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Synthesis and biological evaluation of 4′-[(benzimidazole-1-yl)methyl]biphenyl-2-sulfonamide derivatives as dual angiotensin II/endothelin A receptor antagonists

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ABSTRACT

A series of 4′-[(benzimidazole-1-yl)methyl]biphenyl-2-sulfonamide derivatives (**Ia–Il**) were synthesized and biologically evaluated. It was found that **Ig**, the most active compound, antagonized both Ang II AT_1 and endothelin ET_A receptors (AT_1 IC_{50} = 8.5, ET_A IC_{50} = 8.9 nM), and was more potent than losartan in RHRs with no significant effect on heart rate. The preliminary structure–activity relationships were also discussed in the present paper.

1. Introduction

Antihypertensive drugs with high efficacy and low side-effects remain to be one of the largest unmet medical needs, especially when hypertension is considered to be the portent of the future debilitating cardiovascular disease.[1,2] Most of the currently used antihypertensive agents cannot be used as a single drug therapy because of their limited efficacy,[3] therefore, the development of drugs with multiple therapeutic effects is most desirable. It is well-known that angiotensin II subtype 1 (AT_1) receptor antagonists are clinically useful for the management of hypertension and heart failure,[4,5] and endothelin subtype A (ET_A) receptor antagonists show promising effects in the treatment of the similar indications.[6] Interestingly, some of these two types of antagonists have close similarity in structure. Hence, lots of attempt has been made to hybridize both AT_1 and ET_A receptor antagonistic properties in one molecule. It is anticipated that drugs with dual AT_1 and ET_A receptor antagonistic action could be more effective than the current standard therapies for the treatment of hypertension.

Indeed, L-746,072 (**1**) was first documented to possess an ability to antagonize both AT_1 and ET_A receptors, with IC_{50} values of 24 and 13 nM respectively in ET_A and AT_1 radioligand binding assays.[7] Shortly after, BMS-248360 (**2**)[1] and BMS-346567 (**3**)[8] (Fig. 1) were also proved to be potent dual-action receptor antagonists (DARAs). Compound BMS-248360 as a potential DARA, which had potent affinity for both AT_1 (K_i = 10 nM) and ET_A (K_i = 1.9 nM) receptors.

Additionally, a series of 4′-[(imidazol-1-yl)-methyl] biphenylsulfonamides have been reported to be such dual-action antagonists in the treatment of hypertension, heart failure, and other cardiovascular disorders.[6]

Previously, we designed and synthesized a series of derivatives of 2-alkylbenzimidazoles with *N*-phenyl pyrrolyl-2-tetrazole moiety. Among them, compound **4** is an orally active AT_1 receptor antagonist (IC_{50} = 9.8 nM) that is more potent and efficacious than losartan, which selectively inhibited Ang II-induced contractions of rabbit aortic strips in a competitive manner and had no effect on the contraction induced by norepinephrine (10 nM), KCl (10 nM), and histamine (10 nM).[9] It should be also noted that when we started to prepare our manuscript, the novel AT_1 receptor antagonist azilsartan medoxomil (TAK-491) with benzimidazole moiety has been approved by FDA in 2011.[10] Given that an acidic functionality such as *N*-isoxazolyl substituted sulfonamide is crucial for

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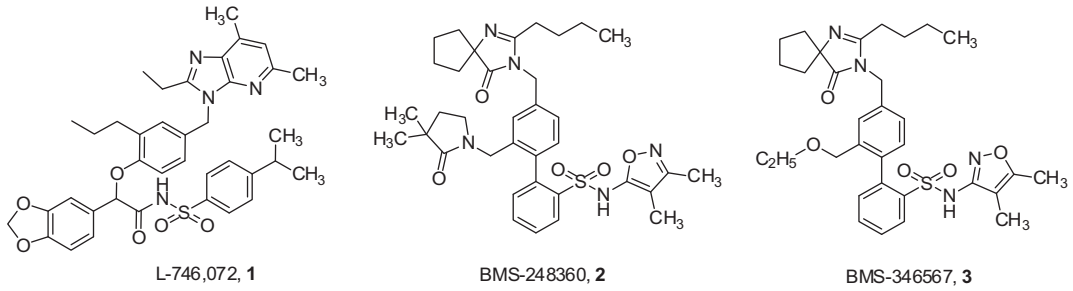


Figure 1. The structures of known DARAs.

