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1,2,3,4-Tetrahydroisoquinoline Derivatives: A New Class of 5-HT$_{1A}$ Receptor Ligands†

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Abstract—Three series of new *N*-substituted 1,2,3,4-tetrahydroisoquinolines with 2-, 3-, and 4-membered alkyl chains (**a, b,** and **c**, respectively) were synthesized, and the effect of some structural modifications on their 5-HT$_{1A}$ receptor affinities and functional properties was discussed. It was found that the volume of the terminal amide substituent was a crucial parameter which determined 5-HT$_{1A}$ receptor affinities of the tested compounds, while the in vivo activity seemed to depend on both the R-volume and the length of a hydrocarbon chain. It was demonstrated that the most active ligands behaved like agonists or partial agonists at post-synaptic 5-HT$_{1A}$ receptors.

Introduction

Classification of multiple types of serotonin (5-HT) receptors, their ligands and their role in various CNS activities, has been reported in several reviews.[1–4] Of different populations of 5-HT receptors, 5-HT$_{1A}$ are the best characterized subtypes. A number of compounds belonging to different chemical classes[5] have a high affinity for these receptors and may act as agonists, partial agonists, or antagonists. 1-Arylpiperazine derivatives constitute one of the largest classes of 5-HT$_{1A}$ receptor ligands and are represented by, for example, buspirone **1**, NAN-190 **2**, and befiperide **3**, or by flesinoxan **4**, which belongs to another class of arylpiperazines (Chart 1).

It is generally accepted that these ligands are recognized by the 5-HT$_{1A}$ receptor due to the presence of a 1-arylpiperazine fragment that possesses two points of interaction with the receptor: a basic nitrogen atom and an aromatic ring, at the distance $d_{Ar-N} = 5.2–5.7$ Å, defined by Hibert et al. as a minimal structure requirement for an interaction with the 5-HT$_{1A}$ binding site.[6,7] In the 1,2,3,4-tetrahydroisoquinoline (THIQ) molecule, this distance (between the basic nitrogen atom and the center of aromatic ring) is shorter ($d_{Ar-N} = 3.77$ Å) than in 1-arylpiperazines. Furthermore, it is too short to form a fairly strong bioactive complex; in effect, THIQ is inactive ($K_i > 50000$ nM) at these receptors.[8] On the other hand, the ionization constant of THIQ at 37 °C ($pK_a = 9.3$)[9] is comparable with that reported for simple 1-arylpiperazines ($pK_a = 7.94–9.14$),[10] and its lipophilicity is also similar to that of many 1-arylpiperazines.[9,10] Thus the above described properties indicate that the nitrogen atom in THIQ may mimic the basic N-4 atom in 1-arylpiperazine at 5-HT$_{1A}$ receptor binding sites.[11] It actually does so, which we demonstrated by replacing the 1-(2-pyrimidyl)piperazine fragment in the buspirone molecule by the THIQ moiety: the obtained derivative **5** retained both the affinity and the functional profile of buspirone.[12] Additionally, the latter result suggested that the basic nitrogen atom and the terminal cycloimide moiety were directly responsible for the formation of a bioactive complex as well as for the functional profile of the buspirone molecule at 5-HT$_{1A}$ receptors.

In line with this finding, we designed and synthesized a series of THIQ derivatives in order to develop a new class of 5-HT$_{1A}$ ligands. To gain an insight into the structural parameters determining their affinity and functional profile at these receptors, we studied the effect of structural variations such as the volume of the terminal amide substituent and the length of the alkyl chain which separates the two crucial pharmacophoric elements.

Key words: 5-HT$_{1A}$ ligands; tetrahydroisoquinoline derivatives; structure–activity relationship.
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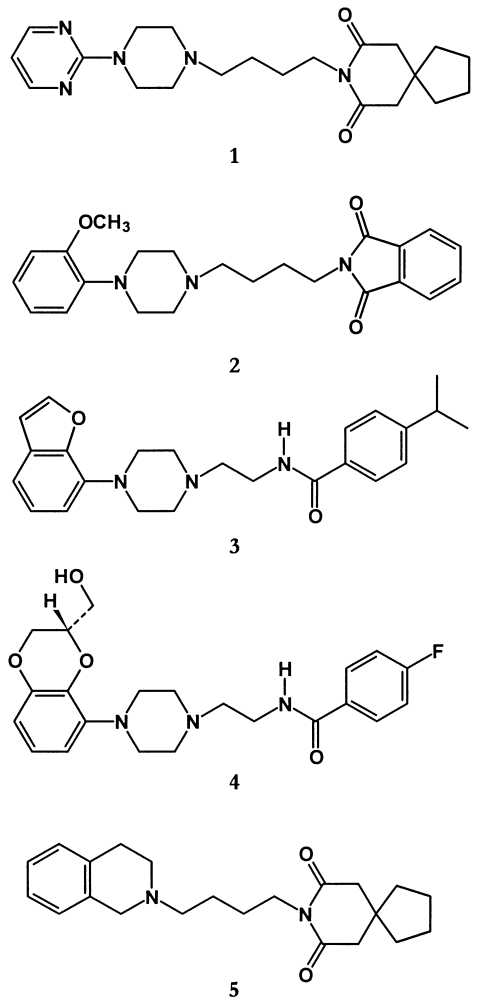


Chart 1.

