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Research Article

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Synthesis, molecular docking and cytotoxic study of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde

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ABSTRACT

The 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde was synthesized by known literature method (Wittig reaction approach) from vanillin. To deduce the anticancer and antibacterial activity of the 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde, it is docked with different biomarkers of cancer cell and bacteria. Grid was generated for each oncoproteins by specifying the active site amino acids. The binding model of best scoring analogue with each protein was assessed from their G-scores and disclosed by docking analysis using the XP visualizer tool. An analysis of the receptor-ligand interaction studies revealed that 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde is most active against 3LAU and 1VOM biomarkers and have the features to prove themselves as anticancer drugs. It shows strong cytotoxicity against human lung (A-459) and breast (MCF-07) cell lines.

Keywords: Benzofurans, Molecular docking, Anticancer, 1VOM, 3LAU, Wittig reaction.

INTRODUCTION

Molecular modelling can accelerate and guide to the chemist or scientist for drug design and contribute to the understanding of the biochemical functions of gene products. These molecular modelling techniques used for the study of organic/inorganic/bio molecules use theoretical and computationally based methods to model or mimic the behavior of molecule/s and have been widely applied for understanding and predicting the behavior of molecular systems [1]. Molecular modelling has become an essential part of contemporary drug discovery processes of new molecules. A traditional approach for drug discovery of molecules relies on step-wise synthesis and screening of large numbers of compounds to optimize activity profiles of molecule which is to act as drug; this is extremely time consuming and costly method takes decades of years. The cost of these processes has increased significantly in recent years [2], and it takes over a decade for a very small fraction of compounds to pass the drug discovery pipeline from initial screening hits or leads, chemical optimization, and clinical trials before launching into the market as drug. The approaches and methodologies used in drug design have changed over time, exploiting and driving new technological advances to solve the varied bottlenecks found along the way. There are several programs used for docking, including DOCK-6, FlexX, GLIDE, GOLD, FRED, and SURFLEX has been assessed and these programs proved to generate reliable poses in numerous docking studies.

Until 1990, the major issues were lead discovery and chemical synthesis of drug-like molecules; the emergence of combinatorial chemistry,[4] gene technology, and high-throughput tests [5,6] has shifted the focus, and poor absorption, distribution, metabolism, and excretion (ADME) properties of new drugs captured more attention [7].

Protein docking is a computational problem to predict the binding of a protein with potential interacting partners. The docking problem can be defined as: Given the atomic coordinates of two molecules, predict their correct bound

association [3], which is the relative orientation and position after interaction. There are three key components in protein docking: (1) representation of the molecules, (2) searching and (3) scoring of the potential solutions.

EXPERIMENTAL SECTION

Docking software used: Maestro 9.9 (Schrodinger). Protein Crystal Structures (PDB ID: **1RJB, 3FDN, 3LAU, 4BBG, 3V3M, 1BAG, 3F8S, 2b4J, 1Z92, 1YC, 4FNY, 2BOU, 1UFQ, 1VOM, 2AZ1, 1KDR, 3MK2, 1TE6, 1P62**). These proteins are characterized by Ramachandran plot.

Table 1: Information of different PDBs

PDB of protein	Worked as	Source
4ASE	Vascular endothelial growth factor receptor 2	Homo sapiens
1YCR	MDM2 bound to the trans-activation domain of p53	Homo sapiens
1Z92	Interleukin-2 with its alpha receptor	Homo sapiens
2b4J	Recognition between hiv-1 integrase and ledgf/p75	Homo sapiens
3F8S	Dipeptidyl peptidase IV (DPP-4) in complex with inhibitor	Homo sapiens
1BAG	Alpha-amylase from bacillus subtilis complexed with maltopentaose	Bacillus subtilis
1RJB (FLT3)	FI cytokine receptor	Homo sapiens
3FDN	Serine/threonine-protein kinase 6	Homo sapiens
3LAU	Arora 2 kinase	Homo sapiens
4BBG	Human kinesin eg5 -like protein kif11	Homo sapiens
3V3M	3C-like proteinase [severe acute respiratory syndrome coronavirus (sars-cov) 3cl protease]	Homo sapiens
1TE6	Gamma enolase [human neuron specific enolase]	Homo sapiens
1VOM	Dictyostelium myosin	Dictyostelium discoideum
2BOU	EGF domains 1,2,5 of human emr2, a 7-tm immune system molecule	Homo sapiens
3MK2	Placental alkaline phosphatase	Homo sapiens
1KDR (Chain A)	Cytidine monophosphate kinase	Escherichia coli
1P62	Deoxycytidine kinase	Escherichia coli
1UFQ	Uridine-cytidine kinase 2	Homo sapiens
2AZ1	Nucleoside diphosphate kinase	Escherichia coli
4FNY	ALK tyrosine kinase receptor	Homo sapiens

1.1. Protocol for ligand-receptor docking:

The three dimensional structures of all proteins were taken from the PDB database. The native autoinducer and all water molecules were removed from basic protein structures. Hydrogen were added using the templates for the protein residues. The three-dimensional structure of the ligand [7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde] was constructed. The ligand was then energy-minimized in the in-built Chem Sketch module of the software.

1.2. Docking:

The active site of each protein were first identified and defined using an eraser size of 5.0 Å. The ligand was docked into the active site separately using the 'Flexible Fit' option. The ligand-receptor site complex was subjected to '*in situ*' ligand minimization which was performed using the in-built CHARMm force field calculation. The nonbond cutoff and the distance dependence was set to 11 Å and (ε = 1R) respectively. The determination of the ligand binding affinity was calculated using the shape-based interaction energies of the ligand with the protein. Consensus scoring with the top tier of s=10% using docking score used to estimate the ligand-binding energies.

2. Study of molecular structure and properties:

Molecular structure has been studied by different molecular programs such as Avogadro, Glide, etc. The molecular parameters such as non-bonded atom bond lengths, bond angles, Drug likeness property has been studied by VEGA ZZ 3.0.3 program.

Fig 1: Van der Waal surface, bond length and bond angles of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde

Van der Waal surfaces **Determination of bond angles**

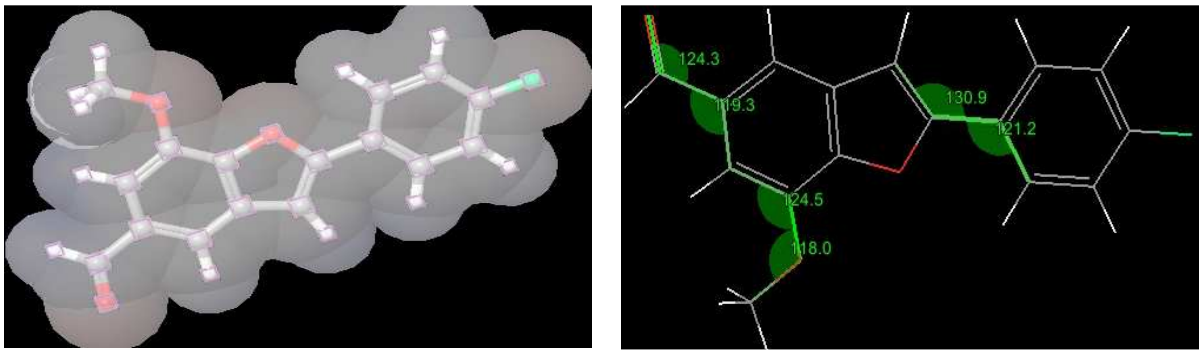
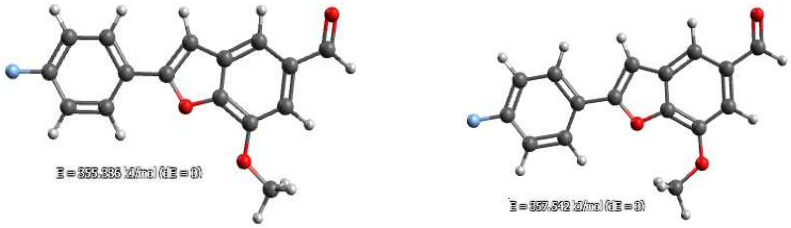


Fig 2: Most stable orientations of groups 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde



Total energy: **355.386** kJ/mol Total energy: **357.542** kJ/mol

Table 2A: Some molecular functions / properties of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde

Molecular formula:	$C_{16}H_{11}FO_3$
Total Energy	**8.5050** kcal/mol.
Molecular weight	**270.262** g/mol.
m/z values	270.07 (100.0%), 271.07 (17.3%), 272.08 (1.4%)
Elemental analysis (% analysis)	C, 71.11; H, 4.10; F, 7.03; O, 17.76
H - donor	**0**
H – bond acceptor	**4**
Energy of HOMO	-11.342 eV
Energy of LUMO	-04.780 eV
Formal charge	0
Gibbs free energy	-126.25 kJ/mol (at 298K & 1atm)
Ovality	1.468665
Partition coefficient	4.579800
Heat of formation	-352.85 kJ/mol (at 298K & 1atm)
Ideal gas thermal capacity	275.235 J/mol.K
Water solubility	0 mg/lit
Stereochemistry	C(8)-C(7): (**Z**)
LogP	3.157 **(n-Octanol/water)**
Mol Refractivity	73.183cm^3/mol
Lipinski Rule	270.069;4;0;;4.58
Henry's Law Constant	6.547
Connolly Accessible Area	481.919 A^2
Num Rotatable Bonds	3 bonds
Polar Surface Area	35.53 A^2
Sum of charges	0.0
Solvation energy	-5.146496 eV
Electrostatic Energy	-75.4876 kcal/mol
Dipole	2.4621 Debye
Membrane energy	0.923756 eV

