Int. J. Pharm. Investigation, 2021;11(2):189-194 *Original Article*

Schiff Base Conjugate of 5-Fluoroisatin with Thiophene-2-Ethylamine and its Mannich Bases: Synthesis, Molecular Docking, and Evaluation of *in vitro* Anti-inflammatory and Anti-tubercular Activity

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ABSTRACT

Objectives: To synthesize Schiff base conjugate of 5-Fluoroisatin with thiophene-2-ethylamine and its Mannich bases and screen for anti-inflammatory and anti-tubercular activity. **Materials and Methods:** The compounds were synthesized by Schiff and Mannich base reactions. The anti-inflammatory activity was studied by analyzing the percentage inhibition of denaturation of Bovine Serum Albumin and anti-tubercular activity using the Almar blue assay against *Mycobacterium tuberculosis* (MTB H37Rv). To understand the interactions of compounds with receptors and pharmacokinetic properties, *in silico* studies were performed. **Results:** The compound ITF 5 (80.08%) showed good anti-inflammatory activity. Also, compound ITF 5 (MIC: 6.25 µg/ml) was found most active against MTB H37Rv (ATCC-27294). **Conclusion:** Compounds showed good anti-inflammatory activities and relatively weak anti-tubercular activities as compared to standards. The compounds ITF 5 with N-phenylpiperazine containing Mannich base showed better anti-inflammatory and anti-tubercular activity as compared to other compounds.

Key words: 5-Fluoroisatin, Anti-inflammatory activity, Docking, Anti-tubercular activity, *In silico* ADME.

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INTRODUCTION

Fluorinated isatin derivatives were extensively investigated for anti-tubercular activity as the prior studies have shown that the substitution of fluorine at the C-5 position of the isatin moiety have a significant effect on anti-tubercular activity.[1-4] Anti-tubercular and anti-inflammatory activities of heterocyclic hybrids with isatin (indole-2,3-dione) are being studied extensively.[5,6] The Mannich bases of isatin shows potent anti-tubercular activities in previous studies.[7,8]

Anti-tubercular and anti-inflammatory properties of isatin and thiophene derivatives have been reported in the literature.[9-14] Tenidap, an indole and thiophene nucleus-containing molecule reached in a clinical trial for the treatment of anti-inflammatory activity.[15,16] The indole derivative with a thiophene-containing fragment at C-3, a halogen group at C-5, and substitutions on the nitrogen atom nitrogen atom present in tenidap, as well as the synthesized molecules of this study. The chemical structure of the tenidap, an anti-inflammatory agent, and the previous studies indicating the significance of fluorinated isatins as anti-tubercular agents encouraged us to design the molecules of the present study.

The molecules that can prevent protein denaturation, are considered desirable candidates for anti-inflammatory drug development. Estimation of inhibition of protein denaturation is one of the methods for studying anti-inflammatory activity.[17-20] The synthesized conjugates were evaluated for their anti-inflammatory activity by analyzing percentage inhibition of denaturation of Bovine Serum Albumin (BSA) and compared with standard diclofenac sodium.

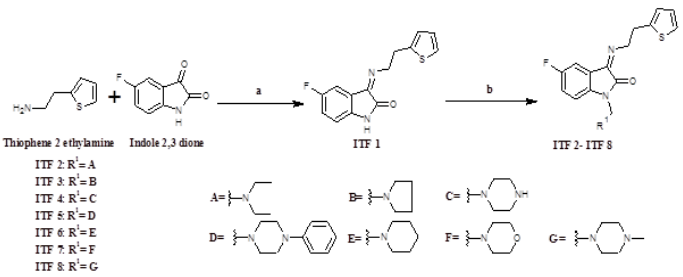
In this research work, we have performed anti-tubercular and anti-inflammatory activity evaluation of ethylene tethered 5-fluoroisatin Schiff's base conjugate with thiophene-2-ethylamine, and further *in silico* docking and Absorption, Distribution, Metabolism, and Excretion (ADME) studies.

