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Original Article

STRUCTURE AFFINITY RELATIONSHIP AND CHARACTERIZATION OF BENZOPORPHYRINS AS POTENT INHIBITORS OF YAP ONCOPROTEIN THROUGH *IN SILICO* EXPERIMENTS

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ABSTRACT

Objective: Yes activated protein (YAP) is a transcriptional coactivator and corepressor that plays a pivotal role in cell proliferation. Amplification of the YAP gene and over expression of the YAP oncoprotein are reported in various human cancer cell lines. Hence inhibition of YAP by small molecular ligands would control cell proliferation.

Methods: Autodock 4.2 was used to dock the designed ligands to the active site of YAP oncoprotein. Structure affinity relationship was carried out for 70 benzoporphyrin derivatives (SAfiR). Based on the SAfiR data, 8 benzoporphyrin derivatives were designed.

Results: 8 compounds were found to potentially inhibit YAP. Verteporfin was taken as the standard with a binding energyof -6.84 kcal/Mol.The designed compounds were found to have binding energy in the range of -8.85 kcal/mol to -7.61 kcal/mol.

Conclusion: Based on the substituent effects and reported pleiotropic property of verteporfin, 8 novel porphyrin derivatives were identified with potent inhibitory effect towards the YAP oncoprotein. Further virtual and high throughput approaches could potent compounds that are specifically targeted towards the YAP peptide.

Keywords: YAP oncoprotein, Benzoporphyrins,

INTRODUCTION

Yes activated protein (YAP) is a transcriptional coactivator and corepressor that plays pivotal role in cell proliferation. YAP is the critical downstream regulatory target in the conserved hippo - tumor suppressor pathway [1]. In the hippo signaling pathway, YAP associates with the TEAD to form a transcription complex, which localizes in the nucleus and promotes and activates the expression of several genes such as G1/S-specific cyclin-E [2, 3]. Thus YAP functions as an oncoprotein and the oncogenic activity of YAP is down regulated by a kinase cascade of upstream events in hippo signaling pathway [4].

However, recent studies have reported down regulated expression of several upstream kinases of the hippo signaling cascade including Mats and Lats in several human cancers such as astrocytoma and soft tissue sarcoma [5, 6]. Further, amplification of the YAP gene was reported in hepatocellular carcinoma cell lines while up regulation of the YAP paralog transcriptional coactivator with PDZ-binding motif, TAZ was found in breast cancers especially in invasive ductal carcinomas [7, 8].

Hence inhibition of YAP by small molecules would be a novel, but rational approach for induction of proliferation arrest. Inhibition of YAP by small molecules prevents its association with the TEAD to form a transcription complex and thereby inhibits its nuclear localization. Druggability of the human yes activated protein has been studied and pharmacological disruption of the TEAD-YAP complex has been shown to suppress the oncogenic activity of YAP. Currently, many small molecules have not been known to inhibit YAP, however, three porphyrinderivatives, namelyverteporfin, protoporphyrin IX and hematoporphyrin have been shown to inhibit and suppress the oncogenic activity of YAP. Verteporfin has been reported to potentially inhibit YAP than protoporphyrin IX and hematoporphyrin [9]. Verteporfin is a drug bearing benzoporphyrin nucleus approved for Photodynamic therapy of sub-fovealchoroidal neovascularization induced by age related macular degeneration. It acts by direct damage of vascular endothelium and blood vessel blockade through oxygen radical production upon irradiation with red laser beam [10]. We studied the effect of various substituents in the porphyrin nucleus by multiple docking analyses with YAP oncoprotein as the target. Based on the substituent effects, novel porphyrin derivatives were identified with potent inhibitory effect towards the YAP oncoprotein.

MATERIALS AND METHODS

In order to understand the effect of various substituents in the benzoporphyrin nucleus, an insilico structure affinity relationship study was carried out. The designed compounds were then individually docked to the active site of the previously prepared target. The parameters set for prediction of binding affinity include binding energy (ΔG), inhibitory constant (Ki), intermolecular energy, internal energy, torsional energy and number of hydrogen bonding interactions established by the ligand with the active site residues of the target.

