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

James E. Audia,* Deborah A. Evrard, Gwyn R. Murdoch, James J. Droste, Jeffrey S. Nissen, Kathy W. Schenck, Pawel Fludzinski, Virginia L. Lucaites, David L. Nelson, and Marlene L. Cohen

Lilly Research Laboratories, A Division of Eli Lilly & Company, Indianapolis, Indiana 46285

Received January 22, 1996[⊗]

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 ($-\log K_B$s >10.0 have been obtained). These high-affinity tetrahydro-β-carboline antagonists are able to discriminate among the $5HT_2$ 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.

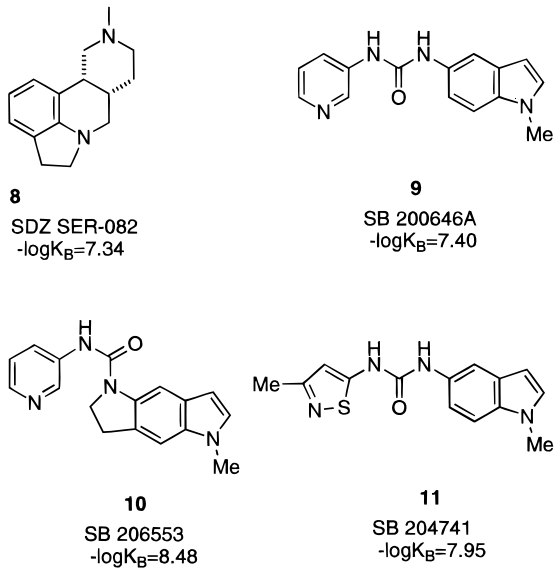
Introduction

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 $5HT_2$ 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



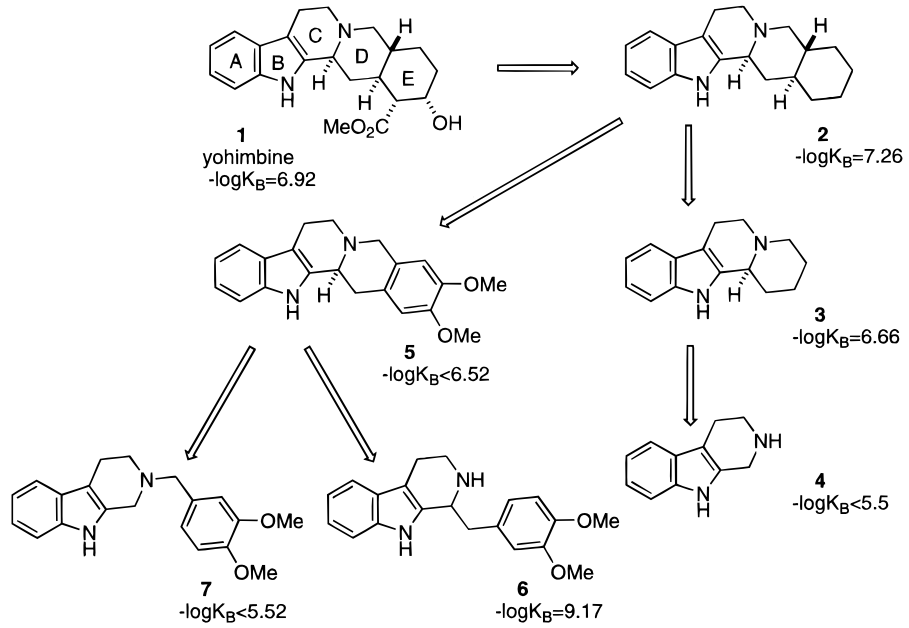
utilizing the serotonin-induced contraction via the $5HT_{2B}$ receptor in rat stomach fundus smooth muscle strips in order to assess directly both affinity and antagonist activity of the newly synthesized ligands (Tables 1−5). We initiated such studies with a survey of several partial structural analogs 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

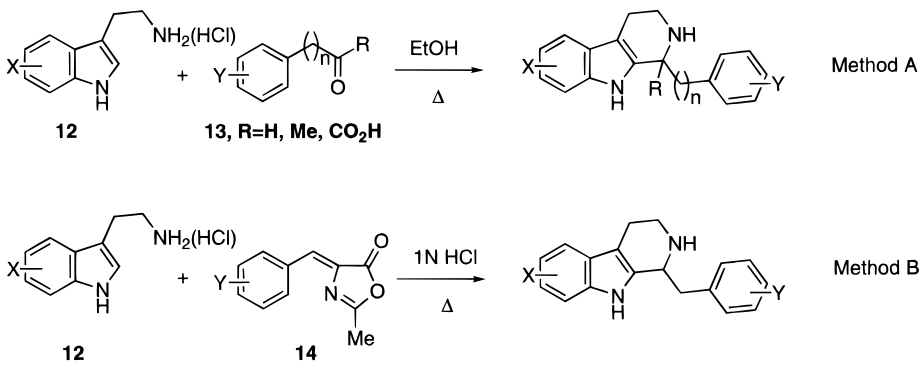
[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1996.

