Research Article

Synthesis, SAR Study, Molecular Docking, HPLC Method Development and Anti-TB study of Novel 3-Chloro-N-{[7-mehtoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline

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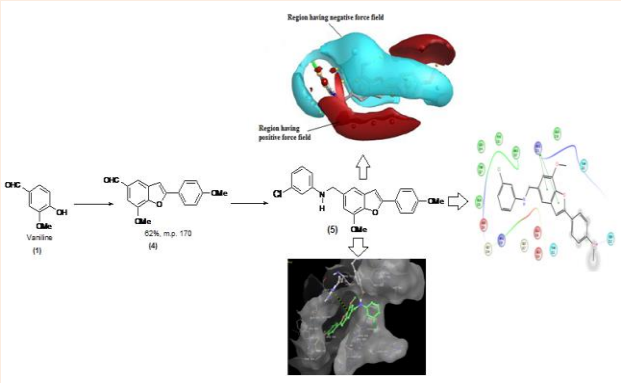
Abstract

7-methoxy-2-[4-(methoxy)phenyl)-l-benzofuran-5-carboxaldehyde was synthesized from Vanillin through series of reactions. The crude product was purified by using column chromatography and subjected to reductive amination and the final product 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline. The amine was purified by column chromatography and characterized by NMR and Mass spectroscopy. It was docked with different 10 PDBs, showing higher docking score with 4ASE and 1RJB. In SAR study molecule shows 56.9% matching of surface properties with ciprofloxacin. HPLC technique involving UV detection was developed and validated for the determination and quantification of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-benzofuran-5-yl]methyl} aniline.



Keywords: SAR study, Mannich reaction, Intramolecular Wittig reaction, Reductive amination, Molecular docking, HPLC

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1. Introduction

In view of the importance of 2-aryl/alkylbenzofuran-5-carboxaldehyde [1] for the synthesis of naturally occurring and synthetic compounds, a number of methods have been reported for their synthesis, some of them are shown in above. 2-Arylbenzofuran-5-carboxaldehyde has been synthesized [1-4] by using organocopper approach. Recently Parthasarathy and Panda have achieved [5] the synthesis of 2-(3-formyl-2,4,6-trimethoxyphenyl)-5-formylbenzofuran using similar approach. 7-Methoxy-2-phenylbenzofuran-5-carboxaldehyde [6] have been synthesized by coupling 5-iodo vanillin with phenylacetylene in presence of Pd-catalyst and copper (I) iodide as co-catalyst.2-Aryl-5-formylbenzofuran [7] derivatives has been synthesized from 2-hydroxyacetophenone by the series of three reaction through oxime intermediate. 2-Cyclopentyl-7-methoxybenzofuran-4-carboxaldehyde [8] has been achieved from isovanillin. The synthesis of 7-methoxy-2-methylbenzofuran-5-carboxaldehyde is useful intermediate for the synthesis of naturally occurring cuamarine has been achieved [9] from vanillin. 2-Alky/2-arylbenzofuran carboaldehydes and their derivatives possessing a wide range of biological activities such as cytotoxic activities [10] against P-388, KB-16, A549, and HT-29 cancer cell lines in vitro, phosphodistearase-IV inhibitor [11], bactericidal activity and fungicidal activity [12]. Benzofuran derivatives are also used for the treatment of bone diseases such as osteoporosis and hyperparathyroidism [13]. Several 2-arylbenzofuran derivatives are shows 5α-reductase inhibiting activity and are used for the prevention and treatment prostatic cancer, prostatic hypertrophy, hirtotism, male pattern baldness, acne, and seborrhea [14].

The transformation of amines or ammonium salts and carbonyl compounds into higher amines is an important method in organic synthesis because of their versatile utility as intermediates for synthesis of pharmaceuticals [15]

and agrochemicals [16]. Generally two reductive amination methods are used, one which is termed as direct reaction which is the conversion of carbonyl functionality to an amine by direct treating a mixture of carbonyl compound and the amine in presence of suitable reducing agents. Another method is stepwise or indirect reactions which involve the conversion of amine from the reduction of imine derivative which is isolated in separate step. The effective reducing methods used for these conversions are catalytic hydrogenation, metal hydride reducing agents, borohydrides and their derivatives, hydrogenated exchange resins, etc have been reported. The catalytic hydrogenation is incompatible with compounds containing carbon-carbon double or triple bonds and other reducible functional groups such as nitro, cyano, furyl, etc groups [17]. Cyanoborohydride and tin hydride reagents are highly toxic and generate toxic byproducts such as HCN, NaCN or organotin compounds [18] during workup. Other hydrides such as $ZnBH_4$, nickel hydride, and PHMS-Ti$(OiPr)_4$ [19] may not be suitable for use of chemoselective reduction of imines having ketone, ester, amide and nitro groups, since these reagents can reduce those functional groups.

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 [20]. 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 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 [20], 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 [22], gene technology, and high-throughput tests [23,24] has shifted the focus, and poor absorption, distribution, metabolism, and excretion (ADME) properties of new drugs captured more attention [25].

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 [27], 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.

Tuberculosis (TB) is a disease of antiquity which is thought to have evolved sometime between the seventh and sixth millennia BC. Current estimates suggest that one third of the world's population are infected resulting in some 2 million deaths per year**.** The introduction of the first drugs for TB treatment some 50 years ago - streptomycin, para-aminosalicylic acid, isoniazid - led to optimism that the disease could be controlled if not eradicated [26]. In conjunction with the spread of HIV infection, tuberculosis is today amongst the worldwide health threats. As resistant strains of Mycobacterium tuberculosis have slowly emerged, treatment failure is too often a fact, especially in countries lacking the necessary health care organization to provide the long and costly treatment adapted to patients. Because of lack of treatment or lack of adapted treatment, at least two million people will die of tuberculosis this year. At the bench level, many M. tuberculosis biochemical transformations have been identified as potential targets for original antituberculosis treatment today. In view of this wealth, it seems, to our probably biased chemist point of view, that the current limiting factor at our level is not anymore the availability of new biochemical targets but may well be the access to original (i.e., untested) chemical compounds.

Chromatographic methods [27] are commonly used for the quantitative and qualitative analysis of raw materials, drug substances, drug products, compounds in biological fluids, etc. The objective of a test method is to generate reliable and accurate data regardless of whether it is for acceptance, release, stability or pharmacokinetics study. Validation of a method is the process by which a method is tested by the developer or user for reliability, accuracy and preciseness of its intended purpose. In modern HPLC systems, the actual separation of chemical components of sample takes place in the column, which is packed with chemically modified 3.5 – 10 μm (often silica) particles. The components of the mixture get separated according to their interactions with particles. Therefore a proper choice of a stationary and mobile phase is essential to reach a desired separation. The quantitative analysis of any compound is easy when its reference compound is available. But for those samples to which reference compound is not available, there is need for structural identification which can be done by chromatographic method. One of them is to run

measurements directly on-line using HPLC as the separation technique with UV detection or mass detection and monitoring at several wavelengths or mass.

