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Synthesis and *Topoisomerase I* inhibitory properties of klavuzon derivatives

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Klavuzon is a naphthalen-1-yl substituted α,β-unsaturated δ-lactone derivative, and is one of the anti-proliferative members of this class of compounds. Asymmetric and racemic syntheses of novel α,β-unsaturated δ-lactone derivatives are important to investigate their potential for the treatment of cancer. In this study, asymmetric and racemic syntheses of heteroatom-substituted klavuzon derivatives are reported. The syntheses were completed by a well-known three-step procedure. Anti-proliferative activity of seven novel racemic klavuzon derivatives were reported against MCF-7, PC3, HCT116 p53+/+ and HCT116 p53−/− cancer cell lines. *Topoisomerase I* inhibitory properties of 5,6-dihydro-2H-pyran-2-one derivatives were also studied.

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1. Introduction

Reactive electrophilic compounds, such as nitrogen mustards, are still in use for chemotherapy of cancer patients [1,2]. Their therapeutic effect is explained by the formation of an irreversible covalent bond between mustards and DNA bases [3]. Similarly, a compound that covalently binds to the active site of proteins may inhibit the enzymatic activity of that protein irreversibly. For example, it was reported that vicinal cysteine 504 and 505 residues are crucial for the catalytic mechanism of human *topoisomerase I* (Topo I) enzyme, a well-known target for anti-cancer drugs [4], and thiol reactive electrophilic compounds may inhibit the human Topo I activity [5,6].

Similarly, it is believed that α,β-unsaturated δ-lactone derivatives can covalently bind to the nucleophilic sites of proteins through a Michael addition reaction, and this is the reason for the resulting biological activity [7]. In the literature, hundreds of compounds-either synthesized in the laboratory or isolated from the natural resources- that have a functional group for this type of reaction have been reported. Styryl lactones bear an α,β-unsaturated δ-lactone pharmacophore in their structures and goniothalamin (GTN, **1**) is a promising example of this class of compounds because of its reported selective anti-proliferative activity on cancer cells [8,9].

To date many syntheses and SAR studies have been carried out for goniothalamin derivatives. The presence of an α,β-unsaturated carbonyl group and a *trans* C=C double bond are essential for the biological activity [10,11]. The (*R*)-isomer is more potent and triggers apoptosis, while the (*S*)-isomer causes autophagy in the cell [12]. In another work, it has been shown that racemic goniothalamin induces TP53 transcription-dependent and -independent apoptosis in hepatocellular carcinoma (HCC)-derived p53 (TP53)-positive and -negative cells [13]. A heteroatom substitution, that donates a pairs of electrons to the benzene ring (**2**–**4**), decreases anti-proliferative activity of goniothalamin. In the same study, compounds having LogP values between +4.4 and +4.7 were more potent [14]. Recently, Barcelos et al. reported that a 2,4-dimethoxy analog (**5**) was approximately twice as potent as goniothalamin (Fig. 1) [15].

Compound **6**, conformationally constrained analog of (*R*)-goniothalamin, and klavuzon (**7**) are α,β-unsaturated δ-lactone derivatives that were first described by our group in 2008. Conformationally constrained analog (**6**) has similar anti-proliferative activity compared to (*R*)-goniothalamin, while klavuzon (**7**) is more anti-proliferative than goniothalamin. Presence of a methyl substitution in the naphthalene-1-yl group (**8** and **9**) further increases its potency (Fig. 1) [16,17].

In this study, asymmetric and racemic preparations of novel derivatives of klavuzon (**10**–**15**) and goniothalamin (**16**) were

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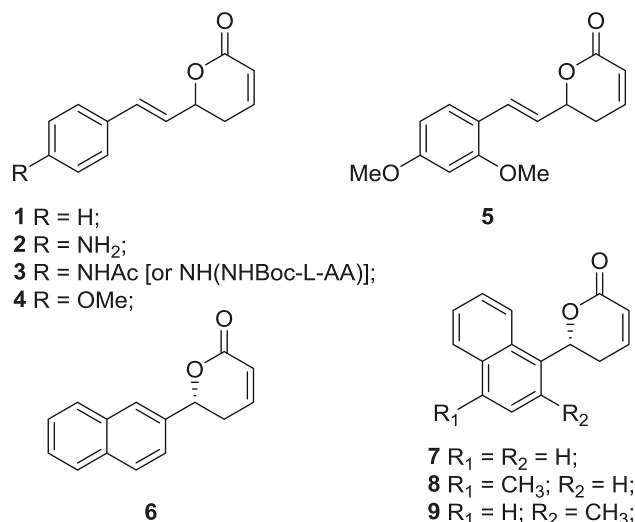


Fig. 1. Structures of heteroatom-substituted goniothalamin (**2**–**5**) and 6-bicycloaryl substituted (*R*)-5,6-dihydro-2H-pyran-2-one (**6**, **9**) derivatives.

studied (Fig. 2). The effects of the heteroatom substitutions on the biological activity of klavuzon were also investigated.

2. Results and discussions

2.1. Chemistry

The syntheses of novel 5,6-dihydro-*2H*-pyran-2-one derivatives (**10**–**16**) were planned by a well-known three-step procedure. Asymmetric or racemic allylation of aldehydes was followed by acrylate aster formation and ring-closing metathesis.

Asymmetric allylation of the 4-dimethylamino-1-naphthaldehyde (**17**) was performed by allyltrimethoxysilane in the presence of *R*-Tol-BINAP.AgF complex at −20 °C to produce *R*-homoallylic alcohol **18** with 69% yield [18]. Chiral HPLC studies with two separate chiral columns were performed to determine the ee% of the product and it showed a single peak, which might be the result of formation of a single enantiomer or poor resolution of enantiomers in the columns. To check the enantiopurity of the formed homoallylic alcohol **(*R*)-18**, it was reacted with acryloyl chloride to form its acrylate ester. Transformation of the obtained alcohol to acrylate ester (***R*-19**) was monitored by TLC [16]. Although the formation of the product was observed in TLC, acrylate ester was highly unstable in silica gel column chromatography. Purification of the crude product gave only elimination product **20** with 40% yield, so the enantiomeric purity of the formed alcohol (***R*-18**) could not be determined. It is likely that donation of the lone pair of electrons from nitrogen atom to the naphthalene was the driving force of this elimination (Scheme 1).

Similarly asymmetric allylation of 4-methyl-1-naphthaldehyde at −20 °C gave compound (***R*)-22** with 56% yield and 82% ee. When the same reaction was performed at room temperature, the enantiomeric excess of the reaction did not change (Scheme 2). All further asymmetric allylation reactions were performed both at −20 °C and room temperature to show the applicability of asymmetric allylation of 1-napthaldehydes at room temperature. Asymmetric allylation of 4-methoxy-1-naphthaldehyde furnished compound **(*R*)-24** with almost the same enantiomeric excess at −20 °C and room temperature (91% and 90% respectively). However, the yields of the products were low compared to the yield of racemic allylation by a TBAT-CuCl mixture [19] (Scheme 3).

Asymmetric allylation of 2-methoxy-1-napthaldehyde and of 4,7-dimethoxy-1-naphthaldehyde gave similar results. The yields of the asymmetric allylation are low compared to racemic allylation and the enantiomeric excess of the reactions were quite similar at −20 °C and room temperature (50% and 55%, respectively, for compound **(*R*)-27**, and 87% and 84%, respectively, for compound **(*R*)-30**). The low enantiomeric excess for compound **(*R*)-27** can be explained by the proximity of the methoxy substituent to the reaction center (Scheme 3 and 4). Surprisingly, it appears that there was no effect of temperature on the enantiomeric excess of the reactions.

To show the asymmetric synthesis of heteroatom-substituted klavuzon derivatives, two chiral homoallylic alcohols, **(*R*)-24** and **(*R*)-27**, were transformed to their acrylate esters and then converted to the lactones by ring-closing metathesis using a first generation Grubbs' catalyst [20]. To minimize the possible elimination reaction, esters were used without purification to produce α,β-unsaturated δ-lactones (Scheme 3).

