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UNLEASHING APOPTOTIC ROLE OF NOVEL QUINOLINE, BENZAMIDE AND NAPHTHOQUINONE DERIVATIVES THROUGH PHARMACOINFORMATIC APPROACHES

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ABSTRACT

One main reason for which available anticancer treatments go impractical is their unwanted intervene in cellular and molecular mechanisms of normal cells and tissues, arbitrating their functions in a manner that ends in secondary ailments. This can be avoided through targeted actions of ligand with tumor proteins. In current study high throughput pharmacoinformatics approaches were implied to identify promising role of novel benzamide, quinoline and naphthoquinone compounds in apoptotic pathway.A series of compounds based on benzamide, quinoline and naphthoquinone groups, was designed and screened for their specific action on potential target protein in apoptosis. Molecular docking analyses of compounds was done after their interaction with protein B-cell lymphoma 2 (BCL-2), and, leads were identified.Pharmacoinformatics tools were applied to check drug likeness, binding affinities, structural properties and ADMET profiles of leads. Affluent observations were recorded and marked effects by lead compounds were observed in activating apoptosis through inhibiting active sites of B-cell lymphoma 2 protein.The obtained inferences in the light of findings will be

used for characterizing synthesized selected leads in their subsequent functional analyses, and, this would be helpful in determining site directed cancer protein inhibitor.

Keywords: Pharmacoinformatics, quinoline, naphthoquinone, molecular docking, apoptosis, BCL-2.

INTRODUCTION

The conventional anticancer agents currently used go challenging for healthy cells too, besides killing tumor cells. Knowledge of adverse effects and toxicities associated with present anticancer agents is imperative for designing and scheming new therapies and active combinations with low or no toxicological profile [1]. Main reason of failure of even potent drug candidate is the disappointed pharmacokinetic and pharmacodynamic profile. Therefore, applying pharmacoinformatics tools for in-silico prediction of pharmacokinetic and pharmacodynamic profiles, before synthesis of compounds, is a relatively innovative way to avoid such failures to happen [2, 3]. A gradual and sustained increase in computational technologies has been seen in recent years. This also involves use of newer and newer approaches of bioinformatics for structure based studies where one molecule acts as ligand and other, mainly of protein origin, serves as the binding site for candidate ligand [4].Around the globe, excessive reserves are being endowed in impediment, diagnosis and management of cancer. A number of studies underwent in past decade on molecular characterization of anticancer agents form plant sources and more focus was put on to design combinatorial therapies, small inhibitory molecules and antibody drug conjugates which have more precise effects on cellular mitochondria.

Berberine, a potent molecule obtained from various Chinese herbs, is a quinoline in nature and belongs to heterocyclic aromatic nitrogen compound. Derivatives of isoquinolines compounds are widely renowned for their extensive application as anesthetic, antihypertensive, vasodilator, anti-proliferative, anti-cancerous, antiarrhythmic, anti-diarrheic, antioxidant and anti-inflammatory [5-8]. This wider spectrum of quinolines's activities may be attributed to their synthetic flexibility which offers an extensive range of structurally sundry products. Amides are another well researched and significant group of compounds having amide linkage, which is a very important functional group. The amide linkage occupies a special place in biochemistry owing to its role as part of number of macromolecules and polymers.

Research studies have reported many substituted benzamides which have different pharmacological activities such as antibacterial, antifungal, antitumor, antipsychotic, and many more [9-12].

In current study derivatives of quinoline, benzamide and quinone were designed computationally and docked with B-cell lymphoma-2 protein which has a vital role in cancer. A variety of cell signals have role in programmed cell death which is manifested by two major executioner pathways; one of which is demonstrated through mechanism of caspases and the other one is characterized through mitochondrial dysfunction. The members of Bcl-2 family are recognized for their decisive role in fate of cell; be it death or survival and, this family contains both pro-apoptotic and anti-apoptotic participants [13]. So, the structural analogs of compounds were analyzed through pharmacoinformatic tools for their site directed actions against Bcl-2 protein.

MATERIALS AND METHODS

Quinoline, benzamide and naphthoquinone compounds were designed with the intent to make them lipophilic to be able to penetrate inside the mitochondria and interact with protein participating in tumor pathways. For this reason structural changes were done and favorable functional groups were added in basic rings thorough computational techniques.

DESIGNING OF COMPOUNDS:

The compounds were designed with the aim of targeted action of basic moiety on cancer protein, and, the changes in the structures of compounds were made after extensive data study. A majority of currently available compounds exhibit their anti-cancerous role through enzyme inhibition whereas; the subcellular functions continue being regulated by the interactions of proteins. For this reason, inhibitory molecules are needed to block the pathways of proteins involved in cancer. Structural amendments were done on precursor berberine molecule to generate quinoline compounds and some of those were joined with vitamin k; a naphthoquinone with known anti-tumor activities. For benzamide derivatives, modifications were done on methyl p-hydroxybenzoate, followed by a series of substitution reactions.

PREPARATION OF TARGET PROTEIN STRUCTURE AND LIGANDS LIBRARY FOR DOCKING PROCESS:

The three dimensional structure of BCL-2 (PDB ID: 4LVT) was taken from Protein Data Bank (PDB). The structure of protein was checked and corrected through t-leap using ff99SB force field. Several

corrections were needed to prepare input files for molecular docking simulation program. All necessary corrections such as addition of hydrogen and missing atoms, removal of irregular amino acids, water molecules, hetero-atoms and bad clashes etc., were made and energy minimization was done by smearing AMBER ff12SB as a force field in Chimera molecular modeling tool. Open babel was used followed by utilizing Hyperchem's MM+ force field and, the 2D structure of protein was converted to mol2 format [14, 15]. Prepared .com file containing three-dimensional Cartesian coordinates of every compound atom, was used as input. Input file was run for optimization geometry procedure. Output file contained compounds with optimized geometry. From a series of given molecules designed computationally, a total of 6 leads were found active for the target protein BCL-2. The library collection was made on mcule online server with their chemical structures.

