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Original Article

Innovative Thiophene Schiff Bases: Synthesis and Evaluation as Antitubercular Agents

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ABSTRACT

Background: Tuberculosis (TB) is a leading airborne disease, impacting millions annually and ranking among the top ten causes of global mortality. Post-COVID-19, TB incidence has increased due to its pulmonary nature, which facilitates infection spread. Current TB treatments primarily control rather than prevent infection and are associated with mycobacterial resistance and significant side effects. **Purpose:** This study aims to design and evaluate thiophene based Schiff bases as potential antitubercular agents targeting polyketide synthase 13 (Pks 13), crucial for mycolic acid production and less prone to resistance. **Materials and Methods:** Thiophene-based Schiff bases were designed based on Structure-Activity Relationship (SAR) analysis and subjected to *in silico* approaches, including molecular docking against Pks 13. Compounds with the best docking scores underwent further *in silico* analysis (ADME, drug-likeness, toxicity). These compounds were synthesized, recrystallized, characterized and evaluated for *in vitro* antitubercular activity using the Microplate Alamar Blue Assay (MABA). **Results:** Compounds Ca3 and Ca5 had the best docking scores (-8.6 and -8.4 kcal/mol) and showed significant antitubercular activity *in vitro* at 25 µg/mL and 12 µg/mL, respectively. *In silico* and *in vitro* results correlated well, indicating strong binding affinity and potency against Pks 13. **Conclusion:** Compounds Ca3 and Ca5 show promise as potent antitubercular agents targeting polyketide synthase 13, supporting further development and optimization of thiophene-based Schiff bases for TB treatment.

Keywords: Polyketide synthase 13, Mycolic acids, Docking, *In silico* analysis, MABA.

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INTRODUCTION

Tuberculosis (TB) is a highly contagious disease caused by *Mycobacterium tuberculosis*, primarily affecting the lungs but also spreading to other parts of the body (extra-pulmonary TB). It spreads through the air via droplets from infected individuals. The death rate from TB has increased in recent years, particularly among those with compromised immune systems such as HIV/AIDS patients, who are 18 times more likely to develop TB. COVID-19 has also contributed significantly to TB-related mortality due to overlapping infection sites.[1,2] In 2022, approximately 1.3 million people died from TB, including 167,000 with HIV, while 10.6 million people globally fell ill with TB.

Despite the availability of antitubercular drugs and ongoing vaccine trials, tuberculosis remains a significant challenge due to antimicrobial resistance. Resistance arises from both intrinsic factors, which occur naturally at high levels and extrinsic factors, including acquired mutations due to sub-optimal drug exposure. Factors such as bacterial load, mutation rates and virulence contribute to this resistance. This has led to the emergence of resistant forms like MDR-TB, XDR-TB and TDR-TB, prompting global concern.[3-5] Identifying essential metabolic pathways and components vital for the organism's survival is critical for developing effective anti-TB drugs.

The cell wall of *Mycobacterium tuberculosis* is rich in mycolic acids, which are crucial for the bacterium's survival and resistance. Among the key enzymes in mycolic acid synthesis, Pks13, a type I polyketide synthase, plays a pivotal role by condensing fatty acid intermediates into mycolic acids.[6-10] Pks13 is less sensitive to resistance and helps form a mycolic acid layer that reduces drug permeability, making it an attractive target for tuberculosis treatment.[11-13] This has led to a focus on discovering new drugs with novel mechanisms, either as independent treatments or in combination.

Heterocyclic rings have shown potent antitubercular activity. This study involves designing thiophene derivatives through a Schiff base reaction. Schiff bases, derived from carbonyl compounds and

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primary amines, are biologically active and found in many natural and synthetic compounds. Although thiophene-containing heterocyclic derivatives have shown promise as antitubercular agents, none are currently on the market, justifying further research and development.[14-17]

Schiff bases have gained importance in medicinal chemistry for their diverse biological activities, including anti-inflammatory, analgesic,[18] anticonvulsant, anticancer, antioxidant, anthelmintic, antimicrobial[19] and antitubercular properties.[20] Those derived from aromatic aldehydes are particularly noted for their antibacterial and antifungal effects. With the rise of multi-drug-resistant and extensively drug-resistant tuberculosis, there is a crucial need for new, effective agents. Research indicates that Schiff bases with aromatic and heterocyclic rings show promising activity against *Mycobacterium tuberculosis* and other microorganisms, making them potential candidates to combat drug resistance in tuberculosis treatment.[15-17]

Computer-Aided Drug Design (CADD) uses structural data from target proteins (structure-based) or known bioactive compounds (ligand-based) to identify potential drug candidates. Virtual screening, a key *in silico* technique, aids in discovering new compounds. Rational drug design within CADD leverages knowledge-driven approaches to elucidate protein-ligand interactions and binding affinities.[21,22] This investigation focuses on evaluating Schiff bases and thiophene heterocyclic amino ester derivatives for their potential antibacterial and anti-mycobacterial activity.

