Synthesis and Renin Inhibitory Activity of Angiotensinogen Analogues Having Dehydrostatine, Leuψ[CH_2S]Val, or Leuψ[CH_2SO]Val at the P_1–P_1' Cleavage Site[1]

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The synthesis and in vitro renin inhibitory potencies of angiotensinogen (ANG) analogues having amide (CONH) bond replacements at P_1–P_1', the Leu-Val cleavage site, corresponding to Leuψ[CH_2S]Val, Leuψ[CH_2SO]Val, and the trans olefinic analogue of statine (Sta), 4(*S*)-amino-6-methyl-2(*E*)-heptenoic acid (dehydrostatine, Dhs), are reported. These are compared to P_1–P_1' Leuψ[CH_2NH]Val-, Sta-, or Phe-Phe-substituted analogues of the same template. The Dhs pseudodipeptide was found to be an adequate mimic of a trans CONH bond and gave a peptide, H-Pro-His-Pro-Phe-His-Dhs-Ile-His-D-Lys-OH, approximately equal in potency to a Phe-Phe-containing inhibitor, but 200-fold less potent than its Sta-substituted congener. That the enhanced potency of the Sta-containing peptide most likely depends on hydrogen bonding as well as tetrahedral geometry is indicated by the 50–100-fold lower potency of the tetrahedral Leuψ[CH_2S]Val and Leuψ[CH_2SO]Val analogues as compared to the Leuψ[CH_2NH]Val-containing congener.

The aspartic proteinase renin catalyzes the first step of the biosynthetic pathway leading to the production of angiotensin II, one of the most potent naturally occurring vasoconstrictive substances known. Angiotensin II has been implicated in the physiological regulation of blood pressure and may also play a role in pathophysiological forms of hypertension.[2,3] It follows that inhibition of renin may be therapeutically beneficial in controlling hypertension and, moreover, that renin inhibitors may constitute a novel class of antihypertensive drugs.[4]

Early work on substrate-based inhibitors using systematic structure–activity studies[5] culminated with the decapeptide, H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys-OH, which was the first inhibitor active in vivo[6] (Figure 1). In this analogue the key to conversion from substrate to inhibitor was the substitution of Phe-Phe for the labile Leu-Val sequence at P_1–P_1'. More recently, modifications at P_1–P_1' have included changes that may mimic the transition state aminol (C[$OH]_2$NH) generated during hydrolytic cleavage of the peptide bond by renin (e.g., Leuψ[CH_2NH]Val and Sta)[7–11] as well as changes of the peptide bond to noncleavable olefinic groups (e.g., Leuψ[*E*-CH=CH]Gly).[12] In many cases these have led to extremely potent and specific inhibitors of renin.[13–19]

We report here the synthesis and in vitro renin inhibitory potencies of compounds with peptide bond replacements at the P_1–P_1' sites corresponding to Leuψ[CH_2S]Val, Leuψ[CH_2SO]Val, and the olefinic analogue of Sta, 4(*S*)-amino-6-methyl-2(*E*)-heptenoic acid, (dehydrostatine, Dhs). These are compared to the Phe-Phe-, Leuψ[CH_2NH]Val-, and statine-containing analogues all within the same Pro-His-Pro-Phe-His-Xaa-Yaa-Ile-His-D-Lys template where Xaa-Yaa is the particular P_1–P_1' substitution. This template is not optimized for maximum potency of renin inhibitors and was chosen so that differences in activity affected by the Xaa-Yaa substitutions could be measured without approaching the limit (concentration of renin in the test plasma) for IC_{50} determinations.

Results and Discussion

Chemistry. Synthesis of Boc-Dhs-OMe containing both trans and cis olefinic bonds in an approximate ratio of 7:3 was accomplished as shown in Scheme I. Boc-L-leucinol was oxidized to Boc-L-leucinal by treatment with pyridine–sulfur trioxide in DMSO[20] followed by reaction with methyl (triphenylphosphoranylidene)acetate. Saponification of the methyl ester yielded the expected trans

Table I. Solid-Phase Synthesis Procedure

step	solvent or reagent	repetitions	time, min
1	CH_2Cl_2	n4	1.5
2	CF_3CO_2H–CH_2Cl_2(1:1)	2[a]	1,5, 25[a]
3	CH_2Cl_2	4	1.5
4	DIPEA–CH_2Cl_2(1:9)	3	5
5	CH_2Cl_2	4	1.5
6	DMF	4	1.5
7	coupling step (see text)	1	variable
8	DMF	4	1.5
9	EtOH	4	1.5

[a] When Boc-His(Tos) was deprotected, three CF_3CO_2H–CH_2Cl_2 treatments are performed for 1.5, 25, and 25 min.

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(1) Abbreviations of amino acids follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature *Eur. J. Biochem.* **1984**, *158*, 9. The ψ[] nomenclature for peptide bond modifications follows the suggestion of Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, Chapter 5. All optically active amino acids are of the L variety unless otherwise specified. The following additional abbreviations are used: ANG, angiotensinogen; Boc, *tert*-butyloxycarbonyl; DCC, dicyclohexylcarbodiimide; Dhs, dehydrostatine; DIPEA, (diisopropylethyl)amine; HBT, 1-hydroxybenzotriazole; Sta, statine.

