Discovery of *N*-Isoxazolyl Biphenylsulfonamides as Potent Dual Angiotensin II and Endothelin A Receptor Antagonists

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The ET_A receptor antagonist (**2**) (*N*-(3,4-dimethyl-5-isoxazolyl)-4′-(2-oxazolyl)-[1,1′-biphenyl]-2-sulfonamide, BMS-193884) shares the same biphenyl core as a large number of AT_1 receptor antagonists, including irbesartan (**3**). Thus, it was hypothesized that merging the structural elements of **2** with those of the biphenyl AT_1 antagonists (e.g., irbesartan) would yield a compound with dual activity for both receptors. This strategy led to the design, synthesis, and discovery of (**15**) (4′-[(2-butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-2′-[(3,3-dimethyl-2-oxo-1-pyrrolidinyl)methyl]-[1,1′-biphenyl]-2-sulfonamide, BMS-248360) as a potent and orally active dual antagonist of both AT_1 and ET_A receptors. Compound **15** represents a new approach to treating hypertension.

Hypertension remains one of the largest unmet medical needs in the 21st century, especially when one considers that hypertension is the portent of future debilitating cardiovascular disease.[1] While many drugs are available for treating the disease, approximately one-third of the hypertensive population is still not adequately treated.[2] Of the more recent avenues explored for treating hypertension, disruption of the effects of either angiotensin II (AII) or endothelin-1 (ET-1) has shown promise. These endogenous vasoactive peptides are among the most potent vasoconstrictors and cell proliferative factors identified to date.[3] AII is the effector molecule of the renin−angiotensin system (RAS), and a large number of AII receptor (AT_1) antagonists, including irbesartan (**3**, Figure 1), have been developed for treating hypertension.[4] The endothelins exert diverse biological effects through two distinct G-protein-coupled receptors termed ET_A and ET_B.[5] A number of selective, as well as nonselective, ET receptor antagonists have been developed, and recent studies have shown that selective blockade of the ET_A receptor may be beneficial in the management of hypertension as well as heart failure.[6] ET_A receptor antagonists may also provide an alternative and effective treatment for hypertension, particularly in patients where RAS blockade has had little therapeutic effect.[7]

Studies in animals have demonstrated that simultaneous blockade of AT_1 and ET_A receptors produces a greater therapeutic benefit than antagonizing either AT_1 or ET_A receptors alone.[8] Losartan and SB-290670 (an ET_A/ET_B nonselective receptor antagonist) each decreased blood pressure, and the combination of losartan and SB-290670 produced an additive reduction in blood pressure compared to either drug alone.[9] In a canine model of renovascular hypertension, the combination of losartan and bosentan (a nonselective ET_A/ET_B receptor antagonist) produced a 40 mmHg reduction in mean blood pressure compared to a 20 mmHg decrease with losartan alone.[10] In a recent model of heart failure in SHR rats, the combination of an AT_1 receptor antagonist with an ET_A receptor antagonist essentially reversed the hypertension and hypertrophy seen in the model. Neither agent alone had an appreciable effect in this model.[11] Thus, concomitant blockade of AT_1 and ET_A receptors should be extremely useful in the treatment of hypertension and other cardiovascular diseases that result from hypertension (i.e., heart failure).

It should be noted that a moderately potent dual AT_1 and ET_A receptor antagonist (**1**, Figure 1) was reported by Walsh and co-workers at Merck in 1995.[12] However, oral activity in this series of compounds had not been demonstrated, and no further development or interest in this compound has been shown. A recent paper described molecular recognition similarities within the AT_1 and ET_A receptors,[13] lending additional motivation that other single combined antagonists could be discovered. In this report, we present a new class of antihypertensive agents: dual action receptor antagonists (DARAs). These agents are potent, *orally active* single-molecule antagonists of both AT_1 and ET_A receptors, which represent a potentially highly effective approach for the treatment of hypertension and the cardiovascular sequelae that follow.

We previously described a series of biphenylsulfonamides as potent, selective, and orally active ET_A receptor antagonists, exemplified by **2** (Figure 1).[14−17] Examination of the core biphenyl framework of these compounds revealed that this class of ET_A antagonists had a structural semblance to the biphenyltetrazole core that predominates the field of AT_1 receptor antagonists, including irbesartan (**3**). Our "road map" (Figure 2) for

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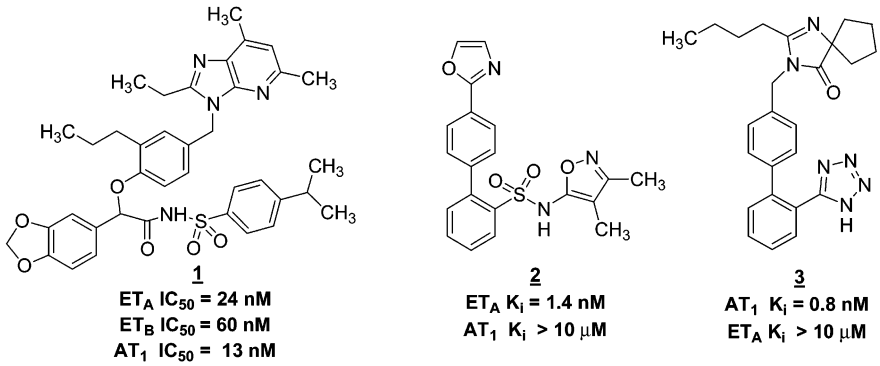


Figure 1.

