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Daniel Becker

Loyola University Chicago, dbecke3@luc.edu

Daniel L. Flynn

Alan E. Moormann

Roger Nosal

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Pyrrolizidine Esters and Amides as 5-HT₄ Receptor Agonists and Antagonists

Daniel P. Becker, Daniel L. Flynn,[§] Alan E. Moormann, Roger Nosal, Clara I. Villamil,[‡]*

Richard Loeffler, Gary W. Gullikson,[†] Chafiq Moummi, Dai-C. Yang

Department of Medicinal Chemistry, Pfizer, 4901 Searle Parkway, Skokie, IL 60077

Department of Pharmacology, Pfizer, 4901 Searle Parkway, Skokie, IL 60077

*To whom correspondence should be addressed. Loyola University, 6525 North Sheridan, Chicago, IL 60626, Phone: 773-508-3089. Fax: 847-730-3181. E-mail dbecke3@luc.edu.

[§]Current address: Deciphera Pharmaceuticals Inc., 1505 Wakarusa Drive, Lawrence, KS 66047. E-mail: dflynn@deciphera.com.

[‡]Current address: Abbott Labs, 100 Abbott Park Road, Abbott Park, IL 60064-6099. E-mail clara.villamil@abbott.com.

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A series of pyrrolizidine esters, amides and ureas were prepared and tested for 5-HT₄ and 5-HT₃ receptor binding, 5-HT₄ receptor agonism in the rat tunica muscularis mucosae (TMM) assay, and for 5-HT₃ receptor-mediated functional antagonism in the Bezold-Jarisch reflex assay. Several pyrrolizidine derivatives were identified with high affinity for the 5-HT₄ receptor including benzamide **12a** (SC-53116), a potent and selective 5-HT₄ partial agonist that exhibits efficacy in promoting antral contractions and activity in promoting gastric emptying in canine models. Also discovered were 5-HT₄ receptor antagonists, including imidazopyridine amide **12h** (SC-53606), which is a potent and selective 5-HT₄ receptor antagonist with a pA₂ value of 8.13 in the rat TMM assay. N-Methyl indole ester **13d** was identified as a potent 5-HT₄ antagonist with a pA₂ value of 8.93. High selectivity was observed for these pyrrolizidine derivatives versus other monoamine receptors including 5-HT₁, 5-HT₂, D₁, D₂, 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-HT₄ receptor was discovered by Baxter, Craig and Clarke² and by Dumuis and Bockaert³ 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 prokinetic⁶ benzamides cisapride **1** and renzapride **2** enhance contractile activity at neuronal 5-HT₄ receptors in the guinea pig ileum was made by Craig and Clarke.⁷ It was later demonstrated by Dumuis and Bockaert that 5-HT₄ receptors mediate the relaxation of smooth muscle of the inner muscularis mucosae of rat esophagus⁸ 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-HT₄ receptors,¹⁰ and an excellent review of the 5-HT₄ receptor and key ligands was recently published.¹¹

Selective ligands for the 5-HT₄ receptor show promise in the treatment of a wide variety of diseases including the 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 which 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 disorders¹⁵ but was withdrawn due to QT prolongation.^{16,17} The 5-HT₄ partial agonist tegaserod (compound **3**, SDZ HTF 919) was approved in 2002 for the treatment of constipation-predominant irritable bowel syndrome (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

We have pursued the exploration of various conformationally-constrained bicyclic and tricyclic amines as ligands for the serotonin 5-HT₄ receptor to treat gastrointestinal motility disorders. Our focus has been on receptor selectivity to avoid potential side effects, and we were particularly cognizant of avoiding the dopamine D₂ receptor, as D₂ antagonism is responsible for extrapyramidal side effects and hyperprolactinaemia observed with the marketed benzamide metoclopramide.²⁵ We were also vigilant of other monoamine receptors, including the 5-HT₂ 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-HT₄ 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-HT₄ 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-HT₄ 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 H-bond of the 2-methoxybenzamides. This approach led to the potent 5-HT₄ 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-HT₃ receptor antagonists

tropisetron³⁴ and granisetron,^{34, 35} particularly considering that tropisetron was shown to be the first surmountable antagonist of 5-HT₄ receptors³ and Buchheit demonstrated that the ester derivative of metoclopramide, SDZ 205-557, is a potent antagonist of 5-HT₄ receptors.³⁶

We were aware of the toxicity of the pyrrolizidine alkaloid natural products³⁷ 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. Based on this premise of structure-specificity we decided to explore the pyrrolizidine nucleus for incorporation into novel 5-HT₄ receptor agonists and antagonists. This paper details our work employing the pyrrolizidine scaffold resulting in the potent 5-HT₄ receptor agonist **12a**, as well as 5-HT₄ receptor antagonist **12h**, and other potent 5-HT₄ 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 Yoshimitsu Nagao³⁸ and summarized in Scheme 1. We analyzed the Mosher ester of **9** by ¹⁹F NMR to confirm the enantiomeric purity which showed the material to be $\geq 99.1\%$ e.e.

