

Potent, Selective Tetrahydro- β -carboline Antagonists of the Serotonin 2B (5HT_{2B}) Contractile Receptor in the Rat Stomach Fundus

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A series of potent, selective 5HT_{2B} receptor antagonists has been identified based upon yohimbine, with SAR studies resulting in a 1000-fold increase in 5HT_{2B} receptor affinity relative to the starting structure ($-\log K_B > 10.0$ have been obtained). These high-affinity tetrahydro- β -carboline antagonists are able to discriminate among the 5HT₂ family of serotonin receptors, with members of the series showing selectivities of more than 100-fold versus both the 5HT_{2A} and 5HT_{2C} receptors based upon radioligand binding and functional assays. As the first compounds reported with such selectivity and enhanced receptor affinity, these tetrahydro- β -carboline antagonists are useful tools for elucidating the role of serotonin acting at the 5HT_{2B} receptor in normal and disease physiology.

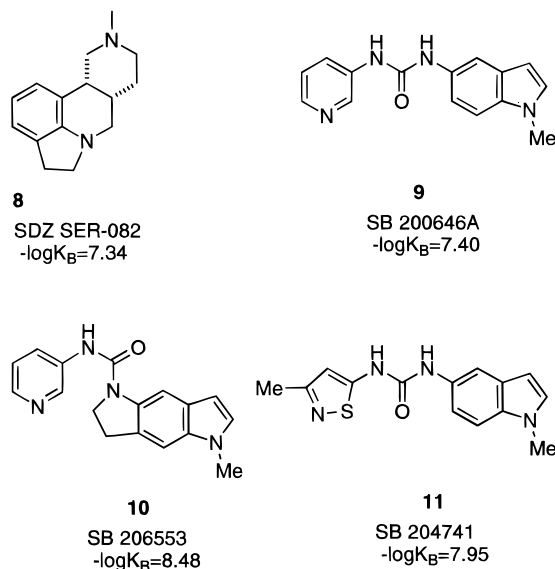
Introduction

Serotonin is a potent contractile agonist in isolated smooth muscle from most tissues. The rat stomach fundus is one of the tissues most sensitive to 5HT-induced contraction with nanomolar concentrations producing a pronounced effect.^{1–8} Considerable effort from these laboratories and others has allowed for the characterization of the stomach fundal receptor, currently designated the 5HT_{2B} receptor.⁹ The rat 5HT_{2B} receptor and its human homolog have been recently cloned, allowing for examination of the binding affinity of receptor agonists and antagonists as well as studies of receptor localization and effector coupling.^{10–14} Such studies have established the correlation for a series of ligands between the cloned 5HT_{2B} receptor and the receptor mediating the contraction in response to 5HT in the rat stomach fundus.¹⁵ Both structurally and pharmacologically, the 5HT_{2B} receptor is similar to the other 5HT₂ receptors.^{9,16}

Recently receptor agonists and antagonists have been identified which begin to differentiate among these closely related receptors (Scheme 1).^{17–20} To date, the compounds reported have achieved useful (>100 -fold) selectivities versus the 5HT_{2A} receptor, while discrimination versus the 5HT_{2C} receptor has proven more challenging and is only attained with agents with lower receptor affinity.

Increasingly selective, high-affinity agents should prove important as tools for the further study of the rat receptor and its human homolog, for which the message has been identified in human brain, liver, heart, kidney, retina, and GI tract.²¹ To that end, we initiated structure–activity relationship (SAR) studies targeted at the development of potent and selective 5HT_{2B} receptor antagonists based upon the tetrahydro- β -carboline alkaloid yohimbine (1). Among its multiple pharmacologic effects, yohimbine has been shown to antagonize 5HT-induced contractions in the rat stomach fundus,²² an observation confirmed in our laboratory ($-\log K_B = 6.92$), although it had 8-fold higher affinity as an α_2 adrenergic receptor antagonist ($-\log K_B = 7.77$).^{23,24} Our SAR studies employed functional data,

Scheme 1

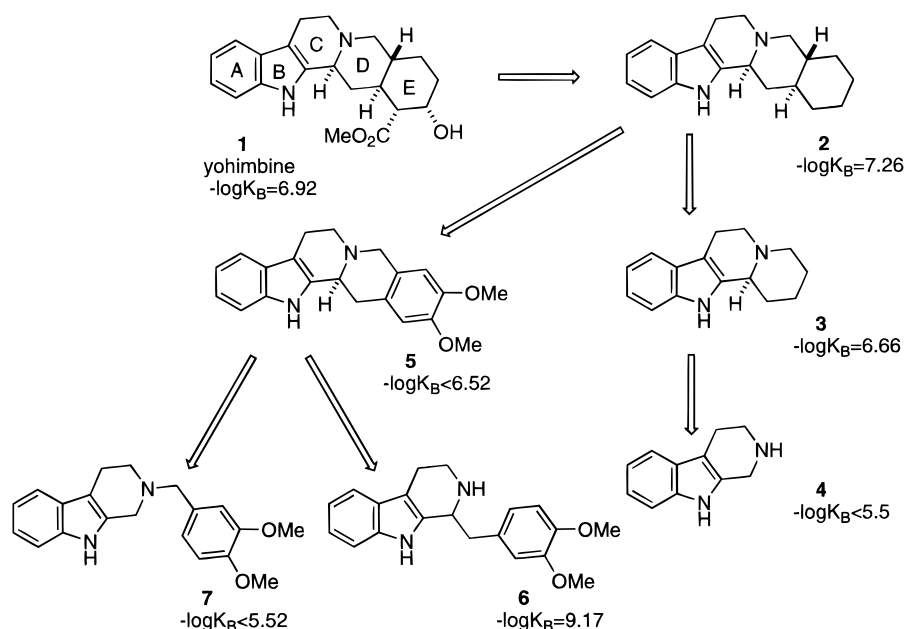


utilizing the serotonin-induced contraction via the 5HT_{2B} receptor in rat stomach fundus smooth muscle strips in order to assess directly both affinity and antagonist activity of the newly synthesized ligands (Tables 1–5). We initiated such studies with a survey of several partial structural analogs of yohimbine (2–7), successively simplifying the molecule, to identify the key structural elements necessary for potent antagonism of 5HT_{2B} receptor-mediated contraction in the rat stomach fundus.

