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Research Article

IN SILICO IDENTIFICATION AND EVALUATION OF HITS FOR THE INHIBITION OF TOPOISOMERASE-II ENZYME VIA STRUCTURE BASED PHARMACOPHORE MAPPING AND VIRTUAL SCREENING

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ABSTRACT

The search for a suitable cancer treatment has been going on for the last few decades for the effective treatment. Now, computational biology has emerged as a novel tool to improve the domain of computer aided drug designing. The present study reports a ligand based pharmacophore computational modeling that elucidates important pharmacophoric features helpful for the inhibition of topoisomerase II activity of cancer. A six featured pharmacophore model of topoisomerase II has been generated via 10 training sets of reported topoisomerase II inhibitors ligands in Molecular Operating Environment 2009.10. pharmacophore constructing tool. The generated pharmacophore model was then validated by the 24 test set database of the reported naphthoquinone inhibitors. Later the validated pharmacophore model was then used to virtually screen the possible hit compounds from the ZINC drug database by using ZINCpharmer tool. The virtually screened hits were filtered by Lipinski's rule of five and further assessed through molecular docking and ADMET studies. The results of docking and interaction studies were validated through binding score analysis and ADMET profiling. Seven hits (ZINC ID's: 00000903, 02570830, 02012726, 00001402, 02040199, 01481831, and 00006923) of different scaffolds having interactions with important active site residues of topoisomerase II were predicted. It can be concluded from the finding of the present study that predicted hits could serve as potential candidates in the development of novel and potent topoisomerase II inhibitors. The present modeling explores the significant role of the predicted hits towards blocking the replication of cancer.

INTRODUCTION

Cancer is the second most common cause of death globally, nearly 1 in 6 deaths is due to cancer [1]. Carcinogenic lacerations have been associated with deficient DNA repair. DNA topoisomerases are enzymes that alter DNA topology by causing and resealing DNA strand breaks. Tumors with high cell proliferation articulate these enzymes more than in normal cells, making topoisomerases good targets for the discovery of novel anticancer drugs [2]. The two types of DNA topoisomerases, i.e. Type I DNA topoisomerases (Topo I) break and rejoin only one of the two strands during catalysis, while Type II topoisomerases (Topo II) catalyze DNA topological changes by breaking both strands of the double helix and transporting another double-stranded DNA segment through the break and then re annealing the break [3]. On the other hand Topo II play an important role in DNA transaction in vivo, including chromosome condensation and segregation, and the removal of the super coils generated during replication and transcription [4] studies in eukaryotes have shown Topo I to be associated with actively transcribed genes [5, 6] whereas, Topo II is required for DNA replication and for successful traverse of mitosis [7-10]. Based on overall survival rates of cancer patients, which make us to vital search for new therapeutic agents to control cancer hopefully, which can be achieved by the development of novel compounds with promising anticancer activity. The numerous natural or synthetic substances containing the quinone nuclei, which is one of well known nuclei can inhibit the activity of topoisomerase. The cytotoxic activity shown by this nuclei is mainly due to inhibition of DNA Topo II, through DNA alkylation or intercalation thereby, inhibiting the heat shock protein HSP90 and as well as through the formation of semiquinones and superoxide radicals, which contribute to the

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production of hydroxyl radical, the main source of oxidative generated damage to cellular DNA [11-13]. It has been established that many of the biological effects of quinone derivatives Such as the antitumor, antiproliferative, antibacterial, anti-inflammatory, antimalarial, antiviral, antifungal and antileishmanial effects depend on their 1,4-NQ pharmacophore group. NQs are molecules widely distributed in nature that possess a broad spectrum of biological activities and especially those containing nitrogen, have promising potential for the treatment of different diseases, including antibacterial, antifungal, antiviral, antiparasitic effects and anticancer activity [14-15]. Moreover, the incorporation of nitrogen and sulfur atoms on C2 and C3 of the 1,4-NQ lead could produce the compounds with diverse biological activities, including anticancer activity [16]. 1,4-NQ derivatives with an amino group at the 2-position have been reported to have good anti-neoplastic, [17,18] carcinostatic actions [19] and bacterial growth inhibition [20]. Based on this above reported literature, we have chosen the naphthoquinone derivatives as a lead ligand to generate the pharmacophore model for virtual screening of the novel hits from ZINC drug database.

The increasing global cancer burdens its health and socio-economic impacts, the presence of drug resistance, etc. leads to the search for newer therapeutic agents with divergent and unique structure and with a mechanism of action possibly different from that of existing drugs are urgently required to tackle the menace. Recent advances in computational biology have noticeably impacted and improved the domain of computer aided drug designing. Due to the high cost and low hit rates practiced during wet lab testing, computational processes and virtual screening have largely aided the drug designing. The development of computational methods and the wide applications of in silico screening were stimulated by the low hit rates and high cost associated with the wet lab [21].

