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An *In Silico* Study of Novel Morpholine Derivatives for Lung Cancer, Non-Hodgkin's Lymphoma and Metastasis Melanoma.

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Abstract

Aim: This study involves identification of effective morpholine derivatives for cancer treatment by insilico analysis using BIOVIA Discovery studio docking tool.

Methods: The compounds were selected from literature survey of fifty new morpholine derivatives. Drug likeness of the selected compounds were evaluated according to Lipinski's rule of five and biological activities of the derivatives were predicted by PASS (Prediction of Activity Spectra for Substances) online software. Compounds which shows Antineoplastic activities including lung cancer,metastasis melanoma, and Non-Hodking's lymphoma were selected for further studies.The molecules then docked against the proteins obtained from the protein data bank.

Result and conclusion: The compounds which show higher docking score are selected . From the insilico designing of new morpholine derivatives it is found that further toxicity studies and invitro studies on these compounds will give a promising anticancer drugs.

Keywords-Anticancer,insilico drug designing,molecular docking,morpholine derivatives.

INTRODUCTION

"Cancer is an abnormal growth of cells which tend to proliferate in an uncontrolled way and, in some cases, to metastasize (spread)" .Among the various type of cancers the frequency of occurrence of lymphoma , melanoma and lung cancer is high. Tobacco usage is one of the main risk factor of lung cancer. The salient abnormalities associated with lung cancer includes abnormalities in growth stimulatory signalling pathway,tumor suppressor gene pathway, and epigenetic changes [1][2]. Epidermal growth factor receptor is the surface cell proteins helps in division and growth of cells[3] , the characteristic abnormal growth and division of cells in cancer is highly associated with EGFR which provides a route to target these protein for cancer treatment[4].The FDA approved drug Gefitinib is an epidermal growth factor receptor tyrosine inhibitor[5][6][7].

The BRAF , a pivotal component of MAPK is a promising target for anticancer therapy."The Ras-Raf-MEK-ERK (ERK) pathway is a logical therapeutic target because it represents a common downstream pathway for several key growth factor tyrosine kinase receptors which are often mutated or over expressed in human cancers".

The mutation in The ras oncogene family is the key factor of pathogenesis of cancer.One of the foremost often detected genetic alterations in cancer is within the ras factor family, that plays an important role within the management of each normal and transformed cell growth.Ras proteins are key intermediates in cell communication, and are the prototypical members of an outsized family of G proteins which cycle between an active (GTP bound) or inactive (GDP bound) state.

Raf was the primary known and most characterised downstream effector enzyme of Ras. The Raf amino acid threonine enzyme family consists of 3 isoforms, Raf-1(C-Raf), A-Raf, and B-Raf[8].Mitogen activated protein kinase pathway is the chief signal transduction pathway which is involved the growth, proliferation and survival of the cells.

Activation of BRAF via mutation results the oncogenic activation of mitogen activated protein kinase pathway lead to metastatic melanoma .The prevalent mutation occurs in BRAF is in the codon 600 result in a substitution of valine (V) for glutamic acid (E) at position 600 (V600E)[9]. Drugs Vemurafenib and Debrafenib are BRAF inhibitors and use for the treatment of malignant melanoma[10][11][12], A skin cancer that begins in cells called melanocytes. Melanocytes can grow together to form benign (not cancerous) moles. A melanoma starts as a collection of cancerous melanocytes.BRAF inhibition has gained effective results in patients with BRAF-mutated melanoma[13]. Its efficacy in other malignancies is currently under evaluation.

Similar to EGFR and BRAF ,Bruton's tyrosine kinase a Tec family protein[14][15][16] is an anticancer drug target for Non-Hodgkin's lymphoma[17] ,is a type of B-cell non-Hodgkin's lymphoma[15][16]. The currently available drugs for mantle cell lymphoma Acalabrutinib and Ibrutinib acts by inhibiting BTK receptor[18].

Heterocyclic compounds serve as building blocks for many anticancer drugs, such as Vemurafenib,Debrafenib,Ibrutinib. Among the heterocyclic compound morpholine is one of the great importance. Morpholine containing drugs have large spectrum of activity includes anticancer, antimicrobial, antidepressant and neuroprotective activities [19][20][21][22][23]. The gefitinib is a morpholine containing anticancer drug use for the treatment of lung cancer.

