Registry No. 1, 6836-22-2; 2, 138113-07-2; 3, 138113-08-3; 4, 139525-77-2; 5, 138112-76-2; 6, 138112-79-5; 7, 138112-99-9; 8, 138112-80-8; 9, 139525-78-3; 10, 139525-79-4; 11, 138112-78-4; 12, 139525-80-7; 13, 138113-03-8; 14, 138113-13-0; 15, 138113-02-7; 16, 138112-77-3; 17, 138112-87-5; 18, 138112-91-1; 19, 138112-95-5; 20, 139564-01-5; $CH_3CH{=}CHC(O)Cl$, 10487-71-5; $CH_3C(O)Cl$, 75-36-5; $CH_3CH_2C(O)Cl$, 79-03-8; $CH_3(CH_2)_2C(O)Cl$, 141-75-3; $CH_3(CH_2)_3C(O)Cl$, 638-29-9; $CH_3(CH_2)_4C(O)Cl$, 142-61-0; $CH_3(CH_2)_5C(O)Cl$, 2528-61-2; $(CH_3)_2CHC(O)Cl$, 79-30-1; c-$C_6H_{11}C(O)Cl$, 2719-27-9; $PhCH_2C(O)Cl$, 103-80-0; PhC(O)Cl, 98-88-4; cyclopropanecarbonyl chloride, 4023-34-1; 5-methoxytryptamine, 608-07-1; melatonin, 73-31-4; cyclobutanecarbonyl chloride, 5006-22-4; 3,5-dichlorobenzoyl chloride, 2905-62-6; 1*H*-indole-2-carbonyl chloride, 58881-45-1.

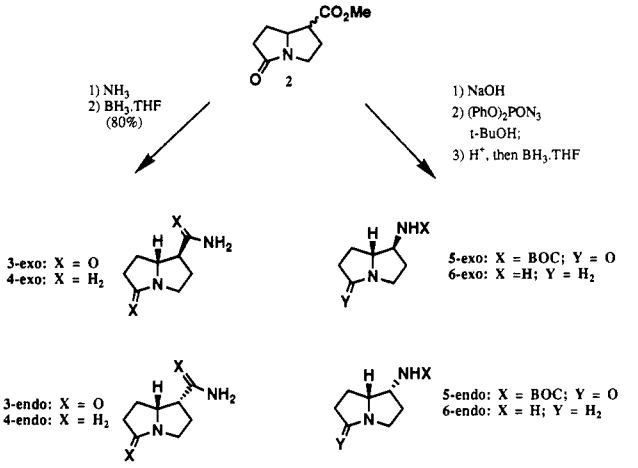
SC-53116: The First Selective Agonist at the Newly Identified Serotonin 5-HT_4 Receptor Subtype†

Serotonin acts as a neurotransmitter, neuromodulator, and hormone in mammals, and it is known to exhibit profound pharmacological activities in the central nervous system, autonomic nervous system, enteric nervous system, and cardiovascular system.[1] Among monoamine neurotransmitters, serotonin is unsurpassed in the number of receptor subtypes identified. Until recently, serotonin was thought to act through receptors subtyped as 5-HT_1, 5-HT_2, and 5-HT_3. Furthermore, even these subtypes have been subclassed to now include 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{3A}, 5-HT_{3B}, and 5-$HT3_{3C}$.[2]

Metoclopramide (Reglan) is a gastrointestinal agent which has been known for some time. Its clinical utilities as an antiemetic and as an upper GI prokinetic agent were originally thought to be due to antagonism at dopamine D_2 receptors (K_i = 113 nM). However, more recently it has been identified as a serotonin 5-HT_3 antagonist (K_i = 240 nM); it is now well established that its utility as an antiemetic (emesis secondary to cytotoxic drugs) is due to its 5-HT_3 antagonist properties.[3,4] Indeed, its interaction with dopamine D_2 receptors is viewed as a clinical liability, causing increased prolactin release and extrapyramidal-like symptoms in medical practice. Regarding utility as a gastrointestinal prokinetic agent, however, interaction with serotonin 5-HT_3 receptors does not adequately explain metoclopramide's activity. Another significant gastroprokinetic agent, cisapride, is believed to act via a serotonergic mechanism in the enteric nervous system. However, its activity does not correlate with interaction at 5-HT_1, 5-HT_2, or 5-HT_3 receptors.[5]

