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11-Phenylundeca-5Z,9Z-dienoic Acid: Stereoselective Synthesis and Dual Topoisomerase I/IIα Inhibition

Vladimir A. D'yakonov[a,*], Lilya U. Dzhemileva[b,c,*], Aleksey A. Makarov[a], Alfiya R. Mulyukova[a], Dmitry S. Baev[d], Elza K. Khusnutdinova[b], Tatiana G. Tolstikova[d] and Usein M. Dzhemilev[a]

V.A. D'yakonov

[a]*Institute of Petrochemistry and Catalysis, Russian Academy of Science, Ufa, Russian Federation;* [b]*Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, Bashkortostan, Russian Federation;* [c]*Department of Immunology and Human Reproductive Health, Bashkir State Medical University, Ufa, Bashkortostan, Russian Federation;* [d]*N.N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russian Federation*

L.U. Dzhemileva

Abstract: (5Z,9Z)-11-Phenylundeca-5,9-dienoic acid was stereoselectively synthesized, based on original cross-cyclomagnesiation of 2-(hepta-5,6-dien-1-yloxy)tetrahydro-2H-pyran and buta-2,3-dien-1-ylbenzene with EtMgBr in the presence of the Cp_2TiCl_2 catalyst giving 2,5-dialkylydenemagnesacyclopentane in 86% yield. The acid hydrolysis of the product and Jones oxidation of the resulting 2-{[(5Z,9Z)-11-phenylundeca-5,9-dien-1-yl]oxy}tetrahydro-2H-pyran afforded (5Z,9Z)-11-phenylundeca-5,9-dienoic acid in an overall yield of 75%. A high inhibitory activity of the synthesized acid with respect to human topoisomerase I (hTop1) and II (hTop2α) was detected. Resorting to the data of molecular docking, a mechanism of inhibition was proposed.

Keywords: Cyclomagnesiation, docking, fatty acids, homogeneous catalysis, novel topoisomerase I and IIα inhibitors, stereoselective synthesis of 5Z,9Z-dienoic acid.

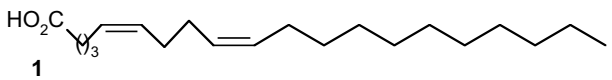
INTRODUCTION

The search for new efficient and low-toxicity antitumor agents is a highly important task of modern medicinal chemistry. Generally, solution of this problem is reduced to the search for new compounds affecting molecular targets that play an important role in carcinogenesis.

Numerous studies in this field demonstrated that topoisomerases I (hTop1) and II (hTop2α) are among the key molecular targets for the development of modern antitumor agents [1-6].

The literature describes quite a few low-molecular-weight chemical compounds [7-15] that are able, owing to their lipophilic nature, to penetrate into the cell nuclei, interact with DNA or block DNA-dependent enzymes and, hence, change indirectly the local conformation of DNA molecules, thus inducing strand cleavage or disturbing the matrix synthesis. The DNA damage results in violation of the cell cycle and cell viability, and, hence, the therapeutic effect is achieved: retardation of proliferation and tumor destruction.

Previously [16, 17], we developed an efficient method for the synthesis of 5*Z*,9*Z*-dienoic acid of high degree of sterechemical purity that exhibited pronounced inhibitory action on human topoisomerase I. In particular, high activity of (5Z,9Z)-5,9-eicosadienoic acid (**1**) as a human topoisomerase I inhibitor at concentrations above 0.1 μM was elucidated.



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We found that the position of the 1Z,5Z-diene group relative to the carboxy group in the synthesized acids has a considerably influence on the inhibition of hTop1 and hTop2α [18].

As a further development of our research, we put forward the idea of introducing a phenyl group into the molecules of stereoisomerically pure higher 5Z,9Z-dienoic acids in question. We assumed that the phenyl group could serve as an electron reservoir, which would provide more active complex-formation of phenyl-substituted acids with the topoisomerase active site or DNA molecule.

In view of the above, we performed stereoselective synthesis of (5*Z*,9*Z*)-11-phenylundeca-5,9-dienoic acid.

