NEW MORPHOLINO ANTHRACYCLINES, MX, MX2, AND MY5

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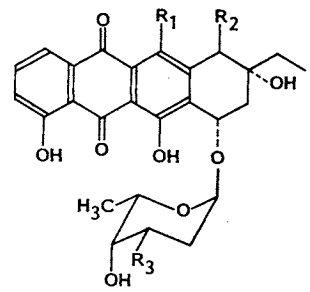
In the course of our screening for new anthracycline derivatives, which are active against both doxorubicin-sensitive (P388/S) and doxorubicin-resistant (P388/ADM) sublines of P388 leukemia, new morpholino anthracyclines, named MX (**1**), MX2 (**2**), and MY5 (**3**) were found to display improved antitumor activities. They were 3′-deamino-3′-morpholino derivatives of 13-deoxocarminomycin (**4**)[1], 13-deoxo-10-hydroxycarminomycin (oxaunomycin) (**5**)[2], and 13-deoxo-11-deoxycarminomycin (4-*O*-demethyl-11-deoxy-13-deoxodaunomycin) (**6**)[3], respectively, and each morpholino compound showed lower potency than its parent anthracycline. In this paper, we describe the preparation, physical properties and preliminary biological data for the new anthracyclines.

The parent compounds were prepared as follows. Compounds **4** and **5** were obtained from the cultured broth of strain RNM134-13, and **6** was from that of strain RU7062. These strains were blocked mutants of *Actinomadura roseoviolacea* 1029-AV1, which produces akrobomycin[4]. The strains were cultured in a 50-liter jar fermentor (Marubishi Co.) containing 25 liters of medium consisted of maltose 4.0%, glucose 0.5%, soybean meal 1.5%, corn steep liquor 1.0%, Polypepton 0.5%, dry yeast 0.2% and $CaCO_3$ 0.4% (pH 7.4) under agitation (200 rpm) at 31°C for 6~8 days. Assays of **4**, **5** and **6** during purification were performed by reverse phase HPLC with a YMC-packed column AM-312 (ODS) (6×150 mm) (Yamamura Chemical Lab.). A mixture of methanol - acetonitrile - 1% triethylamine (adjusted to pH 2.0 with phosphoric acid) (50 : 15 : 35) was used as a mobile phase and run at a flow rate of 2.5 ml/minute. Detection and quantification were carried out at 495 nm or 254 nm using a UV-VIS spectrophotometer, Jasco UVIDEC-100-VI. Anthracycline **4**, **5** and **6** showed single peaks at retention times of 12.3, 1.9 and 6.4 minutes, respectively.

For the preparation of **4**, the fermentation broth of RNM 134-13 was filtered with filter aid at pH 10.0, and the filtrate was passed through a column (10×40 cm) of Diaion PA308 (Mitsubishi Chemical Industries Limited). The column was washed with 6 liters of methanol, and eluted with a mixture of methanol - acetic acid (99: 1). Active fractions were collected, and concentrated to a small volume after adjusting to pH 7.0. The concentrate was dissolved in 10 liters of a mixture of methanol - water (1 : 1), filtered, and subjected to reverse phase preparative HPLC (Yamamura Chemical Lab. 5×50 cm column). Compound **4** was eluted with a mixture of methanol - water adjusted to



1 R_1 = OH R_2 = H R_3 = morpholino (3″, 2″, 5″, 6″)
2 R_1 = R_2 = OH R_3 = morpholino
3 R_1 = R_2 = H R_3 = morpholino
4 R_1 = OH R_2 = H R_3 = NH_2
5 R_1 = R_2 = OH R_3 = NH_2
6 R_1 = R_2 = H R_3 = NH_2