MATERIALS AND METHODS

The Schiff base conjugate (ITF 1) was synthesized by treating thiophene-2-ethylamine with 5-Fluoroisatin in the presence of acetic acid as a catalyst. Mannich bases of synthesized Schiff base were prepared by treating ITF 1 with formaldehyde 37 % and secondary amines. Melting Points were analyzed by using Thiele's tube and are uncorrected. Infrared (IR) spectra were recorded on an IR spectrometer Bruker ALPHA spectrometer. NMR spectra were recorded on Bruker 400 spectrometer in DMSO as a solvent.

Synthesis of isatin and thiophene-2-ethylamine Schiff base (ITF 1)

Equimolar quantities of 5-Fluoroisatin (0.01 M) in 5 ml of warm ethanol and thiophene-2-ethylamine (0.01 M) were mixed. Two drops of acetic acid were added to the mixture and stirred at 500 Revolutions Per minute (RPM) for 6 hr. The resultant yellow solid was washed with ethanol and recrystallized from the chloroform-methanol mixture.



Scheme 1: Synthesis of Schiff's base of 5-Fluoroisatin with thiophene-2-ethylamine and its Mannich bases

Synthesis of Mannich bases of thiophene containing isatin derivatives (ITF 2-8)

A slurry consisting of the ITF 1 (0.001 mol), ethanol (2 ml), and 37% formaldehyde (2 ml) was prepared. Secondary amine (0.001 mol) was added to the above slurry dropwise over 15 min, with cooling and stirring. The reaction mixture was stirred at 60 RPM at room temperature for 1 hr. The reaction mixture was then warmed for 15 min on a steam bath. Then the contents were cooled and the obtained product was recrystallized from chloroform-hexane.[21-24]

5-fluoro-3-((2-(thiophen-2-yl)ethyl)imino)indolin-2-one **(ITF 1)**

Yield: 74%; M.P.: 179-180°C, IR(cm^{-1}): 3243.5191, 3050.4939, 2950.5436, 1731.3630, 1657.4411, 1473.4838, 1354.3680, 1265.5851, 1175.6082, 1041.0658, 830.9277, 746.7976, ^{1}H-NMR (400 MHz, DMSO) δ 10.76 (s, 1H), 7.51 (dd, *J* = 8.6, 2.4 Hz, 1H), 7.24 (dd, *J* = 5.0, 1.1 Hz, 1H), 7.19 (dd, *J* = 9.0, 2.4 Hz, 1H), 6.9 –6.83 (m, 2H), 6.81 (dd, *J* = 8.6, 4.4 Hz, 1H), 4.13 (t, *J* = 6.6 Hz, 2H), 3.27 (t, 2H). ^{13}C-NMR (126 MHz, DMSO) δ 159.62, 153.62, 144.11, 142.68, 136.27, 135.76, 129.83, 127.22, 125.72, 124.65, 114.42, 113.25, 53.26, 31.58, ESI-MS: M+1 *M/z* 275.32.

1-((diethylamino)methyl)-3-(2-(thiophen-2-yl)ethylimino)-5-fluoroindolin-2-one (ITF 2)

Yield: 88%; M.P.: 186-188°C, ^{1}H-NMR (400 MHz, DMSO) δ 7.67–7.57 (m, 2H), 7.30 – 7.18 (m, 2H), 6.97 (dd, *J* = 8.4, 2.2 Hz, 1H), 6.71 (t, *J*= 8 Hz, 1H), 4.52 (s, 2H), 4.31 (t, *J*= 6.5 Hz, 2H), 3.02 (t, *J* = 6.5 Hz, 2H), 2.71 (t, *J* = 6.3 Hz, 4H), 1.08 (t, *J* = 6.2 Hz, 6H).^{13}C-NMR (126 MHz, $CDCl_3$) δ 153.80, 152.11, 141.97, 130.21, 128.94, 127.90, 127.53, 127.38, 127.30, 127.07, 110.55, 107.57, 64.12, 53.38, 38.28, 29.70, 22.00 ESI-MS: M+1 *M/z* 360.91

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-((pyrrolidin-1-yl)methyl) indolin-2-one (ITF 3)

