

Identification of Leads as Topoisomerase-II Inhibitors Using Pharmacophore Mapping

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To cite this article:

Deveeka Zamare, Shraddha Choudhary, Bishwambhar Mishra. Identification of Leads as Topoisomerase-II Inhibitors Using Pharmacophore Mapping. *International Journal of Pharmacy and Chemistry*. Vol. 2, No. 2, 2016, pp. 24-30. doi: 10.11648/j.ijpc.20160202.14

Received: August 27, 2016; **Accepted:** October 12, 2016; **Published:** October 28, 2016

Abstract: DNA topoisomerases are unique enzymes which play a major role in solving the topological problems associated with DNA molecule. Topoisomerase enzymes are highly expressed in cells which undergo rapid and uncontrolled cell divisions. Inhibition of this enzyme represents a major potential therapeutic approach for cancer. The computational tools that have allowed understanding the biological functions of Topoisomerase-II are being applied to understanding drug action. In this study, we have developed a quantitative pharmacophore model based on topoisomerase inhibitors collected from the literature. Molecular Docking Analysis was performed with AutoDock4 Software. Topoisomerase II (PDB-id 2RGR) was selected for finding its potent inhibitor. A comprehensive molecular modelling was performed to identify the pertinent features that could be used as a starting point for design of ligands with increased affinity and target selectivity. According to the pharmacophore mapping, the resulting pharmacophoric points could be used for the generation of new molecule that include all this point and hence will act as a better inhibitor for Topoisomerase II receptor.

Keywords: Topoisomerases, Pharmacophore, Docking, Ligands

1. Introduction

Drug design is one of the most important fields of study in Bioinformatics. The perspective of discovering new drugs based on the knowledge revealed by the human genome and the protein database focused a lot of work and new methods to improve the drug design process. Drug designing on the basis of ligand relies on knowledge of other molecules that can bind to the target of interest from biological origin. These molecules can be used to generate a pharmacophore model having minimum necessary structural characteristics. At the same time, structure-based drug designing relies on knowledge of 3-Dimensional structure of the biological target obtained through analytical methods like x-ray crystallography or Nuclear Magnetic Resonance (NMR) spectroscopy. If the 3-Dimensional structure of a target is not available, it is possible to formulate a homology modelling of the target in accordance with the experimental structure of related

proteins. Using the structure of the biological target, the model drugs with high affinity and selectivity to the target can be designed. Presently, there are various automated computational methodologies are used to predict a new drug for a new target [1, 2].

Many drugs work through interference with the topoisomerases. The broad-spectrum fluoroquinolone antibiotics work by inhibiting the function of bacterial type II topoisomerases. These small molecule inhibitors work as a potential anti-microbial by hijacking the natural ability of topoisomerase to break the chromosomal DNA [3].

In silico methods can help in identifying drug targets via bioinformatics tools. They can also be used to analyze the target structures for possible binding/ active sites, generate candidate molecules, check for their drug likeness, dock these molecules with the target, rank them according to their binding affinities, further optimize the molecules to improve

binding characteristics. The utilization of computers and computational strategies permits all aspects of drug discovery now a days and forms the core of structure-based drug design [4, 5].

The selected ligands begins the searching methods randomly outside the binding site. It will eventually reach the certain conformation by exploring the values of translations, rotations and its internal degree of freedom [6]. Selection and Sorting between good and bad docked conformations are applied through the scoring function.

Pharmacophore mapping is one of the major elements of drug design in the absence of structural data of the target receptor. The tool initially applied to discovery of lead molecules now extends to lead optimization [7, 8]. Pharmacophores can be used as queries for retrieving potential leads from structural databases (lead discovery), for designing molecules with specific desired attributes (lead optimization), and for assessing similarity and diversity of molecules using pharmacophore fingerprints [9].

The present study aims to the generation of Pharmacophoric Map for Topoisomerase II inhibitors using the parameters obtained from Molecular Docking Analysis performed using AutoDock4 Software.

