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# Novel Ametantrone–Amsacrine Related Hybrids as Topoisomerase IIβ Poisons and Cytotoxic Agents

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The precise definition of the structural requirements for effective topoisomerase II poisoning by drug molecules is still an elusive issue. In the attempt to better define a pharmacophoric pattern, we prepared several conjugates combining the chemical features of two well-known topoisomerase II poisons, amsacrine and ametantrone. Indeed, an appropriate fusion geometry, which entails the anthracenedione moiety of ametantrone appropriately connected to the methanesulfonamidoaniline side chain of amsacrine, elicits DNA-intercalating properties, the capacity to inhibit the human topoisomerase IIβ isoform, and cytotoxic activity resembling that of the parent compounds. In addition, the properties of the lateral groups linked to the anthracenedione group play an important role in modulating DNA binding and cell cytotoxicity. Among the compounds tested, **10**, **11**, and **19** appear to be promising for further development.

**Keywords:** Ametantrone / Amsacrine / DNA / Intercalation / Topoisomerase II

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## Introduction

The concept of the pharmacophore as "the ensemble of steric and electronic features necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response" [1, 2] urges the medicinal chemist to identify the essential features that favor selective drug-receptor recognition. In this connection, the case of the enzyme topoisomerase II [3] represents an interesting example where a "transient" pharmacophoric receptor is generated at the interface between a nucleic acid and a protein, when the latter is covalently linked to the former during the catalytic cycle. DNA topoisomerases regulate DNA supercoiling in all living organisms. Type II enzymes introduce double-strand breaks in a DNA duplex cleaving a phosphodiester bond by a transesterification reaction in which an enzymatic tyrosine plays an essential role; then, they force the passage of another duplex through the break and, as a final step, rejoin the breaks by the reverse transesterification reaction. The precise mechanism of the reaction steps is still largely unknown as regards the stereochemistry [4, 5] and, mainly, the time course [6]. Several drugs, among which anthracenediones and acridines deserve special mention, are reported to interfere with the covalent DNA–enzyme intermediate, called the "cleavage complex" [7–9], stabilising it and triggering apoptotic cell death [10–12]. On the other hand, an extensive and irrefutable description of the drug–DNA interaction motif has not been provided yet, and molecular modeling strategies are still widely used to investigate a realistic model [12b]. In addition to this, despite the presence of several drugs reported to be effective against the complex, the quest for new candidates is still open to provide a consistent aid both in the antibacterial and in the anticancer field [12c]. To support this research, structural informations concerning the ligand and,

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in particular, the pharmacophore are of a primary relevance. Given the importance of understanding the molecular mechanism(s) by which topoisomerase II poisons produce anticancer effects [13], we considered two currently used drugs, ametantrone and amsacrine. Ametantrone interacts with the double helix of the deoxyribonucleic acid likely by positioning the two side chains in the DNA major groove and the anthraquinone chromophore intercalated into the double helix with its long axis almost perpendicular to the long axis of base pairs [14–19]. For amsacrine, evidence shows that the 4-methanesulfonamidoaniline chain lies in the minor groove tilted approximately 70° with respect to the base pair plane and the acridine is intercalated with its long axis parallel to the long axis of base pairs with the heterocyclic nitrogen in the center of the major groove [20, 21]. Despite these observations, eukaryotic topoisomerase II-DNA–drug structures are still being investigated and some models were recently described [22a]. In this paper, while the position of amsacrine relative to the nucleic acid confirms previous data, mitoxantrone and ametantrone show the anthraquinone parallel to the long axis of base pairs in contrast with previous reports [22b]. To better understand the relevance of the side chains relative to the one of the chromophore in the interaction of the drug with the polynucleotide we decided to design hybrid compounds derived from the combination of side chains from ametantrone with amsacrine moieties or their analogs maintaining the anthraquinone scaffold. This project can be summarized as in Fig. 1.

Two identical side chains were attached, as in ametantrone, in positions 1 and 4 of the anthracene-9,10-dione planar chromophore while the 4-methanesulfonamidoaniline substituent (with and without a methoxy group in position 2′), characteristic of amsacrine, was linked in position 6. Then, assuming that the anthracene-9,10-dione major axis is perpendicular to the bases major axis as it is in mitoxantrone and anthracycline with DNA [23, 24], the new compounds should interact with DNA positioning the twin chains within the major groove and the 4-methanesulfonamidoaniline substituent in the minor groove, thus reinforcing complex formation and, possibly, exhibiting effective poisoning of topoisomerase II; while if, as stated by Wu [22a], the anthracene-9,10-dione major axis essentially parallels the bases major axis we should observe a decrease in activity in respect to the one of the parent compounds [25]. If so the interactions of side chains with the polynucleotide and the enzyme, and not the stacking of the anthraquinone with the bases, give the major stabilizing contribution to the interaction.

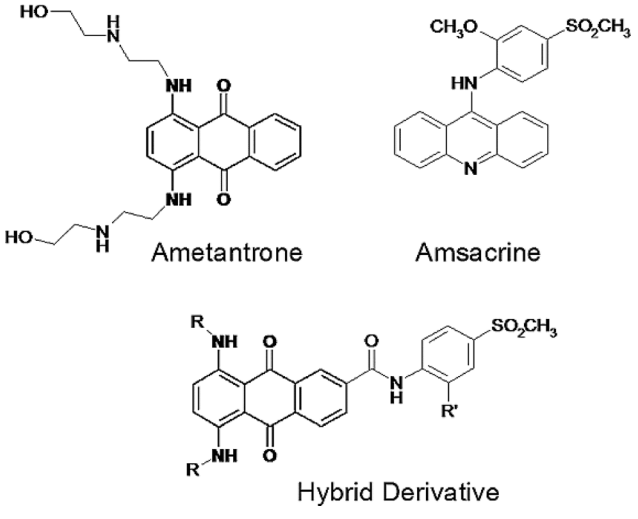



**Figure 1.** Conjugation of ametantrone and amsacrine structural features to give an hybrid derivative.

## Results

### Chemistry

#### *Synthesis*

The chemistry was quite straightforward: the starting synthon, 1,4-dihydroxy-6-carboxyanthracene-9,10-dione, was synthesized from trimellitic anhydride and hydroquinone by a known Friedel Crafts followed by a mild air oxidation [26] as shown in Scheme 1.

Subsequently, the two phenolic groups were protected, the carboxy group activated as chloride and 4-methanesulfonamidoaniline or 2-methoxy-4-methanesulfonamidoaniline added to obtain the desired amide. After deprotecting the phenolic groups we obtained the intermediate **4** or **5**, respectively.

