Binding of Tetrahydrocarboline Derivatives at Human 5-HT$_{5A}$ Receptors

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On the basis of an earlier finding that 5-methyl-5*H*-1,2,3,4-tetrahydropyrido[4,3-*b*]indole (5-methyl-1,2,3,4-tetrahydro-γ-carboline; **1**) binds at murine 5-HT$_{5A}$ receptors, preliminary structure−affinity studies were conducted. The present investigation extends these structure−affinity studies using human 5-HT$_{5A}$ receptors and examined additional analogues of **1**. It was found (a) that there is little interspecies difference for the affinities of these compounds, (b) that an intact 1,2,3,4-tetrahydro-γ-carboline ring system seems optimal and an N^2-(3-(substituted-phenoxy)propyl) moiety results in high affinity, (c) that structurally related 1,2,3,4-tetrahydro-β-carbolines also bind at 5-HT$_{5A}$ receptors, and (d) that all examined derivatives also possess affinity for 5-HT$_{2A}$ receptors. Evidence is provided that 5-HT$_{5A}$ and 5-HT$_{2A}$ receptor affinities probably do not covary and that it might be possible, with continued investigation, to develop analogues with enhanced 5-HT$_{5A}$ selectivity.

Of the seven families of serotonin (5-hydroxytryptamine, 5-HT) receptors (5-HT$_1$−5-HT$_7$), 5-HT$_5$ receptors are perhaps the most poorly understood and the least well investigated and have been referred to as "orphan 5-HT receptors".[1−4] Because there is no direct evidence concerning the existence of functional native receptors, they are most correctly referred to as 5-ht$_5$ receptors.[2,4] Mouse 5-HT$_5$ receptors were first identified by Plassat et al.,[5] in 1992. Shortly thereafter, two murine subpopulations were identified: 5-HT$_{5A}$ and 5-HT$_{5B}$ receptors.[6] Since then, rat 5-HT$_{5A}$[7] and 5-HT$_{5B}$[8] and human 5-HT$_{5A}$[9] receptors have been cloned; human 5-HT$_{5B}$ receptors have not yet been identified. A human 5-HT$_{5B}$ gene has been isolated but apparently does not encode a functional protein because its coding sequence is interrupted by stop codons.[10] 5-HT$_{5A}$ receptors are found primarily in the forebrain, cerebellum, and spinal cord.[2,4] The deduced structure of 5-HT$_{5A}$ receptors is consistent with that of other transmembrane-spanning G-protein-coupled receptors, but the transduction mechanism associated with these receptors has not been unequivocally defined.[2,4] Indeed, Noda et al.,[11] have most recently reported that activation of human 5-HT$_{5A}$ receptors elicits multiple signaling mechanisms and that these receptors regulate neuronal function in a rather complex manner.

It has been speculated that mutations in the gene encoding 5-HT$_{5A}$ receptors might be detrimental to brain development,[6] and that genetic variation in human 5-HT$_{5A}$ receptors could be involved in susceptibility to psychosis or depression.[12,13] 5-HT$_{5A}$-receptor knock-out mice displayed increased locomotor activity and exploration, indicating their possible involvement in these behaviors.[14] There also has been speculation, primarily on the basis of receptor localization, that 5-HT$_{5A}$ receptors could be involved in certain other CNS disorders including Alzheimer's disease,[15] anxiety, and feeding behavior.[5,9]

Relatively little is known about the binding of various agents at 5-HT$_{5A}$ receptors, and no selective agents have been identified. Typical nonselective "serotonin agonists" that bind at human 5-HT$_{5A}$ receptors include 5-HT ($K_i \approx 125$ nM), 5-carboxamidotryptamine ($K_i \approx 20$ nM), and sumatriptan ($K_i \approx 5000$ nM) (see Chart 1 for chemical structures); typical nonselective "serotonin antagonists" that bind include metergoline ($K_i \approx 630$ nM), methysergide ($K_i \approx 100$ nM), and methiothepin (metitepine; $K_i \approx 1$ nM).[9] More information is available about the binding of various agents at rodent than human 5-HT$_{5A}$ receptors, but their pharmacologies, for the few agents examined, appear to be in reasonable agreement.[9] However, this is not always the case; methiothepin, for example, binds with 60-fold lower affinity at mouse versus human 5-HT$_{5A}$ receptors.[9]

Five years ago we undertook a structure−affinity investigation to better understand the binding requirements of various tryptamines at mouse 5-HT$_{5A}$ receptors.[16] Although several high-affinity tryptamines were identified—some with much higher affinity than 5-HT itself—none was selective for 5-HT$_{5A}$ receptors. More recently, we screened >100 nontryptamines in an attempt to identify a novel lead structure for subsequent investigation. One of the compounds to emerge from this screen was 5-methyl-5*H*-1,2,3,4-tetrahydropyrido[4,3-*b*]indole (5-methyl-1,2,3,4-tetrahydro-γ-carboline; **1**). Although the affinity of **1** (K_i = 5300 nM) at 5-HT$_{5A}$ receptors was modest, γ-carbolines typically display reduced affinity for most other populations of 5-HT receptors (with the exception of 5-HT$_2$ receptors), and it was felt that the tricyclic structure might be successfully exploited to develop a reasonably selective ligand.

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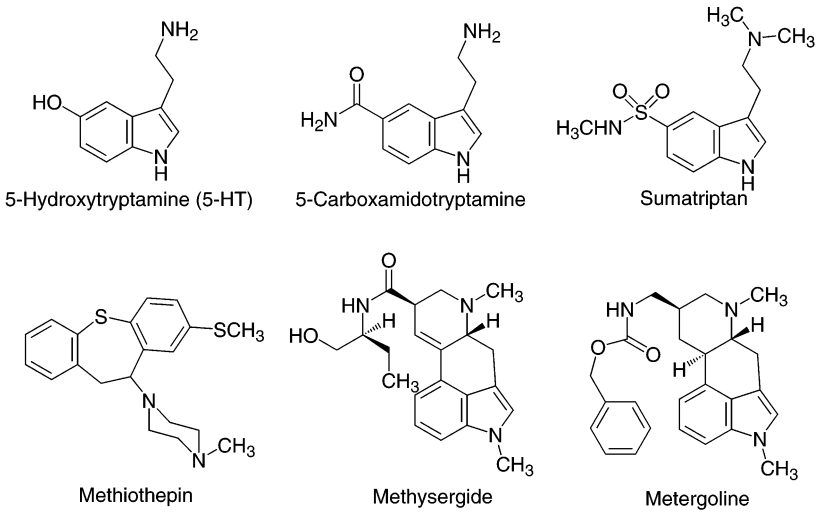
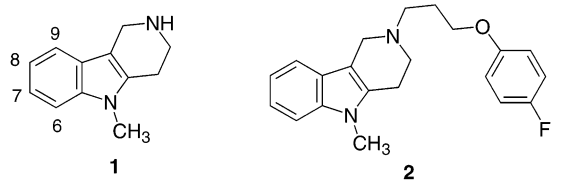
Chart 1. Structures of Some Standard Serotonergic Agents Described in the Text



Initial structure−affinity studies resulted in the identification of **2** (m5-HT$_{5A}$ K_i = 13 nM), a compound with 400-fold higher affinity than **1** at 5-HT$_{5A}$ receptors.[17]

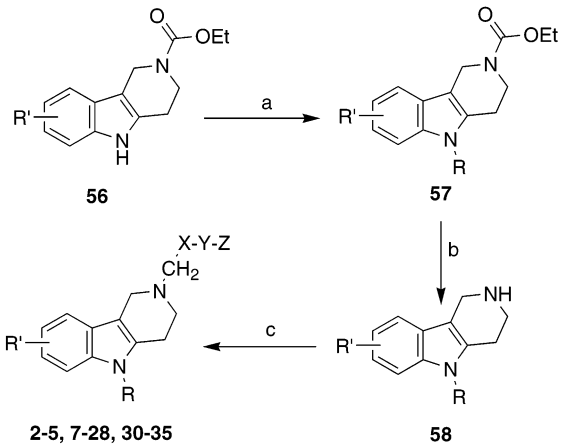


During the course of our investigation human 5-HT$_{5A}$ receptors became available. The purpose of the present investigation was severalfold: (i) to obtain representative human 5-HT$_{5A}$ binding data for selected compounds we had already examined at mouse 5-HT$_{5A}$ receptors, (ii) to determine if the human binding data (K_i values) were similar to the mouse 5-HT$_{5A}$ binding data we had previously reported, (iii) to continue our structure−affinity investigation employing human 5-HT$_{5A}$ receptors, and (iv) to examine the 5-HT$_{2A}$ receptor affinities of selected compounds to determine their 5-HT$_{5A}$ versus 5-HT$_{2A}$ selectivity.

