Structure–Activity Relationship Studies of Central Nervous System Agents. 5. Effect of the Hydrocarbon Chain on the Affinity of 4-Substituted 1-(3-Chlorophenyl)piperazines for 5-HT_{1A} Receptor Site[1]

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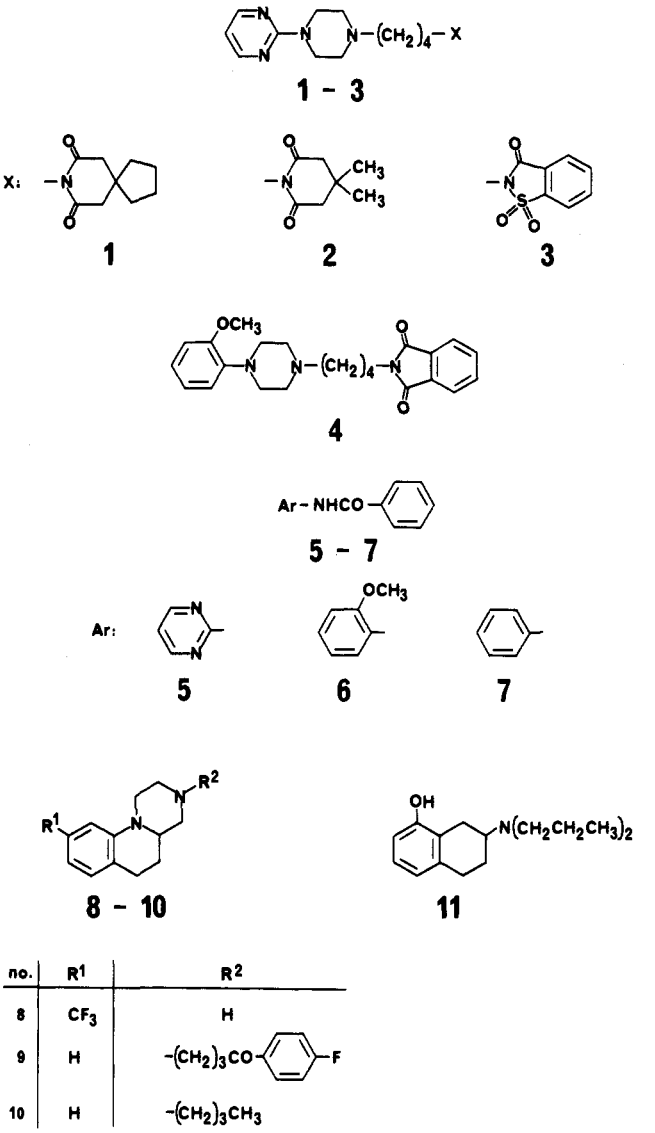
The effect of the hydrocarbon chain of the model 4-substituted 1-(3-chlorophenyl)piperazines **12–31** on their affinity for 5-HT_{1A} receptor sites was investigated. It was found that elongation of the 4-*n*-alkyl chain strongly increases the 5-HT_{1A} affinity of the investigated compounds. The affinity reaches the maximum (K_i = 2.67 nM) for the *n*-hexyl derivative **20**. It was shown that hydrophobic interactions of N-4 substituents of 1-arylpiperazines significantly contribute to their 5-HT_{1A} affinity. The specific binding constant was defined as the receptor affinity of the protonated species of compounds under physiological conditions. The range of K_i^{AH+} = 1–3 × 10^{-11} M is a specific affinity limit of the investigated class of compounds at the 5-HT_{1A} receptor sites.

1-Arylpiperazines produce a variety of behavioral responses and pharmacological effects which directly result from activation of the central serotonin system.[2-5] Furthermore, 1-arylpiperazines have been incorporated in the side chain of some structures, such as buspirone (**1**), gepirone (**2**), ipsapirone (**3**), or NAN-190 (**4**) (Chart I). These compounds (**1–4**) exhibit high affinity for 5-HT_{1A} serotonin receptor sites[6,7] and may form 1-arylpiperazines during biotransformation in vivo. These metabolites are also biochemically and pharmacologically active.[5,8-10] Therefore simple 1-arylpiperazines may constitute a useful tool for evaluating the structure–activity relationships at 5-HT_{1A} receptor sites.