Chemistry

Compounds **6–15** were prepared according to Scheme 1. *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinolines **16**,[12] **17** and **18**[12] were obtained by the Gabriel synthesis. Simple acylation with commercially available acid chlorides in two-phase media afforded compounds **6, 7,** and **8** (Method A-1 or A-2). Amides **9a, 9b, 10a–c, 11b, 12b, 12c, 13a**, and **14a** were obtained by a direct reaction between amines **16–18** and an acid in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (Method B). Compounds **9c, 11a, 11b, 12a, 13b, 13c, 14b, 14c,** and **15a–c** were prepared by acylation of amines according to the Froyen procedure (Method C).[13,14] That one-pot reaction involved preparation of acyl bromide, followed by acylation of the amine in methylene chloride at a room temperature. Some of the compounds were prepared by both B and C methods with a comparable yield (e.g. **15c** 64% and 73%, respectively), but it was easier to separate a product from the reaction mixture by method C. The obtained amides were converted into either hydrochloride or fumarate salts. The structure of the compounds was confirmed by an elemental analysis and ^{1}H NMR spectra. Physicochemical data for all the compounds are collected in Table 1.

Radioligand Binding Studies

The affinity of the investigated compounds for central 5-HT_{1A} and 5-HT_{2A} receptors in vitro were assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetraline) and [^{3}H]-ketanserine, respectively. The results are presented in Table 2.

The investigated derivatives showed a low or a very low 5-HT_{2A} receptor affinity ($300 \leq K_i \leq 10000$ nM), and the majority of them were practically inactive. On the other hand, the 5-HT_{1A} binding constants varied from 10^{-9}M (**14c** and **15c**) to 10^{-5}M for **6b**.

A comparison of the binding data for all series of the compounds (**6a–15a, 6b–15b**, and **6c–15c**) showed that the structure of the hydrocarbon substituent (R) strongly affected the 5-HT_{1A} receptor affinity. All the methyl derivatives (set **6**) were practically inactive ($K_i \geq 4500$ nM) but when the methyl group was replaced with the cyclopropyl moiety, the affinity of **7c** was increased more than fivefold. Further systematic improvement of the activity was observed for the successive sets (**8–15**), as the size of substituent R increased.

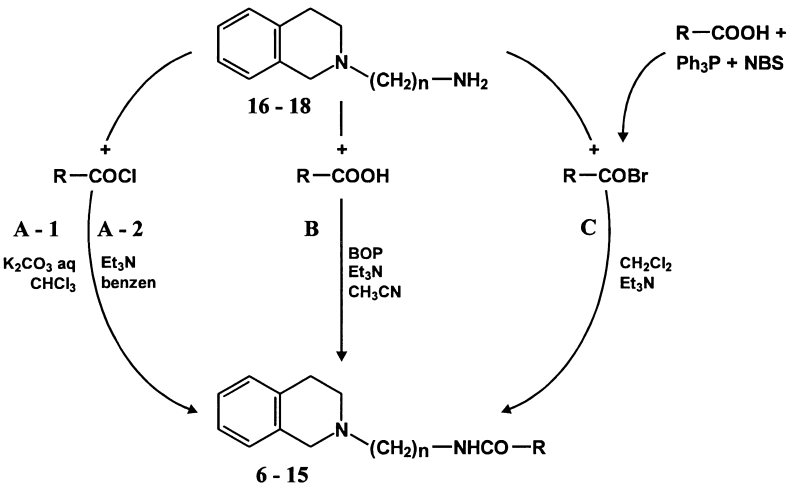
Elongation of the alkyl spacer between THIQ and the amide function (from two to four methylene groups) in each set of compounds (**6–15**) had a different influence on the 5-HT_{1A} affinity but, generally, ligands with the longest chain (series **c**) were more active than their analogues from the two series **a** and **b**. The highest 5-HT_{1A} affinities were observed for sets **12, 14,** and **15**.

In Vivo Experiments

The most active compounds of the three series were tested for their ability to induce a lower lip retraction and serotonin syndrome, or to inhibit those behaviors induced by 8-OH-DPAT, which indicated either stimulation or blockade of 5-HT_{1A} receptors, respectively.

Lower Lip Retraction (LLR) in rats

Compounds **12a–c, 13c, 14c,** and **15a–c** given alone induced the LLR in rats. The most active compounds **14c** (2.5–10 mg/kg) and **15c** (0.625–2.5 mg/kg) induced that effect in a dose-dependent manner, the maximum score being 70% and 90%, respectively, after the highest dose used; in comparison, derivatives **12a** (20 mg/kg), **15a** (5–20 mg/kg), **12b** (10–20 mg/kg), **15b** (10–20 mg/kg), **12c** (10–20 mg/kg) and **13c** (20 mg/kg) caused a weaker LLR (27–63% of maximum score) by themselves at relatively high doses. Compounds **14a** (20 mg/kg) and **14b** (20 mg/kg) given alone were inactive. 8-OH-DPAT (1 mg/kg) induced the LLR, the maximum possible score being 87–93% (Table 3). The effect of 8-OH-DPAT was insignificantly reduced only by compound **14b** (20 mg/kg), whereas derivatives **12a** (10–20 mg/kg), **14a** (20 mg/kg), **15a** (2.5–5 mg/kg), **12b** (10 mg/kg), **15b** (10 mg/kg), **12c** (10 mg/kg), **13c** (10–20 mg/kg) and **14c** (2.5 mg/kg) did not affect the LLR induced by 8-OH-DPAT (Table 3).