In the present study 3-(Morpholino-4-yl)N-phenyl –(substituted)Phenyl propanamide(A) derivatives are designed[24] (Table.1) and found it's biological activities using insilico methods and selected compounds which is having better activity for further toxicity and invitro studies.

Insilico drug designing is one of the cost effective research methodology in drug designing [25].This method includes

structure based drug designing (SBDD) and ligand based drug designing (LBDD). In SBDD The use of three-dimensional structural information gathered from biological targets the frequently used strategies are molecular docking, structure based virtual screening and molecular dynamics. The goal of ligand-protein docking is to predict the predominant binding mode(s) of a ligand with a protein of known three-dimensional structure. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization.

MATERIALS AND METHODS

Protein preparation;

The protein required for docking study is downloaded from the RCSB protein data bank[26]. The receptors (PDB ID = 1M14, 1UWH, 3GEN)[27][28][29]were downloaded in the .pdbformat. The active site of these receptors was identified from the X-ray structure of receptor bound with the ligand.

Generation and optimization of ligands;

The two dimensional structures of morpholine derivatives were drawn by using ACD LAB Chemsketch software [30]and saved into .mol format using OPEN BABEL Software[31].The structures of the compound then converted in to three dimension (3D) using online smile translator and saved in pdb format[32]. Total 10 compounds were selected for further insilico studies.

Molinspiration cheminformation;

The molecular properties including Lipinski rule of five [33][34] and drug likness of the compounds were predicted using Molinspiration cheminformation software[35].

PASS online software;

PASS (Prediction of Activity Spectra for Substances) is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecule. PASS provides simultaneous predictions of many types of biological activity based on the structure of organic compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing

Molecular docking;

Docking Analysis of the selected targets with ligands were analyzed using the docking software Discovery studio 2018. Before docking the targets and ligands were preprocessed for optimizing and minimizing the structure and generating conformers respectively. Library docking is performed for identifying the binding affinity with the targets using Charmm as force field.

RESULT AND DISCUSSION

Molinspiration;

Lipinski's rule of five

Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules

1. Molecular mass less than 500 Dalton
2. High lipophilicity (expressed as LogP less than 5)
3. Less than 5 hydrogen bond donors
4. Less than 10 hydrogen bond acceptors
5. Molar refractivity should be between 40-130.

The table-2 shows the value related to Lipinski's rule of five. From the table-2 it is evident that all the 10 compounds under study obeys Lipinski's rule of five.

Veber's rule[37];

According to Veber's rule the compounds which obey two criteria which include,

1. Rotatable bond count≤10.
2. Polar surface area (PSA) equal to or less than 140 Å are predicted to have good oral bioavailability.

All ten compounds found obey the Veber's rule. Table-3 shows the Rotatable bond count and polar surface area of ten selected compounds.

Table 1 - Analogues

Derivatives	R
MOF 1	4-methylbenzaldehyde
MOF 2	methyl 4-formylbenzoate
MOF 3	2-methoxybenzaldehyde
MOF 4	4-hydroxy-3-methoxy-5-nitrobenzaldehyde
MOF5	2-nitrobenzaldehyde
MOF6	2,2-difluoro-2*H*-1,3-benzodioxole-5-carbaldehyde
MOF7	3-ethoxy-4-hydroxybenzaldehyde
MOF8	2,6-dichloro-3-hydroxy-4-methoxybenzaldehyde
MOF9	2-methoxy-3-methylbenzaldehyde
MOF10	2-formyl –phenyl boronic acid

