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IN-SILICO PREDICTION AND DOCKING STUDIES OF NOVEL SYNTHESIZED BENZOFURAN DERIVATIVES AS ANTI-CANCER ACTIVITY

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Abstract

Amendment on the benzofuran moiety has resulted a various types of derivatives with various biological activities including anticancer and antibacterial activity. With the findings of identifying new anticancer agent an investigation has been done to hybridize various heterocyclic moieties. In this research article I have discussed the molecular docking study of our prepared benzofuran derivatives. Ten novel benzofuran derivatives were subjected to in-silico molecular docking studies to determine the affinity of novel benzofuran derivatives for anticancer targets. The Molecular docking study is the study of how two or more molecular structure (Example- drugs, enzymes and proteins) collaborate with each other. Mainly ten types of benzofuran derivatives (M5a-M5j) were synthesized from (7-chlorobenzofuran-3-yl)hydrazine with substituted benzaldehyde in the presence of sodium acetate at 70^0C at reflux for docking studies. In drug discovery, docking is the genuine pathway for the molecular

interaction of compounds. For the study of molecular docking we have used numerous software's such as molinspiration cheminformatics , swiss target prediction, Chem draw 3D.16.0, PyMOL , Discovery studio, rcsb PDB ,SwissADME and Auto dock vina 1.5.7. By the use of these software's we have estimated the result of our synthesized compounds in the form of binding energy. Novel molecules were privileged on the base of lowest binding energy (-6.9 to -10.4 Calorie/mol) All the novel synthetic derivatives (M5a-M5j) has shown a best negative energy. On the basis of our studies we have concluded that all these prepared compound may be important Pharmacophore against anti-cancer activity.

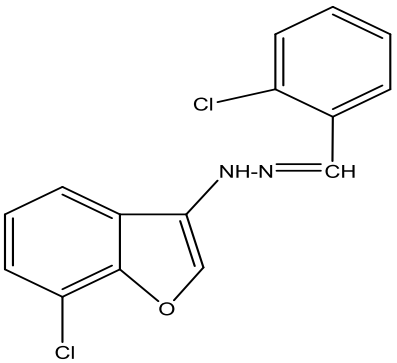
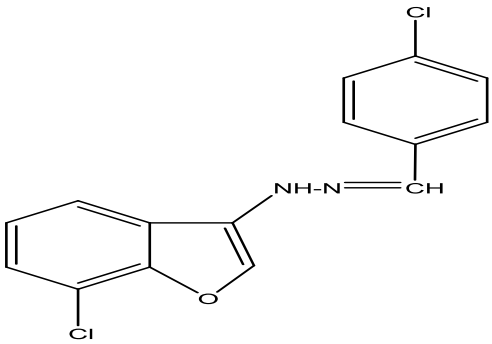
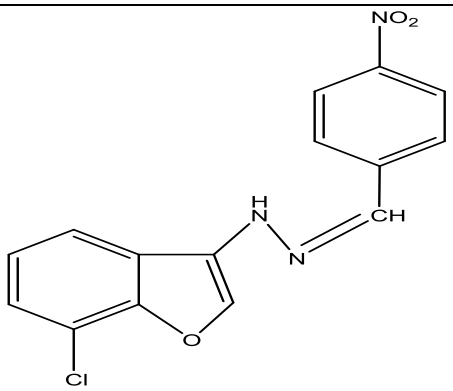
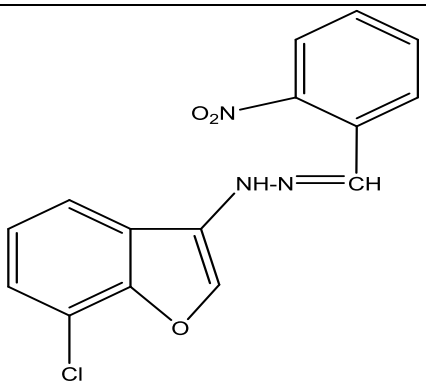
Keywords: Benzofuran moiety, PyMOL, Discovery studio software and Auto dock vina

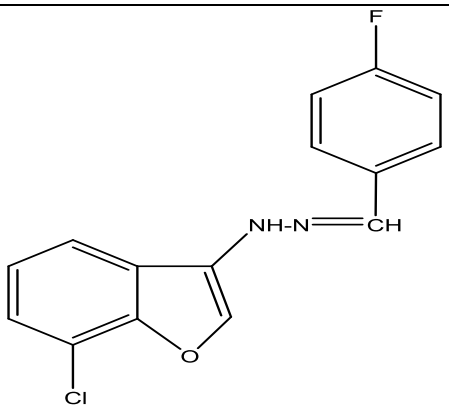
INTRODUCTION:

Malignant tumors are one of the major health diseases that is pressurizing human health and life[1]. It has been examined that worldwide cancer is the foremost cause of death and estimately 10 million people are killing per year because of this cancer only[2]. Prostate, lung, liver and stomach cancer are the most common cancer which are found in men whereas breast and uterus cancer is very common and frequent in women's[3].Therefore it is necessary to find anticancer drugs and treatment to cure these malignant tumors. In this recent era the antitumor activity of benzofuran compounds has fascinated more and more awareness among scientists[1]

Benzofuran is the fused moiety with the molecular formula C8H6O . The biological scenario of these new prepared benzofuran derivatives is more related to the earlier one derivatives. In this article we have analyzed the molecular docking techniques and by this we get to know that how protein interconnect with the ligands[4]. Docking is the most appropriate method in structure based drug design[5]. This research article basically emphasizes the design of new drug derivatives of benzofuran (M5a-M5j) and numerous software's like Pymol, Chem draw 3D, Mol inspiration chem informatics, discovery studio, Auto dock vina and rcsb PDB has been employed for docking of novel synthesize benzofuran derivatives [6,7,8,]