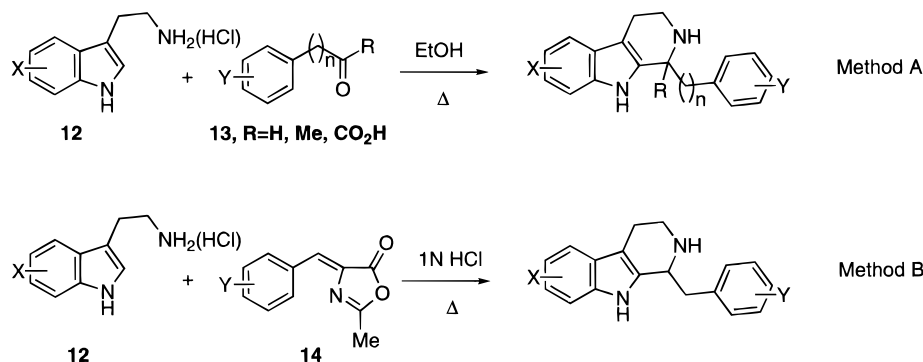
Removal of the ring E ester and hydroxyl functionality from yohimbine to afford the parent pentacycle (2, Scheme 2) greatly simplified the structure and provided the initial impetus for this exercise by virtue of a modest increase in affinity relative to yohimbine (1, Table 1). Further sequential removal of ring E (3) and ring D (4) resulted in a loss of affinity in each case, suggesting a possible role for ring E in the ligand–receptor interactions. The related aryl E-ring yohimbine analog 5 possessed modest affinity. However, excising the methylene linker comprising ring D and exposing a secondary amine (6) resulted in a 100-fold increase in affinity relative to yohimbine. In contrast, the alternative ring

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Scheme 2



Scheme 3



D methylene excision resulted in an inactive tertiary amine analog (7). Our interest in **6** as a template for SAR studies was further heightened by the increased selectivity demonstrated for 5HT_{2B} receptor antagonism versus α_2 receptor antagonism. Whereas yohimbine possessed higher affinity for α_2 receptors as determined by blockade of the UK14301-induced inhibition of the twitch response in the guinea pig ileum, the "partial yohimbine" **6** showed more than 1000-fold selectivity for the 5HT_{2B} receptor blockade in the rat stomach fundus ($-\log K_B$, 5HT_{2B} = 9.17 vs $-\log K_B$, α_2 = 6.13). Thus, excision of ring D, as in **6**, resulted in improved affinity for the 5HT_{2B} receptor with 1000-fold selectivity *vis a vis* the α_2 receptor. On the basis of upon these findings, we pursued a more detailed examination of this "ABCE partial yohimbine" tetracyclic system.

Chemistry

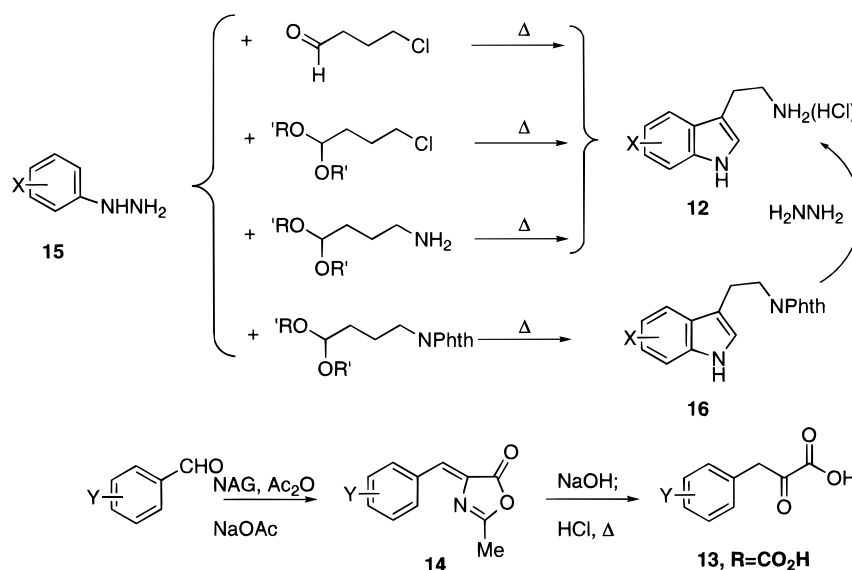
The "partial yohimbines" **2**,²⁵ **3**,²⁶ **4**,²⁷ **5**,²⁸ **6**,²⁹ **7**,³⁰ **17**,³¹ **21**,³² **22**,³³ **26**,³⁴ and **32**³⁵ are known compounds whose syntheses have been reported previously. Novel tetrahydro- β -carbolines (**18**–**20**, **23**–**25**, **27**–**31**, and **33**–**84**) were prepared from their parent tryptamines and suitable carbonyl precursors by two methods. Method A involved a traditional Pictet–Spengler condensation of aldehydes and reactive ketones with the requisite tryptamines.²⁹ Alternatively, method B was employed,

condensing tryptamines directly with aza lactones under our modified hydrolytic Pictet–Spengler protocol (Scheme 3),³⁶ which was developed to increase the breadth of these SAR studies. All tryptamines employed in this study are known compounds and were prepared by literature methods, most frequently Fisher-type indole syntheses utilizing the requisite hydrazine and a suitable 4-aminobutyraldehyde equivalent (Scheme 4).⁴⁸ Aza lactones were prepared from corresponding benzaldehydes by condensation with *N*-acetylglycine in acetic anhydride.³⁷ The aza lactones were converted to the corresponding phenylpyruvic acids for use in method A by a two-step hydrolysis with aqueous sodium hydroxide followed by aqueous hydrochloric acid, or were employed directly in method B. Analytical and physical data for final compounds are reported in Tables 1–3.

