Synthesis and Biological Activity of Carboxylic Acid Replacement Analogues of the Potent Angiotensin Converting Enzyme Inhibitor 5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl-L-proline

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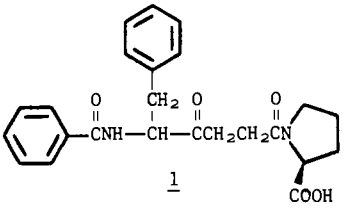
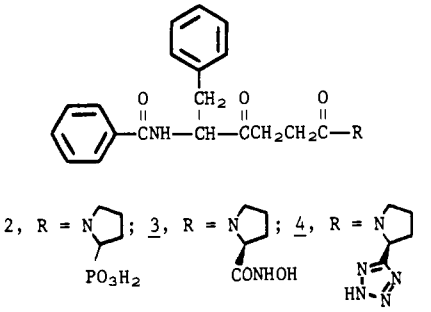
The carboxylic acid group on the proline of 1 was replaced by a phosphoric acid, a hydroxamic acid, and a tetrazole to give compounds **2–4**, respectively. Testing of **2–4** as angiotensin converting enzyme (ACE) inhibitors gave I_{50} values of 100, 1.6, and 22 μM, respectively, compared to 0.07 μM for **1**. A hydroxamic acid derivative of the ketomethylene pentapeptide analogue **18** was then synthesized. This compound, **17**, had an ACE I_{50} of 0.011 μM compared to 0.0076 μM for **18**. Oral administration of 10 mg/kg of **17** to renal hypertensive rats had no effect on blood pressure or heart rate.

Angiotensin converting enzyme (ACE) is responsible for cleaving the carboxy terminal dipeptide from decapeptide angiotensin I to produce the potent vasoconstrictor angiotensin II. Compounds that inhibit this process have been found to act as blood pressure lowering agents in hypertensive animals and man.[1] The ketomethylene analogue **1** of the tripeptide Bz-Phe-Gly-Pro has been shown to be a potent ACE inhibitor with poor in vivo activity as an antihypertensive agent.[2] Studies of radiolabeled derivatives of **1**[3] indicate that after intravenous administration in the rat **1** has only a 10-min half-life in the whole blood due to its rapid excretion in the bile.



1

It was theorized that if one could structurally alter **1** to prevent its rapid excretion in the bile, then a high enough in vivo blood level could be maintained to allow for good ACE inhibition and antihypertensive activity in the rat. Studies of factors that control the biliary excretion of compounds suggest that compounds that are highly concentrated in the bile are usually carboxylic acids with molecular weights of 300 or greater.[4,5] In an attempt to prevent rapid biliary excretion of **1** without greatly lowering its ACE inhibition activity, the carboxylic acid group was replaced by other acidic functionalities to yield compounds **2–4**. The effect on ACE inhibition that these carboxylic acid replacement groups had on analogues of the potent ACE inhibitor captopril[6] is shown in Table I. This table indicates that the carboxylic acid replacement groups chosen for **2–4** do not lower ACE inhibition by more than 10-fold when substituted into captopril analogues. If similar relative ACE inhibition activities are found between



2–4 and **1**, then **2–4** would be good candidates for in vivo studies in hypertensive rats.

Chemical Methods

For the synthesis of compound **2**, (*R,S*)-2-(diethoxyphosphinyl)pyrrolidine[7] (**10**) was condensed with the acid **8** with use of dicyclohexylcarbodiimide as the coupling reagent to yield the diethyl phosphonate ester **13** (Scheme I). Reaction of **8** with trimethylsilyl bromide[7] removed the ester groups to yield **2**.

To prepare **3**, first the hydroxamic acid of *N*-(*tert*-butyloxycarbonyl)-L-proline (**7**) was prepared from *N*-Boc-L-Pro by the procedure described by Petrillo and Ondetti.[8] After cleavage of the Boc group with trifluoroacetic acid, the resulting L-proline hydroxamic acid TFA salt **11** was condensed with the *N*-hydroxysuccinimide ester **9** to yield **3**.

For the synthesis of **4**, the tetrazole derivative of *N*-(carbobenzyloxy)-L-proline (**6**) was first prepared by the method described by Grzonka et al.[9] from the nitrile **5**. After cleavage of the *N*-Cbz blocking group by hydrogenolysis, the resulting tetrazole L-proline derivative **12** was condensed with the mixed anhydride of **8** to yield compound **4**.

Results and Discussion

The ACE inhibition activity of **2–4** is given in Table II. As was seen in Table I with the captopril analogues, the hydroxamic acid analogue **3** is the most potent analogue of **1** that was synthesized. It was not felt however that an ACE inhibitor with an I_{50} of 1.6 μM would show significant activity as an antihypertensive agent in renal hypertensive rats.

