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BMS-187257, A POTENT, SELECTIVE, AND NOVEL HETEROCYCLIC β_3 ADRENERGIC RECEPTOR AGONIST

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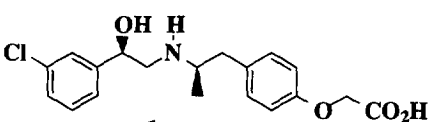
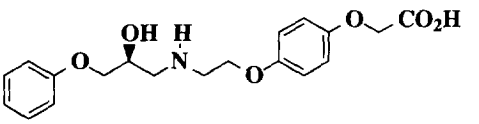
Abstract: Novel heterocyclic β_3 adrenergic receptor agonists[2] were prepared and evaluated for their ability to bind to human β_1, β_2, and β_3 adrenergic receptors. Stimulatory effects on the β_3 adrenergic receptor were also measured. BMS-187257 (**4b**) was found to be a potent and selective β_3 agonist.

Introduction:

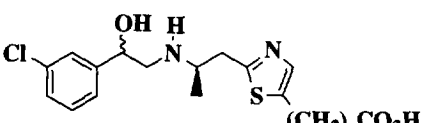
The atypical[3a] or β_3[4] adrenergic receptor (AR) mediates a variety of pharmacological and physiological effects including lipolysis, thermogenesis, and relaxation of intestinal smooth muscle.[5] Consequently, several research groups are engaged in developing selective β_3 agonists for the treatment of gastrointestinal disorders, Type-II diabetes, and obesity.[6] Previous studies suggested that a carboxylic acid moiety properly placed on the right-hand side aromatic ring of β_3 agonists such as arylethanolamine **1** (BRL 37344) and aryloxypropanolamine **2** (ICI 215001) (see structures below) improves selectivity against the β_1 and β_2 ARs.[3a,7] As part of our search for novel and selective β_3 agonists, we explored replacement of the right-hand aryl ring with a heterocycle.[2,8] In this communication, we describe two series of 2,5-disubstituted thiazoles (**3a-b** and **4a-c**), in which the linker length to the carboxyl group was varied. Binding affinities of each compound for all three human beta adrenergic receptors were measured using membranes of Chinese hamster ovary (CHO) cells stably transfected with the appropriate β AR. The abilities of the compounds to stimulate adenylyl cyclase in CHO-β_3 cell membranes were measured as well.



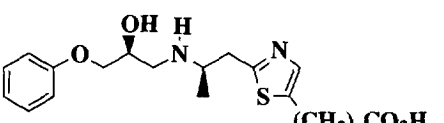
1
BRL 37344



2
ICI 215001



3a n = 2
3b n = 3



4a n = 2
4b n = 3 **(BMS-187257)**
4c n = 4

Materials and Methods:

ICI 215001[7] was generously supplied by ICI Pharmaceuticals. BRL 37344 was prepared by the authors.[3a,3b] CHO cells stably transfected with human β_1, β_2, or β_3 ARs were supplied by Prof. A. D. Strosberg, Laboratoire d'Immuno-Pharmacologie Moléculaire, Institut Cochin de Génétique Moléculaire, Paris, France. Binding studies were performed essentially as reported,[9] except that cell membranes rather than whole cells were used and experiments were conducted at 25 °C rather than 37 °C. Adenylyl cyclase activity was measured in CHO-β_3 cell[9] membranes at 37 °C in a buffer containing 30 mM Tris acetate pH 7.6, 5 mM $MgCl_2$, 5 mM phosphocreatine, 50 U/mL creatine phosphokinase, 1 mM IBMX, 0.2 mM ATP, 1 x 10^7 cpm/mL [α-^{32}P]ATP, 2 μM GTP, and 1 mM EGTA. Radioactive [^{32}P]cAMP produced was isolated by chromatography using sequential Dowex-50 cation exchange and neutral alumina columns and measured using scintillation counting. Sample recovery was determined using [^{3}H]cAMP as an internal standard.[10]

Biological Results and Discussion:

Novel thiazoles **3a-b** and **4a-c** were examined first in the binding assays (see table below). Despite their close structural similarity to BRL 37344, the arylethanolamines **3a** and **3b** exhibited low binding potency and were not selective for the human β_3 AR. In contrast, the aryloxypropanolamines **4a-4c** bound to the β_3 AR with moderate to high affinity. As the number of methylenes in the carboxyl sidechain of the aryloxypropanolamines increased from two to four, β_3 binding potency improved, with a nearly 5-fold increase from two to three methylenes (**4a** to **4b**), and an approximately 2-fold increase from three to four methylenes (**4b** to **4c**). Binding selectivity was maximized in the three methylene analog **4b** (BMS-187257), which exhibited selectivities of about 2-fold for the β_3 AR over both the β_2 and β_1 ARs. Although **4c** was a 2-fold more potent β_3 ligand than BMS-187257 (**4b**), it was also 11-fold more potent at β_2 and thus not selective for β_3. Believing β_3 selectivity to be very important, we considered **4b** to be the optimal compound. BMS-187257, like BRL 37344 and ICI 215001, was a partial agonist in our adenylyl cyclase assay with an intrinsic activity of about 0.7.[11] Its EC_{50} was essentially identical to those of BRL 37344 and ICI 215001. Overall, the in vitro profile of BMS-187257 compares favorably with those of BRL 37344 and ICI 215001.

BINDING CONSTANTS AND STIMULATORY ACTIVITIES IN CHO-β AR MEMBRANES [a]

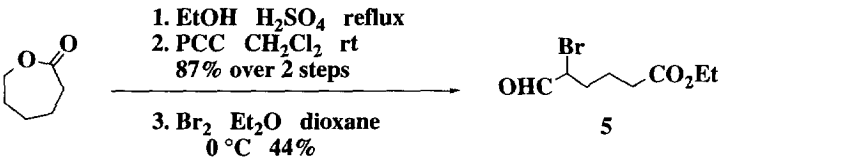
COMPOUND	β_1 K_i	β_2 K_i	β_3 K_i	β_3 EC_{50} (IA)
BRL 37344	**11300**	**630**	**960**	**680 (0.67)**
ICI 215001	**300**	**2350**	**520**	**550 (0.71)**
3a	**58100**	**11400**	**146000**	**27595 (0.39)**
3b	**6400**	**13900**	**36500**	**NT**
4a	**3440**	**1230**	**3530**	**2760 (0.56)**
4b (BMS-187257)	**1300**	**1600**	**760**	**630 (0.72)**
4c	**440**	**150**	**390**	**NT**

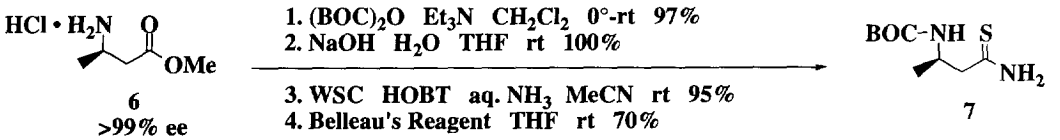
[a] Compounds were assayed for their ability to bind to human β_1, β_2, and β_3 ARs in their respective CHO cell membranes. Binding potency is reported as K_i (nM), the binding inhibition constant, determined by inhibition of [^{125}I]iodocyanopindolol binding (mean, $n \geq 2$). Ability to stimulate adenylyl cyclase activity in CHO-β_3 AR cell membranes is indicated by EC_{50} and intrinsic activity (IA). EC_{50} (nM) is the concentration at which half of the maximal response of the compound was observed. IA is the maximal cyclase activity of the compound as a fraction of that observed with (-)-isoproterenol (IA = 1). NT = not tested.

