Studies Directed toward the Design of Orally Active Renin Inhibitors. 2. Development of the Efficacious, Bioavailable Renin Inhibitor (2*S*)-2-Benzyl-3-[[(1-methylpiperazin-4-yl)sulfonyl]propionyl]-3-thiazol-4-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane (A-72517)

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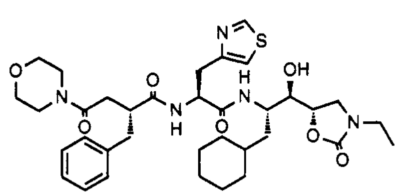
Employing a set of empirical guidelines for the design of well-absorbed renin inhibitors, we have followed two strategies to improve potency while maintaining bioavailability. One process involved incorporation of an extended N-terminal residue bearing a weakly basic substituent and is exemplified by compound **25**. The other approach centered on the inclusion of an N-terminal sulfonamide and culminated in the discovery of inhibitor **32** (A-72517). Both **25** and **32** showed excellent bioavailability in the rat and ferret (>25%) and, while subject to hepatic elimination in the monkey, were efficacious in this species.

In the previous report[1] we described the first phase of our effort to design an orally active renin inhibitor. A detailed investigation of structure–absorption relationships resulted in a set of empirical guidelines for the design of bioavailable renin inhibitors. Optimum structures, exemplified by **1** and **2**, incorporated a single, solubilizing substituent at the C- or N-terminus combined with a lipophilic P_2-site residue. Both **1** and **2** gave unprecedented plasma drug levels upon intraduodenal administration to monkeys and were potent against human plasma renin (IC_{50} = 8.1 and 18 nM, respectively). This potency was



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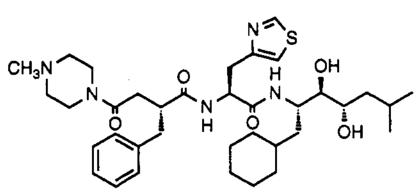

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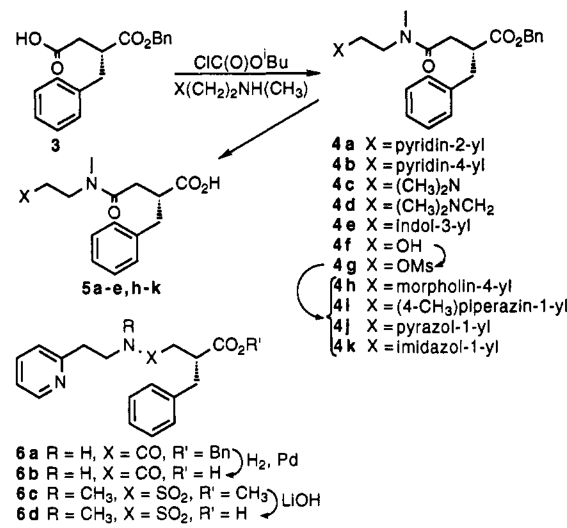
comparable to that of our clinical candidate enalkiren (IC_{50} = 14 nM),[2] which, although not orally bioavailable, was active intravenously. From our extensive clinical investigation of this compound, we realized that a viable drug candidate must not only be well and predictably absorbed from the gastrointestinal tract into the systemic circulation, but it must possess significantly greater in vitro activity than enalkiren. Thus the second phase of our effort to design an orally active renin inhibitor centered on the incorporation of features that would improve in vitro potency. If this refinement could be accomplished without resorting to drastic structural changes, then those characteristics responsible for bioavailability could be maintained, and the resulting inhibitors should be both efficacious and well absorbed.

Scheme I. Synthesis of Extended N-Terminal Groups



Results and Discussion

Synthesis. The syntheses of the various N-terminal residues are outlined in Schemes I and II. These were coupled to the fragments described in the previous report[1] using a water-soluble carbodiimide (EDC) to provide intact inhibitors. Acids **6d**, **12a**, and **12b** were synthesized as racemates. After coupling, the resulting diastereomers were separated chromatographically, and stereochemistry was assigned on the basis of in vitro potency.[3] Although sulfonamide **14** was originally prepared in D,L form,[4] an improved, optically active synthesis was subsequently developed,[5] confirming the stereochemical assignment in this case. Final products, with the exception of **32**, were purified by chromatography on silica gel following an extractive isolation. Inhibitor **32** (A-72517) was isolated by recrystallization of the crude reaction product.

In Vitro Potency and Preliminary Absorption Data. Extended N-Terminus Series. One lead structure in our effort to enhance potency was A-65317.[6] This inhibitor incorporated a substituted ethanolamine residue at the N-terminus that imparted subnanomolar activity against human plasma renin. The corresponding C-terminal

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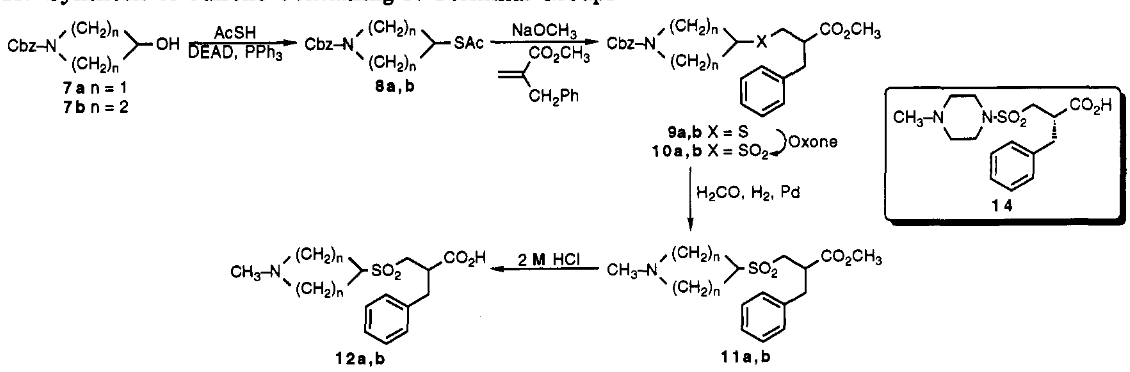
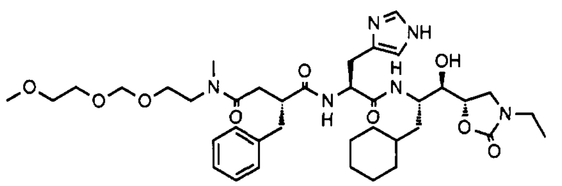
Scheme II. Synthesis of Sulfone-Containing N-Terminal Groups



dipeptide glycol **15** (Table I) maintained excellent in vitro potency. As an initial test of absorption, **15** and subsequent compounds were administered intraduodenally (id) to anesthetized rats. Plasma drug levels were determined by HPLC or a renin inhibition assay[7] from samples taken at 10 and 30 min from both the systemic and portal circulation in the same animals. While this model was insufficient for the determination of bioavailability, the data provided an estimate of both absorption from the intestine and extraction by the liver. As was expected for a histidine-containing compound,[1] **15**, while moderately absorbed from the intestine, exhibited extensive hepatic extraction. Replacement of the P_2-site histidine with thiazol-4-ylalanine **16** significantly increased both the portal and systemic plasma drug levels. Interestingly, truncation of the N-terminus (**17**) had an adverse affect not only on potency but on absorption as well, perhaps as a result of increased lipophilicity in the absence of a solubilizing residue.