Rather than test **3** in vivo, it was decided to make the hydroxamic acid derivative **17** (Scheme II) of the potent ketomethylene pentapeptide analogue **18**.[10] Compound

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(2) Meyer, R. F.; Nicolaides, E. D.; Tinney, F. J.; Lunney, E. A.; Holmes, A.; Hoefle, M. L.; Smith, R. D.; Essenburg, A. D.; Kaplan, H. R.; Almquist, R. G. *J. Med. Chem.* **1981**, *24*, 964.
(3) Almquist, R. G.; Steeger, J.; Jackson, S.; Mitoma, C. *Life Sci.* **1985**, *37*, 299.
(4) Klaassen, C. D.; Eaton, D. L.; Cagen, S. Z. In "Progress in Drug Metabolism"; Bridges, J. W., Chasseaud, L. F., Eds.; Wiley: 1981; Vol. 6, pp 1–75.
(5) Klaassen, C. D.; Watkins, J. B., III *Pharmacol. Rev.* **1984**, *36*, 1.
(6) Petrillo, E. W., Jr.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1.
(7) Petrillo, E. W., Jr.; Spitzmiller, E. R. *Tetrahedron Lett.* **1979**, 4929.
(8) Petrillo, E. W., Jr.; Ondetti, M. A. U.S. Patent 4 284 561, 1981.
(9) Grzonka, Z.; Rekowskas, E.; Tiberek, B. *Tetrahedron* **1971**, *27*, 1783.

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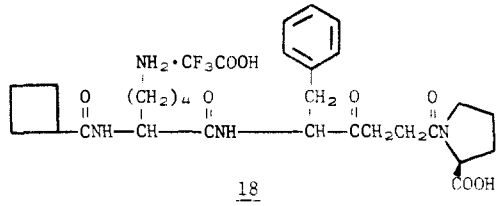


18

18 is also ineffective in lowering blood pressure in renal hypertensive rats and is rapidly excreted into the bile.[10] As can be seen in Table II, compound 17 was a very potent ACE inhibitor in vitro. This compound was tested in six renal hypertensive rats by Pharmakon Labs, Waverly, PA, to evaluate its antihypertensive activity. Oral administration of 10 mg/kg of 17 to the renal hypertensive rats had no effect on blood pressure or heart rate.

Obviously, the replacement of the carboxylic acid group with a hydroxamic acid group does not increase its antihypertensive activity in rats as was hoped. Considering that 17 is a potent ACE inhibitor, it seems likely that like 18 it is rapidly excreted by the liver into the bile. This would mean that the liver's biliary excretion pathway does not distinguish between compounds having a hydroxamic acid or a carboxylic acid group.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-melt instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Mass spectra were taken on a LKB 9000 GC–MS spectrometer. Infrared spectra were taken on a Perkin-Elmer 710B spectrometer. ^{1}H NMR spectra were taken with a Varian EM390 spectrometer. ^{13}C NMR spectra were taken with a Varian XL100 spectrometer. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Preparative TLC was carried out on 20 cm × 20 cm plates from Analtech coated with 1500 μm of silica gel GF. Flash chromatography was conducted on 230–240-mesh silica gel 60 from EM Reagents. Evaporations were performed at 40 °C under house vacuum on a Büchi rotavapor. Elemental analyses were conducted by Galbraith Labs, Knoxville, TN. Some of the compounds could not be totally freed of solvent even on heating under reduced pressure. The elemental analyses of these compounds have been recorded with solvent present. The existence of solvents of crystallization was confirmed by ^{1}H NMR whenever possible.

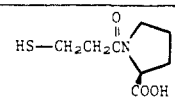
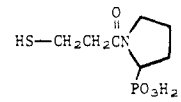
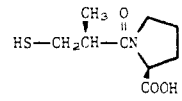
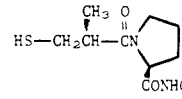
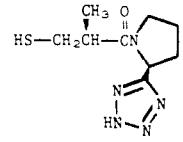
***N*-(Benzyloxycarbonyl)-L-proline Nitrile (5).** Phosphorus oxychloride (1.05 mL) in dichloromethane (2.1 mL) was added over 10 min to a solution of *N*-(benzyloxycarbonyl)-L-proline amide (2.09 g, 8.43 mmol) in dry pyridine (10.5 mL) at −5 to −10 °C under N_2. The mixture was stirred at −5 to −10 °C for 1 h and then it was poured onto ice (50 g) and extracted with diethyl ether (3 × 50 mL). The ether layer was washed with saturated cupric sulfate solution (100 mL) and saturated sodium chloride solution (100 mL), dried ($MgSO_4$), and evaporated in vacuo to give 1.50 g of **5** as a pale yellow oil (77%): R_f 0.63 (ethyl acetate); IR ($CHCl_3$) 3005 (m), 2965 (m), 2900 (m), 2250 (w), 1700 (s), 1590 (w), 1500 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.42 (5 H, s), 5.21 (2 H, s), 4.60 (1 H, m), 3.53 (2 H, m), 2.22 (4 H, m); mass spectrum, *m/e* 230 (M^+), 123 (M − $PhCH_2O$). Anal. ($C_{13}H_{14}N_2O_2$) C, H, N.