Table 2B: Application of VEGA ZZ 3.0.3 for study of Druglikeness property

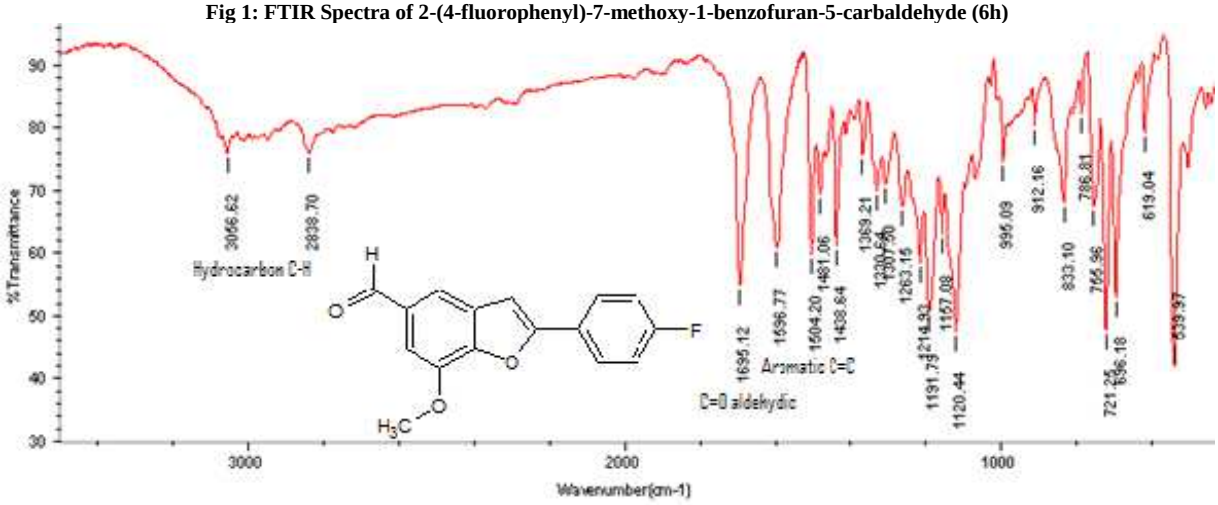
Property		7-methoxy-2-(3-methoxylphenyl)-1-benzofuran-5-carbaldehyde
By using Lipinski rule of five		
Molecular weight	Dalton	270.255
No. of H-bond acceptor	(< 10)	03
No. of H-bond donor	(< 5)	00
Virtual Log P	(< 5)	4.230
Comment		Ok
By using Ghose's rule of five		
Molecular weight	Dalton	270.255
Number of atoms	20 – 70	31
Vertual Log P	-0.4 – 5.6	4.230
Molar refractivity	40 – 130	74.4412
Comment		Ok

3. Experimental Work:

A mixture of phosphonium salt (0.0064 mol), 4-fluorobenzoyl chloride (0.0064 mol) and triethylamine (1.50 g, 0.0148 mol) in toluene (60 ml) was heated under reflux for 4.5 hr. The completion of reaction was confirmed by using TLC. The reaction mixture was cooled to room temperature and adds 40 ml water to it and shake well. The organic layer was separated, washed with 20 ml water and dried by anhydrous sodium salphate. Toluene was distilled off under reduced pressure and the (faint yellow) solid obtained was purified by column chromatography (using 40% ethyl acetate in pet ether as mobile phase) to afford the solid 2-(4-fluorophenyl)-7-methoxy-1-benzofuran-5-carbaldehyde (**6h**) (57 %), yellow crystalline solid, m.p. 126-128 ^{0}C

FT-IR (KBr): 3056, 2838, 1695, 1596, 1504, 1438, 1191, 1120, 833, 721 cm^{-1}. NMR (300 MHz) ($CDCl_3$; δ ppm): $C_{16}H_{11}FO_3$ (Mol. Wt. 270.255 g/mol): 4.02 (s, 3H, OCH_3); 7.01 (s, 1H, Ar-H); 7.21 – 7.38 (m, 3H, Ar-H); 7.58 (t, J = 8Hz, 2H, Ar-H); 7.85 (s, 1H, Ar-H); 9.99 (s, 1H, -CHO). Mass Spectra (M + 1): 271.13

Fig 1: FTIR Spectra of 2-(4-fluorophenyl)-7-methoxy-1-benzofuran-5-carbaldehyde (6h)



3.1. Generation of docking sites:

The binding sites for the docking are generated by using Glide software. The site of the protein having more site score is considered for the docking of ligand. The site which having maximum *site points*, locate on the site in different colours as hydrophobic and hydrophilic maps. The hydrophilic maps are further divided into donor, acceptor, and metal-binding regions. Other properties characterize the binding site in terms of the size of the site, degrees of enclosure by the protein and exposure to solvent, tightness with which the site points interact with the receptor, hydrophobic and hydrophilic character of the site and the balance between them, and degree to which a ligand might donate or accept hydrogen bonds. These all properties are summarized in following table 3.

The docking site scores, size, volume exposure, enclosure, contact, hydrophobic and hydrophilic nature, donor and acceptor ratio of all proteins are shown in table 3.

Table 3: Properties of docking sites of receptors

protein	Site Score	size	D score	volume	exposure	enclosure	contact	phobic	philic	balance	don/acc
3V3M	0.913	75	0.852	258.279	0.611	0.715	0.927	0.473	1.200	0.395	0.510
4BBG	1.040	**223**	1.034	503.867	0.522	0.758	1.035	1.274	1.108	1.150	0.725
3LAU	1.046	116	1.095	437.325	0.609	0.703	0.883	1.245	0.819	1.520	0.749
3FDN	1.047	206	1.02	**760.774**	0.531	0.768	0.964	0.758	1.170	0.648	0.880
1RJB	1.073	100	1.037	195.51	0.492	0.807	**1.124**	0.668	1.186	0.563	0.706
1BAG	0.989	143	0.989	425.663	0.676	0.681	0.849	0.343	1.103	0.311	**0.478**
3F8S	1.009	146	1.012	489.118	0.647	0.711	0.855	0.298	1.089	0.274	0.762
2b4J	1.074	121	1.136	552.321	0.752	0.728	0.860	1.321	0.745	1.773	1.456
1Z92	0.961	95	1.013	316.246	0.749	0.599	0.699	0.396	0.805	0.492	1.427
1YCR	0.755	41	**0.754**	**90.552**	0.653	0.620	0.849	1.171	0.675	1.735	**2.006**
1TE6	1.05	193	0.849	507.64	0.515	0.773	0.993	**0.008**	1.703	**0.004**	0.595
1VOM	1.074	222	1.114	618.772	0.605	0.754	0.934	1.022	0.853	1.198	0.708
2BOU	**0.464**	**16**	0.375	45.962	**0.807**	**0.542**	0.727	0.134	1.000	0.134	1.433
3MK2	0.872	73	0.914	179.389	0.731	0.574	**0.712**	0.632	0.717	0.882	0.623
1KDR	1.047	276	0.963	749.112	0.472	0.768	1.009	0.463	1.343	0.345	0.661
1P62	1.048	200	0.948	372.841	0.438	0.770	1.007	0.49	1.393	0.352	0.520
1UFQ	1.009	176	1.042	756.315	0.656	0.684	0.862	0.51	0.947	0.538	0.931
2AZ1	**1.121**	150	0.958	367.01	**0.385**	**0.879**	1.096	0.397	**1.562**	0.254	0.665
4FNY	1.092	195	**1.161**	426.349	0.556	0.724	0.932	**1.470**	**0.654**	**2.249**	1.858

The docking site score of 2AZ1 (**1.121**) receptor/protein is higher while that of 2BOU (**0.464**) is lowest is indicates that the 2AZ1 protein PDB is more favorable for docking than the others. The size (223) and volume (760.774) available for docking is higher in 4BBG and 3FDN PDBs respectively but exposure to the ligand as compared to 2BOU is lower. The exposure to the ligand is maximum in 2BOU and minimum in 2AZ1 while reverse is the case for the enclosure area, it is higher in 2AZ1 and minimum in 2BOU. The overall contact area to the ligand is higher in 1RJB (1.124). The hydrophobic nature or character and balance between hydrophobic and hydrophilic nature of the active site is higher in 4FNY and 2b4J respectively while that of lower in 1TE6. The hydrophilic nature or character of the active site is higher in 2AZ1 and lower in 4FNY. The ligands having more hydrophilic nature are more tightly binds with 1TE6 and weakly binded to 4FNY (according to the hydrophobic to hydrophilic ratio i.e. balance is higher in 4FNY than lower in 1TE6).