Results and Discussion

After a brief examination of the carbon tether between the tetrahydro- β -carboline and the aromatic ring demonstrated a significant advantage for a single methylene unit (**6**, **17**, **18**), we turned our attention to the preliminary evaluation of substituent effects on the aromatic ring E (Table 1). While the parent tetracycle **19** had approximately 5-fold higher affinity than yohimbine, addition of both electron-withdrawing and electron-donating substituents to ring E resulted in further

Scheme 4

**Table 1.** Physical and Biological Data of Tetrahydro- β -carbolines

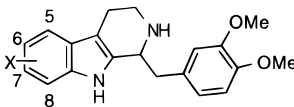
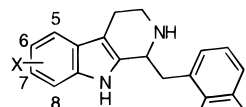
compd	<i>n</i>	Y	mol formula	mp (°C)	meth prep	rat stomach fundus -log K _B
1 (yohimbine)			C ₂₁ H ₂₆ N ₂ O ₃ ·HCl			6.92 ± 0.12(3)
2			C ₁₉ H ₂₄ N ₂			7.26 ± 0.06(3)
3			C ₁₅ H ₁₈ N ₂ ·HCl			6.66 ± 0.24(3)
4			C ₁₁ H ₁₂ N ₂ ·HCl			<5.52(3)
5			C ₂₁ H ₂₂ N ₂ O ₂ ·HCl			<6.52(3)
6	1	3,4-OMe	C ₂₀ H ₂₂ N ₂ O ₂ ·HCl			9.17 ± 0.11(7)
7			C ₂₀ H ₂₂ N ₂ O ₂ ·HCl			<5.52(3)
17	0	3,4-OMe	C ₁₉ H ₂₁ N ₂ O ₂ ·HCl			<6.53(3)
18	2	3,4-OMe	C ₂₁ H ₂₃ N ₂ O ₂ ·HCl			7.07 ± 0.18(6)
19	1	H	C ₁₈ H ₁₈ N ₂ ·HCl	273–4	A	7.61 ± 0.09(3)
20	1	4-Me	C ₁₉ H ₂₀ N ₂ ·HCl	250–1	A	7.84 ± 0.06(3)
21	1	4-OMe	C ₁₉ H ₂₀ N ₂ O·HCl	250–1 dec	A	8.09 ± 0.07(3)
22	1	3-OMe	C ₁₉ H ₂₀ N ₂ O·HCl	256–7	A	8.04 ± 0.20(3)
23	1	3,4-Cl	C ₁₈ H ₁₆ Cl ₂ N ₂ ·HCl	255–7	A	8.22 ± 0.1(3)
24	1	2,5-OMe	C ₂₀ H ₂₂ N ₂ O ₂ ·HCl	222–5 dec	A	7.75 ± 0.07(4)
25	1	3,4,5-OMe	C ₂₁ H ₂₄ N ₂ O ₃ ·HCl·H ₂ O	175–8	A	8.1 ± 0.23(5)
26	1	3,4-OCH ₂	C ₁₉ H ₁₈ N ₂ O ₂ ·HCl	272–4 dec	A	8.4 ± 0.2(3)
27	1	3,4-OEt	C ₂₂ H ₂₆ N ₂ O ₂ ·HCl	210–2	A	8.57 ± 0.11(9)
28	1	2-Cl,3,4-OMe	C ₂₀ H ₂₁ ClN ₂ O ₂ ·HCl	240–1	A	9.27 ± 0.12(9)
29	1	2,3-benzo	C ₂₂ H ₂₀ N ₂ ·HCl	259–60	A	9.35 ± 0.12(9)
30	1	3,4-benzo	C ₂₂ H ₂₀ N ₂ ·HCl	175 dec	A	7.84 ± 0.12(3)

increases in affinity. The most significant positive substituent effects correlated best with steric parameters and indicated that occupancy of the 2-, 3-, and 4-positions was favorable, while substitution at the 5-position was less advantageous. The 1-naphthyl derivative **29** possessed the highest affinity of the tetrahydro- β -carboline antagonists identified thus far. This substituent, along with the 3,4-dimethoxyphenyl (as in **6**), was chosen for the ensuing phase of SAR studies.

We next examined substituent effects on the indole portion of the tetrahydro- β -carboline template. Upon even casual inspection, the superposition of serotonin upon the tetracyclic tetrahydro- β -carboline framework suggests a relationship between C-5 of the natural ligand with C-6 of our antagonist structure. Thus, we were somewhat surprised to discover that introduction

of oxygen substituents at this position (MeO, **31** and BnO, **32**) resulted in considerable loss of affinity relative to **6** (Table 2).⁴⁷ Further examination revealed a trend with halogen substitution at C-6 showing F $\approx$ Cl < Br < H < I, suggesting a negative effect of electron-withdrawing substituents gradually overcome by a positive steric effect. Also noteworthy were the effects of the "soft" or polarizable substituents MeS (**38**) and I (**37**), which resulted in a 5-fold increase in affinity relative to the unsubstituted analogs. Introduction of simple alkyl groups also had a remarkable positive influence upon the 5HT_{2B} receptor antagonist affinity. The beneficial effect of alkyl substituents was limited, however, to those of small size with the greatest effect observed with the 6-Me substituent (**39** and **40**) resulting in a 8-fold increase in affinity. The 6-Et analogs (**42** and **43**) showed improved affinity relative to the