MATERIALS AND METHOD

All solvents were dried (hexane, THF, benzene over Na) and freshly distilled before use. All reactions were carried out under a dry argon atmosphere. ^1H and ^{13}C NMR spectra were obtained using a Bruker AVANCE 400 spectrometer in $CDCl_3$ operating at 400 MHz for ^1H and 100 MHz for ^{13}C and Bruker AVANCE 500 spectrometer in $CDCl_3$ operating

*Address correspondence to these authors at the Institute of Petrochemistry and Catalysis, Russian Academy of Science, Ufa, Russian Federation; Tel: +7(347)2842750; Fax: +7(347)2842750; E-mail: DyakonovVA@gmail.com and Department of Genetics and Fundamental Medicine, Bashkir State University, Ufa, Bashkortostan, Russian Federation; Tel: +7(347)2726370; Fax: +7(347)2736778; E-mail: Dzhemilev@mail.ru

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at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Elemental analyses were measured on a 1106 Carlo Erba apparatus. Mass spectra were obtained on MALDI TOF/TOF spectrometer in a 2,5-dihydroxybenzoic acid matrix and Shimadzu GCMS-QP2010 Plus spectrometer at 70 eV and working temperature 200 °C. Individuality and purity of the synthesized compounds were controlled using of TLC on Silufol UV-254 plates; anisic aldehyde in acetic acid was used as a developer. Column chromatography was carried out on Acrus silica gel (0.060–0.200 мм).

Procedure for the Synthesis of (5Z,9Z)-11-phenylundeca-5,9-dienoic Acid (5)

Diethyl ether (10 mL), 2-(hepta-5,6-dien-1-yloxy) tetrahydro-2H-pyran (10 mmol), buta-2,3-dien-1-ylbenzene (12 mmol), EtMgBr (40 mmol) (as 1.5 M solution in Et_2O), Mg powder (32 mmol), and Cp_2TiCl_2 (0.5 mmol) were charged into a glass reactor with stirring under argon (~0ºC). The reaction mixture was warmed-up to room temperature (20−22°C) and stirred for 6−8 h. Then the reaction mixture was treated with a 5% solution of HCl in H_2O. The 2-{[(5*Z*,9*Z*)-11-phenylundeca-5,9-dien-1-yl]oxy}tetrahydro-2H-pyran (**4**) were extracted with diethyl ether, the extracts were dried with $MgSO_4$, the solvent was evaporated, and the residue was chromatographed on a column (SiO_2, elution with petroleum ether – EtOAc (50:1)). The Jones oxidation of 2-{[(5*Z*,9*Z*)-11-phenylundeca-5,9-dien-1-yl]oxy}tetrahydro-2H-pyran (**4**) furnished (5*Z*,9*Z*)-11-phenylundeca-5,9-dienoic acid (**5**) in 62% yield.

DNA Topoisomerase I Assay

The inhibitory activity and the mechanism of inhibition of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid were determined using the Topoisomerase I Drug Screening Kit TG-1018-2, (Topogen, USA) (the tested compound was added before topoisomerase I). The relaxation of supercoiled DNA under the action of topoisomerase I was carried out as follows: the reaction mixture (20 mL) containing 0.25 mg of the DNA plasmid pHOT (TopoGen, USA), 4 unit of recombinant topoisomerase I (TopoGen, USA), and the tested compound: dienoic acid was incubated in the buffer (35mM Tris-HCl, pH 8.0; 72 mM KCl, 5 mM MgCl, 5 mM dithiothreitol, 5 mM spermidine, and 0,01% bovine serum albumin) for 30 min at 37 °C using a Biosan thermostat (Latvia). The tested compound was introduced in the reaction mixture prior to the addition of the enzyme topoisomerase I. The inhibiting action on topoisomerase I was monitored using the alkaloid camptothecin (TopoGEN, USA). The reaction was terminated by adding sodium dodecyl sulfate up to a concentration of 1%. After addition of a solution (5 mg/mL) of proteinase K (Sigma Chemical Co., USA) (1:10), the reaction mixture was incubated for 15 min at 37 °C. A 0.1 % solution of bromophenol blue (1:10) was added and the samples were electrophoresed in the presence and absence of ethidium bromide. The reaction products were separated in a 1% agarose gel (3 V/cm) for 2-3 h. After the electrophoresis without ethidium bromide, the gels were treated with a solution of ethidium bromide (0.5 mg/mL). The gels were visualized in the UV light in a Infinity VX2 1120/Blue X-Press gel documentation system (Vilber Lourmat, France). The possible action of the tested compounds on supercoiled DNA was checked by performing the reaction without topo I, the tested compounds being added in the same concentrations as in the reaction with the enzyme.

