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DISCOVERY AND DEVELOPMENT OF ARYL-FUSED IMIDAZOLE-BASED ANGIOTENSIN II ANTAGONISTS

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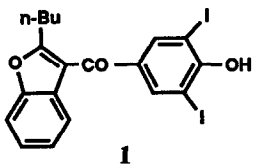
Abstract: A phenolic benzofuran derivative **1** was identified as an angiotensin II receptor antagonist by random screening. Structural modifications led to a novel series of N-(4-hydroxyphenylmethyl)benzimidazoles and imidazo[4,5-b]pyridines, some of which inhibited angiotensin II-induced contractions in rabbit aortic strips with pA_2 values of nearly 9. The related biphenylmethyl derivative E4177 showed potent and long-lasting activity *in vivo*.

The establishment of angiotensin converting enzyme inhibitors as an effective regimen for hypertension has confirmed that the renin-angiotensin system (RAS) plays an important role in blood pressure regulation.[1] As angiotensin II (AII) is the primary effector molecule of the RAS, much research has been directed at antagonizing its effects at its receptors. Although peptide analogs, such as Saralasin[2] are known to be potent receptor antagonists, their clinical use is limited due to short duration of action, poor oral bioavailability, and partial agonistic activity. Here we describe the history of the development of our nonpeptidic AII antagonists, and the structure-activity relationship studies (SARs) related to E4177.[3]

We started our studies by screening ca. 600 compounds for antagonistic activity toward AII receptor using a radio-receptor binding assay in a bovine adrenal cortex membrane preparation specific for AT1 receptor antagonists.[4] We especially selected test compounds from among vasodilators whose mechanisms are unidentified and compounds possessing an imidazole ring or phenol group, since it is thought that histidine and tyrosine may be key amino acids in the structure of AII, involved in its biological activities.



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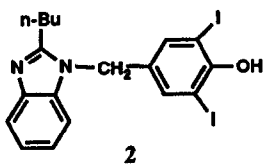

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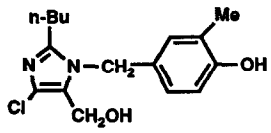

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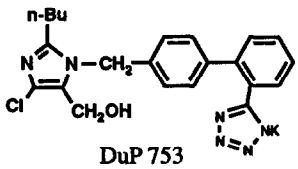


DuP 753

Among the compounds found to bind to AII receptor, we focused on the benzofuran derivative **1**[5] (IC_{50}: 14 μM), a synthetic intermediate of Amiodarone (anti-arrhythmic). Compound **1** has the following similarities in chemical structure to known AII antagonists, **3**[6] and DuP 753[7] ;

(1) A five membered hetero-aromatic ring

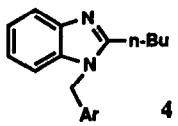
(2) An n-butyl substituent at the 2-position of the hetero-ring

(3) The hetero-ring is joined to the benzene ring via one carbon atom

(4) An acidic proton on the side chain

The replacement of the methanone group by the methylene group and the benzofuran ring by the benzimidazole ring in **1** afforded **2**. Compound **2** gave the high IC_{50} value of 1.5 μM. We assumed that the phenolic proton is essential for AII antagonistic activities. Therefore we investigated substituents of the phenol ring and the linkage between the benzimidazole ring and the phenol ring (Table 1).

Table 1 : Binding Affinities of 2-*n*-Butyl-1-hydroxy-phenylmethylbenzimidazole AII Antagonists



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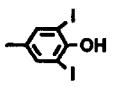
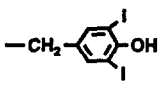
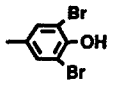
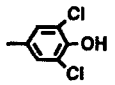
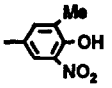
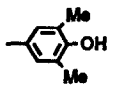
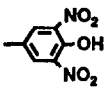
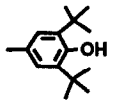
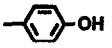
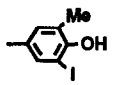
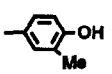
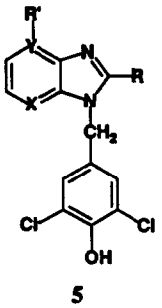
#	Ar	IC_{50}(μM)[4]	#	Ar	IC_{50}(μM)
2		1.5	**4f**		81
4a		2.5	**4g**		33
4b		3.2	**4h**		2.2
4c		4.7	**4i**		1.6
4d		>100	**4j**		8.5
4e		2.2	**4k**		20

The *m*-hydroxy-type compound **4g** had lower affinity than **2**, and the p-hydroxy group was preferable. 2,6-Disubstituted phenols showed higher affinity than mono or non-substituted phenols. Introduction of bulky

tert -butyl groups resulted in substantial loss of affinity. When the linkage between the benzimidazole ring and the phenol ring was replaced by the ethylene group **4f**, the affinity was reduced.

