# Naphtho[2,3-*f*]Indole-5,10-Dione Aminoalkyl Derivatives: A New Class of Topoisomerase I Inhibitors

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Naphtho[2,3-*f*]indole-5,10-dione aminoalkyl derivatives in cytotoxic concentrations inhibit topoisomerase I, which is an important factor of antitumor activity of compounds of this chemical class. The degree of topoisomerase I inhibition with naphtho[2,3-*f*]indole-5,10-dione derivatives depends on the structure and position of active (aminoalkyl) groups. The mechanism of topoisomerase I inhibition with aminoalkylnaphtho[2,3-*f*]indole-5,10-diones differs from specific blocking of the catalytic activity of the enzyme and depends on interactions of these compounds with DNA.

**Key Words:** *naphthoindole diones; topoisomerase I; cytotoxicity; tumor cells*

One of the trends in creation of antitumor drugs with selective action is the search for inhibitors of DNA topoisomerases (enzymes catalyzing the topological restructuring of DNA) [5]. Topoisomerases introduce single- (topoisomerase I; topo I) or double-stranded (topoisomerase II; topo II) DNA breaks ensuring mobility of the DNA strands and modification of DNA conformation essential for (among other things) matrix synthesis and chromosome mobility in mitosis. In addition, these enzymes link DNA breaks, thus restoring its integrity.

Chemical drugs preventing reparation of DNA breaks induce accumulation of damaged DNA molecules, which is an important factor of cell death induction. For example, alkaloid camptothecine interacts with topo I—DNA complex stabilizing it and preventing DNA reparation (specific inhibition of topo I catalytic activity). Along with this mechanism of topo I inhibition, low-molecular DNA ligands modulating DNA conformation and impeding enzyme binding to DNA can prevent topo I function. It is assumed that the main target for anthracycline compounds is topo II [2], but antitumor drugs of this series (for example, doxorubicin) inhibit also topo I [4]. Antitumor effects of compounds inhibiting topo I and topo II were demonstrated in clinical studies [1].

Naphthoindole dione derivatives (Fig. 1) containing active (aminoalkyl) groups in various positions are characterized by high antiproliferative activity. Some compounds cause death of drug-resistant tumor cells [6-8]. Presumably, naphtho[2,3-*f*]indole-5,10-dione derivatives bind to DNA and impair its structure. Indeed, a tryptamine naphthoindole analog containing 2-aminoethyl group in the chromophore position 3 inhibits relaxation of supercoiled DNA when applied in lower concentrations than camptothecine [6].

We studied derivatives of naphtho[2,3-*f*]indole-5,10-dione, an indole analog of anthracycline with cyclohexane cycle replaced by pyrrole.

## MATERIALS AND METHODS

Inhibition of topo I was evaluated by the capacity of the studied compounds to delay relaxation of

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supercoiled DNA. The reaction mixture (20 μl) containing 0.25 μg pHOT plasmid DNA (TopoGen), topo I isolated from calf thymus (1 U, Fermentas), and the studied naphthoindole diones was incubated in buffer (35 mM Tris-HCl, pH 8.0; 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiotreitol, 5 mM spermidine, 0.01% BSA; Sigma) for 30 min at 37°C. The reaction was stopped by adding sodium dodecylsulfate to a final concentration of 1%. After addition of proteinase K, the reaction mixture was incubated for 40 min at 37°C. Analyzed samples were divided into halves and separated by electrophoresis in gel with ethidium bromide (EB; 0.5 μg/ml) in buffer (40 mM Tris-base, 1 mM EDTA, 30 mM glacial acetic acid) or without EB (Fig. 2). In the latter case, the gels were treated with EB solution (0.5 μg/ml) after electrophoresis. The reaction products were separated in 1% agarose gel (3 V/cm) for 4-5 h. The gels were visualized in UV light.

## RESULTS

Naphtho[2,3-*f*]indole-5,10-diones obtained by modification of 4,11-dimethoxynaphtho[2,3-*f*]indole-5,10-dione are represented by two types of compounds. Naphtho[2,3-*f*]indole-5,10-dione 4,11-di(aminoethylamino) derivatives are structural analogs of ametantrone and mitoxantrone (antitumor drugs) and are synthesized by replacement of the methoxy group with ethylene diamine derivatives (compounds 4-7; Fig. 3, *a*) [7]. Other chemicals, 4,11-dihydroxynaphtho[2,3-*f*)-indole-5,10-dione 3-aminomethyl derivatives, were obtained in several stages including Mannich's reaction and methoxy group demethylation (compounds 8-11; Fig. 3, *b*) [8].