Based on the structure – affinity relationship data obtained, novel ligands were designed with better inhibitory activity towards the YAP oncoprotein.

Retrieval and isolation of crystal structure of YAP

Crystal structure of the human YAP oncoprotein was retrieved from the repository of 3D- structural data of large bio-molecules, the protein data bank **(PDB ID: 3KYS).** The YAP domain was segregated from TEAD, since the retrieved crystal structure was the transcription complex formed by the YAP and TEAD proteins. The amino acid sequence of the separated YAP protein was cross validated with its FASTA sequence. The active site of the target protein was predicted using FTSite which employs multiple molecular probes with energy based methods for high accuracy detection of ligand binding sites [11, 12]. The active site of the YAP oncoprotein is comprised of the following amino acids: ASP64, ALA67, LEU68, PHE69, ALA71, VAL80, PRO81, GLN82, THR83, VAL84, PRO85, MET86 and ARG89.

Creation of ligand dataset for structure–affinity relationship

The Verteporfin molecule was taken as the prototype for prediction of the structure affinity relationship. Individual substituents in the benzoporphyrin ring system and the benzoporphyrin ring itself were modified to study the effect of individual groups in ligand binding. A diverse array of electron withdrawing, electron donating, aromatic,

allylic and bioisosteric groups were selected for designing ligands for prediction of structure – affinity relationship. The swissbioisostere database was screened for identifying heterocyclic bioisosteres of acetate [13]. A total of 70 compounds was designed with this approach.

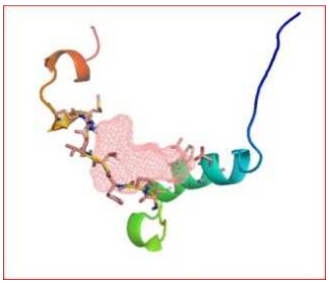


Fig. 1: Active site of the YAP oncoprotein

Prediction of structure–affinity relationship

The ligands designed were individually docked to the active site of the YAP oncoprotein using Autodock 4.2. The ligands were prepared by addition of gasteiger charges and the torsion root was detected. Autodock uses a Lamarckian genetic algorithm and an empirical binding free energy function for prediction of bound conformations of flexible ligands to macromolecular targets [14]. Structure – affinity relationship was established from the difference in binding affinities elucidated through multiple docking analyses.

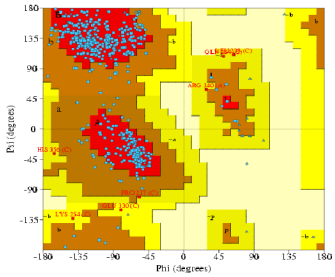
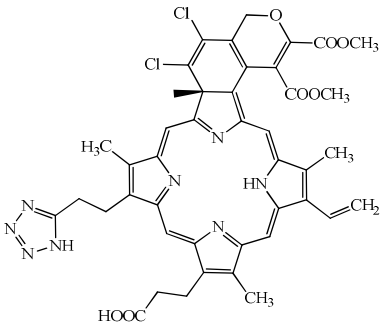


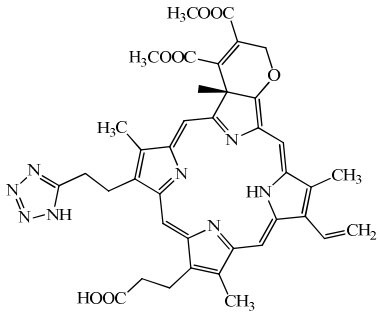
Fig. 2: Ramachandran Plot of the YAP-TEAD transcription complex (PDB id: 3KYS)

