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A convenient synthesis and molecular docking study of novel sulfonamides fused with Betti's bases as DNA Topoisomerase II inhibitors

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ABSTRACT

A series of novel sulfonamides fused with Betti's bases were synthesized and all the synthesized compounds were subjected to molecular docking study against DNA topoisomerase II as it was found to be the target for some of the most active anticancer agents. On the basis of the results obtained from docking studies we concluded that some of the synthesized novel sulfonamide derivatives might show significant anticancer activity by inhibiting DNA topoisomerase II.

Keywords: DNA Topoisomerase II, Molecular Docking, Betti's bases fused sulfonamide derivatives, dock score.

INTRODUCTION

Topoisomerase II is a ubiquitous enzyme that is essential for the survival of all eukaryotic organisms and plays critical roles in virtually every aspect of DNA metabolism. The enzyme unknots and untangles DNA by passing an intact helix through a transient double-stranded break that it generates in a separate helix. Like a magician, topoisomerase II performs tricks with ropes and rings, this enzyme acts as a `molecular Houdini' that unknots and untangles DNA. With deft sleight of-hand, topoisomerase II cleaves both strands of its nucleic acid substrate, passes an intact double helix through the break, and religates the cleaved DNA.(1) Beyond its critical physiological functions, topoisomerase II is the target for some of the most active anticancer agents currently used for the treatment of human malignancies. These drugs smash the topoisomerase II magic act in a unique fashion. Rather than killing cells by preventing the enzyme from performing its molecular rope illusions, they catch topoisomerase II in mid-trick `with scissors in hand' and trigger cell death by increasing levels of enzyme-mediated DNA breaks.(1) As of present the clinical use of catalytic topoisomerase inhibitors as antineoplastic agents is limited to aclarubicin and MST-16. Both of these compounds are preferentially active toward heamatological malignancies and show limited activity toward solid tumours.(2) Despite the wide use of Topo II targeted drugs as antitumour agents, several limitations hamper their benefits. Efforts for improving their clinical efficacy further by overcoming the drug resistance, myelosuppresion and poor bioavailability problems associated with them, were continued to be challenging.(3,4) Therefore, we are endeavouring to develop novel topoisomerase inhibitors with a new scaffold distinct from existing drugs with improved bioactivity and with minimal side effects. Sulfonamides posses many types of biological activities and many of them are widely used in therapy as antibacterial, hypoglycemic, diuretic, anti-carbonic anhydrase and antithyroid agents. Recently, a host of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity in vitro and/or in vivo.(5) Although aromatic/heterocyclic

sulfonamides has been exploited clinically in the treatment of a variety of diseases such as glaucoma, epilepsy, congestive heart failure, mountain sickness, gastric and duodenal ulcers or as diuretic agents, their potential use as antitumour drugs has been little explored up to now.(6) Here we report a study, showing that several sulfonamide derivatives possessing Betti's base may act as effective anticancer agent. This was done by carrying out docking studies of all the synthesized sulfonamide derivatives with crystal structure of DNA Topoisomerase II enzyme.

Designing enzyme inhibitors requires significant amounts of time and laboratory resources. Because only five out of every 40,000 synthesized inhibitors that undergo animal testing actually make it to clinical trials and typically only one of those five are approved, applications of computational techniques are becoming indispensible in the process of drug discovery and development. Virtual screening[VS), or *in silico* modeling, automatically sieves out compounds with incompatible features. Consequently, the portion of time and financial resources spent on fruitless experimental synthesis can be significantly decreased. (7)

When no biochemical or other functional assay is available, VS may be the only way of identifying inhibitors of a specific target. Compared to high throughput screening [HTS), VS is fast and inexpensive. Also, it is conceivable that VS is complementary to HTS, i.e. compounds that are falsely not detected as active [false negatives) in first-round screening of an HTS campaign can be highlighted by their docking scores and therefore be re-tested in the confirmation rounds of a given screening campaign.(8)

