WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 8, 794-805. Research Article ISSN 2277– 7105

INSILICO DRUG DESIGN AND MOLECULAR DOCKING STUDIES OF NOVEL PHTHALAZINE DERIVATIVES FOR ANTICANCER ACTIVITY

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Article Received on
07 May 2019,

Revised on 28 May 2019,
Accepted on 19 June 2019

DOI: 10.20959/wjpr20198-15316

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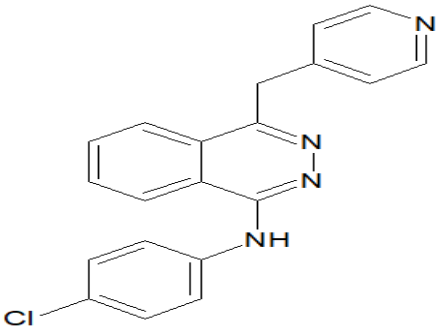
ABSTRACT

Cancer is an important cause of death globally. Every year more cancer cases and cancer death are reported over the world. In order to eradicate the serious disease, a novel phthalazine derivatives (P1-P10) were designed. By using various computational software such as ChemSketch, Molinspiration, PASS, and AdmetSAR the novel para benzaldehyde substituted phthalazine derivatives were designed. The designed compounds having required physicochemical properties, drug-likeness and obeying Lipinski rule of five were selected for molecular docking studies using Biovia discovery studio software 2018. The binding pattern and binding affinity of the novel phthalazine derivatives were determined by targeting the VEGFR-2 as the enzyme in order to rationalize their anticancer activity. Comparing the docked score of all novel series of phthalazine derivatives with a potent VEGFR-2 inhibitor (sorafenib). The phthalazine derivatives P1, P3, P4 P5, P6, P7, P8, P9, and P10 have high docked score and binding affinity than sorafenib (standard drug).

KEYWORDS: Phthalazine, 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1-yl)oxy] acetohydrazide, Anti-cancer activity, VEGFR-2, Molecular docking.

INTRODUCTION

Phthalazines are nitrogen-containing heterocyclic compounds possessing various biological activities such as anticancer[1], antimicrobial[2], anti-inflammatory[3], anticonvulsant, vasodilator, antihypertensive, and antitubercular activity.[4] Hydralazine, budralazine, vatalanib, olaparib, azelastine are the commercially available phthalazine based drugs used for various treatments (fig. 1-4).



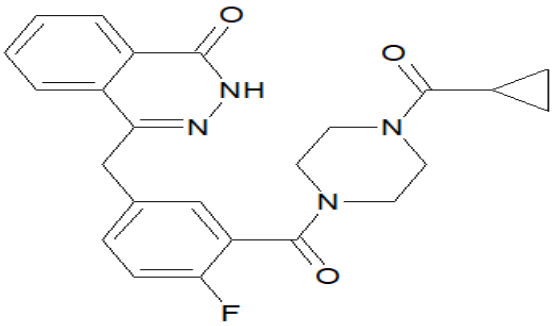
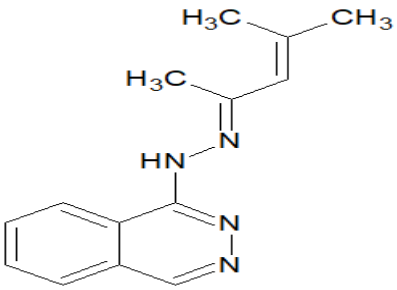


Fig. 1: structure of vatalanib. Fig. 2: structure of olaparib.



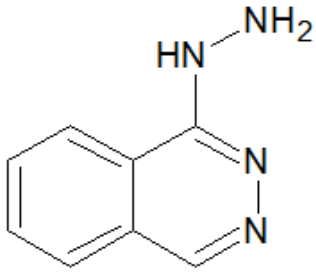


Fig. 3: structure of budralazine. Fig. 4: structure of hydralazine.

