Triazolinones as Nonpeptide Angiotensin II Antagonists. 1. Synthesis and Evaluation of Potent 2,4,5-Trisubstituted Triazolinones[1]

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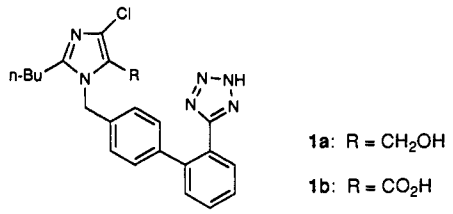
A series of 2,4-dihydro-2,4,5-trisubstituted-3*H*-1,2,4-triazol-3-ones was prepared via several synthetic routes and evaluated as AII receptor antagonists in vitro and in vivo. The preferred compounds contained a [2′-(5-tetrazolyl)biphenyl-4-yl]methyl side chain at N^4 and an n-butyl group at C^5. A number of these bearing an alkyl or aralkyl substituent at N^2 showed in vitro potency in the nanomolar range (rabbit aorta membrane receptor), and several of these, e.g., the 2,2-dimethyl-1-propyl analogue (**54**, IC_{50} = 2.1 nM), effectively blocked the AII pressor response in conscious rats with significant duration (2.5 h at 1 mg/kg orally for **54**). Among analogues possessing aryl substituents at N^2, *ortho* substitution on the phenyl moiety resulted in several derivatives with in vitro potency in the low nanomolar range. One of these, featuring a 2-(trifluoromethyl)phenyl substituent at N^2 (**25**, IC_{50} = 1.2 nM), was effective at 1 mg/kg orally in the rat model, with a duration of >6 h. Implications for hydrophobic and hydrogen-bonding interactions with the AT_1 receptor are discussed.

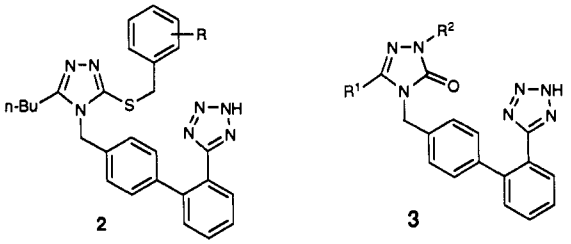
The octapeptide angiotensin II (AII), a powerful vasoconstrictor, is the active hormone of the renin–angiotensin system (RAS).[2] In addition to vasoconstriction, the physiological actions of AII include the regulation of aldosterone secretion and renal reabsorption of sodium. The RAS plays a central role in the regulation of blood pressure and electrolyte balance. The inhibition of this system has been an important target in antihypertension therapy.[3,4] This is exemplified by the success of angiotensin converting enzyme (ACE) inhibitors captopril[5] and enalapril[6] which block the conversion of angiotensin I (AI) to AII. However, in addition to AI, ACE has other substrates including bradykinin and enkephalins.[3,4] Some of the occasional side effects of ACE inhibitors such as dry cough and angioedema have been attributed to this lack of specificity.[7]

The inhibition of the enzyme renin, which acts on angiotensinogen to produce AI, has also been explored extensively.[3] Unfortunately, many potent renin inhibitors suffer from poor oral bioavailability and/or limited duration of action.[3] Since the effects of AII are mediated through cell-surface receptors located on various target organs, inhibition of the RAS at the level of the AII-cell surface receptor interaction could provide a specific approach to block the system with minimal potential side effects.[4] In fact, peptide AII antagonists such as [Phe[4], Tyr[8]]AII[8] and [Sar[1],Ala[8]]AII (saralasin)[9] have been known for some time. However, in addition to being partial agonists, they exhibit poor oral activity and short duration of action.[4] Subsequent to the disclosure by Takeda Laboratories of mildly active imidazole-based nonpeptide AII antagonists,[10] pioneering efforts of the Du Pont group have yielded the clinical candidate losartan, (**1a**, DuP 753)[4b,11-13] and its active metabolite **1b** (EXP3174).[12,14] Both of these compounds are selective for the AT_1 receptor subtype, the site responsible for most of the known physiological effects of AII.[15] More recently, additional



potent AII antagonists from a series of compounds containing imidazo[4,5-*b*]pyridine as the heterocycle (e.g., L-158,809)[16] and another series containing (biphenylylmethoxy)quinoline as the heterocycle (e.g., ICI D8731)[17] have been reported.

In our investigations, we sought to replace the imidazole moiety of **1a,b** by another heterocycle. We have reported potent AII antagonism for certain derivatives of 4*H*-1,2,4-triazoles such as **2**.[18] Initial observations in that series led



to the hypothesis that the aryl portion of the 5-[(arylmethyl)thio] substituents in **2** could interact with a hydrophobic binding region on the AT_1 receptor. To further investigate this issue, 2,4-dihydro-3*H*-1,2,4-triazol-3-ones (triazolinones), exemplified by **3**, were conceived as novel AII antagonists. In these molecules, judicious variations of the N^2-substituent (R^2) could result in a better understanding of the nature of the postulated interaction with the AT_1 receptor in spatial regions adjacent to this position of the heterocyclic ring, an issue thus far not adequately addressed. The oxo moiety at C^3 of **3** was designed as a surrogate for either the sulfur linkage in **2** or the carboxy group in **1b**. The oxo substitution was

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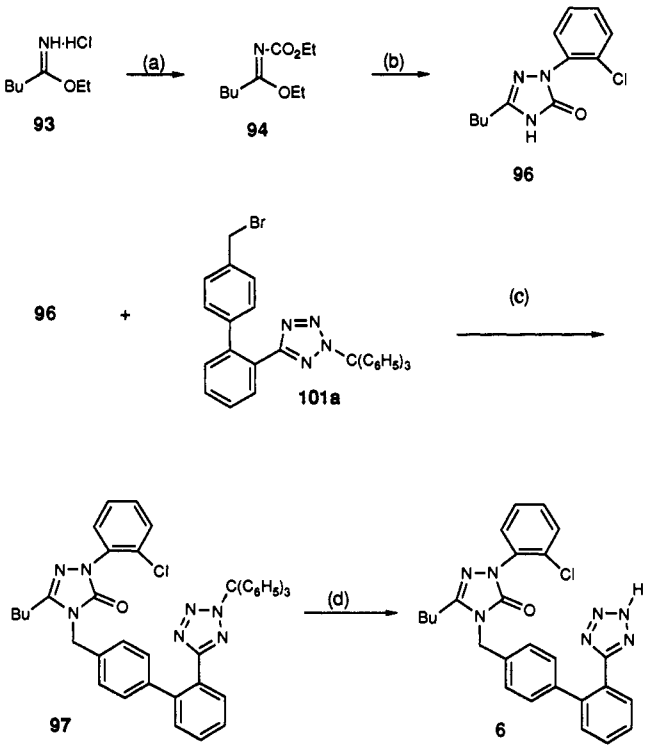
Scheme I[a]



[a] Key: (a) Na_2CO_3, Et_2O; $ClCO_2Et$, NEt_3, CH_2Cl_2, −10 to 20 °C; (b) 2-chlorophenylhydrazine, toluene, 45–50 °C; NEt_3, 90 °C; (c) NaH, DMF, 35 °C; (d) $HOAc/H_2O$, 65 °C.

deemed reasonable in light of some preliminary results from a series of quinazolinone AII antagonists.[19] Therefore, we prepared and evaluated as AII antagonists a series of 2,4,5-trisubstituted triazolinones (**4–92**) related to **3**. In addition to our effort,[20] triazolinone AII antagonists have recently been disclosed by other laboratories.[21,22]

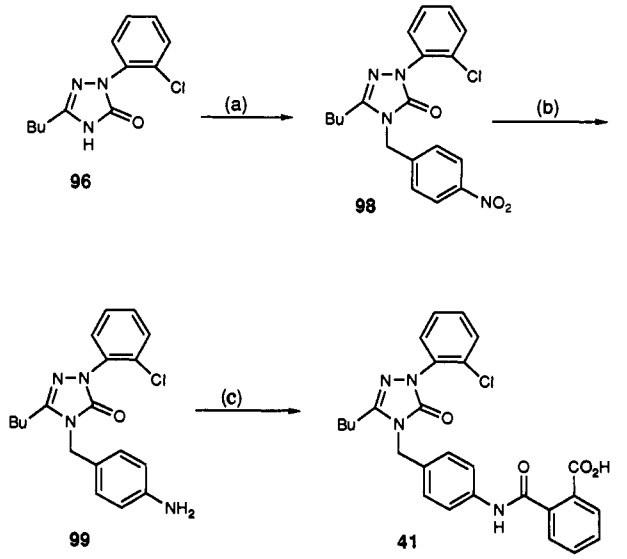
Chemistry

Initially, synthetic efforts focused on the preparation of triazolinones bearing an aryl side chain at N^2 of the triazolinone ring and a (2′-tetrazolylbiphenyl-4-yl)methyl side chain at N^4. The latter structural feature is also present in the reference compounds **1a** and **1b**. In this series, the desired analogues were constructed with the appropriate N^2-aryl moiety in place, followed by elaborations at the N^4 position as indicated in Scheme I. In the example shown, the requisite imidate hydrochloride salt **93**[23] was converted to ethyl *N*-carbethoxyvalerimidate **94** and reacted with 2-chlorophenylhydrazine (**95**)[24] and triethylamine to afford the desired triazolinone **96** in a one-pot reaction.[25] Attachment of the N^4 side chain by alkylation with 5-[4′-(bromomethyl)biphenyl-2-yl]-*N*-trityltetrazole (**101a**)[12] afforded **97**, which was converted[12] to the free tetrazole to yield the target compound **6**.

An example of a triazolinone bearing a 4-[(2-carboxybenzoyl)amino]benzyl side chain at N^4, as in the Du Pont imidazole derivative (EXP6803),[26] was also prepared. As indicated in Scheme II, alkylating the triazolinone **96** with 4-nitrobenzyl bromide gave **98**. Stannous chloride reduction of the nitro group followed by treatment of the resulting aniline **99** with phthalic anhydride afforded the desired analogue **41**.

