Bridged γ-Carbolines and Derivatives Possessing Selective and Combined Affinity for 5-HT_2 and D_2 Receptors

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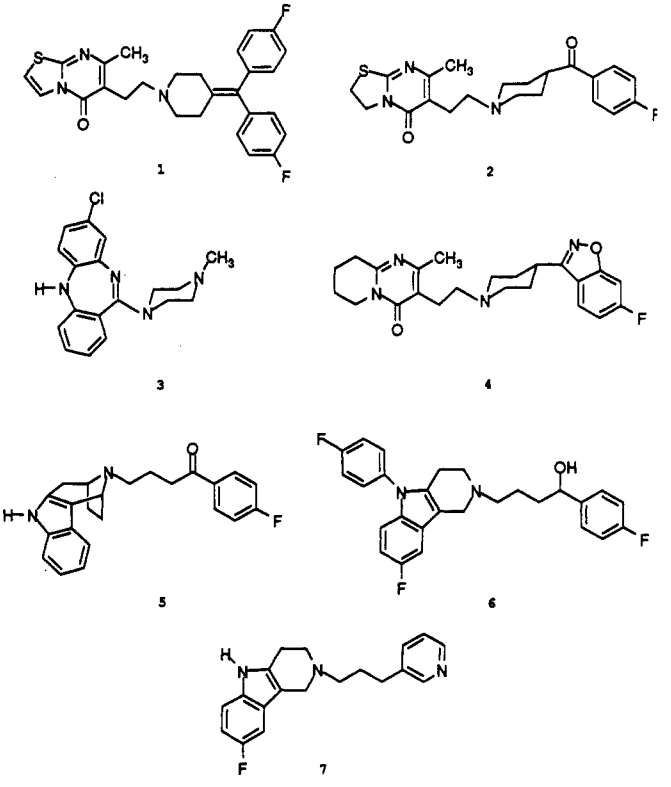
A series of 5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indoles and 6,7,8,9,10,11-hexahydro-7,-11-imino-5*H*-cyclooct[*b*]indoles was prepared. Structural modifications of the lead compound, 11-[4-(4-fluorobenzoyl)propyl]-5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indole (**5**, K_i = 0.82 nM vs [^{3}H]ketanserin) enabled the identification of the functionality necessary for high affinity at serotonin 5-HT_2 and dopamine D_2 receptors in ligand binding studies. The indole ring, as well as the benzoyl or isosteric benzisoxazole moiety, were essential for high affinity. Variations of the length of the side chains resulted in ligands having either selective affinity for the 5-HT_2 receptor or a combination of 5-HT_2 and D_2 affinity. In vivo binding studies were performed on selected members in this series. The most potent member, 2-fluoro-11-[4-(4-fluorobenzoyl)butyl]-5,6,7,8,9,-10-hexahydro-7,10-iminocyclohept[*b*]indole (**36**) had an ED_{50} of <1 mg/kg at the 5-HT_2 and D_2 receptors following oral administration.

Introduction

For the past 3 decades, the use of antipsychotic agents has reduced the number of patients chronically hospitalized in mental institutions and has shifted psychiatric intellectual foundations to a more neurobiological basis.[1] Nevertheless, the discovery of improved therapy for schizophrenia continues to be a major impetus for pharmacological research. Although the positive symptoms (delusions, hallucinations, thought disorders) of schizophrenia can be treated with classical neuroleptic agents, such as chlorpromazine, haloperidol, and fluphenazine, these drugs are less efficacious in attenuating negative symptoms (social withdrawal, apathy, flattened affect), and are also associated with side effects, including involuntary movement disorders or extrapyramidal side effects (EPS). As a consequence, there exists a need to identify side effect free antipsychotic agents which can influence the negative symptoms of schizophrenia.

Several strategies have emerged in search of a more versatile, side effect free therapy. One hypothesis is based on the correlation between clinical improvement of the negative symptoms of schizophrenia with 5-HT_2 receptor blockade.[2] Ritanserin (**1**)[3,4] and setoperone (**2**)[5] (Chart I), potent 5-HT_2 receptor antagonists, have been reported to attenuate some of the negative symptoms of schizophrenia. Antagonism of the 5-HT_2 receptor has also been inferred as a mechanism for reducing EPS.[6] Thus, atypical antipsychotics may produce their psychotherapeutic effects with a lower potential for neurological side effects by interacting with 5-HT_2 receptors. Another approach, which has received considerable attention, has focused on modulation of neural transmission via a combination of antiserotonergic and antidopaminergic activity.[7] The ability of clozapine (**3**) to antagonize 5-HT_2 and to a lesser extent D_2 receptors has been suggested as a major basis for its success in minimizing both positive and negative symptoms without producing significant EPS.[8,9] Another putative atypical antipsychotic, risperidone (**4**), which also possesses potent 5-HT_2 and D_2 antagonist properties, has been reported to ameloriate negative symptoms of schizophrenia.[10,11] Therefore, the right balance of 5-HT_2 and D_2 antagonism may be important for the successful therapeutic treatment of schizophrenia.

Chart I



Recently, we reported the structure–activity relationships (SAR) of a series of bridged γ-carbolines derivatives with respect to the σ receptor.[12] Through further evaluation of this series for potential activity in the central nervous system, we discovered one member, **5**, to possess a high affinity for the 5-HT_2 receptor (K_i = 0.82 nM) and modest affinity for the D_2 receptor (K_i = 275 nM). This information provided a lead structure and the impetus to

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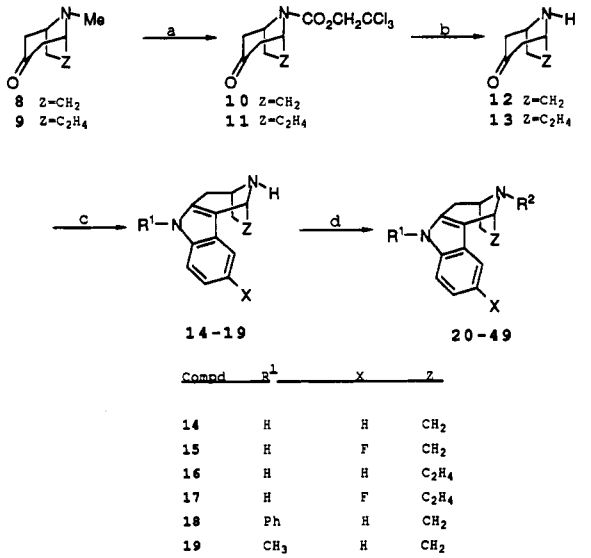
Scheme I [a]



Compd	R^1	X	Z
14	H	H	CH_2
15	H	F	CH_2
16	H	H	C_2H_4
17	H	F	C_2H_4
18	Ph	H	CH_2
19	CH_3	H	CH_2

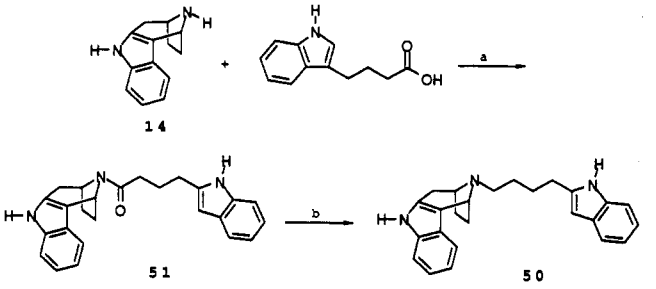
[a] Reagents and conditions: (a) $ClCO_2CH_2CCl_3$, K_2CO_3; (b) Zn, AcOH; (c) Fischer indole; (d) method A: RX, K_2CO_3, DMF, (KI).