MATERIALS AND METHODS

Experimental Section

Materials

Schiff base thiophene derivatives were synthesized by reacting cyclohexanone and substituted aliphatic cyanoacetate in the presence of diethyl-$\boldsymbol{\lambda}^4$-sulfanimine and ethanol which act as catalysts to produce an intermediate compound, further intermediate compound reacts with various types of aromatic aldehyde. The reactants like cyclohexanone and aliphatic cyanoacetate and various aldehydes like bromobenzaldehyde, 1,3-dinitro benzaldehyde, 1-chloro-4-vinyl benzaldehyde, benzaldehyde, 4-chloro benzaldehyde were purchased from Southern India Scientific Corporation Limited Chennai.

Methods

Using conventional techniques, Schiff base thiophene derivatives were synthesized and their melting points determined by the open capillary tube method. Chemical purity was confirmed using thin-layer chromatography. The designed compounds were sketched with Chem3D Pro 12.0 and docked using AutoDock 4.2. Protein preprocessing involved UCSF Chimera, assigning Kollmann charges, generating grid points and performing molecular docking to analyze binding affinities and interactions.[23] Docked complexes were visualized with Biovia Molecular Discovery Studio. The pdb 5v3W protein, validated by resolution and outliers, was selected for docking. The best-ranked compounds underwent *in silico* analyses, including drug-likeness (Molsoft), bioactivity (Molinspiration) and ADMET parameters (pkCSM).[24]

Computational Studies

Molecular Docking

Molecules were designed based on literature surveys and SAR studies, leading to a library of Schiff base compounds created from amino ester derivatives and various aromatic aldehydes. The molecules were then evaluated using *in silico* methods such as docking, ADMET, molecular properties and bioactivity. Molecular docking involved five steps: protein preparation, ligand preparation, grid generation, docking and result analysis. The protein, downloaded from the protein databank in .pdb format, was validated using the Ramachandran plot. Preprocessing included selecting the suitable chain, extracting the co-crystallized ligand, analyzing the active site, generating grid points and minimizing the protein using the OPLS2005 force field. Molecular structures were drawn with ChemDraw Ultra 12.0,[25] minimized with Chem3D Pro 12.0 and scrutinized using the MMFF94 force field. The grid was generated to enclose the active site fully and docking was performed to form macromolecular complexes ranked by energy. Interactions were visualized using Biovia Discovery Studio Visualizer.[26]

In silico Analysis

Molecular properties were analyzed using Molinspiration cheminformatics by sketching the compounds and extracting their SMILES codes. These were then subjected to analysis. The Lipinski rule of five was used, focusing on molecular weight <500 Da, log P≤5, hydrogen bond donors ≤5 and hydrogen bond acceptors ≤10, which correlate with approximately 90% of orally bioavailable drugs reaching phase II clinical trials. Next, the pharmacokinetic profile, including ADMET[27,28] properties (Absorption, Distribution, Metabolism, Excretion and Toxicity), was analyzed using the pkCSM server, considering factors like intestinal permeability and aqueous solubility.

Experimental Section

Synthesis of the Compounds

Various aromatic ketones are treated with the active methylene groups like Ethyl and Methyl cyanoacetate to produce the amino esters in the presence of sulfur and ethanol and further, the amino esters are treated with various aromatic aldehydes to form the corresponding Schiff base product[29-31] and it shown in Figure 1.

Characterization

The melting points and R_f values of the synthesized compounds were preliminarily checked for purity and homogeneity. The final compounds were found to be soluble in organic solvent Ethanol/methanol; compounds were also subjected to FTIR spectral studies, Nuclear Magnetic Resonance (NMR) Spectroscopy, Mass Spectroscopy and Elemental Data Analytical studies for structural elucidation, showed good results indicating successful completion of the reaction and absence of impurities.