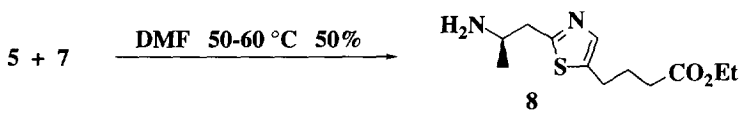
Synthesis[2] of BMS-187257:

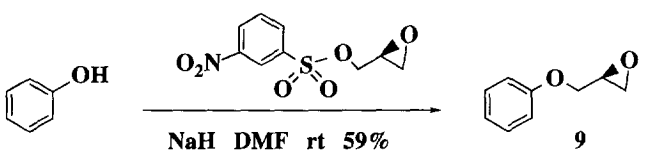
Bromoaldehyde **5** was prepared from caprolactone as shown below by acid catalyzed ethanolysis and PCC oxidation,[12] followed by α-bromination.[13]

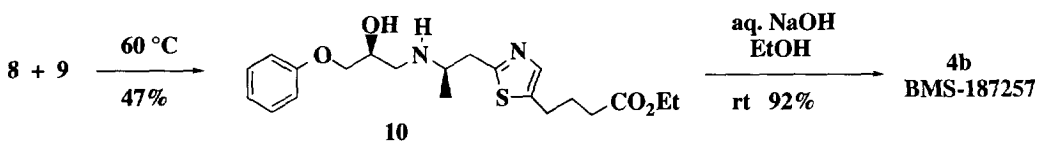
Chiral amino ester **6** was obtained in very high optical purity from methyl acetoacetate by reductive amination using (+)-α-methylbenzylamine, followed by hydrogenolytic removal of the α-methylbenzyl group.[14] N-protection of **6**, conversion to the primary amide, and thionation with Belleau's reagent[15] gave thioamide **7**.











Construction of the thiazole ring was accomplished by condensation of thioamide **7** with bromoaldehyde **5** in warm DMF.[13] The BOC protecting group was conveniently cleaved under the reaction conditions, providing **8** directly.

Chiral epoxide **9**, destined for the left-hand side of BMS-187257, was prepared in high optical purity from (2S)-(+)-glycidyl 3-nitrobenzenesulfonate[16] and sodium phenoxide.

Amine **8** was then coupled (neat) with epoxide **9** in 47% yield to afford **10**. The modest yield of **10** was partly due to formation of bis-alkylated product and the tendency of **8** to self-condense. Saponification of **10** gave **4b** (BMS-187257). This synthetic route is convergent, and the stereochemistry at each chiral center is independently controlled.[2,17]

Conclusions:

We have successfully demonstrated the utility of a 2,5-disubstituted thiazole as the right-hand side aromatic ring in aryloxypropanolamine-type β_3 AR agonists. We have optimized binding selectivity with a three methylene spacer in BMS-187257, which has binding and stimulatory properties that compare well with those of BRL 37344 and ICI 215001.

We are unable to explain why the isosteric replacement was not successful in the arylethanolamine structural type.[18] However, the discrepancy between its success in the aryloxypropanolamine series and its failure in the arylethanolamine series indicates that the ligand-receptor interaction is sufficiently complex that any analysis that considers structural features as isolated entities may have little predictive value.

Acknowledgments:

The authors thank the Bristol-Myers Squibb Pharmaceutical Research Institute Analytical Chemistry Department and Octavian Kocy for MS, HRMS, IR, and elemental analysis data and optical purity determinations. The authors also thank Dr. William Washburn, Dr. Scott Biller, and Dr. Raj N. Misra for helpful discussions.

References and Notes:

1. Current address: University of Medicine & Dentistry of New Jersey, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854-5635.

2. Sher, P. M. U.S. Patent 5321036, 6/14/94. This patent provides the syntheses of all of the heterocyclic compounds described herein (**3a-b** and **4a-c**). As depicted, **3a-b** are mixtures of R,R and S,R stereoisomers (1:1) and **4a-c** are single S,R stereoisomers.

3. (a) Arch, J. R. S.; Ainsworth, A. T.; Cawthorne, M. A.; Piercy, V.; Sennitt, M. V.; Thody, V. E.; Wilson, C.; Wilson, S. *Nature (London)* **1984**, *309*, 163. (b) Cantello, B. C. C.; Smith, S. A. *Drugs of the Future* **1991**, *16*, 797.

4. Emorine, L. J.; Marullo, S.; Briend-Sutren, M.-M.; Patey, G.; Tate, K.; Delavier-Klutchko, C.; Strosberg, A. D. *Science* **1989**, *245*, 1118.

