Antihypertensive (2-Aminoethyl)thiourea Derivatives. 1

Jefferson W. Tilley,* Paul Levitan, Richard W. Kierstead,

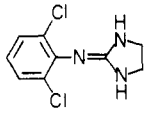
Chemical Research Department

and Michael Cohen

Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received April 7, 1980*

Structure–activity studies were carried out on a series of antihypertensive 1-(2-aminoethyl)-3-(substituted phenyl)thioureas. From this class of compounds, the 2,6-dichlorophenyl analogue **2** was found to have potent oral antihypertensive activity in two hypertensive rat models and the renal hypertensive dog. In addition to its effect on blood pressure, **2** displayed sedative effects which had a marked species specificity.

Clonidine is an orally effective drug for the treatment of moderate and severe hypertension. Although the dose is remarkably low, drowsiness, lethargy, dry or parched oral mucosa, and sedation accompany the observed fall in blood pressure.[1] During the course of synthetic studies in our laboratories directed toward 2-iminoimidazoline structures related to clonidine (1), we had occasion to synthesize the



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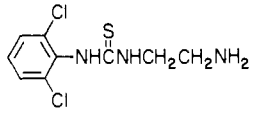


2

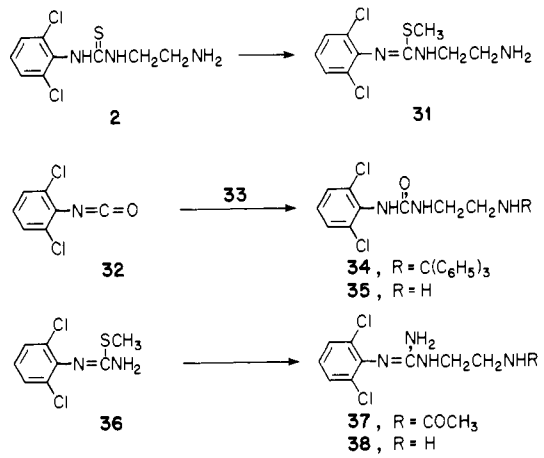
(2-aminoethyl)thiourea **2**. The interesting antihypertensive activity displayed by this compound prompted the survey of similar structures documented in this and the second paper in this series.[2]

The antihypertensive activity of **2** could be due to a metabolic conversion to clonidine (1). Although 1-(2-aminoethyl)-3-phenylthioureas have been shown to serve as chemical intermediates for 2-(phenylamino)-2-imidazolines,[3-5] the pharmacological profile of **2** differs from that of clonidine in some important aspects. In particular, the reduced sedative side effects of **2** in certain animal species encouraged further pharmacological investigation.

Chemistry. The compounds listed in Table I were prepared by reaction of the appropriate isothiocyanates with excess ethylenediamine. The previously unreported 2,6-dibromophenyl isothiocyanate (**13**) was obtained by pyrolysis[6] of the corresponding thiourea, whereas 2,6-difluorophenyl isothiocyanate (**14**) was available from 2,6-difluoroaniline[7] by conventional procedures.[8]

The compounds listed in Table II were prepared by reaction of 2,6-dichlorophenyl isothiocyanate[10] with the appropriate amine, except as described below. Treatment

Scheme I



of an ethanolic solution of the acid **22** (Table II) with a Dowex 50W X 8 cation-ion exchange resin gave the ethyl ester **23**, from which the amide **24** was readily obtained by aminolysis. The amidine **26** was prepared from the imidate derived from the nitrile **25**.

The assignment of **18** as 1-(2,6-dichlorophenyl)-3-[2-(methylamino)ethyl]thiourea rather than the isomeric 3-(2-aminoethyl)-3-methylthiourea rests primarily on mass spectral data which reveal a strong m/z 44 peak for **18** and **19** representing $CH_3NH^+{=}CH_2$, whereas compounds such as **2** possessing a terminal $-CH_2NH_2$ moiety give a strong peak at m/z 30.[13]

The thiourea **2** was treated with methyl iodide in the presence of hydrochloric acid to give the thiopseudourea **31** (Scheme I). The action of phosgene on 2,6-dichloroaniline gave 2,6-dichlorophenyl isocyanate (**32**), essentially as described for the 2,4,6-trichloro compound.[14] However, when **32** was treated with ethylenediamine, an unfavorable mixture of the desired urea **35** and a second compound was formed. The spectral data of the latter compound were consistent with the symmetrical bisurea resulting from attack of a 2nd mol of **32**. In order to circumvent this difficulty, excess ethylenediamine was allowed to react with triphenylmethyl chloride to give, following an extractive workup, nearly pure *N*-(triphenylmethyl)ethylenediamine

