In summary, the azulene derivatives have potent TXA_2/PGH_2 receptor antagonist activities. In this series, compounds having carboxylic acid or the sulfonic acid sodium salt group at the 1-position have greatest activity, with **7b** being the most potent, orally effective, and τ-receptor-selective antagonist without partial agonistic activity. On the basis of these data, we suggest that TXA_2/PGH_2 receptor antagonists derived from azulene derivatives possess good in vitro and in vivo activities.

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Articles

1,2,4-Triazolo[4,3-*a*]pyrazine Derivatives with Human Renin Inhibitory Activity. 1. Synthesis and Biological Properties of Alkyl Alcohol and Statine Derivatives

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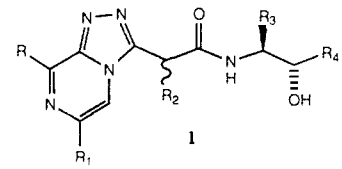
A series of 1,2,4-triazolo[4,3-*a*]pyrazine derivatives with human renin inhibitory activity, which incorporate (1*S*,2*S*)-2-amino-1,3-dicyclohexyl-1-hydroxypropane, statine (Sta), and (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) transition-state mimetics, have been prepared. Structure–activity relationships for renin inhibitory activity in the series are consistent with the 2-[8-isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl]-3-(3-pyridyl)propionic acid moiety **10b** acting as a non-peptidic replacement for the P_4–P_2 (Pro-Phe-His) residues of the natural substrate angiotensinogen. Compounds **12m**, **12o**, and **12q** were potent inhibitors of partially purified human renin (IC_{50} values 1.7, 6.8, and 3.7 nM, respectively), and also effectively lowered blood pressure in anesthetized, sodium depleted marmosets following intravenous administration. On oral administration however, no blood pressure lowering activity could be detected, and absorption studies in bile duct cannulated rats indicate that this may be due primarily to poor oral absorption, rather than rapid biliary excretion. The reason for the observed poor oral activity is not clear, but it seems unlikely that poor aqueous solubility or metabolic instability to gut enzymes are rate-determining, and other factors such as high molecular weight may also be very important.

The renin–angiotensin system is a multiregulated proteolytic cascade of enzyme-mediated events that converts angiotensinogen to angiotensin I (AI), angiotensin II (AII), and angiotensin III (AIII), and so provides a major regulatory mechanism for the control of blood pressure in mammals[1] (Scheme I). Inhibition of angiotensin converting enzyme (ACE), which cleaves AI to AII, has demonstrated that blockade of this system is an effective means of reducing blood pressure in a large majority of hypertensive patients.[2] The conversion of angiotensinogen to AI by the enzyme renin is the first and rate-limiting step in the cascade. Animal studies comparing an ACE inhibitor with a renin inhibitor have shown the two agents to be equieffective.[3] Moreover, renin inhibitors may have the potential for fewer side effects, since, unlike ACE, which hydrolyzes a variety of biologically important peptides,[4] renin is uniquely specific, having angiotensinogen as its only known substrate.[5]



1

Figure 1. General inhibitor structure.

Most of the currently available renin inhibitors, although highly potent in vitro, are peptidic in nature. As a consequence, these compounds tend to suffer from all the

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Scheme I. Renin–Angiotensin System

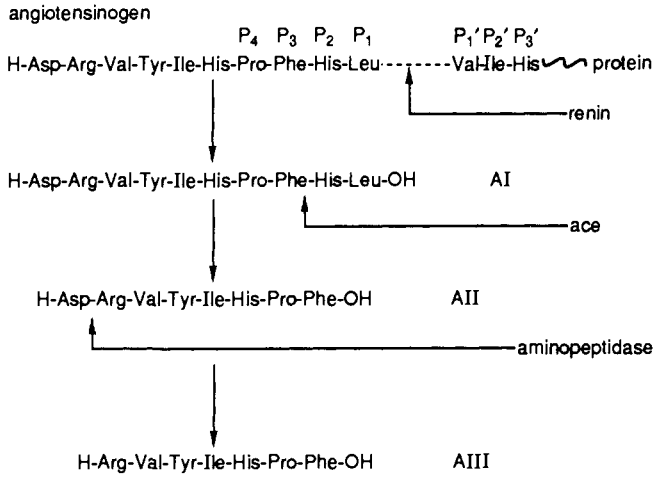


Table I. Physicochemical Data for Intermediates Described in Scheme II

compd no.	mp, °C	formula	anal.
4a	187–188	$C_{13}H_{14}N_2O$	C,H,N
4b	205–207	$C_{14}H_{16}N_2O$	C,H,N
4c	193–195	$C_{12}H_{12}N_3O$	C,H,N
5a	126	$C_{13}H_{16}N_4$	C,H,H
5b	109–110	$C_{14}H_{18}N_4$	C,H,N
5c	164–165	$C_{12}H_{15}N_5$	C,H,N
6a	115–116	$C_{18}H_{22}N_4O_3$	C,H,N
6b	116–118	$C_{19}H_{24}N_4O_3$	C,H,N
6c	141–142	$C_{17}H_{21}N_5O_3$	C,H,N
6d	foam[a]	$C_{13}H_{20}N_4O_3$	-
7a	139–140	$C_{18}H_{20}N_4O_2$	C,H,N
7b	105–107	$C_{19}H_{22}N_4O_2$	C,H,N
7c	130–131	$C_{17}H_{19}N_5O_2$	C,H,N
7d	89–90	$C_{13}H_{18}N_4O_2$	C,H,N
8a	142–143	$C_{23}H_{24}N_6O_2$	C,H,N
8b	110–111	$C_{20}H_{27}N_5O_2$	C,H,N
8c	glass[a]	$C_{23}H_{26}N_6O_2$	-
9a	166–168	$C_{16}H_{16}N_4O_2 \cdot 1.5H_2O$	C,H,N
9b	160[b]	$C_{17}H_{18}N_4O_2$	C,H,N
9c	not isolated[c]	-	-
9d	120–121	$C_{11}H_{14}N_4O_2 \cdot 0.5H_2O$	C,H,N
10a	not isolated[c]	-	-
10b	not isolated[c]	-	-
11	200–205[a]	$C_{21}H_{24}N_8O$	-

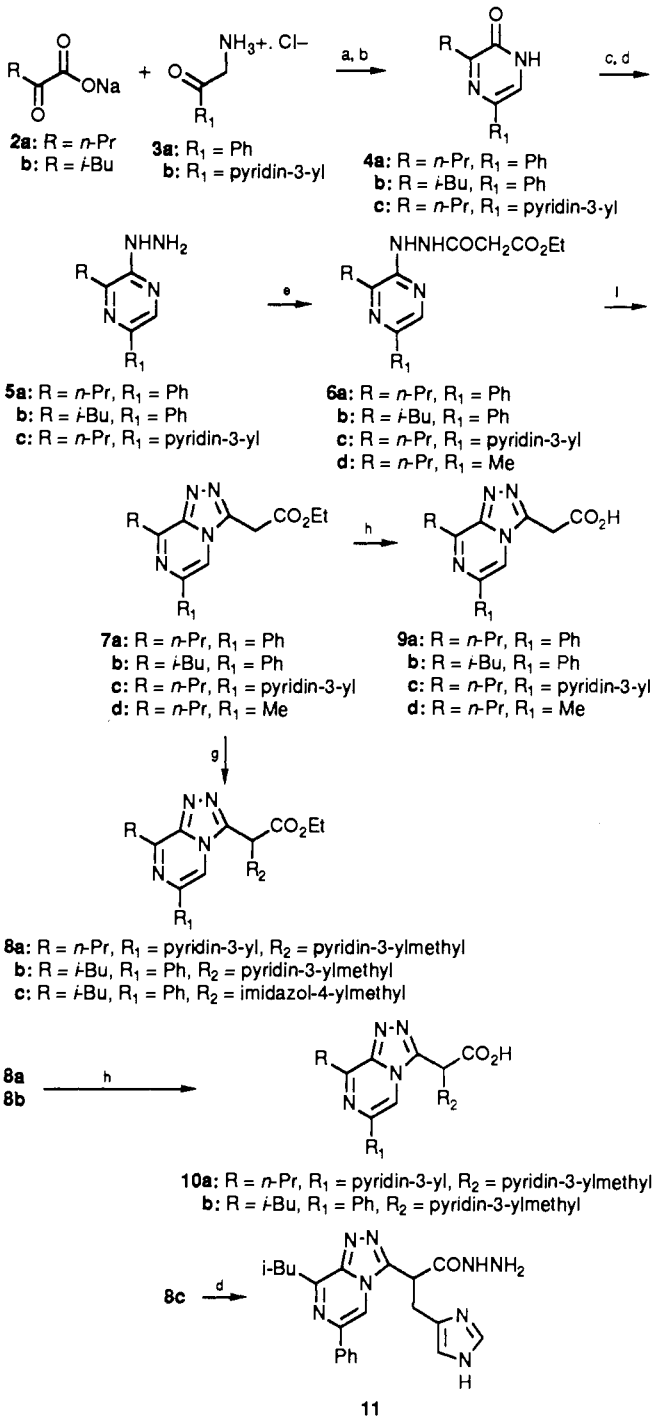
[a] Characterized spectroscopically. [b] Resolidifies and remelts at 181–183 °C. [c] Used directly as the sodium salt.

problems normally associated with peptides such as poor oral activity, proteolytic instability, short duration of action, and rapid excretion.[6] In an attempt to overcome these problems, we have synthesized a series of non-peptidic inhibitors, represented generically by **1** (Figure 1), in which we have combined known transition-state mimetics spanning the P_1–P_4' region of the substrate with novel substituted 1,2,4-triazolo[4,3-*a*]pyrazin-3-ylacetic acid moieties, which appear to replace the P_4–P_2 (Pro-Phe-His) residues of the substrate.

