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Short communication

Annulation of substituted anthracene-9,10-diones yields promising selectively antiproliferative compounds

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ABSTRACT

Anthraquinone derivatives are well-known antiproliferative compounds, and some are currently used in cancer chemotherapy. Some families of annulated anthraquinone analogs have also been examined for antiproliferative activity, but in this regard almost nothing is known of 1-azabenzanthrones (7*H*-dibenzo[*de*,*h*]quinolin-7-ones). A series of 1-azabenzanthrone derivatives, their 2,3-dihydro analogs, and congruently substituted 9,10-anthracenediones were tested against normal human fibroblasts and four human cancer cell lines. Most of the heterocyclic compounds proved to be weakly to moderately antiproliferative with IC_{50} values extending down to 0.86 μM, and exhibited up to 30-fold selectivity between cancer and normal cells. Both 1-azabenzanthrones and 1-aza-2,3-dihydrobenzanthrones were more potent than their anthraquinone counterparts, and almost without exception, the 2,3-dihydro compounds were more potent than the fully aromatic 1-azabenzanthrones.

1. Introduction

Antiproliferative anthraquinones such as ametantrone and its dihydroxy derivative mitoxantrone are historically important antitumor drugs whose usefulness is limited by toxicity concerns, as is also the case with the structurally more complex anthracyclines. One approach that has been explored to overcome such problems is the annulation of anthraquinones or their aza analogs, which might show reduced cardiotoxicity and at the same time be less susceptible to multidrug resistance. Recent examples of these families of compounds, preceded more than two decades ago by the anthrapyrazoles [1], are the 7-oxo-7*H*-dibenz[*f*,*ij*]isoquinolines (3-azabenzanthrones), 7-oxo-7*H*-benzo[*e*]perimidines (1,3-diazabenzanthrones), 3-azabenzanthra-2,7-diones (anthrapyridones) and 1,2-diazabenzanthra-3,7-diones (anthrapyridazones) (Fig. 1) [2–7].

It is somewhat surprising that the 1-azabenzanthrones (7*H*-dibenzo[*de*,*h*]quinolin-7-ones), which are closely related to anthracene-9,10-diones and the above-mentioned annulated analogs, have not been studied to a comparable extent, particularly considering the many publications and patents related to their chemistry as intermediates for the manufacture of dyes and pigments. It is also of interest to note that the 1-azabenzanthrone skeleton occurs naturally in a small group of alkaloids, limited thus far to the family Menispermaceae, where they are generally termed "oxoisoaporphines". Moreover, a couple of these compounds isolated from *Menispermum dauricum* DC, bearing a nitrogen substituent on the polycyclic scaffold (daurioxoisoaporphines, Fig. 2), are toxic to several cell lines and are claimed to be more potent against human mammary cancer cells than the widely used etoposide or VP-16 [8]. We have recently shown that another aminooxoisoaporphine (lakshminine, **6** from *Sciadotenia toxifera* Krukoff & A.C. Sm.) is weakly toxic, as are one of its unnatural positional isomers and their nitro precursors [9]. In connection with possible anticancer activities, the only other exception seems to be a recent paper describing the synthesis, DNA binding and cytotoxicity of a number of 9-aminoalkanamido-1-azabenzanthrones [10].

As an initial attempt to fill this gap, we have synthesized a more extensive series of 1-azabenzanthrones, their 2,3-dihydro analogs, and 9,10-anthracenediones with congruent substitution patterns (Fig. 3), and determined their toxicities versus normal human fibroblasts and four human cancer cell lines.

