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The Influence of Substitution at Aromatic Part of 1,2,3,4-Tetrahydroisoquinoline on In Vitro and In Vivo 5-HT_{1A}/5-HT_{2A} Receptor Activities of Its 1-Adamantoyloaminoalkyl Derivatives

Andrzej J. Bojarski,[a,*] Maria J. Mokrosz,[a] Sijka Charakchieva Minol,[a] Aneta Kozioł,[a] Anna Wesołowska,[b] Ewa Tatarczyńska,[b] Aleksandra Kłodzińska[b] and Ewa Chojnacka-Wójcik[b]

[a]*Department of Medicinal Chemistry Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-143 Kraków, Poland*

[b]*Department of New Drug Research, Institute of Pharmacology, Polish Academy of Sciences, 12 Smętna Street, 31-143 Kraków, Poland*

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Abstract—Further structure–activity relationship (SAR) studies with the 1,2,3,4-tetrahydroisoquinoline (THIQ) class of 5-HT_{1A} ligands led to the synthesis of new 1-adamantoyloaminoalkyl derivatives. The impact of substituent variations in the aromatic part of THIQ moiety on 5-HT_{1A} and 5-HT_{2A} receptor affinities, as well as in vivo functional properties of the investigated compounds were discussed. It was found that those modifications reduced the binding affinity for 5-HT_{1A} receptors (in comparison with unsubstituted THIQ derivatives); however, the majority of new compounds still remained potent 5-HT_{1A} ligands (K_i = 4.9–46 nM) and most of them showed features of partial agonists of postsynaptic 5-HT_{1A} receptors. At the same time, their 5-HT_{2A} receptor affinity was slightly increased (K_i = 40–1475 nM), which resulted in a loss of 5-HT_{2A}/5-HT_{1A} selectivity. 5-Br,8-OCH_3 derivative—the most potent, mixed 5-HT_{1A}/5-HT_{2A} ligand—produced activation of presynaptic 5-HT_{1A} receptors and showed properties of a 5-HT_{2A} receptor antagonist.

Introduction

Since 1994, the 1,2,3,4-tetrahydroisoquinoline (THIQ) moiety has been used as a tool fragment in our systematic SAR studies into the role of individual pharmacophoric groups of long-chain arylpiperazines—serotonin 5-HT_{1A} receptor ligands.[1–3] Although physico-chemical properties of THIQ, important for a ligand–receptor interaction (i.e., lipophilicty and pK_a) are similar to those of 1-arylpiperazines, the compound itself does not show any affinity for the 5-HT_{1A} receptor (K_i > 50,000 nM).[1,4,5] This phenomenon can be explained by the fact that a crucial distance between the center of aromatic ring and the basic nitrogen atom is too short (3.7 Å)[1] to fulfill the requirements specified by Hibert (5.3–5.7 Å) for an interaction with the 5-HT_{1A} receptor binding site.[6,7] On the basis of those properties, we initially applied the THIQ system as a replacement for the arylpiperazine fragment of well-known, potent 5-HT receptor ligands.[3] Those modifications permitted us to draw a conclusion about engagement of the respective pharmacophoric groups in the formation of a ligand–receptor complex.[3] While carrying on our studies with THIQ derivatives, we examined over 60 new compounds for their both 5-HT_{1A} and 5-HT_{2A} receptor activities.[2,3,8–11] In general, all the derivatives that were active at the 5-HT_{1A} receptor were characterized by a high degree of 5-HT_{2A}/5-HT_{1A} selectivity. Those investigations allowed us to distinguish a new THIQ class of 5-HT_{1A} receptor ligands.[10]

Recently, we conducted SAR studies with three series of *N*-substituted THIQs having two-, three- and four-membered alkyl chains. It was found that the volume of terminal hydrocarbon substituents was a key parameter for determination of their 5-HT_{1A} affinities, whereas

*Corresponding author. Tel.: +48-12-637-40-22; fax: +48-12-637-45-00; e-mail: bojarski@if-pan.krakow.pl

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their in vivo activity apparently depended on both the volume and the length of an alkyl spacer.[10] In in vivo models, of all the investigated compounds, adamantyl derivative (**1**) with tetramethylene chain showed the highest 5-HT_{1A} receptor affinity ($K_i = 0.95$ nM) and behaved like a partial agonist of the postsynaptic 5-HT_{1A} receptor. Its two-carbon spacer analogue (**2**) demonstrated a slightly lower but still high 5-HT_{1A} affinity ($K_i = 15$ nM) and was classified as a weak agonist of 5-HT_{1A} receptors.[10]

Until recently, no structural modifications within the THIQ class of ligands have affected the core fragment. To continue our SAR studies with that class of ligands, we focused our attention on substitution at the aromatic part of the THIQ system. We started with substituent variations on the basis of the structure of the previously described adamantyl derivatives **1** and **2**, in order to determine whether such molecular modifications affect the 5-HT_{1A} receptor affinity and selectivity compared to 5-HT_{2A} receptors and their intrinsic activity.

Chemistry

Compounds **20**–**28** were prepared according to Scheme 1.

Commercial benzyl cyanides or aromatic aldehydes were converted into the appropriate 2-phenylethylamines which were cyclized to 1,2,3,4-tetrahydroisoquinolines (THIQ) either in strong acidic media[12] (**3**–**7**), or via the classic Pictet–Spengler reaction[13] (**9**,**10**) (Scheme 1, step 1) or directly by nitration of THIQ[14] (**8**). *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinolines **11**–**19** were obtained by the Gabriel synthesis (Scheme 1, step 2).[3] Simple acylation[10] of **11**–**19** with freshly prepared adamantane acid bromide[15,16] in methylene chloride at a room temperature yielded products **20**–**28** (Scheme 1, step 3).

The structure of both amines **11**–**19** and amides **20**–**28** was confirmed by an elemental analysis (supplementary material) and ^1H NMR spectra. The obtained amides were converted into either fumarate or hydrochloride salts. Physicochemical data concerning final compounds **20**–**28** are collected in Table 1.

