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In silico studies on some of the naturally occurring hydroxy-benzoquinones, naphthaquinones and anthraquinones as potent B-raf inhibitors

Arunkumar B.[1], Annette Fernandez*[1], Shiny P. Laila[1], V. S. Vishnu[2] and Bessy Raj N.[3]

[1]*Department of Chemistry, College of Engineering Trivandrum, University of Kerala, India*
[2]*Government Arts College, Thiruvananthapuram, Kerala, India*
[3]*Government College for Women, Thiruvananthapuram, Kerala, India*

ABSTRACT

BRAF (V-raf murine sarcoma viral homologue B1) is a proto-oncogene which is a member of RAF kinase family of proteins. In this study, the comparative molecular docking of the binding affinity of hydroxy-benzoquinones, naphthaquinones and anthraquinones on crystal structure of mutated B-RAF proteins were carried out using Discovery studio 4.0 software. Docking studies revealed greater affinity of the compounds with the proteins. This may be due to the functional groups present in hydroxyquinones which are responsible for the activity. Pharmacokinetic properties were analysed using TOPKAT software which gave an insight into its ADMET parameters. Since ADMET properties and docking studies gave better results, all the compounds may be used as lead moieties in developing potential drug candidates against mutant B-RAF associated cancers.

Keywords: B-RAF, Hyroxy-benzoquinones, naphthaquinones, anthraquinones, Discovery studio-4.0, Docking, ADMET, TOPKAT.

INTRODUCTION

BRAF which is a member of the RAF family of proteins is the most frequently mutated protein kinase in human cancers **[1].** The RAF family of proteins are classified into 3 types A-RAF, B-RAF and C-RAF. Each form plays a role in the RAS-RAF pathway and B-RAF is the main activator of RAS-RAF-MEK-ERK signal transduction cascade which is depicted in Fig.1. This cascade participates in the regulation of a large variety of processes including apoptosis, cell cycle progression, differentiation, proliferation and transformation to the cancerous state **[2].** B-RAF mutations occur in melanomas, thyroid cancers and colorectal cancer.

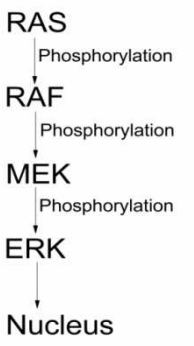

Figure 1 RAS-RAF pathway

Preclinical studies prove that mutations in the BRAF gene allow it to signal independently. As a result mutated BRAF causes overactive signaling via MEK and ERK. This leads to excessive cell proliferation and survival **[3].**

Role of Oncogenic BRAF In Cancer

Oncogenic BRAF can result from mutations in the BRAF gene. Somatic point mutations in BRAF cause the protein to become overactive. This triggers a signaling cascade that can play a role in specific malignancies. Approximately 90% of known BRAF mutations are V600E mutations **[4].** It means the substitution of glutamic acid (E) in the position of Valine (V) at V600E of the protein chain, results in an over expressed activity of mutated BRAF. Other variants include lysine (K), aspartic acid (D), and arginine(R). The V600 point mutation allows BRAF to signal independently of upstream cues and downstream cues **[5].**

As a result of constitutively active BRAF, overactive downstream signaling via MEK and ERK leads to excessive cell proliferation and survival. Independent of growth factors, oncogenic BRAF signaling may lead to increased and uncontrolled cell proliferation and resistance to apoptosis. The over activation of RAS-RAF signaling pathway by the oncogenic BRAF has been influenced by the multiple malignancy and it can be used as a potential therapeutic target in oncology **[6].** There are many tumors like melanoma, papillary thyroid , ovarian, colorectal and prostate tumors which are associated with mutated BRAF **[7].** Many drugs are available to inhibit BRAF mutations but serious side effects and low bioviability are the issues **[8].** Hence less toxic drugs which are of phytochemical origin like hydroxybenzoquinones (embelin and rapanone), naphthaquinones (lawsone, juglone and plumbagin) and anthraquinones (1, 4-dihydroxyanthraquinone and 1, 3, 8-trihydroxyanthraquinone) having wide range of pharmacological actions were selected as B-RAF inhibitors for the present work.

The molecular docking and screening process were carried out using Discovery studio 4.0 software with BRAF kinase protein (pdb id: 4MNF) as the target and the selected hydroxyquinones as the ligands.