It is generally accepted that there are at least two structural features necessary for recognition of ligands by the 5-HT_{1A} site.[7,11-13] One of them is the presence of an aromatic ring in a molecule; the other is a strongly basic nitrogen atom at a distance of 5.2–5.6 Å from the center

Chart I



no.	R^1	R^2
8	CF_3	H
9	H	$-(CH_2)_3CO-C_6H_4-F$
10	H	$-(CH_2)_3CH_3$

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Table I. Affinities of Gepirone (2), Ipsapirone (3), 8-OH-DPAT (11), 4-Substituted Arylpiperazines (12–31), and 1-(*m*-Chlorophenyl)morpholine (32) for [^{3}H]-8-Hydroxy-2-(di-*n*-propylamino)tetralin-Labeled 5-HT$_{1A}$ Sites[a]

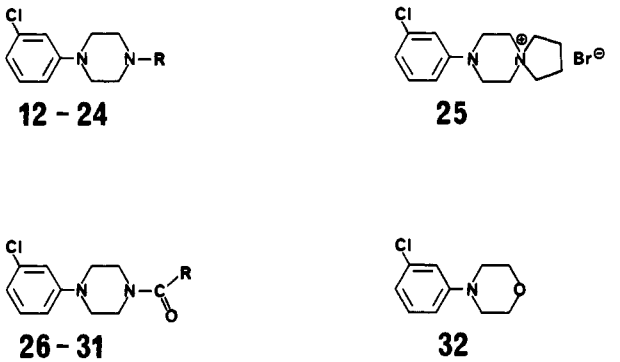
no.	R	K_i, nM	no.	R	K_i, nM
2	gepirone	31.8 ± 1.1	21	$(CH_2)_7CH_3$	11.3 ± 1.2
3	ipsapirone	3.40 ± 0.98	22	$CH_2CH{=}CH_2$	251 ± 6
11	8-OH-DPAT	1.44 ± 0.18	23	$CH_2C_6H_5$[b]	166 ± 29
12	H	143 ± 6	24	CH_2CH_2OH	202 ± 12
13	CH_3	152 ± 4	25	$-(CH_2)_4-$[c]	7220 ± 1460
14	CH_2CH_3	220 ± 10	26	CH_3	>100000
15	$(CH_2)_2CH_3$	122 ± 11	27	CH_2CH_3	30800 ± 3300
16	$CH(CH_3)_2$	159 ± 5	28	$(CH_2)_2CH_3$	49000 ± 3500
17	$(CH_2)_3CH_3$	18.4 ± 4.9	29	$CH(CH_3)_2$	>100000
18	$CH(CH_3)CH_2CH_3$	127 ± 12	30	$CH_2C_6H_5$	14800 ± 1700
19	$(CH_2)_4CH_3$	5.54 ± 0.86	31	C_6H_5	>100000
20	$(CH_2)_5CH_3$	2.67 ± 1.19	32	*c*	>100000

[a] K_i are mean values from at least of three independent experiments. [b] C_6H_5 represents phenyl. [c] See Chart II.

of an aromatic nucleus. The nitrogen atom is coplanar with the aromatic ring, and the electron lone pair is almost perpendicular to the plane of this ring.[11,12] The above structural requirements indicate that both rings of 1-arylpiperazines should adopt a coplanar conformation at 5-HT$_{1A}$ receptor sites. In fact, earlier results of radioligand binding studies with the rigid model structure **8** also confirm the suggestion that the two rings of 1-arylpiperazines are relatively coplanar in the bioactive conformation.[14] Recently we reported quantitative structure–activity relationship (QSAR) results for 5-HT$_1$ and 5-HT$_{1A}$ binding data of simple 1-phenylpiperazine derivatives substituted in the phenyl ring.[15] The results of the QSAR analysis provide substantial support for our hypothesis that not only coplanar conformations of the 1-arylpiperazines are responsible for their activity at 5-HT$_{1A}$ receptors. Although the 1-arylpiperazines with a relatively low barrier of rotation (e.g. meta and para derivatives, and *o*-alkoxy ones) may assume coplanar conformations at 5-HT$_{1A}$ receptors, the others (such as *o*-chloro- or -alkyl-substituted derivatives) should definitely adopt twisted conformations with the electron lone pair of N atoms directed in the aromatic ring plane.

Besides the above-mentioned structural requirements, the contribution of the N-4 side chain to the 5-HT$_{1A}$ affinity of 1-arylpiperazines also seems to be significant.[11-13] It was found that the chain length in structures of the types **1–4** is the critical factor determining their optimal affinity; it was also hypothesized that the four-carbon chain is more likely to act at the receptor as a spacer rather than in a hydrophobic manner.[6,16] It was also reported that the 5-HT$_{1A}$ affinity for a series of N-4-substituted 1-arylpiperazines of the types **1–3** increases with the growing lipophilicity of an amide fragment annelated with the cycloalkyl moiety.[17] Furthermore, Glennon and co-