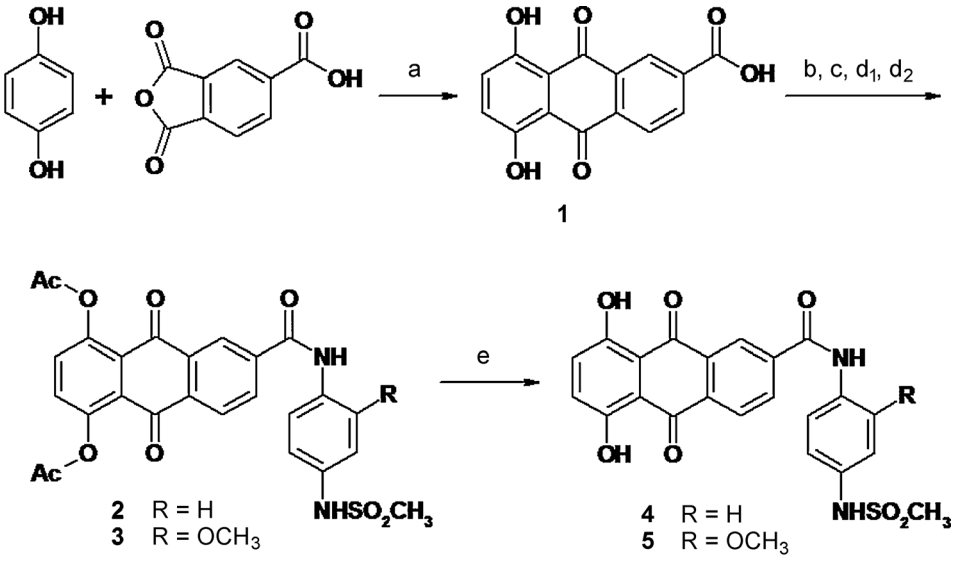
This was reduced to the dihydroderivative, then reacted with the proper primary amine, and finally mildly oxidized to give the compounds **6**–**19**. The side chains were selected according to literature-derived criteria [23–25] and keeping the ametantrone side chain (compounds **14**–**15**) and its oxygen homologous (compounds **12**–**13**) since the basic nitrogen forms an important electrostatic bond between its protonated form and the phosphate anion in the DNA backbone (Scheme 2).

The purity of all final compounds was determined by HPLC as reported in the Supplementary Material. The NMR spectra to check for particular constraints in the side chains positions were also run; an example is reported in the Supplementary Material.

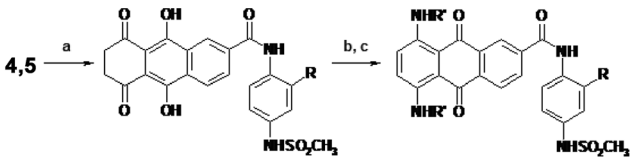
### Cytotoxic activity

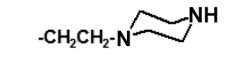
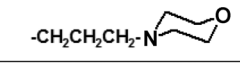
Ametantrone–amsacrine hybrids were tested for their cytotoxic activity toward four human tumor cell lines including examples of ovarian (2008) and breast (MCF-7) cancer, melanoma (A375), and promyelocytic leukemia (HL-60).




**Scheme 1.** Synthesis of compounds **1–5**. Reagents and conditions: (a) $AlCl_3$, NaCl, 150°C, 7 h; (b) acetic anhydride, $H_2SO_4$, reflux, 3 h; (c) $SOCl_2$, DMF, reflux, 3 h; ($d_1$) 4-methanesulfonamidoaniline, triethylamine, $CH_2Cl_2$, reflux, 1.5 h; ($d_2$) 2-methoxy-4-methanesulfonamidoaniline, triethylamine, $CH_2Cl_2$, reflux, 1.5 h; (e) $K_2CO_3$ 5% solution in water, 80°C, 2 h.




| R = H | R' | R = OCH$_3$ |
|---|---|---|
| **6** | -$CH_2CH_2OH$ | **7** |
| **8** | -$CH_2CH_2NHCOCH_3$ | **9** |
| **10** | -$CH_2CH_2CH_2N(CH_3)_2$ | **11** |
| **12** | -$CH_2CH_2OCH_2CH_2OH$ | **13** |
| **14** | -$CH_2CH_2NHCH_2CH_2OH$ | **15** |
| **16** |  | |
| **17** |  | **18** |
| **19** | -$CH_2CH_2N(CH_3)_2$ | |

**Scheme 2.** Synthesis of compounds **6–19**. Reagents and conditions: (a) $Na_2S_2O_4$, $N_2$, $H_2O$, 70–80°C; (b) $NH_2R$, $N_2$, $H_2O$, 100°C, 16 h; (c) $O_2$, $H_2O$, 25°C, 6 h.

Cytotoxicity was evaluated by means of MTT test after 72 h of treatment with increasing concentrations of the tested compounds. For comparison purposes, cytotoxicities of ametantrone and amsacrine were evaluated under the same experimental conditions. $IC_{50}$ values, calculated from dose-survival curves, are reported in Table 1.

Among all newly synthesized ametantrone–amsacrine hybrids, derivatives **9**, **12**, **13**, **17**, and **18** proved to be completely ineffective against all cancer cell lines whereas compounds **7** and **8** showed a negligible *in vitro* antiproliferative activity. Compounds **6**, **14**, **15**, and **16** as well as the intermediates **4** and **5** showed a detectable cytotoxic potency, with $IC_{50}$ values more than 2 orders of magnitude higher than those of ametantrone and roughly 20-fold higher than those recorded with amsacrine. Conversely, compounds **10**, **11**, and **19**, all three bearing tertiary non-cyclic amine side chain, despite retaining a lower antiproliferative activity than ametantrone, showed a cytotoxicity response similar to that of amsacrine with $IC_{50}$ values in the low micromolar range.

Cytotoxic activity of the most promising derivatives **10**, **11**, and **19** was also assessed against two additional human cancer cell lines, LoVo and LoVo MDR, the latter possessing a MultiDrug Resistant (MDR) phenotype. It is well known that acquired MDR, whereby cells become refractory to multiple drugs, poses a most important challenge to the success of anticancer chemotherapy. The resistance of LoVo MDR cells to doxorubicin, a drug belonging to the MDR spectrum, is associated with an overexpression of the multi-specific drug transporters, such as the 170 kDa P-glycoprotein (P-gp) [26].

**Table 1.** Cytotoxicity of the new derivatives on cancer cell lines.

| | $IC_{50}$ [μM] ± SD | | | |
|---|---|---|---|---|
| **Compounds** | **2008** | **A375** | **MCF-7** | **HL60** |
| **4** | 54.23 ± 2.12 | >100 | 64.34 ± 2.23 | 81.32 ± 2.12 |
| **5** | 47.54 ± 1.23 | 54.56 ± 2.11 | 47.45 ± 2.23 | 67.54 ± 3.11 |
| **6** | 66.43 ± 2.21 | >100 | 57.98 ± 3.32 | 69.65 ± 2.54 |
| **7** | 79.43 ± 1.23 | 84.43 ± 1.98 | 96.52 ± 3.43 | 89.22 ± 3.54 |
| **8** | 73.71 ± 2.11 | >100 | 85.43 ± 3.33 | >100 |
| **9** | >100 | >100. | >100 | >100 |
| **10** | 6.33 ± 2.53 | 5.28 ± 1.33 | 6.71 ± 2.02 | 4.34 ± 0.99 |
| **11** | 2.56 ± 1.85 | 2.01 ± 1.12 | 3.33 ± 1.75 | 1.06 ± 0.76 |
| **12** | >100 | >100 | >100 | >100 |
| **13** | >100 | >100 | >100 | >100 |
| **14** | 63.33 ± 3.25 | >100 | 55.43 ± 2.12 | 71.14 ± 1.86 |
| **15** | 42.22 ± 2.87 | 51.21 ± 3.64 | 61.31 ± 2.93 | 63.01 ± 2.23 |
| **16** | 59.11 ± 3.60 | >100 | 69.68 ± 2.46 | 72.32 ± 2.11 |
| **17** | >100 | >100 | >100 | >100 |
| **18** | >100 | >100 | >100 | >100 |
| **19** | 9.31 ± 2.11 | 5.25 ± 2.53 | 7.35 ± 2.63 | 6.17 ± 2.43 |
| Ametantrone | 0.03 ± 0.01 | 0.99 ± 0.12 | 0.51 ± 0.23 | 0.36 ± 0.09 |
| Amsacrine | 4.22 ± 0.76 | 2.32 ± 0.69 | 4.53 ± 0.94 | 1.15 ± 0.64 |

