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

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ABSTRACT

The 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde was synthesised by known literature method (Wittig reaction approach) from vanillin. To deduce the anticancer and antibacterial activity of the 7-methoxy-2-(4-thiomethylphenyl)-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-thiomethylphenyl)-1-benzofuran-5-carbaldehyde is most active against 1BAG and 4FNY biomarkers and have the features to prove themselves as anticancer drugs. It shows strong cytotoxicity against human cell line [lung (A-459) and breast (MCF-07)].

Keywords: Benzofurans, Molecular docking, Anticancer, 1BAG, 4FNY, 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.

MATERIALS AND METHODS

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.

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-thiomethylphenyl)-1-benzofuran-5-carbaldehyde] was constructed. The ligand was then energy-minimized in the in-built ChemSketch 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 forcefield 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.

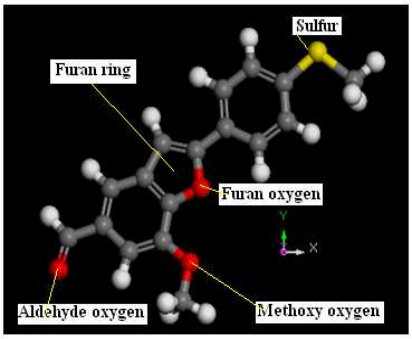
2. Study of molecular structure and properties:

Molecular structure has been studied by different molecular programs such as Avogadro, Glide, DFT, etc. The stable molecular structure, density of state, electron density, HOMO and LUMO are studied by using DFT while molecular

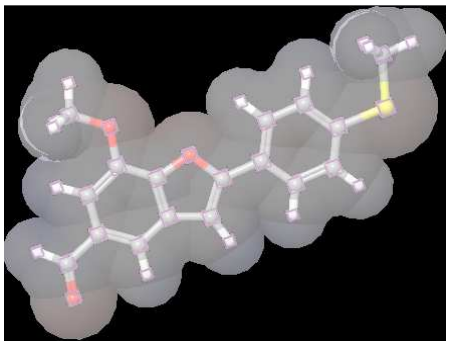
parameters such as non-bonded atom bond lengths, bond angles, Drug likeness property has been studied by VEGA ZZ 3.0.3 program.

Table 1: for the properties of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

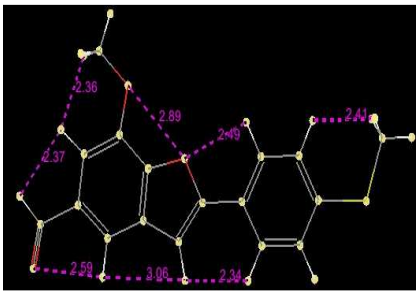
Stable structure



Van der Waal surfaces



Distance between the atoms which are not attached directly



Determination of bond angles

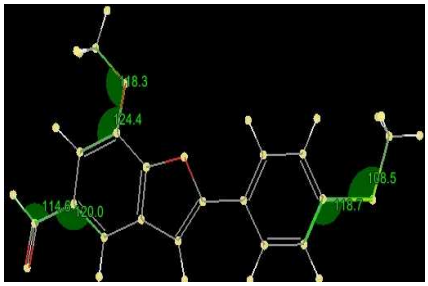


Fig 1: DOS of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

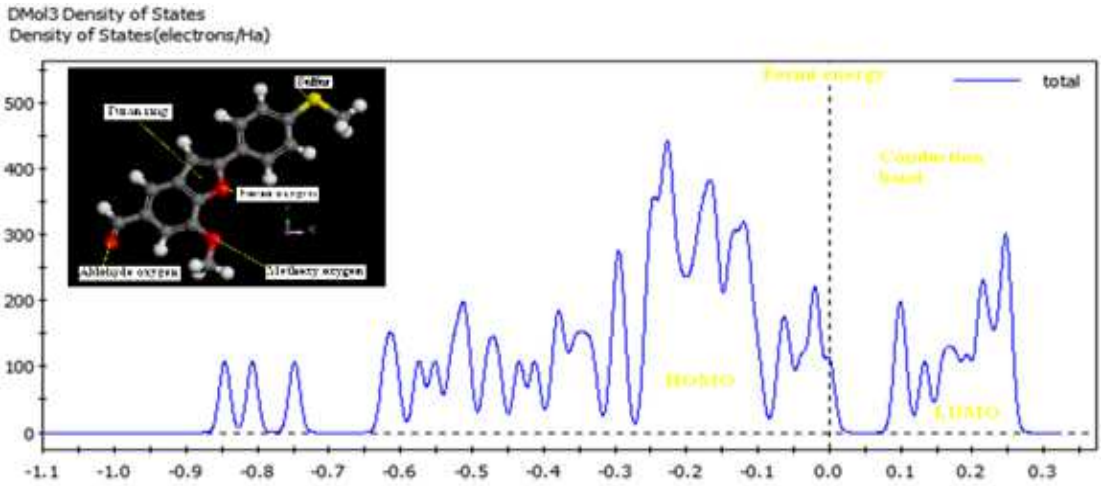


Fig 2: HOMO and LUMO of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

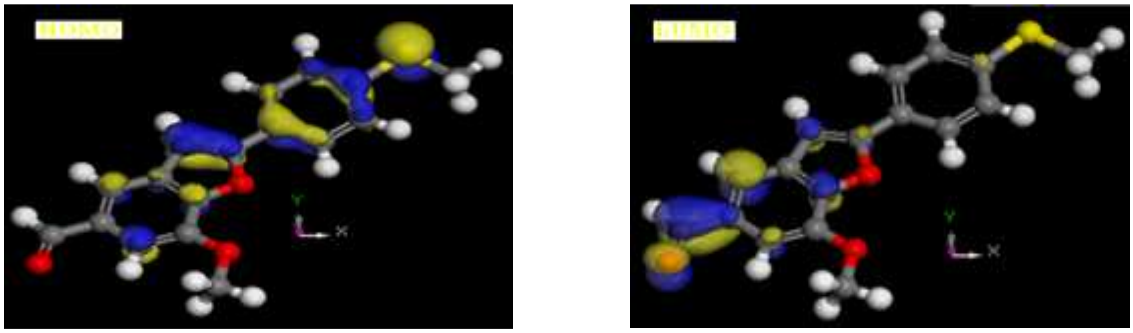


Fig 3: Charge density and electron density over the heteroatoms of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

