2-9-2006

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

Daniel Becker  
*Loyola University Chicago*, dbecke3@luc.edu

Daniel L. Flynn

Alan E. Moormann

Roger Nosal

Author Manuscript
This is a pre-publication author manuscript of the final, published article.

Recommended Citation
Becker, Daniel; Flynn, Daniel L.; Moormann, Alan E.; and Nosal, Roger. Pyrrolizidine Esters and Amides as 5-HT4 Receptor Agonists and Antagonists. *Journal of Medicinal Chemistry*, 49, 3, 2006. Retrieved from Loyola eCommons, Chemistry: Faculty Publications and Other Works, [http://dx.doi.org/10.1021/jm0509501](http://dx.doi.org/10.1021/jm0509501)

© 2006 American Chemical Society

Creative Commons License

This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.
Pyrrolizidine Esters and Amides as 5-HT$_4$ 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.

Received
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, α₁, α₂ 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. 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 kidney⁴,⁵ Initial demonstration that the
gastrointestinal prokinetic benzamides cisapride and renzapride enhance contractile activity at neuronal 5-HT₄ receptors in the guinea pig ileum was made by Craig and Clarke. 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 esophagus and also the cholinergic stimulation of the ascending colon of the guinea pig. New tools including fluorescent antagonists have recently been developed for the study of 5-HT₄ receptors, and an excellent review of the 5-HT₄ receptor and key ligands was recently published.

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 and cognitive disorders. 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. Compound 1 had been marketed for motility disorders but was withdrawn due to QT prolongation. 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). 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. Compound 4, Prucalopride, accelerates colonic transit in healthy subjects but without modification of either gastric emptying or small bowel transit. In patients with severe constipation, the benzamide prucalopride elicited a dose-dependent effect on acceleration of the overall transit time.
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 12a³² 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
tropisetron\textsuperscript{34} and granisetron,\textsuperscript{34,35} particularly considering that tropisetron was shown to be the first surmountable antagonist of 5-HT\textsubscript{4} receptors\textsuperscript{3} and Buchheit demonstrated that the ester derivative of metoclopramide, SDZ 205-557, is a potent antagonist of 5-HT\textsubscript{4} receptors.\textsuperscript{36}

We were aware of the toxicity of the pyrrolizidine alkaloid natural products\textsuperscript{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. Based on this premise of structure-specificity we decided to explore the pyrrolizidine nucleus for incorporation into novel 5-HT\textsubscript{4} receptor agonists and antagonists. This paper details our work employing the pyrrolizidine scaffold resulting in the potent 5-HT\textsubscript{4} receptor agonist \textit{12a}, as well as 5-HT\textsubscript{4} receptor antagonist \textit{12h}, and other potent 5-HT\textsubscript{4} antagonists.

\textbf{Chemistry}

The (S,S)-carbinol (-)-trachelanthamidine \textit{9} was prepared by a diastereoselective alkylation of the chiral tin enolate of 3-(4-chlorobutyryl)-4-(S)-isopropyl-1,3-thiazolidine-2-thione \textit{7} with 5-acetoxy-2-pyrrolidinone as elegantly described by Yoshimitsu Nagao\textsuperscript{38} and summarized in Scheme 1. We analyzed the Mosher ester of \textit{9} by \textsuperscript{19}F NMR to confirm the enantiomeric purity which showed the material to be $\geq 99.1\%$ e.e.

Aminomethylpyrrolizidine \textit{11} was prepared by Mistunobu reaction of \textit{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 alkylation 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-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 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 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-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 30X 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 30X 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 mpk 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 12X 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 analog 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 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 22X 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 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. 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-aminobenzenamides 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 (pA2 = 6.75), whereas addition of the chlorine in the 6-position boosted potency of compound 12h33 by over an order of magnitude (pA2 = 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 (pA2 = 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 pA2 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 (EC50 >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 (pA2 = 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 (pA₂ = 7.15), and potency was markedly increased for the 5-fluoro derivative 13f in 5-HT₄ inhibitory potency (pA₂ = 8.5) as well as binding to the 5-HT₄ and 5-HT₃ receptors (Kᵢ = 700 pM and 10 nM, resp.). Indazole ester 13g had good inhibitory potency (pA₂ = 8.48) and the corresponding N-methyl indazole derivative 13h had comparable potency (pA₂ = 8.56). These indazoles exhibited potent subnanomolar binding to the 5-HT₄ receptor (Kᵢ = 800 pM and 400 pM, respectively), and moderate binding to the 5-HT₃ receptor (Kᵢ = 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, α₁, α₂, β₁, and β₂ 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 pA₂ value of 8.93, which is comparable in potency to the Glaxo antagonist GR-113808 (pA₂ = 9.39).

