(4-Piperidin-1-yl)phenyl Amides: Potent and Selective Human β_3 Agonists

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In search of potent and selective human β_3 agonists as potential drugs for the treatment of human obesity and type II diabetes, a series of (4-piperidin-1-yl)phenyl amides was prepared and evaluated for their biological activity on the human β_3-adrenergic receptor. The leucine derivative **26e** and the reverse amide **33b** were found to be the two most potent and selective compounds in this study. With EC_{50} values of 0.008 and 0.009 μM, respectively, at the β_3 receptor, nearly completely abolished intrinsic activity at either the β_1 or β_2 receptor, and significant thermogenesis effects on human β_3-adrenergic receptor transgenic mice, **26e** and **33b** are among the most potent and selective human β_3 agonists known to date.

Introduction

The β_3-adrenergic receptor (β_3-AR), located on the surface of adipocytes, has been shown to mediate various pharmacological and physiological effects such as lipolysis in white adipocyte tissue (WAT) and thermogenesis in brown adipocyte tissue (BAT).[1] Consequently, a number of laboratories are engaged in developing potent and selective β_3-AR agonists for the treatment of diverse human disease states, such as obesity and type II diabetes. Early β_3-AR agonists, which were developed based on the rodent β_3-AR, are represented by CL 316243,[2] BRL 37344,[3] and CGP 12177A.[4] These compounds have shown antiobesity effects, such as mobilization of fat from WAT depots (lipolysis), increased BAT-mediated thermogenesis, and increased fat oxidation in rodents. In addition to antiobesity effects, they exhibit potent antidiabetic effects (such as an increase in insulin secretion and improvement in insulin-mediated glucose uptake) in rodent model type II diabetes.[5] However, human clinical trials with these early β_3-AR agonists have been disappointing due to a lack of selectivity or potency. Subsequent cloning and expression of the human and rat β_3-ARs indicated a significant difference between the two.[4] This led to the recognition that a cloned human receptor assay would offer major advantages over rodent models for the identification of future β_3-AR agonists.

Second-generation β_3-AR agonists currently under preclinical development are represented by tetrahydroisoquinoline **1**,[6] SB 226552,[7] L 755507,[8a] and L 770644.[8b,c] These highly potent and selective compounds were evaluated in Chinese hamster ovary (CHO) cells expressing the cloned human β_3-AR.

Despite the difference between the human and rat β_3-AR, a recently reported study[1a] demonstrated that treatment of lean volunteers with CL 316243, which was developed in our laboratories based on rodent models, did induce lipolysis and fat oxidation and increased insulin sensitivity. These new findings clearly suggest that desired metabolic effects could be achieved by β_3-AR agonists. This encouraged us to continue the β_3-AR agonists discovery program. In a previous study, (4-piperidin-1-yl)phenyl sulfonamide **2** was found to be a potent (β_3-AR: $EC_{50} = 0.004$ μM, IA = 1.0) and selective (β_1-AR: $EC_{50} = 2.0$ μM; β_2-AR: $EC_{50} = 5.0$ μM) human β_3-AR agonist, but it is a partial agonist at both β_1 (IA = 0.51) and β_2 (IA = 0.45) receptors.[9] In this paper we will describe the synthesis and structure−activity study (SAR) of a variety of (4-piperidin-yl)phenyl amides, leading to the discovery of a leucine derivative **26e** and a reverse amide **33b**, which are among the most potent and selective human β_3-AR agonists reported to date.

Chemistry

The (4-piperidin-1-yl)phenyl amides and related compounds were readily prepared by utilizing reductive amination of piperidones with arylethanolamine **6**.[9] The starting piperidone **5** was prepared by NaOH hydrolysis of the ester **3**[10] followed by HCl-catalyzed hydrolysis of the resulting ketal **4**. Reductive amination of piperidone **5** with arylethanolamine **6** gave the desired product **7** (Scheme 1).

Synthesis of the homologous phenylacetic acid **11** was achieved as illustrated in Scheme 2. The acetophenone **8**[10] was directly converted to the methyl arylacetate **9** by an oxidative rearrangement using thallium(III) nitrate (TTN) in acidic methanol.[11] Ester and ketal hydrolysis followed by reductive amination, as described above, gave the desired analogue **11**.

Phenylpropionic acid **17** was prepared as outlined in Scheme 3. The aldehyde **12**[10] was subjected a Horner−Emmons reaction in the presence of triethylamine to give the phenylacrylate **14**. Basic ester hydrolysis followed by acidic ketal hydrolysis and catalytic hydrogenation, as previously described in Scheme 1, produced the phenylpropionic acid derivative **17**.

Scheme 4 depicts the synthesis of benzyloxyacetic acid **20**. The aldehyde **12** was reduced to the benzyl alcohol **18** by $NaBH_4$. Alkylation of the benzyl alcohol with iodoacetate sodium salt followed by ketal hydrolysis gave the piperidone **19**, which was further converted into the final product **20** by reductive amination.

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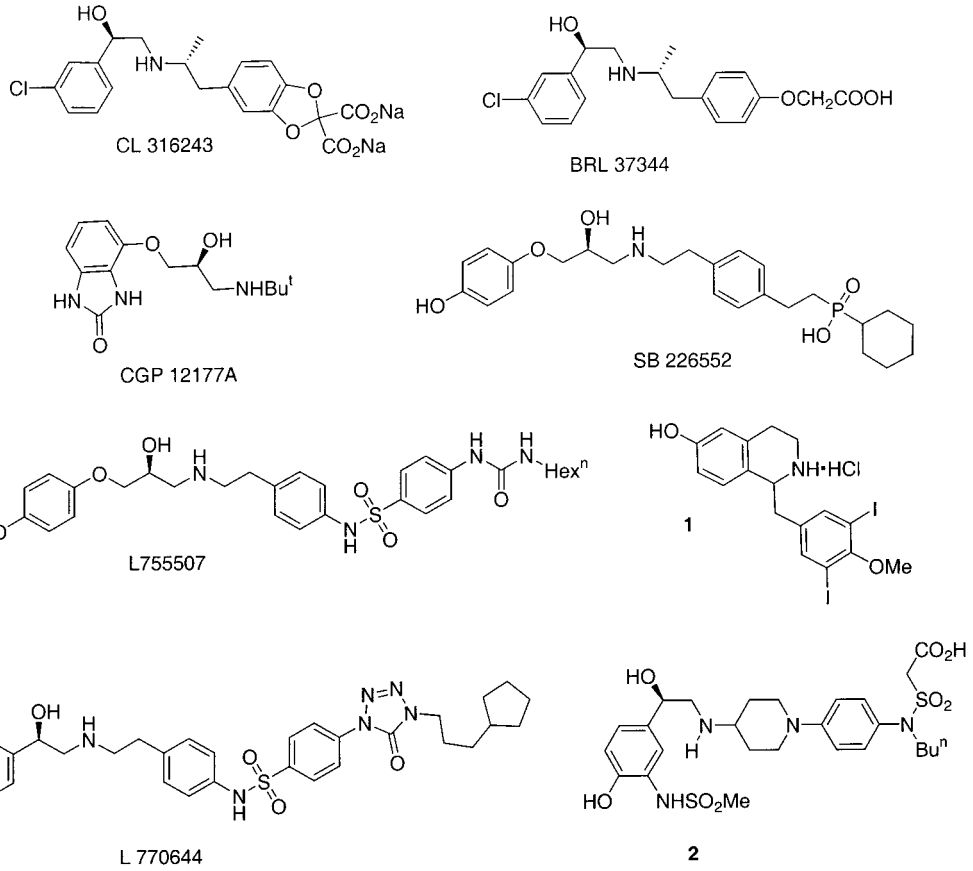
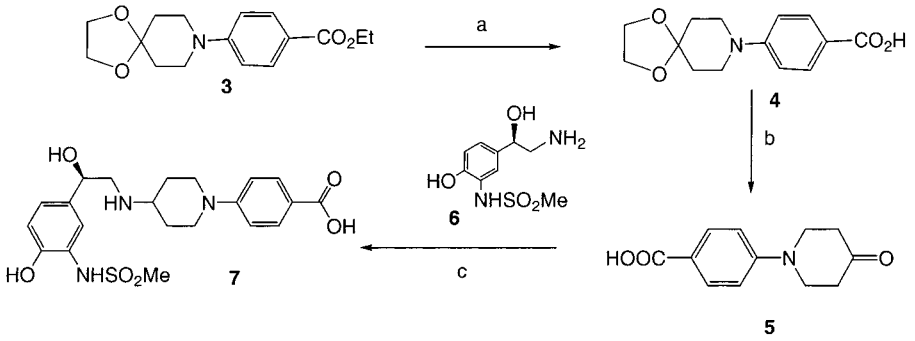
Chart 1

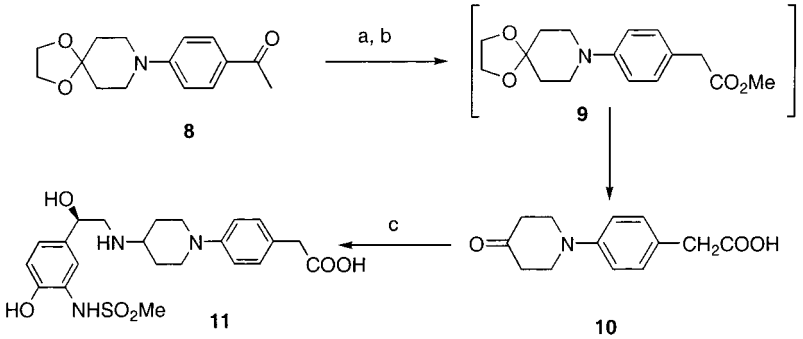


Scheme 1. Synthesis of Benzoic Acid **7**[a]



[a] (a) NaOH, MeOH/THF (1:1), 99%; (b) HCl, 88%; (c) H_2, Pd/C, MeOH, AcOH, 53%.