Ligand design and molecular docking

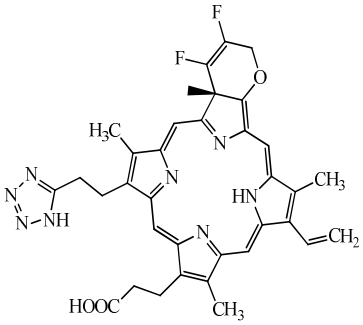
The structure – affinity relationship data was utilized for designing novel ligands. A total of 8 compounds were designed including benzoporphyrins, napthoporphyrins, pyroporphyrins and chromoporphyrins. The designed porphyrin derivatives were docked to the same active site of YAP using the Lamarckian genetic algorithm of Autodock 4.2 with Verteporfin as standard. The compounds designed are given below:



Compound 1



Compound 2



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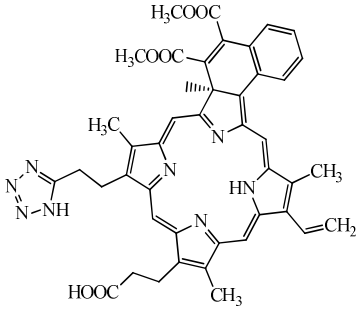
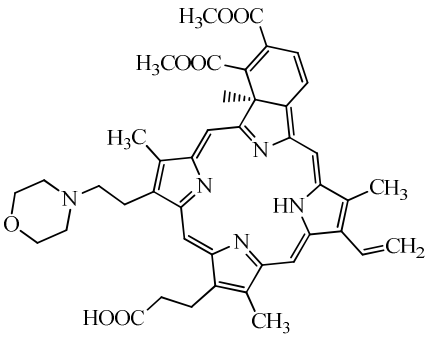
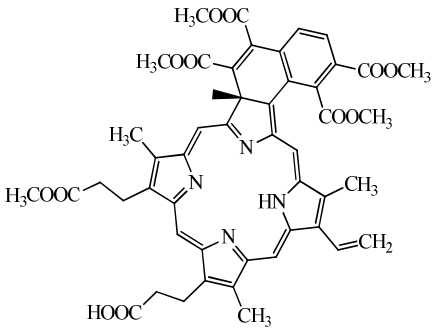


Compound 4



Compound 5



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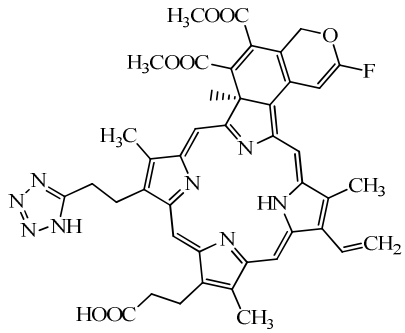
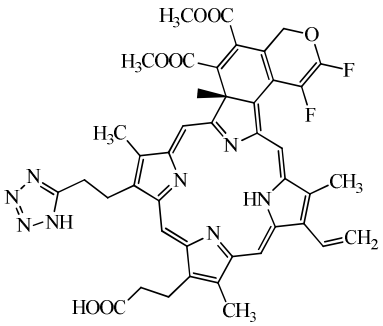


Compound 7



Compound 8

Fig. 3: Compounds designed on the basis of SAFiR data

RESULTS AND DISCUSSION

In order to understand the effect of various substituents in the benzoporphyrin nucleus and thereby arrive at structure – affinity relationship, multiple docking analyses were carried out. The benzoporphyrin nucleus was numbered as below for ease of analysis.