MATERIALS AND METHODS

The crystal structure of mutated B-RAF protein was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank having the PDB code 4MNF (Fig. 2). Chain A was selected for docking. The prepared protein was energy minimized and saved as 4MNF.pdb (Fig. 3). The ligands were designed using Marvinsketch 5.3.0. The ligand preparation was done using discovery studio 4.0. The minimized receptor (BRAF) and ligand was docked with Libdock, a relatively fast algorithm that conducts 'Hotspots' matching with ligand conformation. The binding affinity of the ligands with the protein was compared with that of the standard drug GDC 0879.

(a) Preparation of the protein (Scaffold protein-4MNF)

The X-ray structure of protein containing water molecules and hetero atoms were refined using Accelrys Discovery studio 4.0 and the protein crystal structure was energy optimized after energy minimization. The protein was then saved as 4MNF.pdb and subjected to docking studies.

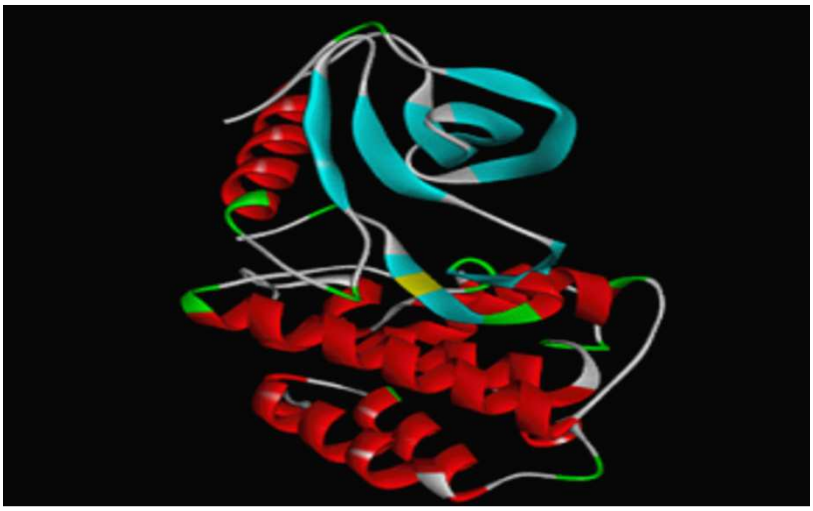

Figure 2 A chain of Ribbon structure of 4MNF

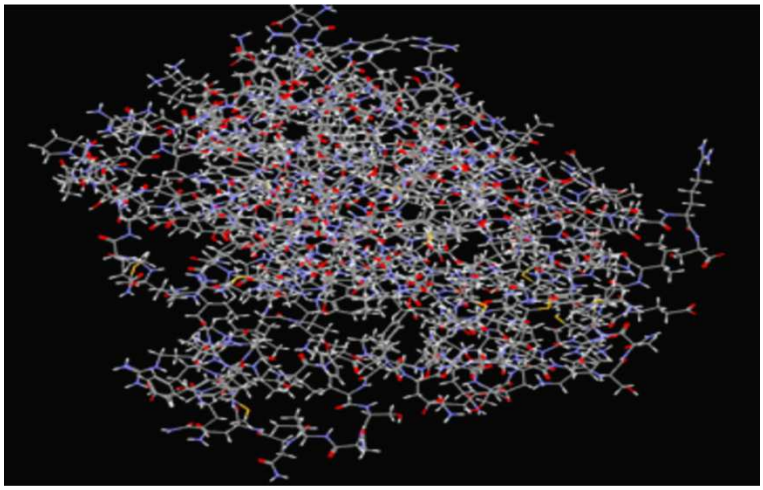

Figure 3 Prepared protein crystal structure 4MNF

(b) Preparation of ligands: hydroxy-benzoquinones, naphthaquinones and anthraquinones.
The structure of the ligands were prepared using Marvinsketch 5.3.0 and saved as sdf file, which are given in Fig. 4 **[9-15].**