Aminomethylpyrrolizidine **11** was prepared by 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 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, 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-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylic acid **21** was prepared by alkylative esterification of 2-aminonicotinic acid **16**, and regioselective chlorination with t-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-HT₄ 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-HT₄ receptor antagonists were also evaluated using the TMM model. Binding to serotonin 5-HT₄ receptors in guinea pig striatum was measured utilizing [³H]-GR113,808 as the ligand. Binding to serotonin 5-HT₃ receptors in rat cortical tissue was measured using [³H]-GR656630. Functional 5-HT₃ antagonism was measured utilizing the von Bezold-Jarisch reflex model.⁴⁰

Pyrrolizidine 5-HT₄ agonists are summarized in Table 1. In the rat TMM assay, the chiral pyrrolizidine benzamide **12a** and its distomer **ent-12a** had EC₅₀ values of 16.5 ± 3.8 nM 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-HT₄ 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 30X more potent in binding to the 5-HT₄ receptor relative to its 5-HT₃ binding (K_i = 152 nM). In contrast to the 30X difference in 5-HT₄ binding, **12a** is only half as potent as **ent-12a** in 5-HT₃ binding. We previously reported a similar trend with compound **6** and its enantiomer, where the 5-HT₄ receptor showed a greater discernment of the antipodes than the 5-HT₃ receptor, although in that case the eutomer was the same compound for both receptors.⁴¹ Functional antagonism of the 5-HT₃ receptor is demonstrated by inhibition

of the von Bezold-Jarisch reflex in mice for both **12a** and **ent-12a** (87% at 10 mpk for both compounds).

The ethoxy benzamide **12b** was prepared in an attempt to increase 5-HT₃ potency, which was successful in boosting 5-HT₃ receptor binding by 6-fold ($K_i = 25$ nM), however, the compound was approximately 12X less potent in the rat 5-HT₄ TMM assay, so 5-HT₃ receptor binding potency was boosted at the expense of 5-HT₄ potency. The methiodide quaternary ammonium salt **12c** was prepared to probe the possibility of synthesizing a potent radiolabeled pyrrolizidine analog employing radiolabeled methyl iodide. The quaternary ammonium salt **12c** was indeed very potent in binding to the 5-HT₄ 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 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-HT₄ receptor binding ($K_i = 183$ pM) and in functional agonism at the 5-HT₄ receptor in the TMM assay ($EC_{50} = 1.5$ nM). It is actually a partial agonist at the 5-HT₄ receptor, with 57% efficacy as an agonist relative to serotonin. N-isopropyl benzimidazolone urea **15a**, which incorporates the benzimidazolone moiety of BIMU 8⁴² and DAU 6215⁴³ exhibited potent binding at the 5-HT₄ receptor ($K_i = 7.0$ nM) but was 22X less potent in 5-HT₄ agonism than **12a**.

The excellent potency of the pyrrolizidine moiety in 5-HT₄ 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-HT₄ 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 between the amide NH and the benzimidazolone carbonyl of **15a**. The 5-HT₄ 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-HT₄ antagonists in the rat TMM assay employing different aromatic groups. All of these compounds were determined to have EC₅₀ 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.⁴⁴ We employed an imidazopyridine aromatic nucleus with the nitrogen lone pair orthogonal to the aromatic pi-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-HT₄ 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 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-HT₄ receptor antagonists. Several imidazopyridines afforded good 5-HT₄ antagonist activity, including 3-ethylimidazopyridine **12f** (pA₂ = 6.56). The role of the second nitrogen atom in antagonist imidazopyridine **12f** relative to inactive indolizine **12e** is noteworthy. The 6-chloro substituent was incorporated in analog **12h** to boost binding based on 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-HT₄ receptor ($K_i = 1.4$ nM). Incorporation of a 3-methyl substituent 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-HT₄ inhibitory potency of compound **12i** ($pA_2 = 7.09$) relative to **12h**. Oxindole urea **15b**, which is structurally related to benzimidazolone 5-HT₄ 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-HT₄ receptor ($K_i = 6.8$ nM).

Pyrrolizidine ester 5-HT₄ receptor antagonists are included in Table 3. The esters were inspired by the work of Buchheit who reported SDZ 205-557, the ester analog of metoclopramide, as the first potent antagonist of 5-HT₄ receptors.³⁶ These compounds all lack agonist activity in the TMM assay ($EC_{50} > 10,000$ nM). Based on the inhibitory potency of 6-chloroimidazopyridine amide **12h** we prepared the corresponding ester **13b** which is a moderate 5-HT₄ receptor antagonist ($pA_2 = 6.75$). Next, we prepared esters incorporating the indole and indazole aromatic moieties present in tropisetron and granisetron, respectively. Attempted 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 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-

HT₄ receptor ($pA_2 = 7.15$), and potency was markedly increased for the 5-fluoro derivative **13f** in 5-HT₄ inhibitory potency ($pA_2 = 8.5$) as well as binding to the 5-HT₄ and 5-HT₃ receptors ($K_i = 700$ pM and 10 nM, resp.). 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-HT₄ receptor ($K_i = 800$ pM and 400 pM, respectively), and moderate binding to the 5-HT₃ receptor ($K_i = 135$ nM and 20 nM, respectively). The N-methyl analog was thus modestly more potent than the N-H analog toward both receptors. Both indazoles were free of binding at 5-HT₁, 5-HT₂, D₁, D₂, α_1 , α_2 , β_1 , and β_2 receptors. Thus, the most potent member of the pyrrolizidine ester 5-HT₄ 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-HT₄/5-HT₃ ligand, with a $K_i = 183$ pM at the 5-HT₄ receptor and $K_i = 5.0$ nM at the 5-HT₃ receptor. This pyrrolizidine ester is free of binding at 5-HT₁, 5-HT₂, D₁, D₂, α_1 , α_2 , β_1 , and β_2 receptors ($K_i > 10$ μ M).

In general, the esters are more potent as 5-HT₄ 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-HT₄ 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 minutes) 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 compound **1** in the canine contractility model are plotted in Figure 4. Pyrrolizidine benzamide **12a** exhibited an ED₅₀ of 0.010 mg/kg iv, whereas benzamide **1** was 5-fold less potent with an ED₅₀ of 0.056 mg/kg iv. Distomer **ent-12a** was approximately 45X less potent than **12a** in promoting gastric antral contractions with an ED₅₀ of 0.45 mg/kg iv. A maximally effective dose of pyrrolizidine **12a** induced antral contractions which 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 gamma scintigraphy. Since 5-HT₄ 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 alpha-adrenergic agonist (2-methyl-3-[(2E)-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**, **ent-12a** and benzamide **1** (Figure 5). Pyrrolizidine benzamide **12a** was shown to be approximately 30X more potent than benzamide **1** in enhancing gastric emptying (ED₅₀ = 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 ED₅₀ of 1.0 mg/kg.

