CHEMICAL MODIFICATION AND ANTITUMOR ACTIVITY OF HERBIMYCIN A. 8,9-EPOXIDE, 7,9-CYCLIC CARBAMATE, AND 17 OR 19-AMINO DERIVATIVES

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(Received for publication May 15, 1984)

Herbimycin A[1] (**1**), a new ansamycin antibiotic isolated from the culture broth of *Streptomyces hygroscopicus* AM-3672, shows herbicidal, anti-tabacco mosaic virus and antitumor[2] activities. This compound has been shown to be a member of the benzoquinone subfamily of the ansamycins.[3,4] Geldanamycin[5,6] and macbecin,[7~9] which are also antitumor ansamycin antibiotics, have been reported to have the structures similar to **1**. Chemical modification of the amide group and the benzoquinone moiety in geldanamycin has been attempted and some derivatives were found to exhibit higher antitumor activity than the mother compound.[10] However, there has been no presentation of the chemical modification of the ansa-chain moiety to date.

In this paper, we describe the synthesis and antitumor activities of 8,9-epoxyherbimycin A (**2**), herbimycin A-7,9-cyclic carbamate (**3**) and the 17 or 19-amino substituted derivatives of **1**, **2** and **3**.

Treatment of **1** with *m*-chloroperbenzoic acid (1.2 equiv) in $CHCl_3$ gave 8,9-epoxyherbimycin A (**2**); MS *m/z* 590 (M, $C_{30}H_{42}N_2O_{10}$), TLC silica gel 60 F_{254} plates (0.2 mm thick, Merck) Rf 0.51 (C_6H_6 - EtOAc, 1: 1), $[\alpha]_D^{23}+126°$ (*c* 0.5, $CHCl_3$), UV λ_{max}^{MeOH} nm (ε) 272 (23,900), IR ν_{max} ($CHCl_3$) cm^{-1} 790 and 750 (assignable to the epoxy group), in 65% yield. The ^1H NMR spectrum (100 MHz $CDCl_3$) of **2** showed a doublet signal (J=9.0 Hz) assignable to the epoxy methine proton (H-9) at δ 2.96, disappearance of a 9-olefinic proton in **1**, and up-field shifts of H-7 and 8-CH_3 proton signals to δ 4.17 and 1.33, respectively. Furthermore, the ^{13}C NMR spectrum (25.1 MHz, $CDCl_3$) showed the disappearance of 8 and 9-olefinic carbon resonances and the appearance of 8 and 9-epoxy carbons newly formed at δ 60.2 and 67.1, respectively. On treatment of **2** with boron trifluoride etherate (3 equiv) in benzene at room temperature, herbimycin A-7,9-cyclic carbamate (**3**); MS *m/z* 590 (M, $C_{30}H_{42}N_2O_{10}$), TLC Rf 0.76 (C_6H_6 - EtOAc, 1: 1), $[\alpha]_D^{23}+135°$ (*c* 0.5, $CHCl_3$), UV λ_{max}^{MeOH} nm (ε) 274 (35,300), was obtained in 59% yield through an S_{N2} attack of the 9-carbamoyl group and epoxide ring opening. The structure of **3** was confirmed from the ^1H and ^{13}C NMR spectral data; δ_H 4.02 (1H, d, J=9.0 Hz, OCONH), 3.76 (1H, dd, J=5.0 and 9.0 Hz, H-9), δ_C 86.8 (C-7), 86.7 (C-8), 56.9 (C-9).

