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Novel Antitubercular Derivatives of Mannich and Schiff Bases of *p*-Aminosalicylic Acid – Rational Design

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Authors' contributions

This work was carried out in collaboration among all authors. Author NBM designed the study, performed computational studies, bench work, wrote the protocol, and wrote the first draft of the manuscript. Authors SD and VSS guided throughout the research, managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the current study is to utilize computational drug design resources to develop and identify promising structural analogs of p-aminosalicylic acid (PAS), to improvise antitubercular activity followed by their synthesis, characterization, and *in-vitro* biological activity determination.
Study Design: Design of the structural analogs of PAS by functional group modification at -COOH and -NH_2 groups followed by *in-silico* prediction of biological activity, toxicity, drug-likeness filters, and molecular docking study to select promising analogs. Synthesis of selected analogs, structural characterization, and screening of biological activity of the same.
Methodology: Using the Prediction of Activity Spectrum of Substances (PASS Online) database, prediction of biological activity was performed for PAS and 22 designed structural analogs of PAS. All these analogs were screened for their drug-likeness properties using Lipinski's rule of five. Later all these 22 analogs were predicted for their toxicities using OSIRIS Property Explorer. At this

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stage, out of 22 analogs, only 6 analogs were selected to go for molecular docking using AutoDock Tools and AutoDock Vina for the determination of their binding energies by comparing with PAS. The selected 6 analogs were synthesized using three-step syntheses. The synthesized analogs were screened for their antitubercular activity using the Microplate Alamar Blue Assay (MABA) method.

Results: Overall 22 structural analogs were designed and screened for their estimated activity (all analogs showed antitubercular activity as primary activity), drug-likeness (all analogs passed), and toxicities (only 10 analogs passed) using computational tools. Out of 22 analogs, 6 analogs were selected and performed molecular docking using AutoDock Vina. All 6 analogs showed better binding affinity than PAS. These 6 analogs (7a-f) were synthesized, characterized, and screened for their *in-vitro* antitubercular activity. Results showed that 5 analogs, 7b-7f, showed excellent antitubercular potency greater than PAS and equipotent activity to that of standard drugs. Analog 7a was found to be less potent than PAS.

Conclusion: Hence, the structural analogs of PAS, 7b-7f, were found to have better antitubercular activity than the lead compound, PAS, and equipotent to that of the standards, streptomycin, ciprofloxacin, and pyrazinamide.

Keywords: p-Aminosalicylic acid; mannich bases; schiff's bases; autodock; analog design.

1. INTRODUCTION

Tuberculosis (Tb) is a venerable disease for ages unknown. Even today, there is no specific drug for the treatment and cure. Due to the discovery of several antibiotics and antibacterial drugs, the drug discovery on antitubercular drugs found poor significance. Since 1983 there is a steep fall in the emergence of approved drugs for Tb treatment [1,2]. PAS was documented to have 6% [3,4] of *Mycobacterium tuberculosis* (*MTb*) resistance that is minimal to any first-line Tb drugs. Clinically, monotherapy of PAS for Tb was also limited in the past 4-6 decades [2]. In general, the process involved in drug discovery is long process, expensive, time consuming, and challenging too. The computational drug discovery tools will help in optimizing this long, expensive, and time-consuming process and act as one of the reliable virtual shortcuts in today's research and development [5]. In this work, with the help of computational drug discovery tools, we had tried to synthesize structural analogs of a forgotten or second-line antitubercular drug, *p*-aminosalicylic acid (PAS), to improvise the potency by incorporating functional group modification concept on the free carboxylic acid group and the free amino group. This lead modification resulted in a total of 22 structural analogs among which 11 were N-acetyl derivatives with Schiff bases (methylidene hydrazides) and 11 were Mannich bases (imidazolyl methylamines) [6-9] with Schiff bases (methylidene hydrazides) (Fig. 1). Instead of opting for direct synthesis of the designed structural analogs without knowing whether they have the activity or not, these derivatives were subjected to predict for their possible biological activity, drug-likeness, and toxicity. Finally, the screened and selected analogs were taken for molecular docking studies with the target, dihydropteroate synthase-I enzyme (DHPS-I), which was downloaded from Protein Databank (PDB) website with PDB ID: 1EYE. Through molecular docking, binding energy for the selected structural analogs was calculated using AutoDock Tools and AutoDock Vina software. Structural analogs-target interactions were visualized through Discovery Studio Visualizer. With the support of these *in-silico* computational drug discovery tools, the finally selected structural analogs of PAS were synthesized and were characterized using infrared (IR), nuclear magnetic resonance (NMR), and mass spectral techniques. Later the characterized analogs were screened for their antitubercular activity using MABA.

