Structure-Affinity Relationship Studies on 5-HT_{1A} Receptor Ligands. 1. Heterobicyclic Phenylpiperazines with N4-Alkyl Substituents

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Structure-affinity relationship (SAR) studies for 5-HT_{1A} receptor site are presented for two series of heterobicyclic phenylpiperazines with *N*4-alkyl substituents: 4-alkyl derivatives of 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (**3**) and 1-(benzo[*b*]furan-7-yl)piperazine (**4**). The linear and branched hydrocarbon chain derivatives up to *n*-decyl were synthesized and evaluated for their ability to displace [^{3}H]-2-(di-*n*-propylamino)-8-hydroxytetralin from its specific binding sites in rat frontal cortex homogenates. All compounds displayed a nanomolar affinity for the 5-HT_{1A} receptor. In both series the *N*-ethyl and *N*-*n*-propyl substituted derivatives have similar affinities, being slightly but statistically significantly less active than the *N*-methyl-substituted derivatives. Elongation of the hydrocarbon chain increases the affinity for the central 5-HT_{1A} receptor site, reaching a local maximum for the *N*-*n*-hexyl-substituted phenylpiperazines **23** (K_i = 0.50 nM) and **39** (K_i = 0.54 nM). Assuming that the arylpiperazine derivatives at the 5-HT_{1A} binding site are in the ionic state, ionization constants were determined in order to evaluate the use of the local inhibition constant, K_i^+, as a more convenient parameter to study the structure–affinity relationships. However, the K_i^+ could not account for the specific N4-substituent effects found.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) and its receptors are involved in many physiological or pathophysiological processes in the brain as well in the periphery. Many serotonin receptor subtypes have become known. The classification of these receptor subtypes, their role in physiological or pathophysiological states, and their respective agonists, partial agonists, and antagonists are reported in several recent reviews.[1-5,78] It is generally accepted that serotonin receptors of the 5-HT_{1A} subtype are also involved in psychiatric disorders like depression and anxiety.

Many compounds of different chemical classes have a high affinity for 5-HT_{1A} receptors[5,6] and may act as agonists, partial agonists, or antagonists. Compounds of the arylpiperazine class of 5-HT_{1A} receptor ligands such as buspirone and ipsapirone (Chart I, **1** and **2**, respectively) are effective antianxiety and antidepressant drugs. Exploration of other new arylpiperazine compounds in search of clinically useful anxiolytics, antidepressants, and antiagressive drugs led to the discovery of eltoprazine (**3**), its benzofuranyl analogue **4**, befiperide (**5**), and flesinoxan (**6**) (see Chart I).

We studied the effect of *N*4-alkyl substitution of eltoprazine and its benzofuranyl analogue **4** (for numbering, see Chart I, compound **3**) on the affinity for the 5-HT_{1A} receptor. At that time no structure–affinity data on a homologous series of *N*4-alkyl-*N*1-arylpiperazines had been published.

In the course of our investigations, data were published by Mokrosz et al.[9] on the ionization constants and the affinity for the 5-HT_{1A} receptor of a homologous series of *N*4-substituted alkyl derivatives of 1-(*m*-chlorophenyl)-piperazine (*m*-CPP, **7**). As these authors proposed the use of the affinity constant of the protonated species (K_i^+) as a more convenient parameter to study structure–affinity relationships, we included this parameter in our studies. In this paper we describe the synthesis of a homologous series of linear and branched-alkyl derivatives of eltoprazine (**3**) and its benzofuranyl analogue **4** and the affinities (K_i and K_i^+) for 5-HT_{1A} receptors obtained by radioligand binding studies.

Chemistry

The *N*4-alkylated phenylpiperazines **18–27**, **34–46** were conveniently synthesized by direct alkylation (methods A and C, see Experimental Section) or by means of a reductive alkylation (methods B and D) of 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (**3**), 1-(benzo[*b*]furan-7-yl)piperazine (**4**), or 1-(*m*-chlorophenyl)piperazine (**7**) with the appropriate alkyl halide or alkyl acid chloride (see Chart II). Physical and spectroscopical properties of the compounds **10–46** are collected in Table I and in the Experimental Section.

The synthetic methods for the preparation of the key intermediates **3** and **4** were taken from the patent application JP 61,152,655 and from the work of van Wijngaarden et al,[10] respectively.

Determination of Ionization Constants, Inhibition Constants and the Local Inhibition Constants

Ionization constants were measured by potentiometric titration[11] in water at temperatures between 20 and 45 °C. The molar fraction of the protonated species (*M*) was calculated from the equation derived from the titration equilibrium

$$BH^+ + H_2O \rightleftharpoons B + H_3O^+ \Rightarrow K_a = \frac{[B][H_3O^+]}{[BH^+]}$$

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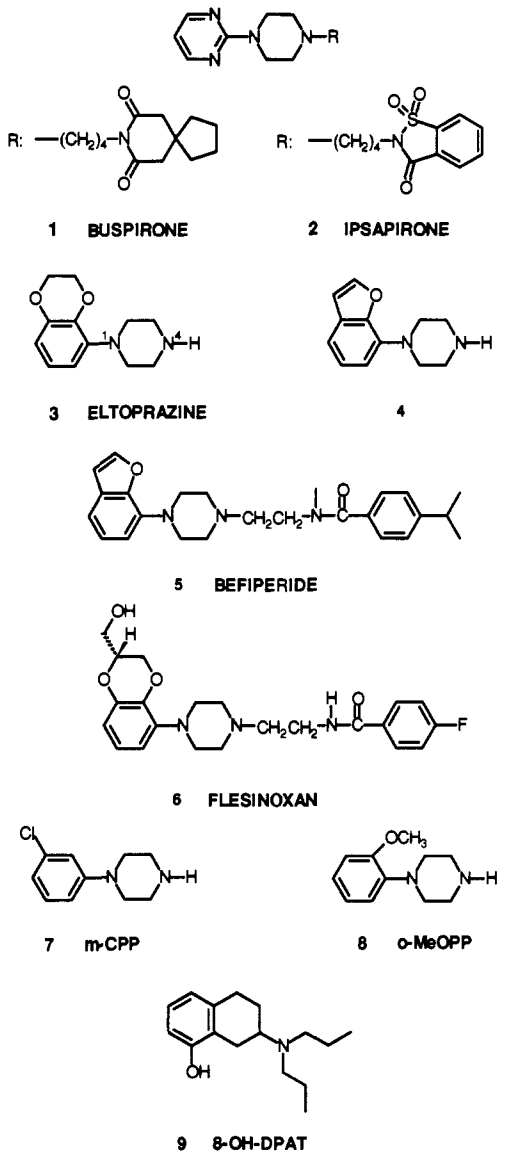
Chart I. Structures of 5-HT_{1A} Receptor Ligands



therefore

$$pK_A = \log \frac{[BH^+]}{[B]} + pH$$

with $[BH^+]/([B] + [BH^+]) = M$

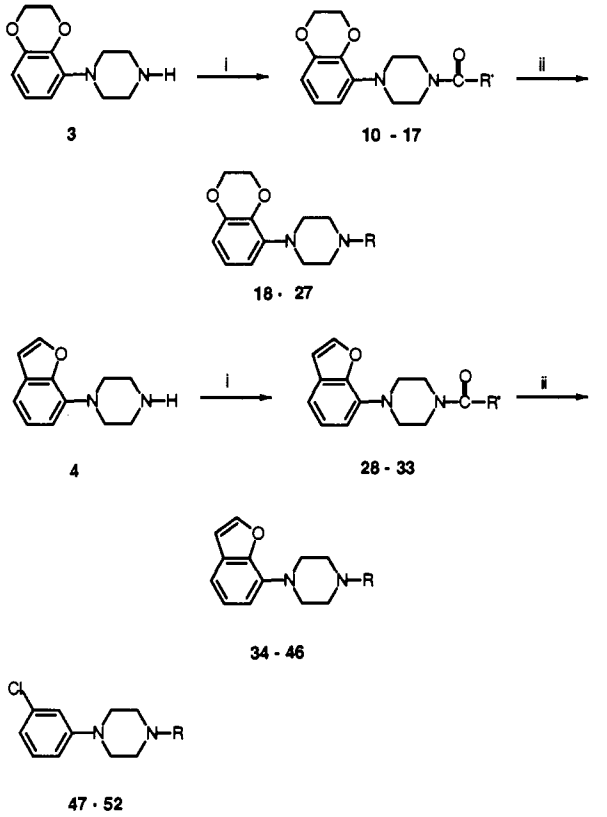
$$M = \frac{1}{1 + 10^{pH-pK_a}} \quad (1)$$

The inhibition constant K_i was derived from the IC_{50} by the Cheng–Prusoff equation

$$K_i = IC_{50}/\left(1 + \frac{L^*}{K_d^*}\right) \quad (2)$$

where IC_{50} is the concentration of the drug necessary to displace 50% of the radioligand [^{3}H]-8-OH-DPAT (**9**) from its specific binding site on the 5-HT_{1A} receptor in rat brain cortex, L* is the concentration of the radioligand used, and K_d* represents the dissociation constant of the radioligand. The receptor binding assay was performed at 37 °C and pH 7.7. Assuming that the arylpiperazine derivatives at the 5-HT_{1A} binding site are in the ionic state, the inhibition constant K_i is transformed into the local

Chart II[a]



[a] Reagents: (i) RCOCl, Et_3N in CH_3CN (ii) $LiAlH_4$ in THF or $NaCNBH_3$ in MeOH Direct alkylation of **3** or **4** gave method C: RBr, iPr_2NEt in CH_3CN.

