Synthesis and Structure−Activity Relationships of Potent and Orally Active 5-HT_4 Receptor Antagonists: Indazole and Benzimidazolone Derivatives

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A series of indole-3-carboxamides, indazole-3-carboxamides, and benzimidazolone-3-carboxamides was synthesized and evaluated for antagonist affinity at the 5-HT_4 receptor in the rat esophagus. The *endo*-3-tropanamine derivatives in the indazole and benzimidazolone series possessed greater 5-HT_4 receptor affinity than the corresponding indole analogues. 5-HT_4 receptor antagonist affinity was further increased by alkylation at N-1 of the aromatic heterocycle. In a series of 1-isopropylindazole-3-carboxamides, replacement of the bicyclic tropane ring system with the monocyclic piperidine ring system or an acyclic aminoalkylene chain led to potent 5-HT_4 receptor antagonists. In particular, those systems in which the basic amine was substituted with groups capable of forming hydrogen bonds showed increased 5-HT_4 receptor antagonist activity. While some of these compounds displayed high affinity for other neurotransmitter receptors (in particular, 5-HT_3, α_1, and 5-HT_{2A} receptors), as the conformational flexibility of the amine moiety increased, the selectivity for the 5-HT_4 receptor also increased. From this series of compounds, we identified LY353433 (1-(1-methylethyl)-*N*-[2-[4-[(tricyclo[3.3.1.$1^{3,7}$]dec-1-ylcarbonyl)amino]-1-piperidinyl]ethyl]-1*H*-indazole-3-carboxamide) as a potent and selective 5-HT_4 receptor antagonist with clinically suitable pharmacodynamics.

Introduction

The neurotransmitter serotonin (5-HT) modulates the activity of the central nervous system and peripheral tissues through its actions at a number of receptor subtypes.[1] Because of the wide range of physiologic and pathophysiologic systems in which 5-HT is known to play a role, considerable attention has centered around the identification of agents which act selectively at each of these receptor subtypes. One of these receptor subtypes, the 5-HT_4 receptor,[2] was first identified in 1988[3] and has recently been cloned.[4] Based on the localization of the 5-HT_4 receptor in gastrointestinal tissue, atrial tissue, urinary bladder tissue, and the central nervous system, a number of therapeutic indications have been proposed for both agonists and antagonists at this receptor. Proposed therapeutic applications for agents that block the 5-HT_4 receptor include the treatment of irritable bowel syndrome, atrial arrhythmias, urinary incontinence, and various diseases of the central nervous system. We were interested in identifying a potent and selective 5-HT_4 receptor antagonist as a possible therapeutic agent.

Although a number of compounds have been identified which block 5-HT_4 receptors, most do not have the requisite selectivity or pharmacodynamic properties which would make them ideal pharmacologic tools or therapeutic agents. Tropisetron (**1**) (Chart 1) was among the first compounds shown to possess 5-HT_4 receptor antagonist activity.[5] However, **1** has higher affinity at 5-HT_3 than 5-HT_4 receptors. The benzamide cisapride (**2**) has been shown to be a partial agonist at 5-HT_4 receptors but also has high affinity for 5-HT_{2A} and α_1 receptors.[6] SDZ 205,557 (**3a**)[7] and LY297524 (**3b**),[8] both esters related to metoclopramide, are reported to be 5-HT_4 receptor antagonists but also have considerable 5-HT_3 receptor antagonist activity.[8] GR113808 (**4**)[9] and SB204070 (**5**)[10] are the first reported 5-HT_4 receptor antagonists with high affinity and selectivity for the 5-HT_4 receptor. However, neither of these compounds has a clinically suitable duration of action following oral administration, presumably because the ester linkage present in both molecules is readily cleaved in vivo.[2c,11] SB207266 (**6**)[12] and RS100235 (**7**)[13] are more recently reported 5-HT_4 receptor antagonists. Both compounds demonstrated improved pharmacodynamics over **4** and **5**, presumably because the amide and ketone systems of **6** and **7**, respectively, are more stable than the ester linkage found in **4** and **5**.

We initiated studies with the goal of identifying structurally distinct, potent, selective, and orally active 5-HT_4 receptor antagonists with a clinically suitable in vivo duration of action. The present report details the structure−activity relationships (SAR), synthesis, and pharmacological characterization of LY353433 (**8**, 1-(1-methylethyl)-*N*-[2-[4-[(tricyclo [3.3.1.$1^{3,7}$]dec-1-ylcarbonyl)amino]-1-piperidinyl]ethyl]-1*H*-indazole-3-carboxamide) a potent, selective, and long-acting compound from this series.[6]

Tropisetron (**1**) and cisapride (**2**) served as starting points for our structure−activity studies. While both compounds have affinity for the 5-HT_4 receptor, neither is particularly potent and both have high affinity for

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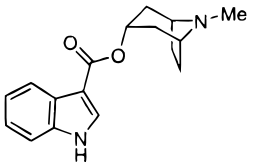
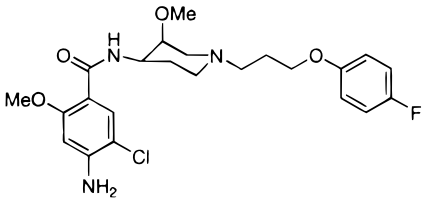
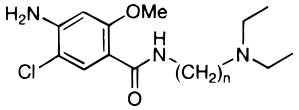
Chart 1



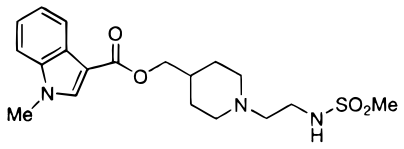
1, tropisetron



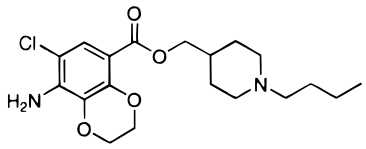
2, cisapride



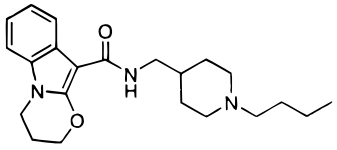
3a: n = 2, SDZ 205,557
3b: n = 3, LY297524



