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Heterocyclic acetamide and benzamide derivatives as potent and selective β_3-adrenergic receptor agonists with improved rodent pharmacokinetic profiles

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ABSTRACT

A series of amide derived β_3-adrenergic receptor (AR) agonists is described. The discovery and optimization of several series of compounds derived from **1**, is used to lay the SAR foundation for second generation β_3-AR agonists for the treatment of overactive bladder.

The β_3-adrenergic receptor (β_3-AR) was discovered in the early 1980s[1] and is expressed in a variety of human tissues including adipose, bladder detrusor, the heart, and the colon.[2] As a result, the β_3-AR has been a drug target for several disease areas including obesity, diabetes, IBS, and overactive bladder (OAB).[3]

L-796568 (**1**), developed in the late 1990s for the treatment of obesity, is a potent and selective agonist of the β_3-AR.[4] Consistent with many compounds of this ethanolamine class, **1** possesses low oral bioavailability in preclinical species.[5] Recently, mirabegron (**2**) achieved proof-of-concept in humans for the treatment of overactive bladder (OAB), and is currently in Phase III clinical trials.[6]

In our efforts to address some of the liabilities associated with these first generation β_3-AR agonists, a series of sulfonamide replacements for **1** were evaluated. A library of over 500 amides was synthesized in an effort to optimize human β_3-AR agonist potency, selectivity over β_1-AR and β_2-AR, and rodent pharmacokinetics in a short period of time.

A library of anilides was synthesized, according to Scheme 1, from intermediate **3**,[7] to explore the SAR of the right side of the molecule. Several analogs of **4** from this library are highlighted in Table 1. Compound **17** showed excellent potency and good selectivity, but general concerns for potential mutagenicity of aminoheteroaromatic groups[8] led to efforts to avoid the aminothiazole moiety. Thiazole **16**, benzopyrazole **11**, and benzotriazole **12** showed good functional activity without this potentially mutagenic functionality. Benzimidazole **8** also showed excellent potency, but possessed no oral bioavailability in rodents. Phenyl pyrimidinones **24** and **26** also demonstrate excellent β_3-AR potency, but possess no oral bioavailability. A comparison of the heterocyclic acetamides revealed that the pyrazole and thiazole offered the most promise for further optimization. In addition, benzamides such as **5** proved to be another potentially interesting lead class. Each of these series was further optimized.

The acetamide linker displayed the best β_3-AR agonist activity with heterocyclic substitution in the initial screens, consistent with a recent report from Astellas (Fig. 1).[12] An examination of acetamide SAR using the benzimidazole moiety as the heterocycle substituent revealed that a methylene linker was optimal (Table 2). Heterocycles directly linked to the carbonyl group (**27**) or with extended tethers (**28** and **30**) showed decreased β_3-AR potency relative to the methylene linker (**8**). In addition, carbamate **29** showed inferior activity relative to the amide linkers of similar tether length. Based on these results, acetamide linkers were selected for further analog optimization.

The SAR of pyrazole acetamide analogs of **4** is summarized in Table 3. All benzopyrazoles (**10, 11**), although potent and selective over β_1- and β_2-ARs, possess low (<5%) oral bioavailability. Removal of the fused phenyl ring (**33**) led to decreased β_3-AR agonist potency. The addition of phenyl groups at the 4 and 5 positions of the pyrazole ring increased potency (**32** and **36**). The addition of a methyl group to the 3 position also improved β_3-AR potency (**34**). Dimethyl analog **35** was both active and selective over β_1-AR and β_2-AR. This analog also possesses improved oral bioavailability in rats (43%).

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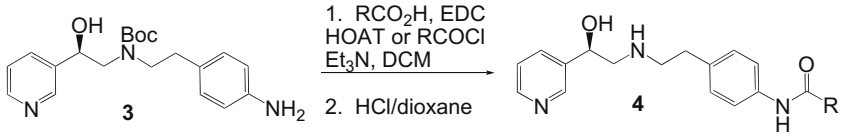
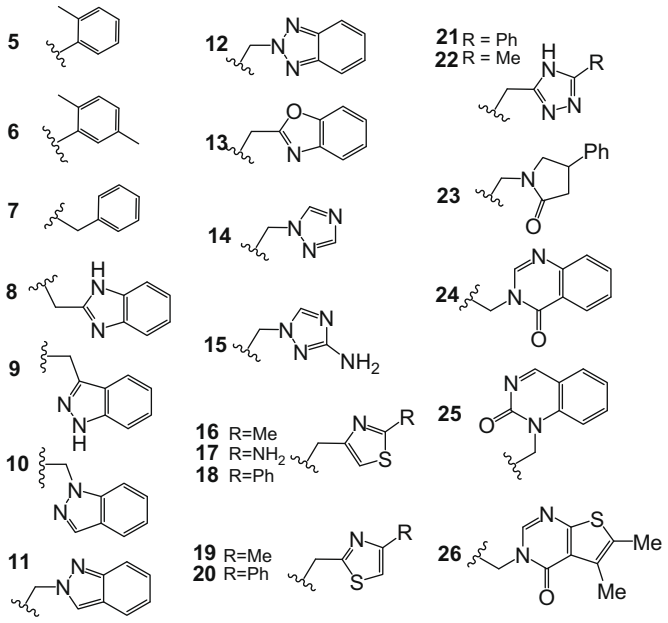


