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Original article

Novel anthraquinone derivatives with redox-active functional groups capable of producing free radicals by metabolism: are free radicals essential for cytotoxicity?

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Abstract – The mode of action of antitumour anthraquinone derivatives (i.e. mitoxantrone) is not clearly established yet. It includes, among others, intercalation and binding to DNA, bioreduction and aerobic redox cycling. A series of anthraquinone derivatives, with potentially bioreducible groups sited in the side chain, have been synthesized and biologically evaluated. Their redox and cytotoxic activities were screened. Derivatives which bear a 2-(dimethylamino)ethylamino substituent, known to confer high DNA affinity, demonstrated cytotoxicity but not redox activity (beside the anthraquinone reduction). Conversely, derivatives which showed redox activity were not cytotoxic toward the P388 cell line. The results suggest that bioreduction is not the main mode of action in the cytotoxicity of anthraquinones. © 1999 Éditions scientifiques et médicales Elsevier SAS

anthraquinones / bioreduction / cytotoxicity

1. Introduction

The broad spectrum of antitumour efficacy of anthracyclines is reflected in their widespread use in cancer chemotherapy [1–3]. Although the mode of their action is not fully established yet, it includes, inter alia: a) the involvement of the quinone component in a redox cycle process [4] leading, through a semiquinone ($AQ^{\bullet-}$) intermediate (by flavin-mediated one-electron reduction), to alkylating intermediates and the generation of reactive oxygen species (ROS), in particular $O_2^{\bullet-}$, H_2O_2 and $^{\bullet}OH$. These species are capable of producing DNA lesions, peroxidative injury to membrane lipids and alteration of subcellular organelles and functional integrity, and b) their intercalative capacity, which either affects DNA structure and transcription to mRNA or alternatively stabilizes the cleavable complex between topoisomerase II and DNA [5].

The anthraquinone (9,10-anthracenedione) skeleton is a central constituent of the anthracyclines (doxorubicin and daunorubicin) and other antineoplastic agents such as mitoxantrone [6, 7], (*figure 1*) and anthrapyrazole derivatives [8]. To gain a wider understanding of the involvement of radicals in the action of anthraquinone-derived

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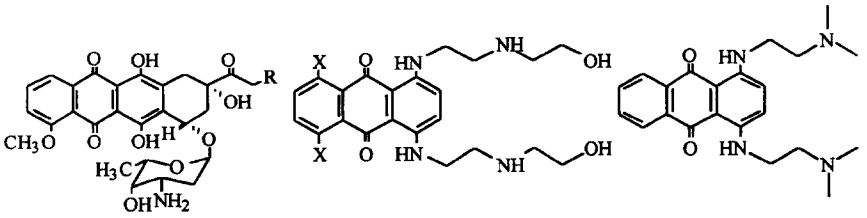
Abbreviations: anh., anhydrous; AQ, anthraquinone; aq., aqueous; CDCl3, deuterochloroform; CV, cyclic voltammetry; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; δ, chemical shift (ppm); DMF, N,N-dimethylformamide; d7-DMF, heptadeutero-N,N-dimethylformamide; d6-DMSO, hexadeuterodimethylsulfoxide; DMSO, dimethylsulfoxide; E^a, anodic redox potential; E^c, cathodic redox potential; EDC, water soluble carbodiimide, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Et3N, triethylamine; EtOAc, ethyl acetate; FAB-MS, Fast Atom Bombardment Mass Spectrometry; Florisil® (U.S. Silica Co.), magnesium silicate particle size 75-150mm; leucoquinizarin, 2,3-dihydro-9,10-dihydroxy-1,4-anthraquinone; MeOH, methanol; mp, melting point; NHS, N-hydroxysuccinimide; Py, pyridine; ROS, reactive oxygen species; RT, room temperature; TLC, thin layer chromatography.



Doxorubicin : R = OH
Daunorubicin : R = H

Mitoxantrone : X = OH
Ametantrone : X = H

37

Figure 1. Selected antineoplastic agents having an anthraquinone moeity.

agents, several related compounds bearing selected characteristic functional groups were designed. The approach was to develop structure-activity relationships (SAR) of anthraquinone analogues with redox-active centres attached to the anthraquinone skeleton through spacer side chains a) at positions 1 and 1,4; and (b) at position 1, together with substituents with DNA-binding affinity at position 4.

If radicals mediate the cytotoxic effect, redox-active groups linked to an anthraquinone skeleton might amplify the anticancer activity. However, if the cytotoxic activity relates only to the intercalative capacity of the anthraquinone structure, it might be affected by groups that enhance binding-affinity to DNA. In the present work, the cytotoxic activity of the derivatives was assessed using the P388 cell line.

2. Results and discussion

2.1. Chemistry

2.1.1. Design of synthetic derivatives

A variety of compounds (derivatives **1–36**), having an additional redox-active centre with potential to be bioactivated, were designed. The characteristic structural feature of the designed substances is a potential redox-active centre in the side chain rather than in the intercalative part of the molecule (*table I*). These compounds were expected to intercalate and undergo activation during their metabolic pathway and thus serve as electron transfer mediators. Bis-bioreductive agents such as bis-nitroimidazoles [9] or N,N′-dioxides [10] have previously demonstrated enhanced cytotoxicity and selectivity towards hypoxic tumour cells. Nitracrine and its N-oxide [11, 12] have been shown to be DNA-directed bioreductive drugs, while the N,N′-dioxide analogue of mitoxantrone [13] has been proposed to be a prodrug, which under hypoxia is bioreductively activated to a DNA-binding agent.

In order to discriminate between the effects of redox-active groups on compounds with high and low DNA-binding affinity, two classes of derivatives were designed: derivatives with cationic substituents (with binding affinity to DNA) and derivatives without cationic groups. The cationic [2-(dimethylamino)ethyl]amino side chain (derivatives **3**, **9**, **29–32**), present in the known cytotoxic compound **37** (*figure 1*) was selected to confer DNA-binding affinity [6]. Unfortunately, part of the non-cationic derivatives (**1**, **4–6**, **10–15**) lack the necessary solubility in aqueous solutions to be appropriately screened, therefore soluble derivatives were designed, via the introduction of the 3-hydroxypropylamino residue (derivatives **2**, **7** and **8**).

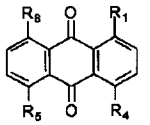
The derivatives may be classified according to the redox-active group introduced:

1) Nitroaromatic derivatives (**6–11**). Nitroaromatics and nitroheterocycles undergo bioactivation [14, 15], under anaerobic conditions, through a series of single electron reductions by a range of flavoenzymes leading to the reduction sequence $RNO_2 \Rightarrow RNO_2^{\bullet-} \Rightarrow RNO \Rightarrow RNHOH \Rightarrow RNH_2$.

2) Pyridine, pyridine N-oxide and pyridinium derivatives (**16–21**, **24–35**). Pyridinium derivatives, like N-methyl-4-phenylpyridinium (MPP^+) [16], are known electron acceptors which undergo one-electron reduction by the following pathways: a) direct one electron transfer and b) a flavin-mediated hydride transfer followed by one electron reduction. The pyridine group might lead to the formation of a pyridyl radical. Pyridine N-oxide derivatives, such as nicotinamide N-oxide, undergo reduction to the corresponding pyridine derivative [17]. During the reduction pathway, the pyridine N-oxide group might be converted to a cytotoxic nitroxide radical intermediate [18].

3) Polyhaloalkyl derivatives (**14–15**). Polyhalogenated alkanes can either undergo oxygenation [19] or reductive

Table I. Anthraquinone derivatives synthesized.



X = $NH(CH_2)_nY$

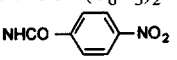
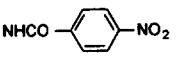
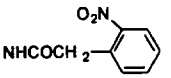
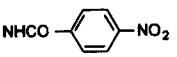
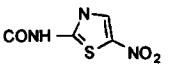
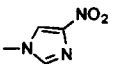
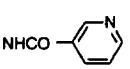
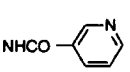
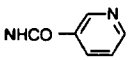
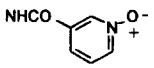
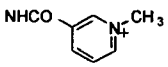
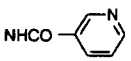
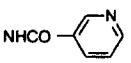
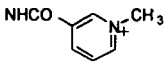
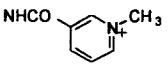
Derivative	R_1	R_4	R_5	R_8
1	X: n = 3, Y = $OC(C_6H_5)_3$	H	H	H
2	X: n = 3, Y = $OC(C_6H_5)_3$	X: n = 3, Y = OH	H	H
3	X: n = 3, Y = $OC(C_6H_5)_3$	X: n = 2, Y = $N(CH_3)_2$	H	H
4	X: n = 3, Y = $OC(C_6H_5)_3$	X: n = 3, Y = $OC(C_6H_5)_3$	H	H
5	X: n = 3, Y = $NHCON(C_6H_5)_2$	H	H	H
6	X: n = 3, Y = 	H	H	H
7	X: n = 3, Y = 	X: n = 3, Y = OH	H	H
8	X: n = 3, Y = 	X: n = 3, Y = OH	H	H
9	X: n = 3, Y = 	X: n = 2, Y = $N(CH_3)_2$	H	H
10	X: n = 3, Y = 	H	H	H
11	X: n = 3, Y = 	H	H	H
12	X: n = 3, Y = $NHCOC_6H_5$	H	H	H
13	X: n = 2, Y = CHO	H	H	H
14	X: n = 3, Y = $NHCOOCH_2CCl_3$	H	H	H
15	X: n = 3, Y = $COOCH_2CCl_3$	H	H	H
16	X: n = 1, Y = 	H	Cl	H
17	X: n = 1, Y = 	H	X: n = 1, Y = 	H
18	X: n = 1, Y = 	H	Cl	H
19	X: n = 1, Y = 	H	X: n = 1, Y = 	H
20		H	H	H
21		OH	H	H
22	$NHCOC_6H_5$	H	H	H
23	$NHCOC_6H_5$	OH	H	H

Table I. Continued.

Derivative	R_1	R_4	R_5	R_8
24	X: n = 2, Y = 	H	H	H
25	X: n = 2, Y = 	H	Cl	H
26	X: n = 2, Y = 	H	X: n = 2, Y = 	H
27	X: n = 2, Y = 	X: n = 2, Y = 	H	H
28	X: n = 2, Y = 	H	H	X: n = 2, Y = 
29	X: n = 1, Y = 	X: n = 2, Y = $N(CH_3)_2$	H	H
30	X: n = 1, Y = 	H	X: n = 2, Y = $N(CH_3)_2$	H
31		X: n = 2, Y = $N(CH_3)_2$	H	H
32		X: n = 2, Y = $N(CH_3)_2$	H	H
33		H	H	H
34			H	H
35			H	H
36	X: n = 3, Y = NH_3^+	X: n = 3, Y = NH_3^+	H	H

dehalogenation by microsomal cytochrome P450 [20–22]. The proposed reductive pathway involves one-electron transfer and halide elimination to generate a carbon-centred radical. The radical formed may either abstract a hydrogen from the close environment (membrane lipid), or react with oxygen to form a reactive peroxy radical, or undergo a second dehalogenation.

4) Triarylmethyl derivatives (**1–4**). Triphenylmethyl radicals are stable carbon-centred radicals, which may be formed during bioreduction (cytochrome P450) of triarylmethane derivatives [23] and may react with oxygen to form a reactive peroxy radical.

