ARTICLE

Synthesis, Molecular Docking, SAR Study and Antituberculosis Activity of 5-Bromo-1-benzofuran-2-carbohydrazide

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ABSTRACT

5-Bromo-1-benzofuran-2-carbohydrazide was synthesized by known literature method from 5-bromosalicylaldehyde. To deduce the antibacterial and anticancer activity of the 5-bromo-1-benzofuran-2-carbohydrazide, 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 5-bromo-1-benzofuran-2-carbohydrazide is most active against 3LAU (Aurora-2 kinase) and 4BBG (human kinesin Eg5) biomarkers and have the features to prove themselves as antituberculosis drugs. The Cramer rules of toxicity predicts the toxicological hazard (when administered orally) from the molecular structure. It shows that it is of class III toxic compound. The antituberculosis studies show that it shows good activity against *Mycobacterium tuberculosis* (H37 RV strain).

KEYWORDS

Benzofuran ethyl ester, Molecular docking, Anticancer, Tuberculosis activity.

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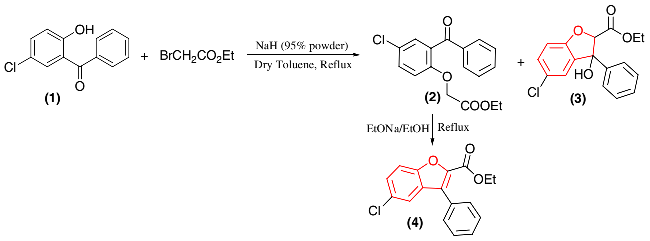
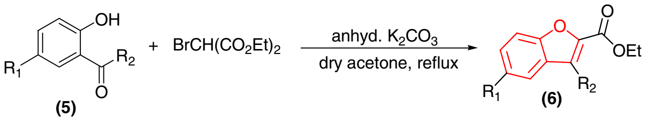
INTRODUCTION

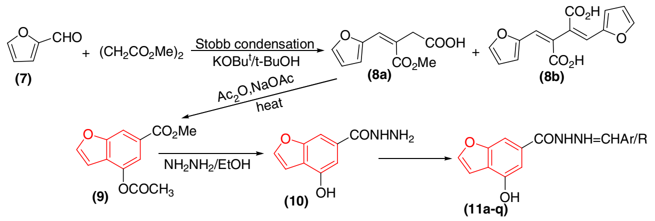
The synthesis of ester of 1-benzofuran-2-carboxylate derivatives was reported by number of researchers, some of them are reported here. The esters can be synthesized by direct condensation of 2-hydroxybenzophenones (**1**) with ethyl 2-bromoacetate in dry toluene in the presence of sodium hydride forming ethyl 2-benzoylphenoxy acetate derivatives (**2**) with very small amount of benzofuran ethyl ester (**3**) which was separated by column chromatography [1].



Searching the literature for the alternative procedure allowing a direct and high yielding synthesis of benzofuran-2-ester (**6**); some of the furochromes were synthesized by the direct cyclization with diethyl bromomalonate in the presence of anhydrous potassium carbonate in dry acetone [2]. The presence of an electron withdrawing group R_1 increased the reactivity of carbonylic function. By contrast, as expected the presence of R_1 as an electron donor group like methyl or lacking of the substitution decreases the electrophilic properties of ketone functionality affording the lower yield of the corresponding benzofuran (< 40 %).

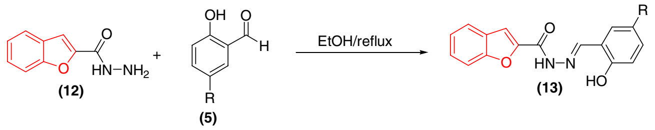


The Schiff bases of 4-hydroxy benzofuran-6-carboxyhydrazide (**10**) and their hydrazone derivatives (**11a-q**) shows moderate to high degree of antibacterial activity when compared to standard drugs [3]. 4-Hydroxy benzofuran-6-carboxyhydrazide has been synthesized from furfuraldehyde (**7**) and dimethyl succinate *via* series of reaction [4].

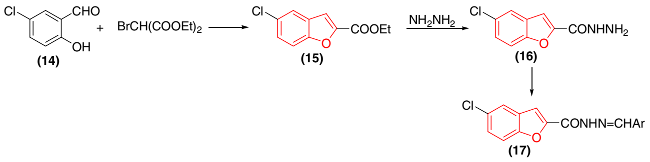


Hydrazides can be synthesized by hydrazinolysis of amine, ester and thioesters [5]. The reaction of hydrazines with acyl chlorides or anhydrides is well known [6]. Novel Schiff bases [4] of 1-benzofuran-2-carbohydride were synthesized and studied their pharmacological activities.

