

Bridgehead-methyl analog of SC-53116 as a 5-HT₄ agonist

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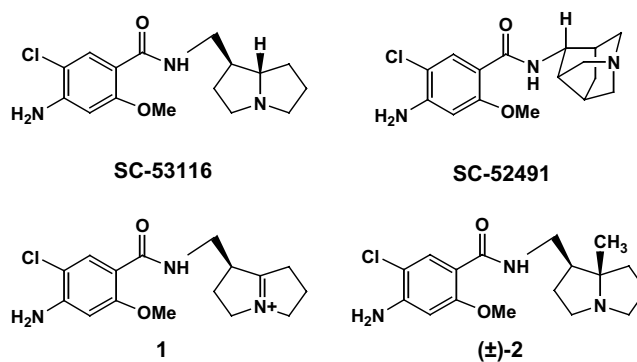
Abstract—Pyrrolizidine benzamide (\pm)-**2**, the bridgehead-methyl analog of **SC-53116**, was prepared and evaluated for 5-HT₄ agonism activity in the rat tunica muscularis (TMM) mucosae assay. Compound (\pm)-**2** has an EC₅₀ of 449 nM in the TMM assay, as compared to 23 nM for **SC-53116**, and 66 nM for the racemate of **SC-53116**.

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We have previously reported our discovery of **SC-53116**, which was the first selective agonist at the 5-HT₄ receptor.¹ **SC-53116** has an ED₅₀ of 23 nM in the tunica muscularis mucosae assay of Craig and Clarke,² and is selective versus other monoamine receptors with a K_i of 152 nM at the 5-HT₃ receptor and K_is of >10,000 nM at the 5-HT₁, 5-HT₂, D₁, D₂, α_1 , α_2 , and β -receptors. The 5-HT₄ receptor was discovered by Clark² and Bockaert³ in the brain and gut, respectively, and is expressed in a wide variety of tissues including brain, heart, bladder, gut, and kidney.⁴ Selective ligands for the receptor show promise in the treatment of diseases including the irritable bowel syndrome, atrial arrhythmia, urinary incontinence, and gastrointestinal motility disorders. An excellent review of the 5-HT₄ receptor and key ligands was recently published.⁵

We have been interested in pursuing 5-HT₄ agonists as gastrointestinal prokinetic agents. Our efforts in this area⁶ have also prompted us to develop several azatri-cyclic benzamides⁷ that are potent 5-HT₄ agonists and 5-HT₃ antagonists, particularly **SC-52491**,⁸ in addition to the pyrrolizidine **SC-53116**.

SC-53116 was a clinical candidate but was halted when it was observed to be positive in the Ames assay when



tested with S9 activation.⁹ We hypothesized that this toxicity was due to oxidation of the pyrrolizidine moiety to the bicyclic iminium ion **1**, which can then function as an alkylating agent. To avoid this liability, we targeted the bridgehead-methyl **SC-53116** analog (\pm)-**2**, as the methyl group would block metabolism to the iminium species. We targeted the racemate initially to test the concept and ensure that sufficient potency is maintained with the additional methyl group.

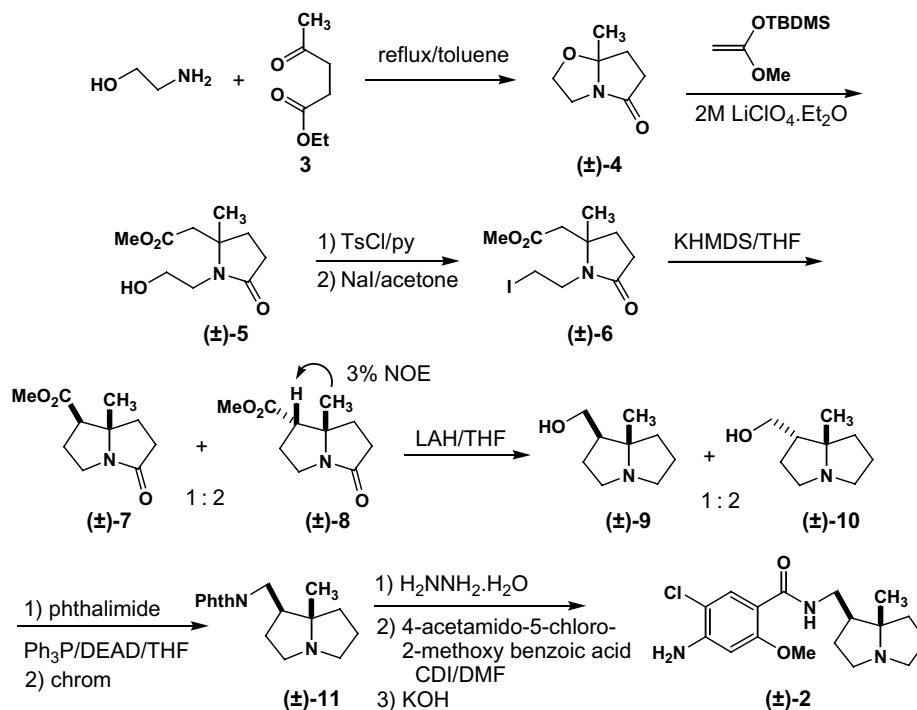
We employed general methodology reported by Burgess and Meyers¹⁰ for introduction of the requisite quaternary carbon at the pyrrolizidinone bridgehead position. As outlined in Scheme 1, ketoester **3** was heated under reflux with ethanolamine to afford (\pm)-**4**¹¹ in 53% yield. The bicyclic lactam (\pm)-**4** was treated with *t*-butyldimethylsilyl methyl acetate ketal¹² in 2 M lithium perchlorate ether¹³ to afford lactam (\pm)-**5** in 30% yield. Attempts with titanium tetrachloride in methylene chloride also afforded (\pm)-**5** but in lower yield. The primary alcohol of (\pm)-**5** was converted to the tosylate in 86% yield by treating with tosyl chloride in pyridine.

