Mitomycin C Analogues with Secondary Amines at Position 7

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A series of mitomycin C analogues with secondary amines at position 7 was prepared from mitomycin A. Eleven of the 20 new compounds in this series were more active than mitomycin C against P-388 murine leukemia, and 2 of these 11 were significantly less leukopenic. The two substituents conferring these superior properties were 4-formylpiperazine and 2-cyanoaziridine. No quantitative correlations could be made among antitumor activities and physicochemical properties of the analogues, although the relative ease of quinone reduction might be related to the good potencies (minimum effective doses) of many of them.

In the preceding articles of this series[1,2] and in related publications from other laboratories,[3-6] a variety of new 7-substituted analogues of mitomycin C have been described. Most of these analogues featured primary amines at position 7, and only a few secondary amines were reported. However, the potency (minimum effective dose) of aziridino analogues, such as **20** (Table I), against four different tumors[1,3-5] and the good activity (prolongation of life) of the 7-piperidino analogue 4 against the Hirosaki ascites sarcoma[3] suggested that the secondary amines should be investigated further. Consequently, we have undertaken the preparation and testing of an extended series of secondary amines. This series includes acyclic compounds and cyclic compounds with three, four, five, and six-membered rings. We also have considered structure–activity relationships among these compounds.

The desired secondary amino analogues were prepared by treating mitomycin A in anhydrous methanol or methylene chloride with the appropriate amine or amine hydrochloride. If an amine hydrochloride was used, excess triethylamine was added.[2] In a few cases, especially with the larger amines, the reaction rate was slow, and a trace of solid potassium carbonate was added to accelerate it. All of the amines were available commercially,[7] except for the substituted aziridines. Table I gives the yields and physical properties of the products.

Acyclic secondary amine analogues prepared included the known dimethylamino compound **1**,[3] the corresponding diethylamino compound **2**, and the [(dimethylamino)-ethyl]methylamino compound **3**. The last compound has analogy to both the highly active (dimethylamino)ethyl analogue reported earlier[2] and the methylpiperazino analogue **12**. A further analogue was the 4-piperidylpiperidino derivative **8**.

Cyclic secondary amines with six-membered rings began with the known piperidino analogue 4[3] and its 3-methyl derivative **5**, 3-hydroxy derivative **6**, and 4,4-dihydroxy derivative **7**. The last compound corresponds to a hydrated carbonyl group at the 4-position of piperidine. The starting amine is formulated this way by the supplier[7] and neither it nor product **7** shows a carbonyl group in the infrared absorption spectrum. Other six-membered-ring compounds were based on isosteric replacement of the 4-methylene group of piperidine with heteroatoms. Thus, the morpholino (**9**), thiomorpholino (**10**), and piperazino (**11**) analogues of mitomycin C were prepared. Among these three compounds, the thiomorpholino analogue **10** definitely is new. The status of the other two is more difficult to determine because of prior disclosure with incomplete characterization in a patent. In this patent, the characterization was based mainly on visible color, ultraviolet absorption, and R_f values.[8] Some of the compounds were prepared on a scale sufficient for antibacterial testing (no antitumor tests were reported), whereas others appear to have been made on a very small scale, just sufficient for characterization as described above. It appears that the piperazino analogue claimed in the patent is the same as our compound **11**. The patented compound has the same color, green, and it was prepared in the same way from mitomycin A and piperazine. A related piperazino analogue that was claimed to be made from mitomycin B also has the expected properties. However, the structure of a similar analogue from porfiromycin, reported to be colorless, is less certain. In our experience, displacement of the amino groups of mitomycin C or porfiromycin by other amines has been unsuccessful despite many attempts. The preparation of morpholino analogue **9**[8] from mitomycin C and morpholine might also be in doubt, especially since it was said to be pink. In contrast, the patented analogues (green color) from mitomycin B and *N*-methylmitomycin A (now called mitomycin F) appear to be valid structures.

The good antitumor activity found for piperazino analogue **11** (Table II) led us to prepare related compounds with 4-substituents. They included the *N*-methyl derivative **12**, the *N*-formyl derivative **13**, and the *N*-(4-acetylphenyl) derivative **14**.

