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Design and Modeling Studies on Liriodenine derivatives as novel topoisomerase II inhibitors

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Abstract: Natural products have been widely used in traditional medicines and are a valuable source for new drug discovery. On the other hand, extensive molecular modeling based on crystallographic data was used to aid the design of synthetic analogues of the natural products. Therefore, in this study, we have proposed the use of molecular modeling and docking techniques to design some potential active agents based on the most effective *aporphine* alkaloids, *liriodenine*, as a novel Inhibitor of topoisomerase II. Then we have predicted possible binding conformation of the agents, which is experimentally not known, using a computational modeling method. Conformations of the designed compounds were optimized through semi-empirical method followed by PM3 calculation by using the HYPERCHEM software. Among all energy minima conformers, the global minimum was selected. Then the crystal of topoisomerase II was obtained from the Protein Data Bank (PDB) server. Finally Docking calculations were carried out using Auto-Dock program. The good interaction of the derivatives and also the Ki (inhibition constant) showed that they can be as potent topoisomerase II inhibitors and act as novel anti cancer agents. We hope this Computational study can offer some useful references in order to understand the inhibition mechanism better so that the molecular designing would be improved and modification of these series of topoisomerase II inhibitors would be more practiced.
Keyword: Topoisomerase II, Natural products, *Aporphine*, Molecular modeling, Docking.

Introduction

Topoisomerase II is a nuclear enzyme whose function, to allow the passage of a double strand of DNA through another one, is essential to dividing cells for disentangling the intertwined sister chromatids after replication [1]. By this action topoisomerase solves topological problems of DNA in replication, transcription, recombination and chromosome condensation as well as decondensation. Type II enzyme cuts and passes double stranded DNA. The mechanism of action of topoisomerase II can be dissected into a series of steps initiated by the binding of DNA to both subunits (S1 and S2) of the enzyme [2]. The classical topoisomerase II targeting substances act by trapping the cleaved G-strand-enzyme intermediate, thus, blocking relegation and enzyme release, leaving the DNA with a permanent double strand break. These substances that lead to higher levels of covalent topoisomerase II-DNA complexes

have been termed topoisomerase II- poisons [3]. They constitute a well established class of antitumor agents used in cancer therapy since decades [4]. On the other hand, the recent identification of *liriodenine* as a strong topoisomerase II inhibitor and a topoisomerase II poison [5]. Based on the previous studies, DNA cleavage mechanism of type II topoisomerase and other potent inhibitors of this enzyme, we selected and designed some structures. For the selection of these skeleton and moieties (Figure1), the following reasons were considered:

a) For general skeleton the flat conformation was required,

b) Lipophil substituent in the 3,4,9 and 10 positions of *liriodenine* was tolerated by the receptor and help to interact better,

c) In addition, it has been shown that hydrogen binding play key role in a Topoisomerase II pocket.

Regarding this issues, the structure of desired inhibitors were built by using HYPERCHEM program. Conformations of the designed compounds were optimized through semi-empirical method followed by PM3 calculation by using the HYPERCHEM software. Among all energy minima conformers, the global minimum was selected. The crystal structure of Topoisomerase II (Figure 2) was obtained from the Protein Data Bank (PDB) server (PDB entry: 3L4K). Then Docking calculations were carried out using AutoDock program (Ver4).

