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Synthesis, SAR, Molecular Docking and Antituberculosis Study of 3-Methyl-1-Benzofuran-2-Carbohydrazide

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3-Methyl-1-benzofuran-2-carbohydrazide was synthesized from 2-hydroxy acetophenone. To deduce the antibacterial and anticancer activity of the 3-methyl-1-benzofuran-2-carbohydrazide, it is docked with different biomarkers of cancer cell and bacteria. 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 3-methyl-1-benzofuran-2-carbohydrazide is most active against 3LAU (Arora 2 kinase) and 1VOM (Dictyostelium myosin) biomarkers and have the features to prove themselves as antituberculosis drugs. The structure of the target molecule compared with ciprofloxacin (antituberculosis drug) and shows 67.4 % structural similarity. Cramer rules of toxicity predicts the toxicological hazard (when administered orally) from the molecular structure. It shows that it is class III toxic compound. The antituberculosis studies show that it shows strong activity (1.6 µg/mL) against mycobacterium tuberculosis (H37 RV strain).

Keywords: Benzofurans, Molecular docking, SAR study, Hydrazones, Tuberculosis activity.

INTRODUCTION

Most of the benzofuran compounds [1,2] frequently occur in natural products and are good chelating agents. The compound amiodarone hydrochloride used as an ideal antiarrhythmic drug [3] contains a 2,3-substituted benzofuran moiety. The synthesis of ester of 1-benzofuran-2-carboxylate derivatives was reported by number of scientists. They can be synthesized by direct condensation of 2-hydroxybenzophenones with ethyl 2-bromoacetate in dry toluene in the presence of sodium hydride, sodium ethoxide in refluxing absolute ethanol [4]. 1-Benzofuran-2-carbohydrazide has been synthesized from salicylaldehyde and ethyl chloroacetate [5]. 5-Chlorobenzofuran-2-carbohydrazide [6] were synthesized from ethyl 5-chlorobenzofuran-2-caboxylate and condensed with various substituted aromatic aldehyde to give Schiff base. 5-Bromosalicylaldehyde was treated with hydroxylamine hydrochloride in N,N-dimethyl formamide under reflux conditions forming 5-bromo salicylonitrile which is further treated with ethyl chloroacetate in anhydrous acetone in presence of anhydrous potassium carbonate forming its ethyl ester. The crude ester was treated with potassium carbonate in DMF under reflux condition forming ethyl 5-bromo-3-amino-1-benzofuran-2-carboxylate [7].

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 behaviour of molecule/s and have been widely applied for understanding and predicting the behaviour of molecular systems [8]. 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 [5,8], including DOCK-6, FlexX, GLIDE, GOLD, FRED, Cresset 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 [9], gene technology and high-throughput tests [10,11] has shifted the focus and poor absorption, distribution, metabolism and excretion (ADME)

properties of new drugs captured more attention [12]. 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 [13], which is the relative orientation and position after interaction.

EXPERIMENTAL

Synthesis of 3-methyl-1-benzofuran-2-carbohydrazide (1b): Dissolve 13.6 g (0.1 mol) of 2-hydroxy acetophenone in 80 mL of acetone and add 20.7 g (0.15 mol) of potassium carbonate slowly with constant string by using mechanical stirrer. Stir the solution for further 0.5 h at room temperature. Add 0.1 mole of ethyl chloroacetate slowly with constant stirring at room temperature and reflux the reaction mixture in water bath for about 6 h. Check completion of reaction by monitoring TLC. After completion of reaction, distilled out acetone under reduced pressure. Dissolve the residue in methanol and add 0.1 mol of potassium carbonate with constant stirring and reflux the reaction mixture in water bath at about 3 h. Distilled out excess methanol under reduced pressure and acidify the reaction mixture with 1 N hydrochloric acid at cold condition. Add water and extract the product with ethyl acetate, wash the organic layer by using sodium bicarbonate and saline water, dry the organic layer by using anhydrous sodium sulphate. Distilled out the ethyl acetate under reduced pressure forming red colour viscous liquid is obtained used it further for hydrazine synthesis. Wash the red colour viscous oil by mixture of toluene and *n*-hexane obtain yellow colour ethyl 3-methyl-1-benzofuran-2-carboxylate.

Dissolve 0.1 mol of ethyl 3-methyl-1-benzofuran-2-carboxylate in methanol add 0.12 mol of hydrazine hydrate with constant stirring and catalytic amount of acetic acid. Reflux the reaction mixture for 6 h, cool the reaction mixture to room temperature and filter out the faint yellow colour solid.

