Discovery of Highly Potent and Selective Biphenylacylsulfonamide-Based β_3-Adrenergic Receptor Agonists and Evaluation of Physical Properties as Potential Overactive Bladder Therapies: Part 5

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As an extension of research conducted on β_3-adrenergic receptor agonists as potential drugs for treating overactive bladder (OAB), novel series containing an acylsulfonamide moiety instead of the carboxylic acid moiety were evaluated. These compounds have been identified as potent and selective human β_3-AR agonists with improved oral bioavailability compared to the previous series. Results of structure−activity relationship (SAR) studies and cassette dosing evaluation in dogs showed several analogues (namely, **6h**, **6j**, **6o**, **7e**, and **9e**) to have an excellent balance of in vitro potency and selectivity, pharmacokinetic (PK) profile, and an in vivo OAB model. Here we examined the relaxation response in dog detrusor muscle strips to a KCl induced tonic concentration. Results showed that the potency of in vitro relaxation response was not mirrored in the potency of the cAMP accumulation in CHO cell lines. Surprisingly, the EC_{50} values of **6e** and **7e** found to induce relaxation of isolated bladder strips were over 50-fold higher than the cAMP accumulation in cell line. In general, increased lipophilicity led to decreased potency for the bladder relaxation compared with cAMP accumulation in CHO cell lines, indicating that lipophilicity is crucial for OAB drug candidates to improve β_3 activity.

Introduction

Overactive bladder (OAB[a]) is defined as urinary urgency with or without urgency incontinence.[1] An estimated 16% of the adult population in the U.S. suffers from OAB, with worldwide numbers steadily increasing.[2] The urinary bladder is innervated by both the sympathetic and parasympathetic nervous systems, and antimuscarinic agents that induce relaxation of the detrusor muscle have been widely used in the treatment of OAB.[3] However, these agents have several disadvantages, including adverse events associated with anticholinergic effects such as dry mouth, constipation, and the potential for voiding difficulty in patients with poorly contractile bladders. Accordingly, a drug lacking these adverse effects due to the blockage of cholinergic neuron would be a significant improvement over current therapy.

Activation of sympathetic nerves contributes to urine storage by relaxing the detrusor muscle via activation of β-adrenoceptors (β-ARs).[4] β-ARs are classified into three types; β_1-, β_2-, and β_3-ARs.[5] The β_3-AR has been demonstrated to mediate several pharmacological and physiological effects such as lipolysis in white adipocytes and thermogenesis (energy expenditure) in brown tissue adipocytes.[6] Both β_1- and β_2-ARs have been identified in the urinary bladder of many species, including humans.[7] In most species, the β_2-AR subtype was proposed to have an important role in relaxation via activation of adenyl cyclase. In humans, however, the detrusor muscle has recently been reported to be the predominant site of β_3-AR mRNA expression. The relaxation induced by adrenergic stimulation of the human detrusor is mediated mainly through β_3-AR activation, suggesting that β_3-ARs may represent a new therapeutic target for treating OAB.[8]

In our previous study, we described a series of first generation biphenyl (FGB) analogues, shown in Figure 1, which exhibited good oral bioavailability and a long plasma half-life.[9] The key to success was finding a benzoic acid moiety and R substitution on the right-hand side (RHS) which maximized β_3-AR activity, selectivity, and bioavailability. Removal of the carboxylic acid from the biphenyl analogue resulted in significantly reduced potency and bioavailability.[10] On the other hand, SAR studies of the R position on the terminal phenyl ring in the FGB analogues indicated that introduction of lipophilic substitute group led to increased potency for β_3-AR activity (O-cyclohexyls **1e**, **2e** > O-isopropyls **1b**, **2b** > O-methyl **1a**), but a decreased oral bioavailability (**1a** > **1b**, **2b** > **1e**, **2e**) while displaying high passive permeability and good stability to liver microsomes. To address this problem, a series of second generation biphenyl (SGB) analogues were developed, with adjustment of physical properties by replacement of the phenyl moiety with pyridine analogues on the left-hand side (LHS), resulting in enhanced potency and maintenance of good oral availability as shown in Table 1.[11] In a previous metabolism study of FGB/SGB analogues conducted in rat and human hepatocytes, we identified two main metabolites: a dealkylated metabolite (M-1) and an acylglucuronide conjugated metabolite

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[a] Abbreviations: β-AR, β-adrenergic receptors; OAB, overactive bladder; FGB, first generation biphenyl; SGB, second generation biphenyl; LHS, left-hand side; RHS, right-hand side; SAR, structure−activity relationship; cAMP, cyclic adenosine monophosphate; ISP, isoproterenol; CHO, Chinese hamster ovary; IVP, intravesical pressure; PAMPA, parallel artificial membrane permeation assay; PK, pharmacokinetic; PB, protein binding.

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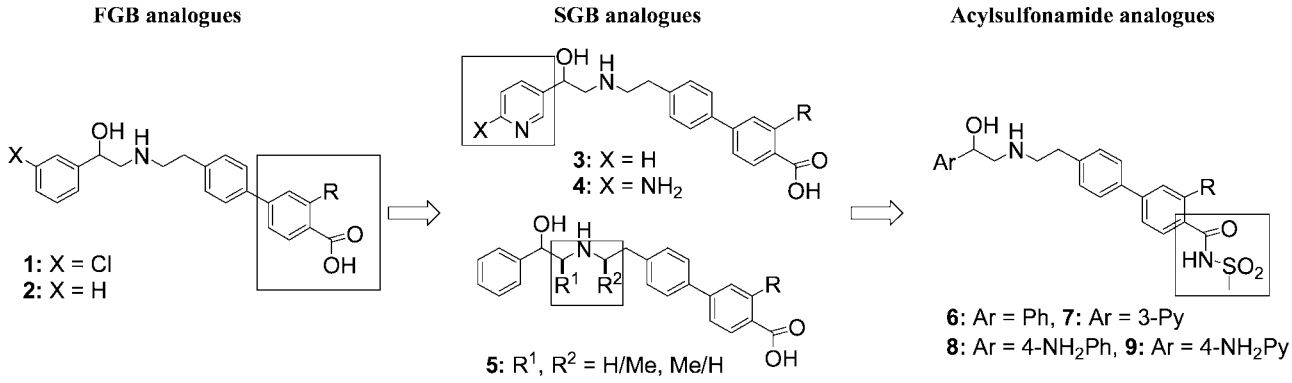
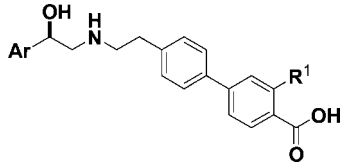


Figure 1. Design and discovery strategy of bipenyl analogues.

Table 1. SAR Results for FGB and SGB Analogues[a]



compd	Ar	R^1	human EC_{50} (nM) β_3	β_2	β_1	dog cassett assay,[b] C_{max} ratio[c]
1a	3-ClPh	O-Me	4.8	>10000	250	1.7
1b		O-iso-Pr	1.1	>10000	720	1.0
1e		O-*c*-Hex	0.46	NT	55	0.0
2b	Ph	O-iso-Pr	2.0	>10000	>1000	0.90
2j		iso-Bu	0.60	>10000	>1000	0.24
2e		O-*c*-Hex	0.30	NT	260	0.0
2f		O-Ph	0.34	NT	980	0.24
2h		NH-iso-Pro	0.29	586	170	0.10
2o		S-iso-Pr	0.56	>10000	>1000	0.32
3b	3-Py	O-iso-Pr	1.5	>10000	>1000	0.65
3j		iso-Bu	0.26	NT	>1000	0.36
3e		O-*c*-Hex	0.26	>10000	480	0.48
4b	3-(6-NH_2Py)	O-iso-Pr	0.19	>10000	130	0.49
4j		iso-Bu	0.066	3200	150	0.36
4e		O-*c*-Hex	0.035	1100	39	0.09

[a] See refs 9 and 11. [b] Dose 0.10 or 0.20 mg/kg po ($n = 2, 3$). [c] The ratio is defined as the C_{max} of each compound divided by the C_{max} of **1b**. The ratio value of **1b** was presented as 1.0.

(M-2) as shown in Scheme 1.[12] Although glucuronide metabolites from phase II metabolism generally enhance elimination in bile and urine, they can also transform into their covalent adducts which can lead to toxicity.[13] In light of these findings, we set out to further explore this series by examining modification utilizing an acylsulfonamide moiety on the RHS instead of the carboxylic acid moiety, a potentially useful strategy to protect against metabolism via acylglucuronide conjugation.

Here, we describe SAR studies conducted on this series as well as report on the pharmacokinetics. We also evaluate the correlation between β_3 activity and the physical properties of this series of biphenyl analogues, which allowed identification of potential clinical drug candidates with no loss of β_3 potency in vitro and in vivo.

Chemistry

The general synthetic route to the target compounds is shown in Scheme 2. Synthesis of the requisite intermediate Boc amine derivatives **10**−**13** was conducted as previously reported,[9,11] and acylsulfonamide derivatives **14** were prepared from the corresponding carboxylic acid by a 1,1′-carbonyldiimidazole-promoted coupling with methansulfonamide followed by treatment with pinacol diborane.[14] Suzuki cross-coupling of Boc amine intermediates **10** with boronic acid derivatives **14** has also been previously described. Target compounds **6a**−**p** were obtained as hydrochloride salts after deprotection the Boc group with 4 N HCl, and pyridine analogues **7** were obtained from **11** by the similar method. The aniline analogue **8** was obtained from **12** through an additional step involving reduction of the nitro group with iron powder in the presence of NH_4Cl. The amino pyridine analogues **9** were prepared by coupling **13** with boronic acids (**14b**,**e**,**o**,**q**) in the presence of a catalytic amount of $PdCl_2(dppf)\cdot CHCl_3$, followed by alkaline hydrolysis and subsequent deprotection of the Boc amine silyl ether using 4 N HCl.