Scheme 1. Synthesis of the investigated compounds: A. **6a–c, 7a–c, 8a–c**; B. **9a, 9b, 10a–c, 11b, 12b, 12c, 13a, 14a**; C. **9c, 11a, 11c, 12a, 13b, 13c, 14b, 14c, 15a–c**.

Table 1. Physical data of the new compounds

Compd	Method[a]	Yield [%]	mp [°C] (recryst. Solvent)[b]	Molecular formula[c]
6a	A-1	55	136–138 (A)	$C_{13}H_{18}N_2O{\cdot}C_4H_4O_4$
6b	A-2	87	104–106 (A)	$C_{14}H_{20}N_2O{\cdot}C_4H_4O_4$
6c	A-1	57	149–150 (A)	$C_{15}H_{22}N_2O{\cdot}HCl{\cdot}0.5H_2O$
7a	A-2	47	196–198 (A)	$C_{15}H_{20}N_2O{\cdot}HCl$
7b	A-1	73	85–86 (A)	$C_{16}H_{22}N_2O{\cdot}C_4H_4O_4{\cdot}0.5\ H_2O$
7c	A-1	37	117–119 (A)	$C_{17}H_{24}N_2O{\cdot}C_4H_4O_4$
8a	A-1	42	179–180 (A)	$C_{15}H_{22}N_2O{\cdot}HCl{\cdot}2H_2O$
8b	A-2	90	97–98 (A)	$C_{16}H_{24}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
8c	A-1	48	113–115 (A)	$C_{17}H_{26}N_2O{\cdot}C_4H_4O_4{\cdot}1.5H_2O$
9a	B	52	145–146 (B)	$C_{16}H_{22}N_2O{\cdot}C_4H_4O_4$
9b	B	46	99–101 (A)	$C_{17}H_{24}N_2O{\cdot}C_4H_4O_4$
9c	C	41	203–305 (A)	$C_{18}H_{26}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
10a	B	41	137–138 (A)	$C_{16}H_{24}N_2O{\cdot}C_4H_4O_4$
10b	B	53	91–93 (A)	$C_{17}H_{26}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
10c	B	60	159–160 (C)	$C_{18}H_{28}N_2O{\cdot}HCl$
11a	C	38	127–129 (A)	$C_{17}H_{24}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
11b	B	66	110–111 (A)	$C_{18}H_{26}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
11c	C	37	131–132 (A)	$C_{19}H_{28}N_2O{\cdot}C_4H_4O_4$
12a	C	62	168–170 (D)	$C_{18}H_{26}N_2O{\cdot}HCl$
12b	B	45	105–107 (A)	$C_{19}H_{28}N_2O{\cdot}C_4H_4O_4$
12c	B	64	158–160 (D)	$C_{20}H_{30}N_2O{\cdot}HCl$
13a	B	49	122–124 (A)	$C_{20}H_{28}N_2O{\cdot}C_4H_4O_4$
13b	C	54	101–103 (A)	$C_{21}H_{30}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
13c	C	40	158–160 (B)	$C_{22}H_{32}N_2O{\cdot}C_4H_4O_4$
14a	B	48	102–104 (E)	$C_{20}H_{28}N_2O{\cdot}C_4H_4O_4$
14b	C	58	110–112 (A)	$C_{21}H_{30}N_2O{\cdot}C_4H_4O_4{\cdot}0.5H_2O$
14c	C	49	124–126 (A)	$C_{22}H_{32}N_2O{\cdot}C_4H_4O_4$
15a	C	74	179–181 (F)	$C_{22}H_{30}N_2O{\cdot}HCl{\cdot}0.5H_2O$
15b	C	75	108–109 (A)	$C_{23}H_{32}N_2O{\cdot}C_4H_4O_4{\cdot}H_2O$
15c	C	64	216–218 (B)	$C_{24}H_{34}N_2O{\cdot}HCl$

[a]The methods of preparation designated by the letters refer to general methods given in the Experimental.
[b]A: (Me_2CO), B: Me_2CO/EtOH, 4/1, C: Me_2CO/EtOH, 9/1, D: Me_2CO/Et_2O, 1/1, E: Me_2CO/EtOH, 1/1, F: Me_2CO/Et_2O, 4/1.
[c]Anal. C, H, N.

Behavioral syndrome in reserpinized rats

Of the investigated compounds, **12c, 13c, 14c**, and **15c** injected in doses of 10–20 mg/kg (**12c, 13c, 14c**) or 2.5–5 mg/kg (**15c**) induced a dose-dependent behavioral syndrome in reserpine-pretreated rats: they produced a flat body posture (the maximum score being 78–94% after the highest dose used) and a weaker forepaw treading (the maximum score being 25–57% after the highest dose used). Compounds **12a–b, 14a–b,** and **15a–b** given alone in doses of 5–20 mg/kg did not evoke changes in the behavior of reserpinized rats (Table 4). 8-OH-DPAT (5 mg/kg) produced a flat body posture and reciprocal forepaw treading in reserpine-pretreated rats, the maximum behavioral score being 14.6 and 13.8, respectively. Compounds **12a** (20 mg/kg), **14b** (20 mg/kg), **12c** (10–20 mg/kg), **13c** (10–20 mg/kg), **14c** (5–20 mg/kg), and **15c** (2.5–5 mg/kg) antagonized dose-dependently the 8-OH-DPAT-induced symptoms, having produced an almost complete (compounds **12c, 13c**, and **14c**) or partial (**12a, 14b**, and **15c**) blockade of the forepaw treading; they also reduced (by 37–73%) the flat body posture after the highest dose used. At the same time, compounds **12b, 14a, 15a,** and **15b** in doses up to 20 mg/kg failed to inhibit the effect induced by 8-OH-DPAT (Table 4).