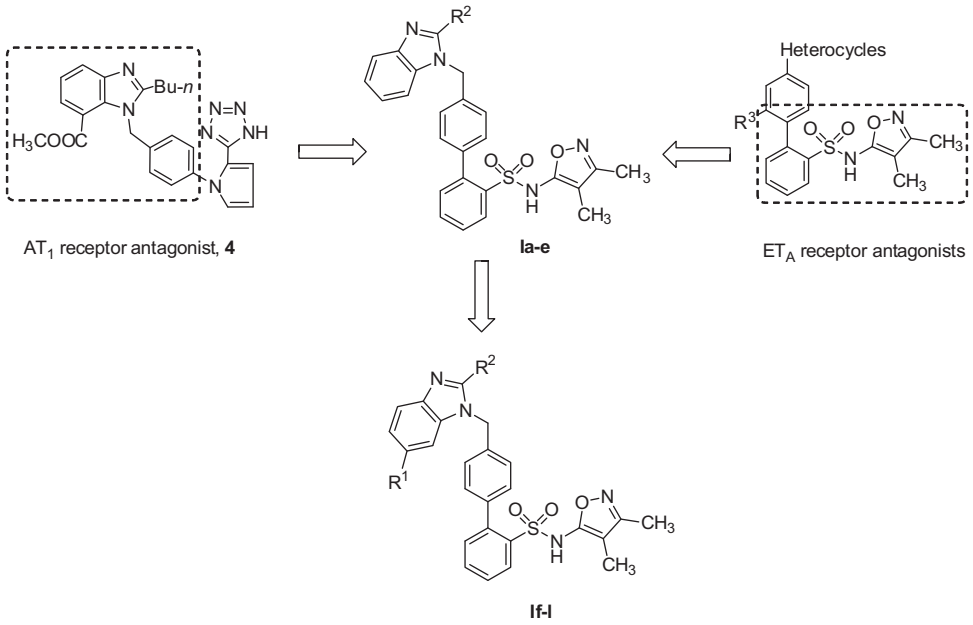


Figure 2. Strategy for the design and optimization of target DARAs.

DARAs **2** and **3**, and that modifications of the classical biphenyl tetrazole moiety in some AT_1 receptor antagonists did not drastically decrease the potency,[11–14] it may be rational to replace *N*-phenyl pyrrolyl-2-tetrazole moiety in **4** by biphenyl-2-[3,4-dimethyl-5-isoxazolyl]-sulfonamide of compound **2** in order to develop single agents with the ability to antagonize both AT_1 and ET_A receptors.

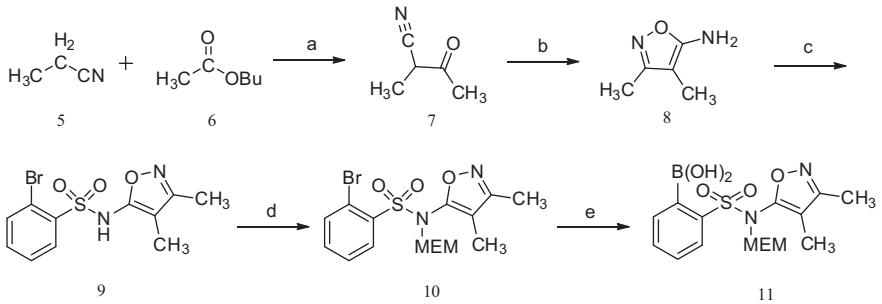
First, a series of 4′-[(benzimidazole-1-yl)methyl]biphenyl-2-sulfonamides derivatives (**Ia–Ie**) were synthesized. The pharmacological results suggested that compound **Ie**, with a butyl group at the 2-position of benzimidazole, was the most potent one in this series. Our efforts were followed by additional optimization and modification at the 6-position of benzimidazole of **Ie** to obtain the series of compounds **If–Il** (Fig. 2).

Herein, we report the synthesis and biological evaluation of 4′-[(benzimidazole-1-yl)-methyl]-biphenyl-2-sulfonamides derivatives (**Ia–Il**).

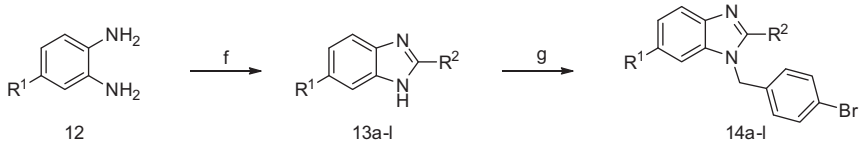
2. Results and discussions

2.1. Chemistry

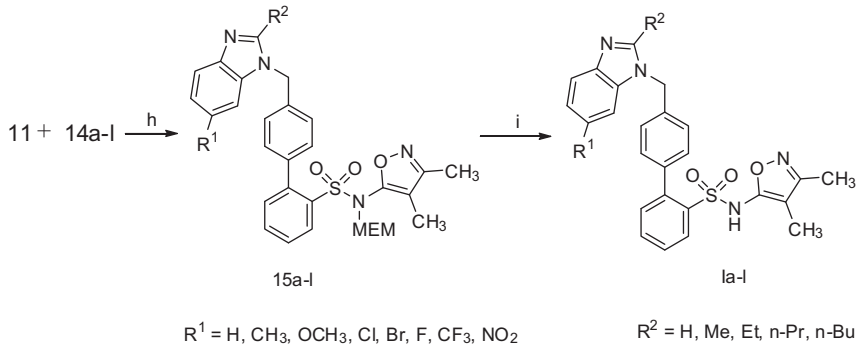
The synthetic route used to synthesize the title compounds is outlined in Schemes 1–3.



Scheme 1. Reagents and conditions: (a) NaOBu, Xylene, reflux, 3 h, 55–67%; (b) $NH_2OH{\cdot}HCl$, $NaOH/H_2O$, reflux, 3 h, 32.7%; (c) 4-bromobenzenesulfonyl chloride; Pyridine, rt, over night, 90%; (d) MEMCl, NaH, THF, 0 °C to rt, 5 h, 70.6%; (e) B(OBu-*n*)$_3$, *n*-BuLi, toluene/THF, −40 °C, 30 min, 38.6%.



