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Original article

Synthesis and in vitro evaluation of 1,8-diazaanthraquinones bearing 3-dialkylaminomethyl or 3-(*N*-alkyl- or *N*-aryl)carbamoyloxymethyl substituent

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Abstract

A series of 1,8-diazaanthraquinone derivatives carrying a 3-dialkylaminomethyl or a 3-(*N*-alkyl or aryl)carbamoyloxymethyl substituent was synthesised and their in vitro cytotoxic activities were evaluated against eight human cancer cell lines (HOP62, SK-OV-3, HCT-15, SF295, MCF7, SNU-354, KB-3-1 and KB-V-1). A number of compounds including **8c**, **8d** and **11c** showed cytotoxic activity comparable to that of doxorubicin against all human cancer cell lines tested. The compounds **8c** and **8d** were 2–100 times more potent than doxorubicin against HCT-15, MCF7 and SNU-354 cancer cell lines. Furthermore, these compounds retained considerable cytotoxic activity against the doxorubicin-resistant cell line KB-V-1, implying their therapeutic potential to treat doxorubicin-resistant tumours. These compounds inhibited topoisomerase II-mediated DNA relaxation in vitro, suggesting that this inhibitory effect be attributable to their cytotoxicity.

Keywords: 1,8-Diazaanthraquinone derivatives; Antitumour agents; Doxorubicin; Topoisomerase II

1. Introduction

Doxorubicin and daunomycin are well-known members of the anthracycline antibiotics and the most commonly prescribed intercalating agents for the treatment of cancer [1]. Doxorubicin (Fig. 1) has a broad spectrum of activity, being particularly efficacious against solid tumours. However its clinical usefulness is limited due to cardiotoxicity developed upon extended therapy, and the appearance of an acquired resistance [2,3]. The development of drug resistance is one of the causes of severe limitation to the chemotherapy in cancer patients.

The azaanthraquinones are a new class of antitumour agents that exhibit promising in vitro and in vivo activity against a wide spectrum of tumour cell lines [4–6]. These are the chromophore-modified analogues of mitoxantrone, a synthetic analogue of doxorubicin. In an effort to develop novel antitumour agents that could overcome the shortcomings of anthracyclines, we recently reported the synthesis and biological evaluation of 3- or 4-substituted-1-azaanthraquinones (**2**) [7–9]. Herein, we report the synthesis and biological evaluation of 1,8-diazaanthraquinone derivatives bearing a 3-dialkylaminomethyl or a 3-(*N*-alkyl or aryl)carbamoyloxymethyl substituent. These analogues (**3**) were designed to explore the effect of an

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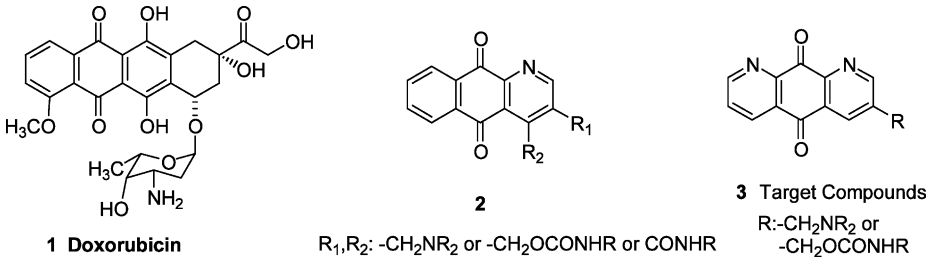


Fig. 1. Structures of Doxorubicin **1**, **2**, and target compounds **3**.

additional nitrogen atom incorporated into the monoazaanthraquinone chromophore. The side chain of the target compounds was designed to have either a basic nitrogen or carbamate.

2. Chemistry

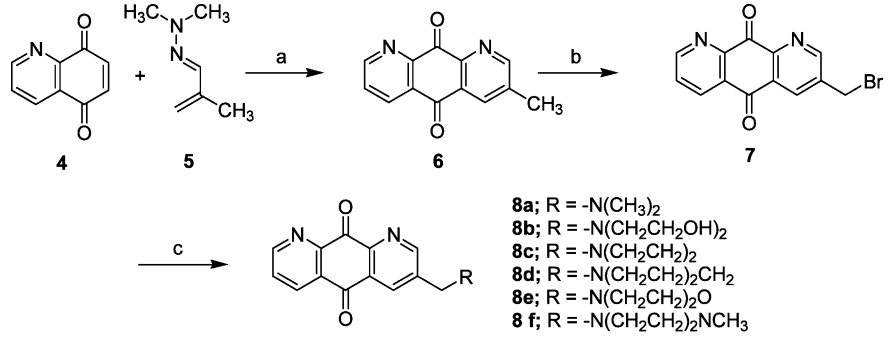
The general synthetic strategy employed to prepare the target compounds was based on the regio-selective lewis acid catalysed hetero Diels–Alder reaction of quinoline-5,8-dione (**4**), which has been previously reported by us [10]. The cycloaddition of dienophile **4** with diene **5** in the presence of $ZnCl_2$ afforded the desired regio-isomer **6** (74%) as a sole product. Having obtained the required 3-methyl-1,8-diazaanthraquinone (**6**), it was treated with *N*-bromosuccinimide (NBS) and a catalytic amount of benzoylperoxide in anhydrous 1,2-dichloroethane at reflux for 48 h with the irradiation of tungsten lamp to give the bromomethyl product **7** in 30% yield.

