

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

# CARBOXYL-PROMOTED ENHANCEMENT OF SELECTIVITY FOR THE $\beta_3$ ADRENERGIC RECEPTOR. NEGATIVE CHARGE OF THE SULFONIC ACID BMS-187413 INTRODUCES $\beta_3$ BINDING SELECTIVITY

Philip M. Sher,\* Arvind Mathur, Liesl G. Fisher,<sup>1</sup> Gang Wu, Stephen Skwish, Inge M. Michel, Steven M. Seiler, and Kenneth E. J. Dickinson

> Bristol-Myers Squibb Pharmaceutical Research Institute, P. O. Box 4000, Princeton, NJ 08543-4000

Abstract: Carboxyl and other negatively charged groups were found to be most effective at producing human  $\beta_3$  adrenergic receptor binding selectivity in 1 (BRL 37344) and related compounds. The sulfonic acid analog 7 (BMS-187413) is a novel and potent  $\beta_3$  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<sup>2</sup> we provided evidence that the carboxyl group of four carboxyl-containing  $\beta_3$  adrenergic agonists enhances the  $\beta_3$  adrenergic receptor (AR) binding selectivity of those compounds. The goals of this paper are to identify the property of the carboxyl group that improves  $\beta_3$  binding selectivity and to disclose other functional groups that can play the same role. Of the compounds previously examined,<sup>2</sup> the selectivity enhancing effect of the carboxyl was strongest for 1 (BRL 37344), as seen when its  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  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),<sup>3</sup> the primary amide (4),<sup>4</sup> the hydroxamic acid (5), the malonic acid (6),<sup>5</sup> the sulfonic acid (7),<sup>6</sup> the phosphinic acid (8),<sup>6,7</sup> the phosphonic acid (9),<sup>6,8</sup> and the phosphonic monoester (10)<sup>6,8</sup> were assayed for their abilities to bind to human  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  ARs using membranes of stably transfected CHO cells. The abilities of the compounds to stimulate adenylyl cyclase in CHO- $\beta_3$  cell membranes were also measured.

- 1  $R = CH_2CO_2H^a$  (BRL 37344)
- 2  $R = CH_2CO_2Me^a$  (BRL 35135)
- $3 \quad \mathbf{R} = \mathbf{CH}_2 \mathbf{CH}_2 \mathbf{OH}^{\mathbf{a}}$
- 4  $R = CH_2CONH_2^a$
- 5  $R = CH_2CONHOH^a$
- $6 \quad \mathbf{R} = \mathbf{CH}(\mathbf{CO}_2\mathbf{H})_2^{\mathbf{b}}$
- 7  $R = CH_2SO_3H^b$  (BMS-187413)
- 8  $\mathbf{R} = \mathbf{CH}_2\mathbf{P}(\mathbf{Me})\mathbf{O}_2\mathbf{H}^{\mathbf{b}}$
- 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  $\beta_1$ ,  $\beta_2$ , or  $\beta_3$  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,<sup>9</sup> 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 <sup>125</sup>ICYP binding using 50 nM radioligand for  $\beta_1$ , 25 nM radioligand for  $\beta_2$ , and 250 nM radioligand for  $\beta_3$ . 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  $\mu$ M.

Adenylyl cyclase activity was measured in CHO- $\beta_3$  cell<sup>9</sup> membranes at 37 °C in a buffer containing 30 mM Tris acetate pH 7.6, 5 mM MgCl<sub>2</sub>, 5 mM phosphocreatine, 50 U/mL creatine phosphokinase, 1 mM IBMX, 0.2 mM ATP, 1 x 10<sup>7</sup> cpm/mL [ $\alpha$ -<sup>32</sup>P]ATP, 2  $\mu$ M GTP, and 1 mM EGTA. Radioactive [<sup>32</sup>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 [<sup>3</sup>H]cAMP as an internal standard.<sup>10</sup> 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.<sup>11</sup> 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<sub>3</sub>, MeOH, NaCN, rt, 78%). Hydroxamic acid **5** was obtained by treatment of BRL 35135 with hydroxylamine (NH<sub>2</sub>OH•HCl, NaOMe, MeOH, 50 °C, 50%). The phosphonic acid **9** and its monoisopropyl ester **10** were prepared as reported.<sup>6</sup>