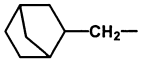
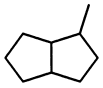
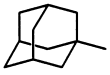
Body temperature in mice

Derivatives **12a–c, 14a–c,** and **15a,b**, given alone in doses of 5–20 mg/kg, did not change the rectal body temperature in mice (data not shown). Compound **15c** administered in doses of 5–10 mg/kg (but not lower) produced a weak hypothermia in mice, which persisted up to 45 min. The maximum hypothermic effect of 0.9 °C ($P < 0.005$) was observed after a dose of 10 mg/kg of **15c**, and it appeared at 30 min after injection. 8-OH-DPAT (5 mg/kg) decreased the rectal body temperature in mice, the maximum hypothermic effect of 1.3 ± 0.2 °C ($P < 0.01$) being observed at 30 min after administration; compound **14b** (which reduced LLR and the behavioral syndrome-induced by 8-OH-DPAT) in doses up to 20 mg/kg did not affect the hypothermic effect of 8-OH-DPAT (data not shown).

Correlation Analysis

The results obtained in the binding experiments suggested that the size of the hydrocarbon substituent R

Table 2. 5-HT_{1A} and 5-HT_{2A} receptor affinity of investigated compounds and volume of hydrocarbon substituent R

R	No.	K_i 5-HT_{1A} ± SEM (nM) n = 2; **a**	n = 3; **b**	n = 4; **c**	K_i 5-HT_{2A} ± SEM (nM) n = 2; **a**	n = 3; **b**	n = 4; **c**	Volume of R [Å^3]
H_3C—	**6**	4400 ± 100	11200 ± 800	5700 ± 400	> 10000	> 10000	> 10000	26.37
	**7**	4350 ± 100	1850 ± 50	900 ± 60	> 10000	> 10000	1750 ± 30	53.44
	**8**	2400 ± 80	1270 ± 50	600 ± 70	> 10000	> 10000	2050 ± 200	60.51
	**9**	1800 ± 70	875 ± 50	170 ± 14	> 10000	> 10000	> 10000	68.65
	**10**	2050 ± 50	870 ± 40	920 ± 30	> 10000	> 10000	7720 ± 130	77.67
	**11**	450 ± 20	580 ± 40	92 ± 9	6450 ± 250	> 10000	4470 ± 120	84.12
	**12**	40 ± 2	36 ± 3	25.5 ± 1.5	470 ± 50	310 ± 10	2610 ± 150	100.27
	**13**	81 ± 3	89 ± 5	77 ± 11	1730 ± 130	1380 ± 20	740 ± 70	122.94
	**14**	54 ± 4	64 ± 15	2.4 ± 0.05	3600 ± 340	5800 ± 600	740 ± 120	123.74
	**15**	15 ± 0.2	68 ± 1	0.95 ± 0.04	640 ± 80	1290 ± 250	452 ± 7	142.14

affected the in vitro activity of the investigated compounds, since enlargement of R from the methyl to the adamantyl moiety strongly increased the 5-HT_{1A} affinity. Glennon et al. postulated that at 5-HT_{1A} binding sites there should exist a region of bulk tolerance, which was capable of accommodating large terminal groups.[15] Our previous results also suggested the existence of a specific hydrophobic pocket at 5-HT_{1A} receptor sites, which could interact with the terminal alkyl moiety of 1-arylpiperazine-type ligands.[16] The calculated ΔV_R and log P_c for the compounds presented in the paper were entirely interdependent, as was indicated by the correlation coefficient r = 0.979 of the linear relationship $\Delta V_R = a + b \log P_c$. Therefore we chose the volume of the hydrocarbon substituent as a basic parameter for a further discussion of the structure–affinity relationship in the investigated derivatives. The Van der Waals volumes ($\Delta V_R = V_R - V_{Me}$) of substituent R are presented in Table 2.

The effect of the substituent volume on the 5-HT_{1A} affinity was significant, and linear correlations were found for all series of the compounds (Fig. 1). Although the presented correlations are statistically significant, the correlation coefficients are not particularly high; however, closer examination of Figure 1 revealed that only single points deviated from the respective regression lines. The potency of compounds **10c** and **13c** was unexpectedly low, whereas derivatives **12a** and **12b** had higher affinities than it was predicted on the basis of the $pK_i(\Delta V_R)$ function.