S0022-2623(96)00062-3 CCC: $12.00

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 ($-\log K_{B,\ 5HT2B} = 9.17$ vs $-\log K_{B,\alpha2} = 6.13$). 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

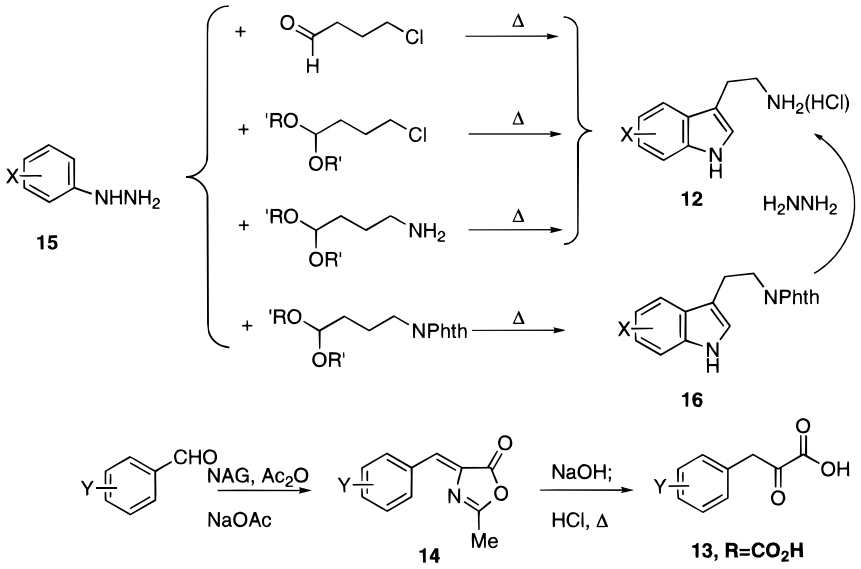
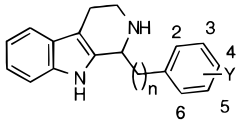


Table 1. Physical and Biological Data of Tetrahydro-β-carbolines

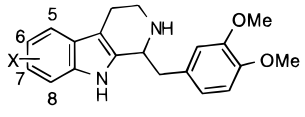


compd	*n*	Y	mol formula	mp (°C)	meth prep	rat stomach fundus $-\log K_B$
1 (yohimbine)			$C_{21}H_{26}N_2O_3 \cdot HCl$			6.92 ± 0.12(3)
2			$C_{19}H_{24}N_2$			7.26 ± 0.06(3)
3			$C_{15}H_{18}N_2 \cdot HCl$			6.66 ± 0.24(3)
4			$C_{11}H_{12}N_2 \cdot HCl$			<5.52(3)
5			$C_{21}H_{22}N_2O_2 \cdot HCl$			<6.52(3)
6	1	3,4-OMe	$C_{20}H_{22}N_2O_2 \cdot HCl$			9.17 ± 0.11(7)
7			$C_{20}H_{22}N_2O_2 \cdot HCl$			<5.52(3)
17	0	3,4-OMe	$C_{19}H_{21}N_2O_2 \cdot HCl$			<6.53(3)
18	2	3,4-OMe	$C_{21}H_{23}N_2O_2 \cdot HCl$			7.07 ± 0.18(6)
19	1	H	$C_{18}H_{18}N_2 \cdot HCl$	273−4	A	7.61 ± 0.09(3)
20	1	4-Me	$C_{19}H_{20}N_2 \cdot HCl$	250−1	A	7.84 ± 0.06(3)
21	1	4-OMe	$C_{19}H_{20}N_2O \cdot HCl$	250−1 dec	A	8.09 ± 0.07(3)
22	1	3-OMe	$C_{19}H_{20}N_2O \cdot HCl$	256−7	A	8.04 ± 0.20(3)
23	1	3,4-Cl	$C_{18}H_{16}Cl_2N_2 \cdot HCl$	255−7	A	8.22 ± 0.1(3)
24	1	2,5-OMe	$C_{20}H_{22}N_2O_2 \cdot HCl$	222−5 dec	A	7.75 ± 0.07(4)
25	1	3,4,5-OMe	$C_{21}H_{24}N_2O_3 \cdot HCl \cdot H_2O$	175−8	A	8.1 ± 0.23(5)
26	1	3,4-OCH_2	$C_{19}H_{18}N_2O_2 \cdot HCl$	272−4 dec	A	8.4 ± 0.2(3)
27	1	3,4-OEt	$C_{22}H_{26}N_2O_2 \cdot HCl$	210−2	A	8.57 ± 0.11(9)
28	1	2-Cl,3,4-OMe	$C_{20}H_{21}ClN_2O_2 \cdot HCl$	240−1	A	9.27 ± 0.12(9)
29	1	2,3-benzo	$C_{22}H_{20}N_2 \cdot HCl$	259−60	A	9.35 ± 0.12(9)
30	1	3,4-benzo	$C_{22}H_{20}N_2 \cdot HCl$	175 dec	A	7.84 ± 0.12(3)