In the present study, a series of novel phthalazine derivatives with potent anticancer activity was designed. Cancer is a serious health problem and the leading cause of death. Some cancer study reveals that the vascular endothelial growth factor receptor[5] (VEGFR-2) plays a pivotal role in the regulation of tumor angiogenesis. Thus VEGFR-2 signaling pathway is considered a good therapeutic target in this study for inhibition of tumor angiogenesis and subsequent tumor growth. During the last decades, there is a growing interest in the design of several phthalazine derivatives for the treatment of cancer[6,7] as potent inhibitors of VEGFR-2. Vatalanib and sorafenib are the potent VEGFR-2 inhibitor and has been approved as an anti-angiogenic drug. Regorafenib and sorafenib showed anticancer activities[8-10] on different cancer cell lines.

Based on these facts, a novel phthalazine derivatives were designed to obtain potent VEGFR-2 inhibitors with good anticancer activity. In this research, 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1-yl)oxy] acetohydrazide[11] took as the phthalazine scaffold (fig. 5).

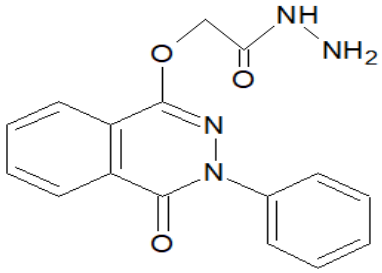


Fig. 5: structure of 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1yl)oxy]acetohydrazide.

From this phthalazine scaffold, different para substituted benzaldehyde (R) was introduced in the amino group of the acetohydrazide moiety to get 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1-yl)oxy] acetohydrazide derivatives (fig. 6).

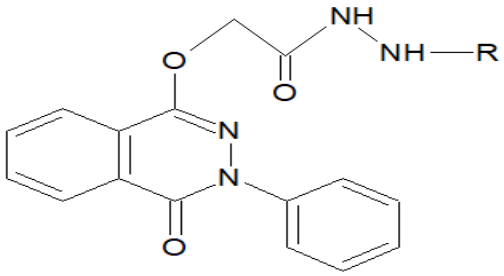


Fig. 6: structure of 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1-yl)oxy]acetohydrazide derivative

The name of para-substituted benzaldehyde are shown in (Table i).

Table I: Name of 4- substituted benzaldehyde.

Name of Compound	Name of 4- Substituted Benzaldehyde
P1	4-nitro benzaldehyde
P2	4-methoxy benzaldehyde
P3	4-dimethylamino benzaldehyde
P4	4-diethyl amino benzaldehyde
P5	4-bromo benzaldehyde
P6	4-fluoro benzaldehyde
P7	4-benzyl benzaldehyde
P8	4-methylthio benzaldehyde
P9	4-(1-Pyrrolidino) benzaldehyde
P10	4-(Trifluoromethoxy) benzaldehyde

MATERIALS AND METHODS

In the rational drug design of novel phthalazine derivatives, different software was used including ACD Lab ChemSketch, Molinspiration, PASS, AdmetSAR, and Discovery studio. ChemSketch[12] was used to draw the structures and for calculating the various molecular descriptors such as molar refractivity, molar volume, parachor, surface tension, polarizability, and log P. In order to determine the drug-likeness and analyzing the Lipinski rule of five[13] Molinspiration[14] were used. Molinspiration is online software, in which the smiles notation of the compounds were used to determine the drug-likeness. Important pharmacokinetic descriptors include octanol-water partition coefficient (log *P*), hydrogen-bond acceptor (HBA), the hydrogen-bond donor (HBD) and molecular weight was determined from molinspiration. These descriptors help to determine the drug-likeness of the compounds by evaluating the Lipinski rule of five. As per Lipinski rule of five, the drug should have log $P \leqslant 5$, molecular weight (MW) < 500 Daltons, HBD $\leqslant 5$ and HBA $\leqslant 10$. Using molinspiration the designed molecules are analyzed whether they obey Lipinski rule of five or not. The probability of anti-cancer activity was predicted using PASS[15] (Prediction of activity spectra for substances) software. The PASS software predicts biological activity, pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters, influence on gene expression. In which the smiles notation of the compounds is used to predict the probability of activity.

The ADMET profile of the compounds was determined using AdmetSAR[16] software. The pharmacokinetic parameters such as absorption, distribution, metabolism, elimination, and toxicity have an important role in the drug design and development process. These parameters are determined using AdmetSAR software.