5-HT₄ agonism data is summarized in Table 4 along with canine antral contractility and canine solid gastric emptying for the pyrrolizidine benzamides **12a**, **ent-12a**, and the benzamide **1**. Pyrrolizidine **12a** is the most potent compound in vitro, as well as in vivo in both canine models motility models. Benzamide **1** has an ED₅₀ of 55 nM in the rat TMM assay, whereas benzamide **2** exhibits an ED₅₀ of 98 nM. Compound **2** is also more potent than benzamide **1** in the antral contractility model, with an ED₅₀ of 0.015 mg/kg iv.

Pyrrolizidine derivatives of the present series are highly selective for the 5-HT₄ receptor. Pyrrolizidine **12a** is very potent as a partial agonist at the 5-HT₄ receptor and in binding to the 5-HT₄ receptor (K_i = 5.2 nM). Compound **12a** exhibits moderate binding at the 5-HT₃ receptor (K_i = 152 nM), but no detectable binding (K_i > 10,000 nM) at any of the other monoamine receptors tested (5-HT₁, 5-HT₂, D₁, D₂, α_1 , α_2 , and β receptors). Imidazopyridine **12h**, as reported previously,³³ is a very potent antagonist at the 5-HT₄ receptor (K_i = 1.4 nM; pA₂ = 8.13) with excellent selectivity versus the other monoamine receptors tested (K_i > 10 μ M). Standards included for comparison are compound **6**, which we have reported to be a potent and selective dual 5-HT₄ agonist and 5-HT₃ receptor antagonist, and compound **1**, which shows potent inhibition of α_1 receptors (K_i = 30 nM) and 5-HT₂ receptors (6.1 nM).

In summary, incorporation of the pyrrolizidine scaffold has resulted in the discovery of potent and selective ligands for the 5-HT₄ receptor, including the potent and selective 5-HT₄ 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 analog 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-HT₄ receptor antagonist³³ as well as the potent N-methylindole 5-HT₄ receptor antagonist **13d**. Related work in these labs on conformationally-constrained amines also led to the discovery of azanoradamantane **6** which is a potent, non-mutagenic and selective dual 5-HT₄ agonist/5-HT₃ 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-chlorobutyl)-4-(S)-isopropyl-1,3-thiazolidine-2-thione **7** with 5-acetoxy-2-pyrrolidinone and subsequent reduction with lithium aluminum hydride as described by Yoshimitsu Nagao³⁸ to afford **9** (3.12 g, 45.5%) as a colorless oil: $[\alpha]_D = +9.2$ ($c = 0.195$ g/dL in CHCl₃; 10 cm); $[\alpha]_{365} = -63.1$ ($c = 0.195$ g/dL in CHCl₃; 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. ¹⁹F 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 was $\geq 99.6\%$ diastereomeric purity, or $\geq 99.1\%$ e.e.

2-[(1S,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl]-1H-isoindole-1,3(2H)-dione (10). To a solution of alcohol **3** (1.44 g, 10.2 mmol) in dry THF (45 mL) was 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 minutes. The reaction was then stirred for 1 h at 0°C and then 16 h at rt. Concentration gave a residue which was purified on silica gel eluting with MeOH (saturated with NH₃)/CHCl₃ (4/96, 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. [α]_D = -2.5°; [α]₃₆₅ = -13.3° (c = 0.285 g/dL in chloroform). ¹H NMR (300 MHz, CDCl₃) 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). ¹³C NMR (75 MHz, CDCl₃) 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. Anal calcd for C₁₆H₁₈N₂O₂·0.4H₂O C 69.24; H, 6.83; N, 10.09. Found C, 69.12; H, 6.56; N, 9.94. HRMS calcd for C₁₆H₁₈N₂O₂ 270.1369, found 270.1369.

(1S,7aS)-Hexahydro-1H-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 rt. 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 X 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, CDCl₃) 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, d₄-MeOD) 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). ¹³C NMR (75 MHz, d₄-MeOD) δ 70.4, 54.4, 53.9, 42.5, 40.7, 29.8, 29.4, 24.3. HRMS MH⁺ calcd for C₈H₁₆N₃ 141.1392, found 141.1394.

4-Amino-5-chloro-N-[(1S-cis-hexahydro-1H-pyrrolizin-1-yl)methyl]-2-methoxybenzenecarboxamide, hydrochloride (12a, SC-53116). To a solution of 4-acetamido-5-chloro-2-methoxy-benzoic 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 rt 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 rt. Concentration gave a yellow oil which was treated with 15% K₂CO₃ (65 mL) and extracted with chloroform (4X). The combined organic extracts were washed successively with water (2X) 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 NH₃)/CHCl₃ (15/85) gave 4-(acetylamino)-N-[(7aS)-hexahydro-1H-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; [α]_D = -8.8° (c = 1.2 g/dL); IR (MIR) 3407, 3310, 1696, 1656 cm⁻¹. ¹H NMR (300 MHz, CDCl₃) 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. Anal calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_3\text{Cl}$ C 59.09, H, 6.61, N, 11.48, Cl, 9.69. Found C, 58.76; H, 6.68; N, 11.42; Cl, 10.02. HRMS calcd for $\text{C}_{18}\text{H}_{24}\text{N}_3\text{O}_3\text{Cl}$ 365.1506, found 365.1514.