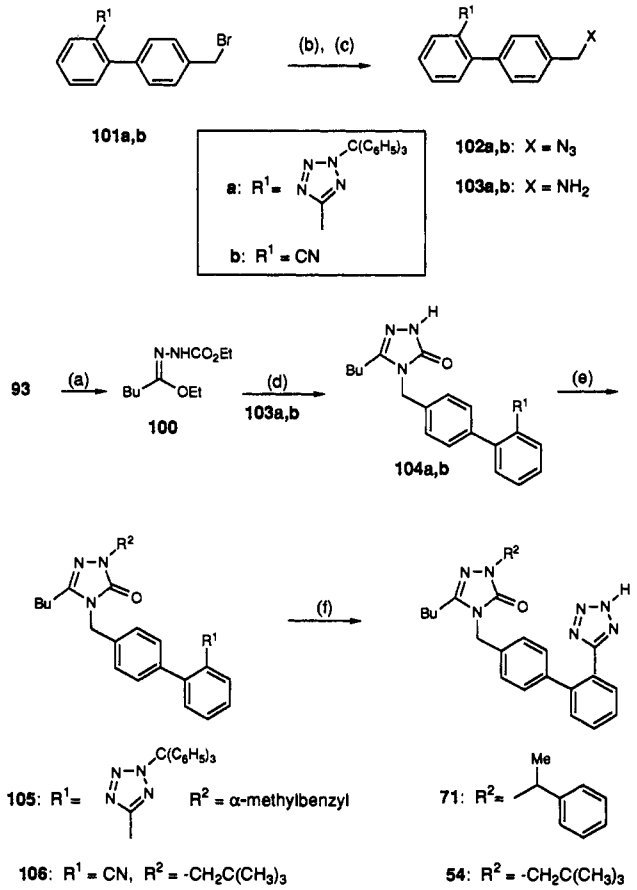
For most of the analogues with nonaryl side chains at N^2, the triazolinone ring was constructed with the appropriately derivatized biphenylylmethyl side chain at N^4 in place, followed by the attachment of the N^2 substituent,

Scheme II[a]



[a] Key: (a) NaH, DMF, 4-nitrobenzyl bromide, 20 °C; (b) $SnCl_2$, HCl, THF, 0–20 °C; (c) phthalic anhydride, THF, 20 °C.

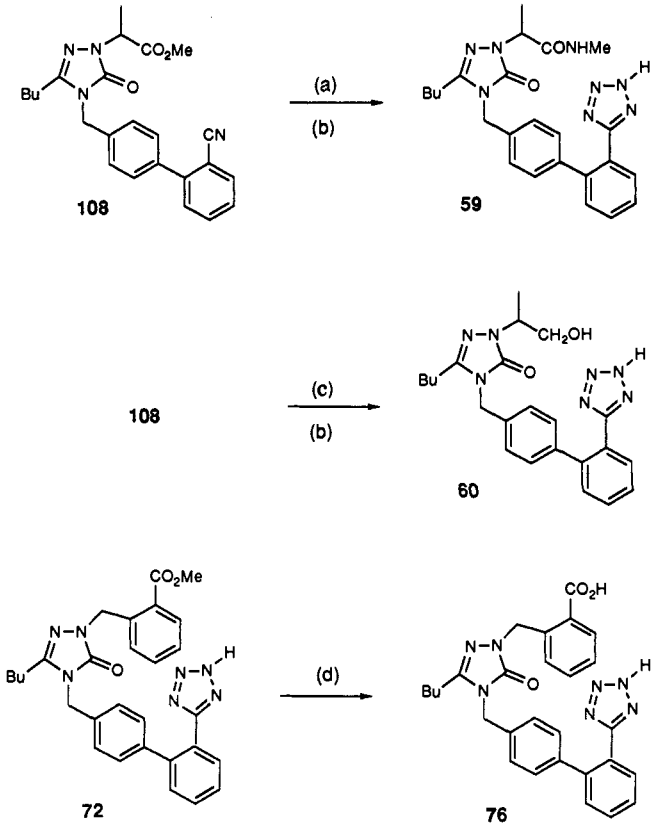
Scheme III[a]



[a] Key: (a) H_2NNHCO_2Et, EtOH, 0–5 °C; (b) LiN_3, DMSO, 20 °C; (c) $P(C_6H_5)_3$, H_2O, THF; (d) EtOH, 80 °C; (e) NaH, DMF, α-methylbenzyl bromide or neopentyl iodide; (f) HOAc, H_2O, 60 °C; or Me_3SnN_3, toluene, 110 °C.

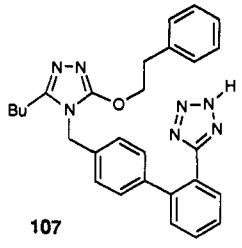
as illustrated in Scheme III. The requisite biarylmethyl amines **103a** and **103b**[18] were prepared in two steps from the corresponding bromides. In the examples shown, the valerimidate hydrochloride **93** was reacted with ethyl carbazate to give the adduct **100**, which, upon heating[27] with **103a,b** in ethanol, was converted to the triazolinones **104a,b**. Alkylation of the anion of **104a,b** with the appropriate alkyl or aralkyl halide gave the corresponding

Scheme IV[a]



[a] Key: (a) H_2NMe (aq), MeOH, reflux; (b) Me_3SnN_3, toluene, reflux; (c) $LiBH_4$, THF, 0–20 °C; (d) NaOH, MeOH; HCl.

N-alkylated products[28] after chromatographic purification. Removal of the trityl protection or conversion of the cyano group to the tetrazole using trimethyltin azide[12] afforded the target compounds **71** and **54**, respectively. In the alkylation step, the products were assigned as N-alkylated[28] based on NMR chemical shifts of the protons on the carbon atoms α to the nitrogen atom.[29a] In the case of **81**, the corresponding O-alkylated isomer, **107**, had been synthesized by an unambiguous route.[18] The relevant hetero-



atom-CH_2 signal of the *N*-phenethyltriazolinone **81** (prepared according to Scheme III) was shifted 0.64 ppm upfield compared to that of the isomeric (phenethyloxy)-triazole **107**.[29b] In addition, the fact that compound **56**, initially prepared via the hydrazine route (Scheme I), was subsequently obtained by the alkylation route (Scheme III) further affirms that under the reaction conditions employed **104a,b** underwent N-alkylation.

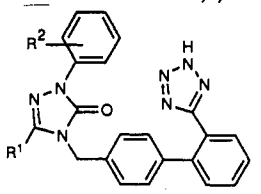
For the preparation of **59** (Scheme IV), compound **108**, an intermediate in the preparation of **61**, was heated at reflux with excess aqueous methylamine to give the corresponding amide. Treatment of this intermediate with trimethyltin azide furnished **59**. Similarly, treatment of **108** with lithium borohydride followed by tetrazole formation afforded **60**. The carboxylic acids **27**, **62**, **64**, **76**, and **77** were prepared by the base hydrolysis of the corresponding methyl esters, shown for **76** in Scheme IV. In addition, several alkyl analogues that were not readily accessible by this route owing to difficulties encountered in the alkylation at N^2 were prepared via the corresponding hydrazines as described in Scheme I.

Biological Results and Discussion

In Vitro AII Antagonism. Triazolinones **4–92** were assessed as AII antagonists by their ability to displace specifically-bound radioligand ^{125}I[Sar^1,Ile^8]AII from the AT_1 receptors in rabbit aorta membrane.[30,18] Two slightly different versions of the assay were used to evaluate these compounds. In one version, the mixture contained 0.2% BSA while in the other, no BSA was present.[31] Several compounds were tested under both sets of conditions as shown in Tables I and II. For virtually all of these compounds, the IC_{50} values obtained from the two assays were essentially equivalent within experimental error (see Experimental Section). The results for compounds **4–40**, bearing aryl substituents at N^2, are listed in Table I. The parent analogue in this series, the unsubstituted phenyl derivative **4**, was about twice as potent as **1a**. Data from **5–16** suggested that, for a given substituent, *ortho* substitution (**5–8**) consistently gave compounds that were more potent than the parent, regardless of the electronic properties of the substituent. *Meta* substitution (**9–12**) gave analogues with somewhat greater or lesser potency than the parent, depending on the substituent. In an extended structure–activity relationship (SAR) study of *para* substitution (**13–19**), encompassing substituents of varying electronic and steric properties, only the *p*-methoxy substituent (**16**) gave substantial improvement over the parent. Results from further SAR of the *ortho* substituents (**20–29**) suggested that among halogens Cl and Br were preferred to F at this position. A number of these compounds had IC_{50} values in the low nanomolar range as exemplified by the *o*-nitro (**7**), *o*-(trifluoromethyl) (**25**) and *o*-isopropyl (**20**) analogues, each gaining close to 20-fold in potency compared to the parent compound **4**. The decreased effectiveness of the *o*-phenyl (**21**) and *o*-benzyl (**22**) derivatives relative to **20** suggested that the optimal size for the *ortho* substituent was approximated by the isopropyl group. The *o*-carbomethoxy analogue (**26**) had potency similar to that of the *o*-methoxy compound (**8**) while the *o*-(dimethylamino) derivative (**29**) was somewhat more effective. The corresponding analogues with polar substituents, *o*-carboxy (**27**) and *o*-amino (**28**), both had poor in vitro potency.

Several polysubstituted phenyl derivatives at N^2 were prepared (**30–32**). The 2,6-dichloro derivative **30** was less than half as effective as the 2-chloro analogue **6**. The 2-nitro-4-methoxy derivative **31** was about equipotent with the 2-nitro compound **7**. The perfluorophenyl analogue **32** was about as potent as the parent compound **4**. One example of an analogue with a heteroaryl group at N^2 was prepared (**42**, Table II), and this 2-pyridyl compound was about one third as potent as the parent **4**. A number of derivatives were prepared with either an *o*-chlorophenyl or an *o*-nitrophenyl group at N^2 and a variety of alkyl or cycloalkyl groups at C^5 to examine the SAR at the latter position (**33–40**). As indicated, branching and/or truncation of the n-butyl side chain to isopentyl, n-propyl, cyclopropylmethyl, or cyclopropyl had deleterious effects on the AT_1 binding affinity. The most dramatic decrease was seen in the examples with R^1 = cyclopropyl (**33**, **38**). Lengthening the butyl chain by one carbon approximately halved the potency in one instance (**37**) and retained it in

Table I. Physical Properties and in Vitro AII Antagonist Potencies of 3*H*-1,2,4-Triazolinones With Aryl Substituents at N^2