Table2 - Calculation of Molecular descriptors by ACD Lab ChemSketch-2018-2.1

Compounds	Molar refractivitycm^3	Molar volumecm^3	Parachor cm^3	Surface tension dynes/cm	Polarizability cm^3
MOF 1	96.08± 0.3 cm^3	277.8 ± 0.3 cm^3	744.7 ± 0.6 cm^3	51.6 ± 0.3 dyne/cm	38.09 ± 0.5 10-24 cm^3
MOF 2	103.03 ± 0.3 cm^3	299.4 ± 3.0 cm^3	810.3 ± 6.0 cm^3	53.6 ± 3.0 dyne/cm	40.84 ± 0.5 $10^{-24}cm^3$
MOF 3	97.94 ± 0.3 cm^3	285.5 ± 3.0 cm^3	765.1 ± 6.0 cm^3	51.5 ± 3.0 dyne/cm	38.82 ± 0.5 $10^{-24}cm^3$
MOF 4	106.36 ± 0.3 cm^3	295.8 ± 3.0 cm^3	837.4 ± 6.0 cm^3	64.1 ± 3.0 dyne/cm	42.16 ± 0.5 $10^{-24}cm^3$
MOF 5	97.80 ± 0.3 cm^3	273.4 ±3.0 cm^3	763.5 ± 6.0 cm^3	60.8 ± 3.0 dyne/cm	38.77 ± 0.5 $10^{-24}cm^3$
MOF 6	97.51 ± 0.4 cm^3	282.7 ± 5.0 cm^3	768.7 ± 6.0 cm^3	54.6 ± 5.0 dyne/cm	38.65 ± 0.5 $10^{-24}cm^3$
MOF 7	104.45 ± 0.3 cm^3	300.5 ± 3.0 cm^3	820.4 ± 6.0 cm^3	55.5 ± 3.0 dyne/cm	41.40 ± 0.5 $10^{-24}cm^3$
MOF 8	109.61 ± 0.3 cm^3	307.9 ± 3.0 cm^3	854.6 ± 6.0 cm^3	59.3 ± 3.0 dyne/cm	43.45 ± 0.5 $10^{-24}cm^3$
MOF 9	99.82 ± 0.3 cm^3	284.0 ± 3.0 cm^3	780.3 ± 6.0 cm^3	56.9 ± 3.0 dyne/cm	39.57 ± 0.5 $10^{-24}cm^3$
MOF 10	97.73 ± 0.4 cm^3	279.8 ± 5.0 cm^3	772.8 ± 6.0 cm^3	58.1 ± 5.0 dyne/cm	38.74 ± 0.5 $10^{-24}cm^3$

Table3 - Analysis of Lipinski's rule of five –Molinspiration Cheminformatics

COMPOUND	Molecular weight(<500D)	Log p (<5)	Hydrogen bond donors(<5)	Hydrogen bond acceptors(<10)	Molar refractivity(40-133) cm^3	Result
MOF-1	324	3.29	1	4	96.08± 0.3	Passed
MOF-2	368	3.02	1	6	103.03 ± 0.3	Passed
MOF-3	340	2.85	1	5	97.94 ± 0.3	Passed
MOF-4	401	2.30	2	9	106.36 ± 0.3	Passed
MOF-5	355	2.75	1	7	99.82 ± 0.3	Passed
MOF-6	390	3.36	1	6	97.51 ± 0.4	Passed
MOF-7	370	2.56	2	6	104.45 ± 0.3	Passed
MOF-8	425	3.62	2	6	109.61 ± 0.3	Passed
MOF-9	356	2.36	2	6	97.73 ± 0.4	Passed
MOF-10	354	1.84	3	6	97.51 ± 0.4	Passed

Table4 - Analysis of Veber's rule-Molinspiration Cheminformatics

Compounds	Rotatable bonds	Polar surface area
MOF1	5	54.71
MOF2	7	99.10
MOF3	7	91.26
MOF4	6	71.03
MOF5	7	71.03
MOF6	5	60.04
MOF7	6	82.03
MOF8	6	50.80
MOF9	7	67.88
MOF10	5	41.57

Table5 - Analysis of biological activity-PASS Online software

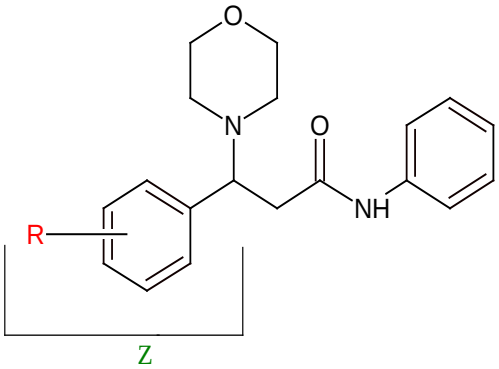
COMPOUND	LUNG CANCER	METASTASIS MELANOMA	NON-HODGKIN'S LYMPHOMA
MOF1	-	-	-
MOF2	-	-	-
MOF3	-	-	-
MOF4	0.314	0.316	-
MOF5	0.395	-	-
MOF6	0.228	0.817	-
MOF7	0.402	0.257	-
MOF8	0.218	0.347	-
MOF9	0.398	0.811	-
MOF10	0.314	-	0.514

