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POTENT, SELECTIVE BENZENESULFONAMIDE AGONISTS OF THE HUMAN β_3 ADRENERGIC RECEPTOR

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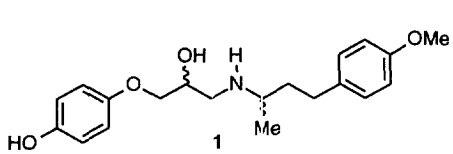
Abstract: A cloned human β_3 adrenergic receptor assay was used to identify phenoxypropanolamine agonist **1**. SAR studies led to the identification of benzenesulfonamide derivative **20**, a 6.3 nM β_3 agonist which shows 30-fold selectivity for β_3 agonist activity over β_1 and β_2 receptor binding. 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.

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 tissue[5–7] 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



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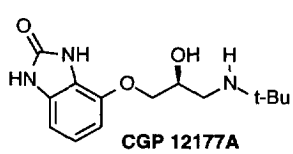

CGP 12177A

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 12177A[20] had a β_3 EC_{50} 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

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

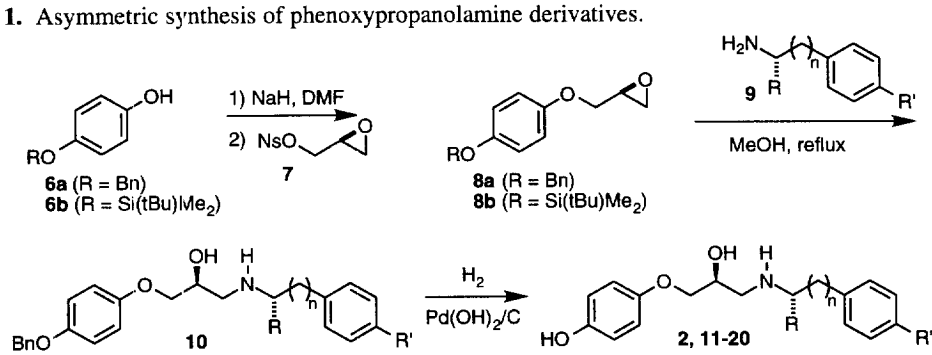
Compound	Configuration of hydroxy center	Configuration of methyl center	β_3 EC_{50} (%act)[a]	β_1 EC_{50} (%act)[a]	β_2 EC_{50} (%act)[a]
1	mixture[b]	mixture[b]	300 (33)	(0 @ 1000)	10 (17)
2	*S*	*S*	200 (50)	(0 @ 1000)	(0 @ 1000)
3	*S*	*R*	600 (55)	(0 @ 1000)	5.0 (25)
4	*R*	*R*	6000 (50)	(0 @ 1000)	30 (25)
5	*R*	*S*	2000 (30)	(0 @ 1000)	(0 @ 1000)

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol; Single point data are reported in parentheses as (%activation @ concentration in nM). [b]NMR 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.**

Scheme 2. Synthesis of sulfonamide derivatives.

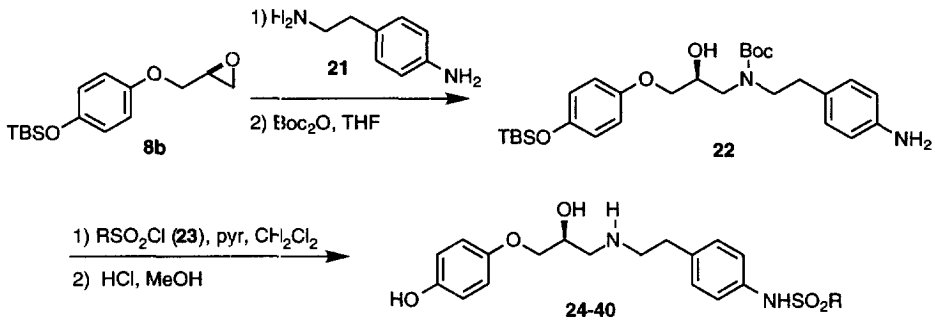
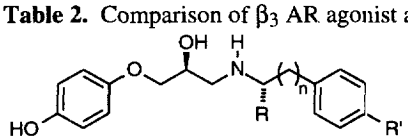


Table 2 summarizes the in vitro activity of diastereomer **2** and related compounds. While these derivatives caused increases in cAMP in CHO cells expressing the human β_3 receptor, they showed little intrinsic activity (0–10% of the maximum stimulation of isoproterenol; data not shown) at the human 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

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



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	H	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	H	OH	30 (48)	900	150
16	1	H	NH_2	440 (49)	1000	500
17	1	H	NHCOMe	27 (22)	25	11
18	1	H	NHCOPh	62 (16)	6.7	230
19	1	H	$NHSO_2Me$	110 (58)	830	220
20	1	H	$NHSO_2Ph$	6.3 (51)	180	190

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol.

[b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-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 (derivatives **16**, **17**, **18**, and **19**, respectively), potency and/or efficacy decreased. In contrast, the benzenesulfonamide 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 IC_{50} 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 μM 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 μM, 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.

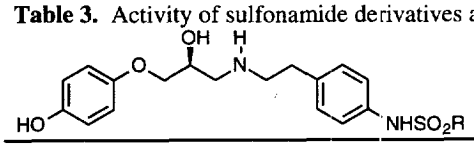
While aliphatic sulfonamides **24–27** were less potent than aromatic sulfonamide lead **20**, the phenethylsulfonamide derivative **29** proved to be equipotent. This suggests that potency and selectivity are not strictly dependent on the acidity of the sulfonamide, which is reduced in aliphatic derivative **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 β1 binding affinity (IC_{50} >10 μM). The isosteric 2-naphthyl derivative **37** also showed decreased binding to β_1 (IC_{50} 9 μM), 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 EC_{50} 1.3 nM) with ≥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. The former, a subnanomolar β_3 agonist, is one of the most 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.

Table 3. Activity of sulfonamide derivatives at the cloned human β_1, β_2, and β_3 receptors.



Compound	R	nM β_3 EC_{50} (%act)[a]	nM β_1 EC_{50} (%act)[a]	β_1 Binding IC_{50}[b] (nM)	nM β_2 EC_{50} (%act)[a]	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_2Ph	39 (70)	(0 @ 1000)	60	(2 @ 1000)	21
29	$(CH_2)_2Ph$	5.9 (85)	(0 @ 1000)	110	(0 @ 1000)	120
30	$(CH_2)_3Ph$	92 (70)	nd[c]	1000	nd[c]	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

[a]Adenylyl cyclase activation given as % of the maximal stimulation with isoproterenol. Single point data are reported in parentheses as (%activation @ concentration in nM). [b]Receptor binding assays were carried out with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol. [c]nd = not determined [d]Data from clone B (see text).

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