The target compounds, **8a**–**8f**, containing 3-dialkylaminomethyl substituent were synthesised by direct substitution reaction with the corresponding dialkylamine as shown in Fig. 2. Treatment of the compound **7** with the appropriate secondary amines in anhydrous dimethylformamide (DMF) afforded *N*,*N*-dimethylamino (**8a**) (92%), *N*,*N*-diethanolamino (**8b**) (80%), pyrrolidino (**8c**) (73%), piperidino (**8d**) (81%), morpholino (**8e**) (84%), and (*N*-methyl)piperazino (**8f**) (70%) methyl derivatives, respectively (Fig. 2).

Another series of the target compounds, **11a**–**11e**, containing 3-(*N*-alkyl- or *N*-aryl)carbamoyloxymethyl substituent was synthesised according to Fig. 3. Treatment of **7** with anhydrous sodium acetate in anhydrous DMF provided **9** in 96% yield. Hydrolysis of the acetate **9** with LiOH in aqueous ethanol afforded 3-hydroxymethyl-1,8-diazaanthraquinone **10** (79%). Hydroxymethyl product **10** was also obtained without isolation of the intermediate **9** (73% in two steps). Treatment of **10** with the corresponding alkyl or arylisocyanates, catalytic amount of dibutyltindiacetate (2–4 drop), and triethylamine in anhydrous dichloromethane afforded *N*-2-chloroethyl **11a** (83%), *N*-ethyl **11b** (74%), *N*-*i*-propyl **11c** (76%), *t*-butyl **11d** (69%), and *N*-phenyl **11e** (81%) carbamoyloxymethyl derivatives, respectively (Fig. 3).

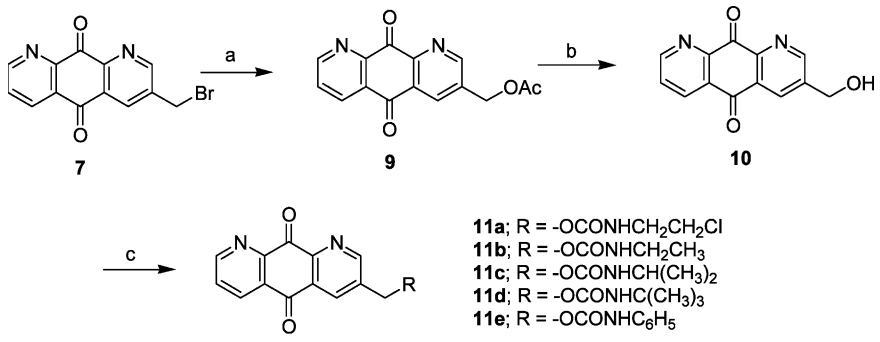
3. Results and discussion

The in vitro cytotoxic activities of the newly synthesised 1,8-diazaanthraquinone derivatives were evaluated against human cancer cell lines originated from lung (HOP62), ovarian carcinoma (SK-OV-3), colon (HCT-15), CNS carcinoma (SF295), breast (MCF7) and liver (SNU-354) according to the protocols developed by the National Cancer Institute [11]. The concentrations of 1,8-diazaanthraquinone derivatives (**6**, **7**, **8a**–**8f**, **10** and **11a**–**11e**) inhibiting cellular growth by 50%, IC_{50} values, are shown in Table 1. The comparative data for doxorubicin are also shown for comparison.



(a; $ZnBr_2$ (1.2 eq), CH_2Cl_2, b; NBS, Benzoylperoxide, $ClCH_2CH_2Cl$, reflux, c; RH, DMF)

Fig. 2. Synthesis of Dialkylaminomethyl-1,8-diazaanthraquinones.



(a; NaOAc, DMF, b; LiOH, c; R-N=C=O, n-$Bu_2Sn(OAc)_2$

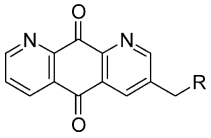
Fig. 3. Synthesis of *N*-Alkyl- or *N*-Arylcarbamoyloxymethyl-1,8-diazaanthraquinones.