Scheme 2. Reagents and conditions: (f) R^2COOH, 4 N HCl, reflux, 2 h, 45.0%; (g) 1-bromo-4-(bromomethyl)benzene, NaH, DMF, 0 °C to rt, 5 h, 64.0%.



Scheme 3. Reagents and conditions: (h) (a) $Pd(OAc)_2$, 2 N Na_2CO_3, Ph_3P, N_2, reflux, 1 h; (b) H_2O, rt, 3 h, 60.0%; (i) EtOH, 6 N HCl, reflux, 2 h, 64%.

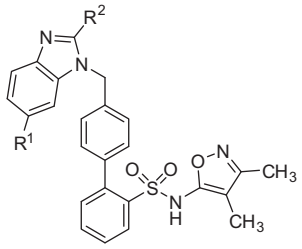
Ethyl cyanide (**5**) reacted with butyl acetate (**6**) in xylene to obtain 2-methyl-3-oxobutanenitrile (**7**). Treatment of **7** with oxammonium hydrochloride in sodium hydroxide solution afforded 3, 4-dimethylisoxazol-5-amine (**8**), which was then subjected to chlorosulfonation with 2-bromobenzene-1-sulfonyl chloride at room temperature to provide *N*-(3,4-dimethyl-5-isoxazolyl)-2-bromobenzene-sulfonamide (**9**). Further reaction with MEM chloride gave the protected benzenesulfonamide **(10)**. The key intermediate 2-borono-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-(methoxyethoxy- methyl)benzenesulfonamide **(11)** was prepared by treating **10** with tributyl borate and *n*-BuLi in THF at −40 °C. Cyclization of the 2-substituent o-phenylenediamines (**12**) with aliphatic carboxylic acids in aqueous 4 N HCl provided imidazolines (**13a–13l**), which were then alkylated with 1-bromo-4-(bromomethyl)benzene to provide benzimidazole derivatives (**14a–14l**). The important intermediates (**11** and **14a–14l**) in propanol were then treated with triphenyl phosphine and palladium(II) acetate under mild condition to give **15a–15l**, followed by MEM deprotection, to provide 4′-[(benzimidazole-1-yl)methyl]biphenyl-2-sulfonamides derivatives **Ia–Il** as target compounds.

2.2. Pharmacological evaluation

2.2.1. In vitro Ang II AT1 and endothelin ETA receptors antagonism

All target compounds were evaluated for Ang II AT_1 and endothelin ET_A receptors antagonism in vitro and the activity was expressed as IC_{50} values (Table 1). In Ang II AT_1 and endothelin ET_A binding assays, the unsubstituted benzimidazole derivative **Ia** was found to be the least active, which indicated that the optimization of benzimidazole is quite necessary. The activity of compounds **Ib–Ie** with alkyl substitution at the 2-position increased obviously with the enlargement of alkyl groups. IC_{50} values of **Ib–Ie** decreased from 45 to 9.9 nM in AT_1 receptor antagonism assays and from 75 to 9.8 nM in ET_A receptor antagonism evaluation respectively, which possibly arose from hydrophobic interactions between different alkyl groups of **Ib–Ie** with AT_1 and ET_A receptors. Especially, compound **Ie** possessing a *n*-butyl group clearly demonstrated the most powerful effect (AT_1 IC_{50} = 9.9 nM, ET_A IC_{50} = 9.8 nM), which was more powerful than AT_1 receptor antagonist reference drug (losartan) and comparable to ET_A receptor antagonist reference drug (bosentan).

Table 1
SAR of 4′-[(benzimidazole-1-yl)methyl]biphenyl-2-sulfonamide derivatives (**Ia–Il**)



Compound	R^1	R^2	AT_1 receptor IC_{50} (nM)	ET_A receptor IC_{50} (nM)
Ia	H	H	92 ± 8.6	99 ± 14
Ib	H	Me	45 ± 2.1	75 ± 6.1
Ic	H	Et	28 ± 1.5	39 ± 4.3
Id	H	*n*-Pr	16 ± 1.4	22 ± 0.9
Ie	H	*n*-Bu	9.9 ± 0.1	9.8 ± 0.3
If	Me	*n*-Bu	9.1 ± 0.2	9.3 ± 0.1
Ig	OMe	*n*-Bu	8.5 ± 0.2	8.9 ± 0.3
Ih	Cl	*n*-Bu	9.8 ± 0.3	9.6 ± 0.4
Ii	Br	*n*-Bu	10 ± 0.7	9.8 ± 0.2
Ij	F	*n*-Bu	10.5 ± 1.0	10 ± 0.9
Ik	CF_3	*n*-Bu	11 ± 0.9	10 ± 0.7
Il	NO_2	*n*-Bu	12 ± 0.6	11 ± 0.8
Losartan	/	/	95 ± 11	/
Bosentan	/	/	/	8.9 ± 0.5

On the basis of combined strong dual receptor blockade of compounds **Ia–Ie**, further optimization of our strategy was to introduce different functional groups at the 6-position of the benzimidazole moiety. Substitution of 6-H with $-CH_3$, $-OCH_3$, –Cl, –Br, –F, $-CF_3$ and $-NO_2$ groups obtained the corresponding compounds **If–Il**. Generally, the title compounds of this series did not bring dramatic changes in their antagonism. As shown in Table 1, **Ig** was found to exhibit slightly better activity (AT_1 IC_{50} = 8.5 nM, ET_A IC_{50} = 8.9 nM) than **Ie**.

