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Molecular docking studies of the interaction between propargylic enol ethers and human DNA topoisomerase IIα

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ABSTRACT

Having identified a novel human DNA topoisomerase IIα (TOP2) catalytic inhibitor from a small and structure-focused library of propargylic enol ethers, we decided to analyze if the chirality of these compounds plays a determinant role in their antiproliferative activity. In this study, we describe for the first time the synthesis of the corresponding enantiomers and the biological evaluation against a panel of representative human solid tumor cell lines. Experimental results show that chirality does not influence the reported antiproliferative activity of these compounds. Docking studies of corresponding enantiomers against TOP2 reinforce the finding that the biological effect is not chiral-dependent and that these family of compounds seem to act as TOP2 catalytic inhibitors.

In the field of drug discovery, the identification of the cellular target of any given molecule is a critical factor. Small molecules typically exert their bioactive effects through interactions with biological targets, which are keys to understanding their mode of action. To date there is no universal systematic process to discover the cellular target or mechanism of action for any given compound. Within this context, we proposed a modular and rational approach, based on phenotypic changes induced in cells by bioactive compounds, which may enable researchers to ascertain the possible biological targets for a given compound in an anticancer screen.[1] As a result of this so-called Phenotypic Drug Discovery approach,[2] we have identified compound **1** (Fig. 1) as a novel human DNA topoisomerase IIα (TOP2) catalytic inhibitor (CI) from a small and structure-focused library of propargylic enol ethers.[3]

Compound **1** is obtained from the reaction of commercially available pentanal and methyl propiolate in a process catalyzed by triethylamine.[4] One consequence of the mechanism of the reaction is that inhibitor **1**, possessing a chiral carbon atom, is obtained as a racemic mixture. Biological systems are chiral entities (e.g., enzymes, receptors, transporters, and DNA) and single enantiomers may be required for effect. The tragic example of thalidomide marked a turning point in drug development and revealed that the physiochemical and biochemical properties of racemic mixtures and individual stereoisomers can differ significantly.[5] Therefore, we planned to evaluate both enantiomers independently in order to determine the effect of chirality on the antiproliferative activity against a panel of representative human solid tumor cell lines. In addition, docking studies were performed to shed light on the binding mode of compound **1** to TOP2.

Due to the shortage of commercially available chiral propargylic alcohols, both enantiomers of inhibitor **1** could not be obtained in a straightforward manner. Fortunately, both enantiomers of 1-octyn-3-ol (**2**) are commercially available. As will be described, compound **2** is the synthetic precursor for compound **3**, an analog of **1** that has one more carbon atom in the alkyl side chain, equally valid for our studies since it displays a good antiproliferative activity in human solid tumor cell lines.[2] In fact, the structure–activity relationship (SAR) study pointed out the important role of both methyl ester groups, while the aliphatic side chain serves as modulator of the biological activity. Therefore, we directed our efforts to the synthesis of (*R*)-**3** and (*S*)-**3**, which is shown in Scheme 1. The enantiomerically pure 1-octyn-3-ols (**2**) were reacted, through oxygen in a hetero-Michael addition reaction, with methyl propiolate to afford the corresponding vinyl ethers. The resulting vinyl ethers were subsequently activated on the terminal alkyne with *n*-BuLi in order to add to methyl chloroformate, affording (*R*)-**3** and (*S*)-**3** in 50–60% overall yield.[6]

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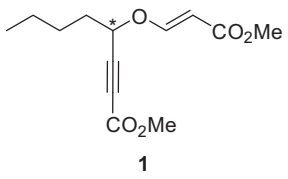

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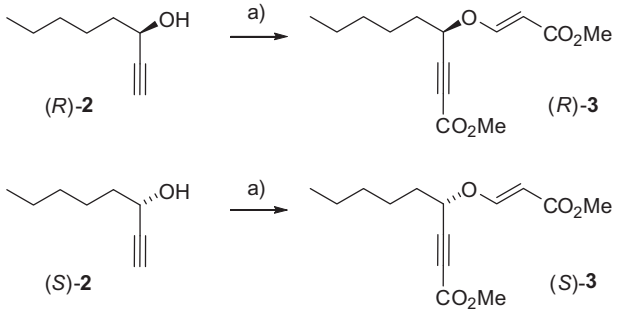
Figure 1. Chemical structure of TOP2 CI inhibitor (*RS*)-**1**.