2. Materials and Methods

2.1. Hardware and Software Specifications

Molecular modeling studies were carried out on a personal computer windows operating system. Virtual screening and Pharmacophore modeling experiments were performed using Schrodinger suite 2009 and modules like section, Ligprep, Quick Prop, Glide, and Virtual Screening progress were employed in the study [10, 11]. TopoisomeraseII enzyme was downloaded from Protein Data Bank having PDB-id 2RGR.

2.2. Visualization Tools

RasMol was used as molecular graphics visualisation tools meant and used primarily for the depiction and exploration of biological macromolecule structures, such as those found within the macromolecule knowledge Bank.

2.3. Cavity Finder

Q-SiteFinder was used as ligand binding site prediction (<http://www.modelling.leeds.ac.uk/qsitefinder/>). It works by binding hydrophobic (CH_3) probes to the protein, and finding clusters of probes with the most favourable

binding energy. It uses the interaction energy between the protein and a straightforward van der Waals probe to find energetically favorable binding sites [12]. Energetically favorable probe sites are clustered consistent with their spatial proximity and clusters are then hierarchical in step with the sum of interaction energies for sites within every cluster.

2.4. Pocket Finder

Pocket-Finder (<http://www.modelling.leeds.ac.uk/pocketfinder/>) was used as pocket detection algorithm based on Ligsite written by Hendlich *et al* (1997). Pocket-Finder works by scanning a probe radius 1.6 angstroms along all gridlines of a grid resolution 0.9 angstroms surrounding the protein [13, 14]. The probe also scans cubic diagonals. Grid points are defined to be part of a site when the probe is within range of protein atoms followed by free space followed by protein atoms. Since the protein is scanned in seven directions, each grid point can be defined to be part of site up to seven times. Grid points are only retained if they are defined to be part of a site at least five times. Pocket Finder uses the same interface as Q-SiteFinder.

2.5. Selection of Ligand on Reference Study

Lead like compounds were identified with IC50 values for Topoisomerase II from various research papers, 11 such compounds of the below listed compounds were sketched using Marvin sketch.

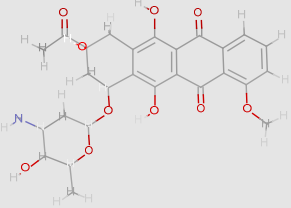
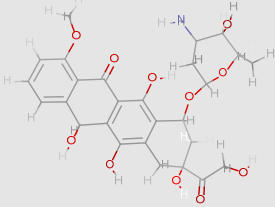
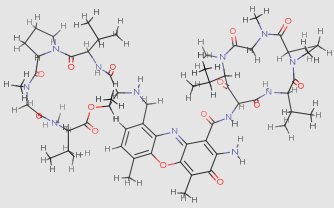
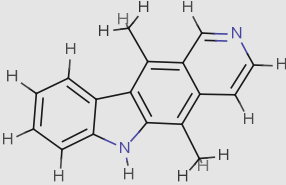
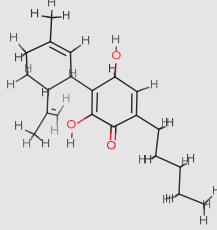
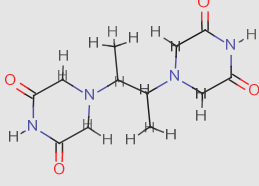
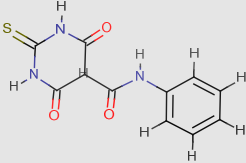
2.6. Docking Studies