inhibition constant K_i^+ by

$$K_i^+ = K_i M \quad (3)$$

Results

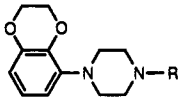
Inhibition Constants. The results of the in vitro binding studies in rat frontal cortex homogenates of the target compounds **3**, **4**, **18–27**, and **34–46** and the data of the reference compounds m-CPP (**7**), 1-(*o*-methoxyphenyl)piperazine (o-MeOPP, **8**), 2-(di-*n*-propylamino)-8-hydroxytetralin (8-OH-DPAT, **9**), and ipsapirone (**2**) measured by displacement of [^{3}H]-8-OH-DPAT from its specific binding sites are summarized in Table II. In the homologous benzofuranyl series of eltoprazine congeners the effect of N4-substitution on the affinity was compared to the affinity of the unsubstituted parent compound **4**. Whereas there is a slight but statistically significant[12] improvement of the affinity by *N*-methyl substitution (compound **34** vs **4**; K_i = 8.1 nM vs 13 nM), substitution with small hydrocarbon chains up to *N*-propyl shows that the *N*-ethyl and *N*-propyl derivatives **35** and **36** have similar affinities (K_i = 23 and 29 nM, respectively), both being slightly but statistically significant less active in comparison to the *N*-methyl derivative **34** (K_i = 8.1 nM). Further elongation of the hydrocarbon chain increases the affinity, which reaches a maximum for the *N*-*n*-hexyl substituent **39** (K_i = 0.54 nM), but further elongation of the *n*-alkyl chain decreases the affinity. The *n*-decyl derivative **41** (K_i = 8 nM) for example is 15 times less potent than compound **39**. Branching the hydrocarbon chain is less favorable for affinity. The *N*-isopropyl and *N*-isobutyl derivatives (compounds **42** and **43**, respectively) are about 2 times less potent than the corresponding

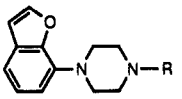
Table I. Physicochemical Properties and Synthesis Methods of Compounds 3, 18–27, 4, and 34–46

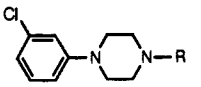
compd	R	formula	prep. method[a]	mp (°C)	C,H,N analysis[b]
3	H	$C_{12}H_{16}N_2O_2 \cdot 0.95HCl$	*c*	256–8	C,H,N
18	CH_3	$C_{13}H_{18}N_2O_2 \cdot 1.80HCl$	A	204–9	C,H,N
19	CH_2CH_3	$C_{14}H_{20}N_2O_2 \cdot 1.20HCl$	B	238	C,H,N
20	$CH_2CH_2CH_3$	$C_{15}H_{22}N_2O_2 \cdot 2.00HCl$	C	201–6	C,H,N
21	$(CH_2)_3CH_3$	$C_{16}H_{24}N_2O_2 \cdot 2.05HCl \cdot 0.50H_2O$	B	205–7	C,H,N,O,Cl
22	$(CH_2)_4CH_3$	$C_{17}H_{26}N_2O_2 \cdot 2.00HCl, 0.10H_2O$	B	195–7	C,H,N,O,Cl
23	$(CH_2)_5CH_3$	$C_{18}H_{28}N_2O_2 \cdot 2.00HCl$	B	198–9	C,H,N
24	$(CH_2)_7CH_3$	$C_{20}H_{32}N_2O_2 \cdot 2.00HCl \cdot 0.12H_2O$	B	223–4	C,H,N,O,Cl
25	$(CH_2)_9CH_3$	$C_{22}H_{36}N_2O_2 \cdot 2.00HCl$	B	195–7	C,H,N
26	CH_2-(c-C_6H_{11})	$C_{19}H_{29}N_2O_2 \cdot 0.30H_2O$	B	88	C,H,N
27	CH_2CH_2-(c-C_6H_{11})	$C_{20}H_{31}N_2O_2 \cdot 2.00HCl \cdot 0.20H_2O$	B	262–4	C,H,N
4	H	$C_{12}H_{14}N_2O_1 \cdot 0.20H_2O$	*d*	194–5	C,H,N
34	CH_3	$C_{13}H_{16}N_2O_1 \cdot HCl \cdot 0.10H_2O$	A	240–1	C,H,N
35	CH_2CH_3	$C_{14}H_{18}N_2O_1 \cdot 2.00HCl \cdot 0.50H_2O$	B	198–9	C,H,N
36	$CH_2CH_2CH_3$	$C_{15}H_{20}N_2O_1 \cdot 1.05H_2O$	C	210–2	C,H,N
37	$(CH_2)_3CH_3$	$C_{16}H_{22}N_2O_1 \cdot 1.94HCl \cdot 0.40H_2O$	B	193–5	C,H,N,O,Cl
38	$(CH_2)_4CH_3$	$C_{17}H_{24}N_2O_1 \cdot HCl$	C	209–10	C,H,N
39	$(CH_2)_5CH_3$	$C_{18}H_{28}N_2O_1 \cdot 2.00HCl \cdot 0.40H_2O$	B	165–8	C,H,N,O,Cl
40	$(CH_2)_7CH_3$	$C_{20}H_{28}N_2O_1 \cdot HCl$	C	183–6	C,H,N
41	$(CH_2)_9CH_3$	$C_{22}H_{34}N_2O_1 \cdot 2.00HCl \cdot 0.47H_2O$	B	132–5	C,H,N,O,Cl
42	$CH(CH_3)_2$	$C_{15}H_{21}N_2O_1 \cdot HCl$	D	212–6	C,H,N
43	$CH_2CH(CH_3)_2$	$C_{16}H_{23}N_2O_1 \cdot 1.50HCl \cdot 0.15H_2O$	D	194–6	C,H,N
44	CH_2-(c-C_3H_5)	$C_{16}H_{20}N_2O_1 \cdot HCl \cdot 0.10H_2O$	B	204–6	C,H,N
45	c-C_6H_{11}	$C_{18}H_{24}N_2O_1 \cdot 1.10HCl$	D	245–7	C,H,N
46	CH_2-(c-C_6H_{11})	$C_{19}H_{26}N_2O_1 \cdot p$-TsOH	B	185–8	C,H,N

[a] Method A: alkylation with methyl tosylate. Method B: reductive alkylation with the appropriate alkyl acid chloride, followed by reduction with $LiAlH_4$. Method C: direct alkylation with the appropiate alkyl halide. Method D: reductive alkylation with $NaCNBH_3$. [b] All values within 0.40% of the calculated theoretical value. [c] Compound 3 was synthesized as described in patent application JP 61,152,655. [d] Compound 4 was synthesized as described in ref 10.

Table II. Displacement of [^{3}H]-2-(Di-*n*-propylamino)-8-hydroxytetralin (9) Binding to Central 5-HT$_{1A}$ Recognition Sites in Rat Frontal Cortex Homogenates by N4-Substituted Heterobicyclic Phenylpiperazines[24]







R	no.	$K_i \pm$ SEM (nM)[a]	no.	$K_i \pm$ SEM (nM)[a]	no.	$K_i \pm$ SEM (nM)[b]
H	3	40 ± 5	4	13 ± 2	7	143 ± 6
CH_3	18	24 ± 7	34	8.1 ± 0.6	47	152 ± 4
CH_2CH_3	19	66 ± 15	35	23 ± 5	48	220 ± 10
$CH_2CH_2CH_3$	20	80 ± 11	36	29 ± 2	49	122 ± 11
$(CH_2)_3CH_3$	21	12 ± 2	37	5.9 ± 1.2	50	18.4 ± 4.9
$(CH_2)_4CH_3$	22	2.2 ± 0.4	38	0.81 ± 0.06	51	5.54 ± 0.86
$(CH_2)_5CH_3$	23	0.50 ± 0.09	39	0.54 ± 0.13	52	2.67 ± 1.19
$(CH_2)_7CH_3$	24	0.61 ± 0.07	40	1.7 ± 0.4	53	11.3 ± 1.2
$(CH_2)_9CH_3$	25	1.0 ± 0.3	41	8.0 ± 2.5		
$CH(CH_3)_2$			42	56 ± 17	54	159 ± 5
$CH_2CH(CH_3)_2$			43	64 ± 8		
CH_2-(c-C_3H_5)			44	20 ± 1		
c-C_6H_{11}			45	64 ± 6		
CH_2-(c-C_6H_{11})	26	11 ± 1	46	7.7 ± 0.4		
CH_2CH_2-(c-C_6H_{11})	27	0.25 ± 0.07				
reference compound						
m-CPP	7	200 ± 60				
oMeOPP	8	170 ± 20				
8-OH-DPAT	9	2.8 ± 0.7				
mCPP-$(CH_2)_4CH_3$	51	7.8 ± 1.9				
ipsapirone	2	5.5 ± 0.8				

[a] $K_i \pm$ SEM (nM) values are based on three to six assays each usings four to six concentrations in triplicate. [b] Data taken from Mokrosz et al.; see ref 9.

unbranched congeners. However, when the isopropyl moiety is replaced by the cyclopropanylmethyl group, yielding **44**, the affinity increases to the level of the unbranched compound **36**. The *N*-cyclohexyl and *N*-cyclohexylmethyl analogues (compounds **45** and **46**) are almost 10 times less potent than the unbranched compounds **37** and **38**, respectively. A similar trend was found in the benzodioxanyl series with respect to the smaller *n*-alkyl substituents, but the affinities found in these derivatives were somewhat lower in comparison to those for the benzofuranyl series. Although in both series maximum affinity is reached in the *n*-hexyl derivatives with similar K_i values (**23**, $K_i = 0.50$ nM; **39**, $K_i = 0.54$ nM), further elongation of the hydrocarbon chain in the benzodioxanyl series did not cause a significant decrease in affnity compared to the *n*-hexyl compound as it does in the benzofuranyl series (see Table II). When the cyclohexylmethyl group in the benzodioxanyl derivative

Table III. Molar Fraction Protonated (M) at 37 °C and pH 7.7 and Local Affinity Constants (K_i^+ in nM) for compounds **3**, **18–22** and **4**, **34–38** and **7**, **51**

R	no.	pK_A	M	K_i^+	no.	pK_A	M	K_i^+	no.	pK_A	M	K_i^+
H	**3**	8.74	0.92	36.6	**4**	8.44	0.85	11.0	**7**	8.36	0.91	131
CH_3	**18**	7.77	0.54	13.0	**34**	7.54	0.41	3.31				
CH_2CH_3	**19**	8.00	0.67	44.0	**35**	7.79	0.55	12.7				
$CH_2CH_2CH_3$	**20**	8.02	0.68	54.1	**36**	7.84	0.56	16.2				
$(CH_2)_3CH_3$	**21**	8.06	0.70	8.35	**37**	7.77	0.54	3.18				
$(CH_2)_4CH_3$	**22**	8.06	0.70	1.53	**38**	>7.7	nd[a]		**51**	>7.2	nd[a]	

[a] No relevant value of the ionization constant pK_A could be obtained; see text.

