(d, J = 16 Hz, 1 H, H-1″ α-pyr), 6.53 (mc, 1 H, H-4″ γ-pyr), 7.09 (s, 1 H, SCH), 7.23 and 7.30 (2 × t, J = 8 Hz, H-5, H-6), 7.37 (d, J = 8 Hz, 1 H, CH), 7.53 (d, J = 8 Hz, 1 H), 7.75 (s, 1 H, H-2); MS m/e 309 (M^+, free base, 100).

Biochemical Methods. (a) Brain Membrane Preparation. Cerebral cortices of male Sprague-Dawley rats (250–300 g) were dissected on ice, weighed, and promptly transferred to 10–15 vol (weight/vol) of ice cold 0.32 M sucrose. The tissue was then homogenized by using ten strokes of a motor driven Teflon/glass homogenizer (Janke and Kunkel) at 500 rpm. The homogenate was centrifuged at 1000g at 4 °C for 10 min and the supernatant then recentrifuged at 48000g, 4 °C for 21 min. The supernatant was discarded and the pellet resuspended in 10–15 vol of 2.5 mM Hepes (pH 7.4 at room temperature) and left to stand at room temperature for 15 min. Finally, the homogenate was recentrifuged for a further 21 min at 48000g, 4 °C, and the resulting pellet stored on ice.

(b) [^{3}H]Q-ICS 205-930 Binding Assay. Cortical membranes were prepared freshly on the day of the assay. Immediately prior to use the pellet was resuspended in 30 vols of the assay buffer (10 mM Hepes containing 10 μM pargyline and 0.1% ascorbate, pH 7.1 at room temperature). The membranes, [^{3}H]Q-ICS 205-930 and displacing drugs were prepared in assay buffer. A 400-μL aliquot of the membrane suspension (approximately 400 μg, protein) was incubated on ice with 0.5–0.7 nM [^{3}H]Q-ICS 205-930 (**36**) plus displacing drug in polypropylene tubes, (final assay volume 1 mL). All assays were carried out in duplicate. Nonspecific binding was defined with 10 μM MDL 72222 (**4**). The reaction was initiated by adding the membrane suspension and was terminated after 15 min of incubation at 4 °C by rapid filtration through Whatman GF/B glass fiber filters by using a Brandel M24-R cell-harvester followed by 2 × 4 mL washes with cold 5 mM Hepes (pH 7.4 at room temperature). The filters had previously been soaked in 0.3% polythylenimine/0/5% Triton X100 for a minimum of 1 h to reduce nonspecific binding. Radioactivity was determined by liquid scintillation counting at 41% efficiency. Potencies for displacement (pIC_{50}) were determined from data obtained by using at least nine concentrations of the displacing ligand by computer-assisted iterative curve fitting.

Acknowledgment. We thank Mr. R. Williams for the determination of pK_a values, Ms. K. Green for preparation of some of the compounds, and Ms. S. Aspley for technical assistance.

Supplementary Material Available: Microanalytical data and NMR data for each compound in Tables 2–5 (9 pages). Ordering information is given on only current masthead page.

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

Robert H. Bradbury* and Janet E. Rivett

Department of Chemistry, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, U.K.
Received March 15, 1990

Two series of 1,2,4-triazolo[4,3-*a*]pyrazine derivatives with human renin inhibitory activity have been synthesized which incorporate the transition-state mimetics (3*S*,4*S*)- and (3*R*,4*S*)-5-cyclohexyl-3,4-diaminopentanoic acid ((*S*)- and (*R*)-CDAPA), and (4*S*)-4-amino-5-cyclohexyl-2,2-difluoro-3-oxopentanoic acid (ACDFOPA). Several compounds in these series, for example **13a**, **19c**, and **19f**, were highly potent inhibitors of partially purified human renin (IC_{50} values of 3.9, 1.6, and 1.4 nM, respectively). The ACDFOPA-based compounds **19c** and **19f** contain no natural amino acid fragments and have molecular weights which compare well with those of previously reported inhibitors of nanomolar in vitro potency. When administered intravenously to anesthetized, sodium-depleted marmosets at doses of 3 mg/kg, compounds **13a** and **19c** caused a marked reduction in mean arterial pressure, but in the same animal model at 30 mg/kg, oral activity was not seen.

Despite the efforts of many research groups during recent years,[2] the target has yet to be achieved of a long-acting, orally effective inhibitor of human renin as a potential alternative to blockade of angiotensin converting enzyme (ACE) for the treatment of hypertension and congestive heart failure. In an attempt to overcome the problems associated with known peptidic inhibitors, such as poor oral absorption, proteolytic instability, short duration of action, and rapid excretion,[3] we sought a nonpeptidic ligand which might bind in the S_4–S_2 region of human renin. As a consequence of the work, we recently reported[1] inhibitors containing a 2-(8-alkyl-6-aryl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropionyl moiety linked to transition-state mimetics such as cyclohexylstatine (ACHPA[4]) and the hydroxyethylene isostere Cha$\overset{OH}{—}$Val.[4] Structure–activity relationships for these series of compounds, for example **1** and **2** (Figure 1), were consistent with the substituted heterocyclic propionyl unit spanning the S_4–S_2 sites of the enzyme and thus acting as

(1) (a) Part 1: Roberts, D. A; Bradbury, R. H.; Brown, D.; Faull, A. W.; Griffiths, D.; Major, J. S.; Oldham, A. A.; Pearce, R. J.; Ratcliffe, A. H.; Revill, J.; Waterson, D. *J. Med. Chem.* **1990**, *33*, 2326. (b) Part 2: Bradbury, R. H.; Major, J. S.; Oldham, A. A.; Rivett, J. E.; Roberts, D. A.; Slater, A. M.; Timms, D.; Waterson, D. *J. Med. Chem.* **1990**, *33*, 2335.

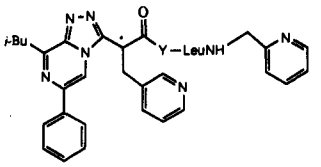
(2) Greenlee, W. J. *Med. Res. Rev.* **1990**, *10*, 173.

(3) Boger, J.; Bennett, C. D.; Payne, L. S.; Ulm, E. H.; Blaine, E. H.; Homnick, C. F.; Schorn, T. W.; Lamont, B. I.; Veber, D. F. *Regul. Peptides* **1985**, *12* (*suppl 4*), 8.

(4) ACHPA = (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid; Cha$\overset{OH}{—}$Val = (2*S*,4*S*,5*S*)-5-amino-6-cyclohexyl-4-hydroxy-2-isopropylhexanoic acid; (*S*)-CDAPA = (3*S*,4*S*)-5-cyclohexyl-3,4-diaminopentanoic acid; (*R*)-CDAPA = (3*R*,4*S*)-5-cyclohexyl-3,4-diaminopentanoic acid; (*RS*)-CDAPA = (3*RS*,4*S*)-5-cyclohexyl-3,4-diaminopentanoic acid; ACDFOPA = (4*S*)-4-amino-5-cyclohexyl-2,2-difluoro-3-oxopentanoic acid; ACDFHPA = (3*R*,4*S*)-4-amino-5-cyclohexyl-2,2-difluoro-3-hydroxypentanoic acid; N_3-Z-N_4-Boc-(*S*)-CDAPA = (3*S*,4*S*)-3-[(benzyloxycarbonyl)amino]-4-[(*tert*-butoxycarbonyl)amino]-5-cyclohexylpentanoic acid; N_3-Z-N_4-Boc-(*R*)-CDAPA = (3*R*,4*S*)-3-[(benzyloxycarbonyl)amino]-4-[(*tert*-butoxycarbonyl)amino]-5-cyclohexylpentanoic acid; N_3-Z-(*S*)-CDAPA = (3*S*,4*S*)-4-amino-3-[(benzyloxycarbonyl)amino]-5-cyclohexylpentanoic acid.

