SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF *N*-ENAMINE DERIVATIVES OF DAUNORUBICIN, 5-IMINODAUNORUBICIN, AND DOXORUBICIN

BARBARA STEFAŃSKA, MARIA DZIEDUSZYCKA, MARIA BONTEMPS-GRACZ and EDWARD BOROWSKI

Department of Pharmaceutical Technology and Biochemistry, Technical University of Gdańsk, 80-952 Gdańsk, Poland

SANTE MARTELLI

Department of Chemical Sciences, University of Camerino, Italy

(Received for publication March 30, 1987)

Eleven *N*-enamine derivatives of daunorubicin and of its 5-imino analogue as well as of doxorubicin have been synthesized and evaluated for antileukemic activity *in vitro* and *in vivo*. Comparison of biological activities of examined compounds with other enamine derivatives of daunorubicin, reported earlier by us, has indicated that the optimal activity is shown by *N*-(1-carboethoxypropen-1-yl-2)daunorubicin.

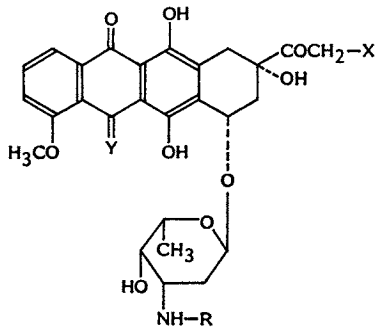
The anthracycline antibiotics daunorubicin (**1**) and doxorubicin (**2**) as broadly used antineoplastic agents, are the object of extensive investigations aimed at the optimization of their pharmacological properties, especially the diminishing of the cardiotoxicity, which is the most undesirable side effect. Among these investigations, the development of new analogues by chemical modification of the biosynthetic product is very important[1,2].

The antitumor activity of **1** and **2** has been reported to be largely due to their ability to bind to nuclear DNA *via* an intercalation mechanism[3,4]. It has been concluded that the protonated 3′-amino group is important as it stabilizes the drug-DNA complex. Therefore modifications of this group may involve substantial changes in the biological activity.

Recently we have reported[5], that the amino group in **1** can be quantitatively substituted with various β-dicarbonyl compounds (acetylacetone, ethyl acetoacetate and 5,5-dimethylcyclohexane-1,3-dione) to give respective *N*-enamine derivatives of **1**. One of these derivatives *N*-(1-carboethoxypropen-1-yl-2)daunorubicin (**3**) exhibits reduced subacute toxicity and cardiotoxicity with retention of antileukemic activity[6,7]. It has been calculated†, that in these *N*-enamine derivatives of **1** the basicity of the 3′-amino function is retained making it possible to bind to DNA. That led us to obtain a series of other enamine derivatives of anthracyclines with different lipophilic properties and varying degree of steric bulk as well as basicity of the 3′-amino group (see Fig. 1). In compounds **5** and **6** the carboethoxy group in the enamine part of **3** was replaced by a carbomethoxy and carbo-*tert*-butoxy group, respectively, compounds **10** and **11** contain an additional carboalkoxy group instead of the methyl group. Compound **9** is an analogue of the earlier obtained **7**[5] with a phenyl group instead of the methyl group at the carbonyl function. Compounds **12** and **13** have the 1-(2-diethoxycarbonyl)-

† Dr. A. TEMPCZYK, University of Gdańsk, private information.

Fig. 1.



1	R=H	X=H	Y=O
2	R=H	X=OH	Y=O
3	R=C(CH$_3$)=CHCOOEt	X=H	Y=O
4	R=C(CH$_3$)=CHCOOEt	X=OH	Y=O
5	R=C(CH$_3$)=CHCOOCH$_3$	X=H	Y=O
6	R=C(CH$_3$)=CHCOO^{t}Bu	X=H	Y=O
7	R=C(CH$_3$)=CHCOCH$_3$	X=H	Y=O
8	R=C(CH$_3$)=CHCOCH$_3$	X=OH	Y=O
9	R=C(CH$_3$)=CHCOPh	X=H	Y=O
10	R=C(COOCH$_3$)=CHCOOCH$_3$	X=H	Y=O
11	R=C(COOEt)=CHCOOEt	X=H	Y=O
12	R=CH=C(COOEt)$_2$	X=H	Y=O
13	R=CH=C(CN)COOEt	X=H	Y=O
14	R=C(CH$_3$)=CHCOOEt	X=H	Y=NH
15	R=C(CH$_3$)=CHCOCH$_3$	X=H	Y=NH

vinyl- and 1-(2-cyano-2-ethoxy)vinyl- substituent at the 3′-amino group, respectively.