Our studies[8] and those of others[9] revealed the crucial role of R and R' on the benzimidazole and the imidazo[4,5-b]- pyridine in imparting high potency. The receptor binding activity of **2** was about two times higher than that of **4b**, but the *in vitro* and *in vivo* AII -antagonistic activities of **2** (pA_2[10] = 7.1, ED_2[11] = 8.0 mg/kg) were lower than those of **4b** (pA_2 = 7.2, ED_2 = 6.5 mg/kg). Thereafter, we evaluated the activities of compounds by means of both *in vitro* and *in vivo* experiments, in order to obtain an indication of the potential clinical usefulness of these compounds. On the basis of the above results, the compounds listed in Table 2 were synthesized and tested for *in vitro* and *in vivo* AII antagonistic activities.

Table 2 : *In Vitro* and *In Vivo* Data for 2,6-Dichlorophenol Derivatives



5

#	X	Y	R	R'	pA_2[10]	ED_2 i.v. (mg/kg)[11]
4b	CH	C	n-Bu	H	7.2	6.5
5a	CH	C	n-Bu	Me	7.7	1.3
5b	N	C	n-Bu	H	7.2	1.9
5c	CH	N	n-Bu	H	6.4	NT[a]
5d	N	C	n-Pr	H	7.5	0.65
5e	CH	N	n-Pr	H	6.1	NT
5f	N	C	Cyclopropyl	H	7.7	0.48
5g	CH	N	Cyclopropyl	H	6.2	NT
5h	N	C	n-Bu	Me	8.0	0.55
5i	N	C	n-Pr	Me	8.9	0.15
5j	N	C	Cyclopropyl	Me	8.7	0.079
DuP 753					8.6	0.28

a) NT = not tested

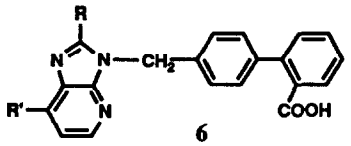
Introduction of a nitrogen atom at the X position increased the activities. On the other hand, introduction of a nitrogen atom at the Y position resulted in a significant decrease in potency. Judging from the pA_2 values[10], methylation (R'=Me) increased the antagonistic activity toward AII 6- to 25-fold. An n-propyl or cyclopropyl substituent at the 2-position gave higher potency than n-butyl. A cyclopropyl substituent showed higher *in vivo*[11] activity than an n-propyl substituent. Compounds **5i**, **5j** are more potent than DuP 753, but their duration of action is short.

With the aim of obtaining both long duration of action and high potency, we also synthesized biphenylmethyl derivatives. Here we will describe SARs for 2 and 7 substituents on the imidazo[4,5-b]pyridine.[9, 12]

Influence of substituents at the 7 position of imidazo[4,5-b]pyridine

Introduction of a methyl group at the 7-position resulted in a significant increase in potency both *in vitro* and *in vivo*, as with the phenol derivatives. The replacement of the methyl group by other groups (**6b**, **6d**, **6e**) was unfavorable (Table 3).

Table 3 : Effects of the 3H-Imidazo-[4,5-b]pyridine 7-Substituent on Biological Activities



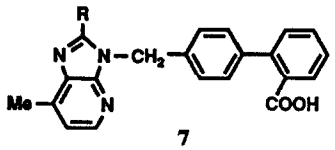
6

#	R'	R	pA_2[10]	ED_2 i.v. (mg/kg)[11]
6a	H	*n*-Pr	7.4	NT
6b	Cl	*n*-Pr	8.1	0.66
6c	Me	*n*-Pr	8.5	0.16
6d	COOH	Cyclopropyl	7.6	NT
6e	CH_2OH	Cyclopropyl	8.0	NT

Influence of substituents at the 2 position of imidazo[4,5-b]pyridine

In the cases of 4-chloroimidazole-5-acetic acid[6b], imidazole[13a] and benzimidazole[13b] derivatives, n-hexyl, n-pentyl and n-butyl at the 2 position are optimal for binding affinity, respectively. For 4-chloro-5-hydroxymethylimidazole derivatives[7], n-propyl was the best. On the other hand, n-propyl and ethyl on the imidazo[4,5-b]pyridine exhibited high activities[9] and the introduction of a cyclopropyl group led to the strongest activity, particularly in the *in vivo* study (Table 4).

Table 4 : Effects of the 7-Methyl-3H-imidazo-[4,5-b]pyridine 2-Substituent on Biological Activities