Finally, racemic syntheses of compounds **10**–**16** were completed (Scheme 5). Briefly, racemic allylation of aldehydes (**17**,

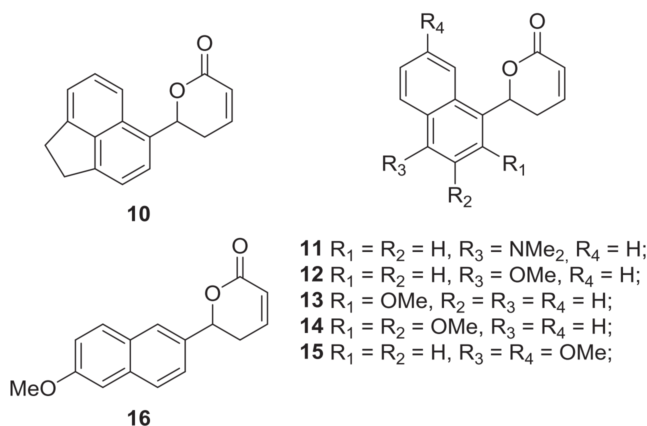
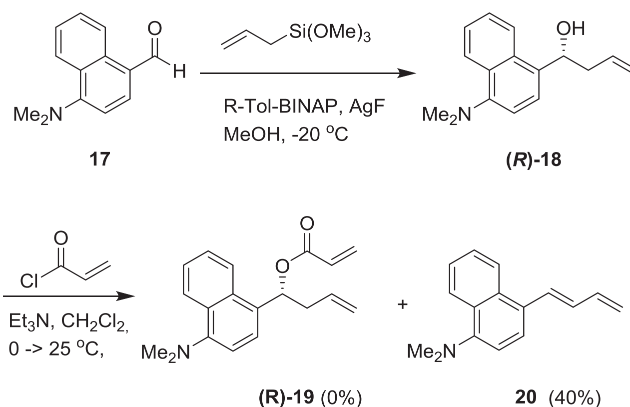
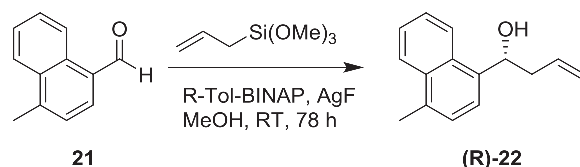


Fig. 2. Proposed klavuzon (**10**–**15**) and goniothalamin (**16**) derivatives.



Scheme 1. Attempts towards the asymmetric synthesis of 4′-dimethylaminoklavuzon.



Scheme 2. Asymmetric allylation of 4-methyl-1-naphthaldehyde at room temperature.

23 R_1 = OMe, R_2 = H; **26** R_1 = H, R_2 = OMe; **(R)-24** R_1 = OMe, R_2 = H; **(R)-27** R_1 = H, R_2 = OMe; **(R)-25** R_1 = OMe, R_2 = H; **(R)-28** R_1 = H, R_2 = OMe; **(R)-12** R_1 = OMe, R_2 = H; **(R)-13** R_1 = H, R_2 = OMe;

Entry	Conditions	% yield, (%ee)					
		(R)-24	(R)-25	(R)-12	(*R*)-27	(*R*)-28	(*R*)-13
1	*R*-Tol-BINAP, AgF, MeOH, RT, 72 h	29, (90)	NI	27*	30, (50)	NI	25*
2	*R*-Tol-BINAP, AgF, MeOH, -20 °C, 75 h	50, (91)	-	-	22, (55)		
3	TBAT, CuCl, THF, RT, 7 h	88, (rac.)			87, (rac.)		

RT: Room Temperature; NI: Not Isolated;* yield for two steps; ee% calculated from chiral HPLC

Scheme 3. Asymmetric and racemic preparations of 4′-methoxyklavuzon (12) and 4′-methoxyklavuzon (13).

29 → **(R)-30**

Entry	Conditions	% yield, (%ee)
		(R)-30
1	*R*-Tol-BINAP, AgF, MeOH, RT, 90 h	13, (84)
2	*R*-Tol-BINAP, AgF, MeOH, -20 °C, 73 h	9, (87)
3	TBAT, CuCl, THF, RT, 5 h	11, (rac.)

Scheme 4. Asymmetric allylation of 4,7-dimethoxy-1-naphttaldehyde (29) at −20 °C and room temperature.

23, **26**, **29**, and **31**–**33**) was successfully completed by adding allyltrimethoxysilane in the presence of TBAT-CuCl mixture. Yields of the reactions were generally high (86–98%), except for 4-dimethylamino1-naphthaldehyde and 4,7-dimethoxy-1-naphthaldehyde (54% and 11%, respectively).

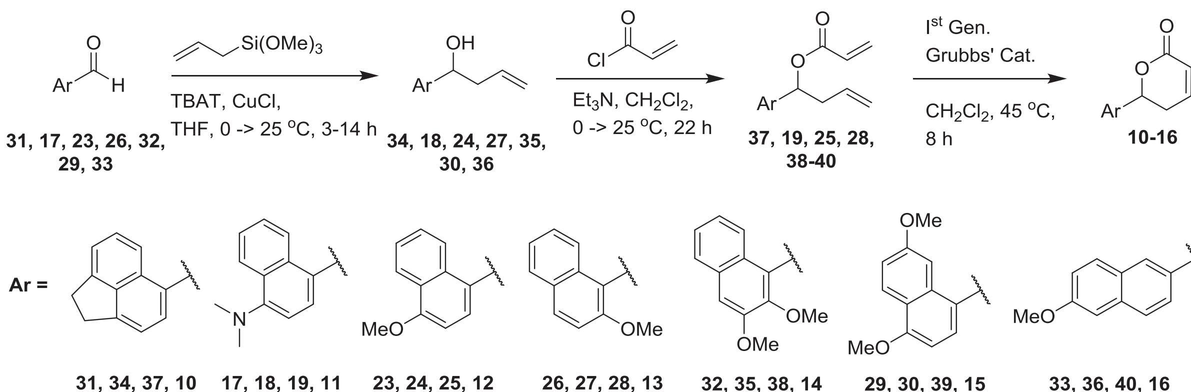
Next, the formed homoallylic alcohols (**18**, **24**, **27**, **30**, and **34**–**36**) were subjected to acrylate ester formation with acryloyl chloride. Yields of the esterification reactions were between 73% and 97%, except for compound **18**, which was used without purification because of its decomposition in the silica gel column.

At the last step of synthesis, ring-closing metathesis reactions of esters (**19, 25**, **28**, and **37**–**40**) were completed with 72–85% yields. Similarly, the crude product of compound **19** was treated with first-generation Grubbs' catalyst to produce lactone **11** with 45% yield (for the last two steps, Scheme 5).

2.2. Biological activity

2.2.1. In vitro anti-proliferative activities of compounds

Anti-proliferative properties of the synthesized compounds **10**–**16** were studied by the MTT assay in four different cancer cell lines (Table 1). Colon cancer cells (HCT116 p53+/+ and HCT116 p53−/−) were more sensitive to the anti-proliferative activity of all tested compounds. A similar result was also reported for racemic goniothalamin treated p53-positive and -negative hepatocellular carcinoma (HCC) cells [13]. It seems that cytotoxic behavior of tested klavuzon derivatives exhibit similarity with that of racemic goniothalamin and cytotoxic activity of both compounds is independent with p53 protein expression. The anti-proliferative effect of com-



Scheme 5. Racemic preparations of klavuzon (10–15) and conformationally constrained goniothalamin (**16**) derivatives.

Table 1
In vitro anti-proliferative activities of compounds **10**–**16** on cancer cell lines in μM.

Compound	MCF-7	PC3	HCT116	
			p53+/+	p53 −/−
(*R*)-1	19.00	4.00	nt	nt
8	0.44	0.05	nt	nt
10	22.42	1.68	0.03	0.01
11	nt	nt	0.12	0.62
12	2.66	1.97	0.10	0.26
13	2.40	1.10	1.36	0.69
14	8.56	0.50	0.35	0.73
15	9.12	3.41	0.25	1.66
16	27.01	20.98	5.06	6.40

nt: not tested, Concentrations, that are needed to inhibit 50% of the cell growth, were determined from nonlinear regression analysis of three separate experiments performed in triplicate by using the GraphPad Prism software ($R^2 > 0.9$).

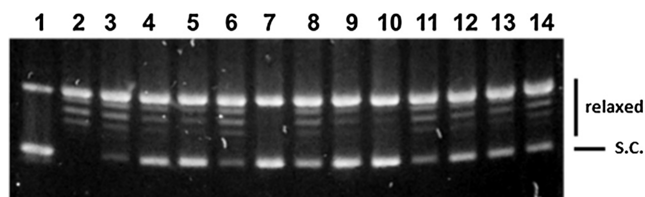


Fig. 3. *Topoisomerase I* catalyzed relaxation of supercoiled DNA in the presence of compounds **(*R*)-GTN**, **6**, **7**, **8** and **9**. Lane 1, pUC19 (Supercoiled DNA, pHOT1 0,25 μg/μL); lane 2, pUC19 + Topo I (1U) + DMSO; lanes 3–4, pUC19 + Topo I + 3 and 50 μM CPT; lanes 5–6, pUC19 + Topo I + 10 and 1 μM (*R*)-goniothalamin; lanes 7–8, pUC19 + Topo I + 10 and 1 μM compound **6**; lanes 9–10, pUC19 + Topo I + 10 and 1 μM compound **7**; lanes 11–12, pUC19 + Topo I + 10 and 1 μM compound **8**; lanes 13–14, pUC19 + Topo I + 10 and 1 μM compound **9**.

pounds **6**–**9** on MCF-7 and PC-3 cancer cell lines was previously reported by our group. Structurally, compound **10** is quite similar to compound **8**. Because both compounds have a hydrophobic carbon at position 4 of naphthalene-1-yl, we expected that compound **10** would possess similar potency compared to compound **8**. Unfortunately, it was found to be less active in MCF-7 and PC3 cancer cell lines, while it had the lowest IC_{50} values for HCT116 p53+/+ and HCT116 p53−/− cancer cell lines.