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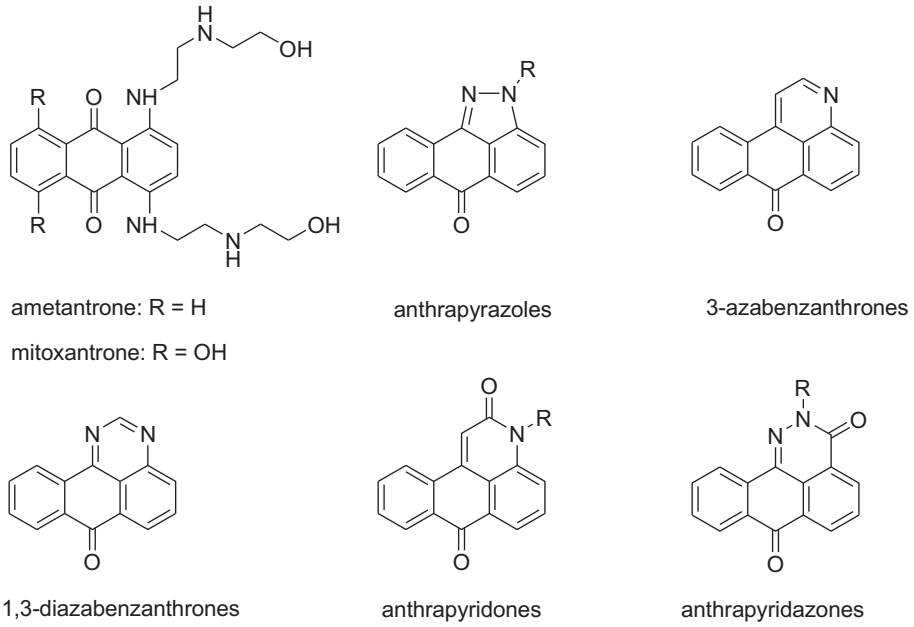


Fig. 1. Some structurally related families of antiproliferative drugs.

2. Results and discussion

2.1. Synthesis

The 1-azabenzanthrones and their 2,3-dihydro derivatives, most of them described previously, required considerably more synthetic effort. Thus, **1A** was obtained starting from *N*-(2-phenylethyl)phthalimide which was reduced and cyclized to afford 5,6,8,12*b*-tetrahydroisoindolo[1,2-*a*]isoquinolin-8-one. This, in turn, was air-oxidized to its 12*b* hydroxy derivative, which by ring-opening and esterification with dimethyl sulfate afforded 1-(2-methoxycarbonylphenyl)-3,4-dihydroisoquinoline, cyclized in sulfuric acid to yield **1A** [11]. **1** was prepared by Pd-catalyzed dehydrogenation of **1A** with air, by analogy with the oxidation of **2A** to **2** [12]. **2A**, **3A** and **4A** were obtained via cyclodehydration of appropriately substituted *N*-phenylethylphthalimidines with polyphosphoric acid, and **2**, **3** and **4** were prepared by the above-mentioned dehydrogenation reaction [12].

Nitration of **2A** with nitric–sulfuric acid mixture in trifluoroacetic acid gave **7A** and **8A**. Amines **5** and **6** were prepared by reduction of **7** and **8**, respectively, with sodium sulfide [9]. Attempts to prepare the amino-2,3-dihydro analogs **5A** and **6A** failed, as the products formed initially by similar reduction of the nitrated **7A** and **8A** were rapidly oxidized in contact with air.

Dinitration of **2A** to afford **9A** required the use of sulfuric acid as solvent. **7**, **8** and **9** were obtained by dehydrogenation of the 2,3-dihydro analogs (**7A**, **8A** and **9A**), as before. Bromination of **1** with molecular bromine in acetonitrile afforded **10**, while the similar reaction of **2** gave a mixture of **11** and **12** [13].

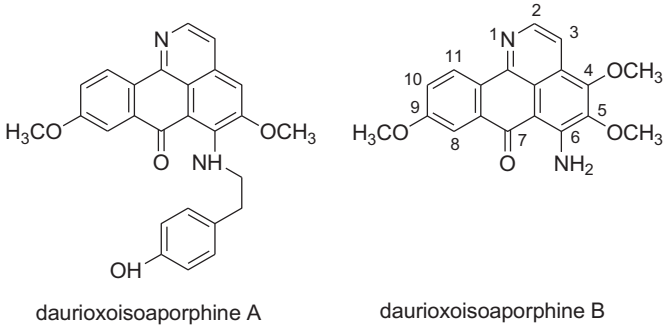


Fig. 2. Structures and numbering scheme of daurioxoisoaporphines A and B.

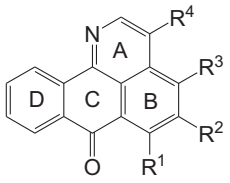
9,10-Anthraquinone **1B** was obtained commercially, and anthracene-9,10-diones **2B**–**9B** were prepared following well-established routes. Thus, 2-methoxyanthracene-9,10-dione (**2B**) was obtained by nucleophilic substitution on the 2-chloro analog [14], **3B** and **4B** were obtained by methylation of alizarin [15,16], and the nitro and amino derivatives (**5B**–**9B**) were prepared by similar routes to those used for the same modifications of the 1-azabenzanthrones and their dihydro analogs [17].