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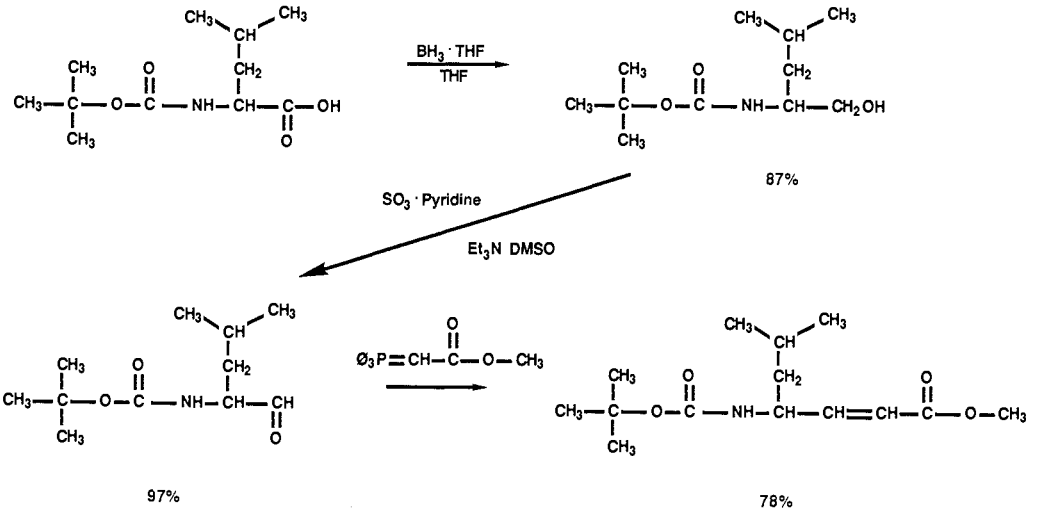
Scheme I



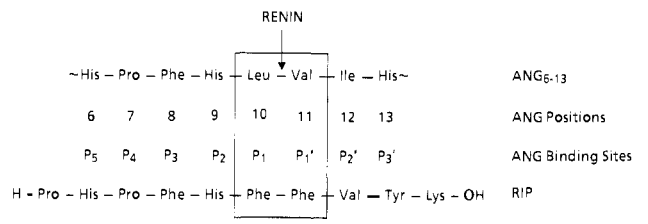
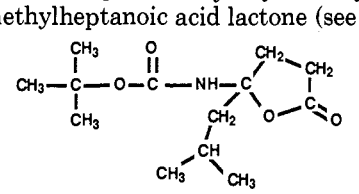


Figure 1.

olefinic acid and 4-[(*tert*-butyloxycarbonyl)amino]-4-hydroxy-6-methylheptanoic acid lactone (see below). No



cis olefinic acid was produced. Boc-Leuψ[CH_2S]Val was synthesized according to the method described by Spatola et al.,[21] and the sulfoxide was obtained by peracetic acid oxidation of the pseudodipeptide-containing final product.

Table II. Renin Inhibitory Activities of Several ANG-Based Peptides

number	compound	IC_{50}, M	relative potency
1	H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-D-Lys-OH	1.0×10^{-5}	1.0
2	H-Pro-His-Pro-Phe-His-Phe-Phe-Ile-His-D-Lys-OH	1.7×10^{-4}	0.6
3	H-Pro-His-Pro-Phe-His-Dhs-Ile-His-D-Lys-OH	6.2×10^{-6}	1.6
4	H–Pro–His–Pro–Phe–His–Sta-Ile-His-D-Lys-OH	2.6×10^{-8}	380
5	H-Pro-His-Pro-Phe-His-Leuψ-[CH_2S]Val-Ile-His-D-Lys-OH	6.0×10^{-6}	1.6
6	H-Pro-His-Pro-Phe-His-Leuψ-[CH_2SO]Val-Ile-His-D-Lys-OH	2.5×10^{-6}	4.0
7	H-Pro-His-Pro-Phe-His-Leuψ-[CH_2NH]Val-Ile-His-D-Lys-OH	4.9×10^{-8}	200

All of the peptides were synthesized by the solid-phase method of synthesis,[22,23] a single cycle of which is given in Table I. Similar to the report by Sasaki and Coy[24] we observed that Boc-Leuψ[CH_2NH]Val could be incorporated into the peptide and extended upon without protection of the secondary amine function. The peptide was cleaved from the resin, and the side-chain protecting groups were removed simultaneously by treatment with HF–anisole (10:1). Final products were purified by gel filtration chromatography and preparative reverse-phase chromatography or partition chromatography.[25,26]

Structure–Activity Studies. All of the ANG-based analogues (Figure 2) were evaluated for their inhibition of human plasma renin by using an in vitro assay described previously.[27] The results shown in Table II are compared to H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-D-Lys-OH, **1**, although the compounds discussed were formally analogues of **2**. The comparison to **1** is necessary because the presence of the human P_2'–P_3' sequence, Ile-His, made compound **2** a substrate for renin ($K_m = 5 \times 10^{-6}$ M, $k_{cat} = 10$