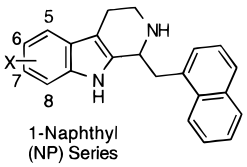
increases in affinity. The most significant positive substituent effects correlated best with steric parameters and indicated that occupancy of the 2-, 3-, and 4-positions was favorable, while substitution at the 5-position was less advantageous. The 1-naphthyl derivative **29** possessed the highest 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 ≈ 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	$C_{20}H_{22}N_2O_2 \cdot HCl$			9.17 ± 0.11(7)
29	NP	H	$C_{22}H_{20}N_2 \cdot HCl$	259−60	A	9.35 ± 0.12(13)
31	DMP	6-OMe	$C_{21}H_{24}N_2O_3 \cdot HCl$	232−3	A	8.48 ± 0.30(5)
32	DMP	6-OBn	$C_{27}H_{28}N_2O_3 \cdot HCl$	232−3	A	6.73 ± 0.40(3)
33	DMP	6-F	$C_{20}H_{21}FN_2O_2 \cdot HCl$	238−40	A	8.49 ± 0.10(3)
34	DMP	6-Cl	$C_{20}H_{21}ClN_2O_2 \cdot C_4H_4O_4 \cdot H_2O$	220−2	B	8.36 ± 0.14(3)
35	NP	6-Cl	$C_{22}H_{19}ClN_2 \cdot HCl$	280−5 dec	B	9.16 ± 0.13(3)
36	DMP	6-Br	$C_{20}H_{21}BrN_2O_2 \cdot C_4H_4O_4$	184−8	B	8.80 ± 0.17(3)
37	DMP	6-I	$C_{20}H_{21}IN_2O_2 \cdot HCl$	270−3 dec	B	9.52 ± 0.17(3)
38	DMP	6-SMe	$C_{21}H_{24}N_2O_2S \cdot HCl \cdot {}^1/_2H_2O$	224−7	A	9.56 ± 0.15(3)
39	DMP	6-Me	$C_{21}H_{24}N_2O_2 \cdot HCl$	245−6 dec	B	9.86 ± 0.14(15)
40	NP	6-Me	$C_{23}H_{22}N_2 \cdot HCl$	276−80 dec	B	9.75 ± 0.11(8)
41	NP	6-CO_2H	$C_{23}H_{20}N_2O_2 \cdot HCl$	284−7 dec	B	<7.5(4)
42	DMP	6-Et	$C_{22}H_{26}N_2O_2 \cdot C_4H_4O_4$	185 dec	B	9.46 ± 0.11(6)
43	NP	6-Et	$C_{24}H_{24}N_2 \cdot C_4H_4O_4$	212−4 dec	B	9.65 ± 0.14(4)
44	DMP	6-*n*-Pr	$C_{23}H_{28}N_2O_2 \cdot C_4H_4O_4$	195−7 dec	B	8.64 ± 0.27(3)
45	DMP	6-*n*-Bu	$C_{24}H_{30}N_2O_2 \cdot C_4H_4O_4$	191−3	B	<7.5(4)
46	DMP	6-*i*-Pr	$C_{23}H_{28}N_2O_2 \cdot C_4H_4O_4$	196−200	B	8.50 ± 0.13(4)
47	DMP	6-c-Hex	$C_{26}H_{32}N_2O_2 \cdot C_4H_4O_4$	208−10 dec	B	<7.5
48	DMP	6-Ph	$C_{26}H_{26}N_2O_2 \cdot HCl$	253−5	B	<7.0
49	DMP	7-OMe	$C_{21}H_{24}N_2O_3 \cdot HCl$	223−4	B	7.31 ± 0.03(3)
50	NP	8-OMe	$C_{23}H_{22}N_2O \cdot C_4H_4O_4$	240−2 dec	B	9.72 ± 0.30(3)
51	NP	8-Br	$C_{22}H_{19}BrN_2 \cdot HCl$	229−35 dec	B	9.10 ± 0.28(3)
52	DMP	8-Me	$C_{21}H_{24}N_2O_2 \cdot C_4H_4O_4$	168 dec	B	9.0 ± 0.07(3)
53	DMP	5-F,6-Me	$C_{21}H_{23}FN_2O_2 \cdot C_4H_4O_4$	191−4	B	9.3 ± 0.12(7)
54	NP	5-F,6-Me	$C_{23}H_{21}FN_2 \cdot C_4H_4O_4$	253−5 dec	B	9.69 ± 0.21(3)
55	DMP	5,7-Me	$C_{22}H_{26}N_2O_2 \cdot HCl$	259−61	B	9.34 ± 0.12(3)
56	DMP	6,7-Me	$C_{22}H_{26}N_2O_2 \cdot C_4H_4O_4$	197−200	B	9.71 ± 0.14(6)
57	DMP	6,8-Me	$C_{22}H_{26}N_2O_2 \cdot HCl$	257−9	B	9.61 ± 0.22(5)
58	DMP	7,8-Me	$C_{22}H_{26}N_2O_2 \cdot HCl$	278−80	B	10.12 ± 0.18(3)
59	DMP	4,8-Me	$C_{22}H_{26}N_2O_2 \cdot HCl$	275−7	B	9.29 ± 0.18(4)
60	DMP	7,8-benzo	$C_{24}H_{24}N_2O_2 \cdot C_4H_4O_4$	187−9 dec	B	9.22 ± 0.05(3)
61	DMP	6,8-F	$C_{20}H_{20}F_2N_2O_2 \cdot C_4H_4O_4$	185−8 dec	B	9.60 ± 0.13(7)
62	DMP	6-Me,8-Br	$C_{21}H_{23}BrN_2O_2 \cdot HCl$	253−5	B	8.92 ± 0.29(4)
63	NP	6-Me,8-Br	$C_{23}H_{21}BrN_2 \cdot C_4H_4O_4$	208−11 dec	B	8.82 ± 0.11(3)
64	DMP	7-Me,8-Br	$C_{21}H_{23}BrN_2O_2 \cdot HCl$	266−7	B	9.02 ± 0.35(3)
65	NP	7-Me,8-Br	$C_{23}H_{21}BrN_2 \cdot HCl$	276−9 dec	B	8.56 ± 0.43(3)

