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Short communication

The first series of 4,11-bis[(2-aminoethyl)amino]anthra[2,3-*b*]furan-5,10-diones: Synthesis and anti-proliferative characteristics

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ABSTRACT

We developed the synthesis of a series of furan-fused tetracyclic analogues of the antitumor agent ametantrone. The reactions included nucleophilic substitution of propoxy groups in 4,11-dipropoxyanthra[2,3-*b*]furan-5,10-diones with ethylenediamines, producing the derivatives of 4,11-diaminoanthra[2,3-*b*]furan-5,10-dione in good yields. Studies of anti-proliferative activity on a panel of mammalian tumor cell lines demonstrated that anthra[2,3-*b*]furan-5,10-diones were the most potent derivatives among heteroarene-fused ametantrone analogues with one heteroatom. We identified several compounds that evoked a growth inhibitory effect at submicromolar concentrations. The anthra[2,3-*b*]furan-5,10-dione **9** with distal methylamino groups was markedly potent against drug-resistant cell lines with P-glycoprotein overexpression or p53 gene deletion. Furthermore, this derivative attenuated *in vitro* topoisomerase I-mediated DNA uncoiling at low micromolar concentrations. These results demonstrate that anthrafurandiones are a new class of heterocyclic anthraquinone derivatives with the properties potentially valuable for anticancer therapy.

1. Introduction

The derivatives and analogues of 1,4-diaminoanthraquinone are the promising class for exploring new chemotherapeutic agents. Mitoxantrone (**1**, Fig. 1) is the antitumor anthraquinone successfully used in patients with hematological and solid malignancies [1]. Ametantrone (**2**, Fig. 1) demonstrated high antitumor activity and low cardiotoxicity in preclinical setting [2]. The aza derivative pixantrone (**3**, Fig. 1) exerted a lower heart toxicity and higher activity than anthracycline antibiotics in chemotherapy of lymphomas [3]. Furthermore, **1** [4] and **3** [5] demonstrated high efficacy in treatment of multiple sclerosis; this efficacy has been attributed to the immunosuppressive activity of these drugs. Recently, new potentially valuable characteristics of **1** have been reported, in particular, an antipox-virus activity [6] and the ability to inhibit tau pre-mRNA splicing [7]. Moreover, among the derivatives of 1,4-diaminoanthraquinones the potent inhibitors of protein kinases have been identified [8,9]. Thus, the aminoanthraquinones definitely have perspective as drug candidates.

The efficacy of anticancer drugs is often limited by drug resistance [10]. Overexpression of ATP binding cassette transporters and loss of function of pro-apoptotic p53 are frequent determinants of altered drug response [11,12]. Therefore, the derivatives with improved activity against drug-resistant tumors are of special interest for drug development [13]. In our earlier work we have reported the synthesis and cytotoxic properties of a series of linear tetracyclic pyrrole [14] and thiophene [15] fused analogues of **2**. In particular, the SAR studies of distal amino groups in substituted anthraquinone derivatives revealed some structural requirements for circumventing drug resistance. We identified the derivatives of naphtho[2,3-*f*]indole-5,10-dione and anthra[2,3-*b*]thiophene-5,10-dione with methylamino groups in the side chains (e.g., compounds **4** [14] and **5** [15], Fig. 1) with remarkable activity against the cell lines with P-glycoprotein (Pgp)-mediated multidrug resistance (MDR) and deletion of p53. This work also pointed to a critical role of the heterocyclic moiety in the cytotoxic potency of these derivatives.

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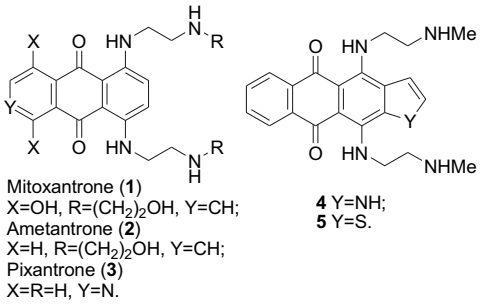
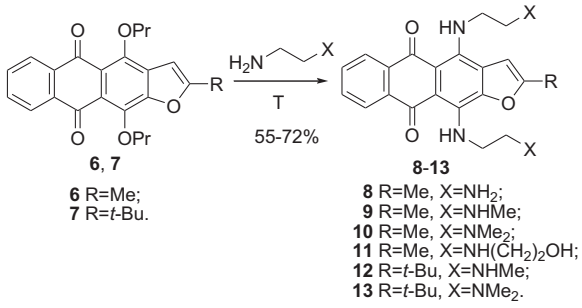


Fig. 1. Structures of anthraquinones **1**, **2** and their heterocyclic analogues **3**–**5**.



