Biarylaniline Phenethanolamines as Potent and Selective β_3 Adrenergic Receptor Agonists

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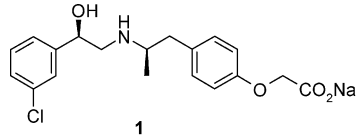
The synthesis of a series of phenethanolamine aniline agonists that contain an aniline ring on the right-hand side of the molecule substituted at the meta position with a benzoic acid or a pyridyl carboxylate is described. Several of the analogues (e.g., **34**, **36**−**38**, **40**, and **44**) have high β_3 adrenergic receptor (AR) potency and selectivity against β_1 and β_2 ARs in Chinese hamster ovary (CHO) cells expressing β ARs. The dog pharmacokinetic profile of some of these analogues showed >25% oral bioavailability and po half-lives of at least 1.5 h. Among the compounds described herein, the 3,3′-biarylaniline carboxylate derivatives **36**, **38** and the phenylpyridyl derivative **44** demonstrated outstanding in vitro properties and reasonable dog pharmacokinetic profiles. These three analogues also showed dose dependent β_3 AR mediated responses in mice. The ease of synthesis and superior dog pharmacokinetics of compound **38** relative to that of **44** in combination with its in vitro profile led us to choose this compound as a development candidate for the treatment of type 2 diabetes.

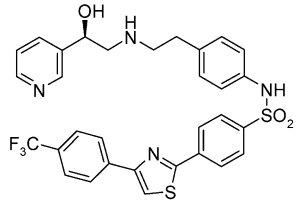
Introduction

A major increase in the prevalence of obesity and type 2 diabetes mellitus and related cardiovascular disorders has led to the search for new pharmacological approaches in the treatment of these conditions.[1] In the early 1980s, the atypical or β_3 adrenoceptor (AR) was first identified as a possible therapeutic opportunity for the treatment of type 2 diabetes and obesity. Interest in this target as a treatment for diabetes was stimulated by findings that compounds such as **1** of the phenethanolamine class possessed thermogenic and antidiabetic properties in rodents[2,3] (Chart 1). Unfortunately, **1** and other β_3 AR agonists discovered during the 1980s were unsuccessful in the clinic, either because of a lack of efficacy or an unfavorable cardiovascular side-effect profile. The clinical failure of early agents related to **1** has been attributed to a lack of sufficient β_3 AR potency and β_1 AR and β_2 AR selectivities resulting from pharmacologic differences between rodent and human receptors.[4] The hypothesis that rodent and human receptor differences are responsible for the lack of human clinical efficacy of **1** and related analogues was supported by the discovery, cloning, and characterization of the human,[5] rat,[6] and mouse[7] β_3 ARs in 1989. An evaluation of the activity of compounds such as **1** on the cloned rodent and human receptors, in fact, uncovered significant interspecies differences in their activities at the three β AR subtypes.[8] The availability of appropriate human receptors has given rise to the design and synthesis of a new generation of β_3 AR agonists with high potency and good selectivity with respect to human β_1 and β_2 ARs exemplified by the potent, selective, and orally bioavailable pyridylethanolamine **2**.[9] Although published clinical data with **2** has failed to confirm that chronic β_3 AR stimulation is an effective approach for the treatment of diabetes,[10] the continued discovery of agents with promising preclinical properties[11] as well as the discovery of possible additional therapeutic applications for β_3 AR agonists for overactive bladder[11c,12] and gastrointestinal disorders[13] have led to continued efforts toward the discovery and exploration of the clinical utilization of these agents.

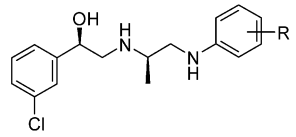
Chart 1. Phenethanolamine β_3 Agonists



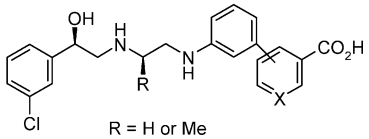
1



2



3a: R = *p*-CH_2CO_2H
3b: R = *m*-$CH_2(CO)NHSO_2$(*p*-tol)



R = H or Me
X = CH or N

4

We previously described efforts in this area that included the disclosure of aniline phenethanolamines exemplified by phenyl acetic acid derivative **3a**, with further optimization efforts resulting in acylsulfonamides such as **3b**.[14] The in vitro potency

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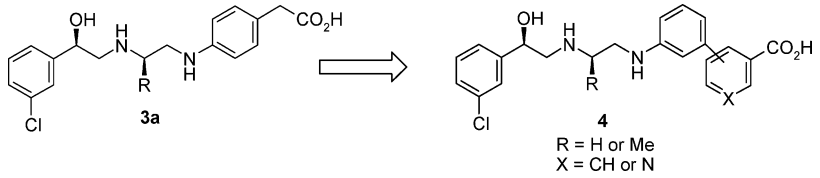
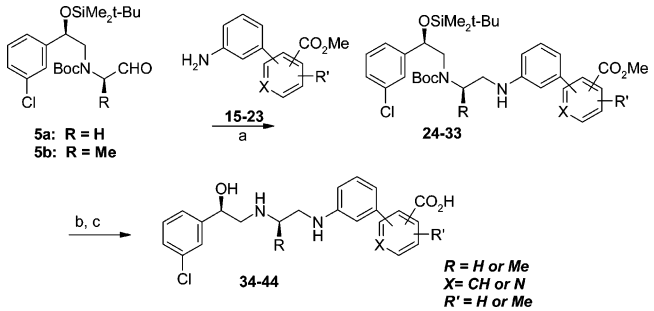


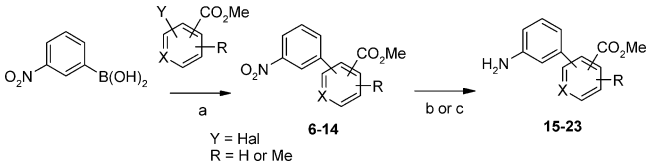
Figure 1. General design of biaryl carboxylic acid derivatives.

Scheme 1. Synthetic Route to Target **34**−**44**[a]



[a] (a) $NaBH(OAc)_3$, (2 equiv) AcOH (cat), CH_2Cl_2 (b) 4 N HCl/dioxane, RT (c) LiOH, 3:1 MeOH/H_2O; sg chromatography.

Scheme 2. Preparation of Biarylanilines **15**−**23**[a]



[a] (a) cat. $Pd(Ph_3P)_4$, 1,4-dioxane, Na_2CO_3, 85 °C (b) H_2, 10% Pd/C, MeOH or EtOAc or THF (c) Tin(II) chloride (1.16 g) EtOAc, 80 °C; aq $NaHCO_3$.

and selectivity of **3a** and related analogues suggested that opportunities exist within this series for further optimization of pharmacokinetic and other characteristics. The glucuronide and taurine conjugates of analogues such as **3a** were major metabolites in the dog, limiting its terminal half-life. Hypothesizing that conformational constraint might slow conjugation by phase II metabolism enzymes leading to enhanced terminal half-lifes, a strategy was implemented to curtail the conformational freedom of the *para*-anilinophenylacetic acid moiety in analogue **3a** (Figure 1). Moreover, the sp^2 hybridization of the carbon atom of an aryl or heteroaryl restraint adjacent to the acid functionality might alter the electronic as well as the steric environment of the acid relative to the sp^3 CH_2 carbon atom of the phenylacetic acid, changing its susceptibility to acyl functionalization by conjugating enzymes. In addition, this rigidification could block the metabolism at other sites in the agonists by altering their occupation of the binding sites of phase I-metabolizing enzymes. Furthermore, conformational confinement could also lead to enhanced potency via a reduction of the entropic penalty to achieve the optimal agonist/receptor interaction. Finally, the constriction of rotational freedom may increase selectivity by preventing the population of rotamers that bind optimally to other adrenergic receptors. In a previous article, we have shown that aniline phenethanolamines containing a carboxylic acid isostere attached to the aniline phenyl ring in the meta position show excellent in vitro profiles.[14] In this article, we describe the identification and characterization of a related series of phenethanolamine anilines containing the meta anilino biaryl carboxylic acid derivatives represented by structure **4**. In addition to having outstanding in vitro profiles, this work has resulted in the discovery of a series of compounds with excellent in vivo and pharmacokinetic properties leading to the identification of the clinical development candidate **38**.

Chemistry. The general synthetic route to β_3 AR agonist targets **4** is shown in Scheme 1. The reductive amination of aldehydes **5a** or **b**, the synthesis of which has been previously described,[14,15] with biaryl anilines **15**−**23** afforded the corresponding Boc amine silyl ether intermediates **24**−**33**.[17] Fully elaborated intermediates **24**−**33** were subjected to a deprotection sequence consisting of the acidic cleavage of the Boc carbamate with the concomitant removal of the silyl ether, followed by hydrolysis of the methyl ester with lithium hydroxide to unmask the carboxylic acid. With the exception of phenethanolamine methyl ester **39**, the methyl ester products that directly resulted from the acid deprotection step were taken directly through the ester saponification step without characterization to give the final biaryl carboxylic acid targets.[17] For final target compounds containing a chiral methyl group adjacent to the phenethanolamine nitrogen (e.g., **34**−**37**, **41**), a minor degree of epimerization occurred either in the final aniline−aldehyde reductive amination or in the final deprotection steps. The diastereomeric composition of purified final targets was determined by ^1H NMR to be at least 80% of the *R*,*R* isomer. For reasons described previously,[14] the presence of minor diastereomers is not expected to significantly impact the β-AR activity or selectivity SAR of the final targets.

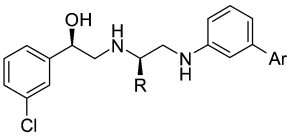
Aniline intermediates **15**−**23** were obtained by reduction of the corresponding nitro derivatives **6**−**14**,[16−18] which were, in turn, obtained through a Suzuki cross-coupling of 3-nitrophenylboronic acid and the appropriate bromophenyl,[19] bromopyridyl,[20−23] or trifluoromethylsulfonylpyridyl[16] carboxylic acid ester. The aryl halide- or triflate-containing derivatives were synthesized from the corresponding carboxylic acid derivatives, which were either commercially available or prepared through known synthetic procedures.[24]

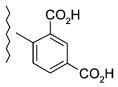
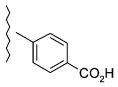
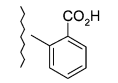
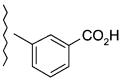
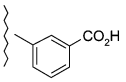
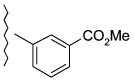
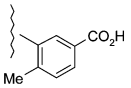
Results and Discussion

The final targets were evaluated as β agonists against cloned human β_3, β_2, or β_1 ARs expressed in Chinese hamster ovary (CHO) cell lines. As described previously,[14] the stimulation of the relevant β ARs was quantified by measuring the accumulation of intracellular cAMP, with results reported as potencies (pEC_{50}) and efficacies (E_{Max}, the fitted maximal response to the compound expressed as a percent of the maximal response) relative to those of the nonselective full β AR agonist isoprenaline (ISO). The results with reference compound **1** are shown for comparison. Compound **1** not only exhibits full, potent agonist activity at the β_3 AR ($pEC_{50} = 7.6$, $E_{Max} = 91\%$) but also exhibits significant agonist activity at the β_2 AR ($pEC_{50} = 7.3$, $E_{Max} = 80\%$).

