Research Article

Homology Modeling and Molecular Docking Studies of Novel Quinazolinone and Benzothiazole Derivatives as DNA Topoisomerase II Inhibitors

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ABSTRACT

DNA topoisomerase enzymes are responsible for the relaxation of DNA torsional strain, untangling of DNA duplexes after replication and also important cancer drug targets. DNA topoisomerase activity is very critical to fundamental cellular processes such as replication, recombination, transcription and DNA repair. The present study combines synthesis of quinazolinone, benzothiazole derivatives and structure-based drug design methods which include homology modeling and molecular docking studies. New compounds 3-(1-(benzo[d]thiazol-2-ylimino)ethyl)-6-methyl-2H-pyran-2,4(3H)-dione (3BTIEMPD), 6-methyl-3-(1-(4-oxo-2-phenylquinazolin-3(4H)-ylimino)ethyl)-2H-pyran-2,4(3H)-dione (MOPQIEP) and 3-(2-hydroxy-3-methoxybenzylideneamino)-2-phenylquinazolin-4-(3H)-one (3-HMBDAPQO) are prepared and characterized on the basis of elemental analyses, mass, IR, ^{1}H-NMR, ^{13}C-NMR and UV-Visible spectra. Homology model of DNA topoisomerase II enzyme (Topo II) was predicted in order to carry out structure-based drug design. The accuracy of the predicted model was further validated by using different computational approaches. Molecular docking study on Topo II was undertaken using synthesized quinazolinone and benzothiazole derivatives. The structural prediction and docking study showed best docking results. Thus the present study of computer-aided drug designing method infers possibility of the title compounds to act as novel drugs against activity of Topo II.

Keywords: DNA Topoisomerase II, Homology modeling and Docking Studies.

INTRODUCTION

DNA topoisomerase II enzymes regulate essential cellular processes by altering the topology of chromosomal DNA and are useful for the condensation of chromosomes and their segregation during mitosis. Different types of DNA topoisomerases occur in human cells and several poisons of topoisomerase I and II are widely used clinically.

DNA Topoisomerase II exists in two isoforms (α isozyme and β isozyme) and are present in mammalian cells that are encoded by distinct genes. The topoisomerase IIα and topoisomerase IIβ enzymes are regulated by post-translational modifications, including sumoylation, ubiquitination and phosphorylation.[2] Anticancer drugs that are DNA topoisomerase II inhibitors are cytotoxic in nature because they form complexes with DNA and DNA topoisomerase II.[3] The isozyme belongs to the type IIA family of DNA topoisomerases that share high sequence homology and similar protein domain organization. Since the precise structure of DNA Topoisomerase II is not known, the structure-function relationship between DNA Topoisomerase II and activators/inhibitors is still unknown. Computational methods to predict protein structure and protein ligand interaction have been successfully applied in biochemical research for decades. Therefore, a model of DNA Topoisomerase II was generated using homology modeling and precise molecular docking studies of synthesized 2-phenyl quinazolinone and 2-amino benzothiazole derivatives with DNA Topoisomerase II were studied using Autodock4.2 software.[4] The ligand binding residues were found to be similar to the predicted active site residues. All the four synthesized 2-phenyl quinazolinone and 2-amino benzothiazole derivatives were found to dock in the vicinity of the predicted active sites of DNA Topoisomerase II. Docking studies provide valuable insight into mechanism of substrate and inhibitor binding to the enzyme active site.

The above observations contemplated us to synthesize some new 2-aminobenzothiazole and 2-phenyl quinazolinone derivatives to explore their binding ability towards the DNA topoisomerase II and also potential biologically active agents. The structures of the newly synthesized quinazolinone and benzothiazole compounds were characterized on the basis of various spectro-analytical techniques such as elemental analyses, IR, mass, UV-Visible, ^{1}H-NMR and ^{13}C-NMR. The present study infers that the title compounds could be investigated further as selective topoisomerase II inhibitors and thus, study confirms the applicability of computer-aided drug designing methods for the discovery of novel topoisomerase II inhibitors.

