we found the tendency that the higher dipole moment increases the affinity at 5-HT_{1A} receptors but this is only a qualitative relationship between these two properties. A tentative suggestion may be drawn however, that the differences in the affinities of the investigated compounds can be attributed to the subtle stereochemical distortions within the heterocyclic fragment.

Finally, concerning 5-HT_{1A} receptor affinity and the selectivity towards 5-HT_{1A}/5-HT_{2A} receptors, we selected two compounds i.e. **5b** and **8b**, to determine their functional profile at 5-HT_{1A} receptors. Compounds **5b** and **8b** (2.5–5 mg/kg i.p.), given alone induced a dose-

Table III. MS data on selected compounds.

No.	M^+	*m/z* intensity
7a, base	486 (18)	470 (20); 345 (8); 319 (47); 290 (24); 209 (100); 166 (25); 70 (25); 55 (19).
7b, base	481 (45)	466 (30); 325 (37); 290 (18); 205 (100); 190 (40); 177 (30); 70 (30); 55 (27).
8a, base	483 (37)	468 (30); 317 (58); 288 (44); 248 (41); 209 (100); 166 (25); 70 (40); 56 (19).
8b, base	479 (80)	464 (20); 287 (58); 248 (28); 205 (100); 190 (41); 62 (30); 136 (28); 70 (25); 56 (19).
9a, base	499 (10)	484 (57); 347 (18); 333 (50); 304 (30); 209 (100); 195 (38); 70 (58); 56 (16).
9b, base	495 (22)	480 (70); 333 (17); 304 (17); 219 (20); 205 (100); 190 (38); 164 (26); 70 (30); 56 (16).

Table IV. 5-HT_{1A} and 5-HT_{2A} receptor affinities of compounds **5–9.**

Compound	K_i ± SEM [nM] [a]		Selectivity
	5-HT_{1A}	5-HT_{2A}	5-HT_{2A}/5-HT_{1A}
5a	25.7 ± 1	126 ± 12	4.9
5b	2.8 ± 0.3	486 ± 31	173
6a	26.7 ± 3.1	330 ± 9	12.3
6b	17.2 ± 0.5	562 ± 37	32.7
7a	42.9 ± 4	182 ± 14	4.2
7b	21.6 ± 0.8	4219 ± 183	195
8a	32.8 ± 1.6	1080 ± 40	32.9
8b	10.4 ± 1.2	3882 ± 418	373
9a	60.3 ± 10.2	359 ± 10	5.9
9b	168 ± 1	5014 ± 6	29.8

[a] Mean values from at least three independent experiments/receptors [16, 17].

dependent LLR in rats, the maximum score being 73% and 77%, respectively, after the highest dose used *(table V)*. The same effect was observed after administration of 8-OH-DPAT (0.25–1 mg/kg s.c.), a full agonist of 5-HT_{1A} receptors; the maximum possible score was 100% after a dose of 1 mg/kg s.c.. Therefore it may be concluded that, like 8-OH-DPAT, the two investigated compounds, **5b** and **8b**, are agonists of postsynaptic 5-HT_{1A} receptors in the behavioral (LLR) model studied. In reserpinized rats, **5b** (5–10 mg/kg) alone did not evoke a behavioral syndrome (flat body posture and/or forepaw treading), whereas **8b** in the highest dose used (10 mg/kg i.p.) induced a flat body posture, the maximum possible score being 50%; however, forepaw treading was not observed after the latter administration. 8-OH-DPAT (5 mg/kg i.p.) induced a flat body posture (97–100% of possible scores) and forepaw treading (88–90% of possible scores) in reserpinized rats. The 8-OH-DPAT-induced forepaw treading was dose-dependently attenuated by **5b** (5–10 mg/kg i.p.) and blocked by (S)-WAY 100135 (10 mg/kg s.c.), a 5-HT_{1A} receptor antagonist, **8b** did not change that effect of 8-OH-DPAT. At the same time, **5b** and **8b** (5–10 mg/kg i.p.) had no influence on the flat body posture induced by 8-OH-DPAT in reserpinized rats; the reference drug, (S)-WAY 100135 (5–10 mg/kg s.c.), slightly reduced (by ca. 40%) that effect of 8-OH-DPAT, the reduction being equal after either dose used *(table VI)*. The above results indicate that in this model (serotonin syndrome) **8b** shows features characteristic of a weak agonist of postsynaptic 5-HT_{1A} receptors, whereas **5b** behaves like antagonist of these receptors. The results of both these behavioral studies permit a conclusion that **8b** (which induced LLR and flat body posture) may be classified as an agonist of postsynaptic 5-HT_{1A} receptors, whereas compound **5b** behaves like a partial agonist of these receptors: it induced LLR and, at the same time, attenuated the forepaw treading induced by 8-OH-DPAT, a 5-HT_{1A} receptor agonist. In those tests,