Table 2
Effects on blood pressure in renal antihypertensive rats

Groups	No. of animals	Dose (mg/kg)	Parameter	Time of observation (min)				
				0 h	2 h	4 h	6 h	8 h
Control	10		SAP (mmHg)	185.50 ± 11.2	193.70 ± 5.0	190.22 ± 7.8	186.45 ± 10.1	184.24 ± 12.8
			⊿		8.20 ± 6.2	4.72 ± 3.4	0.95 ± 1.1	−1.26 ± 1.6
			DAP (mmHg)	145.50 ± 5.4	149.90 ± 7.0	147.12 ± 5.3	146.54 ± 9.2	145.61 ± 10.3
			⊿		4.40 ± 2.5	1.62 ± 2.2	1.04 ± 1.1	0.11 ± 2.3
			HR (BPM)	414.68 ± 10.7	416.90 ± 20.4	410.80 ± 19.4	415.78 ± 20.8	416.80 ± 22.5
			⊿		2.22 ± 10.5	−3.88 ± 15.1	1.10 ± 11.3	2.12 ± 20.6
Losartan	10	20	SAP (mmHg)	198.15 ± 11.2	191.74 ± 14.6	183.04 ± 16.6	180.02 ± 10.5	182.14 ± 11.7
			⊿		−6.41 ± 3.7*	−15.11 ± 6.5**	−18.13 ± 9.8**	−16.01 ± 9.8**
			DAP (mmHg)	155.14 ± 12.4	144.09 ± 10.1	134.99 ± 9.6	133.57 ± 8.1	141.06 ± 10.2
			⊿		−11.05 ± 2.7**	−20.15 ± 7.8***	−21.57 ± 7.8***	−14.08 ± 6.0**
			HR (BPM)	413.75 ± 15.1	407.25 ± 24.1	411.06 ± 16.1	412.80 ± 15.4	410.55 ± 10.2
			⊿		−6.50 ± 19.7	−2.69 ± 15.2	0.95 ± 17.1	−3.2 ± 20.0
Ig	10	20	SAP (mmHg)	192.88 ± 10.7	181.33 ± 10.6	168.92 ± 12.1	168.42 ± 10.5	172.03 ± 12.1
			⊿		−11.55 ± 5.9**	−23.96 ± 5.4***	−24.46 ± 3.1***	−20.85 ± 4.3***
			DAP (mmHg)	152.68 ± 12.5	138.62 ± 10.5	130.58 ± 8.4	127.66 ± 9.2	133.68 ± 9.1
			⊿		−14.06 ± 4.1**	−22.10 ± 6.5***	−25.02 ± 5.6***	−19.00 ± 7.4**
			HR (BPM)	412.40 ± 18.6	408.14 ± 14.9	407.28 ± 22.1	415.62 ± 27.5	417.97 ± 29.2
			⊿		−4.26 ± 9.7	−5.12 ± 10.0	3.22 ± 20.1	5.57 ± 20.8

Each value represents the mean ± SEM (n = **8**).
* Significance levels p <0.1.
* Significance levels p <0.05.
*** Significance levels p <0.01 as compared with the respective control. Changes of BP (HR) (Δ) = BP (HR) after administration−P (HR) before administration.

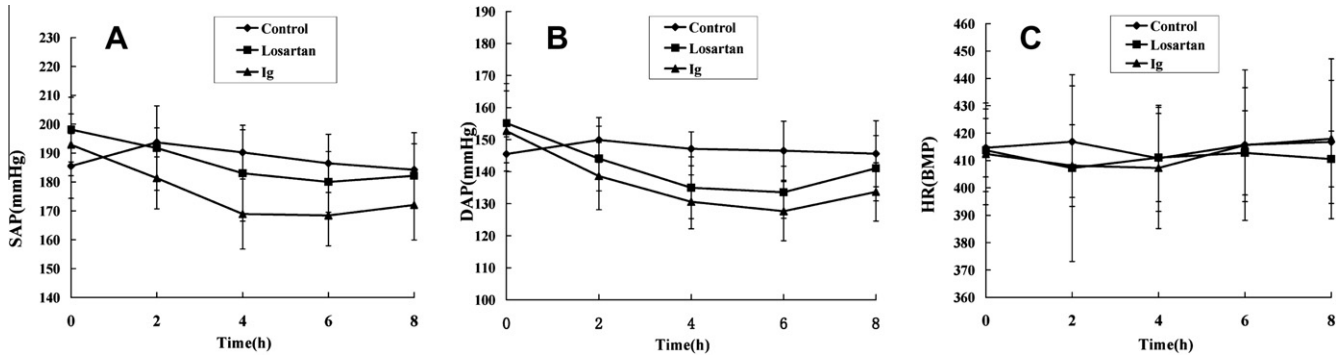


Figure 3. (**A**) The acute antihypertensive activities of compound **Ig** and losartan in RHRs (SAP, systolic arterial pressure); (**B**) The acute antihypertensive activities of compound **Ig** and losartan in RHRs (DAP, diastolic arterial pressure); (**C**) The changes of heart rate (HR) of compound **Ig** and losartan in RHRs.

