Synthesis and Biological Properties of Side-Chain-Modified Bleomycins

Wilhelmus J. Vloon,[†] Cornelis Kruk,[†] Upendra K. Pandit,*[†] Hendricus P. Hofs,[‡] and John G. McVie*[‡]

Organic Chemistry Laboratory, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands, and Antoni van Leeuwenhoekhuis, Het Nederlands Kanker Instituut, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. *Received August 9, 1985*

New side-chain-modified bleomycins (BLMs) **3a–k** have been synthesized by the reaction of demethyl BLM A2 with α-bromoacetamides (**2a–k**). The structures of these BLM analogues have been established by comparison of their NMR spectra with the corresponding spectra of model thiazole derivatives. Mass spectra (FAB) of the modified BLMs are not informative, since the fragmentation patterns exhibit a loss of the modified chain moiety, presumably in the matrix. The purity of the compounds is attested by TLC and HPLC analyses. Biological evaluation of **3a–k** in in vitro (survival of B16 melanoma cells) shows that the compounds are almost as effective as bleomycin. Examination of the effects of **3c**, **3e**, and **3f** on lungs of male mice indicates that the analogues do not exhibit lower pulmonary toxicity than bleomycin.

The bleomycins (Scheme I) constitute a group of glycopeptide antibiotics that display antitumor properties. Following their discovery[1] and evaluation for cancer chemotherapy, a mixture of bleomycins, containing A2 (**1b**, ~70%) and B2 (**1c**, ~20%), has been successfully used in the clinical treatment of several types of cancer, notably Hodgkins disease, squamous cell carcinoma, lymphomas, and tumors of the testis.[2] Long-term administration of bleomycins results in symptoms of pulmonary toxicity, which greatly limit the utility of the drug. The development of modified bleomycins with lower pulmonary toxicity and increased therapeutic effect on human cancer is, consequently, an important challenge. In this paper we present the chemical synthesis and biological evaluation of a series of side-chain-modified bleomycins.

The various bleomycins are characterized by a common glycopeptide bithiazole unit, corresponding to bleomycinic acid (**1a**), and differ in the nature of their side chains, which are constituted by an amide bond between the carboxyl group of **1a** and the terminal amine moiety. The cytotoxic activity of bleomycins is associated with cleavage of DNA,[3a] which is accompanied by preferential release of thymidine (T) residues located to the 3-*O'*-side of cytidine (C) and guanosine (G) units.[3b] The currently accepted mechanism of action of bleomycin invokes specific roles for three regions of the molecule:[4] (a) the sulfonium ion or an equivalent positively charged function, which provides electrostatic interaction with the phosphates; (b) the bithiazole system, which intercalates with the heteroaromatic nucleobases of the DNA; and (c) the region comprising of pyrimidoblamic acid (N_1, $N_{2'}$, CNH_2) and histidine (N_3, α-NHCO), which provides the coordinating sites for an iron atom (Fe(II)), for the formation of a "pseudoheme" system. The latter constitutes the catalytic site of bleomycin where molecular oxygen is activated for mediation of the oxidative fission of DNA. In a study of the pulmonary toxicity of modified BLMs, it has been shown that the "lung fibrosis index" is influenced by structural changes in the terminal amine part of the molecule.[5a-c] These results suggest the need to synthesize and screen a series of BLMs in which the side chain is altered in a systematic fashion, following a specific rationale.

In the present study we have chosen to examine the influence of both the immediate structural environment of the sulfur atom and the intensity of its positive charge on the chemotherapeutic index and on lung toxicity of the modified bleomycin. The decision to tune the charge on the sulfur atom derives from the fact that interaction between DNA and the side chain is electrostatic in character.

An approach to modify BLM A2 with the aforementioned objective consists of replacing one of the methyl groups of the sulfonium moiety by a series of suitably designed ligands in which a specific structural entity is systematically altered. This requires the development of a chemical procedure that selectively alters the side chain without affecting the rest of the molecule. This paper describes one such method and its application to the synthesis of several new bleomycins, together with data on their biological activity.[6]

Results and Discussion

As starting material for our studies we employed "clinically outdated" Blenoxane, generous quantities of which were provided by Bristol-Myers.[7] Blenoxane (~70% BLM A2, ~20% BLM B2, and 10% minor component) was subjected to laborious chromatography[8] to yield pure BLM A2. The latter was converted into demethyl A2 (**1d**), essentially following literature procedure.[9] For the proposed investigation, demethyl BLM A2 (**1d**) constituted the central intermediate from which the various modified bleomycins could be prepared. To this end the reaction of **1d** (as a Cu(II) complex) with a number of electrophiles was investigated. The stringent requirement that the sulfur atom exclusively constitute the site of electrophilic attack was met at a practical level by reagents

[†] University of Amsterdam.
[‡] Het Nederlands Kanker Instituut.