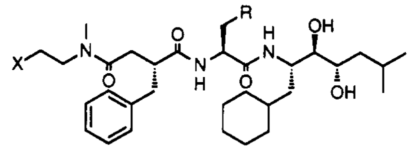
A-65317

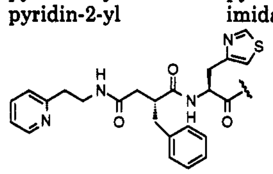
Although inhibitor **16** was potent and was well absorbed in the id rat screening model, this compound proved difficult to formulate due to its moderate aqueous solubility and the lack of a group suitable for salt formation. We therefore decided to incorporate an N-terminal basic residue while maintaining the extended N-terminus. Compound **18**, in which the (methoxyethoxy)methoxy (MemO) residue was replaced with a dimethylamino group, showed apparently good (but variable) absorption from the intestine and low hepatic extraction; however, this modification caused a 6-fold reduction in potency. The corresponding propylenediamine derivative **19** produced lower plasma drug levels (statistically significant differences were observed only for the 30-min portal levels), presumably due to the increased basicity of the N-terminal residue.[1] Substitution of a saturated heterocycle (morpholine) for the dimethylamino group (**20**) maintained good absorption into the systemic circulation while improving potency to a small extent. Again, a minor structural modification that enhanced basicity resulted in lower plasma drug levels (**21**). The best in vitro potencies were obtained when aromatic heterocycles were incorporated (**22–25**). Encouragingly, good systemic plasma levels also were observed in the id rat screening model with these four inhibitors (although results from **23** were variable), suggesting a significant improvement compared to histidine derivative **15**. Inhibitors **20**, **25**, and **26** (vide infra) were chosen for more extensive in vivo evaluation.

Compounds **26–29** further delineated the structure–activity relationships within this series of inhibitors. The importance of the *N*-methyl substituent at the N-terminus and some steric constraints at the site accepting the terminal heterocycle were illustrated by compounds **28** and **29**. As was observed previously,[1] incorporation of imidazol-1-ylalanine at the P_2 site (**27**) was detrimental to potency, and this effect was only partially offset by the extended N-terminus. Substitution of the thiazol-4-ylalanine of **25** with pyrazol-1-ylalanine **26** had little effect on potency, physicochemical properties, or systemic plasma drug levels in the id rat screening model. Portal drug levels, however, differed significantly between these two compounds. This behavior was in contrast to the results obtained with the corresponding N-terminal *N*-methylpiperazine inhibitors[1] where similar portal but differing systemic levels were observed. Thus while the empirical rules delineated in the previous paper provided general guidelines for the design of renin inhibitors with extended N-termini, it was clear that the absorption from the intestine into the systemic circulation was an intricate process that could be affected by subtle structural features.

In Vitro Potency and Preliminary Absorption Data. A-72517 and Related Structures. Our second approach toward maximizing in vitro activity was based upon the report that an N-terminal *tert*-butylsulfonyl residue could impart greater potency than standard carbonyl-derived groups.[8] Replacement of the (4-methylpiperazin-1-yl)-carbonyl of **2** with this residue provided inhibitor **30** (Table II). While a 21-fold enhancement in potency was achieved, this compound exhibited poor absorption, presumably due to its extreme lipophilicity unopposed by a solubilizing group. We therefore decided to incorporate both a tertiary amine and an oxidized sulfur at the N-terminus to provide (1-methylpiperidin-4-yl) sulfone **31**. Results from the corresponding carbonyl analog of **31** had suggested that a piperidine nitrogen was too basic for adequate absorption.[1] Consequently, two strategies were employed to reduce the basicity of the terminal nitrogen: the piperidine was replaced with a piperazine to give inhibitor **32** (A-72517), the sulfonamide analog of **2**, and the ring size was reduced to provide 3-sulfonylazetidine **33**. These tactics placed an electron-withdrawing substituent closer to the terminal nitrogen thereby modulating its basicity. Both **32** and **33** produced plasma drug levels comparable to those observed with **2** in the id rat screening model. Furthermore, **32** proved to be 16-fold more potent than **2** against

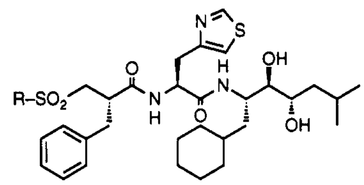
Table I. C-Terminal Glycol Renin Inhibitors Containing an Extended N-Terminus



no.	X	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	assay	10 mg/kg id rat experiments plasma level (ng/mL)[a]: 10 min, systemic	10 min, portal	30 min, systemic	30 min, portal
15	MemO	imidazol-4-yl	0.80	nd[e]	0.18	HPLC	68 ± 27	1900 ± 300	50 ± 15	370 ± 100
16	MemO	thiazol-4-yl	1.6	>4.6	0.18	RI[f]	300 ± 70[g]	3100 ± 700	570 ± 150[g,h]	3100 ± 700[g,h]
17	CH_3O	thiazol-4-yl	4.1	nd	0.063	HPLC	33 ± 33[g]	930 ± 640	33 ± 33[g]	600 ± 400[g]
18	$(CH_3)_2N$	thiazol-4-yl	9.4	3.11	0.74	RI	600 ± 360[i]	1600 ± 200[i]	450 ± 320[i]	780 ± 350[i]
19	$(CH_3)_2NCH_2$	thiazol-4-yl	8.1	2.83	0.46	HPLC	93 ± 50	580 ± 60[g]	47 ± 26	270 ± 80
20	morpholin-4-yl	thiazol-4-yl	4.1	4.15	0.12	HPLC	450 ± 130[j]	nd	570 ± 240	nd
21	(4-Me)piperazin-1-yl	thiazol-4-yl	8.5	3.58	1.0	RI	80 ± 16	580 ± 450	74 ± 30	590 ± 250
22	pyrazol-1-yl	thiazol-4-yl	0.72	>4.2	0.044	HPLC	140 ± 30	1900 ± 800	230 ± 60[k]	2300 ± 500
23	imidazol-1-yl	thiazol-4-yl	1.3	4.01	0.12	HPLC	57 ± 57	400 ± 360	190 ± 80	810 ± 590
24	pyridin-4-yl	thiazol-4-yl	3.0	>4.2	0.012	HPLC	160 ± 10[j]	nd	190 ± 30[k]	nd
25	pyridin-2-yl	thiazol-4-yl	2.6	4.22	0.013	RI	220 ± 100	550 ± 170	230 ± 50[k]	560 ± 180
26	pyridin-2-yl	pyrazol-1-yl	1.6	>4.2	0.014	HPLC	220 ± 60	2200 ± 800	300 ± 50[k]	4500 ± 1300[g]
27	pyridin-2-yl	imidazol-1-yl	14	nd	nd		nd	nd	nd	nd
28			37	nd	nd		nd	nd	nd	nd
29	indol-3-yl	thiazol-4-yl	33	nd	nd		nd	nd	nd	nd

[a] Mean ± SEM, $n = 3$. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] Not determined. [f] Renin inhibition assay. [g] Differs significantly from value directly above ($P < 0.05$). [h] $n = 6$. [i] $n = 2$. [j] 15-min value. [k] Differs significantly from value for 15 ($P < 0.05$).

Table II. C-Terminal Glycol Renin Inhibitors Containing a Sulfone or Sulfonamide N-Terminus



no.	R	IC_{50} (nM)[b]	log P[c]	solubility[d]	assay	10 mg/kg id rat experiments plasma level (ng/mL)[a]: 10 min, systemic	10 min, portal	30 min, systemic	30 min, portal
30	$(CH_3)_3C$	0.87	>4.2	0.0020	RI[f]	22 ± 4	830 ± 200	29 ± 15	500 ± 250
31	1-methylpiperidin-4-yl	4.0	3.61	nd[e]		nd	nd	nd	nd
32	4-methylpiperazin-1-yl	1.1 ± 0.2[a]	4.57	0.00081	RI	280 ± 110[g]	nd	440 ± 150	nd
33	1-methylazetidin-3-yl	3.9	3.89	0.0051	HPLC	510 ± 50	1800 ± 500	550 ± 130	1900 ± 700
34	(2-pyridin-2-ylethyl)methylamine	1.8	>4.5	nd		nd	nd	nd	nd

[a] Mean ± SEM, $n = 3$. [b] Human plasma renin, pH 7.4. [c] Octanol/water, pH 7.4. [d] mg/mL, pH 7.4. [e] Not determined. [f] Renin inhibition assay. [g] 15-min value.

human plasma renin and was selected for additional in vivo evaluation. (While compound **31** was not tested in the id rat screening model, subsequent id monkey experiments shown in Table VI demonstrated that it was indeed less well absorbed than inhibitor **32**.) Finally, compound **34** was designed to accommodate both an extended N-terminus (cf. **25**) and the N-terminal sulfonamide. Although **34** was potent, it was no more so than members of either series demonstrating that the results from the two approaches were not additive.