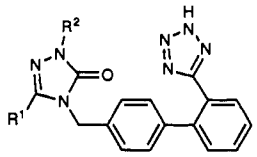
no.	R^1	R^2	method[a]	yield (%)	mp (°C)	formula[b,c]	FAB-MS *m/e* $(M + H)^+$	Rabbit Aorta AT_1 IC_{50} (nM) *d*	*e*
1a								50[f,g]	
1b								6[f,h]	
4	n-Bu	H	A	61	187–188	$C_{26}H_{25}N_7O\cdot1.1H_2O$	452	22	
5	n-Bu	2-Me	A	80	99–101	$C_{27}H_{27}N_7O\cdot0.17CHCl_3$	466	4.1	
6	n-Bu	2-Cl	A	66	94–95	$C_{26}H_{24}ClN_7O\cdot0.33Tol\cdot0.75H_2O$	486	2.4	
7	n-Bu	2-NO_2	A	85	103–105	$C_{26}H_{24}N_8O_3\cdot0.33CHCl_3$	497	0.85	
8	n-Bu	2-OMe	A	40	107–109	$C_{27}H_{27}N_7O_2\cdot1.4H_2O$	482	6.6	
9	n-Bu	3-Me	A	78	152–155	$C_{27}H_{27}N_7O\cdot0.25Tol\cdot0.5H_2O$	466	18	
10	n-Bu	3-Cl	A	54	89–90	$C_{26}H_{24}ClN_7O\cdot0.1CHCl_3$	486	120	
11	n-Bu	3-NO_2	A	71	161–163	$C_{26}H_{24}N_8O_3\cdot0.7H_2O$	497	43	
12	n-Bu	3-OMe	A	75	83–85	$C_{27}H_{27}N_7O_2\cdot0.1Tol\cdot0.6H_2O$	482	15	
13	n-Bu	4-Me	A	87	97–99	$C_{27}H_{27}N_7O\cdot0.1CHCl_3$	466	16	
14	n-Bu	4-Cl	A	87	94–95	$C_{26}H_{24}ClN_7O\cdot0.15CHCl_3$	486	69	
15	n-Bu	4-NO_2	A	63	111–113	$C_{26}H_{24}N_8O_3\cdot0.13CH_2Cl_2$	497	80	
16	n-Bu	4-OMe	A	90	92–94	$C_{27}H_{27}N_7O_2\cdot0.25CH_2Cl_2$	482	5	
17	n-Bu	4-Et	A	95	89–92	$C_{28}H_{29}N_7O\cdot0.1H_2O$	480	27	
18	n-Bu	4-F	A	88	98–100	$C_{26}H_{24}FN_7O\cdot0.4H_2O$	470	21	
19	n-Bu	4-CO_2Me	A	62	195–198	$C_{28}H_{27}N_7O_3\cdot0.5H_2O$	510	33	
20	n-Bu	2-i-Pr	A	58	89–92	$C_{29}H_{31}N_7O\cdot0.1Tol\cdot0.2CH_2Cl_2$	494	1.4	
21	n-Bu	2-Ph	A	62	116–118	$C_{32}H_{29}N_7O\cdot0.7CH_2Cl_2$	528	3.6	
22	n-Bu	2-CH_2Ph	A	53	87–90	$C_{33}H_{31}N_7O\cdot0.4H_2O$	542	11	
23	n-Bu	2-F	A	78	90–92	$C_{26}H_{24}FN_7O\cdot0.4H_2O$	470	7.7	
24	n-Bu	2-Br	A	78	105–108	$C_{26}H_{24}BrN_7O\cdot0.05CH_2Cl_2$	531	2	
25	n-Bu	2-CF_3	A	58	88–90	$C_{27}H_{24}F_3N_7O\cdot0.1CH_2Cl_2\cdot0.1H_2O$	520	1.2	
26	n-Bu	2-CO_2Me	A	91	87–90	$C_{28}H_{27}N_7O_3\cdot0.4CH_2Cl_2$	510	5.6	
27	n-Bu	2-CO_2H	A	75	117–120	$C_{27}H_{25}N_7O_3\cdot0.2Tol\cdot1.5H_2O$	496	115	50
28	n-Bu	2-NH_2	A[i]	25	gum	$C_{26}H_{29}N_8O$	466.2273[j]	100	
29	n-Bu	2-NMe_2	A	78	179–181	$C_{27}H_{28}N_7O_3\cdot0.25Tol\cdot0.5H_2O$	494	3.2	
30	n-Bu	2,6-Cl_2	A	95	117–119	$C_{26}H_{23}Cl_2N_7O\cdot0.33H_2O$	520	5.8	
31	n-Bu	2-NO_2,4-OMe	A	84	102–104	$C_{27}H_{29}N_8O_4\cdot0.7H_2O\cdot0.4CH_2Cl_2$	527	0.74	
32	n-Bu	2,3,4,5,6-F_5	A	75	89–91	$C_{26}H_{20}F_5N_7O$	542	17	
33	c-Pr	2-Cl	A	70	117–119	$C_{25}H_{20}ClN_7O$	469.1398[k]		250
34	n-Pr	2-Cl	A	77	115–117	$C_{25}H_{22}ClN_7O\cdot0.8H_2O$	472	14	20
35	CH_2(c-Pr)	2-Cl	A	80	114–116	$C_{28}H_{22}ClN_7O\cdot0.25CH_2Cl_2$	484		27
36	i-pentyl	2-Cl	A	70	160–162	$C_{27}H_{26}ClN_7O$	500.1191[l]		16
37	n-pentyl	2-Cl	A	79	87–91	$C_{27}H_{26}ClN_7O\cdot0.4H_2O$	500	5.7	
38	c-Pr	2-NO_2	A	34	113–115	$C_{25}H_{20}N_8O_3$	480.1706[m]		95
39	n-Pr	2-NO_2	A	61	113–115	$C_{28}H_{22}ClN_7O\cdot0.25CH_2Cl_2$	483	9.5	21
40	n-pentyl	2-NO_2	A	67	95–98	$C_{27}H_{29}N_8O_3\cdot0.1Tol\cdot0.3CH_2Cl_2$	511	0.93	
41	[nBu	2-Cl]^n	[o]	76	125–127	$C_{27}H_{25}ClN_4O_4\cdot0.2H_2O$	505	570	

[a] A: N^4-substituent was introduced in the penultimate step. See Experimental Section for detailed description of general methods. [b] Analyses for C, H, and N within ±0.4% except where characterized by high-resolution FAB-MS. [c] Tol = toluene. [d] BSA (0.2%) included in the assay mixture. [e] No BSA included in the assay mixture. [f] Data for rabbit aorta membrane receptor assay from ref 16. [g] An IC_{50} of 19 nM (rat adrenal cortical membrane receptor assay) was reported for this compound in ref 12. [h] An IC_{50} of 37 nM (rat adrenal cortical membrane receptor assay) was reported for this compound in ref 14. [i] Obtained by $SnCl_2$ reduction of the nitro precursor. [j] Calcd for $C_{26}H_{27}N_8O$ $(M + H)^+$: 466.2232. [k] Calcd for $C_{25}H_{21}ClN_7O$ $(M + H)^+$: 469.1419. [l] Calcd for $C_{27}H_{27}ClN_7O$ $(M + H)^+$: 500.1966. [m] Calcd for $C_{25}H_{21}N_8O_3$ $(M + H)^+$: 480.1660. [n] For this compound, the (tetrazolylbiphenylyl)methyl side chain at N^4 was replaced by 4-[(2-carboxybenzoyl)amino]benzyl. [o] Synthesized according to Scheme II.

another (**40**). Replacement of the C—C bond between the two rings of the biphenyl group in **6** by an amide bridge gave **41**, which had an IC_{50} of 570 nM. This dramatic loss in potency of more than 200-fold deterred further investigation in the amide-linked series.

The SAR data for analogues bearing nonaryl substituents at N^2 (**43–92**) are presented in Table II. The majority of these compounds had potencies equivalent or superior to that of the reference compound **1a**, although the unsubstituted analogue **43** was somewhat less potent than **1a**. Among the straight-chain alkyl derivatives studied (**44-47**), binding affinity increased with chain length, leading to the potent n-butyl analogue **47**.[32] Branched alkyl and cycloalkyl groups (**48–55**) provided several additional potent compounds with the notable exception of the cyclopropyl analogue. The 2,2,2-trifluoroethyl analogue (**56**) was less effective than the corresponding ethyl compound. Changing the functional group at the β-carbon of the N^2-substituent to nitrile, ester, amide, alcohol, ketone, or acid (**57–66**) failed to improve potency. To follow up on the relatively potent benzyl analogue **67**, a series of related compounds was prepared (**68–80**). Replacement of phenyl in **67** by cyclohexyl gave a slightly more potent compound (**68**). Methylated benzyl derivatives were 2- to 3-fold inferior except the α-methyl analogue **71** which was equipotent with **67**. Substitution with the carbomethoxy group on benzyl (**72–75**) produced less potent analogues, particularly the 3-(carbomethoxy)-benzyl derivative **73**. The corresponding acids of several of these analogues were also less potent (**76–77** vs **67**). Pentafluorobenzyl and naphthylmethyl groups resulted in considerable loss of potency (**78–80**). Surprisingly, re-

Table II. Physical Properties and in Vitro AII Antagonist Potencies of 3*H*-1,2,4-Triazolinones with Nonaryl Substituents at N^2