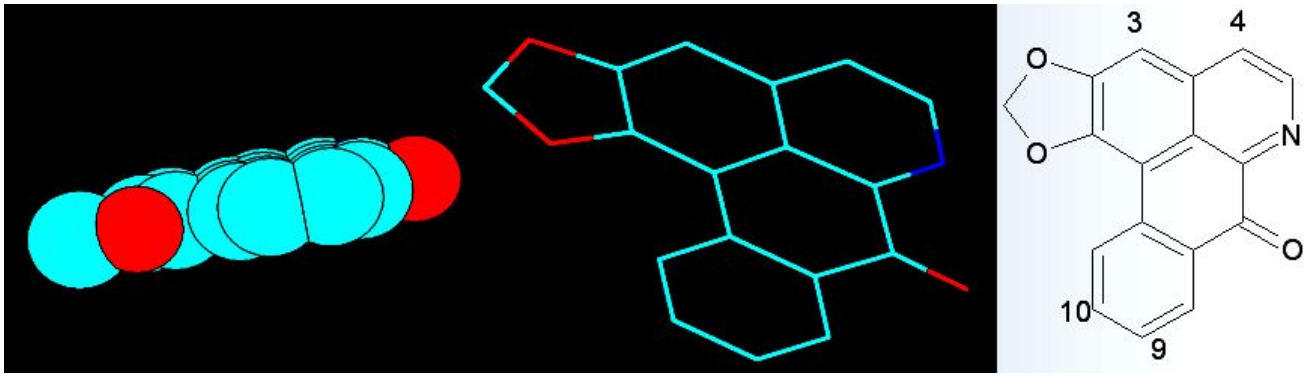


Figure1. Molecular structure of liriodenine. The space-filling and sticks models are shown the planar conformation.

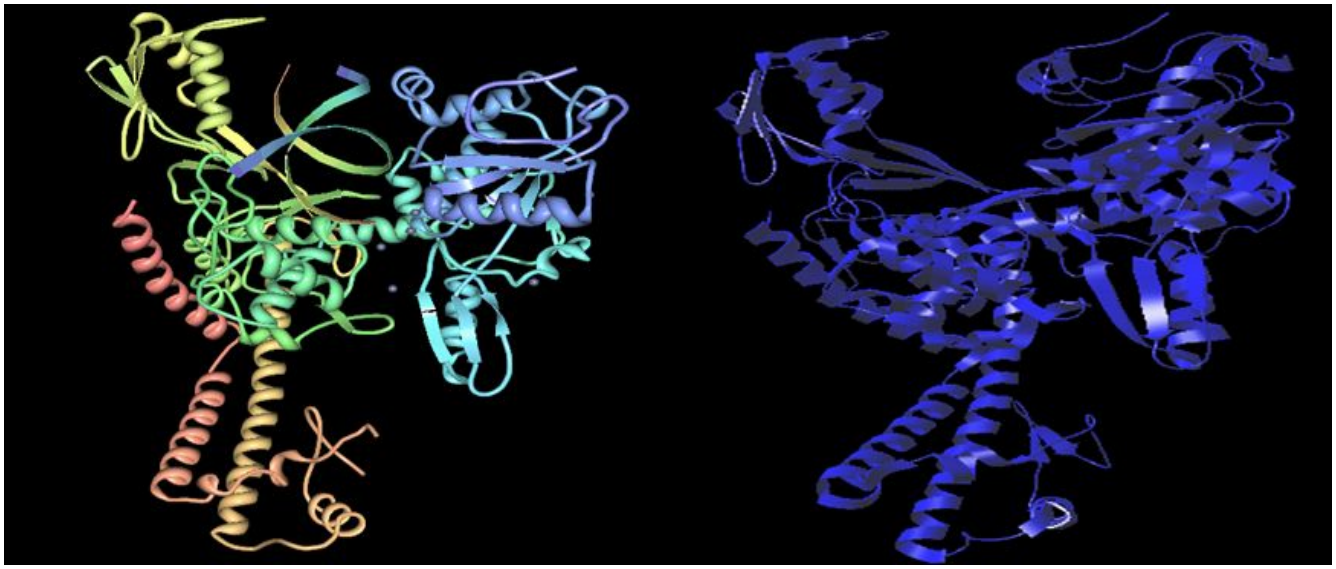


Figure2. The structure of Topoisomerase II enzyme (3L4K).

