## SC-53116: The First Selective Agonist at the Newly Identified Serotonin 5-HT₄ 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-HT₁, 5-HT₂, and 5-HT₃.  Furthermore, even these subtypes have been subclassed to now include 5-HT₁ₐ, 5-HT₁ᵦ, 5-HT₁c, 5-HT₁ᴅ, 5-HT₂ₐ, 5-HT₂ᵦ, and 5-HT₃ₐ, 5-HT₃ᵦ, and 5-HT₃c.[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 D₂ receptors ($K_i = 113$ nM).  However, more recently it has been identified as a serotonin 5-HT₃ 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-HT₃ antagonist properties.[3,4]  Indeed, its interaction with dopamine D₂ 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-HT₃ 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-HT₁, 5-HT₂, or 5-HT₃ receptors.[5]

*Very recently, Bockaert[6] and Clarke[7] independently reported a new serotonin receptor subtype (5-HT₄) in brain and gut tissues, respectively.*  Serotonin is quite potent (EC₅₀ = 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-HT₄ receptor.[8]  Clarke et al. have described several functional models for the 5-HT₄ 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-HT₄

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

**Scheme I.** Preparation of Racemic Pyrrolizidine Side Chains
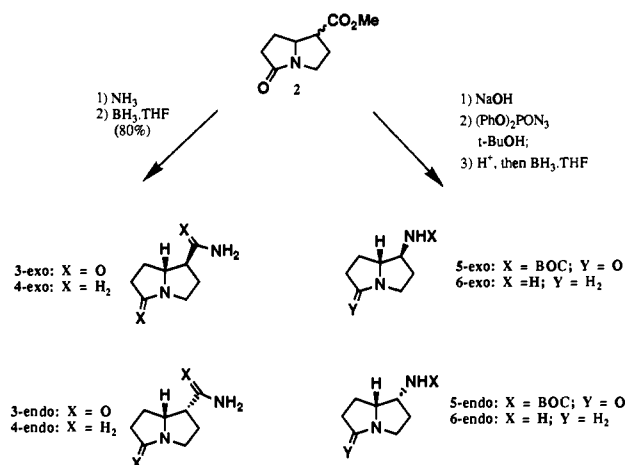


receptor, Bockaert has identified functional 5-HT₄ receptors in the guinea pig hippocampus,[12] and Boddeke and Kalkman have demonstrated that activation of 5-HT₄ receptors in rat hippocampal regions induce an increase in EEG energy.[13]  The design of agents selectively potent

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(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.

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(10) Baxter, G. S.; Craig, D. A.; Clarke, D. E. 5-Hydroxytryptamine₄ 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.

**Scheme II.** Preparation of Enantiopure Pyrrolizidine Side Chains



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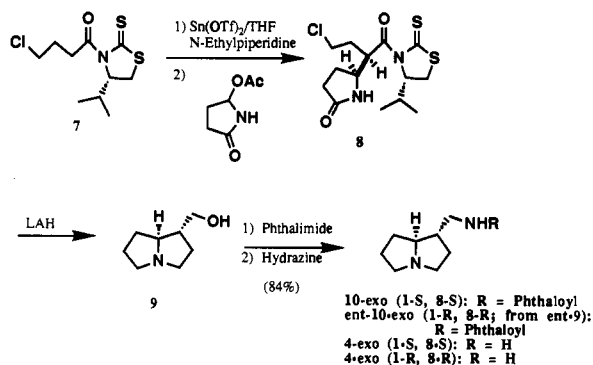


for this 5-HT$_4$ receptor may therefore provide new therapies for both central nervous system and gastrointestinal diseases.