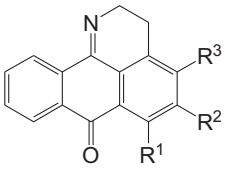
2.2. Pharmacology

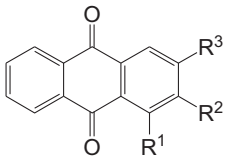
The effects of these compounds on cell proliferation were determined using the MTT reduction assay in five different human cell lines (MRC-5: normal lung fibroblasts; AGS: gastric adenocarcinoma cells; SK-MES-1: lung cancer cells; J82: bladder carcinoma; and HL-60: promyelocytic leukemia cells). The concentrations of the compounds inhibiting cell growth by 50% (IC_{50} values) were obtained adjusting the dose–response curves to a sigmoidal model.

Tables 1–3 show that some of the compounds in these series exhibit IC_{50} values of 4 μM or less, suggesting that they may be of interest for further development. Their selective toxicities toward gastric and bladder cancer cells *vis-à-vis* normal fibroblasts are also promising and, in the case of bladder carcinoma, superior to that of the reference drug (etoposide) (Table 4).

Comparing Tables 1 and 3, it can be seen that annulation of the anthracene-9,10-dione skeleton to 1-azabenzanthrones generally leads to increased antiproliferative potency, with the marked exception of 1,3-dinitro-2-methoxyanthracene-9,10-dione (**9B**), which is fairly toxic to all five cell lines, especially HL-60. Moreover, reduction of these 1-azabenzanthrones to their 2,3-dihydro derivatives (Tables 1 and 2) leads to lower IC_{50} values *vs.* cancer cells, *i.e.* higher potency, in almost every case. Table 4 shows that some of the new compounds are not only antiproliferative with $IC_{50} < 10$ μM (Tables 1–3) but some are also more selective than etoposide *vs.* normal fibroblasts. Of particular interest are the 1-aza-2,3-dihydrobenzanthrones **1A**, **2A** and **3A**, which exhibit comparable potencies to etoposide in gastric adenocarcinoma and bladder carcinoma cells, plus similar to considerably greater selectivity *vs.*







1 $R^1 = R^2 = R^3 = R^4 = H$
2 $R^1 = H$; $R^2 = OMe$; $R^3 = H$; $R^4 = H$
3 $R^1 = OH$; $R^2 = OMe$; $R^3 = R^4 = H$
4 $R^1 = R^2 = OMe$; $R^3 = R^4 = H$
5 $R^1 = H$; $R^2 = OMe$; $R^3 = NH_2$; $R^4 = H$
6 $R^1 = NH_2$; $R^2 = OMe$; $R^3 = R^4 = H$
7 $R^1 = H$; $R^2 = OMe$; $R^3 = NO_2$; $R^4 = H$
8 $R^1 = NO_2$; $R^2 = OMe$; $R^3 = R^4 = H$
9 $R^1 = NO_2$; $R^2 = OMe$; $R^3 = NO_2$; $R^4 = H$
10 $R^1 = R^2 = R^3 = H$; $R^4 = Br$
11 $R^1 = H$; $R^2 = OMe$; $R^3 = H$; $R^4 = Br$
12 $R^1 = H$; $R^2 = OMe$; $R^3 = Br$; $R^4 = H$

1A $R^1 = R^2 = R^3 = H$
2A $R^1 = H$; $R^2 = OMe$; $R^3 = H$
3A $R^1 = OH$; $R^2 = OMe$; $R^3 = H$
4A $R^1 = OMe$; $R^2 = R^3 = H$
5A $R^1 = H$; $R^2 = OMe$; $R^3 = NH_2$
6A $R^1 = NH_2$; $R^2 = OMe$; $R^3 = H$
7A $R^1 = H$; $R^2 = OMe$; $R^3 = NO_2$
8A $R^1 = NO_2$; $R^2 = OMe$; $R^3 = H$
9A $R^1 = NO_2$; $R^2 = OMe$; $R^3 = NO_2$