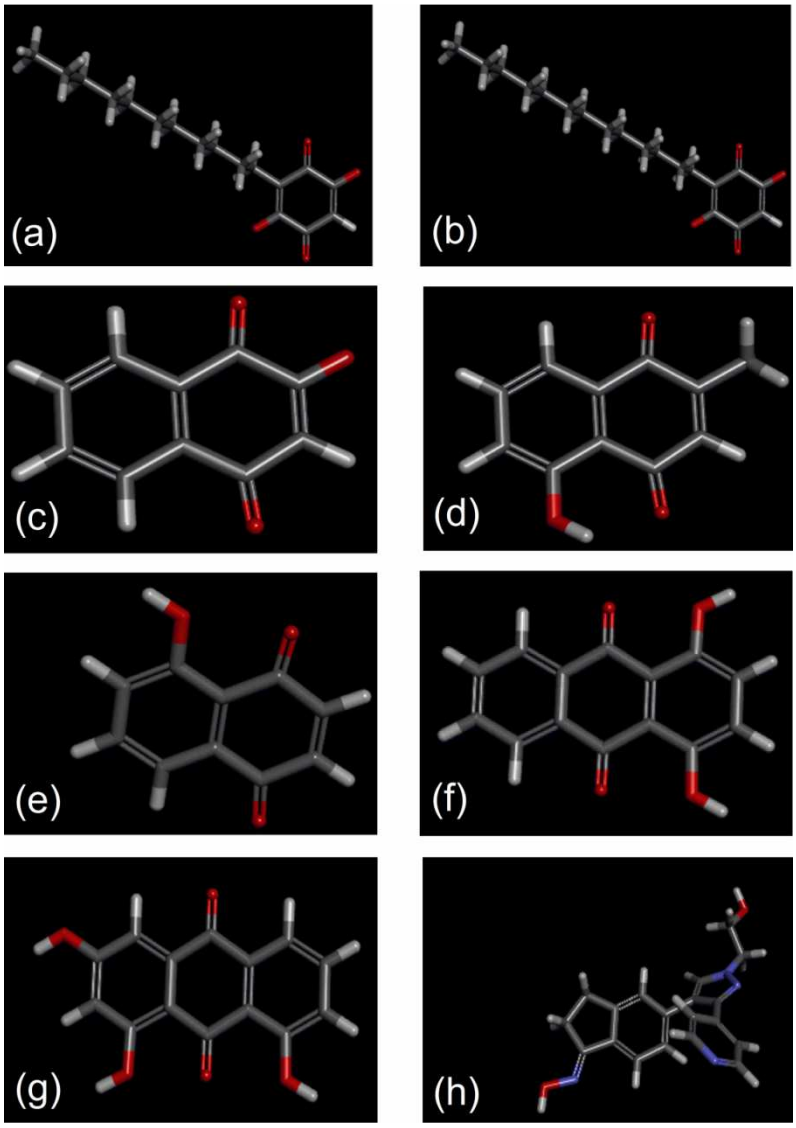

Figure 4 The prepared ligands: (a) Embelin, (b) Rapanone, (c) Lawsone, (d) Plumbagin, (e) Juglone, (f) 1, 4–dihydroxyanthraquinone, (g) 1, 3, 8–trihydroxyanthraquinone, (h) standard drug (GDC 0879)

(c) Docking methodology

The software for molecular docking used in this study was Discovery studio 4.0 (DS 4.0, Accerlrys Inc. SanDiego, CA). The docking between the ligand and protein was evaluated by using Libdock docking program. Libdock is a high-throughput algorithm for docking ligands into an active binding site on the receptor, which is also a site feature docking algorithm. The seven ligands were docked with the binding site of BRAF protein. Ligand conformations were aligned to the receptor interaction sites and the best poses were reported in the end of the docking simulations. Each pose was evaluated according to the Libdock score. The scores obtained from docking studies are given in Table 1.

Table 1 Docking scores of the ligands at the active site of BRAF kinase

Ligands	Amino acid residue				Conf. No.	Libdock score
	Electrostatic interaction	Hydrogen bonding	Hydrophobic interaction	Alkyl and pi-alkyl interaction		
Embelin	LYS A:483	ASP A: 593	LYS A:483		83	120.323
Rapanone	ASPA:594, LYS A:483	ASPA:594, LYS A:483	-	PHE A :583	52	121.04
Lawsone	-	LYS A:483	-	PHE A:583	1	76.2359
Juglone	LYS A:483	LYS A:483, ASP A:594	LYS A:483	PHE A:483	1	80.1346
Plumbagin	LYS A:483	LYS A:483	LYS A:483	PHE A:583	1	82.0419
1,4-dihydroxy anthraquinone	-	ASN A:581 GLN A:612	VAL A:471, PHE A:583	-	1	84.6445
1,3,8-trihydroxyanthraquinone	-	ASP A 594, GLN A:612, GLU A:501	-	TRP A :531	1	94.9814
GDC 0879 (Std drug)	-	GLN A:612, LYS A:483	-	PHE A:583	15	110.335

The various interactions of amino acids with the different ligands as well as their 2D plots are given in Figs. 5-12.