The order protein in the decreasing order of hydrophilic character and increasing order of hydrophobic character is – 1TE6 > 2BOU > 2AZ1 > 3F8S > 1BAG > 1KDR > 1P62 > 3V3M > 1Z92 > 1UFQ > 1RJB > 3FDN > 3MK2 > 4BBG > 1VOM > 3LAU > 1YCR > 2b4J > 4FNY. This indicates that the ligands having more hydrophobic nature are binds easily 4FNY. The hydrogen bond donor/acceptor character ratio is higher in 1YCR (2.006) while lower in 1BAG (0.478) therefore the ligand contains more hydrogen bond acceptor atoms/groups are more tightly binds to 1YCR while those containing hydrogen bond donor atoms/groups are bind to 1BAG. The order protein in the decreasing order of H-bond donor to H-bond acceptor ratio is – 1YCR > 4FNY > 2b4J > 2BOU > 1Z92 > 1UFQ > 3FDN > 3F8S > 3LAU > 4BBG > 1VOM > 1RJB > 2AZ1 > 1KDR > 3MK2 > 1TE6 > 1P62 > 3V3M > 1BAG.

3.2. Molecular docking:

The estimation of binding affinity of the ligand-receptor/protein complex is still a challenging task. Scoring functions (docking score) in docking programs take the ligand-receptor/protein poses as input and provides ranking or estimation of the binding affinity of the pose. These scoring functions require the availability of receptor/protein-ligand complexes with known binding affinity and use the sum of several energy terms such as *van der Waals* potential, electrostatic potential, hydrophobicity and hydrogen bonds in binding energy estimation. The second class consists of *force field-based scoring functions*, which use atomic force fields used to calculate free energies of binding of ligand-receptor/protein complex.

Table 4A: Docking properties of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

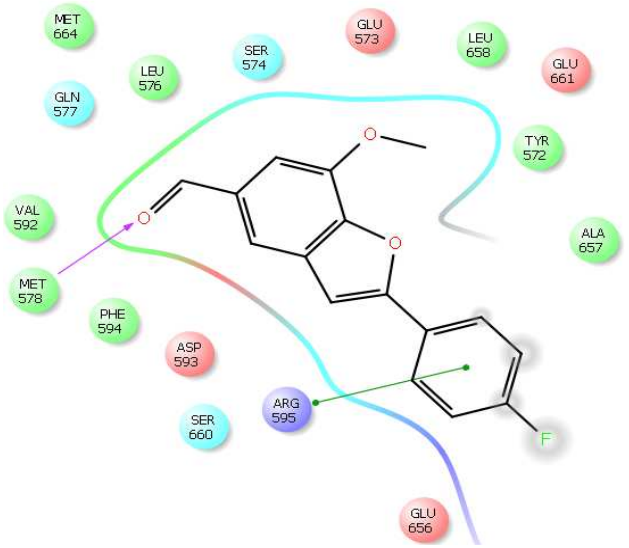
Description	Protein									
	1BAG	**1YCR**	**1Z92**	**2b4J**	**3F8S**	**1TE6**	**1VOM**	**2BOU**	**3MK2**	**1KDR**
Potential Energy OPLS 2005	61.805	61.805	61.805	61.805	61.805	61.805	61.805	61.805	61.805	61.805
RMS Derivative OPLS 2005	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Glide lignum	10	10	10	15	15	9	9	9	9	9
Docking Score	-6.216	-4.305	-4.607	-3.825	-4.176	-3.744	**-6.626**	-4.003	-5.059	-4.402
Glide Ligand efficiency	-0.311	-0.215	-0.23	-0.191	-0.209	-0.187	-0.331	-0.2	-0.253	-0.22
Glide Ligand efficiency sa	-0.844	-0.584	-0.625	-0.519	-0.567	-0.508	-0.899	-0.543	-0.687	-0.597
Glide Ligand efficiency In	-1.556	-1.077	-1.153	-0.957	-1.045	-0.937	-1.658	-1.002	-1.266	-1.102
Glide gscore	-6.216	-4.305	-4.607	-3.825	-4.176	-3.744	-6.626	-4.003	-5.059	-4.402
glide lipo	-1.966	-1.037	-0.923	-0.242	-0.914	**-0.132**	-3.232	-1.436	-2.298	-0.816
glide hbond	0	0	-0.241	-0.033	0	-0.392	-0.139	0	0	-0.326
glide metal	0	0	0	0	0	0	0	0	0	0
glide rewards	-2.41	-1.978	-1.727	-2.054	-1.879	-1.566	-1.935	-1.572	-1.732	-1.828
Glide evdw	-29.612	-23.601	-25.649	-26.315	-25.677	**-21.295**	-32.733	-21.234	-25.163	-28.023
Glide ecoul	-4.034	-2.07	-4.251	-2.587	-2.287	-4.874	0.472	-0.959	-0.076	-1.696
glide erotb	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245
glide esite	0	-0.044	-0.042	-0.038	0	**-0.103**	0	-0.034	-0.004	-0.02
Glide emodel	-45.819	-32.783	-38.451	-34.093	-35.725	-32.705	-42.127	**-27.438**	-33.504	-37.818
Glide energy	-33.646	-25.670	-29.899	-28.901	-27.965	-26.169	-32.261	**-22.193**	-25.239	-29.718
Glide einternal	1.314	0.873	0.288	1.314	0.653	0.552	6.023	0.345	0.217	0.939
glide confnum	2	2	1	2	1	2	1	1	2	2
Glide posenum	104	266	364	163	334	336	327	27	389	71
XP GScore	-6.216	-4.305	-4.607	-3.825	-4.176	-3.744	-6.626	-4.003	-5.059	-4.402
H-Bonds										
pi-pi/pi-cation interactions										

Table 4B: Docking properties of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

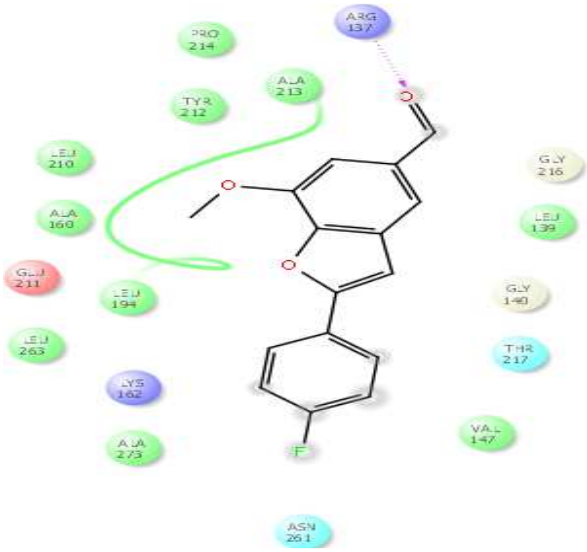
Description	Protein								
	1P62	**1UFQ**	**2AZ1**	**4FNY**	**1RJB**	**3FDN**	**3LAU**	**3V3M**	**4BBG**
Potential Energy OPLS 2005	61.805	61.805	61.805	61.805	61.805	61.805	61.805	61.805	61.805
RMS Derivative OPLS 2005	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Glide lignum	9	9	9	9	7	8	5	6	7
Docking Score	-4.349	-4.509	-4.817	-6.279	-6.024	-5.718	**-6.706**	**-3.514**	-5.657
Glide Ligand efficiency	-0.217	-0.225	-0.241	-0.314	-0.301	-0.286	**-0.335**	**-0.176**	-0.283
Glide Ligand efficiency sa	-0.59	-0.612	-0.654	-0.852	-0.818	-0.776	-0.91	-0.477	-0.768
Glide Ligand efficiency In	-1.088	-1.129	-1.206	-1.572	-1.507	-1.431	-1.678	-0.879	-1.416
Glide gscore	-4.349	-4.509	-4.817	-6.279	-6.024	-5.718	-6.706	-3.514	-5.657
glide lipo	-0.553	-1.28	-0.677	**-3.252**	-1.798	-1.025	-2.828	-1.045	-1.897
glide hbond	-0.208	-0.117	-0.251	0	-0.596	**-0.819**	-0.098	-0.594	-0.045
glide metal	0	0	0	0	0	0	0	0	0
glide rewards	-1.622	-1.615	-1.948	-1.804	-1.728	-1.354	-2.172	-1.224	-1.879
Glide evdw	-27.273	-27.198	-31.202	-29.306	**-33.453**	-30.113	-29.019	-25.001	-30.556
Glide ecoul	**-5.092**	-2.124	-4.063	-0.018	-3.069	-4.995	-2.408	-2.919	**0.007**
glide erotb	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245	0.245
glide esite	-0.084	-0.063	-0.017	-0.001	-0.015	-0.054	-0.041	-0.067	-0.034
Glide emodel	-41.168	-37.604	-43.782	-40.261	**-46.138**	-45.493	-44.786	-37.003	-28.237
Glide energy	-32.365	-29.322	-35.265	-29.324	**-36.522**	-35.108	-31.427	-27.921	-30.549
Glide einternal	1.570	1.154	3.955	0.312	**7.221**	0.323	0.218	**0.110**	0.273
glide confnum	1	1	1	2	2	1	2	1	1
Glide posenum	398	92	294	89	56	84	390	2	3
XP GScore	-4.349	-4.509	-4.817	-6.279	-6.024	-5.718	-6.706	-3.514	-5.657
H-Bonds						1			0
00pi-pi/pi-cation interactions									