Among the heteroatom-substituted klavuzon derivatives, compounds **12** and **13** are analogous to compounds **8** and **9**, respectively. Comparison of their anti-proliferative activity implies that a single methoxy substitution on naphthalene does not enhance the activity as much as a single methyl group does for compounds **8** and **9**. Dimethoxy substitution (**14** and **15**) does not make any significant change in terms of biological activity, and compound **14** was found to be the most active molecule as it had IC_{50} values at nanomolar concentrations in three cancer cell lines.

Besides, compound **16** was considered as the methoxy-substituted conformationally constrained analog of goniothalamin. In this case, a single methoxy substitution dramatically decreased the potency of the compound compared with the IC_{50} values of unsubstituted conformationally constrained goniothalamin analog reported in the literature [16].

Up to date, there are many compounds which possess anti-proliferative activity via interfering with Topo I action mechanism. Topo I poisons, such as camptothecin (CPT) derivatives, reversibly stabilize the Topo I-DNA complex [21] while Topo I catalytic inhibitors, such as DNA intercalators [22,23] or Michael acceptors [5,6,24,25], can only interacts with Topo I or DNA and prohibit Topo I binding to DNA. Among these, Michael acceptors covalently bind themselves to the nucleophilic thiol side chains of the target proteins, and Montaudon and coworkers showed that among eight cysteine residues in human topoisomerase I only Cys504 and Cys505 residues are valuable targets for the inhibition of Topo I [6].

Since goniothalamin and klavuzon have a Michael acceptor in their structure, derivatives of these compounds can also inhibit Topo I activity by covalently binding to the Cys504 and Cys505 residues. Previously synthesis and anticancer property of conformationally constrained (R)-goniothalamin derivatives have been reported by our group [16]. As it can be seen in Fig. 3 (R)-goniothalamin and its conformationally constrained analog (**6**) inhibit Topo I at even 1 μM concentration. Similarly, (R)-klavuzon (**7**), (R)-4′-methylklavuzon (**8**) and (R)-2′-methylklavuzon (**9**) also inhibits Topo I mediated supercoiled DNA relaxation at 1 μM concentration. In general tested compounds showed similar Topo I inhibition potency at 10 μM concentration compared to camptothecin (CPT) at 50 μM concentration.

Newly synthesized heteroatom substituted racemic klavuzon derivatives (**10**–**15**) and conformationally constrained goniothalamin derivative (**16**) can also inhibit Topo I enzyme (Figs. 4 and 5). Replacement of methyl substituent with methoxide in klavuzon structure at 2′-position does not cause any significant effect in Topo I inhibition potency (compounds **9** and **13** in Fig. 4). Interestingly, 4′-dimethylaminoklavuzon (**11**) and 2′,3′-dimethoxyklavu zon (**15**) derivatives inhibits Topo I at higher concentration (50 μM). Preincubation of Topo I enzyme with Michael acceptors may also have influence in supercoiled DNA relaxation assay. Topoisomerase I enzyme was preincubated only with 4′methylklavuzon (**8**) for 0, 1, 5 and 10 min prior to the addition of supercoiled DNA (Fig. 6). While there was not any significant Topo I enzyme inhibition after 0 and 1 min of preincubation, 5 and 10 min of preincubation is enough for Topo I inhibition.

It seems quite difficult to correlate strong antiproliferative activity in cancer cells with inhibition of Topo I relatively at higher doses for klavuzon derivatives. But this may not be the case due to two reasons. Firstly, all supercoiled DNA relaxation assays were performed at pH of 8 as they were advised by the manufacturer. Addition of thiols residues to Michael acceptors can be turned on or off by depending on the pH of the reaction medium. Michael addition reaction is irreversible and extremely slow at low pH while it is reversible and faster at basic pH values [26]. Previously, it was shown that Michael addition of thiols is a time dependent equilibrium equation. After the reaction reach to equilibrium point, concentrations of both starting material and adduct are almost similar [26].

Secondly, amongst the different subcellular compartments only mitochondria has a relatively basic medium (pH = 8) to support a reversible Michael addition reaction. Some other intracellular compartments, such as nucleus, cytosol, endoplasmic reticulum and

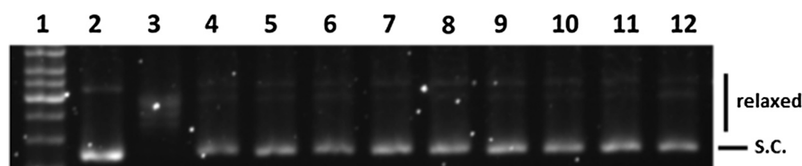


Fig. 4. *Topoisomerase I* catalyzed relaxation of supercoiled DNA in the presence of compounds **12**–**14** and **9**. Lane 1, 1 kB DNA Ladder; Lane 2, pUC19 (Supercoiled DNA, pHOT1 0,25 μg/μL); lane 3, pUC19 + Topo I (1U) + DMSO; lane 4, pUC19 + Topo I + 50 μM CPT; lanes 5–6, pUC19 + Topo I + 10 and 50 μM compound **12**; lanes 7–8, pUC19 + Topo I + 10 and 50 μM compound **9**; lanes 9–10, pUC19 + Topo I + 10 and 50 μM compound **13**; lanes 11–12, pUC19 + Topo I + 10 and 50 μM compound **14**.

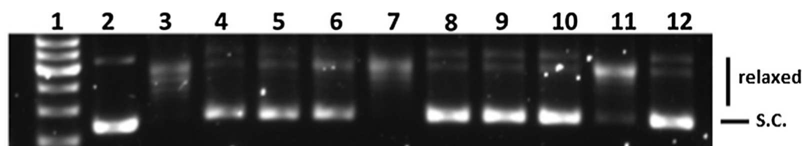


Fig. 5. *Topoisomerase I* catalyzed relaxation of supercoiled DNA in the presence of compounds **10**, **11**, **15**, and **16**. Lane 1, 1 kB DNA Ladder; Lane 2, pUC19 (Supercoiled DNA, Phot1 0,25 μg/μL); lane 3, pUC19 + Topo I (1U) + DMSO; lane 4, pUC19 + Topo I + 50 μM CPT; lanes 5–6, pUC19 + Topo I + 10 and 50 μM compound **16**; lanes 7–8, pUC19 + Topo I + 10 and 50 μM compound **15**; lanes 9–10, pUC19 + Topo I + 10 and 50 μM compound **10**; lanes 11–12, pUC19 + Topo I + 10 and 50 μM compound **11.**

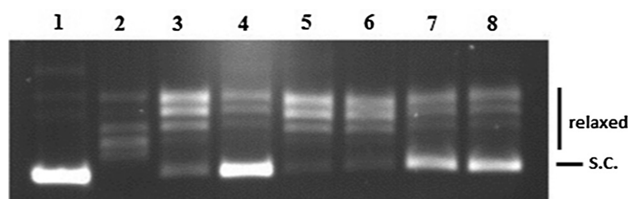


Fig. 6. Time dependent *Topoisomerase I* catalyzed relaxation of supercoiled DNA in the presence of compound **8**: Lane1, pHOT1 (supercoiled DNA, 250 ng); lane 2, relaxed DNA (100 ng); lane3, pHOT1 + Topo I + DMSO; lane4, pHOT1 + Topo I + 50 μM CPT; lanes 5–8, pHOT1 + Topo I pre-incubated with 100 μM compound **8** for 0, 1, 5 and 10 min.

peroxisomes, are between neutral and slightly basic (pH = 7.0–7.2). Remaining compartments, golgi network and lysosome, are quite acidic (pH = 6.7–4.7) [27]. Also there are some evidence that cancer cells may be more acidic [28]. It was reported that even at physiological pH 7.4 Michael addition of glutathione to maleimide gave almost 90% product formation [29]. Hence, outside the mitochondria, all possible Michael addition reactions will be very slow and irreversible. Possible reaction of Michael acceptors with Topo I inside the nucleus would be slower but irreversible. This may require lower doses of klavuzons inside the cell compared to that of *in vitro* supercoiled DNA relaxation assay.

