Pyrrolizidine Esters and Amides as 5-HT$_4$ Receptor Agonists and Antagonists

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A series of pyrrolizidine esters, amides, and ureas was prepared and tested for 5-HT$_4$ and 5-HT$_3$ receptor binding, 5-HT$_4$ receptor agonism in the rat tunica muscularis mucosae (TMM) assay, and for 5-HT$_3$ receptor-mediated functional antagonism in the Bezold−Jarisch reflex assay. Several pyrrolizidine derivatives were identified with high affinity for the 5-HT$_4$ receptor, including benzamide **12a** (SC-53116), a potent and selective 5-HT$_4$ partial agonist that exhibits efficacy in promoting antral contractions and activity in promoting gastric emptying in canine models. Also discovered were 5-HT$_4$ receptor antagonists, including imidazopyridine amide **12h** (SC-53606), which is a potent and selective 5-HT$_4$ receptor antagonist with a pA_2 value of 8.13 in the rat TMM assay. *N*-Methyl indole ester **13d** was identified as a potent 5-HT$_4$ antagonist with a pA_2 value of 8.93. High selectivity was observed for these pyrrolizidine derivatives versus other monoamine receptors, including 5-HT$_1$, 5-HT$_2$, D$_1$, D$_2$, α_1, α_2, and β receptors.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) functions as both a hormone and a neurotransmitter, controlling a host of central and peripheral effects in mammalian systems, and is noteworthy in its diversity of receptors and subtypes.[1] Given the diversity and ubiquitous nature of serotonin receptors, it is imperative to prepare potent and selective 5-HT ligands to enable pharmacological studies of the various receptor subtypes and to serve as drugs to treat diseases that have an etiology in serotonin receptor imbalance.

The 5-HT$_4$ receptor was discovered by Baxter et al.[2] and by Dumuis et al.[3] in the gut and brain, respectively, and is expressed in a wide variety of tissues, including brain, heart, bladder, gut, and kidney[4,5] Initial demonstration that the gastrointestinal prokinetic[6] benzamides cisapride (**1**) and renzapride (**2**) (Figure 1) enhance contractile activity at neuronal 5-HT$_4$ receptors in the guinea pig ileum was made by Craig and Clarke.[7] It was later demonstrated by Dumuis and Bockaert that 5-HT$_4$ receptors mediate the relaxation of smooth muscle of the inner muscularis mucosae of rat esophagus[8] and also the cholinergic stimulation of the ascending colon of the guinea pig.[9] New tools including fluorescent antagonists have recently been developed for the study of 5-HT$_4$ receptors,[10] and an excellent review of the 5-HT$_4$ receptor and key ligands was recently published.[11]

Selective ligands for the 5-HT$_4$ receptor show promise in the treatment of a wide variety of diseases, including irritable bowel syndrome (IBS) and other gastrointestinal motility disorders, urinary incontinence, atrial arrhythmia,[11,12] and cognitive disorders.[13] Gastrointestinal motility disorders are a collection of syndromes that are characterized by a hypofunctional bowel, abnormal motility patterns and transit, and painful gut wall distension. The mechanisms thought to be common to many of these motility disorders is the malfunction of enteric nervous system control of peristalsis at the level of the myenteric plexus.[14] Compound **1** had been marketed for motility disorders[15] but was withdrawn due to QT prolongation.[16,17] The 5-HT$_4$ partial agonist tegaserod (compound **3**, SDZ HTF 919) was approved in 2002 for the treatment of constipation-predominant IBS.[18−20] Tegaserod shows a clear effect on the total colonic transit time in healthy subjects, and a significant improvement in patients with constipation-predominant IBS in a phase III trial.[21] Compound **4**, prucalopride,[22] accelerates colonic transit in healthy subjects but without modification of either gastric emptying or small bowel transit.[23] In patients with severe constipation, the benzamide prucalopride elicited a dose-dependent effect on acceleration of the overall transit time.[24]

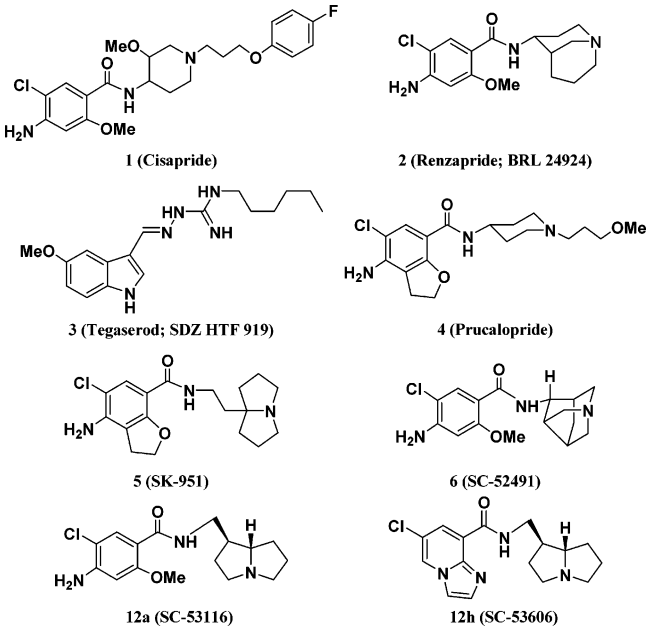

Figure 1. 5-HT4 agonists and antagonists.

We have pursued the exploration of various conformationally constrained bicyclic and tricyclic amines as ligands for the serotonin 5-HT$_4$ receptor to treat gastrointestinal motility disorders. Our focus has been on receptor selectivity to avoid potential side effects, and we were particularly cognizant of

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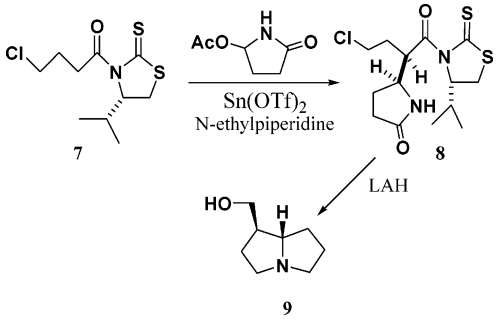
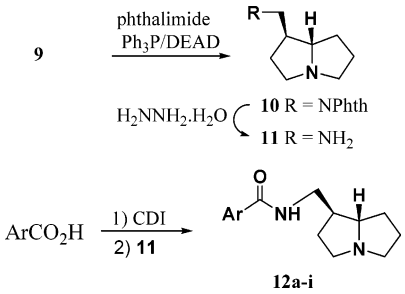
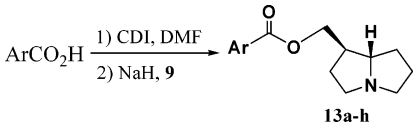
Scheme 1. Preparation of (−)-Trachelanthamidine **9** per Nagao et al.[38]



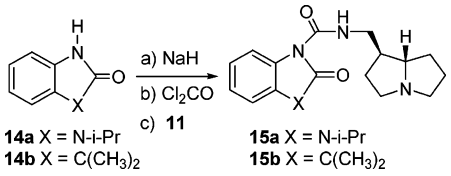
Scheme 2. Preparation of Pyrrolizidine Amides **12a**−**i**



Scheme 3. Preparation of Pyrrolizidine Esters **13a**−**h**

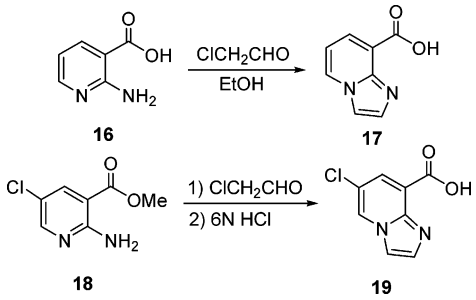


Scheme 4. Preparation of Benzimidazolone **15a** and Oxindole **15b**

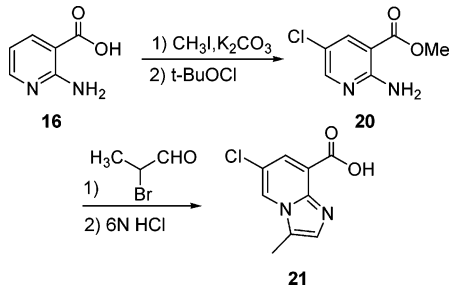


avoiding the dopamine D_2 receptor, as D_2 antagonism is responsible for extrapyramidal side effects and hyperprolactinemia observed with the marketed benzamide metoclopramide.[25] We were also vigilant of other monoamine receptors, including the 5-HT_2 receptor, which is potently inhibited by compound **1**.[26] We were attracted to the pyrrolizidine azabicyclo[3.3.0]-octane ring system and reasoned that this fairly rigid bicyclic amine could provide a fruitful scaffold for preparing potent and selective serotonin 5-HT_4 agonists. A similar approach was used by King in the discovery of renzapride (**2**), which was designed to mimic the higher energy conformers of azabicyclic quinolizidine benzamides of their earlier studies[27] and in particular was designed to lock in the cis form of the ring junction of the azabicycle and reduce the steric bulk extending outward around the basic nitrogen. The Beecham group then also employed a pyrrolizidine scaffold.[28,29] More recently, the 5-HT_4 receptor agonist *meso*-pyrrolizidine **5** (SK-951) has been described, which is a potent gastrointestinal prokinetic agent in rats and dogs.[30,31] Our own successful approach employing the pyrrolizidine moiety led to the potent 5-HT_4 receptor partial agonist **12a**[32] by attaching the pyrrolizidine to the traditional aromatic moiety of prokinetic benzamides **1** and **2**. In addition, we explored the attachment of other aromatic amides to the pyrrolizidine bicyclic amine, seeking to mimic the intramolecular

Scheme 5. Preparation of Imidazol[1,2-*a*]pyridine-8-carboxylic Acid **17** and 6-Chloroimidazo[1,2-*a*]pyridine-8-carboxylic Acid **19**



Scheme 6. Preparation of 6-Chloro-3-methylimidazo[1,2-*a*]pyridine-8-carboxylic Acid **21**



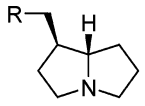
H-bond of the 2-methoxybenzamides. This approach led to the potent 5-HT_4 receptor antagonist **12h**.[33] We also explored both amides and esters of the pyrrolizidine amine moiety with indole and indazole aromatic moieties, inspired by the potent 5-HT_3 receptor antagonists tropisetron[34] and granisetron,[34,35] particularly considering that tropisetron was shown to be the first surmountable antagonist of 5-HT_4 receptors,[3] and Buchheit et al. demonstrated that the ester derivative of metoclopramide, SDZ 205-557, is a potent antagonist of 5-HT_4 receptors.[36]

We were aware of the toxicity of the pyrrolizidine alkaloid natural products[37] and considered the fact that toxicity among pyrrolizidine alkaloids varies dramatically with differences in chemical structure. Certain pyrrolizidine natural products can form highly reactive pyrrole intermediates upon metabolism by CYP3A4, which are responsible for their nascent toxicity. A ring nucleus containing a double bond at the 1,2-position is considered to be essential for toxic effects of the alkaloid, along with additional hydroxyl groups around the nucleus. On the basis of this premise of structure-specificity we decided to explore the pyrrolizidine nucleus for incorporation into novel 5-HT_4 receptor agonists and antagonists. This paper details our work employing the pyrrolizidine scaffold resulting in the potent 5-HT_4 receptor agonist **12a**, as well as 5-HT_4 receptor antagonist **12h** and other potent 5-HT_4 antagonists.

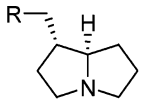
Chemistry

The (*S*,*S*)-carbinol (−)-trachelanthamidine **9** was prepared by a diastereoselective alkylation of the chiral tin enolate of 3-(4-chlorobutyryl)-4(*S*)-isopropyl-1,3-thiazolidine-2-thione **7** with 5-acetoxy-2-pyrrolidinone as elegantly described by Nagao et al.[38] and summarized in Scheme 1. We analyzed the Mosher ester of **9** by ^{19}F NMR to confirm the enantiomeric purity, which showed the material to be ≥99.1% ee. Aminomethylpyrrolizidine **11** was prepared by the Mistunobu reaction of **9** with phthalimide in the presence of triphenylphosphine and diethylazodicarboxylate to afford phthalimide derivative **10**, and the phthalimide moiety was removed with hydrazine to afford **11** (Scheme 2). Coupling of the amine with an aryl carboxylic acid

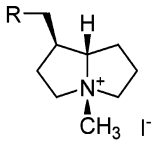
Table 1. Pyrrolizidine 5-HT$_4$ Agonists



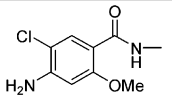
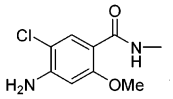
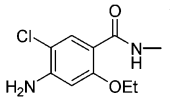
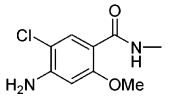
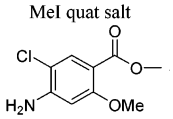
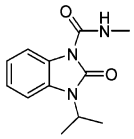
12a-b, 13a, 15a



ent-12a



12c

Entry	R	5-HT$_4$ Binding (K_i, nM)	5-HT$_4$ agonism EC_{50}, nM in rat TMM	5-HT$_3$ Binding (K_i nM)	B-J Reflex: 5HT$_3$ antagonism, % inhib at mpk ip	Other Receptors (IC_{50}, nM)
12a		5.2 (0.5)	16.5 (3.8)	152	87% at 10 57% at 3 7% at 1	>10K for 5HT$_1$, 5HT$_2$, D$_1$, D$_2$, α_1, α_2, β_1, β_2
ent-12a		18% at 500 nM	323 (46)	66 nM	87% at 10 79% at 3 20% at 1	D$_2$ = 3900
12b		…	195	25nM	89% at 10 48% at 3	D$_2$ 5,300
12c		0.350 (0.050)	129 (10)	…	16% at 1000	…
13a	MeI quat salt 	0.183 (0.033)	1.5 (0.2)	K_i = 51 (5)	0% at 10	D$_2$ >10K
15a		7.0 (1.5)	374.2 (171.7)	20.2	87% at 10 62% at 1 0% at 0.3	…

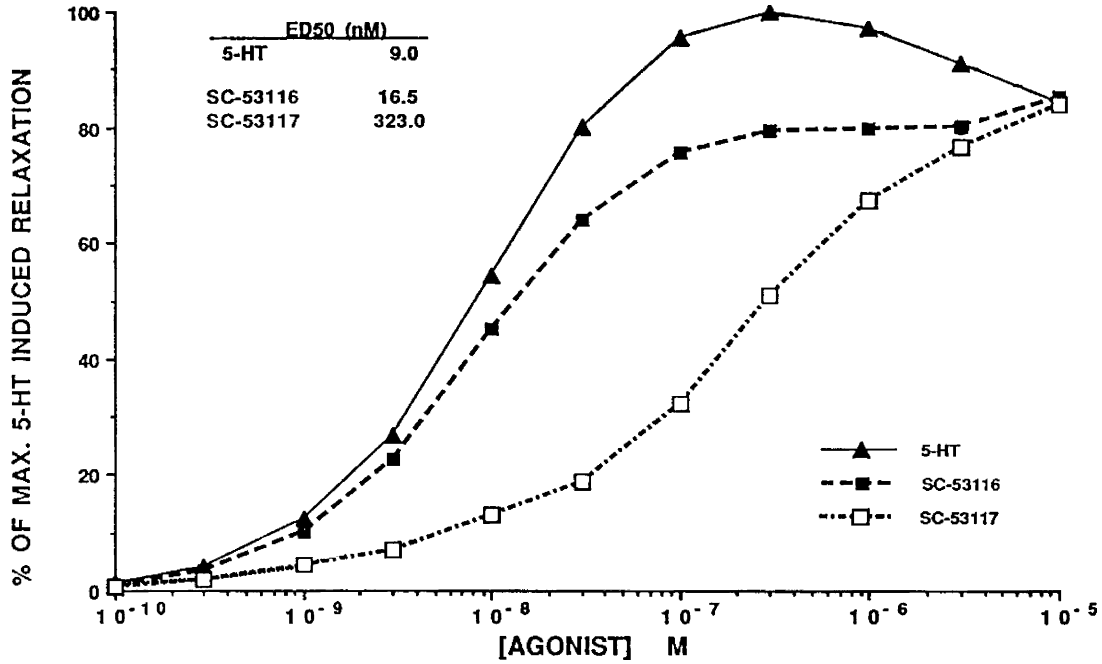
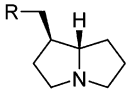


