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Synthesis, molecular docking and cytotoxic study of 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde

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ABSTRACT

The 7-methoxy-2-(3,4-dimethoxyphenyl)-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-(3,4-dimethoxyphenyl)-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-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde is most active against 3LAU (Arora 2 kinase) and 1VOM (Dictyostelium myosin) biomarkers and have the features to prove themselves as anticancer drugs. The Cramer rules of toxicity predicts the toxicological hazard (when administered orally) from the molecular structure. It shows that it is class III toxic compound. Also stereochemistry and molecular parameters are studied by using Avogadro's software. Both MB and MTT assay shows that, 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde is strong cytotoxic against (A-459) human lung cell line than (MCF-07) breast cancer cell line.

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

INTRODUCTION

Molecular modeling 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 modeling 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 modeling 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-(3,4-dimethoxyphenyl)-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. Experimental Work:

A solution of 3,4-dimethoxybenzoic acid (1.8 g, 0.001 mol), thionyl chloride (3.5 g, 0.029 mol) in toluene (32 ml) was refluxed for 3 hr. Toluene and excess thionyl chloride was removed under educed pressure to obtain 3,4-dimethoxybenzoyl chloride (1.9 g).

A mixture of phosphonium salt (4 g, 0.0086 mol), 3,4-dimethoxybenzoyl chloride (1.9 g, 0.0095 mol) and triethylamine (2.0 g, 0.0198 mol) in toluene (80 ml) was heated under reflux conditions for 6 hr. The reaction mixture was cooled to room temperature and adds 20 ml cold water to it. The organic layer was separated, washed with water and dried it by using anhydrous sodium salphate. Distilled the toluene under reduced pressure and the solid product formed was recrystallized from acetone : methanol (8:2) to obtained faint yellow solid 2-(3,4-dimethoxyphenyl)-7-methoxy-1-benzofuran-5-carbaldehyde (1.6 g, 59 %), m.p. 184-85^{0}C.

FT-IR (KBr): 3010, 2836, 2778, 1691, 1612, 1513, 1284, 1168, 1103, 1024, 998, 833 cm^{-1}.
NMR (300 MHz) (CDCl$_3$; δ ppm): $C_{18}H_{16}O_2$ (mol wt: 312.3 g/mol): 3.95 (s, 3H, -OCH$_3$); 4.00 (s, 3H, -OCH$_3$); 4.10 (s, 3H, -OCH$_3$); 6.95 (d, J = 8.4 Hz, 1H, Ar-H); 6.99 (bs, 1H, Ar-H); 7.35 (bs, 1H, Ar-H); 7.37 (d, J = 1.8 Hz, 1H, Ar-H); 7.48 (dd, J = 8.4 & 1.8 Hz, 1H, Ar-H); 7.69 (d, J = 1.8 Hz, 1H, Ar-H); 10.04 (s, 1H, -CHO).

Mass Spectra (M + 1): 313.15

Fig 1: FTIR of 2-(3,4-dimethoxyphenyl)-7-methoxy-1-benzofuran-5-carbaldehyde

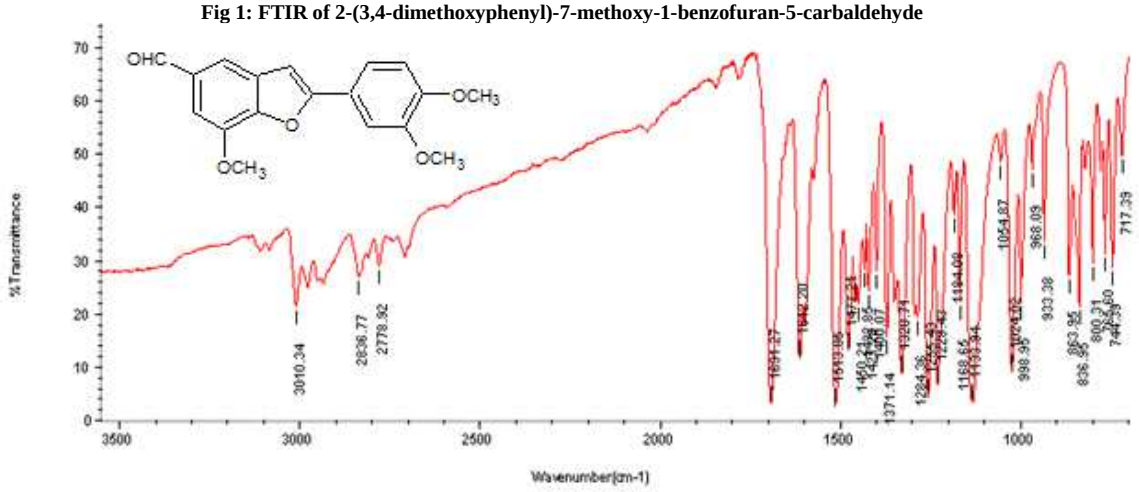
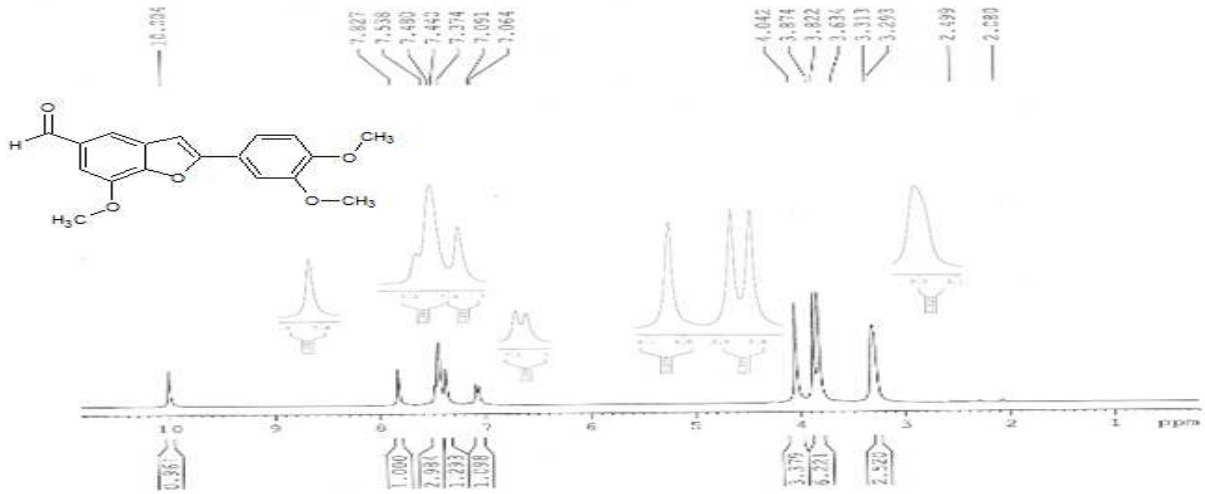
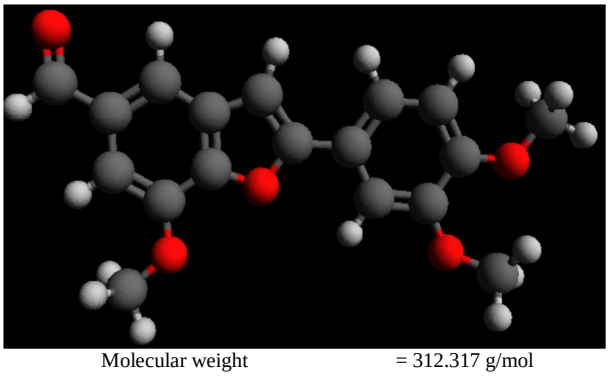
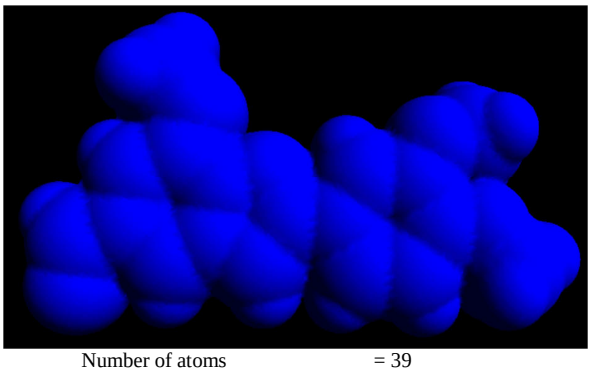