Chart II



workers noted that more complex amide terminus fragments of **1–4** and the related compounds are not necessary for the 5-HT$_{1A}$ affinity, in comparison with the simpler benzamido derivatives **5**, **6**, and **7** (K_i = 60, 2, and 11 nM, respectively).[6] Similar conclusions may be drawn from the 5-HT$_{1A}$ binding data on the structurally related and simple compounds **9** and **10** (K_i = 20 and 170 nM, respectively).[18]

This study shows the influence of the hydrocarbon chain in 4-substituted 1-arylpiperazines on their affinity for 5-HT$_{1A}$ receptor sites. We have chosen 1-(*m*-chlorophenyl)piperazine (mCPP; **12**) as a reference compound (with a moderate affinity for 5-HT$_{1A}$ sites; K_i = 130 ± 10 nM),[6] with regard to a series of the model derivatives **13–32** (Chart II).

5-HT$_{1A}$ Binding Studies

The affinity of compounds **2**, **3**, and **11–32** for central 5-HT$_{1A}$ neuroreceptors in vitro was assessed on the basis of their ability to displace [^{3}H]-8-OH-DPAT. The obtained results are summarized in Table I.

The 5-HT$_{1A}$ affinity of mCPP (**12**) and its 4-methyl (**13**) and 4-isopropyl (**16**) derivatives is essentially the same. The derivative **14**, which contains a 4-ethyl substituent, exhibits a slightly lower 5-HT$_{1A}$ affinity in comparison with the reference compound **12**. Elongation of the 4-*n*-alkyl chain strongly increases the 5-HT$_{1A}$ affinity of the investigated compounds. The affinity reaches its maximum (K_i = 2.67 nM) for the *n*-hexyl derivative **20**, and drops down again (K_i = 11.3 nM) for the *n*-octyl one **21** (Table I). Additional steric hindrance of both the isopropyl and

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Table II. Structural Parameters, Ionization Constants, Percent Ionization, and Calculated Binding Constants of the Protonated Form (K_i^{AH+}) of Derivatives 2, 3, 11–24, and 32

no.	structural parameters σ^{*a}	structural parameters $^1\chi^V$	pK_{a1} at 37 °C	pK_{a2} at 37 °C	% ionization, pH = 7.4, 37 °C	K_i^{AH+}, nM
2		9.521	b	7.64 ± 0.03	63.5	20.2
3		9.476	b	5.25 ± 0.04	0.7	0.024
11		7.296		8.02 ± 0.02	80.6	1.16
12	0.49	4.952	b	8.42 ± 0.02	91.3	131
13	0	5.324	2.82 ± 0.03	8.27 ± 0.03	88.1	134
14	−0.10	5.900	2.48 ± 0.02	7.89 ± 0.02	75.5	166
15	−0.11	6.400	2.39 ± 0.02	7.07 ± 0.03	31.9	38.9
16	−0.19	6.290	2.37 ± 0.02	7.65 ± 0.02	64.0	102
17	−0.13	6.900	2.43 ± 0.03	6.70 ± 0.03	16.6	3.06
18	−0.21	6.828	2.32 ± 0.02	6.84 ± 0.02	21.6	27.4
19	−0.14	7.400	2.46 ± 0.03	6.13 ± 0.04	5.1	0.282
20	−0.15	7.900	2.57 ± 0.03	5.49 ± 0.02	1.2	0.032
21	−0.15	8.900	2.52 ± 0.05	4.42 ± 0.04	0.1	0.011
22	0.23	6.010	2.94 ± 0.04	7.08 ± 0.04	32.4	81.2
23	0.26	7.457	b	4.39 ± 0.02	0.1	0.166
24	0.21	6.009	b	6.92 ± 0.03	24.9	50.3
32			2.33 ± 0.02			

[a] Inductive constants for substituents R, taken from ref 26. [b] Monohydrochloride.

sec-butyl groups at the N-4 atom has either a very weak or no effect on the affinity of the derivatives of the same chain length (cf. 16 and 14, or 18 and 15). The 5-HT_{1A} affinity of 4-allyl (22), 4-benzyl (23), and 4-(β-hydroxyethyl) (24) derivatives has the same range and is comparable with that of 12–14.

The quaternary salt of 25 and the 4-acyl derivatives 27, 28, and 30 show a very low micromolar affinity, whereas other 4-acyl derivatives (26, 29, and 31) and 1-(*m*-chlorophenyl)morpholine (32) do not bind to 5-HT_{1A} receptor sites at all (Table I).