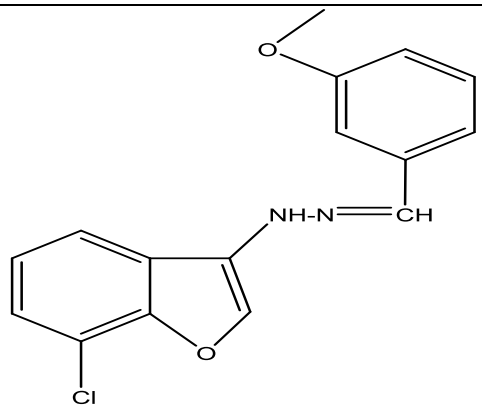
Table 1: Novel synthesized benzofuran derivatives (M5a-M5j) are shown in below table

 1-(7-chlorobenzofuran-3-yl)-2-(2-chlorobenzylidene hydrazine **Compound M5a**	 1-(7-chlorobenzofuran-3-yl)-2-(4chlorobenzylidene) hydrazine **Compound M5b**
 1-(7-chlorobenzofuran-3-yl)-2-(4-nitrobenzylidene)hydrazine **Compound M5c**	 1-(7-chlorobenzofuran-3-yl)-2-(2-nitrobenzylidene)hydrazine **Compound M5d**



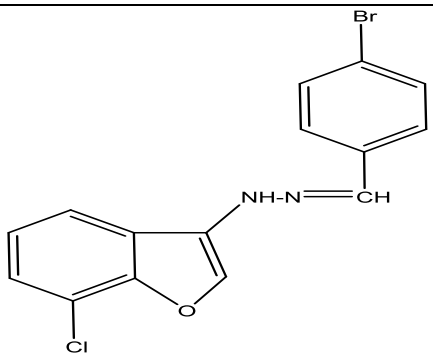
1-(7-chlorobenzofuran-3-yl)-2-(4-fluorobenzylidene)hydrazine

Compound M5e



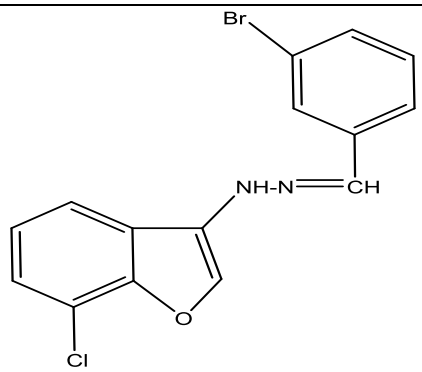
1-(7-chlorobenzofuran-3-yl)-2-(3-methoxybenzylidene)hydrazine

Compound M5f



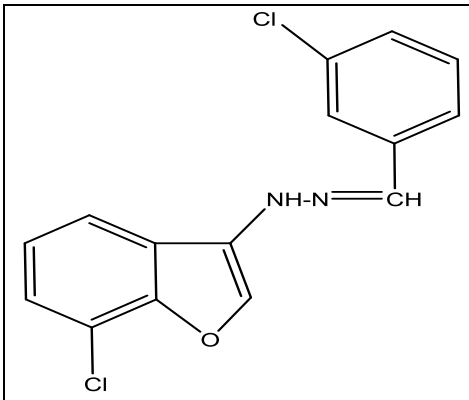
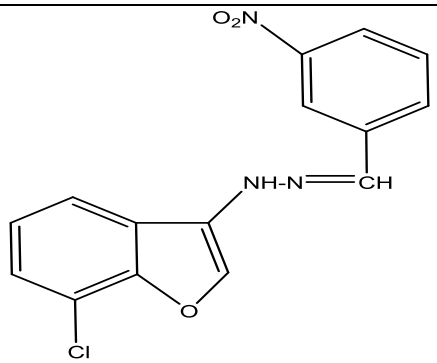
1-(4-bromobenzylidene-2-(7-chlorobenzofuran-3-yl)hydrazine

Compound M5g



1-(3-bromobenzylidene-2-(7-chlorobenzofuran-3-yl)hydrazine

Compound M5h

 1-(7-chlorobenzofuran-3-yl)-2-(3-chlorobenzylidene)hydrazine **Compound M5i**	 1-(7-chlorobenzofuran-3-yl)-2-(3-nitrobenzylidene)hydrazine **Compound M5j**

MATERIAL AND METHODS:

There are some steps used in-silico design and molecular docking studies of novel benzofuran derivatives for their anticancer activity:

Ligand preparation (9, 10)

We developed and selected novel synthetic benzofuran (M5a-M5n) derivatives as ligands. The hypothesized chemical structure of benzofuran derivatives was then drawn in the software chemdraw3D.16.0, and the energy of the generated structures was minimized in the same software. The file was then saved as lig.pdb. Then, lig.pdb will open in Auto Dock Vina 1.5.7, and prepare lig.pdbqt file of ligand.

Protein preparation (11)

Using Swiss target prediction software, we have now predicted the target site (protein) for ligands based on likelihood. Next, we retrieved the PDB file for the target site from protein data bank. Open the pdb file in PyMOL and delete any superfluous atoms or

amino acid chains and refine the structure of protein before saving it as a prot.pdb file. Further the Auto Dock Vina 1.5.7 software will opened the prot.pdb file and created the prot.pdbqt file of the protein.

Molecular Docking:

Preparation of grid box:(9,10)

Setting the grid by Auto Dock Tools is one of the key tasks in docking studies. The best grid structures and coordinates were chosen that covered the whole binding pocket of the target protein. The prot.pdbqt file open by grid macromolecule in autodock and the grid parameter file was saved in the conf file, which was used for docking, once the grid had been calculated.
Grid box dimensions were shown in **Table-1**.

