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Beta 3 Agonists. Part 1: Evolution from Inception to BMS-194449†

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Abstract—Screening of the BMS collection identified 4-hydroxy-3-methylsulfonanilidoethanolamines as full beta 3 agonists. Substitution of the ethanolamine nitrogen with a benzyl group bearing a *para* hydrogen bond acceptor promoted β_3 selectivity. SAR elucidation established that highly selective β_3 agonists were generated upon substitution of C_α with either benzyl to form (*R*)-1,2-diarylethylamines or with aryl to generate 1,1-diarylmethylamines. This latter subset yielded a clinical candidate, BMS-194449 (**35**).[1]

The incidence of obesity and non-insulin dependent diabetes mellitus (Type 2) is increasing at an alarming rate in Western countries.[2,3] Our goal was to utilize β_3 adrenergic mediated thermogenesis to modulate obesity and to lower plasma glucose and insulin levels thereby ameliorating Type 2 diabetes.[4] Treatment of diabetic rodents for 10–15 days with β_3 agonists results in loss of white adipose tissue (WAT), proliferation of brown adipose tissue (BAT), and concurrent normalization of elevated plasma glucose, insulin, nonesterified fatty acid (NEFA), and triglyceride levels.[5] In rodents, activation of β_3 receptors, localized almost exclusively on both brown and white adipocytes, elevates c-AMP levels thereby stimulating lipolysis in WAT and upregulating BAT specific genes.[6] The increased expression of thermogenin, a BAT specific protein that uncouples fatty acid oxidation from oxidative phosphorylation, leads to increased energy expenditure. Elevated NEFA consumption necessitates increased glucose metabolism to maintain homeostasis. The resulting decrease in plasma glucose leads to diminished insulin secretion and peripheral insulin resistance.

Since large adult mammals including man do not have defined BAT depots,[7] our strategy was to utilize full rather than partial β_3 agonists to enhance the probability of eliciting a thermogenic response from whatever BAT is present in man. To minimize β_1 and β_2 side effects, structural features were sought to reduce β_1 and β_2 affinities (K_i) and intrinsic activity (IA). K_i for β_1, β_2, and β_3 and β_3 IA were determined using membranes isolated from CHO cells transfected with human β_1, β_2, or β_3 adrenergic receptors to insure that species dependent differences in β_3 responsiveness did not contribute to clinical failure as was the case for BRL 37344 or CL316243, for which rodent tissues had been employed for SAR elucidation.[3,8,9]

Screening of the Bristol-Myers Squibb compound collection identified a subset of the β_2 selective chemotype, 4-hydroxy-3-methylsulfonanilidoethanolamines that were full β_3 agonists (**1**–**8**).[10] Full adrenergic agonism of this chemotype had been attributed to the bioisosteric relationship between the left-hand portion of this class and the catechol moiety of natural ligands adrenaline and noradrenaline.[11] K_i β_3 for this chemotype was a function of the ethanolamine *N*-substituent; alkyl moieties strongly favored β_2 whereas benzyl or phenethyl promoted β_3 particularly if the aryl ring bore a *p*-methoxyl (see Table 1). Phenethyl containing derivatives **4**–**6**

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†See ref 1.

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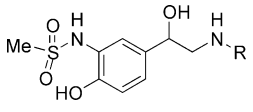
were particularly promising as a starting point in view of the 14-fold increase in selectivity for β_3 relative to β_2 upon progressively increasing the number of methoxy substituents.

We anticipated that replacement of the methoxyl of **5** with an oxyacetic acid residue (**10**) would further increase selectivity for β_3 since we and others had converted *m*-chlorophenylethanolamines to β_3 selective ligands by introduction of an appropriately oriented anionic group.[14] However, this modification diminished β_3 K_i 100-fold relative to **5**; moreover, steric repulsion was not the explanation since the methyl ester **9** bound 16-fold tighter than **10**.[15] Similar results were also obtained for the corresponding *m*-substituted phenoxyacetic acid/ester (data not shown). We attributed these failures to the difference in interactions of the left-hand of these two chemotypes with the adrenergic receptors. Hydroxysulfonanilides being a catechol bioisostere should bind in a specific fashion to fix the molecular orientation; whereas, the loose interactions of the *m*-chlorophenyl moiety of BRL 37344 and CL316243 would be more accommodating.

