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Original paper

Synthesis of aminoanthraquinone derivatives and their *in vitro* evaluation as potential anti-cancer drugs

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Summary — Anthraquinones, monosubstituted by aminoalkylamino side chains at positions 1, 2 or disubstituted at positions 1, 5 or 1, 8 were prepared. Their *in vitro* cytotoxic activity (ED_{50}) was evaluated using: 1) P388 murine leukemia cells and 2) a subline of these cells resistant to doxorubicin (P388/ADR).

The results of the structure—activity relationship analysis indicated that monosubstitution in position 1 or 2 showed a decrease of the activity when compared to adryamicin. Disubstitution in positions 1, 5 by *N,N*-dimethyl ethylenediamine side chain led to optimal activity, whereas the presence of cyclic dialkylamino substituents in the same positions resulted in a corresponding decrease in the anti-tumor activity.

Disubstitution in positions 1,8 did not show any improvement in the cytotoxic activity.

Résumé — **Synthèse de dérivés aminoanthraquinoniques et leur évaluation *in vitro* comme anti-tumoraux potentiels.** *Différentes anthraquinones, monosubstituées en position 1, 2 par des chaînes latérales aminoalkylaminées ou disubstituées en position 1, 5 ou 1, 8 ont été préparées. Leur activité cytotoxique* in vitro (ED_{50}) *a été déterminée sur des cellules leucémiques murines P388 ainsi que sur des cellules résistantes à la doxorubicine (P388/ADR).*

Les résultats des relations structure—activité indiquent que la monosubstitution en position 1 ou 2 entraînent un abaissement de l'activité en comparaison avec l'adryamicine. La disubstitution en position 1, 5 par une chaîne latérale N,N-*diméthylethylènediamine conduit à une activité optimale, tandis que la présence de substituants cycliques dialkylaminés en même position diminue l'activité anti-tumorale.*

La disubstitution en position 1, 8 n'entraîne aucune amélioration de l'activité cytotoxique.

aminoantraquinones / anti-cancer / DNA / intercalation

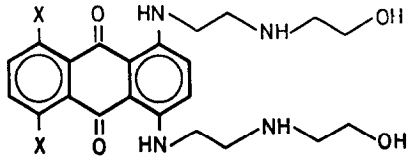
Introduction

The anthracycline antibiotics, daunorubicin and adriamycin [1—11], present broad spectrum activity as anti-neoplastic agents against a variety of clinical and experimental tumors. Although the exact mode of action of these compounds has not yet been fully elucidated, the current view is that among the plausible mechanisms proposed: 1) intercalating [12—17], 2) free radical [18—24] and 3) membrane effects [25—27], the anti-tumor activity of anthracyclines appears to be associated with 1, *i.e.*, their ability to bind to DNA by intercalation. The binding affinity constant (K_{app}) of adriamycin was found to be in the range of 2—5 $\times$ 10^6 M. Possibly, drug—DNA binding, which probably produced a distortion of the DNA structure, plays an important role in blocking RNA [28, 29] and DNA [14] syntheses and in the cleavage and damage of DNA strands [30, 31].

Unfortunately, the use of anthracyclines is limited due to their severe cumulative cardiotoxicity [32—35]. Based upon the idea of the N—O—O triangular atomic arrangement, Adamson [36] proposed the removal of the amino-sugar moiety of the molecule and its replacement by an appropriate amino group. Consequently, many analogs of adriamycin consisting of the anthraquinone skeleton and

Abbreviations: j, k-**Cn**, *j,k* designate the position numbers on the anthraquinone skeleton substituted by side chains. Monosubstituted anthraquinones, $j = 0$, $k = 1, 2$. Disubstituted anthraquinones, $j = 1$, $k = 4, 5, 8$. C denotes CH_2. *n* is the number of CH_2 groups in the alkylamino side chain, $n = 2, 3, 4$. Substituent on the terminal amino groups: **DM**: *N,N*-dimethyl; **DE**: *N,N*-diethyl; **PYR2**: 2-pyridine; **PYR3**: 3-pyridine; **MOR**: morpholine; **PIP**: piperidine; **PYRO**: pyrrolidine; **PIPZ**: piperazine; **NHDE**: *N,N*-bis(2-hydroxy-ethyl); **NHE**: *N*-2-hydroxyethyl.

amino, alkylamino and aminoalkylamino side chains, were synthesized [37—54]. Several of these compounds, such as mitoxantrone and ametantrone, demonstrated remarkable anti-tumor activity.



