Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 12, pp. 1583-1588, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00266-7

CARBOXYL-PROMOTED ENHANCEMENT OF SELECTIVITY FOR THE β_3 ADRENERGIC RECEPTOR. NEGATIVE CHARGE OF THE SULFONIC ACID BMS-187413 INTRODUCES β_3 BINDING SELECTIVITY

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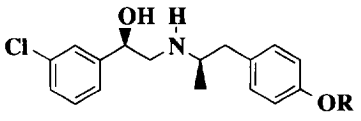
Abstract: Carboxyl and other negatively charged groups were found to be most effective at producing human β_3 adrenergic receptor binding selectivity in **1** (BRL 37344) and related compounds. The sulfonic acid analog **7** (BMS-187413) is a novel and potent β_3 adrenergic agonist that binds selectively, and thus has an in vitro profile that compares favorably with that of BRL 37344. © 1997 Elsevier Science Ltd.

Introduction:

In a preceding paper[2] we provided evidence that the carboxyl group of four carboxyl-containing β_3 adrenergic agonists enhances the β_3 adrenergic receptor (AR) binding selectivity of those compounds. The goals of this paper are to identify the property of the carboxyl group that improves β_3 binding selectivity and to disclose other functional groups that can play the same role. Of the compounds previously examined,[2] the selectivity enhancing effect of the carboxyl was strongest for **1** (BRL 37344), as seen when its β_1, β_2, and β_3 AR binding affinities were compared to those of its methyl ester (**2**, BRL 35135). Therefore, we chose to investigate a series of molecules related to **1** in which the functional group in R was varied. The primary alcohol (**3**),[3] the primary amide (**4**),[4] the hydroxamic acid (**5**), the malonic acid (**6**),[5] the sulfonic acid (**7**),[6] the phosphinic acid (**8**),[6,7] the phosphonic acid (**9**),[6,8] and the phosphonic monoester (**10**)[6,8] were assayed for their abilites to bind to human β_1, β_2, and β_3 ARs using membranes of stably transfected CHO cells. The abilities of the compounds to stimulate adenylyl cyclase in CHO-β_3 cell membranes were also measured.

1 R = CH_2CO_2H[a] (BRL 37344)
2 R = CH_2CO_2Me[a] (BRL 35135)
3 R = CH_2CH_2OH[a]
4 R = CH_2CONH_2[a]
5 R = $CH_2CONHOH$[a]
6 R = $CH(CO_2H)_2$[b]
7 R = CH_2SO_3H[b] (BMS-187413)
8 R = $CH_2P(Me)O_2H$[b]
9 R = $CH_2PO_3H_2$[c]
10 R = $CH_2P(OiPr)O_2H$[c]



a. R,R/S,S racemate
b. R,R isomer
c. R,R/S,R 1:1 mixture

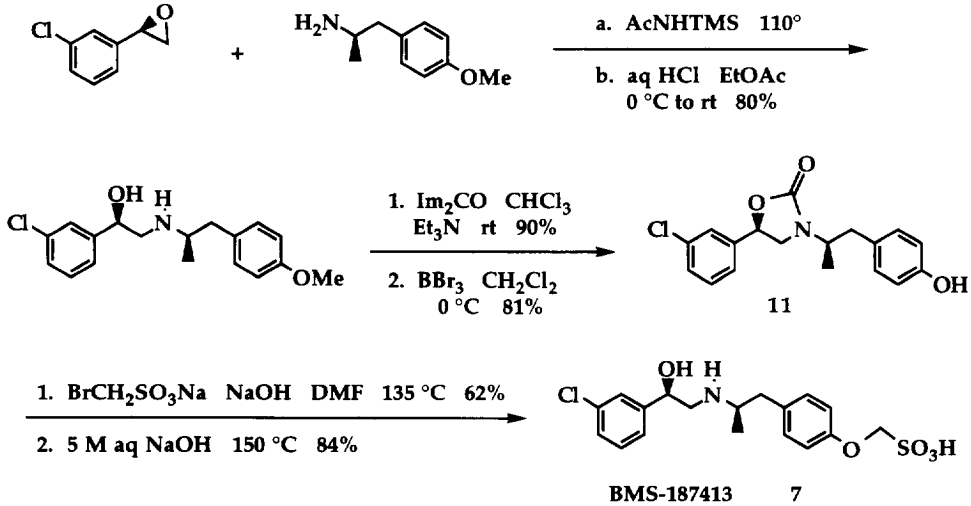
Materials and Methods:

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. Binding constants were determined by inhibition of ^{125}ICYP binding using 50 nM radioligand for β_1, 25 nM radioligand for β_2, and 250 nM radioligand for β_3. Binding constants reported are the mean of at least two determinations using different membrane preparations. Values varied by approximately 20%. For each determination, a seven point binding inhibition curve was constructed employing the test compound at concentrations from 0.1 nM to 100 μM.

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] Maximal cyclase activity was determined with (-)-isoproterenol. Adenylyl cyclase activities were determined in single or duplicate assays. Where duplicated, variability was less than 35%.

BRL 35135 (**1**) and BRL 37344 (**2**) were prepared by literature described routes.[11] Primary alcohol **3** was obtained by reduction of BRL 35135 (LAH, THF, rt, 64%). Primary amide **4** was obtained by ammonolysis of BRL 35135 (NH_3, MeOH, NaCN, rt, 78%). Hydroxamic acid **5** was obtained by treatment of BRL 35135 with hydroxylamine (NH_2OH•HCl, NaOMe, MeOH, 50 °C, 50%). The phosphonic acid **9** and its monoisopropyl ester **10** were prepared as reported.[6]

The synthesis of BMS-187413 (**7**) is shown below. (*R*)-3-chlorostyrene oxide (Kaneka) was coupled with (*R*)-4-methoxyamphetamine[12] via its N-TMS derivative.[13] After protection of the ethanolamine moiety and deprotection of the phenol to **11**, coupling with $BrCH_2SO_3Na$[14] and saponification in a steel bomb provided BMS-187413.



Phosphinic acid **8** was prepared analogously to BMS-187413 from **11** (a. $ClCH_2P(Me)O_2H$, NaOH, DMF, 135 °C, 53%.[15,16] b. 5 M aq NaOH, 150 °C, 68%.). The malonic acid **6** was also obtained from **11** (a. $ClCH(CO_2Me)_2$, Na_2CO_3, acetone, rt.[17] b. aq NaOH, MeOH, rt. c. 5 M aq NaOH, reflux.).[18]

Biological Results and Discussion:

The table below shows human β_1, β_2, and β_3 AR binding data and β_3 stimulatory data for compounds **1–10**. All of the compounds assayed for β_3 stimulatory activity were partial agonists with intrinsic activities (IA) ranging from 0.53 to 0.77.[19] Binding selectivity for β_3 over β_1 ($S_{3/1}$) spanned a 150-fold range with the molecules bearing negatively charged functional groups (carboxylic acid **1**, malonic acid **6**, sulfonic acid **7**, phosphinic acid **8**, phosphonic acid **9**, and phosphonic monoester **10**) being more selective than the molecules bearing polar, uncharged groups (primary alcohol **3**, primary amide **4**, and hydroxamic acid **5**[20]). The least selective compound, ester **2**, was also the least polar. Binding selectivity for β_3 over β_2 ($S_{3/2}$) spanned a 1300-fold range, and again, molecules bearing negatively charged groups were more selective than molecules bearing polar, uncharged functional groups, and the least selective compound (ester **2**) was the least polar. We conclude that polarity, and optimally a full negative charge, enhances binding selectivity for the β_3 AR over the β_1 and β_2 ARs.