Pharmacology

Compounds **20**–**28** were evaluated for in vitro 5-HT_{1A} and 5-HT_{2A} receptor affinities on the basis of their ability to displace [^3H]-8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetraline] and [^3H]-ketanserin, respectively. The results are presented in Table 1.

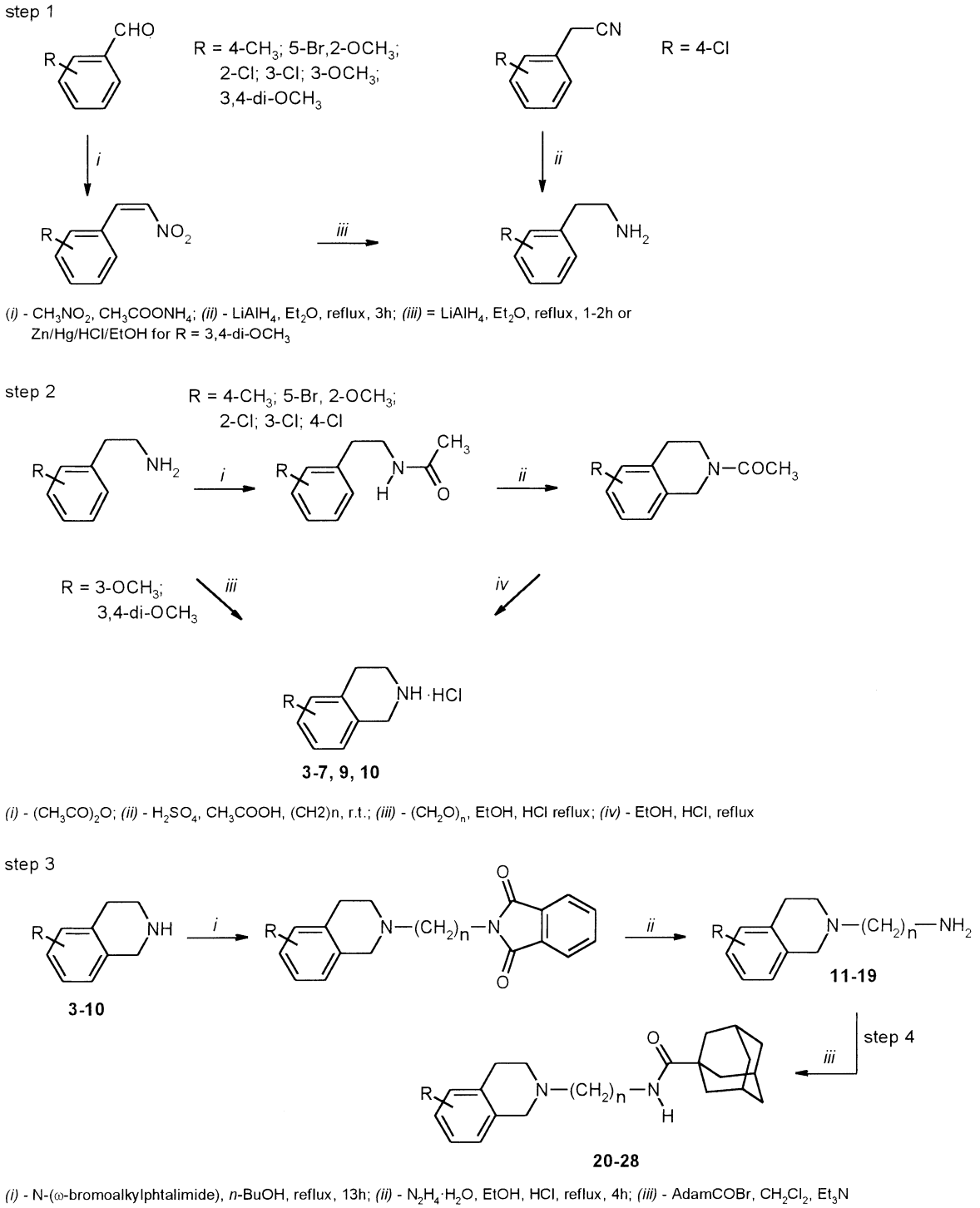
Compounds with the highest affinity for 5-HT_{1A} (**20**–**23**, **25**–**27**) and 5-HT_{2A} (**21**) receptors ($K_i < 50$ nM) were tested in vivo in several commonly used models. It is generally accepted that the hypothermia induced by 8-OH-DPAT (a 5-HT_{1A} receptor agonist) in mice is mediated by presynaptic 5-HT_{1A} receptors,[17,18] whereas the 8-OH-DPAT-induced behavioral syndrome (flat body posture, FBP and forepaw treading, FT) in reserpinized rats[19] and the lower lip retraction (LLR) in rats[20,21] are mediated by postsynaptic 5-HT_{1A} receptors. The ability of the tested compounds to induce hypothermia in mice [which was blocked by WAY 100635(-*N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-piridynyl)cyclohexanecarboxamide, a 5-HT_{1A} antagonist]), FBP, FT and LLR in rats, or to inhibit those effects induced by 8-OH-DPAT (which indicated either stimulation or blockade of 5-HT_{1A} receptors, respectively) was tested. Additionally, the 5-HT_{2A} antagonistic activity of **21** was assessed by testing its ability to antagonize the (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane [(±)-DOI]-induced headtwitches in mice.[22] All the results of in vivo studies are presented in Tables 2–6.