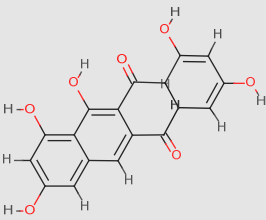
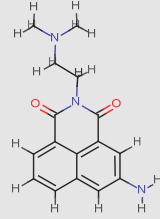
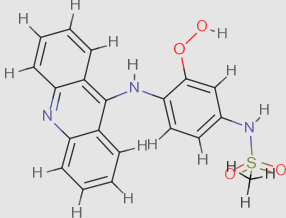
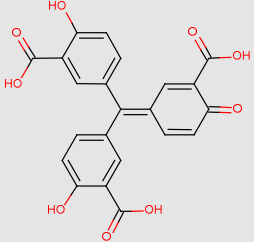
Docking study was performed by using Auto dock 4. The Glide algorithmic program approximates a scientific search of positions, orientations and conformations of the ligand within the enzyme-binding pocket via a series of ranked filters. information of the popular orientation successively could also be used to predict the strength of association or binding affinity between two molecules exploitation as an example scoring functions. the form and properties of the receptor are represented on a grid by many totally different sets of fields, which give increasingly} more correct scoring of the ligand pose [15, 16]. The binding site is outlined by an oblong box confining the translations of the mass centre of the ligand. AutoDock grip maps for each atom type in the ligand, calculated by AutoGrid, 2) a PDBQT file for the ligand, and 3) a docking parameter file (“dpf”) that specifies the files and parameters for the docking calculation.

3. Results and Discussion

3.1. Lead Optimization

Table 1. Lead Optimization study with molecular weight, LogP, IC₅₀ and pIC₅₀ values.

SL NO	Name	Structure	Molecular Wt	LogP	IC ₅₀ (in nM)	pIC ₅₀
1	Daunorubicin		527.52	2.32	500	6.301
2	Doxorubicin		543.519	0.41	1000 to 2000	6 to 5.698
3	Actinomycin-D		1255.44	0.33	0.4	9.397
4	Ellipticine		246.31	3.89	1000	6
5	HU331		328.5	4.46	200	6.698
6	ICRF-193		282.3	-2.24	600	6.221
7	Merbarone		263.27	0.79	40000	4.397

SL NO	Name	Structure	Molecular Wt	LogP	IC ₅₀ (in nM)	pIC ₅₀
8	Saintopin		338.267	1.31	350	6.455
9	Amonafide		283.32	1.10	6050	5.218
10	Amsacrine		393.46	2.93	2000	5.698
11	Aurintricarboxylic acid		422.341	4.05	940	6.026

Lead optimization is a complex, non-linear process. During this stage of drug discovery, the chemical structure of a confirmed hit is refined to improve its drug characteristics with the goal of producing a preclinical drug candidate. Lead optimization is done by using: Marvin Sketch. Different parameters (IC₅₀, pIC₅₀ and log P Values) were optimized. Lead improvement could be a advanced, non-linear method. throughout this stage of drug discovery, the chemical structure of a confirmed hit is refined to enhance its drug characteristics with the goal of producing a presymptomatic drug candidate. Lead improvement is finished by using: Marvin Sketch. completely different parameters (IC₅₀, pIC₅₀ and log P Values) were optimized. The effectiveness of a compound in inhibiting biological or organic chemistry function is expressed by IC₅₀. This quantitative measure indicates how much of a selected drug or different substance (inhibitor) is required to inhibit a given biological process (or element of a process, i.e. an enzyme, cell, cell receptor or microorganism) by 0.5. In different words, it's the 0.5 largest (50%) restrictive concentration (IC) of a substance (50% IC, or IC₅₀). it's usually used as a live of antagonist drug efficiency in pharmacologic analysis. Sometimes, it's additionally regenerate to the pIC₅₀ scale (-log IC₅₀), within which higher

values indicate exponentially larger efficiency (Table 1).