All analogues tested in this study exhibited significant cytotoxic activity. The compounds **8c**, **8d** and **11c** exhibited cytotoxicity comparable to that of doxorubicin against all human cancer cell lines tested. In particular, **8c** and **8d** were 2–100 times more potent than doxorubicin against HCT-15, MCF7 and SNU-354 cancer cell lines. In contrast, the compound **8b** containing the bulky diethanolamine substituent was least potent. This suggests that the bulky side chain may prevent the cytotoxic effect of the compound. The compounds **8e** and **8f** were less potent than the other analogues bearing aminomethyl substituents. This might be ascribed to the additional hetero atom incorporated into the side chain of the derivatives.

The derivatives bearing 3-(*N*-alkyl or aryl)carbamoyloxymethyl side chain exhibited cytotoxic activity comparable to that of doxorubicin. The cytotoxic activity of the compounds decreased in the order of **11c** (*N*-isopropyl-) > **11d** (*N*-*t*-butyl-) > **11b** (*N*-ethyl-) ≅ **11a** (*N*-chloroethyl-) > **11e** (*N*-phenyl-), although the differences were small. The hydroxymethyl compound **10** was less cytoxic than the analogues bearing carbamate.

We next evaluated cytotoxic activities of these compounds against the human epidermoid carcinoma cell

Table 1
In vitro cytotoxic activity of 1,8-diazaanthraquinone derivatives against human cancer cell lines (



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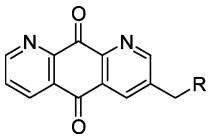
Compd.	R	IC_{50} (μM) [a] of cell lines [b]					
		HOP62	SK-OV-3	HCT-15	SF295	MCF7	SNU-354
6	H	0.015	0.04	0.35	0.25	0.68	0.28
7	Br	0.055	0.3	0.4	0.5	2.64	0.87
8a	$N(CH_3)_2$	0.015	0.025	0.03	0.15	0.65	0.10
8b	$N(CH_2CH_2OH)_2$	1.5	3	1.8	2.2	6.90	15.45
8c	$N(CH_2CH_2)_2$	0.008	0.015	0.03	0.1	0.49	0.075
8d	$N(CH_2CH_2)_2CH_2$	0.003	0.005	0.01	0.07	0.47	0.011
8e	$N(CH_2CH_2)_2O$	0.025	0.1	0.15	0.25	2.95	2.56
8f	$N(CH_2CH_2)_2N{\cdot}CH_3$	0.02	0.045	0.19	0.55	1.40	1.93
10	OH	0.1	0.2	0.4	0.3	3.42	0.60
11a	$OCONHCH_2CH_2Cl$	0.02	0.2	0.15	0.3	1.74	1.22
11b	$OCONHCH_2CH_3$	0.04	0.3	0.2	0.5	1.92	2.52
11c	$OCONHCH(CH_3)_2$	0.006	0.02	0.02	0.1	1.51	2.38
11d	$OCONHC(CH_3)_3$	0.01	0.05	0.04	0.35	1.71	0.58
11e	$OCONHC_6H_5$	0.02	0.2	0.15	1.5	6.90	3.51
Doxorubicin		0.004	0.02	0.1	0.035	1.22	1.33

[a] IC_{50}, concentration of compound (μM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as an average value.

[b] Human cancer cell lines: HOP62 (lung cancer), SK-OV-3 (ovarian carcinoma), HCT-15 (colon cancer), SF295 (CNS carcinoma), MCF7 (breast cancer), SNU-354 (liver cancer).

Table 2
In vitro cytotoxic activity of 1,8-diazaanthraquinone derivatives against human epidermoid carcinoma (KB-3-1) and a subline resistant to doxorubicin (KB-V-1) (



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Compd.	R	IC_{50} (μM) [a] of cell lines [b]		
		KB-3-1	KB-V-1	RI [c]
6	H	0.34	6.37	18.7
7	Br	6.13	6.77	1.1
8a	$N(CH_3)_2$	1.31	3.49	2.7
8b	$N(CH_2CH_2OH)_2$	14.54	62.27	4.3
8c	$N(CH_2CH_2)_2$	1.60	3.17	2.0
8d	$N(CH_2CH_2)_2CH_2$	0.30	2.23	7.4
8e	$N(CH_2CH_2)_2O$	4.58	9.28	2.02
8f	$N(CH_2CH_2)_2N{\cdot}CH_3$	1.01	6.13	6.1
10	OH	3.89	7.30	1.9
11a	$OCONHCH_2CH_2Cl$	4.72	14.80	3.1
11b	$OCONHCH_2CH_3$	2.02	12.63	6.3
11c	$OCONHCH(CH_3)_2$	1.22	6.21	5.1
11d	$OCONHC(CH_3)_3$	1.87	6.43	3.4
11e	$OCONHC_6H_5$	21.19	22.37	1.1
Doxorubicin		0.28	34.10	121.8