3. Conclusions

In this work, it has been shown that enantiomeric excess of the asymmetric allylation of 1-naphthaldehyde derivatives catalyzed by R-Tol-BINAP.AgF complex do not depend on temperature. Heteroatom substitution does not enhance the anti-proliferative activities of klavuzon derivatives, but 2′,3′-dimethoxy–substituted klavuzon (**14**) was the most active compound and caused a 50% inhibition of cell proliferation of three cancer cell lines at nanomolar concentrations. Additionally it is found that both goniothalamin and derivatives of klavuzon inhibit Topo I enzyme.

4. Experimental

4.1. Chemistry part

4.1.1. General procedures

Reagents were commercial grade and were used as supplied. Dichloromethane was distilled over calcium hydride. Tetrahydrofuran was dried by MBraun-SPS-Solvent Purification System. Reactions were monitored by using Merck TLC plates (Silica gel 60 F 254). Chromatographic separations and isolations were performed using Fluka 70–230 mesh silica gel. Solvents, required for SiO_2 column chromatography, were commercial grade and were used as supplied. Solvents, required for HPLC, were HPLC grade and were used as supplied. ^{1}H NMR and ^{13}C NMR data were recorded on a Varian 400-NMR (400 MHz) spectrometer. Chemical shifts for ^{1}H NMR and ^{13}C NMR are reported in δ (ppm). $CDCl_3$ peaks were used as reference in ^{1}H NMR (7.26 ppm) and ^{13}C NMR (77.16 ppm), respectively. MestreNova NMR Processing Software was used for processing NMR spectra. Optical rotations were measured with ADP 410 Digital Polarimeter (Bellingham + Stanley Ltd.). HPLC studies were performed by employing Chiralcel OJ-H column (0.46 cmΦ; ×150 mm) on Agilent 1200 Series instrument.

4.1.2. General procedure for the synthesis of asymmetric homoallylic alcohols (R-18, R-24, R-27, R-30 and R-22)

In a two necked round bottom flask 165 mg of R-Tol-BINAP (0.24 mmol) and 52 mg of AgF (0.41 mmol) were placed and dissolved in 10 mL of anhydrous methanol. Solution was stirred about 20 min under nitrogen atmosphere at room temperature without expose of light. Then solution mixture was cooled down to −20 °C (room temperature for the reactions which was carried out at room temperature) and 691 mg of 4-methyl-1-naphthaldehyde (4.06 mmol) and 719 μL of allyltrimethoxysilane (693 mg, 4.27 mmol) were added to reaction mixture respectively. Final solution was allowed to stir 75 h at −20 °C (room temperature for the reactions which was carried out at room temperature). Then resulting mixture was filtered through celite-silica gel mixture. Purification of crude product by column chromatography on silica gel (1:10 → 1:8 EtOAc/Hexane) furnished desired asymmetric alcohol.

4.1.2.1. (R)-1-(4-(dimethylamino)naphthalen-1-yl)but-3-en-1-ol (R-18). Purification of crude product by column chromatography on silica gel (1:6, EtOAc:Hexane) furnished 167 mg of (*R*)-1-(4-(dime thylamino)naphthalen-1-yl)but-3-en-1-ol as colorless oil, with 54% yield. Rf: 0,29 (1:6, EtOAc:Hexane); $[\alpha]_D^{22}$ +182 (*c* 1.67, CH_2Cl_2); Enantiomeric excess was found as 100% with Chiralcel OJ-H HPLC column (Hexane:Isopropanol, 95:5, 1 mL/min, 320 nm, R_t = 7.70 - min as single peak); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.36–8.31 (m, 1H, naphthalene C5-*H*), 8.09–8.04 (m, 1H, naphthalene C8-*H*), 7.55 (d, *J* = 7.8 Hz, 1H, naphthalene C3-*H*), 7.54–7.49 (m, 2H, naphthalene C6-*H* and C7-*H*), 7.07 (d, *J* = 7.8 Hz, 1H, naphthalene C2-*H*), 6.01–5.88 (m, 1H, =C*H*—), 5.46–5.41 (m, 1H, benzylic *H*), 5.27–5.15 (m, 2H, =CH_2), 2.90 (s, 6H, —N(CH_3)$_2$), 2.79–2.70 (m, 1H, —C*H*H—), 2.69–2.57 (m, 1H, —CH*H*—), 2.42 (s, 1H, —O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.60, 135.20, 133.97, 131.66, 129.01, 125.94, 124.99, 124.85, 123.48, 123.03, 118.01, 113.54, 70.05, 45.34, 42.78.

4.1.2.2. (R)-(+)-1-(4-methoxynaphthalen-1-yl)but-3-en-1-ol (R-24). Purification of crude product by column chromatography on silica gel (1:18, EtOAc:Hexane) furnished 113 mg of (*R*)-(+)-1-(4-methoxynaphthalen-1-yl)but-3-en-1-ol as white solid with 50% yield. Rf: 0.23 (1:6, EtOAc:Hexane); $[\alpha]_D^{22}$ +181 (*c* 0.45, CH_2Cl_2); Enantiomeric excess was found as 91% ee with Chiralcel OJ-H column (90:10 Hexane:Isopropanol, 1 mL/min; 280 nm, R_{t1}: 18.628 min as major peak, R_{t2}: 17.385 min as minor peak); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38–8.32 (m, 1H, naphthalene C5-*H*), 8.06 (d, *J* = 8.7 Hz, 1H, naphthalene C8-*H*), 7.58–7.46 (m, 3H, naphthalene C2-*H*, C6-*H* and C7-*H*), 6.81 (d, *J* = 8.0 Hz, 1H, naphthalene C3-*H*), 6.00–5.86 (m, 1H, =C*H*—), 5.44 (dd, *J* = 8.1, 4.3 Hz, 1H, benzylic *H*), 5.26–5.14 (m, 2H, =CH_2), 4.01 (s, 3H, —OCH_3), 2.80–2.71 (m, 1H, —C*H*H—), 2.68–2.58 (m, 1H, —CH*H*—), 2.11 (s, 1H, —O*H*);

^{13}C NMR (100 MHz, $CDCl_3$) δ 155.21, 135.17, 131.48, 131.41, 126.72, 125.95, 125.04, 123.26, 122.98, 122.89, 118.25, 103.28, 70.15, 55.65, 42.88.

4.1.2.3. (R)-(+)-1-(2-methoxynaphthalen-1-yl)but-3-en-1-ol (R-27). Purification of crude product by column chromatography on silica gel (1:18, EtOAc:Hexane) furnished 50 mg of (*R*)-(+)-1-(2-methoxy naphthalen-1-yl)but-3-en-1-ol as white solid, with 22% yield. Rf: 0.26 (1:6, EtOAc:Hexane); $[\alpha]_D^{25}$ +64 (*c* 0.50, CH_2Cl_2); Enantiomeric excess was found as 55% ee with Chiralcel OJ-H column (90:10 Hexane:Isopropanol, 1 mL/min; 280 nm, R_{t1}: 7.850 min as major peak, R_{t2}: 11.596 min as minor peak); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 8.7 Hz, 1H, naphthalene C8-*H*), 7.82–7.77 (m, 2H, naphthalene C4-*H* and C5-*H*), 7.52–7.46 (m, 1H, naphthalene C6-*H*), 7.39–7.33 (m, 1H, naphthalene C7-*H*), 7.29 (d, *J* = 9.1 Hz, 1H, naphthalene C3-*H*), 5.97–5.85 (m, 1H, =C*H*—), 5.65–5.58 (m, 1H, benzylic *H*), 5.14 (d, *J* = 17.1 Hz, 1H, =C*H*H), 5.06 (d, *J* = 10.2 Hz, 1H, =CH*H*), 4.01 (s, 3H, —OCH_3), 3.91 (s, 1H, —O*H*), 2.91–2.81 (m, 1H, —C*H*H—), 2.70–2.61 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.68, 135.63, 131.72, 129.51, 129.48, 128.75, 126.78, 124.11, 123.77, 123.16, 117.10, 113.37, 69.75, 56.49, 42.34.

