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ACYLIMINOTHIADIAZOLINE DERIVATIVES: NEW, HIGHLY POTENT, AND ORALLY ACTIVE ANGIOTENSIN II RECEPTOR ANTAGONISTS

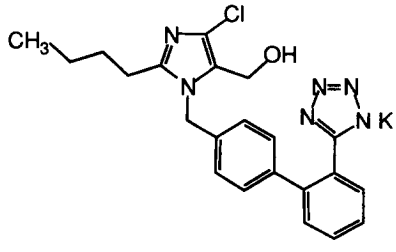
Terukage Hirata*, Jun Nomiyama, Nobuya Sakae, Kouji Nishimura, Masaharu Yokomoto, Satoshi Inoue, Koichi Tamura, Masayasu Okuhira, Hirotaka Amano, and Yoshimitsu Nagao†*

Institute for Medical Research, Wakunaga Pharmaceutical Co., Ltd., 1624 Shimokotachi, Koda-cho, Takata-gun, Hiroshima 739-11, Japan, †Faculty of Pharmaceutical Sciences, The University of Tokushima, Sho-machi, Tokushima 770, Japan

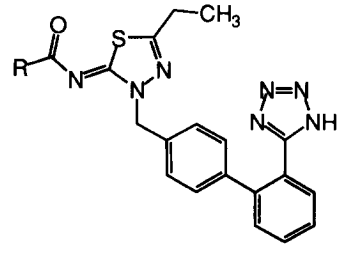
Abstract: Syntheses and pharmacological properties of a new series of acyliminothiadiazoline derivatives **1** are described. These compounds exhibited angiotensin II receptor antagonistic activities *in vitro* and *in vivo*. Among them, the compound **1g** (**KRH-594**) showed the strongest antihypertensive action in renal hypertensive rats after oral administration, and its oral bioavailability in dogs was also found to be high (73%).

The renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure and electrolyte balance.[1] The clinical success of angiotensin-converting enzyme (ACE) inhibitors[2] has proved that the inhibitors of RAS are useful for such diseases as hypertension and congestive heart failure. AII receptor antagonists, which interrupt the binding of AII to its cell surface receptor, act by specific blockade of RAS. The first orally active AII antagonist losartan (DuP 753)[3] was discovered by DuPont, and already launched as an antihypertensive agent in Europe.

In the course of our research for AII receptor antagonists, we found that a new series of acyliminothiadiazoline derivatives **1** exhibited a potent AII receptor antagonism and oral antihypertensive action. Although a large number of losartan analogs bearing other heterocyclic moieties in place of the imidazole have been reported,[4] the acyliminothiadiazoline moiety has never been employed. The chemical structure of acyliminothiadiazoline is unique and there are only a few examples to use this moiety as a part of pharmacologically active compound.[5] In this communication, we describe efficient syntheses and pharmacological properties of the acyliminothiadiazoline derivatives.



Losartan (DuP 753)



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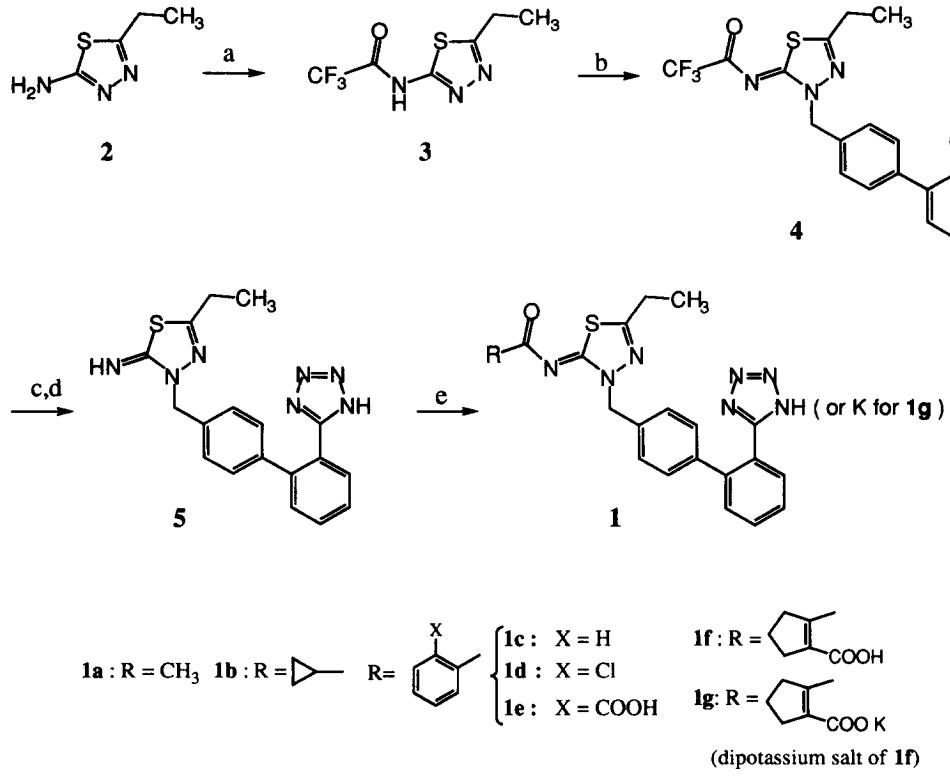
Chemistry

