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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

*n*Z,(*n* + 4)Z-Dienoic fatty acids: a new method for the synthesis and inhibitory action on topoisomerase I and IIα

Vladimir A. D'yakonov[1] · Lilya U. Dzhemileva[2] · Aleksey A. Makarov[1] · Alfiya R. Mulyukova[1] · Dmitry S. Baev[3] · Elza K. Khusnutdinova[4] · Tatiana G. Tolstikova[3] · Usein M. Dzhemilev[1]

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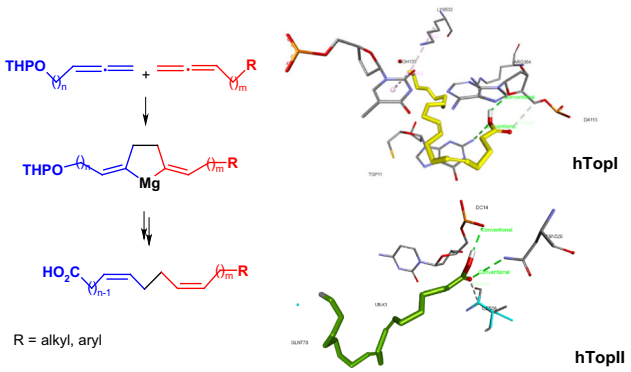
Abstract An original, effective approach to the stereoselective method for the synthesis of higher unsaturated acids containing a 1Z,5Z-diene group in 61–75 % yields and with >98 % selectivity based on the new intermolecular Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of terminal aliphatic and O-containing 1,2-diene with Grignard reagents has been developed. The inhibitory action of the obtained dienoic acids on the human topoisomerase I and II was studied. Resorting to the data of molecular docking, a probable mechanism of inhibition was proposed.

Graphical Abstract



Keywords Fatty acids · Cyclomagnesiation · Homogeneous catalysis · Topoisomerase I and IIα inhibitors · Docking · Stereoselective synthesis of *n*Z,(*n* + 4)Z-dienoic acids

Introduction

The DNA-dependent enzyme topoisomerase, which catalyzes the topological transformations of DNA and plays a key role in all aspects of genome functioning, is one of the most important enzymes that participate in the cell cycle (Pommier, 2009, 2013; Bailly, 2012; Dezhenkova *et al.*, 2014).

Intensive search and selection of natural inhibitors of topoisomerase I and II are in progress, and new synthetic analogs and semisynthetic derivatives of known antitumor compounds able to change the catalytic activity of enzymes by stabilizing the DNA–protein complexes are being developed (Nagarajan *et al.*, 2006; Castelli *et al.*, 2013; Kiselev *et al.*, 2011; Karki *et al.*, 2015).

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✉ Vladimir A. D'yakonov
DyakonovVA@gmail.com

✉ Lilya U. Dzhemileva
Dzhemilev@mail.ru

1 Institute of Petrochemistry and Catalysis of Russian Academy of Sciences, 141 Prospekt Oktyabrya, Ufa, Bashkortostan, Russian Federation 450075

2 Department of Immunology and Human's Reproductive Health, Bashkir State Medical University, 3 Lenin Street, Ufa, Bashkortostan, Russian Federation 450003

3 N. N. Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch, Russian Academy of Sciences, Lavrentjev Avenue 9, Novosibirsk, Russian Federation 630090

4 Department of Genetics and Fundamental Medicine, Bashkir State University, 32 Zaki Validi Street, Ufa, Bashkortostan, Russian Federation 450043

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Previously, several research groups demonstrated that 5*Z*,9*Z*-dienoic fatty acids possess a large potential for solving the above problem and simultaneously they exhibit antimalarial, antituberculous, antimicrobial, and antiviral activities accompanied by low toxicity, which makes this class of compounds a fairly attractive base for the development of modern pharmaceutical drugs (Ayanoglu *et al*., 1983; Carballeira *et al*., 1997; Carballeira, 2008; Djerassi and Lam, 1991; D'yakonov *et al*., 2013a, b; Mena *et al*., 1984; Nemoto *et al*., 1997; Reyes and Carballeira, 1997).

In our opinion, further investigation and application of 5*Z*,9*Z*-dienoic acids aimed at the development of efficient drugs is held up, among other factors, by the lack of preparative methods for their synthesis; the known methods consist of numerous steps (4–20 steps) and give target products in 0.5–15 % yields, most often, as stereoisomer mixtures (Carballeira *et al*., 1999, 2002).

Recently, we developed a stereoselective method for the synthesis of natural and synthetic 5*Z*,9*Z*-dienoic acids in high yields (61–67 %) and with high selectivity (>98 %) based on the new intermolecular catalytic cross-cyclomagnesiation reaction of terminal aliphatic and O-containing 1,2-dienes with Grignard reagents in the presence of the Cp_2TiCl_2 catalyst. Furthermore, high activity of the human topoisomerase I inhibition by (5*Z*,9*Z*)-5,9-eicosadienoic and (5*Z*,9*Z*)-11-phenyl-5,9-undecadienoic acids in concentrations above 0.1 μM was found (D'yakonov *et al*., 2013a, b, 2015).

This paper presents the results obtained as a continuation of the development of new effective methods for the synthesis of higher dienoic acids with different positions of the 1Z,5Z-diene group with respect to the carboxy group and investigation of the relationship between the acid structure and activity in the topoisomerase hTop1 and hTop2α inhibition.

Results and discussion

Relying on the earlier results on cross-cyclomagnesiation of O-containing and terminal aliphatic 1,2-dienes (Dzhemilev *et al*., 2004, 2005; D'yakonov *et al*., 2008, 2012a, b), we developed an efficient versatile method for the synthesis of dienoic acid containing a 1Z,5Z-diene group.

According to the developed strategy of the synthesis of *n*Z,(*n* + 4)Z-dienoic fatty acids, the first step is Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of tetrahydropyran ethers of alkadien-1-ols **1** with terminal aliphatic allenes **2** induced by EtMgBr under the conditions (**1**:**2**:EtMgBr:Mg:[Ti] = 10:12:40:32:0.5; Et_2O, 6 h, 20–22 °C) to afford 2,5-dialkylidenemagnesacyclopentanes, which are then hydrolyzed to give the tetrahydropyran ethers of alkadienols **3**. The Jones oxidation of these products (CrO_3–H_2SO_4, 0 °C, 0.5 h) furnished the target dienoic acids **4a**–**l** with the specified 1Z,5Z position of the diene group with respect to the carboxy group in 61–75 % yields and with stereoselectivity of >98 % (Scheme 1).

During the study of catalytic cyclomagnesiation of 1,2-dienes, we found that the structure of tetrahydropyran ethers of alkadien-1-ols **1** and the length of the terminal 1,2-diene **2** do not influence significantly the yield or selectivity of formation of tetrahydropyran ethers **3**.