In a previous article, the design of the series of potent and selective targets described began with phenylacetic acid derivative **3a**,[14,15] which was found to be a very active but only a moderately selective lead.[14] In that endeavor, our efforts were focused on improving the properties of lead **3a** by replacing the carboxylic acid of **3a** with isosteric functionalities that led to the acylsulfonamide analogues such as **3b**. In the medicinal chemistry strategy described herein, the carboxylic acid was

Table 1. Stimulation of cAMP Accumulation in CHO Cells Expressing Human β_3, β_2, and β_1 ARs by Biphenyl Phenethanolamine Derivatives



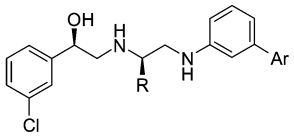
	Structure		β_3	β_2		β_1	
Cmpd	**R**	**Ar**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**β_2 EC_{50}/ β_3 EC_{50}**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**β_1 EC_{50}/ β_3 EC_{50}**
ISO	-	-	8.5 ± 0.3 (112 ± 20)	9.8 ± 0.4 (116 ± 17)	0.1	9.0 ± 0.3 (109 ± 14)	0.3
1	-	-	7.6 ± 0.4 (91 ± 6)	7.3 ± 0.4 (80 ± 15)	2	<6.1[c] (28±11)	>31
3a	-	-	7.8 ± 0.5 (117 ± 7)	7.3 ± 0.3 (90 ± 31)	3.1	7.1 ± 0.4 (24 ± 3)	5.0
3b	-	-	8.2 ± 0.1 (98 ± 20)	<6.0 (16 ± 5)	>158	<5.3 (8 ± 13)	>794
34	Me		9.7 ± 0.6 (96 ± 2)	<5.0[c] (0)	>10000	5.6 ± 0.5 (10 ± 9)	10000
35	Me		7.5 ± 0.4 (103 ± 16)	5.8 ± 0.2 (63 ± 36)	50	6.1 ± 0.4 (16 ± 0.6)	25
36	Me		9.6 ± 0.1 (102 ± 13)	6.0 ± 0.4 (10 ± 7)	3981	6.2 ± 0.3 (13 ± 3)	2512
37	Me		10 ± 0[c] (84 ± 1)	7.3 ± 0.1 (20 ± 2)	501	8.2 ± 0.1 (16 ± 6)	100
38	H		8.4 ± 0.2 (79 ± 10)	5.9 ± 0.6 (4.6 ± 3.1)	316	5.4 ± 0.8 (1.7 ± 3.3)	1000
39	H		6.8 ± 0.2 (88 ± 15)	6.3 ± 0.1 (22 ± 11)	3.1	5.9 ± 0.8 (10 ± 9)	8
40	H		8.8 ± 0.4 (84 ± 7)	6.2 ± 0.7 (8.7 ± 4)	398	6.1 ± 1.0 (13 ± 12)	501

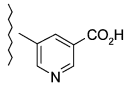
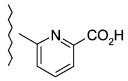
[a] Human β_1, β_2, and β_3 receptors expressed in CHO cells. pEC_{50} = negative log molar drug concentration which produces a cAMP response equal to 50% of its maximal response; $n = 3$ for all compounds except ISO, $n = 25$ and where otherwise indicated. [b] E_{Max} is the fitted maximal value of the concentration−response expressed as a percent of the maximal response to *R*-(−)-isoproterenol (Iso). [c] $n = 2$ experiments. [d] The compound produced a negligible response in these experiments.

retained but was constrained within a biaryl system representated by structure **4** (Figure 1). By retaining the carboxylic acid while limiting its flexibility in this manner, we hypothesized that the position of the carboxylate could be kept in an orientation that not only would provide superior selectivity and potency relative to analogues such **3a** but also would improve the pharmacokinetics of this series.

To execute this strategy, we first prepared a series of biphenyl carboxylic acids (**34**−**40**) in which the position of the carboxylic acid was varied by placing it at the ortho, meta, or para positions of the terminal phenyl ring (Table 1). Because other results in our labs showed superior potency and selectivity being attained when the point of attachment of the terminal substituent of the right-hand side phenyl ring was meta to the aniline nitrogen (e.g., analogue **3b**),[14] only analogues in which the inner phenyl ring has a meta substitution pattern relative to the aniline and the terminal phenyl ring were synthesized. Gratifyingly, in contrast to phenyl acetic acid derivative **3a** and clinical comparator compound **1**, the majority of biphenyl compounds bearing an ortho or meta carboxylic acid on the terminal phenyl ring were not only potent (pEC50 > 7.5) and fully efficacious (E_{Max} > 80%) at the β_3 AR, but also exhibited low activity and poor efficacy at both the β_1 and the β_2 ARs. The position of the carboxylic acid on the aryl substituent appeared to strongly influence the overall β AR profile. Analogues containing an ortho carboxylic acid (**34** and **36**) were particularly potent (pEC50 > 9) and selective (>1000-fold vs β_1 and β_2 ARs, Table 1). However, para analogue **35** showed a lower potency (pEC50

Table 2. Stimulation of cAMP Accumulation in CHO Cells Expressing Human β_3, β_2, and β_1 ARs by Pyridylphenyl Phenethanolamine Derivatives



	Structure		β_3	β_2		β_1	
Cmpd	**R**	**Ar**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**β_2 EC_{50}/ β_3 EC_{50}**	**pEC_{50}[a] ($\%E_{Max}$)[b]**	**β_1 EC_{50}/ β_3 EC_{50}**
3	-	-	7.8 ± 0.5 (117 ± 7)	7.3 ± 0.3 (90 ± 31)	3.1	7.1 ± 0.4 (24 ± 3)	5.0
41	Me		7.5 ± 0.4 (103 ± 16)	5.8 ± 0.2 (63 ± 36)	50	6.1 ± 0.4 (16 ± 6)	25
42	H		7.2 ± 0.2 (121 ± 8)	5.4 ± 0.7 (3 ± 5)	63	5.5 ± 0.9 (3 ± 5)	50
43	H		7.4 ± 0.2 (95 ± 14)	5.0 ± 0.1 (7 ± 12)	251	5.3 ± 0.6 (1 ± 1)	126
44	H		10.0 ± 0.2 (96 ± 12)	<5.0[c] (0)	>10000	5.0 ± 0.1 (9.3 ± 16)	>10000

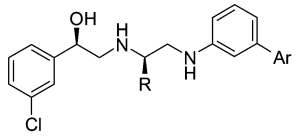
[a] Human β_1, β_2, and β_3 receptors expressed in CHO cells. pEC_{50} = negative log molar drug concentration which produces a cAMP response equal to 50% of its maximal response; $n = 3$ for all compounds except ISO, $n = 25$, and where otherwise indicated. [b] E_{Max} is the fitted maximal value of the concentration−response expressed as a percent of the maximal response to *R*-(−)-isoproterenol(Iso). [c] The compound produced a negligible response in all experiments.

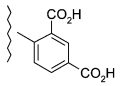
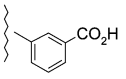
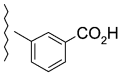
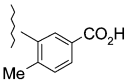
= 7.5) and a poorer selectivity (63 and 16-fold wrt β_1 and the β_2 ARs) compared to that of the meta and ortho substituted derivatives. Relative to the ortho carboxylic acid containing analogue **36**, moving the carboxylic acid to the meta position on the terminal phenyl ring (compound **37**) had little effect on the β_3 AR potency ($pEC50 = 10.0$ vs 9.6) and led to somewhat decreased selectivity, particularly, at the β_1 AR (100-fold vs 2512-fold at β_1). The removal of the chiral methyl group, however, restored high (>300-fold vs β_1 and β_2 AR) selectivity for meta carboxylic acid analogue **38**, which showed minimal stimulation of either the β_1 AR or the β_2 AR (1 and 3% of isoprenaline, respectively). Compound **38**, although somewhat less potent ($pEC_{50} = 8.4$ vs 10) than the corresponding analogue with the chiral *R* methyl group in the chain (**37**), still showed greater activity against the human receptor than the comparator molecules **1** and **3**. On the basis of previous SAR results for compounds of related structures that show the importance of the acid moiety on selectivity,[14,25] it was not surprising that the corresponding methyl ester analogue of compound **38** (analogue **39**) had an overall poor potency ($pEC_{50} = 6.8$) and selectivity (22-fold vs β_2 and 10-fold β_1 AR) profile relative to that of analogue **38**.

A series of biaryl analogues in which the terminal right-hand side phenyl ring was replaced by pyridyl were also synthesized. Given the preference of an ortho or meta carboxylic acid for potency and selectivity, the design focus was placed on ortho and meta carboxylates in these positions relative to the point of attachment of the inner phenyl ring (Table 2). In general, it was found that pyridyl analogues **41**−**43**, while also showing minimal activity against the β_1 or β_2 ARs ($pEC_{50} < \sim 6$), had only modest ($pEC_{50} < \sim 6$) potency at the β_3 AR relative to that of their corresponding phenyl analogues shown in Table 1 (Compare **36** vs **41** or **38** vs **42**). This diminished activity may result from an increased desolvation penalty to remove water molecules associated with the pyridine nitrogen upon receptor binding. In contrast, derivative **44** which contained a carboxylic acid in the ortho relationship relative to the biaryl linkage and the nitrogen in the 3 position of the terminal ring was found to have a very high potency ($pEC_{50} = 10$) and selectivity (>1000-fold vs β_2 and 10-fold β_1 AR). Likely, this pyridine nitrogen forms a favorable hydrogen bond and/or electrostatic charge−charge interaction that compensates for any desolvation penalty. Because **42** also has the pyridine nitrogen meta to the aryl ring, the ortho **44** and meta **42** carboxylates probably bind slightly differently, positioning their respective pyridine nitrogens in favorable (**44**) or neutral/detrimental (**42**) positions relative to agonist activity. The ortho analogue **44** along with several of the biphenyl derivatives was progressed into further studies.

Several of the analogues with the most attractive in vitro profiles were progressed into dog pharmacokinetic (PK) assays. To rapidly assess compounds for further progression, the majority of compounds were evaluated for dog PK assays in a streamlined approach by testing in only one animal. In the case where multiple animals were used (compound **38**), the variability was found to be less than 30% for the parameters reported. In these experiments, potent and selective dicarboxylate analogue **34** was found to have a short terminal half-life ($t_{1/2} < 2$ h) and low exposure ($AUC_{0\rightarrow\infty}$) by iv administration and consequently was not further investigated. Biphenyl analogues **36**−**38** and **40** and phenylpyridyl analogue **44** were evaluated in dog (PK) assays following both iv and po administrations. As can be seen in Table 3, with the exception of pyridyl compound **44**, all of these compounds showed good bioavailabilities in excess of 30% with oral half-lives of 2 h or greater. The steady-state volumes of distribution tended to be low, reflecting the polar nature of these analogues. In particular, desmethyl meta

Table 3. Dog Pharmacokinetic Data (Mean ± SEM) for β_3 Biaryl Acids[a]



Cmpd	R	Ar	$AUC_{0\rightarrow\infty,\,po}$ (ng.h/mL)	CL (mL/min/kg)	V_{SS} (L/kg)	$t_{½,\,po}$ (h)	F (%)
34	Me		NT	9.3	0.99	NT	NT
36	Me		310	11.3	0.37	2.4	40
37	Me		135	7.3	0.45	2.5	28
38	H		595 ±129	2.4 ±0.2	0.34 ±0.07	5.0 ±1.7	43 ±11
40	H		173	7.1	1.37	2.4	37
44	H		161	5.64	0.33	1.7	27

[a] Compounds were dosed intravenously and orally to dogs ($n = 1$ for all compounds except compound **38**, $n = 3$) in 0.025 M aqueous methanesulfonic acid solution with 5% mannitol at a concentration of 0.2 mg/mL to give a dose of 0.2 mg/kg; plasma drug concentrations were determined by LC-MS/MS; NT: Not tested.

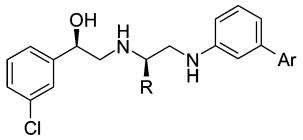
carboxylate biphenyl analogue **38** showed a particularly encouraging profile with low clearance (2.4 mL/min/kg) and an oral half-life of 5 h.