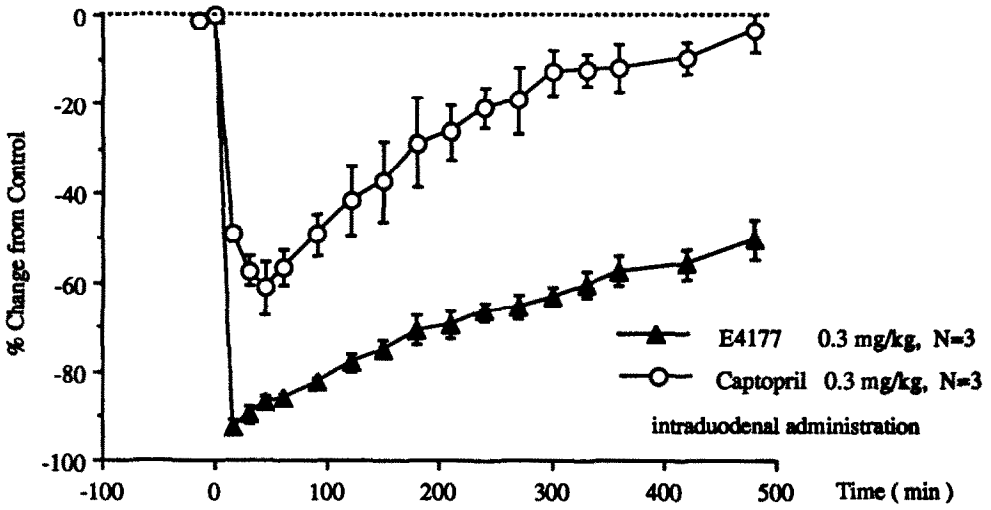


7

#	R	pA_2[10]	ED_2 i.v. (mg/kg)[11]
7a	Me	7.3	1.3
7b	Et	8.9	0.29
7c	*i*-Pr	7.5	0.84
7d	*n*-Pr	8.5	0.16
7e	*n*-Bu	8.1	0.45
E4177	Cyclopropyl	8.7	0.075
7f	Cyclobutyl	8.3	0.25
7g	$-CH_2OMe$	7.1	0.65
7h		8.3	0.35
7i	$-(CH_2)_3OH$	6.3	NT
7k	SMe	8.0	0.97
7l	SEt	8.3	0.41

E4177 (Table 4) was selected for a detailed study.[14] When administered intraduodenally to anesthetized dogs, E4177 showed a stronger and longer-lasting antagonistic activity for AII-induced pressor response than captopril (Figure 1).[15] This compound is currently undergoing clinical evaluation.

Figure 1. Inhibitory Effects of E4177 and Captopril on Pressor Response Induced by Angiotensin I in Anesthetized Dogs



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b) Related work, which appears to have been performed concurrently with this study, has been published: Kim, D.; Mantlo, N.B.; Tata, J.R.; Fitch, K.; Dhanoa, D.; Siegl, P.K.S.; Chang, R.S.L.; Chen,T.; Scheck, S.; Zingaro, G.J.; Kivlighn, S.D.; Greenlee, W.J. "Acidic Phenols; A New Class of Potent Nonpeptidic Angiotensin II Receptor Antagonists" *Bioorg. Med. Chem. Lett.* Symposia-in Print of Angiotensin II Antagonists
4. General procedure for IC_{50}: Specific binding of ^{125}I-[Ile^5]-AII to bovine adrenal cortex membranes was examined in a reaction mixture consisting of adrenal homogenate, ^{125}I-[Ile^5]-AII and test compound or assay buffer (control). The reaction mixture was incubated at 0°C overnight and binding was terminated by rapid filtration through Whatman GF/B glass fiber filters (Whatman International Ltd.). Nonspecific binding was measured in the presence of 1 μM unlabeled human AII.
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10. General procedure for pA_2: Rabbit aortic strips were suspended in tissue baths. Fifty mM KCl was added to induce contraction and the contractile tension was measured with an isometric force transducer. The peak contraction was taken as 100% contracture, and the tissue was washed. Thereafter, AII was added cumulatively to obtain a concentration-response curve. When studying the antagonistic activity of the test compounds, they were added 15 minutes before the addition of AII to observe the shift of the concentration-response curve to the right. The potency of the AII antagonists was evaluated as pA_2, which is defined as the negative common logarithm of the molar concentration of test compounds that shifts the concentration-response curve for AII two-fold to the right. pA_2 values were calculated according to van Rossum's method.
11. General procedure for ED_2: Wistar Kyoto male rats were anesthetized with pentobarbital, and the carotid and jugular were catheterized. The carotid catheter was connected to a pressure transducer and the mean arterial blood pressure was measured. Ganglions were blocked by 10 mg/kg i.v. of pentolinium. After the blood pressure stabilized, 0.003, 0.01, 0.03 or 0.1 μg/kg of AII was administered through the jugular catheter. Then, 0.1 to 10 mg/kg of test compound was administered intravenously, and again 0.003 to 1 μg/kg i.v. of AII was administered. The dose necessary for bringing about a doubled shift of the dose-pressor reaction curve to the right was taken as ED_2.
12. a) Roberts, D.A.; Ratcliffe, A.H.; Gibson, K.H.; Wood, R. *Eur.Pat.Appl.* 399,731, **1990**.
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15. General procedure: Mongrel dogs (10-20 kg) were anesthetized with ketamine hydrochloride, 10 mg/kg i.m. and thiopental sodium, 20 mg/kg i.v.. After tracheal intubation, respiration and anesthesia were maintained using an anesthesia ventilator with enflurane (0.8-1.5%) in a mixture of O_2 and N_2O (1:2). Blood pressure was measured by a micro tip catheter pressure transducer inserted from a femoral artery. Angiotensin I was administered at 300 ng/kg i.v. before the intraduodenal administration of E4177 or captopril (0.3 mg/kg) and subsequently at 15-60 min intervals for 8 hr.

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