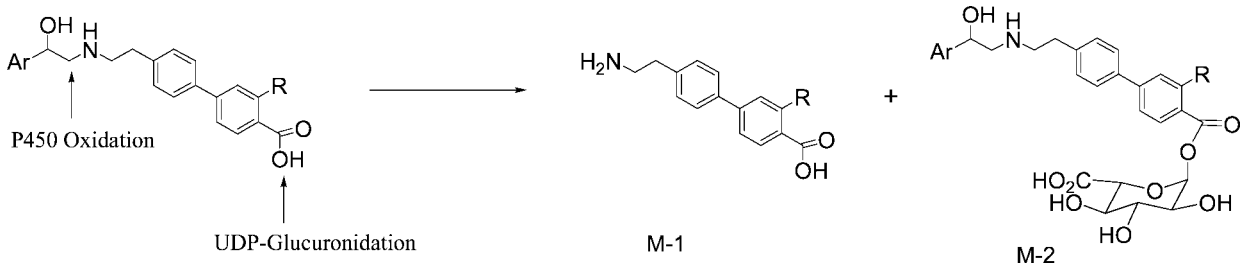
Result and Discussion

All compounds were evaluated for the ability to produce cAMP in Chinese hamster ovary (CHO) cell lines expressing cloned human β_3- and β_1-ARs. Select compounds were also evaluated for human β_2 activity by a similar method. The results for the reference compound, isoproterenol (ISP), a nonselective β-AR agonist, are shown for comparison in Tables 2 and 3. Pharmacokinetic (PK) properties of selected compounds were evaluated by cassette dosing assay in dogs.[15]

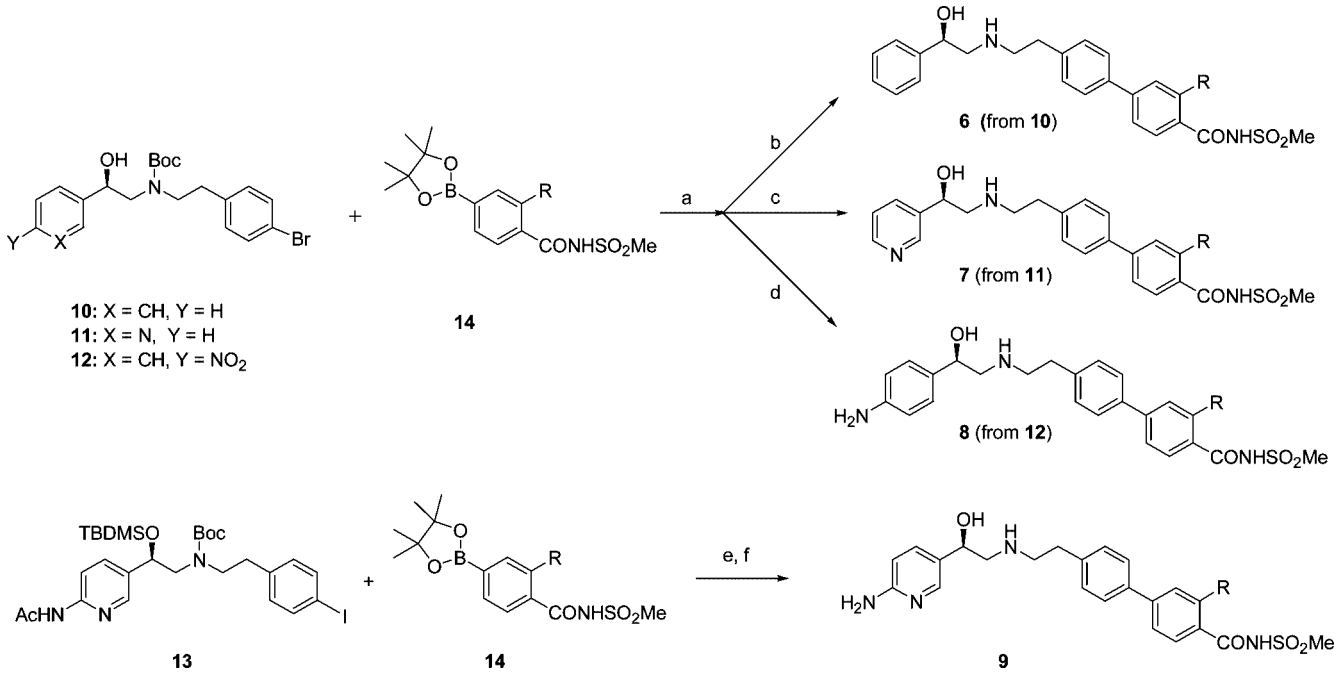
We initially investigated the effect of newly designed acylsulfonamide analogues with a phenyl moiety on the LHS. The modification from a carboxylic acid to the corresponding acylsulfonamide resulted in SAR trends similar to those seen in FGB analogues with regard to β_3 activity and the selectivity for β_1 and β_2. Substitution to the R position of the terminal phenyl ring on the RHS with a more lipophilic group such as an O-linker resulted in increased β_3-AR activity (O-cycloheptyl **6g** > O-cyclohexyl **6e** > O-phenyl **6f** > O-cyclopentyl **6d** > O-isopropyl **6b** > O-methyl **6a**). Substitution with a bulky cyclopentyl group (**6d**) showed a 4.5-fold greater potency than the corresponding analogue substituted with an *n*-pentyl group (**6c**). The N-linker analogues with an isopropyl group (**6h**) and a cyclohexyl group (**6i**) showed greater improvement in β_3-AR activity relative to the O-linker analogues **6b** and **6e**, respectively. The C-linker analogues with isobutyl (**6j**), cyclopentyl (**6k**), and cyclohexyl (**6l**) also showed good activity; however, the most lipophilic compound **6m** was less active than the other C-linker analogues and the corresponding cyclohexyl analogues **6e** and **6i**. Among analogues with an isopropyl group (**6b**, **6h**, **6j** and **6o**), the S-linker **6o** showed the most potent β_3 activity and selectivity for β_1 and β_2.

Of particular note is the fact that all of these compounds displayed remarkable pharmacokinetic properties on dogs.[16] The compounds with O-cyclohexyl (**6e**), NH-isopropyl (**6h**), isobutyl (**6j**), and S-isopropyl (**6o**) showed dramatically improved bioavailability over the corresponding carboxylic acids (**2e**, **2h**, **2j**, and **2o**, respectively), a finding that can be explain by

Scheme 1. Main Metabolites Pathway of FGB and SGB Analogues



Scheme 2. Synthesis Route to Target Compounds[a]



[a] (a) $Pd(PPh_3)_4$, aq $NaHCO_3$, DME, 70 °C; (b) 4 N HCl/AcOEt; (c) 4 N HCl/dioxane; (d) Fe (powder), NH_4Cl, EtOH, reflux, then 4 N HCl/dioxane; (e) $PdCl_2(dppf)\cdot CH_2Cl_2$, dppf, aq Na_2CO_3, toluene, EtOH, 75 °C; (f) 1 N NaOH aq, EtOH, 100 °C, then 4 N HCl/dioxane, MeOH.

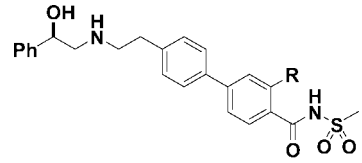
protection from conjugation. Further, compounds **6h** and **6k** also showed good bioavailability despite having a low passive permeability as shown by parallel artificial membrane permeation assay (PAMPA),[17] indicating that uptake following oral administration of these compounds is likely dependent on active transport using a carrier proteins.

We next focused our attention on replacing the phenyl moiety on the LHS. Transformation of the phenyl group to a pyridyl group resulted in slightly increased in β_3-AR activity (3- to 1.5-fold) over the corresponding phenyl group and increased potency for the cyclohexyl substitution over that for the isopropyl substitution. The N- and S-linker compounds with isopropyl (**7h** and **7j**) showed 5-fold more potent activity than the O-linker **7b**. However, the shift to pyridyl analogues led to a decreased bioavailability compared to the phenyl analogues. Only the compound with an O-cyclohexyl group (**7e**) displayed an excellent balance between bioavailability and potency, with improved activity and a PK profile relative to the corresponding phenyl analogue **6e**. On replacement of the phenyl moiety with a 4-aminophenyl moiety on the LHS, compound **8e** showed the same activity and PK profile as the corresponding phenyl compound **6e**. Although the aminopyridyl derivatives on the LHS display greatly increased β_3 activity, the C_{max} level after oral administration is fairly reduced in contrast to the pyridyl derivatives. The compound with an O-cyclohexyl (**10e**) showed the most potent activity among this series, and bioavailability was improved over that of the corresponding SGB analogues.