Scheme 2. Synthesis of Phenylacetic Acid **11**[a]

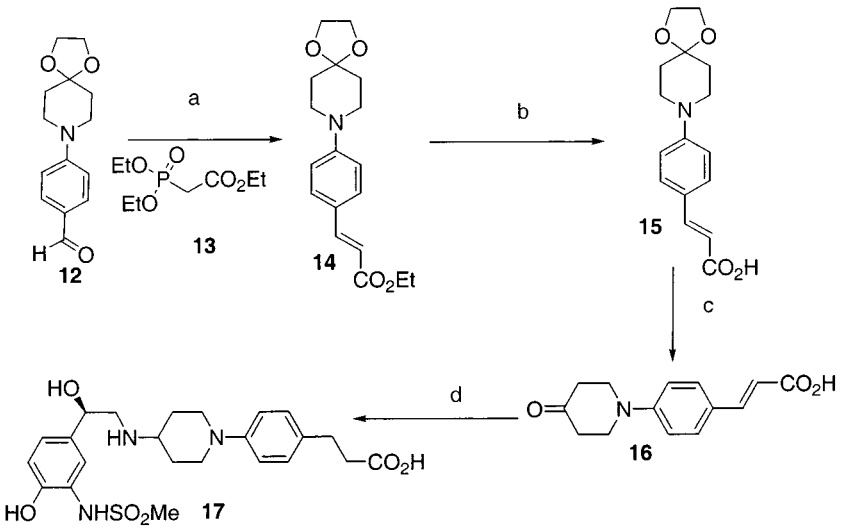


[a] (a) TTN, perchloric acid, MeOH; (b) i. HCl, ii. NH_4OH, 17% over steps a and b; (c) **6**, H_2, Pd/C, MeOH, 5%.

When the ketal **21**[10] was subjected to hydrolysis in the presence of concentrated HCl, the cyano group of **21** was also hydrolyzed to generate the amide **22**, which gave the amide **23** after reductive amination with **6**.

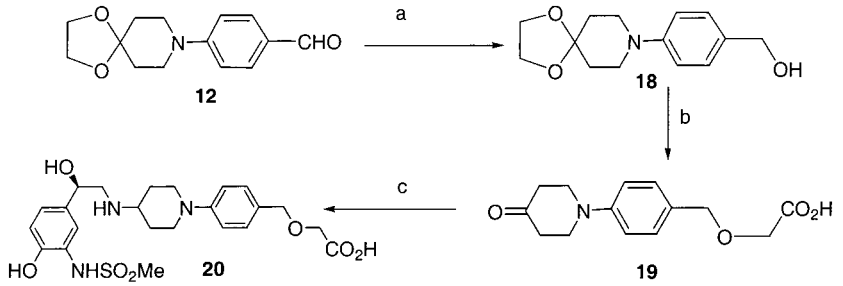
The amino acid derivatives **26a**−**g** were prepared according to Scheme 6. Coupling of the benzoic acid **5** with amino acid esters using EDC as the coupling agent gave the piperidone **24**. The reductive amination of **24**

Scheme 3. Synthesis of **17**[a]



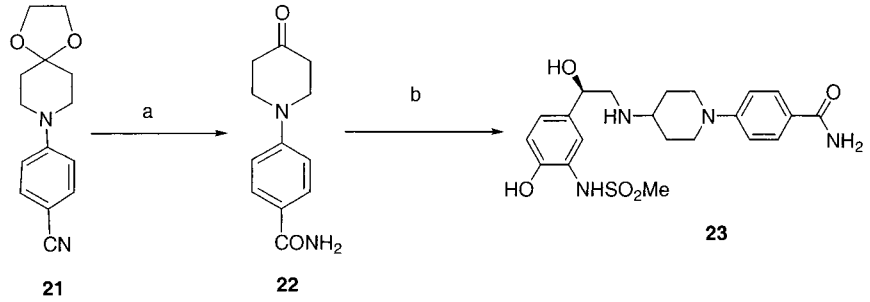
[a] (a) Et_3N, LiBr, THF, 81%; (b) NaOH, 100%; (c) HCl, 90%; (d) **6**, H_2, Pd/C, MeOH, 94%.

Scheme 4. Synthesis of **20**[a]



[a] (a) $NaBH_4$, MeOH, 100%; (b) i. ICH_2CO_2Na, NaH, THF, ii. HCl, 26% over steps i and ii; (c) **6**, H_2, Pd/C, MeOH, 45%.

Scheme 5. Synthesis of **23**[a]



[a] (a) HCl, 82%; (b) **6**, H_2, Pd/C, MeOH, 54%.

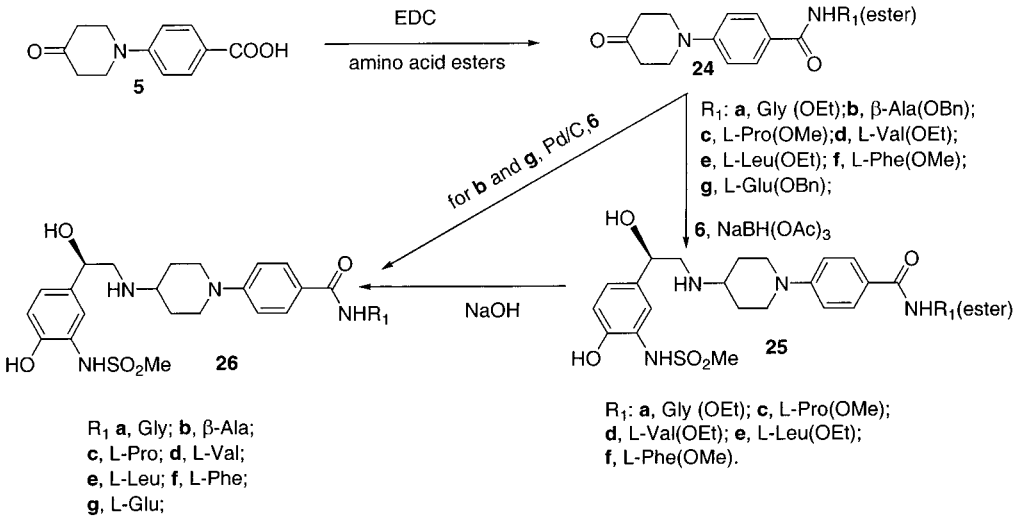
with **6** was carried out with $NaBH(OAc)_3$ in DMF. The target carboxylic acids were prepared by basic hydrolysis of the intermediate alkyl esters **25a**,**c**−**f** or via catalytic hydrogenation of the benzyl esters **24b**,**g** with **6**. The *N*-butylglycine derivative **29** was prepared by a similar route as shown in Scheme 7.

The reverse amide **33** was conveniently prepared as outlined in Scheme 8. Acylation of the aniline **30**[9] with ethyl 3-chloro-3-oxopropionate gave the corresponding amide **31**. Reductive amination followed by basic ester hydrolysis, as previously described in Scheme 6, produced the reverse amide derivative **33**.

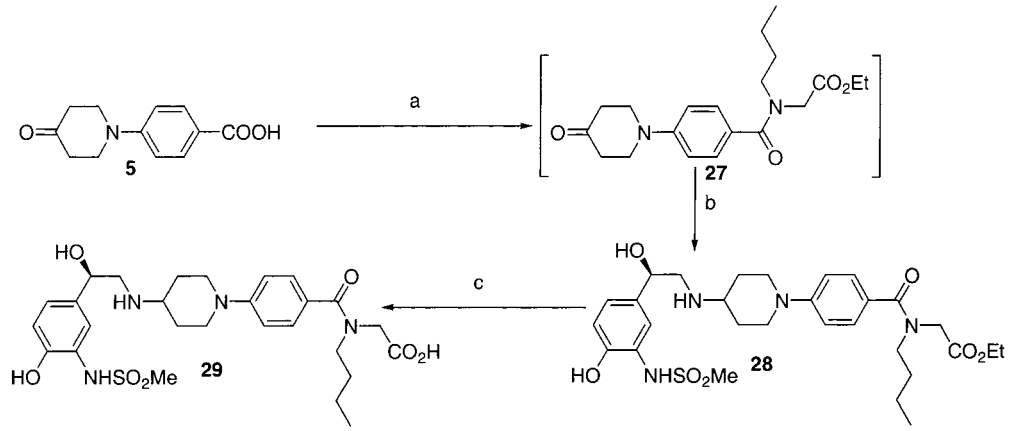
Biological Results and Discussion

The (4-piperidin-1-yl)phenyl amides and related compounds were tested for their in vitro activity[13] in stimulating an increase in cAMP levels in Chinese hamster ovary (CHO) cells expressing the cloned human β_3-, β_2-, and β_1-AR receptors, and the results are summarized in Table 1. As a reference, the parent benzoic acid **7** was tested for activity and selectivity at the human β_3 receptor and was found to be a modestly potent β_3-AR full agonist (EC_{50} = 0.22 μM, IA = 1.20) with greater than 200-fold selectivity versus both the β_2- and β_1-ARs. The homologous analogue **11** showed similar activity at the β_3 receptor with EC_{50} = 0.27 μM and IA = 0.86. Increasing the tether length between the carboxylic acid and the right-hand-side phenyl (phenylpropionic acid **17** and benzoxylacetic acid **20**) resulted in a moderate increased (3−6-fold) activity at the β_3 receptor, but selectivity versus the β_1 or β_2 receptor was decreased. Furthermore, both showed significant agonist activity at both the β_2- and β_1-AR receptors (IA = 0.56−0.92).

Scheme 6. Synthesis of Amino Acid Derivatives 26**a**−**g**

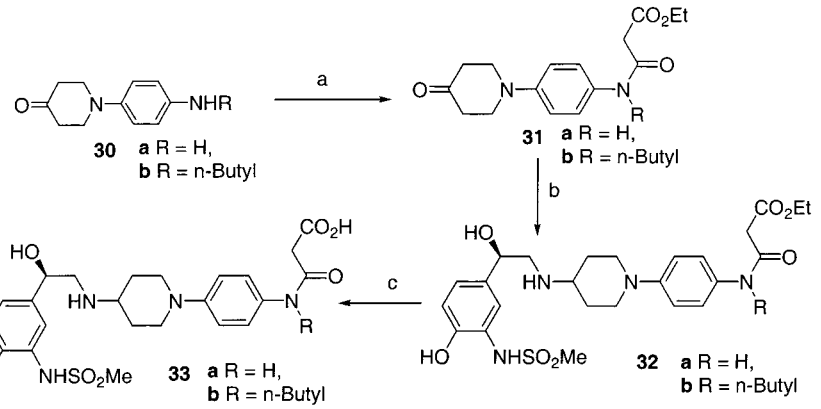


Scheme 7. Synthesis of *N*-Butylglycine Derivative **29**[a]



[a] (a) *N*-Butylglycine ethyl ester,[12] EDC, NMM, CH_2Cl_2; (b) **6**, $NaBH(OAc)_3$, DMF, 10% over steps a and b; (c) NaOH, 60%.