Chemistry

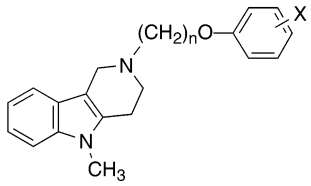
The synthesis of many of the γ-carboline derivatives (i.e., those for which murine 5-HT$_{5A}$ K_i values have been reported) was previously described as shown in Scheme 1.[17] Several additional derivatives were prepared using the same general route by alkylation of **58** (R = CH_3) with the appropriate alkyl halide (Scheme 1), and their physicochemical properties are shown in Table 1. Compounds **38** and **40** were prepared in like manner using 1,2,3,4-tetrahydroisoquinoline and 1-methyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine, respectively, in place of **58**. Compound **40** was prepared from the appropriately substituted pyrrole as shown in Scheme 2; cyclization of formamide **60** afforded pyrrolopyridine **61** which

Scheme 1[a]



[a] Reagents: (a) NaH, RI, DMF; (b) KOH; (c) ClCH$_2$−X−Y−Z, NaI, K_2CO_3.

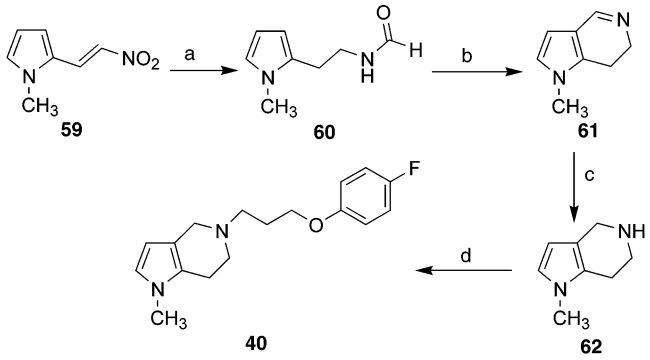
Table 1. Physicochemical Properties of Novel Phenyl-Substituted Tetrahydro-γ-carboline Derivatives



	n	X	mp (°C)	% yield	recrystallization solvent	empirical formula[a]
19	2	4-F	223−225	42	MeOH/Et$_2$O	$C_{20}H_{21}FN_2O$·HCl
25	3	4-NO_2	226−228	46	MeOH/Et$_2$O	$C_{21}H_{23}N_3O_3$·HCl[b]
26	3	4-Cl	234−236	39	MeOH/Et$_2$O	$C_{21}H_{23}Cl\ N_2O$·HCl
27	3	3,4-Cl_2	217−219	19	MeOH/Et$_2$O	$C_{21}H_{22}Cl_2N_2O$·HCl
28	3	4-OMe	215−217	58	MeOH/Et$_2$O	$C_{22}H_{26}N_2O_2$·HCl[c]

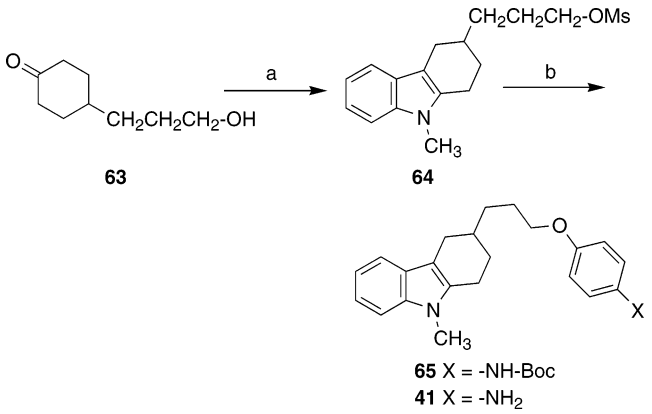
[a] Compounds analyzed within 0.4% of theory for C, H, N. [b] Crystallized with 0.25 mol of H_2O. [c] Crystallized with 0.1 mol of H_2O.

Scheme 2[a]



[a] Reagents: (a) (i) $LiAlH_4$, (ii) HCOOEt; (b) $POCl_3$; (c) $NaBH_4$; (d) (i) ArO(CH$_2$)$_3$Cl, K_2CO_3, (ii) oxalic acid.

Scheme 3[a]



[a] Reagents: (a) (i) MeHNNHPh, HCl, (ii) MsCl; (b) (i) *tert*-butyl (4-hydroxyphenyl)carbamate, K_2CO_3, (ii) HCl/EtOAc.

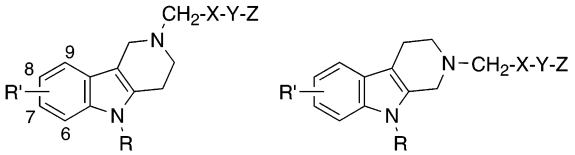
was subsequently reduced with sodium borohydride to **62** and alkylated with the appropriate aryloxyalkyl chloride. Oxime **6** was prepared by reaction of **4** with hydroxylamine, and amine **29** was obtained by reduction of nitro compound **25**.

Gramine analogue **39** was obtained by the condensation of indole-3-carboxaldehyde with 3-(4-fluorophenoxy)propylamine, followed by methylation. Carbazole **41**, the deaza analogue of **29**, was prepared via a Fischer-type cyclization of 4-(3-hydroxypropyl)cyclohexanone; the hydroxy intermediate was mesylated to **64**, compound **64** was allowed to react with N-Boc-protected 4-hydroxyaniline, and the resulting product was deprotected to afford the desired product **41** (Scheme 3). The β-carboline derivatives **53**−**55** were obtained by alkylation of the requisite 1,2,3,4-tetrahydro-β-carboline.