Table 2. Physical and Biological Data of Tetrahydro- β -carbolines

		 3,4 Dimethoxyphenyl (DMP) Series		 1-Naphthyl (NP) Series		
compd	series	X	mol formula	mp (°C)	meth prep	rat stomach fundus -log K_B
6	DMP	H	C ₂₀ H ₂₂ N ₂ O ₂ ·HCl			9.17 ± 0.11(7)
29	NP	H	C ₂₂ H ₂₀ N ₂ ·HCl	259–60	A	9.35 ± 0.12(13)
31	DMP	6-OMe	C ₂₁ H ₂₄ N ₂ O ₃ ·HCl	232–3	A	8.48 ± 0.30(5)
32	DMP	6-OBn	C ₂₇ H ₂₈ N ₂ O ₃ ·HCl	232–3	A	6.73 ± 0.40(3)
33	DMP	6-F	C ₂₀ H ₂₁ FN ₂ O ₂ ·HCl	238–40	A	8.49 ± 0.10(3)
34	DMP	6-Cl	C ₂₀ H ₂₁ ClN ₂ O ₂ ·C ₄ H ₄ O ₄ ·H ₂ O	220–2	B	8.36 ± 0.14(3)
35	NP	6-Cl	C ₂₂ H ₁₉ ClN ₂ ·HCl	280–5 dec	B	9.16 ± 0.13(3)
36	DMP	6-Br	C ₂₀ H ₂₁ BrN ₂ O ₂ ·C ₄ H ₄ O ₄	184–8	B	8.80 ± 0.17(3)
37	DMP	6-I	C ₂₀ H ₂₁ IN ₂ O ₂ ·HCl	270–3 dec	B	9.52 ± 0.17(3)
38	DMP	6-SMe	C ₂₁ H ₂₄ N ₂ O ₂ S·HCl· ¹ / ₂ H ₂ O	224–7	A	9.56 ± 0.15(3)
39	DMP	6-Me	C ₂₁ H ₂₄ N ₂ O ₂ ·HCl	245–6 dec	B	9.86 ± 0.14(15)
40	NP	6-Me	C ₂₃ H ₂₂ N ₂ ·HCl	276–80 dec	B	9.75 ± 0.11(8)
41	NP	6-CO ₂ H	C ₂₃ H ₂₀ N ₂ O ₂ ·HCl	284–7 dec	B	<7.5(4)
42	DMP	6-Et	C ₂₂ H ₂₆ N ₂ O ₂ ·C ₄ H ₄ O ₄	185 dec	B	9.46 ± 0.11(6)
43	NP	6-Et	C ₂₄ H ₂₄ N ₂ ·C ₄ H ₄ O ₄	212–4 dec	B	9.65 ± 0.14(4)
44	DMP	6- <i>n</i> -Pr	C ₂₃ H ₂₈ N ₂ O ₂ ·C ₄ H ₄ O ₄	195–7 dec	B	8.64 ± 0.27(3)
45	DMP	6- <i>n</i> -Bu	C ₂₄ H ₃₀ N ₂ O ₂ ·C ₄ H ₄ O ₄	191–3	B	<7.5(4)
46	DMP	6- <i>i</i> -Pr	C ₂₃ H ₂₈ N ₂ O ₂ ·C ₄ H ₄ O ₄	196–200	B	8.50 ± 0.13(4)
47	DMP	6- <i>c</i> -Hex	C ₂₆ H ₃₂ N ₂ O ₂ ·C ₄ H ₄ O ₄	208–10 dec	B	<7.5
48	DMP	6-Ph	C ₂₆ H ₂₆ N ₂ O ₂ ·HCl	253–5	B	<7.0
49	DMP	7-OMe	C ₂₁ H ₂₄ N ₂ O ₃ ·HCl	223–4	B	7.31 ± 0.03(3)
50	NP	8-OMe	C ₂₃ H ₂₂ N ₂ O·C ₄ H ₄ O ₄	240–2 dec	B	9.72 ± 0.30(3)
51	NP	8-Br	C ₂₂ H ₁₉ BrN ₂ ·HCl	229–35 dec	B	9.10 ± 0.28(3)
52	DMP	8-Me	C ₂₁ H ₂₄ N ₂ O ₂ ·C ₄ H ₄ O ₄	168 dec	B	9.0 ± 0.07(3)
53	DMP	5-F,6-Me	C ₂₁ H ₂₃ FN ₂ O ₂ ·C ₄ H ₄ O ₄	191–4	B	9.3 ± 0.12(7)
54	NP	5-F,6-Me	C ₂₃ H ₂₁ FN ₂ ·C ₄ H ₄ O ₄	253–5 dec	B	9.69 ± 0.21(3)
55	DMP	5,7-Me	C ₂₂ H ₂₆ N ₂ O ₂ ·HCl	259–61	B	9.34 ± 0.12(3)
56	DMP	6,7-Me	C ₂₂ H ₂₆ N ₂ O ₂ ·C ₄ H ₄ O ₄	197–200	B	9.71 ± 0.14(6)
57	DMP	6,8-Me	C ₂₂ H ₂₆ N ₂ O ₂ ·HCl	257–9	B	9.61 ± 0.22(5)
58	DMP	7,8-Me	C ₂₂ H ₂₆ N ₂ O ₂ ·HCl	278–80	B	10.12 ± 0.18(3)
59	DMP	4,8-Me	C ₂₂ H ₂₆ N ₂ O ₂ ·HCl	275–7	B	9.29 ± 0.18(4)
60	DMP	7,8-benzo	C ₂₄ H ₂₄ N ₂ O ₂ ·C ₄ H ₄ O ₄	187–9 dec	B	9.22 ± 0.05(3)
61	DMP	6,8-F	C ₂₀ H ₂₀ F ₂ N ₂ O ₂ ·C ₄ H ₄ O ₄	185–8 dec	B	9.60 ± 0.13(7)
62	DMP	6-Me,8-Br	C ₂₁ H ₂₃ BrN ₂ O ₂ ·HCl	253–5	B	8.92 ± 0.29(4)
63	NP	6-Me,8-Br	C ₂₃ H ₂₁ BrN ₂ ·C ₄ H ₄ O ₄	208–11 dec	B	8.82 ± 0.11(3)
64	DMP	7-Me,8-Br	C ₂₁ H ₂₃ BrN ₂ O ₂ ·HCl	266–7	B	9.02 ± 0.35(3)
65	NP	7-Me,8-Br	C ₂₃ H ₂₁ BrN ₂ ·HCl	276–9 dec	B	8.56 ± 0.43(3)

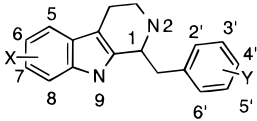
parent structures, but affinity rapidly diminished with increased steric bulk, resulting in a loss of affinity with the 6-*n*Bu (**45**) and 6-*c*Hex (**47**) analogs.