Scheme 1. Reagents and conditions: (a) (i) methyl propiolate, Et_3N, CH_2Cl_2, rt; (ii) *n*-BuLi, THF, −78 °C; (iii) methyl chloroformate.

Table 1
Antiproliferative activity (GI_{50}) against human solid tumor cells of compounds produced via Scheme 1[a]

Cell line (type)	(*R*)-3	(*S*)-3	(*RS*)-3	(*RS*)-1
HBL-100 (breast)	3.3 (±0.5)	2.3 (±0.1)	2.7 (±0.8)	0.47 (±0.25)
HeLa (cervix)	2.6 (±0.1)	2.9 (±0.3)	17 (±1.2)	3.4 (±0.7)
SW1573 (lung)	5.5 (±0.5)	8.4 (±2.2)	1.3 (±0.1)	0.42 (±0.16)
WiDr (colon)	26 (±1.9)	24 (±0.4)	13 (±3.5)	3.6 (±0.6)

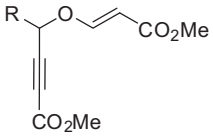
[a] Values are given in μM and are means of two to five experiments; standard deviation is given in parentheses.

Once in hand, both enantiomers were evaluated for their antiproliferative activity. Compounds (*R*)-**3** and (*S*)-**3** were tested against the panel of representative human solid tumor cells HBL-100, HeLa, SW1573, and WiDr using the well-established NCI protocol.[7] The results expressed as GI_{50} were obtained after 48 h of exposure to the compounds and are shown in Table 1. The biological testing demonstrated that both enantiomers had similar activities and the results were comparable with those previously obtained for the racemate (*RS*)-**3**.[2] At this point, we encountered that the biological activity was not affected by the stereochemistry of the chiral center.

In order to gain a better understanding on how propargylic enol ethers might bind to the catalytic site of TOP2, we carried out docking experiments[8] of a series of derivatives into the binding pocket of the α subunit of the previously reported crystal structure of the human TOP2 with bound ADP (PDB ID: 1ZXN).[9] In addition to (*R*)-**1** and (*S*)-**1**, we analyzed the binding to TOP2 of both enantiomers of a series of eleven propargylic enol ethers that differ in the aliphatic side chain, including (*R*)-**3** and (*S*)-**3**. The results are shown in Table 2.

In all cases under study, the calculated docking interaction energy is comparable for both enantiomers and they have very similar docked conformations as shown in Figure 2 for (*R*)-**1** and (*S*)-**1**. Analysis of docked results shows that both enantiomers have almost identical poses inside the protein and establish H-bonds with TOP2 residues Arg-162, Gly-164, Tyr-165, Gly-166 and Phe-373. Additionally, hydrophobic non-bonded interactions are formed with TOP2 residues Asp-86, Glu-87, Val-90, Gly-160, Gly-161, Asn-163, Ile-317, Pro-371, Thr-372 and Lys-378. These docking results further reinforce the previous observation that the biological action is not affected by the stereochemistry of the chiral center. Furthermore, a competitive inhibition mechanism can be proposed for these compounds as they could effectively displace ADP from TOP2 active site (Fig. 2). When considering the alkyl side chain, the docking results show that the best interaction is obtained for the cyclohexyl derivative (entry 12). This is an interesting result, since the SAR study indicated that branched analogs were less favored when compared to linear ones.[2] When comparing GI_{50} values of the racemates, the cyclohexyl derivative was 10 times less active than lead **1**. We cannot discard that other factors might influence the biological activity of this family of propargylic enol ethers and explain the results obtained.