Scheme 8. Synthesis of Reverse Amide Derivative **33**[a]



[a] (a) Ethyl 3-chloro-3-oxopropionate, Et_3N, CH_2Cl_2; (b) **6**, $NaBH(OAc)_3$, DMF; (c) NaOH.

While the amide derivative **23** showed improved (11-fold) potency as compared to **7** at the human β_3 receptor, with $EC_{50} = 0.02\ \mu M$ and 112% intrinsic activity, it was not very selective versus the β_1 receptor (IA = 0.69). A variety of amino acid derivatives were then examined. To our delight, glycine derivative **26a** proved to be a potent agonist of the human β_3 receptor ($EC_{50} = 0.06\ \mu M$, IA = 0.95) with dramatically decreased intrinsic activity at both the β_2- and β_1-ARs (IA = 5% and 4%, respectively). The corresponding ester **25a** showed a moderate increased (4-fold) activity at the β_3 receptor, but it was less selective versus the β_2 (IA = 0.36) and β_1 receptors (IA = 0.25). This result is consistent with a previous finding[14] that carboxylic acids are usually more selective than the corresponding esters.

Effects of alkylation of the amide NH on β_3 agonist activity/selectivity were then examined. *N*-Butyl amide **29** showed $EC_{50} = 0.036\ \mu M$. Only a minor improvement (1.7-fold as compared to **26a**) in its agonist activity was observed. However, it had significant partial agonist

Table 1. In Vitro Activity for Benzoic Acid Derivatives

	EC_{50}, μM (IA)[a]		
compd	β_3-AR	β_2-AR	β_1-AR
L 755507[16]	0.001 (0.96)[b]	(0.02)[b]	0.33 (0.30)[b]
7	0.22 (1.2)[b]	>50 (0.19)	48.7 ± 20 (0.6)
11	0.27 ± 0.05 (0.86)	nd[c]	nd
17	0.039 ± 0.019 (1.1)	4.7 ± 1.2 (0.69)	9.8 ± 0.6 (0.56)
20	0.066 ± 0.019 (0.69)	6.0 ± 0.1 (0.92)	1.6 ± 0.1 (0.75)
23	0.020 ± 0.004 (1.12)	(0.27)	1.2 ± 0.4 (0.69)
25a	0.015 (0.99)[b]	3.2 ± 0.9 (0.36)	4.5 ± 0.3 (0.25)
26a	0.06 (0.95)[b]	(0.04)	(0.05)
26b	0.26 (0.94)[b]	nd	nd
26c	0.098 (1.01)[b]	(0.02)	(0.0)
26d	0.026 (1.1)[b]	>10 (0.27)	>10 (0.31)
26e	0.008 (0.96)[b]	(0.08)[b]	(0.06)[b]
26f	0.004 (1.13)[b]	1.5 (0.41)[b]	43.7 (0.65)[b]
26g	0.92 ± 0.16 (0.88)	nd	nd
29	0.036 ± 0.011 (1.1)	10.5± 0.28 (0.59)	>1 (0.29)
33a	0.13 (1.0)[b]	(0.09)	(0.1)
33b	0.009 (1.1)[b]	(0.06)[b]	(0.04)[b]

[a] β-AR agonistic activities were assessed by measurement of cAMP accumulation levels in CHO cells expressing human β-ARs. The intrinsic activities (IA) were given as a fraction of the maximal stimulation with isoproterenol. All results are based on a single dose−response determination ± SE, unless otherwise indicated. Number of doses: $n = 7$ for β_2 and β_1 dose−response curves; $n = 14$ for β_3 dose−response curves. [b] Results are given as the mean of two or three independent dose−response determinations. [c] nd = not determined.

activity at the β_2 receptor (IA = 0.59) and modest partial agonist activity at the β_1 receptor (IA = 0.29).

More amino acid derivatives were then evaluated. Lengthening the carboxylic acid tether by one carbon atom (β-alanine derivative **26b**) resulted in a moderate decrease (4.3-fold) in β_3-AR activity. Proline derivative **26c**, a conformationally constrained analogue of **26a**, was also less active (EC_{50} = 0.098 μM at the β_3-AR) as compared to the glycine analogue **26a**. While introduction of an isopropyl group at the α-position of glycine led to a branched amino acid derivative (valine **26d**), which had a minor increased β_3 activity (2.3-fold) as compared to the glycine analogue **26a**, introduction of an isobutyl group resulted in a leucine analogue **26e** which proved to be about 8-fold more potent than glycine **26a** at the β_3 receptor. Furthermore, agonist **26e** exhibited a remarkable selectivity profile with IA = 8% for the β_2-AR and 6% for the β_1-AR. This suggests that the spatial arrangement of the alkyl substituent and the carboxylic acid is important for maintaining β_3 agonist activity and selectivity as the isomeric *N*-butyl analogue **29** was about 5-fold less potent at the β_3 receptor and much less selective versus either the β_1- or β_2-AR. Thus, with EC_{50} = 0.008 μM and full agonist activity at the β_3 receptor, and nearly completely abolished intrinsic activity at either the β_1- or β_2-AR, to our knowledge **26e** is among the most potent and selective human β_3-AR agonists reported to date.

Replacement of the isobutyl substituent with the larger benzyl group (phenylalanine derivative **26f**) resulted in a minor increase (2-fold) in β_3-AR agonist activity. However, it was less selective than **26e**. Introduction of another carboxylic acid substituent (glutamic acid **26g**) diminished the β_3-AR activity (EC_{50} = 0.92 μM). All of these results suggest that the combination of a lipophilic alkyl group with the correct spatial orientation and a polar carboxylic acid is important for activity and selectivity for the β_3-AR. This same combination/activity pattern was also observed with the reverse amide series. The parent amide **33a** was a rather modest β_3 agonist (EC_{50} = 0.13 μM), but *N*-butyl derivative **33b** exhibited increased activity (EC_{50} = 0.009 μM, IA = 1.10). A good selectivity profile was seen with both **33a** and **33b**.

Table 2. Thermogenesis and β_1-AR/β_2-AR Binding Data

		binding[b] K_i, μM	
compd	thermogenesis (%)[a]	β_1-AR	β_2-AR
CGP 12177A		0.00025	0.0001
vehicle[c]	0 ± 10		
L 755507	28 ± 5	0.57	0.16
CL 316243	nd[d]	>100	79.2
26a	51 ± 8[17]	>100	11.6
26d	nd	>100	3.6
26e	53 ± 8	>100	3.4
26f	nd	>100	0.48
33b	35 ± 12	>100	>100

[a] Compounds were tested for increased thermogenesis (%) using β_3 human transgenic mice at 10 mg/kg ip. Compounds were considered active if they were able to produce a statistically significant 15% increase in thermogenesis in β_3 transgenic mice. [b] Binding potency is reported as K_i, the binding inhibition constant, determined by inhibition of ^{125}I-iodocyanopindolol. Results are given as the mean of two or three independent experiments. [c] Vehicle is 0.5% methylcellulose:0.1% Tween-80 and injected 0.2 mL/mouse ip. [d] nd = not determined.

Compounds with low cAMP functional activity in β_1- and β_2-ARs may exhibited strong antagonist activity which may cause unwanted side effects.[1] Several of the more selective compounds and three known selective β_3 agonists (CGP 12177A, L 755507, and CL 316243) were evaluated in β_1- and β_2-AR binding assays, and the results are shown in Table 2. In contrast to the known antagonist CGP 12177A, which had very strong binding affinities for β_2 and β_1-ARs, glycine derivative **26a**, three branched amino acid analogues **26d**−**f**, and the reverse amide **33b** all had a binding inhibition constant (K_i) greater than 100 μM at the β_1 receptor site and K_i = 11.6, 3.6, 3.4, 0.48, and >100 μM, respectively, at the β_2 receptor site. These results suggested that **26a, 26d**−**f**, and **33b** had very weak binding affinities to β_2- and β_1-ARs, thus further confirming their selectivity for β_3-AR versus β_1- and β_2-ARs.

Three of the most interesting compounds and the standard L 755507 were examined in vivo for their thermogenesis effect,[15] and the results are included in Table 2. L 755507 has been shown to be active when given iv to monkeys resulting in increased UCP-1 mRNA in BAT.[8a] When given ip to human β_3 transgenic mice at a dose of 10 mg/kg, L 755507 increased thermogenesis by 28%. This confirmed that our β_3 transgenic mice model is giving a result that is comparable to this reference compound when it was given to nonhuman primates. Under our assay conditions, all three new compounds (**25a**,[17] **26e**, **33b**) produced a major effect (more than 35% increase) in thermogensis.

Conclusions

In this study the synthesis and SAR within a series of (4-piperidin-1-yl)phenyl amide based β_3 agonists has been discussed. With EC_{50} = 0.008 and 0.009 μM, respectively, at the human β_3 receptor, nearly completely abolished intrinsic activity at either the β_1 or β_2 receptor, the leucine derivative **26e** and the reverse amide **33b** are the two most potent and selective compounds in this study. These compounds also showed

good thermogenesis effects in human β_3-AR transgenic mice. On the basis of their desirable in vitro and in vivo properties, **26e** and **33b** were chosen for further biological evaluations.

Experimental Section

General. Melting points were determined in open capillary tubes on a Meltemp melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined with a Bruker DPX-300 spectrometer at 300 MHz. Chemical shifts δ are expressed in parts per million relative to the internal standard tetramethylsilane and J values (coupling constant) in Hz. Electrospray (ES) mass spectra were recorded in positive or negative mode on a Micromass Platform spectrometer. Electron impact and high-resolution mass spectra were obtained on a Finnigan MAT-90 spectrometer. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. Chromatographic purifications were performed by flash chromatography using Baker 40-μm silica gel. Thin-layer chromatography (TLC) was performed on Analtech silica gel GHLF 250 M prescored plates.