Docking Analysis of the selected targets with synthetic ligands were analyzed using the docking software Biovia Discovery studio 2018.[17] The designed ligand was docked with target protein human vascular endothelial growth factor receptor- 2(VEGFR-2). Before docking, the targets and ligands were preprocessed for optimizing and minimizing the structure and generating conformers. Library docking is performed for identifying the binding affinity with the target using charmm as a force field. The (VEGFR-2) in complex with a novel 4-amino furopyrimidine were retrieved from PDB with PDB ID: 1YWN with a resolution of 1.71 A0. The protein was preprocessed by removing the bounded ligands and the energy of the protein become minimized to form a stable structure for molecular docking.

RESULTS AND DISCUSSION

The present work revealed the significance of rational designing of novel 2-[(4-oxo-3-phenyl-3,4-dihydro phthalazine-1-yl)oxy] acetohydrazide derivatives.

Estimating molecular descriptors from ACD Lab Chemsketch: Various molecular descriptors like molar volume, parachor, surface tension, polarizability, molar refractivity and log P of novel phthalazine derivatives were computed from ChemSketch (Table ii). All the proposed compounds have the required physicochemical parameters.

Table II: Determination of molecular descriptors using chemsketch.

Name of ligand	MR (cm3)	MV (cm3)	Parachor (cm3)	Surface Tension (dyne/cm)	Polarizability (cm3)	Log P
P1	120.22 ± 0.5	319.7 ± 7.0	892.5 ± 8.0	60.7 ± 7.0	47.66 ± 0.5 10-24	4.50
P2	120.37 ±0.5	336.1 ± 7.0	897.3 ± 8.0	50.7 ± 7.0	47.72 ± 0.5 10-24	4.71
P3	127.36 ± 0.5	355.6 ± 7.0	943.4 ± 8.0	49.5 ± 7.0	50.49 ± 0.5 10-24	4.75
P4	136.58 ± 0.5	387.7 ± 7.0	1020.6 ±8.0	48.0 ± 7.0	54.14 ± 0.5 10-24	4.81
P5	122.11 ± 0.5	327.0 ± 7.0	890.6 ± 8.0	55.0 ± 7.0	48.41 ± 0.5 10-24	4.51
P6	114.43 ± 0.5	317.3 ± 7.0	847.2 ± 8.0	50.7 ± 7.0	45.36 ± 0.5 10-24	4.79
P7	144.27 ± 0.5	398.2 ± 7.0	1062.5 ±8.0	50.6 ± 7.0	57.19 ± 0.5 10-24	4.99
P8	126.79 ± 0.5	344.1 ± 7.0	927.7 ± 8.0	52.7 ± 7.0	50.26 ± 0.5 10-24	4.43
P9	134.40 ± 0.5	360.9 ± 7.0	980.8 ± 8.0	54.5 ± 7.0	53.28 ± 0.5 10-24	4.78
P10	120.70 ± 0.5	350.9 ± 7.0	920.3 ± 8.0	47.2 ± 7.0	47.85 ± 0.5 10-24	4.74

MR – Molar Refractivity, MV- Molar Volume

Determination of drug-likeness from molinspiration: Using Molinspiration, all the proposed compounds obey the Lipinski rule of five. These molecules are better-dug candidate. The pharmacokinetic descriptors and drug-likeness obtained from molinspiration are shown in (Table iii and Table iv).

Table III: Analysis of lipinski rule of five from Molinspiration.

Name of Ligand	miLog P	natoms	MW	nON	nOHNH	nviolations
P1	3.74	33	443.42	10	1	0
P2	3.83	32	428.45	8	1	0
P3	3.88	33	441.49	8	1	0
P4	4.63	35	469.55	8	1	0
P5	4.59	31	477.32	7	1	0
P6	3.94	31	416.41	7	1	0
P7	4.99	37	488.55	7	1	0
P8	4.21	32	444.52	7	1	0
P9	4.28	35	467.53	8	1	0
P10	4.75	35	482.42	8	1	0

Natoms- Number of atoms, MW- Molecular weight, nON- Number of hydrogen bond acceptors, nOHNH- Number of hydrogen bond donors, nviolation- number of violation

Table. IV: Analysis of drug-likeness.

