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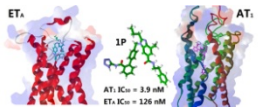

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Synthesis and biological evaluation of 4′-[(benzimidazol-1-yl) methyl] biphenyl-2-amides as dual angiotensin II and endothelin A receptor antagonists

Xiao-Feng Han, Wei-Zhe Xue, Li-Ping Hao and Zhi-Ming Zhou*

By introduced a novel fragment [*N*-(1H-tetrazol-5-yl)-amide], a series of 4′-[(benzimidazol-1-yl)methyl] biphenyl-2-amides (**1a-1w**) was designed, synthesized, and biologically evaluated. **1d**, **1k** and **1p** showed potent antagonistic activities against angiotensin II receptor (AT_1) and endothelin A receptor (ET_A). The evaluation in spontaneous hypertensive rats indicated that the oral activity of compound **1p** was more potent than Irbersartan. Structural biology studies of **1p** exhibited that strong interactions were contributed to the AT_1 and ET_A receptors and the tetrazol-5-ylamide could be an important moiety for the binding to the proteins.

Introduction

Angiotensin II (Ang II) is an octapeptide and potent vasoconstrictor that presents important functions in the pathophysiology of hypertension. Ang II usually affects biological functions by activating selective membrane-bound receptors[1]. Two distinct subtypes of Ang II receptors, namely, AT_1 and AT_2, have been identified, and both are known belong to the G-protein-coupled receptor superfamily (GPCRs)[2]. Numerous studies show that AT_1 receptors are located in various parts of the body and mediate all known effects associated with Ang II, such as vasoconstriction, aldosterone release and other functions that elevate blood pressure[3]. The use of non-peptide-selective AT_1 receptor antagonists has become a globally accepted therapy for hypertension[4,5]. Endothelin-1 (ET-1) is a potent vasoconstrictor that binds to two GPCR members[6], namely, ET_A and ET_B[7]. Previous studies show that the selective blockade of ET_A receptor antagonists may also provide an alternative and effective treatment for hypertension and heart failure[8,9]. Preclinical studies on animals show that simultaneous antagonism of AT_1 and ET_A receptors results in lower blood pressure and greater therapeutic benefits than antagonising either of the receptors individually[10]. Experimental results further show that dual AT_1 and ET_A receptor antagonists (DARAs) are more effective than the current standard therapies for antihypertension and other cardiovascular diseases[11].

Our research efforts over the last decade have concentrated on identifying new types of AT_1 receptor antagonists. The derivatives of 6-substituted carbonyl benzimidazoles were developed in recent years[12]. To develop antihypertensive agents that are more potent than their prototypes, we took advantage of the established structure-activity relationship [13] of 6-substituted aminocarbonyl benzimidazoles (**Fig. 1**, **2**) and incorporated a new *N*-(1H-tetrazol-5-yl)-amide as the isostere of *N*-(3,4-dimethyl-5-isoxazolyl) sulphonamide, which has been described as a key pharmacophore for ET_A receptor antagonists by Tellew [14] (**Fig. 1**, **DARA-3**) meanwhile, the biphenyl moiety with the simplest structure was maintained as the common and crucial skeleton (**Fig. 1**, **1**). The present report describes the synthesis and evaluation of benzimidazoles as DARAs, and further explains the development of the pharmacophore model and docking study to better understand the receptor-ligand interaction.

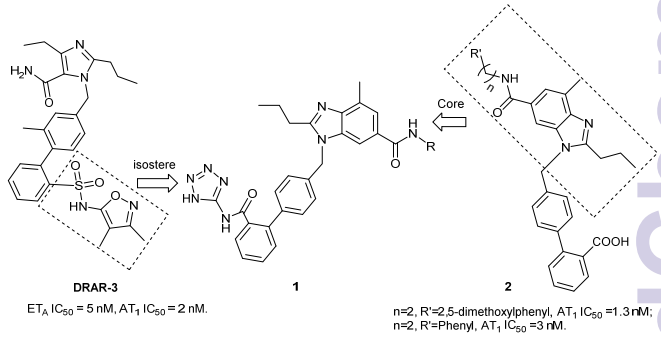

Fig. 1 Strategy for the design of target DARAs.

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Results and discussion

Based on our study of DARA pharmacophore hypothesis[15], the structures of the test set of the target benzimidazoles (**1a** to **1w**) were constructed and their conformational models were generated. By means of the selected DARA hypothesis (**Fig. 2**), the activities of tested compounds were predicted during the Compare/Fit process[16], and the values of the best-fitting conformers were obtained and listed in **Table 1**. This molecular modelling simulation revealed that compounds **1d** (5.916), **1f** (5.467), **1i** (5.469), **1k,** (5.503), **1p** (5.893) and **1t** (5.571) could be considered as promising candidates.