Scheme 1. Synthesis of anthra[2,3-*b*]furan-5,10-diones **8**–**13**.

In the present study we synthesized and tested a series of anthra[2,3-*b*]furan-5,10-dione analogues of **2**. Substitution of pyrrole or thiophene rings in **4** or **5**, respectively, for furan is the classical bioisosteric replacement for optimization of drug candidates [16]. The key role of furanoanthraquinones in the biosynthesis of highly active aflatoxins is known [17]; however, the biological properties of anthra[2,3-*b*]furan-5,10-diones have not been explored. Herein, we report the synthesis of a series of anthra[2,3-*b*]furan-5,10-diones with high growth inhibitory activity for a variety of tumor cell lines and their drug-resistant counterparts. We provide evidence that the introduction of methylamino or guanidine groups into the side chains of anthra[2,3-*b*]furan-5,10-dione may vary the anti-proliferative potency whereas the ability to modulate the activity of DNA-dependent enzyme topoisomerase I (topo I) is primarily attributed to the anthrafurandione moiety.

2. Chemistry

The majority of previously developed efficient methods of preparation of anthrafurandiones [18,19] are inconvenient for the synthesis of 4,11-bis(2-aminoethylamino)anthra[2,3-*b*]furan-5,10-diones. To obtain pyrrole- and thiophene-fused analogues of **2** (compounds **4** and **5**) we have developed the procedure based on the reactions of nucleophilic substitution of 4,11-alkoxy groups of naphtho[2,3-*f*]indole-5,10-diones or anthra[2,3-*b*]thiophene-5,10-dione with ethylenediamines [14,15]. A similar approach was used in the present study for the synthesis of furan analogues. For the corresponding anthrafurandione scaffolds we showed the possibility of nucleophilic substitution of propoxy groups with primary and secondary amines [20]. Starting from quinizarine, 2-methyl- and 2-*tert*-butyl-4,11-dipropoxyanthrafurandiones **6** and **7** were obtained via a 5-stage procedure [20,21]. Next, the series of furan analogues of **2** with various distal amino groups in the side chains and methyl or *tert*-butyl groups in the position 2 of heterocyclic ring were synthesized. The propoxy groups of anthrafurandiones **6** and **7** were readily substituted by treatment with an excess of ethylenediamines, resulting in 4,11-diaminoanthra[2,3-*b*]furan-5,10-dione derivatives **8**–**13** with good yields (Scheme 1). In the ^{1}H NMR spectra of **9** and **12** the triplets of N–H groups attached to the anthrafurandione moiety (12.3 and 11.8 ppm) confirmed the proposed structures.

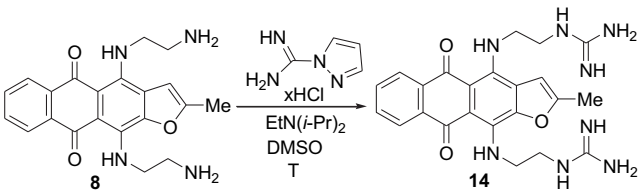
Given that DNA is a major intracellular target of anthraquinone-based compounds, these agents are supposed to interfere with DNA-dependent enzymes [2]. We have designed a novel type of topo I and telomerase inhibitors based on the anthra[2,3-*b*]thiophene-5,10-dione scaffold with delocalized basic groups (e.g., guanidine groups) [15]. To get further insight into SAR of modulators of DNA-dependent enzymes of this chemotype we prepared the furan analogues bearing guanidine residue in the side chains. The distal amino groups in **8** were transformed into the guanidine groups by the treatment with pyrazolocarboxamidine, yielding bis-guanidino derivative **14** (Scheme 2).

The novel anthra[2,3-*b*]furan-5,10-diones were examined for toxicity against mammalian tumor cell lines and isogenic drug-resistant sublines: murine leukemia L1210, T-lymphocyte cell lines Molt4/C8 and CEM, human myeloid leukemia K562 and its Pgp-positive MDR subline K562/4, and colon carcinoma HCT116 and its subline HCT116p53KO with deleted p53 gene. The cytotoxic potencies of compounds (determined by cell growth inhibition) are presented in Table 1 in which IC_{50} values represent the concentrations that inhibited cell proliferation by 50%.