1B $R^1 = R^2 = R^3 = H$
2B $R^1 = H$; $R^2 = OMe$; $R^3 = H$
3B $R^1 = OH$; $R^2 = OMe$; $R^3 = H$
4B $R^1 = R^2 = OMe$; $R^3 = H$
5B $R^1 = H$; $R^2 = OMe$; $R^3 = NH_2$
6B $R^1 = NH_2$; $R^2 = OMe$; $R^3 = H$
7B $R^1 = H$; $R^2 = OMe$; $R^3 = NO_2$
8B $R^1 = NO_2$; $R^2 = OMe$; $R^3 = H$
9B $R^1 = NO_2$; $R^2 = OMe$; $R^3 = NO_2$

Fig. 3. Structures of 1-azabenzanthrones, 1-aza-2,3-dihydrobenzanthrones and similarly substituted anthracene-9,10-diones.

normal cells. The brominated 1-azabenzanthrone **11** is also noteworthy, as it is quite active *vs.* stomach, lung and bladder cancer cells in culture, again with similar or greater selectivity than etoposide.

The recently described series of antiproliferative 9-aminoalkanamido-1-azabenzanthrones is suggested to act primarily via intercalation between DNA base pairs, as is believed to be the case with planar anthracene-9,10-diones such as ametantrone and mitoxantrone [10]. A recent paper describes the X-ray crystallographic structure of 1-aza-6-[(2-hydroxyethyl)amino]benzanthrone, in which the tetracyclic core is quite flat and in addition shows a stacking interaction in the crystal which mimics the stacking interactions of molecules that intercalate between DNA base pairs [18]. Besides, the (protonated) amino side chains of these anthraquinones and 1-azabenzanthrones are expected to enhance DNA binding by interacting with the negatively charged phosphate residues of the nucleic acid [10]. Our active compounds, which are at least as potent as the 9-substituted 1-azabenzanthrones or more so, lack amino side chains. Besides, they do not have the perfectly flat ring system of the wholly aromatic 1-azabenzanthrones and anthraquinones, suggesting that DNA intercalation might not be favored to the same extent.

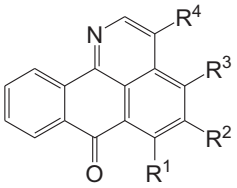
The 1-aza-2,3-dihydrobenzanthrone molecules are expected to depart from strict planarity, and indeed the X-ray diffraction structures of **2A** and **3A**, which are among the most potent of this series, clearly show the nonplanarity of ring A in the crystals [19,20]. Although this feature does not preclude DNA intercalation, it would seem to make it less likely. Nevertheless, the similarly nonplanar aporphine alkaloid dicentrine inhibits topoisomerase II and is active in a DNA unwinding assay, leading to the proposal that in complex with biological molecules this alkaloid might adopt a slightly less stable planar conformation as an "adaptive" DNA intercalator [21], which could also be the case with our compounds.

It may be pointed out that both **2A** and **3A** have been shown to undergo reversible electrochemical reduction, via a semiquinone radical, to their 4-aminophenol analogs [22]. Autoxidation of these reduced forms could lead to the unsurmountable generation of reactive oxygen species via redox cycling, a well-established mechanism of cell toxicity [23]. If this were so, the 1-aza-2,3-dihydrobenzanthrones might be acting as mitochondrial poisons, interfering with electron transfer and causing the disruption of these cellular power plants.

We had shown previously that the introduction of an amino group at C4 (**5**) leads to some toxicity to normal fibroblasts, but negligible effects on the tumor cell lines, while amination at C6 (**6**) results in modest antiproliferative activity in all the tested cell lines with no significant selectivity [9]. The 2,3-dihydro compounds **5A** and **6A** were not tested because they could not be isolated in adequate purity, as described above.

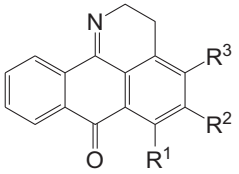
Although 1-aza-5-methoxybenzanthrone (**2**) is practically inactive, its 4-nitro derivative (**7**) is quite potent *vs.* normal and tumor cell lines, while its 6-nitro isomer (**8**) is less so, and the 4,6-dinitro analog (**9**) exhibits intermediate activity in all cell lines. The correspondingly nitrated 2,3-dihydro compounds (**7A**, **8A** and **9A**) show a similar trend to the fully aromatic **7**, **8** and **9**. However, the non-nitrated **2A** somewhat resembles the 4-nitro **7A** in its activities, with the important difference that the nitrated compound is several-fold more toxic to normal cells. These results may be compared with the behavior in the anthraquinone series, where the inactive **2B** acquires fairly weak activities on mononitration at C3 (**7B**), but not at C1 (**8B**). However, anthraquinone **2B** becomes quite

Table 1
In vitro activity (IC_{50}, μM) of 1-azabenzanthrones (boldface numbers) *vs.* normal human fibroblasts (MRC-5), gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), bladder carcinoma (J82), and myelocytic leukemia (HL-60) cells. Each concentration was tested in quadruplicate and repeated three times in separate experiments. Values are expressed as means ± S.E.M.