The sulfonamide of **32** imparted not only enhanced potency, but also crystallinity. While this attribute was advantageous from a synthetic perspective, a less desirable consequence was a 200-fold reduction in solubility (pH 7.4) compared to amide **2**. From the preliminary rat experiments we knew that the HCl salt (formed during formulation) provided sufficient aqueous solubility for intraduodenal administration. To determine an optimum salt for eventual oral administration, both inorganic acids and citric acid derivatives were studied (Table III). We theorized that citrate salts, being highly oxygenated and having the capacity to carry multiple charges, might possess superior solubility characteristics. However even **32i**, being a di-salt and bearing 10 additional oxygen atoms, was not as soluble as the simple mineral acid salts **32a–d**. The monohydrochloride **32b** was 5 orders of magnitude more soluble than the free base and was used in subsequent oral studies.[9]

Absolute Bioavailability and Efficacy. Inhibitors from both structural series were dosed at 10 mg/kg via the intraduodenal route to anesthetized rats and ferrets. In contrast to the screening model, companion intravenous experiments were performed and plasma samples were taken at sufficient time points so that an estimate of absolute bioavailability values could be calculated (Table V). Bioavailability in both species was uniformly high,

Table III. Salts of Compound **32** (A-72517)

no.	acid	formula[a]	solubility[b]
32a	$(HCl)_2$	$C_{35}H_{57}N_5O_6S_2Cl_2$[c]	17
32b	HCl	$C_{35}H_{56}N_5O_6S_2Cl \cdot 0.5H_2O$[d]	10
32c	CH_3SO_3H	$C_{36}H_{59}N_5O_9S_3 \cdot 0.5H_2O$	20
32d	H_3PO_4	$C_{35}H_{58}N_5O_{10}S_2P \cdot 0.75H_2O$[e]	8.7
32e	citric acid	$C_{41}H_{63}N_5O_{13}S_2 \cdot 1.4H_2O$	3.5
32f	Na citrate	$C_{41}H_{62}N_5O_{13}S_2Na \cdot 0.5H_2O$[c]	0.8
32g	K citrate	$C_{41}H_{62}N_5O_{13}S_2K \cdot 0.75H_2O$[c,f]	1.2
32h	choline citrate	$C_{46}H_{76}N_6O_{14}S_2$[g]	0.4
32i	$(HOCH_2)_3CNH_3^+$ citrate	$C_{45}H_{74}N_6O_{16}S_2 \cdot H_2O$	1.2

[a] Analyses for C, H, N, S, Cl, K, Na, P were ±0.4% of expected values (for formulae shown) unless otherwise noted. [b] Aqueous solubility, mg/mL. [c] S not determined. [d] S: calcd, 8.53; found 8.17. [e] P: calcd, 3.79; found, 3.26. [f] K: calcd, 4.12; found, 4.67. [g] S: calcd 6.40; found, 5.74.

Table IV. In Vitro Potency of Inhibitors **25** and **32** against Plasma Renin from Various Species[a]

	IC_{50} (nM)[a]	
species	compound **25**	compound **32**[b]
monkey	0.76	0.24
human	2.6	1.1 ± 0.2
dog	33	110
rat	>10 000	1400

[a] pH 7.4. [b] See ref 9 for data from additional species.

ranging from 35% to 85% in the ferret and from 20% to 80% in the rat. Peak plasma drug levels in both the ferret (400–940 ng/mL) and rat (460–980 ng/mL) were similar to the time-averaged values[10] (290–750 ng/mL, ferret; 310–650, ng/mL rat), indicating steady, prolonged absorption. Since inhibitors **32** and **33** appeared similar in these small animals models, **32** was chosen for additional evaluation on the basis of its superior in vitro potency. (By using alternate protocols for the id rat experiments and the companion iv experiments in which plasma samples were drawn at additional time points, a more accurate estimation of bioavailability for **32** was calculated to be 35 ± 7%.[9]) Interestingly, **25** appeared to give higher plasma levels in the rat than its pyrazol-1-yl analog **26**. Although these differences were not statistically significant, **25** was judged the most promising inhibitor from the extended N-terminus series.

Having achieved highly potent compounds that were bioavailable in small animal models, our next task was to demonstrate that these compounds would indeed prove more efficacious than **1** and **2**. As shown in Table IV, Inhibitors **25** and **32** were selective for primate renin. Consequently, the initial determination of in vivo activity was performed in the salt-depleted cynomolgus monkey. Hypotensive responses, systemic plasma drug levels, and portal plasma drug levels following 1 and 10 mg/kg intraduodenal doses for both compounds are shown in Figure 1 and Table VI. Gratifyingly, the pharmacologic responses were significantly greater than were observed with **1** and **2**. At the larger dose, both **25** and **32** caused a statistically significant fall in mean arterial pressure (MAP) that persisted for more than 2 h. The maximum response for **32** was 32 ± 5 mm Hg (37 ± 7%) compared to 19 ± 3 mm Hg (28 ± 4%) for **25**. Qualitatively,[11] the maximum effects at the two doses were similar while the 10 mg/kg dose showed an apparent longer duration of action for inhibitor **32**. In contrast, both the magnitude and the duration of the response appeared reduced when **25** was given at the lower dose. For comparison, the angiotensin converting enzyme inhibitor captopril ad-

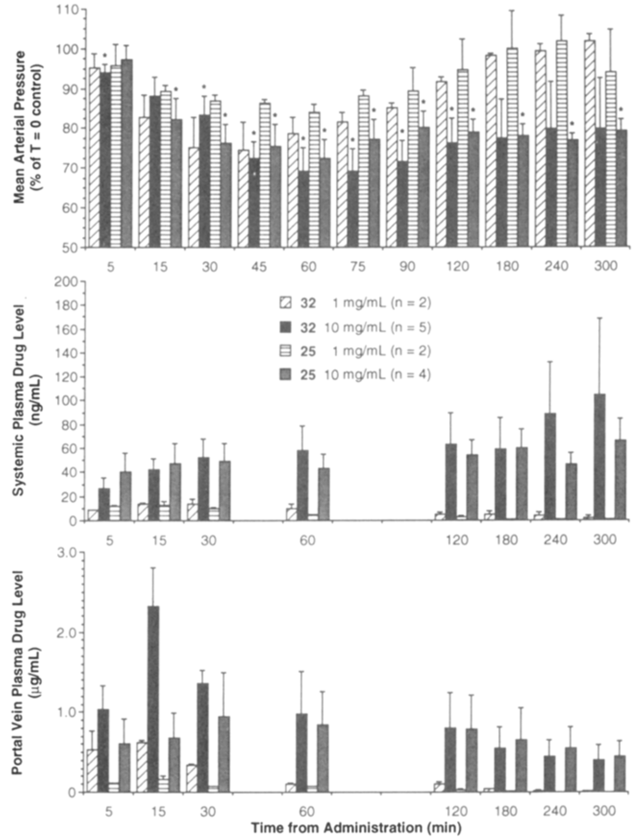


Figure 1. Intraduodenal administration of compounds **25** and **32** to anesthetized, salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM and were considered significantly different from baseline if $P < 0.05$ (*). Plasma drug levels were determined by a renin inhibition assay, see ref 7.

ministered at 10 mg/kg id in this model reduced MAP 29 ± 5 mm Hg (39 ± 4%, $n = 3$, data not shown). Since a complete dose–response relationship was not established for either captopril or **32**, however, the maximum response attainable in the monkey with these compounds remains to be determined.

Figure 2 shows the plasma renin activity (PRA) profile from these id monkey experiments. At the 10 mg/kg doses, PRA was reduced a maximum of 99.8 ± 0.1% and 98.4 ± 1.6% for inhibitors **32** and **25**, respectively, and was still >95% suppressed at the end of the 5-h experiment for both compounds. Dissociation between the hypotensive effects and PRA has been observed with other renin inhibitors[12] and with **32** in the dog,[9] and one explanation is that a renin inhibitor must interact with a second, extraplasma pool of renin in order elicit a pharmacologic response.[13] At the lower 1.0 mg/kg doses, an apparent[11] recovery of PRA toward baseline was observed with both inhibitors.