METHODOLOGY

Sequence alignment and Structure prediction

The FASTA sequence of query protein (Q6LXB5) was retrieved from Expasy Uniprot sequence search (www.expasy.org). Following BLASTp run using NCBI protein BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), "DNA TOPOISOMERASE VI A SUBUNIT" (PDB ID: 1D3Y)

was selected as template sequence (http://www.rcsb.org/pdb).

Both the sequences were subjected to pairwise alignment using ClustalX. The 3D-structure of query protein was predicted by automated homology modeling program, Modeller9.15.[5]

The template and query sequences were aligned carefully to remove potential alignment errors. Loop building was performed by using spdb viewer.[6] The final validation of the model was done by Ramachandran plot analysis.

MODELLER 9.15 was then used to gain satisfactory models; an automated approach to comparative modeling by satisfaction of spatial restrains.[7]

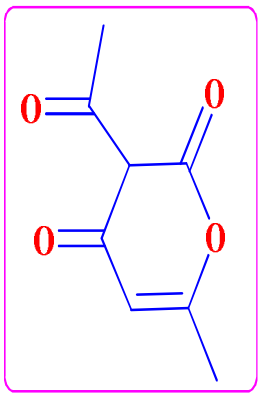
After manually modifying the alignment input file in MODELLER 9.15 to match the template and query sequence, 20 models were generated and were then minimized using the molecular dynamics and simulation procedure CHARMM in MODELLER for each of the primary sequences out of which the models with least modeller objective function were then chosen. The structure and the stereochemistry of the protein was checked by using PROCHECK[8] software, which generates Ramachandran plots with in-depth assessment of Psi/Phi angles and the backbone conformation of the model.

Docking studies

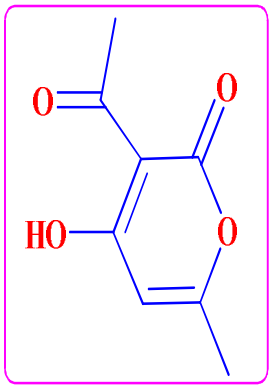
All the synthesized molecules were chosen for molecular docking studies. Molecular docking study was carried out by using Autodock4.2 software, which uses Genetic algorithm (GA). DNA Topoisomerase II protein was loaded into AutoDock Tools (ADT) (http://autodock.scripps.edu/resources) as a receptor and made ready for docking. After loading the protein, hydrogens were added using the edit option in ADT. Initially, the modelled PDB file does not contain any hydrogens. The ligands were docked with DNA Topoisomerase II, a grid for dock search was built for the molecule to find the most probable binding site in DNA Topoisomerase II and to measure its interaction parameters with synthesized molecules. The docking process was carried out in the default parameters of ADT. Active sites has been predicted by submitting the predicted homology model to 3Dligand site, a binding site prediction server (http://www.sbg.bio.ic.ac.uk/3dligandsite/advanced.cgi).[9]

Structures of DHA, 3BTIEMPD, MOPQIEP and 3-HMBDAPQO

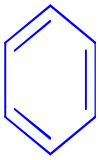
The prepared 3BTIEMPD, MOPQIEP and 3-HMBDAPQO were characterized on the basis of elemental analyses, mass, IR, ^{1}H-NMR, ^{13}C-NMR and UV-Visible spectra. The structures of DHA, 3BTIEMPD, MOPQIEP and 3-HMBDAPQO were shown in below.