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 (4X). The combined organic extracts were washed successively with water (2X) 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, 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. $[\alpha]_{\text{D}} = -10.6^\circ$; $[\alpha]_{365} = -36.7^\circ$ ($c = 0.330$ 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. Analysis calculated for $\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_2\text{Cl}$: 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 liters) 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. IR (MIR) 3323, 3194, 1620, 1594 cm^{-1} . ^1H NMR (300 MHz, d_4 -MeOD) 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, d_4 -MeOD) 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 $\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_2\text{Cl}$ 323.1415, found 323,1400. Analysis calculated for $\text{C}_{16}\text{H}_{22}\text{N}_3\text{O}_2\text{Cl}\cdot\text{HCl}\cdot 0.75\text{H}_2\text{O}$: 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. $[\alpha]_{\text{D}} = -2.6^\circ$; $[\alpha]_{365} = -6.4^\circ$ ($c = 0.47\text{g/dL}$ in methanol).

Amide Coupling General Procedure. We have found that benzamide couplings utilizing 4-amino-5-chloro-2-methoxy-benzoic 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 equivalent) in DMF (freshly opened) is added 1,1'-carbonyldiimidazole (1 equivalent) which results in a vigorous gas evolution. After 0.5 to 1 h at rt a solution of the amine 11 in DMF is added and reaction is stirred for 2 h at rt followed by 2 h at 50°C. Concentration affords a residue which is treated with 15% K_2CO_3 and extracted with chloroform (3X). The combined organic extracts are washed successively with water (2X) 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-[(1R,7aR)-hexahydro-1H-pyrrolizin-1-ylmethyl]-2-methoxybenzamide (ent-12a, SC-53117). This compound was prepared from (+)-trachelanthamidine, prepared according to the method of Nagao³⁸ 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**: Analysis calculated 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. $[\alpha]_D = +0.5^\circ$; $[\alpha]_{365} = +3.3^\circ$ (c = 0.183 g/dL in methanol).

4-Amino-5-chloro-2-ethoxy-N-[(1S,7aS)-hexahydro-1H-pyrrolizin-1-ylmethyl]benzamide hydrochloride (12b). To a solution of 4-amino-5-chloro-2-methoxy-benzoic acid (1 eq) in DMF was added 1,1'-carbonyldiimidazole (1 eq). After 1 h at rt 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**: ¹H NMR (400 MHz, d₄-MeOD) δ 7.77 (1H, s), 6.49 (1H, s), 4.17 (2H, q, J = 7 Hz), 3.96 to 3.91 (1H, m), 3.78 to 3.73 (1H, m), 3.54 to 3.52 (2H, m), 3.47 to 3.41 (1H, m), 3.24 to 3.17 (2H, m), 3.13 to 3.06 (1H, m), 2.43 to 2.34 (1H, m), 2.29 to 2.21 (1H, m), 2.19 to 2.09 (2H, m), 2.07 to 1.99 (1H, m), 1.96 to 1.89 (1H, m), 1.86 to 1.79 (1H, m), 1.48 (3H, t). Analysis calculated for $C_{17}H_{24}N_3O_2Cl \cdot HCl \cdot 0.5H_2O$: C, 53.27; H, 6.84; N, ; Cl, 18.50. Found C, 53.14; H, 6.88; N, 10.87; Cl, 20.07.

(1S,7aS)-1-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]methyl]-4-methylhexahydro-1H-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 μ L, 0.103 mmol) in toluene (50 mL). The reaction flask was wrapped with foil and allowed to stand at rt for 24 h. Filtration afforded the methiodide salt **12c** (35 mg, 75%) as a pale

yellow solid: ^1H NMR (300 MHz, $\text{d}_4\text{-MeOD}$) 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, $\text{d}_4\text{-MeOD}$) 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 $\text{C}_{17}\text{H}_{25}\text{N}_3\text{O}_2\text{I}$: C, 47.45; H, 5.86; N, 9.77. Found C, 47.17; H, 5.92; N, 9.54.

N-[(1S,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl]-1-methyl-1H-indole-3-carboxamide (12d). To a solution of N-methylindole-3-carboxylic acid (1.0 eq) in DMF at rt was added CDI (1 eq). After 1 h a solution of amine 11 (1 eq) in DMF was added and the solution stirred for 16 h at rt. Aqueous workup and chromatography on silica gel afforded the desired indole carboxamide **12d**: $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}$ Anal calcd for $\text{C}_{18}\text{H}_{23}\text{N}_3\text{O}$: C, 72.70; H, 7.80; N, 14.13. Found C, 72.91; H, 7.68; N, 13.92.

N-exo(Tetrahydro-1H-pyrrolizin-4(5H)-ylmethyl)-3-ethylindolizine-1-carboxamide (12e). 3-Ethylindolizine-1-carboxylic acid⁴⁴ (190 mg, 1.0 mmol) was suspended in CHCl_3 (2 mL). Oxalyl chloride (184 μL , 2.1 mmol) and DMF (1 drop) were added and the mixture 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-1H-pyrrolizin-4(5H)-methylamine **11** (140 mg, 1.0 mmol), and triethylamine (279 μL , 2.0 mmol) in CHCl_3 (2 mL) and the mixture was stirred for 18 h. The organic solution was washed with 1N NaOH, 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 hygroscopic solid (190 mg, 63%). ^1H NMR (400 MHz, $\text{d}_4\text{-MeOD}$) δ 8.20 (1H, d, $J = 18$ Hz), 8.02 (1H, t, $J =$

14 Hz), 7.03 to 6.97 (3H, m), 6.80 to 6.75 (1H, m), 4.06 to 3.99 (1H, m), 3.83 to 3.75 (1H, m), 3.67 (2H, d, $J = 6$ Hz), 3.46 to 3.42 (1H, m), 3.25 to 3.18 (1H, m), 3.18 to 3.03 (1H, m), 2.54 to 2.45 (1H, m), 2.37 to 2.29 (1H, m), 2.22 to 2.12 (2H, m), 2.09 to 2.01 (3H, m). Anal calcd for $C_{19}H_{25}N_3O \cdot 2.0 H_2O$: C, 65.68; H, 7.25; N, 12.09. Found C, 65.79; H, 7.56; N, 12.03. MS calcd for $C_{19}H_{25}N_3O$: 311.43, Found: 311.19. Attempts to make the HCl salt resulted in a brown oil, so the compound was tested as the free base.