2. MATERIALS AND METHODS

For sketching chemical structures of PAS and structural derivatives, ChemSketch software was employed. For the prediction of activity, the PASS Online web server had been utilized [10,11]. OSIRIS property explorer application was used for the prediction of toxicity [12-14] of the designed structural analogs. The prediction of activity and toxicity will be carried out by sketching the chemical structure in the window provided in the respective applications. Open Babel GUI used for converting .mol file to .pdb file to take up for molecular docking. Discovery Studio Visualizer, AutoDock Tools, and AutoDock Vina served the purpose of molecular

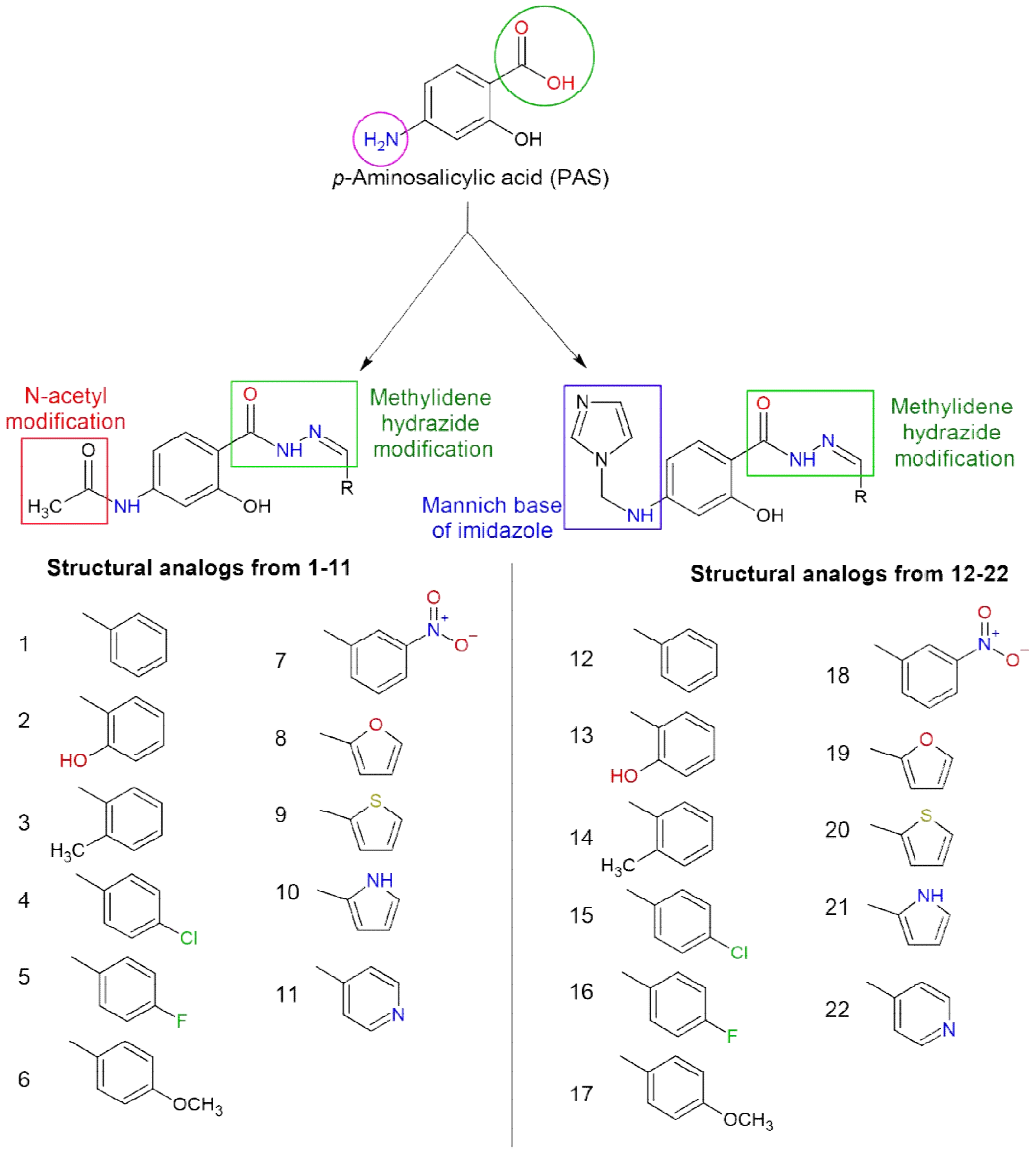


Fig. 1. Functional group modification on carboxylic acid and amino groups of PAS to design 22 analogs

docking. All the reagents and chemicals used in the current work were purchased from Sigma Aldrich. The method employed to determine melting point was an open capillary method and was uncorrected. To monitor the reactions, thin-layer chromatography (TLC) was performed. The Potassium bromide pellet (KBr) technique was used for recording IR spectra. Bruker 400 MHz NMR spectrometer was used for ^{1}H NMR and ^{13}C NMR spectra with DMSO-d6 as solvent. Shimadzu mass spectrometer was used to record mass spectrum using electrospray ionization technique in positive mode. MABA method was selected to carry out an antitubercular assay for the synthesized and characterized structural analogs of PAS [15].

2.1 General Procedure for Molecular Docking

To carry out molecular docking, AutoDock Tools (AutoDock 4.2) and AutoDock Vina [16-24] served the purpose. Supporting software and applications include, ChemSketch, Discovery Studio Visualizer, and Open Babel GUI were employed prepare for and complete the entire