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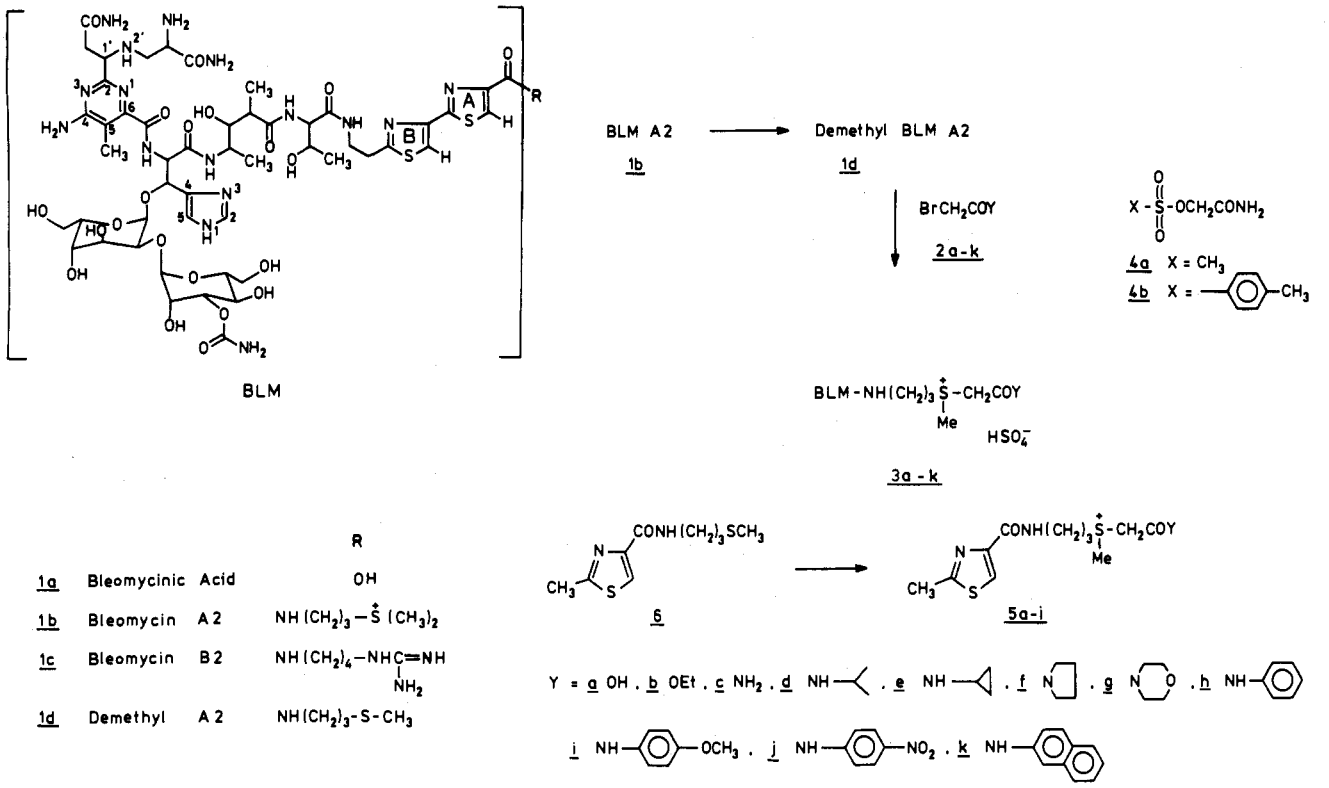
Scheme I



		R
1a	Bleomycinic Acid	OH
1b	Bleomycin A2	$NH(CH_2)_3-\overset{+}{S}(CH_3)_2$
1c	Bleomycin B2	$NH(CH_2)_4-NHC(=NH)NH_2$
1d	Demethyl A2	$NH(CH_2)_3-S-CH_3$

Table I. Selected Chemical Shifts of BLM A2 and BLM Analogues **3a–k**

			thiazole		histidine[b]			
no.[a]	$(CH_3)S^+$	pyr CH_3	5	$t_{R,i}$'s[c]	2	5	CH_2[f]–CONH–Y moiety	pD[c]
1b	2.92	2.00	8.23	8.01	8.08	7.39		4.40
3a	2.93	2.03	8.20	8.02	7.88	7.30		6.16
3b[d]	3.03	2.03	8.21	8.02	7.80	7.27	1.24 (3 H, t, J = 7 Hz, CH_3CO), 4.28 (2 H, q, J = 7 Hz, CCH_2O)	6.00
3c	3.02	2.04	8.23	8.03	7.82	7.30		6.96
3c	3.00	2.03	8.23	8.04	7.97	7.34	1.08–1.16 [6 H, m, $(CH_3)_2C$]	5.75
3e	2.98	2.02	8.21	8.02	7.79	7.27	0.45–0.51 (2 H, m, CHCH), 0.65–0.75 (2 H, m, CHCH), 2.52–2.62 (1 H, m, NCHC)	6.56
3f[e]	2.97	2.01	8.20	8.01	7.79	7.20	1.80–2.00 (4 H, m, CCH_2CH_2C)	6.42
3g[e]	2.99	2.03	8.24	8.05	8.06	7.38		5.44
3h	3.03	2.00	8.11	7.89	7.80	7.26	7.05–7.09 (1 H, m, C_4-H), 7.18–7.26 (4 H, m, C_2-H, C_3-H, C_5-H, C_6-H)	7.07
3i	3.04	1.99	8.08	7.85	7.80	7.26	3.66 (3 H, s, CH_3O), 6.64–6.68 (2 H, m, C_3-H, C_5-H), 7.09–7.11 (2 H, m, C_2-H, C_6-H)	6.90
3j	3.06	1.98	8.07	7.86	7.78	7.29	7.27–7.32 (2 H, m, C_2-H, C_6-H), 7.76–7.80 (2 H, m, C_3-H, C_5-H)	6.10
3k	3.06	1.92	7.94	7.54	7.79	7.26	7.05–7.65 (7 H, m, Ar CH)	6.91