BINDING SITE ANALYSIS OF TARGET BCL-2:

The analysis of the protein binding site of target protein was done, and, for this purpose certified servers were used to estimate binding site moieties. The BLC-2 with UniProt ID P1045, resolution 2.5 °A, R free-value 0.222, R-work value 0.017 of length 166 was docked with all six molecules. The structural weight of the crystal protein complex was 40716.69. It was found that amino acids Gly142A, Tyr199A, Met112A, Tyr105A, Leu134A, Phe101A, Asp108A and Ala146A certainly participated in interactions with all six inhibitors. A binding pocket was seen serving an active site for the efficient binding of protein [16].

MOLECULAR DOCKING:

Docking work was executed using protein-protein docking software through AutoDock 4.2 established in an operating system Linux. All the compounds were analyzed one by one for specific binding conformations on target protein through automated mechanism. Target protein was assigned with polar hydrogen atoms, Gasteiger partial charges and Kollman charges whereas non-polar hydrogens were incorporated for quinolines, quinones and benzamides. Interactions of protein and compounds were foreseen and evaluated. AutoDock 4.2 works proficiently by giving 100 most plausible forecasts out of thousands of contestants on the basis of electrostatic complementarity, geometry of the molecular appearance and hydrophobicity. Post docking analysis was performed and compounds with promising binding energy were designated as the docked pose of quinolines, quinones and

benzamide, with the lowermost energies [16-18].

DRUG LIKENESS IDENTIFICATION:

The concept of drug likeness is an aspect in drug designing, used to identify the drug like properties of a compound, even before it is synthesized. Drug likeness is identified on the basis of certain characteristics such as bioavailability and toxicity [19, 20]. The compounds identified in this study were checked on the basis of Lipinski's rule of five and also their drug likeness properties were found out.

PREDICTION OF ADMET PROPERTIES:

Previously, it has been the practice of the scientists to work on the absorption, distribution, metabolism, elimination and toxicity (ADMET) profile of new compounds at the end of the drug discovery, or during preclinical trials. For this reason, many potent drugs could not reach their final phase owing to their poor pharmacokinetic and pharmacodynamic profiles. In current study, the ADMET properties of the compounds were predicted using AdmetSAR online server [21, 22].

MOLECULAR DOCKING ANALYSIS OF COMPOUNDS WITH TARGET PROTEIN:

Molecular docking studies were done on designed compounds and target protein. All the selected leads designed in this work, found to bind with active binding sites, with high affinities. The docked poses of compounds showed strong interactions with important key residues inside the hydrophobic grooves. The docking analysis was done using Autodock-4.2.

A total of six leads were selected for synthesis on the basis of their drug-like properties as defined under Lipinski's Rule of Five [2], and, their binding energies with target receptor, and, these included; 9-hydroxy-10-methoxy-5,6-dihydro-[1,3]dioxolo [4,5-g] isoquinolino [3,2-a] isoquinolin-7-ium; BBR4, 4,4'-(propane-1,3-diylbis(oxy)) bis (N,N-diethylbenzamide); DP1, 10-methoxy-9-(nicotinoyloxy)-5,6-dihydro-[1,3] dioxolo [4,5-g]isoquinolino [3,2-a] isoquinolin-7-ium; BNA; 10-methoxy-9-((4-methoxybenzoyl) oxy)-5,6-dihydro-[1,3]dioxolo[4,5-g] isoquinolino [3,2-a] isoquinolin-7-ium; BNS, N,N-diethyl-4-(3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl) butanamide ; KGD, and, 10-methoxy-9-((4-(3-methyl-1,4-dioxo-1,2,3,4-tetrahydronaphthalen-2-yl)butanoyl)oxy)-5,6-dihydro-[1,3]dioxolo[4,5-g]isoquinolino[3,2-a]isoquinolin-7-ium chloride; KGB.

RESULTS

The structures of selected and analyzed compounds were drawn on ChemDraw Ultra version 12.0.2. The simplified molecular-input line-entry system (SMILES) formats of structures were taken from ChemDraw Ultra 12.0.2. Compounds structures with their codes and SMILES formats are arranged in table 1. Molecular drug-like characteristics of compounds and, drug-likeness scores along with molecular weights of compounds are given in table 2. The output shows that most of the compounds followed the proposed limit validating their drug likeness, and, obeyed Lipinski's rule of 5. Drug-likeness scores were estimated through Molsoft tool (figure 1). ADMET profiles of compounds were determined through AdmetSAR; online cheminformatics tool. The LogPs of compounds felled in between 2.8 to 5.6 in the present examination. The most hydrophobic molecule amongst all examined compounds was the one with the highest logP value of 5.6. Pharmacokinetics table 3, of all compounds declares that they have good oral bioavailability. The 3D structure of BCl-2 was analyzed for identification of binding sites, structural masses, and interacting residues [16]. AD 4.2 was used for docking of library of ligands with target. This tool predicted the mechanism and way of connection of ligands with the target receptor (figure 2). The docked conformations of the compounds were studied on the basis of their binding affinity with the target, hydrophobic interactions and number of hydrogen bonds (table 4 and figure 3). Binding analysis showed actively bound compounds in the pocket of BCL-2 (figure 3). The pharmacological and compositional parameters of each compound such as hydrogen acceptors and donors and other distinct parameters were taken through Molinspiration.