Ionization Constants

Ionization constants of compounds 2, 3, 11–24, and 32 were determined by a potentiometric titration, and the results are collected in Table II. The pK_{a2} value of mCPP (12), obtained by the potentiometric titration at 37 °C, is in good agreement with the results of a spectrophotometric titration at the same temperature (pK_{a2} = 8.64), reported by Caccia et al.[19] The effect of substituents on the pK_{a1} values of the investigated compounds is relatively small. However, the pK_{a2} values across the entire series of derivatives 12–24 are strongly differentiated. *n*-Octyl (21) and benzyl (23) derivatives have the lowest pK_{a2} (ca. 4.4), and differ from the reference compounds (12) by ca. 4 units (Table II).

Although the electronic effect of unbranched alkyl substituents (from ethyl to *n*-octyl) should be similar, as their inductive constants (σ*, Table II) are very close to one another, the hypothesis about a shielding steric effect, which decreases the second ionization constants of derivatives with a longer chain, is justified. The same can be anticipated for a pair of derivatives containing an allyl (22) and a benzyl (23) substituent. Their electronic effects are almost the same, but a higher steric hindrance of the benzyl group in comparison with the allyl one results in dramatic pK_{a2} changes. The pK_{a2} of the benzyl derivative (23) is ca. 2.7 units lower than that of the allyl one (22). The observed basicity-weakening effect in the series of 1-arylpiperazines 12–15, 17, and 19–24 is attributed to a decreased solvation in both the un-ionized molecules and the ammonium ions, which is possible due to the steric shielding effect of the N-4 substituent.[20–23] Such changes

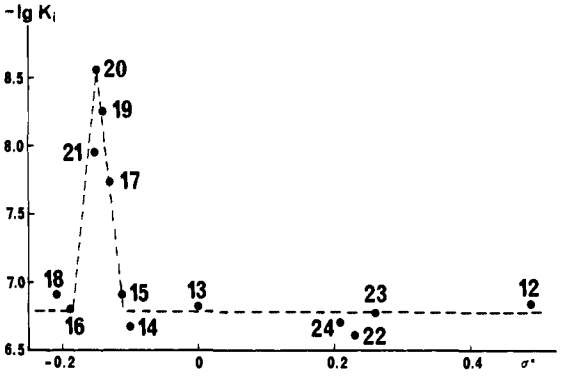

Figure 1. Relationship between the log K_i values and inductive constants (σ*) of the substituents for derivatives 12–24.

in the solvation states increase stability of the un-ionized species, and additionally destabilize the ammonium ions.[20,24]

Derivatives 16 and 18, which contain branched substituents, have higher pK_{a2} values than their unbranched isomers 15 and 17, by 0.58 and 0.14 units, respectively. This typical effect results from introduction of a methyl group into the α-position of the N-4 alkyl chain.[23,25]

Discussion

The electronic effect of N-4 hydrocarbon substituents

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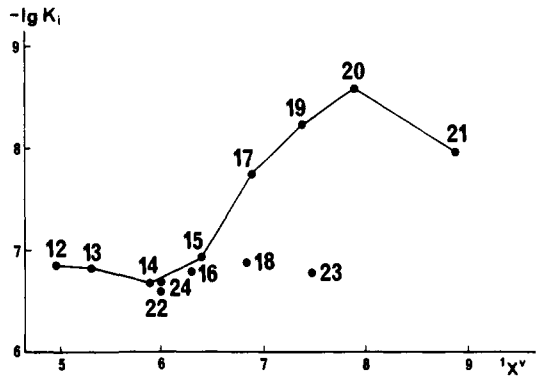

Figure 2. Plot of the log K_i values vs the valence connectivity indices of the first order ($^1\chi^v$) for derivatives 12–24.

on the 5-HT_{1A} affinity of derivatives 12–16, 18, and 22–24 seems to be negligible, as their apparent binding constants (K_i) are within the same, relatively narrow range of 122–251 nM. Therefore, the observed changes in the affinity for 17 and 19–21 cannot be connected with the electronic effects of the N-4 substituents (Table I, Figure 1). The 5-HT_{1A} affinity of 22 and 23, which contain an allyl and a benzyl substituent, respectively, is comparable with that of derivatives 12–16 and 18. This means that there is no π-electron interaction between the double bond or aromatic ring in such an arrangement and the appropriate receptor region. Nor does the terminal hydroxy group of the N-4 substituent does not contribute to the affinity (cf. 24 and 12–15 or 22, Table I). Therefore it may also be anticipated that there is no hydrogen-bonding interaction between the substituent and a specific receptor region. The observed high 5-HT_{1A} affinity of the long-chain derivatives 17 and 19–21 (Figure 1) may be explained only in terms of a lipophilic effect of the N-4 substituents and their hydrophobic interaction with a specific receptor region.

