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Research article

Design synthesis and biological evaluation of thiophene 2- pentafluoro benzamide derivatives as antitubercular agent

Balakrishnan Shanthakumar, Venkatesan Saravanan, Bharath Kumar Chagaleti, Muthu Kumaradoss Kathiravan*

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRMIST, Kattankulathur Chengalpattu district, Tamil nadu, India

ABSTRACT

Tuberculosis, a major contagious air-borne disease, killing millions in a year. Many of the existing anti-tubercular drugs have acquired bacterial resistance. Though they are highly bactericidal but, later on due to several factors like poor patient compliance, incomplete therapy, etc. leading to treatment relapse. This enables researcher to focus on identifying new leads utilizing structure-based drug design which plays a crucial role in drug development. The mycolic acid synthesized by Pks 13 is identified as a perfect target and hence a series of molecules were designed and the best fit ligands with a least energy were picked and analysed for *Insilco* bioactivity and drug likeness. Five best docked Molecules satisfied the Lipinski rule of five, namely the number of hydrogen bond donors, acceptors, log P, total polar surface area and the number of rotatable bonds. The bioactivity was found to be moderate to excellent against the receptor. The target molecules were synthesised *via* amino esters to yield pentafluorinated thiophene derivatives. Among the series, molecule 12 was found to be more potent when tested for antitubercular activity by Microplate Alomar blue assay technique.

Keywords: Amino thiophene esters, Pentafluoro phenyl amide, Structure based drug design, Antitubercular activity, Polyketide synthase 13 thioesters

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Correspondence: Muthu Kumaradoss Kathiravan ✉ mkkathiravan.srmist@gmail.com, **Orcid Id**: 0000-0001-8357-2730

Department of Pharmaceutical Chemistry, SRM College of Pharmacy, SRMIST, Kattankulathur Chengalpattu district, Tamil nadu, India.

INTRODUCTION

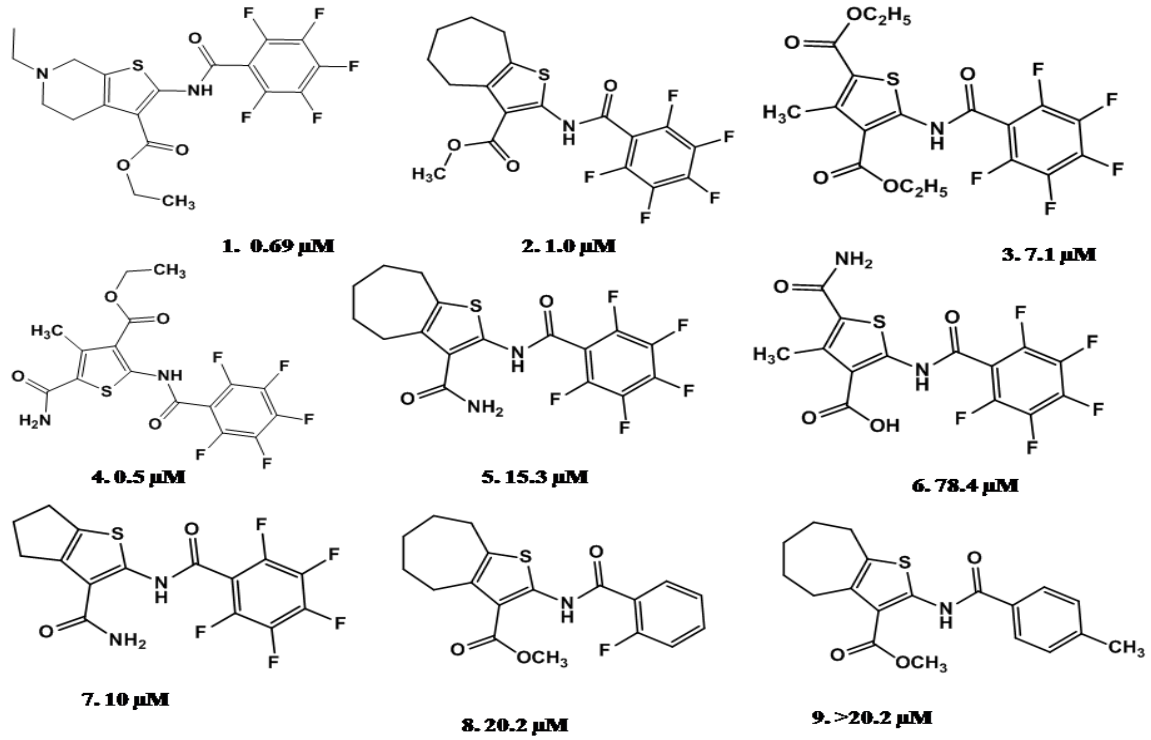
Tuberculosis is the most dreadful bacterial infection that is responsible for increase in death rates every year. The infection usually affects people under poverty, overcrowding environment that becomes the biggest health crisis. According to the WHO report in 2019, around 10 million were infected, 1.2 million deaths were reported among HIV negative people and in addition 208,000 death were found with HIV positive cases. Globally 10 million people were infected per year and probably 1.7 million are at risk of developing a disease [1-3]. Human immune virus and tuberculosis co-infection is a serious problem in countries where TB is endemic. The HIV encounters the T cell which are responsible for immunity and hence mycobacterium, takes this opportunity to attack and develop tuberculosis. It has been estimated that 26-31 times HIV positive people are more likely to develop an infection rather than HIV negative people. Every year, about 1.5 million deaths were accounted for this infection. However, drugs are available and fewer vaccines are under clinical trials, but the disease prevails because of the challenges faced due to microbial resistance. Several factors lie behind the mechanism of resistance to the existing drugs and a failure in therapy is due to both intrinsic and extrinsic mechanism [4,5]. The intrinsic defines a high level of antibiotic resistance that takes place naturally and extrinsic is all about the novel acquired mutations. The mutations usually take place at drug sub optimal concentration and repeated exposure to numerous drug regimens randomly which in turn depends on different factors namely bacterial load, rate of mutations and its vigorousness. This led to alarming rise in the resistant forms of TB like MDR-TB, XDR-TB and TDR-TB has gained worldwide attention [6-8]. Identification of newer metabolic pathways and key components responsible for survival of the organism is the key to success for the identification of anti TB drug candidate. The *M. tuberculosis* cell wall envelope and its constituents were well analysed and identified that the mycolic acids were predominating and paves way for survival and resistance [9,10]. Nearly two key enzymes are involved in mycolic acid synthesis, out of which the polyketide synthase plays a vital role in condensation of two key intermediates of fatty acids which varies in length and finally condensed by the enzyme Pks 13 to form a long chain fatty acid called mycolic acid motiff responsible for virulence and pathogenicity [11,12]. Polyketide synthase 13 contains about five distinct domains and specific functions which are necessary however, the thioesterase domain is responsible for release and acyl transfer reactions that are essentially required for mycolic acid biosynthesis