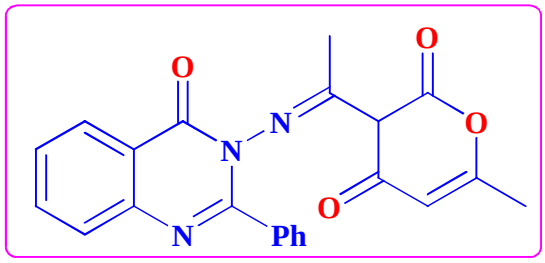


Keto form DHA

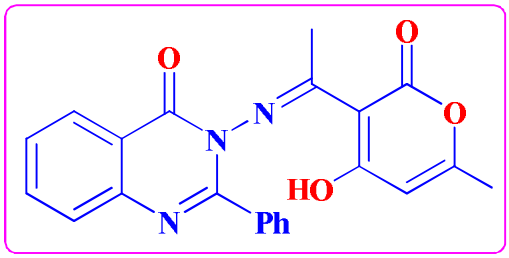


Enol form DHA

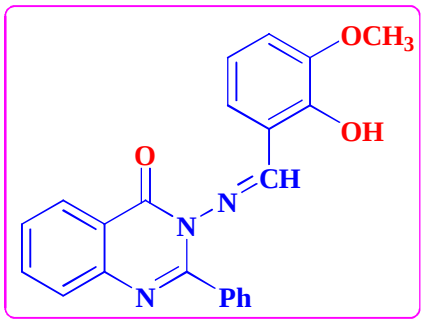




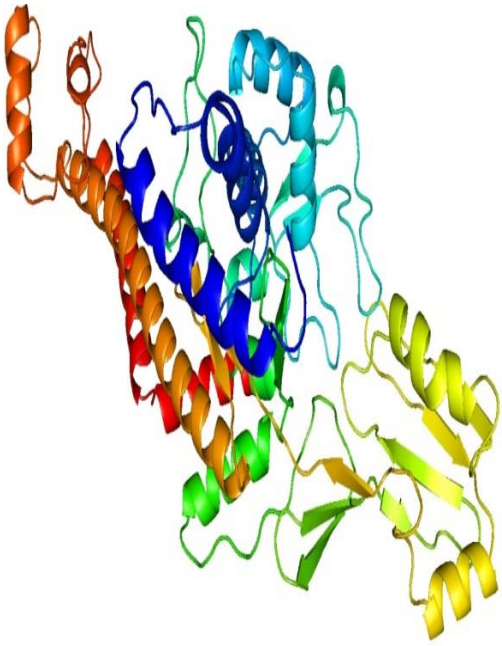
Keto form MOPQIEP



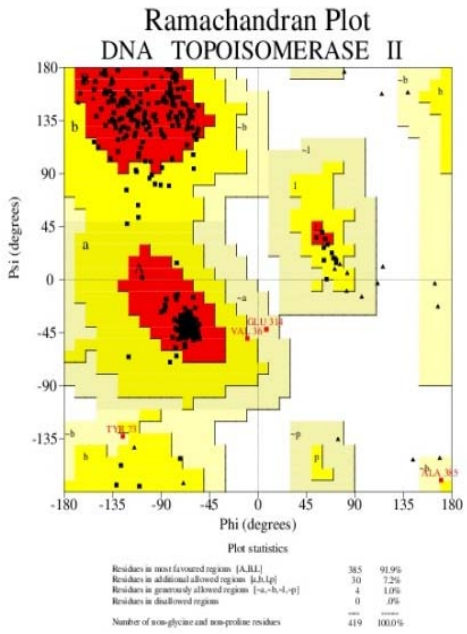
Enol form MOPQIEP



3-HMBDAPQO



Secondary protein structure of DNA Topoisomerase II



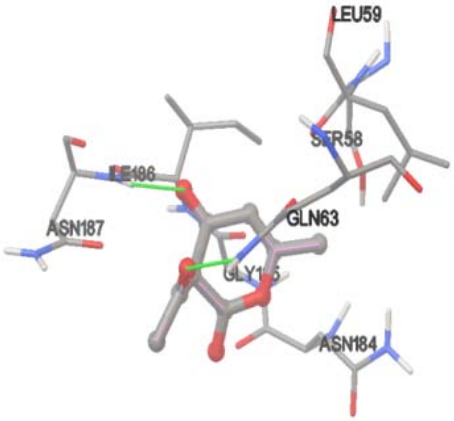
Ramachandran plot of DNA Topoisomerase II

Figure 1: The secondary structure and Ramachandran plot of DNA Topoisomerase II.