Table6 - Molecular docking-Discovery studio

SL NO	COMPOUND MOF7	RECEPTOR	DOCKING SCORE
1	MOF6	1M14	92.48
2	STD DRUG- GEFITINIB	1M14	84.29
3	MOF7	IUWH	122.91
4	VEMURAFENIB	IUWH	135.71
5	MOF10	3GEN	126.15
6	STD DRUG-IBRUTINIB	3GEN	128.84

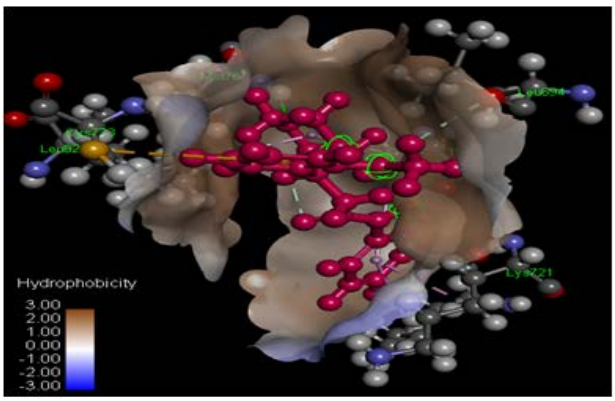
PASS (Prediction of Activity Spectra for Substances);
According to the various interactions of compounds with biological entity a spectrum of biological activities of selected compounds were obtained from PASS online software.The compounds which shows anticancer activities are selected from the ten analogues.The table-4 shows the list of PASS values of compounds which shows anticancer activities. From the data it is found that only one analogue MOF 10 show activity against Non-Hodgking's lymphoma , MOF 4 to 10 shows activity against lung cancer and MOF 4,6,7,8,and 9 shows activity against metastasis melanoma. From this MOF 6, MOF7 and MOF 10 were selected for further docking studies.

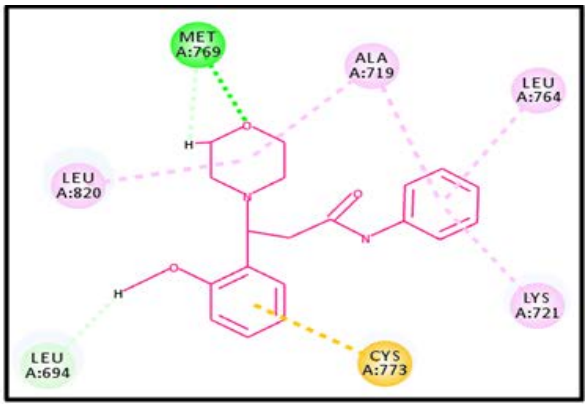
Molecular docking – BIOVIA Discovery studio -2018;
Molecular docking is done using BIOVIA Discovery studio. The proteins (1M14,IUWH and 3GEN)in the pdb format used for the docking. Analogues MOF6, MOF7 and MOF10 shows significant binding affinity with the receptor protein IM14,IUWH and 3GEN respectively when compared with standard drug available in market which act on the same receptor protein. The standard drugs used were Geftinib(Lung cancer) , Vemurafenib (Metastasis melanoma) and Ibrutinib (Non-Hodgkin's lymphoma).
As the analogues give better binding affinity when compared to the standard drug from the result of docking studies we can conclude that further toxicity studies and invivo studies will give a promising drug molecule for cancer treatment.



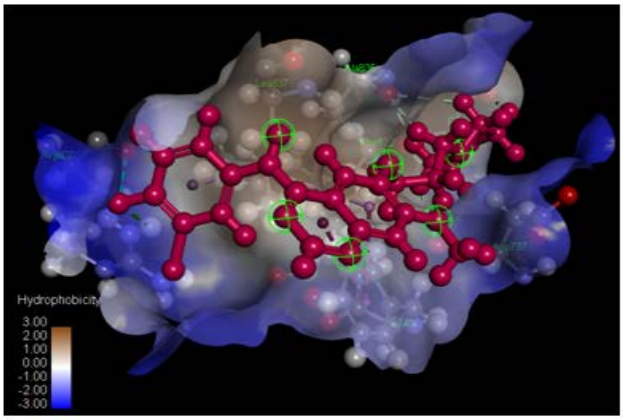
3-(morpholino-4-yl)N-phenyl-3-(substituted) phenyl propanamide