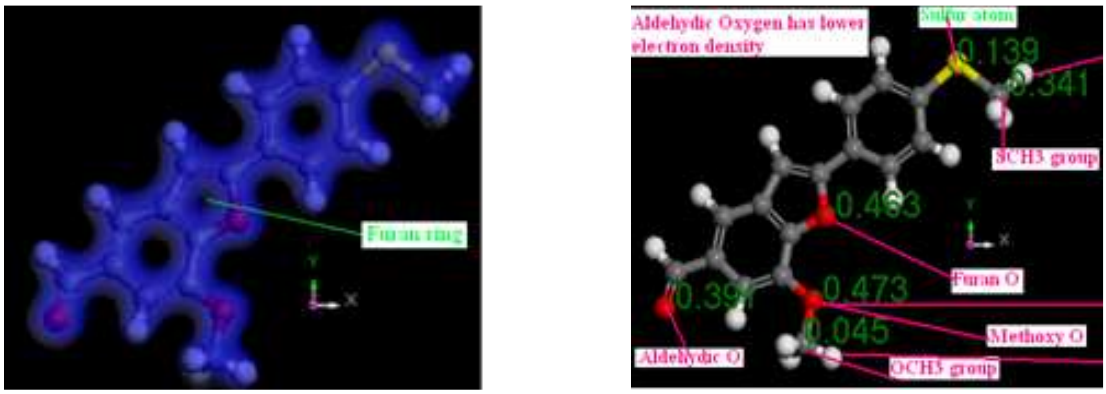


Table 2: Some molecular functions / properties of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

Molecular formula:	$C_{17}H_{14}O_3S$
Total Energy	17.4952 kcal/mol.
Molecular weight	298.356 g/mol.
m/z values	298.07 (100), 299.07 (18.4), 300.06 (4.5), 300.07 (1.6)
Elemental analysis (% analysis)	C – 68.44, H – 4.73, O – 16.09, S – 10.75
H - donor	0
H – bond acceptor	3
Energy of HOMO	-08.663 eV
Energy of LUMO	-04.445 eV
Formal charge	0
Gibbs free energy	109.83 kJ/mol (at 298K & 1atm)
Ovality	1.483393
Partition coefficient	4.995800
Heat of formation	-135.51 kJ/mol (at 298K & 1atm)
Ideal gas thermal capacity	310.588 J/mol.K
Water solubility	0 mg/lit
Stereochemistry	C(8)-C(7): (**Z**)
LogP	3.438
Mol Refractivity	85.874 cm^3/mol
Lipinski Rule	298.066;3;0;4;4.996
Henry's Law Constant	8.15
Connolly Accessible Area	519.757 A^2
Num Rotatable Bonds	4 bonds
Polar Surface Area	35.53 A^2
Sum of charges	0.0
Solvation energy	-4.588405 eV
Electrostatic Energy	-67.1367 kcal/mol
Dipole	2.5238 Debye

Table 3: Application of VEGA ZZ 3.0.3 for study of Druglikeness property

Property		**7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde**
By using Lipinski rule of five		
Molecular weight	Dalton	298.356
No. of H-bond acceptor	(< 10)	03
No. of H-bond donor	(< 5)	00
Virtual Log P	(< 5)	4.697
Comment		Ok
By using Ghose's rule of five		
Molecular weight	Dalton	298.356
Number of atoms	20 – 70	35
Vertual Log P	-0.4 – 5.6	4.697
Molar refractivity	40 – 130	86.8928
Comment		Ok

3. Experimental Work:

7-Methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde is synthesized by known literature method [8]. A mixture of (2-hydroxy-3-methoxy-5-formylbenzyl) triphenylphosphonium chloride (3.5 g, 7.5 mmol), 4-(methylsulfanyl)benzoyl chloride (7.8 mmol) and triethylamine (1.6 g, 16 mmol) in toluene (70 ml) was heated under reflux for 6 hrs. The reaction mixture was cooled to room temperature and water (50 ml) was added. Separate the organic layer by separating funnel and wash by water (2 x 50 ml) and dried over Na_2SO_4. Toluene was removed under reduced pressure and the residue obtained was purified by using silica column chromatography (100-200 mesh, Eluent 20% ethyl acetate in hexane), from the 7-methoxy-2-[4-(thiomethyl)phenyl]-l-benzofuran-5-carboxaldehyde (1.385 g, 58%) as a faint yellow crystalline solid, m.p. 115^0C.

FT-IR (KBr): 2973, 2938, 2834, 2723, 1691, 1648, 1592, 1344, 1218, 1141, 1095, 840 cm^{-1}.; **NMR (300 MHz) (DMSO-D6: ppm) C17H14O3S (mol. Wt. 298.368 g/mol):** 2.532 (s, 3H, SCH3); 4.089 (s, 3H, OCH3); 7.040 (s, 1H, Ar-H); 7.356-7.264 (m, 3H, Ar-H); 7.692 (s, 1H, Ar-H); 7.810-7.784 (d, 2H, Ar-H); 9.997 (s, 1H, CHO).