Results and Discussion

Human versus Mouse 5-HT$_{5A}$ Binding. Human 5-HT$_{5A}$ receptor radioligand binding data were obtained for many of the same γ-carboline derivatives for which we had previously reported mouse 5-HT$_{5A}$ data. The results, along with the previously reported mouse 5-HT$_{5A}$ receptor affinities (K_i values), are shown in Table 2. In general, there was relatively little interspecies difference between K_i values, and a significant correlation (r = 0.901) exists between human and murine 5-HT$_{5A}$ receptor affinities. Figure 1 shows a plot of

Table 2. 5-HT$_{5A}$ and 5-HT$_2$ Receptor Affinities of Tetrahydro-γ- and β-carboline Derivatives **3**−**35** and **53**−**55**, Respectively



3-35 **53-55**

	R	R′	X	Y	Z	mouse 5-HT$_{5A}$ K_i, nM (±SEM)[a]	human 5-HT$_{5A}$ K_i, nM (±SEM)[b]	5-HT$_{2A}$ K_i, nM (±SEM)	5-HT$_{2C}$ K_i, nM (±SEM)
3	H	H	-CH_2CH_2-	C=O	4-F Ph	512	–	–	–
4	CH_3	H	-CH_2CH_2-	C=O	4-F Ph	115	–	6[c]	60[c]
5	CH_3	H	-CH_2CH_2-	C=O	Ph	135	168 (10)	22 (4)	–
6	CH_3	H	-CH_2CH_2-	C=NOH	4-F Ph	–	340 (25)	38 (2)	190 (15)
7	CH_3	H	-CH_2CH_2-	-CH_2-	4-F Ph	95	–	65 (20)	–
8	CH_3	H	-CH_2CH_2-	-CH_2-	Ph	130	–	–	–
9	CH_3	H	-CH_2CH_2-	-CH_2-	4-OMe Ph	90	–	415 (80)	990 (180)
10	CH_3	H	-CH_2-	-CH_2-	Ph	250	–	–	–
11	CH_3	H	-CH_2CH_2-	-$(CH_2)_2$-	Ph	120	174 (6)	130[c]	1000 (60)
12	CH_3	H	-CH_2CH_2-	-$(CH_2)_3$-	Ph	435	–	185 (30)	530 (20)
13	CH_3	H	-CH_2CH_2-	-CH(OH)-	4-F Ph	225	285 (10)	160 (30)	390 (10)
14	CH_3	H	-CH_2-	-CH_2-	OH	1100	1120 (100)	–	–
15	CH_3	H	-CH=CH- (*Z*)	-$(CH_2)_2$-	Ph	330	208 (20)	120 (5)	285 (25)
16	CH_3	H	-CH=CH- (*E*)	-$(CH_2)_2$-	Ph	350	185 (45)	60 (10)	245 (30)
17	CH_3	H	-C≡C-	-$(CH_2)_2$-	Ph	1710	1775 (100)	–	–
18	H	H	-CH_2CH_2-	-O-	4-F Ph	75	–	–	–
2	CH_3	H	-CH_2CH_2-	-O-	4-F Ph	13	55 (5)	30[c]	75[c]
19	CH_3	H	-CH_2-	-O-	4-F Ph	–	930 (100)	175 (15)	235 (15)
20	CH_3	H	-CH_2CH_2-	-O-	CH_2Ph	295	–	225 (20)	650 (80)
21	H	H	-CH_2CH_2-	-O-	Ph	600	–	20 (2)	160 (15)
22	CH_3	H	-CH_2CH_2-	-O-	Ph	25	100 (5)	70[c]	285[c]
23	C_2H_5	H	-CH_2CH_2-	-O-	Ph	75	100 (10)	50 (5)	215 (10)
24	CH_2Ph	H	-CH_2CH_2-	-O-	Ph	875	–	65 (15)	90 (20)
25	CH_3	H	-CH_2CH_2-	-O-	4-NO_2 Ph	–	250	280 (90)	300 (60)
26	CH_3	H	-CH_2CH_2-	-O-	4-Cl Ph	–	200 (5)	115 (20)	–
27	CH_3	H	-CH_2CH_2-	-O-	3,4-Cl_2 Ph	–	200 (25)	195 (15)	–
28	CH_3	H	-CH_2CH_2-	-O-	4-OMe Ph	–	350 (100)	100 (10)	–
29	CH_3	H	-CH_2CH_2-	-O-	4-NH_2 Ph	–	190 (20)	285 (50)	300 (40)
30	CH_3	H	-CH_2CH_2-	-O-	1-naphthyl	650	–	625 (220)	400 (100)
31	CH_3	H	-CH_2CH_2-	-O-	2-naphthyl	655	–	480 (180)	300 (80)
32	CH_3	8-OMe	-CH_2CH_2-	-O-	4-F Ph	98	165 (15)	38 (5)	150 (20)
33	CH_3	6-OMe	-CH_2CH_2-	-O-	4-F Ph	1220	1395 (50)	120 (15)	225 (30)
34	CH_3	8-Cl	-CH_2CH_2-	-O-	4-F Ph	180	270 (50)	130 (15)	300 (60)
35	CH_3	6-Cl	-CH_2CH_2-	-O-	4-F Ph	590	225 (10)	210 (15)	225 (10)
53	H	H	-CH_2CH_2-	C=O	Ph	550 (30)	–	16 (1)	115 (10)
54	H	H	-CH_2CH_2-	-O-	Ph	335 (15)	–	50 (5)	310 (15)
55	CH_3	H	-CH_2CH_2-	-O-	Ph	360 (15)	620 (20)	150 (10)	400 (20)

[a] Murine 5-HT$_{5A}$ K_i values were previously reported where SEM is not shown.[17] [b] For comparison, K_i = 307 (±31) nM for 5-HT. [c] 5-HT$_2$ K_i values previously reported.[17]

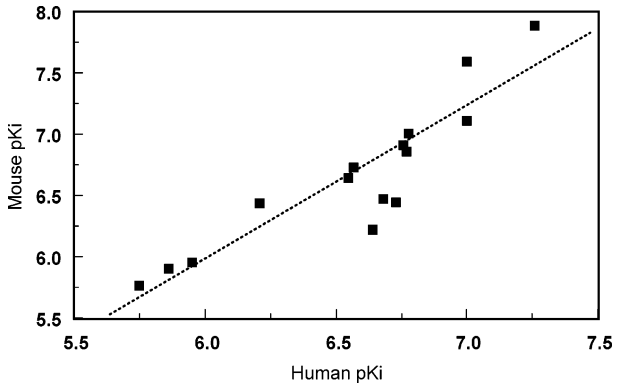


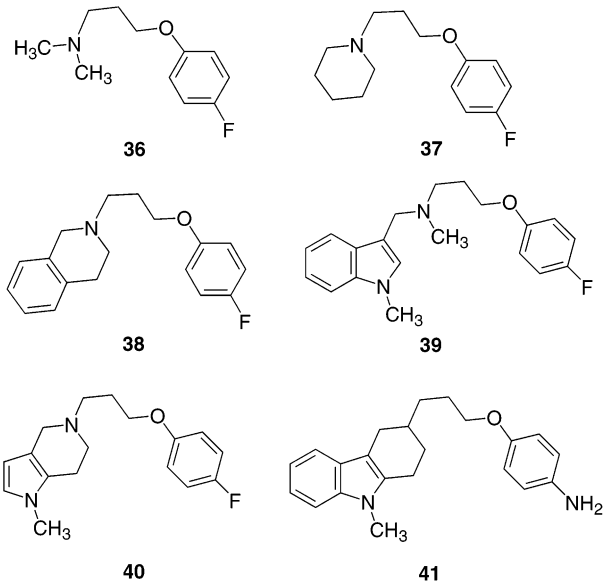
Figure 1. Relationship between human and murine 5-HT$_{5A}$ receptor affinities (p*K*i values; data from Table 2, $r = 0.901$; $n = 15$).

human versus mouse 5-HT$_{5A}$ pK_i values. Rees et al.[9] had previously commented that there was good general agreement between the pharmacology of a small series of agents at mouse and human 5-HT$_{5A}$ receptors. The present findings substantiate this concept for tetrahydrocarboline derivatives. On this basis, it was felt justified in not obtaining human data on all the γ-carbolines previously examined and in making structure−affinity generalizations using mouse data in the few instances where human data were unavailable.