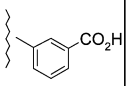
Although the major criteria for the selection of a clinical candidate were a combination of high in vitro β_3 AR potency, good β_1 and β_2 AR selectivity, and reasonable dog pharmacokinetic profile, further validation of the pharmacodynamic potential of members in this series as antidiabetic agents was explored by testing their functional activity in a rodent in vivo model of diabetes. Therefore, the ability of three key compounds to lower the plasma glucose in the *db*/*db* diabetic mouse model was investigated.[26] On the basis of their combination of outstanding in vitro profiles and acceptable dog PK profiles, compounds **36**, **38**, and **44** were selected for profiling in this diabetic model. These compounds were administered to male *db*/*db* mice at the doses indicated in parentheses by oral gavage twice daily for 14 days (Table 4). All three analogues were effective at lowering glucose in a dose dependent manner. In addition to lowering plasma glucose levels, the three compounds were effective at lowering the related parameters of glycosylated hemoglobin (Hb1Ac) (Tables 5). The treated animals also caused a dose dependent decrease in insulin levels, suggesting enhanced insulin sensitivity in treated animals (Table 6). Interestingly, it was found that although the maximum efficacy of these analogues for lowering plasma glucose was similar, compound **38** was considerably less potent than **36** or **44**, providing submaximal efficacy at doses below 100 mg/kg. The reasons for the comparatively high potencies of compounds **36** or **44** compared to that of **38** are not clear but may be, at least in part, a result of the greater potency of these two compounds versus that of **38** at the rodent β_3 AR relative to that at the human receptor.[27]

As a further confirmation of its β_3 AR functional in vivo activity in rodents, analogue **38** was tested for its ability to elicit an in vivo β_3 AR-mediated thermogenic response in the brown fat rich interscapular region of *db*/*db* mice by infrared imaging. As described previously, β_3 AR agonists have been shown to elicit a thermogenic response in this model, causing a temperature increase in the interscapular region of the animal that can be monitored using an infrared camera capable of producing quantifiable image-based readouts.[13,28] Interestingly, compared to the *db*/*db* glucose model, the thermogenesis assay in mice showed a somewhat higher sensitivity toward treatment by derivative **38** because doses of 3, 10, and 30 mg/kg produced significant dose-related increases in thermogenic activity (Figure 2).

Although compounds **36**, **38**, and **44** were all of potential interest as possible development candidates, the superior pharmacokinetics of **38** in terms of its half-life relative to those of the other two compounds led us to focus on this compound as a possible development candidate. To further investigate the progression of compound **38**, it was tested in a bacterial miniscreen assay, which is a version of the Ames assay.[29,30] In this test, several bacterial strains were used with and without metabolism via rat S9 liver fractions. Gratifyingly, this compound showed no indication of having a mutagenic liability from

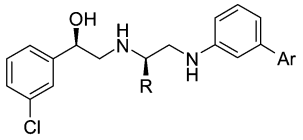
Table 4. The Effects of Chronic Treatment (14 days) with Selected β_3 Agonists on Plasma Glucose in *db*/*db* Mice[a]

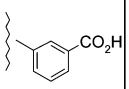


Compound			Plasma glucose (mg/kg)			
No.	**R**	**Ar**	Vehicle	Compound (Dose, mg/kg)		
36	Me		615 ± 24	417 ± 40 (0.03)	238 ± 26 (0.3)	222 ± 12 (3.0)
38	H		589 ± 32	384 ± 56 (10)	207 ± 22 (30)	185 ± 20 (100)
44	H		546 ± 37	351 ± 43 (0.01)	181 ± 8 (0.1)	180 ± 5 (1.0)

[a] Data are shown as mean ± SEM. Male *db*/*db* mice (10 mice/group), 60 days of age, were administered either the vehicle (D-α tocopherol poly(ethylene glycol) 1000 succinate (TPGS) and propylene glycol (PG) (25:75%w/w) for compound **38** or 0.025M methanesulfonic acid for **36** and **44** at the doses indicated in parentheses (BID) by oral gavage in a volume of 5μL/gm body weight twice daily for 14 days. Prior to the start of dosing, 10 mice were anesthetized and exsanguinated by cardiac puncture for baseline measurements (day 0 predose values) of postprandial glucose. See Experimental Section for further details.

Table 5. The Effects of Chronic Treatment (14 days) with Selected β_3 Agonists on Glycosylated Hemoglobin in *db*/*db* Mice[a]

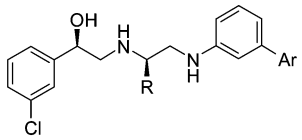


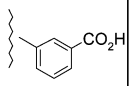
Compound			Glycosylated Hemoglobin (%HbA1c)			
No.	**R**	**Ar**	Vehicle	Compound (Dose, mg/kg)		
36	Me		7.6 ± 0.3	7.2 ± 0.3 (0.03)	5.7 ± 0.3 (0.3)	5.4 ± 0.1 (3.0)
38	H		7.87 ± 0.4	7.3 ± 0.5 (10)	7.6 ± 0.3 (30)	7.06 ± 0.3 (100)
44	H		6.2 ± 0.2	5.6 ± 0.3 (0.01)	5.0 ± 0.2 (0.1)	4.8 ± 0.2 (1.0)

[a] Data are shown as mean ± SEM. Male *db*/*db* mice (10 mice/group), 60 days of age, were administered either the vehicle (D-α tocopherol poly(ethylene glycol)) 1000 succinate (TPGS) and propylene glycol (PG) (25:75% w/w) for compound **38** or 0.025M methanesulfonic acid for **36** and **44** at the doses indicated in parentheses (BID) by oral gavage in a volume of 5μL/gm body weight twice daily for 14 days. Prior to the start of dosing, 10 mice were anesthetized and exsanguinated by cardiac puncture for baseline measurements (day 0 predose values) of glycosylated hemoglobin. See Experimental Section for further details.

this test and was, therefore, considered a viable candidate for further progression. The results of additional pharmacokinetic profiling across the three different species is shown in Table 7. Compound **38** demonstrated a bioavailability of 30, 43, and 46% in rats, dogs, and monkeys and iv half-life values of 2.1, 3.3, and 4.5 h in these species, respectively. Human versus rat and monkey microsomal S9 experiments suggested no indication of enhanced metabolism in human microsomes relative to those of other species examined (data not shown). These findings in combination with the fact that hERG assay results with analogue

Table 6. The Effects of Chronic Treatment (14 days) with Selected β_3 Agonists on Plasma Insulin in *db/db* Mice[a]



Compound			Insulin (ng/ml)			
No.	R	Ar	Vehicle	Compound (Dose, mg/kg)		
36	Me		10.9 ± 3.1	13.7 ± 2.9 (0.03)	6.7 ± 3.0 (0.3)	3 ± 0.6 (3.0)
38	H		5.6 ± 1.2	6.3 ± 1.7 (10)	4.4 ± 1.6 (30)	0.9 ± 0.2 (100)
4	H		21.7 ± 1.6	11.4 ± 1.4 (0.01)	2.8 ± 0.5 (0.1)	1.4 ± 0.2 (1.0)

[a] Data are shown as mean ± SEM. Male *db/db* mice (10 mice/group), 60 days of age, were administered either vehicle (D-α tocopherol poly(ethylene glycol)) 1000 succinate (TPGS) and propylene glycol (PG) (25:75%w/w) for compound **38** or 0.025M methanesulfonic acid for **36** and **44** at the doses indicated in parentheses (BID) by oral gavage in a volume of 5μL/gm body weight twice daily for 14 days. Prior to the start of dosing, 10 mice were anesthetized and exsanguinated by cardiac puncture for baseline measurements (day 0 predose values) of postprandial insulin. See Experimental Section for further details.

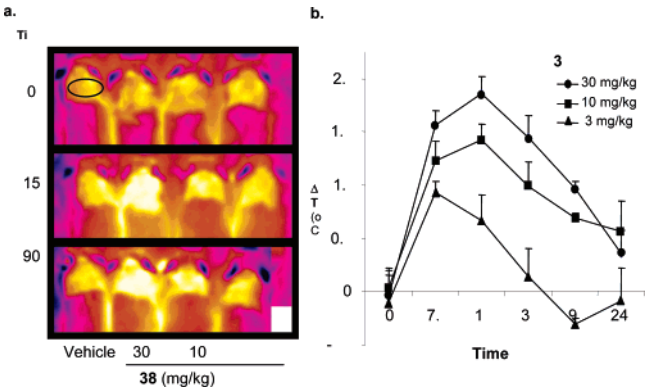


Figure 2. Thermogenesis Response Elicited by Compound **38**.

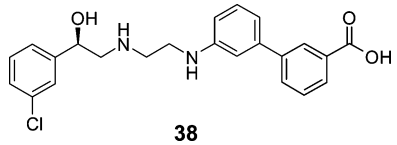
38 indicated no clinical concern for QT-dependent arrhythmogenesis further supported this compound as a clinical development candidate.

Conclusion

Potency and selectivity data from a series of biaryl aniline phenethanolamine β_3 AR agonists in which the both the position of the carboxylate substituent of the terminal aryl ring as well as the aryl ring was varied have been described. The results communicated above demonstrate that the strategy of constraining the right-hand side carboxylic acid substituent within a biaryl aniline scaffold in the right-hand side of the molecule was effective in delivering analogues with outstanding β_3 AR potency and β_1 and β_2 AR selectivity. Although some of the most potent and selective analogues (analogues **34**, **36**, **38**, and **40**) were members of the 3-biphenyl aniline series containing a carboxylic acid at either the ortho or meta position of the terminal phenyl ring, phenylpyridyl analogue **44** also showed an outstanding in vitro profile. The dog pharmacokinetic profile of compounds showing promising in vitro profiles (**34**, **36**-**38**, **40**, and **44**) indicated that all compounds tested had acceptable (>25%) oral bioavailability and oral half-lives of at least 1.5 h.

Among the analogues described herein, 3,3′-biarylaniline carboxylate derivatives **36**, **38** and phenylpyridyl derivative **44** were of particular interest because of a combination of outstanding in vitro properties and acceptable dog PK profiles. Although **38** was less potent than the other two analogues in rodent models of diabetes, all three analogues showed dose dependent β_3 AR mediated responses in mice. These results, in combination with their excellent in vitro profile against cloned human receptors, provide evidence to support the hypothesis that any of these three agents should be capable of stimulating β_3 ARs in a selective manner with respect to β_2 and β_1 ARs at pharmacologically relevant doses. Overall, analogue **38** offered a preferred composite profile compared to that of either analogue **44** or analogue **36** because of a combination of both its relative simplicity in the synthesis of the biaryl aniline right-hand side starting material and its superior dog PK profile. Though a thorough comparison of the two compounds was not made, the longer half-life and lower clearance of compound **38** compared to those of our previously disclosed selective β_3 AR agonists, such as **3b** in dogs, and their lower maximal stimulation of β_2 and β_1 ARs led us to focus on **38** relative to exemplar compounds in the acylsulfonamide series as potential development candidates. Finally, the binding data for compound **38** on β_2 and β_1 ARs suggests a minimal risk of functional antagonism against these receptors at clinically relevant doses (Table 8). This compound was, therefore, chosen for progression into the clinic for evaluation as a drug candidate for treatment of type 2 diabetes and overactive bladder. The results of these clinical studies will be reported in due course.