Among different methods for quantifying the molecular structure of a compound, those derived from the chemical graph theory (e.g. molecular connectivity) appear to be very useful and popular.[27-29] We chose, for the purpose of a structure–activity analysis, valence molecular connectivity indices of the first order $^1\chi^v$, given in Table II and calculated in the usual manner.[27] The $^1\chi^v$ indices efficiently encode the additive and constitutive nature of complex molecules or substituents, including their basic stereochemical properties.[28] These indices well-characterize the hydrocarbon fragment and are sensitive to the chain length, position, and type of the multiple bond or branching.[27,30,31] The molecular connectivity indices are also helpful in estimating the lipophilic character of the substituents and molecules.[27,29]

The plot of the apparent binding constants in the logarithmic scale (log K_i) against the $^1\chi^v$ indices is shown in Figure 2. Although mCPP (12) and its derivatives 13–17

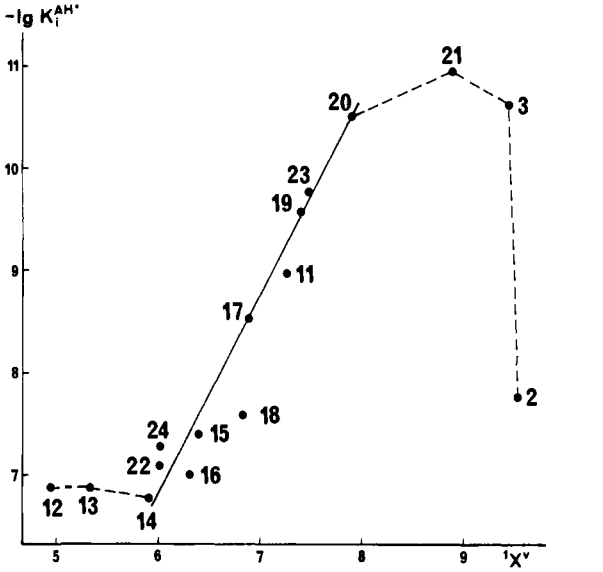

Figure 3. Relationship between the specific binding constants (log K_i^{AH+}) and the valence connectivity indices of the first order ($^1\chi^v$) for derivatives 2, 3, and 11–24.

and 19–21 show a tendency to form an S-shaped curve, the *sec*-butyl (18) and the benzyl (23) derivatives do not fit this curve at all.

It is a well-established view that the presence of a basic nitrogen atom as a protonation center in the molecule is necessary for its binding to 5-HT_{1A} sites.[7,11-13,19,32] In fact, the compounds devoid of the protonation center (26–32), or with the blocked quaternary nitrogen atom (25), do not practically bind to the 5-HT_{1A} sites (Table I). On the other hand, the pK_{a2} values of compounds 12–24 are distinctly differentiated, which means that concentration of the ionized species of the particular derivatives is also different under experimental conditions (pH = 7.4, 37 °C). The percentage ionization of the compounds (Table II) was obtained from eq 1. Furthermore, specific binding con-

$$\%\ \text{ionization} = [1 + 10^{(7.4 - pK_a)}]^{-1} \times 100 \qquad (1)$$

stants of the ionized species (K_i^{AH+}, Table II) depend on the concentration of the protonated form in the solution (expressed as a molar fraction) and can be calculated from eq 2.

$$K_i^{AH+} = K_i/[1 + 10^{(7.4 - pK_a)}] \qquad (2)$$

The effect of hydrophobic interactions between the substituents and the appropriate receptor region can be separated from the lipophilic effect of these substituents, which is responsible for the transmembrane transport phenomena. Hence the relationship between the log K_i^{AH+} values and the $^1\chi^v$ indices shown in Figure 3 represents only the hydrophobic effect of the N-4 substituents on the specific 5-HT_{1A} affinity (K_i^{AH+}) of the analyzed derivatives 12–24.

Contribution of methyl and ethyl groups to the hydrophobic interactions seems to be negligible, as the K_i^{AH+} values of compounds 12–14 are approximately the same. Elongation of the *n*-alkyl chain up to six carbon atoms causes stronger hydrophobic interactions, which result in the observed K_i^{AH+} changes (Table II, Figure 3). The relationship between the log K_i^{AH+} values and the length of the N-4 chain for derivatives 14, 15, 17, 19, and 20 is linear (eq 3). The linear relationship remains statistically