docking process. ChemSketch was used to sketch in the chemical structures and saving as .mol files. Later these .mol files were converted to .pdb files using Open Babel GUI. From the protein data bank website, 1EYE, the target file was downloaded. Using Discovery Studio Visualizer, 1EYE target file was cleaned to remove water, salts, ions, and predocked ligands if any [25]. The cleaned target file was saved in the form of.pdb format. This .pdb file was opened using AutoDock Tools to process it further to be ready to go for docking like the addition of polar hydrogens, minimization of energy, and finally converted as .pdbqt format. Identification and defining a binding site in the processed target file, 1EYE, was performed using GRID option in AutoDock Tools. Then the structural analogs had been energy minimized using AutoDock Tools by determining the number of rotatable bonds, saved into .pdbqt format. At this instance, both, the target, and the structural analogs were subjected to actual molecular docking through AutoDock Vina using command prompt. After this binding energy was calculated for different possible best poses and was arranged in descending order. The output file was saved as .pdbqt which contains the binding poses. This file was used to visualize, determine, and save the binding poses along with binding pockets too using Discovery Studio Visualizer [25].

2.2 Synthetic Procedures

Synthesis of the designed, screened, and selected structural analogs were carried out in three-step syntheses using conventional synthetic methods like reflux and stirring at room temperature. The scheme of the synthesis was represented in Fig. 2.

2.2.1 Synthesis of mannich base derivative of *p*-Aminosalicylic acid(**3**)

Dissolve PAS, **1**, 0.01 mol in 20 ml of methanol, add 0.01 mol of imidazole,**2**, and 2 ml of 37% formaldehyde were added. For 5 hours, the reaction mixture was stirred at room temperature. Add distilled water in excess to the reaction mixture and left overnight. The formed solid was filtered and recrystallized using methanol and dried [26], resulted to give a sepia yellow product, **3**.