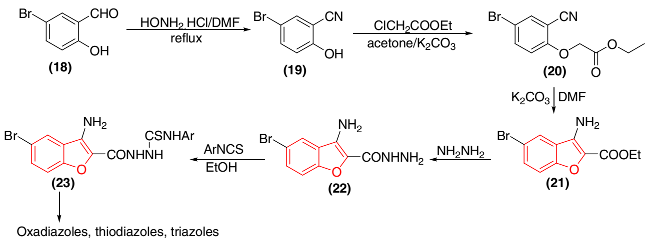
Most of the benzofuran compounds [7] frequently occur in the natural products and are good chelating agents. The compound amiodarone hydrochloride used as an ideal anti-arrhythmic drug [8] contains a 2,3-substituted benzofuran moiety. The microbial activities of ligands are altered many fold on coordination with metal ions [9]. Hence, there is a need for information on coordination compounds of simple benzofuran Schiff bases (**13**) and their metal complexes. In view of the biological importance of Schiff bases and benzofuran it was thought worthwhile to synthesize the benzofuran Schiff bases (**13**) derived from the condensation of benzofuran-2-carbohydrazide (**12**) [10] with 5-methyl-salicylaldehyde and 5-chlorosalicylaldehyde and to prepare their metal complexes with metal ions like Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), UO_2(II) and Th(IV). All the complexes and ligands are screened for their antimicrobial activity.



5-Chlorobenzofuran-2-carbohydrazides (**17**) [11] were synthesized from ethyl 5-chlorobenzofuran-2-caboxylate (**15**) and condensed with various substituted aromatic aldehyde to give Schiff base and obtained compound were cyclized with carbon disulphide and alcoholic potassium hydroxide to obtain various 5-chlorobenzofuran derivatives. Some of them showed good activity against gram positive and gram negative bacteria like *S. aureus* and *E. coli*.



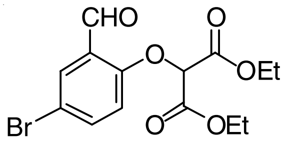
5-Bromosalicylaldehyde (**18**) was treated with hydroxylamine hydrochloride in N,N-dimethyl formamide under reflux conditions for about 20 min forming 5-bromo salicylonitrile (**19**) which is the important precursor for the synthesis of benzofuran skeleton. The 5-bromo salicylonitrile (**19**) was further treated with ethyl chloroacetate in anhydrous acetone in presence of anhydrous potassium carbonate forming its ethyl ester (**20**). The crude ester was treated with potassium carbonate in DMF under reflux condition forming ethyl 5-bromo-3-amino-1-benzofuran-2-carboxylate (**21**) [12]. This ester was converted into hydrazide (**22**) by treating it with hydrazine hydrate in ethanol. It undergoes cyclization under different reaction conditions forming oxadiazoles, triazoles and thiadiazoles.



Many methodologies for the synthesis of benzofuran-2-carboxylic acid skeleton were developed and most of them are based on the reaction of salicylaldehydes with haloacetates followed by cyclization of formylphenoxyacetic acid derivatives intermediates [13-19]. The cyclizations were usually carried out in an alcoholic solution in the presence of a basic catalyst such as sodium ethanolate [13], 1,8-diazobicyclo-[5.4.0]-7-undecane, potassium carbonate [14,15] or phosphine [16]. Moreover, ethyl 5-nitrobenzofuran-2-carboxylate was prepared by cyclocondensation of 2-hydroxy-5-nitrobenzaldehyde with bromomalonic acid [20] as well as palladium catalyzed carbonylative cyclization of *o*-alkynylphenols [21-23]. Hydrazines are the attractive targets compounds for new drug development due to their synthetic and biological versatility including antituberculosis activities [24-28].

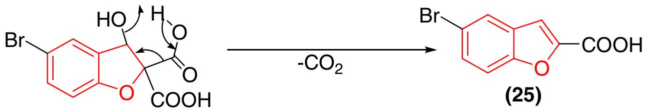
EXPERIMENTAL

5-Bromo-1-benzofuran-2-carbohydrazide was synthesized from 5-bromosalicylaldehyde (**18**) by series of reaction which was synthesized by the bromination of salicylaldehyde using bromine in acetic acid. The bromination of the product was confirmed by the sodium fusion test (Lassanger test). It is treated with diethyl chloromalonate in 2-butanone as solvent in presence of potassium dicarbonate as base. Diethyl chloromalonate was freshly prepared by the chlorination of diethyl malonate by using thionyl chloride and purified by distillation. Potassium carbonate is weak base. It causes deprotonation of 5-bromosalicylaldehyde forming phenoxide which acts nucleophile and shows nucleophilic attack on carbon of diethyl chloromalonate containing chlorine. The unreacted potassium carbonate and potassium chloride were removed by filteration. The organic layer was washed with sulphuric acid to remove if acidic impurity formed distilled out organic solvent under reduced pressure to obtain crude product diethyl (4-bromo-2-formylphenoxy)propanedioate (**24**). The carbon atom of diethyl (4-bromo-2-formylphenoxy)propanedioate (**24**) is more acidic than the carbon atom of diethyl malonate containing chlorine due to more electro negativity of oxygen atom.