The molecular modeling and pharmacologic field the IC₅₀ values are regenerate in to pIC₅₀ values. For this purpose there's formula to convert the IC₅₀ value to pIC₅₀ value with representing the exponent values:-

$$\text{pIC}_{50} = \log 1/\text{IC}_{50} \text{ (A)}$$

$$\text{pIC}_{50} = -\log \text{IC}_{50} \text{ (B)}$$

The higher than formula will modification the IC₅₀ values into wide range of selection to pIC₅₀ slender range. But the IC₅₀ values are given in nanomolar and micromolar scale thus, once more the nanomolar values also ought to enclosed within the pIC₅₀. therefore the formula once more changes in step with nanomolar price, this is often the formula for nanomolar conversion of IC₅₀ values to pIC₅₀ values.

$$\text{pIC}_{50} = -\log \text{IC}_{50} \times 10^{-9} \text{ (C)}$$

These A, B and C are basic formula for the conversion of the IC₅₀ into pIC₅₀ value in nanomolar. The bioassay values are understood as completely different numbers, that take issue from one compound to a different compound even in same assay. pIC₅₀ values is ever-changing the IC₅₀ values

towards interpretation for all QSAR and Pharmacophore analysis [17]. The log P of a compound is constant for a given specific combine of binary compound and organic solvents, and its value are often determined by trial and error by one among many phase-partitioning ways. However, the calculated log P price for a compound in water vs. a straightforward chemical compound like octanol or dissolving agent will offer a guideline for predicting its solubility characteristics in different aqueous and organic solvents.

3.2. Q-site Finder

By using Q-site finder best docking site was estimated as well as its residues in that site with additional information of, protein volume, site volume and binding site co-ordinates which is useful to set the grid during docking are found at this site. Protein molecules best cavity i.e 1st site was found and its corresponding residues are listed below (Figure 1).

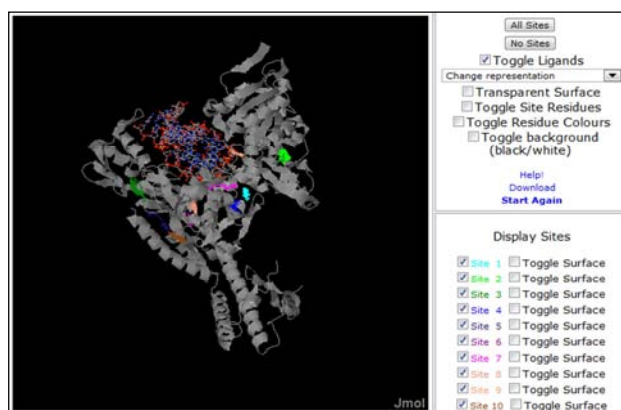


Figure 1. Q-site finder to find the information about binding site for the protein molecules/

3.3. Pocket Finder

The output file obtained for the molecule is visualized

using RasMol. Output File for Topoisomerase II showing pockets (Figure 2).

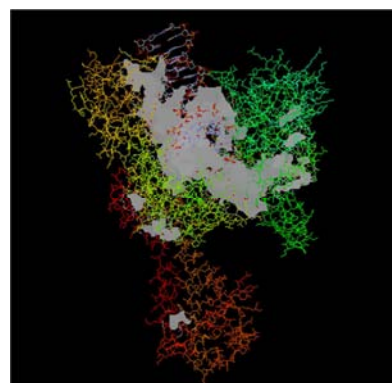


Figure 2. Visualization of Topoisomerase II with RasMol.

3.4. Clustering by using AutoDock4 Software

Graph between Conformations and Binding Energy is obtained from docking analysis, which shows results in the form of clusters (Figure 3).

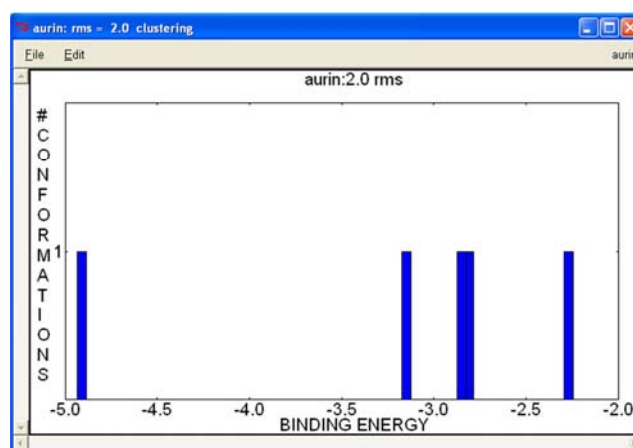


Figure 3. Relationship between Conformations and Binding energy of the lead molecule.