2-Aryl-5-formyl-7-methoxybenzofuran can be synthesized by intramolecular Wittig reaction. It undergoes reductive amination to corresponding aniline derivative which is subjected for the further HPLC and biological analysis. Chromatography was carried out on Waters 2695 separation module HPLC system with Waters 2487 Dual wavelength Absorbance detector and Waters 2998 Photodiode Array Detector using Stainless Steel Column of dimension 15 cm x 4.6 mm packed with octadecylsilane bonded with silica (Make : Phenomenex Prodigy 5 µ ODS 3 100A column is suitable) using filtered and degassed mixture of Buffer (prepared by addition of 1 L purified water and 10 ml of acetic acid and 5 ml Triethylamine) and suitable mobile phases according to the solubility and nature of compounds. The mobile phase was flow of 10 ml/min and effluents was monitored at different wavelengths. The method was validated in terms of Specificity, Linearity and Range, Precision, Accuracy, Intermediated Precision, Solution stability, and Rubustness.

2. Experimental Work

The phosphonium salt **(3)** required for the synthesis of benzofuran **(4)** was synthesized from vanillin **(1)** by the sequence of reaction shown below. The starting compound dimethylaminomethyl **(2)** was synthesized from vanillin using the procedure developed[27] by Sinhababu and Borchardt (using dimethylamine and paraformaldehyde, Mannich reaction). The synthesis of phosphonium salt **(3)** can be achieved by sequence of three steps. A solution of amino compound **(2)** in acetic anhydride was refluxed for 24 hrs to give crude diacetate which is purified and react with HCl to give chloromethyl derivative. It is reacted with triphenylphosphine in dry benzene under reflux condition provided the phosphonium salt decomposed at 254^{0}C. The phosphonium salt **(3)** showed positive $FeCl_3$ coloration and positive 2,4-DNP test. It showed in IR (KBr) spectra; band at 3429, 2735 and 1715 cm^{-1} confirming phenolic –OH and –CHO group. The phosphonium salt **(3)** undergoes condensation[28] with 4-methoxybenzoyl chloride by refluxing in toluene in presence of triethylamine. The reaction was completed in 6 hrs. The crude product was purified by using column chromatography. The resulting 7-methoxy-2-[4-methoxyphenyl]-l-benzofuran-5-carboxaldehyde **(4)** was subjected to reductive amination and the final product 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline **(5)** was purified by column chromatography and characterized by NMR and Mass spectroscopy.

2.1. Synthesis of 4-phenoxybenzoyl chloride

To a stirred solution of 4-methoxybenzoic acid (1.20 g, 7.9 mmol) in 20 ml dichloromethane, add catalytic amount of N,N-dimethylformamide followed by oxylyl chloride (1.20 g, 9.4 mmol) at 0^{0}C. The completion of reaction was confirmed by monitoring TLC time to time. The reaction mixture was stirred for two hours at 0^{0}C. The solvent was evaporated under reduced pressure and the crude product obtained was used in further reaction.

2.2. Synthesis of 7-methoxy-2-[4-(methoxy)phenyl)-l-benzofuran-5-carboxaldehyde (4)

2.2.1. Preparation of 5-dimethylaminomethyl-4-hydroxy-3-methoxybenzaldehyde (2)

Vanillin **(1)** (76 g, 0.5 mol) was added to a well stirred solution of 37% aqueous paraformaldehyde (60 g, 0.75 mol) and 38% aqueous dimethylamine (90 g, 0.75 mol) in methanol (450 ml). The reaction mixture was refluxed for 30 min and the stirred at ambient temperature for 8 hrs. It was then cooled to 5^{0}C and the white granular solid formed was filtered, washed with ice cold acetone (50 ml) and dried under vaccum to give 5-dimethylaminomethyl-4-hydroxy-3-methoxybenzaldehyde **(2)** as a crystalline solid (92 g, 88%) m.p. 140-141^{0}C (lit 139-141^{0}C).

2.2.2. Preparation of (2-hydroxy-3-methoxy-5-formylbenzyl) triphenylphosphonium chloride (3)

A solution of 5-dimethylaminomethyl-4-hydroxy-3-methoxybenzaldehyde **(2)** (10 g, 0.047 mol) in acetic anhydride (50 g, 0.49 mol) was refluxed for 24 hrs. The volatile material was removed by distillation under reduced pressure. The residue crude acetate was cooled and add concentrated hydrochloric acid (45 ml, 0,53 mol) to it gradually. The reaction mixture was stirred at about 1.5 hours at ambient temperature. The chloromethyl derivative formed was extracted by using benzene (2 x 75 ml). The combined benzene layer was washed with water (2 x 50 ml), dried over Na_2SO_4 and evaporate to gives a solid. The solid compound dissolves in benzene (125 ml) and triphenylphosphine (8 g, 0.03 mol) was added to it. The reaction mixture was heated under reflux for 6 hrs. The solid separated was filtered, washed with hot benzene (25 ml) and dried to gives (2-hydroxy-3-methoxy-5-formylbenzyl) triphenylphosphonium chloride **(3)** (17.50 g, 79%), m.p. 254^{0}C (decomp).

2.2.3. Synthesis of 7-methoxy-2-[4-(methoxy)phenyl]-l-benzofuran-5-carboxaldehyde (4)

A mixture of (2-hydroxy-3-methoxy-5-formylbenzyl) triphenylphosphonium chloride (3.5 g, 7.5 mmol), 4-methoxybenzoyl chloride (1.34 g, 7.8 mmol) and triethylamine (1.6 g, 16 mmol), in toluene (70 ml) was heated under reflux for 6 hrs. The reaction mixture was cooled to room temperature and water (50 ml) was added to it. Separate the organic layer by separating funnel and wash it by water (2 x 50 ml) and dried over Na_2SO_4. Toluene was removed under reduced pressure and the residue obtained was purified by using silica column chromatography (100-200 mesh, Eluent 40% ethyl acetate in hexane), from the 7-methoxy-2-[4-(methoxy)phenyl]-l-benzofuran-5-carboxaldehyde (1.385 g, 62%) as a white crystalline solid, m.p. 170^0C.

NMR (300 MHz) (DMSO-D6; δ ppm): 10.01 (s, 1H, -CHO), 7.86 – 7.891 (m, 3H, 3 Aromatic protons), 7.42 (dd, 2H, Aromatic protons), 7.09 (dd, 2H, Aromatic protons), 4.046 (s, 3H, $-OCH_3$), 3.831 (s, 3H, $-OCH_3$).

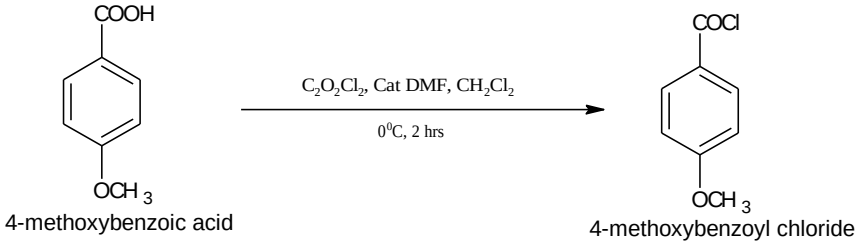
Mass Spectra: (M+1) = 283.