The synthesis of BMS-187413 (7) is shown below. (*R*)-3-chlorostyrene oxide (Kaneka) was coupled with (*R*)-4-methoxyamphetamine<sup>12</sup> via its N-TMS derivative.<sup>13</sup> After protection of the ethanolamine moiety and deprotection of the phenol to 11, coupling with BrCH<sub>2</sub>SO<sub>3</sub>Na<sup>14</sup> 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%.<sup>15,16</sup> b. 5 M aq NaOH, 150 °C, 68%.). The malonic acid 6 was also obtained from 11 (a.  $ClCH(CO_2Me)_2$ , Na<sub>2</sub>CO<sub>3</sub>, acetone, rt.<sup>17</sup> b. aq NaOH, MeOH, rt. c. 5 M aq NaOH, reflux.).<sup>18</sup>

#### **Biological Results and Discussion:**

The table below shows human  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  AR binding data and  $\beta_3$  stimulatory data for compounds 1–10. All of the compounds assayed for  $\beta_3$  stimulatory activity were partial agonists with intrinsic activities (IA) ranging from 0.53 to 0.77.<sup>19</sup> Binding selectivity for  $\beta_3$  over  $\beta_1$  (S<sub>3/1</sub>) 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<sup>20</sup>). The least selective compound, ester 2, was also the least polar. Binding selectivity for  $\beta_3$  over  $\beta_2$  (S<sub>3/2</sub>) 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  $\beta_3$  AR over the  $\beta_1$  and  $\beta_2$  ARs.

COMPOUND		$\beta_1  K_i$	β2 Ki	β3 Ki	S <sub>3/1</sub>	S <sub>3/2</sub>	β <sub>3</sub> EC <sub>50</sub> (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 ( <b>0.</b> 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	<b>60</b> (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)

BINDING CONSTANTS, BINDING SELECTIVITIES, AND STIMULATORY ACTIVITIES a

<sup>a</sup> Compounds were assayed for their ability to bind to human  $\beta_1$ ,  $\beta_2$ , and  $\beta_3$  ARs in their respective CHO cell membranes. Binding potency is reported as  $K_i$  (nM), the binding inhibition constant, determined by inhibition of <sup>125</sup>ICYP binding. Binding selectivity for  $\beta_3$  over  $\beta_n$  is defined by  $S_{3/n} = \beta_n K_i / \beta_3 K_i$ . Ability to stimulate adenylyl cyclase activity in CHO- $\beta_3$  AR cell membranes is indicated by EC<sub>50</sub> and intrinsic activity (IA). EC<sub>50</sub> (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,  $\beta_1$  K<sub>i</sub> and  $\beta_2$  K<sub>i</sub>, that factor into the binding selectivities discussed above, reveals that both  $\beta_1$  and  $\beta_2$  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  $\beta_3$  binding selectivity results in large part from a decrease in  $\beta_1$  and  $\beta_2$  binding affinity.<sup>2</sup>

In contrast to the simple inverse relationship between  $\beta_1$  and  $\beta_2$  binding affinities and the degree of polarity,  $\beta_3$  binding affinity did not reflect the degree of polarity straightforwardly. This is seen by comparing the  $\beta_3$  K<sub>i</sub> 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  $\beta_3$  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  $\beta_3$  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  $\beta_3$  AR binding, this is probably the dominant factor in  $\beta_1$  and  $\beta_2$  AR binding.

Regarding a specific binding interaction with the  $\beta_3$  AR, perhaps a positively charged amino acid residue<sup>21</sup> 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  $\beta_3$  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  $\beta_3$  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  $\beta_3$  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  $\beta_3$  specific binding interaction and desolvation energy cost factors could largely explain the observed relative binding affinities of these molecules for the  $\beta_3$  AR.<sup>23,24</sup>

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  $\beta_3$  binding potency. While the  $\beta_3$  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  $\beta_3$  AR binding selectivity. This selectivity enhancement is largely due to a decrease in  $\beta_1$  and  $\beta_2$  binding affinity. We hypothesize that a combination of  $\beta_3$  specific binding interaction and desolvation energy cost factors

explain the relative binding affinities of compounds 1-10 for the  $\beta_3$  AR. Although all of the molecules examined that bear negatively charged functional groups exhibited some  $\beta_3$  binding selectivity, only the sulfonic acid 7 showed both good  $\beta_3$  binding selectivity and submicromolar  $\beta_3$  binding potency. BMS-187413 (7) is a novel and potent  $\beta_3$  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.