Diethyl 2-(4-bromo-2-formylphenoxy)malonate (**24**)

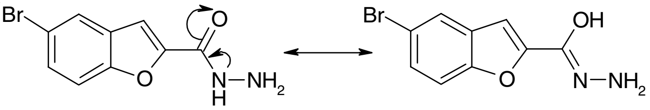
The –CH- group of (**24**) is more acidic than that of diethyl chloromalonate, therefore undergoes deprotonation forming carbanion which shows intramolecular nucleophilic attack on aldehyde group in presence of 10 % alcoholic KOH forming solid dipotassium carboxylate salt. Both the ester groups undergo hydrolysis. The salt was dissolved in 3M sulphuric acid forming free dicarboxylic acid which undergoes dicarboxylation on heating forming 5-bromo-1-benzofuran-2-carboxylic acid (**25**).



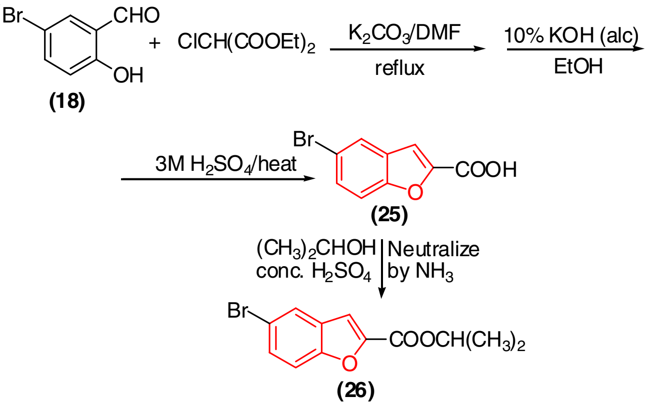
The isopropyl ester of 5-bromo-1-benzofuran-2-carboxylic acid (**25**) was formed by the esterification of (**25**) in presence of excess 2-propanol and concentrated sulphuric acid (as a dehydrating agent). Pour the reaction mixture into ice and neutralized by ammonia; extract the product by using ether and dried by using anhydrous calcium chloride to remove the water from ether. Distilled out ether under reduced pressure forming propan-2-yl 5-bromo-1-benzofuran-2-carboxylate (**26**). The formation of product (**26**) was confirmed by TLC, m.p. and FT-IR spectra. The carbonyl group of ester shows stretching vibration at 1727 cm^{-1} confirms the formation of product (**26**).

The propan-2-yl-5-bromo-1-benzofuran-2-carboxylate (**26**) was treated with hydrazine hydrate in 2-propanol in presence of acetic acid as catalyst. The solid 5-bromo-1-benzofuran-2-carbohydrazide (**27**) obtained was filtered and recrystallized from ethanol. The melting points of all Schiff bases and their starting compounds are recorded by using Labtronics Digital Melting point apparatus. The formation of product (**27**) was confirmed by TLC, m.p., sodium fusion test, FT-IR, NMR spectroscopy. The compound (**27**) shows frequencies at 3322 and 3183 cm^{-1} is due to –NH- and –NH$_2$ stretching vibrations, respectively and 1668 cm^{-1} is due to stretching vibrations of carbonyl group of hydrazide. It does not show any absorption band in the frequency region of carbonyl group of ester.

Hydrazides having two nitrogen atoms available for the donation of electron pair. The lone pair of electrons present over the nitrogen atom of –NH- group is in conjugation with double bond of carbonyl and not easily available for donation but in case of –NH$_2$ group, lone pair is free for donation and easily donated towards electrophilic center like carbonyl group as shown below:

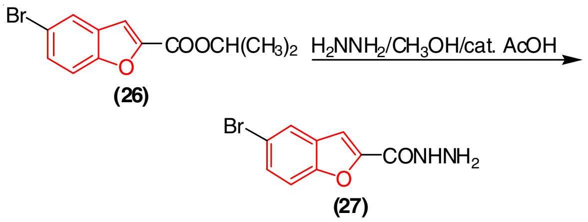


Synthesis of propan-2-yl-5-bromo-benzofuran-2-carboxylate (26): Dissolve 20 g of 5-bromosalicyladehyde (**18**) and 47 g of diethyl chloromalonate (65 % pure, freshly prepared from diethyl malonate and thionyl chloride) in 200 mL of 2-butanone. Add 27 g of potassium carbonate slowly with constant stirring and reflux the given reaction mixture about 4 h. Check the completion of reaction by monitoring TLC time to time. Cool the reaction mixture in ice cold water and filter the salt by Buchner funnel. Wash the residue by 2 × 10 mL of 2-butanone and reject the solid. Combine the filtrate and distilled out the solvent under reduced pressure to obtain solid. Dissolve the crude solid material in dichloromethane and wash the organic layer by dilute sulphuric acid (to remove carbonate impurities present in the solid). Distilled out the organic solvent under reduced pressure to obtain red colour viscous oil. Dissolve the product in 125 mL 10 % potassium hydroxide in ethanol and warm it. Cool the reaction mixture and filter the solid product. Dissolve the crude solid product in 350 mL 3M sulphuric acid and heat it to 80-90 °C in water bath (till evolve of carbon dioxide by product of decarboxylation) till the effervescences of gas get stop. The solid separated was filtered and recrystallized by hot water (**25**).