Fig 2: NMR spectra of 2-(3,4-dimethoxyphenyl)-7-methoxy-1-benzofuran-5-carbaldehyde



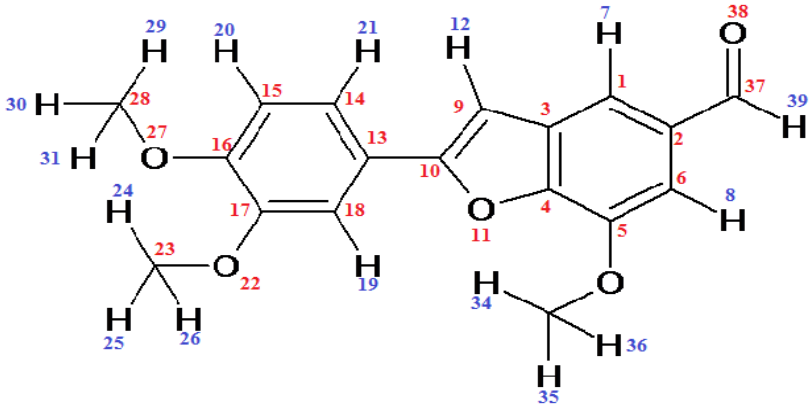
The molecular properties of 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde was calculated by using Avogadro 1.1.1 (Git revision: 3248586).





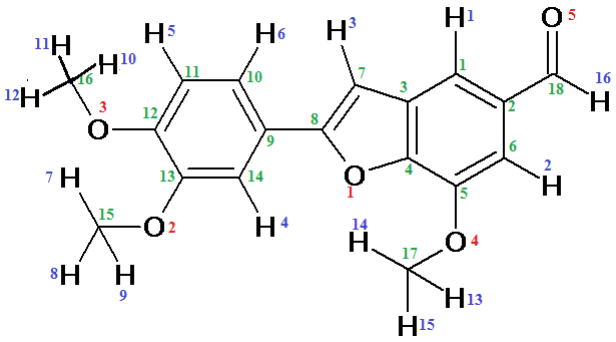
Molecular weight	= 312.317 g/mol	Number of atoms	= 39
Molecular formula	= $C_{18}H_{16}O_5$	Number bonds	= 41
Estimated dipole moment (D)	= 0.897	Rotable bonds	= 03

Partial charges on atoms:



Atom No.	Partial Charge	Atom No.	Partial Charge	Atom No.	Partial Charge
^{11}O	-0.451	^{22}O	-0.491	^{27}O	-0.491
^{32}O	-0.491	^{38}O	-0.296		
Carbon atoms					
^{23}C	0.079	^{28}C	0.079	^{33}C	0.079
^{37}C	0.150	^{1}C	-0.041	^{6}C	-0.006
^{9}C	-0.008	^{14}C	-0.047	^{15}C	-0.016
^{18}C	-0.006	^{2}C	0.018	^{3}C	0.025
^{4}C	0.177	^{10}C	0.137	^{13}C	0.021
Hydrogen atoms					
Methoxy H	0.066	^{39}H	0.108	^{21}H, ^{7}H	0.063
^{8}H,^{12}H,^{19}H,^{20}H	0.066				

Bond lengths of the molecule: The bond length measured in A0 which is used to determine bond order (single, double, triple or partial bond). The distance between the non-bonded atoms in molecule is used to determine orientation of the atom of group and stereochemistry of the molecule. The stereochemistry and planarity of the atoms or groups of the molecule also explain with the help of torsion bond angles.



Some important bond lengths

Bond	Length in A^0	Bond	Length in A^0	Bond	Length in A^0	Bond	Length in A^0
^{18}C-^{5}O	1.2212	^{18}C-^{16}H	1.0855	^{8}C-^{9}C	1.4822	^{4}C-^{1}O	1.3115
^{8}C-^{1}O	1.3228						

Distance between non-bonded atoms in molecule

^{2}H-^{6}H	2.477	^{1}H-^{16}H	3.837	^{1}H-^{5}O	2.583	^{2}H-^{5}O	3.913
^{1}H-^{3}H	2.850	^{2}H-^{13}H	2.458	^{2}H-^{14}H	3.749	^{2}H-^{15}H	2.458
^{3}H-^{6}H	2.277	^{3}H-^{4}H	4.840	^{4}H-^{1}O	2.577	^{4}H-^{2}O	2.563
^{5}H-^{3}O	2.682	^{4}H-^{7}H	4.151	^{4}H-^{8}H	4.576	^{4}H-^{9}H	4.374
^{5}H-^{10}H	2.423	^{5}H-^{11}H	2.429	^{5}H-^{12}H	3.711	^{15}C-^{16}C	4.054
^{1}O-^{4}O	2.867	^{2}O-^{3}O	2.846				

The torsion bond angle explain the stereochemistry of allylic and homoallylic atoms. Some of torsion bond angles of the molecule given below –