(1) Hoefke, W. in "New Antihypertensive Drugs"; Scriabine, A.; and Sweet, C., Ed.; New York Spectrum: New York, 1976; pp 441–459.
(2) Tilley, J. W.; Ramuz, H.; Hefti, F.; Gerold, M. *J. Med. Chem.*, under Notes in this issue.
(3) Stoutland, O.; Helgen, L.; Agre, C. L. *J. Org. Chem.* **1959**, *24*, 818, 884.
(4) Najer, H.; Giudicelli, R.; Sette, J. *Bull. Soc. Chim. Fr.* **1961**, 2114.
(5) Boehringer Ingelheim G.m.b.H., British Patent 1 034 938; *Chem. Abstr.* **1966**, *65*, 12211*b*.
(6) Baxter, J. N.; Cymerman-Craig, J.; Moyle, M.; White, R. A. *J. Chem. Soc.* **1956**, 659.
(7) Roe, A. M.; Burton, R. A.; Willey, G. L.; Baines, M. W.; Rasmussen, A. C. *J. Med. Chem.* **1968**, *11*, 814.
(8) Dyson, G. M.; George, H. J. *J. Chem. Soc.* **1924**, 1702.
(9) De Bennville, P. L.; Moss, J. N.; U.S. Patent 3 950 537.
(10) Rasschaert, A. T.; Benoy, G. J.; van Besauw, J. F.; British Patent 1 131 780; *Chem. Abstr.* **1969**, *70*, 77561*m*.
(11) Loev, B.; Bender, P. E.; Bowman, H.; Helt, A.; McLean, R.; Jen, T. *J. Med. Chem.* **1972**, *15*, 1024.
(12) Winterbottom, R.; Clapp, J. W.; Miller, W. H.; English, J. P.; Roblin, R. O. *J. Am. Chem. Soc.* **1947**, *69*, 1393.
(13) MS data for **2**: m/z 263 (M^+, <1), 228 (contains one chlorine, 59), 221 (contains two chlorines, 66), 30 (100). MS data for **18**: m/z 242 (M – Cl, 18), 203 (contains two chlorines, 95) 44 (100). MS data for **19**: m/z 291 (M^+, <1), 256 (contains one chlorine, 100), 44 (69).
(14) Georges, L. W.; Hamalainen, C. *J. Am. Chem. Soc.* **1949**, *71*, 743.

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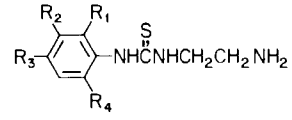
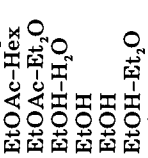
Table I. Antihypertensive Activity of (Aminoethyl)thiourea Derivatives



compd	R_1	R_2	R_3	R_4	yield, %	mp, °C	recryst solvent	formula[a,b]	oral antihypertensive act.[c] SH (rat) dose, mg/kg	SH (rat) effect	DOCA-Na (rat) dose, mg/kg	DOCA-Na (rat) effect
1	(clonidine)								0.5	+++	0.5	+++
2	Cl	H	H	Cl	80	165-167	EtOH	$C_9H_{11}Cl_2N_3S \cdot C_4H_4O_4$	0.5	–	0.5	–
									1	++	1	++
									10	+++	10	+++
3[d]	H	H	H	H	32	196-199	EtOH-Et_2O	$C_{11}H_{17}N_3S \cdot HCl$	10	–		
4	Br	H	H	Br	81	155-157	CH_2Cl_2-Hex	$C_9H_{11}Br_2N_3S$	1	++		
									10	+++	10	+++
5	F	H	H	F	66	80-84	EtOH-Et_2O	$C_9H_{11}F_2N_3 \cdot C_4H_6O_4$ $0.5C_2H_5OH$[e]	10	–	10	–
6[f]	CH_3	H	H	CH_3	84	175-177	EtOH	$C_{11}H_{17}N_3S \cdot C_4H_6O_4$	10	++	10	+++
7	OCH_3	H	H	OCH_3	92	162-163	EtOH	$C_{11}H_{17}N_3O_2S \cdot C_4H_4C_4$	100	–		
8	Cl	Cl	H	H	12	130-132	H_2O	$C_9H_{11}Cl_2N_3S \cdot C_4H_4O_4$	0.5	–	1	–
									1	+++	5	+ (NS)
									10	+++	10	+++
9[g]	Cl	H	CH_3	H	54	151-153	H_2O	$C_{10}H_{14}ClN_3S \cdot C_4H_4O_4$	100	–		
10[h]	CH_3	H	CH_3	H	71	124-126	EtOH	$C_{11}H_{17}N_3S \cdot C_4H_4O_4$	100	–		
11	Cl	H	Cl	Cl	61	168-170	H_2O	$C_9H_{10}Cl_3N_3S \cdot C_4H_4O_4$	10	–		
									100	++		
12	Cl	H	NO_2	Cl	56	172-174	H_2O	$C_9H_{10}Cl_2N_4O_2S \cdot C_4H_4O_4$	10	–		
									50	+++		