N-exo(tetrahydro-1H-pyrrolizin-4(5H)-ylmethyl)-3-ethylimidazo[1,2-a]pyridine-1-carboxamide monohydrochloride (12f). 3-Ethylimidazo[1,5-a]pyridine-1-carboxylic acid⁴⁴ (190 mg, 1.0 mmol) was suspended in $CHCl_3$ (2 mL). Oxalyl chloride (184 μ L, 2.1 mmol) and DMF (1 drop) were added and mixture 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-1H-pyrrolizin-4(5H)-methylamine 11 (140 mg, 1.0 mmol) and triethyl amine (279 μ L, 2.0 mmol) in $CHCl_3$ (2 mL) and the mixture stirred for 18 h. The organic solution was washed with 1N NaOH, 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**: Anal calcd for $C_{18}H_{24}N_4O \cdot HCl \cdot 0.75 H_2O$: 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. MS calcd for $C_{18}H_{24}N_4O$ 312, found 312.

N-exo((4-s,7 α -s)-Tetrahydro-1H-pyrrolizin-4(5H)-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'-carbonyldiimidazole (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 μ L, 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 $\text{MeOH}/\text{CHCl}_3$ containing 0.25% NH_4OH . The filtrate was concentrated and the residue was dissolved in CHCl_3 and 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 $\text{C}_{16}\text{H}_{20}\text{N}_4\text{O} \cdot 2.0\text{H}_2\text{O} \cdot 2.2\text{HCl} \cdot 0.25\text{CHCl}_3$ C: 46.12; H: 6.25; N: 13.24. Found: C: 46.22; H: 6.41; N: 13.43.

N-exo((4-S,7 α -s)-Tetrahydro-1H-pyrrolizin-4(5H)-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'-carbonyldiimidazole (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 $\text{MeOH}/\text{CHCl}_3$ with 0.25% NH_4OH . The filtrate was concentrated and the residue was dissolved in CHCl_3 and 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, $\text{d}_4\text{-MeOD}$) δ 8.83 (1H, d, $J = 2\text{ Hz}$), 7.99 (1H, d, $J = 2\text{ Hz}$), 7.96 (1H, d, $J = 1.3\text{ Hz}$), 7.70 (1H, 1.3 Hz), 4.05 to 3.98 (1H, m), 3.82 to 3.75 (1H, m), 3.689 (2H, d, $J = 6\text{ Hz}$), 3.49 to 3.42 (1H, m), 3.25 to 3.18 (1H, m), 3.16 to 3.08 (1H, m), 2.54 to 2.45 (1H,

m), 2.37 to 2.29 (1H, m), 2.22 to 2.12 (2H, m), 2.09 to 1.86 (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,7 α -s)-Tetrahydro-1H-pyrrolizin-4(5H)-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) were 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), then the solution was concentrated. The residue was dissolved in MeOH (15 mL) and t-butylhypochlorite (570 mg, 5.25 mmol) was added to the reaction mixture and stirred until the yellow color dissipated. Additional t-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

C₇H₇ClN₂O₂ 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% NH₄OH) indicated that the starting material was consumed. The reaction mixture was concentrated and the residue was triturated with acetone, then partitioned between CH₂Cl₂ and 5% aqueous K₂CO₃. The organic layer was dried over MgSO₄ and concentrated. The residue was triturated with Et₂O to yield methyl 6-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylate (5.0 g, 50%) as a solid: IR: 1720 cm⁻¹. Anal calcd for C₁₀H₉ClN₂O₂ 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. MS MH⁺ calcd for C₁₀H₉ClN₂O₂ 225, found 225.

The methyl 6-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylate (5.0 g; 0.022 mol) was suspended in 6N 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-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylic acid **21** (4.9 g) as the HCl salt: IR: 1704 and 3175 cm⁻¹. Anal calcd for C₉H₇ClN₂O₂.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. MS MH⁺ calcd for C₉H₇ClN₂O₂ 211, found 211.

To a solution of 6-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylic acid **21** (247 mg, 1.00 mmol) in DMF (5 mL) was added 1,1'-carbonyldiimidazole (178 mg, 0.011 mol) and the solution stirred for 1 h. Aminomethylpyrrolizidine **11** (120 mg, 0.85 mmol) and Et₃N (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 CHCl₃ and 5% aqueous K₂CO₃. The organic layer was dried over Na₂SO₄ and concentrated. The residue

was purified by preparative thin-layer chromatography eluting with 20/80 MeOH/CHCl₃ containing 0.25% NH₄OH. The filtrate was concentrated and the residue was dissolved in CHCl₃ and filtered through celite and concentrated to yield an oil (144 mg, 43%). This residue was converted to the HCl salt the desired imidazopyridine amide **12i** using a mixture of acetyl chloride/MeOH. ¹H NMR (400 MHz, d₄-MeOD) 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 to 4.03 (1H, m), 3.81 to 3.75 (1H, m), 3.63 (2H, d, J= 6 Hz), 3.51 to 3.45 (1H, m), 3.22 to 3.19 (1H, m), 3.16 to 3.09 (1H, m), 2.68 (3H, d, J= 1 Hz), 2.52 to 2.46 (1H, m), 2.35 to 2.27 (1H, m), 2.25 to 2.14 (2H, m), 2.09 to 2.03 (1H, m), 1.99 to 1.87 (2H, m). Anal calcd for C₇H₈N₂O₂ C: 55.26; H: 5.30; N: 18.41. Found: C: 54.90; H: 5.36; N: 18.26. Anal calcd for C₁₉H₂₆N₄O₂·1.25 H₂O·2.2 HCl·0.25 MeOH 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.