Torsion bond angle property

^{16}H-^{18}C-^{2}C-^{1}C, ^{2}H-^{6}C-^{5}C-^{4}C, ^{14}H-^{15}C-^{4}O-^{5}C, ^{1}H-^{1}C-^{2}C-^{6}C, ^{10}H-^{16}C-^{3}O-^{12}C					± 179.97
^{16}H-^{18}C-^{2}C-^{6}C, ^{2}H-^{6}C-^{5}C-^{4}O, ^{1}H-^{1}C-^{2}C-^{18}C					± 0.0256
^{13}H-^{17}C-^{4}O-^{5}C, ^{15}H-^{17}C-^{4}O-^{5}C, ^{11}H-^{16}C-^{3}O-^{12}C					± 60.96
^{7}H-^{15}C-^{2}O-^{13}C	130.78	^{8}H-^{15}C-^{2}O-^{13}C	13.588	^{9}H-^{15}C-^{2}O-^{13}C	-110.91
^{12}H-^{16}C-^{3}O-^{12}C	-61.129				

The distance between 2H and methoxy hydrogens (7-OCH_3) indicates that two hydrogen atoms (^{13}H and ^{15}H) are at same distance while third hydrogen atom (^{14}H) far away from 2H. Also torsion bond angle of the ^{13}H and ^{15}H with ^{5}C atom has same magnitude but opposite sign indicates that these two hydrogens are not in plane of phenyl ring, but one is above the lane while another is below the plane with 60^0. The torsion angle of ^{14}H indicates that it is present in plane of phenyl ring but anti with respect to ^{5}C i.e. it is forced toward furan ring oxygen atom. The same orientation of the 4'-OCH_3 hydrogen atoms are observed. They are forced away from 3'-OCH_3 hydrogens. All three 3'-OCH_3 hydrogen atoms are not present in same plane of benzene ring. This is because of sterric interactions of hydrogen atoms of other methoxy group and phenyl hydrogens. The space distance of 3'-OCH_3 hydrogen atoms with 4H indicates that they are forced away from the furan ring.

Estimation of toxic hazard: Toxtree [9,10,11] is a full-featured and flexible user-friendly open source application, which is able to estimate toxic hazard by applying a decision tree approach. Toxtree has been designed with flexible capabilities for future extensions in mind (e.g. other classification schemes that could be developed at a future date). It predicts the toxicological hazard (when administered orally) from the molecular structure. This study explain - Carcinogenicity (genotox and nongenotox) and mutagenicity rulebase by ISS, in vitro mutagenicity (Ames test) alerts by ISS, Skin irritation / skin corrosion, Eye irritation and corrosion, Skin sensitization reactivity domains, START Biodegradability, Cytochrome P450-Mediated Drug Metabolism, Structure Alerts for the in vivo micronucleus assay in rodents, Structural Alerts for Functional Group Identification (ISSFUNC), Protein binding Alerts, DNA binding Alerts.

By applying various decision tree approaches to the three dimensional structure of the molecule to estimate their toxic hazards, it shows class III toxicity for oral administration, low probability of a life time cancer risk greater than 1 to 10^6, narcosis or baseline toxicity, negative for nongenotoxic carcinogenicity, structural alert for *S. typhimurium* mutagenicity, non-irritating or corrosive to skin and eyes (predicted lipid solubility is 10%, m.p. 184^0C and water

solubility is 1%), capability to form Schiff bases with skin, persistent chemical (not easily biodegradable), three sites for metabolism, one positive structural alert for the micronucleus assay, and has Michael acceptor sites.

3. 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 colors 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 1.

The docking site scores, size, volume exposure, enclosure, contact, hydrophobic and hydrophilic nature, donor and acceptor ratio of all proteins are shown in table 1.

Table 1: 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

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.

Table 2A: Docking score and other different docking properties of 7-methoxy-2-(3,4-dimethoxyphenyl)-l-benzofuran-5-carboxaldehyde

Description	Protein									
	1RJB	**3FDN**	**3LAU**	**4BBG**	**3V3M**	**1BAG**	**3F8S**	**2b4J**	**1Z92**	**1YCR**
Potential Energy OPLS 2005 = 127.013										
RMS Derivative OPLS 2005 = 0.023										
Glide lignum	10	11	7	11	11	16	12	12	16	17
Docking Score	-5.479	-6.314	**-6.506**	-4.924	-3.663	-5.922	-4.060	-3.554	-4.657	-4.225
Glide Ligand efficiency	-0.238	-0.253	-0.283	-0.197	-0.147	-0.257	-0.177	-0.155	-0.202	-0.169
Glide Ligand efficiency sa	-0.677	-0.738	-0.804	-0.576	-0.428	-0.732	-0.502	-0.439	-0.576	-0.494
Glide Ligand efficiency In	-1.325	-1.497	-1.573	-1.167	-0.868	-1.432	-0.982	-0.859	-1.126	-1.001
Glide gscore	-5.479	-6.314	-6.506	-4.924	-3.663	-5.922	-4.060	-3.554	-4.657	-4.225
glide lipo	-1.683	-1.234	-2.533	-1.956	1.987	-2.135	-0.480	-0.591	-1.495	-1.124
glide hbond	-0.305	-0.873	-0.059	-0.254	-1.186	0.0	-0.080	0.0	0.0	-0.046
glide metal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
glide rewards	-1.432	-1.321	-1.959	-1.236	-1.584	-1.841	-1.410	-1.28	-1.28	-1.478
Glide evdw	-35.502	-34.987	-31.910	-35.998	-26.786	-37.101	-33.008	-26.017	-30.858	-25.990
Glide ecoul	-3.161	-8.011	-3.670	-4.561	-6.300	-1.871	-4.194	-3.823	-3.426	-2.929
glide erotb	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190
glide esite	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	-0.016	0.0
Glide emodel	-47.856	-62.289	-45.489	-46.124	-38.794	-51.613	-45.435	-29.542	-44.602	-34.373
Glide energy	-38.664	-42.998	-35.580	-40.537	-33.087	-38.972	-37.202	-29.839	-34.285	-28.919
Glide einternal	7.470	2.182	9.898	2.159	4.593	2.155	2.306	13.34	0.242	4.911
glide confnum	1	2	1	1	1	2	2	2	2	1
Glide posenum	151	1	325	124	12	349	340	182	198	375
XP GScore	-5.479	-6.314	-6.506	-4.924	-3.663	-5.922	-4.060	-3.554	-4.657	-4.225
H-Bond	0	**2**	1	**2**	1	0	1	0	0	0
pi-pi /pi-cation interactions	1	0	1	1	**2**	1	**4**	1	0	0