Scheme 1. Synthesis of analogs **4**.

Table 1
Comparison of β_3-AR agonist activity and β_1-AR and β_2-AR binding affinity for anilide analogs of **4** and **1**[9]



Compound	β_3 Agonist activity EC_{50}[10] nM (% act)	β_1 Binding affinity IC_{50}[11], μM	β_2 Binding affinity IC_{50}[11], μM
1	3.6 (94)	2.3	2.3
5	297 (40)	>20	>20
6	95 (61)	>20	>20
7	225 (34)	>10	3.0
8	8.8 (48)	6.0	>10
9	19 (94)	>10	9.6
10	48 (97)	15	19
11	14 (67)	>10	3.1
12	24 (73)	13	7.6
13	21 (70)	1.1	0.6
14	693 (81)	>20	>20
15	22 (111)	>20	>20
16	103 (87)	>10	>10
17	6.8 (75)	8.7	8.3
18	174 (75)	1.7	1.5
19	74 (92)	>10	>10
20	86 (73)	1.7	1.1
21	8.0 (98)	>10	>10
22	171 (78)	>20	>20
23	64 (77)	6.7	1.4
24	19 (96)	>10	3.4
25	19 (103)	>10	3.0
26	16 (75)	7.8	3.6

Substitutions α to the amide carbonyl in the pyrazole series were next explored. The addition of a methyl group at this position gave a large boost in potency (**37** and **38**). Only one diastereomer showed full β_3-AR agonist activity (**37**) while the other appeared to be a partial agonist (**38**).[14] The dimethyl analog (**39**) was significantly less potent at β_3-AR. Methylation α to the carbonyl of the dimethyl pyrazole analog (**40**) also afforded a loss in potency, indicating a possible steric interaction between the α-methyl group and the pyrazole 2-methyl group.

The optimization of thiazole analogs is shown in Table 4. The addition of an α methyl group to **17** improved β_3-AR agonist potency (**41**) but *N*-methyl derivatives of **17** lacked β_3-AR agonist activity (**42**). Replacement of the aminothiazole with a methyl thiazole (**16**) furnished a compound which retained some potency and increased oral bioavailability for the first time in this series. Phenyl substitution (**18**) showed decreased $\beta_{1/2}$-AR selectivity and no improvement in potency. The addition of a second methyl group to **16** gave a compound with improved potency, selectivity and rat PK profile (**43**).

Optimization of the benzamide series is described in Table 5. *Ortho* substitutions led to compounds with moderate β_3-AR agonist activity (e.g. **44**). *Meta* (**45**, **57–59**) and *para* (**46**, **60**) substitution,

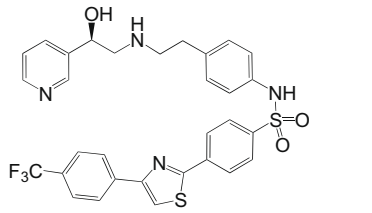
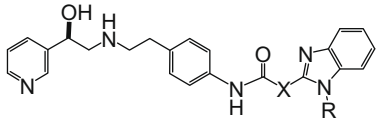


Figure 1. Merck's L-796568 (**1**) and Astellas' mirabegron (**2**).

although showing potent EC_{50}s, led to a loss in receptor activation and a large increase in binding affinity at β_1-AR. Several *ortho* substituted benzamides were synthesized in an attempt to optimize this series. Similar to the acetamide series, imidazole (**49**) and benzimidazole (**55**) substitution improved potency but with a detrimental effect on oral bioavailability in rats. Although marked by high plasma clearance, simple extended tether substituents (**54**, **56**) also showed good potency but with bioavailabilities of 9% and 15%, respectively. Extended tethered heterocycles, such as pyrrole **53**, mitigated the high clearance and improved the potency. Simple heterocyclic substitutions not possessing an NH functionality all showed good potency and PK profiles (**47**, **48**). Simple pyrrolidine **47** in particular showed β_3-AR agonist activity, lower clearance and improved bioavailability for this series. When this pyrrolidine benzamide was additionally substituted at the meta and para positions with a methyl group, compounds with comparable potency and selectivity and improved oral bioavailabilities were found (**61** and **62**).