[a] IC_{50}, concentration of compound (μM) required to inhibit the cellular growth by 50% after 72 h of drug exposure, as determined by the SRB assay. Each experiment was run at least three times, and the results are presented as an average value.
[b] Human cancer cell lines: KB-3-1 (epidermoid carcinoma), KB-V-1 (epidermoid carcinoma multidrug-resistant cell).
[c] Resistance index: IC_{50} of resistance cell line/IC_{50} of sensitive cell line.

line (KB-3-1) and its multi-drug resistant subline (KB-V-1). Their respective IC_{50} values and the resistance index (RI), the IC_{50} against the resistant cell line divided by the IC_{50} against the sensitive cell line, are shown in Table 2. Except for the compounds **6** and **8d**, the remaining analogues were 4–100 times less potent than doxorubicin against the sensitive cell line. The compounds **6** and **8d** showed cytotoxic activity comparable to that of doxorubicin against the sensitive cell line (KB-3-1). Furthermore, it is noteworthy that these compounds retained much of their activity against the resistant cell line (KB-V-1). The compound **8d** possessed the most potent cytotoxic activity against the sensitive and resistant cell lines, with the respective IC_{50} values of 0.30 and 2.23 μM. The RI of **8d** was 7.4, whereas the RI of doxorubicin was >120 (Table 2).

Diazaanthraquinone chromophore was designed to make more π-deficient than the monoazaanthraquinone derivatives (**2**). This would bring a favourable π–π interaction between the diazaanthraquinone derivatives and the base pairs of the rather electron-rich DNA. The incorporation of an additional hetero atom into the 1-azaanthraquinone ring system would increase the residence time of the 1,8-diazaanthraquinone derivatives within DNA or DNA-topoisomerase II complex. This effect would make the diazaanthraquinone derivatives more potent than the 1-azaanthraquinone derivatives.

As expected, the 1,8-diazanthraquinone derivatives were found to be more potent than the 1-azaanthraquinone derivatives (**2**) reported previously by us [7–9]. The compounds such as **8c**, **8d** and **11c** were as potent as doxorubicin (Tables 1 and 2). However, in contrast to doxorubicin, they retained cytotoxic activity against the resistant cell line (Table 2), implying their therapeutic potential for the treatment of doxorubicin-resistant tumours.

In order to identify the site of action, the abilities of some of these potent cytotoxic compounds to intercalate with DNA and/or to inhibit DNA topoisomerase II were examined. As shown in Fig. 4A, none of the tested compounds intercalated with DNA, while doxorubicin, a known intercalator did. However, most of the tested compounds were found to possess the ability to inhibit topoisomerase II-mediated DNA relaxation (Fig. 4B). The extent of this inhibition by compounds **8c**, **8d**, and **11c** was similar or superior to that achieved by etoposide (VP16) which is known to inhibit topoisomerase II. These data suggest that these newly synthesised 1,8-diazaanthraquinone derivatives (at least compounds **8c**, **8d**, and **11c**) exert their cytotoxic effects by inhibiting topoisomerase II.

4. Experimental

4.1. Chemistry

Melting points were determined in an open capillary with Electrothermal IA9100 digital melting point apparatus and are uncorrected. Thin layer chromatography (TLC, silica gel 60 GF_{254}, Merck, Darmstadt) was used to monitor reactions and check product homogeneity. IR spectra were determined with a JASCO FT-IR300E spectrophotometer. ^{1}H-NMR spectra were determined with Bruker DPS300 spectrometer (tetramethylsilane as internal standard). Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments. Results were within $\pm 0.4\%$ of predicted values for all compounds. Commercially available regents and solvents were used without additional purification unless otherwise stated.

4.1.1. 3-Methyl-1,8-diazaanthracene-9,10-dione (6)

Quinoline-5,8-dione (**4**) (477 mg, 3.0 mmol) and $ZnCl_2$ (491 mg, 3.6 mmol) were dissolved in anhydrous dichloromethane (15 ml). The mixture was stirred for 30 min and then 1-(*N*,*N*-dimethylamino)-1-aza-1,3-buta-