Dissolve the crude product (**25**) in 25 mL of isopropyl alcohol and add 18 mL conc. sulphuric acid slowly with constant stirring. Reflux the reaction for about 6 h on water bath. The completion of reaction was confirmed by taking TLC. Cool the reaction mixture and add into 100 g crushed ice slowly with stirring and neutralized by aqueous ammonia solution. Extract the product by ether 2 × 50 mL and dry the organic layer anhydrous calcium chloride. Evaporate the ether in boiling water bath to obtain isopropyl 5-bromo-1-benzofuran-2-carboxylate (**26**) as white solid. Recrystallized it from ethanol. Yield is 72 %. m.p. = 85-87 °C. FTIR (KBr, ν_{max}, cm^{-1}): 3095, 2977, 1727, 1563, 1311, 1182, (3424 cm^{-1} water proton).

Synthesis of 5-Bromo-1-benzofuran-2-carbohydrazide (27): Dissolve 0.300 g (6 mmol) of hydrazine hydrate in 20 mL of isopropyl alcohol; add 1.420 g (5 mmol) of propan-2-yl-5-bromo-1-benzofuran-2-carboxylate (**26**) and catalytic amount of acetic acid. Reflux the reaction mixture to 5 h and cool, the solid product (**27**) separated was filtered. The purity of the product was checked by IR, NMR and mass spectra. m.p. 165 °C and yield 83 %. FT-IR (KBr, ν_{max}, cm^{-1}): 3322, 3183, 1668, 1617, 1317, 1184, 800. NMR (chemical shift in ppm): 12.224 and 8.436 (bs, 1H, -CO-NH); 8.091 (s, 1H, Ar-H); 7.706 (m, 2H, Ar-H); 7.659 (s, 1H, Furan-H); 7.040 (bs, 2H, -NH$_2$). Mass spectra: 91, 253, 254.8 (M -1), 256.9 (M+1, Br is ^{81}Br), 257.9 (M+2).



The strong absorptions in FT-IR spectra of **27** at 3322 and 3183 cm^{-1} are due to symmetric stretching vibrations of N-H bond of –NH-NH$_2$ group of hydrazide. The formation of hydrazide functionality is also confirmed by the carbonyl stretching frequency. The ester carbonyl group of **26** shows absorption at 1727 cm^{-1} which is absent in hydrazide and new absorption band observed at 1668 cm^{-1} indicate the formation of amide –CO- group. The aromatic double bonds show strong absorption bands in 1617-1578 cm^{-1} region.

The NMR spectrum of 5-bromo-1-benzofuran-2-carbohydrazine (**27**) and its Schiff bases was recorded by using 300 MHz NMR instrument by using DMSO-d_6 solvent and TMS is reference compound. The amide proton of hydrazide linkage [-CO-NH-N<] shows two chemical shifts, one at δ 12.224 ppm [due to C(OH)=N-N] and second at δ 8.436 ppm due to (CO-NH-N). The NH$_2$ protons are highly deshielded and therefore shows chemical shift at δ 7.040 ppm. The aromatic proton present at C-4 position (C$_4$-H) is highly deshielded and shows chemical shift at δ 8.091 ppm. The C$_6$-H and C$_7$-H are also deshielded and shows multiplet at δ 7.706 ppm. The furan proton (C$_3$-H) is shielded proton as compared to other aromatic protons even though it is present 2-position with respect to electron-withdrawing –CONHNH$_2$ group because of high average electron density of five membered ring carbon and +R effect of furan oxygen atom.

The presence of one bromine atom in compound **27** has been confirmed from its mass spectra. The compound shows two fragments 254.8 is M-1 (when Br is ^{79}Br) and 256.9 is M+1 (when Br is ^{81}Br) with equal intensity indicate the presence of one bromine atom.

Computational molecular properties: ChemBio3D is used to build, visualize and analyze 3D models of chemical structures. 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.

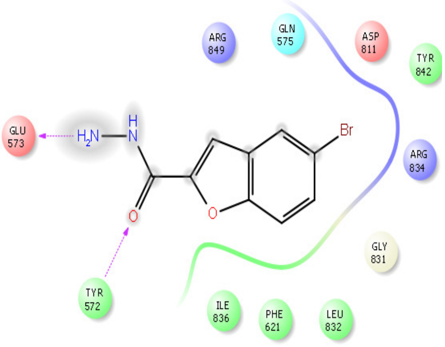
SAR study: Forge (software) is a molecular design and SAR interpretation tool. It will generate detailed 3D models of binding and pharmacophores that will help to define the requirements of the protein of interest, aiding the synthetic chemist in the designing of new actives. It also gives rationale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

Molecular docking: The three dimensional structures of all proteins were taken from the PDB database. The three-dimensional structure of the ligand [5-bromo-1-benzofuran-2-carbohydrazide] was constructed (Fig. 1). The ligand was then energy-minimized in the in-built ChemSketch module of the software.

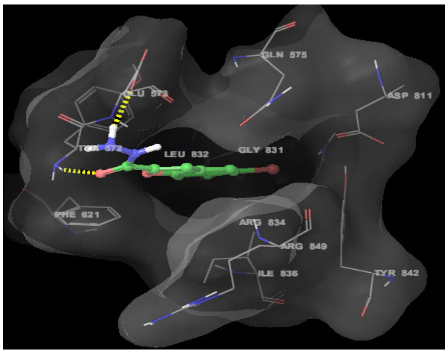
The active site of each protein were first identified and defined using an eraser size of 5.0 Å. The ligand was docked into the active site separately using the 'Flexible Fit' option. The ligand-receptor site complex was subjected to '*in situ*' ligand minimization which was performed using the in-built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

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-bromo-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.