Our examination of the effects of substitution at alternate locations of the indole platform was less detailed, but resulted in some interesting observations. In similar fashion to the 6-OMe analog (**31**), the 7-OMe derivative (**49**) showed diminished affinity relative to the parent. In contrast, however, the 8-OMe analog (**50**) was among the highest affinity compounds. Further divergence of the C-8 SAR from the C-6 pattern of substituent effects showed that substitution by halogen (8-Br, **51**) or alkyl (8-Me, **52**) was without marked effect on affinity. The effects of disubstitution on the indole ring were also briefly investigated, and the results were consistent with our earlier observations. Most striking were the effects resulting from introduction of a 7-methyl substituent, which increased affinity 10-fold in conjunction with the 8-methyl group (**58** versus **52**), whereas it had little effect in conjunction with the 6-methyl (**56**) or the 8-bromo (**64** and **65**) analogs.

Our final efforts were directed to the examination of the ring E substituent effects in the 6-methyltetrahydro- β -carboline series (Table 3). The results were in good

agreement with those obtained in our initial studies on the unsubstituted indole series, with the 3–10-fold increases in affinity attributed to the 6-methyl substituent maintained across a variety of E-ring substitution patterns. Also interesting was the lack of tolerance of alkylation on either nitrogen atom of the tetrahydro- β -carboline, resulting in a 10- to >100-fold loss of affinity (**79**–**82**). Likewise, introduction of C-1 methyl substitution resulted in similar dramatic loss of affinity (**83**).

With the publication of other 5HT_{2B(2C)} receptor antagonists, we sought to identify parallels that might exist among the different series. Unfortunately, only limited comparisons of SAR trends from our series to those observed in the indolylurea or indolonaphthyridine can be made at this time due to a shortage of published SAR data on the latter series. However, SAR data has been published for the 2-methyl-3-ethyl-5-(dimethylamino)indole (“medmain”) series of 5HT_{2B} (“fundal”) receptor antagonists, from which the indolylureas were derived.²⁰ The loss of 5HT_{2B} receptor affinity upon indole N-alkylation suggests that direct indole superposition of the indolylureas (such as **9** or **10**) and the tetrahydro- β -carbolines is unlikely to reflect their relative receptor-binding orientations, since N-alkyla-

Table 3. Physical and Biological Data of Tetrahydro- β -carbolines


compd	X	Y	mol formula	mp (°C)	meth prep	rat stomach fundus -log K _B
39	6-Me	3',4'-OMe	C ₂₁ H ₂₄ N ₂ O ₂ ·HCl	245–6 dec	B	9.86 ± 0.14(15)
40	6-Me	2',3'-benzo	C ₂₃ H ₂₂ N ₂ ·HCl	276–80 dec	B	9.75 ± 0.11(8)
66	6-Me	3'-OH,4'-OMe	C ₂₀ H ₂₂ N ₂ O ₂ ·HCl	272–4	B	9.23 ± 0.24(3)
67	6-Me	2'-Cl,3',4'-OMe	C ₂₁ H ₂₃ ClN ₂ O ₂ ·HCl	244 dec	A	9.80 ± 0.15(4)
68	6-Me	2'-Br,3',4'-OMe	C ₂₁ H ₂₃ BrN ₂ O ₂ ·HCl	272–4 dec	A	9.70 ± 0.22(4)
69	6-Me	2'-NO ₂ ,3',4'-OMe	C ₂₁ H ₂₃ N ₃ O ₄ ·HCl	233 dec	B	9.16 ± 0.21(3)
70	6-Me	2'-NH ₂ ,3',4'-OMe	C ₂₁ H ₂₅ N ₃ O ₂ ·2HCl	214 dec	B	9.02 ± 0.08(4)
71	6-Me	5'-I,3',4'-OMe	C ₂₁ H ₂₃ IN ₂ O ₂ ·C ₄ H ₄ O ₄	163–6 dec	B	8.12 ± 0.16(7)
72	6-Me	5'-NO ₂ ,3',4'-OMe	C ₂₁ H ₂₃ N ₃ O ₄ ·HCl	239–43	B	8.55 ± 0.10(4)
73	6-Me	5'-NH ₂ ,3',4'-OMe	C ₂₁ H ₂₅ N ₃ O ₂ ·2HCl	230–4	B	8.89 ± 0.12(4)
74	6-Me	3',5'-F	C ₁₉ H ₁₈ F ₂ N ₂ ·C ₄ H ₄ O ₄	205 dec	B	9.05 ± 0.03(4)
75	6-Me	3',4'-F	C ₁₉ H ₁₈ F ₂ N ₂ ·C ₄ H ₄ O ₄	216 dec	B	8.47 ± 0.24(5)
76	6-Me	3'-F,4'-OMe	C ₂₀ H ₂₁ FN ₂ O·HCl	277–9	B	9.42 ± 0.18(5)
77	6-Me	3'-CF ₃	C ₂₀ H ₁₉ F ₃ N ₂ ·HCl	252–3	B	8.91 ± 0.17(7)
78	6-Me	3',4'-Me	C ₂₁ H ₂₄ N ₂ ·HCl	285–7 dec	B	9.06 ± 0.27(3)
79	2,6-Me	2'-Cl,3',4'-OMe	C ₂₂ H ₂₅ ClN ₂ O ₂ ·C ₄ H ₄ O ₄	172–6 dec	B	8.40 ± 0.40(3)
80	2,6,9-Me	2'-Cl,3',4'-OMe	C ₂₃ H ₂₇ ClN ₂ O ₂ ·HCl	227–9 dec	B	<7.52(4)
81	2,6-Me	2',3'-benzo	C ₂₄ H ₂₄ N ₂ ·HCl	218–21	B	~7.52(4)
82	6,9-Me	3',4'-OMe	C ₂₂ H ₂₇ N ₂ O ₂ ·HCl	146–50	B	<7.52(4)
83	1,6-Me	3',4'-OMe	C ₂₂ H ₂₆ N ₂ O ₂ ·C ₄ H ₄ O ₄	183–5	B	<7.52(4)
84	6-Me	“perhydro”	C ₁₉ H ₂₆ N ₂ ·HCl	230 dec	A	8.32 ± 0.17(3)