5-HT$_{5A}$ Structure—Affinity Study. Radioligand binding data are shown in Table 2. Our earlier study had identified compound **3** as binding with 10-fold higher affinity than **1**, and showed that N_5-methylation (i.e., **4**) resulted in further 5-fold enhancement of affinity.[17] Because the aryl fluoro group was found to make a minimal contribution to affinity, many subsequently examined compounds lacked this functionality. It had been shown that replacement of the carbonyl group of **5** with an ethylene group (i.e., **11**) had no effect on murine 5-HT$_{5A}$ receptor affinity; the same was found in comparing the human 5-HT$_{5A}$ affinity of **5** ($K_i = 168$ nM) with **11** ($K_i = 174$ nM; Table 2). Introduction of unsaturation to afford the alkenes **15** and **16** ($K_i = 208$ and 185 nM, respectively) also had little influence on affinity whereas the alkyne **17** displayed about 10-fold reduced affinity. As in the mouse assay, replacement of the carbonyl group with an ether oxygen atom was found to be optimal (e.g., compare **5**, $K_i = 168$, with **2**, $K_i = 55$ nM); removal of the fluoro group halved affinity (**22**; $K_i = 100$ nM). Nevertheless, compound **2** displayed 4-fold reduced affinity for human versus murine 5-HT$_{5A}$ receptors. Shortening the alkoxy chain length of **2** by a single methylene group (i.e., **19**; $K_i = 930$ nM) reduced affinity by >15-fold. In general, where comparisons could be made, the effect of chain alteration on human 5-HT$_{5A}$ binding was very similar to what was found for binding at mouse 5-HT$_{5A}$ receptors.

Introduction of aromatic electron donating and electron-withdrawing substituents to the appended phenyl ring (e.g., **25**−**29**) failed to result in enhanced affinity. Introduction of electron-donating and -withdrawing substituents to the γ-carboline ring (e.g., **32**−**35**) had varying effects but failed to significantly enhance human 5-HT$_{5A}$ receptor affinity much in the same manner that it failed to enhance murine 5-HT$_{5A}$ receptor affinity.[17]

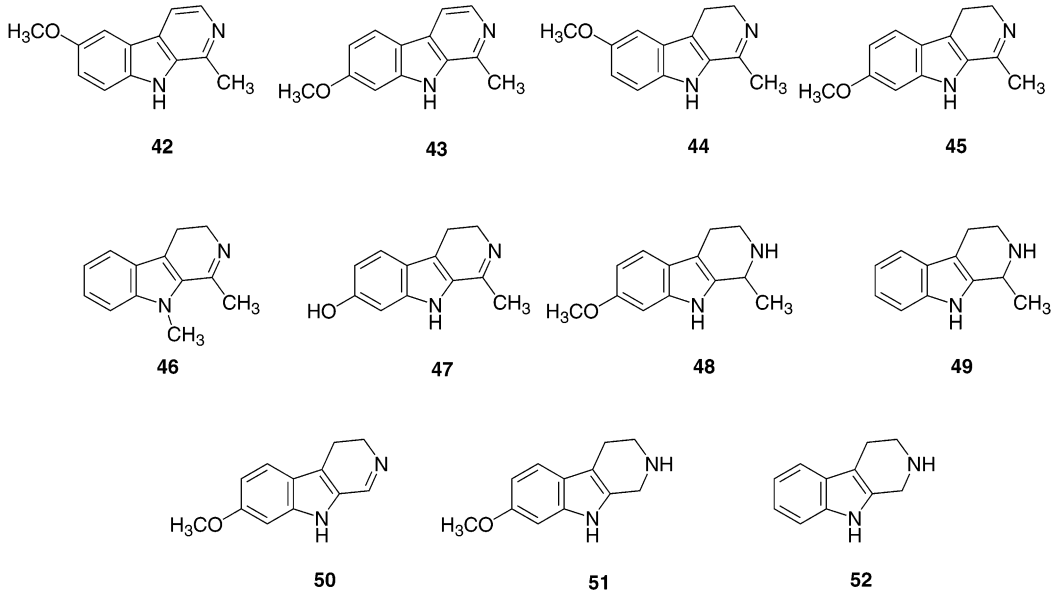
The role of an intact γ-carboline ring was next examined. Compounds **36**−**40** were prepared and evaluated. In compounds **36** and **37** (K_i > 10 000 nM), the tetrahydrocarboline ring was abbreviated to a simpler tertiary amine; lack of affinity indicated that the ring system contributes to binding. With compound **38**, the pyrrole portion of the tetrahydro-γ-carboline ring was excised; the affinity of **38** ($K_i = 400 \pm 30$ nM) was about 8-fold lower than that of its intact parent **2**. The tetrahydropyridyl portion of **2** was ring-opened to provide **39**; here too, affinity was reduced. The affinity of **39** ($K_i = 1080 \pm 200$ nM) was about 20-fold lower than that of **2**. In compound **40** ($K_i = 1920 \pm 370$ nM) the fused phenyl ring of **2** was eliminated, resulting in about a 35-fold decrease in affinity. Taken together, these results suggest that an intact ring system is optimal for the binding of **2** at 5-HT$_{5A}$ receptors.



Even though the presence of an amine moiety might be intuitively expected to be a requirement for binding, the role of the basic γ-carboline nitrogen atom was examined. Compound **41** is the deaza analogue of **29** ($K_i = 190$ nM). Replacement of the basic ring-nitrogen atom with a methylene group (i.e., racemic **41**, K_i >10 000 nM) demonstrates that the amine is required for optimal affinity.

Having established that a tricyclic ring system and an amine are important for binding, and knowing that a chain length of four to five atoms is tolerated, the possibility was explored that the tetrahydro-γ-carboline ring might be replaced by a tetrahydro-β-carboline system. A series of β-carboline derivatives (i.e., pyrido[3,4-*b*]indoles), on hand as a result of other studies,[18] was examined (Chart 2). Compounds **42**−**51** lacked affinity for 5-HT$_{5A}$ receptors (i.e., K_i > 10 000 nM). The individual optical isomers of **48** were examined; both lacked affinity (K_i >10 000 nM). However, removal of all ring substituents to give 1,2,3,4-tetrahydro-β-carboline (**52**) ($K_i = 3200 \pm 400$ nM) resulted in an affinity that was not very different than that of **1**. Consequently, several novel 1-desmethyl tetrahydro-β-carboline derivatives were prepared. The β-carboline derivative **53** ($K_i = 550$ nM; Table 2) was found to bind at murine 5-HT$_{5A}$ receptors with an affinity comparable to that of

Chart 2. Structures of β-Carbolines Examined at 5-HT$_{5A}$ Receptors



3 (K_i = 512 nM) and only severalfold lower than that of **5**, leading to speculation that replacement of the tetrahydro-γ-carboline nucleus with a tetrahydro-β-carboline might be permissible. As a result, tetrahydro-β-carboline derivatives of **21** and **22** (i.e., **54** and **55**; Table 2) were prepared and examined. Compound **54** (m5-HT$_{5A}$ K_i = 335 nM) displayed twice the affinity of its tetrahydro-γ-carboline counterpart (**21**, K_i = 600 nM). Because *N*-methylation had been shown to enhance affinity in the tetrahydro-γ-carboline series, it was expected that the *N*-methyl analogue of **54** (i.e., **55**) might also bind with enhanced affinity. However, **55** (m5-HT$_{5A}$ K_i = 360 nM, h5-HT$_{5A}$ K_i = 620 nM) failed to show this effect. Although tetrahydro-β-carboline derivatives might represent useful templates for future study, they could behave somewhat differently, from a structure−affinity perspective, than their tetrahydro-γ-carboline counterparts.