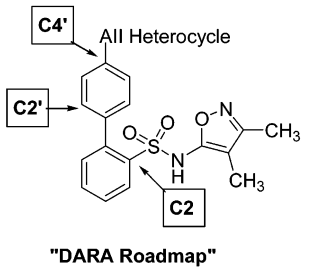


Figure 2. DARA roadmap.

using the template of **2** as an advanced scaffold for the preparation of a DARA required the following conditions.

(a) The isoxazole sulfonamide located at C2 of the biphenyl core (Figure 2) was crucial to ET_A receptor activity,[14] and in any biphenyl DARA, it would be a requirement. The Merck dual ET_A/AT_1 antagonist **1** (Figure 1) relied on an acylsulfonamide for dual receptor antagonism, suggesting that an acylsulfonamide moiety may be an effective carboxylic acid bioisostere for both AT_1 and ET_A receptors.

(b) In all AT_1 receptor antagonists, a hydrogen bond donating heterocycle is present at C4′ of the biphenyl core. The irbesartan imidazolinone "AII-heterocycle" (**7**, Scheme 1) was larger than any of the C4′ substituents typically placed on the biphenyl core of our ET_A receptor antagonists. The ET_A receptor would need to be able to tolerate larger groups at C4′ of the biphenyl scaffold to accommodate those heterocycles that would confer potent AT_1 receptor affinity.

(c) SAR generated in our efforts to find potent ET_A receptor antagonists suggested that substitution at the C2′ position of the biphenyl core of an ET_A antagonist often led to an increase in ET_A receptor affinity. This tack would likely be needed to optimize potency for a DARA.

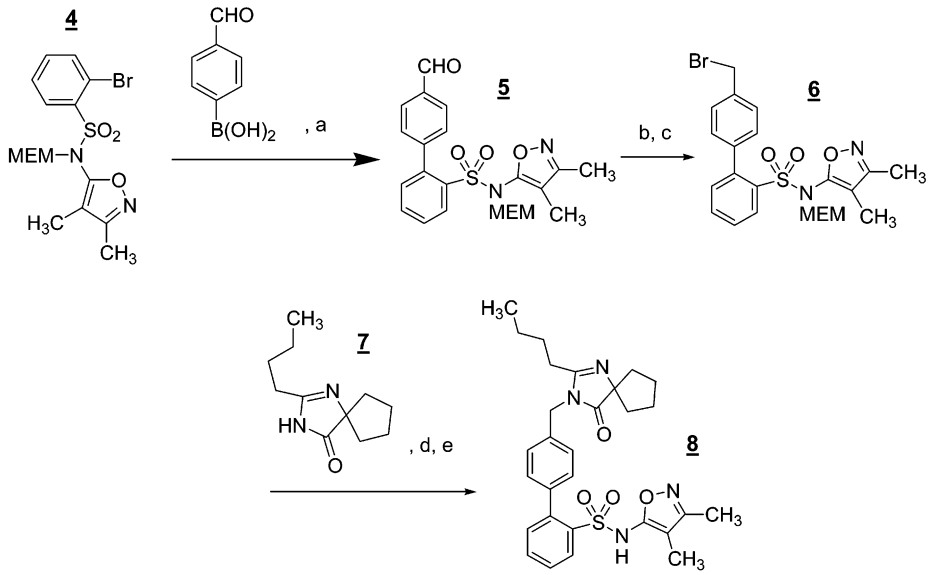
Results and Discussion

Chemistry. Synthesis of the biphenylsulfonamide (**8**), which merged the structures of irbesartan (AT_1 receptor antagonist) and **2** (ET_A receptor antagonist), is shown in Scheme 1. Suzuki coupling of 4-formylphenylboronic acid with the MEM-protected 2-bromobenzenesulfonamide derivative **4**[15] afforded the biphenylaldehyde **5**. Reduction of **5** and conversion of the resulting hydroxyl group to a bromide afforded the bromomethylbiphenyl derivative **6**. Displacement of the bromide in **6** with the anion of the imidazolinone **7**, followed by MEM deprotection, afforded our desired biphenyl DARA target **8** in an overall yield of 20% from **4**.

Previous ET_A receptor antagonist SAR[15,17] suggested that certain C2′ biphenyl substituents could significantly improve ET_A receptor potency. One of the more potent C2′ groups was the (3,3-dimethyl-2-oxopyrrolidino)methyl moiety, which was incorporated into compound **15**. The synthesis of this derivative (**15**) is shown in Scheme 2. The benzyl bromide derivative **10** was obtained from **9**[22] in a three-step sequence: (1) reduction of the nitrile group using DIBAL-H; (2) reduction of the resulting aldehyde to hydroxymethyl using sodium borohydride; (3) conversion of the hydroxymethyl group to the bromomethyl group via treatment with triphenylphosphine and carbon tetrabromide. The overall yield for this three-step sequence was 51%. Reaction of benzyl bromide **10** with the anion of imidazolinone **7**, followed by treatment with aqueous 1 N HCl, provided the key aldehyde **11**. Suzuki coupling of boronic acid **4** with imidazolinone **11** in the presence of catalytic palladium tetrakistriphenylphosphine, followed by the removal of the MEM group using 6 N HCl in refluxing EtOH, afforded **12**, the penultimate intermediate (60% from **10**). Reductive amination of the aldehyde in **12** using 4-amino-2,2-dimethylbutanoic acid hydrochloride **13**[23] and sodium triacetoxyborohydride, followed by cyclization of the resulting crude amino ester (**14**) using diisopropylcarbodiimide in methylene chloride, provided the target compound **15** in 31% yield from **12** (9% overall yield from **9**). Amorphous **15** was crystallized from EtOAc/hexane to provide material from which an X-ray crystal structure was determined (Figure 3). It appears that the sulfonamide −NH− intermolecularly hydrogen-bonds to the pyrrolidinone amide carbonyl via a water molecule in the crystalline lattice.