Compound	IC_{50} (μM)				
	MRC-5	AGS	SK-MES-1	J82	HL-60
1	41.6 ± 2.9	32.9 ± 1.6	>100	93.6 ± 5.7	–
2	>100	>100	>100	>100	–
3	65.1 ± 3.9	45.5 ± 2.7	>100	62.6 ± 3.8	–
4	>100	>100	>100	>100	–
5	37.0 ± 1.9	>100	>100	>100	–
6	17.9 ± 0.7	78.6 ± 3.9	43.3 ± 3.4	37.6 ± 2.6	–
7	10.2 ± 0.5	4.5 ± 0.3	17.1 ± 1.0	25.4 ± 1.3	–
8	80.0 ± 4.8	47.7 ± 2.9	>100	48.3 ± 2.4	–
9	41.8 ± 2.7	36.3 ± 2.5	55.6 ± 3.3	76.6 ± 6.3	22.2 ± 1.3
10	>100	>100	>100	>100	–
11	28.9 ± 2.0	3.3 ± 0.2	9.2 ± 0.5	2.4 ± 0.1	–
12	>100	94.9 ± 6.1	73.9 ± 4.4	>100	–
Etoposide	3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.2	1.8 ± 0.1

Table 2
In vitro activity (IC_{50}, μM) of 1-aza-2,3-dihydrobenzanthrones (numbers followed by letter **A**) *vs.* normal human fibroblasts (MRC-5), gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), bladder carcinoma (J82), and myelocytic leukemia (HL-60) cells. Each concentration was tested in quadruplicate and repeated three times in separate experiments. Values are expressed as means ± S.E.M.

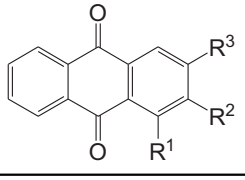


Compound	IC_{50} (μM)				
	MRC-5	AGS	SK-MES-1	J82	HL-60
1A	28.0 ± 1.7	1.5 ± 0.1	28.9 ± 2.0	0.86 ± 0.04	–
2A	85.2 ± 5.1	6.8 ± 0.3	18.2 ± 0.4	3.0 ± 0.2	–
3A	42.4 ± 2.6	3.9 ± 0.2	73.8 ± 4.6	5.8 ± 0.4	–
4A	50.5 ± 3.0	62.1 ± 3.7	17.2 ± 0.9	33.9 ± 2.4	–
5A	–	–	–	–	–
6A	–	–	–	–	–
7A	11.7 ± 0.6	5.7 ± 0.3	23.0 ± 1.4	22.9 ± 1.7	–
8A	65.6 ± 4.6	18.4 ± 1.1	>100	22.8 ± 1.6	–
9A	36.4 ± 2.2	33.3 ± 1.8	25.7 ± 1.8	17.5 ± 1.1	19.5 ± 1.9
Etoposide	3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.2	1.8 ± 0.1

toxic to all cell lines upon introduction of two nitro groups at these two positions (**9B**). These results might be related to the hypothetically different ease of reduction of the nitro groups to anion radical species, as in a number of heterocyclic nitro compounds used or tested as antiparasitic drugs [24]. Nevertheless, lacking electrochemical studies, such a proposal can only be tentative.

Intriguingly, bromination of the inactive 1-aza-5-methoxybenzanthrone (**2**) at C3 to generate **11**, but not at C4, giving **12**, leads to a considerable increase in potency, affording one of the most potent analogs, and by far the most potent (**11**) of the fully aromatic compounds of this series. The lipophilicity of the isomeric **11** and **12** should be almost identical, practically ruling out the hypothesis that differential cell penetration and/or subcellular distribution play a role in their different activities. Therefore, the electronic structures of these isomers might be considered. A bromine atom at the same position of 1-aza-2-methoxybenzanthrone is susceptible to nucleophilic substitution [25], and therefore

Table 3
In vitro activity (IC_{50}, μM) of anthracene-9,10-diones (numbers followed by letter **B**) *vs.* normal human fibroblasts (MRC-5), gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), bladder carcinoma (J82), and myelocytic leukemia (HL-60) cells. Each concentration was tested in quadruplicate and repeated three times in separate experiments. Values are expressed as means ± S.E.M.



