A Series of N4-Imidoethyl Derivatives of 1-(2,3-Dihydro-1,4-benzodioxin-5-yl)piperazine as 5-HT_{1A} Receptor Ligands: Synthesis and Structure–Affinity Relationships

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A series of unsubstituted and substituted succinimido, maleimido, and glutarimidoethyl derivatives of eltoprazine (**3**) was synthesized and tested for affinity for the 5-HT_{1A} receptor in rat brain homogenates. The unsubstituted compounds have a moderate affinity for the receptor, while the affinity considerably increases by substitution at or enlargement of these cyclic ring systems. A good correlation was found between the inhibition constant K_i (expressed as pK_i) and the lipophilicity (clogP). No correlation was observed between the pK_i or pK_i^+ (local inhibition constant) and the basicity of the N4-nitrogen atom.

Introduction

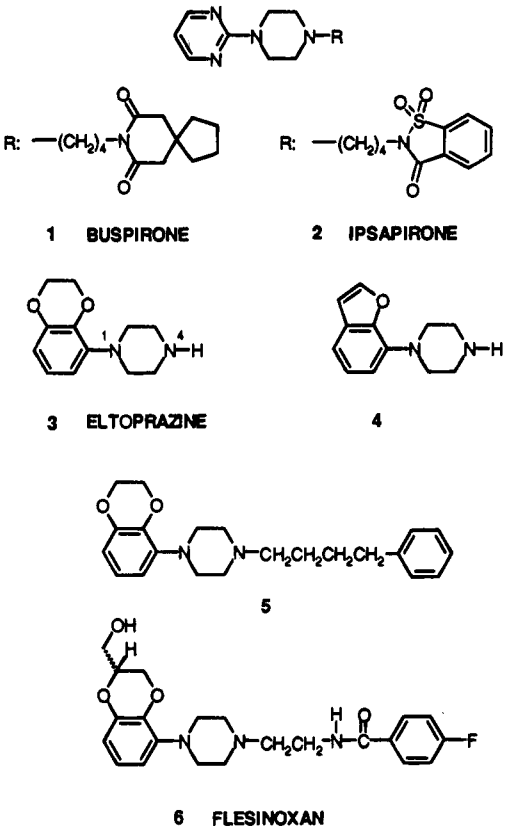
Compounds such as buspirone (**1**) and ipsapirone (**2**) (Chart 1) belonging to the class of heteroarylpiperazines are clinically effective drugs for the treatment of anxiety and depression.[1,2] It is now generally accepted that the clinical effects of these drugs are due to the interaction with 5-HT_{1A} receptors.[3–6] The discovery of other heteroarylpiperazines as potent 5-HT_{1A} ligands such as N4-substituted derivatives of eltoprazine (**3**) and **4**, e.g., the phenylbutyl derivative **5**, and flesinoxan (**6**) has resulted in the pharmacological evaluation of these drugs as potential central nervous system (CNS) drugs.[7–10]

In this paper the synthesis of a new series of N4-imidoethyl derivatives (**7**–**18**) of eltoprazine (**3**) and their affinity for the 5-HT_{1A} receptor are described. The correlation of the inhibition constant pK_i or the local inhibition constant pK_i^+ with the pK_a and clogP was investigated.

Chemistry

The N4-imidoethyl derivatives (**7**–**13** and **15**–**18**) of 1-(2,3-dihydro-1,4-benzodioxin-5-yl)piperazine (**3**) were synthesized by reacting 4-(2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazineethanamine (**20**) (Scheme 1) with the appropriate anhydride in toluene or dioxane in the presence of diisopropylethylamine. Key intermediate **20** was conveniently synthesized by reacting **3** with chloroacetonitrile followed by reduction of the cyano group in a hydrogen atmosphere with Ra Ni as the catalyst. The N4-phthalimidoethyl derivative **14** (Table 2) was synthesized by a different route. In a first attempt, very low yields were obtained when the commercially available *N*-(2-bromoethyl)phthalimide was used in a direct alkylation step. Recently, Guillaumet et al.[11] described similar low yields for the alkylation of 3-(aminomethyl)-2,3-dihydro-1,4-dioxino[2,3-*b*]pyridine with *N*-(2-bromoethyl)glutarimides in which they identified the corresponding iminoamides as the major product. The low yields in our alkylations may also be due to products derived from this side reaction. In a second synthetic approach, the Mitsunobu reaction was successfully used for the preparation of the phthalimidoethyl derivative **14** using diethyl azodicarboxylate and triphenylphosphine (Scheme 1). Physical and spectroscopical properties of the newly synthesized compounds are collected in Table 1 and the Experimental Section, respectively.