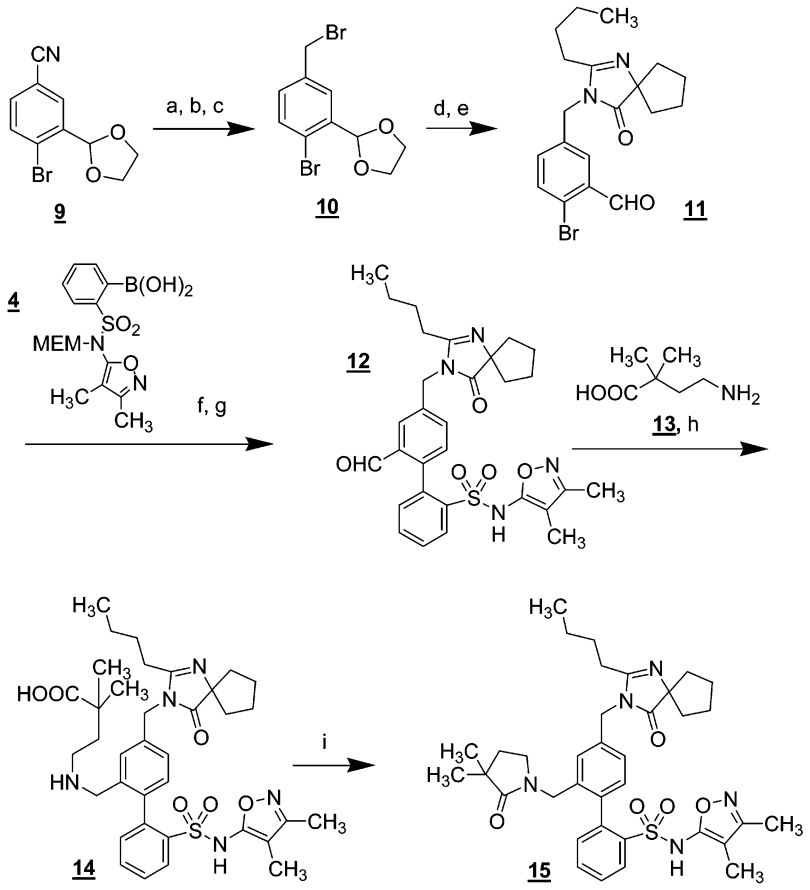
In Vitro Results. Data from radioligand binding studies performed with irbesartan, **2**, **8**, and **15** are summarized in Table 1. The biphenylsulfonamide **8**, which started with the ET_A receptor antagonist core of **2**, was converted to a potent AT_1 receptor ligand via the incorporation of the imidazolinone of irbesartan. Compound **8** was only approximately 6-fold less potent than irbesartan (Table 1).[18] Unfortunately, its ET_A receptor affinity was diminished compared to **2** (approximately 40-fold less potent than **2**, Table 1). AT_1 receptor antagonist SAR clearly suggested the necessity of the imidazolinone heterocycle at C4′ of the biphenyl in **8** in order to achieve potent AT_1 receptor affinity. We were pleased to observe that the acidic isoxazole sulfonamide in **8** clearly substituted for the tetrazole in irbesartan.

Scheme 1[a]



[a] (a) $(Ph_3P)_4Pd$, aqueous Na_2CO_3, EtOH/toluene (80%); (b) $NaBH_4$, MeOH; (c) CBr_4, Ph_3P, DMF (80%, two steps); (d) compound **7**, NaH, DMF; (e) 6 N aqueous HCl/EtOH (32% two steps).

Scheme 2[a]



[a] (a) DIBAL, toluene; (b) $NaBH_4$, MeOH; (c) $(Ph)_3P$, CBr_4, THF (51% from **9**); (d) compound **7**, NaH, DMF; (e) 1 N HCl; (f) compound **4**, $(Ph_3P)_4Pd$, aqueous Na_2CO_3, EtOH/toluene; (g) 6 N aqueous HCl/EtOH (60% from **10**); (h) **13**, sodium triacetoxy borohydride, AcOH, (i) diisopropylcarbodiimide, CH_2Cl_2 (31% from **12**).

This was a significant breakthrough for our efforts because it confirmed our approach toward dual receptor antagonists.

Following this result, compound **15** was prepared because our previous efforts in discovering ET_A receptor antagonists demonstrated that particular substituents located at C2′ of the biphenyl could be used to increase ET_A receptor affinity. In these studies, cyclic amides such as the pyrrolidinone in **15** were especially useful for improving ET_A receptor activity. Thus, modification of **8** to include a pyrrolidinone led to **15**. Compound **15** had potent affinity for *both* AT_1 (K_i = 10 nM) and ET_A

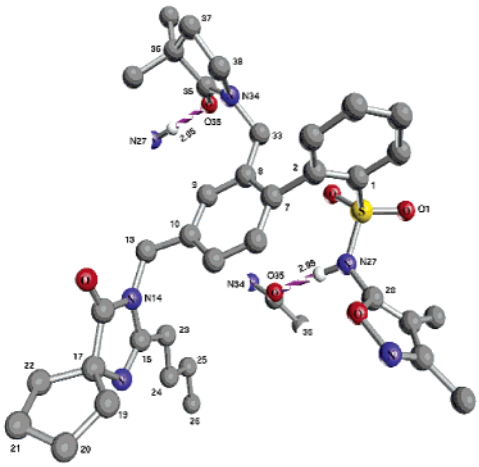


Figure 3. X-ray ORTEP of **15**.