pH 2.0 with phosphoric acid (6 : 4). The active eluate was adjusted to pH 8.0 and extracted twice with a mixture of chloroform - methanol (9 : 1). The organic layer was concentrated to give a pure red powder of **4** (1.0 g). Compound **5** was prepared from the same broth filtrate as **4** by the following procedure. The filtrate was adjusted to pH 3.4 and centrifuged. The supernatant was applied to a Diaion HP-20 (Mitsubishi Chemical Industries Limited) column (10×40 cm), and eluted with a mixture of acetone - 0.01 M HCl-KCl buffer (pH 1.5) (70 : 30). The eluate was combined with the precipitate from centrifugation described above, to which were added acetone and 28% aqueous ammonia to a final concentrations of 75% and 1.5%, respectively. After stirring for 3~4 hours at 20°C, the solution was adjusted to pH 7.2 with H_2SO_4, concentrated to a small volume, and extracted three times with a mixture of chloroform - methanol (9 : 1) at pH 8.0. The organic layer was concentrated to dryness, dissolved in 10 liters of a mixture of methanol - water (1 : 2), and filtered. Further purification was carried out on a preparative HPLC with a reverse phase column (5×50 cm; Yamamura Chemical Lab.). The filtrate was injected into a HPLC apparatus, eluted with a mixture of methanol - phosphoric acid (pH 2.0) (1 : 1), and extracted twice with chloroform at pH 8.0. The combined extracts were concentrated *in vacuo* to yield 2.0 g of **5**. Compound **6** was obtained from the pH 7.5 broth filtrate of the mutant strain RU7062. The filtrate was passed through on Diaion PA308 column, washed with a mixture of methanol - 28% aqueous ammonia (99 : 1), and eluted with a mixture of methanol-acetic acid (99 : 1). The eluate was adjusted to pH 9.0 with 28% aqueous ammonia and allowed to stand at 40°C for 5 hours. The solution was neutralized, and evaporated to a small volume under reduced pressure. The concentrate was extracted twice with chloroform at pH 8.0. The organic layer was evaporated to dryness, the residue was dissolved in 60 liters of a mixture of methanol - water (1 : 1), and purified by the same method as for **5**. Thus, 2.5 g of **6** was obtained as an orange powder.

The ^{1}H NMR, UV, and mass spectra of **4** and **6** were identical with those of 13-deoxocarminomycin[1] and 4-*O*-demethyl-11-deoxy-13-deoxodaunomycin[3], respectively. The optical rotation values of their hydrochlorides also showed good agreement with reported data. Compound **5** was identified as oxaunomycin by direct comparison with an authentic sample.

The synthesis of 3′-deamino-3′-morpholino derivatives of anthracyclines was studied independently by ACTON *et al.*[5] and UMEZAWA *et al.*[6]. ACTON's group applied BORCH's reductive alkylation using diglycol aldehyde and sodium cyanoborohydride. UMEZAWA's group reported the *N*-alkylation using bis(2-iodoethyl) ether and triethylamine in DMF. We applied both methods for the preparation of **1**, **2** and **3**.

13-Deoxocarminomycin (**4**, 135 mg) was treated with diglycol aldehyde (320 mg) and sodium cyanoborohydride (17 mg) in chloroform (15 ml) at room temp for 1 hour to give MX (**1**) in 58% yield: MP 132~135°C (dec); $[\alpha]_D^{22}$ +218° (*c* 0.04, 0.01 N HCl-MeOH); field desorption mass spectra (FD-MS) *m/z* 569 (M, $C_{30}H_{35}NO_{10}$). Unsatisfactory *Anal* data were obtained. UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 234 (683), 252 (545), 292 (158), 492 (261); $\lambda_{max}^{Acidic\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (783), 252 (612), 292 (192), 492 (315); $\lambda_{max}^{Alkaline\ MeOH}$ nm ($E_{1cm}^{1\%}$) 243 (653), 290 (166), 562 (192), 596 (162); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.36~2.46, 2.51~2.59 (6H, 10a-H, 3′-H, 3″-H, 5″-H), 3.65~3.69 (4H, 2″-H, 6″-H).

MX2 (**2**) was prepared from **5** in 48% yield by the above method: MP 160~163°C (dec); $[\alpha]_D^{24}$ +484° (*c* 0.31, 0.01 N HCl-MeOH); FD-MS *m/z* 585 (M, $C_{30}H_{35}NO_{11}$). Unsatisfactory *Anal* data were obtained. UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 234 (821), 252 (478), 290 (153), 492 (295); $\lambda_{max}^{Acidic\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (805), 252 (479), 290 (155), 492 (297); $\lambda_{max}^{Alkaline\ MeOH}$ nm ($E_{1cm}^{1\%}$) 242 (805), 292 (149), 564 (280), 600 (226); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.50~2.59, 2.35~2.44 (5H, 3′-H, 3″-H, 5″-H), 3.65~3.69 (4H, 2″-H, 6″-H).

MY5 (**3**) was obtained from **6** in 37% yield by the above method; MP 180~184°C (dec); $[\alpha]_D^{24}$ + 355° (*c* 0.10, 0.01 N HCl-MeOH); FD-MS *m/z* 553 (M, $C_{30}H_{35}NO_9$). Unsatisfactory *Anal* data were obtained. UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 229 (542), 257 (361), 289 (165) 429 (187); $\lambda_{max}^{Acidic\ MeOH}$ nm ($E_{1cm}^{1\%}$) 228 (553), 257 (371), 291 (169) 431 (193); $\lambda_{max}^{Alkaline\ MeOH}$ nm ($E_{1cm}^{1\%}$) 237 (493), 252 (376), 292 (157), 520 (164); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.37~2.45, 2.52~2.59 (5H, 3′-H, 3″-H, 5″-H), 3.64~3.69 (4H, 2″-H, 6″-H).

