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POTENT, SELECTIVE BENZENESULFONAMIDE AGONISTS OF THE HUMAN β_3 ADRENERGIC RECEPTOR

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Abstract: A cloned human β_3 adrenergic receptor assay was used to identify phenoxypropanolamine agonist 1. SAR studies led to the identification of benzenesulfonamide derivative **20**, a 6.3 nM β_3 agonist which shows 30-fold selectivity for β_3 agonist activity over β_1 and β_2 receptor binding. Further refinement of this lead provided 4-bromo derivative **39**, a subnanomolar agonist with 660-fold and 230-fold selectivity over β_1 and β_2 , respectively. © 1998 Elsevier Science Ltd. All rights reserved.

Activation of the β_3 adrenergic receptor on the surface of adipocytes leads to increases in intracellular cAMP and stimulation of lipolysis. In brown adipose tissue, this serves to up-regulate and activate the mitochondrial uncoupling protein (UCP1), which mediates a proton conductance pathway that uncouples oxidative phosphorylation leading to a net increase in energy expenditure. Increasing metabolic rate with a selective β_3 agonist is an attractive approach for the treatment of obesity.² Selective β_3 agonists have been identified on the basis of their ability to stimulate lipolysis in rat adipocytes^{3,4} or activate rat brown adipose tissue⁵⁻⁷ in the absence of β_1 or β_2 effects; however, weight loss studies with these agents in humans were inconclusive and were complicated by muscle tremors^{8,9} (a β_2 effect) and, in some cases, tachycardia (a β_1 effect).¹⁰ Both the rat^{11,12} and human^{13,14} β_3 receptors have been cloned, and pharmacological differences between the two have been noted.¹⁵ Thus, a cloned human receptor assay would appear to offer major advantages over rodent models for the identification of β_3 agonists which will be both efficacious and free from β_1 and β_2 side-effects in the clinic. Herein we report the development of potent and selective agonists of the human β_3 receptor. Several other reports of human β_3 agonists have recently appeared.¹⁶⁻¹⁸

Selected arylethanolamine and aryloxypropanolamine derivatives from the Merck sample collection were screened for their ability to stimulate increases in cAMP in Chinese hamster ovary (CHO) cells expressing the cloned human β_3 receptor, but not in cells expressing the cloned human β_1 or β_2 adrenergic receptor.¹⁹ This assay led to the identification of phenoxypropanolamine **1**, a 300 nM β_3 partial agonist which showed no agonist



activity at β_1 and low efficacy at β_2 (17% of the maximum response elicited by isoproterenol, a nonselective full agonist). In contrast, the known human β_3 partial agonist CGP 12177A²⁰ had a β_3 EC₅₀ of 5300 nM, with 26% activation. Because the original sample 1 was a mixture of all four diastereomers, the individual isomers were synthesized (see below) and assayed. As expected, diastereomers 2 and 3 (Table 1) with (S)-hydroxy centers

| Compound | Configuration of hydroxy center | Configuration of methyl center | <u>β3 EC50 (%act)</u> ^a | $\beta_1 EC_{50}$ (%act) ^a | $\beta_2 EC_{50} (\% act)^a$ |
|----------|------------------------------------|-----------------------------------|------------------------------------|---------------------------------------|------------------------------|
| 1 | mixtureb | mixtureb | 300 (33) | (0 @ 1000) | 10 (17) |
| 2 | S | S | 200 (50) | (0 @ 1000) | (0 @ 1000) |
| 3 | S | R | 600 (55) | (0 @ 1000) | 5.0 (25) |
| 4 | R | R | 6000 (50) | (0 @ 1000) | 30 (25) |
| 5 | R | S | 2000 (30) | (0@1000) | (0 @ 1000) |

Table 1. Activity of compound 1 and its individual diastereomers at the cloned human β_1 , β_2 , and β_3 receptors.

