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Potent and selective, sulfamide-based human β_3-adrenergic receptor agonists

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Abstract—A series of sulfamide-based analogs related to L-796568 were prepared and evaluated for their biological activity at the human β_3-adrenergic receptor (AR). This modification allows for a significant reduction in molecular weight, while maintaining single-digit nanomolar potencies at the β_3-AR and high selectivities versus the β_1- or β_2-AR.

It has been fifty-five years since the receptor systems that are activated by the endogenous catecholamines, adrenaline, and noradrenaline, were classified into α- and β-subtypes.[1] Twenty years later the β-adrenoceptor (β-AR) classes were further subdivided into the β_1- and β_2-subtypes.[2] In this classification system the lipolytic response (i.e., hydrolysis of fatty acid stores), induced by β-AR agonists in adipocytes, was initially defined as a β_1-AR-mediated response.[2] With the advent of selective β-AR antagonists by the early 1980s it became clear that the lipolytic response was not wholly mediated via the β_1-subtype, leading to the proposal of a third member of the beta-adrenoceptor family (β_3-AR).[3] Drug discovery efforts directed toward optimization of the pharmacological properties associated with the β_3-AR, such as thermogenesis (energy expenditure) in brown adipose tissue and lipolysis in white adipose tissue, lead to the identification of several potent chemotypes (see Fig. 1).[4] Based on the tissue expression of the β_3-AR there is therapeutic potential for these agents in the treatment of obesity, type-II diabetes, and urinary incontinence.

A number of agents were advanced into human clinical trials during the late 1980s and early 1990s, however none of these compounds produced significant responses in the disease endpoints of type-II diabetes or obesity.[4] It was during this period that the human β3-AR was cloned and expressed.[5] Subsequent investigation revealed that the rat β_3-AR pharmacology was significantly different from the human receptor, with the former being significantly more responsive to agonists.[6] Since optimization of the early agonist structures **1** were carried out utilizing the rat receptor, research efforts shifted to identification of agents that were more effective against the human β3-AR.

One of the series to arise from efforts to make more potent agonists of the human β_3-AR was a series of compounds in which the acid moiety of **1** is replaced by a sulfonamide functionality.[7] These efforts ultimately led to the discovery of L-796568 (**2**), which was advanced to human clinical trials. After a single-dose of L-796568 in healthy, overweight to obese male subjects modest increases in energy expenditure (8%) were observed at a dose of 1000 mg.[8] However, upon chronic treatment (28 days) there were no statistically significant differences in energy expenditure or body composition.[9] Pharmacokinetic evaluation of L-796568 revealed that it has low oral bioavailability in preclinical species, with poor absorption being the major factor.[10] This poor absorption is most likely due to the high molecular weight (MW = 625) and lipophilicity ($c\mathrm{Log}P = 5.3$) of L-796568, attributes that have been associated with this pharmacokinetic issue.[11] Unfortunately, the large

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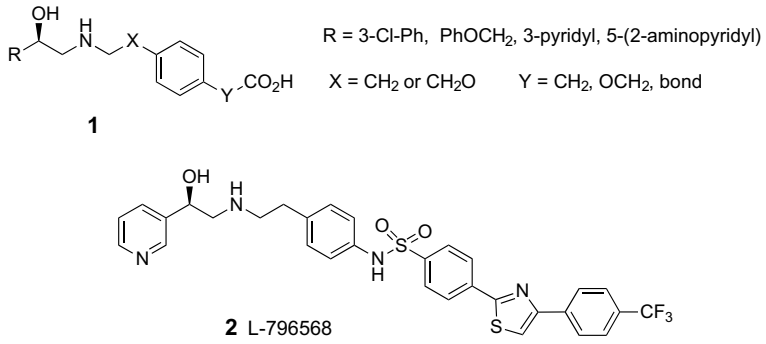
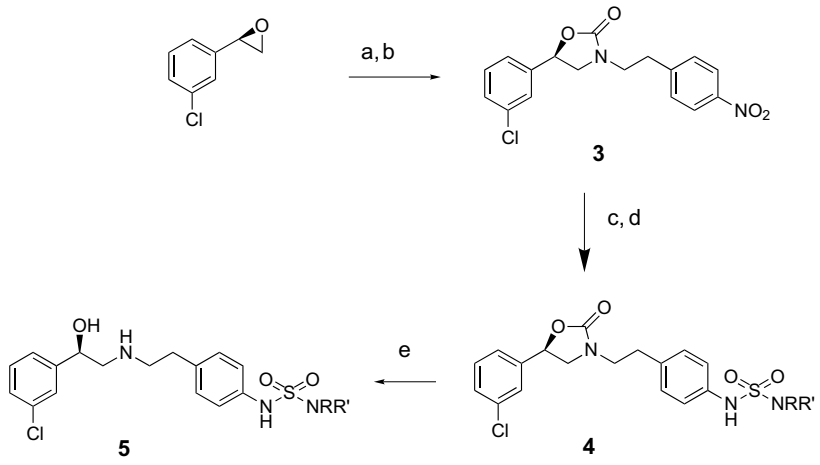