The various ligand-based and structure-based pharmacophore generation methods have been one of the major tools in drug discovery and development and successfully applied in virtual screening, *de novo* design and lead optimization. Among this, the high-throughput screening has been used for finding topoisomerase II inhibitors. In order to search the high active topoisomerase inhibitors which have different scaffolds, we have developed a ligand based pharmacophore model, the obtained pharmacophore models are expected to identify the crucial pharmacophore features of potent topoisomerase II inhibitors. Then these pharmacophore models were used as 3D search queries for the hit chemical compounds search from Zinc drug databases by using online tool ZINCpharmer [22].

This work reports the ligand based pharmacophore modeling to find out the important pharmacophoric features essential for the inhibition of topoisomerase II activity of DNA by virtual screening, drug-likeness predictions, molecular docking, protein-ligand binding interactions, ADMET prediction, binding affinity predictions and binding energy calculations. The identification of 22 novel and potent lead compounds as topoisomerase II inhibitors clearly reflects the significance of this study. Therefore, the aim of this study is related to look for a newer analogue for the treatment of cancer with the help of pharmacophore search, virtual screening and molecular docking of the active hit compounds from the available database. At last, 9 hits were selected, which could have different scaffolds, high estimated activity, and good ADMET properties. Molecular docking was carried out to study the bind modes of these hits with topoisomerase II enzyme, from this result show that the seven hits may act as novel leads for topoisomerase II enzyme inhibitors designing.

METHODS

Software and Online Tool Used

All the chemical compound structures of naphthoquinone derivatives were generated by using Marvin_windows-x64_18.8 version. Marvin Sketch is an advanced chemical tool for illustrating the chemical structures, queries, reactions, and etc. [23]. Then the structures were viewed on Marvin Viewer screen to generate the SMILES notation of the ZINC drug database retrieved compounds. Pharmacophore modeling and molecular docking were accomplished via the Molecular Operating Environment (MOE) 2009.10 software package [24]. The MOE is a comprehensive suite developed by Chemical Computing Group (CCG) Incorporation. It has different tools and computational methods that are applied to structure and fragment based drug designing, pharmacophore discovery, protein structure analysis, data processing and molecular docking and simulations [25].
ZINCpharmer is an online tool for searching the hit compounds from ZINC drug database. A search of 2, 15,407,096 conformations of 22,723,923 compounds classically takes very few minutes. The results can be instantly observed and structures could be downloaded for further analysis. ZINCpharmer support for uploading pharmacophore definitions in load features represented in .PH4 format, used by MOE [22]. The maximum hits results were filtered both in terms of the number of returned results and the properties of the returned results. The number of hits was reduced by specifying a limit of 1 on the number of different orientations returned for each conformation ('Max Hits per Conf'), a limit of 1 on the number of different orientations of different conformations returned for each molecule ('Max Hits per Mol'), the limit was set here as nil on the total number of hits returned ('Max Total Hits') and the returned hits have the best possible root mean squared deviation (mRMSD) as 2 to the query. The properties of the returned hits were reduced by specifying a limit of $\leq$ 500 on molecular weight and $\leq$10 on a rotatable bond of hit screening arena. After applying this filters on ZINCpharmer, there are around 22 hits were downloaded for further screening.

Generation of Ligand Based Pharmacophore Model

Based on IUPAC description, a pharmacophore is an ensemble of steric and electronic features that is necessary to ensure the optimal supra molecular interactions with a specific biological target and to trigger or block its biological response [26]. The pharmacophore constructing tool implemented in MOE 2009.10 suite was used for the generation and visualization of 3D pharmacophore from reported structural data of topoisomerase II ligand compounds. A set of 34 compounds were collected from recently reported literature [27] for the ligand based pharmacophore generation. A total of 10 compounds of them were chosen as the training set to generate the pharmacophore model and other 24 compounds were

chosen as the test set to validate the developed model, structures of these training set compounds were given in Table **1.** The 3D structures of these molecules were built by MOE builder tool. Energy minimization of 3D structures was achieved by MOE energy minimization algorithm with Force Field: MMFF94X parameter. Then a series of energetically reasonable conformations of each training set of 10 compounds were generated using Generate Conformations protocol. Then whole diverse conformations of these training set compounds were used to generate pharmacophore models by using ligand based pharmacophore Generation tool. The significant chemical features for the generation of pharmacophore model were identified, a total of six key features including two hydrogen bond acceptors (Acc), one aromatic center/pi ring center/ hydrophobic center (Aro/PiR/Hyd), one hydrophobic center/hydrogen bond acceptor projection (Hyd/ACC2), one aromatic or pi ring normal/hydrogen bond acceptor projection (PiN/ACC2) and one aromatic center/hydrophobic center (Aro/Hyd) were generated in the resulting pharmacophore model using the default parameters of MOE 2009.10. The generated pharmacophore model was validated through the test database set of 24 known inhibitors of topoisomerase II enzyme [27]. All the compounds of the test database were screened on the six featured ligand based pharmacophore and their mapping modes were analyzed.

couple of rationales: firstly, the validated pharmacophore model was successfully used for appropriate identification of compounds having known inhibitory potential against topoisomerase II and secondly, the importance of this technique to recognize new and effective drug-like items for additional assessment. Similarly, Lipinski's rule of five was used to determine drug-like attributes of the compounds retrieved from the ZINC drug database. The rule illustrates molecular properties that are significant for a drug's pharmacokinetics in the human body. To check the appropriate molecular properties of compounds, their drug scan was executed using the ligand properties checking tool molinspiration server 2011.16 [28]. Drug scan will tell us the potential and effectiveness of these compounds.

