93798-91-5; **11k**, 102131-88-4; **11k**·2HCl, 93798-92-6; **11l**, 93799-54-3; **11l**·2HCl, 93798-90-4; **12** (R^1 = C_6H_5, R^2 = H), 100-52-7; **12** (R^1 = 4-ClC_6H_4, R^2 = H), 104-88-1; **12** (R^1 = 4-$CF_3C_6H_4$, R^2 = H), 455-19-6; **12** (R^1 = 2-$CH_3OC_6H_4$, R^2 = H), 133-02-4; **12** (R^1 = 2-HOC_6H_4, R^2 = H), 90-02-8; **12** (R^1 = 3-HOC_6H_4, R^2 = H), 100-83-4; **12** (R^1 = 4-HOC_6H_4, R^2 = H), 123-08-0; **12** (R^1 = 3-$C_6H_5OC_6H_4$, R^2 = H), 39515-51-0; **12** (R^1 = 4-$C_6H_5OC_6H_4$, R^2 = H), 67-36-7; **12** (R^1 = 2-$C_6H_5CH_2OC_6H_4$, R^2 = H), 5896-17-3; **12** (R^1 = 3-$C_6H_5CH_2OC_6H_4$, R^2 = H), 1700-37-4; **12** (R^1 = 4-$C_6H_5CH_2OC_6H_4$, R^2 = H), 4397-53-9; **12** (R^1 = 2-CH_2=$CHCH_2OC_6H_4$, R^2 = H), 28752-82-1; **12** (R^1 = 3-CH_2=$CHCH_2OC_6H_4$, R^2 = H), 40359-32-8; **12** (R^1 = 4-CH_2=$CHCH_2OC_6H_4$, R^2 = H), 40663-68-1; **12** (R^1 = 2-$H_2NCOCH_2OC_6H_4$, R^2 = H), 24590-06-5; **12** (R^1 = 4-$CH_3CONHC_6H_4$, R^2 = H), 122-85-0; **12** (R^1 = 2,4-$(OCH_3)_2C_6H_3$, R^2 = H), 613-45-6; **12** (R^1 = 3,4-$(OCH_3)_2C_6H_3$, R^2 = H), 120-14-9; **12** (R^1 = 2-HO-3-$CH_3OC_6H_3$, R^2 = H), 148-53-8; **12** (R^1 = 3-OCH_3-4-OHC_6H_3, R^2 = H), 121-33-5; **12** (R^1 = 2-CH_2=$CHCH_2O$-3-$CH_3OC_6H_3$, R^2 = H), 23343-06-8; **12** (R^1 = 3-CH_3O-4-CH_2=$CHCH_2OC_6H_3$, R^2 = H), 22280-95-1; **12** (R^1 = 2-$C_6H_5CH_2O$-3-$CH_3OC_6H_3$, R^2 = H), 2011-06-5; **12** (R^1 = 2-CH_3O-4-$C_6H_5CH_2OC_6H_3$, R^2 = H), 58026-14-5; **12** (R^1 = 2-Et_2NCOCH_2O-3-$CH_3OC_6H_3$, R^2 = H), 102131-57-7; **12** (R^1 = 3,4,5-$(CH_3O)_3C_6H_2$, R^2 = H), 86-81-7; **12** (R^1 = 2-pyrrolyl, R^2 = H), 1003-29-8; **12** (R^1 = 2-pyridyl, R^2 = H), 1121-60-4; **12** (R^1 = 4-pyridyl, R^2 = H), 872-85-5; **12** (R^1 = 2-furyl, R^2 = H), 98-01-1; **12** (R^1 = 2-thienyl, R^2 = H), 98-03-3; **12** (R^1 = 2-ClC_6H_4, R^2 = H), 89-98-5; **12** (R^1 = 3-thienyl, R^2 = H), 498-62-4; **12** (R^1 = CH_3, R^2 = 2-thienyl), 88-15-3; **13a**, 87-62-7; **13b**, 100-46-9; **13c**, 103-67-3; **13d**, 104-86-9; **13e**, 2393-23-9; **13f**, 700-63-0; **13g**, 91-00-9; **13h**, 120-20-7; **13i**, 3731-53-1; **13j**, 31828-71-4; **13k**, 18865-38-8; **13l**, 41708-72-9; **13m**, 102089-62-3; **14a**, 93969-05-2; **14a**·HCl, 93969-11-0; **14b**, 102132-11-6; **14b**·HCl, 93968-90-2; **14c**, 102132-12-7; **14c**·HCl, 93968-93-5; **14d**, 102132-13-8; **14d**·HCl, 93968-91-3; **14e**, 93968-92-4; **14e**·HCl, 97813-47-3; **14f**, 93968-94-6; **14f**·HCl, 93969-07-4; **14g**, 93968-95-7; **14g**·HCl, 93969-08-5; **14h**, 102132-14-9; **14h**·HCl, 93968-96-8; **14i**, 102132-15-0; **14i**·2HCl, 93968-97-9; **14j**, 93968-89-9; **14j**·HCl, 93969-09-6; **14k**, 93969-03-0; **14k**·HCl, 94528-65-1; **14l**, 93969-04-1; **14l**·HCl, 97799-75-2; **14m**, 102132-16-1; **14m**·HCl, 102132-17-2; **16a**, 102132-18-3; **16a**·2HCl, 102132-21-8; **16b**, 102132-19-4; **16b**·2HCl, 102132-22-9; **16c**, 102132-20-7; **16c**·2HCl, 102132-23-0; **17** (Z = Z^1), 85-42-7; **17** (Z = Z^2), 85-43-8; **17** (Z = Z^3), 85-44-9; **18a**, 93799-07-6; **18b**, 93799-08-7; **18c**, 93799-09-8; **18d**, 93799-10-1; **18e**, 93799-11-2; **18f**, 93799-12-3; **19a**, 102132-24-1; **19a**·HCl, 93798-99-3; **19b**, 102132-25-2; **19b**·HCl, 93799-00-9; **19c**, 102132-26-3; **19c**·HCl, 93799-01-0; **19d**, 93799-35-0; **19d**·HCl, 93799-02-1; **19e**, 93799-36-1; **19e**·HCl, 93799-03-2; **19f**, 93799-37-2; **19f**·HCl, 93799-04-3; **19g**, 93799-38-3; **19g**·HCl, 93799-05-4; **21** (R^3 = Me), 525-76-8; **21** (R^3 = Et), 2916-09-8; **22a**, 102132-27-4; **22a**·2HCl, 93799-13-4; **22b**, 93799-39-4; **22b**·2HCl, 93799-14-5; **22c**, 93799-40-7; **22c**·HCl, 93799-15-6; **22d**, 93799-41-8; **22d**·2HCl, 93799-16-7; **22e**, 102132-28-5; **22e**·2HCl, 93799-18-9; **23a**, 93969-06-3; **23a**·HCl, 93968-98-0; **23b**, 102132-29-6; **23b**·HCl, 102132-36-5; **23c**, 102132-30-9; **23c**·HCl, 102132-37-6; **23d**, 102132-31-0; **23d**·2HCl, 102132-38-7; **23e**, 93799-32-7; **23e**·2HCl, 93798-96-0; **23f**, 102132-32-1; **23f**·2HCl, 102132-39-8; **23g**, 102132-33-2; **23g**·2HCl, 102132-40-1; **23h**, 93799-31-6; **23h**·2HCl, 93798-94-8; **23i**, 93799-34-9; **23i**·2HCl, 93798-98-2; **23j**, 102132-34-3; **23**·2HCl, 102132-41-2; **23k**, 93844-07-6; **23k**·HCl, 93799-06-5; **23l**, 102132-35-4; **23l**·2HCl, 93823-97-3; **23m**, 93799-42-9; **23m**·2HCl, 93799-17-8; **24a**, 102132-42-3; **24b**, 102132-43-4; **24c**, 102132-44-5; **24d**, 102132-45-6; **25a**, 102132-46-7; **25b**, 102132-47-8; **26**, 37558-29-5; **27**, 58537-73-8; **28**, 31084-42-1; **29a**, 102132-48-9; **29b**, 102132-49-0; **29c**, 102132-50-3; **29d**, 102132-51-4; **30a**, 102132-52-5; **30b**, 102132-53-6; $H_2NCH_2CH_2NH_2$, 107-15-3; $H_2NCH_2CH_2CH_2NH_2$, 109-76-2; $H_2NCH_2CH_2CH_2CH_2NH_2$, 110-60-1; $H_2NCH_2CH(OH)CH_2NH_2$, 616-29-5; $H_2NCH_2C(CH_3)_2CH_2NH_2$, 7328-91-8; $H_2NCH_2CH(CH_3)NH_2$, 78-90-0; $H_2NCH_2C(CH_3)_2NH_2$, 811-93-8; PCPOH, 87-86-5; EtOCOOH, 541-41-3; 4-pyridinecarbonyl chloride, 14254-57-0; 2-thiophenecarbonyl chloride, 5271-67-0; phtalimide, 85-41-6.