Only one mitomycin analogue with a secondary amine in a five-membered ring, pyrrolidino derivative **15**, was known previously.[4] We prepared it and the corresponding pyrrolino derivative **16**. Commercially supplied 3-pyrroline contains about 25% of pyrrolidine, and both **15** and **16** were obtained from it. The mixture could be separated by fractional crystallization from ether, with pyrrolidino derivative **15** crystallizing first. Related analogues **17–19** were prepared from mitomycin A and 3-hydroxypyrrolidine, thiazolidine, and indoline, respectively. Pyrrole and indole failed to react with mitomycin A. This is not surprising in view of their poor nucleophilicity. However, the sodium salts of pyrrole and indole also failed

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(1) Iyengar, B. S.; Lin, H. J.; Cheng, L.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1981**, *24*, 975–981.
(2) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16.
(3) Usubuchi, I.; Sobajima, Y.; Hongo, T.; Kawaguchi, T.; Sugawara, M.; Matsui, M.; Wakaki, S.; Uzu, K. *Gann* **1967**, *58*, 307–313.
(4) Oboshi, S.; Matsui, M.; Ishii, S.; Masago, N.; Wakaki, S.; Uzu, K. *Gann* **1967**, *58*, 315–321.
(5) Kojima, R.; Driscoll, J.; Mantel, N.; Goldin, A. *Cancer Chemother. Rep.* **1972**, *3*, 121–135.
(6) Imai, R.; Ashizawa, T.; Urakawa, C.; Morimoto, M.; Nakamura, N. *Gann* **1980**, *71*, 560–562.
(7) Aldrich Chemical Co., Milwaukee, WI.
(8) Cosulich, D. B.; Patrick, J. B., Williams, R. P. U.S. Patent 3 332 944, 1967.

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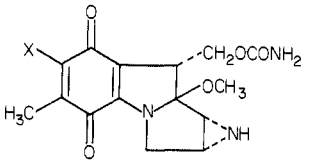
Table I. Properties of Mitosanes with Secondary Amines at Position 7[a]