Yield: 74 %; M.P.: 167-169°C, IR(cm^{-1}): 3190.9644, 2950. 6964, 1731.5390, 1699.1109, 1557.6974, 1434.9658, 1265.6523, 1175.6161, 871.6475, 728.1632, ^{1}H-NMR (400 MHz, DMSO) δ 7.65–7.51 (m, 2H), 7.40–7.20 (m, 2H), 6.90 (t, *J* = 7.9 Hz, 1H), 6.71 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.68 (s, 2H), 4.20 (t, *J* = 6.6 Hz, 2H), 3.28 (t, *J* = 6.6 Hz, 2H), 2.89 – 2.84 (t, *J* = 4.0 Hz, 4H), 1.81 (t, *J* = 4.0 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 183.15, 158.90, 151.59, 151.10, 138.39, 129.16, 125.35, 123.96, 120.08, 117.61, 116.35, 111.76, 62.37, 51.57, 50.74, 49.18, 49.11 ESI-MS: M+1 *M/z* 358.73.

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-((piperazin-1-yl)methyl) indolin-2-one (ITF 4)

Yield: 80%; M.P.: 159-160°C, ^{1}H-NMR (400 MHz, DMSO) δ 7.75 – 7.62 (m, 2H), 7.32 – 7.17 (m, 2H), 6.95 (t, *J* = 8 Hz, 1H), 6.71 (dd, *J* = 8.1, 2.4 Hz, 1H), 4.75 (s, 2H), 4.70–4.65 (m, 1H), 4.31 (t, *J* = 6.6 Hz, 2H), 3.15 (t, *J* = 6.6 Hz, 2H), 2.89 (t, 4H), 2.78 (t, 4H). ^{13}C-NMR (126 MHz, DMSO) δ 179.99, 162.79, 159.88, 151.74, 143.94, 132.82, 127.29, 126.84, 123.58, 121.67, 112.95, 112.69, 66.68, 66.66, 59.51, 53.89, 49.56, ESI-MS: M+1 *M/z* 373.80

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-((4-phenylpiperazin-1-yl) methyl)indolin-2-one (ITF 5)

Yield: 72%; M.P. 181-183°C, IR(cm^{-1}): 3080.0084, 2955.5341, 2922.6091, 1726.0572, 1597.1087, 1463.0840, 1276.7926, 1191.2413, 1056.6402, 988.5214, 730.3508 ^{1}H-NMR (400 MHz, DMSO) δ 7.58 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.32 (td, *J* = 9.0, 2.3 Hz, 1H), 7.27 – 7.19 (m, 2H), 7.10 (t, *J* = 7.9 Hz, 2H), 6.95 – 6.77 (m, 4H), 6.67 (t, *J* = 7.2 Hz, 1H), 4.43 (s, 2H), 4.18 (t, *J* = 6.5 Hz, 2H), 3.29 (t, *J* = 6.5 Hz, 2H), 3.00 (t, 4H), 2.59 (t, *J* = 5.0 Hz, 4H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 168.90, 166.18, 164.17, 163.48, 163.10, 152.18, 137.08, 136.29, 131.90, 131.84, 128.62, 128.29, 124.60, 111.73, 107.04, 102.50, 40.22, 40.10, 31.11, 29.70, 24.13; ESI-MS: M+1 *M/z* 449.85

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-((piperidin-1-yl)methyl) indolin-2-one (ITF 6)

Yield: 67 %; M.P.: 177-179°C, IR(cm^{-1}): 3150. 5396, 2950.5658, 1744.2803, 1651.5392, 1598.2056, 1473.2304, 1257.4454, 908.7893, 810.6097, 720.5053, ^{1}H-NMR (400 MHz, DMSO) δ 7.59–7.50 (m, 2H), 7.32–7.15 (m, 2H), 6.97 (t, *J* = 7.9 Hz, 1H), 6.71 (dd, *J* = 8.5, 2.2 Hz, 1H), 4.71 (s, 2H), 4.10 (t, *J* = 6.6 Hz, 2H), 3.25 (t, *J* = 6.5 Hz, 2H), 2.73 (t, 4H), 1.66 (m, 6H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 169.11, 163.53, 158.72, 141.55, 140.67, 129.41, 128.06, 118.89, 117.01, 115.97, 113.61, 113.16, 66.79, 66.61, 55.02, 54.15, 52.67, 50.94; ESI-MS: M+1 *M/z* 372.88