Mass Spectra: (M+1) = 298.94 and (M + 2) = 299.92.

Table 4: Different properties of proteins at docking site

protein	**Site Score**	**size**	**Dscore**	**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

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 summarised in following table 4.

The docking site scores, size, volume exposure, enclosure, contact, hydrophobic and hydrophilic nature, donor and acceptor ratio of all proteins are shown in table 4.

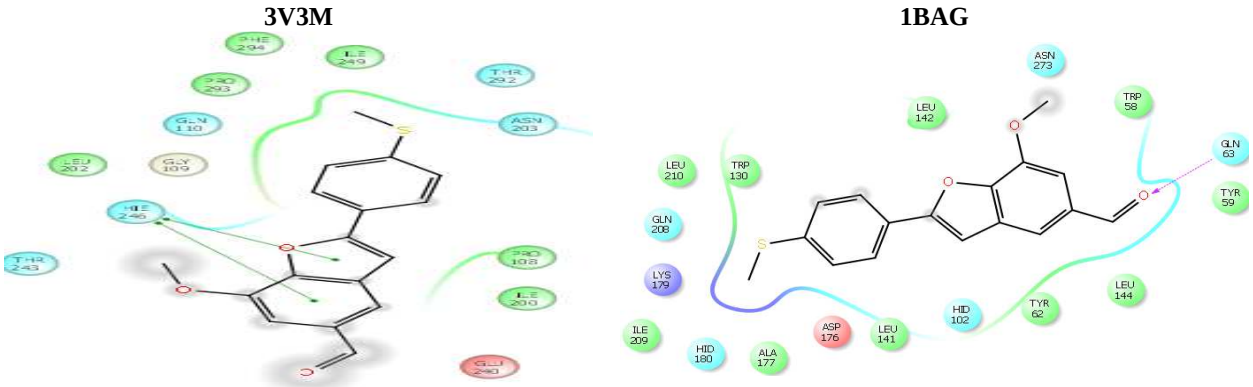
The docking site score of 2AZ1 (**1.121**) receptor/protein is higher while that of 2BOU (**0.464**) is lowest is indicates that the 2AZ1 protein PDB is more favourable for docking than the others. The size (223) and volume (760.774) available for docking is higher in 4BBG and 3FDN PDBs respectively 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.

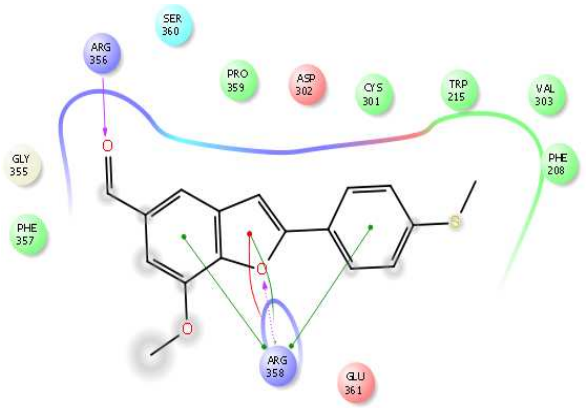
3.2. Molecular docking:

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.

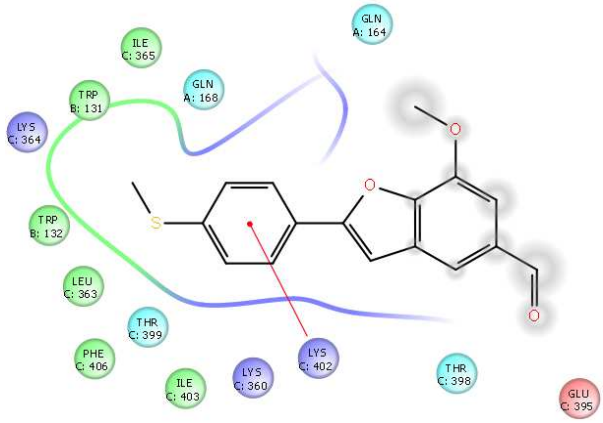
Fig 4: 2D docking image of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde with different proteins