In vitro Antitubercular Activity

The anti-mycobacterial activity of compounds against *M. tuberculosis* was evaluated using the Microplate Alamar Blue Assay (MABA). This method uses a thermally stable reagent and correlates well with proportional and BACTEC radiometric methods. To prevent medium evaporation, 200 µL of sterile deionized water was added to the outer perimeter wells of a 96-well plate. Each well received 100 µL of Middlebrook 7H9 broth and serial dilutions of the compounds were prepared on the plate, with final concentrations ranging from 100 to 0.2 µg/mL. The plates were sealed with parafilm and incubated at 37°C for 5 days. After incubation, 25 µL of a 1:1 mixture of Alamar Blue reagent and 10% Tween 80 were added to each well and incubated for another 24 hr. Blue wells indicated no bacterial growth, while pink indicated growth. The MIC was the lowest concentration preventing the color change from blue to pink. The standard strain used was *M. tuberculosis* (H37 RV strain) ATCC No., with Pyrazinamide, Ciprofloxacin and Streptomycin serving as controls at 3.125 µg/mL, 3.125 µg/mL and 6.21 µg/mL, respectively.[32]

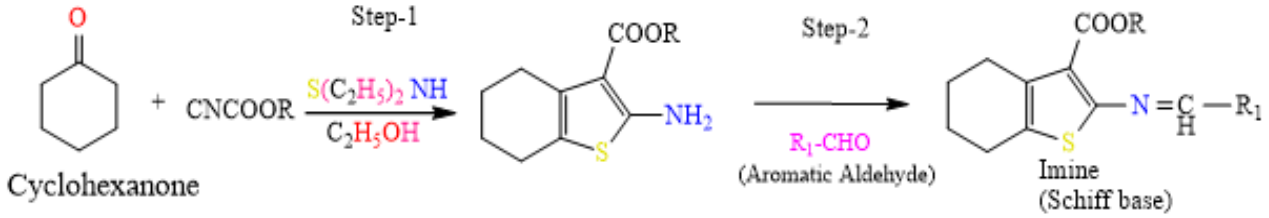
RESULTS

Molecular Docking Studies: *In silico* docking simulations were used to identify ligands with high predicted binding affinity for protein PDB 5V3W. The top-ranked compounds were selected for synthesis and docking results, ranked by lowest energy, are presented in Table 1. Figures 2 and 3 shows the 2D and 3D images of the docked complexes, highlighting ligand-protein interactions.

ADME Properties: The pharmacokinetic properties of 10 designed molecules were evaluated using the pkCSM server for ADMET prediction, focusing on drug-likeness. These properties are detailed in Table 2.

Molecular Properties of Synthesized Compounds: Key molecular properties, such as log P, TPSA, volume, molecular weight, total number of atoms, number of rotatable bonds, hydrogen bond acceptors and hydrogen bond donors, are shown in Table 3.

Bioactivity: The selected compounds were evaluated for bioactivity against various receptors, including nuclear ligand-receptors, G-protein coupled receptors, tyrosine kinase-linked receptors, ion channels and protease and enzyme inhibitors. The results are shown in Table 4.



	Substituents	Name of the substituents
R	CH_3, C_2H_5	Methyl, Ethyl
R_1	C_6H_5Br	Bromobenzene
	$C_6H_5(NO_2)_2$	1,3-dinitrobenzene
	C_8H_7Cl	1-Chloro-4-vinylbenzene
	C_6H_5	Benzene
	C_6H_5OH	Phenol
	$C_6H_5OCH_3$	Anisole
	$C_6H_5C_1$	Chlorobenzene

Figure 1: Scheme of the designed schiff base derivatives.

Table 1: Molecular Docking Results.

Compound Code	Binding energy (K. Cal)	Amino Acids	Type of Interactions
Ca 1	-7.7	TYR B: 1582 ALA B:1583 TYR B: 1637 ALA B: 1586 VAL B: 1614 VAL B: 1618 PRO B :1595	Pi-Sulfur Alkyl Amide-Pi stacked Pi-Alkyl Alkyl Pi-Alkyl Alkyl
Ca 2	-7.6	PHE B: 1670, TYR B:1582 VAL B:1614 TRP B:1579 VAL B:1614 ALA B:1586	Pi-Pi T-Shaped Amide-Pi Stacked Alkyl Amide-Pi Stacked Alkyl Pi-Alkyl
Ca 3	-8.6	TYR B:1582 VAL B:1614 LEU B:1615 ARG B:1634 VAL B:1611 PHE B:1670 MET B:1669	Pi-Pi T-Shaped Alkyl Pi-Alkyl Alkyl Alkyl Hydrogen Bond Vander Waals
Ca 4	-8.2	TRP B:1579 TYP B:1582 VAL B:1614 ASN B:1640	Amide-Pi Stacked Amide-Pi Stacked Alkyl Hydrogen Bond
Ca 5	-8.4	TYR B:1582 TRP B:1579 ALA B:1583 VAL B:1614 ALA B:1586 PRO B:1595	Pi-Sulfur Vander Waals Alkyl Pi-Alkyl Alkyl Vander Waals
Ca 6	-7.2	TYR B:1582 TRP B:1579 VAL B:1618 LEU B:1615	Amide-Pi Stacked Pi-Sulfur Alkyl Alkyl
Ca 7	-7.2	ALA B:1586 VAL B:1614 ALA B:1583 LEU B:1615 ARG B:1634 VAL B:1611 TYR B:1637 TYR B:1582	Alkyl Alkyl Alkyl Pi-Alkyl Alkyl Pi-Alkyl Alkyl Pi-Sigma
Ca 8	-7.2	TYR B:1637 TYR B:1582	Amide-Pi Stacked Amide-Pi Stacked