Table 7. Summary of Pharmacokinetic Data (Mean ± SEM) for Compound **38** in Rats, Dogs, and Monkeys[a]

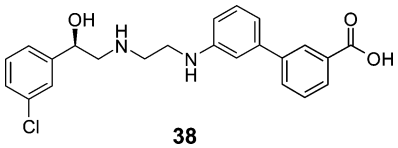


38

species	total clearance (mL/min/kg)	renal clearance (mL/min/kg)	Vss (mL/kg)	$t_{1/2,\ iv}$ (h)	$t_{1/2,\ po}$ (h)	F (%)
rat	18.1 ± 0.4	0.16 ± 0.03	1300 ± 405	2.1 ± 0.1	3.0 ± 0.3	30[b]
dog	2.4 ± 0.2	0.014 ± 0.007	341 ± 72	3.3 ± 0.8	5.0 ± 1.7	43 ± 11
monkey	5.5 ± 0.7	ND	556 ± 51	4.5 ± 1.1	5.2 ± 1.3	46 ± 14

[a] Compound **38** was dosed as a solution intravenously or by oral gavage to rats, dogs, or monkeys ($n = 2$ for rats, $n = 3$ for dogs and monkeys) in 0.025 M aqueous methanesulfonic acid solution with 5% mannitol at a concentration of 0.2 mg/mL (dogs), 1.0 mg/mL (rats), or 0.5 mg/mL (monkeys); plasma drug concentrations were determined by LC-MS/MS. ND: Not determined. Compound **38** concentrations were measured by LC-MS. [b] Rat iv/po was not a crossover-design; therefore, a measure of variability for F is not given. The AUC's for each animal through the po route of administration were within 50% of their average.

Table 8. Binding Data for Compound **38**[a]



38

β_3 AR activity	β_2 AR[a] binding	β_3 functional/β_2 binding[b]	β_1 AR binding[a]	β_3 functional/β_2 binding[b]
8.4 ± 0.2	5.8 ± 0.5	398	6.4 ± 0.5	100

[a] The binding constant pK_i of compound **38** ($n = 3$) against β_2 or β_1 ARs; see Experimental Section. [b] The ratio of the pIC_{50} of the compound for β_3 AR relative to the binding constant for β_2 or β_1 ARs.

Experimental Section

Chemistry. General Methods. Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Unless stated otherwise, the reagents were obtained from commercial sources and were used directly. Reactions involving air- or moisture-sensitive reagents were carried out under a nitrogen atmosphere. The reactions were carried out at ambient temperature unless otherwise indicated. Silica gel (EM Science, 230−400 mesh) was used for chromatographic purification unless otherwise indicated. Anhydrous solvents were obtained from Aldrich (Sure Seal). ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer; the chemical shifts are reported in parts per million (ppm) relative to TMS. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. High performance liquid chromatography (HPLC) was performed on a Beckman 126 with a Beckman 166 UV detector (monitoring at 215 nm) with a Rainin Dynamax-60A C18 83-201-C with a 25 cm × 4.6 mm column, eluting with 5−40% CH_3CN in H_2O with 0.1% TFA buffer and a flow rate of 1.5 mL/min. The retention times are expressed as t_r in minutes. A solvent elution used a gradient consisting of 20:80 A/B to 10:90 A/B over 20 min, where A = 1% aqueous trifluoroacetic acid (TFA) and B = 1% TFA in CH_3CN. Optical rotation values are expressed as $[\alpha]_D$ values. Mass spectra (ms) were obtained using electrospray (positive or negative ion) analyses. ^{1}H NMR analyses were carried out in deuterated chloroform, unless otherwise indicated. Elemental analyses, performed by Atlantic Microlab, Inc. Norcross, GA, were within 0.4% of the theoretical values calculated for C, H, and N.

3′-Nitro-[1,1′-biphenyl]-2,4-dicarboxylic Acid Dimethyl Ester (6). General Suzuki Coupling Procedure. To a stirred mixture of dimethyl 4-bromoisophthalate[19] (1.26 g, 4.61 mmol) and 3-nitrophenylboronic acid (795 mg, 4.76 mmol) in 1,4-dioxane (20 mL) was added $Pd(Ph_3P)_4$ (167 mg, 0.143 mmol) and Na_2CO_3 (710 mg). The mixture was heated overnight at 85 °C, cooled to room temperature, and partitioned between CH_2Cl_2 (100 mL) and 2 M aq Na_2CO_3 (50 mL) containing concentrated NH_4OH (5 mL). The aqueous layer was further extracted twice with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with EtOAc/hexane to provide the product (880 mg, 61% yield) as a tan solid. Electrospray MS (M + Na) 338. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.66 (s, 3H), 3.91 (s, 3H), 7.66−7.82 (m, 3H), 8.30−8.16 (m, 3H), 8.41 (s, 1H). Anal. Calcd for $C_{16}H_{13}NO_6$: C, 60.95; H, 4.16; N, 4.44. Found: C, 60.88; H, 4.21; N, 4.50.

3′-Nitro-[1,1′-biphenyl]-4-carboxylic Acid Methyl Ester (7).[18] A mixture of methyl 4-bromobenzoate (1.00 g, 4.65 mmol) and 3-nitrophenylboronic acid (800 mg, 4.79 mmol) in dioxane (20 mL) with $Pd(PPh_3)_4$ (165 mg, 0.142 mmol) and Na_2CO_3 (710 mg) were subjected to the general procedure described above to provide the product (198 mg, 16% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.98 (s, 3H), 7.59 (t, 1H, $J = 7.7$), 7.61 (t, 1H, $J = 7.9$), 7.80 (d, 1H, $J = 7.9$), 7.91 (d, 1H, $J = 7.7$), 8.22 (dd, 1H, $J = 7.7$, 1.4), 8.33 (ms, 1H), 8.42 (s, 1H).

3′-Nitro-[1,1′-biphenyl]-2-carboxylic Acid Methyl Ester (8). Methyl 2-bromobenzoate (1.53 g, 7.1 mmol), $Pd(PPh_3)_4$ (270 mg, 0.23 mmol) and 3-nitrophenylboronic acid (1.44 g, 8.62 mmol) were reacted according to the general procedure used to obtain intermediate **6** to give the product (1.81 g, 99% yield) as a white solid. ^{1}H NMR (DMSO-d_6) δ 3.61 (s, 3H), 7.51 (d, 1H), 7.58 (t, 1H), 7.69 (m, 3H), 7.88 (d, 1H), 8.24 (d, 1H);

3′-Nitro-[1,1′-biphenyl]-3-carboxylic Acid Methyl Ester (9). Methyl 3-bromobenzoate (2.0 g, 9.3 mmol), $Pd(Ph_3P)_4$ (348 mg, 0.30 mmol), and 3-nitrophenylboronic acid (1.9 g, 11.3 mmol) were reacted according to the general procedure used to obtain intermediate **6** to give the product (2.28 g, 95% yield) as a brown solid, mp 88−90 °C. ^{1}H NMR (400 MHz, $CDCl_3$,) δ 3.96 (s, 3H), 7.57 (t, 1H, $J = 7.7$), 7.64 (t, 1H, $J = 7.9$), 7.81 (d, 1H, $J = 7.9$), 7.94 (d, 1H, $J = 7.7$), 8.09 (d, 1H, $J = 7.7$), 8.23 (dd, 1H, $J = 7.7$, 1.1), 8.30 (s, 1H), 8.48 (t, 1H).

3′-Nitro-[1,1′-biphenyl]-2-methyl-5-carboxylic Acid Methyl Ester (10). The general procedure used to obtain intermediate **6** was employed, starting from methyl 3-bromo-4-methylbenzoate[20] (2.3 g) in toluene (28 mL), $(Ph_3P)_4Pd$ (381 mg), and 3-nitrophenylboronic acid (2.03 g) in MeOH (7 mL) to give the product (605 mg) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.37 (s, 3H), 3.96 (s, 3H), 7.42 (d, 1H, $J = 7.9$), 7.63−7.72 (m, 2H), 7.96 (d, 1H, $J = 1.5$), 8.02 (dd, 1H, $J = 7.9$, 1.5), 8.25 (s, 1H), 8.29 (dd, 1H, $J = 7.6$, 1.7). Anal. Calcd for $C_{15}H_{13}N_1O_4$: 66.41, H, 4.83, N, 5.16. Found: C, 66.36, H, 4.87, 5.15.

Methyl 2-(3-nitrophenyl)-3-pyridinecarboxylate (11). (a). To a stirred, cooled (−78 °C) solution of 2-hydroxy-3-pyridinecarboxylic acid methyl ester[31] (1.12 g) in CH_2Cl_2 was added di-*iso*-propylamine (1.04 g) dropwise. The mixture was stirred for 20 min and trifluoromethanesulfonic anhydride (2.18 g) was added dropwise. After 30 min, the mixture was quenched with water, allowed

to warm to room temperature and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by silica gel chromatography eluting with 1:4 ethyl acetate/hexane to provide 2-(trifluoromethanesulfonyl)oxy-3-pyridinecarboxylic acid methyl ester (1.66 g) as a white solid. Electrospray MS $(ES)^+$: (M + H) 307. ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 7.78 (dd, 1H), 8.58 (dd, 1H), 8.69 (dd, 1H).

(b). The general procedure used to obtain intermediate **6** was employed using 2-(trifluoromethanesulfonyl)oxy-3-pyridinecarboxylic acid methyl ester (506 mg, 1.76 mmol), 3-nitrophenylboronic acid (325 mg, 1.95 mmol), and $Pd(Ph_3P)_4$ (70 mg, 0.06 mmol) to afford, after purification with 4:1 hexane/EtOAc the product (301 mg, 66%) as a tan solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.69 (s, 3H), 7.75 (dd, 1H), 7.94 (dd, 1H), 8.29 (m, 3H), 8.86 (dd, 1H).

3-(3-Nitrophenyl)-5-pyridinecarboxylic Acid Methyl Ester (12).[18] **(a).** 3-Bromo-5-pyridinecarboxylic acid (3.00 g) was converted to the corresponding 3-bromo-5-pyridinecarboxylic acid methyl ester as a pale yellow solid (2.97 g) according to a literature procedure.[22] ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.86 (s, 3H), 7.89 (d, 1H, J = 8.0), 7.94 (t, 1H, J = 8.0), 8.05 (t, 1H, J = 6.8).

(b). The general procedure used to obtain intermediate **6** was employed starting from 3-bromo-5-pyridinecarboxylic acid methyl ester (1.00 g, 4.6 mmol), 3-nitrophenylboronic acid (785 mg, 4.7 mmol), and $Pd(Ph_3P)_4$ (164 mg, 0.14 mmol) to give the desired product (296 mg, 25% yield) as a tan solid. Anal. Calcd for $C_{13}H_{10}N_2O_4$: C, 60.47; H 3.90; N 10.85%. Found: C, 60.61; H, 3.93; N, 10.78%.

6-(3-Nitrophenyl)-2-pyridine-carboxylic Acid Ethyl Ester (13). (a) Sulfuric acid (1.46 mL) was added to a mixture of 2-bromo-6-pyridine-carboxylic acid, ethanol (15 mL), and toluene (30 mL). The reaction was heated at reflux for 16 h, then allowed to cool, and partitioned between $CHCl_3$ and a saturated aq $NaHCO_3$. The aqueous layer was extracted with $CHCl_3$ (2×), and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated to yield 2-bromo-6-pyridine-carboxylic acid ethyl ester as a cloudy, orange oil. The oil was purified by silica gel chromatography with 9:1 hexane/ethyl acetate. The title product was obtained as an oily, white solid (1.31 g). ^{1}H NMR (CD_3OD) δ 1.39 (t, 3 H), 4.41 (q, 2H), 7.79 (d, 2H), 7.85 (t, 1H), 8.08 (d, 1H).

(b). A modified version of the general procedure used to obtain intermediate **6** was employed starting from 2-bromo-6-pyridine-carboxylic acid ethyl ester (1.2 g, 5.21 mmol) 3-nitrophenylboronic acid (1.0 g, 6.0 mmol), and $Pd(Ph_3P)_4$ (181 mg, 0.156 mmol) in toluene (20 mL) and MeOH (5 mL) to give the product (289 mg, 20% yield) as a 2.7:1 mixture of ethyl to methyl esters as judged by ^{1}H NMR spectroscopy. ^{1}H NMR (ethyl ester) (400 MHz, $CDCl_3$) δ 1.47 (t, 2.9H), 4.04 (s, 0.8 H), 4.50 (q, 1.46 H), 7.67 (t, 1H), 7.97−7.99 (m, 2H), 8.10−8.16 (m, 1H), 8.29 (d, 1H), 8.43−8.48 (m, 1H), 8.86−8.87 (m, 1H).