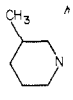
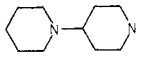
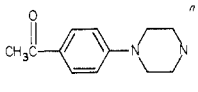
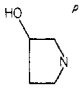
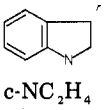
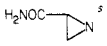
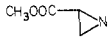
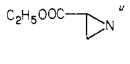
no.	X	yield, %	recrystn solvents	solvent impurity	mp, °C	^{1}H NMR signals for the new substituents; signals for the solvent impurity,[b] δ
1	$(CH_3)_2N$	ref 3				
2	$(C_2H_5)_2N$	36	CH_2Cl_2-CH_3OH	$0.5CH_2Cl_2$	67-70 dec	0.97 (s, 6), 2.50 (q, 4); 5.58 (s)
3	$(CH_3)_2NCH_2CH_2NCH_3$[g]	42	CH_2Cl_2-CH_3OH	$1.5CH_3OH$	79-82 dec	2.16 (s, 9), 2.00-2.69 (m, 4); 3.38 (br s), 4.05 (br s)
4	c-NC_5H_{10}	ref 3				
5		55	CH_3OH-hexane		75-88 dec	0.84 (d, 3), 1.80 (m, 5), 3.4-3.9 (m, 4)
6		58[c]	CH_2Cl_2-CH_3OH		98-101 dec	(Me_2SO-d_6) 0.97-2.13 (m, 4), 2.17-3.13 (m, 4), 3.33-4.33 (m, 1), 5.30 (br s, 1)
7		58[c]	CH_2Cl_2-CH_3OH		107-110 dec	(Me_2SO-d_6) 2.40 (t, 4), 3.13 (m, 6)
8		72[d]	CH_2Cl_2-CH_3OH	$2.5CH_3OH$	79-82 dec	1.18-1.85 (m, 8), 2.05-3.42 (m, 11); 3.40 (br s), 4.05 (br s)
9	c-$O(CH_2CH_2)_2N$[j]	95	CH_2Cl_2-CH_3OH	$0.7CH_2Cl_2$	200 dec	2.90 (t, 4), 3.74 (t, 4); 5.57 (s)
10	c-$S(CH_2CH_2)_2N$[k]	22	$CHCl_3$-CH_3OH	$1H_2O$	90-91 dec	2.81 (t, 4), 3.6 (t, 4)
11	c-$N(CH_2CH_2)_2NH$	ref 8				
12	c-$N(CH_2CH_2)_2N$-CH_3[l]	42	CH_2Cl_2-CH_3OH	$0.25CH_2Cl_2$	84-86 dec	2.27 (s, 3), 2.47 (t, 4), 2.92 (t, 4); 5.57 (s)
13	c-$N(CH_2CH_2)_2N$-CHO[m]	41	CH_2Cl_2-CH_3OH	$0.75CH_3OH$	94-96 dec	2.38-3.05 (m, 4), 3.06-3.88 (m, 4), 8.13 (s, 1); 3.40 (br s), 5.30 (br s, 1)
14		20[d]	CH_2Cl_2-CH_3OH		133-136 dec	2.03 (s, 3), 2.52 (t, 4), 3.41 (t, 4), 6.27-8.13 (dd, 4)
15	c-NC_4H_8	ref 4				
16	c-NC_4H_6[o]	27	$(C_2H_5)_2O$		85 dec	3.82 (m, 4), 5.9 (br s)
17		40[e]	CH_2Cl_2-CH_3OH	$0.4CH_2Cl_2$	82-85 dec	(Me_2SO-d_6) 1.6-2.23 (m, 2), 2.83-3.1 (br s, 5), 4.03-4.3 (m, 1); 5.55 (s)
18		43	CH_2Cl_2-CH_3OH	$0.25CH_2Cl_2$	105-107 dec	2.62 (m, 2), 2.68-3.02 (m, 2), 3.32-4.02 (br s, 2); 5.55 (s)
19		67	$CHCl_3$-CH_3OH[f]		133-137 dec	2.85-3.7 (m, 4), 6.15-7.15 (m, 4)
20	c-NC_2H_4	ref 1				
21		ref 1				
22		37	CH_2Cl_2-CH_3OH		160-167 dec	1.6-2.9 (m, 3), 6.9-7.4 (m, 5)
23		51	CH_2Cl_2-CH_3OH	$0.3CH_2Cl_2$	87-89 dec	1.93 (m, 2), 2.63-3.10 (br s, 1); 5.55 (s)
24		47	$CHCl_3$-CH_3OH[f]		80 dec	2.25-2.75 (m, 3), 6.25-7.15 (d, 2)
25		73	$CHCl_3$-CH_3OH[f]		125-130 dec	2.4-2.75 (m, 3), 3.73 (s, 3)
26		39	$CHCl_3$-acetone[f]		87-91 dec	1.2-1.4 (t, 3), 2.3-2.7 (m, 3), 3.7 (q, 4)

Table I (*Footnotes*)

[a] Analytical results were within ±0.40% of theoretical values for all elements (C, H, and N), except as shown in subsequent footnotes. In some examples, the solvent impurities indicated in the table had to be added to reconcile the calculated and found values for these elements. NMR signals for the solvent impurities are given in the table. It was not possible to obtain exact ratios for protons in the solvent impurities with respect to those in the compound by integrating the spectra. [b] The solvent was $CDCl_3$ unless specified otherwise. [c] Triethylamine (0.5 mL) was added to the reaction. [d] Anhydrous potassium carbonate was added to the reaction. [e] The reaction was conducted in 1:1 CH_2Cl_2-CH_3OH. [f] The product was obtained pure after chromatography on silica gel with 9:1 $CHCl_3$-CH_3OH as solvent. [g] H: calcd, 7.55; found, 6.98. [h] N: calcd, 13.45; found, 12.16. [i] N: not determined. [j] C: calcd, 51.00; found, 51.44. N: calcd, 12.07; found, 10.99. [k] N: calcd, 12.78; found, 12.27. [l] N: calcd, 15.95; found, 14.26. [m] N: calcd, 15.37; found, 14.84. [n] C: calcd, 62.18; found, 61.73. [o] N: calcd, 14.50; found, 12.34. [p] N: calcd, 12.77; found, 12.01. [q] N: calcd, 13.09; found, 12.58. [r] N: calcd, 12.84; found, 12.40. [s] H: calcd, 5.95; found, 5.38. N: calcd, 16.50; found, 15.72. [t] N: calcd, 11.60; found, 10.68. [u] N: calcd, 12.70; found, 12.00.

