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TRIAZOLINONES AS NONPEPTIDE ANGIOTENSIN II ANTAGONISTS. 2. DISCOVERY OF A POTENT AND ORALLY ACTIVE TRIAZOLINONE ACYLSULFONAMIDE[1]

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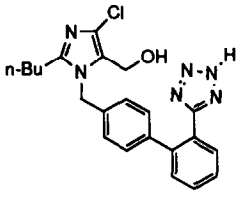
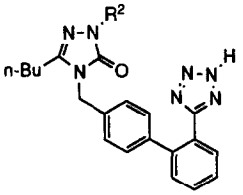
Abstract: A series of trisubstituted triazolinones with a [2'-(N-acylsulfamoyl)biphenyl-4-yl]methyl side chain at N^4 has been prepared. The inhibition of AII pressor responses by these potent AT_1-selective AII antagonists indicated some of them to be superior *in vivo* to their tetrazole counterparts. At 1 mg/kg, **3d** (L-159,913) was effective orally with >4 h duration in dogs and had significant efficacy with >10 h duration i.v. in chimpanzees.

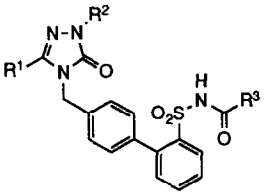
The renin angiotensin system (RAS), which plays a central role in the regulation of blood pressure and electrolyte balance, has angiotensin II (AII) as its principal active hormone.[2] The blockade of the RAS in antihypertensive therapy via angiotensin converting enzyme (ACE) inhibitors is well documented.[3] The rationale for the use of an AII receptor antagonist as an alternative to ACE inhibitors in the treatment of hypertension has been discussed.[4] A number of highly active non-peptide AII antagonists have been reported[5] subsequent to the discovery of losartan (DuP 753, MK-954, **1**),[6] which is in Phase III clinical trials. Recently, we described a series of 2,4-dihydro-3*H*-1,2,4-triazol-3-ones (triazolinones) bearing a (2'-tetrazolylbiphenyl-4-yl)methyl side chain at N^4, such as **2**, as potent AII antagonists.[1a] In order to improve the *in vitro* and/or *in vivo* properties of this class of AII antagonists, we considered replacing the tetrazole by other carboxylic acid bioisosteres such as acylsulfonamides. This substitution seemed reasonable, considering the pK_a of sulfabenzamide (4.6)[7] and that of 5-aryl tetrazoles (estimated to be 5-6).[6a,b] The exchange of tetrazoles by acylsulfonamides has been reported for imidazole-based[8a] and imidazopyridine-based[8b] AII antagonists. In this communication, we describe structure-activity relationship (SAR) studies of a series of triazolinone acylsulfonamides, **3**, leading to a potent and orally active compound selective for the AT_1 receptor.[9]



1 (Losartan, DuP 753)