4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)benzoic Acid (4). A mixture of 4-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)benzoic acid ethyl ester[10] (14 g, 48.1 mmol) in MeOH/THF (1:1, 100 mL) and NaOH (2 N, 150 mL) was refluxed for 2 h. After cooling to room temperature, the solution was acidified with acetic acid and the solid which was formed was collected and dried to give the title compound as a white solid (12.5 g, 99%): mp 170−172 °C; ^{1}H NMR ($CDCl_3$) δ 1.67 (t, J = 5.6 Hz, 4 H), 3.43 (t, J = 5.6 Hz, 4 H), 3.92 (s, 4 H), 6.96 (d, J = 9.0 Hz, 2 H), 7.75 (d, J = 9.0 Hz, 2 H); MS (ES) m/z 264.4 (MH^+); HRMS calcd for $C_{14}H_{17}NO_4$(M^+) 263.1158, found 263.1160. Anal. ($C_{14}H_{17}NO_4$) C, H, N.

4-(4-Oxopiperidin-1-yl)benzoic Acid (5). Compound **4** (12.5 g, 47.5 mmol) was treated with concentrated HCl (500 mL) at room temperature. After 15 h, ~28% NH_4OH was added dropwise and the precipitate was collected by filtration and dried over P_2O_5 to give the title compound as a white solid (9.2 g, 88%): mp 209−211 °C; ^{1}H NMR ($CDCl_3$) δ 2.46 (t, J = 6.1 Hz, 4 H), 3.74 (t, J = 6.1 Hz, 4 H), 7.02 (d, J = 9.0 Hz, 2 H), 7.80 (d, J = 9.0 Hz, 2 H); MS (ES) m/z 219.8 (MH^+); HRMS calcd for $C_{12}H_{14}NO_3$ (MH^+) 220.0974, found 220.0947. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

General Procedures for Preparation of Compounds 7, 11, 17, 20, 23, 25a−f, 26a−f, 33a,b. Method A. A mixture of the piperidone (as specified, 1 equiv), acetic acid (1−5 equiv) and *N*-[5-((1*R*)-2-amino-1-hydroxyethyl)-2-hydroxyphenyl]-methanesulfonamide (**6**)[9] (1 equiv) in EtOH (0.01−0.5 M) was hydrogenated in the presence of 10% Pd/C (5−15 wt %) under H_2 (5−20 psi) for overnight. The catalyst was then removed by filtering through a short pad of silica gel. The filtrate was concentrated and purified by preparative TLC, silica gel chromatography using 0−10% MeOH/CH_2Cl_2 as eluent or recrystallization from MeOH/CH_2Cl_2.

Method B. The piperidone (as specified, 1 equiv) and **6** (1 equiv) were mixed in DMF (0.05−0.3M) and then treated with $NaBH(OAc)_3$ (1.5−5 equiv) and acetic acid (1.5−5 equiv). After stirring at room temperature under a N_2 atmosphere for 1−24 h the mixture was quenched with 1 N NaOH and then poured into a saturated aqueous $NaHCO_3$. The precipitate which formed was collected and purified by preparative TLC or silica gel chromatography using 0−10% MeOH/CH_2Cl_2 as eluent.

Method C. To a stirred solution of the alkyl ester (as specified, 1 equiv) in distilled water (0.01−0.5 M) was added 1 N NaOH (1−10 equiv). The reaction was stirred at room temperature for 2−24 h. The reaction mixture was made acidic (pH 6) with glacial acetic acid, and the solid was collected and dried over P_2O_5. The product was purified (if needed) by preparative TLC, silica gel chromatography using 0−10% MeOH/CH_2Cl_2 as eluent or recrystallization from MeOH/CH_2Cl_2.

4-{4-[(2*R*)-2-Hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}benzoic Acid (7). Method A, **5**, off-white solid, 53% yield: mp >85 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (m, 2 H), 1.80−1.95 (m, 2 H), 2.60−3.00 (m, 5 H), 2.92 (s, 3 H), 3.75−3.85 (m, 2 H), 4.53 (dd, J = 8.3, 4.0 Hz, 1 H), 6.82 (d, J = 8.3 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.01 (dd, J = 8.3, 2.0 Hz, 1 H), 7.19 (d, J = 2.0 Hz, 1 H), 7.73 (d, J = 8.9 Hz, 2 H); MS (ES) m/z 449.9 (MH^+); HRMS calcd for $C_{21}H_{26}N_3O_6S$ ($M - H$)$^-$ 448.1549, found 448.1541. Anal. ($C_{21}H_{27}N_3O_6S\cdot 2H_2O\cdot 0.5MeOH$) C, H, N.

[4-(4-Oxopiperidin-1-yl)phenyl]acetic Acid (10). 1-[4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)phenyl]ethanone[10](2.61 g, 10 mmol) was added to a solution of thallium trinitrate trihydrate[11] (4.44 g, 10 mmol) in MeOH (40 mL) and CH_2Cl_2 (20 mL) containing perchloric acid (70%, 10 mL). After 5 h at room temperature, the precipitated thallium(I) nitrate was removed by filtration and the filtrate was diluted with water (100 mL). The organic phase was separated, the aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were washed with water, dried over Na_2SO_4 and concentrated. The residue was dissolved in concentrated HCl (250 mL) at room temperature. After 3 h the solution pH was adjusted to 5 with ~28% NH_4OH. The aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were washed with water, dried over Na_2SO_4 and concentrated. The product was purified by chromatography on silica gel with EtOAc/hexanes as eluant to give the title compound as a pale yellowish solid (0.4 g, 17%): ^{1}H NMR (DMSO-d_6) δ 2.42 (t, J = 6.0 Hz, 4 H), 3.41 (s, 2 H), 3.56 (t, J = 6.0 Hz, 4 H), 6.98 (d, J = 8.7 Hz, 2 H), 7.12 (d, J = 8.7 Hz, 2 H), 12.20 (s, 1 H); MS (ES) m/z 234.3 (MH^+); HRMS calcd for $C_{13}H_{15}NO_3$ (M^+) 233.1052, found 233.1045. Anal. ($C_{13}H_{15}NO_3$) C, H, N.

(4-{4-[(2*R*)-2-Hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}phenyl)acetic Acid (11). Method A, **10**, pale yellowish solid, 5% yield: mp >140 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.25−1.40 (m, 2 H), 1.75−1.95 (m, 2 H), 2.50−3.60 (m, 7 H), 2.88 (s, 3 H), 3.16 (s, 2 H), 4.47 (dd, J = 8.0, 4.1 Hz, 1 H), 6.78 (d, J = 8.2 Hz, 1 H), 6.84 (d, J = 8.3 Hz, 2 H), 6.95 (dd, J = 8.2, 1.7 Hz, 1 H), 7.05 (d, J = 8.3 Hz, 2 H), 7.16 (d, J = 1.7 Hz, 1 H); MS (ES) m/z 464.1 (MH^+); HRMS calcd for $C_{22}H_{28}N_3O_6S$ ($M - H$)$^-$ 462.1704, found 462.1696. Anal. ($C_{22}H_{29}N_3O_6S\cdot 2H_2O\cdot 0.15AcOH$) C, H, N.

(*E*)-3-[4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)phenyl]acrylic Acid Ethyl Ester (14). To a solution of LiBr (0.53 g, 6 mmol) in THF (5 mL) was added triethyl phosphonoacetate (1.14 g, 5 mmol), followed by Et_3N (0.51 g, 5 mmol). After stirring for 10 min, a solution of 4-(1,4-dioxa-8-azaspiro[4.5]-dec-8-yl)benzaldehyde[10] (1.24 g, 5 mmol) in THF (2 mL) was added, and the mixture was stirred at room temperature for 20 h. Additional triethyl phosphonoacetate (0.57 g, 2.5 mmol) and Et_3N (0.25 g, 2.5 mmol) were added, and stirring was continued for 3 days. The mixture was evaporated, and the residue stirred with 20 mL of water and 5 mL of 1 N HCl. The aqueous suspension was filtered, and the precipitate was washed with water and dried in vacuo to give 1.28 g (81%) of a light yellow solid: mp 115−116 °C; ^{1}H NMR ($CDCl_3$) δ 1.33 (t, 3 H), 1.81 (dd, 4 H), 3.47 (dd, 4 H), 4.00 (s, 4 H), 4.25 (q, 2 H), 6.26 (d, 1 H), 6.89 (d, 2 H), 7.42 (d, 2 H), 7.62 (d, 1 H); MS (ES) m/z 318.0 (MH^+); HRMS calcd for $C_{18}H_{24}NO_4$ (MH^+) 318.1700, found 318.1699. Anal. ($C_{18}H_{23}NO_4$) C, H, N.

(*E*)-3-[4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)phenyl]acrylic Acid (15). Prepared from **14** according to the procedure for **4** as a light yellow solid in 100% yield: mp 221−222 °C; ^{1}H NMR (DMSO-d_6) δ 1.67 (dd, 4 H), 3.40 (dd, 4 H), 3.91 (s, 4 H), 6.26 (d, 1 H), 6.96 (d, 2 H), 7.47 (d, 1 H), 7.50 (d, 2 H), 12.10 (br s, 1 H); MS (ES) m/z 290 (MH^+); HRMS calcd for $C_{16}H_{20}NO_4$ (MH^+) 290.1421, found 290.1421. Anal. ($C_{16}H_{19}NO_4$) C, H, N.

(*E*)-3-[4-(4-Oxopiperidin-1-yl)phenyl]acrylic Acid (16). Prepared from **15** according to the procedure for **5** as a light yellow solid in 90% yield: mp 215 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (t, 4 H), 3.71 (t, 4 H), 6.28 (d, 1 H), 7.01 (d, 2 H), 7.50 (d, 1 H), 7.55 (d, 2 H); MS (ES) m/z 246.3 (MH^+); HRMS calcd for $C_{14}H_{16}NO_3$ (MH^+) 246.1123, found 246.1127. Anal. ($C_{14}H_{15}NO_3\cdot 0.55H_2O$) C, H, N.

3-(4-{4-[(*R*)-2-Hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}phenyl)-propionic Acid (17). Method A, **16**, off-white solid, 94% yield: mp 130−133 °C; ^{1}H NMR (DMSO-d_6) δ 1.30−1.46 (m, 2 H), 1.84−1.95 (m, 2 H), 2.37−2.50 (m, 4 H), 2.60−2.78 (m, 4 H), 2.92 (s, 3 H), 3.53−3.62 (m, 3 H), 4.46−4.55 (m, 1 H), 6.85 (d, 2 H), 6.92−7.10 (m, 4 H), 7.18 (dd, 1 H); MS (ES) *m*/*z* 478.3 (MH^+); HRMS calcd for $C_{23}H_{32}N_3O_6S$ (MH^+) 478.2011, found 478.2013. Anal. ($C_{23}H_{31}N_3O_6S\cdot2.5H_2O$) C, H, N.