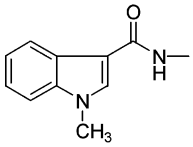
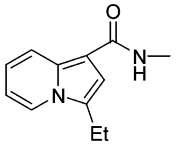
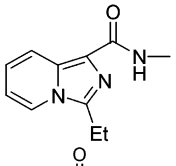
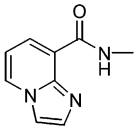
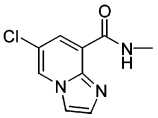
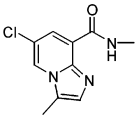
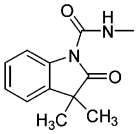
Figure 2. 5-HT$_4$ receptor-mediated relaxation of rat tunica muscularis mucosa by 5-HT, **12a** (SC-53116), and **ent-12a** (SC-53117).

was then typically accomplished with 1,1′-carbonyl diimidazole (CDI) to give the requisite pyrrolizidine amides **12**. Esters of **9** were prepared by treating the imidazolide derived from the appropriate aryl carboxylic acid and CDI with the sodium salt of alcohol **9** to afford esters **13** (Scheme 3). The enantiomer of aminomethylpyrrolizidine **11** and its derivatives (in particular,

Table 2. Pyrrolizidine Amide and Urea 5-HT$_4$ Antagonists



12d-i, 15b

Entry	R	5-HT$_4$ Antagonism pA$_2$ in rat TMM IA at 10^{-7} M
12d		
12e		IA @ 10^{-6} M
12f		6.56
12g		6.75 (0.05)
12h		8.13 (0.06)
12i		7.09 (0.08)
15b		7.0

benzamide **ent-12a**, the enantiomer of **12a**) were prepared from the (*R*)-enantiomer of thiazolidinethione **7**.

Benzimidazolone **15a** and oxindole urea **15b** were prepared by treatment of the sodium salt of the corresponding benzimidazolone **14a** or oxindole **14b** with phosgene followed by the aminomethylpyrrolizidine **11** (Scheme 4). Imidazol[1,2-*a*]pyridine-8-carboxylic acid **17** was prepared by reacting 2-aminonicotinic acid **16** with chloroacetaldehyde, and 6-chloroimidazo-[1,2-*a*]pyridine-8-carboxylic acid **19** was prepared from methyl 2-amino-5-chloronicotinate **18** with chloroacetaldehyde followed by saponification (Scheme 5). Similarly, 6-chloro-3-methylimidazo[1,2-*a*]pyridine-8-carboxylic acid **21** was prepared by alkylative esterification of 2-aminonicotinic acid **16** and regioselective chlorination with *tert*-butyl hypochlorite to afford **20**, followed by conversion to the imidazopyridine with 2-bromopropionaldehyde and hydrolysis of the ester to afford carboxylic acid **21** (Scheme 6). The pyrrolizidine esters and amides of these imidazopyridines were prepared by the general methods described in Schemes 2 and 3.

Results and Discussion

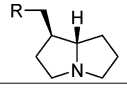
Initial testing of pyrrolizidine 5-HT$_4$ receptor ligands was accomplished using the rat esophageal tunica muscularis mucosal (TMM) tissue,[2,39] as this model allows for determination of full cumulative dose−response curves to agonists. Serotonin 5-HT$_4$ receptor antagonists were also evaluated using the TMM model. Binding to serotonin 5-HT$_4$ receptors in guinea pig striatum was measured utilizing [^{3}H]GR113,808 as the ligand. Binding to serotonin 5-HT$_3$ receptors in rat cortical tissue was measured using [^{3}H]GR656630. Functional 5-HT$_3$ antagonism was measured by utilizing the von Bezold−Jarisch reflex model.[40]

Pyrrolizidine 5-HT$_4$ agonists are summarized in Table 1. In the rat TMM assay, the chiral pyrrolizidine benzamide **12a** and its distomer **ent-12a** had EC$_{50}$ values of 16.5 ± 3.8 and 323 ± 46 nM, respectively. A plot of the TMM activity of **12a** and **ent-12a** relative to serotonin is shown in Figure 2, demonstrating that the pyrrolizidine benzamide **12a** has comparable potency to serotonin (16.5 nM vs 9.0 nM for 5-HT) and has 80% of the efficacy of serotonin in this preparation. The distomer **ent-12a** is 36 times less potent than serotonin. The potency of **12a** relative to its enantiomer is reflected in 5-HT$_4$ receptor binding. Pyrrolizidine **12a** has a K_i value of 5.2 nM, while **ent-12a** inhibits ligand binding by only 18% at 500 nM (Table 1). Benzamide **12a** is approximately 30 times more potent in binding to the 5-HT$_4$ receptor relative to its 5-HT$_3$ binding (K_i = 152 nM). In contrast to the 30× difference in 5-HT$_4$ binding, **12a** is only half as potent as **ent-12a** in 5-HT$_3$ binding. We previously reported a similar trend with compound **6** and its enantiomer, where the 5-HT$_4$ receptor showed a greater discernment of the antipodes than the 5-HT$_3$ receptor, although in that case the eutomer was the same compound for both receptors.[41] Functional antagonism of the 5-HT$_3$ receptor is demonstrated by inhibition of the von Bezold−Jarisch reflex in mice for both **12a** and **ent-12a** (87% at 10 mg/kg for both compounds).

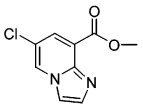
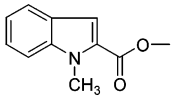
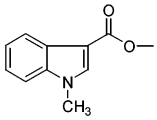
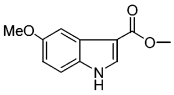
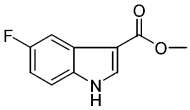
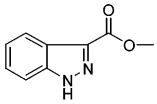
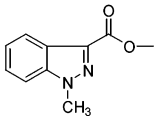
The ethoxy benzamide **12b** was prepared in an attempt to increase 5-HT$_3$ potency, which was successful in boosting 5-HT$_3$ receptor binding by 6-fold (K_i = 25 nM); however, the compound was approximately 12 times less potent in the rat 5-HT$_4$ TMM assay, so 5-HT$_3$ receptor binding potency was boosted at the expense of 5-HT$_4$ potency. The methiodide quaternary ammonium salt **12c** was prepared to probe the possibility of synthesizing a potent radiolabeled pyrrolizidine analogue by employing radiolabeled methyl iodide. The quaternary ammonium salt **12c** was indeed very potent in binding to the 5-HT$_4$ receptor (K_i = 0.35 nM), but despite its greater potency in binding, **12c** was markedly less potent than **12a** as an agonist in the TMM functional assay, presumably due to the membrane transport limitations of the quaternary salt. Compound **13a** is the ester corresponding to **12a** and was the most potent compound tested in our hands for both 5-HT$_4$ receptor binding (K_i = 183 pM) and in functional agonism at the 5-HT$_4$ receptor in the TMM assay (EC$_{50}$ = 1.5 nM). It is actually a partial agonist at the 5-HT$_4$ receptor, with 57% efficacy as an agonist relative to serotonin. *N*-Isopropyl benzimidazolone urea **15a**, which incorporates the benzimidazolone moiety of BIMU 8[42] and DAU 6215,[43] exhibited potent binding at the 5-HT$_4$ receptor (K_i = 7.0 nM) but was 22 times less potent in 5-HT$_4$ agonism than **12a**.

The excellent potency of the pyrrolizidine moiety in 5-HT$_4$ receptor agonism prompted us to consider employing this scaffold for the preparation of antagonists; indeed, we were encouraged in this regard by the potent but partial agonism of benzoate ester **13a**. Agonism of the 5-HT$_4$ receptor may be dependent upon a planar array of the amide bond with the aromatic ring system, enabled by an intramolecular H-bond between the amide NH and the methoxy group as for **12a** or

Table 3. Pyrrolizidine Ester 5-HT$_4$ Antagonists



13b-h

Entry	R	5-HT$_4$ Binding, (K_i, nM)	5-HT$_4$ Antagonism: pA_2 in rat TMM	5-HT$_3$ binding K_i, nM (SEM)	5-HT$_1$, 5-HT$_2$, D_1, D_2, α_1, α_2, β_1, β_2 binding K_i, nM
13b		…	6.75	…	…
13c		…	IA at 10^{-7} M	…	…
13d		0.183 (0.017)	8.93(0.07)	5.0 (1.0)	>10K
13e		…	7.15	…	…
13f		0.70 (0.15)	8.50	10 (1)	…
13g		0.80 (0.83)	8.48	135 (5)	>10K
13h		0.40 (0.033)	8.56	20 (2)	>10K

between the amide NH and the benzimidazolone carbonyl of **15a**. The 5-HT$_4$ agonist efficacy of ester **13a** is lower (57%), apparently due to the inability to form this H-bond and the corresponding conformational mobility.

Table 2 includes pyrrolizidine amides and ureas that are functional 5-HT$_4$ antagonists in the rat TMM assay employing different aromatic groups. All of these compounds were determined to have EC_{50} values for agonism in the rat TMM assay of >10 000 nM. Two of the derivatives tested were determined to be inactive in the rat TMM antagonism assay at concentrations of up to 100−1000 nM, including indole carboxamide **12d** and indolizine **12e**, which borrows the indolizine moiety described by Bermudez et al.[44] We employed an imidazopyridine aromatic nucleus with the nitrogen lone pair orthogonal to the aromatic π-system (compounds **12f**−**i**) pointing toward the amide NH in an attempt to mimic the intramolecular hydrogen bond present in the 2-methoxybenzamide prokinetic 5-HT$_4$ agonists. Additionally, we were intrigued that the bridgehead nitrogen lone pair is partially delocalized into the amide carbonyl as in the 4-aminobenzamides **1** and **2**, making the carbonyl oxygen more electron rich and favoring the coplanarity of the aromatic heterocycle and the amide. The structural similarity to benzofuran amides **4** and **5** is also apparent. Nonetheless, we found that these compounds function as 5-HT$_4$ receptor antagonists. Several imidazopyridines afforded good 5-HT$_4$ antagonist activity, including 3-ethylimidazopyridine **12f** (pA_2 = 6.56). The role of the second nitrogen atom in the antagonist imidazopyridine **12f** relative to inactive indolizine **12e** is noteworthy. The 6-chloro substituent was incorporated in analogue **12h** to boost binding on the basis of structural overlays done with the benzamide moiety of the prokinetic benzamides, assuming a similar binding mode for the two series based on modeling considerations. Unsubstituted imidazopyridine **12g** was moderately potent (pA_2 = 6.75), whereas addition of the chlorine in the 6-position boosted potency of compound **12h**[33] by over an order of magnitude (pA_2 = 8.13). Imidazopyridine **12h** exhibited very potent binding to the 5-HT$_4$ receptor (K_i = 1.4 nM). Incorporation of a 3-methyl substitutent was probed in order to augment potency with an additional hydrophobic interaction from the synthetically accessible imidazopyridine, but further substitution with the 3-methyl group decreased the 5-HT$_4$ inhibitory potency of compound **12i** (pA_2 = 7.09) relative to **12h**. Oxindole urea **15b**, which is structurally related to benzimidazolone 5-HT$_4$ receptor agonist **15a**, is an antagonist that exhibits a pA_2 value of 7.0. As with the imidazopyridines, we originally hoped that the oxindole carbonyl could participate in an intramolecular hydrogen bond with the urea NH. Oxindole **15b** exhibits potent binding to the 5-HT$_4$ receptor (K_i = 6.8 nM).

Pyrrolizidine ester 5-HT$_4$ receptor antagonists are included in Table 3. The esters were inspired by the work of Buchheit et al., who reported SDZ 205-557, the ester analogue of metoclopramide, as the first potent antagonist of 5-HT$_4$ receptors.[36] These compounds all lack agonist activity in the TMM assay (EC_{50} > 10 000 nM). On the basis of the inhibitory potency of 6-chloroimidazopyridine amide **12h**, we prepared the corresponding ester **13b**, which is a moderate 5-HT$_4$ receptor antagonist (pA_2 = 6.75). Next, we prepared esters incorporating the indole and indazole aromatic moieties present in tropisetron and granisetron, respectively. Attachment at the indole 2-position afforded *N*-methyl indole-2-carboxylic ester **13c**, which was inactive, but good antagonism was obtained with 3-substituted

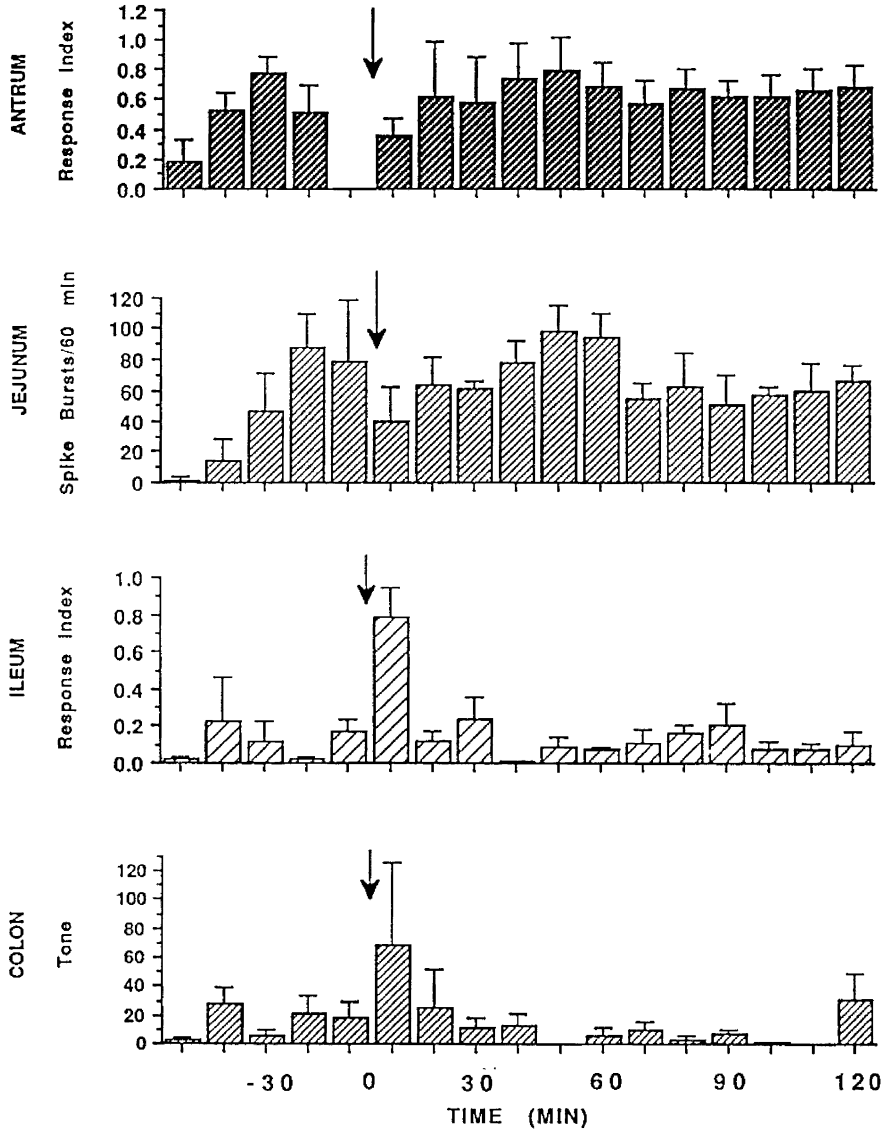


Figure 3. Gastrointestinal response to compound **12a** (0.3 mg/kg, iv) in the dog. Contractile responses of the antrum (panel 1), jejunum (panel 2), ileum (panel 3), and colon (panel 4) before and after 5-HT_4 partial agonist **12a** is given iv to fasted dogs ($n = 4$). A sustained stimulation of antral and jejunal motility occurs with brief augmentation of ileal and colonic contractions.