2.2.2 Synthesis of acid hydrazide fromfMannich base derivative of PAS (**5**)

A solution of **3**, 0.01 mol, Vilsmeier reagent, 0.01 mol, and trimethylamine, 0.03 mol in 100 ml of dichloromethane was prepared and added to the solution containing 0.04 mol hydrazine hydrate, **4**, in 50 ml of dichloromethane. The formed reaction mixture was stirred for 7 hours at room temperature. Wash the mixture with 150 ml of saturated sodium bicarbonate followed by 150 ml of brine and collect the organic layer. The organic layer was dried using anhydrous sodium sulfate. The resulted product was filtered and recrystallized with 95% ethyl alcohol [27] to get a rust yellow product.

2.2.3 Synthesis of mannich base containing methylidene hydrazide derivatives (**7a-f**)

Transfer 0.002 mol of **5** and 25 ml of ethyl alcohol into a round-bottomed flask. Add a solution of aldehydes (**6a-f**), 0.002 mol in 5 ml of ethanol each was added individually in presence of 2-3 drops of acetic acid. Reflux the contents under the water condenser for 4 hours using a water bath. Cool the reaction mixture and pour the contents on crushed ice. Filter the formed products (7a-f) and recrystallized with 95% ethyl alcohol [28].

2.3 *In-vitro* Antitubercular Activity

Microplate Alamar Blue Assay method was preferred to carryout antitubercular activity for the synthesized structural analogs [15,29-32]. By using MABA, the minimum inhibitory concentration (MIC) of structural analogs of PAS was determined. In this *in-vitro* assay, non-virulent vaccine strain, H37 RV strain, of *Mycobacterium tuberculosis* was employed using a sterilized 96-well plate. 200 µl of sterilized water was added to all peripheral walls to avoid desiccation during incubation. Middlebrook 7H9 broth stock, 100 µl was added to the microplate followed by serial dilutions of structural analogs and standards on the plate directly. To the wells containing analogs, add 100 µl of *MTb* suspension to make the final volume to 200 µl. The final concentrations of the test compounds were made to be within the range from 100 to 0.8 µg/ml. The plates were marked, sealed with paraffin, and incubated for 5 days at 37 ^{o}C. After incubating, 25 µl of 1:1 mixture of Alamar Blue reagent and 10% of Tween 80 were added into the wells of the plate and made to incubate for 24 hours. Finally, based on visual color change, the readings were recorded. Bacterial growth was indicated by the pink color in the well and the blue color indicates no bacterial growth [32].