Indole 13d is a very potent dual receptor 5-HT₄/5-HT₃ ligand, with a Kᵢ = 183 pM at the 5-HT₄ receptor and Kᵢ = 5.0 nM at the 5-HT₃ receptor. This pyrrolizidine ester is free of binding at 5-HT₁, 5-HT₂, D₁, D₂, α₁, α₂, β₁, and β₂ receptors (Kᵢ > 10 uM).

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.\textsuperscript{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 ED50 = 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, 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 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.

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 (Ki = 5.2 nM). Compound 12a exhibits moderate binding at the 5-HT$_3$ receptor (Ki = 152 nM), but no detectible binding (Ki > 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, is a very potent antagonist at the 5-HT$_4$ receptor (Ki = 1.4 nM; pA$_2$ = 8.13) with excellent selectivity versus the other monoamine receptors tested (Ki > 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 (Ki = 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 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-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, non-mutagenic 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 Yoshimitsu Nagao\(^ {38}\) to afford 9 (3.12 g, 45.5%) as a colorless oil: \([\alpha]_D = +9.2 ~ (c = 0.195 \text{ g/dL in CHCl}_3; 10 \text{ cm}); [\alpha]_{365} = -63.1 ~ (c = 0.195 \text{ g/dL in CHCl}_3; 10 \text{ cm})\). The Mosher ester was prepared by treatment of the alcohol with the Mosher acid chloride [from (S)-(−)−\(\alpha\)-methoxy−\(\alpha\)-(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 was $\geq 99.6\%$ diastereomeric purity, or
$\geq 99.1\%$ e.e.

2-[(1S,7aS)-Hexahydro-1H-pyrrolizin-1-ylmethyl]-1H-isoinole-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 NH$_3$)/CHCl$_3$ (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$^{-1}$. [$\alpha$]$_D$ = -2.5°; [$\alpha$]$_{365}$ = -13.3° (c = 0.285 g/dL in chloroform). $^1$H
NMR (300 MHz, CDCl$_3$) $\delta$ 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$_3$) $\delta$ 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
C$_{16}$H$_{18}$N$_2$O$_2$·0.4H$_2$O C 69.24; H, 6.83; N, 10.09. Found C, 69.12; H, 6.56; N, 9.94.
HRMS calcd for C$_{16}$H$_{18}$N$_2$O$_2$ 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
16 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. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 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: $^1$H NMR (300 MHz, d$_4$-MeOD) $\delta$ 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, d$_4$-MeOD) $\delta$ 70.4, 54.4, 53.9, 42.5, 40.7, 29.8, 29.4, 24.3. HRMS MH$^+$ calcd for C$_8$H$_{16}$N$_3$ 141.1392, found 141.1394.