Ethyl 3-(3-nitrophenyl)-4-pyridinecarboxylate (14). (a). A mixture of 3-iodo-4-pyridine carboxylic acid (1.45 g, 5.8 mmol, prepared according to the method described by Epsztajn, et al.[24]) in water (50 mL) was treated with solid $NaHCO_3$ (613 mg, 7.3 mmol), and the mixture was concentrated, and the resulting solid was azeotropically dried by adding toluene and concentrating. The resulting residue was taken up in *N*,*N*-DMF (15 mL), and iodomethane (0.36 mL, 5.8 mmol) was added. The mixture was cooled to 0 °C and maintained at this temperature for 24 h. The mixture was concentrated, and the remaining oil was partitioned between $CHCl_3$ and H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to afford an oil that was passed through a plug of silica gel (eluting with $CHCl_3$) to afford methyl 3-iodo-4-pyridinecarboxylate (870 mg, 60% yield) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 3.92 (s, 3H), 7.65 (d, 1H, J = 4.8), 7.94 (s, 1H), 8.57 (d, 1H, J = 4.8), 9.02 (s, 1H).

(b). A solution of methyl 3-iodo-4-pyridinecarboxylate (870 mg, 3.31 mmol) in toluene (16 mL) was treated with a solution of 3-nitrophenyl boronic acid (581 mg, 3.48 mmol) in absolute ethanol (4 mL). The mixture was cooled to −78 °C and evacuated and flushed with nitrogen (3×). A catalytic amount of $Pd(dppf)Cl_2$ (~30 mg) was added, followed by 1 N Na_2CO_3 (4 mL). The mixture was allowed to warm to ambient temperature and heated at 90 °C for 24 h. The mixture allowed to cool to ambient temperature and then concentrated, and the residue was purified by silica gel chromatography (eluting with 7:3 hexanes/EtOAc) to afford 230 mg (30% yield) of the product as a semisolid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (t, 3H, J = 7.2), 4.22 (q, 2H, J = 7.2), 7.63−7.71 (m, 2H), 7.80 (d, 1H, J = 5.1), 8.24 (s, 1H), 8.31 (d, 1H, J = 7.8), 8.71 (s, 1H), 8.82 (d, 1H, J = 5.1).

3′-Amino-[1,1′-biphenyl]-3-carboxylic Acid Methyl Ester (18). To a stirred solution of 3′-nitro-[1,1′-biphenyl]-3-carboxylic acid methyl ester 9 (4.47 g, 17.3 mmol) in anhydrous THF (125 mL) under a blanket of nitrogen was added 10% palladium on activated charcoal (860 mg). The reaction was evacuated and placed under a hydrogen atmosphere and stirred overnight. The reaction mixture was filtered through Celite, and the solvent was removed under reduced pressure to yield a gray oil (4.4 g). The residue was chromatographed on silica, eluting with 3:1 hexane/EtOAc. Concentration of the appropriate fractions provided the product as a white solid (3.5 g, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.75 (s, 2H), 3.93 (s, 3H), 6.68 (dd, 1H, J = 8.0, 2.6), 6.92 (s, 3H), 6.99 (d, 1H, J = 7.2), 7.45−7.49 (m, 2H), 7.47 (t, 1H, J = 8.0), 7.73 (d, 1H, J = 7.6), 7.99 (d, 1H, J = 7.6), 8.23 (s, 1H).

3′-Amino-[1,1′biphenyl]-2,4-dicarboxylic Acid Dimethyl Ester (15). 3′-Nitro-[1,1′biphenyl]-2,4-dicarboxylic acid dimethyl ester **6** (556 mg, 1.77 mmol) was subjected to the hydrogenation procedure used to supply intermediate **18** to give the product (458 mg, 91% yield). ^{1}H NMR (DMSO-d_6) δ 3.64 (s, 3H), 3.88 (s, 3H), 5.21 (s, 2H), 6.41 (d, 1H), 6.52 (s, 1H), 6.56 (d, 1H), 7.06 (t, 1H), 7.54 (d, 1H), 8.10 (d, 1H), 8.17 (s, 1H).

3′-Amino-[1,1′-biphenyl]-4-carboxylic Acid Methyl Ester (16).[32] The hydrogenation procedure used to supply intermediate **18** was employed starting from 3′-nitro-[1,1′-biphenyl]-4-carboxylic acid methyl ester **7** (196 mg, 0.76 mmol) to give the product (170 mg, 99% yield) as a pale yellow solid. Electrospray MS (positive ion): (M + H) 228.

3′-Amino-[1,1′-biphenyl]-2-carboxylic Acid Methyl Ester (17). 3′-Nitro-[1,1′-biphenyl]-2-carboxylic acid methyl ester **8** (1.05 g, 4.08 mmol) was subjected to the hydrogenation procedure used to supply intermediate **18** to give the product (910 mg, 98% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.61 (s, 3H), 3.67 (bs, 2H), 6.61 (s, 1H), 6.64 (t, 1H, J = 7.6), 7.13 (t, 1H, J = 8.0), 7.32−7.36 (m, 2H), 7.42 (dt, 1H, J = 7.2, 1.2), 7.71 (d, 1H, J = 8.0).

3′-Amino-[1,1′-biphenyl]-2-methyl-5-carboxylic Acid Methyl Ester (19). The hydrogenation procedure used to supply intermediate **18** was employed starting from 3′-nitro-[1,1′-biphenyl]-2-methyl-5-carboxylic acid methyl ester **10** (605 mg, 2.23 mmol) to give the product (572 mg, quantitative yield) as a white crystalline solid. Electrospray MS (positive ion): (M + H) 242.5. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (s, 3H), 3.75 (bs, 2H), 3.88 (s, 3H), 6.60 (s, 1H), 6.62−6.64 (m, 2H), 7.18 (t, 1H, J = 7.6), 7.29 (d, 1H, J = 7.6), 7.88 (m, 2H).

2-(3-Aminophenyl)-3-pyridinecarboxylic Acid Methyl Ester (20). 2-(3-Nitrophenyl)-3-pyridinecarboxylic acid methyl ester **11** (293 mg, 1.13 mmol) and 10% Pd/C (30 mg) were used in a procedure similar to that used for intermediate **18** to give the product (275 mg, quantitative yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (s, 3H), 5.19 (s, 2H), 6.58 (dt, 2H), 6.76 (s, 1H), 7.05 (t, 1H), 7.44 (dd, 1H), 8.02 (d, 1H), 8.73 (d, 1H).

5-(3-Aminophenyl)-3-pyridinecarboxylic Acid Ethyl Ester (21). 5-(3-Nitrophenyl)-3-pyridinecarboxylic acid ethyl ester **12** (100 mg, 0.367 mmol) was subjected to the hydrogenation procedure used for intermediate **18** to give the product (19.9 mg, 22% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (t, 3H), 4.43 (q, 2H), 6.75 (dd, 1H), 6.90 (t, 1H), 6.98 (d, 1H), 8.43 (t, 1H), 8.95 (d, 1H), 9.16 (d, 1H).

6-(3-Aminophenyl)-2-pyridine-carboxylic Acid Ethyl Ester (22). 6-(3-Nitrophenyl)-2-pyridine-carboxylic acid ethyl ester **13** (280 mg, a 2.7:1 mixture of ethyl and methyl esters) and tin(II)

chloride (1.16 g) were taken up in EtOAc (10 mL). The mixture was heated at 80 °C for 45 min and then allowed to cool to ambient temperature. The mixture was poured into ice, and saturated aqueous $NaHCO_3$ was added until the mixture attained a pH of approximately 7. Celite and EtOAc were added, and the mixture was stirred for 10 min. The mixture was filtered and placed in a separatory funnel. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated to yield the crude product. Purification by silica gel chromatography (4:1 hexane/EtOAc) afforded the product (126 mg) as a brown oil judged to be a 2.5:1 mixture of the ethyl and methyl esters. Electrospray MS (positive ion); (M + H) 229.2 and 243.2.

5-(3-Aminophenyl)-4-pyridinecarboxylic Acid Ethyl Ester (23). A solution of ethyl 3-(3-nitrophenyl)-4-pyridinecarboxylate **14** (230 mg, 0.84 mmol) and Pd/C (240 mg) was subjected to the hydrogenation procedure used for intermediate **18** with EtOAC substituted as the solvent instead of THF to afford the product (195 mg, 81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02 (t, 3H, J = 6.8), 3.65 (bs, 2H), 4.11 (q, 1H, J = 6.8), 6.58 (d, 1H, J = 1.6), 6.64 (m, 2H), 7.12 (t, 1H, J = 7.6), 7.50 (d, 1H, J = 4.8), 8.60 (d, 1H, J = 4.8), 8.61 (s, 1H).

General Reductive Amination Procedure Between Biarylaniline Carboxylic Acid Esters and 1,1-Dimethylethyl ((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}ethyl)(2-oxoethyl)carbamate (5a) or 1,1-Dimethylethyl ((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}ethyl)[(1*R*)-1-methyl-2-oxoethyl]carbamate (5b). An approximately equimolar ratio of biaryl aniline **15**−**23** and aldehyde **5a** or **b** was dissolved in anhydrous CH_2Cl_2 (~0.25 M) under N_2 and treated with a catalytic (1−10 drops) AcOH. The mixture was allowed to stir for 5−30 min and treated with ~2 equiv of $NaBH(OAc)_3$. The mixture was allowed to stir for 8−36 h and then partitioned between saturated aq $NaHCO_3$ and additional CH_2Cl_2. The mixture was separated, dried over Na_2SO_4, filtered, and concentrated to afford a residue that was purified by silica gel chromatography eluting with hexanes/EtOAc to afford, after the concentration of relevant fractions, the desired intermediate N-Boc amine TBDMS silyl ether product.

3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxyl)carbonyl]amino]propyl]amino]-[1,1′-biphenyl]-2,4-dicarboxylic Acid Dimethyl Ester (24). 3′-Amino-[1,1′-biphenyl]-2,4-dicarboxylic acid dimethyl ester **15** (456 mg, 1.61 mmol) and [2*R*-(*tert*-butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino]-propionaldehyde **5b** (609 mg, 1.38 mmol) were used in the general reductive amination procedure to give the product (339 mg, 38% yield) as a yellow foam. Electrospray MS (positive ion): (M + H) 711.

3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]propyl]amino]-[1,1′-biphenyl]-4-carboxylic Acid Methyl Ester (25). [2*R*-(*tert*-Butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino]-propionaldehyde **16** (340 mg, 0.769 mmol) and 3′-amino-[1,1′-biphenyl]-4-carboxylic acid methyl ester **5b** (168 mg, 0.739 mmol) were reacted according to the above reductive amination procedure to give the product (296 mg, 61%) as a white foam. Electrospray MS (positive ion): (M + H) 653.

3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]propyl]amino]-[1,1′-biphenyl]-2-carboxylic Acid Methyl Ester (26). 3′-Amino-[1,1′-biphenyl]-2-carboxylic acid methyl ester **17** (3.39 g, 14.9 mmol) and [2*R*-(*tert*-butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino]-propionaldehyde **5b** (8.24 g, 18.7 mmol)) were subjected to the standard reductive amination conditions using 7.2 g (34.0 mmol) of $NaBH(OAc)_3$ to give the product as a white foam (6.89 g, 71%). Electrospray MS (positive ion): (M + Na-Boc) 553.

