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BMS-196085: A Potent and Selective Full Agonist of the Human β_3 Adrenergic Receptor

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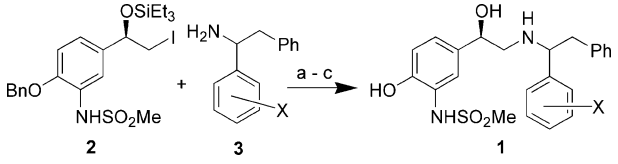
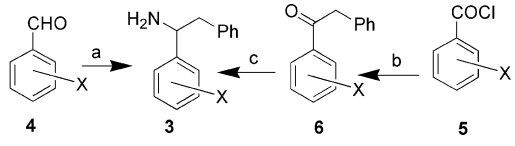
Abstract—A series of 4-hydroxy-3-methylsulfonanilido-1,2-diarylethylamines were prepared and evaluated for their human β_3 adrenergic receptor agonist activity. SAR studies led to the identification of BMS-196085 (**25**), a potent β_3 full agonist ($K_i = 21$ nM, 95% activation) with partial agonist (45%) activity at the β_1 receptor. Based on its desirable in vitro and in vivo properties, BMS-196085 was chosen for clinical evaluation.

Agonist occupancy of the β_3 adrenergic receptor (AR) on the adipose tissue elevates cAMP levels, thereby stimulating lipolysis and upregulating adipose specific genes. The increased expression of uncoupling protein, a brown adipose tissue specific mitochondrial protein, uncouples fatty acid oxidation from oxidative phosphorylation. This process increases heat production with a commensurate boost in energy consumption. β_3 AR agonists represent a novel approach to alter energy utilization and thereby ameliorate obesity and non-insulin dependent diabetes mellitus.[1] The preceding paper[2] described the discovery of hydroxysulfonanilides as a novel class of potent and selective agonists of the human β_3 AR leading up to the discovery of BMS-194449. This paper[3] describes SAR studies in the 4-hydroxy-3-methylsulfonanilido-1,2-diarylethylamine series, leading to the discovery of BMS-196085 (**25**), a β_3 full agonist with a significantly improved profile over BMS-194449, that was selected for clinical evaluation.

The β_3 AR agonists **1** disclosed in this report were prepared by a convergent route comprising coupling of the iodide **2**[2] with various 1,2-diarylethylamines **3** in the presence of diisopropylethylamine followed by sequential removal of the triethylsilyl and the benzyl protecting groups (Scheme 1).[4] The requisite 1,2-diarylethylamines **3** were prepared, as illustrated in Scheme 2, by either sequential treatment of the aldehyde **4** with lithium hexamethyldisilazide and benzyl magnesium chloride[5] or via a two-step sequence utilizing reductive amination of the 1,2-diarylethanone **6** obtained from acid chloride **5** using zinc mediated palladium-catalyzed coupling[6] with benzyl bromide.



Scheme 1. Reagents and conditions: (a) diisopropylethylamine, THF, 140 °C; (b) TFA, aq methanol; (c) H_2, Pd/C, methanol.



Scheme 2. Reagents and conditions: (a) $LiN(SiMe_3)_2$, THF; $PhCH_2MgCl$, THF; aq NH_4Cl; (b) $PhCH_2Br$, Zn, $Pd(PPh_3)_4$, DME; (c) HCO_2NH_4, 160 °C; concd HCl, MeOH.

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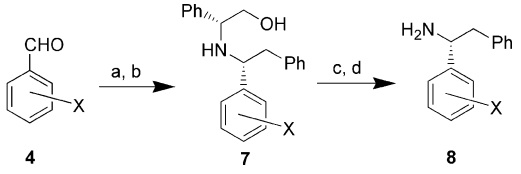
The synthesis of the optically active amine derivatives **8** is shown in Scheme 3.[7] Aldehyde **4** was condensed with (*R*)-phenylglycinol and the resulting imine was added to a solution-suspension of benzylmagnesium chloride and cerium(III) chloride in THF at −45 °C to provide a 10:1 mixture in favor of the desired *R*,*R*-diastereomer **7**, which was obtained in diastereomerically pure form by recrystallization as an HCl salt. Oxidation of the chiral auxiliary with lead tetraacetate generated an imine, which was hydrolyzed to liberate the homochiral amine **8**.[8]