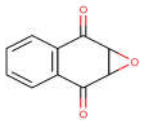
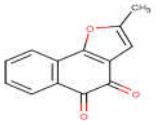
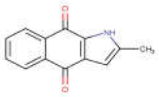
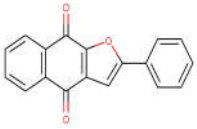
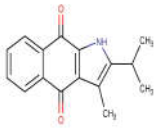
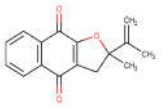
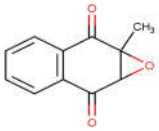
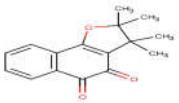
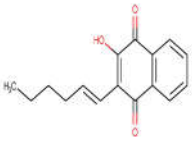
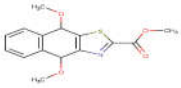
Drug-likeness and ADMET Analysis

For *in-silico* screening of the ADMET profiles of the potential compounds, admetSAR server [28] was used which predicted the ADMET associated properties of the active compounds for various kinds of models.

Admet SAR Predictions

The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and the Toxicity of the target molecules were predicted using admet SAR

Table 1 Chemical Structure of 10 naphthoquinone training set

Compound Code	Chemical Structure	Compound Code	Chemical Structure
1a		4g	
2b		4k	
2e		4o	
3b		4s	
4c		5b	

Pharmacophore Based Database Virtual Screening

The purpose of virtual screening is to find potential leads with different scaffolds and high inhibitory activity to topoisomerase II. This was achieved by using pharmacophore based visual screening of the ZINC drug database by using ZINCpharmer online tool with generated 3D pharmacophore query of the validated pharmacophore model. [22]. This type of screening was assumed due to a

(http://lmmd.ecust.edu.cn/admetsar1/predict/) database which plays an important role in drug discovery. Using admetSAR prediction model, the absorption parameters like Blood-Brain Barrier, Human Intestinal Absorption, Caco-2 Permeability, P-glycoprotein Substrate, P-glycoprotein Inhibitor and Renal Organic Cation Transporter were ensured. The distribution parameter such as Sub cellular localization and metabolism parameters like CYP450 2C9 Substrate, CYP450 2D6 Substrate, CYP450 3A4 Substrate, CYP450 1A2 Inhibitor,

CYP450 2C9 Inhibitor, CYP450 2D6 Inhibitor CYP450 2C19 Inhibitor, CYP450 3A4 Inhibitor and CYP Inhibitory Promiscuity was checked. These are the most important enzymes for the metabolism of drugs in humans. It also predicts the toxicity of Human Ether-a-go-go-Related Gene Inhibition, AMES Toxicity, Carcinogens, Fish Toxicity, Tetrahymena Pyriformis Toxicity, Honey Bee Toxicity, Biodegradation, Acute Oral Toxicity and Carcinogenicity (Three-class).

Molecular Docking

In this study, the crystal structure of Human topoisomerase II beta in complex with DNA and etoposide (PDB codes: 3QX3) [29]. For further evaluation of the hit compounds, all the retrieved compounds from ZINCpharmer database were docked into the binding site of topoisomerase II beta (PDB ID: 3QX3). Original substrate based inhibitor was also test docked into the binding site of topoisomerase II as reference ligand. Removal of water molecules, 3D protonation and energy minimization was carried out using MOE [24] with parameters, force field: MMFF94X+solvation, Gradient: 0.05, Chiral constraint and current geometry. This minimized structure was used as the ligand for docking analysis. The active site of the binding pocket was selected with the help of the MOE site finder tool. For docking simulations, the placement was set as triangular matcher, rescoring was set as London dG, the number of retaining was set as 10 and the refinement was set as forcefield on MOE suite to generate 10 poses of each target ligand confirmations. Most appropriate docked ligand target structure was selected on the basis of higher S-score and Root Mean Square Deviation (RMSD) values. The S-score is the value calculated by built-in scoring functions of MOE on the basis of ligand binding affinity with receptor protein after docking. While RMSD value is generally used to compare the docked conformation with the reference conformation or with other docked conformation. The only compounds that have higher S-score and lower RMSD value than its natural substrates can be developed as potential inhibitors [30].