X = OH = mitoxantrone
X = H = ametantrone

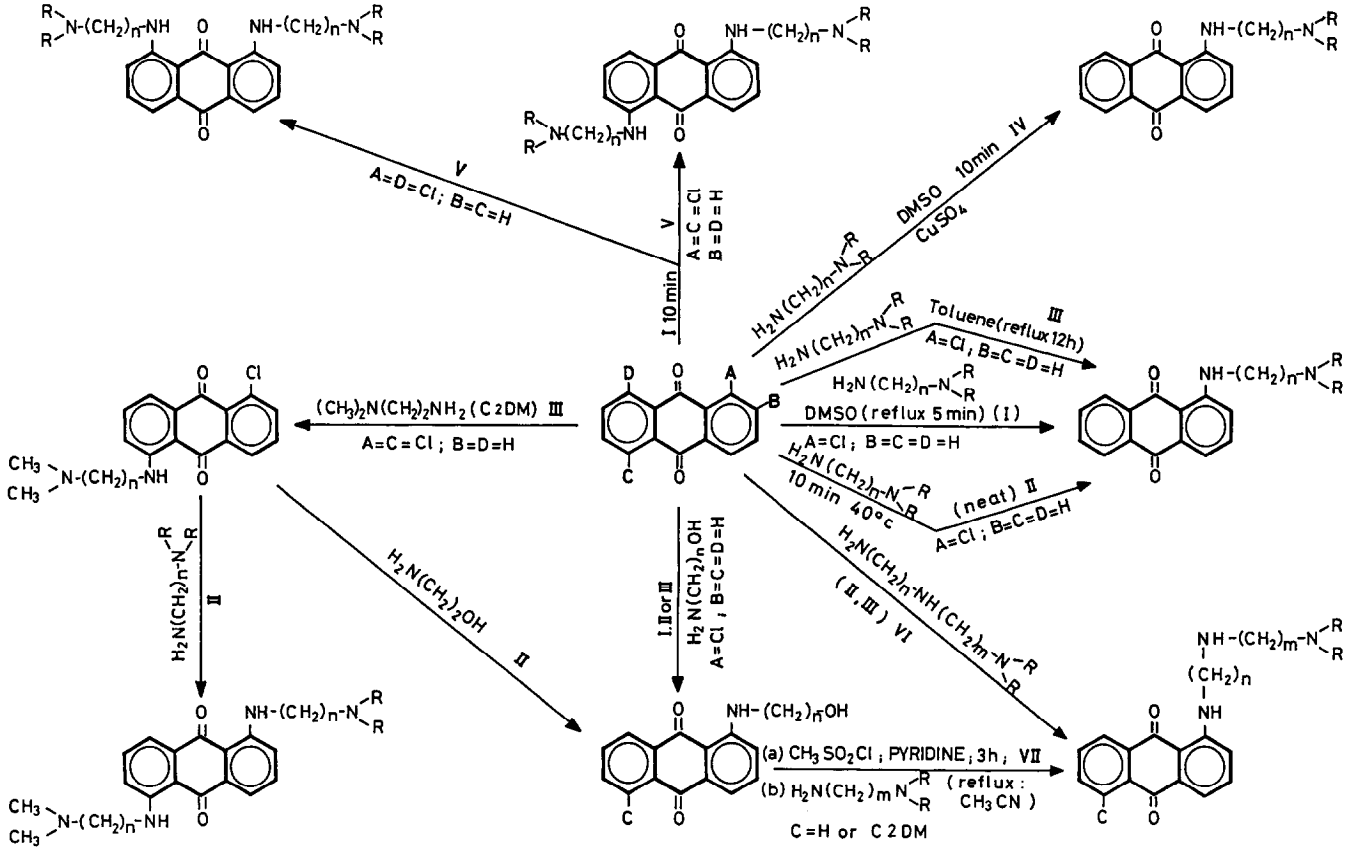
This intensive search for active analogs led to several conclusions about the structural features affecting anti-tumor activity. 1) The necessity of a group in the side chain, accessible for hydrogen bonding. 2) The optimal length of the alkyl group between the two nitrogen atoms in the side chain is 2; increasing the length to 3, decreases activity. 3) Certain substituents on the terminal N-atom, *e.g.*, hydroxyethyl and methyl, increase activity. Bulk substituents, such as propyl, piperazinyl, piperidinyl and morpholidine have little effect. 4) A basic terminal amino group is required. Pyridyl, anilino and *N*-acyl derivatives were inactive. 5) The presence of 2 basic side chains is not necessary and a hydroxyl group may replace one of the aminoalkylamino chains. 6) Positions 1 and 4 of the side chain yield optimal activity. 7) 5,8-Dihydroxy groups augment activity.

It is noteworthy that most of the work reported on the structure—activity relationship of aminoanthraquinone derivatives refers to substituents located at position 1,4. Only a limited number of aminoalkylamino modifications at positions 1,5, 1,8 and 2,6 have been reported.

In order to extend the study on structural requirements for the activity of aminoanthraquinones, we prepared 48 compounds possessing the aminoalkylamino side chain at positions 1,2, 1,5 and 1,8. For comparison, 4 compounds of the 1,4 series were also synthesized.

Chemistry

Based upon the anthraquinone skeleton, the aminoalkylamino derivatives can be readily prepared *via* general methods of nucleophilic substitution reactions. These include: a) substitution of hydroxyanthraquinones [41, 48, 57], b) substitution of leukohydroxyanthraquinones [40, 41, 48, 55, 56], and c) substitution of chloroanthraquinones. In this study, we adopted method (c), which is also employed in other aromatic systems [52—54, 58—62].