5-HT$_{5A}$ versus 5-HT$_2$ Selectivity. Our earlier investigation showed that several tetrahydro-γ-carbolines bind at 5-HT$_{2A}$ receptors and that some of them displayed higher affinity for 5-HT$_{2A}$ than 5-HT$_{5A}$ receptors;[17] however, only a few analogues were examined at that time. In the present investigation it was found that **1** binds equally well at rat 5-HT$_{2A}$ (K_i = 3100 ± 5 nM) and murine 5-HT$_{5A}$ (K_i = 5300 nM) receptors. Examination of an extended series of such compounds (Table 2) shows that, indeed, all compounds bind at rat 5-HT$_{2A}$ receptors and, although generally with somewhat lower affinity, at rat 5-HT$_{2C}$ receptors. In general, there is little to no 5-HT$_{5A}$ selectivity; the 4-methoxy-substituted compound **9**, and its ether counterpart **28**, displayed maximal (only about 4-fold) preference for 5-HT$_{5A}$ receptors over 5-HT$_{2A}$ receptors. Will it be possible to divorce 5-HT$_2$ character from 5-HT$_{5A}$ character? Are 5-HT$_{5A}$ and 5-HT$_2$ structure−affinity relationships inextricably linked; that is, do 5-HT$_{5A}$ and 5-HT$_2$ K_i values covary? Figure 2 shows the relationship between 5-HT$_{5A}$ and 5-HT$_{2A}$ affinity for the tetrahydro-γ-carbolines examined; although there is a trend toward covariance, the correlation coefficient (r = 0.520) is not particularly impressive. Hence, it might be possible to separate the two activities. Due to the reversal in selectivity seen with **9** and **28** compared to most of the other compounds, further exploration of the distal phenyl group portion of these molecules seems warranted and might lead to improved selectivity.

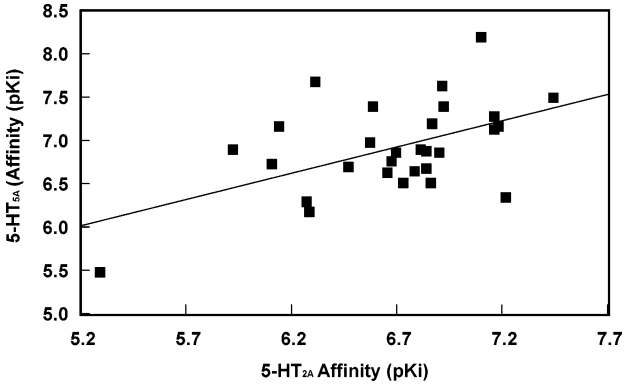


Figure 2. Relationship between 5-HT$_{5A}$ versus 5-HT$_{2A}$ receptor affinities for the tetrahydro-γ-carboline derivatives shown in Table 2 (r = 0.520; n = 29). Where human 5-HT$_{5A}$ data were unavailable, murine data were used.

Summary. 5-HT$_{5A}$ receptors represent a poorly investigated family of serotonin receptors for which no selective agent has yet been identified. Screening of various compounds identified tetrahydro-γ-carboline **1** as a possible lead structure and a structure−affinity investigation was undertaken. Our initial studies employed mouse 5-HT$_{5A}$ receptors and subsequent investigations used human 5-HT$_{5A}$ receptors once they became available. For 15 tetrahydro-γ-carboline derivatives, it was found that there is little interspecies difference in binding affinities. A total of >50 tetrahydrocarboline-related derivatives was prepared and examined in order to formulate structure−affinity relationships. The general conclusion is that compounds of type **2** bind with nanomolar affinity at 5-HT$_{5A}$ receptors, that an intact ring-system is important, that the tetrahydro-γ-carboline ring might be replaced by a tetrahydro-β-carboline ring (although few examples were explored), but that most of the compounds also bind at 5-HT$_{2A}$ receptors. While it seems that most structural modifications had

similar effect on 5-HT$_{5A}$ and 5-HT$_{2A}$ receptor affinity, a comparison of K_i values showed only a modest correlation. Hence, continued work with these compounds, with emphasis on the terminal phenyl portion of the molecule, might result in improved selectivity.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded with a Varian EM-390 spectrometer, and peak positions are given in parts per million (δ) downfield from tetramethylsilane as the internal standard. Microanalyses were performed by Atlantic Microlab (GA) for the indicated elements, and the results are within 0.4% of the calculated values. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040−0.063 mm, Merck) by flash chromatography. Reactions and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel precoated F$_{254}$ Merck plates.

5-Methyl-2-(4-oximino-(4-fluorophenyl)butyl)-5*H*-1,2,3,4-tetrahydropyrido[4,3-*b*]indole Hydrochloride (6). A solution of compound **4** (free base)[17] (0.10 g, 0.28 mmol) in a mixture of EtOH (1 mL) and H_2O (0.5 mL), in which $NH_2OH\cdot$HCl (0.04 g, 0.60 mmol) and NaOH (0.06 g) had been dissolved, was allowed to stir at room temperature for 30 min. The mixture was evaporated to dryness, H_2O (25 mL) was added, and the whole was extracted with CH_2Cl_2 (2 × 25 mL). The combined organic portion was dried ($MgSO_4$), and solvent was evaporated under vacuum to yield a crude product. Purification was achieved using column chromatography (silica gel; EtOAc/MeOH; 9:1). A solution of the free base in anhydrous Et_2O was treated with ethereal HCl to give 0.08 g (70%) of **6** as a white powder following recrystallization from an anhydrous MeOH/Et_2O mixture; mp 215−217 °C. ^{1}H NMR (CD_3CN): δ 1.78−1.90 (m, 2H, CH_2), 2.58−2.67 (t, J = 7.8 Hz, 2H, CH_2), 2.80−2.92 (m, 6H, 3CH_2), 3.60−3.70 (bs, 5H, CH_2 and NCH_3), 6.98−7.19 (m, 4H, Ar−H), 7.30−7.40 (m, 2H, Ar−H), 7.68−7.76 (m, 2H, Ar−H). Anal. Calcd ($C_{22}H_{24}FN_3O\cdot$HCl$\cdot$0.25H_2O) C, H, N.

2-((4-Aminophenoxy)propyl)-5-methyl-5*H*-1,2,3,4-tetrahydropyrido[4,3-*b*]indole Hydrochloride (29). A mixture of **25** (free base) (0.32 g, 0.89 mmol) and $SnCl_2\cdot 2H_2O$ (2.40 g, 10 mmol) in absolute EtOH (10 mL) was heated at 70 °C for 3 h under N_2. The reaction mixture was allowed to cool to room temperature and poured into ice−water. The slurry was extracted with EtOAc (2 × 30 mL). The combined organic portion was dried ($MgSO_4$), solvent was evaporated under vacuum, and the crude product was purified by column chromatography (silica gel; EtOAc/MeOH; 9:1). The solid material in anhydrous MeOH was treated with methanolic HCl. Recrystallization from an anhydrous MeOH/Et_2O mixture gave 0.17 (45%) of **29** as a white powder; mp 227 °C (decomposes). ^{1}H NMR (CD_3OD): δ 2.40−2.51 (m, 2H, CH_2), 3.30−3.36 (m, 2H, CH_2), 3.60−3.68 (m, 2H, CH_2), 3.72−3.76 (s, 3H, NCH_3), 3.90−4.10 (bs, 2H, CH_2), 4.19−4.26 (t, J = 5.7 Hz, 2H, CH_2), 4.40−4.52 (bs, 2H, CH_2), 7.08−7.23 (m, 6H, Ar−H), 7.40−7.46 (d, J = 7.5 Hz, 1H, Ar−H), 7.48−7.52 (d, J = 7.5 Hz, 1H, Ar−H). Anal. Calcd ($C_{21}H_{25}N_3O\cdot$2HCl$\cdot$1.25H_2O) C, H, N.