4.1.2.4. (R)-(+)-1-(4,7-dimethoxynaphthalen-1-yl)but-3-en-1-ol (R-30). Purification of crude product by column chromatography on silica gel furnished (*R*)-(+)-1-(4,7-dimethoxynaphthalen-1-yl)but-3-en-1-ol was collected as white solid with 9% yield. Rf: 0,08 (1:8, EtOAc:Hexane); $[\alpha]_D^{25}$ +163 (*c* 0.16, CH_2Cl_2); Enantiomeric excess was found as 87% ee with Chiralcel OJ-H column (90:10 Hexane:Isopropanol, 1 mL/min; 280 nm, R_{t1}: 10.189 min as major peak, R_{t2}: 12.268 min as minor peak); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 9.2 Hz, 1H, naphthalene C5-*H*), 7.48 (d, *J* = 8.0 Hz, 1H, naphthalene C6-*H*), 7.35 (d, *J* = 2.1 Hz, 1H, naphthalene C8-*H*), 7.17–7.11 (m, 1H, naphthalene C2-*H*), 6.66 (dd, *J* = 8.1, 3.0 Hz, 1H, naphthalene C3-*H*), 5.99–5.87 (m, 1H, =C*H*—), 5.33 (dd, *J* = 8.0, and 4.6 Hz, 1H, benzylic *H*), 5.21 (d, *J* = 17.2 Hz, 1H, =C*H*H), 5.19–5.14 (m, 1H, =CH*H*), 3.97 (s, 3H, —OCH_3), 3.92 (s, 3H, —OCH_3), 2.79–2.70 (m, 1H, —C*H*H—), 2.69–2.59 (m, 1H, —CH*H*—), 2.10 (s, 1H, —O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.18, 155.34, 135.29, 132.90, 130.12, 124.51, 124.10, 120.99, 118.04, 116.74, 102.44, 101.36, 70.53, 55.53, 55.35, 42.37.

4.1.2.5. (R)-(+)-1-(4-methylnaphtalen-1-yl)but-3-en 1-ol (R-22). Purification of crude product by column chromatography on silica (1:10 → 1:8 EtOAc/Hexane) furnished 479 mg of (*R*)-1-(4-methylnaphtalen-1-yl)but-3-en 1-ol as light yellow oil with 56% yield. Rf: 0.25 (1:6, EtOAc:Hexane); $[\alpha]_D^{24}$ +223 (*c* 1.91, CH_2Cl_2); Enantiomeric excess was found as 82% with Chiralcel OJ-H HPLC column (Hexane:Isopropanol, 99:1, 1 mL/min, 280 nm, R_{t1} = 15.217 min as minor peak, R_{t2} = 15.937 min as major peak); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.14–8.09 (m, 1H, naphthalene C8-*H*), 8.08–8.03 (m, 1H, naphthalene C5-*H*), 7.58–7.51 (m, 3H, naphthalene C3-*H*, C6-*H* and C7-*H*), 7.34 (d, *J* = 7.3 Hz, 1H, naphthalene C2-*H*), 6.01–5.88 (m, 1H, =C*H*—), 5.55–5.49 (m, 1H, benzylic *H*), 5.26–5.16 (m, 2H, =CH_2), 2.81–2.73 (m, 1H, —C*H*H—), 2.70 (s, 3H, —CH_3), 2.67–2.57 (m, 1H, —CH*H*—), 2.12 (s, 1H, —O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 137.59, 134.89, 133.47, 132.60, 130.22, 126.04, 125.34, 125.07, 124.70, 123.41, 122.46, 117.41, 69.76, 42.58, 19.38.

4.1.3. General procedure for the synthesis of racemic homoallylic alcohols (34, 18, 24, 27, 35, 30 and 36)

A mixture of 20 mg of CuCl (0.20 mmol) and 111 mg of TBAT (0.20 mmol) in 5 mL of THF was stirred for 1 h at room temperature. After cooling the mixture in an ice bath, 505 μL of allyltrimethoxysilane (486 mg, 3.00 mmol) and 372 mg of 6-methoxy-1-naphthaldehyde (2.00 mmol) were added and then the cooling bath was removed. After starting material disappeared on TLC, 2 M HCl aq. in MeOH (1:1) was added for desilylation. Resulting mixture was extracted with ethyl acetate and collected organic phase washed with brine (2 × 40 mL) dried with $MgSO_4$. After removing excess solvent under vacuum, crude product was purified by column chromatography on silica gel (1:10 → 1:6, EtOAc/Hexane) to give desired alcohol.

4.1.3.1. 1-(1,2-Dihydroacenaphthylen-5-yl)but-3-en-1-ol (34). Purification of crude product by column chromatography yielded 193 mg of 1-(1,2-dihydroacenaphthylen-5-yl)but-3-en-1-ol as light yellow oil, with 86% yield. Rf: 0.32 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 8.4 Hz, 1H, naphthalene C8-*H*), 7.57 (d, *J* = 7.1 Hz, 1H, naphthalene C2-*H*), 7.48 (dd, *J* = 8.3, 7.0 Hz, 1H, naphthalene C7-*H*), 7.33–7.27 (m, 2H, naphthalene C3-*H* and C6-*H*), 6.02–5.80 (m, 1H, =C*H*—), 5.41 (brs, 1H, benzylic *H*), 5.27–5.11 (m, 2H, =CH_2), 3.45–3.34 (m, 4H, —CH_2CH_2—), 2.81–2.57 (m, 2H, —CH_2—), 2.19 (s, 1H, —O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.73, 145.91, 139.64, 135.47, 135.10, 135.08, 130.20, 128.92, 128.02, 127.92, 124.65, 119.33, 119.02, 118.98, 118.24, 70.55, 42.96, 30.66, 30.03.

4.1.3.2. 1-(4-(Dimethylamino)naphthalen-1-yl)but-3-en-1-ol (18). Purification of crude product by column chromatography yielded 129 mg of 1-(4-(dimethylamino)naphthalen-1-yl)but-3-en-1-ol as colorless oil, with 54% yield. Rf: 0.29 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data were consistent with previously reported values in 4.1.2.1.

4.1.3.3. 1-(4-Methoxynaphthalen-1-yl)but-3-en-1-ol (24). Purification of crude product by column chromatography yielded 400 mg of 1-(4-methoxynaphthalen-1-yl)but-3-en-1-ol as colorless oil, with 88% yield. Rf: 0.23 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data were consistent with previously reported values in 4.1.2.2.

4.1.3.4. 1-(2-Methoxynaphthalen-1-yl)but-3-en-1-ol (27). Purification of crude product by column chromatography yielded 398 mg of 1-(2-methoxynaphthalen-1-yl)but-3-en-1-ol as colorless oil, with 87% yield. Rf: 0.26 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data were consistent with previously reported values in 4.1.2.3.

4.1.3.5. 1-(2,3-Dimethoxynaphthalen-1-yl)but-3-en-1-ol (35). Purification of crude product by column chromatography yielded 245 mg of 1-(2,3-dimethoxynaphthalen-1-yl)but-3-en-1-ol as white solid, with 95% yield. Rf: 0.23 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, *J* = 7.4 Hz, 1H, naphthalene C8-*H*), 7.76–7.66 (m, 1H, naphthalene C5-*H*), 7.45–7.32 (m, 2H, naphthalene C6-*H* and C7-*H*), 7.13 (s, 1H, naphthalene C4-*H*), 6.01–5.86 (m, 1H, =C*H*—), 5.67–5.56 (m, 1H, benzylic *H*), 5.22–5.07 (m, 2H, =CH_2), 3.99 (s, 3H, —OCH_3), 3.98 (s, 3H, —OCH_3), 3.62 (brs, 1H, —O*H*), 2.92–2.80 (m, 1H, —C*H*H—), 2.72–2.61 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.66, 147.21, 135.38, 131.69, 130.58, 127.43, 126.63, 125.32, 124.24, 124.06, 117.52, 107.50, 69.78, 61.74, 55.69, 43.13.

4.1.3.6. 1-(4,7-Dimethoxynaphthalen-1-yl)but-3-en-1-ol (30). Purification of crude product by column chromatography yielded 57 mg of 1-(4,7-dimethoxynaphthalen-1-yl)but-3-en-1-ol as white solid, with 11% yield. Rf: 0.09 (1:8, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data were consistent with previously reported values in 4.1.2.4.

4.1.3.7. 1-(6-Methoxynaphthalen-2-yl)but-3-en-1-ol (36). Purification of crude product by column chromatography yielded 449 mg of 1-(6-methoxynaphthalen-2-yl)but-3-en-1-ol as white solid, with 98% yield. Rf: 0.22 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77–7.68 (m, 3H, naphthalene C1-*H*, C4-*H* and C8-*H*), 7.45 (dd, *J* = 8.6, 1.7 Hz, 1H, naphthalene C3-*H*), 7.18–7.11 (m, 2H, naphthalene C5-*H* and C7-*H*), 5.90–5.77 (m, 1H, =C*H*—), 5.23–5.11 (m, 2H, =CH_2), 4.91–4.83 (m, 1H, benzylic *H*), 3.92 (s, 3H, —OCH_3), 2.65–2.55 (m, 2H, —CH_2—), 2.11 (brs, 1H, —O*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.85, 139.16, 134.64, 134.26, 129.57, 128.88, 127.21, 124.71, 124.59, 119.08, 118.53, 105.89, 73.60, 55.46, 43.89.