2.2.2. In vivo antihypertensive activity of compound Ig

Biological evaluation in vitro demonstrated that compound **Ig** was the most potent DARA among the titled compounds. As an interesting new entity, compound **Ig** was selected for further evaluation of antihypertensive effects in vivo. Its influence on blood pressure (BP) was analyzed in renal antihypertensive rats (RHRs) under ethyl carbamate (EC) anesthesia (1.0 g kg^{-1} ip) compared to the reference drug losartan. After the intravenous administration at 20 mg kg^{-1}, **Ig** reduced the blood pressure significantly (Table 2). This trend continued throughout the remaining time of the study. The average SAP of the RHRs treated with **Ig** was reduced by almost 10–20 mmHg for about 8 h, which was obviously superior to the SAP reduction achieved by losartan (Fig. 3). Meanwhile, the maximum antihypertensive effect on DAP of compound **Ig** was prominently more effective than that of losartan. Heart rates recorded taken from RHRs proved that **Ig**, just the same as losartan, did not cause noticeable changes of HR. Preliminary biological evaluation in vivo showed that compound **Ig** was promising enough for further investigation.

3. Conclusions

In summary, by merging together the key structural element of AT_1 receptor antagonist **4** with the key structural moiety of biphenylsulfonamide ET_A receptor antagonist (**BMS-248360**), a series of 4′-[(benzimidazole-1-yl) methyl]biphenyl- 2-sulfonamides derivatives (**Ia–Ie**) were designed and synthesized. Compound **Ie** had an IC_{50} of 9.8 nM for functional antagonism of ET_A receptor, and displayed IC_{50} of 9.9 nM in AT_1 receptor antagonism assays.

To analyze the influence of electron-donating and electron-withdrawing groups on the aimed molecules, compounds **If–Il** were further designed and obtained. The results revealed that the derivatives of this series exhibited powerful antagonism on both AT_1 and ET_A receptors, better or equal than either losartan or bosentan respectively. The most prospective compound **Ig** was found to have the most effective antagonism for Ang II AT_1 and endothelin ET_A receptors, exhibiting more potent activity (AT_1 IC_{50} = 8.5 nM, ET_A IC_{50} = 8.9 nM) than losartan and equivalent activity to bosentan.

Preliminary SAR suggested that the activities of the compounds with alkyl substitution at the 2-position of benzimidazole increased obviously with the enlargement of the alkyl groups. Especially, compounds possessing the *n*-butyl group clearly demonstrated the most potent inhibitory effect. This suggests a hydrophobic pocket of receptor into which an aliphatic chain of the proper length, for example, *n*-butyl group, fits tightly.

Further substitution with electron-withdrawing groups (–Cl, –Br, –F, $–CF_3$, $–NO_2$) and electron-donating groups ($–CH_3$ and $–OCH_3$) at

the 6-position of benzimidazoles did not increased the activities notablely. Strongly electron-withdrawing ones (–F, $-CF_3$, $-NO_2$) reduced the antagonistic activity of both receptors slightly compared to the weekly electron-withdrawing ones (–Cl, –Br). On the contrary, the electron-donating groups ($-CH_3$ and $-OCH_3$) are favorable in improving the activity slightly, especially compound **Ig** bearing methoxyl moiety.

Biological evaluation in vivo suggested that **Ig** is more potent and efficacious than losartan in RHRs, with no significant impact on heart rate. These results suggested that further optimization of this series of derivatives could yield highly-potent, orally active dual receptor antagonists.

4. Experimental

4.1. Chemistry

Most chemicals and solvents were of analytical grade and, when necessary, were purified and dried by standard methods. Melting points were taken on an XT-4 micro melting point apparatus and uncorrected. IR spectra were recorded in KBr on a Nicolet Impact 410 grating infrared spectrophotometer (ν_{max} in cm^{-1}) and ^1H NMR spectra were recorded with 300 or 500 MHz.

Spectrometers in the indicated solvents (TMS as internal standard): the values of the chemical shifts are expressed in δ values (ppm) and the coupling constants (J) in Hz. High-resolution mass spectra were recorded using Agilent QTOF 6520.

Purity of all tested compounds was $\geqslant$95%, as estimated by HPLC analysis. The major peak of the compounds analyzed by HPLC accounted for $\geqslant$95% of the combined total peak area when monitored by a UV detector at 254 nm. Flash chromatography was done on Merck silica gel 60 (230–400 mesh).

4.1.1. 2-Methyl-3-oxobutanenitrile (7)

To the solution of butyl acetate (116.1 g, 1 mol) in 150 mL xylene, NaOBu (48.1 g, 0.5 mol) was added, followed by ethyl cyanide (27.5 g, 0.5 mol). The resulting mixture was heated to reflux for 3 h, and cooled to room temperature, adjusting pH to 2.5–3 with acid hydrochloric acid and adding 100 mL water. After partitioned, water layer was extracted with toluene (3 × 80 mL), and the combined organic extracts were washed with water, dried over $MgSO_4$ and concentrated under reduced pressure, followed by atmospheric distillation at 145–146 °C to afford 27 g compound **7** as a yellowish oil in yield of 55–67%.