The structures of compounds **3** and **4** were confirmed by one (^1H, ^{13}C)- and two-dimensional (COSY, NOESY, HSQC, HMBC) NMR experiments and by mass spectrometry.

The stereochemical purity and *cis*-configuration of the double bonds in the resulting 1,5-dienes were proved based on the presence of high-field signals of internal allylic carbon atoms at ~27 ppm in the ^{13}C NMR spectrum, indicating the presence of *cis*-interaction with the outer allylic carbon atoms (Levy and Nelson, 1972).

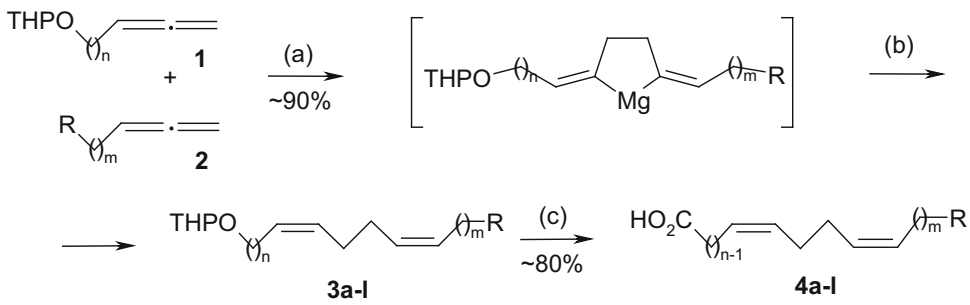
Considering the results we obtained previously and the published data about the inhibitory activity of 5*Z*,9*Z*-dienoic acids with respect to human topoisomerase I and about exceptionally high inhibitory activity of (5Z,9Z)-5,9-eicosadienoic acid with respect to hTop1, we attempted to elucidate the effect of the position of the 1Z,5Z-diene group relative to the carboxy group in the synthesized acids on the human topoisomerase I and II inhibitory activity (Carballeira *et al*., 1999; Carballeira, 2008; Nemoto *et al*., 1997; Mena *et al*., 1984). Note that by the beginning of our studies, no data on human topoisomerase II inhibition by dienoic fatty acids have been reported.

In the next stage, we studied the ability of dienoic acids **4a**–**l** (Table 1) to inhibit the topoisomerase I and IIα enzymes in vitro in the relaxation of supercoiled plasmid DNA under standard conditions (Figs. 1, 2, respectively).

This study allowed us not only to find active inhibitors of topoisomerase I and II among these compounds but also to elucidate the relationship between their structure and inhibitory activity, which determines most promising routes of chemical modification of the compounds in order to enhance their chemotherapeutic properties.

The increase in the concentration of the dienoic acid added from 50 to 250 μM induced a gradual decrease in the number of topoisomers formed and increase in the fractions of both the superhelical DNA and the open ring form, which is indicative of retardation of the relaxation process, i.e., of a decrease in the topoisomerase I activity. Without compounds being studied in the system, this effect is not observed (lanes 3, 4). In the presence of acids **4a**, **g**–**l**, noticeable inhibition was already observed at 50 μM concentration, which was manifested as retention of the residual amounts of supercoiled DNA in comparison with the supercoiled DNA as negative control. All six

Scheme 1 New approach to the synthesis of *n*Z,(*n* + 4)Z-dienoic acids **4**



(a): EtMgBr, Mg, [Ti]; (b): H_3O^+; (c) Jones oxidation. [Ti] = Cp_2TiCl_2
(R = Me) n = 2: m = 11 (**a**);
n = 4: m = 5 (**b**), 9 (**c**), 11 (**d**), 13 (**e**), 17 (**f**);
n = 5: m = 8 (**g**);
n = 6: m = 7 (**h**);
n = 10: m = 3 (**i**), 11 (**k**).
(R = Ph) n = 4: m = 1 (**l**)

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

	Acid	Binding affinity (hTop1), kcal/mol	Binding affinity (hTop IIα + DNA), kcal/mol	Binding affinity (DNA), kcal/mol
1.	(3Z,7Z)-Ecosa-3,7-dienoic acid (**4a**)	−6.0	−4.8	−5.4
2.	(5Z,9Z)-Hexadeca-5,9-dienoic acid (**4b**)	−5.8	−4.8	−4.8
3.	(5Z,9Z)-Ecosa-5,9-dienoic acid (**4c**)	−5.9	−5.0	−5.2
4.	(6Z,10Z)-Ecosa-6,10-dienoic acid (**4g**)	−6.2	−5.0	−5.1
5.	(7Z,11Z)-Ecosa-7,11-dienoic acid (**4h**)	−5.9	−5.3	−5.1
6.	(11Z,15Z)-Ecosa-11,15-dienoic acid (**4i**)	−5.8	−5.0	−5.0
7.	(11Z,15Z)-Octacosa-11,15-dienoic acid (**4k**)	−5.8	−4.5	−4.1
8.	(5Z,9Z)-11-Phenyl-5,9-undecadienoic acid (**4l**)	−7.0	−5.4	−5.4

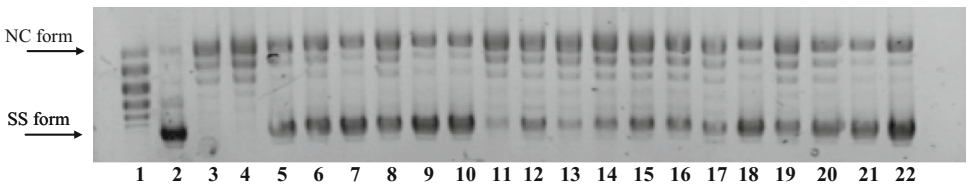


Fig. 1 Electropherogram of the products of in vitro relaxation of supercoiled plasmid DNA under the action of topoisomerase I (Topogen, USA) in the presence of (5Z,9Z)-11-phenyl-5,9-undecadienoic acid (**4l**), (3Z,7Z)-ecosa-3,7-dienoic acid (**4a**), (6Z,10Z)-ecosa-6,10-dienoic acid (**4g**), (7Z,11Z)-ecosa-7,11-dienoic acid (**4h**), (11Z,15Z)-ecosa-11,15-dienoic acid (**4i**), and (11Z,15Z)-octacosa-11,15-dienoic acid (**4k**) (the compound was introduced prior to the addition of topoisomerase I). (*Lane 1*) relaxed plasmid DNA (pHOT-1); (*lane 2*) supercoiled plasmid DNA (pHOT1); (*lane 3*) supercoiled plasmid DNA + topoisomerase I (1 unit); (*lane 4*) supercoiled plasmid DNA + topoisomerase I + DMSO (1 μM); (*lanes 5–7*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4l** at concentration of 50, 100, 250 μM; (*lanes 8–10*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4a** at concentration of 50, 100, 250 μM μM; (*lanes 11–13*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4g** at concentration of 50, 100, 250 μM; (*lanes 14–16*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4h** at concentration of 50, 100, 250 μM; (*lanes 17–19*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4i** at concentration of 50, 100, 250 μM; (*lanes 20–22*) supercoiled plasmid DNA + topoisomerase I (1 unit) + compound **4k** at concentration of 50, 100, 250 μM