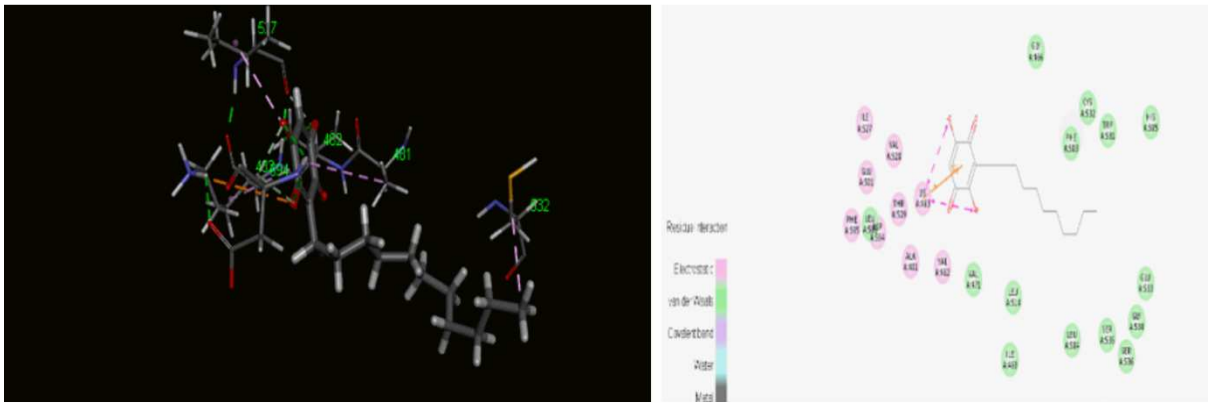

Figure 5 Docking of Embelin with 4MNF and the interactions with amino acid residues