**4-Amino-5-chloro-N-[(1S-cis-hexahydro-1H-pyrrolizin-1-yl)methyl]-2-methoxybenzene carboxamide, 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% K$_2$CO$_3$ (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 NH$_3$)/CHCl$_3$ (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; $[\alpha]_D$ = -8.8° (c = 1.2 g/dL); IR (MIR) 3407, 3310, 1696, 1656 cm$^{-1}$. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 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 C$_{16}$H$_{24}$N$_3$O$_3$Cl 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. HRMS calcd for C$_{16}$H$_{24}$N$_3$O$_3$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. $\left[\alpha\right]_D = -10.6^\circ; \left[\alpha\right]_{365} = -36.7^\circ$ (c = 0.330 g/dL in chloroform). $^1$H 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 C$_{16}$H$_{22}$N$_3$O$_2$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, d₄-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). ¹³C NMR (75 MHz, d₄-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 C₁₆H₂₂N₃O₂Cl 323.1415, found 323,1400. Analysis calculated for C₁₆H₂₂N₃O₂Cl·HCl·0.75H₂O: 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. [α]D = -2.6°; [α]₃65 = -6.4° (c = 0.47g/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 acetamidine 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% K₂CO₃ 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 NH₃)/CHCl₃ (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 Nagao 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_{3}O_{2}Cl: 1.2HCl: 0.8H_{2}O: 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. [α]_D = +0.5°; [α]_{365} = +3.3° (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, d_{4}-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_{3}O_{2}Cl:HCl:0.5H_{2}O: 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 uL, 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: $^1$H NMR (300 MHz, d$_4$-MeOD) $\delta$ 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, d$_4$-MeOD) $\delta$ 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$_3$O$_2$: 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: C18 H23 N3 O Anal calcd for C$_{18}$H$_{23}$N$_3$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 acid$^{44}$ (190 mg, 1.0 mmol) was suspended in CHCl$_3$ (2 mL). Oxaly chloride (184 uL, 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 uL, 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$_2$CO$_3$, 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, d$_4$-MeOD) $\delta$ 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 2.1 (3H, m). Anal calcd for C₁₉H₂₅N₃O: 2.0 H₂O: C, 65.68; H, 7.25; N, 12.09. Found C, 65.79; H, 7.56; N, 12.03. MS calcd for C₁₉H₂₅N₃O: 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-lH-pyrrolizin-4(5H)-ylmethyl)-3-ethylimidazo[1,2-a]pyridine-l-carboxamide monohydrochloride (12f). 3-Ethylimidazo[1,5-a]pyridine-l-carboxylic acid⁴⁴ (190 mg, 1.0 mmol) was suspended in CHCl₃ (2 mL). Oxalyl chloride (184 uL, 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 CHCl₃ was added a solution of exo-tetrahydro-lH-pyrrolizin-4(5H)-methylamine 11 (140 mg, 1.0 mmol) and triethyl amine (279 uL, 2.0 mmol) in CHCl₃ (2 mL) and the mixture stirred for 18 h. The organic solution was washed with 1N NaOH, brine, dried over K₂CO₃, filtered and concentrated to give a crude oil which was chromatographed on silica gel eluting with 5% CH₃OH(NH₃)/CHCl₃ 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 C₁₈H₂₄N₄O.HCl:0.75H₂O 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 C₁₈H₂₄N₄O 312, found 312.

N-exo((4-s,7ααα)-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’-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 Et₃N (560 µL, 4.0 mmol) was added to the reaction mixture and 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 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 C₁₆H₂₀N₄O.2.0H₂O: 2.2 HCl: 0.25 CHCl₃ C: 46.12; H: 6.25; N: 13.24. Found: C: 46.22; H: 6.41; N: 13.43.

N-exo((4-S,7ααα)-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’-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 Et₃N (2.5 mL) was added to the reaction mixture and 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 with 0.25% NH₄OH. The filtrate was concentrated and the residue was dissolved in CHCl₃ 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, d₄-MeOD) 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 to 3.98 (1H, m), 3.82 to 3.75 (1H, m), 3.689 (2H, d, J= 6 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,
N-exo((4-s,7\(\alpha \alpha \alpha \alpha\)-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\(_2\)CO\(_3\) (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\(_2\)Cl\(_2\) containing 0.1% NH\(_4\)OH. The resulting solution was concentrated and the residue was suspended in Et\(_2\)O, 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\(_2\)Cl\(_2\) containing 0.1% NH\(_4\)OH) indicated that the starting material was consumed. The reaction mixture was concentrated and the residue was partitioned between CH\(_2\)Cl\(_2\) 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\(_2\)Cl\(_2\)/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, 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-chloroimidazo[1,2-a]pyridine-3-methyl-8-carboxylate (5.0 g, 50%) as a solid: IR: 1720 cm\(^{-1}\). 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. MS MH\(^{+}\) calcd for C_{10}H_9ClN_2O_2 225, 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 C_{9}H_7ClN_2O_2.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 C_{9}H_7ClN_2O_2 211, 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 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/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 C₂₇H₂₈N₂O₂ C: 55.26; H: 5.30; N: 18.41. Found: C: 54.90; H: 5.36; N: 18.26. Anal calcd for C₁₉H₂₆N₄O₂·1.25H₂O·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.  

(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(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. HRMS calcd for C$_{16}$H$_{21}$N$_2$O$_3$Cl 325.1319, found 325.1298. Anal calcd for C$_{16}$H$_{21}$N$_2$O$_3$Cl:HCl:0.25H$_2$O: C, 52.54; H, 6.06; N, 7.66; Cl, 19.39. Found C, 52.44; H, 6.06; N, 7.66; Cl, 19.18. $^1$H NMR (300 MHz, d$_4$-MeOD) $\delta$ 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, d$_4$-MeOD) $\delta$ 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 CHCl$_3$ (3X). The combined organic extracts were washed with water (2X) and brine and dried over Na$_2$SO$_4$. 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; 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 µL, 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, CD3OD) 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 C18H22N2O2 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
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, d$_4$-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 C$_{18}$H$_{22}$N$_2$O$_2$.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%.