RESULTS AND DISCUSSION

The vital search for the novel therapeutic lead to control cancer hopefully could be attained by the development of novel compounds with promising anticancer activity. Different strategies have been used so far to search out potent inhibitors for cancer topoisomerase II enzyme including rational discovery of potential agents based on non-competitive binding, structure based virtual screening, ligand based virtual screening, synthesizing rationally designed substrate-based cyclopeptide or peptidomimetics, virtual screening and scaffold hopping, screening natural products and small compound libraries [21]. From the literature reports, the substances containing the quinone nuclei can inhibit the activity of topoisomerase II through DNA alkylation or intercalation. [11-13]. It has been established that many of the biological effects of quinone derivatives such as the antitumor, antiproliferative, antibacterial, anti-inflammatory, antimalarial, antiviral, antifungal and antileishmanial effects depend on their NQ pharmacophore group [14-20]. Keeping in view of the above discussion, we have chosen the naphthoquinone derivatives as a lead ligand to generate the pharmacophore model for virtual screening of the novel hits from ZINC drug database. So, the present study focuses on the pharmacophore based virtual screening followed by drug likeness prediction and molecular docking approach. This is the novel methodology and exposed potential compounds which strongly bind with topoisomerase II active site by hindering its replication and could be further used as drug lead compounds.

Generation and Validation of Ligand Based Pharmacophore Model

The ligand based pharmacophore model of the 10 training sets of previously reported topoisomerase II inhibitors was generated by using MOE 2009.10 pharmacophore constructing tool. Binding interactions induce significant chemical features which were taken into account for the creation of the pharmacophore model. By using default parameters of MOE, there are six key features including two hydrogen bond acceptors (Acc), one aromatic center/pi ring center/ hydrophobic center (Aro/PiR/Hyd), one hydrophobic center/hydrogen bond acceptor projection (Hyd/ACC2), one aromatic or pi ring normal/hydrogen bond acceptor projection (PiN/ACC2) and one aromatic center/hydrophobic center (Aro/Hyd) were generated in the resulting pharmacophore model (Fig. 2). The created pharmacophore system was validated via the test database sets of 24 known inhibitors of topoisomerase II. All inhibitors of the test database along with their mapping modes were evaluated on the basis of six featured ligand based pharmacophore model.The evaluation resulted in 22 out of 24 most active compounds as hits. These hits showed the mapping of 986 entries various conformations of six features created pharmacophore system. None of the inactive was mapped to any feature of ligand based pharmacophore system. The results from the test database revealed the accuracy of the generated pharmacophore model.

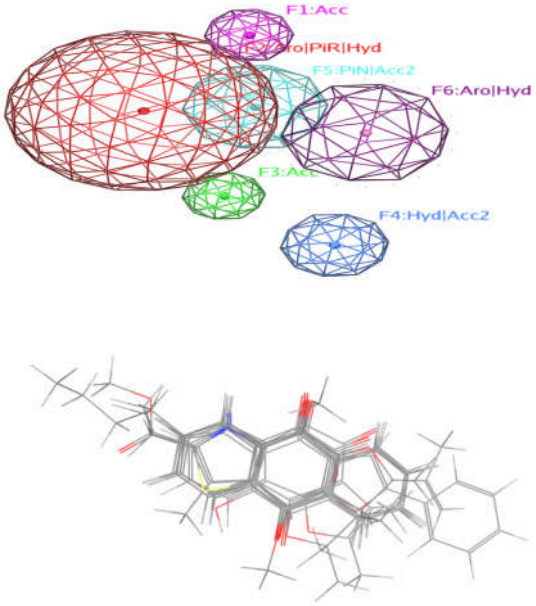


Fig 1 Three dimensional pharmacophoric features and flexi alignment generated from the ligand of topoisomerase II inhibitors

Table 2 ADMET profiling results for selected hit compounds

ZINC ID	Blood brain barrier	Human intestinal absorption	Caco-2 Permeability	P-glycoprotein Inhibitor	Renal Organic Cation Transporter
			Absorption		
00000903	BBB+	HIA+	Caco2+	NI	I
02570830	BBB+	HIA+	Caco2+	NI	I
00003876	BBB+	HIA+	Caco2+	I	NI
02012726	BBB+	HIA+	Caco2+	NI	NI
00001402	BBB+	HIA+	Caco2+	NI	I
02040199	BBB+	HIA+	Caco2+	NI	NI
01481831	BBB+	HIA+	Caco2-	NI	NI
02015503	BBB+	HIA+	Caco2+	NI	NI
00006923	BBB+	HIA+	Caco2+	NI	NI
			Metabolism		
ZINC ID	**CYP450 1A2 Inhibitor**	**CYP450 2C9 Inhibitor**	**CYP450 2D6 Inhibitor**	**CYP450 2C19 Inhibitor**	**CYP450 3A4 Inhibitor**
00000903	I	I	NI	I	NI
02570830	I	I	NI	I	NI
00003876	I	NI	NI	NI	NI
02012726	NI	I	NI	I	I
00001402	I	I	NI	I	NI
02040199	NI	I	NI	NI	I
01481831	NI	I	NI	I	I
02015503	NI	I	NI	NI	I
00006923	NI	NI	NI	NI	NI
			Toxicity		
ZINC ID		**AMES Toxicity**		**Carcinogens**	
00000903		Non AMES toxic		Non Carcinogens	
02570830		Non AMES toxic		Non Carcinogens	
00003876		Non AMES toxic		Non Carcinogens	
02012726		Non AMES toxic		Non Carcinogens	
00001402		Non AMES toxic		Non Carcinogens	
02040199		Non AMES toxic		Non Carcinogens	
01481831		Non AMES toxic		Non Carcinogens	
02015503		Non AMES toxic		Non Carcinogens	
00006923		Non AMES toxic		Non Carcinogens	