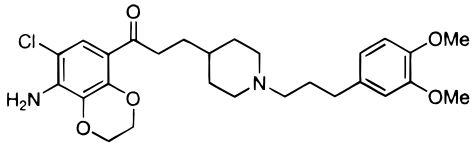
4, GR113808



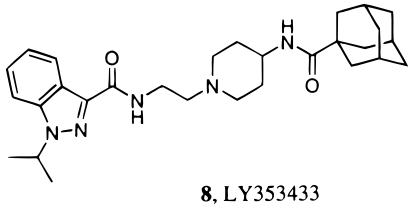
5, SB204070



6, SB207266

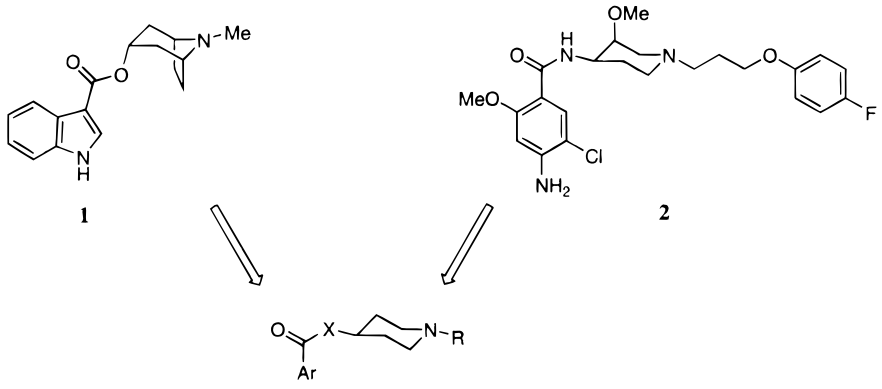


7, RS100235



8, LY353433

Chart 2



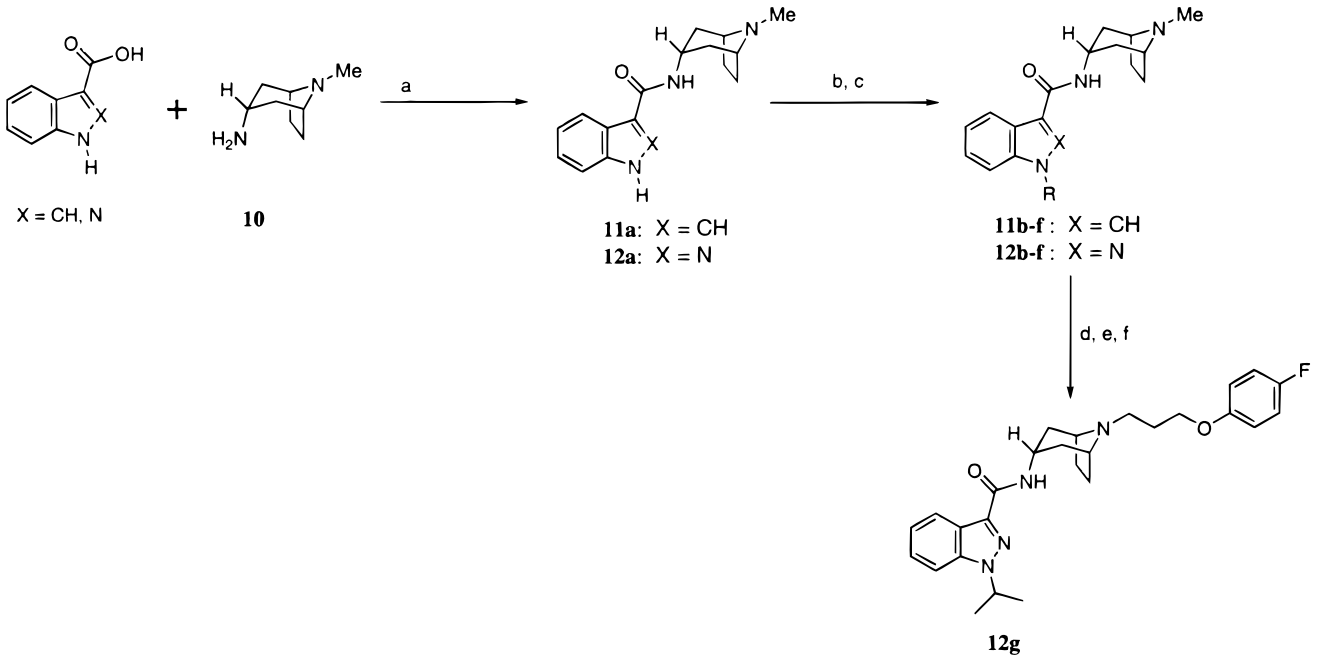
1 **2** **9**

other receptors. A common pharmacophore (**9**) can be drawn for the two compounds which is centered around a piperidine ring system (Chart 2). For tropisetron, the piperidine is further rigidified into a tropane ring system. In each case, an aromatic acyl group is connected to the 4-position of the piperidine through either an ester or amide linkage. The basic nitrogen of the piperidine is also a site for substitution. We explored the modification of each of these elements of the proposed pharmacophore: the aromatic acyl group, the piperidine ring system, and the substituent on the basic nitrogen. Because of the expected lability of the ester linkage in compounds such as tropisetron, we focused our synthetic efforts on compounds in which the acyl group was connected to the piperidine ring through an amide linkage.

Chemistry

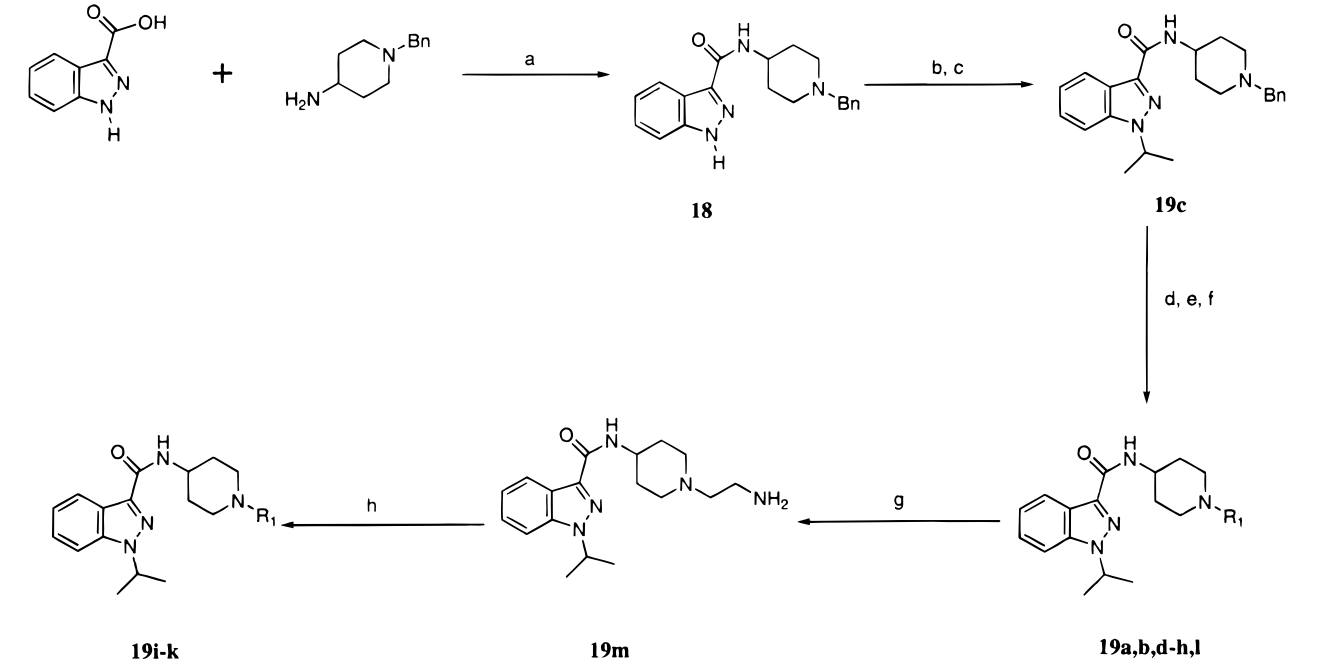
The synthesis of the indolecarboxamides **11a**−**f** and the indazolecarboxamides **12a**−**f** is outlined in Scheme 1. Treatment of indole-3-carboxylic acid with oxalyl chloride provided the corresponding acid chloride which was reacted with the known tropanamine **5**[14] to give **11a**. Alkylation at N-1 was achieved by sequential treatment with sodium hydride and an alkyl halide to yield **11b**−**f**. The analogous indazoles were prepared by carbonyldiimidazole-mediated coupling between indazole-3-carboxylic acid[15] and **10** to give **12a**. Alkylation

Scheme 1[a]



[a] (a) X = CH, oxalyl chloride, THF; X = N, 1,1′-carbonyldiimidazole, DMF; (b) NaH, DMF; (c) RX; (d) MeCH(Cl)OCOCl, CH_2Cl_2; (e) MeOH, heat; (f) 3-(4-fluorophenoxy)-1-(tosyloxy)propane, Na_2CO_3, DMF.

Scheme 2[a]

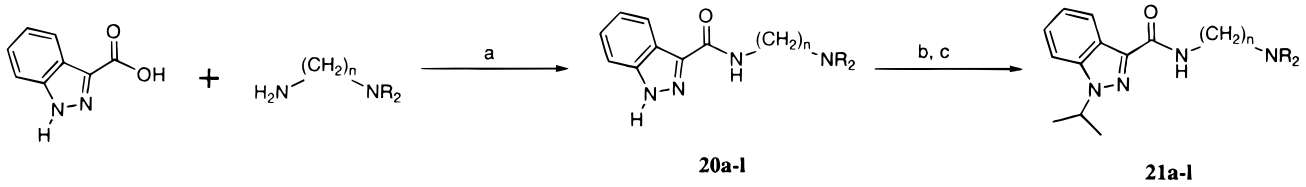


[a] (a) 1,1-Carbonyldiimidazole, DMF; (b) NaH, DMF; (c) *i*-PrI; (d) MeCH(Cl)OCOCl, CH_2Cl_2; (e) MeOH, heat; (f) R_1X, Na_2CO_3, DMF; (g) NH_2NH_2, MeOH, heat; (h) MsCl or PhCOCl or 1-adamantanecarbonyl chloride.

as before yielded the corresponding N-1 alkyl derivatives **12b**−**f** with good regiocontrol.[16] Modified von Braun demethylation of **12e** with 1-chloroethyl chloroformate provided the corresponding desmethyltropane which was alkylated with 3-(4-fluorophenoxy)-1-(tosyloxy)-propane to give **12g**. Amide **14** was prepared from 1-naphthalenecarboxylic acid and **10** using carbonyldiimidazole as the activating agent. Benzimidazolones **13a**−**f**,[17] amide **15**,[18] and zatosetron (**16**)[19] were prepared as outlined in the literature. By analogy with **12g**, von Braun degradation of **13e** followed by alkylation provided **13g**. Reaction of 1-(chlorocarbonyl)benzimidazolone[17] with 4-amino-*N*-methylpiperidine and with 4-amino-*N*-(1-(3-(4-fluorophenoxy)propyl))piperidine provided the desired amides which were alkylated at *N*-1 with isopropyl iodide to yield compounds **17a**,**b**.

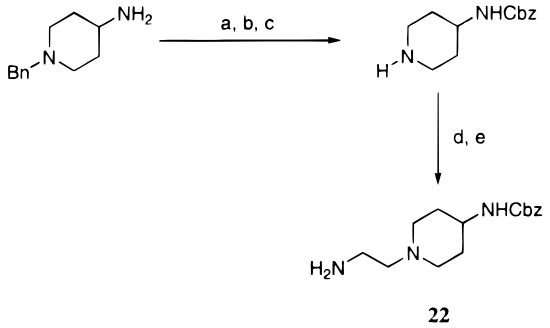
Amide **18** underwent regioselective alkylation at N-1 with sodium hydride and isopropyl iodide to yield **19c** (Scheme 2). Debenzylation gave the secondary amine **19a** which was alkylated under standard conditions to provide **19b,d**−**h,l**. Hydrazinolysis of the phthalimidoethyl derivative **19h** produced the aminoethyl intermediate **19m** which was reacted with methanesulfonyl chloride, benzoyl chloride, and adamantane-1-carbonyl

Scheme 3[a]



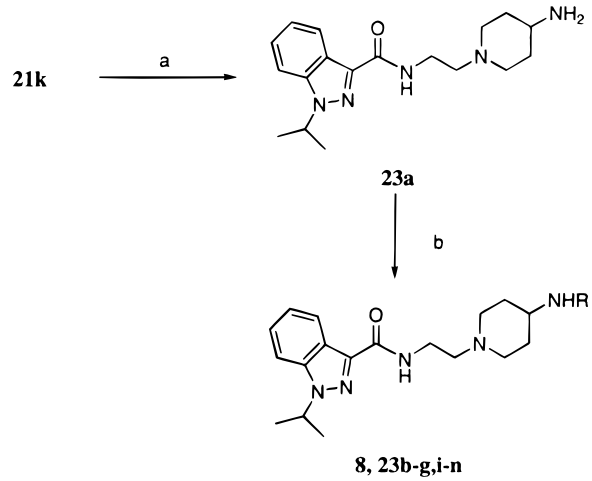
[a] (a) 1,1-Carbonyldiimidazole, DMF; (b) NaH, DMF; (c) *i*-PrI.