Compound Code	Binding energy (K. Cal)	Amino Acids	Type of Interactions
Ca 9	-7.4	SER B:1636 TYR B:1637 TYR B:1582 VAL B:1611	Pi-Sigma Amide-Pi Stacked Amide-Pi Stacked Alkyl
Ca 10	-8.0	ALA B: 1583 VAL B:1614 ILE B:1597 TRP B:1579 TYR B:1582 TYR B:1637	Alkyl Pi-Alkyl Alkyl Alkyl Pi-Sigma Alkyl

Table 2: **Pharmacokinetic and toxicity Prediction.**

	Absorption		Distribution		Metabolism		Excretion		Toxicity	
Compound code	Water Solubility	Intestinal absorption	VDss (Human)	Fraction Unbounded	CYP2 D6	CYP3A4	Total clearance	Renal OCT2 Substrate	Max total clearance	Oral Rat Acute T
Ca1	-6.48	92.31	0.61	0	No	Yes	-0.05	No	0.34	2.68
Ca2	-6.16	92.79	0.49	0	No	Yes	-0.04	No	0.27	2.75
Ca3	-5.44	98.77	0.35	0	No	Yes	0.29	No	-0.76	2.74
Ca4	-5.22	98.01	0.31	0	No	No	0.34	No	-0.76	2.69
Ca5	-6.68	91.79	0.57	0	No	Yes	-0.01	No	0.15	2.74
Ca6	-4.89	92.86	0.18	0.05	No	Yes	-0.08	No	0.08	2.29
Ca7	-5.24	92.26	0.26	0.04	No	Yes	-0.13	No	0.16	2.23
Ca8	-5.38	95.34	0.44	0	No	Yes	0.14	Yes	0.16	2.50
Ca9	-6.25	93.03	0.52	0	No	Yes	0.08	Yes	0.26	2.66
Ca10	-5.57	95.42	0.30	0	No	Yes	0.06	Yes	0.07	2.62
Isoniazid	-1.65	92.61	-0.35	0.72	No	No	0.72	No	1.16	2.30

Table 3: **Molecular Properties of Synthesized Compounds.**

Compound Code	Log P	TPSA	Natomas	MW	Nrotb	Volume	Hydrogen Bond Acceptor	Hydrogen Bond Donor
Ca1	5.38	38.67	23	392.32	5	305.01	4	0
Ca2	5.00	38.67	22	378.29	3	288.20	4	0
Ca3	4.41	130.32	28	403.42	6	333.79	8	0
Ca4	4.04	130.32	27	389.39	5	316.99	8	0
Ca5	5.62	38.67	24	359.88	4	311.27	4	0
Isoniazid	-0.97	68.01	10	137.14	1	122.56	3	2

Antitubercular Activity by MABA Test

The antitubercular activity for the synthesized compounds was performed by microplate alamar blue assay method and the results were tabulated in Table 5.

Characterization

Ethyl2-((2,4-bromobenzylidene) amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (1)

Yield 76%, Melting point: 145°C; IR (Cyclohexane cm^{-1}): 3310 (N-H stretching), 760 (Thiophene, C-H bending), 1710 (C=O

Table 4: Bioactivity of the Synthesized Compounds.

Compound Code	GPCR Ligand	Ion channel	Kinase Inhibitor	Nuclear Receptor Ligand	Protease Inhibitor	Enzyme Inhibitor
Ca1	-0.36	-0.47	-0.60	-0.58	-0.50	-0.26
Ca2	-0.34	-0.49	-0.58	-0.62	-0.49	-0.27
Ca3	-0.64	-0.95	-0.68	-0.85	-0.86	-0.85
Ca4	-0.63	-0.49	-0.65	-0.91	-0.84	-0.46
Ca5	-0.37	-0.75	-0.67	-0.65	-0.70	-0.38
Isoniazid	-1.39	-1.45	-1.05	-2.23	-1.23	-0.66

Table 5: Anti-tubercular activity for synthesized compound.