[4-(1,4-Dioxa-8-azaspiro[4.5]dec-8-yl)phenyl]methanol (18). $NaBH_4$ (3.78 g, 100 mmol) was added in four portions to a stirred solution of 4-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)-benzaldehyde[10] (4.94 g, 20 mmol) in MeOH /THF (1:1, 100 mL) at 0 °C and the resulting solution was stirred at 0 °C for 30 min and at room temperature for 1 h. CH_2Cl_2 was added and the solution was washed with water and dried over Na_2SO_4. The solvents were removed and the residue solidified upon standing (5.0 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.84 (t, J = 5.7 Hz, 4 H), 3.33 (t, J = 5.7 Hz, 4 H), 3.99 (s, 4 H), 4.59 (d, J = 5.8 Hz, 2 H), 6.93 (d, J = 8.7 Hz, 2 H), 7.24 (d, J = 8.7 Hz, 2 H); MS (ES) *m*/*z* 249.9 (MH^+); HRMS calcd for $C_{14}H_{20}NO_3$ (MH^+) 250.1443, found 250.1434. Anal. ($C_{14}H_{19}NO_3\cdot0.18H_2O$) C, H, N.

[4-(4-Oxopiperidin-1-yl)benzyloxy]acetic Acid (19). A solution of **18** (3.49 g, 14 mmol) in THF (100 mL) was treated with 60% NaH in mineral oil (0.50 g, 21 mmol) and heated to a gentle reflux for 2 h. After cooling to room temperature, iodoacetic acid sodium salt (2.91 g, 14 mmol) was added in one portion. The resulting mixture was heated to a gentle reflux for another 2 h. After cooling to room temperature the reaction was quenched by careful addition of water and then partitioned between water and CH_2Cl_2. The aqueous layer was acidified with acetic acid and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, and concentrated. The residue was treated with concentrated HCl (150 mL) at room temperature. After 3 h the solution pH was adjusted to 4 with ∼28% NH_4OH. The aqueous layer was extracted with CH_2Cl_2, and the combined organic phases were washed with water, dried over Na_2SO_4 and concentrated to give a gum (**19**) (0.95 g, 26%): ^{1}H NMR ($CDCl_3$) δ 2.55 (t, J = 6.1 Hz, 4 H), 3.61 (t, J = 6.1 Hz, 4 H), 4.10 (s, 2 H), 4.56 (s, 2 H), 6.95 (d, J = 8.6 Hz, 2 H), 7.29 (d, J = 8.6 Hz, 2 H); MS (ES) *m*/*z* 264.3 (MH^+).

(4-{4-[(2*R*)-2-Hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}benzyloxy)-acetic Acid (20). Method A, **19**, off-white solid, 45% yield: mp >145 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.30−1.50 (m, 2 H), 1.80−2.00 (m, 2 H), 2.60−2.80 (m, 5 H), 2.90 (s, 3 H), 3.50−3.70 (m, 2 H), 3.65 (s, 2 H), 4.52 (s, 2 H), 4.54 (dd, J = 8.3, 3.8 Hz, 1 H), 6.75−6.90 (m, 3 H), 7.00 (dd, J = 8.3, 2.0 Hz, 1 H), 7.02−7.20 (m, 3 H); MS (ES) *m*/*z* 494.2 (MH^+); HRMS calcd for $C_{23}H_{30}N_3O_7S$ ($M - H)^-$ 492.1810, found 492.1804. Anal. ($C_{23}H_{31}N_3O_7S\cdot H_2O\cdot CH_2Cl_2$) C, H, N.

4-(4-Oxo-1-piperidin-1-yl)benzamide (22). A suspension of 4-(1,4-dioxa-8-azaspiro[4.5]dec-8-yl)benzonitrile (15.0 g, 61.5 mmol)[10] in 150 mL of concentrated HCl was stirred at room temperature overnight. The solution was neutralized with 5 N NaOH and the solid that formed was collected to give the title compound (11.0 g, 82%) as a white solid: mp 186−188 °C; ^{1}H NMR ($CDCl_3$) δ 2.58 (t, J = 6.1 Hz, 4 H), 3.74 (t, J = 6.1 Hz, 4 H), 5.76 (brs, 2 H), 6.94 (d, J = 9.0 Hz, 2 H), 7.74 (d, J = 9.0 Hz, 2 H); MS (ES) *m*/*z* 218.8 (MH^+); HRMS calcd for $C_{12}H_{14}N_2O_2$ (M^+) 218.1055, found 218.1051. Anal. ($C_{12}H_{14}N_2O_2$) C, H, N.

4-{4-[(2*R*)-2-Hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}benzamide (23). Method A, **22**, white solid, 54% yield: mp >75 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.2−1.40 (m, 2 H), 1.70−1.90 (m, 2 H), 2.55−2.95 (m, 5 H), 2.97 (s, 3 H), 3.70−3.85 (m, 2 H), 4.48 (dd, J = 8.0, 4.3 Hz, 1 H), 6.81 (d, J = 8.2 Hz, 1 H), 6.91 (d, J = 9.0 Hz, 2 H), 6.99 (dd, J = 8.2, 2.0 Hz, 1 H), 7.17 (d, J = 2.0 Hz, 1 H), 7.67 (br s, 2 H), 7.72 (d, J = 9.0 Hz, 2 H); MS (ES) *m*/*z* 448.9 (MH^+); HRMS calcd for $C_{21}H_{29}N_4O_5S$ (MH^+) 449.1853, found 449.1853. Anal. ($C_{21}H_{28}N_4O_5S\cdot0.8AcOH$) C, H, N.

General Procedure for Preparation of Compounds 24a−f. A mixture of 4-(4-oxopiperidin-1-yl)benzoic acid (**5**) (1 equiv), 1-[3-(dimethylamino)propyl-3-ethylcarbodiimide hydrochloride (1.1−1.5 equiv) and the amine salt (as specified, 1−1.5 equiv) was stirred in CH_2Cl_2 (0.01−0.3 M). *N*-Methylmorpholine (2−5 equiv) was added dropwise and the mixture was stirred for 2−24 h. The mixture was then washed with 0.05 N HCl and water. The resulting solution was dried with $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography using 0−10% MeOH/CH_2Cl_2 as eluant.

Ethyl {[4-(4-Oxo-1-piperidinyl)benzoyl]amino}acetate (24a). Glycine ethyl ester hydrochloride, pale yellowish solid, 66% yield: mp 98−99 °C; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.1 Hz, 3 H), 2.43 (t, J = 6.1 Hz, 4 H), 3.72 (t, J = 6.1 Hz, 4 H), 3.95 (d, J = 5.8 Hz, 2 H), 4.10 (q, J = 7.1 Hz, 2 H), 7.04 (d, J = 8.9 Hz, 2 H), 7.77 (d, J = 8.9 Hz, 2 H), 8.63 (t, J = 5.8 Hz, 1 H); MS (ES) *m*/*z* 305.2 (MH^+); HRMS calcd for $C_{16}H_{20}N_2O_4$ (M^+) 304.1423, found 304.1422. Anal. ($C_{16}H_{20}N_2O_4\cdot0.2H_2O$) C, H, N.

3-[4-(4-Oxopiperidin-1-yl)benzoylamino]propionic Acid Benzyl Ester (24b). β-Alanine benzyl ester *p*-tosylate, white solid, 41% yield: mp 87−89 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (t, J = 6.0 Hz, 4 H), 2.63 (t, J = 6.9 Hz, 2 H), 3.40−3.50 (m, 2 H), 3.71 (t, J = 6.0 Hz, 4 H), 5.10 (s, 2 H), 6.74 (d, J = 8.9 Hz, 2 H), 7.30−7.40 (m, 5 H), 7.73 (d, J = 8.9 Hz, 2 H), 8.29 (t, J = 5.5 Hz, 1 H); MS (ES) *m*/*z* 381.3 (MH^+); HRMS calcd for $C_{22}H_{24}N_2O_4$ (M^+) 380.1737, found 380.1755. Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

Methyl 1-[4-(4-Oxo-1-piperidinyl)benzoyl]-L-prolinate (24c). L-Proline methyl ester hydrochloride, pale yellowish solid, 54% yield: mp 79−81 °C; ^{1}H NMR (DMSO-d_6) δ 1.70−1.95 (m, 4 H), 2.20−2.40 (m, 1 H), 2.41 (t, J = 6.0 Hz, 4 H), 3.32 (s, 3 H), 3.69 (t, J = 6.0 Hz, 4 H), 4.35−4.50 (m, 2 H), 7.01 (d, J = 8.7 Hz, 2 H), 7.49 (d, J = 8.7 Hz, 2 H); MS (ES) *m*/*z* 331.2 (MH^+); HRMS calcd for $C_{18}H_{22}N_2O_4$ (M^+) 330.1580, found 330.1572. Anal. ($C_{18}H_{22}N_2O_4\cdot0.2H_2O$) C, H, N.

Ethyl 1-[4-(4-Oxo-1-piperidinyl)benzoyl]-L-valinate (24d). L-Valine ethyl ester hydrochloride, pale yellowish solid, 72% yield: mp 51−52 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (d, J = 6.8 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H), 1.20 (t, J = 7.1 Hz, 3 H), 2.10−2.30 (m, 1 H), 2.43 (t, J = 6.0 Hz, 4 H), 3.72 (t, J = 6.0 Hz, 4 H), 4.00−4.30 (m, 3 H), 4.46 (t, J = 5.1 Hz, 1 H), 7.05 (d, J = 8.9 Hz, 2 H), 7.82 (d, J = 8.9 Hz, 2 H), 8.21 (d, J = 7.9 Hz, 1 H); MS (ES) *m*/*z* 347.3 (MH^+); HRMS calcd for $C_{19}H_{26}N_2O_4$ (M^+) 346.1892, found 346.1896. Anal. ($C_{19}H_{26}N_2O_4\cdot0.25H_2O$) C, H, N.