Table II. Antitumor Activity, Leukopenia, and Reduction Potentials of Mitosanes with Secondary Amines at Position 7

	P-388 murine leukemia[a]				leukopenia % change in WBC at opt dose on day 3[b]	
	max % T/C		opt dose,	MED,		
no.	compd	mit C	mg/kg	mg/kg		$E_{1/2}$,[c] V
1	238	325	25.6	0.2		
2	222	211	25.6	1.6		−0.26, −0.45[d]
3	267	211	25.6	0.8		−0.33, −0.42[d]
4	183	322	12.8	0.2		−0.27
5	238	319	25.6	0.2		
6	250	161	12.8	0.2	−55	−0.26
7	231	219	25.6	0.2		−0.28
8	194	200	12.8	1.6	−29	−0.28
9	271	224	25.6	0.4	−82	−0.39
10	211	178	3.2	0.4	−44	
11	200	288	12.8	0.2		−0.26
12	261 (2)	183	3.2	0.2	−34	−0.24
13	294	224	6.4	0.2	−16	−0.23, −0.40[d]
14	161	200	3.2	0.4	−40	
15	>333 (3)	161	25.6	0.4	−64	−0.35
16	216	268	12.8	0.2	−13	
17	163	219	25.6	0.8		−0.37
18	222	183	25.6	0.2	−69	−0.39
19	150	288	12.8	0.8		
20	266 (2)	300	1.6	0.025	−11	−0.31
21	233	250	3.2	0.1	−24	−0.32
22	311	200	3.2	0.2	−38	
23	339 (2)	228	12.8	0.2	−2	−0.30
24	206	233	6.4	0.8		
25	147	288	6.4	1.6		
26	133	233	12.8	12.8		
mit C	161-300		3.2	0.2	−42	−0.45

[a] Determined at Bristol Laboratories, Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted ip in CDF_1 female mice. Six mice were used at each dose of the mitosane, and 10 control mice were injected with saline. A control group of six mice at each dose received mitomycin C in the same experiment: MST = median survival time; max effect (% T/C) = MST treated/MST control × 100 at the optimal dose (opt dose); MED = minimum effective dose (% T/C 125); TR = therapeutic ratio (opt dose/MED). The number of 30-day survivors at the optimal dose is given in parentheses beside the maximum effect. Complete testing results, including each dose used for therapeutic effect, number of 5- and 30-day survivors, and average weight difference between the test and control group, are available as supplementary material (see paragraph at end of paper). [b] For a complete description of this assay, see Bradner, W. T.; Schurig, J. E. *Cancer Treat. Rev.* **1981**, *8*, 93-102. [c] Determined by differential pulse polarography on a Model 174A EGAG polarographic analyzer. The electrolyte was 1.0 M KCl solution, and the standard was 10^{-3} $CdCl_2$ in 1.0 M KCl. Mitomycin analogues were 10^{-3} M in 1.0 M KCl. $E_{1/2}$ values are given relative to the saturated calomel electrode. The following conditions were used: potential scan, 0.1 V/in.; potential scan rate, 1 mV/s; voltage range, 1.5 V; initial potential, −0.1 or −0.2 V; modulation amplitude, 25; rate of mercury drops, 60/min. [d] The polarographs for these compounds were anomalous. The expected peaks were small and followed by large peaks at a higher $E_{1/2}$.

to react with mitomycin A in methanol.

Attempts to prepare mitomycin analogues having secondary amines in four-membered rings met with frustration. We were able to prepare new green substances from mitomycin A and both azetidine and azetidine-2-carboxylic acid. Unfortunately, it was not possible to obtain reasonable combustion analysis on either substance, and both were inactive in the P-388 leukemia assay.