Materials and Methods

a) Molecular Modeling

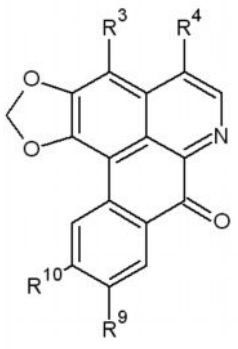
The chemical structures of *liriodenine* (Table.1) were constructed using Hyperchem software (version 7, Hypercube Inc.) .Semi-empirical molecular orbital calculations (PM3) of the structure were performed using the Hyperchem program and the among all energy minima conformers, the global minimum of compounds were consider in docking calculations. And also, Superimposition main pharmacophores of recognized Topoisomerase inhibitor compounds and our potent compound was performed.

b) Docking study:

Docking studies were carried out by using the program AUTODOCK 4. This program starts with a ligand molecule in an arbitrary conformation, orientation and position and finds favorable dockings in a protein-binding site using both simulating annealing and genetic algorithms. The program AutoDockTools (ADT), which has been released as an extension suite to the Python Molecular Viewer, was used to prepare the protein and the ligand.For the macromolecule (Topoisomerase II, that was generated by resorting to multi body molecular dynamics simulations, was downloading from the PDB bank server [PDB entry 3L4K]), polar hydrogens were added, and then Kollman United Atom charges and atomic solvation parameters were assigned.

The grid maps of docking studies were computed using the AutoGrid4 included in the Autodock4 distribution. Grid center was placed on the active site which was obtained by trial and error and the previous study done by Schmidt, B.H. et al (Nature 2010) and 40x40x40 points with grid spacing of 0.375 were calculated(Figure 3).

Table1. Structures of derivatives



Compounds	R_3	R_4	R_9	R_{10}
1	F	H	F	H
2	Cl	H	Cl	H
3	OCH_3	H	OCH_3	H
4	OC_2H_5	H	OC_2H_5	H
5	H	F	H	F
6	H	Cl	H	Cl
7	H	OCH_3	H	OCH_3
8	H	OC_2H_5	H	OC_2H_5
9	F	OCH_3	F	OCH_3
10	F	OC_2H_5	F	OC_2H_5