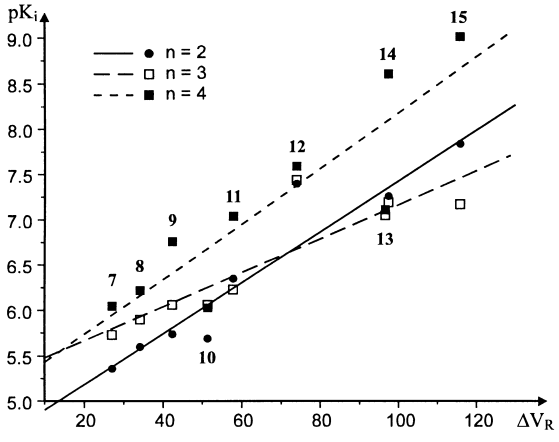


Figure 1. The linear relationships between pK_i and ΔV_R for all three series of compounds: n = 2: $pK_i = 0.0280\ \Delta V_R + 4.62$; N = 9, r = 0.946, $F_{001} = 26.23$; n = 3: $pK_i = 0.0189\ \Delta V_R + 5.29$; N = 9, v = 0.888, $F_{001} = 26.23$; n = 4: $pK_i = 0.0307\ \Delta V_R + 5.13$; N = 9, r = 0.890, $F_{001} = 26.38$.

Discussion

In our previous study we analyzed a group of *N*-(2-methoxyphenyl)piperazine (*o*-OMe-PhP) derivatives with similar modifications within the amide fragment.[16]

Table 3. Induction by **12a–c, 13c, 14a–c,** and **15a–c** Lower Lip Retraction[a] (LLR) and the effect of investigated compounds on the 8-OH-DPAT-induced LLR[b] in rats

Treatment	Dose, mg/kg	Induction[a] mean ± SEM	Inhibition[b] LLR score
Vehicle	—	0.1 ± 0.1	2.8 ± 0.3
12a	10	0.3 ± 0.2	2.3 ± 0.2
	20	0.9 ± 0.2[c]	2.2 ± 0.3
14a	20	0.3 ± 0.1	2.8 ± 0.2
Vehicle	—	0.1 ± 0.1	2.5 ± 0.3
15a	2.5	0.4 ± 0.2	2.8 ± 0.3
	5	1.1 ± 0.3[d]	2.7 ± 0.2
	10	1.7 ± 0.2[d]	NT
	20	1.7 ± 0.2[d]	NT
Vehicle	—	0.1 ± 0.1	2.5 ± 0.3
12b	10	0.7 ± 0.2[c]	2.6 ± 0.2
	20	1.5 ± 0.2[d]	NT
Vehicle	—	0.1 ± 0.1	2.6 ± 0.2
14b	10	0.1 ± 0.1	2.5 ± 0.2
	20	0.5 ± 0.2	1.3 ± 0.3[e]
15b	10	0.9 ± 0.2[c]	2.7 ± 0.3
	20	1.9 ± 0.2[d]	NT
Vehicle	—	0.1 ± 0.1	2.6 ± 0.2
12c	10	0.7 ± 0.2[c]	1.8 ± 0.2
	20	1.9 ± 0.2[c]	NT
13c	10	0.6 ± 0.2	2.7 ± 0.3
	20	1.3 ± 0.2[d]	2.3 ± 0.2
Vehicle	—	0.1 ± 0.2	2.5 ± 0.2
14c	2.5	1.2 ± 0.2[d]	2.4 ± 0.3
	5	1.8 ± 0.3[d]	NT
	10	2.1 ± 0.3[d]	NT
Vehicle	—	0.1 ± 0.1	—
15c	0.625	1.6 ± 0.3[d]	NT
	1.25	2.3 ± 0.3[d]	NT
	2.5	2.7 ± 0.2[d]	NT

[a]The investigated compounds were administered ip 15 min before the test.
[b]The investigated compounds were administered ip 45 min before 8-OH-DPAT (1 mg/kg sc).
[c]$P < 0.05$.
[d]$P < 0.01$ versus vehicle.
[e]$P < 0.01$ versus vehicle + 8-OH-DPAT. NT = not tested.

Compounds with a 2- and 4-carbon spacer, examined in that study, always showed a higher affinity for 5-HT_{1A} receptors in comparison to the respective THIQ analogues (Tables 2 and 5).[16–19] Occasionally, the differences in the binding were fairly large (> 200 times **14a** versus **22a**, and **10c** versus **19b**), but in the case of the most potent THIQ derivatives they narrowed to 3.8 (**14c** versus **22b**) and 2.3 times (**15c** versus **23b**). This general phenomenon, which consists in diminishing the activity of THIQ derivatives versus *o*-OMe-PhP analogues can be attributed to the properties of the fragments themselves. As has been mentioned at the beginning of the paper, THIQ is totally inactive ($K_i > 50000$ nM) at 5-HT_{1A} receptors, while the unsubstituted *o*-OMe-PhP is known to be a fairly potent 5-HT_{1A} receptor ligand ($K_i = 168$ nM).[20] Substitution of both those amines with *n*-hexyl chains results in the enhancement of the affinities due to hydrophobic interactions ($K_i = 1240$ nM and 1.5 nM, respectively),[8,20] yet the difference is striking enough. Weaker interactions between the amine moiety and the receptor binding sites are compensated by hydrophobic forces brought about by the terminal hydrocarbon substituent; thus, the effect of its size is much easier to trace in a series of the THIQ derivatives. As a result, the affinities of the THIQ derivatives **14c** and **15c** containing the most bulky substituents are comparable to those of their *o*-OMe-PhP analogues. Moreover, as shown by a correlation analysis, a linear relationship between the 5-HT_{1A} affinity and the volume of the R fragment exists in all the three series of the investigated compounds. The observed deviations are limited to single points. The unexpectedly high affinities of **12a** and **12b** may suggest that the cyclohexyl substituent is particularly well accommodated at the receptor hydrophobic pocket, and that its distance from the basic nitrogen atom does not seem to play any role.