Table 4. 5HT_{2A} and 5HT_{2C} Receptor Binding Affinities and Rat 5HT_{2B} (Fundus) Affinity of Tetrahydro- β -carbolines

compd	-log K _i		-log K _B 5HT _{2B}	ratio 2B/2A	ratio 2B/2C
	5HT _{2A}	5HT _{2C}			
1 (yohimbine)	5.79 ± 0.26(3)	<5.0	6.92 ± 0.12(3)	13.5	83.0
6 (LY23728)	7.18 ± 0.08(3)	6.90 ± 0.07(3)	9.17 ± 0.11(7)	97.7	186.2
39	7.65 ± 0.09(3)	7.92 ± 0.05(3)	9.86 ± 0.14(15)	162.2	87.1
40	8.45 ± 0.15(3)	8.68 ± 0.10(3)	9.75 ± 0.11(8)	20.2	11.8
54	8.57 ± 0.20(3)	9.02 ± 0.20(3)	9.69 ± 0.21(3)	13.2	4.68
56	8.33 ± 0.07(3)	8.51 ± 0.03(3)	9.71 ± 0.14(6)	24.0	15.8
58 (LY287375)	8.06 ± 0.12(3)	8.11 ± 0.08(3)	10.12 ± 0.18(3)	114.8	102.3
64 (LY288345)	8.53 ± 0.20(3)	8.69 ± 0.14(3)	9.02 ± 0.35(3)	3.09	2.14
67 (LY266097)	7.71 ± 0.12(3)	7.61 ± 0.04(3)	9.80 ± 0.15(4)	123.0	154.9
68	7.78 ± 0.16(3)	7.73 ± 0.05(3)	9.70 ± 0.22(4)	83.2	93.3
8, SDZ SER-082	6.2 ± 0.1–0.2 ^a	7.8 ± 0.1–0.2 ^b	7.34 ± 0.1–0.2 ^c	13.8	0.35
9, SB 200646A	<5.2 ^d	6.86 ± 0.07 ^e	7.40 ± 0.06 ^f	>158.5	3.50
10, SB 206553	5.79 ± 0.01 ^g	8.00 ± 0.02 ^h	8.48 ± 0.05 ⁱ	489.8	3.02
11, SB 204741	<5.2 ^d	5.82 ± 0.02 ^e	7.95 ± 0.11 ^f	>562	135

^a Binding affinity, rat cortex, [³H]ketanserin, ref 18. ^b Binding affinity, pig choroid plexus, [³H]mesulergine, ref 18. ^c Rat stomach fundus, ref 18. ^d Binding affinity, human clone, [³H]ketanserin, ref 19. ^e Binding affinity, human clone, [³H]mesulergine, ref 19. ^f Rat stomach fundus, ref 19. ^g Binding affinity, human clone, [³H]ketanserin, ref 17. ^h Binding affinity, human clone, [³H]mesulergine, ref 17. ⁱ Rat stomach fundus, ref 17.

tion of the indolylureas had little effect on affinity. Likewise, the strong preference for the secondary amine over the tertiary amine analogs in the tetrahydro- β -carboline series would suggest a rather different environment around the basic amine for the receptor-bound ligand than that experienced by the indolonaphthyridine (**8**). Further comparison among the series awaits publication of more detailed SAR data for the indolylureas and indolonaphthyridines.

Having identified 5HT_{2B} receptor antagonists with high affinity, we examined the 5HT_{2A} and 5HT_{2C} receptor binding affinity of selected compounds from the series as a measure of selectivity within the 5HT₂ receptor family. Table 4 illustrates the comparison of radioligand binding data obtained at antagonist-labeled rat 5HT_{2A} and mouse 5HT_{2C} receptors with functional rat 5HT_{2B} receptor antagonism. Interestingly, compound **64** (LY288345) emerged as a high-affinity, non-selective 5HT₂ receptor antagonist. For many of the

remaining potent members of the series, however, there was 80- to >180-fold selectivity for the 5HT_{2B} receptor relative to both the 5HT_{2A} and 5HT_{2C} receptors. Thus, these compounds represent the first high affinity antagonists to demonstrate substantial selectivity for 5HT_{2B} receptor *vis a vis* both closely related 5HT₂ receptor subtypes. Because of our awareness of the issues surrounding the use of mixed data types (radioligand binding versus functional studies, species differences, etc.) and to support further the observed selectivity profile, we examined a set of compounds for their functional 5HT_{2A} receptor antagonism. To avoid species differences, we utilized blockade of the serotonin-induced contraction of the rat jugular vein (Table 5) for *in vitro* assessment of the 5HT_{2A} receptor-mediated effects. The data obtained therein are also consistent with 50–500-fold selectivity for the 5HT_{2B} receptor-mediated response and are in good agreement with our binding data.