^aAdenylyl cyclase activation given as % of the maximal stimulation with isoproterenol; Single point data are reported in parentheses as (% activation @ concentration in nM). ^bNMR analysis indicated a 4:3 mixture of (S^*, S^*) and (S^*, R^*) isomers.

were more potent than the corresponding (R) isomers 4 and 5. While none of the diastereomers showed agonist activity at the β_1 receptor, compounds 3 and 4, which contain (R)-methyl groups, showed partial agonist activity at the β_2 receptor. Again, the (S)-hydroxy diastereomer 3 was more potent than the (R)-hydroxy derivative 4. Thus SAR studies were initiated in the more potent and selective (S,S) series.

The synthesis of (S,S) diastereomer 2 is illustrated is Scheme $1.^{21,22}$ Benzyloxyphenol **6a** was treated with sodium hydride in dimethylformamide and the resultant sodium salt was alkylated with commercially available (S)-glycidyl 3-nitrobenzenesulfonate (7) to provide epoxide **8a**. Treatment with the requisite amine **9** (R = Me, n = 2, R' = OMe)²³ in refluxing methanol followed by deprotection with hydrogen over Pearlman's catalyst gave the desired phenoxypropanolamine **2** (R = Me, n = 2, R' = OMe). Diastereomers **3–5** were synthesized in an analogous fashion starting with the appropriate stereoisomers of glycidyl sulfonate **7** and amine **9**. Structural analogs **11**, **12**, and **14–20** (Table 2) were synthesized from epoxide **8a** and the appropriate amine **9**. Compound **13** was synthesized from benzyl ether **10** (R = Me, n = 1, R' = OH) by treatment with carbonyl diimidazole to form the corresponding oxazolidinone. Alkylation of the phenol with methyl iodide and potassium carbonate in refluxing acetone followed by hydrolysis of the oxazolidinone with 1 N aqueous sodium hydroxide in ethanol at 90 °C provided benzyl ether **10** (R = Me, n = 1, R' = OMe), which was deprotected as described above to give the desired product **13**.

Scheme 1. Asymmetric synthesis of phenoxypropanolamine derivatives.



An alternate route was developed for the synthesis of sulfonamide analogs 24-40 (Table 3).²⁴ Epoxide **8b** was synthesized from the corresponding *tert*-butyldimethylsilyloxyphenol **6b** (Scheme 1) as described above for the benzyl derivative. As illustrated in Scheme 2, treatment with 4-aminophenethylamine (21) followed by

protection of the resultant secondary amine with one equivalent of di-*tert*-butyldicarbonate in tetrahydrofuran gave aniline 22. Acylation with the appropriate sulfonyl chloride 23 in dichloromethane with excess pyridine and removal of both the silyl ether and *tert*-butyl carbamate protecting groups using methanolic hydrogen chloride provided the desired products 24–40.





Table 2 summarizes the in vitro activity of diastereomer 2 and related compounds. While these derivatives caused increases in cAMP in CHO cells expressing the human β_3 receptor, they showed little intrinsic activity (0–10% of the maximum stimulation of isoproterenol; data not shown) at the human β_1 or β_2 receptor. Competitive binding assays revealed they were antagonists at these receptors. In fact compound 2 was 10- to 20-fold selective for β_1 and β_2 binding over β_3 agonist activity. Removal of the methyl group on the amino chain gave derivative

| HU ~ | | ₹ R | | | | |
|----------|---|-----|----------------------|--|--|---|
| Compound | n | R | R' | nM β ₃ EC ₅₀ (%act) | β_1 Binding IC ₅₀ (nM) | β_2 Binding IC ₅₀ (nM) |
| 2 | 2 | Me | OMe | 200 (50) | 10 | 20 |
| 11 | 2 | Н | OMe | 600 (24) | 15 | 63 |
| 12 | 2 | Me | OH | 12 (37) | 15 | 10 |
| 13 | 1 | Me | OMe | 1000 (52) | nd | nd |
| 14 | 1 | Me | OH | 25 (60) | 81 | 84 |
| 15 | 1 | н | OH | 30 (48) | 900 | 150 |
| 16 | 1 | н | NH ₂ | 440 (49) | 1000 | 500 |
| 17 | 1 | Н | NHCOMe | 27 (22) | 25 | 11 |
| 18 | 1 | Н | NHCOPh | 62 (16) | 6.7 | 230 |
| 19 | 1 | Н | NHSO ₂ Me | 110 (58) | 830 | 220 |
| 20 | 1 | Н | NHSO ₂ Ph | 6.3 (51) | 180 | 190 |

Table 2. Comparison of β_3 AR agonist activity and β_1 and β_2 AR binding affinity for analogs of 1.