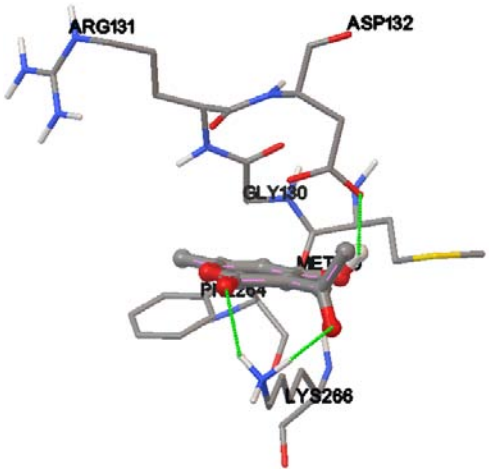
Table 1: Ramachandran plot statistics

	No. of Residues	Percentage (%)
Residues in Most Favoured region	385	91.9%
Residues in additional allowed region	30	7.2%
Residues in generously allowed region	4	1.0%
Disallowed region	0	0%
Number of non-glycine and non-proline residues	419	100.0%

a) Interaction of DHA keto & Enol forms with DNA Topoisomerase II

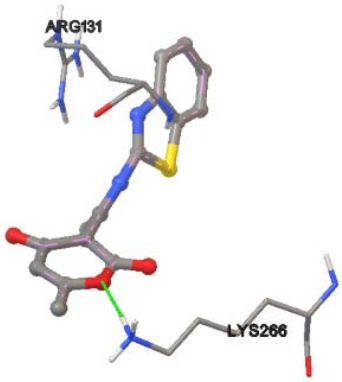


Keto form of DHA

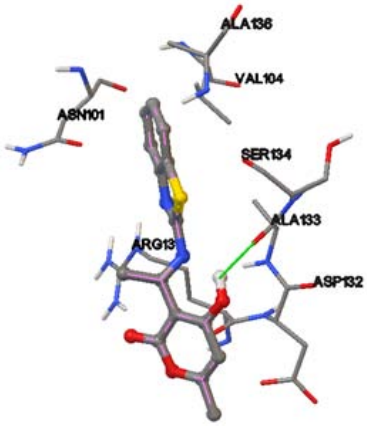


Enol form of DHA

b) Interaction of 3BTIEMPD keto & Enol forms with DNA Topoisomerase II

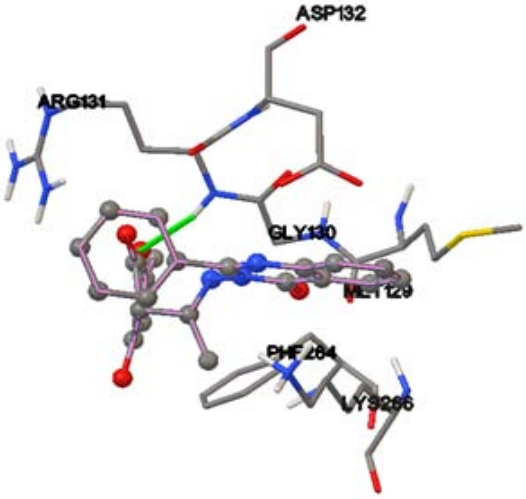


Keto form of 3BTIEMPD

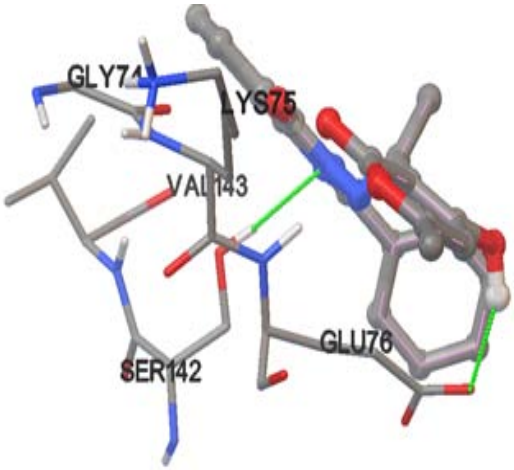


Enol form of 3BTIEMPD

c) Interaction of MOPQIEP Keto & Enol forms with DNA Topoisomerase II

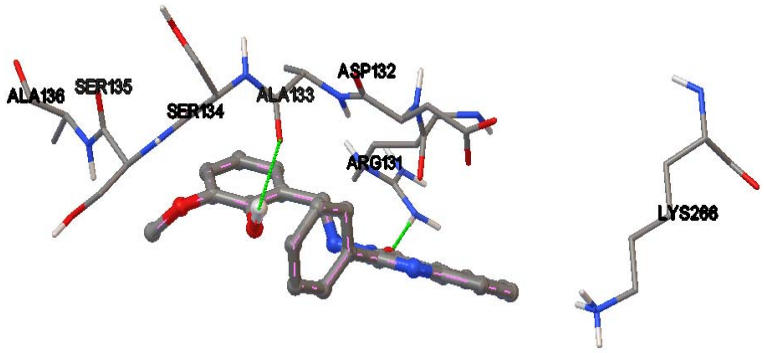


Keto form of MOPQIEP



Enol form MOPQIEP

d) Interaction of 3-HMBDAPQO Molecular form with DNA Topoisomerase II



Molecular form of 3-HMBDAPQO