(1R,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl 4-amino-5-chloro-2-methoxybenzoate hydrochloride (13a). To NaH solid (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/CHCl₃ afforded the requisite imidazolide (780 mg, 78%) as colorless crystals, mp 122-122.5°C.] The reaction was allowed to warm to rt over 2 h. Water was added and the mixture extracted with CHCl₃. The combined organic extracts were washed with water (2X) and brine and dried over Na₂SO₄. Concentration gave a solid (362 mg) which was purified by chromatography on

silica gel eluting with i-PrOH(NH₃)/CHCl₃ 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. HRMS calcd for C₁₆H₂₁N₂O₃Cl 325.1319, found 325.1298. Anal calcd for C₁₆H₂₁N₂O₃Cl:HCl:0.25H₂O 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. ¹H NMR (300 MHz, d₄-MeOD) 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). ¹³C NMR (75 MHz, d₄-MeOD) 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.

(1R,7aS)-Hexahydro-1H-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 rt over 3 h. Concentration gave a residue that was quenched with water (20 mL) and extracted with CHCl₃ (3X). The combined organic extracts were washed with water (2X) and brine and dried over Na₂SO₄. 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; ¹H NMR (300 MHz, CDCl₃) 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 uL, 19.6 mg, 0.279 mmol) to MeOH (1.5 mL)]. After 1 h at rt 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. ¹H NMR (300 MHz, d₄-MeOD) 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). ¹³C NMR (75 MHz, d₄-MeOD) 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 C₁₆H₁₈N₃O₂Cl 321.1040, found 321.1049. Anal calcd for C₁₆H₁₈N₃O₂Cl.HCl:0.25H₂O 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-1H-indole-2-carboxylic acid-(1R,7aS)-1-(hexahydro-pyrrolizin-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 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 NH₃)/CHCl₃ (10/90) gave the ester (170 mg, 53%) as a solid: ¹H NMR (300 MHz, CD₃OD) 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 μ L, 1.00 mmol) to MeOH]. After 1 h at rt 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%). ^1H NMR (300 MHz, CD_3OD) 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 $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_2$ 298.1673, found 298.1686.

(1R,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl 1-methyl-1H-indole-3-carboxylate (13d). To N-methylindole-3-carboxylic acid⁵³ (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 rt for 3h. Water (20 mL) and ice (30 g) were added and the mixture was extracted with chloroform (3X). 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 rt 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

NH₃)/CHCl₃ (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: ¹H NMR (400 MHz, d₄-MeOD) δ 8.45 (1H, d, J= 6 Hz), 8.00 (1H, s), 7.46 (1H, d J= 6 Hz), 7.3 to 7.2 (2H, m), 4.45 to 4.34 (2H, m), 4.14 to 4.08 (1H, m), 3.862 (3H, s), 3.83 to 3.77 (1H, m), 3.51 to 3.45 (1H, m), 3.26 to 3.13 (2H, m), 2.64 to 2.55 (1H, m), 2.38 to 2.30 (1H, m), 2.27 to 2.15 (2H, m), 2.11 to 1.93 (3H, m). Analysis calculated for C₁₈H₂₂N₂O₂·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%.

(1R,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl 5-methoxy-1H-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 hours 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 NH₃)/CHCl₃ (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 rt the solution was concentrated and resulting solid was triturated with diethyl ether and then dried to give the desired

hydrochloride salt **13e** (56 mg, 46%). ^1H 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 $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_3\cdot\text{HCl}\cdot 0.33\text{H}_2\text{O}$: 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.

(1R,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl 5-fluoro-1H-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 stirred for 16 hours. Solvent was concentrated to give 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 rt the solution was concentrated and resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13f** (133 mg, 74%). Anal calcd $\text{C}_{17}\text{H}_{19}\text{N}_2\text{O}_2\text{F}\cdot\text{HCl}\cdot 0.33\text{H}_2\text{O}$: 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. ^1H 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).

(1S,7aR)-Hexahydro-1H-pyrrolizin-1-ylmethyl 1H-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 rt and 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 was stirred for 16 h. Solvent was concentrated to give desired ester as an oil. Purification on silica gel eluting with MeOH (saturated with NH₃)/CHCl₃ (5/95) gave the ester as a solid (205 mg, 68%). ¹H NMR (300 MHz, CDCl₃) 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 C₁₆H₁₉N₃O₂ 285.1474, Found: 285.1500. Anal calcd for C₁₆H₁₉N₃O₂·0.5H₂O 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 rt the solution was concentrated and resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13g** (137 mg, 76%): ¹H NMR (400 MHz, d₄-MeOD) 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 to 4.49 (2H, m), 4.16 (1H, t, J= 6 Hz), 3.87 to 3.82 (1H, m), 3.54 to 3.48 (2H, m), 3.27 to 3.17 (1H, m), 2.73 to 2.64 (1H, m), 2.43 to 2.36 (1H, m), 2.32 to 2.17 (2H, m), 2.10 to 1.98 (3H, m). HRMS calcd for C₁₆H₁₉N₃O₂: 285.1496, found: 285.1511. Anal calcd for C₁₆H₁₉N₃O₂·HCl·0.15H₂O: 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.