Table 2B: Docking score and other different docking properties of 7-methoxy-2-(3,4-dimethoxyphenyl)-l-benzofuran-5-carboxaldehyde

Description	Protein								
	4FNY	**2BOU**	**1UFQ**	**1VOM**	**2AZ1**	**1KDR**	**3MK2**	**1TE6**	**1P62**
Potential Energy OPLS 2005 = 127.013									
RMS Derivative OPLS 2005 = 0.023									
Glide lignum	6		6	6	6	6	6	6	6
Docking Score	-6.127		-4.993	**-6.397**	-4.938	-3.985	-3.449	-3.665	-4.242
Glide Ligand efficiency	-0.266		-0.217	-0.278	-0.215	-0.173	-0.15	-0.159	-0.184
Glide Ligand efficiency sa	-0.758		-0.617	-0.791	-0.611	-0.493	-0.427	-0.453	-0.525
Glide Ligand efficiency In	-1.482		-1.207	-1.547	-1.194	-0.964	-0.834	-0.886	-1.026
Glide gscore	-6.127		-4.993	-6.397	-4.938	-3.985	-3.449	-3.665	-4.242
glide lipo	-1.941	**Does not dock**	-1.333	-2.988	-0.785	-0.32	-0.332	-0.300	-0.491
glide hbond	-0.32		-0.322	-0.145	-0.238	-0.191	-0.098	-0.168	-0.268
glide metal	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0
glide rewards	-2.23		-1.28	-1.397	-1.646	-1.712	-1.357	-1.28	-1.28
Glide evdw	-28.695		-28.043	-31.82	-33.401	-33.433	-23.48	-25.253	-31.241
Glide ecoul	-2.615		-5.483	-3.108	-5.261	-1.867	-4.524	-5.38	-4.881
glide erotb	0.190		0.190	0.190	0.190	0.190	0.190	0.190	0.190
glide esite	0.0		-0.024	0.0	0.0	0.0	0.0	-0.038	-0.1
Glide emodel	-43.026		-45.410	-48.816	-47.093	-43.332	-31.758	-37.389	-46.014
Glide energy	-31.311		-33.526	-34.928	-38.662	-35.3	-28.004	-30.633	-36.123
Glide einternal	0.228		0.416	0.958	7.064	1.532	5.915	2.795	1.231
glide confnum	1		2	1	2	1	1	2	1
Glide posenum	25		369	399	210	234	157	380	58
XP GScore	-6.127		-4.993	-6.397	-4.938	-3.985	-3.449	-3.665	-4.242
H-Bonds	1		3	1	1	1	1	**2**	1
pi-pi/pi-cation interactions	0		0	2	0	**6**	0	0	**4**

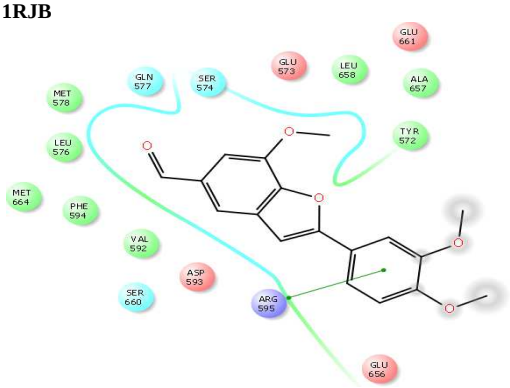
4. Molecular docking:

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

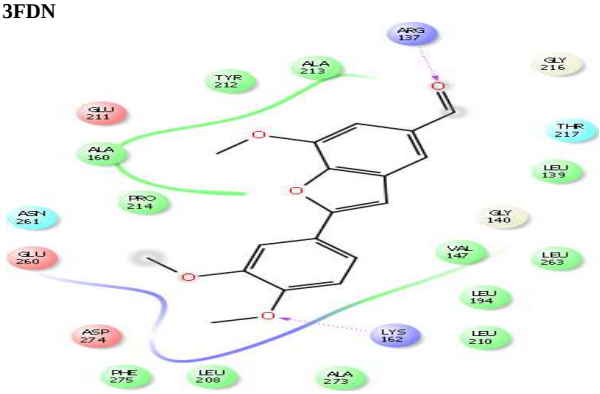
binding of ligand-receptor/protein complex. The docking score and other different docking properties of 7-methoxy-2-(3,4-dimethoxyphenyl)-l-benzofuran-5-carboxaldehyde are shown in table 2A and table 2B.

The docking images of 7-methoxy-2-(3,4-dimethoxyphenyl)-l-benzofuran-5-carboxaldehyde with different PDBs are shown below.