2-(3-(4-Fluorophenoxy)propyl)-1,2,3,4-tetrahydroisoquinoline Hydrochloride (38). A mixture of 1,2,3,4-tetrahydroisoquinoline (0.14 g, 1 mmol), 3-(4-fluorophenoxy)propyl chloride (0.19 g, 1 mmol), K_2CO_3 (0.50 g, 3.7 mmol), and a catalytic amount of NaI in MeCN (10 mL) was heated at reflux for 24 h. The reaction mixture was filtered, and the filtrate was evaporated to dryness under reduced pressure. The residue was suspended in H_2O (25 mL) and extracted twice with Et_2O (25 mL). The combined ethereal extract was evaporated under vacuum, and the crude product was purified by column chromatography (silica gel; CH_2Cl_2 followed with EtOAc/MeOH; 1:1). The free base was dissolved in anhydrous MeOH and treated with methanolic HCl. Recrystallization from anhydrous MeOH/Et_2O afforded 0.14 g (43%) of the title compound as white crystals; mp 195−196 °C. ^{1}H NMR (CD_3OD): δ 2.30−2.42 (m, 2H, CH_2), 3.20−3.30 (t, J = 8.1 Hz, 2H, CH_2), 3.50−3.58 (t, J = 7.8 Hz, 2H, CH_2), 3.62−3.72 (bs, 2H, CH_2), 4.10−4.18 (t, J = 6.0 Hz, 2H, CH_2), 4.48−4.58 (bs, 2H, CH_2), 6.90−7.09 (m, 4H, Ar−H), 7.22−7.36 (m, 4H, Ar−H). Anal. Calcd ($C_{18}H_{20}FNO$·HCl) C, H, N.

***N*-[3-(4-Fluorophenoxy)propyl]-*N*-methyl-*N*-(1-methyl-1*H*-indol-3-yl)methylamine Oxalate (39).** A mixture of indole-3-carboxaldehyde 97% (0.44 g, 3 mmol), 3-(4-fluorophenoxy)propylamine[19] (0.51 g, 3 mmol), and $NaBH_3CN$ 95% (0.19 g, 3 mmol) in anhydrous MeOH (20 mL) was allowed to stir at room temperature for 48 h. Solvent was evaporated under vacuum, H_2O (25 mL) was added, and the mixture was basidified to pH 10 with 10% KOH and extracted with Et_2O (4 × 25 mL). The combined organic portion was dried ($MgSO_4$), solvent was evaporated under vacuum, and the crude product was purified by column chromatography (silica gel; EtOAc/hexane; 9:1) to give 0.35 g (39%) of the intermediate secondary amine as an oil. IR (neat): 3411 cm^{-1}. Sodium hydride 95% (0.06 g, 2.2 mmol) was added to a stirred solution of the amine (0.30 g, 1 mmol) in dry DMF (10 mL) under N_2. Methyl iodide (0.60 g, 4.2 mmol) was added, and the reaction mixture was allowed to stir at room temperature for 30 min. The resultant solution was poured into ice/H_2O (50 mL) and extracted with Et_2O (3 × 25 mL). The combined organic portion was dried ($MgSO_4$), and solvent was evaporated under vacuum to afford a crude product that was purified by column chromatography (silica gel; EtOAc/MeOH; 9:1). The free base of **39** in anhydrous CH_2Cl_2 was treated with oxalic acid in anhydrous Et_2O. Recrystallization from anhydrous MeOH/Et_2O afforded 0.19 g (45%) of **39** as a pink powder; mp 141−143 °C. ^{1}H NMR (CD_3OD): δ 2.22−2.33 (bs, 2H, CH_2), 2.87−2.94 (s, 3H, CH_3), 3.33−3.50 (bs, 2H, CH_2), 3.84−3.90 (s, 3H, CH_3), 4.13−4.20 (bs, 2H, CH_2), 4.55−4.63 (bs, 2H, CH_2), 6.80−6.90 (m, 2H, Ar−H), 6.97−7.06 (t, J = 8.7 Hz, 2H, Ar−H), 7.17−7.26 (t, J = 9.3 Hz, 1H, Ar−H), 7.27−7.37 (t, J = 7.8 Hz, 1H, Ar−H), 7.46−7.53 (d, J = 9.0 Hz, 2H, Ar−H), 7.70−7.78 (d, J = 8.4 Hz, 1H, Ar−H). Anal. Calcd ($C_{20}H_{23}FN_2O\cdot C_2H_2O_4\cdot$0.25$H_2O$) C, H, N.

5-[3-(4-Fluorophenoxy)propyl]-1-methyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine Oxalate (40). A mixture of 1-methyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine (**62**) (0.15 g, 1.1 mmol), 3-(4-fluorophenoxy)propyl chloride (0.21 g, 1.1 mmol), K_2CO_3 (0.50 g, 3.5 mmol), and a catalytic amount of NaI in DMF (10 mL) was heated at reflux for 10 h. The reaction mixture was allowed to cool to room temperature, concentrated under high vacuum, and diluted with water (25 mL). The mixture was extracted with CH_2Cl_2 (3 × 25 mL), the combined organic extract was dried ($MgSO_4$), solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (silica gel; CH_2Cl_2 followed with EtOAc/MeOH; 9:1). The product was treated with oxalic acid in an anhydrous CH_2Cl_2/Et_2O mixture to form the salt. Recrystallization from anhydrous MeOH/Et_2O gave 0.15 g (36%) of **40** as orange crystals; mp 130−132 °C. ^{1}H NMR (CD_3OD): δ 2.26−2.36 (m, 2H, CH_2), 3.00−3.07 (t, J = 6.0 Hz, 2H, CH_2), 3.45−3.53 (t, J = 8.1 Hz, 2H, CH_2), 3.55−3.59 (s, 3H, NCH_3), 3.60−3.72 (m, 2H, CH_2), 4.08−4.15 (t, J = 5.7 Hz, 2H, CH_2), 4.30−4.37 (bs, 2H, CH_2), 5.95−5.90 (d, J = 2.7 Hz, 1H, CH), 6.67−6.70 (d, J = 3.0 Hz, 1H, CH), 6.91−6.98 (m, 2H, Ar−H), 7.00−7.07 (m, 2H, Ar−H). Anal. Calcd ($C_{17}H_{21}FN_2O\cdot C_2H_2O_4\cdot$0.25$H_2O$) C, H, N.

(±)3-[3-(4-Aminophenoxy)propyl]-9-methyl-1,2,3,4-tetrahydrocarbazole Hydrochloride (41). A saturated solution of HCl (g) in EtOAc (1 mL) was added to **65** (0.2 g, 0.46 mmol) in EtOAc (3 mL), and the reaction mixture was allowed to stir at room temperature for 18 h. The precipitated material was collected by filtration and recrystallized from anhydrous MeOH/Et_2O to give 0.15 g (87%) of **41** as a white powder; mp 176−178 °C. ^{1}H NMR (DMSO): δ 1.48−1.62 (m, 4H, 2CH_2), 1.74−1.84 (m, 2H, CH_2), 1.98−2.09 (m, 1H, CH), 2.18−2.30 (m, 1H, CH), 2.66−2.89 (m, 3H, CH_2, and CH), 3.57−3.62 (s, 3H, N−CH_3), 3.97−4.05 (t, J = 6.3 Hz, 2H, CH_2), 6.91−7.09 (m, 4H, Ar−H), 7.18−7.25 (m, 2H, Ar−H), 7.30−7.39 (t, J = 6.0 Hz, 2H, Ar−H). Anal. Calcd ($C_{22}H_{26}N_2O\cdot$HCl$\cdot$0.25H_2O) C, H, N.