Docking procedures allows virtually screening a database of compounds and predict the strongest binder based on various scoring functions. It gives way in which two molecules such as drugs and an enzyme receptor fit together and dock to each other well.(9) Molecular docking is an intuitively appealing solution when the three dimensional [3D) structure of the target protein is available. The prediction of binding conformation and pose by docking simulations for a compound library to the same target protein would quite likely produce well-aligned models for the ligands, while revealing the essential features responsible for their activities. It has been found that docking results are accurate enough for appropriately aligning the ligands and developing reliable QSAR models.(10) Since there is ample structural information available in the protein data bank [PDB) for the DNA topoisomerase II, a combination of receptor docking and 3D-QSAR approaches would be desired for the investigation of the ligand libraries as DNA topoisomerase II inhibitors, however only limited studies had been reported on this subject. Due to our interest in the development of novel anticancer agents, in this study, we report the synthesis of some novel sulfonamide derivatives with different Betti's bases hoping that these new compounds might show significant anticancer activity by inhibiting DNA topoisomerase II.

Increasing intermittence of mammalian tumors and severe side-effects of chemotherapeutic agents reduce the clinical efficacy of a large variety of anticancer drugs that are currently being used. Thus, there is always a constant need to develop alternative or symbiotic anticancer drugs with minimal side-effects. One important strategy to develop effective anticancer agents is to study the structure activity relationship of the synthesized compounds virtually. Thus, the objectives of present work are [i) to synthesize new sulfonamide derivatives using different Betti's bases and [ii) to explore the binding modes of these compounds at the active site of DNA Topoisomerase II by docking studies, ergo predicting and exploring the most active compound as anticancer agent.

MATERIALS AND METHODS

All the reagents, solvents and catalyst are of analytical grade purchased from a commercial source and used directly. All the melting points were determined by open tube capillaries method and are uncorrected. The purity of compounds was checked routinely by TLC (0.5mm thickness) using silica gel-G coated Al-plates (Merck, 60F254) and spots were visualized under UV light. IR spectra were recorded on a Bruker FTIR-αE; ^{1}H NMR (400 MHz) spectra and ^{13}C NMR (100 MHz) spectra of the synthesized compounds were recorded on a Bruker-Avance II 400, Varian-Gemini spectrophotometer using DMSO-*d6* solvent and TMS as the internal standard. EI-MS spectra were determined on an liquid chromatography quadrupole (LCQ) ion trap mass spectrometer *(Thermo Fisher, San Jose, CA, USA)* equipped with an EI source.. The elemental analysis (C, H, N, and S) of compounds was performed on Perkin Elmer 2400 CHN analyzer. The values found for C, H, N, and S was within ±0.5% of the theoretical ones. The results were found to be in good agreement with the calculated values.

General procedures for the synthesis of substituted 1-(amino(phenyl)methyl)naphthalen-2-ol 3(a-j): To a stirring suspension of 2-naphthol (10 mmol) in ethanol (15 mL), the appropriate aromatic aldehyde (10 mmol) and

ammonia in excess (10 mmol) were added. The reaction mixture was stirred at ambient temperature for 5-6hrs. The precipitated product was separated by filteration. The precipitate was washed with cold EtOH, dried, and purified by recrystallization from EtOH.(11)

General procedures for the synthesis of substituted N-(4-(N-((2-hydroxynaphthalen-1-yl)(phenyl)methyl)sulfamoyl)phenyl)acetamide 5(a-j): To the solution of substituted 1-(amino(phenyl)methyl)naphthalen-2-ol **3(a-j)** (1 equiv) in DMF triethyl amnie (1 equiv) were added slowly. To this mixture acetamido benzene-sulfonyl chloride **4** (1 equiv) were added with stirring. The mixture was stirred at 150 ^{o}C for 10-12 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, it was quenched with ice water and the precipitated product was filtered off. The sulfonamide was recrystallized from ethanol.