search for novel potential atypical antipsychotics which possess both serotonergic and dopaminergic antagonistic properties. In order to discern the important interactions necessary for binding at the 5-HT_2 receptor, it was important to identify the key pharmacophoric elements of **5**. Perusal of the literature revealed the γ-carboline nucleus to be a structural unit in several biologically active compounds, many of which demonstrate potential antipsychotic activity.[13] In fact, the clinically active neuroleptic flutroline (**6**)[14,15] has a very similar structure to that of **5**, the most obvious differences between **5** and **6** being the semirigid γ-carboline skeleton, the N-aryl substituted indole nucleus, and the reduced butyrophenone moiety. More recently, the γ-carboline gevetroline (**7**) demonstrated preclinical neuroleptic activity and low EPS liability.[16,17] The conformational constraints, induced by the ethylene bridge of **5**, allowed the further assessment of the importance of the aromatic ring-basic nitrogen distance and the nitrogen out-of-plane distance. The present work describes the structural modifications and structure–activity relationships of a series of bridged conformationally constrained γ-carbolines with focus on the in vitro and in vivo binding affinity at the 5-HT_2 and D_2 receptors.

Chemistry

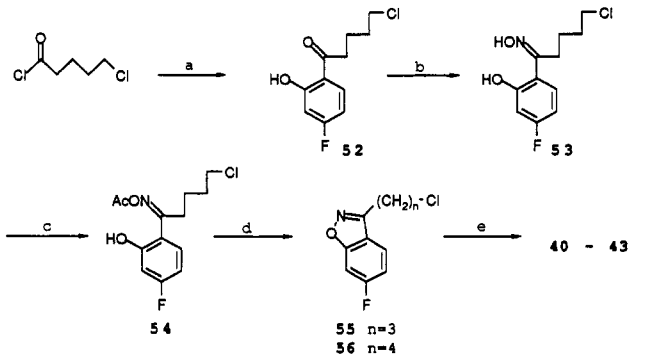
Bridged γ-carbolines **20–49** were prepared as outlined in Scheme I. The synthesis of the key intermediates **14**, **15**, and **18** was recently described beginning with commercially available tropinone.[12] Similarly, intermediate **19** was prepared by reacting 1-methyl-1-phenylhydrazine with nortropinone (**12**). Intermediates **16** and **17** were prepared via readily available pseudopelletierine (**9**).[18] Demethylation of **9** was performed using the procedure of Montzka et al.[19] Application of Fischer indole methodology allowed for the final construction of the desired γ-carboline intermediates **16** and **17**.[12,20] Alkylation of intermediates **14–19** was achieved by reaction with the appropriately substituted alkyl bromides or chlorides (in the presence of KI) in dimethylformamide as described in the Experimental Section (method A). Coupling of **14** with 3-indolebutyric acid gave **51**, which was reduced with

Scheme II[a]



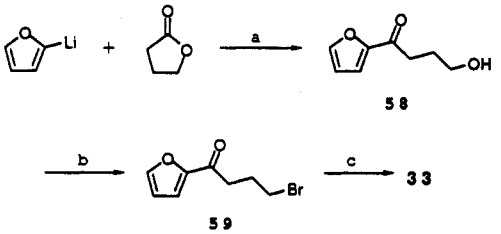
[a] Reagents and conditions: (a) EDC, CH_2Cl_2; (b) LAH, Δ.

Scheme III[a]



[a] Reagents and conditions: (a) 3-fluorophenol, BF_3·Et_2O; (b) NH_2OH·HCl, pyr.; (c) Ac_2O, Δ; (d) K_2CO_3, DMF; (e) method A.

Scheme IV[a]



[a] Reagents and conditions: (a) THF, −78 °C; (b) PPh_3, CBr_4, CH_2Cl_2; (c) **15**, method A.

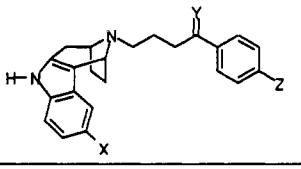
lithium aluminum hydride to give **50** (Scheme II, method B). Treatment of γ-carbolines **15** and **17** (method A) with the previously reported benzisoxazole alkylating agent **55**[21] afforded benzisoxazoles **40** and **42**. The homologous benzisoxazole side chain **56** was prepared in a similar fashion; it was reacted with **15** and **17** to afford **41** and **43**, respectively (Scheme III, Table VI). Metalation of furan[28] with *n*-butyllithium, followed by reaction with excess valerolactone at −78 °C, afforded the known alcohol **58**.[23] Conversion to the corresponding bromide, **59**, followed by reaction with **15** gave the desired furan **33** (Scheme IV).

Results and Discussion

Affinity of all target compounds for 5-HT_2 and D_2 receptors was determined by their ability to displace [^{3}H]-ketanserin and [^{3}H]sulpiride, respectively (Tables I–VIII). The in vitro and in vivo radioligand binding methods are described in detail in the Experimental Section.

As depicted in Table I, modification of the original lead γ-carboline, **5**, at the three different positions shown led to analogues with decreased affinity for 5-HT_2 and D_2 receptors. A 27-fold decrease in affinity at the 5-HT_2 receptor was observed when the carbonyl functionality was removed (**5** vs **23**). The importance of the fluorine atom in **5** was manifested from its comparison to **22**,

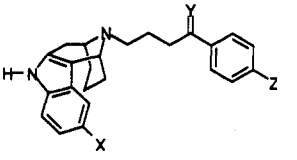
Table I. Structure-Affinity-Relationships of **5**



compd[a]	X	Y	Z	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
5[e]	H	O	F	0.82 ± 0.14	275 ± 75
20	F	O	F	1.77 ± 0.19	231 ± 38
21[e]	H	H_2	H	56.9 ± 12.5	735 ± 13
22	H	O	H	6.55 ± 0.35	540 ± 76
23[e]	H	H_2	F	22.1 ± 4.3	1160 ± 8
24[e]	F	H_2	H	76.6 ± 3.8	769 ± 208
ritanserin				0.82 ± 0.11	93 ± 21
haloperidol				26.5 ± 2.5	0.47 ± 0.05

[a] All compounds were prepared in racemic form. [b] The K_i values for Tables I-VII were generated as described in the Experimental Section. Values represent the mean of two to five separate determinations ±SEM. [c] Versus [^{3}H]ketanserin. [d] Versus [^{3}H]sulpiride. [e] Previously prepared in ref 12.