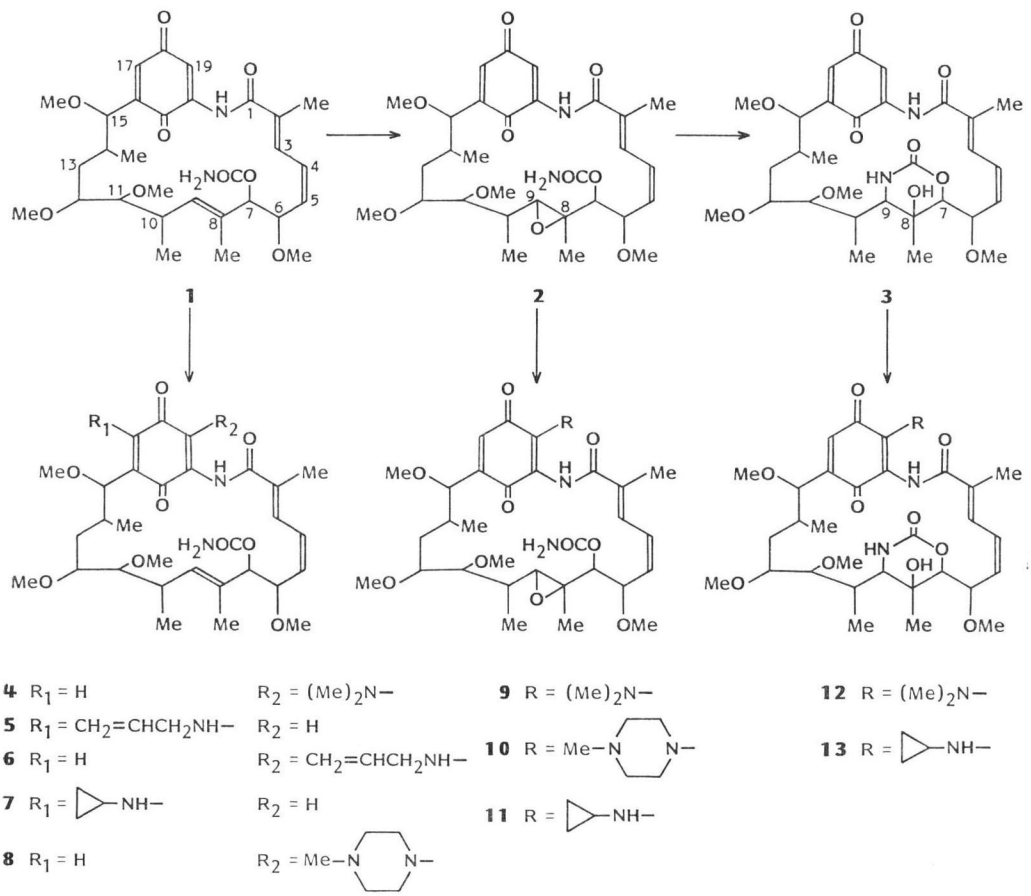
Treatment of **1**, **2** and **3** with substituted amines such as dimethylamine, allylamine, cyclopropylamine and methylpiperazine in benzene afforded the corresponding 17 or 19-substituted derivatives (**4**~**13**), respectively. This can be explained by MICHAEL addition of the nucleophile at position 17 or 19 followed by subsequent air oxidation of the resulting intermediate dihydroquinone. The structure of the amino derivatives were confirmed by the following ^1H and ^{13}C NMR spectral data.

19-Dimethylaminoherbimycin A (**4**): MS m/*z* 617 (M, $C_{32}H_{47}N_3O_9$); TLC Rf 0.28 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}-61.5°$ (*c* 0.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 259 (20,600); ^1H NMR ($CDCl_3$) δ 6.40 (1H, d, J=2.0 Hz, H-17), 4.34 (1H, br s, H-15), 3.17 (6H, s, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 133.6 (C-17), 118.8 (C-19), 42.4 ($N(CH_3)_2$).

17-Allylaminoherbimycin A (**5**): MS *m/z* 629 (M, $C_{33}H_{47}N_3O_9$); TLC Rf 0.60 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}-150°$ (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 247 (12,500) and 338 (11,700); ^1H NMR ($CDCl_3$) δ 7.64 (1H, m, NH), 6.96 (1H, d, J=2.0 Hz, H-19), 4.48 (1H, s, H-15); ^{13}C NMR ($CDCl_3$) δ 116.9 (CH_2=*C*H), 109.1 (C-19), 108.2 (*C*H_2=CH), 48.3 ($NHCH_2$).