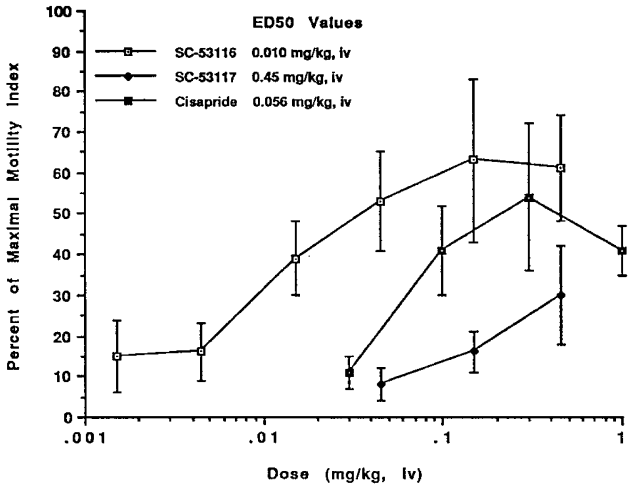


Figure 4. Stimulation of gastric antral contractions by **12a** (SC-53116), **ent-12a** (SC-53117), and **1** (cisapride) given in phase 1 of the MMC cycle as the percentage of maximal activity seen during phase 3 of the MMC.

indoles and indazoles, particularly with *N*-methyl indole-3-carboxylic ester **13d**, which exhibited exceptional potency as an antagonist. 5-Methoxyindole-3-carboxylate ester **13e** exhibited moderate inhibitory potency at the 5-HT_4 receptor ($pA_2 = 7.15$), and potency was markedly increased for the 5-fluoro derivative **13f** in 5-HT_4 inhibitory potency ($pA_2 = 8.5$) as well as binding to the 5-HT_4 and 5-HT_3 receptors ($K_i = 700$ pM and 10 nM, respectively). Indazole ester **13g** had good inhibitory potency ($pA_2 = 8.48$) and the corresponding *N*-methyl indazole derivative **13h** had comparable potency ($pA_2 = 8.56$). These indazoles exhibited potent subnanomolar binding to the 5-HT_4 receptor ($K_i = 800$ and 400 pM, respectively) and moderate binding to the 5-HT_3 receptor ($K_i = 135$ and 20 nM, respectively). The *N*-methyl analogue was thus modestly more potent than the N−H analogue toward both receptors. Both indazoles were free of binding at 5-HT_1, 5-HT_2, D_1, D_2, α_1, α_2, β_1, and β_2 receptors. Thus, the most potent member of the pyrrolizidine ester 5-HT_4 receptor antagonist series summarized in Table 3 is *N*-methyl indole-3-carboxylic ester **13d** with a pA_2 value of 8.93, which is comparable in potency to the Glaxo antagonist GR-113808 ($pA_2 = 9.39$). Indole **13d** is a very potent dual receptor 5-HT_4/5-HT_3 ligand, with a $K_i = 183$ pM at the 5-HT_4 receptor and $K_i = 5.0$ nM at the 5-HT_3 receptor. This

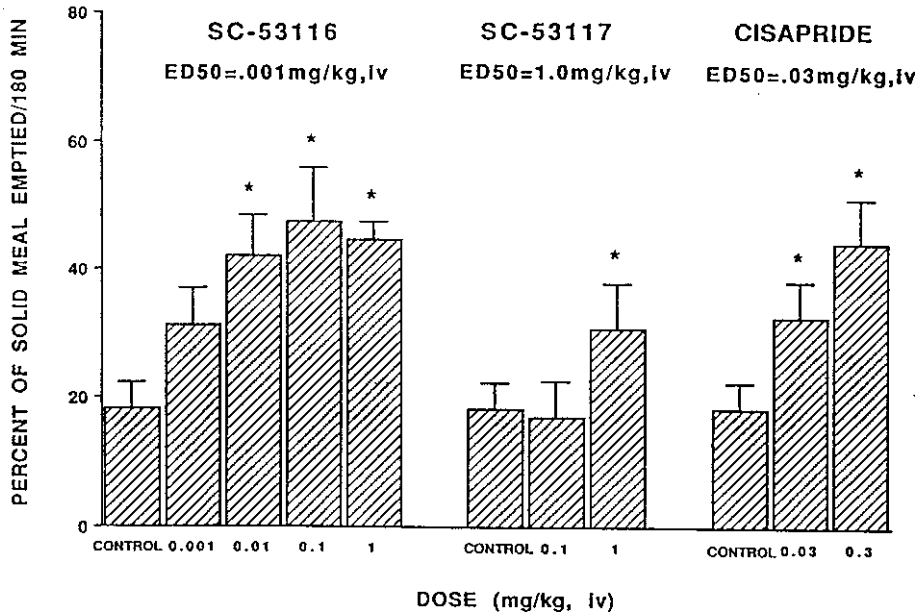


Figure 5. Enhancement of gastric emptying of radiolabeled solid meals by **12a** (SC-53116), **ent-12a** (SC-53117), and **1** (cisapride).

Table 4. Comparison of Enantiomers **12a** and **ent-12a** with **1** and **2**

assay	**1**	**2**	**12a**	**ent-12a**
5-HT$_4$ agonism rat TMM (EC_{50}, nM)	55 ± 8	98 ± 14	17 ± 4	323 ± 46
antral contractility in fasted dog (ED_{50}, mg/kg, iv)	0.048	0.015	0.010	0.45
solid gastric emptying (ED_{50}, mg/kg, iv)	0.03	active, but full dose-response not performed	0.001	1.0

pyrrolizidine ester is free of binding at 5-HT$_1$, 5-HT$_2$, D$_1$, D$_2$, α_1, α_2, β_1, and β_2 receptors (K_i > 10 μM).

In general, the esters are more potent as 5-HT$_4$ antagonists than the corresponding amides, although the present work only allows for two direct comparators. Specifically, *N*-methyl indole ester **13d** is more potent than the *N*-methyl indole carboxamide **12d**. In contrast, the 6-chloroimidazopyridine amide **12h** is more potent than the corresponding ester **13b**.

In vivo 5-HT$_4$ agonism was examined in conscious dogs by recording contractile activity of the distal region of the stomach (antrum), as well as along other portions of the gastrointestinal tract. Test compounds were intravenously administered to fasted animals during phase I (a period of quiescence that lasts about 50−80 min) of the interdigestive migrating motor complex (MMC). The maximal contractile activity that occurred during phase III of the MMC was used to normalize the motility response to the compound. Pyrrolizidine amide **12a** stimulated sustained antral and jejunal contractile activity as well as a brief augmentation of ileal and colonic contractions (Figure 3).

The antral responses to intravenous dosing of **12a**, **ent-12a**, and **1** in the canine contractility model are plotted in Figure 4. Pyrrolizidine benzamide **12a** exhibited an ED_{50} of 0.010 mg/kg iv, whereas benzamide **1** was 5-fold less potent with an ED_{50} of 0.056 mg/kg iv. Distomer **ent-12a** was approximately 45 times less potent than **12a** in promoting gastric antral contractions with an ED_{50} of 0.45 mg/kg iv. A maximally effective dose of pyrrolizidine **12a** induced antral contractions that were approximately 60% of the maximum phase 3 contractions, compared to 54% for compound **1** and 69% for compound **2** (not shown).

In vivo gastrointestinal prokinetic efficacy was also determined in a functional model for gastroparesis employing γ-scintigraphy. Since 5-HT$_4$ agonists enhance solid gastric emptying largely by increasing the force of contraction of the gastric antrum, the canine antral contractile response was used to select compounds for the gastroparesis model. In the gastroparesis model, an α-adrenergic agonist (2-methyl-3-[(2*E*)-pyrrolidin-2-ylideneamino]phenol) inhibits food-induced gastric antral and duodenal contractions, thus mimicking motor abnormalities characteristic of clinical dysmotilities.[45] Enhancement of gastric emptying in the gastroparesis model is demonstrated by pyrrolizidines **12a** and **ent-12a** and benzamide **1** (Figure 5). Pyrrolizidine benzamide **12a** was shown to be approximately 30 times more potent than benzamide **1** in enhancing gastric emptying (ED_{50} = 0.001 mg/kg vs an E_{D50} = 0.03 mg/kg for compound **1**. As expected, distomer **ent-12a** was less potent than both benzamide **1** and pyrrolizidine **12a**, with an ED_{50} of 1.0 mg/kg.

5-HT$_4$ agonism data is summarized in Table 4 along with canine antral contractility and canine solid gastric emptying for the pyrrolizidine benzamides **12a** and **ent-12a** and the benzamide **1**. Pyrrolizidine **12a** is the most potent compound in vitro, as well as in vivo, in both canine motility models. Compound **12a** has an ED_{50} in the fasted dog antral contractility model of 0.010 mg/kg, iv, and exhibits an ED_{50} of 0.001 mg/kg, iv in the canine solid gastric emptying model. Benzamide **1** has an

Table 5. Receptor Profiling of Compounds: EC_{50} or K_i

compd	EC_{50} (nM): 5-HT4 agonism	K_i (nM)								
		5-HT$_4$	5-HT$_1$	5-HT$_2$	5-HT$_3$	D$_1$	D$_2$	α_1	α_2	β
1	54.7	17	>1K	6.1	134	1700	227	30	4500	>10K
12a	16.5	5.2	>10K	>10K	152	>10K	>10K	>10K	>10K	>10K
12h	>10K	1.4	>10K	>10K	259	>10K	>10K	>10K	>10K	>10K
6	51	29	>10K	>10K	1.2	>10K	>10K	>10K	>10K	>10K

ED_{50} of 55 nM in the rat TMM assay, whereas benzamide **2** exhibits an ED_{50} of 98 nM. Compound **2** is also more potent than benzamide **1** in the antral contractility model, with an ED_{50} of 0.015 mg/kg iv relative to 0.048 mg/kg iv for compound **1**.

Pyrrolizidine derivatives of the present series are highly selective for the 5-HT_4 receptor. Pyrrolizidine **12a** is very potent as a partial agonist at the 5-HT_4 receptor and in binding to the 5-HT_4 receptor (K_i = 5.2 nM) (Table 5). Compound **12a** exhibits moderate binding at the 5-HT_3 receptor (K_i = 152 nM), but no detectible binding (K_i > 10 000 nM) at any of the other monoamine receptors tested (5-HT_1, 5-HT_2, D_1, D_2, α_1, α_2, and β receptors). Imidazopyridine **12h**, as reported previously,[33] is a very potent antagonist at the 5-HT_4 receptor (K_i = 1.4 nM; pA_2 = 8.13) with excellent selectivity versus the other monoamine receptors tested (K_i > 10 μM). Standards included for comparison are compound **6**, which we have reported to be a potent and selective dual 5-HT_4 agonist and 5-HT_3 receptor antagonist, and compound **1**, which shows potent inhibition of α_1 receptors (K_i = 30 nM) and 5-HT_2 receptors (6.1 nM).

In summary, incorporation of the pyrrolizidine scaffold has resulted in the discovery of potent and selective ligands for the 5-HT_4 receptor, including the potent and selective 5-HT_4 agonist **12a**, with excellent efficacy in canine antral motility and gastric emptying models. Toxicological profiling of **12a** revealed that the compound is active in the Ames assay after S9 activation, so the compound was not pursued as a clinical candidate. It is interesting to note that metabolic activation giving rise to mutagenicity appears to be enantiospecific, as the enantiomer **ent-12a** is not mutagenic with or without S9 activation. The potency and efficacy of this series has prompted analogue work to avoid metabolic activation by bridgehead-methyl substitution.[46] It is worth noting in this context that pyrrolizidine **5** is substituted at the bridgehead carbon, which would not permit the formation of a pyrrole-containing metabolite. Nevertheless, pyrrolizidine **12a** represents a useful pharmacological tool.[47−50] Work employing the pyrrolizidine scaffold also resulted in the discovery of imidazopyridine **12h**, which is a potent and selective 5-HT_4 receptor antagonist,[33] as well as the potent *N*-methylindole 5-HT_4 receptor antagonist **13d**. Related work in these labs on conformationally constrained amines also led to the discovery of azanoradamantane **6**, which is a potent, nonmutagenic, and selective dual 5-HT_4 agonist/5-HT_3 antagonist.[41,51,52]

Experimental Section

(−)-Trachelanthamidine (9). The (*S*,*S*)-carbinol (−)-trachelanthamidine **9** was prepared by a diastereoselective alkylation of the chiral tin enolate of 3-(4-chlorobutyryl)-4(*S*)-isopropyl-1,3-thiazolidine-2-thione 7 with 5-acetoxy-2-pyrrolidinone and subsequent reduction with lithium aluminum hydride as described by Nagao et al.[38] to afford **9** (3.12 g, 45.5%) as a colorless oil: $[\alpha]_D$ = +9.2 (c = 0.195 g/dL in $CHCl_3$, 10 cm); $[\alpha]_{365}$ = −63.1 (c = 0.195 g/dL in $CHCl_3$, 10 cm). The Mosher ester was prepared by treatment of the alcohol with the Mosher acid chloride [from (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid and thionyl chloride] and pyridine. ^{19}F NMR analysis of the peak at −172.17 ppm and the absence of the peak at −172.29 ppm from the diastereomeric standard showed that the material had ≥99.6% diastereomeric purity, or ≥99.1% ee.

2-[(1*S*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl]-1*H*-isoindole-1,3(2*H*)-dione (10). To a solution of alcohol **3** (1.44 g, 10.2 mmol) in dry THF (45 mL) were added triphenylphosphine (5.35 g, 20.4 mmol) and phthalimide (3.00 g, 20.4 mmol). The solution was then cooled to 0 °C and diethylazodicarboxylate (3.21 mL, 3.55 g, 10.4 mmol) was added dropwise over 15 min. The reaction was then stirred for 1 h at 0 °C and then 16 h at room temperature. Concentration gave a residue which was purified on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (4/96 and then 6/94) to afford the desired phthalimide **10** (2.12 g, 82%) as a pale yellow crystalline solid: mp 72−73 °C; IR (MIR) 1763, 1706.5 cm^{-1}; $[\alpha]_D$ = −2.5°; $[\alpha]_{365}$ = −13.3° (c = 0.285 g/dL in chloroform); ^1H NMR (300 MHz, $CDCl_3$) δ 7.86 (2H, m), 7.73 (2H, m), 3.76 (2H, d, J = 7.1 Hz), 3.28 (1H, m), 3.21 (1H, m), 2.94 (1H, dt, J = 10.2, 5.9 Hz), 2.51 (2H, m), 2.12 (1H, m), 1.99 (1H, m), 1.90−1.63 (3H, m), 1.43 (1H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.8, 133.5, 131.5, 122.7, 67.7, 54.6, 53.9, 44.9, 40.3, 31.6, 31.0, 25.8. HRMS calcd for $C_{16}H_{18}N_2O_2$ 270.1369, found 270.1369. Anal. Calcd for $C_{16}H_{18}N_2O_2 \cdot 0.4H_2O$: C, 69.24; H, 6.83; N, 10.09. Found: C, 69.12; H, 6.56; N, 9.94.