Results and Discussion

As has been mentioned elsewhere, the THIQ class of 5-HT_{1A} receptor ligands is originally derived from the arylpiperazine family. The most frequently used structural modifications of both simple and long-chain arylpiperazines are concerned with introduction of various substituents in the aryl part. The influence of a substituent position (*ortho*, *meta* and *para*) and its character (electron-donating or-withdrawing) on the 5-HT_{1A} receptor activity of ligands has already been described in a number of papers.[23–28] These studies indicate that the *para* position is unfavorable for 5-HT_{1A} receptor interactions,[24,26,28] especially for such electron-withdrawing substituents as fluorine or the nitro group.[24,27] On the other hand, introduction of Cl, OCH_3, CF_3 or F to the *ortho* or *meta* positions generally has a positive influence on the 5-HT_{1A} receptor binding constant.[24,26] In our earlier studies only an unsubstituted THIQ system was used; at present, like in the case of arylpiperazines, we modified the THIQ aromatic part by introducing to positions 5, 6, 7 or 8 substituents of a different nature. Since a simple, unsubstituted THIQ molecule is inactive at 5-HT_{1A}, we decided to use adamantane derivatives (**1** and **2**) with a pronounced 5-HT_{1A} receptor activity as basic structures for the pilot studies described herein.

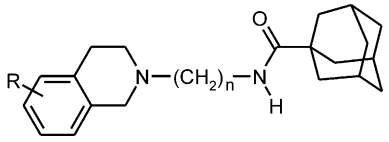
The majority of the tested derivatives (**20**–**23**, **25**–**27**; Table 1) showed a high affinity for 5-HT_{1A} receptors ($K_i = 4.9$–46 nM), lower than their parent compound **1**, though, which exhibits a subnanomolar activity ($K_i = 0.95$ nM).[10] A significant decrease in the 5-HT_{1A} affinity was observed for **24** and **28** ($K_i = 169$ and 149 nM, respectively) compared with the initial values obtained for the unsubstituted counterparts **1** and **2** ($K_i = 15$ nM).[10] The above results suggest that both the character and the position of THIQ substituents have only a minor effect on the 5-HT_{1A} affinity. The only exception was found for position 7, where the introduction of a chlorine atom significantly reduced the binding affinity for 5-HT_{1A} receptors (**24**, $K_i = 169$ nM) compared to 7-methyl (**20**) and 7-nitro (**25**) analogues ($K_i = 4.9$ and 24 nM, respectively).

Almost all the investigated compounds displayed a moderate to low affinity ($K_i = 99–1475$ nM) for 5-HT_{2A} receptors, except for the 8-Br-5-OCH_3 derivative (**21**; $K_i = 40$ nM) which showed a substantially higher in vitro activity than did the parent derivative (**1**; $K_i = 452$ nM). Since such substitution in the aromatic ring of THIQ improves the 5-HT_{2A} affinity, we have synthesized an analogue of the most potent compound **21** with a shorter hydrocarbon chain. The same tendency was observed for the resultant derivative **28** (a tenfold increase in the 5-HT_{2A} receptor affinity). Since modifications of the THIQ structure were designed with reference to phenylpiperazine (PhP), some basic superposition studies were carried out (Fig. 1). In order to obtain the best possible fit between the rigid THIQ system and PhP, only a co-planar conformation of phenyl and piperazine rings was taken into account. As found by Dijkstra et al.,[29] such an assumption is plausible in the case of *meta* and *para* substitution of PhP, i.e., when steric effects are negligible and the energy of such a rotamer is close to the global energy minimum. However, in the case of *ortho*-substituted PhP, only twisted



Scheme 1. Synthesis of the investigated compounds **20**–**28**.

Table 1. Structure, physical data of the new compounds and 5-HT_{1A} and 5-HT_{2A} binding affinities for **1**, **2** and **20**–**28**



Compd	R	*n*	Yield (%)[a]	Mp (C°) (Cryst. solv.)[b]	Molecular formula[c]	$K_i \pm$SEM (nM)	
						5-HT_{1A}	5-HT_{2A}
1[d]	H	4				0.95±0.04	452±7
20	7-CH_3	4	60	158–159 (A)	$C_{25}H_{36}N_2O{\cdot}C_4H_4O_4{\cdot}H_2O$	4.9±0.7	99±24
21	5-OCH_3, 8-Br	4	39	164–165 (B)	$C_{25}H_{35}N_2O_2Br{\cdot}C_4H_4O_4$	6.2±0.4	40±1
22	5-Cl	4	55	241–243 (B)	$C_{24}H_{33}N_2OCl{\cdot}HCl$	46±18	122±11
23	6-Cl	4	70	160–162 (B)	$C_{24}H_{33}N_2OCl{\cdot}C_4H_4O_4$	5.0±0.4	317±8
24	7-Cl	4	70	154–156 (B)	$C_{24}H_{33}N_2OCl{\cdot}C_4H_4O_4{\cdot}0.5H_2O$	169±8	180±8
25	7-NO_2	4	25	117–119 (B)	$C_{24}H_{33}N_3O_3{\cdot}C_4H_4O_4{\cdot}0.5H_2O$	24±1	481±47
26	6-OCH_3	4	65	193–195 (B)	$C_{25}H_{36}N_2O_2{\cdot}HCl{\cdot}H_2O$	34±7	1475±34
27	6,7-di-OCH_3	4	37	188–890 (B)	$C_{26}H_{38}N_2O_3{\cdot}C_4H_4O_4{\cdot}0.5H_2O$	42±3	1140±3
2[a]	H	2				15±0.2	640±80
28	5-OCH_3, 8-Br	2	30	226–228 (B)	$C_{23}H_{31}N_2O_6Br{\cdot}C_4H_4O_4{\cdot}H_2O$	149±13	63±4

[a]Yield of step 4.
[b]A: $(CH_3)_2CO$; B: $(CH\pm_3)_2CO/C_2H_5OH$, 9:1.
[c]Anal. C, H, N.
[d]Data obtained from ref 10.