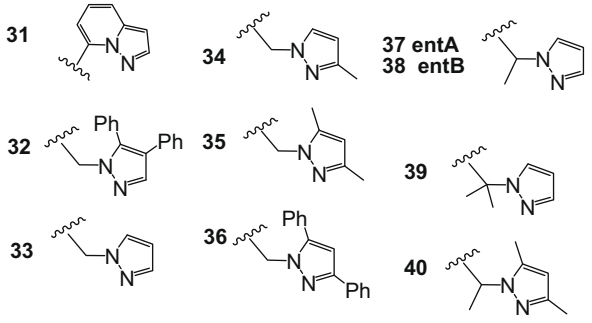
In summary, the synthesis of a library of amides as sulfonamide replacements on the right side of the ethanolamine core of **1** led to the discovery of leads in three different classes.[15] Pyrazole **37**, thiazole **43**, and benzamides **61** and **62** showed good potency, selectivity and much improved pharmacokinetic profiles over previous analogs from the sulfonamide series (e.g., **1**). These compounds are the first β_3-AR agonist anilides in this series reported to possess oral bioavailabilities in rats above 20% while possessing good selectivities over

Table 2
Varying tethers and substitution to the benzimidazole[9]



Compound	X	R	β_3 Agonist activity EC_{50}[10], nM (% act)	β_1 Binding affinity IC_{50}[11], μM	β_2 Binding affinity IC_{50}[11], μM
27	Bond	H	283 (40)	1.1	8.6
8	$-CH_2-$	H	8.8 (48)	6.0	>10
28	$-(CH_2)_2-$	H	2647 (41)	>20	>20
29	$-OCH_2-$	H	>10 k (11)	>10	0.84
30	$-(CH_2)_2-$	CH_3	454 (60)	>20	11

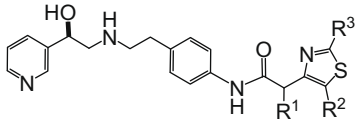
Table 3
Comparison of β_3-AR agonist activity, β_1-AR and β_2-AR binding affinity, and rat PK profiles for select pyrazole derivatives[9]



Compound	β_3 Agonist activity EC_{50}[10], nM (% act)	β_1/β_2 Binding affinity IC_{50}[11], μM	Rat PK	
			Cl_p (mL/min/kg)/$t_{1/2}$[a] (h)	F[a] (%)
31	261 (66)	0.6/16	—	—
32	580 (84)	>20/>20	52/2.7	22
33	218 (64)	>20/>20	—	—
34	241 (88)	>20/>20	—	—
35	123 (92)	>20/>20	46/1.0	43
36	498 (72)	3.6/>20	—	—
37	27 (98)	>20/>20	85/1.6	12
38	10 (49)	>20/>20	56/1.9	26
39	3768 (109)	>20/>20	—	—
40	1404 (50)	>20/>20	—	—

[a] '—' indicates not tested; compounds were dosed as cassette mixtures.[13]

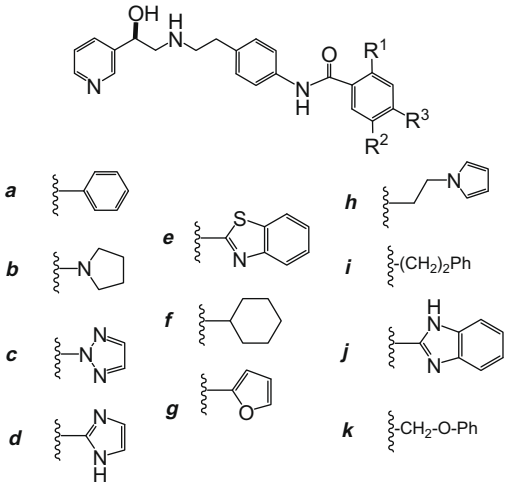
Table 4
Comparison of β_3-AR agonist activity, β_1 and β_2 binding affinity, and PK profile for select thiazole derivatives[9]