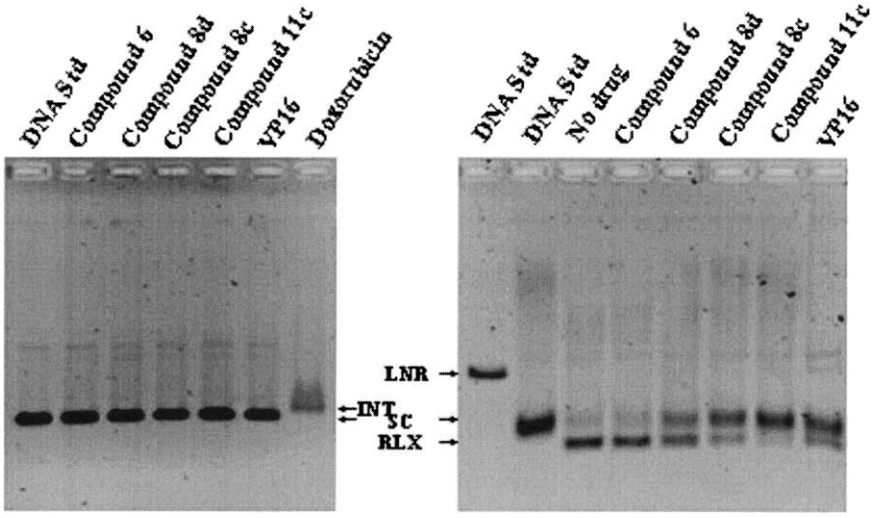

Fig. 4. Effect of several compounds on DNA intercalation (A) and Topo II-mediated DNA relaxation (B). Two DNA standards are shown for reference: linearised pRYG (LNR) and negatively supercoiled pRYG (SC). The positions of intercalated pRYG (INT) and relaxed pRYG (RLX) are also indicated.

diene (**5**) (404 mg, 3.6 mmol) was added dropwise. The mixture was stirred for 24 h under nitrogen atmosphere. The reaction mixture was diluted with dichloromethane (100 ml) and washed with saturated sodium bicarbonate solution (50 ml × 3). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (3% methanol in dichloromethane) to give **6** as a brown solid (497 mg, 74%): m.p.(dec.) 214–216 °C; IR (KBr) 1690, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.19 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.01 (s, 1H), 8.67 (dd, $J = 7.9$ Hz, $J = 2$ Hz, 1H), 8.45 (s, 1H), 7.80 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 2.61 (s, 3H). Anal. Calc. for $C_{13}H_8N_2O_2$: C, 69.64; H, 3.60; N, 12.49%. Found: C, 69.80; H, 3.62; N, 12.51.

4.1.2. 3-Bromomethyl-1,8-diazaanthracene-9,10-dione (7)

3-Methyl-1,8-diazaanthracene-9,10-dione (**6**) (336 mg, 1.50 mmol) was dissolved in dichloroethane (60 ml). NBS (1.342 g, 7.50 mmol) and benzoylperoxide (30 mg) were added to the solution. The mixture was refluxed for 48 h under nitrogen atmosphere. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography (50% ethyl acetate in dichloromethane → 4% methanol in dichloromethane) to give **7** as a yellow solid (137 mg, 30%): m.p. 185–186 °C; IR (KBr) 2925, 1699, 1671, 1581 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.20 (m, 2H), 8.70 (m, 2H), 7.82 (m, 1H), 4.65 (s, 2H). Anal. Calc. for $C_{13}H_7BrN_2O_2$: C, 51.51; H, 2.33; N, 9.24%. Found: C, 51.44; H, 2.40; N, 9.28. Starting material (120 mg, 26%) was recovered from this reaction and reused.

4.1.3. 3-(N,N-Dialkylamino)methyl-1,8-diazaanthracene-9,10-diones (8a–8f)

4.1.3.1. General procedure. 3-Bromomethyl-1,8-diazaanthracene-9,10-dione (**7**) (50 mg, 0.17 mmol) was treated with an appropriate dialkylamine in DMF at room temperature for 3 h. The solvent was removed in vacuo and the resulting residue was purified by flash column chromatography on silica gel, using methanol–dichloromethane (1:9) as the eluent to give a product.

*4.1.3.2. 3-(N,N-Dimethylamino)methyl-1,8-diazaanthracene-9,10-dione (**8a**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 31 mg of 40% dimethylamine in H_2O (0.30 mmol) was obtained 41 mg of **8a** (92%) as a brown solid: m.p. 188–189 °C; IR (KBr) 2970, 1697, 1668, 1579 cm^{-1}; 1H-NMR (DMSO-d_6) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.08 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.51 (s, 1H), 7.93 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 3.68 (s, 2H), 2.23 (s, 6H). Anal. Calc. for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 15.72%. Found: C, 67.51; H, 4.92; N, 15.70.