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



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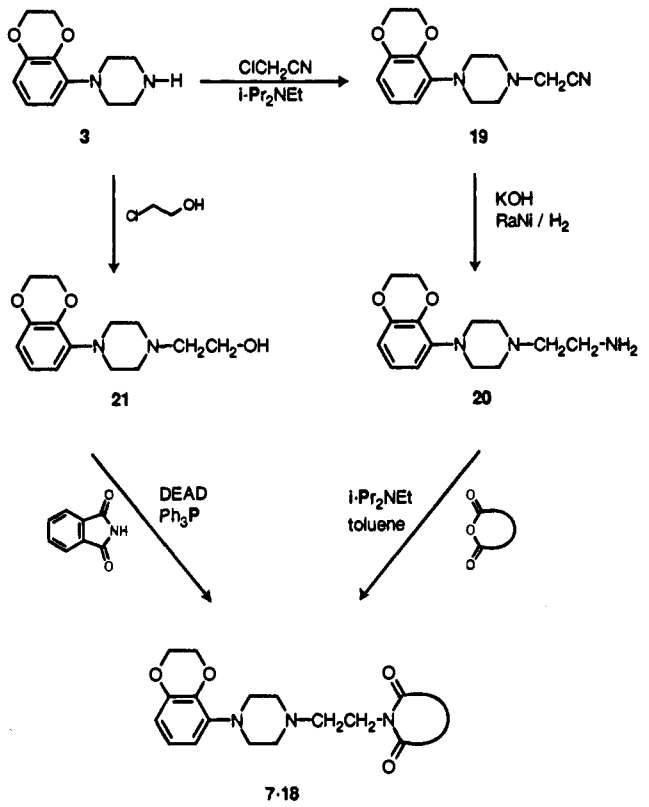
Scheme 1. General Method for the Synthesis of Compounds **7**–**13** and **15**–**18**[a]



[a] Compound 14 was synthesized from the N4-hydroxyethyl-substituted eltoprazine and phthalimide in a Mitsunobu reaction; see the Experimental Section.

Table 1. Physicochemical Properties[a] and Synthesis Methods of Compounds **7**–**18**

compd	formula	yield[b] (%)	mp (°C)
7	$C_{18}H_{23}N_3O_4$	59	142–4
8	$C_{19}H_{25}N_3O_4 \cdot 1.05H_2O$	61	120–2
9	$C_{22}H_{29}N_3O_4 \cdot 2.00HCl$	65	222–4
10	$C_{22}H_{27}N_3O_4 \cdot 2.00HCl$	70	224–6
11	$C_{18}H_{23}N_3O_4 \cdot 0.40H_2O$	80	127–9
12	$C_{19}H_{23}N_3O_4 \cdot 2.00HCl$	39	207–10
13	$C_{22}H_{27}N_3O_4 \cdot 2.30HCl$	67	245–7
14	$C_{22}H_{23}N_3O_4 \cdot C_4H_4O_4$	63[c]	189–91
15	$C_{23}H_{25}N_3O_4 \cdot 0.50H_2O$	67	156–8
16	$C_{19}H_{25}N_3O_4$	16	132
17	$C_{21}H_{29}N_3O_4 \cdot 2.00HCl$	70	218–20
18	$C_{23}H_{31}N_3O_4 \cdot 1.00C_7H_8S_1O_3$	47	184–5

[a] All C, H, N analyses are within 0.4% of the theoretical value, except for compound **8**; H: calcd, 7.22; found, 6.70. [b] All compounds were synthesized from 4-(2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazineethanamine, trihydrochloride (**20**) and the appropriate anhydride in toluene or dioxane using diisopropylethylamine. [c] Compound 14 was synthesized by the Mitsunobu reaction of **21** and phthalimide; see the Experimental Section.

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

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

$$K_i = IC_{50}/(1 + L^*/K_d^*)$$

where IC_{50} is the concentration of the drug necessary to displace 50% of the radioligand [^{3}H]-8-OH-DPAT from its specific binding site on the 5-HT_{1A} receptor in rat frontal cortex homogenates at 37 °C and pH 7.7, L^* is the concentration of the radioligand used, and K_d^* represents the dissociation constant of the radioligand. The results are expressed as pK_i values (−log K_i, nM).

Ionization constants were measured by potentiometric titration[12] in water at 37 °C. The molar fraction (M) of the protonated species (M) was calculated from the equation derived from the titration equilibrium:

$$M = 1/(1 + 10^{pH-pK_a})$$

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 inhibition constant K_i^+ by

$$K_i^+ = K_i M$$

clogP was calculated using the DAYLIGHT software.[13]

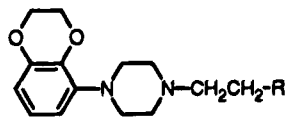
Results and Discussion

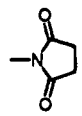
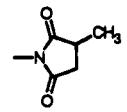
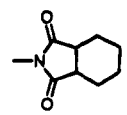
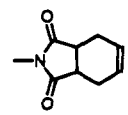
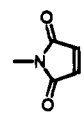
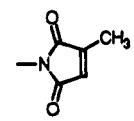
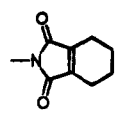
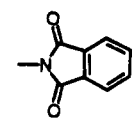
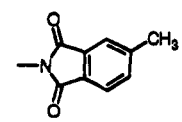
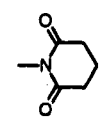
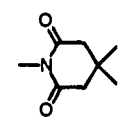
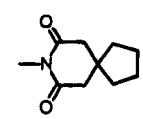
The results of the in vitro binding studies in rat frontal cortex homogenates of the target compounds **7**–**18** are summarized in Table 2. Succinimidyl compound **7** has only a moderate affinity for the 5-HT_{1A} receptor (pK_i = 6.96). Methylation of the succinimidyl ring results in the racemic derivative **8** (pK_i = 6.82). Enlargement of the methylsuccinimidyl moiety of **8** to the *cis*-hexahydrophthalimide derivative **9** enhances affinity 40 times. Introduction of a double bond located at positions 5–6 slightly decreases the affinity (compound **10**, pK_i = 8.00). The unsaturated maleic analog (**11**) of the saturated parent compound **7** shows a 9-fold decrease in affinity (pK_i = 6.01 vs 6.96). Methylation of the maleic ring results in a considerable enhancement of the affinity (**12**, pK_i = 7.20). The affinity is even further enhanced by the enlargement of the methylmaleic group to a 4,5,6,7-tetrahydrophthalimide moiety (**13**, pK_i = 8.19). Aromatization of the tetrahydrophthalimide ring to a phthalimide ring (compound **14**) however leads to a 5-fold decrease in affinity. This loss in affinity can be completely recovered by methylation of the phthalimide ring in the 5-position as in compound **15**. Ring expansion of the five-membered succinimide ring in **7** to the six-membered glutarimide ring in **16** seems unfavorable for affinity. However 4,4-dimethylation of the glutarimide ring of **16** enhances the affinity from pK_i = 6.46 to pK_i = 8.33 (**17**, Table 2). Conversion of the dimethylglutarimide ring of **17** to the azaspirodecanedione analog **18** enhances the affinity even further to pK_i = 9.15.