It was stated, that the cardiotoxic and mutagenic side effects of **1** appear to be greatly reduced in the 5-imino-derivative[8]. Therefore we have prepared also 5-imino-*N*-(1-carboethoxypropen-1-yl-2)- and 5-imino-*N*-(penten-2-one-4-yl-2)daunorubicin, as 5-imino-derivatives of **3** and **7**, respectively. To synthesize various *N*-enamine derivatives of **1** and **2** (see Fig. 1; **3**~**13**) the respective antibiotics were reacted with an excess of β-diketones, alkyl acetylenedicarboxylates, acetoacetic esters, ethoxymethylene malonate and/or ethoxymethylene cyanoacetate. To obtain the compounds **14** and **15** (see Fig. 1) 5-iminodaunorubicin was used as substrate. All reactions took place in mild conditions. TLC of the crude products indicated only traces of impurities, however if alkyl acetylenedicarboxylates were used as reaction reagents corresponding products **10** and **11** were obtained in admixture with other unidentified derivatives of **1**. The obtained *N*-enamine derivatives **3**~**15** were purified by means of column chromatography on molecular sieves or silica gel and the yields were in the range 45 to 70% with the exception of **4** that was obtained in 35% yield. The structures proposed for compounds **3**~**15** were demonstrated by their spectral data (UV-visible, IR and ^{1}H NMR) as well as their MS determination (field desorption (FD) technique) and elemental analysis data (see Table 1). The electronic absorption spectra in the visible region were identical with that of the parent compounds; the IR spectra showed in the 1725~1580 cm^{-1} region several strong bands characteristic for free and chelated carbonyl groups; the IR spectrum of **13** indicated additionally the C≡N band at 2220 cm^{-1}.

Table 1. Physico-chemical properties of enamine derivatives of daunorubicin, 5-iminodaunorubicin and doxorubicin.