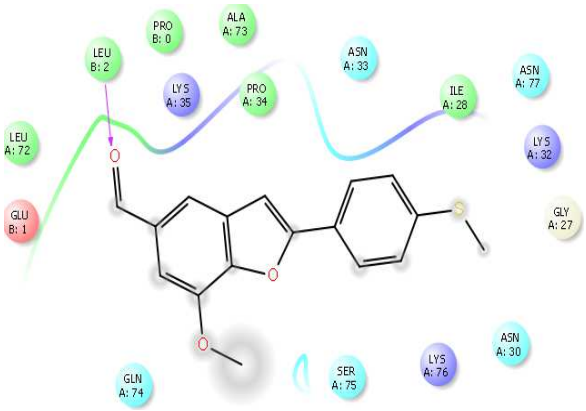
3F8S



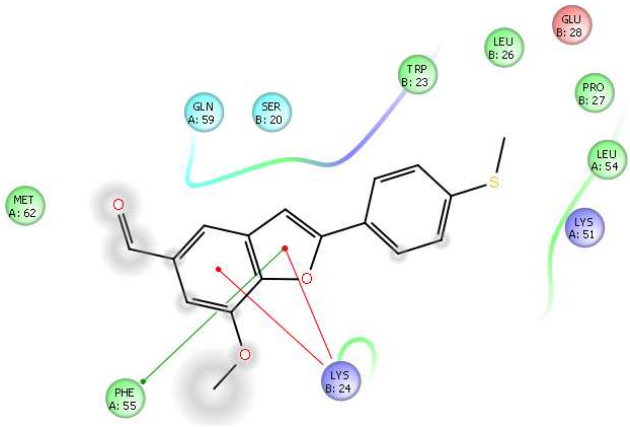
2b4J



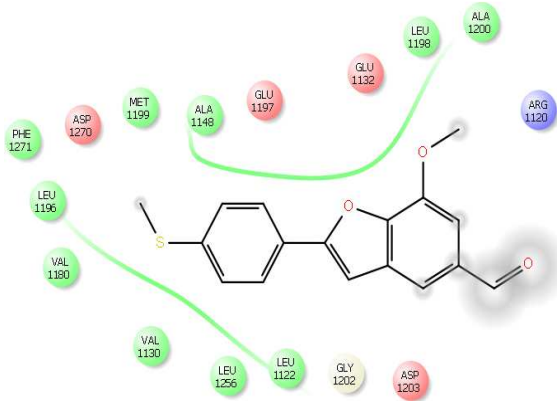
1Z92



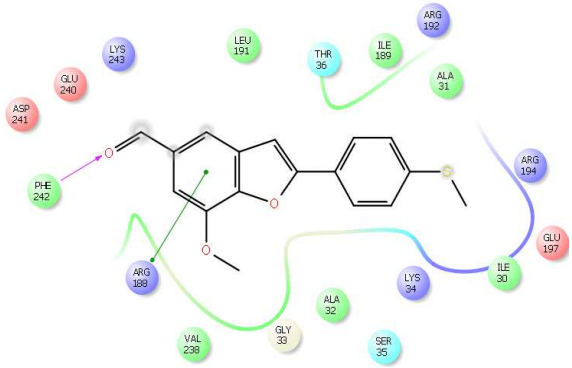
1YCR



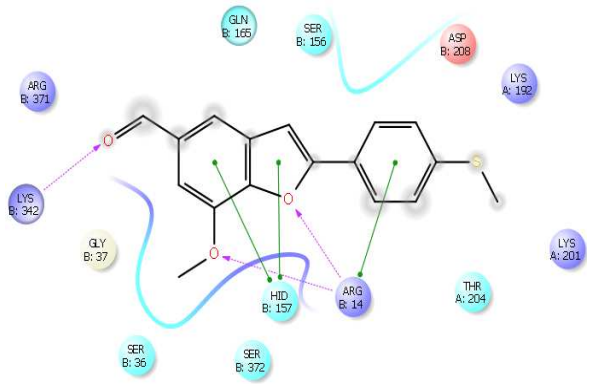
4FNY



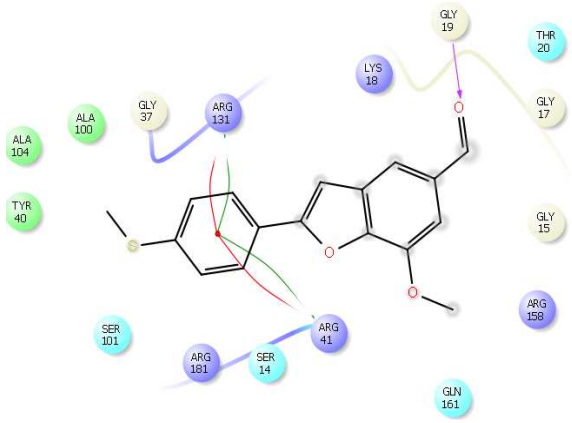
1P62



1TE6



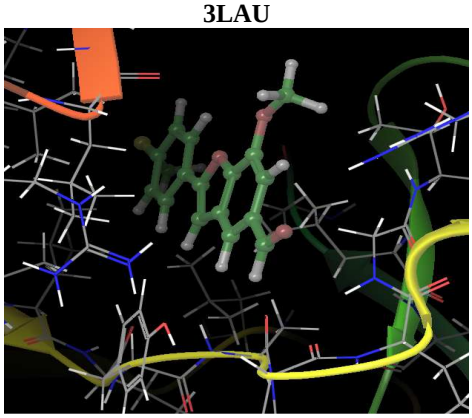
1KDR



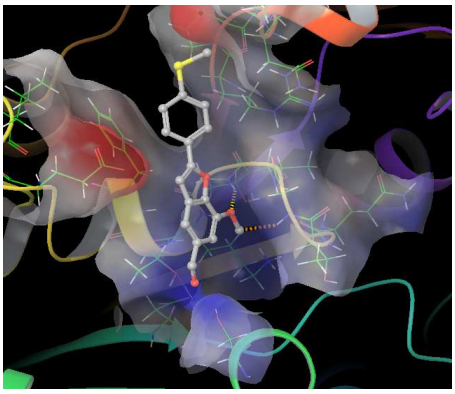
2BOU: Does not dock

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

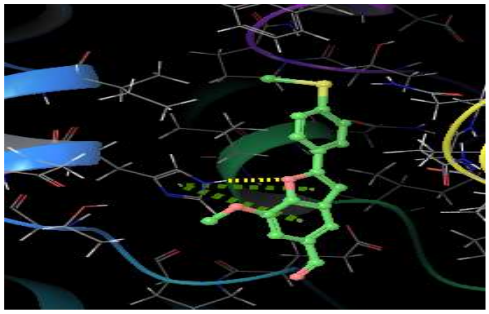
3LAU



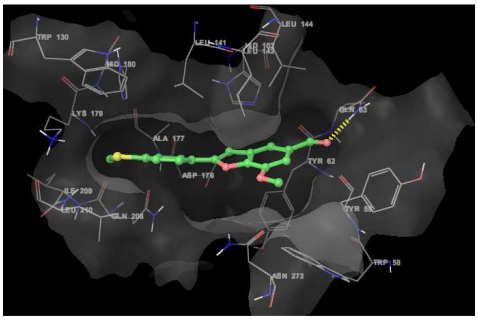
4BBG