(1*S*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethylamine Dihydrochloride (11). To a solution of phthalimide **10** (4.11 g, 15.2 mmol) in ethanol (60 mL) was added hydrazine hydrate (3.70 g, 74 mmol) and the reaction was stirred for 15 h at room temperature. The resulting suspension was concentrated to give a residue. To the residue was added 4 N KOH (50 mL, presaturated with NaCl) and the mixture was extracted with chloroform (15 × 20 mL). The combined extracts were dried over sodium sulfate and concentrated to give the title amine **11** (2.13 g, 100%) as a yellow semisolid, which may be stored under argon at −30°, if necessary, before use in the subsequent step: ^1H NMR (300 MHz, $CDCl_3$) δ 3.13 (2H, m), 2.95 (1H, m), 2.74 (2H, m), 2.56 (2H, m), 2.03 (1H, m), 1.98−1.63 (4H, m), 1.62−1.43 (2H, m). Dihydrochloride salt of **11**: ^1H NMR (300 MHz, MeOD-d_4) δ 3.97 (1H, m), 3.79 (1H, m), 3.46 (1H, m), 3.29−3.03 (3H, m), 2.51−2.34 (2H, m), 2.32−1.78 (5H, m); ^{13}C NMR (75 MHz, MeOD-d_4) δ 70.4, 54.4, 53.9, 42.5, 40.7, 29.8, 29.4, 24.3; HRMS MH^+ calcd for $C_8H_{16}N_3$ 141.1392, found 141.1394.

4-Amino-5-chloro-*N*-[(1*S*-*cis*-hexahydro-1*H*-pyrrolizin-1-yl)-methyl]-2-methoxybenzenecarboxamide, Hydrochloride (12a, SC-53116). To a solution of 4-acetamido-5-chloro-2-methoxybenzoic acid (3.70 g, 15.2 mmol) in DMF (15 mL, freshly distilled under high vacuum) was added 1,1′-carbonyldiimidazole (2.46 g, 15.2 mmol), which resulted in a vigorous gas evolution. After 40 min at room temperature a solution of the free base amine **11** (2.13 g, 15.2 mmol) in DMF (6 mL) was added and reaction was stirred for 40 h at room temperature. Concentration gave a yellow oil which was treated with 15% K_2CO_3 (65 mL) and extracted with chloroform (4×). The combined organic extracts were washed successively with water (2×) and brine and dried over sodium sulfate to give a pale yellow solid (5.9 g). Purification on silica gel (170 g) eluting with MeOH (saturated with NH_3)/$CHCl_3$ (15/85) gave 4-(acetylamino)-*N*-[(7a*S*)-hexahydro-1*H*-pyrrolizin-1-ylmethyl]-5-chloro-2-methoxybenzamide (4.89 g, 88%) as a colorless solid after drying for 1 h at 100 °C: mp 131−132 °C; $[\alpha]_D$ = −8.8° (c = 1.2 g/dL); IR (MIR) 3407, 3310, 1696, 1656 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.33 (1H, br s), 8.16 (1H, s), 8.12 (1H, s), 7.98 (1H, t, J = 5 Hz), 3.95 (3H, s), 3.47 (2H, br s), 3.19 (2H, m), 2.94 (1H, m), 2.56 (2H, m), 2.28 (3H, s), 2.19−1.47 (7H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5, 163.2, 155.9, 135.0, 131.3, 117.0, 114.2, 104.2, 68.0, 55.8, 54.3, 53.9, 45.2, 42.4, 31.3, 31.2, 25.5, 24.2; HRMS calcd for $C_{18}H_{24}N_3O_3Cl$ 365.1506, found 365.1514. Anal. Calcd for $C_{18}H_{24}N_3O_3Cl$: C, 59.09, H, 6.61, N, 11.48, Cl, 9.69. Found: C, 58.76; H, 6.68; N, 11.42; N, 10.02.

A solution of the acetamide (4.88 g, 13.3 mmol) in ethanol (670 mL) was treated with potassium hydroxide (4.49 g, 80 mmol) and heated under reflux for 2 h. Concentration gave a residue to which was added water (150 mL), and the resulting mixture was extracted with chloroform (4×). The combined organic extracts were washed successively with water (2×) and brine and dried over sodium sulfate. Concentration gave the desired benzamide as a solid (4.21 g, 98%) which was purified on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (12/88 and then 16/84) to afford the pure free base of **12a** (3.86 g, 90%) as a colorless solid: mp 165.5−166.5 °C; $[\alpha]_D$ = −10.6°; $[\alpha]_{365}$ = −36.7° (c = 0.330 g/dL in chloroform); ^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, s), 7.80 (1H, t, J = 6 Hz), 6.30 (1H, s), 4.40 (2H, s), 3.90 (3H, s), 3.47 (2H, m),

3.19 (2H, m), 2.98 (1H, m), 2.57 (2h, m), 2.10−1.47 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5, 157.3, 146.9, 132.7, 111.8, 111.2, 97.6, 68.5, 55.9, 54.8, 54.3, 45.7, 42.7, 31.7, 31.6, 25.9. Anal. Calcd for $C_{16}H_{22}N_3O_2Cl$: C, 59.34; H, 5.85; N, 12.98; Cl, 10.95. Found: C, 59.09; H, 6.71; N, 12.84; Cl, 11.18.

To a suspension of the free base of **12a** (3.52 g, 10.87 mmol) in methanol (10 mL) was added a solution of HCl (10.87 mmol) in methanol [prepared by the addition of acetyl chloride (0.76 g, 10.9 mmol) to methanol (10 mL)]. The resulting solution was concentrated to give a solid which was redissolved in methanol (5 mL) and added slowly to diethyl ether (3 L) with rapid stirring. The resulting suspension was stored at 0 °C for 16 h. Filtration gave the desired hydrochloride salt of **12a** (3.63 g, 88%) as a colorless solid: mp 103−105 °C; $[\alpha]_D = -2.6°$; $[\alpha]_{365} = -6.4°$ ($c = 0.47$ g/dL in methanol); IR (MIR) 3323, 3194, 1620, 1594 cm^{-1}; ^{1}H NMR (300 MHz, MeOD-d_4) δ 7.79 (1H, s), 6.52 (1H, s), 3.98 (1H, m), 3.93 (3H, s), 3.76 (1H, ddd, $J = 3.0$, 7.2, 10.8 Hz), 3.53 (2H, d, $J = 6.3$ Hz), 3.20 (1H, m), 3.10 (1H, td, $J = 11.4$, 6.0 Hz), 2.39 (1H, m), 2.30−1.79 (6H, m); ^{13}C NMR (75 MHz, MeOD-d_4) δ 167.7, 159.6, 133.2, 111.5, 111.2, 98.5, 72.3, 56.6, 55.8, 55.5, 46.5, 31.2, 31.1, 25.8; HRMS calcd for $C_{16}H_{22}N_3O_2Cl$ 323.1415, found 323,1400. Anal. Calcd for $C_{16}H_{22}N_3O_2Cl{\cdot}HCl{\cdot}0.75H_2O$: C, 51.41; H, 6.61; N, 11.24; Cl, 18.97. Found: C, 51.58; H, 6.70; N, 10.94; Cl, 18.57.

Amide Coupling General Procedure. We have found that benzamide couplings utilizing 4-amino-5-chloro-2-methoxybenzoic acid generally proceed very well without acetamide protection of the 4-amino group. According to this general procedure, to a solution of carboxylic acid (1 equiv) in DMF (freshly opened) is added 1,1′-carbonyldiimidazole (1 equiv), which results in a vigorous gas evolution. After 0.5−1 h at room temperature a solution of the amine **11** in DMF is added and reaction is stirred for 2 h at room temperature followed by 2 h at 50 °C. Concentration affords a residue which is treated with 15% K_2CO_3 and extracted with chloroform (3×). The combined organic extracts are washed successively with water (2×) and brine and dried over sodium sulfate. Concentration affords the desired product, which may be purified by crystallization or may be pure enough to carry on directly to the hydrochloride salt formation as described above for **12a**. Alternatively, purification on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (15/85) affords the pure coupled material as the free base which is then converted to the hydrochloride salt.

4-Amino-5-chloro-*N*-[(1*R*,7a*R*)-hexahydro-1*H*-pyrrolizin-1-ylmethyl]-2-methoxybenzamide (ent-12a, SC-53117). This compound was prepared from (+)-trachelanthamidine, prepared according to the method of Nagao et al.[38] and continued as above for the enantiomeric series beginning with 3-(4-chlorobutyryl)-4(*R*)-isopropyl-1,3-thiazolidine-2-thione to afford **ent-12a**: $[\alpha]_D = +0.5°$; $[\alpha]_{365} = +3.3°$ ($c = 0.183$ g/dL in methanol). Anal. Calcd for $C_{16}H_{22}N_3O_2Cl{\cdot}1.2HCl{\cdot}0.8H_2O$: C, 50.31; H, 6.54; N, 11.00; Cl, 20.42. Found: C, 50.45; H, 6.62; N, 10.67; Cl, 20.40.

4-Amino-5-chloro-2-ethoxy-*N*-[(1*S*,7a*S*)-hexahydro-1*H*-pyrrolizin-1-ylmethyl]benzamide Hydrochloride (12b). To a solution of 4-amino-5-chloro-2-methoxybenzoic acid (1 equiv) in DMF was added 1,1′-carbonyldiimidazole (1 equiv). After 1 h at room temperature a solution of the amine **5** in DMF was added. After the reaction was complete, aqueous workup as for **12a** and chromatography on silica gel afforded the requisite amide **12b**: ^{1}H NMR (400 MHz, MeOD-d_4) δ 7.77 (1H, s), 6.49 (1H, s), 4.17 (2H, q, $J = 7$ Hz), 3.96−3.91 (1H, m), 3.78−3.73 (1H, m), 3.54−3.52 (2H, m), 3.47−3.41 (1H, m), 3.24 to3.17 (2H, m), 3.13−3.06 (1H, m), 2.43−2.34 (1H, m), 2.29−2.21 (1H, m), 2.19−2.09 (2H, m), 2.07−1.99 (1H, m), 1.96−1.89 (1H, m), 1.86−1.79 (1H, m), 1.48 (3H, t). Anal. Calcd for $C_{17}H_{24}N_3O_2Cl{\cdot}HCl{\cdot}0.5H_2O$: C, 53.27; H, 6.84; N, 10.96; Cl, 18.50. Found: C, 53.14; H, 6.88; N, 10.87; Cl, 20.07.

(1*S*,7a*S*)-1-{[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-methyl}-4-methylhexahydro-1*H*-pyrrolizinium Iodide (12c). To a solution of the free base of **12a** (32.5 mg, 0.10 mmol) in toluene (5 mL) was added a solution of MeI (12.5 μL, 0.103 mmol) in toluene (50 mL). The reaction flask was wrapped with foil and allowed to stand at room temperature for 24 h. Filtration afforded the methiodide salt **12c** (35 mg, 75%) as a pale yellow solid: ^{1}H NMR (300 MHz, MeOD-d_4) δ 7.79 (1H, s), 6.53 (1H, s), 3.95 (3H, s), 3.88 (1H, dd, $J = 8.1$, 3.4 Hz), 3.78−3.63 (3H, m), 3.63−3.56 (2H, m), 3.45 (1H, m), 3.23 (3H, s), 2.52 (1H, m), 2.40−2.13 (4H, m), 2.12−1.90 (2H, m); ^{13}C NMR (75 MHz, MeOD-d_4) δ 167.7, 159.5, 150.5, 133.1, 111.4, 111.1, 98.5, 83.8, 67.2, 66.3, 56.7, 53.5, 47.7, 41.8, 31.3, 29.3, 25.1. Anal. Calcd for $C_{17}H_{25}N_3O_2I$: C, 47.45; H, 5.86; N, 9.77. Found: C, 47.17; H, 5.92; N, 9.54.

***N*-[(1*S*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl]-1-methyl-1*H*-indole-3-carboxamide (12d).** To a solution of *N*-methylindole-3-carboxylic acid (1.0 equiv) in DMF at room temperature was added CDI (1 equiv). After 1 h, a solution of amine **11** (1 equiv) in DMF was added and the solution stirred for 16 h at room temperature. Aqueous workup and chromatography on silica gel afforded the desired indole carboxamide **12d**. Anal. Calcd for $C_{18}H_{23}N_3O$: C, 72.70; H, 7.80; N, 14.13. Found: C, 72.91; H, 7.68; N, 13.92.

***N*-*exo*-(Tetrahydro-1*H*-pyrrolizin-4(5*H*)-ylmethyl)-3-ethylindolizine-1-carboxamide (12e).** 3-Ethylindolizine-1-carboxylic acid[44] (190 mg, 1.0 mmol) was suspended in $CHCl_3$ (2 mL). Oxalyl chloride (184 uL, 2.1 mmol) and DMF (1 drop) were added, and the mixture was stirred for 2 h. The reaction mixture was concentrated in vacuo, azeotroping once with toluene. To the residue dissolved in $CHC1_3$ was added a solution of *exo*-tetrahydro-1*H*-pyrrolizin-4(5*H*)-methylamine **11** (140 mg, 1.0 mmol) and triethylamine (279 uL, 2.0 mmol) in $CHCl_3$ (2 mL) and the mixture was stirred for 18 h. The organic solution was washed with 1 N NaOH and brine, dried over K_2CO_3, filtered, and concentrated to give a crude oil. Purification on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (30/70) gave the desired compound **12e** as a very hydroscopic solid (190 mg, 63%): ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.20 (1H, d, $J = 18$ Hz), 8.02 (1H, t, $J = 14$ Hz), 7.03−6.97 (3H, m), 6.80−6.75 (1H, m), 4.06−3.99 (1H, m), 3.83−3.75 (1H, m), 3.67 (2H, d, $J = 6$ Hz), 3.46−3.42 (1H, m), 3.25−3.18 (1H, m), 3.18−3.03 (1H, m), 2.54−2.45 (1H, m), 2.37−2.29 (1H, m), 2.22−2.12 (2H, m), 2.09−1.86 (3H, m); MS calcd for $C_{19}H_{25}N_3O$ 311.43, found 311.19. Anal. Calcd for $C_{19}H_{25}N_3O{\cdot}2.0H_2O$: C, 65.68; H, 7.25; N, 12.09. Found: C, 65.79; H, 7.56; N, 12.03. Attempts to make the HCl salt resulted in a brown oil, so the compound was tested as the free base.