3. Biological results and discussion

Almost all final derivatives of anthra[2,3-*b*]furan-5,10-dione (except guanidine derivative **14**) markedly inhibited the growth of L1210, Molt4/C8 and CEM cell lines (Table 1). Compound **9** with methylamino groups in the side chains demonstrated the activity close to doxorubicin. Substitution of the methyl group in the position 2 of the chromophore for a *tert*-butyl group (compounds **12**, **13**) led to a 5–10-fold decrease of growth inhibitory potency. Guanidination of the amino group in the side chain of **8** yielded compound **14** with 2–3 orders of magnitude lower cytotoxicity. Comparison of the furan analogue **9** and structurally related derivatives of naphtho[2,3-*f*]indole-5,10-dione **4** and anthra[2,3-*b*]thiophene-5,10-dione **5** (that are less potent) demonstrated the key role of the heteroatom in the anti-proliferative activity of tetracyclic analogues of **2** (Table 1).

The structure of distal amino groups is especially important for circumventing transmembrane transport-associated drug resistance. The derivatives **8** and **11** with amino and ethanolamino groups in the side chains were less potent for the Pgp-positive MDR subline K562/4 than **9**, **10**, **12** and **13** with methylamino and particularly dimethylamino groups (Table 1). Substitution of the methyl group in the position 2 of the chromophore of **9** for a bulky and hydrophobic *tert*-butyl group (compound **12**) significantly increased the anti-proliferative potency for K562/4 subline. Thus, the resistance index (RI) for 2-*tert*-butyl derivative **12** was 10 times smaller than the RI for 2-methyl analogue **9** with similar structure of side chains. These results can be explained by a higher hydrophobicity of *tert*-butyl derivative **12**. In the case of pair analogues **10**



Scheme 2. Synthesis of bis(guanidine) derivative **14**.

Table 1
Cell growth inhibition (IC_{50},[a] μM) by anthra[2,3-*b*]furan-5,10-dione derivatives **8**–**14** and reference compounds **4**, **5** and doxorubicin (**Dox**).

Compd	L1210	Molt4/C8	CEM	K562	K562/4	RI[b]	HCT116	HCT116p53KO	RI[c]
8	0.45 ± 0.08[a]	0.75 ± 0.2	1.2 ± 0.3	0.1 ± 0.08	6.3 ± 0.4	63	1.1 ± 0.2	0.6 ± 0.03	0.55
9	0.15 ± 0.01	0.11 ± 0.02	0.15 ± 0.02	0.12 ± 0.04	4.2 ± 0.3	35	1.6 ± 0.04	0.8 ± 0.02	0.5
10	0.51 ± 0.16	0.21 ± 0.08	0.44 ± 0.1	0.6 ± 0.05	1.0 ± 0.1	1.7	7.5 ± 0.4	2.6 ± 0.5	0.3
11	0.42 ± 0.11	0.72 ± 0.22	0.74 ± 0.2	0.8 ± 0.1	>50	>62	0.8 ± 0.01	0.8 ± 0.02	1
12	1.4 ± 0.1	0.6 ± 0.3	1.5 ± 0.1	0.11 ± 0.01	0.4 ± 0.15	4	2.8 ± 0.1	1.2 ± 0.1	0.4
13	2.7 ± 0.3	1.1 ± 0.07	1.65 ± 0.2	1.2 ± 0.1	1.8 ± 0.4	1.6	7.5 ± 0.5	5.9 ± 1.1	0.8
14	>500	>500	35 ± 3.7	19 ± 3.2	20 ± 4.2	1	17 ± 1	27 ± 1	1.6
4	1.2 ± 0.1	0.87 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	2.5 ± 0.4	3.1	11.1 ± 0.4	11.3 ± 0.4	1.0
5	0.95 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.06	0.7 ± 0.2	1.4	8.3 ± 0.2	5.3 ± 0.3	0.6
Dox	0.37 ± 0.07	0.20 ± 0.02	0.06 ± 0.01	0.12 ± 0.03	9.7 ± 0.9	81	0.11 ± 0.03	0.52 ± 0.1	4.7

[a] IC_{50}, μM, mean ± S.D. of 3 experiments.
[b] RI, resistance index = IC_{50}(K562/4)/IC_{50}(K562).
[c] RI, resistance index = IC_{50}(HCT116p53KO)/IC_{50}(HCT116).

and **13** the above modification only slightly changed RI for *tert*-butyl derivative **13** (RIs = 1.7 and 1.6, respectively). Importantly, **10** and **12** attenuated the growth of wild type K562 cells at the same range of concentrations as doxorubicin; however, unlike doxorubicin, **10** and **12** were more toxic for K562/4 subline (Table 1). Thus, these anthrafurandiones were more potent against MDR cells than the reference drug doxorubicin.