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-(morpholinomethyl)indolin-2-one (ITF 7)

Yield 89%; M.P.: 168-169°C, IR(cm^{-1}):: 3190.5240, 2949.5435, 1731.1042, 1685.6272, 1656.7267, 1600.7740, 1558.4428, 1542.3421 1522.2959. ^{1}H-NMR (400 MHz, DMSO) δ 7.59–7.50 (m, 2H), 7.30–7.18 (m, 2H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.71 (dd, *J* = 8.1, 2.4 Hz, 1H), 4.65 (s, 2H), 4.36 (t, *J* = 6.5 Hz, 2H), 3.62 (t, 4H), 3.20 (t, *J* = 6.5 Hz, 2H), 2.83 (t, 4H). ESI-MS: M+1 *M/z* 374.87.

3-(2-(thiophen-2-yl)ethylimino)-5-fluoro-1-((4-methylpiperazin-1-yl) methyl)indolin-2-one (ITF 8)

Yield: 78%; M.P. 167-169°C, IR(cm^{-1}): 3051.5657, 2823.3506, 1728.9289, 1602.9915, 1496.1849, 1268.4834, 919.2086, 793.4854, ^{1}H-NMR (400 MHz, DMSO) δ 7.65–7.56 (m, 2H), 7.34–7.22 (m, 2H), 6.95 (t, *J* = 7.8 Hz, 1H), 6.71 (dd, *J* = 8.4, 2.4 Hz, 1H), 4.73 (s, 2H), 4.45 (t, *J* = 6.5 Hz, 2H), 3.36 (t, *J* = 6.5 Hz, 2H), 2.71 (t, 4H), 2.61 (t, 4H), 2.31 (s, 3H). ^{13}C-NMR (126 MHz, $CDCl_3$) δ 168.77, 157.63, 153.60, 142.38, 140.53, 137.35, 128.94, 128.87, 127.12, 127.06, 126.69, 112.78, 59.60, 42.19, 40.62, 29.72, 22.08, 15.07, ESI-MS: M+1 *M/z* 387.66

In vitro anti-inflammatory activity

The anti-inflammatory activity of conjugates was investigated by analyzing percentage inhibition of denaturation of the BSA technique[19,25-28] using the reported method. The sample containing test compound and 1% aqueous solution of the BSA fraction was prepared and the pH of the mixture was adjusted to pH 6.3 using a small amount of dilute hydrochloric acid. (0.05 ml of saline solution was used in place of the drug in the control sample). Further, test samples were incubated for 20 min, and then, the mixture was heated to 57°C for 20 min. After cooling and the turbidity of the sample was measured at 660 nm using a spectrophotometer. The diclofenac sodium was used as the standard drug. The anti-inflammatory activities of the conjugates were estimated based on the percentage of inhibition of albumin denaturation using the following equation,

% inhibition = [(control absorbance-sample absorbance)/control absorbance]*100

Antitubercular activity

The anti-tubercular activity of conjugates was performed against *Mycobacterium tuberculosis* (H37 RV strain, ATCC 27294). Sterile deionized water of 200µl was added to outer perimeter wells to reduce evaporation of medium in the test wells during incubation. The wells plate filled with 100 µl of the Middlebrook 7H9 broth and serial dilution of samples were made on the plate. The final compound concentrations investigated were 100 to 0.2 µg/ml. Plates were covered with parafilm and incubated at 37°C for five days. After that, 25µl of prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hr. The blue color in the well indicated no bacterial growth, and the pink color indicated the growth of strain. The MIC values were calculated to study the inhibition at minimum concentrations.[29]

Molecular docking

The molecular docking study was performed on the PyRx suite based on the Autodock program[30,31] and visualization in Discovery studio visualizer v19.1.0.18287. For docking analysis, PDB: 3LN1 was selected from the RCSB protein data bank (*https://www.rcsb.org*) with a **resolution:** 2.4 Å. PDB: 3LN1 is a structure of celecoxib structure bound at the COX-2 binding site. For docking analysis, the protein file was prepared by adding missing atoms and residues in the receptor structure. Grid for docking study was selected where the co-crystallized was ligand bound. Interactions exhibited with the designed molecules and co-crystallized ligand with protein were studied.