no.	R^1 [a]	R^2 [a]	method [b]	yield (%)	mp (°C)	formula [a,c]	FAB-MS, *m/e* $(M + H)^+$	Rabbit Aorta AT_1 IC_{50} (nM) *d*	*f*
42	n-Bu	2-pyridyl	B	14	120–123	$C_{25}H_{24}N_8O \cdot 0.63CH_2Cl_2$	453	79	
43	n-Bu	H	B	86	215–216	$C_{20}H_{21}N_7O \cdot 0.4H_2O$	376	60	
44	n-Bu	Me	B	51	202–203	$C_{21}H_{23}N_7O$	390	70	90
45	n-Bu	Et	B	29	197–198	$C_{22}H_{25}N_7O \cdot 0.2H_2O$	404	10	
46	n-Bu	n-Pr	B	47	155–156	$C_{23}H_{27}N_7O \cdot 0.2H_2O$	418	8.2	
47	n-Bu	n-Bu	B	43	175–177	$C_{24}H_{29}N_7O \cdot 0.3H_2O \cdot 0.1CH_2Cl_2$	432	2.9 [f]	5
48	n-Bu	i-Pr	B	86	187–188	$C_{23}H_{27}N_7O \cdot 0.75H_2O$	418	7.7	
49	n-Bu	c-Pr	B	34	79–81	$C_{23}H_{25}N_7O$	415.2104 [g]		100
50	n-Bu	i-Bu	B	84	>80 (gradual)	$C_{24}H_{29}N_7O \cdot 0.6H_2O$	432	3.2	
51	n-Bu	CH_2(c-Pr)	B	80	168–170	$C_{24}H_{27}N_7O \cdot 0.1Tol \cdot 0.8H_2O$	430		16
52	n-Bu	s-Bu	B	91	79–81	$C_{24}H_{29}N_7O \cdot 0.5H_2O \cdot 0.25CHCl_2$	432	1.8	2.1
53	n-Bu	t-Bu	A	81	180–182	$C_{24}H_{29}N_7O \cdot 0.7H_2O$	432		23
54	n-Bu	CH_2(t-Bu)	C	48	88–90	$C_{25}H_{31}N_7O \cdot 0.67H_2O$	446		2.1
55	n-Bu	c-Pn	B	45	226–227	$C_{25}H_{29}N_7O \cdot 0.4H_2O$	444		1.8
56	n-Bu	CH_2CF_3	A	89	66–70	$C_{22}H_{22}F_3N_7O \cdot 0.3Tol \cdot H_2O$	456		76
57	n-Bu	CH_2CN	B	32	86–88	$C_{23}H_{22}N_8O \cdot 0.25CH_2Cl_2$	415		37
58	n-Bu	CH_2CO_2Me	B	53	148–150	$C_{23}H_{25}N_7O_3 \cdot 0.1Et_2O \cdot 0.75H_2O$	448	17	
59	n-Bu	CHMeCONHMe	C	43	153–155	$C_{24}H_{29}N_8O_2$	461.2427 [h]		95
60	n-Bu	$CHMeCH_2OH$	C	51	114–116	$C_{23}H_{27}N_7O_2 \cdot H_2O \cdot 0.15CH_2Cl_2$	434		24
61	n-Bu	$CHMeCO_2Me$	B	79	69–71	$C_{24}H_{27}N_7O_3 \cdot 0.6H_2O$	462		11
62	n-Bu	$CHMeCO_2H$	B	81	105–109	$C_{23}H_{25}N_7O_3$	448.2110 [i]		26
63	n-Bu	$CH(CO_2Me)CHMe_2$	B	74	gum	$C_{28}H_{31}N_7O_3$	490.2575 [j]		52
64	n-Bu	$CH(CO_2H)CHMe_2$	B	72	105–107	$C_{25}H_{29}N_7O_3$	476.2423 [k]		70
65	n-Bu	c-Pn(2-OH)	C	56	122–125	$C_{25}H_{29}N_7O \cdot 0.4CH_2Cl_2$	460		12
66	n-Bu	CH_2COPh	B	84	89–92	$C_{28}H_{27}N_7O_2 \cdot CH_3OH$	494		12
67	n-Bu	Bn	B	94	112–113	$C_{27}H_{27}N_7O \cdot 0.3Tol \cdot 0.75H_2O$	466	4.6	4.6
68	n-Bu	CH_2(c-Hex)	B	78	180–181	$C_{27}H_{33}N_7O \cdot 0.2H_2O$	472	2.9	
69	n-Bu	(2-Me)Bn	B	91	192–194	$C_{28}H_{29}N_7O \cdot 0.2Tol \cdot 0.13CHCl_3$	480	11	
70	n-Bu	(3-Me)Bn	B	78	72–74	$C_{28}H_{29}N_7O \cdot 0.17Tol \cdot 0.2CHCl_3$	480	12	
71	n-Bu	(α-Me)Bn	B	75	163–164	$C_{28}H_{29}N_7O \cdot 0.1CHCl_3$	480	4.9	
72	n-Bu	(2-CO_2Me)Bn	B	95	79–80	$C_{28}H_{29}N_7O_3 \cdot 1.25H_2O$	524	11	
73	n-Bu	(3-CO_2Me)Bn	B	97	82–86	$C_{28}H_{29}N_7O_3 \cdot 0.25CHCl_3$	524	110	
74	n-Bu	(4-CO_2Me)Bn	B	84	81–84	$C_{28}H_{29}N_7O_3 \cdot 0.15CHCl_3$	524	23	
75	n-Bu	(α-CO_2Me)Bn	B	73	92–94	$C_{28}H_{29}N_7O_3 \cdot H_2O \cdot 0.1Tol$	524	12	
76	n-Bu	(2-CO_2H)Bn	B	95	114–117	$C_{28}H_{27}N_7O_3 \cdot 2H_2O \cdot 0.1Tol$	510	490	14
77	n-Bu	(α-CO_2H)Bn	B	48	121–124	$C_{28}H_{27}N_7O_3$	509.2118 [l]	68	50
78	n-Bu	$CH_2C_6F_5$	B	91	82–85	$C_{27}H_{22}F_5N_7O \cdot 0.4CH_2Cl_2 \cdot 0.15Tol$	556	32	
79	n-Bu	CH_2(α-Naph)	B	45	90–93	$C_{31}H_{29}N_7O$	515.2403 [m]		33
80	n-Bu	CH_2(β-Naph)	B	49	98–100	$C_{31}H_{29}N_7O \cdot 0.7H_2O \cdot 0.15CH_2Cl_2$	516		27
81	n-Bu	$(CH_2)_2Ph$	B	88	71–73	$C_{28}H_{29}N_7O \cdot 0.5H_2O$	480		0.9
82	n-Bu	$(CH_2)_3Ph$	B	66	63–65	$C_{29}H_{31}N_7O \cdot 0.6H_2O$	494		11
83	n-Bu	CH_2O(p-Cl-C_6H_4)	B	69	77–79	$C_{27}H_{26}ClN_7O_2$	516.1933 [n]		50
84	n-Bu	CH_2SPh	B	75	170 dec	$C_{27}H_{27}N_7OS$	497.2032 [o]		16
85	n-Bu	$(CH_2)_3OCONMePh$	B	68	56–58	$C_{31}H_{34}N_8O_3 \cdot 0.8H_2O$	567		7.4
86	n-Bu	$(CH_2)_3NMeCO_2Bn$	B	33	49–51	$C_{32}H_{36}N_8O_3 \cdot H_2O$	581		60
87	n-Bu	$(CH_2)_3NBnCO_2Bn$	B	62	61–63	$C_{38}H_{40}N_8O_3 \cdot 0.5H_2O \cdot 0.15CH_2Cl_2$	657		30
88	n-Bu	CH_2-(2′-CN-biphenyl-4-yl)	B	76	100–101	$C_{34}H_{30}N_8O \cdot H_2O$	567		680
89	n-Bu	CH_2-(2′-tetrazolylbiphenyl-4-yl)	B	91	150–151	$C_{34}H_{31}N_{11}O$	609.2639 [p]		10
90	c-Pr	$(CH_2)_2C_6H_5$	B	94	91–92	$C_{27}H_{25}N_7O \cdot 0.6H_2O$	464		130
91	CH_2(c-Pr)	$(CH_2)_2C_6H_5$	B	66	164–166	$C_{28}H_{27}N_7O \cdot 0.15CH_2Cl_2 \cdot 0.25CH_3OH$	478		14
92	i-Pn	$(CH_2)_2C_6H_5$	B	87	82–84	$C_{29}H_{31}N_7O$	494		3.1

[a] Bn = benzyl, Naph = naphthyl, Pn = pentyl, Tol = toluene. [b] A: N^4-substituent was introduced in the penultimate step. B: N^2-substituent was introduced in the penultimate step. C: trimethyltin azide route was used. See Experimental Section for detailed description of general methods. [c] Analyses for C, H, and N within ±0.4% except where characterized by high-resolution FAB-MS. [d] BSA (0.2%) included in the assay mixture. [e] No BSA included in the assay mixture. [f] An IC_{50} of 1.8 nM (rat adrenal cortical membrane receptor assay) was reported for this compound in ref 32. [g] Calcd for $C_{28}H_{29}N_7O$ $(M + H)^+$: 415.2122. [h] Calcd for $C_{24}H_{29}N_8O_2$ $(M + H)^+$: 461.2414. [i] Calcd for $C_{28}H_{29}N_7O_3$ $(M + H)^+$: 448.2097. [j] Calcd for $C_{28}H_{32}N_7O_3$ $(M + H)^+$: 490.2567. [k] Calcd for $C_{25}H_{30}N_7O_3$ $(M + H)^+$: 476.2410. [l] Calcd for $C_{28}H_{29}N_7O_3$ $(M + H)^+$: 509.2177. [m] Calcd for $C_{31}H_{30}N_7O$ $(M + H)^+$: 515.2435. [n] Calcd for $C_{27}H_{27}ClN_7O_2$ $(M + H)^+$: 516.1915. [o] Calcd for $C_{27}H_{29}N_7OS$ $(M + H)^+$: 497.2000. [p] Calcd for $C_{34}H_{32}N_{11}O$ $(M + H)^+$: 609.2716.

placement of benzyl by phenethyl (81) gave a 5-fold improvement in potency over 67. This was followed by the preparation of the homologue 82 and compounds 83 and 84 where one methylene group was replaced by a heteroatom. Unfortunately, these all gave a marked decrease in potency. Compounds 85–87 were prepared to investigate the tolerance of polar functional groups in positions distal to N^2. Compared to the carbamate 85, the reversed carbamates 86 and 87 had inferior potency. To probe the effects of bulk and length of the side chain at N^2, two derivatives containing the biphenylylmethyl group at that position were prepared (88, 89). With an

IC_{50} of 10 nM, the latter, bearing a second tetrazole moiety, was surprisingly potent compared to the cyano analogue **88**. In terms of variations at C^5 (i.e., R^1), available data (**90–92**) implied that, parallel to findings in the N^2-aryl series, branching or truncation of the n-butyl group resulted in a considerable loss of potency.

The AT_1 receptor-ligand model advanced by the Du Pont group[12,26] for the imidazole-based antagonists **1a,b** suggested multiple interactions. These include (1) an ionic interaction between a cationic group on the receptor and the acidic substituent on the *ortho*-position of the distal ring of the biphenyl side chain, (2) a hydrogen bond between a proton donor on the receptor and a proton acceptor on the C^5-substituent of the imidazole, and (3) hydrophobic associations between lipophilic pockets on the receptor and the alkyl chain at C^2 and the chloro substituent at C^4. More recently, SAR studies of triazoles[18] have suggested the existence of a major hydrophobic area on the receptor more distant from the heterocyclic binding region. The present results on triazolinones lend some support to both of these hypotheses. First of all, for triazolinones with the core structure **3**, the putative hydrophobic association between a lipophilic region on the receptor and the side chain at C^5 was optimized by an n-butyl group, with little tolerance for deviation. Secondly, the oxygen at C^3 of the triazolinone ring could fulfill the role of a proton acceptor in a hydrogen bond with the receptor, an interaction suggested in the Du Pont model. Similar interactions have been invoked to explain the marked enhancement of binding affinity seen in the imidazo[4,5-*b*]pyridines compared to benzimidazoles, which lack the pyrido nitrogen at that position.[16] In contrast, the role of hydrogen bonding at an equivalent position of the triazole AII antagonists (e.g., **2**) has been questioned.[18]

Unlike imidazole- and triazole-based antagonists, triazolinones seem to be very sensitive with regard to the length of the side chain at N^4. While the (tetrazolylbiphenylyl)methyl side chain gave very potent compounds such as **6**, an amide bridge between the two rings of the biphenyl group, as in compound **41**, reduced the binding affinity drastically.