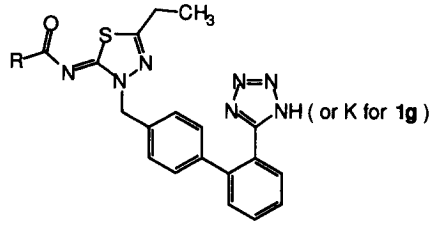
A straightforward synthetic pathway of **1a-f** starting from aminothiadiazole **2** is illustrated in Scheme 1. Acylation of aminothiadiazole **2** with trifluoroacetic anhydride gave trifluoroacetamidothiadiazole **3**. Regioselective preparation of the desired key intermediate, trifluoroacetyliminothiadiazoline **4** was efficiently achieved by exploitation of the trifluoroacetamido **3**.[6] Namely, treatment of **3** with commercially available 4-bromomethyl-2'-biphenylnitrile in the presence of K_2CO_3 in DMF afforded exclusively the compound **4** in 78% yield.[6] The cyano group of **4** was readily converted to a tetrazole group by reaction with trimethyltin azide (Me_3SnN_3) followed by detrifluoroacetylation with aq. NaOH to give iminothiadiazoline **5**. The compound **5** was then allowed to react with several acylchlorides or acid anhydrides to give the corresponding acyliminothiadiazolines **1a-f**. Dipotassium salt **1g**[7] was obtained by treatment of **1f** with ethanolic KOH in a quantitative yield.

Scheme 1



Reagents: (a) $(CF_3CO)_2O$, toluene, (88%); (b) 4-bromomethyl-2'-biphenylnitrile, K_2CO_3, DMF, (78%); (c) Me_3SnN_3, toluene, reflux and then conc. HCl; (d) aq. NaOH, THF, reflux, (58%, 2 steps); (e) RCOCl, pyridine CH_2Cl_2 or acid anhydride, DMF, (60-90%).

Table 1. Inhibitory Effects of AII Receptor Antagonists on Specific Binding of [^{125}I]AII and Pressor Response Induced by AII in Rats



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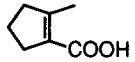
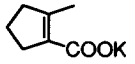
Compd.	R	Binding affinity[a] IC_{50} (nM) mean ± SEM[c]	Dose mg/kg, po	Inhibition of pressor response[b] mean ± SEM (%) at 6 h	at 24 h	n
1a	CH_3	7.20±1.0	3	11.3±13.9	-10.3±8.3	3
1b		6.50±1.5	3	7.8±14.4	-12.4±11.8	3
1c		37±4	3	4.9±1.1	-0.4±4.6	3
1d		3.60±0.5	3	21.3±7.9	-11.3±12.2	3
1e		0.66±0.04	3	48.1±7.6	23.2±7.0	3
1f		0.44±0.01	0.3	22.4±6.0	15.0±6.2	8
			1	36.4±10.3	36.6±8.8	4
			3	39.5±9.7	63.1±2.1	3
1g (KRH-594)		0.44±0.09	0.1	11.4±2.8	-0.7±5.4	5
			0.3	45.4±2.5	20.7±5.6	5
			1	72.1±4.2	57.0±3.6	5
Losartan (DuP 753)		9.8±1.4	1	19.3±5.4	7.3±4.6	5
			3	50.1±6.2	27.9±4.7	6
			10	74.2±3.9	57.4±5.5	5
EXP 3174[d]		0.59±0.11		N.T.[e]		

[a] Rat liver membranes (type 1 receptor); see ref. 9 for experimental details. [b] Percent inhibition of the AII (100 ng/kg, iv)-induced pressor response at 6 and 24 h after oral administration; see ref. 12 for experimental details. [c] n = 3. [d] EXP 3174 is an active metabolite of losartan. [e] N.T. for not tested.

Biological results and Discussion

The *in vitro* binding affinities of **1a-f** were determined by their ability to displace the specific binding [^{125}I]-

AII from receptors (type 1 receptor[8]) in rat liver membranes, and the results were summarized in Table 1.[9] The high binding affinity (IC_{50} = 7.2 nM) of compound **1a**, which we had first synthesized in this series, prompted us to prepare a large number of acyliminothiadiazoline derivatives. Replacement of the acetyl group in the compound **1a** by cyclopropylcarbonyl group, benzoyl group, or *ortho*-chlorobenzoyl group provided each corresponding compound **1b**, **1c**, or **1d**. The binding affinity of cyclopropyl carbonyl derivative **1b** was similar to that of the initial compound **1a**. Although benzoyl derivative **1c** was less active than acetyl derivative **1a**, surprisingly *ortho*-chlorobenzoyl derivative **1d** was found to increase the binding affinity.