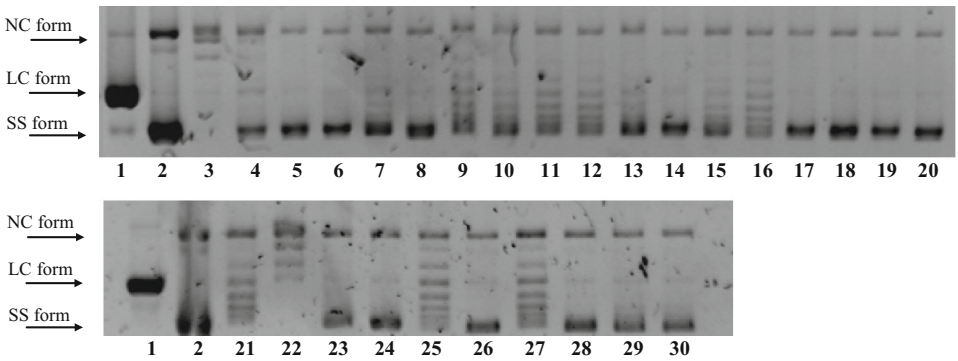


Fig. 2 DNA topoisomerase II inhibitory activity of compound **5**. Electropherogram of the topoisomerase I induced relaxation products of 250 ng of plasmid DNA (pHOT1) in vitro in the presence of acids **4a**, **4c**, **4g–l**. (*Lane 1*) linear DNA (pHOT-1); (*lane 2*) supercoiled plasmid DNA (pHOT1); (*lane 3*) supercoiled plasmid DNA + topoisomerase II (1 unit); (*lane 4*) supercoiled plasmid DNA + topoisomerase II + etoposide (100 μM)—positive control; (*lanes 5*, *6*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4l** at concentration of 0,1 and 1 μM; (*lanes 7–10*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4a** at concentration of 0.1, 1, 50, 100 μM; (*lanes 11–14*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4g** at concentration of 0.1, 1, 50, 100 μM; (*lanes 15–18*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4i** at concentration of 0.1, 1, 50, 100 μM; (*lanes 19*, *20*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4h** at concentration of 0.1, and 1 μM; (*lanes 23–26*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4c** at concentration of 0.1, 1, 0.01, 100 μM; (*lanes 27–30*) supercoiled plasmid DNA + topoisomerase II (1 unit) + compound **4k** at concentration of 0.1, 1, 50 and 100 μM

unsaturated dienoic acids behaved almost in the same way in the concentration range studied, exerting inhibitory action on topoisomerase I in concentrations of 50 μM and above.

The results of our experiments indicate that (5Z,9Z)-11-phenyl-5,9-undecadienoic (**4l**), (3Z,7Z)-eicosa-3,7-dienoic (**4a**), (6Z,10Z)-eicosa-6,10-dienoic (**4g**), (7Z,11Z)-eicosa-7,11-dienoic (**4h**), (11Z,15Z)-eicosa-11,15-dienoic (**4i**), (11Z,15Z)-octacosa-11,15-dienoic (**4k**) acids suppress the catalytic activity of topoisomerase I even when present in micromolar concentrations. The mechanism of interaction of dienoic acids with topoisomerase is still not entirely clear. Presumably, their action includes both stabilization of the DNA–topo I covalent complex (specific inhibition) and competition of the dienoic acid and the enzyme for the DNA-binding sites (non-specific inhibition).

Figure 2 presents the results of electrophoresis of the products of pHOT1 relaxation induced by topoisomerase II in the presence of acids **4a**, **c**, **g–l**. Among the range of acids, (5Z,9Z)-eicosa-5,9-dienoic (**4c**), (7Z,11Z)-eicosa-7,11-dienoic (**4h**), and (5Z,9Z)-11-phenyl-5,9-undecadienoic (**4l**) acids proved to be more potent topoisomerase II inhibitors than the other acids. Their inhibiting concentration was 0.1 μM. An increase in the concentration of these acids leads to a decrease in the number of topoisomers formed and to only partial relaxation of the supercoiled DNA form. Acid **4h** has a fairly strong inhibitory action (0.1 μM concentration) on topoisomerase II, while its inhibitory action on topoisomerase I is manifested at higher concentration (50 μM).

To clarify the mechanism of action of the obtained compounds, computer molecular modeling was performed using a three-dimensional binding model of the tested compound with the topoisomerase I, IIα active sites and with DNA obtained by crystallographic methods (Staker *et al*., 2002; Uytterhoeven *et al*., 2002; Wu *et al*., 2013; Rappe *et al*., 1992) (the model was based on X-ray diffraction data, PDB ID: 1Z2B). The molecular docking of the nine tested compounds into the topoisomerase I, IIα, and DNA-binding sites was carried out. The study was performed using the AutoDock Vina program (taking account of the conformational mobility of the ligands). Topoisomerase–ligand–DNA complexes with the best values of scoring functions calculated by the indicated program were used (Table 1).

It is known that upon interaction of inhibitors with the labile topoisomerase I–DNA and topoisomerase II–DNA complexes, camptothecin and etoposide stabilize the complexes and thus prevent ligation of single- or double-strand DNA cleavages, and, hence, they specifically inhibit the catalytic activity of topo I and topo II. Meanwhile, low-molecular-weight DNA ligands that change the DNA conformation and/or hamper enzyme binding to the duplex are also able to prevent the formation of DNA topoisomers by retarding the enzyme catalytic cycle. Being suppressors of enzyme operation, topo I activity inhibitors of this type act by a non-specific inhibition mechanism.

The result of the molecular docking of acids **4a**, **c**, **g–l** in the topoisomerase I–ligand model provides the following conclusions. The low topoisomerase I inhibitory activity of unsaturated acids with hydrocarbon chain length of 20

carbon atoms and various positions of the diene system confirms the importance of double-bond positions at the carbon atoms 5 and 9, as (5Z,9Z)-eicosa-5,9-dienoic and (5Z,9Z)-11-phenyl-5,9-undecadienoic acids are most active with respect to this enzyme. Therefore, it is noteworthy that the ability to inhibit topoisomerase II is most pronounced for (5Z,9Z)-11-phenyl-5,9-undecadienoic acid, (7Z,11Z)-eicosa-7,11-dienoic acid, and (11Z,15Z)-eicosa-11,15-dienoic acid. The data we obtained attest to a special role of double-bond position in the hydrocarbon chain or the electron-donating phenyl group present in position 11 (Fig. 3).