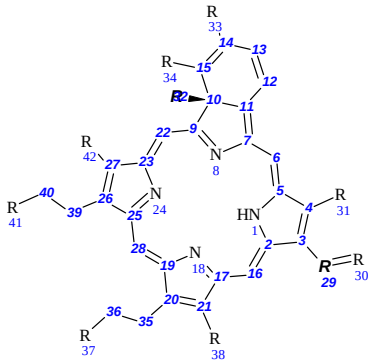


Fig. 4: Benzoporphyrin nucleus subjected for SAFiR analysis

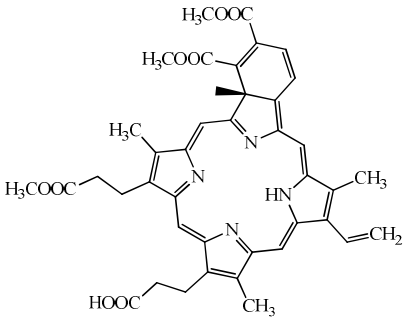


Fig. 5: Verteporfin

Structure–affinity relationship of benzoporphyrin

Positions 33, 34

1. Replacement of the acetate groups with weak electron withdrawing groups has little or negligibly no change in binding affinity. In case of replacement with electronegative groups which are weakly electron withdrawing such as halogens, a mild increase in binding affinity is observed as electronegativity decreases. However, replacement with fluorine considerably increases the binding affinity. Replacement with moderate electron withdrawing groups such as Nitrile has better binding affinity. However, a fall in the binding affinity is observed upon replacement by strong electron withdrawing groups. The fall in binding affinity is because of the decreased electron density in the benzoporphyrin ring system conferred by strong electron withdrawing groups.

2. Similarly, replacement of the acetate groups with weak electron donating groups increases the binding affinity whereas a decrease in binding affinity is observed with moderate and strong electron donating groups probably due to an increase in electron cloud within the benzoporphyrin ring system.

3. In conclusion, both strong electron donating and withdrawing groups disfavor binding and an optimum electron density in the benzoporphyrin ring system is required for activity.

4. Increasing the length of the side chain bearing these groups decreases the binding affinity. i. e Homologation of the side chain has negative effects.

Positions 31, 38, 42

The methyl groups at positions 31, 38 and 42 are essential for activity. Though replacement with diverse groups does not completely retard the binding affinity, a considerable decrease in binding affinity was observed.

Position 41

1. Replacement of the acetate group at position 41 with chemically diverse groups such as methyl, halo, cyano, amino etc. decreases the binding affinity.

2. However, replacement with heterocyclic bioisosteres of acetate increases the binding affinity considerably.

3. The ethyl side chain bearing the acetate group is essential for activity. Homologation or decreasing the side chain length decreases the binding affinity. Altering the side chain length alters the ligands rotational flexibility, producing a conformationally disfavored orientation.

4. De-esterification of the acetate group to the carboxyl group decreases the binding affinity.

Position 37

1. Sites 37 and 41 are identical to some extent, but differ in their bonding. However, replacement of the carboxyl group with complex heterocycleswas not found to increase the binding affinity as observed with site 37.

2. Esterification of the carboxyl side chain at position 37 decreases the binding affinity suggesting that this carboxyl group is essential for activity.

Benzene ring system

1. The benzene ring attached to the porphyrin ring system is suitable for substitutions and replacements. Conversion of the benzene ring to six membered heterocycle 2H-pyran considerably increases the binding affinity.

2. However, a decrease in binding affinity was observed with other six membered heterocycles such as pyridine, thiopyran etc.

3. Conversion of the benzene ring to naphthalene and chromene highly increases the binding affinity.

4. Substitutions in the so formed naphthalene or chromene ring systems were also found to potentially increase the binding affinity.

5. However, direct substitution in the dimethyl phthalate ring system decreases the binding affinity.

Position 32

1. The methyl group at position 32 is essential for activity. However a considerable increase in binding affinity is observed when the cis orientation of the methyl group is converted to trans.

Position 3

1. The ethylene side chain at position 3 is essential for activity. Conversion of the olefinic side chain to alkane or alkyne yielded compounds with decreased binding affinity.

2. Replacement of the side chain with diverse groups and increasing the chain length through conjugation decreases the binding affinity.

Positions 22, 28, 16, 6

Any substitution at positions 22, 28, 16, 6 considerably decreases the binding affinity.

The binding energy data of various ligands docked for prediction of structure – affinity relationship are enlisted in table 1. Only the site considered is substituted with a specific group for a ligand considered,

whereas the other sites in the ligand were retained as in Verteporfin. Verteporfin was considered as the standard and the binding energy of Verteporfin was found to be -6.84kcal/Mol.