Table 1: Compounds structures with their codes and SMILES formats

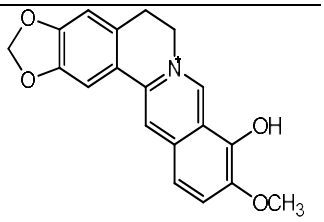
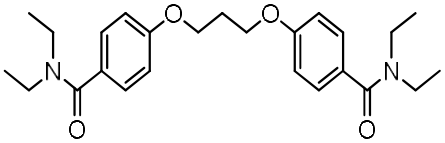
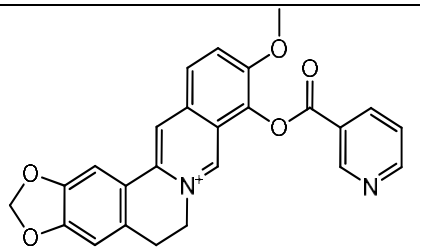
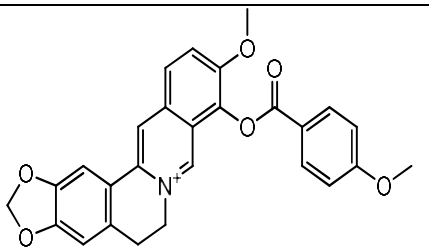
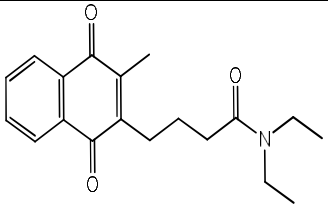
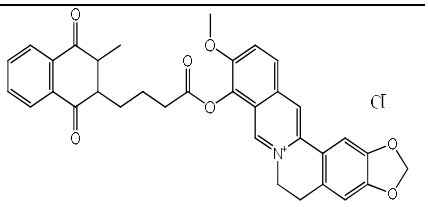
Compound	SMILE format	Structure
BBR4	**OC1=C(OC)C=CC(C1=C2)=CC3=[N+]2CCC4=CC(OCO5)=C5C=C43**	
DP1	**O=C(N(CC)CC)C1=CC=C(OCCCOC2=CC=C(C(N(CC)CC)=O)C=C2)C=C1**	
BNA	**COC1C(OC(C2C=CC=NC=2)=O)=C2C(C=C3[N+](=C2)CCC2=C3C=C3C(=C2)OCO3)=CC=1**	
BNS	**O=C(C1=CC=C(OC)C=C1)OC2=C3C=[N+](C4=CC3=CC=C2OC)CCC5=C4C=C(OCO6)C6=C5**	
KGD	**CC1=C(CCCC(N(CC)CC)=O)C(=O)C2=C(C=CC=C2)C1=O**	
KGB	**CC1=C(CCCC(OC2=C3C(C=C4[N+](=C3)CCC3=C4C=C4C(=C3)OCO4)=CC=C2OC)=O)C(=O)C2=C(C=CC=C2)C1=O**	

Table 2: Drug like properties of compounds.

Compd.	MW.	LogP	LogS	HBA	HBD	RB	PSA	Violations	R-value	No. of Atoms	Heavy Atoms	Drug-Likeness Score
BBR4	322.333	2.7933	-2.778	5	1	1	51.8	0	90.405	40	24	0.93
DP1	426.547	4.4985	-3.5985	6	0	14	59.08	0	123.584	65	31	0.15
BNA	427.427	3.7019	-3.0516	7	0	4	70.76	0	117.577	51	32	1.67
BNS	456.465	4.3155	-2.9235	7	0	5	67.1	0	126.274	56	34	1.32
KGD	313.39	3.4208	-3.9769	4	0	7	54.45	0	90.602	46	23	0.65
KGB	562.586	5.5591	-3.2376	8	0	7	92.01	2	156.431	70	42	1.03

Drug likeness properties of compounds are tabulated where; Mw: Molecular weight; HBA: Hydrogen Bond Acceptor; HBD: Hydrogen Bond Donor; RB: Rotatable bonds; PSA: Polar Surface Area.

Table 3: Predicted pharmacokinetics profiles of compounds.

ADMET Property	BBR4	DP1	BNS	BNA	KGD	KGB
BBB	+	+	+	+	+	+
HIA	-	+	+	-	+	-
Caco-2 Permeability	+	+	+	+	+	+
P- glycoprotein Substrate	-	+	-	-	+	+
Substrate Inhibitor	-	+	-	-	+	-
P- glycoprotein Inhibitor	-	-	-	-	-	+
ROCT	+	-	+	-	+	-
CYP450 2C9						
Substrate	-	-	-	-	-	-
Inhibitor	-	-	-	-	-	-
CYP450 2D6						
Substrate	-	-	-	-	-	-
Inhibitor	+	-	+	+	-	+
CYP450 3A4						
Substrate	+	+	+	+	+	+
Inhibitor	-	-	+	+	+	+
CYP450 1A2 Inhibitor	+	-	+	+	+	+
CYP450 2C19 Inhibitor	-	-	-	-	+	-
CYP IP	High	High	High	High	High	High
HERG Inhibition						
HERG-1	Weak	Weak	Weak	Weak	Weak	Weak
HERG-II	-	-	-	-	+	-
Ames Toxicity	-	-	-	-	-	-
Carcinogens	-	-	-	-	-	-
TPT	High	High	High	High	High	High
HBT	Low	Low	Low	Low	Low	Low
Biodegradation	-	-	-	-	-	-
AOT	III	III	III	III	III	III
Carcinogenicity (Three-class)	-	-	-	-	-	-
Aqueous Solubility; Log S	-2.778	-3.5985	-2.9235	-3.0516	-3.9769	-3.2376
Caco-2 Permeability; Log Papp, cm/s	0.9921	1.232	1.2645	1.2015	1.4037	0.8457
TPT;pIGC$_{50}$, µg/L	0.3542	0.454	0.3362	0.419	0.4949	0.3604

Abbreviations used: ADMET: Absorption Distribution Metabolism Elimination and Toxicity; BBB: Blood Brain Barrier; HIA: Human Intestinal Absorption; ROCT: Renal Organic Cationic Transporter; CYP450: Cytochrome P450; HERG: Human Ether-a-go-go Related Gene; TPT: Tetrahymena Pyriformis Toxicity; HBT: Honey Bee Toxicity; AOT: Acute Oral Toxicity

