



Stereoselective synthesis of 11-phenylundeca-5Z,9Z-dienoic acid and investigation of its human topoisomerase I and II α inhibitory activity



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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 Cp₂TiCl₂ catalyst giving 2,5-dialkyldenemagnesiumcyclopentane in 86% yield. The acid hydrolysis of the product and the Jones oxidation of the resulting 2-[(5Z,9Z)-11-phenylundeca-5,9-dien-1-yl]oxytetrahydro-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 (hTop2 α) was determined.

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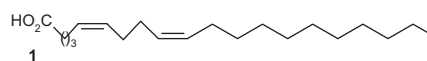
The search for new efficient and low-toxicity antitumor agents is a highly important task of modern medicinal chemistry. Generally, a solution of this problem is reduced to the search for new compounds affecting molecular targets that play an important role in carcinogenesis.

Numerous studies in this field demonstrated that topoisomerase I (hTop1) and topoisomerase II (hTop2 α) 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 the 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 5Z,9Z-dienoic acid of high stereochemical 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 μ M was elucidated.



We found that the position of the 1Z,5Z-diene group relative to the carboxyl group in the synthesized acids has a considerable influence on the inhibition of hTop1 and hTop2 α .¹⁸

As a further development of our research, we put forward the idea of introducing a phenyl group into 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 a DNA molecule.

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

According to the previously developed¹⁶ strategy for the synthesis of 5Z,9Z-dienoic acids, this was performed via 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 Cp₂TiCl₂ catalyst under

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chosen experimental conditions (2:3:EtMgBr/Mg/[Ti] = 10:12:40:32:0.5, Et₂O, 8 h, 20–22 °C). Acid hydrolysis of the reaction mixture gave 2-[[[(5Z,9Z)-11-phenylundeca-5,9-dien-1-yl]oxy]tetrahydro-2H-pyran (4) in 86% yield. The Jones oxidation of this product furnished previously undescribed (5Z,9Z)-11-phenylundeca-5,9-dienoic acid (5) in 75% yield (Scheme 1).

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

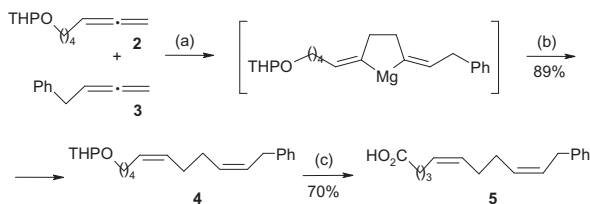
The next stage was to investigate the inhibitory action of acid 5 on human topoisomerase I activity *in vitro* in the relaxation of supercoiled plasmid DNA under standard conditions (Fig. 1).

The results presented in Figure 1 (A and B) indicate that in the relaxation reaction of supercoiled plasmid DNA in which the topoisomerase I (Topogen, USA) activity is inhibited by the added acid 5 (in this e.g., 1 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 decreases from 10 to 0.06 μM.

Since under electrophoresis in the absence of ethidium bromide the migration rate of nicked or open and closed forms of circular plasmid DNA in the gel are close, it becomes difficult to identify them.

Therefore, the same reaction products were examined in the gel containing an intercalator additive, ethidium bromide (0.5–1.0 μg/mL) (Fig. 1B).

In the presence of acid 5, as in the case with camptothecin, one could notice the line of open circular form. However, its intensity was lower than in the presence of camptothecin, which is a highly selective topoisomerase I inhibitor.



Scheme 1. Stereoselective synthesis of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid. Reagents: (a) EtMgBr, Mg, [Cp₂TiCl₂]; (b) H₃O⁺; (c) Jones oxidation.