26 (K_i = 11 nM) was moved further away from the N4 nitrogen atom by one methylene group, the affinity for the 5-HT_{1A} receptor increased significantly, resulting in a K_i of 0.25 nM for compound **27**. Finally, comparing the results found in the benzodioxanyl and benzofuranyl series with the N4-substitution effects found in the m-CPP series, again a similar trend was found, although the m-CPP series was the lesser active of the three series investigated. Smaller *N*-*n*-alkyl substituents show that the *N*-ethyl derivative **48** is a lesser active compound in the m-CPP series. *N*-Methyl derivative **41** has a similar affinity as the unsubstituted parent molecule m-CPP **7**. The *N*-propyl derivative **49** shows a slightly higher affinity, whereas further elongation of the hydrocarbon chain resulted in an increase in the affinity, which reached its maximum for the *n*-hexyl derivative of m-CPP, compound **52**, as also found in the benzofuranyl and benzodioxanyl series.

Local Inhibition Constants. The ionization constants (pK_A) were determined for compounds **3**, **18–22** in the benzodioxanyl series, compounds **4**, **34–38** in the benzo[*b*]furanyl series, and compounds **7** and **51** in the m-CPP series (see Table III). In the benzodioxanyl- and the benzofuranylpiperazine series the ionization constant decreases about 1 log unit by N4-methylation of the N4-unsubstituted arylpiperazines. There is an increase of the ionization constants of ca. 0.3 log units in the *N*4-ethyl derivatives **19** and **35**. Further elongation does not affect the pK_A values. For compounds **38** and **51**, no relevant pK_A value could be obtained. Due to the low solubility of the free base, both compounds start to precipitate when the first equivalent point is reached and the second equivalent of base is added to the solution. When precipitation takes place, one can then only give an estimation of the ionization constant as calculated from the first datapoints of the titration curve. Generally, a lower pK_A value will be obtained when precipitation of the free base takes place. For 4-(*n*-pentylbenzofuranyl)-piperazine **38** this estimation is pK_A > 7.7, for the *N*-*n*-pentyl derivative of m-CPP, compound **51**, this pK_A is >7.2. When we did not take the precipitation into account, a value of approximately 6.3 for the pH at 50% volume of the titrant consumption at the equivalence point was found for the *N*-*n*-pentyl derivative of m-CPP, compound **51**. The pK_A values of the compounds with exception of **38** and **51** were used to calculate the molar fraction (M) of the protonated species at 37 °C and pH 7.7 (Table III). At this temperature and pH alkylated piperazines are about 40–70% protonated, the nor derivatives are about 90% protonated. The local affinity constant K_i^+, obtained by multiplying the M values by the K_i values (Table II)

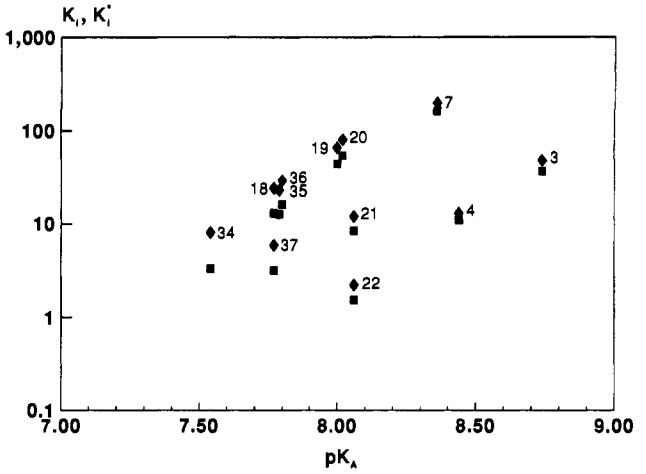


Figure 1. Relation between pK_A measured at 37 °C and the inhibition constant K_i (♦) and the local inhibition constant K_i^+ (■) for compounds **3**, **18–22** and **4**, **34–37** and **7**.

are summarized in Table III. It is obvious that in both series the SAR using K_i^+ values is very similar to the SAR obtained with K_i values. This is further illustrated in Figure 1, showing K_i and K_i^+ values as a function of the pK_A value.

The results of a temperature dependent pK_A determination can be analyzed with classical thermodynamic equations.[13] The standard Gibbs free energy change ($\Delta G°$) of association was calculated from the equation

$$\Delta G° = -RT \ln K_A \quad (4)$$

were R is the gas constant (1.99 cal/mol·K), T is the temperature in Kelvin, and K_A is the ionization constant. Changes in the standard Gibbs free energy reflect changes in the enthalpy ($\Delta H°$) as well as changes in the entropy ($\Delta S°$) according to

$$\Delta G° = \Delta H° - T\Delta S° \quad (5)$$

The standard enthalpy change ($\Delta H°$) was calculated from van 't Hoff plots of the dependence of K_A on temperature between 20 and 45 °C (293–318 K). The slope of a van 't Hoff plot (ln K_A vs $1/T$) equals $-\Delta H°/R$. Once $\Delta G°$ and $\Delta H°$ were determined as described above, the standard entropy change ($\Delta S°$) was calculated from eq 5. The results are useful in calculating the contribution of the individual entropy and enthalpy term to ΔG. Such calculations give more detailed information about the substituent effect on the pK_A value.

The results of the temperature dependent pK_A determinations of compounds **3**, **18–22**, **4**, and **7** are presented in Table IV. The van 't Hoff plots for compounds **3**, **7**, and **22** are illustrated in Figure 2. The contribution of the

Table IV. Ionization Constants (pK_A values) Determined by Potentiometric Titration in Aqueous Solution for Compounds **3**, **18–22** and **4**, **34–38** and **7**, **51**

compound	no.	T = 20 °C	T = 25 °C	T = 30 °C	T = 37 °C	T = 45 °C
BD-H	**3**	9.18	8.99	8.88	8.74	8.55
BD-CH_3	**18**	8.07			7.77	
BD-CH_2CH_3	**19**	8.34			8.00	
BD-$CH_2CH_2CH_3$	**20**	8.36			8.02	
BD-$(CH_2)_3CH_3$	**21**	8.41			8.06	
BD-$(CH_2)_4CH_3$	**22**	8.41	8.27	8.21	8.06	7.91
BF-H	**4**	8.84			8.44	
BF-CH_3	**34**				7.54	
BF-CH_2CH_3	**35**				7.79	
BF-$CH_2CH_2CH_3$	**36**				7.84	
BF-$(CH_2)_3CH_3$	**37**				7.77	
BF-$(CH_2)_4CH_3$	**38**				>7.7[a]	
mCPP-H	**7**	8.72	8.60	8.46	8.36	8.12
mCPP-$(CH_2)_4CH_3$	**51**	>7.4[a]	>7.4[a]	>7.5[a]	>7.2[a]	>7.0[a]

[a] Because of the low solubility of the free base of compounds **38** and **51** during the titration, no relevant value of the ionization constant could be obtained. BD = N4-substituted 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine, BF = N4-substituted 1-(benzo[*b*]furan-7-yl)piperazine, mCPP = N4-substituted 1-(*m*-chlorophenyl)piperazine.

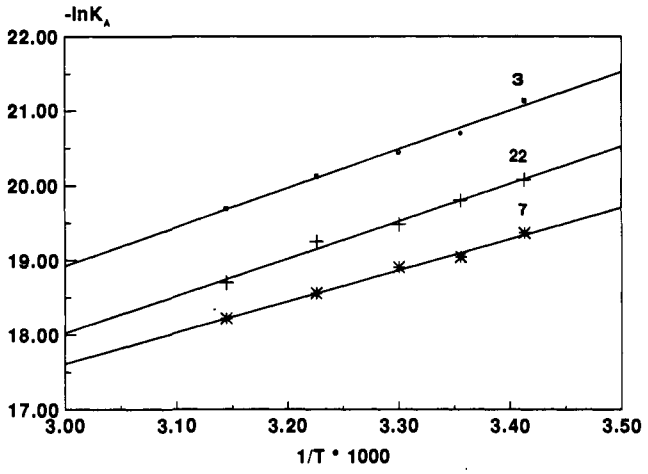


Figure 2. van 't Hoff plots of the dependence of K_A on temperature for compounds **3**, **7**, and **22**.