*4.1.3.3. 3-(N,N-Diethanolamino)methyl-1,8-diazaanthracene-9,10-dione (**8b**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 38 mg of diethanolamine (0.36 mmol) was obtained 43 mg of **8b** (80%) as a yellow solid: (43 mg, 80%): m.p. 173–174 °C; IR (KBr) 3395, 3044, 1697, 1675, 1582 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.09 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.51 (s, 1H), 7.93 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 4.48 (t, $J = 5.1$ Hz, 2H), 3.95 (s, 2H), 3.50 (m, 4H), 2.61 (t, $J = 5.9$ Hz, 4H). Anal. Calc. for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04%. Found: C, 60.12; H, 4.35; N, 14.10.

*4.1.3.4. 3-Pyrrolidinomethyl-1,8-diazaanthracene-9,10-dione (**8c**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 26 mg of pyrrolidine (0.36 mmol) was obtained 30 mg of **8c** (73%) as a brown solid: m.p. 157–158 °C; IR (KBr) 2986, 1701, 1665, 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.03 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J =$ 1.9 Hz, 1H), 8.45 (s, 1H), 7.92 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 3.85 (s, 2H), 2.59 (t, $J = 3.2$ Hz, 4H), 1.74 (t, $J = 3.2$ Hz, 4H). Anal. Calc. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33%. Found: C, 69.80; H, 5.17; N, 14.30.

*4.1.3.5. 3-Piperidinomethyl-1,8-diazaanthracene-9,10-dione (**8d**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 31 mg of piperidine (0.36 mmol) was obtained 41 mg of **8d** (81%) as a brown solid: m.p. 169–170 °C; IR (KBr) 2934, 1701, 1665, 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.10 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J =$ 1.9 Hz, 1H), 8.52 (s, 1H), 7.92 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 3.77 (s, 2H), 2.56 (m, 4H), 1.53 (m, 6H). Anal. Calc. for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67%. Found: C, 70.12; H, 5.51; N, 13.90.

*4.1.3.6. 3-Morpholinomethyl-1,8-diazaanthracene-9,10-dione (**8e**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 31 mg of morpholine (0.36 mmol) was obtained 43 mg of **8d** (84%) as a red–brown solid: m.p. 202–203 °C; IR (KBr) 2959, 1700, 1673, 1579 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.12 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.05 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J =$ 1.9 Hz, 1H), 8.47 (s, 1H), 7.92 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 3.89 (s, 2H), 3.61 (s, 4H), 2.50 (s, 4H). Anal. Calc. for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.58%. Found: C, 66.10; H, 4.91; N, 13.55.

*4.1.3.7. 3-(4-Methylpiperazino)methyl-1,8-diazaanthracene-9,10-dione (**8f**).* Following the general procedure, from 50 mg of **7** (0.17 mmol) and 36 mg of 4-methylpiperazine (0.36 mmol) was obtained 28 mg of **8d** (70%) as a brown solid: m.p. 192–193 °C; IR (KBr) 2995, 1696, 1671, 1577 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.02 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.45 (s, 1H), 7.92 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 3.74 (s, 2H), 2.27 (m, 8H), 2.19 (s, 3H). Anal. Calc. for $C_{18}H_{18}N_4O_2$: C, 67.07; H, 5.63; N, 17.38%. Found: C, 67.10; H, 5.67; N, 17.30.

*4.1.4. 3-Acetyloxymethyl-1,8-diazaanthracene-9,10-dione (**9**)*

3-Bromomethyl-1,8-diazaanthracene-9,10-dione (7) (100 mg, 0.33 mmol) was treated with an anhydrous sodium acetate (56 mg, 0.68 mmol) in anhydrous DMF (10 ml) for 24 h under nitrogen atmosphere. The reaction mixture was diluted with water (100 ml), extracted with dichloromethane (3×25 ml). Organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (4% methanol in dichloromethane) to give **9** as a yellow solid (96 mg, 96%): m.p. 202–203 °C; IR (KBr) 2996, 1734, 1700, 1667, 1577, cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 8.82 (s, 1H), 8.56 (dd, $J = 7.9$ Hz, $J =$ 1.9 Hz, 1H), 8.40 (s, 1H), 7.58 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 4.98 (s, 2H), 2.20 (s, 3H). Anal. Calc. for $C_{15}H_{10}N_2O_4$: C, 63.83; H, 3.57; N, 9.93%. Found: C, 63.77; H, 3.59; N, 9.95.

*4.1.5. 3-Hydroxymethyl-1,8-diazaanthracene-9,10-dione (**10**)*

3-Acetyloxymethyl-1,8-diazaanthracene-9,10-dione (**9**) (200 mg, 0.83 mmol) was treated with lithium hydroxide (70 mg, 1.66 mmol) in 80% ethanol solution (20 ml). The mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography (5% methanol in dichloromethane) to give **10** as a yellow solid (157 mg, 79%): m.p. 248–249 °C; IR (KBr) 3366, 2997, 1695, 1673, 1583 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.12 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 8.82 (s, 1H), 8.57 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.40 (s, 1H), 7.58 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 5.42 (s, 1H), 4.98 (s, 2H). Anal. Calc. for $C_{13}H_8N_2O_3$: C, 65.00; H, 3.36; N, 11.66%. Found: C, 65.11; H, 3.42; N, 11.59.