2-((4-Oxo-4-phenyl)butyl)-1,2,3,4-tetrahydro-9*H*-pyrido-[3,2-*b*]indole Hydrochloride (53). Sodium carbonate (0.80 g, 5.8 mmol) and KI (0.01 g, 0.06 mmol) were added to a solution of 1,2,3,4-tetrahydro-9*H*-pyrido[3,2-*b*]indole (1.0 g, 5.8 mmol) in DMF (10 mL), 4-chloro-1-phenyl-1-butanone (0.93 mL, 5.8 mmol) was added to the stirred reaction mixture in a dropwise manner, and stirring was allowed to continue at 65 °C for 3.5 h under an N_2 atmosphere. The reaction mixture was allowed to cool to room temperature and poured into H_2O (50 mL), extracted with EtOAc (3 × 25 mL), and the combined EtOAc portion was combined and evaporated to afford a yellow oil. The oil was purified by column chromatography with CH_2Cl_2:MeOH (0% MeOH increasing to 2% MeOH) as the eluent to afford 0.02 g (11%) of an off-white solid. The HCl salt was prepared and recrystallized from MeOH; mp 254−255 °C. ^{1}H NMR (DMSO-d_6) δ 3.01−3.06 (m, 2H), 3.23−3.28 (m, 2H), 3.34−3.39 (m, 2H), 3.41−3.50 (bs, 1H), 3.79−3.83 (bs, 1H), 4.41−4.63 (m, 2H), 7.01−7.15 (m, 2H), 7.37−7.40 (d, 1H, J = 8.3 Hz), 7.47−7.58 (m, 3H), 7.64−7.69 (m, 1H), 7.98−8.01 (m, 2H), 11.05 (s, 1H). Anal. Calcd ($C_{21}H_{22}N_2O$·HCl·0.1MeOH) C, H, N.

2-(3-Phenoxypropyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,2-*b*]indole Hydrochloride (54). Solid sodium carbonate (0.80 g, 5.8 mmol) and KI (0.01 g, 0.06 mmol) were added to a solution of 1,2,3,4-tetrahydro-9*H*-pyrido[3,2-*b*]indole (1.0 g, 5.8 mmol) in DMF (10 mL), 1-bromo-3-phenoxypropane (0.91 mL, 5.8 mmol) was added to the stirred mixture in a dropwise manner, and stirring was allowed to continue at 65 °C for 3.5 h under an N_2 atmosphere. The reaction mixture was cooled to room temperature, poured into H_2O (50 mL), and extracted with EtOAc (3 × 25 mL), and the combined EtOAc portion was evaporated to afford a yellow oil. The oil was purified by column chromatography with EtOAc:hexanes (8:2) as the eluent to afford 1.29 g (72%) of an off-white solid; mp 121−123 °C. The HCl salt was prepared and recrystallized from MeOH; mp 252−253 °C. ^{1}H NMR (DMSO-d_6) δ 2.29−2.34 (m, 2H), 2.99−3.07 (m, 2H), 3.39−3.45 (m, 3H), 3.80 (bs, 1H), 4.08−4.12 (t, 2H, J = 6 Hz), 4.44−4.58 (m, 2H), 6.93−6.97 (m, 3H), 7.00−7.05 (m, 1H), 7.09−7.l4 (m, 1H), 7.28−7.39 (m, 3H), 7.46−7.49 (d, 1H, J = 7.7 Hz), 11.14 (s, 1H). Anal. Calcd ($C_{20}H_{22}N_2O$·HCl) C, H, N.

9-Methyl-2-(3-phenoxypropyl)-1,2,3,4-tetrahydro-9*H*-pyrido[3,2-*b*]indole Hydrochloride (55). Sodium hydride (60% dispersion, 0.04 g, 1.1 mmol) was added to a solution of 2-(3-phenoxypropyl)-2,3,4,9-tetrahydro-1*H*-β-carboline (**54**; 0.30 g, 1.0 mmol) in DMF (5 mL) at 0 °C. The reaction mixture was allowed to stir at 0 °C for 15 min under an N_2 atmosphere. Methyl iodide (0.15 g, 1.1 mmol) in DMF (2 mL) was added to the mixture in a dropwise manner, and stirring was continued at room temperature under an N_2 atmosphere for 1.5 h. The reaction mixture was poured into H_2O (25 mL) and extracted with EtOAc (3 × 25 mL); the combined EtOAc portion was evaporated to afford an oil that was purified by column chromatography with CH_2Cl_2:MeOH (99:1) as the eluent to afford 0.21 g (67%) of an yellow oil. The HCl salt was prepared and recrystallized from MeOH; mp 221−224 °C. ^{1}H NMR (DMDO-d_6) 2.35−2.39(m, 2H), 2.97−3.16(m, 2H), 3.45(s, 3H), 3.67−3.68(m, 3H), 3.76−3.84(m, 1H), 4.10−4.14(m, 2H), 4.45−4.50(d, 1H, J = 14.07), 4.78−4.83(d, 1H, J = 14.07), 6.93−6.98(m, 3H), 7.03−7.09(m, 1H), 7.15−7.21(m, 1H), 7.27−7.34(m, 2H), 7.44−7.52(m, 2H). Anal. Calcd ($C_{21}H_{24}N_2O$·HCl) C, H, N.

1-Methyl-2-(2-nitrovinyl)-1*H*-pyrrole (59). The title compound was synthesized according to the general procedure described by Spadoni et al.[20] A solution of 1-methyl-2-pyrrolecarboxaldehyde (1.00 g, 9 mmol) and NaOAc (0.34 g, 4 mmol) in CH_3NO_2 (10 mL) was heated at reflux for 1.5 h under N_2. The reaction mixture was allowed to cool to room temperature, EtOAc (25 mL) was added, and the organic phase was decanted. The organic phase was washed with H_2O (2 × 25 mL) and dried (Na_2SO_4), solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (silica gel; CH_2Cl_2). Recrystallization from anhydrous CH_2Cl_2/Et_2O gave 1.02 g (75%) of the nitrovinyl compound as pale-yellow crystals; mp 99−100 °C (lit.[21] mp 101−102 °C).

1-Methyl-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine (61). Nitrovinyl compound **59** (1.00 g, 6.5 mmol) was added portionwise to a stirred suspension of $LiAlH_4$ (1.00 g, 26 mmol) in dry THF (40 mL) under N_2. The reaction mixture was allowed to stir at room temperature for 5 h and chilled to 0 °C, and the excess $LiAlH_4$ was destroyed by the dropwise addition of water. The mixture was purified by column chromatography (silica gel; EtOAc:MeOH; 9:1) to give 0.55 g (69%) of the amine as an oil. Employing the general method of Herz et al.,[22] a mixture of the amine (0.7 g, 5.6 mmol) and ethyl formate (30 mL) was heated at reflux for 7 h. Solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (silica gel; EtOAc:MeOH; 5:1) to give 0.52 g (60%) of formamide **60** as an oil. ^{1}H NMR ($CDCl_3$): δ 2.81−2.89 (t, J = 6.6 Hz, 2H, CH_2), 3.54−3.64 (m, 5H, CH_2 and CH_3), 5.74−5.86 (bs, 1H, NH, D_2O exchangeable), 5.94−6.02 (m, 1H, CH), 6.08−6.14 (m, 1H, CH), 6.56−6.66 (m, 1H, CH), 8.16−8.22 (s, 1H, CH). Formamide **60** (0.5 g, 3.3 mmol) in refluxing toluene (15 mL) was treated in a dropwise manner with a solution of $POCl_3$ (0.5 mL) in toluene. After heating at reflux for a total of 3 h, the reaction mixture was allowed to cool to room temperature and was washed several times with hot H_2O (3 × 25 mL). The combined aqueous portion was washed with CH_2Cl_2 (3 × 15 mL) and basified by the addition of KOH. Extraction with CH_2Cl_2 (3 × 25 mL) gave 0.16 g (36%) of **61** an oil. The compound was used in the preparation of **62** without further purification. ^{1}H NMR ($CDCl_3$): δ 2.78−2.87 (t, J = 8.4 Hz, 2H, CH_2), 3.59−3.63 (s, 3H, CH_3), 3.86−3.95 (t, J = 8.7 Hz, 2H, CH_2), 6.29−6.33 (d, J = 3 Hz, 1H, CH), 6.57−6.62 (d, J = 3 Hz, 1H, CH), 8.29−8.36 (s, 1H, CH).

