Synthesis and Antibacterial Activity of 2,2′-Dithiobis(benzamide) Derivatives against *Mycobacterium* Species

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A series of compounds, which are analogues of 2,2′-dithiobis(benzamide), were synthesized and tested for in vitro antibacterial activity against *Mycobacterium tuberculosis* H37Rv including resistant strains against streptomycin, kanamycin, or isonicotinic acid hydrazide. MICs of these compounds against atypical mycobacteria, *Mycobacterium kansasii* and *Mycobacterium intracellulare* were also examined. Structure–activity relationships were found in a series of (acyloxy)alkyl ester derivatives depending upon the length of alkyl carbon chain. The MIC of the most potent compound, 2,2′-dithiobis[*N*-[3-(decanoyloxy)propyl]benzamide] [**56**] was superior or at least equivalent to streptomycin, kanamycin, and ethanbutol. All the compounds showed no cross-resistance between the current antitubercular agents.

Some 2,2′-dithiobis(benzamide) analogues were synthesized and reported to possess antibacterial activity in 1959.[1] 2,2′-Dithiobis(benzamide) [1] and 2,2′-dithiobis(*N*-methylbenzamide) were reported to possess bactericidal activity against *Staphylococcus aureus*, *Bacillus subtilis*, and other microorganisms[2] while 2,2′-dithiobis(*N*-butylbenzamide) was reported to be useful as an antiseptic for cosmetics.[3] We have previously described the synthesis of several alkyl derivatives of dithiobis(benzamide) and have shown [**35**] to be a relatively nontoxic inhibitor of platelet aggregation.[4] Although streptomycin (SM), kanamycin (KM), isonicotinic acid hydrazide (INH), ethanbutol (EB), and rifampicin (RFP) have been developed as effective agents in tuberculosis chemotherapy, mycobacteria are known to acquire easily resistance to the drugs. Moreover, the conventional antitubercular agents exhibit only limited activity against atypical mycobacteria. Several additional studies[5–8] have been so far attempted to develop new tuberculostatic compounds. Such a background has prompted us to expand the evaluation of 2,2′-dithiobis(benzamide) analogues for their antibacterial activity against *Mycobacterium* species.

This paper describes the syntheses, structures, and physicochemical properties of 2,2′-dithiobis(benzamide) analogues. The in vitro antibacterial spectrum of the compounds against *Mycobacterium tuberculosis* H37Rv including strains resistant to SM, KM, and INH will be reported as well as antibacterial activity against *Mycobacterium kansasii* and *Mycobacterium intracellulare*. In the series of (acyloxy)alkyl derivatives, the structure–activity relationships relating to the length of the alkyl side chain will be discussed.

Chemistry. The principal synthetic routes for the preparation of these compounds are outlined in Charts I and II and described in detail in the Experimental Section.

2,2′-Dithiobis(benzoic acid)[2] was converted to the acid chloride by treatment with thionyl chloride ($SOCl_2$), and the resulting acid chloride was then subjected to reactions with ammonia water, primary and/or secondary amines, and heterocyclic amino compounds in a suitable solvent such as dioxane or tetrahydrofuran to give amide compounds (Chart I).

(Hydroxymethyl)benzamide [**31**] was obtained by the reaction of benzamide [1] with formalin (37%). *N*-(Hydroxymethyl)-1,2-benzisothiazolin-3-one [**74**] and its 1,1-dioxide derivative [**75**] were prepared from 1,2-benzisothiazolin-3-one and saccharin in a similar manner as above, respectively.

N-(2-Hydroxyethyl)- [**76**] and *N*-(2-hydroxypropyl)-1,2-benzisothiazolin-3-one [**77**] derivatives were prepared from 2,2′-dithiobis[(hydroxyalkyl)benzamide] derivatives through the cleavage of the S–S bond by bromination and then ring closure with triethylamine.

[(Acyloxy)alkyl]benzamide derivatives were synthesized by the Schotten–Baumann reaction with the respective acyl chloride and (hydroxyalkyl)benzamide (Chart II). Structures of the compounds that do not appear in Charts I and II are shown in Chart III, and the yields and physical data of new 2,2′-dithiobis(benzamides) synthesized in this study are shown in Table I.

Biology. All the compounds synthesized here were tested in vitro for their ability to inhibit the growth of *M. tuberculosis* H37Rv, *M. kansasii*, and *M. intracellulare*. Some compounds were also tested against SM, KM, and INH resistant strains of *M. tuberculosis* H37Rv. The antimycobacterial activities of the 2,2′-dithiobis(benzamide) derivatives, as well as the standard antimycobacterial drugs SM, KM, INH, EB, and RFP are summarized in Table II.

Among alkyl derivatives [**1–10**], 2,2′-dithiobis-[*N*-(2-ethylhexyl)benzamide] [**5**] showed the most potent antimycobacterial activity. With the aromatic or heterocyclic compounds, most of the phenyl [**11, 30**], substituted phenyl [**12–29**], piperazino [**60–65**], and morpholino [**66**] derivatives showed weak antibacterial activity. Exceptions included compounds **15, 17, 20, 21,** and **29**, which inhibited *M. tuberculosis* H37Rv with MIC values lower than 3.13 μg/mL.

Hydroxyalkyl [**31–43**] and alkoxyalkyl [**44–46**] derivatives generally showed more potent antibacterial activity