Pepstatin Analogues as Novel Renin Inhibitors

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Pepstatin analogues corresponding to the general formula A-X-Y-Sta-Ala-Sta-R were synthesized in solution phase. Various changes in the nature of the A, X, and Y groups were made to improve the inhibitory potency against human plasma renin activity. The results were interpreted by use of the active-site model based on the sequence of human angiotensinogen. The *tert*-butyloxycarbonyl group and the isovaleryl group were found to be the most effective acyl groups (A). The analogues having a Phe residue in place of Val[1] (X) and His or an amino acid with an aliphatic side chain such as norleucine or norvaline in the Y position showed the highest inhibition of human plasma renin activity with IC_{50} values of about 10^{-8} M. Esterification or amidification of the carboxyl group of the C-terminal statine did not change the inhibitory potency. The selectivity for rat, dog, pig, and monkey plasma renin of the most interesting compounds was studied.

Renin is an aspartyl proteinase that cleaves the circulating protein angiotensinogen releasing angiotensin I. This decapeptide is in turn the substrate for the converting enzyme, a zinc metallocarboxydipeptidase, which generates the pressor octapeptide angiotensin II. Blockage of the liberation of angiotensin II by inhibition of the converting enzyme (ACE) has led to the development of powerful antihypertensive agents such as captopril[1,2] and enalapril maleate.[3] Substances inhibiting the preceding step, i.e., cleavage of angiotensinogen by renin should also play an important role in the control of hypertension.[4]

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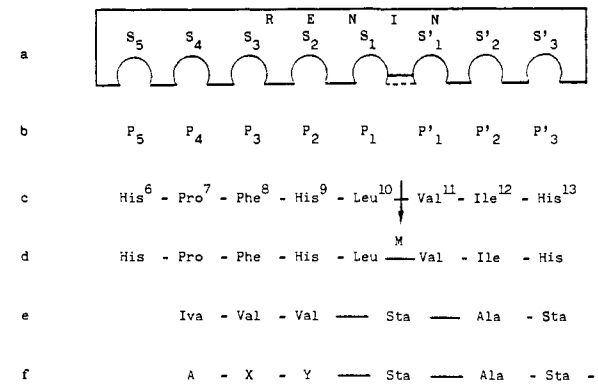


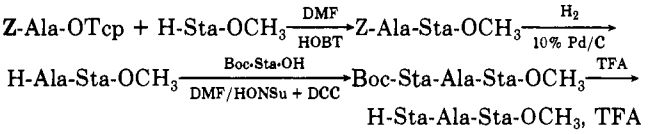
Figure 1. Putative interactions of substrate and inhibitors with the active-site model of human renin. [a,b]S_1, S_2, etc. are the binding subsites for the amino acid residues P_1, P_2, etc. to the left of the scissile amide bond, and S'_1, S'_2, etc. are binding subsites for the amino acid residues P'_1, P'_2, etc. according to Schechter and Berger (Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 898). [c]*Sequence* of the 6–13 fragment of human angiotensinogen. The arrow indicates the cleavage site by renin. [d]*Renin* inhibitors with M = CH_2NH or $CHOHCH_2$. [e]*Sequence* of the natural inhibitor pepstatin. [f]*Sequence* of the pepstatin derivatives studied in this paper.

Two different approaches have been followed by several investigators in the design of renin inhibitors. The first approach is based on chemical modification of the natural substrate of the enzyme either by replacement of some constitutive amino acids or by introduction of peptidase-resistant groups at the level of the peptide bond to be cleaved. Thus, competitive inhibitors of porcine renin have been developed by replacement of the scissile Leu^{10}-Leu^{11} bond in the 5–13 C-terminal fragment of porcine angiotensinogen by the Phe^{10}-Phe^{11} unit.[5,6] Following the strategy of the active-site model used in the design of ACE inhibitors[1,3] or enkephalin-degrading enzyme inhibitors,[7,8] Szelke et al.[9] have replaced the Leu^{10}-Leu^{11} peptide bond with the reduced amino isostere ($-CH_2NH-$) in the 6–13 fragments of pig or horse angiotensinogens, leading therefore to modified peptides classified as transition-state enzyme inhibitors.[10] Recently, more potent inhibitors of human renin have been obtained by introduction of the hydroxy isostere linkage ($-CHOHCH_2-$) in place of the scissile Leu^{10}-Val^{11} amide bond in the human angiotensinogen sequence His-Pro-Phe-His-Leu-Val-Ile-His.[11,12] According to the mechanism of action of peptidases, this latter series of inhibitors can be considered as transition-state analogues.[13]

An alternative approach in the design of renin inhibitors is based on appropriate modification of natural inhibitors of aspartyl proteinases such as pepstatin,[14] Iva-Val-Val-Sta-Ala-Sta (Iva = isovaleryl group), a highly potent pepsin inhibitor[13] containing two unusual amino acids [Sta = (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid]. Extensive studies on the inhibition of aspartyl proteinases by pepstatin analogues modified at the level of the central Sta residue[15] and X-ray studies on the complex formed between aspartyl proteinases and pepstatin[16] or a shorter fragment[17] have shown that the 3(*S*)-hydroxyl group of statine very likely mimics the tetrahedral intermediate for amide-bond hydrolysis[13] and that the statine moiety can be considered as an analogue of a dipeptide.[13,18,19] According, Boger et al.[19] introduced a statine moiety in place of the scissile Leu^{10}-Leu^{11} or Leu^{10}-Val^{11} dipeptide units in the 6–13 fragments of pig or human angiotensinogens and obtained potent inhibitors of renin.