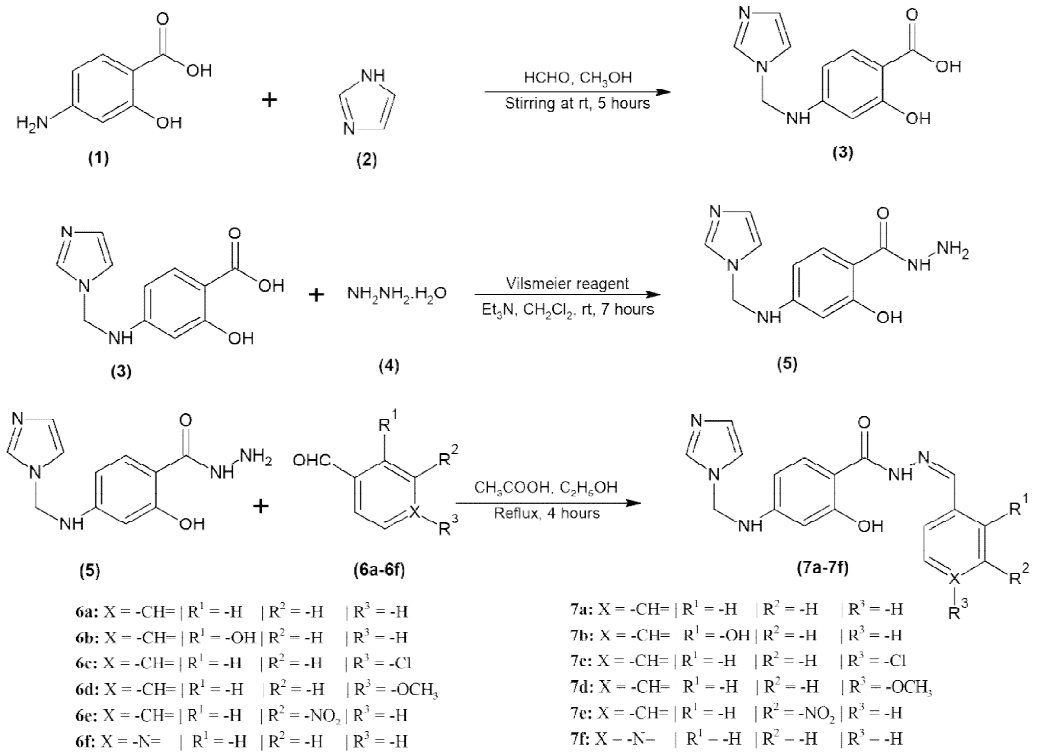


Fig. 2. Synthetic scheme for the synthesis of designed, screened, and structural analogs of PAS

3. RESULTS AND DISCUSSION

3.1 Prediction of Activity

Prediction of biological activity of the designed structural analogs was carried out using PASSOnline. The probability of being active (Pa) value for all 22 structural analogs showed superior antituberculosis or antituberculosic activity than PAS, the lead molecule as their primary activity (Table 1).

3.2 Prediction of Drug-likeness

For the determination of drug-likeness properties of the designed structural analogs, www.swissadme.ch web resource was used. In this web page, chemical structures of the analogs were sketched and converted to Simplified Molecular Input Line Entry System (SMILES) notation to obtain the results. Lipinski's rule of five drug-likeness filters was used to screen the analogs. All the 22 structural analogs passed drug-likeness filters (Table 2).

Following are the filters of Lipinski's Rule of Five:

- Molecular weight ≤ 500
- MLogP ≤ 4.15
- Number of hydrogen bond donors ≤ 10
- Number of hydrogen bond acceptors ≤ 5

3.3 *In-silico* Prediction of Toxicity

The prediction of toxicity for the designed structural analogs was performed using the OSIRIS property explorer application. Toxicities predicted include tumorigenic, mutagenic, irritant properties, and reproductive effects. Out of 22 analogs, analogs ranging from 12-18 and 20-22 showed no indication of toxicities (Table 3). Analogs from 1-11 including PAS showed medium to high risk of toxicities.

3.4 Molecular Docking Studies

Molecular docking was performed for the screened and selected 6 structural analogs, 12, 13, 15, 17, 18, and 22 (during synthesis labelled as 7a-7f respectively) with the drug target, DHPS-I obtained from protein data bank with PDB ID: 1EYE. All the 6 structural analogs

showed better binding energy than the lead molecule PAS. The binding energy of PAS was found to be -5.5 Kcal/mol and the binding energy of the structural analogs falls within the range of -7.0 to -7.9 Kcal/mol. This indicates the optimized structural analogs are having high binding affinity to DHPS-I than the lead molecule, PAS (Table 4). The protein-ligand binding interactions with the interacting amino acids were given in Table 4 and the best binding poses were given in Figs. 3 and 4. After functional group modification, the number of interacting amino acids of the target also increased with each structural analog than PAS indicating improved binding.

Table 1. Predicted antitubercular activity of designed structural analogs using PASSOnline

Derivatives	Pa Value	Derivatives	Pa Value
PAS	**0.612**	12	0.857
1	0.894	13	0.853
2	0.891	14	0.858
3	0.897	15	0.854
4	0.877	16	0.818
5	0.838	17	0.860
6	0.876	18	0.901
7	0.937	19	0.911
8	0.938	20	0.778
9	0.796	21	0.685
10	0.759	22	0.889
11	0.932		