Table 5. Rat 5HT_{2B} and Rat 5HT_{2A} Affinity of Tetrahydro- β -carboline Antagonists

compd	-log K _B	
	5HT _{2B} (rat stomach fundus)	5HT _{2A} (rat jugular vein)
1 (yohimbine)	6.92 $\pm$ 0.12(3)	6.37 $\pm$ 0.11(3)
6	9.17 $\pm$ 0.11(7)	7.43 $\pm$ 0.09(6)
39	9.86 $\pm$ 0.14(15)	7.21 $\pm$ 0.20(4)
40	9.75 $\pm$ 0.11(8)	8.07 $\pm$ 0.10(8)
67	9.80 $\pm$ 0.15(4)	8.14 $\pm$ 0.10(6)

For comparative purposes, it is apparent that certain compounds from the present series have considerably higher affinity for the 5HT_{2B} receptor in the rat stomach fundus than previously reported 5HT_{2B} receptor antagonists. Additionally, compounds **6** (LY23728), **58** (LY287375), and **67** (LY266097) are more than 100-fold selective for the rat 5HT_{2B} receptor versus both the 5HT_{2A} and 5HT_{2C} receptors as demonstrated both by radioligand binding and by functional measures. These compounds should prove useful in elucidating the role of serotonin acting at the 5HT_{2B} receptors *vis a vis* 5HT_{2A} or 5HT_{2C} receptors in normal and disease physiology. Ultimately, such agents could have therapeutic potential in the treatment of anxiety,³⁸ migraine,^{14,39–41} and other disorders.

Conclusions

A series of potent tetrahydro- β -carboline 5HT_{2B} receptor antagonists has been identified on the basis of the yohimbine platform, with SAR studies resulting in 1000-fold increase in 5HT_{2B} receptor affinity for several compounds relative to yohimbine. High-affinity compounds from this series have been identified which are able to discriminate among the 5HT₂ family of serotonin receptors with >100-fold selectivity for 5HT_{2B} receptors over both 5HT_{2A} and 5HT_{2C} receptors as demonstrated by radioligand binding and functional studies. More detailed pharmacological investigation of members of this series will be reported in due course.

Experimental Section

General. Solvents and reagents were obtained from commercial suppliers and were used without further purification except as noted. Benzaldehydes were purified by recrystallization from suitable solvents (hexanes, diethyl ether) or by vacuum distillation prior to use. The tryptamines employed were obtained from commercial suppliers or were prepared by literature methods. Melting points were determined in open capillary tubes on a Gallencamp melting point apparatus and are uncorrected. ¹H NMR were determined at 300 MHz in CDCl₃ or DMSO-*d*₆ and chemical shifts reported downfield of TMS. Elemental analyses were performed by the Physical Chemistry Department of Eli Lilly and Company. TLC analysis was conducted on silica gel plates visualized with UV at 254 nm and/or staining with *p*-anisaldehyde solution and heating. Preparative chromatography was carried out on silica gel using modified flash chromatography conditions of Still.⁴²

General Procedure for the Condensation of Carbonyl Compounds with Tryptamines (Method A). To a solution of tryptamine hydrochloride (1.0 g, 5.0 mmol) in 50 mL of absolute ethanol was added phenylacetaldehyde (702 μ L, 6.0 mmol) [alternatively, phenylpyruvic acids were employed in equivalent fashion], and the solution was heated to reflux for 24–72 h under an atmosphere of nitrogen. The reaction was allowed to cool to room temperature and was concentrated under reduced pressure. The residue was dissolved in 5% 2-propanol/chloroform and made basic by addition of saturated

sodium carbonate solution. The layers were separated, and the aqueous layer was back extracted with 2 volumes of 5% 2-propanol/chloroform. The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on silica, eluting with methanol/ or 2-propanol/chloroform solutions (in the presence of 0.1% ammonium hydroxide solution). Fractions containing product were pooled and concentrated under reduced pressure. The free base was dissolved in diethyl ether/methanol and treated either with excess dry HCl gas to form the hydrochloride salt or with 1 equiv of maleic acid to prepare the maleate salt. The resulting salt was isolated by filtration, washed with 2-propanol, and dried under reduced pressure.

General Procedure for the Hydrolytic Condensation of Aza Lactones with Tryptamines: Method B, with Free Base Isolation, Preparation of Maleate Salt. To 20 mL of a 1.0 N solution of hydrochloric acid was introduced 1.14 g (5.41 mmol) of 5-methyltryptamine hydrochloride followed by 2.00 g (7.21 mmol) of aza lactone. Stirring was initiated, and the mixture was heated to reflux for 12–72 h under an atmosphere of nitrogen. The reaction was allowed to cool to room temperature, diluted with 5% 2-propanol/chloroform, and made basic by addition of saturated sodium bicarbonate solution. The layers were separated, and the aqueous layer was back extracted with 2 volumes of 5% 2-propanol/chloroform. The combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. The residue was purified by flash chromatography on silica, eluting with methanol/ or 2-propanol/chloroform solutions (in the presence of 0.1% ammonium hydroxide solution). Fractions containing product were pooled and concentrated under reduced pressure. The free base was dissolved in diethyl ether/methanol and treated with 1 equiv of maleic acid with stirring. The resulting salt was isolated by filtration, washed with 2-propanol, and dried under reduced pressure.

Method B, with Direct Preparation of Hydrochloride Salt. To 20 mL of a 1.0 N solution of hydrochloric acid was introduced 1.0 g (4.75 mmol) of 5-methyltryptamine hydrochloride followed by 1.1–1.5 equiv of the appropriate aza lactone. Stirring was initiated, and the mixture was heated to reflux for 12–72 h under an atmosphere of nitrogen. The reaction mixture was allowed to cool to room temperature, and the resulting precipitate was collected by filtration. The solid was washed with cold water, triturated with 2-propanol, washed with diethyl ether, and dried in a vacuum oven. The tetrahydro- β -carboline was isolated as its hydrochloride salt with acceptable purity for biological evaluation. Physical data are summarized in Tables 1, 2, and 3. Table 6, containing MS and elemental analysis can be found in the Supporting Information.