It has been demonstrated in other series of arylpiperazines that the presence of a basic nitrogen atom is essential for affinity. For example, Mokrosz et al.[14] have synthesized N4-substituted (*m*-chlorophenyl)piperazines (*m*-CPP) in which the basicity of the N4-nitrogen was strongly modified. In contrast to the N4-alkyl-substituted compounds, the corresponding N4-acyl derivatives only showed a very low affinity for the 5-HT_{1A} receptor.

As within our homologous N4-substituted benzodioxanylpiperazine series the affinity for the 5-HT_{1A} receptor strongly varies (pK_i = 6.01–9.15) and the structures resemble the *m*-CPP derivatives investigated by Mokrosz et al. with respect to the phenylpiperazine moiety, we investigated the possible relation of the basicity of the N4-nitrogen atom and the inhibition constant pK_i.

Table 2. Displacement of [^{3}H]-2-(Di-*n*-propylamino)-8-hydroxytetralin: Binding to 5-HT$_{1A}$ Recognition Sites in Rat Frontal Cortex Homogenates of Compounds **7**–**18**



R	no.	pK$_i$[a]	±	SEM
	**7**	6.96	±	0.08
	**8**	6.82	±	0.06
	**9**	8.42	±	0.09
	**10**	8.00	±	0.10
	**11**	6.01	±	0.08
	**12**	7.20	±	0.04
	**13**	8.19	±	0.02
	**14**	7.49	±	0.05
	**15**	8.32	±	0.08
	**16**	6.46	±	0.09
	**17**	8.33	±	0.07
	**18**	9.15	±	0.05

[a] pK$_i$ values are based on three to six assays using four to six concentrations in triplicate. For the reference compounds, the following values were found. Buspirone (**1**), pK$_i$ = 7.83; ipsapirone (**2**), pK$_i$ = 8.26; eltoprazine (**3**), pK$_i$ = 7.40; and flesinoxan (**6**), pK$_i$ = 8.77.

Table 3. Ionization Constants (pK_a), Molar Fraction Protonated (*M*) at 37 °C and pH 7.7, Local Affinity Constants (pK_i^+), and Calculated log *P* Values of Compouneds **7**–**18**

compd	pK_a	M	pK_i^+	clogP
7	6.16	0.03	8.51	2.38
8	6.16	0.03	8.38	2.91
9	6.07	0.02	9.64	3.82
10	6.05	0.02	9.85	3.33
11	5.90	0.02	7.82	2.41
12	6.30	0.04	9.84	2.93
13	6.30	0.04	8.62	3.84
14	nd			3.68
15	6.10	0.02	9.93	4.18
16	6.40	0.05	7.78	2.95
17	6.42	0.05	9.63	3.98
18	6.51	0.06	9.15	4.37

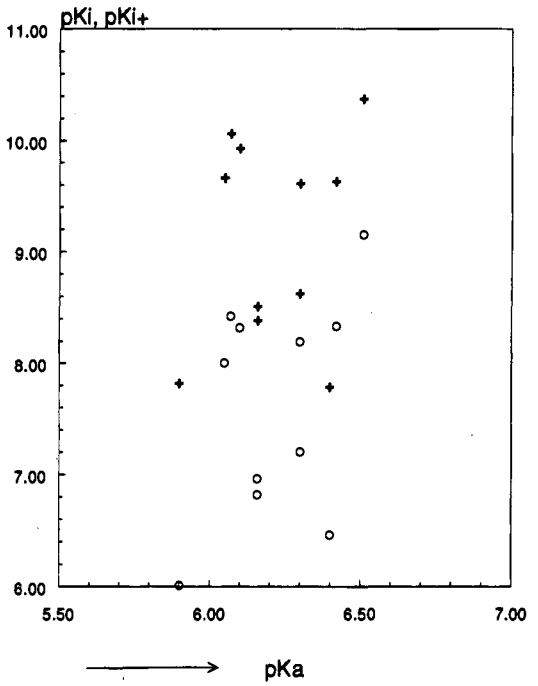


Figure 1. Relation between the inhibition constant (pK_i, ○), the local inhibition constant (pK_i^+, +), and the ionization constant (pK_a) determined in water at 37 °C for compounds **7**–**13** and **15**–**18**.