SAR analysis of N^2-aryl triazolinones **4–40** supports the notion of a lipophilic pocket on the receptor in the vicinity of the binding site for the 4-chloro substituent of **1a,b** assuming superposition of the triazolinone and the imidazole rings. However, the present study suggests that this pocket is sizable, capable of accommodating phenyl groups with rather bulky *ortho*-substituents and resulting in binding affinity enhancements of as much as 50-fold over **1a**. The receptor binding data for the series of analogues containing N^2-(*ortho*-substituted aryl) groups demonstrate that hydrophobic *ortho*-substituents (e.g., **25** and **20**, with π = 0.88 for CF_3, and π = 1.53 for isopropyl)[33] are especially favorable, although slightly polar substituents can also be effective (e.g., **7**, with π = −0.28 for NO_2). Highly polar substituents apparently interact poorly with the receptor, as exemplified by the *o*-carboxy compound **27** (π = −4.36 for CO_2^-) and the *o*-amino analogue **28** (π = −1.23 for NH_2). Compound **27** was 20-fold less effective than the more hydrophobic *o*-carbomethoxy derivative **26** (π = −0.01 for CO_2Me), while compound **28** was 30-fold less potent than the more lipophilic *o*-(dimethylamino) analogue **29** (π = 0.18 for NMe_2).

Additional important hydrophobic interactions at least three atoms removed from the triazolinone heterocycle are suggested by the data presented in Table II. Thus, derivatives with aralkyl or medium-length alkyl side chains at N^2 tended to be more potent than analogues with compact substituents at that position (compare **47**, **52**, **54**, **67**, and **81** with **44**, **49**, and **53**). In most cases, the potency gained in this manner was not as dramatic as that achieved by the substituted phenyl groups discussed above. Compound **81**, the phenethyl derivative, is an exception. The subnanomolar binding affinity of this compound rivals that of the most potent analogues in the substituted phenyl derivatives such as **7** and **25**.

Some indication of the space being filled by the potent *N*-aryl or -aralkyl triazolinones may be provided by the molecular modeling shown in Figure 1. At present, the bioactive conformation has not been established for any nonpeptide AII antagonist. A complication inherent in the computer modeling of compounds of this type is that multiple low-energy conformations are possible, differing in the biphenylyltetrazole side chain with regard to torsion angles and, therefore, orientation relative to the heterocycle.[17,34] From our molecular modeling, one alignment of this side chain that emerged as a low-energy conformation, shared by each of the structures, was selected for overlays.[35] The considerable increment in potency upon addition of a suitable *ortho*-substituent to the N^2-phenyltriazolinone **4**, as in the trifluoromethyl derivative **25**, could be attributed to the favorable contact of this substituent with an adjacent region of the receptor, although a partial conformational restriction about the *N*-phenyl bond may also be involved. A similar affinity enhancement is achieved by extension of the side chain at N^2 from phenyl to phenethyl, as in **81**, presumably allowing access to a lipophilic site somewhat distant from N^2. An overlay of molecular models of **25** and **81** (Figure 1a) suggests that it is unlikely that the benzene ring of the phenethyl side chain in **81** would occupy the same region of space as the trifluoromethyl group in **25**. This raises the possibility that either the putative lipophilic site is quite extensive or that there are two distinct binding regions accessible from N^2. The large difference in potency between **81** (0.9 nM) and the isomeric (phenethyloxy)triazole **107** (63 nM)[18] may stem, at least in part, from the unfavorable positioning of the aralkyl moiety in the latter (Figure 1b). The *O*-phenethyl side chain of **107** simply may not be able to reach the "remote" hydrophobic binding region that binds the *N*-phenethyl substituent of **81**.

In Vivo Pharmacology. The inhibition of the pressor response to AII challenge by a number of tetrazolyl triazolinones in conscious, normotensive rats was evaluated, and representative data are shown in Table III. Via the intravenous route at 1 mg/kg, the N^2-aryl triazolinones **7**, **20**, and **25** as well as N^2-alkyl derivatives **48** and **54** had peak inhibitions similar to that of **1a** and significant durations of action. The alcohol–acid pair **60** and **62** also showed a good iv profile but at a higher dose, reflective of their lower in vitro potency. Among the remainder of the compounds tested, some were effective AII antagonists by iv administration. Regardless of the nature of the side chain at N^2 or C^5, all of these had characteristically short duration of action when given intravenously. This situation was illustrated well by compound **47**.[32] In our in vivo studies, **47** was effective intravenously as an AII antagonist, with peak inhibition of 83% at 1 mg/kg. However, its duration of action at this dose (0.5 h) was short and inferior to that of analogues such as **20**, **25**, **48**,

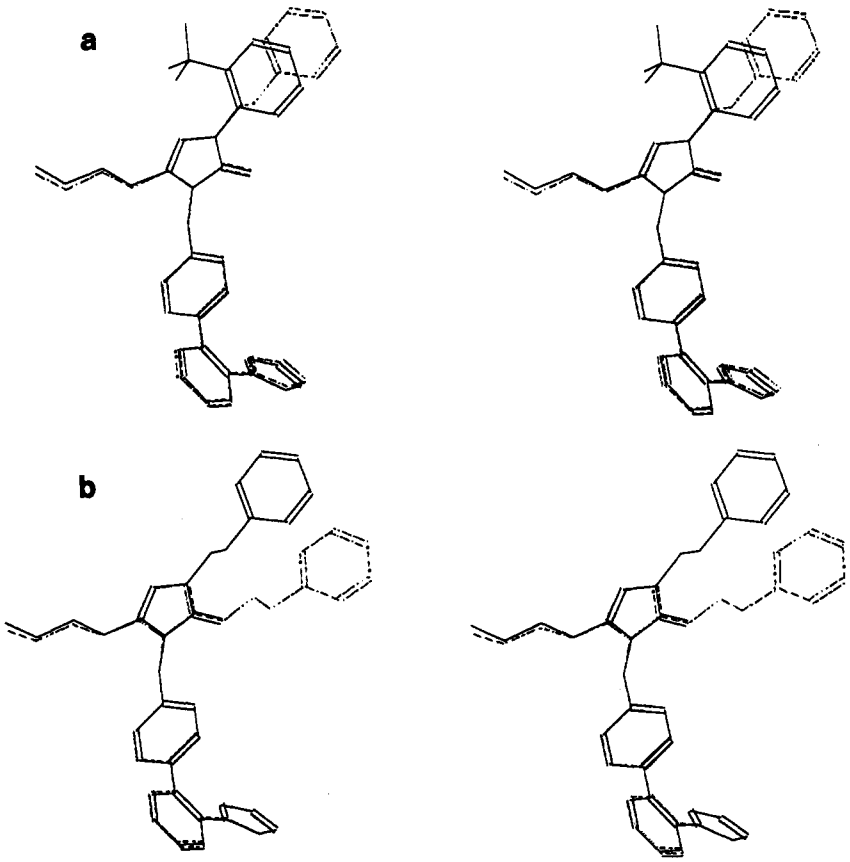


Figure 1. Stereoviews of computer-generated, energy-minimized conformations of AII antagonists: (a) overlay of triazolinones **25** (solid) and **81** (dotted); (b) overlay of triazolinone **81** (solid) and triazole **107** (dotted).

Table III. Inhibition of AII Pressor Response by Triazolinone Derivatives in Conscious, Normotensive Rats

no.	dose (mg/kg)	route	peak inhibn (%)	duration[a] (h)	N^b
1a	1	iv	78 ± 6	>6	4
	1	po	58 ± 8	>2	6
	3	po	94 ± 2	>4.5	4
1b	0.3	iv	100 ± 0	>29	2
	1	po	72 ± 10	>7	2
5	1	iv	74 ± 4	0.6 ± 0.2	2
6	1	iv	80 ± 3	0.5 ± 0.1	2
	1	po	34 ± 6	NA	2
7	1	iv	84 ± 2	1.1 ± 0.4	2
	1	po	63 ± 7	5.2 ± 0.2	2
16	1	iv	59 ± 11	0.4 ± 0.1	2
20	1	iv	71 ± 4	4 ± 1	2
	1	po	22 ± 14	NA[c]	2
25	1	iv	73 ± 7	1.5 ± 0.6	4
	1	po	66 ± 5	>6	4
29	1	iv	74 ± 12	0.3 ± 0.2	2
39	1	iv	60	0.5	1
46	1	iv	62 ± 6	0.3 ± 0.2	2
47	1	iv	83 ± 4	0.5 ± 0	2
48	1	iv	92 ± 0	2.9 ± 2.6	2
	1	po	20 ± 9	ND[d]	6
50	1	iv	84 ± 4	0.6 ± 0.4	2
54	1	iv	92 ± 4	3.2 ± 1.8	2
	1	po	53 ± 9	2.5 ± 0.5	2
60	3	iv	86 ± 2	>4	2
62	3	iv	90 ± 0	4.2 ± 0.2	2
	3	po	62 ± 9	1.8 ± 1.4	2
67	1	iv	58 ± 10	0.4 ± 0.3	2
81	1	iv	74 ± 13	ND[d]	2

[a] Time from onset of action until significant (i.e., ≥30%) inhibition of pressor response is no longer observed. [b] Number of animals treated. [c] NA = not active. [d] ND = not determined.

and **54**. Compared to **47**, each of these four compounds at 1 mg/kg exhibited superior iv duration (up to 4 h), and several were more effective (up to 92% peak inhibition) in inhibiting the AII pressor response.

Some derivatives, including **7**, **25**, and **54**, showed significant oral activity. In particular, at 1 mg/kg po, both the N^2-[2-(trifluoromethyl)phenyl] analogue **25** and the N^2-(2-nitrophenyl) analogue **7** exhibited efficacy similar to that of losartan (**1a**), with a duration of action of several hours.

Conclusions

An extensive series of 2,4-dihydro-2,4,5-trisubstituted-3*H*-1,2,4-triazolinones has been prepared by several synthetic routes and evaluated as AII antagonists in vitro and in vivo. The most active of these had (1) an n-butyl substituent at C^5, (2) a [2′-(5-tetrazolyl)biphenyl-4-yl]-methyl side chain at N^4, and (3) an *ortho*-substituted phenyl group, an alkyl chain with at least four carbons, or an aralkyl moiety at N^2. A number of these had IC_{50} values in the low nanomolar range when assayed against the AT_1 receptor from the rabbit aorta membrane. For good in vivo (iv) and in vitro potency, appropriate *ortho*-substituted aryl or alkyl substituents at N^2 were essential, as shown by analogues **7**, **20**, **25**, **48**, and **54**. Apparently, compounds with these structural features participate well in ionic, hydrogen-bonding, and hydrophobic interactions with the AT_1 receptor. Two compounds, **7** and **25**, exhibited effective antihypertensive activity with substantial duration of action orally at 1 mg/kg in a conscious rat model.

Experimental Section

Melting points (uncorrected) were determined in open capillary tubes with a Thomas-Hoover apparatus. ^{1}H NMR spectra were recorded on Varian XL-400, XL-300, or XL-200 spectrometers, using tetramethylsilane as internal standard. Positive ion fast

atom bombardment (FAB) or electron impact (EI) mass spectra (MS) were obtained on Varian MAT 731, Finnigan MAT 90, JEOL HX110, and Varian MAT 212 instruments. Flash column chromatography was carried out on EM Science silica gel 60 (230–400 mesh). Compounds showed satisfactory purity by TLC on Analtech silica gel GHLF plates (visualized by UV light at 254 nm and/or by 1% ceric sulfate in 10% aqueous H_2SO_4) in the indicated solvent systems. Elemental combustion analyses, where indicated only by the elements, were within ± 0.4% of theoretical values. Many of the compounds were unavoidably analyzed as solvates, owing to their tendency to retain solvent under nondestructive drying conditions. Where solvation is indicated, the presence of solvent in the analytical sample was verified by NMR.