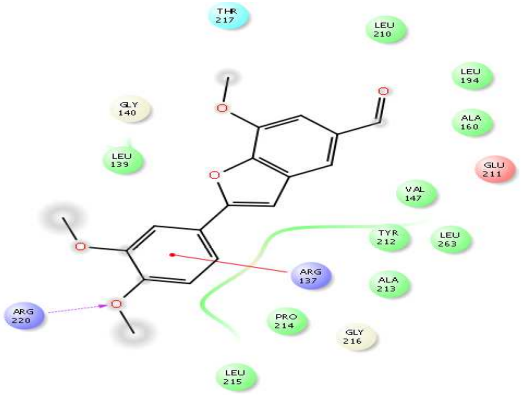
1RJB



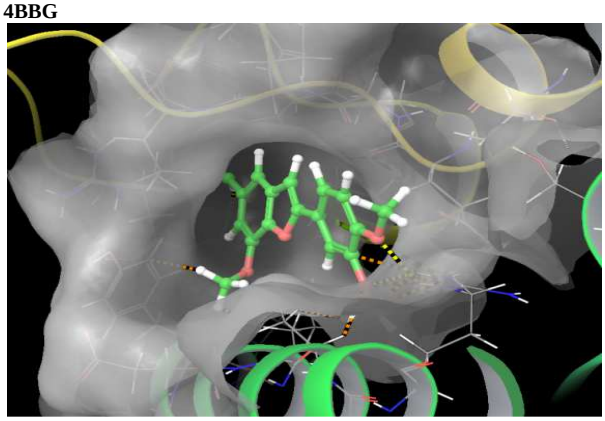
3FDN



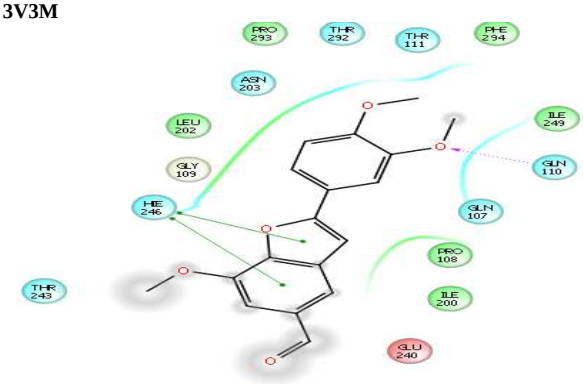
3LAU



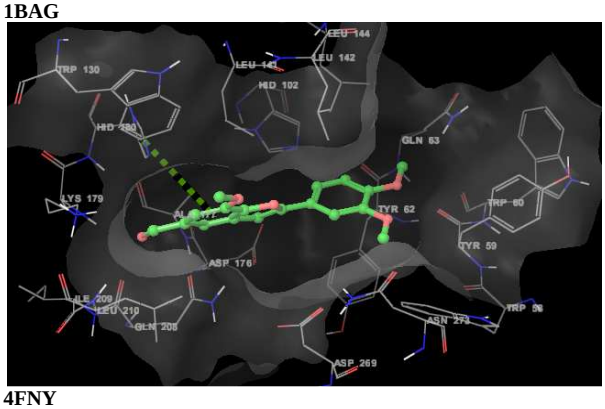
4BBG



3V3M

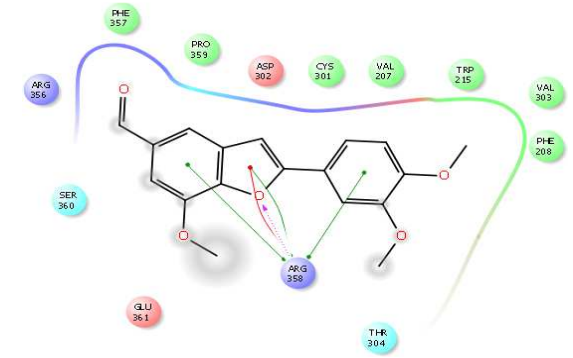


1BAG

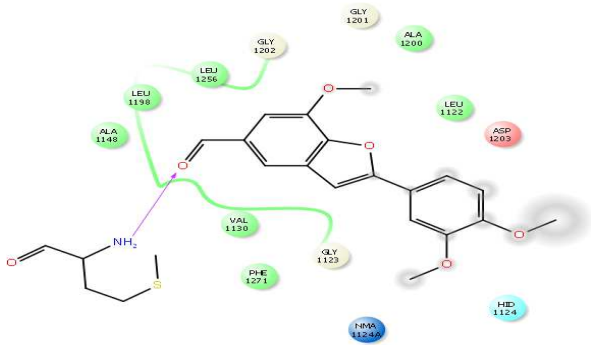


3F8S

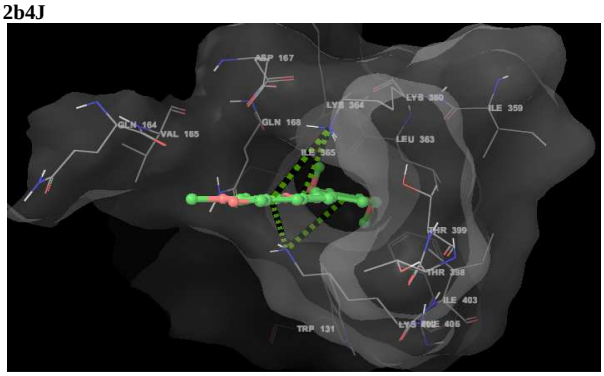
4FNY



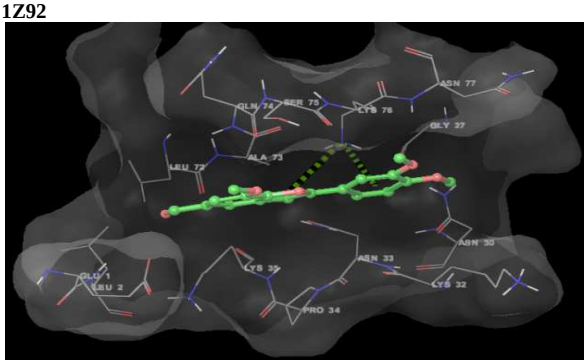
2b4J



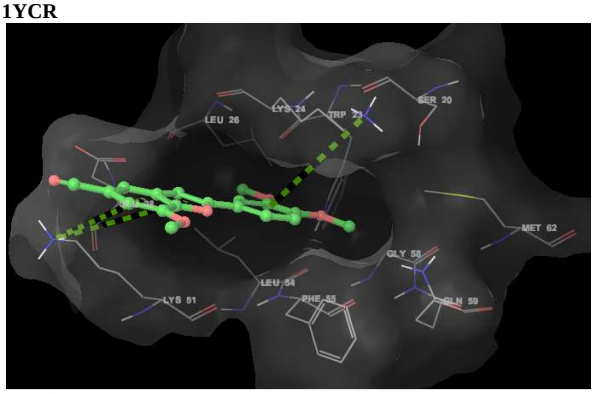
1Z92



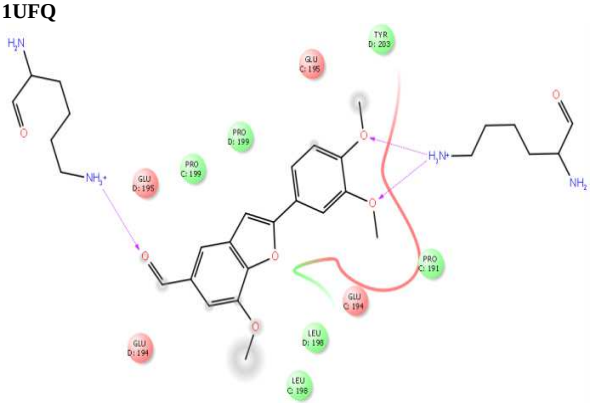
1YCR



2BOU
Does not dock

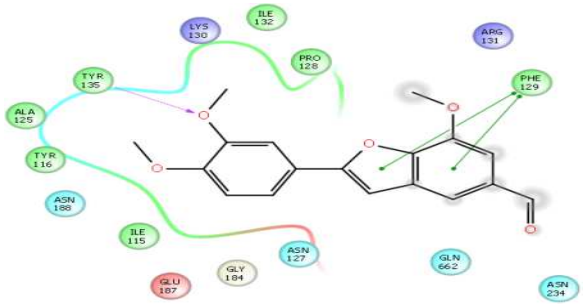


1UFQ

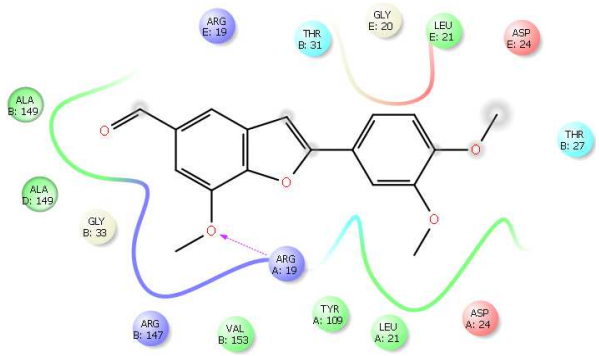


2AZ1

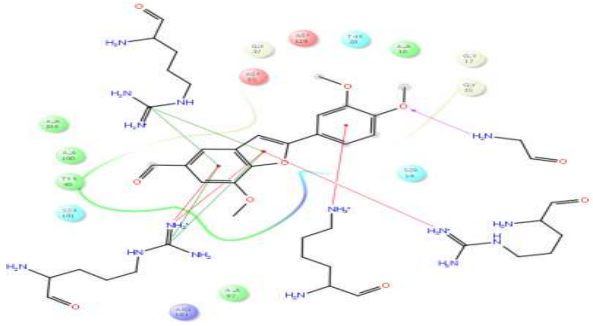
1VOM



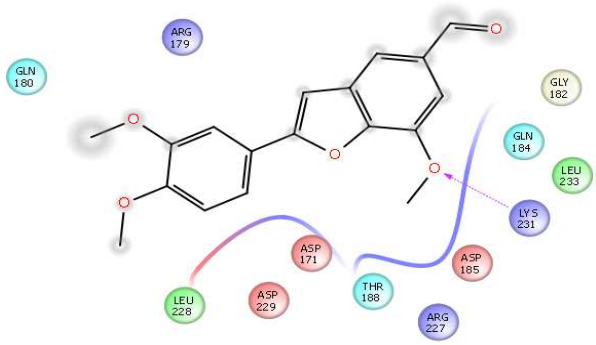
1KDR



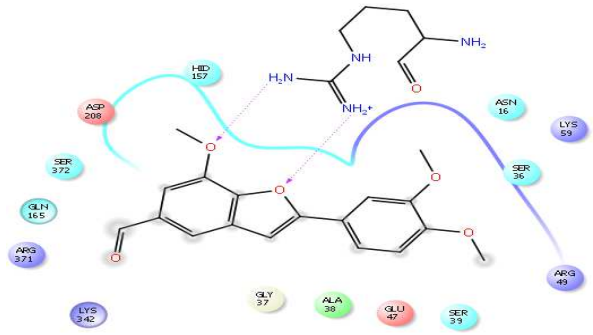
3MK2



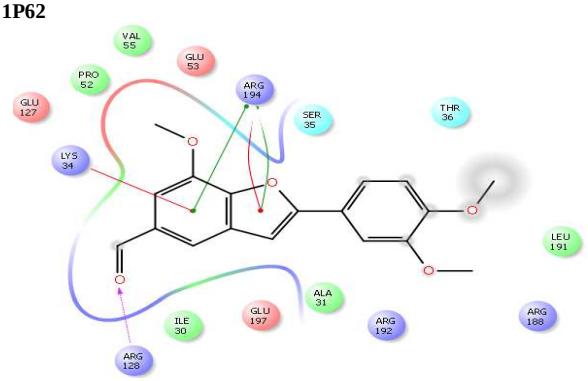
1TE6

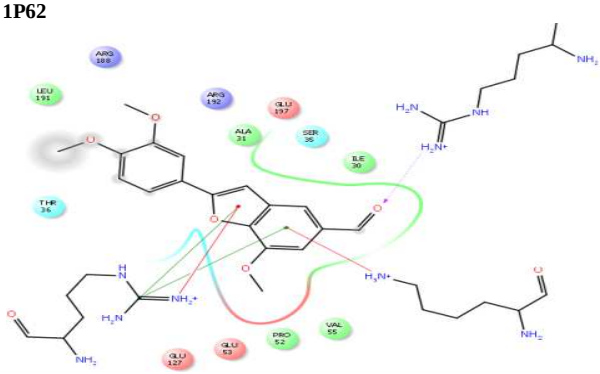


1P62



1P62





5. 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.

MTT Assay: After twenty-four hours of seeding, the medium was removed and then the cells were incubated for 24 hours with DMEM with the absence and/or the presence of various concentration of test compound. Test compound was added at various concentrations ranging from 0.1 to 10 mg/ml. After incubation, 100 µl of MTT reagent was added into each well.