2.1.2. Synthesis

Method A: this method involves nucleophilic displacement of 1-chloro-, 1,5-dichloro- and 1,8-dichloroanthraquinone by alkanamine derivatives to form mono- and bis-substituted anthraquinones [7, 24]. The alkanamine derivatives in this class (*figure 2*) were 1,3-propanediamine, 3-aminopropanol, 4-aminobutyric acid and various aminoalkylpyridine derivatives. This method led to a direct (one step) synthesis of the 1-ethylamino and 1-methylamino side chains bearing 2′-pyridyl or 3′-pyridyl residues (derivatives **16**, **18**, **24** and **25**). The symmetrically substituted 1,5- and 1,8-bis(pyridyl-

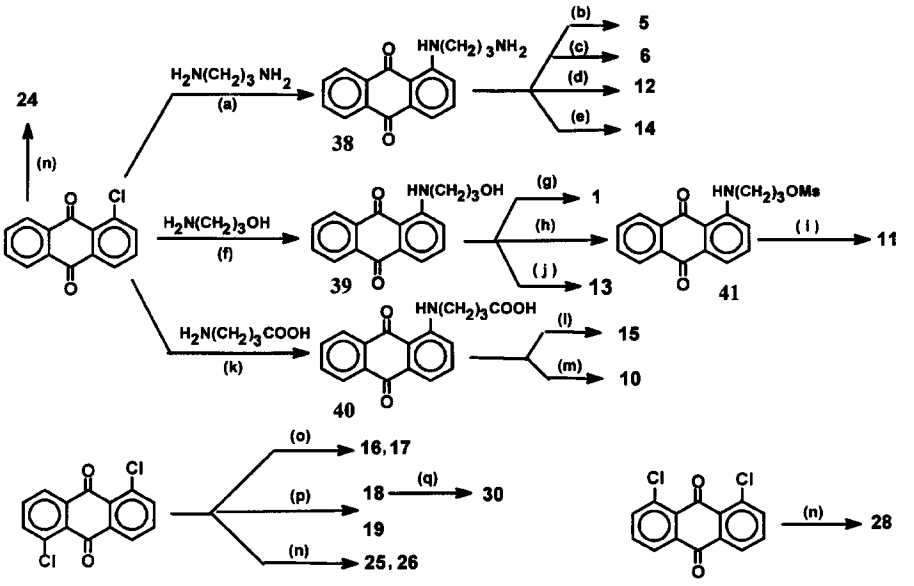


Figure 2. (a) 1,3-diaminopropane, toluene, reflux, 18 h; (b) diphenylcarbamyl chloride, CH_2Cl_2, R.T., 3 h; (c) 4-nitrobenzoic acid, DMF, Et_3N, NHS, water soluble carbodiimide (WSCI), 3 d; (d) benzoyl chloride, Py, R.T., 18 h; (e) 2,2,2-trichloroethyl chloroformate, Et_3N, CH_2Cl_2, R.T., 90 min; (f) 3-aminopropanol, n-butanol, reflux, 4 h; (g) chlorotriphenylmethane, Py, 80 °C, 8 h; (h) methanesulfonyl chloride, Py, CH_2Cl_2, R.T., 48 h; (i) 4-nitroimidazole, DMF:CH_2Cl_2 (1:2), DBU, reflux, 12 h; (j) pyridinium chlorochromate / silica gel, CH_2Cl_2, 90 min, R.T. in ultrasound bath; (k) 4-aminobutyric acid, DMSO, Et_3N, 150 °C, 90 min; (l) 2,2,2-trichloroethanol, DCC, R.T., 3 h; (m) 2-amino-5-nitrothiazole, DMF, Et_3N, NHS, WSCI, R.T., 3 d; (n) 2-(2-aminoethyl)pyridine, DMSO, 150 °C, 10 min; (o) 3-(aminomethyl)pyridine (neat), 40–50 °C, 36 h; (p) 2-(aminomethyl)pyridine (neat), 40–50 °C, 36 h; (q) N,N-dimethylethylenediamine (neat), 55 °C, 72 h.

alkylamino)anthraquinones (derivatives **17**, **19**, **26** and **28**) were obtained under the same reaction conditions. The non-symmetrically substituted derivative **30** was prepared in a two-step reaction, where the intermediate derivative **18** was further treated with N,N-dimethylethylenediamine. Other monosubstituted derivatives (*figure 2*) derived from conversion of 3-amino-, 3-hydroxy- and 3-carboxypropylamino side chains (intermediates **38–40**) to their final defined moieties. Intermediate **38** was carbamoylated (**5**), benzoylated (**6** and **12**) and formylated (**14**) via conventional methods. Intermediate **39** was tritylated to yield **1**. In addition, it was oxidized by pyridinium chlorochromate (on silica gel) to form **13** [25]. Derivative **11** was obtained in two steps by mesylation of **39** to get **41**, which underwent nucleophilic substitution by 4-nitroimidazole in the presence of DBU as a strong base. The carboxy group in **40** was amidated (**10**) and esterified (**15**).

Method B: this method is related to the synthesis of symmetrically and non-symmetrically substituted 1,4-bis(alkylamino)anthraquinones (*figure 3*). In the case of the symmetrically substituted analogues, the usual methodology for synthesis is the treatment of leucoquinizarin [6, 24, 26, 27] with a two molar excess of the appropriate amine under nitrogen atmosphere followed by air oxidation. The derivatives **27** and **36** were thus prepared in good yield. Derivatives **2** and **4** were prepared in two steps: synthesis of the intermediate **42** from leucoquinizarin and 3-aminopropanol, followed by tritylation of the hydroxy groups in the side chains.

The non-symmetrically 1,4-substituted analogues were obtained in a "one pot" sequential treatment of leucoquinizarin [26, 28] with two amines (intermediates **43–45** and derivative **29**). The two side chains of the non-symmetrically substituted compounds thus prepared serve two goals. One side arm is designed toward its engagement in bioreductive processes by itself (derivative **29**), or after substitution with bioreductive groups (derivatives **3**, **7–9**) and the second enhances solubility (3-aminopropanol) or DNA-binding affinity (N,N-dimethylethylenediamine).

Method C: this method involves an amidation [29, 30] of 1-amino-, 1,4-diamino-, 1-amino-4-hydroxy- and 1-amino-4-[2-(dimethylamino)ethylamino] anthraquinone (*figure 4*). In general, this was accomplished by treatment of the 1-aminoanthraquinone derivatives with benzoyl

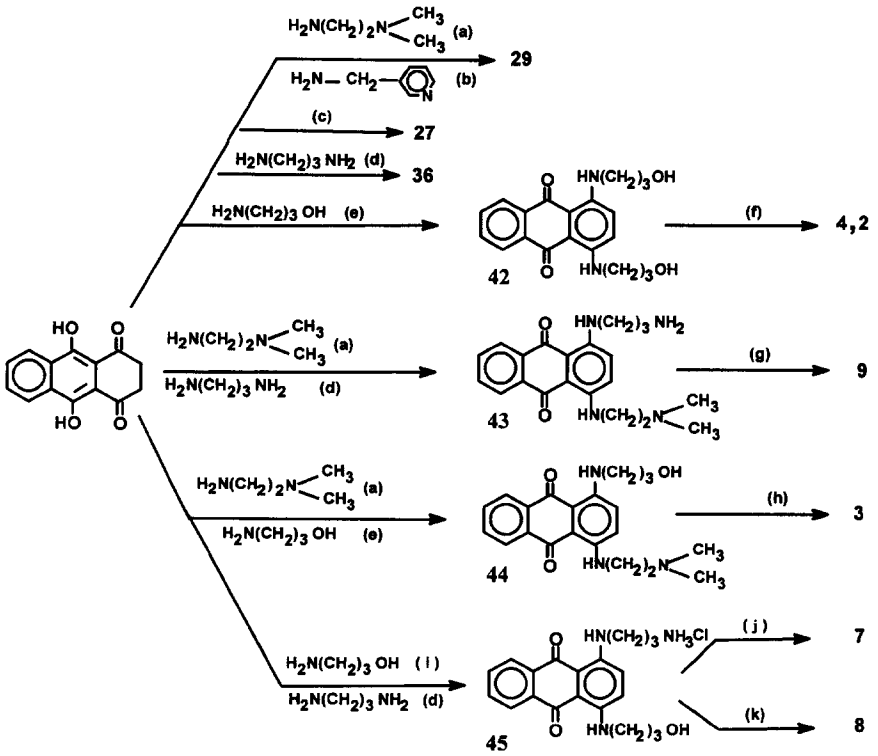


Figure 3. (a) N,N-dimethylethylenediamine, MeOH, N_2, 50 °C, 1 h; (b) 3-(aminomethyl)pyridine, N_2, 50 °C, 2 h; air 18 h; (c) 2-(2-aminoethyl)pyridine, MeOH, N_2, 50 °C, 1 h; air, 18 h; (d) 1,3-diaminopropane, MeOH, N_2, 50 °C, 2 h; air, 18 h; (e) 3-aminopropanol, MeOH, N_2, 50 °C, 2 h; air, 18 h; (f) chlorotriphenylmethane, Py, 80 °C, 6 h; (g) 4-nitrobenzoic acid, DMF, Et_3N, NHS, WSCI, RT, 3 d; (h) chlorotriphenylmethane, Py, 80 °C, 3 h; (i) 3-aminopropanol, MeOH, N_2, 50 °C, 1 h; (j) 4-nitrobenzoyl chloride, DMF, Et_3N, R.T., 12 h; (k) 2-nitrophenylacetyl chloride, DMF, Et_3N, R.T., 12 h.

chloride (derivatives **22**, **23**), nicotinoyl chloride (derivatives **20**, **21**, **31**, **34**) and nicotinoyl chloride N-oxide (derivative **32**) in toluene under reflux. The monopyridinium and the bispyridinium salts (derivatives **33** and **35**) were obtained from derivatives **20** and **34** respectively, by a direct methylation of the pyridine residue with methyl iodide (neat). Intermediate **46**, which was employed as a starting material for the synthesis of **31** and **32**, was prepared according to the guidelines presented in the synthesis of non-symmetrically 1,4-substituted anthraquinones (*figure 3*): leucoquinizarin was treated first with N,N-dimethylethylenediamine, then with gaseous ammonia in one reaction vessel and finally oxidized.

2.1.3. Redox activity assayed by cyclic voltammetry (CV)

Reduction of the anthraquinone moiety: the reduction sweep (cathodic) for most of the derivatives in DMF consisted of two major reversible (or quasi-reversible) reduction waves (*table II*). The electrochemical transitions consisted of two consecutive one-electron reduction steps [31] of anthraquinone (AQ) to generate first the semiquinone radical anion $AQ^{\bullet -}$ and then the dianion AQ^{-2}. Derivatives with an amido group attached to the anthraquinone skeleton (**20–22**, **31–35**) displayed less negative values (from –0.48 V to –0.73 V) for the first reduction wave than the amino derivatives (**1–18**, **24–30**, **42–44**, **46**), which displayed values from –0.85 V to –1.20 V. This recurring difference in the redox potential between the two series of compounds can be attributed either to the difference between the electron withdrawal effect of the substituents of the two systems, or to possible perturbation due to the acyl substituent, to hydrogen bonding between the amino group in position 1 and the quinonic oxygen atom. Concerning the substituent polar effect, it is known that electron-donating substituents, such as alkylamino groups, shift more than electron-withdrawing substituents such as the amido

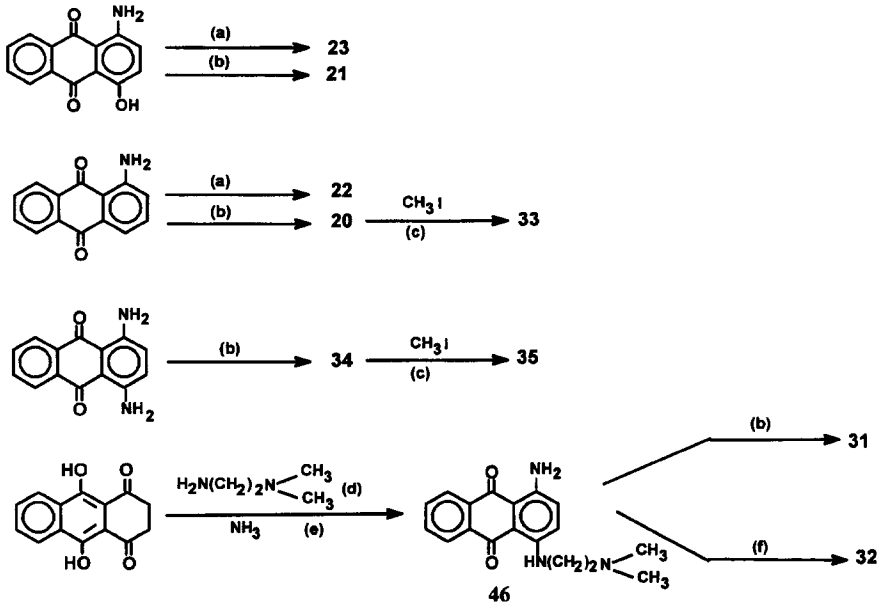


Figure 4. (a) benzoyl chloride, toluene, reflux, 24 h; (b) nicotinoyl chloride, toluene, reflux, 24 h; (c) iodomethane, reflux, 24 h; (d) N,N-dimethylethylenediamine, MeOH, N_2, 50 °C, 1 h; (e) NH_3 (gas) bubbled 5 min, R.T., then sealed for 2 d, R.T.; (f) nicotinoyl chloride N-oxide, toluene, reflux, 24 h.

groups, the reduction potential of the anthraquinone to negative values [32]. Referring to the second explanation to the above phenomenon, it is assumed that in aprotic solvents an internal hydrogen bond is formed between the quinonic oxygen and the amino group at positions 1, 4, 5 and 8. Accordingly, this would result in a more negative reduction wave potential. Acylation of the amino group at these positions modulates the function of the hydrogen bond. In the amide form, the carbonyl group of the side chain tends to compete with the quinonic oxygen for hydrogen bonding. Thus, the quinone will be less resistant to reduction.