The id bioavailability of **32** from the 10 mg/kg monkey experiments was 5.1 ± 2.2% when compared to a 1.0 mg/kg iv dose. This value was a modest improvement compared to other reported renin inhibitors in the monkey (except for **2**). Nevertheless, bioavailability and systemic plasma levels were lower than was observed in either the ferret or rat, reflecting either poor absorption from the intestine or efficient hepatic extraction. Since samples taken from the portal vein yielded drug levels that were significantly higher than the corresponding arterial levels (as was also true for **25**), it was obvious that **32** was well absorbed from the intestine in all three species, but was

Table V. Plasma Drug Levels and Absolute Bioavailability of Selected Renin Inhibitors Dosed Intraduodenally at 10 mg/kg in Ferrets and Rats

					systemic plasma level[a]		
no.[b]	species	duration (h)	n	assay	AUC[c]	peak (ng/mL)	bioavailability (%)[d]
20	ferret	3	2	RI[e]	1200 ± 500	860 ± 370	85 ± 38
25	ferret	3	2	RI	1100 ± 200	510 ± 160	68 ± 19
25	rat	2	3	RI	1300 ± 300	980 ± 220	27 ± 8
26	ferret	3	2	RI	870 ± 140	400 ± 20	35 ± 9
26	rat	2	3	HPLC	760 ± 170	460 ± 120	20 ± 4
32	ferret[f]	2	6	RI	1500 ± 600	940 ± 320	82 ± 32
32	rat	2	7	RI	620 ± 170	470 ± 110	23 ± 6
33	ferret	3	2	RI	2000 ± 100	790 ± 60	80 ± 10
33	rat	2	2	HPLC	1300 ± 700	850 ± 360	80 ± 45

[a] Mean ● SEM. [b] See Tables I and II for structures. [c] Integrated area under the plasma drug level–time curve, ng h/mL. [d] Compared to 0.3 mg/kg (ferret) or 1.0 mg/kg (rat) iv dose. [e] Renin inhibition assay. [f] Data taken from ref 9.

Table VI. Selected Renin Inhibitors Dosed Intraduodenally at 10 mg/kg in Monkeys

			plasma level[a]				
		hypotension[b]	portal		systemic		
no.[c]	n	peak	AUC[d]	peak (ng/mL)	AUC[d]	peak (ng/mL)	bioavailability
25	4	28 ± 4	3400 ± 1700	1200 ± 500	260 ± 70	70 ± 17	3.1 ± 1.1[e]
31	2	23 ± 7	530 ± 200	550 ± 360	110 ± 10	72 ± 20	1.8 ± 0.1[e]
32	5	37 ± 7	3800 ± 1400	2400 ± 500	340 ± 140	130 ± 60	5.1 ± 2.2[f]

[a] Mean ± SEM, determined by a renin inhibition assay. [b] % Change from predose baseline pressure. [c] See Tables I and II for structures. [d] Integrated area under the plasma drug level–time curve, ng h/mL. [e] Compared to 0.3 mg/kg iv dose. [f] Compared to 1.0 mg/kg iv dose, data taken from ref 9.

Table VII. Physical Data and Synthetic Methods for Renin-Inhibiting Compounds

no.[a]	formula[b]	chromatography solvent[c]
15	$C_{38}H_{61}N_5O_8{\cdot}0.5H_2O$	3–4
16	$C_{38}H_{60}N_4O_8S$	1–2.5
17	$C_{35}H_{54}N_4O_6S$	1
18	$C_{36}H_{57}N_5O_5S{\cdot}0.75H_2O$	3.5–4
19	$C_{37}H_{59}N_5O_5S{\cdot}0.5H_2O$	5–6
20	$C_{38}H_{59}N_5O_6S{\cdot}0.5H_2O$	1.5–3
21	$C_{39}H_{62}N_6O_5S{\cdot}0.5H_2O$	3.5–7
22	$C_{37}H_{54}N_6O_5S{\cdot}0.5H_2O$	1–3
23	$C_{37}H_{54}N_6O_5S{\cdot}H_2O$	2.5
24	$C_{39}H_{55}N_5O_5S{\cdot}0.5H_2O$	2–5
25	$C_{39}H_{55}N_5O_5S{\cdot}0.5H_2O$	1.3–2
26	$C_{39}H_{56}N_6O_5{\cdot}0.5H_2O$	1.5–2
27	$C_{39}H_{56}N_6O_5{\cdot}0.5H_2O$	2.3
28	$C_{38}H_{53}N_5O_5S{\cdot}2.3H_2O$[d]	5[e]
29	$C_{42}H_{57}N_5O_5S{\cdot}0.5H_2O$	1.2
30	$C_{34}H_{53}N_3O_6S_2$	1
31	$C_{36}H_{56}N_4O_6S_2$	3–5
32	$C_{35}H_{55}N_5O_6S_2$	not required
33	$C_{34}H_{52}N_4O_6S_2{\cdot}0.25H_2O$	3–4
34	$C_{38}H_{55}N_5O_6S_2{\cdot}1.25H_2O$	1.8

[a] See Tables I and II for structures. [b] Analyses for C, H, N were ±0.4% of expected values (for formulae shown) unless otherwise noted. [c] % Methanol in chloroform. [d] H: calcd, 7.92; found, 7.33. [e] % Methanol in dichloromethane.

subject to first-pass hepatic elimination in the monkey. Subsequent experiments demonstrated that **32** was notably bioavailable in the dog (10 mg/kg id bioavailability = 33 ± 12%) and that, in each of the four species, similar bioavailabilities and plasma drug levels were observed following either id or oral dosing (10 mg/kg po bioavailability = 24 ± 9%, rat; 32 ± 14%, ferret; 8.1 ± 1.0%, monkey, 53 ± 8%, dog).[9] Furthermore, the excellent oral absorption in the dog allowed both efficacy and a dose–response relationship to be demonstrated despite its reduced potency in this species.[9]

Through a systematic examination of structure–absorption relationships outlined in this and the preceding paper, we have clearly demonstrated that the low bioavailability of previously reported peptide-based renin inhibitors can be augmented without sacrificing in vitro

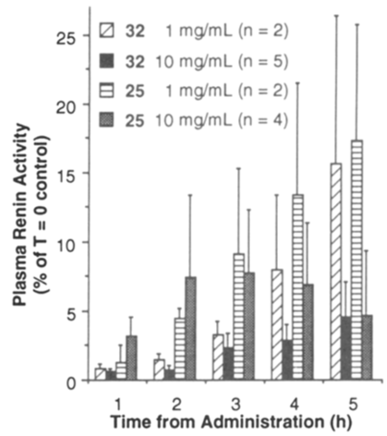


Figure 2. Plasma renin activity following intraduodenal administration of compounds **25** and **32** to anesthetized, salt-depleted cynomolgus monkeys.

potency or in vivo efficacy. Inhibitor **32** (A-72517) is currently being evaluated in the clinic. Preliminary data suggest this compound is absorbed into the systemic circulation of human subjects. Definitive studies assessing the oral bioavailability and antihypertensive activity of A-72517 are presently underway, and results will be reported in due course.

Experimental Section

Solvents and other reagents were reagent grade and were used without further purification unless otherwise noted. Final product solutions were dried over anhydrous Na_2SO_4 (unless otherwise noted) prior to evaporation on a rotary evaporator. Tetrahydrofuran was distilled from sodium benzophenone, and methylene chloride was distilled from CaH_2. NMR spectra were recorded at 300 MHz and are expressed in parts per million (ppm) downfield from tetramethylsilane as an internal standard. Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck) eluting with 5–10 psi air pressure. Thin-layer chromatography was done on silica gel plates (E. Merck), and components were visualized with ninhydrin or phosphomolybdic acid reagents. The following solvent systems were used: 50% ethyl acetate/50% hexane (I), 20% ethyl acetate/

80% hexane (II), 85% chloroform/15% methanol (III), 25% acetic acid/25% *n*-butanol/25% ethyl acetate/25% water (IV), ethyl acetate (V), 90% chloroform/10% methanol (VI), 90% ethyl acetate/10% methanol (VII). Chromatography of final compounds was performed using the solvent systems outlined in Table VII.