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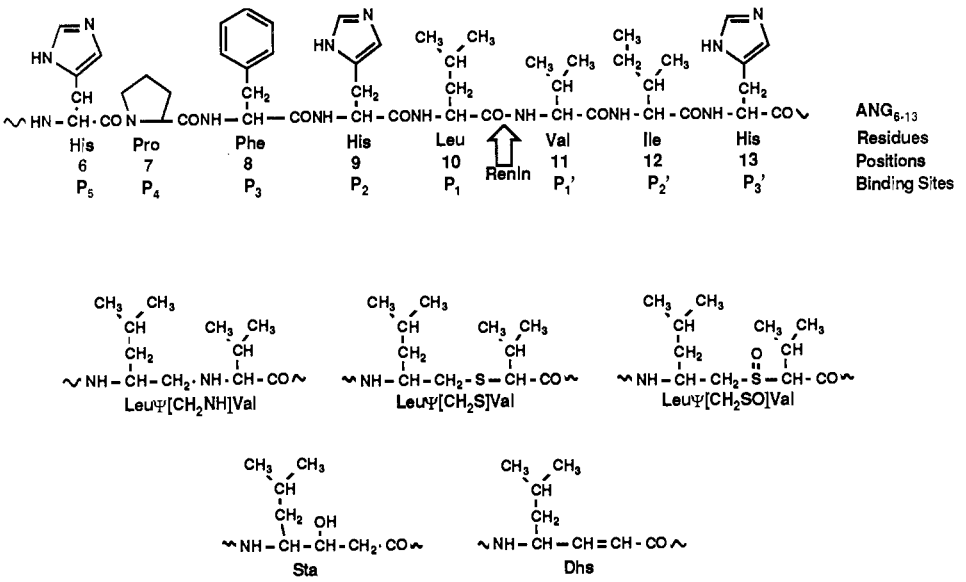

Figure 2.

min^{-1}). It appears that the trans olefinic bond in Dhs was a possible mimic of the trans form of the P_1–P_1' peptide bond since peptide **3** possessed a potency about equal to that of **1**. Similar to a report on a series of renin inhibitors having dehydrohydroxyethylene pseudodipeptides at P_1–P_1',[28] the presence of an α,β-unsaturated amide in **3** did not lead to irreversible inactivation through covalent bond formation. However, as based on current hypotheses for the mechanism of action of aspartyl proteinases,[29,30] such nucleophilic addition reaction would not necessarily be expected. Peptide **3** was about 200 times less potent than the Sta-containing analogue, **4**, whose tetrahedral geometry mimics the transition state during the hydrolysis of the substrate Leu-Val peptide bond. However, tetrahedral geometry may not be the only requirement for high potency. The analogues containing Leuψ[CH_2S]Val and Leuψ[CH_2SO]Val (**5** and **6**, respectively) are at least 50-fold less active than analogue **7** containing Leuψ[CH_2NH]Val. In the recently reported[29] high-resolution crystallographic structure of the aspartic proteinase from *Rhizopus chinensis* with the inhibitor H-D-His-Pro-Phe-His-Pheψ[CH_2NH]Phe-Val-Tyr-OH bound to it, a hydrogen bond between the P_1' N atom and $O^{\delta 2}$ of the active aspartate Asp-218 was implicated. It is very likely that a similar situation exists for renin. This hydrogen bond is unavailable to Dhs, Leuψ[CH_2S]Val, or Leuψ[CH_2SO]Val and most probably accounts for the diminished potency of **3**, **5**, and **6**. In conclusion, the trans olefin, thioether, and corresponding sulfoxide appear to be acceptable mimics of a peptide bond in applications where hydrogen bonding is not critical for high binding affinity.

Experimental Section

General Procedures. All melting points were determined in open capillary tubes and are reported uncorrected. Thin-layer chromatography was performed on precoated plates of silica gel G-60 F-254 (E. Merck). Compounds were applied in loads of at least 40 μg and developed for 10–15 cm in the following solvent systems (all by volume): A, $CHCl_3$–EtOAc (4:1); B, CH_2Cl_2–EtOAc–HOAc (36:6:1); C, EtOAc–hexane (3:7). Visualization was performed by examination under UV light or by exposing the plate to chlorine vapors followed by starch–KI spray (1% each) or by ninhydrin spray. High-pressure liquid chromatography was performed on a Beckman HPLC equipped with a Model 165 dual wavelength detector. Amino acid analyses[31] were performed on a Dionex D-500 amino acid analyzer following hydrolysis in degassed 6 M HCl at 110 °C. Solid-phase synthesis was performed on a Beckman 990B synthesizer, Synthor 1000, or in manual apparatus. ^1H NMR was performed on a Varian EM390 or XL200 or on a Bruker-500 MHz instrument. ^{13}C NMR was performed on the Bruker instrument at 125.7 MHz. Optical rotations were taken on a Perkin-Elmer Model 241 instrument. Fast atom bombardment mass spectrometry (FAB-MS) was determined on a Varian-MAT-CH5-DF or a VG-2AB-2F mass spectrometer.