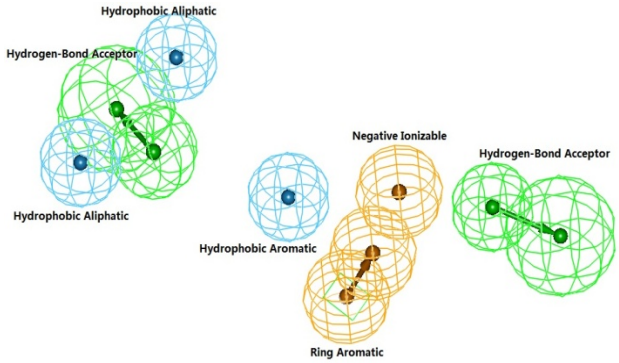


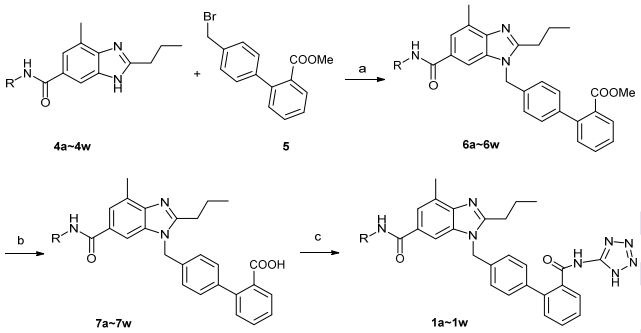
Fig.2 The selected pharmacophore hypothesis Hypo-DARA.

Table 1 Fit values on the pharmacophore and *in vitro* experimental data of compounds (**1a-1w**).

	R	Fit value	AT_1 IC_{50}(nM)[a]	ET_A IC_{50}(nM)[a]
1a	2-(pyrrolidin-1-yl)ethyl	5.180	69.4±6.1	7100
1b	2-(piperidin-1-yl)ethyl	5.274	67.5±7.3	>20000
1c	2-(4-methylpiperazin-1-yl)ethyl	5.291	53.5±4.9	9100
1d	2-morpholinoethyl	5.916	44±4.2	165±33
1e	*n*-propyl	5.266	363±31	>20000
1f	isopropyl	5.467	161±27	>20000
1g	*n*-butyl	5.327	439±85	>20000
1h	*tert*-butyl	5.235	390±76	>20000
1i	phenyl	5.469	73.7±7.6	14000
1j	2-methoxyphenyl	5.151	347±29	886±131
1k	benzyl	5.503	82±8.1	10±2.2
1l	2-methoxybenzyl	5.305	73.3±5.8	7200
1m	3-methoxybenzyl	5.324	53.9±4.3	625±98
1n	4-methoxybenzyl	5.439	549.8±87	>20000
1o	3,4-dimethoxybenzyl	5.274	32.9±4.1	10000
1p	2-phenylethyl	5.893	3.9±0.4	126±25
1q	2-methoxyphenethyl	5.346	26.1±3.7	7000
1r	3-methoxyphenethyl	5.296	36.9±5.5	>20000
1s	4-methoxyphenethyl	5.142	87.8±7.3	13000
1t	2,5-dimethoxyphenethyl	5.571	30.7±4.8	>20000
1u	3,4-dimethoxyphenethyl	5.234	93±10.7	>20000
1v	2-fluorophenethyl	5.138	90±8.4	11000
1w	4-fluorophenethyl	5.281	140±11.4	>20000
Losartan	----	----	16.2±1.2	**----**
Bosentan	**----**	----	----	8.4±1.1

a. Each value is the mean ± SEM of three determinations.

The target compounds (**1a** to **1w)** were synthesized according to the route described in **Scheme 1**, and all the benzimidazole precursors (**4** to **7**) were prepared following our own methods previously described[12,17] with good yield. In the last step, **7a to 7w** were converted to the N-(1H-tetrazol-5-yl)-amides (**1a** to **1w)** with anhydrous 5-amino-tetrazole in DMF. All final compounds were identified by MS, ^{1}H-NMR, ^{13}C-NMR and elemental analysis.



Scheme 1. Reagents and conditions: (a) t-BuOK, DMF, r.t., 6h; (b) KOH, CH_3OH; 2h; (c) anhydrous 5-amino-tetrazole, HATU, DMF, 2h.