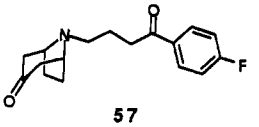
Table II. Binding Affinity Data of Propylene-Bridged Analogues **25–30**



compd[a]	X	Y	Z	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
25	H	O	F	0.85 ± 0.29	38 ± 6
26	F	O	F	2.76 ± 0.55	200 ± 47
27	H	H_2	H	103 ± 22	670 ± 40
28	H	H_2	F	24.5 ± 2.0	973 ± 62
29	F	H_2	H	172 ± 55	1872 ± 181
30	F	H_2	F	143 ± 31	1965 ± 50

[a–d] See footnotes of Table I.

revealing an 8-fold decrease in affinity at the 5-HT$_2$ receptor. Complete removal of the butyrophenone moiety (e.g. **14**) resulted in virtually complete loss of affinity at the 5-HT$_2$ (K_i = 12.6 μM) and D$_2$ receptors (K_i > 10 μM). Another dramatic effect was observed when the indole ring of **5** (e.g. **57**) was removed, resulting in a 650-fold loss



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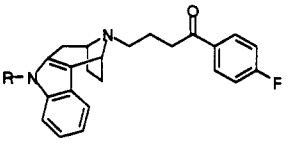
in affinity for the 5-HT$_2$ receptor (K_i = 533 nM) and low affinity for the D$_2$ receptor (K_i = 1.3 μM). These results indicate the importance of both the indole and benzoyl functionalities to achieve high affinity for both 5-HT$_2$ and D$_2$ receptors. The propylene-bridged analogue **25** had similar affinity for the 5-HT$_2$ receptor to that of **5** (Table II). However, a 7-fold increase in affinity was observed for the D$_2$ receptor, suggesting that the D$_2$ receptor is more sensitive to this structural change as compared to the 5-HT$_2$ receptor. Similar to that observed for **5**, removal of the carbonyl in **25** (e.g. **28**, Table II) resulted in an approximate 30-fold decrease in affinity at the 5-HT$_2$ receptor. Substitution of a methyl or phenyl group onto the indole nitrogen of **5** (e.g. **31** and **32**, Table III) resulted in a 3- and 400-fold decrease in affinity, respectively. Two examples of heteroaromatic ring replacements for the

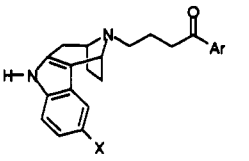
Table III. Binding Affinity Data of *N*-Substituted Indole Derivatives of **5**



compd[a]	R	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
5	H	0.82 ± 0.14	275 ± 75
31	CH_3	2.83 ± 0.10	287 ± 155
32	Ph	330 ± 106	840 ± 8

[a–d] See footnotes of Table I.

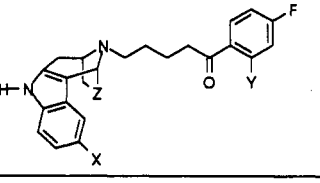
Table IV. Binding Affinity Data for Ethylene Bridged γ-Carbolines **22**, **33**, and **34**



compd[a]	Ar	X	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
22	Ph	H	6.55 + 0.35	540 ± 76
33		F	264 ± 28	643 ± 161
34		H	27.2 ± 9.2	550 ± 13

[a–d] See footnotes of Table I.

Table V. Binding Affinity Data for Chain-Extended Homologues of **5** and **25**

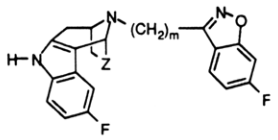


compd[a]	X	Y	Z	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
35	H	H	CH_2	2.81 ± 0.94	4.43 ± 0.85
36	F	H	CH_2	2.98 ± 0.70	2.77 ± 1.04
37	H	H	C_2H_4	20.9 ± 2.0	43.5 ± 1.5
38	F	H	C_2H_4	59.8 ± 24	72.0 ± 9.0
39	F	F	CH_2	15.7 ± 6.2	34.1 ± 22.9

[a–d] See footnotes of Table I.

phenyl ring of **22** demonstrate the importance of this functionality to achieve high affinity for the 5-HT$_2$ receptor. As indicated in Table IV, incorporation of a furan or thiophene ring in place of a phenyl ring led to a loss in affinity at the 5-HT$_2$ receptor. Elaboration of the chain length of the butyrophenone to the valerophenone moiety achieved a remarkable increase in affinity at the D$_2$ receptor (Table V). A 62-fold increase in affinity (**35** vs **5**) at the D$_2$ receptor was observed at the expense of only a 3-fold decrease in affinity at the 5-HT$_2$ receptor, resulting in a 212-fold decrease in receptor selectivity. This is somewhat surprising since the butyrophenone moiety has been accepted as an optimized side chain for the D$_2$ receptor.[24] Nevertheless, again this suggests that the D$_2$ receptor is more sensitive than the 5-HT$_2$ receptor to structural changes. In contrast, the semirigid 5-aryltetrahydro-γ-carbolines, reported by Harbert,[15] were observed to lose activity when the valerophenone moiety

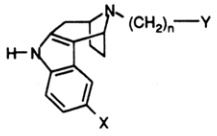
Table VI. Binding Affinity Data of Benzisoxazole Derivatives **40–43**

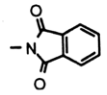
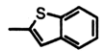
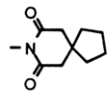


compd[a]	Z	m	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
40	CH_2	3	8.54 ± 1.85	308 ± 28
41	CH_2	4	7.98 ± 1.87	2.70 ± 0.7
42	C_2H_4	3	13.4 ± 2.4	248 ± 11
43	C_2H_4	4	133 ± 102	131 ± 57

[a–d] See footnotes of Table I.

Table VII. Binding Affinity Data for Compounds **44–50**



compd[a]	X	Y	n	K_i (nM)[b] 5-HT$_2$ receptor[c]	K_i (nM)[b] D$_2$ receptor[d]
44	F		4	1245 ± 135	1006 ± 94
45	H		4	879 ± 98	2630
46	H		4	284 ± 6	635 ± 58
47	H		4	610 ± 190	416 ± 6
48	H		4	2255 ± 1534	3007 ± 912
49	H		3	213 ± 65	1155 ● 125
50	H		4	329 ± 11	853 ± 198

[a–d] See footnotes of Table I.

was attached to the γ-carboline nucleus. Interestingly, the propylene-bridged derivatives **37** and **38** were 7- and 20-fold less potent than their ethylene-bridged analogues, **35** and **36**, respectively. The benzisoxazole bioisosteres, **40–43** (Table VI), resulted in compounds having similar affinity for 5-HT$_2$ and D$_2$ receptors with respect to their benzoyl analogues (e.g. **20** vs **40**; **26** vs **42**; **36** vs **41**; **38** vs **43**). As indicated in Table VII, side chains lacking the benzoyl moiety or benzisoxazole ring resulted in a significant loss in affinity at both 5-HT$_2$ and D$_2$ receptors.

Assessment of selected compounds for in vivo potency with respect to interactions with 5-HT$_2$ and D$_2$ receptors in rat brain following oral administration is shown in Table VIII. In general, the ED$_{50}$ values obtained from in vivo binding experiments correlated well with K_i values obtained from in vitro binding methods (compare results on Table VIII with Tables I, II, and V). Interestingly, though

Table VIII. *In Vivo* Binding Data of Selected Compounds

compd	ED$_{50}$ (mg/kg, po) 5-HT$_2$ receptors inhibition of [^{3}H]ketanserin[a]	ED$_{50}$ (mg/kg, po) D$_2$ receptors inhibition of [^{3}H]raclopride[a]
5	20.9 (14.9–29.1)	NT
20	41.1 (30.0–56.3)	NT
22	12% inhibition @ 25 mg/kg	NT
25	16.3 (9.1–29.0)	NT
26	5.89 (3.12–11.06)	>15
35	7.32 (2.08–25.8)	6.55 (2.83–15.2)
36	0.65 (0.25–1.68)	0.92 (0.35–2.45)
ritanserin	0.14 (0.08–0.27)	>15
haloperidol	>10	0.036 (0.017–0.076)

[a] The ED$_{50}$ values were determined as described in detail in the Experimental Section. Values in parentheses represent 95% confidence limits.