Sample	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
Ca1	S	S	R	R	R	R	R	R
Ca2	S	S	R	R	R	R	R	R
Ca3	S	S	S	R	R	R	R	R
Ca4	S	S	R	R	R	R	R	R
Ca5	S	S	S	S	R	R	R	R

stretching), 1180 (C-O stretching), 2920 (C-H stretching), 1590 (C=N stretching), 1480 (C=C stretching), 630 (C-Br stretching); 1H NMR(400 MHz, DMSO): 7.65 (d, 2H, Ar-H, J≈8 Hz), 7.50 (d, 2H, Ar-H, J≈8 Hz,), 8.40 (s, 1H), 1.90 (m, 4H, CH_2), 2.60 (m, 4H, CH_2), 4.20 (q, 2H, OCH_2, J≈7 Hz), 1.30 (t, 3H, CH_3, J≈7 Hz). Compound -1 Molecular formula: $C_{17}H_{16}BrNO_2S$, Molecular weight: 392.31, Elemental Analysis: C (53.97), H (4.26), Br (21.12), N (3.70), O (8.46), S (8.48).

Methyl 2-{[(4bromophenyl) methylidene] amino}4,5, 6,7-tetrahydrobenzo[b] thiophene-3carboxylate (2)

Yield 75%, Melting point: 163°C, IR (Cyclohexane cm^{-1}): 1730-1750 (C=O stretching), 1600-1620 (C=C stretching), 1650-1690 (C-N stretching), 500-600 (C-Br stretching), 690-740 (C-S stretching), 2850-2950 (C-H stretching), 3000-3100 (Aromatic C-H stretching); 1H NMR(400 MHz, DMSO): 7.2-7.8 (m, 4H), 8.0-8.5 (s, 1H, CH=N), 3.5-4.0 (s, 3H,OCH_3), 1.2-2.5 (m, 8H). Compound-2 Molecular formula: $C_{18}H_{18}BrNO_2S$, Molecular weight: 364.26, Elemental Analysis: C (55.11), H (4.62), Br (20.37), N (3.57), O (8.16), S (8.17).

Ethyl 2-{[(2,4-dinitrobenzylidene) amino)- 4,5,6,7-tetrahydrobenzo[b]thiophene-3carboxylate (3)

Yield 85%, Melting point: 136°C, IR (Cyclohexane cm^{-1}): 1730-1750 (C=O stretching), 1600-1620 (C=C Aromatic stretching), 1630-1680 (C-N stretching), 1510-1560, 1320-1350 (NO_2 stretching), 690-740 (C-S stretching), 2850-2950 (Alkyl C-H stretching), 3000-3100 (Aromatic C-H stretching); 1H NMR(400 MHz, DMSO): 7.4-8.0 (m, 4H), 8.0-8.5 (s, 1H, CH=N), 1.2-1.4 (d, 3H, CH_2CH_3, 1.5-2.5 (m, 8H, CH_2 groups); Compound-3 Molecular formula: $C_{18}H_{17}N_3O_6S$, Molecular weight: 403.41, Elemental Analysis: C (53.59), H (4.25), N (10.42), O (23.80), S (7.95).

Methyl 2-((2,4-dinitrobenzylidene) amino)- 4,5,6,7-tetrahydrobenzo[b]thiophene-3carboxylate (4)

Yield 72%, Melting point: 141°C, IR (Cyclohexane cm^{-1}): 1730-1750 (C=O stretching), 1600-1620 (C=C Aromatic stretching), 1630-1680 (C-N stretching), 1510-1560, 1320-1350 (NO_2 stretching), 690-740 (C-S stretching), 2800-2950 (Methyl stretching), 3000-3100 (Aromatic C-H stretching); ^{1}H NMR (400 MHz, DMSO): 7.4-8.0 (m, 4H), 8.0-8.5 (s,1H, CH=N), 2.0-2.5 (s, 3H, CH_3), 1.5-2.5 (m, 8H, CH_2); Compound-4 Molecular formula: $C_{17}H_{15}N_3O_6S$, Molecular weight: 389.38, Elemental Analysis: C (52.44), H (3.88), N (10.79), O (24.65), S (8.23).

Methyl 2-((3-(4-chlorophenyl) allylidene) amino)- 4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxylate (5)

Yield 84%, Melting point: 185°C, IR (Cyclohexane cm^{-1}): 1730-1750 (C=O stretching), 1600-1620 (C=C Aromatic stretching), 1630-1680 (C-N stretching), 1600-1650 (C=C stretching,), 700-800 (C-Cl stretching), 690-740 (C-S stretching), 2800-2950 (C-H stretching), 3000-3100 (Aromatic C-H stretching); ^{1}H NMR (400 MHz, DMSO): 7.0-8.0 (m, 5H, Aromatic protons), 5.5-6.5 (m, 2H, $CH=CH_2$), 8.0-8.5 (s, 1H, CH=N), 2.0-2.5 (s, 3H,CH_3), 1.5-2.5 (m, 8H, CH_2 groups); Compound-5 Molecular Formula: $C_{19}H_{18}ClNO_2S$ Molecular weight: 359.87, Elemental Analysis: C (63.41), H (5.04), N (3.89), O (8.89), S (8.91), Cl (9.85).