[13-15]. This critical situation enables the research in tuberculosis to focus on identifying new leads with novel mechanism of action either to acts as solely or as a combined regimen to express its synergistic action in inhibiting the organism.

The current trends in drug discovery process eventually utilizes Computer aided drug design [16-20] whereas Structure based drug design, executes information from the target, where the molecules are designed based on the literature survey. Thiophene based heterocycles have been reported recently for antitubercular activity as small molecule inhibitors [21]. Regina Wilson et al (2013) developed antitubercular thiophenes which act on *M. tuberculosis* by an uncharacterized mechanism of action showing Pks 13 inhibition in mycolic acid biosynthesis Two molecules containing thiophene nucleus namely Molecule 2 and Molecule 4 showed excellent sterilizing action on *M. tb* and drug-resistant strains. However, Molecule 4 with amide and methyl substitution showed higher activity at 0.5 μM than Molecule 2 with cycloheptanone at 1.0 μM. The results revealed high-level inhibition of mycolic acid biosynthesis and an increase in levels of mycolic acid precursors. Molecules that possessed amide group were precisely found to have excellent Pks 13 inhibition, whereas amino esters derivatives prepared from cyclopentanones and cycloheptanones with different methyl esters showed decline in activity.

Sandeep Thanna et al (2013) synthesized a series of 2-amino – 4,5,6,7- tetrahydro thieno [2,3-c] pyridines versus M. tb strains that are sensitive and resistant towards the existing antitubercular drug (7) with MIC of 0.69 μM by microplate alamar blue assay method. From the investigation of thiophene cores, N-ethyl cyclohexanones with ethyl amino esters coupled with Penta fluor derivatives were found to have excellent Pks 13 inhibition, whereas the amino esters prepared from N-butyloxy carbonyl with benzoyl chloride and acetyl chloride result in a decrease in activity. The molecules synthesized using similar amino esters with 3, 5-trifluoromethyl benzoyl chloride does not exhibit satisfactory Pks 13 inhibition. While molecules with Pentafluoro phenyl group shows an increase in activity whereas 2-Fluoro phenylamide and 4- Fluoro phenylamide showed a decline in activity and the molecules were shown in Figure (1) along with their MIC values.