2.3. Synthesis of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline (5)

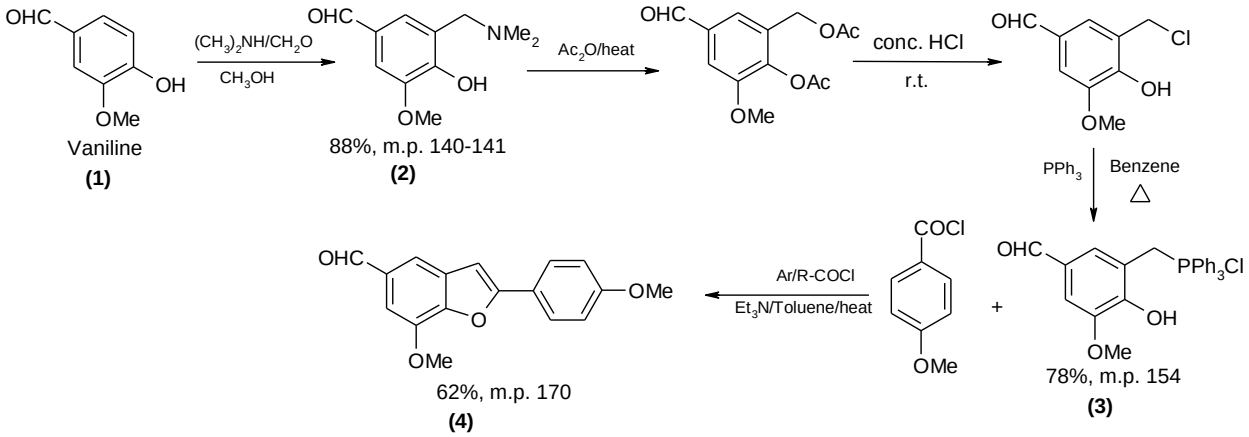
To a stirred solution of 7-methoxy-2-[4-(methoxy)phenyl)-l-benzofuran-5-carboxaldehyde **(4)** (130 mg, 0.48 mmol) and 3-chloroaniline (61 µl, 0.57 mmol) in ethylene dichloride (5 ml), add catalytic amount of acetic acid. Stirred the reaction for 2 hours at ambient temperature. The completion of reaction was confirmed by monitoring TLC time to time. After completion of reaction, add sodium triacetoxyborohydride (202 mg) and stirred the reaction mixture overnight at room temperature. Reaction mixture was quenched by saturated solution of sodium bicarbonate and product was extracted with (25ml x 2) ethyl acetate. Combined the organic layers and washed with brine (saturated NaCl), dried anhydrous sodium sulfate. Concentrate the organic layer and residue obtained was purified by column chromatography (silica, 100-200 mesh, Eluent 30% ethyl acetate in hexane) obtain 150 mg 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline (**5**) having yield 83% and m.p 127-28^0C.

NMR (300 MHz) ($CDCl_3$; δ ppm): 7.82 (s, 1H), 7.79 (s, 1H), 7.26 (s, 1H), 7.13 (m, 1H), 6.97 (dd, 2H), 6.83 (s, 1H), 6.76 (m, 1H), 6.65 (dd, 2H), 6.51 (m, 1H), 4.35 (s, 2H, $-CH_2-N$), 4.146 (bs, NH), 4.039 (s, 3H), 3.860 (s, 3H).

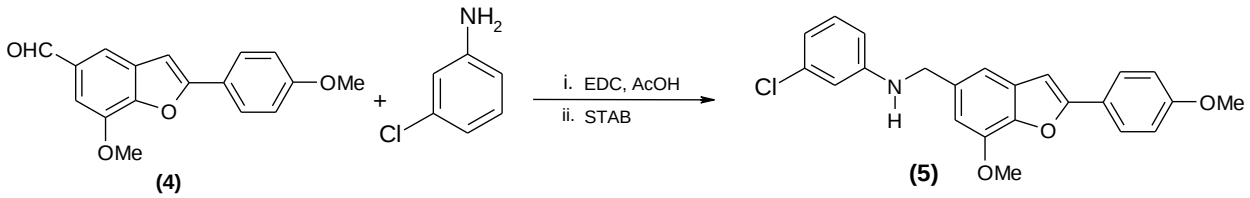
Mass Spectra: (M + 3) = 395.47, (M + 2) = 394.15, M^+ = 392.21.



Scheme 1 Synthesis of 4-methoxybenzoyl chloride



Scheme 2 Synthesis of 7-methoxy-2-[4-(methoxy)phenyl)-l-benzofuran-5-carboxaldehyde (4)



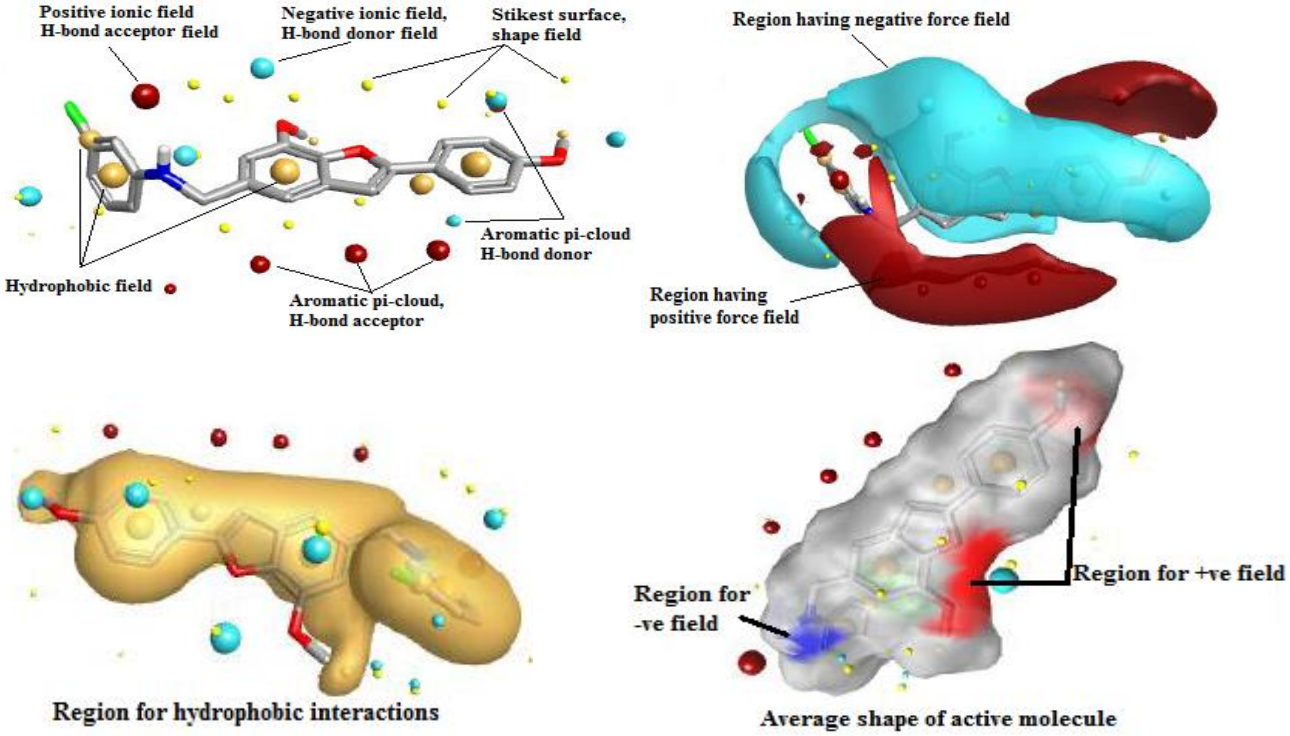
Scheme 3 Synthesis of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline (5)