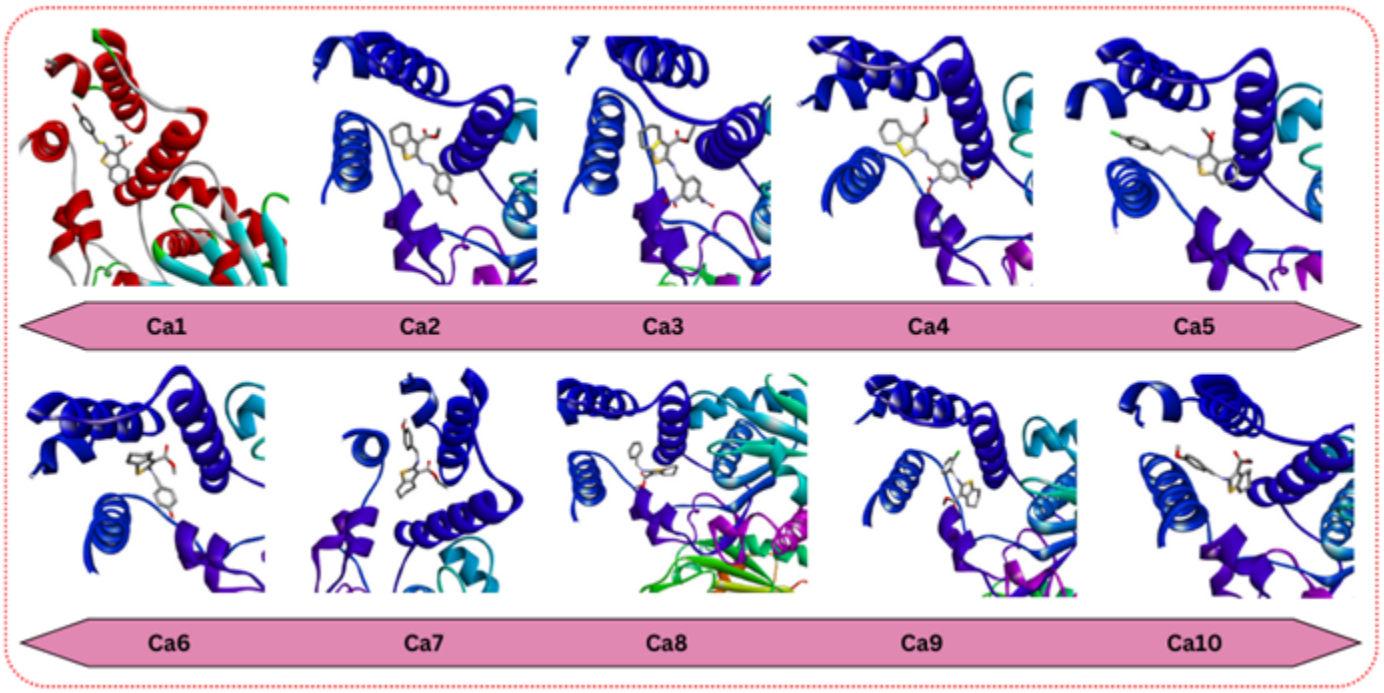


Figure 2: 3D Interactions of Ca1 -Ca 10.