(4*S*)-4-[(*tert*-Butyloxycarbonyl)amino]-6-methylhept-2-enoic Acid Methyl Ester (Boc-Dhs-OMe). Boc-leucinol (2.17 g, 10 mmol) was dissolved in 25 mL of anhydrous DMSO (25 mL) containing triethylamine (4.17 mL, 30 mmol). The solution was stirred with a magnetic stirrer under nitrogen. An ice bath was applied, and a solution of sulfur trioxide–pyridine (4.77 g, 30 mmol) in anhydrous DMSO was added quickly in one portion. After 45 s the ice bath was removed, and the solution was stirred for 20 min under nitrogen at ambient temperature. The solution was poured into an ice slurry (150 mL) and extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with 10% citric acid, water, and 5% $NaHCO_3$ (2 × 75 mL each) and then dried over Na_2SO_4. The drying agent was filtered off and the filtrate was evaporated to dryness under reduced pressure. After being flushed with nitrogen, the product, Boc-leucinal,[32] weighed 2.08 g (97% yield). ^1H NMR showed an aldehyde proton at δ 9.7, the area of which when compared with the six methyl protons centered at δ 0.95 indicated complete conversion from Boc-leucinol. The oily Boc-leucinal was dissolved in MeOH (40 mL), and methyl (triphenylphosphoranylidene)acetate (4.0 g, 12 mmol) was added as a solid. The reaction was followed by TLC (system A) and appeared complete in 2 h. An ice–water slurry (100 mL) was added, and the mixture was extracted with Et_2O (2 × 200 mL). The combined Et_2O extracts were washed with water and dried over Na_2SO_4. After the drying agent was filtered off and the solvent was evaporated, 5.45 g of sticky white solid was left. This was purified by flash chromatography[33] on a 3 × 20 cm column of silica gel 60 (43–60 μm) that had been equilibrated with toluene. The sample was loaded in toluene, and the column was eluted with toluene (100 mL), toluene–EtOAc (19:1, 300 mL), and finallly toluene–EtOAc (9:1, 200 mL). The eluate was collected in 15-mL fractions, and the product was detected by TLC (system A). Fractions containing an apparently single

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component at R_f 0.5 were pooled and evaporated to give 2.11 g (78% yield) of the titled compound as a colorless oil. ^{1}H NMR showed a complex pattern for the vinyl proton region (δ 5.57–7), the area of which, when compared to the methyl protons from the ester functionality, are in a ratio of 2:3. The resonance at δ 6.9 was a quartet J's of 16 and 3 Hz corresponded to 0.7 H. The multiplet at δ 5.6–6.3 shows J's of 3, 12, and 16 Hz and corresponded to 1.3 H. Thus the ^{1}H NMR tends to indicate a Z/E ratio of about 3:7. HPLC on a Merck Hibar EC column at 2 mL/min in H_2O–CH_3CN (1:1) containing 0.2% CF_3CO_2H showed two peaks emerging at 7.13 and 7.69 min in a ratio of 67:33, respectively.

***N*-(*tert*-Butyloxycarbonyl)-(*E*)-(4*S*)-4-amino-6-methylhept-2-enoic Acid (Boc-Dhs-OH).** Boc-Dhs-OMe (2.0 g, approximately 7.5 mmol) was dissolved in MeOH (50 mL) and H_2O (10 mL) followed by 1 M NaOH (8 mL). Progress of the saponification was followed by HPLC on a Synchropack RP-P column in H_2O–CH_3CN containing 0.2% CF_3CO_2H with a gradient going from 30% to 60% CH_3CN over 2–14 min at a flow rate of 1.5 mL/min. The reaction was complete in 20 h. Water (100 mL) was added, and the MeOH was evaporated. The aqueous phase was extracted with Et_2O and cooled, EtOAc (100 mL) was added, and then the mixture was made acidic with concentrated HCl. The phases were separated, and the aqueous phase was extracted once more with EtOAc (100 mL). The combined EtOAc phases were washed with H_2O until the washes were pH 6 and then dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was evaporated in vacuo to give 2.01 g of colorless oil. The entire batch was subjected to flash chromatography on a 3 × 20 cm column of silica gel G (43–60 μm) that had been equilibrated with CH_2Cl_2. The column was eluted with CH_2Cl_2 (100 mL) and then with CH_2Cl_2–EtOAc–HOAc (36:6:1). The eluate was collected in 15-mL fractions. Product was detected by TLC (system B), and fractions comprising the main component were pooled and evaporated with added toluene to give 0.71 g of colorless oil that crystallized upon standing. This material was recrystallized from toluene–hexane to give 430 mg (21.5% yield): mp 110–111 °C; $[\alpha]_D$ −20.6 °C (c 2.03, $CDCl_3$). HPLC as described above showed the product to be 99.3% one component by UV at 220 and 250 nm: ^{1}H NMR ($CDCl_3$) δ 0.95 (d, 6 H), 1.1–1.8 (m, 12 H with sharp singlet at 1.4), 4.1–4.7 (broad peak, 2 H), 5.9 (d, 1 H), 6.95 (q, 1 H). Anal. ($C_{13}H_{23}NO_4$) C, H, N. A second crop of 200 mg (10%) was obtained from less pure fractions from flash chromatography (mp 106–109 °C after recrystallization).

Another component (390 mg), which crystallized upon standing, was isolated from early fractions. Although it was expected that this would be the Z isomer, low-field ^{1}H NMR revealed the absence of vinyl protons. High-field ^{1}H NMR showed δ 0.73 (d, J = 6.70 Hz, 6 H), 1.24 (s, 9 H), 1.96 (heptet, J = 6.70 Hz, 1 H), 2.14 (d, J = 6.70 Hz, 2 H), 2.40 (t, J = 6.60 Hz, 2 H), 2.53 (t, J = 6.60 Hz, 2 H). ^{13}C NMR revealed, in addition to the two carbonyl carbons at 157.76 and 176.73 ppm for the Boc and carboxylic acid functions, respectively, two quaternary carbons, one at 79.69 ppm corresponding to the Boc group and another at 94.34 ppm. High-resolution FAB-MS showed $[M + H]^+$ of 258.1692, which corresponded to $C_{13}H_{24}NO_4$ or $C_{13}H_{23}NO_4$ for m. When taken with IR data (carbamate NH at 3288 cm^{-1}, amide II at 1549 cm^{-1}, carbamate C=O at 1725 cm^{-1}, and lactone carbonyl at 1746 cm^{-1}) and the nonacidic character of the molecule, 4-[(*tert*-butyloxycarbonyl)amino]-4-hydroxy-6-methylheptanoic acid lactone most closely fits the data.