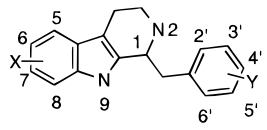
parent structures, but affinity rapidly diminished with increased steric bulk, resulting in a loss of affinity with the 6-nBu (**45**) and 6-cHex (**47**) analogs.

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	$C_{21}H_{24}N_2O_2 \cdot HCl$	245−6 dec	B	9.86 ± 0.14(15)
40	6-Me	2′,3′-benzo	$C_{23}H_{22}N_2 \cdot HCl$	276−80 dec	B	9.75 ± 0.11(8)
66	6-Me	3′-OH,4′-OMe	$C_{20}H_{22}N_2O_2 \cdot HCl$	272−4	B	9.23 ± 0.24(3)
67	6-Me	2′-Cl,3′,4′-OMe	$C_{21}H_{23}ClN_2O_2 \cdot HCl$	244 dec	A	9.80 ± 0.15(4)
68	6-Me	2′-Br,3′,4′-OMe	$C_{21}H_{23}BrN_2O_2 \cdot HCl$	272−4 dec	A	9.70 ± 0.22(4)
69	6-Me	2′-NO_2-3′,4′-OMe	$C_{21}H_{23}N_3O_4 \cdot HCl$	233 dec	B	9.16 ± 0.21(3)
70	6-Me	2′-NH_2-3′,4′-OMe	$C_{21}H_{25}N_3O_2 \cdot 2HCl$	214 dec	B	9.02 ± 0.08(4)
71	6-Me	5′-I,3′,4′-OMe	$C_{21}H_{23}IN_2O_2 \cdot C_4H_4O_4$	163−6 dec	B	8.12 ± 0.16(7)
72	6-Me	5′-NO_2,3′,4′-OMe	$C_{21}H_{23}N_3O_4 \cdot HCl$	239−43	B	8.55 ± 0.10(4)
73	6-Me	5′-NH_2,3′,4′-OMe	$C_{21}H_{25}N_3O_2 \cdot 2HCl$	230−4	B	8.89 ± 0.12(4)
74	6-Me	3′,5′-F	$C_{19}H_{18}F_2N_2 \cdot C_4H_4O_4$	205 dec	B	9.05 ± 0.03(4)
75	6-Me	3′,4′-F	$C_{19}H_{18}F_2N_2 \cdot C_4H_4O_4$	216 dec	B	8.47 ± 0.24(5)
76	6-Me	3′-F,4′-OMe	$C_{20}H_{21}FN_2O \cdot HCl$	277−9	B	9.42 ± 0.18(5)
77	6-Me	3′-CF_3	$C_{20}H_{19}F_3N_2 \cdot HCl$	252−3	B	8.91 ± 0.17(7)
78	6-Me	3′,4′-Me	$C_{21}H_{24}N_2 \cdot HCl$	285−7 dec	B	9.06 ± 0.27(3)
79	2,6-Me	2′-Cl,3′,4′-OMe	$C_{22}H_{25}ClN_2O_2 \cdot C_4H_4O_4$	172−6 dec	B	8.40 ± 0.40(3)
80	2,6,9-Me	2′-Cl,3′,4′-OMe	$C_{23}H_{27}ClN_2O_2 \cdot HCl$	227−9 dec	B	<7.52(4)
81	2,6-Me	2′,3′-benzo	$C_{24}H_{24}N_2 \cdot HCl$	218−21	B	~7.52(4)
82	6,9-Me	3′,4′-OMe	$C_{22}H_{27}N_2O_2 \cdot HCl$	146−50	B	<7.52(4)
83	1,6-Me	3′,4′-OMe	$C_{22}H_{26}N_2O_2 \cdot C_4H_4O_4$	183−5	B	<7.52(4)
84	6-Me	"perhydro"	$C_{19}H_{26}N_2 \cdot HCl$	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