3. Sar Study

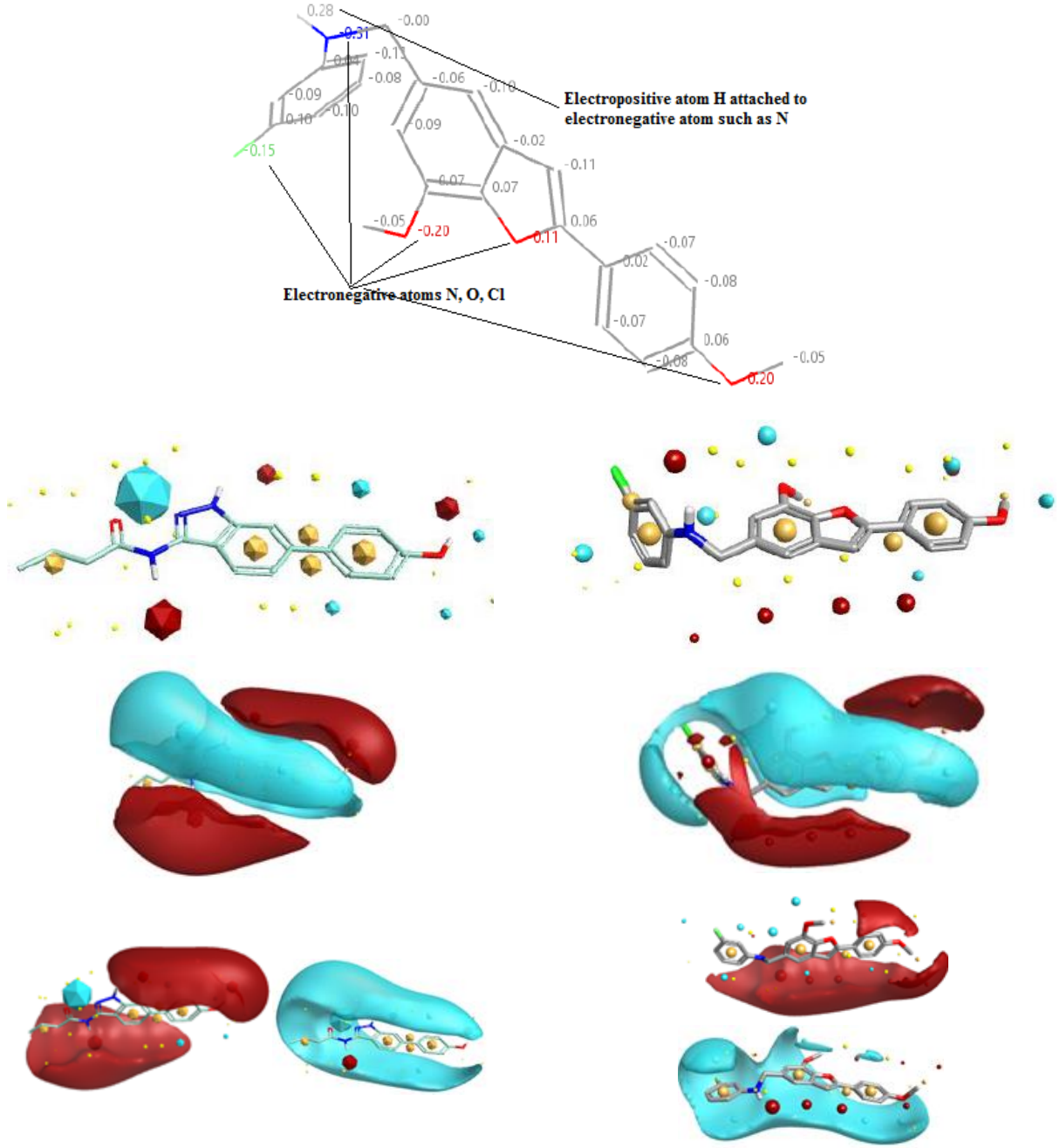
Forge (software) is a molecular design and SAR interpretation tool. It will generate detailed 3D models of binding and pharmacophores that will help to define the requirements of the protein of interest, aiding the synthetic chemist in the designing of new actives. It also gives rationale for the polarization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

Fore describe the molecules based on their molecular fields not on their structure. The interaction between a ligand and a protein involves electrostatic fields and the surface properties (e.g. H-bonding, hydrophobic surface, etc). If any two molecules binds to a common active sites tends to make a similar interactions with protein and hence have highly similar field properties. Accordingly, using these properties to describe molecules is a powerful tool for the medicinal chemist as it concentrates on the aspects of the molecules that are important for biological activity.

Forge condense the molecular field down to a set of points around the molecule termed field points. The field points are local extrema of the electrostatic, van der Waals and hydrophobic potential of the molecule. They have size/strength information associated with them i.e. all H-bond donor are not treated the same; some make stronger bonds than the others. The bigger fields points are generated by charged groups such as ammonium or carbonyl group or highly polar group. The colours of the field points indicates – Blue – Negative field points (like to interact with positives or H-bond donor present on protein); Red – Positive field points (like to interact with negatives or H-bond acceptors present on protein); Gold or Orange – Hydrophobic field points (describe the regions with high polarizability or hydrophobicity); Yellow – van der Waal field points (describe possible surface or rdW interactions). In general, ionic groups including those forming hydrogen bonding; give rise to the strongest electrostatic field. Aromatic groups encode both electrostatic and hydrophobic fields. Aliphatic groups give rise to hydrophobic and surface points but are essential electrostatically neutral.



Activity atlas of Forge actually performs three types of analysis: useful for quantitative information can be gained from 3D model. This model shows that what 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 (red or blue) 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 (yellow) contributions shows the regions where the active molecules is general make hydrophobic interactions with the target of interest.