[a] Analyses for C, H, N were within ±0.4% for all compounds, except as indicated. [b] $C_4H_4O_4$ = maleic acid, $C_4H_6O_4$ = succinic acid. [c] – = <10%; + = 10–15%; ++ = 15–20%; +++ = >20% fall in systolic blood pressure 6–24 h after dosing. NS indicates the observed effect was not statistically significant at the $p < 0.05$ level. [d] The free base of 3 is known.[3] [e] C: calcd, 45.15; found, 44.70. [f] The free base of 6 is known.[4] [g] The free base of 9 is known.[5] [h] The free base of 10 has been disclosed.[9]

Table II. Antihypertensive Activity of 2,6-Dichlorophenylthioureas



compd	R	yield, %	mp, °C	solvent	formula[a,b]	oral antihypertensive act.[c] SH (rat) dose, mg/kg	SH (rat) effect	DOCA-Na (rat) dose, mg/kg	DOCA-Na (rat) effect
15	NH_2[d]		156-158		$C_7H_7Cl_2N_3S$	60	-	60	+++
16	$NHNH_2$	95	193-194	THF-Hex	$C_7H_{10}Cl_2N_2OS$	250	-		
17	$NH(CH_2)_2OH$	78	133-136	PrOH-H_2O	$C_9H_{13}Cl_2N_3S{\cdot}C_4H_4O_4$	175	-		
18	$NH(CH_2)_2NHCH_3$	44	128-130	H_2O	$C_{10}H_{15}Cl_2N_3S{\cdot}C_4H_4O_4$	100	-		
19	$N(CH_3)(CH_2)_2NHCH_3$	84	167-168	EtOH-H_2O	$C_{11}H_{15}Cl_2N_3S{\cdot}C_4H_4O_4$	85	-		
20	$NH(CH_2)_2N(C_2H_5)_2$	50	156-157	MeOH-Et_2O	$C_{13}H_{19}Cl_2N_3S{\cdot}HCl$	45	+ (NS)		
21	$NH(CH_2)_3NH_2$	14	121-122	EtOAc-Hex	$C_{10}H_{13}Cl_2N_3S$	200	++		
22	$NH(CH_2)_2CO_2H$	32	178-180	EtOAc-Et_2O	$C_{10}H_{12}Cl_2N_2O_2S$	100	-		
23	$NH(CH_2)_2CO_2C_2H_5$	67	125-135	EtOH-H_2O	$C_{12}H_{14}Cl_2N_2O_2S$	100	-		
24	$NH(CH_2)_2CONH_2$	85	193-195	EtOH	$C_{10}H_{11}Cl_2N_3OS$	100	-		
25	$NH(CH_2)_2CN$	82	144-146	EtOH	$C_{10}H_9Cl_2N_3S$	75	-		
26	$NH(CH_2)_2C(=NH)NH_2$	36	208-210	EtOH-Et_2O	$C_{10}H_{12}Cl_2N_4S{\cdot}HCl$	100	-		
27	$NH(CH_2)_2SO_2NH_2$[e]	91	173-176	EtOH	$C_9H_{11}Cl_2N_3O_2S_2$	100	++		
28	$c\text{-}N(CH_2CH_2)_2NCH_3$	99	235-243	MeOH-Et_2O	$C_{12}H_{15}Cl_2N_3S{\cdot}HCl$	60	-		
29	$NH(CH_2)_2\text{-}c\text{-}NC_5H_{10}$	83	205-208	MeOH-Et_2O	$C_{14}H_{19}Cl_2N_3S{\cdot}HCl$	30	-		
30	$NH(CH_2)_2\text{-}c\text{-}N(CH_2CH_2)_2N\text{-}CH_3$	56	214-216	MeOH-Et_2O	$C_{14}H_{20}Cl_2N_4S{\cdot}2HCl$	160	++ (NS)		

[a] $C_4H_4O_4$ = maleic acid. [b] Satisfactory analyses (±0.4%) were obtained for C, H, and N on all new compounds. [c] - = <10%; + = 10-15%; ++ = 15-20%; +++ = >20% fall in systolic blood pressure 6 to 24 h after dosing. NS indicates that the results was not statistically significant at the $p < 0.05$ level. [d] Lit.[11] mp 157-159. [e] For synthesis of starting amine, see ref 12.

Table III. Antihypertensive Activity of 2,6-Dichlorophenyl Derivatives[a]

compd	SH (rat) dose, mg/kg	SH (rat) effect	DOCA-Na (rat) dose, mg/kg	DOCA-Na (rat) effect
31	10	++	10	++
35	250	-	10	-
37	250	-	10	-
38	250	+	10	+ (NS)

[a] - = <10%; + = 10-15%; ++ = 15-20%; +++ = >20% fall in systolic blood pressure 6 to 24 h after dosing. NS indicates that the result was not statistically significant at the $p < 0.05$ level.