2: R^2 = aryl, alkyl

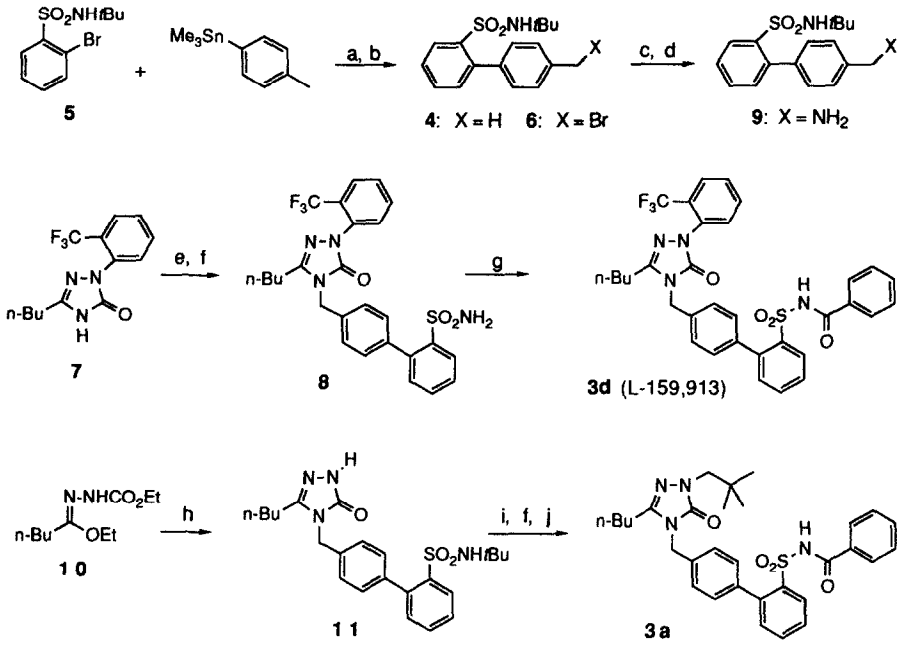


3

Chemistry

Compounds **3a-h** (Table 1) were synthesized according to one of two routes, depending on the nature of R^2 in **3**. For either route, the biaryl compound **4** was required. This material was prepared in 70% yield[8a] by a Pd(II)-catalyzed cross-coupling reaction between 2-bromobenzenesulfonamide **5** and *p*-toyltrimethyltin.[10] Bromination

of **4** provided the biarylmethyl bromide **6**. Alkylation of the previously described triazolinone **7**[1a] with compound **6** provided the free sulfonamide **8**, after removal of the *t*-butyl group by TFA. In the example shown, acylation of this material with benzoyl chloride under standard conditions provided the desired N^2-aryl triazolinone acylsulfonamide **3d**.[11] For the preparation of N^2-alkyl compounds, the intermediate **6** was derivatized to the corresponding amine **9** via reduction of the intermediate azide. Reaction of **9** with the substituted hydrazone **10**[1a] provided the corresponding triazolinone **11**, unsubstituted at N^2. Alkylation of **11**[12] followed by removal of the *t*-butyl group and acylation of the free sulfonamide[8b] provided the desired N^2-alkyltriazolinone acylsulfonamide **3a**.



a: $Pd(PPh_3)_2Cl_2$, DMF, 90°C, 6 h; 70%. b: NBS, AIBN, CCl_4, reflux, 4 h; 78%. c: LiN_3, DMSO; 72%.

d: PPh_3, H_2O, THF; 86%. e: NaH, DMF, **6**; 74%. f: TFA, anisole, rt; 85%. g: PhCOCl, pyr, rt; 75%.

h: **9**, EtOH, 80°C; 47%. i: NaH, DMF, $I\text{-}CH_2CMe_3$, 90°C; 74%. j: $PhCO_2H$, Im_2CO, DBU, THF; 96%.

In Vitro and *In Vivo* Structure-Activity Relationships

Triazolinones **3a-h** (Table 1) were assessed as AII antagonists *in vitro* by their ability to competitively block specific binding of the radioligand $^{125}I[Sar^1,Ile^8]AII$ to the AT_1 receptors in a rabbit aorta membrane preparation as previously described.[13] The inhibition of the pressor response to exogenous AII challenge in conscious, normotensive rats was evaluated according to established protocols.[14,13c] Initially, we assayed four benzoylsulfonamides, **3a-d**, and compared them to the corresponding tetrazoles **2a-d**.[1a] As shown in Table 1, several benzoylsulfonamides were more potent *in vitro* than the corresponding tetrazoles, leading to a subnanomolar compound **3d**. *In vivo* at 1 mg/kg i.v., the benzoylsulfonamides in the first two pairs were less

potent and had shorter duration of action than the corresponding tetrazoles, but in the last two pairs, they showed better duration of action than the tetrazoles. Based on these data, **3d** was chosen for further derivatization.