Table 2. Interactions of Docked Compound from AutoDock4 Software.

SL NO	Best Docked Ligand	Binding Energy	H-Bonds	Length	Residues	H-Bond Energy
1	Ellipticine	-6.71	No H-Bond			
2	Daunorubicin	-6.44	2	1.628 2.215	Daunorubicine:A:MOL0:H :2RGR:A:LEU654:O 2RGR:A:GLY660:HN: Daunorubicine:A:MOL0:O	2.594 -4.124
3	Amsacrine	-6.03	No H-Bond			
4	Amonafide	-6.0	1	1.987	2RGR_grid_A:HIS529:HN:Amonafide:A:MOL0:O	-4.756
5	Saintopin	-5.8	2	2.193 2.002	Saintopin:A:MOL0:H: 2RGR_grid_A:LYS651:O 2rgr_grid:A:ASN:688:HD22:Saintopin:A:MOL0:O	-1.169 -4.781
6	ICRF-193	-5.7	2	2.094 1.84	ICRF193:A:MOL0:H: 2RGR_grid_A:ASP528:O 2RGR_grid_A:HIS529:HE2:ICRF-193:MOL0:O	-4.309 -3.92
7	Doxorubicin	-5.08	No H-Bond			
8	Merbarone	-4.85	1	2.106	2RGR_grid_A:ILE984:HN:Merbarone:A:MOL0:O	-4.446
9	HU331	-3.82	1	2.007	2RGR_grid_A:GLN599:HN:HU331:A:MOL0:O	-6.01
10	Actinomycin-D	-3.77	1	2.175	2RGR_grid_A:ARG1015:HH21:Actinomycin-D:A:MOL0:O	-2.649

Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes (topoisomerase I and II), that are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands throughout the normal cell cycle. In recent years, topoisomerases became widespread targets for cancer chemotherapy treatments. It's thought that topoisomerase block the ligation step of the cell cycle, generating single and double stranded breaks that damage the integrity of the genome. Introduction of those breaks afterward cause apoptosis and death. Topoisomerase II(2RGR) was taken as the target molecule. Study of its binding sites and cavity was done using Q-site Finder and Pocket Finder (Table 2).

Later, the inhibitors of Topoisomerase II were selected as Lead Molecules for Docking and Pharmacophore Mapping. Ten lead molecules were selected and further optimized using Marvin Sketch and the logP values for each were calculated using the same. The IC₅₀ values for the leads were referred from different research [18, 19, 20, 21] papers which were further converted into pIC₅₀ values using Sanjeev's Lab [22]. On the basis of their Docking Analysis and Pharmacophore Mapping performed using AutoDock4 software the binding Energy and number of Hydrogen Bonds, Length of Hydrogen Bonds, Residues involved and Energy of Hydrogen Bond were obtained. After the Docking Analysis the lead molecule Ellipticine was found with the least binding energy (-6.71) with no Hydrogen Bond. The lead molecule Actinomycin-D was found with the least binding energy -3.77.

4. Conclusion

According to the pharmacophore mapping, the resulting pharmacophoric points can be used for the generation of new molecule which will include all this point and hence will act as a better inhibitor for Topoisomerase II receptor. On the basis of the whole docking analysis performed a 3-dimensional QSAR (Quantative Structural Activity Relationship) can be generated which will be receptor as well as ligand Specific.

Acknowledgement

The authors want to acknowledge the financial support and laboratory facilities from Sreenidhi Institute of Science and Technology, Hyderabad and Rajiv Gandhi Institute of IT and Biotechnology, Pune to carry out this research work.

Conflict of Interest

The authors declare that they have no conflict of interest.

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