$IC_{50}$ values were calculated by four parameter logistic model ($p < 0.05$). Cells ($3–8 \times 10^4$/mL) were treated for 72 h with increasing concentrations of the tested compounds. SD, standard deviation.

Cytotoxicity was assessed after a 48-h exposure by the MTT test. Cross-resistance profiles were evaluated by means of the resistance factor (RF), which is defined as the ratio between $IC_{50}$ values calculated for resistant cells and those arising from chemosensitive ones (Table 2).

Derivatives **10**, **11**, and **19** showed a similar pattern of response across the parental (LoVo cells) and the resistant (LoVo MDR) subline and allowed the calculation of RF values about 25 times lower than that obtained with doxorubicin, suggesting that these new derivatives may not be deemed as potential MDR substrates.

## DNA binding mode

Spectroscopic data indicated that the tested derivatives efficiently interact with calf thymus DNA. Unfortunately, extensive drug aggregation processes prevented a precise quantification of the binding affinity. Qualitatively, compound **10** appeared to exhibit the most effective binding to the nucleic acid and no precipitation was observed in the range of measured concentrations, so it was considered to study its interaction with DNA. In addition, the same compound was able to intercalate into DNA at micromolar concentration as shown by the unwinding assays performed in the presence of the enzyme topoisomerase I. In fact, the effects of intercalation antagonize enzyme-induced relaxation of the supercoiled plasmid. An example is reported in Fig. 2. Indeed, compound **10** shows interference with the relaxation process at concentrations between 1 and 2 μM, whereas compound **14** along with the other congeners (not shown) is not effective up to 20 μM.

**Table 2.** MDR cross-resistance profiles.

| **Compounds** | **LoVo $IC_{50}$ [μM] ± SD** | **LoVo MDR $IC_{50}$ [μM] ± SD** | **RF** |
|---|---|---|---|
| **10** | 4.34 ± 1.12 | 5.32 ± 1.15 | 1.2 |
| **11** | 3.42 ± 1.01 | 4.43 ± 1.65 | 1.3 |
| **19** | 5.23 ± 1.44 | 5.32 ± 2.36 | 1.0 |
| Ametantrone | 0.74 ± 1.23 | 5.77 ± 2.65 | 7.8 |
| Doxorubicin | 1.48 ± 0.33 | 43.46 ± 3.32 | 29.4 |

$IC_{50}$ values were calculated by four parameter logistic model ($p < 0.05$). Cells ($5 \times 10^4$/mL) were treated for 48 h with increasing concentrations of tested compounds. Cytotoxicity was assessed by MTT test. SD, standard deviation.

## Topoisomerase II poisoning

Topoisomerase II poisoning tests showed that only derivative **10** was a good inhibitor of the topoisomerase II activity, producing 100% inhibition at a concentration below 5 μM (same as amsacrine), the others not being effective up to 25 μM.

Compound **10** was further investigated for activity against the human enzyme isoforms α and β. An *in vitro* DNA cleavage assay was performed and the fraction of linearized plasmid DNA was quantified in the presence of different drug concentrations.

We compared the test compound to the known topoisomerase poison VM26 as a positive control and to derivative **4**, lacking the ametantrone side chains, as a negative control. The latter compound had no effect on topoisomerase IIβ (Fig. 3A, lanes 3–5). On the opposite, derivative **10** has a clear effect producing 30–40% of the linearized form of plasmid DNA. Moreover, **10** appears to be remarkably more potent than VM26 since 1 μM of the former produces an effect similar to 50 μM of the latter (compare lane 2 to lane 6 in Fig. 3A). Much lower DNA cleavage was detected when using the topoisomerase IIα isoform (not shown). Hence, compound **10** shows selectivity for the β isofom of the enzyme.

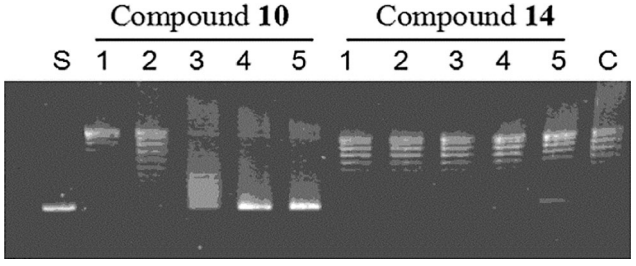



**Figure 2.** Topoisomerase I relaxation reactions of supercoiled pBR322 performed in the presence of increasing concentrations of compounds **10** and **14** (lane 1, 1.25 μM; lane 2, 2.5 μM; lane 3, 5 μM; lane 4, 10 μM; and lane 5, 20 μM). S, supercoiled pBR 322; C, relaxed pBR 322 obtained incubating the plasmid DNA with the enzyme in the absence of drug.

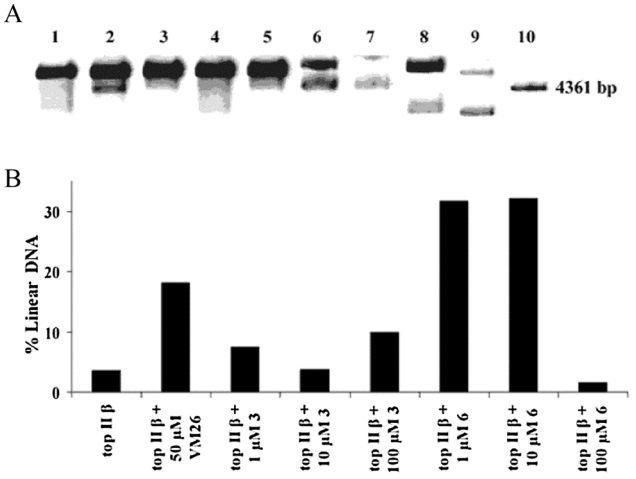



**Figure 3.** DNA cleavage results. (A) Lanes are: 1, top IIβ; 2, top IIβ + 50 μM VM26; 3, top IIβ + 1 μM **4**; 4, top IIβ + 10 μM **4**; 5, top IIβ + 100 μM **4**; 6, top IIβ + 1 μM **10**; 7, top IIβ + 10 μM **10**; 8, top IIβ + 100 μM **10**; 9, supercoiled pBR322 DNA; 10, *Hin*dIII-linearized pBR322 DNA. (B) DNA cleavage levels as determined by analyses of gels such as the one shown in panel (A).