Table No-1: Dimensions of grid box of compounds M5a-M5j(9)

Compoun name		M5a	M5b	M5c	M5d	M5e
Grid dimension	Size x	40	40	40	40	40
	Size y	40	40	40	40	40
	Size z	40	40	40	40	40
	Center x	-46.194	104.103	80.910	97.231	5.768
	Center y	9.869	129.442	129.109	34.115	3.696
	Center z	1.711	47.871	46.996	99.406	26.471

Compoun name		M5f	M5g	M5h	M5i	M5j
Grid dimension	Size x	40	40	40	40	40
	Size y	40	40	40	40	40
	Size z	40	40	40	40	40
	Center x	-20.569	32.782	21.498	22.804	12.615
	Center y	22.726	32.099	23.124	123.403	119.781
	Center z	-20.493	-2.286	-3.598	45.119	59.935

The complete set of prepared files, including lig.pdb, lig.pdbqt, prot.pdb, prot.pdbqt, and conf.txt, were saved and the molecular docking results were then acquired in the form of a log file and all ligand.pdbqt. The ligand site which is having the highest negative binding energy was opened in the PyMOL software and then save as complex file in pdb format. Further that complex file get opened in discovery studio to see the interaction between ligand and protein in the form of 3D and 2D diagram.

Table 2: IUPAC Name Of Synthesized Derivatives Or Ligands With Smile File

The novel synthesized derivatives or ligand, IUPAC name and smile file given in the below **Table-2** by cheminformatics

S.No	Ligand Name	IUPAC Name	Smile File
1.	M5a	1-(7-chlorobenzofuran-3-yl)-2-(2-chlorobenzylidene hydrazine	Clc1ccccc1C=NNc2coc3c(Cl)cccc23
2.	M5b	1-(7-chlorobenzofuran-3-yl)-2-(4-chlorobenzylidene) hydrazine	Clc3ccc(C=NNc1coc2c(Cl)cccc12)cc3

3.	M5c	1-(7-chlorobenzofuran-3-yl)-2-(4-nitrobenzylidene)hydrazine	O=N(=O)c3ccc(C=NNc1coc2c(Cl)cccc12)cc3
4.	M5d	1-(7-chlorobenzofuran-3-yl)-2-(2-nitrobenzylidene)hydrazine	O=N(=O)c1ccccc1C=NNc2coc3c(Cl)ccccc23
5.	M5e	1-(7-chlorobenzofuran-3-yl)-2-(4-fluorobenzylidene)hydrazine	Fc3ccc(C=NNc1coc2c(Cl)cccc12)cc3
6.	M5f	1-(7-chlorobenzofuran-3-yl)-2-(3-methoxybenzylidene)hydrazine	COc3cccc(C=NNc1coc2c(Cl)cccc12)c3
7.	M5g	1-(4-bromobenzylidene-2-(7-chlorobenzofuran-3-yl)hydrazine	Clc2cccc3c(NN=Cc1ccc(Br)cc1)coc23
8.	M5h	1-(3-bromobenzylidene-2-(7-chlorobenzofuran-3-yl)hydrazine	Clc2cccc3c(NN=Cc1cccc(Br)c1)coc23
9.	M5i	1-(7-chlorobenzofuran-3-yl) -2-(3-chlorobenzylidene)hydrazine	Clc3cccc(C=NNc1coc2c(Cl)cccc12)c3
10.	M5j	1-(7-chlorobenzofuran-3-yl) -2-(3-nitrobenzylidene)hydrazine	O=N(=O)c3cccc(C=NNc1coc2c(Cl)ccccc12)c3

The novel synthesized derivatives or Ligands (M5a-M5n) were drawn by Chem Draw3D 16.0 and the prepared file of lig.pdb and lig.pdbqt of the derivatives (M5a- M5n) does not exist in any record because it was a recently drawn molecule. In **Table -3** selected target sites, Interacting residues, and PDB Ids by Swiss target prediction and ligands compatibility with PDB is shown.

Table -3: Showing promising PDB Ids and target of the compounds (M5a- M5j) (9)

S. No	Compound Name	Target	Interacting residues	Common Nane	PDB Id
1.	M5a	Kinase	LEU A:642, TYR A:653,ILE A:885, ASP A:954, MET A:887, GLY A:953, LYS A:621, PHE	EIF2AK3	4G31

			A:943, VAL A:651, VAL A:606, ALA A:619, PHE A:955		
2.	M5b	Family AG Protein coupled receptor	ILE A:1379, LEU A:1355, PHE A:1276, ILE A:1174, ALA A:1189, ALA A:1171, VAL A:1192	ADORA1	5n2S
3.	M5c	Family AG Protein coupled receptor	TRP A:1237, LEU A:1162, PRO A:1191	ADORA1	5n2S
4.	M5d	Nuclear Receptor	LYS A:362, VAL A:364, PRO A:365, GLY A:366, ASP A:369, ALA A:307	ESR1	1A52
5.	M5e	Nuclear Receptor	ARG A:301, LEU A:294, PHE A:311, ALA A:257, LEU A:253, LEU A:431, ILE A:328, HIS A:430	ESR2	1HJ1
6.	M5f	Kinase	LEU A:243, GLU A:242, LEU A:244, ALA A:189, LEU A:167, PHE A:241, VAL A:175, VAL A:225, VAL A:324, LYS A:191	CLK4	6FYV
7.	M5g	Oxidoreductase	ALA A:199, LEU A:391, PHE A:200, HIS A:388, TRP A:387, TYR A:385, ASN A:382, HIS A:386, HIS A:207	PTGS2	1CXZ
8.	M5h	Oxidoreductase	LEU A:408, VAL A:444, LEU A:391, VAL A:295, GLN A:203, HIS A:388, PHE A:210, HIS A:207, TYR A:385, ALA A:202	PTGS2	1CXZ
9.	M5i	Family AG Protein coupled receptor	ILE A:23, ALA A:448, SER A:24, ARG A:51, GLY A:443, CYS A:406, PHE A:352, TYR A:444, TYR A:407	CHRM1	2bxs

10.	M5j	Family AG Protein coupled receptor	THR A:435, SER A:24, ILE A:23, GLY A:443, TYR A:407, PHE A:352, GLY A:67, CYS A:406, TYR A:444, ARG A:51	CHRM1	2bxs

Table 4: Molecular docking results with hydrogen bond, hydrophobic interaction and pi-pi interaction of novel benzofuran compounds in the form of binding energies (9)

The molecular docking was done by Auto Dock Vina 1.5.7 and the result predicted by Auto Dock Vina is in the form of binding energy which is shown in the below table i.e **Table No-4**

Docking score more negative values indicates higher binding affinity [12].Docking is the study of how two or more molecular structures (for example,enzymes, drugs and proteins) interact. **Table NO:4** Shows the binding energy of the compounds whereas **Figure 1-10** shows the remarkable docking interaction of ligand-Target.