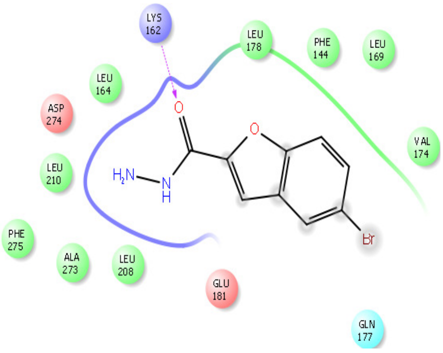
Antimycobacterium activity: Tuberculosis (TB) is a lung infection caused mainly by *Mycobacterium tuberculosis* (MTB). Antibiotics are most effective against actively growing *M. tuberculosis*, as these persistent organisms exhibit a phenotypic drug resistance; *i.e.*, their resistance is not associated with genetic changes but with their extant metabolic state. The anti Mycobacterium activity of hydrazide should be assessed against *M. tuberculosis* using micro plate Alamar Blue assay (MABA). Finally we conclude the activity of the hydrazide as they were active or not. This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with



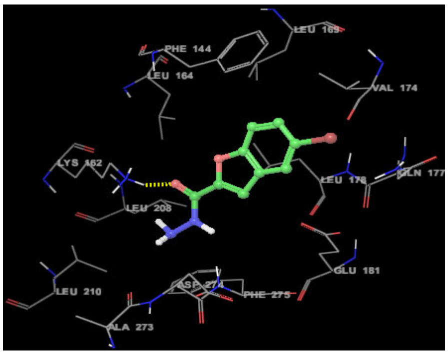
1RJB



1RJB



3FDN



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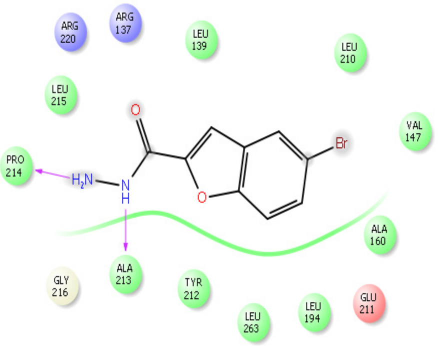
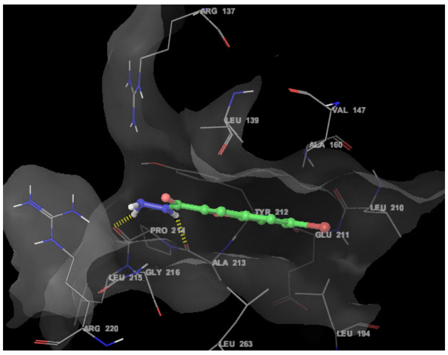


3LAU



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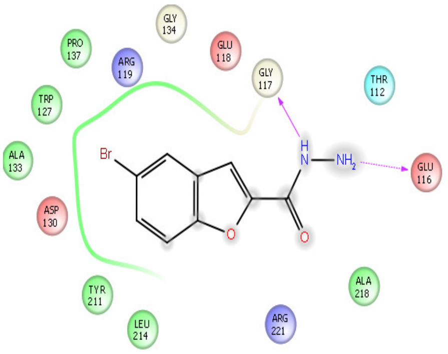
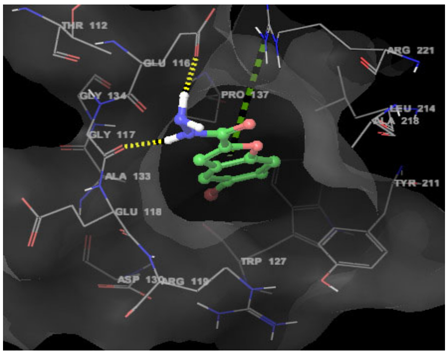
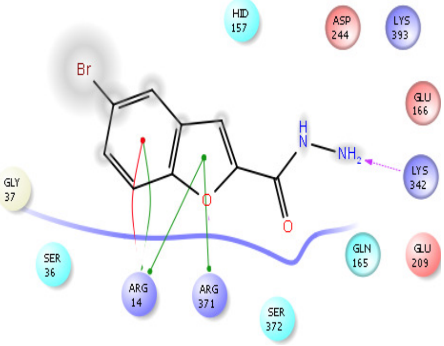