DNA Topoisomerase II Assay

The inhibitory activity and the mechanism of inhibition of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid were determined using the Topoisomerase IIα Drug Screening Kit TG1009-2, (Topogen, USA). (the tested compound was added before topoisomerase IIα). The relaxation of supercoiled DNA under the action of topoisomerase IIα was carried out as follows: the reaction mixture (20 mL) containing 0.25 mg of the DNA plasmid pHOT (TopoGen, USA), 4 unit of recombinant topoisomerase IIα (TopoGen, USA), and the tested compound: dienoic acid was incubated in the Topo II Assay Buffer (Topo II Buffer is supplied as a 10x Stock solution in two parts: 10X Incomplete Topo II Assay Buffer A contains the following: 0.5M Tris-HCl (pH 8.0), 1.5M NaCl, 0.1M $MgCl_2$, 5 mM dithiothreitol and 10X ATP Buffer B contains 20 mM ATP in water) for 30 min at 37 °C using a Biosan thermostat (Latvia). The tested compound was introduced in the reaction mixture prior to the addition of the enzyme topoisomerase IIα. The inhibiting action on topoisomerase IIα was monitored using the etopozide (TopoGEN, USA). The reaction was terminated by adding sodium dodecyl sulfate up to a concentration of 1%. After addition of a solution (5 mg/mL) of proteinase K (Sigma Chemical Co., USA) (1:10), the reaction mixture was incubated for 15 min at 37 °C. A 0.1 % solution of bromophenol blue (1:10) was added and the samples were electrophoresed in the presence and absence of ethidium bromide. The reaction products were separated in a 1% agarose gel (3 V/cm) for 2-3 h. After the electrophoresis without ethidium bromide, the gels were treated with a solution of ethidium bromide (0.5 mg/mL). The gels were visualized in the UV light in a Infinity VX2 1120/Blue X-Press gel documentation system (Vilber Lourmat, France). The possible action of the tested compounds on supercoiled DNA was checked by performing the reaction without topo IIα, the tested compounds being added in the same concentrations as in the reaction with the enzyme.

EXPERIMENTAL

(5Z,9Z)-11-Phenylundeca-5,9-dienoic Acid (5)

Yield = 62% (1.69 g), as a colorless oil. R_f = 0.52 (hexan-EtOAc – 5:1). IR: 3395, 3010, 2925, 2856, 1741, 1657, 1466, 1385, 1365, 1238, 1035, 734 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ − 1.70-1.76 (m, 2H), 2.13-2.40 (m, 8H, C<u>H</u>$_2$CH=), 3.43 (d, 2H, C<u>H</u>$_2$-Ph, *J* = 7.5 Hz), 5.39-5.63 (m, 4H, C<u>H</u>=C<u>H</u>), 7.20-7.33 (m, 5H, Ph) ppm. ^{13}C NMR ($CDCl_3$, 125 MHz): δ 24.6 C(3), 26.5 C(4), 27.3 C(7,8), 33.4 C(2), 33.5 C(11), 125.9 C(15), 128.4 C(13,17), 128.5 C(14,16), 128.6 C(5), 128.9 C(10), 130.1 C(9), 130.4 C(6), 141.1 C(12), 179.8 C(1) ppm. Anal. Calcd for $C_{17}H_{22}O_2$: C, 79.03; H, 8.58. Found: C, 78.84; H, 8.60.

RESULTS AND DISCUSSIONS

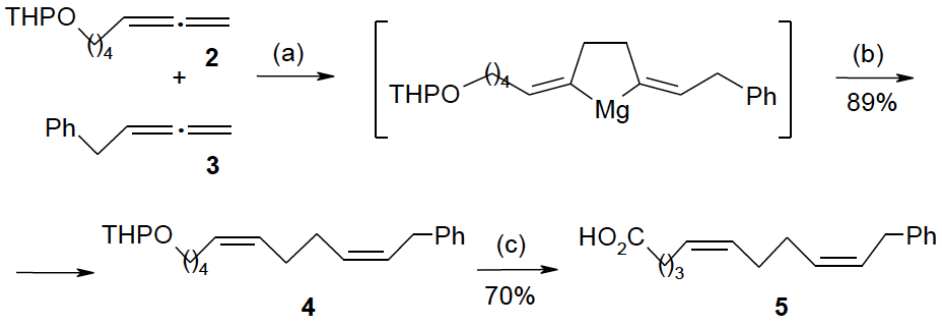
According to the previously developed [16] strategy for the synthesis of 5*Z*,9*Z*-dienoic acids, this was done by intermolecular cross-cyclomagnesiation of 2-(hepta-5,6-