Scheme 4[a]



[a] (a) Cbz-Cl, NaOH, H_2O, CH_2Cl_2; (b) MeCH(Cl)OCOCl, CH_2Cl_2; (c) MeOH, heat; (d) *N*-(2-bromoethyl)phthalimide, K_2CO_3, DMF; (e) $NH_2NH_2 \cdot H_2O$, EtOH, heat.

Scheme 5[a]



[a] (a) H_2, Pd/C, MeOH; (b) RCOCl, NEt_3, THF; or MsCl, NEt_3, THF.

chloride to yield **19i**, **19j**, and **19k**, respectively. Amides **21a**−**l** were prepared by carbonyldiimidazole-mediated acylation of the corresponding aminoalkyl derivative with indazole-3-carboxylic acid followed by regioselective alkylation with sodium hydride and isopropyl iodide (Scheme 3).

For the synthesis of **21k**, the required aminoethyl derivative, **22**, was prepared according to Scheme 4. Schotten−Bauman acylation of 4-amino-1-benzylpiperidine with benzyl chloroformate followed by removal of the benzyl group using von Braun conditions produced 4-((carbobenzyloxy)amino)piperidine. Alkylation with *N*-(2-bromoethyl)phthalimide and treatment with hydrazine to remove the phthalimide protecting group yielded **22**. Hydrogenolytic cleavage of the carbobenzyloxy protecting group of **21k** provided **23a** which served as a key synthetic intermediate (Scheme 5). Functionalization of **23a** under standard conditions provided compounds **8** and **23b**−**g,i**−**n**.

Results and Discussion

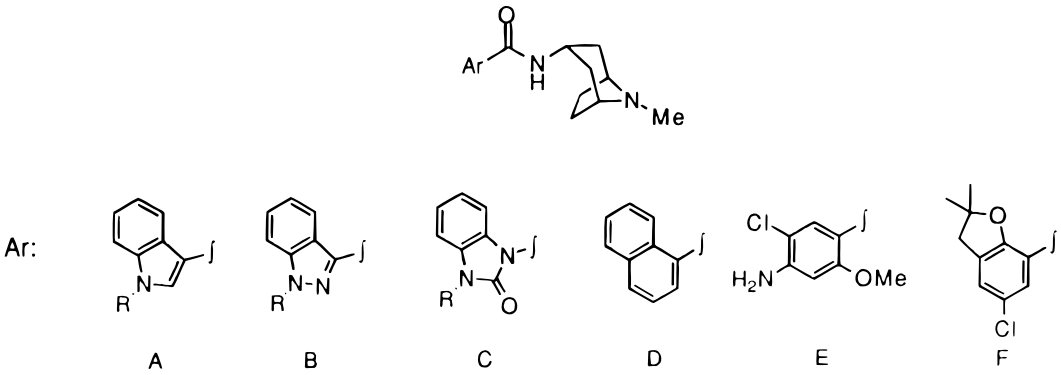
In the carbamylcholine-contracted rat esophagus preparation, serotonin produces a concentration-dependent relaxation mediated through activation of 5-HT_4 receptors.[8,20] The 5-HT_4 receptor antagonist affinity of compounds was evaluated by their ability to block serotonin-induced relaxation in carbamylcholine (10^{-6} M)-contracted esophagus. Tissues were challenged with serotonin (1 μM), and relaxation of the tissue in the presence of a given concentration of the antagonist or vehicle was measured as reported in Tables 1−5. In the presence of vehicle, 1 μM serotonin produced 68.8 ± 4.3% (N = 10) relaxation of the carbamylcholine (10^{-6} M)-contracted rat esophagus. Thus, the greater the antagonist affinity, the less relaxation produced by serotonin (1 μM).

Modification of the Aromatic Acyl Group. Using the amide analogue of tropisetron, **11a**, as our starting point, our initial goal was to study the effect of modification of the aromatic acyl group on 5-HT_4 receptor antagonist affinity. Our examination focused on a series of bicyclic aromatic amides. Replacement of the ester functionality in tropisetron by an amide linkage led to a significant decrease in 5-HT_4 receptor affinity (Table 1, **11a**). This finding is consistent with a study of metoclopramide analogues in which ester derivatives possessed an order of magnitude higher affinity than the corresponding amide analogues at the 5-HT_4 receptor.[21] Alkylation at N-1 of the indole-3-carboxamide ring (Table 1, **11b**−**f**) resulted in compounds which were slightly more potent than tropisetron in inhibiting serotonin-induced relaxation of the rat esophagus. The potency of these 1-alkylindole derivatives increased with the size of the N-1 substituent, the 1-isopropylindole derivative **11e** being the most potent of the 5-HT_4 receptor antagonists. Extending the alkyl group further as in the *sec*-butyl analogue **11f** led to a significant decrease in 5-HT_4 receptor affinity.

Replacement of the indole C-2 with a nitrogen atom (Table 1, **12a**−**f**) provided a series of indazole analogues that possessed higher 5-HT_4 receptor antagonist affinity than the corresponding indole counterparts. While the unsubstituted indazole **12a** was only weakly active as a 5-HT_4 receptor antagonist, methylation at N-1 led to a significant increase in antagonist potency. Similar to the indole derivatives, incorporation of larger N-1 alkyl substituents increased 5-HT_4 receptor antagonist affinity.

Since the *N*-ethyl (**13c**, BIMU-1) and *N*-isopropyl (**13e**, BIMU-8) derivatives were reported to possess affinity for the 5-HT_4 receptor,[22] we included the *N*-acylbenzimidazolone derivatives **13a**−**f** in our study. Interestingly, *N*-alkylbenzimidazolones possessed 5-HT_4 receptor antagonist activity in the rat esophagus similar to that of the indazole series (Table 1, **13a**−**f**). The

Table 1



compd no.	Ar	R	molecular formula	solvent of crystallization	mp (°C)	5-HT$_4$ receptor antagonist activity (% relaxation to 10^{-6} M 5-HT ± SEM (no. of determinations)) antagonist concentration 10^{-8} M	3×10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M
1									33.9 ± 3.9(3)	26.4 ± 5.4(13)
2									7.0 ± 2.0 (3)	20.3 ± 3.3(3)
11a	A	H	$C_{17}H_{21}N_3O\cdot C_4H_4O_4$	EtOAc−MeOH	207				60.0 ± 4.0(4)	
11b	A	Me	$C_{18}H_{23}N_3O\cdot C_4H_4O_4$	EtOAc−MeOH	205−208				47.3 ± 11.0(4)	
11c	A	Et	$C_{19}H_{25}N_3O\cdot C_2H_2O_4$	EtOAc−MeOH	200−203				44.8 ± 10.1(4)	
11d	A	*n*-Pr	$C_{20}H_{27}N_3O\cdot C_2H_2O_4$	EtOAc−MeOH	182−183				34.3 ± 9.1(3)	
11e	A	*i*-Pr	$C_{20}H_{27}N_3O\cdot C_2H_2O_4$	EtOAc−MeOH	207−208				25.3 ± 4.1(4)	
11f	A	s-Bu	$C_{21}H_{29}N_3O\cdot C_2H_2O_4$	EtOAc−MeOH	174−175				57.8 ± 7.8(4)	
12a	B	H	$C_{17}H_{20}N_4O$	EtOAc	228−230					50.5 ± 15.9(4)
12b	B	Me	$C_{17}H_{22}N_4O$	Et_2O	123−124					21.0 ± 7.1(3)
12c	B	Et	$C_{18}H_{24}N_4O\cdot C_2H_2O_4$	EtOAc−MeOH	232−233				6.7 ± 0.3(3)	
12d	B	*n*-Pr	$C_{19}H_{26}N_4O\cdot C_2H_2O_4$	EtOAc−MeOH	214−215				2.5 ± 1.0(4)	
12e	B	*i*-Pr	$C_{19}H_{26}N_4O\cdot C_2H_2O_4$	EtOAc−MeOH	185−187	43.2 ± 8.0(5)		9.0 ± 4.1(4)	8.0 ± 2.0(5)	
12f	B	*s*-Bu	$C_{20}H_{28}N_4O\cdot C_4H_4O_4$	EtOAc−cyclohexane	130−132		46.3 ± 5.6(4)			
13b	C	Me	$C_{17}H_{22}N_4O_2\cdot HCl$	EtOAc−MeOH	>250				7.3 ± 4.3(4)	
13c	C	Et	$C_{18}H_{24}N_4O_2\cdot HCl$	acetone	227−230		37.5 ± 10.1(4)			
13d	C	*n*-Pr	$C_{19}H_{26}N_4O_2\cdot HCl$	MeOH	222−223		30.7 ± 15.1(3)		4.3 ± 2.0(4)	
13e	C	*i*-Pr	$C_{19}H_{26}N_4O_2\cdot HCl$	MeOH	138−140	55.0 ± 10.9(4)	13.8 ± 8.7(4)		2.7 ± 1.5(3)	
13f	C	*s*-Bu	$C_{20}H_{28}N_4O_2\cdot C_2H_2O_4$	Et_2O	205				8.8 ± 4.4(5)	
14	D		$C_{19}H_{22}N_2O\cdot C_7H_8O_3S$	EtOH	238−239					76.5 ± 7.0(4)
15	E		$C_{16}H_{22}N_3O_2\cdot C_4H_4O_4$	EtOAc−MeOH	172				5.4 ± 2.5(5)	2.8 ± 1.5(4)
16	F								37.0 ± 7.7(7)	22.2 ± 6.3(6)

effect of N-alkylation on the benzimidazolones was also similar to the indazole series with the *N*-isopropyl derivative **13e** providing potent inhibitors of serotonin-induced relaxation in the rat esophagus.