[a] All spectra are recorded in D_2O at 298 K on a Bruker WM-500 spectrometer. The δ values are given in ppm and were related to the same spectral reference as the δ value of sodium (trimethylsilyl)[2,2,3,3-d_4]propionate in D_2O at 300 K. [b] pD-dependent signals. [c] pD = meter reading + 0.40.[12] [d] After 48 h at pD 6.00 at 293 K more than 60% decomposition into **3a**. At pD 2.80 slight decomposition. [e] The chemical shift of some protons of the γ moiety are not assignable in the spectrum since their signals fall under (v/v); bulk of the protons between 3.4 and 4.1 ppm. [f] These protons show $K_i = [(t_{R,i}/t_{R,0})$ fast exchange against D_2O.

of the type XCH_2COY, especially when the reaction was carried out in acidic solution (pH 1.6). The reaction of Co(III) complex of **1d** with 1-[*p*-(bromoacetamido)-phenyl]ethylenedinitrilotetraacetic acid has been reported earlier.[10a] After the present work was completed, the reactions of Co(III) and Cu(II) complexes of **1d** with 1-[*p*-(bromoacetamido)phenyl]ethylenedinitrilotetraacetic acid and 1-[*p*-(bromoacetamido)benzyl]ethylenedinitrilotetraacetic acid were reported in a patent.[10b]

When **1d** (Cu(II) complex) was allowed to react with bromoacetamides **2a–k** (see the Experimental Section), the corresponding sulfonium salts (modified bleomycins **3a–k**) were obtained in fair yields (~60%, Scheme I). Analogous reactions were also observed between Cu(II)–**1d** and electrophiles **4a** and **4b**, although these proceeded at a slower rate. The structures of the new BLMs are based upon the following evidence. In the first instance, a complete interpretation of the ^1H and ^{13}C NMR spectra of BLM A2 was carried out by high-resolution (500 MHz) ^1H and ^{13}C NMR, in conjunction with two-dimensional Fourier transform techniques.[11] NMR spectra of BLMs **3a–k** were correlated with that of BLM A2. Furthermore, thiazole derivatives **5a–i** (Scheme I) were prepared (see the Experimental Section) as models of BLMs **3a–i** for purposes of comparative NMR study. The salient data for the BLM analogues are provided in Table I, while that for the model thiazoles are given in the Experimental Section. As expected, comparison of the data shows similarities in two specific regions of the spectra of the bleo-

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Table II

no.	yield,[a] %	mp, °C	Rf[b]	$t_{R,i}$,[c] s	K_i[d]
1b			0.36	230	0.59
1c			0.66	310	1.13
1d			0.78	1498	9.33
3a	80	180 dec	0.58	193	0.33
3b	64	180 dec	0.42		
3c	79	175 dec	0.54	207	0.42
3d	64	175 dec	0.52	422	1.91
3e	75	177 dec	0.51	310	1.13
3f	75	172 dec	0.36	364	1.51
3g	68	180 dec	0.43	270	0.86
3h	80	180 dec	0.52	1022	6.05
3i	77	170 dec	0.50	974	5.71
3j	82	190 dec	0.47	1097	6.57
3k	72	180 dec	0.51	>40 min	>15.55

[a] All compounds are white powders except **3j** (light yellow). [b] TLC, eluents methanol, 10% ammonium acetate = 1:1 (v/v) on SiO_2. [c] Retention times of the Cu(II) complexes; column, adsorbosphere, 3 μM, C18, 150 × 4.6 mm; eluents, 1% ammonium acetate/H_2O–methanol = 62.5:37.5 (v/v); flow rate 0.6 mL/min; detector, Pye Unicam variable wavelength; apparatus, Perkin-Elmer Series 2. [d] Capacity factor $K_i = [(t_{R,i}/t_{R,0}) - 1]$.