Benzyl (2*R*)-2-Benzyl-3-[[(2-pyridin-2-ylethyl)methylamino]carbonyl]propionate (4a). To acid 3[14] (500 mg, 1.7 mmol) in dichloromethane (7 mL) at −10 °C was added *N*-methylmorpholine (0.20 mL, 1.8 mmol) followed by isobutyl chloroformate (0.22 mL, 1.7 mmol). After 5 min 2-[2-(methylamino)ethyl]pyridine (0.23 mL, 1.7 mmol) was added and the mixture was stirred at −10 °C for 15 min and at ambient temperature for 2 h. The solvent was evaporated and the residue was taken up in ethyl acetate which was washed with saturated $NaHCO_3$, water, and brine and then was dried and evaporated. Chromatography of the residue with 1.3–1.5% methanol in chloroform afforded 0.57 g (81%) of an oil: TLC (V) R_f = 0.15, (VI) R_f = 0.57; ^{1}H NMR ($CDCl_3$) δ 8.57–8.43 (m, 1 H), 7.63–7.51 (m, 1 H), 7.38–6.98 (m, 12 H), 5.20–5.00 (4 d, total 2 H), 3.80–3.52 (m, 2 H), 3.38–3.18 (m, 1 H), 3.08–2.57 (envelope, 5 H), 2.86, 2.82 (2 s, total 3 H), 2.30, 2.18 (2 dd, total 1 H); MS *m/e* $(M + H)^+$ 417.

Benzyl (2*R*)-2-Benzyl-3-[[(2-pyridin-4-ylethyl)methylamino]carbonyl]propionate (4b). Prepared according to the method for **4a** in 77% yield following chromatography with 1.5% methanol in chloroform: TLC (III) R_f = 0.58; ^{1}H NMR ($CDCl_3$) δ 8.55–8.45 (m, 2 H), 7.39–6.95 (m, 12 H), 5.20–5.01 (4 d, total 2 H), 3.60–2.55 (envelope, 8 H), 2.89, 2.82 (2 s, total 3 H), 2.30, 2.14 (2 dd, total 1 H); MS *m/e* $(M + H)^+$ 417.

Benzyl (2*R*)-2-Benzyl-3-[[[2-(dimethylamino)ethyl]methylamino]carbonyl]propionate (4c). Prepared according to the method for **4a** in 80% yield following chromatography with 2% methanol in chloroform: TLC (III) R_f = 0.33; ^{1}H NMR ($CDCl_3$) δ 7.38–7.10 (m, 10 H), 5.15 (2 d, total 1 H), 5.05 (d, 1 H), 3.62–2.20 (envelope, 9 H), 2.93, 2.89 (2 s, total 3 H), 2.26, 2.18 (2 s, total 6 H); MS *m/e* $(M + H)^+$ 383.

Benzyl (2*R*)-2-Benzyl-3-[[[3-(dimethylamino)propyl]methylamino]carbonyl]propionate (4d). Prepared according to the method for **4a** in 81% yield following chromatography with 4–6% methanol in chloroform: TLC (III) R_f = 0.18; ^{1}H NMR ($CDCl_3$) δ 7.38–7.10 (m, 10 H), 5.15, 5.14 (2 d, total 1 H), 5.05, 5.04 (2 d, total 1 H), 3.62–2.15 (envelope, 9 H), 2.92, 2.86 (2 s, total 3 H), 2.27, 2.14 (2 s, total 6 H), 1.82–1.55 (m, 2 H); MS *m/e* $(M + H)^+$ 397.

Benzyl (2*R*)-2-Benzyl-3-[[(2-indol-3-ylethyl)methylamino]carbonyl]propionate (4e). Prepared according to the method for **4a** in 100% yield following chromatography with 35% ethyl acetate in hexane: TLC (V) R_f = 0.55; ^{1}H NMR ($CDCl_3$) δ 7.95 (br, 1 H), 7.61, 7.51 (2 d, total 1 H), 7.40–6.88 (envelope, 14 H), 5.20–4.98 (4 d, total 2 H), 3.72–2.00 (envelope, 9 H), 2.93, 2.81 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 455.

Benzyl (2*R*)-2-Benzyl-3-[[(2-morpholin-4-ylethyl)methylamino]carbonyl]propionate (4h). To compound **4f**[6] (1.02 g, 2.87 mmol) in CH_2Cl_2 (10 mL) at −78 °C was added triethylamine (0.66 mL, 4.7 mmol) and methanesulfonyl chloride (0.37 mL, 4.8 mmol). After 90 min, morpholine (0.75 mL, 8.6 mmol) was added and the mixture was warmed to ambient temperature and then was heated at reflux for 2 h. The solvent was evaporated, and the residue was suspended in ethyl acetate which was washed with saturated $NaHCO_3$ solution, water, and brine and then was dried and evaporated. Chromatography of the residue with 5% methanol in ethyl acetate afforded 0.95 g (78%) of an oil: TLC (III) R_f = 0.55, (VII) R_f = 0.21; ^{1}H NMR ($CDCl_3$) δ 7.37–7.10 (m, 10 H), 5.16, 5.15 (2 d, total 1 H), 5.04 (d, 1 H), 3.73–3.60 (m, 4 H), 3.60–2.30 (envelope, 13 H), 2.94, 2.90 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 425. Anal. ($C_{25}H_{32}N_2O_4 \cdot 0.75H_2O$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[[2-(4-methylpiperazin-1-yl)ethyl]methylamino]carbonyl]propionate (4i). Prepared according to the method for **4h** in 74% yield following chromatography with 1–3% methanol in chloroform: TLC (III) R_f = 0.24; ^{1}H NMR ($CDCl_3$) δ 7.53–7.10 (m, 10 H), 5.16, 5.15 (2 d, total 1 H), 5.04 (2 d, total 1 H), 3.60–2.30 (envelope, 17 H), 2.93, 2.89 (2 s, total 3 H), 2.28 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 438.

Benzyl (2*R*)-2-Benzyl-3-[[(2-pyrazol-1-ylethyl)methylamino]carbonyl]propionate (4j). Prepared according to the method for **4h** (22-h reaction) in 98% yield following chromatography with 0.5–1% methanol in chloroform: TLC (III) R_f = 0.71, (V) R_f = 0.24; ^{1}H NMR ($CDCl_3$) δ 7.51, 7.40 (2 d, total 1 H), 7.38–7.18 (m, 10 H), 7.17–7.08 (m, 1 H), 6.21, 6.13 (2 dd, total 1 H), 5.20–4.99 (4 d, total 2 H), 4.28–4.18 (m, 2 H), 3.80–2.20 (envelope, 7 H), 2.78, 2.52 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 406. Anal. ($C_{24}H_{27}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[(2-imidazol-1-ylethyl)methylamino]carbonyl]propionate (4k). Prepared according to the method for **4h** (18-h reaction) in 89% yield following chromatography with 1% methanol in chloroform: TLC (III) R_f = 0.51; ^{1}H NMR ($CDCl_3$) δ 7.42 (s, 1 H), 7.40–7.00 (m, 11 H), 6.89 (s, 1 H), 5.18 (d, 1 H), 5.09 (d, 1 H), 4.10–4.00 (m, 2 H), 3.67–3.55 (m, 1 H), 3.55–3.43 (m, 1 H), 3.38–3.27 (m, 1 H), 3.08 (dd, 1 H), 2.85–2.60 (m, 2 H), 2.59 (s, 3 H), 2.27 (dd, 1 H); MS *m/e* $(M + H)^+$ 406.