Figure 1: Thiophene containing molecules with antitubercular activity



1. *N*-(6-ethyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)-2,3,4,5,6-pentafluorobenzamide

2. methyl 2-(2,3,4,5,6-pentafluorobenzamido)-5,6,7,8-tetrahydro-4*H* cyclohepta[*b*]thiophene-3-carboxylate

3. diethyl 3-methyl-5-(2,3,4,5,6-pentafluorobenzamido) thiophene-2,4-dicarboxylate

4. 2-ethyl 4-methyl 3-carbamoyl-5-(2,3,4,5,6-pentafluorobenzamido) thiophene-2,4-dicarboxylate

5. 2-(2,3,4,5,6-pentafluorobenzamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxamide

6. 5-carbamoyl-4-methyl-2-(2,3,4,5,6-pentafluorobenzamido) thiophene-3-carboxylic acid

7. 2-(2,3,4,5,6-pentafluorobenzamido)-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxamide

8. methyl 2-(2-fluorobenzamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate

9. methyl 2-(4-methylbenzamido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate

Molecules with the top score in docking will be considered as better lead for designing new molecules which acts against Pks 13. Further, the Molecules were scrutinized for good binding affinity through docking. Molecular complexes with potential binding interactions and least energy was subjected to ADMET prediction. The molecules were also checked for various physicochemical properties and toxicities. Finally, the best fit Molecules were opted for synthesis and evaluation of anti-tubercular activity.

The present study deals with the identification of new inhibitors against Pks 13 under the SBDD that utilizes the available Molecules from existing literature led us to investigate by SAR and docking analysis to design more novel thiophene scaffolds. The designing is brought about by showing diverse substitutions, where cyclic ketones of both aliphatic / aromatic namely benzyl piperidinones, cyclohexanone, cyclopentanones, along with alkyl cyano ester like ethyl and methyl cyano acetate were employed resulting in library of Molecules. Then, it was computationally evaluated, synthesized selectively and screened them for antimycobacterial activity.

MATERIALS AND METHODS

Structure based drug design approach is applied for developing Pks13 inhibitors. Various computational procedures can be implemented during the stages of drug discovery. One of the best preliminary methods from SBDD is molecular docking as it exposes favourable amino acid interactions, with the ligand and its configuration expressed as ligand poses [22]. The interaction of the protein with the ligand is termed as binding efficiency and it is quantified as scoring function [23]. Molecules with the least global minimum and a low RMSD value will be selected for synthesis. About 250 molecules possessing thiophene scaffold were designed with different substitutions based on the literature survey. It was then assigned for docking interaction to identify the best molecules which exhibits higher binding affinity. Molecules with an excellent binding poses with resembling interactions are then viewed using Biovia molecular discovery.

Docking Studies

In order to analyse the binding efficiency, docking studies were performed using Autodock vina version 1.5.7[24]. The target receptor was extracted from the protein data bank, Pks 13 5V41. Proteins and ligands were prepared by adding polar hydrogens, aiding Kollman charges and Gasteiger charges respectively. Binding energies were calculated, and interactions were seen from best poses using discovery studio visualiser. Molecules showing best binding affinity and interactions with least energy were selected for synthesis [25].

Insilico ADMET Analysis

Protox II was used to access the various toxicities like carcinogenicity, mutagenicity, hepatotoxicity, for the selected Molecules. Then it was also analyzed for Lipinski's rule for drug likeness and bioactivity scores using Molinspiration [26].

Reactants and Reagents

All the reactants and solvents were commercially picked up from Merck, SISCO laboratories and Sigma Aldrich. Analytical grade solvents are used for recrystallization. Pre-coated TLC plates (alumina sheet) are used to carry out thin layer chromatography. Ultraviolet light is used for detecting the spots. Digital melting point apparatus was used to examine the Melting point for the synthesis of Molecules [27].

Synthesis Procedure

Step 1 Synthesis of thiophene 2-amino esters

To the weighed quantity of 0.1 mole of alpha methylene carbonyl Molecule, sulphur and alkyl cyano acetate were dissolved in 20 ml of ethanol were mixed and stirred together. To this well stirred mixture diethyl amine (0.125 mole) were added drop wise and stirring for about 3 hours at ambient temperature [28]. Upon completion, the reaction mixture was refrigerated overnight and on the next day the separated solid was filtered, washed with 20 ml of chilled aqueous methanol. The brown solid obtained was dissolved in dichloro methane and washed with saturated ammonium chloride 20 ml, deionized water 20 ml and saturated sodium chloride 20 ml. The dichloro methane layer was separated and anhydrous sodium sulphate was added and filtered. The solvent was evaporated to get the crude product and purified by recrystallization from cold methanol to obtain a yellow solid.

