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Discovery of New DNA Topoisomerase II Inhibitors using Structure Based Virtual Screening Method

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Abstract: DNA topoisomerases are proved therapeutic targets of antibacterial and anticancer drugs. Structures of topoisomerase–DNA and inhibitor ternary complexes have revealed the exact binding sites and mechanisms of topoisomerase poisons. There are two isoforms of Human Topoisomerase II; α and β. Both of them perform similar functions and their levels differ depending on the replicative activity and type of tissue. Topo IIα is preferentially expressed in proliferating cells. Thus, selective Topo IIα inhibitors have been of particular interest in cancer therapy, as they may represent a more targeted approach to highly proliferative cells. In this study, we use structure-based virtual screening method with molecules which are commercially available in the ZINC database. Docking studies were performed by Glide module available in Schrödinger software, to obtain an efficient collection of hit molecules ligand filtration was also done by employing Lipinski's "rule of five" and pharmacokinetic properties were tested using Qikprop module. From approximately ten thousand compounds from Zinc database we selected 4 top chemical structures with suitable ADME/Tox properties and good inhibiting profile for topo II. Thus compounds 1-4 could be the promising inhibitors of human topo IIα enzyme.

Keywords: anticancer activity, docking, topoisomerase, virtual screening.

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INTRODUCTION

Topoisomerases are ubiquitous enzymes in key cellular processes such as DNA replication, transcription, recombination, and repair processes such as supercoiled, relaxed, catenated, and knotted DNA (1). All type of topoisomerases shows their biochemical functions by catalyzing DNA cleavage and relegation (2). DNA topoisomerases are proved therapeutic targets of antibacterial and anticancer drugs. Structures of topoisomerase–DNA and inhibitor ternary complexes have revealed the exact binding sites and mechanisms of topoisomerase poisons. α and β Human Topoisomerase II are two available isoforms. Both of them perform similar functions and their levels differ depending on the replicative activity and type of tissue (3-6). Human topo IIα and β sharing a similar tertiary structure and primary sequence but they distribute in various cells and tissues. They also show various cellular functions, topo IIα overexpressed in proliferating cells and generally located in the nuclear plasma. Topoisomerase IIβ plays apparent roles in transcriptional regulation, cell development, and differentiation, but not essential for cell proliferation and survival. Although human topo IIα relaxes negatively supercoiled plasmid slower than positively supercoiled plasmids, but topo IIβ is not. Because of all these reasons, topo IIα seems to be the more attractive target for new anticancer drugs. Thus selective Topo IIα inhibitors have been of particular interest in

cancer therapy, as they may represent a more targeted approach to highly proliferative cells (7-10).

Recently, computational protein–ligand docking process has been used to predict the affinities and optimal binding modes of the compounds with the target proteins. Ligand docking method enables the visualization of an optimal complex that can be predicted from a target protein structure and a candidate drug compound. Structure-based virtual screening method focus on the therapeutic targets three-dimensional (3D) information for docking method. In order to select the hits that exhibit chemical, structural and electronic characteristics, docking procedures are used. The information of the target protein can be obtained from *in silico* technique or experimental data. In order to exploring lead compounds for target proteins all these advantages have encouraged the usage of computational methods in drug discovery (11-13).

Human topo IIα has a homodimer structure and its monomer is composed of 1531 amino acids including four sections DNA-gate, N-gate, C-gate, and CTD (14). The X-ray crystallographic structure of this enzyme (PDB: 5GWK) is available in Protein Data Bank (www.rcsb.org) and we used this structure for *in silico* studies (15). In this work, we use structure-based drug design method, in order to predict the binding modes and calculate the ADME/Tox properties to propose new anti-cancer candidates which have suitable properties to be promising oral human topo IIα inhibitors.

MATERIAL AND METHODS

Structure-based virtual screening method focus on the therapeutic targets 3D information. In order to select the hits that exhibit chemical, structural and electronic characteristics, docking procedures are used. The information of the target protein can be obtained from *in silico* technique or experimental data (13). Docking calculations were performed using Schrödinger 2018-2, with Maestro 11.5 and the Glide module (16-18).

Protein Preparation

The X-ray crystallographic structure of human topoisomerase II enzyme complex with etoposide (PDB: 5GWK) was obtained from Protein Data Bank (www.rcsb.org) and prepared for docking process. To prepare the enzyme (PDB: 5GWK), we used the protein preparation wizard module. We use OPL5-2005 force field and pH =7.0 to minimize hydrogen atoms. Bond orders were assigned, with zero order bonds to disulfide bonds and metals as well.

Ligand preparation

For virtual screening study, 10,241 commercially available compounds were obtained from ZINC database. All of these ligands were prepared by using Schrödinger, LigPrep module. The bond angles and bond orders were assigned after ligand minimization step. For the minimization OPLS 2005 force field was used. In order to keep the ligands in the right protonation state in biological conditions, epik option was used.