Scheme 1.

Table I. *In vitro* activity of aminoalkylaminoanthraquinones against leukemia P388 cells and *E. coli*.

No.	Compound	P388 ED_{50} (μM) S/ADR[a]	P388 ED_{50} (μM) R/ADR[b]	*E. coli* IC_{50} (μM)	No.	Compound	P388 ED_{50} (μM) S/ADR	P388 ED_{50} (μM) R/ADR	*E. coli* IC_{50} (μM)
	Adriamycin	0.04	1	1					
1	1-C2	3.4	5.1	20	**27**	1,5-C2PIP	0.57	1.25	
2	1-C3	5.6	10	100	**28**	1,5-C2PYRO	0.61	1.5	
3	1-C4	11	13	1860	**29**	1,5-C2NHE	0.35	3	
4	1-C2DM	6	6	400	**30**	1-C2, 5-C2DM	4.3	6	
5	1-C3DM	6	6	750	**31**	1-C3, 5-C2DM	1.6	1.9	
6	1-C2C2	6			**32**	1-C3DM, 5-C2DM	0.46	0.88	
7	1-C2C3	6			**33**	1-C3MOR, 5-C2DM	1.6	8—10	
8	1-C3C2	6			**34**	1-C1, 5-C2DM	1	1.9	
9	1-C3C3	6			**35**	1,8-C2	8.7	1.7	200
10	2-C2	8.9	14.5	150	**36**	1,8-C3	8.4	4.3	340
11	2-C3	4.6	11.5	150	**37**	1,8-C4	1.8	8.1	632
12	2-C4	2.4	5	256	**38**	1,8-C2DM	1.2	2.4	200
13	2-C2DM	0.94	4		**39**	1,8-C3DM	3	3	250
14	2-C3DM	1.35	10		**40**	1,8-C2MOR	1.8	4.8	1800
15	2-C2PIPZ	4.9	10.5		**41**	1,8-C5M	10	10	
16	1,5-C2	1	6.2		**42**	1,3-C2PIP	1.6	3	60
17	1,5-C3	4.7	18.5	100	**43**	1,8-C2PYRO	1.2	2.4	40
18	1,5-C4	6.8	14.5	25	**44**	1,8-C3NHDE	25	80	105
19	1,5-C2DM	0.12	0.27	120	**45**	1,8-C2PYR2	8	10	1168
20	1,5-C3DM	0.51	4.8	40	**46**	1-C2DM, 8-C1	1	1.9	1272
21	1,5-C2DE	1.45	2	450	**47**	1-C2DM, 3-C3	1	3	
22	1,5-C8NHDE	1.1	84	159	**48**	1-C2NHE, 8Cl	0.8	1.6	
23	1,5-C2PYR2	3	3		**49**	1-C3, 4-OH	3	6	
24	1,5-C1PYR3	9	10		**50**	1,4-C3	3	3	
25	1,5-C2MOR	1.6	2.9		**51**	1,4-C2DM	0.03	0.24	
26	1,5-C3MOR	5	10		**52**	1,4-C2NHE	< 0.01	0.74	
					53	5-FU	0.7	0.59	

[a]Adriamycin-sensitive cells.
[b]Adriamycin-resistant cells.

Scheme 1 delineates the synthetic pathways of the target compounds. Although monosubstituted aminoanthraquinones can be prepared according to one of the three routes I, II and III, the preferred trajectory depends upon the starting material. 1-Chloroanthraquinone is aminated by either one of the above mentioned pathways. However, monoamination of 1,5 or 1,8 dichloroanthraquinone requires mild conditions which are achieved *via* route III or alternatively by route II. Route I, in this case will lead to the disubstituted product. Amination of 2-chloroanthraquinone is accomplished only at elevated temperature, dimethyl sulfoxide (DMSO), reflux for 5—10 min. Prolonged heating extends side reactions and, thus, introduces the need of a tedious process of purification. The addition of an equivalent amount of $CuSO_4$ to the reaction mixture improves the yield. The asymmetric amino disubstituted derivatives, at positions 1,5 or 1,8 were synthesized *via* a two step reaction: route II followed by route III.

Diethylenetriamine was introduced by reacting 1-chloroanthraquinone with ethanolamine, according to routes I, II or III. The isolated product, 2-hydroxyethyl-1-amino anthraquinone, was treated with methanesulfonylchloride in pyridine to produce the methanesulfonate derivative and, subsequently, ethylenediamine in acetonitrile was added to form the final product.

Structure—activity relationship (SAR)

Fifty-two different aminoalkylaminoanthraquinone derivatives were evaluated for their cytocidic effect (ED_{50}) on P388 murine leukemia cells both sensitive to and resistant to adriamycin. The bacteriocidic activity (ID_{50}) of some of these compounds on *E. coli* was also studied. The results are given in Table I.