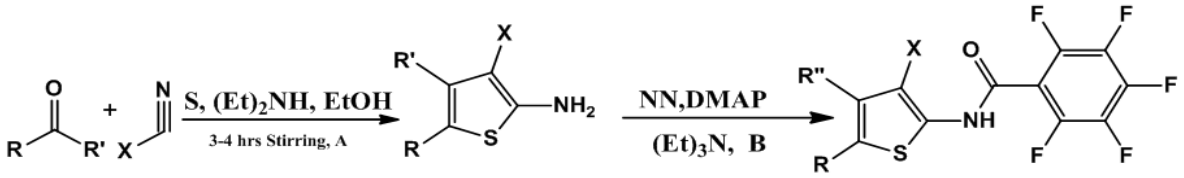
Step 2 Synthesis of penta fluoro derivatives from amino esters

Intermediate from step 1 was dissolved in dichloro methane and cooled to 0^0C and N,N-dimethyl amino pyridine and triethyl amine was added. To this pentafluoro benzoyl chloride was added drop wise and allowed to stir for 12 hours at room temperature. The solvent was evaporated to dryness to obtain a crude solid and purified by methanol to get pure pentafluoro derivatives [29] and the synthetic scheme was depicted in Figure (2). The progress of the reaction was monitored by TLC, melting point was analysed by Melting point apparatus by open capillary tubes and are uncorrected.

R, R' = Aryl/Cyclic Ketones, X = $CH_3CH_2OOCCH_2CN$-, CH_3OOCCH_2CN

A = Room temperature, B = Penta fluoro benzoyl chloride.

Figure 2: Synthetic Scheme



Characterisation

The synthesised compounds were characterised by IR, Mass and NMR spectroscopy. The infrared spectrum was recorded in a Perkin Elmer FT-IR spectrometer by KBr pellet technique. ^{1}H NMR spectrum was recorded in a FT mode NMR spectrometer and the mass spectrum was recorded in an electron ionisation- mass spectrometer (m/z).

Invitro Antituberculosis Activity

The antituberculosis activity for the synthesized molecules was tested with H37Rv by MABA technique. This method usually employs a chemical substance which is heat resistant, non-toxic exhibits a fine proportion and association with BACTEC radiometric technique. It consists of sterile 96 well plate, in which the peripheral micro plates were filled with 200µl of sterile water found completely devoid of ions. It was done essentially to circumvent drying of the medium while incubation.

The 96-well plate was filled with 100µl of Middle Brook 7H9 broth, and the Molecules were serially diluted on the plate. The drugs in the final samples ranged from 100 to 0.2 g/ml. Parafilm was used to cover and seal the plates, which then incubated at 37°C for five days. The plate was filled with 25µl of recently prepared 1:1 Alomar Blue reagent and 10% tween 80, which was incubated for 24 hours. A well indicating blue colour represents inhibiting the growth of bacteria and a pink colour represents the growth of bacteria [30]. The minimal drug dose which maintained to alter the blue colour towards pink, was considered as the minimum inhibitory concentration (MIC).Standard Strain used - ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain)- ATCC No- µg/ml, Ethambutol – 1.6 µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin – 0.8 µg/ml, Streptomycin- 0.8µg/ml.

RESULTS AND DISCUSSION

Docking Results

Thiophene containing Molecules were reported to have antitubercular activity as cited in the literature. From which an inhouse library of hundred and fifty Molecules containing thiophene nucleus were designed with the diverse in chemical substitutions. It includes alicyclic ketones, aryl alicyclic ketones with five to six membered rings in adjunct with a methyl and ethyl cyano ester. The molecules were analyzed for binding efficiency and results were interpreted in the Table (1). Out of which only five Molecules had excellent binding affinity towards pks13 target than the molecule 2 and Molecule 4 (reference molecules containing thiophene nucleus) shown in Figure (1). The molecules were distinct from other molecules being exempted from higher members of cyclic ketones and alkyl cyano ester resulting in the category of small molecule thiophene inhibitors. It was then justified by poor activity with molecules possessing more than six membered and higher ester moieties. The docked poses and its binding interactions were shown in Figure (3).

The molecule 10 showed interaction with TYR1582 towards sulphur atom (pi-sulfur), PHE1670, PHE1585 with (pi-pi) stacking with the aromatic ring, VAL1614 towards penta fluoro aromatic ring (pi-sigma) interaction. Similarly, molecule 11 containing heterocyclic ring interacts with ALA1586, ALA1583, and VAL1614 showing pi-alkyl and pi-sigma interactions respectively. Benzyl group showed (pi-pi) stacking with TYR1582, and Vander Waals interactions with ALA1617, ALA1596 towards halogenated aromatic ring.