Table No:4-Showing binding energies of the novel benzofuran compounds

S. No	Compound Name	Binding Energies (K calories/mol)	No. of Hydrogen bonds	Hydrophobic Interactions	Pi-Pi interaction
1.	M5a	-9.5	1	LEU:957, ILE:650,ALA:643	TYR:653, GLY:953
2.	M5b	-7.7	-	VAL:1188, LEU:1170, TYR:1117, GLU:1277	PHE:1276
3.	M5c	-6.4	-	PHE:1241	-

4.	M5d	-6.8	3	GLN:375, PHE:367, ARG:363, ALA:318, LEU:310	-
5.	M5e	-8.3	3	GLU:260, LEU:256, THR:254, MET:291, PHE:332, MET:295	PHE:311
6.	M5f	-8.5	-	GLY:245, GLY:168, ASP:325, GLU:206	-
7.	M5g	-8.9	2	GLN:203, LEU:390, THR:206, THR:212, PHE:210	-
8.	M5h	-9.8	-	PHE:395, PHE:404, ALA:199	-
9.	M5i	-9.8	1	GLY:434, GLY:22, THR:52, MET:445, GLY:67	TYR:407, TYR:444
10.	M5j	-10.6	5	GLY:434, GLY:22, THR:408, THR:52, GLY:66	TYR:444

Molecular docking is done to estimate the conformation of interaction of ligands-Target.

Some compounds has shown a remarkable docking interaction in the below figure **[Figure:1 -10** are showing 2D and 3D interaction of M5a-M5j