***N*-*exo*-(Tetrahydro-l*H*-pyrrolizin-4(5*H*)-ylmethyl)-3-ethylimidazo[1,2-*a*]pyridine-l-carboxamide Monohydrochloride (12f).** 3-Ethylimidazo[1,5-*a*]pyridine-l-carboxylic acid[44] (190 mg, 1.0 mmol) was suspended in $CHCl_3$ (2 mL). Oxalyl chloride (184 uL, 2.1 mmol) and DMF (1 drop) were added, and the mixture was stirred for 2 h. The reaction mixture was concentrated in vacuo, azeotroping once with toluene. To the residue dissolved in $CHCl_3$ was added a solution of *exo*-tetrahydro-l*H*-pyrrolizin-4(5*H*)-methylamine **11** (140 mg, 1.0 mmol) and triethylamine (279 uL, 2.0 mmol) in $CHCl_3$ (2 mL) and the mixture stirred for 18 h. The organic solution was washed with 1 N NaOH and brine, dried over K_2CO_3, filtered, and concentrated to give a crude oil which was chromatographed on silica gel eluting with 5% $CH_3OH(NH_3)$/$CHCl_3$ to give 110 mg (35%) of desired compound as the free base. The HCl salt **12f** was prepared in the same manner as for **12a**: MS calcd for $C_{18}H_{24}N_4O$ 312, found 312. Anal. Calcd for $C_{18}H_{24}N_4O{\cdot}HCl{\cdot}0.75H_2O$: C, 57.91; H, 7.24; N, 15.01; Cl, 12.35. Found: C, 58.15; H, 6.95; N, 14.95; Cl, 12.25.

***N*-*exo*-((4*S*,7α*S*)-Tetrahydro-1*H*-pyrrolizin-4(5*H*)-yl)imidazo[1,2-*a*]pyridine-8-carboxamide (12g).** Imidazol[1,2-*a*]pyridine-8-carboxylic acid monohydrochloride **17** (198 mg, 1.00 mmol) and 1,1′-carbonyldiimidizole (178 mg, 1.1 mmol) were dissolved in DMF (5 mL) and stirred for 1 h. A solution of the aminomethylpyrrolizidine **11** (120 mg, 0.85 mmol) in Et_3N (560 μL, 4.0 mmol) was added to the reaction mixture and stirred for 1 h before concentrating. The residue was partitioned between $CHCl_3$ and 5% aqueous K_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by preparative thin-layer chromatography eluting with 20/80 MeOH/$CHCl_3$ containing 0.25%

NH_4OH. The filtrate was concentrated and the residue was dissolved in $CHCl_3$, filtered through Celite, and concentrated to yield the imidazopyridine amide (144 mg, 43%) as an oil. This residue was converted to the pyrrolizidine hydrochloride salt **12g** using a mixture of acetyl chloride/MeOH: IR 1579, 1659, 1697, 3049 and 3325 cm^{-1}. Anal. Calcd for $C_{16}H_{20}N_4O \cdot 2.0H_2O \cdot 2.2HCl \cdot 0.25CHCl_3$: C, 46.12; H, 6.25; N, 13.24. Found: C, 46.22; H, 6.41; N, 13.43.

***N-exo*-((4*S*,7α*S*)-Tetrahydro-1*H*-pyrrolizin-4(5*H*)-yl)-6-chloroimidazo[1,2-*a*]pyridine-8-carboxamide Dihydrochloride (12h, SC-53606).** 6-Chloroimidazo[1,2-*a*]pyridine-8-carboxylic acid monohydrochloride **19** (1.39 g; 6.0 mmol) and 1,1′-carbonyldiimidizole (972 mg, 6.0 mmol) were dissolved in DMF (10 mL) and stirred for 1 h. A solution of aminomethylpyrrolizidine 11 (800 mg, 5.7 mmol) in Et_3N (2.5 mL) was added to the reaction mixture and stirred for 1 h before concentrating. The residue was partitioned between $CHCl_3$ and 5% aqueous K_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by preparative thin-layer chromatography eluting with 20/80 MeOH/$CHCl_3$ with 0.25% NH_4OH. The filtrate was concentrated and the residue was dissolved in $CHCl_3$, filtered through Celite, and concentrated to yield the amide (1.42 g) as an oil. This residue was converted to the HCl salt using a mixture of acetyl chloride/MeOH to afford **12h**: IR 1652 cm^{-1}; ^1H NMR (400 MHz, MeOD-d_4) δ 8.83 (1H, d, $J = 2$ Hz), 7.99 (1H, d, $J = 2$ Hz), 7.96 (1H, d, $J = 1.3$ Hz), 7.70 (1H, 1.3 Hz), 4.05−3.98 (1H, m), 3.82−3.75 (1H, m), 3.689 (2H, d, $J = 6$ Hz), 3.49−3.42 (1H, m), 3.25−3.18 (1H, m), 3.16−3.08 (1H, m), 2.54−2.45 (1H, m), 2.37−2.29 (1H, m), 2.22−2.12 (2H, m), 2.09 to1.86 (3H, m). Anal.calcd for $C_{16}H_{19}ClN_4O \cdot H_2O \cdot HCl$: C, 51.48; H, 5.94; N, 15.01; Cl, 19.00. Found: C, 51.32; H, 5.64; N, 14.88; Cl, 18.86.

***N-exo*-((4*S*,7α*S*)-Tetrahydro-1*H*-pyrrolizin-4(5*H*)-yl)-6-chloroimidazo[1,2-*a*]pyridine-3-methyl-8-carboxamide (12i).** A suspension of 2-aminonicotinic acid **16** (5.0 g; 0.036 mol) and K_2CO_3 (5.0 g; 0.36 mol) in DMF (50 mL) was heated to reflux, and then the solution was cooled to ambient temperature. Iodomethane (5.1 g, 2.2 mL, 0.036 mol) was then added to the mixture and the solution was stirred for 18 h. The mixture was filtered and concentrated. The residue was filtered through a pad of silica, eluting with 5/95 EtOH/CH_2Cl_2 containing 0.1% NH_4OH. The resulting solution was concentrated and the residue was suspended in Et_2O, filtered, and concentrated to yield methyl 2-aminonicotinate (3.2 g).

The methyl 2-aminonicotinate (800 mg, 0.00525 mol) was dissolved in MeOH (15 mL). HCl gas was passed over the solution until the solution was acidic (pH 2) and then the solution was concentrated. The residue was dissolved in MeOH (15 mL), *tert*-butylhypochlorite (570 mg, 5.25 mmol) was added to the reaction mixture and it was stirred until the yellow color dissipated. Additional *tert*-butyl hypochlorite was added until TLC (5/95 EtOH/CH_2Cl_2 containing 0.1% NH_4OH) indicated that the starting material was consumed. The reaction mixture was concentrated and the residue was partitioned between CH_2Cl_2 and 5% aqueous $NaHCO_3$. The organic layer was washed with 5% aqueous sodium thiosulfate, dried over $MgSO_4$, and concentrated. The solid residue was suspended in 1:1 CH_2Cl_2/hexane, filtered, washed with hexane, and suction dried to yield methyl 2-amino-5-chloronicotinate **20** (250 mg, 26%): mp 139−40 °C. Anal. Calcd for $C_7H_7ClN_2O_2$: C, 45.60; H, 3.78; N, 15.01; Cl, 19.00. Found: C, 44.72; H, 3.75; N, 15.00; Cl, 19.20.

Methyl 2-amino-5-chloronicotinate **20** (7.0 g, 0.045 mol) and 2-bromopropionaldehyde (15.8 g, 0.116 mol) were combined in EtOH (100 mL) and refluxed until TLC (5/95 EtOH/toluene containing 0.1% NH_4OH) indicated that the starting material was consumed. The reaction mixture was concentrated, and the residue was triturated with acetone and then partitioned between CH_2Cl_2 and 5% aqueous K_2CO_3. The organic layer was dried over $MgSO_4$ and concentrated. The residue was triturated with Et_2O to yield methyl 6-chloro-3-methylimidazo[1,2-*a*]pyridine-8-carboxylate (5.0 g, 50%) as a solid: IR 1720 cm^{-1}; MS MH^+ calcd for $C_{10}H_9ClN_2O_2$ 225, found 225. Anal. Calcd for $C_{10}H_9ClN_2O_2$: C, 53.47; H, 4.04; N, 12.47; Cl, 15.78. Found: C, 53.14; H, 4.06; N, 12.37; Cl, 16.03.

The methyl 6-chloroimidazo[1,2-*a*]pyridine-3-methyl-8-carboxylate (5.0 g; 0.022 mol) was suspended in 6 N HCl (50 mL) and heated to reflux for 2 h. The reaction mixture was concentrated to near dryness. The residue was suspended in acetone and the solid filtered and washed with acetone to yield 6-chloro-3-methylimidazo-[1,2-*a*]pyridine-8-carboxylic acid **21** (4.9 g) as the HCl salt: IR 1704 and 3175 cm^{-1}; MS MH^+ calcd for $C_9H_7ClN_2O_2$ 211, found 211. Anal. Calcd for $C_9H_7ClN_2O_2 \cdot HCl$: C, 43.75; H, 3.26; N, 11.34; Cl, 28.70. Found: C, 43.54; H, 3.16; N, 11.26; Cl, 28.74.

To a solution of 6-chloro-3-methylimidazo[1,2-*a*]pyridine-8-carboxylic acid **21** (247 mg, 1.00 mmol) in DMF (5 mL) was added 1,1′-carbonyldiimidizole (178 mg, 0.011 mol) and the solution stirred for 1 h. Aminomethylpyrrolizidine **11** (120 mg, 0.85 mmol) and Et_3N (560 μL, 4.0 mmol) were added to the reaction mixture and the solution was stirred for 1 h before concentrating. The residue was partitioned between $CHCl_3$ and 5% aqueous K_2CO_3. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by preparative thin-layer chromatography eluting with 20/80 MeOH/$CHCl_3$ containing 0.25% NH_4OH. The filtrate was concentrated and the residue was dissolved in $CHCl_3$, filtered through Celite, and concentrated to yield an oil (144 mg, 43%). This residue was converted to the HCl salt of the desired imidazopyridine amide **12i** using a mixture of acetyl chloride/MeOH: ^1H NMR (400 MHz, MeOD-d_4) δ 9.10 (1H, d, $J = 2.5$ Hz), 8.56 (1H, d, $J = 2.5$ Hz), 7.89 (1H, d, $J = 1$ Hz), 4.08−4.03 (1H, m), 3.81−3.75 (1H, m), 3.63 (2H, d, $J = 6$ Hz), 3.51−3.45 (1H, m), 3.22−3.19 (1H, m), 3.16−3.09 (1H, m), 2.68 (3H, d, $J =$ 1 Hz), 2.52−2.46 (1H, m), 2.35−2.27 (1H, m), 2.25−2.14 (2H, m), 2.09−2.03 (1H, m), 1.99−1.87 (2H, m). Anal. Calcd for $C_7H_8N_2O_2$: C, 55.26; H, 5.30; N, 18.41. Found: C, 54.90; H, 5.36; N, 18.26. Anal. Calcd for $C_{19}H_{26}N_4O_2 \cdot 1.25H_2O \cdot 2.2HCl \cdot 0.25MeOH$: C, 46.55; H, 6.18; N, 12.41; Cl, 25.12. Found: C, 46.87; H, 5.94; N, 12.47; Cl, 25.34.

(1*R*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 4-Amino-5-chloro-2-methoxybenzoate Hydrochloride (13a). To solid NaH (52 mg of 60% dispersion, washed with hexane, 31 mg NaH) was added a solution of alcohol **9** (185 mg, 1.31 mmol) in DMF (2 mL) at 0 °C. After the effervescence was complete (0.5 h) this sodium alcoholate was added to the imidazolide of 4-amino-5-chloro-2-methoxybenzoic acid (330 mg, 1.31 mmol) at 0 °C. [The imidazolide was prepared by the addition of CDI (656 mg, 4.05 mmol) to 4-amino-5-chloro-2-methoxybenzoic acid (800 mg, 3.97 mmol); aqueous workup and recrystallization from ether/$CHCl_3$ afforded the requisite imidazolide (780 mg, 78%) as colorless crystals, mp 122−122.5 °C.] The reaction was allowed to warm to room temperature over 2 h. Water was added and the mixture extracted with $CHCl_3$. The combined organic extracts were washed with water (2×) and brine and dried over Na_2SO_4. Concentration gave a solid (362 mg) which was purified by chromatography on silica gel eluting with i-PrOH(NH_3)/$CHCl_3$ to afford the free base ester (129 mg, 30%). To a solution of the free base (117 mg, 0.36 mmol) in MeOH (1.5 mL) was added HCl/MeOH [prepared by the addition of acetyl chloride (23 uL, 25 mg, 0.36 mmol) to MeOH (1.5 mL)] to afford the desired monohydrochloride salt of ester **13a** (98 mg, 72%) as a beige solid: mp 191−193 °C; ^1H NMR (300 MHz, MeOD-d_4) δ 7.73 (1H, s), 6.48 (1H, s), 4.37 (1H, dd, $J = 11.3$, 5.4 Hz), 4.28 (1H, dd, $J = 11.3$, 6.6 Hz), 4.06 (1H, m), 3.82 (3H, s), 3.78 (1h, m), 3.48 (1H, m), 3.29−3.10 (2H, m), 2.54 (1H, m), 2.38−1.85 (6H, m); ^{13}C NMR (75 MHz, MeOD-d_4) δ 166.3, 162.0, 151.6, 134.0, 110.4, 108.0, 98.8, 71.8, 64.9, 56.3, 55.9, 55.6, 45.3, 31.0, 30.1, 25.6; HRMS calcd for $C_{16}H_{21}N_2O_3Cl$ 325.1319, found 325.1298. Anal. Calcd for $C_{16}H_{21}N_2O_3Cl \cdot HCl \cdot 0.25H_2O$: C, 52.54; H, 6.20; N, 7.66; Cl, 19.39. Found: C, 52.44; H, 6.06; N, 7.66; Cl, 19.18.

(1*R*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 6-Chloroimidazo[1,2-*a*]pyridine-8-carboxylate (13b). To NaH (91 mg of 60%, 2.30 mmol, washed with hexane) was added a solution of alcohol **9** (162 mg, 1.15 mmol) in DMF (2 mL) at 0 °C. After 0.5 h the solution of this sodium salt was added to the imidazolide of imidazopyridine carboxylic acid [prepared by adding added CDI (186 mg, 1.15 mmol) to a solution of 6-chloroimidazo[1,2-*a*]-

pyridine-8-carboxylic acid **19** (267 mg, 1.15 mmol) in DMF (2 mL) at 0 °C.] After 0.5 h at 0 °C, the solution was allowed to warm to room temperature over 3 h. Concentration gave a residue that was quenched with water (20 mL) and extracted with $CHCl_3$ (3×). The combined organic extracts were washed with water (2×) and brine and dried over Na_2SO_4. Concentration gave a pale yellow oil (210 mg) which was purified by recrystallization from EA to afford the desired pyrrolizidine ester free base (155 mg, 42%) as yellow crystals: mp 116−117 °C; IR (MIR) 3100, 1690, 1537, 1494, 1307, 1277 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (1H, d, J = 1.8 Hz), 7.88 (1h, d, J = 1.8 Hz), 7.80 (1H, s), 7.65 (1H, s), 4.46 (2H, m), 3.36 (1H, q, J = 6.5 Hz), 3.23 (1H, t, J = 7.6 Hz) 3.00 (1h, dt, J = 10.2, 5.9 Hz), 2.60 (2H, m), 2.21 (1H, m), 2.14 (1H, m), 1.98 (1H, m), 1.90−1.73 (3H, m), 1.62 (1H, m). To a solution of this free base (89.1 mg, 0.28 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (17.8 μL, 19.6 mg, 0.279 mmol) to MeOH (1.5 mL)]. After 1 h at room temperature, the solution was concentrated and the resulting solid was triturated with EA and then dried to afford the requisite imidazopyridine ester **13b** (92 mg, 92%) as an off-white powder: mp 192 °C (dec); IR (MIR) 3400, 3093, 2969, 2479, 1721, 1543, 1304, 1282, 1187 cm^{-1}; ^{1}H NMR (300 MHz, MeOD-d_4) δ 8.99 (1H, d, J = 1.8 Hz), 8.14 (1H, d, J = 1.4 Hz), 8.07 (1H, d, J = 1.1 Hz), 7.83 (1H, s), 4.57 (1H, dd, J = 11.1, 3.5 Hz), 4.48 (1H, dd, J = 11.1, 2.3 Hz), 4.29 (1H, td, J = 8.8, 0.8 Hz), 3.84 (2H, AB m), 3.55 (1H, m), 3.22 (1H, m), 2.91 (1H, m), 2.63 (1H, m), 2.46−1.87 (4H, m); ^{13}C NMR (75 MHz, MeOD-d_4) δ 164.3, 142.1, 134.7, 133.1, 131.1, 121.2, 119.6, 116.2, 72.1, 70.7, 55.4, 55.2, 42.7, 30.3, 29.2, 24.6; HRMS calcd for $C_{16}H_{18}N_3O_2Cl$ 321.1040, found 321.1049. Anal. Calcd for $C_{16}H_{18}N_3O_2Cl$·HCl·0.25H_2O: C, 53.27; H, 5.45; N, 11.65; Cl, 19.66. Found: C, 53.06; H, 5.36; N, 11.57; Cl, 19.54.

