Design, Synthesis, and Biological Evaluation of 6-Benzoxazole
Benzimidazole Derivatives with Antihypertension ActivitiesZhuo Wu,[†] Xiao-Lu Bao,[†] Wei-Bo Zhu,[†] Yan-Hui Wang,[†] Nguyen Thi Phuong Anh,[†] Xiao-Feng Wu,[‡] Yi-Jia Yan,^{*,‡} and Zhi-Long Chen^{*,†,§,||}[†]Department of Pharmaceutical Science and Technology, College of Chemistry and Biology, Donghua University, Shanghai 201620, China[‡]Shanghai Xianhui Pharmaceutical Co., Ltd., Shanghai 200433, China

Supporting Information

ABSTRACT: A series of new angiotensin II receptor 1 antagonists were prepared. They displayed nanomolar affinity to AT₁ receptor and could decrease blood pressure efficiently in spontaneously hypertensive rats. Among them, compounds **1b** and **2b** could reduce the blood pressure with more or equal potency compared to Losartan. So, compounds **1b** and **2b** could be considered as potential antihypertension drug candidates.

KEYWORDS: Hypertension, antihypertension drug, angiotensin II receptor 1 antagonist

Hypertension is one of the most shared cardiovascular diseases in the world, which has significant morbidity and mortality.^{1–3} There are about 200 million hypertension patients in the world, and it is increasing by 10% every year. The renin angiotensin system (RAS) consists of a cascade of enzymatic reactions which plays a crucial role in the electrolyte/fluid homeostasis and regulation of blood pressure.^{3–6} Angiotensin II receptor 1 antagonist (ARB) is a novel class of antihypertensive drug with the characteristics of high potency, long-term effect, and low toxicity.^{7,8} Several ARBs have been discovered and clinically used after modification of Losartan (Figure 1), which is the first drug used in clinics.^{8–10} These ARBs can block the effects of angiotensin II by selectively antagonizing the AT₁ receptor.^{10,11} The angiotensin II receptor blockers, regarded as antihypertensive drugs, have developed rapidly and occupied the main position of the market over the past 30 years.

Structure–activity relationships of Losartan have been reported.¹² A lipophilic liner alkyl group at the 2-position of imidazole which will be associated with hydrophobic pocket of the AT₁ receptor, and an acidic group like COOH and tetrazole at the ortho position of the biphenyl group which will bind to a basic position of the receptor, are required for potent antihypertension activity. The biphenyl moiety, which acts as a

Figure 1. Binding profile of Losartan, **1** and **1b/2b**, where L1 is lipophilic pockets and H is the H-bond donor site.

spacer connecting the acidic group with other features, could be replaced by other similar spacers such as *N*-phenyl indole.¹⁰ The imidazole ring could also be replaced by other heterocyclic rings, such as benzimidazole to act as an acceptor in a hydrogen-bonding interaction with the receptor.^{13–16} Further studies of the benzimidazole derived AT₁ antagonists showed that periphery substitution to the benzimidazole ring with basic heterocycles could result in potent AT₁ antagonist

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like telmisartan.¹³ A methyl group in position 4 of the benzimidazole ring will be helpful to reduce the metabolism rate *in vivo*.

In a previous paper,¹⁷ we reported an effective and long-lasting antihypertension compound (**1**) with dibenzimidazole, indole, and benzoic acid moieties. Here a series of 6-benzoxazole substituted benzimidazole derivatives with 1,4- or 1,5-disubstituted indoles moieties (**1a–f**, **2a–f**) (Figure 1) were prepared and evaluated.

RESULTS AND DISCUSSION

Chemistry. Compounds **1a–f** and **2a–f** were synthesized by the multistep procedure outlined in Schemes 1–3.

Scheme 1. Reagents and Reaction Conditions: (a) 2-Fluor Benzonitrile, K₂CO₃, DMF, Reflux; (b) NaBH₄, EtOH, THF, Ice Bath; (c) NaOH, DMF, Reflux; (d) CH₃I, Dichloromethane; (e) HBr/AcOH (35%), Dichloromethane, Ice Bath

Scheme 2. Reagents and Reaction Conditions: (a) RCOCl, Et₃N, DCM; (b) Fuming HNO₃, −15 °C; (c) Raney Ni, N₂H₄·H₂O; (d) CH₃COOH, Reflux; (e) NaOH, H₂O, MeOH, Reflux; (f) 2-Amino-3-methylphenol/2-Amino-6-methylphenol, PPA, 150 °C

As shown in Scheme 1, indole-4-formaldehyde **3a** or indole-5-formaldehyde **3b** was reacted with 2-Fluor benzonitrile to afford compound **4a** or **4b** in yield about 89% according to our previously reported methods.¹⁷ **4a** or **4b** was hydrogenated with sodium borohydride in the mixed solution of ethanol and tetrahydrofuran at 0 °C to provide alcohol **5a** or **5b** in yield about 93% or 85%. After hydrolyzation of **5a** or **5b** by sodium hydroxide in boiling DMF in yield of 87% or 94%, the resulting acid **6a** or **6b** was reacted with iodomethane in dichloromethane to give methyl benzoate compounds **7a** or **7b** in yield 96% or 93%. Compound **8a** or **8b**, which was obtained after the bromination of compounds **7a** or **7b** with HBr saturated AcOH in yield 90% or 87%, was used in subsequent reactions.