From the data, it appears that several structural features, such as side chain length, type of substituents on the terminal nitrogen atom, number of side chains and substituent positions (1,2, 1,5, 1,8) on the anthraquinone skeleton, play an important role in modulating the activity as follows:

Position 1

1) Increasing the chain length between the nitrogen atoms from 2 methylene groups to 3 or 4 decreases the activity 1.6- and 3.2-fold, respectively. The optimal number was 2 carbon atoms (compounds **1**, **2**, **3**). 2) A terminal primary amine was more active than a tertiary amine (**1** and **4**). 3) Insertion of an additional ethylamino group onto the terminal side chain nitrogen atom did not increase activity and, in some cases, slightly decreased it (**1**, **2**, **6**, **7**, **8**, **9**). In general, all derivatives on position 1 were 80—150-fold less active than adriamycin. The most active compound in this set was 1-C2 (**1**), which was 85 times less active than adriamycin.

Position 2

1) In contrast to the observation at position 1, no similar relationship between the length of the alkyl side chain at position 2 and activity was noted. When the terminal group was a primary amine, activity increased with the increase of chain length (**10**, **11**, **12**). However, in the case of the terminal dimethyl alkylamino group (**13**, **15**), the effect was in the opposite direction. 2) Attachment of an additional aminoalkyl group (piperazine **15**) proved disadvantageous. 3) Terminal tertiary amino groups were more active than primary amino groups.

The most active compound observed in this series (side chain at position 2) was 2-C2DM (**13**), which was 24 times less active than adriamycin.

Positions 1, 5

1) The presence of two basic side chains in positions 1 and 5 generally augmented activity (compare: **16**, **17**, **18**, with **1**, **2**, **3**; **19**, **20** with **4**, **5** and **25**, **26** with **4**, **5**). 2) The optimal chain length of the various homologues was 2 methylene groups. 3) (a) The terminal nitrogen atom should be highly basic: a pyridyl group significantly reduced activity. (b) A dimethyl amino group on the terminal nitrogen atom was most effective (**19**). Replacing the methyl groups by ethyl (**21**) decreased activity. (c) Cyclic dialkyl amino substituents (piperidine **27**; pyrrolydine **28**) slightly reduced activity. In this series, the less basic cyclic amine (morpholine **25**) was the least active. (d) The *N*-(hydroxyethyl) derivative **3** was not the most potent compound. However, its activity was comparable to that of the dimethylamino derivative **19**. (e) Replacing the dimethyl groups on the terminal nitrogen by two hydroxyethyl residues abolished activity.

4) In the case of non-symmetrical compounds composed of different side chains on the anthraquinone nucleus, it is noteworthy that the introduction of a 2-dimethylaminoethyl amino group into one of the side chains improved activity. Compounds: 1-C3,5-C2DM **31**, 1-C3DM, 5-C2DM **32** and, 1-C3MOR,5-C2DM **33** were more active than their counterparts: 1,5-C3 **17** and 1,5-C3DM **20**, and 1,5-C3MOR **25** by a factor of: 2.9, 1.1 and 3.1, respectively. The most potent compounds in the 1,5 series: 1,5-C2DM **19**, 1,5-C3DM **20**, 1,5-C2PIP **27**, 1,5-C2PYRO **28**, 1,5-C2NHE **29** and 1-C3DM,5-C2DM **32** contain the ethylenediamine moiety, with the exception of compound **20**. The respective compounds are 3, 13, 14, 15, 9 and 11-fold less active against P388 leukemia cells *in vitro*.

Position 1,8

Substituents on the 1,8 positions did not exhibit any significant enhancement in activity. In several cases the activities of the 1,8 derivatives was similar to those of the monosubstituted series. However, the SAR in these derivatives led to conclusions similar to those reached about the other series: 1) the terminal nitrogen atom was essential for the maintainance of high activity; 2) 2 carbon atoms between the nitrogens were superior to 3, and 3) the terminal nitrogen should be highly basic and tertiary.

The most potent compounds of the 1,8 substituted compounds (**46**, **47**) were still less active than adriamycin by a factor of 25.

Position 1,4

For comparison, some 1,4 derivatives were introduced (compounds **49**—**52**). From the data, it was inferred that substituents with low activity at positions 1,5 or 1,8 did not enhance activity if inserted into position 1,4 of the anthraquinone skeleton. However, substituents exhibiting high activity at other positions (**19**: 1,5-C2DM; **29**: 1,5-C2NHE) became significantly more active at position 1,4. Compound **50** (1,4-C2DM) was 4 times more active than **19** and the activity of compound **51** (ametantrone) was at least 35 times greater than that of its respective 1,5 analogue.

We can, therefore, conclude that active substituents at the 1,4 position are superior to other positions, probably due to a better mode of binding to DNA.

Pharmacology

Activity on adriamycin-resistant cells

The aminoalkylaminoanthraquinones were also tested on P388 leukemia cells resistant to adriamycin (R/ADR). It can be seen (Table I) that with the exception of compound **36**, the adriamycin-resistant cells were cross resistant to aminoalkylamino derivatives and had higher ED_{50} values (up to 8-fold) when compared to the parent P388 cell line. Only in 10% of the tested compounds was there no difference between the cytotoxic effects measured in both cell types.