	$-\log K_i$				
compd	$5HT_{2A}$	$5HT_{2C}$	$-\log K_B$ $5HT_{2B}$	ratio 2B/2A	ratio 2B/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, [^{3}H]ketanserin, ref 18. [b] Binding affinity, pig choroid plexus, [^{3}H]mesulergine, ref 18. [c] Rat stomach fundus, ref 18. [d] Binding affinity, human clone, [^{3}H]ketanserin, ref 19. [e] Binding affinity, human clone, [^{3}H]mesulergine, ref 19. [f] Rat stomach fundus, ref 19. [g] Binding affinity, human clone, [^{3}H]ketanserin, ref 17. [h] Binding affinity, human clone, [^{3}H]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 $5HT_2$ 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, nonselective $5HT_2$ 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 $5HT_2$ 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

	$-\log K_B$	
compd	$5HT_{2B}$ (rat stomach fundus)	$5HT_{2A}$ (rat jugular vein)
1 (yohimbine)	6.92 ± 0.12(3)	6.37 ± 0.11(3)
6	9.17 ± 0.11(7)	7.43 ± 0.09(6)
39	9.86 ± 0.14(15)	7.21 ± 0.20(4)
40	9.75 ± 0.11(8)	8.07 ± 0.10(8)
67	9.80 ± 0.15(4)	8.14 ± 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 $5HT_2$ 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. ^{1}H NMR were determined at 300 MHz in $CDCl_3$ 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 μL, 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*-Acetylglycine (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 = CO_2H). 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; $CaCl_2$,1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37 °C and equilibrated with 95% O_2−5% CO_2. Jugular veins and stomach strips were placed under optimum resting force (1*g* and 4*g*, 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 ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50}. 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 $5HT_{2C}$ receptor[43] and the rat $5HT_{2A}$ receptors[44] 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 ^{32}P-labeled cDNA probe followed by radioligand binding assays.

Radioligand Binding Assays. Cell membranes were prepared for binding studies as previously described[15] 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 (7.5−15) × 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 described[46] using [^{3}H]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 [^{3}H]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 μM pargyline, 0.2 nM [^{3}H]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 μM mianserin. K_i values for the compounds were calculated using the Cheng−Prusoff equation[48] as previously described.[15]

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

References

(1) Cohen, M. L.; Fludzinski, L. A. Contractile serotoninergic receptor in rat stomach fundus. *J. Pharmacol. Exp. Ther.* **1987**, *243*, 264−9.

(2) Baez, M.; Yu, L.; Cohen, M. L. Pharmacological and molecular evidence that the contractile response to serotonin in rat stomach fundus is not mediated by activation of the 5-hydroxytryptamine$_{1C}$ receptor. *Mol. Pharmacol.* **1990**, *38*, 31−7.

(3) Baxter, G. S.; Murphy, O. E.; Blackburn, T. P. Further characterization of 5-hydroxytryptamine receptors (putative). *Br. J. Pharmacol.* **1994**, *112*, 323−31.