We report herein the synthesis of a new series of inhibitors of renin based on structural analogy of pepstatin. We hypothesized that the dipeptide unit isovaleryl-Val-Val of pepstatin is probably poorly recognized by renin and decided to replace the Val-Val dipeptide by two amino acids having a better affinity for the subsites S_3 and S_2 in the catalytic center of renin. As a model we used the sequence Phe-His, which precedes the scissile Leu-Val in the human renin substrate fragment His-Pro-Phe-His-

Scheme I[a]



[a] Z = benzyloxycarbonyl group, OTcp = 2,4,5-trichlorophenoxy group, DMF = dimethylformamide as solvent, HOBT = *N*-hydroxybenzotriazol, Boc = *tert*-butyloxycarbonyl, HONSu = *N*-hydroxysuccinimide, ONSu = succinimidooxy group, DCC = dicyclohexylcarbodiimide, TFA = trifluoroacetic acid, BOP = benzotriazolyloxytris(dimethylamino)phosphonium hexafluorophosphate, DIPEA = diisopropylethylamine.

Scheme II

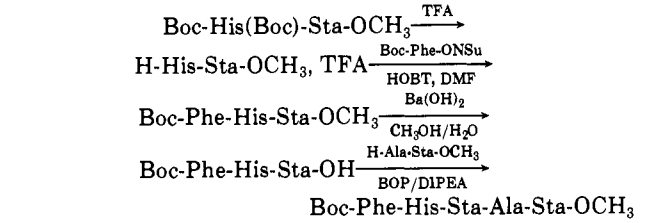


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Table I. Chemical Characteristics and IC_{50} Values for the Pepstatin Analogues Having the General Formula A-X-Y-Sta-Ala-Sta-R

no.	A	X	Y	R	formula	M	strategy	TLC,[a] R_f	aa anal.[b]	purity[c]	human plasma renin[d]
pepstatin	Iva	Val	Val	OH							1.4×10^{-5}
1	Z	Phe	Phe	OH	$C_{45}H_{61}O_{10}N_5$	832	A	0.5[β]	2.10 (2), 1.98 (2), 0.98 (1)	96	10^{-6}
2	Z	Phe	Phe	OCH_3	$C_{46}H_{63}O_{10}N_5$	846	A	0.35[α]	2.10 (2), 1.98 (2), 0.98 (1)	98	3.5×10^{-6}
3	Z	Phe	D-Phe	OCH_3	$C_{46}H_{63}O_{10}N_5$	846	A	0.31[α]	2.00 (2), 2.14 (2), 0.97 (1)	96	$>10^{-5}$
4	Boc	Phe	Phe	OCH_3	$C_{43}H_{65}O_{10}N_5$	812	A	0.24[α]	2.00 (2), 1.93 (2), 1.04 (1)	95.5	1.9×10^{-7}
5	Boc	D-Phe	Phe	OCH_3	$C_{43}H_{65}O_{10}N_5$	812	A	0.45[α]	1.92 (2), 2.07 (2), 1.00 (1)	98	$>10^{-5}$
6	Boc-Tau	Phe	Phe	OCH_3	$C_{45}H_{70}O_{12}N_6S$	919	A	0.41[α]	Tau-Phe 0.99 (1), 1.02 (1), 2.02 (2), 0.97 (1)	97.2	1.8×10^{-7}
7	acetyl	Phe	Phe	OCH_3	$C_{40}H_{59}O_9N_5$	754	A	0.86[β]	2.00 (2), 2.05 (2), 1.02 (1)	97	1.4×10^{-6}
8	t-$BuCH_2CO$	Phe	Phe	OCH_3	$C_{44}H_{67}O_9N_5$	810	A	0.40[α]	2.01 (2), 2.04 (2), 1.05 (1)	97	4.8×10^{-7}
9	Iva	Phe	Phe	OCH_3	$C_{43}H_{64}O_9N_5$	795	A	0.72[β]	1.92 (2), 2.04 (2), 1.03 (1)	95.5	3.2×10^{-7}
10	Iva	Phe	Phe	NH_2	$C_{42}H_{63}O_8N_6$	780	A	0.76[β]	2.01 (2), 2.04 (2), 0.95 (1)	99.7	1.9×10^{-7}
11	$C_6H_5SO_2$	Phe*[e]	Phe	OCH_3	$C_{44}H_{61}O_{10}N_5S$	852	A	0.71[β]	1.05 (1), 1.95 (2), 1.01 (1), *	96	4.4×10^{-7}
12	Adoc	Phe	Phe	OCH_3	$C_{49}H_{70}O_{10}N_5$	889	A	0.26[α]	2.04 (2), 1.97 (2), 1.05 (1)	95	3.0×10^{-6}
13	Z	Phe	Trp	OCH_3	$C_{48}H_{64}O_{10}N_6$	885	A	0.26[α]	0.99 (1), 0.92 (1), 2.05 (2), 0–0.97 (1)	96	1.4×10^{-6}
14	Z-Tau	Phe	His	OCH_3	$C_{45}H_{66}N_8O_{12}S$	943	B	0.48[β]	Tau-Phe 1.09 (1), 0.96 (1), 1.99 (2), 0.97 (1)	95.5	1.1×10^{-7}
15	Boc	Phe	His	OCH_3	$C_{40}H_{62}O_{12}N_7$	801	B	0.25[β]	0.98 (1), 0.99 (1), 2.01 (2), 1.01 (1)	98.4	2.7×10^{-8}
16	Z	Phe	Pro	OCH_3	$C_{39}H_{63}O_{10}N_5$	762	A	0.43[α]	0.93 (1), 0.92 (1), 2.10 (2), 0.98 (1)	96	10^{-5}
17	Boc	(NCH_3)Phe*	Phe	OCH_3	$C_{44}H_{67}O_{10}N_5$	826	A	0.33[α]	0.9 (1), 2.02 (2), 0.98 (1), *	97.7	$>10^{-5}$
18	Boc	(αCH_3)Phe*	Phe	OCH_3	$C_{44}H_{67}O_{10}N_5$	826	A	0.34[α]	0.92 (1), 2.02 (2), 0.96 (1), *	98.8	$>10^{-5}$
19	Iva	Phe	Lys	OCH_3	$C_{40}H_{68}O_9N_6HBr$	858	B	0.27[β]	0.95 (1), 0.98 (1), 2.05 (2), 0.98 (1)	96.8	$>10^{-5}$
20	Iva	Phe	Gly	OCH_3	$C_{36}H_{59}O_9N_5$	705	A	0.26[α]	0.93 (1), 0.98 (1), 1.98 (2), 1.05 (1)	94.5	4.7×10^{-6}
21	Iva	Phe	Ala	OCH_3	$C_{37}H_{61}O_9N_5$	719	A	0.76[β]	0.92 (1), 1.98 (2), 2.05 (2)	95.8	4.7×10^{-7}
22	Z	Phe	Val	OCH_3	$C_{42}H_{63}O_{10}N_5$	798	A	0.5[α]	1.03 (1), 0.99 (1), 1.98 (2), 1.00 (1)	95.2	1.8×10^{-7}
23	Boc	D-Phe	Val	OCH_3	$C_{39}H_{66}O_{10}N_5$	764	A	0.23[α]	0.97 (1), 1.08 (1), 1.96 (2), 1.00 (1)	96	10^{-5}
24	Boc	Phe	Nva	OCH_3	$C_{39}H_{65}O_{10}N_5$	764	A	0.8[β]	1.01 (1), 1.00 (1), 2.01 (2), 0.99 (1)	96	5.1×10^{-8}
25	acetyl	Phe	Nva	OCH_3	$C_{35}H_{61}O_9N_5$	694	A	0.72[β]	1.01 (1), 1.00 (1), 2.02 (2), 1.02 (1)	97	2.6×10^{-7}
26	Iva	Phe	Nva	OCH_3	$C_{38}H_{66}O_9N_5$	736	A	0.3[α]	1.02 (1), 1.00 (1), 1.96 (2), 1.02 (1)	96	4.2×10^{-8}
27	Iva	Phe	Abu	OCH_3	$C_{37}H_{64}O_9N_5$	722	A	0.22[α]	1.05 (1), 0.95 (1), 1.96 (2), 1.06 (1)	97.5	2.0×10^{-7}
28	Iva	Phe	Leu	OCH_3	$C_{39}H_{68}O_9N_5$	750	A	0.71[β]	0.95 (1), 1.01 (1), 2.00 (1), 1.02 (1)	98	7.5×10^{-8}
29	Z	Phe	Ile	OCH_3	$C_{43}H_{65}O_{10}N_5$	812	A	0.41[α]	1.03 (1), 0.98 (1), 2.03 (2), 0.96 (1)	96.5	10^{-6}
30	H	Phe	Nle	OCH_3	$C_{34}H_{60}O_8N_5$	666	A	0.5[α]	1.01 (1), 0.96 (1), 2.01 (2), 1.05 (1)	96	7.7×10^{-6}
31	Boc	Phe	Nle	OH	$C_{39}H_{65}O_{10}N_5$	764	A	0.10[α]	1.01 (1), 0.96 (1), 2.02 (2), 1.07 (1)	98	3.0×10^{-8}
32	Iva	Phe	Nle	OH	$C_{39}H_{65}O_9N_5$	748	A	0.83[β]	0.98 (1), 1.00 (1), 2.04 (2), 0.99 (1)	95.5	2.8×10^{-8}
33	Boc	Phg	Phe	OCH_3	$C_{42}H_{63}O_{10}N_5$	798	A	0.23[α]	0.95 (1), 1.05 (1), 2.04 (2), 0.96 (1)	96.5	$>10^{-5}$
34	Boc	Trp	Trp	OCH_3	$C_{47}H_{65}O_{10}N_7$	888	A	0.13[α]	1.88 (2), 1.97 (2), 0.98 (1)	98	1.5×10^{-7}
35	Boc	D-Trp	Trp	OCH_3	$C_{47}H_{65}O_{10}N_7$	888	A	0.70[β]	1.87 (2), 2.04 (2), 0.99 (1)	97	$>10^{-5}$
36	Boc	Trp	D-Trp	OCH_3	$C_{47}H_{65}O_{10}N_7$	888	A	0.25[α]	1.84 (2), 1.91 (2),	96.5	$>10^{-5}$