Molecular formula: $C_{10}H_{10}N_2O_2$; Colour: faint yellow solid; Yield: 62 %; m.p.: 135 °C. FT-IR (in KBr): 3313, 3224, 3002, 1650, 1608, 1521, 1450, 1361, 1301, 1272, 1145, 964, 875, 717 cm^{-1}. NMR spectra (δ in ppm): 9.810 (s, 1H, NH-CO); 7.70 (d, 1H, Ar-H); 7.53 (d, 1H, Ar-H); 7.44 (t, 1H, Ar-H); 7.31 (t, 1H, Ar-H); 4.50 (bs, 2H, NH2); 2.49 (s, 3H, Ar-CH_3). Mass spectra: 191.14 (M + 1).

Estimation of toxic hazard: Toxtree is a full-featured and flexible user-friendly open source application, which is able to estimate toxic hazard by applying a decision tree approach. By applying these decision tree approaches to the three dimensional structure of 3-methyl-1-benzofuran-2-carbohydrazide 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 genotoxic and nongenotoxic carcinogenicity, structural alert for *S. typhimurium* mutagenicity, non-irritating or corrosive to skin and eyes (predicted lipid solubility is 10 % 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.

Antimycobacterium activity: The antimycobacterial activity of hydrazide should be assessed against *M. tuberculosis* using micro plate Alamar Blue assay (MABA).This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile de-ionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of the Middlebrook 7H9 broth and serial dilution of compounds was made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for 5 days. After this time, 25 µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10 % between 80 % was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

Strain used for antituberculosis study [14] is *M. tuberculosis* (H37 RV strain): ATCC No – 27294. The standard or reference used for the antituberculosis study are pyrazinamide, streptomycin and ciprofloxacin.

COMPUTATIONAL METHODS

ChemBio3D is used to build, visualize and analyze 3D models of chemical structures. Using the *ab initio* and semi-empirical quantum mechanics, Gaussian predicts the minimum energies, molecular structures, vibrational frequencies, optimum properties, IR/Raman spectra, NMR spectra and chemical properties of molecules and reactions in a variety of chemical environments. Gaussian can be applied to both stable compounds and compounds that are difficult or impossible to observe experimentally such as short-lived intermediates and transition structures. CS MOPAC performs semi-empirical calculations on atoms and molecules to determine details of molecular structures and properties.

AutoDock is an automated docking tool that helps to predict how small molecules bind to a receptor of known 3D structure. ChemBio3D maintains an interface to AutoDock to perform the docking calculation. CONFLEX can be used to search for chemically significant conformers in flexible molecules and displays the conformers as fragments in your model. pKa, log S and log P for predicting acid dissociation constants, aqueous solubility and octanol/water distribution coefficients of chemical compounds are computational calculator modules based on Molecular Networks' chemoinformatics platform MOSES.

SAR study: Forge (software) is a molecular design and SAR interpretation tool. It will generate detailed 3D models of binding and pharmacophores that will help to define the requirements of the protein of interest, aiding the synthetic chemist in the designing of new actives. It also gives rationale for the polarization of the molecules for synthesis.

Activity atlas of Forge actually performs three types of analysis: useful for quantitative information can be gained from 3D model. This model shows that the average active molecule

looks like by making an analysis of what have in common the active molecules in the data set. The average electrostatic of actives shows the region where the active molecule in general shows either a positive or a negative field. As this field is associated with a high biological activity, new molecules that show either positive or negative fields in the same field should also be active. The average hydrophobic of the actives contributions shows the regions where the active molecules is general make hydrophobic interactions with the target of interest.

RESULTS AND DISCUSSION

The electron density present on the heteroatoms of the molecule is carbonyl group oxygen atom of –$CONHNH_2$ group is -0.42, furan oxygen is -0.10, nitrogen of –CONH- group (-0.24) and –NH_2 nitrogen is -0.50. These electron distribution indicates the strength of hydrogen bonding is N (NH_2) > O (-CONH-) > N (-CONH-) > O (furan ring). The hydrogen (0.28) attached to the – NH_2 group is more electropositive than the hydrogen (0.21) attached to -NH the nitrogen atom which are able to form hydrogen bonding. This can be confirm from NMR spectra and IR-spectra signal intensity. The force field points of the 3-methyl-1-benzofuran-2-carbohydrazide was compared with ciprofloxacin (antituberculosis, considered as reference molecule) is 0.674 which quite good but with respect to ciprofloxacin, target molecule shows better results. The docking score table indicate that 3-methyl-1-benzofuran-2-carbohydrazide is more active against 3LAU (docking score -6.726) and 1VOM (docking score -5.760) while is less active against 3V3M (docking score -3.925).