Very recently, Bockaert[6] and Clarke[7] independently reported a new serotonin receptor subtype (5-HT_4) in brain and gut tissues, respectively. Serotonin is quite potent (EC_{50} = 109 nM,[6] 2.8 nM[7]) as an agonist at this receptor, which is positively coupled to adenylate cyclase. Bockaert et al. reported that the gastroprokinetic activity of several agents, including metoclopramide, zacopride, cisapride, and renzapride, could be correlated with agonist activity at this 5-HT_4 receptor.[8] Clarke et al. have described several functional models for the 5-HT_4 receptor in gut tissues,[9,10] and the activities of gastroprokinetic agents were attributed to agonism at this new serotonin receptor.[11] Regarding CNS pharmacology of the 5-HT_4 receptor, Bockaert has identified functional 5-HT_4 receptors in the guinea pig hippocampus,[12] and Boddeke and Kalkman have demonstrated that activation of 5-HT_4 receptors in rat hippocampal regions induce an increase in EEG energy.[13] The design of agents selectively potent

Scheme I. Preparation of Racemic Pyrrolizidine Side Chains



† Dedicated to Professor Lester A. Mitscher on the occasion of his sixtieth birthday.

(1) For a timely review, see: *Serotonin Receptor Subtypes: Basic and Clinical Aspects*; Wiley-Liss, Inc.: New York, 1991.
(2) Peroutka, S. J. *The Molecular Pharmacology of 5-Hydroxyamine Receptor Subtypes. Serotonin Receptor Subtypes: Basic and Clinical Aspects*; Wiley-Liss, Inc.: New York, 1991; pp 65–80.
(3) Miner, W. D.; Sanger, G. J.; Turner, D. H. Evidence that 5-Hydroxytryptamine$_3$ Receptors Mediate Cytotoxic Drug and Radiation-evoked Emesis. *Br. J. Cancer* **1987**, 159–162.
(4) Andrews, P. L. R.; Rapeport, W. G.; Sanger, G. J. Neuropharmacology of Emesis Induced by Anti-cancer Therapy. *Trends Pharmacol. Sci.* **1988**, *9*, 334–341.
(5) Schuurkes, J. A. J.; Van Nueten, J. M. 5-HT3 Serotonergic Mechanisms and Stimulation of Gastrointestinal Motility by Cisparide. *Gastroenterology* **1988**, *94*, A415.
(6) Dumuis, A.; Bouhelal, R.; Sebben, M.; Cory, R.; Bockaert, J. A Non-classical 5-Hydroxytryptamine Receptor Positively Coupled with Adenylate Cyclase in the Central Nervous System. *Mol. Pharmacol.* **1988**, *34*, 880–887.
(7) Craig, D. A.; Clarke, D. E. Pharmacological Characterization of a Neuronal Receptor for 5-Hydroxytryptamine in Guinea Pig Ileum with Properties Similar to the 5-Hydroxytryptamine$_4$ Receptor. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 1378–1386.
(8) Dumuis, A.; Sebben, M.; Bockaert, J. The Gastrointestinal Prokinetic Benzamide Derivatives are Agonists at the Non-Classical 5-HT Receptor (5-HT4) Positively Coupled to Adenylate Cyclase in Neurons. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1989**, *340*, 403–410.
(9) Craig, D. A.; Eglen, R. M.; Walsh, L. K. M.; Perkins, L. A.; Whiting, R. L.; Clarke, D. E. 5-Methoxytryptamine and 2-Methyl-5-Hydroxytryptamine Induced Desensitization as a Discriminative Tool for the 5-HT3 and Putative 5-HT4 Receptors in Guinea Pig Ileum. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1990**, *342*, 9–16.
(10) Baxter, G. S.; Craig, D. A.; Clarke, D. E. 5-Hydroxytryptamine$_4$ Receptors Mediate Relaxation of the Rat Oesophageal Tunica Muscularis Mucosae. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 439–446.
(11) Craig, D. A.; Clarke, D. E. Peristalsis Evoked by 5-HT and Renzapride: Evidence for Putative 5-HT4 Receptor Activation. *Br. J. Pharmacol.* **1991**, *102*, 563–564.
(12) Bockaert, J.; Sebben, M.; Dumuis, A. Pharmacological Characterization of 5-Hydroxytryptamine4 (5-HT4) Receptors Positively Coupled to Adenylate Cyclase in Adult Guinea Pig Hippocampal Membranes: Effect of Substituted Benzamide Derivatives. *Mol. Pharmacol.* **1990**, *37*, 408–411.
(13) Boddeke, H. W. G. M.; Kalkman, H. O. Zacopride and BRL-24924 Induce an Increase in EEG-Energy in Rats. *Br. J. Pharmacol.* **1990**, *101*, 281–284.