Compound	R^1	R^2/R^3	β_3 Agonist activity EC_{50}[10], nM (% act)	β_1/β_2 Binding affinity IC_{50}[11], μM	Rat PK	
					Cl_p (mL/min/kg)/$t_{1/2}$[a] (h)	F[a] (%)
16	H	H/Me	103 (87)	>10/>10	11/1.4	15
41	Me	H/NH_2	1.9 (88)	>20/>20	10/20	0
42	H	H/NHMe	978 (39)	16/0.7	—	—
43	H	Me/Me	37 (86)	>20/>20	51/1.6	32

[a] '—' indicates not tested; compounds were dosed as cassette mixtures.[13]

Table 5
Comparison of β_3-AR agonist activity, β_1 and β_2 binding affinity, and PK profile for select benzamide derivatives[9]



Compound	R^1	R^2/R^3	β_3 Agonist activity EC_{50}[10], nM (% act)	β_1/β_2 Binding affinity IC_{50}[11], μM	Rat PK	
					Cl_p (mL/min/kg)/$t_{1/2}$[a] (h)	F[a] (%)
44	*a*	H/H	179 (76)	>20/13	—	—
45	H	*a*/H	184 (52)	0.4/4.9	—	—
46	H	H/*a*	187 (32)	0.06/1.9	—	—
47	*b*	H/H	67 (86)	5.3/>20	58/1.3	9.6
48	*c*	H/H	76.2 (88)	>10/>10	39/1.6	7.5
49	*d*	H/H	21 (115)	>10/>10	30/14	0
50	*e*	H/H	36 (94)	>20/5.0	143/1.2	4.7
51	*f*	H/H	42 (81)	3.0/6.0	158/1.5	7.5
52	*g*	H/H	86 (86)	>20/>20	—	—
53	*h*	H/H	24 (74)	6.9/12	75/1.6	6.4
54	*i*	H/H	32 (74)	1.7/2.4	132/1.4	9.2
55	*j*	H/H	35 (67)	>10/1.7	106/3.2	0
56	*k*	H/H	47 (66)	4.7/9.8	165/1.7	15
57	H	*n*/H	6.9 (21)	0.6/>10	—	—
58	H	*d*/H	10 (21)	0.4/4.1	—	—
59	H	*i*/H	37 (21)	0.4/4.0	—	—
60	H	H/*i*	105 (30)	0.1/1.9	—	—
61	*b*	Me/H	73 (77)	15/12	74/1.8	24
62	*b*	H/Me	76 (97)	16/19	33/2.3	12

R^1, R^2, and R^3 are selected from the groups ***a*–*k***.
[a] '—' indicates not tested; compounds were dosed as cassette mixtures.[13]

β_1-AR and β_2-AR (up to 1000-fold). These compounds have provided leads for further optimization of the ethanolamine core and have established the SAR foundation for application to a second generation of β_3-AR agonists. Further efforts to incorporate the SAR described in this report into new structure classes and further information of the optimal stereochemical configuration at the α-methyl chiral center will be disclosed in future communications.