Grid preparation

The active site of the topo II enzyme, was defined for generating the grid in Maestro. The grid box was limited to the size of 20 Å at the active site. Firstly, docking procedure was validated by extracting ligand etoposide from the binding site and re-docking it to the topo II (PDB: 5GWK). Glide had successfully reproduced the experimental binding conformations of etoposide in topo II enzyme with an acceptable root-mean-square deviation (RMSD) value of 0.42Å.

Virtual Screening

Docking studies were carried out using high throughput virtual screening (HTVS) option, SP screening (standard-precision) and XP screening (extra-precision) mode of Glide module, respectively. We considered ring conformations, nitrogen inversions, input partial charges and, for amides, a penalty for nonplanar conformations was applied. Epik state penalties were added to docking scores. We did not use any similarities or constraints for the docking calculations. 10,241 commercially available compounds from ZINC database were screened. The compounds were redocked via postprocessing. The best pose was output on the basis of Glide score. After visual inspection we retained four inhibitor candidates, compounds 1-4 (ZINC000131302839, ZINC000119841605, ZINC000131302897, ZINC000119841475). Docking scores of these compounds were shown in Table 1.

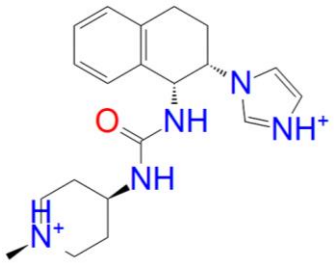
ADME/Tox Analyses

To obtain an efficient *in silico* collection of hit molecules, ligand filtration was done by employing Lipinski's "rule of five" and ADME properties using Qikprop module of Schrödinger (19). Calculated ADME properties of the comp. 1-4 (ZINC000131302839, ZINC000119841605, ZINC000131302897, ZINC000119841475) which have best docking scores, were shown in Table 2. This analysis includes, brain/blood partition coefficient (QPlog BB), aqueous solubility (QPlog S), total solvent accessible surface area (SASA),

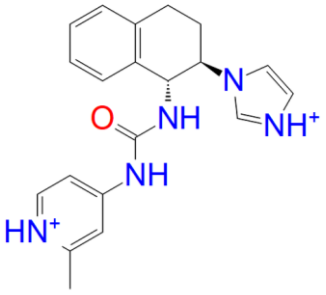
octanol/water partition coefficient (QP log Po/w), predicted apparent MDCK cell permeability (QPMDCK), Lipinski Rule of 5 violations, and human oral absorption.

RESULT AND DISCUSSION

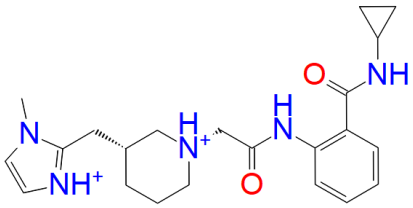
In this study, we use structure based virtual screening method with 10,241 commercially available compounds in the ZINC database. To obtain an efficient collection of hit molecules, docking studies were performed using Glide module available in Schrödinger software, ligand filtration was also done by employing Lipinski "rule of five" using Qikprop module (12, 13). Docking scores of the best topo II inhibitor candidate compounds are shown in Table 1 and QikProp Properties Predictions topo II inhibitor candidate compounds were also shown in Table 2. According to the docking studies, binding energies of the comp. 1-4 (ZINC000131302839, ZINC000119841605, ZINC000131302897, ZINC000119841475) were found −12.692, −12.417, −11.082, −11.058 respectively, and all of these compounds showed better docking score than standard drug etoposide (−10.193). All of the pharmacokinetic properties conducted by Qikprop were within the permissible range. From approximately ten thousand compounds from Zinc database we selected 4 top chemical structures with suitable ADME/Tox properties and good inhibiting profile for human topo IIα. The structures of the compounds 1-4 are shown in Figure 1.



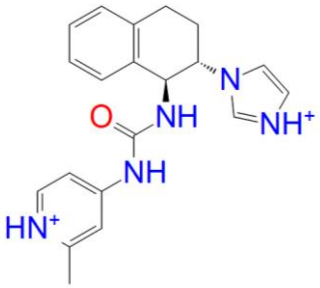
Compound 1 (ZINC000131302839)



Compound 2 (ZINC000119841605)



Compound 3 (ZINC000131302897)



Compound 4 (ZINC000119841475)

Figure 1 Structures of the topo II inhibitor candidate compounds

Table 1. Docking scores of the topo II inhibitor candidate compounds.

Code	Docking Score	Glide Score
Comp. 1 (ZINC000131302839)	-12.692	-12.885
Comp. 2 (ZINC000119841605)	-12.417	-13.093
Comp. 3 (ZINC000131302897)	-11.082	-11.403
Comp. 4 (ZINC000119841475)	-11.058	-11.734
Etoposide	-10.193	-10.193

Table 2. QikProp Properties Predictions topo II inhibitor candidate compounds.