Table I (Continued)

no.	A	X	Y	R	formula	M	strategy	TLC,[a] R_f	aa anal.[b]	purity[c]	human plasma renin[d]
37	Z	Trp	Val	OCH_3	$C_{44}H_{64}O_{10}N_6$	831	A	0.30[α]	0.90 (1), 1.00 (1), 2.03 (2), 1.01 (1)	96	1.5×10^{-7}
38	Boc	Trp	His	OCH_3	$C_{42}H_{62}O_{10}N_8$	839	B	0.36[β]	0.91 (1), 0.98 (1), 2.08 (2), 0.93 (1)	95.6	1.4×10^{-7}
39	Z	Tyr	Val	OCH_3	$C_{42}H_{63}O_{11}N_5$	814	A	0.21[α]	0.99 (1), 0.99 (1), 2.01 (2), 1.02 (1)	95.7	2.2×10^{-6}
40	Boc	Ile	His	OCH_3	$C_{37}H_{64}O_{10}N_7$	760	B	0.30[β]	0.97 (1), 1.00 (1), 1.98 (2), 1.02 (1)	96.4	3.5×10^{-7}
41	Z	Tau	Val	OCH_3	$C_{35}H_{59}O_{11}N_5S$	758	A	0.31[β]	Tau Val 1.02 (1), 1.98 (2), 1.02 (1)	96.5	$>10^{-5}$
42	Boc	Ada*	His	OCH_3	$C_{44}H_{74}O_{10}N_7$	861	B	0.48[β]	*Ada, 0.95 (1), 2.04 (2), 1.01 (1)	96	2.5×10^{-7}

[a] Solvent systems for TLC development: (α) chloroform/methanol/acetic acid, 95:5:3 (v/v), (β) chloroform/methanol/acetic acid, 80:15:5 (v/v). [b] Amino acid analysis. The data in this column should be interpreted as follows: Taking compound 1 as an example, Z-Phe-Phe-Sta-Ala-Sta-OH, 2.10 (2), 1.98 (2), 0.98 (1) correspond to Phe, Sta, and Ala, respectively. Theoretical values are written in parentheses. [c] Polypeptide purity was determined by the ratio of the main peak to the sum of the integration of all peaks recorded by HPLC at 210 nm. [d] IC_{50} determined at pH 7.4. [e] (*) The amino acid content could not be determined by use of the standard procedure.

Leu-Val-Ile-His[20] and therefore occupies the subsites S_3 and S_2 (Figure 1).

We synthesized peptides with the general formula A-X-Y-Sta-Ala-Sta-R, where A is an acyl group, X and Y natural or unusual α-amino acids, and R an OH, O-alkyl, or NH_2 residue. By this approach we were able to select analogues that were potent inhibitors of human renin. Some of the analogues also inhibited monkey, dog, pig, and rat renin. Part of this work was reported previously at the Eighth American Peptide Symposium.[21] At that conference Boger described a related structure Boc-Phe-His-Sta-Leu-Sta-OH,-NH_2,[22] which he synthesized following an approach[19] different from the one described in the present paper.

Synthesis

The peptides reported in the present paper are listed in Table I. They were synthesized in solution phase with H-Sta-OCH_3 as the starting material.[23] Most of the peptides were synthesized according to strategy A beginning with fragment H-Sta-Ala-Sta-OCH_3 synthesized as shown in Scheme I. The rest of the amino acids were introduced stepwise with use of amino acids that were protected and activated according to classical techniques of peptide synthesis. An example of the complete synthesis of an analogue by strategy A is given in the Experimental Section for product **32**.

Peptides having His or Lys in position Y were synthesized according to strategy B illustrated in Scheme II. With respect to the synthesis of product **10**, which has a carboxamido function at the carboxy terminus, strategy B was also followed but with one modification; namely, H-Ala-Sta-NH_2 was used instead of H-Ala-Sta-OCH_3.

Results

The IC_{50} values for inhibition of human plasma renin activity are shown in Table I.

Influence of the Nature of the Acyl Group A on Renin Inhibition. The influence of the acyl group A on the inhibition of renin was investigated with use of a homogeneous series of compounds in which the dipeptide unit Phe-Phe (corresponding to X-Y) was kept constant. Iva in compound **9**, Boc in compound **4**, and Boc-taurine (Boc-Tau) in compound **6** led to analogues with similar potencies, whereas introduction of the following groups, Z in **2**, acetyl in **7**, and (adamantyloxy)carbonyl (Adoc) in **12**, led to about 10 times less potent analogues. The favorable role of the acyl group A for the interaction with the renin active site is clearly shown by the reduced affinity following its removal in compound **30**.

Influence of the Nature of Y on Renin Inhibition. In the series of compounds numbered **1–32**, the results in Table I show that the amino acids in position Y that gave the most active pepstatin analogue inhibitors of renin are His in compound **15**, norvaline (Nva) in **24** and **26**, Leu in **28**, and norleucine (Nle) in **31** and **32**. The IC_{50} values for renin inhibition by these analogues were in the range of $2.5–7.5 \times 10^{-8}$ M. Other than His in position Y, amino acids having a hydrophobic side chain gave the most active analogues. For those amino acids with an aliphatic side chain, the maximum activity was obtained with Nle and Nva. Shortening of this chain led to a decrease in activity particularly remarkable with Ala (**21**) and Gly (**20**).