In IR spectra, the carbonyl group (carbon-oxygen double bond) appears in many interacting compounds and this bond acts like a well behaved localized vibration. The carbonyl group of the hydrazide shows absorption in the region at 1650 cm^{-1}. The H–N bonds of the hydrazide group shows stretching vibration in the region 3313-3224 cm^{-1}. The region below 1000 cm^{-1} often reveals strong bands that are useful for the characterizing aromatic compounds. The benzofuran ring shows strong absorption band in the region of 1050-980 cm^{-1} whereas C=C bonds of the aromatic ring shows absorption band in the region of 1610-1520 cm^{-1}. In NMR spectra, the NH-CO proton shows singlet at 9.810 ppm while that of –NH_2 protons at 4.50 ppm. The methyl proton shows singlet at 2.49 ppm. Finally, the formation of the molecule was confirmed by the mass spectra, it shows (m + 1) peak at 191.14.

The molecule containing one -CO–NH-NH_2 and one –CH_3 group which will be confirmed by its NMR spectrum, the –NH_2 protons showing broad singlet at 4.50 ppm, the –CH_3 hydrogen shows singlet at 2.49 ppm while –NH proton shows singlet at 9.810 ppm. The presence of -$NHNH_2$ group can also be confirm from FT-IR spectra of molecule. The strong absorption in FT-IR spectra of molecule at 3313, 3224 and 3002 (hydrazide) cm^{-1} is due to symmetric stretching vibrations of N-H bond. The carbonyl group of amide (-CO-NH-) linkage shows strong absorption at 1650 cm^{-1} while aromatic double bonds show strong absorption bands in 1602 and 1521 cm^{-1}. The 3-methyl-1-benzofuran-2-carbohydrazide molecule containing two oxygen atoms, one is member of benzofuran ring, another is part of hydrazide group, both having different chemical environment and therefore different electron density around them.

The carbonyl group oxygen atom of –$CONHNH_2$ group (-0.42) has more electron density while that of furan oxygen (-0.10). The nitrogen of –CONH- group has electron density -0.24 and that of –NH_2 nitrogen is -0.50. These electron distribution indicates the strength of hydrogen bonding is N (NH_2) > O (-CONH-) > N (-CONH-) > O (furan ring). The hydrogen (0.28) attached to the – NH_2 group is more electropositive than the hydrogen (0.21) attached to -NH the nitrogen atom which are able to form hydrogen bonding. The oxygen and nitrogen atom are act as H-bond bond donor while hydrogen atom attached to nitrogen and oxygen atom acts as H-bond acceptor. The phenyl and furan rings shows mixed hydrophobic and electrostatic character and is reflected in a combination of in plane positive field points, π-cloud points and hydrophobic points are at its center. Field point score with respect to ciprofloxacin: 0.674.

The force field points of the 3-methyl-1-benzofuran-2-carbohydrazide was compared with ciprofloxacin as reference molecule. The molecular field similarity between them was found to be 0.674 which was quite good.

Density functional study of 3-methyl-1-benzofuran-2-carbohydrazide

Minimum binding energy of 3-methyl-1-benzofuran-2-carbohydrazide: The structure of 3-methyl-1-benzofuran-2-carbohydrazide obtained at minimum binding energy. The white colour ball indicates presence of hydrogen, red ball indicates presence of oxygen, blue ball indicates presence of nitrogen and gray colour ball is of carbon (Fig. 1). The minimum binding energy of the molecule is calculated from the HOMO-LUMO energy gap (from density of states) *i.e.,* band gap.

Density of states of 3-methyl-1-benzofuran-2-carbohydrazide (DOS): The DFT calculation has been performed for the determination of electronic density of state of molecule