0022-2623/91/1834-0151$02.50/0

Table I. Characterization and in Vitro Human Renin Inhibition of Compounds **13a–d**

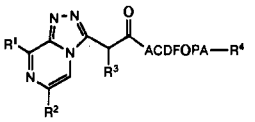


13

no.	Y[a]	config at C*	mp, °C	formula[b]	human renin IC_{50},[c] nM
13a	(*S*)-CDAPA	more polar epimer	116–120	$C_{46}H_{60}N_{10}O_3 \cdot 3H_2O$[d]	3.9
13b	(*S*)-CDAPA	less polar epimer	165–170	$C_{46}H_{60}N_{10}O_3 \cdot 1.5H_2O$	100
13c	(*R*)-CDAPA	more polar epimer	118–122	$C_{46}H_{60}N_{10}O_3 \cdot 1.5H_2O$	6.1
13d	(*R*)-CDAPA	less polar epimer	120–125	$C_{46}H_{60}N_{10}O_3 \cdot H_2O$	120

[a] See ref 4 for definitions of (*R*)- and (*S*)-CDAPA. [b] Analyses for C, H, N were correct with ±0.4% unless otherwise stated. [c] Test details are described in ref 1a. [d] C, N, H, calcd, 7.7; found, 7.1.

Table II. Characterization and in Vitro Human Renin Inhibition of Compounds **19a–f**[a]



19

no.	R^1	R^2	R^3	R^4	mp, °C	formula	FABMS[b] calcd	FABMS[b] found	human renin IC_{50},[c] nM
19a	*i*-Bu	Ph	H	LeuNH–pyridin-3-yl	90–95	$C_{40}H_{51}F_2N_8O_4$	745.4001	745.4067	1.9
19b	*i*-Bu	Ph	H	$NHCH_2CH_2CH(CH_3)_2$	78–80	$C_{33}H_{45}F_2N_6O_3$	611.3521	611.3556	38
19c	*n*-Pr	pyridin-3-yl	H	$NHCH_2CH_2CH(CH_3)_2$	67–69	$C_{31}H_{42}F_2N_7O_3$	598.3317	598.3322	1.6
19d	*n*-Pr	pyridin-3-yl	H	$NHCH_2CH(CH_3)CH_2CH_3$[d]	79–81	$C_{31}H_{42}F_2N_7O_3$	598.3317	598.3339	1.7
19e	*n*-Pr	pyridin-3-yl	pyridin-3-yl methyl[e]	$NHCH_2CH_2CH(CH_3)_2$	80–82	$C_{37}H_{46}F_2N_8O_3$	689.3739	689.3797	1.8
19f	*n*-Pr	pyridin-3-yl	H	$NHCH_2CH_3$	81–83	$C_{28}H_{36}F_2N_7O_3$	556.2848	556.2840	1.4

[a] See ref 4 for definition of ACDFOPA. [b] High-resolution FABMS corresponding to $(M + H)^+$. [c] Test details are described in ref 1a. [d] *S* configuration. [e] Ca. 1:1 mixture fo diastereoisomers at asymmetric center substituted by pyridin-3-ylmethyl.

a novel, nonpeptidic replacement for the substrate amino acid residues Pro-Phe-His. Molecular modeling studies relating to the proposed binding mode were reported in our previous paper.[1b]

Both **1**[1a] and **2**[1b] showed good in vitro activity against partially purified human renin. When administered intravenously to anesthestized, sodium-depleted marmosets, low doses of these compounds caused a marked and sustained reduction in mean arterial pressure (MAP). On oral evaluation in the same animal model, however, disappointing results were seen. Compound **1** at 50 mg/kg produced no effect on MAP, and while **2** at 30 mg/kg elicited a modest fall in MAP, a crude comparison of oral and intravenous efficacy suggested the oral bioavailability of **2** to be significantly below that needed for a clinically useful agent.

As discussed in our previous papers,[1] we believe that the poor oral bioavailability of these compounds reflects shortcomings in oral absorption, rather than metabolic instability or rapid biliary clearance. In particular, high molecular weight (829 for **1**, 696 for **2**) could be a key factor limiting oral absorption. In attempting to reduce this parameter from inhibitor **1** as starting point, we chose to link our putative nonpeptidic P_4–P_2 ligand to other known transition-state mimetics. Thus, as outlined previously,[1b] in deriving inhibitor **2** from **1** one of the two key structural changes was replacement of ACHPA by Cha$\overset{OH}{—}$Val. The tighter binding of the latter transition-state mimetic permitted a modest reduction in molecular weight by truncation at the C-terminus of the inhibitor. Since, as mentioned above, compound **2** does show oral activity (albeit at a poor level), we were encouraged to pursue further alternative transition-state mimetics. These, we

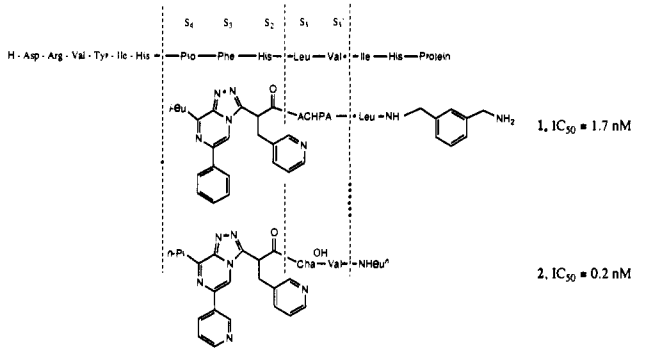


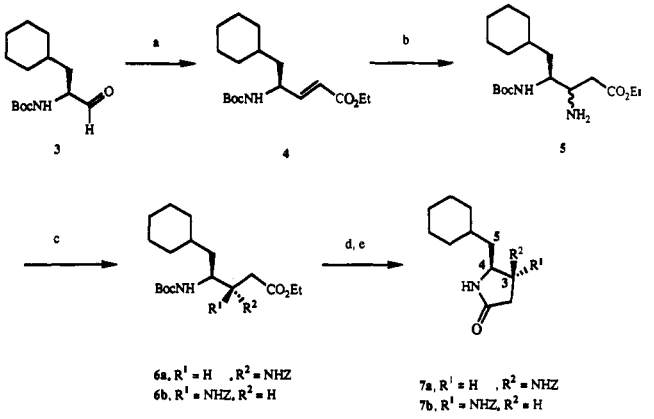
Figure 1. Human angiotensinogen and the structures of inhibitors **1** and **2** (see ref 4 for definitions of ACHPA and Cha$\overset{OH}{—}$Val).

hoped, might similarly allow a reduction in molecular weight, and thereby like Cha$\overset{OH}{—}$Val provide a basis for improving oral activity compared with that of **1**.