(**33**). Although this material was obtained as a gum, its NMR spectra was clean except for traces of solvent and, in particular, showed no detectable amounts of ethylenediamine or the symmetrical product resulting from reaction of ethylenediamine with 2 equiv of triphenylmethyl chloride. Reaction of this material with the isocyanate **32** then gave the urea **34** in 70% yield, from which the desired **35** was liberated through the action of ethanolic hydrochloric acid. Treatment of the isothiourea **36**[15] with a threefold excess of *N*-acetylethylenediamine[16] gave a 49% yield of the guanidine **37**, which on heating with concentrated hydrochloric acid afforded a quantitative yield of **38**.

Results and Discussion

Blood-pressure activity was measured in groups of 6 to 12 conscious spontaneously hypertensive (SH) or deoxycorticosterone–sodium hypertensive (DOCA–Na) rats. The animals were dosed orally with the test substances, and blood pressure was determined prior to and 1, 3, 6, and 24 h post drug administration using the tail-cuff method. Although the active compounds produced decreases in blood pressure starting with the second hour, the maximal changes occurred 6–24 h post drug.

The results summarized in Tables I–III indicate that the most interesting compounds possess a primary amino group linked to a substituted phenylthiourea by a two-carbon chain. Of the 1-(2-aminoethyl)thioureas with variations in the aromatic ring (Table I), the 2,6-dibromo (**4**), 2,6-dimethyl (**6**), and 2,3-dichloro (**8**) analogues were comparable to **2** in antihypertensive effect, while the others were only marginally active or inactive. Extension of the chain length to three carbons (**21**, Table II) led to a marked reduction in potency. The thiopseudoureido (**31**) analogue of **2** (Table III) was slightly less active than the latter compound at a dose of 10 mg/kg, while the corresponding guanidino compound **38** was substantially less active, especially in the SH rat model.

The thiourea **2** appeared to be typical of the more potent compounds and was selected for further pharmacological investigation. The oral antihypertensive activity of **2** (0.1, 1.0, and 10.0 mg/kg) was evaluated in six unanesthetized, renal hypertensive dogs. While there were no significant changes at the lower dose, a dose of 1 mg/kg produced a 27% decrease in systolic blood pressure with no change in heart rate. The duration of the effect was 2–5 h. The highest dose (10 mg/kg) caused a 31% decrease in blood pressure, accompanied by a 52% decrease in heart rate. A 0.3 mg/kg oral dose of clonidine in this animal model decreased blood pressure by 28% and heart rate by 43% with a duration of more than 6 h.

It is widely known that sedation is a prominent side effect accompanying the clinical use of clonidine. There-

(15) Zeile, K.; Hauptmann, K.-H.; Stahle, H.; U.S. Patent 3 202 660.
(16) Hill, A. J.; Aspinall, S. R. *J. Am. Chem. Soc.* 1939, *61*, 822.

Table IV. Comparison of Pharmacological Activity of the Thiourea 2 with Clonidine (1)

compd	species	oral dose, mg/kg[a] reduction of BP	sedation	therapeutic ratio[b]
2	rat	2.40	0.69[c]	0.28
1	rat	0.06	0.11[c]	1.83
2	rat	2.40	1.7[d]	0.71
1	rat	0.06	0.18[d]	3.0
2	dog	1.0	30-60[e]	30-60
1	dog	0.3	0.25-0.50[e]	0.8-1.7
2	monkey		>64[d]	
1	monkey		0.7[d]	

[a] Calculated dose required to cause a 20% fall in blood pressure or sedation. BP = blood pressure. [b] Therapeutic ratio = oral dose of compound producing sedation/reduction in blood pressure. [c] Decreased spontaneous motor activity. [d] Increase in foot shock rate. [e] See text.

fore, additional studies were made to determine the behavioral effects of clonidine and 2 in three animal species (Table IV). In normotensive rats, reduction in spontaneous motor activity was measured during a 10-min period, 1 h after dosing, using an electronic motility meter. The ED_{50} values (95% fiducial limits) estimated from the dose–response curves were 0.11 (0.08–0.17) mg/kg for clonidine and 0.69 (0.39–1.16) mg/kg for 2. In contrast to clonidine in which the sedative dose is approximately twice the antihypertensive dose, 2 was actually three to four times more potent as a sedative than as an antihypertensive agent.