Table 1: Structure affinity relationship of Benzoporhyrins – substituent effects

Position	Group in veteporfin	Substituent group	Binding energy(kcal/Mol)
33, 34	$-COOCH_3$	-F	-7.23
		-Cl	-6.61
		-Br	-6.62
		-I	-6.93
		$-CF_3$	-5.59
31, 38, 42	$-CH_3$	$-N(CH_3)_2$	-4.28
		-Cl	-5.05
		$-COOCH_3$	-5.97
		$-NH_2$	-4.15
		$-OCH_3$	-3.12
		$-C_6H_5$	-5.48
		Removed*	-4.9
		SO_3CH_3	-4.45
41	$-COOCH_3$	$-CH_3$	-6.31
		-Cl	-5.39
		-CN	-5.42
		$-NH_2$	-5.13
		Tetrazole	-7.51
		Ethyl side chain bearing the acetate group was homologated to butyl	-5.08
		Ethyl side chain bearing the acetate group was shortened to methyl	-6.62
		Removed*	-6.17
37	COOH	$-COOCH_3$	-5.3
		Tetrazole	-5.69
Benzene ring system	Benzene	Pyridine	-5.62
		2*H*-pyran	-7.55
		2*H*-thiopyran	-4.97
		Napthalene	-7.28
12	H	-Cl	-4.88
32			-7.4
3	$-CH=CH_2$	$-CH_2-CH_3$	-4.22
			-5.28
		-Cl	-5.95
22, 28, 16, 6	-H	-F	-4.77
		$-OCH_3$	-4.3

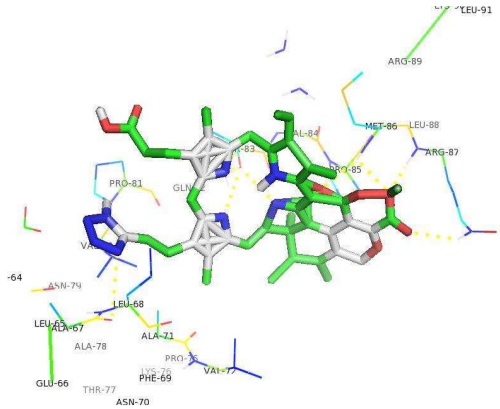
Table 2: Molecular docking analysis of designed porphyrin derivatives

Identifier	ΔG	kI	Intermolecular energy	Internal energy	Torsional energy	No. of hydrogen bonds	Interacting residues
Compound 1	-8.85	327.31 nm	-11.83	-1.32	2.98	1	THR 83
Compound 2	-7.68	2.36um	-10.36	-0.84	2.68	3	THR 83, ARG 87
Compound 3	-7.61	2.64um	-10.0	-0.79	2.39	1	THR 83
Compound 4	-8.41	687.42 nm	-10.79	-1.24	2.39	3	MET 86, ARG 87
Compound 5	-7.09	6.31um	-9.48	-0.21	2.39	1	ARG 87
Compound 6	-8.0	1.37um	-11.28	-0.84	3.28	1	ARG 87
Compound 7	-7.68	2.36um	-10.06	-0.51	2.39	2	THR 83
Compound 8	-7.61	2.65um	-9.99	-0.82	2.39	1	ARG 87
Vertepofin	-6.84	9.66um	-10.72	-0.35	3.88	2	ARG 87

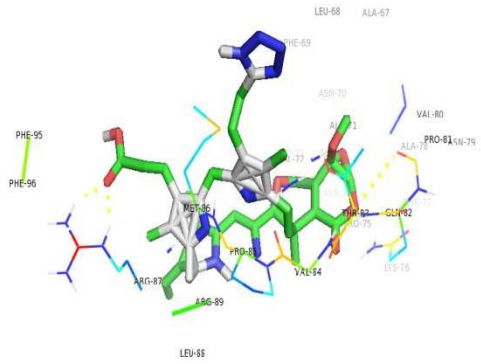
Molecular docking analysis

The compounds designed based on the structure affinity relationship data obtained were individually docked to the active site of YAP oncoprotein with Verteporfin as the standard. All the 8 compounds were found to potentially inhibit YAP.