Fig 4: 2D docking image of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different proteins

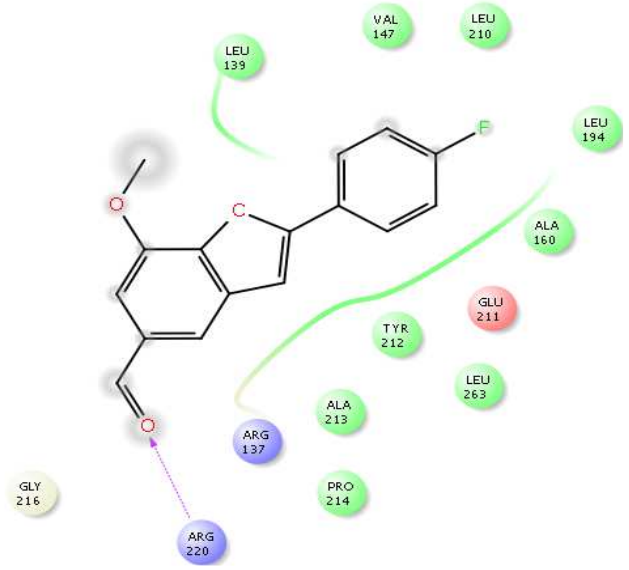
1RJB



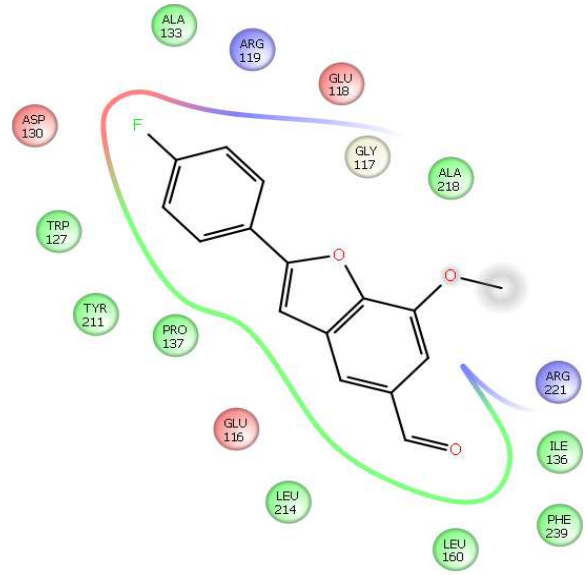
3FDN



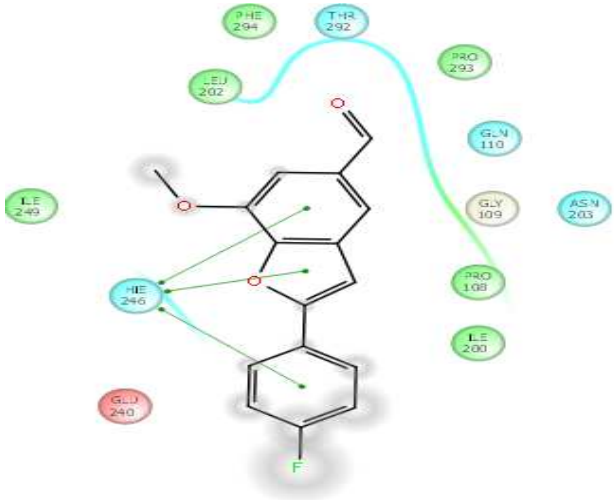
3LAU



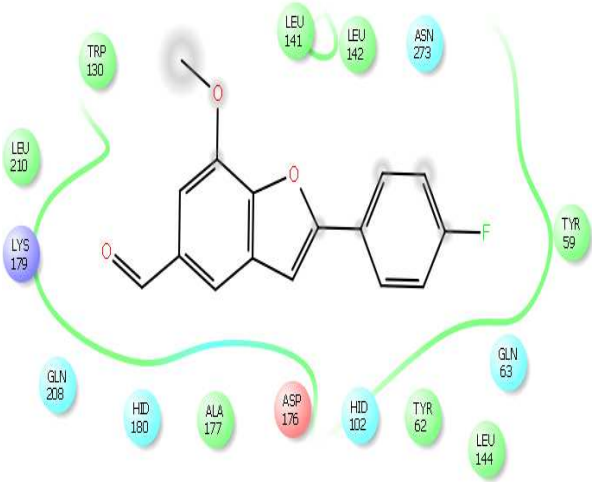
4BBG



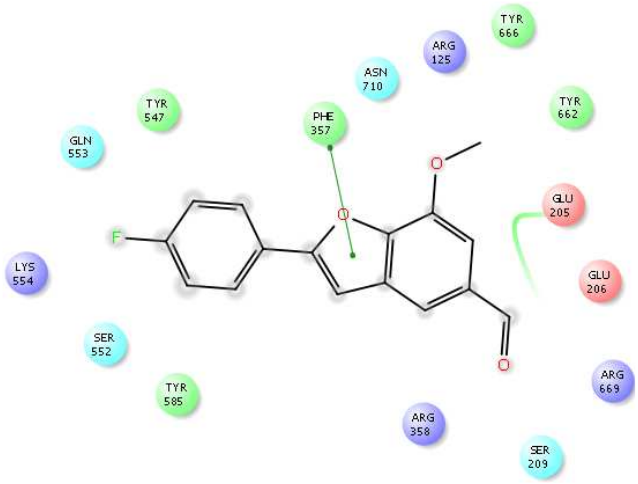
3V3M



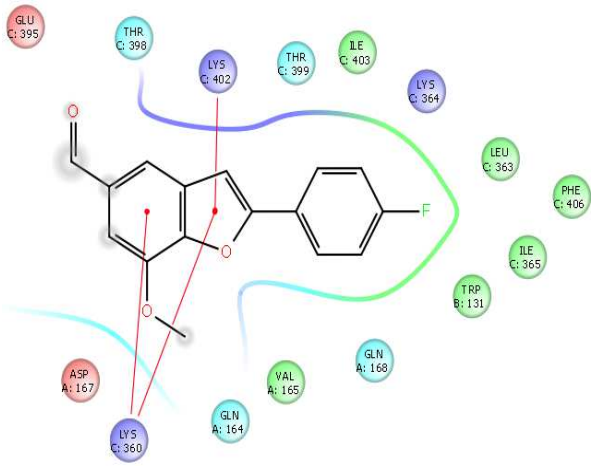
1BAG



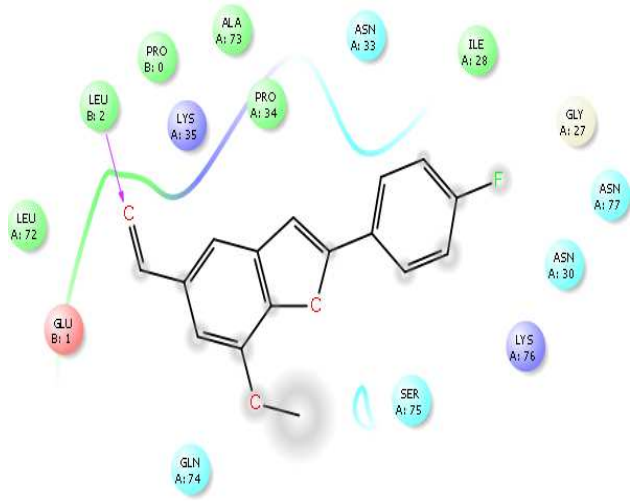
3F8S



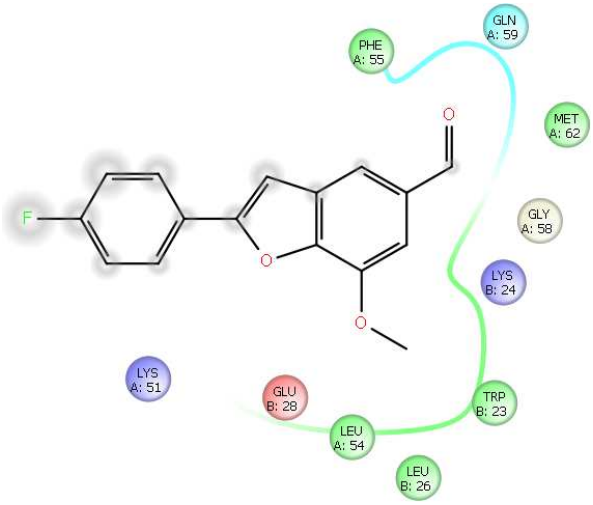
2b4J



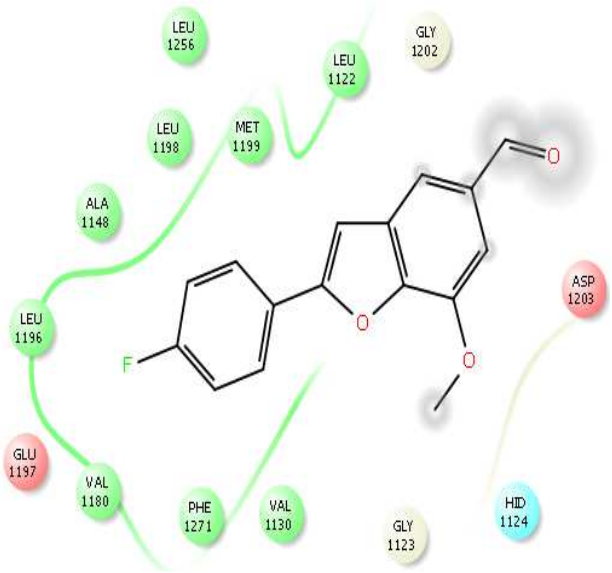
1Z92



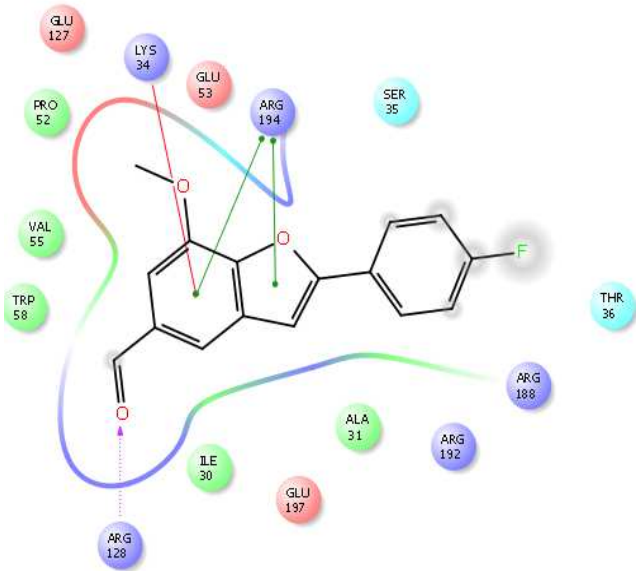