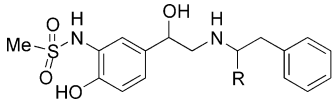
Table 1. Comparison of β_3 AR agonist activity and selectivity versus β_1 and β_2 for compounds **1**–**10**[12,13]



Compd	R	β_3 K_i (nM)	β_3 IA (% act)	Selectivity[b]	
				vs β_1	vs β_2
1[a]	*i*-Pr	4100	109	2	0.6
2[a]	*t*-Amyl	3700	125	0.3	0.03
3[a]	Adamantyl	11,000	110	15	0.04
4[a]	CH_2CH_2Ph	1100	91	2	0.07
5[a]	CH_2CH_2Ph-4-OMe	480	93	4	0.3
6[a]	CH_2CH_2Ph-3,4-$(OMe)_2$	550	110	4	1
7[a]	CH_2Ph	12,700		2	0.25
8[a]	CH_2Ph-4-OMe	1550	95	22	2
9[a]	CH_2CH_2Ph-4-OCH_2CO_2Me	3300	79	0.7	0.01
10[a]	CH_2CH_2Ph-4-OCH_2CO_2H	49,000	77	2	0.06
BRL 37344		660	67	18	1
CL316243		20,000	45	20	11
36[a]	H	17,000	93	0.6	0.1

[a]Racemic mixture.
[b]Selectivity is defined as the ratio of β_1 K_i or β_2 K_i to β_3 K_i.

Table 2. Comparison of β_3 AR agonist activity and selectivity versus β_1 and β_2 for compounds **11**–**17**[12,13]



Compd	R	β_3 K_i (nM)	β_3 IA (% act)	Selectivity[b]	
				vs β_1	vs β_2
11[a]	$(CH_2)_4CO_2H$	87,000	57	1	0.3
12[a]	$(CH_2)_4CO_2Me$	570	89	26	5
13[a]	pentyl	320	77	8	0.5
14[a]	$(CH_2)_4CH_2OH$	480	72	10	0.5
15[a]	$(CH_2)_4CONHMe$	1600	104	7	0.5
16[a]	cyclohexyl	4300	80	7	0.7
17[a]	Ph	360	133	15	3
28	(±) CH_2Ph	1500		6	0.3

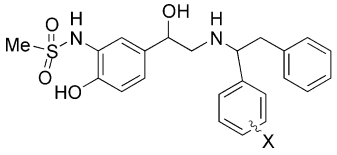
[a]Diastereomeric mixture.
[b]Selectivity is defined as the ratio of β_1 K_i or β_2 K_i to β_3 K_i.

Before abandoning this approach, **11**, bearing a conformationally mobile valeric acid side chain as a Cα substituent of **4,** was synthesized to maximize the probability of achieving proper positioning of the anionic charge (Table 2).[16] Despite this feature, β_3 affinity of **11** was diminished relative to that of **4**; whereas, that of the corresponding methyl ester **12** was so enhanced that **12** became β_3 selective. This effect was unique to the methyl valerate side chain since the corresponding hydrocarbon **13**, alcohol **14**, and amide **15** were β_2 selective. In the course of exploring the SAR of this α appendage, the chain was cyclized to generate **16** and subsequently aromatized to form **17**. Although **16** bearing a cyclohexyl moiety was β_2 selective, **17** containing a planar phenyl was β_3 selective.

Discovery that the 1,2-phenylethylamine moiety of **17** significantly enhanced β_3 affinity of hydroxysulfonanilidoethanolamines was a critical advance. Small lipophilic or modestly polar *para* and/or *meta* substituents of the α aryl ring increased β_1 and β_2 affinity 2- to 4-fold. Commensurate increases were found for β_3 affinity unless the *para* substituent was methoxy (**18**). The 10- to 12-fold further enhancement of β_3 affinity due to the *para* methoxyl of **18**, **20**, and **26** was not emulated by the methyls of **22**, chlorines of **23** or ethyls of **24.** The *para* methoxyl was the salient feature since an additional *meta* methoxyl (**20**) modestly enhanced β_3 binding whereas *ortho* substitution (**21**) decreased β_3 affinity 2-fold (Table 3).