Chemistry

The 1,2,4-triazolo[4,3-*a*]pyrazin-3-ylacetic acid intermediates (Table I) required for the preparation of the various inhibitors listed in Tables II and III, were synthesized as shown in Scheme II. Standard amide coupling of the appropriately substituted α-keto acids **2a,b** with the

Scheme II[a]



[a] (a) DCC/CH_2Cl_2; (b) NH_4OAc/EtOH; (c) $COCl_2$/toluene/THF; (d) $H_2NNH_2 \cdot H_2O$/DMSO; (e) $ClCOCH_2CO_2Et$/EtOAc; (f) PTSA/toluene; (g) NaH/DMF/R_2–Cl; (h) NaOH/EtOH.

α-amino ketones **3a,b**, followed by direct reaction of the intermediate α-keto amides with ammonium acetate in ethanol under reflux gave the hydroxypyrazines **4a–c**. This method appears to be superior to previous syntheses of these ring systems,[7] which utilized various glyoxals and the appropriate amino acid amides as starting materials, giving rise to regioisomeric mixtures of products. Chlorination of **4a–c** and reaction of the intermediate chloropyrazine derivatives with hydrazine hydrate gave the hydrazinopyrazines **5a–c**, which were acylated with ethylmalonyl chloride to afford the acylhydrazines **6a–d**. The hydra-

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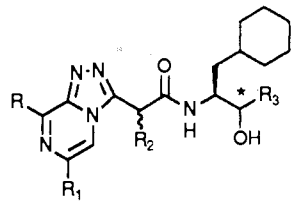
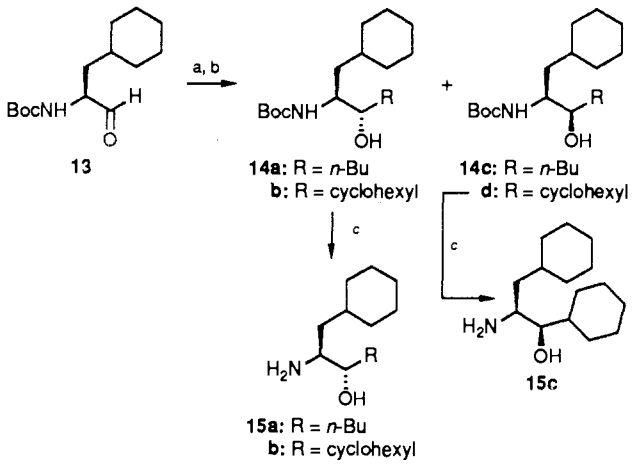
Table II. Characterization and in Vitro Human Renin Inhibition of Compounds **12a–h**



no.	R	R_1	R_2	R_3	configuration at C*	mp, °C	formula[a]	human renin IC_{50} nM[b]
12a	*n*-Pr	Me	H	*n*-Bu	*S*	128–132	$C_{24}H_{39}N_5O_2 \cdot 0.5H_2O$	>100 000
12b	*n*-Pr	Me	H	C_6H_{11}	*S*	147–150	$C_{26}H_{41}N_5O_2$	32 000
12c	*n*-Pr	Ph	H	C_6H_{11}	*S*	196–198	$C_{31}H_{43}N_5O_2$	1 920
12d	*i*-Bu	Ph	H	C_6H_{11}	*S*	188–192	$C_{32}H_{45}N_5O_2$	1 100
12e	*i*-Bu	Ph	H	C_6H_{11}	*R*	100–105	$C_{32}H_{45}N_5O_2 \cdot 0.5H_2O$	>100 000
12f	*i*-Bu	Ph	imidazol-4-ylmethyl[c]	C_6H_{11}	*S*	146–150	$C_{36}H_{49}N_7O_2 \cdot H_2O$	330
12g	*i*-Bu	Ph	pyridin-3-ylmethyl[c]	C_6H_{11}	*S*	210–214	$C_{38}H_{50}N_6O_2$	290
12h	*n*-Pr	pyridin-3-yl	H	C_6H_{11}	*S*	204–208	$C_{30}H_{42}N_6O_2$	48

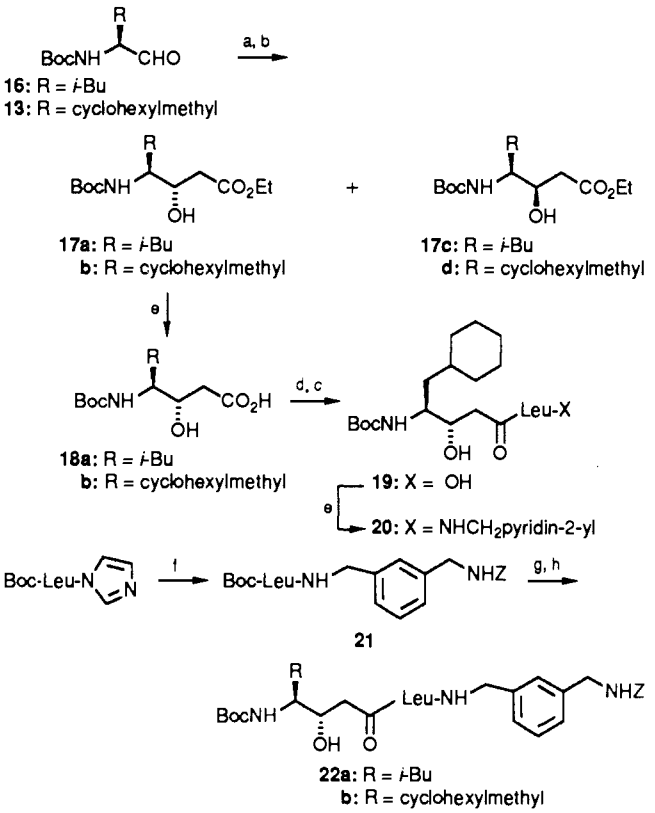
[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] Purified human kidney renin was incubated with human angiotensinogen. Test details are described in the Experimental Section. [c] Compounds **12f** and **12g** are diastereoisomeric mixtures (ca. 1:1).

Scheme III.[a] Preparation of Amines **15a–c**



[a] (a) RMgBr/ether; (b) flash chromatography on silica; (c) HCl/dioxane.

Scheme IV.[a] Preparation of Amines **20**, **22a**, and **22b**



[a] (a) $LiCH_2CO_2Et/ZnBr_2$; (b) chromatographic separation; (c) NaOH/EtOH; (d) $Me_2N(CH_2)_3NCNEt \cdot HCl$/HOBT/H-Leu-OMe/$Et_3N$; (e) $Me_2N(CH_2)_3NCNEt \cdot HCl$/HOBT/$Et_3N$/2-(aminomethyl)pyridine; (f) *m*-(Z-aminomethyl)benzylamine/CH_2Cl_2; (g) HCl/dioxane; (h) $Me_2N(CH_2)_3NCNEt \cdot HCl$/HOBT/$Et_3N$/either **18a** or **18b**.

zinopyrazine precursor to **6d** is a known compound.[8] Cyclization under acidic conditions gave the 1,2,4-triazolo[4,3-*a*]pyrazin-3-ylacetic acid derivatives **7a–d** which were hydrolyzed directly to the acids **9a–d**. Compounds **7a–c** were also alkylated with the appropriate chloromethyl heterocycles to give **8a–c**. Hydrolysis of the esters **8a,b** then gave the acids **10a,b**, while the α-imidazol-4-ylmethyl derivative **8c** was converted to the hydrazide **11**.

The amine derivatives used in the synthesis of **12a–h** were prepared as shown in Scheme III. Thus addition of *n*-butylmagnesium bromide to aldehyde **13**[9] gave a mixture of **14a** and **14c** which were separated by chromatography. Deprotection of **14a** then gave the amino alcohol **15a**. Similarly, addition of cyclohexylmagnesium bromide to **13**, followed by chromatographic separation of **14b** and **14d** and deprotection, gave **15b** and **15c**.