The ionization constants (pK_a) of the compounds are summarized in Table 3. The imidoethyl-substituted phenylpiperazines **7**–**18** have pK_a values in the range of 5.90–6.51. As could be expected, the pK_a is only slightly influenced by the different substituents in this series. These small differences in the pK_a values result in a small differentiation of the molar fraction of the protonated species M at 37 °C and pH 7.7. It can be concluded from the pK_a values that the imidoethyl derivatives are only slightly protonated under these conditions (2–6%). The local inhibition constant pK_i^+ values calculated from *M* and pK_i are summarized in Table 3. The relation between the pK_a value and the inhibition constant (pK_i) and the local inhibition constant (pK_i^+) is illustrated in Figure 1. These data show that for a given pK_a value the affinity ranges over 2–3 decades. Although the pK_i^+ values are in a somewhat more narrow range in comparison with the K_i values (7.78–10.37 vs 6.01–9.15 nM), it appears that there is no correlation between the pK_a and pK_i^+ or pK_i. Therefore pK_i^+ seems not to be a more convenient parameter than pK_i itself for the description of the specific structure–affinity relationships found.

The lack of a dynamic range in the pK_a values might lead to the conclusion that the basicity of the N4-nitrogen center is not an important factor during the process of interaction between the synthesized ligands and the 5-HT_{1A} receptor. As there is a general consensus that basicity is an essential factor in the binding of basic ligands to the 5-HT_{1A} receptor, this conclusion does not seem justified. It seems more appropriate to conclude that the data only show that the synthesized compounds all have a comparable basicity but on the other hand do not have a comparable affinity for the 5-HT_{1A} receptor. Furthermore, the possibility that the potentiometric titration in water at 37 °C is not a good measure of the pK_a values can also not be ruled out. It is generally accepted that during the binding process changes in the solvatation of ligands during the formation of the ligand–receptor complex take place. These changes in the solvatation will normally result in a reduction of the number of hydration molecules. Therefore, a determination of the pK_a value in water not necessarily has to result in representative value for the local pK_a values during the binding process. If the local pK_a values were known, a more straightforward relationship between local pK_a and pK_i or pK_i^+ could possibly emerge.

However it is obvious that within the narrow range of pK_a's in our series basicity per se is not a factor contributing to the differences in pK_i of the compounds under investigation, and so it seems likely that physicochemical properties other than pK_a values may have a considerable effect on affinity of the compounds for the 5-HT_{1A} receptor. Hence it seems plausible to investigate the possibility of a correlation between the affinity of the compounds and their lipophilicity. We therefore calculated the log P values (clogP) of compounds **7**–**18**. The clogP values are summarized in Table 3 and range from 2.38 to 4.37. The unsubstituted succinic, maleic, and glutaric imides **7**, **11**, and **16** have clogP values of 2.38, 2.41, and 2.95, respectively. The values increase significantly by substitution at or enlargement of these cyclic ring systems by substituents of a more hydrophobic nature as, e.g., in **9** (clogP = 3.82), **13** (clogP = 3.84), and **18** (clogP = 4.37). The enhancement of the lipophilicity also results in an increase in affinity for the 5-HT_{1A} receptor (Table 2). Figure 2 shows the relationship between the pK_i values and the clogP values. The equation pK_i = 3.32 + 1.26clogP (n = 12, r^2 = 0.83) obtained by regression analysis describes this relationship and indicates that the suboptimal affinity of **7**, **11**, and **16** for the 5-HT_{1A} receptor can be well compensated for by additional hydrophobic interactions. Furthermore, for the homologous series of compounds investigated, the pK_i values are predominantly driven by the hydrophobic character of the N4-substituents. Within the present series of compounds, the conclusions derived from the data are of a more general nature, and it is not possible to address specific regions of the receptor, not even when the racemic **8** would be separated into its enantiomers. This is best illustrated by Figure 3. When the symmetry in the N4-imidoethyl fragment in compound **9** is taken into account, it becomes clear that no differentiation can be made with respect to the absolute orientation of the imidoethyl fragments. Due to this symmetry, the orientation represented by **9** is energetically equal to

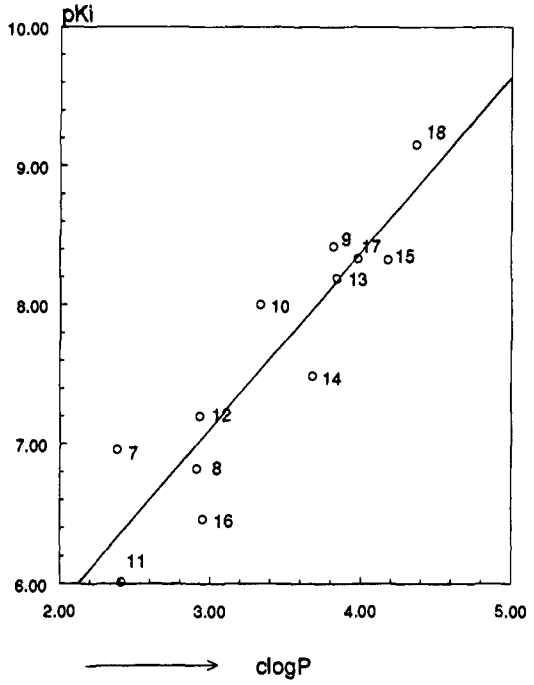


Figure 2. Relation between the inhibition constant (pK_i) and the calculated log P (clogP) for the imide derivatives **7**–**18**.