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Table I. Yields and Physical Data for New 2,2'-Dithiobis(benzamides)

no.	yield, %	solvent[a]	mp, °C	IR (KBr), cm^{-1}	anal. C, H, N[b]
[5]	94.1	EtOH	147–148.5	3290, 2950, 1630	$C_{30}H_{44}N_2S_2O_2$
[6]	86.3	EtOH	211–212	3260, 1625, 1540	$C_{34}H_{36}N_2S_2O_2$[c]
[7]	74.8	EtOH	>240	2900 (br), 1610	$C_{26}H_{36}N_4S_2O_2Cl_2$
[8]	89.6	EtOH	182–185	3280, 1625	$C_{24}H_{20}N_2S_2O_4$
[10]	94.3	DIOX	185–189	1620, 1380	$C_{30}H_{44}N_2S_2O_2$
[16]	70.4	DMF	>240	3250, 1640	$C_{34}H_{36}N_2S_2O_2$
[18]	93.2	Me_2CO	>230	3300, 1700, 1640	$C_{30}H_{24}N_2S_2O_6$
[19]	75.5	DMF	>240	3100–3000 (br), 1650, 1590, 1510	$C_{32}H_{24}N_6S_6O_6$
[20]	86.3	Me_2CO	206–208	3300, 2960, 1640, 1515	$C_{34}H_{36}N_2S_2O_2$[d]
[22]	94.3	MDG	>230	3250, 1640, 1495	$C_{34}H_{22}N_2S_2O_2Br_2$
[23]	78.1	Me_2SO–MeOH	>230	3280, 1640, 1500	$C_{34}H_{22}N_2S_2O_2Cl_2$
[24]	84.0	DMF	165–168	3200, 1630, 1500	$C_{34}H_{24}N_2S_2O_4$
[25]	76.2	DMF–MeOH	155–159	3400–3200 (br), 1640, 1620, 1530	$C_{34}H_{24}N_2S_2O_4$
[26]	90.6	DMF	233–235	3290, 1635, 1525	$C_{36}H_{28}N_2S_2O_2$
[27]	87.1	DMF	195–196	1650, 1520	$C_{32}H_{22}N_4S_2O_2$
[28]	80.6	DMF	>240	3320, 1660, 1500	$C_{34}H_{22}N_4S_2O_6$[e]
[29]	64.8	DMF–MeOH	241–244	3275, 1640, 1630, 1530	$C_{42}H_{34}N_4S_2O_2$
[30]	56.3	MeOH	203–205	1655, 1590, 1490	$C_{38}H_{28}N_2S_2O_2$
[34]	74.0	DMF–EtOH	181–183	3300 (br), 1620	$C_{22}H_{30}N_4S_2O_4$
[37]	88.4	EtOH	169–171	3290, 1635, 1535	$C_{22}H_{28}N_2S_2O_4$
[39]	90.0	Me_2CO	126–128	3250 (br), 1625, 1550	$C_{24}H_{32}N_2S_2O_6$
[40]	65.2	MeOH	149–151	3300 (br), 1620, 1520	$C_{30}H_{28}N_2S_2O_8$
[43]	93.5	EtOH	>240	3250, 1650, 1555	$C_{18}H_{18}N_2S_2O_4Cl_2$
[45]	75.3	MeOH	135–136	3280, 1630, 1530	$C_{22}H_{28}N_2S_2O_6$
[47]	69.4	*i*-PrOH	>45	2930, 1690, 1625	$C_{44}H_{68}N_2S_2O_6$
[48]	78.5	*i*-PrOH	55–60	2910, 1700, 1630, 1540	$C_{52}H_{82}N_2S_2O_6$
[49]	80.0	*i*-PrOH	115–118	3320, 2930, 2860, 1740, 1635	$C_{42}H_{64}N_2S_2O_6$
[50]	83.2	*i*-PrOH	119–121	3315, 2930, 2860, 1735, 1630	$C_{50}H_{80}N_2S_2O_6$
[51]	70.5	EtOH	127–129	3300, 2975, 1735, 1630, 1545	$C_{24}H_{28}N_2S_2O_6$
[52]	85.6	EtOH	79–81	3280, 2975, 1730, 1635, 1550	$C_{28}H_{36}N_2S_2O_6$
[53]	95.0		viscous liq	3330, 2970, 1730, 1635, 1540	$C_{32}H_{44}N_2S_2O_6$
[54]	90.2		viscous liq	3350, 2975, 2950, 1735, 1640	$C_{34}H_{48}N_2S_2O_6$
[55]	94.2		viscous liq	3325, 2940, 2875, 1730, 1640	$C_{36}H_{52}N_2S_2O_6$
[56]	83.6	Me_2CO	92–94	3300, 2940, 2840, 1740, 1640	$C_{40}H_{60}N_2S_2O_6$[f]
[57]	85.6	EtOH	91–93	3290, 2930, 2855, 1740, 1630	$C_{44}H_{68}N_2S_2O_6$[g]
[58]	90.2	MeOH	101–103	3290, 2925, 2855, 1740, 1635	$C_{52}H_{84}N_2S_2O_6$
[59]	85.4	MeOH	107–109	3290, 2925, 2850, 1740, 1635	$C_{60}H_{100}N_2S_2O_6$
[68]	90.4	EtOH	146–150	3280, 1755, 1635, 1530	$C_{22}H_{24}N_2S_2O_6$
[69]	92.0	EtOH	187–189	3300, 1690, 1630, 1530	$C_{24}H_{30}N_4S_2O_6$
[70]	91.5	DMF–MeOH	>240	1670, 1600, 1455	$C_{32}H_{26}N_4S_4O_4$
[71]	80.3	DIOX	225–227	1800, 1735, 1680, 1290	$C_{30}H_{16}N_2S_2O_6$[h]
[72]	87.6	BENZ	168–170	1710, 1680	$C_{26}H_{16}N_6S_2O_2$
[73]	50.0	Me_2CO	176–177	3250, 1640	$C_{20}H_{24}N_2S_2O_4$
[74]	33.7	EtOH	>140	3330, 1620, 2650 (br)	$C_8H_7NSO_2$
[75]	31.5	EtOH	>125	3250, 1740, 1600, 1330,	$C_8H_7NSO_4$
[76]	81.2	EtOH	76–78	3300, 1630, 1590	$C_{10}H_{11}NSO_2$

[a] Solvents used for recrystallization: EtOH, ethanol; DIOX, dioxane; DMF, dimethylformamide; Me_2CO, acetone; MDG, diethylene glycol monomethyl ether; Me_2SO, dimethyl sulfoxide; *i*-PrOH, 2-propanol; BENZ, benzene. [b] All compounds gave satisfactory C, H, and N analysis unless otherwise indicated. [c] C: calcd, 71.80; found, 70.95. [d] C: calcd, 71.80; found 71.16. [e] C: calcd, 63.15; found, 63.66. [f] C: calcd, 65.90; found 65.22. [g] C: calcd, 67.31; found, 67.74. [h] C: calcd, 63.82; found 62.99. [i] C: calcd, 61.40; found 60.83.

than the aromatic derivatives. In the (acyloxy)alkyl [47–59] derivatives, some compounds [54–57] showed the most potent activity against *M. tuberculosis* H37Rv of the series of dithiobis(benzamide) derivatives synthesized and tested. These active compounds were effective against SM, KM, and INH resistant strains of *M. tuberculosis* H37Rv, too, exhibiting no cross-resistance between the current antimycobacterial agents.