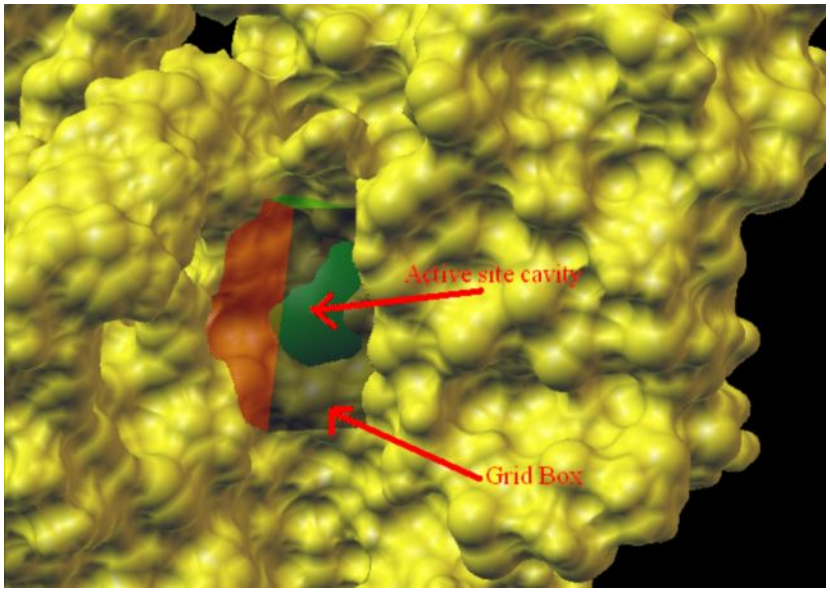


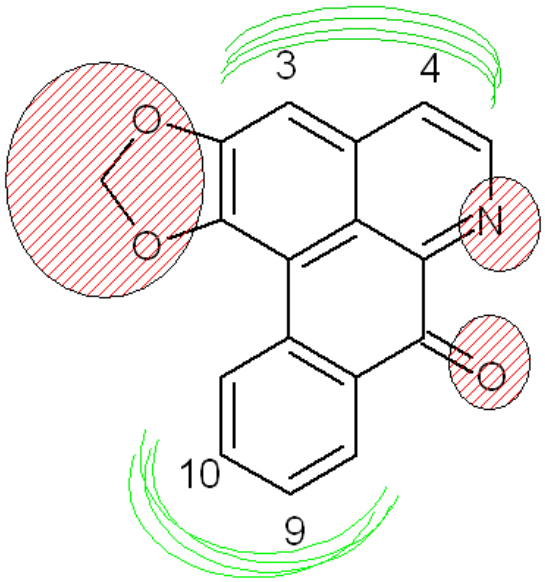
Figure3. The active site and grid box of the enzyme.

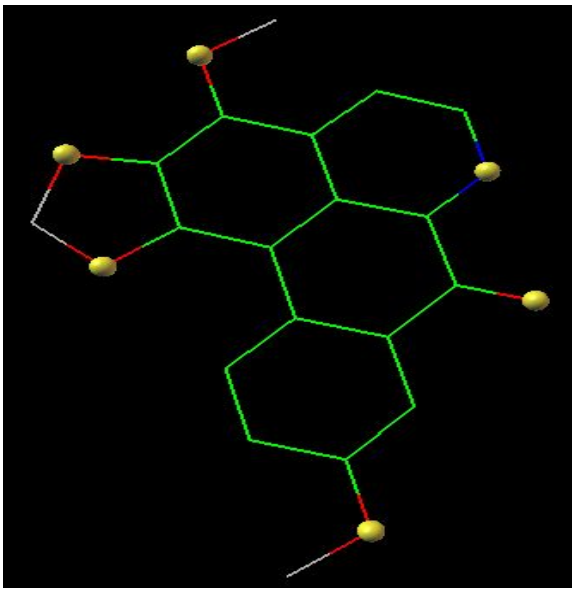
The GA-LS method was adopted to perform the molecular docking. The parameters for GA were defined as follows: a maximum number of 250,000 energy evaluations; a maximum number of generations of 27,000; mutation and crossover rates of 0.02 and 0.8, respectively. Pseudo-Solis & Wets parameters were used for local search and 300 iterations of Solis & Wets local search were imposed. The number of docking runs was set to 50. Both Autogrid and Autodock computations were performed on Cygwin. After docking, all structures generated were assigned to clusters based on a tolerance of 1 A ° all-atom RMSD from the lowest-energy structure. Hydrogen bonding and hydrophobic interactions between docked potent agents and macromolecule were analyzed using ADT (Version 1.50).

Results

Molecular geometry of the designed compounds has been calculated by the semi-empirical method using PM3. Now, Based on the results obtained from the superimposition of the compounds on the main pharmacophore belonged to Topoisomerase II inhibitor (*liriodenine*), which is evaluated by Sung Ho Woo et al, we expected potential H-bond acceptor sites created by the oxygen of carbonyl, oxygenes of dioxolane and nitrogen. Furthermore, it is anticipated that the aromatic groups in the newly designed compounds play key role in charge transfer interaction. And also, we inserted the lipophil moieties (fluorine and chlorine) and methoxy and ethoxy in 3,4,9 and 10 positions to optimize LogP and improve H-bond sites (Figure 4a,4b).

Flexible docking of all data sets used for the computational study was carried out on the active site of Topoisomerase II. To verify and compare the model we docked *liriodenine* as known Topoisomerase inhibitor as well as the designed agents. The interactions were shown as follow. The orientation of the most potent compounds (Comp.3 and Comp8) , in the active site of Topoisomerase II were shown by ADT software (Figure5) [6]. This molecular modeling shows that in Comp3 (Figure 5a), the O methoxy groups make noticeable hydrogen bonding interactions with both the NH2 of LYS603 (distance=2.196A^0) and the NH2 of DNA polymer chain (DG4) (distance= 1.911, 1.943 A^0). The charge transfer interaction between ARG475 and Comp8 is shown below by the twin red circles in Figure 5b, also in the same image the hydrogen bonding of NH2 group (LYS603) with the oxygen of ethoxy (distance= 2.092A^0) is mentioned. In the Figure 5b hydrogen bonding of ethoxy oxygen with the NH2 of DNA base (DG4) (distance=2.081)is illustrated.