(1S,7aR)-Hexahydro-1H-pyrrolizin-1-ylmethyl 1-methyl-1H-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 hours. Solvent was removed in vacuo

to give an oil. Purification on silica gel eluting with MeOH (saturated with NH₃)/CHCl₃ (10/90) gave the ester as a solid (196 mg, 62%). ¹H NMR (300 MHz, CDCl₃) 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 C₁₇H₂₁N₃O₂: 299.1616, found 285.1632. Anal calcd for C₁₇H₂₁N₃O₂·0.2H₂O: 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 rt the solution was concentrated and resulting solid was triturated with diethyl ether and then dried to give the desired hydrochloride salt **13h** (174 mg, 92%). Anal calcd C₁₇H₂₁N₃O₂·HCl·0.15 H₂O: 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. HRMS calcd C₁₇H₂₁N₃O₂: 299.1649, found: 299.1648. ¹H NMR (400 MHz, CDCl₃) 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).

cis-N-[(Hexahydro-1H-pyrrolizin-1-yl)methyl]-2,3-dihydro-3-(1-methylethyl)-2-oxo-1H-benzimidazole-1-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-1-(1-methylethyl)-2H-benzimidazol-2-one⁵⁴ (176 mg, 1.00 mmol) was added to the suspension. This mixture was stirred 15 minutes before adding 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 Et₃N (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/CHCl₃ containing 0.25% NH₄OH. The product was rinsed from the silica with 9/95 NH₄OH/MeOH. The filtrate was concentrated and the residue was dissolved in CHCl₃ and filtered through celite and concentrated to afford the benzimidazolone **15a** (157 mg, 50%). The product was converted to the HCl salt by dissolving 36 uL of acetyl chloride in 5.0 mL of MeOH and adding this solution to the product, then concentrating to dryness: ¹H NMR (400 MHz, d₄-MeOD) 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 to 3.96 (1H, m), 3.82 to 3.74 (1H, m), 3.58 to 3.55 (2H, m), 3.50 to 3.42 (1H, m), 3.25 to 3.18 (1H, m), 3.16 to 3.08 (1H, m), 2.49 to 2.39 (1H, m), 2.36 to 2.26 (1H, m), 2.25 to 2.17 (1H, m), 2.11 to 2.03 (1H, m), 2.01 to 1.93 (1H, m), 1.91 to 1.82 (1H, m), 1.54 (2H, d, J= 7 Hz). MS MH⁺ calcd for C₁₉H₂₆N₄O₂ 343, found 343. Anal calcd for C₁₉H₂₆N₄O₂·HCl·H₂O 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-1H-pyrrolizin- 1S-yl)methyl]-2-oxo-1H-indole-1-carboxamide, hydrate hydrochloride (15b). To sodium hydride (214 mg, 5.6 mmol, washed 2X with hexane) suspended in THF (1 mL) was added 1,3-dihydro-3,3-dimethyl-2H-indol-2-one⁵⁵ (226 mg, 1.4 mmol) and the reaction was stirred for 10 minutes. 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 hour. 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 hours. 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: Analysis calculated 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. HRMS calculated for $C_{19}H_{25}N_3O_2$: 327.1947, found 327.1930. 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: 1H NMR (400 MHz, d_4 -MeOD) δ 8.10 (1H, d, J = 2 Hz), 7.33 (1H, d, J = 2Hz), 7.28 (1H, t, J = 2 Hz), 7.19 (1H, t, J = 2 Hz), 4.02 to 3.96 (1H, m), 3.79 to 3.74 (1H, m), 3.53 (2H, d, J = 6.4 Hz), 3.49 to 3.43 (1H, m), 3.24 to 3.18 (1H, m), 3.15 to 3.08 (1H, m), 2.48 to 2.38 (1H, m), 2.33 to 2.25 (1H, m), 2.22 to 2.13 (2H, m), 2.08 to 1.02 (1H, m), 1.99 to 1.92 (1H, m), 1.90 to 1.83 (1H, m), 1.41 (6H, s). Analysis calculated 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. MS calculated for $C_{19}H_{25}N_3O_2$: 327.1947, found: 327.1939.

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 and 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 $C_8H_6N_2O_2 \cdot HCl$ 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 and 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-2,5 β -methano-1H-3aS,3a α ,6a α -cyclopenta[c]pyrrol-4 α -yl)-2-methoxybenzamide, monohydrochloride (6, SC-52491).

Compound **6** was prepared initially by synthesis involving resolution as previously described⁴¹ then via an enantioselective synthesis commencing with an asymmetric Diels-Alder reaction.⁵²

Serotonin 5-HT₃ Receptor Binding

Displacement of [³H]-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 [³H]-GR65630 in the presence or absence of graded

concentrations test compound for 60 min at 22°C. One micromolar 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 [³H]-GR65630 to the 5-HT₃ receptor.

Serotonin 5-HT₄ Receptor Binding

Serotonin 5-HT₄ receptor binding in guinea pig striatum utilizing [³H]-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: [³H]-5HT for 5-HT₁-like receptors; [³H]-ketanserin for 5-HT₂ receptors; [³H]-SCH23390 for D₁ receptors; [³H]-spiperone for D₂ receptors; and [³H]-prazosin for α₁-adrenergic receptors.

In Vitro Functional Assay for Serotonin 5-HT₄ agonism in the Rat TMM (tunica muscularis mucosae) Assay

Serotonin 5-HT₄ agonism was measured in the rat esophagus in vitro preparation as reported by Baxter.^{2,57} Agonist activity was determined 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 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 μ M) to inhibit monoamine oxidase. Following a 30 min equilibrium period, tissues were isometrically contracted with carbachol (3 μ M) to obtain a tonic contraction. A stable plateau was obtained within 20 min when test compound was added cumulatively to relax the muscle strip. EC₅₀ values were obtained for each agonist in tissues from rats.