Anhydrous tetrahydrofuran (THF) was obtained by distillation from sodium/benzophenone ketyl under N_2, or purchased from Aldrich Chemical Co. Reagent-grade CH_2Cl_2, DMF, DMSO, MeOH, EtOH, and toluene were dried over 3-Å molecular sieves. Reactions were routinely conducted under N_2 (bubbler) unless otherwise indicated.

Method A. Ethyl *N*-Carbethoxyvalerimidate (94). Ethyl valerimidate hydrochloride (**93**)[23] (12.7 g, 76.7 mmol) was dissolved in K_2CO_3 (aqueous, 33% w/w) and extracted with 3 × 40 mL of ether. The combined ether layers were dried over Na_2SO_4, filtered, and concentrated in vacuo to give 7.09 g (72%) of ethyl valerimidate free base as a clear oil which was used directly in the ensuing reaction: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, *J* = 7 Hz, 3 H), 1.24 (t, *J* = 7 Hz, 3 H), 1.31 (m, 2 H), 1.50 (m, 2 H), 2.19 (t, *J* = 7.5 Hz, 2 H), 4.06 (q, *J* = 7 Hz, 2 H), 6.84 (br s, 1 H).

A solution of 6.5 g (50.3 mmol) of ethyl valerimidate free base, prepared above, in 90 mL of dry CH_2Cl_2 was treated with 7.71 mL (5.60 g, 55.3 mmol) of triethylamine. The resulting solution was stirred at −10 °C in an ice–salt bath as a solution of 4.81 mL (5.46 g, 50.3 mmol) of ethyl chloroformate in 10 mL of CH_2Cl_2 was added dropwise over 25 min. Upon completion of addition, the cooling bath was removed, and the mixture was stirred at room temperature for 2 h. After evaporation of the solvent in vacuo, the residue was taken up in hexane and filtered to remove triethylamine hydrochloride. Concentration of the filtrate yielded 7.08 g (70%) of the desired product as a yellow oil, suitable for use in the next step without further purification. The NMR spectrum indicated a mixture of syn and anti isomers. TLC (98:2 CH_2Cl_2/MeOH) showed a close pair of spots: R_f 0.48, 0.52; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (distorted t, *J* = 7.5 Hz, 3 H), 2.15–2.35 (m, 8 H), 2.4–2.65 (m, 2 H), 2.19, 2.35 (t, *J* = 7.5 Hz, 2 H total), 4.0–4.2 (m, 4 H); EI-MS *m/e* 201 (M)$^+$.

5-n-Butyl-2-(2-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (96). To a solution of 285 mg (2.0 mmol) of 2-chlorophenylhydrazine (**95**) (generated from the hydrochloride by partitioning between ether and 1 N Na_2CO_3) in 6 mL of toluene was added 442 mg (2.2 mmol) of **94**, and the mixture was heated at 45–50 °C for 1.5 h. Subsequently, 307 μL (223 mg, 2.2 mmol) of triethylamine was added, and the reaction mixture was stirred at 90 °C overnight. The dark red solution was cooled to room temperature and concentrated in vacuo. Flash chromatography of the residue (gradient elution with 0.6–2.0% MeOH in CH_2Cl_2) afforded 257 mg (51%) of the product as an off-white solid: mp 103–104 °C; TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.92 (t, *J* = 7 Hz, 3 H), 1.38 (m, 2 H), 1.68 (m, 2 H), 2.57 (t, *J* = 7.5 Hz, 2 H), 7.3–7.55 (m, 4 H), 12.04 (br s, 1 H); FAB-MS *m/e* 252 (M + H)$^+$. Anal. ($C_{12}H_{14}ClN_3O$) C, H, N.

5-n-Butyl-2-(2-chlorophenyl)-2,4-dihydro-4-[[2′-(*N*-trityltetrazol-5-yl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (97). A mixture of 125 mg (0.497 mmol) of **96**, 21 mg (0.522 mmol) of sodium hydride (60% in oil), and 0.5 mL of dry dimethylformamide (DMF) was stirred at 40–50 °C for 20 min. After the mixture was cooled to 35 °C, a solution of 299 mg (0.536 mmol) of **101a**[12] in 1.0 mL of DMF was added and stirring continued at 35 °C for 2.5 h before the reaction mixture was cooled to room temperature and concentrated in vacuo. The residue obtained was treated with 20 mL of H_2O and extracted with 3 × 12 mL of ethyl acetate (EtOAc). The combined organic layers were washed with water and brine and dried over Na_2SO_4. After filtration and concentration of the filtrate in vacuo, the crude product was flash chromatographed (gradient elution with 20–40% EtOAc in hexane) to yield 248 mg (69%) of the desired material as a glassy solid: mp >69 °C (gradual); TLC in 99:1 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (t, *J* = 7 Hz, 3 H), 1.28 (m, 2 H), 1.56 (m, 2 H), 2.35 (t, *J* = 7.5 Hz, 2 H), 4.78 (s, 2 H), 6.90 (d, *J* = 7.5 Hz, 6 H), 7.06, 7.12 (d, *J* = 8 Hz, each 2 H), 7.2–7.55 (m, 16 H), 7.93 (d, *J* = 8 Hz, 1 H); FAB-MS *m/e* 728 (M + H)$^+$; high-resolution FAB-MS *m/e* 728.2939 [calcd for $C_{45}H_{39}ClN_7O$ (M + H)$^+$ 728.2904].

5-n-Butyl-2-(2-chlorophenyl)-2,4-dihydro-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (6). A mixture of 130 mg (0.179 mmol) of **97**, 1.6 mL of glacial acetic acid, and 1.0 mL of H_2O was stirred at 65 °C overnight and then cooled to room temperature. After removal of the volatiles in vacuo, the residue was azeotroped with toluene and then flash chromatographed (gradient elution with 5–10% MeOH in CH_2Cl_2) to give 63 mg (73%) of the product as a glassy solid after azeotroping with toluene: mp 94–95 °C; TLC in 90:10:0.1 CH_2Cl_2/MeOH/AcOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, *J* = 7 Hz, 3 H), 1.37 (m, 2 H), 1.63 (m, 2 H), 2.49 (t, *J* = 7.5 Hz, 2 H), 4.86 (s, 2 H), 7.1, 7.6 (m, 11 H), 7.97 (d, *J* = 7.5 Hz, 1 H); FAB-MS *m/e* 486 (M + H)$^+$. Anal. ($C_{28}H_{24}ClN_7O \cdot 0.75H_2O \cdot 0.33$-toluene) C, H, N.

5-n-Butyl-2-(2-chlorophenyl)-2,4-dihydro-4-(4-nitrobenzyl)-3*H*-1,2,4-triazol-3-one (98). A mixture of 400 mg (1.59 mmol) of **96**, 76 mg (1.59 mmol) of sodium hydride (60% in oil), and 3.5 mL of dry DMF was stirred at room temperature for 2.5 h before 447 mg (2.07 mmol) of 4-nitrobenzyl bromide was added in one portion. After being stirred at room temperature for 3.5 h, the reaction mixture was quenched with 10 mL of H_2O and extracted with 3 × 20 mL of EtOAc. The combined organic layers were washed with water and brine and dried over Na_2SO_4. After filtration and concentration of the filtrate in vacuo, the crude product was flash chromatographed (gradient elution with 0.5–1.0% MeOH in CH_2Cl_2) to yield 434 mg (71%) of the desired material as a pale yellow oil: TLC in 99:1 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.90 (t, *J* = 7 Hz, 3 H), 1.38 (m, 2 H), 1.68 (m, 2 H), 2.47 (t, *J* = 7.5 Hz, 2 H), 5.02 (s, 2 H), 7.37–7.58 (m, 6 H), 8.27 (d, *J* = 6.7 Hz, 2 H); FAB-MS *m/e* 387 (M + H)$^+$; high-resolution FAB-MS *m/e* 387.1217 [calcd for $C_{19}H_{20}ClN_4O_3$ (M + H)$^+$ 387.1224].

4-(4-Aminobenzyl)-5-n-butyl-2-(2-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (99). To a solution of 295 mg (0.763 mmol) of **98** in 5 mL of dry THF stirred in an ice bath was added dropwise a solution of 1.72 g (7.63 mmol) of stannous chloride dihydrate in 2 mL of concentrated HCl. The ice bath was removed, and the mixture was stirred at room temperature for 1 h. It was then poured into a vigorously stirred mixture of 10 mL of 50% NaOH (aqueous) and 30 g of ice. After 20 min the mixture was extracted with 2 × 20 mL of Et_2O and then with 3 × 20 mL of EtOAc. The combined organic extracts were dried (Na_2SO_4), filtered, and concentrated in vacuo. Flash chromatography of the residue (elution with 2.5% MeOH in CH_2Cl_2) yielded 216 mg (79%) of the desired product as a yellow oil: TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, *J* = 7.4 Hz, 3 H), 1.35 (m, 2 H), 1.56 (m, 2 H), 2.44 (t, *J* = 7.8 Hz, 2 H), 4.78 (s, 2 H), 6.70 (d, *J* = 8.3 Hz, 2 H), 7.12 (d, *J* = 8.4 Hz, 2H), 7.31–7.34 (m, 2 H), 7.45–7.51 (m, 2 H); FAB-MS *m/e* 357 (M + H)$^+$; high-resolution FAB-MS *m/e* 357.1461 [calcd for $C_{19}H_{22}ClN_4O$ (M + H)$^+$ 357.1483].

5-n-Butyl-4-[4-[(2-carboxybenzoyl)amino]benzyl]-2-(2-chlorophenyl)-2,4-dihydro-3*H*-1,2,4-triazol-3-one (41). A solution of 84 mg (0.236 mmol) of **99** in 2 mL of dry THF was treated with 35 mg (0.236 mmol) of phthalic anhydride. This reaction mixture was stirred at room temperature for 3 h in a stoppered flask. The residue obtained after evaporation of volatiles in vacuo was flash chromatographed (gradient elution with 5–15% MeOH in CH_2Cl_2) to give 90 mg (76%) of the desired material as a white powder: mp 125–127 °C; TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.84 (t, *J* = 7.2 Hz, 3 H), 1.34 (m, 2 H), 1.52 (m, 2 H), 2.53 (m, 2 H), 4.88 (s, 2 H), 7.27 (d, *J* = 8.4 Hz, 2 H), 7.49–7.71 (m, 8 H), 7.88 (d, *J* = 7.4 Hz, 1 H), 10.4 (s, 1 H); FAB-MS *m/e* 505 (M + H)$^+$. Anal. ($C_{27}H_{25}ClN_4O_4 \cdot 0.2H_2O$) C, H, N.