Table 2. The effect of the tested compounds and WAY 100635 on the body temperature in mice

Treatment	Dose (mg/kg)	$\Delta t \pm$ SEM (°C)			
		30 min	60 min	90 min	120 min
Vehicle	—	−0.1±0.1	−0.1±0.1	−0.1±0.1	−0.1±0.1
20	10	0.4±0.2	0.4±0.2	0.4±0.3	0.5±0.2
	20	−0.8±0.2[a]	−0.4±0.2	−0.3±0.1	−0.1±0.1
Vehicle	—	−0.2±0.1	−0.1±0.1	−0.1±0.1	−0.1±0.1
21	10	−0.7±0.2[a]	−0.6±0.1[a]	−0.4±0.1	−0.4±0.1
	20	−1.7±0.2[b]	−1.0±0.2[b]	−0.7±0.1[b]	−0.5±0.1
Vehicle	—	−0.2±0.1	−0.3±0.2	−0.1±0.2	−0.2±0.1
22	10	−1.2±0.2[a]	−1.0±0.2[a]	−0.5±0.2	−0.3±0.1
	20	−1.1±0.2[a]	−0.9±0.2[a]	−0.3±0.2	−0.1±0.2
Vehicle	—	−0.1±0.1	−0.1±0.1	−0.1±0.1	0.1±0.1
23	10	−0.1±0.2	0.3±0.1	0.5±0.2	0.5±0.1
	20	−0.1±0.1	0.2±0.1	0.4±0.2	0.4±0.1
Vehicle	—	−0.3±0.1	−0.3±0.1	−0.3±0.1	−0.2±0.1
25	10	−0.2±0.1	−0.7±0.2	−0.7±0.2	−0.1±0.2
	20	−0.3±0.1	−0.1±0.1	−0.6±0.2	0.1±0.2
Vehicle	—	−0.2±0.1	−0.2±0.1	−0.1±0.1	−0.2±0.1
26	10	−1.4±0.3[b]	−0.6±0.1	−0.5±0.1	−0.2±0.1
	20	−1.8±0.3[b]	−0.8±0.2[a]	−0.6±0.2	−0.3±0.3
Vehicle	—	0.0±0.1	−0.1±0.1	−0.1±0.1	−0.1±0.1
27	10	0.2±0.2	−0.1±0.1	−0.1±0.1	0.2±0.1
	20	0.1±0.1	0.1±0.1	0.0±0.1	0.1±0.1
Vehicle	—	0.0±0.1	0.1±0.1	0.1±0.1	0.0±0.1
WAY 100635	0.03	0.1±0.1	0.2±0.1	0.2±0.1	0.1±0.1
	0.1	0.2±0.1	0.2±0.1	0.1±0.1	0.2±0.1

The investigated compounds were administered (ip) 30 min before the test. The absolute mean initial body temperatures were within a range of 36.5±0.5 °C.
[a]$p < 0.05$.
[b]$p < 0.01$ versus vehicle.

Table 3. The effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by **20**, **21**, **22**, and **26** in mice

Treatment and dose (mg/kg)	$\Delta t \pm$SEM (°C)	
	30 min	60 min
Vehicle + vehicle	−0.1±0.0	−0.1±0.1
Vehicle + **20** (20)	−1.4±0.3[b]	−0.5±0.2
WAY 100635 + **20** (20)	−1.9±0.3[b]	−0.6±0.2
Vehicle + vehicle	−0.1±0.1	0.1±0.1
Vehicle + **21** (20)	−1.7±0.2[b]	−0.7±0.2[b]
WAY 100635 + **21** (20)	−0.8±0.2[b,B]	0.1±0.2[b,B]
Vehicle + vehicle	−0.1±0.1	−0.1±0.1
Vehicle + **22** (10)	−1.1±0.3[a]	−0.7±0.2
WAY 100635 + **22** (10)	−0.2±0.3[A]	−0.1±0.2
Vehicle + vehicle	−0.1±0.1	−0.1±0.1
Vehicle + **26** (10)	−1.4±0.2[b]	−0.4±0.1
WAY 100635 + **26** (10)	−0.2±0.2[A]	0±0.1

WAY 100635 was administered (sc) 15 min before investigated compounds (ip). The absolute mean initial body temperatures were within a range of 36.1±0.5 °C.
[A]$p < 0.05$.
[B]$p < 0.01$ versus vehicle + investigated compounds.
[a]$p < 0.05$.
[b]$p < 0.01$ versus vehicle + vehicle.

or even perpendicular conformations are possible. Thus even if 5 or 8 positions of THIQ correspond to the *ortho* substitution of PhP (Fig. 1), direct comparisons between the respective substituents are not feasible.

Furthermore, as can be seen in Figure 1, positions 6 and 7 of THIQ do not precisely mimic either *meta* or *para* substitution of PhP. The above arguments may help to explain why substitution of the THIQ system does not change the 5-HT_{1A} affinity like in the case of the phenylpiperazine series. On the other hand, such structural changes lead to some improvement in the 5-HT_{2A} receptor affinity and, in consequence, all those compounds display a poorer 5-HT_{2A}/5-HT_{1A} receptors selectivity.

As has already been mentioned in the introduction, compound **1** (devoid of substituents in the THIQ skeleton) behaved like a postsynaptic 5-HT_{1A} partial agonist, whereas its presynaptic activity was almost negligible.[10]

Table 4. The effect of **23**, **25**, **27** and WAY 100635 on the 8-OH-DPAT(5 mg/kg)- induced hypothermia in mice