Ethyl 1-[4-(4-Oxo-1-piperidinyl)benzoyl]-L-leucinate (24e). L-Leucine ethyl ester hydrochloride, white flakes, 76% yield: mp 75−77 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, J = 6.2 Hz, 6 H), 1.30 (t, J = 7.1 Hz, 3 H), 1.60−1.80 (m, 3 H), 2.56 (t, J = 6.0 Hz, 4 H), 3.72 (t, J = 6.0 Hz, 4 H), 4.22 (q, J = 7.1 Hz, 2 H), 4.80−4.90 (m, 1 H), 6.42 (d, J = 8.2 Hz, 1 H), 6.94 (d, J = 9.0 Hz, 2 H), 7.77 (d, J = 9.0 Hz, 2 H); MS (ES) *m*/*z* 361.3 (MH^+); HRMS calcd for $C_{20}H_{28}N_2O_4$ (M^+) 360.2049, found 360.2081. Anal. ($C_{20}H_{28}N_2O_4$) C, H, N.

Methyl 1-[4-(4-Oxo-1-piperidinyl)benzoyl]-L-phenylalaninate (24f). L-Phenylalanine methyl ester hydrochloride, pale yellowish solid, 75% yield: mp 30 °C; ^{1}H NMR (DMSO-d_6) δ 2.41 (t, J = 6.0 Hz, 4 H), 3.05−3.20 (m, 2 H), 3.71 (t, J = 6.0 Hz, 4 H), 4.55−4.65 (m, 1 H), 6.97 (d, J = 8.9 Hz, 2 H), 7.10−7.45 (m, 5 H), 7.73 (d, J = 8.9 Hz, 2 H), 8.52 (d, J = 7.8 Hz, 1 H); MS (ES) *m*/*z* 381.3 (MH^+); HRMS calcd for $C_{22}H_{25}N_2O_4$ (MH^+) 381.1809, found 381.1807. Anal. ($C_{22}H_{24}N_2O_4\cdot0.4H_2O$) C, H, N.

Dibenzyl 1-[4-(4-Oxo-1-piperidinyl)benzoyl]-L-glutamate (24g). L-Glutamic acid dibenzyl ester *p*-tosylate, white solid, 45% yield: mp 64−66 °C; ^{1}H NMR (DMSO-d_6) δ 1.90−2.20 (m, 4 H), 2.42 (t, J = 6.0 Hz, 4 H), 3.72 (t, J = 6.0 Hz, 4 H), 4.45−4.55 (m, 1 H), 5.07 (s, 2 H), 5.13 (s, 2 H), 7.03 (d, J = 9.0 Hz, 2 H), 7.25−7.40 (m, 8 H), 7.85 (d, J = 9.0 Hz, 2 H), 8.49 (d, J = 9.0 Hz, 2 H); MS (ES) *m*/*z* 529.1 (MH^+); HRMS calcd for $C_{31}H_{33}N_2O_6$ (MH^+) 529.2339, found 529.2331. Anal. ($C_{31}H_{32}N_2O_6$) C, H, N.

Ethyl [(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)amino]acetate (25a). Method B, **24a**, white solid, 69% yield: mp >140 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20 (t, J = 7.1 Hz, 3 H), 1.20−1.35 (m, 2 H), 1.75−1.90 (m, 2 H), 2.50−2.90 (m, 5 H), 2.90 (s, 3 H), 3.70−3.85 (m, 2 H), 3.93 (d, J = 5.8 Hz, 2 H), 4.10 (q, J = 7.1 Hz, 2 H), 4.47 (dd, J = 7.9, 4.4 Hz, 1 H), 6.79 (d, J = 8.3 Hz, 1 H), 6.90−7.00 (m, 3 H), 7.16 (d, J = 2.0 Hz, 1 H), 7.72 (d, J = 8.9 Hz, 2 H), 8.58 (t, J = 5.8 Hz, 1 H); MS (ES) m/z 535.2 (MH^+); HRMS calcd for $C_{25}H_{35}N_4O_7S$ (MH^+) 535.2221, found 535.2216. Anal. ($C_{25}H_{34}N_4O_7S\cdot0.4CH_2Cl_2\cdot1.2MeOH$) C, H, N.

Methyl 1-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-prolinate (25c). Method B, **24c**, off-white solid, 58% yield: mp >80 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (m, 2 H), 1.75−1.95 (m, 4 H), 2.15−2.30 (m, 2 H), 2.50−2.90 (m, 5 H), 2.90 (s, 3 H), 3.55−3.80 (m, 7 H), 4.40−4.55 (m, 2 H), 6.81 (d, J = 8.3 Hz, 1 H), 6.92 (d, J = 8.7 Hz, 2 H), 6.98 (dd, J = 8.3, 2.0 Hz, 1 H), 7.17 (d, J = 2.0 Hz, 1 H), 7.43 (d, J = 8.7 Hz, 2 H); MS (ES) m/z 561.3 (MH^+); HRMS calcd for $C_{27}H_{37}N_4O_7S$ (MH^+) 561.2377, found 561.2369. Anal. ($C_{27}H_{36}N_4O_7S\cdot0.7CH_2Cl_2$) C, H, N.

Ethyl *N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-valinate (25d). Method B, **24d**, off-white solid, 59% yield: mp >85 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.92 (d, J = 6.8 Hz, 3 H), 0.97 (d, J = 6.8 Hz, 3 H), 1.17 (t, J = 7.1 Hz, 3 H), 1.20−1.40 (m, 2 H), 1.75−1.95 (m, 2 H), 2.05−2.20 (m, 1 H), 2.55−2.90 (m, 5 H), 2.90 (s, 3 H), 3.70−3.85 (m, 2 H), 4.00−4.20 (m, 2 H), 4.22 (t, J = 7.7 Hz, 1 H), 4.48 (dd, J = 8.0, 4.4 Hz, 1 H), 6.80 (d, J = 8.2 Hz, 1 H), 6.95 (d, J = 8.9 Hz, 2 H), 6.98 (dd, J = 8.2, 2.0 Hz, 1 H), 7.17 (d, J = 2.0 Hz, 1 H), 7.76 (d, J = 8.9 Hz, 2 H), 8.17 (d, J = 7.9 Hz, 1 H); MS (ES) m/z 577.2 (MH^+); HRMS calcd for $C_{28}H_{41}N_4O_7S$ (MH^+) 577.2690, found 577.2682. Anal. ($C_{28}H_{40}N_4O_7S\cdot2.1H_2O$) C, H, N.

Ethyl *N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-leucinate (25e). Method B, **24e**, off-white solid, 78% yield: mp >85 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.86 (d, J = 6.3 Hz, 3 H), 0.92 (d, J = 6.3 Hz, 3 H), 1.18 (t, J = 7.1 Hz, 3 H), 1.20−1.40 (m, 2 H), 1.45−1.95 (m, 5 H), 2.55−2.92 (m, 5 H), 2.92 (s, 3 H), 3.70−3.85 (m, 2 H), 4.10 (q, J = 7.1 Hz, 2 H), 4.40−4.55 (m, 2 H), 6.82 (d, J = 8.3 Hz, 1 H), 6.94 (d, J = 8.9 Hz, 2 H), 7.00 (dd, J = 8.3, 2.0 Hz, 1 H), 7.18 (d, J = 2.0 Hz, 1 H), 7.75 (d, J = 8.9 Hz, 2 H), 8.33 (d, J = 7.7 Hz, 1 H); MS (ES) m/z 591.3 (MH^+); HRMS calcd for $C_{29}H_{43}N_4O_7S$ (MH^+) 591.2847, found 591.2840. Anal. ($C_{29}H_{42}N_4O_7S\cdot0.6CH_2Cl_2$) C, H, N.

Methyl *N*-(4-{4-[((2*R*)-2-hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-phenylalaninate (25f). Method B, **24f**, off-white solid, 59% yield: mp >98 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (m, 2 H), 1.80−1.90 (m, 2 H), 2.05−2.90 (m, 5 H), 2.91 (s, 3 H), 3.00−3.20 (m, 2 H), 3.70−3.80 (m, 2 H), 4.47 (dd, J = 8.0, 4.3 Hz, 1 H), 4.50−4.65 (m, 1 H), 6.81 (d, J = 8.2 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.00 (dd, J = 8.2, 2.0 Hz, 1 H), 7.10−7.30 (m, 6 H), 7.67 (d, J = 8.9 Hz, 2 H), 8.47 (d, J = 7.8 Hz, 1 H); MS (ES) m/z 611.2 (MH^+); HRMS calcd for $C_{31}H_{39}N_4O_7S$ (MH^+) 611.2534, found 611.2525. Anal. ($C_{31}H_{38}N_4O_7S\cdot0.85CH_2Cl_2$) C, H, N.

[(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)amino]acetic Acid (26a). Method C, **25a**, white solid, 92% yield: mp >85 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (m, 2 H), 1.75−1.90 (m, 2 H), 2.55−2.90 (m, 5 H), 2.80 (s, 3 H), 3.49 (d, J = 4.5 Hz, 2 H), 3.70−3.80 (m, 2 H), 4.43 (dd, J = 7.9, 4.4 Hz, 1 H), 6.70 (d, J = 7.4 Hz, 1 H), 6.81 (dd, J = 7.4, 2.0 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.12 (d, J = 2.0 Hz, 1 H), 7.54 (t, J = 4.5 Hz, 1 H), 7.64 (d, J = 8.9 Hz, 2 H); MS (ES) m/z 507.2 (MH^+); HRMS calcd for $C_{23}H_{31}N_4O_7S$ (MH^+) 507.1908, found 507.1912. Anal. ($C_{23}H_{30}N_4O_7S\cdot3H_2O\cdot0.7CH_2Cl_2$) C, H, N.

***N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-β-alanine (26b).** Method A, **24b**, white solid, 42% yield: mp >170 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.45 (m, 2 H), 1.80−1.95 (m, 2 H), 2.43 (t, J = 7.0 Hz, 2 H), 2.60−2.90 (m, 5 H), 2.90 (s, 3 H), 3.00−3.40 (m, 2 H), 3.70−3.85 (m, 2 H), 4.50 (dd, J = 8.0, 4.2 Hz, 1 H), 6.82 (d, J = 8.3 Hz, 1 H), 6.92 (d, J = 8.9 Hz, 2 H), 7.01 (dd, J = 8.3, 1.9 Hz, 1 H), 7.18 (d, J = 1.9 Hz, 1 H), 7.68 (d, J = 8.9 Hz, 2 H), 8.26 (t, J = 5.2 Hz, 1 H); MS (ES) m/z 521.2 (MH^+); HRMS calcd for $C_{24}H_{33}N_4O_7S$ (MH^+) 521.2064, found 521.2056. Anal. ($C_{24}H_{32}N_4O_7S\cdot MeOH$) C, H, N.