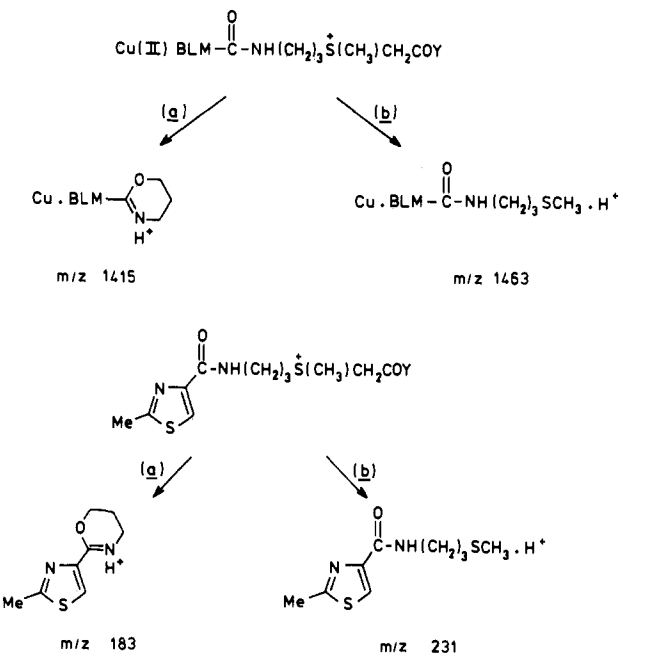
mycins and the model thiazole derivatives. These regions are the sulfonium methyls (CH_3S^+) and the amide chain moieties (CH_2COY). The purity of the BLMs (**3a–k** was established by TLC and HPLC analyses. The retention times ($t_{R,i}$), the capacity factor (K_i), and the R_f values for the compounds are presented in Table II. It is noteworthy that the K_i values for BLM analogues bearing an aromatic group (**3h–k**) are considerably higher and lie in a different range from those with an aliphatic or an alicyclic chain. This can be due to either an enhancement of the lipophilic character or a change in conformation of the molecule, as a consequence of the aryl substituent in the BLM chain.

Mass spectroscopy has been shown to be helpful in characterizing bleomycins[13,14] and mixtures of bleomycins.[15] Recently, Nibbering and co-workers in our Department have succeeded in determining the precise masses of the Cu(II) complexes of bleomycin A2 [$(M + Cu - 2H)^+$, $C_{55}H_{82}N_{17}O_{21}S_3{}^{63}Cu$ calcd, 1475.4329; found, 1475.4334] and demethyl A2 [$(M + Cu)^+$, $C_{54}H_{81}N_{17}O_{21}S_3{}^{63}Cu$ calcd, 1462.4251; found, 1462.4248] by fast atom bombardment (FAB) mass spectroscopy employing Cs_6I_5 as reference. In contrast to these results, it has not been possible to measure the precise mass of bleomycin analogues **3a–k**, due to their relative instability. In all cases, fragments corresponding to m/z 1415 (path a, Scheme II) and m/z 1463 (path b, Scheme II) were observed, thus making the analogues indistinguishable by mass spectroscopy. This behavior can be explained on the basis of metal ion reduction and loss of the (modified) side chain.[16] It is likely that these fragmentations occur by reactions with the molecules of the matrix. An analogous decomposition behavior has been observed for the model compounds, the corresponding pathways a and b leading to fragments m/z 183 and m/z 231, respectively (Scheme II).

Biological Results

The effect of bleomycins **3a–k** on the survival of B16 mouse melanoma cells in vitro, as compared to bleomycin, is shown by the data in Table III. The data show that

Scheme II



the modified bleomycins are as effective as bleomycin itself in this screening model.

Pulmonary toxicity studies were carried out on three compounds, namely, **3c**, **3e**, and **3f**, which represent examples of analogues containing a primary, secondary, and tertiary amide group. Male (C57BL/RijXCBA/Rij) F1 hybrid mice were administered subcutaneously blenoxane (as reference) and **3c**, **3e**, and **3f**, at a dose level of 15 mg/kg twice weekly for 4 weeks. Eight weeks after initiation of the treatment (five mice per drug), the mice were sacrificed.[17] Although scoring of fibrosis is hazardous due to sampling errors and the difficulty of comparing patchy severe fibrosis with minor but diffuse interstitial fibrosis, histopathology screening was used to test the drugs. In each of the groups of five mice treated at equivalent concentrations of bleomycin or analogue, at least one mouse showed abnormal patterns of fibrosis. This varied in intensity and distribution from mouse to mouse, but no trend was obvious in favor of any one of the drugs. The conclusion was drawn from these studies that the analogues gave rise to fibrosis in the same order as the parent and thus were not attractive for clinical testing. The results of these studies showed that analogues did not cause lower pulmonary toxicity than bleomycin.