Treatment and dose (mg/kg)	$\Delta t \pm$SEM (°C)			
	15 min	30 min	45 min	60 min
Vehicle + vehicle	-0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.4 ± 0.1
Vehicle + 8-OH-DPAT	-1.1 ± 0.3^{b}	-1.1 ± 0.2^{b}	-0.7 ± 0.2^{b}	-0.4 ± 0.3^{a}
23 (20) + 8-OH-DPAT	-0.5 ± 0.2^{A}	-0.7 ± 0.2^{b}	-0.3 ± 0.2	-0.3 ± 0.2
Vehicle + vehicle	-0.3 ± 0.1	0.1 ± 0.1	-0.2 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT	-1.7 ± 0.1^{b}	-1.5 ± 0.3^{a}	-0.8 ± 0.2^{b}	-0.7 ± 0.2^{a}
25 (20) + 8-OH-DPAT	-1 ± 0.2^{bB}	-0.9 ± 0.4^{a}	-0.8 ± 0.3^{a}	-0.7 ± 0.2^{a}
Vehicle + vehicle	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT	-1.2 ± 0.2^{b}	-1 ± 0.2^{b}	-0.8 ± 0.2^{b}	-0.4 ± 0.2
27 (20) + 8-OH-DPAT	-0.3 ± 0.2^{B}	0.1 ± 0.1^{B}	-0.2 ± 0.2^{A}	0.2 ± 0.2
Vehicle + vehicle	-0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Vehicle + 8-OH-DPAT	-1.2 ± 0.1^{b}	-1 ± 0.1^{b}	-0.7 ± 0.1^{b}	-0.2 ± 0.1
WAY 100635(0.03) + 8-OH-DPAT	-0.2 ± 0.1^{B}	-0.4 ± 0.1^{B}	-0.2 ± 0.1^{A}	-0.1 ± 0.1
WAY 100635(0.1) + 8-OH-DPAT	-0.1 ± 0.1^{B}	-0.1 ± 0.1^{B}	0.1 ± 0.1^{B}	0.2 ± 0.1

The investigated compounds were administered (ip) 45 min before 8-OH-DPAT (sc). WAY 100635 was administered (sc) 15 min before 8-OH-DPAT. The absolute mean initial body temperatures were within a range of 36.2 ± 0.5 °C.
$^{A}p < 0.05$.
$^{B}p < 0.01$ versus vehicle + 8-OH-DPAT.
$^{a}p < 0.05$.
$^{b}p < 0.01$ versus vehicle + vehicle.

Table 5. Induction of lower lip retraction (LLR) by the tested compounds and WAY 100635 (A) and their effect on the 8-OH-DPAT (1 mg/kg)-induced LLR (B) in rats

Treatment	Dose (mg/kg)	Mean ± SEM LLR score	
		A	B
Vehicle	—	0.1 ± 0.1	2.7 ± 0.1
20	5	0.9 ± 0.3^{a}	1.5 ± 0.0^{b}
	10	1.8 ± 0.3^{b}	1.6 ± 0.2^{b}
	20	1.8 ± 0.2^{b}	1.2 ± 0.2^{b}
Vehicle	—	0 ± 0	2.8 ± 0.2
21	10	0 ± 0	2.6 ± 0.3
	20	0.2 ± 0.1	2.3 ± 0.3
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
22	5	1.1 ± 0.2^{b}	2.3 ± 0.3
	10	1.2 ± 0.2^{b}	1.5 ± 0.1^{a}
	20	1.3 ± 0.2^{b}	1.3 ± 0.3^{a}
Vehicle	—	0.1 ± 0.1	2.8 ± 0.2
23	5	0.7 ± 0.3	2.4 ± 0.4
	10	1.6 ± 0.2^{b}	1.8 ± 0.2^{a}
	20	2.1 ± 0.3^{b}	1.8 ± 0.2^{a}
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
25	10	1 ± 0.1^{b}	1.8 ± 0.2^{b}
	20	1.8 ± 0.2^{b}	2.2 ± 0.2
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
26	10	0.9 ± 0.2^{a}	2.4 ± 0.2
	20	1.7 ± 0.2^{b}	2.2 ± 0.2
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
27	10	0 ± 0	2 ± 0.3^{a}
	20	0.1 ± 0.1	1.3 ± 0.2^{b}
Vehicle	—	0.1 ± 0.1	2.8 ± 0.1
WAY 100635	0.01	0 ± 0	2.1 ± 0.2
	0.03	0.1 ± 0.1	0.9 ± 0.4^{b}
	0.1	0.1 ± 0.1	0.3 ± 0.2^{b}

The investigated compounds were administered (ip) 15 min before test (A) or 45 min before 8-OH-DPAT (sc) (B).
$^{a}p < 0.05$.
$^{b}p < 0.01$ versus vehicle (A) or versus vehicle + 8-OH-DPAT (B).

Of the compounds tested in vivo, **21**, **22** and **26**—like 8-OH-DPAT—induced hypothermia in mice (Table 2), which was attenuated (**21**) or blocked (**22**, **26**) by WAY 100635 (Table 3). Compound **20** also showed a hypothermic effect in mice (Table 2), which was not changed by 5-HT_{1A} antagonist; thus reasons other than stimulation of 5-HT_{1A} receptors seemed to be responsible for the hypothermia induced by that compound. On the other hand, compounds **23**, **25** and **27**—like WAY 100635 — did not change body temperature in mice (Table 2), but attenuated (**23**, **25**) or blocked (**27**)—like WAY 100635—the hypothermia induced by 8-OH-DPAT (Table 4). These results indicate that in the hypothermia model compounds **21**, **22** and **26** behaved like presynaptic 5-HT_{1A} agonists, whereas **23**, **25** and in particular **27** showed properties of presynaptic 5-HT_{1A} antagonists. Like 8-OH-DPAT, compounds **20**, **22**, **23**, **25** and **26** evoked LLR in rats (Table 5); furthermore, **20**, **22** and **23** induced a behavioral syndrome in reserpinized rats (Table 6), the strongest 5-HT_{1A} agonistic-like effect being observed after injection of compound **23** in a dose of 5–20 mg/kg. On the other hand, **20** and **22**–**27**—like WAY 100635—inhibited LLR and/or the behavioral syndrome induced by 8-OH-DPAT (Table 6). The above results show that **20**, **22**, **23**, **25**, and **26** are postsynaptic 5-HT_{1A} partial agonists, whereas **27** behaves like a weak pre- and postsynaptic 5-HT_{1A} antagonist. Unexpectedly, compound **21** with high 5-HT_{1A} affinity and properties of a presynaptic 5-HT_{1A} agonist seems to be devoid of a postsynaptic 5-HT_{1A} activity in vivo as it neither induced LLR, FBP or FT in rats, nor inhibited these symptoms induced by 8-OH-DPAT. Compound **21** demonstrated 5-HT_{2A} antagonistic properties towards the (±)-DOI-induced head twitches in mice: it dose-dependently inhibited the effect of (±)-DOI; its ED_{50} value was 18 (12–27) mg/kg.