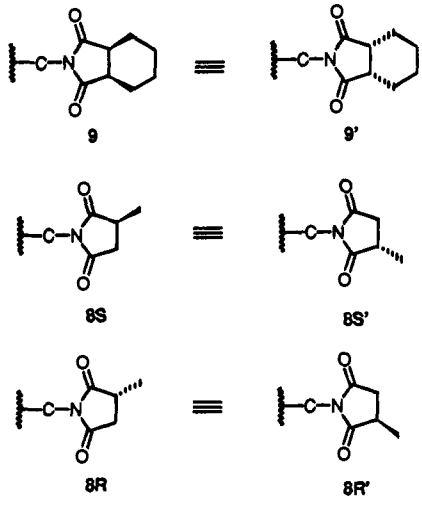


Figure 3. Comparison of the rotamers of compound **9** with the rotamers of the enantiomers **8R** and **8S** of compound **8**.

the orientation represented by **9'**. When the enantiomers **8S** and **8R** are considered and compared with the rotamers **9** and **9'**, it is obvious that each of the orientations illustrated by **8S** and **8R** can be superimposed on **9** and **9'**, respectively. The same can be applied for the rotamers of **8S** and **8R** (represented by **8S'** and **8R'**), which can be superimposed on **9'** and **9**, respectively.

More rigid derivatives with respect to the hydrocarbon ethyl chain are needed in order to investigate this structural mode of interaction in depth.

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 analyses were performed at the TNO laboratory of Organic Chemistry, Utrecht, The Netherlands, or Mikroanalytisches Labor Pascher, Remagen-Bandorf, Germany, and are within 0.4% of the theoretical values unless

stated otherwise. Thin-layer chromatography (TLC) was run on Merck silica gel 60 F-254 plates. For column chromatography, Merck silica gel type 60 (size 70–230 and 230–400 mesh, respectively) was used. Starting materials were used as high-grade commercial products. All reactions were performed under a nitrogen atmosphere. Compounds **7**–**13** and **15**–**18** were synthesized and purified according to the general procedure as described hereafter for compound **7** unless stated otherwise.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-2,5-pyrrolidinedione (7). A solution of 4-(2,3-dihydro-1,4-benzodioxin-5-yl)-1-piperazineethanamine, trihydrochloride (**20**; 3.00 g, 8.1 mmol), diisopropylethylamine (DIPEA; 4.65 mL, 26.7 mmol), and succinic anhydride (0.96 g, 9.72 mmol) in dioxane (50 mL) was heated at reflux for 16 h. After being cooled to 20 °C and concentrated in vacuo, the residue was taken up in EtOAc (100 mL) and washed with H_2O (25 mL). The organic layer was dried (Na_2SO_4) and concentrated. The crude **7** was purified by column chromatography (EtOAc–MeOH, 9:1). The obtained oil crystallized on standing: yield 1.65 g (59%); mp 142–4 °C; ^{1}H NMR ($CDCl_3$) δ 2.61 (t, 2 H, NCH_2CH_2succ, J = 6), 2.69 (m, 4 H, CH_2 pip), 2.72 (s, 4 H, succinimide), 3.03 (m, 4 H, CH_2 pip), 3.69 (t, 2 H, NCH_2CH_2-succ, J = 6), 4.24, 4.32 (m, 2 H, Bzd H-2,3), 6.52 (dd, 1 H, Bzd H-6, J = 8, 1), 6.58 (dd, 1 H, Bzd H-8, J = 8, 1), 6.77 (t, 1 H, Bzd H-7, J = 8). Anal. ($C_{18}H_{23}N_3O_4$) C,H,N.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-3-methyl-2,5-pyrrolidinedione (8). A solution of the piperazineethanamine **20** and methylsuccinic anhydride in toluene was heated at reflux for 16 h. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 97:3) and crystallized by treatment with diisopropyl ether: yield 61%; mp 120–2 °C; ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 3 H, $CHCH_3$, J = 7), 2.32 (m, 1 H, imid H-4), 2.55–2.76 (cluster, 6 H, CH_2 pip, NCH_2CH_2), 2.81–3.14 (cluster, 6 H, CH_2 pip, imid H-3,4), 3.67 (m, 2 H, $CH_2CH_2N(C{=}O)_2$), 4.23 (m, 2 H, Bzd H-2,3), 4.31 (m, 2 H, Bzd H-2,3), 6.52 (dd, 1 H, Bzd H-6, J = 8, 1), 6.59 (dd, 1 H, Bzd H-8, J = 8, 1), 6.77 (t, 1 H, Bzd H-7, J = 8). Anal. ($C_{19}H_{25}N_3O_4 \cdot 1.05H_2O$) C,H,N.

2-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]hexahydro-1*H*-isoindole-1,3(2*H*)-dione, Dihydrochloride (9). Compound **9** was prepared from *cis*-hexahydrophthalic anhydride and the piperazineethanamine **20**. The free base was converted to its dihydrochloride salt: yield 65%; mp 222–4 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 1.28–1.48 (cluster, 4 H, imid H-5,6), 1.58–1.82 (cluster, 4 H, imid H-4,7), 3.06–3.30 (cluster, 6 H, imid H-3a,7a, H_{ax} CH_2 pip), 3.37 (q, 2 H, $^+NHCH_2CH_2$, J = 6), 3.51 (m, 2 H, H_{eq} CH_2 pip), 3.66 (m, 2 H, H_{eq} CH_2 pip), 3.82 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 6), 4.25 (m, 4 H, Bzd H-2,3), 6.49 (dd, 1 H, Bzd H-6, J = 8, 1), 6.56 (dd, 1 H, Bzd H-8, J = 8, 1), 6.74 (t, 1 H, Bzd H-7, J = 8), 11.15 (br, 1 H, $^+$NH). Anal. ($C_{22}H_{29}N_3O_4 \cdot 2.00HCl$) C,H,N.