H-Pro-His-Pro-Phe-His-Dhs-Ile-His-D-Lys-OH (3). From 1.5 g of chloromethylated polystyrene-1%-divinyl benzene resin to which Boc-D-Lys(Cl-*Z*) had been esterified by the cesium salt method[34] to the extent of 0.5 mmol of lysine per gram of resin, the peptide resin was extended in a stepwise manner by successively coupling Boc-His(Tos)-OH, Boc-Ile-OH, Boc-Dhs-OH, Boc-His(Tos)·DCHA, Boc-Phe-OH, Boc-Pro-OH, Boc-His-(Tos)·DCHA, and Boc-Pro-OH. One coupling cycle consisted of the nine steps outlined in Table I. Solvent and reagent volumes were 20 mL. In a typical cycle the Boc-amino acid (2.0 mmol) was preactivated with dicyclohexylcarbodiimide (DCC) (1.0 mmol) in CH_2Cl_2 to form the symmetrical anhydride. After the dicyclohexylurea was filtered off and the solvent was evaporated, the anhyride was dissolved in DMF and added to the peptide resin at step 7 of the cycle. In the case of Boc-Dhs-OH, 1.0 mmol was preactivated in DMF with DCC (1.0 mmol) in the presence of 1-hydroxybenzotriazole (1.0 mmol, HBT).[35] Whenever Boc-His(Tos)·DCHA was coupled to the peptide resin, the dicyclohexylamine salt form was activated with (benzotriazolyloxy)-(trisdimethylamino)phosphonium hexafluorophosphate (LeBop).[36] Also, whenever the Boc group was removed from an N-terminal His residue, two 25-min treatments with 50% CF_3CO_2H–CH_2Cl_2 were used instead of a single 25-min treatment. Completeness of coupling was monitored by the ninhydrin test,[37] and double couplings were performed if required. After removal of the terminal Boc group, 1.80 g of peptide resin was recovered.

The entire batch of peptide resin was treated in HF containing 10% anisole (by volume) for 1 h at 0 °C. After evaporation of the HF, the peptide and resin were triturated with Et_2O, and the peptide was extracted from the resin with CF_3CO_2H. After the resin was filtered off, the volume of filtrate was reduced to 5 mL by evaporation, and the peptide was precipitated by addition of Et_2O to yield 700 mg of crude peptide. A portion (320 mg) was subjected to gel filtration chromatography on a 2.2 × 90 cm column of Sephadex G-15 eluting with 50% aqueous HOAc. The eluant was monitored at 260 nm and collected in 2.3-mL fractions. Fractions (48–73) comprising the main component were pooled, reduced to 20 mL by rotary evaporation, diluted with H_2O, and lyophilized (240 mg). A portion of this material (104 mg) was further purified by high-performance reverse-phase chromatography on a 2.2 × 54 cm column of Vydac C_{18} (25 μm). The column was eluted at 4 mL/min with H_2O–CH_3CN (containing 0.2% CF_3CO_2H) by using a gradient of 18–25% CH_3CN over 2.5 h. Fractions comprising the main component were pooled for maximum purity, rather than yield, to give 13 mg. By HPLC on a 4.1 × 240 mm Synchropak RP-P column, the product eluted as a single peak in H_2O–CH_3CN (0.2% CF_3CO_2H) going from 18% to 25% CH_3CN over 10 min. Amino acid analysis: Pro, 1.99; Ile, 0.98; Phe, 1.05; His, 2.92; Lys, 1.06. FAB mass spectroscopy showed $[M + H]^+$ at m/z 1151.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-[(*p*-tolylsulfonyl)oxy]-4-methylpentane. Boc-L-leucinol (2 g, 9.2 mmol) was dissolved in pyridine (15 mL). To the stirred cold (ice bath) solution was added tosyl chloride (3.5 g, 184 mmol) in three equal portions. The solution was stored 12 h at −20 °C than at 4 °C for 24 h and poured into 150 mL of ice slurry. The pH was adjusted to 2.5 with 6 N HCl, and the resulting mixture was extracted with EtOAc (3 × 100 mL). The combined EtOAc extracts were washed with H_2O and saturated NaCl solution and then dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was evaporated under reduced pressure, leaving a colorless oil. The crude product was purified by column chromatography on silica gel with hexane–CH_2Cl_2 (1:1). Combination of appropriate fractions yielded 2.9 g (82%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 7.20–7.80 (4 H, m), 4.40–4.70 (1 H, broad), 3.50–4.10 (3 H, m), 2.3 (3 H, s), 1.10–1.70 (3 H, m), 1.3 (9 H, s), 0.6–0.9 (6 H, m). Anal. ($C_{18}H_{29}NSO_5$) C, H, N.

