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Research paper

Discovery of novel, potent and low-toxicity angiotensin II receptor type 1 (AT_1) blockers: Design, synthesis and biological evaluation of 6-substituted aminocarbonyl benzimidazoles with a chiral center

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ABSTRACT

Novel angiotensin II receptor type 1 (AT_1) blockers bearing 6-substituted carbamoyl benzimidazoles with a chiral center were designed and synthesized as the first step to develop new antihypertensive agents and understand their pharmacodynamic and pharmacokinetic properties. The newly synthesized compounds were tested for their potential ability to displace [^{125}I] Sar^1 Ile^8-Ang II, which was specifically bound to human AT_1 receptor. Radioligand binding assays revealed nanomolar affinity in several compounds under study. The IC_{50} values of nine ligands were higher than those of Losartan. The screening of decreased blood pressure in spontaneous hypertensive rats displayed that compound **8S** (IC_{50} = 5.0 nM) was equipotent with Losartan, whereas compounds **13R** (IC_{50} = 7.3 nM), **14R** (IC_{50} = 6.3 nM), and **14S** (IC_{50} = 3.5 nM) were slightly ahead of Losartan, and the most significant activity was demonstrated by compound **8R** (IC_{50} = 1.1 nM). Candidate **8R** was identified for its excellent efficacy in antihypertension and fairly low toxicity based on plasma analyses, toxicology studies, and chronic oral tests. Finally, compound **8R** exhibited strong and multiple interactions with target active sites of the theoretical AT_1 receptor model in docking study.

1. Introduction

Hypertension is a lifelong condition, and patients with high blood pressure need to take medicine on a daily basis. Given the advancements in society worldwide, hypertension presents a challenge not only to people in Western countries but also to the entire world. For instance, the hypertensive morbidity rate of Chinese adults may reach 30%, as predicted by the Centers for Disease Control of China. Therefore, the effectiveness and safety of antihypertensive drugs, which were developed for several years, such as diuretics in the 1960s [1], β-blockers in the 1970s [2], angiotensin converting enzyme inhibitors [3] and calcium channel antagonists [4] in the 1980s, angiotensin II receptor blockers (ARBs) in the 1990s [5], and most recently, renin inhibitors [6], should be urgently promoted. ARBs, as a first-line therapy for clinical hypertension, are known to be safe and efficacious agents. However, their use also poses risks, such as abnormal liver function, renal insufficiency [7], or even cancer [8]. Thus, better and safer ARBs should be explored and developed.

Our research efforts in the last decade were focused on developing anti-hypertensive compounds with high efficiency and low toxicity. Considering our understanding of benzimidazole derivatives in the field of medicinal chemistry [9], we were curious about the effect of this kind of structure, which showed good activity at positions 5–7 [10]. Among these reports, a carboxylic acid function and its derivatives at the 5 or 7 position were favourable for Ang II antagonism. In our previous report [11], the derivatives of 6-aminocarbonyl and 6-acylamino benzimidazoles were developed and found to have favourable antagonistic activity via *in vitro* and *in vivo* studies. Specifically, 4′-((4-methyl-6-(phenethylcarbamoyl)-2-*n*-propyl-1H-benzimidazol-1-yl)methyl)-[1,1′-biphenyl]-2-carboxylic acid (Fig. 1, **I**), which recently passed the acute toxicity (LD_{50} = 12 g/kg), genotoxicity (negative) and long-term (chronic) toxicity tests, showed extremely ideal results for toxicology studies.

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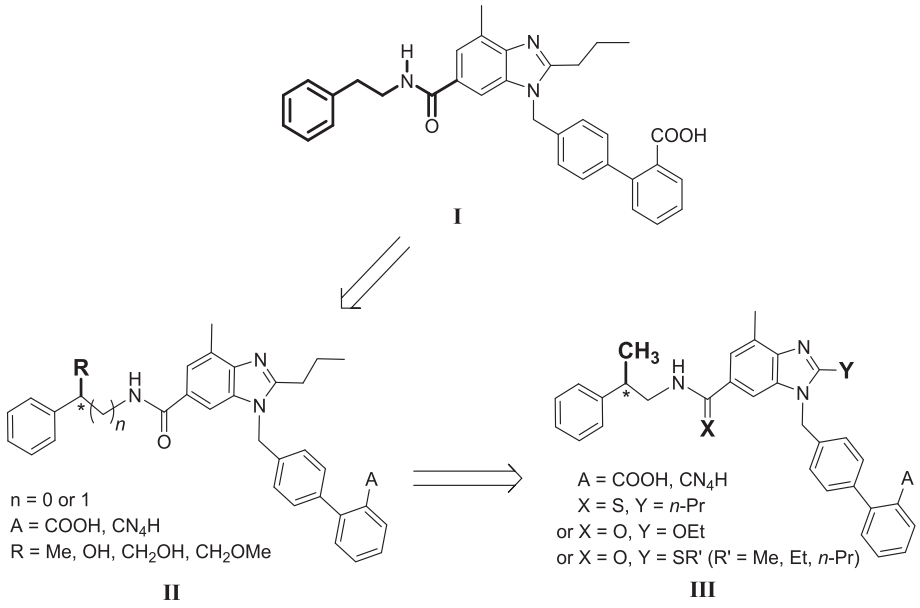

Fig. 1. Strategy for the design of target ARBs.