The amine derivatives used in the synthesis of **12i–o** and **12r** (Table III) were prepared as shown in Scheme IV. The statine ((3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; Sta) and (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA) derivatives (**17a,b**) were prepared with use of a modification[10] of existing procedures.[9,11] Thus condensation of aldehydes **16** and **13** with the zinc enolate of ethyl acetate at −78 °C furnished the required 3*S*,4*S* compounds **17a,b** in moderate yield and high (>95:5) diastereoselectivity over the 3*R*,4*S* isomers

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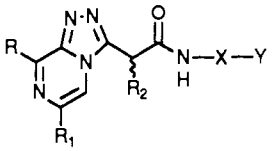
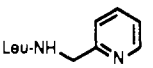
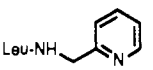
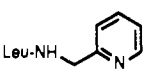
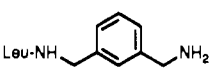
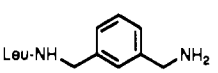
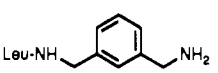
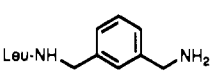
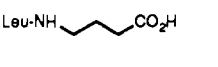
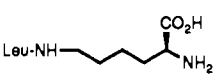
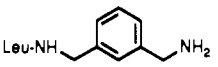
Table III. Characterization and in Vitro Human Renin Inhibition of Compounds **12i–r**



no.	R	R_1	R_2	X	Y	mp, °C	formula[a]	human renin IC_{50} nM[b]
12i	*i*-Bu	Ph	H	ACHPA		110–114	$C_{40}H_{54}N_8O_4 \cdot 2H_2O$	63
12j	*i*-Bu	Ph	pyridin-3-ylmethyl (less polar epimer)	ACHPA		115–120	$C_{46}H_{59}N_9O_4 \cdot H_2O$	640
12k	*i*-Bu	Ph	pyridin-3-ylmethyl (more polar epimer)	ACHPA		115–120	$C_{46}H_{59}N_9O_4 \cdot 2H_2O$	1.0
12l	*i*-Bu	Ph	pyridin-3-ylmethyl (less polar epimer)	ACHPA		136–142	$C_{48}H_{63}N_9O_4$[c]	190
12m	*i*-Bu	Ph	pyridin-3-ylmethyl (more polar epimer)	ACHPA		125–130	$C_{48}H_{63}N_9O_4 \cdot H_2O$	1.7
12n	*i*-Bu	Ph	pyridin-3-ylmethyl (less polar epimer)	Sta		156–164	$C_{45}H_{59}N_9O_4 \cdot 2H_2O$	130
12o	*i*-Bu	Ph	pyridin-3-ylmethyl (more polar epimer)	Sta		155–164	$C_{45}H_{59}N_9O_4 \cdot 4H_2O$	6.8
12p	*i*-Bu	Ph	pyridin-3-ylmethyl (more polar epimer)	ACHPA		180–184	$C_{44}H_{60}N_8O_6 \cdot 2H_2O$	1.9
12q	*i*-Bu	Ph	pyridin-3-ylmethyl (more polar epimer)	ACHPA		160–170	$C_{46}H_{65}N_9O_6 \cdot 4H_2O$	3.7
12r	*n*-Pr	pyridin-3-yl	pyridin-3-ylmethyl (more polar epimer)	ACHPA		142–146	$C_{46}H_{60}N_{10}O_4 \cdot 3.5H_2O$	1.6

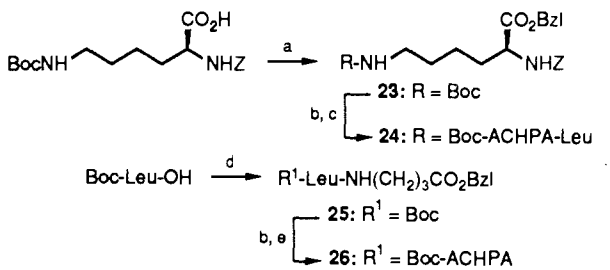
[a] Analyses for C, H, N were correct within ±0.4% unless otherwise stated. [b] Purified human renin was incubated with human angiotensinogen. Test details are described in the Experimental Section. [c] Characterized spectroscopically.

17c,d, which were removed by chromatography. Hydrolysis of **17a,b** gave the acids **18a,b**. Compound **19** was prepared by standard amide coupling methodology with **18b** and leucine methyl ester, followed by base hydrolysis. Coupling of **19** with 2-aminomethylpyridine then gave **20**. Similarly, coupling of Boc-Leu-imidazolide with *m*-[[(benzyloxycarbonyl)amino]methyl]benzylamine gave **21** which was deprotected and coupled with **18a,b** to give the amine derivatives **22a,b**.

The amine derivatives used in the synthesis of **12p,q** were prepared as shown in Scheme V. Thus compound **23**, derived by benzylation of (2*S*)-2-[(benzyloxycarbonyl)amino]-6-[(*tert*-butyloxycarbonyl)amino]hexanoic acid,[12] was deprotected and coupled with **19** to furnish **24**. Similarly, coupling of Boc-Leucine with benzyl 4-aminobutyrate gave **25**, which was deprotected and coupled with **18b** to give **26**.

The final compounds **12a–e**, **12g**, and **12h** described in Table II were prepared by amide coupling of the appropriate acids **9a–d** and **10b** with amines **15a–c**. Compound **12f** was prepared by acyl azide coupling of the hydrazide **11** with the amine **15b**. The compounds described in Table III were prepared as follows: **12i–k** were synthesized by amide coupling of the acids **9b** and **10b** with the amine derivative obtained by deprotection of **20**; **12j,k** were formed as a diastereoisomeric mixture which was separated chromatographically, compounds **12l–r** were prepared in

Scheme V.[a] Preparation of Amine Derivatives **24** and **26**



[a] (a) K_2CO_3/BzlBr/acetone; (b) HCl/dioxane; (c) $Me_2N(CH_2)_3NCNEt \cdot HCl$/DMF/HOBT/$Et_3N$/**19**; (d) $Me_2N(CH_2)_3NCNEt \cdot HCl$/DMF/HOBT/$Et_3N$/$H_2N(CH_2)_3CO_2Bzl$; (e) $Me_2N(CH_2)_3NCNEt \cdot HCl$/DMF/HOBT/$Et_3N$/**18b**.

two steps by firstly amide coupling of the acids **10a,b** with the amine derivatives obtained by deprotection of **22a,b**, **24**, and **26**, followed by chromatographic separation of the diastereoisomeric mixtures; hydrogenolytic removal of the benzyloxycarbonyl and benzyl protecting groups by using either phase-transfer hydrogenation (**12l–p**, **12r**) or catalytic hydrogenation over palladized charcoal (**12q**), then furnished the final products.

In Vitro Renin Inhibition

Previous work, both in our laboratories, and elsewhere,[13]

(12) Schnabel, E. *Liebig's Ann. Chem.* **1967**, *702*, 188.

(13) Luly, J. R.; Dellaria, J. F.; Plattner, J. J. European Patent, 172,346, 1986.

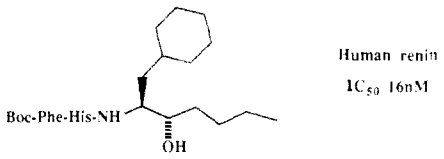


Figure 2. Typical structure of an acylated dipeptide renin inhibitor incorporating an alkyl alcohol type transition state mimetic.

has demonstrated that moderately potent renin inhibitors (IC_{50}s ca. 10 nM) can be obtained by incorporating alkyl alcohol type transition-state mimetics (eg. **15a,b**) into the acylated dipeptide sequence Boc-Phe-His... (Figure 2). However, compounds of this type appear to suffer from all the inherent drawbacks associated with peptides in vivo. In an attempt to overcome these problems, we decided to synthesize a number of amides derived from various heterocyclic acetic and propionic acids and the amine **15a**, with the objective of identifying a non-peptidic lead compound. Out of more than 70 compounds prepared, only one, the 1,2,4-triazolo[4,3-*a*]pyrazine derivative **12a**, demonstrated very weak but consistent activity in vitro (Table II). Encouraged by this observation, the cyclohexyl analogue **12b** was synthesized and exhibited slightly improved activity, and replacement of the heterocyclic 6-methyl group by phenyl (**12c**) gave a further improvement. A variety of functional groups were incorporated at the 8-position of the heterobicycle (data not shown) but the isobutyl group was chosen for further study, since it was found to be readily accommodated and easily synthetically accessible (**12d**). In order to test whether the *S* secondary hydroxyl group of **12d** was binding to the active site of the enzyme in a stereospecific fashion, the *R* isomer was synthesized (**12e**), and indeed, demonstrated the expected fall in activity. This observation is consistent with the importance of the 3(*S*)-hydroxyl group of previously described statine containing inhibitors.[14] Structural comparison of these compounds with the corresponding peptidic series indicated that the disubstituted 1,2,4-triazolo[4,3-*a*]pyrazin-3-acetyl moiety might be mimicking the Pro-Phe-His residues of the natural substrate. If this was the case, we reasoned that substitution of the methylene group α to the heterocycle with a group to mimic the side chain of the histidine residue might be beneficial. Incorporation of an imidazol-4-ylmethyl group at this position (**12f**) did indeed improve potency and this was maintained in the more readily accessible α-pyridin-3-ylmethyl analogue (**12g**). For synthetic convenience, all the remaining α-substituted analogs (**12j–r**) incorporated this same feature. A wide variety of substituted phenyl and heteroaryl variants of the heterobicyclic 6-position were synthesized (data not shown). From these studies, the 6-pyridin-3-yl derivative (**12h**) was found to confer the largest gain in in vitro potency over the parent compound **12d**.