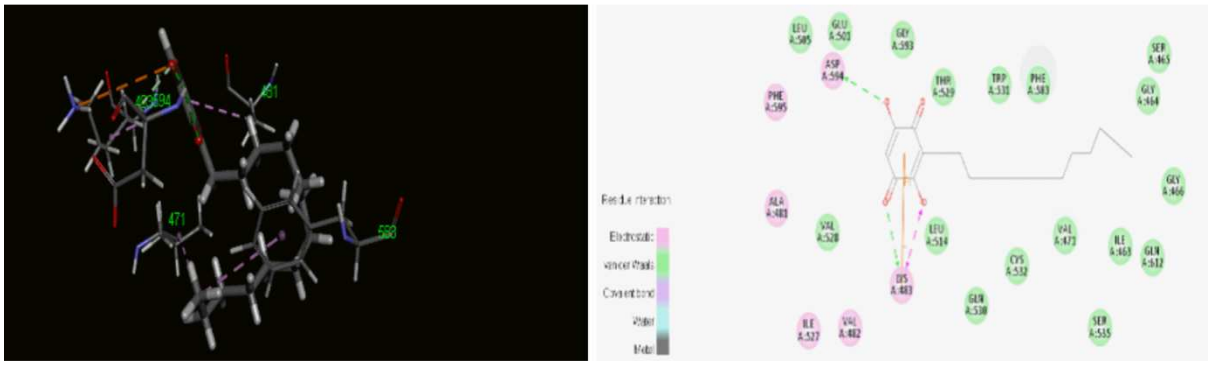

Figure 6 Docking of Rapanone with 4MNF

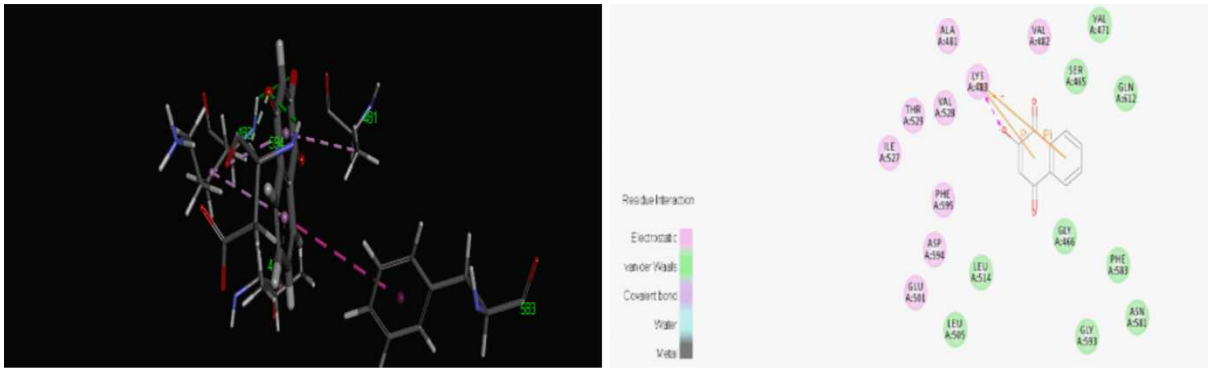

Figure 7 Docking of Lawsone with 4MNF

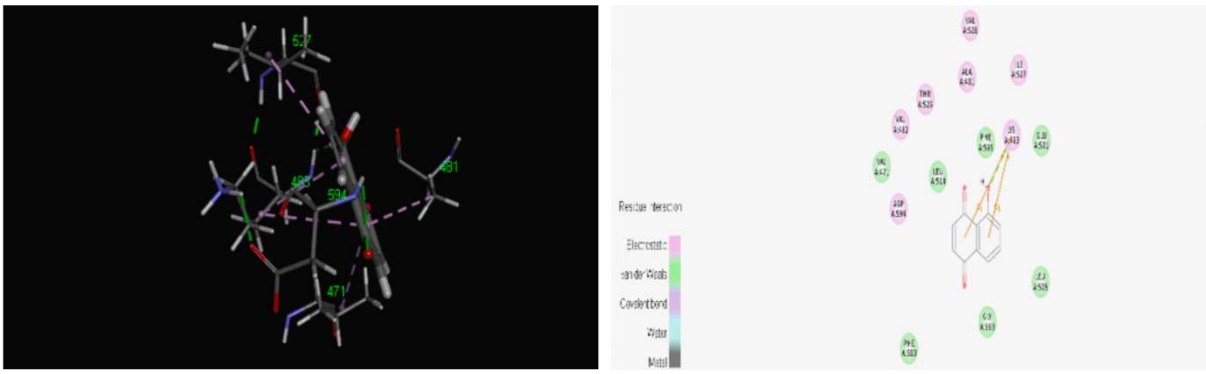

Figure 8 Docking of Juglone with 4MNF

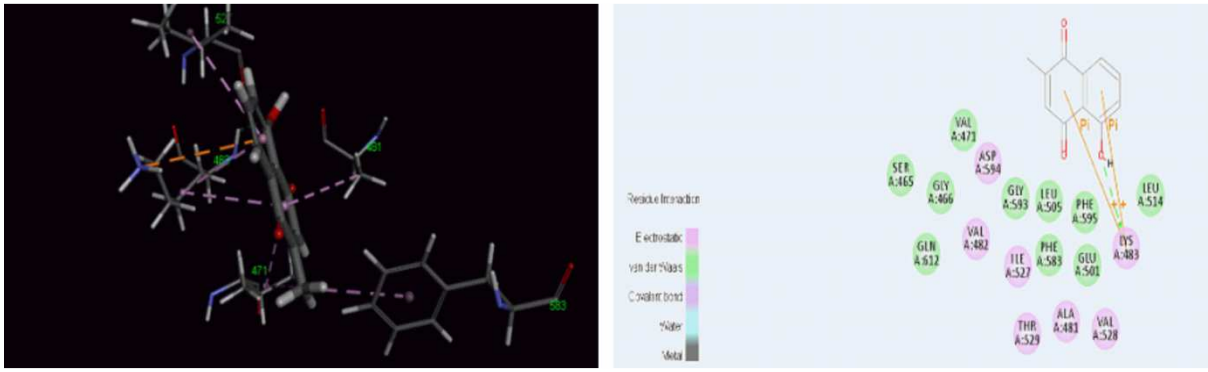

Figure 9 Docking of Plumbagin with 4MNF

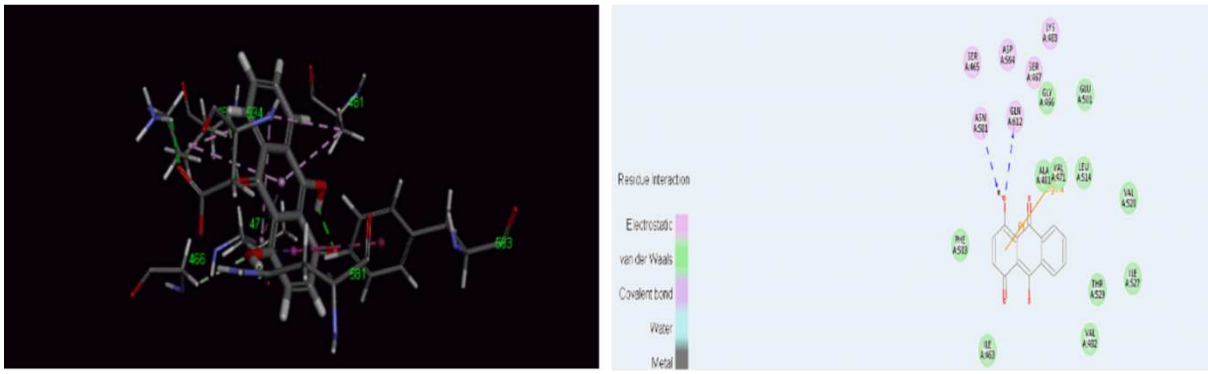

Figure 10 Docking of 1, 4-dihydroxyanthraquinone with 4MNF

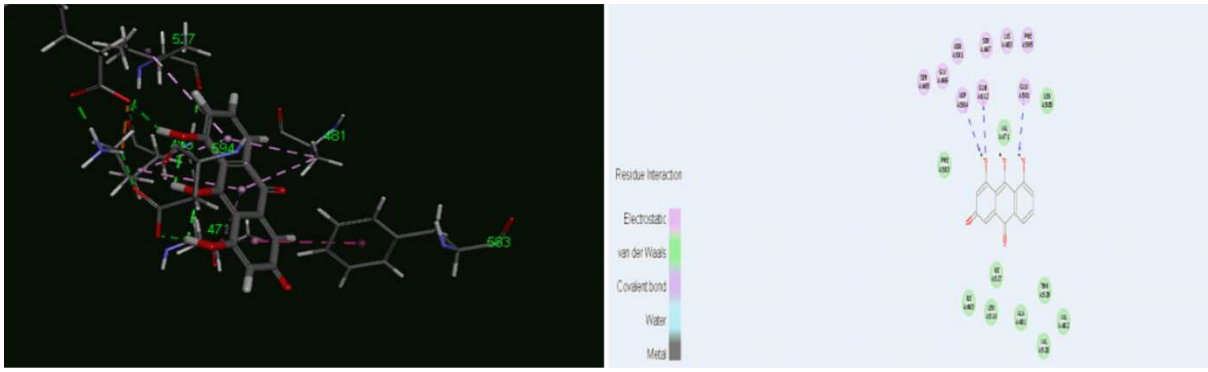

Figure 11 Docking of 1, 3, 8 Trihydroxyanthraquinone with 4MNF

Figure 12 Docking of GDC0879 with 4MNF

(d) ADMET Studies using TOPKAT

The bioavailability and drug likeness screening were evaluated by using TOPKAT, a module of Discovery studio 4.0. Using TOPKAT, the aqueous solubility, blood brain barrier penetration, cytochrome 450 2D6 binding, hepatotoxicity, intestinal absorption and plasma protein binding were evaluated by the molecular modelling software. The ADMET scores are depicted in Table 2.