Effective poisoning was also observed with compounds **11** and **19**, which did not cause substantial DNA precipitation in the presence of the enzymes.

Next, we determined the ability of the new drugs to inhibit the catalytic activity of both human topoisomerase II isoforms by a relaxation assay (Fig. 4).

In this assay, similar catalytic amounts of the two human isozymes were used, corresponding to about fivefold less proteins than in the DNA cleavage assay. Again, **10** was substantially more active than **4** on topoisomerase IIβ. Moreover, the latter isoform was more sensitive to **10** than the topoisomerase IIα form. Altogether, the biochemical tests show that **10** is an effective poison of human topoisomerase II with selective recognition of the β form.

## Discussion

A set of new molecules was designed, synthesized, and tested starting from the idea of rationally combining the features of two chemical structures sharing common molecular target(s) and mechanism of action [27–31] and so contribute to a better definition of the yet ill-defined pharmacophore of topoisomerase II poisons and possibly identify novel useful leads. Two intercalating drugs, ametantrone and amsacrine, were chosen since the details of their molecular mechanism of action are still only partially understood, and their modes of DNA binding foresee a different intercalation geometry and location of the side-chain groups [17–19, 31], hence generating different contact points at the nucleic acid–enzyme interface. The hybrid derivatives maintained the anthraquinone ring system, showing side chains of the amsacrine and ametantrone type bound at positions 2, 5, and 8 of the aromatic intercalating chromophore.

Preliminary modeling studies using a simplified pharmacophore consensus strategy [32, 33] suggest that the hybrids would fit within the cleavable complex. Moreover, the amide bond connecting the 4-methanesulfonamidoaniline substituent to the anthraquinone appears to represent a reasonable linker. The hybrid compounds were assembled by a convergent synthesis: the anthraquinone chromophore and the 4-methanesulfonamidoaniline were prepared first and then joined; the 1 and 4 side chains were introduced into the anthraquinone nucleus using a known reaction strategy.

To assess the contribution of the planar part of the molecule with respect to the side chains the novel hybrids were tested for their DNA-binding efficiency, topoisomerase II inhibition, and cell cytotoxicity against a panel of cancer cell lines. The results showed that a precise combination of the side chain substituents can give effective compounds despite their lower cytotoxicity compared to the parent compounds ametantrone and amsacrine as shown in Table 1. Indeed, derivatives **10**, **11**, and **19** were able to poison topoisomerase II and kill cancer cells. In fact, a clear-cut connection exists between DNA binding, topoisomerase II poisoning, and antiproliferative activity, which suggests that the new drugs are characterized by the same mechanism of action as the parent drugs. If these findings confirm that the hybrid strategy can be successful to obtain active congeners, the overall outcome shown by the new compounds points to the requirement of strict spatial relationships to elicit a biological response. SAR analysis shows that a protonable group must be present at positions 1

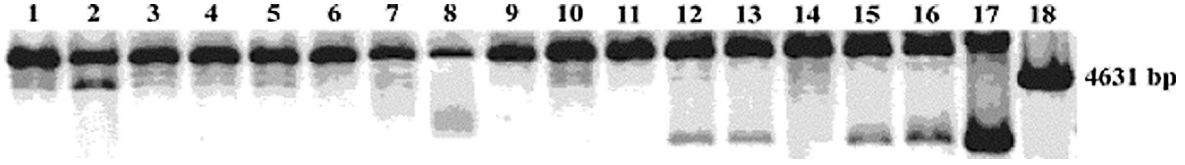



**Figure 4.** Inhibition of DNA topoisomerase II catalytic activity. Lanes are: 1, top IIα; 2, top IIα + 50 μM VM26; 3, top IIα + 1 μM **4**; 4, top IIα + 5 μM **4**; 5, top IIα + 10 μM **4**; 6, top IIα + 1 μM **10**; 7, top IIα + 5 μM **10**; 8, top IIα + 10 μM **10**; 9, top IIβ; 10, top IIβ + 50 μM VM26; 11, top IIβ + 1 μM **4**; 12, top IIβ + 5 μM **4**; 13, top IIβ + 10 μM **4**; 14, top IIβ + 1 μM **10**; 15, top IIβ + 5 μM **10**; 16, top IIβ + 10 μM **10**; 17, supercoiled pBR322 DNA; 18, *Hin*dIII-linearized pBR322 DNA.

and 4 located at a distance of three methylene groups (in compounds **10** and **11**) or two methylene groups (in compound **19**) from the nitrogen linked to the planar ring system. Interestingly, the presence of the ametantrone side chain hydroxyethylamino ethyl groups (compounds **14** and **15**) does not produce better results. Addition of a second protonable nitrogen in the side chains (compound **16**) is also detrimental. The cytotoxicity data show a close matching between compounds **10**, **11**, and **19** and amsacrine. This is in line with the suggestion that the side-chain contacts between drug and enzyme (the planar portion stacks on the DNA) play an important role in modulating drug potency and implies that the moiety located in the minor groove represents a key determinant. Biochemical studies show that compounds **10**, **11**, and **19** specifically inhibit and poison the β isoform of human topoisomerase II, but behave as poor inhibitors of the α isoform of the enzyme. This is in line with the isolation of amsacrine-resistant mutants with modified β isoforms [34] and further supports the role played by the amsacrine side chain even when connected to a different planar system. In addition, the presence of the β isoform in non-proliferating cells suggests that drug preferentially aimed at this isoenzyme might be active in slow-growing tumors, generally poorly responsive to chemotherapy [35]. Finally, it is worth recalling the lack of cross-resistance (Table 2) with anthracycline resistant cells, which confirms the prevalence of the amsacrine related portion of the hybrids in recognizing the topoisomerase II-DNA cleavable complex and, as a consequence, in affecting cytotoxic response. The biochemical and biological data collected show that neither the planar chromophore nor the side chains alone are able to direct the strength of the interaction between the small molecule and the macromolecular complex. The above data on active derivatives prove the concept that building new interfacial inhibitors based on the combination of fragments from active molecules is viable, but in this case the concept of pharmacophore must be extended beyond the one of a decorated nucleus.

## Experimental

### Chemistry

Commercially available chemicals were purchased from Aldrich, and used as received, unless stated. All air-sensitive manipulations were conducted according to Schlenk line techniques, using dry nitrogen and glassware. If required, solvents were dried prior to use. For work-up and chromatographic purification, commercial grade solvents were used; chromatographic separations were carried out using silica gel 60 (230–400 mesh, Grace Davisil). In addition, semi-preparative and preparative purification of the derivatives was carried out on Isolera One, an automated flash chromatography system provided by Biotage (Upsala, Sweden); the chromatography was carried out using disposable cartridges made of silica gel as stationary phase and bench solvents as mobile phase.

$^{1}H$ and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker Avance III 400 MHz and a Bruker AMX 300 MHz spectrometers. All spectra were recorded at room temperature, and the solvent for a particular spectrum is given in parentheses. Chemical shifts are reported in ppm and are relative to TMS internally referenced to the residual solvent peak. The multiplicity of signals is reported as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination of any of these.

High-resolution mass spectra were recorded on a ESI-TOF Mariner from Perseptive Biosystem (Stratford, Texas, USA), using electrospray (ES) ionization.