In an attempt to further improve in vitro potency, the alternative ACHPA transition-state mimetic was incorporated in conjunction with a more hydrophilic C-terminal group[15] to give **12i**, and again potency was improved relative to **12d**. Incorporation of an α-pyridin-3-ylmethyl group furnished diastereoisomers **12j** and **12k**, the latter, more polar, compound being some 60-fold more potent than its epimer. This result reinforced our view that the α-pyridin-3-ylmethyl group was binding to the S_2 site of the enzyme and led us to hypothesize that the absolute configuration at the α-carbon of **12k**, must be *S*, as in the natural substrate. However, we have no definitive chemical evidence for this. We were also keen to decrease the lipophilicity of the series in order to optimize physical properties such as aqueous solubility. With this in mind, the previously reported[15] [*m*-(aminomethyl)benzyl]amino C-terminal group was incorporated in **12l** and **12m**, the latter, more polar, isomer this time being >100-fold more potent that its epimer. Similar results were obtained in the statine derivatives **12n** and **12o**, the latter compound being somewhat less potent than **12m**, consistent with previous results[9] in peptidic series. Acidic (**12p**) or zwitterionic (**12q**) termini could also be incorporated without undue loss in potency. Disappointingly, replacement of the 6-phenyl group by the previously described 6-pyridin-3-yl heterocycle in **12r**, failed to give the improvement in potency seen previously in **12h**.

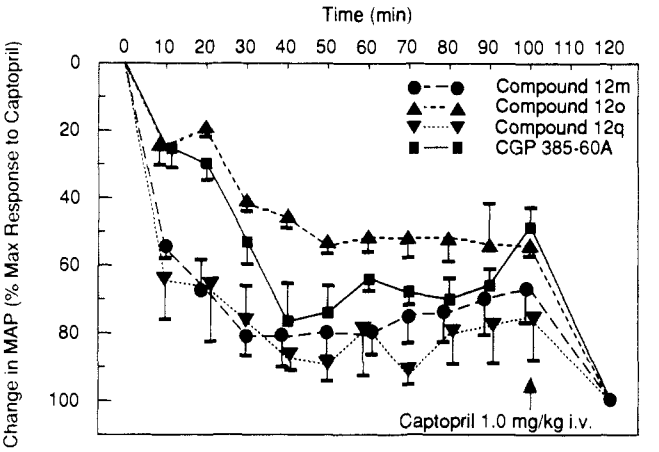


Figure 3. Effects on mean arterial pressure (MAP) of CGP 385-60A, compounds **12m**, **12o**, and **12q**, dosed at 3 mg/kg iv to anesthetized, sodium-depleted marmosets. Effects on MAP are expressed as percentages of the response to captopril (1.0 mg/kg iv). Mean SE values are shown (n = 3).

Pharmacological Evaluation

Several of the above series of compounds (**12k**, **12m**, **12o–r**) were chosen for evaluation of hypotensive efficacy in anesthetized, sodium depleted marmosets. Typical responses for this group of compounds following intravenous administration are illustrated for **12m**, **12o**, and **12q** in Figure 3, using a supramaximal dose of captopril as an internal standard. The fall in mean arterial pressure (MAP) produced by captopril in these experiments was 44.3 ± 2.7 mm. The hypotensive activity in this model of the recently described[16,17] CGP 385-60A is also included for comparison. All three compounds, **12m**, **12o**, and **12q**, caused significant falls in MAP, of similar magnitude to CGP 385-60A. In addition, all three compounds maintained these falls in MAP for the whole duration of the experiment.

Disappointingly, when these compounds were administered orally (50 mg/kg) to the anesthetized, sodium-depleted marmoset, no changes in MAP were detected.

(14) Boger, J.; Lohr, N. S.; Ulm, E. H.; Poe, M.; Blaine, E. H.; Fanelli, G. M.; Lin, T.-Y.; Payne, L. S.; Schorn, T. W.; LaMont, B. I.; Vassil, T. C.; Stabilito, I. I.; Veber, D. F.; Rich, D. H.; Bopari, A. S. *Nature (London)* **1983**, *303*, 81.

(15) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. *J. Med. Chem.* **1988**, *31*, 1918.

(16) Buhlmayer, P.; Caselli, A.; Fuhrer, W.; Goschke, R.; Rasetti, V.; Rueger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. *J. Med. Chem.* **1988**, *31*, 1839.

(17) De Gasparo, M.; Cumin, F.; Nussberger, J.; Guyenne, T. T.; Wood, J. M.; Menard, J. *Br. J. Clin. Pharmac.* **1989**, *27*, 587.

Table IV. Biliary Excretion of Compound **12o** after Dosing at 10 mg/kg Intravenously to Conscious Rats with Indwelling Bile Duct Cannulae

time after dosing (h)	concentration of compound **12o** in bile, μg/rat per h (n = 3)[a]
0–1	114.2 ± 46.5
1–2	335.7 ± 15.9
2–3	303.4 ± 17.6
3–4	251.2 ± 13.8
4–5	112.9 ± 18.6
5–6	66.7 ± 8.7
6–24	4.8 ± 1.1

[a] Calculated % dose excreted in bile over 24 h, 63.7 ± 2.9%.

Moreover, neither compound **12m** nor **12o** demonstrated any inhibition of plasma renin activity at this oral dose. In order to ascertain whether the lack of oral activity of **12o** was the result of poor absorption/gut metabolism or rapid biliary excretion, the compound was dosed to bile duct cannulated rats in a crossover fashion (10 mg/kg iv and 10 mg/kg orally), and bile samples taken over a 24-h period were analyzed by HPLC (Table IV). Following the iv administration, 63.7 ± 2.9% (n = 3) of the original dose appeared in the bile as unchanged drug, which was eliminated at a fairly constant rate over the first 4 h. By contrast, following oral administration of **12o**, no unchanged drug was detected in the bile, despite the HPLC detection limit being ca. 0.02% of the original dose. Our conclusion from these studies is that **12o** was either metabolized in the gut or never absorbed. A similar study in bile duct cannulated rats with **12m** gave exactly the same result, i.e. no oral absorption. The aqueous solubility of **12m** is poor (32 μg/mL) and this may be the dominant factor in its poor oral activity. However, the statine containing analogue **12o** is significantly more soluble (400 μg/mL), consistent with previous observations.[15] In order to ascertain whether metabolic instability in the rat gut was the route of the problem, several examples of this chemical series were incubated in rat gastric juice for 90 min. No significant degradation occurred indicating stability to gut enzyme (results not shown). By contrast, several peptide derivatives of the type illustrated in Figure 2 were rapidly hydrolyzed between the Phe-His residues in this same enzyme system.

Summary

This paper describes a novel series of 1,2,4-triazolo[4,3-a]pyrazine derivatives which display potent inhibition of human renin in vitro. Structure–activity relationships in the series indicate that the trisubstituted 1,2,4-triazolo[4,3-a]pyrazin-3-acetyl moiety may be mimicking the P_4–P_2 residues (Pro-Phe-His) of the natural substrate. These compounds caused marked reductions in mean arterial pressure in anesthetized, sodium-depleted marmosets, following intravenous administration. However, on oral administration, the compounds show no activity, and absorption studies in bile duct cannulated rats indicate that this may be due primarily to poor oral absorption, rather than rapid biliary excretion. The reason for the observed poor absorption is not clear, but it seems unlikely that poor aqueous solubility or metabolic instability to gut enzymes are rate-determining, and that other factors such as high molecular weight may also be important. Further work in this series, which attempts to address this latter problem, is described in the following paper.

Experimental Section

All operations were carried out at ambient temperature unless otherwise stated. Tetrahydrofuran (THF) was dried by distillation from calcium hydride. CH_2Cl_2 was dried by distillation from P_2O_5. All evaporations were carried out at below 50 °C by using a Büchi rotary evaporator. Melting points were taken on a Buchi apparatus with glass capillary tubes and are not corrected. NMR spectra were recorded on Bruker WM200, WM250 or WM400 instruments and are reported as δ values (parts per million) relative to Me_4Si as an internal standard. Chemical ionization mass spectra (CIMS) were recorded on a VG 12-12 quadrapole or a VG 70-250 SE spectrometer. Positive fast-atom bombardment mass spectra (FABMS) were determined on a VG ZAB 2-SE or a VG modified AE1/Dratos MS9 spectrometer. IR spectra were determined on a Perkin-Elmer 1420 spectrophotometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of the theoretical values.