The 7-aziridino analogue (**20**) of mitomycin C is known for its high potency (minimum effective dose) against experimental tumors.[1,3-5] Decreases in both potency and antitumor effectiveness resulted when a methyl group was added to the 7-aziridine as in analogue **21**. In order to determine whether this decrease was related to the size, lipophilicity, or electron-donation property of the methyl group, we prepared a series of analogues in which the 7-aziridino group was substituted with electron-withdrawing groups of various sizes and lipophilicities. They included phenyl (**22**), cyano (**23**), carboxamido (**24**), carbomethoxy (**25**), and carbethoxy (**27**). The required 2-substituted aziridines were not available commercially, but they were prepared by the following literature methods: 2-phenylaziridine was obtained from lithium aluminum hydride reduction of the oxime of phenylacetaldehyde;[9] 2-cyanoaziridine was made from dibromopropionitrile and ammonia;[10] the 2-carboxylic acid esters of aziridine were

(9) Kotera, K.; Miyazaki, S.; Takahashi, H.; Okada, T.; Kitahonoki, K. *Tetrahedron* **1968**, *24*, 3681-3696.

(10) Burgin, K.; Enderer, K. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 151-152.

prepared by heating the α-chloro-β-alanine esters in base;[11] and aziridine-2-carboxamide was obtained by ammonolysis of the methyl ester.[11] Alternative routes to these compounds were unsuccessful.

Biological Activity. Table II gives the activities of the secondary amine analogues against P-388 lymphocytic leukemia in mice. The assays were not all run concurrently, but a mitomycin C standard was included in each experiment. Therefore, compounds should not be compared directly with each other, but compared on the basis of how each one related to mitomycin C. From Table II it is evident that the secondary amines are a potent and efficaceous group of mitomycin analogues. Among the 20 new compounds in this table, 11 are better than mitomycin C in prolonging the life span, and 7 of these 11 have equal or greater potency (minimum effective dose) than mitomycin C. Their leukopenic effects seem to be random, with some compounds more leukopenic and some less leukopenic than mitomycin C. In the latter group, compounds **13**, **16**, and **23** are noteworthy.

Both of the two new acyclic analogues **2** and **3** prolong life more than mitomycin C, whereas the dimethylamino analogue **1** does not; however, they are considerably less potent than these known compounds. This loss of potency could be caused by a number of factors, including size and lipophilicity. The prototype six-membered-ring analogue, piperidino derivative **4**, was previously found to be inactive against L1210 leukemia[5] but highly active against the Hirosaki ascites sarcoma in mice.[3] Table II shows it to be inferior to mitomycin C against P-388 leukemia. However, all of the related compounds **5**–**14** have better activity than **4**, and six of them are better than mitomycin C in this assay. Among these six, the 4-methylpiperazino (**12**) and 4-formylpiperazino (**13**) analogues were clearly superior to mitomycin C in prolongation of life. They also appeared to have diminished leukopenia. The known five-membered-ring prototype **15** had been found inactive against sarcoma 180[4] and less active than mitomycin C and many analogues against the Hirosaki ascites sarcoma.[3] Consequently, it was a surprise to find its exceptionally good activity against P-388 leukemia. It was, however, less potent and more leukopenic than mitomycin C. None of the analogues of **15** in Table II showed exceptionally good activity, although pyrroline **16** had low leukopenia, and thiazolidine **18** was slightly better than mitomycin C in prolonged life. Two of the substituted aziridines, phenylaziridine analogue **22** and cyanoaziridine analogue **23**, showed outstanding antitumor activity, and the latter compound was not leukopenic. They were less potent than the parent aziridino analogue **20**, but equal in potency to mitomycin C.

Two of the more active analogues described above, **13** and **23**, were evaluated against B-16 melanocytic melanoma in mice. As shown in Table III, they were superior to mitomycin C in this assay, particularly in the number of cures (tumor-free survivors on day 62).

Attempts at correlating the antitumor potencies of the compounds listed in Table II with their physicochemical properties are not easy because of the large number of variations in the 7-substituent. One highly significant property is that all of the compounds are readily reducible to the corresponding hydroquinones, a step considered essential for their bioactivation.[12] Mitomycin C has a polarographic half-wave reduction potential of −0.45 V, and typical primary amine analogues have potentials of −0.44 to −0.41 V.[2] The compounds in Table II have potentials of −0.39 to −0.23 V, which are considerably smaller in magnitude than those of the primary amines. This effect probably accounts for the good potencies of many of the compounds in Table II. The reason for such potentials is uncertain, but the most likely explanation is steric inhibition of delocalization of electrons from the nitrogen into the quinone ring. Particularly small (in magnitude) potentials for the acyclic and six-membered-ring compounds are consistent with this explanation. The small potentials of the aziridino analogues are caused by a different phenomenon, the tendency of the three-membered ring to retain its electrons in order to increase its stability. This tendency is apparent in other properties, such as the relatively high infrared absorption frequency of amides with aziridino nitrogens.[13] Within each ring size the reduction potentials are nearly the same, and one would hope that the antitumor potencies could be correlated quantitatively with important properties such as partition coefficient and substitutent size. Unfortunately, we are unable to find any broad correlations. Even the six-membered-ring compounds, which form the largest and most rational subset of secondary amines, do not show any consistent trends based on these physical properties. Some of the analogues with the largest 7-substitutents, including bipiperidyl (**8**), indolyl (**19**), and carbethoxyaziridinyl (**26**), show low potencies (Table II). Substituent size was cited as an important factor in a previous attempt at structure–activity correlation among mitomycin analogues.[14]