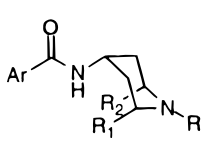
The effect of *N*-isopropyl substitution might have been anticipated, since substitution of a heteroaromatic ring with an isopropyl group has led to increases in antagonist potency at other 5-HT receptor subtypes, as well. In a series of ergoline derivatives, N-1 isopropyl derivatives had higher affinity for the rat 5-HT$_{2A}$ receptor and were more potent 5-HT$_{2A}$ receptor antagonists than their corresponding N-1 unsubstituted derivatives.[23] Further, isopropyl substitution of tryptamine derivatives served to convert agonist activity to antagonist activity at the 5-HT$_{2B}$ receptor in the rat stomach fundus and at the rat 5-HT$_{2A}$ receptor.[23, 24]

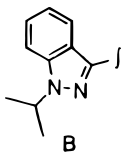
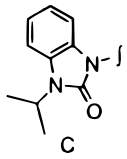
The 1-naphthalenecarboxamide **14** and the dihydrobenzofuran derivative zatosetron (**16**) are two bicyclic aromatic amides which displayed only weak 5-HT$_4$ receptor antagonism in the concentrations tested. The tropane analogue of cisapride, **15**, has been previously characterized as a potent 5-HT$_3$ receptor antagonist,[18] and we found that **15** was also a potent 5-HT$_4$ receptor antagonist.

The increased 5-HT$_4$ receptor affinity of **15** and the indazole and benzimidazolone derivatives over the acylindole and naphthalenecarboxylic acid derivatives can be understood in terms of increased conformational rigidity of these systems. The amide N−H can participate in an intramolecular hydrogen bond with the methoxy group of **15**, the lone pair of N-2 in the indazole amides **12a**−**f**, and the carbonyl of the benzimidazolone amides **13a**−**f**. This hydrogen-bonding interaction enforces coplanarity of the aromatic ring with the atoms of the amide linkage. While the less active indole and naphthalene systems have similar steric requirements as the indazole and benzimidazolone systems, they cannot form a similar rigidifying intramolecular hydrogen bond. The importance of analogous intramolecular hydrogen bonding interactions has been demonstrated with a series of dopamine D2 receptor antagonists and serotonin 5-HT$_3$ receptor antagonists.[18,25] While zatosetron can theoretically form an intramolecular hydrogen-bond, the *gem*-dimethyl substitution on the dihydrobenzofuran ring may provide sufficient steric bulk that the group can no longer participate in the required binding interactions with the 5-HT$_4$ receptor.

Modification of the Tropane Group. These studies identified 1-isopropylindazole and 1-isopropylbenzimidazolone as platforms producing compounds with relatively high affinity as 5-HT$_4$ receptor antagonists. To improve the affinity and selectivity of these derivatives, we incorporated structural elements of cisapride by replacing the tropane ring system with the piperidine

Table 2



Ar:  B  C

compd no.	Ar	R_1	R_2	R[a]	molecular formula	solvent of crystallization	mp (°C)	5-HT_4 receptor antagonist activity (% relaxation to 10^{-6} M 5-HT ± SEM (no. of determinations)) antagonist concentration 3×10^{-8} M	10^{-7} M	10^{-6} M
12g	B	$-CH_2-CH_2-$		X	$C_{27}H_{33}FN_4O_2 \cdot HCl$	2-PrOH	245−247		22.2 ± 6.1(5)	17.7 ± 5.3(6)
19b	B	H	H	Me	$C_{17}H_{24}N_4O \cdot C_2H_2O_4$	2-PrOH	185	37.3 ± 3.2(3)		
19l	B	H	H	X	$C_{25}H_{31}FN_4O_2 \cdot HCl$	EtOAc−MeOH	174−175	36.0 ± 6.2(6)	15.7 ± 5.5(6)	7.5 ± 3.5(8)
13g	C	$-CH_2-CH_2-$		X	$C_{27}H_{33}FN_4O_3 \cdot HCl$	EtOAc−MeOH	235−236		29.5 ± 6.0(6)	2.1 ± 6.5(6)
17a	C	H	H	Me	$C_{17}H_{24}N_4O_2 \cdot HCl$	EtOAc−MeOH	203−205		40.8 ± 11.4(4)	7.5 ± 2.7(4)
17b	C	H	H	X	$C_{25}H_{31}FN_4O_3 \cdot HCl$	EtOAc	210−212		62.0 ± 13.9(6)	

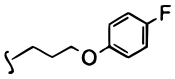
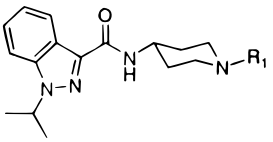
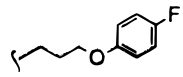
[a] X = 

Table 3



compd no.	R_1[a]	molecular formula	solvent of crystallization	mp (°C)	5-HT_4 receptor antagonist activity (% relaxation to 10^{-6} M 5-HT ± SEM (no. of determinations)) antagonist concentration 10^{-8} M	3×10^{-8} M	10^{-7} M	10^{-6} M
19a	H	$C_{16}H_{22}N_4O \cdot HCl$	EtOAc−MeOH	145				32.0 ± 11.3(4)
19c	CH_2Ph	$C_{23}H_{28}N_4O \cdot HCl$	EtOAc−MeOH	209−212	38.5 ± 9.0(4)	23.3 ± 8.9(4)	24.5 ± 7.9(6)	3.85 ± 2.8(4)
19d	$(CH_2)_2Ph$	$C_{24}H_{30}N_4O \cdot C_2H_2O_4$	EtOH	188				1.3 ± 1.3(3)
19e	$(CH_2)_3Ph$	$C_{25}H_{32}N_4O \cdot C_2H_2O_4$	EtOH	184				3.7 ± 2.7(3)
19f	$(CH_2)_4Ph$	$C_{26}H_{34}N_4O \cdot C_2H_2O_4$	EtOH	167				4.0 ± 0.6(3)
19g	$(CH_2)_5Ph$	$C_{27}H_{36}N_4O \cdot HCl$	EtOAc	133				43.7 ± 8.5(6)
19h	$(CH_2)_2Phth$	$C_{26}H_{29}N_5O_3 \cdot C_2H_2O_4$	EtOAc−MeOH	148−150				7.5 ± 3.9(4)
19i	$(CH_2)_2NHMs$	$C_{19}H_{29}N_5O_3S \cdot C_4H_4O$	EtOH	180			16.0 ± 4.8(4)	6.8 ± 5.4(4)
19j	$(CH_2)_2NHCOPh$	$C_{25}H_{31}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	115			28.0 ± 8.7(4)	6.0 ± 2.7(5)
19k	$(CH_2)_2NHCOAd$	$C_{29}H_{41}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	124			38.5 ± 11.8(4)	1.0 ± 1.0(4)
19b	Me	$C_{17}H_{24}N_4O \cdot C_2H_2O_4$	2-PrOH	185		37.3 ± 3.2(3)		
19l	X	$C_{25}H_{31}FN_4O_2 \cdot HCl$	EtOAc−MeOH	174−175		36.0 ± 6.2(6)	15.7 ± 5.5(6)	7.5 ± 3.5(8)

[a] Phth = phthalimido; Ms = methanesulfonyl; Ad = 1-adamantyl; X = 

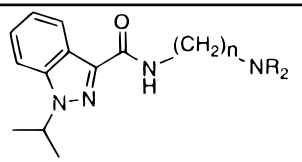
ring system and by incorporating the *p*-fluorophenoxypropyl side chain into the compounds.

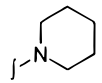
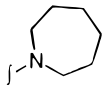
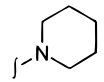
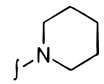
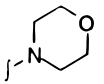
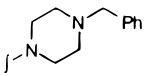
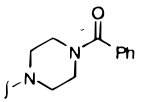
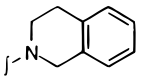
In the indazole series, incorporation of a *p*-fluorophenoxypropyl side chain (**12g**) led to a modest decrease in 5-HT_4 receptor antagonist activity. However, the piperidine analogue of this compound, **19l**, retained most of the activity of the original tropane derivative and was equipotent with the *N*-methylpiperidine derivative **19b** (Table 2). In contrast, modifying the benzimidazolone analogue **13e** by either replacing the tropane nucleus with the piperidine system (**17a**) or incorporating the *p*-fluorophenoxypropyl side chain (**13g**) decreased activity. When both of these modifications were made, the resulting compound, **17b**, was inactive as a 5-HT_4 receptor antagonist at 10^{-7} M. Since the 1-isopropylindazole series was more tolerant to these modifications, we focused subsequent efforts on this series.