dien-1-yloxy)tetrahydro-2H-pyran (**2**) and buta-2,3-dien-1-ylbenzene (**3**) with EtMgBr in the presence of activated Mg and Cp_2TiCl_2 catalyst under the conditions (**2**:**3**:EtMgBr:Mg:[Ti] = 10:12:40:32:0.5, Et_2O, 8h, 20-22 ^oC). Acid hydrolysis of the reaction mixture gave 2-{[(5*Z*,9*Z*)-11-phenylundeca-5,9-dien-1-yl]oxy}tetrahydro-2H-pyran (**4**) in 86% yield. The Jones oxidation of this product furnished previously undescribed (5*Z*,9*Z*)-11-phenylundeca-5,9-dienoic acid (**5**) in 75% yield (Scheme **1**).

The structures of the resulting compounds **4** and **5** were reliably proved by ^1H and ^{13}C NMR spectroscopy and mass spectrometry.

The next stage was to investigate the inhibitory action of acid **5** on human topoisomerase I activity *in vitro* in the relaxation of supercoiled plasmid DNA under standard conditions (Fig. **1**).

The presented in Fig. **1** (**A** and **B**) indicate that in the relaxation results reaction of supercoiled plasmid DNA in which the topoisomerase I (Topogen, USA) activity is inhibited by the added (5Z,9Z)-11-phenylundeca-5,9-dienoic acid (in this example, 4 enzyme units are inhibited by 0.1 μM of the compound under study), the residual amount of the supercoiled plasmid DNA decreases and the number of topoisomers formed (lanes 5-11) increases, as the concentration of the added compound successively increases from 0.1 μM to 500 μM. When the concentration was 0.1 to 50 μM, accumulation of mainly the supehelical plasmid form was observed upon the addition of ethidium bromide to the gel (Fig. **1B**, lanes 5-8), while at concentrations above



Scheme 1. Stereoselective synthesis of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid. Reagents: (**a**) EtMgBr, Mg, [Cp_2TiCl_2]; (**b**) H_3O^+; (**c**) Jones oxidation.