5-[1-(Benzyloxycarbonyl)pyrrolidin-2-yl]tetrazole (6). A mixture of **5** (230 mg, 1.00 mmol), sodium azide (72 mg, 1.04 mmol), ammonium chloride (59 mg, 1.10 mmol), and dry DMF (0.75 mL) was stirred at 90–95 °C under nitrogen for 6 h. The mixture was poured onto ice (10 g), acidified to pH 2 with diluted HCl, and extracted with $CHCl_3$ (3 × 10 mL). The $CHCl_3$ layer was washed with water (10 mL) and saturated sodium chloride solution 10 mL, dried (Na_2SO_4), and evaporated in vacuo to give 322 mg of crude material. This was purified by preparative TLC, eluting with acetic acid/EtOAc (1:99), to give 273 mg of **5** (100%), which was used without further purification: R_f 0.50 (acetic acid/EtOAc, 1:99); IR ($CHCl_3$) 3120 (m), 3000 (s), 2970 (s), 2900 (s), 1670 (s), 1590 (w), 1540 (m), 1500 (m) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.32 (5 H, s), 5.20 (3 H, m), 3.59 (2 H, m), 2.20 (4 H, m); mass spectrum, *m/e* 274 ($M + H^+$), 273 (M^+), 230 (M − HN_3).

5-Pyrrolidin-2-yltetrazole (12). Compound **6** (273 mg, 1.0 mmol) and 10% palladium on charcoal (55 mg) in acetic acid/water (9:1, 15 mL) was stirred under H_2 at room temperature for 4 h. The mixture was filtered through Celite and the filtrate was evaporated in vacuo to give 189 mg of crude material, which was recrystallized from CD_3CO_2D/Et_2O to give 95 mg of **12** (68%): mp 272–273 °C; IR (neat) 3400 (br, m), 2940 (s), 2540 (br s), 2000 (br m), 1705 (s) cm^{-1}; ^{1}H NMR (CD_3CO_2D) δ 5.26 (1 H, m), 3.67 (2 H, m), 2.28 (4 H, m); mass spectrum, *m/e* 139 (M^+), 111 (M − N_2). Anal. ($C_5H_9N_5 \cdot {}^1/_5H_2O$) C, H, N.

5-[1-[5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl]-pyrrolidin-2-yl]tetrazole (4). Ethyl chloroformate (0.03 mL, 0.308 mmol) was added to a solution of **8**[11] (100 mg, 0.308 mmol) and triethylamine (0.043 mL, 0.308 mmol) in $CHCl_3$ (1 mL) at −5 °C with stirring under N_2. After 10 min a solution of **12** (47 mg, 0.339 mmol) and triethylamine (0.047 mL, 0.339 mmol) in dry DMF (1 mL) was added and the mixture was vigorously stirred for 30 min at 0 °C followed by a further 3 h at room temperature. The mixture was poured into ice-cold 1 N HCl (10 mL) and was extracted with $CHCl_3$ (3 × 10 mL). The $CHCl_3$ layer was washed with water (10 mL) and saturated sodium chloride solution (10 mL), dried (Na_2SO_4), and evaporated in vacuo to give 266 mg of crude material. This was heated in a drying pistol overnight to remove DMF and purified by preparative TLC, eluting with acetic acid/EtOAc (1:99), to give 59 mg of **4** (43%): R_f 0.17 (acetic acid/EtOAc, 1:99); IR ($CHCl_3$) 3400 (w), 2985 (m), 2945 (m), 2910 (m), 2860 (m), 1710 (m), 1640 (s), 1575 (m), 1505 (s) cm^{-1}; ^{1}H NMR

Table I. ACE Inhibition Activity of Acid Replacement Analogues of Captopril

compd	ACE inhibn:[a] I_{50}, μM
	0.20
	1.7
 (captopril)	0.023
	0.022
	0.26

[a] Results taken from ref 6.

Table II. Inhibition Results with Porcine Plasma Angiotensin Converting Enzyme[a]

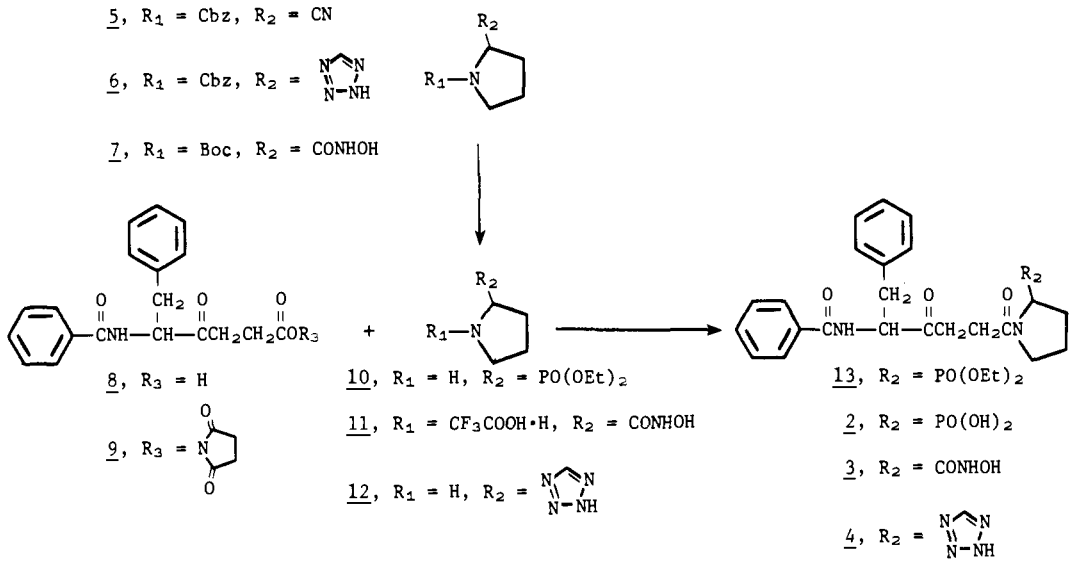
compd	I_{50},[b] μM	compd	I_{50},[b] μM
1	0.07	4	22
2	100	17	0.011
3	1.6	18	0.0076