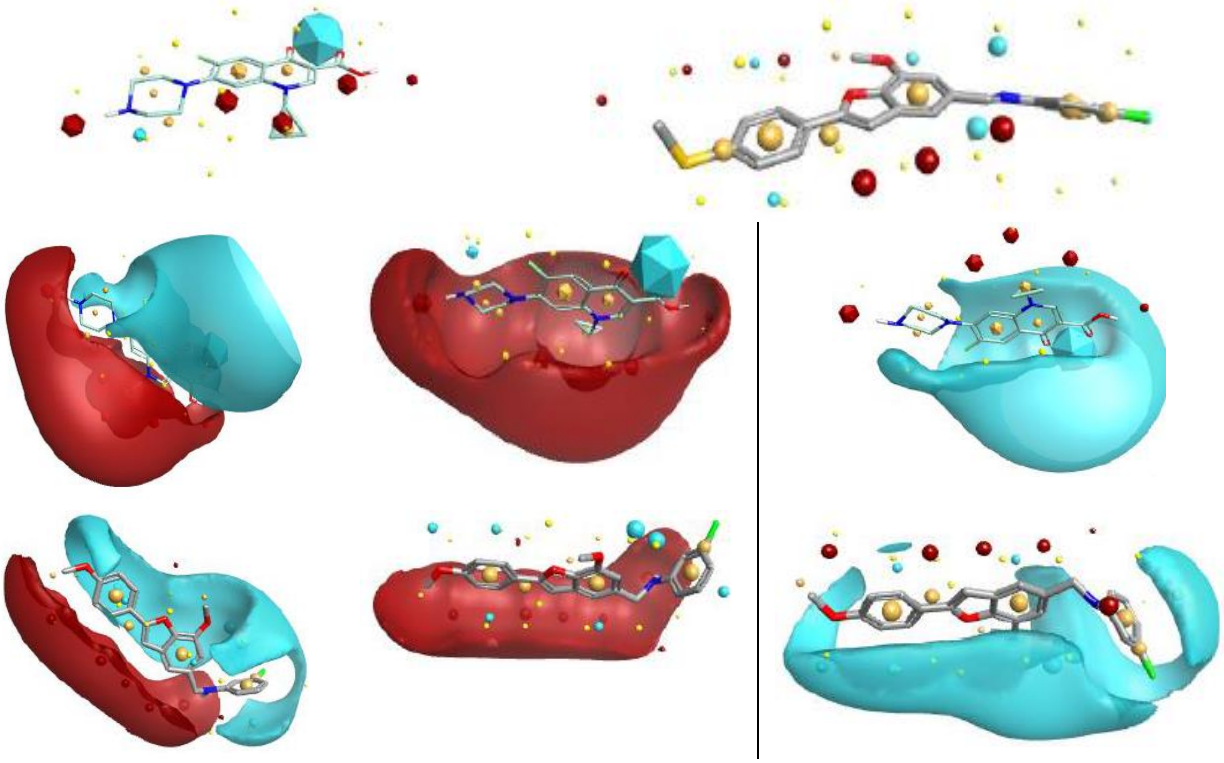


Comparison or structure similarity score with respect to molecular similarity: 0.687

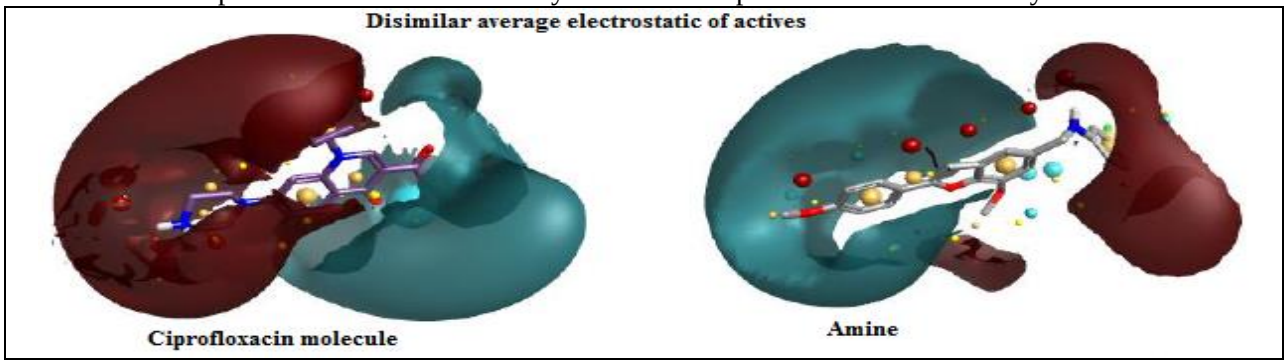
Figure 1 Comparison with N-[6-(4-hydroyphenyl)-1H-indazol-3-yl]-butanamide (docked with 3LAU)

The methoxy oxygen atom (-0.20) has more electron density than furan oxygen (-0.11) and lower than amine nitrogen atom (-0.31) therefore the strength of hydrogen bonding is N (amine) > O (methoxy) > O (furan). The chlorine atom (-0.15) has higher electron density than furan oxygen atom. The amine (H-N) hydrogen (0.28) is most electropositive atom in the molecule is able to form hydrogen bonding. The molecule does not have any highly polarized group or charged group or atom, therefore field points of molecule does contain any large force field points but it contain secondary amine nitrogen, oxygen in methoxy form and a part of benzofuran ring. The methoxy oxygen and nitrogen atom are act as H-bond bond donor while hydrogen atom attached to nitrogen 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, pi-cloud points are located above and below the plane of the rings and hydrophobic points are at its center.

The force field points of the N-[6-(4-hydroyphenyl)-1H-indazol-3-yl]-butanamide (docking ligand with 3LAU; considered as reference molecule) was compared with 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**). The molecular field similarity between them was found to be 0.687 which was quite good. It indicates that the aniline derivative (**5**) also get docked easily at the active site but not perfectly because of dissimilarity of electrostatic, hydrophobic and van der Waal field. Also, the average shape of aniline derivative (**5**) is very much higher than reference molecule which explains less docking score of the molecule.