Compound	IC_{50} (μM)				
	MRC-5	AGS	SK-MES-1	J82	HL-60
1B	>100	>100	>100	>100	>100
2B	>100	>100	>100	>100	>100
3B	>100	>100	>100	>100	>100
4B	>100	>100	>100	>100	>100
5B	>100	48.9 ± 3.4	56.8 ± 3.4	42.9 ± 3.9	>100
6B	>100	>100	>100	>100	>100
7B	95.0 ± 5.5	82.5 ± 5.8	78.4 ± 4.9	62.0 ± 4.2	35.6 ± 2.5
8B	>100	>100	>100	>100	>100
9B	11.4 ± 0.7	11.7 ± 0.8	15.4 ± 1.0	18.1 ± 1.5	4.1 ± 0.3
Etoposide	3.9 ± 0.2	0.36 ± 0.02	2.5 ± 0.2	2.8 ± 0.2	1.8 ± 0.1

Table 4
Selectivity ratios (based on IC_{50} values) of some 1-azabenzanthrones (boldface numbers), 1-aza-2,3-dihydrobenzanthrones (numbers followed by letter **A**) and anthracene-9,10-diones (numbers followed by letter **B**), and etoposide, comparing their activities *vs.* gastric adenocarcinoma (AGS), lung cancer (SK-MES-1), bladder carcinoma (J82), and myelocytic leukemia (HL-60) cells with their activities *vs.* normal human fibroblasts (MRC-5). $IC_{50\ (MRC\text{-}5)}/IC_{50\ (tumor\ cell\ line)} \leq 1$ is nonselective.

Compound	$IC_{50\ (MRC\text{-}5)}/IC_{50\ (tumor\ cell\ line)}$			
	AGS	SK-MES-1	J82	HL-60
1A	19	1.0	33	–
2A	13	4.7	28	–
3A	11	0.6	7.3	–
7	2.3	0.6	0.4	–
7A	2.1	0.5	0.5	–
9B	1.0	0.7	0.6	2.8
11	8.8	3.1	12	–
Etoposide	11	1.6	1.4	2.2

reaction of **11** with a cellular nucleophile might explain this unexpected result, either due to damage to some essential component of the cell or to the generation of a more toxic 1-azabenzanthrone.

2.3. Conclusion

In summary, our preliminary exploration of the 1-azabenzanthrone scaffold in regard of its antiproliferative properties has uncovered several compounds that are sufficiently potent to warrant further study, with IC_{50} values in the same range as the important anticancer drug etoposide, and, in comparison with the normal fibroblast line, with up to 30-fold selectivity for the J82 bladder carcinoma cell line, for which etoposide is practically unselective.

3. Experimental section

3.1. Chemistry

3.1.1. General experimental procedures

All reagents and solvents were commercially available from Sigma–Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) and were used without further purification. Melting points are uncorrected and were determined with a Reichert Galen III hot-plate microscope equipped with a DUAL JTEK Dig-Sense thermocouple thermometer. ^1H and ^{13}C NMR spectra were recorded at 300 or 400 MHz and 75 or 100 MHz, respectively, on Bruker Avance 300 or AMX 400 spectrometers, using $CDCl_3$, D_2O or DMSO-d_6 as solvent. The chemical shifts are reported as δ (ppm) downfield from TMS for ^1H NMR and relative to the central DMSO-d_6 resonance (39.5 ppm) for ^{13}C NMR. Coupling constants (J) are given in Hz. EIMS were run on a Thermo Finnigan MAT 95XP instrument, with electron impact ionization at 70 eV and with perfluorokerosene as reference. Purities of the compounds subjected to biological testing were >95% in every case (quantitative ^1H NMR or HPLC). Elemental analyses were done with a Fisons Instruments EA-1108 apparatus. Most of the syntheses are either classical or have recently been described in detail [13].