It has been shown that several AII antagonists possessing two acidic groups exhibited high binding affinity.[10] Therefore, we replaced the chlorine atom in **1d** by carboxyl group. As anticipated, the binding affinity of *ortho*-carboxybenzoyl derivative **1e** was 6-fold higher than that of the *ortho*-chlorobenzoyl derivative **1d**. Consequently, replacement of the phenyl ring in **1e** with 1-cyclopentene provided compound **1f** and elevated the binding affinity. The binding affinity (IC_{50} = 0.44 nM) of **1f** was the highest among the test compounds as shown in Table 1.[11]

The *in vivo* potency of the acyliminothiadiazoline derivatives was evaluated by assessing the inhibition of pressor responses induced by AII (100 ng/kg, iv) in conscious normotensive rats.[12] The results are summarized in Table 1 and Fig. 1. Several compounds were orally active, among which the inhibitory effect of dipotassium salt **1g** was the most potent. Namely, **1g** was *ca.* 10-fold more potent than losartan and had a long duration as shown in Fig. 1. The antihypertensive effects of several acyliminothiadiazolines **1d-g** were also determined in renal hypertensive rats.[13] As shown in Table 2, **1g** exhibited the strongest antihypertensive action among the test compounds. The result was well consistent with that of Table 1.

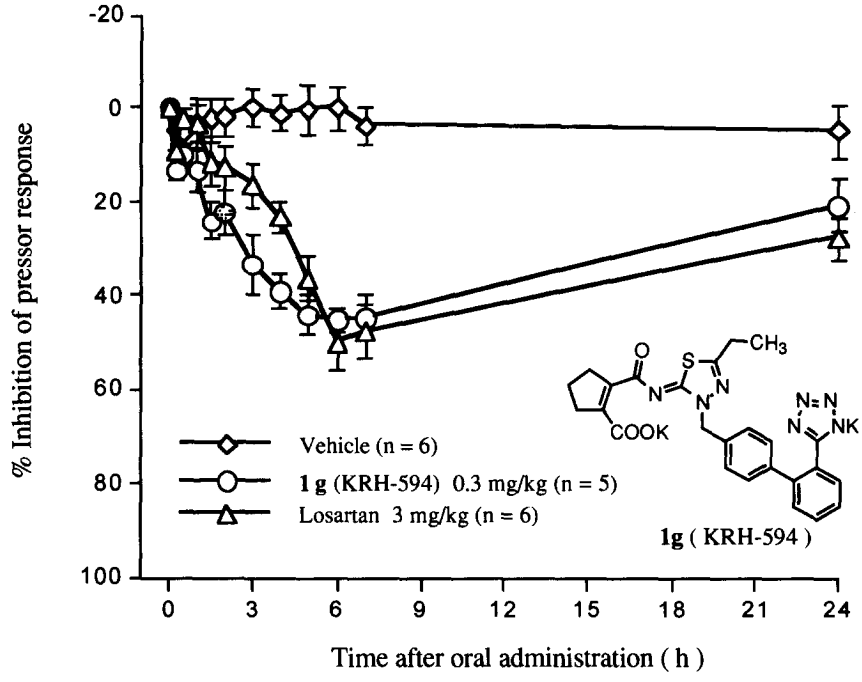
Subsequently, in order to investigate the possibility of **1g** as the antihypertensive agent, we examined the oral bioavailability (BA) of **1g** in dogs[14] and the oral BA of **1g** was found to be high (73%). Also, its active metabolite was not detected in this experiment suggesting that **1g** was not a prodrug such as losartan. Due to its high oral BA in dogs, we decided finally to select **1g** for further investigation as a clinical candidate.

Table 2. Antihypertensive Effect of Acyliminothiadiazolines in Renal Hypertensive Rats[a]

Compd.	Acyl group	Blood pressure reduction[b] Dose (mg/kg, po)	mean±SEM (%)	n
1d	o-Chlorobenzoyl	10	20.2±2.8	3
1e	o-Carboxybenzoyl	3	25.1±3.1	5
		10	33.8±5.5	8
1f	2-Carboxyl-1-	1	33.5±2.4	5
	cyclopentenecarbonyl	3	44.3±2.2	4
1g	2-Potassium oxycarbonyl	0.3	27.2±2.5	5
(KRH-594)	-1-cyclopentenecarbonyl	1	38.4±3.2	5
Losartan		3	27.4±2.6	4
(DuP 753)		10	40.4±3.1	5

[a] See ref. 13 for experimental details. [b] At peak.