The results of the molecular docking analysis are given in table 2.



Compound 1



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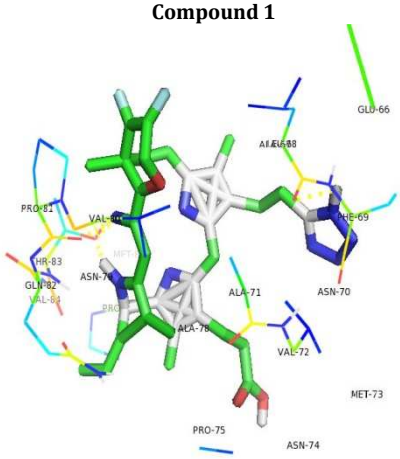

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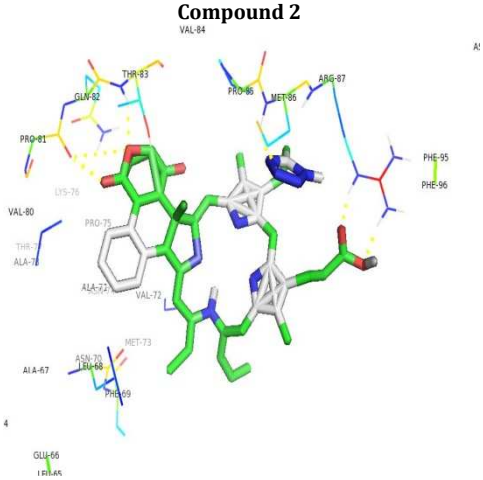

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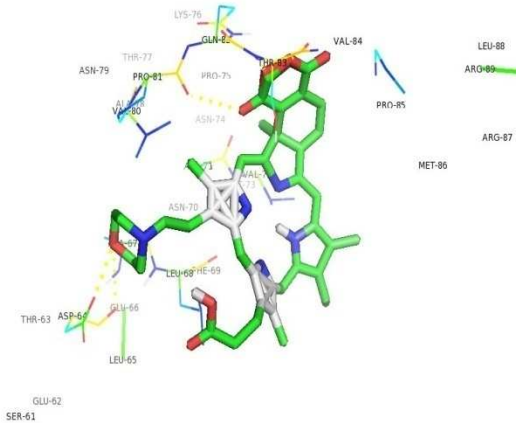
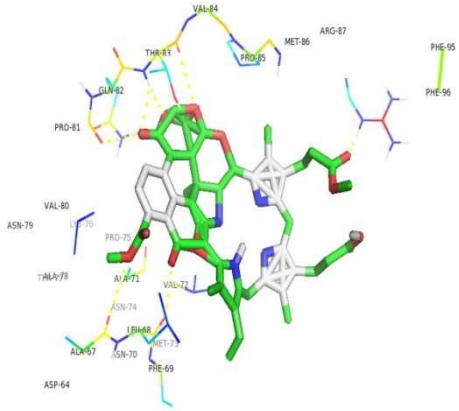


Compound 5



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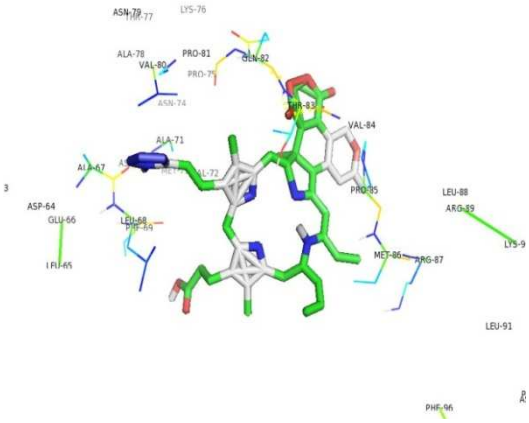