Conclusions

Thus, for the first time, we developed an efficient highly stereoselective synthesis of unsaturated fatty acids containing a 1Z,5Z-diene group in high yields based on the use of original intermolecular Cp_2TiCl_2-catalyzed cross-cyclomagnesiation of terminal aliphatic and O-containing 1,2-dienes with Grignard reagents as the key step. The synthesized acids were found to be low-molecular-weight ligands, which simultaneously hamper enzyme binding to both the duplex and DNA and thus retard the catalytic cycle of topoisomerase. These topo I activity inhibitors functioning as enzyme operation suppressors operate by a non-specific inhibition mechanism.

Experimental

General methods

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 at 500 MHz for ^1H 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).

General procedure for the synthesis of dienoic acids

Diethyl ether (10 mL), tetrahydropyran ether of alkadien-1-ol (**1**) (10 mmol), 1,2-diene (**2**) (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 (~0 °C). 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 tetrahydropyran ethers of alkadienols (**3**) 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 (0 °C, 0.5 h) of tetrahydropyran ethers of alkadienols (**3**) furnished dienoic acid (**4**) in 61–74 % yields. The acids was chromatographed on a column [SiO_2, elution with petroleum ether—EtOAc (5:1)].

2-(Eicosa-3Z,7Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3a)*** Yield = 81 %, as a colorless oil. $n_d^{20} = 1.4684$. $R_f = 0.45$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3007, 2927, 2856, 1730, 1455, 1380, 1364, 1260, 1200, 1137, 1033, 769,

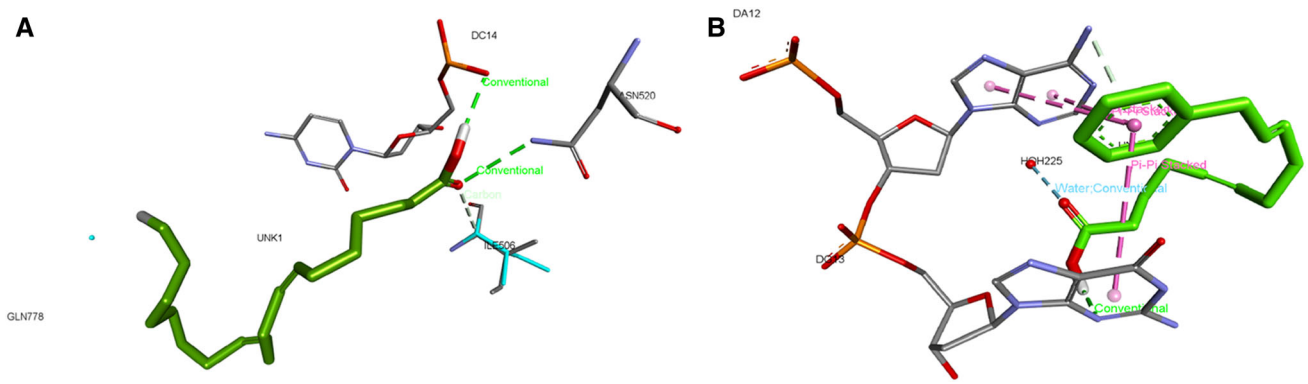


Fig. 3 Docking of (7Z,11Z)-eicosa-7,11-dienoic acid (**a**) and (5Z,9Z)-11-phenyl-5,9-undecadienoic acid (**b**) into the DNA-binding site (N-gate) to topoisomerase IIα (most of hydrogen atoms are omitted)

669 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.89$ (t, 3H, CH_3, $J = 7.2$ Hz), 1.27–1.30 (m, 20H, CH_2), 1.49–1.86 (m, 6H, CH_2), 1.94–2.13 (m, 8H, CH_2), 3.38–3.87 (m, 4H, CH_2), 4.59 (t, 1H, CH, $J = 3.6$ Hz), 5.35–5.46 (m, 4H, CH=). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-20), 19.5 (C-23), 22.6 (C-19), 25.5 (C-24), 27.2 (C-5,6)(2C), 27.5 (C-2), 28.0 (C-9), 29.0 (C-10), 29.3 (C-12), 29.4 (C-13), 29.5 (C-14,15), 29.6 (C-16,17), 29.7 (C-11), 30.7 (C-22), 31.8 (C-18), 62.2 (C-25), 67.0 (C-1), 98.6 (C-21), 125.9 (C-3), 128.9 (C-7), 130.4 (C-4), 131.2 (C-8). MALDI TOF: 378.5 $[M]^+$. Anal. Calcd. for $C_{25}H_{46}O_2$: C, 79.30; H, 12.50. Found: C, 78.94; H, 12.44.

2-(Hexadeca-5Z,9Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3b)*** Yield = 86 %, as a colorless oil. $n_d^{20} = 1.4831$. $R_f = 0.41$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3005, 2924, 2853, 1441, 1380, 1353, 1200, 1182, 1159, 1137, 1121, 1078, 1034, 992, 971, 905, 869, 815 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, 3H, CH_3, $J = 7.2$ Hz), 1.26–1.85 (m, 18H, CH_2), 2.00–2.07 (m, 8H, CH_2), 3.38–3.87 (m, 4H, CH_2), 4.56 (t, 1H, CH, $J = 3.2$ Hz), 5.34–5.38 (m, 4H, CH=). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$ (C-16), 19.6 (C-19), 22.6 (C-15), 25.5 (C-20), 26.4 (C-3), 27.0 (C-4), 27.2 (C-2), 27.3 (C-7), 27.4 (C-8), 29.4 (C-11), 29.7 (C-13), 30.7 (C-18), 31.8 (C-14), 62.1 (C-21), 67.4 (C-1), 98.7 (C-17), 129.0 (C-9), 129.4 (C-6), 129.9 (C-5), 130.3 (C-10). MALDI TOF: 322.5 $[M]^+$. Anal. Calcd. for $C_{21}H_{38}O_2$: C, 84.51; H, 11.88. Found: C, 78.41; H, 11.69.