2-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-3a,4,7,7a-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione, Dihydrochloride (10). Compound **10** was prepared using *cis*-1,2,3,6-tetrahydrophthalic anhydride and the piperazineethanamine **20**. The free base was converted to its dihydrochloride salt: yield 70%; mp 224–6 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 2.21 (m, 2 H), 2.44 (m, 2 H), 3.12–3.43 (cluster, 8 H, H_{ax} CH_2 pip, $^+NHCH_2CH_2$, imid H-3a,7a), 3.50 (m, 2 H, H_{eq} CH_2 pip), 3.64 (m, 2 H, H_{eq} CH_2 pip), 3.81 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 6), 4.25 (m, 4 H, Bzd H-2,3), 5.86 (m, 2 H, imid H-5,6), 6.49 (dd, 1 H, Bzd H-6, J = 8, 1), 6.56 (dd, 1 H, Bzd H-8, J = 8, 1), 6.74 (t, 1 H, Bzd H-7, J = 8), 11.35 (br, 1 H, $^+$NH). Anal. ($C_{22}H_{27}N_3O_4 \cdot 2.00HCl$) C,H,N.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-1*H*-pyrrole-2,5-dione (11). Compound **11** was prepared from the piperazineethanamine **20** and maleic anhydride: yield 80%; mp 127–9 °C; ^{1}H NMR ($CDCl_3$) δ 2.61 (t, 2 H, NCH_2CH_2, J = 7), 2.68 (m, 4 H, CH_2 pip), 3.02 (br, 4 H, CH_2 pip), 3.69 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 7), 4.23 (m, 2 H, Bzd H-2,3), 4.32 (m, 2 H, Bzd H-2,3), 6.52 (dd, 1 H, Bzd H-6, J = 8, 1), 6.58 (dd, 1 H, Bzd H-8, J = 8, 1), 6.70 (s, 2 H, imid H-3,4), 6.76 (t, 1 H, Bzd H-7, J = 8). Anal. ($C_{18}H_{23}N_3O_4 \cdot 0.40H_2O$) C,H,N.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-3-methyl-1*H*-pyrrole-2,5-dione, Dihydrochloride (12). Compound **12** was prepared using the piperazineethanamine **20** and citraconic anhydride. Purification was accomplished by column chromatography (petroleum ether (60–80 °C)–acetone, 3:2). The free base was converted to its dihydrochloride salt: yield 39%; mp 207–10 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 2.03 (d, 3 H, CH_3, J = 2), 3.12 (m, 2 H, H_{ax} CH_2 pip), 3.24 (m, 2 H, $H_{ax}CH_2$ pip), 3.38 (m, 2 H, $^+NHCH_2$-CH_2), 3.52 (m, 2 H, H_{eq} CH_2 pip), 3.66 (m, 2 H, H_{eq} CH_2 pip), 3.87 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 6), 4.25 (m, 4 H, Bzd H-2,3), 6.50 (dd, 1 H, Bzd H-6, J = 8, 1), 6.56 (dd, 1 H, Bzd H-8, J = 8, 1), 6.63 (q, 1 H, imid H-4, J = 2), 6.74 (t, 1 H, Bzd H-7, J = 8), 11.0 (br, 1 H, $^+$NH). Anal. ($C_{19}H_{23}N_3O_4 \cdot 2.00HCl$) C,H,N.

2-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-4,5,6,7-tetrahydro-1*H*-isoindole-1,3(2*H*)-dione, Dihydrochloride (13). Compound **13** was prepared using 3,4,5,6-tetrahydrophthalic anhydride and the piperazineethanamine **20**. The free base was converted to its dihydrochloride salt: yield 67%; mp 245–7 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 1.72 (m, 4 H, imid H-5,6), 2.27 (m, 4 H, imid H-4,7), 3.13 (m, 2 H, H_{ax} CH_2 pip), 3.24 (m, 2 H, H_{ax} CH_2 pip), 3.36 (br, 2 H, $^+NHCH_2CH_2$), 3.51 (m, 2 H, H_{eq} CH_2 pip), 3.65 (m, 2 H, H_{eq} CH_2 pip), 3.66 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 6), 4.24 (m, 4 H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, J = 8, 1), 6.56 (dd, 1 H, Bzd H-8, J = 8, 1), 6.74 (t, 1 H, Bzd H-7, J = 8), 11.0 (br, 1 H, $^+$NH). Anal. ($C_{22}H_{27}N_3O_4 \cdot 2.30HCl$) C,H,N.