Experimental Section

All melting points are uncorrected. IR spectra were recorded on an Unicam SP 200 or Perkin-Elmer 257 spectrometer. The absorptions are given in cm^{-1}. ^1H NMR spectra were run on Varian Associates Model HA-100, Bruker WM-250, and Bruker WM-500 instruments. The chemical shifts (δ) are given in ppm with Me_4Si as an internal standard; however, the δ values of all spectra measured in D_2O were related to the same spectral reference as the δ value of 3-(trimethylsilyl)[2,2,3,3-d_4]propionate in D_2O at 300 K. For the resonance signals the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. Spin–spin coupling constants are given in hertz. Field-desorption and electron-impact mass spectra were obtained with a Varian MAT-711 spectrometer. Fast atom bombardment (FAB) mass spectrometry was carried out on a VG Analytical ZAB-HF mass spectrometer, an instrument with reverse

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Table III. A Comparative Study of the Effect of Bleomycin and Analogues **3b–k** on the Survival of B16 Melanoma Cells in Vitro[a]

compound	dose, μg/mL	% survival	compound	dose, μg/mL	% survival
bleomycin	10	39 ± 1	**3g**	10	51 ± 2
	100	28 ± 1		100	28 ± 2
3b	10	43 ± 2	**3h**	10	45 ± 4
	100	27 ± 1		100	27 ± 3
3c	10	38 ± 2	**3i**	10	40 ± 3
	100	21 ± 2		100	28 ± 2
3d	10	41 ± 3	**3j**	10	46 ± 3
	100	23 ± 3		100	30 ± 2
3e	10	38 ± 3	**3k**	10	48 ± 6
	100	28 ± 2		100	32 ± 2
3f	10	35 ± 4			
	100	29 ± 6			

[a] B16 mouse melanoma cells from log phase cultures were suspended in fresh medium [Dulbecco's modified minimum essential Eagle medium with 10% newborn calf serum (Gibco) at 5×10^4 cells/mL. For the experiments, six multiwell plates were employed, with 2 mL of the cell suspension in each well. After 24 h the test agents were incubated with the B16 melanoma cells for 21 h. After treatment, the cells were washed twice and fresh medium added, 2 mL/well. Forty-eight hours after the start of the incubation, the cells were trypsinized and counted on a Sysmex microcell counter. The number of cells surviving a particular drug treatment could be estimated by comparing counts of untreated cells with those of treated cells. Viability was estimated by Trypan blue exclusion. Two doses were tested: 10 μg/mL and 100 μg/mL. Percent survival was calculated according to the formula T/C × 100. T = treated cells, C = untreated control. The number is the mean of three samples.

geometry, fitted with a high-field magnet, and coupled to a VG 11-250 data system. The samples were loaded in glycerol–water–methanol solution on a stainless steel probe and bombarded with xenon atoms having 8-keV energy.

N-Substituted α-Bromoacetamides. General Procedure.[18a,b] α-Bromoacetyl chloride (1 equiv), the amine (1 equiv), and triethylamine (1 equiv) were allowed to react at −50 to −15 °C in chloroform or ether as solvent. The reaction mixture was allowed to warm up to room temperature, the precipitated amine hydrochloride filtered off, the solvent removed under reduced pressure, and the residual amide purified by distillation, sublimation, or crystallization.

2d[19] (44%): white needles, mp 62–63 °C; IR (KBr) 3280, 2970, 1644, 1550 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15 (6 H, d, J = 6.5 Hz, 2 CH_3), 3.79 (2 H, s, CH_2Br), 3.7–4.2 (1 H, m, CHN), 5.9 (1 H, NH).

2e[20] (60%): white needles, mp 116–117 °C; IR ($CHCl_3$) 3415, 2990, 1665, 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.40–0.90 (4 H, m, CH_2CH_2), 2.5–2.9 (1 H, m, CHN), 3.86 (2 H, s, CH_2Br), 6.2–6.9 (1 H, NH).

2f[18b] (36%): white needles, mp 38–41 °C; IR ($CHCl_3$) 3000, 2980, 1640, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.7–2.3 (4 H, m, CH_2CH_2) 3.35–3.80 (4 H, CH_2NCH_2), 4.05 (2 H, s, CH_2Br).

2g[18a] (66%): bp 103–104 °C (0.1 mm); IR ($CHCl_3$) 3000, 2970, 2920, 2900, 2860, 1645, 1460, 1440 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.45–4.0 (8 H, m, 2 NCH_2CH_2O), 3.9 (2 H, s, CH_2Br).

2h[19] (85%): mp 132–134 °C; IR (KBr) 3290, 3200, 3145, 3100, 1655, 1618, 1608, 1598, 1550 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.05 (2 H, s, CH_2Br), 7.09, 7.34, 7.61 (5 H, 3 m, Ar H); MS (C_8H_8BrNO), calcd, 212.9790; found, 212.9790.

2i[21] (88%): mp 129–130 °C; IR (KBr) 3280, 2950, 1655, 1615, 1600, 1545, 1505 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.74 (3 H, s, OCH_3), 4.03 (2 H, s, CH_2Br), 6.92, 7.52 (4 H, 2 d, J = 9 Hz, Ar H), 10.28 (1 H, s, NH); MS ($C_9H_{10}BrNO_2$), calcd, 242.9902; found, 242.9899.