Docking studies

Molecular docking studies were performed and dock score values were combined with hydrogen bonds formed with the surrounding amino acid residues. In order to compare the binding affinity of the of synthesized sulfonamide derivatives, we docked them into the empty binding site of the experimentally known crystal structure of the human DNA topoisomerase II as a target for anticancer compounds.(12) All docking studies were performed using "**ArgusLab 4.0.1**".

Preparation of protein structure

The crystal structure of the DNA topoisomerase II (PDB code- pdb1oit.ent) was retrieved from the Protein Data Bank (http://www.pdb.org). All bound waters ligands and cofactors were removed from the protein.

Preparation of the ligand structures

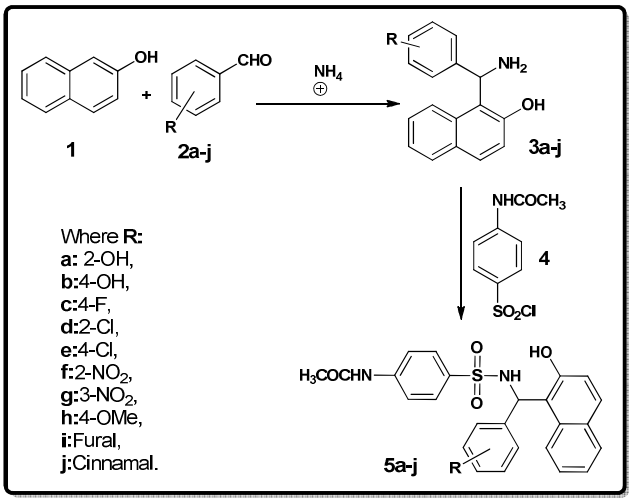
A set of 10 substituted sulfonamides synthesized, was designed to bind with DNA topoisomerase II, ChemDraw 3D structures were constructed using ChemDraw 3D pro12.0 software, and then they were energetically minimized with MM2, Jop Type with *show every iterations* and minimum RMS gradient of 0.01, and saved as pdb file. The synthesized compounds considered for the study are listed in Table 1.

Protein-ligand docking using ArgusLab 4.0.1

DNA topoisomerase II protein was docked against the compounds synthesized using ArgusLab 4.0.1 (Mark A. Thompson, Planaria Software LLC, Seattle, WA, USA, http://www.arguslab.com). The interaction was carried out to find the favorable binding geometries of the ligand with the protein. Docking simulations were performed by selecting "ArgusDock" as the docking engine. The selected residues of the receptor were defined to be a part of the binding site. A spacing of 0.4 Å between the grid points was used and an exhaustive search was performed by enabling "regular precision" option in Docking precision menu, "Dock" was chosen as the calculation type, "flexible" for the ligand, and the "AScore" was used as the scoring function. A maximum of 150 poses were allowed to be analyzed. The AScore function, with the parameters read from the AScore prm file, was used to calculate the binding energies of the resulting docked structures. All the compounds in the dataset were docked into the active site of DNA topoisomerase II protein, using the same protocol. The docking poses saved for each compound were ranked according to their dock score function. The pose having the highest dock score was selected for further analysis.

RESULTS AND DISCUSSION

All novel sulfonamides containing Betti's bases as DNA topoisomerase II inhibitors **(5a-5j)** described herein were synthesized following the synthetic pathway depicted in Scheme 1. The reaction of different 1-(amino(phenyl)methyl)naphthalen-2-ol **(3a-j)** with 4-acetamidobenzene-1-sulfonyl chloride was carried out in the presence of triethylamine and dimethylformamide as a solvent. The chemical structures, colour, melting point and yield of all the synthesized compounds **(5a-j)** are given in Table 1. The reactions were monitored by thin layer chromatography (TLC) and the crude products were purified by recrystallization with ethanol.



Scheme 1.: General protocol for synthesis of N-(4-(N((2-hydroxynaphthelen-1-yl)phenyl)methyl) Sulfamoyl)phenyl)acetamide derivatives.