2-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-1*H*-isoindole-1,3(2*H*)-dione, (*E*)-2-Butenedioate (14). To a solution of triphenylphosphine (3.93 g, 15.0 mmol) in dry THF (30 mL) was added dropwise DEAD (2.56 mL, 15.0 mmol). After 15 min of stirring at 20 °C, a solution of **21** (2.00 g, 7.6 mmol) and phthalimide (1.15 g, 7.8 mmol) in THF (30 mL) was added dropwise. Stirring was continued for 16 h. After evaporation of the solvent, the residue was purified by column chromatography (methyl *tert*-butyl ether). The obtained red oil was converted into its fumarate. The salt was recrystallized from EtOH: yield 2.46 g (63%); mp 189–91 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 2.58–2.72 (cluster, 6 H, CH_2 pip, NCH_2-CH_2), 2.93 (m, 4 H, CH_2 pip), 3.77 (t, 2 H, NCH_2CH_2phth, J = 6), 4.22 (m, 4 H, Bzd H-2,3), 6.41 (dd, 1 H, Bzd H-6, J = 8, 1), 6.47 (dd, 1 H, Bzd H-8, J = 8, 1), 6.60 (s, 2 H, fumaric acid), 6.68 (t, 1 H, Bzd H-7, J = 8), 7.80–7.90 (cluster, 4 H). Anal. ($C_{22}H_{23}N_3O_4 \cdot C_4H_4O_4$) C,H,N.

2-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-5-methyl-1*H*-isoindole-1,3(2*H*)-dione (15). Compound **15** was prepared by using 4-methylphthalic anhydride and the piperazineethanamine **20**. Purification was accomplished by stirring with 2-propanol/diisopropyl ether (1:1): yield 1.5 g (67%); mp 156–8 °C; ^{1}H NMR ($CDCl_3$) δ 2.51 (s, 3 H, $ArCH_3$), 2.65–2.76 (cluster, 6 H, NCH_2CH_2, CH_2 pip), 3.02 (br, 4 H, CH_2 pip), 3.84 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 7), 4.23 (m, 2 H, Bzd H-2,3), 4.31 (m, 2 H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, J = 8, 1), 6.57 (dd, 1 H, Bzd H-8, J = 8, 1), 6.75 (t, 1 H, Bzd H-7, J = 8), 7.50 (m, 1 H, imid H-6), 7.65 (m, 1 H, imid H-4), 7.72 (d, 1 H, imid H-7, J = 8). Anal. ($C_{23}H_{25}N_3O_4 \cdot 0.50H_2O$) C,H,N.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-2,6-piperidinedione (16). Compound **16** was prepared by using the piperazineethanamine **20** and glutaric anhydride. The crude product was purified by column chromatography (CH_2Cl_2–MeOH, 97:3) and crystallized by treatment with diisopropyl ether: yield 0.31 g (16%); mp 132 °C; ^{1}H NMR ($CDCl_3$) δ 1.95 (m, 2 H, imid H-4), 2.55 (t, 2 H, NCH_2-CH_2, J = 7), 2.66 (t, 4 H, imid H-3,5, J = 6), 2.71 (br, 4 H, CH_2 pip), 3.05 (br, 4 H, CH_2 pip), 3.97 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 7), 4.24 (m, 2 H, Bzd H-2,3), 4.32 (m, 2 H, Bzd H-2,3), 6.53 (dd, 1 H, Bzd H-6, J = 8, 1), 6.58 (dd, 1 H, Bzd H-8, J = 8, 1), 6.77 (t, 1 H, Bzd H-7, J = 8). Anal. ($C_{19}H_{25}N_3O_4$) C,H,N.

1-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-4,4-dimethyl-2,6-piperidinedione, Dihydrochloride (17). Compound **17** was prepared from 4,4-dimethylglutaric anhydride and the piperazineethanamine **20**. The free base was converted to its dihydrochloride salt: yield 70%; mp 218–20 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 1.03 (s, 6 H), 2.65 (s, 4 H, imid H-3,5), 3.15 (m, 2 H, H_{ax} CH_2 pip), 3.21 (m, 2 H, H_{ax} CH_2 pip), 3.31 (q, 2 H, $^+NHCH_2CH_2$, J = 6), 3.51 (m, 2 H,

H_{eq} CH_2 pip), 3.68 (m, 2 H, H_{eq} CH_2 pip), 4.09 (t, 2 H, $CH_2CH_2N(C{=}O)_2$, J = 6), 4.25 (m, 4 H, Bzd H-2,3), 6.49 (dd, 1 H, Bzd H-6, J = 8, 1), 6.56 (dd, 1 H, Bzd H-8, J = 8, 1), 6.74 (t, 1 H, Bzd H-7, J = 8), 10.85 (br, 1 H, $^+$NH). Anal. ($C_{21}H_{29}N_3O_4 \cdot 2.00HCl$) C,H,N.

8-[2-[4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazinyl]-ethyl]-8-azaspiro[4.5]decane-7,9-dione, *p*-Toluenesulfonate (18). Compound **18** was prepared from **20** (3.00 g, 8.1 mmol) and 3,3-tetramethyleneglutaric anhydride (1.63 g, 9.72 mmol) in dioxane. The obtained crude oil was purified by column chromatography (EtOAc). The free base was converted to its *p*-toluenesulfonate. The salt was recrystallized from EtOH–petroleum ether (60–80 °C): yield 2.25 g (47%); mp 184–5 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (m, 4 H, cyclopentyl), 1.65 (m, 4 h, cyclopentyl), 2.35 (s, 3 H, CH_3phenyl), 2.80 (s, 4 H, $CH_2(C{=}O)N(C{=}O)CH_2$), 3.02–3.51 (cluster, 8 H), 4.09 (m, 2 H, H_{eq} CH_2 pip), 4.20 (m, 2 H, $NCH_2CH_2N(C{=}O)_2$), 4.24 (m, 2 H, Bzd H-2,3), 4.29 (m, 2 H, Bzd H-2,3), 6.43 (br, 1 H), 6.67 (d, 1 H, Bzd H-8, J = 8), 6.76 (t, 1 H, Bzd H-7, J = 8), 7.17 (m, 2 H, arom H *p*-toluenesulfonate), 7.74 (m, 2 H, arom H *p*-toluenesulfonate). Anal. ($C_{23}H_{31}N_3O_4 \cdot 1.00C_7H_8S_1O_3$) C,H,N.