Interestingly, in contrast to the furan derivative **9** that showed an unexpectedly low potency for K562/4 subline (RI = 35), pyrrole and thiophene analogues **4** and **5** were much more toxic for these MDR cells (RIs 3.1 and 1.4, respectively). One may suggest that the derivatives with highly hydrophilic primary and secondary amino groups in the side chains (**8** and **11**) might be effluxed from the cells by the pumps such as Pgp. If this is the case, a diminished hydrophilicity achievable via introduction of methylamino and especially dimethylamino groups (**9**, **10**, **12**, **13**) into the side chains should increase the ability to circumvent transporter-mediated MDR.

It is known that p53 plays a major role in chemotherapy-induced apoptosis, and p53 inactivation has been associated with a lower response of tumor cells to DNA damaging agents including doxorubicin [22]. To reveal a role of p53 in anti-proliferative activity of anthra[2,3-*b*]furan-5,10-diones, we tested these compounds against the colon carcinoma HCT116 cell line and its isogenic subline HCT116p53KO with deleted p53 gene. The modification of the heterocyclic moiety resulted in a 10-fold increase of growth inhibitory activity of anthra[2,3-*b*]furan-5,10-diones for HCT116 cells compared with thiophene and pyrrole analogues (compare **9** versus **4** and **5**, Table 1). The distinctive feature of all tetracyclic analogues of **2**, including anthra[2,3-*b*]furan-5,10-dione, naphtho[2,3-*f*]indole-5,10-diones and anthra[2,3-*b*]thiophene-5,10-dione, is the ability to induce p53-independent cell death. Indeed, almost all anthra[2,3-*b*]furan-5,10-diones (except guanidine derivative **14**) were equally or even preferentially cytotoxic for the $p53^{-/-}$ subline than for the parental $p53^{+/+}$ cells (RI < 1 while for doxorubicin RI = 4.7; Table 1). Thus, the selected anthra[2,3-*b*]furan-5,10-diones, in particular, **9** and **12**, were remarkably potent for tumor cells with the determinants of altered drug response, that is, Pgp expression or p53 deletion.

Finally, we investigated novel heterocyclic anthraquinone derivatives with structurally similar chromophores and side chains for the ability to interfere with DNA-dependent enzymes vital for tumor cell biology. The thiophene analogues of **2** (e.g., **5**) inhibited topo I-mediated relaxation of supercoiled DNA [15]. Therefore, we tested anthra[2,3-*b*]furan-5,10-diones **9**, **14** and the reference compound **5** (the latter being the most potent topo I inhibitor among the series of anthra[2,3-*b*]thiophene-5,10-diones) for the ability to modulate topo I activity. Fig. 2 shows the topoisomers of pHot plasmid generated in the presence of **9**, **14** and **5**. Interestingly, anthrafurandiones with different structure of side chains and different cytotoxicity evoked similar effects on topo I-mediated DNA relaxation. At 0.5 μM **9** and **14** attenuated DNA relaxation only partially. At 0.1 μM and 0.5 mM anthra[2,3-*b*]thiophenediones **5** attenuated topo I activity to a somewhat greater extent than anthrafurandiones **9** and **14**. The enzymatic activity was completely blocked at 2.5 μM of each compound (Fig. 2). These results demonstrate the decisive role of the structure of the heterocyclic moiety in modulation of topo I activity.

However, the anti-proliferative activities of **9**, **14** and **5** differed substantially (Table 1), therefore no direct correlation between topo I inhibition *in vitro* and cell growth retardation by the analogues of **2** was found. The reason for this discrepancy remains to be elucidated. One may suppose that changes of amphiphilicity might alter the intracellular distribution of compounds, so the nuclear localization would be limited whereas the hydrophobic compartments, that is, plasma membrane and/or membranes of organelles, would entrap certain amounts of the drug. In this scenario the cytotoxic effect of the compound might be less pronounced regardless of its potency to inhibit topo I *in vitro*.

In summary, a series of anthra[2,3-*b*]furan-5,10-diones was synthesized and evaluated for the ability to inhibit tumor cell growth and modulate topo I. Introduction of distal amino groups into the side chains modifies the characteristics of derivatives. The selected compounds showed excellent anti-proliferative activity against a variety of mammalian tumor cell lines including drug-resistant sublines. Importantly, we provide evidence for efficient structural modifications that yield the potent topo I inhibitors with attenuated cytotoxicity.