In this paper we describe the synthesis and biological properties of representative inhibitors utilizing a primary amine[5] or a hydrated ketone[6] as the transition-state mimetic. It has been shown previously, through introduction of the dipeptide replacements aminodeoxystatine[5,7] and

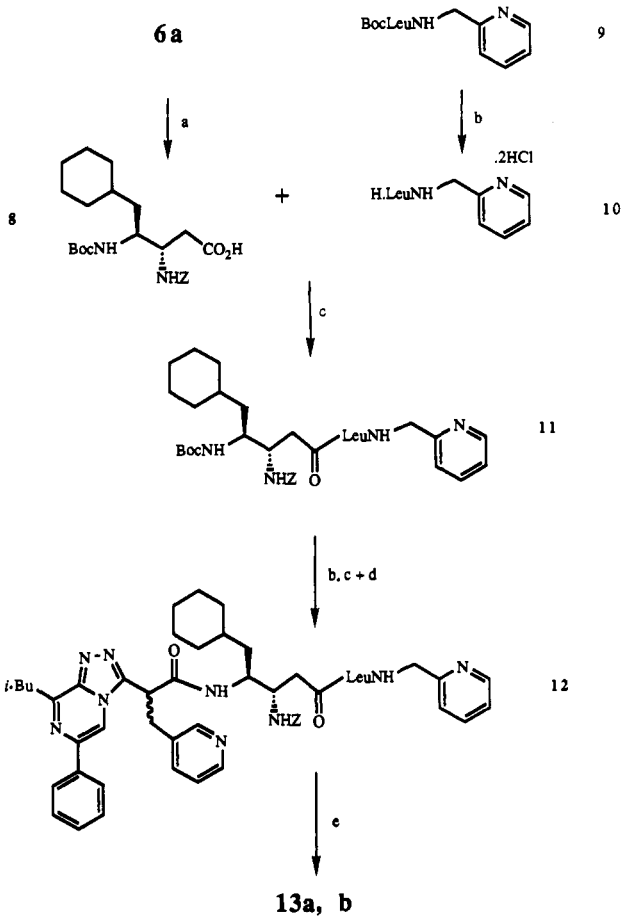
(5) (a) Jones, M.; Sueiras-Diaz, J.; Szelke, M.; Leckie, B.; Beattie, S. *Peptides, Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds; Pierce Chemical Comp.: Rockford, II, 1985; p 759. (b) Arrowsmith, R. J.; Carter, K.; Dann, J. G.; Davies, D. E.; Harris, C. J.; Morton, J. A.; Lister, P.; Robinson, J. A.; Williams, D. J. *J. Chem. Soc. Chem. Commun.* **1986**, 755.
(6) Gelb, M. H.; Suaren, J. P.; Abeles, R. H. *Biochemistry* **1985**, *24*, 1813.

Scheme I[a]



[a] (a) $Ph_3P{=}CHCO_2ET/THF$; (b) $NH_3/EtOH/100$ °C; (c) ZCl/Et_3N/DMAP/CH_2Cl_2/0 °C; (d) HCl/dioxane; (e) *i*-Pr_2NEt/dioxane/100 °C.

Scheme II[a]



[a] (a) NaOH/dioxane/H_2O; (b) HCl/Dioxane; (c) HOBT/Et_3N/HCl·$Me_2N(CH_2)_3N{=}C{=}NEt$/DMF; (d) **17d** (see Scheme III); (e) NH_4O_2CH/Pd/C/EtOH.

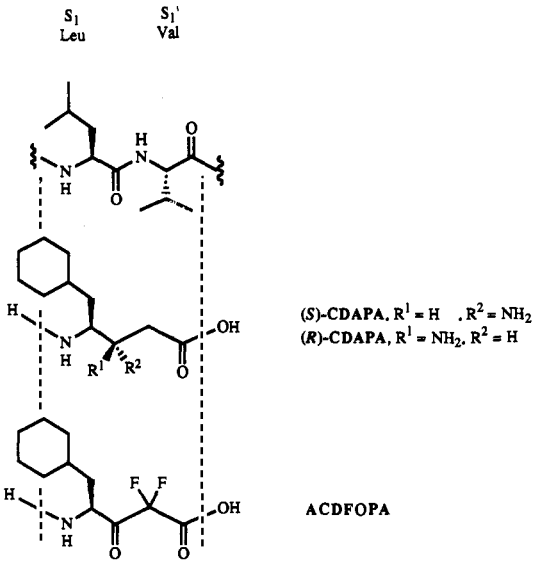
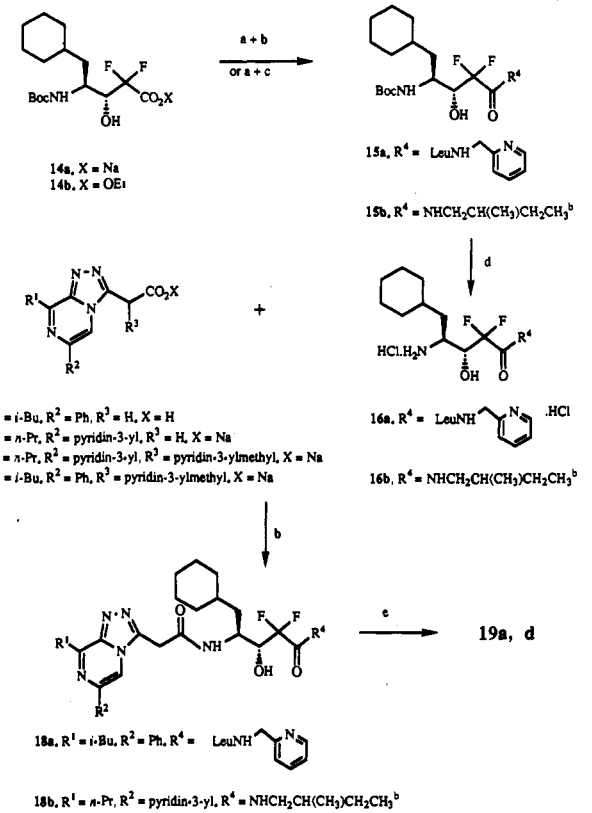


Figure 2. Structures of the transition-state mimetics (*S*)-CDAPA, (*R*)-CDAPA and ACDFOPA (see ref 4 for definitions).

Scheme III[a]



[a] (a) R^4NH_2; (b) HOBT/Et_3N/HCl·$Me_2N(CH_2)_3N{=}C{=}NEt$/DMF; (c) $Me_2NC(=NH)NMe_2/CHCl_3$/heat; (d) HCl/dioxane; (e) Dess–Martin periodinane/CH_2Cl_2.
[b] *S* configuration.

difluorostatone,[8] that potent inhibitors of human renin can be obtained by incorporation of such functionality into substrate-derived sequences. Based on the well-established[8a,9] preference for a cyclohexylmethyl side chain at the P_1 position in renin inhibitors, the compounds reported here contain, as replacements for the scissile dipeptide fragment Leu-Val, the corresponding P_1-cyclohexylmethyl

(7) (a) Thaisrivongs, S.; Schostarez, H. J.; Pals, D. T.; Turner, S. R. *J. Med. Chem.* **1987**, *30*, 1837. (b) Schostarez, H. J. *J. Org. Chem.* **1988**, *53*, 3628.
(8) (a) Thaisrivongs, S.; Pals, D. T.; Kati, W. M.; Turner, S. R.; Thomasco, L. M.; Watt, W. *J. Med. Chem.* **1986**, *29*, 2080. (b) Fearon, K.; Spaltenstein, A.; Hopkins, P. B.; Gelb, M. H. *J. Med. Chem.* **1987**, *30*, 1617.
(9) (a) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; Lamont, B. L.; Lin, T.-Y.; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779. (b) Buhlmeyer, 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.

variants (*S*)- and (*R*)-CDAPA[4] and ACDFOPA[4] (Figure 2).

Chemistry

Compounds **13a–d** and **19a–f** prepared during the course of this work are listed in Tables I and II. Their syntheses are illustrated by the routes to **13a,b** and **19a,d** outlined in Schemes I–III.