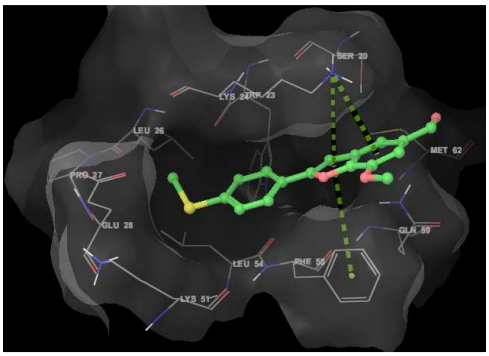
3V3M



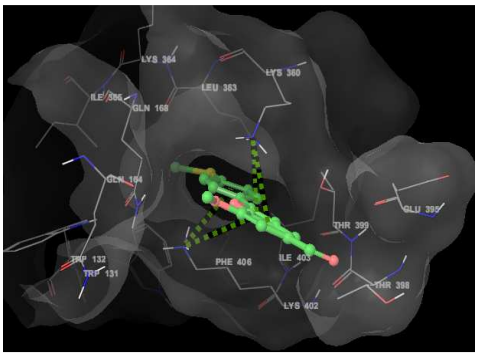
1BAG



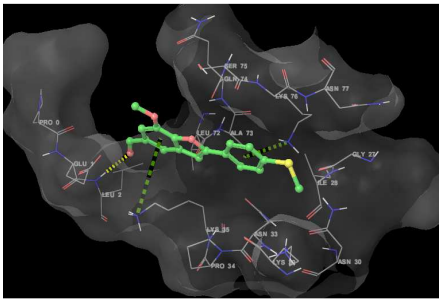
1YCR



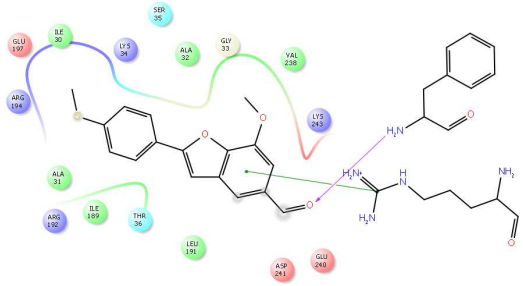
2b4J



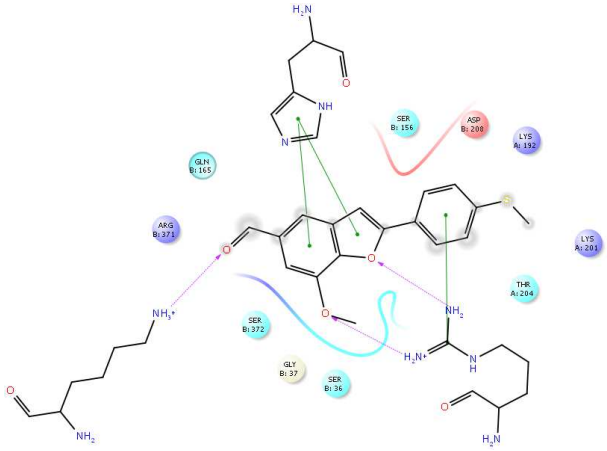
1Z92



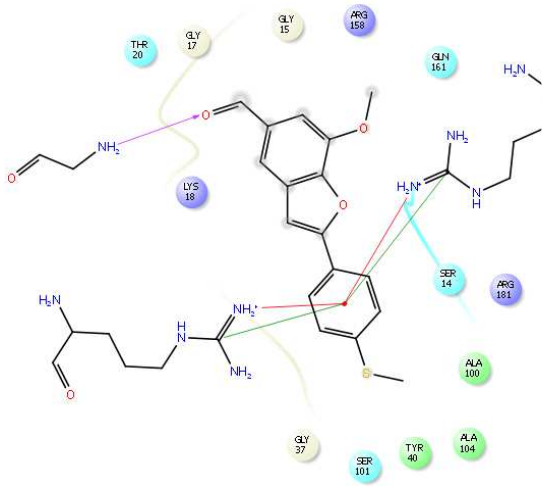
1P62



1TE6



1KDR



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	89.80	99.26	92.44	92.43
7.5	79.83	85.15	70.47	65.56
5.0	54.48	60.68	46.22	52.73
2.5	36.53	44.21	35.37	45.57
1.0	30.27	37.15	28.00	31.40
0.50	20.87	27.26	19.56	21.54
0.25	11.47	14.56	8.44	11.28
0.10	4.63	0.91	3.08	1.82