1-(Cyanomethyl)-4-(2,3-dihydro-1,4-benzodioxin-5-yl)-piperazine (19). To a solution of the free base of eltoprazine (**3**; 26.2 g, 120 mmol), NaI (0.1 g, catalytic), and DIPEA (25.1 mL, 144 mmol) in DMF (200 mL) was added dropwise chloroacetonitrile (9.11 mL, 144 mmol) in DMF (30 mL). After being heated at reflux for 2 h, the solvent was evaporated and the residue was taken up in EtOAc (250 mL) and washed with H_2O (2 × 50 mL). The organic layer was dried (Na_2SO_4) and concentrated: yield 24.0 g (78%); brown oil; ^{1}H NMR ($CDCl_3$) δ 2.78 (m, 4 H, CH_2 pip), 3.12 (m, 4 H, CH_2 pip), 3.56 (s, 2 H, NCH_2CN), 4.21–4.34 (m, 4 H, Bzd H-2,3), 6.51 (dd, 1 H, Bzd H-6, J = 8, 1), 6.60 (dd, 1 H, Bzd H-8, J = 8, 1), 6.77 (t, 1 H, Bzd H-7, J = 8).

4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazine-ethanamine, Trihydrochloride (20). To a solution of **19** (24.0 g, 93.0 mmol) in EtOH (600 mL) was added KOH (2.60 g, 46.0 mmol) in H_2O (10 mL) and Ra Ni catalyst (0.25 g). Hydrogenation at 20 °C and 1 atm was complete after 24 h. The catalyst was filtered off over Hyflo, and the filtrate was concentrated in vacuo. The obtained oil was taken up in EtOAc (100 mL) and washed with H_2O (25 mL). The organic layer was dried (Na_2SO_4) and concentrated, giving the free base of **20**, which was obtained pure after flash chromatography (THF–MeOH–NH_4OH, 68:30:2). The oil was converted to its trihydrochloride salt: yield 19.4 g (56%); mp 218–20 °C; ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 3.10–3.63 (cluster, 10 H), 3.71 (m, 2 H, H_{eq} CH_2 pip), 4.26 (m, 4 H, Bzd H-2,3), 6.53 (dd, 1 H, Bzd H-6, J = 8, 1), 6.58 (dd, 1 H, Bzd H-8, J = 8, 1), 6.76 (t, 1 H, Bzd H-7, J = 8), 8.65 (br, 3 H, $^+NH_3$), 11.5 (br, 1 H, $^+$NH).

4-(2,3-Dihydro-1,4-benzodioxin-5-yl)-1-piperazine-ethanol (21). A solution of **3** (10.0 g, 39.0 mmol), DIPEA (16.8 mL, 97.0 mmol), NaI (0.1 g, catalytic), and 2-chloroethanol (4.10 mL, 61.0 mmol) in dry CH_3CN (100 mL) was heated at reflux for 16 h. After being concentrated in vacuo, the residue was taken up in EtOAc (200 mL) and washed with H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated: yield 8.0 g (78%); ^{1}H NMR (DMSO–$CDCl_3$, 4:1) δ 2.45 (t, 2 H, NCH_2CH_2OH, J = 6), 2.56 (br, 4 H, CH_2 pip), 2.98 (br, 4 H, CH_2 pip), 3.54 (q, 2 H, NCH_2CH_2OH, J = 6), 4.21 (m, 4 H, Bzd H-2,3), 4.36 (t, 1 H, CH_2CH_2OH, J = 6), 6.44 (dd, 1 H, Bzd H-6, J = 8, 1), 6.48 (dd, 1 H, Bzd H-8, J =8, 1), 6.69 (t, 1 H, Bzd H-7, J = 8).

Ionization Constant Determination. pK_a's were determined by use of a calibrated (37 °C, buffers pH 4, 7, and 9; Merck) glass pH electrode with an internal reference electrode (Metrohm 6.0203.100) by potentiometric titration[12] using an automatic titrator (Metrohm Titroprocessor E670). The titration was performed with 0.05 M sodium hydroxide (of low carbonate content) by constant volume addition of 0.05 mL at 37 °C which was controlled at ±1 °C under a nitrogen atmosphere. Substance (30 mg) was dissolved in 40 mL of 0.1 M potassium chloride in carbonate free water, giving a concentration of 0.002–0.005 M. From several points of the titration curve, a pK_a value was calculated according to $pK_a = pH + \log[(V_{eq} - V_d)/V_d]$, were V_{eq} is the volume titrant used at the equivalence point and V_d is the volume titrant used at the data point, and the results were averaged. In the case of precipitation during titration, only data points obtained before the start of precipitation were used. The standard deviation of repeated determinations was estimated 0.02. The standard deviation of selected data points of each pK_a determination (5–15 data points collected from the titration curve) was 0.01.

Biochemistry: Receptor Binding Assay. The binding assay was carried out as described.[15] Thus, the radioligand-binding studies were performed on rat frontal cortex homogenates using [^{3}H]-2-(di-*n*-propylamino)-8-hydroxytetralin as radioligand at 37 °C and pH 7.7.

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