The method for evaluation of topo I activity is based on the relationship between electrophoretic mobility DNA and conformations of supercoiled, relaxed, and circular (open and closed) DNA. The composition of topoisomers (relaxation products of supercoiled DNA) changed in the presence of compounds 4-10: the number of partially relaxed forms was significantly higher than the content of completely relaxed DNA. This means that all new compounds inhibit relaxation of supercoiled DNA. Compounds 5 and 7 containing ethylene diamine residue with 2-hydroxyethyl and methyl groups in the side chains exhibited a strong inhibitory effect. Activities of compounds 8-11 with the active group in position 3 was somewhat lower (Fig. 2, *a*). The most active inhibitor was compound 11 containing a quinuclidine residue in the side chain: in a concentration of 5 μM it completely inhibited DNA relaxation (gel photograph is not presented).

As the positions of open and closed circular DNA forms coincided in electrophoresis without EB (Fig. 2, *a*), they were separated by electrophoresis with EB in gel and buffer. The open circular form migrated slower under these conditions (accumulation of this form indicates specificity of inhibition of DNA relaxation) [3]. Comparison of rows without inhibitors and with 10 and 100 μM camptothecine showed accumulation of the open circular form indicating specificity of inhibition (Fig. 2, *b*). Compounds 4-10 (each in a concentration of 2.5 μM) exhibited no effect of this kind, and hence, the studied naphthoindole diones presumably prevented topo I-mediated DNA relaxation nonspecifically. Hence, camptothecine and naphthoindole dione derivatives 4-11 inhibit topo I activity, but the mechanisms of inhibition principally differ.

This assumption is confirmed by similarity of electrophoretic mobility of topoisomers formed in the presence of intercalator EB and compounds 4-7 (Fig. 2, *c*). Suppression of DNA relaxation in the presence of EB (1 μM) was shown by a rapidly

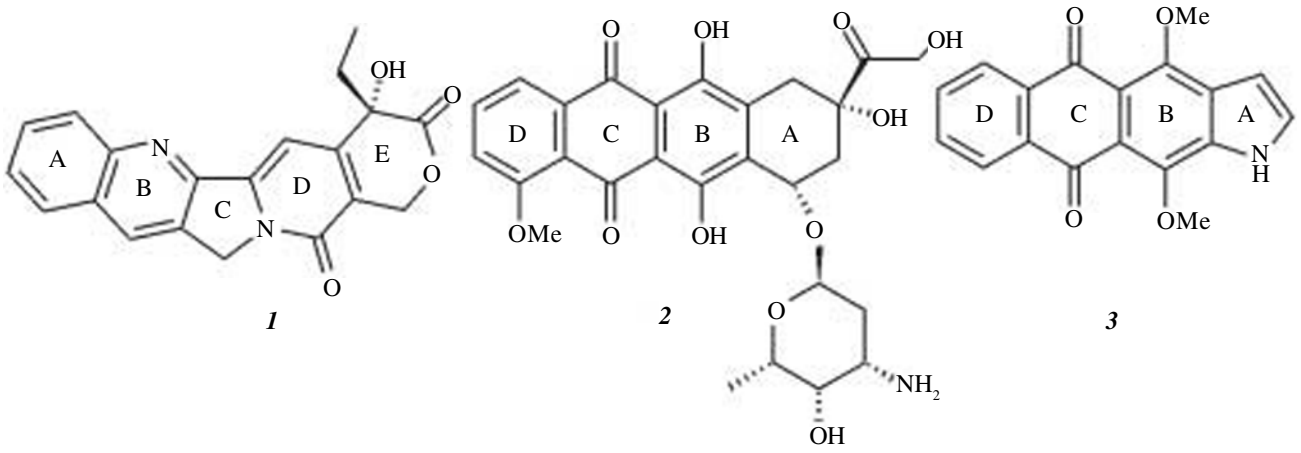


**Fig. 1.** Formulas of compounds used in experiment. *1*) camptothecine; *2*) doxorubicin; *3*) 4,11-dimethoxynaphtho[2,3-*f*]indole-5,10-dione.