(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 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 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%). \(^1\)H NMR (300 MHz, CD\(_3\)OD) \(\delta\) 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\(_2\)O\(_3\):HCl:0.33H\(_2\)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 \(\mu\)l, 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 C\(_{17}\)H\(_{19}\)N\(_2\)O\(_2\)F:HCl:0.33 H\(_2\)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. \(^1\)H NMR (300 MHz, CD\(_3\)OD) \(\delta\) 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·HCl: 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·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·H₂O: 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, 1H), 2.69 (m, 1H), 2.40 (m, 1H), 2.22 (m, 5H), 2.05 (m, 1H).

**cis-N-[(Hexahydro-1H-pyrrolizin-yl)methyl]-2,3-dihydro-3-(1-methylethyl)-2-oxo-lH-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-(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/CHCl$_3$ containing 0.25% NH$_4$OH. The product was rinsed from the silica with 9/95 NH$_4$OH/MeOH. The filtrate was concentrated and the residue was dissolved in CHCl$_3$ 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: $^1$H NMR (400 MHz, d$_4$-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 C$_{19}$H$_{26}$N$_4$O$_2$ 343, found 343. Anal calcd for C$_{19}$H$_{26}$N$_4$O$_2$.HCl.H$_2$O 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-one$^{55}$ (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$_2$CO$_3$ solution, dried over K$_2$CO$_3$, filtered and concentrated in vacuo to give crude desired product as an oil. Purification on silica gel eluting with 10% CH$_3$OH(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$_3$O$_2$:0.4H$_2$O 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$_3$O$_2$: 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: $^1$H NMR (400 MHz, d$_4$-MeOD) $\delta$ 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$_3$O$_2$.HCl:0.25H$_2$O 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$_3$O$_2$: 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$_2$Cl$_2$/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$_8$H$_6$N$_2$O$_2$.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<sub>9</sub>H<sub>7</sub>N<sub>2</sub>O<sub>2</sub>.HCl.H<sub>2</sub>O: 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 hydrochloride salt (6.3 g, 75.5%) as a solid: IR: 1679 and 3281 cm<sup>-1</sup>. Anal calcd for C<sub>8</sub>H<sub>5</sub>N<sub>2</sub>O<sub>2</sub>.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-2,5ββββ-methano-1H-3aS,3aαααα,6aαααα-cyclopenta[c]pyrrol-4α-yl)-2-methoxybenzamide, monohydrochloride (6, SC-52491). Compound 6 was prepared initially by synthesis involving resolution as previously described<sup>41</sup> then via an enantioselective synthesis commencing with an asymmetric Diels-Alder reaction.<sup>52</sup>

Serotonin 5-HT<sub>3</sub> Receptor Binding

Displacement of [<sup>3</sup>H]-GR65630 from brain cortex obtained from male Wistar rats was done by the method of Kilpatrick.<sup>56</sup> Cortical membrane preparations (0.04 mg) were incubated with 0.2 nM [<sup>3</sup>H]-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 $[^3\text{H}]-\text{GR65630}$ to the 5-HT$_3$ receptor.

**Serotonin 5-HT$_4$ Receptor Binding**

Serotonin 5-HT$_4$ receptor binding in guinea pig striatum utilizing $[^3\text{H}]-\text{GR113},808$ 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: $[^3\text{H}]-5\text{HT}$ for 5-HT$_1$-like receptors; $[^3\text{H}]-\text{ketanserin}$ for 5-HT$_2$ receptors; $[^3\text{H}]-\text{SCH23390}$ for D$_1$ receptors; $[^3\text{H}]-\text{spiperone}$ for D$_2$ receptors; and $[^3\text{H}]-\text{prazosin}$ for $\alpha_1$-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.$^{2,57}$ 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 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 (tunica muscularis mucosae) 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.$^{39}$ Cumulative dose-
response curves for agonists interacting with 5-HT$_4$ 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 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 to 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\textsubscript{50} values were calculated as the concentrations causing 50\% of this maximal effect. EC\textsubscript{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\textsubscript{2} value ± S.E.M. of the antagonist.\textsuperscript{58} The pA\textsubscript{2} value for single antagonist concentrations in individual tissues was also calculated using the method of MacKay: pA\textsubscript{2} = -\log (antagonist concentration [mol/l]) + \log (DR-1). A mean pA\textsubscript{2} value ± S.E.M. was calculated from the individual pA\textsubscript{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,\textsuperscript{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.\textsuperscript{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 α2-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 \( \alpha_2 \)-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
descrining 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 \( \alpha_2 \)-
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 µg 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.