Table V. Thermodynamic Parameters Derived from van 't Hoff Plots As Determined from Eqs 4 and 5 for compounds **3**, **4**, **7**, and **18–22**[a]

compound	no.	$\Delta G°$ (310 K) (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (310 K) (eu)	$T\Delta S°$ (310 K) (kcal/mol)
BD-H	**3**	12.382	10.744	−0.0053	−1.638
BD-CH_3	**18**	11.008	7.325	−0.0119	−3.683
BD-CH_2CH_3	**19**	11.334	8.302	−0.0098	−3.032
BD-$CH_2CH_2CH_3$	**20**	11.362	8.302	−0.0099	−3.060
BD-$(CH_2)_3CH_3$	**21**	11.419	8.546	−0.0093	−2.873
BD-$(CH_2)_4CH_3$	**22**	11.419	8.546	−0.0093	−2.873
BF-H	**4**	11.957	9.767	−0.0071	−2.191
mCPP	**7**	11.844	8.790	−0.0099	−3.053

[a] BD = N4-substituted (2,3-dihydro-1,4-benzodioxin-5-yl)piperazine, BF = N4-substituted (benzo[*b*]furan-7-yl)piperazine, mCPP = 1-(*m*-chlorophenyl)piperazine. The thermodynamic parameters for compounds **3**, **7** and **22** were calculated from the van 't Hoff plot containing five temperature dependent pK_A determinations; all other compounds were titrated at 20 and 37 °C only. See Table IV.

individual enthalpy and entropy term to the free energy term is summarized in Table V. The $T\Delta S$ contribution to ΔG and thus to the pK_A for the unsubstituted phenylpiperazines is 25.8% for compound **7**, 18.3% for compound **4**, and 13.2% for compound **3**. The contribution $T\Delta S$ increases by N-methylation (compound **18**, 33%). Elongation of the *N*-alkyl hydrocarbon chain up to *N*-*n*-hexyl results in a contribution of about 26% for all the compounds tested (**19–22**). These results demonstrate that the *N*-*n*-alkyl substituents on the benzodioxanylphenylpiperazine series affect the pK_A value in a similar way. The substitution pattern of the phenyl moiety of the arylpiperazines slightly effects the pK_A in a different way, as demonstrated for the unsubstituted piperazines.

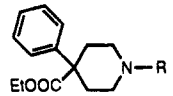
Discussion

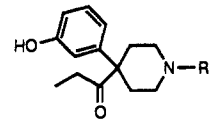
Our results indicate that N-alkylation of the benzodioxanylpiperazine **3** and its benzofuranyl analogue **4** significantly influences the affinity for 5-HT$_{1A}$ receptors. In both series the affinity decreases slightly in the *N*-ethyl- and *N*-*n*-propylsubstituted derivatives and increases strongly by further elongation of the hydrocarbon chain reaching a local maximum for the *N*-*n*-hexyl-substituted phenylpiperazines. Branching is less favorable for 5-HT$_{1A}$ affinity. Our data parallels the results found by Mokrosz,[9] who reported the effect of N-alkylation of mCPP (**7**) on the affinity for 5-HT$_{1A}$ receptors. However, in contrast to the proposal of Mokrosz to use local inhibition constants (K_i^+) as a more convenient parameter to discuss structure–affinity relationships, this could not be confirmed by our experiments. The SAR using K_i^+ was very similar to that of the SAR using K_i values. The pK_A value for m-CPP found by Mokrosz (pK_A = 8.42) is in good agreement with the value found in our laboratories: pK_A 8.36. However, for the *N*-*n*-pentyl-substituted m-CPP derivative **51** Mokrosz reported a pK_A value of 6.13. Due to the low solubility of the free base of compound **51**, we could not determine a relevant pK_A value. If we did not consider the precipitation during the titration, a pK_A value of 6.3 for the pH at 50% volume of the titrant consumption at the equivalence point was found, which is approximately the value Mokrosz reported. For compound **51** and also for compound **38**, the estimated pK_A values will be >7.2 and >7.7, respectively. In our opinion these values are more reliable.

Using molecular connectivity indices, Mokrosz concluded that the high affinity for the 5-HT$_{1A}$ receptor of the long-chain derivatives of m-CPP could be explained by a favorable hydrophobic interaction of these side chains with the receptor. However, addition of one methylene group to a linear hydrocarbon chain reflects in a constant increase in these indices. Due to this constant increment, these indices cannot account for the nonlinear SAR found in our series, indicating that the contribution of the nonspecific hydrophobic interaction is not the major component in the interaction between the synthesized ligands and the 5-HT$_{1A}$ binding site.

The results of the structure–affinity relationship studies in our phenylpiperazine series is also seen in two series of N-alkylated phenylpiperidines displaying affinity for opiate receptors. Pert and Snyder[14] reported the affinity data for the opiate receptor in vitro (Table VI, Figure 3) and the analgesic potency in mice in a series of eight *N*-*n*-alkyl homologues of meperidine. The affinity decreases when the *N*-methyl group of meperidine is displaced by larger groups as *N*-ethyl or *N*-propyl but increases again from *N*-butyl onward. The highest affinity was found for the *N*-*n*-heptyl and *N*-*n*-octyl congeners of meperidine. No explanation for this phenomenon was offered by the authors. Wilson and Rogers[15] investigated the correlation between opiate receptor binding results and analgesic potency in mice for a series of *N*-*n*-alkyl homologues of ketobemidone. The affinity for the opiate receptor was

Table VI. Displacement of [^{3}H]Naloxone, Opiate Receptor Affinity for *N*-alkyl Homologues of Meperidine[a] and Ketobemidone Series[b]





homologues	Meperidine series	ketobemidone series
CH_3	meperidine 0.50	ketobemidone 7–10
CH_2CH_3	5.00	400
$(CH_2)_2CH_3$	4.00	200
$(CH_2)_3CH_3$	0.90	50
$(CH_2)_4CH_3$	0.40	8
$(CH_2)_5CH_3$	0.20	20
$(CH_2)_6CH_3$	0.055	20
$(CH_2)_7CH_3$	0.03	200
$(CH_2)_8CH_3$	0.15	700
$(CH_2)_9CH_3$		500

[a] Data taken from Pert and Snyder, see ref 14. [b] Data taken from Wilson and Rogers, see ref 15. [c] Inhibition of opiate receptor binding is presented as the concentration of drug which reduced specific [^{3}H]naloxone binding by 50% in the absence of sodium, the mean of two determinations varying less than 30%.

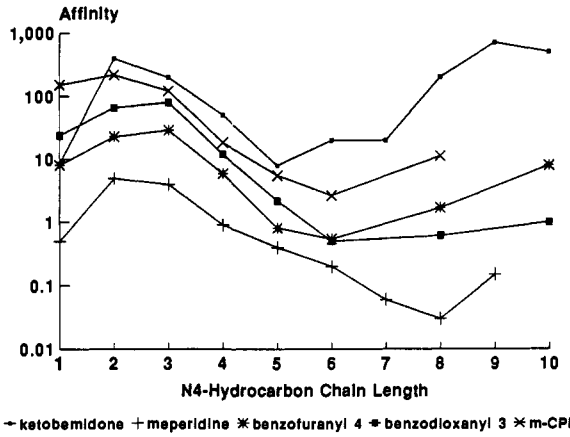


Figure 3. Relation between *N*4-hydrocarbon chain length and affinity for *N*4-*n*-alkyl-substituted phenylpiperidines and phenylpiperazines. For the ketobemidone and meperidine series the affinity is expressed as the IC_{50} in μM for opiate receptor inhibition [data taken from Pert and Snyder (see ref 14) and from Wilson and Rogers (see ref 15)]. The N4-alkyl-substituted Phenylpiperazine data were taken from Table II and are expressed as the K_i in nM determined by displacing [^{3}H]-8-OH-DPAT from its specific sites in rat frontal cortex homogenates.

determined as described by Pert and Snyder.[14] The results are shown in Table VI and Figure 3. The *N*-*n*-alkyl structure–affinity relationships are similar to those described by Pert and Snyder. There is again a decrease in affinity for the *N*-ethyl and *N*-propyl derivatives compared to the parent compound ketobemidone and an increase in affinity when the hydrocarbon chain is further elongated to *n*-butyl. The highest affinity in this series was found for the *N*-*n*-pentyl congener while longer alkyl chains led to a gradual decrease in affinity, the *N*-*n*-octyl having the same affinity as the *N*-*n*-propyl derivative. For these effects no explanation was given.

The results, as published by the above mentioned authors, are in good agreement with our data, as shown in Figure 3.

Recently, models of the central 5-HT_{1A} receptor site have been presented. These models can be classified as models which are defined by ligand-affinity data[17,18] or as models based on receptor protein data in combination with ligand-affinity data. The models based on ligand-affinity data mostly consider the fitting of compounds of different chemical classes which all interact with the same receptor. The regions of the binding site described by these ligand models are often based on smaller molecules, preferably with rigid fragments in order to define the pharmacophore. Such models cannot be developed very well with molecules containing flexible substituents, such as longer *N*-alkyl chains (as present in our series of arylpiperazines), and therefore are of limited use for structure–affinity predictions.

The reported three-dimensional protein models of the 5-HT_{1A} receptor transmembrane regions are based on the analysis of their primary amino acid sequence, site-directed mutagenesis, ligand-binding data, and the experimental structure of bacteriorhodopsin as described by Hibert et al.[19,20] and Kuipers et al.[21] These models constitute a working hypothesis in order to discuss structure–affinity relationships. The exact fit of heterobicyclic arylpiperazines in these models has not been published; therefore, a possible explanation derived from these models for the specific *N*-*n*-alkyl effects found in the investigated series cannot be postulated without becoming highly speculative. However, taking the above mentioned remarks into account, a possible explanation for the specific structure–affinity relations found can be derived from the postulated 3D models of the 5-HT_{1A} receptor. Smaller 5-HT_{1A} ligands, e.g. 5-hydroxytryptamine, bearing no or small *N*-alkyl substituents, fit well into the receptor cavity region and interact with well-defined amino acids located in the transmembrane regions III, IV, and V.[21] Assuming that the unsubstituted arylpiperazines **3**, **4**, and **7** in our series interact with the receptor binding site in a similar way, these ligands also fit into the receptor cavity region. When these arylpiperazines are derivatized into their *N*-ethyl or *N*-propyl analogues, the ligands can no longer completely fit into the receptor cavity. The decrease in affinity found for the *N*-ethyl and *N*-propyl derivatives could be the result of steric hindrance of the *N*-alkyl substituent of the ligands with an amino acid group located near the active site. Further elongation of the hydrocarbon chain results in an increase in the affinity, reaching a local maximum approximately for the *N*-*n*-hexyl substituent. As the nonspecific interaction of the *N*4-alkyl substituent effects found in our series does not account for the observed structure–affinity relationships, the possibility remains that longer *N*-alkyl substituents contribute in a specific manner. So the loss in affinity caused by the steric hindrance of the smaller *N*-alkyl substituents could be well compensated by a positive effect if an accessory binding site is reached by larger alkyl substituents. This additional interaction is likely to be a hydrophobic interaction with another transmembrane region located near the binding site. An interaction with this accessory binding site would lead to an increase in affinity when the hydrophobicity of the *N*-alkyl substituent is increased as already demonstrated in the benzodioxanyl series: the *N*-cyclohexylethyl derivative **27** (K_i = 0.25 nM) is 2 times more potent than the corresponding *N*-*n*-hexyl compound **23** (K_i = 0.50 nM).