For preparation of **13a,b**, we required the protected diamino ester **6a**. Analogously to a published synthesis of aminodeoxystatine derivatives,[5b] **6a** was readily obtained in three steps from Boc-cyclohexylalaninal (**3**) by a sequence involving formation of α,β-unsaturated ester **4**, followed by Michael addition of ammonia (Scheme I). Without purification, the resulting free amine **5** was treated with (benzyloxy)carbonyl chloride to give a ca. 1:1 mixture of diastereoisomers **6a,b** which could be separated by flash chromatography. In common with the earlier synthesis of aminodeoxystatine derivatives,[5b] we assume this sequence to proceed without racemization at the asymmetric center derived from **3**.

The relative stereochemistry of **6a,b** was established by conversion to the corresponding γ-lactams,[7b,8a] with the less polar 3*S*,4*S* diastereoisomer **6a** forming *cis*-lactam **7a** and the more polar 3*R*,4*S* diastereoisomer **6b** giving *trans*-lactam **7b**. Like the γ-lactams derived from (3*R*,4*S*)- and (3*S*,4*S*)-2,2-difluorostatones,[8a] **7a,b** could be distinguished on the basis of their ^{13}C NMR spectra. The signals from C_5 (statine numbering) for *cis*- and *trans*-lactams **7a,b** appeared at δ 37.6 and 42.2, respectively, an upfield shift in **7a** relative to **7b** being fully consistent with the cis disposition of the two substituents[10] on the γ-lactam ring of **7a**. Similarly, the signals from C_3 and C_4 also showed an upfield shift due to steric compression (**7a**, C_3 δ 50.0, C_4 δ 54.8; **7b**, C_3 δ 52.8, C_4 δ 59.0).

Elaboration of **6a** to **13a,b** was achieved by standard deprotection and coupling procedures as summarized in Scheme II. Ester hydrolysis followed by coupling of the resulting carboxylic acid **8** with **10** provided **11**, which was deprotected and acylated with **17d**[1a] to yield **12** as a ca. 1:1 mixture of diastereoisomers. Finally, cleavage of the Z group gave **13a,b** which were separated by chromatography.

For synthesis of difluorostatone derivatives **19a,d**, standard deprotection and coupling sequences were again employed (Scheme III) to obtain the precursor alcohols **18a,b**, the starting materials **14a,b** being readily available[8a] in the 3*R*,4*S* configuration from Reformatsky reaction of Boc-cyclohexylalaninal with ethyl bromodifluoroacetate followed by ester hydrolysis. In the route to **18b**, the initial intermediate **15b** could be obtained directly from ester **14b** by heating with (*S*)-2-methylbutylamine in the presence of tetramethylguanidine. The final conversion of **18a,b** to **19a,d** was performed with the Dess–Martin periodinane,[11] a reagent which has been reported[12] to be highly effective for oxidation of fluorinated alcohols.

Analysis of the ^1H-decoupled ^{13}C NMR spectrum of **19d** showed the compound to be isolated as a ca. 1:1 mixture of free and hydrated ketone, consistent with previously reported[6,8b] behavior of proteolytic enzyme inhibitors containing difluorostatone. In DMSO-d_6, triplets of approximately equal intensity were evident at δ 197.8 and 94.9, corresponding[6] to the carbon atoms of the free and hydrated carbonyl group, respectively, and close line doubling of several other signals was also seen. Upon addition of CD_3CO_2D, both the signal at δ 94.9 and the line doubling were lost, in accord with predominance of the free ketone under acidic conditions. The absence of line doubling in the ^{13}C NMR spectrum of **19d** in DMSO-d_6 and CD_3CO_2D was also consistent with the final oxidation step proceeding without racemization at the labile asymmetric center[8a] adjacent to the ketonic function, although we have no definitive evidence in support of this conclusion.

In Vitro Renin Inhibition

The amine-based inhibitors **13a–d** (Table I) all incorporate in the heterocyclic P_4–P_2 replacement the substituents which were found to be optimal for the corresponding series of compounds derived from cyclohexylstatine.[1a] In vitro structure–activity relationships for the diastereoisomers **13a–d** were consistent with previous data in two important respects. Firstly, the more polar isomers **13a,c** at the asymmetric center α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle showed greater than 20-fold higher potency than the less polar isomers **13b,d**. In common with our earlier series of inhibitors,[1] we hypothesize that the pyridin-3-ylmethyl substituent at this asymmetric center binds to the S_2 site of the enzyme and that the configuration must therefore be *S* as in the natural substrate, although again we have no definite proof of this assignment. Secondly, as demonstrated by the comparable affinity of **13a** and **13c**, tight binding to the enzyme was seen with both the *R* and *S* diastereoisomers of the amine-based transition-state mimetic. Similar results have been reported for peptide-derived inhibitors containing (*R*)- and (*S*)-aminodeoxystatine,[5] and an explanation of the data put forward which proposes that the *R* isomer has an additional ionic interaction of the amino function at the enzyme active site.[5b]

Compounds **13a,c** showed activity of the same order as the corresponding inhibitors based on cyclohexylstatine,[1a] and thus represented another example of inhibitors in which the substituted 1,2,4-triazolo[4,3-*a*]pyrazin-3-ylpropionyl moiety appears to act as a P_4–P_2 replacement. Further analogues with extended polar C-terminal groups also displayed nanomolar inhibitory potency (data not shown). However, by analogy with our earlier inhibitors,[1a] we judged that truncation of these compounds at the C-terminus would result in a significant fall in activity. With the objective of reducing molecular weight, we therefore turned our attention to difluorostatone-based inhibitors, which might be expected[8] to show greater in vitro potency.

Compound **19a** (Table II) fulfilled this expectation, despite lacking a putative P_2 substituent[1] at the methylene group adjacent to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle. With an IC_{50} of 1.9 nM, this compound is ca. 30-fold more potent that the equivalent inhibitor derived from cyclohexylstatine.[1a] The significant increase in binding obtained by introduction of the difluorostatone transition-state mimetic encouraged us to reduce the size of the inhibitor at the C-terminus (Table II). Truncation to isoamylamide **19b** resulted in a ca 20-fold loss in affinity, but like our earlier series of analogues containing a hydroxyethylene isostere as the transition-state mimetic,[1b] the activity could be regained by replacing the 6-phenyl substituent by pyridin-3-yl (**19c**). From our previous work[1a] on optimization of substituents in the 1,2,4-triazolo[4,3-*a*]pyrazine ring, the change of the 8-substituent from isobutyl to *n*-propyl in going from **19b** to **19c** is not significant. The corresponding (*S*)-2-methylbutylamide **19d** showed an equally high level of in vitro activity. However, introduction of the P_2 pyridin-3-ylmethyl substituent was of no benefit (**19e**). This contrasting behavior

(10) Jager, Y.; Buss, V. *Liebigs Ann. Chem.* **1980**, 101.
(11) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155.
(12) Linderman, R. J.; Groves, D. M. *J. Org. Chem.* **1989**, *54*, 661.