After SAR examination, we selected **6h**, **6j**, **6o**, **7e**, and **9e** for PK profile evaluation. Table 4 shows the metabolic stability as measured by in vitro clearance using liver microsomes. Overall, all compounds showed good stability to dog, monkey, and human microsomes, and poor stability to rat microsomes. Table 5 shows the pharmacokinetic profiles in rats, dogs, and monkeys. The phenyl analogue with an S-isopropyl group (**6o**) and the pyridyl analogue with an O-cyclohexyl group (**7e**) displayed good oral bioavailability in all species (rats, $F = 41\%$, 25%; dogs, $F = 62\%$, 81%; monkeys, $F = 24\%$, 38%, respectively) and a moderate plasma half-life in dogs ($t_{1/2\beta} = 4.8$, 5.5 h, respectively). Isobutyl analogue **6j** displayed similar good PK profiles in rats and dogs (rats, $F = 40\%$; dogs, $F = 67\%$). However, the phenyl analogue with an N-isopropyl (**6h**) and the aminopyridyl analogue with an O-cyclohexyl (**9e**) displayed good oral bioavailability in dogs (dogs, $F = 45\%$ and 54%, respectively) but poor bioavailability in monkeys.

We then examined the inhibitory effect on a carbachol-induced increase of intravesical pressure (IVP) in anesthetized dog OAB model.[18] Table 6 shows in vitro potency of these compounds as well as FGB and SGB series for human and dog β_3-AR activity in CHO cell lines, with in vivo inhibition results as well. Phenyl analogues on the LHS with an O-isopropyl group (**6b**), an

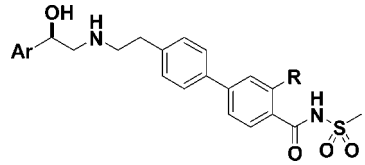
Table 2. Right Hand-SAR Results and β_3-AR Activity and Pharmacokinetic Profiles for Acylsulfonamide Analogues[a]



		human EC_{50} (nM)[b]			pharmacokinetic parameters in dogs[b,c]					
					po ($n = 3$)		iv ($n = 3$)			
compd	R	β_3	β_2	β_1	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	$t_{1/2}$ (hr)	CL_{tot} ((mL/min)/kg)	F^d (%)	PAMPA[e]/ clogP[f]
6a	O-Me	18.0 ± 0.39	NT[h]	730 ± 159	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	7.6/3.38
6b	O-iso-Pr	3.10 ± 0.10	NT[h]	>1000	33.4 ± 1.4	561.5 ± 60.6	12.0 ± 2.0	2.3 ± 0.1	75	15/4.22
6c	O-*n*-Pen	2.80 ± 0.99	NT[h]	160 ± 24	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]/5.50
6d	O-*c*-Pen	0.62 ± 0.11	NT[h]	750 ± 70	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]/4.86
6e	O-*c*-Hex	0.43 ± 0.06	NT[h]	>1000	22.2 ± 3.6	363.4 ± 41.3	7.8 ± 1.2	3.0 ± 0.1	70	24/5.41
6f	O-Ph	0.60 ± 0.08	NT[h]	>1000	70.3 ± 13.2	756.5 ± 203.6	4.4 ± 0.1	2.2 ± 0.3	98	18/5.26
6g	O-*c*-Hep	0.38 ± 0.04	NT[h]	220 ± 10	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]/5.97
6h	NH-iso-Pr	0.60 ± 0.05	>10000	260 ± 55	8.6 ± 0.7	95.8 ± 1.9	6.3 ± 1.3	7.5 ± 1.6	45	0.8/4.52
6i	NH-*c*-Hex	0.14 ± 0.01	NT[h]	900 ± 10	3.2 ± 1.0	33.5 ± 7.3	3.9 ± 0.2	9.9 ± 0.5	22	4.4/5.71
6j	iso-Bu	0.46 ± 0.08	>10000	>1000	24.9 ± 1.9	210.5 ± 22.6	3.5 ± 0.2	5.3 ± 0.8	67	6.8/4.85
6k	*c*-Pen	0.41 ± 0.03	NT[h]	>1000	17.1 ± 0.53	211.1 ± 25.7	4.7 ± 0.2	4.0 ± 1.1	50	0.32/4.65
6l	*c*-Hex	0.33 ± 0.12	NT[h]	>1000	13.1 ± 1.1	105.6 ± 1.9	NT[h]	NT[h]	NT[h]	1.2/5.21
6m	CH-*c*-Hex	0.54 ± 0.08	NT[h]	370 ± 30	27.9 ± 2.9	230.0 ± 43.1	3.9 ± 0.1	4.9 ± 0.4	70	NT[h]/6.04
6n	S-*n*-Pro	1.40 ± 0.05	NT[h]	>1000	8.1 ± 1.0	91.9 ± 12.5	NT[h]	NT[h]	NT[h]	2.7/4.25
6o	S-iso-Pro	0.32 ± 0.03	>10000	>1000	39.8 ± 7.8	440.6 ± 91.2	4.3 ± 0.3	2.9 ± 0.3	75	2.2/4.03
6p	S-*c*-Hex	0.50 ± 0.06	NT[h]	840 ± 154	8.0 ± 2.2	56.3 ± 9.0	NT[h]	NT[h]	NT[h]	12.5/5.23
1b	O-iso-Pr	1.10 ± 0.10	>1000	720 ± 160	13.0 ± 1.1	149 ± 8.7	9.9 ± 0.4	4.9 ± 0.6	45	>30/3.30
ISP[g]		0.97 ± 0.14	2.0 ± 0.9	0.84 ± 0.02						

[a] Details of experimental methods are in refs 9 and 11. [b] The results are the mean ± SE ($n = 3$) or are presented as the average of two experiments. [c] Cassette assay data. Dose of 0.10 mg/kg po and iv. [d] F = bioavailability. [e] See ref 17. [f] Biobyte ClogP, version 4.3. [g] ISP = isoproterenol. [h] NT = not tested.

Table 3. Left Hand-SAR Results and β_3-AR Activity and Pharmacokinetic Profiles for Acylsulfonamide Analogues[a]



			human EC_{50} (nM)[b]			pharmacokinetic parameters in dogs[b,c]					
						po ($n = 3$)		iv ($n = 3$)			
compd	Ar	R	β_3	β_2	β_1	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	$t_{1/2}$ (h)	CL_{tot} ((mL/min)/kg)	F^d (%)	PAMPA[e]/ clogP[f]
7b	3-Py	O-iso-Pr	1.0 ± 0.08	NT[h]	130 ± 20	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]/2.72
7e	3-Py	O-*c*-Hex	0.13 ± 0.005	>10000	800 ± 40	34.5 ± 3.8	384.6 ± 57.7	5.5 ± 0.4	3.2 ± 0.3	81	15/3.92
7h	3-Py	NH-iso-Pr	0.20 ± 0.02	NT[h]	320 ± 35	1.9 ± 1.1	10.4 ± 5.2	2.7 ± 0.3	16.4 ± 2.4	12	0.10/3.02
7i	3-Py	NH-*c*-Hex	0.062 ± 0.001	>10000	>1000	10.3 ± 2.2	28.4 ± 9.3	1.3 ± 0.6	12.4 ± 4.9	21	1.5/4.22
7j	3-Py	iso-Bu	0.32 ± 0.03	NT[h]	490 ± 20	2.6 ± 1.1	12.0 ± 9.4	0.6 ± 0.1	35.9 ± 5.9	21	0.50/3.35
7k	3-Py	*c*-Pen	0.29 ± 0.02	NT[h]	>1000	1.4 ± 0.3	13.4 ± 0.6	1.4 ± 0.2	21.8 ± 4.3	18	0.41/3.16
7o	3-Py	S-iso-Pro	0.19 ± 0.01	NT[h]	240 ± 35	5.3 ± 0.7	50.9 ± 22.4	4.3 ± 0.7	5.4 ± 0.7	14	1.4/2.54
8b	4-(NH_2Ph)	O-iso-Pr	5.5 ± 0.70	NT[h]	>1000	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]	NT[h]/2.99
8e	4-(NH_2Ph)	O-*c*-Hex	0.50 ± 0.04	NT[h]	>1000	17.5 ± 1.0	212.5 ± 14.4	5.7 ± 0.3	5.0 ± 0.2	68	3.8/4.19
9q	3-(6-NH_2Py)	O-Et	0.68 ± 0.02	NT[h]	93 ± 2.0	4.8 ± 1.0	36.5 ± 6.3	NT[h]	NT[h]	NT[h]	NT[h]/2.09
9b	3-(6-NH_2Py)	O-iso-Pr	0.14 ± 0.02	NT[h]	200 ± 35	3.9 ± 0.6	44.4 ± 6.9	1.6 ± 0.02	22.9 ± 1.6	36	NT[h]/2.40
9e	3-(6-NH_2Py)	O-c-Hex	0.029 ± 0.003	290	140 ± 10	2.5 ± 0.3	26.7 ± 1.0	6.7 ± 1.3	34.9 ± 2.5	54	0.60/3.59
9o	3-(6-NH_2Py)	S-iso-Pr	0.080 ± 0.012	1200	390 ± 35	0.9 ± 0.1	3.8 ± 2.1	NT[h]	NT[h]	NT[h]	NT[h]/2.21
ISP[g]			0.97 ± 0.14	2.0 ± 0.9	0.84 ± 0.02						

[a] Details of experimental methods are in refs 9 and 11. [b] The results are the mean ± SE ($n = 3$) or are presented as the average of two experiments. [c] Cassette assay data. Dose of 0.10 mg/kg p.o and iv. [d] F = bioavailability. [e] See ref 17. [f] Biobyte ClogP, version 4.3. [g] ISP = isoproterenol. [h] NT = Not Test.