Influence of the Nature of X on Renin Inhibition. Peptides showing high inhibitory activity (Y = Phe, Trp, Val, His) were modified in position X. The introduction of hydrophobic amino acids such as Trp in compounds **34**, **37**, and **38**, Ile in **40**, and adamantylalanine (Ada) (**42**) gave active analogues (IC_{50} of about 10^{-7} M). On the contrary, as in the series **1–32**, a configuration inversion, such as compound **35**, was highly unfavorable. Substitution of X by (NCH_3)Phe in compound **17**, (αCH_3)Phe in compound **18**, phenylglycine (Phg) in compound **33**, Tyr in compound **39**, or Tau in compound **41** led to compounds with markedly low or no activity. On the other hand, compound **42**, despite the introduction of the sterically hindered adamantyl moiety, remains as potent as compound **38** and only 10 times less active than **15**.

Influence of the Nature of R on Renin Inhibition. Since this position does not have much influence on the inhibition of plasma renin activity, most of the pepstatin derivatives described are the methyl ester.

Species Specificity in Renin Inhibition. Table II shows the species specificity of plasma renin inhibition by some of the analogues. The results show differences among the species of renin tested. Compounds **15**, **34**, **38**, **40**, and **42** were found to have a lower inhibitory power for rat renin than for the other species studied.

Discussion

Efficient renin inhibition by modified analogues of the substrate requires compounds able to interact with the extended $S_4–S'_3$ part of the enzyme active site. Indeed, modified peptides only able to fit the $S_4–S'_1$[24] or $S_1–S'_3$[25] subsites of renin exhibited inhibitory potencies inferior to

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Table II. Species Specificity of Some Pepstatin Analogues

A-X-Y-Sta-Ala-Sta-R					inhibition of plasma renin activity: IC_{50}				
no.	A	X	Y	R	man	rat[a]	monkey[b]	hog	dog[c]
pepstatin	Iva	Val	Val	OH	1.4×10^{-5}	8.3×10^{-6}	1.5×10^{-5}	1.0×10^{-6}	2.0×10^{-6}
4	Boc	Phe	Phe	OCH_3	1.9×10^{-7}	1.9×10^{-7}	1.3×10^{-7}	5.2×10^{-8}	7.6×10^{-8}
15	Boc	Phe	His	OCH_3	2.7×10^{-8}	1.2×10^{-6}	6.0×10^{-8}	1.5×10^{-7}	5.7×10^{-8}
17	Boc	(NCH_3)Phe	Phe	OCH_3	1.5×10^{-5}	1.7×10^{-5}	1.5×10^{-5}		
22	Z	Phe	Val	OCH_3	1.8×10^{-7}	3.8×10^{-8}	1.4×10^{-8}	3.1×10^{-8}	9.3×10^{-8}
26	Iva	Phe	Nva	OCH_3	4.2×10^{-8}	8.8×10^{-7}	3.0×10^{-8} [d]	4.7×10^{-8}	5.8×10^{-8}
32	Iva	Phe	Nle	OH	2.8×10^{-8}	6.0×10^{-7}	1.8×10^{-8} [e], 5.3×10^{-8}	3.4×10^{-8}	4.8×10^{-8}
34	Boc	Trp	Trp	OCH_3	1.7×10^{-7}	1.0×10^{-6}	1.5×10^{-7}		
37	Z	Trp	Val	OCH_3	1.5×10^{-7}	2.2×10^{-8}	2.5×10^{-8}	1.5×10^{-8}	4.2×10^{-8}
38	Boc	Trp	His	OCH_3	1.4×10^{-7}	1.3×10^{-6}	1.1×10^{-7}		
40	Boc	Ile	His	OCH_3	3.5×10^{-7}	1.4×10^{-5}		6.2×10^{-6}	3.4×10^{-6}
42	Boc	Ada	His	OCH_3	2.5×10^{-7}	2.2×10^{-6}		6.0×10^{-7}	6.5×10^{-7}

[a] Sprague-Dawley. [b] *Papio Hamadryas*. [c] Mongrel dogs. [d] *Callithrix jacchus* (common marmoset). [e] *Cynomolgus* monkey.

10^{-7} M. In contrast, the use of statine as an analogue of the dipeptide tetrahedral intermediate led to highly potent renin inhibitors characterized by a sequence of five residues with an acylated N-terminal amino acid and a statine moiety assumed to bind the two successive S_1–S'_1 subsites of renin.[19,21,22] In addition to their facile synthesis, these inhibitors are expected to be more resistant to nonspecific peptidases. Indeed, acylation of the N-terminal amino acid inhibits the cleavage by aminopeptidases while incorporation into the sequence of one or two unusual hydroxyl-containing amino acids such as statine[19,21,22] or related structures[26] probably bestows to these molecules resistance to endopeptidases. Therefore, a structure–activity study was performed to optimize the inhibitory potency for human renin of a pepstatin-derived series of compounds, A-X-Y-Sta-Ala-Sta-OH.

Differences in natural pepstatins occur at the level of the acyl moiety (acetyl, propionyl, *n*-butyryl, isovaleryl, *n*-caproyl, ...). Aoyagi et al.[27,28] showed that the inhibition of renin is slightly lowered by a reduction in the size of the acyl group A. In our present series, the absence of the A group (compound **30**) leads to a drastic loss of affinity, illustrating that efficient binding to renin requires interactions with the S_4 subsite. The latter is expected to bind the proline moiety present in the natural substrate, and it is interesting to note that the more efficient inhibitors contain a Boc (**4**) or Iva (**9**) group that corresponds roughly to the spatial dimension of the proline ring. Nevertheless, increase in the size of A as in **2** (Z group) or **12** (Adoc group) induced only a 10-fold decrease in affinity. Taken together, these features are in agreement with the occurrence of a relatively large hydrophobic pocket in the S_5–S_3 region of aspartyl proteinases[16–18] including human renin.[29,30]

Thus, at the level of the S_3 subsite corresponding to the binding site of X, the replacement of Phe by Trp (compound **38**) and even by the larger Ada residue (compound **42**) induced only a relatively weak decrease in the inhibitory potency. Similarly, a 10-fold increase in affinity for human renin was recently observed by replacement of the Phe moiety by its naphthylalanine analogue in Z-[3-(1′-naphthyl)-Ala]-His-leucinal.[2] This large and hydrophobic S_5–S_3 region probably accounts for the inhibition of human renin by cyclic, conformationally restricted analogues of angiotensinogen,[29,30] characterized by a β-turn involving the P_5–P_2 His-Pro-Phe-His sequence. This bent structure with Pro and Phe at the corner of the chain reversal seems to occur also in the natural substrate.[31] On the other hand, a large hydrophobic pocket corresponding to the S_3 subsite was also found in other aspartyl proteinases such as penicillinopepsin[17] and *Rhizopus chinensis*.[16] All these results could explain the good affinity of compounds with large side chains in the X position, such as in compounds **38**, **40**, and **42**, and suggest that the bulkier P_3 moiety could be accommodated. Nevertheless, flexibility of the side chain of the P_3 residue to fit the corresponding S_3 subsite is required as clearly shown by the drastic loss of potency of **33** (with a Phg residue) vs. **4**. A change in the configuration of the X residue such as in compounds **5** and **35** inhibits the enzyme recognition, supporting the expected stereoselectivity of the binding to the peptidase. The loss of affinity induced by N-methylation (**17**) or C-methylation (**18**) of the Phe residue could be due to unfavorable conformations of the modified compounds hindering the binding to the renin active site.