5. Arch, J. R. S.; Kaumann, A. J. *Med. Res. Rev.* **1993**, *13*, 663.

6. Howe, R. *Drugs of the Future* **1993**, *18*, 529.

7. Howe, R.; Rao, B. S.; Holloway, B. R.; Stribling, D. *J. Med. Chem.* **1992**, *35*, 1751. ICI 215001 is the active (S) isomer component of ICI 201651 (a racemate).

8. See also Alig, L.; Müller, M. U.S. Patents 5064863, 4871755, 4743604, 4652679, and 4585796. These workers reported replacement of the right-hand aryl ring with a thiophene. This modification led to active compounds, but because biological data was reported at only a single dose in vivo, it was impossible to gain an understanding of structure-activity relationships at the receptor level. However, this indication that the right-hand aryl ring might be replaced by a heterocycle, coupled with the novelty and synthetic accessiblity of our thiazole analogs, prompted the study described herein.

9. Tate, K. M.; Briend-Sutren, M.-M.; Emorine, L. J.; Delavier-Klutchko, C.; Marullo, S.; Strosberg, A. D. *Eur. J. Biochem.* **1991**, *196*, 357. Blin, N.; Camoin, L.; Maigret, B.; Strosberg, A. D. *Mol. Pharm.* **1993**, *44*, 1094. See also reference 11.

10. Salomon, Y.; Londos, C.; Rodbell, M. *Anal. Biochem.* **1974**, *58*, 541.

11. BRL 37344 has been reported to be a partial agonist in adenylyl cyclase assays where human β_3 - CHO cell membranes, rather than whole cells, were used: Fève, B.; Emorine, L. J.; Lasnier, F.; Blin, N.; Baude, B.; Nahmias, C.; Strosberg, A. D.; Pairault, *J. Biol. Chem.* **1991**, *266*, 20329. Liggett, S. B. *Mol. Pharm.* **1992**, *42*, 634. Granneman, J. G.; Lahners, K. N.; Chaudry, A. *Mol. Pharm.* **1993**, *44*, 264. Arch, J. R. S.; Wilson, S. *Int. J. Obesity* **1996**, *20*, 191.

12. Huckstep, M.; Taylor, R. J. K.; Caton, M. P. L. *Synthesis* **1982**, 881.

13. Poittevin, A.; Torelli, V. U.S. Patent 4053618, 10/11/77.

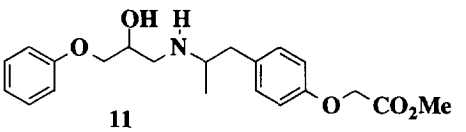
14. Jolidon, S.; Meül, T. U.S. Patent 4585887, 4/29/86.

15. Made by heating P_4S_{10} in excess diphenyl ether at 165-170 °C. See Lajoie, G.; Lépine, F.; Maziak, L.; Belleau, B. *Tetrahedron Lett.* **1983**, *24*, 3815. This reagent gave higher yields than Lawesson's reagent.

16. Recrystallization of this reagent from ethanol increased its optical purity to over 99% from approximately 92% as purchased (Aldrich). The reaction with phenoxide proceeded with a loss of only 0.2% ee. See Klunder, J. M.; Onami, T.; Sharpless, K. B. *J. Org. Chem.* **1989**, *54*, 1295.

17. Spectral data were fully consistent with the structures depicted.

18. Although the discrepancy between the success of the isosteric replacement in the aryloxypropanolamine series and its failure in the arylethanolamine series might appear to be particularly surprising in light of the greater structural similarity between arylethanolamines **3a-b** and BRL 37344 than between aryloxypropanolamines **4a-c** and ICI 215001 (focus on the chain linking the aromatic ring to the nitrogen atom), it should be noted that **11**, like BRL 37344 and ICI 215001, is a potent and selective β_3 agonist in vivo.[7] Aryloxypropanolamines **4a-c** bear the same structural relationship to the carboxylic acid of **11** (its presumed active metabolite) that arylethanolamines **3a-b** bear to BRL 37344.



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