In vitro Ang II receptor 1 and endothelin receptor A binding assays were performed to evaluate the affinity of target compounds and the results were expressed as IC_{50} (**Table 1**). Losartan and Bosentan were taken as positive control drugs in the assays respectively. From all the IC_{50} values in Table 1, 23 candidate compounds **1a** to **1w** display generally good activity to AT_1 receptor. On the contrary, their performance varies with that of the ET_A receptor. Unlike the relatively close fit values, there are significant activity cliffs within the results of ET_A activity, e.g. benzyl to Ph (1400×loss of potency) or benzyl to 4-methoxybenzyl (>2000×loss). It is probably because the molecules with the reported fragment of N-(3,4-dimethyl-5-isoxazolyl)-sulphonamide were chosen for the training set and test set of the DARA pharmacophore hypothesis[15]. However, this new SAR data could enrich the structural diversity and the fitting experiences necessary to improve this model in further study. Some of the tested molecules containing saturated heterocyclic structures fail to exhibit good binding affinity to ET_A receptor except for **1d**, which has a moderate value (AT_1 IC_{50}=44 nM, ET_A IC_{50}=165 nM) possibly because of the introduced oxygen atom. Aliphatic amides **1e** to **1h** cannot produce acceptable results. The compounds **1i** to **1w**, which start with the AT_1 receptor antagonist core[12], show good activity at the AT_1 receptor and partly moderate binding affinity at the ET_A receptor. The compound **1k** demonstrates high ET_A receptor affinity and good AT_1 receptor binding. It suggests that the tetrazol amide in **1k** apparently substituted for the pyrimidine sulphonamide in Bosentan. In addition, the phenylethylamides **1p** were more active (AT_1 IC_{50}=3.9 nM) than Losartan was (AT_1 IC_{50}=16.2 nM). The methoxyl substituents of amides maintain the AT_1 receptor antagonism but decrease the ET_A receptor antagonism. This result may be attributed to the high electron density on the benzene ring of the amide substituents that might promote the binding with the AT_1 receptor. Furthermore, the replacement of the methoxyl group by fluorin does not improve the activity (compound **1s** vs. **1w**). With regard to the ET_A receptor, the steric congestion affects the binding affinity between the

compounds and the ET_A receptor. When 6-amide substituent groups on the benzimidazoles of the tested compounds are kept a certain length, the groups display better antagonistic activity with decrease in bulk, because the relatively small groups could easily enter the cavity pocket of the ET_A receptor and generate the hydrophobic interaction. Compared to the isostere of isoxazolyl sulphonamides from our previous work[15], though it appears that only several structures of the tetrazol amide provide a better ET_A activity than the isoxazolyl sulphonamide, for example 2-morpholinoethyl, phenyl and 2-phenylethyl, the acylamino tetrazole still provides a good new option and direction for deep study as the isostere of its traditional structure. To sum up, three of the synthesised compounds **1d**, **1k** and **1p** demonstrate obvious and balanced dual antagonism to AT_1 and ET_A receptors in accord with the fit values, and **1p** exhibits slightly better activity than **1d** and **1k.**

The most potent DARA **1p** in *in vitro* binding assays was selected to further evaluate its antihypertensive effects on *in vivo* models. As shown in **Fig. 3**, after oral administration of **1p** (20 mg/kg) and Irbesartan (20 mg/kg) to spontaneous hypertensive rats (SHRs) [19], the maximal reduction of compound **1p** obtained at 6h was observed with 24mmHg on the mean blood pressure (BMP), while the reductive effect of Irbesartan reached 20 mmHg at 4h. Although both compound **1p** and Irbesartan could show the antihypertensive effect in 24h, the duration of lowering BMP over 20 mmHg was kept for 4 hours by **1p**, compared with only 1 hour by Irbesartan[20]. Furthermore, the heart rate (HR) was as effectively controlled by **1p** as Irbesartan (**Fig. 3**). Therefore, compound **1p** was evidently superior to Irbesartan.

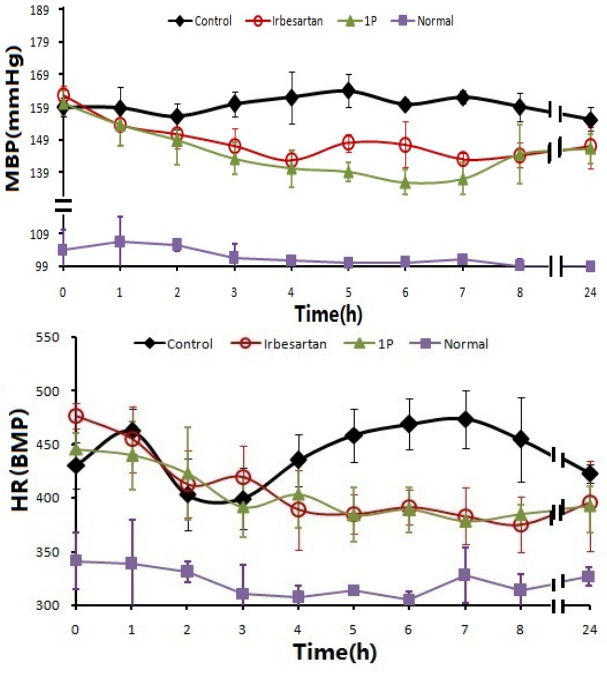


Fig. 3 Effects of **1p** and Irbersartan (20 mg/kg po) on MAP and HR in conscious SHRs after oral administration.