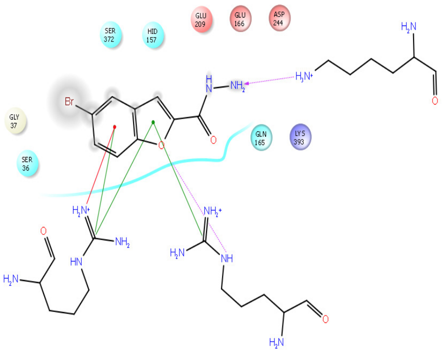
4BBG



4BBG



1TE6



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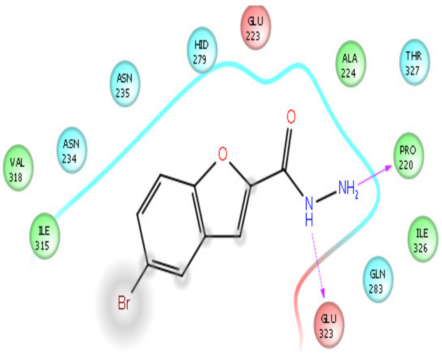
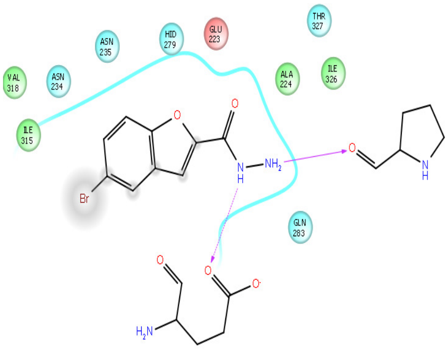


1VOM



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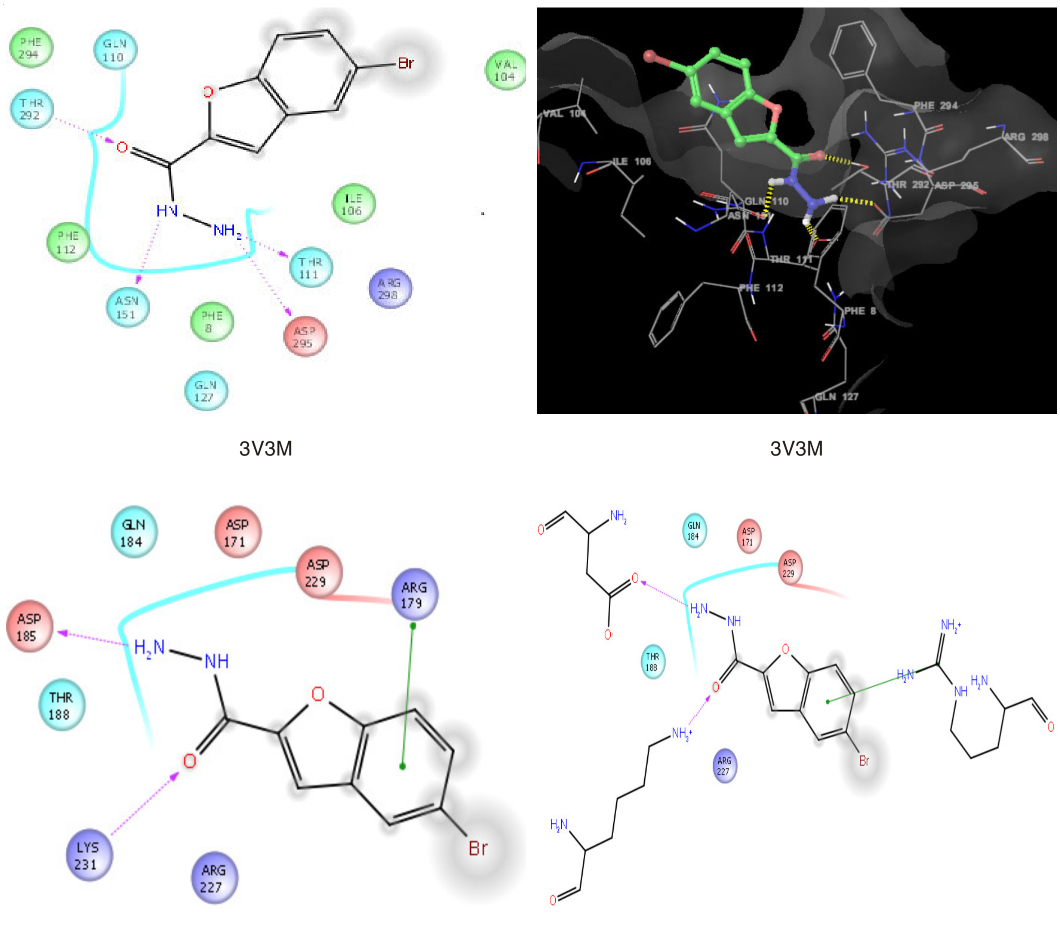

3V3M 3V3M

3MK2 3MK2

Fig. 1. Docking images of 5-bromo-1-benzofuran-2-carbohydrazide with different PDBs

proportional and BACTEC radiometric method. In brief, 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 middle-brook 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 five 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 [29] is *M. tuberculosis* (H37 RV strain): ATCC No-27294. 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, 6.25 and 3.125 µg/mL, respectively while that of target compound is **25** µg/mL.

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 confirming from NMR spectra and IR-spectra signal intensity. The force field points of the 3-bromo-1-benzofuran-2-carbohydrazide was compared with ciprofloxacin (antituberculosis; considered as reference molecule) is 0.694 which quite good but with respect to ciprofloxacin, target molecule shows better results. The docking score table indicate that 3-bromo-1-benzofuran-2-carbohydrazide is more active against 3LAU (docking score -7.194) and 4BBG (docking score -6.419) while is less active against 3MK2 (docking score -3.640).