Table V. The induction of lower lip retraction (LLR) by **5b, 8b** and 8-OH-DPAT in rats.

Compounds	Dose (mg/kg)	LLR score (mean ± SEM)
Vehicle	–	0.1 ± 0.1
5b	2.5	1.2 ± 0.2
	5.0	2.2 ± 0.2 [b]
Vehicle	–	0.1 ± 0.1
8b	2.5	1.2 ± 0.2 [a]
	5.0	2.3 ± 0.2 [b]
Vehicle	–	0.1 ± 0.1
8-OH-DPAT	0.25	1.5 ± 0.1 [a]
	0.5	2.1 ± 0.5 [b]
	1.0	3.0 ± 0.0 [b]

5b and **8b** were administered (i.p.) and 8-OH-DPAT (s.c.), 15 min before the test. Each group consisted of 6 rats. [a] $p < 0.05$, [b] $p < 0.01$ vs. respective vehicle group (Newman–Keuls test).

Table VI. The induction of serotonin syndrome by **5b, 8b** and (S)-WAY 100135 (A) and the effect of compounds **5b, 8b** and (S)-WAY 100135 on the 8-OH-DPAT-induced syndrome (B) in reserpinized rats.

Compounds	Dose (mg/kg)	Mean ± SEM behavioral score			
		A		B	
		Flat body posture	Forepaw treading	Flat body posture	Forepaw treading
Vehicle	–	0.2 ± 0.1	0.2 ± 0.1	14.5 ± 0.3	13.5 ± 0.2
5b	5.0	0.1 ± 0.1	0.3 ± 0.1	13.0 ± 0.7	9.5 ± 1.0 [a]
	10.0	0.1 ± 0.1	0.3 ± 0.1	12.9 ± 1.0	5.9 ± 0.8 [b]
Vehicle	–	0.3 ± 0.1	0.2 ± 0.1	14.5 ± 0.3	13.5 ± 0.2
8b	5.0	0.1 ± 0.1	0.5 ± 0.1	15.0 ± 0.0	11.4 ± 0.5
	10.0	7.6 ± 1.6 [b]	0.3 ± 0.1	13.1 ± 1.6	11.9 ± 1.0
Vehicle	–	0.2 ± 0.1	0.2 ± 0.1	15.0 ± 0.0	13.2 ± 0.9
(S)-WAY 100135	5.0	0.2 ± 0.1	0.2 ± 0.1	9.0 ± 1.0 [b]	5.5 ± 0.6 [b]
	10.0	0.3 ± 0.1	0.2 ± 0.1	9.7 ± 1.0 [b]	1.0 ± 0.3 [b]

Reserpine (1 mg/kg, s.c.) was administered 18 h before the test. A: **5b**, **8b** (i.p.) and (S)-WAY 100135 (s.c.) were administered 3 min before the test. B: **5b**, **8b** (i.p.) and (S)-WAY 100135 (s.c.) were administered 45 min before 8-OH-DPAT (5 mg/kg, i.p.). Each group consisted of 6 rats. [a] $p < 0.05$, [b] $p < 0.01$ vs. respective vehicle group (Newman–Keuls test).

the same functional profile, characteristic of **5b**, was described for ipsapirone, a well-known partial agonist [14, 20, 21], and a potent anxiolytic drug. Compounds **5b** and **8b** (2.5–10 mg/kg), given alone, produced dose-dependent hypothermic responses in mice; after the highest dose of **5b** and **8b**, the decrease in body temperature mediated by 5-HT_{1A} receptors was back to the control level within 60 min. 8-OH-DPAT (1.25–5 mg/kg), used as a reference compound, also evoked hypothermia in mice, which lasted up to 45 min after its administration *(table VII)*. The hypothermic effects observed after **5b** (10 mg/kg) and **8b** (5 mg/kg) administration were attenuated and abolished (30 min after **8b** administration), respectively, by (S)-WAY-100135 (10 mg/kg). The 8-OH-DPAT (5 mg/kg)-induced hypothermia in mice was completely blocked by (S)-WAY 100135 (10 mg/kg) *(table VIII)*.This finding indicates that, like 8-OH-DPAT, **5b** and **8b** may be agonists of presynaptic 5-HT_{1A} receptors.