The treatment of **4** with bis(2-iodoethyl) ether and triethylamine in DMF gave two major derivatives, **1** in 11% yield and **2** in 15% yield. The

Table 1. ^{13}C Chemical shift assignments[a] of **1**~**3** (in $CDCl_3$, ppm).

Carbon	1	2	3
1	119.49	119.72	120.07
2	136.88	137.16	137.19
3	124.60	124.97	124.68
4	162.50	162.72	162.49
5	190.46	190.84[b]	192.67
6	(156.98)	156.77[b]	161.90
7	70.91	71.05	71.10
8	36.92	32.89	37.42
9	69.42	71.89	70.16
10	36.47	66.59	42.96
11	(157.24)	157.24[b]	121.46
12	186.06	186.25[b]	181.56
13	35.27	30.39	35.02
14	7.45	6.58	7.39
4a	116.05	116.02	115.86
5a	(111.01)	112.15[b]	(113.69)
6a	(134.91)	134.91[b]	(113.59)
10a	138.49	138.60	146.02
11a	(110.13)	111.54[b]	(130.67)
12a	(133.43)	133.34[b]	(132.54)
1′	100.97	101.32	100.88
2′	27.76	27.78	27.79
3′	58.50	58.50	58.54
4′	65.13	65.13	65.16
5′	66.36	66.49	66.33
6′	16.99	16.97	17.00
2″	67.09	67.09	67.08
3″	49.67	49.68	49.68
5″	49.67	49.68	49.68
6″	67.09	67.09	67.08

[a] Chemical shift assignments were made on the bases of ^{1}H-^{13}C chemical shift correlated two dimensional NMR. Values in parentheses may be interchangeable.

[b] The assignments were made on the basis of incorporation study using [2-^{13}C]acetate.

result indicates that hydroxylation occurred at a C-10 position under the condition described above. The same treatment of **5** and **6** afforded only the corresponding 3′-deamino-3′-morpholino derivatives, **2** and **3**, respectively. The assignments of ^{13}C NMR signals of **1**, **2** and **3** are shown in Table 1.

Growth inhibitory effects of **1**~**6** on P388/S and P388/ADM *in vitro* were tested in comparison with doxorubicin. The results are summarized in Table 2. The morpholino compounds, **1**, **2** and **3** were not cross-resistant with doxorubicin in P388/ADM subline, but the parent compounds, **4** and **5** cross-resistant. The derivatives had a

Table 2. Growth inhibitory effect on P388/S and P388/ADM cells *in vitro*[a].

Compound	IC_{50} (ng/ml)		RF[b]
	P388/S	P388/ADM	
Doxorubicin	26.3	3,800	144
1	35.0	35.0	1.0
2	12.9	15.4	1.2
3	180.3	169.4	0.9
4	12.2	62.0	5.1
5	2.2	80.6	36.6
6	170.6	258.8	1.5

[a] IC_{50} values were determined on day 3 culture.

[b] Resistance factor (RF): IC_{50} for resistant subline/IC_{50} for sensitive subline.

Table 3. Antitumor activity against P388 leukemia in mice[a].

Compound	Dose (mg/kg)	ILS (%)	TI[b]
MX (**1**)	1.32	18	3.9
	1.98	40	
	2.96	57	
	4.44	70	
	6.67	111	
	10.0	51	
MX2 (**2**)	0.29	10	8.9
	0.44	43	
	0.66	62	
	0.99	73	
	1.48	89	
	3.33	140	
	5.00	35	
MY5 (**3**)	7.9	26	7.0
	11.9	46	
	17.8	52	
	26.7	59	
	40	98	
	60	142	
	90	−15	
ADM	1.76	23	6.5
	2.63	40	
	3.95	68	
	5.93	79	
	8.89	90	
	13.3	140	
	20	43	

[a] P388 Leukemia cells (10^6/mouse) were inoculated ip on day 0. The compounds were administrated iv on days 1 and 5.

[b] Therapeutic index (TI) was calculated from the equation: TI=(Dose for ILS max)/(dose for ILS 30).

slightly lower potency than their parent compounds against a P388/ADM subline. Table 3 shows the antitumor activity of **1**~**3** and doxorubicin against P388 leukemia in CDF_1 mice. MX2 (**2**) and MY5 (**3**) were found to be more effective than doxorubicin. The LD_{50} of **1**, **2** and **3** by intravenous injection in mice was 12.3, 5.58 and 72 mg/kg, respectively.

Further studies on the biological activities of **1**~**3** are in progress and will be reported in subsequent paper.

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