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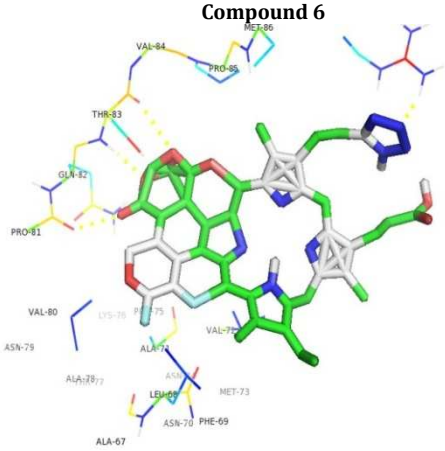
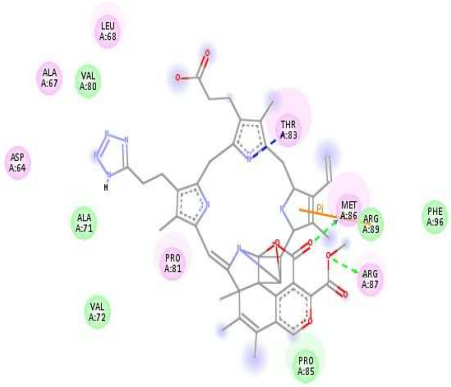


Compound 8

Fig. 6: Docked ligand poses



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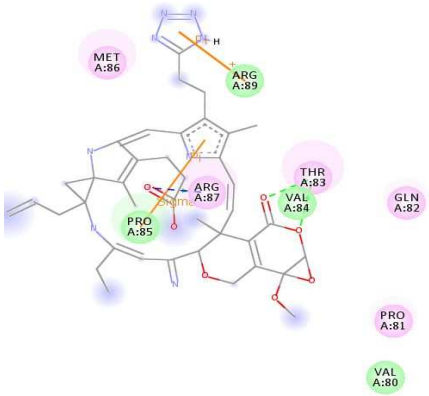

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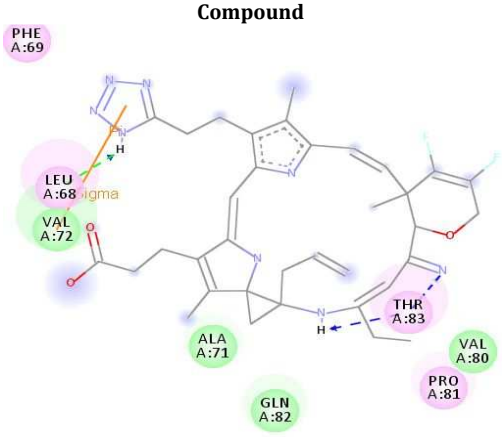

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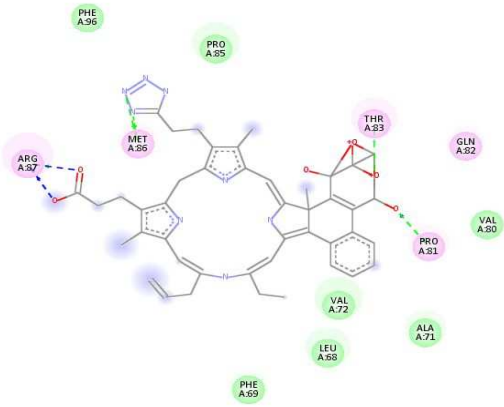

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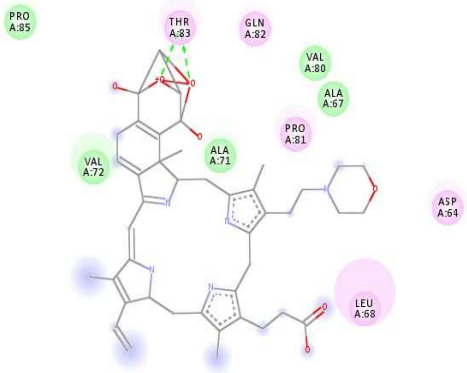