In conclusion, it should be mentioned that the previously described derivatives possessing the same as **5b** and **8b**, heteroaromatic terminal fragments and 1-phenylpiperazine moiety were classified as typical postsynaptic 5-HT_{1A} antagonists [9]. The replacement of 1-phenyl by 1-(o-methoxyphenyl)piperazine, which is usually present in 5-HT_{1A} receptor antagonist i.e. MP-3022 or WAY-100135, surprisingly resulted in the ligands with high intrinsic activity, which demonstrated dominating agonistic properties at 5-HT_{1A} receptors. In fact, the

Table VII. The effect of **5b, 8b** and 8-OH-DPAT on the body temperature in mice.

Compounds	Dose (mg/kg)	$\Delta t \pm SEM$ (°C) [a]			
		15 min	30 min	45 min	60 min
Vehicle	–	0.2 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2
5b	2.5	–0.5 ± 0.2 [b]	–0.1 ± 0.2	–0.0 ± 0.1	0.3 ± 0.1
	5.0	–0.4 ± 0.1 [b]	0.1 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
	10.0	–0.7 ± 0.2 [c]	–0.6 ± 0.2 [c]	–0.2 ± 0.2 [b]	0.1 ± 0.2
Vehicle	–	–0.1 ± 0.2	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
8b	2.5	–1.3 ± 0.3 [c]	–0.7 ± 0.2	–0.5 ± 0.3	–0.3 ± 0.2
	5.0	–1.6 ± 0.2 [c]	–1.0 ± 0.3 [b]	–0.6 ± 0.2	–0.2 ± 0.2
Vehicle	–	0.1 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	0.1 ± 0.2
8-OH-DPAT	1.25	–0.3 ± 0.2	–0.6 ± 0.3 [b]	–0.1 ± 0.1	–0.1 ± 0.2
	2.5	–0.3 ± 0.2	–0.6 ± 0.3 [b]	–0.3 ± 0.2	–0.4 ± 0.2
	5.0	–1.0 ± 0.2 [c]	–1.2 ± 0.2 [c]	–0.9 ± 0.2 [b]	–0.6 ± 0.2

5b, **8b** and 8-OH-DPAT (i.p.) were administered 15 min before the test. Each group consisted of 7–8 mice. [a] The absolute mean initial body temperatures were within a range of 36.3 ± 0.5 °C; [b] $p < 0.05$, [c] $p < 0.01$ vs. respective vehicle group (Dunnett's test).

Table VIII. The effect of (S)-WAY 100135 on the **5b**-, **8b**- and 8-OH-DPAT-induced hypothermia in mice.

Compounds	Dose (mg/kg)	$\Delta t \pm$ SEM (°C) [a]			
		15 min	30 min	45 min	60 min
Vehicle	–	–0.3 ± 0.2	–0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
5b	–	–1.7 ± 0.3 [c]	–1.4 ± 0.3 [b]	–0.9 ± 0.2	–0.3 ± 0.3
(S)-WAY 100135	10	–0.7 ± 0.2 [B]	–0.5 ± 0.2 [A]	0.1 ± 0.2	0.2 ± 0.1
Vehicle	–	–0.3 ± 0.2	–0.2 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
8b	–	–1.4 ± 0.3 [c]	–1.0 ± 0.2	–0.2 ± 0.2	0.3 ± 0.2
(S)-WAY 100135	10	–0.8 ± 0.2	–0.1 ± 0.5 [A]	–0.1 ± 0.2	0.2 ± 0.2
Vehicle	–	0.3 ± 0.1	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.3
8-OH-DPAT	–	–1.3 ± 0.3 [c]	–1.6 ± 0.2 [c]	–1.8 ± 0.4 [c]	–1.4 ± 0.3 [c]
(S)-WAY 100135	10	0.2 ± 0.2 [B]	0.0 ± 0.2 [B]	0.3 ± 0.2 [B]	0.1 ± 0.2 [B]