The similarity of this effect to that observed with methyl ester **12** suggested that the effect was due to an appropriately oriented alkoxy fragment attached to a sp^2 carbon. We suggest that the enhanced affinity is not steric

Table 3. Comparison of β_3 AR agonist activity and selectivity versus β_1 and β_2 for compounds **18**–**28**[12,13]



Compd	X	β_3 K_i (nM)	β_3 IA (% act)	Selectivity[b]	
				vs β_1	vs β_2
18[a]	4-OMe	44	99	60	8
19[a]	3-OMe	230	99	16	2
20[a]	3,4-$(OMe)_2$	33	105	69	8
21[a]	2,4-$(OMe)_2$	70	102	37	3
22[a]	3,4-$(Me)_2$	133	79	43	3
23[a]	3,4-$(Cl)_2$	414	97	9	1
24[a]	3,4-$(Et)_2$	520	91	6	0.5
26	(*R*,*R*) 3,4-$(OMe)_2$	11	112	63	6
27	(*R*,*S*) 3,4-$(OMe)_2$	320	93	63	6

[a]Diastereomeric mixture.
[b]Selectivity is the ratio of β_1 K_i or β_2 K_i to β_3 K_i.

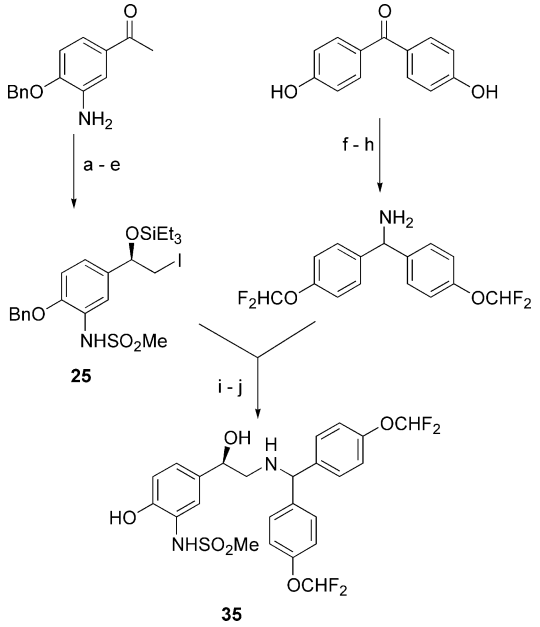
or electronic in origin, but rather stems from ability of *p*-methoxy moiety of the α aryl ring to function as an H-bond acceptor. Although proper spatial presentation of two aryl rings are required to achieve full benefit, this effect is also operative for *N*-benzyl- and *N*-phenethyl-hydroxysulfonanilidoethanolamines since β_3 affinity increased 8- and 3-fold upon *para* methoxylation of respectively **7** and **4** to generate **8** and **5**. Thus, in this instance, β_3 selectivity was induced by increasing β_3 affinity whereas β_3 selectivity had been previously achieved for BRL 37344, CL316243, and other related agonists by introduction of an acidic moiety that disfavored β_1 and β_2 binding.[13]

Knowing that β_3 agonists required a *R* configuration at the hydroxylic center,[10] the *R*,*R* and *R*,*S* diastereomers **26** and **27** comprising **20** were prepared by a route analogous to that outlined in Scheme 1, entailing sequential alkylation of racemic 2-phenyl-1-(3,4-dimethoxyphenyl)ethyl amine with TBS protected (*R*)-iodohydrin **25**, deprotection, and HPLC separation of diastereomers.[17] The β_3 affinity for the *R*,*R* diastereomer **26** was 30-fold greater than that of the *R*,*S* isomer **27** thereby establishing the preferred spatial orientation of the rings. A similar pattern for β affinities and β_3 IA was observed for 17 other *R*,*R*/*R*,*S* pairs of diastereomers (data not shown).[18]