Keywords: Serotonin; 5-HT₄; Prokinetic; SC-53116; Pyrrolizidine.

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Scheme 1. Synthesis of (±)-2.

Treatment of the tosylate with sodium iodide under Finkelstein conditions afforded the primary iodide (±)-6.

A variety of bases were employed to effect closure of (±)-6, with potassium hexamethyldisilazide giving the best yield of (±)-7 and (±)-8 in a combined 70% yield. The *exo* and *endo* methyl esters were isolated in a 1:2 ratio, with the *endo* (±)-8 as the main component, as determined by NOE.¹⁴ Reduction with lithium aluminum hydride gave a mixture of alcohols (±)-9 and (±)-10, and this mixture was converted directly to the phthalimides under Mitsunobu conditions, allowing chromatographic isolation of the requisite *exo* isomer (±)-11 in 24% yield. Deprotection of (±)-11 with hydrazine gave the free amine in quantitative yield, which was coupled directly with 5-acetamido-4-chloro-2-methoxybenzoic acid utilizing carbonyl diimidazole to afford the desired benzamide. The acetamide was removed with potassium hydroxide in ethanol under reflux to afford the desired benzamide isomer (±)-2 in 66% yield.

Thus (±)-2, the bridgehead-methyl analog of SC-53116, was tested for agonist activity at the 5-HT₄ receptor in the rat tunica muscularis mucosae assay. The potency for the compound was good, at 449 nM (±185). However, this was approximately 7X less potent than SC-49518, the racemate of SC-53116, which has an EC₅₀ of 66 nM ± 11 nM. Due to the loss of potency, the bridgehead methyl analog (±)-2 was not pursued further. The azatricycle benzamide compounds that we developed^{7,8} are potent, efficacious, and safe, so we turned our attention to those molecules. Specifically, SC-52491 was negative in the Ames assay at the highest concentrations tested, either with or without S9 activation.¹⁵

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- SC-53116 was tested for mutagenic activity in a GLP study using the Ames Salmonella/microsome assay with five strains of Salmonella typhimurium (TA1535, TA100, TA1538, TA98, and TA97) in the presence and absence of a rat liver homogenate metabolic activation system (S9)

- over test article concentrations ranging from 7.2 to 3600 $\mu\text{g}/\text{plate}$. Significant test article-related increases of 4X in the number of revertant colonies were observed in strain TA98 with activation at 3600 $\mu\text{g}/\text{plate}$. A 2–3X increase was observed with activation in strain TA100 between 720 μg and 3600 $\mu\text{g}/\text{plate}$, and a 2–6X increase was observed with activation in strain TA1538 also between 720 μg and 3600 $\mu\text{g}/\text{plate}$. Significant increases in numbers of revertant colonies were not observed in the test without S9 activation.
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 14. Patricia Finnegan is gratefully acknowledged for help with the NOE experiment.
 15. **SC-52491** was tested versus five strains of *Salmonella typhimurium* (TA97, TA98, TA100, TA1535, and TA1538) in the presence and absence of rat liver homogenated metabolic activation system (S9) over **SC-52491** concentrations ranging from 50 to 7500 $\mu\text{g}/\text{plate}$. There was no evidence of mutagenicity by **SC-52491** in any of the strains tested.