Table 3 outlines the effect of modification of the substituent on the piperidine nitrogen of **19b**. *N*-Methyl substitution of the secondary amine increased 5-HT_4 receptor antagonist affinity by greater than 10-fold relative to the unsubstituted amine **19a**. Likewise, incorporation of a benzyl, phenethyl, phenylpropyl, or phenylbutyl group led to compounds with improved 5-HT_4 receptor antagonist activity (**19c**−**f**). Further lengthening of the side chain to the phenylpentyl group (**19g**) reduced 5-HT_4 receptor antagonism. Thus, relatively large groups were well-tolerated at the piperidine nitrogen. Since the mesylamidoethyl side chain of the known 5-HT_4 receptor antagonist GR113808 (**4**) is available to participate in a hydrogen bond, we wished to incorporate that and similar functionality into the series. As anticipated, the mesylamidoethyl derivative **19i** and its corresponding phthalimido (**19h**), benzamido (**19j**), and adamantylamide (**19k**) analogues were all potent 5-HT_4 receptor antagonists.

Piperidine Replacement Strategy. In general, we had found that modification of the rigid tropane skeleton

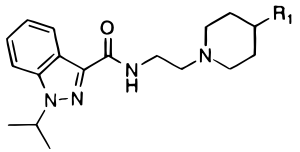
Table 4



Compound number	n	NR2	Molecular formula	solvent of crystalization	melting point (°C)	5HT4 Receptor Antagonist Activity: In vitro (% relaxation to 10^{-6} M 5HT ± SEM (number of determinations)) Antagonist concentration 10^{-7} M	$3 \cdot 10^{-7}$ M	10^{-6} M	10^{-5} M	Ex vivo [a] (% relaxation to 10^{-6} M 5HT ± SEM (number of determinations))
21a	2	NEt_2	$C_{17}H_{26}N_4O \cdot C_2H_2O_4$	EtOAc	128-130			26.8 ± 9.2 (4)		
21b	2		$C_{17}H_{24}N_4O \cdot HCl$	EtOAc-MeOH	210-212			4.3 ± 1.3 (3)		51 ± 4 (3)
21c	2		$C_{18}H_{26}N_4O \cdot HCl$	EtOAc-MeOH	>250	22.0 ± 9.9 (5)			6.6 ± 1.5 (5)	38 ± 4 (5)
21d	2		$C_{19}H_{28}N_4O \cdot HCl$	EtOAc-MeOH	216-218			4.8 ± 1.9 (4)		52 ± 2 (3)
21e	3		$C_{19}H_{28}N_4O \cdot HCl$	EtOAc-MeOH	156-158	27.3 ± 11.8 (3)	14.3 ± 4.8 (6)	6.3 ± 3.7 (4)		15 ± 4 (3)
21f	4		$C_{20}H_{30}N_4O \cdot C_2H_2O_4$	2-PrOH	143	43.3 ± 21.5 (2)		3.3 ± 0.6 (4)		49 ± 6 (4)
21g	2		$C_{17}H_{24}N_4O_2 \cdot HCl$	EtOAc-MeOH	201-203	42.3 ± 8.3 (3)		19.8 ± 7.7 (5)		
21h	2		$C_{24}H_{31}N_5O \cdot 2HCl$	2-PrOH	242			9.1 ± 2.3 (7)		
21i	2		$C_{24}H_{29}N_5O_2 \cdot C_2H_2O_4$	EtOAc-MeOH	105			19.8 ± 4.2 (4)		
21j	2		$C_{22}H_{26}N_4O \cdot C_2H_2O_4$	EtOH	191	20.3 ± 6.9 (4)				41 ± 3 (3)

[a] Compounds tested at 0.1 mg/kg po.

Table 5



compd no.	R_1	molecular formula	solvent of crystallization	mp (°C)	5-HT_4 receptor antagonist activity: in vitro (% relaxation to 10^{-6} M 5-HT ± SEM (no. of determinations)) antagonist concentration 10^{-8} M	3×10^{-8} M	10^{-7} M	10^{-6} M	ex vivo[a] (% relaxation to 10^{-6} M 5-HT ± SEM (no. of determinations)
21c	H	$C_{18}H_{26}N_4O \cdot HCl$	EtOAc−MeOH	>250			22.0 ± 9.9(5)		38 ± 4(5)
23a	NH_2	$C_{18}H_{27}N_5O \cdot 2C_2H_2O_4$	MeOH−H_2O	232				36.8 ± 9.8(4)	41 ± 1(3)[b]
23b	NHCOMe	$C_{20}H_{29}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	176	51.3 ± 13.1(4)	33.5 ± 8.7(4)		6.8 ± 0.6(4)	19.1 ± 1(3)
23c	NHCOEt	$C_{21}H_{31}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	169			11.3 ± 2.9(3)		36 ± 4(3)
23d	NHCO(*n*-Pr)	$C_{22}H_{33}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	122			26.5 ± 6.4(4)		51 ± 5(3)
23e	NHCO(*n*-Bu)	$C_{23}H_{35}N_5O_2$	Et_2O	107−109		51.0 ± 7.6(4)	25.6 ± 8.5(5)		38 ± 4(5)
23f	NHCO(*i*-Pr)	$C_{22}H_{33}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	191		34.8 ± 7.6(4)	9.0 ± 2.4(5)		45 ± 2(3)
23g	NHCO(*t*-Bu)	$C_{23}H_{35}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	194	50.5 ± 7.8(6)	35.0 ± 8.6(4)	7.8 ± 3.4(5)	3.3 ± 2.3(3)	30 ± 4(5)
8	NHCOAd[c]	$C_{29}H_{41}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	228	48.1 ± 4.5(3)	23.2 ± 4.7(5)	11.0 ± 3.5(6)		27 ± 5(4)
23i	NHCOPh	$C_{25}H_{31}N_5O_2 \cdot C_2H_2O_4$	2-PrOH	165	34.3 ± 13.8(4)		14.7 ± 2.2(3)	2.0 ± 0.6(3)	50 ± 7(6)[d]
23j	$NHCOCH_2Ph$	$C_{26}H_{33}N_5O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	168			23.7 ± 6.9(3)	5.6 ± 1.6(5)	39 ± 4(3)
21k	$NHCOOCH_2Ph$	$C_{26}H_{33}N_5O_3 \cdot C_2H_2O_4$	EtOAc−MeOH	145				2.8 ± 2.8(4)	21 ± 3(6)
23k	NHCONHPh	$C_{25}H_{32}N_6O_2 \cdot C_2H_2O_4$	EtOH	198				11.0 ± 3.1(3)	33.7 ± 7(6)
23l	NHCONH(*p*-F-Ph)	$C_{25}H_{31}FN_6O_2 \cdot C_2H_2O_4$	EtOAc−MeOH	158			44.8 ± 8.5(4)	6.3 ± 3.8(3)	45.5 ± 5(3)
23m	$NHCONHCH_2Ph$	$C_{26}H_{34}N_6O_2 \cdot C_2H_2O_4$	EtOH	168				3.3 ± 0.3(3)	30 ± 3(3)
23n	$NHSO_2Me$	$C_{19}H_{29}N_5O_3S \cdot C_2H_2O_4$	EtOAc−MeOH	204				7.3 ± 2.3(4)	29 ± 5(4)
21l	CH_2Ph	$C_{25}H_{32}N_4O \cdot C_2H_2O_4$	EtOAc	149			42.0 ± 13.4(4)	16.4 ± 3.3(5)	26 ± 3(3)

[a] Compounds tested at 0.1 mg/kg, po, unless otherwise noted. [b] Compound tested at 3.0 mg/kg, po. [c] Ad = 1-adamantyl. [d] Compound tested at 1.0 mg/kg, po.

to the less rigid piperidine system increased 5-HT_4 receptor antagonist activity. To explore further increases in the flexibility of the side chain, replacement of the piperidine ring with the acyclic diethylaminoethyl group (**21a**) resulted in a compound with only moderate 5-HT_4 receptor antagonist activity (Table 4). However, replacement of the diethylamino group with a cyclic amine (**21b**−**d**) significantly increased activity, similar to previous observations with a series of metoclopramide ester analogues.[26] Increasing the length of the linking methylene chain to three and four carbons (**21e,f**) did not further increase 5-HT_4 receptor antagonist activity. Substitution with a morpholine (**21g**) or a substituted piperazine (**21h,i**) group slightly decreased 5-HT_4 receptor affinity, whereas the benzo-fused piperidine system **21j** was well-tolerated and equipotent with the parent piperidine system.

Pursuing our hypothesis that hydrogen bonding increased 5-HT_4 receptor antagonist activity, we proposed that incorporation of groups capable of hydrogen bond formation into **21c** would increase 5-HT_4 receptor affinity. We chose to attach these groups through C-4 of the piperidine of **21c** since the resulting derivatives would, because of the symmetry of the system, consist of a single stereoisomer. The 5-HT_4 receptor antagonist activity of this series is outlined in Table 5. Although the 4-aminopiperidine analogue **23a** had lower affinity than the parent piperidine derivative as a 5-HT_4 receptor antagonist, a series of aliphatic amide derivatives of the 4-aminopiperidine (**23b**−**g, 8**) were potent 5-HT_4 receptor antagonists in vitro. We propose that the amide groups interact with the 5-HT_4 receptor in an area of steric tolerance since substituents as large as *tert*-butyl and 1-adamantyl gave rise to potent 5-HT_4 receptor antagonists. The benzamide (**23i**) and phenacyl (**23j**) derivatives were also 5-HT_4 receptor antagonists as were the carbamate **21k**, the urea derivatives **23k**−**m**, and the methanesulfonamide **23n**. Because the 4-benzylpiperidine analogue **21l** was less potent than the other 4-substituted analogues, the C-4 substituent likely interacts with the 5-HT_4 receptor in a hydrogen-bonding interaction rather than strictly a hydrophobic interaction.