(2*S*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-[((2*S*)-2-mercapto-3-methylbutyryl)oxy]-4-methylpentane (Boc-Leuψ[CH$_2$S]Val). To an argon-purged solution of KOH (0.63 g, 11 mmol) in H_2O (3 mL) was added (2*S*)-2-mercapto-3-methylbutyric acid[38] (0.5 g, 3.75 mmol) in DMF (2 mL) followed by (2*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-[(*p*-tolylsulfonyl)-oxy]-4-methylpentane (1.67 g, 4.75 mmol). After being stirred under argon for 96 h at room temperature, the solution was poured

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into 100 mL of cold H_2O, and the mixture was washed with Et_2O (2 × 50 mL). The aqueous phase was saturated with citric acid and extracted with EtOAc (3 × 50 mL). The combined EtOAc phase was washed with H_2O and saturated NaCl solution and then dried over $NaSO_4$. The drying agent was filtered off, and the solvent was evaporated, leaving an oily residue. The product was purified by column chromatography on silica gel with MeOH–$CHCl_3$ (5:95) to give 0.90 g (72% yield) of title compound: ^1H NMR ($CDCl_3$) δ 9.2 (1 H, broad), 4.1 (1 H, broad), 2.9–3.2 (1 H, q), 2.75 (1 H, d), 1.80–2.20 (3 H, m), 1.20–1.70 (3 H, m), 1.40 (9 H, s), 0.7–1.1 (12 H, m); FAB-MS $[M + H]^+$ at m/z 333; $[\alpha]_D$ −95.7° (*c* 1.5, MeOH).

H-Pro-His-Pro-Phe-His-Leuψ[CH_2S]Val-Ile-His-D-Lys-OH (5). From 1.1 g of Boc-D-Lys(Cl-*Z*) resin described above, the title compound was synthesized as outlined in Table I. In this case coupling steps were performed by preactivating 2 mmol of Boc-pretreated amino acid with 2 mmol of HBT and 2.2 mmol of DCC in DMF for 20 min. This preactivation mixture was filtered, added to the resin, and shaken until the ninhydrin test indicated complete reaction. Histidine was coupled as Boc-His(Tos)·DCHA with LeBop. The peptide was cleaved from the resin with HF–anisole (10:1, v/v), extracted, precipitated, and isolated as described for **3** to yield 410 mg of crude product. The entire batch was subjected to gel filtration chromatography in 30% HOAc to give 370 mg of partially purified material. A portion (170 mg) was purified by reverse-phase chromatography on the column described above using a linear gradient of CH_3CN in H_2O (containing 0.1% CF_3CO_2H) going from 16% to 18% CH_3CN over 2.5 h. Fractions containing the main component were pooled, and the peptide was isolated by lyophilization, 122 mg. HPLC on a 4.1 × 240 mm Synchropak RP-P column under the same conditions as for **3** showed the product to elute as a single peak. Amino acid analysis: Pro, 2.01; Ile, 0.98; Phe, 1.02; His, 2.97; Lys, 1.00. FAB-MS $[M + H]^+$ at m/z 1227.

Pro-His-Pro-Phe-His-Leuψ[CH_2SO]Val-Ile-His-D-Lys (6). A portion of Pro-His-Pro-Phe-His-Leuψ[CH_2S]Val-Ile-His-D-Lys (40 mg, 0.032 mmol) was dissolved in HOAc (1 mL) containing 30% H_2O_2 (25 μL). Progress of the oxidation was followed by HPLC and deemed complete after 20 min. After evaporation of the solvent under reduced pressure, the product was purified by reverse-phase chromatography on a 2.5 × 13 cm column of Vydac-C_{18} by using a linear gradient of CH_3CN in H_2O (containing 0.2% CF_3CO_2H) from 12% to 28% CH_3CN. The eluate was monitored by UV at 220 nm, fractions containing the main component were pooled, and the peptide was isolated by lyophlization; 32 mg. HPLC as above showed a single peak. Amino acid analysis: Pro, 1.99; Ile, 0.96; Phe, 1.0; His, 2.9; Lys, 1.00. FAB-MS $[M + H]^+$ at m/z 1243.

H-Pro-His-Pro-Phe-His-Sta-Ile-His-D-Lys-OH. From 1.0 g of Boc-D-Lys(Cl-*Z*) resin, the title compound was synthesized as described for **5**. Boc-Sta was coupled without protection of the hydroxyl function. The peptide was cleaved from the resin, extracted, precipitated, and isolated as previously described to yield 450 mg of crude product. A portion (200 mg) was purified by reverse-phase chromatography on a 2.5 × 32 cm column of Vydac-C_{18} by using a linear gradient of CH_3CN in H_2O (containing 0.2% CF_3CO_2H) from 5% to 30% CH_3CN. Peptide was detected by monitoring the eluate at 220 nm, and fractions comprising the main peak were pooled and lyophilized, 165 mg. Single peak by HPLC as above. Amino acid analysis: Pro, 1.90; Ile, 0.94; Phe, 1.0; His, 2.8; Lys, 1.05. FAB-MS $[M + H]^+$ at m/z 1169.