In silico ADME and Drug-likeness study

The factor affecting ADME such as Hydrogen Bond Donors (HBD), Hydrogen Bond Acceptors (HBA), Gastrointestinal (GI) absorption, Log P, CYP2C19 inhibition, CYP2C9 inhibition, CYP3A4 inhibition, etc, were analyzed on the online server SwissADME (http://www.swissadme.ch/).[32,33]

RESULTS

Chemistry

Schiff's base was synthesized by treating dissolved 5-Fluoroisatin in warm ethanol with thiophene-2-ethylamine using two drops of glacial acetic acid (GAA) as a catalyst. (Scheme 1) The conjugates ITF 2- ITF 8 were synthesized by reacting synthesized Shiff base with an excess of 37% formaldehyde and secondary amines equimolar to Schiff base. The yields of synthesized compounds were in the range of 67–88%.

Anti-inflammatory activity

Synthesized conjugates were tested for their *in vitro* anti-inflammatory activity against BSA. Among the synthesized conjugates, ITF 5 showed the highest percentage inhibition. The compounds ITF 1-ITF 8 showed good percentage inhibition as compared to standard. (Figure 1) (Table 1).

The compounds showed weak to good antitubercular activity against the strain of MTB $H37R_v$. The compound containing N-phenylpiperazine Mannich base (ITF 5) exhibited inhibition of growth above 6.25 µg/ml. (Table 2)

Docking

The Molecular docking study of conjugates was carried out using COX-2 enzyme structure as target (PDB: 3LN1). The binding site contains VAL509, ALA513, LEU520, PHE367, PHE191, PHE195, SER516, TYR371, VAL335, GLY512, PHE504, MET508, LEU338, ALA502, ARG499, and HIS75 in the proximity of 4 Å.

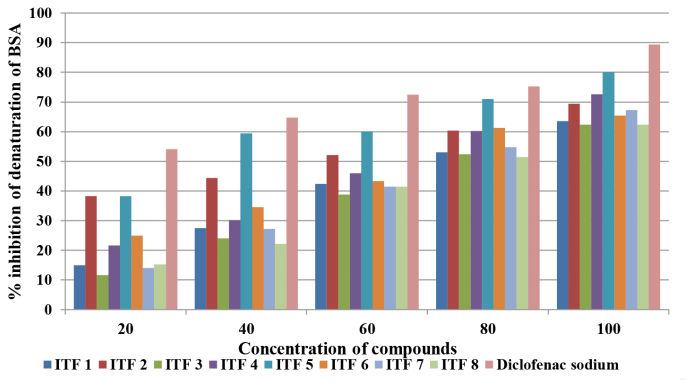


Figure 1: Anti-inflammatory activity of compounds.

Table 1: Anti-inflammatory activity of the compounds.

Comp.	% inhibition of BSA denaturation					Binding energy*
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL	
ITF 1	14.95	27.52	42.38	53.00	63.53	-8.4
ITF 2	38.23	44.33	52.12	60.35	69.46	-8.2
ITF 3	11.68	23.98	38.84	52.38	62.38	-8.2
ITF 4	21.68	30.08	45.92	60.17	72.56	-9.3
ITF 5	38.23	59.38	60.08	70.97	80.08	-8.7
ITF 6	24.95	34.60	43.27	61.23	65.39	-8.2
ITF 7	14.07	27.16	41.50	54.77	67.25	-8.4
ITF 8	15.22	22.21	41.50	51.50	62.38	-7.9
Diclofenac sodium	54.15	64.69	72.47	75.22	89.38	-9.3

%: Percentage, *: Lower binding energy values indicate higher docking score

In silico ADME

All of the compounds were found to be within the permissible drug-likeness parameters, which include M.W., HBD, HBA, and log P.[34] The conjugates were predicted to have high gastrointestinal absorption. Except conjugate ITF 4, all the compounds are predicted as (Blood Brain Barrier) BBB permeants.