2j[21] (65%): mp 174–175 °C; IR (KBr) 3275, 3230, 3170, 3110, 1675, 1620, 1560, 1490, 1335 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.12 (2 H, s, CH_2Br), 7.83, 8.26 (4 H, 2 d, J = 9 Hz, Ar H), 11.0 (1 H, s, NH); MS ($C_8H_7BrN_2O_3$), calcd, 257.9640; found, 257.9643.

2k[22] (71%): mp 129–131 °C; IR (KBr) 3280, 1655, 1590, 1560 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.12 (2 H, s, CH_2Br), 7.35–7.64, 7.84–7.93 (6 H, 2 m, Ar H), 8.32 (1 H, d, J = 1.4 Hz, Ar H), 10.62 (1 H, s, NH); MS ($C_{12}H_{10}BrNO$), calcd, 262.9954; found, 262.9950.

Bleomycin Analogues (3a–k). General Procedure. A mixture of the Cu(II) complex of demethylbleomycin A2 and an excess (10–50-fold) of bromoacetamide, in H_2O–H_2SO_4 (pH 1.6) for the nonaromatic and in H_2O–H_2SO_4 (pH 1.6) + 50% Me_2SO for the aromatic acetamides, was allowed to stand in a thermostated bath (35 °C) for 10 h (the reaction being monitored by TLC for minimum concentration of both demethyl A2 and decomposition products). Any undissolved bromoacetamide was filtered off over a glass filter and the residue thoroughly washed with H_2O–H_2SO_4 (pH 1.6). The combined filtrates are neutralized by filtration over a Dowex 44 (OH^-) column. The neutral filtrate was chromatographed over a column packed with CM-Sephadex C-25 (Pharmacia, column bed length 23 cm, diameter 3 cm) with a linear salt gradient consisting of 450 mL of 0.02 N Na_2SO_4 and 450 mL of 0.4 N Na_2SO_4 (degassed). The fractions containing the bleomycin derivative were brought on an Amberlite XAD-2 (23 cm × 13 cm) column, which was degassed before use. The copper was removed by eluting with 4% Na_2EDTA solution, followed by washing with 0.2 N Na_2SO_4 to remove EDTA and finally with degassed bidistilled water. The copper free product was eluted with CH_3OH–0.01 M H_2SO_4 (1/1, v/v). The methanol was evaporated at room temperature or lower and the acidic residue neutralized by filtration over a Dowex 44 (OH^-) column and lyophilized. The ^1H NMR and other relevant data on the bleomycin analogues **3a–k** are presented in Tables I and II.

Thiazole-4-carboxamides 5a–i. General Procedure. A mixture of 2-methyl-4-[[[3-(methylthio)propyl]amino]-carbonyl]thiazole[23] (**6**) (1 equiv) and the bromoacetamide (2 equiv) in CH_3OH–H_2SO_4–H_2O or in H_2SO_4–H_2O was stirred at 37 °C for 8 h. A few milliliters of dilute HCl (pH 1.6) was added to the mixture and the precipitate formed filtered off and washed with dilute HCl. The filtrate was extracted with $CHCl_3$ to remove the acetamide, the water layer was neutralized by passing it over a Dowex 44 (OH^-) column, and the bromide and sulfate ions were exchanged for chloride ion by filtration over a column packed with IRA-400 (Cl^-). Lyophilizing of the aqueous solution yielded the sulfonium salts **5a–i**. The compounds were chromatographed with EtOAc–EtOH (3:1) on Al_2O_3.

5a (24%): viscous hygroscopic liquid; ^1H NMR (D_2O, pD 4.0) δ 2.07–2.19 (2 H, m, CCH_2C), 2.68 (3 H, s, $ArCH_3$), 2.88 (3 H, s, CH_3S^+), 3.20–3.50 (2 H, m, CCH_2S^+), 3.55 (2 H, t, J = 6.5 Hz, NCH_2C), 4.06–4.22 (2 H, AB, J_{AB} = 15.7 Hz, $^+SCH_2CO$), 8.00 (1 H, s, Ar H); MS (MH^+, $C_{11}H_{17}N_2S_2O_3$), calcd, 289.0680; found, 289.0702.

5b (50%): viscous oil; ^1H NMR (D_2O, pD 6.7) δ 1.23 (3 H, t, J = 7 Hz, CH_3C), 2.10–2.21 (2 H, m, CCH_2C), 2.67 (3 H, s, Ar CH_3),

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3.00 (3 H, s, CH_3S^+), 3.30–3.58 (4 H, m, CCH_2S, NCH_2C), 4.25 (2 H, q, J = 7 Hz, CCH_2CO), 8.00 (1 H, s, Ar H); MS (M^+, $C_{13}H_{21}N_2S_2O_3$), calcd, 317.0994; found, 317.1001.

5c (29%): viscous oil; ^1H NMR (D_2O, pD 5.6) δ 2.13–2.25 (2 H, m, CCH_2C), 2.72 (3 H, s, Ar CH_3), 3.00 (3 H, s, CH_3S^+), 3.40–3.61 (2 H, m, CCH_2S), 3.59 (2 H, t, J = 6.4 Hz, NCH_2C), 4.41–4.52 (2 H, AB, J_{AB} = 15.7 Hz, SCH_2CO), 8.04 (1 H, s, Ar H); MS (M^+, $C_{11}H_{18}N_3S_2O_2$), calcd, 288.0840; found, 288.0803.