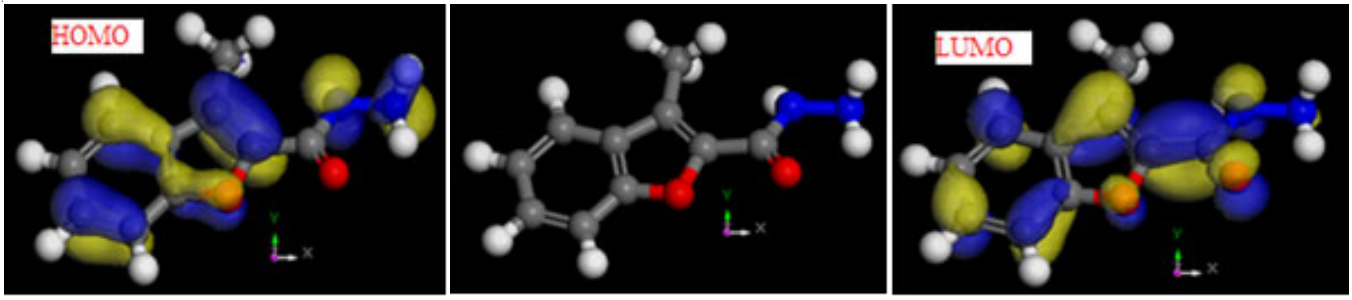


Fig. 1. Structure of 3-methyl-2-carbohydrazide

in terms of electron density in k space. It gives energy in terms of Fermi Energy, not in term of absolute energy. The density of state (DOS) of 3-methyl-1-benzofuran-2-carbohydrazide shows conduction band indicates the molecule has electrical conductivity property. The density of state is also used to calculate minimum energy required for the excitation of electrons from HOMO to LUMO (for electronic transition). The density of state of the molecule also shows HOMO of the molecule is completely filled as shown in Fig. 2.

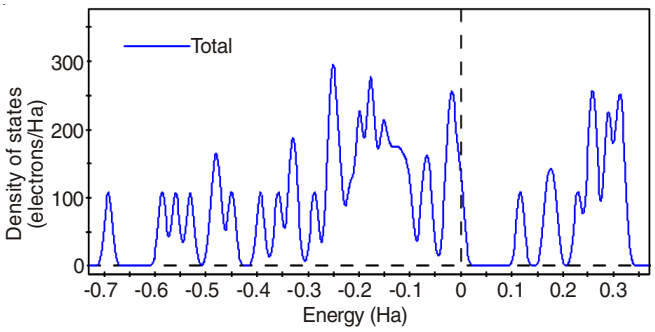


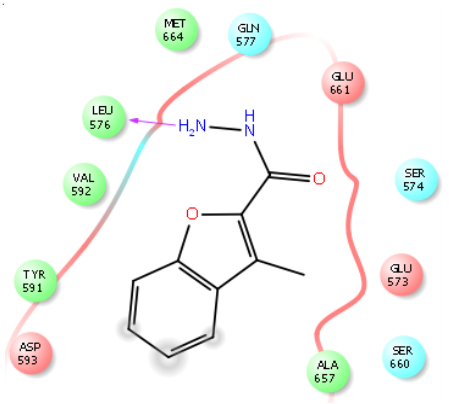
Fig. 2. Density of state 3-methyl-1-benzofuran-2-carbohydrazide

Molecular docking: The three dimensional structures of all proteins were taken from the PDB database. The three-dimensional structure of the ligand [3-methyl-1-benzofuran-2-carbohydrazide] was constructed (Fig. 3). The ligand was then energy-minimized in the in-built ChemSketch module of the software.

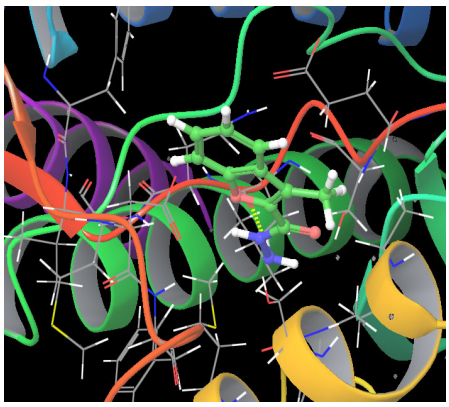
The active site of each protein were first identified and defined using an eraser size of 5.0 E. The ligand was docked into the active site separately using the 'Flexible Fit' option. The ligand-receptor site complex was subjected to '*in situ*' ligand minimization which was performed using the in-built *CHARMm forcefield* calculation. The non-bond cutoff and the distance dependence was set to 11 E and (ε = 1R) respectively. 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.

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.