Conclusions

We have shown that the *N*4-alkyl substituents of heterobicyclic phenylpiperazines influence significantly their ability to bind to 5-HT_{1A} receptors. Hydrocarbon chain substitution up to *N*4-propyl shows that *N*-methyl substitution gives a slightly higher affinity in comparison

with the unsubstituted phenylpiperazines. The *N*-ethyl and *N*-propyl derivatives have similar affinities, both being slightly but statistically significantly less active in comparison to the *N*-methyl derivatives. Further elongation of the hydrocarbon chain strongly increases the affinity for 5-HT$_{1A}$ receptors, reaching a local maximum affinity approximately for the *N*4-*n*-hexyl derivatives. Evaluation of the local inhibition constant K_i[+] in order to establish the use of this parameter as a more convenient parameter to use in comparison to the inhibition constant itself for structure–affinity relationship studies led to the conclusion that this is not justified in the series we have investigated.

The *N*4-hydrocarbon structure–affinity relations found are likely to be a mixture of specific and nonspecific hydrophobic interactions with 5-HT$_{1A}$ receptors. The assumption that the interaction is only due to a nonspecific hydrophobic component does not account for the SAR results found.

As already reported for the peripheral benzodiazepine receptor binding site[22] and for the β-adrenergic receptor site,[23] a thermodynamic analysis of the drug–receptor interaction for the 5-HT$_{1A}$ receptor subtype in combination with agonistic and antagonistic data may give more detailed information about the specific interactions of the *N*4-alkyl derivatives as described.

Experimental Section

Chemistry. Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker WP-200 or AM400 instrument. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane; coupling constants (*J*) are in hertz. Elemental analysis was performed at the TNO laboratory of Organic Chemistry, Utrecht, The Netherlands, and were within 0.4% of the theoretical values. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. For normal pressure and flash chromatography, Merck silica gel type 60 (size 70–230 and 230–400 mesh, respectively) was used. Unless stated otherwise, starting materials were used as high-grade commercial products. Commercially unavailable acid chlorides were prepared from the corresponding acids with thionyl chloride. 1-(2,3-Dihydro-1,4-benzodioxin-5-yl)piperazine hydrochloride (**3**) and 1-(7-benzo[*b*]furanyl)piperazine hydrochloride (**4**) were prepared according to the patent application JP 61,152,655 and the procedure reported by van Wijngaarden et al.,[10] respectively. All reactions were performed under a nitrogen atmosphere.

General Method A. 1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-methylpiperazine, Dihydrochloride (18). To a suspension of **3** (1.70 g, 6.60 mmol) in CH_3CN (15 mL) was added diisopropylethylamine (2.68 mL, 15.0 mmol). After 10 min of stirring at 20 °C, methyl tosylate (0.6 mL, 3.98 mmol) was added at 0 °C and the reaction mixture was stirred for 3 h. Then another 0.6 mL (3.98 mmol) of methyl tosylate was added. Stirring was continued at 20 °C for 2 days. After evaporation of the solvent, the residue was taken up in dichloromethane (25 mL), washed with H_2O (15 mL) and 2 N NaOH (15 mL). The organic layer was dried (Na_2SO_4) and concentrated, giving crude **18** as the free base, which was obtained pure after normal-pressure chromatography (CH_2Cl_2–MeOH, 9:1). The product was converted to its dihydrochloride salt: 0.3 g (20%); mp 204–9 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 2.83 (d, 3 H, CH_3, *J* = 5), 3.08, 3.22 (m, 2 H, H_{ax} CH_2 pip), 3.45–3.54 (4 H, H_{eq} CH_2 pip), 4.25 (m, 4H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, *J* = 8 and 2), 6.57 (dd, 1 H Bzd H-8, *J* = 8 and 2), 6.75 (t, 1 H, Bzd H-7, *J* = 8), 11.0 (br, 1 H, NH^+). Anal. ($C_{13}H_{18}N_2O_2$·1.80 HCl) C, H, N.

General Method B. 1-Acetyl-4-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (10). To a solution of **3** (7.5 g, 29.0 mmol) in CH_3CN (100 mL) was added triethylamine (8.49 mL, 61.0 mmol). After cooling to 0 °C, acetyl chloride (2.28 mL, 32.0 mmol) in CH_3CN (40 mL) was added dropwise. After 2 h of stirring at 20 °C the precipitate was filtered off and the filtrate concentrated. The oil was purified with column chromatography (ether) giving 6.8 g (89%) of **10**: ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3 H, CH_3), 3.03 (m, 4 H, CH_2 pip), 3.63, 3.78 (m, 2 H, CH_2 pip), 4.29 (m, 4 H, Bzd H-2,3), 6.55 (dd, 1 H, Bzd H-6, *J* = 8 and 2), 6.67 (dd, 1 H, Bzd H-8, *J* = 8 and 2), 6.82 (t, 1 H, Bzd H-7, *J* = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-ethylpiperazine, Dihydrochloride (19). To $LiAlH_4$ (300 mg, 7.89 mmol) suspended in anhydrous THF (30 mL) was added dropwise at 0 °C **10** (2.0 g, 7.63 mmol) in anhydrous THF (60 mL). After stirring overnight at 20 °C, the mixture was treated cautiously with H_2O (0.3 mL), 2 N NaOH (0.6 mL), and H_2O (0.6 mL) at 0 °C. The residue was filtered over Hyflow after 30 min of stirring at an ambient temperature. The filtrate was washed with H_2O (25 mL) and evaporated. The crude oil was purified with column chromatography (CH_2Cl_2–MeOH, 7:3). The obtained product was converted to its dihydrochloride salt: yield 0.8 g (33%); mp 238 °C; ^{1}H NMR ($CDCl_3$) δ 1.55 (t, 3 H, CH_2CH_3, *J* = 7), 3.26 (m, 2 H, $^+NHCH_2CH_3$), 3.59, 3.65 (m, 2 H, H_{eq} CH_2 pip), 4.34, 4.52 (m, 2 H, Bzd H-2,3), 4.37, 5.04 (m, 2 H, H_{ax} CH_2 pip), 6.93 (t, 1 H, Bzd H-7, *J* = 8), 7.02 (dd, 1 H, Bzd H-6, *J* = 8 and 2), 7.75 (dd, 1 H, Bzd H-8, *J* = 8 and 2), 13.7 (br, 1 H, NH^+). Anal. ($C_{14}H_{20}N_2O_2$·1.20 HCl) C, H, N.

General Method C. 1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-propylpiperazine, Dihydrochloride (20). To a suspension of **3** (1.4 g, 5.46 mmol) in CH_3CN (15 mL) was added *n*-propyl bromide (0.69 mL, 7.64 mmol) and diisopropylethylamine (1.95 mL, 10.9 mmol). The solution was refluxed for 3 h. After evaporation, the residue was taken up in methylene chloride (40 mL) and the solution washed with 2 N NaOH (15 mL) and H_2O (15 mL). The organic layer was dried (Na_2SO_4) and concentrated, giving crude **20** as the free base, which was purified with column chromatography (CH_2Cl_2–MeOH, 9:1). The oil was converted to its dihydrochloride salt: yield 0.56 g (31%); mp 201–6 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.95 (t, 3 H, $CH_2CH_2CH_3$, *J* = 7), 1.80 (m, 2 H, $CH_2CH_2CH_3$), 3.07 (m, 2 H, $CH_2CH_2CH_3$), 3.08–3.62 (8 H, CH_2 pip), 4.24 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, *J* = 8 and 2), 6.56 (dd, 1 H, Bzd H-8, *J* = 8 and 2), 6.75 (t, 1 H, Bzd H-7, *J* = 8), 10.9 (br, 1 H, NH^+). Anal. ($C_{15}H_{22}N_2O_2$·2.00 HCl) C, H, N.

General Method D. 1-(7-Benzofuranyl)-4-isopropylpiperazine, Hydrochloride (42). To a solution of **4** (1.50 g, 6.28 mmol) in MeOH (20 mL) was added HOAc (0.36 mL, 6.29 mmol), NaOAC (0.52 g, 6.34 mmol), and acetone (1.0 mL, 13.55 mmol). After 30 min of stirring at 20 °C, $NaCNBH_3$ (0.38 g, 6.28 mmol) was added, stirring was continued at 20 °C, and then another portion of $NaCNBH_3$ (0.38 g, 6.28 mmol) was added. After 3 h the solution was acidified with concentrated HCl to pH < 2 and concentrated in vacuo. The residue was taken up in 6 N NaOH (pH > 10) and extracted with ether (4 × 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The crude oil was purified by flash chromatography (CH_2Cl_2–MeOH, 98:2). The free base was converted to its hydrochloride salt: yield 80%; mp 212.0–6 °C; ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 6 H, $CH(CH_3)2$), 3.05–4.00 (9 H: 1 H, $CH(CH_3)_2$ and 8 H CH_2 pip), 6.77 (dd, 1 H, Bzf H-6, *J* = 8 and 1), 6.77 (d, 1 H, Bzf H-3, *J* = 2), 7.14 (t, 1 H, Bzf H-5, *J* = 8), 7.27 (dd, 1 H, Bzf H-4, *J* = 8 and 1), 7.59 (d, 1 H, Bzf H-2, *J* = 2), 12.34 (br, 1 H, NH^+). Anal. ($C_{15}H_{21}N_2O_1$·HCl) C, H, N.