Table III. Comparative Activities of Compounds 13 and 23 and Mitomycin C against B-16 Melanoma in Mice[a]

no.	dose,[b] mg/(kg day)	effect = MST, % T/C	cures[c]	av wt change, g
13	6	88	2/10	−0.7
	4	310	6/10	−1.0
	3	310	6/10	−0.4
	2	275	5/10	−0.1
	1	210	1/10[d]	0.0
23	5	toxic		−1.3
	3	65	0	0.0
	2	307	5/10	0.0
mit C	4	200	0	−0.1
	3	165	0	−0.1
	2	152	0	0.5
	1	155	0	0.2

[a] Determined according to standard NCI protocol at Bristol Laboratories. [b] Injections given ip on days 1, 4, and 7. The vehicle was Me_2SO + BS. Ten mice were used at each dose. [c] Tumor-free survivors on day 62. [d] There also was one tumored survivor.

Experimental Section

Melting points were recorded on a Mel-Temp melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian (EM 360, 60 MHz) NMR spectrometer with tetramethylsilane as the standard. Elemental analyses were performed by the Analytical Center, University of Arizona, Tucson, AZ, or by Chemalytics, Inc., Tempe, AZ.

Preparation of Mitomycin C Analogues (General Method). A solution of mitomycin A (100 mg of 0.286 mmol) in 8 mL of anhydrous methanol was stirred with 0.6 mmol of amine or amine hydrochloride (in the case of amine hydrochloride, 0.5 mL of triethylamine was added to the reaction mixture) until thin-layer chromatography showed that the conversion was complete or no longer progressing. In those cases where the reaction was slow, anhydrous potassium carbonate (25 mg) was added as a promoter.

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During the reactions the solutions turned from plum colored to green. The solvent was removed by evaporation under reduced pressure, and the crude products were purified by preparative thin-layer chromatography using precoated silica gel plates (20 × 20 cm and 2-mm thickness) as adsorbent and $CHCl_3$–MeOH (9:1, v/v) as the developing solvent. The products were recrystallized from CH_2Cl_2–MeOH or other solvents specified in Table I. This table also gives the yields and properties of the products.

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Registry No. 1, 4049-15-4; 2, 86689-59-0; 3, 86689-60-3; 4, 4154-16-9; 5, 86689-61-4; 6, 84397-50-2; 7, 86689-62-5; 8, 86689-63-6; 9, 17287-49-9; 10, 84397-25-1; 11, 17269-53-3; 12, 84397-44-4; 13, 86689-64-7; 14, 86689-65-8; 15, 4349-74-0; 16, 84397-42-2; 17, 86689-66-9; 18, 84397-43-3; 19, 84397-26-2; 20, 4117-86-6; 21, 86709-35-5; 22, 86689-67-0; 23, 84397-24-0; 24, 86689-68-1; 25, 86689-69-2; 26, 86689-70-5; $(C_2H_5)_2NH$, 109-89-7; $(CH_3)_2NCH_2CH_2NHCH_3$, 142-25-6; c-$O(CH_2CH_2)_2NH$, 110-91-8; c-$S(CH_2CH_2)_2NH$, 123-90-0; c-$HN(CH_2CH_2)_2NCH_3$, 109-01-3; c-$HN(CH_2CH_2)_2NCHO$, 7755-92-2; mitomycin A, 4055-39-4; 3-methylpiperidine, 626-56-2; 3-hydroxypiperidine, 6859-99-0; 4,4-dihydroxypiperidine, 73390-11-1; 4-piperidylpiperidine, 4897-50-1; *N*-(4-acetylphenyl)piperazine, 51639-48-6; 3-pyrroline, 109-96-6; 3-hydroxypyrrolidine, 40499-83-0; thiazolidine, 504-78-9; indoline, 496-15-1; 2-phenylaziridine, 1499-00-9; 2-cyanoaziridine, 33898-53-2; 2-carboxamidoaziridine, 5950-35-6; 2-methoxycarbonylaziridine, 5950-34-5; 2-ethoxycarbonylaziridine, 5950-36-7.