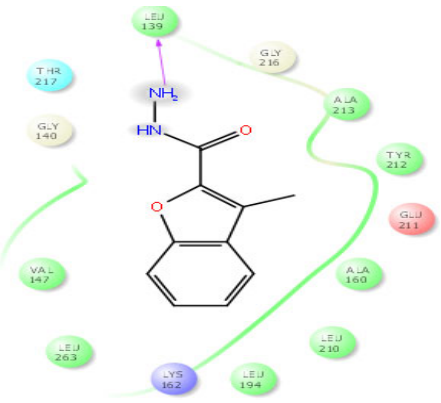
The docking site score of 1VOM (1.074) and 1RJB (1.073) receptor/protein is higher while that of 2BOU (0.464) is lowest is indicates that the 1VOM and 1RJB proteins PDB are more favourable for docking than the others. The size of 4BBG (223) and 1VOM (222) are higher while volume of 3FDN (760.77)



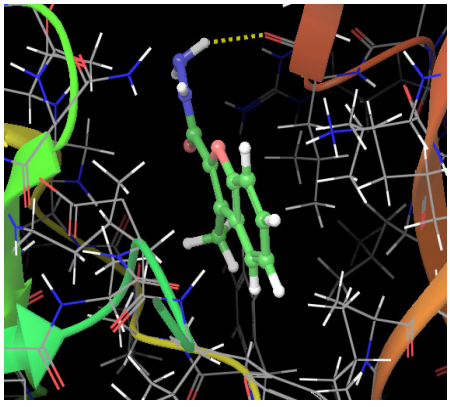
1RJB



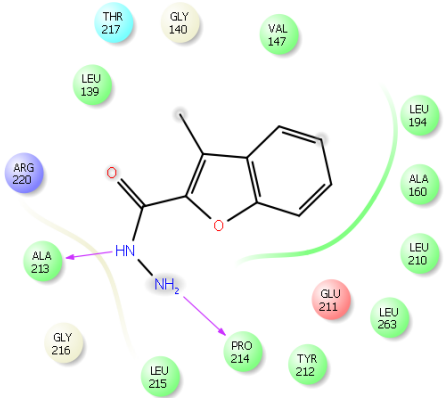
1RJB



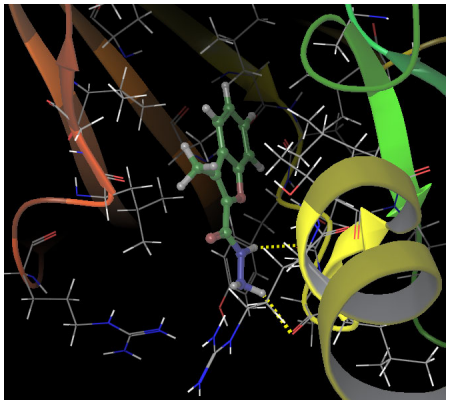
3FDN



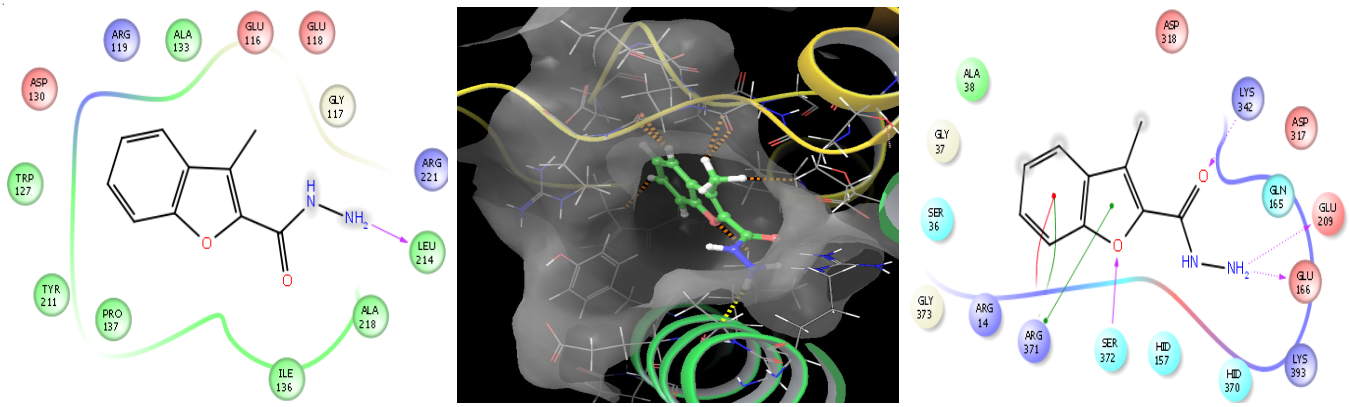
3FDN

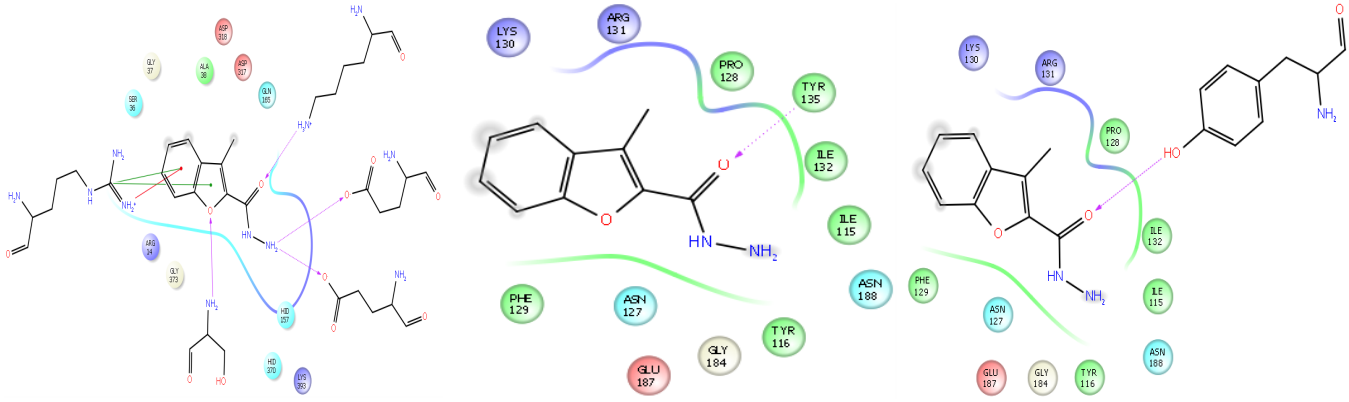


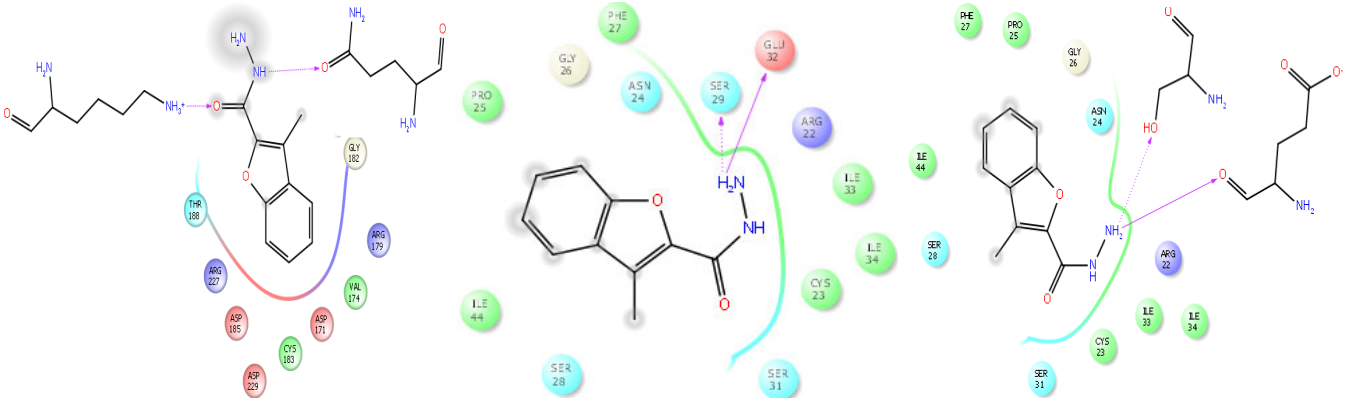
3LAU



3LAU







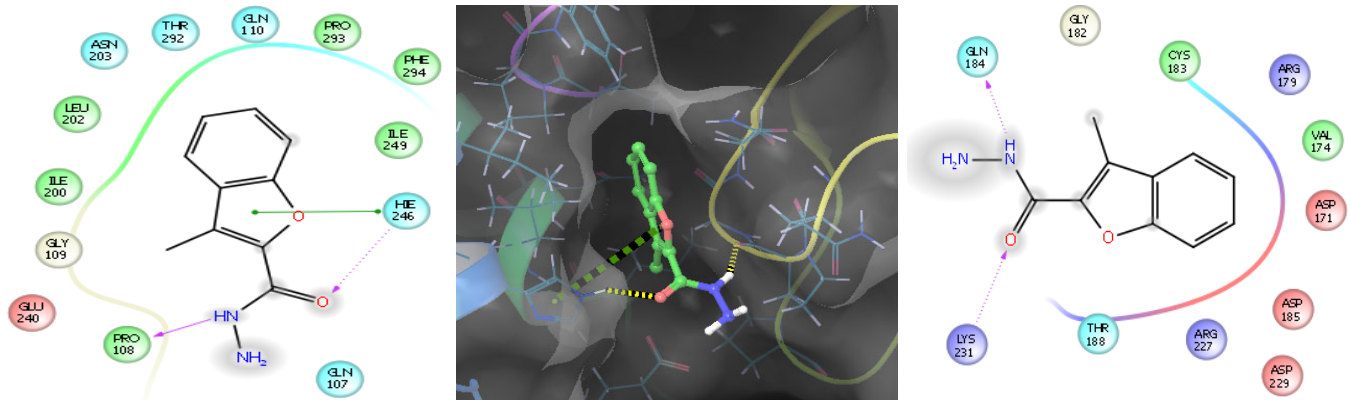


Fig. 3. Docking images of 3-methyl-1-benzofuran-2-carbohydrazide with different PDBs

and 1VOM (618.77) available for docking is higher but exposure to the ligand as compared to 3LAU and 3V3M is lower. The exposure to the ligand is maximum in 2BOU and 3V3M and minimum in 1RJB while reverse is the case for the enclosure area, it is higher in 1RJB and 1TE6 while minimum in 3MK2. The overall contact area to the ligand is higher in 1RJB (1.124) and 1TE6 (0.993). The hydrophobic nature or character and balance between hydrophobic and hydrophilic nature of the active site is higher in 4BBG and 3LAU respectively while that of lower in 1TE6 (0.008). The hydrophilic nature or character of the active site is higher in 1TE6 (1.703) and lower in 3MK2 (0.717). The ligands having more hydrophilic nature are more tightly binds with 3MK2 and weakly binded to 1TE6.

The order of protein in the decreasing order of hydrophilic character and increasing order of hydrophobic character is - 1TE6 > 2BOU > 3V3M > 1RJB > 3FDN > 3MK2 > 4BBG > 1VOM > 3LAU. This indicates that the ligands having more hydrophobic nature are binds easily 3LAU. The hydrogen bond donor/acceptor character ratio is higher in 2BOU (1.433) and 3FDN (0.880) while lower in 3V3M (0.510) therefore the ligand contains more hydrogen bond acceptor atoms/groups are more tightly binds to 2BOU and 3FDN while those containing hydrogen bond donor atoms/groups are bind to 3V3M. The order protein in the decreasing order of H-bond donor to H-bond acceptor ratio is –2BOU > 3FDN > 3LAU > 4BBG > 1VOM > 1RJB > 3MK2 > 1TE6 > 3V3M. 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 docking score and other different docking properties of 3-methyl-1-benzofuran-2-carbohydrazide are shown in Table-1.

The docking score table indicate that 3-methyl-1-benzofuran-2-carbohydrazide is more active against 3LAU (docking score -6.726) and 1VOM (docking score -5.760) while is less active against 3V3M (docking score -3.925). 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 hydrazide and amino acid residues of the protein are only observed in 1TE6 (-0.191) and 3MK2 (-0.075) but these are totally absent in 3LAU, 3FDN, 1RJB, 3V3M, 2BOU, 1VOM and 4BBG. The hydrazide shows higher polar interactions with 1TE6 PDB.

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 3-methyl-1-benzofuran-2-carbohydrazide. 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 hydrazide-protein 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 Glide evdw of the interaction in decreasing order is as 1RJB > 3LAU > 1VOM > 4BBG > 1TE6 > 3FDN > 2BOU > 3V3M > 3MK2.

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 hydrazide-1RJB complex. The