Ethyl Valerate Carbethoxyhydrazone (100). To a solution of 7.0 g (25.3 mmol) of ethyl valerimidate hydrochloride (**93**)[23] in 35 mL of dry ethanol at −78 °C was added dropwise a solution of 25 g (23 mmol) of ethyl carbazate in 35 mL of dry ethanol. Precipitation occurred during the addition, which took 20 min and was accompanied by a mild exotherm. The mixture was

allowed to stand at 5 °C for 60 h and then filtered. The filtrate was concentrated, and the residue was flash chromatographed (elution with 1.5% MeOH in CH_2Cl_2), yielding 3.06 g (61%) of a clear oil: TLC in 97:3 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.91 (t, J = 7 Hz, 3 H), 1.2–1.4 (m, 8 H), 1.4–1.6 (m, 2 H), 2.2–2.4 (m, 2 H), 3.95–4.3 (m, 4 H), 6.91, 8.11 (br s, 1 H total); FAB-MS m/e 217 (M + H)$^+$.

Method B. 5-[4′-(Azidomethyl)biphenyl-2-yl]-*N*-trityltetrazole (102a). To a stirred suspension of 9.20 g (16.5 mmol) of 5-[4′-(bromomethyl)biphenyl-2-yl]-*N*-trityltetrazole (**101a**)[12] in 50 mL of dry DMSO was added 1.01 g (20.6 mmol) of freshly pulverized lithium azide, and the mixture was stirred at room temperature. Within a few minutes virtually all of the solid had dissolved, accompanied by a mild exotherm. After 4 h the solid which precipitated was collected on a filter and washed with methanol, water, and again with methanol. This material was air-dried overnight and then dried at 70 °C in vacuo to give 8.47 g (99%) of a tan solid of satisfactory purity by TLC for use in the next step: mp 139–140.5 °C dec; TLC in 9:1 hexane/EtOAc; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.22 (s, 2 H), 6.90 (d, J = 8 Hz, 6 H), 7.04, 7.14 (d, J = 8 Hz, each 2 H), 7.2–7.55 (m, 12 H), 7.96 (dd, J = 8 Hz, 1 Hz, 1 H); FAB-MS m/e 243 (trityl)$^+$.

5-[4′-(Aminomethyl)biphenyl-2-yl]-*N*-trityltetrazole (103a). To a stirred solution of 4.37 g (8.40 mmol) of **102a** in 20 mL of dry THF at room temperature was added 2.21 g (8.41 mmol) of triphenylphosphine in small portions over 10 min. After 2 h, 302 μL (302 mg, 16.8 mmol) of H_2O was added, and the reaction mixture was stirred for another 23 h. Removal of the volatiles in vacuo gave the crude product as a gum which was flash chromatographed (gradient elution with 1.5–10% MeOH in CH_2Cl_2) to give 3.45 g (83%) of the desired product as a white foam: mp 134–136 °C dec; TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (br s, 2 H), 3.75 (s, 2 H), 6.88 (d, J = 8 Hz, 6 H), 7.03, 7.09 (d, J = 8 Hz, each 2 H), 7.2–7.5 (m, 12 H), 7.95 (dd, J = 8 Hz, 1 Hz, 1 H); FAB-MS m/e 494 (M + H)$^+$; high-resolution FAB-MS m/e 494.2366 [calcd for $C_{33}H_{28}N_5$ (M + H)$^+$ 494.2345]. An analytical sample, after trituration with Et_2O, was obtained as white crystals: mp 138–139 °C dec. Anal. ($C_{33}H_{27}N_5 \cdot 0.5H_2O$) C, H, N.

5-n-Butyl-2,4-dihydro-4-[[2′-(*N*-trityltetrazol-5-yl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (104a). A mixture of 1.20 g (2.43 mmol) of **103a**, 683 mg (3.16 mmol) of **100**, and 5 mL of ethanol was stirred at 80 °C. All of the solid dissolved within 15 min, and precipitation began after about 2 h. After 3.5 h the mixture was cooled to room temperature and concentrated. The residue was flash chromatographed (gradient elution with 1–5% MeOH in CH_2Cl_2) to afford 628 mg (42%) of the desired compound as a white powder: mp 177–178 °C; TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (t, J = 7 Hz, 3 H), 1.26 (m, 2 H), 1.51 (m, 2 H), 2.27 (t, J = 7.5 Hz, 2 H), 4.69 (s, 2 H), 6.90 (d, J = 7.5 Hz, 6 H), 6.99, 7.13 (d, J = 8 Hz, each 2 H), 7.2–7.5 (m, 12 H), 7.91 (dd, J = 8 Hz, 1.5 Hz, 1 H), 9.03 (s, 1 H); FAB-MS m/e 618 (M + H)$^+$; high-resolution FAB-MS m/e 618.2963 [calcd for $C_{39}H_{36}N_7O$ (M + H)$^+$ 618.2981].

5-n-Butyl-2,4-dihydro-2-(α-methylbenzyl)-4-[[2′-(*N*-trityltetrazol-5-yl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (105). A mixture of 50 mg (0.081 mmol) of **104a**, 11.7 mg (0.243 mmol) of sodium hydride (60% in oil), and 160 μL of dry DMF was stirred at room temperature for 2 h. Next, 111 μL (150 mg, 0.31 mmol) of (1-bromoethyl)benzene was added, and stirring was continued for an additional 3 h at room temperature. The reaction was quenched by careful addition of 0.8 mL of H_2O and then extracted with 3 × 1.5 mL of EtOAc. The combined organic layers were washed with 2 × 2 mL of H_2O and then with brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. Flash chromatography of the residue (elution with 0.6% MeOH in CH_2Cl_2) gave 49 mg (84%) of the desired compound as an oil: TLC in 99:1 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.82 (t, J = 7.4 Hz, 3 H), 1.25 (m, 2 H), 1.56 (m, 2 H), 1.75 (d, J = 7.1 Hz, 3 H), 2.25 (t, J = 7.2 Hz, 2 H), 4.62, 4.70 (d, J = 16 Hz, each 1 H), 5.49 (q, J = 7.1 Hz, 1 H), 6.89 (d, J = 7.8 Hz, 6 H), 6.96, 7.06 (d, J = 8 Hz, each 2 H), 7.2–7.5 (m, 12 H), 7.91 (d, J = 7 Hz, 1 H); FAB-MS m/e 722 (M + H)$^+$; high-resolution FAB-MS m/e 722.3632 [calcd for $C_{47}H_{44}N_7O$ (M + H)$^+$ 722.3608].

5-n-Butyl-2,4-dihydro-2-(α-methylbenzyl)-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (71). A mixture of 40 mg (0.055 mmol) of **105**, 1.0 mL of glacial acetic acid, and 0.5 mL of H_2O was stirred overnight at room temperature. The cooled, filtered solution was concentrated in vacuo, and the residue was reconcentrated from toluene. The resulting crude product was flash chromatographed (gradient elution with 4–10% MeOH in CH_2Cl_2) to give, after coevaporation with chloroform, 20 mg (75%) of the desired product as a white powder: mp 162.5–164 °C; TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, J = 7.3 Hz, 3 H), 1.32 (m, 2 H), 1.60 (m, 2 H), 1.72 (d, J = 7.1 Hz, 3 H), 2.42 (t, J = 7.5 Hz, 2 H), 4.70, 4.78 (d, J = 16 Hz, each 1 H), 5.37 (q, J = 7.1 Hz, 1 H), 7.13 (apparent s, 4 H), 7.2–7.4 (m, 6 H), 7.51–7.58 (m, 2 H), 8.03 (d, J = 7.3 Hz, 1 H); FAB-MS m/e 480 (M + H)$^+$. Anal. ($C_{28}H_{29}N_7O \cdot 0.1CHCl_3$) C, H, N.

Method C. 5-n-Butyl-4-[(2′-cyanobiphenyl-4-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (104b). A mixture of 400 mg (1.92 mmol) of 4′-(aminomethyl)-2-biphenylcarbonitrile (**103b**),[18] 457 mg (2.12 mmol) of **100**, and 7 mL of ethanol was stirred at 50 °C for 3 h and then at 80 °C for 2 days. The mixture was cooled and concentrated in vacuo. The residue was flash chromatographed (gradient elution with 1.5–5% MeOH in CH_2Cl_2) to give 591 mg (93%) of the desired product as a colorless oil: TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85 (t, J = 7.2 Hz, 3 H), 1.35 (m, 2 H), 1.60 (m, 2 H), 2.42 (t, J = 7.2 Hz, 2 H), 4.87 (s, 2 H), 7.35 (d, J = 7 Hz, 2 H), 7.50 (m, 4 H), 7.62 (d, J = 8.5 Hz, 1 H), 7.75 (d, J = 8.5 Hz, 1 H), 9.13 (br s, 1 H); FAB-MS m/e 333 (M + H)$^+$. Anal. ($C_{20}H_{20}N_4O \cdot 0.33H_2O$) C, H, N.

5-n-Butyl-4-[(2′-cyanobiphenyl-4-yl)methyl]-2,4-dihydro-2-(2,2-dimethyl-1-propyl)-3*H*-1,2,4-triazol-3-one (106). A mixture of 100 mg (0.30 mmol) of **104b**, 29 mg (0.60 mmol) of sodium hydride (60% in oil), and 600 μL of dry DMF was stirred at room temperature for 2 h. Next, 250 μL (1.88 mmol) of 1-iodo-2,2-dimethylpropane was added, and stirring was continued overnight at 90 °C. After the mixture was cooled to room temperature, volatiles were evaporated, and the residue was coevaporated with toluene. Flash chromatography of the crude product (gradient elution with 0.5–2.0% MeOH in CH_2Cl_2) gave 93 mg (77%) of the desired compound as a clear oil: TLC in 98:2 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J = 7.3 Hz, 3 H), 1.02 (s, 9 H), 1.36 (m, 2 H), 1.55 (m, 2 H), 2.43 (t, J = 7.1 Hz, 2 H), 3.62 (s, 2 H), 4.90 (s, 2 H), 7.34–7.78 (m, 8 H); FAB-MS m/e 403 (M + H)$^+$; high-resolution FAB-MS m/e 403.2265 [calcd for $C_{25}H_{31}N_4O$ (M + H)$^+$ 403.2259].