Although most of the dithiobis(benzamide) compounds inhibited weakly atypical mycobacteria (*M. kansasii*, *M. intracellulare*), the (acyloxy)alkyl [53–56] derivatives showed exceptionally very potent activity against the atypical mycobacteria.

Among the (acyloxy)alkyl derivatives, the relationship between the length of the alkyl side chain and in vitro antibacterial activity against *M. tuberculosis* H37Rv is shown in Table III. In alkyl side chain *m*, the compound possessing three carbon atoms [57] was more potent than the compound with two carbon atoms [49]. In alkyl side chain *n*, six to eight carbon atoms [55, 56] were thought to be the most suitable length for exhibiting potent antibacterial activities. The antibacterial activity against *M. tuberculosis* H37Rv, including resistant strains, and atypical mycobacteria of the most potent (acyloxy)alkyl [54–57] derivatives showed them to be superior or at least equivalent to SM, KM, or EB. Against sensitive strains, however, they were inferior to INH and RFP.

The acute toxicities of the compounds [5], [20], and [29] in mice administered orally were >250, >500, and >1000 mg/kg, respectively. Although the mode of action of the compounds has not yet been established, the fact that [54–57] did not inhibit any bacteria other than mycobacteria (data not shown) suggested that a certain interaction might exist between the compounds and some outer-envelope components specific to mycobacteria. This requires further investigations, however, to determine whether or not this is indeed the case.

Experimental Section

Chemistry. Melting points were in open capillaries in a Shibata melting point apparatus and are uncorrected. Infrared spectra were recorded on a Hitachi 260-30 IR instrument.

Table II. Antibacterial Activity of 2,2′-Dithiobis(benzamide) Derivatives against *Mycobacterium* Species[a]

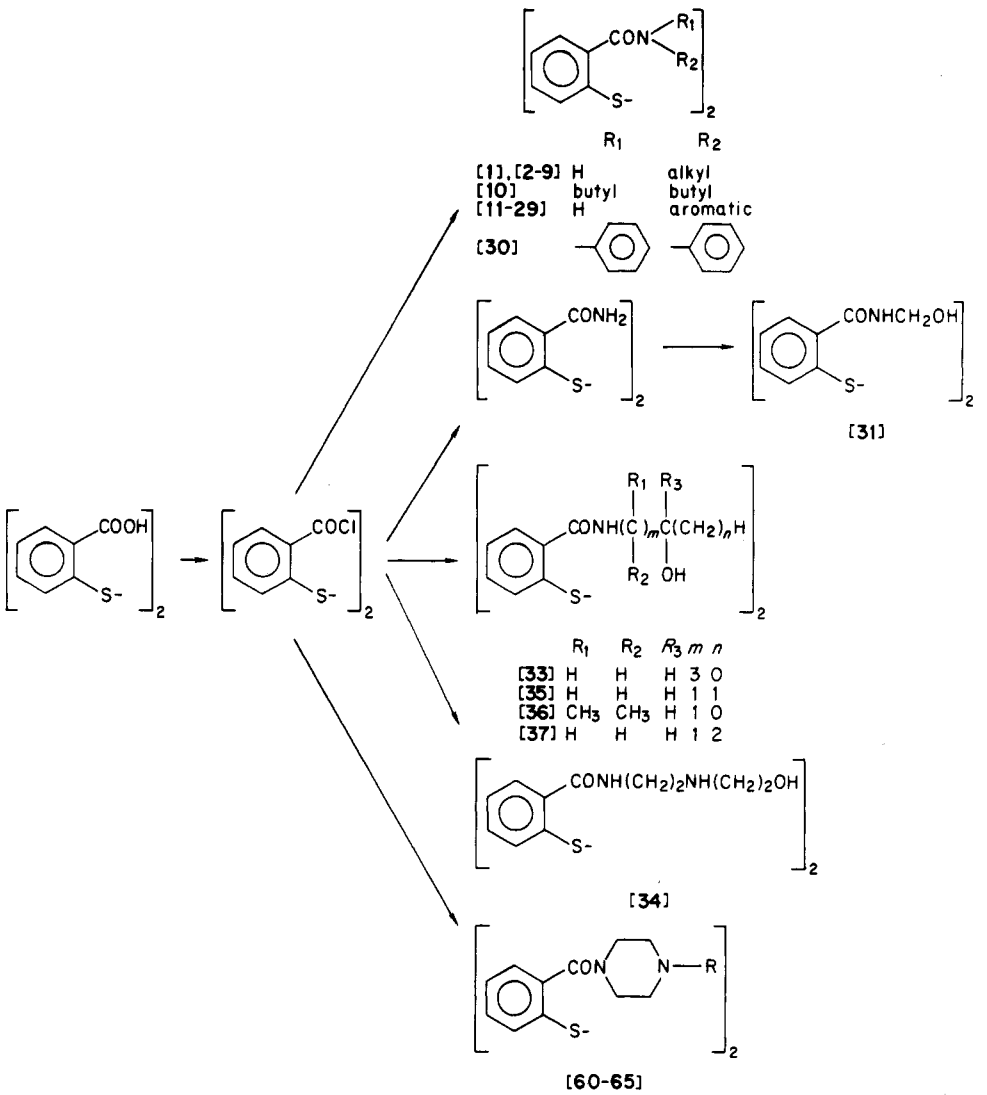
	MIC, μg/mL					
no.	*M. tub.*[1] H37Rv	*M. tub.*[2] SM^r	*M. tub.*[3] KM^r	*M. tub.*[4] INH^r	*M. kans.*[5]	*M. intr.*[6]
[1]	12.5	6.25	6.25	6.25	25	25
[2]	12.5	3.13	6.25	3.13	12.5	25
[3]	3.13				25	50
[4]	100				>100	>100
[5]	1.56	1.56	1.56	1.56	3.13	50
[6]	100				>100	>100
[7]	100				>100	>100
[8]	25	12.5	12.5	12.5	25	25
[9]	12.5				100	>100
[10]	6.25				25	25
[11]	25				50	>100
[12]	100				>100	>100
[13]	12.5	3.13	6.25	3.13	25	25
[14]	25				100	>100
[15]	3.13				12.5	25
[16]	>100	>100	>100	>100	>100	>100
[17]	3.13	3.13	6.25	3.13	25	25
[18]	100	100	100	100	100	100
[19]	100	100	50	100	50	100
[20]	0.78	1.56	1.56	0.78	12.5	12.5
[21]	1.56	1.56	1.56	1.56	6.25	6.25
[22]	12.5	12.5	12.5	12.5	50	>100
[23]	12.5	25	12.5	12.5	50	>100
[24]	50	50	50	12.5	50	50
[25]	12.5	12.5	6.25	12.5	25	50
[26]	>100	>100	>100	>100	>100	>100
[27]	25	50	50	12.5	100	>100
[28]	50	50	50	50	50	>100
[29]	3.13	1.56	3.13	1.56	50	>100
[30]	>100				>100	>100
[31]	6.25				25	25
[32]	6.25				25	25
[33]	6.25				25	25
[34]	25				>100	>100
[35]	1.56				25	25
[36]	12.5	12.5	12.5	6.25	12.5	12.5
[37]	12.5	12.5	12.5	12.5	12.5	12.5
[38]	12.5	12.5	12.5	12.5	25	25
[39]	25				25	100
[40]	100	>100	100	100	>100	>100
[41]	100				100	>100
[42]	12.5				25	25
[43]	6.25				25	50
[44]	6.25				12.5	12.5
[45]	3.13				12.5	12.5
[46]	1.56				12.5	12.5
[47]	3.13				50	100
[48]	25				>100	>100
[49]	6.25	3.13	3.13	3.13	25	100
[50]	50				100	100
[51]	12.5	12.5	12.5	6.25	25	25
[52]	6.25	6.25	6.25	3.13	6.25	25
[53]	3.13	3.13	3.13	3.13	0.39	12.5
[54]	0.78	1.56	1.56	1.56	0.39	12.5
[55]	0.39	0.78	0.39	0.39	0.78	12.5
[56]	0.39	0.78	0.39	0.39	0.39	12.5
[57]	0.78	0.78	0.78	0.78	12.5	>100
[58]	50				100	>100
[59]	>100	>100	>100	>100	>100	>100
[60]	25				50	>100
[61]	>100				>100	>100
[62]	100				>100	>100
[63]	>100				>100	>100
[64]	100				>100	>100
[65]	12.5				50	25
[66]	12.5				>100	>100
[67]	>100	>100	>100	>100	>100	>100
[68]	25	12.5	25	12.5	25	25
[69]	25	25	25	25	25	25
[70]	>100	>100	>100	>100	>100	>100
[71]	>100	>100	>100	>100	>100	>100
[72]	>100	>100	>100	>100	>100	>100
[73]	100	>100	>100	>100	>100	>100
[74]	12.5	12.5	12.5	6.25	12.5	12.5