[a] The enzyme assay used is described in ref 11. [b] All values are the average of results obtained in two or more experiments.

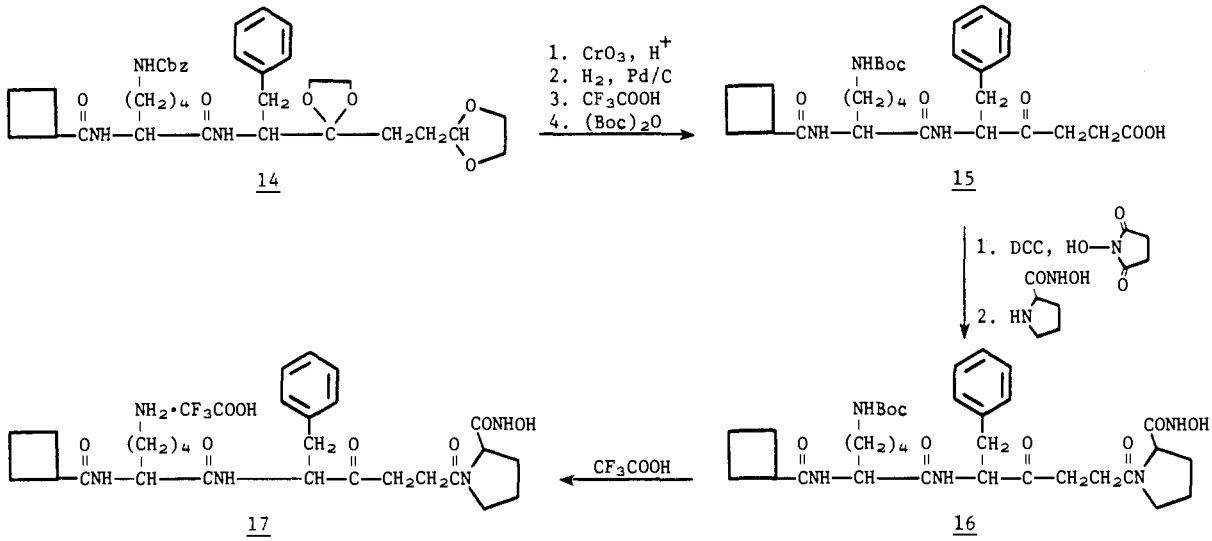
(10) Almquist, R. G.; Jennings-White, C.; Chao, W.-R.; Steeger, T.; Wheeler, K.; Rogers, J.; Mitoma, C. *J. Med. Chem.*, previous paper in this issue.

(11) Almquist, R. G.; Chao, W.-R.; Ellis, M. E.; Johnson, H. L. *J. Med. Chem.* **1980**, *23*, 1392.

Scheme I



Scheme II



(CDCl$_3$) δ 11.83 (3 H, br), 7.70 (2 H, m), 7.27 (10 H, m), 5.32 (1 H, m), 4.95 (1 H, m), 3.56 (2 H, m), 3.12 (2 H, m), 2.85 (2 H, m), 2.56 (2 H, m), 2.00 (7 H, s); mass spectrum, *m/e* 446 (M^+), 428 ($M - H_2O$), 418 ($M - N_2$), 403 ($M - HN_3$). Anal. ($C_{24}H_{26}N_6O_3 \cdot CH_3CO_2H \cdot H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-L-proline Hydroxylamide (7).** Ethyl chloroformate (0.94 mL, 10.0 mmol) was added dropwise over 5 min at −10 °C under N_2 to a stirred solution of *N*-(*tert*-butyloxycarbonyl)-L-proline (2.15 g, 10.0 mmol) and triethylamine (1.40 mL, 10.0 mmol) in dry THF (20 mL). The mixture was stirred at −10 °C for 20 min and then a solution was added that had been prepared by filtering under suction a mixture of DMF (30 mL), hydroxylamine hydrochloride (988 mg, 14.2 mmol), and triethylamine (2.00 mL, 14.2 mmol). The mixture was stirred at room temperature for 16 h, was poured into ice-cold saturated NH_4Cl solution (100 mL), and was extracted with EtOAc (2 × 100 mL). The EtOAc layer was washed with saturated NaCl solution 100 mL, dried (Na_2SO_4), and evaporated in vacuo to give 628 mg of crude material. The aqueous phases were combined and extracted with $CHCl_3$ (2 × 100 mL), which was dried (Na_2SO_4) and evaporated in vacuo to give another 1.20 g of crude material. The crude material was recrystallized from CH_3OH/EtOAc/hexane to give 934 mg of **7** (41%). Another 80 mg of **7** (3%) was obtained by purifying the mother liquors by preparative TLC, eluting with CH_3OH/CH_2Cl_2 (1:9): mp 164–167 °C; R_f 0.30 (CH_3OH/CH_2Cl_2, 1:9); IR ($CHCl_3$) 3400 (w), 3235 (br m), 2975 (m), 2930 (m), 2880 (m), 1680 (s) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.60 (2 h, br), 4.17 (1 H, br), 3.46 (2 H, m), 2.06 (4 H, m), 1.47 (9 H, s); mass spectrum (DCI–NH_3), *m/e* 231 ($M + H^+$), 215 ($M - CH_3$). Anal. ($C_{10}H_{18}N_2O_4 \cdot {}^1/_4H_2O$) C, H, N.