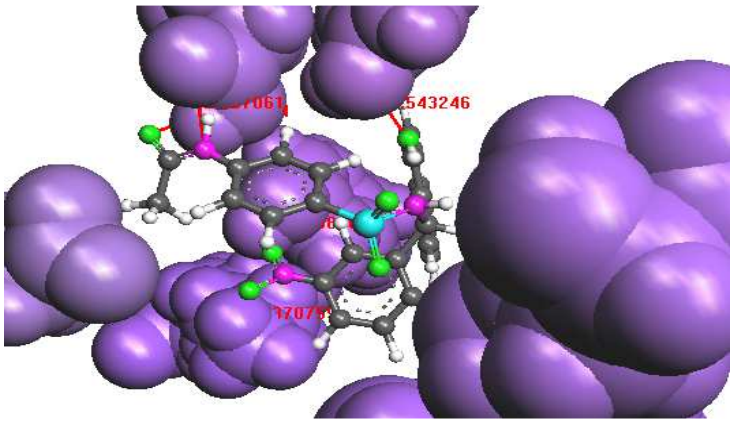


Fig. 1 Binding pose of 5g in the active site of DNA Topoisomerase II.

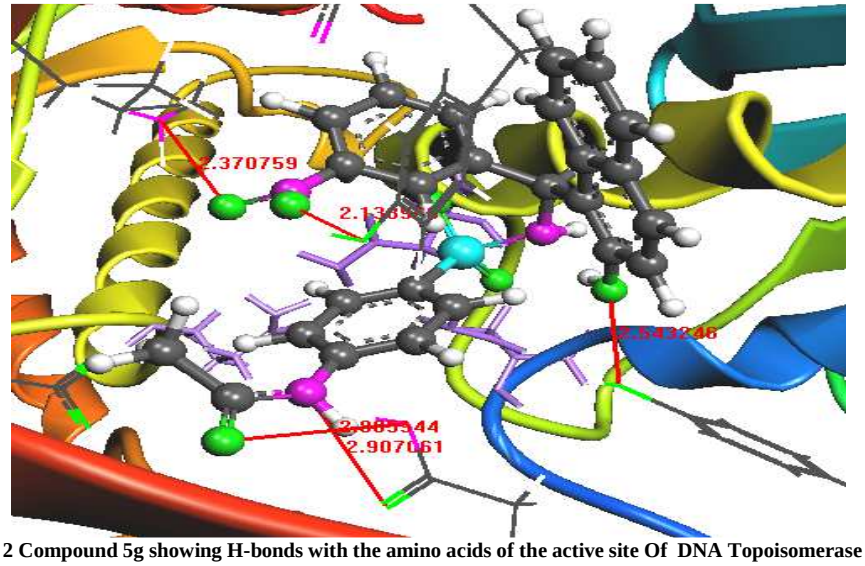


Fig. 2 Compound 5g showing H-bonds with the amino acids of the active site Of DNA Topoisomerase II.

Table 1 Some physiochemical properties of synthesized compounds 5(a-j).