The results presented in *table II* for **37** are in accord with those previously described [31], although the two sets of experiments were not carried out in the same solvent (DMF instead of DMSO). However, a third wave at –1.54 V was observed, in analogy to other alkylaminoanthraquinone derivatives assayed (*table II*). It is plausible to assign the peaks at –1.13 V and –1.58 V to the first and the second electron reduction wave of the quinone structure. The reversible peak at –0.79 V seems also to be related to the first reduction process, although its specific implication is obscure. It can be speculated that this reduction peak is associated with internal hydrogen bonding (NH-----O=C) equilibrium which is faster than the scan rate of 50 mV/s and thus, the quinone is reduced in its two forms. An alternative possibility is that the two less negative peaks (–0.79 V and –1.13 V) correspond to the first and the second electron reduction steps, while the third reduction step is attributed to a rapid complex formation between species involved in the course of the CV reduction [33]. In the case of hypericin [34], the two cathodic waves (in DMF) at –1.14 V and –1.53 V, ascribed to the redox steps of the quinone part, are in good agreement with the data presented in *table II* for the cathodic peaks of alkylaminoanthraquinones. Electrochemical reduction of anthrapyrazole (in DMF) [35], leading to the formation of a semiquinone-type radical, also occurs at a highly negative redox potential of –1.30 V which is in the range of the tested alkylaminoanthraquinones.

Redox-active substituent effect: most of the nitro-derived compounds (**6–10**) showed an additional quasi-reversible reduction wave (shoulder) at –1.6 V. This reduction potential is attributed to the nitro group since a similar reduction wave pattern was observed for methyl 4-nitrobenzoate (**47**) (*table II*). An additional reversible reduction wave (shoulder) was observed for the nitro derivatives **7**, **9** and **10** at –0.8 V to –1.0 V, which is not related explicitly to the anthraquinone or the nitro groups. This wave (shoulder) is presumably related to intramolecular redox activity between two reductive linked species (nitro and quinone groups). Since the reduction potential of the nitro group resides in the range of the two

Table II. Redox potential of anthraquinone derivatives measured by cyclic voltammetry.

Derivative	E^{c}_{p1} [a]	E^{a}_{p1} [b]	E^{c}_{p2} [a]	E^{a}_{p2} [b]	E^{c}_{p3} [a]	E^{a}_{p3} [b]	E^{c}_{p4} [a]	E^{a}_{p4} [b]
1,4-Ametantrone	– 0.79		– 1.09	– 1.03	– 1.51	– 1.43		
1,5-Ametantrone	– 1.03	– 0.96	– 1.53	– 1.51				
1,5-Dichloroanthraquinone	– 0.77	– 0.69	– 1.40	– 1.21				
1-Aminoanthraquinone	– 0.97	– 0.92	– 1.52					
1	– 0.96	– 0.93	– 1.58	– 1.24				
2	– 1.08	– 1.03	– 1.55	– 1.48				
3	– 1.13	– 1.08	– 1.59	– 1.53				
4	– 1.13	– 1.06	– 1.62	– 1.55				
5	– 1.00	– 0.85	– 1.53sh [d]	– 1.38	– 1.63	– 1.58		
6	– 0.98	– 0.90	– 1.53sh		– 1.65	– 1.53		
7	– 1.00	– 0.95	– 1.15	– 1.08	– 1.58sh	– 1.40	– 1.70	– 1.53
8	– 1.15	– 1.08	– 1.58	– 1.47	– 1.67			
9	– 1.03sh	– 0.98	– 1.14	– 1.09	– 1.60sh	– 1.55	– 1.72	
10	– 0.83sh	– 0.70	– 0.95	– 0.89	– 1.55	– 1.50		
11	– 0.95	– 0.90	– 1.48	– 1.40				
12	– 0.95	– 0.90	– 1.58	– 1.45				
13	– 0.73sh		– 1.05	– 0.95	– 1.75	– 1.45		
14	– 0.98	– 0.93	– 1.50	– 1.39				
15	– 0.93	– 0.88						
16	– 0.88	– 0.80	– 1.47	– 1.38				
17	– 0.71		– 0.93	– 0.89	– 1.60	– 1.53		
18	– 0.67sh		– 0.87	– 0.80	– 1.46	– 1.38		
20	– 0.67	– 0.61	– 1.27	– 1.22				
21	– 0.52	– 0.44	– 1.07	– 0.98	– 1.35			
22	– 0.68	– 0.62	– 1.27	– 1.20				
24	– 0.93	– 0.84	– 1.38	– 1.31				
25	– 0.65		– 0.90	– 0.80	– 1.40	– 1.32		
26	– 0.70		– 1.05	– 0.97				
27	– 1.07	– 0.98	– 1.53	– 1.43				
28	– 0.99	– 0.90	– 1.48	– 1.35				
29	– 0.75sh – 0.85sh		– 1.05	– 1.03	– 1.60	– 1.50		
30	– 0.72sh		– 1.02	– 0.96	– 1.64	– 1.58		
31	– 0.77	– 0.70	– 1.01		– 1.32	– 1.24		
32	– 0.72	– 0.68	– 1.24	– 1.18				
33	– 0.61	– 0.53	– 1.01sh		– 1.21	– 1.10		
35	– 0.40		– 0.71	– 0.67	– 1.29	– 1.16		
36	– 0.70		– 0.95	– 0.85	– 1.13			
37 [c]	– 0.79	– 0.75	– 1.13	– 1.08	– 1.58	– 1.50		
42	– 0.80		– 1.14	– 1.08	– 1.62	– 1.53		
43	– 1.08	– 1.05	– 1.65	– 1.55				
44	– 0.93		– 1.20	– 1.13	– 1.62sh		– 1.70	– 1.65
45	– 0.55		– 1.03	– 0.83				
46	– 0.76		– 1.14	– 1.11	– 1.58	– 1.47		
47	– 0.95	– 0.80	– 1.55sh	– 1.30	– 1.70	– 1.52		

[a] E^{c}_{p}: cathodic peak potential (V); [b] E^{a}_{p}: anodic peak potential (V); [c] see scheme 1; [d] sh: shoulder.

reduction potentials of the anthraquinone molecule, it seems most plausible that the nitro group plays a role in some intramolecular electron transfer processes.

Derivative **33** displayed an additional shoulder at –1.0 V, which could be due to the reduction of the pyridinium ring, in compliance with the reduction potential of $NADP^{+}$ in DMSO [36].

For derivative **5**, which contains a diphenylureido component, an additional quasi-reversible reduction wave (shoulder) was noticed at –1.5 V. This shoulder

Table III. Concentration of synthetic derivatives effective in inhibiting the growth rate of the P388 murine leukaemia cells and macrophage-like J774.2 cells by 50 % (ED_{50}).

Derivatives	ED_{50} (μM)	Derivatives	ED_{50} (μM)	
	P388		P388	J 774.2
1	> 10	**17**	>> 7	
2	6.0	**18**	>> 20	
3	2.8	**19**	>> 4	
4	> 10	**20**	> 6	
5	10	**21**	> 1.3	
6	10	**22**	>> 3	
7	> 10	**23**	> 0.8	
8	10	**29**	1.2	8.6
9	5	**30**	1.6	1.6
10	3.7	**31**	0.086	0.5
11	> 10	**32**	0.043	0.097
12	10	**33**	>> 20	
13	3	**35**	12	
14	> 10	**36**	0.37	
15	2.4	**37**	0.044	0.097
16	>> 20	**46**	0.87	0.5

might be associated with the effect of the ureido group on the quinone structure since N,N-diphenyl-N′-propylurea did not exhibit any detectable peak during the CV sweep.

Other attached functional groups as triphenylmethoxy (**1–4**), formyl (**13**), trichloromethyl (**14**, **15**) and pyridine (**16–21**, **24–31**, **34**) did not exhibit any additional reduction wave, which can be assigned exclusively to these groups. One plausible explanation is that their reduction wave is hidden by the anthraquinone wave.

2.2. Biological activity

2.2.1. Cytotoxic activity of the anthraquinone derivatives in cell culture

All the derivatives synthesized were evaluated for cytotoxic activity against P388 murine leukaemia cells [37, 38]. Those compounds which have shown activity were then evaluated against murine macrophage-like J774.2 cells [39] (*table III*).

Derivatives **31** and **32**, which are non-symmetrically substituted at the 1,4-positions of the anthraquinone, showed high cytotoxic activity comparable to **37**, as described in the literature [6, 40]. The N-oxide of the nicotinoyl group neither increased nor lowered the cytotoxicity. The intermediate **46** and derivative **36** had significant cytotoxic activity. All the other derivatives did not show any significant activity. Some of them precipitated in the medium due to their low solubility in aqueous solutions, thus preventing an accurate assessment of their cytotoxic potential.

The presence of at least one cationic substituent, like 2-(dimethylamino)ethylamino, which confers DNA-binding affinity, was essential for cytotoxicity. However, its presence does not account for the differences in cytotoxicity: a cationic derivative (**9**) was not cytotoxic, suggesting that other unknown factors contribute to cytotoxicity. Derivatives **29** and **31** both possess a cationic substituent, though **31** is ten times more potent than **29**, suggesting that amidoanthraquinones (with less negative reduction potentials) are more cytotoxic than aminoanthraquinones. All the non-cationic derivatives, even those soluble in aqueous solutions, were not cytotoxic. These results reaffirm that the presence of at least one cationic group is required for cytotoxicity and suggest that the bioreduction is not the predominant mode of action in the cytotoxicity of anthraquinones.

In conclusion, new anthraquinone derivatives with potential bioreducible groups were synthesized. Two derivatives (**31** and **32**) showed high cytotoxicity toward the P388 cell line. The presence of at least one cationic substituent, like 2-(dimethylamino)ethylamino in these derivatives, which confers DNA-binding affinity, was essential for cytotoxicity. However, one cationic derivative (**9**) was not cytotoxic, suggesting that other unknown factors contribute to cytotoxicity. All the non-cationic derivatives, even those soluble in aqueous solutions, were not cytotoxic. These results reaffirm that the presence of at least one cationic group is required for cytotoxicity and suggest that the bioreduction is not the predominant mode of action in the cytotoxicity of anthraquinones.

3. Experimental protocols

Materials: solvents were purchased from Biolab (Jerusalem, Israel) and Frutarom (Haifa, Israel). Chemical reagents were bought from Aldrich (Milwaukee, WI). Unless otherwise stated, the experiments were conducted at room temperature.

^{1}H NMR (300 MHz) and ^{13}C NMR spectra (75.429 MHz) were obtained on a Varian VXR-300S (Palo Alto, CA) spectrometer. Chemical shifts are reported as ppm using tetramethylsilane (TMS) as internal standard. Mass spectra were obtained with an LKB 2091 mass spectrometer using electron impact ionization (70 eV). The ion source was heated to 220 °C and the sample was inserted by direct inlet without external heating. FAB-MS were carried out by the Mass Spectra Laboratory, Faculty of Chemistry, Technion, Haifa. IR spectra were recorded on a Perkin Elmer FT-2000 model. Solid samples were pressed into KBr pellets or dispersed in Nujol onto NaCl plates. Melting points were observed on an Electrothermal apparatus (Electrothermal, England) and are uncorrected. Elemental analyses were performed by the Microanalytical Laboratory at the Hebrew University, Givat Ram, Jerusalem. Analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values.

3.1. Chemistry

3.1.1. 1-[(3-Aminopropyl)amino]-9,10-anthracenedione (**38**)

To a solution of 10 g (41 mmol) of 1-chloroanthraquinone in toluene (350 mL), 14 mL (0.17 mol) of 1,3-diaminopropane were added and the mixture was refluxed for 18 h to a deep red suspension. The reaction mixture was concentrated under vacuum and the residue partitioned between CH_2Cl_2 (400 mL) and water (150 mL). The organic layer was washed with water (100 mL) and then the product was extracted with aq. 1 M HCl (2 × 200 mL) to the aqueous layer. The organic layer was discarded. The acidic aqueous solution was made alkaline with 10% NaOH and the resulting suspension was extracted several times with CH_2Cl_2 (1 L), the organic layer was washed once with water, dried over anh. $MgSO_4$ and evaporated to dryness (yield: 7.2 g, 63%). An analytical sample was obtained by column chromatography either on Florisil® (eluent: MeOH:EtOAc 1:1) or neutral alumina grade I (eluent: MeOH: CH_2Cl_2 5:95), followed by recrystallization from EtOAc. M.p. 155–158 °C. IR (cm^{-1}, KBr) 3 327 (NH), 1 668 (quinone C=O). ^{1}H NMR (d_6-DMSO) δ 9.80 (t, 1H), 8.25 (dd, 2H), 7.90–8.02 (m, 2H), 7.74 (t, 1H), 7.52 (d, 1H), 7.37 (d, 1H), 3.45 (q, 2H), 2.78 (t, 2H), 1.84 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 187.9, 186.9, 155.5, 139.6, 138.5, 137.9, 137.4, 136.4, 130.4, 130.2, 122.7, 118.9, 115.9, 44.0, 43.0, 36.5. MS m/z (rel. intensity, %): 280 ([M]$^{\bullet+}$, 15), 238 ([M–C_2H_4N]$^{\bullet+}$, 100), 237 ([M–C_2H_5N]$^{\bullet+}$, 96). Anal $C_{17}H_{16}N_2O_2$ (C, H, N).