L-Proline Hydroxylamide Hydrotrifluoroacetate (11). Trifluoroacetic acid (2 mL) was added to a solution of **7** (230 mg, 1.00 mmol) in CH_2Cl_2 (2 mL) and the mixture was stirred at room temperature for 16 h. The mixture was evaporated in vacuo to give 394 mg of crude material. This was partitioned between $CHCl_3$ (10 mL) and water (10 mL), and the aqueous phase was separated and lyophilized to give 276 mg of **11** as a pale brown oil, which was used without further purification: R_f 0.2 (butanol/acetic acid/water, 1:1:1); IR (thin film) 3400 (s), 3145 (s), 2960 (s), 2765 (s), 1665 (s) cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.09 (4 H, br), 4.25 (1 H, m), 3.40 (2 H, m), 2.08 (4 H, m); mass spectrum, *m/e* 131 (M^+).

Succinimidyl 5(*S*)-Benzamido-4-oxo-6-phenylhexanoate (9). *N*-Hydroxysuccinimide (127 mg, 1.10 mmol) was added to a solution of **8** (325 mg, 1.00 mmol) in CH_2Cl_2 (5 mL) and DMF (1.5 mL) with cooling in ice and stirring under nitrogen. To this was added a solution of dicyclohexylcarbodiimide (227 mg, 1.10 mmol) in CH_2Cl_2 (1 mL) and the mixture was stirred at room temperature for 16 h. The mixture was filtered under suction and the solid was washed with $CHCl_3$ (5 mL). The combined filtrates were evaporated in vacuo to give the crude product (464 mg). This was recrystallized from $CHCl_3$/hexane to give 358 mg of **9**. The mother liquors were evaporated in vacuo and recrystallized from $CHCl_3$/hexane to give another 40 mg of product. The total yield of **9** was thus 398 mg (94%): mp 145–146 °C; IR ($CHCl_3$) 3400 (w), 2995 (m), 2925 (w), 1817 (m), 1785 (m), 1720 (s), 1712 (m), 1660 (s), 1600 (m), 1580 (m), 1510 (m) cm^{-1}; ^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ 7.73 (2 H, m), 7.42 (3 H, m), 7.27 (6 H,

s), 4.96 (1 H, t, J = 7 Hz), 3.17 (2 H, m), 2.93 (4 H, m), 2.83 (4 H, s); mass spectrum, m/e 422 (M^+). Anal. ($C_{23}H_{22}N_2O_6$) C, H, N.

5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl-L-proline Hydroxylamide (3). A solution of **11** (58 mg, 0.237 mmol) in dry DMF (0.58 mL) was added to a solution of **9** (100 mg, 0.237 mmol) in dry dichloromethane (5 mL) at 0 °C under nitrogen with stirring. Triethylamine (0.073 mL, 0.520 mmol) was added and the mixture was stirred at room temperature for 24 h. The mixture was poured into ice-cold water (20 mL) and was extracted with CH_2Cl_2 (2 × 20 mL). The CH_2Cl_2 extract was washed with saturated sodium chloride solution (20 mL), dried (Na_2SO_4), and evaporated in vacuo to give 97 mg of crude **3**. This was purified by preparative TLC, eluting with CH_3OH/CH_2Cl_2 (1:9), to give 66 mg of **3** (64%) as a white solid: mp 164–166 °C; R_f 0.49 (CH_3OH/CH_2Cl_2, 1:9); IR ($CHCl_3$) 3275 (br m), 3020 (m), 2945 (m), 2900 (m), 1720 (m), 1660 (s), 1585 (m), 1515 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 10.15 (1 H, br), 7.72 (2 H, m), 7.30 (10 H, m), 5.00 (1 H, m), 4.41 (1 H, m), 3.53 (2 H, m), 3.23 (2 H, m), 2.90 (2 H, m), 2.53 (2 H, m), 2.05 (4 H, m); ^{13}C NMR ($CDCl_3/(CD_3)_2SO$) δ 200.0, 170.3, 138.0, 131.2, 129.0, 128.1, 127.3, 126.2, 79.8, 78.4, 76.9, 60.2, 58.0, 46.9, 41.6, 40.7, 39.8, 38.8, 37.9, 35.3, 34.1, 29.3, 28.2, 24.3; mass spectrum (DCI–NH_3), m/e 438 ($M + H^+$), 377 ($M - CONHOH$). Anal. ($C_{24}H_{27}N_3O_5$) C, H, N.