3′-{[(2*R*)-2-(((2*R*)-2-(3-Chlorophenyl)-2-{[(1,1-dimethylethyl)-(dimethyl)silyl]oxy}ethyl){[(1,1-dimethylethyl)oxy]carbonyl}-amino)propyl]amino}-3-biphenylcarboxylic Acid Methyl Ester (27). To a stirred solution of 3′-amino-[1,1′-biphenyl]-3-carboxylic acid methyl ester **18** (0.844 g, 3.71 mmol) and 1,1-dimethylethyl ((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)(dimethyl)silyl]-oxy}ethyl)[(1*R*)-1-methyl-2-oxoethyl]carbamate **5b** (1.73 g, 3.39 mmol) in anhydrous CH_2Cl_2 (15 mL) was added 2 drops of AcOH. The mixture was allowed to stir for 15 min and treated with $NaBH(OAc)_3$ (1.71 g, 8.07 mmol). The reaction was quenched with saturated aq $NaHCO_3$, and CH_2Cl_2 was added. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield a white foam. The residue was purified by silica gel chromatography and eluted with 9:1 hexane/EtOAc to provide the title compound as a white foam (1.90 g, 86% yield). R_f (5:1 Hex/EtOAc) = 0.55. The product was used in the next step without further characterization.

(*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[1,1′-biphenyl]-3-carboxylic Acid Methyl Ester (28). To a stirred solution of 3′-amino-[1,1′-biphenyl]-3-carboxylic acid methyl ester **18** (3.0 g, 13.2 mmol) and (*R*)-[(*tert*-butoxycarbonyl)-[2-(*tert*butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino}acetaldehyde **5a** (8.2 g, 19.2 mmol) in anhydrous CH_2Cl_2 (65 mL) was added acetic acid (8 drops). After stirring for twenty-five minutes, $NaBH(OAc)_3$ (5.6 g, 26.4 mmol) was added and the reaction stirred overnight. The reaction was quenched with saturated aqueous $NaHCO_3$, and CH_2Cl_2 was added. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield a white foam. The residue was purified by silica gel chromatography and eluted with 9:1 hexane/EtOAc to provide the title compound as a white foam (5.62 g, 65% yield). Electrospray MS (positive ion): (M + H) 640.0.

(*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[1,1′-biphenyl]-2-methyl-5-carboxylic Acid Methyl Ester (29). A mixture of 3′-amino-[1,1′-biphenyl]-2-methyl-5-carboxylic acid methyl ester (4.19 g, 17.4 mmol) and {2*R*-(*tert*-butoxycarbonyl)-[2-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino}acetaldehyde **5a** (7.0 g, 16.4 mmol) were subjected to the general reductive amination procedure using 6.6 g (31.1 mmol) of $NaBH(OAc)_3$ in 60 mL of CH_2Cl_2 to give the product (7.21 g, 67% yield) as a white foam. Electrospray MS (positive ion): (M + H) 653.3.

2-[3-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]propyl]amino]phenyl]-3-pyridinecarboxylic Acid Methyl Ester (30). 2-(3-Aminophenyl)-3-pyridinecarboxylic acid methyl ester **20** (273 mg, 1.20 mmol) and {2*R*-(*tert*-butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino}propionaldehyde **5b** (504 mg, 2.23 mmol) were reacted according to the above procedure to give the product (339 mg, 43% yield) as a white foam. Electrospray MS (positive ion): (M + H) 654.

(*R*)-5-[[2-[[2-(3-Chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[phenyl]-3-pyridinecarboxylic Acid Ethyl Ester (31). A mixture of 5-(3-Aminophenyl)-3-pyridinecarboxylic acid methyl ester **21** (0.19 g, 0.83 mmol) and {2*R*-(*tert*-butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino}acetaldehyde **5a** (0.249 mg, 0.582 mmol) were subjected to the above reductive amination procedure to give the product (260 mg, 70% yield) as a yellow foam. Electrospray MS (positive ion): (M + H) 639.8.

(*R*)-6-[[2-[[2-(3-chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[phenyl]-2-pyridine-carboxylic Acid Ethyl Ester (32). A 1:2.5 mixture of 6-(3-aminophenyl)-2-pyridine-carboxylic acid methyl ester, 6-(3-aminophenyl)-2-pyridinecarboxylic acid ethyl ester **22** (126 mg, 0.55 mmol), and (*R*)-[(*tert*-butoxycarbonyl)-[2-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino]-acetaldehyde **5a** (490 mg, 1.14 mmol) was subjected to the typical reductive amination procedure to give the product (263 mg, 75% yield) as a yellow oil judged to be a 1:2.5 mixture of the methyl and ethyl esters. Electrospray MS (positive ion): (M + H-BOC) 539.9 and 553.9.

(*R*)-5-[[2-[[2-(3-Chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[phenyl]-3-pyridinecarboxylic Acid Ethyl Ester (33). Methyl 3-(3-aminophenyl)-4-pyridinecarboxylate **23**(0.112 g, 0.49 mmol) and

{2*R*-(*tert*-butoxycarbonyl)-[2*R*-(*tert*-butyldimethylsilyloxy)-2-(3-chlorophenyl)ethyl]amino}acetaldehyde **5a** (0.249 mg, 0.582 mmol) were subjected to the above reductive amination procedure to give the product (317 mg, 85% yield) as a yellow foam. Electrospray MS (positive ion): (M + H) 639.8.

3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-hydroxyethyl]amino]propyl]-amino]-[1,1′-biphenyl]-2,4-dicarboxylic Acid (34). 3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]propyl]amino]-[1,1′-biphenyl]-2,4-dicarboxylic acid dimethyl ester **24** (655 mg, 0.92 mmol) was covered with 4 N hydrochloric acid in 1,4-dioxane (5 mL) and stirred for 2 h. The mixture was concentrated with a rotary evaporator to leave the hydroxylamine methyl ester HCl salt intermediate as an uncharacterized semisolid residue, which was dissolved in MeOH (3.0 mL) and treated with a solution of $LiOH·H_2O$ (256 mg, 6.25 mmol) in H_2O (1 mL). The mixture was stirred for 16 h and then concentrated to leave the crude product as a lithium salt. Purification by silica gel chromatography eluting with (60:40:2.2 $CHCl_3$/MeOH/concentrated NH_4OH) gave product **34** as a yellow solid (302 mg, 70% overall) judged to be at least a 27:1 ratio of diastereomers determined by the integration of the methyl doublets by ^1H NMR. Electrospray MS (positive ion): (M + H) 469.1. ^1H NMR (400 MHz, DMSO-d_6): δ 1.13 (d, 3H, $J = 6.0$), 2.79 (t, 1H, $J = 10.0$), 3.03 (m, 2H), 3.21 (m, 2H), 5.07 (d, 1H, $J = 7.6$), 5.80 (bs, 1H), 6.55 (d, 1H, $J = 8.0$), 6.64 (d, 1H, $J = 8.0$), 6.97 (s, 1H), 7.06 (t, 1H, $J = 7.6$), 7.27−7.35 (m, 4H), 7.44 (s, 1H), 7.80 (dd, 1H, $J = 8.0$, 1.6), 7.88 (d, 1H, $J = 1.6$). HPLC (C18): 94.2% purity, 8.71 min retention time using a 30−80% acetonitrile−water with 0.1% trifluoroacetic acid gradient mobile phase with detection by absorbance at 254 nM. Anal. ($C_{25}H_{25}Cl_1N_2O_5·2H_2O$): C, H, N.

3′-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-hydroxyethyl]amino]propyl]-amino]-[1,1′-biphenyl]-4-carboxylic Acid (35). A mixture of 3′-[[2*R*-[[2-(3-chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]propyl]amino]-[1,1′-biphenyl]-4-carboxylic acid methyl ester **25** (289 mg, 0.45 mmol) in 4 N hydrochloric acid in 1,4-dioxane (4 mL) was stirred for 1.5 h. The mixture was diluted with Et_2O and stirred for 20 min to give a viscous residue. The solvent was decanted from the residue, and the residue was dried under vacuum. This material was dissolved in 3:1 MeOH/H_2O (10 mL), treated with $LiOH·H_2O$ (120 mg, 2.86 mmol), and stirred overnight. The mixture was concentrated under reduced pressure and chromatographed on silica eluting with MeOH/CH_2Cl_2/88% NH_4OH (15:85:1.5) to give the title compound as a white solid (31 mg, 18% yield), judged by ^1H NMR to be at least a 25:1 mixture of diastereomers determined by the integration of the methyl doublets. Electrospray MS (positive ion): (M + H) 425.0. HPLC (C18): 98.35% purity, 12.7 min retention time using a 10−100% acetonitrile−water with 0.1% trifluoroacetic acid. ^1H NMR (300 MHz, DMSO-d_6): δ 1.13 (d, 1H, $J = 6.0$), 2.81−2.93 (m, 2H), 3.08−3.30 (m, 5H), 4.79 (dd, 1H, $J = 8.7$, 3.6), 5.85 (bs, 1H), 6.61 (d, 1H, $J = 8.4$), 6.84 (d, 1H, $J = 7.8$), 6.90 (s, 1H, 7.16 (t, 1H, $J = 7.8$), 7.27−7.34 (m, 5H), 7.42 (s, 1H), 7.61 (d, 1H, $J = 8.4$), 7.98 (d, 1H, $J = 8.1$).

3′-[((2*R*)-2-{[(2*R*)-2-(3-Chlorophenyl)-2-hydroxyethyl]amino}-propyl)amino]-2-biphenylcarboxylic Acid (36). A mixture of methyl 3′-{[(2*R*)-2-(((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)-(dimethyl)silyl]oxy}ethyl){[(1,1-dimethylethyl)oxy]carbonyl}amino)-propyl]amino}-2-biphenylcarboxylate **26** (6.89 g, 10.55 mmol) and 4 N HCl/dioxane (40 mL) was stirred at room temperature for 2 h. The mixture was concentrated to ca. 50% volume with a rotary evaporator, and Et_2O was added to supply the intermediate hydroxyphenylethylamine methyl ester hydrochloride (5.34 g) as a white solid. This material was dissolved in MeOH (63 mL), and 3.68 g (87.8 mmol) of $LiOH·H_2O$ in H_2O (21 mL) was added. The mixture was heated at 45 °C for 16 h. The mixture was allowed to cool to room temperature and concentrated with a rotary evaporator. Purification of the residue by silica gel chromatography (92:15:1 to 30:15:1 $CHCl_3$/MeOH/concentrated NH_4OH) afforded 2.0 g (45% yield) of product **36** as a white solid, judged by ^1H NMR integration of the methyl doublet to be a 4.6:1 mixture of diastereomers. Electrospray MS (positive ion): (M + H) 425. ^1H NMR (400 MHz, CD_3OD): δ 1.30 (d, 3H, $J = 6.4$), 3.05 (dd, 1H, $J = 12.4$, 10.4), 3.26−3.37 (m, 3H); 3.61 (septet, 1H, $J = 6.4$); 5.05 (dd, 1H, $J = 10.4$, 2.8); 6.60 (d, 1H, $J = 8.0$); 6.80 (d, 1H, $J = 7.6$); 6.91 (s, 1H), 7.12 (t, 1H, $J = 8.0$); 7.24−7.34 (m, 6H), 7.40 (d, 1H, $J = 8.8$), 7.46 (s, 1H). Anal. ($C_{24}H_{25}Cl_1N_2O_3·H_2O$): C, H, N.