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$$\log K_i^{AH+} = 4.66\ (\pm 0.61) - 1.91\ (\pm 0.09)^1\chi^v \quad (3)$$

compounds **14**, **15**, **17**, **19**, **20**

$$n = 5,\ r = 0.997,\ s = 0.140,\ F_{0.001} = 468$$

highly significant even after inclusion of the derivatives with the α-branched (**16**, **18**), allyl (**22**), benzyl (**23**), and β-hydroxyethyl (**24**) substituents in the correlation (eq 4).

$$\log K_i^{AH+} = 4.24\ (\pm 1.24) - 1.84\ (\pm 0.18)^1\chi^v \quad (4)$$

compounds **14–20**, **22–24**

$$n = 10,\ r = 0.963,\ s = 0.386,\ F_{0.001} = 101$$

Further elongation of the hydrocarbon chain is not linear, and the specific 5-HT_{1A} affinity shows a tendency to reach a constant value (Figure 3).

The results presented above indicate that the hydrophobic interactions of the N-4 substituents of 1-arylpiperazines significantly contribute to their 5-HT_{1A} affinity. In other words, the region of the 5-HT_{1A} sites with which the N-4 substituents interact must be capable of accommodating a chain of six to eight carbon atoms in an active manner. The above model was verified using gepirone (**2**), ipsapirone (**3**) and 8-OH-DPAT (**11**) as a probe. The latter compounds are known to be selective and potent 5-HT_{1A} agents. The specific 5-HT_{1A} affinity (K_i^{AH+}) of **2**, **3**, and **11** was calculated from eq 2, and the obtained values are differentiated due to diverse concentrations of the protonated form in the solution under experimental conditions (cf. the pK_a values and the percentage ionization for **2**, **3**, and **11**; Table II). Compounds **3** and **11** fit very well the S-shaped curve shown in Figure 3. However, the specific affinity of **2** is relatively low. This means that the interaction between the N-4 substituent and the hydrophobic region of the receptor sites is very limited and may be interpreted as a result of the two effects. One of them seems to be a different conformation of the complex N-4 substituent in the solution, as the pK_{a2} value of **2** is relatively high in comparison with other long-chain derivatives. Gepirone molecule (**2**) is the biggest in size ($^1\chi^v > 9.5$) of all the investigated compounds. Therefore the limit of the bulk tolerance in the hydrophobic region of the 5-HT_{1A} site may be regarded as the other possible effect.

Conclusions

In summary, we have found that the hydrophobic interactions of the N-4 substituents of 1-arylpiperazines significantly contributes to their 5-HT_{1A} affinity. At this time, the nature of the interaction of the complex N-4 substituents of the selective 5-HT_{1A} agents of types 1–4 seems to be more understandable. Our results indicate that the carbon chain in 1–4 does not act as a spacer, but is more likely to interact with the receptor in a hydrophobic manner. It also seems that the size of a molecule within the range of $^1\chi^v = 9$–9.5 is the limit of the bulk tolerance in the 5-HT_{1A} hydrophobic region.

In our opinion, the specific K_i^{AH+} affinity defined by eq 2 is a more convenient parameter to discuss some structure–affinity relationship problems than the directly determined binding constant K_i. Moreover, the range of $K_i^{AH+} = 1$–3×10^{-11} M is the specific affinity limit of the investigated class of compounds at the 5-HT_{1A} receptor sites.

Experimental Section

Melting points were determined on a Böetius apparatus and are uncorrected. Electron impact mass spectra (70 eV) were taken with an LKB 2091 instrument. ^{1}H NMR spectrum of **25** was obtained on a Varian EM-360L (60 MHz) in DMSO-d_6 solution with Me_4Si as an internal reference. Elemental analyses were performed in the Institute of Organic Chemistry PAS (Warsaw, Poland), and were within ±0.3% of the theoretical values.

The following compounds were synthesized by published procedures: 1-(3-chlorophenyl)-4-methylpiperazine[33] (**13**), 1-(3-chlorophenyl)-4-(2-hydroxyethyl)piperazine[34] (**24**), 4-acetyl-1-(3-chlorophenyl)piperazine[34] (**26**), 4-benzoyl-1-(3-chlorophenyl)-piperazine[34] (**31**), and *N*-(3-chlorophenyl)morpholine[35] (**32**). Gepirone (**2**, Bristol-Meyers Co.) and 8-OH-DPAT (**11**, RBI) were commercial products. Ipsapirone (**3**) was obtained from Troponwerke.