1YCR



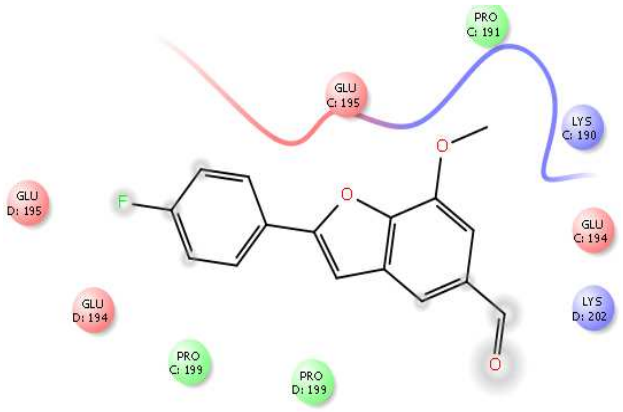
4FNY



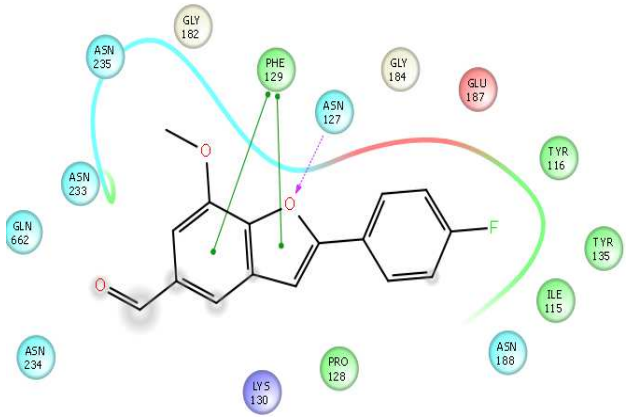
1P62



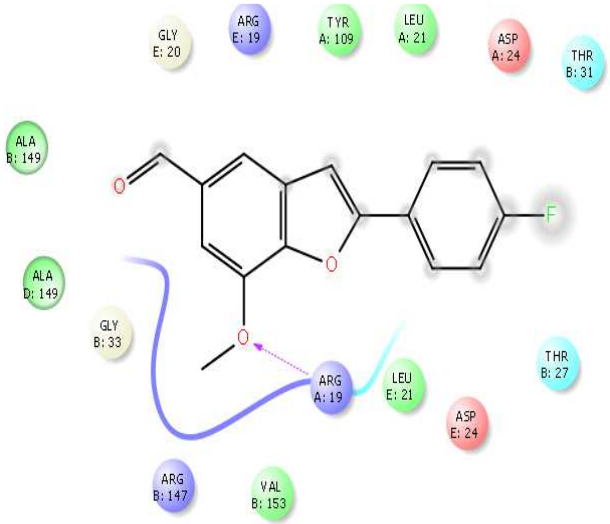
1UFQ



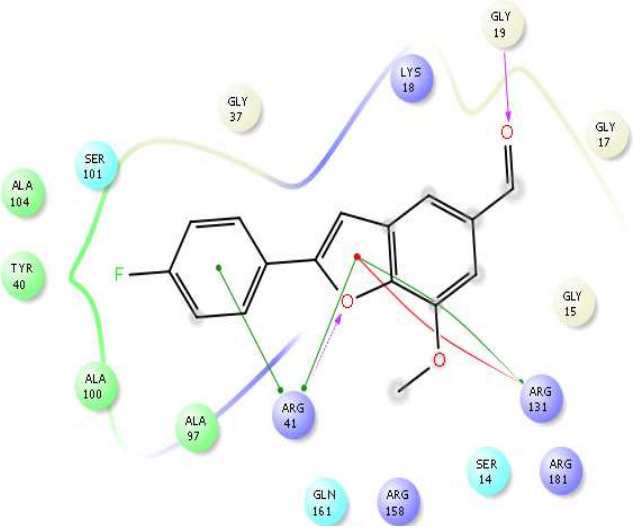
1VOM



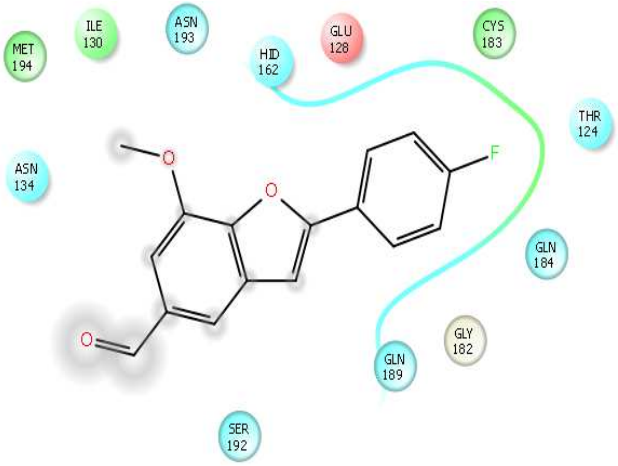
2AZ1



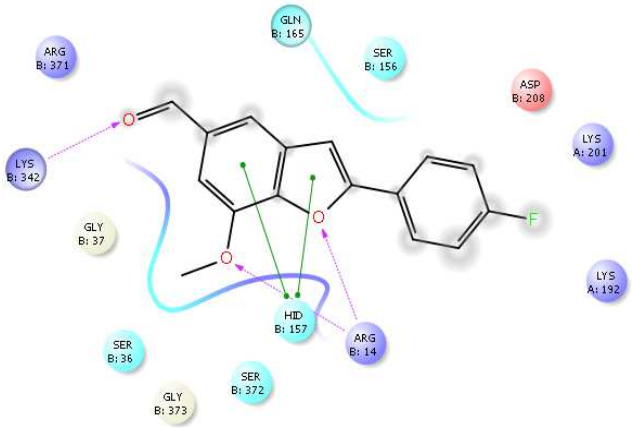
1KDR



3MK2



1TE6



2BOU

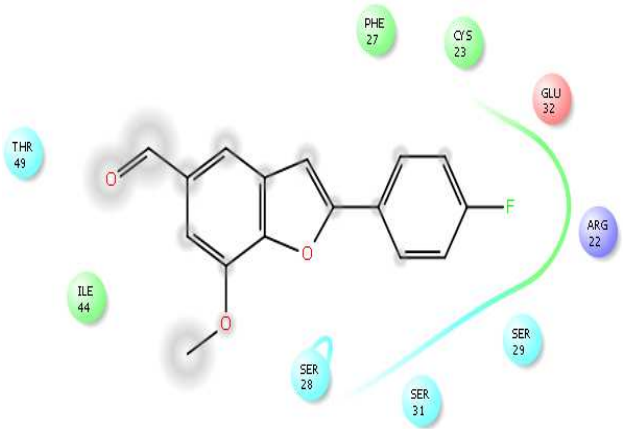
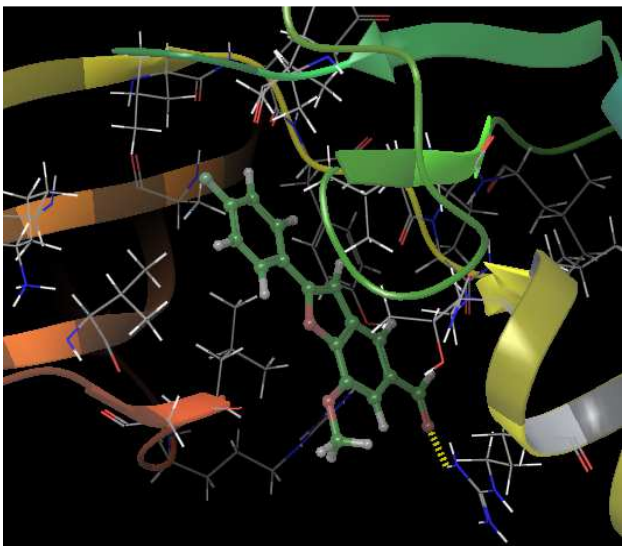
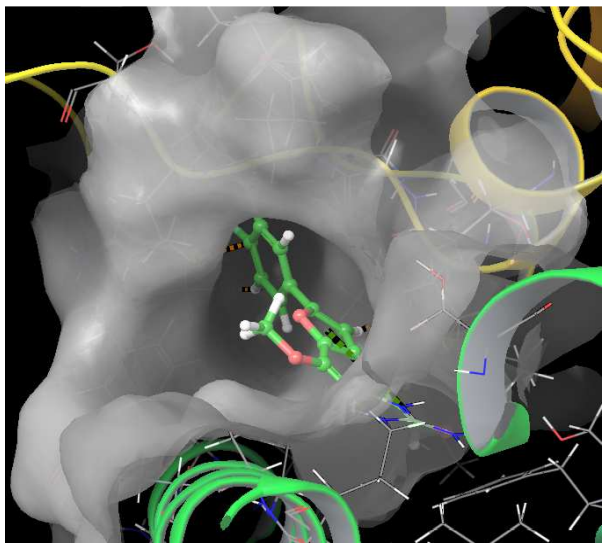


Fig 5: 3D docking image of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different proteins