RESULTS AND DISCUSSION

The stable three dimensional structure with minimum energy shows that the methyl group of C_7-OMe group is projected away from the furan oxygen atom while that of $C_{4'}$-SMe group is goes slightly out of plane of molecule. The density of state (DOS) of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde shows conduction band indicates the molecule has electrical conductivity property. The DOS is also used to calculate minimum energy required for the excitation of electrons from HOMO to LUMO (for electronic transition) which will confirm from the UV-spectra of the molecule. The energy of HOMO is 0.0216Ha (0.058 eV) and that of LUMO is 0.0781 Ha (0.2115 eV). The energy difference (0.15355 eV) between HOMO and LUMO is less than 3 eV indicates that the molecule shows conductivity property at T = 0K. The molecule containing one $–OCH_3$ group and one $–SCH_3$ group which gives two different singlets for 3H (protons) in its NMR spectrum, one at 2.532 (s, 3H, SCH_3) is due to thiomethyl protons and at 4.089 (s, 3H, OCH_3) is due to methoxy protons. The oxygen atom of $–OCH_3$ group attached to benzofuran ring at 7-position having total electron charge is (-0.473e) the carbon atom of methoxy carbon is + 0.045e while the total electronic charge present on sulfur atom and carbon atom of thiomethyl group is -0.139e and -0.341e respectively. Therefore $–CH_3$ protons of methoxy group present at 7-position of benzofuran ring is deshielded as compared to $–CH_3$ protons of thiomethyl protons. The methyl protons of methoxy group shows singlet at 2.532 ppm while that of methoxy group shows singlet at 4.089 ppm.

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 table indicate that 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde is more active against 1BAG (docking score -7.190) and 4FNY (docking score -6.761) while is less active against 3V3M (docking score -3.095) and 2b4J (docking score -3.126). 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.

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 3MK2 (-0.054), 1Z92 (-0.040), 1TE6 (-0.029), and 1YCR (-0.001) but these are totally absent in 4FNY. The aldehyde shows higher polar interaction 1TE6, 4BBG, 1Z92, 1VOM, 1BAG, 3V3M, 2b4J and 1KDR proteins PDBs. This is one of the reason for the higher docking score of aldehyde in 1BAG. Also the molecule containing hydrogen bond donor atoms (3) and hydrogen bond accepting nature of 1BAG 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 hybridised oxygen atoms (carbonyl, ether and aromatic) capable of forming P → L type of hydrogen bonding during interaction. The backbone of MET, ARG, LEU and GLY amino acids and side chain of ARG, GLN and LYS forming hydrogen bonding with ligand.

Table 6: Table of don/acc ratio, docking score, glide esite and polar interactions of 7-methoxy-2-(4-thiomethylphenyl)-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	-6.043	0	01 (MET578) (with backbone)	ARG595, ARG595
3FDN	0.880	-5.41	0	--	--
3LAU	0.749	-6.266	0	--	--
4BBG	0.725	-3.638	0	01 (GLY110) (with backbone)	--
3V3M	0.510	**-3.095**	0	--	HIE246, HIE246
1BAG	**0.478**	**-7.190**	0	01 (GLN63) (with side chain)	--
3F8S	0.762	-4.416	0	02 (ARG356) (with backbone), (ARG358) (with side chain)	ARG358 (with three rings pi-pi and with one ring pi-cation interactions
2b4J	1.456	**-3.126**	0	--	C-LYS402
1Z92	1.427	-4.993	-0.04	01 (B-LEU2) (with backbone)	--
1YCR	**2.006**	-4.682	-0.001	--	A-PHE55, B-LYS24, B-LYS24
4FNY	1.858	**-6.761**	0	--	--
2BOU	1.433	**Does not dock**			
1UFQ	0.931	-4.105	0	--	--
1VOM	0.708	-5.195	0	--	--
2AZ1	0.665	-4.808	0	--	--
1KDR	0.661	-4.081	0	01 (GLY19) (with backbone)	ARG41, ARG131, ARG41, ARG131
3MK2	0.623	-3.758	-0.054	--	--
1TE6	0.595	-3.727	-0.029	**03** (B-LYS242, B-ARG14, B-ARG14) (with side chain)	B-HID157, B-ARG14, B-HID157
1P62	0.520	-6.521	0	01 (PHE242) (with backbone)	ARG188

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 stabilisation 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 of the interaction in decreasing order is as 1RJB > 1VOM > 1BAG > 2AZ1 > 3LAU > 1UFG > 1P62 > 3F8S > .....

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 aldehyde has higher glide energy during the interaction with PBDs in the decreasing order as 1RJB > 4BBG > 1VOM > 1BAG > 2AZ1 > 3F8S > 1P62 > 1UFQ > .....

Along with major interactions, there are some other interactions such polar interactions (faint blue colour), hydration sites (orange, interaction with water), electrostatic interactions (blue and pink) and hydrophobic interaction (major weak interaction with maximum number of amino acids) present between the ligand-protein complex.

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 interaction with same amino acids of 1P62, 1TE6, 1KDR, 3MK2, 1AZ1, 1Z92, 2b4J, and 3UFQ 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, 1BAG, 4FNY and 1VOM whereas is absent with 3V3M.