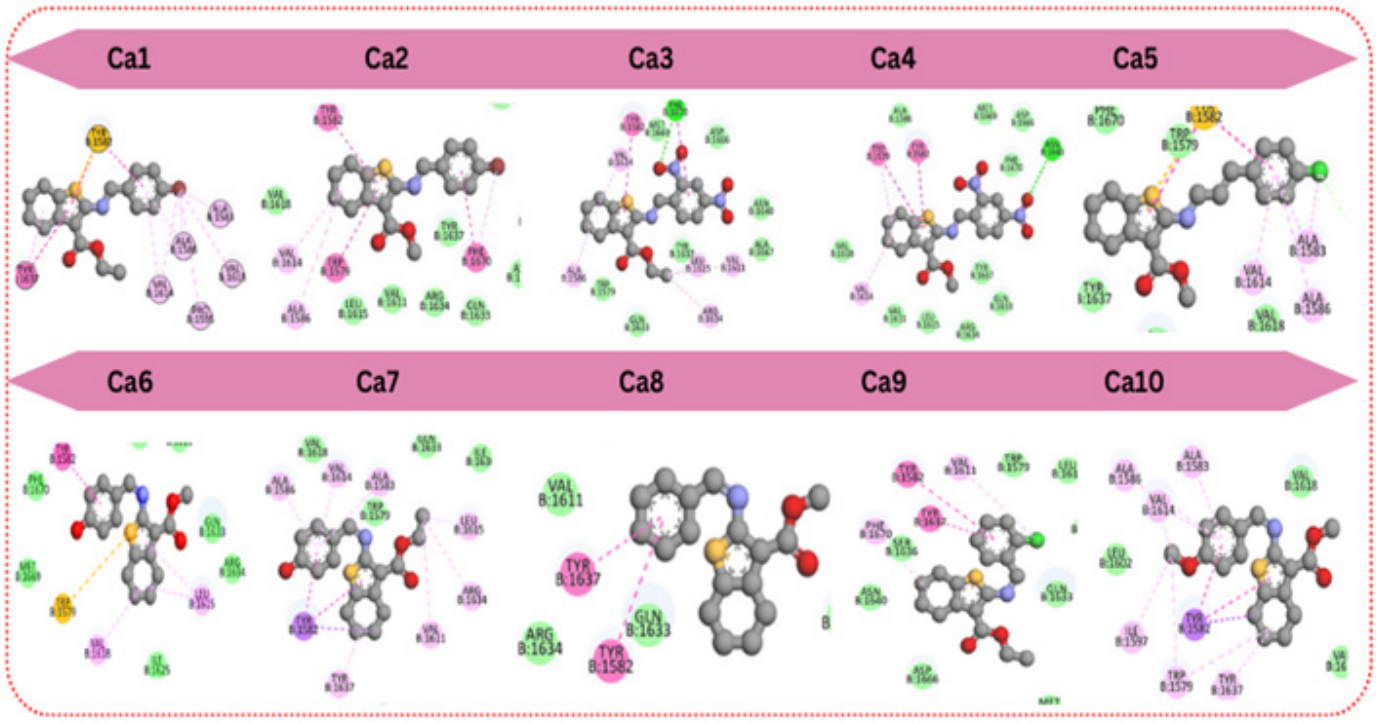


Figure 3: 2D Interactions of Ca1 -Ca 10.

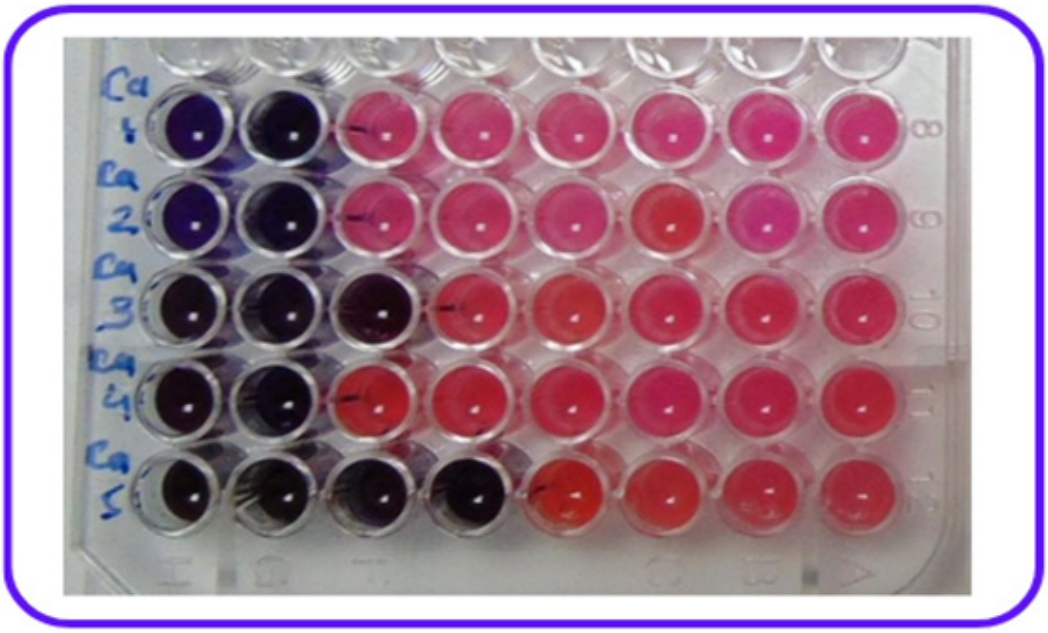


Figure 4: Anti-tubercular activity by MABA test.

DISCUSSION

Ten novel compounds were designed and subjected to docking studies. Compounds Ca3 and Ca5 exhibited the lowest binding energies and highest binding affinities. Ca3 had a binding energy of -8.6 kcal/mol, interacting with amino acids TYR B:1582, VAL B:1614, LEU B:1615, ARG B:1634, VAL B:1611, PHE B:1670 and MET B:1669 through pi-pi stacking, T-shaped interactions, alkyl, pi-alkyl, conjugated hydrogen bonding and van der Waals forces. Ca5 had a binding energy of -8.4 kcal/mol, interacting with TYR B:1582, TRP B:1579, ALA B:1583, VAL B:1614, ALA B:1586 and PRO B:1595 through Pi-Sulfur, Van der Waals, alkyl and pi-alkyl interactions. The docking results, ranked by lowest energy, are shown in Table 1 and Figures 2 and 3 present the 2D and 3D images of the docked complexes, highlighting the ligand-protein interactions.