This study for the first time implicates anthra[2,3-*b*]furan-5,10-diones as anticancer drug candidates. Comparison of anti-proliferative activity of **4**, **5** and **9**, the compounds with similar structure of the

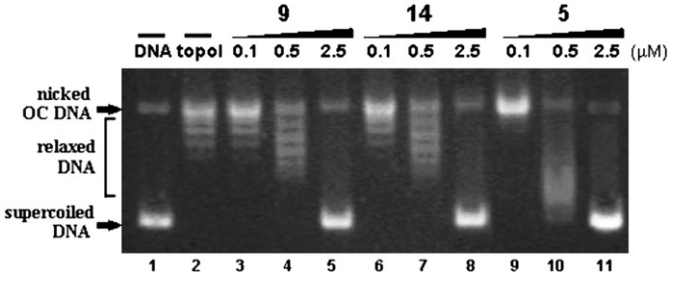


Fig. 2. DNA uncoiling in the presence of anthra[2,3-*b*]furan-5,10-diones **9**, **14** and anthra[2,3-*b*]thiophene-5,10-dione **5**. Shown is the electrophoretic mobility of untreated supercoiled pHot plasmid DNA (lane 1), the plasmid DNA treated with topo I in the absence of drugs (lane 2) or the plasmid DNA treated with topo I in the presence of 0.1, 0.5 or 2.5 μM of **9** (lanes 3–5), **14** (lanes 6–8) or **5** (lanes 9–11). After electrophoresis the gel was stained with ethidium bromide and visualized under UV light. The experiments were performed 3 times with essentially the same results.

side chains, demonstrates that the heteroatom in the heterocyclic analogues of **2** significantly affected activity against the majority of tested cancer cell lines. The anti-proliferative activity decreased depending on the heterocycle: furan > thiophene > pyrrole. Thus, our systematic studies of aminoanthraquinone-based compounds led to identification of 4,11-bis[(2-aminoethyl)amino]anthra[2,3-*b*] furan-5,10-diones as the most potent among heteroarene-fused ametantrone analogues with single heteroatom. These compounds emerge as a clinically promising class of heterocyclic derivatives of aminoanthraquinones.

4. Experimental section

4.1. General information

NMR spectra were registered on a Varian VXR-400 instrument operated at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR). Chemical shifts were measured in DMSO-d_6, $CDCl_3$ or D_2O using tetramethylsilane as internal standard. Analytical TLC was performed on Silica Gel F_{254} plates (Merck) and column chromatography on Silica Gel Merck 60. Melting points were determined on a Buchi SMP-20 apparatus and are uncorrected. High-resolution mass spectra were recorded with electron-spray ionization on a Bruker Daltonics microOTOF-QII instrument. UV spectra were recorded on Hitachi-U2000 spectrophotometer. HPLC was performed using Shimadzu Class-VP V6.12SP1 system. All solutions were dried over Na_2SO_4 and evaporated at reduced pressure on a Buchi-R200 rotary evaporator at the temperature below 45 °C. All products were vacuum dried at room temperature. All solvents, chemicals and reagents were obtained commercially and used without purifications. Anthra[2,3-*b*]furan-5,10-diones **6** and **7** were prepared as described by us [20,21].

4.2. Experimental procedures

4.2.1. 4,11-Bis(2-aminoethylamino)-2-methylanthra[2,3-b]furan-5,10-dione dihydrochloride (*8*)

A mixture of 4,11-dipropoxyanthra[2,3-*b*]furan-5,10-dione (**6** [20]; 0.20 g, 0.53 mmol) and ethylenediamine (3.0 mL) was heated at 50 °C for 1.5–2 h. During this time, the yellow color of the reaction mixture changed to dark blue, and after the complete conversion of **6** (as determined by TLC) the solution was cooled and quenched with water. Aqueous solution of HCl (1%) was added to reach pH 8.0, the solution was saturated with NaCl, and the product was extracted with warm *n*-butanol (3 × 30 mL). The extract was washed twice with brine, dried and evaporated. The residue was purified by column chromatography using chloroform–methanol–concd NH_4OH (10:2:0 → 10:4:1) as eluting solvent. The purified residue was dissolved in warm 1 N HCl and re-precipitated with acetone. The precipitated crystals were filtered, washed with acetone and dried to yield 0.13 g (55%) dark blue powder of dihydrochloride **8**, mp 259–261 °C; free base mp 178–180 °C; HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 16.6 min, purity 96%. ^{1}H NMR (400 MHz, D_2O) δ 7.26 (m, 2H, H^{6,9}), 7.19 (m, 2H, H^{7,8}), 6.05 (s,1H, H^3), 3.69 (t, 2H, J = 5.8 Hz, HNCH_2), 3.51 (t, 2H, J = 5.6, HNCH_2), 3.21 (m, 4H, CH_2N), 2.12 (s, 3H, Me). HRMS (ESI) calculated for $C_{21}H_{23}N_4O_3$ $[M+H]^+$ 379.1765, found 379.1748. Analysis calculated for $C_{21}H_{22}N_4O_3.2HCl.2H_2O$: C 51.75, H 5.79, N 11.50. Found: C 52.02, H 5.89, N 11.73.