(4a) **(4b)**

Figure (4a). General structure of designed compounds (Red circles show H-bond sites and green lines present effective sites in LogP balance and additional. H-bonds. (4b) Yellow points show the acceptor H binding of the methoxy moiety and other groups of compound 3.

(a)

(b)

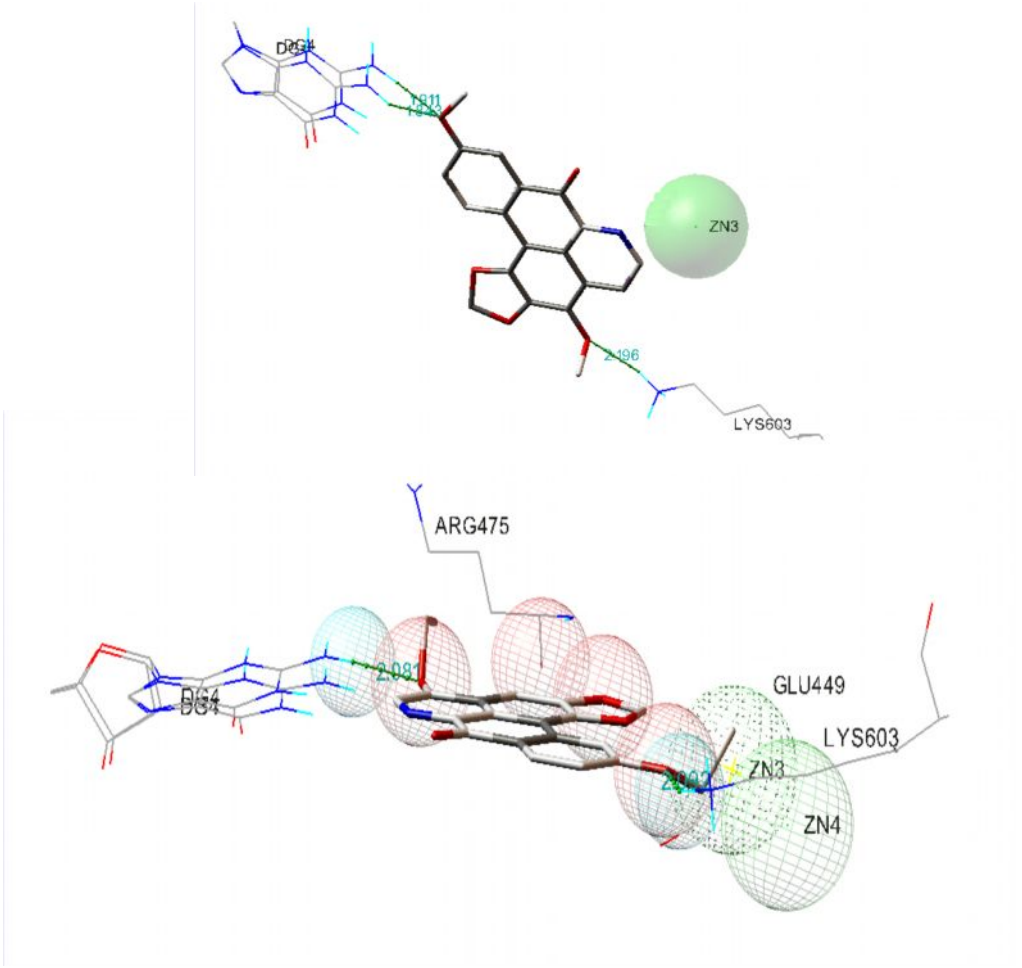


Figure5. Docked structures of Comp3(a), Comp 8 (b) in Model of Topoisomerase II. Our designed compounds are displayed as sticks, and Hydrogen bonds are represented with dashed green lines.(Docking study by using ADT program and Topoisomerase II model obtained from PDB server).