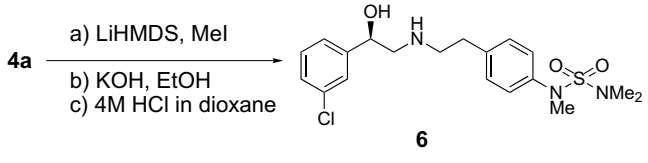
Figure 1.



Scheme 1. Reagents and conditions: (a) 4-nitrophenethylamine, N,O-bis(trimethylsilylamide), DMSO, 75 °C; (b) carbonyldiimidazole, CH_2Cl_2, 57% overall; (c) stannous chloride, EtOH, reflux, 100%; (d) RR′NSO_2Cl, pyridine, 1,2-dichloroethane −35 to 60 °C, 69% for **4d**; (e) KOH, EtOH, 80 °C, 40% for **5d**.

sulfonamide functionalities found in this structural class are necessary for the β_3-AR binding affinity and selectivity. Based on these results a series of sulfamide-containing analogs were investigated to determine where this pharmacophore modification would potentially enhance β_3-AR binding affinity/selectivity, allowing for reductions in molecular weight of the resulting agonists.

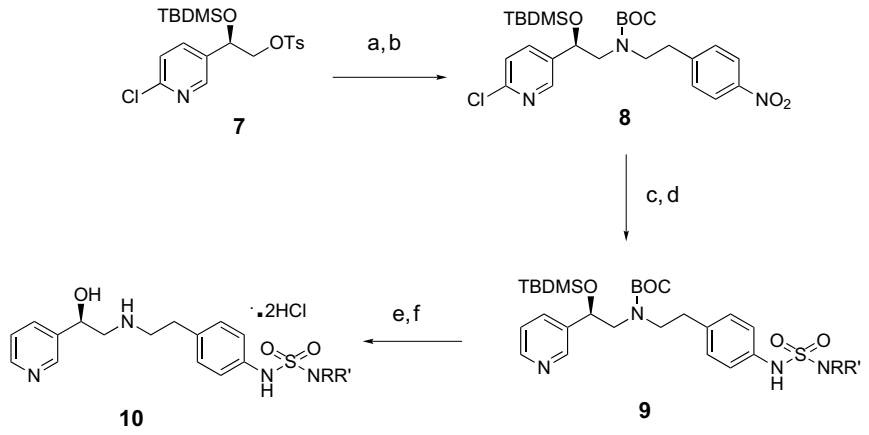
Synthetic routes to these sulfamide-based derivatives are detailed in Schemes 1–4. For the chlorophenyl analogs (Scheme 1), reaction of commercially available (*R*)-3-chlorophenylstyrene oxide with excess 4-nitrophenethylamine and reaction with carbonyldiimidazole afforded oxazolidinone **3**. Reduction of the nitro group and reaction with the appropriate sulfamoyl chloride,[12] followed by deprotection provided **5**. The N-methyl derivative **6** was prepared via Eq. 1.



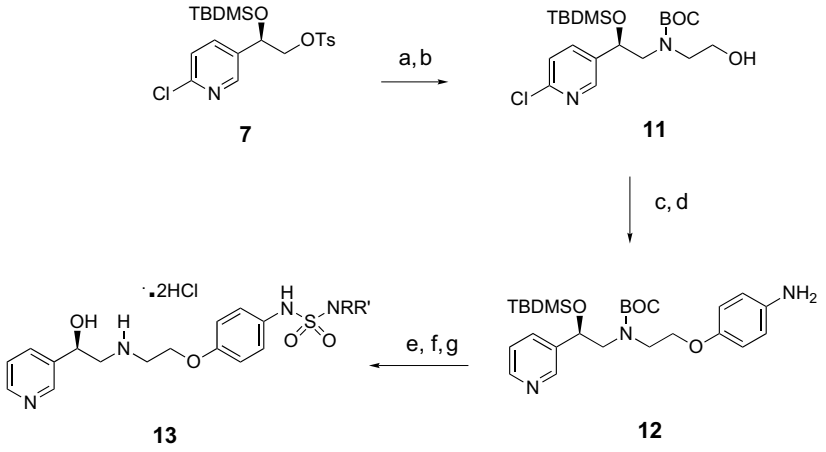
(1)