TABLE 1. SAR OF TRIAZOLINONE ACYLSULFONAMIDES AND TETRAZOLES

				In Vitro Binding[a]	In Vivo Functional Assay[b]		
Compound	R^1	R^2	R^3	AT_1 IC_{50} (nM)	Dose (mg/kg)/ Route	Peak Inhibition,%	Duration, (h)
2a[c]	——	CH_2CMe_3	——	2.1	1.0 / i.v.	92±4	3.2±1.8
3a	n-Bu	CH_2CMe_3	C_6H_5	3.3	1.0 / i.v.	50±3	0.5±0
2b[c]	——	(2-Cl)C_6H_4	——	2.4[d]	1.0 / i.v.	80±3	0.5±0.1
3b	n-Bu	(2-Cl)C_6H_4	C_6H_5	1.4	1.0 / i.v.	28±12	<1
2c[c]	——	(2,6-diCl)C_6H_3	——	5.8[d]	1.0 / i.v.	80±2	0.9±0.1
3c	n-Bu	(2,6-diCl)C_6H_3	C_6H_5	2.6	1.0 / i.v.	63±0	1.5±0.5
2d[c]	——	(2-CF_3)C_6H_4	——	0.78	1.0 / i.v.	73±7	1.5±0.6
3d	n-Bu	(2-CF_3)C_6H_4	C_6H_5	0.43	1.0 / i.v.	73±6	3.5±1.5
					1.0 / p.o.	65±2	>3.5
3e	n-Bu	(2-CF_3)C_6H_4	$COCF_3$	0.80	1.0 / i.v.	82±3	0.5±0
3f	n-Bu	(2-CF_3)C_6H_4	$COCHMe_2$	0.80	1.0 / i.v.	100±0	>6
					1.0 / p.o.	45±1	1.3±0
3g	n-Bu	(2-CF_3)C_6H_4	$(CH_2)_6CH_3$	2.9	1.0 / i.v.	NA[e]	NA
3h	n-Bu	(2-CF_3)C_6H_4	$CH_2C_6H_5$	1.6	1.0 / i.v.	48±5	0.2±0.04

a: Displacement of specifically-bound ^{125}I[Sar^1,Ile^8]AII from a rabbit aorta membrane AT_1 receptor preparation.
b: Inhibition of pressor response induced by exogenously administered AII (0.1 μg/kg i.v.) in conscious normotensive rats.
c: This compound was reported in reference 1a. d: 0.2% BSA was present in the binding assay buffer. e: Not Active

Data from compounds **3d-h** show some SAR at the acylsulfonamide site.[15] Small aliphatic R^3 groups with differing electronic properties such as trifluoromethyl and *i*-propyl resulted in analogs with subnanomolar potency, while more extended groups (**3g-h**) were less favored. *In vivo*, the trifluoroacetyl analog **3e** was very effective at 1 mg/kg but it had short duration of action, as was the case with the phenylacetyl derivative **3h**. Compound **3g**, containing a long aliphatic chain at R^3, was not active at this dose. The *i*-butyryl compound **3f** was very effective i.v. but had an unsatisfactory p.o. profile. Based on its *in vitro* and *in vivo* properties in the rat, compound **3d** was selected for further evaluation.

Table 2: In Vitro IC_{50} (nM) Values of 3d (L-159,913) on ^{125}I[Sar^1,Ile^8]AII Binding in Various Tissues

Receptor Subtype	AT_1			AT_2
SOURCE / TISSUE	Rabbit	Rat	Human	Rat
Aorta	0.43	NT[a]	NT	NT
Adrenal	0.50	0.33	2.5[b]	270
Brain	0.55	0.31	NT	300

a: NT = not tested. b: in the presence of 0.2% BSA

In Vitro and In Vivo Pharmacology of 3d (L-159,913)

The *in vitro* binding affinities of the triazolinone N-benzoylsulfonamide **3d** (L-159,913) at the AT_1 receptor were consistently subnanomolar in adrenal and brain tissue preparations from the rabbit and the rat (Table 2).[13] This compound was a reversible and apparently competitive antagonist of the vascular (rabbit aorta) AT_1 receptor as determined by Scatchard analysis of the specific binding

of ^{125}I[Sar^1,Ile^8]AII,[13b] with an inhibition constant (K_i) of 1.7 nM.[16] The AT_2 IC_{50} value of this compound in a rat midbrain preparation[17] was determined to be 300 nM, making it selective for the AT_1 receptor. However, compared to the corresponding tetrazolyl compound **2d** (AT_2 IC_{50} = 23 μM), the benzoylsulfonamide **3d** was 75-fold more potent for the AT_2 receptor. In terms of specificity for AII receptors, **3d** was inactive in radioligand binding assays at high concentrations (>1μM) for the oxytocin, endothelin, vasopressin or neurotensin receptors. In *in vitro* functional assays,[13b] **3d** demonstrated specificity for antagonism of contractions produced by AII in the rat pulmonary artery: at a concentration (2.0 nM) effective for AII antagonism, the concentration-response curves or maximal contractile responses to epinephrine were not significantly affected.