The main point in Figure 6, is the interaction of reference inhibitor (*liriodenine*). The molecular modeling confirmed the design concepts by showing that the oxygen of dioxolane moiety forms a hydrogen bonding interaction with the NH2 of LYS603 (distance=2.135A0) in reference inhibitor .

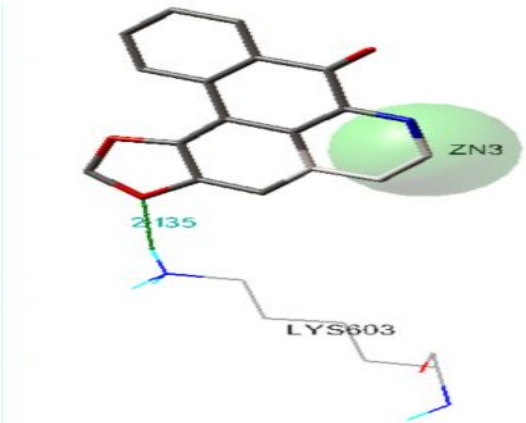


Figure6. Docked structures of reference Inhibitor (liriodenine) in Model of Topoisomerase II that is displayed as sticks , and Hydrogen bonds are represented with dashed green lines.(Docking study by using Auto Dock Tool program and Topoisomerase II model obtained from PDB server).

Also the predicted binding energies and inhibitory constant of these inhibitors are listed in Table 2.

Table2:Docking results by using AutoDock4 software

Compounds	Binding energy[1]	K_i [2]
1	-8.34	7.75
2	-8.90	2.99
3	-9.34	1.42
4	-9.01	2.48
5	-8.20	9.79
6	-9.04	2.37
7	-9.24	1.67
8	-9.56	0.98
9	-8.84	3.32
10	-8.81	3.47
Liriodenine	-8.23	9.30

1) The predicted binding energy (Kcal/mol)
2) The predicted inhibitory constant (10^{-7} molar)

Based on the results it is clear that almost all designed compounds were more potent than *liriodenine*. But there are some important points:
1- Regarding that methoxy and ethoxy moieties have H-bond site they have the best inhibitory effects. (comp. 3,4,7 and 8)
2- The lipophil moieties due to their charge transfer interaction have better Ki than *liriodenine*. (comp. 1,2 and 6)
3- The results of comp 9 and 10 show that there is no enough space in active site pocket due to the existence of both moieties. (H-bond site and lipophil)

All in all the results anticipate that the optimized structure that can be tolerated by the active site should only have one moiety in each side plus results exhibit that the H-bond role is more crucial than the charge transfer interaction.
By considering the obtained results and also focusing on these observations we can expect the designed compounds as novel topoisomerase II inhibitors which have the power to act as anti cancer agents.

Conclusion

To sum up, in this research we have got to this point that these compounds are theoretically active as anti cancer agents by inhibiting the topoisomerase II. Our suggestion is that the Topoisomerase II enzyme is a suitable target for developing novel compounds in the field of natural products which will definitely represent valuable candidates for the new, cancer therapy, especially against strains resistant to conventional chemotherapeutic treatments.

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References

[1] J.C. Wang, Cellular roles of DNA topoisomerases: a molecular perspective., Nat. Rev. Mol. Cell Biol., 2002,3, 430-40.
[2] Jan G. Hengstler et al., Dietary topoisomerase II-poisons: contribution of soy products to infant leukemia? , EXCLI Journal, 2002,1,8-14 .
[3] J.V. Walker and J.L. Nitiss, DNA topoisomerase II as a target for cancer chemotherapy. Cancer Invest 2002, 20,570-89.
[4] K.R. Hande, Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta ,1998, 1400,173–184.
[5] SH Woo, MC Reynolds, NJ Sun, JM Cassady and RM Snapka, Inhibition of topoisomerase II by liriodenine. Biochem Pharmacol ,1997,54, 467–473.
[6] Docking studies were performed using Autodock softwareVersion 4.0.

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