Figure 1: Ligand (M5a) and protein (4G31) 2-D and 3-D interaction images

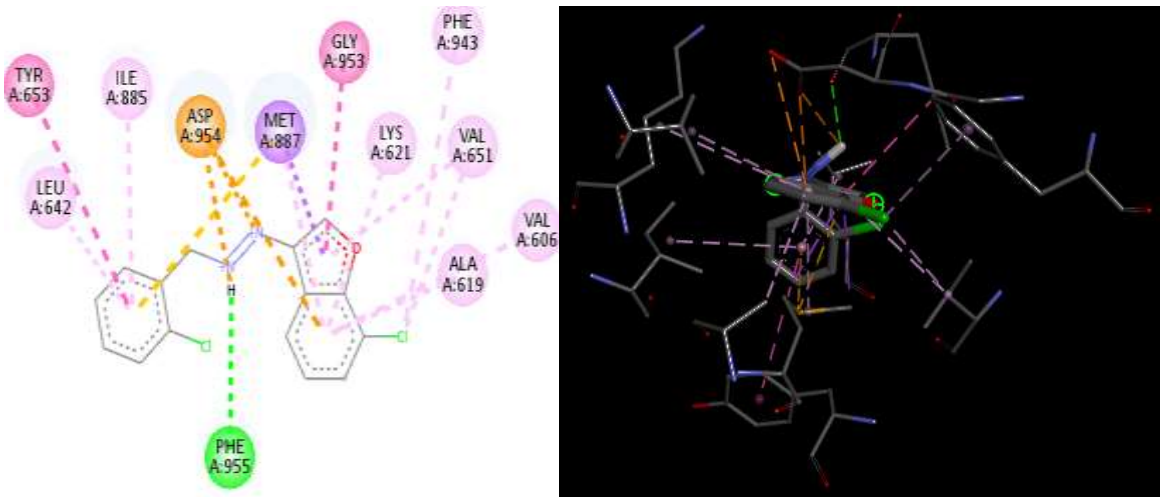


Figure 2: Ligand (M5b) and protein (5n2S) 2-D and 3-D interaction images

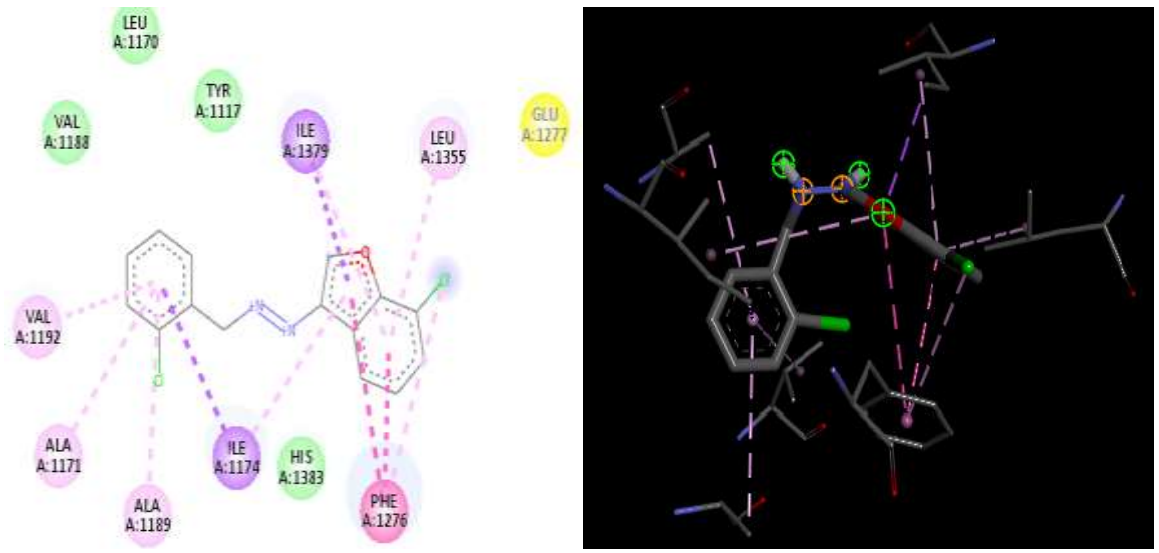


Figure 3: Ligand (M5c) and protein (5n2S) 2-D and 3-D interaction images

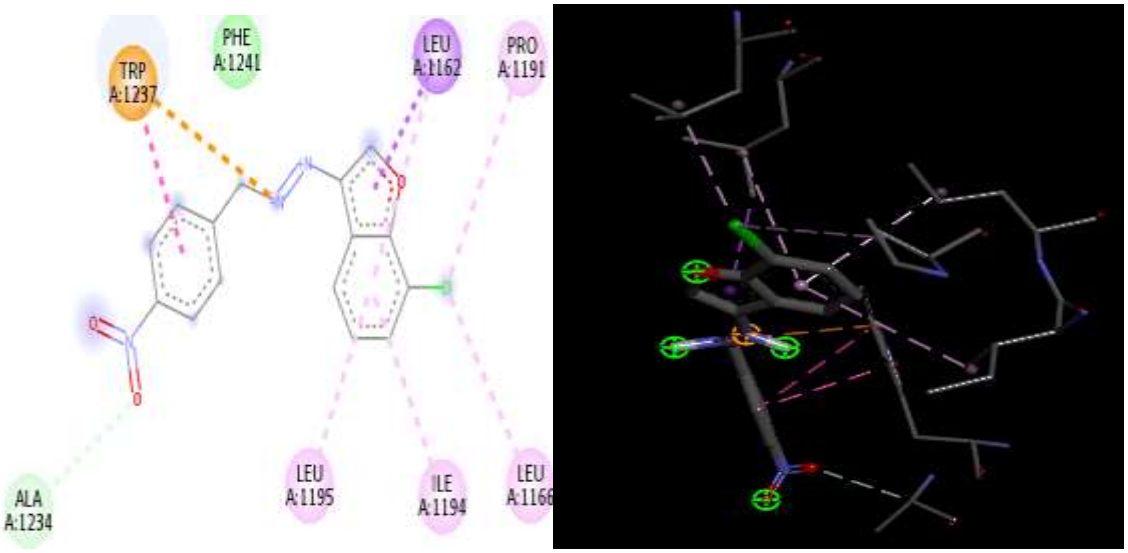