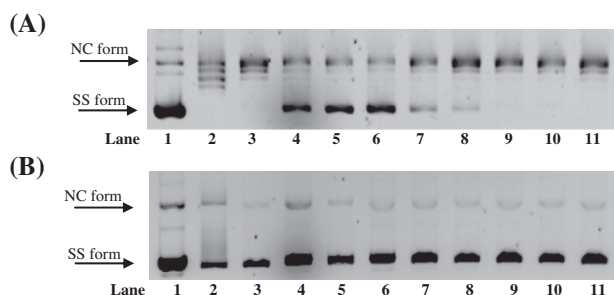


Figure 1. DNA topoisomerase I inhibitory activity of compound 5. Electrophoretogram of the topoisomerase I induced relaxation products of 250 ng of plasmid DNA (pHOT1) *in vitro* in the presence of compound 5. (A) Photographs without ethidium bromide stained gels are shown. (lane 1) supercoiled plasmid DNA (pHOT1); (lane 2) relaxed plasmid DNA (pHOT-1); (lane 3) supercoiled plasmid DNA+topoisomerase I (1 unit); (lane 4) supercoiled plasmid DNA+topoisomerase I+camptothecin (100 μM)–positive control; (lanes 5–11) supercoiled plasmid DNA+topoisomerase I (1 unit)+compound 5 at concentration of 10, 5, 1, 0.5, 0.1, 0.08, 0.06 μM. (B) Photographs of ethidium bromide stained gels are shown. (lane 1) supercoiled plasmid DNA (pHOT1); (lane 2) relaxed plasmid DNA (pHOT-1); (lane 3) supercoiled plasmid DNA+topoisomerase I (1 unit); (lane 4) supercoiled plasmid DNA+topoisomerase I+camptothecin (100 μM)–positive control; (lanes 5–11) supercoiled plasmid DNA+topoisomerase I (1 unit)+compound 5 at concentration of 10, 5, 1, 0.5, 0.1, 0.08, 0.06 μM.

The topoisomerase inhibition can be associated with the direct interaction of compound 5 with the DNA molecule thus altering its molecular shape (conformation), in contrast to the mechanism of action of camptothecin.

Since it is known that some substances interact with DNA, the electrophoretic mobility of the DNA molecules, which observed in our experiments, may be caused not only by changing the activity of topo I, but also due to the influence of dienoic acids themselves on the conformation of the DNA molecule.

Therefore, it was necessary to determine the concentration range, in which the studied compounds can influence the electrophoretic mobility of DNA.

In order to detect whether synthesized compounds 1 and 5 induce conformational changes in the DNA helix and whether there is a relationship between the plasmid–DNA binding affinity of the acids, we investigated their capacity to remove and reverse the supercoiling of closed circular pHOT1 plasmid DNA as assessed by electrophoretic mobility measurements on agarose gels (Fig. 2). As generally accepted, DNA cleavage is controlled by relaxation of the supercoiled circular conformation form (SS form) of plasmid pHOT1 DNA to the nicked circular form (NC form) and the linear conformation form (LC form). In gel electrophoresis experiments effected on the supercoiled circular conformation, the fastest migration will be observed for DNA of SS form. Following the cleavage of one strand, the supercoil will relax to produce the slower moving nicked conformation. If both strands are cleaved, a linear conformation will be generated that migrates in between.

Figure 2 shows that compound 5 can cleave plasmid pHOT1 DNA rather effectively, in a region of micromolar concentrations, as evidenced by the decrease of the SS Form and the increase of NC form and appearance of LC form. In the electrophoretograms the untreated pHOT1 plasmid DNA, which is a mixture of mainly covalently closed circular form and a small amount of open circular form bands, was used as the control (lane 1). With increasing acid 5 concentrations from 5 to 10 μmol (Fig. 5, lanes 7 and 8) the amount of nicked DNA increases to the advantage of the supercoiled DNA. For the two compounds the degree of interaction with DNA follows in compound 5. Significantly, in experiments on pHOT1 plasmid DNA, only the compound 5 brought about efficient DNA cleavage.

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 acid 5 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. 3).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. 3).

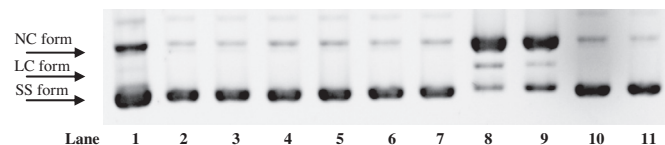


Figure 2. Modification of gel electrophoretic mobility of pHOT1 supercoiled plasmid DNA when incubated (30 min, 37 °C) with various concentrations of compounds 1, 5, camptothecin and etoposide (VP16). Concentrations (in μM) are as follows: (lane 1) untreated pHOT1 supercoiled plasmid DNA; (lanes 2–5, incubated with 1): 5, 50, 250, 500; (lanes 6–9, incubated with 5): 5, 50, 250, 500; (lane 10, incubated with camptothecin) 50; (lane 11, incubated with etoposide) 50. The top and the bottom bands correspond to open circular form, covalently closed circular form, and linear conformation form of plasmids, respectively.