3.1.2. N-[3-(9,10-Dihydro-9,10-dioxo-1-anthracenylamino)propyl]-N′,N′-diphenylurea (**5**)

To a suspension of 0.6 g (2.1 mmol) of **38** in CH_2Cl_2 (40 mL), 0.8 g (3.5 mmol) of diphenylcarbamyl chloride were added and the mixture was stirred at room temperature for 3 h. The solution was diluted with CH_2Cl_2 (100 mL), washed with water (2 × 50 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: EtOAc:CH_2Cl_2 10:90) giving a red solid (yield: 0.5 g, 50%), which was recrystallized from EtOAc. M.p. 205–208 °C. IR (cm^{-1}, KBr) 3 315 (NH), 1 657 (C=O). ^{1}H NMR ($CDCl_3$) δ 9.72 (bt, 1H), 8.22–8.25 (m, 2H), 7.68–7.79 (m, 2H), 7.51–7.61 (m, 2H), 7.30–7.36 (m, 4H), 7.24–7.28 (m, 4H), 7.16–7.22 (m, 2H), 7.02–7.05 (m, 1H), 4.72 (t, 1H), 3.45 (q, 2H), 3.38 (q, 2H), 1.99 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 185.8, 184.6, 157.0, 152.3, 143.4 (2C), 136.0, 135.6, 135.3, 134.6, 133.6, 130.1 (4C), 128.0 (4C), 127.4, 127.3, 126.9 (2C), 118.4, 116.4, 113.9, 41.3, 39.5, 30.4. MS m/z (rel. intensity, %): 475 ([M]$^{\bullet+}$, 11), 307 ([M–$C_{12}H_{10}N$]$^{\bullet+}$, 97), 300 (100). Anal $C_{30}H_{25}N_3O_3$ (C, H, N).

3.1.3. 1-[3-(4′-Nitrobenzamido)propylamino]-9,10-anthracenedione (**6**)

To a solution of 0.67 g (4 mmol) of 4-nitrobenzoic acid in DMF (100 mL), triethylamine (1 mL), 0.58 g (5 mmol) of N-hydroxysuccinimide (NHS) and 0.96 g (5 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were added. The solution was stirred at room temperature for 1 h and then 0.56 g (2 mmol) of **38** were added and the mixture was stirred overnight. Then 0.38 g (2 mmol) of EDC were added and the mixture was stirred for a further 48 h. The mixture was filtered and the solid was washed with CH_2Cl_2 and discarded. The filtrate was successively washed with 0.5 M HCl (2 × 100 mL) and water (5 × 50 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: EtOAc: CH_2Cl_2 20:80) giving a red solid (yield: 0.65 g, 76%), which was recrystallized from acetone. M.p. 220–222 °C. IR (cm^{-1}, KBr) 3 280 (NH), 1 663 (quinone C=O), 1 625 (C=O). ^{1}H NMR (d_6-DMSO) δ 9.84 (t, 1H), 9.04 (t, 1H), 8.38 (d, 2H), 8.22 (m, 2H), 8.18 (d, 2H), 7.90–8.00 (m, 2H), 7.74 (t, 1H), 7.52 (d, 1H), 7.37 (d, 1H), 3.56 (q, 4H), 2.04 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 187.8, 186.7, 168.6, 155.2, 152.8, 144.1, 139.5, 138.3, 138.2, 137.9,

137.3, 136.2, 132.6 (2C), 130.3, 130.1, 127.3 (2C), 122.5, 118.9, 116.0, 44.0, 41.4, 32.3. MS m/z (rel. intensity, %): 429([M]$^{\bullet+}$, 70), 264 ([M–$C_7H_5N_2O_3$]$^{\bullet+}$, 59), 259 (100). Anal $C_{24}H_{19}N_3O_5$ (C, H, N).

3.1.4. 1-[(3-Benzamidopropyl)amino]-9,10-anthracenedione (**12**)

To a solution of 0.56 g (2 mmol) of **38** in Py (20 mL), 0.9 mL (8 mmol) of benzoyl chloride were added dropwise and the mixture was stirred for 18 h at room temperature. The mixture was then partitioned between CH_2Cl_2 (100 mL) and 1 M HCl (50 mL). The organic layer was successively washed with 1 M HCl (4 × 50 mL), 5% $NaHCO_3$ (50 mL) and water, dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on Florisil® (eluent: EtOAc:CH_2Cl_2 1:1) giving a red solid (yield: 0.4 g, 52%), which was recrystallized from EtOAc. M.p. 226–229 °C. IR (cm^{-1}, KBr) 3 306 (NH), 2 920 (CH_2), 2 853 (CH_2), 1 668 (quinone C=O), 1 627 (C=O). ^{1}H NMR ($CDCl_3$) δ 9.84 (bt, 1H), 8.22–8.26 (m, 2H), 7.75 (d, 2H), 7.70–7.79 (m, 2H), 7.53–7.62 (m, 2H), 7.36–7.46 (m, 3H), 7.08 (d, 1H), 6.36 (bt, 1H), 3.68 (q, 2H), 3.49 (q, 2H), 2.12 (m, 2H). MS m/z (rel. intensity, %): 384([M]$^{\bullet+}$, 78), 264 ([M–C_7H_6NO]$^{\bullet+}$, 71), 236 ([M–$C_9H_{10}NO$]$^{\bullet+}$, 100). Anal $C_{24}H_{20}N_2O_3$ (C, H, N).

3.1.5. 2′,2′,2′-Trichloroethyl 3-[(9,10-dihydro-9,10-dioxo-1-anthracenyl)amino]propyl carbamate (**14**)

To a solution of 0.42 g (1.5 mmol) of **38** and triethylamine (0.3 mL) in CH_2Cl_2 (50 mL), 0.28 mL (2 mmol) of 2,2,2-trichloroethyl chloroformate were added, and the solution was stirred at room temperature for 90 min. The mixture was washed with water (2 × 50 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: CH_2Cl_2) giving a red solid (yield: 0.43 g, 63%), which was recrystallized from EtOAc. M.p. 140–142 °C. IR (cm^{-1}, KBr) 3 348 (NH), 2 949 (CH_2), 2 863 (CH_2), 1 738 (C=O), 1 666 (quinone C=O). ^{1}H NMR ($CDCl_3$) δ 9.75 (bt, 1H), 8.22–8.25 (m, 2H), 7.68–7.79 (m, 2H), 7.51–7.61 (m, 2H), 7.02–7.05 (d, 1H), 5.22 (bt, 1H), 4.75 (s, 2H), 3.40–3.50 (m, 4H), 2.03 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 185.2, 183.8, 154.7, 151.5, 135.4, 134.9, 134.8, 134.0, 133.0, 126.7 (2C), 117.6, 115.9, 113.3, 95.6, 74.6, 40.2, 39.1, 29.4. MS m/z (rel. intensity, %): 454 ([M]$^{\bullet+}$, 9), 307 ([M–$C_2H_2Cl_3O$]$^{\bullet+}$, 38), 301 (100). Anal $C_{20}H_{17}Cl_3N_2O_4$ (C, H, Cl, N).

3.1.6. 1-[(3-Hydroxypropyl)amino]-9,10-anthracenedione (**39**)

To a solution of 5 g (21 mmol) of 1-chloroanthraquinone in n-butanol (100 mL), 6.3 g (85 mmol) of 3-aminopropanol were added and the solution was heated to reflux for 4 h. The solution was evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: EtOAc:CH_2Cl_2 10:90) giving a red solid (yield: 2.5 g, 43%), which was recrystallized from EtOAc. M.p. 185–188 °C. IR (cm^{-1}, KBr) 3 300 (OH), 2 920 (CH_2), 2 868 (CH_2), 1 664 (quinone C=O). ^{1}H NMR($CDCl_3$) δ 9.78 (bt, 1H), 8.22–8.26 (m, 2H), 7.69–7.76 (m, 2H), 7.51–7.58 (m, 2H), 7.10 (d, 1H), 3.88 (t, 2H), 3.49 (q, 2H), 2.02 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 184.6, 183.8, 151.8, 135.3, 135.0, 134.7, 133.9, 133.0, 132.9, 126.7 (2C), 117.8, 115.7, 113.1, 60.4, 39.8, 31.9. MS m/z (rel. intensity, %): 281([M]$^{\bullet+}$, 36), 236 ([M–C_2H_5O]$^{\bullet+}$, 100). Anal $C_{17}H_{15}NO_3$ (C, H, N).

3.1.7. 1-[3-(Triphenylmethoxy)propylamino]-9,10-anthracenedione (**1**)

A solution of 0.56 g (2 mmol) of **39** and 0.67 g (2.4 mmol) of chlorotriphenylmethane in Py (30 mL) was heated to 80 °C for 8 h. The mixture was then partitioned between CH_2Cl_2 (100 mL) and 1 M HCl (50 mL). The organic layer was successively washed with 1 M HCl (4 × 30 mL), 5% $NaHCO_3$ (30 mL) and water, dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: petroleum ether 40–60 °C:CH_2Cl_2 80:20) giving a red solid (yield: 0.68 g, 65%), which was recrystallized from acetone. M.p. 180–183 °C. IR (cm^{-1}, KBr) 3 059 (arom. CH), 2 918 (CH_2), 2 872 (CH_2), 1 667 (quinone C=O). ^{1}H NMR ($CDCl_3$) δ 9.72 (bt, 1H), 8.22–8.25 (m, 2H), 7.72–7.78 (dt, 1H), 7.66–7.71 (dt, 1H), 7.56–7.59 (dd, 1H), 7.47–7.53 (t, 1H), 7.42–7.46 (m, 6H), 7.24–7.29 (m, 6H), 7.17–7.22 (m, 3H), 7.06–7.09 (dd, 1H), 3.48 (q, 2H), 3.26 (t, 2H), 2.03 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 184.6, 183.8, 151.8, 144.2 (3C), 135.2, 135.1, 134.7, 133.9, 133.1, 132.8, 128.7 (6C), 127.8 (6C), 126.9 (3C), 126.7, 126.6, 117.9, 115.6, 113.0, 86.4, 60.8, 40.1, 29.9. MS m/z (rel. intensity, %): 523([M]$^{\bullet+}$, 5), 280 ([M–$C_{19}H_{15}$]$^{\bullet+}$, 100), 274 ([$C_{20}H_{18}O$]$^{\bullet+}$, 100). Anal $C_{36}H_{29}NO_3$ (C, H, N).

3.1.8. 1-[3-[(Methylsulfonyl)oxy]propylamino]-9,10-anthracenedione (**41**)

To a solution of 0.8 g (2.8 mmol) of **39** in CH_2Cl_2 (50 mL) and Py (1.2 mL), 0.5 mL (6.5 mmol) of methanesulfonyl chloride were added and the solution was stirred for 48 h. The mixture was washed with 1 M HCl (2 × 50 mL) and water (50 mL), dried over anh. $MgSO_4$ and evaporated to a red solid (yield: 1 g, 100%). M.p. 125–128 °C. IR (cm^{-1}, KBr) 2 921 (CH_2), 2 851 (CH_2), 1 665 (quinone C=O), 1 197 (S=O). ^{1}H NMR ($CDCl_3$) δ 8.22–8.28 (m, 2H), 7.69–7.80 (m, 2H), 7.55–7.65 (m,

2H), 7.08 (d, 1H), 4.43 (t, 2H), 3.55 (t, 2H), 3.08 (s, 3H), 2.18–2.26 (m, 2H). MS m/z (rel. intensity, %): 359([M]$^{\bullet+}$, 16), 236 ([M–$C_3H_7O_3S$]$^{\bullet+}$, 100).