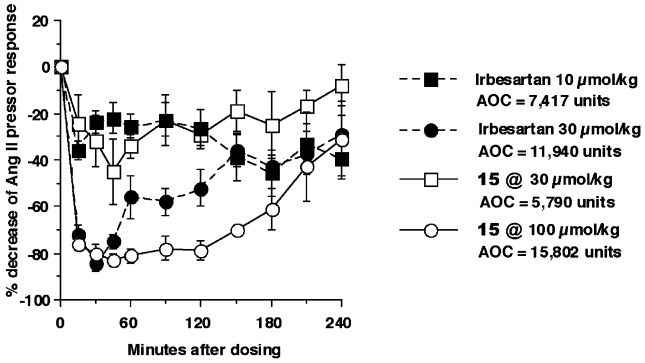


Figure 4. Effect of **15** (po, $n = 4$ per dose) and irbesartan (po, $n = 4$ per dose) on the Ang II pressor response up to 4 h after oral dosing. Area over curve (AOC) indicates potency and duration of action.

Table 1.

compound	AT_1 K_i (nM) rat	AT_1 K_i (nM) human	hET_A K_i (nM)
3 (Irbesartan)	0.8 ± 0.2	1.1 ± 0.3	$>10^4$
2	$>10^4$	$>10^4$	1.4 ± 0.2
8	4.7 ± 0.4	ND	39 ± 6
15	6.0 ± 1.0	10 ± 1.0	1.9 ± 0.4

($K_i = 1.9$ nM) receptors (Table 1). Thus, AT_1 receptor potency was maintained as we added the necessary structural features to enhance ET_A receptor affinity. The affinity of compound **15** for the rat AT_1 (6.0 nM) and rat ET_A receptor (1.9 nM, not shown) was similar to the affinity observed for the human receptors. Compound **15** showed no activity against AT_2 and ET_B receptor subtypes ($K_i > 10$ μM; data not shown).

In Vivo Evaluation. Since our ultimate goal was the discovery of an oral agent to effectively compete with the established AT_1 and ET_A receptor antagonists, our in vivo efforts were immediately focused on the oral activity of **15**.

In conscious normotensive rats, an intravenous injection of human AII causes a rapid and transient increase in mean blood pressure that is inhibited by orally administered AT_1 receptor antagonists (AII pressor). Irbesartan (**3**) given orally produced a dose-related sustained inhibition in the AII pressor test (Figure 4).[19] It should be noted that an ET_A receptor antagonist such as **2** was inactive in this test (data not shown). When "big"-ET-1 (bET-1) is given to normotensive rats, a sustained increase in mean blood pressure can be observed, and this increase in blood pressure can be blocked by orally administered ET_A receptor antagonists such as **2** (ET pressor, Figure 5).[20] It should be noted that irbesartan is inactive in this test (data not shown). While irbesartan was active only in blocking the pressor effect of angiotensin II and **2** was active only in blocking bET-1 pressor effect, **15** was uniquely effective as an inhibitor in both Ang II and bET-1 pressor studies when administered *orally* (Figures 4 and 5). These findings represent landmark results because they clearly demonstrated that dual antagonism of ET_A and AT_1 receptors has been achieved simultaneously upon oral delivery. AII and bET-1 pressor dose response studies (iv and po, Table 2) revealed that orally administered **15** was approximately 2-fold less potent as an AT_1 receptor antagonist compared with irbesartan and also 2-fold less potent as an ET_A receptor antagonist compared with **2**.

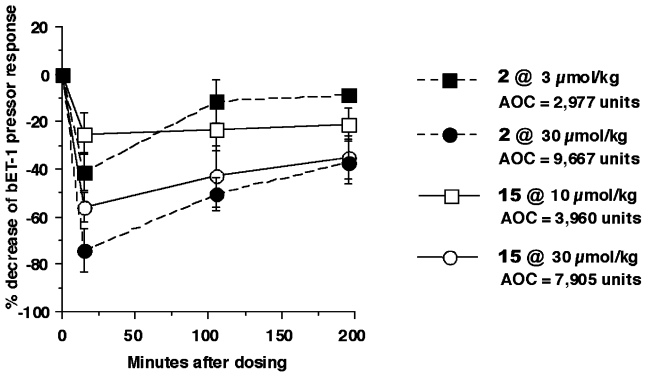


Figure 5. Effect of **15** (po) and **2** (po) on the bET-1 pressor response up to 3 h after oral dosing ($n = 4$ per dose). Area over curve (AOC) indicates potency and duration of action.