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.
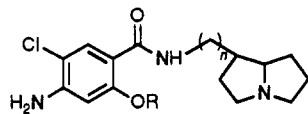


SC-53116 is 1B (1-*S*,8-*S* isomer; *n* = 1; R = Me)

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

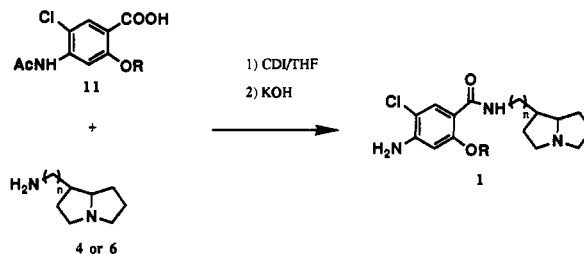**Table I.** Serotonin 5-HT$_4$ Agonist and 5-HT$_3$ Antagonist Activities of Substituted Pyrrolizidines 1[a]



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 $\mu$M ICS-205930. [b] Compound 1H has all aromatic ring substituents = hydrogen.

ent-9 were prepared in >99% optical purity by the method of Nagao.[15] Treatment of 9 or ent-9 with phthalimide/Ph$_3$P/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.

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 $\mu$M) and fluoxetine (1 $\mu$M) were included in the buffer to prevent uptake of serotonin as well as pargyline (10 $\mu$M) to inhibit monoamine oxidase.

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**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$_{50}$ | %E[a] | 5-HT$_3$ | 5-HT$_1$ | 5-HT$_2$ | D$_1$ | D$_2$ | $\alpha_1$ | $\alpha_2$ | $\beta$ |
|---|---|---|---|---|---|---|---|---|---|---|
| SC-53116 (1B) | 23.7 (4.9) | 93 | 152 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 |
| cisapride | 54.7 (0.8) | 105 | 134 | >10 000 | 6.1 | 1700 | 227 | 30 | 4500 | >10 000 |
| renzapride | 44.0 (6.9) | 98 | 5.3 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 |
| zacopride (S)-isomer | 173.0 (4.0) | 93 | 0.23 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 |
| zacopride (R)-isomer | 505.0 (73.0) | 96 | 8.0 | >10 000 | 415 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 |
| BIMU-8 | 40.2 (5.5) | >111 | 7.0 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 | >10 000 |

[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); $\alpha_1$ (rat whole brain; prazosin); $\alpha_2$ (rat cortex; rauwolscine); $\beta$ (rat whole brain; dihydroalprenolol).

Following a 30-min equilibrium period, tissues were isometrically contracted with carbachol (3 $\mu$M) to obtain a tonic contraction. A stable plateau was obtained within 20 min when test compound was added cumulatively to relax the muscle strip. 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$_{50}$ = 24 100 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 p$A_2$ values of 6.3 and 6.4 when evaluated against SC-53116 and serotonin, respectively.[16] These values are consistent with other published p$A_2$ 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 cisapride (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 $\mu$M in binding studies at 5-HT$_1$, 5-HT$_2$, dopamine D$_1$, D$_2$, $\alpha_1$, $\alpha_2$, and $\beta$ adrenergic monoamine receptors. By contrast, cisapride exhibits potent receptor interactions at the serotonin 5-HT$_2$ ($K_i$ = 6.1 nM) and $\alpha_1$ 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

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(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-HT₃ receptor-mediated bradycardic response to serotonin with ED₅₀ values of 0.25, 0.005, and 0.01 mg/kg ip. By contrast, the pyrrolizidine **1B** exhibited an ED₅₀ of 3.0 mg/kg ip, consistent with the modest $K_i$ of 152 nM in the 5-HT₃ binding assay.

In conclusion, the substituted pyrrolizidines **1** afford a novel series of potent and selective 5-HT₄ agonists. **1B** is the most potent and selective agent yet reported for agonism at this newly identified serotonin receptor. While exhibiting potency (ED₅₀ = 23 nM) similar to serotonin in the 5-HT₄ rat esophagus model, it is only modestly active at the 5-HT₃ 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 (IC₅₀ > 10 000 nM) for dopamine D₂, 5-HT₂, or α₁ adrenergic receptors. The in vitro 5-HT₄ 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-HT₄ receptors in various CNS and gastrointestinal diseases.

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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]

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In the late 1970s sodium orthovanadate (Na₃VO₄, 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 (VO₄³⁻) 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(H₂O)₅]SO₄·(H₂O)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

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