4.1.4. Synthesis of acrylate esters (37, 19, 20, 25, 28, 38–40)

A solution of 439 mg of 1-(6-methoxynaphthalen-2-yl)but-3-en-1-ol (1.92 mmol) in 12 mL of CH_2Cl_2 was cooled down to 0 °C, then 313 μL of acryloyl chloride (3.85 mmol) and 1.05 mL of triethylamine (7.53 mmol) were added sequentially. The mixture was allowed to warm to room temperature and stirred until alcohol were consumed under nitrogen atmosphere. The final mixture was filtered through celite, poured into water and extracted with CH_2Cl_2. Combined organic phase concentrated under vacuum and purification of crude product by column chromatography on silica gel (EtOAc:Hexane, 1:10) gave the corresponding acrylate ester.

4.1.4.1. 1-(1,2-Dihydroacenaphthylen-5-yl)but-3-en-1-yl acrylate (37). Purification of crude product by column chromatography yielded 173 mg of 1-(1,2-dihydroacenaphthylen-5-yl)but-3-en-1-yl acrylate as light yellow oil with 80% yield. Rf: 0.65 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 8.4 Hz, 1H, naphthalene C8-*H*), 7.54–7.47 (m, 2H, naphthalene C2-*H* and C7-*H*), 7.31 (d, *J* = 6.9 Hz, 1H, naphthalene C3-*H*), 7.27 (d, *J* = 7.7 Hz, 1H, naphthalene C6-*H*), 6.54 (t, *J* = 6.8 Hz, 1H, benzylic *H*), 6.45 (d, *J* = 17.3 Hz, 1H, —COCH=C*H*H), 6.24–6.14 (m, 1H, —COC*H*=CH$_2$), 5.86–5.74 (m, 2H, —C*H*= and —COCH=CH*H*), 5.13 (d, *J* = 17.1 Hz, 1H, =C*H*H), 5.06 (d, *J* = 10.2 Hz, 1H, =CH*H*), 3.44–3.33 (m, 4H, —CH_2CH_2—), 2.95–2.78 (m, 2H, —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.57, 146.70, 146.60, 139.67, 133.78, 131.67, 130.92, 129.10, 128.77, 128.19, 126.06, 119.46, 119.29, 118.89, 118.01, 73.24, 40.29, 30.60, 30.10.

4.1.4.2. 1-(4-(Dimethylamino)naphthalen-1-yl)but-3-en-1-yl acrylate (19). Purification was not performed. It was used without further purification in next step. R_f: 0.52 (1:6, EtOAc:Hexane).

4.1.4.3. (E)-4-(buta-1,3-dien-1-yl)-N,N-dimethylnaphthalen-1-amine (20). Purification of crude product by column chromatography on silica gel furnished 22 mg of (*E*)-4-(buta-1,3-dien-1-yl)-*N,N*-dimethylnaphthalen-1-amine as colorless oil with 40% yield as elimination product. Rf: 0.75 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32–8.25 (m, 1H, naphthalene C5-*H*), 8.18–8.12 (m, 1H, naphthalene C8-*H*), 7.61 (d, *J* = 7.9 Hz, 1H, naphthalene C2-*H*), 7.55–7.49 (m, 2H, naphthalene C6-*H* and C7-*H*), 7.32 (d, *J* = 15.2 Hz, 1H, —PhC*H*=), 7.07 (d, *J* = 7.9 Hz, 1H, naphthalene C3-*H*), 6.86–6.76 (m, 1H, =C*H*—CH=), 6.72–6.62 (m, 1H, =CH—C*H*=), 5.37 (d, *J* = 16.7 Hz, 1H, =C*H*H), 5.21 (d, *J* = 9.9 Hz, 1H, =CH*H*), 2.91 (s, 6H, —N(CH$_3$)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.03, 137.80, 132.49, 131.09, 129.96, 129.31, 128.80, 126.05, 125.14, 124.83, 124.15, 123.67, 117.07, 113.96, 45.30.

4.1.4.4. 1-(4-Methoxynaphthalen-1-yl)but-3-en-1-yl acrylate (25). Purification of crude product by column chromatography yielded 348 mg of 1-(4-methoxynaphthalen-1-yl)but-3-en-1-yl acrylate as white solid with 73% yield. Rf: 0.49 (1:8, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.33 (d, *J* = 8.4 Hz, 1H, naphthalene C5-*H*), 8.13 (d, *J* = 8.4 Hz, 1H, naphthalene C8-*H*), 7.57 (ddd, *J* = 8.5, 6.8, 1.5 Hz, 1H, naphthalene C6-*H*), 7.52–7.47 (m, 2H, naphthalene C3-*H* and C7-*H*), 6.80 (d, *J* = 8.0 Hz, 1H, naphthalene C2-*H*), 6.63–6.55 (m, 1H, benzylic *H*), 6.44 (dd, *J* = 17.3, 1.5 Hz, 1H, —COCH=C*H*H), 6.19 (dd, *J* = 17.3, 10.4 Hz, 1H, —COC*H*=CH$_2$), 5.87–5.71 (m, 2H, —COCH=CH*H* and —C*H*=), 5.15–5.02 (m, 2H, =CH_2), 4.00 (s, 3H, —OCH_3), 2.88–2.81 (m, 2H, —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.58, 155.64, 133.84, 131.63, 130.89, 128.83, 127.81, 126.97, 126.00, 125.16, 124.70, 123.20, 122.89, 117.97, 103.19, 72.89, 55.65, 40.34.

4.1.4.5. 1-(2-Methoxynaphthalen-1-yl)but-3-en-1-yl acrylate (28). Purification of crude product by column chromatography yielded 420 mg of 1-(2-methoxynaphthalen-1-yl)but-3-en-1-yl acrylate as white solid, with 90% yield. Rf: 0.43 (1:8, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (d, *J* = 8.4 Hz, 1H, naphthalene C8-*H*), 7.82–7.76 (m, 2H, naphthalene C4-*H* and C5-*H*), 7.52–7.45 (m, 1H, naphthalene C6-*H*), 7.38–7.32 (m, 1H, naphthalene C7-*H*), 7.27 (d, *J* = 8.8 Hz, 1H, naphthalene C3-*H*), 6.97 (dd, *J* = 8.3, 6.8 Hz, 1H, benzylic *H*), 6.39 (dd, *J* = 17.3, 1.3 Hz, 1H, —COCH=C*H*H), 6.15 (dd, *J* = 17.3, 10.4 Hz, 1H, —COC*H*=CH$_2$), 5.87–5.74 (m, 2H, —C*H*= and —COCH=CH*H*), 5.08 (ddd, *J* = 17.1, 3.1, and 1.6 Hz, 1H, =C*H*H), 5.01 (ddt, *J* = 10.2, 1.9, and 1.1 Hz, 1H, =CH*H*), 3.99 (s, 3H, —OCH_3), 3.15–3.04 (m, 1H, —C*H*H—), 2.88–2.77 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.59, 155.04, 134.19, 132.14, 130.59, 130.43, 129.93, 128.95, 128.92, 126.41, 124.87, 123.51, 120.77, 117.49, 114.00, 69.97, 57.30, 39.16.

4.1.4.6. 1-(2,3-Dimethoxynaphthalen-1-yl)but-3-en-1-yl acrylate (38). Purification of crude product by column chromatography yielded 270 mg of 1-(2,3-dimethoxynaphthalen-1-yl)but-3-en-1-yl acrylate as white solid 97% yield. Rf: 0.41 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.41–8.37 (m, 1H, naphthalene C8-*H*), 7.74–7.69 (m, 1H, naphthalene C5-*H*), 7.41–7.34 (m, 2H, naphthalene C6-*H* and C7-*H*), 7.14 (s, 1H, naphthalene C4-*H*), 6.89 (dd, *J* = 8.4, 6.8 Hz, 1H, benzylic *H*), 6.41 (d, *J* = 17.4 Hz, 1H, —COCH=C*H*H), 6.16 (dd, *J* = 17.4, 10.3 Hz, 1H, —COC*H*=CH$_2$), 5.87–5.75 (m, 2H, —C*H*= and —COCH=CH*H*), 5.12–5.00 (m, 2H, =CH_2), 4.03 (s, 3H, —OCH_3), 3.98 (s, 3H, —OCH_3), 3.15–3.05 (m, 1H, —C*H*H—), 2.87–2.77 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.57, 151.72, 147.54, 133.93, 131.95, 130.85, 128.76, 127.62, 127.05, 126.64, 125.11, 125.08, 124.03, 117.84, 107.99, 70.62, 61.24, 55.69, 39.40.