(2*R*)-2-Benzyl-3-[[(2-pyridin-2-ylethyl)methylamino]carbonyl]propionic Acid (5a). Compound **4a** (1.18 g, 2.83 mmol) and 10% Pd/C (0.54 g) in methanol (25 mL) were stirred under a hydrogen atmosphere for 2 h. The mixture was filtered and evaporated to afford 0.84 g (91%) of a foam: TLC (III) R_f = 0.32; ^{1}H NMR ($CDCl_3$) δ 8.52–8.47 (m, 1 H), 7.64–7.56 (m, 1 H), 7.34–6.98 (m, 7 H), 3.82–2.40 (envelope, 9 H), 2.89, 2.73 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 327. Anal. ($C_{19}H_{22}N_2O_3 \cdot 0.5H_2O$) C, N, H: calcd, 6.91; found, 6.43.

(2*R*)-2-Benzyl-3-[[(2-pyridin-4-ylethyl)methylamino]carbonyl]propionic Acid (5b). Prepared according to the method for **5a** in 100% yield: mp 88–92 °C; TLC (III) R_f = 0.29; ^{1}H NMR ($CDCl_3$) δ 8.55–8.43 (m, 2 H), 7.33–6.92 (m, 7 H), 3.75–2.25 (envelope, 9 H), 2.87, 2.75 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 327.

(2*R*)-2-Benzyl-3-[[[2-(dimethylamino)ethyl]methylamino]carbonyl]propionic Acid (5c). Prepared according to the method for **5a** in 96% yield: ^{1}H NMR ($CDCl_3$) δ 7.30–7.13 (m, 5 H), 3.65–2.20 (envelope, 9 H), 2.96, 2.88 (2 s, total 3 H), 2.46, 2.23 (2 s, total 6 H); MS *m/e* $(M + H)^+$ 293. Anal. ($C_{16}H_{24}N_2O_3 \cdot 0.9H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[[3-(dimethylamino)propyl]methylamino]carbonyl]propionic Acid (5d). Prepared according to the method for **5a** in 100% yield: ^{1}H NMR ($CDCl_3$) δ 7.32–7.13 (m, 5 H), 3.75–2.15 (envelope, 9 H), 2.95, 2.87 (2 s, total 3 H), 2.47, 2.31 (2 s, total 6 H), 1.88–1.65 (m, 2 H); MS *m/e* $(M + H)^+$ 307.

(2*R*)-2-Benzyl-3-[[(2-indol-3-ylethyl)methylamino]carbonyl]propionic Acid (5e). Prepared according to the method for **5a** in 84% yield: TLC (III) R_f = 0.44; ^{1}H NMR ($CDCl_3$) δ 8.49, 8.40 (2 br s, total 1 H), 7.56, 7.48 (2 d, total 1 H), 7.39, 7.35 (2 d, total 1 H), 7.30–6.82 (m, 8 H), 3.80–2.00 (envelope, 9 H), 2.91, 2.65 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 365.

(2*R*)-2-Benzyl-3-[[(2-morpholin-4-ylethyl)methylamino]carbonyl]propionic Acid (5h). Prepared according to the method for **5a** in 94% yield: TLC (III) R_f = 0.12; ^{1}H NMR ($CDCl_3$) δ 7.33–7.17 (m, 5 H), 3.70–3.60 (2 t, total 4 H), 3.60–2.15 (envelope, 13 H), 2.92, 2.86 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 335. Anal. ($C_{18}H_{26}N_2O_4 \cdot 0.5H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[[2-(4-methylpiperazin-1-yl)ethyl]methylamino]carbonyl]propionic Acid (5i). Prepared according to the method for **5a** in 80% yield: ^{1}H NMR ($CDCl_3$) δ 7.31–7.14 (m, 5 H), 3.70–2.20 (envelope, 17 H), 2.91, 2.88 (2 s, total 3 H), 2.33, 2.31 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 348.

(2*R*)-2-Benzyl-3-[[(2-pyrazol-1-ylethyl)methylamino]carbonyl]propionic Acid (5j). Prepared according to the method for **5a** in 98% yield: TLC (III) R_f = 0.34; ^{1}H NMR ($CDCl_3$) δ 7.51, 7.45 (2 d, total 1 H), 7.40–7.15 (m, 6 H), 6.22, 6.19 (2 dd, total 1 H), 4.38–4.10 (m, 2 H), 3.85–2.15 (envelope, 7 H), 2.82, 2.49 (2 s, total 3 H); MS *m/e* $(M + H)^+$ 316. Anal. ($C_{17}H_{21}N_3O_3 \cdot 0.5H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[(2-imidazol-1-ylethyl)methylamino]carbonyl]propionic Acid (5k). Prepared according to the method for **5a** in 96% yield: TLC (III) R_f = 0.08; ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1 H), 7.32–7.15 (m, 5 H), 7.14 (s, 1 H), 6.93 (s, 1 H), 4.36–4.23 (m, 1 H), 4.09 (dt, 1 H), 3.99 (dt, 1 H), 3.40–2.60 (envelope, 5 H), 2.60 (major), 2.37 (2 s, total 3 H), 2.10 (dd, 1 H); MS *m/e* $(M + H)^+$ 316. Anal. ($C_{17}H_{21}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Benzyl (2*R*)-2-Benzyl-3-[[(2-pyridin-2-ylethyl)amino]carbonyl]propionate (6a). To acid 3[14] (500 mg, 1.68 mmol) in dimethylformamide (18 mL) was added 2-(2-aminoethyl)pyridine (226 mg, 1.84 mmol), 1-hydroxybenzotriazole (726 mg, 5.37 mmol), and *N*-methylmorpholine (0.20 mL, 1.8 mmol). The mixture was cooled to −23 °C and treated with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC, 386 mg, 2.01 mmol). After stirring 2 h at −23 °C and 15 h at ambient temperature, the mixture was poured into saturated $NaHCO_3$ solution and was extracted into ethyl acetate which was washed with water and brine and then was dried and evaporated. Chromatography of the residue with ethyl acetate afforded 566 mg (84%) of an oil: TLC (V) R_f = 0.24; ^{1}H NMR ($CDCl_3$) δ 8.51 (dt, 1 H), 7.60 (dt, 1 H), 7.37–7.05 (m, 12 H), 6.43 (br, 1 H), 5.09 (d, 1 H), 5.02 (d, 1 H), 3.71–3.53 (m, 2 H), 3.33–3.22 (m, 1 H), 2.98 (dd, 1 H), 2.98–2.85 (m, 2 H), 2.81 (dd, 1 H), 2.50 (dd, 1 H), 2.28 (dd, 1 H); MS *m/e* (M + H)$^+$ 403. Anal. ($C_{25}H_{26}N_2O_3{\cdot}0.5H_2O$) C, H, N.

(2*R*)-2-Benzyl-3-[[(2-pyridin-2-ylethyl)amino]carbonyl]propionic Acid (6b). Prepared according to the method for **5a** in 87% yield: ^{1}H NMR ($CDCl_3$) δ 8.43 (dt, 1 H), 8.20 (br s, 1 H), 7.62 (dt, 1 H), 7.35–7.05 (m, 7 H), 3.60–3.50 (m, 2 H), 3.50–2.20 (envelope, 7 H).

Methyl 2-Benzyl-3-[[(2-pyridin-2-ylethyl)methylamino]sulfonyl]propionate (6c). To methyl 2-benzyl-3-(chlorosulfonyl)propionate[5] (149 mg, 0.538 mmol) in dichloromethane (5 mL) at −10 °C was added 2-[2-(methylamino)ethyl]pyridine (0.078 mL, 0.56 mmol) and triethylamine (0.090 mL, 0.64 mmol). After 30 min the solvent was evaporated and the residue was taken up in ethyl acetate which was washed with saturated $NaHCO_3$ solution, water, and brine and then was dried and evaporated. Chromatography of the residue with 66–100% ethyl acetate in hexane afforded 146 mg (72%) of an oil: TLC (V) R_f = 0.30; ^{1}H NMR ($CDCl_3$) δ 8.52 (dt, 1 H), 7.61 (dt, 1 H), 7.33–7.10 (m, 7 H), 3.66 (s, 3 H), 3.58–3.47 (m, 2 H), 3.39 (dd, 1 H), 3.26–3.15 (m, 1 H), 3.08–2.98 (m, 3 H), 2.91 (dd, 1 H), 2.86 (dd, 1 H), 2.75 (s, 3 H); MS *m/e* (M + H)$^+$ 377.