Table 4: Molecular interactions of compounds with target BCL-2

Compound	Interacting Residues	Hydrogen Bonds Residues/ Distance A°	Number of Hydrophobic Bonds	Total No. of Bonds	Binding Energy of compound
BBR4	**Asp108, Phe101, Glu149, Phe109, Val153, Ala146, Phe150, Val130, Asp137, Arg143, Glu133, Met112, Leu134**	***Asp137/ 4.60***	**2**	**3**	**-7.7**
DP1	**Tyr199, Gly142, Ala97, Trp141, Phe109, Asp108, Arg143, Met112, Val130, Phe150, Leu134, Ala146, Val145, Phe195, Leu198, Phe101, Tyr105**	***NIL***	**10**	**10**	**-7.1**
BNA	**Val130, Glu133, Phe150, Glu149, Val153, Phe109, Phe101, Asp108, Gly142, Arg143, Asn140, Leu134, Ala146, Met112**	***Arg143/ 5.14, Asn140/ 5.42, Glu133/ 3.53***	**6**	**9**	**-8.3**
BNS	**Val130, Phe150, Glu149, Val153, Phe101, Asp108, Phe109, Glu133, Gly142, Asn140, Met112, Ala146, Arg143, Leu134**	***Glu133/ 3.91, Phe109/ 4.33***	**7**	**9**	**-8.6**
KGD	**Trp141, Phe195, Tyr199, Val145, Gly142, Asn140, Arg104, Asp100, Ala146, Arg143, Leu198, Ala97, Phe101, Tyr105**	***Gly142/ 3.66***	**4**	**5**	**-7.4**
KGB	**Phe109, Val153, Glu149, Phe150, Leu134, Arg143, Asn140, Asp108, Phe101, Gly142, Arg104, Val145, Ala97, Phe195, Tyr199, Leu198, Ala146, Met112, Tyr105**	***Leu198/ 4.32***	**4**	**5**	**-10.9**

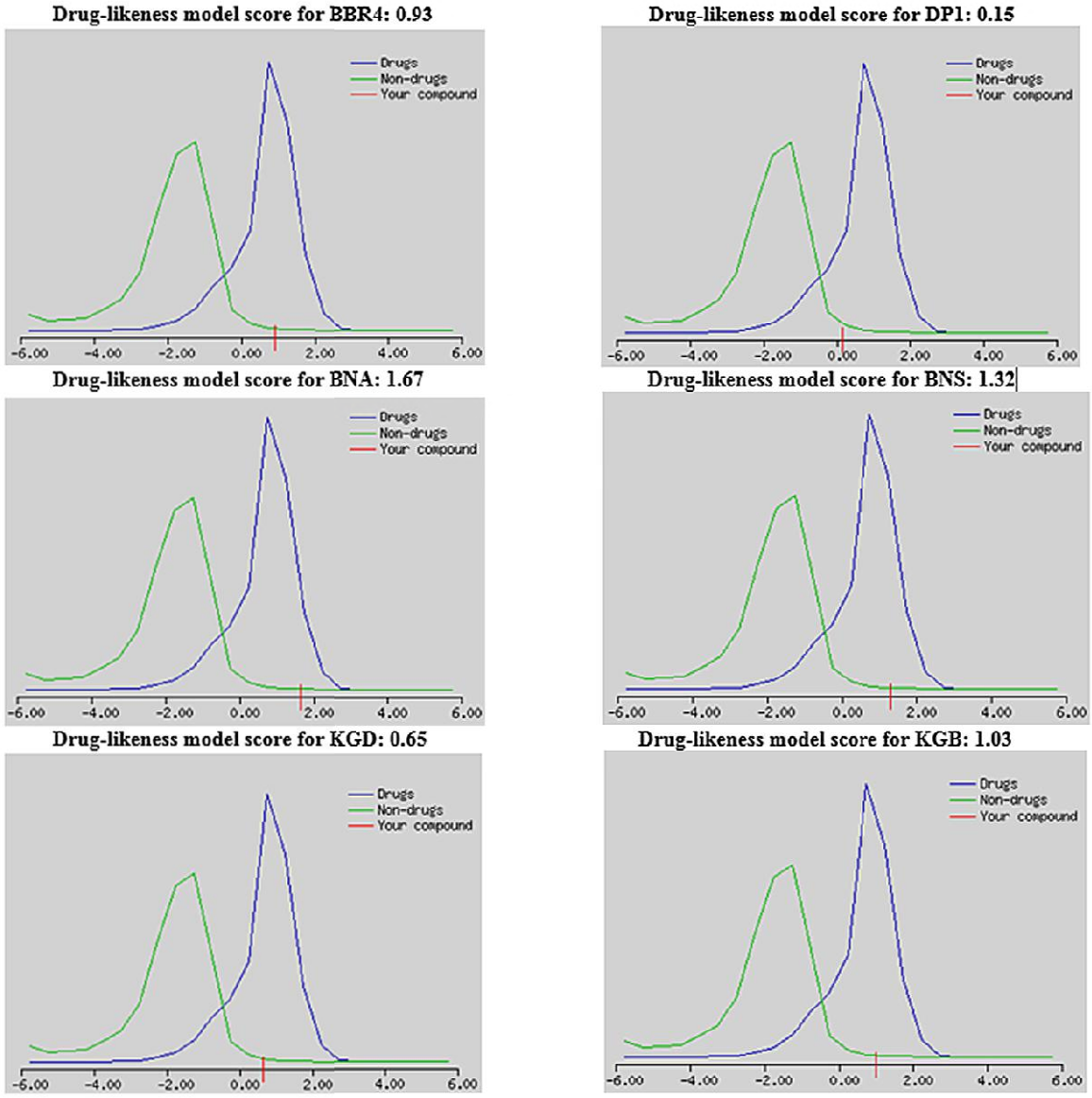


Figure1. Drug likeness scores of compounds: Scores were estimated through Molinspiration software.