These plates were incubated again for 4 hours in CO_2 incubator at 37^0C. MTT reagent was decanted and 100 µl of DMSO was added as the stopping reagent. The plate was incubated again for 30 minutes in dark.

The resulting MTT-products were determined by measuring the absorbance at 595 nm using multimode reader. Cytotoxic concentration 50 (CC50) values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

MB Assay: After the incubation period, the medium was removed and the cells were washed three times with 200µl PBS. Subsequently, 100µl of a solution of 0.5% (w/v) methylene blue in 50% (v/v) ethanol/water was added to each well. After 3 minutes at RT, the plates were inverted briefly to allow most of the strains to dry away.

Give three washes of distilled water, after the final rinse, the wells were drained by inverting the plates on a sheet of blotting paper of 100µl/well of 1% (w/v) SDS. The absorbance was measured at 620 nm after 1 hour.

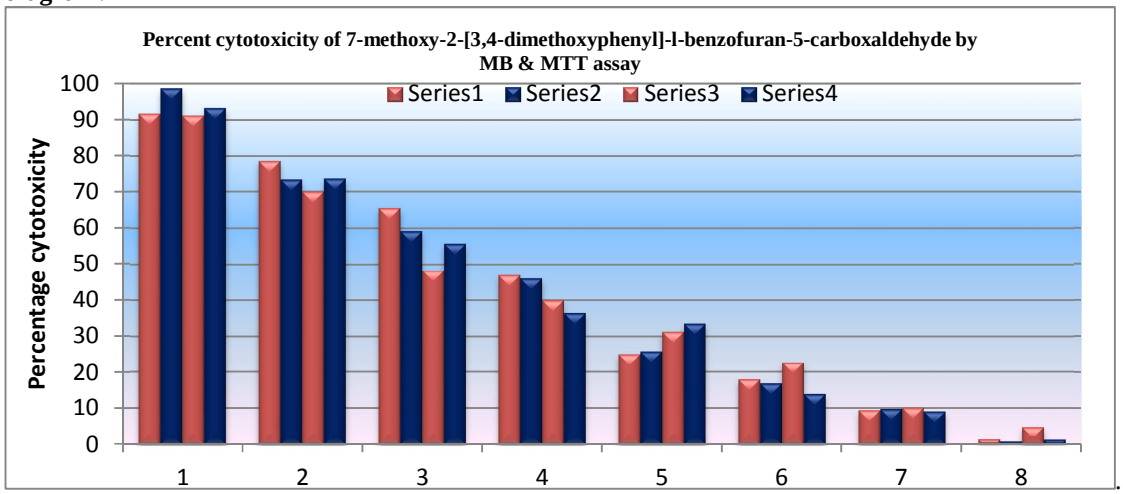
Observations: After the 30 minutes incubation in dark, the formation of purple colour was observed in positive control after which reading were taken at 520 nm on multimode reader.