(4) Cohen, M. L.; Wittenauer, L. A. Relationship between serotonin and tryptamine receptors in the rat stomach fundus. *J. Pharmacol. Exp. Ther.* **1985**, *233*, 75−9.

(5) Cohen, M. L.; Schenck, K. W.; Colbert, W.; Wittenauer, L. Role of 5HT2 receptors in serotonin-induced contractions of nonvascular smooth muscle. *J. Pharmacol. Exp. Ther.* **1985**, *232*, 770−4.

(6) Cohen, M. L.; Colbert, W. E. Relationship between receptors mediating serotonin (5HT) contractions in the canine basilar artery to 5HT1, $5HT_2$ and rat stomach fundus 5HT receptors. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 713−18.

(7) Cohen, M. L.; Wittenauer, L. A. Further evidence that the serotonin receptor in the rat stomach fundus is not $5HT_{1A}$ or $5HT_{1B}$. *Life Sci.* **1986**, *38*, 1−5.

(8) Secrest, R. J.; Schoepp, D. D.; Cohen, M. L. Comparison of contractions to serotonin, carbamylcholine and prostaglandin $F_{2\alpha}$ in rat stomach fundus. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 971−8.

(9) Humphrey, P. P. A.; Hartig, P.; Hoyer, D. A. A Proposed New Nomenclature of 5HT Receptors. *Trends Pharmacol. Sci.* **1992**, *14*, 233.

(10) Foguet, M.; Hoyer, D.; Pardo, L. A.; Parekh, A.; Kluxen, F. W.; Kalkman, H. O.; Stuehmer, W.; Luebbert, H. Cloning and functional characterization of the rat stomach fundus serotonin receptor. *EMBO J.* **1992**, *11*, 3481−7.

(11) Kursar, J. D.; Nelson, D. L.; Wainscott, D. B.; Cohen, M. L.; Baez, M. Molecular cloning, functional expression, and pharmacological characterization of a novel serotonin receptor (5- hydroxytryptamine$_{2F}$) from rat stomach fundus. *Mol. Pharmacol.* **1992**, *42*, 549−57.

(12) Kursar, J. D.; Nelson, D. L.; Wainscott, D. B.; Baez, M. Molecular cloning, functional expression, and mRNA tissue distribution of the human 5-hydroxytryptamine$_{2B}$ receptor. *Mol. Pharmacol.* **1994**, *46*, 227−34.

(13) Foguet, M.; Nguyen, H.; Le, H.; Lubbert, H. Structure of the mouse $5HT_{1C}$, $5HT_2$ and stomach fundus serotonin receptor genes. *NeuroReport* **1992**, *3*, 345−8.

(14) Schmuck, K.; Ullmer, C.; Engels, P.; Luebbert, H. Cloning and functional characterization of the human $5HT_{2B}$ serotonin receptor. *FEBS Lett.* **1994**, *342*, 85−90.

(15) Wainscott, D. B.; Cohen, M. L.; Schenck, K. W.; Audia, J. E.; Nissen, J. S.; Baez, M.; Kursar, J. D.; Lucaites, V. L.; Nelson, D. L. Pharmacological characteristics of the newly cloned rat 5-hydroxytryptamine$_{2F}$ receptor. *Mol. Pharmacol.* **1993**, *43*, 419−26.

(16) Nelson, D. L. The $5HT_2$ Subfamily of Receptors: Pharmacological Challenges. *Med. Chem. Res.* **1993**, *3*, 306.

(17) Forbes, I. T.; Ham, P.; Booth, D. H.; Martin, R. T.; Thompson, M.; Baxter, G. T.; Blackburn, T. P.; Glen, A.; Kennett, G. A.; Wood, M. D. 5-Methyl-1-(3-pyridylcarbamoyl)-1,2,3,5-tetrahydropyrrolo[2,3-f]indole: A Novel $5HT_{2C}$/$5HT_{2B}$ Receptor Antagonist with Improved Affinity, Selectivity, and Oral Activity. *J. Med. Chem.* **1995**, *38*, 2524−2530.

(18) Nozulak, J.; Kalkman, H. O.; Floerscheim, P.; Hoyer, D.; Schoeffter, P.; Buerki, H. R. (+)-cis-4,5,7a,8,9,10,11,11a-Octahydro-7H-10-methylindolo[1,7- bc][2,6]- naphthyridine: A $5HT_{2C}$/$_{2B}$ Receptor Antagonist with Low $5HT_{2A}$ Receptor Affinity. *J. Med. Chem.* **1995**, *38*, 28−33.