1-Methyl-1*H*-indole-2-carboxylic Acid (1*R*,7a*S*)-1-(Hexahydropyrrolizin-1-yl)methyl Ester (13c). To indole 2-carboxylic acid (186 mg, 1.06 mmol) dissolved in DMF (2.5 mL) was added 1,1′-carbonyldiimidazole (172 mg, 1.06 mmol) at ambient temperature and the mixture stirred. After 1.5 h, alcohol **3** (150 mg, 1.06 mmol) in DMF (0.5 mL) was added and the reaction was stirred for 16 h. Solvent was evaporated to give the desired ester as an oil. Purification on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (10/90) gave the ester (170 mg, 53%) as a solid: ^{1}H NMR (300 MHz, CD_3OD) δ 7.68 (m, 1H), 7.37 (d, 2H), 7.35 (d, 1H), 7.16 (t, 1H), 7.10 (s, 1H), 4.64 (dd, 2H), 4.09 (m, 1H), 3.37 (q, 1H), 3.24 (t, 1H), 3.00 (m, 1H), 2.61 (m, 2H), 2.20−1.77 (m, 6H), 1.964 (septet, 1H). To a solution of this free base (150 mg, 0.50 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (72 μL, 1.00 mmol) to MeOH]. After 1 h at room temperature the solution was concentrated and resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13c** (140 mg, 83%): ^{1}H NMR (300 MHz, CD_3-OD) δ 7.64−7.56 (d, 1H), 7.49 (d, 1H), 7.35 (d, 1H), 7.33 (s, 1H), 7.12 (t, 1H); 4.44 (qq, 2H), 4.10 (m, 1H), 4.05 (s, 3H), 3.82 (m, 1H), 3.49 (m, 1H), 3.26−3.18, (m, 2H); 2.62 (m, 1H), 2.36 (m, 2H), 2.21−2.29 (m, 2H), 1.98−2.02 (m, 2H); HRMS calcd for $C_{18}H_{22}N_2O_2$ 298.1673, found 298.1686.

(1*R*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 1-Methyl-1*H*-indole-3-carboxylate (13d). To *N*-methylindole-3-carboxylic acid[53] (2.0 g, 11 mmol) in DMF (10 mL) was added 1,1′-carbonyldiimidazole (1.78 g, 11 mmol) and the solution was stirred at room temperature for 3 h. Water (20 mL) and ice (30 g) were added, and the mixture was extracted with chloroform (3×). The combined organic extracts were washed with brine and dried over magnesium sulfate. Concentration gave the intermediate imidazolide (2.4 g, 93%), which was used directly.

To a suspension of sodium hydride (312 mg of 60% NaH, 7.8 mmol; washed with hexane) in DMF (5 mL) at 0 °C was added a solution of alcohol **9** (1.1 g, 7.8 mmol) in DMF (6 mL). The resulting mixture was stirred at 0 °C for 0.5 h, after which time a solution of the imidazolide (1.76 g, 7.8 mmol) in DMF (5 mL) was added. The reaction was allowed to warm to room temperature for 16 h. The mixture was then concentrated to give a solid which was chromatographed on silica gel (50 g) eluting with MeOH (saturated with NH_3)/$CHCl_3$ (5/95) to give the free base of **13d** (2.03 g, 87%). To a solution of this free base (2.0 g, 6.70 mmol) in methanol (10 mL) was added methanolic HCl [prepared by the addition of acetyl chloride (0.48 mL, 6.7 mmol) to methanol (10 mL) at 0 °C]. Concentration gave a foam which was redissolved in a minimum amount of methanol and added dropwise to diethyl ether (700 mL) with vigorous stirring. Filtration gave a beige solid (2.02 g) which contained the desired indole carboxylate as well as imidazole hydrochloride. This material was recrystallized from diethyl ether/ethanol to give the title compound **13d** (1.19 g, 60%) as a colorless solid: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.45 (1H, d, J = 6 Hz), 8.00 (1H, s), 7.46 (1H, d J = 6 Hz), 7.3−7.2 (2H, m), 4.45−4.34 (2H, m), 4.14−4.08 (1H, m), 3.862 (3H, s), 3.83−3.77 (1H, m), 3.51−3.45 (1H, m), 3.26−3.13 (2H, m), 2.64−2.55 (1H, m), 2.38−2.30 (1H, m), 2.27−2.15 (2H, m), 2.11−1.93 (3H, m). Anal. Calcd for $C_{18}H_{22}N_2O_2$·HCl: C, 64.61; H, 6.92; N, 8.37; Cl, 10.59. Found: C, 64.31; H, 7.24; N, 8.65; Cl, 10.95. Purity by HPLC 99.9%.

(1*R*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 5-Methoxy-1*H*-indole-3-carboxylate Hydrochloride (13e). To 5-methoxyindole-3-carboxylic acid (203 mg, 1.06 mmol) dissolved in DMF (2.5 mL) was added 1,1′-carbodiimidazole (172 mg, 1.06 mmol) at ambient temperature. After 1.5 h alcohol **9** (150 mg, 1.06 mmol) in DMF (0.5 mL) was added and the reaction was stirred for 16 h. The solvent was concentrated to afford an oil. Purification on silica gel (20 g) eluting with MeOH (saturated with NH_3)/$CHCl_3$ (10/90) gave the ester as a solid (120 mg, 36%). To a solution of this free base (110 mg, 0.35 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (50 μL, 0.70 mmol) to MeOH]. After 1 h at room temperature the solution was concentrated and the resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13e** (56 mg, 46%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.95 (s, 1H), 7.54 (d, 1H), 7.32 (d, 1H), 6.87 (dd, 1H), 4.41 (m, 2H), 4.09 (m, 1H), 3.84 (s, 3H), 3.79 (m, 1H), 3.49 (m, 1H), 3.19 (m, 2H), 2.61 (m, 1H), 2.36 (m, 2H), 2.21 (m, 2H), 2.03 (m, 2H). Anal. Calcd $C_{18}H_{22}N_2O_3$·HCl·0.33H_2O: C, 60.59; H, 6.68; N, 7.85; Cl, 9.94. Found: C, 60.78; H, 6.54; N, 7.81; Cl, 9.69.

(1*R*,7a*S*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 5-Fluoro-1*H*-indole-3-carboxylate Hydrochloride (13f). To 5-fluoroindole-3-carboxylic acid (190 mg, 1.06 mmol) dissolved in DMF (2.5 mL) was added 1,1′-carbodiimidazole (172 mg, 1.06 mmol) at ambient temperature. After 1.5 h, alcohol **9** (150 mg, 1.06 mmol) in DMF (0.5 mL) was added and the mixture stirred for 16 h. Solvent was concentrated to give the desired ester as an oil. Purification on silica gel (20 g) eluting with MeOH (saturated with NH_3)/$CHCl_3$ (10/90) gave the ester as a solid (170 mg, 53%). To a solution of this free base (160 mg, 0.53 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (75 μL, 1.06 mmol) to MeOH]. After 1 h at room temperature, the solution was concentrated and the resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13f** (133 mg, 74%): ^{1}H NMR (300 MHz, CD_3OD) δ 8.05 (s, 1H), 7.69 (dd, 1H), 7.42 (dd, 1H), 6.99 (t, 1H), 4.42 (m, 2H), 4.09 (m, 1H), 3.80 (m, 1H), 3.49 (m, 1H), 3.19 (m, 2H), 2.62 (m, 1H), 2.36 (m, 2H), 2.22 (m, 2H), 2.02 (m, 2H). Anal. Calcd $C_{17}H_{19}N_2O_2F$·HCl·0.33H_2O: C, 59.32; H, 6.03; N, 8.14; Cl, 10.36. Found: C, 59.58; H, 6.13; N, 8.20; Cl, 10.36.

(1*S*,7a*R*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 1*H*-Indazole-3-carboxylate (13g). To indazole-3-carboxylic acid (172 mg, 1.06 mmol) dissolved in DMF (3 mL) was added 1,1′-carbodiimidazole (172 mg, 1.06 mmol) at room temperature and the mixture stirred for 1.5 h, after which time alcohol **9** (150 mg, 1.06 mmol) in DMF (0.5 mL) was added and the reaction stirred for 16 h. Solvent was concentrated to give desired ester as an oil. Purification on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (5/95) gave the ester as a solid (205 mg, 68%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.08 (s, 1H), 8.14 (d, 1H), 7.62 (d, 1H), 7.36 (t, 1H), 7.22 (t, 1H), 4.49 (m, 2H), 3.54 (q, 1H), 3.39 (m, 1H), 3.11 (quintet, 1H), 2.68 (m, 1H), 2.29 (m, 1H), 2.15 (m, 1H), 2.02 (septet, 1H), 1.85

(m, 4H), 1.67 (s, 1H); HRMS calcd for $C_{16}H_{19}N_3O_2$ 285.1474, found 285.1500. Anal. Calcd for $C_{16}H_{19}N_3O_2 \cdot 0.5H_2O$: C, 65.29; H, 6.85; N, 14.28. Found: C, 65.39; H, 6.56; N, 14.01. To a solution of this free base (160 mg, 0.56 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (80 μL, 1.12 mmol) to MeOH]. After 1 h of stirring at room temperature the solution was concentrated and the resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13g** (137 mg, 76%): ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.14 (1H, d, J = 8.2 Hz), 6.62 (1H, d, 8.5 Hz), 7.46 (1H, t, J = 8 Hz), 7.32 (1H, t, J = 8 Hz), 4.59−4.49 (2H, m), 4.16 (1H, t, J = 6 Hz), 3.87−3.82 (1H, m), 3.54−3.48 (2H, m), 3.27−3.17 (1H, m), 2.73−2.64 (1H, m), 2.43−2.36 (1H, m), 2.32−2.17 (2H, m), 2.10−1.98 (3H, m). HRMS calcd for $C_{16}H_{19}N_3O_2$ 285.1496, found 285.1511. Anal. Calcd for $C_{16}H_{19}N_3O_2 \cdot HCl \cdot 0.15H_2O$: C, 59.22; H, 6.31; N, 12.95; Cl, 10.93. Found: C, 59.13; H, 6.34; N, 12.88; Cl, 11.06.

(1*S*,7a*R*)-Hexahydro-1*H*-pyrrolizin-1-ylmethyl 1-Methyl-1*H*-indazole-3-carboxylate (13h). To *N*-methylindazole-3-carboxylic acid (186 mg, 1.06 mmol) dissolved in DMF (2 mL) was added 1,1′-carbodiimidazole (172 mg, 1.06 mmol) at ambient temperature. After stirring for 1.5 h, the alcohol **9** (150 mg, 1.06 mmol) in DMF (0.5 mL) was added and the reaction stirred for 16 h. Solvent was removed in vacuo to give an oil. Purification on silica gel eluting with MeOH (saturated with NH_3)/$CHCl_3$ (10/90) gave the ester as a solid (196 mg, 62%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (d, 1H), 7.47 (d, 2H), 7.34 (t, 1H), 4.49 (m, 2H), 4.18 (S, 3H), 3.37 (q, 1H), 3.21 (m, 1H), 2.99 (m, 1H), 2.59 (m, 2H), 2.37 (m, 1H), 2.16 (m, 1H), 2.16 (m, 1H), 1.99 (septet, 1H), 1.81 (m, 2H), 1.61 (sextet, 1H); HRMS calcd for $C_{17}H_{21}N_3O_2$ 299.1616, found 285.1632. Anal. Calcd for $C_{17}H_{21}N_3O_2 \cdot 0.2H_2O$: C, 67.39; H, 7.12; N, 13.87. Found: C, 67.54; H, 6.99; N, 13.74. To a solution of this free base (168 mg, 0.56 mmol) was added HCl/MeOH [prepared by the addition of acetyl chloride (80 μL, 1.12 mmol) to MeOH]. After 1 h at room temperature the solution was concentrated and the resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13h** (174 mg, 92%): HRMS calcd $C_{17}H_{21}N_3O_2$ 299.1649, found 299.1648; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, 1H), 7.69 (d, 1H), 7.51 (t, 1H), 4.52 (qq, 2H), 4.16 (S, 3H), 4.13 (m, 1H), 3.84 (m, 1H), 3.50 (m, 1H), 3.22 (m, 2H), 2.69 (m, 1H), 2.40 (m, 1H), 2.22 (m, 5H), 2.05 (m, 1H). Anal. Calcd $C_{17}H_{21}N_3O_2 \cdot HCl \cdot 0.15H_2O$: C, 60.31; H, 6.64; N, 12.41; Cl, 10.47. Found: C, 60.28; H, 6.76; N, 12.40; Cl, 10.71.

***cis*-*N*-[(Hexahydro-1*H*-pyrrolizin-l-yl)methyl]-2,3-dihydro-3-(1-methylethyl)-2-oxo-l*H*-benzimidazole-l-carboxamide (15a).** A dispersion of 60% NaH/mineral oil (80 mg, 0.002 mol) was washed with hexane and suspended in THF. Solid 1,3-dihydro-l-(l-methylethyl)-2*H*-benzimidazol-2-one[54] (176 mg, 1.00 mmol) was added to the suspension. This mixture was stirred for 15 min before being added to a solution of 2.5 mL (0.004 mol) of 20% phosgene in toluene/2.5 mL THF. The resulting mixture was filtered through Celite and concentrated. The residue was dissolved in THF (5.0 mL) and a solution of aminomethylpyrrolizidine **11** (140 mg, 1.0 mmol) was added in Et_3N (0.5 mL). This mixture was stirred for 1 h, filtered, and concentrated. The residue was purified by preparative thin-layer chromatography eluting with 30/70 MeOH/$CHCl_3$ containing 0.25% NH_4OH. The product was rinsed from the silica with 9/95 NH_4OH/MeOH. The filtrate was concentrated and the residue was dissolved in $CHCl_3$, filtered through Celite, and concentrated to afford the benzimidazolone **15a** (157 mg, 50%). The product was converted to the HCl salt by dissolving 36 μL of acetyl chloride in 5.0 mL of MeOH and adding this solution to the product and then concentrating to dryness: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.11 (1H, dd, J = 8.1, 0.8 Hz), 7.32 (1H, dd, J = 8.1, 0.8 Hz), 7.22 (1H, d of t, J = 8.1, J = 1 Hz), 7.13 (1H, d of t, J = 8.1, J = 1 Hz), 4.70 (1H, sep, J = 7 Hz), 4.04−3.96 (1H, m), 3.82−3.74 (1H, m), 3.58−3.55 (2H, m), 3.50−3.42 (1H, m), 3.25−3.18 (1H, m), 3.16−3.08 (1H, m), 2.49−2.39 (1H, m), 2.36−2.26 (1H, m), 2.25−2.17 (1H, m), 2.11−2.03 (1H, m), 2.01−1.93 (1H, m), 1.91 to1.82 (1H, m), 1.54 (2H, d, J = 7 Hz); MS MH^+ calcd for $C_{19}H_{26}N_4O_2$ 343, found 343. Anal. Calcd for $C_{19}H_{26}N_4O_2 \cdot HCl \cdot H_2O$: C, 57.50; H, 7.32; N, 14.12; Cl, 8.93. Found: C, 57.44; H, 7.42; N, 13.87; Cl, 8.93.