4.2.2. 2-Methyl-4,11-bis(2-(methylamino)ethylamino)anthra[2,3-b]furan-5,10-dione (*9*)

This compound was prepared similarly from anthrafurandione **6** [20] and *N*-methylethylenediamine (50 °C, 3–3.5 h). Dark blue needles, yield 63%. Free base mp > 138–140 °C (from benzene–*n*-heptane mixture (1:4)); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.32 (t, 1H, J = 4.5 Hz, NH), 11.84 (t, 1H, J = 5.2 Hz, NH), 8.32 (m, 2H, H^{6,9}), 7.61 (m, 2H, H^{7,8}), 6.67 (s, 1H, H^3), 4.01 (dd, 2H, ^1J = 5.8, ^2J = 4.5 Hz, HNCH_2), 3.79 (dd, 2H, ^1J = 5.8, ^2J = 5.2 Hz, HNCH_2), 3.00 (m, 4H, 2CH_2NHMe), 2.54 (s, 6H, 2NHMe), 1.41 (s, 3H, Me). Dihydrochloride: mp > 260 °C; HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 13.2 min, purity 98%. ^{1}H NMR (400 MHz, D_2O) δ 7.42 (m, 2H, H^{6,9}), 7.31 (m, 2H, H^{7,8}), 6.20 (s, 1H, H^3), 3.77 (t, 2H, J = 6.5 Hz, HNCH_2), 3.61 (t, 2H, J = 6.5 Hz, HNCH_2), 3.32 (m, 4H, 2CH_2N), 2.84 (s, 3H, NMe), 2.81 (s, 3H, NMe), 2.20 (s, 3H, Me). HRMS (ESI) calculated for $C_{23}H_{27}N_4O_3$ $[M+H]^+$ 407.2078, found 407.2083.

4.2.3. 4,11-Bis(2-(dimethylamino)ethylamino)-2-methylanthra[2,3-b]furan-5,10-dione (*10*)

A mixture of anthrafurandione **6** [20] (0.10 g, 0.31 mmol) and *N*,*N*-dimethylethylenediamine (3.0 mL) was heated at 50 °C for 4–5 h. After the complete conversion of **6** (as determined by TLC) the reaction mixture was cooled, quenched with water, and pH was adjusted to 8.0 by 1% aqueous HCl. The product was extracted with ethyl acetate (4 × 30 mL). The residue obtained after the evaporation of the extract was purified by column chromatography with chloroform–methanol (10:1 → 2:1) as eluent. The solid product was crystallized from benzene–*n*-heptane mixture (1:4) and dried to afford anthrafurandione **10** (0.10 g, 64%) as dark blue needles, mp 174–175 °C; HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 13.7 min, purity 99%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.29 (t, 1H, J = 5.4 Hz, NH), 11.81 (t, 1H, J = 5.5 Hz, NH), 8.37 (m, 2H, H^{6,9}), 7.64 (m, 2H, H^{7,8}), 6.79 (d, 1H, J = 0.9 Hz, H^3), 4.04 (dd, 2H, ^1J = 5.4, ^2J = 7.0 Hz, HNCH_2), 3.81 (dd, 2H, ^1J = 5.5, J = 6.9 Hz, HNCH_2), 2.73 (t, 2H, J = 7.0 Hz, CH_2NMe$_2$), 2.71 (t, 2H, J = 6.9 Hz, CH_2NMe$_2$), 2.45 (s, 3H, Me^2), 2.38 (s, 6H, NMe$_2$), 2.37 (s, 6H, NMe$_2$). HRMS (ESI) calculated for $C_{25}H_{31}N_4O_3$ $[M+H]^+$ 435.2391, found 435.2407. Analysis calculated for $C_{25}H_{30}N_4O_3$: C 69.10, H 6.96, N 12.89. Found: C 69.25, H 6.80, N 12.67.