4.1.2. 3,4-Dimethylisoxazol-5-amine (8)

To a mixture of 2-methyl-3-oxobutanenitrile (7) (9.7 g, 0.1 mol), hydroxylamine hydrochloride (7 g, 0.1 mol) and water (20 mL), a solution of NaOH in water (4 g, 10 mL) was added dropwise at 40 °C. Then the mixture was heated to reflux for 3 h, cooled and partitioned. The organic layer stood overnight and was filtrated to give compound **8** as a white crystal (3.66 g), 32.7%, mp 123–125 °C.

4.1.3. *N*-(3,4-Dimethyl-5-isoxazolyl)-2-bromobenzenesulfonamide (9)

3,4-Dimethylisoxazol-5-amine (1.32 g, 11.74 mmol) was added to a solution of 2-bromobenzene-1-sulfonyl chloride (3.0 g, 11.74 mmol) in pyridine and the mixture was stirred at room temperature overnight. The resulting mixture was first mixed with ice water (150 mL) and filtrated by vacuum. The pH of the filtrate was then adjusted to 2 with 6 N hydrochloric acid and then filtrated again. The residue was purified by flash column chromatography with *n*-hexane/ethyl acetate/acetic acid (200:200:1, v/v/v) as eluent to afford **9** as a white crystal in yield of 90%, mp 125–126 °C.

4.1.4. 2-Bromo-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-(methoxyethoxy-methyl)benzenesulfonamide (10)

Compound **9** (1.53 g, 0.046 mol) was dissolved in THF, then below 0 °C NaH (0.445 g, 60% in mineral oil, 0.011 mol) was added in batches to the solution of **9** and the reaction solution was stirred under nitrogen for 15 min. Then MEMCl (0.87 g, 0.056 mol) was added and stirred at room temperature for 5 h. The mixture was concentrated, diluted with water (25 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were concentrated and purified by flash column chromatography with *n*-hexane/ethyl acetate (3:1, v/v) to afford a white solid 1.37 g in yield of 70.6%, mp 63–65 °C.

4.1.5. 2-Borono-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-(methoxyethoxy-methyl)benzenesulfonamide (11)

At −40 °C and under nitrogen, *n*-BuLi (4.8 mL, 11.8 mmol) was added dropwise to the mixture of toluene (13 mL), THF (8 mL), tributyl borate (3.2 mL, 11.8 mmol) and compound **10** (3.30 g, 7.9 mmol). The reaction mixture was stirred for 30 min, then its temperature increased slowly to −20 °C. 2 N hydrochloric acid (8 mL) was added and the temperature was rised to room temperature. The water layer was basified to pH 7 with sodium hydroxide solution and extracted with THF (3 × 10 mL). The combined organic extracts were concentrated and purified by flash column chromatography with petroleum ether/EtOAc (1:3, v/v) to afford a red mucoid material in yield of 38.6%. This material was used without further purification.

4.1.6. General procedure for the preparation of 1*H*-benzimidazole (13a–13l)

An appropriately 2-substituent *o*-phenylenediamines (50 mmol) and derivatives of methanoic acid (60 mmol) were added to 4 N hydrochloric acid (60 mL). The resulting mixture was heated to reflux for 2 h and basified with ammoniae aqua. The mixture was then filtered and the filter cake was washed with water and recrystallized with 95% alcohol to provide **13a–13l** as white solids in yields of 60–75%.

4.1.7. General procedure for the preparation of 1-(4-bromobenzyl)-benzimidazole (14a–14l)

NaH (0.48 g, 60% in mineral oil, 12.0 mmol) was added in batches to a solution of 13 in DMF (10 mL) at 0 °C, and the mixture was stirred for 15 min. A solution of 1-bromo-4-(bromomethyl)benzene (1.0 g, 4.0 mmol) in DMF (5 mL) was then added and the mixture was stirred for 5 h at room temperature. The mixture was poured into cold saturated NaCl solution, filtered, and the filter cake was recrystallized with 95% alcohol to give **14a–14l** as white solid in yields of 45–65%.

4.1.8. General procedure for the preparation of 4′-[(1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)-*N*-(methoxyethoxymethyl)[1,1′-biphenyl]-2-sulfonamide (15a–15l)

Under nitrogen, the intermediates **11** (1 mmol) and **14** (1.6 mmol) were dissolved in propanol (10 mL), then triphenyl phosphine (2 mg, 0.01 mmol), palladium(II) acetate (8 mg, 0.03 mmol) and 2 N sodium carbonate solution (1 mL) were added. The mixture was heated to reflux for 1 h, and then reacted for an additional 3 h at room temperature. EtOAc (5 mL) was added, the water layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were concentrated and purified by flash column chromatography with *n*-hexane/ethyl acetate (1:3, v/v) to give **15a–15l** as pale yellow oil in yields of 30–50%.