TABLE-1
DOCKING SCORE AND OTHER DIFFERENT DOCKING PROPERTIES OF 3-METHYL-1-BENZOFURAN-2-CARBOHYDRAZIDE

Description	Proteins								
	1RJB	3FDN	3LAU	4BBG	3V3M	2BOU	1TE6	1VOM	3MK2
Potential Energy OPLS 2005 = 46.347, 47.088									
RMS Derivative OPLS 2005 = 0.009									
Glide lignum	3	2	12	2	2	2	2	2	2
Docking score	-5.590	-5.182	**-6.726**	-4.842	**-3.925**	-4.943	-4.476	-5.760	-5.356
Glide ligand efficiency	-0.399	-0.370	**-0.480**	-0.346	**-0.280**	-0.353	-0.320	-0.411	-0.383
Glide ligand efficiency sa	-0.962	-0.892	**-1.158**	-0.834	**-0.676**	-0.851	-0.771	-0.992	-0.922
Glide ligand efficiency In	-1.536	-1.424	**-1.848**	-1.331	**-1.079**	-1.358	-1.230	-1.583	-1.472
Glide gscore	-5.590	-5.182	**-6.726**	-4.842	**-3.925**	-4.943	-4.476	-5.760	-5.350
Glide lipo	-1.212	-0.684	**-2.405**	-0.678	-0.876	-0.518	**0.0**	-1.837	-1.621
Glide hbond	-0.320	**-1.105**	-0.315	-0.984	-0.157	-0.827	-0.487	-0.227	-0.263
Glide metal	0.0	0.0	0.0	0.0	0.0	0.0	**0.0**	0.0	0.0
Glide rewards	-2.562	-2.015	**-2.753**	-2.042	**-1.894**	-2.514	-2.112	-2.511	-2.112
Glide evdw	**-28.426**	-22.275	-27.412	-24.074	-20.113	-22.989	-23.989	-25.400	-17.580
Glide ecoul	-3.454	**-1.101**	-2.171	-2.670	-4.233	-2.522	**-6.197**	-2.395	-5.65
Glide erotb	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444	0.444
Glide esite	0.0	0.0	0.0	0.0	0.0	0.0	**-0.191**	0.0	-0.075
Glide emodel	**-41.868**	-31.666	-40.156	-36.563	-31.836	-32.072	-37.819	-37.243	**-31.085**
Glide energy	**-31.880**	-23.376	-29.584	-26.745	-24.346	-25.511	-30.187	-27.795	-23.244
Glide einternal	1.296	0.062	1.184	**0.025**	0.127	**1.554**	1.454	1.307	0.791
Glide confnum	2	5	2	1	1	2	2	2	1
Glide posenum	157	10	305	08	**4**	230	176	116	**399**
XP GScore	-5.590	-5.182	**-6.726**	-4.842	**-3.925**	-4.943	-4.476	-5.760	-5.350
H-Bond	01	01	02	01	02	02	04	01	02
π-π/π-cation interactions	0	0	0	0	01	00	03	00	00

decreasing order of the glide energy of 3-methyl-1-benzofuran-2-carbohydrazide with PBDs in the decreasing order as 1RJB > 1TE6 > 3LAU > 1VOM > 4BBG > 2BOU > 3V3M > 3FDN > 3MK2.

The above docking images [electrostatic interactions (blue)] shows that two amino acids in all proteins as ARG and LYS shows positive interactions. 3-Methyl-1-benzofuran-2-carbohydrazide shows stronger such interaction with same amino acids of 4BBG, 3V3M, 3MK2 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 3LAU, 3FDN and 2BOU whereas is absent with 1RJB and 3V3M.

The above docking images [electrostatic interactions (pink)] shows that two amino acids in all proteins as ASP and GLU shows negative interactions. 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, 4BBG, 1TE6 and 3MK2 PDBs shows maximum number of such type of interactions with 3-methyl-1-benzofuran-2-carbohydrazide while these interactions are weaker with 3FDN, 3LAU, 2BOU, 1VOM and 3V3M.

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 3-methyl-1-benzofuran-2-carbohydrazide and amino acid residue at the docking site in the order of 3LAU > 1VOM > 3MK2 > 1RJB > 3V3M > 3FDN > 2BOU > 4BBG PDBs at the neutral pH = 7. At lower pH, hydrazide get protonated and its lipophilicity character goes on decreasing. The hydrazide shows weaker lipophilic and hydrophobic attraction in 1TE6.

The electron rich π-system (containing electron donating group) are generally interact with other electron deficient π-system having electron withdrawing group. These are denoted by green colour and are called as hydrophobic interactions. Also, electron rich π-centre interacts with cation (denoted by dark blue colour) and electron deficient centre interact with anion (denoted by pink colour). The 3-methyl-1-benzofuran-2-carbohydrazide shows the π-π interactions with the amino acid residue containing aromatic ring or π-electrons, the amino acids such as ARG (C=N bond) and PHE, HIE and HID (aromatic ring) shows such interactions with it. The π-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 π-electrons of aromatic rings. 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 1TE6 and 3MK2 PDBs are shows weak interaction with 3-methyl-1-benzofuran-2-carbohydrazide which can be explained by their low docking score.

The antimycobacterium activity of hydrazide should be assessed against *M. tuberculosis* using micro plate alamar blue assay (MABA). The standard or reference used for the antituberculosis study are pyrazinamide, streptomycin and ciprofloxacin and their standard values for the antituberculosis test which was performed her are - 3.125 µg/mL, 6.25 µg/mL and 3.125 µg/mL respectively while that of target compound is 6.25 µg/mL.

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