Table 6. Induction of behavioral syndrome by the tested compounds and WAY 100635 (A) and their effect on the 8-OH-DPAT (5 mg/kg)-induced behavioral syndrome (B) in reserpine-pretreated rats

Treatment	Dose (mg/kg)	$\Delta t \pm$SEM behavioral score			
		A		B	
		FBP	FT	FBP	FT
Vehicle	—	0.1±0.1	0.1±0.1	13.5±0.7	13±0.7
20	5	6.3±0.7[b]	0.5±0.5	7.2±0.8[b]	6.3±0.8[b]
	10	7±1.0[a]	0.4±0.4	4.5±1.1[b]	3.3±1.1[b]
Vehicle	—	0±0	0±0	12.5±0.2	12.2±0.4
21	20	0±0	0±0	11.5±0.7	11.2±0.7
Vehicle	—	0.1±0.1	0.1±0.1	12.2±0.5	2±0.8
22	5	3.2±1.2	0±0	10.5±0.8	7.7±1.1[b]
	10	7±1.6[a]	0.7±0.3	6.2±1.3[b]	5±0.8[b]
	20	7.2±1.3[b]	0.8±0.3	1.8±0.7[b]	3.4±0.8[b]
Vehicle	—	0.1±0.1	0.1±0.1	13.2±0.6	13.2±0.8
23	5	4.8±1.3[b]	4.2±0.6[b]	11.5±0.6	9.8±1.1[a]
	10	9.5±1.2[b]	4.7±1.1[b]	9.3±0.8	4.7±1.0[b]
	20	12±1.4[b]	5.3±1.1[b]	7.8±0.8[b]	3.8±0.8[b]
Vehicle	—	0±0	0±0	13±0.3	12.2±0.5
25	20	1.5±1	1.5±0.6	11.7±0.8	11±1.2
Vehicle	—	0±0	0±0	13±0.3	12.2±0.5
26	10	0±0	0±0	9.8±0.7[a]	9.5±0.9
	20	1±0.6	0.8±0.5	6±1.1[b]	5.8±0.8[b]
Vehicle	—	0±0	0±0	13±0.3	12.2±0.5
27	20	0±0	0±0	11.8±0.8	9.7±0.6
Vehicle	—	0±0	0±0	13.7±0.4	12±0.7
WAY 100635	0.01	0±0	0±0	13.2±0.5	8.3±1.2[a]
	0.03	0.1±0.1	0±0	3.3±0.6[b]	1.7±0.8[b]
	0.1	0±0	0±0	0.8±0.4[b]	1.2±0.7[b]

FBP, flat body posture, FT, forepaw treading.
Reserpine (1 mg/kg, sc) was administered 18 h before the tests. The investigated compounds were administered (ip) 3 min before the test (A) or 60 min before 8-OH-DPAT (sc) (B).
[a] $p < 0.05$.
[b] $p < 0.01$ versus vehicle (A) or versus vehicle + 8-OH-DPAT (B).

As follows from in vivo studies, modifications of the THIQ part may have some influence on the intrinsic activity of the tested compounds. Most derivatives (**20**, **22**, **23**, **25** and **26**) retain features of postsynaptic 5-HT_{1A} partial agonists, and in relation to presynaptic 5-HT_{1A} receptors they behave like agonists (**21**, **22**, **26**) or antagonists (**23**, **25**). Introduction of two methoxy groups in positions 6 and 7 (**27**) altered the pharmacological profile, since compound **27** can be regarded as a weak pre- and postsynaptic 5-HT_{1A} antagonist. Interestingly, analogue **21**—a mixed 5-HT_{1A}/5-HT_{2A} ligand—produced in vivo activation of presynaptic 5-HT_{1A} receptors only; at the same time, it showed properties of a 5-HT_{2A} receptor antagonist.

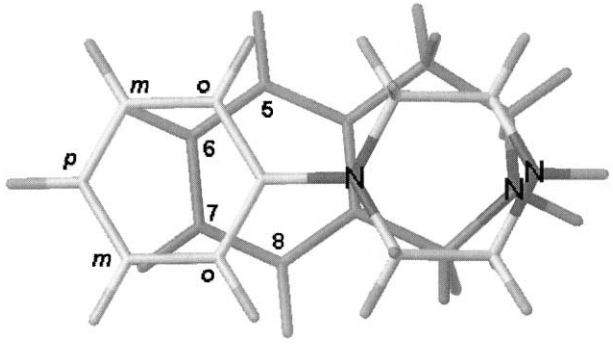


Figure 1. Superposition of 1-phenylpiperazine (coplanar conformation) and 1,2,3,4-tetrahydroisoquinoline (dark grey); Sybyl software ver. 6.6 (Tripos Inc.).

Conclusions

The previously described adamantyl derivatives of unsubstituted THIQ (**1** and **2**) showed a high 5-HT_{1A} receptor affinity and 5-HT_{2A}/5-HT_{1A} selectivity.[10] Results of the present study indicate that substitution in the aromatic part of the THIQ moiety reduces 5-HT_{1A} receptor affinity and simultaneously slightly increases 5-HT_{2A} receptor affinity, which leads to a loss of 5-HT_{2A}/5-HT_{1A} selectivity. Introduction of $-OCH_3$ and –Br in positions 5 and 8, respectively, appears to be particularly profitable for the mixed 5-HT_{1A}/5-HT_{2A} ligand (**21**) yielded. Such a dual in vitro activity together and the pharmacological profile (a presynaptic 5-HT_{1A} agonist/5-HT_{2A} antagonist) may suggest possible psychotropic properties. It seems a good idea to focus further studies on the substituent variations in positions 5 and 8 in order to confirm the obtained results using other parent (unsubstituted) compounds.