The degree of purity of the compounds synthesized throughout this investigation was assayed by HPLC, using a Varian Pro-Star system equipped with a Biorad 1706 UV–VIS detector and an Agilent C-18 column (5 μm, 4.6 mm × 150 mm). Water (A) and acetonitrile (B) were used as mobile phase with an overall flow rate of 1 mL/min and starting (90% A–10% B), then 15 min (10% A–90% B), then 20 min (10% A–90% B), then 21 min (90% A–10% B), finally 25 min (90% A–10% B).

#### *5,8-Dihydroxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**4**)*

A suspension of 5,8-diacetoxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4-methanesulfonylamino-phenyl)-amide (4.4 g, 8.1 mmol) in 80 mL of 5% aqueous potassium carbonate was heated at 80°C for 2 h. The reaction mixture was cooled and made acidic with hydrochloric acid, then extracted with ethyl acetate. The combined organic phases were dried over $Na_2SO_4$ and the solvent was evaporated to afford compound (**4**) as red solid (3.4 g, yield 92%).

$^{1}H$ NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 10.74 (s, 1H), 9.63 (s 1H), 8.82 (d, $J = 1.7$ Hz, 1H), 8.48 (dd, $J = 8.2$ Hz, $J = 1.7$ Hz, 1H), 8.41 (d, $J = 8.2$ Hz, 1H), 7.78 (d, $J = 8.9$ Hz, 2H), 7.49 (s, 2H), 7.24 (d, $J = 8.9$ Hz, 2H), 2.98 (s, 3H); $^{13}C$ NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 181.2, 164.8, 154.6, 154.5, 137.6, 137.1, 133.8, 133.4, 127.6, 126.5, 125.9, 123.5, 122.6, 122.4, 120.7, 120.4, 117.2, 116.5, 41.5; HMRS calcd. for $C_{22}H_{17}N_2O_7S$ $[M+H]^+$, 453.0605; found, 453.0632; m.p. >350°C.

#### *5,8-Dihydroxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**5**)*

A suspension of 5,8-diacetoxy-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2-methoxy-4-methanesulfonylamino-phenyl)-amide (5.1 g, 9 mmol) in 80 mL of 5% aqueous potassium carbonate was heated at 80°C for 2 h. The reaction mixture was cooled and made acidic with hydrochloric acid, then extracted with ethyl acetate. The combined organic phases were dried over $Na_2SO_4$ and the solvent was evaporated to afford compound **5** as red solid (4.22 g, yield 97%). $^{1}H$ NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 10.05 (s, 1H), 9.97 (s, 1H), 8.77 (d, $J = 1.7$ Hz, 1H), 8.37 (dd, $J = 8.2$ Hz, $J = 1.7$ Hz, 1H), 8.27 (d, $J = 8.2$ Hz, 1H), 7.56 (d, $J = 8.6$ Hz, 1H), 7.30 (s, 2H), 6.93 (d, $J = 2.3$ Hz, 1H), 6.82 (dd, $J = 8.6$ Hz, $J = 2.3$ Hz, 1H), 3.80 (s, 3H), 2.99 (s, 3H); $^{13}C$ NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.0, 164.8, 154.6, 154.5, 153.6, 137.5, 137.0, 134.4, 133.8, 130.8, 130.1, 127.5, 123.6, 123.5, 123.4, 120.7, 120.6, 114.2, 108.8, 99.7, 55.9,

41.5; HMRS calcd. for $C_{23}H_{19}N_2O_8S$ $[M+H]^+$, 483.4745; found, 483.4723; m.p. 316–318°C.

### *General procedure for the synthesis of derivatives **6**–**19** from **4** or **5***

To a suspension of **4** or **5** (0.5 g, 1.1 mmol) in 50 mL water and potassium carbonate (0.5%) in $N_2$ atmosphere, $Na_2S_2O_4$ (1.0 g, 5.7 mmol) was added. The reaction mixture was heated at 100°C and $Na_2S_2O_4$ (0.5 g, 2.9 mmol) and the proper primary amine were added (11 mmol). The reaction mixture was then refluxed overnight. Then the mixture was cooled at room temperature and air was introduced for 6 h. Water was added until complete precipitation of the product and the solid was filtered out and dried. The crude product was then purified by column chromatography.

### *5,8-Bis-(2-hydroxy-ethylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**6**)*

Yield: 300 mg (50%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.15–11.10 (m, 2H), 10.64 (s, 1H), 9.86 (s, 1H) 8.83 (d, $J = 1.7$ Hz, 1H), 8.37 (d, $J = 8.2$ Hz, 1H), 8.28 (dd, $J = 8.2$ Hz, $J = 1.7$ Hz, 1H), 7.78 (d, $J = 8.9$, 2H), 7.56 (s, 2H), 7.22 (d, $J = 8.9$ Hz, 2H), 5.00 (br s, 2H), 3.69 (t, $J = 5.3$ Hz, 4H), 3.56 (dt, $J = 5.5$ Hz, $J = 5.3$ Hz, 4H), 2.97 (s, 3H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.1, 180.8, 162.3, 139.8, 139.2, 132.5, 132.0, 131.9, 131.2, 130.0, 129.6, 127.9, 126.3, 125.9, 125.2, 122.3, 117.8, 116.0, 110.0, 61.7, 61.0, 45.2, 45.1, 41.3; HMRS calcd. for $C_{26}H_{25}N_4O_7S$ $[M+H]^+$, 540.1521; found, 540.1429; m.p. 246–249°C.

### *5,8-Bis-(2-hydroxy-ethylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**7**)*

Yield: 400 mg (63%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.13 (t, $J = 5.5$ Hz, 2H), 10.44 (s, 1H), 9.87 (s, 1H), 8.80 (d, $J = 1.8$ Hz, 1H), 8.35 (d, $J = 8.1$ Hz, 1H), 8.27 (dd, $J = 8.1$ Hz, $J = 1.8$ Hz, 1H), 7.76 (d, $J = 8.7$ Hz, 1H), 7.54 (s, 2H), 6.96 (d, $J = 2.2$ Hz, 1H), 6.83 (dd, $J = 8.7$ Hz, $J = 2.2$ Hz, 1H), 5.01 (br s, 2H), 3.82 (s, 3H), 3.72 (t, $J = 5.2$ Hz, 4H), 3.56 (dt, $J = 5.5$ Hz, $J = 5.2$ Hz, 4H), 3.02 (s, 3H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 181.2, 181.1, 162.2, 139.6, 139.0, 132.3, 132.0, 131.6, 131.2, 131.0, 130.5, 130.2, 129.4, 127.8, 126.3, 125.5, 125.1, 122.4, 117.9, 116.0, 110.1, 61.1, 61.0, 55.4, 45.2, 45.0, 41.0; HMRS calcd. for $C_{27}H_{29}N_4O_8S$ $[M+H]^+$, 570.3548; found, 570.3429; m.p. >350°C.