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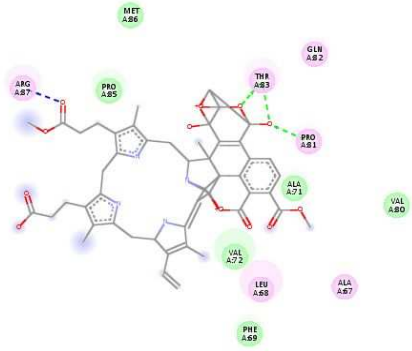

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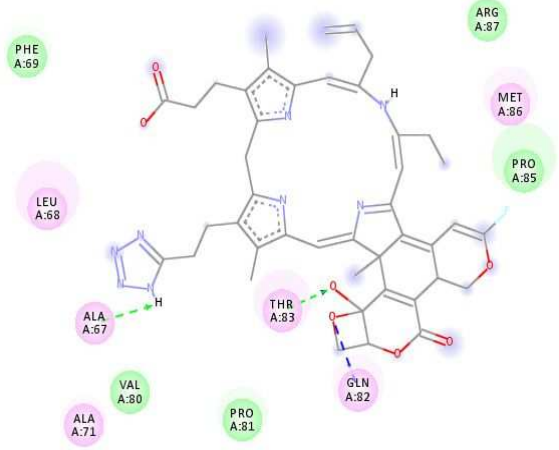

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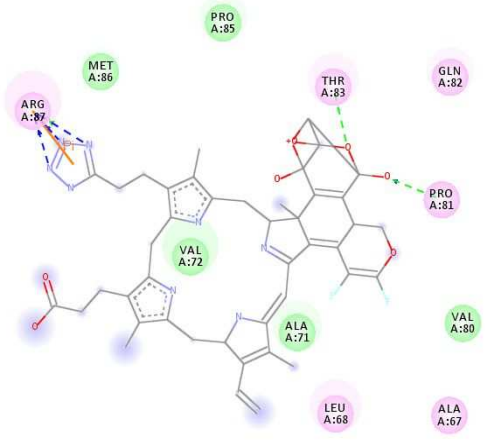

Compound

Fig. 7: Ligand interaction maps

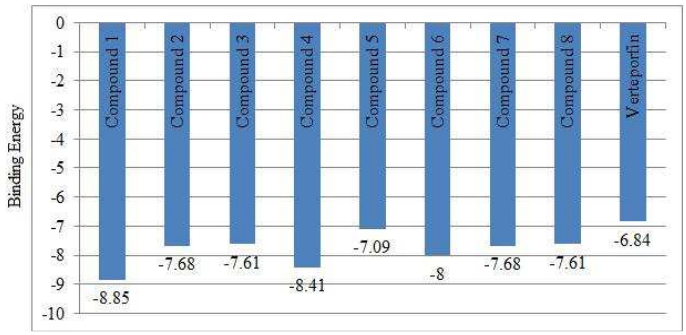

Fig. 8: Binding energy data

CONCLUSION

Cancer is a disease with a high mortality rate. In spite of the diverse array of anti-cancer drugs available, the mortality rate yet remains at peak and the available anti-cancer drugs also have their limitations because of potential adverse effects associated with them. This is because most of the conventional anti – cancer drugs are not molecularly targeted and hence they are not specific. In this study, novel ligands have been designed to inhibit YAP oncoprotein which has been reported to be over expressed in various cell lines. Further virtual and high throughput approaches coupled with genome wide screening could yield better ligands against the molecular targets of cancer. Since these drugs are molecularly targeted, they should only be used in patients in whom YAP oncoprotein is over expressed. Hence pharmacogenetic screenings should be done before therapeutic intervention with these molecules for control of cell proliferation.

CONFLICT OF INTERESTS

Declared None

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