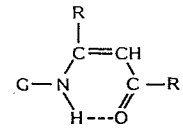
Compound No.	Formula[a]	MP (°C, dec)	^{1}H NMR ($CDCl_3$ **4**~**13**; DMSO-d_6 **14** and **15**)		IR (KBr) cm^{-1}	FD-MS *m/z* (relative intensity, %)
			14-H	*N*-Substituent		
4	$C_{33}H_{37}O_{13}N$	183~185	4.76 (2H, s)	8.75 (1H, d, NH), 4.39 (1H, s, CH=), 4.04 (2H, q, OCH_2CH_3), 1.82 (3H, s, CH_3C=), 1.2 (3H, t, OCH_2CH_3)	1720, 1715, 1670, 1610, 1590	655 ($(M)^+$, 100), 656 ($(M+1)^+$, 60)
5	$C_{32}H_{35}O_{12}N$	157~159	2.41 (3H, s)	8.78 (1H, d, NH), 4.41 (1H, s, CH=), 3.69 (3H, s, $COOCH_3$), 1.85 (3H, s, CH_3C=)	1700, 1630, 1600, 1580	625 ($(M)^+$, 100)
6	$C_{35}H_{41}O_{12}N$	169~171	2.41 (3H, s)	8.78 (1H, d, NH), 4.48 (1H, s, CH=), 1.87 (3H, s, CH_3C=), 1.27 (9H, s, $C(CH_3)_3$)	1720, 1650, 1640, 1620~1600, 1580	667 ($(M)^+$, 95), 668 ($(M+1)^+$, 100), 669 ($(M+2)^+$, 30)
8	$C_{32}H_{35}O_{12}N$	210~213	4.78 (2H, s)	10.8 (1H, d, NH), 4.8 (1H, s, CH=), 1.89 (6H, s, 2×CH_3C=)	1715, 1630, 1610, 1590	625 ($(M)^+$, 100), 626 ($(M+1)^+$, 80)
9	$C_{37}H_{37}O_{11}N$	164~168	2.41 (3H, s)	11.51 (1H, d, NH), 7.6 (5H, m, COC_6H_5), 5.58 (1H, s, CH=), 1.93 (3H, s, CH_3C=)	1720, 1620~1600, 1590	670 ($(M-1)^+$, 70), 671 ($(M)^+$, 100), 672 ($(M+1)^+$, 20)
10	$C_{33}H_{35}O_{14}N$	154~157	2.41 (3H, s)	8.28 (1H, d, NH), 5.15 (1H, s, CH=), 3.71 and 3.66 (6H, 2s, 2×$COOCH_3$)	1750~1730, 1680, 1620, 1590	669 ($(M)^+$, 100)
11	$C_{35}H_{39}O_{14}N$	136~139	2.40 (3H, s)	7.95 (1H, d, NH), 5.12 (1H, s, CH=), 4.05 (4H, q, 2×$COOCH_2CH_3$), 1.2 (6H, t, 2×$COOCH_2CH_3$)	1735, 1670, 1630~1620, 1595	695 ($(M)^+$, 100), 696 ($(M+1)^+$, 65), 697 ($(M+2)^+$, 30)
12	$C_{35}H_{39}O_{14}N$	134~136	2.41 (3H, s)	8.0 (1H, m, NH), 7.55 (1H, s, CH=), 4.15 (4H, q, 2×$COOCH_2CH_3$), 1.21 (6H, t, 2×$COOCH_2CH_3$)	1710, 1680, 1660, 1640, 1620, 1580	697 ($(M)^+$, 100)
13	$C_{33}H_{34}O_{12}N_2$	156~158	2.41 (3H, s)	7.93 (1H, m, NH), 7.54 (1H, s, CH=), 4.14 (2H, q, $COOCH_2CH_3$), 1.22 (3H, t, $COOCH_2CH_3$)	1720, 1690, 1640, 1595	650 ($(M)^+$, 100)
14	$C_{33}H_{38}O_{11}N_2$	160~162	2.31 (3H, s)	8.8 (1H, d, NH), 4.28 (3H, s, CH=), 4.0 (2H, q, OCH_2CH_3), 1.8 (3H, s, CH_3C=), 1.18 (3H, t, OCH_2CH_3)	1720, 1715, 1655, 1645, 1600, 1590	638 ($(M)^+$, 100)
15	$C_{32}H_{36}O_{10}N_2$	181~183	2.30 (3H, s)	4.82 (1H, s, CH=), 1.82 (6H, s, 2×CH_3C=)	1715, 1640, 1600, 1585	608 ($(M)^+$, 100), 609 ($(M+1)^+$, 30)

[a] All compounds gave elemental analyses in agreement with their structure.

It was considered on the basis of UV, IR and ^{1}H NMR spectra analysis, that simple enamine derivatives of glycosylamines are intramolecularly bonded, β-amino-α,β-unsaturated ketones[9~11] (Fig. 2). Similar structures of chelated *N*-enamine derivatives **3**~**15** were deduced from the ^{1}H NMR spectra (the position of NH signals at very low fields δ 8~11).

In the reaction of **1** with ethyl ethoxymethylene cyanoacetate we have obtained two major products. One of them was isolated in pure form by means of column chromatography on silica gel. Its ^{1}H NMR spectrum showed the signal at δ 7.93 assigned to the amino proton NH. The remaining was isolated again as mixture of both compounds in similar proportions. Its ^{1}H NMR spectrum besides the signal at δ 7.93 revealed another one at δ 5.38 due to the $-CH\begin{matrix}CO\\CN\end{matrix}$ proton. On the basis of these data and MS determination (identical mass ions) we concluded that the synthesized derivative appears in two tautomeric forms as Scheme 1.

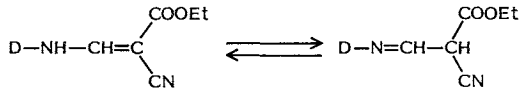
Fig. 2.



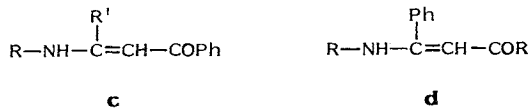
G = Glycosyl

R, R' = Alkyl or alkoxyl

Scheme 1.



D = Residue of daunorubicin



c **d**

R' = H, CH_3

R = Residue of the sugar

The reaction between a primary amine and an unsymmetrical β-dicarbonyl compound can, in principle, give rise to two isomeric enamines (structure **c** or **d**). It has been well established for condensates of benzoylacetaldehyde and/or 1-phenyl-1,3-butanedione with 2-amino-2-deoxyglucose, that these substances react with the carbonyl group of benzoylacetaldehyde and 1-phenyl-1,3-butanedione which is further away from the phenyl group (structure **c**[12]).