2-Benzyl-3-[[(2-pyridin-2-ylethyl)methylamino]sulfonyl]propionic Acid (6d). To compound **6c** (143 mg, 0.380 mmol) in dioxane (2 mL) at 0 °C was added LiOH monohydrate (30.0 mg, 0.715 mmol) in water (0.5 mL). After 90 min the reaction was quenched with 2.0 M HCl (0.36 mL, 0.72 mmol) and concentrated. The mixture was taken up in brine and extracted into 25% 2-propanol in chloroform which was dried and evaporated to afford 118 mg (85%) of a foam: TLC (III) R_f = 0.30; MS *m/e* (M + H)$^+$ 363.

1-(Benzyloxycarbonyl)-3-hydroxyazetidine (7a). 1-(Diphenylmethyl)-3-hydroxyazetidine[15] (1.00 g, 4.18 mmol) and 10% Pd/C (0.50 g) in methanol (10 mL) were stirred under a hydrogen atmosphere for 20 h. The mixture was filtered and evaporated, and the residue was dissolved in methylene chloride and cooled to 0 °C. After addition of triethylamine (0.64 mL, 4.6 mmol) and benzyl chloroformate (0.60 mL, 4.2 mmol), the reaction was stirred at room temperature for 90 min. The mixture was evaporated, taken up in ethyl acetate, washed with 2 M HCl, saturated $NaHCO_3$ solution and brine, and then was dried and evaporated. Chromatography of the residue with 50–60% ethyl acetate in hexane afforded 0.38 g (43%) of a colorless oil: TLC (I) R_f = 0.13; ^{1}H NMR ($CDCl_3$) δ 7.39–7.29 (m, 5 H), 5.10 (s, 2 H), 4.70–4.59 (m, 1 H), 4.26 (dd, 1 H), 4.23 (dd, 1 H), 3.91 (dd, 1 H), 3.88 (dd, 1 H), 2.15 (d, 1 H); MS *m/e* (M + H)$^+$ 208.

3-(Acetylthio)-1-(benzyloxycarbonyl)azetidine (8a). To triphenylphosphine (4.40 g, 16.8 mmol) in THF (25 mL) at −78 °C was added diethyl azodicarboxylate (2.60 mL, 16.5 mmol) in THF (15 mL). After 7 min thiolacetic acid (1.25 mL, 17.5 mmol) in THF (15 mL) was added followed by, after 7 min, **7a** (2.789 g, 13.46 mmol) in THF (25 mL). The reaction was stirred at −78 °C for 1 h and then at ambient temperature for 20 h. The mixture was evaporated and chromatographed with 20% ethyl acetate in hexane affording 3.250 g (91%) of a white solid: mp 94.5–95.5 °C; TLC (II) R_f = 0.17; ^{1}H NMR ($CDCl_3$) δ 7.41–7.28 (m, 5 H), 5.09 (s, 2 H), 4.48 (d, 1 H), 4.44 (d, 1 H), 4.26–4.15 (m, 1 H), 3.92 (d, 1 H), 3.89 (d, 1 H), 2.34 (s, 3 H); MS *m/e* (M + H)$^+$ 266. Anal. ($C_{13}H_{15}NO_3S$) C, H, N.

4-(Acetylthio)-1-(benzyloxycarbonyl)piperidine (8b). Prepared according to the method for **8a** in 74% yield following chromatography with 10–30% ethyl acetate in hexane: TLC (II) R_f = 0.27; ^{1}H NMR ($CDCl_3$) δ 7.40–7.29 (m, 5 H), 5.12 (s, 2 H), 4.00–3.87 (br m, 2 H), 3.70–3.57 (m, 1 H), 3.22–3.08 (m, 2 H), 2.32 (s, 3 H), 1.99–1.85 (br m, 2 H), 1.65–1.48 (br m, 2 H); MS *m/e* (M + H)$^+$ 294. Anal. ($C_{15}H_{19}NO_3S{\cdot}0.75H_2O$) C, N, H: calcd, 6.73; found 6.14.

Methyl 2-Benzylacrylate. 2-Benzylacrylic acid[16] (12.00 g, 74.00 mmol) in methanol (240 mL) was treated with $BF_3{\cdot}Et_2O$ (24 mL, 200 mmol). The mixture was heated at reflux for 14 h, and then the solvent was evaporated. The residue was dissolved in ether, and the mixture was washed with water (5×, until the washes tested neutral), saturated $NaHCO_3$ solution, and brine, and then was dried and evaporated to afford 12.25 g (94%) of a mobile oil: TLC (II) R_f = 0.54; ^{1}H NMR ($CDCl_3$) δ 7.34–7.17 (m, 5 H), 6.23 (dd, 1 H), 5.47 (dd, 1 H), 3.73 (s, 3 H), 3.63 (s, 2 H); MS *m/e* (M + H)$^+$ 177.

Methyl 2-Benzyl-3-[[1-(benzyloxycarbonyl)azetidin-3-yl]thio]propionate (9a). Sodium bis(trimethylsilyl)amide (0.75 mL, 0.75 mmol, 1.0 M in THF) was added to methanol (3 mL), and this solution was added to **8a** (205.0 mg, 0.773 mmol) in methanol (3 mL). After 45 min, methyl 2-benzylacrylate (150.0 mg, 0.851 mmol) in methanol (2 mL) was added. After 45 min the reaction was quenched with 2 M HCl (0.38 mL, 0.76 mmol), evaporated, and chromatographed with 20% ethyl acetate in hexane to afford 280.6 mg (91%) of a colorless oil: TLC (II) R_f = 0.13; ^{1}H NMR ($CDCl_3$) δ 7.40–7.10 (m, 10 H), 5.08 (s, 2 H), 4.29 (dd, 1 H), 4.27 (dd, 1 H), 3.84 (dd, 1 H), 3.81 (dd, 1 H), 3.66 (s, 3 H), 3.63–3.53 (m, 1 H), 3.00 (dd, 1 H), 2.90–2.72 (m, 3 H), 2.63 (dd 1 H); MS *m/e* (M + H)$^+$ 400.

Methyl 2-Benzyl-3-[[1-(benzyloxycarbonyl)piperidin-4-yl]thio]propionate (9b). Prepared according to the method for **9a** in 70% yield following chromatography with 15–20% ethyl acetate in hexane: TLC (II) R_f = 0.21; ^{1}H NMR ($CDCl_3$) δ 7.38–7.12 (m, 10 H), 5.12 (s, 2 H), 4.17–3.93 (br m, 2 H), 3.65 (s, 3 H), 3.05–2.11 (envelope, 8 H), 1.93–1.77 (m, 2 H), 1.53–1.38 (m, 2 H); MS *m/e* (M + H)$^+$ 428.

Methyl 2-Benzyl-3-[[1-(benzyloxycarbonyl)azetidin-3-yl]sulfonyl]propionate (10a). Compound **9a** (276.0 mg, 0.691 mmol) in methanol (6 mL) and water (5 mL) was treated with oxone (1.27 g, 2.07 mmol). After 14 h the mixture was diluted with methanol, filtered, and concentrated to about 5 mL. After neutralization with solid K_2CO_3, the mixture was extracted into ethyl acetate which was washed with saturated $NaHCO_3$ solution, water, and brine and then was dried and evaporated to afford 295.9 mg (99%) of a colorless oil: TLC (I) R_f = 0.18; ^{1}H NMR ($CDCl_3$) δ 7.40–7.10 (m, 10 H), 5.09 (s, 2 H), 4.35–4.22 (m, 2 H), 4.25 (dd, 1 H), 4.12 (dd, 1 H), 3.92–3.80 (m, 1 H), 3.73 (s, 3 H), 3.44 (dd, 1 H), 3.38–3.27 (m, 1 H), 3.14 (dd, 1 H), 2.92 (dd, 1 H), 2.87 (dd, 1 H); MS *m/e* (M + H)$^+$ 432.