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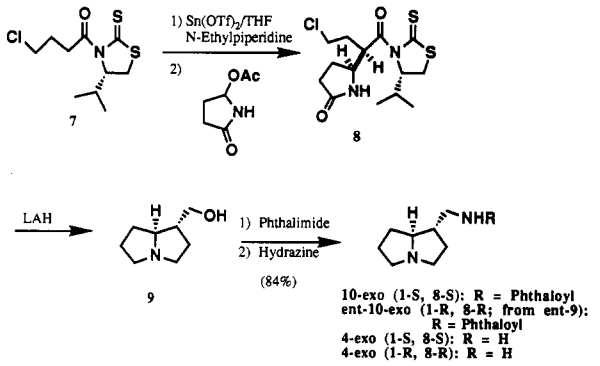
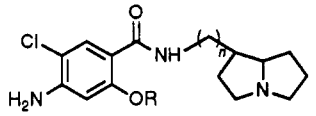
Scheme II. Preparation of Enantiopure Pyrrolizidine Side Chains



for this 5-HT_4 receptor may therefore provide new therapies for both central nervous system and gastrointestinal diseases.

Currently available drug candidates (cisapride, renzapride, zacopride, metoclopramide), while being agonists at the 5-HT_4 receptor, are either modestly active (metoclopramide, zacopride) or nonselective (renzapride, cisapride) due to potent interactions at other monoamine receptors (vide infra). Regarding our efforts to discover selective serotonin 5-HT_4 agonists, *we report herein the discovery of substituted pyrrolizidines 1 [specifically SC-53116; 1B] which surpass the potency and selectivity of other benzamides (metoclopramide, zacopride, renzapride, and cisapride) and the benzimidazolone (BIMU-8) as agonists at the newly described serotonin 5-HT_4 receptor.* Additionally, structure–activity relationships have been developed in this series whereby chemical manipulation allows either 5-HT_4 agonism or 5-HT_3 antagonism to be selectively enhanced.

From a design perspective, we utilized the principle of conformational restriction to produce agents more potent and selective than the weak 5-HT_4 agonist metoclopramide. Among several candidate series, the substituted pyrrolizidines 1 emerged as very promising.



SC-53116 is **1B** (1-*S*,8-*S* isomer; $n = 1$; R = Me)

Schemes I–III illustrate the synthetic routes used to prepare the substituted pyrrolizines 1. The diastereomeric pyrrolizidine esters **2** were prepared by the method of Celerier et al.[14] Racemic substituted pyrrolizidines **4** and **6** were prepared as illustrated in Scheme I. Pyrrolizidine ester **2** was utilized in one of two ways. Amidation (NH_3, MeOH) gave rise to diastereomeric amides **3-exo** and **3-endo**, which were separated by flash chromatography and individually reduced with BH_3 to afford the (pyrrolizidin-1-ylmethyl)amines **4-exo** and **4-endo**. Alternatively the diastereomeric esters **2** were saponified and then treated with diphenyl phosphorazidate/*t*-BuOH to give rise to the rearranged BOC-amines **5-exo** and **5-endo**, which were separated by chromatography. BOC removal (trifluoroacetic acid) followed by reduction (BH_3) afforded the desired pyrrolizidin-1-amines **6-exo** and **6-endo**.