### *5,8-Bis-(2-acetylamino-ethylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**8**)*

Yield: 300 mg (44%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 10.97 (t, $J = 5.5$ Hz, 2H), 10.67 (s, 1H), 9.78 (s, 1H), 8.80 (d, $J = 1.7$ Hz, 1H), 8.37 (d, $J = 8.3$ Hz, 1H), 8.29 (dd, $J = 8.3$ Hz, $J = 1.7$ Hz, 1H), 7.79 (d, $J = 8.9$ Hz, 2H), 7.62 (s, 2H), 7.23 (d, $J = 8.9$ Hz, 2H), 3.58 (t, $J = 5.5$ Hz, 4H), 3.44–3.39 (m, 4H), 2.97 (s, 3H), 1.83 (s, 6H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 181.9, 181.1 162.6, 155.3, 152.9, 139.8, 139.0, 132.8, 132.7, 131.2, 131.0, 130.6, 129.4, 127.8, 126.5, 125.8, 125.1, 122.1, 117.4, 116.5, 110.5, 62.7, 61.0, 44.9, 45.0, 41.2, 40.9, 40.2; HMRS calcd. for $C_{30}H_{33}N_6O_7S$ $[M+H]^+$, 621.2126; found, 621.2106; m.p. 248–250°C.

### *5,8-Bis-(2-acetylamino-ethylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**9**)*

Yield: 300 mg (44%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.07 (t, $J = 5.3$ Hz, 2H), 11.04 (s, 1H), 9.89 (s, 1H), 8.87 (d, $J = 1.3$ Hz, 1H), 8.35 (dd, $J = 8.2$ Hz, $J = 1.3$ Hz, 1H), 8.28 (d, $J = 8.2$ Hz, 1H), 7.82 (d, $J = 8.4$ Hz, 1H), 7.66 (s, 2H), 6.96 (d, $J = 2.3$ Hz, 1H), 6.85 (dd, $J = 8.4$ Hz, $J = 2.3$ Hz, 1H), 4.09–4.04 (m, 2H), 3.82 (s, 3H), 3.65 (t, $J = 5.3$ Hz, 4H), 3.56–3.51 (m, 4H), 2.98 (s, 3H), 2.29 (s, 6H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.0, 181.9, 162.5, 155.2, 151.9, 138.9, 138.6, 133.2, 133.1, 132.6, 132.1, 131.2, 130.9, 130.8, 129.4, 126.8, 126.4, 125.6, 125.0, 123.1, 118.6, 115.5, 110.2, 62.6, 61.2, 56.0, 44.6, 44.0, 41.1, 40.6, 40.2; HMRS calcd. for $C_{31}H_{35}N_6O_8S$ $[M+H]^+$, 651.7586; found, 651.7545; m.p. 229–230°C.

### *5,8-Bis-(3-dimethylamino-propylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**10**)*

Yield: 300 mg (44%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.09 (t, $J = 5.5$ Hz, 2H), 10.64 (s, 1H), 9.64 (s, 1H), 8.79 (d, $J = 1.7$ Hz, 1H), 8.38 (d, $J = 8.3$ Hz, 1H), 8.28 (dd, $J = 8.3$ Hz, $J = 1.7$ Hz, 1H), 7.68 (d, $J = 8.9$ Hz, 2H), 7.57 (s, 2H), 7.22 (d, $J = 8.9$ Hz, 2H), 3.53–3.48 (m, 4H), 2.97 (s, 3H), 2.39 (t, $J = 6.3$ Hz, 4H), 2.20 (s, 12H), 1.81–1.73 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 180.2, 180.1, 164.8, 147.4, 147.0, 138.6, 137.0, 135.4, 134.9, 134.7, 131.7, 131.6, 126.9, 125.7, 122.5, 121.8, 121.3, 108.7, 108.5, 56.8, 56.4, 45.7, 45.6, 45.5, 45.4, 45.3, 27.5, 27.4; HMRS calcd. for $C_{32}H_{41}N_6O_5S$ $[M+H]^+$, 621.8163; found, 621.8188; m.p. 250–252°C.

### *5,8-Bis-(3-dimethylamino-propylamino)-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**11**)*

Yield: 350 mg (48%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.05 (t, $J = 5.3$ Hz, 2H), 9.88 (s, 1H), 9.70 (s, 1H), 8.81 (d, $J = 1.3$ Hz, 1H), 8.37 (d, $J = 8.2$ Hz, 1H), 8.28 (dd, $J = 8.2$ Hz, $J = 1.3$ Hz, 1H), 7.63 (d, $J = 8.4$ Hz, 1H), 7.58 (s, 2H), 6.96 (d, $J = 2.3$ Hz, 1H), 6.85 (dd, $J = 8.4$ Hz, $J = 2.3$ Hz, 1H), 3.82 (s, 3H), 3.53–3.44 (m, 4H), 3.04 (s, 3H), 2.36 (t, $J = 5.1$ Hz, 4H), 2.18 (s, 12H), 1.86–1.81 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.8, 153.6, 138.2, 138.0, 137.6, 137.0, 134.4, 133.8, 130.8, 130.1, 127.6, 123.4, 117.7, 117.6, 114.2, 110.8, 110.7, 108.8, 99.7, 56.5, 56.3, 55.9, 45.9, 45.8, 42.8, 42.7, 41.5, 26.6, 26.5; HMRS calcd. for $C_{33}H_{43}N_6O_6S$ $[M+H]^+$, 651.8463; found, 651.8452; m.p. 218–219°C.

### *5,8-Bis-[2-(2-hydroxy-ethoxy)-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**12**)*

Yield: 350 mg (50%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.09 (t, $J = 5.5$ Hz, 2H), 10.85 (s, 1H), 9.89 (s, 1H), 8.83 (d, $J = 1.6$ Hz, 1H), 8.38 (d, $J = 8.2$ Hz, 1H), 8.29 (dd, $J = 8.2$ Hz, $J = 1.6$ Hz, 1H), 7.78 (d, $J = 8.9$ Hz, 2H), 7.58 (s, 2H), 7.24 (d, $J = 8.9$ Hz, 2H), 4.65 (m, 2H), 3.72 (t, $J = 4.9$ Hz, 4H), 3.66 (t, $J = 4.9$ Hz, 4H), 3.54–3.45 (m, 4H), 2.97 (s, 3H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.8, 147.3, 138.2, 134.5, 133.4, 132.9, 128.2, 126.6, 122.9, 122.4, 122.1, 121.9, 121.0, 120.5, 108.7, 108.4, 72.4, 72.3, 69.7, 69.4, 61.5, 61.4, 43.1, 43.0, 42.5; HMRS calcd. for $C_{30}H_{35}N_4O_9S$ $[M+H]^+$, 627.1974; found, 627.2019; m.p. 261–263°C.