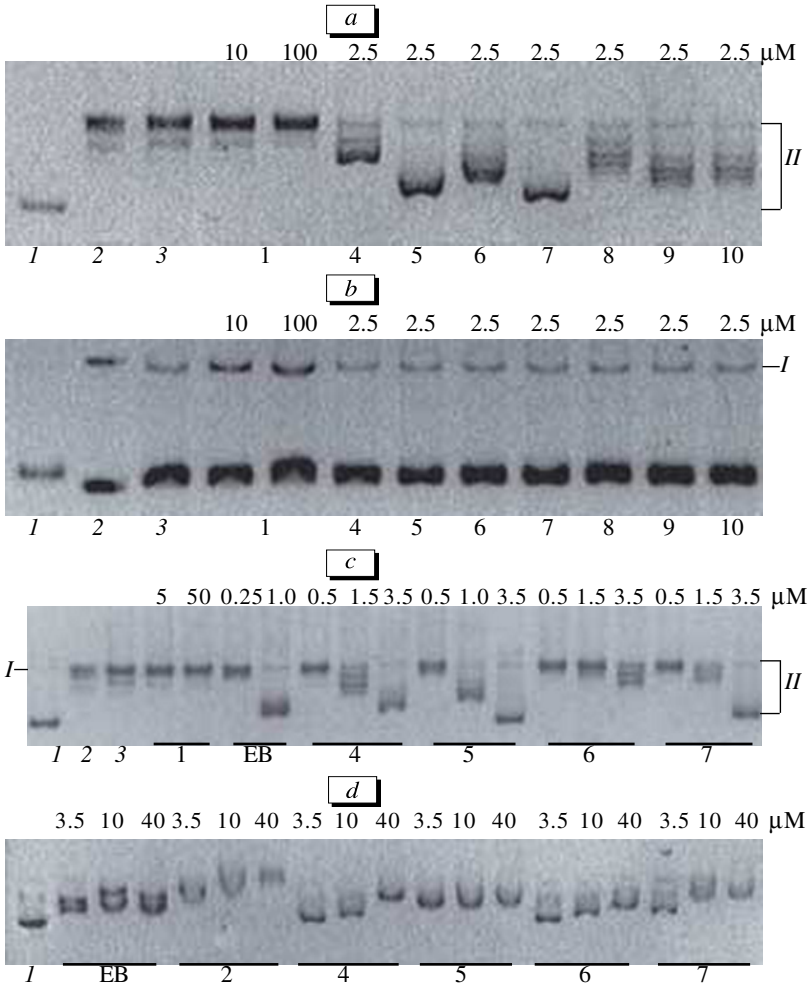


**Fig. 2.** Effects of naphthoindole dione derivatives on topo I activity. Mobility of PHot plasmid DNA in gel after DNA relaxation in the presence of new naphthoindole dione derivatives and control intercalators. *a*) electrophoresis without EB; *b*) electrophoresis with EB; *c*) compounds 4-7 in concentrations of 0.5-3.5 μM; *d*) without topo I in reaction mixture. *1*) supercoiled DNA; *2*) relaxed DNA; *3*) supercoiled DNA and topo I. *I*) open circular plasmid form; *II*) topoisomers. *1*) camptothecine; *2*) doxorubicin. *a-d*) electrophoresis: *a*) no EB, compounds 4-10 in a concentration of 2.5 μM; *b*) with EB, compounds 4-10 in a concentration of 2.5 μM; *c*) no EB, compounds 4-7 in concentrations changing from 0.5 to 3.5 μM; *d*) no EB, compounds 4-7 without topo I in reaction mixture.

migrating intensive band. Increase in the concentrations of compounds 4-7 also inhibited DNA relaxation, which was seen from accumulation of more mobile topoisomers. Compounds 4, 5, and 7 completely inhibited DNA relaxation in a concentration of 3.5 μM (Fig. 2, *c*). Similarity of the effects of reference drugs and naphthoindole diones on DNA migration suggests that the studied derivatives, similarly as EB, interact with DNA. Due to this reaction, naphthoindole dione derivatives inhibit topo I-mediated relaxation of supercoiled DNA.