3.1.9. 1-[3-(4′-Nitro-1H-imidazol-1′-yl)propylamino]-9,10-anthracenedione (**11**)

To a solution of 0.8 g (2.2 mmol) of **41** in CH_2Cl_2 (40 mL) and DMF (20 mL), 0.75 mL (5 mmol) of DBU and 0.5 g (4.4 mmol) of 4-nitroimidazole were added and the mixture was refluxed for 12 h. The mixture was then partitioned between CH_2Cl_2 (100 mL) and water (50 mL). The organic layer was washed with water (5 × 30 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: EtOAc) giving a red solid (yield: 0.6 g, 72%), which was recrystallized from EtOAc. M.p. 201–203 °C. IR (cm^{-1}, KBr) 1 656 (quinone C=O). ^{1}H NMR (d_6-DMSO) δ 9.72 (t, 1H), 8.61 (s, 1H), 8.26 (dd, 2H), 8.01 (s, 1H), 7.92–8.04 (m, 2H), 7.75 (t, 1H), 7.54 (d, 1H), 7.35 (d, 1H), 4.33 (t, 2H), 3.50 (q, 2H), 2.32 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 188.0, 186.8, 155.0, 141.3, 139.6, 138.4, 138.3, 137.9, 137.4, 136.3, 130.3, 130.2, 125.5, 122.5, 119.1, 116.2, 49.5, 43.4, 33.2. MS m/z (rel. intensity, %): 376([M]$^{\bullet+}$, 20), 236 ([M–$C_5H_6N_3O_2$]$^{\bullet+}$, 61), 135 (100). Anal $C_{20}H_{16}N_4O_4$ (C, H, N).

3.1.10. 1-[(3-Oxopropyl)amino]-9,10-anthracenedione (**13**)

A finely-ground mixture of 0.86 g (4 mmol) of pyridinium chlorochromate and 0.86 g of silica gel was suspended in CH_2Cl_2 (30 mL). 0.56 g (2 mmol) of **39** were added and the suspension was kept in an ultrasound bath for 90 min. The mixture was filtered through a short column of Celite and silica gel and eluted with CH_2Cl_2 giving a red solid (yield: 0.23 g, 41%), which was recrystallized from EtOAc. M.p. 165–168 °C. IR (cm^{-1}, KBr) 1 718 (C=O), 1 663 (quinone C=O). ^{1}H NMR ($CDCl_3$) δ 9.90 (t, 1H, J = 1Hz), 9.78 (bt, 1H), 8.22–8.25 (m, 2H), 7.68–7.79 (m, 2H), 7.56–7.64 (m, 2H), 7.08 (d, 1H), 3.71 (q, 2H, J = 6Hz), 2.95 (dt, 2H, J = 6Hz, J = 1Hz). MS m/z (rel. intensity, %): 279([M]$^{\bullet+}$, 7), 236 ([M–C_2H_3O]$^{\bullet+}$, 22), 223 ([M–C_3H_4O]$^{\bullet+}$, 100). Anal $C_{17}H_{13}NO_3$ (C, H, N).

3.1.11. 1-[(3-Carboxypropyl)amino]-9,10-anthracenedione (**40**)

A suspension of 4.8 g (20 mmol) of 1-chloroanthraquinone in DMSO (100 mL) and triethylamine (8 mL) was heated to solution and 4.1 g (40 mmol) of 4-aminobutyric acid were added. The mixture was heated to 150 °C for 90 min. After cooling it was partitioned between CH_2Cl_2 (300 mL) and aq. 0.1 M HCl (250 mL). The organic layer was washed with water (4 × 100 mL) to extract DMSO and then the product was extracted with aq. 0.1 M NaOH (2 × 200 mL) to the aqueous layer. The organic layer was discarded. The basic aqueous solution was acidified with 1 M HCl and the resulting suspension was filtered through sintered glass. The red solid was dried in an oven (yield: 1.4 g, 23%). An analytical sample was obtained by column chromatography on silica gel (eluent: EtOAc:CH_2Cl_2 30:70), followed by recrystallization from EtOAc. M.p. 214–215 °C. ^{1}H NMR (d_6-DMSO) δ 9.82 (bt, 1H), 8.27 (dd, 2H), 7.94–8.01 (m, 2H), 7.76 (t, 1H), 7.54 (d, 1H), 7.40 (d, 1H), 3.52 (q, 2H), 2.48 (t, 2H), 1.99 (m, 2H). ^{13}C NMR (d_6-DMSO) δ 188.0, 186.8, 178.0, 155.3, 139.6, 138.4, 138.3, 137.9, 137.4, 136.3, 130.4, 130.2, 122.5, 119.0, 116.0, 45.4, 35.0, 28.1. MS m/z (rel. intensity, %): 309 ([M]$^{\bullet+}$, 82), 291 ([M–H_2O]$^{\bullet+}$, 100), 264 ([M–CHO_2]$^{\bullet+}$, 30), 236 ([M–$C_3H_5O_2$]$^{\bullet+}$, 87).

3.1.12. 4-[(9,10-Dihydro-9,10-dioxo-1-anthracenyl)amino]-N-(5′-nitrothiazol-2′-yl) butanamide (**10**)

To a solution of 0.46 g (1.5 mmol) of **40** in DMF (15 mL), triethylamine (0.5 mL), 0.34 g (3 mmol) of NHS and 0.38 g (2 mmol) of EDC were added. The solution was stirred at room temperature for 3 h and then 0.43 g (3 mmol) of 2-amino-5-nitrothiazole were added and the mixture was stirred for 18 h. Then 0.38 g (2 mmol) of EDC were added and the mixture was stirred for further 48 h. The mixture was partitioned between EtOAc (150 mL) and water (100 mL). The organic layer was successively washed with 0.5 M HCl (2 × 50 mL) and water (4 × 50 mL), dried over anh. $MgSO_4$ and evaporated to a red solid (yield: 0.6 g, 90%), which was recrystallized from dioxane-methanol. M.p. 245–247 °C. IR (cm^{-1}, KBr) 3 225 (NH), 1 661 (quinone C=O) 1 622 (C=O). ^{1}H NMR (d_7-DMF) δ 9.80 (t, 1H), 8.42 (s, 1H), 8.10–8.20 (m, 2H), 7.80–7.94 (m, 2H), 7.67 (t, 1H), 7.48 (d, 1H), 7.36 (d, 1H), 3.56 (q, 2H), 2.84 (t, 2H), 2.18 (m, 2H). ^{13}C NMR (d_7-DMF) δ 185.0, 183.7, 173.6, 162.9, 152.4, 142.8, 136.2, 135.3, 135.1, 134.9, 134.0, 133.4, 127.1, 126.8, 119.2, 115.8, 113.4, 42.5, 33.6, 24.6. MS m/z (rel. intensity, %): 436([M]$^{\bullet+}$, 16), 292 ([M–$C_3H_2N_3O_2S$]$^{\bullet+}$, 25), 232 (100). Anal $C_{21}H_{16}N_4O_5S$ (C, H, N, S).

3.1.13. 1-[3-[(2′,2′,2′-Trichloroethoxy)carbonyl]propylamino]-9,10-anthracenedione (**15**)

To a suspension of 0.31 g (1 mmol) of **40** in CH_2Cl_2 (10 mL) containing Py (0.15 mL) and 0.2 mL (2 mmol) of 2,2,2-trichloroethanol, were added 0.2 g (1mmol) of DCC and the mixture was stirred for 3 h at room temperature. The suspension was filtered to remove DCU

and washed with CH_2Cl_2 (50 mL). The combined filtrates were washed successively with 1 M HCl (30 mL), 5% $NaHCO_3$ (20 mL) and water, dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: petroleum ether 40–60 °C:CH_2Cl_2 50:50) giving a red solid (yield: 0.23 g, 52%), which was recrystallized from EtOAc. M.p. 106–109 °C. IR (cm^{-1}, KBr) 2 954 (CH_2), 2 864 (CH_2), 1 754 (C=O), 1 667 (quinone C=O). ^1H NMR ($CDCl_3$) δ 9.80 (bt, 1H), 8.24–8.28 (m, 2H), 7.68–7.79 (m, 2H), 7.54–7.62 (m, 2H), 7.06–7.12 (d, 1H), 4.78 (s, 2H), 3.48 (q, 2H), 2.68 (t, 2H), 2.16 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 185.2, 183.7, 171.3, 151.6, 135.4, 135.0, 134.8, 134.0, 133.0, 126.8, 126.7, 117.7, 115.9, 113.3, 94.9, 74.1, 41.9, 31.3, 24.2. MS m/z (rel. intensity, %): 439 ($[M]^{\bullet+}$, 7), 292 ($[M-C_2H_2Cl_3O]^{\bullet+}$, 10), 236 ($[M-C_5H_6Cl_3O_2]^{\bullet+}$, 100). Anal $C_{20}H_{16}Cl_3NO_4$ (C, H, Cl, N).

3.1.14. 1-[2-(2′-Pyridinyl)ethylamino]-9,10-anthracenedione (**24**)

A suspension of 2.4 g (10 mmol) of 1-chloroanthraquinone in DMSO (50 mL) was heated to dissolution and then 6 mL (50 mmol) of 2-(2-aminoethyl)pyridine were added. The mixture was heated to 150 °C for 10 min and, after the addition of cold water (150 mL), the product was collected by filtration. The red solid was purified by flash chromatography on silica gel (eluent: EtOAc:CH_2Cl_2 20:80) giving a red solid (yield: 2 g, 60%), which was recrystallized from methanol. M.p. 122–124 °C. ^1H NMR (d_6-DMSO) δ 9.75 (bt, 1H), 8.56 (d, 1H), 8.15 (d, 2H), 7.86 (m, 2H), 7.74 (t, 1H), 7.67 (t, 1H), 7.45 (d, 1H), 7.39 (d, 1H), 7.34 (d, 1H), 7.26 (dd, 1H), 3.80 (q, 2H), 3.15 (t, 2H). MS m/z (rel. intensity, %) 328 ($[M]^{\bullet+}$, 22), 236 ($[M-C_6H_6N]^{\bullet+}$, 00). Anal $C_{21}H_{16}N_2O_2$ (C, H, N).

3.1.15. (Pyridinylmethylamino)-9,10-anthracenedione derivatives (**16–19**)

The above compounds were prepared according to the following general method: a suspension of 3 g (10.8 mmol) of 1,5-dichloroanthraquinone in 20 mL of an appropriate (aminomethyl)pyridine was heated to 40–50 °C for 36 h. Then the mixture was poured into water (100 mL) and the precipitate was collected by filtration, dissolved in CH_2Cl_2 and dried over anh. $MgSO_4$. Upon removal of the solvent the products were isolated by column chromatography on silica gel (gradient elution from CH_2Cl_2 to CH_2Cl_2:MeOH 95:5) and recrystallized from chloroform-methanol (1:1).

3.1.15.1. 1-Chloro-5-[(3′-pyridinyl)methylamino]-9,10-anthracenedione (**16**)

Yield: 52%. M.p. 192 °C. ^1H NMR (d_6-DMSO) δ 9.95 (t, 1H, NH-AQ), 8.65 (s, 1H, H-2′), 8.5 (d, 1H, H-6′), 8.25 (d, 1H, H-8), 7.9 (m, 2H, H-6,7), 7.8 (d, 1H, H-4), 7.65 (t, 1H, H-3), 7.4 (dd, 2H, H-4′,5′), 7.2 (d, 1H, H-2), 4.7 (d, 2H, NH–CH_2). MS m/z (rel. intensity, %) 348 ($[M]^{\bullet+}$, 81), 108 (100). Anal $C_{20}H_{13}ClN_2O_2$ (C, H, Cl, N).

3.1.15.2. 1,5-Bis[(3′-pyridinyl)methylamino]-9,10-anthracenedione (**17**)

Yield: 25%. M.p. 225 °C. ^1H NMR (d_6-DMSO) δ 10.0 (t, 2H, NH-AQ), 8.65 (s, 2H, H-2′), 8.5 (d, 2H, H-6′), 7.8 (d, 2H, H-4,8), 7.6 (t, 2H, H-3,7), 7.5 (d, 2H, H-4′), 7.4 (dd, 2H, H-5′), 7.15 (d, 2H, H-2,6), 4.7 (d, 4H, NH–CH_2). MS m/z (rel. intensity, %): 420 ($[M]^{\bullet+}$, 58), 327 (100). Anal $C_{26}H_{20}N_4O_2$ (C, H, N).

3.1.15.3. 1-Chloro-5-[(2′-pyridinyl)methylamino]-9,10-anthracenedione (**18**)

Yield: 53%. M.p. 172 °C. ^1H NMR (d_6-DMSO) δ 10.2 (t, 1H, NH-AQ), 8.6 (d, 1H, H-6′), 8.25 (d, 1H, H-8), 7.86 (m, 2H, H-6,7), 7.8 (t, 1H, H-5′), 7.63 (t, 1H, H-3), 7.42 (d, 1H, H-4), 7.37 (d, 1H, H-3′), 7.33 (dd, 1H, H-4′), 7.2 (d, 1H, H-2), 4.75 (d, 2H, NH–CH_2). MS m/z (rel. intensity, %): 348 ($[M]^{\bullet+}$, 100), 107 (89). Anal $C_{20}H_{13}ClN_2O_2$ (C, H, Cl, N).