O-cyclohexyl group (**6e**), and an O-phenyl group (**6f**) all showed less inhibitory activity after intravenous administration than the FGB analogue with an O-isopropyl group (**2b**), although the dog EC_{50} of **6e** was 3.5-fold improved. The N-linker with an isopropyl group (**6h**), C-linker with an isobutyl group (**6j**), and S-linker with an isopropyl group (**6o**) all showed potent in vivo activity (**6h**, EC_{50} = 7.1 nM; **6j**, EC_{50} = 14.2 nM; **6o**, EC_{50} = 8.2 nM). The pyridyl analogue with an O-cyclohexyl group (**7e**) also exhibited improved activity compared with phenyl analogue **6e**; however, the in vivo EC_{50} value of 26.5 nM was lower than those of **6h** and **6o** despite similar in vitro dog β_3-AR activity and lower dog protein binding. The aminopyridyl analogue on the LHS (**9e**) had the most potent in vivo activity among the selected compounds, with an EC_{50} = 1.2 nM.

Table 4. In Vitro Metabolism in Liver Microsomes CL_{int} ((mL/min)/kg)[a]

compd	rat	dog	monkey	human
6h	36.0	3.3	4.7	4.6
6j	NT[b]	ND[c]	NT[b]	ND[c]
6o	84.3	ND[c]	ND[c]	ND[c]
7e	46.9	5.4	3.4	2.2
9e	10.3	ND[c]	3.5	ND[c]

[a] Each compound was incubated at 37 °C with live microsomes from rats, dogs, monkeys, and humans in the presence of the NADPH-generating system. Results are presented as the average of two to three experiments. [b] NT = not tested. [c] ND = not determined (<0.1 (mL/min)/kg).

The ED_{50} values of acylsulfonamide derivatives were improved over those of the corresponding carboxylic acid derivatives, suggesting that this improvement was due to improvement of pharmacokinetic properties (as in **6j** vs **2j**, **6o** vs **2o**, **7e** vs **3e**). However, the EC_{50} value of compound **7e** showed 7-fold less activity compared to compound **3e**. To investigate this discrepancy more carefully, we examined the relaxation response in dog detrusor muscle strips in a KCl induced tonic concentration.[19] Differences in EC_{50} values between cAMP accumulation and bladder relaxation for each compound are shown in Table 6. Our findings suggested that the potency of in vitro relaxation response was not mirrored by the corresponding potency of the cAMP accumulation in CHO cell lines. In particular, the EC_{50} values for the relaxation of isolated dog bladder strips of **6e** and **7e** were over 50-fold higher than values for cAMP accumulation in the dog CHO cell line, which may explain the lower activity observed in vivo.

We also investigated the correlation of in vitro β_3 activity using 84 biphenyl analogues.[20] Figure 2 shows a plot of the disparity index[21] between cAMP accumulation in the dog CHO cell line and the relaxation of isolated dog bladder strips versus lipophilicity (clogP and clogD).[22] This reveals that the lipophilic properties of the compounds account for the general trend seen in the difference in pEC_{50} values. Increasing lipophilicity of the biphenyl analogues led to a decrease in potency for the bladder relaxation compared to cAMP accumulation in CHO cell.

In addition, we investigated the direct correlation between β_3 activity and each structural transformation on RHS or LHS using matched molecular pairs of molecules (Figure 3).[23] Table 7 shows the corresponding change in potency when exchanging an isopropyl moiety on the terminal phenyl ring of the RHS for a cyclohexyl moiety. Although this exchange results in markedly improved in pEC_{50} values of the β_3 activity of cAMP accumulation in humans and dogs, the improvement observed in pEC_{50} values for relaxation in dog bladder strips failed to meet expectations. Similarly, Table 8 indicates the effect on potency after exchanging the phenyl moiety on the LHS with various aromatic moieties. Replacement of the phenyl moiety with a more lipophilic (+0.7 units of clogP) Cl-phenyl moiety showed slightly improved potency for human and dog cAMP level but decreased potency for bladder relaxation with an overall mean change of approximately 0.91 units (pEC_{50}). Exchanging the phenyl group for a pyridyl group (1.5 units reduction in lipophilicity) led to a loss of potency for dog cAMP with a mean change of 0.39 units but allowed for maintenance of dog bladder relaxation with 0.08 units. Exchanging the phenyl group for an aminopyridyl group resulted in an improvement of 0.63 units in potency for dog bladder relaxation, despite a loss in potency for dog cAMP, with an overall mean change of 0.31 units. Lipophilicity in this aminopyridyl analogue was reduced by the presence of a polar amine group on the pyridine ring. Given the value of human cAMP, these results highlight that the aminopyridyl analogue has promising pharmacological properties for use in the human bladder.

These findings raise the issue that increased lipophilicity leads to opposing affect from other types of interaction in isolated dog bladder strips, reducing the compound's overall efficacy. Lipophilicity underlying the structural properties seems to be complementary properties, and increasing lipophilicity would also affect protein binding or result in undesirable drug targets.[24] With regard to drug design, increasing potency without increasing lipophilicity is important in achieving an optimum design.

Conclusions

In the present study, we described potent and selective β_3 agonists in novel series that contain an acylsulfonamide moiety instead of a carboxylic acid moiety and demonstrated their improved PK profile over the FGB and SGB series. Results of our SAR study and cassette dosing showed that several analogues (namely, **6h**, **6j**, **6o**, **7e**, and **9e**) displayed an excellent balance of potency, selectivity, and PK profiles. In a carbachol-induced IVP model in dogs, these compounds showed improved ED_{50} or both improved in vivo ED_{50} and EC_{50} values. On the basis of their attractive pharmacological profiles, these compounds may merit consideration for use in OAB treatment. Further, to clarify the relationship between in vitro and in vivo activity, we evaluated the relaxation response of biphenyl analogues in dog detrusor muscle strips. Managing lipophilicity was found to be a crucial aspect of drug design for improving β_3 activity. Increased lipophilicity of the biphenyl analogues was found to reduce the compound's efficacy in relaxation of isolated dog bladder strips, regardless of the nature of the cAMP accumulation of the compounds. These findings will be useful in identifying clinical

Table 5. Pharmacokinetic Profiles of Selected Members of Acylsulfonamide Analogues[a]

		po (n = 3)			iv (n = 3)		
compd	species	dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-24h} (ng·h/mL)	$t_{1/2\beta}$ (h)	CL_{tot} ((mL/min)/kg)	F (%)[b]
6h	rat	0.32	14.9 ± 1.1	60.9 ± 5.1	4.1 ± 0.9	16.9 ± 4.0	20
	dog	0.10	8.6 ± 0.7	95.8 ± 1.9	6.3 ± 1.3	7.5 ± 1.6	45
	monkey	0.32	2.7 ± 1.3	19.3 ± 4.1	3.1 ± 0.5	25.9 ± 4.8	10
6j	rat	0.32	6.4 ± 0.8	64.8 ± 2.9	2.5 ± 0.5	35.2 ± 9.7	40
	dog	0.10	24.9 ± 1.9	210.5 ± 22.6	3.5 ± 0.2	5.3 ± 0.8	67
6o	rat	0.32	9.9 ± 0.8	108.3 ± 12.5	2.3 ± 0.2	19.6 ± 4.2	41
	dog	0.10	18.2 ± 3.1	214.9 ± 63.0	4.8 ± 0.34	5.1 ± 1.0	62
	monkey	0.32	15.7 ± 3.8	143.0 ± 9.2	5.8 ± 0.3	9.2 ± 1.1	24
7e	rat	0.32	17.0 ± 2.7	72.2 ± 6.2	3.0 ± 0.90	17.1 ± 0.13	25
	dog	0.10	34.5 ± 3.8	384.6 ± 57.7	5.5 ± 0.4	3.2 ± 0.3	81
	monkey	0.32	35.1 ± 10.2	272.4 ± 90.0	4.7 ± 0.2	7.5 ± 1.4	38
9e	rat	0.32	3.3 ± 0.6	6.3 ± 2.9	1.7 ± 0.7	36.4 ± 0.6	5
	dog	0.1	2.5 ± 0.3	26.7 ± 1.0	6.7 ± 1.3	34.9 ± 2.5	54
	monkey	0.32	0	0	ND	52.0 ± 14.0	0

[a] The results are shown as the mean ± SE (n = 3). Cassette assay data except the dog PK data of **6o**. ND = not determined. [b] F = bioavailability.

Table 6. Inhibitory Effect of β_3 Agonists on Increase in IVP, Induced by Carbachol in Anesthetized Dogs[a]

	in vitro[b]		in vivo				
				id[b]			
	human β_3 EC_{50} (nM)	dog β_3 EC_{50} (nM)	iv (10 μg/kg),[c] inhibition (%)	ED_{50} (μg/kg)	EC_{50} (nM)	PB[d]	bladder-relaxation,[e] EC_{50} (nM)
6b	3.1 ± 0.1	4.9 ± 2.0	51	NT	NT	NT	NT
6e	0.43 ± 0.06	0.83 ± 0.08	58	NT	NT	NT	>50
6f	0.60 ± 0.08	2.9 ± 1.0	35	NT	NT	NT	NT
6h	0.60 ± 0.05	1.1 ± 0.05	>70	34.9 ± 17.5	7.1 ± 0.9	90	1.2 ± 0.2
6j	0.46 ± 0.08	0.88 ± 0.12	>70	24.7 ± 6.6	14.2 ± 1.4	92	2.6 ± 1.7
6o	0.32 ± 0.03	0.68 ± 0.05	>70	14.5 ± 6.6	8.2 ± 0.2	90	11.4 ± 4.2
7e	0.13 ± 0.005	1.4 ± 0.05	>70	16.2 ± 4.6	26.5 ± 0.8	83	62.0 ± 3.8
9e	0.029 ± 0.003	1.2 ± 0.2	>70	14.3 ± 5.2	1.2 ± 0.06	76	4.4 ± 1.2
2b	2.0 ± 0.06	2.9 ± 0.4	>70	25.9 ± 6.9	20.0 ± 4.0	86	6.8 ± 0.2
2j	0.60 ± 0.12	2.2 ± 0.1	NT	45.9 ± 26.9	18.7 ± 5.6	86	8.7 ± 0.1
2o	0.56 ± 0.10	1.2 ± 0.1	NT	47.0 ± 19.2	11.2 ± 3.9	NT	11.0 ± 0.5
3e	0.26 ± 0.02	1.3 ± 0.2	NT	65.0 ± 31.6	4.0 ± 0.5	89	14.1 ± 0.8
4j	0.066 ± 0.004	3.2 ± 0.4	NT	16.1 ± 9.5	1.8 ± 1.0	80	3.6 ± 0.1

[a] Details of experimental methods are in refs 9 and 11. NT = not tested. [b] The results are shown as the mean ± SE ($n \geq 3$). [c] The results are shown as the average of two experiments ($n = 2$). [d] PB = dog protein binding ($n = 2$). [e] The relaxing effect on the KCl-induced dog bladder strips ($n = 3$). See Experimental Section.