5-HT₄ Receptor Antagonism in the Rat TMM (tunica muscularis mucosae) Assay

Antagonist activity of compounds at 5-HT₄ receptors was determined in a manner similar to the *in vitro* agonism activity described by Gullikson.³⁹ Cumulative dose-response curves for agonists interacting with 5-HT₄ receptors of rat TMM were done according to the method of Baxter.² Male Sprague-Dawley rats (300 - 400 g) from Charles River Laboratories (Wilmington, MA) were asphyxiated with CO₂ 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 to 3 mN of tension for 60 min before the study. The tissues were suspended in and washed with continuously oxygenated (95% O₂/5% CO₂) Tyrode's buffer which contained fluoxetine (1 μ M) and corticosterone (30 μ M) to prevent tissue uptake of 5-HT, methysergide (1 μ M) to block 5-HT₁ and 5-HT₂ receptors, and pargyline (100 μ M) 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 μ M of carbachol. Relaxant responses to cumulative additions of 5-HT or 5-HT₄ 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. EC₅₀ values were calculated as the concentrations causing 50% of this maximal effect. EC₅₀ 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 pA₂ value ± S.E.M. of the antagonist.⁵⁸ The pA₂ value for single antagonist concentrations in individual tissues was also calculated using the method of MacKay: $pA_2 = -\log(\text{antagonist concentration [mol/l]}) + \log(\text{DR}-1)$. A mean pA₂ value ± S.E.M. was calculated from the individual pA₂ 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 i.p. (mg/kg) to a group of 3 mice. Thirty minutes later, a 5-HT (0.25 mg/kg i.v.)-induced bradycardia was recorded in pentobarbital anesthetized animals. A greater than 50 percent (>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 cm, 4 cm 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 two weeks to recover and were trained to stand quietly in Pavlov slings. Dogs were fasted for 18-24 hours 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 minutes and consists of 45-60 minutes of motor quiescence (Phase I) 30-45 minutes of intermittent activity (Phase II) and 10-15 minutes 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.^{45,60} Dogs weighing 15-25 kg were trained to stand quietly in Pavlov slings for 3-4 hours, and consistent control emptying responses

were obtained prior to use in gastric emptying experiments with the test compounds. The solid meal consisted of 2 cooked scrambled eggs which were divided into 1 cm sized pieces and mixed with beef stew. One mCi of Tc-99m sulfur colloid was incorporated into the eggs prior to cooking. The dogs were fasted for at least 24 hours prior to the study and were fed the solid meal by intragastric tube. To delay normal gastric emptying 0.030 mg/kg of α_2 -adrenergic agonist 2-methyl-3-[(2E)-pyrrolidin-2-ylideneamino]phenol was administered immediately following the meal. The test compounds were given via intravenous injection 45 minutes prior to feeding.

A Siemens 370 ZLC gamma camera with high resolution low energy collimator was used to acquire left lateral images during emptying studies. Acquisition times were 3 min/frame for 180 min 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 per 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 3 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 4X in the number of revertant colonies were observed in strain TA98 with activation at 3600 µg/plate. A 2-3X increase was observed with activation in strain TA100 between 710 ug and 3600 µg/plate, and a 2-6X increase was observed with activation in strain TA1538 also between 720 µg and 3600 µg/plate. Significant increases in 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 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 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 tester strains with or without activation. These data support the conclusion that **ent-12a** is not mutagenic under the conditions of this test system.

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Figure 1. 5-HT₄ Agonists and Antagonists

1 (Cisapride)

2 (Renzapride; BRL 24924)

3 (Tegaserod; SDZ HTF 919)

4 (Prucalopride)

5 (SK-951)

6 (SC-52491)

12a (SC-53116)

12h (SC-53606)

Scheme 1. Preparation of (-)-trachelanthamidine **9** per Nagao³⁸

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**

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-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylic acid **21**

Figure 2. 5-HT₄ receptor-mediated relaxation of rat tunica muscularis mucosa by 5-HT, **12a** (SC-53116) and **ent-12a** (SC-53117).

Figure 3. Gastrointestinal response to compound **12a** (0.3 mpk, 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-HT₄ 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.

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

Table 1. Pyrrolizidine 5-HT₄ agonists

<div><div> </div><div> </div><div> </div></div>						
		12a-b, 13a, 15a				
			ent-12a			
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		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	...

Table 2. Pyrrolizidine amide and urea 5-HT₄ antagonists

Entry	R	5-HT ₄ Antagonism pA ₂ in rat TMM IA at 10 ⁻⁷ M
12d-i, 15b
12d		IA at 10 ⁻⁷ M
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

Table 3. Pyrrolizidine ester 5-HT₄ antagonists

<div style="display: flex; align-items: center; justify-content: center;"> <div style="margin-left: 10px;">13b-h</div> </div>					
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

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 , mpk, iv)	0.048	0.015	0.010	0.45
Solid Gastric Emptying (ED ₅₀ , mpk, iv)	0.03	Active, but full dose-response not performed	0.001	1.0

Table 5. Receptor profiling of compounds: EC50, nM [5-HT₄ agonism] or Ki, nM [all others]

Cmpd	5-HT ₄ agonism	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

Pyrrolizidine Esters and Amides as 5-HT₄ Agonists and Antagonists

Daniel P. Becker,^{*} Daniel L. Flynn,[§] Alan E. Moormann, Roger Nosal, Clara I. Villamil,[‡]

Richard Loeffler, Gary W. Gullikson,[†] Chafiq Moummi, Dai-C. Yang