2-(Eicosa-5Z,9Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3c)*** Yield = 88 %, as a colorless oil. $R_f = 0.46$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 2926, 2853, 1660, 1441, 1382, 1354, 1200, 1180, 1159, 1125, 1078, 1034, 769, 676 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.89$ (t, 3H, $C\underline{H}_3$, $J = 7.2$ Hz), 1.25–1.80 (m, 26H, $C\underline{H}_2$), 2.01–2.06 (m, 8H, $C\underline{H}_2CH$=), 3.41–3.89 (m, 4H, $C\underline{H}_2$–O), 4.61 (m, 1H, O–$\underline{CH}$–O), 5.37–5.48 (m, 4H, $C\underline{H}$=$C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-20), 19.5 (C-23), 22.7 (C-19), 25.5 (C-24), 27.2 (C-3), 27.5 (C-4), 27.9 (C-2), 29.3 (C-11), 29.4 (C-7), (C-8), 29.5 (C-17), 29.6 (C-16), 29.4 (C-13), 29.5 (C-12), 29.6 (C-15), 29.7 (C-14), 30.7 (C-22), 31.9 (C-18), 62.1 (C-25), 67.0 (C-1), 98.6 (C-21), 125.9 (C-9), 128.9 (C-6), 130.4 (C-5), 131.2 (C-10). MALDI TOF: 378.5 $[M]^+$. Anal. Calcd. for $C_{25}H_{46}O_2$: C, 79.30; H, 12.50. Found: C, 78.98; H, 12.42.

2-(Docosa-5Z,9Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3d)*** Yield = 90 %, as a colorless oil. $R_f = 0.44$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 2925, 2851, 1654, 1445, 1380, 1352, 1200, 1180, 1159, 1135, 1121, 1078, 1034, 767, 662 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.89$ (t, 3H, $C\underline{H}_3$, $J = 6.8$ Hz), 1.26–1.82 (m, 30H, $C\underline{H}_2$), 2.01–2.17 (m, 8H, $C\underline{H}_2CH$=), 3.39–3.90 (m, 4H, $C\underline{H}_2$–O), 4.59 (t, 1H, O–$\underline{CH}$–O, $J = 4$ Hz), 5.36–5.48 (m, 4H, $C\underline{H}$=$C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$ (C-22), 19.6 (C-25), 22.6 (C-21), 25.5 (C-26), 25.8 (C-3), 26.2 (C-4), 26.4 (C-2), 27.0 (C-11), 27.2 (C-7), 27.4 (C-8), 29.3 (C-19), 29.4 (C-18), 29.5 (C-16), 29.6 (C-17,19), 29.7 (C-12,13,14,15), 30.7 (C-24), 31.9 (C-20), 62.1 (C-27), 67.5 (C-1), 98.7 (C-23), 129.0 (C-9), 129.4 (C-6), 129.9 (C-5), 130.3 (C-10). MALDI TOF: 406.6 $[M]^+$. Anal. Calcd. for $C_{27}H_{50}O_2$: C, 79.74; H, 12.39. Found: C, 79.48; H, 12.18.

2-(Tetracosa-5Z,9Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3e)*** Yield = 92 %, as a colorless oil. $R_f = 0.43$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 2925, 2851, 1445, 1380, 1352, 1200, 1180, 1159, 1135, 1121, 1078, 1034, 992, 970, 905, 3005, 867, 812 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, 3H, $C\underline{H}_3$, $J = 6.8$ Hz), 1.26–1.84 (m, 34H, $C\underline{H}_2$), 2.00–2.07 (m, 8H, $C\underline{H}_2CH$=), 3.37–3.88 (m, 4H, $C\underline{H}_2$–O), 4.57 (t, 1H, O–$\underline{CH}$–O, $J = 4$ Hz), 5.36–5.40 (m, 4H, $C\underline{H}$=$C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-24), 19.5 (C-27), 22.6 (C-23), 25.5 (C-28), 25.8 (C-3), 26.2 (C-4), 26.3 (C-2), 27.0 (C-11), 27.2 (C-7), 27.3 (C-8), 29.3 (C-21), 29.4 (C-20), 29.5 (C-16), 29.6 (C-17,19), 29.7 (C-12,13,19), 29.7 (C-14,15,18), 30.7 (C-26), 31.9 (C-22), 62.1 (C-29), 67.4 (C-1), 98.7 (C-25), 129.0 (C-9), 129.4 (C-6), 129.8 (C-5), 130.3 (C-10). MALDI TOF: 434.7 $[M]^+$. Anal. Calcd. for $C_{29}H_{54}O_2$: C, 80.12; H, 12.52. Found: C, 79.88; H, 12.48.

2-(Octacosa-5Z,9Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3f)*** Yield = 92 %, as a colorless oil. $R_f = 0.43$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 2925, 2851, 1445, 1380, 1352, 1200, 1180, 1159, 1135, 1121, 1078, 1034, 992, 970, 905, 3005, 867, 812 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.87$ (t, 3H, $C\underline{H}_3$, $J = 6.8$ Hz), 1.26–1.84 (m, 42H, $C\underline{H}_2$), 2.00–2.07 (m, 8H, $C\underline{H}_2CH$=), 3.37–3.88 (m, 4H, $C\underline{H}_2$–O), 4.57 (t, 1H, O–$\underline{CH}$–O, $J = 4$ Hz), 5.36–5.40 (m, 4H, $C\underline{H}$=$C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$ (C-28), 19.6 (C-31), 22.7 (C-27), 25.5 (C-32), 25.8 (C-3), 26.2 (C-4), 26.4 (C-2), 27.0 (C-11), 27.2 (C-7), 27.4 (C-8), 29.3 (C-21), 29.4 (C-20), 29.5 (C-16,22-24), 29.6 (C-17,19,25), 29.66 (C-12,13,19), 29.69 (C-14,15,18), 30.7 (C-30), 31.9 (C-26), 62.1 (C-33), 67.4 (C-1), 98.8 (C-29), 129.0 (C-9), 129.4 (C-6), 129.9 (C-5), 130.3 (C-10). MALDI TOF: 490.5 $[M]^+$. Anal. Calcd. for $C_{33}H_{62}O_2$: C, 80.75; H, 12.73. Found: C, 80.58; H, 12.68.

2-(Eicosa-6Z,10Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3g)*** Yield = 91 %, as a colorless oil. $n_d^{20} = 1.4837$. $R_f = 0.37$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3005, 2925, 2853, 1441, 1380, 1353, 1200, 1182, 1159, 1136, 1121, 1078, 1034, 729, 664 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.89$ (t, 3H, CH_3, $J = 6.8$ Hz), 1.26–1.34 (m, 20H, CH_2), 1.51–1.66 (m, 6H, CH_2), 2.01–2.16 (m, 8H, CH_2), 3.38–3.86 (m, 4H, CH_2), 4.56 (t, 1H, CH, $J = 4$ Hz), 5.33–5.41 (m, 4H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz):

δ = 14.1 (C-20), 19.6 (C-23), 22.6 (C-19), 25.5 (C-24), 26.3 (C-3), 27.2 (C-12), 27.3 (C-5) 27.4 (C-8, 9), 29.1 (C-13), 29.5 (C-14-17), 29.6 (C-4), 29.7 (C-2), 30.8 (C-22), 31.1 (C-5), 31.6 (C-18), 62.2 (C-25), 67.5 (C-1), 98.8 (C-21), 129.1 (C-10), 129.2 (C-6), 130.2 (C-11), 130.3 (C-7). MALDI TOF: 378.5 $[M]^+$. Anal. Calcd. for $C_{25}H_{46}O_2$: C, 79.30; H, 12.50. Found: C, 79.08; H, 12.44.