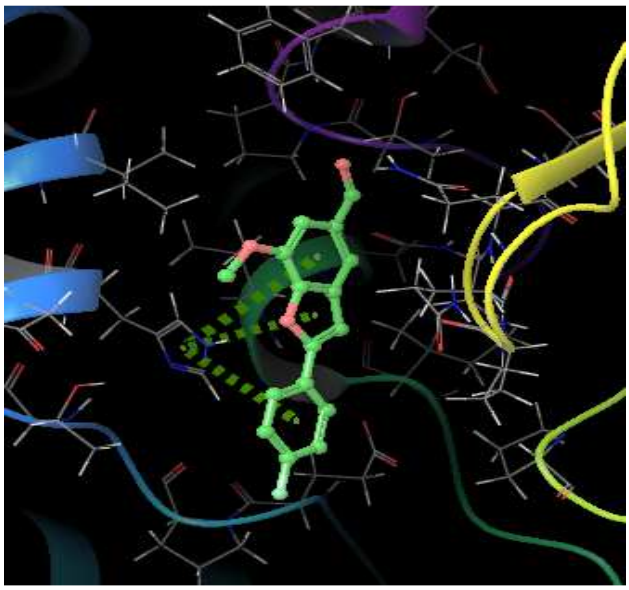
3LAU



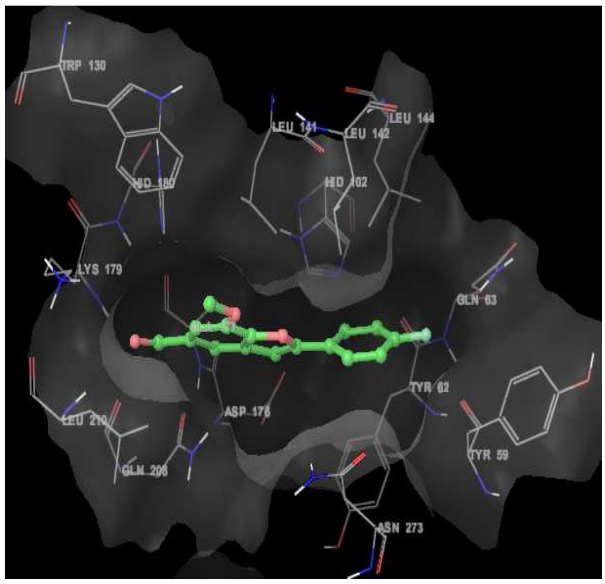
4BBG



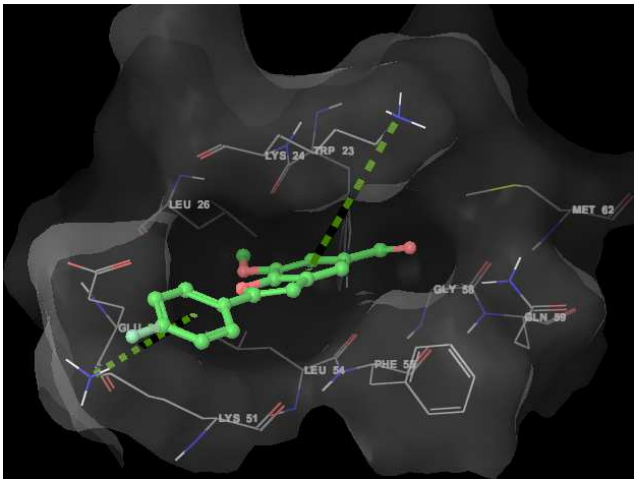
3V3M



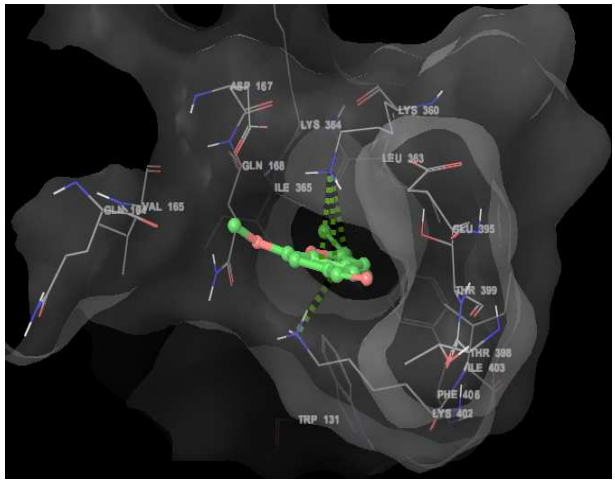
1BAG



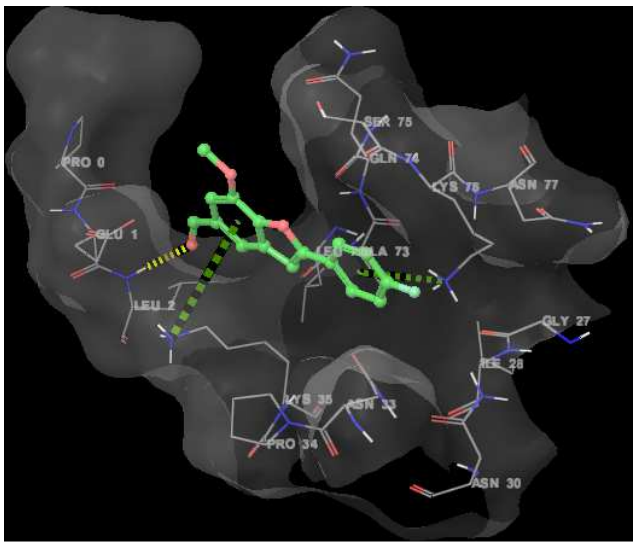
1YCR



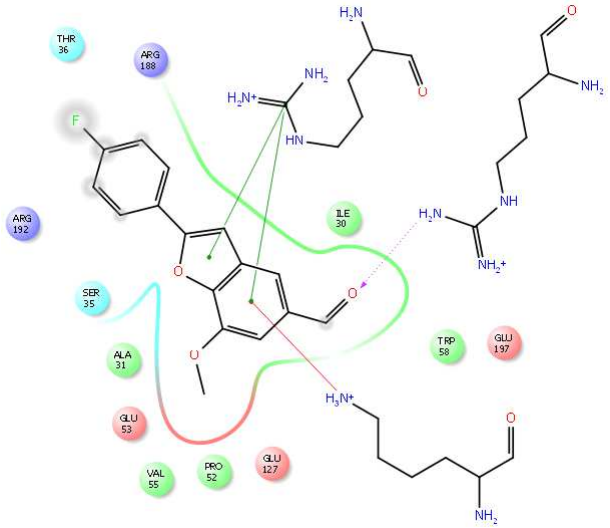
2b4J



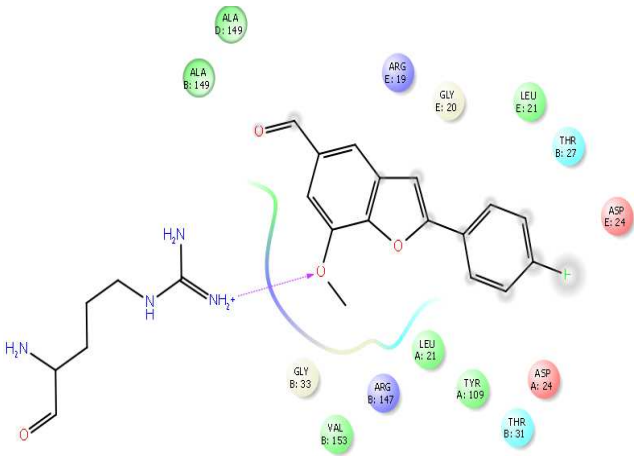
1Z92



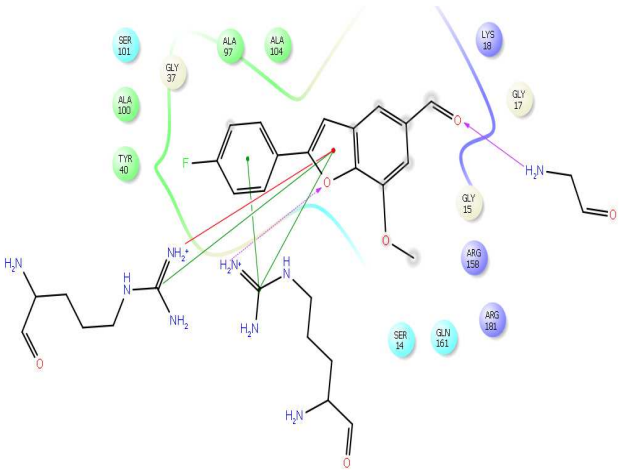
1P62



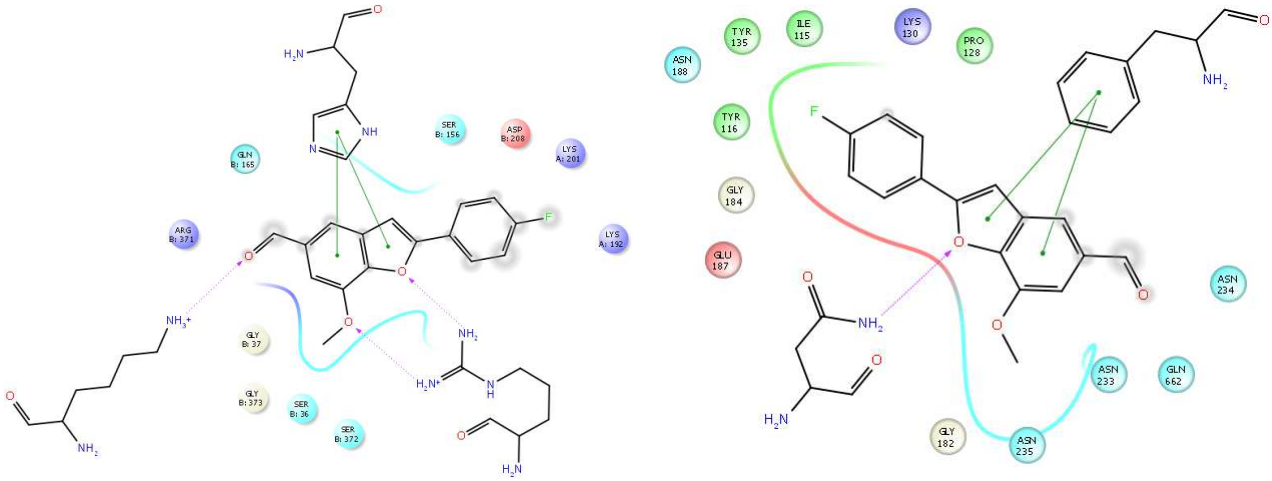
2AZ1



1KDR



1TE6 **1VOM**



4. Cytotoxic study:

Lung cancer cell line (A459) and Breast cancer cell lines (MCF-07) was selected as a test system because it is a commonly available cancer cell lines. It has been historically shown to be a suitable cell line module for cytotoxicity studies. The study was conducted in based on the in house standardized method and available literature to determine the cytotoxicity of test compound. The cancerous cell line viz. Breast (MCF - 07) and Lung (A - 549) were procured from National Center of Cell Science, Pune. The cells were allowed to acclimatize to the experimental laboratory conditions for a period of five days by regular pass aging of cells. Cell pass aging was done in the cell culture experimental room. Before the start of experiment the room was sterilized by keeping UV on for 20 minutes. The culture flasks were kept in 5% CO_2 incubator at 37^0C. The experimental room was cleaned and mopped daily with Liquid disinfectant. Each column was dedicated for specific test compound while two columns were used as cell control and two as positive control. Cells were exposed to the test compound for the period of around 18-24 hours.

Samples were freshly prepared in DMEM without phenol Red and then appropriate dilutions were prepared just prior to start of study. Cell viability assay was performed as per the standard procedure. The obtained data was subjected to statistical evaluation. CC50 values were calculated as the concentrations that show 50% inhibition of proliferation on the cell line.

Table 5: Percent cytotoxicity

Conc. mg/ml	MTT assay		MB assay	
	A – 459 cells	MCF - 07 cells	A – 459 cells	MCF - 07 cells
10	90.08	**95.03**	**92.97**	92.56
7.5	70.71	69.15	64.31	69.88
5.0	59.89	52.68	49.57	47.06
2.5	42.23	33.85	36.71	24.64
1.0	24.28	23.50	30.28	20.86
0.50	14.31	15.97	18.49	9.79
0.25	8.33	3.74	9.78	6.55