Experimental

Chemistry

Melting points were determined using an Electrothermal Digital Melting Point IA9000 apparatus and were uncorrected. ^{1}H NMR spectra were obtained with a Varian EM-360L (60 MHz) in a $CDCl_3$ solution with TMS as an internal standard; the values of chemical shifts (δ) are given in ppm. The starting R-substituted 1,2,3,4-tetrahydroisoquinolines were obtained according to the published procedure, that is compounds **3**–**7**,[12] **8**[14] and **9**, **10**.[13] Methods describing preparation of compounds **11**–**19**[3] and **20**–**28**[10] had been previously published.

General procedure for preparation of compounds 11–19

A mixture of *N*-(2-chloroethyl)- or *N*-(4-bromobutyl)-phthalimide (3 mmol), substituted 1,2,3,4-tetrahydroisoquinoline **3**–**10** (0.4 g, 3 mmol), and *n*-butanol (30 mL) was refluxed for 13 h. Then the reaction mixture was filtered off, and the solvent was evaporated to give a crude product, which was purified by crystallization.

A solution of phtalimide derivative (free bases, 1.2 mmol) and hydrazine (0.5 mL, 12 mmol) in 99.8% ethanol (15 mL) was refluxed for 1 h. The reaction mixture was cooled down and treated with an additional amount of 99.8% ethanol (15 mL) and concentrated HCl (1.3 mL). Then the reaction mixture was refluxed for 4 h and left overnight in a refrigerator. The precipitate was filtered off, and the solvent was evaporated. The residue was treated with *n*-hexane (20 mL) and NH_3 (aqueous, 15 mL). The solution was extracted with $CHCl_3$ (3×15 mL). the organic layer was dried over anhydrous K_2CO_3, and the solvents were evaporated to give a product (**11**–**19**) which was used without further purification.

General procedure for preparation of compounds 20–28

1-Adamantanecarboxylic acid (1.5 mmol) was dissolved in methylene chloride (3 mL) and triphenylphosphine (1.5 mmol) was added on stirring. After 5 min *N*-bromo-succinimide (NBS 1.6 mmol) was added in portions and after that the mixture was stirred for 0.5 h at room temperature. Then the solution of *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinoline (**11**–**19**) (1.4 mmol) and triethylamine (1.7 mmol) in methylene chloride (1 mL) was added dropwise. The reaction mixture was stirred for 3 h at room temperature and left overnight. Then it was diluted with $CHCl_3$ (10 mL) and washed with 20% aqueous NaOH (5 mL), water (5 mL) and dried over anhydrous $MgSO_4$. The inorganic precipitate was filtered off, the solvents were evaporated and the amides (**20**–**28**) were separated by column chromatography using SiO_2 and $CHCl_3$ followed by $CHCl_3$–MeOH, 95:5.

2-[4-(1-Adamantoylamino)butyl]-7-methyl-1,2,3,4-tetrahydroisoquinoline (20). Oil, yield 60%. ^{1}H NMR 1.4–2.1 (m, 19H), 2.2 (s, 3H, CH_3), 2.4–2.7 (m, 2H, CH_2), 2.7–3.1 (m, 4H), 3.1–3.4 (m, 2H, CH_2N), 3.7 (s, 2H, CH_2), 6.2 (br. s, 1H, NH), 6.9 (s, 1H, arom.), 7.0 (s, 2H, arom). Mp (salt) 158–159 °C. Anal. ($C_{25}H_{36}N_2O\cdot C_4H_4O_4\cdot H_2O$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-8-bromo-5-methoxy-1,2,3,4-tetrahydroisoquinoline (21). Oil, yield 39%. ^{1}H NMR 1.4–2.1 (m, 19H), 2.4–3.0 (m, 6H), 3.2–3.5 (m, 2H, CH_2N), 3.6 (s, 2H, CH_2), 3.8 (s, 3H, OCH_3), 6.1 (br s, 1H, NH), 6.7 (d, $J=8$ Hz, 1H, arom.), 7.4 (d, $J=8$ Hz, 1H, arom). Mp (salt) 164–165 °C. Anal. ($C_{25}H_{35}BrN_2O_2\cdot C_4H_4O_4$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-5-chloro-1,2,3,4-tetrahydroisoquinoline (22). Oil, yield 55%. ^{1}H NMR 1.4–2.1 (m, 19H), 2.4–3.0 (m, 6H), 3.2–3.5 (m, 2H, CH_2N), 3.7 (s, 2H, CH_2), 6.0 (br s, 1H, NH), 7.0–7.6 (m, 3H, arom). Mp (salt) 241–243 °C. Anal. ($C_{24}H_{33}ClN_2O\cdot HCl$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-6-chloro-1,2,3,4-tetrahydroisoquinoline (23). Oil, yield 70%. ^{1}H NMR 1.4–2.2 (m, 19H), 2.4–3.0 (m, 6H), 3.1–3.5 (m, 2H, CH_2N), 3.7 (s, 2H, CH_2), 6.0 (br s, 1H, NH), 7.0–7.3 (m, 3H, arom). Mp (salt) 160–162 °C. Anal. ($C_{24}H_{33}ClN_2O\cdot C_4H_4N_4$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-7-chloro-1,2,3,4-tetrahydroisoquinoline (24). Oil, yield 70%. ^{1}H NMR 1.4–2.2 (m, 19H), 2.4–2.9 (m, 6H), 3.1–3.5 (m, 2H, CH_2N), 3.6 (s, 2H, CH_2), 6.1 (br s, 1H, NH), 7.1 (s, 3H, arom). Mp (salt) 154–156 °C. Anal. ($C_{24}H_{33}ClN_2O\cdot C_4H_4N_4\cdot 0.5\ H_2O$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-7-nitro-1,2,3,4-tetrahydroisoquinoline (25). Oil, yield 25%. ^{1}H NMR 1.4–2.3 (m, 19H), 2.5–3.3 (m, 6H), 3.3–3.8 (m, 2H, CH_2N), 3.7 (s, 2H, CH_2), 5.8 (br s, 1H, NH), 7.3 (d, $J=7$ Hz, 1H, arom), 7.4 (s, 1H, arom), 8.1 (d, $J=7$ Hz, 1H, arom). Mp (salt) 117–119 °C. Anal. ($C_{24}H_{33}N_3O_3\cdot C_4H_4O_4$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-6-methoxy-1,2,3,4-tetrahydroisoquinoline (26). Oil, yield 65%. ^{1}H NMR 1.5–2.2 (m, 19H), 2.3–3.1 (m, 6H), 3.1–3.5 (m, 2H, CH_2N), 3.5 (s, 2H, CH_2), 3.8 (s, 3H, OCH_3), 6.0 (br s, 1H, NH), 6.5–7.0 (m, 3H, arom). Mp (salt) 193–195 °C. Anal. ($C_{25}H_{36}N_2O_2\cdot HCl\cdot H_2O$) C, H, N.

