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Research paper

Design, synthesis and evaluation of novel potent angiotensin II receptor 1 antagonists

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ABSTRACT

A series of new angiotensin II (Ang II) receptor 1 antagonists were designed, synthesized and evaluated. All compounds showed nanomolar affinities for the angiotensin II type 1 receptor in radioligand binding assays and could reduce blood pressure significantly in spontaneously hypertensive rats(SHRs). From which, compound **2b** displayed higher affinity binding to angiotensin II type 1 receptor at the same order of magnitude to irbesartan with an IC_{50} value of 1.26 ± 0.08 nM in radioligand binding assays. **2b** showed an efficient and long-lasting effect in reducing blood pressure, the maximal reducing responses were 40.62 ± 4.08 mmHg of MBP at 15 mg/kg and 28.39 ± 2.09 mmHg at 10 mg/kg in SHRs, 39.56 ± 4.83 mmHg at 15 mg/kg and 29.05 ± 2.20 mmHg at 10 mg/kg in RHRs, the significant antihypertensive effect lasted beyond 12 h both in SHRs and in RHRs. In the single-dose pharmacokinetic experiments, compound **2b** could be absorbed efficiently and metabolized smoothly in Wistar rats after oral administration. The values of C_{max}, T_{max}, AUC_{0-72} and MRT_{0-72} were 885.61 ± 432.7 ng/mL, 5.67 ± 1.51 h, 6110.28 ± 7398.33 ng/mL h and 7.87 ± 2.30 h at 10 mg/kg, 2945.55 ± 1543.67 ng/mL, 4.33 ± 0.82 h, 26473.62 ± 12217.16 ng/mL h and 10.24 ± 6.94 h at 15 mg/kg, 5759.03 ± 1331.75 ng/mL, 5 ± 1.10 h, 89488.44 ± 18413.15 ng/mL·h and 12.89 ± 2.0 h at 30 mg/kg respectively. The $T_{1/2}$ values of the three groups were similar, about 9–10 h. Compound **2b** was distributed into tissues rapidly and extensively after oral administration. The level of it was the highest in the liver, followed by in spleen, kidney, and the lowest in brain. The acute toxicity assays of **2b** proved its low acute toxicity with an LD_{50} value of 1551.71 mg/kg, and no toxicity reaction appeared at dose of 1200.00 mg/kg. These encouraging results make compound **2b** an effective, long-lasting and safe anti-hypertensive drug candidate and worthy of further investigation.

1. Introduction

Hypertension, defined as raised blood pressure greater than or to 140 mmHg systolic or 90 mmHg diastolic, is a serious health problem associated with an increased risk of death, stroke, and metabolic syndromes including insulin resistance and lipid abnormalities [1,2]. It is recognized as one of the leading risk factors for human morbidity and mortality [3,4]. As the population ages and the prevalence of contributing factors such as obesity, sedentary lifestyle and smoking rise, hypertension has currently affected approximately one billion adults globally and it may increase to 1.56 billion by the year 2025 [5]. Despite global awareness of hypertension, its consequences and the availability of effective therapeutics, an estimated 32% of hypertensive patients remain untreated [6].

The rennin angiotensin system (RAS) plays a central role in the pathophysiology of hypertension, electrolyte balance and blood volume [7]. Angiotensin II (Ang II), an octapeptide formed within the RAS from angiotensin I by angiotensin converting enzyme (ACE), is the biologically active component of the rennin-

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angiotensin system and is responsible for most of the peripheral effects of this system and it is one of the most powerful vasoconstrictors [8,9]. Angiotensin II receptor blockers (ARBs) are the newest class of approved antihypertensive agents and have rapidly become established as one of the leading therapeutic drugs in the management of hypertension [10,11]. At present, many efficient and safe ARBs have been widely used in the treatment of hypertension, such as losartan, valsartan, irbesartan, candesartan, telmisartan and so on.

Irbesartan, a potent, long-acting angiotensin II receptor 1 antagonist, decreases blood pressure effectively in a dose-dependent manner in patients with mild-to-moderate hypertension [12,13]. Here a series of new compounds (compound **1a**–**c**, **2a**–**d**) (Fig. 1) were designed and synthesized taking irbesartan as lead compound. The dominant conformations of compound **1b**, **2b** and irbesartan were shown as Fig. 2 from computer software Spartan 10. According to their overlay conformations, it is indicated that they align well with each other. And the affinities of this series of compounds to AT_1 receptor were tested by specific radioligand binding assay *in vitro*. The anti-hypertension effects of them were evaluated in spontaneously hypertensive rats (SHRs) *in vivo*. The anti-hypertension effects in renal hypertensive rats (RHRs), plasma pharmacokinetics, tissue distribution in Wistar rats and acute toxicity in ICR mouse of compound **2b** were further investigated.

2. Results and discussion

2.1. Chemistry

The preparation of compounds **1a**–**c** and **2a**–**d** was performed by means of a multistep procedure described in Schemes 1 and 2.

The synthesis of **1a**–**c** was accomplished starting from the commercially available 1-amino-cyclopentanecarboxamide (**3**), which reacted with different acyl chlorides in DCM using Et_3N as base to give amide **4a**–**d**. **4a**–**d** were cyclized to produce spiroazacyclic compounds **5a**–**d** with 10 M KOH in MeOH. 4-Methylindole **6a** was *N*-protected by acylation with benzoyl chloride to give (4-methyl-1H-indol-1-yl) (phenyl) methanone **7a**, which was brominated with NBS to produce (4-bromomethyl-1H-indol-1-yl) (phenyl) methanone **8a**. **8a** was substituted by **5a**–**d** and then de-protected in the presence of K_2CO_3 to generate indole compounds **9a**–**c** which were then coupled with 2-fluoro-benzonitrile to give benzonitrile **10a**–**c**. The target compounds **1a**–**c** were acquired after the formation of tetrazole using **10a**–**c** with sodium azide and chlorotributylstannane in DMF. At each stage of the synthetic sequence the product was isolated, purified by column chromatography and characterized by NMR and mass spectrum techniques.