I: Inhibitor, NI: Non inhibitor

Table 3 Lipiniski's drug like screening and bioactivity score of the selected 9 hits according to Molinspiration Cheminformatics

				Lipiniski's drug like screening					
ZINC ID	**Mi logp**	**TPSA**	**No.of atoms**	**MW**	**N.ON**	**N.OHNH**	**N. Violations**	**nrotb**	**Volume**
00000903	2.29	43.08	22	308.77	4	0	0	1	263.23
02570830	3.17	43.08	22	393.70	4	0	0	1	271.83
00003876	3.15	37.62	23	307.40	4	0	0	3	295.47
02012726	3.83	49.85	26	368.82	5	0	0	1	313.67
00001402	3.03	43.08	23	342.86	4	0	0	2	287.31
02040199	-0.34	76.91	30	414.43	8	1	0	4	362.02
01481831	0.58	109.81	29	453.90	8	0	0	6	342.34
02015503	-0.34	76.91	30	414.43	8	1	0	4	362.02
00006923	0.17	44.31	23	314.40	4	2	0	6	302.37
Reference ligand (3QX3)	0.70	160.86	42	588.56	13	3	2	5	493.51
				Bioactivity score					
ZINC ID	**GPCR ligand**	**Ion channel modulator**		**Kinase inhibitor**	**Nuclear receptor ligand**		**Protease inhibitor**		**Enzyme inhibitor**
00000903	0.68	0.61		-0.38	-0.51		-0.37		-0.17
02570830	0.78	0.35		-0.41	-0.81		-0.56		-0.14
00003876	0.16	0.19		0.17	-0.56		-0.38		-0.12
02012726	-0.02	-0.25		-0.40	-0.23		-.30		-0.15
00001402	0.89	0.21		-0.32	-0.81		-0.56		-0.19
02040199	-0.04	-0.17		-0.49	-0.30		-0.46		-0.09
01481831	0.12	-0.37		-0.32	-0.26		-0.22		-0.16
02015503	-0.04	0.17		-0.49	-0.30		-0.46		-0.09
00006923	0.08	-0.07		-0.04	0.01		-0.06		0.07
Reference ligand (3QX3)	0.18	-0.48		-0.38	-0.33		0.12		0.30

Pharmacophore .PH4 Based Database Screening

By using the validated pharmacophore model, the best hit compounds with similar features and novel structural conformations were screened from the ZINC drug database of ZINCpharmer online tool. The six featured Pharmacophore generated model based virtual screening on ZINC drug database resulted in an around 22 structurally diverse hits. For the further evolution of drug ability of these hit compounds, Lipinski's rule of five was used. These rules state that drug-like molecules should contain log p-value <5, molecular weight <500 Da, hydrogen bond acceptors <10 and hydrogen bond donors <5. A deviation from these rules results in poor permeation or absorption of the compounds [28]. Only 18 hits of topoisomerase II inhibitors were able to pass the criteria of Lipinski's rule of five and were further analyzed.

ADMET Analysis

An admetSAR is helpful for *in silico* screening of ADMET profiles for the potent lead hit compounds along with 10 training set database compounds for further comparison [28]. ADMET (absorption, distribution, metabolism, elimination, toxicity) analysis is important in drug design. Some properties including human intestinal absorption, aqueous solubility levels, BBB penetration levels, CYP2D6 inhibition and hepatotoxicity of these 18 compounds were analyzed. Molecules only with the following properties can be selected out as final hits where the Brain-Blood ratio must be less than 0.3 : 1, and they must be unlikely to inhibit CYP2D6 enzyme, unlikely to cause dose-dependent liver injuries, aqueous solubility level log(Sw) must be more than -4.0 and less than 0.0, and should have moderate or good intestinal absorption. After adding these restrictions, only 9 molecules meet the conditions and were accepted through ADMET test [31]. The information of these nine hits is listed in Table 2. BBB positive implying the ability of the compound to permeate the BBB while BBB negative implying inability of the compound to do so. Only nine hit compounds were accepted through ADMET test. The Lipiniski's drug like screening results and bioactivity score of these 9 hit compounds were shown in Table 3**.** Chemical structures of the finally selected 9 hit compounds are shown in Fig. 2**.**