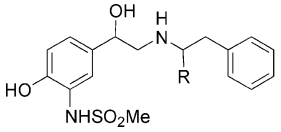
Previous results[2] from our laboratories indicated that the hydroxysulfonanilido-ethanolamine moiety confers full agonist activity at the human β_3 receptor with the 1,2-diarylethylamine promoting high affinity and greater selectivity for binding to the β_3 adrenoceptor over β_1 and β_2 receptors. The parent compound **9** showed reasonable potency, selectivity and full agonist activity for the human β_3 receptor (Table 1). Further SAR studies revealed that *R*,*R* diastereomers were preferred since they provided the most favorable β_3 potency and selectivity profile in this series.[2] The nature of the α aryl ring in the 1,2-diarylethylamine was quite important since replacement with a thiazole or a thiophene resulted in β_2-selective compounds; whereas, certain heterocycles such as a pyridine or benzo[b]thiophene maintained selectivity.

The potency of compounds in this series was significantly influenced by the *para* substituent *R* on the α phenyl ring in the 1,2-diarylethylamine moiety (Table 2). A methyl ester (**18**) or a primary carboxamide (**21** and **22**) provided substantially increased affinity for the β_3 receptor. The β_3 binding selectivity of **20**, **21**, and **23** versus the β_1 receptor is noteworthy.



Scheme 3. Reagents and conditions: (a) (*R*)-2-phenylglycinol, $CDCl_3$; (b) $PhCH_2MgCl$, $CeCl_3$, THF; (c) $Pb(OAc)_4$, methanol; (d) aq HCl, methanol.

Table 1. Activity of compounds at the cloned human β adrenoceptors[9]

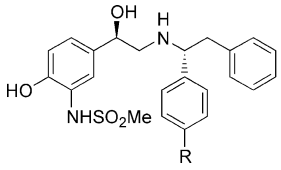


Compd	R	β_3 Binding K_i (nM)	β_3 IA (% act)	Selectivity[a]	
				vs β_1	vs β_2
9	Phenyl	360	133	15	3
10	4-Pyridyl	700	129	30	8
11	2-Thiazolyl	2400	45	4	0.6
12	3-Thienyl	129	110	1	0.3
13	5-Benzo[b]thienyl	230	90	19	2

[a]Binding selectivity is defined as K_i β_1/K_i β_3 or K_i β_2/K_i β_3.

Further SAR in this series established the paradigm that a sterically less demanding hydrogen bond acceptor, such as a methoxyl (**24**) or a benzyloxy group (**28**), in the *para* position of the α aryl ring enhances binding to the β_3 receptor with no significant effect on the affinity for the β_1 or β_2 receptor (Table 3). The reduced potency and selectivity of compound **29** may be attributed to an increase in steric bulk and, secondarily, to the reduced basicity of the oxygen attached to the phenyl ring. Progressive increase in electronegativity of the substituent attached to the phenolic oxygen ultimately abolished the enhanced β_3 affinity. Compare **24**, **25**, and **26**, where a methoxyl or a difluoromethoxy group is favored in the *para*-position of the α aryl ring while a trifluorometh-

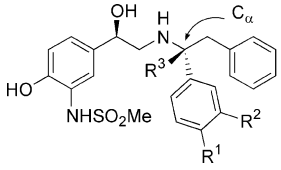
Table 2. Activity of compounds at the cloned human β adrenoceptors[9]



Compd	R	β_3 Binding K_i (nM)	β_3 IA (% act)	Selectivity	
				versus β_1	versus β_2
14[a]	F	836	100	11	1
15[a]	SO_2Me	440	129	53	3
16	CN	210	107	30	4
17	CO_2H	8600	123	17	6
18	CO_2Me	85	98	42	5
19	$CONMe_2$	800	98	25	0.4
20	CONHMe	120	102	79	3
21	$CONH_2$	70	108	90	8
22	CH_2CONH_2	74	106	17	2
23	CONHOH	147	106	537	4

[a]Mixture of four diastereomers.