Apart from the comparison of the in vitro results, it was interesting to keep the track of the influence of amine fragment changes on the in vivo activity. In the present study we evaluated the effects attributed to stimulation of postsynaptic, rather than presynaptic, 5-HT_{1A} receptors. It is generally accepted that the 8-OH-DPAT-induced lower lip retraction (LLR) in rats,[21–23] and the behavioral syndrome (flat body posture and forepaw trading) in reserpinized rats[24] are mediated by 5-HT_{1A} postsynaptic receptors. Administration of 5-HT_{1A} partial agonists induces the LLR[21] and the behavioral syndrome in rats;[24,25] on the other hand ipsapirone practically does not induce such a syndrome.[26,27] Moreover, the majority of partial agonists (e.g. buspirone and ipsapirone) reduce the behavioral syndrome induced by 8-OH-DPAT.[12,26,27]

Compounds **21a** and **23a**, described previously,[16] were classified as 5-HT_{1A} postsynaptic receptor agonists. As could be expected from the binding data, their THIQ analogues (**12a** and **15a**) were less active and induced the LLR at higher doses; furthermore, they evoked neither the flat body posture nor the forepaw treading in rats. Additionally, compound **12a** inhibited the 8-OH-DPAT-induced forepaw treading, but not the flat body posture in rats, whereas **15a** did not affect the 8-OH-DPAT-induced behavioral syndrome. The results obtained in both those tests permit a conclusion that **12a** behaves like a weak partial agonist, while **15a** can be regarded as a weak agonist at postsynaptic 5-HT_{1A} receptors. Thus replacement of *o*-OMe-PhP by THIQ in the two methylene group series (**21a** versus **12a** and **23a** versus **15a**) did not significantly change the pharmacological profile, affecting only the potency of the compounds.

The results of our in vivo experiments indicate that regardless of the R substituent, all the ligands **12c–15c**, behave like 5-HT_{1A} partial agonists, with a different potency, though. Compound **21b**[19] (an analogue of **12c**) is also classified as a partial agonist, while **23b**[17] (RK-153) is regarded as a postsynaptic antagonist. It should be stressed, that substitution of *o*-OMe-PhP in **23b** with THIQ yields the equipotent ligand **15c**, with a different pharmacological profile, though.

The shortening of the spacer length in set **15** results in a loss of postsynaptic antagonistic properties, since **15a** and **b** show only a weak agonistic activity. On the

Table 4. Induction by **12a–c, 13c, 14a–c,** and **15a–c** the behavioral syndrome[a] and effect of the investigated compounds on the 8-OH-DPAT: induced behavior[b] in reserpine-pretreated rats

Treatment	Dose, mg/kg	Induction[a] mean ± SEM LLR score		Induction[b] mean ± SEM LLR score	
		Flat body posture	Forepaw treading	Flat body posture	Forepaw treading
Vehicle	—	0	0	14.0 ± 0.3	11.8 ± 1.0
12a	20	0	0	11.6 ± 0.8	5.0 ± 1.6[c]
14a	20	0	0	12.1 ± 0.4	9.4 ± 0.6
Vehicle	—	0	0	14.0 ± 0.6	11.6 ± 1.1
15a	5	0	0	13.3 ± 0.6	11.0 ± 1.3
	10	0	0	13.0 ± 0.6	8.5 ± 1.3
	20	0	0	13.0 ± 0.6	10.3 ± 1.1
Vehicle	—	0	0	14.0 ± 0.3	11.8 ± 1.0
12b	20	0.5 ± 0.5	1.3 ± 0.3	12.7 ± 0.6	10.5 ± 0.9
14b	20	0	0	8.8 ± 0.9[c]	7.8 ± 0.9
15b	20	1.7 ± 0.7	0	11.3 ± 0.8	10.8 ± 0.8
Vehicle	—	0	0	13.8 ± 0.3	11.3 ± 0.4
12c	10	13.1 ± 0.9[d]	3.0 ± 0.7	4.3 ± 0.3[c]	3.1 ± 0.7[c]
	20	14.3 ± 0.5[d]	4.8 ± 0.6[d]	5.8 ± 0.8a[c]	0.8 ± 0.3[c]
13c	10	7.5 ± 1.0[d]	0.6 ± 0.4	8.6 ± 0.9[c]	7.8 ± 1.0[c]
	20	14.3 ± 0.5[d]	3.2 ± 0.6	3.8 ± 1.1[c]	1.0 ± 0.8[c]
Vehicle	—	0	0	14.6 ± 0.4	13.8 ± 0.6
14c	5	9.8 ± 0.8[d]	2.7 ± 0.6	8.9 ± 0.9[c]	7.5 ± 0.8[c]
	10	12.2 ± 1.1[d]	5.6 ± 1.0[d]	5.7 ± 1.0[c]	5.8 ± 0.4[c]
	20	13.0 ± 0.9[d]	6.5 ± 0.9[d]	6.3 ± 1.0[c]	3.3 ± 0.5[c]
15c	2.5	9.8 ± 1.8[d]	5.0 ± 0.6[d]	7.8 ± 0.7[c]	8.0 ± 1.0[c]
	5	11.8 ± 0.7[d]	7.3 ± 0.8[d]	4.8 ± 1.4[c]	5.8 ± 0.7[c]