### *5,8-Bis-[2-(2-hydroxy-ethoxy)-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**13**)*

Yield: 290 mg (40%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.07 (t, $J$ = 5.3 Hz, 2H), 11.04 (s, 1H), 10.64 (s, 1H), 8.79 (d, $J$ = 1.3 Hz, 1H), 8.35 (dd, $J$ = 8.2 Hz, $J$ = 1.3 Hz, 1H), 8.28 (d, $J$ = 8.2 Hz, 1H), 7.62 (d, $J$ = 8.4 Hz, 1H), 7.65 (s, 2H), 6.96 (d, $J$ = 2.3 Hz, 1H), 6.85 (dd, $J$ = 8.4 Hz, $J$ = 2.3 Hz, 1H), 3.82 (s, 3H), 3.68–3.61 (m, 8H), 3.54–3.48 (m, 8H), 3.03 (s, 3H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.0, 164.9, 153.6, 138.2, 138.1, 137.6, 137.1, 134.4, 133.8, 130.8, 130.1, 127.6, 123.4, 117.7, 117.6, 114.2, 110.7, 110.6, 108.8, 99.7, 72.4, 72.3, 70.6, 70.5, 61.5, 61.4, 55.9, 44.2, 44.0, 41.5; HMRS calcd. for $C_{31}H_{37}N_4O_{10}S$ $[M+H]^+$, 625.1974; found, 625.2019; m.p. 220–221°C.

### *5,8-Bis-[2-(2-hydroxy-ethylamino)-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**14**)*

Yield: 300 mg (43%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.60 (t, $J$ = 5.5 Hz, 2H), 10.59 (s, 1H), 10.21 (s, 1H), 8.81 (d, $J$ = 1.7 Hz, 1H), 8.35 (d, $J$ = 8.2 Hz, 1H), 8.22 (dd, $J$ = 8.2 Hz, $J$ = 1.7 Hz, 1H), 7.79 (d, $J$ = 8.9 Hz, 2H), 7.57 (s, 2H), 7.24 (d, $J$ = 8.9 Hz, 2H), 4.95 (t, $J$ = 4.9 Hz, 2H), 3.65–3.56 (m, 8H), 3.48–3.41 (m, 4H), 2.97 (s, 3H), 2.87–2.81 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.8, 138.2, 137.6, 133.8, 133.4, 130.8, 130.2, 127.6, 125.9, 122.4, 122.3, 117.7, 117.5, 116.5, 116.4, 110.8, 110.7, 61.6, 61.5, 52.1, 52.0, 49.5, 49.4, 49.1, 49.0, 41.5; HMRS calcd. for $C_{30}H_{37}N_6O_7S$ $[M+H]^+$, 625.2439; found, 625.2453; m.p. 219–220°C.

### *5,8-Bis-[2-(2-hydroxy-ethylamino)-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**15**)*

Yield: 340 mg (50%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.72 (t, $J$ = 5.4 Hz, 1H), 11.54 (s, 1H), 10.89 (s, 1H), 8.28 (d, $J$ = 1.3 Hz, 1H), 8.15 (dd, $J$ = 8.2 Hz, $J$ = 1.3 Hz, 1H), 7.68 (d, $J$ = 8.2 Hz, 1H), 7,22 (d, $J$ = 8.4 Hz, 1H), 7.05 (s, 2H), 6.55 (d, $J$ = 2.1 Hz, 1H), 6.42 (dd, $J$ = 8.4 Hz, $J$ = 2.1 Hz, 1H), 3.82 (s, 3H), 3.65–3.58 (m, 8H), 3.01 (s, 3H), 2.97–2.92 (m, 4H), 2.01–1.96 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.9, 153.6, 138.2, 138.0, 137.5, 137.1, 134.4, 133.8, 130.7, 130.1, 127.6, 123.4, 117.7, 117.6, 114.2, 110.9, 110.7, 108.8, 99.7, 61.6, 61.5, 55.9, 52.1, 52.0, 50.2, 49.3, 49.1, 49.0, 41.4; HMRS calcd. for $C_{31}H_{39}N_6O_8S$ $[M+H]^+$, 655.8499; found, 655.8345; m.p. 235–238°C.

### *5,8-Bis-[2-piperazin-1-yl-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**16**)*

Yield: 300 mg (40%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.09 (t, $J$ = 6.4 Hz, 1H), 10.62 (s, 1H), 8.81 (d, $J$ = 1.7 Hz, 1H), 8.37 (d, $J$ = 8.2 Hz, 1H), 8.26 (dd, $J$ = 8.2 Hz, $J$ = 1.7 Hz, 1H), 7.79 (d, $J$ = 8.9 Hz, 2H), 7.58 (s, 2H), 7.22 (d, $J$ = 8.9 Hz, 2H), 2.97 (s, 3H), 2.89–2.84 (m, 8H), 2.68–2.63 (m, 4H), 1.85–1.82 (m, 12H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.3, 174.5, 165.1, 146.1, 137.5, 137.0, 136.1, 133.4, 127.2, 126.2, 126.4, 123.4, 122.2, 121.8, 121.5, 121.4, 121.2, 108.4, 108.0, 56.9, 53.8, 53.2, 53.1, 46.7, 46.3, 46.0, 45.8, 45.7; HMRS calcd. for $C_{34}H_{41}N_8O_5S$ $[M+H]^+$, 675.4124; found, 675.4128; m.p. 247–249°C.

### *5,8-Bis-[N-amino-propyl-morpholinyl]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**17**)*

Yield: 400 mg (51%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.05 (t, $J$ = 6.5 Hz, 1H), 10.62 (s, 1H), 9.98 (s, 1H), 8.85 (d, $J$ = 1.7 Hz, 1H), 8.37 (d, $J$ = 8.2 Hz, 1H), 8.28 (dd, $J$ = 8.2 Hz, $J$ = 1.7 Hz, 1H), 7.80 (d, $J$ = 8.9, 2H), 7.58 (s, 2H), 7.22 (d, $J$ = 8.9 Hz, 2H), 3.60–3.56 (m, 8H), 3.55 (d, $J$ = 5.5 Hz, 4H), 2.97 (s, 3H), 2.39–2.34 (m, 8H), 1.83 (t, $J$ = 6.5 Hz, 4H), 1.66–1.58 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.9, 138.2, 138.0, 137.6, 137.0, 133.8, 130.7, 130.2, 127.6, 125.9, 122.4, 117.7, 117.6, 116.3, 116.1, 110.7, 110.6, 66.8, 66.7, 53.7, 53.5, 51.9, 51.8, 42.8, 42.7, 41.5, 27.2, 27.1: HMRS calcd. for $C_{36}H_{45}N_6O_7S$ $[M+H]^+$, 705.1569; found, 705.1547; m.p. 238–240°C.

### *5,8-Bis-[N-amino-propyl-morpholinyl]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(2′-methoxy-4′-methanesulfonylamino-phenyl)-amide (**18**)*

Yield: 400 mg (49%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.05 (t, $J$ = 6.5 Hz, 1H), 10.79 (s, 1H), 9.89 (s, 1H), 8.81 (d, $J$ = 1.7 Hz, 1H), 8.37 (d, $J$ = 8.2 Hz, 1H), 8.26 (dd, $J$ = 8.2 Hz, $J$ = 1.7 Hz, 1H), 7.80 (d, $J$ = 8.9 Hz, 1H), 7.58 (s, 2H), 7.22 (dd, $J$ = 8.9 Hz, $J$ = 2.3 Hz, 1H), 6.98 (d, $J$ = 2.3 Hz, 1H), 3.65–3.61 (m, 8H), 3.55–3.49 (m, 4H), 3.40 (s, 3H), 2.97 (s, 3H), 2.37–2.32 (m, 8H), 1.81–1.76 (m, 4H), 1.63–1.56 (m, 4H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.9, 138.2, 138.0, 137.4, 137.1, 134.4, 133.8, 130.9, 130.1, 127.6, 123.4, 117.7, 117.5, 114.2, 110.7, 110.6, 108.8, 99.7, 66.8, 66.7, 55.9, 53.8 53.7, 53.6, 51.9, 51.8, 42.8, 42.7, 41.5, 27.2, 27.1; HMRS calcd. for $C_{37}H_{47}N_6O_8S$ $[M+H]^+$, 735.4124; found, 735.4128; m.p. 219–220°C.