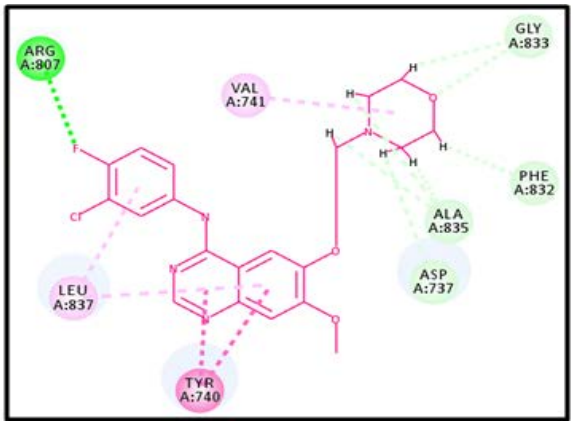
1M14 interaction with MOF 7



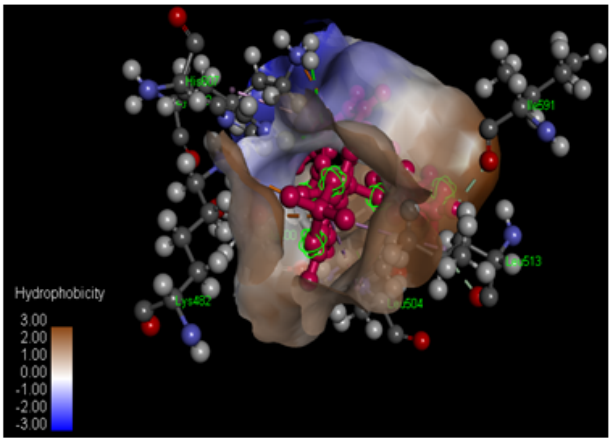


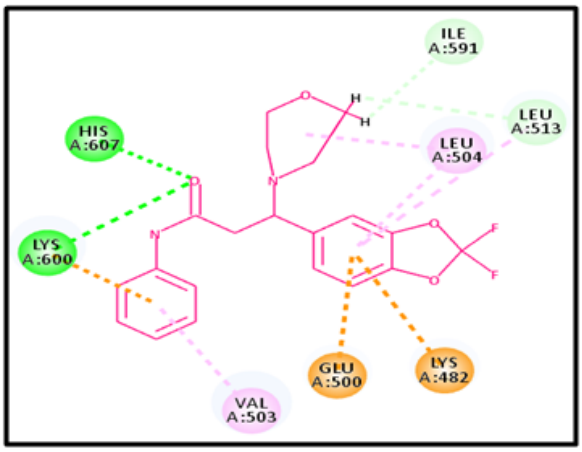
1M14 interaction with Gefitinib



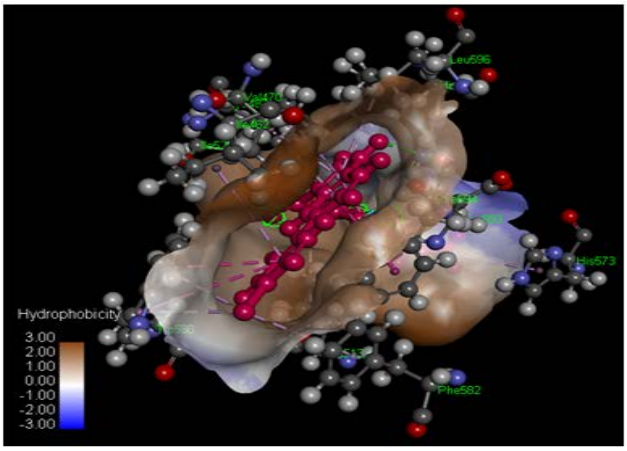


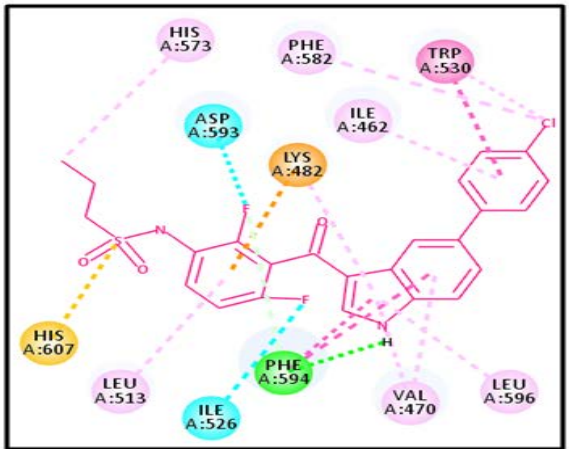
IUWH interaction with MOF-6



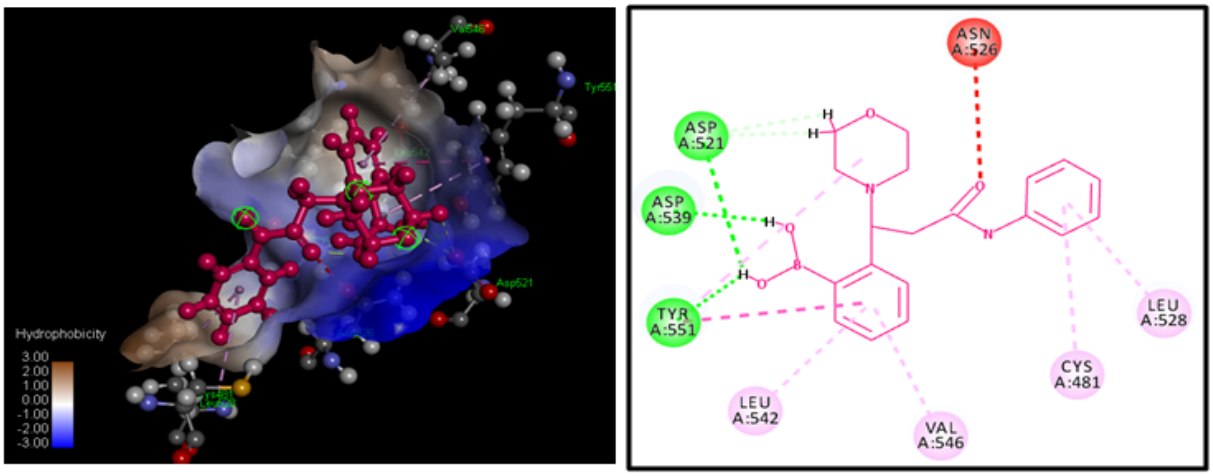


IUWH interaction with Vemurafenib

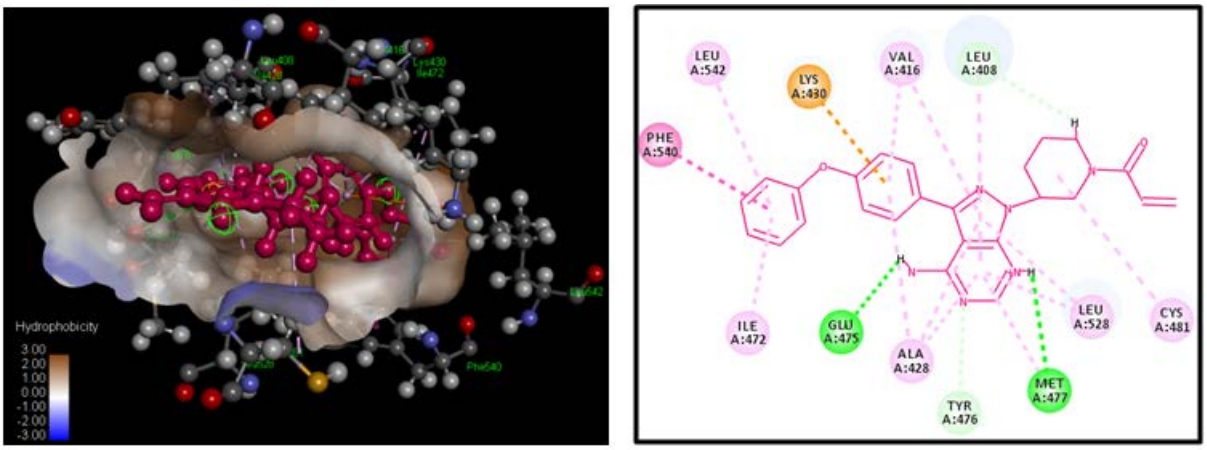




3GEN interaction with MOF – 10



3GEN interaction with Ibrutinib



CONCLUSION

Morpholine is a heterocyclic compound which is the building block of many biologically active molecule. Geftinib is a morpholine containing drug for lung cancer treatment .In the current study new morpholine analogues are designed and its activity against different types of cancer is evaluated using insilico method .Even though the PASS value is less for the analogues they showed better binding affinity in the docking analysis when compared with the standard drug . So the in silico studies indicate the relevance of the work and further investigation can be done in future.

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