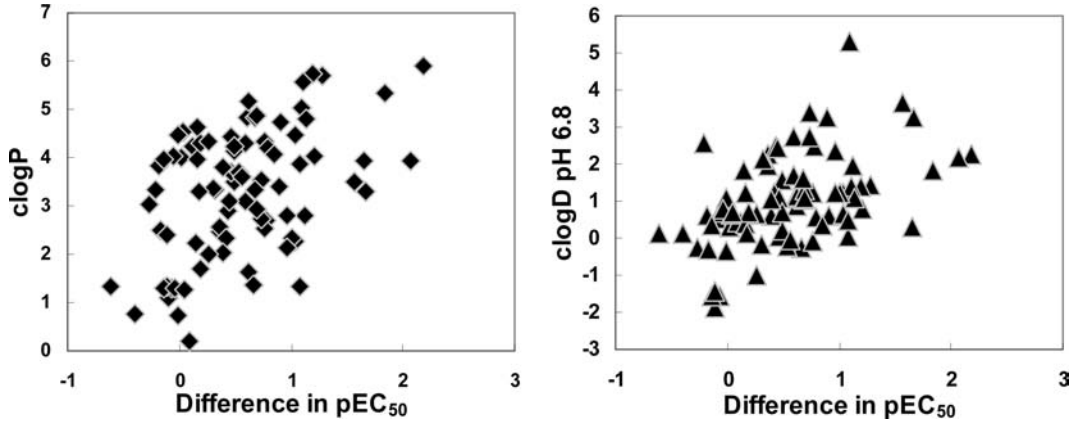


Figure 2. Plot of effect of target compounds on disparity index, showing pEC_{50}(cAMP accumulation on dogs) − pEC_{50}(bladder relaxation on dogs) vs lipophilicity: clogP and clogD, pH 6.8.

candidates that provide best-in-class properties in addition to an acceptable safety profile.

Experimental Section

Chemistry. General Methods. Reactions involving air- or moisture-sensitive reagents were carried out under a nitrogen atmosphere. If not specified, reactions were carried out at ambient temperature. Silica gel (Kanto Chemical, 63−210 μm) was used for chromatographic purification unless otherwise indicated. Anhydrous solvents were obtained from commercial sources. Proton NMR spectra were recorded on a Bruker BioSpin Avance 400 or DPX 200. Values in ppm relative to tetramethylsilane are given. The following abbreviations are used to describe peak patterns when appropriate: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra were recorded with Micromass LCT. Chemical purity was given by HPLC analysis with a Shiseido Capcell pack C18 column (detection at 254 nm), confirming >95% purity. Results of elemental analysis were recorded with Perkin-Elmer 2400II and were within 0.4% of the theoretical values calculated for C, H, and N unless otherwise noted.

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-3-methoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6a). Compound **6a** was synthesized from **10** according to procedure A. Anal. for $C_{25}H_{29}ClN_2O_5S$. ESI-MS (*m*/*z*): 469 $(M + H)^+$. ^1H NMR (200 MHz, DMSO-d_6) δ: 2.99−3.27 (6H, m), 3.36 (3H, s), 3.98 (3H, s), 4.95−5.06 (1H, m), 6.23 (1H, d, $J = 4.0$ Hz), 7.29−7.44 (9H, m), 7.65−7.8 (3H, m).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-3-isopropoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6b). Compound **6b** was synthesized from **10** according to procedure A. Anal. for $C_27H_{35}ClN_2O_5S$. ESI-MS (*m*/*z*): 497 $(M + H)^+$. NMR (200 MHz, DMSO-d_6) δ: 1.37 (6H, d, $J = 5.7$ Hz), 3.06−3.25 (6H, m), 3.38 (3H, s), 4.97−5.00 (2H, m), 6.23 (1H, broad s), 7.28−7.48 (9H, m), 7.72−7.79 (3H, m).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)-3-(pentyloxy)biphenyl-4-carboxamide Hydrochloride (6c). Compound **6c** was synthesized from **10** according to procedure A. Anal. for $C_{29}H_{37}ClN_2O_5S$. ESI-MS (*m*/*z*): 562 $(M + H)^+$. NMR (200 MHz, DMSO-d_6) δ: 0.91 (6H, d, $J = 5.4$ Hz), 1.25−1.58 (4H, m), 1.75−1.85 (2H, m), 3.0−3.35 (6H, m), 3.36 (3H, s), 4.24 (2H, t, $J = 5.4$ Hz), 4.95−5.03 (1H, m), 6.23 (1H, broad s), 7.3−7.5 (9H, m), 7.73−7.76 (3H, m).

3-(Cyclopentyloxy)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6d). Compound **6d** was synthesized from **10** according to procedure A. Anal. for $C_{29}H_{35}ClN_2O_5S \cdot 0.2H_2O$. ESI-MS (*m*/*z*): 560 $(M + H)^+$. NMR (200 MHz, DMSO-d_6) δ: 1.6−2.1 (8H, m), 1.75−1.85 (2H, m), 3.0−3.35 (6H, m), 3.37 (3H, s), 4.97−5.01 (1H, m), 5.22 (1H, m), 6.21 (1H, broad s), 7.3−7.5 (9H, m), 7.72−7.74 (3H, m).

3-(Cyclohexyloxy)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide hydrochloride (6e). Typical Procedure A. (1) To a solution of *tert*-butyl [2-(4-bromophenyl)ethyl][(2*R*)-2-hydroxy-2-phenylethyl]carbamate (550 mg) in 1,2-dimethoxyethane (10 mL) was added 2-cyclohexyloxy-*N*-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-

Table 7. Demonstrating the Effect on Potency of Changing *i*-Pro to *c*-Hex on RHS[a]

compd		h-β_3 (pEC_{50})		d-β_3 (pEC_{50})		d-mag (pEC_{50})[b]		pEC_{50} (*c*-Hex) − pEC_{50}(*i*-Pro)		
i-Pro →	*c*-Hex	*i*-Pro	*c*-Hex	*i*-Pro	*c*-Hex	*i*-Pro	*c*-Hex	h-β_3	d-β_3	d-mag
3b	**3e**	8.8	9.6	8.0	8.9	8.1	8.3	+0.8	+0.9	+0.2
6h	**6i**	9.2	9.9	9.0	9.2	8.9	7.9	+0.7	+0.2	−1.0
8h	**8i**	9.7	10.2	8.6	9.0	8.9	8.9	+0.5	+0.4	0
9b	**9e**	9.9	10.5	8.0	8.9	8.1	8.4	+0.6	+0.9	+0.3

[a] Set of compounds illustrated in Figure 3. [b] The relaxing effect on the KCl-induced dog bladder strips.

Table 8. Demonstrating the Effect on Potency of Changing Aryl Moiety on LHS[a]

		h-β_3		d-β_3		d-mag[d]		
Ar	no. of pairs	mean[b]	SD[c]	mean[b]	SD[c]	mean[b]	SD[c]	mean change in clogP[e]
3-ClPh	3	+0.13	0.18	+0.25	0.19	−0.91	0.51	0.7
3-Py	8	+0.31	0.18	−0.39	0.18	+0.08	0.21	−1.5
3-(6-NH_2Py)	4	+1.13	0.17	−0.31	0.13	+0.63	0.36	−1.8

[a] Set of compounds illustrated in Figure 3. [b] The mean value of (pEC_{50} for Ar-B) − (pEC_{50} for Ph-B). [c] Standard deviation. [d] The relaxing effect on the KCl-induced dog bladder strips. [e] The mean of the distribution of values of (clogP for Ar-B) − (clogP for Ph-B).

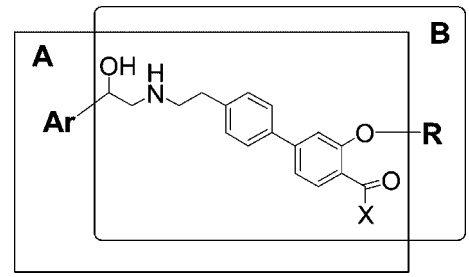


Figure 3. Matched molecular pairs for substitution on biphenyl analogues in Tables 7 and 8.

benzamide (275 mg), tetrakis(triphenylphosphine)palladium (55 mg), and aqueous solution of sodium carbonate (2 M, 2.0 mL), and the mixture was stirred at 80 °C for 6 h under nitrogen. The mixture was diluted with ethyl acetate, washed with 1 N aqueous hydrochloride solution, water, and brine, dried over magnesium sulfate, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 2/1) to give *tert*-butyl [(2*R*)-2-hydroxy-2-phenylethyl][2-(3′-(3-cyclohexyloxy)-4′-{[(methylsulfonyl)amino]carbonyl}-4-biphenylyl)ethyl]carbamate (380 mg).