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-(1-oxobutyl)piperazine (11). Compound **11** was prepared from **3** and butanoyl chloride (method B). Purification was by column chromatography (EtOAc–MeOH, 88:12): yield 94%; oil; ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3 H, CH_2CH_3, *J* = 7), 1.69 (m, 2 H, $CH_2CH_2CH_3$), 2.35 (t, 2 H, C=OCH_2CH_2, *J* = 8), 3.02 (cluster, 4 H, CH_2 pip), 3.65, 3.80 (m, 2 H, CH_2 pip), 4.26, 4.34 (m, 2 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, *J* = 8 and 1), 6.63 (dd, 1 H, Bzd H-8, *J* = 8 and 1), 6.79 (t, 1 H, Bzd H-7, *J* = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-(1-oxopentyl)piperazine (12). Compound **12** was prepared from the free base of **3** and pentanoyl chloride (method B). Purification was by column chromatography (THF–MeOH–NH_4OH, 92:7.5:0.5): yield 57%; oil; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3 H, CH_2CH_3, *J* = 7), 1.39 (m, 2 H, $CH_2CH_2CH_3$), 1.65 (m, 2 H, $CH_2CH_2CH_2$), 2.37 (t, 2 H, C=OCH_2CH_2, *J* = 7), 3.02 (cluster, 4 H, CH_2 pip), 3.65, 3.80 (m, 2 H, CH_2 pip), 4.30 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6,

J = 8 and 2), 6.63 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.79 (t, 1 H, Bzd H-7, J = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-(1-oxohexyl)piperazine (13). Compound 13 was prepared from 3 and hexanoyl chloride (method B). Purification was by column chromatography (methyl-*tert*-butyl ether): yield 69%; oil; ^{1}H NMR ($CDCl_3$) δ 0.91 (t, 3 H, CH_2CH_3, J = 7), 1.29–1.40 (cluster, 4 H, $CH_2CH_2CH_2CH_3$), 1.66 (m, 2 H, $CH_2CH_2CH_2$), 2.37 (t, 2 H, $C{=}OCH_2CH_2$, J = 8), 3.02 (m, 4 H, CH_2 pip), 3.64, 3.80 (m, 2 H, CH_2 pip), 4.26, 4.34 (m, 2 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.63 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.79 (t, 1 H, Bzd H-7, J = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-(1-oxooctyl)piperazine (14). Compound 14 was prepared from the free base of 3 and octanoyl chloride (method B). Purification was by column chromatography (THF–MeOH–NH_4OH, 92:7.5:0.5): yield 77%; oil; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_2CH_3, J = 7), 1.25–1.39 (cluster, 8 H, $CH_2(CH_2)_4CH_3$), 1.65 (m, 2 H, $C{=}OCH_2CH_2CH_2$), 2.36 (t, 2 H, $C{=}OCH_2CH_2$, J = 7), 3.02 (cluster, 4 H, CH_2 pip), 3.65, 3.80 (m, 2 H, CH_2 pip), 4.30 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.63 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.79 (t, 1 H, Bzd H-7, J = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-(1-oxodecyl)piperazine (15). Compound 15 was prepared from the free base of 3 and decanoyl chloride (method B). Column chromatography was not applied: yield 80%; oil; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_2CH_3, J = 7), 1.2–1.4 (12 H, $CH_2(CH_2)_6CH_3$), 1.65 (m, 2 H, $CH_2CH_2CH_2$), 2.36 (t, 2 H, $C{=}OCH_2CH_2$, J = 7), 3.02 (m, 4 H, CH_2 pip), 3.64, 3.79 (m, 2 H, CH_2 pip), 4.29 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.62 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.78 (t, 1 H, Bzd H-7, J = 8).

1-(1-Cyclohexylcarbonyl)-4-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (16). Compound 16 was prepared from the free base of 3 and cyclohexylcarbonyl chloride (method B): yield 54%; oil; ^{1}H NMR ($CDCl_3$) δ 1.20–1.86 (cluster 10 H, cyclohexane CH_2), 2.51 (m, 1 H, H_{ax} cyclohexane), 3.03 (cluster, 4 H, CH_2 pip), 3.68, 3.80 (m, 2 H, CH_2 pip), 4.26, 4.34 (m, 2 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.63 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.79 (t, 1 H, Bzd H-7, J = 8).

1-(Cyclohexylacetyl)-4-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (17). Compound 17 was prepared from the free base of 3 and cyclohexylacetyl chloride (method B): yield 53%; oil; ^{1}H NMR ($CDCl_3$) δ 0.9–1.90 (cluster, 11 H, cyclohexane CH_2), 2.26 (d, 2 H, $C{=}OCH_2$, J = 7), 3.02 (cluster, 4 H, CH_2 pip), 3.66, 3.81 (m, 2 H, CH_2pip), 4.26, 4.34 (m, 2 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.63 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.79 (t, 1 H, Bzd H-7, J = 8).

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-butylpiperazine, Dihydrochloride (21). Compound 21 was prepared from 11 (method B). Column chromatography was not applied; the dihydrochloride salt was recrystallized from 2-propanol: yield 64%; mp 205–7 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.95 (t, 3 H, CH_2CH_3, J = 7), 1.36 (m, 2 H, $CH_2CH_2CH_3$), 1.75 (m, 2 H, $CH_2CH_2CH_2$), 3.05–3.24 (cluster, 6 H: 2 H, $^+NHCH_2CH_2$ and 4 H, CH_2 pip), 3.52 (cluster, 4 H, H_{eq} CH_2 pip), 4.25 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8 and 1), 6.56 (dd, 1 H, Bzd H-8, J = 8 and 1), 6.75 (t, 1 H, Bzd H-7, J = 8), 10.85 (br, 1 H, NH^+). Anal. ($C_{16}H_{24}N_2O_2$·2.05HCl·0.50H_2O) C, H, N, O, Cl.

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-pentylpiperazine, Dihydrochloride (22). Compound 22 was prepared from 12 (method B). Column chromatography was not applied: yield 59%; mp 195–7 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1), δ 0.92 (t, 3 H, CH_2CH_3, J = 7), 1.33 (cluster, 4 H, $CH_2(CH_2)_2CH_3$), 1.78 (m, 2 H, $CH_2CH_2CH_2$), 3.09 (m, 2 H, $^+NHCH_2CH_2$), 3.18, 3.52 (cluster, 4 H, CH_2 pip), 4.25 (cluster, 4 H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, J = 8 and 1), 6.56 (dd, 1 H, Bzd H-8, J = 8 and 1), 6.75 (t, 1 H, Bzd H-7, J = 8), 11.0 (br, 1 H, NH^+). Anal. ($C_{17}H_{26}N_2O_2$·2.00HCl·0.10H_2O) C, H, N, O, Cl.

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-hexylpiperazine, Dihydrochloride (23). Compound 23 was prepared from 13 (method B). Column chromatography was not applied; the dihydrochloride salt was recrystallized from 2-propanol: yield 41%; mp 198–9 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, CH_2CH_3, J = 7), 1.28–1.47 (cluster, 6 H, $CH_2(CH_2)_3CH_3$), 1.92 (m, 2 H, $CH_2CH_2CH_2$), 3.12 (m, 2 H, $^+NHCH_2CH_2$), 3.62 (cluster, 4 H, H_{eq} CH_2 pip), 4.29–4.47 (cluster, 4 H: 2 H, CH_2 pip, 2 H, Bzd H-2,3), 4.52 (m, 2 H, Bzd H-2,3), 5.07 (m, 2 H, H_{ax} CH_2 pip), 6.93 (t, 1 H, Bzd H-8, J = 8), 7.02 (d, 1 H, Bzd H-7, J = 8), 7.75 (d, 1 H, Bzd H-4, J = 8), 13.55 (br, 1 H, NH^+). Anal. ($C_{18}H_{28}N_2O_2$·2.00HCl) C, H, N.

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-octylpiperazine, Dihydrochloride (24). Compound 24 was prepared from 14 (method B). Column chromatography was not applied: yield 58%; mp 223–4 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, CH_2CH_3, J = 7), 1.20–1.45 (cluster, 10 H, $CH_2(CH_2)_5CH_3$), 1.93 (m, 2 H, $CH_2CH_2CH_2$), 3.12 (m, 2 H, $^+NHCH_2CH_2$), 3.55–3.68 (cluster, 4 H, H_{eq} CH_2 pip), 4.34, 4.52 (m, 2 H, Bzd H-2,3), 4.39, 5.08 (m, 2 H, H_{ax} CH_2 pip), 6.93 (t, 1 H, Bzd H-7, J = 8), 7.03 (dd, 1 H, Bzd H-6, J = 8 and 2), 7.76 (dd, 1 H, Bzd H-8, J = 8 and 2), 13.6 (br, 1 H, NH^+). Anal. ($C_{20}H_{32}N_2O_2$·2.00HCl·0.12H_2O) C, H, N, O, Cl.

1-(2,3-Dihydro-1,4-benzodioxin-5-yl)-4-*n*-decylpiperazine, Dihydrochloride (25). Compound 25 was prepared from 15 (method B). Purification was by column chromatography (CH_2Cl_2–MeOH–NH_4OH, 96.8:3.0:0.2): yield 28%; mp 195–7 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.87 (t, 3 H, CH_2CH_3, J = 7), 1.2–1.35 (cluster, 14 H, $CH_2(CH_2)_7CH_3$), 1.76 (m, 2 H, $CH_2CH_2CH_2$), 3.08 (m, 2 H, $^+NHCH_2CH_2$), 3.12–3.22 (cluster, 4 H, CH_2 pip), 3.46–3.58 (cluster, 4 H, CH_2 pip), 4.25 (m, 4 H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.57 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.75 (t, 1 H, Bzd H-7, J = 8), 11.0 (br, 1 H, NH^+). Anal. ($C_{22}H_{36}N_2O_2$·2.00HCl) C, H, N.