Code	Molecular Weight	Percent Human Oral Absorption	SASA	QPlog BB	QPlog S	QPlog Po/w	QPMDCK	Rule of Five
Comp. 1	353.466	91.03	630.508	0.278	-3.575	2.663	346.748	0
Comp. 2	347.419	100	648.084	-0.372	-5.438	3.457	866.085	0
Comp. 3	395.503	95.33	683.098	-0.168	-3.572	3.457	252.772	0
Comp. 4	347.419	100	649.387	-0.365	-5.475	3.468	886.651	0
Etoposide	588.564	47.524	773.724	-1.481	-3.453	0.606	111.019	2

According to the docking results; comp. 1 (ZINC000131302839) revealed H-bonds with deoxythymidine DT9, and Glu461; pi-pi stacking with deoxyadenosine DA12, deoxyguanosin DG13; salt bridges with deoxycytidine DC8 and Glu 461; comp. 2 (ZINC000119841605) revealed H-bond with Glu461; π-π stacking with deoxythymidine DT9 and deoxyguanosine DG13; salt bridges with deoxythymidine DT9 and Glu 461; comp. 3 (ZINC000131302897) revealed H-bonds with deoxythymidine DT9, and Gly488; π - π stacking with deoxyadenosine DA12, deoxyguanosine DG13; salt bridges with deoxythymidine DT9, π -cation interaction with deoxythymidine DT9, deoxyguanosine DG10 and deoxyadenosine DA12; comp. 4 (ZINC000119841475) revealed H-bonds with deoxythymidine DT9; π - π stacking with deoxythymidine DT9, and deoxyguanosine DG13; salt bridges with deoxythymidine DT9 and Glu 461 and etoposide revealed H-bond with deoxyguanosine DG13, and Asp463; π - π stacking with deoxyguanosine DG13; π - cation interactions with Arg487 (Figure 2). According to the docking studies, it can be concluded that compounds 1-4 showed better docking scores than standard drug etoposide. The binding energies of compounds 1-4 were found −12.692, −12.417, −11.082, −11.058 respectively, and for etoposide it was −10.193. These results showed that, compounds 1-4 have strong interactions with human topo IIα and they could be the promising inhibitors of this enzyme, thus compounds 1-4 were selected for the further studies as human topo IIα candidate drugs.

According to the Qikprop Properties Predictions, the human oral absorption percentage of the selected compounds was in the appropriate excretion range of 91 to 100%. Compound 2, and compound 4 showed 100% oral absorption. For selected lead compounds, the partition coefficient (QP log Po/w) was within the permissible range of 0.6-3.47. SASA and brain/blood partition coefficient (QP log BB) were also found to be within satisfactory range. Violations of Lipinski's rule of five were also calculated (20). For all selected compounds have no violations of the Lipinski's Rule of 5, thus indicating their potential as a drug-like molecule. Additionally, compounds are in the acceptable range for predicted apparent MDCK cell permeability (QPMDCK) and predicted aqueous solubility (QPLog S). Table 1 showed some pharmacokinetic properties calculated for compound 1-4 by Qikprop simulation.