Using pkCSM server results, predicted water solubility (log P) ranged from -6.48 to -4.89, indicating moderate to low water solubility. Intestinal absorption was high (91.79 to 98.77) and the Volume of distribution (Vd) ranged from 0.18 to 0.61, suggesting wider distribution for compounds with lower Vd values. Compounds showed varying interactions with metabolic enzymes Cytochrome P2D6 and P3A4. Total renal clearance ranged from -0.08 to 0.34, suggesting moderate clearance, with oral rat acute toxicity between 2.23 to 2.75 indicating moderate oral clearance rates in rats. Compounds with desirable pharmacokinetic properties and good molecular docking results were selected for further studies.

The compounds' LogP values ranged from 4.04 to 5.62, indicating moderate to low water solubility. TPSA values ranged from 38.67 to 130.2 Å², suggesting moderate polarity; compounds with lower TPSA may have better passive membrane diffusion and higher oral absorption, while higher TPSA compounds may have lower diffusion due to greater polarity. The molecular weights ranged from 359.88 to 403.42 Da and the hydrogen bond acceptors, donors and numbers of rotatable bonds were within the acceptable range for orally administered drugs according to the Lipinski Rule of Five.

Compounds with values ranging from -0.34 to -0.64 indicate weak to moderate binding affinity with GPCR, with lower values suggesting weaker binding. Ion channel interaction showed stronger inhibition at -0.47 and weaker inhibition at -0.95. For IC_{50} values, a lower value (closer to -0.58) indicates a stronger inhibitor, requiring a lower concentration for 50% inhibition, while a higher value (closer to -0.68) indicates a weaker inhibitor. Inhibition of protease enzymes ranged from -0.50 to -0.91, indicating weak inhibition. Similarly, enzyme inhibition values ranged from -0.26 to -0.85, also indicating weak inhibition.

Molecules with favorable docking, ADME predictions and biological activity were synthesized. Cyclohexanone (0.1 moles) reacted with ethyl cyanoacetate, methyl cyanoacetates, sulfur and diethyl amine (0.125 moles) in 20 mL of ethanol. The mixture was stirred for 3 hr at room temperature and refrigerated overnight. After adding 20 mL of ice-cold water, an amino ester was obtained. In the second step, the amino ester reacted with various aromatic aldehydes in 10 mL of ethanol to yield an imine base. Melting points ranged from 136-185°C, with yields between 72% and 85%. Reaction completion was monitored by TLC using n-hexane and ethyl acetate. The R_f values varied and ethanol was used for recrystallization.

It shows that the values range between the concentration ranging from 0.8-100 µg/mL. The compounds Ca3 and Ca5 were found to be active at 25 µg/mL and 12 µg/mL respectively whereas Ca1, Ca2 and Ca4 were active only at 50 µg/mL concentration. The color change from blue to pink in the microtitre plate is scored as growth, whereas blue indicates no growth and it is shown in Figure 4.

CONCLUSION

In this study, a series of new thiophene derivatives were synthesized using Schiff base reaction and screened for *in silico* antitubercular activity. All compounds satisfied Lipinski's Rule of Five, indicating good oral bioactivity. Molecular docking revealed that compounds Ca3 and Ca5 had the best interactions with the target, showing the lowest binding energy. These compounds were optimized for future development. This investigation suggests that these thiophene derivatives can be used as leads for novel antitubercular agents.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TB: Tuberculosis; **Pks13:** Polyketide Synthase 13; **ADMET:** Absorption Distribution Metabolism Excretion Toxicity; **MABA:** Microplate Alamar Blue Assay; **HIV:** Human Immunodeficiency Virus; **MDR-TB:** Multidrug-resistance Tuberculosis; **XDR-TB:** Extensively drug-resistance Tuberculosis; **TDR-TB:** Total drug-resistance tuberculosis; **M.tb:** *Mycobacterium tuberculosis*; **CADD:** Computer-aided Drug Design; **PDB:** Protein Data Bank; **SAR:** Structural Activity Relationship; **MMFF94:** Merck Molecular Force Field; **OPLS2005:** Optimized Potentials for Liquid Simulations; **FTIR:** Fourier Transform Infrared Spectroscopy; **NMR:** Nuclear Magnetic Resonance; **TPSA:** Topological Polar Surface Area; **TLC:** Thin layer Chromatography.

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