Moreover, the application and preparation of chiral drugs are two of the current research directions of medicine development in industrial settings, but ARBs with an asymmetric centre have been rarely reported [12]. Thus, in the present report, encouraged by the discovery of the best compound in our previous work [11b], we hypothesised that the insertion of a chiral substituent group (Fig. 1, **II**) can improve the specificity of anti-hypertensive efficacy of parent structure **I**. Inspired by the success of Candesartan [13] and Fimasartan [14], oxygen or sulphur atom was also introduced at position 2 or 6 of benzimidazole to yield the same results (Fig. 1, **III**). Compound **8R** was selected for detailed *in vivo*, pharmacokinetic, toxicologic and molecular docking studies based on the results from the binding and functional assays.

2. Result ant discussion

2.1. Chemistry

Target compounds **1**–**10** were synthesized following the route described in Scheme 1, and the starting material 4-methyl-2-*n*-propyl-1H-benzimidazole-6-carboxylic acid (**21**) used in this scheme was prepared from 3-methyl-4-nitrobenzoic acid based on reported methods [15]. This compound was converted to acyl chloride with thionyl dichloride under reflux conditions; the acyl chloride was then coupled with different amines to obtain an acylamide compound **22** yielding 90%–95%. Subsequently, the acylamides were alkylated with 5-(4′-bromomethyl-1,1′-biphenyl-2-yl)-1-triphenylmethyl-1H-tetrazole or methyl 4′-(bromomethyl) biphenyl-2-carboxylate by using potassium tert-butoxide in DMF to obtain the corresponding products in yields of 60%–70%. Finally, the hydrolysis of methyl carbonate and the removal of the trityl group of tetrazole by using aqueous sodium hydroxide in boiling EtOH were accomplished with >70% yield. Moreover, **24e** and **24f** were synthesized through the methylation of **24c** and **24d** with methyl iodide and sodium hydride before deprotection.

Encouraged by the evaluation results (Table 1), the next structural exploration was conducted on the basis of chiral 2-phenylpropan-1-amine $\mathbf{R_{1g}}$ and $\mathbf{R_{1h}}$ to develop more potential compounds. As shown in Scheme 2, thionation of **24g** and **24h** with Lawesson's reagent in toluene at reflux condition [16] provided thioamide derivatives **25g** and **25h**, which were treated with aqueous sodium hydroxide in boiling EtOH to yield **11** and **12**, respectively, and containing a thioamide group at the 6-position of benzimidazole.

The starting material 2-ethoxy-4-methyl-1H-benzimidazole-6-carboxylic acid **26** and 4-methyl-2-(alkylthio)-1H-benzimidazole-6-carboxylic acid **29** were accumulated in gram scale without column chromatographic purification in Chaturvedula's and our own routes [17]. Compounds **13**–**20** were prepared as described in Schemes 3 and 4. The three steps for targeting molecules were the same as those mentioned above in Scheme 1. However, limited by the susceptibility to acids, compound **26** was coupled with chiral 2-phenylpropan-1-amine $\mathbf{R_{1g}}$ and $\mathbf{R_{1h}}$ through mild condensation reaction to obtain 2-ethoxy-4-methyl-*N*-(2-phenylpropyl)-1H-benzimidazole-6-carboxamide **27**, and the ideal dehydrating agent was N,N′-carbonyldiimidazole, which can reach about 73%–88% yield.

The last three synthesis steps of compounds **1**–**20** followed the same methods. As an example of compounds **8S** and **8R**, the optically pure 2-phenylpropan-1-amines underwent condensation/alkylation/deprotection sequence without any loss of optical activity (Fig. 2).

All the target compounds were identified by ^{1}H NMR, ^{13}C NMR, and HRMS. Furthermore, the structure of compound **14R** was confirmed via X-ray crystallography [18] (Fig. 3).

2.2. In vitro Ang II antagonism

All the target compounds were evaluated for their *in vitro* Ang II receptor 1 binding affinity in the competitive inhibition of [^{125}I] Ang II binding to the angiotensin II receptor type 1 (AT_1) by using a conventional ligand-binding assay as described previously [19]. The results were expressed in IC_{50} values, which represented the concentration of each compound inhibiting the [^{125}I] Ang II binding to the receptor by 50%. Compounds **1**–**10** were also investigated *in vitro* by using the contractile response of isolated rabbit aortic strips in a functional assay. Losartan and compound **I** were used as positive control drugs in all the assays.

As expected, the binding assay results showed that all the compounds displayed an obvious degree of inhibition activity in