3-Isobutyl-5-phenylpyrazin-2-ol (4b). A solution of 1,3-dicyclohexylcarbodiimide (157.0 g, 761 mmol; DCC) in dichloromethane (200 mL) was added dropwise over 2 h to an efficiently stirred mixture of powdered sodium 4-methyl-2-oxopentanoate (115.5 g, 836 mmol), 2-aminoacetophenone hydrochloride (130.4 g, 760 mmol), and 1-hydroxybenzotriazole hydrate (103.0 g, 762 mmol; HOBT) in dichloromethane (1000 mL). The mixture was stirred overnight, and the insoluble dicyclohexylurea was removed by filtration. The filtrate was concentrated in vacuo to 500 mL. After further filtration, the filtrate was washed with saturated sodium hydrogen carbonate solution (500 mL), water (500 mL), and saturated sodium chloride solution (500 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo to give the intermediate 4-methyl-2-oxo-*N*-(2-oxo-2-phenylethyl)pentanamide as a foam (183.6 g) which was used without purification: NMR ($CDCl_3$) 1.0 (d, 6 H), 2.2 (m, 1 H), 2.8 (d, 2 H), 4.75 (d, 2 H), 7.4–7.7 (m, 3 H), 7.9 (br, 1 H), 8.0 (m, 2 H). The crude product was taken into ethanol (1200 mL) and heated under reflux with ammonium acetate (183.6 g, 2.38 mol) for 3.5 h. After the solution was allowed to stand overnight, the resulting precipitate was filtered off to give **4b** as pale yellow needles (115.8 g, 67%): mp 205–207 °C; NMR ($CDCl_3$) 1.1 (d, 6 H), 2.45 (m, 1 H), 2.8 (d, 2 H), 7.4 (m, 3 H), 7.6 (s, 1 H), 7.8 (m, 2 H). Anal. ($C_{14}H_{16}N_2O$) C, H, N.

Compounds **4a** and **4c** were prepared by analogous procedures with sodium 2-oxopentanoate and either 2-aminoacetophenone hydrochloride or 3-(α-aminoacetyl)pyridine as the starting materials.

2-Hydrazino-3-isobutyl-5-phenylpyrazine (5b). A solution of phosgene in toluene (20% w/v; 220 mL, 425 mmol) was added to a solution of **4b** (39.7 g, 174 mmol) in tetrahydrofuran (THF; 200 mL) and the solution was heated under reflux for 4 h. Volatile material was removed in vacuo, and the residue was dissolved in ether (500 mL). The solution was washed with saturated sodium hydrogen carbonate solution (500 mL), water (500 mL), and saturated sodium chloride solution (500 mL). The organic phase was dried ($MgSO_4$), and the solvent removed in vacuo to give the intermediate 2-chloro-3-isobutyl-5-phenylpyrazine as a brown oil (44 g), which was used without purification: NMR ($CDCl_3$) 1.0 (d, 6 H), 2.3 (m, 1 H), 2.85 (d, 2 H), 7.4 (m, 3 H), 7.9 (m, 2 H). The crude product was taken into dimethyl sulfoxide (DMSO; 150 mL) and heated under reflux for 2 h with hydrazine hydrate (36 mL, 696 mmol) under an atmosphere of argon. The solution was poured onto ice–water (500 mL); the precipitated solid was collected by filtration, washed well with water, and dried in vacuo to afford **5b** (18.8 g, 45%): mp 109–110 °C (ex methanol); NMR (DMSO-d_6) 1.0 (d, 6 H), 2.3 (m, 1 H), 2.65 (d, 2 H), 7.4 (m, 3 H), 8.0 (m, 2 H), 8.6 (s, 1 H). Anal. ($C_{14}H_{18}N_4$) C, H, N.

Compounds **5a** and **5c** were prepared by analogous procedures with **4a** and **4c** as starting materials. In the case of **4c**, it was necessary to use phosphorus oxychloride in place of phosgene in toluene to effect the chlorination reaction.

***N*-[[(Ethoxycarbonyl)methylene]carbonyl]-*N'*-(3-isobutyl-5-phenylpyrazin-2-yl)hydrazine (6b).** A solution of **5b** (66.4 g, 291 mmol) in ethyl acetate (700 mL) was stirred at 0 °C during the addition of ethyl malonyl chloride (40.6 g, 270 mmol) over 10 min. After the solution was stirred for 1 h, the precipitated solid was removed by filtration, washed with ethyl acetate (200 mL), and suspended in chloroform (500 mL). A solution of sodium hydrogen carbonate (45.3 g) in water (500 mL) was added to the stirred suspension. The organic layer was separated, washed with water (500 mL), followed by saturated sodium chloride solution (500 mL), and dried ($MgSO_4$). The solvent was removed in vacuo,

and the residue was triturated with ether/hexane (1:1 v/v; 500 mL) to give **6b**, as a white solid (54.0 g, 52%): mp 116–118 °C; NMR ($CDCl_3$) 1.0 (d, 6 H), 1.3 (t, 3 H), 2.4 (m, 1 H), 2.7 (d, 2 H), 3.5 (s, 2 H), 4.3 (q, 2 H), 7.2 (br, 1 H), 7.4 (m, 3 H), 7.9 (m, 2 H), 8.4 (s, 1 H), 9.7 (br, 1 H). Anal. ($C_{19}H_{24}N_4O_3$) C, H, N.

Compounds **6a**, **6c**, and **6d** were prepared by analogous procedures with **5a** and **5c** as starting materials. The hydrazinopyrazine precursor to **6d** is a known compound.[8] **6d** was isolated as a foam: NMR ($CDCl_3$) 1.0 (t, 3 H), 1.3 (t, 3 H), 1.8 (q, 2 H), 2.4 (s, 3 H), 2.7 (d, 2 H), 3.5 (s, 2 H), 4.25 (q, 2 H), 7.0 (br, 1 H), 7.8 (s, 1 H), 9.6 (br, 1 H); EIMS *m/e* 280 (M^+), 262, 234, 193, 165, 150, 134.

Ethyl (8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)acetate (7b). A solution of **6b** (53.0 g, 149 mmol) and *p*-toluenesulfonic acid monohydrate (PTSA; 2.8 g) in toluene (500 mL) was heated under reflux for 2.5 h. The solvent was removed in vacuo, and the residue was dissolved in chloroform (300 mL). The solution was washed with saturated sodium hydrogen carbonate solution (300 mL), water (300 mL), and saturated sodium chloride solution (300 mL). The dried ($MgSO_4$) solution was evaporated in vacuo, and the residue was trituated with ether/hexane (1:1 v/v; 300 mL) to give **7b** as a white solid (37.1 g, 74%): mp 105–107 °C; NMR ($CDCl_3$) 1.0 (d, 6 H), 1.2 (t, 3 H), 2.6 (m, 1 H), 3.2 (d, 2 H), 4.2 (q, 2 H), 4.3 (s, 2 H), 7.4 (m, 3 H), 7.9 (m, 2 H), 8.1 (s, 1 H). Anal. ($C_{19}H_{22}N_4O_2$) C, H, N.

Compounds **7a**, **7c**, and **7d** were prepared by analogous procedures with **6a**, **6c**, and **6d**, respectively, as the starting materials.

(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-acetic Acid (9b). Aqueous sodium hydroxide solution (2 M, 15 mL) was added to a solution of **7b** (4.5 g, 13.3 mmol) in ethanol (75 mL), and the mixture was stirred for 1 h. Volatile material was removed in vacuo, the residue was dissolved in water (250 mL) and the solution was washed with ethyl acetate (50 mL), cooled to 0 °C and acidified to pH 3 with 1 M hydrochloric acid. The precipitated solid was dried in vacuo to afford **9b** as a white solid (3.9 g, 95%): mp 160 °C (resolidifies and remelts at 181–183 °C); NMR (DMSO-d_6) 1.0 (d, 6 H), 2.5 (m, 1 H), 3.15 (d, 2 H), 4.4 (s, 2 H), 7.5 (m, 3 H), 8.1 (m, 2 H), 9.0 (s, 1 H). Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

Compounds **9a** and **9d** were prepared by analogous procedures with **7a** and **7d** as starting materials. Compound **9c** was not isolated as the free acid but was prepared as the sodium salt by hydrolysis as described for **7b**, but by using exactly 1 molar equiv of sodium hydroxide solution. Removal of volatile material in vacuo then gave the sodium salt of **9c** which was used directly without purification.

Ethyl 2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-(3-pyridyl)propionate (8b). Sodium hydride (0.202 g, 8.4 mmol) was added to a solution of **7b** (1.35 g, 3.99 mmol) in DMF (10 mL) with stirring at 0 °C under an atmosphere of argon. When evolution of hydrogen had ceased (ca. 15 min), a solution of 3-(chloromethyl)pyridine hydrochloride (0.722 g, 4.4 mmol) in DMF (5 mL) was added dropwise over 5 min. After stirring for 2 h, the mixture was added to ice-cold 0.5 M hydrochloric acid (60 mL). The solution was washed with ether (50 mL) and then basified by the addition of solid sodium carbonate. The resulting emulsion was extracted with ethyl acetate (2 × 50 mL), and the combined extracts were washed with water (50 mL) and saturated sodium chloride solution (50 mL), and dried ($MgSO_4$). The solvent was removed in vacuo, and the residue was purified by flash chromatography on silica (Merck art. 9385) eluting with methanol/dichloromethane (3:97 v/v), to give **8b** as a white powder (1.1 g, 75%): mp 110–111 °C (after trituration with ether); NMR ($CDCl_3$) 1.1 (d, 6 H), 1.2 (t, 3 H), 2.6 (m, 1 H), 3.3 (d, 2 H), 3.75 (ddd, 2 H), 4.2 (q, 2 H), 4.5 (t, 1 H), 7.2 (dd, 1 H), 7.5 (m, 3 H), 7.6 (m, 1 H), 7.9 (dd, 1 H), 8.2 (s, 1 H), 8.4 (dd, 1 H), 8.6 (d, 1 H). Anal. ($C_{20}H_{27}N_5O_2$) C, H. N.