3.1.15.4. 1,5-Bis[(2′-pyridinyl)methylamino]-9,10-anthracenedione (**19**)

Yield: 30%. M.p. 223–224 °C. ^1H NMR (d_6-DMSO) δ 10.3 (t, 2H, NH-AQ), 8.62 (d, 2H, H-6′), 7.8 (t, 2H, H-5′), 7.63 (t, 2H, H-3,7), 7.5 (d, 2H, H-4.8), 7.43 (d, 2H, H-3′), 7.3 (dd, 2H, H-4′), 7.15 (d, 2H, H-2,6), 4.7 (d, 4H, NH–CH_2). MS m/z (rel. intensity,%): 420 ($[M]^{\bullet+}$, 100), 328 (49).

3.1.16. 1-Chloro-5-[2-(2′-pyridinyl)ethylamino]-9,10-anthracenedione (**25**) *and 1,5-Bis[2-(2′-pyridinyl) ethylamino]-9,10-anthracenedione* (**26**)

A suspension of 2.7 g (10 mmol) of 1,5-dichloroanthraquinone in DMSO (50 mL) was heated to dissolution and then 12 mL (100 mmol) of 2-(2-aminoethyl)pyridine were added. The mixture was heated to 150 °C for 10 min and, after the addition of cold water (150 mL), the product was collected by filtration. The residue was purified by flash chromatography on silica gel (first eluent: EtOAc:CH_2Cl_2 3:7, second eluent: EtOAc) giving two red products: **25** (yield: 0.8 g, 22%) and **26** (yield: 1.4 g, 35%), which were recrystallized from methanol. **25**: M.p. 167–171 °C. ^1H NMR (d_6-DMSO) δ 9.60 (bt, 1H), 8.55 (d, 1H), 8.20 (d, 1H), 7.87 (d, 1H), 7.82 (t, 1H), 7.74 (t, 1H), 7.67 (t, 1H), 7.39 (d,

1H), 7.37 (d, 1H), 7.32 (d, 1H), 7.26 (dd, 1H), 3.78 (q, 2H), 3.15 (t, 2H). MS m/z (rel. intensity, %) 362 ($[M]^{\bullet+}$, 40), 270 ($[M-C_6H_6N]^{\bullet+}$, 100). Anal $C_{21}H_{15}ClN_2O_2$ (C, H, Cl, N). **26**: M.p. 148–152 °C. Anal $C_{28}H_{24}N_4O_2$ (C, H, N).

3.1.17. 1-[2-(Dimethylamino)ethylamino]-5-[2′-(pyridinyl)methylamino]-9,10-anthracenedione (**30**)

A solution of **18** (600 mg, 1.72 mmol) in N, N-dimethylethylenediamine (15 mL) was heated at 55 °C for 72 h. Then, cold water was added and the resulting solid was filtered and dissolved in CH_2Cl_2. The solution was washed with water (to extract the free amine) and dried over anh. $MgSO_4$. After removal of the solvent under reduced pressure the crude material was subjected to column chromatography on silica gel (gradient elution from CH_2Cl_2 to (95:5) CH_2Cl_2:MeOH) and recrystallized from $CHCl_3$:MeOH (1:1) (yield: 495 mg, 72%). M.p. 162 °C. ^1H NMR (d_6-DMSO) δ 10.3 (s, 1H, $NHCH_2CH_2$), 9.78 (s, 1H, NH–CH_2), 8.63 (d, 1H, H-6′), 7.82 (t, 1H, H-5′), 7.64 (m, 2H, H-3,7), 7.46 (m, 3H, H-4,8, H-3′), 7.34 (dd, 1H, H-4′), 7.18 (d, 1H, H-7), 7.13 (d, 1H, H-2), 4.75 (d, 2H, $NHCH_2$), 3.3 (q, 2H, $NHCH_2CH_2$), 2.6 (t, 2H, $NHCH_2CH_2$), 2.22 (s, 6H, N–CH_3). MS m/z (rel. intensity, %): 400 ($[M]^{\bullet+}$, 3), 354 (100). Anal $C_{24}H_{24}N_4O_2$ (C, H, N).

3.1.18. 1,8-Bis[2-(2′-pyridinyl)ethylamino]-9,10-anthracenedione (**28**)

A suspension of 2.7 g (10 mmol) of 1,8-dichloroanthraquinone in DMSO (50 mL) was heated to dissolution and then 12 mL (100 mmol) of 2-(2-aminoethyl)pyridine were added. The mixture was heated to 150 °C for 10 min and, after the addition of cold water (150 mL), the product was collected by filtration. The residue was purified by flash chromatography on silica gel (eluent: EtOAc:CH_2Cl_2 20:80) giving a violet solid (yield: 1.3 g, 30%), which was recrystalized from methanol. M.p. 182–185 °C. ^1H NMR (d_6-DMSO) δ 9.62 (bt, 2H), 8.56 (d, 2H), 7.74 (t, 2H), 7.58 (t, 2H), 7.38 (m, 4H), 7.27 (m, 4H), 3.72 (q, 4H), 3.15 (t, 4H). Anal $C_{28}H_{24}N_4O_2$ (C, H, N).

3.1.19. 1,4-Bis[(3-hydroxypropyl)amino]-9,10-anthracenedione (**42**)

A suspension of 2 g (8 mmol) of leucoquinizarin in MeOH (20 mL) was de-gassed by stirring at 5 °C under N_2 and then 6.3 mL (82 mmol) of 3-aminopropanol were added dropwise. The mixture was heated to 50 °C for 1 h, cooled and then aerated overnight. The mixture was evaporated and the residue was purified by chromatography on Florisil® (eluent: MeOH:EtOAc 5:95) giving a blue solid (yield: 1 g, 35%), which was recrystallized from EtOAc. M.p. 161–162 °C. IR (cm^{-1}, KBr) 3 300 (OH), 2 934 (CH_2), 2 862 (CH_2), 1 645 (quinone C=O). ^1H NMR ($CDCl_3$) δ 10.83 (bt, 2H), 8.30–8.34 (m, 2H), 7.66–7.70 (m, 2H), 7.24 (s, 2H), 3.86–3.91 (q, 4H), 3.54–3.60 (q, 4H), 1.98–2.06 (m, 4H).

3.1.20. 1,4-Bis[3-(triphenylmethoxy)propylamino]-9,10-anthracenedione (**4**) *and 1-[(3-Hydroxypropyl)amino]-4-[3-(triphenylmethoxy)propylamino]-9,10-anthracenedione* (**2**)

A solution of 0.41 g (1.1 mmol) of **42** and 0.56 g (2 mmol) of chlorotriphenylmethane in Py (15 mL) was heated to 80 °C for 6 h. The mixture was then partitioned between CH_2Cl_2 (100 mL) and 1 M HCl (50 mL). The organic layer was successively washed with 1 M HCl (4 × 50 mL), 5% $NaHCO_3$ (50 mL) and water, dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on Florisil® (eluent: CH_2Cl_2) giving two blue solids **4** (yield: 0.3 g, 32%) and **2** (yield: 0.4 g, 61%), which were recrystallized from MeOH. **4**: M.p. 114–118 °C. IR (cm^{-1}, KBr) 3 060 (arom. CH), 2 922 (CH_2), 2 850 (CH_2), 1 644 (quinone C=O). ^1H NMR ($CDCl_3$) δ 10.78 (bt, 2H), 8.30–8.34 (m, 2H), 7.68–7.72 (m, 2H), 7.40–7.44 (d, 12H), 7.17–7.30 (m, 20H), 3.56 (q, 4H), 3.27 (t, 4H), 2.03 (m, 4H). Anal $C_{58}H_{50}N_2O_4$ (C, H, N). **2**: M.p. 152–156 °C. IR (cm^{-1}, KBr) 3 500 (OH), 3 059 (arom. CH), 2 924 (CH_2), 2 854 (CH_2), 1 643 (quinone C=O). ^1H NMR ($CDCl_3$) δ 10.84 (bt, 1H), 10.75 (bt, 1H), 8.29–8.35 (m, 2H), 7.66–7.70 (m, 2H), 7.40–7.44 (m, 6H), 7.25–7.30 (m, 6H), 7.17–7.24 (m, 5H), 3.87 (q, 2H), 3.56 (q, 4H), 3.26 (t, 2H), 1.99–2.04 (m, 4H). Anal $C_{39}H_{36}N_2O_4$ (C, H, N).

3.1.21. 1,4-Bis[2-(2′-pyridinyl)ethylamino]-9,10-anthracenedione (**27**)

A suspension of 1 g (4 mmol) of leucoquinizarin in MeOH (20 mL) was de-gassed by stirring at 5 °C under N_2 and then 4.8 mL (40 mmol) of 2-(2-aminoethyl)pyridine were added dropwise. The mixture was heated to 50 °C for 1 h, cooled and then aerated overnight. The mixture was evaporated and the residue was purified by flash chromatography on silica gel (eluent: MeOH:EtOAc 10:90) giving a blue solid (yield: 0.55 g, 30%), which was recrystallized from methanol. M.p. 115–117 °C. ^1H NMR (d_6-DMSO) δ 9.82 (bt, 2H), 8.55 (d, 2H), 8.19 (d, 2H), 7.77 (m, 2H), 7.72 (dd, 2H), 7.54 (s, 2H), 7.38 (d, 2H), 7.25 (dd, 2H), 3.88 (q, 4H), 3.15 (t, 4H). MS m/z (rel. intensity, %) 448($[M]^{\bullet+}$), 263. Anal $C_{28}H_{24}N_4O_2$ (C, H, N).

3.1.22. 1,4-Bis[(3-aminopropyl)amino]-9,10-anthracenedione.2HCl (**36**)

A suspension of 10.5 g (42 mmol) of leucoquinizarin in MeOH (100 mL) was de-gassed by stirring at 5 °C under N_2 and then 32 mL (0.42 mol) of 1,3-diaminopropane were added dropwise. The mixture was heated to 50 °C for 1 h, cooled and then aerated overnight. After evaporation the residue was dissolved in CH_2Cl_2 (100 mL) and 17.9 g (82 mmol) of di-*tert*-butyl dicarbonate in CH_2Cl_2 (20 mL) were added dropwise. The mixture was stirred for 1 h, then evaporated and the residue was purified by chromatography on Florisil® (eluent: EtOAc:CH_2Cl_2 10:90) giving a blue solid. The solid was dissolved in MeOH (90 mL) containing conc. HCl (10 mL), refluxed for 1 h and evaporated giving a blue solid (yield: 4 g, 22%), which was recrystallized from methanol. M.p. 88–92 °C. IR (cm^{-1}, KBr) 2 926 (NH_3^+), 1 642 (quinone C=O) 1 594 (NH_3^+). ^1H NMR (CD_3OD) δ 8.48–8.54 (m, 2H), 7.96–8.00 (m, 2H), 7.72 (s, 2H), 3.82 (t, 4H), 3.32 (t, 4H), 2.22 (m, 4H).

3.1.23. 1,4-Bis[2-(dimethylamino)ethylamino]-9,10-anthracenedione (**37**)

Compound **37** was prepared according to the procedure described in the literature [6] (yield: 70%). M.p. 172–173 °C. ^1H NMR ($CDCl_3$) δ 10.77 (s, 2H, $NHCH_2CH_2$), 8.38 (d, 2H, **H**-5,8), 7.7 (m, 2H, **H**-6,7), 7.26 (s, 2H, **H**-2,3), 3.50 (q, 4H, $NHCH_2CH_2$), 2.70 (t, 4H, $NHCH_2CH_2$), 2.40 (s, 12H, $N-CH_3$).