Comparison or structure similarity score with respect to structure similarity: 0.569



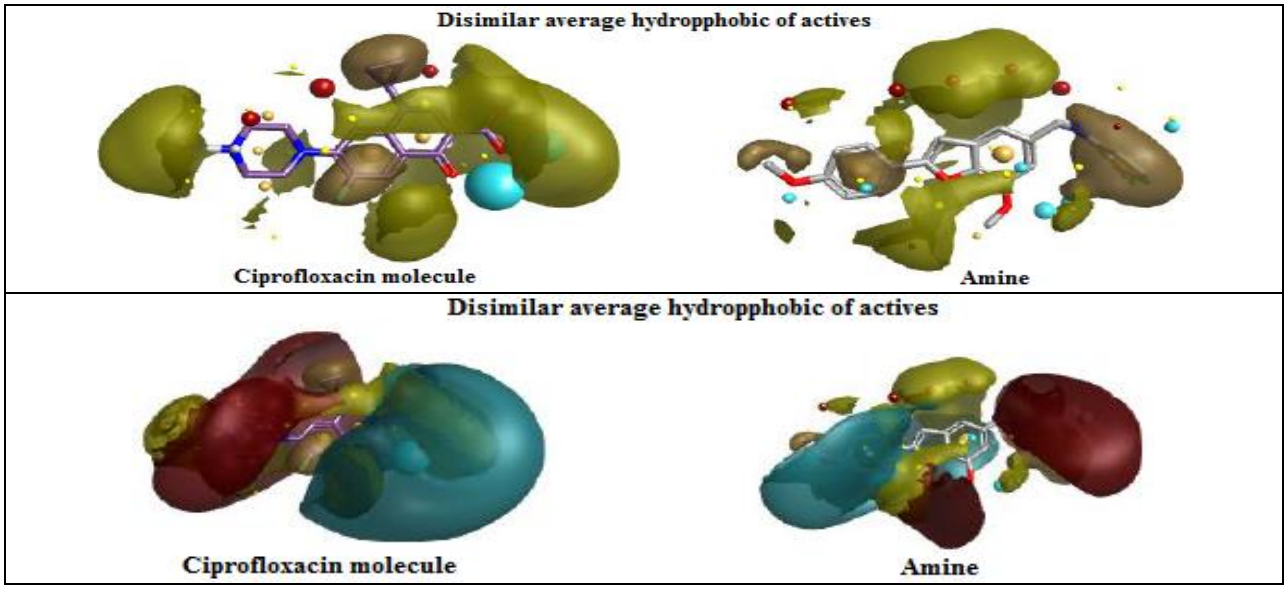


Figure 2 Comparison with Ciprofloxacin (Anti-TB drug)

The force field points of the ciprofloxacin (anti-TB; considered as reference molecule) was compared with 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**). The molecular field similarity between them was found to be 0.569 which was quite good. The reference molecule containing highly polarized group such as carboxylic acid and amino group which are exist in zwitter ion form at neutral conditions, increases it molecular electrostatic potential. Such highly ionized groups are not present in the aniline derivative (**5**); therefore molecular field similarity score is comparatively low. Most of the negative and positive ionic field strength of aniline derivative (**5**) is observed in opposite site than expected one.

4. Molecular 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 (**5**) [3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline] was constructed. 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 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.

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 has 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. The docking site score of 1VOM (**1.074**) and 1RJB (1.073) receptor/protein is higher while that of 3MK2 (**0.872**) is lowest is indicates that the 1VOM and 1RJB proteins PDB are more favorable 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 max in 3LAU 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 protein in the decreasing order of hydrophilic character and increasing order of hydrophobic character is – 4BBG > 3LAU > 1VOM > 3FDN > 4ASE > 1RJB > 3MK2 > 3V3M > 1TE6. This indicates that the ligands having more hydrophobic nature are binds easily 1TE6. The hydrogen bond donor/acceptor character ratio is higher in 3FDN (0.880) while lower in 3V3M (0.510) therefore the ligand contains more hydrogen bond acceptor atoms/groups are more tightly binds to 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 –3FDN > 3LAU > 4BBG > 1VOM > 1RJB > 4ASE > 3MK2 > 1TE6 > 3V3M.

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 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**) are shown in following table.

Table 1 Docking score and other different docking properties of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (5)

Description	**Proteins**								
	1RJB	**3FDN**	**3LAU**	**4ASE**	**3V3M**	**4BBG**	**1TE6**	**1VOM**	**3MK2**
Potential Energy OPLS 2005 = 98.474/99.448 (1VOM)									
RMS Derivative OPLS 2005 = 0.021/0.006 (1VOM)									
Glide lignum	3	3	3	3	3	3	3	3	3
Docking Score	-6.577	-5.328	-5.758	**-8.199**	-4.716	-5.684	-3.151	-6.220	-4.161
Glide Ligand efficiency	-0.235	-0.190	-0.206	**-0.293**	-0.168	-0.203	-0.104	-0.222	-0.149
Glide Ligand efficiency sa	-0.713	-0.578	-0.625	**-0.889**	-0.511	-0.616	-0.322	-0.675	-0.451
Glide Ligand efficiency In	-1.518	-1.230	-1.329	**-1893**	-1.089	-1.312	-0.727	-1.436	-0.961
Glide gscore	-6.577	-5.328	-5.758	**-8.199**	-4.716	-5.684	-3.151	-6.220	-4.161
glide lipo	-3.389	-2.397	-2.624	**-4.878**	-1.892	-2.221	-0.177	-3.427	-1.579
glide hbond	-0.231	-0.076	-0.245	0.0	-0.160	-0.354	-0.286	-0.184	**-0.348**
glide metal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
glide rewards	-0.724	-0.724	-0.852	**-1.378**	-0.724	-1.021	-0.724	-0.724	-0.724
Glide evdw	-42.790	-40.613	-38.124	**-46.968**	-34.214	-34.578	-30.698	-33.192	-25.937
Glide ecoul	-3.969	-4.030	-4.240	-0.507	-4.280	-5.754	-5.750	**-1.077**	-4.732
glide erotb	0.504	0.504	0.504	0.504	0.504	0.504	0.504	0.504	0.504
glide esite	-0.002	0.0	0.0	0.0	**-0.091**	0.0	-0.071	-0.001	-0.008
Glide emodel	-63.245	-55.313	-57.370	**-66.657**	-46.565	-51.910	-42.706	-55.206	-38.265
Glide energy	-46.758	-44.643	-42.364	**-47.625**	-38.494	-40.333	-36.448	-40.270	-30.669
Glide einternal	2.904	6.181	1.150	9.201	6.138	**9.65**	3.981	1.438	1.763
glide confnum	29	24	18	33	**34**	19	18	20	14
Glide posenum	131	272	133	264	265	345	**267**	214	45
XP GScore	-6.577	-5.328	-5.758	**-8.199**	-4.716	-5.684	-3.151	-6.220	-4.161
H-Bond	01	00	01	00	01	01	**03**	00	02
pi-pi /pi-cation interactions	01	01	03	01	00	02	**04**	00	02