Compound **8a** was prepared by an analogous procedure with **7c** as the starting material. Compound **8c** was prepared similarly by alkylation of **7b** with 4-chloromethylimidazole: NMR (DMSO-d_6) 1.02 (d, 6 H), 1.10 (t, 3 H), 2.48 (m, 1 H), 4.10 (m, 2 H), 5.10 (dd, 1 H), 6.75 (s, 1 H), 7.4–7.6 (complex m, 4 H), 8.07 (dd, 2 H), 8.82 (s, 1 H).

Hydrolysis of compounds **8a,b** utilizing an identical procedure with that used for the preparation of **9c** above gave the sodium salts of acids **10a,b**.

Compound **8c** was converted into the corresponding hydrazide **11** by reaction with hydrazine hydrate in ethanol at reflux. **8c**: glass, mp 200–205 °C; NMR (DMSO-d_6/CD_3CO_2D) 1.02 (d, 6 H), 2.52 (m, 1 H), 3.16 (d, 2 H), 3.44 (dd, 1 H), 3.65 (dd, 1 H), 4.70 (dd, 1 H), 6.96 (s, 1 H), 7.4–7.6 (complex m, 3 H), 7.96 (s, 1 H), 8.07 (m, 1 H); CIMS *m/e* 405 $(M + H)^+$, 390, 373, 347.

(1*S*,2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-hydroxy-1,3-dicyclohexylpropane (14b) and (1*R*,2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1,3-dicyclohexyl-1-hydroxypropane (14d). A solution of cyclohexylmagnesium bromide [prepared from magnesium (6.43 g, 265 mmol) and cyclohexyl bromide (43.2 g, 265 mmol) in dry ether (130 mL)] was added at −20 °C to a solution of **13**[9] (12.14 g, 48 mmol) in dry ether (120 mL) under an atmosphere of argon. The mixture was allowed to warm to room temperature and stirred for 18 h. After the addition of ice (50 g), saturated ammonium chloride (50 mL) was added, and the mixture was extracted with ether (4 × 75 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed in vacuo to give an oil which was chromatographed on silica (Merck art. 9385) with ethyl acetate/hexane (15:85, v/v) as eluant to afford first the less polar product **14b** as an oil (9.8 g, 61%): NMR ($CDCl_3$) 0.80–1.95 (m, 24 H), 1.45 (s, 9 H), 3.15 (m, 1 H), 3.85 (m, 1 H), 4.65 (m, 1 H); EIMS *m/e* 340 $(M + H)^+$, 284, 266, 240, 226. Further elution of the column gave the more polar isomer **14d** as an oil (0.90 g, 5.5%): NMR ($CDCl_3$) 0.7–2.05 (m, 24 H), 1.45 (s, 9 H), 3.35 (m, 1 H), 3.85 (m, 1 H), 4.70 (m, 1 H).

Compounds **14a** and **14c** were prepared by an analogous method. **14a**: NMR (DMSO-d_6) 0.8 (m, 4 H), 1.0–2.0 (m, 23 H), 3.5 (m, 1 H), 4.3 (d, 1 H), 6.1 (d, 1 H). CIMS (NH_3/N_2) *m/e* 314 $(M + H)^+$, 296, 258, 214.

Ethyl (3*S*,4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-5-cyclohexyl-3-hydroxypentanoate (17b). Lithioethyl acetate was generated in THF (200 mL) from *n*-butyllithium (280 mL; 1.6 M solution in hexane, 450 mmol), diisopropylamine (63 mL, 450 mmol), and ethyl acetate (43.9 mL, 450 mmol), at −78 °C under an atmosphere of argon. After the mixture was warmed to −60 °C, a solution of anhydrous zinc bromide (101 g, 450 mmol) in THF (200 mL) was added. A solution of **13**[9] (16.03 g, 63 mmol) in THF (50 mL) was added to the stirred solution of the zinc enolate, and the mixture was allowed to warm to room temperature overnight. After cooling to 0 °C, saturated ammonium chloride solution/acetic acid (200 mL, 9:1) was added, and the mixture was extracted with ether (4 × 500 mL). Removal of volatile material in vacuo gave an oil the NMR spectrum of which (DMSO-d_6) indicated almost complete conversion to the 3*S*,4*S* isomer **17b** (O*H*, 4.75 δ, N*H* 6.20 δ) with no detectable amount of the 3*R*,4*S* isomer **17d**. This oil was chromatographed on silica with cyclohexane/acetone (9:1, v/v) as eluant to afford **17b** as a solid (11.91 g, 51%), mp 59–60 °C (ex hexane), $[\alpha]^{22}_D$ −32.3° (*c* = 1, methanol). Further elution of the column yielded a second mixed fraction (3.4 g, 10%) consisting of a mixture of **17b** and **17d** in an ca. 4:1 ratio by NMR (DMSO-d_6): **17d** (3*R*,4*S*) O*H*, δ 5.05, N*H*, 6.45. The relative stereochemistry of **17b** and **17d** was proven by deprotection and conversion to the corresponding oxazolidinones.[10]

Compound **17a** was prepared in a similar fashion with **16** as starting material. Compounds **18a,b** were prepared by hydrolysis of **17a,b** by using previously described methods.[9,11]

Boc-ACHPA-Leu-OH (19). HOBT (0.297 g, 2.2 mmol) was added to a stirred solution of leucine methyl ester hydrochloride (0.39 g, 2.2 mmol), triethylamine (0.77 mL, 5.5 mmol), Boc-ACHPA-OH (**18b**, 0.7 g, 2.2 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.63 g, 3.3 mmol), and the mixture was stirred for 18 h. Water (10 mL) was added, the mixture was extracted with ethyl acetate (3 × 25 mL), and the combined extracts were washed with saturated brine (20 mL), and dried ($MgSO_4$). Removal of solvents in vacuo gave an oil which was chromatographed on silica (Merck art. 9385), with hexane/ethyl acetate (55.45 v/v) as eluant to give Boc-ACHPA-Leu-OMe as an oil (0.805 g, 83%). This material was taken without further purification into methanol (7 mL), 2 M sodium hydroxide solution (3 mL, 6 mmol) was added, and the solution was stirred for 1.5 h. Solvent was removed in vacuo, and the residue was partitioned between water (10 mL) and ether (20 mL). The aqueous phase was acidified to pH 4 with citric acid solution (20% w/v) and extracted with methylene chloride (4 × 25 mL),

and the combined organic extracts were dried ($MgSO_4$). Volatile material was removed in vacuo to afford **19** as a foam (0.66 g, 85%): NMR ($CDCl_3$) 0.75–1.85 (complex m, 16 H), 0.95 (d, 6 H), 2.5 (dd, 2 H), 3.65 (m, 1 H), 3.95 (m, 1 H), 4.55 (m, 1 H), 4.88 (d, 1 H), 5.4–7.3 (br, 1 H), 7.32 (d, 1 H); EIMS *m/e* $(M + H)^+$ 429, 385, 373, 329, 198, 170, 126.

Compound **20** was prepared by analogous amide coupling of **19** with 2-(aminomethyl)pyridine. **20** (foam): NMR (DMSO-d_6/CD_3CO_2D) 0.9 (dd, 6 H), 1.0–1.3 (m, 6 H), 1.4 (s, 9 H), 1.5–1.85 (m, 10 H), 2.3 (m, 2 H), 3.6 (m, 1 H), 3.85 (m, 1 H), 4.25–4.45 (m, 3 H), 7.25 (m, 2 H), 7.75 (ddd, 1 H), 8.5 (d, 1 H). Anal. ($C_{28}H_{46}N_4O_5 \cdot 0.5H_2O$) C, H, N.