Continuous avoidance behavior was evaluated in male Charles River C-D rats and squirrel monkeys which were previously trained to press an avoidance lever at a stable rate to postpone the onset of foot shocks. Failure to depress the lever at the required rate resulted in a 5-s duration foot shock every 20 s. Drug-induced decreases in the rate of depression of the avoidance lever (avoidance rate) and increases in the number of foot shocks/hour were recorded. Both decreases in avoidance rate of 12% and increases in foot shocks of 4/hour over controls were significant ($p \leq 0.05$).[17]

Clonidine and 2 were tested orally in the same animals, with control behavior being monitored before, between, and after the test sessions. In the rat, the mean doses of clonidine and 2 required to provoke a moderate (25%) decrease in avoidance rate were 0.17 and 2.2 mg/kg, respectively. The rate of foot shocks/hour was increased moderately (10 more than control) by 0.18 mg/kg of clonidine and 1.71 mg/kg of 2. The therapeutic ratios of sedative to antihypertensive doses of clonidine and 2 in the rat were 3.0 and 0.71, respectively (Table IV), as determined by assessing continuous avoidance behavior.

In four squirrel monkeys, a mean dose of 0.70 mg/kg of clonidine caused a moderate (25%) decrease in avoidance rate and a moderate increase in foot shocks/hour (10 more than control). One of the four monkeys showed a moderate (25%) decrease in avoidance rate at a dose of 28 mg/kg of 2. Only insignificant to minimal (15% decrease) effects on avoidance rate were observed in the remaining three animals at doses of 2 up to 64 mg/kg, and no increases in foot shocks were observed in any of the four even at the highest dose level. From an assessment of continuous avoidance behavior, 2 is 10–12 times less potent as a sedative than clonidine in the rat, while in the monkey it is >90 times less potent.

(17) Heise, G. A.; Boff, E. *Psychopharmacologia* **1962**, *3*, 264.

In a blind study, dogs were dosed with either clonidine or 2 and were observed for qualitative signs of sedation such as decreased activity compared to controls, ataxia, and sleeping. In this animal model, 2 was markedly less sedative than in the rat. The dose required to cause moderate to marked sedation was 30–60 mg/kg; with clonidine, comparable sedative effects were observed with 0.25–0.50 mg/kg.

In summary, the thiourea derivatives typified by 2 lower blood pressure in a variety of animal models. The cardiovascular effects of 2 in two hypertensive rat models are similar to those seen with clonidine, while in the renal hypertensive dog less bradycardia is seen with 2 at equally effective antihypertensive doses. The sedative effects of 2 show a marked species specificity.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on Varian T-60, A-60, or XL-100 instruments using Me_4Si as an internal standard. Mass spectra were recorded on a Varian CH-5 or CEC 21-103 spectrometers. Infrared spectra were recorded on Beckman Instruments IR-9 or Perkin-Elmer Model 137 spectrophotometers. Concentration refers to evaporation of the solvent under aspirator pressure on a rotary evaporator. Spectral (IR, MS, NMR) data were compatible with the assigned structures in all cases.

1-(2-Aminoethyl)-3-(2,6-dichlorophenyl)thiourea (2). A solution of 947 g (4.64 mol) of 2,6-dichlorophenyl isothiocyanate[10] in 5.0 L of dry THF was added over 30 min to 6.2 L (92.8 mol) of ethylenediamine while maintaining the reaction temperature below 15 °C. The reaction mixture was allowed to warm to room temperature overnight and was evaporated to a heavy oil. The oil was dissolved in 6 L of 3 N hydrochloric acid and was washed with 8 × 1 L of dichloromethane. The aqueous layer was adjusted to pH 8 by the careful addition of solid sodium hydroxide as the product precipitated to give 983 g (80%) of 2, mp 129–131 °C. Recrystallization from ethyl acetate gave mp 135–137 °C. Anal. ($C_9H_{11}Cl_2NS$) C, H, N.

The maleate salt was prepared by treatment of an ethanolic solution of 2 with 1.1 equiv of maleic acid. The other (aminoethyl)thioureas listed in Table I were prepared by a similar sequence.

2,6-Dibromophenyl Isothiocyanate (13). Following the general method described in "Organic Syntheses",[18] 90 g (0.36 mol) of 2,6-dibromoaniline was converted to 101.0 g (91%) of 2,6-dibromophenylthiourea, mp 186–188 °C, which was suspended in 1 L of chlorobenzene. The mixture was refluxed for 20 h and, after cooling and filtration, the solvent was evaporated. The resulting tan solid was taken up in hexane, filtered hot, and allowed to cool as 64.3 g (68%) of 13, mp 63–65 °C, separated. The analytical sample was obtained from hexane, mp 65–66 °C. Anal. ($C_7H_3Br_2NS$) C, H, N.

2,6-Difluorophenyl Isothiocyanate (14). A suspension of 11.0 g (0.0859 mol) of 2,6-difluoroaniline[7] in 100 mL of 1 N hydrochloric acid and 50 mL of dichloromethane was stirred mechanically as 6.8 mL (0.0892 mol) of thiophosgene was added. After 3 h at room temperature, the layers were separated and the aqueous phase was extracted with 2 × 100 mL of dichloromethane. The combined organic layers were washed with water, dried ($MgSO_4$), and evaporated. The resulting oil was distilled and the fraction boiling 160–165 °C (100 mm) was collected: yield 11.1 g (78%); IR ($CHCl_3$) 2050 cm^{-1} (N=C=S). Molecular ion calcd for $C_7H_3F_2NS$: 170.9954. Found: 170.9868.