0.10	2.92	1.38	1.47	1.28

RESULTS AND DISCUSSION

The PBD 1YCR has more hydrogen bond donor character while the PDB 1BAG has more hydrogen bond accepting character at the docking site. The docking score of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde against different PDBs suggest that, it is more active against 3LAU (docking score -6.706) and 1VOM (docking score -6.626) while is less active against 3V3M (docking score -3.514) and 1YE6 (docking score -3.744). There are number of types of interactions observed between ligand and receptor such as hydrogen bonding, pi-pi interactions, ion-pi interactions, hydrophobic and hydrophilic interactions, ionic interactions, van der Waal interactions, etc along with steric interactions determine the docking score.

Table 6: Table of don/acc ratio, docking score, glide esite and polar interactions of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

Proteins	Description of property and amino acid information				
	don/acc at the docking site	Docking score	Glide esite	No. of hydrogen bonds (amino acid residues)	Polar interactions (amino acid residues) (π-π, π-cation)
1RJB	0.706	-6.024	-0.015	01 (MET578) (with backbone)	ARG595
3FDN	0.880	-5.718	-0.054	01 (ARG137) (with side chain)	--
3LAU	0.749	**-6.706**	-0.041	01 (ARG220) (with side chain)	--
4BBG	0.725	-5.657	-0.034	--	--
3V3M	0.510	**-3.514**	-0.067	--	HIE 246 (with three rings)
1BAG	**0.478**	-6.216	0	--	--
3F8S	0.762	-4.176	0	--	PHE357
2b4J	1.456	-3.825	-0.038	--	C-LYS360, C-LYS 402
1Z92	1.427	-4.607	-0.042	01 (B-LEU2) (with backbone)	--
1YCR	**2.006**	-4.305	-0.044	--	--
4FNY	1.858	-6.279	-0.001	--	--
2BOU	1.433	-4.003	-0.034	--	--
1UFQ	0.931	-4.509	-0.063	--	--
1VOM	0.708	-6.626	0	01 (ASN127) (with side chain)	PHE129, PHE129
2AZ1	0.665	-4.817	-0.017	01 (A-ARG19) (with side chain)	--
1KDR	0.661	-4.402	-0.020	02 (GLY19) (with backbone), (ARG41) (with side chain)	ARG41, ARG131, ARG41, ARG131
1P62	0.520	-4.349	-0.084	01 (ARG128) (with side chain)	ARG194 (with two rings), LYS34
3MK2	0.623	-5.059	-0.004	--	--
1TE6	0.595	-3.744	**-0.103**	03 (B-ARG14) (with side chain, two bonding), (B-LYS342) (with side chain)	B-HID 157, B-HID 157

Table 7: Table of glide evdw, glide energy, electrostatic and polar interactions 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