Supplementary Material Available: Full screening data for compounds submitted to the P-388 assay (Table II) (5 pages). Ordering information is given on any current masthead page.

Inhibition of Renin by Angiotensinogen Peptide Fragments Containing the Hydroxy Amino Acid Residue 5-Amino-3-hydroxy-7-methyloctanoic Acid

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The 3*R*,5*S* and 3*S*,5*S* diastereoisomers of the hydroxy amino acid 5-amino-3-hydroxy-7-methyloctanoic acid (AHMOA) were synthesized from L-leucine and then incorporated into various peptide fragments of angiotensinogen to give the following polypeptides: AHMOA-Val-Phe-OCH_3, His-AHMOA-Val-Phe-OCH_3, and AHMOA-Ile-His-OCH_3. These compounds were tested in an in vitro renin assay system for their ability to inhibit either hog kidney renin or human amniotic renin. The most active analogue of the series was (3*R*,5*S*)-AHMOA-Val-Phe-OCH_3 (**16**). Against hog kidney renin, this compound possessed a $K_i = 1.7 \times 10^{-4}$ M, while against human amniotic fluid, **16** had a $K_i = 0.95 \times 10^{-4}$ M. The analogues AHMOA-Val-Phe-OCH_3 and His-AHMOA-Val-Phe-OCH_3 exhibited noncompetitive kinetics when the 3*R*,5*S* isomer of AHMOA was employed and competitive kinetics when the 3*S*,5*S* diastereoisomer of AHMOA was used.

Because of the important role that the renin–angiotensin system plays in the regulation of blood pressure and blood volume in both physiological and pathophysiological states, there has been an ongoing effort by many investigators to develop antagonists of the renin–angiotensin system as either pharmacological tools or as therapeutic agents.[1-3] Inhibition of renin has been thought to be one means by which the renin–angiotensin system could be blocked, since the reaction between renin and its substrate, angiotensinogen, is the rate-limiting step in the sequence of enzymatic reactions that make up the renin–angiotensin system.

We have been attempting to develop new types of renin inhibitors by utilizing an approach that involves the design and synthesis of compounds that might mimic the postulated transition state of the renin–angiotensinogen reaction. Although renin's mechanism of catalysis remains unknown, two models have been proposed as a result of studies that have been conducted on renin and related aspartyl proteases.[4-7] In one model, it is postulated that the carboxyl group of one of the aspartic acid residues known to be present within the active site of renin attacks the Leu[10] amide carbonyl group. A general-base mechanism of hydrolysis is postulated for model 2. In this model, the carbonyl carbon of the scissile peptide bond is attacked by a molecule of water. The result in both cases is the formation of a tetrahedral intermediate (Figure 1) wherein the Leu[10] carbonyl group is transformed into a hydroxyl group and the amide nitrogen atom of residue 11 begins to take on the characteristics of an amino nitrogen.

In previous studies aimed at mimicking the postulated tetrahedral intermediates shown in Figure 1, we modified the known substrate analogue inhibitor Leu-Leu-Val-Phe-OCH_3[8] in two ways. In one modification the N-terminal leucyl residue of Leu-Leu-Val-Phe-OCH_3 was replaced with various α-hydroxyalkanoyl residues.[9] This modification yielded inhibitors of renin that were up to five times more active than Leu-Leu-Val-Phe-OCH_3. A second modification consisted of substituting the hydroxy amino acid residue 3-amino-2-hydroxy-5-methylhexanoic acid for the leucyl residues of Leu-Leu-Val-Phe-OCH_3.[10] This modification yielded inhibitors that exhibited competitive or noncompetitive kinetics, depending on the

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