5-n-Butyl-2,4-dihydro-2-(2,2-dimethyl-1-propyl)-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (54). A mixture of 80 mg (0.20 mmol) of **106**, 1 mL of dry toluene, and 143 mg (0.697 mmol) of trimethyltin azide was stirred and heated at 110 °C for 2 days. After the mixture was cooled to room temperature, toluene was removed under reduced pressure, and the residue was taken up in 3 mL of methanol. This solution was treated with 1 g of silica gel and stirred for 5 h at room temperature. Upon removal of the methanol, the resulting powder was made into a slurry in CH_2Cl_2 and applied to a flash chromatography column (gradient elution with 2–20% MeOH in CH_2Cl_2) to yield 43 mg (48%) of the desired material as a foam: mp 88–90 °C; TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, J = 7.3 Hz, 3 H), 0.92 (s, 9 H), 1.33 (m, 2 H), 1.57 (m, 2 H), 2.41 (t, J = 7.7 Hz, 2 H), 3.50 (s, 2 H), 4.78 (s, 2 H), 7.16 (apparent s, 4 H), 7.39–7.42 (m, 1 H), 7.52–7.59 (m, 2 H), 8.00 (d, J = 6.3 Hz, 1 H); FAB-MS m/e 446 (M + H)$^+$. Anal. ($C_{25}H_{31}N_7O \cdot 0.67H_2O$) C, H, N.

5-n-Butyl-2,4-dihydro-2-[1-(*N*-methylcarbamoyl)ethyl]-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (59). A solution of 100 mg (0.239 mmol) of 5-n-butyl-[2-(1-carbomethoxy)ethyl]-4-[(2′-cyanobiphenyl-4-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazol-3-one (**108**, prepared as described for **106** except that methyl 2-bromopropionate was used as the alkylating agent) in 0.5 mL of MeOH was treated with excess aqueous methylamine (~1.0 mL), and the resulting mixture was heated at reflux for 2 days. After the mixture was cooled to room temperature, the volatiles were removed in vacuo, and the residue was flash chromatographed (gradient elution with 0.5–1.0% MeOH in CH_2Cl_2) to give 79 mg (79%) of the amide as a colorless oil: TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88 (t, J = 7.3 Hz, 3 H), 1.35 (m, 2 H), 1.57 (m, 2 H), 1.62 (d, J = 7.3 Hz, 3 H), 2.45 (t, J = 7.4 Hz, 2 H), 2.80 (d, J = 4.8 Hz, 3 H), 4.90 (s, 2 H), 6.50 (m, 1 H), 7.33–7.36 (m, 2 H), 7.45–7.56

(m, 4 H), 7.60–7.70 (m, 1 H), 7.74–7.77 (m, 2 H); FAB-MS *m/e* 418 (M + H)$^+$; high-resolution FAB-MS *m/e* 418.2228 [calcd for $C_{24}H_{28}N_5O_2$ (M + H)$^+$ 418.2243].

Treatment of this amide with trimethyltin azide according to the procedure for the preparation of **54** gave **59** as a white powder in 43% yield after flash chromatography (gradient elution with 5–30% MeOH in CH_2Cl_2): mp 153–155 °C; TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.81 (t, *J* = 7.3 Hz, 3 H), 1.27 (m, 2 H), 1.42 (m, 2 H), 1.46 (d, *J* = 7.3 Hz, 3 H), 2.41 (t, *J* = 7.1 Hz, 2 H), 2.58 (d, *J* = 6.4 Hz, 3 H), 4.62 (m, 1 H), 4.80 (s, 2 H), 7.10–7.20 (m, 4 H), 7.42–7.70 (m, 4 H), 7.70–8.0 (m, 1 H); FAB-MS *m/e* 461 (M + H)$^+$; high-resolution FAB-MS *m/e* 461.2427 [calcd for $C_{24}H_{29}N_5O_2$ (M + H)$^+$ 461.2414].

5-n-Butyl-2,4-dihydro-2-[1-(hydroxymethyl)ethyl]-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (60). To a solution of 125 mg (0.299 mmol) of **108** (see procedure for **59**) in 1.0 mL of dry THF at 0 °C was added dropwise 450 μL (0.90 mmol) of a 2.0 M solution of lithium borohydride in THF. Upon completion of addition, the mixture was warmed to room temperature over 2 h before being cooled to 0 °C again and quenched with 2 mL of MeOH. Subsequently, 1.5 mL of HOAc was added followed by 10 mL of H_2O, and the organic material was extracted with 3 × 10 mL of EtOAc. The combined organic layers were washed with H_2O and brine and dried (Na_2SO_4). After filtration and removal of volatiles in vacuo, the residue was flash chromatographed (elution with 1.0% MeOH in CH_2Cl_2) to give 107 mg (91%) of the alcohol as a colorless oil: TLC in 95:5 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87 (t, *J* = 7.3 Hz, 3 H), 1.33 (m, 2 H), 1.40 (d, *J* = 6.8 Hz, 3 H), 1.55 (m, 2 H), 2.11 (br s, 1 H), 2.40 (t, *J* = 7.6 Hz, 2 H), 3.79–3.85 (m, 1 H), 3.91–3.96 (m, 1 H), 4.33–4.38 (m, 1 H), 4.87 (s, 2 H), 7.32–7.35 (m, 2 H), 7.41–7.55 (m, 4 H), 7.60–7.67 (m, 1 H), 7.74 (d, *J* = 7.7 Hz, 1 H); FAB-MS *m/e* 391 (M + H)$^+$; high-resolution FAB-MS *m/e* 391.2135 [calcd for $C_{23}H_{27}N_4O_2$ (M + H)$^+$ 391.2134].

Treatment of this alcohol with trimethyltin azide according to the procedure for the preparation of **54** gave **60** as a white powder in 51% yield after flash chromatography (gradient elution with 5–30% MeOH in CH_2Cl_2): mp 114–116 °C; TLC in 90:10 CH_2Cl_2/MeOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86 (t, *J* = 7.2 Hz, 3 H), 1.24 (d, *J* = 6.7 Hz, 3 H), 1.32 (m, 2 H), 1.54 (m, 2 H), 2.43 (t, *J* = 7.7 Hz, 2 H), 3.61–3.78 (m, 2 H), 4.40–4.53 (m, 1 H), 4.78 (d, *J* = 16 Hz, 1 H), 4.92 (d, *J* = 16 Hz, 1 H), 7.07–7.20 (m, 4 H), 7.41–7.60 (m, 3 H), 7.95 (d, *J* = 6.6 Hz, 1 H); FAB-MS *m/e* 434 (M + H)$^+$. Anal. ($C_{23}H_{27}N_7O_2 \cdot H_2O \cdot 0.15CH_2Cl_2$) C, H, N.

5-n-Butyl-2-(2-carboxybenzyl)-2,4-dihydro-4-[[2′-(5-tetrazolyl)biphenyl-4-yl]methyl]-3*H*-1,2,4-triazol-3-one (76). To a solution of 20 mg (0.038 mmol) of the methyl ester **72** in 200 μL of dry THF was added 84 μL (0.084 mmol) of 1 N NaOH in MeOH. The mixture was stirred overnight at room temperature and then evaporated to dryness. The residual solid was dissolved in 1.5 mL of MeOH and treated with 90 μL of 1 N HCl in MeOH until pH 2 was reached. The solvent was evaporated, and the residue was leached with $CHCl_3$. After removal of insoluble solid (NaCl) by filtration through glass wool, the filtrate was concentrated in vacuo and then reconcentrated from toluene. The residue was dried overnight in the presence of P_2O_5 to give 20 mg (95%) of the titled compound as a stiff, white foam: mp 114–117 °C; TLC in 90:10:0.1 CH_2Cl_2/MeOH/AcOH; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.84 (t, *J* = 7.5 Hz, 3 H), 1.30 (m, 2 H), 1.55 (m, 2 H), 2.43 (t, *J* = 7.5 Hz, 2 H), 4.77 (s, 2 H), 5.27 (s, 2 H), 7.10 (m, 4 H), 7.2–7.6 (m, 6 H), 7.92, 7.96 (d, *J* = 8 Hz, each 1 H); FAB-MS *m/e* 510 (M + H)$^+$. Anal. ($C_{28}H_{27}N_7O_3 \cdot 2H_2O \cdot 0.1$-toluene) C, H, N.

Rabbit Aorta AT_1 Receptor Binding Assay.[30,18] Rabbit aorta membrane pellets, prepared as previously described,[30] were suspended in binding buffer. The original version of the assay also included 0.2% bovine serum albumin (BSA).

Test compounds were dissolved at 2.7 mM in 1:1 DMSO–MeOH and serially diluted to five concentrations bracketing the IC_{50}. All binding assays were performed in duplicate tubes. To each incubation tube was added 10 μL of ^{125}I[Sar^1,Ile^8]AII at a final concentration of 20–40 pM and 10 μL of one of the following: (a) buffer vehicle (for total binding), (b) unlabeled 1 μM [Sar^1,Ile^8]AII (for nonspecific binding), or (c) the test compound solution (for displacement of specific binding). Finally, 250 μL of the above membrane preparation was added to each tube. The tubes were mixed and incubated in a water bath at 37 °C for 90 min. The mixture, after dilution with wash buffer, was filtered immediately under reduced pressure. The filters were washed with wash buffer and the radioactivity associated with the membrane collected was measured.[18] After correction for nonspecific binding, the bound radioactivity in the presence of a given concentration of test compound was compared to specific binding in the control to determine the percent inhibition. The concentration required to inhibit specific binding of ^{125}I[Sar^1,Ile^8]AII to the receptor by 50% (IC_{50}) was calculated using nonlinear regression analysis of the displacement curves. On the basis of the results of several standard compounds having three or more determinations, the standard error (expressed as percent of mean) of the IC_{50} measurement in this assay is estimated to be less than 30%. In some cases the reported IC_{50} values represent an average of two or more determinations from separate assays.

Evaluation of AII Antagonists in Conscious, Normotensive Rats.[18] Male Sprague–Dawley rats were anesthetized with methohexital sodium and surgically instrumented with catheters for (a) measurements of arterial blood pressure and heart rate, (b) administration of AII, and (c) intravenous administration of test compound, as appropriate. The incisions were sutured, and the rats were allowed to recover overnight prior to testing. Angiotensin II and methoxamine were each dissolved in saline solution and administered in injection volumes of 0.5 mL/kg iv in the appropriate vehicles as described previously.[18]

The responsiveness of the rat was verified by initial challenge with methoxamine followed by bolus injections of AII at 15-min intervals. Provided that AII responses were consistent, the test compound in its vehicle was administered intravenously or orally. AII was then given at fixed time points for as long as the test compound exhibited activity. At the conclusion of AII challenges, the catheter was flushed, and methoxamine was administered as a control.

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. For each compound at a given dose, the peak percent inhibition and duration of action were determined, based on averaged results from two or more rats in most cases. A 30% inhibition of the AII pressor response is considered significant in this assay. The duration of action for a single bolus dose of the test compound is defined as the time from onset of activity until the inhibition of the AII-induced increase in MAP falls below 30% and remains at <30% for two subsequent AII challenges.

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