Enantiopure **4-exo** was prepared as illustrated in Scheme II. Trachelanthamidine (**9**) and its enantiomer **ent-9** were prepared in >99% optical purity by the method of Nagao.[15] Treatment of **9** or **ent-9** with phthalimide/Ph_3P/DEAD (Mitsunobu reaction) afforded the phthalimides **10** or **ent-10**. Hydrazinolysis gave **4-exo**(1-*S*,8-*S*) and **4-exo**(1-***R***,8-***R***), respectively. Coupling of racemic or enantiopure **4** or **6** with benzoic acid **11** gave rise to the target compounds **1** as shown in Scheme III.

Scheme III. Coupling of Pyrrolizidine Side Chains with Benzoic Acid 11

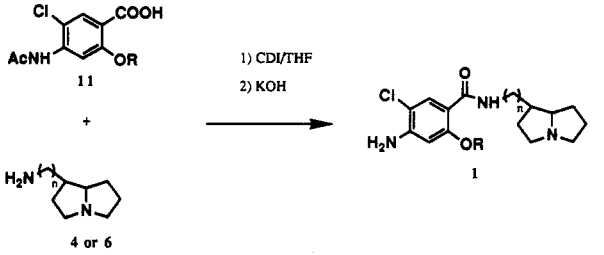
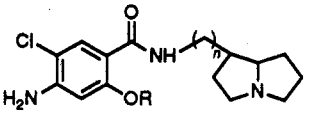


Table I. Serotonin 5-HT_4 Agonist and 5-HT_3 Antagonist Activities of Substituted Pyrrolizidines 1[a]



1

entry	R	n	stereochem	5-HT4 agonism: ED_{50}, nM (SEM)	5-HT3 binding: K_i, nM (SEM)
serotonin				16 (2)	390 (17)
metoclopramide				24,100 (6246)	240 (5)
1A	Me	1	exo (racemic)	64 (1)	104 (14)
1B	Me	1	exo (1-*S*,8-*S*)	23 (3)	152 (1)
1C	Me	1	exo (1-*R*,8-*R*)	323 (46)	66 (7)
1D	Et	1	exo (racemic)	1377 (747)	25 (3)
1E	Me	1	endo (racemic)	280 (23)	278 (24)
1F	Me	0	exo (racemic)	187 (63)	14 (2)
1G	Et	0	exo (racemic)	2270 (764)	8 (1)
1H[b]		0	exo (racemic)	>10000	>200
1J	Me	0	endo (racemic)	3450 (1190)	96 (5)
1K	Et	0	endo (racemic)	>10000	99 (9)

[a] Assay conditions: 5-HT_4 agonism: ability of compounds to relax carbachol-induced contractions of rat tunica muscularis mucosae (see text for experimental details). ED_{50} values for each compound represents 50% of its own maximum. 5-HT_3 binding: binding was determined using brain cortices from male rats and ^{3}H-GR65630 (0.2 nM) as the radioligand. Nonspecific binding determined in the presence of 1 μM ICS-205930. [b] Compound **1H** has all aromatic ring substituents = hydrogen.

Serotonin 5-HT_4 agonism was measured in the rat esophagus in vitro preparation as reported by Baxter et al.[10] Briefly, 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 were removed. The inner tunica muscularis mucosa was mounted under 0.2–0.3 g of tension in a tissue bath containing oxygenated Tyrode's solution at 37 °C. Corticosterone 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.

(14) Celerier, J. P.; Haddad, M.; Jacoby, D.; Lhommet, G. Heterocyclization of Primary Amines with Highly Activated Cyclopropanes: A New Route to Isoretronecanol. *Tetrahedron Lett.* **1987**, *28*, 6597.

(15) Nagao, Y.; Dai, W. M.; Ochiai, M.; Tsukagoshi, S.; Fujita, E. Highly Diastereoselective Alkylation of Chiral Tin(II) Enolates onto Cyclic Acyl Imines. An Efficient Asymmetric Synthesis of Bicyclic Alkaloids Bearing a Nitrogen Atom Ring Juncture. *J. Org. Chem.* **1990**, *55*, 1148–1156.