Table II (Continued)

no.	MIC, μg/mL					
	M. tub.[1] H37Rv	*M. tub.*[2] SM^r	*M. tub.*[3] KM^r	*M. tub.*[4] INH^r	*M. kans.*[5]	*M. intr.*[6]
[75]	>100	100	100	>100	>100	100
[76]	12.5	12.5	12.5	12.5	25	12.5
[77]	12.5	12.5	12.5	12.5	25	12.5
SM	0.78	>100	3.13	0.78	3.13	12.5
KM	3.13	6.25	>100	6.25	25	6.25
INH	0.05	0.05	0.05	100	1.56	6.25
EB	0.78	0.78	1.56	0.78	12.5	12.5
RFP	0.05	0.025	0.05	0.025	0.05	0.2

[a] Key: (1) *Mycobacterium tuberculosis* H37Rv; (2) *M. tuberculosis* H37Rv SM resistant; (3) *M. tuberculosis* H37Rv KM resistant; (4) *M. tuberculosis* H37Rv INH resistant; (5) *Mycobacterium kansasii* Braunel; (6) *Mycobacterium intracellulare* Uyeda.

Chart I



2,2'-Dithiobis[*N*-(2-hydroxybutyl)benzamide] [37]. To a solution of 2,2'-dithiobis(benzoyl chloride) (3.43 g) in dioxane (30 mL) was added dropwise below 15 °C a solution of 1-amino-2-butanol (3.57 g) in dioxane (10 mL). After addition, the solution was stirred for 2 h. The reaction mixture was poured over ice in a beaker. A white precipitate was filtered, and the filtrate was washed with water and air-dried. [37] was obtained as white crystals upon recrystallization from ethanol; 3.96 g (88.4%).

2,2'-Dithiobis[*N*-[1,1-bis(hydroxymethyl)propyl]benzamide] [39]. To a solution of 2,2'-dithiobis(benzoyl chloride) (3.43 g) in tetrahydrofuran (THF, 30 mL) was added dropwise below 10 °C for 1 h a THF solution (20 mL) of 2-amino-2-ethyl-1,3-propanediol (4.80 g). After additional stirring for 2 h at room temperature, the mixture was poured into ice water in a beaker. A precipitate was filtered, and the filtrate was washed with water and air-dried at room temperature. [39] was obtained as a white powder upon recrystallization from acetone; 4.57 g (90%). [34] and [40] were prepared in a similar manner as above. [43] was synthesized in a similar manner as above using 2,2'-dithiobis(5-chlorobenzoyl chloride) as the starting material.

2,2'-Dithiobis[*N*-(2-ethylhexyl)benzamide] [5]. A solution of 2-ethylhexylamine (5.2 g) in dioxane (20 mL) was added to a suspension of 2,2'-dithiobis(benzoyl chloride) (3.43 g) in dioxane (20 mL) below 15 °C for 1 h. After addition, the mixture was stirred for more than 2 h at room temperature. Then, the reaction mixture was poured into ice water in a beaker. The generated precipitate was filtered, and the filtrate was washed with water and air-dried. A white crystalline powder was obtained by recrystallization from ethanol; 4.98 g (94.1%). [6] and [8] were prepared in a manner similar to that above.