Anti-bacterial effect

Although only 25 compounds were tested for bacteriocidic activity, it can be seen that the structural features required to attain high activity against *E. coli* parallel those necessary for the activity against P388 leukemia cells.

Substituents on position 1, indeed, demonstrated a similar pattern for both systems: the most active compound was 1-C2. However, substituents on position 2 exhibited a very low activity. For substituents on positions 1,5: in contrast to the effects observed in the P388 system, a primary amine on the terminal side chain was more active than a tertiary amine and a long side chain (C4) was superior to a short one. The most active compound in this series was 1,5-C4 **18**, which was less active than adriamycin by a factor of 25. Substitutents on the 1,8 positions were, in general, less active than those on the 1,5 positions.

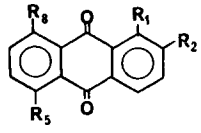
Experimental protocols

Melting points were determined with a Thomas—Hoover capillary melting point apparatus, IR spectra on a Perkin—Elmer 547 infrared spectrophotometer, UV spectra on a Varian 635 UV—VIS spectrophotometer and ^1H NMR spectra were obtained in $CDCl_3$, D_2O or DMSO-d_6 using a Brucker W.P. 60 or a WH 300 spectrometer. Physical data for compounds **1**—**47** are reported in Table II.

Analytical thin—layer chromatography (TLC) was carried out on silica gel using ethanol:chloroform:ammonium hydroxide (70:25:5). Separation and purification of products were carried out as follows:

A. After completion of the reaction, the cooled reaction mixture was treated with chloroform. The chloroformic layer was separated and washed successively with 2% NH_4OH until the aqueous phase became colorless. The chloroform layer was washed further with a saturated solution of NaCl followed by water and then dried over K_2CO_3 and filtered. The filtrate was acidified by 2—3 drops of H_2SO_4 and the product, in salt form, was removed and washed with acetone. The

Table II. Physical data of aminoalkylaminoanthraquinone derivatives.



No.	Compound	R: R_2=R_5=R_8=H; R_1=	FORMULA	Analysis	M.P. °C	Method	Recryst. from	Yield %
1	1-C2	$HN(CH_2)_2NH_2$	$C_{16}H_{14}N_2O_2.HCl$	C,H,N,Cl	278	(I)B	C(1)v	50
2	1-C3	$HN(CH_2)_3NH_2$	$C_{17}H_{16}N_2O_2.HCl$	C,H,N,Cl	298	(II)B	C(1)v	50
3	1-C4	$HN(CH_2)_4NH_2$	$C_{18}H_{18}N_2O_2$	C,H,N	97	(I)C	C(1)i	40
4	1-C2DM	$HN(CH_2)_2N(Me)_2$	$C_{18}H_{18}N_2O_2.HCl$	C,H,N,Cl	266	(III)B	C(1)v	75
5	1-C3DM	$HN(CH_2)_3N(Me)_2$	$C_{19}H_{20}N_2O_2.HCl$	C,H,N,Cl	248	(II)B	C(1)v	70
6	1-C2C2	$HN(CH_2)_2HN(CH_2)_2NH_2$	$C_{18}H_{19}N_3O_2$	C,H,N	119	(VII)C,D	C(1)v	50
7	1-C2C3	$HN(CH_2)_2NH(CH_2)_3HN_2$	$C_{19}H_{21}N_3O_2$	C,H,N	110	(VII)C,D	C(1)v	60
8	1-C3C2	$HN(CH_2)_3NH(CH_2)_2NH_2$	$C_{19}H_{21}N_3O_2.3H_2O$	C,H,N	129	(VII)C,D	C(1)v	60
9	1-C3C3	$HN(CH_2)_3NH(CH_2)_3NH_2$	$C_{20}H_{23}N_3O_2$	C,H,N	116	(VII)C,D	C(1)v	60

No.	Compound	R: R_1=R_5=R_8=H; R_2=	FORMULA	Analysis	M.P. °C	Method	Recryst. from	Yield %
10	2-C2	C2	$C_{16}H_{14}N_2O_2$	C,H,N	178	(IV)C	C(1)i	60
11	2-C3	C3	$C_{17}H_{16}N_2O_2.HCl$	C,H,N	279	(IV)C	C(1)i	60
12	2-C4	C4	$C_{18}H_{18}N_2O_2.1/2H_2SO_4$	C,H,N	269dec	(IV)A	C(1)i	25
13	2-C2DM	C2DM	$C_{18}H_{18}N_2O_2$	C,H,N	134	(IV)C,D	C(1)iv	60
14	2-C3DM	C3DM	$C_{19}H_{20}N_2O_2$	C,H,N	129	(IV)C,D	C(1)iv	30
15	2-C2PIPZ	$HN(CH_2)_2$-N◯NH	$C_{18}H_{16}N_2O_2.HCl$	C,H,N,Cl	308dec	(IV)B	C(1)iv	35

homogeneity of the material was examined on TLC and, if necessary, it was repurified in the same manner.