The S_2 subsite of renin is assumed to bind the His^9 amino acid of human angiotensinogen. Accordingly, compounds bearing this amino acid in the Y position (Table I) display stronger inhibitory potency (compounds **15**, **38**, **40**, **42**). Moreover, the replacement of His in **15** by Gly (compound **20**) leads to 200-fold decrease in affinity, suggesting that interaction with the S_2 subsite is a crucial requirement for strong binding to renin. Furthermore, the replacement of the His moiety in position Y by Pro (compound **16**) led to a strong loss of affinity. Nevertheless, the selectivity of the S_2 subsite of renin is not very stringent since compound **4** with Y = Phe is only 10 times less potent than **15** in which Y = His. As expected, interaction with the S_2 subsite requires correct orientation of the interacting residue as shown by the loss of activity following the replacement of L-Phe in **2** by D-Phe in **3**. However, the most interesting result is the very high inhibitory potency of compounds bearing a hydrophobic amino acid in the Y position. The highest activity was obtained with amino acids bearing linear aliphatic side chains such as Nle (**31**, **32**) or Nva (**24**, **26**) more active than Abu (**27**), Ala (**21**), and Gly (**20**). The decreased potency produced by introduction of Ile (**29**) in place of Leu (**28**) might be due

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to unfavorable steric factors. Furthermore, the preference of the S_2 subsite in human renin for compounds bearing a hydrophobic amino acid in the Y position is suggested by the weak affinity of **19**, a compound characterized by an hydrophilic aminated side chain in the Y position.

Reports in the literature[9,29,32-36] on the species specificity of renin inhibitors only describe substrate analogues modified in positions P'_1, P'_2, P'_3, ..., but not in positions P_5–P_1. The logic for developing this type of renin inhibitors probably lies in the fact that all natural renin substrates have the sequence His-Pro-Phe-His-Leu; the species differ in the nature of the amino acids in the carboxyl-terminal direction of the substrate (P'_1...). In the present paper, however, we have showed a species specificity in the P_4–P_2 region. Such a finding is probably not so surprising since the inhibitory potency of pepstatin itself is species dependent.[19] We made the same observation. In particular, we showed that compound **15**, which has a His residue in position P_2, was markedly less inhibitory for rat renin (IC_{50} = 1.2 × 10^{-6} M) than for renin from other species (IC_{50} = 2.7–6.6 × 10^{-8} M). Compounds **38**, **40**, and **42**, which also have a His residue in position P_2, showed a similar type of species specificity. On the contrary, compounds **22** and **37**, which have a Val residue in position P_2, were found to be more active against rat renin. In vivo, preliminary experiments carried out with compound **32** in two primate species showed that this compound has powerful hypotensive activity.[37] In support of renin inhibition as a novel strategy for correcting arterial hypertension are the encouraging results obtained with a human antirenin monoclonal antibody.[38,39]

Experimental Section

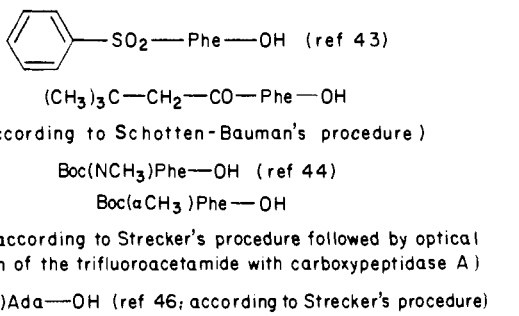
Chemical Synthesis. General Remarks. Solvents were removed by evaporation by using a rotary distillation apparatus at a bath temperature of 30–40 °C. Thin-layer chromatography (TLC) was performed on silica gel plates (Merck 60 F-254); spots of compounds were revealed by standard spray techniques (ninhydrin, Pauly, Reindel-Hoppe, iodine, etc.). Solvent systems for TLC development were (ratios expressed as v/v) chloroform/methanol/acetic acid, α = 95:5:3 and β = 80:15:5. Amino acid analyses were performed on a Beckman 119 BL amino acid analyzer on samples that were hydrolyzed (110 °C, 20 h) in 6 N HCl (analytical grade) in sealed, evacuated tubes. Values obtained for Trp were not corrected because of partial decomposition of this amino acid. High-pressure liquid chromatography was performed with a Varian 5.000 apparatus equipped with a Varian loop injector (10 μL), a Varian pump, a spectromonitor LDC multiwavelength detector, and a Sefram recorder. The chromatographic conditions were as follows: 300 × 3.9 mm column filled with Bondapak C18 adsorbent (10-μm particles), a mixture of triethylammonium phosphate (TEAP), pH 3, mixed with acetonitrile (43%) as eluant, flow rate 4 mL min^{-1} at 3000 psi, detector set at λ = 210 nm and range = 0.2 AUFS, attenuation of recorder 8. ^1H NMR was performed at 250 MHz with a Brucker WM 250 apparatus in Me_2SO as solvent. An example of the interpretation of an RMN spectrum is given in Table III for product **32**.

Table III. NMR Spectrum of Compound **32**, Iva-Phe-Nle-Sta-Ala-Sta-OH[a]

δ	aspect	integration	attribution	
0.58–0.91	m[b]	21 H	$^6C(CH_3)_2$	Sta
			CH_3	Nle
			$^3C(CH_3)_2$	Iva
1.04–1.93	m	18 H	$^5CH_2$-^6CH	Sta
			CH_3	Ala
			$^2CH_2$-^3CH	Iva
			$CH_2CH_2CH_2$	Nle
1.93–2.29	m	4 H	$^2CH_2$	Sta
2.56–3.04	m	2 H	$^3CH_2$	Phe
3.64–4.61	m	7 H	^4CH-^3CH	Sta
			^2CH	Phe
			^2CH	Ala
			^2CH	Nle
4.6–5.1	m	2 H	OH	Sta
7.06–7.29	m	5 H	arom	
7.29–7.45	m	2 H	2 NH	
7.83	d[c]	1 H	NH	
8	2 d	2 H	NH	

[a] ^1H RMN/Me_2SO at 250 MHz, internal reference Me_2SO. [b] Multiplet = m. [c] doublet = d.

Chart I



Protected and Activated Amino Acids. The protected and activated amino acid were prepared according to classical techniques of peptide chemistry.[40]

Z-Tau-Phe-OH. To a solution of phenylalanine methyl ester prepared from the corresponding hydrogen chloride form (16 g, 85 mmol) in THF (50 mL) was added a solution of Z-Tau-Cl[41] (34 mmol) in THF (50 mL). The reaction mixture was left overnight at room temperature. Next, the precipitate was filtered off and the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate (150 mL) and the solution was washed successively with 2 × 50 mL of each of the following: 5% aqueous $K_2SO_4/KHSO_4$ (pH 2), saturated NaCl, 5% aqueous $NaHCO_3$, and saturated NaCl. After drying ($MgSO_4$) and evaporation of the organic phase, we obtained a residue that recrystallized from benzene/pentane to give Z-Tau-Phe-OCH_3. Yield 12.73 g, (89%); NMR confirmed that it was the desired compound; mp 93 °C. The preceding compound (250 mg, 0.59 mmol) was dissolved in 5 mL of DMF. Two equivalents of 1 N NaOH was added, and the pH of the medium was kept at 13 for 1 h at room temperature.