(2) To a solution of the product (89 mg) in AcOEt (2 mL) was added hydrochloric acid in AcOEt solution (4 N, 4 mL) at room temperature, and the mixture was stirred at the same temperature for 2 h. The resultant solid was collected by filtration and dried to give 3-(cycloheyloxy)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)-4-biphenylcarboxamide hydrochloride (76 mg). Anal. for $C_{30}H_{37}ClS$. ESI-MS (*m*/*z*): 537$(M + H)^+$. NMR (200 MHz, DMSO-d_6) δ: 1.32−1.81 (8H, m), 1.89−2.02 (2H, m), 2.98−3.30 (6H, m), 3.38 (3H, s), 4.75−4.87 (1H, m), 4.98−5.03 (1H, m), 6.23 (1H, d, J = 3.8 Hz), 7.31−7.42 (9H, m), 7.71−7.80 (3H, m).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)-3-phenoxybiphenyl-4-carboxamide Hydrochloride (6f). Compound **6f** was synthesized from **10** according to procedure A. Anal. for $C_{30}H_{31}ClN_2O_5S\cdot 3H_2O$. ESI-MS (*m*/*z*): 531 $(M + 1)^+$. NMR (200 MHz, DMSO-d_6) δ: 3.0−3.3 (6H, m), 3.33 (3H, s), 4.82 (1H, m), 5.02 (1H, m), 6.20 (1H, m), 7.1−7.5 (12H, m), 7.5−7.9 (5H, m).

3-(Cycloheptyloxy)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6g). Compound **6g** was synthesized from **10** according to procedure A. Anal. for $C_{31}H_{39}ClN_2O_5S\cdot 0.2H_2O$. ESI-MS (*m*/*z*): 588 $(M + 1)^+$. NMR (200 MHz, DMSO-d_6) δ: 1.4−1.84 (10H, m), 2.02−2.12 (2H, m), 2.98−3.30 (6H, m), 3.38 (3H, s), 4.75−4.87 (1H, m), 4.98−5.03 (1H, m), 6.21 (1H, d, J = 3.8 Hz), 7.33−7.43 (9H, m), 7.71−7.80 (3H, m).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-3-(isopropylamino)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (6h). Compound **6h** was synthesized from **10** according to procedure A. Anal. for $C_{27}H_{35}Cl_2N_3O_4S\cdot H_2O$. ESI-MS (*m*/*z*): 494.2 $(M - H)^-$. ^{1}H NMR (200 MHz,) δ: 1.24 (6H, d, J = 6.2 Hz), 2.98−3.32 (6H, m), 3.37 (3H, s), 3.89−3.98 (1H, m), 5.51 (1H, d, J = −192.6 Hz), 6.86 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 7.3−7.42 (7H, m), 7.7 (2H, d, J = 8.1 Hz), 7.83 (1H, d, J = 8.4 Hz), 8.94 (1H, b.s), 9.35 (1H, b s).

3-(Cyclohexylamino)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (6i). Compound **6i** was synthesized from **10** according to procedure A. Anal. for $C_{30}H_{39}Cl_2N_3O_4S\cdot 4H_2O$. ESI-MS (*m*/*z*): 536.2 $(M + H)^+$. ^{1}H NMR (200 MHz,) δ: 1.14−1.66 (8H, m), 1.91−1.99 (2H, m), 3.08−3.37 (6H, m), 3.37 (3H, s), 3.55−3.72 (1H, m), 5 (1H, d, J = 7.6 Hz), 6.83 (1H, d, J = 8.2 Hz), 6.97 (1H, s), 7.32−7.42 (7H, m), 7.68 (2H, d, J = 8 Hz), 7.82 (1H, d, J = 8.4 Hz), 8.93 (1H, b s), 9.29 (1H, b s).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-3-isobutyl-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6j). Compound **6j** was synthesized from **10** according to procedure A. Anal. for $C_{28}H_{35}ClN_2O_4S$. ESI-MS (*m*/*z*):493 $(M - H)^-$. NMR (400 MHz, DMSO-d_6) δ: 0.87 (6H, d, J = 6.5 Hz), 1.82−1.86 (1H, m), 2.73 (2H, d, J = 7.0 Hz), 3.02−3.08 (3H, m), 3.19−3.23 (3H, m), 3.36 (3H,s), 4.95−5.00 (1H, m), 6.22 (1H, br), 7.32−7.41 (7H, m), 7.53−7.61 (3H, m), 7.70 (2H, d, J = 8.0 Hz), 8.83 (1H, br), 9.12 (1H, br).

3-Cyclopentyl-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6k). Compound **6k** was synthesized from **10** according to procedure A. Anal. for $C_29H_{35}ClN_2O_4S\cdot 0.2H_2O$. ESI-MS (*m*/*z*):505 $(M - H)^-$. NMR (200 MHz, DMSO-d_6) δ: 1.65−1.81 (6H, m), 1.99−2.05 (2H, m), 3.04−3.33 (7H, m), 3.38 (3H, s), 4.95−5.00 (1H, m), 6.22 (1H, d, J = 3.8 Hz), 7.31−7.42 (7H, m), 7.46−7.58 (2H, m), 7.67 (1H, d, J = 7.3 Hz), 7.69 (2H, d, J = 8.1 Hz), 8.86 (1H, br), 9.10 (1H, br), 12.21 (1H, br).

3-Cyclohexyl-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6l). Compound **6l** was synthesized from **10** according to procedure A. Anal. for $C_{30}H_{37}ClN_2O_4S$. ESI-MS (*m*/*z*): 519 $(M - H)^-$. NMR (200 MHz, DMSO-d_6) δ: 1.28−1.84 (10H, m), 2.84−2.95 (2H, m), 3.04−3.24 (6H, m), 3.38 (3H, S), 4.95−5.00 (1H, m), 6.23 (1H, d, J = 3.8 Hz), 7.31−7.40 (7H, m), 7.42−7.71 (5H, m), 8.86 (1H, br), 9.10 (1H, br), 12.2 (1H, br).

3-(Cyclohexylmethyl)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}-ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6m). Compound **6m**was synthesized from **10** according to procedure A. Anal. for $C_{31}H_{39}ClN_2O_4S\cdot 0.5H_2O$. ESI-MS (*m*/*z*):533 $(M - H)^-$. NMR (200 MHz, DMSO-d_6) δ: 0.91−1.17 (5H, m),

1.53−1.72 (6H, m), 2.74 (2H, d, J = 6.3 Hz), 3.05−3.31 (6H, m), 3.37 (3H, s), 4.93−5.00 (1H, m), 6.22 (1H, d, J = 3.8 Hz), 7.28−7.42 (7H, m), 7.51−7.61 (3H, m), 7.69 (2H, d, J = 8.1 Hz), 8.90 (1H, br), 9.22 (1H, br).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)-3-(propylsulfanyl)biphenyl-4-carboxamide Hydrochloride (6n). Compound **6n** was synthesized from **10** according to procedure A. Anal. for $C_{27}H_{33}ClN_2O_4S_2$. ESI-MS (m/z):511 (M − H)$^-$. NMR (200 MHz, DMSO-d_6) δ: 1.25 (3H, t, J = 7.3 Hz), 1.57−1.68 (2H, m), 3.03 (2H, t, J = 7.2 Hz), 3−3.34 (6H, m), 3.37 (3H, s), 4.96−5.01 (1H, m), 6.23 (1H, d, J = 3.8 Hz), 7.3−7.42 (7H, m), 7.54 (1H, d, J = 8.1 Hz), 7.63−7.67 (2H, m), 7.73 (2H, d, J = 8.2 Hz).

4′-(2-{[(2*R*)-2-Hydroxy-2-phenylethyl]amino}ethyl)-3-(isopropylsulfanyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6o). Compound **6o** was synthesized from **10** according to procedure A. Anal. for $C_{27}H_{33}ClN_2O_4S_2$. ESI-MS (m/z): 511 (M − H)$^-$. NMR (200 MHz, DMSO-d_6) δ: 1.26 (6H, d, J = 6.6 Hz), 3.00−3.30 (6H, m), 3.65 (1H, m), 4.95−5.00 (1H, m), 6.22 (1H, d, J = 3.7 Hz), 7.30−7.42 (7H, m), 7.61 (2H, s), 7.70−7.74 (3H, m).

3-(Cyclohexylsulfanyl)-4′-(2-{[(2*R*)-2-hydroxy-2-phenylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Hydrochloride (6p). Compound **6p** was synthesized from **10** according to procedure A. Anal. for $C_{30}H_{37}ClN_2O_4S_2$. ESI-MS (m/z): 551 (M − H)$^-$. NMR (200 MHz, DMSO-d_6) δ: 1.14−1.99 (10H, m), 3.04−3.42 (6H, m), 3.36 (3H, s), 4.94−4.99 (1H, m), 6.22 (1H, d, J = 3.8 Hz), 7.28−7.42 (7H, m), 7.6 (2H, s), 7.69 (1H, s), 7.73 (2H, s), 8.85 (1H, b s), 9.04 (1H, b s), 12.19 (1H, b s).