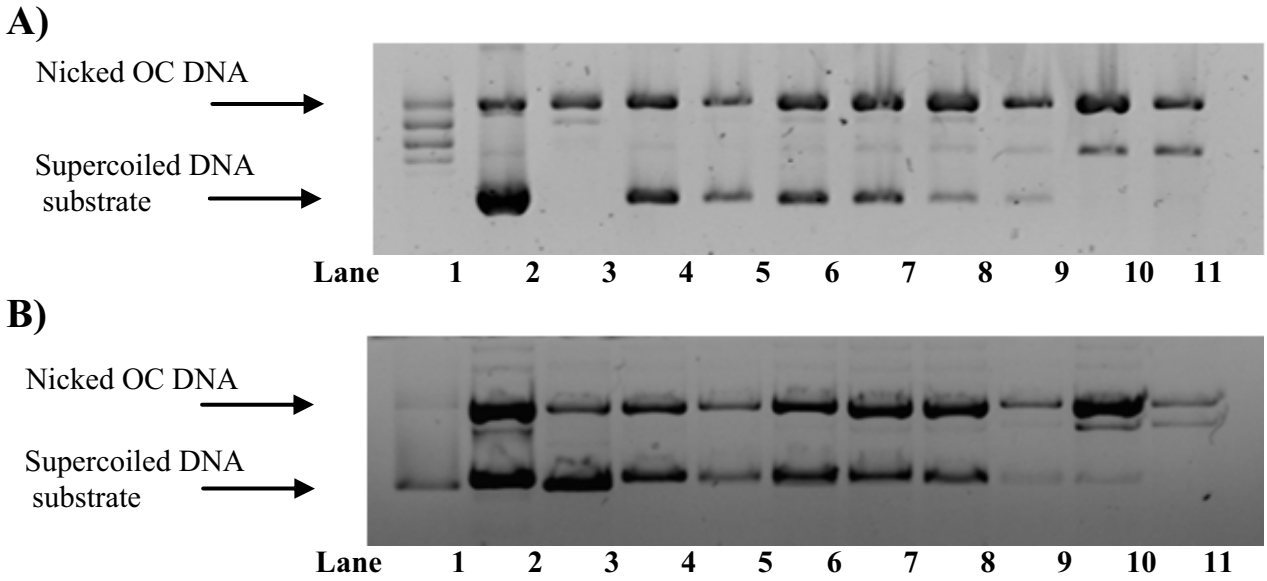


Fig. (1). Electrophoregram of the products of relaxation of supercoiled. Electrophoregram of the topoisomerase I induced relaxation products of 250 ng of plasmid DNA (pHOT1) *in vitro* (Topoisomerase I Drug Screening Kit TG-1018-2, Topogen, USA) in the presence of (5*Z*,9*Z*)-11-phenylundeca-5,9-dienoic acid. **A**) Photographs without ethidium bromide stained gels are shown. (lane 1) relaxed plasmid DNA (pHOT-1); (lane 2) supercolied plasmid DNA (pHOT1); (lane 3) supercolied plasmid DNA + topoisomerase I (4 unit); (lane 4) supercolied plasmid DNA + topoisomerase I + camptothecin (100μM) – positive control; (lanes 5-11) supercolied plasmid DNA + topoisomerase I (4 unit) + compound **5** at concentration of 0.1, 1, 20, 50, 100, 250, 500 μM. **B**) Photographs of ethidium bromide stained gels are shown. (lane 1) relaxed plasmid DNA (pHOT-1); (lane 2) supercolied plasmid DNA (pHOT1); (lane 3) supercolied plasmid DNA + topoisomerase I (4 unit); (lane 4) supercolied plasmid DNA + topoisomerase I + camptothecin (100μM) – positive control; (lanes 5-11) supercolied plasmid DNA + topoisomerase I (4 unit) + compound **5** at concentration of 0.1, 1, 20, 50, 100, 250, 500 μM.

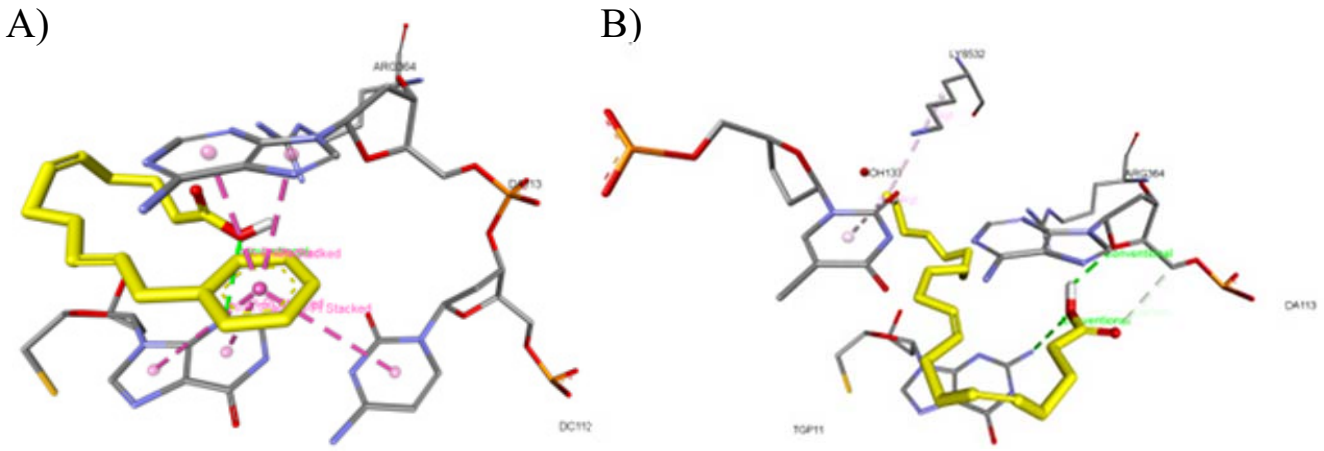


Fig. (2). Docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid **5** (**A**) and (5*Z*,9*Z*)-5,9-eicosadienoic acid **1** (**B**) in the topoisomerase I active site (the hydrogen bonds are shown by dashed lines).

50 μM (Fig. **1B**, lanes 9-11), an increase in the amount of the open ring form was detected. These results led us to the conclusion that (5Z,9Z)-11-phenylundeca-5,9-dienoic acid suppresses the catalytic activity of topo I; however, its action is dose-dependent, and when present in concentration of 50 μM and higher, it is also able to affect the formation of covalent complexes between DNA and topo I. Probably, the inhibitory action of acid **5** is determined by its high DNA binding affinity, which apparently hampers the topo I interaction with DNA specific sequences or this compound locally changes the DNA conformation. This assumption was supported by the data of molecular docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid performed using the refined computer model of the binding site of the compound in question with topoisomerase I active site obtained by crystallographic methods (Fig. **2**) [19].