Figure 3: Interaction of a) DHA, b) 3BTIEMPD, c) MOPQIEP and d) 3-HMBDAPQO with DNA Topoisomerase II

Table 2: Docking interactions of title compounds and their binding energy values

Compound Name	Name of the source protein	Interacting amino acids	Grid X-Y-Z coordinates	Free energy ΔG (Kcal/Mol)
Keto form of DHA	DNA Topoisomerase II	Ile186, Gln63	55.411, 28.471, 21.961	-5.00
Enol form of DHA	DNA Topoisomerase II	ASP132, Lys266	55.411, 28.471, 21.961	-4.50
Enol form MOPQIEP	DNA Topoisomerase II	Glu76, Ser142	55.411, 28.471, 21.961	-4.82
Keto form of MOPQIEP	DNA Topoisomerase II	Arg131	55.411, 28.471, 21.961	-4.89
Keto form of 3BTIEMPD	DNA Topoisomerase II	Lys266	55.411, 28.471, 21.961	-4.90
Enol form of 3BTIEMPD	DNA Topoisomerase II	Ala133	55.411, 28.471, 21.961	-5.77
Molecular form 3-HMBDAPQO	DNA Topoisomerase II	Arg131, Ala133	55.411, 28.471, 21.961	-4.86

RESULTS AND DISCUSSION

Structural evaluation

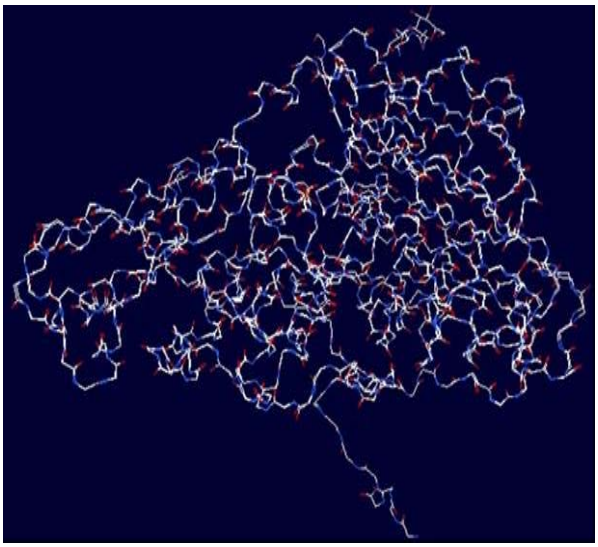


Figure 2: The secondary structure alignment obtained between the query and template sequence in