General Procedure for the Synthesis of 1-Substituted 3-(2,6-Dichlorophenyl)thioureas (Table II). A solution of 2,6-dichlorophenyl isothiocyanate[10] in dichloromethane or THF was treated with 1 equiv of the appropriate monofunctional amine or an excess of a difunctional amine and was stirred overnight at room temperature. The product thioureas either separated from the reaction mixture or were obtained by evaporation of the

(18) Frank, R. L.; Smith, P. V. In "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 735.

solvent and were crystallized or converted to a salt form for characterization.

1-(2-Carboxyethyl)-3-(2,6-dichlorophenyl)thiourea (22). To a stirred suspension of 58 g (0.651 mol) of β-alanine and 90 mL (0.648 mol) of triethylamine in 600 mL of DMF was added 100 g (0.490 mol) of 2,6-dichlorophenyl isothiocyanate.[10] After 18 h at room temperature, the reaction mixture was diluted with 2.5 L of water as a solid impurity separated. Acidification of the filtrate with hydrochloric acid precipitated 79.21 g of **22**, mp 169–172 °C. Recrystallizations from ethanol and ethyl acetate–hexane gave 46.07 g (32%), mp 178–180 °C.

1-[2-(Carboethoxy)ethyl]-3-(2,6-dichlorophenyl)thiourea (23). A solution of 8.72 g (0.0297 mol) of **22** in 1 L of ethanol was stirred for 48 h over 10 g of Dowex-50W X 8 cation-exchange resin. The reaction mixture was filtered and evaporated to give 9.73 g of **23**, mp 122–131 °C. Recrystallization from ethanol–water gave 6.5 g (67%), mp 125–135 °C.

1-(2-Carbamoylethyl)-3-(2,6-dichlorophenyl)thiourea (24). A solution of 10 g (0.0311 mol) of **23** in 250 mL of concentrated ammonium hydroxide was stirred for 48 h. The precipitate amounted to 3.25 g (36%) of **24**, mp 193–195 °C. Concentration of the filtrate afforded a further 3.70 g of **24** (41%), mp 187–193 °C.

1-[2-(Aminoiminomethyl)ethyl]-3-(2,6-dichlorophenyl)-thiourea Hydrochloride (26). A solution of 15.0 g (0.0547 mol) of **25** (Table II), 3.5 mL (0.0603 mol) of ethanol, and 2.1 g (0.050 mol) of dry hydrogen chloride was held at 0 °C for 5 days and 1.75 mL of ethanol and 1.05 g of hydrogen chloride dissolved in 3 mL of THF were added. After 2 days, the reaction mixture was evaporated to dryness and the residue was taken up in 200 mL of ammonium hydroxide. The resulting precipitate was collected, treated with ethanolic hydrochloric acid, and crystallized from ethanol to give 6.38 g (37%) of **26**, mp 206–209 °C. The analytical sample was obtained from ethanol–ether (Table II).

1-(2-Aminoethyl)-3-(2,6-dichlorophenyl)-2-(methylthio)-pseudourea Dihydrochloride (31). A solution of 12.68 g (0.048 mol) of **2** in 80 mL of ethanol, 4.0 mL of concentrated hydrochloric acid, and 12 mL (0.092 mol) of iodomethane was heated to reflux for 2 h. On cooling, the mixture was poured onto 200 mL of water, and sufficient 4 N sodium hydroxide was added to bring the pH to 11. The aqueous solution was extracted with 3 × 150 mL of dichloromethane, and the combined organic layers were washed with 1 × 100 mL of water, dried (K_2CO_3), and concentrated. The resulting gum gave a solid on standing, mp 74–79 °C, the free base of **31**. The material was too labile for purification and was acidified with hydrochloric acid and crystallized from ethanol–ether to give 14.19 g (84%) of **31**, mp 179–187 °C. Two crystallizations from ethanol–ether gave the analytical sample, mp 179–184 °C. Anal. ($C_{10}H_{13}Cl_2N_3S$·2HCl) C, H, N.

2,6-Dichlorophenyl Isocyanate (32). Following the general procedure of Georges and Hamalainen,[14] a suspension of 35.35 g (0.178 mol) of 2,6-dichloroaniline hydrochloride in 250 mL of chlorobenzene (dried over molecular sieves) was stirred mechanically as phosgene was bubbled through in a continuous stream. The temperature was gradually raised to reflux over 4 h, and the mixture finally became homogeneous after a further 30 min. A 50-mL portion of the chlorobenzene was distilled, the bath temperature was allowed to drop to 80 °C, and the bulk of the solvent was removed under reduced pressure (60 mm). The residue was allowed to stand at room temperature overnight and was distilled; the fraction boiling 104–105 °C, 19.63 g (59%), consisted of analytically pure **32**, which solidified on standing, mp 40–45 °C. Anal. ($C_7H_3Cl_2NO$) C, H, N.