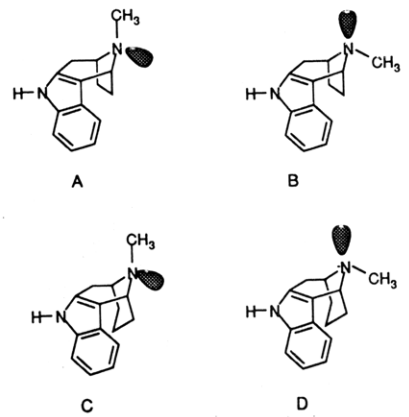
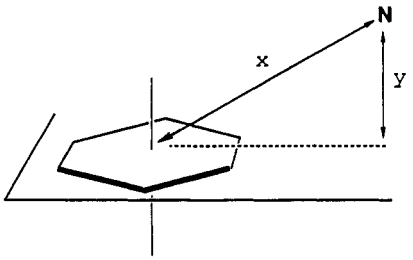


Figure 1. Invertomers of ethylene- and propylene-bridged γ-carbolines.

addition of a fluorine atom into the indole ring had little effect on in vitro affinity for 5-HT$_2$ and D$_2$ receptors (**35** vs **36**), in vivo binding studies revealed a 11-fold increase in potency following oral administration.

As indicated in Tables I and II, ligands which possess the butyrophenone side chain appear to be optimized with respect to the 5-HT$_2$ receptor, irrespective of the alkyl bridge incorporated into the γ-carboline skeleton, whereas, ligands which possess the valerophenone side chain appear to be optimized with respect to the D$_2$ receptor (Tables V and VI), though still possessing high affinity for the 5-HT$_2$ receptor. In contrast, those valerophenone ligands which contain the propylene-bridged γ-carboline moiety exhibited significantly less affinity than the ethylene-bridged γ-carboline analogues for the D$_2$ receptor. In order to further understand the differences observed for the high affinity D$_2$ ligands **35**, **36**, and **41** with respect to the propylene-bridged analogues **37**, **38**, and **43**, molecular modeling calculations were performed. Two parameters commonly believed to be pertinent to the dopamine D$_2$ receptor interaction are the aromatic ring–basic nitrogen distance (X) and the nitrogen out-of-plane elevation (Y) relative to the aromatic ring. In order to simplify molecular mechanical calculations, the rigid γ-carboline nucleus was focused upon by replacing the common valerophenone side chain with a methyl group. Shown in Figure 1 are the invertomers (**A–D**) of the ethylene- and propylene-bridged γ-carbolines (the propylene-bridged γ-carbolines were assumed to be in the chair conformation). As indicated in Table IX, the average distances from the aromatic ring to the basic nitrogen (X_{AVG}) were very similar (<1% variance) between the ethylene and propylene series (X_{AVG}

Table IX. Structural Parameters Based on Molecular Mechanical Energy Calculations



structure	X (Å)	X_{AVG} (Å)	Y (Å)	Y_{AVG} (Å)	strain energy (kcal/mol)
A	5.19		0.85		21.49
		5.16		0.87	
B	5.12		0.89		21.98
C	5.22		0.73		6.58
		5.19		0.75	
D	5.16		0.76		7.65

Table X. Bridged γ-Carboline Derivatives

compd	formula[a]	mp, °C	recryst solvent	% yield[b]
20	$C_{23}H_{22}N_2F_2O \cdot C_2H_2O_4$[c]	126–128	acetone	65
21	$C_{23}H_{26}N_2 \cdot HCl$[c]	*f*	acetone	88
22	$C_{23}H_{24}N_2O \cdot C_2H_2O_4$	156–158	acetone	58
23	$C_{23}H_{25}N_2F \cdot C_2H_2O_4$	120–121	Et_2O	72
24	$C_{23}H_{25}N_2F \cdot C_2H_2O_4$	161–162	THF/EtOAc	67
25	$C_{26}H_{25}N_2FO \cdot C_2H_2O_4$[c]	104–108	EtOH/Et_2O	82
26	$C_{24}H_{24}N_2F_2O \cdot C_2H_2O_4$	120–124	EtOH/Et_2O	90
27	$C_{24}H_{28}N_2 \cdot C_2H_2O_4$[c]	92–95	EtOH/Et_2O	77
28	$C_{24}H_{27}N_2F \cdot C_2H_2O_4$	156–160	EtOH/Et_2O	96
29	$C_{24}H_{27}FN_2 \cdot C_2H_2O_4$	110–115	Et_2O	76
30	$C_{24}H_{26}N_2F_2 \cdot C_2H_2O_4$	175–176	EtOH/Et_2O	67
31	$C_{24}H_{25}N_2F \cdot C_2H_2O_4$	78–83	THF/Et_2O	72
32	$C_{29}H_{27}N_2OF \cdot C_2H_2O_4$	183–184	EtOAc	67
33	$C_{21}H_{21}N_2FO_2 \cdot C_2H_2O_4$[c]	144–146	THF	63
34	$C_{21}H_{22}N_2OS \cdot C_2H_2O_4$	165–167	acetone	49
35[g]	$C_{24}H_{25}N_2O \cdot C_2H_2O_4$	92–95	Et_2O	86
36[g]	$C_{24}H_{24}N_2F_2O \cdot C_2H_2O_4$[c]	175–177	EtOH/Et_2O	81
37[g]	$C_{25}H_{27}N_2FO \cdot C_2H_2O_4$[c]	78–81	EtOH/Et_2O	70
38[g]	$C_{25}H_{26}N_2F_2O \cdot C_2H_2O_4$	91–93	EtOH/Et_2O	76
39[g]	$C_{24}H_{23}N_2O \cdot C_2H_2O_4$[d]	68–74	THF/Et_2O	67
40	$C_{23}H_{21}N_2FO \cdot C_2H_2O_4$	119–127	THF	72
41	$C_{24}H_{23}N_3F_2 \cdot C_2H_2O_4$	169–173	THF	73
42	$C_{24}H_{23}N_3FO \cdot C_2H_2O_4$[c]	97–102	THF/Et_2O	80
43	$C_{25}H_{25}N_3F_2O \cdot C_2H_2O_4$	92–96	THF/Et_2O	69
44	$C_{27}H_{26}N_3FOS \cdot C_2H_2O_4$	115–122	THF	84
45	$C_{25}H_{25}N_3O \cdot C_2H_2O_4$	134–136	THF	90
46	$C_{25}H_{27}N_3 \cdot C_2H_2O_4$[e]	136–140	EtOAc	56
47	$C_{25}H_{26}N_2S \cdot C_2H_2O_4$	92–95	EtOH/Et_2O	88
48	$C_{26}H_{33}N_3O$[c]	149–152	EtOAc	64
49	$C_{24}H_{25}N_3 \cdot C_2H_2O_4$[c]	130–133	EtOAc	68
50	$C_{25}H_{27}N_3 \cdot C_2H_2O_4$	99–102	THF/Et_2O	82[h]
57	$C_{17}H_{20}NFO_2 \cdot C_2H_2O_4$	157–163	THF	66

[a] $C_2H_2O_4$ represents oxalic acid. All new compounds analyzed correctly (±0.4%) for C, H, N. [b] Yields refer to method A unless otherwise noted; see the Experimental Section. [c] 0.5-Hydrate. [d] 0.25-Hydrate. [e] 1.25-Hydrate. [f] Amorphous solid. [g] The alkylating agents, 5-bromo-1-(4′-fluorophenyl)-1-pentanone (**60**) and 5-chloro-1-(2′,4′-difluorophenyl)-1-pentanone (**61**) were synthesized as described in the Experimental Section. [h] See method B in the Experimental Section.