Table 1: Binding Energy of Docked Molecules

Molecule Code	Docking Affinity(Kcal/mol)
10	-8.1
11	-8.2
12	-9.1
13	-9.2
14	-8.0
2	-7.8
4	-7.9

The molecule 12 shows interactions with PHE1670 showing Vander Waals interaction, PHE1585 towards halogenated ring, PHE1590 showing pi-sulphur interaction, TYR1637, and VAL1611 showing pi alkyl with ethyl ester. The Molecule 13 interacts with PHE1690, pi-sigma and ALA1586, VAL1618, with pi-alkyl interaction. Halogenated rings showing Vander Waals interaction with ILE1630, and phenyl ring with TYR1637 showing pi-pi stacking interactions. The Molecule 14 shows pi-alkyl interactions withALA1583, ALA1586, Vander Waals interaction with TYR1582, VAL 1611 and TYR1579, TYR1637 with the halogenated ring showing pi-pi interactions.

Drug likeness

The analysis according to Lipinski revealed that all Molecules showed good results in relations to MW, log P, HBD, HBA, n of violations, no of rot bonds <10, and their TPSA was less than 140 A, which is tabulated in Table (2).

Figure 3: Binding Interactions of molecules synthesised by SBDD Approach

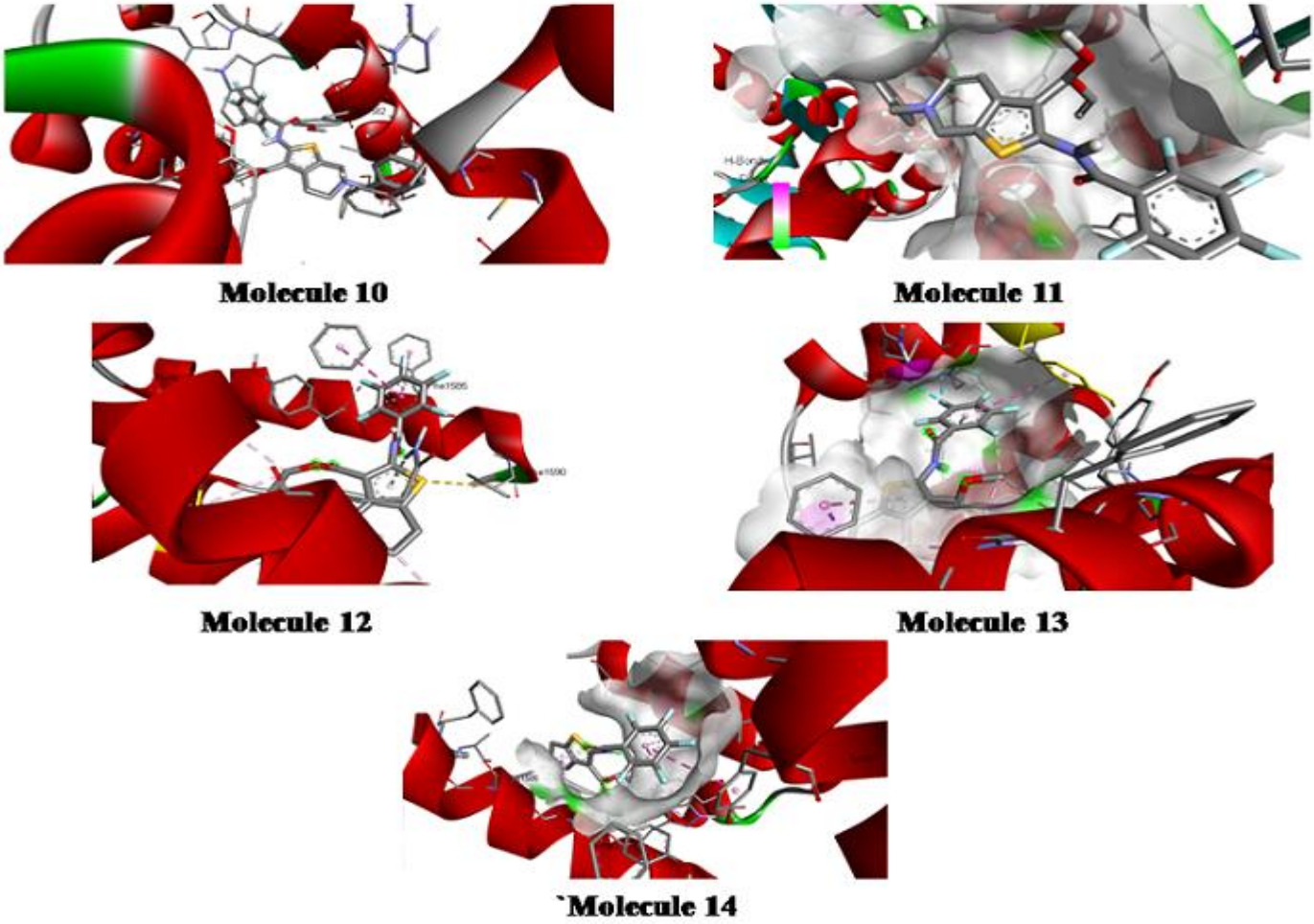


Table 2: Properties of Drug Likeness for the docked Molecules

Molecule Code	milogP	TPSA	natoms	MW	nON	nOHNH	nviolations	nrotb	volume
10	5.20	58.64	34	496.08	5	1	2	6	405.88
11	5.58	58.64	35	510.47	5	1	2	7	422.68
12	4.45	55.40	28	419.37	4	1	0	5	319.80
13	4.08	55.40	27	405.34	4	1	0	4	303.10
14	3.94	55.40	27	405.34	4	1	0	5	303.10
2	4.58	55.40	28	419.37	4	1	0	4	319.90
4	2.71	98.50	28	422.33	6	3	0	6	310.37

Bioactivity

All the selected molecules show moderate to excellent bioactivity score with respect to all types of Receptor-Ligand interactions of which molecule 13 and 14 shows excellent activity towards nuclear receptor ligand which is shown in Table (3).

Table 3: *In silico* Bioactivity Score for the docked Molecules

Molecule code	GPCR Ligand	Ion Channel Modulator	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
10	-0.28	0.70	-0.32	-0.58	-0.41	-0.22
11	-0.30	-0.68	-0.36	-0.54	-0.42	-0.24
12	-0.55	-0.83	-0.45	-0.63	-0.67	-0.34
13	0.53	0.87	0.41	0.67	0.66	0.31
14	0.70	0.90	0.52	0.75	0.83	0.41
2	-0.50	-0.83	-0.39	-0.64	-0.62	-0.29
4	-0.62	-0.93	-0.35	-0.78	-0.74	-0.39

ADMET Analysis