(19) Forbes, I. T.; Jones, G. E.; Murphy, O. E.; Holland, V.; Baxter, G. S. N-(1-Methyl-5-indolyl)-N′-(3-methyl-5-isothiazolyl)urea: A Novel, High-Affinity $5HT_{2B}$ Receptor Antagonist. *J. Med. Chem.* **1995**, *38*, 855−7.

(20) Fludzinski, P.; Wittenauer, L. A.; Schenck, K. W. 2,3-Dialkyl-(dimethylamino)indoles: interaction with $5HT_1$, $5HT_2$, and rat stomach fundal serotonin receptors. *J. Med. Chem.* **1986**, *29*, 2415−18.
(21) Bonhaus, D. W.; Bach, C.; DeSouza, A.; Salazar, R. H. R.; Matsuoka, B. D.; Zuppan, P.; Chan, H. W.; Eglen, R. M. The pharmacology and distribution of human 5-hydroxytryptamine2B ($5HT_{2B}$) receptor gene products: comparison with $5HT_{2A}$ and $5HT_{2C}$ receptors. *Br. J. Pharmacol.* **1995**, *115*, 622−8.
(22) Clineschmidt, B. V.; Reiss, D. R.; Pettibone, D. J.; Robinson, J. L. Characterization of 5-hydroxytryptamine receptors in rat stomach fundus. *J. Pharmacol. Exp. Ther.* **1985**, *235*, 696−708.
(23) Goldberg, M. R.; Robertson, D. Yohimbine: a pharmacological probe for study of the α_2-adrenoreceptor. *Pharmacol. Rev.* **1983**, *35*, 143−80.
(24) Grossman, F.; Manji, H. K.; Potter, W. Z. Platelet α_2-adrenoreceptors in depression: a critical examination. *J. Psychopharmacol.* **1993**, *7* (1), 4−18.
(25) Toke, L.; Honty, K.; Szantay, C. Total synthesis of (+)-yohimbine and (-)-β-yohimbine. *Chem. Ber.* **1969**, *102*, 3248−59.
(26) Loewe, M. F.; Meyers, A. I. An asymmetric synthesis of indole alkaloids. *Tetrahedron Lett.* **1985**, *26*, 3291−4.
(27) Rommelspacher, H.; Coper, H.; Strauss, S. On the mode of formation of tetrahydro-β-carbolines. *Life Sci.* **1976**, *18*, 81−8.
(28) Kametani, T.; Takeshita, M.; Satoh, F. Syntheses of heterocyclic compounds. DLI. Syntheses of 1-benzyl-1,2,3,4-tetrahydro-β-carboline and hexadehydroyohimbane derivatives under the condition of the Pictet-Spengler reaction. *Yakugaku Zasshi* **1974**, *94*, 261−4.
(29) Onda, M.; Kawanishi, M. Analogs of Rauwolfia alkaloids. *J. Pharm. Soc. Jpn.* **1956**, *76*, 966−8.
(30) Rehse, K.; Piesker, G.; Kehr, W.; Wachtel, H. Neuropsychotropic activity of the dopamine-analogous 1,2,3,4-tetrahydro-β-carbolines. Part 2. Alteration of activity by substituents. *Arch. Pharm.* **1979**, *312* (7), 597−603.
(31) Hayashi, M.; Horiguchi, A.; Kawashima, Y.; Watanabe, Y.; Hatayama, K. Preparation of tetrahydro-β-carboline derivatives as lipid peroxidation inhibitors and antiulcer agents. JP 03287586, December 18, 1991.
(32) Shavel, J., Jr.; Morrison, G. C. Cis-syn-5,6,7,7a,8,8a,9,10,12,-12a-decahydro-7-methyl-11H-benz[b]indolo[3,2,1-ij][1,5]naphthyridin-11-one. US 3492304, June 24, 1968.
(33) Kametani, T.; Kajiwara, M.; Fukumoto, K. Syntheses of heterocyclic compounds. DXLVII. Synthesis of a yohimban derivative by thermolysis. *Tetrahedron* **1974**, *30*, 1053−8.
(34) Taborsky, R. G.; McIsaac, W. M. Synthesis and preliminary pharmacology of some 9H-pyrido-[3,4-b]indoles (β-carbolines) and tryptamines related to serotonin and melatonin. *J. Med. Chem.* **1964**, *7*, 135−41.
(35) Gaignault, J. C.; Frechet, D.; Nedelec, L. 1-(Indolylmethyl)-1,2,3,4-tetrahydroisoquinolines and 1-benzyl-2,3,4,9-tetrahydro-(1H)-β-carbolines prepared from tryptamine or from its derivatives. *Ann. Pharm. Fr.* **1978**, *36*, 401−8.
(36) Audia, J. E.; Droste, J. J.; Nissen, J. S.; Murdoch, G. R.; Evrard, D. A. "Pictet-Spengler-Like" Synthesis of Tetrahydro-β-carbolines Under Hydrolytic Conditions. Direct Use of aza lactones as Phenylacetaldehyde Equivalents. *J. Org. Chem.*, in press.