3′-[((2*R*)-2-{[(2*R*)-2-(3-chlorophenyl)-2-hydroxyethyl]amino}-propyl)amino]-3-biphenylcarboxylic Acid (37). A mixture of methyl 3′-{[(2*R*)-2-(((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)-(dimethyl)silyl]oxy}ethyl){[(1,1-dimethylethyl)oxy]carbonyl}amino)-propyl]amino}-3-biphenylcarboxylate **27** (1.90 g, 2.91 mmol) in 4 N HCl/dioxane (10 mL) was stirred for 5 h. The reaction mixture was treated with Et_2O (100 mL), and the resulting precipitate was collected by suction filtration to afford, after drying in vacuo, the hydroxylamine methyl ester HCl salt (1.35 g) as a white precipitate. This material was dissolved in MeOH (16 mL), and a solution of $LiOH·H_2O$ (562 mg, 13.41 mmol) in H_2O (5.5 mL) was added. The resulting mixture was stirred at room temperature for 7 h and concentrated with a rotary evaporator, and the residue was purified by silica gel chromatography (75:15:1 $CHCl_3$/MeOH/concentrated NH_4OH) to supply product **37** (867 mg, 72% overall yield) as a white solid. An analysis of the methyl doublet by ^1H NMR showed this material to be a 4.6:1 ratio of diastereomers with the major as the (*R*,*R*) isomer. ^1H NMR (400 MHz, CD_3OD) (**37**, major diastereomer) δ 1.35 (d, 3H, $J = 6.4$), 3.10 (dd, 1H, $J = 12.4$, 10.0), 3.18 (dd, 1H, $J = 12.4$, 3.2), 3.30−3.54 (m, 3H), 4.93 (dd, 1H, $J = 10.0$, 3.2), 6.65 (d, 1H, $J = 7.6$), 6.94 (d, 1H, $J = 6.8$), 6.96 (s, 1H), 7.18 (t, 1H, $J = 8.0$), 7.26−7.34 (m, 3H), 7.36 (t, 1H, $J = 7.6$), 7.42 (s, 1H), 7.61 (d, 1H, $J = 7.6$), 7.87 (d, 1H, $J = 7.2$), 8.17 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) (major diastereomer): δ 17.70, 48.25, 52.62, 53.91, 70.64, 111.36, 112.01, 115.11, 125.31, 126.48, 127.54, 127.90, 128.61, 129.32, 130.25, 130.48, 130.55, 133.51, 134.65, 141.14, 141.64, 147.05, 150.00, 169.22. Anal. ($C_{24}H_{25}Cl_1N_2O_3·1H_2O$): C, H, N.

(*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]ethyl]-amino]-[1,1′-biphenyl]-3-carboxylic Acid (38). (a). (*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]ethyl]amino]-[1,1′-biphenyl]-3-carboxylic Acid Methyl Ester Dihydrochloride (39). A solution of (*R*)-3′-[[2-[[2-(3-chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[1,1′-biphenyl]-3-carboxylic acid methyl ester (23.8 g, 37.2 mmol mg) in 4 N hydrochloric acid in dioxane (80 mL) was stirred for 3 h. Diethyl ether was added, and the mixture was stirred for 20 min. The resulting solid was collected by suction filtration to afford 15.72 g (92% yield) of the product as a white solid. $C_{24}H_{25}Cl_1N_2O_3$: MH^+ calcd, 425.1632; found, 425.1635 Δ0.3 mmu. ^1H NMR (400 MHz, DMSO-d_6) δ 3.00−3.30 (m, 4H), 3.47 (t, 2H, $J = 6.4$), 3.85 (s, 3H), 4.20 (bs, 2H), 4.97 (d, 1H, $J = 10.0$), 6.67 (d, 1H, $J = 7.2$), 6.87 (d, 1H, $J = 7.2$), 6.89 (s, 1H), 7.21 (t, 1H, $J = 8.0$), 7.32−7.39 (m, 3H), 7.43 (s, 1H), 7.57 (t, 1H, $J = 8.0$), 7.86 (d, 1H, $J = 7.6$), 7.90 (d, 1H, $J = 7.6$), 8.10 (s, 1H).

(b). (*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2-hydroxyethyl]amino]ethyl]amino]-[1,1′-biphenyl]-3-carboxylic Acid (38). To a solution of the (*R*)-3′-[[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]amino]ethyl]-amino]-[1,1′-biphenyl]-3-carboxylic acid methyl ester hydrochloride (4.12 g) in MeOH (60 mL) was added a solution of $LiOH·H_2O$ (2.08 g) in water (20 mL). The mixture was stirred for 16 h, and 1 N hydrochloric acid was added until the mixture was neutral. The mixture was decanted, and the residue was purified by flash silica chromatography eluting with 6:2:0.1 $CHCl_3$/MeOH/concentrated NH_4OH to afford a viscous oil. Trituration with ether and washing with water afforded product **38** as a white solid (2.22 g). ^1H NMR (400 MHz, CD_3OD) δ 3.09 (dd, 1H, $J = 12.4$, 10.0), 3.23 (m, 1H), 3.25 (t, 2H, $J = 6.0$) 3.50 (t, 2H, $J = 6.0$), 4.97 (dd, 1H, $J = 10.0$, 3.2), 6.59 (s, 1H), 6.62 (d, 1H, $J = 8.0$), 6.66 (d, 1H, $J = 8.0$), 7.17 (t, 1H, $J = 7.6$), 7.22 (d, 1H, $J = 8.0$), 7.28−7.33 (m, 3H), 7.45 (s, 1H), 7.78 s, 1H), 7.79 (t, 1H, $J = 7.6$). ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.49, 48.10, 56.69, 70.70, 111.21, 112.07, 115.09, 125.30, 126.45, 127.53, 127.88, 128.61, 129.42, 130.27, 130.58, 130.72, 133.50, 141.08, 141.74, 147.17, 149.95, 168.92. $C_{23}H_{23}Cl_1N_2O_3$: MH^+ calcd, 411.1475; found, 411.1495. Anal. ($C_{23}H_{23}Cl_1N_2O_3·0.46H_2O$): C, H, N.

(*R*)-3′-[[2-[[2-(3-Chlorophenyl)-2- hydroxyethyl]amino]ethyl]-amino]-[1,1′-biphenyl]-2-methyl-5-carboxylic Acid (40). A mixture of methyl 3′-{[2-(((2*R*)-2-(3-chlorophenyl)-2-{[(1,1-dimethylethyl)(dimethyl)silyl]oxy}ethyl){[(1,1-dimethylethyl)oxy]carbonyl}amino)ethyl]amino}-3-biphenylcarboxylate **29** (7.21 g, 11.0 mmol) in 4 N HCl/dioxane (30 mL) was stirred at room temperature for 1.5 h. The mixture was treated with 5:1 hexanes/EtOAc (100 mL), and the resulting white precipitate was collected by suction filtration and dried in vacuo to give the amino alcohol methyl ester intermediate hydrochloride (4.83 g) as a white solid, which was taken into the next step without further characterization. This material was dissolved in MeOH (62 mL), and a solution of LiOH·H_2O (2.11 g, 51.6 mmol) in H_2O (20 mL) was added. The mixture was stirred at room temperature for 20 h. The mixture was concentrated with a rotary evaporator to leave a gummy residue that was purified by silica gel chromatography (30:15:1 $CHCl_3$/MeOH/concentrated NH_4OH) to give, after trituration of the resulting material by MeOH, 2.80 g (69% yield) of the product as a white solid. HRMS $C_{24}H_{25}Cl_1N_2O_5$: MH^+ calcd, 425.1632; found, 425.1638. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.24 (s, 3H), 3.09 (dd, 1H, J = 12.0, 10.0), 3.22−3.29 (m, 3H), 3.50 (t, 1H, J = 6.4), 4.97 (dd, 1H, J = 10.4, 3.2), 6.59 (s, 1H, 6.62 (d, 1H, J = 8.0), 6.66 (d, 1H, J = 8.0), 7.17 (t, 1H, J = 7.6), 7.22 (d, 1H, J = 8.0), 7.28−7.33 (m, 3H), 7.45 (s, 1H), 7.78 (s, 1H), 7.79 (d, 1H), J = 7.6). ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.00, 42.49, 48.16, 56.63, 70.73, 111.68, 113.27, 117.16, 125.31, 126.46, 127.57, 128.45, 129.55, 130.58, 130.74, 131.10, 133.50, 140.58, 141.91, 142.91, 149.34, 168.15. Anal. ($C_{24}H_{25}Cl_1N_2O_3$·1.5H_2O·0.25$CHCl_3$): C, H, N.

2-[3-[[2*R*-[[2-(3-Chlorophenyl)-2*R*-hydroxyethyl]amino]propyl]amino]phenyl]-3-pyridinecarboxylic Acid (41). The procedure above was used starting from 2-[3-[[2*R*-[[2-(3-chlorophenyl)-2*R*-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)-carbonyl]amino]-propyl]amino]phenyl]-3-pyridinecarboxylic acid methyl ester **30** (420 mg, 0.64 mmol), 4 N hydrochloric acid in 1,4-dioxane (4 mL) and LiOH·H_2O (295 mg, 7.0 mmol) in 3:1 THF/H_2O (3 mL) to give, after purification by silica gel chromatography (30:15:1 $CHCl_3$/MeOH/concentrated NH_4OH), the product as a yellow solid (268 mg, 98% yield), judged by ^{1}H NMR to be at least a 40:1 ratio of diastereomers by the integration of the methyl doublet. Electrospray MS (positive ion): (M + H) 426.1. HPLC (C18): 95.5% purity, 4.79 min retention time using a 30−80% acetonitrile−water with 0.1% trifluoroacetic acid gradient mobile phase with detection by absorbance at 254 nM. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.17 (d, 3H, J = 7.0); 2.87 (m, 2H), 3.05 (m, 1H); 3.31 (m, 2H); 5.30 (d, 1H, J = 11.6); 6.60 (d, 1H, J = 10.8); 7.01 (d, 1H, J = 10.0); 7.09 (d, 1H, J = 10.0), 7.12−7.36 (m, 5H); 7.46 (s, 1H); 7.60 (dd, 1H, J = 9.6, 2.0), 8.46 (dd, 1H = 6.0, 2.0). Anal. ($C_{23}H_{24}Cl_1N_3O_3$· 0.75 H_2O): C, H, N, Cl.

(*R*)-5-[3-[[2-[[2-(3-Chlorophenyl)-2- hydroxyethyl]amino]ethyl]amino]phenyl]- 3-pyridinecarboxylic Acid (42). The procedure above was used starting from (*R*)-5-[3-[[2-[[2-(3-chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]-amino]ethyl]amino]phenyl]-3-pyridinecarboxylic acid methyl ester **31** (251 mg, 0.39 mmol), 4 N hydrochloric acid in 1,4-dioxane (10 mL) and LiOH·H_2O (96 mg, 2.28 mmol) in 3:1 THF/H_2O (20 mL) to give, after purification using similar methods as those above, the product (115 mg, 71% yield) as a yellow solid. HRMS: $C_{22}H_{22}$-$Cl_1N_3O_3$: MH^+ calcd, 412.1428; found, 412.1425. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.86 (t, 1H, J = 10.0), 3.00−3.03 (m, 2H), 3.30−3.35 (m, 3H), 4.86 (d, 1H, J = 6.4), 6.05 (bs, 1H), 6.62 (d, 1H, J = 8.0), 6.83 (d, 1H, J = 7.6), 6.87 (s, 1H), 7.18 (t, 1H, J = 7.6), 7.28−7.36 (m, 3H), 7.41 (s, 1H), 8.28 (s, 1H), 8.81 (d, 1H, J = 2.0), 8.93 (d, 1H, J = 1.6). Anal. ($C_{22}H_{22}Cl_1N_3O_3$·1.5$CHCl_3$· 2H_2O): C, H, N.

6-{3-[(2-{[(2*R*)-2-(3-Chlorophenyl)-2-hydroxyethyl]amino}-ethyl)amino]phenyl}-2-pyridinecarboxylic Acid (43). A 2.5:1 mixture of (*R*)-6-[3-[[2-[[2-(3-chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-phenyl]-2-pyridinecarboxylic acid ethyl ester and methyl ester **32** (263 mg, 0.39 mmol), 4 N hydrochloric acid in dioxane (10 mL), and LiOH·H_2O (65 mg, 1.54 mmol) in (3:1) MeOH/water (40 mL) gave the product (30 mg, 19% yield) as a yellow solid. HRMS: $C_{22}H_{22}N_3O_3Cl$: MH^+ calcd, 412.1428; found, 412.1436. ^{1}H NMR (400 MHz, CD_3OD-d_4) δ 3.07 (t, 1H, J = 10.4), 3.24−3.33 (m, 3H), 3.59 (t, 2H, J = 6.4), 4.98 (dd, 1H, J = 10.4, 2.8), 6.70 (bs, 1H), 7.18 (d, 2H, J = 4.4), 7.26−7.31 (m, 3H), 7.42 (s, 1H), 7.61 (s, 1H), 7.80 (d, 1H, J = 7.6), 7.85 (t, 1H, J = 7.6), 7.93 (d, 1H, J = 7.6).

