6-21-2004

Bridgehead-methyl Analog of SC-53116 as a 5-HT4 Agonist

Daniel Becker
Loyola University Chicago, dbecke3@luc.edu

Daniel L. Flynn

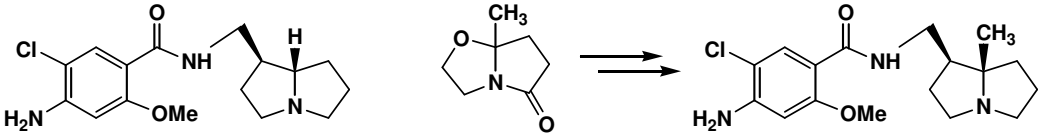
Clara I. Villamil

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

Bridgehead-Methyl Analog of SC-53116 as a 5-HT4 Agonist

Daniel P. Becker*, Daniel L. Flynn, and Clara I. Villamil
Department of Medicinal Chemistry, Pfizer Research & Development, 4901 Searle Parkway, Skokie, IL 60077

Pyrrolizidine **(±)-2**, the bridgehead-methyl analog of **SC-53116**, was prepared and evaluated for 5-HT_4 agonism activity in the rat tunica muscularis (TMM) mucosae assay. Compound **(±)-2** has an EC_{50} of 449 nM in the TMM assay, as compared to 23 nM for **SC-53116**, and 66 nM for the racemate of **SC-53116**.



SC-53116 **(±)-5** **(±)-2**

Bridgehead-Methyl Analog of SC-53116 as a 5-HT4 Agonist

Daniel P. Becker*, Daniel L. Flynn[§], and Clara I. Villamil[‡]

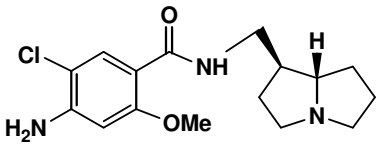
Department of Medicinal Chemistry, Pfizer Research & Development, 4901 Searle Parkway, Skokie, IL 60077

[*]To whom correspondence should be addressed. Current address: Department of Chemistry, Loyola University, 6525 North Sheridan Road, Chicago, IL 60626, dbecke3@luc.edu
[§]Current address: Deciphera Pharmaceuticals Inc., 1505 Wakarusa Drive, Lawrence, KS 66047 USA
[‡]Current address: Abbott Labs, 100 Abbott Park Road, Abbott Park, IL 60064-6099

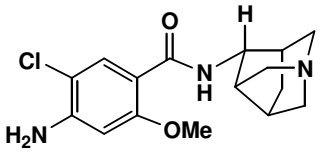
Abstract—Pyrrolizidine benzamide **(±)-2**, the bridgehead-methyl analog of **SC-53116**, was prepared and evaluated for 5-HT_4 agonism activity in the rat tunica muscularis (TMM) mucosae assay. Compound **(±)-2** has an EC_{50} of 449 nM in the TMM assay, as compared to 23 nM for **SC-53116**, and 66 nM for the racemate of **SC-53116**.

We have previously reported our discovery of **SC-53116**, which was the first selective agonist at the 5-HT_4 receptor.[1] **SC-53116** has an ED_{50} 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-HT_3 receptor and K_i's of >10,000 nM at the 5-HT_1, 5-HT_2, D_1, D_2, α_1, α_2 and β-receptors. The 5-HT_4 receptor was discovered by Clark[2] and Bockaert[3] 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-HT_4 receptor and key ligands was recently published.[5]

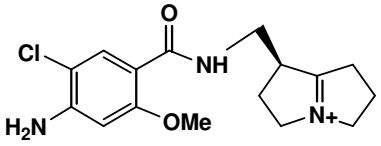
We have been interested in pursuing 5-HT_4 agonists as gastrointestinal prokinetic agents. Our efforts in this area[6] have also prompted us to develop several azatricyclic benzamides[7] that are potent 5-HT_4 agonists and 5-HT_3 antagonists, particularly **SC-52491**,[8] in addition to the pyrrolizidine **SC-53116**.