Figure 2-I

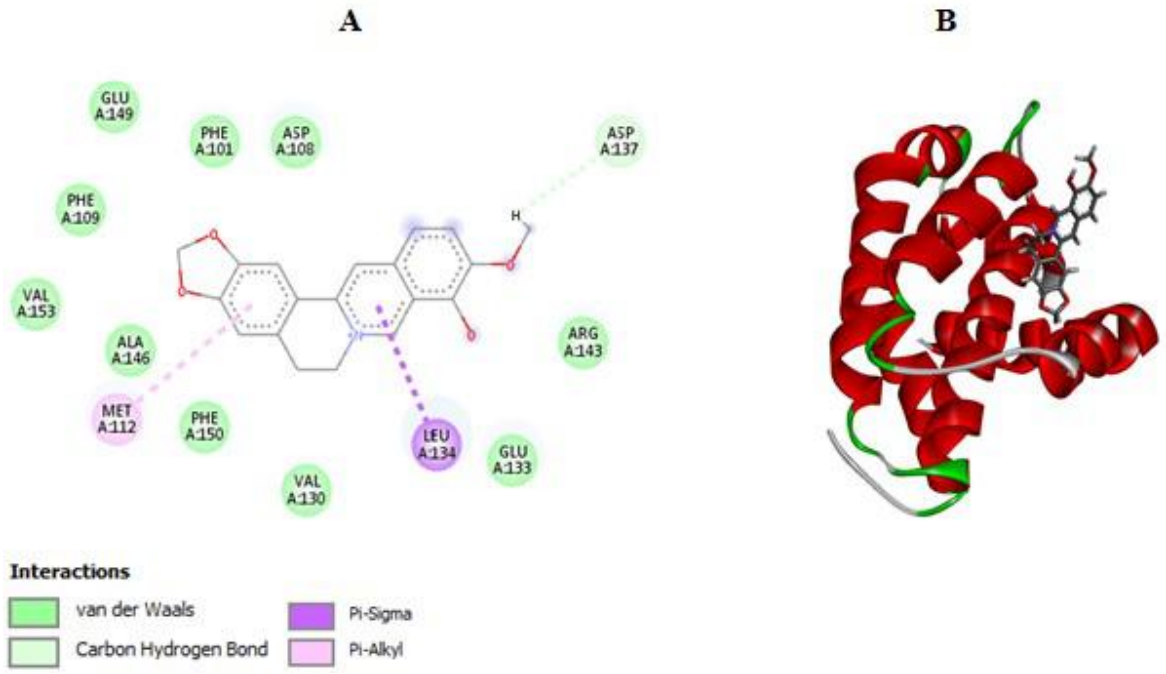
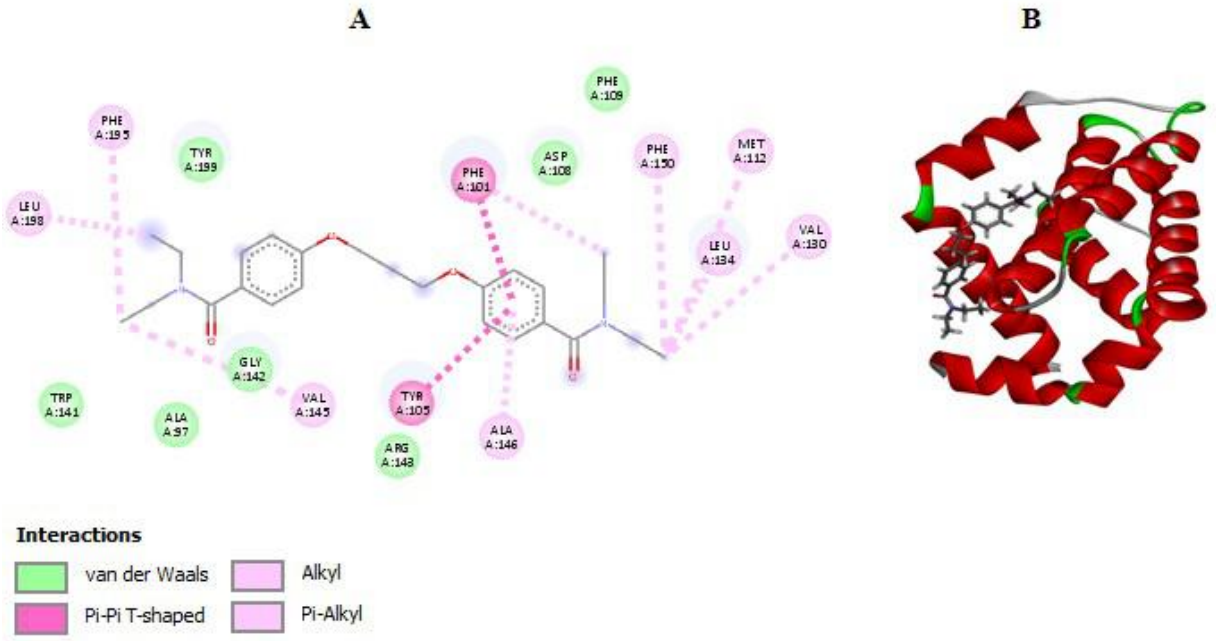