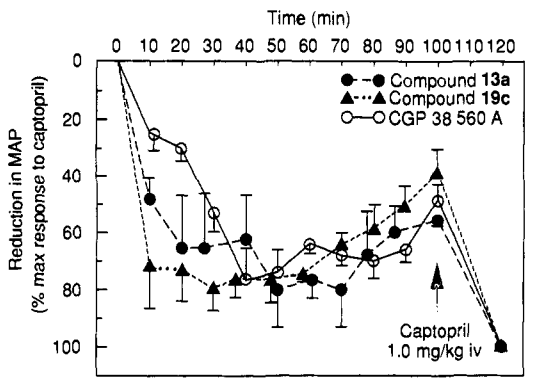


Figure 3. Effects of compounds **13a**, **19c**, and CGP 38 560 A after intravenous dosing at 3 mg/kg to anesthetized, sodium-depleted marmosets. Effects on mean arterial pressure (MAP) are expressed as a percentage of the maximum response to 1 mg/kg captopril, dosed intravenously 120 min after administration of the compounds. Mean ± SE values are shown (n = 3).

compared with our earlier series of compounds[1] may reflect subtle changes in inhibitor conformation arising from binding of the hydrated ketone to the catalytic aspartates in the enzyme. Among our various series of compounds,[1] this represents the only case where such an anomaly has been seen.[13] Finally, with ethylamide **19f** a further reduction in size was achieved without loss of potency. It is noteworthy that the molecular weight of this compound (557) compares favorably with values typically in excess of 600 for previously reported inhibitors with nanomolar potency.[2]

Pharmacological Evaluation

The more potent of the compounds listed in Tables I and II were evaluated for hypotensive efficacy in anesthetized, sodium-depleted marmosets. Typical responses for the compounds following a bolus intravenous administration of 3 mg/kg are illustrated in Figure 3 for **13a** and **19c**, using a supramaximal dose of captopril as an internal standard. The fall in MAP produced by captopril in these experiments was 44.1 ± 7.3 mm. For comparison, the hypotensive activity in this model of the recently described[9b] CGP 38 560 A is also included. Like our previous compounds, both **13a** and **19c** caused significant falls in MAP, which were maintained for the duration of the experiment. When administered orally in the same animal model at a dose of 30 mg/kg, however, neither compound significantly reduced MAP. A similar lack of response was seen with the lower molecular weight inhibitor **19f**, despite this compound also showing comparable activity to **19c** after intravenous dosing. Although **19c** produced a ca. 80% suppression of plasma renin activity (PRA) lasting up to 4 h (data not shown), presumably consistent with a modest degree of oral bioavailability,[14] disappointingly, the lower molecular weight analogue **19f** showed no such effect.

As discussed in our previous papers,[1] we again suspect this lack of oral activity to reflect poor oral absorption, rather than metabolic instability or rapid biliary clearance. At the outset of the work described here, we hypothesized that high molecular weight, for example 829 for compound **1**, could be a key factor mitigating against oral absorption. In comparison to **1**, with inhibitors such as **19c** and **19f** (MW 599 and 557, respectively) a significant reduction in this parameter has been achieved without loss of in vitro potency, but no useful oral activity has resulted. We conclude that for these series of nonpeptidic renin inhibitors high molecular weight may not be the only property precluding oral absorption, and in a subsequent publication we will describe efforts to address this issue involving alternative bicyclic heterocycles in place of the 1,2,4-triazolo[4,3-a]pyrazine moiety.

(13) The effect of omitting the P_2 substituent in previous series of renin inhibitors based on fluorostatone has not been reported. See ref 8.

(14) The limitations of using PRA measurements to quantify in vivo renin inhibition has recently been discussed in relation to a clinical study: Jeunemaitre, X.; Menard, J.; Nussberger, J.; Guyene, T. T.; Brunner, H. R.; Corvol, P. *Am. J. Hypertens.* **1989**, *2*, 219.

Summary

This paper describes novel inhibitors of human renin with nanomolar potency, arising from combination of a previously described heterocyclic P_4–P_2 replacement with aminodeoxystatine- and difluorostatone-based isosteres of the scissile amide bond. Use of the difluorostatone-derived transition-state mimetic led to compounds containing no natural amino acid fragments and with molecular weights which compare well with previously reported inhibitors of namomolar potency. When administered intravenously to anesthetized sodium-depleted marmosets at doses of 3 mg/kg, the compounds caused marked reductions in mean arterial pressure, but in the same animal model at doses of 30 mg/kg, oral activity was not seen.

Experimental Section

All operations were carried out at ambient temperatures unless otherwise stated. All evaporations were done at below 50 °C with a Büchi rotary evaporator. Flash chromatography was performed on silica (Merck Kieselgel, Art. 9385). Melting points were taken on a Büchi apparatus using glass capillary tubes and are uncorrected. ^{1}H and ^{13}C 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. Electron-impact mass spectra (EIMS) 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/Kratos MS9 spectrometer. The experimental procedures for the biological tests have been described previously.[1a]

Ethyl (2*E*,4*S*)-4-[(*tert*-Butoxycarbonyl)amino]-5-cyclohexyl-2-pentenoate (4). A solution of Boc-cyclohexylalaninal[9a] (15.0 g, 58.8 mmol) and (carbethoxymethylene)triphenylphosphorane (20.5 g, 58.8 mmol) in THF (200 mL) was left to stand overnight. The solvent was removed by evaporation and the residue was purified by flash chromatography, eluting with EtOAc/hexane (1:4 v/v), to give **4** (18.1 g, 95%), as an oil which slowly crystallized on standing: mp 53–54 °C (from hexane); ^{1}H NMR (DMSO-d_6) δ 0.8–1.4 (complex m, 20 H), 1.55–1.8 (complex m, 5 H), 4.1 (q, 2 H), 4.2 (m, 1 H), 5.7 (dd, J = 1.5, 15 Hz, 1 H), 6.8 (dd, J = 6, 15 Hz, 1 H), 7.0 (br d, 1 H); EIMS m/e 326 (M + H)$^+$, 270, 226, 180. Anal. ($C_{18}H_{31}NO_4$) C, H, N.

N_3-Z-N_4-Boc-(*S*)-CDAPA-OCH$_2$CH$_3$ (6a) and N_3-Z-N_4-Boc-(*R*)-CDAPA-OCH$_2$CH$_3$ (6b). A solution of **4** (18.1 g, 55.7 mmol) in absolute EtOH (500 mL) was saturated with NH_3 gas and then heated at 100 °C in an autoclave for 16 h. Volatile material was removed by evaporation to give intermediate amino ester **5** (18.6 g, 98%), as a clear oil which was used without purification.

Crude **5** (18.6 g, 54.4 mmol) was dissolved in CH_2Cl_2 (170 mL), and Et_3N (5.5 g, 54.4 mmol) and 4-(dimethylamino)pyridine (66 mg, 0.54 mmol) were added. The solution was cooled to 0 °C and with stirring a solution of benzyl chloroformate (9.3 g, 54.4 mmol) in CH_2Cl_2 (30 mL) was added dropwise over 30 min. The mixture was stirred at 0 °C for 1 h and then water was added. The organic phase was separated, washed with saturated brine (200 mL), and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by flash chromatography, eluting with EtOAc/hexane (1:4 v/v), to give initially **6a** (2.6 g), as a clear oil: ^{1}H NMR (DMSO-d_6) δ 0.7–1.8 (complex m, 25 H), 2.4 (m, 2 H), 3.7 (m, 1 H), 4.0 (m, 3 H), 5.0 (d, 1 H), 5.05 (d, 1 H), 6.4 (br d, 1 H), 7.0 (br d, 1 H), 7.3 (m, 5 H); FABMS m/e 477 (M + H)$^+$, 421, 377,

343, 287, 91, 57. Anal. ($C_{26}H_{41}N_2O_6$) C, H, N. Further elution of the chromatography column provided **6b** (2.4 g), as a white solid: mp 125–126 °C (from hexane); ^{1}H NMR (DMSO-d_6) δ 0.7–1.8 (complex m, 25 H), 2.3 (dd, 1 H), 2.45 (dd, 1 H), 3.5 (m, 1 H), 3.75 (m, 1 H), 4.0 (q, 2 H), 5.0 (s, 2 H), 6.6 (br d, 1 H), 7.2 (br d, 1 H), 7.3 (m, 5 H). Anal. ($C_{26}H_{41}N_2O_6$) C, H, N. Intermediate fractions from the chromatography column contained a mixture of **6a** and **6b** (4.0 g). Total yield of **6a** and **6b** was 9.0 g (38% from **4**).