General Procedure for Preparation of Aza Lactones 14. A solution of 0.16 mol of the aromatic carboxaldehyde (freshly distilled or recrystallized before use) was dissolved in 147 mL of acetic anhydride. *N*-Acetyltryptamine (19.0 g, 0.16 mol) was added followed by sodium acetate (13.1 g, 0.16 mol), and the mixture was heated to 100 °C in an oil bath for 4 h. The flask containing the reaction mixture was allowed to remain in the oil bath under continued stirring during a slow overnight cooling. During this time, the aza lactone precipitated, typically as an orange/red granular solid. The solid was isolated by filtration. Washing with 100 mL of cold diethyl ether and drying under reduced pressure afforded aza lactone of suitable purity for use in subsequent synthetic operations.

General Procedure for Preparation of Phenylpyruvic Acids (13, R = CO₂H). A suspension of aza lactone (0.125 mol) in 200 mL of 1.0 N sodium hydroxide solution was heated on a steam bath until homogeneous. The solution was acidified with 5 N HCl solution until acidic by Congo Red paper. Concentrated HCl (30 mL) was added followed by water to a final volume of 500 mL, and the mixture was heated to reflux for 4 h. The mixture was cooled to 4 °C, and the product acid was isolated by filtration. Washing with water and drying under reduced pressure afforded phenylpyruvic acid of suitable purity for use in subsequent operations (method A).

Isolation of Tissue for Receptor Antagonist Studies.

Male Wistar rats (150–375 g; Harlan Sprague–Dawley, Inc.) were sacrificed by cervical dislocation, and longitudinal sections of the stomach fundus were prepared for *in vitro* examination. Four preparations were obtained from one rat stomach fundus. In some experiments, external jugular veins from the rats were dissected free of connective tissue, cannulated *in situ* with polyethylene tubing (PE-50, o.d. = 0.97 mm), and placed in Petri dishes containing Krebs' bicarbonate buffer (see below). The tips of two 30-gauge stainless steel hypodermic needles bent into an L-shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was tied with thread to a stationary rod and the upper thread was tied to a transducer. Stomach strips and vascular rings were mounted in organ baths containing 10 mL of modified Krebs' solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; CaCl₂, 1.6; KH₂PO₄, 1.2; MgSO₄, 1.2; dextrose, 10.0; NaHCO₃, 24.8. Tissue bath solutions were maintained at 37 °C and equilibrated with 95% O₂–5% CO₂. Jugular veins and stomach strips were placed under optimum resting force (1g and 4g, respectively) and were allowed to equilibrate for approximately 1 h before exposure to drugs. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and a microscale accessory attachment.

Determination of Apparent 5HT_{2A} and 5HT_{2B} Receptor Antagonist Dissociation Constants. After control cumulative contractile responses to serotonin were obtained in the stomach fundus (5HT_{2B}) and jugular vein (5HT_{2A}), the tissues were incubated with an appropriate concentration of antagonist for 1 h. Contractile responses to serotonin were then repeated in the presence of the antagonist. Only one antagonist concentration was examined in each tissue. Apparent antagonist dissociation constants (K_B) were determined for each concentration of antagonist according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1) \quad (1)$$

where [B] is the concentration of the antagonist and dose ratio is the ED₅₀ of the agonist in the presence of the antagonist divided by the control ED₅₀. These results were then expressed as the negative logarithm of the K_B (i.e., $-\log K_B$).

Membrane Preparation for Cloned Cells. Full-length cDNA clones encoding the mouse 5HT_{2C} receptor⁴³ and the rat 5HT_{2A} receptors⁴⁴ were inserted into the eukaryotic expression vector phd.⁴⁵ AV12 cells (Syrian hamster fibroblasts, ATCC no. CRL 9595) were transformed with the receptor-vector constructs, as previously described,⁴⁶ using the calcium phosphate coprecipitation method. Methotrexate or hygromycin resistance was used to select for stably transformed cell clones which were isolated and tested for receptor expression by cytoplasmic dot hybridization using a ³²P-labeled cDNA probe followed by radioligand binding assays.

Radioligand Binding Assays. Cell membranes were prepared for binding studies as previously described¹⁵ with the final resuspension in 67 mM Tris-HCl, pH 7.4. For the cells containing the 5HT_{2A} receptor this resuspension was equivalent to approximately $(7.5\text{--}15) \times 10^6$ cells/mL of buffer, and for the 5HT_{2C} containing cells it was 2×10^6 cells/mL. Measurement of the 5HT_{2A} receptors was carried out as previously described⁴⁶ using [³H]ketanserin. The assay volume was 0.8 mL with the following final composition: 50 mM Tris-HCl, pH 7.6, 100 nM prazosin, and 0.5 nM [³H]ketanserin. The 5HT_{2C} receptor binding assay was adapted from that originally described by Pazos et al.⁴⁷ The final assay volume was 0.8 mL containing 10 μ M pargyline, 0.2 nM [³H]mesulergine, and 50 mM Tris-HCl, pH 7.6. For both assays competing compounds were added to generate six-point competition curves spanning 10^{-10} – 10^{-5} M. Tubes were incubated at 37 °C for 15 min for the 5HT_{2A} receptor and 30 min for the 5HT_{2C} receptor assays, and the incubations were terminated by filtration through GF/B filters. Washing of the filters and determination of bound radioactivity were determined as

previously described.¹⁵ The level of nonspecific binding for both assays was determined in the presence of 3 μ M mianserin. K_i values for the compounds were calculated using the Cheng–Prusoff equation⁴⁸ as previously described.¹⁵

Supporting Information Available: Table 6, containing MS and elemental analysis for compounds **19–84** and ¹H NMR (300 MHz) spectra for tetrahydro- β -carbolines **19–84** (69 pages). Ordering information is given on any current masthead page.

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