^aAdenylyl cyclase activation given as % of the maximal stimulation with isoproterenol.

^bReceptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ¹²⁵I-iodocyanopindolol.

11, with decreased β_3 agonist activity; however, when the methoxy group of 2 was replaced with a hydroxy group, the resultant phenol derivative 12 showed a 10-fold increase in β_3 agonist activity. Since potent binding to β_1 and β_2 was maintained, this derivative was completely nonselective. Shortening the aminoalkyl chain to give phenethyl amine 13 led to a decrease in β_3 activity. In this series, when the methoxy group was replaced with a phenol (compound 14), β_3 activity increased and both β_1 and β_2 binding affinities decreased. Compound 15, which lacks the α -methyl substituent, shows 30-fold and 5-fold selectivity for β_3 agonist activity over β_1 and β_2 binding, respectively. When the right-side phenol was replaced with other substituents capable of forming hydrogen bonds such as aniline, acetamide, benzamide, and methyl sulfonamide (derivative 20 showed a dramatic increase in both potency and selectivity. This 6.3 nM β_3 agonist is 30-fold selective over both β_1 and β_2 binding, and binds to the β_3 receptor with an IC₅₀ of 17 nM. In addition, it is a potent stimulator of lipolysis in vitro in both human and rhesus adipose tissue, but not in adipocytes from rats.²⁵

A variety of aliphatic and aromatic sulfonamide derivatives were synthesized, some of which are shown in Table 3. Activation of the β_3 receptor ranged from 55-100%; however, we do not consider these differences to be biologically significant. In cloned β_3 receptor assays, efficacy varies with receptor expression levels,²⁶ which are low in our assay,¹⁹ and thus may underestimate lipolytic effects in vivo. In general, these compounds had no significant agonist activity at the β_2 receptor. In the β_1 clone (clone A) originally used in the assay, these derivatives showed no significant activation of the receptor at 1 μ M with the exception of cyclohexanesulfonamide 27. Subsequently, several derivatives were assayed in a CHO cell line containing a more highly coupled β_1 clone (clone B). While full agonists such as isoproterenol show similar activity in both clones, aromatic sulfonamides 33 and 38–40, which showed no agonist activity in clone A at 1 μ M, do appear to be partial agonists in clone B. Because it is not apparent which clone better reflects the in vivo situation, clone B, a "worse-case" clone, has replaced clone A in our screening assay.

While aliphatic sulfonamides 24–27 were less potent than aromatic sulfonamide lead 20, the phenethylsulfonamide derivative 29 proved to be equipotent. This suggests that potency and selectivity are not strictly dependent on the acidity of the sulfonamide, which is reduced in aliphatic derivative 29. Shortening or lengthening the phenyl tether to give benzyl and phenylpropyl sulfonamides 28 and 30 led to decreased activity. SAR studies on derivatives 29 and 20 were initiated; however only the latter led to compounds with improved potency and selectivity.

As illustrated for chloro series **31–33**, substitution at the para position of the benzenesulfonamide ring of lead **20** was generally favorable, while substitution at ortho and meta positions resulted in a decrease in β_3 agonist activity. Activation of the β_3 receptor was relatively insensitive to electronic effects on the ring. The 4-chloro, 4-methyl, and 4-methoxy derivatives **33**, **34**, and **35** all have similar potency to the unsubstituted lead compound **20**; however, chloro derivative **33** shows a 10-fold decrease in binding affinity for the β_1 receptor and is thus more selective. While 3,4-dichlorobenzene sulfonamide **36** is 10-fold less potent at β_3 , it does maintain excellent selectivity over β_1 binding affinity (IC₅₀ > 10 μ M). The isosteric 2-naphthyl derivative **37** also showed decreased binding to β_1 (IC₅₀ 9 μ M), but was 10-times more potent than the 3,4-dichloro derivative at β_3 . The related 3-quinoline derivative **38** showed increased potency (β_3 EC₅₀ 1.3 nM) with \geq 200-fold selectivity over both β_1 and β_2 binding. Both the 4-bromo and 4-iodophenyl derivatives **39** and **40** were also highly potent and selective compounds reported

to date, with 660-fold and 230-fold selectivity for efficacy at β_3 over β_1 and β_2 binding, respectively. Moreover, it is 440-fold selective for β_3 agonist activity over partial agonist activity at β_1 .