Statistical Evaluation of Results: Raw data was processed and analyzed for reporting group means, standard deviations and standard error with significance between the controls and the treated groups using statistical software such as Graph Pad prism 5.0 Version (Online Free Trial version). The mean values of all the parameters are rounded of based on the accuracy of the individual values and given in the summary tables.

Table 3: Percent cytotoxicity of 7-methoxy-2-[3,4-dimethoxyphenyl]-l-benzofuran-5-carboxaldehyde

X axis	Conc. mg/ml	MTT assay		MB assay	
		A – 459 cells (Series 1)	MCF - 07 cells (Series 2)	A – 459 cells (Series 3)	MCF - 07 cells (Series 4)
1	**10**	91.79	**98.79**	91.23	93.37
2	**7.5**	**78.69**	73.38	70.07	73.66
3	**5.0**	**65.59**	59.26	48.36	55.84
4	**2.5**	**47.07**	46.09	40.06	36.53
5	**1.0**	25.14	25.85	31.35	**33.56**
6	**0.50**	18.02	16.91	**22.77**	13.98
7	**0.25**	9.47	9.85	**10.18**	9.11
8	**0.10**	1.50	0.91	**4.82**	1.42

Bar diagram:



CC50 values were calculated as the concentrations that show 50% inhibition of proliferation on the cell line. The CC50 values obtained for the test compound is as follows –

Table 4: CC50 values of 7-methoxy-2-[3,4-dimethoxyphenyl]-l-benzofuran-5-carboxaldehyde

MTT assay		MB assay	
A – 459 cells	**MCF - 07 cells**	**A – 459 cells**	**MCF - 07 cells**
1.98	1.93	1.59	1.48

RESULT AND DISCUSSION

The docking score table indicate that 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde is more active against 3LAU (docking score -6.506) and 1VOM (docking score -6.397) while is less active against 2b4J (docking score -3.554) and 3MK2 (docking score -3.449). 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 5: Table of don/acc ratio, docking score, glide esite and polar interactions of 7-methoxy-2-(3,4-dimethoxyphenyl)-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	-5.479	0.0	--	ARG595 (pi-pi)
3FDN	0.880	-6.314	0.0	(ARG137 & LYS162) (with side chain)	--
3LAU	0.749	**-6.506**	0.0	(ARG220) (with side chain)	ARG137 (pi-cation)
4BBG	0.725	-4.924	0.0	2 (ARG221) (with side chain)	ARG221(pi-pi)
3V3M	0.510	-3.663	0.0	(GLN110) (with side chain)	HIE246, HIE246 (pi-pi)
1BAG	**0.478**	-5.922	0.0	--	HID180 (pi-pi)
3F8S	0.762	-4.060	0.0	(ARG358) (with side chain)	ARG358 (3 pi-pi), ARG358 (pi-cation)
2b4J	1.456	**-3.554**	0.0	--	C-LYS360 (pi-cation)
1Z92	1.427	-4.657	-0.016	--	--
1YCR	**2.006**	-4.225	0.0	--	--
4FNY	1.858	-6.127	0.0	MET1199) (with backbone)	--
2BOU	**Does not dock with ligand**				
1UFQ	0.931	-4.993	-0.024	3 (C-LYS202 & D-LYS202) (with side chain)	--
1VOM	0.708	-6.397	0.0	(TYR135) (with side chain)	PHE129, PHE129 (pi-pi)
2AZ1	0.665	-4.938	0.0	(A-ARG19) (with side chain)	--
1KDR	0.661	-3.985	0.0	(GLY19) (with backbone)	ARG41, ARG131 (2 pi-pi each); ARG131 (2), ARG92, LYS18 (pi-cation)
3MK2	0.623	-3.449	0.0	(LYS231) (with side chain)	--
1TE6	0.595	-3.665	-0.038	2 (ARG14) (with side chain)	--
1P62	0.520	-4.665	-0.001	(ARG128 - with side chain)	ARG194 (2 pi-pi), ARG194 & LYS34 (pi-cation)

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 1P62 (-0.001), 1TE6 (-0.038), 1UFQ (-0.024) and 1Z92 (-0.016) but these are totally absent in 1YCR. The aldehyde shows higher polar interactions with 1P62, 1KDR, 3F8S, 1UFQ, 4BBG, 3V3M, 1VOM, 3FDN, and 3LAU proteins PDBs. This is one of the reason for the higher docking score of aldehyde in 3LAU and 1VOM. Also the molecule containing five hydrogen bond donor atoms and hydrogen bond donating nature of 1VOM and 3LAU at docking site is comparable (≈ 0.7). The docking score of aldehyde during docking with 1VOM and 3LAU was higher (even though they forming one hydrogen bonding and weaker 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 amino acids of backbone of PDBs such as MET, ARG, LEU, TYR and GLY and side chain of the amino acids such as ARG, GLN and LYS are forming hydrogen bonding with aldehyde.