Docking studies were performed to evaluate the binding modes of **1p** in a theoretical human AT_1 receptor protein (PDB ID: 1ZV0)[21] and the modelled ET_A protein[22,23]. Hydrophobic groups are positioned in a lipophilic cavity pocket formed by Phe208, Leu119 and Phe249 (**Fig. 4A**). One H-bond interaction was formed between the acidic tetrazole ring and O–H of Tyr184 (2.55 Å distance), then another was between the oxygen of tetrazol amide and N-H of Asn200. Compared with ET_A model[24], the single interaction caused by the formation of an H-bond between the tetrazole ring and the N-H of Gln165 (2.58 Å distance) could be observed in the hydrophilic pocket from Gln165, Lys166 and Gly170 (**Fig. 4B**). However, the effect of docking on the ET_A model was not expected, given the insufficient depth of the ligand pose into the corresponding protein cavity. Therefore, it seems that **1p** docking to the AT_1 receptor was more evident and had stronger interactions with the target sites compared with docking to the ET_A receptor, and this finding was consistent with the experimental binding result of **1p**. More important, the fragment of tetrazol amide played a key role both in AT_1 and ET_A antagonism.

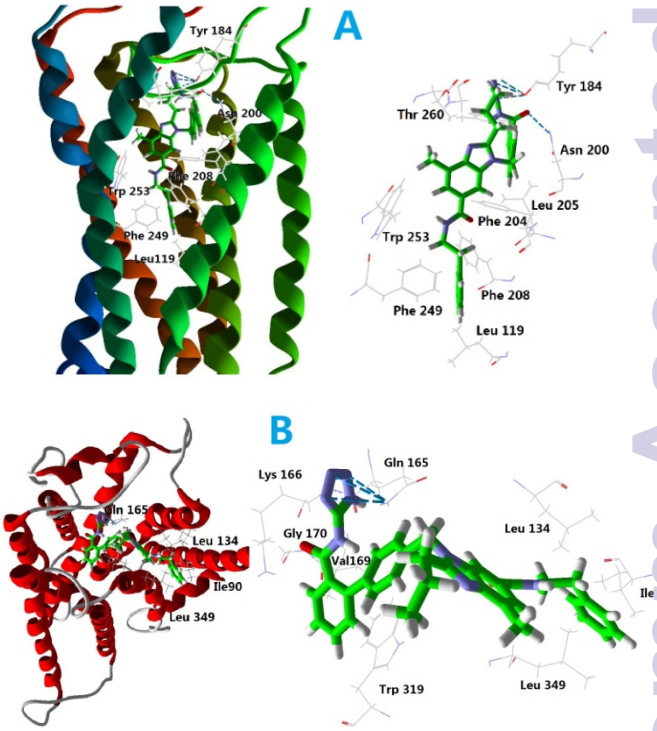


Fig. 4A The detailed representation of docking **1p** to AT_1. Compound **1p** are represented in sticks and colored by atom types (carbon: green, oxygen: red, nitrogen: blue, H-bond: sky-blue). **Fig. 4B** Model of the compound **1p** bound to ET_A. Compound **1p** are represented in sticks and colored by atom types (carbon: green, oxygen: red, nitrogen: blue, H-bond: sky-blue).

Conclusions

By introducing the novel and potent N-(1H-tetrazol-5-yl)-amide fragment to the biphenyl ring, a benzimidazole-based series of DARAs was designed and synthesised. In the *in vitro* dual receptor binding assay, **1d, 1k** and **1p** showed satisfactory antagonistic biological activity towards AT_1 and ET_A receptors. Compound **1p**, which notably showed anti-hypertensive activity in SHRs, proved to be more efficacious than Irbesartan. Furthermore, the **1p** docking

study revealed that the relatively higher antihypertensive activity was attributed to excellent fitting as a result of stronger lipophilic and hydrophilic interactions between ligand **1p** and AT_1 receptor pockets, whereas the relatively weaker activity to ET_A resulted from the single hydrophilic interaction of the tetrazole ring of the same ligand with the ET_A receptor residue.

Acknowledgements

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Graphical abstract