5d (52%): viscous liquid; ^1H NMR (D_2O, pD 4.9) δ 1.09–1.16 (6 H, m, $(CH_3)_2C$), 2.10–2.25 (2 H, m, CCH_2C), 2.73 (3 H, s, Ar CH_3), 3.00 (3 H, s, CH_3S^+), 3.40–3.60 (2 H, m, CCH_2S), 3.59 (2 H, t, J = 6.3 Hz, NCH_2C), 3.85–4.00 (1 H, m, NCH(C)), 8.05 (1 H, s, Ar H); MS (M^+, $C_{14}H_{24}N_3S_2O_2$), calcd, 330.1310; found, 330.1320.

5e (80%): viscous liquid; ^1H NMR (D_2O, pD 6.6) δ 0.50–0.60 and 0.75–0.84 (2 × 2 H, m, CHCH), 2.10–2.25 (2 H, m, CCH_2C), 2.58–2.70 (1 H, m, NCH(C)), 2.74 (3 H, s, Ar CH_3), 3.01 (3 H, s, CH_3S^+), 3.40–3.60 (2 H, m, CCH_2S), 3.61 (2 H, t, J = 6.4 Hz, NCH_2C), 8.07 (1 H, s, Ar H); MS (M^+, $C_{14}H_{22}N_3S_2O_2$), calcd, 328.1153; found, 328.1132.

5f (48%): viscous liquid; ^1H NMR (D_2O, pD 5.2) δ 1.80–2.00 (4 H, m, CCH_2CH_2C), 2.10–2.27 (2 H, m, CCH_2C), 2.71 (3 H, s, Ar CH_3), 2.98 (3 H, s, CH_3S^+), 3.30–3.61 (8 H, m, CCH_2S, NCH_2C, NCH_2CCCH_2N), 8.04 (1 H, s, Ar H); MS (M^+, $C_{15}H_{24}N_3S_2O_2$), calcd, 342.1310; found, 342.1330.

5g (55%): viscous liquid; ^1H NMR (D_2O, pD 2.9) δ 2.10–2.28 (2 H, m, CCH_2C), 2.72 (3 H, s, Ar CH_3), 2.98 (3 H, s, CH_3S^+), 3.30–3.90 (12 H, m, CCH_2S, NCH_2C, NCH_2CH_2O), 8.05 (1 H, s, Ar H); MS (M^+, $C_{15}H_{24}N_3S_2O_3$), calcd, 358.1259; found, 358.1240.

5h: hygroscopic solid (71%): mp 78–81 °C; IR (KBr) 3000–3500 (salt bands), 1630–1680 (amide I), 1550 (amide II), 1600, 1500 (aromatic); ^1H NMR (D_2O, pD 4.3) δ 2.10–2.30 (2 H, m, CCH_2C), 2.64 (3 H, s, Ar CH_3), 3.04 (3 H, s, CH_3S^+), 3.45–3.61 (4 H, m, CCH_2S, NCH_2C), 7.20–7.30 (1 H, m, C_4-H), 7.34–7.42 (4 H, m, C_2-H, C_3-H, C_5-H, C_6-H), 7.95 (1 H, s, Ar H); MS (M^+, $C_{17}H_{22}N_3S_2O_2$), calcd, 364.1153; found, 364.1138; Anal. ($C_{17}H_{22}N_3S_2O_2Cl\cdot H_2O$) C, H, N.

5i (80%): hygroscopic white powder; mp 76–79 °C; IR (KBr) 3000–3500 (salt bands), 1640–1670 (amide I), 1545 (amide II), 1510 (aromatic); ^1H NMR (D_2O, pD 6.7) δ 2.10–2.30 (2 H, m, CCH_2C), 2.62 (3 H, s, Ar CH_3), 3.05 (3 H, s, CH_3S^+), 3.45–3.61 (4 H, m, CCH_2S, NCH_2C), 3.80 (3 H, s, CH_3O), 6.90 (2 H, AB, J_{AB} = 9.0 Hz, C_3-H, C_5-H), 7.28 (2 H, AB, J_{AB} = 9.0 Hz, C_2-H, C_6-H), 7.91 (1 H, s, Ar H); MS (M^+, $C_{18}H_{24}N_3S_2O_3$), calcd, 394.1259; found, 394.1245. Anal. ($C_{18}H_{24}N_3S_2O_3Cl\cdot H_2O$) C, H, N.