Figure 4: Ligand (M5d) and protein (1A52) 2-D and 3-D interaction images

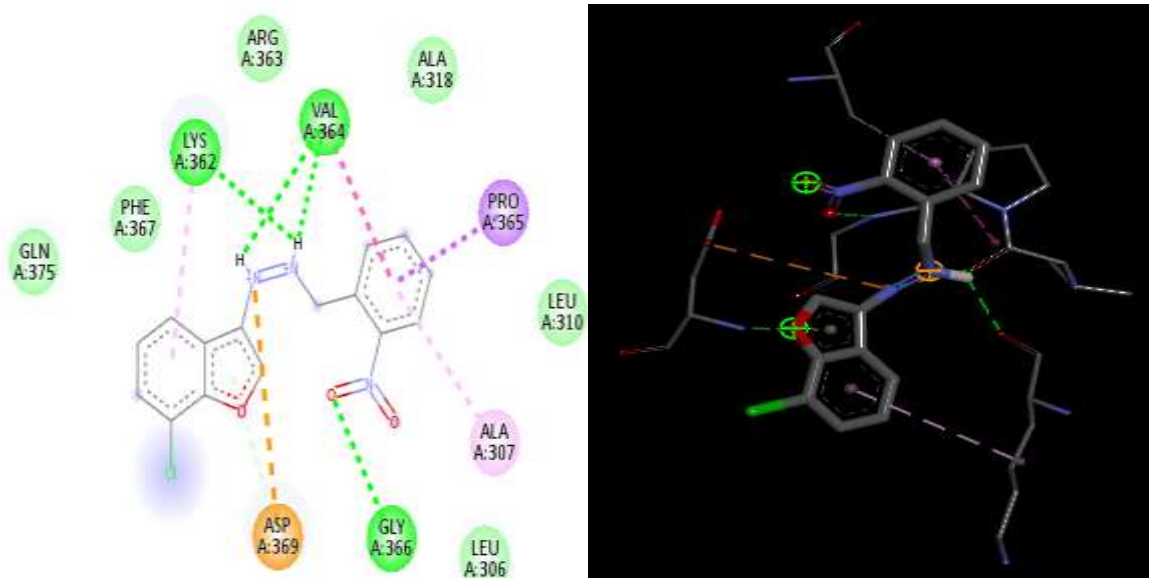


Figure 5: Ligand (M5e) and protein (1HJ1) 2-D and 3-D interaction images

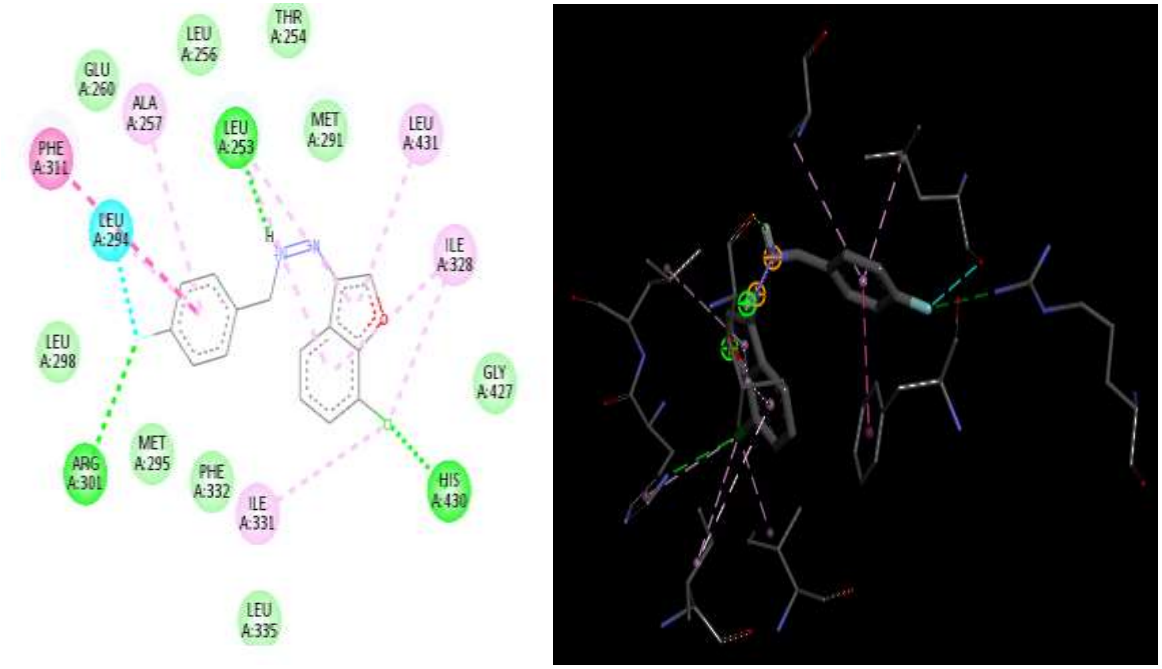


Figure 6: Ligand (M5f) and protein (6FYV) 2-D and 3-D interaction images

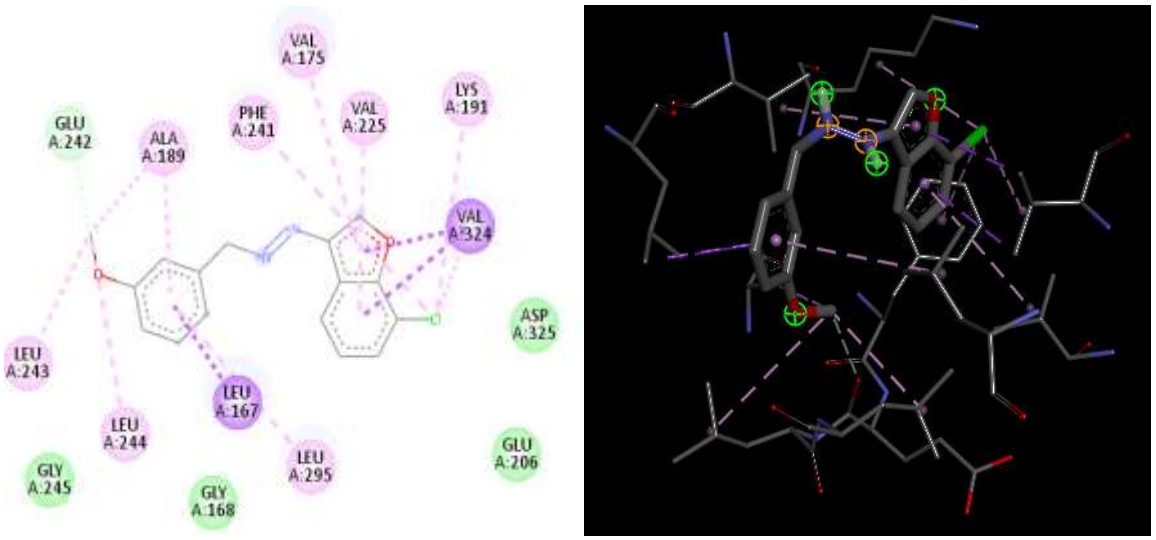