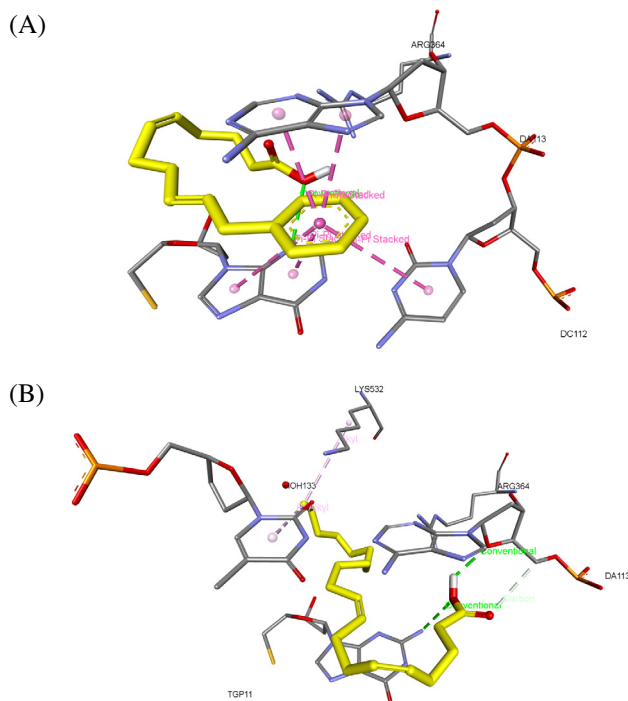


Figure 3. Docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid **5** (A) and (5Z,9Z)-5,9-eicosadienoic acid **1** (B) in the topoisomerase I active site (the hydrogen bonds are shown by dashed lines, yellow—ligand structure).

A comparison of the location of acid **5** and (5Z,9Z)-5,9-eicosadienoic acid we synthesized previously,¹⁶ which also exhibited a high hTop1 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. 3A and B). The replacement of the alkyl chain in the initial 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, acid **5** interacts more tightly with DNA as compared with the acid **1** having long alkyl chains (Fig. 2B, Table 1). The molecular docking of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid **5** and (5Z,9Z)-5,9-eicosadienoic acid **1** and some minor-groove binding ligands (distamycin, netropsin, and DAPI) performed in appropriate computer model of interaction with DNA²¹ 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 acids **1** and **5** with DNA, hydrogen bonds are formed only through carboxy groups (Fig. 4). 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 Figure 4.

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 I.²² The IC_{50} value of acid **1** is 0.81 μ M and the IC_{50} value of acid **5** is 0.67 μ M that is consistent

Table 1

Minimum binding energies of the tested compounds with topoisomerase I, II α and DNA minor groove

Active compound	Predicted binding energy (hTop1), kcal/mol	Predicted binding energy, kcal/mol (DNA)	Predicted binding energy (hTop II α +DNA), kcal/mol
Acid 1	-5.9	-5.4	-5
Acid 5	-7	-5.4	-5.4

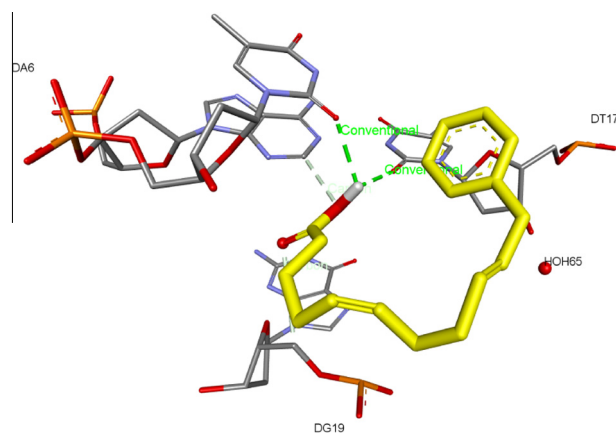


Figure 4. Docking of acid **5** in DNA minor groove (some hydrogen bonds are shown by dashed lines, yellow—ligand structure).