Table 2. Drug-likeness properties of designed structural analogs of PAS

Derivatives	Molecular Weight	MLogP*	Hydrogen Bond Acceptors	Hydrogen Bond Donors	Drug-likeness Filter
PAS	153.14	-0.70	3	3	Passed
1	297.31	1.74	4	3	Passed
2	313.31	1.21	5	4	Passed
3	311.34	1.98	4	3	Passed
4	331.75	2.25	4	3	Passed
5	315.30	2.13	5	3	Passed
6	327.33	1.45	5	3	Passed
7	342.31	0.85	6	3	Passed
8	287.27	0.08	5	3	Passed
9	303.34	0.88	4	3	Passed
10	286.29	0.08	4	4	Passed
11	298.29	0.30	5	3	Passed
12	335.36	1.33	4	3	Passed
13	351.35	0.80	5	4	Passed
14	349.38	1.56	4	3	Passed
15	369.80	1.83	4	3	Passed
16	353.35	1.71	5	3	Passed
17	365.38	1.04	5	3	Passed
18	380.35	0.47	6	3	Passed
19	325.32	0.09	5	3	Passed
20	341.38	0.88	4	3	Passed
21	324.33	0.09	4	4	Passed
22	336.34	0.30	5	3	Passed

**MLogP: Lipophilicity*

Table 3. Toxicity risk prediction of designed compounds using OSIRIS property explorer

Derivatives	Toxic Risk Mutagenic	Tumorigenic	Irritant	Reproductive
PAS	**High**	**High**	**High**	**Medium**
1	Medium	Medium	High	No indication
2	Medium	Medium	High	No indication
3	Medium	Medium	High	No indication
4	Medium	Medium	High	No indication
5	Medium	Medium	High	No indication
6	Medium	Medium	High	No indication
7	Medium	Medium	High	No indication
8	Medium	Medium	High	No indication
9	Medium	Medium	High	No indication
10	Medium	Medium	High	No indication
11	Medium	Medium	High	No indication
12	No indication	No indication	No indication	No indication
13	No indication	No indication	No indication	No indication
14	No indication	No indication	No indication	No indication
15	No indication	No indication	No indication	No indication
16	No indication	No indication	No indication	No indication
17	No indication	No indication	No indication	No indication
18	No indication	No indication	No indication	No indication
19	High	No indication	No indication	No indication
20	No indication	No indication	No indication	No indication
21	No indication	No indication	No indication	No indication
22	No indication	No indication	No indication	No indication

Table 4. AutoDock Vina binding energies of selected structural analogs of PAS (7a-f) with 1EYE as target

Derivatives	Synthetic Analog Code	Binding Energy (Kcal/mol)	Definitions of Binding Site
PAS	**PAS**	**-5.5**	ASN A:189, LYS A:213, ARG A:214, PHE A:215
12	7a	-7.0	ASN A:189, LYS A:213, ARG A:214, ARG A:215, ARG A:233
13	7b	-7.8	ASP A:21, GLY A:50, ASP A: 86, MET A:88, VAL A:107, PHE A:182, LYS A:213, ARG A:253, HIS A:255
15	7c	-7.6	ASP A:21, ASP A:86, MET A:88, VAL A:107, HIS A:141, PHE A:182, LYS A:213, ARG A:253, HIS A:255
17	7d	-7.7	ASP A:21, GLY A:50, GLU A:65, ASP A:86, MET A:88, VAL A:107, HIS A:141, PHE A:182, LYS A:213, ARG A:253, HIS A:255
18	7e	-7.9	VAL A:11, ASP A:21, HIS A:141, THR A:185, ALA A:186, LYS A:213, ARG A:214, ARG A:253, HIS A:255
22	7f	-7.5	VAL A:11, ASP A:21, GLY A:181, LYS A:213, ARG A:214, ARG A:253, HIS A:255