= 5.16 Å vs 5.19 Å, respectively). However, the difference observed in the nitrogen out-of-plane distances (Y_{AVG}) appears to be significant (0.87 Å vs 0.75 Å), indicating a 17% increase in the elevation of the nitrogen above the aromatic ring by replacing the propylene with a ethylene bridge. As a consequence of the large increase in steric strain generated by fusing a five-membered ring versus a six-membered ring onto the γ-carboline skeleton, a puckering effect of the nitrogen above the ring was observed. Interestingly, the distance Y_{AVG} for structures **A** and **B** (i.e. 0.87 Å) is in good agreement with the value of 0.9 Å published for the potent D_2 receptor antagonist, (+)-dexclamol.[25]

Summary

We have discovered a series of bridged γ-carbolines which are selective for the 5-HT_2 receptor and are capable of being structurally modified to possess a combination of 5-HT_2 and D_2 receptor affinity. The key pharmacaphores were identified through structural modifications. The γ-carboline nucleus and benzoyl moiety or benzisoxazole ring were all observed to be essential for high affinity at both 5-HT_2 and D_2 receptors. Selectivity for the 5-HT_2 receptor versus the D_2 receptor appears to be determined by a delicate balance between the nitrogen out-of-plane elevation and the distance from the basic nitrogen to the benzoyl or benzisoxazole moieties. The highest selectivity for the 5-HT_2 receptor was observed with the butyrophenone analogues **5** (D_2/5-HT_2 = 335) and **20** (D_2/5-HT_2 = 130). γ-Carbolines which possessed both the ethylene bridge and valerophenone side chain were observed to have high affinity at both the 5-HT_2 and D_2 receptors. Molecular modeling calculations suggest that the nitrogen out-of-plane distance may be the more critical parameter when comparing the ethylene- and propylene-bridged γ-carboline skeletons versus affinity at the D_2 receptor. The conformational restraints, imparted on the γ-carboline skeleton by the alkyl bridge, allowed further clarification for the structural requirements of the 5-HT_2 and D_2 receptors in order to design selective, high-affinity ligands of the 5-HT_2 and D_2 receptors. The resolution and preparation of selected members in this series are currently being carried out in order to assess enantioselectivity and will be reported shortly.

Experimental Section

Chemistry. Melting points were determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. ^1H NMR was recorded with a General Electric QE300 spectrometer or Bruker AC 400-MHz spectrometer using tetramethylsilane as an internal standard. ^{13}C spectra was recorded with a Bruker AC 400 MHz at a frequency of 100.627 MHz. IR spectra were obtained on a Beckman FT 1300 spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Inc. of Atlanta, GA. Thin-layer chromatography (TLC) was performed using coated fluorescent 0.25-mm silica gel plates (Merck, Kieselgel 60 F-254). Shown below is an example of each method described in Table IX.

2,2,2-Trichlorocarboxynorpseudopelletierine (11) was prepared according to the method of Montzka[19] using pseudopelletierine[18] to afford a crystalline solid: yield 76.3%: mp 95–96 °C (EtOAc/hexane). Anal. ($C_{11}H_{14}Cl_3NO_3$) C, H, N.

Norpseudopelletierine (13).[19] To a suspension of zinc (67.2 g, 1.03 g-atom) in glacial acetic acid (40 mL) at 65 °C was slowly added a solution of **11** (80.9 g, 0.257 mol) in glacial acetic acid (120 mL) over 1 h. An additional 40 mL of acetic acid was used to rinse the addition funnel. The temperature of the reaction mixture was maintained above 60 °C until gas evolution had ceased. The reaction was allowed to cool to ambient temperature followed by the addition of water (40 mL). The reaction mixture was filtered, and the zinc salts were washed with water (50 mL). The glacial acetic acid was removed under vacuum by rotoevaporator (60 °C). The resultant orange oil was slowly added to 80 mL of ammonium hydroxide at 5 °C using 80 mL of water to aid in the transfer. An additional 80 mL of ammonium hydroxide was added followed by the addition of 200 mL of 15% aqueous sodium hydroxide. The solution was extracted with methylene chloride (6 × 300 mL), dried over sodium sulfate, filtered, and

concentrated to an oil. The crude product was dissolved in 200 mL of ether to which 310 mL of 1 N HCl in ether was added. The precipitate was filtered and washed with ether (75 mL) and then dried in vacuo to afford a white crystalline solid: yield 38.7 g (86%): mp 216–219 °C (HCl, Et_2O); ^1H NMR (HCl, DMSO-d_6) δ 1.24–1.35 (1H, m), 1.57 (1H, d, J = 15.0 Hz), 1.74 (2H, d, J = 14.0 Hz), 1.96–2.03 (2H, m), 2.44 (2H, d, J = 17.2 Hz), 3.08 (2H, dd, J = 6.6, 17.2 Hz), 3.94 (2H, s), 9.82 (1H, bs), 10.12 (1H, bs); ^{13}C NMR (HCl, $CDCl_3$) δ 14.43, 27.30, 42.59, 47.89, 204.05./ Anal. ($C_8H_{10}NO$·HCl) C, H, N.