^{1}H NMR spectrum of the enamine part of the condensation product of **1** with 1-phenyl-1,3-butanedione (compound **9**) is similar with that of 2-deoxy-2-[2-(4-oxo-4-phenyl-2-butenyl)amino]-D-glucose[9]. It allowed us to propose the structure of compound **9** as in Fig. 1. We have observed also, that in the reaction of dibenzoylmethane with **1** performed in the same and more drastic conditions, as we have used in the reaction of 1-phenyl-1,3-butanedione with **1** no enamine products were formed. This is an additional argument for the structure indicated in Fig. 1.

Biological Activity and Discussion

The synthesized *N*-enamine derivatives of **1**, **2** and of 5-iminodaunorubicin were tested for the growth inhibition of L1210 cells *in vitro* and against P388 leukemia *in vivo*. It has been found that all the examined compounds exhibit cytotoxicity *in vitro* of about one order of magnitude lower than the parent antibiotics. The *in vivo* activity of the daunorubicin derivatives **5**, **6** and **9**~**11** was comparable with that of **1** (T/C 130% at the dose 25 mg/kg) whereas **12** and **13** were less active (T/C ~100% at the same level dose). Doxorubicin derivative **4** has shown lower activity than doxorubicin (**2**) itself, and **8** approaches the activity of **2** (T/C 160% at the dose 12.5 mg/kg). These preliminary data demonstrated that structural changes in the enamine part of the synthesized *N*-enamine derivatives of

1 and **2** does not cause essential changes in antileukemic activity. However the examined compounds appear to be less toxic than the parent drugs[6,7]. The comparison of *in vivo* antitumor activity and toxicity of the series of *N*-enamine derivatives of **1** synthesized with the data obtained for previously described compounds of this group[5~7] indicate that the optimal activity is shown by *N*-(1-carboethoxy-propen-1-yl-2)daunorubicin (**3**). *N*-Enamine derivatives of 5-iminodaunorubicin (**14** and **15**) are less active than their daunorubicin analogues (**3** and **7**). It was disappointing because 5-iminodaunorubicin demonstrated antileukemic activity similar with that of **1**[8].

Experimental

Instrumental Analysis

MP was determined with a Kofler hot plate apparatus and are uncorrected. IR spectra were recorded on a UR-10 Zeiss Spectrometer in KBr pellets. ^{1}H NMR spectra were recorded on a Varian 90 MHz spectrometer using TMS as internal standard and are reported as ppm. Splitting patterns are designed as: s; singlet, d; doublet, t; triplet, m; multiplet. A Beckman spectrometer was used for UV spectral determinations. MW was determined by mass spectrometry (FD technique) on a Varian Mat 711 instrument. The instrumental conditions were the following; wire heating current 5~20 mA, ion source temp 70~100°C, accelerating voltage 4~6 kV. Column chromatography was performed on Silica gel 60 (70~230 mesh, Merck) and on molecular sieves Sephadex LH-20.

Synthesis

General Procedure: A sample of 1 mmol of daunorubicin, doxorubicin or 5-iminodaunorubicin (as free base) in 50 ml of a mixture of $CHCl_3$ - MeOH (10 : 1) was stirred with a 2~3-equivalents of the appropriate reagent except for the synthesis of compounds **6** and **12** where a 6-molar excess was necessary. The reaction was carried out under nitrogen in room temp or 40°C. The reaction times carried from 1 hour (compounds **10** and **11**), 3~5 hours (compounds **5**, **9** and **12~15**) to 10 hours (compounds **3**, **4**, **6** and **7**). After evaporation of the reaction mixture (*in vacuo*) to a small volume the crude product was isolated by precipitation with petroleum ether and purified by means of column chromatography using molecular sieves (Sephadex LH-20) or silica gel. However the application of the last column packing caused loss of yields about 15% (compound **6** could be purified only by means of molecular sieves because of its instability). The following solvent systems were used for following compounds: **10** and **11**, $CHCl_3$ - acetone (7 : 1); **12** and **13**, benzene - acetone (4 : 1); **14**, benzene - acetone (2 : 1); **15**, $CHCl_3$ - MeOH (30 : 1).