The $CaCO_2$ permeability for the selected Molecules is within the limits (>0.90) directly relating to their excellent permeability. The intestinal absorption was also found to be 89% which is more than 30%, indicates excellent absorption. The molecules crosses BBB permeability however, their CNS penetration is poor as its values are less than -3.5. Molecules 10 and 14, possess

hepatotoxicity and AMES were negative indicating as they are non-carcinogenic and non-mutagenic. CYP3A4 is one of the most

common and versatile enzyme among CYP45D family. CYP2D6 catalysis mostly 25% of the clinically important drugs. The table (4) explains that all the molecules showed good ADMET properties like excellent CaCO2 permeability and water solubility which in turn shows good intestinal absorption, and clearance. The molecules were found to be devoid of AMES toxicity and poor CNS permeability. whereas with molecules 13 and 14 shows little hepatotoxic than the other molecules.

Table 4: ADMET Prediction of the docked Molecules

ADMET Properties	MOLECULE CODE						
	10	**11**	**12**	**13**	**14**	**2**	**4**
$CaCO_2$ Permeability	1.645	1.601	1.617	1.298	1.375	1.265	1.323
Intestinal Absorption	90.377	89.379	90.43	90.232	91.326	90.42	90.33
BBB Permeability	1.19	1.315	1.281	1.371	-1.544	-1.412	1.26
CNS Permeability	-2.693	-2.857	-2.749	-2.743	-2.919	-2.642	-2.512
Total Clearance	0.045	0.041	-0.073	0.178	-0.135	0.033	0.023
Hepatotoxicity	YES	NO	NO	NO	YES	YES	YES
AMES	NO	NO	NO	NO	NO	YES	YES
Water Solubility	-4.913	-5.055	-5.312	-5.129	-4.893	-5.83	-5.33
CYP2D6	NO	NO	NO	NO	NO	NO	NO
CYD3A4	YES	NO	YES	YES	YES	NO	YES

Synthesis

Active methylene containing groups, ethyl and methyl cyanoacetate were reacted with appropriate ketones such as cyclopentanone, cyclohexanone and benzyl piperidones in the presence of sulphur and diethyl amine using ethanol as solvent at room temperature for six hours. The substitutions are X is ethyl and methyl cyano acetate and R, R' are cyclocyclohexanone, cyclopentanone and benzyl piperidinones. The reaction mixture was refrigerated overnight to yield corresponding amino esters [21]. The amino esters were then treated with pentafluoro benzoyl chloride, N, N-DMAP and triethyl amine to yield the target molecule. The progress of the reaction was monitored by TLC. The molecular weight of the synthesized molecule was from 405-519 with melting point in the range of 110-125^{0}C. The molecules were lime yellow to dark brown. The physiochemical properties of the synthesized molecules were shown in Table (5).