5-HT_3, 5-HT_{2A}, and α_1 Adrenergic Receptor Selectivity. Throughout the development of this SAR directed toward obtaining high affinity 5-HT_4 receptor antagonists, careful attention was paid to the specificity of the 5-HT_4 receptor interactions. We had chosen tropisetron and cisapride as the leads for these studies. Since tropisetron is a potent 5-HT_3 receptor antagonist[27] and cisapride has high affinity for 5-HT_{2A} and α_1 receptors,[6] we initially focused our attention on interactions with these three receptors.

As the SAR progressed, selected compounds were tested as 5-HT_3 receptor antagonists in the guinea pig ileum. Serotonin induces a contraction in this tissue which is blocked by 5-HT_3 receptor antagonists.[27] The affinities of selected compounds are found in Table 6. The tropane derivatives tropisetron (**1**) and zatosetron (**16**) potently block 5-HT_3 receptors in the guinea pig ileum with pK_b's of 8.06 and 8.78, respectively.[28] Potent 5-HT_3 receptor antagonist activity was also seen for **12e** and **13e**, the tropane analogues in the indazole and benzimidazolone series. Since many high-affinity 5-HT_3 receptor antagonists have a basic nitrogen constrained within a polycyclic framework,[29] we proposed that conformationally labile derivatives would have lower affinity at the 5-HT_3 receptor. Indeed, the piperidine **19b** had 10-fold lower 5-HT_3 receptor affinity than its tropane analogue, **12e**. In **21c**, the flexibility of the system is further increased by replacing the piperidine ring with the acyclic ethylene linker, and this compound showed a further decrease in 5-HT_3 receptor affinity with a pK_b of 5.8. By replacement of the tropane system of **12e** with the more flexible piperidine or acyclic systems, we significantly minimized the interactions of these compounds with the 5-HT_3 receptor.

Table 6. 5-HT_3 Receptor Affinity of Selected Compounds

compd no.	5-HT_3 receptor affinity[a] (pK_b)
1	8.06 ± 0.05(16)[b]
16	8.78 ± 0.10(10)[b]
12e	7.4 ± 0.59(4)
13e	7.5 ± 0.29(3)
19b	6.3 ± 0.80(3)
21c	5.8 ± 0.3(4)

[a] Determined from compound's ability to block serotonin-induced contraction of the guinea pig ileum.[28] [b] Data from ref 28.

Table 7. α_1 Adrenergic Receptor and 5-HT_2 Receptor Affinity of Selected Compounds

	α_1 receptor affinity		5-HT_2 receptor affinity[c]
compd no.	pK_b (nM)[a]	pK_i (nM)[b]	pK_b (nM)
2	6.7		7.89
19l	6.7	7.69	6.8
19c	<5.0	<4.0	5.5
19d	7.68		
19e	5.8		
19f	6.5		
19g	5.6		
19j	5.8	6.62	
19i	<5.0	<4.0	
19h			<5.0
21b		<4.0	
21c	4.9	4.8	<5.0
21d		5.0	
21e		<4.5	
21f		5.2	
23b		<4.0	
8		4.3	
21k	<5.0	5.9	

[a] As determined by block of norepinephrine-induced contraction of the rat aorta.[31] [b] As determined by displacement of [^{3}H]prazosin from rat whole brain.[30] [c] As determined by block of serotonin-induced contraction of the rat aorta.[31]

We then explored the interactions of compounds containing the piperidine and acyclic linkers with α_1 adrenergic receptors. The affinity for the α_1 adrenergic receptor was determined by displacement of [^{3}H]prazosin from rat whole brain tissue[30] or by blockade of norepinephrine-induced contractions of rat aorta.[31] The results are reported in Table 7. Cisapride (**2**) blocked α_1 receptors in the rat aorta with a pK_b of 6.7. Compound **12g**, the piperidine derivative containing the *p*-fluorophenoxypropyl side chain, was a similarly potent α_1 receptor blocker. Compound **19d**, in which the *p*-fluorophenoxypropyl group is replaced by a phenethyl group, possessed 10-fold higher affinity than cisapride as an α_1 receptor blocker. Increasing the length of the phenylalkylene side chain decreased α_1 receptor affinity (**19e**−**g**), but none of these compounds had pK_b's lower than 5.6. On the other hand, the *N*-benzylpiperidine

analogue **19c** had virtually no affinity for the α_1 receptor. While the (mesylamidoethyl)piperidine derivative **19i** was not an α_1 receptor antagonist, the analogous benzamidoethyl derivative **19j** did block α_1 receptors. In the acyclic-linked series, none of the compounds studied (**8**, **21b**−**f,k, 23b**) had significant affinity for the α_1 adrenergic receptor.

The 5-HT_{2A} receptor antagonist activity of selected compounds was determined by their ability to block the contractile effect of serotonin in the rat aorta (Table 7).[31] Cisapride (**2**) was an antagonist in this assay with a pK_b of 7.89. The *N*-(*p*-fluorophenoxypropyl)piperidine derivative **19l** was also a 5-HT_{2A} receptor antagonist but with 10-fold lower affinity than cisapride. Replacing the *p*-fluorophenoxypropyl side chain with a benzyl (**19c**) or phthalimidoethyl (**19h**) group led to further decreases in 5-HT_{2A} receptor affinity. In the acyclic series, **21c** was inactive as a 5-HT_{2A} receptor blocker in the concentrations tested.

The trend from these selectivity studies is clear. In progressing from the highly constrained tropane skeleton through the less rigid piperidine ring system and onto the flexible acyclic systems, we were able to progressively increase the selectivity of these compounds for the 5-HT_4 receptor versus 5-HT_3, α_1 adrenergic, and 5-HT_{2A} receptors. Next, we focused our attention on the oral in vivo activity of these acyclic derivatives and, to a lesser extent, on selected substituted piperidines that possessed the highest 5-HT_4 receptor affinity and selectivity.

In Vivo 5-HT_4 Receptor Antagonist Activity following Oral Administration. During these SAR studies, our interest centered upon obtaining a potent and orally active 5-HT_4 antagonist with a long duration of in vivo activity for clinical studies. However, convenient and well-documented functional consequences of 5-HT_4 receptor activation were not readily available in vivo. Although 5-HT_4 receptor antagonist activities can be estimated by several complex gastrointestinal assays in dogs[12,32] and by tachycardia in pigs,[33] we utilized ex vivo rat esophageal relaxation to serotonin as a simple, efficient comparative estimate of the in vivo 5-HT_4 receptor antagonist activity of these compounds. We selected 3 h after oral administration of antagonist to rats to remove the esophagus and challenge with serotonin ex vivo. The time (3 h) was selected to minimize any mechanical effects of the gavage on the esophagus and to measure activity at a time that would support a relatively long duration of action necessary for clinical utility.[34]

Of the cyclic amines studied (Table 4) several were evaluated for ex vivo activity. Of these, **21e** showed excellent activity to inhibit ex vivo serotonin-induced relaxation of the rat esophagus after oral administration of 0.1 mg/kg. However, **21e** did possess some modest affinity at both alpha$_2$ and muscarinic receptors which precluded our further interest (data not shown).

A number of the 4-substituted piperidinoethyl derivatives, in particular **23a**,**g**, **8**, **21k**,**l**, and **23m**,**n**, were effective in blocking 5-HT_4 receptors ex vivo following oral administration. The selectivity profile and duration of action of compound **8** dictated further investigation and development of this analogue.[35] Thus, compound **8** has been identified as a potent and selective 5-HT_4 receptor antagonist with pharmacodynamic properties suitable for clinical development.

Experimental Section

General Experimental. All solvents and reagents were used as commercially available. Moisture- and/or air-sensitive reactions were run under a positive pressure of nitrogen. The solvent system used for flash chromatographic purification is reported in parentheses. "(NH_4OH)" indicates that ca. 0.1% ammonium hydroxide solution was added to the eluting solvent. Analytical thin-layer chromatography (TLC) was performed on 0.25-mm Merck silica gel 60 F254 plates. R_f's are reported using the same solvent system that was used for the flash column. Melting points were determined on a Hoover-Thomas Uni-Melt capillary melting point apparatus and are uncorrected. NMRs were recorded at 300 MHz. Chemical shifts are reported in parts per million downfield (δ) from tetramethylsilane in the form: chemical shift (multiplicity, coupling constant, number of protons). *J* values are reported in hertz (Hz). Mass spectra were recorded using field desorption (FD) ionization on a Varian MAT 731 mass spectrometer. Mass spectral data are reported in the form: *m/e* of parent ion (relative intensity). Elemental analyses were performed by the Physical Chemistry Research department of the Lilly Research Laboratories.