Compound	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
P1	-0.56	-0.67	-0.50	-0.90	-0.70	-0.49
P2	-0.48	-0.69	-0.43	-0.86	-0.64	-0.45
P3	-0.44	-0.65	-0.37	-0.81	-0.61	-0.42
P4	-0.40	-0.63	-0.37	-0.78	-0.59	-0.43
P5	-0.54	-0.72	-0.46	-0.96	-0.71	-0.49
P6	-0.46	-0.67	-0.39	-0.86	-0.64	-0.45
P7	-0.35	-0.59	-0.32	-0.67	-0.47	-0.30
P8	-0.51	-0.71	-0.49	-0.86	-0.60	-0.45
P9	-0.39	-0.59	-0.34	-0.79	-0.54	-0.40
P10	-0.37	-0.51	-0.39	-0.62	-0.48	-0.38

GPCR - G-protein-coupled receptors

Prediction of anti cancer activity using PASS software

In this study the anti cancer activity was predicted by PASS software.

Prediction of ADMET profile from AdmetSAR software: By analyzing the results all the phthalazine derivatives have positive results for penetration across BBB and human intestinal absorption (HIA). The ligand P5 and P6 are non AMES toxic rest of compounds are AMES toxic. All the phthalazine derivatives are non carcinogens (Table v).

Table. V: Prediction of ADMET.

Name of ligand	BBB		HIA		AMES Toxicity		Carcinogen	
	Results	Probability	Results	Probability	Results	Probability	Results	Probability
Sorafenib	BBB+	0.8510	HIA+	0.9649	Non AMES Toxic	0.8143	Non-carcinogens	0.8684
P1	BBB+	0.8898	HIA+	0.9236	AMES toxic	0.7889	Non-carcinogens	0.7642
P2	BBB+	0.8578	HIA+	0.9630	AMES toxic	0.6227	Non-carcinogens	0.7938
P3	BBB+	0.9164	HIA+	0.9910	AMES toxic	0.5623	Non-carcinogens	0.7042
P4	BBB+	0.9406	HIA+	0.9964	AMES toxic	0.5297	Non-carcinogens	0.5772
P5	BBB+	0.9616	HIA+	0.9825	Non AMES toxic	0.5343	Non-carcinogens	0.7450

P6	BBB+	0.9737	HIA+	0.9877	Non AMES toxic	0.5229	Non-carcinogens	0.7315
P7	BBB+	0.9722	HIA+	0.9855	AMES toxic	0.5449	Non-carcinogens	0.7751
P8	BBB+	0.8831	HIA+	0.9751	AMES toxic	0.5279	Non-carcinogens	0.7651
P9	BBB+	0.9878	HIA+	0.9915	AMES toxic	0.5278	Non-carcinogens	0.7781
P10	BBB+	0.9400	HIA+	0.9909	AMES toxic	0.5303	Non-carcinogens	0.7226

BBB - blood-brain barrier, HIA-human intestinal absorption

Molecular Docking: The active site residues were selected based on the position of the ligand bounded in the structure. The active site residues are Glu915, Cys917, Glu883, and Asp1044. The phthalazine derivatives docked at one of the critical amino acid residues was Glu883 with good docking score (fig. 7-16).

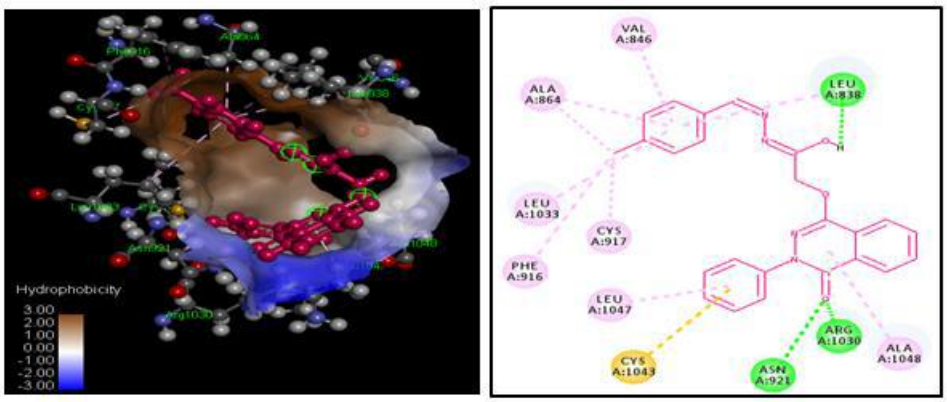


Fig. 7: Docking image of P1to 1YWN.