***N*$_3$-Z-(*S*)-CDAPA, γ-Lactam (7a).** A solution of **6a** (250 mg, 0.53 mmol) in 4 M hydrogen chloride in dioxane (10 mL) was left to stand for 2 h. Volatile material was removed by evaporation and the residue was dissolved in dioxane (10 mL). *N,N*-Diisopropylethylamine (102 mg, 0.79 mmol) was added and the solution was heated under reflux for 6 h. The solution was concentrated and the residue was partitioned between $CHCl_3$ (20 mL) and 1 M H_2SO_4 (20 mL). The organic phase was separated, washed with H_2O (20 mL) and saturated brine (20 mL), and dried ($MgSO_4$). The solvent was evaporated and the residue was purified by flash chromatography, eluting with MeOH/CH_2Cl_2 (7:93 v/v), to give **7a** (96 mg, 52%), as a white powder: mp 112 °C; ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (complex m, 13 H), 2.2 (dd, 1 H), 2.6 (dd, 1 H), 3.8 (m, 1 H), 4.5 (m, 1 H), 5.1 (m, 2 H), 5.6 (br d, 1 H), 6.8 (br s, 1 H), 7.3 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 25.8, 25.9, 26.2, 32.3, 33.9, 34.1, 36.9, 37.6, 50.0, 54.8, 66.7, 127.8, 128.0, 128.3, 136.2, 155.8, 175.4. Anal. ($C_{19}H_{26}N_2O_3$) C, H, N.

***N*$_3$-Z-(*R*)-CDAPA, γ-Lactam (7b).** Similar to preparation of **7a**, Boc-cleavage of **6b** followed by cyclization gave **7b**, as a white powder, mp 144 °C: ^{1}H NMR ($CDCl_3$) δ 0.8–1.8 (complex m, 13 H), 2.2 (dd, 1 H), 2.7 (dd, 1 H), 3.5 (m, 1 H), 4.0 (m, 1 H), 5.1 (s, 2 H), 5.7 (br d, 1 H), 6.9 (br s, 1 H), 7.3 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 25.8, 25.9, 26.2, 32.5, 33.7, 34.3, 36.7, 42.2, 52.8, 59.0, 66.7, 127.9, 128.0, 128.4, 136.1, 155.7, 175.3. Anal. ($C_{19}H_{26}N_2O_3$), C, N.; H: calcd, 7.9; found, 8.4.

***N*$_3$-Z-*N*$_4$-Boc-(*S*)-CDAPA (8).** A 1 M NaOH solution (7.2 mL, 7.2 mmol) was added to a solution of **6a** (850 mg, 1.8 mmol) in dioxane (15 mL) and the solution was left to stand for 4 h. Volatile material was removed by evaporation and the residue was dissolved in water (5 mL). The solution was cooled to 0 °C and acidified to pH 3 with 1 M hydrochloric acid. The precipitated solid was collected and dried under vacuum to give **8** (750 mg, 95%), as a white powder which was used in the next stage without further purification: ^{1}H NMR (DMSO-d_6) δ 0.7–1.8 (complex m, 22 H), 2.3 (m, 2 H), 3.7 (m, 1 H), 3.9 (m, 1 H), 4.95 (d, 1 H), 5.05 (d, 1 H), 6.4 (br d, 1 H), 6.95 (br d, 1 H), 7.3 (m, 1 H).

Boc-Leu Pyridin-2-ylmethylamide (9). A solution of Boc-LeuOH (3.0 g, 13.0 mmol), 2-(aminomethyl)pyridine (1.41 g, 13.0 mmol), 1-hydroxybenzotriazole hydrate (HOBT, (1.75 g, 13.0 mmol), and dicyclohexylcarbodiimide (2.68 g, 13.0 mmol) in CH_2Cl_2 (30 mL) was left to stand overnight. The insoluble dicyclohexylurea was removed by filtration and the filtrate was washed with saturated $NaHCO_3$ (30 mL), water (30 mL), and saturated brine (30 mL). The organic phase was dried ($MgSO_4$) and then concentrated to give **9** (3.57 g, 86%), as a foam which was used in the next stage without purification: ^{1}H NMR (DMSO-d_6) δ 0.9 (d, 6 H), 1.0–1.8 (complex m, 12 H), 4.0 (m, 1 H), 4.35 (d, 2 H), 6.9 (br d, 1 H), 7.25 (m, 2 H), 7.7 (m 1 H), 8.4 (br t, 1 H), 8.5 (d, 1 H).

***N*$_3$-Z-*N*$_4$-Boc-(*S*)-CDAPA-Leu Pyridin-2-ylmethylamide (11).** A solution of **9** (530 mg, 1.65 mmol) in 4 M hydrogen chloride in dioxane (15 mL) was left to stand for 2 h. Volatile material was removed by evaporation and the resulting intermediate **10** was dissolved in DMF (20 mL). Carboxylic acid **8** (740 mg, 1.65 mmol) was added, followed by Et_3N (490 mg, 4.85 mmol), HOBT (233 mg, 1.65 mmol), and 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDC, 317 mg, 1.65 mmol). The solution was left to stand overnight and then volatile material was removed by evaporation. The residue was partitioned between EtOAc (30 mL) and saturated $NaHCO_3$ (30 mL). The organic phase was separated, washed with H_2O (30 mL) and saturated brine (30 mL), and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by flash chromatography, eluting with MeOH/CH_2Cl_2 (1:19 v/v), to give **11** (1.08 g, 52%), as a foam: ^{1}H NMR (DMSO-d_6) δ 0.6–1.8 (complex m, 31 H), 2.3 (m, 2 H), 3.7 (m, 1 H), 4.0 (m, 1 H), 4.25 (m, 1 H), 4.35 (d, 2 H), 4.9 (d, 1 H), 5.05 (d, 1 H), 6.5 (br d, 1 H), 6.8 (br d, 1 H), 7.2 (m, 2 H), 7.3 (m, 5 H), 7.7 (m, 1 H), 8.0 (d, 1 H), 8.3 (br t, 1 H), 8.45, (d, 1 H); FABMS *m/e* 652 (M + H)$^+$, 552, 225, 109, 86.

***N*$_3$-Z-*N*$_4$-[(2*RS*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]-(*S*)-CDAPA-Leu Pyridin-2-ylmethylamide (12).** By an analogous procedure to that described for the deprotection of **9**, the Boc group was cleaved from **11** (510 mg, 0.82 mmol) and the intermediate amine hydrochloride was acylated with carboxylic acid sodium salt **17d**[1a] (345 mg, 0.82 mmol) using Et_3N (166 mg, 1.64 mmol), HOBT (110 mg, 0.82 mmol), and EDC (156 mg, 0.82 mmol). This provided **12** (434 mg, 57%), as a foam: ^{1}H NMR (DMSO-d_6, CD_3CO_2D) δ 0.4–1.8 (complex m, 28 H), 2.2 (m, 2 H), 2.45 (m, 1 H), 3.15 (m, 2 H), 3.4–3.8 (complex m, 2 H), 4.0 (m, 2 H), 4.2–4.5 (complex m, 3 H), 4.7–5.1 (complex m, 3 H), 7.2–7.5 (complex m, 11 H), 7.75 (m, 2 H), 8.05 (m, 2 H), 8.4 (m, 3 H), 8.6 (dd, 1 H), 8.8 (2 × s, ratio 1:1, total 1 H); FABMS *m/e* 935 (M + H)$^+$, 384, 358, 293, 109.