Chart II

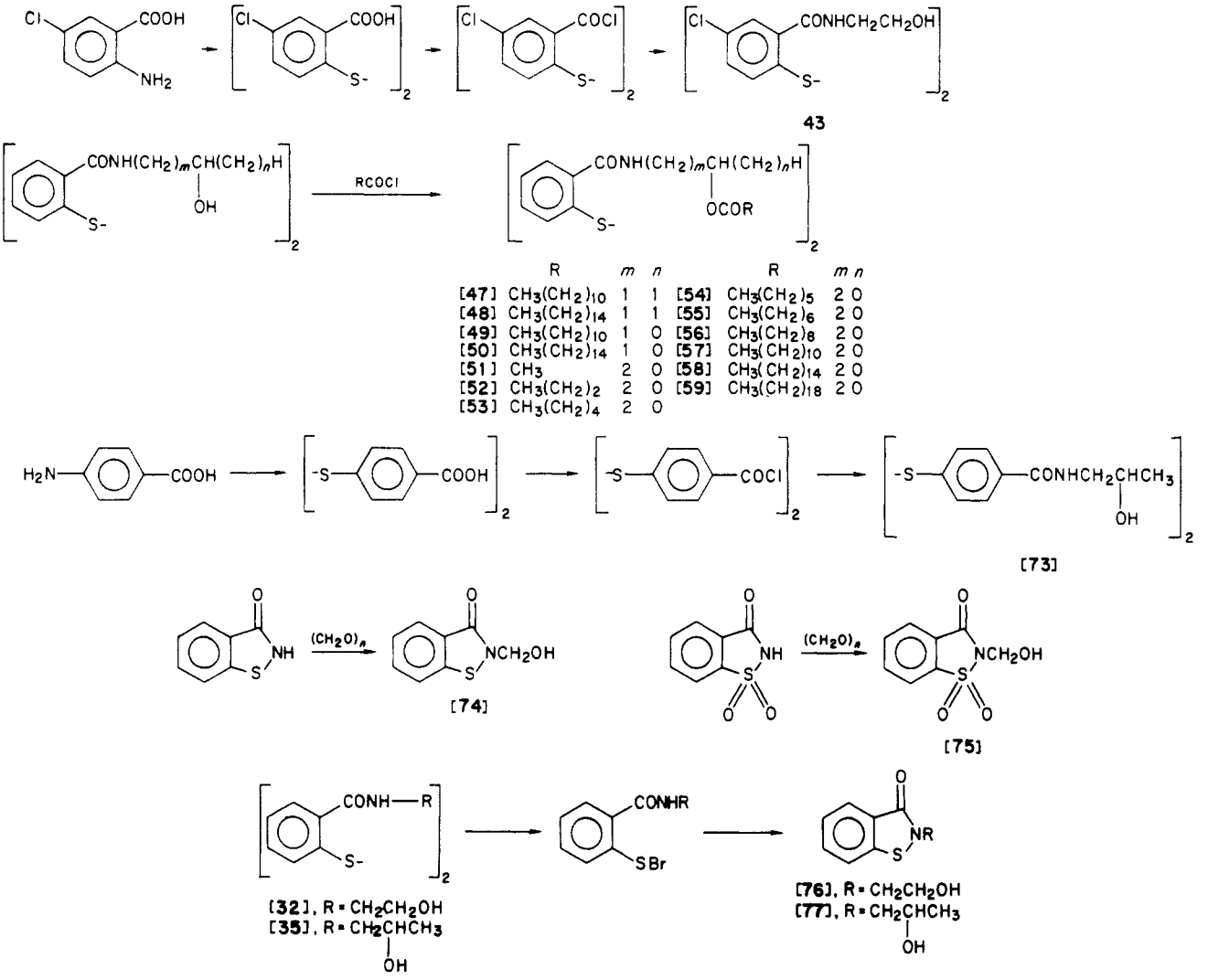
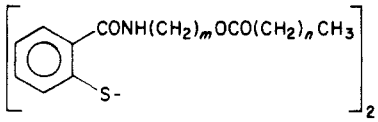


Table III. Relation of the Length of the Alkyl Side Chain and MIC in (Acyloxy)alkyl Derivatives



no.	no. of C atoms: m	no. of C atoms: n	MIC, μg/mL: vs. *Mycobacterium tuberculosis* H37Rv
[49]	2	10	6.25
[57]	3	10	0.78
[51]	3	0	12.5
[52]	3	2	6.25
[53]	3	4	3.13
[54]	3	5	0.78
[55]	3	6	0.39
[56]	3	8	0.39
[57]	3	10	0.78
[58]	3	14	50
[59]	3	18	>100

2,2′-Dithiobis[*N*-(4-piperidinylmethyl)benzamide] Dihydrochloride [7]. To a solution of 2,2′-dithiobis(benzoyl chloride) (3.43 g) in THF was added a THF solution of 4-(aminomethyl)piperidine (2.28 g) dropwise below 10 °C with stirring, and the stirring was continued for 2 h at room temperature. The reaction mixture was filtered, washed with cold acetone, and air-dried. The precipitate was recrystallized from ethanol. A pale brown crystalline product was obtained; 4.27 g (74.8%).

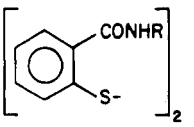
2,2′-Dithiobis[*N*-(2,2-dimethoxyethyl)benzamide] [45]. To a suspension of 2,2′-dithiobis(benzoyl chloride) (3.43 g) in dioxane (20 mL) was added dropwise below 15 °C a dioxane solution of aminoacetaldehyde dimethyl acetal (4.50 g). After 2 h of stirring at room temperature, the reaction mixture was poured over ice in a beaker. The precipitate was filtered, and the filtrate was washed with water and air-dried at room temperature. [45] was obtained as a white crystalline powder by recrystallization from methanol; 3.62 g (75.3%).

2,2′-Dithiobis[*N*-[(ethoxycarbonyl)methyl]benzamide] [68]. To a solution of 2,2′-dithiobis(benzoyl chloride) in THF was added dropwise below 10 °C a THF solution of a glycine ethyl ester (4.3 g). After additional stirring for 2 h at room temperature, the reaction mixture was poured into ice water in a beaker. The precipitate was filtered, and the filtrate was washed with water. [68] was obtained as white needles by recrystallization from ethanol; 4.31 g (90.4%).