Molecular Docking

Algorithms of molecular docking technique as compared to pharmacophore modeling are more advanced, composite and computationally challenging. Thus, molecular docking has more potential to accurately predict binding affinities of screening hits as well as potentially elucidate lead structures with novel modes of binding [32,33]. Together with the pharmacophore modeling and virtual screening, it is believed that molecular docking has great promise in drug discovery. Hence, for further improvement of the retrieved hit inhibitors, the Lipinski's rules passed compounds were docked into the binding site of topoisomerase II beta (PDB ID: 3QX3) using the MOE docking suite 2009.10 to discover the binding pattern of small molecules against their targets. The best docked results of compounds were saved in a separate database. On the basis of the docking score, top 7 compounds were taken for further assessment (Fig. 2). The molecules having vital interactions with most of the significant catalytic triad of topoisomerase II enzyme were selected as capable hits. From docked structures, 7 out of 9 hits showed essential interactions with the catalytic triad of the target protein which is further compared with their ligand inhibitor of topoisomerase II inhibitor (3QX3) which is downloaded from protein database bank [29] (Table 4).

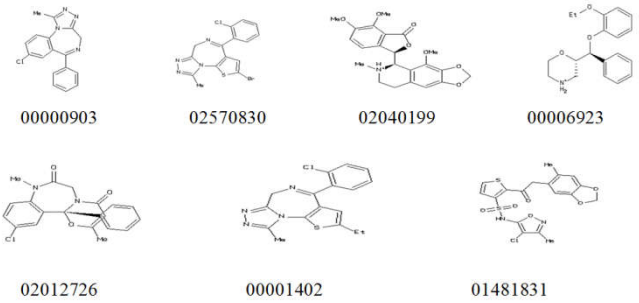


Fig 2 2D structures of finally retrieved hits from ZINC database. The numbers are representing their ZINC database IDs

Interaction Analysis of Finally Selected Hit Compounds

The S-score is the mathematical method used to calculate the strength of interaction between receptor protein and ligand after they have been docked. On the basis of S-score, binding energy and affinity, it is possible to conclude that these finally selected compounds have potential interaction with catalytic triad residues and fulfill the requirements to be the drug lead. The compound having ZINC ID 00001402 was placed at the top as it had potential interactions such as side chain acceptor on Arg 945, Lys 739, Arene Cation interaction on Arg 945, backbone donor on Gly 812 and backbone acceptor on Glu 870 residues of the binding pocket and has a docking score of -15.3673 kcal/mol. The compound having ZINC ID 02570830 showed the highest docking score of -20.6346 kcal/mol whereas, ZINC ID 02015503 and 00003876 were placed at least and it had very fewer interactions with receptor as shown in below Table 4. Docking conformations and pharmacophore mapping of the selected ZINC hit compounds were given in Table 4. The Interaction of ZINC ID 02570830 hit with a generated pharmacophoric feature from topoisomerase II inhibitors shown in Fig. 3.

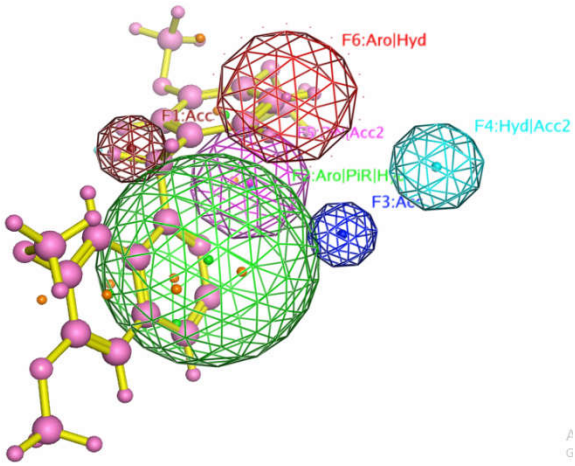


Fig 3 Interaction of ZINC ID 02570830 with generated pharmacophoric feature from topoisomerase II inhibitors

Table 4 Docking results for the returned hits from ZINC Drug Database with protein 3QX3

ZINC ID	S	rmsd_refine	E_conf	E_place	E_score1	E_refine	No. of conf.	Residues interacting with ligand
00000903	-12.7827	3.0465	93.518	-24.3359	-10.4003	-12.7827	9	SCA- Arg 945; SCD-Glu 870
02570830	-20.6346	2.3089	18.7327	-34.3280	-12.9576	-20.6346	10	SCA -Arg 945; Metal Contact - Lys 739
00003876	-6.6537	1.8820	74.2359	-47.4850	-11.1924	-6.6537	7	Arene Cation- Arg 945
02012726	-15.3307	2.7065	49.4589	-49.0631	-12.7409	-15.3307	10	SCA – Lys 739, Arg 945; BBA- Glu 870
00001402	-15.3673	1.5172	52.1973	-77.4860	-12.5247	-15.3673	8	SCA – Arg 945, Lys 739; Arene Cation – Arg 945; BBD- Gly 812; BBA- Glu 870
02040199	-17.9925	0.9713	-71.3793	-42.9708	-16.0661	-17.9925	9	SCA – Thr 783, Lys 739
01481831	-12.1969	2.2076	52.1071	-74.9466	-12.4549	-12.1969	9	SCA –Arg 945, Lys 814
02015503	-0.0156	1.9716	69.9658	-60.7347	-11.0879	-0.0156	6	SCA – Lys 739
00006923	-19.9583	5.3405	-21.2088	-72.8671	-12.8978	-19.9583	9	SCA – Lys 814; Arene Cation - Lys 739
Reference Ligand	-16.6899	4.2066	112.3437	-27.2667	-9.4361	-16.6899	10	SCA – Thr 783, Lys 739, Asn 786; Arene Cation – Lys 739; BBD – Glu 870