***N*-[5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl]-2-(diethoxyphosphinyl)pyrrolidine (13).** A mixture of 5(*S*)-benzamido-4-oxo-6-phenylhexanoic acid[11] (**8**; 1.00 g, 3.07 mmol) and 2-(diethoxyphosphinyl)pyrrolidine[7] (**10**; 0.637 g, 3.07 mmol) in HPLC-grade CH_2Cl_2 (12 mL) and dry dimethylformamide (3 mL) was stirred in an ice bath and dicyclohexylcarbodiimide (0.633 g, 3.07 mmol) in CH_2Cl_2 (5 mL) was added. The mixture was stirred at ice bath temperature for 30 min and at room temperature for 40 h. The mixture was cooled in an ice bath and filtered. The filtrate was washed successively with ice-cold 2 N HCl (30 mL), saturated aqueous $NaHCO_3$ (50 mL), and saturated aqueous NaCl (50 mL). The CH_2Cl_2 layer was dried (Drierite) and evaporated to a clear oil, which solidified on standing. This solid was triturated in cold EtOAc (6.0 mL) and filtered. The filtrate was purified by flash chromatography on silica gel (80 g) with 2% CH_3OH in $CHCl_3$ as the eluting solvent. Fractions corresponding to product R_f 0.40 (5% CH_3OH in $CHCl_3$) were combined and evaporated to **13** as a pale yellow syrup: 1.05 g (66.4%); ^1H NMR ($CDCl_3$) δ 1.30 (m, 6, $P(OCH_2CH_3)_2$), 4.13 (m, 4, $P(OCH_2CH_3)_2$), 5.05 (m, 1), 7.28 (s, 5, phenyl), 7.32 (s, <2, $CHCl_3$), 7.80–7.35 (m, 5, benzoyl). Anal. ($C_{27}H_{35}N_2O_6P \cdot {}^1/_5CHCl_3$) C, H, N.

***N*-[5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl]-2-phosphonopyrrolidine (2).** A solution of **13** (0.903 g, 1.75 mmol) in CH_2Cl_2 (30 mL) was stirred under a nitrogen atmosphere and cooled in an ice bath while bromotrimethylsilane (0.926 mL, 7.00 mmol) was added. The mixture was stirred at ice-bath temperature for 30 min and at room temperature for 4.5 h. After evaporation, the resulting residue was again dissolved in CH_2Cl_2 (30 mL) and treated at room temperature with bromotrimethylsilane (0.926 mL, 7.00 mmol) for 60 h. The mixture was then evaporated to a pale yellow solid. This solid was mixed with water (60 mL) and dissolved by addition of saturated $NaHCO_3$ solution. The aqueous mixture was extracted with $CHCl_3$ (100 mL). The aqueous layer was mixed with $CHCl_3$ (150 mL) and acidified to pH 1 by addition of concentrated HCl. After shaking, the $CHCl_3$ layer was separated and the aqueous layer was extracted further with $CHCl_3$ (50 mL). These last two $CHCl_3$ layers were combined, washed with saturated NaCl solution (2 × 150 mL), and dried (Na_2SO_4). Evaporation yielded a white foam, 0.578 g. This foam was dissolved in $CHCl_3$ (30 mL) and diethyl ether (10 mL) and added dropwise to stirring ether (300 mL). After cooling of the mixture in the refrigerator, a flocculant white precipitate was collected by filtration and dried to a white amorphous solid (**2**): 0.445 g (55%); mp sinters 140 °C, melts 156–246 °C; ^1H NMR ($CDCl_3$) δ 5.00 (br s, 1), 7.24 (s, 5, phenyl), 7.77–7.33 (m, 5, benzoyl), 9.50 (br s, 3, $COOH \cdot H_2O$). Anal. ($C_{23}H_{27}N_2O_6P \cdot H_2O$) C, H, N.