***N*$_4$-[(2*S*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]-pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]-(*S*)-CDAPA-Leu Pyridin-2-ylmethylamide (13a)[15] and *N*$_4$-[(2*R*)-2-(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)-3-pyridin-3-ylpropanoyl]-(*S*)-CDAPA-Leu Pyridin-2-ylmethylamide (13b).[15]** Ammonium formate (504 mg, 8.0 mmol) and 10% Pd/C catalyst (250 mg) were added to a solution of **12** (373 mg, 0.40 mmol) in absolute EtOH (10 mL). The mixture was stirred for 2 h and then the catalyst was removed by filtration through Celite. The filtrate was evaporated and the residue was partitioned between $CHCl_3$ (20 mL) and water (20 mL). The organic phase was separated, washed with saturated brine (20 mL), and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by flash chromatography, eluting with aqueous NH_3 (SG 880)/MeOH/$CHCl_3$ on a gradient from 1:19:180 (v/v) to 1:19:80 (v/v), to give initially **13b** (57 mg 17%) as a white powder: mp 165–170 °C (softens from 155 °C) (after trituration with ether); ^{1}H NMR (DMSO-d_6, CD_3CO_2D) δ 0.7–1.8 (complex m, 28 H), 2.5–2.8 (complex m, 3 H), 3.2 (d, 2 H), 3.4–3.7 (complex m, 3 H), 4.1 (m, 1 H), 4.35 (m, 1 H), 4.5 (s, 2 H), 4.9 (dd, 1 H), 7.3 (m, 3 H), 7.5 (m, 3 H), 7.75 (m, 2 H), 8.05 (d, 1 H), 8.1 (d, 1 H), 8.4 (dd, 1 H), 8.5 (dd, 1 H), 8.6 (d, 1 H), 8.8 (s, 1 H); FABMS *m/e* calcd for $C_{46}H_{60}N_{10}O_3$ + 1 801.4928, found 801.4913. Anal. ($C_{46}H_{60}N_{10}O_3 \cdot 1.5H_2O$) C, N; H: calcd, 7.7; found, 7.1. Further elution of the chromatography column provided **13a** (71 mg, 21%), as a white powder: mp 116–120 °C (softens from 110 °C) (after trituration with ether); ^{1}H NMR (DMSO-d_6, CD_3CO_2D) δ 0.7–1.8 (complex m, 28 H), 2.2–2.5 (complex m, 3 H), 3.2 (d, 2 H), 3.55 (m, 1 H), 3.7 (m, 2 H), 4.1 (m, 1 H), 4.5 (m, 3 H), 4.85 (dd, 1 H), 7.3 (m, 3 H), 7.5 (m, 3 H), 7.7 (m, 2 H), 8.05 (d, 1 H), 8.1 (d, 1 H), 8.4 (dd, 1 H), 8.45 (dd, 1 H), 8.55 (d, 1 H), 8.9 (s, 1 H); FABMS *m/e* calcd for $C_{46}H_{60}N_{10}O_3$ + 1 801.4928, found 801.4950. Anal. ($C_{46}H_{60}N_{10}O_3 \cdot 3H_2O$) C, H, N.

Compounds **13c** and **13d** (Table I) were prepared by an analogous sequence starting from **6b**.

Boc-ACDFHPA-Leu Pyridin-2-ylmethylamide (15a). By an analogous procedure to that described for the preparation of **12**, compound **9** (340 mg, 1.06 mmol) was deprotected and acylated with carboxylic acid sodium salt **14a**[8a] (394 mg, 1.06 mmol) to give **15a** (478 mg, 81%), as a foam: ^{1}H NMR ($CDCl_3$) δ 0.7–2.1 (complex m, 31 H), 3.9 (m, 1 H), 4.1 (m, 1 H), 4.55 (m, 3 H), 5.1 (br d, 1 H), 7.1 (br d, 1 H), 7.3 (m, 3 H), 7.7 (m, 1 H), 7.85 (br, 1 H), 8.5 (dd, 1 H); FABMS *m/e* 555 (M + H)$^+$, 499, 451, 222, 129, 109, 86.

***N*-[(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-*a*]pyrazin-3-yl)acetyl]-ACDFHPA-Leu Pyridin-2-ylmethylamide (18a).** By an analogous procedure to that described for the preparation of **11**, compound **15a** (305 mg, 0.55 mmol) was deprotected and the resulting intermediate **16a** was acylated with carboxylic acid **17a**[1a] (171 mg, 0.55 mmol) to give **18a** (315 mg, 77%), as a foam: ^{1}H NMR (DMSO-d_6, CD_3CO_2D) δ 0.6–1.8 (complex m, 28 H), 2.45 (m, 1 H), 3.15 (d, 2 H), 4.0–4.5 (complex m, 7 H), 7.1 (m, 2 H), 7.3 (m, 4 H), 8.05 (d, 1 H), 8.1 (d, 1 H), 8.3 (dd, 1 H), 8.8 (s, 1

(15) Provisional assignment of stereochemistry at the asymmetric center α to the 1,2,4-triazolo[4,3-*a*]pyrazine heterocycle. See the Results and Discussion.

H); FABMS m/e 747 $(M + H)^+$, 294.

N-[(8-Isobutyl-6-phenyl-1,2,4-triazolo[4,3-a]pyrazin-3-yl)acetyl]-ACDFOPA-Leu Pyridin-2-ylmethylamide (19a). The Dess-Martin periodinane[11] (390 mg, 1.57 mmol) was added to a solution of **18a** (317 mg, 0.42 mmol) in CH_2Cl_2 (10 mL) and the mixture was stirred for 2.5 h. A solution of $Na_2S_2O_3$ (200 mg, 2.98 mmol) in saturated $NaHCO_3$ (5 mL) was added and the mixture was stirred vigorously for 0.5 h. The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 10 mL). The compound organic solutions were washed with H_2O (10 mL) and saturated brine (10 mL) and dried ($MgSO_4$). The solvent was removed by evaporation and the residue was purified by flash chromatography, eluting with $MeOH/CH_2Cl_2$ (1:19 v/v), to give **19a** (158 mg, 50%), as a white powder: mp 90-95 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) δ 0.7-1.8 (complex m, 28 H), 2.5 (m, 1 H), 3.15 (d, 2 H), 4.2-4.5 (complex m, 6 H), 4.9 (dd, 1 H), 7.25 (m, 2 H), 7.5 (m, 3 H), 7.7 (m, 1 H), 8.1 (dd, 1 H), 8.45 (dd, 1 H), 8.8 (s, 1 H); FABMS m/e calcd for $C_{40}H_{51}F_2N_8O_4$ + 1 745.4001, found 745.4067. Anal. ($C_{40}H_{50}F_2N_8O_4 \cdot 0.5H_2O$) C, H; N: calcd, 14.9; found, 14.2.

Boc-ACDFHPA (S)-2-Methylbutylamide (15b). A solution of 14b[8a] (300 mg, 0.79 mmol), (S)-2-methylbutylamine (172 mg, 1.97 mmol), and 1,1,3,3-tetramethylguanidine (91 mg, 0.79 mmol) in $CHCl_3$ (5 mL) was heated under reflux for 2 h. Volatile material was removed by evaporation and the residue was purified by flash chromatography, eluting with EtOAc/hexane (3:7 v/v), to give **15b** (338 mg, 83%), as a foam: ^1H NMR ($CDCl_3$) δ 0.9-1.9 (complex m, 31 H), 3.2 (m, AB X, 2 H), 3.8-4.1 (m, 2 H), 4.7 (br d, 1 H), 6.9 (br, 1 H); FABMS m/e 421 $(M + H)^+$, 365, 321.