References


42. Dumuis, A.; Sebben, M.; Monferini, E.; Nicola, M.; Turconi, M.; Ladinsky, H.; Bockaert, J. Azabicycloalkyl benzimidazolone derivatives as a novel class of potent


**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 Nagao$^{38}$
Scheme 2. Preparation of pyrrolizidine amides 12a-i

\[ \text{ArCO}_2\text{H} \xrightarrow{1) \text{CDI}} \text{ArNH} \xrightarrow{2) \text{11}} \]

\[ \text{10} \quad R = \text{NPhth} \]
\[ \text{11} \quad R = \text{NH}_2 \]
Scheme 3. Preparation of pyrrolizidine esters 13a-h

ArCO₂H 1) CDI, DMF 2) NaH, 9 → Ar CO₂H

13a-h
Scheme 4. Preparation of benzimidazolone 15a and oxindole 15b

14a $X = \text{N-i-Pr}$  
14b $X = \text{C(CH}_3\text{)}_2$  
15a $X = \text{N-i-Pr}$  
15b $X = \text{C(CH}_3\text{)}_2$
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-HT$_4$ 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-HT$_4$ agonists

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>5-HT$_4$ Binding ($K_i$, nM)</th>
<th>5-HT$<em>4$ Agonism EC$</em>{50}$, nM in Rat TMM</th>
<th>5-HT$_3$ Binding ($K_i$, nM)</th>
<th>B-J Reflex: 5HT$_3$ Antagonism, % Inhib at mpk ip</th>
<th>Other Receptors (IC$_{50}$, nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12a</td>
<td>ClH$<em>2$NO$</em>{Me}$</td>
<td>5.2 (0.5)</td>
<td>16.5 (3.8)</td>
<td>152</td>
<td>87% at 10</td>
<td>&gt;10K for 5HT$_1$, 5HT$_2$, D$_1$, D$_2$, α$_1$, α$_2$, β$_1$, β$_2$</td>
</tr>
<tr>
<td>ent-12a</td>
<td>ClH$<em>2$NO$</em>{Me}$</td>
<td>18% at 500 nM</td>
<td>323 (46)</td>
<td>66 nM</td>
<td>87% at 10</td>
<td>89% at 10</td>
</tr>
<tr>
<td>12b</td>
<td>ClH$<em>2$NO$</em>{Et}$</td>
<td>...</td>
<td>195</td>
<td>25nM</td>
<td>89% at 10</td>
<td>D$_2$ 5,300</td>
</tr>
<tr>
<td>12c</td>
<td>ClH$<em>2$NO$</em>{Me}$</td>
<td>0.350 (0.050)</td>
<td>129 (10)</td>
<td>...</td>
<td>16% at 1000</td>
<td>...</td>
</tr>
<tr>
<td>13a</td>
<td>ClH$<em>2$NO$</em>{Me}$</td>
<td>0.183 (0.033)</td>
<td>1.5 (0.2)</td>
<td>$K_i = 51$ (5)</td>
<td>0% at 10</td>
<td>D$_2$ &gt;10K</td>
</tr>
<tr>
<td>15a</td>
<td>ClH$<em>2$NO$</em>{Me}$</td>
<td>7.0 (1.5)</td>
<td>374.2 (171.7)</td>
<td>20.2</td>
<td>87% at 10</td>
<td>87% at 10</td>
</tr>
</tbody>
</table>

---

61
### Table 2. Pyrrolizidine amide and urea 5-HT\textsubscript{4} antagonists

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>5-HT\textsubscript{4} Antagonism</th>
</tr>
</thead>
<tbody>
<tr>
<td>12d</td>
<td></td>
<td>pA\textsubscript{2} in rat</td>
</tr>
<tr>
<td>12d</td>
<td></td>
<td>TMM</td>
</tr>
<tr>
<td>12d</td>
<td></td>
<td>IA at 10\textsuperscript{-7} M</td>
</tr>
<tr>
<td>12e</td>
<td></td>
<td>IA @ 10\textsuperscript{-6} M</td>
</tr>
<tr>
<td>12f</td>
<td></td>
<td>6.56</td>
</tr>
<tr>
<td>12g</td>
<td></td>
<td>6.75 (0.05)</td>
</tr>
<tr>
<td>12h</td>
<td></td>
<td>8.13 (0.06)</td>
</tr>
<tr>
<td>12i</td>
<td></td>
<td>7.09 (0.08)</td>
</tr>
<tr>
<td>15b</td>
<td></td>
<td>7.0</td>
</tr>
</tbody>
</table>
Table 3. Pyrrolizidine ester 5-HT$_4$ antagonists