B. The same as **A**, but HCl gas was passed through the solution instead of a few drops of H_2SO_4.

C. The products were isolated as their free base. The procedure is similar to that of **A** but no acid was added or passed through. Instead, the washed and dried chloroformic layer was then evaporated to yield a solid or oily residue. The crude material was then crystallized from the following solvents: 1) monosubstituted anthraquinones: i: chloroform—ethanol—NH_4OH (25:70:5), ii: chloroform—methanol/NH_3 (saturated) (60:40), iii: acetone, iv: ethylacetates, v: water/ethanol; 2) disubstituted anthraquinones: i: methanol—chloroform (50:50), ii: methanol/NH_3 (saturated), iii: chloroform, iv: acetone, v: hot methylated spirit (5% methanol in ethanol) containing 2 eq of maleic acid, vi: ethylacetate—chloroform (70:30).

D. The reaction mixture was evaporated to dryness. The residue was dissolved in a small volume of solvent (DMSO; MeOH or EtOH) and subjected to a celite column 50 × 3 cm. The column was eluted subsequently with petroleum ether, chloroform and methanol (200 ml each). The chloroform and methanol fractions were combined and evaporated. The residue was then dissolved in DMSO (80 mg/ml) loaded onto a thick—layer chromatograph (2 × 200 × 200 mm), and developed 3 times with chloroform and finally with chloroform—methanol—NH_4OH (84:15:1). The appropriate band was cut and eluted subsequently with chloroform, methanol and methanol—NH_4OH (99:1). The combined solution was evaporated under reduced pressure and the residue crystallized from one of the solvent compositions listed in **C**.

E. i. The reaction mixture was concentrated and subjected to low pressure column chromatography on silica (130 × 2.5 cm). The product was eluted with MeOH—$CHCl_3$ (10:90) at a pressure of 80—10 psi.

ii. After the initial treatment (Ei) the reaction mixture was subjected to dry column chromatography on florisil (ratio of material to florisil is 1:400) and developed with methanol—NH_4OH (99:1). The desired band was cut out in sections and eluted with $CHCl_3$, MeOH and MeOH—NH_4OH (99:1). The combined eluates were evaporated and the residue was crystallized from one of the solvents listed in **C**.

Aminoalkylaminoanthraquinones were synthesized according to the following general methods.

Method I: 1-[(2-aminoethyl)amino]anthraquinone

4.85 g (20 mmol) of 1-chloroanthraquinone were dissolved in 50 ml of DMSO and heated to reflux. 6 ml of ethylene diamine (90 mmol) were added and kept under reflux for 5—10 min. Following this, the color changed from yellow to dark red. The solvent was then evaporated

Table II. (Continued).

No.	Compound	R $R_2 = R_8 = H$ $R_1 = R_5 =$	FORMULA	Analysis	M.P. °C	Method	Recryst. from	Yield %
16	1,5–C2	C2	$C_{18}H_{20}N_4O_2$	C,H,N	140	(V)D	C(1)iv	70
17	1,5–C3	C3	$C_{20}H_{24}N_4O_2.H_2O$	C,H,N	181	(V)D	C(1)i	80
18	1,5–C4	C4	$C_{22}H_{28}N_4O_2.C_4H_4O_4.H_2O$	C,H,N	182	(V)C(2)v	C(2)v	45
19	1,5–C2DM	C2DM	$C_{22}H_{28}N_4O_2$	C,H,N	191	(V)Eii	C(2)i	75
20	1,5–C3DM	C3DM	$C_{24}H_{32}N_4O_2$	C,H,N	95	(V)Eii	C(2)i	80
21	1,5–C2DE	$HN(CH_2)_2N(Et)_2$	$C_{28}H_{40}N_4O_2.1/2H_2O$	C,H,N	162	(V)Ei	C(2)i	80
22	1,5–C3NHDE	$HN(CH_2)_3N(CH_2CH_2OH)_2$	$C_{28}H_{40}N_4O_6$	C,H,N	126	(V)C	C(2)iv	72
23	1,5–C2PYR2	$HN(CH_2)_2$– 	$C_{28}H_{24}N_4O_2$	C,H,N	168	(V)C	C(2)i	20
24	1,5–C1PYR3	$HNCH_2$— 	$C_{26}H_{20}N_4O_4.H_2O$	C,H,N	226	(V)C,D	C(2)iii	70
25	1,5–C2 MOR	$HN(CH_2)_2$– 	$C_{26}H_{32}N_4O_4$	C,H,N	204	(V)C	C(2)i	68
26	1,5–C3 MOR	$HN(CH_2)_3$– 	$C_{27}H_{34}N_4O_4$	C,H,N	170	(V)C,D	C(2)iii	70
27	1,5–C2 PIP	$HN(CH_2)_2$– 	$C_{28}H_{36}N_4O_2$	C,H,N	174	(V)C	C(2)i	60
28	1,5–C2 PYRO	$HN(CH_2)_2$– 	$C_{26}H_{32}N_4O_2$	C,H,N	147	(V)C	C(2)i	45
29	1,5–C2 NHE	$HN(CH_2)_2NH(CH_2)_2OH$	$C_{22}H_{28}N_4O_4.1/2H_2O$	C,H,N	185	(V)C	C(2)i	60