***N*-(Triphenylmethyl)ethlenediamine (33).** To a solution of 200 mL of ethylenediamine in 200 mL of dichloromethane was added a solution of 71.93 g (0.258 mol) of triphenylmethyl chloride in 300 mL of dichloromethane with ice-bath cooling over the course of an hour. The resulting mixture was stirred at room temperature overnight, the layers were allowed to separate, and the cloudy lower layer was poured onto 400 mL of ice and water. The aqueous solution was extracted with 2 × 300 mL of ether and 3 × 300 mL of benzene, and the combined organic layers were washed with 2 × 300 mL of water and 1 × 300 mL of saturated sodium chloride, dried (K_2CO_3), and evaporated under high vacuum to a heavy oil, 86.4 g (110%), which was used directly: NMR ($CDCl_3$) δ 1.43 (s, 3), 2.23 (t, J = 6 Hz, 2), 2.77 (3, J = 6 Hz, 2), 7.27 (m, 15); MS, $m/$ 302 (M^+, 1); 243 (100); 165 (38).

1-(2,6-Dichlorophenyl)-3-[2-[(triphenylmethyl)amino]-ethyl]urea (34). To a solution of 31.04 g (0.103 mol) of **33** in 100 mL of THF was added a solution of 19.3 g (0.103 mol) of **32** in 100 mL of THF. After 30 min at room temperature, the mixture was concentrated, triturated with boiling ethanol, and filtered to give 18.49 g (37%) of **34**, mp 214–217 °C partly melts, 262–265 °C. The filtrate gave two additional crops on cooling and concentration, totaling 18.86 g (37%): mp 211–217 °C partly melts, 262–265 °C. Recrystallization of a sample from dichloromethane–ethyl acetate–hexane gave the analytical sample, mp 216–220 °C partly melts, 265–268 °C. Anal. ($C_{28}H_{25}Cl_2N_3O$) C, H, N.

1-(2-Aminoethyl)-3-(2,6-dichlorophenyl)urea Hydrochloride (35). A solution of 35.35 g of **34** in 350 mL of ethanol and 15 mL of concentrated hydrochloric acid was heated to reflux for 1.5 h. On cooling, the mixture was concentrated and partitioned between 700 mL of water and 200 mL of ether. The aqueous layer was washed with 1 × 200 mL of ether and was evaporated to dryness, giving 20.55 g of **35**, mp 282–287 °C. Recrystallization from aqueous ethanol–ether gave the analytical sample as a partial hydrate, mp 283–287 °C. Anal. ($C_9H_{11}Cl_2N_3O$·HCl·0.4H_2O) C, H, N, H_2O.

1-[2-(Acetylamino)ethyl]-2-(2,6-dichlorophenyl)guanidine (37). A solution of 45.0 g (0.124 mol) of 1-(2,6-dichlorophenyl)-2-(methylthio)pseudourea (**36**)[15] and 44.75 g (0.438 mol) of *N*-acetylethylenediamine[16] in 35 mL of amyl alcohol was heated at a bath temperature of 180 °C for 3 h. On cooling, the mixture was partitioned between 250 mL of 1 N sodium hydroxide and 250 mL of dichloromethane. The aqueous layer was extracted with 2 × 250 mL of dichloromethane, and the combined organic layers were washed with water, dried (K_2CO_3), and evaporated to an oil. Trituration with ether gave 16.56 g (46%) of **37**, mp 186–193 °C.

Further addition of ether to the filtrate gave an additional 1.01 g (3%), mp 184–193 °C. Two crystallizations from methylene chloride–hexane gave the analytical sample, mp 196–198 °C. Anal. ($C_{11}H_{14}Cl_2N_4O$) C, H, Cl; N: calcd, 19.38; found, 20.28.

Addition of 0.5 equiv of succinic acid to an aliquot and recrystallization from ethanol–ether gave the hemisuccinate mp 127–133 °C. Recrystallization from ethanol–ether gave mp 127–133 °C. Anal. ($C_{11}H_{14}Cl_2N_4O$·0.5$C_4H_6O_4$) C, H, N.

1-(2-Aminoethyl)-2-(2,6-dichlorophenyl)guanidine Dihydrochloride (38). A solution of 16.57 g (0.0573 mol) of **37** in 150 mL of concentrated hydrochloric acid was refluxed for 16 h and was concentrated under reduced pressure. Trituration with ethanol gave 24.06 g of a white solid, which was recrystallized from aqueous ethanol–ether to give 14.18 g (77%) of **38**, mp 154–160 °C partly melt, 222–224 °C clear. On addition of ether the filtrate gave an additional 4.81 g (26%), mp 155–160 °C partly melt, 221–224 °C clear. A sample of the first crop was submitted for analysis. Spectral data (IR, NMR) are in accord with the assigned structure and indicate persistent traces of ethanol. Anal. ($C_9H_{12}Cl_2N_4$·2HCl) C, H; N: calcd, 17.51; found, 17.08.