Table 2 ADMET scores of the various ligands

Ligands	ADMET BBB	ADMET BBB level	ADMET Absorption level	ADMET Solubility	ADMET solubility level	ADMET_EXT Hepatotoxicity	ADMET_EXT CYP2D6	ADMET_EXT PPB	ADMETAlog P98	ADMET PSA_2D
Embelin	0.068	1	0	-3.69	3	-11.304	-1.30154	-0.59458	4.62	76.232
Rapanone		4	1	-4.261	2	-13.3086	-0.98938	-0.34515	5.533	76.232
Lawsone	-0.654	3	0	-1.915	4	-4.56655	-9.02452	-2.5644	1.22	55.417
Juglone	-0.563	3	0	-2.162	3	-2.76189	-7.35757	-3.42125	1.515	55.417
Plumbagin	-0.425	2	0	-2.659	3	-5.3918	-6.56528	-2.5675	1.962	55.417
1,4-dihydroxyanthraquinone	-0.642	3	0	-3.087	3	4.17092	-3.07329	-1.9434	20324	76.232
1,3,8-Trihydroxyanthraquinone	-1.046	3	0	-2.812	3	5.24781	-3.28708	-7.15164	2.082	97.048
GDC089	-0.689	3	0	-3.284	3	2.12641	-4.3181	0.236556	2.407	80.824