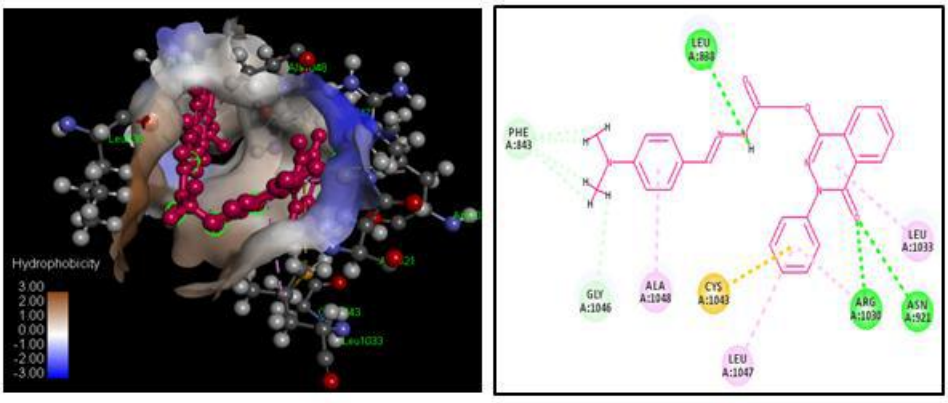


Fig. 8: Docking image of P3 to 1YWN.

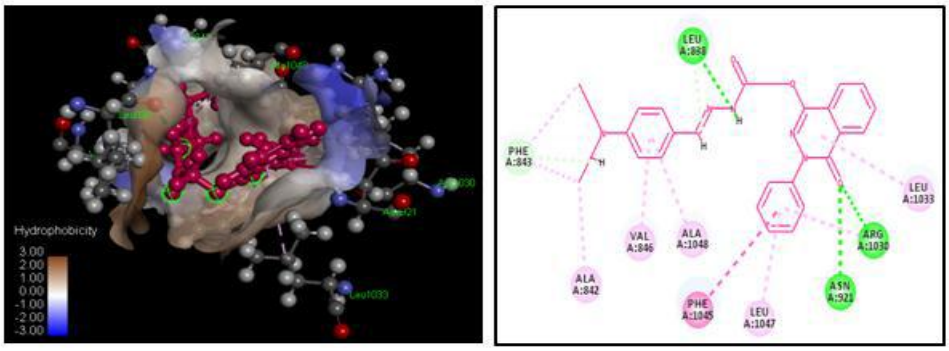


Fig. 9: Docking image of P4 to 1YWN.

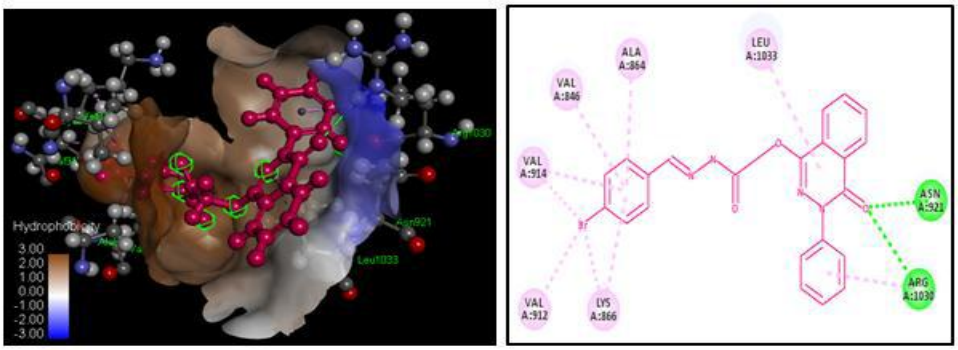


Fig. 10: Docking image of P5 to 1YWN.