5(*S*)-[[*N*$^\epsilon$-(Butyloxycarbonyl)-*N*$^\alpha$-(cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoic Acid (15). A solution of **14**[10] (2.33 g, 3.65 mmol) in acetone (250 mL) was stirred in an ice bath while CrO_3 (3.65 g, 36.5 mmol) in 35% H_2SO_4 (60 mL) was added dropwise over 30 min. The reaction mixture was then stirred at ice-bath temperature for 1 h. The mixture was then poured into a separatory funnel containing H_2O (180 mL) and $CHCl_3$ (500 mL). After shaking, the $CHCl_3$ layer was separated, and the aqueous layer was extracted twice more with $CHCl_3$ (2 × 250 mL). The three $CHCl_3$ extracts were combined and washed successively with H_2O (500 mL) and 8% NaCl solution (300 mL). The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to a white solid foam, 2.35 g. This solid foam was dissolved in acetic acid (50 mL) and 2 N HCl (1.82 mL, 3.64 mequiv) plus 10% palladium on carbon (1.50 g) was added. This mixture was stirred under 1 atm of hydrogen gas for 16 h and then filtered through Celite. The filtrate was evaporated to a bluish liquid. This liquid was mixed with 90% aqueous trifluoroacetic acid (15 mL). After stirring at room temperature for 7 h, the reaction mixture was mixed with H_2O (50 mL) and evaporated at 2 mmHg under vacuum at 40 °C to a pale green syrup. After a second evaporation from H_2O (70 mL), the resulting syrup was lyophilized twice from H_2O (2 × 70 mL) to a pale green fluffy solid. This solid was dissolved in H_2O (15 mL)–dimethylformamide (20 mL) and di-*tert*-butyl dicarbonate (0.919 mL, 4.00 mmol) was added. This mixture was stirred in an ice bath and triethylamine (1.01 mL, 7.30 mmol) was added. After standing at room temperature for 5 h, the mixture was evaporated at 1 mmHg under vacuum and 40 °C to a yellow syrup. This syrup was partitioned between H_2O (80 mL) and $CHCl_3$ (80 mL). The $CHCl_3$ layer was separated and washed successively with 10% citric acid solution (100 mL) and saturated NaCl solution (80 mL). The $CHCl_3$ layer was dried (Na_2SO_4) and evaporated to an off-white solid, 1.79 g. This solid was crystallized twice from $CHCl_3$–Et_2O and then once from EtOAc to white solid **15**, 0.965 g. Purification of the mother liquors by silica gel preparative TLC followed by crystallization from EtOAc yielded an additional 0.145 g of **15**: mp 123–125 °C; $[\alpha]^{23}_D$ −58.4° (*c* 1.0, absolute EtOH); R_f 0.40 (10% MeOH in $CHCl_3$); mass spectrum (EI), m/e 532 ($M + H^+$). Anal. ($C_{28}H_{41}N_3O_7$) C, H, N.

5(*S*)-[[*N*$^\epsilon$-(Butyloxycarbonyl)-*N*$^\alpha$-(cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoyl-L-proline Hydroxylamide (16). Solutions of **15** (0.800 g, 1.50 mmol) in CH_2Cl_2 (15 mL) and *N*-hydroxysuccinimide (0.356 g, 1.72 mmol) in dry dimethylformamide (5 mL) were combined and stirred in an ice bath while dicyclohexylcarbodiimide (0.200 g, 1.74 mmol) in CH_2Cl_2 (5 mL) was added. The mixture was stirred at ice-bath temperature for 30 min and at room temperature for 20 h. The reaction mixture was then cooled in an ice bath and filtered. The filtrate was evaporated at 1 mmHg to a semisolid yellow syrup. This syrup was mixed with CH_2Cl_2 (~2.0 mL) and filtered into a flask containing **11** (0.552 g, 2.26 mmol) in dry dimethylformamide (4.0 mL). This mixture was stirred in an ice bath and triethylamine (0.55 mL, 3.95 mmol) was added. This mixture was stirred at room temperature for 5 h and then evaporated at 40 °C at 1 mmHg under vacuum to an orange syrup. This syrup was partitioned between EtOAc (25 mL) and H_2O (30 mL). The EtOAc layer was dried (Na_2SO_4) and evaporated to a pale yellow solid. The solid was triturated in refluxing EtOAc (10 mL) and cooled to 5 °C, and white slushy crystals were collected by filtration and dried to a white solid. This solid was crystallized from EtOAc–$CHCl_3$ to white crystals of **16**, 0.392 g, mp 172 °C gas↑. Crystallization of the mother liquor from EtOAc–$CHCl_3$ yielded more **16**: 0.202 g (total yield 62%); ^1H NMR ($CDCl_3$–CD_3OD, 40:1) δ 1.42 (s, 9, Boc), 7.20 (s, 5, phenyl). Anal. ($C_{33}H_{49}N_5O_8$) C, H, N.

5(*S*)-[[*N*$^\alpha$-(Cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoyl-L-proline Hydroxylamide Trifluoroacetic Acid Salt (17). A solution of **16** (0.200 g, 0.311 mmol) in 50% trifluoroacetic acid in CH_2Cl_2 (5 mL) was left to stand for 15 min at room temperature. It was then evaporated to an oily pink residue. This residue was partitioned between $CHCl_3$ (50 mL) and H_2O (50 mL). The H_2O layer was washed with EtOAc (50 mL) and evaporated at 40 °C at 1 mmHg to a pink residue. This residue was reevaporated from H_2O (50 mL) and then it was lyophilized from H_2O (30 mL) to a fluffy pink solid. This solid was crystallized from MeOH (2.0 mL)–EtOAc (20 mL) to **16** as a pale pink solid: 0.165 g (82%); mp 164–167 °C dec; mass spectrum (DCI), m/e 544 ($M + H^+$). Anal. ($C_{30}H_{42}F_3N_5O_8$) C, H, N.