Table 2. Effect of Irbesartan, **2**, and **15** Dosed in the Ang II and bET-1 Pressor Test

study	irbesartan	**2**	**15**
Ang II pressor test (iv): ED_{50} (μmol/kg)	0.3	no effect	1.4
Ang II pressor test (po): ED_{50} (μmol/kg)	16	no effect	39
bET-1 pressor test (iv): ED_{50} (μmol/kg)	no effect	0.9	2.1
bET-1 pressor test (po): ED_{50} (μmol/kg)	no effect	10	26

Table 3. Pharmacokinetic Properties of **15** in Male Rats

parameter	iv (mean ± SD, $n = 6$)	po (mean ± SD, $n = 6$)
dose (μmol/kg)	10	10
$AUC_{0-\infty}$ (μM h)	50 ± 24	19 ± 6
Cl (mL min^{-1} kg^{-1})	3.8 ± 1.4	
V_{ss} (L kg^{-1})	1.4 ± 0.4	
$t_{1/2}$ (h)	5.5 ± 1.2	3.0 ± 0.5
MRT (h)	6.4 ± 1.6	5.0 ± 0.5
C_{max} (μM)		3.1 ± 1.4
T_{max} (h)		0.7 ± 0.4
bioavailability (%)		38

Concomitant with the demonstration of the dual pharmacodynamic activity of **15** after oral dosing, we also studied the pharmacokinetic profile of **15** in rats (Table 3). The DARA (**15**) was found to be orally bioavailable in rats (%F = 38) with excellent oral exposure ($C_{max} = 3.1$ μM) and reasonable elimination profile ($T_{1/2} = 5.5$ h). The systemic plasma clearance rate was slow (Cl = 3.8 mL min^{-1} kg^{-1}), and the steady-state volume of distribution was moderate (1.4 L kg^{-1}), indicating significant extravascular distribution. These

results clearly bode well for **15** as a potential oral human pharmaceutic.

In summary, compound **15** (BMS-248360) represents the first *orally active* DARA that blocks both AT_1 and ET_A receptors with potency comparable to the potency of both irbesartan (AT_1 receptor antagonist) and **2** (ET_A receptor antagonist). When dosed orally in normotensive rats, **15** is slightly less potent than irbesartan (as an AT_1 receptor antagonist) at blocking the hypertensive effects of intravenously administered AII. Compound **15** is also slightly less potent than **2** (as an ET_A receptor antagonist) at blocking the hypertensive effects of intravenously administered "big" ET. Nonetheless, **15** represents a new potential antihypertensive treatment that is capable of simultaneously blocking both AT_1 and ET_A receptors, two receptors that are independent antihypertensive targets. Given the reasonable pharmacokinetic profile of **15** in rats, the compound truly represents a potential revolutionary approach to treating hypertension.

Experimental Section

Melting points were recorded on a Thomas-Hoover capillary apparatus and are uncorrected. All chemical experiments were run under a positive pressure of argon. All solvents and reagents were used as obtained. Solutions were dried with magnesium sulfate unless otherwise noted. Proton NMR (^{1}H NMR) and carbon NMR (^{13}C NMR) spectra were recorded on JEOL FX-270 and GX-400 spectrometers with tetramethylsilane as an internal standard. Chromatography was performed under flash conditions using EM Science silica with 0.040−0.063 mm particle size. Analytical and preparative HPLC were performed on YMC columns (S-5, 120A ODS, 4.6 mm × 150 mm; S-10, 120A ODS, 30 mm × 500 mm) with MeOH/water gradients containing 0.1% trifluoroacetic acid.

***N*-(3,4-Dimethyl-5-isoxazolyl)-4′-formyl-*N*-methoxyethoxymethyl-[1,1′-biphenyl]-2-sulfonamide (5).** To a stirred solution of 2-bromo-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*′-(methoxyethoxymethyl)benzenesulfonamide **4** [15] (4.0 g, 9.54 mmol) and 4-formylphenylboronic acid (1.86 g, 12.4 mmol) in toluene (50 mL) and 95% EtOH (25 mL) under argon was added tetrakis(triphenylphosphine)palladium(0) (1.10 g, 0.95 mmol), followed by 2 M aqueous sodium carbonate (20 mL). The reaction mixture was heated at 85 °C for 2.5 h under argon, cooled, and diluted with EtOAc (100 mL). The organic liquid was separated and washed with H_2O and brine, then dried and concentrated. The residue was chromatographed on silica gel using 2:1 hexane/EtOAc to afford **5** (3.4 g, 80%) as a colorless gum: ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 3H), 2.15 (s, 3H), 3.31 (s, 3H), 3.45 (m, 2H), 3.68 (m, 2H), 4.34 (s, 2H), 7.21−8.01 (m, 8H), 10.09 (s, 1H).

***N*-(3,4-Dimethyl-5-isoxazolyl)-4′-bromomethyl-*N*-methoxyethoxymethyl-[1,1′-biphenyl]-2-sulfonamide (6).** To a stirred solution of **5** (3.4 g, 7.65 mmol) in MeOH (75 mL) was added $NaBH_4$ (347 mg, 9.18 mmol). The reaction mixture was stirred at room temperature for 2 h and was concentrated. The residue was diluted with EtOAc (200 mL), washed with H_2O and brine, and then dried and concentrated. The residue was dissolved in DMF (40 mL). Carbon tetrabromide (4.51 g, 12.24 mmol) was added, followed by triphenylphosphine (3.21 g, 12.24 mmol). The resulting mixture was stirred at 0 °C for 5 h, diluted with EtOAc, washed with H_2O and brine, and then dried and concentrated. The residue was chromatographed on silica gel using 3.5:1 hexane/EtOAc to afford **6** (3.12 g, 80%) as a colorless gum: ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 3H), 2.14 (s, 3H), 3.31 (s, 3H), 3.45 (m, 2H), 3.68 (m, 2H), 4.22 (s, 2H), 4.55 (s, 2H), 7.26−7.97 (m, 8H).