Boc-Leu-*m*-[[(benzyloxycarbonyl)amino]methyl]benzylamide (21). A solution of *m*-(aminomethyl)benzylamine (13.6 g, 100 mmol) in methylene chloride (500 mL) was stirred at 0 °C during the dropwise addition over 0.5 h of the solution of Boc-Leu-imidazolide[18] (5.32 g, 20 mmol) in methylene chloride (50 mL). After stirring for 2 h, the solvent was removed in vacuo, and the residue was partitioned between water (200 mL) and ether (500 mL). The organic phase was washed with sodium carbonate solution (2 × 5 mL; 0.5 M) and saturated sodium chloride solution (10 mL) and dried ($MgSO_4$). Volatile material was removed in vacuo to give an oil (5.53 g) which was taken directly into acetone (50 mL) and cooled to 0 °C. Sodium hydrogen carbonate (8.4 g, 100 mmol) was added to the stirred solution, followed by benzyl chloroformate (3.41 g, 20 mmol), and the mixture was stirred for 3 h. Volatile material was removed in vacuo and the residue was partitioned between water (100 mL) and ethyl acetate (100 mL). The aqueous phase was further extracted with ethyl acetate (2 × 50 mL), and the combined organic extracts were dried ($MgSO_4$). The solvent was evaporated in vacuo, and the oily residue was chromatographed on silica (Merck art. 9385), with toluene/ethyl acetate (2:1 v/v) as eluant to afford **21** (7.2 g, 74%): mp 92–95 °C; NMR (DMSO-d_6/CD_3CO_2D) 0.90 (dd, 6 H), 1.40 (s, 9 H), 1.4–1.74 (m, 3 H), 4.10 (m, 1 H), 4.25 (s, 2 H), 4.35 (s, 2 H), 5.10 (s, 2 H), 7.10–7.4 (m, 9 H). Anal. ($C_{27}H_{38}N_3O_5$) C, H, N.

Boc-ACHPA-Leu-*m*-[[(benzyloxycarbonyl)amino]methyl]benzylamide (22b). A solution of **21** (2.75 g, 5.68 mmol) was stirred for 1.5 h in a solution of 4 M hydrogen chloride in dioxane (25 mL). Volatile material was evaporated in vacuo, and the residue was coupled with **18b** by using an analogous procedure to that used for the preparation of **19**, to give **22b** as a foam (1.539 g, 40%): NMR ($CDCl_3$) 0.95 (m, 6 H), 1.0–1.9 (m, 17 H), 1.45 (s, 9 H), 2.2–2.5 (m, 2 H), 3.55 (br s, 1 H), 3.85 (br s, 1 H), 4.25–4.55 (m, 4 H), 4.75 (d, 1 H), 5.15 (s, 2 H), 5.50 (br s, 1 H), 6.55 (br s, 1 H), 7.0 (br s, 1 H), 7.1–7.4 (m, 9 H); FABMS *m/e* 681 $(M + H)^+$, 581, 547, 384.

Compound **22a** was made by an analogous coupling procedure between **21** and **18a**. **22a** (oil): NMR (DMSO-d_6) 0.85 (m, 12 H), 1.1–1.8 (m, 5 H), 1.35 (s, 9 H), 2.2 (m, 2 H), 3.5 (br s, 1 H), 3.8 (br s, 1 H), 4.1–4.3 (m, 4 H), 4.6 (d, 1 H), 5.05 (s, 2 H), 6.20 (d, 1 H), 7.05–7.4 (m, 9 H), 7.75 (br t, 1 H), 7.90 (d, 1 H), 8.35 (br t, 1 H); FABMS *m/e* 641 $(M + H)^+$, 541, 507, 384, 271, 140.

N^{α}-Z-N^{ϵ}-Boc-Lys-OBzl (23). A solution of (2*S*)-2-[[(benzyloxy)carbonyl]amino]-6-[(*tert*-butyloxycarbonyl)amino]hexanoic acid[12] (4.2 g, 11.05 mmol), potassium carbonate (4.6 g, 33.3 mmol), and benzylbromide (1.45 mL, 12 mmol) in acetone (50 mL) was heated under reflux for 2.5 h. The cooled solution was filtered through Celite, the filtrate was evaporated in vacuo, and the residue was recrystallized from ether/hexane (1:1 v/v) to give **23** as a white solid (4.5 g, 86%): mp 76–79 °C; NMR (DMSO-d_6/CD_3CO_2D) 1.25–1.45 (m, 13 H), 1.70 (m, 2 H), 2.90 (t, 2 H), 4.10 (m, 1 H), 5.05 (s, 2 H), 5.15 (s, 2 H), 7.35 (m, 10 H). Anal. ($C_{26}H_{34}N_2O_6$) C, H, N.

N^{α}-Z-Lys-[N^{ϵ}-(Boc-ACHPA-Leu)]-OBzl (24). A solution of **23** (1.65 g, 3.5 mmol) in 4 M hydrogen chloride in dioxane (20 mL) was stirred at room temperature for 3 h. Volatile material was removed in vacuo, and the residue was taken into DMF (10 mL) and added to a stirred solution of **19** (1.50 g, 3.5 mmol), HOBT (0.51 g, 3.8 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.0 g, 5.2 mmol), and triethylamine (1.2 mL, 8.5 mmol) in DMF (20 mL). After 18 h, the mixture was poured onto saturated brine (100 mL), extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were washed with 1 M sodium hydrogen carbonate solution (20 mL), 20% w/v citric acid solution (20 mL), and saturated brine (20 mL). The dried ($MgSO_4$) solution was evaporated in vacuo to furnish an oil which was chromatographed on silica (Merck art. 9385) with ethyl acetate/hexane (60:40 v/v) as eluant to give **24** as a foam (1.50 g, 56%): NMR (DMSO-d_6/CD_3CO_2D) 0.80 (d, 3 H), 0.90 (d, 3 H), 1.0–1.8 (m, 23 H), 2.25 (m, 2 H), 3.05 (m, 2 H), 3.55 (m, 1 H), 3.80 (m, 1 H), 4.05 (m, 1 H), 4.20 (m, 1 H), 5.10 (m, 4 H), 7.30 (m, 1 H); EIMS *m/e* 781 $(M + H)^+$, 681.

Compound **25** was prepared by an analogous procedure to that of **19**, starting from benzyl 4-aminobutyrate.[19] **25** (oil): Anal ($C_{22}H_{34}N_2O_5 \cdot 0.5H_2O$) C, H, N.

Boc-ACHPA-Leu-Gaba-OBzl (26). Compound **26** was prepared by deprotection of **25** in situ (by analogy with the preparation of **24**) and coupling with **19**. **26** (oil): NMR ($CDCl_3$) 0.94 (6 H, m), 1.05–1.75 (m, 9 H), 1.44 (s, 9 H), 1.85 (t, 2 H), 2.4 (m, 3 H), 3.28 (q, 2 H), 3.6 (br s, 1 H), 3.95 (br s, 1 H), 4.45 (br m, 1 H), 4.80 (m, 1 H), 5.12 (s, 2 H), 6.4 (br s, 1 H), 6.75 (br s, 1 H), 7.3–7.4 (m, 5 H); FABMS *m/e* 604 $(M + H)^+$, 504, 414, 307, 126.

General Coupling Procedure for the Preparation of Compounds 12a–e and 12g–k. The general procedure used in the preparation of compounds **12a–e** and **12g–k** is illustrated by the following example.

(1*S*,2*S*)-2-[(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)acetamido]-1,3-dicyclohexyl-1-hydroxypropane (12d). Deprotection of **14b** (0.281 g, 0.83 mmol) was carried out in 4 M hydrogen chloride in dioxane, as described previously for the preparation of **22b**. The crude product was taken directly into DMF (7 mL), and **9b** (0.26 g, 0.83 mmol), HOBT (0.112 g, 0.83 mmol), triethylamine (115 μL, 0.83 mmol), and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.24 g, 1.24 mmol) were added, and the solution was stirred for 18 h at room temperature. Volatile material was removed in vacuo, and the residue was partitioned between saturated brine (20 mL) and ethyl acetate (50 mL). The aqueous phase was further extracted with ethyl acetate (2 × 25 mL), and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to yield a solid which was chromatographed on silica (Merck art. 9385), with methanol/ethyl acetate (2:98, v/v) as eluant to give **12d** as a solid (0.14 g, 32%), mp 188–192 °C. Anal. ($C_{32}H_{45}N_5O_2$) C, H, N.

Compounds **12j,k** were formed as a diastereoisomeric mixture and were separated chromatographically.

(1*S*,2*S*)-2-[(2*RS*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-imidazol-4-ylpropionamido]-1,3-dicyclohexyl-1-hydroxypropane (12f). Deprotection of **14b** (0.102 g, 0.3 mmol) was carried out as described above. Hydrogen chloride (6 M) in dioxane (0.17 mL, 1.0 mmol) was added to a stirred suspension of the hydrazide **11** (0.1 g, 0.25 mmol) in DMF (5 mL) at −20 °C. *tert*-Butyl nitrite (0.034 g, 0.3 mmol) was added, and the resulting solution was maintained at −20 °C for 0.25 h. After addition of the deprotected amino alcohol (described above) in DMF (1 mL), the solution was adjusted to pH 7 by the addition of triethylamine (0.226 g, 2.2 mmol). After the solution was allowed to stand overnight at 0 °C, volatile material was removed in vacuo, and the residue was partitioned between ethyl acetate (10 mL) and water (10 mL). The organic phase was washed with saturated sodium chloride solution (10 mL) and dried ($MgSO_4$). The residue was chromatographed on silica (Merck art. 9385) with methanol/ethyl acetate (1:4, v/v) as eluant to afford **12f** as a white powder (0.043 g, 28%): mp 146–150 °C; NMR (DMSO-d_6/CD_3CO_2D) 0.9–1.8 (complex m, 30 H), 2.44 (m, 1 H), 2.88 (m, 1 H), 3.10 (t, 2 H), 3.36 (m, 1 H), 3.71 (m, 1 H), 3.94 (m, 1 H), 4.88 (m, 1 H), 7.09, 7.11 (both s, 1:1 ratio, total 1 H), 7.4–7.6 (complex m, 3 H), 8.10 (m, 2 H), 8.17, 8.20 (both s, 1:1 ratio, total 1 H) 8.98, 9.08 (both s, 1:1 ratio, total 1 H). Anal. ($C_{36}H_{49}N_7O_2 \cdot H_2O$) C, H, N.