Table 3. Activity of compounds at the cloned human β adrenoceptors[9]



Compd	R^1	R^2	R^3	β_3 Binding K_i (nM)	Selectivity	
					vs β_1	vs β_2
24[a]	OMe	H	H	44	59	8
25	$OCHF_2$	H	H	21	36	5
26[b]	OCF_3	H	H	210	25	2
27[b]	OCH_2CF_3	H	H	84	83	1
28[b]	OCH_2Ph	H	H	70	58	2
29[b]	OPh	H	H	990	4	0.5
30	OCH_2O		H	8	52	8
31	$OCHF_2$	$OCHF_2$	H	38	37	0.4
32	$OCHF_2$	Me	H	69	6	0.6
33	$OCHF_2$	H	Me	9	12	14

[a]Mixture of four diastereomers.
[b]Mixture of *R*,*R* and *R*,*S* diastereomers.

oxyl is not, since the hydrogen bond acceptor ability of the latter is drastically reduced. The difluoromethoxy group also provides additional metabolic stability by minimizing the propensity for the *N*-dealkylation pathway to produce 4-hydroxy-3-methylsulfonoanilidoethanolamine.[2]

Substitution at the *meta* position of the α phenyl ring is not beneficial since it tends to increase binding to the β_2 receptor. For example, difluoromethoxy (**31**) or a methyl (**32**) substitution at the *meta*-position in the α phenyl ring reduced the binding selectivity ratio (K_i β_2/K_i β_3) of **25** about 10-fold. Although introduction of a methyl group on the α carbon resulted in a significant improvement in β_3 potency (**33**), this effect was accompanied by an even greater increase in affinity for the β_1 receptor.

For compounds with β_3 binding selectivity, intrinsic activity (IA) at the β_1 receptor, measured as a percentage of the maximal increase in the beating rate of an isolated spontaneously beating guinea pig atrium induced by isoproterenol,[10] was found to be a useful in vitro predictor of tachycardia in primates. Increasing lipophilicity was beneficial in reducing β_1 agonist activity in this series (Table 4). Replacing the α phenyl ring with a naphthalene (**36** vs **25**) provided significant reduction in the β_1 IA with concurrent improvement in β_3 binding and the selectivity profile. Similar substitutions in the closely related 3-pyridine series resulted in improved β_3 potency and selectivity but the reduced lipophilicity of these compounds contributed to higher β_1 agonist activity.

Selected compounds were evaluated in vivo in anesthetized African green monkeys to determine the margin of separation between β_3-mediated lipolysis and β_1- or β_2-dependent events as measured by the changes in heart rate and serum potassium levels, respectively. Changes in plasma concentrations of non-esterified fatty acids (NEFA), free glycerol and potassium were determined 30 min after intravenous (iv) administration. Changes in heart rate were monitored over the 30 min period. Compounds **25** and **30** elicited dose-related increases in NEFA levels (ED_{50} = 0.02 mg/kg for both **25** and **30**), achieving the maximal response evoked by isoproterenol. The lipolysis was unchanged in the presence of 0.1 mg/kg of propranolol, a potent β_1 and β_2 antagonist, thus indicating that it was mediated through the β_3 adrenoceptor. However, despite the greater than 50-fold binding selectivity for the β_3 versus β_1 receptor, these compounds produced tachycardia at a dose of 0.1 mg/kg, which was attributed to the activation of β_1 AR. Indeed, the tachycardia was abolished upon co-administration with 0.1 mg/kg of propranolol.

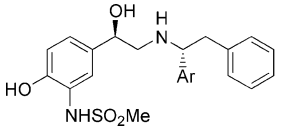
In vivo selectivity of the most promising β_3 agonists, based on their in vitro profile and metabolic considerations, was measured in African green monkeys by increasing the dose progressively (0.01, 0.02, 0.1, 0.5, and 2.5 mg/kg) until the onset of statistically significant β_1- or β_2-mediated side effects. Although all the compounds induced maximal lipolysis comparable to that induced by isoproterenol, the naphthalene containing compounds **34** and **36** exhibited a 10-fold reduction in potency relative to the phenyl series, as shown in Table 5. Compound **25** (BMS-196085) provided the best profile with about 5-fold and 25-fold in vivo selectivity versus β_1 and β_2 adrenergic receptors, respectively.