Methyl 2-Benzyl-3-[[1-(benzyloxycarbonyl)piperidin-4-yl]sulfonyl]propionate (10b). Prepared according to the method for **10a** in 97% yield: TLC (I) R_f = 0.28; ^{1}H NMR ($CDCl_3$) δ 7.40–7.12 (m, 10 H), 5.12 (s, 2 H), 4.40–4.22 (br m, 2 H), 3.71 (s, 3 H), 3.47 (dd, 1 H), 3.40–3.29 (m, 1 H), 3.13 (dd, 1 H), 2.97–2.65 (m, 5 H), 2.10–1.60 (envelope, 4 H); MS *m/e* (M + H)$^+$ 460.

Methyl 2-Benzyl-3-[(1-methylazetidin-3-yl)sulfonyl]propionate (11a). Compound **10a** (270.8 mg, 0.628 mmol) and 10% Pd/C (150 mg) in methanol (6 mL) were treated with aqueous formaldehyde (0.25 mL, 3.3 mmol, 37 wt %) and stirred under a hydrogen atmosphere for 20 h. The mixture was filtered and evaporated to afford 194.3 mg (99%) of a colorless oil: TLC (III) R_f = 0.60; ^{1}H NMR ($CDCl_3$) δ 7.37–7.12 (m, 5 H), 3.77 (dd, 1 H), 3.71 (s, 3 H), 3.56 (dd, 1 H), 3.50–3.38 (m, 4 H), 3.36–3.26 (m, 1 H), 3.12 (dd, 1 H), 2.96 (dd, 1 H), 2.88 (dd, 1 H), 2.32 (s, 3 H); MS *m/e* (M + H)$^+$ 312.

Methyl 2-Benzyl-3-[(1-methylpiperidin-4-yl)sulfonyl]propionate (11b). Prepared according to the method for **11a** in 91% yield: mp 97–98 °C; TLC (I) R_f = 0.56; ^{1}H NMR ($CDCl_3$) δ 7.37–7.13 (m, 5 H), 3.70 (s, 3 H), 3.45 (dd, 1 H), 3.43–3.30 (m, 2 H), 3.12 (dd, 1 H), 3.02–2.87 (m, 5 H), 2.78–2.65 (m, 1 H), 2.27 (s, 3 H), 2.08–1.73 (m, 4 H); MS *m/e* (M + H)$^+$ 340.

2-Benzyl-3-[(1-methylazetidin-3-yl)sulfonyl]propionic Acid Hydrochloric Acid Salt (12a). Compound **11a** (2.12 g, 6.81 mmol) in 2 M HCl (12 mL) was stirred at 75 °C for 20 h. The mixture was washed with ether, evaporated with water chasers, and lyophilized to afford 2.08 g (91%) of a white foam: TLC (IV) R_f = 0.50; ^{1}H NMR (CD_3OD) δ 7.35–7.17 (m, 5 H), 3.68–3.58 (m, 2 H), 2.95 (s, 3 H); MS m/e (M + H − HCl)$^+$ 298. Anal. ($C_{14}H_{20}NO_4SCl\cdot1.25H_2O$) C, N, H: calcd, 6.36; found, 5.78.

2-Benzyl-3-[(1-methylpiperidin-4-yl)sulfonyl]propionic Acid Hydrochloric Acid Salt (12b). Prepared according to the method for **12a** in 98% yield: TLC (IV) R_f = 0.46; ^{1}H NMR (CD_3OD) δ 7.36–7.20 (m, 5 H), 3.70–2.90 (envelope, 10 H), 2.88 (s, 3 H), 2.40–2.30 (m, 1 H), 2.22–2.10 (m, 1 H), 2.04–1.85 (m, 2 H); MS m/e (M + H − HCl)$^+$ 326.

(2S)-*N*-[2-Benzyl-3-[(1-methylpiperazin-4-yl)sulfonyl]propionyl]-3-thiazol-4-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane, A-72517 (32). To acid **14**[5] (1.000 g, 3.064 mmol), H-3-thiazol-4-yl-L-alanine amide of (2*S*,3*R*,4*S*)-2-amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane[17] (1.110 g, 2.792 mmol), and 1-hydroxybenzotriazole (1.022 g, 7.563 mmol) in dimethylformamide (20 mL) was added *N*-methylmorpholine (0.35 mL, 3.2 mmol). The mixture was cooled to −23 °C and treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC, 0.760 g, 3.96 mmol). After 2 h at −23 °C and 14 h at ambient temperature, the reaction was poured into saturated $NaHCO_3$ solution (100 mL) and extracted into ethyl acetate (2 × 50 mL) which was washed with water (2 × 50 mL) and brine (50 mL) and then was dried over Na_2SO_4 and evaporated to afford 1.94 g. Recrystallization from ethanol (15 mL)/hexane (90 mL) afforded 1.559 (79%) of a white solid: mp 169–170 °C; TLC (VI) R_f = 0.40; ^{1}H NMR ($CDCl_3$) δ 8.73 (d, 1 H), 7.43 (d, 1 H), 7.37–7.16 (m, 6 H), 6.23 (d, 1 H), 4.63 (dd, 1 H), 4.29–4.17 (m, 2 H), 3.47–3.34 (m, 2 H), 3.31–2.81 (m, 11 H), 2.49 (d, 1 H), 2.47–2.38 (m, 4 H), 2.30 (s, 3 H), 1.98–1.81 (m, 1 H), 1.73–0.70 (envelope, 15 H), 0.95 (s, 3 H), 0.87 (s, 3 H); IR 1655, 1505 cm^{-1}; MS m/e (M + H)$^+$ 706. Anal. ($C_{35}H_{55}N_5O_6S_2$) C, H, N.

(2*S*)-2-Benzyl-3-[[(1-methylpiperazin-4-yl)sulfonyl]propionyl]-3-thiazol-4-yl-L-alanine Amide of (2*S*,3*R*,4*S*)-2-Amino-1-cyclohexyl-3,4-dihydroxy-6-methylheptane Monohydrochloride (32b). Acetyl chloride (216.8 mg, 2.762 mmol) was taken up in ethanol (6 mL) and allowed to stand for 2 h. This mixture was added to a solution of **32** (1.9898 g, 2.819 mmol) in ethanol (50 mL) at 10–20 °C. After 15 min, the solvent was evaporated and the residue was triturated and stirred with ether to afford 1.9305 g (92%) of a fine white powder. Anal. ($C_{35}H_{56}N_5O_6S_2Cl\cdot0.5H_2O$) C, H, N, Cl, S: calcd, 8.53; found, 8.17.

Salts **32a** and **32c–i** were prepared similarly.

Physicochemical Properties. log *P* values and aqueous solubilities were determined as described previously.[6]

In Vitro and in Vivo Data. Potencies against human plasma renin[12] and monkey plasma renin[6] were determined as described previously. Compounds for id testing were formulated as follows: the test compound was dissolved (40 mg/mL) in ethanol, 110 mol % of HCl was added, and the solution was diluted with an aqueous solution of hydroxypropyl methylcellulose to a final ethanol concentration of 25% (10 mg/mL in the test compound). Compounds for iv testing were formulated as follows: the test compound was dissolved (30 mg/mL) in ethanol, 110 mol % of HCl was added, and the solution was diluted with an aqueous solution of 5% dextrose to a final ethanol concentration of 10% (3 mg/mL in the test compound). Dosing via the intraduodenal or intravenous routes was performed as described previously.[12] Plasma samples for determination of drug levels were drawn at the following time points: iv rat, 3, 10, 30, 60, 90, 120 min; id rat, 15, 30, 60, 120 min; iv ferret 1, 5, 10, 20, 30, 60, 120 min; id ferret 5, 15, 30, 60, 120, 180 min. Plasma drug levels were determined via a renin inhibition assay[7] or by HPLC,[1] and selected plasma samples from the monkey were assayed for plasma renin activity.[18]

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