BINDING CONSTANTS, BINDING SELECTIVITIES, AND STIMULATORY ACTIVITIES [a]

COMPOUND	β_1 K_i	β_2 K_i	β_3 K_i	$S_{3/1}$	$S_{3/2}$	β_3 EC_{50} (IA)
1 (carboxylic acid)	**11300**	**630**	**960**	**12.**	**0.66**	**680 (0.67)**
2 (methyl ester)	**170**	**3.2**	**1150**	**0.15**	**0.0028**	**420 (0.69)**
3 (primary alcohol)	**1250**	**260**	**2140**	**0.58**	**0.12**	**750 (0.77)**
4 (primary amide)	**590**	**170**	**3700**	**0.16**	**0.046**	**1380 (0.60)**
5 (hydroxamic acid)	**310**	**112**	**1960**	**0.16**	**0.057**	**1610 (0.66)**
6 (malonic acid)	**243000**	**17100**	**11100**	**22.**	**1.5**	**NT**
7 (sulfonic acid)	**7600**	**2250**	**806**	**9.4**	**2.8**	**60 (0.57)**
8 (phosphinic acid)	**140000**	**73700**	**19900**	**7.0**	**3.7**	**NT**
9 (phosphonic acid)	**>100000**	**39700**	**55300**	**>1.8**	**0.72**	**2640 (0.53)**
10 (phosphonic monoester)	**139000**	**42600**	**18800**	**7.4**	**2.3**	**610 (0.59)**

[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}ICYP binding. Binding selectivity for β_3 over β_n is defined by $S_{3/n} = \beta_n K_i / \beta_3 K_i$. 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.

Examination of the binding constants, β_1 K_i and β_2 K_i, that factor into the binding selectivities discussed above, reveals that both β_1 and β_2 binding affinity were highest for the least polar compound, ester **2**, lower for the molecules bearing polar, uncharged functional groups, and lowest for the molecules bearing negatively charged groups. Thus, the positive effect of polarity, and optimally negative charge, on β_3 binding selectivity results in large part from a decrease in β_1 and β_2 binding affinity.[2]

In contrast to the simple inverse relationship between β_1 and β_2 binding affinities and the degree of polarity, β_3 binding affinity did not reflect the degree of polarity straightforwardly. This is seen by comparing the β_3 K_i values for acid **1**, ester **2**, and phosphonic acid **9**. It is tempting to explain the low binding potency of phosphonic acid **9** by citing its highly polar, doubly charged functional group; yet, acid **1** bound as avidly as ester **2**, despite being more polar. We suspect that the functional group in **R** impacts *two* factors that determine β_3 binding affinity. One is desolvation of the functional group upon binding; the other is the ability of the functional group to bind to a specific site on the β_3 AR. Both of these factors may be analyzed in terms of functional group polarity. With respect to desolvation, the higher the degree of polarity, the greater the desolvation energy cost for bringing the functional group into the receptor. In addition to being an important factor in β_3 AR binding, this is probably the dominant factor in β_1 and β_2 AR binding.

Regarding a specific binding interaction with the β_3 AR, perhaps a positively charged amino acid residue[21] can productively bind polar, and optimally negatively charged, functional groups, providing a limited amount of binding energy. This hypothetical binding interaction would offset the desolvation energy cost of bringing the functional group into the receptor. Thus, the observed similar β_3 binding affinities of acid **1** and ester **2** could reflect a situation where, relative to the ester group of **2**, the carboxyl group of **1** provides greater specific binding interaction energy and costs a similarly larger amount of energy to desolvate. The same argument could explain why primary alcohol **3**, primary amide **4**, hydroxamic acid **5**, and sulfonic acid **7** all have β_3 binding affinities close to that of ester **2**. On the other hand, the phosphonic acid **9** may have bound with lower affinity because its specific binding interaction does not as fully compensate for the much greater energy cost inherent in desolvating the doubly charged, phosphonic acid group. By the same argument, doubly charged malonic acid **6**[22] would also be expected to have lower β_3 binding affinity, as was observed. The reason for the lower binding affinities of phosphinic acid **8** and phosphonic monoester **10** is unclear, but it is possible that their desolvation energy costs are very high, despite being only singly charged, because of their highly polar P-O bonds. Thus, the combination of β_3 specific binding interaction and desolvation energy cost factors could largely explain the observed relative binding affinities of these molecules for the β_3 AR.[23,24]

Turning to the issue of which functional group(s) can play the same role as the carboxyl, all of the molecules examined here that bear negatively charged functional groups exhibited binding selectivities in the same range as those of carboxylic acid **1**. However, only the sulfonic acid **7** also exhibited comparable β_3 binding potency. While the β_3 stimulatory potency of sulfonic acid **7** was substantially greater than that of carboxylic acid **1**, its intrinsic activity was marginally lower. The reason for these differences remains unclear, and it is not known how these differences would affect activity in humans. Overall, BMS-187413 (**7**) has in vitro properties comparable to those of BRL 37344 (**1**).