Proteins	Description of property and amino acid information				
	Glide evdw	Glide energy	Electrostatic interactions (blue)	Electrostatic interactions (pink)	Polar interactions (amino acid residues)
1RJB	**-33.453**	**-36.522**	ARG595	GLU573, ASP593, GLU656, GLU661	SER574, GLN577, SER660
3FDN	-30.113	-35.108	ARG137, LYS162	GLU211	THR217, ASN261
3LAU	-29.019	-31.427	ARG137, ARG220	GLU211	--
4BBG	-30.556	-30.549	ARG119, ARG221	GLU116, GLU118, ASP130	--
3V3M	-25.001	-27.921	--	GLU240	GLN63, ASN203, HIE246, THR292
1BAG	-29.612	-33.646	LYS179	ASP176	GLN63, HID102, HID180, GLN208, ASN273
3F8S	-25.677	-27.965	ARG125, ARG358, LYS554, ARG669	GLU205, GLU206	SER209, SER552, GLN553, ASN710
2b4J	-26.315	-28.901	C-LYS360, C-LYS364, C-LYS402	A-ASP167, C-GLU395	A-GLN164, A-GLN168, C-THR398, C-THR399
1Z92	-25.649	-29.899	A-LYS35, A-LYS76	B-GLU1	A-ASN30, A-ASN33, A-GLN74, A-SER75, A-ASN77
1YCR	-23.601	-25.670	A-LYS51, B-LYS24	B-GLU28	A-GLN59
4FNY	-29.306	-29.324	--	GLU1197, ASP1203	HID1124
2BOU	-21.234	**-22.193**	ARG22	GLU32	SER28, SER29, SER31
1UFQ	-27.198	-29.322	C-LYS190, D-LYS202	C-GLU194, C-GLU195, D-GLU194, D-GLU195	--
1VOM	-32.733	-32.261	LYS130	GLU187	ASN127, ASN188, ASN233, ASN234, ASN235, GLN662
2AZ1	-31.202	-35.265	A-ARG19, B-ARG147, E-ARG19	A-ASP24, E-ASP24	B-THR27, B-THR31
1KDR	-28.023	-29.718	LYS18, ARG41, ARG131, ARG158, ARG181	--	SER14, SER101, GLN161
3MK2	-25.163	-25.239	--	GLU128	THR124, ASN134, HID162, GLN184, GLN189, SER192, ASN193
1TE6	**-21.295**	-26.169	A-LYS192, A-LYS201, B-ARG14, B-LYS342, B-ARG371	B-ASP208	B-SER36, B-SER156, B-HID157, B-GLN165, B-SER372
1P62	-27.273	-32.365	LYS34, ARG128, ARG188, ARG192, ARG194	GLU53, GLU127, GLU197	SER35, THR37

Glide esite explains the polar interaction in the active site between ligand and amino acid residue at the docking site after recombination. The polar interactions between the aldehyde and amino acid residues of the protein are only observed in all PDBs except 1BAG, 3F8S and 1VOM. The aldehyde shows higher polar interaction in 1TE6, 1P62, 3V3M, 1UFQ and 3FDN proteins PDBs. This is one of the reason for the higher docking score of aldehyde in

1VOM. Also the molecule containing three hydrogen atom acceptors and hydrogen atom donor character of 4FNY at docking site is higher. The docking score of aldehyde during docking with 4FNY is higher (even though there is absence of hydrogen bonding and stronger pi-cation/anion interactions and polar interactions) because the molecule is completely fit into docking site with minimum internal strain and deformation of the geometry.

The aldehyde does not have any hydrogen atom which is capable of forming L (ligand)→P (protein) hydrogen bonding. It contains sp^2 and sp^3 hybridized oxygen atoms (carbonyl, ether and aromatic) capable of forming P → L type of hydrogen bonding during interaction. The backbone of ALA and GLY amino acids and side chain of ARG, GLN, TYR, ASN and LYS forming hydrogen bonding with ligand.

Glide evdw explains the van der Waal energy of the complex of ligand and amino acid residue at the docking site after recombination. The comparison between glide evdw and glide energy shows that van der Waal energy shows major contribution than coulombic energy for the stabilization of complex. The van der Waal interaction is depends on surface area (polar and non-polar) of the ligand, as surface area increases, van der Waal energy increases and vice versa. The contribution of glide evdw into the docking score is considerable. The Glide evdw suggested that interactions are higher with 1RJB while least with 1TE6.

Glide energy is summation of coulomb and van der Waal energy of interaction. The glide energy table indicates that, the comparatively coulombic force and van der Waal interactions (energies) are higher for the aldehyde-1RJB complex. This is due to higher surface area (both polar and non-polar) of 1RJB available for interaction with aldehyde.

The table 7 [**Electrostatic interactions (blue)**] shows that, two amino acids in all proteins as ARG and LYS shows positive interactions (hydrogen bonding between proton of protein and O/N of ligand or electrostatic interaction between positive centre of protein and negative / electron density of ligand). Both the amino acids containing amino group in their side chain which is capable of forming such type of interactions in neutral or protonated forms. Benzofuran aldehyde shows stronger such type of interaction with same amino acids of 1P62, 1KDR, 1TE6 and 3F8S indicates that orientation of the molecule does not change during docking in major extend by the changing of skeleton or functional group. But such type of interaction is weaker in 1RJB and 1BAG whereas is absent with 3V3M, 3MK2 and 4FNY.

The table 7 [**Electrostatic interactions (pink)**] shows that, two amino acids in all proteins as ASP and GLU shows negative interactions (hydrogen bonding between proton of ligand and oxygen of protein or electrostatic interaction between positive centre of ligand and negative / electron density of protein). Both the amino acids containing carboxylic acid group in their side chain which is capable of forming such type of interactions in neutral or deprotonated form. This type interaction depends on the number of positive charge centre present in the ligand molecules and number of donor amino acids present in the docking site. 1RJB, 1UFQ, 4BBG and 1P62 PDBs shows maximum number of such type of interactions with aldehyde while 3FDN, 3LAU, 3V3M, 1BAG, 1Z92, 1YCR, 3MK2 and 2BOU shows minimum number of such interactions and are absent in 1KDR.

Benzofuran aldehyde molecule is hydrophobic in nature, even though it has strong region for hydrogen bonding, pi-pi interactions and hydrophobic interactions. This interaction would trigger the change in orientation of structure and their groups during binding. The group of aldehyde such as C=O, -O-, aromatic –O- groups/atoms are capable for the formation of hydrogen bonding. The aromatic ring and $–CH_3$ group put some limitations in the packing of micellar rearrangement as well as reducing the chance of forming hydrogen bonding with amino acids residue of protein.

Glide lipo explains the lipophilic and lipophobic attraction between ligand and amino acid residue at the docking site after recombination. The molecule is undissociated and thus available for penetration through various lipid barriers. The rate of penetration is strongly depends on the lipophilicity of the drug molecule in its unionised form. The lipophilic-hydrophilic balance plays very important role in passive transport and active transport along with drug metabolism. As length of hydrophobic chain increases, both partion coefficient and anaesthetic potency increases. Lipophilic and phobic attraction between aldehyde and amino acid residue at the docking site is stronger with 4FNY, 1VOM, 3LAU, 1BAG, 3MK2 PDBs at the neutral pH = 7. At lower pH, amine get protonated and its lipophilicity character goes on decreasing. The aldehyde shows weaker lipophilic and hydrophobic attraction with 1TE6, 2b4J, 1P62, 2AZ1, 1KDR and 3F8S.

Table 8: Table of glide lipo and polar interactions of 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs, hydrophobic and hydrophilic character of PDBs

Proteins	Description of property and amino acid information				
	phobic	philic	Glide lipo	Pi-pi interactions (green)	Pi-cation interactions (pink)
1RJB	0.668	1.186	-1.798	ARG595	--
3FDN	0.758	1.170	-1.025	--	--
3LAU	1.245	0.819	-2.828	--	--
4BBG	1.274	1.108	-1.897	--	--
3V3M	0.473	1.200	-1.045	HIE246, HIE246, HIE246	--
1BAG	0.343	1.103	-1.966	--	--
3F8S	0.298	1.089	-0.914	PHE357	--
2b4J	1.321	0.765	-0.242	--	C-LYS360, C-LYS360, C-LYS402
1Z92	0.396	0.805	-0.923	--	--
1YCR	1.171	0.675	-1.037	--	--
4FNY	**1.470**	**0.654**	**-3.252**	--	--
2BOU	0.134	1.000	-1.436	--	--
1UFQ	0.510	0.947	-1.28	--	--
1VOM	1.022	0.853	-3.232	PHE129, PHE129	--
2AZ1	0.397	1.562	-0.677	--	--
1KDR	0.463	1.343	-0.816	ARG41, ARG41, ARG131	ARG131
3MK2	0.632	0.717	-2.298	--	--
1TE6	**0.008**	**1.703**	**-0.132**	B-HID157, B-HID157	--
1P62	0.49	1.393	-0.553	ARG194, ARG194	LYS34

The electron rich pi-system (containing electron donating group) are generally interact with other electron deficient pi-system having electron withdrawing group. These are denoted by green colour and are called as hydrophobic interactions. Also, electron rich pi-centre interacts with cation (denoted by dark blue colour) and electron deficient centre interact with anion (denoted by pink colour). The benzofuran aldehyde shows the pi-pi interactions with the amino acid residue containing aromatic ring or pi electrons, the amino acids such as ARG (C=N bond) and PHE, HID & HIE (aromatic ring) shows such interactions with aldehyde. The pi-cation interaction are shown by those amino acid residue containing free cation or partial positive charge centre in their side chain such as LYS and ARG, both containing amino groups which get protonated and forming quaternary ammonium cation which get interact with pi-electrons of aldehyde. The polar hydroxyl group (hydrogen having partial positive charge/oxygen having partial negative charge/lone pair of electrons of oxygen) interact with aromatic ring. These type of interactions are depends on the orientation of the molecule in the docking site and amino acid arrangement in the same.

Based on the results of MTT and MB assay, it is concluded that 7-methoxy-2-(4-fluorophenyl)-1-benzofuran-5-carbaldehyde was toxic against breast cancer cell line and cancerous lung cell line. It may act as good pharmacore as anticancer.

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