4.1.9. General procedure for the preparation of 4′-[(1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ia–Il)

Compound **15** (2.75 mmol) was dissolved in 95% EtOH (10 mL), then 6 N hydrochloric acid (10 mL) was added. The mixture was heated to reflux for 2 h and the pH was adjusted to 4 with hydrochloric acid. The resulting solution was concentrated and purified by flash column chromatography with CH_2Cl_2/MeOH (20:1, v/v) to give **Ia–Il** as white solids in yields of 30–80%.

4.1.9.1. 4′-[(1*H*-Benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1'-biphenyl]-2-sulfonamide (Ia). White solid, 64.0%, mp 212–214 °C. IR(KBr/cm^{-1}): 2353, 1650, 1496, 1336, 1165. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.47 (s,1H), 7.18–8.00 (m, 12H), 5.55 (s, 2H), 2.01 (s, 3H), 1.55 (s, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{25}H_{23}N_4O_3S$: 459.1485, found 459.1488.

4.1.9.2. 4′-[(2-Methyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ib). White solid, 46.5%, mp 214–215 °C. IR(KBr/cm^{-1}): 2924, 1616, 1467, 1341, 1168. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.10–8.01 (m, 12H), 5.53 (s, 2H), 2.63 (s, 3H), 1.98 (s, 3H), 1.54 (s, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{26}H_{25}N_4O_3S$: 473.1642, found 473.1640.

4.1.9.3. 4′-[(2-Ethyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ic). White solid, 72.3%, mp 221–223 °C. IR(KBr/cm^{-1}): 2946, 1658, 1464, 1337, 1167. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.22–8.06 (m, 12H), 5.81 (s, 2H), 3.23–3.30 (m, 2H), 2.03 (s, 3H), 1.59 (s, 3H), 1.39–1.44 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{27}H_{27}N_4O_3S$: 487.1798, found 487.1795.

4.1.9.4. 4′-[(2-Propyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Id). White solid, 38.1%, mp 228–230 °C. IR(KBr/cm^{-1}): 2967, 1615, 1464, 1340, 1164. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.07–8.00 (m, 12H), 5.55 (s, 2H), 2.84-2.89 (t, *J* = 7.4 Hz, 2H), 1.99 (s, 3H), 1.77–1.84 (m, 2H), 1.55 (s, 3H), 0.95-1.00 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{28}H_{29}N_4O_3S$: 501.1955, found 501.1958.

4.1.9.5. 4′-[(2-Butyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ie). White solid, 40.8%, mp 229–232 °C. IR(KBr/cm^{-1}): 2956, 1615, 1464, 1316, 1163. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.98–8.00 (m, 12H), 5.50 (s, 2H), 2.86-2.89 (t, *J* = 7.4 Hz, 2H), 1.84 (s, 3H), 1.74-1.77 (m, 2H), 1.43 (s, 3H), 1.39-1.42 (m, 2H), 0.88–0.91 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) m/z: calcd for $C_{29}H_{31}N_4O_3S$: 515.2111, found 515.2115.

4.1.9.6. 4′-[(2-Butyl-6-methyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (If). White solid, 54.1%, mp 245–247 °C. IR(KBr/cm^{-1}): 2960, 1616, 1468, 1320, 1165. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.90–8.05 (m, 11H), 5.60 (s, 2H), 2.86–2.88 (t, *J* = 7.4 Hz, 2H), 2.45 (s, 3H), 1.86 (s, 3H), 1.75-1.77 (m, 2H), 1.45 (s, 3H), 1.38–1.41 (m, 2H), 0.87–0.90 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{30}H_{33}N_4O_3S$: 529.2268, found 529.2271.

4.1.9.7. 4′-[(2-Butyl-6-methoxy-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ig). White solid, 60.3%, mp 230–231 °C. IR(KBr/cm^{-1}): 2952, 1620, 1465, 1332, 1165. ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.77–7.99 (m, 11H), 5.52 (s, 2H), 3.82 (s, 3H), 2.84-2.86 (t, *J* = 7.4 Hz, 2H), 1.82 (s, 3H), 1.74-1.77 (m, 2H), 1.42 (s, 3H), 1.37–1.40 (m, 2H), 0.88–0.92 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{30}H_{33}N_4O_4S$: 545.2217, found 545.2214.

4.1.9.8. 4′-[(2-Butyl-6-chloro-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ih). White solid, 59.8%, mp 256–258 °C. IR(KBr/cm^{-1}): 2948, 1610, 1470, 1328, 1168. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.22–8.19 (m, 11H), 5.60 (s, 2H), 2.86–2.89 (t, *J* = 7.4 Hz, 2H), 1.91 (s, 3H), 1.76-1.79 (m, 2H), 1.55 (s, 3H), 1.39-1.44 (m, 2H), 0.90-0.95 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{29}H_{30}ClN_4O_3S$: 549.1722, found 549.1726.

4.1.9.9. 4′-[(2-Butyl-6-bromo-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ii). White solid, 53.5%, mp 253–255 °C. IR(KBr/cm^{-1}): 2928, 1615, 1460, 1320, 1164. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.20–8.21 (m, 11H), 5.55 (s, 2H), 2.86–2.89 (t, *J* = 7.4 Hz, 2H), 1.90 (s, 3H), 1.76–1.78 (m, 2H), 1.54 (s, 3H), 1.39–1.43 (m, 2H), 0.89-0.93 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{29}H_{30}BrN_4O_3S$: 593.1217, found 593.1212.