5b (10 mg/kg, i.p.), **8b** (5 mg/kg, i.p.) and 8-OH-DPAT (5 mg/kg, i.p.) were administered 15 min before the test. (S)-WAY 100135 (s.c.) was administered 45 min before the experiment. Each group consisted of 7–8 mice. [a] The absolute mean initial body temperatures were within a range of 36.3 ± 0.5 °C; [b] $p < 0.05$, [c] $p < 0.01$ vs. respective vehicle group; [A] $p < 0.05$, [B] $p < 0.01$ vs. respective **5b**, **8b** or 8-OH-DPAT group (Dunnett's test).

observed in vivo activity of compounds **5b** and **8b**, resulting from their high affinity for 5-HT_{1A} receptors, permits to classify **5b** as presynaptic agonist and postsynaptic partial agonist, while **8b** as pre- and post-synaptic agonist of 5-HT_{1A} receptors. It should be stressed also that different functional activity of these two compounds can be attributed to the enlargement of the π-system in **8b** vs. **5b** and/or diminished flexibility of the heterocyclic fragment.

5. Experimental protocol

5.1. Chemistry

The m.p.'s were uncorrected. All compounds were analyzed for C, H, N.

^{1}H-NMR: Varian 200 BB (200 MHz) apparatus using TMS as internal standard, MS: AMD-604 (70 eV) with direct inlet. TLC was performed on Merck plates (Kieselgel 60 F_{254}), solvent A: benzene + acetone (7 + 3), solvent B: benzene + acetone (1 + 1), solvent C: methanol + CH_3COOH (3 + 7), solvent D: chloroform + methanol + CH_3COOH (7 + 2 + 1). solvent E: benzene + acetone + methanol (1 + 1 + 1).

*5.2. Synthesis of compounds **5** and **6** (general procedure)*

A solution of 1-(3'-chlorophenyl)piperazine hydrochloride or 1-(2'-methoxyphenyl)piperazine hydrochloride (0.01 mol) in 10 mL of 30% NaOH was extracted with chloroform (4 × 20 mL). The combined organic layers were dried over anhydrous K_2CO_3, filtered off and evaporated. The oily residue was treated with 30 mL of

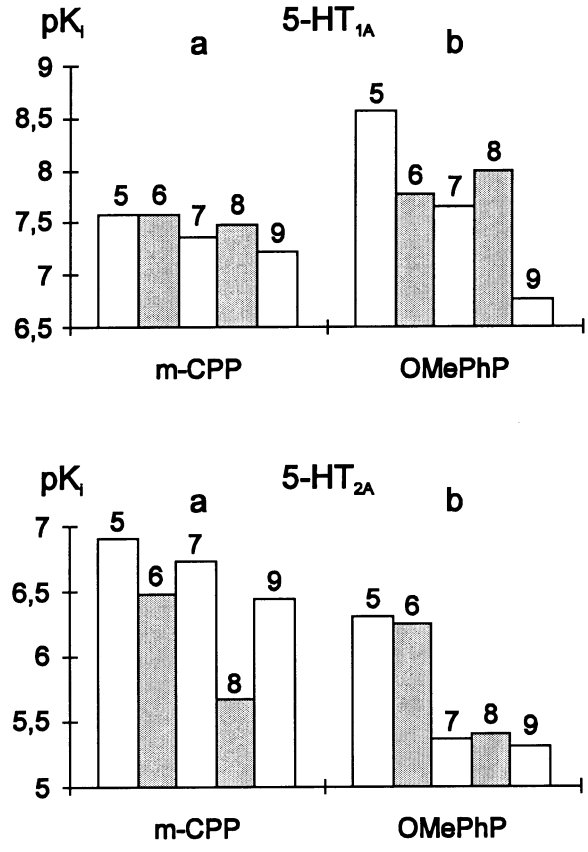


Figure 4. A relationship between the pK_i values and different arylpiperazine fragments.

anhydrous toluene then 0.0045 mol of anhydrous K_2CO_3 and 0.0045 mol of γ-bromopropyl substrates were added in one portion. The reaction mixture was refluxed for 6 h (compounds **5a,b**) or 10 h (compounds **6a,b**). The inorganic salts were filtered off from the hot mixture and the solvent was evaporated to dryness. To the residue 10 mL H_2O was added, the crystalline product (**5a,b**) was filtered off washed with water dried and used for the salt preparation. In the case of compounds **6a,b**, oily residues after treating with H_2O were obtained.