3-{3-[(2-{[(2*R*)-2-(3-Chlorophenyl)-2-hydroxyethyl]amino}-ethyl)amino]phenyl}-4-pyridinecarboxylic Acid (44). To a solution of (*R*)-5-[[2-[[2-(3-chlorophenyl)-2-[[(*tert*-butyl)dimethylsilyl]-oxy]ethyl][(*tert*-butoxy)carbonyl]amino]ethyl]amino]-[phenyl]-3-pyridinecarboxylic acid ethyl ester **33** (316.6 mg, 0.494 mmol) in CH_2Cl_2 (10 mL) was added 4 N HCl in 1,4-dioxane (5 mL). The mixture was stirred at room temperature for 16 h. The solvent was decanted to leave a semisolid residue that was triturated with diethyl ether. The resulting residue was dissolved in 4 mL of 3:1 MeOH/H_2O, and LiOH·H_2O (166 mg, 3.95 mmol) was added. The mixture was stirred for 3 h and concentrated to leave a residue that was dissolved in 30:15:1 $CHCl_3$/MeOH/concentrated NH_4OH. The insoluble solids were filtered away, then the filtrate was concentrated, and the residue was purified by silica gel chromatography (76:15:1 $CHCl_3$/MeOH/concentrated NH_4OH) to give 180.7 mg (89% yield) of product as a pale yellow powder. ^{1}H NMR (400 MHz, CD_3OD) δ 3.10 (dd, 1H, J = 11.6, 10.8), 3.18−3.31 (m, 3H), 3.47 (t, 2H, J = 6.4), 4.99 (dd, 1H, J = 10.8, 2.4), 6.66 (d, 1H, J = 8.0), 6.80 (d, 1H, J = 7.6), 6.89 (s, 1H), 7.18 (t, 1H, J = 8.0), 7.27−7.33 (m, 3H), 7.40 (d, 1H, J = 4.8), 7.44 (s, 1H), 8.44 (d, 1H, J = 4.4), 8.50 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 42.28, 54.21, 67.99, 112.54, 113.11, 117.14, 121.84, 125.29, 126.46, 128.17, 129.72, 130.89, 133.72, 134.32, 138.81, 144.21, 144.3, 145.22, 148.47, 148.90, 150.52, 170.80. Anal. ($C_{22}H_{22}N_3O_3$-Cl·0.5H_2O): C, H, N.

Biological Methods. 1. In Vitro Functional Assays. In these experiments, the β_3 AR clone of Granneman and co-workers was employed.[29] Chinese hamster ovary (CHO) cells expressing human β_1, β_2, or β_3 ARs were grown in DMEM/F12 (with pyroxidine·HCl, 15 mM HEPES, and l-glutamine) supplemented with 10% heat-inactivated FBS, 500 μg/mL of G418, 2 mM l-glutamine, 100 units of penicillin G, and 100 μg of streptomycin sulfate. One confluent flask of cells was trypsinized and resuspended in the above medium at a concentration of 30−40 000 cells/100 μL and plated into 96-well flat bottom plates. The cells were then used for assay within 18−24 h. The medium was aspirated from each well and replaced with 180 μL of DMEM/F12 with 500 mM IBMX. The plate was then placed back in the incubator for 30 min. Drugs were then added to the wells (20 μL, 100× the required final concentration) for 60 min. The responses were determined by measuring cAMP levels of a 20 μL sample of extracellular media using a scintillation proximity based radioimmunoassay (NEN Flashplates).

2. Binding Assays. Human recombinant Sf9 cells expressing the cloned human β_1 and β_2 receptors were obtained using the method of Smith and Teitler.[33] Receptor binding assays were carried out using the radioligand [^{125}I]cyanopindolol at a concentration of 150 pM and the compound of interest at six concentrations ranging from 0.21 to 50 μM. Binding reactions were carried out for 1 h and 30 min for β_1 and β_2 receptors, respectively, at 22 °C and terminated by filtration through glass fiber filters (GF/B, Packard). Bound radioactivity was measured with a scintillation counter (Topcount, Packard) using a liquid scintillation cocktail (Microscint 0, Packard).

3. Pharmacokinetic Studies in Dogs. Pharmacokinetic studies of individual compounds were conducted in fasted male beagle dogs (weight range: 8−12 kg) after iv or oral administration, separated by at least a one week washout period. On the morning of study, each compound was dissolved in 0.025 M methanesulfonic acid containing 5% mannitol at a concentration of 0.2 mg/mL. For iv dosing, each compound was dosed intravenously via a cephalic vein cannula at a dose level of 0.2 mg/kg body weight (5 min infusion period). Blood was collected via a second cephalic vein cannula at

0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2.5, 4, 6, 8, and 24 h. For oral dosing, the compounds were administered orally via a feeding tube, and blood was collected via a cephalic vein at 0, 0.25, 0.5, 0.75, 1, 1.5, 2.5, 4, 6, 8, and 24 h. The resulting serum samples were prepared by protein precipitation with acetonitrile and analyzed for compound content by LC/MS/MS. The compounds were eluted from a Hypersil BDS C18 column (30 × 1 mm, 3μm) at ambient temperature by a gradient mobile phase of 3−99% acetonitrile in 5 mM ammonium acetate buffer at pH 4.5 and a flow rate of 80 μL/min. Detection was by multiple reaction monitoring (MRM) on a Sciex API III+ with argon as the collision gas. Data reduction was performed using Sciex MacQuan software. The pharmacokinetic parameters were caculated from the iv and oral serum concentration versus time profiles by noncompartmental methods using WinNonlin Professional Version 3.0 (Pharsight Corp. Mountain View, CA).

4. Pharmacokinetic Studies in Monkeys. The study was conducted in male, cynomolgus monkeys (weight range 4.2−6.0 kg). Three monkeys were intravenously dosed with a solution of 0.5 mg/mL of compound **38** on Day 1. One week later, the same three monkeys received a solution of 1.0 mg/mL solution of compound **38** in 0.025% methanesulfonic acid with 5% mannitol by oral gavage. In all experiments, the blood samples (2 mL) were drawn via an indwelling venus cannula into a syringe at 5, 15, 30, and 45 min and 1, 1.5, 2, 2.5, 4, 6, 8, 12, and 24 h post dosing. The serum was separated from the red blood cells by centrifugation, frozen at −20 °C, and stored for analysis.

5. Pharmcokinetic Studies in Rats. Four male Han Wistar rats (weight range 262−272 kg) were surgically fitted with jugular vein cannulae, and two of these rats were additionally fitted with a femoral vein cannulae, 48 h prior to dosing. The rats were fasted overnight prior to dosing. On the morning of dosing, compound **38** was dissolved in 0.025 M methanlesulfonic acid with 5% mannitol at a concentration of 1 mg/mL. Two rats were intravenously given **38** via the femoral vein cannula at a dose of 1 mg/kg bodyweight. The other two rats received **38** by oral gavage at a dose of 1 mg/kg body weight. The blood samples were collected from the jugular vein cannula before dosing at at 5 (iv only), 15, 30, and 45 min and 1, 1.5, 2.5, 4, 6, 8 12 (oral only), and 24 h post dosing. The serum was separated from the red blood cells by centrifugation, frozen at −20 °C, and stored for analysis.

6. Antidiabetic Efficacy Studies. The antidiabetic efficacy of compounds **36**, **38**, and **44** were evaluated using the *db*/*db* mouse as a model of type 2 diabetes. This mouse model has been characterized as hyperinsulemic, hyperglycemic, and hypertriglyceridemic with elevated levels of circulating nonesterified fatty acids. The mice have a point mutation in chromosome 4 that encodes the high affinity leptin receptor and, thus, exhibit severe hyperphagia. The animals are obese by 3−4 weeks of age. Circulating insulin concentrations are increased at 10 days of age, peaking at 6−10 times higher than those of lean controls by 2−3 months of age. The rates of lipogenesis and gluconeogenesis are also increased. There is a precipitous decline in circulating insulin concentrations, progression to ketosis, weight loss, and death by 5−10 months.

The data (mean ± SEM) from chronic efficacy studies with compounds **36**, **38**, and **44** performed in male *db/db* mice (10 mice/group; 60 days of age at onset of dosing) are shown in Tables 4, 5, and 6. All compounds were administered twice daily (BID) by oral gavage for 14 days. The reconstitution vehicles for the in vivo studies were a solution of D-α tocopherol poly(ethylene glycol) 1000 succinate (TPGS) and propylene glycol (PG) (25:75% w/w) with water as the diluant for compound **38** and 0.025 M methanesulfonic acid for compounds **36** and **44**. Dosing solutions were prepared fresh daily.

Prior to the start of dosing, 10 mice were anesthetized and exsanguinated by cardiac puncture for baseline measurements (day 0 predose values) of postprandial glucose, glycosylated hemoglobin, and insulin. Subsequently, on days 7 and 14 of dosing, the mice from each dose group were sacrificed, and the blood samples for the analytes listed above were obtained. Body weights were measured throughout the study, and there were no significant effects of any of the compounds on body weight gain in these studies. All serum biochemical measurements were made using an ILAB600 automated chemistry Analyzer (Instrumentation Laboratories). Glycosylated hemoglobin measurements were performed on a ColumnMateAnalyzer (Helena Laboratories, Beaumont, TX). Serum insulin measurements were performed by electrochemiluminescence using an Origen (Igen International, Gathersburg, MD).

7. Rodent Infrared Thermography Studies.[14,28] CD-1 mice (CD-1(ICR)BR) were anesthetized with isoflurane and shaved to expose the interscapular region before IR imaging. The animals were orally dosed, and at the appropriate times, they were anesthetized and scanned. Animals were dosed by oral gavage with either the vehicle (0.025 M methanesulfoxide at 10 mL/kg) or the test compound (10 mL/kg (vol), 1 mg/kg, 0.1 mg/kg, and 0.01 mg/kg (concentration)). The mice were placed into a manifold with nose-ports for continual delivery of isoflurane. To maintain body core temperature during scanning, the rodents were placed onto a tightly regulated heating table (37 °C + 0.1). The heating table was housed in an isothermal, nonreflective chamber (24 °C ± 0.1, 50% relative humidity). Upon closure of the chamber door, heat emissions from the areas of interest were acquired using a high-resolution InSb IR scanning detector (AGEMA Thermovision 900, Thermogenic Imaging, Billerika, MA) mounted 30 cm above the area of interest. Images were recorded at 1-min intervals for 5 min. A frame-averaging rate of 16 frames/s was used for each designated time point. Acquired images were analyzed for average temperatures using a GlaxoSmithKline (RTP, NC) image processing software application (RoboImage). Data were expressed as either average temperature/area or Δtemperature/area (drug treated − vehicle treated). Data were calculated as the mean and standard error of the mean from experiments performed on 8−10 animals per treatment group. Two tailed t-tests were performed to calculate *p* values. Correlation coefficients were determined by regression analysis using Sigma Plot.

8. Ames Mutagenicity Studies. The bacterial mutagenicity assays were performed as described,[29,30] with the following modifications. Twelve-well multiple tissue culture plates (Corning. Corning, NY) were used for bacterial strains TA98 and TA100. DMSO was used as the solvent (20 μL/well), and the compounds were tested from 5 to 400 μg/well. Compound **38** was tested with (10% v/v) and without rat liver S9.

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

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