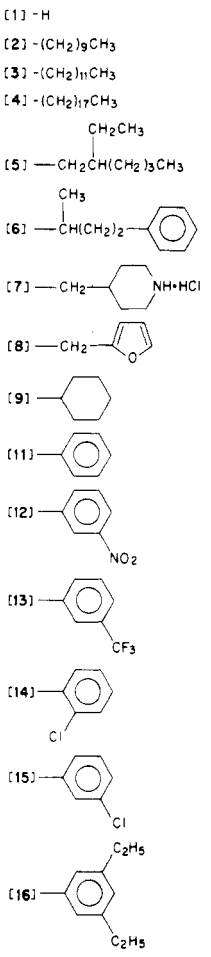
2,2′-Dithiobis[*N*-[2-[(*N*-ethylcarbamoyl)oxy]ethyl]benzamide] [69]. To a solution of 2,2′-dithiobis[*N*-(2-hydroxyethyl)benzmide] [32] (3.9 g) in dioxane (30 mL) was added dropwise below 20 °C for 10 min ethyl isocyanate (1.4 g) in dioxane (5 mL). After the addition, the mixture was heated at 98–105 °C for 2 h. After the completion of the reaction, the flask was cooled and then the precipitate appeared. Water (20 mL) was added, and the mixture was stirred. The precipitate was filtered under suction, and the filtrate was washed with water and dried. Pure [69] was obtained by recrystallization from ethanol; 4.92 g (92.0%).

2,2′-Dithiobis[*N*-[2-(lauroyloxy)propyl]benzamide] [47]. To a solution of 2,2′-dithiobis[*N*-(2-hydroxypropyl)benzamide] (4.21 g) [35] in dichloromethane (30 mL) was added dropwise with stirring below 20 °C for 30 min a dichloromethane solution (20

Chart III. Structures of 2,2′-Dithiobis(benzamide) Derivatives



R



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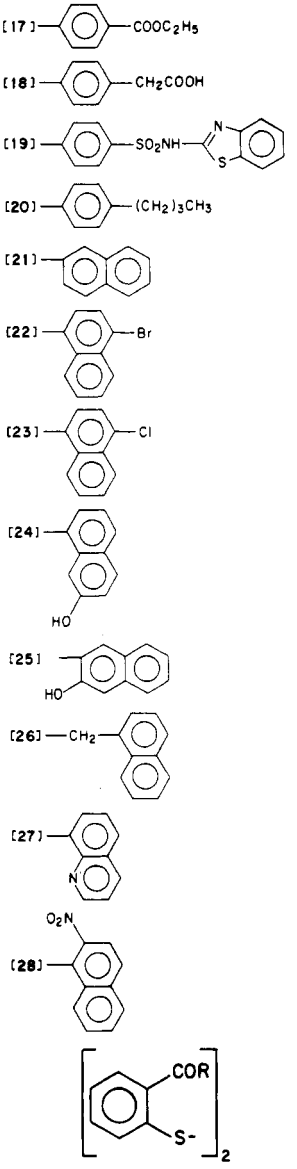

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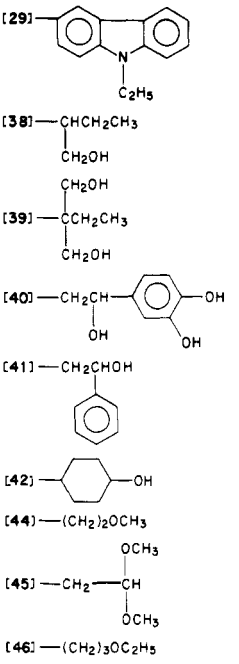

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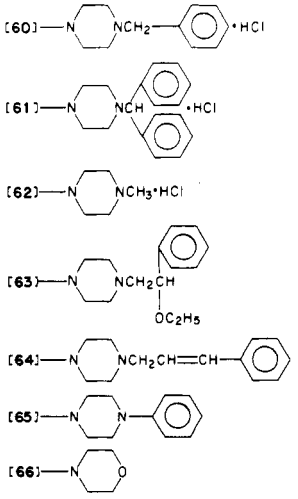

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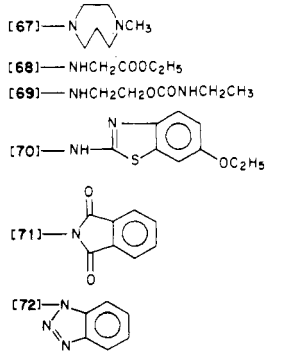

mL) of lauroyl chloride (4.38 g). The reaction mixture was allowed to stir for 30 min, and 1.60 g of pyridine was added to the solution below 20 °C during 1 h. After that, stirring was continued for 2 h. Then, the reaction mixture was washed with a 100-mL portion of a NaCl-saturated solution and dried over Na_2SO_4, and the solvent was evaporated at reduced pressure. The residue was recrystallized from 2-propanol. A white crystalline substance was obtained; 5.45 g (69.4%).

2,2′-Dithiobis[*N*-[2-(palmitoyloxy)ethyl]benzamide] [50]. To a solution of 2,2′-dithiobis[*N*-(2-hydroxyethyl)benzamide] (3.92 g) [**32**] in dichloromethane (30 mL) was added dropwise below 20 °C a solution of palmitoyl chloride (5.5 g) in dichloromethane. After addition, the reaction mixture was allowed to stir at room temperature for 30 min, and pyridine (1.6 g) was added to the solution below 20 °C. The reaction was continued for 2 h at room temperature. After that, the solution was washed with NaCl-saturated water (100 mL), dried over Na_2SO_4, and concentrated at reduced pressure. The residue was recrystallized from 2-propanol, and a white crystalline powder was obtained; 7.24 g (83.2%). [**48–49**] and [**51–59**] were prepared in a manner similar to that above.

2,2′-Dithiobis[*N*-(3,5-diethylphenyl)benzamide] [16]. To a solution of 2,2′-dithiobis(benzoyl chloride) (3.43 g) in dioxane (30 mL) was added dropwise below 15 °C with stirring a solution of 3,5-diethylaniline (5.97 g) in dioxane. After addition, the reaction mixture was stirred for 2 h at room temperature and was poured into ice water in a beaker. The precipitate was air-dried and recrystallized from dimethylformamide (DMF), and a white crystalline product was obtained; 4.0 g (70.4%). [**18–20**] and [**70–72**] were prepared in a manner similar to that above.