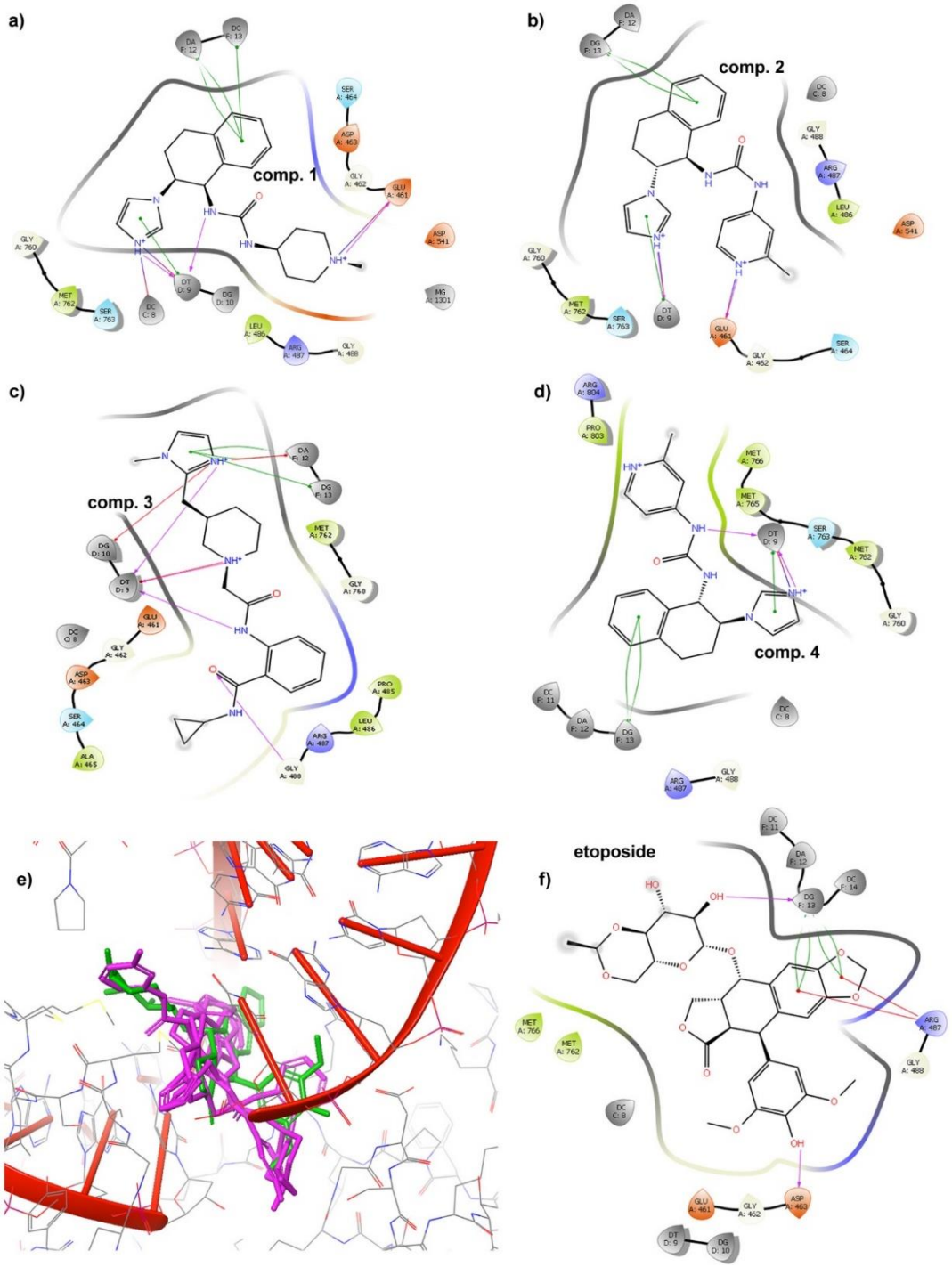


Figure 2 **a)** Docked position of comp.1 (ZINC000131302839): compound revealed H-bonds with deoxythymidine DT9, and Glu461; π- π stacking with deoxyadenosine DA12, deoxyguanosine DG13; salt bridges with deoxycytidine DC8 and Glu 461. **b)** Docked position of compound 2 (ZINC000119841605): compound revealed H-bond with Glu461; π - π stacking with deoxythymidine DT9 and deoxyguanosine DG13; salt bridges with deoxythymidine DT9 and Glu 461. **c)** Docked position of compound 3 (ZINC000131302897): compound revealed H-bonds with deoxythymidine DT9, and Gly488; π - π stacking with deoxyadenosine DA12, deoxyguanosine DG13; salt bridges with deoxythymidine DT9, π -cation interaction with deoxythymidine DT9 , deoxyguanosine DG10 and deoxyadenosine DA12. **d)** Docked position of comp.4 (ZINC000119841475): compound revealed H-bonds with deoxythymidine DT9; π - π stacking with deoxythymidine DT9, and deoxyguanosine DG13; salt bridges with deoxythymidine DT9 and Glu 461. **e)** Structure of topoisomerase IIα (pdb ID:5gwk) complex with etoposide (green) and docking poses of inhibitor candidate compounds (magenta) in active site. **f)** Docked position of etoposide: compound revealed H-bond with deoxyguanosine DG13, and Asp463; π - π stacking with deoxyguanosine DG13; π -cation interactions with Arg487.

CONCLUSION

Virtual screening methods have been an important tool for new hit compound search. In this study, from approximately ten thousand compounds from Zinc database, it was possible to select 4 top chemical structures with good inhibiting profile for topo II. According to the docking studies, it can be concluded that compounds 1-4 (ZINC000131302839, ZINC000119841605, ZINC000131302897, ZINC000119841475) showed better docking score than standard drug etoposide. The binding energies of compounds 1-4 were found −12.692, −12.417, −11.082, −11.058 respectively, and for etoposide it was −10.193. These compounds showed strong interactions with human topo IIα, they bound to the active site residues of the enzyme and DNA. Besides, all of the predicted pharmacokinetic properties conducted by Qikprop were within the permissible range. As a conclusion, we selected 4 top chemical structures with suitable ADME/Tox properties and good inhibiting profile for topo II, thus compounds 1-4 could be the promising inhibitors of topoisomerase IIα enzyme.

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