Compounds **5a** and **6a** were obtained previously in the reaction of γ-bromopropyl substrates (synthesized by other methods) with piperazine derivatives, using different solvent and reaction conditions [23].

Hydrochloride salts were prepared as follows: the crystalline bases (**5a,b**) or oily residues (**6a,b**) were dissolved in conc. HCl, the solution was evaporated to dryness then isopropanol was added. The separated crystals of the salts were recrystallized from isopropanol.

*5.3. Synthesis of compounds **7–9** (general procedure)*

The free bases 1-(3'-chlorophenyl)piperazine and 1-(2'-methoxyphenyl)piperazine were obtained by extraction from their hydrochlorides (0.01 mol) by analogical manner as for compounds **5** and **6**. The oily residue after evaporation of chloroform was treated with 10 mL of 2-methoxyethanol then 0.005 mol of γ-chloropropyl substrate was added and the reaction mixture was refluxed for 9 h. After refrigerating (–15 °C) for 12 h the precipitated product was filtered off, washed with small amount of cold anhydrous ethanol and with H_2O. The crude free base was recrystallized.

*5.4. Hydrochlorides of compounds **7–9***

The suspension of the base (1.0 g) in 20 mL of anhydrous ethanol was saturated with gaseous HCl. The mixture was cooled on the water–ice bath. The compounds was dissolved, after few minutes the salt was precipitated. The product was filtered off, washed with anhydrous ethanol and recrystallized.

5.5. Pharmacology

5.5.1. 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 5-HT_{2A} receptors according to the published procedures [22]. The following radioligands were used: [^{3}H]-8-OH-DPAT (190 Ci/mmol, Amersham) and [^{3}H]-ketanserin (60 Ci/mmol, NEN Chemicals) for 5-HT_{1A} and 5-HT_{2A} 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 triplicates, were used.

5.5.2. In vivo experiments

The experiments were performed on male Wistar rats (250–300 g) or male Albino–Swiss mice (22–26 g). The animals were kept at ambient temperature (20 ± 1 °C) on a natural day–night cycle (September–January), 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.), (S)-N-tert-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropanamide ((S)-WAY 100135, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences), reserpine (Ciba) and the investigated hydrochloride salts of **5b** and **8b** were used in the form of freshly prepared aqueous solutions.

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

5.5.2.1. Induction of lower lip retraction (LLR) in rats

The LLR was conducted according to the method described by Berendsen et al. [14]. The animals were individually placed in cages, having been scored three times at 15, 30 and 45 min after administration of **5b** (i.p.), **8b** (i.p.) and 8-OH-DPAT (s.c.) 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.

5.5.2.2. Induction of behavioral syndrome in reserpinized rats

The animals were individually placed in cages 5 min before injection of **5b** (i.p.), **8b** (i.p.) and (S)-WAY 100135 (s.c.). Observation sessions, lasting 45 sec each, began 3 min after administration of the investigated compounds, and were repeated every 3 min. Flat body posture and reciprocal forepaw treading were scored using a ranked intensity scale, where 0 = absent, 1 = equivocal, 2 = present, and 3 = intense, according to Tricklebank et al. [15]. The maximum score, summed up over five observation periods, amounted to 15 for each symptom per animal. Reserpine (1 mg/kg, s.c.) was administered 18 h before the test.

In a separate experiment, the effect of **5b**, **8b** and (S)-WAY 100135 on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg, i.p.) was estimated. The tested compounds were administered 45 min before 8-OH-

DPAT. Observation sessions began 3 min after injection of 8-OH-DPAT.

5.5.2.3. Induction of hypothermia 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 **5b** (i.p.), **8b** (i.p.) and 8-OH-DPAT (i.p.).

In a separate experiment, the effect of (S)-WAY 100135 (10 mg/kg, s.c.) on the hypothermia induced by **5b** (10 mg/kg, i.p.), **8b** (5 mg/kg, i.p.) or 8-OH-DPAT (5 mg/kg, i.p.) was investigated. (S)-WAY 100135 was administered 30 min before the tested compounds, the rectal body temperature of mice was measured 15, 30, 45 and 60 min after their injection.

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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