3.1.1.1. 2,3-Dihydro-5-methoxy-4,6-dinitro-7H-dibenzo[de,h]quinolin-7-one (1-aza-5-methoxy-4,6-dinitrobenzanthrone, ***9A****).* To a solution of **2A** (0.5 g, 1.9 mmol) in concentrated H_2SO_4 (2 mL), H_2SO_4/HNO_3 1:1 (5 mL) was added carefully, stirring at room temperature for 2 h. The reaction mixture was poured into water (50 mL), made alkaline with $NaOH_{(aq)}$ (pH 8–9) and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water and dried over Na_2SO_4. The solvent was evaporated leaving a dark brown residue that was chromatographed on silica gel (AcOEt) to afford **9A** 0.407 g (61%); Mp 170–171 °C; ^1H NMR ($CDCl_3$) δ 2.94 (2H, t, J = 7.9 Hz, H2 or 3), 3.97 (3H, s, OCH_3), 4.21 (2H, t, J = 7.8 Hz, H3 or 2), 7.80 (1H, t,

J = 7.6 Hz, H10 or 9), 7.91 (1H, t, J = 7.4 Hz, H9 or 10), 8.13 (1H, d, J = 7.8 Hz, H11), 8.37 (1H, d, J = 7.8 Hz, H8).

3.1.1.2. 5-Methoxy-4,6-dinitro-7H-dibenzo[de,h]quinolin-7-one (1-aza-5-methoxy-4,6-dinitrobenzanthrone, ***9****).* A solution of **9A** in toluene (10.0 mL) was stirred under reflux, in the presence of air, with Pd/C (0.1 g) for 24 h. The suspension was filtered, and the solution was concentrated to dryness to leave the crude dehydrogenated product, 72% yield; Mp 197–200 °C; ^{1}H NMR ($CDCl_3$) δ, 4.16 (3H, s, OCH_3), 7.58 (1H, d, J = 5.9 Hz, H3), 7.73 (1H, t, J = 7.8 Hz, H11), 7.89 (1H, t, J = 7.6 Hz, H10 or 9), 8.33 (1H, t, J = 7.1 Hz, H9 or 10), 8.89 (1H, d, J = 7.8 Hz, H8), 8.94 (1H, d, J = 5.9 Hz, H2).

3.2. In vitro testing

3.2.1. Cell culture and MTT assay

The MTT reduction assay was used to determine the viability of MRC-5 normal human lung fibroblasts (ATCC, CCL-171), AGS human gastric adenocarcinoma cells (ATCC, CRL-1739), SK-MES-1 human lung cancer cells (ATCC, HTB-58), J82 human bladder carcinoma cells (ATCC, HTB-1), and HL-60 human myelocytic leukemia (ATCC, CCL-240) from the American Type Culture Collection (Manassas, VA, USA). MRC-5, J82, and SK-MES-1 cells were grown in Eagle's Minimum Essential Medium, containing 2 mM L-glutamine, 1 mM sodium pyruvate and 1.5 g/L $NaHCO_3$.

AGS cells were grown in Ham's F-12 medium supplemented with 2 mM L-glutamine and 1.5 g/L $NaHCO_3$. HL-60 cells were grown in RPMI 1640 medium containing 1 mM sodium pyruvate and 2 g/L $NaHCO_3$. All media were additionally supplemented with 10% heat-inactivated fetal bovine serum, 100 IU/mL penicillin G, and 10 μg/mL streptomycin. Cells were sub-cultured once a week and the medium was changed every two days. The cells were stored in liquid nitrogen in media with 10% glycerol added, and their viability after thawing was higher than 90%, as assessed by the trypan blue exclusion test. For the assay, cells were plated in 96-well plates (100 μL/well) at a density of 5×10^4 cells/mL. One day after seeding, the cells were treated with the medium containing the compounds at concentrations ranging from 0 to 100 μM, first dissolved in DMSO (final concentration of 1%), diluted with complete medium, and incubated for 72 h in a humidified incubator with 5% CO_2 in air at 37 °C, after which the MTT reduction assay was performed as described previously [26]. Etoposide (98% purity, Sigma–Aldrich, St. Louis, MO, USA) was used as a positive control. Each experiment was carried out three times in quadruplicate. The IC_{50} value was obtained adjusting the dose–response curve to a sigmoidal model

$$\left(a + (b-a)/1 + 10^{(x-c)}\right), \text{ where c } = \log \mathrm{IC}_{50}.$$

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