2-(Eicosa-7Z,11Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3h)*** Yield = 93 %, as a colorless oil. n_d^{20} = 1.4841. R_f = 0.38 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3005, 2925, 2853, 1441, 1380, 1353, 1200, 1182, 1159, 1136, 1121, 1078, 1034, 729, 664 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.89 (t, 3H, CH_3, J = 6.8 Hz), 1.26–1.34 (m, 20H, CH_2), 1.50–1.65 (m, 6H, CH_2), 2.02–2.16 (m, 8H, CH_2), 3.38–3.88 (m, 4H, CH_2), 4.57 (t, 1H, CH, J = 4 Hz), 5.35–5.40 (m, 4H, CH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1 (C-20), 19.6 (C-23), 22.6 (C-19), 25.5 (C-24), 26.2 (C-3), 27.2 (C-13), 27.3 (C-6) 27.4 (C-9),(C-10), 29.1 (C-14), 29.5 (C-15-17), 29.6 (C-4), 29.7 (C-2), 30.8 (C-22), 31.2 (C-5), 31.9 (C-18), 62.2 (C-25), 67.5 (C-1), 98.8 (C-21), 129.0 (C-11), 129.1 (C-7), 130.2 (C-12), 130.2 (C-8). MALDI TOF: 378.5 $[M]^+$. Anal. Calcd. for $C_{25}H_{46}O_2$: C, 79.30; H, 12.50. Found: C, 79.08; H, 12.44.

2-(Eicosa-11Z,15Z-dien-1-yloxy)tetrahydro-2H-pyran ***(3i)*** Yield = 90 %, as a colorless oil. R_f = 0.44 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 2925, 2853, 1662, 1441, 1381, 1354, 1202, 1181, 1159, 1125, 1078, 1033, 765, 670 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, 3H, C<u>H</u>$_3$, J = 7.0 Hz), 1.25–1.82 (m, 26H, C<u>H</u>$_2$), 2.02–2.06 (m, 8H, C<u>H</u>$_2$CH=), 3.41–3.88 (m, 4H, C<u>H</u>$_2$–O), 4.62 (m, 1H, O–<u>CH</u>–O), 5.38–5.46 (m, 4H, C<u>H</u>=C<u>H</u>). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1 (C-20), 19.5 (C-23), 22.6 (C-19), 26.8 (C-24), 27.2 (C-17), 27.5 (C-10), 27.8 (C-14), 27.9 (C-13), 29.3 (C-8), 29.4 (C-4,5), 29.5 (C-7), 29.5 (C-3), 29.6 (C-6), 29.7 (C-2), 30.6 (C-22), 30.9 (C-9), 31.2 (C-18), 62.2 (C-25), 67.2 (C-1), 98.6 (C-21), 129.6 (C-11), 129.9 (C-16), 130.4 (C-12), 130.3 (C-15). MALDI TOF: 378.5 $[M]^+$. Anal. Calcd. for $C_{25}H_{46}O_2$: C, 79.30; H, 12.50. Found: C, 78.71; H, 12.45.

2-[(11-Phenylundeca-5Z,9Z-dien-1-yl)oxy]tetrahydro-2H-pyran ***(3l)*** Yield = 86 % (2.88 g), as a colorless oil. n_d^{20} = 1.5311. R_f = 0.45 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3390, 2938, 2870, 1762, 1661, 1453, 1352, 1261, 1200, 1120, 1075, 1032, 747, 699 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.27–1.89 (m, 10H, C<u>H</u>$_2$), 2.08–2.26 (m, 6H, C<u>H</u>$_2$CH=), 3.48 (d, 2H, C<u>H</u>$_2$–Ph, J = 6.8 Hz), 3.52–3.92 (m, 4H, C<u>H</u>$_2$–O, J = 6.8 Hz), 4.58 (t, 1H, J = 3.6 Hz), 5.41–5.65 (m, 4H, C<u>H</u>=C<u>H</u>), 7.22–7.36 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 19.8 (C-20), 25.5 (C-21), 27.2 (C-4), 27.3 (C-2), 27.4 (C-7), 27.5 (C-8), 30.8 (C-19), 33.6 (C-11), 62.3 (C-22), 67.5 (C-1), 98.8 (C-18), 125.9 (C-15), 128.2 (C-13,17), 128.4 (C-14,16), 128.5 (C-9), 129.2 (C-6), 129.6 (C-5), 130.3 (C-10), 141.1 (C-12). MALDI TOF: 328.4 $[M]^+$. Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.44; H, 9.82. Found: C, 80.02; H, 9.73.

(3Z,7Z)-Ecosa-3,7-dienoic acid ***(4a)*** Yield = 61 %, as a colorless oil. R_f = 0.56 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2920, 2855, 1740, 1660, 1465, 1385, 1365, 1240, 1035, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, 3H, C<u>H</u>$_3$, J = 7.2 Hz), 1.23-1.31 (m, 20H, C<u>H</u>$_2$), 2.02–2.12 (m, 6H, C<u>H</u>$_2$CH=), 3.16 (d, 2H, C<u>H</u>$_2$–COOH, J = 6 Hz), 5.37–5.63 (m, 4H, C<u>H</u>=). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1 (C-20), 22.7 (C-19), 26.9 (C-5), 27.3 (C-6), 27.9 (C-9), 29.0 (C-10), 29.2 (C-12), 29.3 (C-13), 29.4 (C-14,15), 29.7 (C-16,17), 29.8 (C-11), 31.9 (C-18), 34.1(C-2), 120.4 (C-3), 128.4 (C-7), 130.9 (C-4), 133.3 (C-8), 178.5 (C-1). Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.44; H, 11.68.

(5Z,9Z)-Hexadeca-5,9-dienoic acid ***(4b)*** Yield = 69 %, as a colorless oil. R_f = 0.49 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3006, 2928, 2856, 1743, 1655, 1464, 1385, 1365, 1238, 1038, 969, 727 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, 3H, CH_3, J = 7.2 Hz); 1.30–1.32 (m, 8H, CH_2); 1.70 (q, 2H, CH_2, J = 7.6 Hz); 2.01–2.14 (m, 8H, =CH–C<u>H</u>$_2$); 2.37 (t, 2H, CH_2, J = 7.2 Hz); 5.33–5.46 (м, 4H, CH=). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.1 (C-16), 22.6 (C-15), 24.6 (C-3), 26.5 (C-11), 27.3 (C-7,8), 27.4 (C-4), 28.9 (C-12), 29.7 (C-13), 31.8 (C-14), 33.6 (C-2), 128.6 (C-10), 128.9 (C-9), 130.5 (C-5), 130.6 (C-6), 180.3 (C-1). MALDI TOF: 252.4. Anal. Calcd. for $C_{16}H_{28}O_2$: C, 76.14; H, 11.18. Found: C, 76.01; H, 11.05.