4′-[(2-Butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-[1,1′-biphenyl]-2-sulfonamide (8). NaH (56 mg, 60% in mineral oil,1.4 mmol) was added to a stirred solution of 2-*n*-butyl-1,3-diazaspiro[4.4]non-1-en-4-one (**7**)[21] (150 mg, 0.65 mmol) in DMF (0.5 mL) at 0 °C. The resulting mixture was stirred at room temperature for 20 min and was cooled to 0 °C. A solution of **6** (255 mg, 0.5 mmol) in 0.5 mL of DMF was then added, and the mixture was stirred at room temperature for 3 h. The mixture was then diluted with EtOAc, washed with H_2O and brine, and then dried and concentrated. The residue was chromatographed on silica gel using 3:4 hexane/EtOAc to afford 4′-[(2-butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-methoxyethoxymethyl-[1,1′-biphenyl]-2-sulfonamide (202 mg) as a gum.

This material was dissolved in 95% EtOH (10 mL), 6 N aqueous HCl (10 mL) was added, and the resulting solution was heated at reflux for 1 h. The reaction mixture was concentrated, and the pH of the solution was adjusted to pH 8 using a saturated solution of sodium bicarbonate. The reaction solution was then reacidified to pH 5 with glacial acetic acid, and the mixture was extracted with EtOAc. The combined organic extracts were washed with H_2O and brine, then dried and concentrated. The residue was purified by preparative HPLC on an ODS S10 column using 31% solvent A (10% MeOH, 90% H_2O, 0.1%TFA) and 69% solvent B (90% MeOH, 10% H_2O, 0.1%TFA) to provide **8** as a white solid (85 mg, 0.16 mmol, 32%, for two steps): mp 83−86 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, J = 7.0 Hz, 3H), 1.33 (m, 2H), 1.60 (m, 2H), 1.85−2.10 (m, 11H), 2.13 (s, 3H), 2.35 (m, 2H), 4.75 (s, 2H), 7.21−8.01 (m, 8H). Anal. ($C_{29}H_{34}N_4O_4S$) C, H, N, S.

2-[(2′-Bromo-5′-bromomethyl)phenyl)]-1,3-dioxolane (10). DIBAL-H (1.0 M solution in toluene, 445 mL, 445 mmol) was added over 30 min to a solution of 2-[(2′-bromo-5′-cyano)-phenyl)]-1,3-dioxolane **9**[22] (103 g, 404 mmol) in toluene (2.0 L) at −78 °C. The solution was allowed to warm to 0 °C. After 1 h, a solution of Rochelle's salt (125 g) in water (200 mL) was added, and the mixture was allowed to warm to room temperature and was then stirred vigorously for 16 h. The organic layer was separated and concentrated, and the residue was partitioned between ethyl acetate and 1 N aqueous hydrochloric acid. The organic layer was washed with a saturated solution of sodium bicarbonate, dried over sodium sulfate, and then concentrated to give 70.5 g of 2-[(2′-bromo-5′-formyl)-phenyl)]-1,3-dioxolane as a yellow solid, which was used without further purification.

Sodium borohydride (3.66 g, 96.7 mmol) was added to a solution of crude 2-[(2′-bromo-5′-formyl)phenyl)]-1,3-dioxolane (49.7 g, 193 mmol) in absolute ethanol (1300 mL) at 0 °C. After 2 h, a solution of 10% aqueous sodium dihydrogen phosphate (50 mL) was added, and the resulting mixture was stirred and allowed to warm to room temperature. The mixture was then concentrated and partitioned between ethyl acetate and saturated aqueous sodium bicarbonate. The organic layer was separated, dried, and concentrated to give 49.0 g of crude 2-[(2′-bromo-5′-hydroxymethyl)phenyl)]-1,3-dioxolane as a yellow oil, which was used without further purification.

To a stirred solution of this material in THF (500 mL) at 0 °C was added carbon tetrabromide (69.0 g, 208 mmol), followed by triphenylphosphine (52.7 g, 199 mmol), which was added in portions over 15 min. The mixture was stirred for 2 h, and then a saturated solution of sodium bicarbonate (20 mL) was added, and the mixture was allowed to warm to room temperature and was then concentrated. Ether (500 mL) was added, and the resulting mixture was filtered. The filtrate was dried over magnesium sulfate and concentrated. The residue was chromatographed on silica gel (8:1 hexanes/ethyl acetate as eluant) to give **10** as a white solid (31.1 g, 51% yield from 2-[(2′-bromo-5′-cyano)phenyl)]-1,3-dioxolane, **9**). ^{1}H NMR ($CDCl_3$) δ 4.1−4.2 (m, 4H), 4.45 (s, 2H), 6.06 (s, 1H), 7.26 (dd, J = 2, 8 Hz, 1H), 7.54 (d, J = 8 Hz, 1H), 7.62 (d, J = 2 Hz, 1H).