# **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.
- Sher, P. M.; Fisher, L. G.; Skwish, S.; Michel, I. M.; Seiler, S. M.; Washburn, W. N.; Dickinson, K. E. J. Med. Chem. Res., in press.
- 3. Duckworth, D. M. US Patent 4 382 958, 1983; Chem. Abstr. 1982, 97, 162563.
- 4. Ainsworth, A. T.; Smith, D. G. US Patent 4 338 333, 1982; Chem. Abstr. 1981, 95, 61726.
- 5. Beeley, L. J.; Smith, R. A. G. World Patent Application 94/24090, 1994; *Chem. Abstr.* 1995, *122*, 31110.
- 6. Sher, P. M.; Mathur, A. US Patent 5 491 134, 1996; Chem. Abstr. 1996, 124, 343660.
- 7. Beeley, L. J.; Berge, J. M. World Patent Application 95/07284, 1995; Chem. Abstr. 1995, 123, 144275.
- Beeley, L. J.; Berge, J. M.; Jarvest, R. L. World Patent Application 94/02493, 1994; *Chem. Abstr.* 1994, 120, 323858.
- (a) Tate, K. M.; Briend-Sutren, M.-M.; Emorine, L. J.; Delavier-Klutchko, C.; Marullo, S.; Strosberg, A. D. Eur. J. Biochem. 1991, 196, 357. (b) Blin, N.; Camoin, L.; Maigret, B.; Strosberg, A. D. Mol. Pharm. 1993, 44, 1094. See also references 19a, 19b, 19c, and 19e.
- 10. Salomon, Y.; Londos, C.; Rodbell, M. Anal. Biochem. 1974, 58, 541.
- 11. Cantello, B. C. C.; Smith, S. A. Drugs of the Future 1991, 16, 797.
- 12. Nichols, D. E.; Barfknecht, C. F.; Rusterholz, D. B.; Benington, F.; Morin, R. D. J. Med. Chem. 1973, 16, 480.
- 13. Atkins, R. K.; Frazier, J.; Moore, L. L.; Weigel, L. O. Tetrahedron Lett. 1986, 27, 2451.
- 14. Petigara, R. B.; Yale, H. L. J. Het. Chem. 1974, 11, 331.

- 15. Moedritzer, K. J. Am. Chem. Soc. 1961, 83, 4381.
- 16. Lee, S-F.; Henrick, C. A. US Patent 4 536 355, 1985; Chem. Abstr. 1986, 104, 225044.
- 17. Takemura, K. H.; Pulickal, M.; Hoff, F. O. J. Org. Chem. 1971, 36, 3646.
- 18. Spectral data were fully consistent with the structures depicted.
- BRL 37344 has sometimes been reported to be a partial agonist in human β<sub>3</sub> CHO cell adenylyl cyclase assays, particularly where cell membranes, rather than whole cells, were used. See (a) Fève, B.; Emorine, L. J.; Lasnier, F.; Blin, N.; Baude, B.; Nahmias, C.; Strosberg, A. D.; Pairault, J. Biol. Chem. 1991, 266, 20329. (b) Liggett, S. B. Mol. Pharm. 1992, 42, 634. (c) 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. (d) Dolan, J. A.; Muenkel, H. A.; Burns, M. G.; Pellegrino, S. M.; Fraser, C. M.; Pietri, F.; Strosberg, A. D.; Largis, E. E.; Dutia, M. D.; Bloom, J. D.; Bass, A. S.; Tanikella, T. K.; Cobuzzi, A.; Lai, F. M.; Claus, T. H. J. Pharm. Exp. Ther. 1994, 269, 1000. (e) Wilson, S.; Chambers, J. K.; Park, J. E.; Ladurner, A.; Cronk, D. W.; Chapman, C. G.; Kallender, H.; Browne, M. J.; Murphy, G. J.; Young, P. W. J. Pharm. Exp. Ther. 1996, 279, 214.
- 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.
- Birnberg, G. H.; Epstein, J. W.; Walker, G. E.; Claus, T. H.; Largis, E. E.; Burns, M. G. 207<sup>th</sup> American Chemical Society National Meeting, San Diego, CA, MEDI 230, March 13-17, 1994.
- 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  $\beta_3$  binding affinity, as the molecules bearing larger functional groups tend to exhibit lower  $\beta_3$  binding potency.
- We note that some of the compounds tested herein were single R,R isomers and others were 1:1 24. 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  $\beta$  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  $\beta$  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.