Figure 1. Inhibitory Effects of Oral Administration of 1g (KRH-594) and Losartan on AII-induced Pressor Response in Conscious Normotensive Rats. Data represent the mean±SEM



Conclusion

In conclusion, we clarified that the acyliminothiadiazoline derivatives **1** were new and highly potent AII receptor antagonists. Among them, compound **1g** (KRH-594) exhibited the most potent AII receptor antagonistic activity in *in vitro* and *in vivo* and the good oral efficacy in renal hypertensive rats. Here, it is characteristic that **1g** is not a prodrug and the oral BA of **1g** in dogs is among the highest class in those of the AII receptor antagonists on development. Thus, **1g** has been chosen as a hopeful candidate toward the clinical evaluation for hypertension on the basis of its pharmacological profiles.

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(1) (a) Timmermans, P. B. M. W. M.; Wong, P. C.; Chiu, A. T.; Herblin, W. F. *Trend. Pharmacol. Sci.* **1991**, *12*, 25-62.
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(4) (a) Wexler, R. R.; Greenlee, W. J.; Irvin, J. D.; Goldberg, M. R.; Prendergast, K.; Smith, R. D.; Timmermans, P. B. M. W. M. *J. Med. Chem.*, **1996**, *39*, 625.

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(6) In general, alkylation of 2-acylamido-1,3,4-thiadiazoles except for 2-trifluoroacetamido derivatives **3** gives a mixture of the endo *N*- and exo *N*-alkylated products. See, Hirata, T.; Shiro, M.; Nagao, Y. *Heterocycles* accepted for publication.

(7) Physical data for compound **1g** (KRH-594) are described as follows. mp >280 ℃ (EtOH-H_2O); ^1H NMR (270 MHz, D_2O) δ 1.23 (t, J = 7.7 Hz, 3 H), 1.95-2.00 (m, 2 H), 2.66-2.86 (m, 6 H), 5.49 (s, 2 H), 7.04 (d, J = 8.0 Hz, 2 H), 7.31 (d, J = 8.0 Hz, 2 H), 7.37-7.39 (m, 1 H), 7.51-7.62 (m, 3 H); IR (KBr) 1642, 1570 cm^{-1}; Anal. Calcd for $C_{25}H_{21}N_7O_3SK_2$, C : 51.97, H : 3.66, N : 16.97, Found, C : 51.90, H : 3.70, N : 16.97.

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(9) The binding assay was peformed by the method described in ref. 9. Robertson, M. J.; Barnes, J. C.; Drew, G. M.; Clark, K. L.; Marshall, F. H.; Michel, A.; Middlemiss, D.; Ross, B. C.; Scopes, D.; Dowle, M. D. *Br. J. Pharmacol.* **1992**, *107*, 1173.

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(11) The structure-activity relationships at the 5'-position of thiadiazoline ring were also investigated. The derivatives having an ethyl substituent were found to exhibit the highest binding affinity.

(12) Protocol for *in vivo* testing in conscious rats: Male Sprague-Dawley rats aged 8-9 weeks and weighing 250-350 g (Charles River Laboratories) were anesthetized with pentobarbital (40 mg/kg, ip), and the left femoral artery and vein were cannulated. The femoral artery catheter was connected to a pressure transducer (TP400T, Nihon Kohden, Tokyo Japan) coupled to a polygraph monitor mean arterial blood pressure. AII (100 ng/kg) was injected three times in the femoral vein to establish the control response. Test compounds were then administered orally at a constant volume of 5 ml/kg. Thereafter, AII was injected repeatedly at given times. The pressor response to AII at each dose of the test compounds was compared with that obtained for the pretreatment control.

(13) Protocol for *in vivo* testing in RHRs: Male Sprague-Dawley rats aged 6 weeks and weighing 250-350 g (Charles River Laboratories) were anesthetized with pentobarbital (40 mg/kg, ip). The left kideny artery was constricted with a silver clip (internal diameter 0.43 mm) and the right kidney was left intact. 4-7 weeks after the constriction, the animals were anesthetized with pentobarbital, and the left femoral artery was cannulated.The rats were permitted to recover overnight from anesthesia and allowed regular rat chow and water ad libitum. The experiments were started the next day. Their blood pressure was measured as described in ref. 12. The rats with mean arterial pressures of 160 to 190 mmHg were used. Test compounds were then administered orally (po) at a constant volume 5 ml/kg.

(14) The oral bioavailability of **1g** in fasted beagle dogs was calculated as AUCpo/AUCiv x100. Serum concentrations of **1g** after a single oral or intravenous administration (3 mg/kg) were determined by reverse phase high-performance liquid chromatography.

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