No.	Compound	R $R_2=R_8=H$ $R_1=$	$R_5=$	FORMULA	Analysis	M.P. °C	Method	Recryst. from	Yield %
30	1–C2, 5–C2DM	R_1=C2	R_5=C2DM	$C_{20}H_{24}N_4O_2$	C,H,N	182	(VI)C	C(1)ii	60
31	1–C3, 5–C2DM	R_1=C3	R_5=C2DM	$C_{21}H_{27}N_4O_2.1/2H_2O$	C,H,N	161	(VI)C,D	C(2)i	60
32	1–C3DM, 5–C2DM	R_1=C3DM	R_5=C2DM	$C_{23}H_{30}N_4O_2.2H_2O$	C,H,N	98	(VI)C,D	C(2)i	60
33	1–C3MOR, 5–C2DM	R_1=C3MOR	R_5=C2DM	$C_{25}H_{32}N_4O_3$	C,H,N	129	(VI)C,D	C(2)i	60
34	1–Cl, 5–C2DM	R_1=Cl	R_5=C2DM	$C_{18}H_{17}N_2O_2Cl$	C,H,N	196	(III)Eii	C(1)iv	70

under reduced pressure to a volume of 10 ml and the residue treated according to the procedure outlined in **B**. The final product was crystallized from solution C(1)v.

Method II: 1-[(3-aminopropyl)amino]anthraquinone·HCl
25 ml of 1,3 diaminopropane (300 mmol) were added to 4.85 g (20 mmol) of 1-chloroanthraquinone (25 ml). The solution warmed to 40°C for 10—15 min and then cooled to — 10°C for 20 h. The precipitate formed was removed by filtration and treated as described in **B**. The final product in a salt form was recrystallized from solvent C(1)v.

Method III: 1-[(2-dimethylaminoethyl)amino]anthraquinone·HCl
4.85 g (20 mmol) of 1-chloroanthraquinone was dissolved in 200 ml of dry toluene containing 6 ml of *N,N*-dimethylethylenediamine (61 mmol). The solution was refluxed for 12—16 h, the solvent evaporated and the resulting material then treated as described in **B**. The precipitated salt was recrystallized twice from C(1)v.

Method IV: 2-[(2-aminoethyl)amino]anthraquinone
4.8 g (30 mmol) of anhydrous cupric sulfate was added to a stirred solution of 5 g (20.6 mmol), 2-chloroanthraquinone and 10 ml (150 mmol) of diaminoethane in 100 ml of DMSO. The mixture was heated to reflux for 10—15 min and filtered while hot. The cupric sulfate was washed with 5 ml of DMSO and the combined solutions treated according to **C** and recrystallized from C(1)i.

Method V: 1,5-bis[(3-dimethylaminopropyl)amino]anthraquinone
3 g (11 mmol) of 1,5-dichloroanthraquinone was dissolved in 50 ml of DMSO and heated to reflux. 10 ml of *N,N*-dimethylamino propylamine (79.6 mmol) were added to the hot solution and reflux continued for an additional 15 min. The reaction mixture was then cooled and treated according to **E**.

Method VI: 1-[(2-aminoethylamino)]-5-[(2-dimethylaminoethyl)amino]-anthraquinone (1-C2, 5-C2DM)

Table II. (Continued).

No.	Compound	R R_2=R_5=H R_1 = R_8 =	FORMULA	Analysis	M.P. °C	Method	Recryst. from	Yield %
35	1,8–C2	C2	$C_{18}H_{20}N_4O_2.H_2SO_4$	C,H,N	320	(V)A	C(1)v	30
36	1,8–C3	C3	$C_{20}H_{24}N_4O_2.H_2O$	C,H,N	165	(V)C	C(1)iv	70
37	1,8–C4	C4	$C_{21}H_{26}N_4O_2.H_2O.C_4H_4O_4$	C,H,N	160	(V)C(2)v	C(2)v	25
38	1,8–C2DM	C2DM	$C_{22}H_{28}N_4O_2.1/2H_2O$	C,H,N	148	(V)Eii	C(2)i	65
39	1,8–C3DM	C3DM	$C_{24}H_{32}N_4O_2.2H_2O.HCl$	C,H,N	280	(V)B	C(1)v	50
40	1,8–C2MOR	C2MOR	$C_{26}H_{32}N_4O_4$	C,H,N	173	(V)C	C(2)iv	85
41	1,8–C5M	$HN(CH_2)_5CH_3$	$C_{26}H_{34}N_2O_2.H_2O$	C,H,N	120	(V)C	C(2)iii	50
42	1,8–C2PIP	C2PIP	$C_{28}H_{36}N_4O_2.1/2H_2O$	C,H,N	133	(V)C	C(2)i	50
43	1,8–C2PYRO	C2PYRO	$C_{26}H_{32}N_4O_2$	C,H,N	125	(V)C	C(2)i	75
44	1,8–C3NHDE	C3NHDE	$C_{28}H_{40}N_4O_6H_2O$	C,H,N	108	(V)C	C(2)iv	60
45	1,8–C2PYR2	C2PYR2	$C_{28}H_{24}N_4O_2$	C,H,N	179	(V)C	C(2)i	80
46	1–C2DM, 8–Cl	R_1=C2DM, R_8=Cl	$C_{20}H_{24}N_4O_2$	C,H,N	106	(III)C	C(1)iii	15
47	1–C2DM, 8–C3	R_1=C2DM, R_8= C3	$C_{21}H_{26}N_4O_2$	C,H,N	149	(VI)C,D	C(2)vi	15