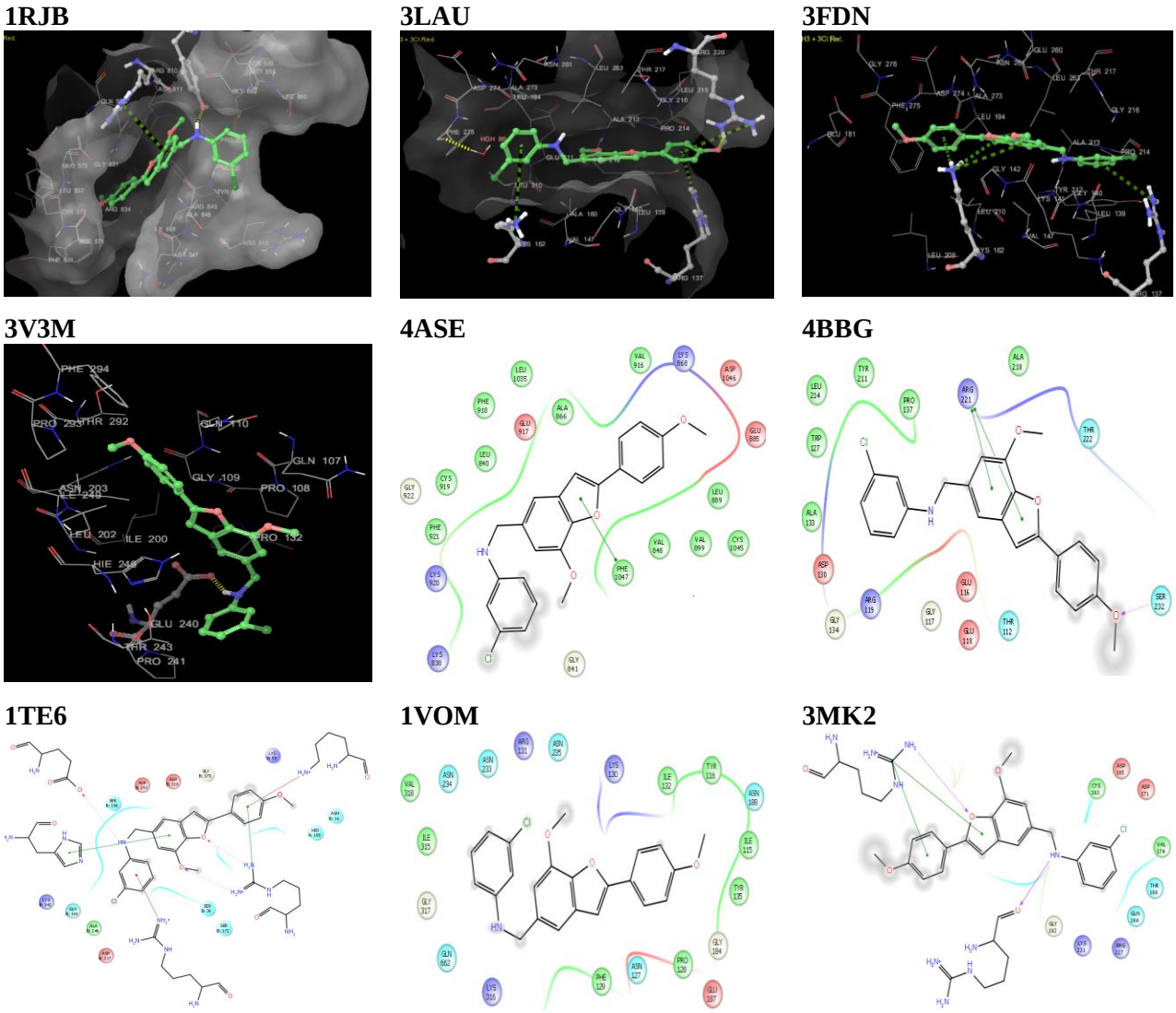


Figure 3 Docking images of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (5) with different PDBs

The docking score table indicate that 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**) is more active against 4ASE (docking score -8.199) and 1RJB (docking score -6.577) while is less active against 1TE6 (docking score -3.151). There are number of types of interactions observed between ligand and receptor such as hydrogen bonding, pi-pi interactions, ion-pi interactions, hydrophobic and hydrophilic interactions, ionic interactions, van der Waal interactions, etc along with steric interactions determine the docking score. Glide esite explains the polar interaction in the active site between ligand and amino acid residue at the docking site after recombination. The polar interactions between the aldehyde and amino acid residues of the protein are only observed in 3V3M (-0.091), 1TE6 (-0.071), 3MK2 (-0.008), 1RJB (-0.002) and 1VOM (-0.001) but these are totally absent in 3LAU, 3FDN, 4ASE, and 4BBG. The aldehyde shows higher polar interactions with 3V3M and 1TE6 proteins PDBs. The aniline derivative (**5**) have only one hydrogen atom attached to nitrogen atom which is capable of forming L (ligand)→P (protein) hydrogen bonding. It contains sp^3 hybridized oxygen and nitrogen atoms 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 substituted aniline (**5**). **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 Glide evdw of the interaction in decreasing order is as 4ASE > 1RJB > 3FDN > 3LAU > 4BBG > 3V3M > 1VOM > 1TE6 > 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 aniline-4ASE complex. This is due to higher surface area (both polar and non-polar) of 4ASE available for interaction with aniline (**5**). The aniline derivative (**5**) has higher glide energy during the interaction with PBDs in the decreasing order as 4ASE > 1RJB > 3FDN > 3LAU > 4BBG > 1VOM > 3V3M > 1TE6 > 3MK2.

The above docking images [**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 is capable of forming such type of interactions in neutral or protonated forms. Substituted aniline (**5**) shows stronger such interaction with same amino acids of 1TE6, 3MK2, 1RJB, 3FDN, 4ASE, 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 4BBG whereas is absent with 3V3M.

The above docking images [**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 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, 1TE6, 4ASE and 3FDN PDBs shows maximum number of such type of interactions with aldehyde while these interactions are weaker with 3LAU, 1RJB, 3MK2, 1VOM, and 3V3M shows minimum number of such interactions.

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 aniline derivative (**5**) and amino acid residue at the docking site in the order of 4ASE > 1VOM > 1RJB > 3LAU > 3FDN > 4BBG > 3V3M > 3MK2 > 1TE6 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 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 aniline derivative (**5**) 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 it. 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 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 3V3M and 1VOM shows weak interaction with 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**) which can be explained by their low docking score. It does show any kind of docking interactions with 2BOU.

5. 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. 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 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline (**5**) 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.

6. Anti-Mycobacterium Activity

Tuberculosis (TB) is a lung infection caused mainly by Mycobacterium tuberculosis (M. tuberculosis [MTB]). It is considered to be one of the most contagious and deadly diseases and is a major threat for public health. 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 structures of the developing tuberculosis lesions may effectively define the metabolic status of their bacterial inhabitants, and it has been speculated that at least four significant subpopulations of bacteria exist for which different drugs could be efficacious. These might include active growers that may be killed by isoniazid (INH), those with sporadic metabolic bursts that could be killed by rifampicin (RIF), a population with low metabolic activity that is considered likely to experience acidic surroundings and hypoxia that may be susceptible to pyrazinamide (PZA), and finally dormant bacilli that are not killed by any current agents. These complex phenomena are poorly understood and add a further barrier to the already formidable challenges associated with drug development and treatment of the disease. Despite its superlative early bactericidal activity (EBA), INH is no more effective than other drugs after this period and RIF becomes the most significant bactericidal drug. Its activity against sporadically active M. tuberculosis is crucial for preventing relapses, and INH then serves to limit the emergence of RIF resistance. Because of its apparent ability to kill a subset of bacteria not killed by the other drugs, supposed sporadically active organisms subject to an hypoxic and possibly acidic environment, PZA represents an important component of combination therapy. Phenazinamine derivatives closely related to the anti-leprosy drug clofazimine, CFM 42 [(E)-N,5-bis(4-chlorophenyl)-3-(isopropylimino)-3,5-dihydrophenazin-2-amine, etc are active against a range clinical *M. tbc.* isolated including MDR strains [28]. Clofazimine and its derivatives stimulate intracellular synthesis of hydrogen peroxide which inhibits the multiplication of the cells because of binding to the guanine in DNA. The biological activity like antibacterial and antifungal of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline (**5**) will be studied using the conventional methods reported in the literature. The anti Mycobacterial 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 proportional and BACTEC radiometric method. Briefly, 200µl 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 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% between 80% was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