For the corresponding analogs containing the 3-pyridyl 'head group' the ethanolamine core was constructed via alkylation of tosylate **7** [13] with 4-nitrophenethylamine and protection of the secondary amine as the *tert*-butylcarbamate (Scheme 2). Conversion of the nitro group to the sulfamide functionality and deprotection of the ethanolamine core afforded analogs **10**. Preparation of homologs of **10** in which the phenethylamine portion is replaced by phenoxyethylamine (**13**) is outlined in Scheme 3. Reaction of **7** with ethanolamine and protection of the amine afforded **11**. Incorporation of the phenoxy-based sulfamide substituent proceeded via Mitsunobu coupling with 4-nitrophenol and reduction of the nitro group. Reaction with sulfamoyl chlorides and deprotection afforded targets **13**.

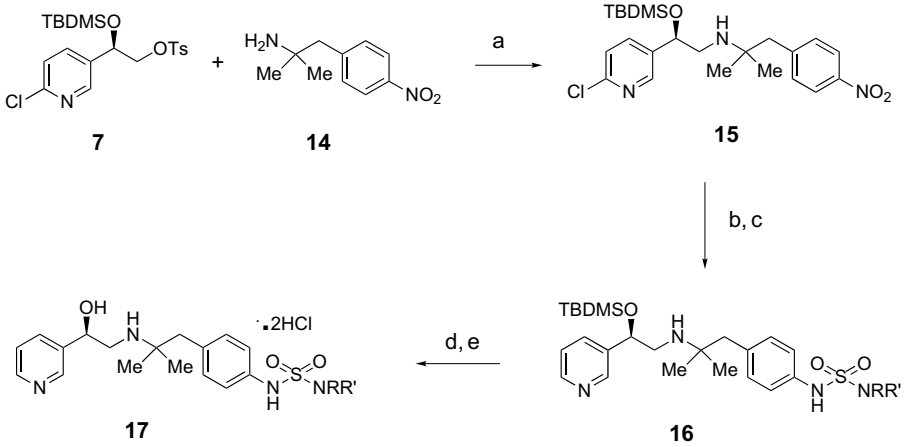
Scheme 4 details the synthetic route to β,β-dimethylphenethylamine-based derivatives **17**. Condensation of hindered amine **14**[14] with tosylate **7** could be achieved by heating at 100 °C for 48 h in DMSO. Attempted protection of the amino functionality as the *tert*-butylcarbamate failed due to steric hindrance. This result suggested that selective reaction with the aniline might be possible, thus obviating the need for protection of the secondary amine. Reduction of the nitro functionality of **15**, followed by reaction with sulfamoyl chlorides provided moderate to good yields of the monosulfamides



Scheme 2. Reagents and conditions: (a) 4-nitrophenethylamine, DMSO, 100 °C; (b) $(Boc)_2O$, THF, 45% overall; (c) 10% Pd/carbon, H_2, ethyl acetate, 77%; (d) $RR'NSO_2Cl$, pyridine, 1,2-dichloroethane, −35 to 60 °C, 55% for **9d**; (e) tetrabutylammonium fluoride, THF; (f) 4 M HCl in dioxane 87% overall for **10d**.



Scheme 3. Reagents and conditions: (a) ethanolamine, diisopropylethylamine, DMSO, 80 °C; (b) $(Boc)_2O$, THF, 87% overall; (c) 4-hydroxynitrophenol, diisopropylazo-dicarboxylate, triphenylphosphine, THF; (d) 10% Pd/C, ammonium formate, MeOH, 50% overall; (e) $RR'NSO_2Cl$, pyridine, 1,2-dichloroethane −35 to 60 °C; (f) tetrabutylammonium fluoride, THF; (g) 4 M HCl in dioxane, 70% overall for **13b**.



Scheme 4. Reagents and conditions: (a) diisopropylethylamine, DMSO, 100 °C, 48 h, 63%; (b) 10% Pd/C, ammonium formate, MeOH, 93%; (c) $RR'NSO_2Cl$, pyridine, 1,2-dichloroethane −35 to 60 °C, 40% for **16j**; (d) tetrabutylammonium fluoride, THF; (e) HCl, dioxane/MeOH 90% overall for **17j**.