General Procedure for the Synthesis of 5,6,7,8,9,10-Hexahydro-7,10-iminocyclohept[*b*]indoles and 6,7,8,9,10,11-Hexahydro-7,11-imino-5*H*-cyclooct[*b*]indoles (14–19). Indoles **14**, **15**, and **18** were prepared as previously described.[12] Indoles **16**, **17**, and **19** were prepared accordingly. **16**: 68% yield; mp 68–72 °C (EtOH/Et_2O, free base); ^1H NMR (DMSO-d_6, free base) δ 1.24 (1H, m), 1.38 (1H, m), 1.61 (1H, m), 1.64 (1H, m), 1.92 (2H, m), 3.10–3.45 (4H, m), 3.83 (1H, s), 4.74 (1H, s), 6.95 (1H, t, J = 7.6 Hz), 7.03 (1H, t, J = 7.0 Hz), 7.31 (1H, t, J = 8.0 Hz), 7.40 (1H, d, J = 7.6 Hz). Anal. ($C_{14}H_{16}N_2$·$C_2H_2O_4$·0.5H_2O) C, H, N. **17**: 74% yield; mp 200–201 °C (free base, CH_2Cl_2); ^1H NMR (DMSO-d_6, free base) δ 1.18–1.22 (1H, m), 1.30 (1H, m), 1.46–1.55 (2H, m), 1.74–1.81 (2H, m), 2.47 (1H, d, J = 17 Hz), 3.06 (1H, dd, J = 7, 17 Hz), 3.42 (1H, m), 4.17 (1H, s), 6.77 (1H, app t, J = 8.5 Hz), 7.08 (1H, dd, J = 1.5, 10 Hz), 7.22 (1H, dd, J = 4.6, 8.6 Hz), 10.83 (1H, s). Anal. ($C_{14}H_{15}FN_2$) C, H, N. **19**: 47% yield; mp 100–104 °C (free base, $CHCl_3$); ^1H NMR ($CDCl_3$, free base) δ 1.53–1.60 (1H, m), 1.96–2.02 (2H, m), 2.05–2.19 (m, 2H), 2.49 (1H, dd, J = 1.0, 16.1 Hz), 3.11 (1H, dd, J = 4.5, 16.1 Hz), 3.54 (3H, s), 4.03–4.06 (1H, m), 4.54 (1H, d, J = 5.1 Hz), 7.07 (1H, dt, J = 1.0, 7.0 Hz), 7.13 (1H, dt, J = 1.3, 7.0 Hz), 7.23 (1H, d, J = 8.1 Hz), 7.48 (1H, d, J = 7.7 Hz); ^{13}C NMR ($CDCl_3$, free base) δ 29.12, 29.78, 33.17, 36.71, 51.93, 52.85, 108.81, 116.90, 117.37, 118.95, 120.41, 124.51, 131.99, 136.48. Anal. ($C_{14}H_{16}N_2$·$C_2H_2O_4$) C, H, N.

5-Chloro-4′-fluoro-2′-hydroxyvalerophenone (52).[21] To a mixture of boron trifluoride etherate (64 mL, 0.51 mol) at 0 °C was added 3-fluorophenol (18.0 g, 0.16 mol), and 5-chlorovaleryl chloride (50.0 g, 0.32 mol). The reaction mixture was stirred at 0 °C for 15 min and then heated at 130 °C for 20 h. The reaction was cooled to 0 °C and water (200 mL) was slowly added and stirred for 10 min. The organic layer was removed and the aqueous layer extracted with ethyl acetate (2 × 125 mL). The organic layers were combined and washed with water (2 × 200 mL) and brine (200 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed in vacuo to afford a dark oil. Filtration through a short column of silica afforded a red oil (49.9 g). This material was allowed to stand in a freezer for 6 days to afford a yellow solid. This material was washed with hexane and filtered to afford a crystalline pale yellow solid (5.9 g). The mother liquor was filtered to afford another 8.6 g of product: total yield, 14.5 g (39.3%); mp 45–47 °C; ^1H NMR ($CDCl_3$) δ 1.84–1.96 (4H, m), 3.00 (2H, t, J = 6.9 Hz), 3.60 (2H, t, J = 6.1 Hz), 6.60–6.64 (1H, m), 6.66 (1H, dd, J = 10.3, 2.5 Hz), 7.77 (1H, dd, J = 6.4, 9.0 Hz), 12.62 (1H, d, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$) δ 21.51, 31.86, 37.33, 44.56, 105.13 (d, J = 23.5 Hz), 107.24 (d, J = 22.8 Hz), 116.40 (d, J = 2.0 Hz), 132.18 (d, J = 12.1 Hz), 165.08 (d, J = 15.1 Hz), 167.36 (d, J = 256.6 Hz), 204.69 (C=O); IR ($CHCl_3$) 1642 cm^{-1}. Anal. ($C_{11}H_{12}ClFO_2$) C, H, N.

5-Chloro-4′-fluoro-2′-hydroxyvalerylphenone oxime (53).[21] A mixture of pyridine (13 mL), **52** (5.75 g, 0.025 mol), and hydroxylamine hydrochloride (1.89 g, 0.027 mol) was stirred at room temperature for 2 days. The reaction mixture was poured into 1 N aqueous HCl (50 mL), stirred for 5 min, and then extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water and brine, dried over anhydrous magnesium sulfate, filtered, and evaporated in vacuo to yield a yellow oil which crystallized overnight. The solid was washed with hexane to afford a pale yellow crystalline solid: yield, 4.81 g (78.6%); mp 88–90 °C; ^1H NMR ($CDCl_3$) δ 1.75–1.83 (2H, m), 1.88–1.95 (2H, m), 2.88 (2H, t, J = 7.8 Hz), 3.59 (2H, t, J = 6.4 Hz), 6.60–6.65 (1H, m), 6.68 (1H, dd, J = 2.6, 10.3 Hz), 7.38 (1H, dd, J = 6.4, 8.9 Hz), 7.41 (1H, s), 11.55 (1H, s); ^{13}C NMR ($CDCl_3$) δ 23.80, 23.96, 32.19, 44.43, 104.70 (d, J = 24.2 Hz), 106.73 (d, J = 22.1 Hz), 113.91 (d, J = 3.1 Hz), 128.90 (d, J = 10.8 Hz), 159.98 (d, J = 12.9 Hz), 162.24, 164.02 (d, J = 250.0 Hz). Anal. ($C_{11}H_{13}ClFNO_2$) C, H, N.

5-Chloro-4′-fluoro-2′-hydroxyvalerylphenone *O*-acetyloxime (54).[21] A mixture of acetic anhydride (3.0 mL) and **53** (4.7 g, 0.019 mol) was heated at 60 °C for 1.5 h. The reaction mixture was allowed to cool to room temperature and dissolved in ethyl acetate (50 mL). The organic mixture was washed with saturated aqueous sodium bicarbonate, water, and brine. The organic layer was dried over anhydrous magnesium sulfate and filtered, and the solvent was removed in vacuo to afford an orange oil. The crude material was purified by flash chromatography (ethyl acetate/hexane, 20 to 40%) to afford a yellow oil: yield, 4.43 g (84.5%); ^1H NMR ($CDCl_3$) δ 1.80–1.86 (2H, m), 1.88–1.95 (2H, m), 2.25 (3H, s), 2.91 (2H, t, J = 7.7 Hz), 3.60 (2H, t, J = 6.1 Hz), 6.63–6.68 (1H, m), 6.74 (1H, dd, J = 2.6, 10.4 Hz), 7.43 (1H, dd, J = 6.3, 9.0 Hz), 11.65 (1H, d, J = 1.7 Hz); ^{13}C NMR ($CDCl_3$) δ 19.22, 24.27, 25.73, 31.95, 44.05, 105.37 (d, J = 24.1 Hz), 107.01 (d, J = 22.5 Hz), 112.70, 129.90 (d, J = 11.0 Hz), 161.26 (d, J = 13.2 Hz), 165.10 (d, J = 251.4 Hz), 166.33, 166.61; IR ($CHCl_3$) 1782 cm^{-1}.