[a]Reserpine (1 mg/kg sc) and investigated compounds (ip) were administered 18 h and 3 min, respectively, before the test.
[b]Reserpine (1 mg/kg sc) and investigated compounds (ip) were administered 18 h and 60 min, repectively, before 8-OH-DPAT (5 mg/kg sc). 8-OH-DPAT was administered 3 min before test.
[c]$P < 0.01$ versus vehicle + 8-OH-DPAT.
[d]$P < 0.01$ versus vehicle.

contrary, in set **14**, the shortening to a 3-membered spacer results in a very weak, postsynaptic 5-HT_{1A} antagonistic activity of **14b**. Further shortening of the hydrocarbon chain yields **14a** inactive in vivo. In set **12**, derivatives with the even number of methylene groups (**12a, c**) show properties of partial agonists, while the odd analogue **12b** behaves like a weak postsynaptic agonist.

The hypothermic effect observed in mice after administration of 5-HT_{1A} agonists or partial agonists is attributed to the presynaptic 5-HT_{1A} receptor stimulation.[28,29] Of all the compounds tested in vivo, only **15c** given alone evokes a weak decrease in the body temperature in mice. Thus it may be assumed that activation of presynaptic 5-HT_{1A} receptors is responsible for their influence on body temperature; however, this assumption has not been fully documented as yet. Compound **14b** shows a weak postsynaptic antagonistic activity, but does not change the hypothermia induced by 8-OH-DPAT in mice. Therefore it seems that the presynaptic activity of compounds tested in this model is negligible.

Our in vivo results indicate that THIQ amide derivatives generally show properties of weak agonists (**12b, 15a,b**) or partial agonists (**12a, c, 13c, 14c, 15c**) of postsynaptic 5-HT_{1A} receptors.

Conclusions

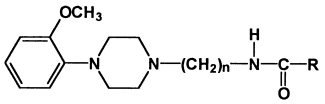
In this study we successfully continued to search for new 5-HT_{1A} receptor ligands in a group of 1,2,3,4-tetrahydroisoquinoline derivatives. In fact, we found some potent 5-HT_{1A} receptor ligands within sets **12–15**. We showed that the volume of the terminal hydrocarbon substituent (R) was directly responsible for stabilization of the bioactive complex with 5-HT_{1A} receptor, and, that that relationship was linear. Moreover, even small changes in ligand–receptor interactions, brought about by the substituent, were clearly visible in the THIQ series, since they were not obscured by interactions in the amine part, as they are in the case of arylpiperazine derivatives. It should be stressed that the length of the spacer seems to influence the in vivo activity, since the series **c** compounds were the most active ones. On the other hand, in this series, the activity of compounds is also attributed to the R substituent; the bigger the volume of R, the higher the in vivo activity of the compound. It is also of interest that compound **15c** and its *o*-OMe-PhP analogue **23b** (RK-153)[17] have a comparable 5-HT_{1A} affinity, but a different pharmacological profile.

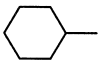
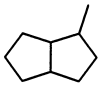
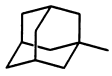
Experimental

Chemistry

Melting points were determined on a Boetius apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian EM-360L (60 MHz) in the $CDCl_3$ solution with Me_4Si as an internal reference (data not shown). Elemental analyses were performed in the Institute of Organic Chemistry PAS (Warsaw, Poland), and were within ±0.4% of the theoretical values.

Table 5. 5-HT_{1A} Receptor affinities of compounds **19a,b–23a,b**



R	n = 2		n = 4	
	No.	$K_i \pm SEM$ [nM]	No.	$K_i \pm SEM$ [nM]
	**19a**[a]	73 ± 6	**19b**[b]	1.00 ± 0.002
	**20a**[c]	4.7 ± 0.5	**20b**[c]	1.88 ± 0.22
	**21a**[a]	2.1 ± 0.3	**21b**[d]	0.6 ± 0.1
	**22a**[c]	0.24 ± 0.07	**22b**[c]	0.63 ± 0.05
	**23a**[a]	0.21 ± 0.02	**23b**[b]	0.40 ± 0.03

[a]Ref 16.
[b]Ref 17.
[c]Ref 18.
[d]Ref 19.

The starting amines were synthesized by published procedures: *N*-(2-aminoethyl)-1,2,3,4-tetrahydroisoquinoline[12] (**16**), *N*-(4-aminobutyl)-1,2,3,4-tetrahydroisoquinoline[12] (**18**) and *N*-(3-aminopropyl)-1,2,3,4-tetrahydroisoquinoline (**17**) obtained in the same manner is characterized below. All carboxylic acids and acyl chlorides were commercial products (Aldrich).

2-(3-Aminopropyl)-1,2,3,4-tetrahydroisoquinoline (17). Yield 76%; ^1H NMR δ 1.2 (s, 2H, NH_2), 1.7 (m, 2H, CH_2), 2.3–3.0 (m, 8H, 4 CH_2), 3.6 (s, 2H, CH_2), 6.9–7.2 (m, 4H, arom).