Table II. Receptor Profiling of SC-53116 and Other Reported 5-HT$_4$ Agonists: ED_{50} (5-HT$_4$) or K_i (All Others) Values (nM)

entry	5-HT$_4$ ED_{50}	5-HT$_4$ %E[a]	5-HT$_3$	5-HT$_1$	5-HT$_2$	D$_1$	D$_2$	α_1	α_2	β
SC-53116 (1B)	23.7 (4.9)	93	152	>10000	>10000	>10000	>10000	>10000	>10000	>10000
cisapride	54.7 (0.8)	105	134	>10000	6.1	1700	227	30	4500	>10000
renzapride	44.0 (6.9)	98	5.3	>10000	>10000	>10000	>10000	>10000	>10000	>10000
zacopride (S)-isomer	173.0 (4.0)	93	0.23	>10000	>10000	>10000	>10000	>10000	>10000	>10000
zacopride (R)-isomer	505.0 (73.0)	96	8.0	>10000	415	>10000	>10000	>10000	>10000	>10000
BIMU-8	40.2 (5.5)	>111	7.0	>10000	>10000	>10000	>10000	>10000	>10000	>10000

[a] %E relative to serotonin = 100%. Tissues and ^{3}H-radioligands used in binding assays: 5-HT$_3$ (rat cortex; GR65630); 5-HT$_1$ (rat cortex; 5-HT); 5-HT$_2$ (rat whole brain; ketanserin); D$_1$ (rat striata; SCH 23390); D$_2$ (rat striata; domperidone); α_1 (rat whole brain; prazosin); α_2 (rat cortex; rauwolscine); β (rat whole brain; dihydroal prenolol).

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. ED_{50} values were obtained for each agent in tissues from five rats.

Serotonin acts at this 5-HT$_4$ receptor to cause relaxation of carbachol precontracted smooth muscle strips. Serotonin exhibits potent agonism (ED_{50} = 16 nM) in this preparation, while metoclopramide exhibits only weak agonism (ED_{50} = 24100 nM). See Table I. We have found that the racemic benzamide **1A** is very potent as a 5-HT$_4$ agonist in this model, exhibiting an ED_{50} of 64 nM. The potency of **1A** is quite comparable to that found for the substituted benzamides cisapride (ED_{50} = 55 nM) and renzapride (ED_{50} = 44 nM) and the benzimidazolone BIMU-8 (ED_{50} = 40 nM), while being decidedly more potent than the individual (*S*)- or (*R*)-enantiomers of zacopride (ED_{50} = 173 and 505 nM, respectively). Moreover, the 1-*S*,8-*S* enantiomer **1B** is even more potent (ED_{50} = 23 nM) at the 5-HT$_4$ receptor, rivaling the potency of serotonin itself. By contrast, the 1-*R*,8-*R* enantiomer **1C** is less active at the 5-HT$_4$ receptor (Table I, ED_{50} = 323 nM).

1B is a more potent agonist at the 5-HT$_4$ receptor than are cisapride, renzapride, (*S*)- and (*R*)-zacopride, and BIMU-8 in this rat tunica muscularis mucosae preparation (Table II). Like cisapride, renzapride, and zacopride, compound **1B** is a full agonist in this preparation (93% of the maximum efficacy of serotonin). ICS-205930 acted as a weak competitive antagonist, exhibiting pA_2 values of 6.3 and 6.4 when evaluated against SC-53116 and serotonin, respectively.[16] These values are consistent with other published pA_2 values for ICS-205930 in this[10] and other[7,8] 5-HT$_4$ functional assays.

Hadley et al.[17] have reported conformationally restricted quinolizidine substituted benzamides which increased basal intragastric pressure in rats. In particular, BRL-20627 exhibited activity comparable to that of metoclopramide. Dumuis et al.[8] have recently reported that BRL-20627 and metoclopramide are of comparable modest potency (pEC_{50} = 5.4) compared to cispapride (7.14), renzapride (6.90), and zacopride (5.95) in the 5-HT$_4$ functional model in mouse colliculi neurons. The present study suggests that the pyrrolizidine (SC-53116) nucleus is superior to the quinolizidine nucleus for conferring serotonin 5-HT$_4$ agonism in the benzamide series.