Figure 2-II



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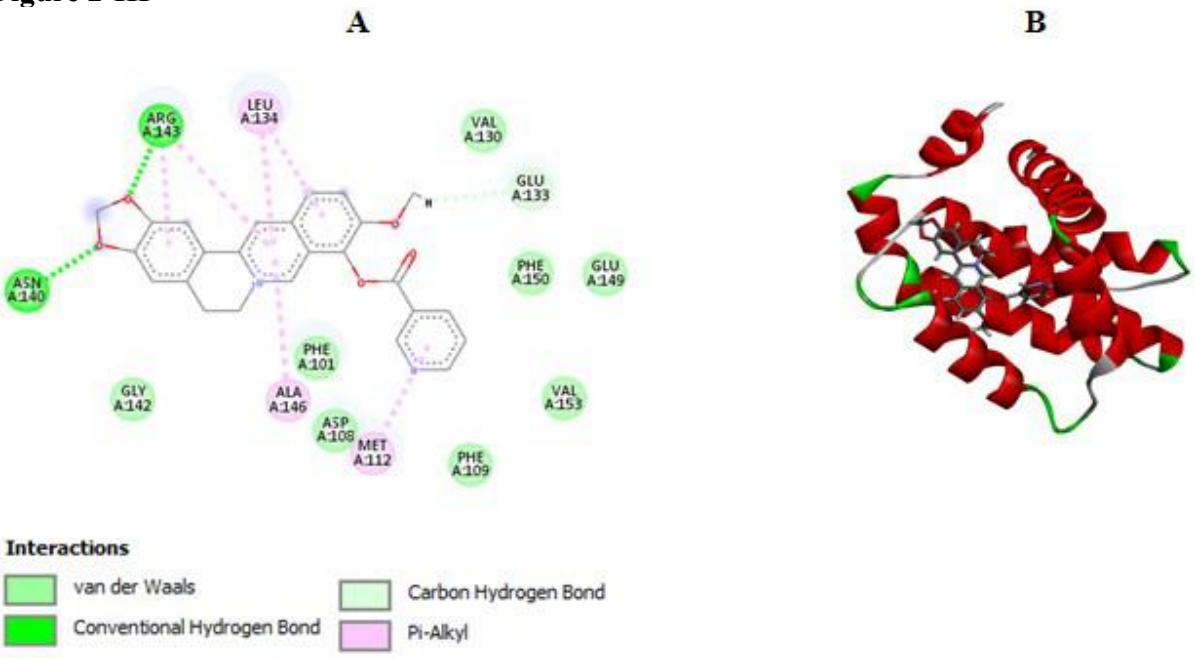
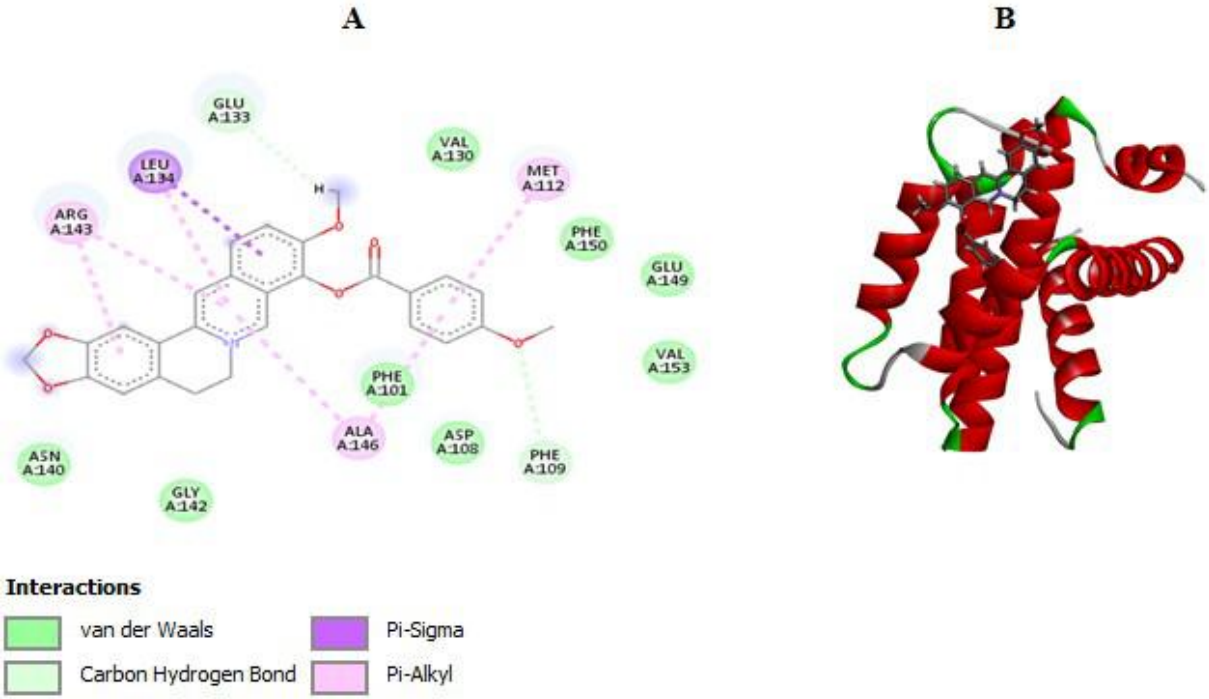


Figure 2-IV



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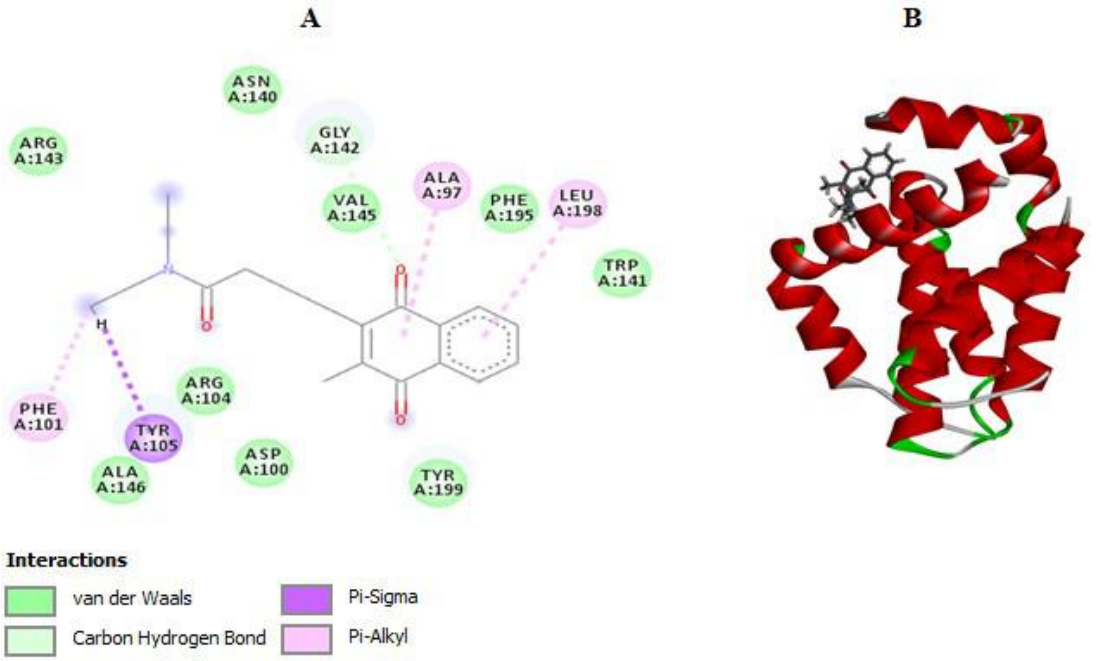