Conclusions:

We have identified the negative charge of the carboxyl group as its property that enhances β_3 AR binding selectivity. This selectivity enhancement is largely due to a decrease in β_1 and β_2 binding affinity. We hypothesize that a combination of β_3 specific binding interaction and desolvation energy cost factors

explain the relative binding affinities of compounds **1–10** for the β_3 AR. Although all of the molecules examined that bear negatively charged functional groups exhibited some β_3 binding selectivity, only the sulfonic acid **7** showed both good β_3 binding selectivity and submicromolar β_3 binding potency. BMS-187413 (**7**) is a novel and potent β_3 adrenergic agonist that binds selectively, and thus has an in vitro profile that compares favorably with that of BRL 37344 (**1**).

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 acknowledge Dr. William Washburn, Dr. Scott Biller, and Dr. Raj N. Misra for helpful discussions.

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

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20. It is likely that the hydroxamic acid is largely undeprotonated at the pH of the assay buffer: Challis, B. C.; Challis, J. A. In *Comprehensive Organic Chemistry*; Sutherland, I. O., Ed.; Pergamon: New York, 1979; Vol 2, pp 957—1065. Bauer, L.; Exner, O. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 376. Monzyk, B.; Crumbliss, A. L. *J. Org. Chem.* **1980**, *45*, 4670.

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22. Malonic acids, especially α-aryloxymalonic acids, are doubly deprotonated at the pH of the assay buffer (pH 7.4). See Roberts, S. M. In *Comprehensive Organic Chemistry*; Sutherland, I. O., Ed.; Pergamon: New York, 1979; Vol 2, pp 739—778.

23. Steric interactions may also play a role in determining β_3 binding affinity, as the molecules bearing larger functional groups tend to exhibit lower β_3 binding potency.

24. We note that some of the compounds tested herein were single *R,R* isomers and others were 1:1 mixtures of *R,R* isomers with either *S,R* or *S,S* isomers. We believe that our analyses in which mixtures and single isomers are considered together are, nevertheless, valid for the following reasons. Based on general SAR of β AR ligands and the specific SAR of Beecham's compounds, we believe that only the *R,R* isomers of the compounds described in this paper are pharmacologically relevant. See, respectively (a) Ruffolo, R. R., Jr. *Drug Design and Discovery* **1993**, *9*, 351. (b) Muzzin, P.; Boss, O.; Mathis, N.; Revelli, J.-P.; Giacobino, J.-P.; Willcocks, K.; Badman, G. T.; Cantello, B. C. C.; Hindley, R. M.; Cawthorne, M. A. *Mol. Pharm.* **1994**, *46*, 357. (c) Oriowo, M. A.; Chapman, H.; Kirkham, D. M.; Sennitt, M. V.; Ruffolo, R. R., Jr.; Cawthorne, M. A. *J. Pharm. Exp. Ther.* **1996**, *277*, 22. *S,S* and *S,R* isomers in mixtures with *R,R* isomers can be considered inert. Compounds described herein which are 1:1 mixtures ought to have exhibited binding constants twofold higher than those that would have been measured had the pure *R,R* isomers been tested. (Reference 24c clearly shows in several assays that BRL 37344 (**1**), a *R,R/S,S* racemate, is half as active as its *R,R* component isomer, and that the *R,S* isomer, the *S,R* isomer, and the *S,S* isomer are all essentially inert.) Since this argument applies to binding to all β ARs, binding selectivities observed for the mixtures are the same as those that would have been measured with pure *R,R* isomers. Correction for the distortion of binding constants, by adjusting the binding constants of some compounds by a factor of 2, does not alter our overall interpretation of the data.

(*Received in USA* 21 *February* 1997; *accepted* 19 *May* 1997)