Figure 7: Ligand (M5g) and protein (1CXZ) 2-D and 3-D interaction images

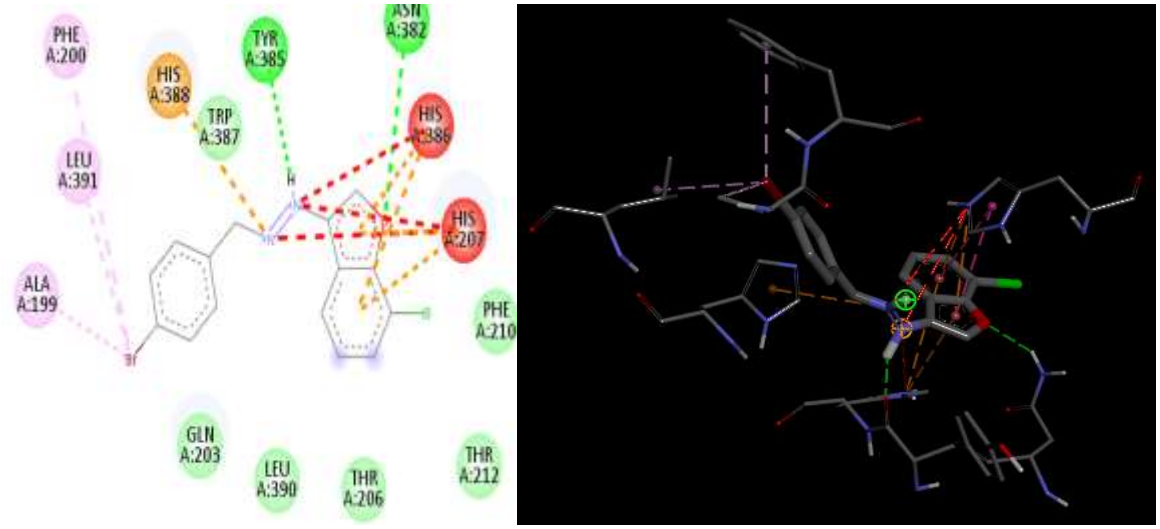


Figure 8: Ligand (M5h) and protein (1CXZ) 2-D and 3-D interaction images

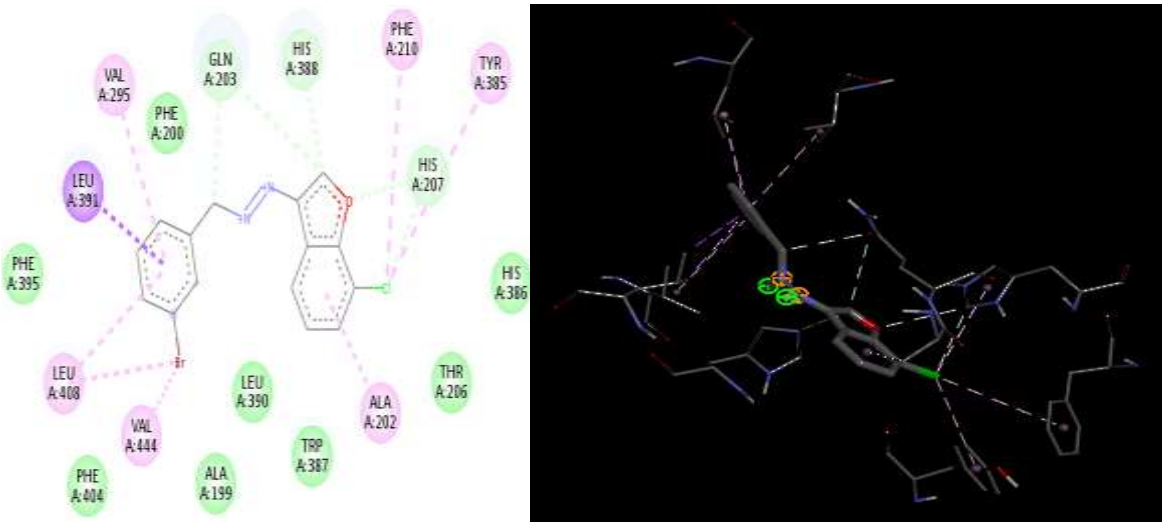


Figure 9: Ligand (M5i) and protein (2bxs) 2-D and 3-D interaction images

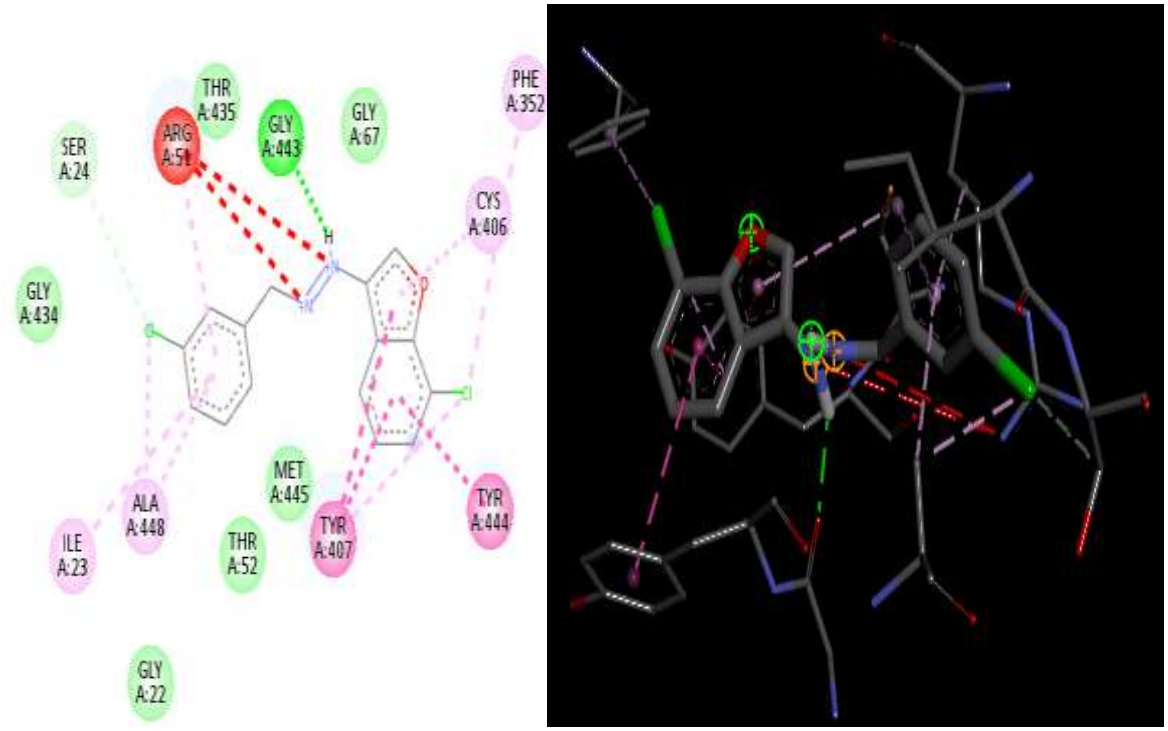
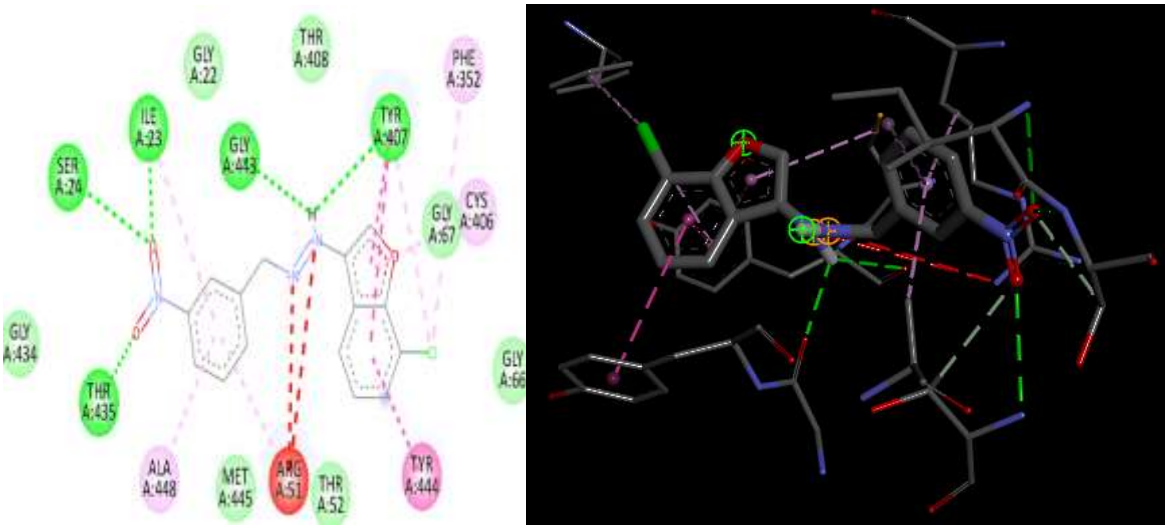