In conclusion, SAR studies on screening lead 1 have led to the identification of a potent and selective series of aryl sulfonamide human β_3 adrenergic receptor agonists. The most potent of these, bromo derivative **39**, is a subnanomolar β_3 agonist with >200-fold selectivity over β_1 and β_2 binding. With the availability of these potent and selective agonists of the human receptor, it now should be possible to determine whether appropriate β_3 agonists are useful for increasing energy expenditure and inducing weight loss in primates.

| но | Į | NHSO₂R | | | | |
|----------|------------------------------------|--|--|--|--|--|
| Compound | R | nM β_3 EC ₅₀ (%act) ^a | $\begin{array}{c} nM \ \beta_1 \ EC_{50} \\ (\%act)^a \end{array}$ | β_1 Binding IC ₅₀ ^b (nM) | $\begin{array}{c} nM \ \beta_2 \ EC_{50} \\ (\%act)^a \end{array}$ | β_2 Binding IC ₅₀ ^b (nM) |
| 24 | nPro | 60 (61) | (4 @ 1000) | 1000 | (4 @ 1000) | 500 |
| 25 | nPent | 4000 (80) | (5 @ 1000) | 1000 | (0 @ 1000) | 250 |
| 26 | isoPro | 25 (66) | (0 @ 1000) | 100 | (0 @ 1000) | 58 |
| 27 | cHex | 1500 (100) | 1200 (27) | 2000 | (0 @ 1000) | 3000 |
| 28 | CH ₂ Ph | 39 (70) | (0 @ 1000) | 60 | (2 @ 1000) | 21 |
| 29 | (CH ₂) ₂ Ph | 5.9 (85) | (0 @ 1000) | 110 | (0 @ 1000) | 120 |
| 30 | (CH ₂) ₃ Ph | 92 (70) | ndc | 1000 | ndc | 130 |
| 31 | 2-Cl-Ph | 54 (75) | 3 (15) | 610 | (2 @ 1000) | 100 |
| 32 | 3-Cl-Ph | 15 (100) | (8 @ 1000) | 1000 | (26 @ 1000) | 370 |
| 33 | 4-Cl-Ph | 4.8 (55) | 450 (54) ^d | 1000 | (0 @ 1000) | 280 |
| 34 | 4-Me-Ph | 2.6 (81) | (0 @ 1000) | 150 | (9 @ 1000) | 170 |
| 35 | 4-OMe-Ph | 4.3 (76) | (2 @ 1000) | 140 | (9 @ 1000) | 190 |
| 36 | 3,4-(Cl) ₂ -Ph | 58 (74) | (0@1000) | >10,000 | (0 @ 1000) | 3000 |
| 37 | 2-Naphthyl | 5.4 (100) | (0 @ 1000) | 9000 | (0 @ 1000) | 630 |
| 38 | 3-Quinolinyl | 1.3 (73) | 300 (24) ^d | 500 | (0 @ 1000) | 240 |
| 39 | 4-Br-Ph | 0.77 (86) | 340 (42) ^d | 510 | (0 @ 1000) | 180 |
| 40 | 4-I-Ph | 1.6 (73) | 320 (37) ^d | 170 | (0 @ 1000) | 120 |

| Table 3. | Activity of sulfonamide derivatives | at the cloned hu | man β_1, β_2, a | and β_3 receptors. |
|----------|---|------------------|---------------------------|--------------------------|
| | ЮН Н | | | |
| ^ | | | | |
| | $\gamma^{*} \vee \vee \gamma^{*} \vee \gamma^{*} \vee \gamma^{*}$ | | | |

^aAdenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. Single point data are reported in parentheses as (%activation @ concentration in nM). ^bReceptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ¹²⁵I-iodocyanopindolol. ^cnd = not determined ^dData from clone B (see text).

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