4-[(2-*n*-Butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-2-formylbromobenzene (11). Sodium hydride (60% dispersion in mineral oil, 9.65 g, 241 mmol, 2.5 equiv) was added in portions over 15 min to a mixture of 2-*n*-butyl-1,3-diazaspiro-[4.4]non-1-en-4-one hydrochloride (**7**) (18.7 g, 96.5 mmol, 1.0 equiv) in DMF (400 mL) at 0 °C. The mixture was stirred and allowed to warm to room temperature over 15 min. To this

mixture was added via cannula a solution of **10** (31.1 g, 96.5 mmol) in DMF (100 mL). After being stirred for14 h, the mixture was concentrated in vacuo and partitioned between ethyl acetate (500 mL) and 10% aqueous sodium dihydrogen phosphate (300 mL). The organic layer was dried over sodium sulfate and concentrated to give an orange oil (42.7 g), which was used without further purification.

A solution of this material (6.0 g) in THF (180 mL) and 1 N hydrochloric acid (30 mL) was heated at 65 °C for 1.5 h. The mixture was cooled and then partitioned between a saturated solution of sodium carbonate (75 mL) and ethyl acetate (200 mL). The organic layer was removed, dried over sodium sulfate, and concentrated to give **11** as a crude yellow oil (8.2 g). This material was used without further purification. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, $J = 7$ Hz, 3H), 1.32 (quintuplet, $J = 7$ Hz, 2H), 1.57 (quintuplet, $J = 7$ Hz, 2H), 1.8 (m, 2H), 1.9−2.0 (m, 6H), 2.28 (t, $J = 7$ Hz, 2H), 4.67 (s, 2H), 7.26 (dd, $J = 2$, 8 Hz, 1H), 7.64 (d, $J = 8$ Hz, 1H), 7.71 (d, $J = 2$ Hz, 1H), 10.34 (s, 1H).

4′-[(2-Butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-2′-formyl-*N*-(3,4-dimethyl-5-isoxazolyl)-[1,1′-biphenyl]-2-sulfonamide (12). To a stirred solution of compound **11** (1.53 g, 3.93 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.272 g, 0.24 mmol) in toluene (20 mL) under argon was added a 2 M aqueous solution of sodium carbonate (8.0 mL), followed by 2-borono-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*′-(methoxyethoxymethyl)benzenesulfonamide **4**[15] (1.81 g, 4.71 mmol) in 95% EtOH (10 mL). The resulting mixture was heated at reflux for 2 h, diluted with water (50 mL), and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed once with brine (50 mL), dried, and evaporated. The residue was chromatographed on silica gel using hexanes/EtOAc (2:1) to afford 4′-[(2-butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-2′-formyl-*N*-[(2-methoxyethoxy)methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-[1,1′-biphenyl]-2-sulfonamide (1.98 g, 77%) as a colorless gum.

To a solution of this compound (1.98 g, 3.04 mmol) in 95% ethanol (20 mL) was added 6 N aqueous hydrochloric acid (20 mL), and the resulting mixture was heated at reflux for 1 h. The mixture was then concentrated and diluted with water (10 mL). The solution was neutralized using a saturated solution of sodium bicarbonate and then was reacidified to pH 4 using glacial AcOH. The mixture was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were washed once with brine (50 mL), dried, and evaporated. Chromatography of the residue on 50 g of silica gel using hexanes/EtOAc (1:1) provided **12** (1.26 g, 73%) as a white solid. ^{1}H NMR ($CDCl_3$) δ 0.87 (t, $J = 7.2$ Hz, 3H), 1.35 (m, 2H), 1.59 (m, 2H), 1.82 (s, 3H), 1.96−2.37 (m, 8H), 2.14 (s, 3H), 2.35 (m, 2H), 4.78 (ABq, $J = 16.2$, 14.1 Hz, 2H), 7.36−8.04 (m, 7H), 9.66 (s, 1H).

4′-[(2-Butyl-4-oxo-1,3-diazaspiro[4.4]non-1-en-3-yl)methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-2′-[(3,3-dimethyl-2-oxo-1-pyrrolidinyl)methyl]-[1,1′-biphenyl]-2-sulfonamide (15). To a solution of **12** (110 mg, 0.20 mmol) in CH_2Cl_2 (4 mL) was added 4-amino-2,2-dimethylbutanoic acid hydrochloride (**13**)[23] (98 mg, 0.59 mmol) and 3 Å molecular sieves, followed by glacial acetic acid (35 mg, 0.59 mmol) and then sodium acetate (48 mg, 0.59 mmol). The resulting mixture was stirred at room temperature for 10 min, and then $NaB(AcO)_3H$ (124 mg, 0.59 mmol) was added. The reaction mixture was stirred for 2 h, diluted with EtOAc, and filtered. The filtrate was washed with H_2O and brine, then dried and concentrated to afford compound **14**, which was used directly without any further purification.

This material (**14**) was dissolved in CH_2Cl_2 (6 mL), and 1,3-diisopropylcarbodiimide (32 mg, 0.25 mmol) was added. The resulting reaction mixture was stirred at room temperature for 2 h and diluted with CH_2Cl_2, washed with H_2O and brine, and then dried and concentrated. The residue was purified by preparative HPLC to provide **15** as a white solid (40 mg, 31%): mp 104−110 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.0$ Hz, 3H), 1.08 (s, 3H), 1.14 (s, 3H), 1.36 (m, 2H), 1.61 (m, 2H), 1.75−2.06 (m, 13H), 2.17 (s, 3H), 2.39 (m, 2H), 4.18 (m, 2H), 4.71 (m, 2H), 7.02−7.93 (m, 7H); ^{13}CNMR ($CDCl_3$) δ 7.82, 11.91, 14.79, 23.36, 25.50, 25.61, 27.11, 28.81, 29.88, 35.33, 38.42, 41.48, 44.59, 46.24, 46.47, 109.29, 125.15, 125.76, 129.68, 130.58, 131.76, 133.20, 134.07, 137.15, 138.27, 139.11, 139.57, 155.81, 162.68, 162.91, 181.25, 187.83. Anal. ($C_{36}H_{45}N_5O_5S$) C, H, N, S.