4.1.9.10. 4′-[(2-Butyl-6-fluoro-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ij). White solid, 66.7%, mp 262–264 °C. IR(KBr/cm^{-1}): 3015, 1608, 1480, 1335, 1166. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.00–8.10 (m, 11H), 5.56 (s, 2H), 2.85–2.88 (t, *J* = 7.4 Hz, 2H), 1.92 (s, 3H), 1.78–1.80 (m, 2H), 1.56 (s, 3H), 1.38–1.42 (m, 2H), 0.90–0.94 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{29}H_{30}FN_4O_3S$: 533.2017, found 533.2019.

4.1.9.11. 4′-[(2-Butyl-6-trifluoromethyl-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Ik). White solid, 58.9%, mp 270–272 °C. IR(KBr/cm^{-1}): 3010, 1636, 1475, 1332, 1170. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.18–8.25 (m, 11H), 5.60 (s, 2H), 2.85–2.89 (t, *J* = 7.4 Hz, 2H), 1.96 (s, 3H), 1.73–1.76 (m, 2H), 1.54 (s, 3H), 1.37–1.40 (m, 2H), 0.88–0.92 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{30}H_{30}F_3N_4O_3S$: 583.1985, found 583.1988.

4.1.9.12. 4′-[(2-Butyl-6-nitro-1*H*-benzimidazole-1-yl)-methyl]-*N*-(3,4-dimethyl-5-isoxazolyl)[1,1′-biphenyl]-2-sulfonamide (Il). White solid, 42.6%, mp 276–278 °C. IR(KBr/cm^{-1}): 2968, 1606, 1498, 1325, 1158. ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.22–8.85 (m, 11H), 5.56 (s, 2H), 2.87–2.89 (t, *J* = 7.4 Hz, 2H), 1.98 (s, 3H), 1.78–1.81 (m, 2H), 1.55 (s, 3H), 1.40–1.43 (m, 2H), 0.90–0.98 (t, *J* = 7.4 Hz, 3H). HR-MS (ESI, M+H) *m*/*z*: calcd for $C_{29}H_{30}N_5O_5S$: 560.1962, found 560.1960.

4.2. Pharmacological evaluation

4.2.1. Angiotensin II receptor (AT_1) binding assay

Membrane fractions or bovine adrenal cortex were prepared by modification of the method of Maeda et al.[15] The freshly isolated bovine adrenal cortex was homogenized in ice-cold medium containing 10 mM sodium phosphate buffer (pH 7.4), 30 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), 1 μM (*p*-amidinophenyl) methanesulfonyl fluoride HCl (*p*-APMSF), and 0.02% NaN_3. The homogenate was layered on a 41% sucrose solution and centrifuged at 95000*g* for 60 min. The interfacial band between the supernatant and the sucrose portion was collected. The membrane fraction was washed by centrifugation at 95000*g* for 20 min. The pellet obtained was used as the source of AT_1 receptor.

Binding of [^{125}I] AngII to membranes was performed at 22 °C for 120 min in 96-well plates. Each 200 μL of incubated solution

contained the following (final concentration): 20 mM Tris HCl (pH 7.4), 120 mM NaCl, 5 mM $MgCl_2$, 0.05% bovine serum albumin (BSA), 1 μM *p*-APMSF, 0.5 mM EDTA, 0.1 mM DTT, 0.1 nM [^{125}I] Ang II, the test compound and membrane preparations (10 ug of protein/well). At the end of incubation, bound complex was trapped on filters (GF/C) and washed with cold Tris buffer (pH 7.4; 3 × 250 μL). Filter disks were dried, punched out, and counted in an *r*-counter. Specific binding was defined as total binding minus nonspecific binding, which was estimated in the presence of 1 μM unlabeled Ang II. The IC_{50} of an inhibitor was determined as the concentration that displaced the specifically bound [^{125}I] Ang II by 50%.

4.2.2. Endothelin receptor subtype A (ET_A) binding assay

ET_A receptor affinity was determined using CHO-K1 cells expressing the human endothelin A receptor as described previously by Murugesan et al.[1]

4.2.3. Antihypertensive effects in the spontaneously hypertensive rats (RHRs)

Male rats were anesthetized with ethyl carbamate (EC) anesthesia (1.0 g kg^{-1} ip). The abdominal cavity was opened by midline incision. A solid silver clip with an internal diameter of 0.2 mm was applied to the left renal artery as close as possible to the aorta. Care was taken that the artery rests at the basis of the slit and that a visible blood flow remains in the artery behind the clip. The contralateral kidney was not disturbed. The rats were allowed to recover for several weeks in an individual cage.

Then an aortic cannula was inserted via the left femoral artery for measuring blood pressure and a caval cannula inserted via the left femoral vein for injecting the test compound. The other end of the venous cannula was led under the skin and exteriorized at the back of the neck. A rat was placed in an individual cage after surgery and fasted for 24 h. On the next day, the aortic cannula was connected to a pressure transducer (San-ei 45277, Japan); the mean blood pressure was recorded on a polygraph (San-ei 7747, Japan); and the test compound was administered by iv at 20 mg kg^{-1}. The blood pressure and heart rate were monitored for up to 8 h postdose.

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