1-(Cyclohexylmethyl)-4-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (26). Compound 26 was prepared from 16 (method B). Purification was by column chromatography (THF–MeOH–NH_4OH, 92:7.5:0.5): yield 22% of the free base as white crystals; mp 88 °C; ^{1}H NMR ($CDCl_3$) δ 0.82–1.85 (cluster, 11 H, cyclohexane), 2.19 (d, 2 H, NCH_2CH, J = 7), 2.58, 3.08 (br, 4 H, CH_2 pip), 4.24, 4.32 (m, 2 H, Bzd H-2,3), 6.54 (dd, 1 H, Bzd H-6, J = 8 and 2), 6.59 (dd, 1 H, Bzd H-8, J = 8 and 2), 6.77 (t, 1 H, Bzd H-7, J = 8). Anal. ($C_{19}H_{29}N_2O_2$·0.30H_2O) C, H, N.

1-(2-Cyclohexylethyl)-4-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine dihydrochloride (27). Compound 27 was prepared from 17 (method B). Purification was by column chromatography with a gradient (ether, CH_2Cl_2–MeOH, 7:3): yield 21%; mp 262–4 °C; ^{1}H NMR ($CDCl_3$) δ 0.90–1.90 (cluster, 13 H, cyclohexane and CH_2 cyclohexane), 3.16 (m, 2 H, $^+NHCH_2CH_2$), 3.60 (m, 4 H, H_{eq} CH_2 pip), 4.30–4.55 (cluster, 6 H; 4 H, Bzd H-2,3 and 2 H, H_{ax} CH_2 pip), 5.07 (m, 2 H, H_{ax} CH_2 pip), 6.93 (t, 1 H, Bzd H-7, J = 8), 7.03 (d, 1 H, Bzd H-6, J = 8), 7.75 (d, 1 H, Bzd H-8, J = 8), 13.55 (br, 1 H, NH^+). Anal. ($C_{20}H_{31}N_2O_2$·2.00HCl·0.20H_2O) C, H, N.

1-Acetyl-4-(7-benzofuranyl)piperazine (28). Compound 28 was prepared from the free base of 4 and acetyl chloride (method B): yield 96%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 3 H, CH_3), 3.28, 3.33, 3.70, 3.86 (m, 2 H, CH_2 pip), 6.75 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.78 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.25 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.62 (d, 1 H, Bzf H-2, J = 2).

1-(7-Benzofuranyl)-4-(1-oxobutyl)piperazine (29). Compound 29 was prepared from the free base of 4 and butanoyl chloride (method B). Purification was by column chromatography (ether–light petroleum ether, 1:1): yield 95%; yellow oil; ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3 H, CH_2CH_3, J = 7), 1.70 (m, 2 H, $CH_2CH_2CH_3$), 2.37 (t, 2 H, $CH_2CH_2CH_3$, J = 7), 3.29 (cluster, 4 H, CH_2 pip), 3.69, 3.86 (m, 2 H, CH_2 pip), 6.73 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.75 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.23 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.61 (d, 1 H, Bzf H-2, J = 2).

1-(7-Benzofuranyl)-4-(1-oxohexyl)piperazine (30). Compound 30 was prepared from the free base of 4 and hexanoyl chloride (method B). Column chromatography was not applied: yield 80%; oil; ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 3 H, CH_2CH_3, J = 7), 1.3–1.4 (cluster, 4 H, $CH_2(CH_2)_2CH_3$), 1.67 (m, 2 H, $C{=}OCH_2CH_2(CH_2)_2CH_3$), 2.39 (t, 2 H, $C{=}OCH_2CH_2$, J = 7), 3.31 (cluster, 4 H, CH_2 pip), 3.72, 3.87 (m, 2 H, CH_2 pip), 6.76 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.77 (d, 1 H, Bzf H-3, J = 2), 7.16 (t, 1 H, Bzf H-5, J = 8), 7.25 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.62 (d, 1 H, Bzf H-2, J = 2).

1-(7-Benzofuranyl)-4-(1-oxodecyl)piperazine (31). Compound 31 was prepared from the free base of 4 and decanoyl chloride (method B). Purification was by column chromatog-

raphy (ether–light petroleum ether, 1:1): yield 100%; oil; ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, CH_2CH_3, J = 7), 1.2–1.4 (cluster, 12 H, $CH_2(CH_2)_6CH_3$), 1.66 (m, 2 H, C=O$CH_2CH_2(CH_2)_6CH_3$), 2.37 (t, 2 H, C=O$CH_2(CH_2)_7CH_3$, J = 7), 3.26 (cluster, 4 H, CH_2 pip), 3.67, 3.85 (m, 2 H, CH_2 pip), 6.71 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.74 (d, 1 H, Bzf H-3, J = 2), 7.13 (t, 1 H, Bzf H-5, J = 8), 7.23 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.59 (d, 1 H, Bzf H-2, J = 2).

1-(7-Benzofuranyl)-4-(cyclohexylcarbonyl)piperazine (33). Compound **33** was prepared from the free base of **4** and cyclohexylcarbonyl chloride (method B). Column chromatography was not applied: yield 67%; oil; ^{1}H NMR ($CDCl_3$) δ 1.20–1.88 (cluster 10 H, cyclohexane CH_2), 2.53 (m, 1 H, H_{ax} cyclohexane), 3.30 (cluster, 4 H, CH_2 pip), 3.74, 3.87 (m, 2 H, CH_2 pip), 6.75 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.77 (d, 1 H, Bzf H-3, J = 2), 7.16 (t, 1 H, Bzf H-5, J = 8), 7.25 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.62 (d, 1 H, Bzf H-2, J = 2).

1-(7-Benzofuranyl)-4-methylpiperazine, Hydrochloride (34). Compound **34** was prepared from **4** (method A). Purification was by flash chromatography (CH_2Cl_2–MeOH, 95:5): yield 54% of the monohydrochloride salt; mp 240–1 °C; ^{1}H NMR ($CDCl_3$) δ 2.90 (d, 3 H, CH_3, J = 4), 3.22, 3.73 (m, 2 H, H_{ax} CH_2 pip), 3.63, 3.87 (m, 2 H, H_{eq} CH_2 pip), 6.79 (d, 1 H, Bzf H-3, J = 2), 6.82 (br dd, 1 H, Bzf H-6), 7.17 (t, 1 H, Bzf H-5, J = 8), 7.31 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.61 (d, 1 H, Bzf H-2, J = 2), 13.1 (br, 1 H, NH^+). Anal. ($C_{13}H_{18}N_2O_1$·1.00HCl·0.10H_2O) C, H, N.

1-(7-Benzofuranyl)-4-ethylpiperazine, Dihydrochloride (35). Compound **35** was prepared from **28** (method B). Purification was by column chromatography (EtOAc): yield 65% of the dihydrochloride salt; mp 198–9 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 1.36 (t, 3 H, CH_2CH_3), 3.16–3.40 (cluster, 6 H: 2 H, $^+NHCH_2$-CH_3 and 4 H, CH_2 pip), 3.62, 3.92 (m, 2 H, H_{eq} CH_2 pip), 6.82 (d, 1 H, Bzf H-6, J = 8), 6.90 (d, 1 H, Bzf H-3, J = 2), 7.16 (t, 1 H, Bzf H-5, J = 8), 7.26 (d, 1 H, Bzf H-4, J = 8), 7.90 (d, 1 H, Bzf H-2, J = 2), 11.2 (br, 1 H, NH^+). Anal. ($C_{14}H_{18}N_2O_1$·2.00HCl·0.50H_2O) C, H, N.

1-(7-Benzofuranyl)-4-*n*-propylpiperazine, Hydrochloride (36). Compound **36** was prepared from **4** and *n*-propyl bromide (method C). Purification by flash chromatography (CH_2Cl_2–MeOH, 94:6): yield 68% of the monohydrochloride salt; mp 210–2 °C; ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3 H, $CH_2CH_2CH_3$, J = 7), 2.02 (m, 2 H, $CH_2CH_2CH_3$), 3.00 (m, 2 H, $CH_2CH_2CH_3$), 3.04–3.90 (br, 8 H, CH_2 pip), 6.78 (d, 1 H, Bzf H-3, J = 2), 6.78 (dd, 1 H, Bzf H-6, J = 8 and 1), 7.16 (t, 1 H, Bzf H-5, J = 8), 7.29 (dd, 1 H, Bzf H-4, J = 8 and 2 Hz), 7.59 (d, 1 H, Bzf H-2, J = 2), 12.8 (br, 1 H, NH^+). Anal. ($C_{15}H_{20}N_2O_1$·1.05HCl) C, H, N.

1-(7-Benzofuranyl)-4-*n*-butylpiperazine, Dihydrochloride (37). Compound **37** was prepared from **4** by the method desribed for **19**. Column chromatography was not applied: yield 74%; mp 193–5 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.96 (t, 3 H, CH_2CH_3), 1.37 (m, 2 H, $CH_2CH_2CH_3$), 1.78 (m, 2 H, CH_2CH_2-CH_2), 3.14 (m, 2 H, $^+NHCH_2CH_2$), 3.26, 3.35 (m, 2 H, H_{ax} CH_2 pip), 3.62, 3.91 (m, 2 H, H_{eq} CH_2 pip), 6.83 (d, 1 H, Bzf H-6, J = 8), 6.91 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.26 (d, 1 H, Bzf H-4, J = 8), 7.92 (d, 1 H, Bzf H-2, J = 2), 11.15 (br, 1 H, NH^+). Anal. ($C_{16}H_{22}N_2O_1$·1.94HCl·0.40H_2O) C, H, N, O, Cl.