***exo*-2,3-Dihydro-3,3-dimethyl-*N*-[(hexahydro-l*H*-pyrrolizin-1*S*-yl)methyl]-2-oxo-1*H*-indole-1-carboxamide, Hydrate Hydrochloride (15b).** To sodium hydride (214 mg, 5.6 mmol, washed twice with hexane) suspended in THF (1 mL) was added 1,3-dihydro-3,3-dimethyl-2*H*-indol-2-one[55] (226 mg, 1.4 mmol) and the reaction was stirred for 10 min. The resulting suspension was added to a solution of 20% phosgene in toluene (5.50 mL, 11.2 mmol) in THF (5 mL) and the reaction was stirred for 1 h. The reaction mixture was filtered through Celite and concentrated in vacuo to give an oil. To a solution of the oil in THF (5 mL) was added a solution of aminomethylpyrrolizidine **11** (200 mg, 1.4 mmol) and triethylamine (200 uL, 1.4 mmol) in THF (2 mL) and the reaction was stirred for 18 h. The solution was then diluted with chloroform, washed with saturated K_2CO_3 solution, dried over K_2CO_3, filtered, and concentrated in vacuo to give crude desired product as an oil. Purification on silica gel eluting with 10% $CH_3OH(NH_3)$/$CHCl_3$ gave the free base of the benzimidazolone (193 mg, 42%) as a solid: HRMS calcd for $C_{19}H_{25}N_3O_2$ 327.1947, found 327.1930. Anal. Calcd for $C_{19}H_{25}N_3O_2 \cdot 0.4H_2O$: C, 68.19; H, 7.77; N, 12.56. Found: C, 68.06; H, 7.74; N, 12.44. The free base (180 mg, 0.550 mmol) was converted to the hydrochloride salt by treatment with methanolic HCl to give the hydrochloride salt **15b** (128 mg, 64%) as a solid: ^{1}H NMR (400 MHz, MeOD-d_4) δ 8.10 (1H, d, J = 2 Hz), 7.33 (1H, d, J = 2 Hz), 7.28 (1H, t, J = 2 Hz), 7.19 (1H, t, J = 2 Hz), 4.02−3.96 (1H, m), 3.79−3.74 (1H, m), 3.53 (2H, d, J = 6.4 Hz), 3.49−3.43 (1H, m), 3.24−3.18 (1H, m), 3.15 to3.08 (1H, m), 2.48−2.38 (1H, m), 2.33−2.25 (1H, m), 2.22−2.13 (2H, m), 2.08−1.02 (1H, m), 1.99−1.92 (1H, m), 1.90−1.83 (1H, m), 1.41 (6H, s); MS calcd for $C_{19}H_{25}N_3O_2$ 327.1947, found 327.1939. Anal. Calcd for $C_{19}H_{25}N_3O_2 \cdot HCl \cdot 0.25H_2O$: C, 61.95; H, 6.98; N, 11.41; Cl, 9.62. Found: C, 61.54; H, 6.92; N, 11.36; Cl, 9.90.

Imidazol[1,2-*a*]pyridine-8-carboxylic Acid Monohydrochloride (17). 2-Aminonicotinic acid **16** (14.1 g; 0.102 mol) and chloroacetaldehyde [45% aqueous solution] (8.6 g, 100 mmol) were dissolved in EtOH (100 mL) and heated to reflux. The reaction was monitored by TLC with 30% MeOH/CH_2Cl_2/1.0% HOAc, and additional chloroacetaldehyde was added until the starting material was consumed. The reaction mixture was concentrated and the solid filtered, washed with EtOH, and suction dried to yield imidazol-[1,2-*a*]pyridine-8-carboxylic acid monohydrochloride **17** (17.5 g, 88%) as a solid: IR 1582, 1651, 1687, 3049 and 3320 cm^{-1}. Anal. Calcd for $C_8H_6N_2O_2 \cdot HCl$: C, 48.38; H, 3.55; N, 14.10; Cl, 17.85. Found: C, 48.16; H, 3.59; N, 13.95; Cl, 17.50.

6-Chloroimidazo[1,2-*a*]pyridine-8-carboxylic Acid (19). Methyl 2-amino-5-chloronicotinate **18** (7.0 g; 0.045 mol) and 45% aqueous chloroacetaldehyde (10.5 g; 0.06 mol) were dissolved in EtOH (100 mL) and heated to reflux for 3 h. The reaction mixture was concentrated and the solid filtered, washed with EtOH, and suction dried to yield methyl 6-chloroimidazo[1,2-*a*]pyridine-8-carboxylate (9.5 g, 80%) as a solid. Anal. Calcd for $C_9H_7N_2O_2 \cdot HCl \cdot H_2O$ C, 40.78; H, 3.80; N, 10.57; Cl, 26.75. Found: C, 40.68; H, 3.74; N, 10.19; Cl, 26.85.

The methyl 6-chloroimidazo[1,2-*a*]pyridine-8-carboxylate (9.5 g, 0.036 mol) was combined with 6N HCl (100 mL) and heated to reflux for 2 h. The reaction mixture was concentrated to near dryness. The residue was suspended in acetone and the solid filtered and washed with acetone to yield 6-chloroimidazo[1,2-a]pyridine-8-carboxylic acid **19** hydrochloride salt (6.3 g, 75.5%) as a solid: IR 1679 and 3281 cm^{-1}. Anal. Calcd for $C_8H_5N_2O_2 \cdot HCl$: C, 41.23; H, 2.60; N, 12.02; Cl, 30.42. Found: C, 40.95; H, 2.43; N, 12.16; Cl, 30.67.

(+)-4-Amino-5-chloro-*N*-(hexahydro-2,5β-methano-1*H*-3a*S*,-3aα,6aα-cyclopenta[*c*]pyrrol-4α-yl)-2-methoxybenzamide, Monohydrochloride (6, SC-52491). Compound **6** was prepared initially by synthesis involving resolution as previously described[41] and then via an enantioselective synthesis commencing with an asymmetric Diels−Alder reaction.[52]

Serotonin 5-HT$_3$ Receptor Binding. Displacement of [^{3}H]-GR65630 from brain cortex obtained from male Wistar rats was

done by the method of Kilpatrick.[56] Cortical membrane preparations (0.04 mg) were incubated with 0.2 nM [^{3}H]GR65630 in the presence or absence of graded concentrations test compound for 60 min at 22 °C. A 1 μM solution of tropisetron was used for nonspecific binding to the membranes. Membranes were filtered, washed three times, and counted to obtain binding displacement curves and determine specific binding of [^{3}H]GR65630 to the 5-HT$_3$ receptor.

Serotonin 5-HT$_4$ Receptor Binding. Serotonin 5-HT$_4$ receptor binding in guinea pig striatum utilizing [^{3}H]GR113,808 was performed by MDS Pharma Services (formerly Panlabs Taiwan) according to the method of Grossman and Kilpatrick.[57]

Selectivity Binding Assays. The following radioligands were used for receptor profiling studies: [^{3}H]5HT for 5-HT$_1$-like receptors, [^{3}H]ketanserin for 5-HT$_2$ receptors, [^{3}H]SCH23390 for D$_1$ receptors, [^{3}H]spiperone for D$_2$ receptors, and [^{3}H]prazosin for α_1-adrenergic receptors.

In Vitro Functional Assay for Serotonin 5-HT$_4$ Agonism in the Rat TMM (Tunica Muscularis Mucosae) Assay. Serotonin 5-HT$_4$ agonism was measured in the rat esophagus in vitro preparation as reported by Baxter et al.[2,57] Agonist activity was determined by utilizing relaxation of carbachol-contracted rat tunica muscularis mucosae. One 2-cm segment of intrathoracic esophagus proximal to the diaphragm was removed from male rats weighing approximately 300 g, and the outer muscle layers were removed. The inner tunica muscularis mucosa was mounted under 0.2−0.3 g of tension in a tissue bath containing oxygenated Tyrode's solution at 37 °C. Cortisterone acetate (30 μM) and fluoxetine (1 μM) were included in the buffer to prevent uptake of serotonin, as well as pargyline (10 μM) to inhibit monoamine oxidase. Following a 30-min equilibrium period, tissues were isometrically contracted with carbachol (3 μM) to obtain a tonic contraction. A stable plateau was obtained within 20 min when test compound was added cumulatively to relax the muscle strip. EC$_{50}$ values were obtained for each agonist in tissues from rats.

5-HT$_4$ Receptor Antagonism in the Rat TMM Assay. Antagonist activity of compounds at 5-HT$_4$ receptors was determined in a manner similar to the in vitro agonism activity described by Gullikson et al.[39] Cumulative dose−response curves for agonists interacting with 5-HT$_4$ receptors of rat TMM were done according to the method of Baxter et al.[2] Male Sprague−Dawley rats (300−400 g) from Charles River Laboratories (Wilmington, MA) were asphyxiated with CO_2 and the TMM were isolated from 2-cm segments of rat esophagus obtained orad to the diaphragm. The TMM was mounted in a 37 °C tissue bath under 2−3 mN of tension for 60 min before the study. The tissues were suspended in and washed with continuously oxygenated (95% O_2/5% CO_2) Tyrode's buffer which contained fluoxetine (1 μM) and corticosterone (30 μM) to prevent tissue uptake of 5-HT, methysergide (1 μM) to block 5-HT$_1$ and 5-HT$_2$ receptors, and pargyline (100 μM) to prevent oxidation of 5-HT by monoamine oxidase. Selected concentrations of antagonist were added to the tissue bath 5 min after TMM were contracted with 3 μM of carbachol. Relaxant responses to cumulative additions of 5-HT or 5-HT$_4$ agonists (from 10^{-10} to 10^{-5} M in half-log increments at 2.5-min intervals) were started 20 min after addition of the carbachol. Agonist ability to relax TMM was expressed relative to the maximum relaxant response by the agonist in the absence of antagonist. EC$_{50}$ values were calculated as the concentrations causing 50% of this maximal effect. EC$_{50}$ ratios (dose ratios) from the agonist concentration−response curves in the absence and presence of three increasing concentrations of antagonist for each tissue were calculated. Dose ratios from at least four tissues for each of the three antagonist concentrations were used for Schild plot analysis to determine the mean pA value ± SEM of the antagonist.[58] The pA_2 value for single antagonist concentrations in individual tissues was also calculated using the method of MacKay: pA_2 = −log(antagonist concentration [mol/1]) + log(DR − 1). A mean pA_2 value ± SEM was calculated from the individual pA_2 values from at least four tissues for a single concentration of the antagonist.

von Bezold−Jarisch Reflex Assay. According to the method of Saxena and Lawang,[40] the test sample was administered ip (mg/kg) to a group of three mice. Thirty minutes later, a 5-HT (0.25 mg/kg iv)-induced bradycardia was recorded in pentobarbital-anesthetized animals. A greater than 50% (>50) reduction in the bradycardic response relative to vehicle-treated control mice is considered significant.

Antral Motility in Conscious Fasted Dogs. Gastric antral contractile activity is stimulated by prokinetic drugs which enhance gastric emptying of solid food.[59] This contractile activity is thought to enhance gastric emptying by more rapidly reducing food particle size for passage through the pylorus. The ability of the test compound to increase the frequency and/or amplitude of the contractile activity is therefore a measure of GI prokinetic activity of compounds. Mongrel dogs of either sex weighing 15−25 kg were surgically implanted with strain gauge force transducers on the gastric antrum at 6, 4, and 2 cm from the gastroduodenal junction. Strain gauge transducers were also implanted on the ileum and proximal colon. Silver monopolar electrodes were implanted on the jejunum. The dogs were allowed at least 2 weeks to recover and were trained to stand quietly in Pavlov slings. Dogs were fasted for 18−24 h prior to each experiment to record a pattern of antral contractile activity characteristic of the fasted state called the migrating motor complex (MMC). The period of the MMC cycle is approximately 90−120 min and consists of 45−60 min of motor quiescence (phase I), 30−45 min of intermittent activity (phase II), and 10−15 min of intense contractile activity (phase III). A control MMC period is recorded prior to compound administration to obtain the length of the quiescent phase I period. Compound is given intravenously at the end of phase III of the control MMC cycle, and the subsequent phase I period is examined for the ability of the compound to produce contractions. A compound is considered active if it produces any contractile activity during the normally quiescent phase I. A motility index of cumulative activity consisting of frequency and amplitude components is used to quantitatively describe the total contractile activity of a time period after compound administration equal to the control MMC period.

Canine Gastric Emptying In Vivo Model. Determination of the effects of test compounds on gastric emptying of solid meals in nonsedated dogs was done in separate experiments in an α_2-adrenergic model of gastroparesis as described by Gullikson et al.[45,60] Dogs weighing 15−25 kg were trained to stand quietly in Pavlov slings for 3−4 h, and consistent control emptying responses were obtained prior to use in gastric emptying experiments with the test compounds. The solid meal consisted of two cooked, scrambled eggs which were divided into 1 cm sized pieces and mixed with beef stew. Then 1 mCi of Tc-99m sulfur colloid was incorporated into the eggs prior to cooking. The dogs were fasted for at least 24 h prior to the study and were fed the solid meal by intragastric tube. To delay normal gastric emptying, 0.030 mg/kg of the α_2-adrenergic agonist 2-methyl-3-[(2*E*)-pyrrolidin-2-ylideneamino]phenol was administered immediately following the meal. The test compounds were given via intravenous injection 45 min prior to feeding.

A Siemens 370 ZLC γ-camera with a high-resolution low-energy collimator was used to acquire left lateral images during emptying studies. Acquisition times were 3 min/frame for 180 min of solid emptying. Disappearance of contents from the stomach region of interest was plotted over time to obtain emptying curves. The amount of solid meal remaining in the stomach at the end of each experiment and the fractional solid emptying rate (% emptied/min) were calculated from linear regression equations describing solid emptying. Emptying measurements obtained from one replicate of each drug treatment were compared to the means of at least three control responses for the α_2-adrenergic agonist in solid emptying studies.

Ames Mutagenicity Assay. Pyrrolizidine benzamide **12a** was tested for mutagenic activity in a GLP study using the Ames salmonella/microsome assay with five strains of *Salmonella typhimurium* (TA1535, TA100, TA1538, TA97, and TA98) in the presence and absence of a rat liver homogenate metabolic activation system (S9) over test article concentrations ranging from 7.2 to 3600 μg/plate. Significant test-article-related increases of 4 times

in the number of revertant colonies were observed in strain TA98 with activation at 3600 μg/plate. A 2−3 times increase was observed with activation in strain TA100 between 710 and 3600 μg/plate, and a 2−6 times increase was observed with activation in strain TA1538 also between 720 and 3600 μg/plate. Significant increases in the numbers of revertant colonies were not observed in the test without S9 activation.

Pyrrolizidine benzamide **ent-12a** was tested for mutagenic activity in a GLP study using the Ames salmonella/microsome assay with five strains of *S. typhimurium* (TA1535, TA100, TA1538, TA97, and TA98) in the presence and absence of a rat liver homogenate metabolic activation system (S9) over test article concentrations ranging from 10 to 5000 μg/plate. There were no test-article-related increases in the number of revertant colonies or cytotoxic effects observed in any of the tested strains with or without activation. These data support the conclusion that **ent-12a** is not mutagenic under the conditions of this test system.