BMS-196085 emerged as the lead candidate from this series and was chosen for further evaluation. BMS-196085 is 190-fold more potent for binding to the human (K_i = 21 nM) versus murine (K_i = 4,000 nM) β_3 adrenoceptor. It is a partial agonist for the stimulation of adenylate cyclase activity in CHO cells transfected with the human β_1 (IA = 63%) and β_2 (IA = 45%) receptors. In addition, this compound is a partial agonist (IA = 45%) at the β_1 receptor in the guinea pig atria assay.

BMS-196085 showed a high volume of distribution (10 ± 5 L/kg), moderate clearance (36 ± 9 mL/min/kg) and a half-life of 7.3 ± 1.9 h upon iv administration to African green monkeys. Extensive glucuronidation resulted in poor (< 5%) oral systemic bioavailabilty. In vitro studies showed that the phenolic and benzylic hydroxyls were highly susceptible to glucuronidation.

BMS-196085 was administered via subcutaneous osmotic mini-pumps for 2 weeks in obese hyperglycemic ob/

Table 4. Activity of compounds at the cloned human β adrenoceptors[9]



Compd	Ar	β_3 Binding (IA) K_i (nM) (% act)	Selectivity vs β_1	vs β_2	β_1 IA (% act)[a]
30	3,4-Methylenedioxyphenyl	8 (94)	52	8	80
25	4-Difluoromethoxyphenyl	21 (95)	36	5	45
34	1-Napthyl	43 (105)	33	6	40
35	4-Methoxy-1-napthyl	26 (85)	46	9	22
36	4-Difluoromethoxy-1-napthyl	2 (100)	275	9	23
37	6-Methoxy-3-pyridyl	25 (101)	180	30	70
38	2,6-Dimethoxy-3-pyridyl	15 (94)	61	13	60

[a]β_1 intrinsic activity is given as a percent of the maximal increase in the beating rate of an isolated spontaneously beating guinea pig atrium induced by isoproterenol.

Table 5. In vivo selectivity in African green monkeys (iv)

Compd	Lipolysis ED_{50} (mg/kg)	Margin before onset of tachycardia[a]	Margin before onset of hypokalemia[a]
25	0.02	5	25
30	0.02	1	25
34	0.25	2	2
36	0.25	2	2

[a]Margin before onset of tachycardia or hypokalemia was defined as the lowest dose required to produce statistically significant event divided by the ED_{50} for lipolysis.

ob mice to test its efficacy in a rodent model of obesity and diabetes. A dose-dependent reduction in plasma NEFA and free glycerol was observed. In addition, 3.4 mg/kg/day of BMS-196085 significantly reduced plasma glucose concentrations from 399 ± 53 mg/dl in vehicle-treated mice to 207 ± 6 mg/dl. Thus, chronic administration of BMS-196085 produced desirable lowering of plasma glucose and fatty acids in a murine model of obesity-induced diabetes. This is consistent with previous studies demonstrating that chronic treatment with β_3 AR agonists results in proliferation of brown adipose tissue, with an upregulation of the β_3 AR, which is associated with a decrease in plasma glucose, insulin and NEFA levels.[11]

While the low oral bioavailability of BMS-196085 precluded development as an oral agent, the combination of potency, low clearance, and moderate (12 $\mu g/cm^2/h$) transdermal flux across human cadaver skin suggested that transdermal delivery might be a viable means to assess whether long term exposure to a β_3 agonist would increase resting metabolic rate in man.[12] In an initial clinical proof-of-concept study, bolus iv administration of BMS-196085 resulted in elevation of plasma levels of free fatty acids at doses that did not produce any β_1- or β_2-mediated side effects. However, 2-week continuous iv infusion of BMS-196085 up to doses resulting in tachycardia failed to produce statistically significant changes in the resting metabolic rate.[13]

In summary, this paper describes the study of 4-hydroxy-3-methylsulfonanilido-1,2-diarylethylamines as human β_3 adrenergic receptor agonists. The study culminated in the discovery of BMS-196085, a potent β_3 full agonist with good selectivity for β_3 over β_1 and β_2 receptors. However, efforts to demonstrate clinical efficacy with this compound were not successful.

Acknowledgements

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