(37) Knabe, J.; Suggau, R. Dihydroisoquinoline rearrangement. 16. Reaction in the β-carboline series analogous to the rearrangement of1,2-dihydroisoquinolines. *Arch. Pharm.* **1973**, *306*, 500.
(38) Kennett, G. A.; Pittaway, K.; Blackburn, T. P. Evidence that $5HT_{2C}$ receptor antagonists are anxiolytic in the rat Geller-Seifter model of anxiety. *Psychopharmacology* **1994**, *114* (1), 90−6.
(39) Kalkman, H. O. Is migraine propylactic activity caused by $5HT_{2B}$ or $5HT_{2C}$ receptor blockade? *Life Sci.* **1994**, *54*, 641−4.
(40) Fozard, J. R.; Kalkman, H. O. 5-Hydroxytryptamine (5HT) and the initiation of migraine: new perspectives. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1994**, *350*, 225−9.
(41) Fozard, J. R. The 5-hydroxytryptamine-nitric oxide connection: the key link in the initiation of migraine? *Arch. Int. Pharmacodyn. Ther.* **1995**, *329*, 111−19.
(42) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(43) Yu, L.; Nguyen, H.; Le, H.; Bloem, L. J.; Kozak, C. A.; Hoffman, B. J.; Snutch, T. P.; Lester, H. A.; Davidson, N.; Lubbert, H. The mouse $5HT_{1C}$ receptor contains eight hydrophobic domains and is X-linked. *Mol. Brain Res.* **1991**, *11*, 143−149.
(44) Liu, J.; Chen, Y.; Kozak, C. A.; Yu, L. The $5HT_2$ serotonin receptor gene Htr-2 is tightly linked to ES-10 on mouse chromosome 14. *Genomics* **1991**, *11*, 231−234.
(45) Grinnell, B. W.; Berg, D. T.; Walls, J.; Yan, B. Trans-activated expression of fully gamma-carboxylated human protein C, an antithrombotic factor. *Bio/tech* **1987**, *5*, 1189−1192.
(46) Johnson, M. P.; Loncharich, R. J.; Baez, M.; Nelson, D. L. Species variations in transmembrane region V of the 5-hydroxytryptamine Type 2A receptor alter the structure-activity relationship of certain ergolines and tryptamines. *Mol. Pharmacol.* **1994**, *45*, 277−286.
(47) Pazos, A.; Hoyer, D.; Palacios, J. M. Mesulergine, a selective serotonin-2 ligand in the rat cortex, does not label these receptors in porcine and human cortex: evidence for species differences in brain serotonin-2 receptors. *Eur. J. Pharmacol.* **1985**, *106*, 531−538.
(48) Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (Ki) and the concentratioon of an inhibitor that causes 50% inhibition (I50) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(49) 5-Methoxytryptamine has equally high affinity at the rat $5HT_{2B}$ receptor as has serotonin (9.15 versus 10.2 nM) and is a fully effective agonist (EC_{50} 4.7 nM, 97.1% of 5HT max. Vs 23.3 nM).[15]
(50) For the first reports of direct synthesis of tryptamines via a Fischer-type synthesis, see: Suvorov, N. N.; Gordeev, E. N.; Vasin, M. V. Indole Derivatives. CI. Synthesis and biological activity of some tryptamines. *Khim. Geterotsikl. Soedin.* **1974**, *11*, 1496−501; *Chem. Abstr.* **1975**, *82*, 139888. Grandberg, I. I.; Bobrova, N. I. Indoles. XLVI. Arylhydrazine salts and bisulfite derivatives of g-halocarbonyl compounds in the synthesis of tryptamines. *Khim. Geterotsikl. Soedin.* **1974**, *8*, 1085−8; *Chem. Abstr.* **1975**, *82*, 31202. For a recent application of this methodology, see: Chen, C.; Senanayake, C. H.; Bill, T. J.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. Improved Fischer Indole Reaction for the Preparation of N, N-Dimethyltryptamines: Synthesis of L-695, 894, a Potent $5HT_{1D}$ Receptor Agonist. *J. Org. Chem.* **1994**, *59*, 3738−41.

JM960062T