4.1.4.7. 1-(4,7-Dimethoxynaphthalen-1-yl)but-3-en-1-yl acrylate (39). Purification of crude product by column chromatography yielded 56 mg of 1-(4,7-dimethoxynaphthalen-1-yl)but-3-en-1-yl acrylate as white solid with 82% yield. Rf: 0.45 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, *J* = 9.2 Hz, 1H, naphthalene C5-*H*), 7.47 (d, *J* = 8.1 Hz, 1H, naphthalene C2-*H*), 7.41 (d, *J* = 2.5 Hz, 1H, naphthalene C8-*H*), 7.14 (dd, *J* = 9.2, 2.5 Hz, 1H, naphthalene C6-*H*), 6.67 (d, *J* = 8.1 Hz, 1H, naphthalene C3-*H*), 6.57–6.51 (m, 1H, benzylic *H*), 6.44 (dd, *J* = 17.3, 1.5 Hz, 1H, —COCH=C*H*H), 6.17 (dd, *J* = 17.3, 10.4 Hz, 1H, —COC*H*=CH$_2$), 5.87–5.74 (m, 2H, —C*H*= and —COCH=CH*H*), 5.17–5.10 (m, 1H, =C*H*H), 5.10–5.04 (m, 1H, =CH*H*), 3.98 (s, 3H, —OCH_3), 3.94 (s, 3H, —OCH_3), 2.91–2.82 (m, 2H, —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.70, 158.49, 155.87, 133.93, 133.24, 130.97, 128.80, 126.35, 125.57, 124.56, 121.04, 118.00, 117.18, 102.43, 101.35, 72.82, 55.58, 55.42, 39.74.

4.1.4.8. 1-(6-Methoxynaphthalen-2-yl)but-3-en-1-yl acrylate (40). Purification of crude product by column chromatography yielded 455 mg of 1-(6-methoxynaphthalen-2-yl)but-3-en-1-yl acrylate as colorless oil with 84% yield. Rf: 0.52 (1:6, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77–7.70 (m, 3H, naphthalene C1-*H*, C4-*H* and C8-*H*), 7.46 (dd, *J* = 8.4, 1.7 Hz, 1H, naphthalene

C3-*H*), 7.16 (dd, *J* = 8.9, 2.5 Hz, 1H, naphthalene C7-*H*), 7.13 (d, *J* = 2.4 Hz, 1H, naphthalene C5-*H*), 6.44 (dd, *J* = 17.3, 1.5 Hz, 1H, —COCH═C*H*H), 6.18 (dd, *J* = 17.3, 10.4 Hz, 1H, —COC*H*═CH_2), 6.07–6.00 (m, 1H, benzylic *H*), 5.83 (dd, *J* = 10.4, 1.5 Hz, 1H, —COCH═CH*H*), 5.81–5.68 (m, 1H, —C*H*═), 5.15–5.01 (m, 2H, ═CH_2), 3.92 (s, 3H, —OCH_3), 2.86–2.75 (m, 1H, —C*H*H—), 2.75–2.65 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.55, 158.05, 135.16, 134.44, 133.42, 130.91, 129.68, 128.81, 128.71, 127.25, 125.84, 125.05, 119.18, 118.23, 105.84, 75.70, 55.45, 40.76.

4.1.5. Synthesis of 5,6-dihydro-2H-pyran-2-ones (Klavuzon derivatives) (10–16)

To a solution of 434 mg of 1-(6-methoxynaphthalen-2-yl)but-3-en-1-yl acrylate (1.54 mmol) in 154 mL of CH_2Cl_2 (0.01 M) was heated to 45 °C, and 130 mg of 1st Generation Grubbs' catalyst (0.16 mmol) in 16 mL of CH_2Cl_2 (0.01 M) was added to the solution. The resulting mixture was heated at reflux until all starting material consumed. At the end of the reaction, solution was cooled down to room temperature and concentrated under vacuum. Purification of crude product by column chromatography on silica gel (EtOAc:Hexane, 1:5 → 1:3) furnished the corresponding 5,6-dihydro-2*H*-pyran-2-one.

4.1.5.1. 6-(1,2-Dihydroacenaphthylen-5-yl)-5,6-dihydro-2H-pyran-2-one (10). Purification of crude product by column chromatography yielded 109 mg of 6-(1,2-dihydroacenaphthylen-5-yl)-5,6-dihy dro-2*H*-pyran-2-one as white solid with 80%. Rf: 0.33 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71–7.65 (m, 1H, naphthalene C8-*H*), 7.63–7.57 (m, 1H, naphthalene C2-*H*), 7.52–7.45 (m, 1H, naphthalene C6-*H*), 7.36–7.27 (m, 2H, naphthalene C3-*H* and C7-*H*), 7.07–6.99 (m, 1H, —COCH═C*H*—), 6.24–6.17 (m, 1H, benzylic *H*), 6.12–6.04 (m, 1H, —COC*H*═CH—), 3.41 (s, 4H, —CH_2CH_2—), 2.93–2.67 (m, 2H, —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.62, 147.36, 146.96, 145.46, 139.60, 129.86, 128.49, 126.04, 121.79, 119.70, 118.99, 118.98, 118.61, 77.33, 31.26, 30.65, 30.15; HRMS: $C_{17}H_{14}O_2Na$ found as [M+Na]$^+$: 273.0891 (Calculated for [M+Na]$^+$: 273.0891).

4.1.5.2. 6-(4-(Dimethylamino)naphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (11). Purification of crude product by column chromatography yielded 65 mg of 6-(4-(dimethylamino)naphthalen-1 -yl)-5,6-dihydro-2*H*-pyran-2-one as yellow jelly compound with 45% yield (overall yield from alcohol). Rf: 0.31 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34–8.24 (m, 1H, naphthalene C5-*H*), 7.99–7.89 (m, 1H, naphthalene C8-*H*), 7.62–7.45 (m, 3H, naphthalene C3-*H*, C6-*H* and C7-*H*), 7.10–6.97 (m, 2H, naphthalene C5-*H* and —COCH═C*H*—), 6.24–6.08 (m, 2H, benzylic *H* and —COC*H*═CH—), 2.92–2.71 (m, 8H, —N$(CH_3)_2$ and —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.66, 151.96, 145.49, 131.58, 129.11, 128.09, 126.57, 125.40, 125.16, 124.63, 123.14, 121.83, 113.39, 77.07, 45.31, 31.09; HRMS: $C_{17}H_{17}NO_2Na$ found as [M+Na]$^+$: 290.1158 (Calculated for [M+Na]$^+$: 290.1157).

4.1.5.3. 6-(4-Methoxynaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (12). Purification of crude product by column chromatography yielded 272 mg of 6-(4-methoxynaphthalen-1-yl)-5,6-dihydro-2*H*-pyran-2-one as white solid with 86% yield. Rf: 0.29 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.37–8.32 (m, 1H, naphthalene C5-*H*), 7.94 (d, *J* = 8.4 Hz, 1H, naphthalene C8-*H*), 7.61–7.45 (m, 3H, naphthalene C2-*H*, C6-*H* and C7-*H*), 7.07–6.98 (m, 1H, —COC*H*═CH—), 6.81 (d, *J* = 8.1 Hz, 1H, naphthalene C3-*H*), 6.22–6.17 (m, 1H, benzylic *H*), 6.10 (dd, *J* = 11.8, 4.2 Hz, 1H, —COC*H*═CH—), 4.01 (s, 3H, —OCH_3), 2.90–2.79 (m, 1H, —C*H*H—), 2.78–2.69 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.64, 156.11, 145.50, 131.28, 127.22, 125.98, 125.86, 125.30, 124.87, 123.09, 122.59, 121.76, 103.14, 77.04, 55.71, 31.08; HRMS: $C_{16}H_{14}O_3Na$ found as [M+Na]$^+$: 277.0832 (Calculated for [M+Na]$^+$: 277.0841).

4.1.5.4. 6-(2-Methoxynaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (13). Purification of crude product by column chromatography yielded 290 mg of 6-(2-methoxynaphthalen-1-yl)-5,6-dihydro-2*H*-pyran-2-one as white solid with 81% yield. Rf: 0.26 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (d, *J* = 8.7 Hz, 1H, naphthalene C8-*H*), 7.85 (d, *J* = 9.1 Hz, 1H, naphthalene C4-*H*), 7.80 (d, *J* = 8.1 Hz, 1H, naphthalene C5-*H*), 7.50–7.43 (m, 1H, naphthalene C6-*H*), 7.38–7.32 (m, 1H, naphthalene C7-*H*), 7.26 (d, *J* = 9.1 Hz, 1H, naphthalene C3-*H*), 7.05 (ddd, *J* = 9.9, 6.2, 2.0 Hz, 1H, —COCH═C*H*—), 6.54 (dd, *J* = 13.2, 4.3 Hz, 1H, benzylic *H*), 6.20 (dd, *J* = 9.8, 2.8 Hz, 1H, —COC*H*═CH—), 3.94 (s, 3H, —OCH_3), 3.31–3.20 (m, 1H, —C*H*H—), 2.49–2.40 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.94, 154.92, 146.32, 132.15, 131.35, 129.75, 129.01, 126.88, 124.47, 123.75, 121.48, 118.04, 113.18, 73.62, 56.85, 29.53; HRMS: $C_{16}H_{14}O_3Na$ found as [M+Na]$^+$: 277.0845 (Calculated for [M+Na]$^+$: 277.0841).