General procedure A-1. Preparation of derivatives 6a, 6c, 7b, 7c, 8a, and 8c. *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinoline (1.5 mmol) was dissolved in chloroform (10 mL) and a 20% aq K_2CO_3 (10 mL). Then acyl chloride (2.2 mmol) was added on stirring at room temperature and the reaction mixture was left overnight. The organic layer was separated, washed with water (2×5 mL), and dried over anhydrous $MgSO_4$. The inorganic precipitate was filtered off, the solvent was evaporated and the crude product was purified using column chromatography (SiO_2/$CHCl_3$-CH_3OH, 95/5 for **6a, 7b, 7c, 8a**, SiO_2/$CHCl_3$-CH_3OH, 9/1 for **6c**, Al_2O_3/$CHCl_3$-*n*-hexane, 2/1 for **7a**, and Al_2O_3/$CHCl_3$ for **8c**). Free bases were dissolved in acetone (5–7 mL), treated either with an excess of diethyl ether saturated with dry, gaseous HCl or equimolar amount of fumaric acid dissolved in acetone, and kept in refrigerator to give colorless crystalline products.

General procedure A-2. Preparation of compounds 6b, 7a, and 8b. To the slightly warm (30–40 °C) solution of *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinoline (1.5 mmol) and triethylamine (1.5 mmol) in benzene (20 mL) acyl chloride (1.55 mmol) was added and the mixture was stirred for 4 h at room temperature and then left overnight. Benzene solution was washed with a 20% aqueous NaOH (3×10 mL) and dried over anhydrous $MgSO_4$. The inorganic precipitate was filtered off, the solvent evaporated and the residue was purified using column chromatography (Al_2O_3/$CHCl_3$ for **6b, 8b** and Al_2O_3/$CHCl_3$-*n*-hexane, 2/1 for **7a**). The appropriate salts were prepared according to the general procedure A-1.

General procedure B. Preparation of derivatives 9a, 9b, 10a–c, 11b, 12b, 12c, 13a, and 14a. The appropriate acid (1 mmol) was dissolved in acetonitrile (15 mL) and BOP (1 mmol) was added on stirring. To the homogenous solution triethylamine (2 mmol) was added followed by solution of *N*-(ω-aminoalkyl)-1,2,3,4-tetrahydroisoquinoline in acetonitrile (15 mL). Stirring was continued for 6 h at room temperature and the mixture was left overnight. The solvent was evaporated, the oily residue was dissolved in $CHCl_3$ (30 mL) and then washed with 10% aqueous NaOH (3×20 mL), water (20 mL) and dried over anhydrous $MgSO_4$. The crude amides were purified by column chromatography (Al_2O_3/$CHCl_3$-*n*-hexane, 2/1 for **9a, 9b, 10b, 12b, 13a, 14a** and Al_2O_3/$CHCl_3$ for **10a, 10c, 11b, 12c**). The appropriate salts were prepared according to the general procedure A-1.

General procedure C. Preparation of derivatives 9c, 11a, 11c, 12a, 13b, 13c, 14b, 14c, and 15a–c. The appropriate carboxylic 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 (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% aq 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 were separated by column chromatography using SiO_2 and $CHCl_3$ followed by $CHCl_3$/MeOH, 95/5.

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.[30] The following radioligands were used: [^3H]-8-OH-DPAT (224 Ci/mmol, Amersham) and [^3H]-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.

In vivo experiments

The experiments were performed on male Wistar rats (250–300 g) or male Albino-Swiss mice (20–25 g). The animals were kept at room temperature (20 ± 1 °C) on a natural day–night cycle (January–June) and housed under standard laboratory conditions. They had free access to food (Bacutil pellets) and tap water before the experiment. Each experimental group consisted of 6–8 animals/dose, and all the animals were used only once. 8-Hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.), reserpine (Ciba) and the investigated salts of **12a–c, 14a–c,** and **15c** were used in the form of freshly prepared aqueous solutions. The salts of **15a, 15b,** and **13c** were administered as suspensions in 1% Tween 80.

Lower Lip Retraction (LLR) in rats

The LLR was assessed according to the method described by Berendsen et al.[21] The rats were individually placed in cages, having been scored three times at 15, 30, and 45 min after ip administration of **12a–c, 13c, 14a–c**, and **15a–c** as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The total maximum score amounted to 3/rat. In a separate experiment the effect of compounds **12a–c, 13c, 14a–c,** and **15a–b** on LLR induced by 8-OH-DPAT (1 mg/kg sc) was tested, they were administered ip 45 min before 8-OH-DPAT and animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

Behavioral syndrome in reserpinized rats

Reserpine (1 mg/kg sc) was administered 18 h before the test. The rats were individually placed in the experimental cages 5 min before injection of **12a–c, 13c, 14a–c,** and **15a–c**. Observation sessions, lasting 45 s each, began 3 min after the 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/animal/symptom.[24] The effect of **12a–c, 13c, 14a–c,** and **15a–c** on the behavioral syndrome induced by 8-OH-DPAT (5 mg/kg sc) in reserpinized rats was estimated in an independent experiment. The compounds were administered ip 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.

Body temperature in mice

The effects of **12a–c, 13c, 14a–c,** and **15a–c** given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 15, 30, 45, and 60 min after the administration. In an independent experiment the effect of **14b** on 8-OH-DPAT (5 mg/kg ip)-induced hypothermia was tested. Compound **14b** was administered 30 min before 8-OH-DPAT, the rectal body temperature was measured 15, 30, 45, and 60 min after the injection of 8-OH-DPAT. The results were expressed as a change in the body temperature, (Δt) with respect to the basal body temperature, as measured at the beginning of the experiment.

Statistics

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

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