1-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-proline (26c). Method C, **25c**, white solid, 47% yield: mp >220 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.40 (m, 2 H), 1.75−1.95 (m, 4 H), 2.50−2.90 (m, 7 H), 2.62 (s, 3 H), 3.50−4.00 (m, 5 H), 4.35−4.45 (m, 1 H), 6.60 (d, J = 8.3 Hz, 1 H), 6.79 (d, J = 8.7 Hz, 2 H), 6.90 (dd, J = 8.2, 1.4 Hz, 1 H), 7.06 (d, J = 1.4 Hz, 1 H), 7.37 (d, J = 8.6 Hz, 2 H); MS (ES) m/z 545.6 ($M - H)^-$; HRMS calcd for $C_{26}H_{33}N_4O_7S$ ($M - H)^-$ 545.2075, found 545.2064; HPLC purity 95.5% at 5.3 min (pH 3 phosphate/ACN).

***N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-valine (26d).** Method C, **25d**, pale yellowish solid, 27% yield: mp >135 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.90 (d, J = 7.3 Hz, 3 H), 0.92 (d, J = 7.3 Hz, 3 H), 1.45−1.55 (m, 2 H), 1.90−2.05 (m, 2 H), 2.05−2.20 (m, 1 H), 2.60−2.94 (m, 5 H), 2.94 (s, 3 H), 3.70−3.85 (m, 2 H), 4.11 (t, J = 6.0 Hz, 1 H), 4.65−4.75 (m, 1 H), 6.86 (d, J = 8.3 Hz, 1 H), 6.91 (d, J = 8.9 Hz, 2 H), 7.06 (dd, J = 8.3, 2.0 Hz, 1 H), 7.22 (d, J = 2.0 Hz, 1 H), 7.65−7.75 (m, 3 H); MS (ES) m/z 549.3 (MH^+); HRMS calcd for $C_{26}H_{35}N_4O_7S$ ($M - H)^-$ 547.2231, found 547.2229. Anal. ($C_{26}H_{36}N_4O_7S\cdot2.4H_2O$) C, H, N.

***N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-leucine (26e).** Method C, **25e**, white solid, 45% yield: mp >177 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.87 (d, J = 6.3 Hz, 3 H), 0.90 (d, J = 6.3 Hz, 3 H), 1.35−1.75 (m, 5 H), 1.90−2.00 (m, 2 H), 2.60−2.90 (m, 5 H), 2.94 (s, 3 H), 3.65−3.80 (m, 2 H), 4.25−4.35 (m, 1 H), 4.60−4.65 (m, 1 H), 6.85 (d, J = 8.3 Hz, 1 H), 6.90 (d, J = 8.9 Hz, 2 H), 7.05 (dd, J = 8.3, 1.9 Hz, 1 H), 7.21 (d, J = 1.9 Hz, 1 H), 7.73 (d, J = 8.9 Hz, 2 H), 7.98 (d, J = 7.7 Hz, 1 H); MS (ES) m/z 561.3 ($M - H)^-$; HRMS calcd for $C_{27}H_{37}N_4O_7S$ ($M - H)^-$ 561.2377, found 561.2381. Anal. ($C_{27}H_{38}N_4O_7S\cdot0.35CH_2Cl_2$) C, H, N.

***N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}benzoyl)-L-phenylalanine (26f).** Method C, **25f**, pale yellowish solid, 74% yield: mp >160 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.40−1.50 (m, 2 H), 1.90−2.05 (m, 2 H), 2.50−3.50 (m, 7 H), 2.95 (s, 3 H), 3.69−3.75 (m, 2 H), 4.30−4.45 (m, 2 H), 4.70−4.75 (m, 1 H), 6.83 (d, J = 8.8 Hz, 2 H), 6.88 (d, J = 8.3 Hz, 1 H), 7.05−7.30 (m, 7 H), 7.63 (d, J = 8.8 Hz, 2 H), 7.93 (br d, J = 6.6 Hz, 1 H); MS (ES) m/z 597.1 (MH^+); HRMS calcd for $C_{30}H_{35}N_4O_7S$ ($M - H)^-$ 595.2231, found 595.2232. Anal. ($C_{30}H_{36}N_4O_7S\cdot2.5H_2O$) C, H, N.

***N*-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]piperidin-1-yl}benzoyl)-L-glutamic Acid (26g).** Method A, **24g**, white solid, 42% yield: mp >230 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.40−1.60 (m, 2 H), 1.90−2.10 (m, 4 H), 2.31 (t, J = 7.5 Hz, 2 H), 2.65−3.20 (m, 7 H), 3.00 (s, 3 H), 3.85−4.00 (m, 2 H), 4.25−4.35 (m, 1 H), 4.65−4.80 (m, 1 H), 6.88 (d, J = 8.4 Hz, 1 H), 6.95 (d, J = 8.7 Hz, 2 H), 7.08 (dd, J = 8.4, 1.2 Hz, 1 H), 7.25 (d, J = 1.8 Hz, 1 H), 7.74 (d, J = 8.7 Hz, 2 H), 8.14 (d, J = 7.2 Hz, 1 H); MS (ES) m/z 579.1 (MH^+); HRMS calcd for $C_{26}H_{35}N_4O_9S$ (MH^+) 579.1973, found 579.1955. Anal. ($C_{26}H_{34}N_4O_9S\cdot CH_2Cl_2$) C, H, N.

[Butyl(4-{4-[(2*R*)-2-hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}benzoyl)amino]acetic Acid Ethyl Ester (28). A mixture of **5** (0.30 g, 1.37 mmol), 1-[3-(dimethylamino)propyl-3-ethylcarbodiimide

hydrochloride (0.53 g, 2.74 mmol) and *N*-butylglycine ethyl ester[12] (0.44 g, 2.7 mmol) was stirred in CH_2Cl_2 (70 mL). *N*-Methylmorpholine (0.28 g, 2.74 mmol) was added dropwise and the mixture was stirred overnight. The mixture was then washed with 0.05 N HCl and water. The resulting solution was dried with $MgSO_4$ and concentrated to give a gum (**27**). The gum was dissolved in DMF (15 mL) and then treated with **6** (0.25 g, 1 mmol), $NaBH(OAc)_3$ (0.85 g, 4 mmol) and acetic acid (0.3 mL). After stirring at room temperature under a N_2 atmosphere for 2 h, the mixture was poured into a saturated aqueous $NaHCO_3$. The aqueous layer was extracted with *n*-butanol and the concentrated gum was purified by silica gel chromatography ($MeOH/CH_2Cl_2$) to give the title compound as a white solid (60 mg, 10%): mp >60 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.70−1.60 (m, 12 H), 1.75−1.95 (m, 2 H), 2.50−3.50 (m, 7 H), 2.91 (s, 3 H), 3.60−3.75 (m, 2 H), 4.00−4.15 (m, 4 H), 4.47 (dd, J = 8.0, 4.3 Hz, 1 H), 6.81 (d, J = 8.5 Hz, 1 H), 6.90−7.00 (m, 3 H), 7.05−7.25 (m, 3 H); MS (ES) m/z 591.3 (MH^+); HRMS calcd for $C_{29}H_{43}N_4O_7S$ (MH^+) 591.2847, found 591.2840.

[Butyl(4-{4-[(2*R*)-2-hydroxy-2-(4-hydroxy-3-methanesulfonylaminophenyl)ethylamino]piperidin-1-yl}benzoyl)-amino]acetic Acid (29). Method C, **28**, pale yellowish solid, 60% yield: mp >95 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.70−1.70 (m, 9 H), 1.90−2.10 (m, 2 H), 2.50−3.50 (m, 7 H), 2.94 (s, 3 H), 3.60−3.85 (m, 4 H), 4.75−4.90 (m, 1 H), 6.80−7.40 (m, 8 H); MS (ES) m/z 561.5 $(M - H)^-$; HRMS calcd for $C_{27}H_{37}N_4O_7S$ $(M - H)^-$ 561.2377, found 561.2388. Anal. ($C_{27}H_{38}N_4O_7S\cdot 2H_2O$) C, H, N.

Ethyl 3-Oxo-3-[4-(4-oxo-1-piperidinyl)anilino]propanoate (31a). To a stirred solution, under N_2 atmosphere, of 1-(4-aminophenyl)piperidin-4-one (**30a**)[9] (10.0 g, 38 mmol), and Et_3N (15.35 g, 150 mmol) in CH_2Cl_2 (100 mL) was added ethyl 3-chloro-3-oxopropionate (6.32 g, 42 mmol). The reaction was stirred for 18 h. The reaction mixture was concentrated down in vacuo and purified by flash silica gel chromatography eluting with 1:1 EtOAc/hexanes to give an orange solid (0.86 g, 7%): ^{1}H NMR ($CDCl_3$) δ 1.32 (t, J = 7.2 Hz, 3 H), 2.55 (t, J = 6 Hz, 4 H), 3.46 (s, 2 H), 3.56 (t, J = 6 Hz, 4 H), 4.22 (q, J = 7.2 Hz, 2 H), 6.93 (d, J = 9.0 Hz, 2 H), 7.47 (d, J = 9.0 Hz, 2 H), 9.09 (s, 1 H); MS (ES) m/z 305.3 (MH^+, 100%).

Ethyl 3-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}anilino)-3-oxopropanoate (32a). Method B, **31a**, yellowish solid, 36% yield: mp >120 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7.0 Hz, 3 H), 1.25−1.45 (m, 2 H), 1.75−1.95 (m, 2 H), 2.50−2.70 (m, 5 H), 2.92 (s, 3 H), 3.33 (s, 2 H), 3.45−3.60 (m, 2 H), 4.09 (q, J = 7.0 Hz, 2 H), 4.48 (dd, J = 8.1, 4.2 Hz, 1 H), 6.80 (d, J = 8.3 Hz, 1 H), 6.90 (d, J = 9.0 Hz, 2 H), 7.00 (dd, J = 8.3, 2.0 Hz, 1 H), 7.18 (d, J = 2.0 Hz, 1 H), 7.39 (d, J = 9.0 Hz, 2 H), 9.92 (s, 1 H); MS (ES) m/z 535.3 (MH^+); HRMS calcd for $C_{25}H_{35}N_4O_7S$ (MH^+) 535.2221, found 535.2208. Anal. ($C_{25}H_{34}N_4O_7S\cdot 1.5H_2O$) C, H, N.