4′-(2-{[(2*R*)-2-Hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-3-isopropoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (7b). Typical Procedure B. (1) Suzuki coupling was performed with **11** instead of **10** in the same procedure as procedure A.

(2) To a solution of the product (100 mg) in 1,4-dioxane (2 mL) was added hydrochloric acid in 1,4-dioxane solution (4 N, 4 mL) at room temperature, and the mixture was stirred at the same temperature for 2 h. The solution was evaporated under reduced pressure. The resultant solid was washed with ether and collected to give 4′-(2-{[(2*R*)-2-hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-3-isopropoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide dihydrochloride (92 mg). Anal. for $C_{26}H_{33}Cl_2N_3O_5S\cdot1.5H_2O$. ESI-MS ($m/z$): 498 (M + H)$^+$. NMR (200 MHz, DMSO-$d_6$) δ: 1.30 (6H, d, J = 6.0 Hz), 2.9−3.4 (6H, m), 3.48 (3H, s), 4.6−5.3 (2H, m), 7.2−8.0 (8H, m), 8.4−8.9 (3H, m).

3-(Cyclohexyloxy)-4′-(2-{[(2*R*)-2-hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (7e). Compound **7e** was synthesized from **11** according to procedure B. Anal. for $C_{29}H_{37}Cl_2N_3O_5S\cdot1.3H_2O$. ESI-MS ($m/z$): 538 (M + 1)$^+$. NMR (200 MHz, DMSO-$d_6$) δ: 1.2−2.1 (10H, m), 3.0−3.6 (5H, m), 4.81 (1H, m), 5.20 (1H, m), 7.2−7.4 (4H, m), 7.7−7.7.9 (3H, m), 7.9−8.0 (1H, m), 8.46 (1H, m), 8.82−8.89 (2H, m).

4′-(2-{[(2*R*)-2-Hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-3-(isopropylamino)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Trihydrochloride (7h). Compound 7 h was synthesized from **11** according to procedure B. Anal. for $C_{26}H_{35}Cl_3N_4O_4S\cdot2.1H_2O$. ESI-MS ($m/z$): 495.2 (M − H)$^-$. NMR (200 MHz, DMSO-$d_6$) δ: 1.24 (6H, d, J = 3.1 Hz), 3.07−3.11 (2H, m), 3.18−3.31 (3H, m), 3.37 (3H, s), 3.37−3.43 (1H, m), 3.9−3.93 (1H, m), 5.35 (1H, dd, J = 1.5, 4.4 Hz), 6.86 (1H, d, J = 4.2 Hz), 6.97 (1H, s), 7.38 (2H, d, J = 4.1 Hz), 7.7 (2H, d, J = 4.1 Hz), 7.83 (1H, d, J = 4.2 Hz), 8.08 (1H, dd, J = 2.8, 4 Hz), 8.6 (1H, d, J = 4 Hz), 8.89 (1H, d, J = 2.8 Hz), 8.95 (1H, s), 9.34 (1H, br), 9.44 (1H, br).

3-(Cyclohexylamino)-4′-(2-{[(2*R*)-2-hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Trihydrochloride (7i). Compound **7i** was synthesized from **11** according to procedure B. Anal. for $C_{29}H_{39}Cl_3N_4O_4S\cdot1.8H_2O$. ESI-MS ($m/z$): 535.2 (M − H)$^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 1.61−1.67 (8H, m), 1.91−1.99 (2H, m), 2.99−3.48 (6H, m), 3.55−3.72 (1H, m), 6.84 (1H, d, J = 8.4 Hz), 6.97 (1H, s), 7.38 (2H, d, J = 8.2 Hz), 7.69 (2H, d, J = 8.1 Hz), 7.82 (1H, d, J = 8.4 Hz), 8.08 (1H, dd, J = 5.7, 8.1 Hz), 8.61 (1H, d, J = 8.2 Hz), 8.89 (1H, d, J = 5.5 Hz), 8.95 (1H, s), 9.34 (2H, b s).

4′-(2-{[(2*R*)-2-Hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-3-isobutyl-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (7j). Compound **7j** was synthesized from **11** according to procedure B. Anal. for $C_{27}H_{35}Cl_2N_3O_4S\cdot2H_2O$. ESI-MS ($m/z$): 494 (M − H)$^-$. NMR (200 MHz, DMSO-$d_6$) δ: 0.87 (6H, d, J = 6.5 Hz), 1.77−1.87 (1H, m), 2.74 (2H,d, J = 7.0 Hz), 3.06−3.37 (6H, m), 3.57 (3H, s), 5.22−5.25 (1H, m), 7.37−7.73 (8H, m), 7.82−7.89 (1H, m), 8.31−8.35 (1H, m), 8.77 (1H, d,J = 4.3 Hz), 8.83 (1H, s), 9.14 (1H, br), 9.26 (1H, br), 12.2 (1H, br).

3-Cyclopentyl-4′-(2-{[(2*R*)-2-hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (7k). Compound **7k** was synthesized from **11** according to procedure B. Anal. for $C_{28}H_{35}Cl_2N_3O_4S_2\cdot2H_2O$. ESI-MS ($m/z$): 507 (M − H)$^-$. NMR (200 MHz, DMSO-$d_6$) δ: 1.65−1.81 (6H, m), 1.99−2.05 (2H, m), 3.06−3.36 (7H, m), 3.40 (3H, s), 5.24−5.28 (1H,m), 7.37−7.71 (8H, m), 7.88−7.95 (1H, m), 8.38−8.42 (1H, m), 8.80 (1H, d, J = 4.3 Hz), 8.86 (1H, s), 9.20 (1H, br), 9.33 (1H, br), 12.2 (1H, br).

4′-(2-{[(2*R*)-2-Hydroxy-2-pyridin-3-ylethyl]amino}ethyl)-3-(isopropylsulfanyl)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (7o). Compound **7o** was synthesized from **11** according to procedure B. Anal. for Anal. for $C_{26}H_{33}Cl_2N_3O_4S_2\cdot1.8H_2O$. ESI-MS ($m/z$): 514 (M + H)$^+$. NMR (200 MHz, DMSO-$d_6$) δ: 1.26 (6H, d, J = 6.6 Hz), 2.99−3.75 (10H, m), 4.93−5.08 (1H, m), 6.33 (1H, bs), 7.36−7.45 (4H, m), 7.58 (1H, s), 7.69 (3H, d, J = 7.5 Hz), 8.01 (1H, d, J = 8.4 Hz), 8.55 (1H, dd, J = 1.5, 4.8 Hz), 8.62 (1H, d, J = 1.7 Hz).

4′-(2-{[(2*R*)-2-(4-Aminophenyl)-2-hydroxyethyl]amino}ethyl)-3-isopropoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (8b). Compound **8b** was synthesized from **12** according to procedure C. Anal. for $C_{27}H_{35}Cl_2N_3O_5S\cdot1.2H_2O$. ESI-MS ($m/z$): 512 (M + H)$^+$. NMR (200 MHz, DMSO-$d_6$) δ: 1.31 (6H, d, J = 6.0 Hz), 3.0−3.3 (6H, m), 3.34 (3H, s), 5.02 (1H, m), 7.1−7.5 (8H, m), 7.6−7.9 (3H, m), 8.9 (1H, m), 9.2 (1H, m).

4′-(2-{[(2*R*)-2-(4-Aminophenyl)-2-hydroxyethyl]amino}ethyl)-3-(cyclohexyloxy)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (8e). Typical Procedure C. (1) *tert*-Butyl [2-[3′-(cyclohexyloxy)-4′-[[(methylsulfonyl)amino]carbonyl]-4-biphenylyl]-ethyl][(2*R*)-2-hydroxy-2-(4-nitrophenyl)ethyl]carbamate was obtained from **12** in a method similar to procedure A.

(2) A mixture of the product (281 mg), iron powder (69.1 mg), ammonium chloride (11 mg), ethanol (4.2 mL), and water (1.4 mL) was refluxed for 1 h. After the mixture was cooled to room temperature, the insoluble solid was filtered off through a Celite pad and washed with ethyl acetate (20 mL). The filtrate was washed with brine (20 mL) and dried over magnesium sulfate. Filtration followed by evaporation gave a yellow foam (271 mg) which was chromatographed on silica gel (eluent, hexane/ethyl acetate) to give *tert*-butyl [(2*R*)-2-(4-aminophenyl)-2-hydroxyethyl][2-[3′-(cyclohexyloxy)-4′-[[(methylsulfonyl)amino]carbonyl]-4-biphenylyl]ethyl]carbamate (104 mg) as a pale-yellow solid.

(3) To a solution of the product (81.8 mg) in dioxane (1 mL) was added 4 N hydrogen chloride in ethyl acetate (1 mL), and the mixture was stirred at room temperature for 3 h. The precipitates were collected by filtration, washed with ethyl acetate, and dried under reduced pressure to give 4′-(2-{[(2*R*)-2-(4-aminophenyl)-2-hydroxyethyl]amino}ethyl)-3-(cyclohexyloxy)-*N*-(methylsulfonyl)-biphenyl-4-carboxamide dihydrochloride (64.5 mg) as an off-white solid. Anal. for $C_{30}H_{39}Cl_2N_3O_5S\cdot1.4H_2O$. ESI-MS ($m/z$): 550 (M − H)$^-$. NMR (400 MHz, DMSO-$d_6$,) δ: 1.31−1.64 (6H, m), 1.69−1.77 (2H, m), 1.92−1.98 (2H, m), 3.01−3.26 (6H, m), 3.39 (3H, s), 4.79−4.84 (1H, m), 4.99 (1H, dd, J = 2.2, 10.3 Hz), 7.25 (2H, d, J = 7.7 Hz), 7.35−7.45 (6H, m), 7.74 (2H, d, J = 8.1 Hz), 7.78 (1H, d, J = 8.1 Hz), 8.91 (1H, br), 9.25 (1H, br), 9.74 (1H, br), 11.2 (1H, br).