N-(1-Benzylpiperidin-4-yl)-1*H*-indazole-3-carboxamide (18). To a solution of 1*H*-indazole-3-carboxylic acid (8.11 g, 50 mmol) in DMF (140 mL) under a nitrogen atmosphere was added in one portion 1,1′-carbonyldiimidazole (8.92 g, 55 mmol). The resulting solution was warmed at 60 °C for 2 h and then cooled to room temperature before adding a solution of 4-amino-1-benzylpiperidine (9.51 g, 50 mmol) in DMF (20 mL). The resulting solution was heated at 60 °C for 2 h. The DMF was evaporated under reduced pressure and the residue dissolved in CH_2Cl_2 (ca. 250 mL). This solution was washed sequentially with water, 1 N NaOH solution, water, and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was recrystallized twice from EtOH to give the product as light-yellow crystals (10.63 g, 76%); mp 198−200 °C. ^{1}H NMR (DMSO-d_6): 1.60−1.84 (m, 4H), 1.97−2.11 (m, 2H), 2.77−2.90 (m, 2H), 3.48 (s, 2H), 3.77−3.94 (m, 1H), 7.19−7.45 (m, 7H), 7.6 (d, 1H, J = 9.0 Hz), 8.10−8.20 (m, 2H), 13.55 (s, 1H). MS: 334 (100). Anal. ($C_{20}H_{22}N_4O$) C,H,N.

N-(1-Benzylpiperidin-4-yl)-1-isopropylindazole-3-carboxamide Hydrochloride (19c). To a solution of *N*-(1-benzylpiperidin-4-yl)-1*H*-indazole-3-carboxamide (9.94 g, 29.7 mmol) in DMF (200 mL) under a nitrogen atmosphere was added sodium hydride (60% dispersion in mineral oil, 1.2 g, 30 mmol), and the resulting mixture was allowed to stir at room temperature for 3 h. The reaction mixture was cooled to about 20 °C, and 2-iodopropane (3.3 mL, 33 mmol) was added. The resulting solution was allowed to stir at room temperature for about 18 h. The DMF was evaporated under reduced pressure, and the residue was dissolved in EtOAc (300 mL). This solution was washed sequentially with 10% aqueous sodium carbonate solution, water, and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give an oil which crystallized. The crude product was purified via preparative HPLC (silica gel, CH_2Cl_2, 0−2% MeOH, 0.5% NH_4OH) to give 8.77 g (78%) of product. A sample of this product (753 mg, 2 mmol) was dissolved in EtOH (30 mL) and treated with ethanolic HCl solution. The mixture was evaporated under reduced pressure, and the residue was stirred in about 40 mL of Et_2O for 2 h. The resulting precipitate was collected by filtration and recrystallized from MeOH/EtOAc to give the hydrochloride salt (478 mg, 58%), mp 209−212 °C. ^{1}H NMR (DMSO-d_6): 1.56 (d, 6H, J = 8.0 Hz), 1.94−2.12 (m, 4H), 3.00−3.45 (m, 4H), 3.97−4.17 (m, 1H), 4.30 (d, 2H, J = 6.0 Hz), 5.08 (septet, 1H, J = 7.0 Hz), 7.27 (t, 1H, J = 8.0 Hz), 7.38−7.70 (m, 6H), 7.80 (d, 1H, J = 9.0 Hz), 8.15 (d, 1H, J = 9.0 Hz), 8.34 (d, 1H, J = 8.0 Hz), 10.36 (br s, 1H). MS: 376 (100). Anal. ($C_{23}H_{29}N_4ClO$) C,H,N.

N-(Piperidin-4-yl)-1-isopropylindazole-3-carboxamide Hydrochloride (19a). 1-Chloroethyl chloroformate (2.86

g, 20 mmol, 2 equiv) was added dropwise to a solution of *N*-(1-benzylpiperidin-4-yl)-1-isopropylindazole-3-carboxamide hydrochloride (3.76 g, 10 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and then heated to reflux for 1 h. The reaction mixture was concentrated under vacuum and the residue dissolved in MeOH (50 mL) and heated to reflux for 1 h. The reaction mixture was concentrated to give a white solid which was recrystallized from MeOH/EtOAc to give the product (2.92 g, 91%), mp 145 °C. ^{1}H NMR (DMSO-d_6): 1.57 (d, 6H, J = 6.0 Hz), 1.80−2.05 (m, 4H), 2.93−3.01 (m, 2H), 4.05−4.20 (m, 1H), 5.08 (septet, 1H, J = 8.0 Hz), 7.25 (t, 1H, J = 8.0 Hz), 7.44 (t, 1H, J = 8.0 Hz), 7.80 (d, 1H, J = 9.0 Hz), 8.30 (d, 1H, J = 9.0 Hz), 8.85 (br s, 1H). MS: 286 (100). Anal. ($C_{16}H_{23}N_4ClO$) C,H,N.

***N*-(1-(2-(Phthalimid-1-yl)ethyl)piperidin-4-yl)-1-isopropylbenzimidazole-3-carboxamide Oxalate (19h).** To a solution of *N*-(piperidin-4-yl)-1-isopropylindazole-3-carboxamide hydrochloride (2.24 g, 6.9 mmol) in DMF (35 mL) under a nitrogen atmosphere was added sodium carbonate (2.94 g, 28 mmol). N-(2-Bromoethyl)phthalimide (1.76 g, 6.9 mmol) in DMF (10 mL) was then added, and the resulting solution was heated at 100 °C for about 18 h. The DMF was evaporated under reduced pressure to provide a residue which was taken up in EtOAc (ca. 250 mL). The solution was washed with water and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 3.08 g of crude product. The free base was purified via flash chromatography (silica gel, EtOAc/EtOH, 100:5) to give a viscous oil (2.14 g, 68%). A sample of the above oil was converted to the oxalate salt in warm EtOAc and recrystallized from MeOH/EtOAc to give the title product as colorless crystals, mp 148−150 °C. ^{1}H NMR (DMSO-d_6): 1.55 (d, 6H, J = 7.0 Hz), 1.68−2.06 (m, 4H), 2.63−2.88 (m, 2H), 2.97−3.20 (m, 2H), 3.25−3.55 (m, 2H), 3.78−4.15 (m, 4H), 5.08 (septet, 1H, J = 7.0 Hz), 7.25 (t, 1H, J = 8.0 Hz), 7.42 (t, 1H, J = 8.0 Hz), 7.68−8.02 (m, 5H), 8.03−8.31 (m, 2H). MS: 459 (100). Anal. ($C_{28}H_{31}N_5O_7$) C,H,N.

***N*-(1-(2-Aminoethyl)piperidin-4-yl)-1-isopropylindazole-3-carboxamide (19m).** To a solution of *N*-(1-(2-(phthalimid-1-yl)ethyl)piperidin-4-yl)-1-isopropylindazole-3-carboxamide (1.86 g, 4.0 mmol) in EtOH (80 mL) at 60 °C was added hydrazine hydrate (2.0 mL), and the resulting solution was stirred at 60 °C for about 4 h. The reaction mixture was cooled to 0 °C and filtered. The filtrate was evaporated under reduced pressure, and to the residue was added 1 N NaOH solution. This mixture was extracted four times with Et_2O. The extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give a colorless solid (1.21 g, 92%) which was used in the next step without further purification.

***N*-(1-(2-((*N*′-Methylsulfonyl)amino)ethyl)piperidin-4-yl)-1-isopropylindazole-3-carboxamide Maleate (19i).** To a solution of *N*-(1-(2-aminoethyl)piperidin-4-yl)-1-isopropylindazole-3-carboxamide (264 mg, 0.8 mmol) in THF (5 mL) under a nitrogen atmosphere at about 15 °C were added triethylamine (0.12 mL, 0.84 mmol) and methanesulfonyl chloride (0.06 mL, 0.8 mmol). The resulting mixture was allowed to stir for about 18 h at room temperature. The mixture was filtered and the filtrate evaporated under reduced pressure to give a cloudy oil. The maleate salt was made in warm EtOAc and was recrystallized from about 10 mL of EtOH to give the title product as colorless crystals (250 mg, 60%) mp 180 °C. ^{1}H NMR (DMSO-d_6): 1.56 (d, 6H, J = 6.0 Hz), 1.80−2.15 (m, 4H), 2.94−3.68 (m, 13H), 3.98−4.20 (m, 1H), 5.10 (septet, 1H, J = 7.0 Hz), 6.05 (s, 2H), 7.22−7.38 (m, 2H), 7.45 (t, 1H, J = 7.0 Hz), 7.80 (d, 1H, J = 9.0 Hz), 8.17 (d, 1H, J = 9.0 Hz), 8.22−8.40 (m, 1H). MS: 407 (100). Anal. ($C_{23}H_{33}N_5O_7S$) C,H,N.

1-Benzyl-4-((carbobenzyloxy)amido)piperidine. A solution of benzyl chloroformate (2 equiv, 144 mmol, 24.4 g) in CH_2Cl_2 (100 mL) was added to a mixture of 4-amino-1-benzylpiperidine (72 mmol, 13.7 g) in CH_2Cl_2 (200 mL) and 1 N NaOH solution (100 mL) at room temperature. The reaction mixture was stirred vigorously for 20 min, poured into water, and extracted with CH_2Cl_2. The extract was dried (Na_2SO_4) and concentrated to yield 36.3 g of crude product as an orange oil. Flash chromatography (2:1 ether/hexanes (NH_4OH)) provided the pure product (R_f 0.21, 15.3 g, 65%) as a colorless oil which solidified upon standing. Recrystallization of a sample of this material (Et_2O/hexanes) gave the product as fine white needles, mp 78−80 °C. ^{1}H NMR ($CDCl_3$): 1.46 (br q, 2H, J = 11), 1.92 (br d, 2H, J = 11), 2.11 (br t, 2H, J = 11), 2.79 (br d, 2H, J = 11), 3.49 (s, 2H), 3.42−3.60 (m, 1H), 4.58−4.70 (m, 1H), 5.07 (s, 2H), 7.20−7.45 (m, 10H). MS: 324 (100). Anal. ($C_{20}H_{24}N_2O_2$) C,H,N.