Computer simulation demonstrates that, owing to relatively small size of the molecule (as compared with camptothecin) [20], (5Z,9Z)-11-phenylundeca-5,9-dienoic acid easily fits, as expected, into the relatively capacious protein cavity of the topoisomerase I active site (Fig. **2**).

A comparison of the location of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid and (5*Z*,9*Z*)-5,9-eicosadienoic acid we synthesized previously [16], which also exhibited a high hTopI inhibiting activity, in the topoisomerase I active site, indicates that the lipophilic moiety of acid **5**, like acid **1**, forms favorable hydrophobic contacts with Arg364 (Fig. **2A**,**B**). The replacement of the alkyl chain in the initial (5*Z*,9*Z*)-5,9-eicosadienoic acid **1** molecule by the lipophilic phenyl moiety brings about active involvement of the phenyl π-system into the stacking (π-π interaction) with the aromatic structure of nucleotides (adenine). Stacking is known to be the principal type of noncovalent interaction in the DNA structure stabilizing the helical configuration. As a result, (5Z,9Z)-11-phenylundeca-5,9-dienoic acid interacts more tightly with DNA as compared with the acids having long alkyl chains (Fig. **2B**, Table **1**). The molecular docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid **5** and (5*Z*,9*Z*)-5,9-eicosadienoic acid **1** and some minor-groove binding ligands (distamycin, netropsin, and DAPI) performed in appropriate computer model of interaction with DNA [21] demonstrated that both distamycin and netropsin molecules interact with purines of the DNA molecule, first of all, through the nitrogen atoms located between the imidazole groups (Suppl. Information, Table **1**). The hydrophobic interaction of DAPI with the DNA purine groups occurs, according to our data, in a somewhat different way, namely, hydrogen bonds are formed between the purines and the benzimidazole group. Upon the reaction of (5Z,9Z)-11-phenylundeca-5,9-dienoic and (5*Z*,9*Z*)-5,9-eicosadienoic acids with DNA, hydrogen bonds are formed only through carboxy groups (Fig. **3**). The spatial hydrophobic interactions of acids **1** and **5** are, in our opinion, not highly developed, as the long chains of the dienoic acids do not tend to be arranged along the DNA minor grooves but are folded to form compact structures, as it is shown in Fig. **3**.

The IC_{50} value, which represents the concentration of inhibitor that prevents the 50% amount of supercoiled DNA from being relaxed, was used to compare the inhibitory effect of acids **1** and **5** on the activity of topoisomerase 1. The IC_{50} value of acid **1** is 0.81 μM and the IC_{50} value of acid **5** is 0.67 μM that is consistent with the published data on the inhibitory activity of natural 5*Z*,9*Z*-dienoic acids [22].

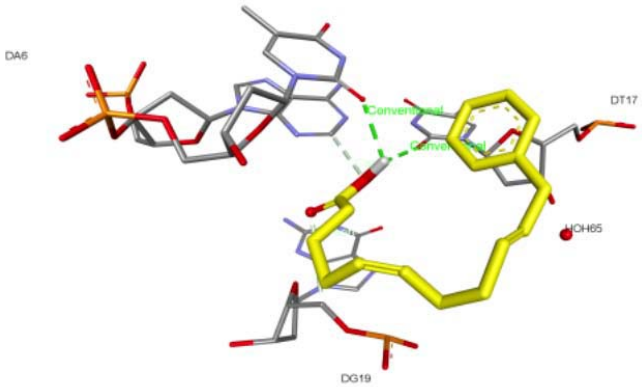


Fig. (3). Docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid DNA minor groove (some hydrogen bonds are shown by dashed lines).

Table 1. Minimum binding energies of the tested compounds with topoisomerase I, IIα and DNA minor groove.

Active Compound	Predicted Binding Energy (hTop1), kcal/mol	Predicted Binding Energy, kcal/mol (DNA)	Predicted Binding Energy (hTop IIα + DNA), kcal/mol
Acid **1**	-5.9	-5.4	-5
Acid **5**	-7	-5.4	-5.4

The above data provide the assumption that the mechanism of hTopI inhibition by (5Z,9Z)-11-phenylundeca-5,9-dienoic acid, unlike the mechanism of action of (5*Z*,9*Z*)-5,9-eicosadienoic acid, includes both the stabilization of the DNA-topo I covalent complex (specific inhibition) and competition of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid with topoisomerase I for DNA binding sites (non-specific inhibition).