S - The final score, rmsd_refine- The root mean squar deviation between the pose before refinement and the pose after refinement, E_conf- The energy of the conformer. E_place - Score from the placement stage, E_score1- Score from the rescoring stage(s), E_refine- Score from the refinement stage and No. of conf- number of conformations generated by ligand, SCA- side chain acceptor, SCD-side chain donor, BBA- back bone acceptor, BBD- back bone donor

CONCLUSION

The present study focuses on ligand based pharmacophore modeling, computational screening, Lipinski's drug likeliness screening, ADMET prediction and molecular docking of ZINC drug database hit compounds against topoisomerase II. As an outcome of the study, seven compounds from ZINC drug database (ZINC ID's: 00000903, 02570830, 02012726, 00001402, 02040199, 01481831, and 00006923) have shown strong bindings with a catalytic triad of topoisomerase II which is further compared with that of reference ligand which is isolated from the Protein ID 3QX3. This study also explores that these compounds can be utilized as potential and strong drug candidates against cancer on the basis of drug profiling. The findings will be useful as they provide insight into the effectiveness of the drug before its manufacturing and testing on a pilot scale in the pharmaceutical industry (for *in vivo* drug design and development). Hence, it can be concluded that in future these compounds can serve as a strong and potential drug leads against cancer on the basis of significant Lipinski's drug likeliness screening, ADMET prediction and molecular docking binding affinity score against topoisomerase II enzyme.

Conflict of Interest

The authors have declared that no competing interest exists.

References

1. GBD 2015 Risk Factors Collaborators. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388:2016; 1659-1724
2. Liu LF. DNA topoisomerase poisons as antitumor drugs. Annu. Rev. Biochem. 1989; 58: 351–375
3. Boonyalai N, Sittikul P, Pradidphol N, Kongkathip N. Biophysical and molecular docking studies of naphthoquinone derivatives on the ATPase domain of human Topoisomerase II. Biomed. Pharmacothera. 2013; 67: 122–128
4. Berger JM, Wang JC. Recent developments in DNA topoisomerase II structure and mechanism. Curr. Opin. Struc. Biol. 1996; 6: 84–90
5. Gilmour DS, Elgin SC. Localization of specific topoisomerase I interactions within the transcribed region of active heat shock genes by using the inhibitor camptothecin. Mol. Cell. Biol. 1987; 7: 141–148
6. Zhang H, Wang JC, Liu LF. Involvement of DNA topoisomerase I in transcription of human ribosomal RNA genes. Proc. Natl. Acad. Sci. 1988; 85: 1060–1064
7. Yang L, Wold MS, Li JJ, Kelly TJ, Liu LF. Roles of DNA topoisomerases in simian virus 40 DNA replication in vitro. Proc. Natl. Acad. Sci. 1987; 84: 950–954
8. DiNardo S, Voelkel K, Sternglanz R. DNA topoisomerase II mutant of Saccharomyces cerevisiae: topoisomerase II is required for segregation of daughter molecules at the termination of DNA replication. Proc. Natl. Acad. Sci. 1984; 81: 2616–2620
9. Holm C, Goto T, Wang JC, Botstein D. DNA topoisomerase II is required at the time of mitosis in yeast. Cell. 1985; 41: 553–563
10. Uemura T, Yanagida M. Isolation of type I and II DNA topoisomerase mutants from fission yeast: single and double mutants show different phenotypes in cell growth and chromatin organization. Embo. 1984; J 3: 1737–1744
11. Pelageev DN, Dyshlovoy SA, Pokhilo ND, Denisenko VA, Borisova KL, Amsberg G.K, Bokemeyer C, Fedorov SN, Honecker F, Anufriev VP. Quinone–carbohydrate nonglucoside conjugates as a new type of cytotoxic agents: Synthesis and determination of in vitro activity. Eur. J. Med. Chem. 2014; 77: 139–144
12. Cadet J, Wagner JR. Oxidatively generated base damage to cellular DNA by hydroxyl radical and one-electron