4-((Carbobenzyloxy)amido)piperidine Hydrochloride. A solution of 1-chloroethyl chloroformate (2 equiv, 84.4 mmol, 12.5 g) in CH_2Cl_2 (25 mL) was added to a solution of 1-benzyl-4-((carbobenzyloxy)amido)piperidine (42.2 mmol, 13.7 g) in CH_2Cl_2 (200 mL) at room temperature. After stirring at room temperature for 1 h, the reaction mixture was concentrated, dissolved in MeOH (200 mL), and heated to reflux for 1 h. The reaction mixture was concentrated to give a white solid. Recrystallization (MeOH/EtOAc) gave the title compound as a white solid (7.56 g, 67%), mp 210.0−210.5 °C. ^{1}H NMR (DMSO-d_6): 1.61 (br q, 2H, J = 11), 1.84 (dd, 2H, J = 3, 14), 2.76−2.96 (m, 2H), 3.14 (br d, 2H, J = 13), 3.47−3.60 (m, 1H), 5.47 (s, 2H), 7.22−7.34 (m, 5H), 7.51 (d, 1H, J = 7), 8.97 (br s, 1H), 9.19 (br s, 1H). MS: 234 (100), 235 (40). Anal. ($C_{13}H_{18}N_2O_2$·HCl) C,H,N.

1-(2-*N*-Phthalimidoethyl)-4-((benzyloxycarbonyl)amino)piperidine. Sodium carbonate (2.83 g, 26.6 mmol) was added to a solution of 4-((carbobenzyloxy)amido)piperidine hydrochloride (2.07 g, 7.6 mmol) and *N*-(2-bromoethyl)phthalimide (1.94 g, 7.6 mmol) in DMF (40 mL) and the reaction mixture stirred at 100 °C for 18 h. The reaction mixture was concentrated, diluted with water, and extracted with EtOAc. The extracts were washed with water and brine, dried (Na_2SO_4), and concentrated to give a solid. Crystallization from EtOH provided the desired product as colorless crystals (1.58 g, 51%), mp 159−161 °C. ^{1}H NMR (DMSO-d_6): 1.20−1.38 (m, 2H), 1.60−1.77 (m, 2H), 1.93−2.10 (m, 2H), 2.76−2.94 (m, 2H), 3.62−3.78 (m, 2H), 5.00 (s, 2H), 7.12−7.24 (m, 1H), 7.24−7.45 (m, 5H), 7.77−7.97 (m, 4H). MS: 407 (100). Anal. ($C_{23}H_{25}N_3O_4$) C,H,N.

***N*-(2-(4-((Benzyloxycarbonyl)amino)-1-piperidinyl)-ethyl)-1-isopropylindazole-3-carboxamide Oxalate (21k).** The following steps were performed without purification of the intermediates.

1-(2-*N*-Aminoethyl)-4-((benzyloxycarbonyl)amino)piperidine (22). A mixture of 1-(2-*N*-phthalimidoethyl)-4-((benzyloxycarbonyl)amino)piperidine (11.9 g, 29.2 mmol), hydrazine hydrate (15.8 mL, 16.4 g, 32.5 mmol), and EtOH (600 mL) was heated to reflux for 4 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated. The resulting residue was taken up in 1 N NaOH solution. This mixture was saturated with NaCl and extracted with Et_2O. The ether extracts were dried (Na_2SO_4) and concentrated to provide the desired product as an oil (8.19 g, 100%) which was used directly in the subsequent reaction. MS: 278 (100).

***N*-(2-(4-((Benzyloxycarbonyl)amino)-1-piperidinyl)-ethyl)-1*H*-indazole-3-carboxamide (20k).** 1,1′-Carbonyldiimidazole (4.70 g, 29 mmol) was added to a solution of 1*H*-indazole-3-carboxylic acid (4.70 g, 29 mmol) in DMF (120 mL) and the reaction mixture stirred at room temperature for 4 h. A solution of 1-(2-*N*-aminoethyl)-4-((benzyloxycarbonyl)amino)-piperidine (8.1 g, 29 mmol) in DMF (18 mL) was added dropwise and the reaction mixture stirred at room temperature for 18 h. The reaction mixture was concentrated to remove the DMF and the residue taken up in water and extracted with EtOAc. The extract was washed with water and brine, dried (Na_2SO_4), and concentrated to the desired product (11.17 g, 91%) which was sufficiently pure for use in the next reaction, mp 184−187 °C. MS: 422 (100).

***N*-(2-(4-((Benzyloxycarbonyl)amino)-1-piperidinyl)-ethyl)-1-isopropylindazole-3-carboxamide Oxalate (21k).** Sodium hydride (60% dispersion in mineral oil, 1.05 g, 26.3 mmol) was added to a solution of *N*-(2-(4-((benzyloxycarbonyl)-

amino)-1-piperidinyl)ethyl)-1*H*-indazole-3-carboxamide (11.1 g, 26.3 mmol) in DMF (100 mL) and the reaction mixture stirred at room temperature for 4 h. The reaction was cooled to 15 °C, 2-iodopropane (2.9 mL, 5.13 g, 29 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 18 h. The DMF was removed in vacuo and the residue dissolved in EtOAc and water. The EtOAc layer was washed sequentially with 10% sodium carbonate solution, water and brine and evaporated to give the crude product. Flash chromatography (5% MeOH in CH_2Cl_2) provided the product as an oil (10.6 g, 87%). Crystallization of the product as the oxalate salt provided colorless crystals, mp 149−151 °C. ^{1}H NMR (DMSO-d_6): 1.55 (d, 6H, J = 8.0 Hz), 1.55−1.75 (m, 1H), 1.85−2.02 (m, 2H), 2.60−4.40 (m, 12H), 5.03 (s, 2H), 5.10 (septet, 1H, J = 7.0 Hz), 7.23−7.55 (m, 7H), 7.82 (d, 1H, J = 9.0 Hz), 8.18 (d, 1H, J = 9.0 Hz), 8.40−8.50 (m, 1H). MS: 463 (100). Anal. ($C_{28}H_{35}N_5O_7$) C,H,N.

N-[2-(4-Amino-1-piperidinyl)ethyl)-1-isopropylindazole-3-carboxamide dioxalate (23a). A solution of *N*-(2-(4-((benzyloxycarbonyl)amino)-1-piperidinyl)ethyl)-1-isopropylindazole-3-carboxamide (8.71 g, 18.8 mmol) in EtOH (135 mL) was hydrogenated over 5% palladium on carbon (3.0 g) at 60 psi and room temperature for 18 h. The reaction mixture was filtered to remove the catalyst and concentrated to give an oil (5.0 g, 81%). Crystallization of this oil as the dioxalate salt gave colorless crystals, mp 232 °C. ^{1}H NMR (DMSO-d_6): 1.55 (d, 6H, J = 7.0 Hz), 1.60−1.80 (m, 2H), 1.90−2.10 (m, 2H), 2.50−2.70 (m, 2H), 2.80−3.00 (m, 2H), 3.05−3.24 (m, 1H), 3.24−3.40 (m, 2H), 3.48−3.67 (m, 2H), 5.10 (septet, 1H, J = 7.0 Hz), 7.28 (t, 1H, J = 8.0 Hz), 7.82 (d, 1H, J = 9.0 Hz), 8.18 (d, 1H, J = 9.0 Hz), 8.30−8.42 (m, 1H). MS: 329 (100). Anal. ($C_{22}H_{31}N_5O_9$) C,H,N.

***N*-(2-(4-(1-Adamantylcarboxamido)-1-piperidinyl)ethyl)-1-isopropylindazole-3-carboxamide Oxalate (8).** 1-Adamantanecarbonyl chloride (199 mg, 1 mmol) was added to a solution of *N*-(2-(4-amino-1-piperidinyl)ethyl)-1-isopropylindazole-3-carboxamide (330 mg, 1.0 mmol) and triethylamine (0.15 mL, 1.07 mmol) in THF (10 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, stirred for 18 h, and filtered. The filtrate was concentrated to give an oil. Crystallization of the oxalate salt (MeOH/EtOAc) gave the title compound as colorless crystals (267 mg, 46%), mp 228 °C. ^{1}H NMR (DMSO-d_6): 1.55 (d, 6H, J = 8.0 Hz), 1.56−1.90 (m, 16H), 1.95 (br s, 3H), 2.80−3.05 (m, 2H), 3.05−3.20 (m, 2H), 3.30−3.55 (m, 2H), 3.55−3.70 (m, 2H), 3.70−3.90 (m, 1H), 5.10 (septet, 1H, J = 7.0 Hz), 7.23−7.32 (m, 1H), 7.27 (t, 1H, J = 8.0 Hz), 7.46 (t, 1H, J = 8.0 Hz), 7.80 (d, 1H, J = 9.0 Hz), 8.18 (t, 1H, J = 9.0 Hz), 8.40−8.55 (m, 1H). MS: 491 (100). Anal. ($C_{31}H_{43}N_5O_6$) C,H,N.

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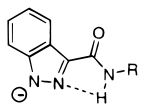

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(35) See ref 6 for a detailed report on the pharmacology of **8**.

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