Subsequently we studied the inhibitory action of acid **5** with respect to human topoisomerase IIα *in vitro* in the relaxation of supercoiled plasmid DNA under standard conditions (Fig. **4**).

The results presented in Fig. **4** (**A** and **B**) indicate that in the relaxation of supercoiled plasmid DNA in which topoisomerase IIα (Topogen, USA) is inhibited by (5Z,9Z)-11-phenylundeca-5,9-dienoic acid (in this particular case, 4 enzyme units are inhibited by 0.1 μM of the compound, lanes 4-11), successive increase in the concentration of the initial acid from 0.1 to 500 μM is accompanied by accumulation of mainly the superhelical plasmid (Fig. **1B**, lanes 4-12, ethidium bromide gel). This suggests that (5Z,9Z)-11-phenylundeca-5,9-dienoic acid suppresses the catalytic activity of hTopIIα and simultaneously affects the formation of covalent complexes of DNA and topoisomerase IIα. Apparently, the inhibitory action of acid **5** on topoisomerase IIα is determined by both the higher affinity for DNA (docking results, Fig. **5**) and more active interaction with the topoisomerase IIα catalytic site due to the presence of the aromatic ring in the molecule. Our assumption is confirmed by molecular docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic performed using the refined computer model of the binding site of the compound in question with topoisomerase IIα active site and DNA obtained by crystallographic methods (Table **1**) [23].