19-Allylaminoherbimycin A (**6**): MS m/*z* 629 (M, $C_{33}H_{47}N_3O_9$); TLC Rf 0.45 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}-124°$ (*c* 0.1, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 247 (13,100) and 335 (9,000); ^1H NMR ($CDCl_3$) δ 7.45 (1H, m, NH), 6.48 (1H, d, J=1.8

Scheme 1.



Hz, H-17), 4.46 (1H, br s, H-15).

17-Cyclopropylaminoherbimycin A (**7**): MS *m/z* 629 (M, $C_{33}H_{47}N_3O_9$); TLC Rf 0.60 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}$ −138° (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 245 (12,000); ^{1}H NMR ($CDCl_3$) δ 7.60 (1H, br d, NH), 6.48 (1H, d, *J*=2.0 Hz, H-17), 4.48 (1H, br s, H-15).

19-Methylpiperazinoherbimycin A (**8**): MS *m/z* 672 (M, $C_{35}H_{52}N_4O_9$); TLC Rf 0.63 ($CHCl_3$ - MeOH, 5: 1); $[\alpha]_D^{23}$ −101° (*c* 0.1, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 257 (21,300); ^{1}H NMR ($CDCl_3$) δ 6.52 (1H, d, *J*=1.8 Hz, H-17), 4.39 (1H, br s, H-15), 2.29 (3H, s, =NCH_3); ^{13}C NMR ($CDCl_3$) δ 133.0 (C-17), 121.5 (C-19), 54.2, 49.2 (NHCH), 46.0 (=NCH_3).

19-Dimethylamino-8,9-epoxyherbimycin A (**9**): MS *m/z* 633 (M, $C_{32}H_{47}N_3O_{10}$); TLC Rf 0.22 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}$ +96° (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 268 (19,000) and 325 (1,000); ^{1}H NMR ($CDCl_3$) δ 6.37 (1H, d, *J*=2.0 Hz, H-17), 4.47 (1H, d, *J*=2.0 Hz, H-15), 3.05 (6H, s, $N(CH_3)_2$): ^{13}C NMR ($CDCl_3$) δ 126.4 (C-17), 117.4 (C-19), 67.9 (C-9), 60.1 (C-8), 42.2 ($N(CH_3)_2$).

19-Methylpiperazino-8,9-epoxyherbimycin A (**10**): MS *m/z* 688 (M, $C_{35}H_{52}N_4O_{10}$); TLC Rf 0.43 (C_6H_6 - EtOAc, 1: 5); $[\alpha]_D^{23}$ +98° (*c* 0.2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 261 (23,000); ^{1}H NMR ($CDCl_3$) δ 6.42 (1H, d, *J*=2.0 Hz, H-17), 4.50 (1H, d, *J*=2.0 Hz, H-15), 2.33 (1H, s, =NCH); ^{13}C NMR ($CDCl_3$) δ 130.9 (C-17), 119.5 (C-19), 68.8 (C-9), 60.6 (C-8), 56.2, 49.8 (=NCH_2), 46.9 (=NCH_3).

19-Cyclopropylamino-8,9-epoxyherbimycin A (**11**): MS *m/z* 645 (M, $C_{33}H_{47}N_3O_{10}$); TLC Rf 0.15 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}$ +135° (*c* 0.3, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 265 (21,500); ^{1}H NMR ($CDCl_3$) δ 6.68 (1H, br d, NH), 6.50 (1H, d, *J* = 1.8 Hz, H-17), 4.57 (1H, br s, H-15).

19 - Dimethylaminoherbimycin A-7, 9-cyclic

Table 1. Antitumor activity of herbimycin A derivatives on Ehrlich ascites carcinoma.

Compound	Total dose (mg)	Dose (mg/kg×days)	T/C (%)	No. of cured mice*/total*
1	5	0.5×10	109	0/4
2	200	20 ×10	141	1/3
3	500	50 ×10	141	1/3
4	100	11 × 9	122	0/3
5	100	11 × 9	131	0/3
6	100	11 × 9	141	0/3
7	200	22 × 9	110	0/3
8	500	50 ×10	141	2/3
9	500	55.6× 9	95	0/5
10	750	75 ×10	141	2/3
11	200	22 × 9	114	0/3
12	50	5.5× 9	110	0/3
13	200	22 × 9	114	0/3

* No. of mice without accumulation of abdominal ascites at day 31.