Table 2
Docking results of propargyl enol ethers against TOP2



Entry	R	Docking interaction energy (kcal mol^{-1})	
		S Enantiomer	*R* Enantiomer
1	Me	−6.3	−6.2
2	Et	−6.2	−6.2
3	*n*Pr	−5.9	−6.3
4	*c*Pr	−6.3	−6.5
5	*i*Pr	−6.4	−6.5
6	*n*Bu	−6.7	−6.6
7	*i*Bu	−6.3	−6.6
8	*s*Bu	−6.7	−6.6
9	*t*Bu	−6.6	−6.8
10	*n*Pent	−6.4	−6.3
11	*n*Hex	−6.3	−6.4
12	*c*Hex	−7.1	−7.2

In conclusion, the concise enantioselective synthesis of both enantiomers of compound **3** starting from the appropriate 1-octyn-3-ol was accomplished by standard procedures. Both enantiomers, previously postulated to be human TOP2 catalytic inhibitors, showed almost identical antiproliferative effects against four diverse human solid tumor cell lines. Docking studies of the enantiomers from a set of compounds from this series, against TOP2, reinforce the conclusion that their antiproliferative activity is not chiral-dependent and suggest that they act as TOP2 CIs. Further modification toward quantitative structure–activity relationship studies, which will be followed by structure-based calculations, is currently underway to better understand structural features needed for increasing their antiproliferative activities.

Acknowledgments

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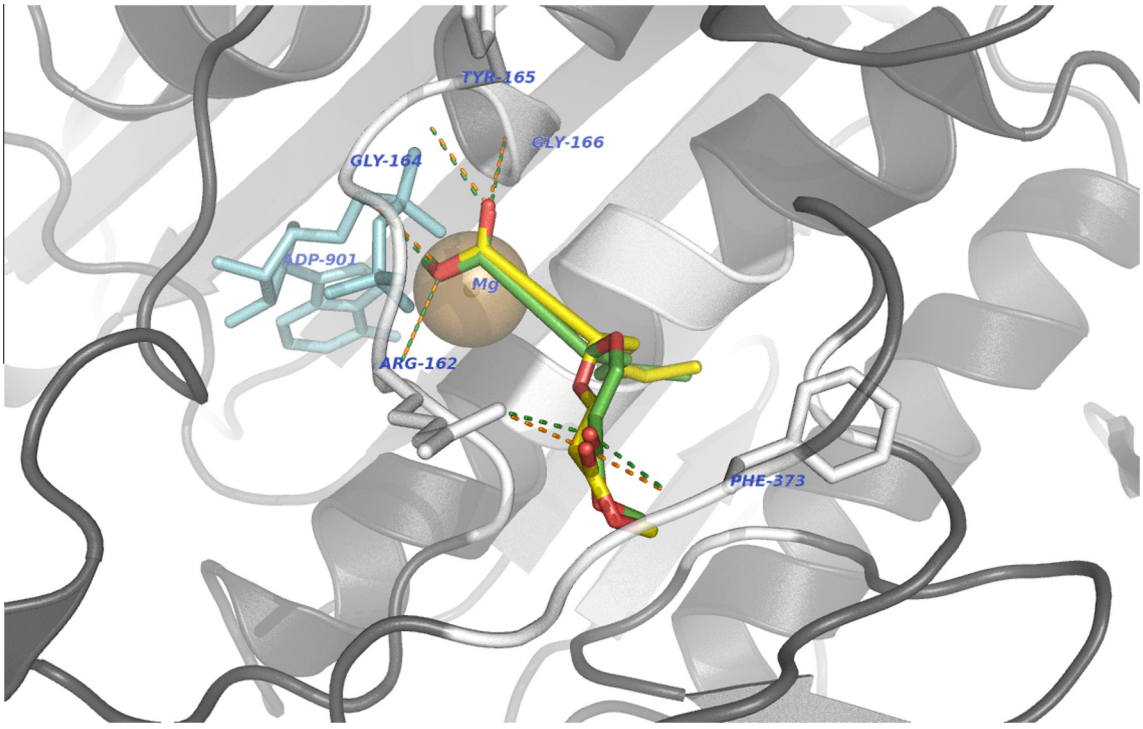

Figure 2. Binding modes of compounds (*R*)-**1** (green) and (*S*)-**1** (yellow) into the catalytic site of TOP2 represented as a ribbon model (PDB ID: 1ZXN).[6] Only amino acids that establish H-bonds (dashed lines) with inhibitors are labeled. An overlay of (*R*)-**1** (docked pose) and (*S*)-**1** (docked pose) with ADP (cyan, X-ray crystal pose) is shown in the catalytic site of TOP2 represented as cartoon.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.07.055.