4′-(2-{[(2*R*)-2-(6-Aminopyridin-3-yl)-2-hydroxyethyl]amino}ethyl)-3-ethoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide (9q). Typical Procedure D. (1) A mixture of *tert*-butyl [(2*R*)-2-hydroxy-2-phenylethyl][2-(4-iodophenoxy)ethyl]carbamate (300 mg), 2-ethoxy-*N*-(methylsulfonyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide (174 mg), [1,1′-bis(diphenylphosphino)ferrocene]-

dichloropalladium(II) complex with dichloromethane (1:1, 62 mg), 1,1′-bis(diphenylphosphino)ferrocene (42.1 mg), *N*,*N*-dimethylformamide (6 mL), and 2 N sodium carbonate solution (1.01 mL) was stirred at 80 °C for 3 h. After cooling to room temperature, the mixture was quenched by the addition of pH 6.86 buffer (30 mL) and extracted with ethyl acetate (20 mL × 1, 10 mL × 1). The extracts were combined and washed with pH 6.86 buffer (20 mL × 2) and brine (20 mL) and dried over magnesium sulfate. Filtration followed by evaporation gave a light-brown solid which was chromatographed on silica gel (eluent, hexane/ethyl acetate) to give *tert*-butyl [(2*R*)-2-[6-(acetylamino)-3-pyridinyl]-2-[[*tert*-butyl(dimethyl)silyl]oxy]ethyl][2-[3′-ethoxy-4′-[[(methylsulfonyl)amino]carbonyl]-4-biphenylyl]ethyl]carbamate (259 mg) as a white solid.

(2) To a solution of the product (237 mg) in ethanol (2.4 mL) was added 1 N sodium hydroxide (3.14 mL), and the mixture was refluxed for 18 h. After cooling to room temperature, the mixture was quenched by the addition of 1 N hydrochloric acid (3.14 mL) and the solvent was removed by evaporation.

(3) To the residue were added 4 N hydrogen chloride in dioxane (4 mL) and methanol (1 mL), and the mixture was stirred at room temperature for 15 h. The solvent was concentrated in vacuo, and the residue was dissolved in water (5 mL) and treated with activated carbon. After stirring for 2 h, the mixture was filtered and the pH of the filtrate was adjusted to 7 by the addition of 1 N NaOH. The precipitates were collected by filtration, washed with water, and dried under reduced pressure at 50 °C to give 4′-[2-[[(2*R*)-2-(6-amino-3-pyridinyl)-2-hydroxyethyl]amino]ethyl]-3-ethoxy-*N*-(methylsulfonyl)-4-biphenylcarboxamide (123 mg) as an off-white solid. Anal. for $C_{25}H_{30}N_4O_5S \cdot 2.8H_2O$. ESI-MS (*m*/*z*): 497 $(M - H)^-$. NMR (400 MHz, DMSO-d_6) δ: 1.27 (3H, t, $J = 7.0$ Hz), 2.88−3.14 (9H, m), 4.15 (2H, q, $J = 7.0$ Hz), 4.67 (1H, t, $J = 6.6$ Hz), 5.73 (1H, br), 5.92 (2H, br), 6.43 (1H, d, $J = 8.4$ Hz), 7.18−7.20 (2H, m), 7.32 (2H, d, $J = 8.4$ Hz), 7.37 (1H, dd, $J = 2.2$, 8.4 Hz), 7.46 (1H, d, $J = 8.4$ Hz), 7.65 (2H, d, $J = 8.4$ Hz), 7.88 (1H, d, $J = 2.2$ Hz).

4′-(2-{[(2*R*)-2-(6-Aminopyridin-3-yl)-2-hydroxyethyl]amino}ethyl)-3-isopropoxy-*N*-(methylsulfonyl)biphenyl-4-carboxamide (9b). Compound **9b** was synthesized from **13** according to procedure D. Anal. for $C_{26}H_{32}N_4O_5S \cdot 2.7H_2O$. ESI-MS (*m*/*z*): 511 $(M - H)^-$. NMR (400 MHz, DMSO-d_6) δ: 1.27 (6H, d, $J = 6.2$ Hz), 2.88−2.99 (4H, m), 2.98 (3H, s), 3.06−3.13 (2H, m), 4.63−4.73 (1H, m), 5.71 (1H, br), 5.91 (2H, brs), 6.43 (1H, d, $J = 8.4$ Hz), 7.18−7.22 (2H, m), 7.32 (2H, d, $J = 8.4$ Hz), 7.37 (1H, dd, $J = 2.6$, 8.4 Hz), 7.47 (1H, d, $J = 7.7$ Hz), 7.63 (2H, d, $J = 8.4$ Hz), 7.88 (1H, d, $J = 2.6$ Hz), 8.02 (2H, br).

4′-(2-{[(2*R*)-2-(6-Aminopyridin-3-yl)-2-hydroxyethyl]amino}ethyl)-3-(cyclohexyloxy)-*N*-(methylsulfonyl)biphenyl-4-carboxamide Dihydrochloride (9e). Compound **9e** was synthesized from **13** according to procedure D. Anal. for $C_{29}H_{38}Cl_2N_4O_5S \cdot 3H_2O$. ESI-MS (*m*/*z*): 551 $(M - H)^-$. NMR (400 MHz, DMSO-d_6) δ: 1.27−1.37 (3H, m), 1.43−1.58 (3H, m), 1.70−1.79 (2H, m), 1.82−1.91 (2H, m), 2.86−2.97 (2H, m), 2.99 (3H, s), 3.00−3.17 (4H, m), 4.47−4.53 (1H, m), 4.65−4.68 (1H, m), 5.73 (1H, br), 5.92 (2H, brs), 6.44 (1H, d, $J = 8.4$ Hz), 7.18−7.21 (2H, m), 7.32 (2H, d, $J = 8.4$ Hz), 7.37 (1H, dd, $J = 2.2$, 8.4 Hz), 7.46 (1H, d, $J = 8.4$ Hz), 7.63 (2H, d, $J = 8.4$ Hz), 7.88 (1H, d, $J = 2.2$ Hz), 8.21 (2H, br).

4′-[2-[[(2*R*)-2-(6-Amino-3-pyridinyl)-2-hydroxyethyl]amino]ethyl]-3-(isopropylthio)-*N*-(methylsulfonyl)-4-biphenylcarboxamide (9o). Compound **9o** was synthesized from **13** according to procedure D. Anal. for $C_{26}H_{32}N_4O_4S_2$. ESI-MS (*m*/*z*): 527 $(M - H)^-$. NMR (400 MHz, DMSO-d_6) δ: 1.26 (6H, d, $J = 6.6$ Hz), 2.96−3.24 (9H, m), 3.64 (1H, heptuplet, $J = 6.6$ Hz), 4.77 (1H, m), 5.97 (1H, d, $J = 3.7$ Hz), 6.03 (2H, s), 6.47 (1H, d, $J = 8.4$ Hz), 7.36−7.42 (3H, m), 7.48 (1H, d, $J = 8.4$ Hz), 7.61 (1H, s), 7.66−7.70 (3H, m), 7.90 (1H, d, $J = 2.2$ Hz), 8.74 (2H, br).

Relaxation Response in a KCl Induced Tonic Concentration in Dog Detrusor Muscle Strips. Female beagle dogs were anesthetized with pentobarbital. The urinary bladder was collected, and top, trigon, and mucosa were removed. Strips of the detrusor of about 2 mm width and 8 mm length were prepared. Detrusor strips were suspended in orgon baths containing 25 mL of oxygenated Krebs−Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2mM KH_2PO_4, 25 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11.1 mM glucose) at 37 °C. The tension of the strips was measured isometrically with a force displacement transducer coupled to a carrier amplifier. Resting tension was adjusted to 0.5 g.

KCl (20 nM) was added at about 30 min intervals. After confirmation of reproducibility of the response, test compound was added 15 min prior to addition of KCl. The protocol was noncumulative with rinse cycles between each concentration of the test compounds. Test compound was added several times, increasing from the lowest concentration. Contractile responses were expressed as a percentage of the contraction before addition of the test compound.

The compound was dissolved in DMSO (10^{-2} M), then diluted with distilled water and added at final concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M. Vehicle was added at corresponding concentrations. Volumes added were all 25 μL.

Percentage inhibition was expressed as the mean ± SE. EC_{50} values were calculated by linear regression analysis, if possible. Statistical analysis was performed using analysis of variance, based on randomized block design, followed by Dunnutt's multiple comparisons.

In Vitro Metabolism. Incubation mixtures (0.5 mL) contained rat hepatocytes (2 × 106 cells/mL) and 100 mM phosphate buffer (pH 7.4). After preincubation at 37 °C for 5 min, the reaction was started by addition of **1** (substrate concentration of 10 μM). Incubations were carried out at 37 °C for 120 min, and the reactions were stopped by adding acetonitrile (0.5 mL). The mixtures were vortexed and centrifuged at 22000*g* for 5 min. The supernatant was removed and analyzed by LC/MS/MS.

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Supporting Information Available: Biological materials and methods and combustion analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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