General Procedure A. Preparation of Derivatives 11–23. To a solution of 1-(3-chlorophenyl)piperazine (**12**; 0.98 g, 5 mmol) in acetone (30 mL) were added anhydrous K_2CO_3 (2 g) and KI (0.2 g). Then a solution of the appropriate alkyl bromide, allyl bromide, or benzyl chloride (6 mmol) in acetone (5 mL) was added dropwise at room temperature. The mixture was stirred for 6–8 h at room temperature for primary alkyl, allyl, and benzyl halides, or under reflux for secondary alkyl bromides. Then the mixture was filtered off, the solvent was evaporated, and the residue was purified using silica gel chromatography with *n*-hexane/$CHCl_3$ (1:1). The product was dissolved with acetone (5 mL) and treated with an excess of Et_2O, saturated with HCl. Resultant salts were recrystallized from acetone.

4-Ethyl-1-(3-chlorophenyl)piperazine dihydrochloride (**14**·2HCl): yield 89%; mp 212–213 °C; MS (free base) *m/e* 224 (50, M^+), 226 (14, M^+), 209 (25), 84 (41), 70 (21), 57 (100), 56 (31). Anal. ($C_{12}H_{17}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-*n*-propylpiperazine dihydrochloride (**15**·2HCl): yield 90%; mp 216–218 °C; MS (free base) *m/e* 238 (33, M^+), 240 (8, M^+), 209 (100), 84 (10), 70 (66), 56 (24). Anal. ($C_{13}H_{19}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-(1-methylethyl)piperazine dihydrochloride (**16**·2HCl): yield 78%, mp 166–168 °C; MS (free base) *m/e* 238 (31, M^+), 240 (9, M^+), 223 (100), 84 (19), 70 (7), 56 (46). Anal. ($C_{13}H_{19}ClN_2$·2HCl) C, H, N.

4-*n*-Butyl-1-(3-chlorophenyl)piperazine dihydrochloride (**17**·2HCl): yield 88%; mp 189–190 °C; MS (free base) *m/e* 252 (43, M^+), 254 (14, M^+), 209 (100), 84 (12), 70 (44), 56 (21). Anal. ($C_{14}H_{21}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-(1-methylpropyl)piperazine dihydrochloride (**18**·2HCl): yield 75%, mp 163–165 °C; MS (free base) *m/e* 252 (11, M^+), 254 (3, M^+), 223 (100), 84 (17), 70 (6), 56 (22). Anal. ($C_{14}H_{21}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-*n*-pentylpiperazine dihydrochloride (**19**·2HCl): yield 85%; mp 156–157 °C; MS (free base) *m/e* 266 (19, M^+), 268 (6, M^+), 209 (100), 84 (3), 70 (31), 56 (13). Anal. ($C_{15}H_{23}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-*n*-hexylpiperazine dihydrochloride (**20**·2HCl): yield 89%; mp 171–173 °C; MS (free base) *m/e* 280 (42, M^+), 282 (14, M^+), 209 (100), 84 (4), 70 (56), 56 (19). Anal. ($C_{16}H_{25}ClN_2$·2HCl) C, H, N.

1-(3-Chlorophenyl)-4-*n*-octylpiperazine dihydrochloride (**21**·2HCl): yield 84%; mp 177–180 °C; MS (free base) *m/e* 308 (19, M^+), 310 (6, M^+), 209 (100), 84 (2), 70 (20), 56 (17). Anal. ($C_{18}H_{29}ClN_2$·2HCl) C, H, N.

4-Allyl-1-(3-chlorophenyl)piperazine dihydrochloride (**22**·2HCl): yield 92%; mp 209–211 °C; MS (free base) *m/e* 236 (81, M^+), 238 (25, M^+), 140 (49), 139 (39), 138 (29), 84 (12), 70 (20), 56 (62), 27 (100). Anal. ($C_{13}H_{17}ClN_2$·2HCl) C, H, N.

4-Benzyl-1-(3-chlorophenyl)piperazine hydrochloride (**23**·HCl): yield 87%; mp 220–223 °C; MS (free base) *m/e* 286 (35, M^+), 290 (12, M^+), 146 (24), 119 (47), 91 (100), 56 (36). Anal. ($C_{17}H_{19}ClN_2$·HCl) C, H, N.

8-(3-Chlorophenyl)-5-azonia-8-azaspiro[4.5]decane Bromide (**25**). A solution of 1-(3-chlorophenyl)piperazine (**12**; 2.95 g, 15 mmol), 1,4-dibromobutane (3.85 g, 18 mmol), and anhydrous K_2CO_3 (5 g) in 99.8% ethanol (30 mL) was refluxed for 10 h. The

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mixture was filtered off, the solvent was evaporated, and the residue was recrystallized from acetone/ethanol (1:1) yielding **25** (80%): mp 223-225 °C; MS *m/e* 330 (10, M^+), 332 (12, M^+), 209 (100), 84 (31), 70 (37), 56 (17); ^{1}H NMR δ 2.25 (m, 2 CH_2), 3.80 (m, 4 CH_2), 7.20 (m, 4 H arom). Anal. ($C_{14}H_{20}BrClN_2$) C, H, N.