Radioligand Binding Studies. a. Angiotensin II. Human AT_1 receptor affinity was determined using a standard binding assay as previously described.[18] Human recombinant AT_1 receptor membranes (cat. no. 6110533, BioSignal Inc.) with 0.8 pmol/mg membrane protein at a concentration of 1.1 mg/mL were diluted from 0.25 to 7.25 mL with buffer (50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, and 0.1% bovine serum albumin). Diluted membranes (150 μL) were incubated with 10 μL of radioligand (0.2 nM [^{125}I]-Sar-Ile-angiotensin II) and 10 μL of 0.1% DMSO vehicle in buffer, or with increasing concentrations of compound or 10 μM angiotensin II. The membranes were incubated for 60 min at 37 °C. Bound and free radioligands were separated and counted as described previously.[18] [^{125}I]-Sar-Ile-angiotensin II binding to rat aortic smooth muscle cells (RASMC) was performed similarly to the human AT1 binding assay. RASMCs were plated in 96-well plates (Costar) for 48 h in HEPES-buffered Dulbecco's modified Eagles medium (DMEM) containing 10% fetal calf serum to achieve 90% confluency. At the time of the assay, RASMCs were incubated with DMEM, 0.1% bovine serum albumin, and 0.01% sodium azide. Increasing concentrations of compound (50 μL) or 10 μM Ang II (50 μL) or 0.1% DMSO vehicle in buffer (50 μL), 0.2 nM [^{125}I]-Sar-Ile-angiotensin II (50 μL), and 100 μL of buffer made up the final 200 μL volume. Angiotensin II binding was performed at room temperature for 2 h on a shaker. The assay was stopped by withdrawing the assay mixture from the wells and quickly rinsing of the wells twice with 200 μL of cold PBS. Then 200 μL of 1% Triton X-100 solution containing 0.1% BSA in distilled water was added for 15 min at room temperature to solubilize the cells. After solubilization, 150 μL of the mixture was transferred to tubes and placed in a Cobra γ counter (Packard). IC_{50} values were determined by curve-fitting software (Sigma Plot).

b. Endothelin. CHO-K1 cells expressing the human endothelin A or endothelin B receptor were cultured in Ham's F12 media (Gibco/BRL, Grand Island, NY) with 10% fetal bovine serum (Hyclone), supplemented with 300 μg/mL Geneticin (G-418 Gibco, Grand Island, NY) and maintained at 37 °C with 5% CO_2 in a humidified incubator. Twenty-four hours prior to assay, the cells were treated with 0.25% trypsin-EDTA and were seeded in Falcon 96-well tissue culture plates at a density of 1.8×10^4 cells/ well. For the binding assay, culture media was aspirated from each well and the monolayers were washed with 50 μL of PBS (Mg^{2+}- and Ca^{2+}-free). The binding assay was performed in a total volume of 125 μL consisting of assay buffer (50 mM Tris, pH 7.4, including 1% BSA and 2 μM phosphoramidon) and 25 μL of either 500 nM ET-1 (to define nonspecific binding) or competing drug. The reaction was initiated with the addition of 25 μL of 0.25 nM [^{125}I]-ET-1 (NEN). Incubation was carried out with gentle orbital shaking at 4 °C for 4 h (equilibrium binding conditions). The reaction was terminated by aspiration of the reaction buffer and two subsequent washes with cold PBS (Mg^{2+}- and Ca^{2+}-free). The cells were dissociated by the addition of 100 μL of 0.5 N NaOH followed by incubation for 40 min. Samples were then transferred into tubes for counting in a Cobra γ counter (Packard). Data were analyzed with curve-fitting software (Sigma Plot).

In Vivo Rat Pressor Studies. a. Angiotensin II. Pressor studies were undertaken as described previously.[15] In conscious normotensive rats with in-dwelling carotid artery and jugular vein catheters, an intravenous (iv) injection of human Ang II causes a rapid and transient increase in mean blood pressure lasting 3 min (i.e., a pressor response) that is inhibited by AT_1 receptor antagonists. The first bolus iv injection of Ang II (100 ng/kg) served as the control response. Irbesartan (10 and 30 umol/kg) was given by oral gavage, and the rats were rechallenged with Ang II at various intervals up to 240 min. Irbesartan produced a dose-related sustained inhibition over 4 h in the Ang II pressor test. **15** (30 and 100

μmol/kg) dosed orally also significantly decreased the Ang II pressor response over 4 h. The difference between the maximum blood pressure increase before and after the drug was reported as the percent inhibition of the Ang II pressor effect.

b. Endothelin. This study was performed as previously described.[15] Intravenous injection of big endothelin-1 (bET-1, 1.0 nmol/kg) into conscious normotensive rats causes a gradual and sustained increase in blood pressure that is inhibited by ET_A receptor antagonists. The initial ET-1 challenge was preceded by vehicle administration to establish a control response to the agonist. Compounds **2** (3 and 30 μmol/kg) and **15** (10 and 30 μmol/kg) were administered after control ET-1 pressor response and at 15, 105, and 195 min intervals after drug dosing ET-1 was injected iv.

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