Table 1. β-Adrenergic activities for series **5**[15]

	NRR′	β_3-AR EC_{50} μM (IA)	β_2-AR IA @ 30 μM	β_1-AR IA @ 30 μM
2		0.003 (92)	1	1
5a	Dimethylamino	0.299 (75)	6	5
5b	Cyclohexylamino	0.464 (63)	7	10
5c	N-Cyclohexyl-N-methylamino	0.537 (62)	5	6
5d	Piperidinyl	0.617 (84)	5	4
6	Dimethylamino	9.9 (105)	1	2

16. Deprotection of the alcohol moiety then afforded the desired aminoalcohols **17**.

Early β_3-AR agonist programs identified the 3-chlorophenyl moiety to be a good pharmacophore replacement for the catechol group of the endogenous hormones.[7] As a baseline, sulfamide targets **5** were initially prepared and tested for β_3-AR agonist activity and selectivity versus β_1- and β_2-AR. These analogs would also serve the role of beginning to define the structure–activity-relationship (SAR) around the sulfamide functionality. As mentioned above, the focus of sulfamide substituent SAR was focused on analogs derived from low molecular weight amines. As can be seen from the examples in Table 1, both 2°- and 3°-sulfamide-based analogs containing the 3-chlorophenylethanolamine core were 100–200-fold less potent than **2**. Alkylation of the aniline nitrogen of the sulfamide functionality leads to a further order of magnitude reduction in potency (**6** vs **5a**).

Next, we investigated incorporation of the 3-pyridylethanolamine core found in **2** (Table 2). While this modification does not produce enhanced β_3-AR functional potency for the dimethylamino (**10a**) and cyclohexylamino (**10b**) sulfamide analogs, other 3°-sulfamides such as **10c** and **10d** exhibit 4–6-fold improvements in potency. Based on the in vitro profile of the piperidinyl analog **10d** a series of functionalized piperidine derivatives were investigated. Incorporation of lipophilic substituents into either the 3-, 4-, or 5-positions (**10e–g**) of the piperidine ring result in further improvements in agonist potency. β_3-AR agonist potencies were now in the 20–30 nM range and selectivities versus β_1- and β_2-AR were >1000-fold. Before exploring further improvements in the in vitro profile of this series, through modifications of the sulfamide functionality, the SAR of the phenylethyl linker region of these compounds was evaluated.

Previous efforts (unpublished) in our laboratories had shown for certain series of β_3-AR agonists that replacement of the two carbon linker between the secondary amine and phenyl group (attached to the sulfamide functionality in this case) with an ethoxy-based linker provided up to an order of magnitude improvement in potency. This modification was incorporated into the current series, utilizing both a relatively small sterically demanding sulfamide substituent (**13a**) and the more sterically demanding 4-methylpiperidine derivative **13b**. Incorporation of the oxygen atom in the linker leads to a significant loss in potency relative to the ethyl-linked homologs (Table 3).

Pitha and co-workers showed that incorporation of a quaternary carbon in the phenethyl linker next to the secondary amine of β-AR agonists can produce dramatic increases in potency.[14] This work was carried out in a rat reticulocyte binding assay (presumably assessing mostly β_1-AR binding) and does not address the potential for such a modification to impact β_3-AR binding. However, extension of this concept to the sulfamide series had a positive impact on β_3-AR agonist activity (Table 4). The better analogs, such as the *cis*-3,5-dimethylpiperdinyl-**17e** and anilino-based **17f** were single-digit nanomolar, full agonists of the human β_3-AR. This structural modification did not enhance β_1- or β_2-AR agonism, with selectivities of >5000-fold for the more potent analogs.

Analogs **17a–f** did not significantly increase oxygen consumption (surrogate measure of energy expenditure) in rats when dosed orally. This is presumably the result of their high intrinsic clearances. For example, the rat

Table 3. β-Adrenergic activities for series **13**[15]

	NRR′	β_3-AR EC_{50} μM (IA)	β_2-AR EC_{50} μM (IA)	β_1-AR EC_{50} μM (IA)
13a	Dimethylamino	0.943 (96)	4	3
13b	4-Methylpiperidinyl	1.36 (98)	7	5

Table 2. β-Adrenergic activities for series **10**[15]