4.2.4. 4,11-Bis(2-(2-hydroxyethylamino)ethylamino)-2-methylanthra[2,3-b]furan-5,10-dione dihydrochloride (*11*)

This compound was prepared from anthrafurandione **6** [20] and 2-[(2-hydroxyethyl)amino]ethylamine as described for anthrafurandione **8** (50 °C, 2–3 h). Yield 56%, mp > 260 °C (ethanol); HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 12.9 min, purity 98%. ^{1}H NMR (400 MHz, D_2O) δ 7.44 (m, 2H, H^{6,9}), 7.32 (m, 2H, H^{7,8}), 6.21 (s, 1H, H^3), 4.01 (m, 4H, 2CH_2OH), 3.82 (t, 2H, J = 6.2 Hz, HNCH_2), 3.67 (t, 2H, J = 6.2 Hz, HNCH_2), 3.35 (m, 8H, 2HN(CH_2)$_2$), 2.21 (s, 3H, Me). HRMS (ESI) calculated for $C_{25}H_{31}N_4O_5$. $[M+H]^+$ 467.2289, found 467.2308.

4.2.5. 2-tert-Butyl-4,11-bis(2-(methylamino)ethylamino)anthra[2,3-b]furan-5,10-dione (*12*)

This compound was prepared from anthrafurandione **7** [21] and *N*-methylethylenediamine as described for **8** (65 °C, 2–3 h). Dark blue needles, yield 72%, mp 112–114 °C; dihydrochloride mp > 260 °C; HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 12.7 min, purity 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.39 (t, 1H, J = 5.0 Hz, NH), 11.91 (t, 1H, J = 5.8 Hz, NH), 8.33 (m, 2H, H^{6,9}), 7.62 (m, 2H, H^{7,8}), 6.73 (s, 1H, H^3), 4.08 (dd, 2H, ^1J = 5.8, ^2J = 5.8 Hz, HNCH_2), 3.90 (dd, 2H, ^1J = 5.0, ^2J = 5.8 Hz, HNCH_2), 3.09 (t, 2H, J = 5.8 Hz, CH_2NHMe), 3.07 (t, 2H, J = 5.8 Hz,

CH_2NHMe), 2.59 (s, 3H, NHMe), 2.58 (s, 3H, NHMe), 1.41 (s, 9H, *t*-Bu). HRMS (ESI) calculated for $C_{26}H_{33}N_4O_3$ $[M+H]^+$ 449.2547, found 449.2550.

4.2.6. 2-tert-Butyl-4,11-bis(2-(dimethylamino)ethylamino)anthra[2,3-b]furan-5,10-dione (**13**)

This compound was prepared from anthrafurandione **7** [21] and *N*,*N*-dimethylethylenediamine as described for anthrafurandione **10** (65 °C, 3–4 h). Dark blue needles, yield 68%, mp 156–157 °C; dihydrochloride mp > 260 °C; HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 17.1 min, purity 96%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.35 (t, 1H, J = 5.1 Hz, NH), 11.83 (t, 1H, J = 5.5 Hz, NH), 8.40 (m, 2H, $H^{6,9}$), 7.67 (m, 2H, $H^{7,8}$), 6.84 (d, 1H, J = 0.9 Hz, H^3), 4.09 (dd, 2H, ^1J = 5.5, ^2J = 6.6 Hz, HNCH_2), 3.88 (dd, 2H, ^1J = 5.1, ^2J = 6.6 Hz, HNCH_2), 2.77 (m, 4H, 2CH_2NMe_2), 2.40 (s, 6H, NMe_2), 2.39 (s, 6H, NMe_2), 1.43 (s, 9H, *t*-Bu). HRMS (ESI) calculated for $C_{28}H_{37}N_4O_3$ $[M+H]^+$ 477.2860, found 477.2893.

4.2.7. 4,11-Bis[(2-guanidinoethyl)amino]-2-methylanthra[2,3-b]furan-5,10-dione dihydrochloride (**14**)