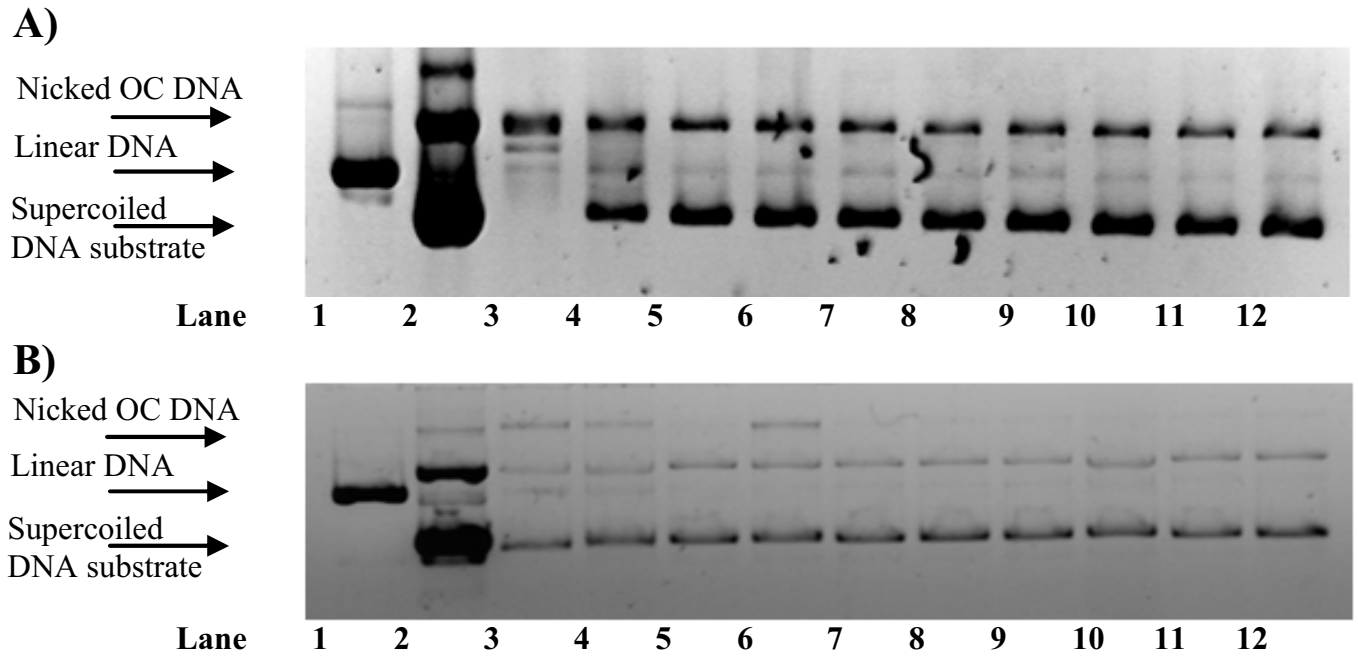


Fig. (4). Electrophoregram of the relaxation products of 250 ng of plasmid DNA (pHOT1) *in vitro* formed under the action of topoisomerase II (Topoisomerase IIα Drug Screening Kit TG1009-2 250, Topogen, USA) in the presence of (5*Z*,9*Z*)-11-phenylundeca-5,9-dienoic acid (the compound was added prior to the addition of the topoisomerase II enzyme). **A**) Photographs without ethidium bromide stained gels are shown. (lane 1) linear DNA (pHOT-1); (lane 2) supercolied plasmid DNA (pHOT1); (lane 3) supercolied plasmid DNA + topoisomerase II (4 unit); (lane 4) supercolied plasmid DNA + topoisomerase II + etoposide (100μM) – positive control; (lanes 5-12) supercolied plasmid DNA + topoisomerase II (4 unit) + compound **5** at concentration of 0.1, 1, 20, 50, 80, 100, 250, 500 μM. **B**) Photographs of ethidium bromide stained gels are shown. (lane 1) linear DNA (pHOT-1); (lane 2) supercolied plasmid DNA (pHOT1); (lane 3) supercolied plasmid DNA + topoisomerase II (4 unit); (lane 4) supercolied plasmid DNA + topoisomerase II + etoposide (100μM) – positive control; (lanes 5-12) supercolied plasmid DNA + topoisomerase II (4 unit) + compound **5** at concentration of 0.1, 1, 20, 50, 80, 100, 250, 500 μM.

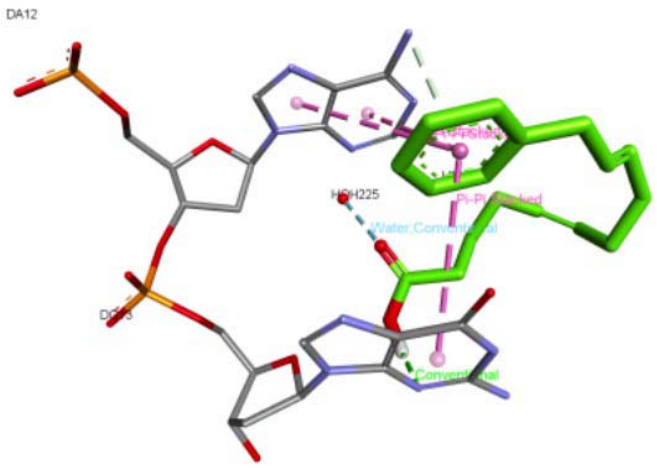


Fig. (5). Docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid in the DNA binding site (N-gate) to topoisomerase IIα (most of hydrogen atoms are omitted).

According to computer simulation, the phenyl radical of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid is actively involved in the stacking with the aromatic systems of the purine moieties of the DNA molecule. The phenyl radical is located between the purines in the plane parallel to their aromatic systems. Apparently this interaction is caused by the fact that the acid resides in the receptor binding pocket formed by topoisomerase II and DNA in which the electron density of the tested compound is somewhat displaced (Fig. **5**).

Comparison of the positions of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid and mitoxantrone in the active site of the topoisomerase IIα and DNA model indicates that the lipophilic aromatic group present in acid **5**, lile the anthraquinone moiety of mitoxantrone, interacts with the purines and pyrimidines of DNA through additional π -interactions. Mitoxantrone also forms favorable hydrophobic contacts with Asn520, Glu522, Gln778, and Arg503.

It is known from the literature [24] that the mechanism of action of mitoxantrone is similar to that of anthracyclines: they increase the concentration of the covalent intermediate (DNA–topoisomerase II) in the cell and thus lock topoisomerase II as a covalent adduct and leave DNA in the two-strand cleavage state hidden by the enzyme molecule. Apparently, the inhibition mechanism of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid has some differences from mitoxantrone, namely, it is stabilization of topoisomerase II as the intermediate complex due to possible change in the DNA modification.

CONCLUSION

Thus, we developed an original method for the synthesis of previously undescribed (5Z,9Z)-11-phenylundeca-5,9-dienoic acid in a high yield and with a high stereoselectivity (>98%). This acid has a clear-cut inhibitory action on topoisomerases I and IIα *in vitro*. The *in silico* studies demonstrated that replacement of the alkyl chain in the 5Z,9Z-dienoic acid by the phenyl group gives rise to a dual inhibition by both the specific mechanism involving stabilization of the DNA-TopI covalent complexes and nonspecific mechanism *via* competing reactions between acid **5** and topoisomerase I for DNA binding sites.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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