(5Z,9Z)-Ecosa-5,9-dienoic acid ***(4c)*** Yield = 73 %, as a colorless oil. R_f = 0.55 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2920, 2855, 1740, 1660, 1465, 1385, 1365, 1240, 1035, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.90 (t, 3H, C<u>H</u>$_3$, J = 7.2 Hz), 1.28–1.32 (m, 16H, C<u>H</u>$_2$), 1.72 (k, 2H, C<u>H</u>$_2$, J = 7.2 Hz), 2.01–2.15 (m, 8H, C<u>H</u>$_2$CH=), 2.38 (t, 2H, C<u>H</u>$_2$–COOH, J = 7.6 Hz), 5.35–5.46 (m, 4H, C<u>H</u>=C<u>H</u>). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.2 (C-20), 22.7 (C-19), 24.6 (C-3), 26.5 (C-11), 27.3 (C-7,8), 27.4 (C-4), 29.3 (C-17), 29.4 (C-16), 29.6 (C-13), 29.7 (C-15), 29.7 (C-14), 31.9 (C-18), 33.5 (C-2), 128.6 (C-10), 128.9 (C-9), 130.5 (C-6), 130.6 (C-5), 180.1 (C-1). Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.32; H, 11.51.

(5Z,9Z)-Docosa-5,9-dienoic acid ***(4d)*** Yield = 74 %, as a colorless oil. R_f = 0.51 (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2925, 2856, 1741, 1655, 1466, 1385, 1365, 1238, 1035, 724 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): δ = 0.91 (t, 3H, C<u>H</u>$_3$, J = 6.8 Hz), 1.28–1.33 (m, 20H, C<u>H</u>$_2$), 1.72 (k, 2H, C<u>H</u>$_2$, J = 7.2 Hz), 2.03–2.15 (m, 8H,

$C\underline{H}_2CH=$), 2.38 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.2$ Hz), 5.38–5.44 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-22), 22.7 (C-21), 24.6 (C-3), 26.5 (C-11), 27.3 (C-7,8), 27.4 (C-4), 29.34 (C-19), 29.37, 29.57 2C, 29.7 2C, 29.74 (C-13–18), 31.9 (C-20), 33.4 (C-2), 128.6 (C-10), 128.9 (C-9), 130.5 (C-6), 130.6 (C-5), 180.1 (C-1). MALDI TOF: 336.5. Anal. Calcd. for $C_{22}H_{40}O_2$: C, 78.51; H, 11.98. Found: C, 78.21; H, 11.92.

(5Z,9Z)-Tetracosa-5,9-dienoic acid (***4e***) Yield = 72 %, as a colorless oil. $R_f = 0.51$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2925, 2856, 1741, 1657, 1466, 1385, 1365, 1238, 1035, 734 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.90$ (t, 3H, $C\underline{H}_3$, $J = 6.8$ Hz), 1.28–1.35 (m, 24H, $C\underline{H}_2$), 1.72 (k, 2H, $C\underline{H}_2$, $J = 7.2$ Hz), 2.02–2.13 (m, 8H, $C\underline{H}_2CH=$), 2.37 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.2$ Hz), 5.37–5.43 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-24), 22.7 (C-23), 24.6 (C-3), 26.5 (C-11), 27.3 (C-7,8), 27.4 (C-4), 29.3 (C-21), 29.4 (C-20), 29.4 (C-16), 29.6 (C-17,19), 29.7 (C-12,13,19), 29.7 (C-14,15,18), 31.9 (C-22), 33.4 (C-2), 128.6 (C-10), 128.9 (C-9), 130.6 (C-6), 130.6 (C-5), 179.7 (C-1). MALDI TOF: 364.6. Anal. Calcd. for $C_{24}H_{44}O_2$: C, 79.06; H, 12.16. Found: C, 78.71; H, 12.05.

(5Z,9Z)-Octacosa-5,9-dienoic acid (***4f***) Yield = 74 %, as a colorless oil. $R_f = 0.52$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3011, 2925, 2856, 1741, 1659, 1466, 1380, 1365, 1238, 1030, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.90$ (t, 3H, $C\underline{H}_3$, $J = 6.8$ Hz), 1.28–1.35 (m, 24H, $C\underline{H}_2$), 1.72 (k, 2H, $C\underline{H}_2$, $J = 7.2$ Hz), 2.02–2.13 (m, 8H, $C\underline{H}_2CH=$), 2.37 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.2$ Hz), 5.37–5.43 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-28), 22.7 (C-27), 24.6 (C-3), 26.5 (C-11), 27.3 (C-7,8), 27.4 (C-4), 29.3 (C-21), 29.4 (C-20), 29.4 (C-16), 29.6 (C-17,19), 29.7 (C-12,13,19,23), 29.7 (C-14,15,18,24,25), 31.9 (C-26), 33.4 (C-2), 128.6 (C-10), 128.9 (C-9), 130.6 (C-6), 130.6 (C-5), 179.7 (C-1).

(6Z,10Z)-Ecosa-6,10-dienoic acid (***4g***) Yield = 73 %, as a colorless oil. $R_f = 0.53$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2920, 2855, 1740, 1660, 1465, 1385, 1365, 1240, 1035, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.90$ (t, 3H, $C\underline{H}_3$, $J = 7.2$ Hz), 1.25–1.71 (m, 18H, $C\underline{H}_2$), 2.02–2.11 (m, 8H, $C\underline{H}_2CH=$), 2.37 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.6$ Hz), 5.35–5.44 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-20), 22.7 (C-19), 26.8 (C-3), 27.2 (C-12), 27.3 (C-5) 27.4 (C-8, 9), 29.1 (C-13), 29.2 (C-14), 29.4 (C-16), (C-15,17), 29.6 (C-4), 29.7 (C-2), 31.9 (C-18), 33.9 (C-18), 129.0 (C-10), 129.4 (C-6), 129.8 (C-11), 130.5 (C-7), 179.5 (C-1). Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.71; H, 11.70.