Boc-Leuψ[CH_2NH]Val-OBzl. Boc-leucinal (30 mmol), prepared as described for Boc-Dhs, dissolved in 75 mL of anhydrous tetrahydrofuran was added to Val-OBzl (31 mmol) under inert atmosphere followed by 4 g of 4-Å molecular sieves. After 30 min, 1.85 g (31 mmol) of sodium cyanoborohydride was added, and the pH was adjusted to 7 with HOAc. After 30 min, CH_2Cl_2 (200 mL) and H_2O (100 mL) were added, and the molecular sieves were filtered off. The phases of the filtrate were separated, and the CH_2Cl_2 phase was extracted with H_2O and then dried over Na_2SO_4. After the drying agent was filtered off, the solvent was evaporated in vacuo, and the residue was subjected to flash chromatography on a 4 × 30 cm column of silica gel 60 (230–400 mesh) in EtOAc–hexane (1:4, v/v). The eluate was collected in 30-mL fractions, and the product was detected by TLC (system C). Fractions corresponding to the main component were pooled and evaporated in vacuo. The product crystallized upon standing: 6.9 g (52% yield); mp 68–69 °C; $[\alpha]^{23}_D$ −36.8° (*c* 1.5, CH_3OH). Single spot TLC R_f (C) 0.48; ^1H NMR showed the ratio between the Val and Leu methyl groups (δ 0.95, 12 H) and the aromatic protons (δ 7.4, s, 5 H) to be the expected value of 2.6:1. Anal. ($C_{23}H_{38}N_2O_4$) C, H, N.

Boc-Leuψ[CH_2NH]Val-OH. The title compound was prepared by hydrogenation of the benzyl ester as previously described.[19]

H-Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-D-Lys-OH (7). With 1.67 g of Boc-D-Lys(Cl-Z) resin as the starting material, solid-phase synthesis was carried out as shown in Table I with the same preactivation coupling protocol as described for the synthesis of **5** but with only 1.4 equiv of amino acids. For the coupling of Boc-Leuψ[CH_2NH]Val-OH, 1.4 equiv were suspended in CH_2Cl_2–DMF (1:1) and added to the resin followed by 1.4 equiv of HBT and 1.5 equiv of DCC. After being mixed overnight, the suspension had dissolved, and the ninhydrin test indicated complete reaction. In order to verify the completeness of the coupling reaction, a small amount of tripeptide resin and pentapeptide resin were treated with HF–anisole (10:1), and the crude products were compared by HPLC. No detectable tripeptide was present in the crude pentapeptide. The synthesis was continued to yield 2.37 g of protected peptide resin. A portion (1.18 g) was treated with HF–anisole and the peptide was extracted and precipitated as described earlier for **3** to yield 420 mg of crude product. A portion (50 mg) was subjected to reverse-phase chromatography on a 2.2 × 28 cm column of Vydac-C_{18} by using a linear gradient of CH_3CN in H_2O (containing 0.2% CF_3CO_2H) going from 10% to 30% CH_3CN. Peptide material was detected by monitoring the eluate at 254 nm, and fractions containing the product were pooled for maximum purity rather than quantity to yield 14 mg. HPLC (conditions as described for **3**) showed a single peak. Amino acid analysis: Pro, 2.09; Ile, 0.4; Phe, 1.01; His, 2.82; Lys 1.08. The bond between Leuψ[CH_2NH]Val and Ile hydrolyzes very slowly. The ratio for Ile increases to 0.7 after 120 h of hydrolysis. FAB-MS $[M + H]^+$ at m/z 1210.

H-Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-D-Lys-OH (1). The synthesis was begun from 1.67 g of Boc-D-Lys(Cl-Z) resin as outlined in Table I by using the same coupling protocol as described for the synthesis of **5**. The 2,6-dichlorobenzyl group was used to protect the side chain of tyrosine. A 1.56-g portion of the protected peptide resin was treated with HF–anisole and the peptide was extracted and precipitated with Et_2O as described previously for **3** to yield 742 mg of crude product. The crude peptide was converted to the acetate salt by dissolving in 30% HOAc and stirring with 10 mL of IR-45 resin (OAc^- form). After the resin was filtered off and diluted with water, the peptide was recovered by lyophilization. A portion (100 mg) was dissolved in 2 mL of the upper and 2 mL of lower phases of the solvent system 1-BuOH–toluene–HOAc–1.5% pyridine in H_2O and applied to a 3 × 68 cm column of Sephadex G-25 (fine) that had previously been equilibrated with the lower and the upper phases of the solvent system. The column was eluted with upper phase, and the eluate was collected in 10-mL fractions. Peptide was detected by the method of Lowry et al.[39] The product emerged with a peak maximum at R_f 0.21. Tubes comprising the product were pooled, the organic solvents were evaporated, and the aqueous phase was lyophilized to give 40 mg of product. HPLC as for **3** showed a single sharp peak. Amino acid analysis: Pro, 2.14; Val, 0.95; Tyr, 0.98; Phe, 2.91; His, 2.02; Lys, 1.00.

H-Pro-His-Pro-Phe-His-Phe-Phe-Ile-His-D-Lys-OH (2) From 2.9 g of Boc-D-Lys(Cl-Z) resin, the peptide was synthesized as outlined in Table I by using the same coupling protocol as described for the synthesis of 1. A portion (2.0 g) of the protected peptide resin was treated with HF–anisole, washed with Et_2O, and extracted with aqueous HOAc to yield 640 mg of crude product. A portion (140 mg) was dissolved in 3 mL of DMF and chromatographed on a 2.5 × 30.0 cm column of Vydac C-18, which had been previously equilibrated with CH_3CN–H_2O (1:9 containing 0.2% CF_3CO_2H). The peptide was eluted from the column by using a linear gradient of increasing CH_3CN from 10% to 33%,

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detected at 220 nm, and the eluate was collected in 5-mL fractions. Tubes comprising the product were pooled, the organic solvent was evaporated, and the aqueous phase was lyophilized to give 58 mg of title compound. HPLC as for **3** showed a single sharp peak. Amino acid analysis: Pro, 2.03; Ile, 0.79; Phe, 3.0; His, 3.06; Lys, 1.04.