4.1.5.5. 6-(2,3-Dimethoxynaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (14). Purification of crude product by column chromatography yielded 187 mg of 6-(2,3-dimethoxynaphthalen-1-yl)-5,6-dihydro-2*H*-pyran-2-one as white solid with 82% yield. Rf: 0.28 (1:2, EtOAc: Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.2 Hz, 1H, naphthalene C8-*H*), 7.73 (d, *J* = 7.5 Hz, 1H, naphthalene C5-*H*), 7.43–7.33 (m, 2H, naphthalene C6-*H* and C7-*H*), 7.20 (s, 1H, naphthalene C4-*H*), 7.06 (ddd, *J* = 9.8, 6.2, 1.9 Hz, 1H, —COCH═C*H*—), 6.43 (dd, *J* = 13.2, 4.3 Hz, 1H, —COC*H*═CH—), 6.22 (dd, *J* = 9.9, 2.8 Hz, 1H, benzylic *H*), 4.00 (s, 3H, —OCH_3), 3.94 (s, 3H, —OCH_3), 3.34–3.23 (m, 1H, —C*H*H—), 2.51–2.41 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.61, 151.53, 147.94, 146.17, 131.90, 127.72, 126.57, 125.46, 124.93, 124.61, 124.50, 121.43, 108.80, 74.23, 61.85, 55.80, 29.92; HRMS: $C_{17}H_{17}O_4$ found as [M+H]$^+$: 285.1128 (Calculated for [M+H]$^+$: 285.1127).

4.1.5.6. 6-(4,7-Dimethoxynaphthalen-1-yl)-5,6-dihydro-2H-pyran-2-one (15). Purification of crude product by column chromatography yielded 38 mg of 6-(4,7-dimethoxynaphthalen-1-yl)-5,6-dihydro-2*H*-pyran-2-one as white solid with 97% yield. Rf: 0.22 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 9.2 Hz, 1H, naphthalene C5-*H*), 7.53 (d, *J* = 8.1 Hz, 1H, naphthalene C2-*H*), 7.22 (d, *J* = 2.4 Hz, 1H, naphthalene C8-*H*), 7.16 (dd, *J* = 9.2, 2.5 Hz, 1H, naphthalene C6-*H*), 7.04 (ddd, *J* = 9.7, 6.0, 2.4 Hz, 1H, —COC*H*═CH—), 6.69 (d, *J* = 8.1 Hz, 1H, naphthalene C3-*H*), 6.23–6.16 (m, 1H, benzylic *H*), 6.02 (dd, *J* = 11.9, 3.9 Hz, 1H, —COC*H*═CH—), 3.99 (s, 3H, —OCH_3), 3.92 (s, 3H, —OCH_3), 2.94–2.82 (m, 1H, —C*H*H—), 2.78–2.67 (m, 1H, —CH*H*—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.65, 158.68, 156.36, 145.54, 132.91, 125.82, 124.86, 124.64, 121.80, 121.12, 116.77, 102.54, 101.36, 77.23, 55.66, 55.50, 30.64; HRMS: $C_{17}H_{16}O_4$ found as 284.1061 (Calculated: 284.0849).

4.1.5.7. 6-(6-Methoxynaphthalen-2-yl)-5,6-dihydro-2H-pyran-2-one (16). Purification of crude product by column chromatography yielded 332 mg of 6-(6-methoxynaphthalen-2-yl)-5,6-dihydro-2*H*-pyran-2-one as white solid with 85% yield. Rf: 0.25 (1:2, EtOAc:Hexane); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82–7.70 (m, 3H, naphthalene), 7.46 (dd, *J* = 8.5, 1.8 Hz, 1H, naphthalene), 7.20–7.10 (m, 2H, naphthalene), 6.98 (ddd, *J* = 9.8, 5.8, 2.6 Hz, 1H, —COC*H*═CH—), 6.19–6.13 (m, 1H, benzylic *H*), 5.58 (dd, *J* = 11.4, 4.5 Hz, 1H, —COC*H*═CH—), 3.92 (s, 3H, —OCH_3), 2.79–2.60 (m, 2H, —CH_2—); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.28, 158.30, 145.03, 134.67, 133.64, 129.71, 128.64, 127.51, 125.28, 124.32, 121.87, 119.49, 105.87, 79.55, 55.47, 31.79; HRMS: $C_{16}H_{14}O_3Na$ found as [M+Na]$^+$: 277.0849 (Calculated for [M+Na]$^+$: 277.0841).

4.2. Biological activity part

4.2.1. MTT cell viability assay

To investigate the anti-proliferative activity of the compounds, 95 μL of cell suspension was inoculated into 96-well microculture plates (GreinerBio-One) at 1x10^4 cells density per well in culture media containing FBS, penicillin/streptomycin. Compounds were dissolved in dimethyl sulfoxide (DMSO), filter sterilized, diluted at the appropriate concentrations with the culture medium. In all well, 1% DMSO concentration was fixed. Dilutions of compounds were freshly prepared before each experiments. After 24 h cultivation for cell attachment, compounds were added at the final concentration between 50 to 0.01 μM for triplicate assay. Cells were incubated with the compounds for 48 h and anti-proliferative effects were determined by tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) based colorimetric assay. Briefly; 4 h before the end of incubation period, medium of the cells was removed and wells were washed by pre-warmed phosphate-buffered saline (PBS) to remove any trace of compounds and to prevent color interference during optical density (OD) determination. MTT (Sigma) stock solution (5 mg/mL PBS) was diluted at 1:10 ratio into complete culture media, 100 μL of MTT dilution was added into each well and incubated. After 3.5 h plates were centrifuged at 1800 rpm for 10 min at room temperatures to avoid accidental removal of formazan crystals. Crystals were dissolved with 100 μL DMSO. The absorbance was determined at 540 nm. Results were represented as a percentage viability and IC50 values are calculated by using GraphPad Prism 5 software.

4.2.2. Topo I mediated supercoiled DNA relaxation assay

Reaction mixtures were prepared according to this order: Distilled water; (needed to bring volume up to 20 μL final), 10X Assay Buffer (350 mM Tris-HCl, (pH 8.0), 720 mM KCl, 50 mM $MgCl_2$, 50 mM DTT, 50 mM spermidine), 0.1% bovine serum albumin, supercoiled DNA (pHOT1, 250 ng) topoisomerase I enzyme (1U, Topogen) and tested compounds in 2 μL DMSO.

The samples were incubated for 30 min at 37 °C. 5 μL of loading dye was added to stop the reaction. A 1% agarose gel was prepared using 1X TAE buffer and samples were loaded after 1% agarose gel has been solidified. (50X TAE buffer: 242 g Tris base, 57.1 mL glacial acetic acid, 100 mL 0.5 M EDTA). Gel was run from 1 to 2.5 V/cm until dye front reaches the end of the gel. It was stained with 0.5 μg/mL ethidium bromide (15 min room temperature) and destained with distilled water for 15 min room temperature. Gels were monitored with UV transluminator.

4.2.3. Time dependent Topo I inhibition assay

In order to determine the effects of compounds on Topo I enzyme, Topoisomerase I Drug Screening kit (Topogen) assay was performed. The experiment was designed to indicate time dependent Topo I inhibition. In a centrifuge tube, reaction mixture was prepared as followed; final volumes were completed to 20 μL with dH_2O, 2 μL TGS buffer (10X TGS; TGS Buffer (1X) is 10 mM Tris-HCl pH 7.9, 1 mM EDTA, 0.15 M NaCl, 0.1% BSA, 0.1 mM Spermidine, 5% glycerol), 2U of Topo I enzyme (Topogen) and 2 μL of 1 mM compound **8** in DMSO. Then Topo I enzymes were pre-incubated with 4′-methylklavuzon at 37 °C for different times (0, 1, 5 and 10 min). Afterwards, 1 μL supercoiled DNA (pHOT1 concentration of 0.25 μg/mL; 25 μg pHOT1 DNA in 100 μL TE buffer, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA) added to tubes and then tubes were incubated at 37 °C for 30 min. After incubation reactions were terminated by adding 10% sodium dodecyl sulfate (SDS), final concentrations of SDS were 1%, 2 μL of loading dye was added. Samples were loaded to 1% agarose gel and run 1–2.5 V/cm until dye reached end of gel. Gel was stained with ethidium bromide 0.5 μg/mL (EtBr) hold on shaker for 30 min and destained with dH_2O for 15 min on shaker. Gels were monitored with UV transluminator.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2017.02.012.

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