Table 6: Table of glide evdw, glide energy, electrostatic and polar interactions 7-methoxy-2-(3,4-dimethoxyphenyl)-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	-35.502	-38.664	ARG595	GLU573, ASP593, GLU661	SER574, GLN577, SER660
3FDN	-34.987	-42.998	ARG137, LYS162	GLU211, GLU260, ASP274	THR217, ASN261
3LAU	-31.910	-35.580	ARG137, ARG220	GLU211	THR217
4BBG	-35.998	-40.537	ARG119, ARG221	GLU116, GLU118, ASP130, GLU215	THR112
3V3M	-26.786	-33.087	--	GLU240	GLN107, GLN110, THR111, ASN203, THR243, HLE246, THR292
1BAG	**-37.101**	-38.972	LYS179	ASP176, ASP269	GLN63, HID102, HID180, GLN208, ASN273
3F8S	-33.008	-37.202	ARG356, ARG358	ASP302, GLU361	Thr304, SER360
2b4J	-26.017	-29.839	C-LYS360, C-LYS364, C-LYS402	A-ASP167	A-GLN164, A-GLN168, C-THR398, C-THR399
1Z92	-30.858	-34.285	A-LYS32, A-LYS35, A-LYS76	B-GLU1	A-ASN30, A-ASN33, A-GLN74, A-SER75, A-ASN77
1YCR	-25.990	-28.919	A-LYS51, B-LYS24	B-GLU28	A-GLN59, B-SER20
4FNY	-28.695	-31.311	--	ASP1203	HID1124
2BOU	**Does not dock with ligand**				
1UFQ	-28.043	-33.526	C-LYS202, D-LYS202	C-GLU194, C-GLU195, D-GLU194, D-GLU195	--
1VOM	-31.820	-34.928	LYS130, ARG131	GLU187	ASN127, ASN188, ASN234, GLN662
2AZ1	-33.401	-38.662	A-ARG19, B-ARG147, E-ARG19	A-ASP24, E-ASP24	B-THR27, B-THR31
1KDR	-33.433	-35.300	LYS18, ARG41, ARG92, ARG131, ARG181	ASP35, ASP129	SER14, THR20, SER101
3MK2	**-23.480**	-28.004	ARG179, ARG227, LYS231	ASP171, ASP185, ASP229	GLN180, GLN184, THR188
1TE6	-25.253	-30.633	ARG14, ARG49, LYS59, LYS342, ARG371	GLU47, ASP208	ASN16, SER36, SER39, HID157, GLN165, SER372
1P62	-31.241	**-36.123**	LYS34, ARG128, ARG188, ARG192, ARG194	GLU53, GLU127, GLU197	SER35, THR36

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 of the interaction in decreasing order is as 1BAG > 4BBG > 1RJB > 3FDN > 1KDR > 2AZ1 > 3F8S > 3LAU >1VOM > 1P62 > 1Z92 > 4FNY >.....

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-3FDN complex. This is due to higher surface area (both polar and non-polar) of 3FDN available for interaction with aldehyde. The aldehyde has higher glide energy during the interaction with PBDs in the decreasing order as 3FDN > 4BBG > 1BAG > 1RJB > 2AZ1 > 3F8S > 1P62 > 3LAU > 1KDR > 1VOM > 1Z92 >.....

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 6 [**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 1KDR, 1TE6, 1P62, 3MK2, 2AZ1, 1Z92, 3FDN, 2b4J, 3F8S, 3LAU and 1VOM 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, and 4FNY.

The table 6 [**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. 4BBG, 1UFQ, 3MK2 and 1P62 PDBs shows maximum number of such type of interactions with aldehyde while these interactions are weaker with 3FDN, 3LAU, 3V3M, 1BAG, 4FNY, 2BOU, 1VOM, and 3MK2 shows minimum number of such interactions.

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.

Table 7: Table of glide lipo and polar interactions of 7-methoxy-2-(3,4-dimethoxyphenyl)-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	-1.683	TYR572, LEU576, MET578, VAL592, PHE594, MET664 ALA657, LEU658,.	GLU573, ASP593, GLU661
3FDN	0.758	1.170	-1.234	LEU139, VAL147, ALA160, LEU194, LEU208, LEU210, TYR212, ALA213, PRO214, LEU263, ALA273, PHE275	GLU211, GLU260, ASP274
3LAU	1.245	0.819	-2.533	LEU139, VAL147, ALA160, LEU194, LEU210, TYR212, ALA213, PRO214, LEU215, LEU263	GLU211
4BBG	1.274	1.108	-1.956	TRP127, ALA133, PRO137, LEU160, LEU170, TYR211, LEU214, ALA218	GLU116, GLU118, ASP130, GLU215
3V3M	0.473	1.200	-1.987	PRO108, GLY109, ILE200, LEU202, ILE249, PRO293, PHE294	GLU240
1BAG	0.343	1.103	-2.135	TRP58, TYR59, TRP60, TYR62, TRP130, LEU141, LEU142, LEU144, LAL177, ILE209, LEU210	ASP176, ASP269
3F8S	0.298	1.089	-0.480	VAL207, PHE208, TRP215, CYS301, VAL303, PHE357, PRO359	ASP302, GLU361
2b4J	1.321	0.765	-0.591	A-VAL162, B-TRP131, C-ILE359, C-LEU363, C-ILE365, C-ILE403, C-PHE406	A-ASP167
1Z92	0.396	0.805	-1.495	A-PRO34, B-LEU2, A-LEU72, A-ALA73	B-GLU1
1YCR	1.171	0.675	-1.124	B-TRP23, B-LEU26, A-LEU54, A-PHE55, A-MET62	B-GLU28
4FNY	1.470	0.654	-1.941	LEU1122, VAL1130, ALA1148, LEU1198, MET1199, ALA1200, LEU1256, LEU1271	ASP1203
2BOU	Does not dock with ligand				
1UFQ	0.510	0.947	-1.333	C-PRO191, C-LEU198, C-PRO199, D-LEU198, D-PRO199, D-TYR203	C-GLU194, C-GLU195, D-GLU194, D-GLU195
1VOM	1.022	0.853	**-2.988**	ILE115, TYR116, ALA125, PRO128, PHE129, ILE132, TYR135	GLU187
2AZ1	0.397	1.562	-0.785	A-LEU21, E-LEU21, A-TYR109, B-ALA-149, D-ALA149, B-VAL153, E-LEU21	A-ASP24, E-ASP24
1KDR	0.463	1.343	-0.320	ALA16, TYR40, ALA97, ALA100, ALA104	ASP35, ASP129
3MK2	0.632	0.717	-0.332	LEU228, LEU233	ASP171, ASP185, ASP229
1TE6	0.008	1.703	**-0.300**	ALA38	GLU47, ASP208
1P62	0.490	1.393	-0.491	ILE30, ALA31, PRO52, VAL55, LEU191	GLU53, GLU127, GLU197

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 unionized 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 1VOM > 3LAU > 1BAG > 3V3M > 4BBG > 4FNY > 1RJB > 1Z92 > 1UFQ > 3FDN > 1YCR >... 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 2AZ1, 2b4J, 1P62, 3F8S, 3MK2, 1KDR .. whereas is totally weak in 1TE6.

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 3V3M and 1KDR shows weak interaction with 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde which can be explained by their low docking score. The aldehydes does shows any kind of docking interactions with 2BOU.

Based on the results of MTT and MB assay, it is concluded that 7-methoxy-2-(3,4-dimethoxyphenyl)-1-benzofuran-5-carbaldehyde more toxic against lung cell line than cancerous breast cancer cell line.

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