Many of the earlier reported benzamides (metoclopramide, zacopride, renzapride) had been reported to possess serotonin 5-HT$_3$ antagonist properties. Therefore, we investigated the structure–activity relationships for both 5-HT$_4$ agonism and 5-HT$_3$ antagonism in this pyrrolizidine series. Compounds **1A–C** all exhibit weak binding constants in a 5-HT$_3$ radiolabeled binding assay[18] (K_i's ranging from 66 to 152 nM; Table I). **1B**, while exhibiting an ED_{50} of 23.7 nM in the 5-HT$_4$ functional assay, exhibits a K_i of 152 nM in binding studies at the 5-HT$_3$ receptor. Consistent with this binding data, **1B** only weakly antagonizes the 5-HT$_3$-mediated bradycardic response in mice (vide infra).

Modest structural manipulation of **1A** leads to profound effects on activity. Thus substitution of an ethyl ether for the methyl ether on the aromatic nucleus (**1D**) produces divergent effects on 5-HT$_4$ and 5-HT$_3$ affinity. While **1D** is 22-fold less potent than **1A** as a 5-HT$_4$ agonist, it is approximately 4-fold more potent as a 5-HT$_3$ ligand. A similar trend toward reduction in 5-HT$_4$ agonism was observed for **1F** vs **1G** and **1J** vs **1K**. It appears that increased steric bulk on the ortho ether of the aromatic ring is deleterious to 5-HT$_4$ agonism, while 5-HT$_3$ affinity is either increased or unaffected. That the presence of the fully substituted aromatic ring is essential for activity is suggested by compound **1H** which is virtually inactive in both 5-HT$_4$ and 5-HT$_3$ assays.

In addition, we observed that the pyrrolizidin-1-amine analogues **1F–K** (n = 0) are less active in the 5-HT$_4$ functional assay than the (pyrrolizidin-1-ylmethyl)amine analogues **1A–E** (n = 1), while the reverse SAR trend is observed for 5-HT$_3$ binding affinity. Indeed, selective 5-HT$_3$ antagonists could be produced by combining these features (overall length and ether substitution pattern). Compound **1G** (SC-52246) is a quite potent 5-HT$_3$ antagonist (K_i = 8 nM) while being very weak in the 5-HT$_4$ assay (ED_{50} = 2270 nM).

As stated above, a second objective of this effort was to produce a potent 5-HT$_4$ agonist which was devoid of activity at other monoamine receptors. *Table II illustrates the selectivity exhibited by 1B for the serotonin 5-HT$_4$ receptor.* In addition to showing selectivity in 5-HT$_4$ vs 5-HT$_3$ affinity, **1B** is inactive at concentrations up to 10 μM in binding studies at 5-HT$_1$, 5-HT$_2$, dopamine D$_1$, D$_2$, α_1, α_2, and β adrenergic monoamine receptors. By contrast, cisapride exhibits potent receptor interactions at the serotonin 5-HT$_2$ (K_i = 6.1 nM) and α_1 adrenergic (K_i = 30 nM) receptors. Additionally, cisapride interacts modestly with dopamine D$_2$ sites (K_i = 227 nM). While renzapride (*S*)-zacopride and BIMU-8 are without effect at 5-HT$_1$, 5-HT$_2$, dopaminergic, and adrenergic receptors, they exhibit potent receptor interactions at the serotonin 5-HT$_3$ subtype (K_i's = 5.3, 0.23, and 7.0 nM, respectively). This

(16) Gullikson, G. W. *Eur. J. Pharmacol.*, in press.

(17) Hadley, M. S.; King, F. D.; McRitchie, B.; Turner, D. H.; Watts, E. A. Substituted Benzamides with Conformationally-Restricted Side Chains. 1. Quinolizidine Derivatives as Selective Gastric Prokinetic Agents. *J. Med. Chem.* **1985**, *28*, 1843–1847.