Table 5: Physiochemical Properties of the Synthesized Molecules

Molecule Code	Molecular Formula	Molecular Weight	TLC Solvent System	Melting point °C	% Yield	Solvent of Recrystallisation
10	$C_{23}H_{17}F_5N_2O_3S$	496.44	Hexane and Ethyl acetate	113	90	Methanol
11	$C_{24}H_{19}F_5N_2O_3S$	510.10	Hexane and Ethyl acetate	105	90	Methanol
12	$C_{18}H_{14}F_5NO_3S$	419.06	Hexane and Ethyl acetate	110	85	Methanol
13	$C_{17}H_{12}F_5NO_3S$	405.04	Hexane and Ethyl acetate	107	85	Methanol
14	$C_{17}H_{12}F_5NO_3S$	405.33	Hexane and Ethyl acetate	115	75	Methanol
Reference molecule 2	$C_{18}H_{14}F_5NO_3S$	419.36	Hexane and Ethyl acetate	110	85	Methanol
Reference molecule 4	$C_{16}H_{11}F_5N_2O_4S$	422.32	Hexane and Ethyl acetate	115	90	Methanol

Characterisation

Molecule 10 Amide C= O (1666), C-F (1278), N-H (3450) KBr (cm^{-1}); ^{1}H NMR ($CDCl_3$) δ ppm. δ (7.06- 7.14 s, Ar-CH) 5H, δ(8.0 NH) 1H, δ(3.88, s, CH_3, 3H, δ2.65 (d, CH_2) 2H,δ3.62 (s, CH_2-N) 4H, δ 2.69 (d, CH_2) 2H. m/z at 496.08.

Molecule 11 Amide C= O (1606), C-F (1252), N-H (3451) KBr (cm^{-1}); ^{1}H NMR ($CDCl_3$) δ ppm. δ (7.06- 7.14 s, Ar-CH) 5H, δ(8.0 NH) 1H, δ(3.88, s, CH_3, 3H, δ 4.29 (d, CH_2 2H), δ2.65 (d, CH_2) 2H, δ3.62 (s, CH_2-N) 4H, δ 2.69 (d, CH_2) 2H. m/z at 510.10.

Molecule 12 Amide C= O (1663), C-F (1261), N-H (3456) KBr (cm^{-1}); ^{1}H NMR ($CDCl_3$) δ ppm. δ2.55 (s, CH_2) 2H, δ1.62 (s, CH_2) 2H, δ4.29 (d, CH_2) 2H, δ (8.0 NH) 1H, δ1.30 (s, CH_3) 3H. m/z at 419.06.

Molecule 13 Amide C= O (1656), C-F (1268), N-H (3450) KBr (cm^{-1}); ^{1}H NMR ($CDCl_3$) δ ppm. δ2.55 (s, CH_2) 2H, δ1.62 (s, CH_2) 2H, δ (8.0 NH) 1H, δ1.30 (s, CH_3) 3H m/z at 405.04.

Molecule 14 Amide C= O (1666), C-F (1266), N-H (3451) KBr (cm^{-1}); ^{1}H NMR ($CDCl_3$) δ ppm. δ1.30 (s, CH_3) 3H, δ4.29 (d, CH_2), δ2.55 (d, CH_2) 4H, δ 1.95 (s, CH_2) 2H. m/z at 405.04.

***In-vitro* Antitubercular Activity**

The antitubercular activity by MABA method for the synthesised molecules were expressed in µg/ml shown in Table (6) and is shown in Figure (4). The strains used were ***Mycobacterium tuberculosis*** (Vaccine strain, H37RV strain) - ATCC No- 27294 and the standards were Isoniazid- 1.6 µg/ml, Ethambutol - 1.6 µg/ml, Pyrazinamide- 3.125µg/ml, Rifampicin - 0.8 µg/ml and Streptomycin- 0.8µg/ml.