N-[(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-a]pyrazin-3-yl)acetyl]-ACDFHPA (S)-2-Methylbutylamide (18b). By an analogous procedure to that described for the preparation of 11, compound **15b** (263 mg, 0.63 mmol) was deprotected and the resulting intermediate **16b** was acylated with carboxylic acid sodium salt **17b**[1a] (201 mg, 0.63 mmol) and Et_3N (64 mg, 0.63 mmol), HOBT (84.5 mg, 0.63 mmol), and EDC (120 mg, 0.63 mmol). This provided **18b** (143 mg, 38%), as a foam: ^1H NMR (DMSO-d_6, CD_3CO_2D) δ 0.7-1.8 (complex m, 25 H), 2.0 (m, 2 H), 2.9 (dd, 1 H), 3.1 (dd, 1 H), 3.3 (t, 2 H), 4.0 (dt, 1 H), 4.2 (m, 1 H), 4.3 (m, CH_2CO partially exchanged with CD_3CO_2D), 7.55 (dd, 1 H), 8.45 (dt, 1 H), 8.65 (m, 1 H), 8.95 (s, 1 H), 9.3 (m, 1 H); FABMS m/e 600 $(M + H)^+$.

N-[(8-Propyl-6-pyridin-3-yl-1,2,4-triazolo[4,3-a]pyrazin-3-yl)-acetyl]-ACDFOPA (S)-2-Methylbutylamide (19d). By an analogous procedure to that described for the preparation of **19a**, oxidation of **18b** gave **19d** in 55% yield, as a white powder: mp 79-81 °C (after trituration with ether); ^1H NMR (DMSO-d_6, CD_3CO_2D) δ 0.7-1.8 (complex m, 25 H), 2.0 (m, 2 H), 2.95 (m, AB X, 2 H), 3.3 (t, 2 H), 4.3 (dd, 2 H), 4.9 (dd, 1 H), 7.6 (dd, 1 H), 8.45 (dt, 1 H), 8.65 (m, 1 H), 9.0 (s, 1 H), 9.3 (m, 1 H); ^{13}C NMR (DMSO-d_6, CD_3CO_2D) δ 11.3, 14.2, 17.1, 20.0, 25.9, 26.2, 26.4, 26.8, 31.6, 33.9, 34.1, 34.5, 36.0, 45.1, 52.6, 110.6 (t, J = 184 Hz, CF_2), 112.8, 124.4, 132.2, 134.3, 135.6, 145.0, 147.5, 150.0, 155.8, 160.9 (t, J = 18 Hz, CF_2CONH), 167.3, 197.8 (t, J = 18 Hz, $CHCOCF_2$); ^{13}C NMR (DMSO-d_6): additional signal at 94.9 (t, J = 18 Hz, $CHC(OH)_2CF_2$) and closely spaced line doubling of many other signals; FABMS, m/e calcd for $C_{31}H_{42}F_2N_7O_3$ + 1 598.3317, found 598.3339. Anal. ($C_{31}H_{41}F_2N_7O_3 \cdot 0.5H_2O$) C, H; N: calcd, 16.2; found, 15.6.

Acknowledgment. We thank J. S. Major, A. A. Oldham, and D. A. Roberts for helpful discussions, B. Wright for advice on interpretation of ^{13}C NMR spectra, and the following for performing the biological testing: C. Bath, K. J. Taylor (in vitro renin inhibition), E. Kelly, and A. McWilliam (pharmacological evaluation).

Synthesis and Antirhinovirus Activity of 8-Substituted Analogues of 6-(Dimethylamino)-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine

James L. Kelley,*,† James A. Linn,† and J. W. T. Selway‡

Division of Organic Chemistry, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709, and Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, U.K. *Received May 18, 1990*

Several 8-substituted analogues of 6-(dimethylamino)-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine (1) were synthesized and tested for activity against rhinovirus type 1B. Among 16 8-substituted analogues, the 8-amino (3) and 8-bromo (2) analogues were most active with IC_{50}s of 0.36 and 1.4 μM, respectively, under conditions where 1 had an IC_{50} of 0.03 μM.

Introduction

The rhinoviruses, which are recognized as the most important causative agents of the common cold,[1] are inhibited in vitro by a wide variety of chemical structures.[2-4] Despite the many reports of in vitro antirhinovirus activity, no agent has consistently exhibited significant clinical efficacy.[2,3,5] We previously reported the potent in vitro antirhinovirus activity of a series of 9-benzyl-6-(dimethylamino)purines.[6-9] One of the most active compounds against rhinovirus serotype 1B was 6-(dimethylamino)-9-(4-methylbenzyl)-2-(trifluoromethyl)-9*H*-purine (1), which had an IC_{50} of 0.03 μM.

Structure-activity studies addressed the effects of substituent variation at the purine 2-position,[8] on the 9-benzyl moiety,[9] and at the purine 6-substituent.[10] In an effort to develop an agent with a broad spectrum of antirhinovirus serotype activity, a series of 6-anilino-9-benzyl-2-chloro- or 2-(trifluoromethyl)purines were studied,[11,12] but the best compound—6-(3-fluoroanilino)-9-(3-fluorobenzyl)-2-(trifluoromethyl)-9*H*-purine—exhibited in vivo properties incompatible with further drug development.[12] To explore further the structure-activity relationship of

† Burroughs Wellcome Co.
‡ Wellcome Research Laboratories.

(1) Douglas, R. G., Jr. In *Antiviral Agents and Viral Diseases of Man*, 2nd ed.; Galasso, G. J., Merigan, T. C., Buchanan, R. A., Eds.; Raven: New York, 1984; pp 313-367.
(2) Kelley, J. L. *Annu. Rep. Med. Chem.* **1984**, *19*, 117.
(3) Ahmad, A. L. M.; Tyrrell, D. A. J. *Antiviral Res.* **1986**, *6*, 241.
(4) Al-Nakib, W.; Tyrrell, D. A. J. *Antiviral Res.* **1987**, *8*, 179.
(5) Al-Nakib, W.; Higgins, P. G.; Barrow, G. I.; Tyrrell, D. A. J.; Andries, K.; Bussche, G. V.; Taylor, N.; Janssen, P. A. J. *Antimicrob. Agents Chemother.* **1989**, *33*, 522.
(6) Kelley, J. L.; Miller, C. A.; Selway, J. W. T.; Schaeffer, H. J. *Eur. J. Med. Chem.* **1988**, *23*, 319.
(7) Kelley, J. L.; Linn, J. A.; Krochmal, M. P.; Selway, J. W. T. *J. Med. Chem.* **1988**, *31*, 2001.
(8) Kelley, J. L.; Linn, J. A.; Selway, J. W. T. *J. Med. Chem.* **1989**, *32*, 218.
(9) Kelley, J. L.; Linn, J. A.; Krochmal, M. P.; Selway, J. W. T. *J. Med. Chem.* **1989**, *32*, 1757.
(10) Kelley, J. L.; Linn, J. A.; Selway, J. W. T. *Eur. J. Med. Chem.* **1990**, *25*, 131.
(11) Kelley, J. L.; Linn, J. A.; Selway, J. W. T. *J. Med. Chem.* **1990**, *33*, 1360.
(12) Kelley, J. L.; Linn, J. A.; Selway, J. W. T. *Eur. J. Med. Chem.* **1990**, *25*, 623.

0022-2623/91/1834-0157$02.50/0