(18) The assay used is essentially that of Kilpatrick. See Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. Identification and Distribution of 5-HT3 Receptors in Rat Brain Using Radioligand Binding. *Nature* **1987**, *330*, 746–748.

binding interaction translates functionally to antagonism of the Bezold–Jarisch reflex in vivo. Thus in mice, renzapride, zacopride, and BIMU-8 antagonized the 5-HT_3 receptor-mediated bradycardic response to serotonin with ED_{50} values of 0.25, 0.005, and 0.01 mg/kg ip. By contrast, the pyrrolizidine **1B** exhibited an ED_{50} of 3.0 mg/kg ip, consistent with the modest K_i of 152 nM in the 5-HT_3 binding assay.

In conclusion, the substituted pyrrolizidines **1** afford a novel series of potent and selective 5-HT_4 agonists. **1B** is the most potent and selective agent yet reported for agonism at this newly identified serotonin receptor. While exhibiting potency (ED_{50} = 23 nM) similar to serotonin in the 5-HT_4 rat esophagus model, it is only modestly active at the 5-HT_3 receptor (K_i = 152 nM) compared to zacopride (0.23 nM), renzapride (5.3 nM), and BIMU-8 (7.0 nM). Moreover, unlike metoclopramide and cisapride, **1B** exhibits little affinity (IC_{50} > 10000 nM) for dopamine D_2, 5-HT_2, or α_1 adrenergic receptors. The in vitro 5-HT_4 agonist property of **1B** correlates well with potent gastrointestinal prokinetic activity in vivo, the results of which will be reported in due course. **1B** will undoubtedly be a useful agent for probing the role of 5-HT_4 receptors in various CNS and gastrointestinal diseases.

Supplementary Material Available: Physical data for intermediates and final products (7 pages). Ordering information is given on any current masthead page.

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Bis(maltolato)oxovanadium(IV) Is a Potent Insulin Mimic

Diabetes is a mammalian condition in which the amount of glucose in the blood plasma is abnormally high.[3] The condition can be acutely life-threatening and, in addition, patients with diabetes suffer from a number of secondary complications, for example, atherosclerosis, microangiopathy, renal disease and failure, cardiac disease, and diabetic retinopathy and other ocular disorders including blindness. Millions of sufferers control diabetes by daily insulin administration and/or diet. Insulin replacement is the easiest method of controlling chronic diabetes; however, insulin is not orally active and must be taken by injection. There is great interest in orally active insulin mimics, particularly vanadium compounds.[4]

In the late 1970s sodium orthovanadate (Na_3VO_4, vanadium(V)) was found by Cantley and co-workers to be a potent inhibitor of Na^+,K^+-ATPase.[5] Shortly thereafter, the same group showed that vanadate taken up by red blood cells was reduced to vanadium(IV) (the vanadyl ion $[V{=}O]^{2+}$) in the cytoplasm.[6] Since then, there has been significant research on the glycosidic function of vanadium (mostly as vanadate) because cardiac glycosides are known to inhibit specifically Na^+,K^+-ATPase. A natural outgrowth of this has been the study of vanadium and diabetes.[4] The insulin-like effect of vanadate ion (VO_4^{3-}) has been known since 1980[7] and is currently under active investigation. The same group showed in the same paper that the insulin-like stimulation of glucose oxidation in rat adipocytes was due to the vanadyl ion. Our group demonstrated that vanadate, administered in drinking water, reduced elevated blood glucose to normal and restored depressed cardiac performance in rats made diabetic with streptozocin (STZ) in 1985.[8] Interest in the insulin-mimicking effect of vanadate and vanadyl has burgeoned since Sakurai and co-workers showed that vanadate is reduced in vivo to vanadyl.[9] Significant drawbacks to vanadate are that it is poorly absorbed from the gastrointestinal tract into the blood and that it is toxic; administered concentrations are, therefore, close to the toxic level in order to manifest the insulin-mimicking effect in animals.