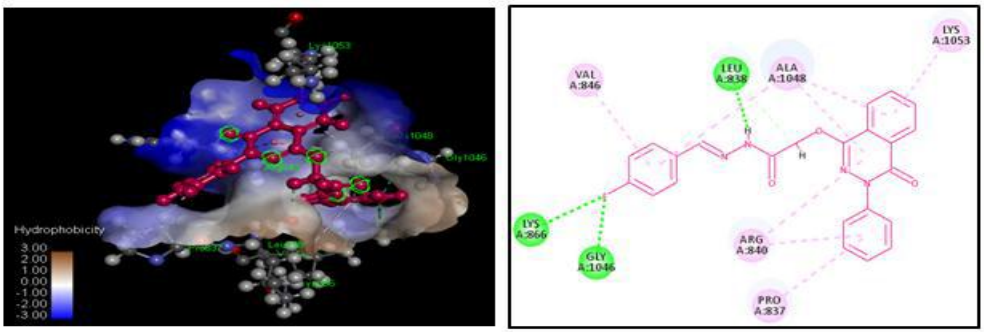


Fig. 11: Docking image of P6 to 1YWN.

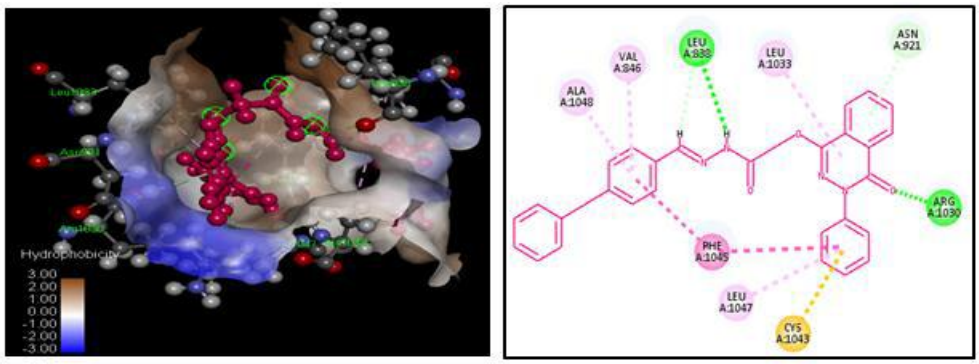


Fig. 12: Docking image of P7 to 1YWN.

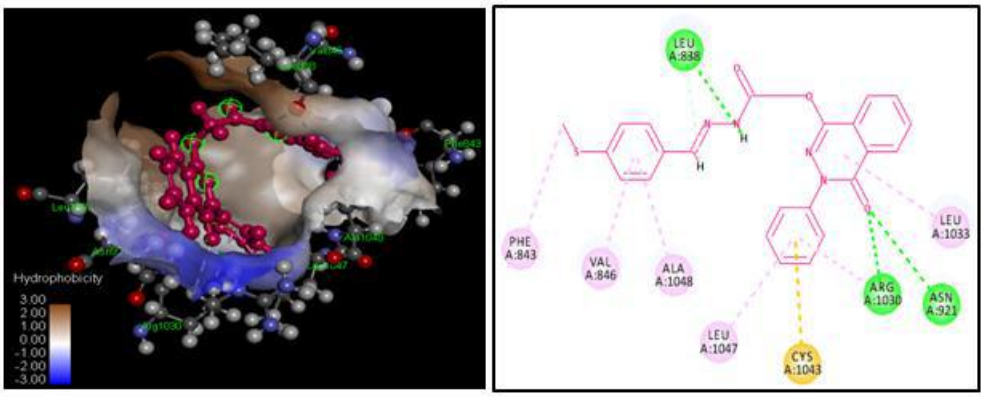


Fig. 13: Docking image of P8 to 1YWN.

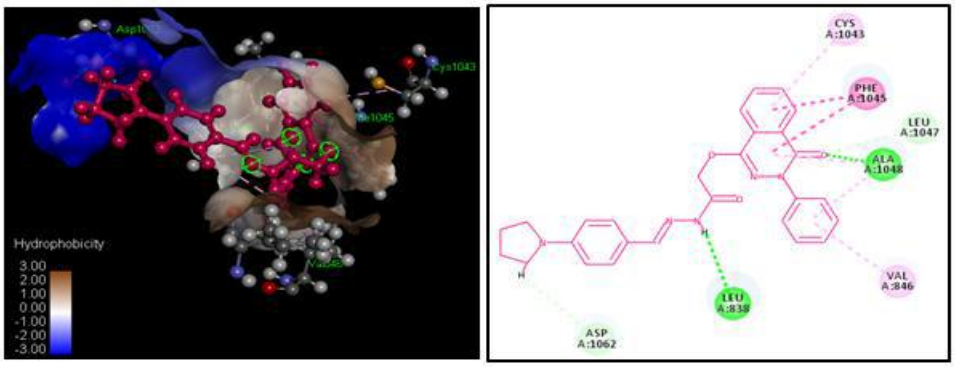


Fig. 14: Docking image of P9 to 1YWN.