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9. β_3-AR EC_{50}'s and activation and β_1-AR and β_2-AR binding affinities are generally reported as means of $n \geqslant 2$, with standard deviations $\leqslant$50% of the mean. The comparison of β_3-AR EC_{50}'s and β_1-AR and β_2-AR IC_{50}'s is used as an assessment of selectivity (see Ref. 4a).
10. The ability of compounds to activate the human β_3-AR receptor was measured using a CHO cell line stably expressing the receptor (Naylor, E. M.; Parmee, E. R.; Colandrea, V. J.; Perkins, L.; Brockunier, L.; Candelore, M. R.; Cascieri, M. A.; Colwell, L. F., Jr.; Deng, L.; Feeney, W. P.; Forrest, M. J.; Hom, G. J.; MacIntyre, D. E.; Strader, C. D.; Tota, L.; Wang, P.-R.; Wyvratt, M. J.; Fisher, M. H.; Weber, A. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 755). The cell line used expressed β_3-AR at levels very similar to those observed in the human bladder detrusor muscle (unpublished observation). To quantify the amount of cAMP released following β_3-AR activation, the LANCE cAMP kit (Perkin–Elmer), a time-resolved fluorescence resonance energy transfer immunoassay, was used. Compounds were serially diluted in DMSO and an aliquot added to either 384-well or 96-well micro titer plates in assay buffer (5 mM HEPES, 0.1% BSA in Hank's Balanced Salt Solution). The reaction was initiated by the addition of 6000 cells per well in assay buffer that also contained a cAMP specific antibody labeled with Alexa Fluor 647 and a phosphodiesterase inhibitor (IBMX). Following a 30 min incubation at room temperature, the cells were lysed by the addition of LANCE detection buffer which contained a europium-labeled cAMP tracer. Fluorescence was measured following a one hour incubation at room temperature using a Perkin–Elmer Envision plate reader, exciting at 340 nm and measuring emission at 615 nm and 665 nm. For each assay, a cAMP standard curve was included and used to convert fluorescence readings directly to cAMP amounts. The values were then normalized to isoproterenol, a known full agonist of β_3-AR, which was titrated in every assay and the EC_{50} determined using a custom in-house data analysis package. Along with EC_{50}, the percent maximum activation relative to isoproterenol is reported.
11. The binding affinity of compounds to the β_1-AR and β_2-AR receptors was determined in a standard competition binding assay using membranes prepared from recombinant cells expressing either β_1-AR or β_2-AR (Forrest, M. J.; Hom, G.; Bach, T.; Candelore, M. R.; Cascieri, M. A.; Strader, C.; Tota, L.; Fisher, M. H.; Szumiloski, J.; Ok, H. O.; Weber, A. E.; Wyvratt, M.; Vicario, P.; Marko, O.; Deng, L.; Cioffe, C.; Hegarty-Friscino B.; MacIntyre E. *Eur. J. Pharmacol.* **2000**, *407*, 175). WGA-PVT SPA beads (GE Amersham; 150 μg) were mixed with either 2 μg β_1-AR or 1 μg β_2-AR membranes per well of a 384-well plate in assay buffer (50 mM Tris, pH 7.4, 5 mM $MgCl_2$, 2 mM EDTA, 3% glycerol and 0.1% BSA) containing a protease inhibitor cocktail (Sigma #P8340). Beads and membranes were then allowed to pre-incubate for 20 min at room temperature. Compounds were next serially diluted in DMSO and an aliquot added to each well in assay buffer. The reaction was then initiated by the addition of ^{125}I-cyanopindolol (2200 Ci/mmol; Perkin–Elmer) at a final concentration of 40 pM. The assay plates are incubated for 3–4 h at room temperature and then counted in a Wallac Scintillation Counter (Perkin–Elmer). Total binding is measured in the absence of compound while maximal inhibition is determined by the addition of 10 μM (final concentration) unlabeled cyanopindolol in control wells. All counts are then normalized to percent inhibition and the IC_{50} determined using a custom in-house data analysis package.
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13. Male Sprague-Dawley rats were dosed with compound in solution intravenously at 0.5 mg/kg and orally at 1 mg/kg using EtOH:PEG vehicle. In cassette dosing, four compounds were dosed simultaneously with a control (For a review on the theory of cassette dosing see: White, R. E.; Manitpisitkul, P. *Drug Metab. Disp.* **2001**, *29*, 957)
14. *Experimental procedure for the preparation of* **37** *and* **38**: To a stirred, room temperature mixture of *rac*-2-(1*H*-pyrazol-1-yl)propanoic acid (58.8 mg, 0.420 mmol), HOBT (0.769 mL, 0.462 mmol), EDC (80 mg, 0.42 mmol) and DIEA (0.147 mL, 0.839 mmol) in DMF (3 mL) was added **3** (150 mg, 0.420 mmol, Ref. 7). The reaction mixture was stirred at room temperature for 18 h then diluted with EtOAc and washed with saturated aqueous sodium bicarbonate, water and then brine. The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The resulting crude Boc-protected intermediate was dissolved in EtOAc (2 mL) and 2 M HCl in ether (2 mL) was added. The reaction mixture was stirred at room temperature for 2 h and concentrated to dryness. The product was purified by reverse phase HPLC (0.1% NH_4OH in H_2O/MeCN) to give 132 mg of the free base mixture of stereoisomers. Separation of the two stereoisomers was accomplished using SFC (OD-H column, eluting with 20% MeOH w/0.1% Et_3N in CO_2 at 50 mL/min (100 bar) to give **38** (33 mg, 0.088 mmol, 21% from **3**) as the first eluting isomer and **37** (45 mg, 0.12 mmol, 29% from **3**) as the second eluting isomer. ESI-MS calculated for $C_{21}H_{25}N_5O_2$: 379.20. Found 380.14 (M+H, **38**) and 380.15 (M+H, **37**). The absolute stereochemistry of the methyl group α to the carbonyl was not determined.
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