Figure 10: Ligand (M5j) and protein (2bxs) 2-D and 3-D interaction images



Novel Benzofuran Derivatives explanatory analysis by SwissADME

Novel benzofuran prepared derivatives analysis is done by SwissADME(www.swiss adme.ch/) to predict drug likeness and ADME profile, using the smile files of ligands is shown in **Table no.5**

Table N0.-5 Benzofuran prepared derivatives explanatory analysis by Swiss ADME(**9**)

Code of compound	**M.W≤ 500 Daltons**	**i-LOG-P**	**MLOG-P ≤ 5**	**TPSA**	**HBA≤ 5**	**HBD ≤ 10**	**N-rotb**	**nLV ≤ 2**	**GIAB**
M5a	305.16	3.01	3.54	37.53^0A	2	1	3	0	High
M5b	305.16	2.94	3.54	37.53^0A	2	1	3	0	High
M5c	315.71	2.34	2.76	83.35^0A	4	1	4	0	High
M5d	315.71	2.33	2.76	83.35^0A	4	1	4	0	High
M5e	288.70	2.78	3.42	37.53^0A	3	1	3	0	High
M5f	300.74	3.02	2.69	46.76^0A	3	1	4	0	High
M5g	349.61	3.07	3.66	37.53^0A	2	1	3	0	High
M5h	349.61	3.06	3.66	37.53^0A	2	1	3	0	High
M5i	305.16	2.92	3.54	37.53^0A	2	1	3	0	High
M5j	315.71	2.31	2.76	83.35^0A	4	1	4	0	High
M5k	315.71	2.35	2.76	83.35^0A	4	1	4	0	High
M5l	315.71	2.35	2.76	83.35^0A	4	1	4	0	High
M5m	326.26	1.80	1.29	129.17^0 A	6	1	5	0	Low
M5n	326.26	1.77	1.29	129.17^0 A	6	1	5	0	Low

M.W-molecular weight in gm/mol, Liphophilicity expressed as **LOG-P**, **iLOG-P**-implicit log-P method, **WLOG-P** –Wildman and Crippen method development, **T P S A**-Topological polar surface area, **H B A** Hydrogen bond acceptor, **H B D**- Hydrogen bond donor, **N - rotb**- number of rotatable bond, **nLV**- Lipinski violation, **GIAB**- Gastro-intestinal absorption, **%-AB** – Absorption percentage

Novel benzofuran derivatives bioactivity score by molinspiration tool(9)

By entering the smiles file of a novel compounds, in molinspiration tool, it leads to forecast the bioactivity score of synthesized compounds**(Displayed in Table no. 6)**

Table no-6: Bioactivity score of designed compounds by molinspiration software

Code of compounds	GPCR Ligands	Ion-channel modulators	Kinase inhibitors	Nuclear receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
M5a	-0.66	-0.97	-0.77	-1.03	-1.01	-0.42
M5b	-0.60	-0.91	-0.76	-0.97	-0.98	-0.42
M5c	-0.66	-0.90	-0.79	-0.93	-0.97	-0.50
M5d	-0.72	-0.92	-0.87	-0.86	-0.99	-0.58
M5e	-0.59	-0.93	-0.71	-0.94	-1.00	-0.43
M5f	-0.69	-0.98	-0.74	-0.89	-0.97	-0.46
M5g	-0.72	-1.01	-0.80	-1.11	-1.12	-0.51
M5h	-0.74	-1.02	-0.84	-1.14	-1.15	-0.52
M5i	-0.59	-0.90	-0.77	-0.98	-0.98	-0.41
M5j	-0.68	-0.91	-0.79	-0.93	-0.98	-0.52

Bioactive score if ≥0.00 then considerable biological activity, if between -0.50 to 0.0 then sensible activity, if less than -0.50 then in-active

Pass data of the selected compounds(9)

To anticipate the anticancer activity, the smile files of chosen compounds was entered into the PASS online web server (http://www.way2drug.com/). The summary is shown in **Table No.7**

Table No-7: Pass data of the selected compounds

	Antineoplastic	
Code of compound	**Pa**	**Pi**
M5a	0.309	0.070
M5b	0.314	0.067
M5c	0.271	0.115
M5d	0.258	0.138
M5e	0.304	0.075
M5f	0.281	0.100
M5g	0.256	0.142
M5h	0.254	0.145
M5i	0.307	0.072
M5j	0.272	0.113

Pa- Probability of activities, Pi- Probability of in activities

Preferable compounds, including M5a, M5b, M5e and M5i, have greater potential to exert anti-cancer activity, according to the PASS prediction.

Conclusion:

The present study can be summarized as 10 novel benzofuran derivatives (M5a-M5j), molecular docking is done by using Chem draw 3D 16.0, molinspiration Chem

informatics, Discovery studio, Pymol, Swiss target prediction software. By the use of these software we get to know the binding energy of compounds and by the binding energy we estimate the highest binding affinity. Compounds that have a higher negative charge of energy demonstrate greater binding to the target location and all the synthesized benzofuran derivatives has shown great negative binding energy (-7.7 to -10.7 Kcal/mol) hence these synthetic compounds i.e. M5a, M5b, M5i, can be useful as a lead molecule in cancer research and development according to the PASS prediction prospects

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NO

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