3.1.24. 1-[(3-Aminopropyl)amino]-4-[[2-(dimethylamino)ethyl]amino]-9,10-anthracenedione (**43**)

A suspension of 3 g (12 mmol) of leucoquinizarin in MeOH (250 mL) was de-gassed by stirring at 5 °C under N_2 and then 4.9 mL (44 mmol) of N,N-dimethylethylenediamine in MeOH (10 mL) were added dropwise. The de-gassed mixture was heated to 50 °C for 1 h, cooled and 2 mL (25 mmol) of 1,3-diaminopropane were added dropwise. The mixture was heated again to 50 °C for 2 h, cooled and then aerated overnight. After evaporation the residue was dissolved in MeOH (100 mL) and 4 g (18 mmol) of di-*tert*-butyl dicarbonate in MeOH (10 mL) were added dropwise. The mixture was stirred for 8 h, then evaporated and the residue was purified by chromatography on Florisil® (eluent: MeOH:EtOAc 10:90) giving a blue solid. The solid was dissolved in MeOH (90 mL) containing conc. HCl (10 mL) and the mixture was refluxed for 1 h, evaporated and dissolved in water (150 mL). The aqueous solution was washed with CH_2Cl_2 (50 mL) to eliminate apolar impurities and then basified with 5% NaOH and extracted with EtOAc (4 × 150 mL). The organic layer was washed with water (100 mL), dried over anh. $MgSO_4$ and evaporated to a blue solid (yield: 2.5 g, 51%), which was recrystallized from EtOAc. M.p. 108–112 °C. IR (cm^{-1}, KBr) 3 380 (NH), 2 940 (CH_2), 2 860 (CH_2), 2 819 (CH_3), 2 770 (CH_3), 1 645 (quinone C=O). ^1H NMR (d_6-DMSO) δ 10.84 (m, 2H), 8.22–8.26 (m, 2H), 7.77–7.80 (m, 2H), 7.51 (s, 2H), 3.51 (q, 4H), 2.71 (t, 2H), 2.54 (t, 2H), 2.24 (s, 6H), 1.73–1.79 (m, 2H).

3.1.25. 1-[2-(Dimethylamino)ethylamino]-4-[3-(4′-nitrobenzamido)propylamino]-9,10-anthracenedione (**9**)

To a solution of 0.84 g (5 mmol) of 4-nitrobenzoic acid in DMF (30 mL), triethylamine (2 mL), 0.69 g (6 mmol) of NHS and 1.9 g (10 mmol) of EDC were added. The solution was stirred at room temperature for 18 h, then 0.73 g (2 mmol) of **43** in DMF (10 mL) were added and the mixture was stirred for 24 h. Then 0.38 g (2 mmol) of EDC were added and the mixture was stirred for a further 48 h. The mixture was partitioned between CH_2Cl_2 (150 mL) and water (100 mL). The organic layer was successively washed with water (5 × 50 mL) and 5% $NaHCO_3$ (50 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: MeOH:EtOAc 10:90) giving a blue solid (yield: 0.65 g, 65%), which was recrystallized from EtOAc. M.p. 218–221 °C. IR (cm^{-1}, KBr) 3 280 (NH), 2 920 (CH_2), 2 855 (CH_2), 2 817 (CH_3), 2 767 (CH_3), 1 640 (quinone C=O). ^1H NMR ($CDCl_3$) δ 10.72 (bt, 1H), 10.68 (bt, 1H), 8.22–8.26 (m, 1H), 8.10–8.14 (m, 1H), 8.04 (d, 2H), 7.78 (d, 2H), 7.58–7.64 (m, 2H), 7.16 (s, 2H), 6.64 (bt, 1H), 3.66 (q, 2H), 3.52 (q, 2H), 3.44 (q, 2H), 2.62 (t, 2H), 2.28 (s, 6H), 2.24–2.32 (m, 2H). Anal $C_{28}H_{29}N_5O_5$ (C, H, N).

3.1.26. 1-[2-(Dimethylamino)ethylamino]-4-[3-(hydroxypropyl)amino]-9,10-anthracenedione (**44**)

A suspension of 3 g (12 mmol) of leucoquinizarin in MeOH (200 mL) was de-gassed by stirring at 5 °C under N_2 and then 4.9 mL (44 mmol) of N,N-dimethylethylenediamine in MeOH (10 mL) were added dropwise. The de-gassed mixture was heated to 50 °C for 45 min, cooled and 2 mL (25 mmol) of 3-aminopropanol were added dropwise. The mixture was heated again to 50 °C for 2 h, cooled and then aerated overnight. After evaporation the residue was purified by chromatography on Florisil® (eluent: MeOH:EtOAc 5:95) giving a blue solid (yield: 2 g, 45%), which was recrystallized from EtOAc. M.p. 149–150 °C. IR (cm^{-1}, KBr) 3 400 (OH), 2 943 (CH_2), 2 862 (CH_2), 2 821 (CH_3), 2 770 (CH_3), 1 643 (quinone C=O). ^1H NMR (d_6-DMSO) δ 10.88 (bt, 1H), 10.84 (bt, 1H), 8.23–8.27 (m, 2H), 7.78–7.81 (m, 2H), 7.50 (s, 2H), 4.69 (t, 1H), 3.58 (q, 2H), 3.54 (q, 4H),

2.57 (t, 2H), 2.25 (s, 6H), 1.80–1.84 (m, 2H). MS m/z (rel. intensity, %): 367 ($[M]^{\bullet+}$, 73), 309 ($[M-C_3H_6O]^{\bullet+}$, 100), 238 ($[M-C_7H_{15}NO]^{\bullet+}$, 25).

3.1.27. 1-[2-(Dimethylamino)ethylamino]-4-[3-(triphenylmethoxy)propylamino]-9,10-anthracenedione (**3**)

A solution of 0.41 g (1.1 mmol) of **44** and 0.72 g (2.6 mmol) of chlorotriphenylmethane in Py (20 mL) was heated to 80 °C for 3 h. The mixture was then partitioned between EtOAc (600 mL) and water (200 mL). The organic layer was successively washed with 1 M HCl (100 mL) and water (2 × 50 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by chromatography on Florisil® (eluent: EtOAc) giving a blue solid (yield: 0.35 g, 52%), which was recrystallized from CH_2Cl_2/petroleum ether. M.p. 101–103 °C. IR (cm^{-1}, KBr) 3 060 (arom. CH), 2 944 (CH_2), 2 862 (CH_2), 2 820 (CH_3), 2 769 (CH_3), 1 644 (quinone C=O). ^1H NMR ($CDCl_3$) δ 10.76 (bt, 1H), 10.70 (bt, 1H), 8.29–8.37 (m, 2H), 7.67–7.70 (m, 2H), 7.41–7.45 (m, 6H), 7.24–7.29 (m, 8H), 7.17–7.22 (m, 3H), 3.56 (q, 4H), 3.26 (t, 2H), 2.74 (t, 2H), 2.40 (s, 6H), 2.02 (m, 2H). Anal $C_{40}H_{39}N_3O_3$ (C, H, N).

3.1.28. 1-[(3-Aminopropyl)amino]-4-[(3-hydroxypropyl)amino]-9,10-anthracenedione hydrochloride (**45**)

A suspension of 6 g (25 mmol) of leucoquinizarin in MeOH (300 mL) was de-gassed by stirring at 5 °C under N_2 and then 6.8 mL (89 mmol) of 3-aminopropanol in MeOH (20 mL) were added dropwise. The de-gassed mixture was heated to 50 °C for 1 h, cooled and 4.1 mL (50 mmol) of 1,3-diaminopropane were added dropwise. The mixture was heated again to 50 °C for 2 h, cooled and then aerated overnight. After evaporation the residue was dissolved in MeOH (100 mL) and 8 g (37 mmol) of di-*tert*-butyl dicarbonate in MeOH (20 mL) were added dropwise. The mixture was stirred overnight, then evaporated and the residue was purified by chromatography on Florisil® (eluent: EtOAc) giving a blue solid. The solid was dissolved in MeOH (90 mL) containing conc. HCl (10 mL) and the mixture was refluxed for 1 h and evaporated giving a blue solid (yield: 2.5 g, 25%), which was recrystallized from MeOH. M.p. 193–195 °C. IR (cm^{-1}, KBr) 3 385 (OH), 2 926 (NH_3^+), 1 630 (quinone C=O), 1 593 (NH_3^+). ^1H NMR (CD_3OD) δ 8.15–8.23 (m, 2H), 7.90–7.95 (m, 2H), 7.70 (d, 1H), 7.50 (d, 1H), 4.00 (t, 2H), 3.65 (q, 4H), 3.35 (t, 2H), 2.30 (m, 2H), 2.25 (m, 2H). Anal $C_{20}H_{24}ClN_3O_3$ (C, H, Cl, N).

3.1.29. 1-[(3-Hydroxypropyl)amino]-4-[3-(4′-nitrobenzamido)propylamino]-9,10-anthracenedione (**7**)

To a solution of 0.46 g (0.9 mmol) of **45** in DMF (250 mL) and triethylamine (0.5 mL) was added 0.3 g (1.6 mmol) of 4-nitrobenzoyl chloride and the mixture was stirred overnight. The mixture was then partitioned between CH_2Cl_2 (100 mL) and water (50 mL). The organic layer was washed with water (5 × 30 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: MeOH:EtOAc 5:95) giving a blue solid (yield: 0.22 g, 49%), which was recrystallized from EtOAc. M.p. 184–186 °C. IR (cm^{-1}, KBr) 3 420 (OH), 3 323 (NH), 2 917 (CH_2), 2 850 (CH_2), 1 642 (quinone C=O), 1 625 (C=O). ^1H NMR (d_6-DMSO) δ 10.92 (bt, 1H), 10.89 (bt, 1H), 8.91 (bt, 1H), 8.27 (d, 2H), 8.18–8.24 (m, 2H), 8.06 (d, 2H), 7.76–7.79 (m, 2H), 7.51 (s, 2H), 4.66 (t, 2H), 3.44–3.59 (m, 6H), 1.95 (m, 2H), 1.81 (m, 2H). Anal $C_{27}H_{26}N_4O_6$ (C, H, N).

3.1.30. 1-[(3-Hydroxypropyl)amino]-4-[3-(2′-nitrophenylacetamido)propylamino]-9,10-anthracenedione (**8**)

To a solution of 0.46 g (0.9 mmol) of **45** in DMF (50 mL) and triethylamine (0.5 mL) 0.44 g (2.2 mmol) of 2-nitrophenylacetyl chloride were added and the mixture was stirred overnight. The mixture was then partitioned between CH_2Cl_2 (100 mL) and water (50 mL). The organic layer was washed with water (5 × 30 mL), dried over anh. $MgSO_4$ and evaporated to dryness. The residue was purified by flash chromatography on silica gel (eluent: MeOH:EtOAc 5:95) giving a blue solid (yield: 0.20 g, 43%), which was recrystallized from EtOAc. M.p. 182–185 °C. ^1H NMR (d_6-DMSO) δ 10.89 (bt, 2H), 8.23–8.27 (m, 2H), 8.21 (bt, 1H), 8.00 (d, 1H), 7.77–7.80 (m, 2H), 7.67 (t, 1H), 7.48–7.55 (m, 2H), 7.50 (s, 2H), 4.66 (t, 2H), 3.89 (s, 2H), 3.48–3.59 (m, 6H), 1.77–1.82 (m, 4H). Anal $C_{28}H_{28}N_4O_6$ (C, H, N).

3.1.31. 1-[2-(Dimethylamino)ethylamino]-4-[(3′-pyridinyl)methylamino]-9,10-anthracenedione (**29**)

Compound **29** was prepared from leucoquinizarin (2.5 g, 10.3 mmol) in MeOH (125 mL) according to the procedure outlined for **43** and **44**. The first step involved the addition of N,N-dimethylethylenediamine (4 mL, 37.1 mmol) and in the second stage 3-(aminomethyl)pyridine (2.1 mL, 20.6 mmol) was added. After air bubbling (overnight) and removal of the solvent, the obtained product was purified by column chromatography on silica gel (gradient elution from EtOAc to (90:10) EtOAc:MeOH, followed by EtOAc:MeOH:Et_3N (88:10:2). The product was recrystallized from (90:10) EtOAc:hexane (yield: 70%). M.p. 160–161 °C. ^1H NMR (d_6-DMSO) δ 11.05 (t, 1H, $NHCH_2$), 10.73 (t, 1H, $NHCH_2CH_2$), 8.63 (s, 1H, H-2′), 8.5 (d, 1H, H-6′), 8.24 (m, 2H, H-5,8), 7.76 (m, 3H, H-7,6, H-4′), 7.45 (s, 2H, H-2,3), 7.39 (dd, 1H, H-5′), 4.78 (d, 2H, $NHCH_2$), 3.47

(q, 2H, $NHCH_2CH_2$), 2.54 (t, 2H, $NHCH_2CH_2$), 2.23 (s, 6H, N–CH_3). Anal $C_{24}H_{24}N_4O_2$ (C, H, N).