Figure 2-VI

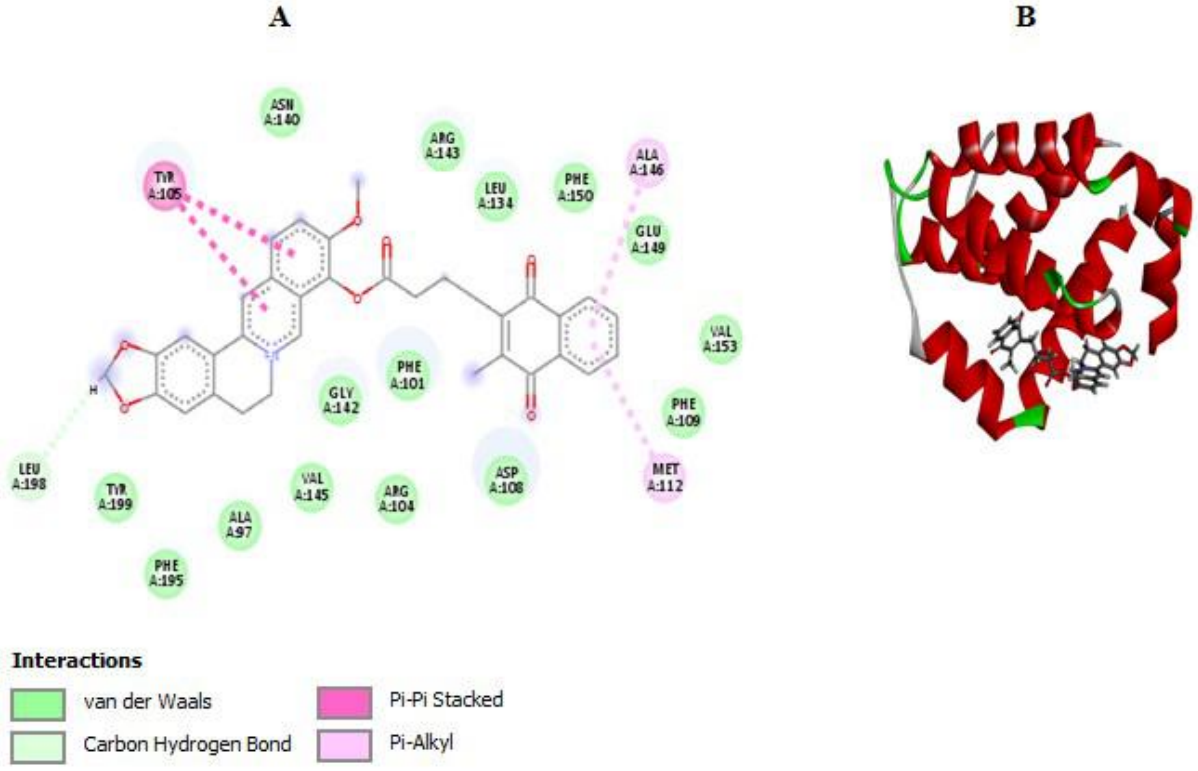

Figure:02. Bound compounds with the target receptor. Whereas figures 2-I, 2-II, 2-III, 2-IV, 2-V and 2-VI represent binding patterns of compounds BBR4, DP1, BNA, BNS, KGD and KGB respectively. A shows the interaction of ligands with the binding residues and B is the bound pose of ligand with the target.