<table>
<thead>
<tr>
<th>Entry</th>
<th>R</th>
<th>5-HT$_4$ Binding, (K$_i$, nM)</th>
<th>5-HT$_4$ Antagonism: pA$_2$ in rat TMM</th>
<th>5-HT$_3$ binding $K_i$, nM (SEM)</th>
<th>5-HT$_1$, 5-HT$_2$, D$_1$, D$_2$, $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$ binding $K_i$, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>13b</td>
<td>Cl</td>
<td>...</td>
<td>6.75</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>13c</td>
<td></td>
<td>...</td>
<td>IA at 10$^{-7}$ M</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>13d</td>
<td></td>
<td>0.183 (0.017)</td>
<td>8.93 (0.07)</td>
<td>5.0 (1.0)</td>
<td>&gt;10K</td>
</tr>
<tr>
<td>13e</td>
<td></td>
<td>...</td>
<td>7.15</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>13f</td>
<td></td>
<td>0.70 (0.15)</td>
<td>8.50</td>
<td>10 (1)</td>
<td>...</td>
</tr>
<tr>
<td>13g</td>
<td></td>
<td>0.80 (0.83)</td>
<td>8.48</td>
<td>135 (5)</td>
<td>&gt;10K</td>
</tr>
<tr>
<td>13h</td>
<td></td>
<td>0.40 (0.033)</td>
<td>8.56</td>
<td>20 (2)</td>
<td>&gt;10K</td>
</tr>
</tbody>
</table>
Table 4. Comparison of enantiomers 12a and ent-12a with 1 and 2.

<table>
<thead>
<tr>
<th>Assay</th>
<th>1</th>
<th>2</th>
<th>12a</th>
<th>ent-12a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT(<em>4) Agonism Rat TMM (EC(</em>{50}), nM)</td>
<td>55±8</td>
<td>98±14</td>
<td>17 ±4</td>
<td>323±46</td>
</tr>
<tr>
<td>Antral Contractility in Fasted Dog (ED(_{50}), mpk, iv)</td>
<td>0.048</td>
<td>0.015</td>
<td>0.010</td>
<td>0.45</td>
</tr>
<tr>
<td>Solid Gastric Emptying (ED(_{50}), mpk, iv)</td>
<td>0.03</td>
<td>Active, but full dose-response not performed</td>
<td>0.001</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Table 5. Receptor profiling of compounds: EC50, nM [5-HT$_4$ agonism] or Ki, nM [all others]

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>5-HT$_4$ agonism</th>
<th>5-HT$_4$</th>
<th>5-HT$_1$</th>
<th>5-HT$_2$</th>
<th>5-HT$_3$</th>
<th>D$_1$</th>
<th>D$_2$</th>
<th>$\alpha_1$</th>
<th>$\alpha_2$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54.7</td>
<td>17</td>
<td>&gt;1K</td>
<td>6.1</td>
<td>134</td>
<td>1700</td>
<td>227</td>
<td>30</td>
<td>4500</td>
<td>&gt;10K</td>
</tr>
<tr>
<td>12a</td>
<td>16.5</td>
<td>5.2</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>152</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
</tr>
<tr>
<td>12h</td>
<td>&gt;10K</td>
<td>1.4</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>259</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>29</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>1.2</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
<td>&gt;10K</td>
</tr>
</tbody>
</table>
Pyrrolizidine Esters and Amides as 5-HT$_4$ Agonists and Antagonists

Daniel P. Becker,* Daniel L. Flynn,§ Alan E. Moormann, Roger Nosal, Clara I. Villamil,‡
Richard Loeffler, Gary W. Gullikson, † Chafiq Moumni, Dai-C. Yang

\[
\begin{align*}
\text{Ar} & \text{O} \quad \text{Ar} & \text{O} \\
\text{N} & \text{H} & \text{N} & \text{H}
\end{align*}
\]