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The pH was lowered to 4 with 1 N HCl. The solvent was evaporated to dryness, and the residue was used without purification.

The intermediates given in Chart I were prepared according to known techniques.

Synthesis of Iva-Phe-Nle-Sta-Ala-Sta-OH (32). Strategy A. Z-Ala-Sta-OCH_3. H-Sta-OCH_3,TFA (1.44 g, 4.8 mmol) *N*-ethylmorpholine (NEM; 595 mg, 4.8 mmol), Z-Ala-OTcp (2.06 g, 5.28 mmol), and 1-hydroxybenzotriazol (HOBT; 1.29 g, 4.8 mmol) were dissolved in DMF (35 mL) at room temperature. The pH was maintained between 6 and 7 during the reaction by addition of *N*-ethylmorpholine. The reaction mixture was stirred at room temperature for 48 h, and then the solvent was evaporated in high vacuum (40 °C). The residual oil was dissolved in ethyl acetate (50 mL) and washed with 5% aqueous $KHSO_4/K_2SO_4$ (pH 2), saturated NaCl, 5% aqueous $NaHCO_3$, and 5% aqueous NaCl. The organic phase was dried ($MgSO_4$) and the solvent evaporated. The residue was triturated in ether. After 1 h at 0 °C, the solid was filtered and dried. Yield 1.47 g, (86%); mp 117–120 °C.

Boc-Sta-Ala-Sta-OCH_3. The preceding product (1.46 g, 4.1 mmol) was dissolved in methanol (20 mL). Ammonium formate (1.03 g, 16.4 mmol) was added at room temperature and the mixture stirred until dissolution. Next, 400 mg of 10% Pd/C was added and the reaction mixture stirred. A TLC control showed that the reaction was terminated in 5 min. The catalyzer was eliminated by filtration. The solution was passed through an Amberlite (OH^-) IR 45 column and the solvent evaporated. The residue was triturated in a minimum of ether and the solvent evaporated. After addition of a minimum of methylene chloride and elimination of insoluble material by filtration and evaporation, a white powder was obtained. Yield 810 mg (75%); mp 123–134 °C. A second batch was synthesized under the same conditions. This substance (960 mg, 3.6 mmol) was dissolved in methylene chloride (500 mL) at room temperature. Boc-Sta-OH (1 g, 3.6 mmol), *N*-hydroxysuccinimide (HONSu; 420 mg, 3.6 mmol), and dicyclohexylcarbodiimide) (DCC; 750 mg, 3.6 mmol) were added. The reaction mixture was stirred for 7 h at room temperature. The dicyclohexylurea (DCU) precipitate was removed by filtration. The organic solution was with 5% aqueous $KHSO_4/K_2SO_4$ (pH 2) and 5% aqueous $NaHCO_3$ and then dried ($MgSO_4$). Evaporation of the solvent gave a residue that was dissolved in a minimum of $CHCl_3/CH_3OH$ (97.5/2.5, v/v) and chromatographed on a column 24 cm × 2.5 cm) filled with Merck silica gel HR type 60 (70–230 mesh) in $CHCl_3/CH_3OH$. Elution with the same $CHCl_3/CH_3OH$ mixture gave 1 g of pure product (mp 95–98 °C) and fractions that were recycled. Global yield 67%.

Iva-Phe-Nle-Sta-Ala-Sta-OH. Boc-Sta-Ala-Sta-OCH_3 (1 g, 1.9 mmol) was solubilized in $CHCl_3$ (5 mL). TFA (10 mL) was added. After 30 min, the solvent was evaporated and the residual oil treated with a minimum of ether. The solid was filtered. Yield 850 mg (85%). *N*-Ethylmorpholine (NEM; 391 mg, 3.4 mmol) was added to a mixture of the preceding product (531 mg, 0.1 mmol), Boc-Nle-ONSu (393 mg, 1.2 mmol), and HOBT (162 mg, 1.2 mmol) in 10 mL of DMF. The final mixture was stirred for 48 h at room temperature, and the pH was maintained at 6–7 by addition of NEM. At the end of the reaction period, the solvent was evaporated. The residue was dissolved in a minimum of ethyl acetate. The solution was washed successively with 5% aqueous $K_2SO_4/KHSO_4$ (pH 2), saturated NaCl, 5% aqueous $NaHCO_3$, and saturated NaCl. The organic phase was dried ($MgSO_4$). The residue obtained by evaporation of the solvent was crystallized in an ether–pentane mixture. Yield 570 mg (70%). The Boc group was removed by TFA/CH_2Cl_2 treatment as described above. The TFA salt of H-Nle-Sta-Ala-Sta-OCH_3 (150 mg, 0.23 mmol) and HOBT (36 mg, 0.27 mmol) were added to dioxane (3 mL) containing NEM (89 mg, 0.77 mmol). Iva-Phe-ONSu (91, 1 mg, 0.27 mmol) was added and the reaction mixture stirred for 24 h at room temperature. The precipitate was filtered, washed in dioxane and then in ether, and dried. Yield 130 mg (73%). Transformation to the free acid: Iva-Phe-Nle-Sta-Ala-Sta-OCH_3 (100 mg, 0.13 mmol) was dissolved in DMF (100 mL) at room temperature. Distilled water (1 mL) and 1 N NaOH (0.26 mL, 2 equiv) were added, and the reaction mixture was stirred for 30 min. Next, 1 N HCl (0.26 mL) was added to adjust the reaction medium to pH 5. The solvent was evaporated and the residue was triturated in water. The solid was filtered, washed with water and ether, and dried. Yield 75 mg (76%). The characteristics of this compound (**32**) are reported in Table I. The NMR spectrum was determined at 250 MHz in Me_2SO (Table III).

Synthesis of Boc-Phe-His-Sta-Ala-Sta-OCH_3 (15). Strategy B. Boc-Phe-His-Sta-OCH_3. Boc-His(Boc)-OH,DCHA (800 mg, 1.5 mmol) was dissolved in CH_2Cl_2 (80 mL). To this solution was added H-Sta-OCH_3,TFA (306 mg, 1.5 mmol). After dissolution, BOP[47] (600 mg, 1.5 mmol) and DIPEA (233 mg, 1.8 mmol) were added. The reaction medium was stirred for 24 h at room temperature. The pH was maintained between 6 and 7 by addition of DIPEA. The solvent was evaporated and the residue was dissolved in a minimum of ethyl acetate and chromatographed on a column (60 cm × 3 cm) of Merck silica gel HR type 60 (70–230 mesh) with ethyl acetate as eluant. The pure fractions (one spot by TLC) were grouped and evaporated. Yield 610 mg (77%). NMR confirmed that the solid product was the desired compound. To this product (350 mg, 0.66 mmol) was added TFA (5 mL) at room temperature. After 30 min, the medium was evaporated to dryness. The TFA salt was triturated in dioxane (50 mL) and NEM (237 mg, 2.0 mmol) was added. After verification that the pH of the solution was between 6 and 7, HOBT (89 mg, 0.66 mmol) and Boc-Phe-ONSu (260 mg, 0.72 mmol) were added. The pH was adjusted to 6–7 with NEM if necessary. After 24 h at room temperature, the solvent was evaporated and the residue was dissolved in a minimum of ethyl acetate and chromatographed under the same conditions as previously described. The fractions containing the pure product (one spot by TLC) were grouped and evaporated. Yield 130 mg (34%). NMR confirmed that it was the desired compound. Transformation to the free acid: Boc-Phe-His-Sta-OCH_3 (450 mg, 0.78 mmol) was dissolved in methanol (20 mL) at room temperature. Distilled water (5 mL) and barium hydroxide (200 mg) were added. After 45 min, barium hydroxide (100 mg) was added. After 90 min, CO_2 was flushed into the reaction mixture for 30 min. The solution was filtered through Celite. The solvents were evaporated, and the residual was triturated in ether. Yield 400 mg (91.7%).