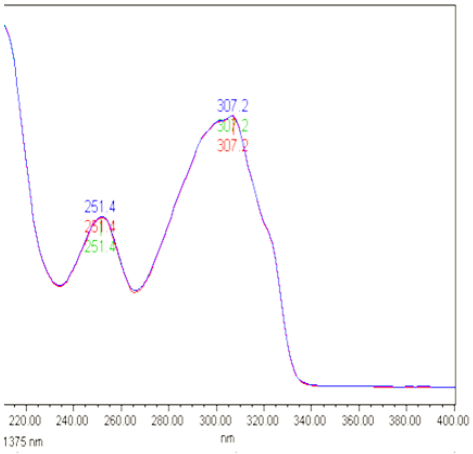
Strain used for anti-TB study is *M. Tuberculosis* (H37 RV strain): ATCC No – 27294. The standard or reference used for the anti-TB study are pyrazinamide, streptomycin and ciprofloxacin and their standard values for the anti-TB test which was performed her are - 3.125 µg/ml, 6.25 µg/ml and 3.125 µg/ml respectively while that of target compound is 25 µg/ml.

7. Hplc Analysis

Analytical method used for Assay of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline **(5)** (in % w/w) on as is basis by using High performance liquid chromatography technique is validated. Validation is carried out on Waters Alliance 2695 (quaternary gradient with VWD) separation module HPLC system with Water 2487 Dual wavelength Absorbance detector. The column used was a stainless steel column of dimensations 15 cm x 4.6 mm, packed with octadecylsilane bonded to porous silica (Make : Water X-terra column is suitable). Detector used was ultraviolet [UV and photodiode array (PDA) detector]. The method details are as follows:

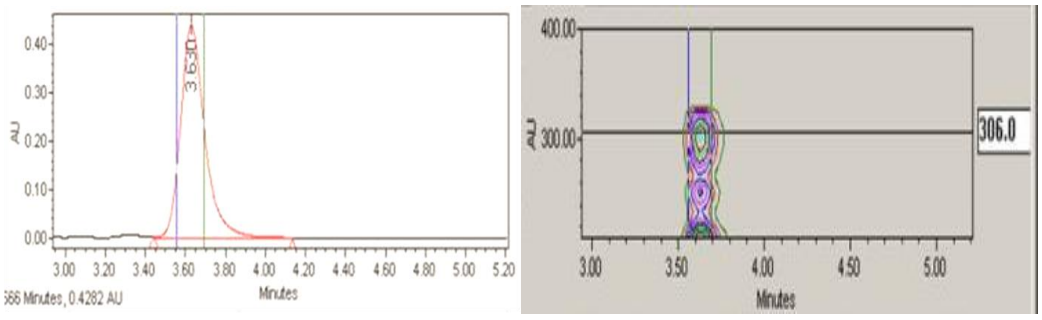
Flow rate: 1.0 ml/min; Detector wavelength: 306 nm; Column Oven temperature 32^0C; Injection Volume: 10 µl.
Mobile phase: Methanol and Acetonitrile (1 : 1) proportion and add 0.2 part of Water; Diluent: Methanol.
Apart from above mentioned method, several other methods are also tried out but due to longer Retention time and co-elution the above method is selective and specific.

Peak purity of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline, λ_{max} 307.2 nm



Sr. No.	Image	Retention time (min)	Info	Max. Absorbance (au)	Baseline Correction	Source Name	Lambda Max (nm)
1	Blue	3.558	Extracted	0.19745	Ok	Cl-Derivative	210.1
2	Red	3.630	Peak 1	0.59198	Ok	Cl-Derivative	210.1
3	green	3.692	Extracted	0.31414	Ok	Cl-Derivative	210.1

Figure 4 Excitation spectra of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline



Retention Time (min)	Purity Angle	Purity Threshold	Area (µV*sec)	% Area	Hight (µV)	Int Type	Peak Type
3.630	0.082	0.249	3690167	100	440183	VB	Cl-derivative

Figure 5 Peak purity of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl}aniline, λ_{max} 307.2 nm

8. Result and Discussion:

The force field points of the ciprofloxacin (anti-TB; considered as reference molecule) was compared with 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**). The molecular field similarity between them was found to be 0.569 which was quite good. The docking score table indicate that 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl]methyl} aniline (**5**) is more active against 4ASE (docking score -8.199) and 1RJB (docking score -6.577) while is less active against 1TE6 (docking score -3.151).

We have devised a convenient method for the synthesis of synthetically useful 4-(methoxy)phenyl-7-methoxybenzofuran-5-carboxaldehyde **(4)** from the easily available vanillin **(1)** through 5-methylaminomethyl-4-hydroxy-3-methoxybenzaldehyde **(2)**.

In PMR (DMSO-D6) spectrum of compound **(4)** exhibited a singlet at 4.046 and 3.831 which showed the presence of two $–OCH_3$ group; first of benzofuran nucleus and second of 4-methoxypheny group attached at 2-position. The signal at 10.018 (singlet) indicate the presence of –CHO group. The mass spectrum gives 100% abidance at 283.05 is the signal due to (M + 1) fragment.

In PMR ($CDCl_3$) spectrum of compound **(5)** exhibited a singlet at 4.039 and 3.860 which showed the presence of two $–OCH_3$ group; first of benzofuran nucleus and second of 4-methoxypheny group attached at 2-position. The new signal observed at 4.146 (broad, singlet) indicate the presence of –NH- group. Also the another new signal is observed at 4.350 (singlet) for two protons indicate the linkage $–CH_2$-N which is observed by the reductive amination of aldehyde and aniline. The mass spectrum gives 100 % abidance at 392.21 is the base peak (M^+). The new peaks at 394.15 (50%) and 395.47 (30%) is due to (M + 2) and (M + 3) fragments indicate the presence of one chlorine atom in the compound **(5)**. The minimum inhibitory concentration for anti-TB activity is 25 µg/ml.

The HPLC analysis of 3-Chloro-N-{[7-methoxy-2-(4-methoxyphenyl)-1-benzofuran-5-yl] methyl}aniline (**5**) shows that the compound has λ_{max} 307.2 nm with retention time 3.630 with % peak area 100% in methanol and methanol:acetonitrile:water as mobile phase.

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