5 g (0.018 mol) of 1,5-dichloroanthraquinone and 3.2 g (0.036 mol) of dimethylaminoethylenediamine were dissolved in 200 ml of toluene and refluxed for 12—16 h. The solvent was removed and the residue was subjected to florisil column chromatography (50 × 3 cm). The column was eluted first with ethylacetate to remove the non-reacting 1,5-dichloroanthraquinone and followed by chloroform :ethanol (50:50) in order to elute the 1-chloro-5-[(2-dimethylaminoethyl)amino]anthraquinone (1,5-C2DMCl). The solvent was evaporated and the final product was collected and dried.

3 g of 1,5-C2DMCl (0.009 mol) were dissolved in 25 ml of ethylenediamine. The solution was treated according to method II and the 1-C2,5-C2DM was recrystallized from C(1)ii.

Method VII: 1-[2-ethylamino(3-propylamino)amino]anthraquinone

5 g (21 mmol) of 1-chloroanthraquinone and 5 g (0.085 mol) of ethanolamine were dissolved in 50 ml of butanol. The reaction mixture was refluxed for 3 h, concentrated to a volume of 20 ml, cooled to — 10°C and the precipitate 1-[(hydroxyethyl)amino]anthraquinone, 1-C20H, was collected and recrystallized from ethylacetate.

3 g (0.011 mol) of 1-C2OH and 1.3 g (11.3 mmol) of methanesulfonylchloride were dissolved in 20 ml of pyridine and stirred for 3 h at room temperature. 40 ml of water were added and the precipitate was removed by filtration and recrystallized from methanol.

2 g (6 mmol) of the product and 3 g (40 mmol) of 1,3-diaminopropane were dissolved in acetonitrile and refluxed for 1 h. The progress of the reaction was followed by TLC and, upon completion, water was added. The target material was extracted several times with chloroform. The chloroform was then dried ($MgSO_4$), evaporated and the residue was recrystallized from methanol—water.

Bioassay

Cell culture

P388 murine leukemia cells sensitive to (S/ADR) and a subline resistant to doxorubicin (R/ADR), were propagated continuously in suspension culture as previously described [63]. Cells were grown in Roswell Park Memorial Institute 1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (GIBCO), 10 μM 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO.). 50 units/ml of penicillin base and 50 μg/ml of streptomycin base (both from GIBCO). Cell growth was assessed by measuring cell density in a Coulter counter (Coulter Electronics Ltd., Harpenden, Hertfordshire, U.K.). An inoculum of cells was transferred to fresh medium once every 4 days, in order to maintain growth in the exponential phase. Initial cell density was $1 \times 10_5$ cells/ml and after 4 days in culture it became $1—2 \times 10_6$ cells/ml. Cell growth rates were calculated from the culture densities measured once a day for 4 days.

Determination of drug sensitivity

The sensitivity of a cell line to a given drug or drug combination was assessed as follows: cells were cultured in the presence of various drug concentrations for 4 days and the slope of the log cell density *versus* time plot was calculated by linear regression analysis. The growth rate at each drug concentration was expressed as a percentage of the control growth rate. In this manner dose—effect curves were produced and used to determine the drug concentration effective in inhibiting the growth rate by 50% (*ED*II). The doxorubicin ED_{50} for the drug sensitive and the drug resistant cell lines ranged from $2—6 \times 10^{-8}$ M and from $1—2 \times 10^{-6}$ M, respectively. No change in the drug sensitivity of either cell line was observed during 4 years of continuous *in vitro* culture. The ED_{50} values obtained have a standard error of less than 10% of the mean.

Growth of E. coli *B. in the presence of inhibitor*

5 ml of Deiwis medium were inoculated with a single bacterial colony and incubated at 37°C overnight with continuous vigorous shaking. The following morning, 5 ml of Deiwis medium were inoculated with 500 μl of the overnight culture and again incubated with continuous vigorous shaking until the culture reached its late log phase (OD_{600} = 0.6). A set of tubes containing various concentrations of inhibitor in 0.9 ml of Deiwis medium and a control tube (without inhibitor) were inoculated at 37°C and shaken until the control tube reached an OD_{600} of 0.6. Solutions were diluted 10 000 and 1000-fold and 10 μl of each was spread onto an agar plate containing minimal medium (4 dilutions on each plate). The colonies were counted and the number of cells in 1 ml of culture was calculated. The ID_{50} was determined from a plot of % growth (control = 100%) as a function of inhibitor concentration.

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