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6. Synthesis of *(E)-methyl 4-((3-methoxy-3-oxoprop-1-en-1-yl)oxy)non-2-ynoate (**3**)*. Propargylic alcohol **2** (0.1 g, 0.8 mmol) and methyl propiolate (0.1 g, 1.2 mmol) were dissolved in CH_2Cl_2 (5 mL). Then, the mixture was cooled to 0 °C and triethylamine (0.02 mL, 0.16 mmol) was added. The reaction mixture was allowed to stir for 45 min, after which time the solvent and excess reagents were removed under reduced pressure. The crude product was used in the next step without further purification as follows. The crude was dissolved in dry THF (5 mL) under inert atmosphere and cooled down to −78 °C. Then, *n*-butyl lithium (2.5 M in hexanes, 0.12 mL, 1.2 mmol) was added and the reaction mixture was stirred for 30 min at −78 °C. Methyl chloroformate (0.1 mL, 1.2 mmol) was added and the reaction was allowed to warm and was stirred for 3 h at room temperature. The reaction was quenched with saturated NH_4Cl (2 mL) and extracted with ethyl acetate (3 × 5 mL). The combined organic phases were dried with $MgSO_4$, filtered, concentrated and the crude was purified by chromatographic column to give the final product in 50–60% yield. Colorless oil; (*R*)-**3**: $[\alpha]_D^{25}$ +154 (*c* 0.97, $CHCl_3$); (*S*)-**3**: $[\alpha]_D^{25}$ −159 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.87 (t, 3H, *J* = 7.0 Hz), 1.25–1.32 (m, 4H), 1.41–1.49 (m, 2H), 1.79–1.93 (m, 2H), 3.68 (s, 3H), 3.76 (s, 3H), 4.61 (t, 1H, *J* = 6.6 Hz), 5.35 (d, 1H, *J* = 12.5 Hz), 7.52 (d, 1H, *J* = 12.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8, 22.3, 24.4, 31.1, 34.5, 51.2, 52.9, 70.5, 78.5, 83.0, 99.1, 153.1, 159.8, 167.6; IR ($CHCl_3$, cm^{-1}) 2955.4, 2865.4, 2241.8, 1716.9, 1646.4, 1437.1, 1257.3, 1187.9; MS, *m*/*z* (relative intensities) 268 (M^+, 2.1), 167 (100), 135 (44), 107 (97), 79 (82), 59 (38). HRMS (ESI): Calcd for $C_{14}H_{20}O_5$ $[M+Na]^+$ 291.1203; found 291.1204.
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8. *Molecular modelling and docking simulations:* All calculations were performed in commodity PCs running Windows 7 or Linux Ubuntu 11.10 operating systems. Open Babel[9] was used to manipulate the various file formats of ligands. PyMol from DeLano[10] was used for visual inspection of results and graphical representations. All the tested compounds were drawn and minimized using AM1 semi-empirical method, with a gradient energy minimization method until the energy change between steps was lower than 0.01 kcal mol^{-1} using Hyperchem 8.0 (Hypercube, Inc., Florida, USA). The algorithm used was the Polak-Ribiere (conjugate gradient). The protein structure was downloaded from the Protein Data Bank and for the purpose of the docking calculations we removed the ADP, water molecules and Mg ion present in the crystal structure. We added polar hydrogen atoms to the protein chain before running any docking calculations. AutoDock Vina[11] was used for docking calculations. Docking was performed with the prepared ligand files against the TOP2 crystal structure with grid box dimensions of 30 × 30 × 30 Å. The docking accuracy, meaning the AutoDock Vina parameter 'exhaustiveness', was set to 100 and all the output files were visually inspected to check the correctness of obtained docked conformations.
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