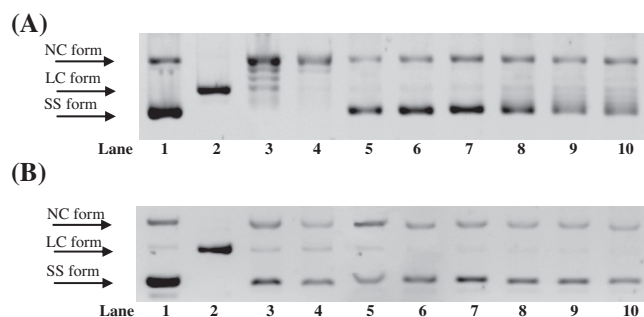


Figure 5. DNA topoisomerase II inhibitory activity of compound **5**. Electrophoretogram of the topoisomerase I induced relaxation products of 250 ng of plasmid DNA (pHOT1) in the presence of compound **5**. (A) Photographs without ethidium bromide stained gels are shown. (lane 1) supercoiled plasmid DNA (pHOT1); (lane 2) linear DNA (pHOT-1); (lane 3) supercoiled plasmid DNA+topoisomerase II (1 unit); (lane 4) supercoiled plasmid DNA+topoisomerase II+etoposide (100 μ M)—positive control; (lanes 5–11) supercoiled plasmid DNA+topoisomerase II (1 unit)+compound **5** at concentration of 5, 1, 0.5, 0.1, 0.08, 0.06 μ M. (B) Photographs of ethidium bromide stained gels are shown. (lane 1) supercoiled plasmid DNA (pHOT1); (lane 2) relaxed plasmid DNA (pHOT-1); (lane 3) supercoiled plasmid DNA+topoisomerase II (1 unit); (lane 4) supercoiled plasmid DNA+topoisomerase II+etoposide (100 μ M)—positive control; (lanes 5–11) supercoiled plasmid DNA+topoisomerase II (1 unit)+compound **5** at concentration of 5, 1, 0.5, 0.1, 0.08, 0.06 μ M.

with the published data on the inhibitory activity of natural 5Z,9Z-dienoic acids.²³

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

The results presented in Figure 5 (A and B) indicate that in the relaxation of supercoiled plasmid DNA in which topoisomerase II α is inhibited by acid **5** (in this particular case, 1 enzyme units are inhibited by 0.08 μ M of the compound, lanes 5–10), successive decrease in the concentration of the initial acid from 5 to

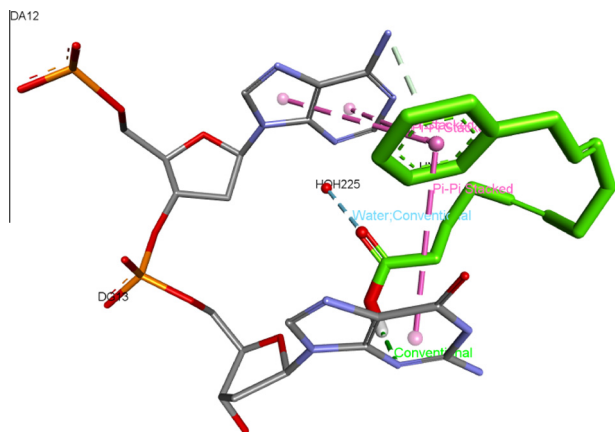


Figure 6. Docking of acid **5** in the DNA binding site (N-gate) to topoisomerase II α (most of hydrogen atoms are omitted).

0.06 μM is accompanied by accumulation of mainly the super-helical plasmid (Fig. 5B, lanes 5–10, ethidium bromide gel).

In the separation of the products of this reaction by gel electrophoresis with ethidium bromide (Fig. 5B) in the presence of acid **5**, we observed a dose-dependent accumulation of the linear form of the plasmid, which is characteristic of the so-called topo II poisons or specific inhibitors.

Additional methods of analysis are needed to confirm this specific mechanism of the enzyme inhibition involving compound **5**.

Apparently, the inhibitory action of acid **5** on topoisomerase II α is determined by both the higher affinity for DNA (docking results, Fig. 6) and more active interaction with the topoisomerase II α catalytic site due to the presence of the aromatic ring in the molecule. Our assumption is confirmed by molecular docking of acid **5** performed using the refined computer model of the binding site of the compound in question with topoisomerase II α active site and DNA obtained by crystallographic methods (Table 1).²⁴

According to computer simulation, the phenyl radical of acid **5** 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. 6).

It is known from the literature²⁵ 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.

Comparison of the positions of (5Z,9Z)-11-phenylundeca-5,9-dienoic acid and mitoxantrone in the active site of the topoisomerase II α and DNA model indicates that the lipophilic aromatic group present in acid **5**, like 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 (see Supplementary material).

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 II α in vitro.

This study allowed us not only to find the active inhibitors of topoisomerases I and II α among the compounds tested, but also to identify the relationship between their structure and inhibitory activity. The results of our experiments showed that (5Z,9Z)-11-phenylundeca-5,9-dienoic acid and (5Z,9Z)-eicosa-5,9-dienoic acid taken in the submicromolar concentrations are able to inhibit the catalytic activity of topo I and topo II, wherein the first compound is a more potent inhibitor of enzymes.

The results, which were obtained in the study of the influence of acids on the electrophoretic mobility of the DNA, provide evidence that acid **5** has a definite effect on the mobility of the DNA, which becomes visible at concentrations of 250 μM and above.

The mechanism of interaction between dienoic acids and enzymes (topo I and topo II) is still not quite clear. Presumably, the impact of dienoic acids can include the stabilization of the covalent complex of the DNA with topo I, and also the competition between topoisomerases and dienoic acids for the DNA binding sites.

Acknowledgments

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Supplementary data

Supplementary data (methods section, NMR spectra, high resolution figures, additional docking studies) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2015.04.011>.

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