Table 7: Table of glide evdw, glide energy, electrostatic and polar interactions 7-methoxy-2-(4-thiomethylphenyl)-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	**-37.726**	**-39.118**	ARG595	GLU573, ASP593, GLU656, GLU661	SER574, GLN577, SER660
3FDN	-27.851	-31.042	ARG137, LYS162	GLU211, ASP274	THR217, ASN261
3LAU	-33.534	-34.491	ARG137, ARG220	GLU211	THR217
4BBG	-26.570	-38.507	ARG26, LYS111	GLU118	ASN29, GLN106, THR107, THR109, THR112, SER232
3V3M	-21.256	-23.277	--	GLU240	GLN110, ASN203, THR243, HIE246, THR292
1BAG	-34.027	-38.334	LYS179	ASP176	GLN63, HID102, HIG180, GLN208, ASN273
3F8S	-32.268	-37.394	ARG356, ARG358	ASP302, GLU361	SER360
2b4J	-25.119	-27.230	C-LYS360, C-LYS364, C-LYS402	C-GLU395	A-GLN164, A-GLN168, C-THR398, C-THR399
1Z92	-30.575	-31.180	A-LYS32, A-LYS35, A-LYS76	B-GLU1	A-ASN30, A-ASN33, A-GLN74, A-SER75, A-ASN77
1YCR	-27.490	-28.008	A-LYS51, B-LYS24	B-GLU28	A-GLN59, B-SER20
4FNY	-31.970	-32.110	ARG1120	GLU1132, GLU1197, ASP1270	--
2BOU	**Does not dock**				
1UFQ	-33.141	-35.769	A-ARG210, D-LYS201, D-LYS202	A-ASP156, A-ASP158, C-GLU194, C-GLU195	A-THR157
1VOM	-36.523	-38.338	LYS190	GLU223, GLU323	ASN219, ASN235, HID279, GLN283, THR327
2AZ1	-33.772	-37.675	A-ARG19, B-ARG147, E-ARG19	A-ASP24, A-GLU30, E-ASP24	B-THR27, B-THR31
1KDR	-29.788	-33.219	LYS18, ARG41, ARG131, ARG158, ARG181	--	SER14, THR20, SER101, GLN161
3MK2	-23557	-24.635	LYS131, ARG179, ARG227, LYS240	ASP171, ASP185, ASP229, GLU236	GLN184, THR188, SER192
1TE6	-25.052	-30.810	A-LYS192, A-LYS201, B-ARG14, B-LYS342, B-ARG371	B-ASP208	A-THR204, B-SER36, B-SER156, B-HID157, B-GLN165, B-SER372
1P62	-32.403	-36.846	LYS34, ARG188, ARG192, ARG194, LYS243	GLU197, GLU240, ASP241	SER35, THR36

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, 3MK2, 4FNY, 2AZ1, and 1P62 PDBs shows maximum number of such type of interactions with aldehyde while 1YCR, 3LAU, 3V3M, 1BAG, 2b4J, 1Z92 and 4BBG 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 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 in the order of 4FNY > 3LAU > 1P62 > 1BAG > 1YCR > 1RJB > ... 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 in the order with 2b4J < 4BBG < 3V3M < 1KDR < 3F8S < 1UFQ < 2AZ1 < .. whereas is totally absent in 1TE6.

Table 8: Table of glide lipo and polar interactions of 7-methoxy-2-(4-thiomethylphenyl)-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	-2.008	ARG595	ARG595
3FDN	0.758	1.170	-1.254	--	--
3LAU	1.245	0.819	-2.985	--	--
4BBG	1.274	1.108	-0.458	--	--
3V3M	0.473	1.200	-0.460	HIE246, HIE246	--
1BAG	0.343	1.103	-2.438	--	--
3F8S	0.298	1.089	-0.602	ARG358, ARG358, ARG358	ARG358
2b4J	1.321	0.765	-0.265	--	C-LYS402
1Z92	0.396	0.805	-1.324	--	--
1YCR	1.171	0.675	-2.015	A-PHE55	B-LYS24, B-LYS24
4FNY	1.470	0.654	-3.748	--	--
2BOU	DOES NOT DOCK				
1UFQ	0.510	0.947	-0.692	--	--
1VOM	1.022	0.853	-1.608	--	--
2AZ1	0.397	1.562	-0.733	--	--
1KDR	0.463	1.343	-0.565	ARG41, ARG131	ARG41, ARG131
3MK2	0.632	0.717	-1.249	--	--
1TE6	0.008	1.703	0	B-ARG14, B-HID157, B-HID157	--
1P62	0.49	1.393	-2.737	ARG188	--

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, HIE and HID (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. The 2BOU does dock with 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde (does not shows any interactions). Based on the results of MTT and MB assay, it is concluded that 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde more toxic on breast cancer cell line and cancerous lung cell line.

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SUPPORTING DATA

Table 9A: Docking properties of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

Description	Protein									
	1RJB	**3FDN**	**3LAU**	**4BBG**	**3V3M**	**1BAG**	**3F8S**	**2b4J**	**1Z92**	**1YCR**
Potential Energy OPLS 2005	64.615	64.615	64.615	64.615	64.615	64.615	64.615	64.615	64.615	64.615
RMS Derivative OPLS 2005	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022
Glide lignum	6	4	3	6	4	11	6	6	11	11
Docking Score	-6.043	-5.41	-6.266	-3.638	-3.095	-7.19	-4.416	-3.126	-4.993	-4.682
Glide Ligand efficiency	-0.288	-0.285	-0.298	-0.173	-0.163	-0.342	-0.210	-0.149	-0.238	-0.223
Glide Ligand efficiency sa	-0.794	-0.76	-0.823	-0.478	-0.435	-0.945	-0.58	-0.411	-0.656	-0.615
Glide Ligand efficiency In	-1.494	-1.372	-1.549	-0.899	-0.785	-1.778	-1.092	-0.773	-1.234	-1.158
Glide gscore	-6.043	-5.41	-6.266	-3.638	-3.095	-7.19	-4.416	-3.126	-4.993	-4.682
glide lipo	-2.008	-1.254	-2.985	-0.458	-0.46	-2.438	-0.602	-0.265	-1.324	-2.015
glide hbond	-0.358	-0.35	0	0	0	-0.32	-0.152	0	-0.241	0
glide metal	0	0	0	0	0	0	0	0	0	0
glide rewards	-1.788	-1.324	-1.667	-1.589	-1.992	-2.29	-1.487	-1.494	-1.375	-1.420
Glide evdw	-37.726	-27.851	-33.534	-26.570	-21.256	-34.027	-32.268	-25.119	-30.575	-27.490
Glide ecoul	-1.392	-3.191	-0.958	-11.94	-2.021	-4.307	-5.126	-2.111	-4.605	-0.518
glide erotb	0.206	0.276	0.206	0.206	0.276	0.206	0.206	0.206	0.206	0.206
glide esite	0	0	0	0	0	0	0	0	-0.040	-0.001
Glide emodel	-48.510	-36.672	-47.421	-37.325	-32.675	-52.814	-43.591	-32.348	-45.780	-35.425
Glide energy	-39.118	-31.042	-34.491	-38.507	-23.277	-38.334	-37.394	-27.230	-31.180	-28.008
Glide einternal	8.930	1.592	1.444	2.299	0.074	1.988	7.633	0.499	0.649	0.858
glide confnum	1	1	2	1	1	2	1	1	2	1
Glide posenum	4	4	296	1	1	86	347	199	33	223
XP GScore	-6.043	-5.41	-6.266	-3.638	-3.095	-7.19	-4.416	-3.126	-4.993	-4.682
H-Bonds	1	0	0	1	0	1	**2**	0	1	0
pi-pi/pi-cation interactions	2	0	0	0	2	0	**4**	1	0	3