### *5,8-Bis-[2-N,N-dimethylamino-ethylamino]-9,10-dioxo-9,10-dihydro-anthracene-2-carboxylic acid(4′-methanesulfonylamino-phenyl)-amide (**19**)*

Yield: 400 mg (61%). $^1$H NMR [300 MHz, $(CD_3)_2SO$] $\delta$ 11.12 (t, $J$ = 5.2 Hz, 1H), 10.64 (s, 1H), 8.98 (s, 1H), 8.65 (d, $J$ = 1.7 Hz, 1H), 8.38 (d, $J$ = 8.2 Hz, 1H), 8.26 (dd, $J$ = 8.2 Hz, $J$ = 1.7 Hz, 1H), 7.80 (d, $J$ = 8.9, 2H), 7.54 (s, 2H), 7.21 (d, $J$ = 8.9 Hz, 2H), 2.97 (s, 3H), 2.39–2.34 (m, 8H), 1.83 (s, 12H); $^{13}$C NMR [75 MHz, $(CD_3)_2SO$] $\delta$ 182.2, 182.1, 164.7, 138.3, 138.2, 137.5, 137.0, 133.4, 130.8, 127.6, 125.9, 122.5, 122.4, 117.7, 117.5, 116.6, 116.4, 110.7, 110.5, 58.1, 58.0, 46.7, 45.7, 45.6, 45.5, 41.5; HMRS calcd. for $C_{30}H_{37}N_6O_5S$ $[M+H]^+$, 593.7853; found, 593.7547; m.p 243–245°C.

## Experiments with human cells

### *Chemicals*

Compounds **3**–**19** obtained were dissolved in dimethyl sulfoxide just before the experiments; calculated amounts of drug solution were added to the growth medium containing cells to a final solvent concentration of 0.5%, which had no discernible effect on cell killing. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), amsacrine, and ametantrone were obtained from Sigma Chemical Co. (St. Louis, USA).

### *Cell cultures*

Human breast (MCF-7) carcinoma along with melanoma (A375) and promyelocytic leukemia (HL-60) cell lines were obtained from ATCC (Rockville, MD). Human ovarian cancer cell line (2008) was kindly provided by Prof. G. Marverti (Department of Biomedical

Science of Modena University, Italy). LoVo human colon-carcinoma cell line and its derivative multidrug-resistant subline (LoVo MDR) were kindly provided by Prof. F. Majone (Department of Biology of Padova University, Italy).

Cell lines were maintained in the logarithmic phase at 37°C in a 5% carbon dioxide atmosphere using the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units/mL penicillin and 50 μg/mL streptomycin), and 2 mM L-glutamine: (i) RPMI-1640 medium (Euroclone) for HL60, 2008, and MCF-7 cells; (ii) DMEM (Dulbecco's modified Eagle's) medium (Euroclone) for A375 cells; (iii) F-12 Ham's (Sigma Chemical Co) for LoVo and LoVo MDR cells (LoVo MDR culture medium also contained 0.1 μg/mL doxorubicin).

### *Cytotoxicity assay*

The growth inhibitory effect toward tumor cell lines was evaluated by means of MTT (tetrazolium salt reduction) assay [23]. Briefly, between 3 and $8 \times 10^3$ cells, dependent upon the growth characteristics of the cell line, were seeded in 96-well microplates in growth medium (100 μL). After 24 h, cells were treated for 48 or 72 h with the appropriate concentrations of the compound. Each well was added with 10 μL of a 5 mg/mL MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) saline solution, and after additional 5 h of incubation, 100 μL of a sodium dodecylsulfate (SDS) solution in HCl 0.01 M was added. After overnight incubation, the absorbance of each well was detected by a microplate reader (Biorad 680, Milan, Italy) at 570 nm. Mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted versus drug concentration. $IC_{50}$ values represent the drug concentrations that reduced the mean absorbance at 570 nm to 50% of those in the untreated control wells.

## Unwinding experiments

Supercoiled pBR322 DNA (0.15 μg) was incubated with 1 U of topoisomerase I (Invitrogen) in 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 100 μM EDTA, and 30 μg/mL BSA in the presence/absence of increasing concentrations of tested ligands. After o.n. incubation at 37°C, NaCl was added to the samples to a final concentration of 0.15 M and, finally, extracted with an equal volume of 1:1 PhOH/$CHCl_3$ buffered to pH 7.5 with 50 mM Tris-HCl. The aqueous phases were loaded on 1% agarose gel, and run in TAE (40 mM Tris, 18 mM acetic acid, and 1 mM EDTA). The reaction products were visualized by ethidium bromide staining.

## Inhibition of topoisomerase II activity

The inhibition of topoisomerase II activity was studied using a purified enzyme from *Drosophila melanogaster* embryos (USB, Amersham Italia S.r.l., Milano, Italy) [20]. pBR322 DNA (0.125 μg) was incubated for 15 min at 37°C in the presence of 2 units topoisomerase II (1 unit is defined as the activity capable of relaxing 0.3 μg of supercoiled DNA) in the reaction buffer containing 10 mM Tris-HCl (pH 7.9), 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 15 μg/mL BSA, and 1 mM ATP. Aliquots of compound solution (2 μL) in DMSO (4.5 μM) were added to reach the following final concentrations: 10, 20, 25, and 50 μM. A suitable amount of reaction buffer was then added to every sample to reach the final volume of 20 μL. The reaction was blocked by adding 7 mM EDTA (3 μL) containing 0.77% SDS. Bromophenol blue (2 μL) containing 15% of glycerol was added to the samples, which were then analyzed by agarose gel (0.7%) electrophoresis containing TAE (40 mM Tris-sodium acetate, pH 8.2; 1 mM EDTA) for 90 min; the gel was stained for 1 h in aqueous ethidium bromide (0.5 μg/mL). The bands were detected by exposure to an UV transilluminator TM36 (UVP, Inc., San Gabriel, CA, USA) and recorded with an Uvitec GAS9200 camera equipped with UVIdoc software.

## DNA cleavage assays

DNA cleavage reactions were performed with recombinant human topoisomerase II isoforms, purified as described [25]. pBR322 plasmid (230 ng) was incubated with the same enzyme amounts in the presence of different drug concentrations, 1 mM ATP, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, 10 mM $MgCl_2$, 0.01 mg/mL BSA, 10 mM DTT, and 150 mM KCl. The reactions were incubated for 30 min at 37°C, and then stopped with 0.1% SDS and 0.5 mg/mL proteinase K (Sigma) for 30 min at 53°C. DNA samples were then run in 1% agarose gels, and stained with ethidium bromide. DNA bands were quantified with ImageQuant 5.2 software (Amersham) with a local average background correction.

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