Isolation of Bacteria

Two most widely used culture media are Egg based Lowenstein Jensen medium and the Middlebrook series of agars (7H10 and 7H11). They are solid phase broths. Among these two medium Lowenstein Jensen media is efficient used to identify the growth rate, whereas the Middlebrook promotes faster bacterial growth. MGIT 960 system (Mycobacterial growth indicator tube) has capable of testing 960 samples. This system is based on fluorescence detection of mycobacterial growth in a tube containing Middlebrook

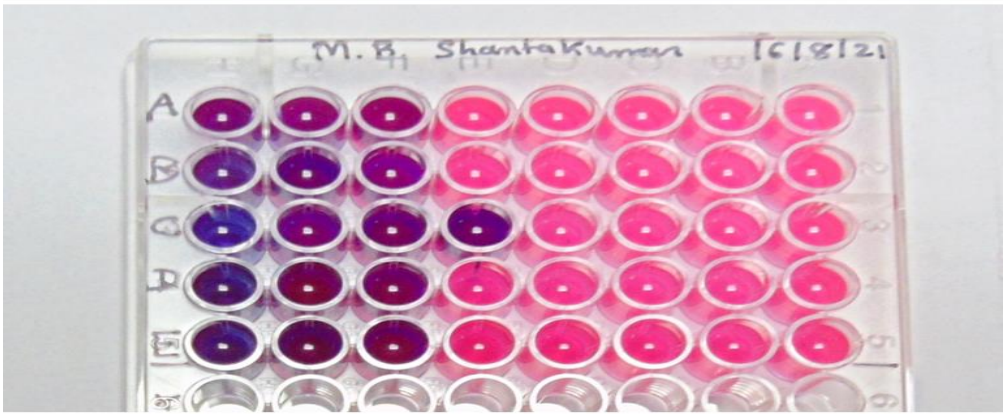
7H9 medium together with fluorescence quenching based oxygen sensor. During isolation some of the tubes may also contains contaminants. Such MGIT tubes must be decontaminated and the process is called as Re-decontamination.

Table 6: *Invitro* Antitubercular Activity of Synthesised Molecules

Molecules	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
10	S	S	S	R	R	R	R	R
11	S	S	S	R	R	R	R	R
12	S	S	S	S	R	R	R	R
13	S	S	S	R	R	R	R	R
14	S	S	S	R	R	R	R	R

Molecule 12 is found to be active at 12.5µg/ml whereas molecules 10,11,13 and 14 are active at 25 µg/ml. From the structural investigations, the synthesized molecule 12 possess six membered cyclic ketones with thiophene -3-ethyl ester moiety proved to have promising antitubercular activity and it is even extended to cycloheptanone and cyclo-octanone systems. The five membered cyclic ketones like cyclopentanone and more than five membered ring systems with a methylene linker connecting other heterocyclic rings shows comparatively decrease in activity. Molecule 10 and 11 was designed using 4-benzyl piperidones with methyl and ethyl cyano ester respectively. Finally, they are benzoylated to give pentafluorinated thiophene derivatives. The molecules were active at 25 µg/ml and does not show any change in activity. Molecule 13 and 14 were designed from cyclopentanones and cyclohexanones with methyl and ethyl ester showing activity at 25 µg/ml. From the results, it is evidenced that molecules designed from six membered cyclic ketones with ethyl ester retained the activity. However, the ester group can further be explored to expect increase in activity whereas higher members of aromatic ketones showed decrease in activity. Hence, the molecule 12 can be taken as lead and further optimized with different ester functionalities coupled with cyclic carbonyl systems which would ultimately results in newer leads with the improved therapeutic activity.

Figure 4: *Invitro* antitubercular activity, Pink – Growth, Blue – No growth



CONCLUSION

In modern drug discovery, CADD ranks topmost, a part of which SBDD is involved in the study where, it utilizes the previous reported literature information about the molecules and receptors. Based on this, ligands were designed and selected by molecular docking. Top five molecules were selected with excellent binding affinity by virtue of its key interaction and ranked by scoring least energy. The best docked molecular protein complex exhibiting least energy than the reference molecule 2 and molecule 4. The best docked poses were visualized by bio via discovery visualizer and identified the various kinds of amino acid interactions. Further, the molecules were passed through *in silico* filters like Swiss ADME inorder to rule out the various toxicities. It was found to be devoid of vital toxicities. They were also tested for drug likeness and bioactivity by molinspiration. All these molecules passed the Lipinski's rule of five and found to be active against various kinds of receptor channels. Finally, the molecules were taken for synthesis by two step reaction. First, the gewald's reaction to produce substituted amino esters which upon benzoylation to give pentafluoro substituted thiophene derivatives. All the synthesized molecules were solid and recrystallized from methanol with the percentage yield ranging from 75 to 90% and melting point in the range of 100-120 °C. These molecules were evaluated for invitro antitubercular assay by microplate Alamar blue assay method. It was found that molecule 12 exhibits higher activity at 12.5 microgram per ml than the other molecules. But when compared with standard regimens it is moderately active. Therefore, molecule 12, will be further scrutinized to design using various substituted aryl/cyclic aryl ketones to execute a strong lead in future.

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