Biological Testing. Oral antihypertensive activity was evaluated in two conscious rat models of hypertensive vascular disease. In the DOCA–Na hypertensive male rat (10–12 weeks old) hypertension was produced by unilateral nephrectomy and subcutaneous implantation of a 25-mg deoxycorticosterone acetate pellet. Animals were placed in individual cages after surgery and maintained on an 0.9% sodium chloride solution. Animals were fed a rat standard chow diet ad libitum. Two weeks were allowed for development of hypertension, i.e., systolic blood pressure above 200 mmHg. The spontaneous hypertensive (SH) male rat derived from a Wistar–Okamoto strain (12–15 weeks old), obtained from in-house breeding facilities, was used as a second animal model. In both the SH and DOCA–Na hypertensive rat, systolic blood pressure was measured indirectly from the tail of unanesthetized rats.[19] All rats were heated for 5–10 min at 37–38 °C and then restrained in holders. A pneumatic pulse transducer (piezoelectric crystal and occluding cuff) was used to measure blood pressure. The transducer and occluding cuff were coupled to a two-channel

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Sanborn recorder. Control blood-pressure readings were taken prior to and at 1, 3, 6, and 24 h after drug administration. All compounds were administered orally in acacia (6%). Six to twelve rats were used for each drug. Data were analyzed for statistical significance using Student's t test (paired comparison; $p < 0.05$).

Renal hypertensive dogs were surgically prepared by removing one kidney and wrapping the contralateral kidney in cellophane.[20] All dogs were used 6–8 weeks after recovery from the anesthesia.

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Synthesis and Biological Activity of a Ketomethylene Analogue of a Tripeptide Inhibitor of Angiotensin Converting Enzyme

Ronald G. Almquist,* Wan-Ru Chao, Marie E. Ellis, and Howard L. Johnson

Bio-Organic Chemistry Laboratory, SRI International, Menlo Park, California 94025. *Received May 3, 1979*

An analogue of a tripeptide inhibitor of angiotensin converting enzyme, Bz-Phe-Gly-Pro, has been synthesized in which the amide bond connecting phenylalanine and glycine has been replaced by a ketomethylene group. This nonpeptide analogue, **20**, shows more potent converting enzyme inhibiting activity, I_{50} = 0.07 μM, than Bz-Phe-Gly-Pro, I_{50} = 9.4 μM, or than the orally active D-3-mercapto-2-methylpropanoyl-L-proline (captopril, 1), I_{50} = 0.30 μM. Compound **20** has a K_i of 1.06×10^{-7} and either competitive or noncompetitive enzyme kinetics depending on what substrate is used in the converting enzyme assay. In tests for inhibition of angiotensin I induced contractions in the guinea pig ileum, **20** has one-tenth the activity of 1.

Angiotensin converting enzyme (ACE) is responsible for the conversion of the decapeptide angiotensin I to the potent vasopressor angiotensin II (an octapeptide). In addition, the enzyme's ability to hydrolyze the potent vasodepressor bradykinin is considered one of bradykinin's major pathways for inactivation.[1] These combined actions of the converting enzyme may play a role in blood pressure regulation.

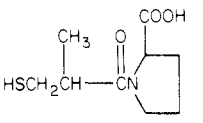
Inhibition of this enzyme by pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ 20881) isolated from snake venom[2,3] lowers blood pressure in animal models of renovascular hypertension[4,5] and in humans with various forms of hypertension.[6-9] The lack of oral activity of this nonapeptide, however, has limited its use as a therapeutic drug for the treatment of hypertension.

Recently, Ondetti et al.[10] reported on the development of an extremely potent orally active inhibitor of the converting enzyme D-3-mercapto-2-methylpropanoyl-L-proline (SQ 14225 or captopril, 1). This compound has shown similar but more potent activity in angiotensin converting enzyme assays,[10,11] as well as in animal studies,[10,12,13] than its predecessor pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro.

Table I. Inhibition Results with Porcine Plasma Angiotensin Converting Enzyme

inhibitor	I_{50}, μM[a]
pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ 20881)	0.22
pGlu-Lys-Trp-Ala-Pro (SQ 20475)	0.73
Phe-Ala-Pro (3)	1.4
Phe-Gly-Pro (11)	20
Bz-Phe-Gly-Pro (12)	9.4
Ts-Phe-Gly-Pro (8)	67
1	0.30
20	0.07

[a] All values are the average of results obtained in two or more experiments.



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