3.1.32. 1-(Nicotinamido)-9,10-anthracenedione (**20**)

Nicotinoyl chloride (5.7 g, 40 mmol) was added to a solution of 1-aminoanthraquinone (3 g, 13.5 mmol) in toluene (150 mL) and refluxed for 24 h. The solvent was then removed and the residue was dissolved in CH_2Cl_2. The organic layer was washed with 5% aqueous Na_2CO_3 to remove the excess of acyl chloride, dried over anh. $MgSO_4$, concentrated and the residue chromatographed on Florisil® column eluting with CH_2Cl_2. The product was recrystallized from $CHCl_3$ (yield: 88%). M.p. 244 °C. IR (cm^{-1}, KBr) 3 172, 3 111 (NH), 1 698 (C=O), 1 637 (quinone C=O). ^1H NMR (d_6-DMSO) δ 13.2 (s, 1H, NHCO), 9.37 (s, 1H, H-2′), 9.24 (d, 1H, H-2), 9.00 (d, 1H, H-6′), 8.53 (d, 1H, H-4′), 8.36 (d, 2H, H-5,8), 8.06–8.18 (m, 4H, H-3,4,6,7), 7.84 (dd, 1H, H-5′). MS m/z (rel. intensity, %): 328 ($[M]^{\bullet+}$, 39), 106 (100). Anal $C_{20}H_{12}N_2O_3$ (C, H, N).

3.1.33. 1-(Nicotinamido)-9,10-anthracenedione methiodide (**33**)

A suspension of 100 mg (0.3 mmol) of **20** in 10 mL of iodomethane was refluxed for 24 h. Acetone (60 mL) was added and the suspension was stirred for 1 h at room temperature. The precipitate was filtered off and triturated several times with diethyl ether to afford the salt (yield 60%). M.p. 243–245 °C. IR (cm^{-1}, KBr) 3 185 (NH), 3 031 (CH_3), 1 695 (C=O), 1 666 (quinone C=O), 1 634. ^1H NMR (d_6-DMSO) δ 12.98 (s, 1H, NHCO), 9.62 (s, 1H, H-2′), 9.25 (d, 1H, H-6′), 9.11 (d, 1H, H-4′), 8.95 (d, 1H, H-2), 8.44 (m, 1H, H-5′), 8.23 (m, 2H, H-5,8), 8.08 (m, 2H, H-3,4), 8.00 (m, 2H, H-6,7), 4.5 (s, 3H, $^+NCH_3$). Anal $C_{21}H_{15}IN_2O_3$ (C, H, N).

3.1.34. 1-(Benzamido)-9,10-anthracenedione (**22**)

To a solution of 1.5 g (6.7 mmol) of 1-aminoanthraquinone in 100 mL toluene, 2.3 mL (20.1 mmol) of benzoyl chloride were added and refluxed for 24 h. The product was worked up as described for compound **20** (yield: 54%). M.p. 244 °C. ^1H NMR ($CDCl_3$) δ 13.01 (s, 1H, NHCO), 9.39 (d, 1H, H-2), 8.35 (d, 2H H-5,8), 8.18 (d, 2H, H-2′,6′), 8.13 (d, 1H, H-4), 7.85 (m, 3H, H-3,6,7), 7.62 (m, 3H, H-3′,4′,5′). MS m/z (rel. intensity, %): 327 ($[M]^{\bullet+}$, 23), 106 (100). Anal $C_{21}H_{13}NO_3$ (C, H, N).

3.1.35. 1-Hydroxy-4-(nicotinamido)-9,10-anthracenedione (**21**)

Compound **21** was prepared as described for compound **20**, employing 2.8 g (12 mmol) of 1-amino-4-hydroxyanthraquinone in 100 mL toluene and 2.5 g (18 mmol) nicotinoyl chloride (yield: 23%). M.p. 254 °C. ^1H NMR (d_6-DMSO) δ 13.01 (s, 2H, OH; NHCO), 9.24 (s, 1H, H-2′), 9.07 (d, 1H, H-2), 8.87 (d, 1H, H-6′), 8.41 (d, 1H, H-4′), 8.31 (m, 2H, H-5,8), 8.01 (m, 2H, H-6,7), 7.71 (dd, 1H, H-5′), 7.6 (d, 1H, H-3). MS m/z (rel. intensity, %): 344 ($[M]^{\bullet+}$, 81), 103 (100). Anal $C_{20}H_{12}N_2O_4$ (C, H, N).

3.1.36. 1-Benzamido-4-hydroxy-9,10-anthracenedione (**23**)

To a solution of 2.5 g (10.45 mmol) of 1-amino-4-hydroxyanthraquinone in 100 mL toluene, 1.46 mL (12.54 mmol) of benzoyl chloride were added and refluxed for 24 h. The product was worked up as described for compound **20** (yield: 92%). M.p. 248–249 °C. ^1H NMR ($CDCl_3$) δ 13.28 (m, 2H, NHCO, OH), 9.38 (d, 1H, H-2), 8.38 (m, 2H, H-5,8), 8.17 (d, 2H, H-2′), 7.83 (m, 2H, H-6,7), 7.6 (m, 3H, H-3′,4′), 7.42 (d, 1H, H-3). MS m/z (rel. intensity, %): 342 ($[M]^{\bullet+}$, 37), 105 (100). Anal $C_{21}H_{13}NO_4$ (C, H, N).

3.1.37. 1,4-Bis(nicotinamido)-9,10-anthracenedione (**34**)

Compound **34** was prepared according to the general procedure described above for compound **20** employing nicotinoyl chloride (10.7 g, 76 mmol) and 1,4-diaminoanthraquinone (3 g, 13 mmol) in 100 mL toluene (yield: 39%). M.p. 140–145 °C. ^1H NMR (d_6-DMSO) δ 13.31 (s, 2H, NHCO), 9.27 (s, 2H, H-2′), 9.24 (s, 2H, H-2,3), 8.87 (d, 2H, H-6′), 8.41 (d, 2H, H-4′), 8.32 (dd, 2H, H-5,8), 7.97 (dd, 2H, H-6,7), 7.69 (dd, 2H, H-5′).

3.1.38. 1,4-Bis(nicotinamido)-9,10-anthracenedione dimethiodide (**35**)

Compound **35** was obtained from **34** by the procedure described for **33** (yield: 85%). M.p. 311 °C. ^1H NMR (d_6-DMSO) δ 12.92 (s, 2H, NHCO), 9.63 (s, 2H, H-2′), 9.25 (d, 2H, H-6′), 9.13 (d, 2H, H-4′), 8.97 (s, 2H, H-2,3), 8.46 (dd, 2H, H-5′), 8.26 (dd, 2H, H-5,8), 8.15 (dd, 2H, H-6,7), 4.5 (s, 6H, ^+N–CH_3). MS (+FAB) m/z (rel. intensity, %): 605 ($[M–I]^{\bullet+}$, 16), 478 ($[M–2I]^{\bullet+}$, 53), 477 ($[M–I–HI]^{\bullet+}$, 100). Anal $C_{28}H_{22}I_2N_4O_4$ (C, H, I, N).

3.1.39. 1-Amino-4-[[2-(dimethylamino)ethyl]amino]-9,10-anthracenedione (**46**)

The general procedure described above for compounds **43** and **44** was followed starting with leucoquinizarin (5 g, 20.6 mmol) and N,N-dimethylethylenediamine (8.2 mL, 74.3 mmol) in MeOH (150 mL, 50 °C, 45 min). After cooling to room temperature, NH_3 (g) was bubbled through the solution for 5 min. The reaction vessel was sealed and kept at room temperature for 48 h. The desired product was purified by column chromatography on silica gel (eluent: EtOAc), followed by recrystallization from

EtOAc (yield: 40%). M.p. 143 °C. ^{1}H NMR (d_6-DMSO) δ 10.8 (s, 1H, $NHCH_2CH_2$), 8.4 (bs, 2H, NH_2), 8.23 (m, 2H, H-5,8), 7.8 (m, 2H, H-6,7), 7.3–7.4 (2d 2H, H-2,3), 3.5 (q, 2H, $NHCH_2CH_2$), 2.58 (t, 2H, $NHCH_2CH_2$), 2.2 (s, 6H, N–CH_3). MS m/z (rel. intensity, %): 309 ([M]$^{\bullet+}$, 100), 250 (84). Anal $C_{18}H_{19}N_3O_2$ (C, H, N).

3.1.40. 1-[[2-(Dimethylamino)ethyl]amino]-4-(nicotinamido)-9,10-anthracenedione (**31**)

Nicotinoyl chloride (1.6 g, 11 mmol) was added to a solution of **46** (3 g, 9.7 mmol) in 100 mL toluene and refluxed for 24 h. The product was worked up as described for compound **20** and purified by column chromatography on silica gel (gradient elution from EtOAc to (95:5) EtOAc:MeOH, followed by (94:5:1) EtOAc:MeOH:Et_3N) and recrystallization from (90:10) CH_2Cl_2-petroleum ether (yield: 45%). M.p. 218–220 °C. ^{1}H NMR (d_6-DMSO) δ 13.26 (s, 1H, AQNHCO), 10.36 (t, 1H, $NHCH_2CH_2$), 9.23 (s, 1H, H-2′), 8.96 (d, 1H, H-2), 8.85 (d, 1H, H-6′), 8.38 (d, 1H, H-4′), 8.24 (dd, 2H, H-5,8), 7.88 (m, 2H, H-6,7), 7.69 (dd, 1H, H-5′), 7.55 (d, 1H, H-3), 3.52 (q, 2H, $NHCH_2CH_2$), 2.65 (t, 2H, $NHCH_2CH_2$), 2.3 (s, 6H, N–CH_3). MS m/z (rel. intensity, %): 414 ([M]$^{\bullet+}$, 32), 105 (100). Anal $C_{24}H_{22}N_4O_3$ (C, H, N).

3.1.41. 1-[[2-(Dimethylamino)ethyl]amino]-4-(nicotinamido)-9,10-anthracenedione N-oxide (**32**)

The product was prepared from nicotinoyl chloride N-oxide (1.9 g, 12 mmol) and **46** (3 g, 9.7 mmol) as described for **31** (yield: 59%). M.p. 195–196 °C: ^{1}H NMR (d_6-DMSO) δ 13.02 (s, 1H, AQNHCO), 10.38 (t, 1H, $NHCH_2CH_2$), 8.84 (d, 1H, H-2), 8.7 (s, 1H, H-2′), 8.49 (d, 1H, H-4′), 8.25 (dd, 2H, H-5,8), 7.9 (m, 3H, H-6,7, H-6′), 7.7 (t, 1H, H-5′), 7.54 (d, 1H, H-3), 3.4 (q, 2H, $NHCH_2CH_2$), 2.58 (t, 2H, $NHCH_2CH_2$), 2.25 (s, 6H, N–CH_3). MS (+FAB) m/z (rel. intensity, %): 431 ([M + H]$^{\bullet+}$, 100). MS (+DCI): 431 ([M + H]$^{\bullet+}$, 18), 415 ([M + H–16]$^{\bullet+}$, 100). MS (–DCI): 430 (M$^{\bullet-}$, 47), 414 (100). Anal $C_{24}H_{22}N_4O_4$ (C, H, N).

3.1.42. Cyclic voltammetry (CV)

The CV measurements were performed using a BAS model CV-58 cyclic voltammeter (West Lafayette, Indiana) equipped with a glassy carbon working electrode, a Pt wire auxiliary electrode and an Ag/AgCl reference electrode. The measurements were carried out between –2 V and 2 V. The samples (1 mM) were dissolved in high-purity DMF, containing 0.1 M tetrabutylammonium perchlorate as supporting electrolyte. The solutions were bubbled with high-purity nitrogen for 20 min before the measurements to remove interfering oxygen. CV measurements were performed mostly at a scan rate of 50 mV/s.

3.2. Biological evaluation

3.2.1. Cytotoxic activity in cell culture

3.2.1.1. Cell culture

A: the P388 murine leukaemia cell line was a generous gift from Dr A. Ramu, Department of Radiation and Clinical Oncology, Hadassah University Hospital, Jerusalem, Israel. Cells were grown in RPMI 1640 medium (GIBCO), supplemented with 10% heat-inactivated foetal calf serum (GIBCO), 10 μM 2-mercaptoethanol, 50 units/mL of penicillin base and 50 μg/mL of streptomycin base in a controlled air mixture containing 5% CO_2 humidified atmosphere at 37 °C. An inoculum of cells was transferred to fresh medium once every 4 d in order to maintain growth in the exponential phase. Cell growth was assessed by measurement of cell density in a Coulter counter. Initial cell density was 1×10^5 cells/mL and after 4 d it became $1–2 \times 10^6$ cells/mL.

B: J774.2 macrophage-like cell line. The cells were grown in complete DMEM, supplemented with 10% horse serum according to published procedures [39].

3.2.1.2. Sensitivity to anthraquinone derivatives

The anthraquinone derivatives were dissolved in DMF. Cells were cultured in the presence of various drug concentrations for 72 h. The final DMF concentration (0.2% v/v) was proved not to be cytotoxic against the cell culture. The initial and final cell densities were measured. The growth rate at each drug concentration was expressed as the percentage of the control growth rate. The concentration of drug effective in inhibiting the growth rate by 50% (ED_{50}) was determined according to previously described protocols [24].

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