General Procedure B. Preparation of Derivatives 27-30. To a solution of 1-(3-chlorophenyl)piperazine (**12**; 0.4 g, 2 mmol) in toluene (30 mL) was added anhydrous K_2CO_3 (0.5 g). Then a solution of the appropriate acid chloride (2.5 mmol) in toluene (10 mL) was added dropwise, and the mixture was stirred for 2-3 h at room temperature. The mixture was treated with water (30 mL) and warmed up to 50 °C, and the organic layer was separated. Then the solvent was evaporated, and the residue was purified using silica gel chromatography with *n*-hexane/$CHCl_3$ (1:1). The product was dissolved with acetone (5 mL) and treated with an excess of Et_2O, saturated with HCl. Resultant salts were recrystallized from acetone/ethanol mixture.

1-(3-Chlorophenyl)-4-propionylpiperazine hydrochloride (27·HCl): yield 85%; mp 148-150 °C; MS (free base) *m/e* 252 (36, M^+), 254 (10, M^+), 166 (100), 154 (32), 56 (76). Anal. ($C_{13}H_{17}ClN_2O$·HCl) C, H, N.

4-Butanoyl-1-(3-chlorophenyl)piperazine hydrochloride (28·HCl): yield 90%; mp 123-125 °C; MS (free base) *m/e* 266 (1, M^+), 196 (20), 154 (100), 56 (12). Anal. ($C_{14}H_{19}ClN_2O$·HCl) C, H, N.

1-(3-Chlorophenyl)-4-(2-methylpropionyl)piperazine hydrochloride (29·HCl): yield 87%; mp 143-145 °C; MS (free base) *m/e* 266 (25, M^+), 268 (8, M^+), 166 (100), 154 (29), 56 (95). Anal. ($C_{14}H_{19}ClN_2O$·HCl) C, H, N.

1-(3-Chlorophenyl)-4-(phenylacetyl)piperazine hydrochloride (30·HCl·$^1/_2H_2O$): yield 85%; mp 129-131 °C; MS (free base) *m/e* 314 (51, M^+), 315 (16, M^+), 195 (19), 166 (100), 154 (39), 56 (58). Anal. ($C_{18}H_{19}ClN_2O$·HCl·$^1/_2H_2O$) C, H, N.

pK_a Measurements. Ionization constants were determined by a potentiometric titration[36] at 37 ± 0.1 °C. The pK_a values were calculated from the experimental data using the ENZFITTER program.[37]

Binding Experiments. Radioligand receptor binding studies were performed in the rat brain (hippocampus), according to the published procedure.[38] A radioligand used in the binding assays was [^{3}H]-8-OH-DPAT.

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Electrophilic Opioid Ligands. Oxygen Tethered α-Methylene-γ-lactone, Acrylate, Isothiocyanate, and Epoxide Derivatives of 6β-Naltrexol

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O^6-Ether derivatives of 6β-naltrexol in which the ether substituent includes various electrophilic groups have been synthesized in an effort to examine structure-activity requirements at the 6β-substituent for receptor affinity and irreversibility of binding in opioid receptor preparations. A series of tethered 6β-ethers having terminal epoxides, α-methylene-γ-lactones, and an isothiocyanate group were prepared. The stereochemistry of the α-methylene-γ-lactones was established by convergent synthesis of their reduction products from epoxides of known absolute stereochemistry. In general, the tested compounds showed comparable affinity and selectivity for the receptor subtypes. All were found with high affinity for μ-sites. The terminal epoxide ether diastereomers **8** and **9** were not bound irreversibly in the assay for total opioid receptors. The α-methylene-γ-lactone diastereomers **10** and **11**, and their O^{14}-acetyl precursors **20** and **21**, respectively, varied in their irreversible effects, but where noted these effects were μ-site selective. Methacrylate ether **7** and isothiocyanate ether **12** were bound irreversibly at both μ- and δ-sites.

A number of irreversible opioid ligands have been synthesized and their receptor binding properties have been examined.[1] Among these agents are many analogues of naltrexone (**1**), a nearly pure opioid receptor antagonist, which has a high affinity and selectivity for μ-binding sites. Incorporated into these compounds is a reactive electrophilic functionality as a C-6 substituent for covalent binding to opioid receptors. β-Chlornaltrexamine (β-CNA) (**2**), β-funaltrexamine (β-FNA) (**3**), and other compounds derived from 6β-naltrexamine have shown interesting binding properties in opioid receptor preparations.[2-4] Of

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