	NRR′	β_3-AR EC_{50} μM (IA)	β_2-AR IA @ 30 μM	β_1-AR IA @ 30 μM
2		0.003 (92)	1	1
10a	Dimethylamino	0.265 (84)	11	6
10b	Cyclohexylamino	1.46 (71)	0	0
10c	N-Cyclohexyl-N-methylamino	0.180 (67)	16	15
10d	Piperidinyl	0.099 (77)	6	7
10e	4-Methylpiperidinyl	0.036 (80)	8	6
10f	4-Phenylpiperidinyl	0.023 (100)	2	2
10g	*cis*-3,5-Dimethylpiperidinyl	0.024 (95)	17	11

Table 4. β-Adrenergic activities for series **17**[15]

	NRR′	β_3-AR EC_{50} μM (IA)	β_2-AR IA @ 30 μM	β_1-AR IA @ 30 μM
2		0.003 (92)	1	1
17a	Dimethylamino	0.038 (98)	7	10
17b	4-Methylpiperidinyl	0.010 (91)	6	12
17c	4-Phenylpiperidinyl	0.047 (106)	9	20
17d	Cyclohexylamino	0.025 (110)	19	17
17e	*cis*-3,5-Dimethylpiperidinyl	0.004 (89)	11	7
17f	Anilino	0.007 (107)	8	29
17g	2-Methoxyethyl-amino	0.165 (103)	24	28
17h	N-Methylpiperizinyl	0.041 (79)	4	7
17i	N-Benzylpiperizinyl	0.018 (105)	13	46
17j	*cis*-3,5-Dimethylmorpholinyl	0.008 (85)	8	10

and human microsomal intrinsic clearances for **17e** are 466 and 130 mL/min/kg, respectively. Since the in vitro clearances tended to track with the molecular weight/lipophilicity of the sulfamide group, a series of analogs were prepared in which polar/ionic functionality was incorporated into the sulfamide. A few representative examples are provided in Table 4 (**17g–j**). Incorporation of small alkoxy (**17g**) or tertiary amine (**17h**) containing sulfamide functionalities lead to significantly reduced clearances, though they also suffered significant reductions in β_3-AR agonist activity. Adding additional lipophilicity (**17i**) to the tertiary amine of **17h** enhances the functional response, but not surprisingly reacquires microsomal lability. Substitution of the 4-methylene substituent of the high potency 3,5-dimethylpiperidinyl analog **17e** with an oxygen atom providing morpholine **17j**, maintains single-digit nanomolar potency and excellent selectivity. This activity profile for **17j** is further confirmed through β-AR binding studies that demonstrate high β_3-AR binding affinity (7 nM), with relatively weak binding to the β_1-AR (9750 nM) and β_2-AR (1400 nM).

More importantly, the rat and human microsomal intrinsic clearances of this compound are reduced to 165 and 11 mL/min/kg, respectively. With a molecular weight of 463 and a $c\mathrm{Log}P = 2.6$ there is an expectation that this compound should also not have the oral absorption issues associated with **2**.

Increases in oxygen consumption in the rat has been utilized as a surrogate endpoint for determination of increased energy expenditure produced by β_3-AR agonists.[16] When dosed intraperitoneally at 30 mg/kg, **17j** produces a $38 \pm 6\%$ increase in oxygen consumption relative to the control animals.[17] This effect was dose responsive with 10 mg/kg resulting in a $26 \pm 3\%$ increase. When dosed orally in rats at 30 mg/kg, **17j** elicits a $35 \pm 3\%$ increase in oxygen consumption. That this oral response is similar to that seen from intraperitoneal administration supports the hypothesis that **17j** has good oral absorption.

In conclusion, this study has detailed the discovery of a novel series of highly potent and selective β_3-AR agonists. These compounds have physiochemical properties that are projected to lead to good oral absorption, which is supported by preliminary in vivo efficacy studies.

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17. Animals are removed from general housing first thing in the morning (7:00–7:30 am) and are deprived of food and

water for the length of the oxygen consumption measurements. Animals are weighed (310–350 g), marked, and placed into individual activity monitored chambers ($17'' \times 17'' \times 5''$). The system is calibrated and the run started (8:00–8:30 am). Oxygen consumption measurements are made every 10 min for 3 h, at which time the animals are dosed with test compounds (**17j** dosed as the monotosylate salt) or vehicle ($n = 4$ each). Oxygen consumption measurements continue for 2 h. Oxygen consumption values associated with periods of high ambulatory activity (>100 counts/10 min) are excluded from all calculations, as well as the first 5 values of the run and the first value after dosing (settle-down periods).