3-(4-Chlorobutyl)-6-fluoro-1,2-benzisoxazole (56).[21] A solution of **54** (4.32 g, 0.149 mol) and potassium carbonate (2.57 g, 0.0186 mol) in DMF (13 mL) was stirred for 5 h at room temperature. The reaction mixture was then poured into water (200 mL) and extracted with ethyl acetate (2 × 150 mL). The organic layer was washed with water (2 × 150 mL) and brine (150 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed in vacuo to afford a yellow oil: yield, 3.42 g (100%); ^1H NMR ($CDCl_3$) δ 1.88–1.96 (2H, m), 1.98–2.08 (2H, m), 3.01 (2H, t, J = 7.3 Hz), 3.60 (2H, t, J = 6.3 Hz), 7.08 (1H, dt, J = 2.1, 8.8 Hz), 7.25 (1H, dd, J = 2.0, 8.5 Hz), 7.61 (1H, dd, J = 5.1, 8.7 Hz); ^{13}C NMR ($CDCl_3$) δ 24.34, 24.71, 31.81, 44.38, 97.42 (d, J = 26.8 Hz), 112.57 (d, J = 25.6 Hz), 118.06, 122.13 (d, J = 13.0 Hz), 157.86, 163.62, 164.28 (d, J = 250.8 Hz); IR ($CHCl_3$) 3024, 2954, 2870, 2414, 1617, 1523, 1491, 1417, 1386 cm^{-1}.

1-(2-Furanyl)-4-hydroxy-1-butanone (58).[22,23] To a solution of furan (2.0 g, 29.4 mmol) in tetrahydrofuran (33 mL) at 0 °C was added *n*-butyllithium (32.3 mmol) and allowed to stir for 1 h. The solution was then cannulated into another flask containing γ-butyrolactone (5.06 g, 58.8 mmol) in tetrahydrofuran (15 mL) at −78 °C. The reaction mixture was stirred for 1 h and allowed to warm to room temperature over 30 min. 1N HCl (30 mL) was added, and the layers were partitioned. The aqueous layer was extracted with ethyl acetate (3 × 75 mL), and the organic layers were combined and washed with a brine solution. The organic layer was then dried over anhydrous magnesium sulfate and filtered, and the solvent was removed under vacuum to afford a red oil. The product was purified by flash chromatography (ethyl acetate/CH_2Cl_2, 1:1) to afford an orange oil: yield, 2.10 g (46%); ^1H NMR δ 1.90–2.03 (2H, m), 2.99 (2H, t, J = 7.0 Hz), 3.73 (2H, t, J = 6.0 Hz), 6.54 (1H, dd, J = 1.8, 3.6 Hz), 7.22 (1H, d, J = 3.4 Hz), 7.59 (1H, bs).

1-(2-Furanyl)-4-bromo-1-butanone (59). To a solution of **58** (672 mg, 4.40 mmol) and carbon tetrabromide (2.48 g, 7.48 mmol) in methylene chloride (15 mL) was added a solution of triphenylphosphine (1.96 g, 7.47 mmol) in methylene chloride (15 mL) over 10 min. The reaction mixture was allowed to stir for 3 h and then partitioned between methylene chloride (100 mL) and water (25 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered and the solvent removed under vacuum. Purification by chromatography (flash silica, ethyl acetate/hexane, 5% to 15%) afforded an oil: yield, 810 mg (85%); ^1H NMR δ 2.26–2.32 (2H, m), 3.05 (2H, t, J = 7.1 Hz), 3.53 (2H, t, J = 6.4 Hz), 6.55 (1H, dd, J = 1.6, 3.5 Hz), 7.23 (1H, dd, J = 0.7, 3.5 Hz), 7.60 (1H, t, J = 0.9 Hz); IR ($CHCl_3$) 3029, 2970, 2365, 2342, 1674, 1568, 1468, 1399, 1291, 1250, 1220, 1165, 1093, 1036, 1018, 880 cm^{-1}.

5-Bromo-1-(4′-fluorophenyl)-1-pentanone (60). To a solution of (4-fluorophenyl)magnesium bromide (0.09 mol, 1.13 eq) in dry ether (35 mL) cooled to 0 °C was added 5-bromovaleronitrile (10.0 g, 61.7 mmol) in ether (70 mL) over 10 min. The mixture was stirred at 0 °C for 15 min and then stirred at room temperature for 1 h. The reaction mixture was cooled to 0 °C and 1 N HCl (100 mL) was slowly added. The layers were separated, and the aqueous layer was washed with ethyl acetate (2 × 150 mL). The combined organic layers were washed with brine, dried over

anhydrous sodium sulfate, and filtered, and the solvent was removed under vacuum to afford a green oil. Purification by chromatography (flash silica, ethyl acetate/hexane, 1:20) gave a green solid: yield, 10.53 g (66%); mp 29–31 °C; ^{1}H NMR ($CDCl_3$) δ 1.86–2.00 (4H, m), 2.99 (2H, t, J = 7.0 Hz), 3.46 (2H, t, J = 6.4 Hz), 7.11–7.17 (2H, m), 7.96–8.02 (2H, m); IR ($CHCl_3$) 3021, 3016, 2957, 2399, 1684, 1599, 1504, 1223, 935, 841 cm^{-1}.

5-Chloro-1-(2',4'-difluorophenyl)-1-pentanone (61). To a suspension of magnesium turnings (1.56 g, 64 mmol) in dry ether (20 mL) under an atmosphere of nitrogen at 0 °C was slowly added a solution of 1-bromo-4-chlorobutane (10.0 g, 59.0 mmol) in ether (25 mL). After the addition was complete, the mixture was stirred at room temperature for 1 h. A solution of 2,4-difluorobenzonitrile (7.32 g, 53 mmol) in ether (25 mL) was added at 0 °C and stirred at room temperature for 3 h. The reaction mixture was acidified with 10% aqueous HCl to a pH of 2 and stirred for 1 h. The layers were separated, and the aqueous layer was washed with ethyl acetate (2 × 200 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed under vacuum to afford a brown oil. Purification by chromatography (flash silica, ethyl acetate/hexane, 1:20) gave a yellow oil (3.34 g) which after standing for 5 days afforded colorless crystals: yield, 1.80 g (15%); ^{1}H NMR ($CDCl_3$) δ 1.83–1.91 (4H, m), 2.97–3.01 (2H, m), 3.58 (2H, t, J = 6.2 Hz), 6.85–6.90 (1H, m), 6.97 (1H, dt, J = 2.4, 8.3 Hz), 7.93 (1H, dt, J = 6.6, 8.6 Hz); IR ($CHCl_3$) 3026, 2952, 2368, 2344, 1685, 1610, 1496, 1430, 1268, 1242, 1211, 1147, 1100, 974, 856 cm^{-1}.

General Procedure for N-Alkylation. 2-Fluoro-11-[3-(6-fluoro-1,2-benzisoxazol-3-yl)propyl]-5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indole (40). Method A. 3-Chloro-1-(6-fluoro-1,2-benzisoxazol-3-yl)propane[21] (649 mg, 3.0 mmol), 2-fluoro-5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indole (15) (537 mg, 2.5 mmol), potassium carbonate (866 mg, 6.3 mmol), and potassium iodide (150 mg) were combined in DMF (12 mL) and heated at 80 °C for 5 h. The reaction mixture was partitioned between ethyl acetate (100 mL) and water (30 mL). The organic layer was separated and washed with water (2 × 100 mL), dried over anhydrous magnesium sulfate, and filtered, and the solvent was removed under vacuum. Purification by flash chromatography ($MeOH/CH_2Cl_2$, 2% to 7%) afforded a light tan foam: yield, 708 mg (72%); mp 119–127 °C (oxalate, THF). Anal. ($C_{23}H_{21}N_3OF_2$).