Supporting Information Available: Results from elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Hartig, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol. Rev.* **1994**, 157−203.

(2) Baxter, G. S.; Craig, D. A.; Clarke, D. E. 5-Hydroxytryptamine$_4$ receptors mediate relaxation of rat esophageal tunica muscularis mucosae. *Naunyn-Schmiedeberg*'s *Arch. Pharmacol*. **1991**, 439.

(3) Dumuis, A.; Sebbon, M.; Bockaert, J. The gastrointestinal prokinetic benzamide derivatives are agonists at the nonclassical 5-HT (5-HT$_4$) receptor coupled to adenylate cyclase in neurones. *Naunyn-Schmiedeberg*'s *Arch. Pharmacol*. **1989**, 403−410.

(4) Eglen, R. M.; Wong, E. H. F.; Dumuis, A.; Bockaert, J. Central 5-HT$_4$ receptors. *Trends Pharmacol. Sci*. **1995**, 391−397.

(5) Hedge, S.; Eglen, R. Peripheral 5-HT$_4$ receptors. *FASEB J*. **1996**, 1398−1407.

(6) Song, C. W.; Lee, K. Y.; Kim, C. D.; Chang, T. M.; Chey, W. Y. Effect of cisapride and renzapride on gastrointestinal motility and plasma motilin concentration in dogs. *J. Pharmacol. Exp. Ther*. **1997**, 1312−1316.

(7) Craig, D.; Clarke, D. E. Pharmacological characterization of a neuronal receptor for 5-hydroxytryptamine in guinea pig ileum with properties similar to the 5-hydroxytryptamine receptor. *J. Pharmacol. Exp. Ther*. **1990**, 1378−1386.

(8) Ronde, P.; Ansanay, H.; Dumuis, A.; Miller, R.; Bockaert, J. Homologous desensitization of 5-hydroxytryptamine$_4$ receptors in rat esophagus: Functional and second messenger studies. *J. Pharmacol. Exp. Ther*. **1995**, 977−983.

(9) Briejer, M. R.; Akkermans, L. M.; Meulemans, A. L.; Lefebvre, R. A.; Schuurkes, J. A. Cisapride and a structural analogue, R 76, 186, are 5-hydroxytryptamine$_4$ (5-HT$_4$) receptor agonists on the guinea-pig colon ascendens. *Naunyn-Schmiedeberg*'s *Arch. Pharmacol*. **1993**, 464−470.

(10) Berque-Bestel, I.; Soulier, J. L.; Giner, M.; Rivail, L.; Langlois, M.; Sicsic, S. Synthesis and Characterization of the First Fluorescent Antagonists for Human 5-HT$_4$ Receptors. *J. Med. Chem.* **2003**, 2606−2620.

(11) Langlois, M.; Fischmeister, R. 5-HT$_4$ receptor ligands: Applications and new prospects. *J. Med. Chem*. **2003**, 319−344.

(12) Kauman, A. J. Do human atrial 5-HT$_4$ receptors mediate arrhythmias? *Trends Pharmacol. Sci*. **1994**, 451−455.

(13) Meneses, A.; Hong, E. Effects of 5-HT$_4$ receptor agonists and antagonists in learning. *Pharmacol. Biochem. Behav*. **1997**, 347−351.

(14) Mertz, H. Visceral Hypersensitivity. *Alim. Pharm. Ther*. **2003**, *5*, 623−633.

(15) McGallum, R. W. Review of the current status of prokinetic agents in gastroenterology. *Am. J. Gastroenterol*. **1985**, 1008−1016.

(16) Colatsky, T. J.; Argentieri, T. M. Potassium channel blockers as antiarrhythmic drugs. *Drug Dev. Res*. **1994**, 235−249.

(17) Ahmad, S. R.; Wolfe, S. M. Cisapride and torsades de pointes. *Lancet* **1995**, 508.

(18) Camilleri, M. Review article: Tegaserod. *Aliment. Pharmacol. Ther*. **2001**, 277−289.

(19) Rivkin, A. Tegaserod maleate in the treatment of irritable bowel syndrome: A clinical review. *Clin. Ther*. **2003**, 1952−1974.

(20) Anon. Tegaserod maleate, zelmac. *Drugs Future* **2001**, 99−100.

(21) Wagstaff, A. J.; Frampton, J. E.; Croom, K. F. Tegaserod: A review of its use in the management of irritable bowel syndrome with constipation in women. *Drugs* **2003**, 1001−1120.

(22) Anon. Prucalopride, R-93877, R-093877, resolor. *Drugs Future* **2000**, 761−763.

(23) Bouras, E. P.; Camilleri, M.; Burton, D. D.; McKinzie, S. Selective stimulation of colonic transit by the benzofuran 5-HT$_4$ agonist, prucalopride, in healthy humans. *Gut* **1999**, 682−686.

(24) Bouras, E. P.; Camilleri, M.; Burton, D. D.; Thomforde, G.; McKinzie, S.; Zinsmeister, A. R. Prucalopride accelerates gastrointestinal and colonic transit in patients with constipation without a rectal evacuation disorder. *Gastroenterology* **2001**, 354−360.

(25) Kataria, M.; Traub, M.; Marsden, C. D. Extrapyramidal side-effects of metoclopramide. *Lancet* **1978**, 1254−1255.

(26) Kii, Y.; Ito, T. Effects of 5-HT$_4$−Receptor Agonists, Cisapride, Mosapride Citrate, and Zacopride, on Cardiac Action Potentials in Guinea Pig Isolated Papillary Muscles. *J. Cardio. Pharm.* **1997**, *5*, 670−675.

(27) Hadley, M. S.; King, F. D.; McRitchie, B.; Turner, D. H.; Watts, E. A. Substituted benzamides with conformationally restricted side chains. 1. Quinolizidine derivatives as selective gastric prokinetic agents. *J. Med. Chem.* **1985**, *28*, 3−1847.

(28) Hadley, M. S.; King, F. D.; McRitchie, B.; Smith, D. M. Turner, D. H. Substituted Benzamides with Conformationally Restricted Side Chains. 3. Azabicyclo[x.y.0]Derivatives as Gastric Prokinetic Agents. *Bioorg. Med. Chem. Lett.* **1992**, *9*, 1147−1152.

(29) King, F. D.; Hadley, M. S.; Joiner, K. T.; Martin, R. T.; Sanger, G. J.; Smith, D. M.; Smith, P.; Turner, D. H.; Watts, E. A. Substituted Benzamides with Conformationally Restricted Side Chains. 5. Azabicyclo[*x.y.z*] Derivatives as 5-HT$_4$ Receptor Agonists and Gastric Motility Stimulants. *J. Med. Chem*. **1993**, 683−689.

(30) Takeda, M.; Tsukamoto, K.; Mizutani, Y.; Suzuki, T.; Taniyama, K. Identification of SK-951, a novel benzofuran derivative, as an agonist to 5-HT$_4$ receptors. *Jpn. J. Pharmacol.* **1999**, 203-212.

(31) Takeda, M.; Tsukamoto, K.; Yamano, M.; Uesaka, H. Effects of SK-951, a benzofuran derivative, as a prokinetic agent in rats and dogs. *Jpn. J. Pharmacol.* **1999**, 292−297.

(32) Flynn, D. L.; Zabrowski, D. L.; Becker, D. P.; Nosal, R.; Villamil, C. I.; Gullikson, G. W.; Moummi, C.; Yang, D. C. The first selective agonist at the newly identified serotonin 5-HT$_4$ receptor subtype. *J. Med. Chem.* **1992**, 1486-1489.

(33) Yang, D. C.; Goldstin, B.; Moormann, A. E.; Flynn, D. L.; Gullikson, G. W. SC-53606, a potent and selective antagonist of 5-hydroxytryptamine$_4$ receptor in isolated rat esophageal tunica muscularis mucosae. *Pharmacol. Exp. Ther.* **1993**, 1339−1347.

(34) Simpson, K.; Spencer, C. M.; McClellan, K. J. Tropisetron: An update of its use in the prevention of chemotherapy-induced nausea and vomiting. *Drugs* **2000**, *6*, 1297−1315.

(35) Plosker, G. L.; Goa, K. L. Granisetron. A review of its pharmacological properties and therapeutic use as an antiemetic. *Drugs* **1991**, *5*, 805−824.

(36) Buchheit, K. H.; Gamse, R.; Pfannkuche, H. J. SDZ 205−557, a selective antagonist at 5-HT4 receptors in the isolated guinea pig ileum. *Eur. J. Pharmacol.* **1991**, 373−374.

(37) Denham, A. Using herbs that contain pyrrolizidine alkaloids. *Eur. J. Herb. Med.* **1996**, *3*, 27−38.

(38) Nagao, Y.; Dai, W. M.; Ochiai, M.; Tsukagoshi, S.; Fujita, E. Highly Diastereoselective Alkylation of Chiral Tin(II) Enolates onto Cyclic Acyl Imines. An Efficient Asymmetric Synthesis of Bicyclic Alkaloids Bearing a Nitrogen Atom Ring Juncture. *J. Org. Chem.* **1990**, *55*, 8−1156.

(39) Gullikson, G. W.; Virina, M. A.; Loeffler, R. F.; Yang, D. C.; Goldstin, B.; Flynn, D. L.; Moormann, A. E. Gastrointestinal motility responses to the S and R enantiomers of zacopride, a 5-HT$_4$ agonist and 5-HT$_3$ antagonist. *Drug Dev. Res.* **1992**, 405−417.

(40) Saxena, P. R.; Lawang, A. A comparison of cardiovascular and smooth muscle effects of 5-hydroxytrpytamine and 5-carboxamidotryptamine, a selective agonist of 5-HT$_1$ receptors. *Arch. Int. Pharmacodyn*. **1985**, 235−252.

(41) Becker, D. P.; Nosal, R.; Zabrowski, D. L.; Flynn, D. L. Synthetic Strategies for the Construction of Enantiomeric Azanoradamantanes. *Tetrahedron* **1997**, 1−20.

(42) Dumuis, A.; Sebben, M.; Monferini, E.; Nicola, M.; Turconi, M.; Ladinsky, H.; Bockaert, J. Azabicycloalkyl benzimidazolone derivatives as a novel class of potent agonists at the 5-HT$_4$ receptor positively coupled to adenylate cyclase in brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, 245−251.

(43) Dumuis, A.; Gozlan, H.; Sebben, M.; Ansanay, H.; Rizzi, C. A.; Turconi, M.; Monferini, E.; Giraldo, E.; Schiantarelli, P.; Ladinsky, H.; Bockaert, J. Characterisation of a novel 5-HT$_4$ receptor antagonist of the azabicycloalkyl benzimidazolone class: DAU 6285. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1992**, 264−269.

(44) Bermudez, J.; Fake, C. S.; Joiner, G. F.; Joiner, K. A.; King, F. D.; Miner, W. D.; Sanger, G. J. 5-Hydroxytryptamine (5-HT$_3$) receptor antagonists. 1. Indazole and indolizine-3-carboxylic acid derivatives. *J. Med. Chem.* **1990**, *7*, 1924−1929.
(45) Gullikson, G. W.; Virina, M. A.; Loeffler, R.; Erwin, W. D. Alpha-2 adrenergic model of gastroparesis: Validation with Renzapride, a stimulator of motility. *Am. J. Physiol.* **1991**, G426-G432.
(46) Becker, D. P.; Flynn, D. L.; Villamil, C. I. Bridgehead-methyl analogue of SC-53116 as a 5-HT$_4$ agonist. *Bioorg. Med. Chem. Lett.* **2004**, 3073−3075.
(47) Matsumoto, M.; Kojima, T.; Togashi, H.; Mori, K.; Ohashi, S.; Ueno, K.; Yoshioka, M. Differential characteristics of endogenous serotonin-mediated synaptic transmission in the hippocampal CA1 and CA3 fields of anaesthetized rats. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2002**, 570−577.
(48) Matsumoto M, Togashi H, Mori K, Ueno K, Ohashi S, Kojima T, Yoshioka M. Evidence for involvement of central 5-HT(4) receptors in cholinergic function associated with cognitive processes: Behavioral, electrophysiological, and neurochemical studies. *J. Pharmacol Exp Ther* **2001**, 676−682.
(49) Hayes, E. S.; Adaikan, P. G.; Ratnam, S. S.; Ng, S. C. 5-HT$_4$ receptors in isolated human corpus cavernosum? *Int. J. Impot. Res.* **1999**, 219−225.
(50) Lambert, H. W.; Lauder, J. M. Serotonin receptor agonists that increase cyclic AMP positively regulate IGF−I in mouse mandibular mesenchymal cells. *Dev. Neurosci.* **1999**, 105−112.
(51) Becker, D. P.; Goldstin, B.; Gullikson, G. W.; Loeffler, R.; Moormann, A.; Moummi, C.; Nosal, R.; Spangler, D.; Villamil, C. I.; Yang, D. C.; Zabrowski, D. L.; Flynn, D. L. Design and Synthesis of Agonists and Antagonists of the Serotonin 5-HT$_4$ Receptor Subtype, in Perspectives in Receptor Research. In *Pharmacochemistry Library, Amsterdam: B;* Elsevier Science: New York, 1996; Vol. 24, pp 99−120.
(52) Becker, D. P.; Husa, R. K.; Moormann, A. E.; Villamil, C. I.; Flynn, D. L. Enantioselective synthesis of dual 5-HT$_3$/5-HT$_4$ serotonergic azanoradamantane SC-52491. *Tetrahedron* **1999**, 11787−11802.
(53) Buttery, C. D.; Jones, R. G.; Knight, D. W. Preparation of 2,3-disubstituted indoles from indole-3-carboxylic acids and amides by a-deprotonation. *J. Chem. Soc., Perkin Trans. 1* **1993**, 1425.
(54) Vernin, G.; Domlog, H.; Siv, C.; Metzger, J.; El-Shafei, A. K. Synthesis of 1-alkyl and 1,3-dialkyl-2-benzimidazolones from 1-alkenyl-2-benzimidazolones using phase-transfer catalysis technique. *J. Heterocycl. Chem.* **1981**, 85−89.
(55) Robertson, D. W.; Krushinski, J. H.; Beedle, E. E.; Wyss, V.; Pollock, G. D.; Wilson, H.; Kauffman, R. F.; Hayes, J. S. Dihydropyridazinone cardiotonics: The discovery and inotropic activity of 1,3-dihydro-3,3-dimethyl-5-(1,4,5,6-tetrahydro-6-oxo-3-pyridazinyl)-2*H*-indol-2-one. *J. Med. Chem.* **1986**, *29*, 2−1840.
(56) Kilpatrick, G. J.; Jones, B. J.; Tyen, M. G. Identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. *Nature* **1987**, 746−748.
(57) Grossman, C. J.; Kilpatrick, G. J.; Bunce, K. T. Development of a radioligand binding assay for 5-HT$_4$ receptors in guinea-pig and rat brain. *Br. J. Pharmacol.* **1993**, 618−624.
(58) Arunlakshana, O.; Schild, H. O. Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* **1959**, 48−58.
(59) Jacoby, H. I.; Brodie, D. A. Gastrointestinal actions of metoclopramide, an experimental study. *Gastroenterology* **1967**, 676−684.
(60) Gullikson, G. W.; Loeffler, R. F.; Virina, M. A. Relationship of serotonin-3 receptor antagonist activity to gastric emptying and motor-stimulating actions of prokinetic drugs in dogs. *J. Pharmacol. Exp. Ther.* **1991**, 103−110.

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