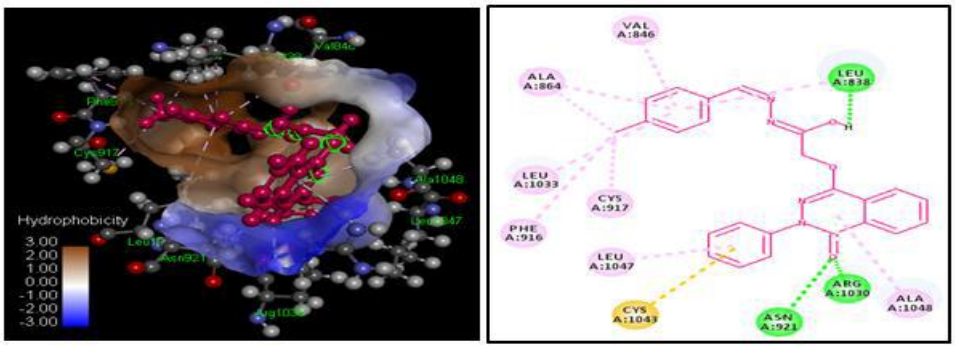


Fig. 15: Docking image of P10 to 1YWN.

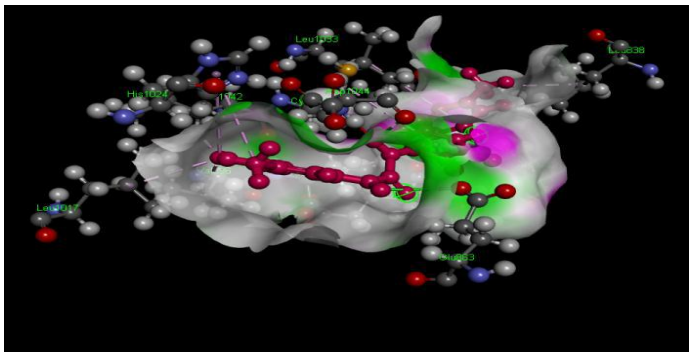


Fig. 16: Docking image of Sorafenib to 1YWN.

The docking score obtained from software explained that almost all phthalazine derivatives except P2 have good binding affinity, hydrogen bond interaction and docking score than the standard drug sorafenib (Table vi).

Table. VI: Determination of docking score and interacting amino acid Residues.

Sl No.	Name of the ligand	Interacting Residues	Docking score
1	P1	Asp1044, Glu883	114.566
2	P2	Leu 838, Arg 1030, Asn 921.	82.1537
3	P3	Lys786, Phe1045,Glu883,Arg840,Arg1049	128.754
4	P4	Leu 838, Arg 1030, Asn 921.	121.293
5	P5	Glu883, Cys1043,Asp1044, Glu883	116.515
6	P6	Glu883, Cys1043,Asp1044,Glu883	116.894
7	P7	Glu883, cys1043,Glu883,Asp1044	134.452
8	P8	Leu 838, Gly841, Leu 838, Gly1046	116.472
9	P9	Ala 1048, Leu 838	111.659
10	P10	Asp1044, Glu883, Glu883, Asp1044	114.566
11	Sorafenib	Cys917,Glu883,Asp1044, Glu915	97.9192

CONCLUSION

In this research study, the insilico drug design was carried out to get a potent anti-cancer drug. The in silico studies were performed on ten novel phthalazine analogs by means of ACD Lab ChemSketch 12.0, Molinspiration, PASS, AdmetSAR and Discovery studio.

Based on the data obtained from the softwares all the novel phthalazine derivatives have required physicochemical properties, drug-likeness and obeying Lipinski rule of five. From the docking results all phthalazine derivatives except P2 having higher docking score and better binding affinity towards the VEGFR-2 active site than standard drug sorafenib. These analogs can be subjected to further detail anticancer screening for consideration as a potent drug candidate.

ACKNOWLEDGMENTS

The authors thank the Department of Computational Biology and Bioinformatics, University of Kerala, Kariavattom North Campus for carrying out the docking analysis.

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