RESULTS AND DISCUSSION

Table I represents the Libdock scores of the various ligands. The docking was performed using the software discovery studio 4.0. The seven ligands were successfully docked into the predicted binding cavity of protein BRAF (4MNF) using Libdock module. GDC0897 was taken as the standard drug. Multiple conformations were generated for each compound. Among the ligands, embelin and rapanone and were found to have good Libdock scores when compared with that of the standard GDC 0879. 1, 3, 8-trihydroxy anthraquinone was comparable with that of the standard. The Libdock ranking follows the order rapanone > embelin > GDC >1, 3, 8-trihydroxyanthraquinone >1,4-dihydroxyanthraquinone > plumbagin > juglone > lawsone. According to the Libdock algorithm, higher the docking score, higher the strength and vice versa. Hence it can be inferred that the ligand, rapanone had the most binding affinity among the screened ligands when compared with the drug.

The amino acids which interact with embelin were found to be LYS A: 483 through electrostatic force of attraction and hydrophobic interaction (pi-alkyl). ASP A: 593 amino acid residue interact with embelin through H-bonding. In rapanone, the amino acids ASP A: 594 and LYS A: 483 interact through H-bonding as well as through electrostatic force of attraction. PHE A: 583 were found to have pi-alkyl and alkyl interactions with rapanone. Lawsone interacts with LYS A: 483 through H bonding, with PHE A: 583 through pi-pi T shaped and pi-alkyl interactions. In Plumbagin, the aminoacid residue LYS A: 483 was found to have H-bonding, electrostatic (pi-cation) and hydrophobic (pi-alkyl) interactions. It also interacts with PHE A: 583 through pi-alkyl hydrophobic interactions. Juglone was found to interact with LYS A: 483 through H-bonding and through hydrophobic interactions (pi-alkyl). It also interacts with ASP A: 594 through H-bonding. In 1,4-dihydroxy anthraquinone, VAL A:471 and PHE A:583 have hydrophobic interactions while ASN A:581 and GLN A:612 have H-bonding interactions. In 1, 3, 8-trihydroxy anthraquinone, the amino acids ASP A: 594, GLN A: 612 and GLU A: 501 have H-bonding interactions while TRP A: 531 was found to have pi-pi stacking interactions.

In the standard drug GDC 0879, the amino acids GLN A: 612, LYS A: 483 interact through H-bonding while PHE A: 583 through pi-pi stacked, pi-pi T-shaped and pi-alkyl interactions. Thus this insilico study predicts that rapanone and embelin are good inhibitors of the BRAF protein.

Prediction of ADME parameters are given in Table 2. The computer aided toxicity predictor TOPKAT was used to predict the cellular toxicity of the ligands under study. The BBB level were in the range 0-4, showing high penetration to no penetration. Most of the ligands under study have medium penetration and rapanone was found to have the least penetration. Ideal aqueous solubility level is 3. Five of the seven ligands were in the ideal level. ADMET descriptors indicate that the ligands are easily absorbed, have low probability of causing hepatotoxicity and are non inhibitors of CYP2D6 enzyme. Thus the phyto ligands used in this study were predicted to have low risk of possible side effects.

CONCLUSION

The ADMET properties give an idea of the pharmacokinetic parameters which a lead molecule must have and the docking studies give an insight to how well the ligands are bound to the amino acids by various interactions in the active site. From the library of the seven hydroxyquinones which were used as ligands, embelin and rapanone exhibited better binding affinities with the mutated BRAF protein when compared with that of the standard drug GDC0879. Thus the docking and ADMET studies help to predict the development of potential lead molecules. The above studies can be further substantiated by in vitro wet lab studies which are under progress.

CONTRIBUTION OF THE AUTHORS

AKN designed data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, cleaned and analysed the data. AF analysed the data , revised and prepared the paper. SPL is initiating the wet lab studies. VSV monitored the data and revised the paper. BRN has collected the data regarding the standard drug. All authors read and approved the final version that is being sent for publication. The authors declare that there is no conflict of interests regarding the publication of this paper. Also, they declare that this paper or part of it has not been published elsewhere.