Blood Pressure Measurements in Renal Hypertensive Rats. The testing of compounds for antihypertensive activity in renal hypertensive rats was performed by Pharmakon Laboratories in Waverly, PA. The testing method they employed is described below.

Hypertension of renal origin was produced in rats by placing a silver clip around the left renal artery near the aorta and leaving the contralateral kidney intact. Several weeks later, the rats were cannulated for blood pressure monitoring by the method of Weeks and Jones.[8] Rats with mean blood pressure greater than 160 mmHg were used for the studies. Four rats received the test compound orally in a 0.25% methylcellulose aqueous solution at 5 mL/kg. Two rats were administered the 0.25% methylcellulose aqueous solution alone at 5 mL/kg orally and served as the controls. Systolic, diastolic, and mean blood pressure and heart rate were monitored prior to dosing and hourly for 8 h and at 24 h after test or control article administration.

Registry No. **2**, 96792-01-7; **3**, 96792-02-8; **4**·HOAc, 96792-14-2; **5**, 63808-36-6; **6**, 33876-20-9; **7**, 96792-04-0; **8**, 74075-31-3; **9**, 96792-05-1; **10**, 73858-58-9; **11**, 96792-06-2; **12**, 33878-70-5; **13**, 96792-07-3; **14**, 96792-08-4; **15**, 96792-09-5; **16**, 96792-10-8; **17**, 96792-12-0; **17** (free base), 96792-11-9; $(BOC)_2O$, 24424-99-5; ACE, 9015-82-1; *N*-(benzyloxycarbonyl)-L-proline amide, 34079-31-7; *N*-(*tert*-butyloxycarbonyl)-L-proline, 15761-39-4; hydroxylamine hydrochloride, 5470-11-1; succinimidyl 5-(*S*)-[[N^{ϵ}-(*tert*-butyloxycarbonyl)-N^{α}-(cyclobutylcarbonyl)-L-lysyl]amino]-4-oxo-6-phenylhexanoate, 96792-13-1.

Synthesis of Xanthines as Adenosine Antagonists, a Practical Quantitative Structure–Activity Relationship Application

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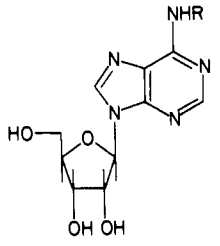
A set of 56 8-phenylxanthines, previously tested for adenosine antagonism (adenosine A_1 receptor affinity), was analyzed by quantitative structure–activity relationship (QSAR) techniques. The resulting QSAR revealed that (1) the most potent receptor binders had already been made in this series and thus suggested the termination of synthesis of compounds with additional phenyl substituents to increase potency and (2) potency was much more strongly affected by changes in ortho than para phenyl substitution. On the basis of this study, an additional 20 compounds were synthesized that contained primarily para substituents designed to increase aqueous solubility. High potency was maintained among the resulting sulfonamide derivatives (as predicted by the QSAR), and aqueous solubility was dramatically increased. Furthermore, in vitro antagonism of an adenosine receptor mediated physiological effect was demonstrated.

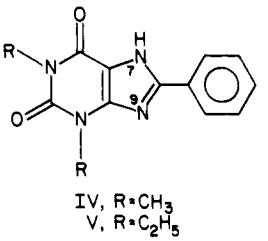
Xanthines have long been known to cause a variety of physiological effects. The central nervous system (CNS) stimulatory properties of caffeine have been utilized for centuries. In addition, tachycardia[1] and bronchodilation[2] are responses elicited by this class of compounds. Inhibition of phosphodiesterase in the heart, brain, and lungs has been postulated[1,3] as the mechanism by which xanthines elicit these effects.

Recently, the role of xanthines as antagonists of adenosine (I) binding has emerged as an alternate explanation for these effects. In vitro, xanthines antagonize a number



I, R=H
II, R=cyclohexyl
III, R=(*R*)-1-methyl-2-phenylethyl

of effects produced by adenosine and the adenosine deaminase resistant analogues N^6-cyclohexyladenosine (II) and (*R*)-N^6-(1-methyl-2-phenylethyl)adenosine ((*R*)-PIA, III).[4] The effects of adenosine and these analogues are mediated by extracellular adenosine receptors that can be divided into two subtypes, A_1 and A_2, which inhibit and stimulate, respectively, adenylate cyclase. Because of the relatively low affinity (micromolar) of caffeine and theophylline at adenosine receptors, 8-phenyltheophylline (IV) and 1,3-diethyl-8-phenylxanthine (V) were developed.[5] They possess at least 25-fold higher affinities at adenosine receptors and act as antagonists in vitro.[5a]



IV, R=CH_3
V, R=C_2H_5

Other pharmacological studies using xanthines as adenosine antagonists support this explanation.[6] Xanthine antagonism of endogenous adenosine in the brain has

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