Boc-Phe-His-Sta-Ala-Sta-OCH_3. H-Ala-Sta-OCH_3 (106 mg, 0.41 mmol) was dissolved in DMF (10 mL). To this solution were added Boc-Phe-His-Sta-OH (230 mg, 0.41 mmol) in DMF (10 mL) containing DIPEA (53 mg, 0.41 mmol) and then a solution of BOP (268 mg, 0.60 mmol) in DMF (10 mL) containing DIPEA (76 mg, 0.60 mmol). After the mixture was stirred, the pH was adjusted to 6–7 with DIPEA. After 24 h at room temperature, the reaction medium was evaporated to dryness in a high vacuum. The residue was treated with water and extracted with CH_2Cl_2. The organic phase was washed successively with 5% aqueous $NaHCO_3$ and water and then dried ($MgSO_4$) and evaporated. The residue was dissolved in a minimum of $CH_3OH/CHCl_3$ (2:98, v/v) and chromatographed on a Merck silica gel 60 (70–230 mesh) column (35 cm × 2 cm) using a mixture of $MeOH/CHCl_3$ with a methanol gradient from 2:98 (v/v) to 10:90 (v/v). The pure fractions were grouped evaporated and triturated in ether. Yield 150 mg (45%). The characteristics of this product are reported in Table I. The NMR spectrum is in agreeement with the structure.

Inhibition Studies. The pepstatin analogues were solubilized according to the method described by Gardes et al.[48] To evaluate the renin inhibitory power of the test substances, we used a pool of human plasma rich in renin. This plasma pool was incubated at 37 °C in a pH 7.4 phosphate buffer in the presence of increasing

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concentrations of test substance. Angiotensin I liberated during the course of the reaction was measured by RIA with use of a commercial kit (Clinical Assays Travenol) according to the method of Haber.[49] An angiotensinase inhibitor, phenylmethanesulfonyl fluoride, was added to the reaction medium. For the monkey, dog, hog, and rat, plasma renin activity (PRA) was stimulated by furosemide. The results expressed as IC_{50} values (molar concentration of test product causing 50% inhibition of PRA) were determined by nonlinear regression (logit against log concentration) with five to seven concentrations of test substance. The reproductibility of the IC_{50} values was within ±5.8%, evaluated by using a weighted equation. Pepstatin was tested in each experiment in at least three concentrations near the IC_{50} value.

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Structure–Activity Study of 6-Substituted 2-Pyranones as Inactivators of α-Chymotrypsin

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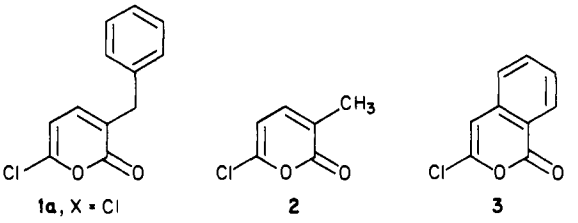
A series of 2-pyranones, bearing halogens or electron-withdrawing groups at the 6-position and alkyl, aryl, or aralkyl groups at positions 3, 4, and 5, were synthesized to investigate their binding to and inactivation of chymotrypsin. Both binding and inactivation by 2-pyranones are sensitive to substitutions on positions 3, 4, 5, and 6. Binding was poorest with alkyl substituents on position 3 and best with phenyl substitution, with benzyl or benzyl-like substitution falling in between. The sequence of binding of 6-substituted pyrones is Cl > Br > H > CF_3. 6-Chloro-2-pyranones bearing 4-phenyl or 3-(2-naphthylmethyl) substituents effected rapid inactivation of chymotrypsin, while those having 3-benzyl or 3-(1-naphthylmethyl) substituents gave slow inactivation and those with 3-phenyl or 3-alkyl substituents gave no inactivation. Only the 6-halopyrones demonstrated inactivation, with chloro-substituted ones acting faster than bromo-substituted ones.

A few 6-chloro-2-pyranones (**1a**, **2**),[1] including the benzopyranones of Powers[2] (**3**), have been reported to inactivate chymotrypsin. While the original premise in designing these inactivators was that the pyrones might act as mechanism-based (suicide) inactivators[1a]—they would then acylate an active-site nucleophile—their inhibition was found to be temporary, and recent evidence suggests that it is due to slow turnover of a stable acyl enzyme.[1b] Nevertheless, the 2-pyrone nucleus offers interesting possibilities as the basis for the development of mechanism-based inactivators, targeted toward serine proteases, and a structure–activity study of the system would be useful. However, little work addressing the binding requirements or the ring activation needed for substrate activity thus far has appeared.



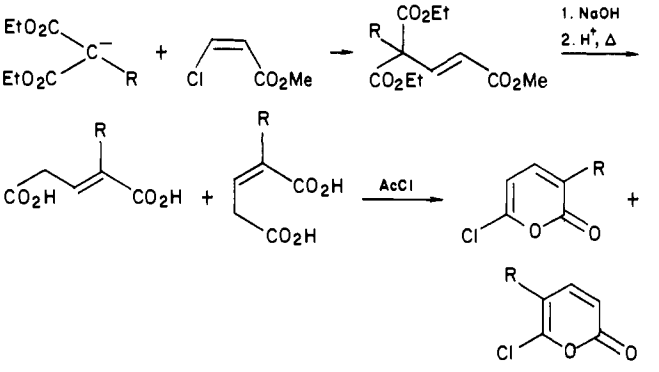
1a, X = Cl **2** **3**

In this report, we describe the preparation of a variety of 2-pyranones, substituted with alkyl, aryl, and aralkyl groups at carbons 3, 4, and 5 and bearing a hydrogen or halogen at carbon 6. We find that all of these substituents have a major effect on the binding affinity and inactivation potency of these pyrones toward chymotrypsin.

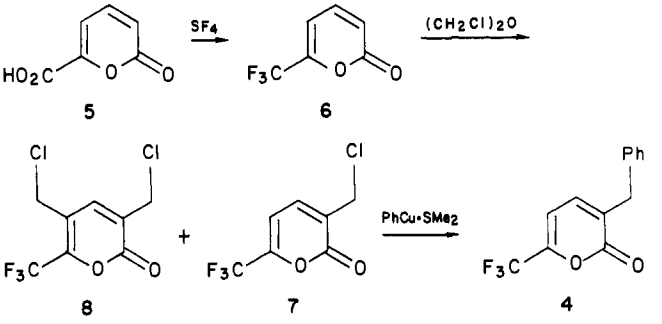
Results and Discussion

The structures of the substituted 2-pyrones prepared for this study are summarized in Table I.

Scheme I



Scheme II



Chemistry. Substituted Glutaconic Acids. The synthesis of substituted 6-halo-2-pyranones is dependent upon the availability of similarly substituted glutaconic acid precursors.[3] It was necessary to modify the classical synthesis of these glutaconic acids to allow a wider range of pyrone substitutions (Scheme I): The appropriately substituted diethyl malonate sodium salt was heated at 80 °C with methyl *cis*-2-chloroacrylate[4] in THF; the re-

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