Plasma Renin Inhibitory Activity Testing. Lyophlized human plasma with 0.1% EDTA was obtained commercially (New England Nuclear). The angiotensin I generation step utilized 250 μL of plasma, 2.5 μL of phenylmethylsulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor in a 1% Tween 80 in water vehicle. Incubation was for 90 min at 37 °C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Clinical Assays). Plasma renin activity values for inhibitor tubes were compared to control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values, which were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition. Details of the assay are given in ref 27.

Acknowledgment. We gratefully acknowledge G. Skala for synthetic assistance, J. Nielsen and L. Baczynskyj for FAB mass spectroscopy, P. A. Meulman for IR spectroscopy, and P. Lupina for excellent secretarial work.

Registry No. 1, 95190-14-0; **2**, 103436-26-6; **3**, 114423-48-2; 4, 103477-15-2; **5**, 114423-49-3; **6**, 114443-30-0; **7**, 114489-13-3; BOC-Dhs-OMe, 114423-50-6; BOC-(*Z*)-Dhs-OMe, 114423-51-7; BOC-Dhs-OH, 101670-81-9; BOC-D-Lys(Cl-Z)-OH, 57096-11-4; BOC-His(Tos)-OH, 35899-43-5; BOC-Ile-OH, 13139-16-7; BOC-His(Tos)-OH·DCHA, 65057-34-3; BOC-Phe-OH, 13734-34-4; BOC-Pro-OH, 15761-39-4; BOC-Leuψ[CH_2S]Val-OH, 114443-31-1; BOC-Sta-OH, 58521-49-6; H-Val-OBzl, 21760-98-5; BOC-Leuψ-[CH_2NH]Val-OBzl, 82252-38-8; BOC-Leuψ[CH_2NH]Val-OH, 82252-39-9; Ph_3P=CHCOOMe, 2605-67-6; (*S*)-HOOCCH(SH)-$CHMe_2$, 114423-53-9; BOC-leucinol, 82010-31-9; BOC-leucinal, 58521-45-2; 4-[(*tert*-butyloxycarbonyl)amino]-4-hydroxy-6-methylheptanoic acid lactone, 114423-52-8; (2*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-[(*p*-tolylsulfonyl)oxy]-4-methylpentane, 112157-30-9; renin, 9015-94-5.

Polycyclic Aryl- and Heteroarylpiperazinyl Imides as 5-HT_{1A} Receptor Ligands and Potential Anxiolytic Agents: Synthesis and Structure–Activity Relationship Studies

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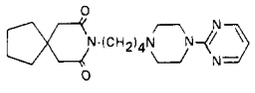
A series of polycyclic aryl- and heteroarylpiperazinyl imides were prepared and tested in various receptor-binding and behavioral tests. Parameters measured included in vitro inhibition of D_2 and 5-HT_{1A} receptor binding, inhibition of apomorphine (APO) induced stereotyped and climbing behavior, and activity in blocking conditioned avoidance responding (CAR). Several compounds demonstrated moderate to high affinity for the 5-HT_{1A} receptor binding site; compounds **27** and **36** containing the serotonin mimetic (*o*-methoxyphenyl)piperazinyl moiety and compounds **42** and **50** containing the 2-pyrimidinylpiperazinyl moiety displayed the highest affinity, being equal to that of the 5-HT_{1A} agonist 8-OH-DPAT (K_i = 1–1.3 nM). In addition to affinity at 5-HT_{1A} binding sites, many compounds were active in blocking CAR. Compound **34**, 2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]hexahydro-4,7-etheno-1*H*-cyclobut[*f*]isoindole-1,3(2*H*)-dione, demonstrated 3 times the activity of buspirone, blocking CAR in rats with an AB_{50} of 13 mg/kg. It also displayed high affinity for the 5-HT_{1A} receptor (K_i = 16 nM), which is at least 20 times higher than its affinity for D_2 (K_i = 345 nM) and 5-HT_2 (K_i = 458 nM) receptors. Compound **34** was selected for further preclinical and pharmacokinetic evaluations for possible development as an anxiolytic agent. Structure–activity relationships within this series are discussed.

In the last decade serotonin (5-HT) has been implicated in anxiety, depression, insomnia, and other behavioral disorders.[1-3] Multiple subtypes of 5-HT_1 receptors have been recently identified with radioligand binding assays which include 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptor subtypes.[4] In recent years, a growing body of literature has attributed the activity of the non-benzodiazepine anxiolytic agents buspirone (**1**) and ipsapirone (**2**) to their selective activation of the 5-HT_{1A} receptor.[5-7] 5-HT_{1A} receptors are found predominantly in the hippocampus and dorsal raphe nucleus[4] and are sensitive to spiperone and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT).[8] Preclinical evidence for a 5-HT_{1A} receptor mediated mechanism of anxiolysis of non-benzodiazepine drugs such as **1** and **2** has been provided mainly through in vitro and in vivo radioligand binding assays.[6]



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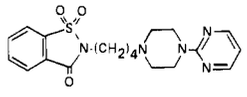

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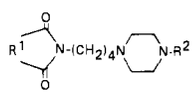

3

The launch of buspirone (**1**) has created much interest in alternative, non-benzodiazepine treatment of anxiety, and consequently, synthetic efforts have been devoted by us and others in an attempt to develop potent ligands with high affinity and selectivity for the 5-HT_{1A} receptor subtype.

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