DISCUSSION

The synthesis procedures of isatin Schiff and Mannich bases reported in the literature were referred to synthesize the molecule of the present research work.[21-24] The synthesis of Schiff base conjugate of 5-Fluoroisatin with thiophene-2-ethylamine and its Mannich bases was efficiently accomplished using the reported method. The Schiff base conjugate was synthesized by commonly used procedures. Glacial acetic acid as catalyst and followed by continuous stirring was done to synthesize the Schiff base of 5-Fluoroisatin and thiophene-2-ethylamine. For the synthesis of Mannich bases, the very slow speed of stirring (60 RPM) and slow addition of secondary amine with cooling in an ice bath is important while performing the reaction followed by recrystallization from chloroform and non-polar solvents like hexane or petroleum ether. Some modifications in reported methods were performed to carry out synthesis with good yield. The singlet at 4.5 to 5.0 δppm having integration of two hydrogen equivalents in ^{1}H NMR confirms the Mannich base formation.

In IR spectra the peak at the carbonyl region shows the presence of -N-C=O group of the 5-Fluoroisatin moiety.

Indomethacin and tenidap are known NSAIDs having indole nucleus and having substituents at C-3, C-5, and the nitrogen atom of indole moiety.[35,36] The molecules of the present research work are designed based on studying these features. The interaction of clinically proven anti-inflammatory medicines with certain proteins has shown that these medicines actively inhibit the denaturation of protein.[19] The estimation of compounds potential of inhibition of denaturation Bovine Serum Albumin is considered a method for anti-inflammatory activity study.[25] The Piperazine containing compounds ITF 4, ITF 5, and ITF 8 showed 72.56, 80.08, and 62.38 % inhibition, respectively, where the standard drug diclofenac sodium showed 89.38 % inhibition of denaturation of bovine serum albumin. (Table 1) It can be concluded from the structure-activity (SAR) analysis that the substitution of N-phenyl Piperazine containing Mannich bases favors the inflammatory activity of synthesized Schiff base conjugate of 5-Fluoroisatin with thiophene-2-ethylamine. The compound containing methyl group at the nitrogen of piperazine leads to decrement in activity as compared to unsubstituted nitrogen atom having molecule. (Figure 1)

Table 2: Antitubercular activity of compounds.

Sr. No.	Sample code	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
1	ITF 1	S	S	R	R	R	R	R	R
2	ITF 2	S	S	R	R	R	R	R	R
3	ITF 3	S	S	R	R	R	R	R	R
4	ITF 4	S	S	S	R	R	R	R	R
5	ITF 5	S	S	S	S	S	R	R	R
6	ITF 6	S	S	R	R	R	R	R	R
7	ITF 7	S	S	S	S	R	R	R	R
8	ITF 8	S	S	S	S	R	R	R	R
9	Isoniazid	S	S	S	S	S	S	S	R

S: Sensitivity, R: Resistance, Sensitivity at lower concentration indicates better activity

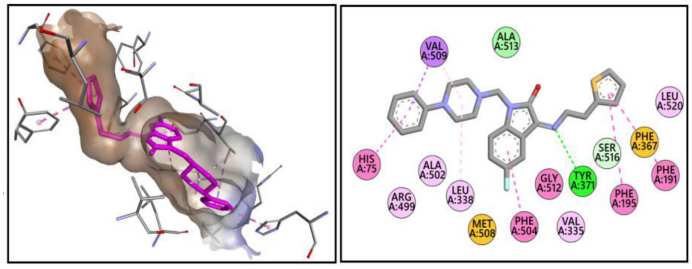


Figure 2: Docking pose and 2D interaction diagram of ITF 7.