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REFERENCES

[1] G Bollag; P Hirth; J Tsai; J Zhang; PN Ibrahim; H Cho; W Spevak; C Zhang; Y Zhang; G Habets; EA Burton; B Wong; G Tsang; BL West; B Powell; R Shellooe; , A Marimuthu; H Nguyen; KY Zhang; DR Artis; J Schlessinger; F Su; B Higgins; R Iyer; D' K Andrea; A Koehler; M Stumm; PS Lin; RJ Lee; J Grippo; I Puzanov; KB Kim; A Ribas; GA McArthur; JA Sosman; PB Chapman; KT Flaherty; X Xu; KL Nathanson; K Nolop, *Nature*, **2010**,467, 596-599.

[2] R Robert, *Biochem. Biophys. Res. Commun*, **2010**, 399, 313-317.

[3] Mc JA Cubrey; LS Steelman; L Abrams; JT Lee; F Chang; FE Bertrand; PM Navolanic; DM Terrian; RA Franklin; ,B D'Assoro; JL Salisbury; MC Mazzarino; F Stivala; M Libra. *Adv. Enzyme Regul.*, **2006**, 46, 249-279.

[4] KP Hoeflich; S Herter; J Tien; L Wong; L Berry; J Chan; C O'Brien; Z Modrusan; S Seshagiri; M Lackner; H Stern; E Choo; L Murray; LS Friedman; M Belvin, *Cancer Res*, **2009**, 69, 3042-3051.

[5] H Davies; GR Bignell; C Cox; P Stephens; S Edkins; S Clegg; J Teague; H Woffendin; MJ Garnett; W Bottomley; N Davis; E Dicks; R Ewing; Y Floyd; K Gray; S Hall; R Hawes; J Hughes; V Kosmidou; A Menzies; C Mould; A Parker; C Stevens; S Watt; S Hooper; R Wilson; H Jayatilake; H Jayatilake; BA Gusterson; C Cooper; J Shipley; D Hargrave; Pritchard-Jones; K N Maitland; G Chenevix-Trench; GJ Riggins; DD Bigner; G Palmieri; A Cossu; A Flanagan; A Nicholson; JW Ho; SY Leung; ST Yuen; BL Weber; HF Seigler; TL Darrow; H Paterson; R Marais; CJ Marshall; R Wooster; MR Stratton; PA Futreal; *Nature*, **2002**, 417, 949-954.
[6] M Libra; G Malaponte; PM Navolanic; P Gangemi; V Bevelacqua; L Proietti; B Bruni; F Stivala; MC Mazzarino; S Travali; JA McCubrey; *Cell Cycle*, **2005**, 4, 1382-1384.
[7] MS Brose; P Volpe; M Feldman; M Kumar; I Rishi; R Gerrero; E Einhorn; M Herlyn; J Minna; A Nicholson; JA Roth; SM Albelda; H Davies; C Cox; G Brignell; P Stephens; PA Futreal; PA Wooster; MR Stratton; BL Weber. *Cancer Res*, **2002**, 62(23), 6997-7000.
[8] MA Rahman; A Salajegheh; RA Smith; AKY Lam. *Crit. Rev. in Oncology/ Hematology*, **2014**, 90, 220-232
[9]S Kantham; *Int. Preclinic. Pharmaceut. Res. (IJPPR)*. **2010**, 1, 35-38.
[10] D Yuvaraj; Mandavkar; SJ Sunil, *Afr. J. Pharm. Pharmacol.*, **2011**, 5(25), 2738-2747.
[11] J Smit ha, UK Patil**.** , *Indian J. Nat. Prod.Resour*. **2010**, 4, 430-437.
[12] A Ashnagar , A Shiri. *Int. J Chem Tech Res.*, **2011**,3,1941-1944.
[13] PL Shiny; F Annette; DVK Rema; VS Vishnu; KB Arun; RPK Kavitha; P D Archana.*IJRRPAS*. **2015**, 5, 1173-1188 .
[14] SN Aiyar; MK Jain; M Krishnamurti. TR Seshadri. *Phytochem*, **1964**, vol 3, 335-339.
[15] C Sina; T Ion; A Gheorghe; B Adrian; *Not. Bot. Horti.Agrobot Cluj-Na* **2011**, 39(1): 237-240.