The preparation of **2a**–**d** was similar to **1a**–**c**.

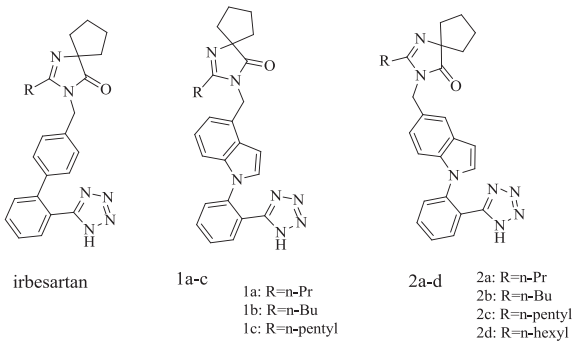

Fig. 1. Chemical structure of irbesartan and compounds **1a**–**c**, **2a**–**d**.

2.2. Biological evaluation

2.2.1. Radioligand binding assays

Immunohistochemical staining showed that smooth muscle actin was expressed in cytoplasm (Fig. 3), which indicated that the obtained cells were vascular smooth muscle cells. The radioligand binding assays showed that all the compounds have nanomolar affinity for the AT_1 receptor subtype (Table 1, Fig. 4). Generally, compounds **1b** and **2b** had much stronger inhibitory ability than compounds **1a**, **1c**, **2a**, **2c** and **2d** in binding assays which indicated that compounds **1b** and **2b** might have better antihypertensive activity *in vivo*. And among all the compounds, compound **2b** showed higher affinity to the angiotensin AT_1 receptor with an IC_{50} value of 1.26 ± 0.08 nM than irbesartan whose IC_{50} value was 1.46 ± 0.34 nM.

2.2.2. In vivo anti-hypertension effects of the compounds

In spontaneously hypertensive rats (SHRs), the effects of compounds **1a**–**c**, **2a**–**d** (15 mg/kg) and irbesartan (15 mg/kg) on the mean blood pressure (MBP) *in vivo* after oral administration were shown in Fig. 5. The results indicated that all the new compounds, especially compound **1b** and **2b,** could decrease blood pressure significantly under the dose of 15 mg/kg compared with the negative control group. The maximal response of compound **1b** at 15 mg/kg was observed at 3 h after oral administration. It lowered 40.57 ± 2.90 mmHg of MBP and the significant ($p < 0.01$) antihypertensive effect sustained for at least 12 h. Among these compounds, **2b** has the strongest antihypertensive effect, whose maximal response lower 40.62 ± 4.08 mmHg MBP at 15 mg/kg and 28.39 ± 2.26 mmHg at 10 mg/kg at 4 h after oral administration in SHRs (Fig. 6a). The antihypertensive effects in RHRs were tested to be coincident with which in SHRs (Fig. 6b). No influence of these compounds to the heart rates of rats was observed. These results suggested that the anti-hypertensive effects of compound **1b** and **2b** were superior to irbesartan, especially **2b**, which were worthy of further investigation.

2.2.3. Pharmacokinetic characteristics of compound 2b

The HPLC chromatograms of compound **2b** in plasma and liver tissues were showed in Fig. 7 and Fig. 8 respectively. The concentrations of compound **2b** in rat plasma samples obtained from 6 male Wistar rats orally administered with **2b** solution at 10–30 mg/kg were quantified. The pharmacokinetic parameters were shown in Table 2. The mean peak plasma concentration-time curve of **2b** was shown in Fig. 9. The maximum concentration (C_{max}) value ranged from 855 ng/mL at 10 mg/kg to 5759.03 ng/mL at 30 mg/kg. The area under the concentration-time curve from 0 to 72 h ($AUC_{0\sim72}$) ranged from 6110.28 ng/mL·h to 89488.44 ng/mL·h. The elimination half-lives ($T_{1/2}$) ranged from 9 h to 13 h. Dose dependency of $AUC_{0\sim\infty}$ and C_{max} (10–30 mg/kg) was assessed and both $AUC_{0\sim\infty}$ and C_{max} met the dose proportionality criteria(Fig. 10).

The compound levels in heart, liver, spleen, kidney and brain of Wistar rats at 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72 h post oral administration of compound **2b** at 10–30 mg/kg were shown in Fig. 11. The pharmacokinetic parameters were shown in Table 3. It could be found that **2b** was extensively distributed in rat tissues. It was accumulated especially in liver which exhibited the highest concentration ranged from 720.73 ng/g at 10 mg/kg to 1995.52 ng/g at 30 mg/kg, followed by spleen ranged from 348.02 ng/g at 10 mg/kg to 1881.59 ng/g at 30 mg/kg. The level of compound **2b** was lowest in the brain, which ranged from 37.16 ng/g to 196.47 ng/g. As compound **2b** was detectable in brain, it was likely that **2b** was able to penetrate the blood-brain barrier (BBB). For all tissues, compound **2b** showed continuous increase at 0–4 h and decrease from 6 to 72 h post administration.