The Mannich bases of isatin-Schiff base conjugates are reported in literature mainly for their potent anti-tubercular activities.[22,23] The substitution of morpholine and piperazine containing substituents as Mannich base substrate also improves the anti-tubercular activity substantially. The Mannich bases derivatives containing substituents like pyrrolidine, diethylamine, and piperidine did not show improvement in inhibition of growth of MTB H37Rv strain. The N-phenyl piperazine containing compound ITF 5 (inhibition up to 6.25 µg/ml) shows the highest activity where standard drug isoniazid showed inhibition of growth of MTB H37Rv strain up to 1.6 µg/ml. From the SAR it can be stated that hydrophobic groups like phenyl ring at the nitrogen atom of piperazine ring may improve the anti-tubercular activity (Table 2).

A significant number of hydrophobic sites were detected during the binding site study, indicating that the binding site has a high potential for hydrophobic interaction. Conjugates (ITF 1- ITF 8) have shown significant interactions at the binding site amino acids. (Figure 2) The binding energies were found in the range of -7.9 to -9.3. The most active compound ITF 5 showed a better binding energy score as compared to other compounds.

More than half of the candidates in drug development failed due to ADME insufficiencies. To avoid such failures, ADME screens are necessary to analyse for the compounds in the discovery process to eliminate those that are likely to fail later.[37] To evaluate the drug-likeness and ADME various parameters were studied. All of the compounds were found to be within the permissible according to Lipinski's rule of five. All the conjugates were predicted as inhibitors of CYP2C19, CYP2C9, and CYP3A4 enzymes. (Table 3)

CONCLUSION

The conjugate ITF 5 was found to be the most effective against MTB h37Rv and to inhibit BSA denaturation better than any other synthesized compound. The compound ITF 5 showed 80.08 % inhibition of BSA denaturation at 100 µg/mL and showed the inhibition of growth of *MTB* h37Rv up to 6.25 µg/ml. It can be concluded that the Mannich bases of 5-Fluoroisatin-thiophene-2-ethylamine Schiff base conjugate with

Table 3: *In silico* ADME properties of the compounds.

Comp.	M. W.	HBA	HBD	iLOGP	GI Absorption	BBB permeant	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP3A4 inhibitor
ITF 1	274.31	3	1	2.45	High	Yes	Yes	Yes	Yes	Yes
ITF 2	359.46	4	0	3.42	High	Yes	Yes	Yes	Yes	Yes
ITF 3	357.45	4	0	3.15	High	Yes	Yes	Yes	Yes	Yes
ITF 4	372.46	5	1	3.35	High	No	Yes	Yes	Yes	Yes
ITF 5	448.56	4	0	3.62	High	Yes	No	Yes	Yes	Yes
ITF 6	371.47	4	0	3.9	High	Yes	Yes	Yes	Yes	Yes
ITF 7	373.44	5	0	3.63	High	Yes	Yes	Yes	Yes	Yes
ITF 8	386.49	5	0	3.71	High	Yes	Yes	Yes	Yes	Yes

piperazine containing compounds and hydrophobic substituents on Piperazine ring could improve the anti-TB and anti-inflammatory activities. Docking analysis revealed that the compounds show good interactions with the COX-2 enzyme binding site.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

ADME: Absorption, Distribution, Metabolism, and Excretion; **MTB:** *Mycobacterium tuberculosis*; **SAR:** Structure-Activity Relationship; **BSA:** Bovine Serum Albumin; **MP:** Melting Point; **IR:** Infra-Red; **UV:** Ultraviolet; **COX-2:** Cyclooxygenase-2; **RPM:** Revolutions Per Minute; **NMR:** Nuclear Magnetic Resonance; **MS:** Mass Spectroscopy; **CHN:** Carbon, Hydrogen, Nitrogen; **^{1}H -NMR:** Proton Nuclear Magnetic Resonance; **^{13}C-NMR:** Carbon-13 Nuclear Magnetic Resonance; **$CDCl_3$:** Deuterated Chloroform; DMSO: Dimethyl Sulfoxide; **PDB:** Protein Data Bank; **M.W.:** Molecular Weight; **IlogP:** Partition Coefficient; **HBA:** Hydrogen Bond Acceptors; **HBD:** Hydrogen Bond Donors.

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