TABLE 3. INHIBITION OF PRESSOR RESPONSE TO EXOGENOUS AII[a] BY 3d (L-159,913)

A: *IN VIVO* POTENCIES (Conscious Animal Models): ED_{50} (mg/kg)[b]

	Rat		Rhesus Monkey		Dog	
Compound	i.v.	p.o.	i.v.	p.o.	i.v.	p.o.
3d (L-159,913)	0.51 (0.44-0.58)	0.72 (0.56-0.91)	0.16 (0.11-0.22)	5.7 (4.8-6.8)	0.073 (0.058-0.090)	0.86 (0.66-1.3)
Losartan	0.28 (0.15-0.50)	0.66 (0.44-0.98)	0 32 (0.18-0.58)	10 (7.0-15)	ND[c]	ND

B: INTRAVENOUS DURATION OF ACTION

	Conscious Rat			Conscious Rh. Monkey			Conscious Dog			Anesthetized Chimpanzee			
		Peak			Peak			Peak			Inhibition, %			
Compound	Dose[d]	Inh.%	Dur[e]	Dose	Inh.%	Dur	Dose	Inh.%	Dur	Dose	3h[f]	10h	24h
3d (L-159,913)	1.0	73	3.5	0.3	80	0 6	1.0	100	>6	1 0	77	49	19
Losartan	1.0	78	>6	1 0	76	0.6	3.0	76	ND	1.0	66	44	13

[a]: A bolus dose of 0.1 μg/kg i v. of AII was used AII receptor inhibition was assessed by percentage of inhibition of AII-induced pressor responses.
[b]: ED_{50} values were calculated from responses with two or three doses of each compound; 95% confidence levels are presented in parentheses.
[c]: ND = Not Determined. The active metabolite of losartan is not readily formed in the dog.
[d]: Dose in mg/kg. [e]: Duration in hours [f]: Time (hours) post i.v. injection of test compound.

In a conscious rat model, by oral or intravenous administration, **3d** (L-159,913) inhibited AII-induced pressor responses without changing basal blood pressure, heart rate or the pressor response to methoxamine. The inhibition of pressor response to exogenous AII challenges by **3d** was examined in the conscious normotensive rat, rhesus monkey, and dog (Table 3A) according to protocols described previously.[14] In the rat, at 1 mg/kg p.o., this compound exhibited 65% peak inhibition with >3.5 h duration of action. Compared to losartan, it was somewhat less potent but had a p.o./i.v. ratio of 1.4 vs. 2.4 for losartan. The oral bioavailability of **3d** was determined to be 44%[18] in the rat, which compares favorably with 33% for losartan.[6d] In the rhesus monkey, at 10 mg/kg p.o., **3d** showed 76% peak inhibition of the pressor response with >4 <24 h duration of action. In this model, it was more potent than losartan and it demonstrated oral bioavailability equivalent to that of losartan,

based on the p.o./i.v. ratio (Table 3A, B). However, both compounds had short duration of action i.v. in the monkey. In the dog, **3d** was quite potent following intravenous and oral administration. At 1.0 mg/kg p.o., it produced >4 <24 h duration of action with a 73% peak inhibition of the AII pressor response. At 1.0 mg/kg i.v., it demonstrated 100% peak inhibition of the pressor response and a duration of action of >6 <24 h (Table 3B). At 1.0 mg/kg i.v., **3d** was a selective and potent inhibitor of AII-induced pressor response in anesthetized chimpanzees.[19] The time-response profile for **3d** was similar to that of losartan. At 10 h post-i.v. injection, this compound was still active with 49% inhibition of basal AII pressor response. In this model, both losartan and **3d** had >10 h duration of action (Table 3B).

In summary, we have prepared and evaluated as AII antagonists a series of trisubstituted triazolinones with a [[2'-(N-acylsulfamoyl)biphenyl-4-yl]methyl] side chain at N^4 (**3**) and discovered a potent compound L-159,913 (**3d**), which was characterized by the following: selectivity for the AT_1 receptor with subnanomolar IC_{50} values; oral activity with good duration of action in conscious rat, dog, and rhesus monkey models; 44% oral bioavailability in the rat; significant efficacy and >10 h duration in anesthetized chimpanzees at 1 mg/kg i.v. Overall, this compound compared well with losartan but is structurally distinct from it and is not a prodrug. It has been selected for further investigations.