Table 9B: Docking properties of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde with different receptor or protein PDBs

Description	Protein								
	4FNY	**2BOU**	**1UFQ**	**1VOM**	**2AZ1**	**1KDR**	**3MK2**	**1TE6**	**1P62**
Potential Energy OPLS 2005	64.615	64.615	64.615	64.615	64.615	64.615	64.615	64.615	64.615
RMS Derivative OPLS 2005	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022	0.022
Glide lignum	15		15	15	15	15	15	15	15
Docking Score	-6.761		-4.105	**-5.195**	-4.808	-4.081	-3.758	-3.727	-6.521
Glide Ligand efficiency	-0.322		-0.195	-0.247	-0.229	-0.194	-0.179	-0.177	-0.311
Glide Ligand efficiency sa	-0.888		-0.539	-0.683	-0.632	-0.536	-0.494	-0.490	-0.857
Glide Ligand efficiency In	-1.672		-1.015	-1.284	-1.189	-1.009	-0.929	-0.921	-1.612
Glide gscore	-6.761		-4.105	-5.195	-4.808	-4.081	-3.758	-3.727	-6.521
glide lipo	-3.748		-0.692	-1.608	-0.733	-0.565	-1.249	0	-2.737
glide hbond	0		-0.16	0	-0.253	-0.293	0	-0.387	-0.301
glide metal	0		0	0	0	0	0	0	0
glide rewards	-1.6	**Does**	-1.408	-1.694	-1.754	-1.425	-1.375	-1.375	-1.375
Glide evdw	-31.970	**not dock**	-33.141	-36.523	-33.772	-29.788	-23.557	-25.052	-32.403
Glide ecoul	-0.141		-2.627	-1.815	-3.904	-3.43	-1.078	-5.758	-4.443
glide erotb	0.206		0.206	0.206	0.206	0.206	0.206	0.206	0.206
glide esite	0		0	0	0	0	0	-0.054	-0.029
Glide emodel	-45.131		-44.085	-50.251	-47.193	-41.966	-30.675	-36.631	-50.404
Glide energy	-32.110		-35.769	-38.338	-37.675	-33.219	-24.635	-30.810	-36.846
Glide einternal	0.626		2.481	0.972	4.019	0.360	0.898	4.610	4.129
glide confnum	2		2	1	2	1	2	1	2
Glide posenum	65		357	315	396	301	112	98	11
XP GScore	-6.761		-4.105	-5.195	-4.808	-4.081	-3.758	-3.727	-6.521
H-Bonds	0	--	0	0	0	1	0	**3**	1
pi-pi/pi-cation interactions	0	--	0	0	0	4	0	**3**	1

Table 10: Molecular properties of 7-methoxy-2-(4-thiomethylphenyl)-1-benzofuran-5-carbaldehyde

mol MW	**dipole**	**SASA**	**Donor HB**	**Accpt HB**
298.356	3.201	555.971	0	3.75
Potential Energy-OPLS-2005	**RMS Derivative-OPLS-2005**	**volume**	**dip^2/V**	**Glob**
64.615	0.045	955.889	0.010722	0.844082
FOSA	**FISA**	**PISA**	**WPSA**	**ACxDN^.5/SA**
185.869	77.432	250.372	42.299	0
QPpolrz	**QPlogPC16**	**QPlogPoct**	**QPlogPw**	**QPlogPo/w**
32.565	9.665	12.763	6.334	3.624
QPlogS	**CIQPlogS**	**QPlogHERG**	**QPPCaco**	**QPlogBB**
-4.485	-4.569	-5.249	1826.47	-0.238
QPPMDCK	**QPlogKp**	**IP(eV)**	**Human Oral Absorption**	**Percent Human Oral Absorption**
1617.463	-1.777	8.612	3	100
SAfluorine	**SAamideO**	**PSA**	**#NandO**	**Rule Of Five**
0	0	52.303	3	0
Rule Of Three	**EA(eV)**	**#metab**	**QPlogKhsa**	**#ringatoms**
0	0.887	2	0.262	15
#in34	**#in56**	**#noncon**	**#nonHatm**	**Jm**
0	15	0	21	0.163