Present study reports that the protein DNA Topoisomerase II is having high degree of homology with (PDB ID: 1D3Y), was used as a template with identity of amino acid sequence 80%. Atomic resolution of its X-ray crystal structure is 2.0 Å. The PROCHECK software is used for validation of modelled protein; it generates a number of files which list complete residue by residue data and the assessment of the overall quality of the generated structure as compared to well refined structures of the same resolution. The Ramachandran plot of DNA Topoisomerase II generated in the present study shows 385 amino acid residues (91.9%) in most favorable region with 30 amino acid residues (7.2%) falling into additionally allowed regions and with 4 amino acids (1.0%) falling into generously allowed regions and with no amino acids falling in disallowed region. These results clearly indicate that the generated models are much more sophisticated and more conformationally stable. The secondary structure and Ramachandran plot of DNA Topoisomerase II of the predicted model is shown in Fig. 1.

The secondary structure alignment obtained between the query and template sequence is shown in Fig 2. The query (Q6LXB5) is subjected for homology modeling by Modeller 9.15. Root mean square deviation (RMSD) value between the template and predicted model was found to be 0.202 indicative of a very good model. The G-factors indicates the quality of covalent and bond angle distance. The predicted model was subjected to PROCHECK analysis

to determine psi and phi torsion angles, the comparable Ramachandran plot characteristics.

Docking Results

The structural interactions between DNA Topoisomerase II with 4 inhibitors were docked separately. Molecular docking studies were commonly used for predicting binding modes to proteins and their binding energies of ligands. Initially, ligand binding pockets were detected by using online 3DLigandsite. The amino acid residues were present in the binding pocked are Gln124, Met129, Asp132, Ala133, Ser134, and Thr289.

Molecular docking study was performed by using AUTODOCK4.2 which was a suite of automated docking tools and was used to predict the affinity, activity, binding orientation of ligand with the target protein and to analyze best conformations, the protein with DHA, MQPQIEP**,** 3BTIEMPD and 3-HMBDAPQO were loaded individually into ADT and evaluate ten finest conformations. In the present investigation we focused mainly on the binding energy values of the title compounds. DHA, MQPQIEP and 3BTIEMPD molecules which exit in keto and enol form. The interactions of ligand with protein were computed for both forms and the interacting amino acids, binding energy and coordinates are given in table 2. The comparison of free energies corresponding to binding of title compounds with target protein reveals that the keto and enol forms of 3BTIEMPD exhibited lower free energy value indicating more thermodynamically favoured interaction than other compounds under study.

CONCLUSION

The compounds 3-(1-(benzo[d]thiazol-2-ylimino) ethyl)-6-methyl-2H-pyran-2, 4(3H)-dione (3BTIEMPD), 6-methyl-3-(1-(4-oxo-2-phenylquinazolin-3(4H)-ylimino)ethyl)-2Hpyran-2,4(3H)-dione (MOPQIEP) and 3-(2-hydroxy-3-methoxybenzylideneamino)-2-phenylquinazolin-4-(3H)-one (3-HMBDAPQO) were prepared and characterized by using various spectro analytical techniques. The predicted 3D model of DNA topoisomerase II shows 91.9% in the core region and is more stable and reliable. All the molecules showed good interactions with newly modeled DNA topoisomerase II indicating the affirmative outcome for future planning of in *vitro* and *in vivo* studies.

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