oxidants: Similarities and differences. Arch. Biochem. Biophys. 2014; 557: 47–54
13. Verma RP. Anti-cancer activities of 1,4-naphthoquinones: A QSAR study. Anticancer Agents Med. Chem. 2006; 6: 489–499
14. De Castro SL, Emery FS, Silva Júnior EN. Synthesis of quinoidal molecules: Strategies towards bioactive compounds with an emphasis on lapachones. Eur. J. Med. Chem. 2013; 69: 678–700
15. Castellanos JRG, Prieto JM, Heinrich M. Red lapacho (tabebuia impetiginosa)—A global ethnopharmacological commodity. J. Ethnopharmacol. 2009; 121 1–13
16. Delarmelina Daltoé MRD, Cerri MF, Madeira KP, Rangel LBA, Lacerda Júnior V, Romão W, Tarantod AG, Greco SJ. Synthesis, Antitumor Activity and Docking of 2,3-(Substituted)-1,4-Naphthoquinone Derivatives Containing Nitrogen Oxygen and Sulfur. J. Braz. Chem. Soc. 2015; 26: S1-S20
17. Zee-Cheng RK, Cheng CC. Structure-activity relationship study of anthraquinones: 1,4-dihydroxy-5,8-bis[[2-(2-hydroxyethoxy)ethyl]amino]- Inhibition of Topoisomerases by Benz[f]indole-4,9-diones 1949 9,10-anthracenedione, an analog of an established antineoplastic agent. J. Pharm. Sci. 1982; 71: 708–709
18. Zee-Cheng RK, Mathew AE, Xu PL, Northcutt RV, Cheng CC. Structural modification study of mitoxantrone (DHAQ). Chloro-substituted mono-and bis [(aminoalkyl)amino]anthraquinones. J. Med. Chem. 1987; 30: 1682–1686
19. Petersen S, Gauss W, Kiehne H, Juhling L. Derivatives of 2-amino-1,4-naphthoquinone as carcinostatic agents. Z. Krebsforsch. 1969; 72: 162–175
20. Silver RF, Holmes HL. Synthesis of some 1,4-naphthoquinones and reaction relating to their use in the study of bacterial growth inhibition. Canadian J. Chem. 1968; 46: 1859
21. Muhammad TQ, Saleha K, Usman AA, Muhammad RJ, Farooq A, Muhammad AA, Anwar HG. Discovery of Novel Dengue NS2B/NS3 Protease Inhibitors Using Pharmacophore Modeling and Molecular Docking Based Virtual Screening of the ZINC Database. Inter. J. Pharmacol. 2016; 12: 621-632
22. David RK, Carlos JC. ZINCPharmer: pharmacophore search of the ZINC database. Nucleic Acids Research. 2012; 40: W409–W414
23. Marvin Sketch Peter C. Marvin View: molecule applets for the World Wide Web. In: Third international electronic conference on synthetic organic chemistry (ECSOC-3). 1999; 1–30
24. Molecular Operating Environment (MOE), 2009.10; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, 2018; H3A 2R7
25. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr. Opin. Struct. Biol. 1996; 6: 402-406
26. Wermuth CG, Ganellin CR, Lindberg P, Mitscher LA. Glossary of terms used in medicinal chemistry (IUPAC Recommendations 1998). Pur. Appl. Chem. 1998; 70: 1129–1143
27. Acuña J, Piermattey J, Caro D, Bannwitz S, Barrios L, López J, Ocampo Y, Ivas-Reyes R, Aristizábal F, Gaitán R, Müller K, Franco L. Synthesis, Anti-Proliferative Activity Evaluation and 3D-QSAR Study of Naphthoquinone Derivatives as Potential Anti-Colorectal Cancer Agents. Molecules. 2018; 23: 186
28. Prabha T, Sivakumar T. Drug design and in-silico study of sulphadiazine Schiff base analogues for their potential claim as anti-mycobacterial ligand. Int. J. Pharma. Bio. Sci. 2018; 9: 106-112
29. Chyuan-Chuan W, Tsai-Kun L, Lynn F, Li-Ying L, Te-Sheng L, Yu-Jen Y, Tien-Jui Y, Chia-Wang C, Nei-Li C. Structural Basis of Type II Topoisomerase Inhibition by the Anticancer Drug Etoposide. Science. 2011; 333: 459-462
30. Yang SY. Pharmacophore modeling and applications in drug discovery: Challenges and recent advances. Drug Discov. Today. 2010; 15: 444-450
31. Jia F, Lu Z, Tao L, Xiang-Yang T. Pharmacophore Modeling, Virtual Screening and Molecular Docking Studies for Discovery of Novel Akt2 Inhibitors. Inter. J. Med. Sci. 2013; 10: 265-275
32. Ma DL, Chan Leung DSHCH. Molecular docking for virtual screening of natural product databases. Chem. Sci. 2011; 2: 1656-1665
33. Prabha Thangavelu, Sivakumar Thangavel, Design, synthesis, and docking of sulfadiazine schiff base scaffold for their potential claim as Inha enoyl-(acyl-carrier-protein) reductase inhibitors. Asian J. Pharm. Clin. Res. 2018; 11(10): 233-237

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