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 1668 cm^{-1}. The strong absorption in FT-IR spectra of at 3322 and 3183 cm^{-1} is due to symmetric stretching vibrations of N-H bond of –NH-NH$_2$ group of hydrazide. 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 amide proton of hydrazide linkage [-CO-NH-N<] shows two chemical shifts, one at δ 12.224 ppm (due to C(OH)=N-N) and second at δ 8.436 ppm (is due to –CO-NH-N). The –NH_2 protons are highly deshielded and therefore shows chemical shift at δ 7.040 ppm. The presence of one bromine atom in the **6** has been confirmed from its mass spectra. The compound shows two fragments 254.8 is M-1 (when Br is ^{79}Br) and 256.9 is M+1 (when Br is ^{81}Br) with equal intensity.

Minimum binding energy of 5-bromo-1-benzofuran-2-carbohydrazide: It is the structure of 5-bromo-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, brown ball indicates bromine and gray colour ball is carbon. The minimum binding energy of the molecule is calculated from the HOMO-LUMO energy gap (from density of states) *i.e.* band gap or given directly.

Density of states (DOS) of 5-bromo-1-benzofuran-2-carbohydrazide: The DFT calculation has been performed for the determination of electronic density of states of molecule 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 5-bromo-1-benzofuran-2-carbohydrazide shows conduction band indicates the molecule has electrical conductivity property. The density of states 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.

Electron density of 5-bromo-1-benzofuran-2-carbohydrazide: The molecule containing one -CO–NH-NH_2 group which will be confirmed by its NMR spectrum, the –NH_2 protons showing broad singlet at 7.040 ppm while –NH proton shows singlet at 12.224 and 8.436 ppm. The –CONH- proton shows two signals because of tautomerism effect. The amide proton of hydrazide linkage [-CO-NH-N<] shows two chemical shifts, one at δ 12.224 ppm (due to C(OH)=N-N) and second at δ 8.436 ppm (is due to CO-NH-N). The presence of –$NHNH_2$ group can also be explain from FT-IR spectra of molecule. The strong absorption in FT-IR spectra of molecule at 3322 and 3183 cm^{-1} is due to symmetric stretching vibrations of N-H bond of –NH-NH_2 group of hydrazide. The carbonyl group of amide (-CO-NH-) linkage shows strong absorption at 1668 cm^{-1} while aromatic double bonds show strong absorption bands in 1617 and 1578 cm^{-1}. The 5-bromo-1-benzofuran-2-carbohydrazide molecule containing two oxygen atoms, one is member of benzofuran ring and another is hydrazide group oxygen, both having different chemical environment and therefore different electron density around them. The hydrazide oxygen (-0.461e) having higher electron density than benzofuran ring oxygen (-0.407e) because of +R effect of NH group (the lone pair of nitrogen is in conjugation with carbonyl π-bond and also tautomerism). The nitrogen of NH_2 group (-0.304e) having higher electron density than NH (-0.260e) of –CO-NH- group because of tautomerism effect. This also explains higher electron density of hydrazide oxygen atom. The lower electron density of hydrogen atom of aromatic rings can also explain from its NMR spectra. The aromatic proton present at C-4 position (C_4-H) is highly deshielded and shows chemical shift at δ 8.091 ppm. The C_6-H and C_7-H are also deshielded and shows multiplet at δ 7.706 ppm.

Reactivity of 5-bromo-1-benzofuran-2-carbohydrazide against nucleophile and electrophile: 5-Bromo-1-benzofuran-2-carbohydrazide has benzofuran ring which contains benzofuran skeleton with –$CONHNH_2$ (hydrazide) group at C_2 and bromine atom at C_5 position. The five membered rings is more electron rich centre than fused benzene ring. The electron density present on nitrogen atom (-NH_2 group) (-0.304 e) and oxygen atom (-0.461 e) of hydrazide are easily donated towards electrophile. The electron density present over the nitrogen atom (-NH- group) is less as compared to electron density of nitrogen atom present in the form of -NH_2 group is lower indicates tautomerism effect. Therefore –NH_2 group (nucleophilic in nature) can easily donate electron density towards electrophile while amide –NH- is neutral. The electron density of five membered ring oxygen is less than amide indicates that electron density of furan-oxygen is part of aromatic sextet while lone pair of nitrogen or π-electron density is of carbonyl group is shifted towards hydrazide-oxygen atom.

The attack of nucleophile takes place at C_2 carbon atom of benzofuran ring with ring opening reaction. The nucleophile reacts at carbonyl carbon atom forming corresponding addition reaction products. The nucleophile also shows substitution reaction at C_5 carbon atom with displacement of bromine atom.

The attack of electrophile takes place preferentially on the nitrogen atom of –NH_2 group forming condensed or substituted product. The electrophile also shows attack at C_3 carbon atom forming substitution product. The electrophile also shows attack at C_4 and C_6 depending on reaction condition forming substitution product.