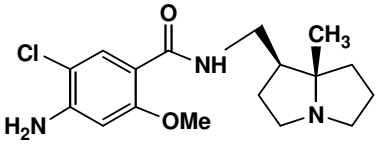
SC-53116



SC-52491



1

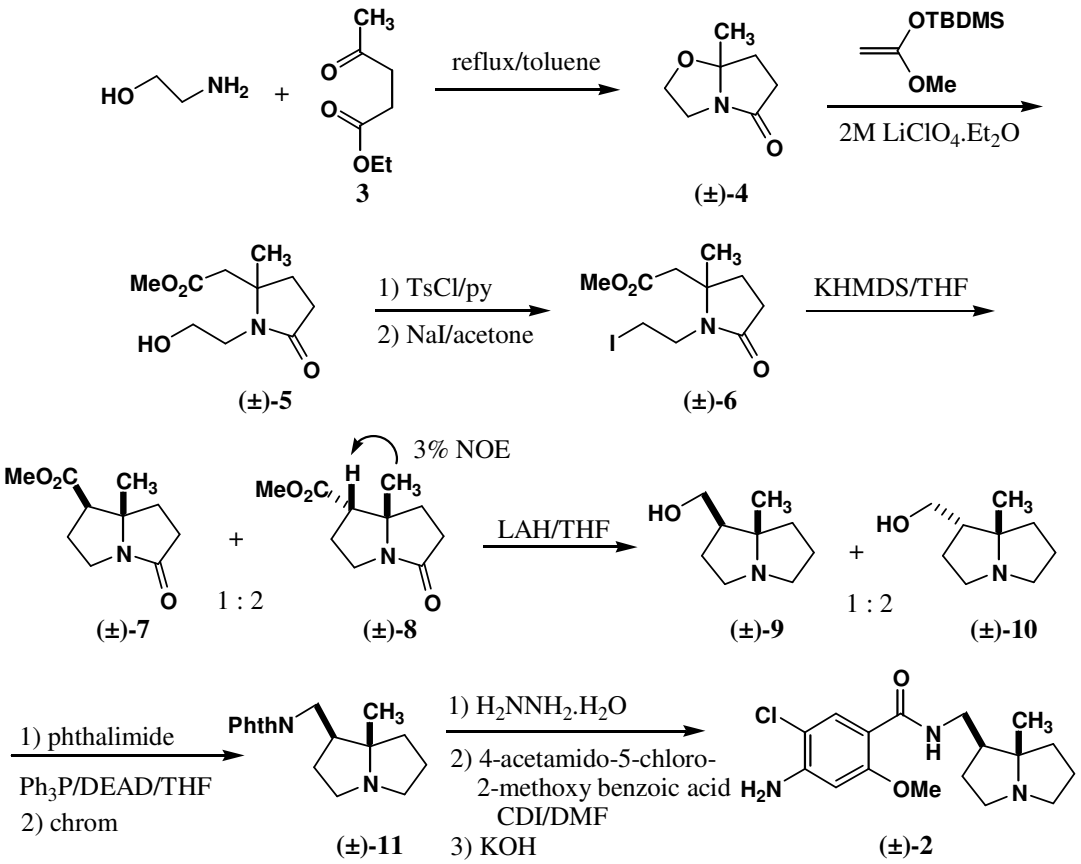


(±)-2

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 **(±)-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 Meyers[10] 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 **(±)-4**[11] in 53% yield. The bicyclic lactam **(±)-4** was treated with t-butyldimethylsilyl methyl acetate ketal[12] in 2M lithium perchlorate ether[13] to afford lactam **(±)-5** in 30% yield. Attempts with titanium tetrachloride in methylene chloride also afforded **(±)-5** but in lower yield. The primary alcohol of **(±)-5** was converted to the tosylate in 86% yield by treating with tosyl chloride in pyridine. Treatment of the tosylate with sodium iodide under Finkelstein conditions afforded the primary iodide **(±)-6**.