Compounds **3~9** were purified on molecular sieves (Sephadex LH-20; solvent system, $CHCl_3$ - MeOH (2 : 1)).

Biological Activity: The antitumor activity *in vitro* was expressed as the concentration of compound required to inhibit by 50% the growth of L1210 leukemia cells (ED_{50}).

ED_{50} values were estimated 48 hours after incubation of L1210 cells with the tested compounds in RPMI 1640 medium supplemented with 5% fetal calf serum[13]. *In vivo* data are the results of screening performed under the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland, U.S.A.

CDF mice were injected ip with 10^6 P388 lymphocytic leukemia cells on day 0 and treated ip on days 5, 9 and 13 with drug dose specified. T/C is the ratio of medium survival time expressed as percent of untreated controls.

Acknowledgments

We acknowledge the financial support of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw (Project CPBR-11.5.) and of Italian Ministero della Publica Istruzione (Fondi 60%). These authors thank Dr. MATTHEW SUFFNESS from the Division of Cancer Treatment, NCI, Bethesda, Maryland, U.S.A. for arranging the *in vivo* biological testing.

References

1) ACTON, E.; C. MOSHER & J. GRUBER: Approaches to more effective anthracyclines by analog synthesis and evaluation. *In* Anthracycline Antibiotics. *Ed.*, H. S. ELKHADEM, pp. 119~139, Academic Press Inc., New York, 1982
2) ACTON, E. M.; G. L. TONG, C. W. MOSHER & R. L. WOLGEMUTH: Intensely potent morpholinyl anthracyclines. J. Med. Chem. 27: 638~645, 1984
3) AUBEL-SADRON, G. & D. LONDOS-GAGLIARDI: Daunorubicin and doxorubicin, anthracycline antibiotics, a physicochemical and biological review. Biochimie 66: 333~352, 1984
4) DI MARCO, A. & F. ARCAMONE: DNA complexing antibiotics daunomycin, adriamycin and their derivatives. Arzneim. Forsch. 25: 368~375, 1975
5) STEFAŃSKA, B.; E. BOROWSKI, L. FALKOWSKI & S. MARTELLI: Synthesis and antileukemic activity of some novel *N*-substituted derivatives of daunorubicin. Pol. J. Chem. 60: 525~529, 1986
6) KLEINROK, Z.; G. RAJTAR, B. CHMIELEWSKI, A. CHODKOWSKA, D. CHIBOWSKI, Z. SIEZIENIEWSKA & Z. SAWINIEC: Comparison of subacute toxicity of rubidomycin and its four derivatives. Arch. Immunol. Ther. Exp. 34: 251~257, 1986
7) MAZERSKA, Z.; B. WOYNAROWSKA, B. STEFAŃSKA, E. BOROWSKI & S. MARTELLI: Antitumor activity of new N-substituted daunorubicin derivatives. Drugs Exp. Clin. Res. 13: 345~359, 1987
8) TONG, G. L.; D. W. HENRY & E. M. ACTON: 5-Iminodaunorubicin. Reduced cardiotoxic properties in an antitumor anthracycline. J. Med. Chem. 22: 36~39, 1979
9) SÁNCHEZ, A. G.; M. T. ALDARE & J. V. DEL PINO: Reactions of glycosylamines with β-diketones and with benzoylacetaldehyde. Carbohydr. Res. 10: 19~33, 1969
10) SÁNCHEZ, A. G. & A. C. VENTULÁ: Enamines derivied from 2-amino-2-deoxy-D-glucose and acetoacetic esters. Carbohydr. Res. 17: 275~286, 1971
11) SÁNCHEZ, A. G.; M. G. GUOLLÉN, E. P. RAMOS & A. C. VENTULÁ: Enamine and pyrrole formation from 2-amino-2-deoxy-D-glucose and dimethyl acetylenedicarboxylate. Carbohydr. Res. 35: 39~47, 1974
12) SÁNCHEZ, A. G. & M. G. GUILLÉN: Reactions of 2-amino-2-deoxy-D-glucose with benzoylacetaldehyde and 1-phenyl-1,3-butanedione. Carbohydr. Res. 3: 486~501, 1967
13) KONOPA, J.; A. MATUSZKIEWICZ, B. HRABOWSKA & K. ONOSZKO: Cucurbitacines, cytotoxic and antitumor substances from Bryonia alba L. Arzneim. Forsch. 24: 1741~1743, 1974