*4.1.6. 3-(N-Alkyl- or aryl-) carbamoyloxymethyl-1,8-diazaanthracene-9,10-diones (**11a**–**11e**)*

4.1.6.1. General procedure. 3-Hydroxymethyl-1,8-diazaanthracene-9,10-dione (**10**) was treated with an appropriate alkyl- or aryl-isocyanate, triethylamine and dibutyltin diacetate (2 drop) in anhydrous dichloromethane (10 ml) at room temperature for 24 h. The solvent was removed in vacuo and the resulting residue was purified by flash column chromatography on silica gel, using methanol–dichloromethane (1:19) as the eluent to give a product.

*4.1.6.2. 3-(N-2-Chloroethyl)carbamoyloxymethyl-1,8-diazaanthracene-9, 10-dione (**11a**).* Following the general procedure, from 40 mg of **10** (0.17 mmol) and 44 mg of 2-chloroethyl isocyanate (0.42 mmol), 25 mg of triethylamine (0.25 mmol) and dibutyltin diacetate (2 drop) for 21 h was obtained 49 mg of **11a** (83%) as a yellow–green solid: m.p. 230–231 °C; IR (KBr) 3343, 2993, 1700, 1669, 1540 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.10 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.06 (s, 1H), 8.56 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.50 (s, 1H), 7.91 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 7.77 (brs, NH), 5.32 (s, 2H), 3.62 (t, $J = 6.1$ Hz, 2H), 3.37 (m, 2H). Anal. Calc. for

$C_{16}H_{12}ClN_3O_4$: C, 55.58; H, 3.50; N, 12.15%. Found: C, 55.80; H, 3.62; N, 12.01.

*4.1.6.3. 3-(N-Ethyl)carbamoyloxymethyl-1,8-diazaanthracene-9,10-dione (**11b**).* Following the general procedure, from 40 mg of **10** (0.17 mmol) and 60 mg of ethyl isocyanate (0.84 mmol), 25 mg of triethylamine (0.25 mmol) and dibutyltin diacetate (3 drop) for 24 h was obtained 39 mg of **11b** (74%) as a green solid: m.p. 204–205 °C; IR (KBr) 3333, 2965, 1730, 1684, 1672, 1590 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.11 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.07 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.50 (s, 1H), 7.93 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 5.34 (s, 1.9H), 5.03 (brs, NH), 3.26 (q, $J = 8.1$ Hz, 2H), 1.18 (t, $J = 6.7$ Hz, 3H). Anal. Calc. for $C_{16}H_{13}N_3O_4$: C, 61.73; H, 4.21; N, 13.50%. Found: C, 61.80; H, 4.24; N, 13.41.

*4.1.6.4. 3-(N-Isopropyl)carbamoyloxymethyl-1,8-diazaanthracene-9,10-dione (**11c**).* Following the general procedure, from 40 mg of **10** (0.17 mmol) and 36 mg of isopropyl isocyanate (0.42 mmol), 25 mg of triethylamine (0.25 mmol) and dibutyltin diacetate (2 drop) for 24 h was obtained 42 mg of **11c** (76%) as a yellow solid: m.p. 216–217 °C; IR (KBr) 3357, 2994, 1700, 1522 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.12 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.08 (s, 1H), 8.58 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.50 (s, 1H), 7.93 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 7.45 (brs, NH), 5.29 (s, 2H), 3.61 (m, 1H), 1.09 (d, $J = 6.7$ Hz, 6H). Anal. Calc. for $C_{17}H_{15}N_3O_4$: C, 62.76; H, 4.65; N, 12.92%. Found: C, 62.80; H, 4.62; N, 13.01.

*4.1.6.5. 3-(N-t-Butyl)carbamoyloxymethyl-1,8-diazaanthracene-9,10-dione (**11d**).* Following the general procedure, from 40 mg of **10** (0.17 mmol) and 42 mg of *t*-butyl isocyanate (0.42 mmol), 25 mg of triethylamine (0.25 mmol) and dibutyltin diacetate (2 drop) for 22 h was obtained 40 mg of **11d** (69%) as a yellow solid: m.p. 190–192 °C; IR (KBr) 3374, 1733, 1676, 1582 cm^{-1}; ^{1}H-NMR ($CDCl_3$/TMS) δ 9.20 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 9.13 (s, 1H), 8.68 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 8.60 (s, 1H), 7.82 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 5.23 (s, 2H), 4.91 (brs, NH), 1.36 (s, 9H). Anal. Calc. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38%. Found: C, 63.80; H, 5.04; N, 12.41.