General Procedure for the Preparation of Compounds 12l–r. The general procedure used in the preparation of compounds **12l–r** is illustrated by the following example.

N-[(2*R*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]-

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pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]-ACHPA-Leu-*m*-(aminomethyl)benzylamide (12l)[20] and *N*-[(2*S*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]-ACHPA-Leu-*m*-(aminomethyl)benzylamide (12m).[20] **22b** (0.85 g, 1.25 mmol) was deprotected as described above (hydrogen chloride in dioxane) and the crude amine hydrochloride was added to a solution of the sodium salt of **10b** (0.529 g, 1.25 mmol) in DMF (20 mL). Triethylamine (0.126 g, 1.25 mmol), HOBT (0.169 g, 1.25 mmol), and ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.24 g, 1.25 mmol) were added, and the solution was stirred for 18 h at 20 °C. Volatile material was removed in vacuo, and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was washed with saturated sodium hydrogen carbonate (50 mL), saturated sodium chloride solution (50 mL), and dried ($MgSO_4$). Volatile material was removed in vacuo to give an oil which was chromatographed on silica (Merck art. 9385) with methanol/dichloromethane (1:19, v/v) as eluant to give first the less polar Z-protected product corresponding to the compound **12l** (0.415 g, 35%). Further elution of the column gave the more polar Z-protected product corresponding to compound **12m** (0.338 g, 27%), both of which were deprotected without further purification as described below.

Palladized charcoal (10%) (0.16 g) and ammonium acetate (0.416 g, 6.6 mmol) were added to a stirred solution of the more polar Z-protected isomer referred to above (0.321 g, 0.33 mmol) in absolute ethanol (15 mL). After stirring for 1 h at 20 °C, the catalyst was removed by filtration through Celite, and the solvent was removed in vacuo. The residue was taken into chloroform (20 mL) and washed successively with water (10 mL), 1 M sodium hydrogen carbonate solution (10 mL), and saturated sodium chloride solution (10 mL), annd dried ($MgSO_4$). The solvent was evaporated to afford a foam (0.290 g) which was chromatographed on silica (Merck art. 9385) with aqueous ammonia (S.G. 0.880)/methanol/chloroform (1:19:180) as eluant to give **12m** as a white solid (0.19 g, 69%): mp 125–130 °C; NMR (DMSO-d_6/CD_3CO_2D) 0.8–1.8 (complex m, 30 H), 2.52 (m, 1 H), 3.12 (ddd, 2 H), 3.48 (dd, 1 H), 3.76 (m, 3 H), 4.03 (s, 2 H), 4.30 (m, 3 H), 4.86 (dd, 1 H), 7.2–7.6 (complex m, 8 H), 7.83 (dt, 1 H), 8.12 (m, 2 H), 8.42 (dd, 1 H), 8.60 (m, 1 H), 9.16 (s, 1 H). Anal. ($C_{48}H_{63}N_9O_4 \cdot 1.5H_2O$) C, H, N.

The less polar Z-protected isomer referred to above was treated in an exactly analogous fashion to that described above to furnish **12l** as a white solid: mp 136–142 °C; NMR (DMSO-d_6/CD_3CO_2D) 0.7–1.7 (complex m, 29 H), 2.05 (m, 1 H), 2.52 (m, 1 H), 3.06 (ddd, 2 H), 3.36 (dd, 1 H), 3.57 (dd, 1 H), 3.80 (m, 2 H), 4.01 (s, 2 H), 4.10 (t, 1 H), 4.30 (dd, 2 H), 4.94 (dd, 1 H), 7.2–7.6 (complex m, 8 H), 7.73 (dt, 1 H), 8.13 (m, 2 H), 8.40 (dd, 1 H), 8.49 (m, 1 H), 8.91 (s, 1 H); FABMS (DMSO/glycerol) *m/e* 830 $(M + H)^+$, 384, 356, 293.

Compound **12q** was prepared by an analogous procedure to that described above except that the final hydrogenolytic removal of the benzyl- and Z- groups was carried out by hydrogenation over 10% palladized charcoal in absolute ethanol. The product was then isolated by filtration through Celite and evaporation in vacuo. **12q**: white powder; mp 160–170 °C; NMR (DMSO-d_6/CD_3CO_2D) 0.5–2.5 (complex m, 35 H), 2.6–3.9 (complex m, 12 H), 4.25 (m, 2 H), 4.85 (m, 2 H), 8.45 (m, 1 H), 8.65 (m, 1 H), 9.2 (s, 1 H). Anal. ($C_{46}H_{65}N_9O_6 \cdot 4H_2O$) C, H, N.

Renin Inhibition Methodology. Partially purified human renal renin[21] was kindly supplied by Dr. Brenda Leckie (MRC Blood Pressure Unit, Glasgow, UK) and was assayed at pH 7.0 in phosphate buffer by using human angiotensinogen partially purified from volunteer plasma by ammonium sulphate fractionation and DEAE-Sepharose chromatography.[22] Test compounds were dissolved and diluted as necessary in dimethyl sulfoxide (DMSO). The drug solutions were then diluted in buffer such that the DMSO concentration was 3% v/v. The final incubation mixture (150 μL) contained the following components: 50 mM phosphate buffer, pH 7.0; 3 mM disodium ethylenediaminetetraacetate (EDTA), 3 mM 8-hydroxyquinoline hemisulfate (8-HQ), 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 1.0% w/v sodium azide, 0.1% w/v bovine serum albumin, and 1% v/v DMSO. The substrate concentration used was between 0.3 and 0.5 times the apparent K_m for the reaction, and the renin concentration was such that the angiotensin generation rate was 5–10 ng/mL per hour. Reactions were allowed to proceed for 120 min at 30 °C and were terminated by cooling on ice. Samples were then assayed for angiotensin I content in the presence of 100 μM pepstatin as described previously[23] by using commercially available ^{125}I-angiotensin II (New England Nuclear) and anti-angiotensin I-BSA antibody (Miles Scientific). Under the conditions used, angiotensin generation rate was linear, and, at the highest concentrations used, the test compounds did not cross-react with the anti-angiotensin antibody. Angiotensin generation rate in the presence of test compound was compared to the control rate in the presence of vehicle. Results were calculated as percentage inhibition of the control reaction rate. IC_{50} values (concentration for 50% inhibition of the renin-catalyzed reaction) were determined from the relationship between percentage inhibition and inhibitor concentration. Test concentrations were chosen to bracket the expected IC_{50} and to cover at least 4 orders of magnitude. The presence of 1% DMSO in the incubation mixture had no significant effect on the renin activity.

Pharmacological Evaluation. Marmosets (*Callithrix jacchus*, ICI Alderley Park) were depleted of sodium for 2 days by treatment with furosemide 25 mg/kg per day po and a low-sodium diet. This regimen stimulated renin secretion and elevated basal plasma renin activity. On the third morning a final dose of furosemide was administered, 1 h prior to anesthesia with Inactin 120 mg/kg ip followed by a 10 mg/kg per h iv maintenance infusion. The arterial pressure (BP) was measured from a catheter in the carotid artery via a Gould Statham P23 pressure transducer and Lectromed M19 chart recorder. The jugular vein was cannulated for drug injection and anesthetic infusion. Blood samples (250 μL) were collected, via a femoral arterial cannula, for subsequent determination of plasma renin activity (PRA) as described previously.[23]

Compounds were administered iv or po. Changes in BP and PRA were determined. At the end of each experiment, captopril 1 mg/kg iv was injected and the change in BP recorded. Each marmoset served as its own control to minimize the number of primates used.

Detection of Compounds in Bile by High-Performance Liquid Chromatography (HPLC). Alderley Park Wistar rats were prepared surgically with indwelling bile duct cannulae exteriorized at the back of the neck. Compounds were dosed to separate groups of rats by the iv or oral route and bile was collected under free flow for subsequent analysis.

Bile aliquots of 200 μL were diluted to 1.0 mL with distilled water and subjected to solid phase extraction with C18 Bond-Elut cartridges. Columns were washed with 2 mL distilled water and eluted with 2 mL of 0.1% trifluoroacetone in methanol. The eluate was evaporated to dryness, taken up in 0.5–1.0 mL of HPLC solvent, and injected onto a C8 Zorbex column. Column effluent was monitored at 250 nM.

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(20) Stereochemical assignment at the carbon α to the heterocycle is only tentative.

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