2,2′-Dithiobis[*N*-(4-bromonaphth-1-yl)benzamide] [22]. A solution of 4-bromo-1-naphthylamine (8.90 g) in dioxane was added dropwise to a suspension of 2,2′-dithiobis(benzoyl chloride) (3.43 g) in dioxane (30 mL) below 15 °C for 30 min. The reaction mixture was stirred for 2 h at 40 °C. Then, the mixture was poured into ice water in a beaker. The precipitate was filtered, and the filtrate was washed with water and dried. A pale violet crystalline product was obtained by recrystallization from diethylene glycol monomethyl ether (MDG); 6.73 g (94.3%). [**23–29**] were prepared in a manner similar to that above.

2,2′-Dithiobis(*N*,*N*-di-*n*-butylbenzamide) [10]. To a suspension of 2,2′-dithiobis(benzoyl chloride) (3.43 g) in dioxane was added dropwise with stirring below 15 °C 4 equiv of di-*n*-butylamine. After stirring for 3 h at room temperature, the mixture was poured into ice water in a beaker. The precipitate was filtered, and the filtrate was washed with water and acetone and air-dried. The filtrate was recrystallized from dioxane. A white crystalline product was obtained; 5.0 g (94.3%). [**30**] was prepared in a manner similar to that above.

***N*-(Hydroxymethyl)-1,2-benzisothiazolin-3-one [74].** 1,2-Benzisothiazolin-3-one (1.5 g) and 37% formalin in 50 mL of methanol was refluxed for 5 h. After completion of the reaction, the solution was concentrated at reduced pressure. The residue was solidified after standing for 1 day. The solid was recrystallized from ethanol, and [**74**] was obtained as white powder, 0.61 g (33.7%).

***N*-(Hydroxymethyl)-1,2-benzisothiazolin-3-one 1,1-Dioxide [75].** A mixture of saccharin (0.91 g) and 37% formalin (4.05 g) in ethanol (40 mL) was refluxed for 4 h. After completion of the reaction, the reaction mixture was concentrated at reduced pressure and the residue was allowed to stand for few days in a refrigerator. The viscous oil was solidified, and the solid was recrystallized from ethanol, obtaining a white crystalline product, 0.67 g (31.5%).

***N*-(2-Hydroxypropyl)-1,2-benzisothiazolin-3-one [77].** To a solution of 2,2′-dithiobis[*N*-(2-hydroxypropyl)benzamide] [**35**] (21 g) in dichloromethane (150 mL) was added dropwise below 10 °C bromine (4 g). Thirty minutes later, triethylamine (10.18 g) was added under cooling. After that, the reaction mixture was heated at 50 °C and stirred for 30 min. After completion of the reaction, the solution was washed with water (300 mL) and dried with Na_2SO_4 and the solvent was evaporated at reduced pressure. The residual solid was recrystallized from ethanol, and a white crystalline powder was obtained; 17.0 g (81.2%).

4,4′-Dithiobis[*N*-(2-hydroxypropyl)benzamide] [73]. A solution of 2-propanolamine (2.96 g) in dioxane (5 mL) was added dropwise below 15 °C for 30 min to a solution of 4,4′-dithiobis(benzoyl chloride) (3.43 g) prepared from *p*-aminobenzoic acid in a manner similar to that for *o*-aminobenzoic acid[9] in dioxane (20 mL). After that, the mixture was stirred at 40–50 °C for 2 h. Then, the mixture was poured into ice water with stirring. The precipitate was filtered, and the filtrate was washed with water and dried. The solid was recrystallized from acetone. [**73**] (2.1 g) was obtained (50%).

The syntheses and physical data of [**1–3**], [**9**], [**17**], [**21**], [**66**],[2] [**13, 14**], [**31**], [**33**], [**35, 36**], [**38**], [**41, 42**], [**46**], [**60, 61**], [**63–65**], [**67**],[4] [**32**], [**44**],[10] [**4**],[11] [**11**],[12] [**12**],[13] [**15**],[14] [**62**],[15] and [**76**][16] were reported previously in each references.

Biology. Test Organisms. The following microorganisms were provided by the Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association: 1. *M. tuberculosis* H37Rv; 2. *M. tuberculosis* H37Rv SM resistant; 3. *M. tuberculosis* H37Rv KM resistant; 4. *M. tuberculosis* H37Rv INH resistant; 5. *M. kansasii* Braunel; 6. *M. intracellulare* Uyeda.

Medium and Inoculum. Test organisms were maintained on a slant of 1% Ogawa medium (EIKEN Chemicals) containing KH_2PO_4 1 g, sodium glutamate 1 g, whole egg 200 mL, glycerol 6 mL, and 2% Malachite Green solution 6 mL/L. One loopful of fresh mycerium was transferred from the slant into Dubos liquid medium (EIKEN Chemicals) which contained KH_2PO_4 1.3 g, $NaHPO_4$ 2.2 g, asparagine 2 g, peptone 5.2 g, $MgSO_4 \cdot 7H_2O$ 0.1 g, ferric ammonium citrate 10 mg, $ZnSO_4 \cdot H_2O$ 0.1 mg, $CuSO_4 \cdot 5H_2O$ 0.1 mg, $CaCl_2 \cdot 2H_2O$ 0.5 mg, Malachite Green 2 mg, Tween 80 0.5 g/L (pH 6.2) supplemented with 10% (v/v) of Albumine for Tuberculosis medium (EIKEN Chemicals) containing bovine albumin 5%, glycerol 5%, glucose 7.5%, and NaCl 0.9%, pH 6.8. The seeded liquid medium was incubated stationary at 37 °C for 4 days. The grown broth was diluted to 6×10^5 cfu/mL by the same medium for preparing the inoculum.