Figure 3-I

A B

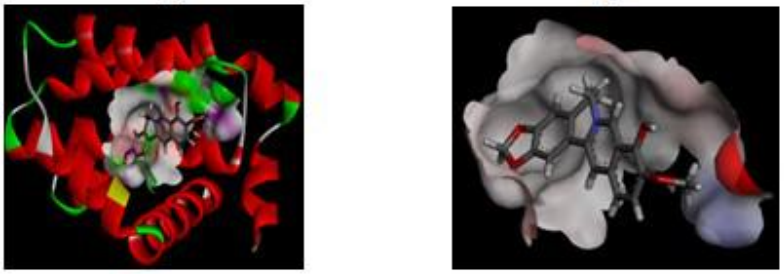

Figure 3-II

A B

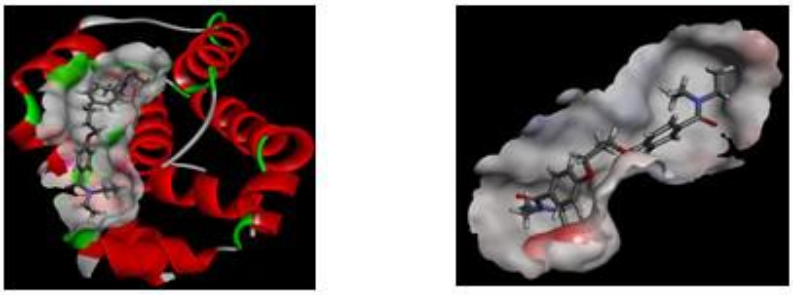

Figure 3-III

A B

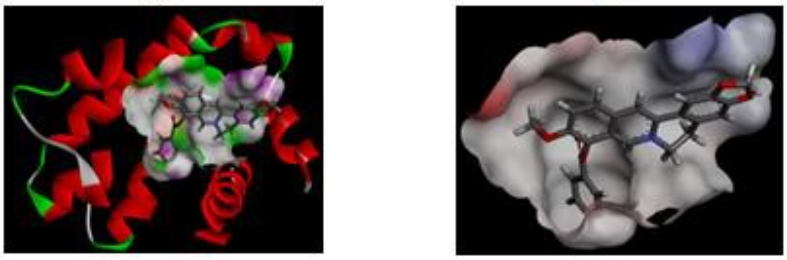

Figure 3-IV

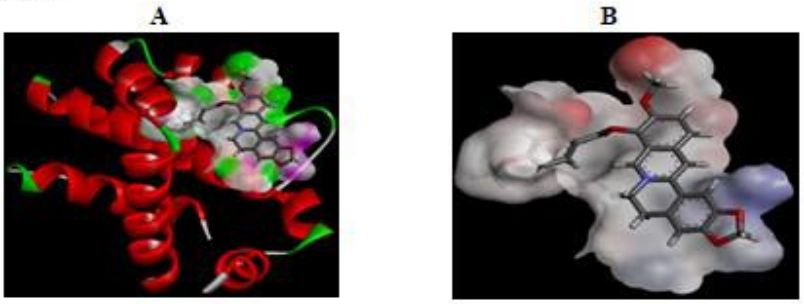

Figure 3-V

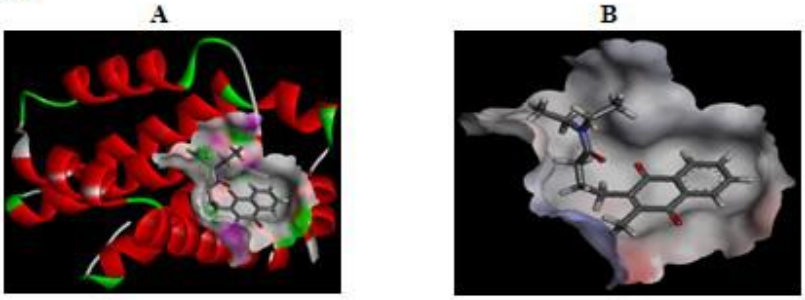

Figure 3-VI

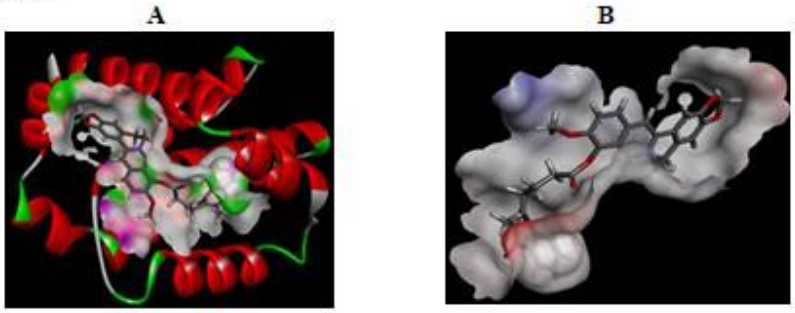

Figure:03. Binding pockets and receptor surface interactions of ligands BBR4, DP1, BNA, BNS, KGD and KGB (figures 3-I, 3-II, 3-III, 3-IV, 3-V and 3-VI respectively) are shown with BCL-2. Whereas, A is the 3D representation of hydrogen bonds during receptor-ligand interaction and B shows the ligand binding receptor pockets.

DISCUSSION

In the current study, a library of novel compounds based on quinoline, benzamide and naphthoquinone groups was designed through structural modification and exploiting combinatorial techniques. High through-put docking tools and advanced pharmaco-informatics techniques were used to describe their proposed mechanistic pathway, pharmacokinetic and pharmacodynamic profiles. The library of molecules was designed through alterations in the side chains of the basic precursor moieties. Some combinatorial compounds were also designed by combining quinoline moieties with vitamin K, in order to identify synergistic effects, as vitamin k has some

reported evidences for anti-tumor effects [23-25, 10, 12].

Enormous achievements have been seen in drug discovery researches that effectively utilize in-silico techniques. Now it's a proven truth that these technologies have successfully offered their roles in the field of drug discovery. All derived and combinatorial compounds were pre-screened prior to synthesis and docked against BCL-2 by using online server mcule and AutoDock 4.2 [2].

Substantial research work has been conducted on quinones, quinolines and benzamides for studying their interactions with target BCL-2.

Some of the authors recommend the decrease in level of BCL-2 by berberine, benzamide and naphthoquinone and also present the proofs for apoptosis in various cancers including squamous cancer of tongue, breast cancer, liver, cervix, colon, epidermoid, glioblastoma and gastric carcinomas. These molecules validate their role in cell induced cytotoxicity, apoptosis, autophagy and cell cycle regulation [26-28]. Target protein Bcl-2 had drawn our attention to exploit the docking pattern of designed molecules, since the precursor compounds show ample data of in-vitro and in-vivo studies against Bcl-2 [29-39].

Noteworthy docking energies were exhibited by compounds after binding with target BCL-2 (table 4). The combinatorial compound based on quinoline and quinone rings presented highest binding score among all compounds i.e., -10.9 K cal/mol. Binding analyses of synthesized compounds were done with target protein Bcl-2 and total bonds along with hydrophobic bonds were analyzed for their binding residues. All bonds between DP1 and Bcl-2 were hydrophobic.

Molinspiration online server was used for identifying pharmacological and compositional characteristics of each compound. The LogPs of compounds felled in between 2.8 to 5.6 in the present examination. LogP is directly proportional to hydrophobicity, and the drug with higher LogP does not readily excrete and remains intact in the biological fluids for longer period thus increasing desired effects. The most hydrophobic molecule amongst all examined compounds was KGB with the highest LogP value of 5.6. Among all studied compounds, KGB was also found inhibitor of P-glycoprotein transporter. All the compounds examined do not show any mutagenic and carcinogenic properties and possess good bio availabilities. The drug-likeness model scores were estimated through Molsoft online server and those were

found in good range for all compounds (table 2).

Some of the potent drugs do face failure due to their poor pharmacokinetics. In silico prediction of pharmacokinetics & pharmacodynamics profile is a fashioned behavior to estimate ADMET profile which proves to be economical and time saving as well. Compounds quoted in this study possessed positive pharmacokinetics and pharmacodynamics which were predicted with various ADMET servers. AdmetSAR online software is basically used for toxic properties prediction specially carcinogenicity and mutagenicity. ADMET analysis was performed for all quinones and benzamides through AdmetSAR server for exploring their properties. Blood brain barrier penetration, aqueous solubility, human intestine absorption and cytochrome P450 inhibition are such type of properties which are effectively predicted by AdmetSAR server [40-42].

CONCLUSION

The novel quinoline, benzamide, quinone analogs and combinatorial compounds, designed through in-silico methods were pre-screened through pharmacoinformatic techniques. Noticeable ADMET profiles were predicted of all molecules; BBR4, DP1, BNA, BNS, KGD and KGB. The strong binding energies, hydrogen bonds and hydrophobic interactions exhibited by lead compounds prove them powerful inhibitors of Bcl-2 protein. Compounds were found promisingly proficient for Bcl-2 protein and the affluent results were compelling enough to execute the wet lab work. The compounds were also analyzed for the other central proteins which participate in the array of cancer development and metastasis. The results obtained through those assays will be shared in future publications.

DATA AVAILABILITY

Since the work is based on pharmacoinformatic techniques which included some bioinformatics based online servers and tools like protein data bank, mcule online server and AutoDock 4.2 for docking, discovery studio tool for visualization, AdmetSAR for ADMET profile and Molinspiration online server for drug-likeness score. All of these are available online and can be accessed by readers.

CONFLICTS OF INTEREST

Authors declare that there is no conflict of interest regarding the publication of this project.

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CONFLICT OF INTEREST

Authors declares no conflict of interest

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