Scheme 3. Reagents and Reaction Conditions: (a) NaH, DMF, 50 °C; (b) NaOH, H₂O, CH₃OH, Reflux

Table 1. IC₅₀ and K_i Values of Compounds **1a–f, **2a–f**, and Losartan (*P < 0.05, **P < 0.01 vs Losartan)**

Compound	R ₁	R ₂	R ₃	IC ₅₀ ± SEM (nM)	K _i (nM)
1a	Et	Me	H	10.3 ± 1.6	7.5 ± 0.8
1b	n-Pr	Me	H	4.0 ± 0.5**	2.9 ± 0.2
1c	n-Bu	Me	H	9.9 ± 0.9	7.2 ± 0.6
1d	Et	H	Me	12.1 ± 0.3	8.8 ± 1.2
1e	n-Pr	H	Me	5.3 ± 1.1**	3.8 ± 0.9
1f	n-Bu	H	Me	10.5 ± 1.8	7.6 ± 1.2
2a	Et	Me	H	12.0 ± 1.7	8.7 ± 1.6
2b	n-Pr	Me	H	4.4 ± 0.2**	3.2 ± 0.2
2c	n-Bu	Me	H	10.7 ± 2.3	7.7 ± 1.7
2d	Et	H	Me	13.6 ± 1.5	9.8 ± 0.2
2e	n-Pr	H	Me	5.6 ± 0.8**	4.0 ± 0.6
2f	n-Bu	H	Me	9.7 ± 0.9*	7.0 ± 0.5
Losartan				14.6 ± 1.6	10.5 ± 1.2

Figure 2. Inhibitory effects of compounds **1b, **2b**, and Losartan on the specific binding of ¹²⁵I-Ang II to AT₁ receptors in VSMCs.**

The benzimidazoles **10a–c** were prepared from the commercially available methyl 4-amino-3-methylbenzoate (**9**) through a four-step procedure reported by our laboratory as shown in Scheme 2.^{17,18} Phenylamine **9** was reacted with acyl chlorides in dichloromethane and then nitrated with fuming HNO₃. The resulting nitrobenzene was hydrogenated with Raney Ni and then cyclized in acetic acid to produce benzimidazoles **10a–c**. **10a–c** were hydrolyzed by 2 M

Figure 3. Effects of **1b**, **2b**, and Losartan on MBP of SHR (n = 6) (*P < 0.05, **P < 0.01 vs Control).

sodium hydroxide solution in methanol; the resulting acids were condensed and cyclized with 2-amino-3-methylphenol/2-amino-6-methylphenol in the presence of polyphosphoric acid to give benzimidazole derivatives **11a–f** in yield 38.5%–55.2%. **11a–f** were alkylated with indole methyl bromides **8a** or **8b** in DMF to generate **12a–f** and **13a–f** in yields over 40% and then hydrolyzed with 2 M sodium hydroxide solution in methanol to afford **1a–f** or **2a–f** with yield 40.3% to 49.0%.

Biological Evaluation. Radioligand Binding Assays. The binding affinities of synthesized compounds for Ang II receptor 1 *in vitro* were detected using vascular smooth muscle cells in a competition radioligand binding assay with [¹²⁵I] Ang II and as described previously.^{13,19} As expected, they all displayed nanomolar affinity to the AT₁ receptor (Table 1, Figure 2). Of these compounds, **1b**, **2b**, **1e**, **2e**, and **2f** exhibited higher affinity to AT₁ receptor than Losartan (**1b**: IC₅₀ = 4.0 ± 0.5 nM, K_i = 2.9 ± 0.2 nM; **2b**: IC₅₀ = 4.4 ± 0.2 nM, K_i = 3.2 ± 0.2 nM and losartan IC₅₀ = 14.6 ± 1.6 nM, K_i = 10.5 ± 1.2 nM). It was also found that compounds with a 1,4-disubstituted indole moiety have relative higher binding ability than with a 1,5-disubstituted indole moiety. The receptor affinity was not significantly influenced by methyl substituents, regardless of their position of R₂ or R₃. In these compounds the n-propyl residue in position 2 of the benzimidazole gave higher affinity than other alkyl groups.

In Vivo Antihypertension Effects. All compounds (5 mg/kg, 10 mg/kg) were tested for antihypertension effects *in vivo* to the mean blood pressure (MBP) in a spontaneously hypertensive rats (SHRs) model after oral administration. These compounds could decrease blood pressure significantly at 5 mg/kg and 10 mg/kg (Figure 3 and Figure 4 in the Supporting

Information). The maximal MBP reduction of **1b** was 54.57 ± 0.05 mmHg, which was more potent than Losartan, and **2b** was 41.91 ± 6.34 mmHg, which was almost equal to Losartan (42.50 ± 4.64 mmHg) at the dose of 10 mg/kg, respectively. The significant (p < 0.01) antihypertensive effect of **1b** and **2b** was sustained for at least 24 h. No influence was observed for these compounds on the heart rates of rats (Figure 3).

CONCLUSION

A series of new 6-substituted benzimidazoles indole derivatives with 1, 4-disubstituted or 1, 5-disubstituted indole moiety and benzoic moiety were designed and synthesized. They could bond to the AT₁ receptor and decrease blood pressure significantly. Among them, compounds **1b** and **2b** exhibited effective antagonistic activities against angiotensin II receptor 1 and could reduce the blood pressure with equal or more potency compared to Losartan. So compounds **1b** and **2b** could be considered as potential antihypertension drug candidates and deserved further investigation.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.8b00335.

Experimental procedures for the biological experiments and synthetic procedures and characterization data for the compounds (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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