Ethyl3-(Butyl-4-{4-[((2*R*)-2-hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}anilino)-3-oxopropanoate (32b). To a stirred mixture of 1-[4-(butylamino)phenyl]-4-piperidinone (**30b**;[9] 2.46 g, 10 mmol) in 30 mL of CH_2Cl_2, Et_3N (1.81 mL, 13 mmol), and DMAP (catalytic amount) was added ethyl 3-chloro-3-oxopropionate (2.18 g, 13 mmol) dropwise. The reaction was stirred for 18 h. The reaction mixture was filtered, concentrated in vacuo, taken up in methylene chloride, and washed with saturated sodium bicarbonate two times and dried over sodium sulfate to give ethyl 3-[butyl-4-(4-oxo-1-piperidinyl)anilino]-3-oxopropanoate (**31b**) as a yellow oil (3.84 g, 68%): MS (ES) m/z 361.3 (MH^+, 100%). The title compound was prepared from **31b** and **6** as described in method B to give 29% of the compound as a tan solid: mp 172−174 °C; ^{1}H NMR (DMSO-d_6) δ 0.81 (t, J = 7.0 Hz, 3 H), 1.11 (t, J = 7.0 Hz, 3 H), 1.20−1.50 (m, 6 H), 1.80−2.00 (m, 2 H), 2.65−2.90 (m, 5 H), 2.93 (s, 3 H), 3.09 (s, 2 H), 3.56 (t, J = 6.8 Hz, 2 H), 3.65−3.80 (m, 2 H), 3.97 (q, J = 7.0 Hz, 2 H), 4.55−4.65 (m, 1 H), 6.85 (d, J = 8.3 Hz, 1 H), 6.96 (d, J = 9.0 Hz, 2 H), 7.00−7.10 (m, 3 H), 7.20 (d, J = 2.0 Hz, 1 H); MS (ES) m/z 591.2 (MH^+); HRMS calcd for $C_{29}H_{42}N_4O_7S$ (M^+) 590.2774, found 590.2847. Anal. ($C_{29}H_{42}N_4O_7S\cdot H_2O$) C, H, N.

3-(4-{4-[((2*R*)-2-Hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}anilino)-3-oxopropanoic Acid (33a). Method C, **32a**, pale gray solid, 59% yield: mp >170 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.30−1.45 (m, 2 H), 1.80−1.95 (m, 2 H), 2.50−2.80 (m, 5 H), 2.90 (s, 2 H), 2.91 (s, 3 H), 3.45−3.60 (m, 2 H), 4.48−4.53 (m, 1 H), 6.80−6.90 (m, 3 H), 7.00 (d, J = 8.3 Hz, 1 H), 7.18 (d, J = 1.6 Hz, 1 H), 7.38 (d, J = 9.0 Hz, 2 H), 11.50−11.60 (m, 1 H); MS (ES) m/z 505.3 $(M - H)^-$; HRMS calcd for $C_{23}H_{31}N_4O_7S$ (MH^+) 507.1908, found 507.1895. Anal. ($C_{23}H_{30}N_4O_7S\cdot CH_2Cl_2\cdot AcOH$) C, H, N.

3-(Butyl-4-{4-[((2*R*)-2-hydroxy-2-{4-hydroxy-3-[(methylsulfonyl)amino]phenyl}ethyl)amino]-1-piperidinyl}anilino)-3-oxopropanoic Acid (33b). Method C, **32b**, tan solid, 19%: mp >200 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.20−1.50 (m, 6 H), 1.70−1.95 (m, 2 H), 2.60−2.80 (m, 5 H), 2.83 (s, 2 H), 2.91 (s, 3 H), 3.45−3.70 (m, 4 H), 4.55−4.65 (m, 1 H), 6.81 (d, J = 8.0 Hz, 1 H), 6.90 (d, J = 8.7 Hz, 2 H), 7.09 (d, J = 8.7 Hz, 2 H), 7.07 (s, 1 H); MS (ES) m/z 563.2 (MH^+); HRMS calcd for $C_{27}H_{39}N_4O_7S$ (MH^+) 563.2461, found 563.2526. Anal. ($C_{27}H_{38}N_4O_7S\cdot 0.4CH_2Cl_2\cdot 0.7AcOH$) C, H, N.

Pharmacological Studies. In Vitro Functional Assays. CHO cells expressing either human β_1-, β_2-, or β_3-AR subtypes were used as previously described.[13] Clones expressing receptor levels of 70−110 fmol/mg protein were used in the assays. CHO cells were grown in 24-well tissue culture plates in Dulbecco's modified Eagle media (DMEM) with 10% fetal bovine serum, MEM nonessential amino acids, penicillin−streptompycin and geneticin. On the day of assay, growth medium was replaced with preincubation media (DMEM; Gibco, #1199-065) and incubated for 30 min at 37 °C. Preincubation medium was replaced with 0.2 mL treatment medium containing DMEM media containing 250 μM IBMX (isobutyl-1-methylxantine) plus 1 mM ascorbic acid with test compound dissolved in DMSO. Test compounds were tested over a concentration range of 10^{-9} to 10^{-5} M for β_3-AR cells and 10^{-8} to 10^{-4} M for β_1- and β_2 -AR transfected cells. Isoproterenol (10^{-5} M) was used as an internal standard for comparison of activity. Cells were incubated at 37 °C on a rocker for 30 min with the β_3-AR cells and 15 min for β_1- and β_2-AR cells. Incubation was stopped with the addition of 0.2 N HCl and neutralized with 2.5 N NaOH. The plates, containing the cells and neutralized media, were stored at −20 °C until ready to assay for cAMP using the SPA assay kit (Amersham). Data collected from the SPA assay was analyzed as a percent of the maximal isoproterenol response at 10^{-5} M. Activity curves were plotted using the SAS statistical and graphics software. EC_{50} values were generated for each compound and the maximal response developed for each compound was compared to the maximal response of isoproternol at 10^{-5} M from the following formula: intrinsic activity (IA) = (% activity of compound)/(% activity of isoproterenol).

***β*$_1$- and *β*$_2$-AR Binding Assays.** CHO cells, permanently transfected with either human β_1- or β_2-AR, were grown to 60−75% of confluency and harvested using nonenzymatic buffers. Intact CHO cells were incubated in 96-well plates using [^{125}I]-iodocyanopindolol (ICYP) as the radioligand. Assays were in duplicate using 25 000 cells for β_1-AR and 30 000 cells for β_2-AR incubations. PBS buffer, containing 0.1% BSA and 1 mM l-ascorbic acid, was used to dilute the cells and ICYP. The β_3-AR agonists to be tested were dissolved in DMSO to a concentration of 10 mM and then diluted to appropriate concentrations in the PBS−BSA buffer. Nonspecific binding was determined by addition of 2 μM D-propranol. The total assay volume was 0.3 mL and was incubated at room temperature for 45 min with gentle shaking (30 cycles/min). The assay was terminated by filtering on Unifilter −96, GF/C plates (Packard) using a Filtermate-196 cell harvester (Packard). The filters were dried, 30 μL of Microscint-20 added and counted on a Topcount microplate scintillation counter (Packard). Duplicate values were averaged and counts for nonspe-

cific binding subtracted. Data were analyzed for IC_{50} and K_i values using GraphPad Prism software (GraphPad Software, Inc., San Diego, CA).

Measurement of Thermogenesis in Transgenic Mice. Female FVB β_3-AR transgenic mice[15] were used to determine in vivo activity. Compounds were tested for increased thermogenesis using the Oxymax indirect calorimeter (Columbus Instruments, Columbus, OH). Eight fed mice are weighed in pairs and placed in 4 chambers, 2/chamber, for 3 h to obtain baseline O_2 and CO_2 values. The relative gas content of each chamber was sampled and recorded at 10−12-min intervals. For each sample, energy expenditure values were calculated by the Oxymax and expressed as kcal/h. After 3 h of baseline measurement, the mice were removed, treated with drugs, and replaced in the chambers. The β_3 agonists were injected at doses of 10 mg/kg (ip). Compounds in 10 mM or 10 mg/mL DMSO solutions were suspended in 0.5% methylcellulose:0.1% Tween-80 and injected (ip). Posttreatment kcal/h values were taken between 40 min and 2.5 h after dosing. The 6−10 sample sections of the pretreatment and posttreatment periods that appeared to best represent stable resting thermogenesis were selected. Each of these sample values was corrected for body weight and used such that each pair of mice served as its own baseline for both *T*-test and percent increase in thermogenesis calculations. An ANOVA and a one-sided *T*-test (H1: posttreatment > pretreatment) were performed using the SAS software modified to down-weight extreme values. The mean baseline value for each chamber was subtracted from the mean posttreatment value for that chamber. This baseline-subtracted value was divided by the mean baseline value and multiplied by 100 to obtain a percent increase in thermogenesis for each chamber. The combined mean percent increase and the standard error of the mean for each chamber were calculated using Excel software. Compounds were considered active if they were able to produce a statistically significant 15% increase in thermogenesis in β_3 transgenic mice.

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Supporting Information Available: Elemental analysis data for novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) To compare the β_3 activity and selectivity of our new compounds with a known selective human β_3 agonist, the in vitro activity of L 755507, under our assay conditions, is incorporated into the data set (Table 1, reported data[8a] for L 755507: β_3 EC_{50} = 0.00043 ± 0.00031 μM, IA = 0.52; β_2 EC_{50} > 10 μM, IA = 0.02; β_1 EC_{50} = 0.58 ± 0.30 μM, IA = 0.30).

(17) We occasionally submitted the esters instead of the acids for in vivo experiments simply because the esters are easier to purify than the acids. We anticipate that the ester **25a**, a prodrug of the acid, should have a similar activity in thermogenesis effect to **26a**. Presumably, the esters were rapidly hydrolyzed to the corresponding acids upon injection. We have observed esters to have similar in vivo activity as the corresponding acids. For example, the methyl ester of **26e** produced a 47 ± 10% thermogenesis effect which is comparable to that of the acid (53 ± 8%).

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