Acknowledgments

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11. a) This and all other compounds discussed were characterized by mp, mass spectrum (FAB), 300 MHz or 400MHz ^{1}H-NMR, and elemental analyses (C, H, N; within ±0.4% of theoretical values) or high resolution mass spectrum. b) The chemical characterization of **3d**, L-159,913, is as follows: mp 91-93°C; FAB-MS *m/e* 673 $(M+K)^+$. 300 MHz ^{1}H-NMR ($CDCl_3$) δ 0.87 (t, *J*=7.3 Hz, 3H), 1.36 (m, 2H), 1.63 (m, 2H), 2.47 (t, *J*=7.5 Hz, 2H), 4.86 (s, 2H), 7.11 (d, *J*=7.8 Hz, 2H), 7.2-7.7 (m, 13H), 7.75 (d, *J*=7.3 Hz, 1H), 8.25 (br s, 1H), 8.39 (d, *J*=7.4 Hz, 1H). Anal. ($C_{33}H_{29}F_3N_4O_4S \cdot 0.33CH_2Cl_2$) C, H, N.

12. In this step, the product isolated was assigned as the N-alkylated compound based on comparison of ^{1}H-NMR chemical shifts with related compounds from the tetrazole series (see ref. 1a).

13. a) Except where noted, no BSA was added to the binding assay buffer (see ref 1a, and 13c). The standard error (expressed as percent of mean) of the IC_{50} measurement in this assay has been estimated to be <30%, based on the results of several standard compounds having 3 or more determinations. In some cases, the reported IC_{50} values represent an average of two or more determinations from separate assays. b) Chang, R. S. L.; Siegl, P. K. S.; Clineschmidt, B. V.; Mantlo, N. B.; Chakravarty, P. K.; Greenlee, W. J.; Patchett, A. A.; Lotti, V. J. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 133; c) Ashton, W. T.; Cantone, C. L.; Chang, L. L.; Hutchins, S. M.; Strelitz, R. A.; MacCoss, M.; Chang, R. S. L.; Lotti, V. J.; Faust, K. A.; Chen, T.-B.; Bunting, P.; Schorn, T. W.; Kivlighn, S. D.; Siegl, P. K. S. *J. Med. Chem.* **1993**, *36*, 591.

14. a) Male Sprague-Dawley rats were used. At least two animals were evaluated in all cases. A ≥30% inhibition of the AII pressor response was considered significant in this assay. The duration of action for a single bolus dose of the test compound was defined as the time from onset of activity until the inhibition of the AII-induced increase in mean arterial pressure fell below 30% and remained at <30% for two subsequent AII challenges. ED_{50} values were calculated from responses with at least three doses of each compound. b) Siegl, P. K. S.; Chang, R. S. L.; Mantlo, N. B.; Chakravarty, P. K.; Ondeyka, D. L.; Greenlee, W. J.; Patchett, A. A.; Sweet, C. S.; Lotti, V. J. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 139.

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16. These assays were run in the presence of 0.2% bovine serum albumin (BSA). The AT_1 IC_{50} value (rabbit aorta) of **3d** in the presence of 0.2% BSA was 3 nM.

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18. The oral bioavailability of [^{3}H]L-159,913 in male Sprague-Dawley rats was calculated as AUC_{oral}/AUC_{iv} using plasma levels for the 24 h period after oral and i.v. administratration (5 mg/kg) in water. Stearns, R. A.; Miller, R. R.; Chiu, S. H. L. Unpublished results.

19. The test compound was injected as an i.v. bolus (1.0 mg/kg) after establishing reproducible baseline pressor responses to Arg^8-vasopressin (AVP, 0.05 μg/kg) and AII (0.1 μg/kg). AII was given at fixed time intervals of 15- or 30-min until 180 min and at 10 h and 24 h post-i.v. injection of the test compound. From measurement of the change in mean arterial pressure (ΔMAP) upon AII challenge, the percent inhibition of the AII pressor response in the presence of test compound was calculated at each time point. AVP challenges were used as controls to confirm specificity of the AII receptor antagonist.

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