Since naphthoindole diones react with DNA, electrophoretic mobility of DNA molecules can be determined by not only topo I activity, but also the effects of DNA ligands on DNA conformation (and hence, mobility). We studied the effects of compounds 4-7 on mobility of supercoiled DNA without topo I. Control DNA intercalators doxorubicin and EB inhibited electrophoretic mobility of the plasmid (Fig. 2, *d*). However, used in concentrations, in which naphthoindole diones 4, 6, and 7 inhibited DNA relaxation (Fig. 2, *c*), these compounds virtually did not modify plasmid migration. These compounds (Nos. 4, 6, and 7) inhibited plasmid migration only when used in concentrations surpassing the range of DNA relaxation inhibition (Fig. 2, *d*). Only the naphthoindole analog of ametantrone (compound 5), used in concentrations inhibiting topo I activity, inhibited DNA migration. Hence, inhibition of the plasmid migration in the presence of derivatives 4, 6, and 7 was due to inhibition of topo I, whereas direct effect on the mobility of supercoiled DNA for derivative 5 cannot be excluded.

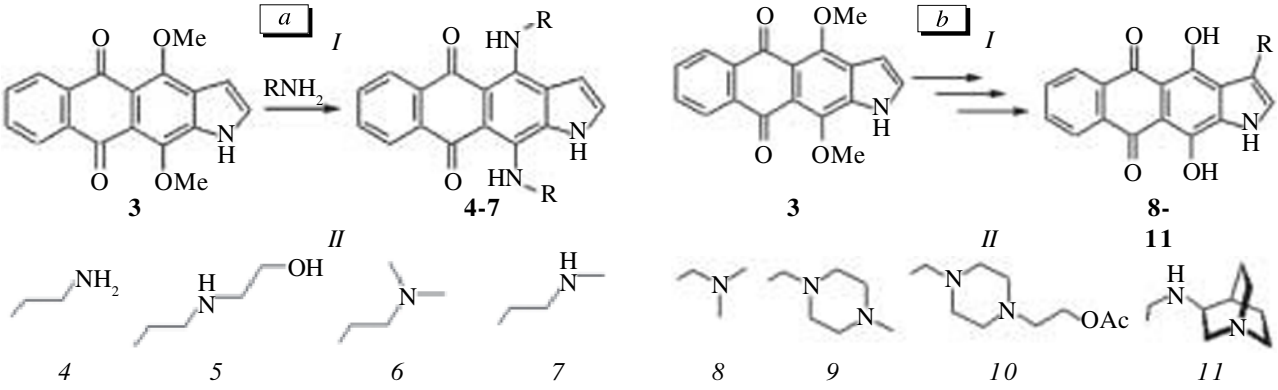


**Fig. 3.** Schemes of synthesis of naphtho[2,3-*f*]-indole-5,10-dione 4,11-di(amino)derivatives 4-7 (*a*) and 4,11-dihydroxynaphtho[2,3-*f*]-indole-5,10-dione 3-aminomethyl derivatives 8-11 (*b*). Scheme of synthesis (*I*) and introduced radicals (*II*).

The topo I-inhibitory activity of the new compounds was evaluated using the $IC_{50}$ index (the concentration of inhibitor preventing enzyme relaxation of 50% supercoiled DNA). The content of supercoiled DNA completely transformed in relaxed form (reaction without inhibitor) corresponded to 100% enzyme activity [9]. $IC_{50}$ for the most active naphthoindole diones was about 1.5 μM for compound 4, 0.5-1.5 μM for compound 5, 1.5-3.5 μM for compound 7, and >3.5 μM for compound 6.

Hence, new naphthoindole dione derivatives in micromolar concentrations inhibit topo I-mediated relaxation of DNA. The mechanism of inhibition differs from that realized with the use of catalytic blocker camptothecine and is presumably associated with direct reaction of naphthoindole diones with DNA. It is important that the range of concentrations in which compounds 4-11 inhibited topo I, coincided with the interval of concentrations causing death of K562 human leukemia cells [6-8]. In addition, compounds 7 and 11 (the most potent inhibitors of topo I) proved to be highly toxic for 60 human tumor cell strains (according to screening data of National Institute of Cancer Research, USA). It seems that suppression of topo I activity is the leading (but presumably not the only one) cytotoxicity factor for compounds of this chemical class.

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