Determination of MIC. The minimum inhibitory concentrations (MIC) were determined by an agar dilution technique. Serial twofold dilutions of the compounds (KM, SM, INH, and EB were dissolved in sterilized water, all of the other compounds were in dimethyl sulfoxide) were added to Dubos agar (2%) medium (EIKEN Chemicals) containing 10% (v/v) of Albumine for Tuberculosis medium, and 10 mL of the compound-supplemented agar medium was poured into sterile Petri dishes (90 mm in diameter). After the agar solidified, the diluted inoculum (6×10^5 cfu/mL) was applied to the agar surface with a Typing apparatus (Muto Kikai). The plates were sealed by Scotch tape for prevention from dryness and incubated at 37 °C for 14 days. The MIC was read as the smallest concentration of drug that inhibited growth of each microorganism.

Registry No. **1**, 2527-57-3; **2**, 63956-26-3; **3**, 78010-07-8; **4**, 78010-08-9; **5**, 88848-47-9; **6**, 63956-36-5; **7**, 98064-09-6; **7** (free base), 81416-54-8; **8**, 98051-77-5; **9**, 2620-89-5; **10**, 78010-09-0; **11**, 2527-63-1; **12**, 19602-86-9; **13**, 96835-63-1; **14**, 50383-29-4; **15**, 2634-29-9; **16**, 98051-78-6; **17**, 90520-54-0; **18**, 96835-64-2; **19**, 98064-10-9; **20**, 90520-53-9; **21**, 2527-65-3; **22**, 98064-11-0; **23**, 98051-79-7; **24**, 98051-80-0; **25**, 98051-81-1; **26**, 98051-82-2; **27**, 98051-83-3; **28**, 98051-84-4; **29**, 90520-55-1; **30**, 78010-10-3; **31**, 78010-11-4; **32**, 7765-80-2; **33**, 78010-12-5; **34**, 78010-19-2; **35**, 73845-37-1; **36**, 96835-56-2; **37**, 96835-55-1; **38**, 96835-54-0; **39**, 81419-25-2; **40**, 98051-85-5; **41**, 78010-30-7; **42**, 78010-34-1; **43**, 78468-94-7; **44**, 33353-20-7; **45**, 78010-26-1; **46**, 78010-27-2; **47**, 78010-17-0; **48**, 78010-18-1; **49**, 78010-13-6; **50**, 78010-14-7; **51**, 88848-48-0; **52**, 88848-49-1; **53**, 88848-50-4; **54**, 88848-51-5; **55**, 88848-52-6; **56**, 88848-53-7; **57**, 78010-15-8; **58**, 78010-16-9; **59**, 98051-86-6; **60**, 73845-35-9; **60** (free base), 78010-29-4; **61**, 98051-87-7; **61** (free base), 78010-25-0; **62**, 98051-88-8; **62** (free

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Supplementary Material Available: NMR data for compounds [5], [8], [10], [24], [25], [28–30], [34], [47], [48], [50], [52], [53], [57], [58], [68], [69], and [73] (2 pages). Ordering information is given on any current masthead page.

Renin Inhibitors. Syntheses of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine[1]

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Analogues of the renin octapeptide substrate were synthesized in which replacement of the scissile dipeptide with (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (statine, Sta) transformed the substrate sequence into potent, transition-state analogue, competitive inhibitors of renin. Synthesis and incorporation of the cyclohexylalanyl analogue of Sta, (3*S*,4*S*)-4-amino-5-cyclohexyl-3-hydroxypentanoic acid (ACHPA), gave the most potent inhibitors of renin yet reported, including *N*-isovaleryl-L-histidyl-L-prolyl-L-phenylalanyl-L-histidyl-ACHPA-L-leucyl-L-phenylalanyl amide [Iva-His-Pro-Phe-His-ACHPA-Leu-Phe-NH_2, **3**], with renin inhibitions of $K_i = 1.6 \times 10^{-10}$ M (human kidney renin), $IC_{50} = 1.7 \times 10^{-10}$ M (human plasma renin), $IC_{50} = 1.9 \times 10^{-9}$ M (dog plasma renin), and $IC_{50} = 2.1 \times 10^{-8}$ M (rat plasma renin). This inhibitor **3**, containing ACHPA, was 55–76 times more potent vs. human renin than the comparable Sta-containing inhibitor **1** and 17 times more potent vs. dog renin than **1**. Inhibitor **3** lowered blood pressure in sodium-deficient dogs, with in vivo potency 19 times that shown by **1**, in close agreement with the relative in vitro potencies. Structure–activity results are presented that show the minimal N-terminus for these inhibitors. An ACHPA-containing pentapeptide, *N*-[(ethyloxy)carbonyl]-L-phenylalanyl-L-histidyl-ACHPA-L-leucyl-L-phenylalanyl amide [Etoc-Phe-His-ACHPA-Leu-Phe-NH_2, **8**], retained subnanomolar inhibitory potency. Molecular modelling studies are described that suggested the design of ACHPA.

The renin–angiotensin system (RAS) is a multiregulated proteolytic cascade that produces two potent pressor and aldosteronogenic peptides: an octapeptide, angiotensin II (AII), and a heptapeptide, angiotensin III (AIII).[2,3] Although the exact role that the RAS plays in the maintenance of normal blood pressure is unclear, it has been demonstrated that the pharmacological interruption of the RAS can lower blood pressure in a large majority of hypertensive patients.[4,5] Specifically, inhibitors have been developed of angiotensin converting enzyme,[6] the enzyme that cleaves the inactive decapeptide angiotensin I (AI) to yield AII. These angiotensin converting enzyme inhibitors may be a major advance in the treatment of hypertension and congestive heart failure.[5]

The first proteolytic step in the RAS is the renin enzyme reaction, in which the decapeptide AI is cleaved from a protein substrate, angiotensinogen. Competitive inhibitors of renin based upon the substrate peptide sequence have been reported from several laboratories.[7–12] We have described[13–15] the design of inhibitors of renin, with potencies around 10 nM ($K_i = 10^{-8}$ M), which are analogues of the minimum substrate octapeptide and which contain

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(1) Abbreviations follow IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides; *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations used are as follows: DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; Boc, *tert*-butyloxycarbonyl; Poc, (isopropyloxy)-carbonyl; Etoc, (ethyloxy)carbonyl; POA, phenoxyacetyl; TFA, trifluoroacetic acid; TEA, triethylamine; Iva, isovaleryl; Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; AHPPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid; ACHPA, (3*S*,4*S*)-4-amino-3-hydroxy-5-cyclohexylpentanoic acid.
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