2-[4-(1-Adamantoylamino)butyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (27). Oil, yield 37%. ^{1}H NMR 1.5–2.2 (m, 19H), 2.5–2.7 (m, 2H), 2.7–3.0 (m, 4H), 3.2–3.5 (m, 2H, CH_2N), 3.6 (s, 2H, CH_2), 3.9 (s, 6H, $2OCH_3$), 6.1 (br s, 1H, NH), 6.6 (d, $J=4$ Hz, 2H, arom). Mp (salt) 188–190 °C. Anal. ($C_{26}H_{38}N_2O_3\cdot C_4H_4O_4\cdot 0.5H_2O$) C, H, N.

2-[2-(1-Adamantoylamino)ethyl]-8-bromo-5-methoxy-1,2,3,4-tetrahydroisoquinoline (28). Oil, yield 30%. ^{1}H NMR 1.5–2.2 (m, 15H), 2.5–3.2 (m, 6H), 3.2–3.5 (m, 2H, CH_2N), 3.6 (s, 2H, CH_2), 3.8 (s, 3H, OCH_3), 6.2 (br s, 1H, NH), 6.6 (d, $J=8$ Hz, 1H, arom), 7.3 (d, $J=8$ Hz, 1H, arom). Mp (salt) 226–228 °C. Anal. ($C_{23}H_{31}BrN_2O_2\cdot C_4H_4O_4\cdot H_2O$) C, H, N.

In vitro studies—binding experiments

Radioligand binding experiments were conducted in rat hippocampus for 5-HT_{1A} receptors, and in the cortex for 5-HT_{2A} receptors according to the published procedures.[30] The radioligands used were [^{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 the tested compounds were used in concentrations of 10^{-10}–10^{-3} M, run in triplicate.

In vivo studies

The experiments were carried out on male Wistar rats (260–300 g) and male Albino Swiss mice (24–26 g). The animals were kept at an ambient temperature of $20\pm1\,^{\circ}C$, and had free access to food (standard laboratory pellets, LSM) and tap water. All the tests were carried out in the light phase of a natural light–dark cycle (from January to June), between 9 am and 2 pm (±)-DOI (hydrochloride, RBI), 8-OH-DPAT (hydrobromide, RBI), reserpine (Ciba, ampoules) and WAY 100635 (synthesized by Dr. J. Boksa, Institute of Pharmacology, PAS, Kraków) were disolved in saline. The investigated salts of **20**–**23** and **25**–**27** were used in the form of a freshly prepared suspension in 1% Tween 80. 8-OH-DPAT, reserpine and WAY 100635 were injected subcutaneously (sc); **20**–**23**, **25**–**27** and (±)-DOI were given intraperitoneally (ip) in a volume of 2 ml/kg (rats) or 10 ml/kg (mice). Each group consisted of 6–9 animals. The obtained data were analyzed by Dunnett's test.

The effect on the body temperature in mice

Rectal body temperature (measured with an Ellab thermometer) was recorded in mice 30, 60, 90 and 120 min after injection of the tested compounds. In a separate experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by **20**, **21**, **22** or **26** was investigated. Rectal body temperature of mice was measured 30 and 60 min after injection of the drugs tested. WAY 100635 was given 15 min before the tested compounds. In another experiment, the effect of **23**, **25**, **27** or WAY 100635 on the 8-OH-DPAT (5 mg/kg)-induced hypothermia was tested. The rectal body temperature of mice was measured 15, 30, 45 and 60 min after 8-OH-DPAT administration. The tested compounds and WAY 100635 were administered 45 and 15 min, respectively, before 8-OH-DPAT.

Lower lip retraction in rats

LLR was assessed according to the method described by Berendsen et al.[20] The rats were individually placed in cages (30×25×25 cm) and were scored three times (at 15, 30 and 45 min after the tested compounds or 8-OH-DPAT) as follows: 0 = lower incisors invisible, 0.5 = partly visible, 1 = clearly visible. The summed up maximum scores amounted to 3 for each rat. The effect of the tested compounds and WAY 100635 on the LLR induced by 8-OH-DPAT (1 mg/kg) was assessed in a separate experiment. The investigated compounds were administered 45 min before 8-OH-DPAT, and the animals were scored at 15, 30 and 45 min after 8-OH-DPAT administration.

Behavioral syndrome in reserpinized rats

The rats were individually placed in cages (30×25×25 cm) 5 min before injection of the tested compounds or 8-OH-DPAT. Observation sessions, lasting 45 s each, began 3 min after drug 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. The maximum score, summed up over five observation periods, amounted to 15 for each symptom/animal.[19] The effect of the tested compounds and WAY 100635 on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg) was assessed in a separate experiment. The investigated compounds were administered 60 min before 8-OH-DPAT, and the animals were scored at 3, 6, 9, 12 and 15 min after 8-OH-DPAT administration. Reserpine (1 mg/kg) was given 18h before the test.

Head twitches in mice

In order 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 prior to treatment. Head twitches were induced in mice by (±)-DOI (2.5 mg/kg). Immediately after the treatment, the number of head twitches was counted for 30 min.[22] The tested compounds or saline were administered 60 min before (±)-DOI.

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