11-[4-(3-Indolyl)butyl]-5,6,7,8,9,10-Hexahydro-7,10-iminocyclohept[*b*]indole (50). Method B. 5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indole (14) (750 mg, 3.78 mmol), 3-indolebutyric acid (846 mg, 4.16 mmol), and 1-[3-(dimethylamino)propyl]ethylcarbodiimide (798 mg, 4.16 mmol) were combined in anhydrous methylene chloride (30 mL) at room temperature and allowed to stir for 12 h. To the reaction mixture was added 1.5 N HCl (15 mL), and the layers were separated. The organic layer was washed with 1 N NaOH (20 mL), dried over anhydrous magnesium sulfate, and filtered and the solvent removed under vacuum. The crude product was quickly passed through a short column (flash silica, $MeOH/CH_2Cl_2$, 1:20) to afford 1.22 g (84%) of **51** as a foam. To a solution of **51** (1.0 g, 2.61 mmol) in anhydrous THF (15 mL) was added a 1 M solution of lithium aluminum hydride (13 mL) at room temperature. The reaction mixture was heated to reflux for 3 h and allowed to cool to room temperature. Water (450 μL) was cautiously added followed by 3 N NaOH (450 μL) and more water (1.5 mL). The solid precipitate was filtered and washed with warm THF (40 mL). The solvent was removed followed by purification by flash chromatography ($MeOH/CH_2Cl_2$, 1:20) to afford **50** as a white foam: yield, 790 mg (82%); mp 99–102 °C (oxalate, Et_2O/THF). Anal. ($C_{25}H_{27}N_3 \cdot C_2H_2O_4$).

Serotonin 5-HT$_2$ Assay. The potency of compounds to inhibit the specific binding of [^{3}H]ketanserin to serotonin 5-HT$_2$ receptors was determined as detailed previously.[26] Rat cortical synaptosomal membranes were prepared on the day of assay. Animals (male, Sprague–Dawley rats) were sacrificed by decapitation; the frontal cortex was removed and homogenized in 10 volumes (w/v) of ice-cold 0.32 M sucrose using a motor-driven Teflon pestle fitted to a glass tube. The synaptosomal pellet was suspended in 50 mM Tris HCl (pH 7.6 at 25 °C) to a concentration of 7.5 mg of original wet weight/mL. For the binding reaction, 400 μL of this tissue suspension was added to polypropylene tubes containing 50 mM Tris HCl and [^{3}H]ketanserin (final concentration of 1 nM) in a final assay volume of 0.5 mL. Following a 15-min incubation at 37 °C, the binding reaction was terminated by rapid vacuum filtration over Whatman GF/B filters, presoaked in a solution of 0.1% (w/v) of aqueous polyethyleneimine (PEI), followed by 3 × 4 mL washes with ice-cold 50 mM Tris HCl. Nonspecific binding was assessed in the presence of 10 μM methylsergide. All incubations were performed in triplicate and IC_{50} values were determined using log logit analysis utilizing 6–10 concentrations of test compound. The K_i values were determined using the Cheng–Prusoff equation,[27] $K_i = IC_{50}/(1 + L/K_D)$, where K_D = 2 nM.

Dopamine D$_2$ Assay. The affinity of compounds of interest for the dopamine D$_2$ receptor was determined by their ability to displace [^{3}H]sulpiride according to a previously reported method.[28] Briefly, on the day of the assay, rats were sacrificed by decapitation; the corpus striatum was dissected and homogenized in 20 volumes of 50 mM Tris HCl (pH 7.5, 25 °C). The homogenate was centrifuged (4 °C) and the resulting pellet was resuspended in 20 volumes of fresh buffer containing 100 mM NaCl to a tissue concentration of 3.75 mg of wet weight/mL. A portion of the suspension (800 μL) was added in triplicate to the tubes containing 3 nM [^{3}H]sulpiride and the test compound. The final assay volume was 1 mL; haloperidol (10 μM) was used to determine nonspecific binding. Following a 60-min incubation at 25 °C, the reactions were terminated by rapid filtration over Whatman GF/B filters which had been pretreated in a solution of 0.3% (w/v) of aqueous PEI. Filters were washed three times using 5 mL of cold buffer and processed using standard procedures to determine radioactivity. The IC_{50} values were obtained from concentration–response curves in which 7–10 concentrations of test compound were examined. The K_i values were determined using the Cheng–Prusoff equation.[27]

5-HT$_2$ in Vivo Binding. The potency of compounds to inhibit 5-HT$_2$ binding in vivo was determined in male CF-1 mice (25–30 g) injected (po) with varying doses of test compound, or with ketanserin (10 mg/kg; ip), in a volume of 0.01 mL/g body weight. Thirty minutes after ip injection, and 60 min after po administration, the animals were injected with 0.05 μCi/g of body weight of [^{3}H]ketanserin via the tail vein in a volume of 100 μL. Fifteen minutes later the animals were sacrificed by decapitation; the forebrain was removed and placed in a tube containing 200 volumes of ice-cold 50 mM Tris HCl (pH 7.4). The tissue was homogenized with a polytron for approximately 10 s at a setting of 5–6. A portion of the homogenate was placed into a scintillation vial containing 1.5 mL of Solvable tissue solubilizer and 15 mL of Formula 989 (Du Pont NEN). Another portion of the homogenate was rapidly filtered over Whatman GF/B filters. Filters containing membrane-bound radioactivity were washed twice with ice cold buffer and placed into scintillation vials containing 15 mL of scintillation cocktail. Radioactivity was quantified by liquid scintillation spectrometry.

For each animal tested, the percent membrane bound [^{3}H]-ketanserin was calculated by dividing the dpm bound to the filter by the total dpm in the tissue sample. For each experiment, specific binding was defined by the percent membrane bound for control animals minus the percent membrane bound for animals injected with 10 mg/kg ketanserin (nonspecific binding). At least four doses of a compound were evaluated. Inhibition of [^{3}H]ketanserin binding by test compounds was calculated as the ratio of specific binding in drug-treated mice relative to specific binding in vehicle-treated mice. The ED_{50} (with 95% confidence limits) was calculated by least squares linear regression after log transformation of dose.

D$_2$ in Vivo Binding. Striatal dopamine (D$_2$) binding was measured in vivo using [^{3}H]raclopride (specific act. = 84.3 Ci/mmol). Mice were administered haloperidol 30 min (ip) or test compound 60 min (po) prior to the injection (iv) of [^{3}H]raclopride (0.05 μCi/g of body weight). After 15 min the mice were sacrificed by decapitation; the brain was removed and the corpus striatum removed. Each striatum was solubilized in 1.5 mL of Solvable tissue solubilizer, after which radioactivity was quantified by liquid scintillation spectrometry. Nonspecific binding was determined in animals pretreated with haloperidol (2 mg/kg).

Inhibition of specific [^{3}H]raclopride binding and calculation of ED_{50} values were determined as for [^{3}H]ketanserin binding.

Computational Methods. The semiempirical calculations were performed on a Silicon Graphics 4D120 GTXB computer using the AM1 Hamiltonian in the program MOPAC,[29] version 5.0. The geometry of the structure was optimized with respect to all geometric variables, and the criteria for terminating the optimizations (electronic and geometric) were increased by a factor of 100 over the default settings.

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