1-Methyl-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine (62). A mixture of $NaBH_4$ (0.05 g, 1.3 mmol) in H_2O (2.5 mL) was added in a dropwise manner to a stirred solution of 1-methyl-6,7-dihydro-1*H*-pyrrolo[3,2-c]pyridine (**61**) (0.15 g, 1.1 mmol) in MeOH (5 mL) at 0 °C. After 1 h, the mixture was washed with brine and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic portion was dried ($MgSO_4$) and solvent evaporated under reduced pressure to give 0.23 g of a solid product that was used in the synthesis of **40** without further purification. ^{1}H NMR ($CDCl_3$): δ 2.54−2.62 (t, J = 6.0 Hz, 2H, CH_2), 3.14−3.22 (t, J = 6.0 Hz, 2H, CH_2), 3.48−3.54 (s, 3H, CH_3), 3.84−3.89 (s, 2H, CH_2), 5.90−5.95 (d, J = 3 Hz, 1H, CH), 6.50−6.54 (d, J = 3 Hz, 1H, CH).

(±)3-[3-(Methanesulfonyl)propyl]-9-methyl-1,2,3,4-tetrahydrocarbazole (64). Fischer-type cyclization of 4-(3-hydroxypropyl)cyclohexanone[23] using *N*-methyl-*N*-phenylhydrazine was used to obtain the corresponding carbazole derivative. A mixture of *N*-methyl-*N*-phenylhydrazine (0.85 g, 6.5 mmol), 75% EtOH (60 mL), and 3 N HCl (1.2 mL) was heated at reflux. 4-(3-Hydroxypropyl)cyclohexanone (**63**) (1.04 g, 6.5 mmol) was added in a dropwise manner, and the reaction mixture was heated at reflux for 3 h. Heat was removed, and the reaction was allowed to stir at room-temperature overnight. Solvent was removed under reduced pressure, and the crude product was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic portion was dried (Na_2SO_4), solvent was evaporated under reduced pressure, and the crude product purified by column chromatography (silica gel; CH_2Cl_2:MeOH; 20:1) to give 1.40 g (88%) of the tetrahydrocarbazole intermediate. Methansulfonyl chloride (0.42 mL) and Et_3N (0.95 mL) were added to a solution of this intermediate (1.1 g, 4.5 mmol) in anhydrous CH_2Cl_2 (25 mL) at −10 °C, and the mixture was allowed to stir at −10 °C for 15 min. The mixture was washed with H_2O (2 × 25 mL), the organic layer was separated and dried ($MgSO_4$), and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (silica gel; CH_2Cl_2) to give 1.42 g (98%) of the mesylate which was used without further characterization in the synthesis of **65**.

(±)3-[3-(4-*tert*-Butylcarbamylphenoxy)propyl]-9-methyl-1,2,3,4-tetrahydrocarbazole (65). A mixture of mesylate

64 (0.64 g, 2.0 mmol), (4-hydroxyphenyl)carbamic acid *tert*-butyl ester[24] (0.42 g, 2.0 mmol), and K_2CO_3 (1.00 g, 7.0 mmol) in MeCN (30 mL) was heated at reflux for 18 h. The reaction mixture was allowed to cool to room temperature, concentrated under vacuum, and extracted twice with CH_2Cl_2 (30 mL). The combined organic portion was dried ($MgSO_4$), solvent was evaporated under reduced pressure, and the crude product was purified by column chromatography (silica gel; CH_2Cl_2:MeOH; 20:1). Recrystallization from anhydrous MeOH give 0.45 g (52%) of **65** as a white powder; mp 139−141 °C. ^{1}H NMR ($CDCl_3$): δ 1.42−1.46 (s, 9H, $3CH_3$), 1.48−1.58 (m, 4H, $2CH_2$), 1.70−1.92 (m, 2H, CH_2), 1.98−2.07 (m, 1H, CH), 2.22−2.34 (m, 1H, CH), 2.63−2.73 (m, 2H, CH_2), 2.82−2.92 (m, 1H, CH), 3.53−3.57 (s, 3H, N−CH_3), 3.86−3.94 (t, J = 6.6 Hz, 2H, CH_2), 6.21−6.27 (bs, 1H, NH), 6.74−6.81 (m, 2H, Ar−H), 6.96−7.13 (t, J = 7.3 Hz, 1H, Ar−H), 7.04−7.12 (t, J = 6.9 Hz, 1H, Ar−H), 7.14−7.22 (m, 3H, Ar−H), 7.36−7.41 (d, J = 7.5 Hz, 1H, Ar−H). Anal. Calcd ($C_{27}H_{34}N_2O_3$·HCl) C, H, N.

Radioligand Binding Assay. The 5-HT$_{5A}$ radioligand binding studies were performed as previously described.[17] Tritiated (+)lysergic acid diethylamide (LSD) was used to label murine 5-HT$_{5A}$ receptors (0.5 nM) and human 5-HT$_{5A}$ receptors (1 nM) (cell line generously donated by Dr. R. Hen) and 1 μM LSD was used to determine nonspecific binding. The 5-HT$_2$ binding assays were also conducted according to published procedures.[25] In the 5-HT$_2$ binding assays, either 0.5 nM [^{3}H]-ketanserin (5-HT$_{2A}$) or 2.0 nM [^{3}H]mesulergine (5-HT$_{2C}$) were used as radioligand; ketanserin (10 μM, 5-HT$_{2A}$) and mesulergine (1 μM, 5-HT$_{2C}$) were used to determine nonspecific binding. The general procedure is as follows: NIH-3T3 cells stably transfected with rat 5-HT$_{2A}$ receptors (donated by Dr. David Julius) and A-9 cells stably transfected with rat 5-HT$_{2C}$ receptors (donated by Dr. Beth Hoffman) were grown to confluence, suspended in 50 mM Tris−HCl buffer, and centrifuged at 12 000*g* for 30 min. The pellet was resuspended in buffer and centrifuged for an additional 20 min. Assay buffer used in the experiments consisted of 50 mM Tris-HCl, 0.5 mM EDTA, 10 mM $MgCl_2$, and 0.1% ascorbate (pH 7.4). After resuspension in assay buffer, 1-mL membrane aliquots (∼10 μg protein measured by bicinchoninic assay) were added to each tube containing 1 mL of assay buffer, radioligand, and test compound. Competition experiments were performed in triplicate in a 2.0-mL volume. Typically, 11 concentrations of test agent (10^{-10} to 10^{-5} M) were evaluated, except where the compound displayed <30% inhibition at 10 000 nM in which case it was not further examined. Membranes were incubated for 30 min at 37 °C, filtered on Schleicher and Schuell (Keene, NH) glass fiber filters (presoaked in 0.1% polyethyleneimine), and washed with 10 mL of buffer. The filters were counted in an Ecoscint liquid scintillation counter at 40% efficiency. Competition experiments were plotted and analyzed using GraphPad Prism. K_i values were determined from the Cheng−Prusoff equation: $K_i = IC_{50}/(1 + [D]/K_D)$.[26] The results reflect a minimum of three replicate determinations.

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