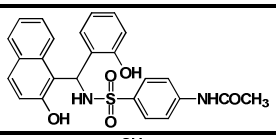
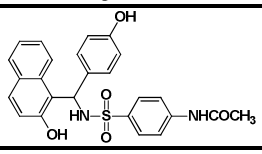
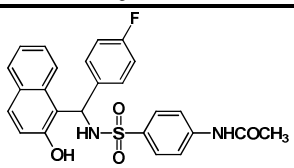
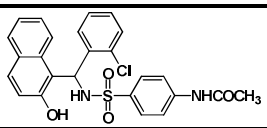
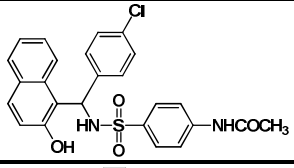
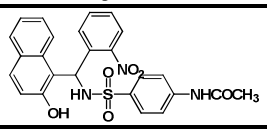
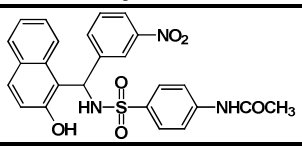
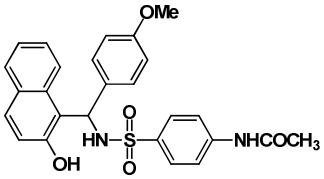
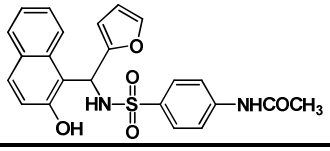
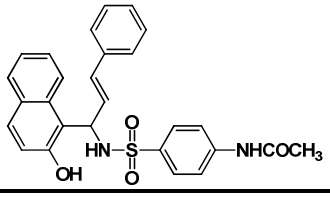
Sr. No.	R(a-j)	PRODUCT	COLOUR	M. P. (°C)	YIELD (%)
5a	2-OH		Grey	125-130	74
5b	4-OH		Light brown	120-122	65
5c	4-F		Off white	126-130	63
5d	2-Cl		Light brown	132-135	72
5e	4-Cl		Grey	128-130	75
5f	2-NO_2		Orange	120-124	80
5g	3-NO_2		Orange-red	130-135	78
5h	4-OMe		Grey	130-132	69
5i	Fural		Dark green	147-150	66
5j	Cinnamal		Maroon-red	133-135	68

Table 2 Binding scores and amino acid interactions of the docked compounds on the active site of DNA topoisomerase II.

COMP-OUND	DOCK SCORE (Kcal/mol)	NO. OF H-BONDS	INVOLVED GROUPS OF AMINO ACID	ATOM OF LIGAND INVOLVED	LENGTH OF H-BOND (Å)
5a	-8.50	7	Gly-108…….NH	O of SO_2	2.14
			Tyr-25…….OH	O of OH	2.36
			Arg-21…….NH	O of OH	2.40
			Pro-17…….OH	O of OH	2.77
			Tyr-25…….OH	N of NH	2.82
			Arg-21…….NH	O of OH	2.87
			His-94……..NH	O of C=O	2.98
5b	-10.48	4	Glu-594……..OH	O of OH	2.08
			Tyr-600……..OH	O of SO_2	2.27
			Tyr-25……..OH	O of SO_2	2.42
			Leu-544…….NH	O of C=O	2.73
5c	-9.90	3	Tyr-600…….OH	O of OH	2.68
			Tyr-600…….OH	O of SO_2	2.89
			Asp-174……OH	N of NH	2.99
5d	-10.66	2	Tyr-600…….OH	O of SO_2	2.29
			Asn-5978…..NH	N of NH	2.99
5e	-10.01	4	Lys-583……NH	O of C=O	2.40
			Tyr-600…….OH	N of NH	2.72
			Tyr-600…….OH	O of C=O	2.96
			Tyr-600…….OH	O of SO_2	2.99
5f	-11.05	4	Asn-598…….NH	O of SO_2	2.45
			Tyr-579…….OH	O of SO_2	2.73
			Tyr-600…….OH	O of OH	2.89
			Tyr-579…….OH	O of OH	2.99
5g	-11.40	5	Tyr-579…….OH	O of NO_2	2.13
			Lys-583……NH	O of NO_2	2.37
			Tyr-600…….OH	O of OH	2.54
			Asn-598…….NH	O of C=O	2.80
			Asn-598…….OH	N of NH	2.90
5h	-10.34	4	Lys-548……NH	O of C=O	2.05
			Glu-546…….OH	O of OH	2.72
			Asn-543…….OH	N of NH	2.78
			Leu-603…….OH	N of NH	2.99
5i	-9.91	4	Tyr-579…….OH	O of SO_2	2.65
			Tyr-579…….OH	O of SO_2	2.80
			Tyr-600…….OH	O of OH	2.80
			Tyr-600…….OH	N of NH	2.99
5j	-10.69	3	Leu-544…….NH	O of SO_2	2.03
			Gly-406…….NH	O of OH	2.30
			Asn-397…….NH	O of C=O	2.72
Quercetin	-8.47	4	Gly-397…….OH	O of OH	1.99
			Ala-292…….NH	O of OH	2.13
			Arg-396…….NH	O of OH	2.33
			Gly-291……..OH	O of OH	2.96
Resveratrol	-10.02	1	Phe-542……..OH	O of OH	2.39