Molecular docking: 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 indicate 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) 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 5-bromo-1-benzofuran-2-carbohydrazide are shown in Table-1.

The docking score table indicate that 3-bromo-1-benzofuran-2-carbohydrazide is more active against 3LAU (docking score -7.194) and 4BBG (docking score -6.419) while is less active against 3MK2 (docking score -3.640). Glide site 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 1RJB (-0.260), 1TE6 (-0.142), 1VOM (-0.096) and 3V3M (-0.096) but these are totally absent in 3LAU. The hydrazide shows higher polar interactions with 1TE6 and 1RJB 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 5-bromo-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 3LAU > 4BBG > 1VOM > 1RJB > 3FDN > 1TE6 > 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 decreasing order of the glide energy of 5-bromo-1-benzo-furan-2-carbohydrazide with PBDs in the decreasing order as 3LAU > 1VOM > 4BBG > 1RJB > 1TE6 > 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. 5-Bromo-1-benzofuran-2-carbohydrazide shows stronger such interaction with same amino acids of 1TE6 and 3MK2 indicates that orientation of the molecule does not change during docking in major extend

TABLE-1
DOCKING SCORE AND OTHER DIFFERENT DOCKING PROPERTIES OF 5-BROMO-1-BENZOFURAN-2-CARBOHYDRAZIDE

Description	Proteins							
	1RJB	3FDN	3LAU	4BBG	3V3M	1TE6	1VOM	3MK2
Potential Energy OPLS 2005 = 38.979								
RMS Derivative OPLS 2005 = 0.007/0.005								
Glide lignum	2	2	2	2	2	1	1	1
Docking score	-5.145	-4.089	**-7.194**	-6.419	-5.556	-3.858	-5.09	**-3.640**
Glide ligand efficiency	-0.368	-0.292	**-0.514**	-0.458	-0.397	-0.276	-0.364	**-0.260**
Glide ligand efficiency sa	-0.886	-0.704	**-1.238**	-1.105	-0.956	-0.664	-0.877	**-0.627**
Glide ligand efficiency In	-1.414	-1.124	**-1.977**	-1.764	-1.527	-1.060	-1.400	**-1.000**
Glide score	-5.154	-4.089	**-7.194**	-6.419	-5.556	-3.858	-5.096	**-3.640**
Glide lipo	-0.965	-0.442	**-2.468**	-2.114	-0.913	**0.0**	-0.786	-0.235
Glide H bond	-0.392	-0.160	-0.608	-0.433	**-0.727**	**-0.016**	-0.208	-0.270
Glide metal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glide rewards	-1.852	-1.753	**-2.235**	-1.748	-1.670	-1.670	-1.859	**-1.670**
Glide evdw	-24.223	-22.475	**-29.153**	-25.874	-18.674	-20.539	-25.386	**-16.553**
Glide ecoul	-4.664	-3.576	**-2.835**	-5.027	**-8.106**	-6.686	-5.876	-4.139
Glide erotb	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glide esite	**-0.260**	-0.074	**0.0**	-0.077	-0.096	-0.142	-0.096	-0.017
Glide emodel	-37.654	-33.023	-44.908	**-45.322**	-36.935	-34.398	-40.853	**-26.195**
Glide energy	-28.888	-26.051	**-31.988**	-30.901	-26.777	-27.225	-31.262	**-20.692**
Glide einternal	0.187	0.225	1.509	0.138	0.239	**0.117**	1.080	0.598
Glide confnum	1	1	1	1	1	1	1	1
Glide posenum	106	**25**	127	67	**369**	250	306	98
XP GScore	-5.154	-4.089	**-7.194**	-6.419	-5.556	-3.858	-5.096	**-3.640**
H-Bond	02	01	02	02	04	01	02	02
π-π/π-Cation interactions	00	00	00	00	00	04	00	01

by the changing of skeleton or functional group. But such type of interaction is weaker in 1RJB, 3LAU, 3FDN, 4BBG and 3V3M whereas is absent with 1VOM.

The above docking images [electrostatic interactions (pink)] show 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. 4BBG, 1TE6 and 3MK2 PDBs shows maximum number of such type of interactions with 5-bromo-1-benzofuran-2-carbohydrazide while these interactions are weaker with 1RJB, 3FDN, 3LAU, 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 5-bromo-1-benzofuran-2-carbohydrazide and amino acid residue at the docking site in the order of 3LAU > 4BBG > 1RJB > 3V3M > 1VOM > 3FDN > 3MK2 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). 5-Bromo-1-benzofuran-2-carbohydrazide shows the π-π interactions with 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 inter-actions 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 weaker hydrophobic interaction while that of exist strongly with 3LAU and 3FDN with 5-bromo-1-benzofuran-2-carbohydrazide which can be explained from their docking score.

The antimycobacterium activity of hydrazide assessed against *M. tuberculosis* using micro plate Alamar Blue assay (MABA). The standard or reference used for the anti-tuberculosis study are pyrazinamide, streptomycin and ciprofloxacin and their standard values for the antituberculosis test which was performed here are -3.125, 6.25 and 3.125 µg/mL, respectively while that of target compound is 25 µg/mL.

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