*4.1.6.6. 3-(N-Phenyl)carbamoyloxymethyl-1,8-diazaanthracene-9,10-dione (**11e**).* Following the general procedure, from 40 mg of **10** (0.17 mmol) and 50 mg of phenyl isocyanate (0.42 mmol), 25 mg of triethylamine (0.25 mmol) and dibutyltin diacetate (2 drop) for 22 h was obtained 50 mg of **11d** (81%) as a light yellow solid: m.p. 250–252 °C; IR (KBr) 3358, 2994, 1726, 1669, 1542 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 9.86 (brs, NH), 9.06 (s, 1H), 9.02 (dd, $J = 5.1$ Hz, $J = 1.9$ Hz, 1H), 8.50 (s, 1H), 8.47 (dd, $J = 7.9$ Hz, $J = 1.9$ Hz, 1H), 7.83 (dd, $J = 7.9$ Hz, $J = 5.1$ Hz, 1H), 7.37 (m, 2H), 7.18 (m, 2H), 6.91 (m, 1H), 5.34 (s, 2H). Anal. Calc. for $C_{20}H_{13}N_3O_4$: C, 66.85; H, 3.65; N, 11.69%. Found: C, 66.70; H, 3.56; N, 11.71.

4.2. Biological activity

4.2.1. Materials

For the in vitro assays RPMI 1640 medium was obtained form Gibco BRL. Dimethyl sulphoxide (DMSO) and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

4.2.2. Cell culture

Eight human cancer cell lines, HOP62, SK-OV-3, HCT-15, SF295, MCF7, SNU-354, KB-3-1 and KB-V-1 were used in this study. HOP62, SK-OV-3, HCT-15, SF295, MCF7 and SNU-354 cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum and 2 mM L-glutamine. Human oral epidermoid cancer KB-3-1 cells and KB-V-1 cells were grown in Dulbecco's modified Eagles medium containing 2 mM L-glutamine and 10% fetal calf serum. KB-V-1 cells were maintained in continuous presence of 1 μM vinblastine. All cells were grown at 37 °C in humidified atmosphere with 5% CO_2 and 95% air.

4.2.3. In vitro cytotoxicity assay

Cell numbers were measured indirectly by sulphorhodamine B (SRB) method according to the NCI (USA)'s protocol [11]. Briefly, cells were plated into 96 well plates at a density of 2×10^3 cells per well. Next day (day 0), compounds of interest dissolved in DMSO/media were added with the concentration ranges of 1 nM–10 μM. The final concentration of DMSO was $<$ 0.1%. Seventy-two hours later (day 3), cells were fixed with 10% trichloroacetic acid (TCA) for overnight at 4 °C. The TCA-treated cells were extensively washed with distilled water and dried in the air. Then, SRB solution (0.4% in 1% acetic acid) was added to each well at room temperature for 1 h. Bound dye was solubilised with 10 mM Tris after washing the wells with 1% acetic acid, and absorbances at 690 nm were measured using a microplate reader. The absorbance values measured at day 0 were subtracted from the absorbance values at day 3.

4.2.4. DNA intercalation and Topo-II inhibition assay

For DNA intercalation assay, the electrophoretic migration of supercoiled pRYG DNA (250 ng) premixed with 100 μM of each compound was analysed by using 1% agarose gel in 4 × TAE buffer (160 mM Tris, 4.6 ml l^{-1} glacial acetic acid, and 4 mM EDTA). After electrophoresis at 2 V cm^{-1} for 5 h, DNA bands were stained with 0.5 μg ml^{-1} of ethidium bromide.

Topoisomerase II-mediated DNA relaxation assays were performed by using Topoisomerase II Drug Screening Kit (TopoGEN, Inc., USA). Briefly, supercoiled pRYG DNA (80 ng) was mixed with each compound (50 μM) including VP16 as a positive control, followed by the addition of purified human topoisomerase II (4 units). All of the mixtures contained 30 mM Tris–HCl, pH 7.6, 3 mM ATP, 15 mM mercaptoethanol, 8 mM $MgCl_2$, 60 mM NaCl, and 0.5% DMSO in a total volume of 20 μl. Reactions were initiated by incubation at 37 °C for 30 min, and terminated by 2 μl of 10% SDS. Electrophoresis was carried out by using 1% agarose gel in 4 × TAE buffer supplemented with ethidium bromide (0.5 μg ml^{-1}) at 2 V cm^{-1} for 5 h.

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