Compounds **28** and **29** (Tables 2 and 4, respectively) were prepared in an attempt to exploit the ability of a neighboring aryl ring to promote β_3 selectivity while concurrently symmetrizing the amine substituent to reduce the number of chiral centers. Unfortunately, the *N*-1,3-diphenyl-2-propylamine derivative **28** was β_2 selective, presumably due to increased conformational flexibility of the 1,3-diarylpropyl substituent. Consequently, this series was not further pursued.

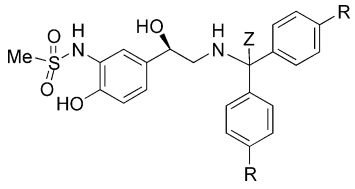
In contrast, since constraints inherent in the *N*-benzhydryl moiety maintained β_3 selectivity for **29**, subsequent synthetic efforts focused on the 1,1-diarylmethylamine series. This effort, exploiting the influence of hydrogen bond accepting *para* substituents on activity at both the β_1 and β_3 receptors, culminated with **34** and **35** (Table 4). Subsequently, incorporation of strongly electron donating substituents was shown to be undesirable. Both **33** and **34** were more prone to undergo metabolic cleavage and solvolysis resulting in formation of a non-selective α and β adrenergic agonist, 4-hydroxy-3-methylsulfonanilidoethanolamine[11] **36** (Table 1). Methylation of C_α (Z = Me) was not beneficial since β_1 and β_2 affinity of **37** and **38** preferentially increased over β_3. In addition, the probability of acid catalyzed formation of **36** was enhanced. As illustrated by **39** (Z = $CONH_2$), quaternizing C_α with an electronegative moiety reduced affinities at all three β receptors, possibly due to the inductive effect attenuating the ethanolamine basicity. The increase in β_3 affinity and IA correlated with hydrogen bonding capabilities of the *para* substituent. Increasing substituent lipophilicity modulated β_1 IA as illustrated by generation of a weak partial β_1 agonist by replacement of the methoxyl of **34** with $OCHF_2$ of **35**.

In vivo potency and the functional margin of separation between β_3 mediated effects versus β_1 or β_2 dependent events were determined by iv administration of promising β_3 agonists to ketamine anesthetized African green



Scheme 1. Reagents and conditions: (a) CH_3SO_2Cl, pyridine, 93%; (b) $CuBr_2$, EtOAc, $CHCl_3$, 73%; (c) (*R*)-2-methyl-CBS-oxazaborolidine, BH_3·THF, −10 °C, 86%; (d) NaI, acetone, 92%; (e) Et_3SiCl, imidazole, DMAP, DMF, 70%; (f) $CHClF_2$, 30% aq NaOH/*i*PrOH, 65 °C, 84%; (g) NH_2OH, 20 °C, 95%; (h) Zn, HOAc; 90%; (i) diisopropylethylamine, THF, 140 °C; NH_4F, THF, 80%; (j) H_2, Pd/C, methanol, 80%.

Table 4. β_1, β_2, and β_3 binding affinity and IA of compounds **29**–**39**[12,13]



Compd	R	Z	K_i β_3 (nM)	IA β_3 (%)	Selectivity vs β_1	Selectivity vs β_2	IA β_1 (%)[b]
29[a]	H	H	5800	44	5	5	
30	$CONMe_2$	H		43			
31[a]	CH_2OMe	H	980	78	46	13	
32	F	H	2800	70	3	2	48
33	NHAc	H	300	96	17	3	88
34	OMe	H	81	100	17	22	85
35	$OCHF_2$	H	160	77	8	7	24
37	OMe	Me	20	79	3	8	79
38	$OCHF_2$	Me	81	94	2	2	
39	OMe	$CONH_2$	220	106	3	10	

[a]Racemic mixture.
[b]β_1 IA was determined by measuring the acceleration in contraction of spontaneously beating guinea pig atria relative to that induced by isoproterenol.[19]