(7Z,11Z)-Ecosa-7,11-dienoic acid (***4h***) Yield = 72 %, as a colorless oil. $R_f = 0.53$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2920, 2855, 1740, 1660, 1465, 1385, 1365, 1240, 1035, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.91$ (t, 3H, $C\underline{H}_3$, $J = 7.2$ Hz), 1.21–1.32 (m, 16H, $C\underline{H}_2$), 1.72 (k, 2H, $C\underline{H}_2$, $J = 7.2$ Hz), 2.01–2.15 (m, 8H, $C\underline{H}_2CH=$), 2.37 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.6$ Hz), 5.31–5.59 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-20), 22.7 (C-19), 24.6 (C-3), 27.0 (C-13), 27.2 (C-6), 27.3 (C-9), 27.4 (C-10), 29.3 (C-14), 29.4 (C-16,17), 29.6 (C-15), 29.7 (C-2), 29.7 (C-5), 31.9 (C-18), 34.2 (C-4), 129.1 (C-11), 129.5 (C-7), 130.2 (C-12), 130.4 (C-8), 180.5 (C-1). Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.64; H, 11.79.

(11Z,15Z)-Ecosa-11,15-dienoic acid (***4i***) Yield = 73 %, as a colorless oil. $R_f = 0.55$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3015, 2920, 2854, 1740, 1662, 1465, 1385, 1365, 1241, 1035, 737 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.91$ (t, 3H, $C\underline{H}_3$, $J = 7.2$ Hz), 1.29–1.35 (m, 16H, $C\underline{H}_2$), 1.65 (m, 2H, $C\underline{H}_2$), 2.03–2.10 (m, 8H, $C\underline{H}_2CH=$), 2.37 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.6$ Hz), 5.39–5.40 (m, 4H, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.0$ (C-20), 22.4 (C-19), 24.7 (C-2), 26.7 (C-17), 27.3 (C-10), 27.4 (C-13,14), 29.1 (C-8), 29.3 (C-3), 29.4 (C-4,5), 29.5 (C-7), 29.7 (C-6), 31.9 (C-18), 33.9 (C-9), 129.1 (C-11), 129.2 (C-16), 130.3 (C-12,15), 179.8 (C-1). Anal. Calcd. for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.32; H, 11.51.

(11Z,15Z)-Octacosa-11,15-dienoic acid (***4k***) Yield = 71 %, as a colorless oil. $R_f = 0.54$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3010, 2920, 2855, 1740, 1660, 1465, 1385, 1365, 1240, 1035, 735 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 0.91$ (t, 3H, $C\underline{H}_3$, $J = 7.2$ Hz), 1.21–1.32 (m, 34H, $C\underline{H}_2$), 1.72 (k, 2H, $C\underline{H}_2$, $J = 7.2$ Hz), 2.01–2.14 (m, 8H, $C\underline{H}_2CH=$), 2.34 (t, 2H, $C\underline{H}_2$–COOH, $J = 7.6$ Hz), 5.31–5.59 (m, 4H, $C\underline{H}=$). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 14.1$ (C-28), 22.7 (C-27), 24.7 (C-2), 27.3 (C-17,10), 27.4 (C-13,14), 29.1 (C-25), 29.2 (C-3), 29.3 (C-24), 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7 (2C), 29.7 (4C) (C-4-9,18-23), 31.2 (C-26), 129.1 (C-11), 129.2 (C-16), 130.3 (C-12), 130.4 (C-15), 178.8 (C-1).

(5Z,9Z)-11-Phenylundeca-5,9-dienoic acid (***4l***) Yield = 75 %, as a colorless oil. $R_f = 0.52$ (hexan-EtOAc—5:1). IR ($CHCl_3$) υ_{max} 3395, 3010, 2925, 2856, 1741, 1657, 1466, 1385, 1365, 1238, 1035, 734 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 1.70$–1.76 (m, 2H), 2.13–2.40 (m, 8H, $C\underline{H}_2CH=$), 3.43 (d, 2H, $C\underline{H}_2$–Ph, $J = 7.5$ Hz), 5.39–5.63 (m, 4H, $C\underline{H}=C\underline{H}$), 7.20–7.33 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 24.7$ (C-2), 26.5 (C-4), 27.3 (C-7), 33.4 (C-8), 33.6 (C-11), 125.9 (C-15), 128.3 (C-13,17), 128.4 (C-14,16), 128.5 (C-9), 128.6 (C-6), 130.1 (C-5), 130.4 (C-10), 141.1 (C-12), 179.8 (C-1). Anal. Calcd. for $C_{24}H_{44}O_2$: C, 79.03; H, 8.58. Found: C, 78.84; H, 8.60.

Topoisomerase I inhibitory activity

The inhibitory activity of acids was 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 μL) containing 0.25 μg of the DNA plasmid pHOT (TopoGen, USA), 1 unit of recombinant topoisomerase I (TopoGen, USA), and the tested compound: dienoic acid was incubated in the buffer (35 mM 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 40 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 4–6 h. After the electrophoresis without ethidium bromide, the gels were treated with a solution of ethidium bromide (0.5 μg/mL). The gels were visualized in the UV light in a Gel Doc^{TM} EZ System (Bio-Rad, USA). 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.

Topoisomerase IIα inhibitory activity

The inhibitory activity of acids was 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 μL) containing 0.25 μg of the DNA plasmid pHOT (TopoGen, USA), 1 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 10× stock solution in two parts: 10× incomplete topo II assay buffer A contains the following: 0.5 M Tris–HCl (pH 8.0), 1.5 M NaCl, 0.1 M $MgCl_2$, 5 mM dithiothreitol and 10× 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 etoposide (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 40 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 4–6 h. After the electrophoresis without ethidium bromide, the gels were treated with a solution of ethidium bromide (0.5 μg/mL). The gels were visualized in the UV light in a Gel Doc^{TM} EZ System (Bio-Rad, USA). 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.

Docking studies

The docking analysis of molecules was carried out using Autodock Vina (Rappe *et al*., 1992). Ligand molecules were sketched in 3D format using OpenBabel module of PyRx GUI. Universal Force Field minimization algorithm (Trott and Olson, 2010) was used to produce low-energy conformers. The structural coordinates of the human topoisomerase I enzyme with ligand camptothecin (PDB ID:1K4T), human topoisomerase IIa with ligand mitoxantrone (PDB ID: 4G0V), and DNA minor groove with ligands distamycin, netropsin, and DAPI (PDB ID 1K2Z) were obtained from the protein databank (PDB). Redocking of minimized molecules of camptothecin, mitoxantrone, and distamycin into 1K4T, 4G0V, and 1K2Z models was done to validate the docking algorithms of AutoDock Vina. The lowest energy conformations were selected, and the ligands interactions in binding site of enzymes were determined. The UCSF Chimera 1.8, Accelrys Discovery Studio Visualizer 4.0, and PoseView 1.1.2 were utilized for docking and interaction visualization.

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