Table 2. Antitumor activity of 19-methylpiperazino-8,9-epoxyherbimycin A (**10**).

Tumor	Mouse		Inoculum site	Median survival days of control	Dose (mg/kg× days)	T/C (%)
Sarcoma 180						
(ascites form)	ICR	1×10^5	ip	22	100 ×5	200
(solid)	ICR	1×10^6	sc	36	12.5×5	167
Ehrlich carcinoma	*dd*Y	2.5×10^6	ip	17	100 ×5	205
IMC carcinoma	CDF_1	3×10^5	ip	12	200 ×5	358
Meth-A fibrosarcoma	BALB/c	1×10^5	ip	29	100 ×5	103
B-16 melanoma	BDF_1	1×10^5	sc	22	25 ×5	155
Lewis lung carcinoma	BDF_1	1×10^5	sc	29	200 ×5	93
P-388 leukemia	CDF_1	1×10^5	ip	12	50 ×5	91
L-1210 leukemia	CDF_1	1×10^5	ip	7	100 ×5	114

Tumors were inoculated sc or ip into mice on day 0. Various doses (12.5~200 mg/kg/day) of an agent were administered ip daily from day 1 to day 5. The observation was terminated at day 60.

carbamate (**12**): MS *m/z* 633 (M, $C_{32}H_{47}N_3O_{10}$); TLC Rf 0.71 (EtOAc); $[\alpha]_D^{23}$ −78° (*c* 0.1, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 258 (23,000); ^{1}H NMR ($CDCl_3$) δ 6.40 (1H, d, *J*=1.9 Hz, H-17), 4.36 (1H, br s, H-15), 3.16 (6H, s, $N(CH_3)_2$); ^{13}C NMR δ 136.1 (C-17), 115.4 (C-19), 86.4 (C-8), 58.8 (C-9), 43.0 ($N(CH_3)_2$).

19-Cyclopropylaminoherbimycin A-7,9-cyclic carbamate (**13**): MS *m/z* 645 (M, $C_{33}H_{47}N_3O_{10}$); TLC Rf 0.21 (C_6H_6 - EtOAc, 1: 1); $[\alpha]_D^{23}$ −132° (*c* 0.1, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 246 (25,000); ^{1}H NMR ($CDCl_3$) δ 6.40 (1H, d, *J*=1.8 Hz, H-17), 4.36 (1H, d, *J*=1.8 Hz, H-15), 5.10 (1H, br d, NH).

The introduction of an amino group at the 17-position in compounds **5** and **7** was established by the appearance of singlet signals of H-15 and H-19 (the signals of both protons of **1** appeared as doublets arising from long-range couplings between H-15 and H-17/H-17 and H-19). On the other hand, a long-range coupling between H-15 and H-17 remained in the 19-amino derivatives (**4**, **6** and **8**~**13**).

To evaluate the antitumor activity of these derivatives, Ehrlich carcinoma cells (2.5×10^6) were inoculated ip to *dd*Y mice on day 0. Mice received various doses of herbimycin A derivatives for 9 or 10 successive days. Antitumor activity was expressed as T/C (%) only at the optimal dose for each derivative: "T" is median survival days of the treated group and "C" is that of the control group. Accumulations of

abdominal ascites were also observed to determine the therapeutic effect on ascites tumor at day 31 when the experiment was terminated.

As shown in Table 1, although herbimycin A (**1**) did not possess strong antitumor activity, 8,9-epoxyherbimycin A (**2**), herbimycin A-7,9-cyclic carbamate (**3**) and the amino derivatives (**4**, **5**, **8** and **10**) showed life prolongation of tumor bearing mice. The antitumor activity of **10** against several murine tumors was further examined.

As seen from Table 2, a derivative (**10**) showed marked antitumor activity against Sarcoma 180 (both ascitic and solid forms), Ehrlich and IMC carcinomas, and B-16 melanoma. The introduction of a methylpiperazino group to the 19 position of the benzoquinone nucleus resulted in high antitumor activity.

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