Table 5. Response of African green monkeys to iv injection of **26** and **35**[a]

Compd	Lipolysis ED_{50} (mg/kg)	β_1 Margin before onset of tachycardia	β_2 Margin before decrease of serum K^+
26	0.03	< 3	3
35	0.08	> 6; < 12	> 60

[a]The margin of separation was the ratio of the dose that produced the onset (statistically significant) of a β_1 or β_2 event to the ED_{50} for lipolysis.

monkeys. β_1 agonist activity was reflected by tachycardia; β_2, by a decrease in serum K^+ levels;[20] β_3, by an increase in non-esterified fatty acids (NEFA). Failure to see blunting of lipolysis upon co-administration of the β_3 agonist and 0.1 mg/kg propranolol, a nonselective β_1 and β_2 antagonist, confirmed that the lipolytic response of the most promising 1,2-diarylethylamine **26** and 1,1-diarylmethylamine **35** were β_3 mediated.

The safety margin (Table 5) of the 1,1-diarylmethylamine analogue **35** (BMS-194449) was superior than that of the more potent 1,2-diarylethylamine **26**. In this primate model **35** appeared to be a partial β_3 agonist since a maximum NEFA elevation of 0.9 mEquiv/L induced by **35** was less than the 1.3 mEquiv/L typically induced by a full agonist such as **26**.

The rat PK profile of **20** and **35** were not favorable; oral bioavailability was less than 1–2%. Subsequent studies with portal vein and bile duct cannulated rats revealed that 70% of the drug administered was converted primarily to a mixture of two monoglucuronides of the benzylic and phenolic hydroxyls along with minor amount of the *N*-glucuronide of the sulfonamido moiety. This transformation primarily occurred during transit of the gut wall; however, hepatic glucuronidation was also a factor. Although oxidative metabolism was a very minor pathway, some *N*-dealkylation of **35** did occur to generate the *R* enantiomer of **36**. Despite this poor prognosis for oral activity, we continued to pursue this chemotype convinced that the combination of full β_3 IA and high β_3 affinity offered the best opportunity to ascertain whether β_3 agonists could elicit a sustained robust thermogenic response in man.

Intravenous administration of **35** to 6 volunteers produced no separation between the onset of lipolysis and β_2 mediated prolongation of QT interval, suggesting that lipolysis was either β_2 mediated or that no separation existed between doses producing β_2 and β_3 responses.[21] Further studies with BMS-194449 were terminated.

Acknowledgements

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References and Notes

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12. Receptor binding assays were run in triplicate with membranes prepared from CHO cells expressing the cloned human receptor in the presence of ^{125}I-iodocyanopindolol; β_3 AR agonist activity was assessed by measurement of cAMP accumulation levels in CHO membranes expressing human β_3 AR.[13] IA was given as percent of the maximal stimulation with isoproterenol.
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14. This approach exploited the finding that β_3 affinity is unaffected by an appropriately oriented negative charge whereas β_1 and β_2 affinities are diminished.[13]
15. Compounds **9**–**29** were prepared by sequentially treating the corresponding amine with the previously described phenacyl bromide[11] generated in step b of Scheme 1 for 30 min in MeCN, followed by $NaBH_4$ reduction in EtOH, and hydrogenolysis over Pd/C in MeOH. Spectral data were fully consistent with structures.
16. The amine precursor for **12** was obtained by sequential treatment of cyclohexyl oxime dianion with (a) BnCl; (b) poly H_3PO_4 at 130 °C; (c) concd HCl/MeOH.
17. Absolute configuration of **27** determined by X-ray.
18. The 1,2-diarylethylamine series is the subject of the following paper. Gavai, A. V.; Sher, P. M.; Mikkilineni, A. B.; Poss, K. M.; McCann, P. J.; Girotra, R. N.; Fisher, L. G.; Wu, G.; Bednarz, M. S.; Mathur, A.; Wang, T. C.; Sun, C. Q.; Slusarchyk, D. A.; Skwish, S.; Allen, G. T.; Hillyer, D. E.;

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