Scheme 1: Synthesis of **(±)-2**



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-HT_4 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]

References and Notes

1. Flynn, D. L.; Zabrowski, D. L.; Becker, D. P.; Nosal, R.; Villamil, C. I.; Gullikson, G. W.; Moummi, Ch. Yang, Dai-C. *J. Med. Chem.,* **1992**, *35*, 1486.
2. Baxter, G. S.; Craig, D. A.; Clarke, D. E. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *343*, 439.
3. Dumuis, A.; Sebbon, M.; Bockaert, J. Naunyn Schmiedebergs *Arch. Pharmacol.* **1989**, *340*, 403.
4. (a) Berque-Bestel, I.; Soulier, J.-L.; Giner, M.; Rivail, L.; Langlois, M.; Sicsic, S.; *J. Med. Chem.* **2003**, *46*, 2606. (b) Eglen, R. M.; Wong. E. H. F.; Dumuis, A.; Bockaert, J. *Trends Pharmacol. Sci*. **1995**, *16*, 391. (c) Hedge, S.; Eglen, R. *FASEB J*. **1996**, *10*, 1398.
5. Langlois, M.; Fischmeister, R. *J. Med. Chem*. **2003**, *46*, 319.
6. Becker, D. P.; Goldstin, B.; Gullikson, G. W.; Loeffler, R.; Moormann, A.; Moummi, C.; Nosal, R.; Spangler, D.; Villamil, C. I.; Yang, D.-C.; Zabrowski, D. L.; Flynn, D. L. "Design and Synthesis of Agonists and Antagonists of the Serotonin 5-HT_4 Receptor Subtype" , in "Perspectives in Receptor Research", ed. D. Giardina et. al. , *Pharmacochemistry Library, Vol. 24*, Elsevier Science B. V.: Amsterdam, **1996**, pp. 99-120.
7. Becker, D. P.; Nosal, R.; Villamil, C. I.; Gullikson, G.; Moummi, C.; Yang, D.-C. Flynn, D. L. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2149.
8. (a) Becker, D. P.; Husa, R. K.; Moormann, A. E.; Villamil, C. I.; Flynn, D. L. *Tetrahedron* **1999**, *55*, 11787. (b) Becker, D. P.; Nosal, R.; Zabrowski, D. L.; Flynn, D. L. *Tetrahedron* **1997**, *53*, 1. (c) Flynn, D. L.; Becker, D. P.; Spangler, D. P.; Nosal, R.; Gullikson, G. W.; Moummi, C.; Yang, D.-C. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1613.
9. **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 μg/plate.

Significant test article-related increases of 4X in the number of revertant colonies were observed in strain TA98 with activation at 3600 μg/plate. A 2-3X increase was observed with activation in strain TA100 between 710 ug and 3600 μg/plate, and a 2-6X increase was observed with activation in strain TA1538 also between 720 μg and 3600 μg/plate. Significant increases in numbers of revertant colonies were not observed in the test without S9 activation.

10. Burgess, L. E.; Meyers, A. I. *J. Am. Chem. Soc.* **1991**, *113*, 9858.
11. Wedler, C.; Schick, H.; Scharfenberg-Pfeiffer, D., Reck, G. *Liebigs Ann. Chem.* **1992**, 29.
12. Heathcock, C. H.; Davidsen, S. K.; Hug, K. T.; Flippin, L. A. *J. Org. Chem.* **1986**, *51*, 3027.
13. Grieco, P. A.; Nunes, J. J.; Gaul, M. D. *J. Am. Chem. Soc.,* **1990**, *112*, 4595.
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 μg/plate. There was no evidence of mutagenicity by **SC-52491** in any of the strains tested.