Dock score values combined with hydrogen bonds formed with the surrounding amino acid residues help to predict the correct binding geometry for each binder at the active site. In order to compare the binding affinity of the synthesized sulfonamide derivatives, we docked them into the empty binding site of the experimentally known crystal structure of the DNA topoisomerase II as a target for anticancer compounds. The binding affinities of the ligand was evaluated with energy score (kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand enzyme complex. Number and length of the hydrogen bond were used to assess the binding models. The results of the docking studies, dock scores, and involved amino acids interacted ligand moieties, number of hydrogen bonds and hydrogen bond length for each compound and the reference native ligand are listed in Table 2. Figure 1 is the image showing binding pose of **5g** in the active site of DNA Topoisomerase II and figure 2 is the image of compound **5g** showing H-bonds with the amino acids of the active site Of DNA Topoisomerase II. Analysis of docking results revealed that:

(1) The topoisomerase II inhibitors, quercetin and resveratrol fits in the active site of DNA topoisomerase II protein and has dock score -8.47 and -10.02kcal/mol respectively (Table S2). Quercetin forms four hydrogen bonds between

O of the OH group with GLY 397, ALA 292, ARG 396 and Asp 175 of bond length 1.99 Å, 2.13 Å, 2.33 Å and 2.96 Å respectively whereas resveratrol forms only one hydrogen bond between PHE 542 with O of the OH group of bond length 2.39 Å.

(2) For sulfonamide derivatives **(5a-j)** (dock scores, -8.50 to -11.40 kcal/mol) a high negative score was estimated for the 3-NO_2 substituent (compound **5g)**, the most active compound in the series with **5** hydrogen bonds, while the other derivative with 2-hydroxy (compound **5a**) was found to have little less negative dock scores with seven hydrogen bonds .

(3) Inspection of the binding mode also demonstrated that all compounds show from one to seven hydrogen bonds with the enzyme active site residue. Also all the target compounds elicited quite good binding affinities to the active site of DNA topoisomerase II as compared to quercetin and resveratrol.

CONCLUSION

The objective of the present study was to synthesize and investigate the anticancer activity of some novel compounds carrying the biologically active sulfonamide moieties via molecular docking. Most of the synthesized compounds showed good binding affinity with better dock score than Quercetin and Resveratrol as reference drug, especially compounds **5a, 5b, 5f** and **5g**. N-(4-(N((2-hydroxynaphthelen-1-yl)phenyl)methyl) Sulfamoyl) phenyl)acetamide derivatives were synthesized and characterized by analytical IR, 1H NMR, and mass spectral studies. All the compounds were virtually screened for their antiancer property via molecular docking using Arguslab 4.0.1. Docking study of synthesized sulfonamide derivatives proved them potential DNA topoisomerase II inhibitors although a systemic biochemical study is necessary to confirm the findings. When synthesized sulfonamide derivatives were docked with DNA topoisomerase II then few among 10 synthesized molecules were found better as compared to the reference drugs. So it could be concluded that these derivatives may be proved to be the good inhibitors of DNA topoisomerase II for the anticancer activity. On comparing the chemical structure of synthesized derivatives with reference drugs, a pre-existing DNA topoisomerase II inhibitor; there is no structural similarity found, so it is concluded that this system may be proved a novel class of this category.

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