1-(7-Benzofuranyl)-4-*n*-pentylpiperazine, Hydrochloride (38). Compound **38** was prepared from **4** and *n*-pentyl bromide by the method described for **20**; reflux time was 18 h. Purification was by column chromatography (CH_2Cl_2–MeOH, 96:4): yield 67%; mp 209–10 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3 H, CH_2CH_3, J = 7), 1.30–1.45 (cluster, 4 H, $CH_2(CH_2)_2CH_3$), 1.96 (m, 2 H, $CH_2CH_2CH_2$), 3.03 (m, 2 H, $^+NHCH_2CH_2$), 3.13, 3.74 (m, 2 H, H_{ax} CH_2 pip), 3.65, 3.85 (m, 2 H, H_{eq} CH_2 pip), 6.77 (cluster, 2 H, Bzf H-3,6), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.28 (d, 1 H, Bzf H-4, J = 8), 7.59 (d, 1 H, Bzf H-2, J = 2), 12.7 (br, 1 H, NH^+). Anal. ($C_{17}H_{24}N_2O_1$·HCl) C, H, N.

1-(7-Benzofuranyl)-4-*n*-hexylpiperazine, Dihydrochloride (39). Compound **39** was prepared from **30** (method B). Column chromatography was not applied: yield 53%; mp 165–8 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.91 (t, 3 H, CH_2CH_3), 1.28–1.40 (cluster, 6 H, $CH_2(CH_2)_3CH_3$), 1.80 (m, 2 H, $^+NHCH_2CH_2$-CH_2), 3.13 (m, 2 H, $^+NHCH_2CH_2$), 3.26, 3.37 (m, 2 H, H_{ax} CH_2 pip), 3.63, 3.91 (m, 2 H, H_{eq} CH_2 pip), 6.82 (dd, 1 H, Bzf H-6, J = 8 and <1), 6.90 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.26 (dd, 1 H, Bzf H-4, J = 8 and <1), 7.91 (d, 1 H, Bzf H-2, J = 2), 11.2 (br, 1 H, NH^+). Anal. ($C_{18}H_{26}N_2O_1$·2.00HCl·0.40H_2O) C, H, N, O, Cl.

1-(7-Benzofuranyl)-4-*n*-octylpiperazine, Hydrochloride (40). Compound **40** was prepared from **4** and *n*-octyl bromide (method C); reflux time was 18 h. Purification was by flash chromatography (CH_2Cl_2–MeOH, 96:4): yield 68%; mp 183–6 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, CH_2CH_3, J = 7), 1.18–1.44 (cluster, 10 H, $CH_2(CH_2)_5CH_3$), 1.96 (m, 2 H, $CH_2CH_2CH_2$), 3.03 (m, 2 H, $^+NHCH_2CH_2$), 3.13 (br, 2 H, H_{ax} CH_2 pip), 3.58–3.92 (cluster, 6 H, CH_2 pip), 6.77 (cluster, 2 H, Bzf H-3,6), 7.16 (t, 1 H, Bzf H-5, J = 8), 7.28 (d, 1 H, Bzf H-4, J = 8), 7.60 (d, 1 H, Bzf H-2, J = 2), 12.75 (br, 1 H, NH^+). Anal. ($C_{20}H_{26}N_2O_1$·HCl) C, H, N.

1-(7-Benzofuranyl)-4-*n*-decylpiperazine, Dihydrochloride (41). Compound **41** was prepared from **31** (method B). Column chromatography was not applied: yield 65%; mp 132–5 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 0.88 (t, 3 H, CH_2CH_3, J = 7), 1.2–1.4 (14 H, $CH_2(CH_2)_7CH_3$), 1.79 (m, 2 H, $CH_2CH_2CH_2$), 3.12 (m, 2 H, $^+NHCH_2CH_2$), 3.25, 3.34 (m, 2 H, H_{ax} CH_2 pip), 3.62, 3.90 (m, 2 H, H_{eq} CH_2 pip), 6.82 (d, 1 H, Bzf H-6, J = 8), 6.90 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.26 (d, 1 H, Bzf H-4, J = 8), 7.91 (d, 1 H, Bzf H-2, J = 2), 11.1 (br, 1 H, NH^+). Anal. ($C_{22}H_{34}N_2O_1$·2.00HCl·0.47H_2O) C, H, N, O, Cl.

1-(7-Benzofuranyl)-4-(2-methylpropyl)piperazine, Hydrochloride (43). Compound **43** was prepared from **4** and isobutyraldehyde (method D); the mixture was heated for a night at 40 °C: yield 61%; mp 194–6; ^{1}H NMR ($CDCl_3$) δ 1.18 (d, 6 H, $CH_2CH(CH_3)_2$, J = 7), 2.29 (m, 1 H, $CH_2CH(CH_3)_2$), 2.92 (d, 2 H, $CH_2CH(CH_3)_2$, J = 7), 3.0–4.0 (8 H, CH_2 pip), 6.76 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.77 (d, 1 H, Bzf H-3, J = 2), 7.13 (t, 1 H, Bzf H-5, J = 8), 7.27 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.58 (d, 1 H, Bzf H-2, J = 2), 12.35 (br, 1 H, NH^+). Anal. ($C_{18}H_{23}N_2O_1$·1.50HCl·0.15H_2O) C, H, N.

1-(7-Benzofuranyl)-4-(cyclopropylmethyl)piperazine, Hydrochloride (44). Compound **44** was prepared from **4** and cyclopropylcarbonyl chloride (method B). Purification was by flash chromatography (CH_2Cl_2–MeOH, 95:5). The resulting product was reduced with $LiAlH_4$ (method B). Purification was by flash chromatography (CH_2Cl_2–MeOH, 95:5): yield of the monohydrochloride salt 59%; mp 204–6 °C; ^{1}H NMR ($CDCl_3$) δ 0.46, 0.79 (m, 2 H, CH_2 cyclopropyl), 1.37 (m, 1 H, CH cyclopropyl), 2.98 (t, 2 H, $^+NHCH_2CH$), 3.17 (m, 2 H, H_{ax} CH_2 pip), 3.6–3.9 (6 H, CH_2 pip), 6.74 (d, 1 H, Bzf H-3, J = 2), 6.76 (dd, 1 H, Bzf H-6, J = 8 and 1), 7.13 (t, 1 H, Bzf H-5, J = 8), 7.26 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.57 (d, 1 H, Bzf H-2, J = 2), 12.63 (br, 1 H, NH^+). Anal. ($C_{16}H_{20}N_2O_1$·HCl·0.10H_2O) C, H, N.

1-(7-Benzofuranyl)-4-(cyclohexyl)piperazine, Hydrochloride (45). Compound **45** was prepared from **4** and cyclohexanone (method D). Column chromatography was not applied, the HCl salt was recrystallized from EtOAc: yield 67%; mp 245–7 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 1.0–2.3 (cluster, 10 H, cyclohexane CH_2), 3.1–4.02 (cluster, 9 H, CH_2 pip + CH cyclohexane), 6.81 (dd, 1 H, Bzf H-6, J = 8 and 1), 6.89 (d, 1 H, Bzf H-3, J = 2), 7.15 (t, 1 H, Bzf H-5, J = 8), 7.26 (dd, 1 H, Bzf H-4, J = 8 and 1), 7.90 (d, 1 H, Bzf H-2, J = 2), 11.0 (br, 1 H, NH^+). Anal. ($C_{18}H_{24}N_2O_1$·1.10HCl) C, H, N.

1-(7-Benzofuranyl)-4-(cyclohexylmethyl)piperazine *p*-toluenesulfonate (46). Compound **46** was prepared from **33** (method B). Purification was by column chromatography (EtOAc). The free base was converted to its *p*-toluenesulfonic acid salt: yield 38%; mp 185–8 °C; ^{1}H NMR ($CDCl_3$) δ 1.01–1.92 (cluster, 11 H, cyclohexane), 2.33 (s, 3 H, CH_3 tosylate), 2.97 (t, 2 H, $^+NHCH_2CH$, J = 6), 3.11, 3.66 (m, 2 H, H_{ax} CH_2 pip), 3.80 (m, 4 H, H_{eq} CH_2 pip), 6.74 (d, 1 H, Bzf H-6, J = 8), 6.77 (d, 1 H, Bzf H-3, J = 2), 7.15 (cluster, 3 H, 1 H: Bzf H-5 and 2 H, tosylate), 7.29 (d, 1 H, Bzf H-4, J = 8), 7.59 (d, 1 H, Bzf H-2, J = 2), 7.77 (m, 2 H, tosylate), 10.8 (br, 1 H, NH^+). Anal. ($C_{19}H_{26}N_2O_1$·*p*-TsOH) C, H, N.

Ionization Constant Determination. pK_A's were determined by use of a calibrated (used temperature, buffers pH 4, 7, and 9, Merck) glass pH electrode with an internal reference electrode (Metrohm 6.0203.100) by potentiometric titration[11] using an automatic titrator (Metrohm Titroprocessor E670). The

titration was performed with 0.05 mol/L sodium hydroxide (of low carbonate content) by constant volume addition of 0.05 mL at the desired temperature which was controlled at ±1 °C under a nitrogen atmosphere. A 30-mg portion of substance was dissolved in 40 mL of 0.1 mol/L potassium chloride in carbonate free water, giving a concentration of 0.002–0.005 mol/L. From several points of the titration curve a pK_A value was calculated according to $pK_A = pH + \log((V_{eq} - V_d)/V_d)$, where V_{eq} is the volume titrant used at the equivalence point and V_d is the volume titrant used at the datapoint, and the results were averaged. In case of precipitation during titration only datapoints obtained before start of precipitation were used. The standard deviation of repeated determinations was estimated at 0.02. The standard deviation of selected datapoints of each pK_A determination (5–15 datapoints collected from the titration curve) was 0.01.

The method used is suitable for determination of pK_A values approximately greater than 4, therefore relevant pK_{A1} values of the measured hydrochloride salts of the phenylpiperazines could not be obtained, all pK_{A1} values were found to be 3, by assumption these values are lower then $pK_{A1} = 3$.

Biochemistry. Receptor Binding Assay. The binding assay was carried out as described.[24] Thus, the radioligand binding studies were performed on rat frontal cortex using [^{3}H]-2-(di-*n*-propylamino)-8-hydroxytetralin as radioligand.

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