Subsequent work by McNeill et al.[10-14] has shown that vanadyl administered orally as aqueous vanadyl sulfate $[VO(H_2O)_5]SO_4{\cdot}(H_2O)_x$ will also lower blood glucose and blood lipids in STZ diabetic rats and will prevent secondary complications of diabetes such as cataracts and cardiac dysfunction. Vanadyl sulfate is less toxic than vanadate but is also poorly absorbed. There have been

(1) Faculty of Pharmaceutical Sciences.
(2) Department of Chemistry.
(3) Atkinson, M. A.; Maclaren, N. K. What Causes Diabetes? *Sci. Am.* **1990**, *263*, 62–71.
(4) Reviewed in: Shechter, Y. Insulin-Mimetic Effects of Vanadate: Possible Implications for Future Treatment of Diabetes. *Diabetes* **1990**, *39*, 1–5. Shechter, Y.; Meyerovitch, J.; Farfel, Z.; Sack, J.; Bruck, R.; Bar-Meir, S.; Amir, S.; Degani, H.; Karlish, S. J. D. Insulin Mimetic Effects of Vanadium. In *Vanadium in Biological Systems: Physiology and Biochemistry*; Chasteen, N. D., Ed.; Kluwer Academic Publishers: Dordrecht, 1990; pp 129–142.
(5) Cantley, L. C., Jr.; Josephson, L.; Warner, R.; Yanagisawa, M.; Lechene, C.; Guidotti, G. Vanadate is a Potent (Na,K)-ATPase Inhibitor Found in ATP Derived from Muscle. *J. Biol. Chem.* **1977**, *252*, 7421–7423.
(6) Cantley, L. C., Jr.; Aisen, P. The Fate of Cytoplasmic Vanadium: Implications on (Na,K)-ATPase Inhibition. *J. Biol. Chem.* **1979**, *254*, 1781–1784.
(7) Shechter, Y.; Karlish, S. J. D. Insulin-like Stimulation of Glucose Oxidation in Rat Adipocytes by Vanadyl(IV) Ions. *Nature (London)* **1980**, *284*, 556–558.
(8) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. Effect of Vanadate on Elevated Blood Glucose and Depressed Cardiac Performance of Diabetic Rats. *Science* **1985**, *227*, 1474–1477.
(9) Sakurai, H.; Shimomura, S.; Fukuzawa, K.; Ishizu, K. Detection of Oxovanadium(IV) and Characterization of its Ligand Environment in Subcellular Fractions of the Liver of Rats Treated with Pentavalent Vanadium(V). *Biochem. Biophys. Res. Commun.* **1980**, *96*, 293–298.
(10) Ramanadham, S.; Mongold, J. J.; Brownsey, R. W.; Cros, G. H.; McNeill, J. H. Oral Vanadyl Sulfate in the Treatment of Diabetes Mellitus in Rats. *Am. J. Physiol.* **1989**, *257*, H904-H911.
(11) Ramanadham, S.; Brownsey, R. W.; Cros, G. H.; Mongold, J. J.; McNeill, J. H. Sustained Prevention of Myocardial and Metabolic Abnormalities in Diabetic Rats Following Withdrawal from Oral Vanadyl Treatment. *Metabolism* **1989**, *38*, 1022–1028.
(12) Pederson, R. A.; Ramanadham, S.; Buchan, A. M. J.; McNeill, J. H. Long-Term Effects of Vanadyl Treatment on Streptozocin-Induced Diabetes in Rats. *Diabetes* **1989**, *38*, 1390–1395.
(13) Ramanadham, S.; Cros, G. H.; Mongold, J. J.; Serrano, J. J.; McNeill, J. H. Enhanced in vivo Sensitivity of Vanadyl-Treated Diabetic Rats to Insulin. *Can. J. Physiol. Pharmacol.* **1990**, *68*, 486–491.
(14) Ramanadham, S.; Heyliger, C.; Gresser, M. J.; Tracey, A. S.; McNeill, J. H. The Distribution and Half-Life for Retention of Vanadium in the Organs of Normal and Diabetic Rats Orally Fed Vanadium(IV) and Vanadium(V). *Biol. Trace Element Res.* **1991**, *30*, 119–124.

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