2-Methyl-4-[[[3-(methylthio)propyl]amino]carbonyl]-thiazole[23] (**6**): pale yellow viscous liquid; bp 160 °C (0.01 mmHg) (Kugelrohr); IR ($CHCl_3$) 3400 (NH), 2980, 2910 (CH), 1650 (amide I), 1540 (amide II) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.82–1.93 (2 H, m, CCH_2C), 2.07 (3 H, s, CH_3S), 2.54 (2 H, t, J = 7 Hz, CH_2S), 2.66 (3 H, s, Ar CH_3), 3.46–3.54 (2 H, m, CCH_2N), 7.40 (1 H, br, NH), 7.89 (1 H, s, Ar H); MS (M^+, $C_9H_{14}N_2S_2O$), calcd, 230.0547; found, 230.0532. Anal. ($C_9H_{14}N_2S_2O$) N: calcd, 12.16; found, 11.73.

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Registry No. **2a**, 79-08-3; **2b**, 105-36-2; **2c**, 683-57-8; **2d**, 75726-96-4; **2d**·HCl, 104240-92-8; **2e**, 77600-79-4; **2e**·HCl, 104240-93-9; **2f**, 90892-09-4; **2f**·HCl, 104240-94-0; **2g**, 40299-87-4; **2g**·HCl, 104240-95-1; **2h**, 5326-87-4; **2h**·HCl, 104240-96-2; **2i**, 29182-87-4; **2i**·HCl, 104240-97-3; **2j**, 3598-91-2; **2j**·HCl, 104240-98-4; **2k**, 22344-79-2; **2k**·HCl, 104240-99-5; **3a**, 104320-74-3; **3b**, 104321-40-6; **3c**, 104320-75-4; **3d**, 104241-10-3; **3e**, 104320-76-5; **3f**, 104241-12-5; **3g**, 104320-77-6; **3h**, 104241-14-7; **3i**, 104241-16-9; **3j**, 104241-18-1; **3k**, 104241-20-5; **5a**, 104241-00-1; **5b**, 104241-01-2; **5c**, 104241-02-3; **5d**, 104241-03-4; **5e**, 104241-04-5; **5f**, 104241-05-6; **5g**, 104241-06-7; **5h**, 104241-07-8; **5i**, 104241-08-9; **6**, 104266-58-2; α-bromoacetyl chloride, 22118-09-8; demethylbleomycin A2 (Cu(II) complex), 71801-39-3; 2-propanamine, 75-31-0; cyclopropanamine, 765-30-0; pyrrolidine, 123-75-1; morpholine, 110-91-8; benzenamine, 62-53-3; 4-methoxybenzenamine, 104-94-9; 4-nitrobenzenamine, 100-01-6; 2-naphthalenamine, 91-59-8.

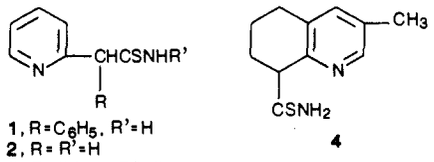
Synthesis and Antisecretory and Antiulcer Activities of Derivatives and Analogues of 2-(2-Pyridyl)tetrahydrothiophene-2-carbothioamide

Jean-Claude Aloup,† Jean Bouchaudon,† Daniel Farge,*† Claude James,† Jean Deregnaucourt,‡ and Monique Hardy-Houis‡

Rhône-Poulenc Santé, Centre de Recherches de Vitry, 94403 Vitry-sur-Seine, Cedex, France. *Received February 10, 1986*

New thioamide derivatives of 2-(2-pyridyl)tetrahydrothiophene-2-carbothioamide (**29**) and related compounds (in which the tetrahydrothiophene ring was replaced by tetrahydrothiopyran, tetrahydrofuran, 1,3-dithiane, or 1,3-oxathiane and where the pyridine ring was replaced by other nitrogen heterocycles) were synthesized and tested for their antisecretory and antiulcer activities. These thioamides were prepared according to one of the following methods: (i) reaction of an isothiocyanate with the carbanion of the corresponding cyclic precursor (for secondary thioamides); (ii) reaction of ammonia or an amine with the dithio ester prepared from the same precursor (for primary, secondary, and tertiary thioamides). These thioamides were evaluated by the Shay method to measure their antisecretory activity and by the stress-induced-ulcer method to test their antiulcer activity. Structure–activity relationships are discussed. *N*-Methyl-2-(2-pyridyl)tetrahydrothiophene-2-carbothioamide (R.P. 40749, **30**) exhibited activities that were at least 10 times higher than those reported for cimetidine.

Thioacetamide derivatives bearing the pyridine nucleus (for example, 2-phenyl-2-(2-pyridyl)thioacetamide (**1**, SC 15396),[1] 2-(2-pyridyl)thioacetamide (**2**, CMN 131),[2] *N*-methyl-2-methoxy-2-(2-pyridyl)thioacetamide (**3**, SKF 59377),[3] and 3-methyl-5,6,7,8-tetrahydroquinoline-8-carbothioamide (**4**, Tiquinamide)[4]) are known to possess non-anticholinergic antisecretory properties.



1, R = C_6H_5, R' = H
2, R = R' = H
3, R = OCH_3, R' = CH_3
4

A structural feature common to these compounds is the

† Chemistry Department.
‡ Biology Department.

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