To a stirring solution of free base of **8** (95 mg, 0.25 mmol) in DMSO (10.0 mL) ethyldiisopropylamine (1.0 mL, 6.0 mmol) and 0.5 g (3.4 mmol) hydrochloride of pyrazole-1-carboxamidine were added. The mixture was stirred for 5 h at 60 °C and then cooled. The product was precipitated with acetone and collected by filtration. The blue solid was re-precipitated twice from hot water with acetone, washed with acetone and, after drying, yielded dihydrochloride **15** (82 mg, 74%); mp > 250 °C (dec.); HPLC Kromasil-100-5-mkm C-18 column (4 × 250 mm, LW = 260 nm), eluent: A – H_3PO_4 (0.01 M), B – MeCN; gradient B 10 → 50% (30 min), elution time 14.9 min, purity 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.13 (t, 1H, J = 5.0 Hz, NH), 11.63 (t, 1H, J = 6.2 Hz, NH), 8.2 (t, 1H, J = 5.3 Hz, NH), 8.18 (m, 2H, 6-H, 9-H), 8.05 (t, 1H, J = 5.8 Hz, NH), 7.71 (m, 2H, 7-H, 8-H), 7.62 (br s, 4H, NH_2), 7.23 (br s, 4H, NH_2), 6.98 (s, 1H, H^3), 3.88 (m, 2H, HNCH_2), 3.75 (m, 2H, HNCH_2), 3.53 (m, 4H, CH_2NH), 2.42 (s, 3H, Me); ^{13}C NMR (100 MHz, DMSO-d_6) δ 179.77 (C═O), 179.18 (C═O), 169.34 (C), 157.61 (C), 157.37 (C), 147.07 (C), 144.38 (C), 136.86 (C), 134.10 (C), 133.90 (C), 123.18 (C), 106.48, (C), 105.32 (C), 131.96 (CH), 131.80 (CH), 125.71 (CH), 125.52 (CH), 105.55 (CH), 43.85 (CH_2), 43.77 (CH_2), 41.84 (CH_2), 40.99 (CH_2), 13.72 (CH_3); UV (ethanol) λ_{max} (log ε) 235 (4.2), 261 (4.6), 285 sh (4.0), 344 sh (3.7), 523 sh (3.8), 555 (4.3), 599 (4.4) nm. HRMS (ESI) calculated for $C_{23}H_{27}N_8O_3$ $[M+H]^+$ 463.2201, found 463.2215.

4.3. Cell lines, drug treatment and cytotoxicity assays

The K562 human leukemia cell line (American Type Culture Collection; ATCC, Manassas, VA) and its Pgp-positive subline K562/4 selected for survival in the continuous presence of doxorubicin (gift of A. Saprin), the HCT116 colon carcinoma cell line (ATCC) with wild type p53 ($p53^{+/+}$) and the HCT116p53KO subline (both *p53* alleles deleted [23]) (generated in B.Vogelstein lab, Johns Hopkins University, Baltimore, MD; gift of B.Kopnin) were cultured in Dulbecco modified Eagle's medium supplemented with 5% fetal calf serum (HyClone, Logan, UT), 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C, 5% CO_2 in humidified atmosphere. The murine leukemia L1210, human lymphocyte Molt4/C8 and CEM cell lines were cultured in RPMI-1640 supplemented with 10% fetal calf serum, 0.075% $NaHCO_3$ and 2 mM L-glutamine. Cells in logarithmic phase of growth were used in all experiments. All reagents were from Sigma–Aldrich, St. Louis, MO unless specified otherwise. Novel compounds were dissolved in 10% aqueous DMSO as 10 mM stock solutions followed by serial dilutions in water immediately before experiments. The cytotoxicity was determined in a formazan conversion assay (MTT-test). Briefly, cells (5×10^3 in 190 μL of culture medium) were plated into a 96-well plate (Becton Dickinson, Franklin Lakes, NJ) and treated with 0.1% DMSO (vehicle control) or with increasing concentrations of compounds (each dose in duplicate) for 48 h (L1210 cells) or 72 h (all other cell lines). After the completion of drug exposure, 50 μg of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were added into each well for an additional 2 h. Formazan was dissolved in DMSO, and the absorbance at λ = 540 nm was measured. The cytotoxicity at a given drug concentration was calculated as the percentage of absorbance in wells with drug-treated cells to that of vehicle control cells (100%). The IC_{50} (50% growth inhibitory concentration) was defined as the concentration of the compound that inhibited MTT conversion by 50%. In some experiments (L1210, Molt4/C8 and CEM) cells were counted in a Coulter counter.

4.4. Topo I assay

The ability of compounds **9**, **14** and **5** to modulate the activity of topo I *in vitro* was determined in a DNA relaxation assay [15]. Briefly, 1-2 units of purified topo I (Amersham Biosci., UK) were incubated with 0.25 μg of supercoiled pHot plasmid DNA in the buffer (35 mM Tris–HCl, pH 8.0; 72 mM KCl; 5 mM $MgCl_2$; 5 mM dithiotreitol; 2 mM spermidine, 0.01% bovine serum albumin) in the presence of 0.1% DMSO (vehicle control) or compounds **9**, **14** and **5** at 37 °C for 30 min. The reaction was terminated by the addition of sarcosyl (up to 1%). DNA topoisomers were resolved by electrophoresis in 1% agarose gel (3 h, 70 V) in the buffer containing 40 mM Tris–acetate, pH 7.6 and 1 mM ethylenediamine tetraacetic acid disodium salt. The gels were stained with ethidium bromide after electrophoresis.

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