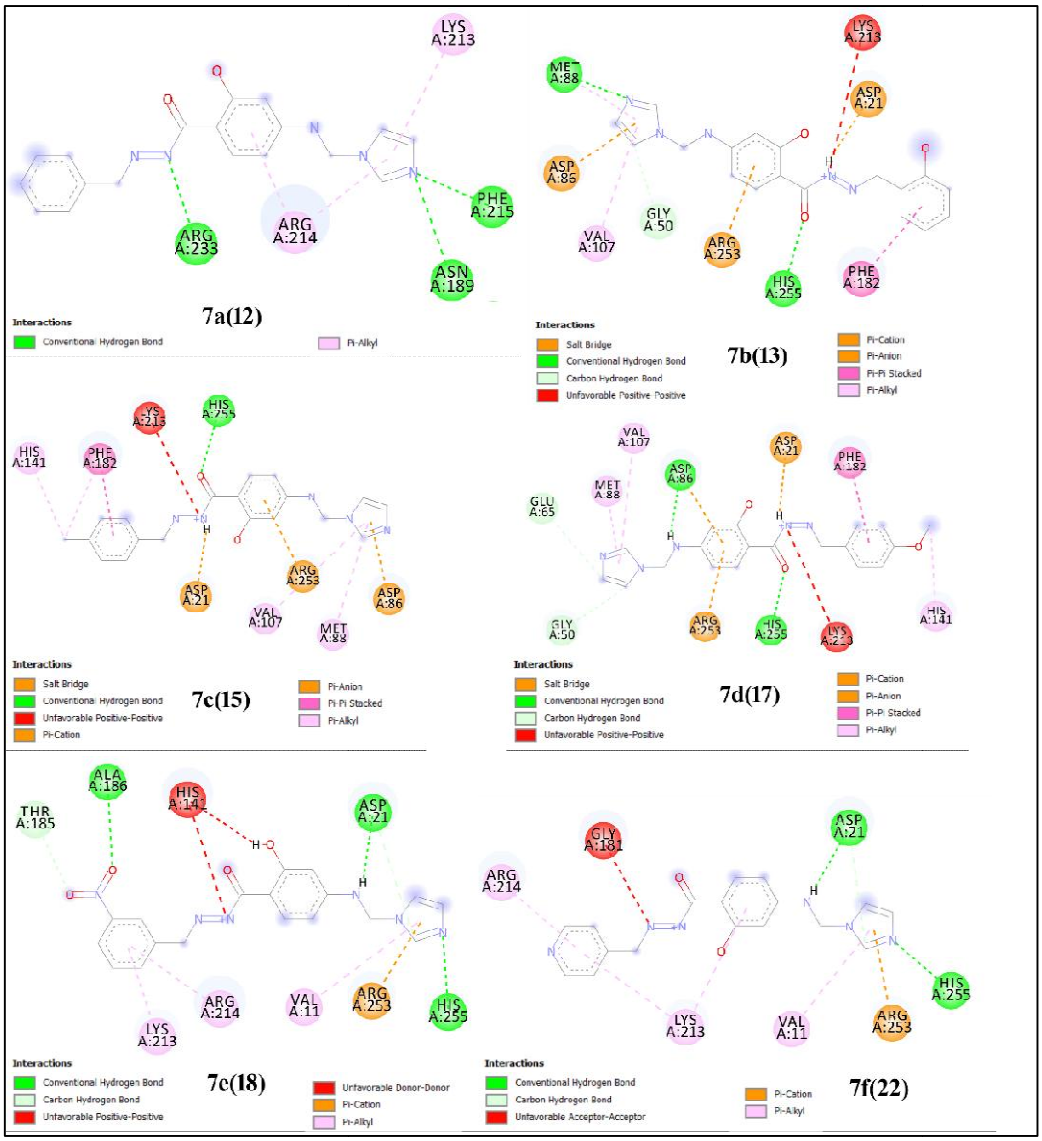


Fig. 3. 2D binding poses of 7a-7f, structural analogs of PAS along with bound amino acids

3.5 Spectral Data

Compound 7a

Yield:81.2%, **m.p. (^{0}C):**230-232, Solid and Golden yellow, **IR (KBr) cm^{-1}:**3325, 3050, 2948, 1621, 1585, 1483, 1444, 1377, 1102; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.40 (s, 1H), 11.57 (s, 1H), 7.93 (t, *J* = 1.7 Hz, 1H), 7.79 (t, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.67 – 7.58 (m, 2H), 7.49 – 7.37 (m, 4H), 7.30 (dd, *J* = 4.0, 1.8 Hz, 1H), 7.06 (dd, *J* = 4.0, 1.6 Hz, 1H), 6.18 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.13 (d, *J* = 1.9 Hz, 1H), 5.49 (d, *J* = 3.8 Hz, 2H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.64, 160.41, 149.89, 147.58, 135.51, 134.14, 131.05, 130.31, 130.19, 128.75, 127.62, 117.79, 112.31, 104.70, 100.01, 57.30; **Mass:** (M+1) - 336.25

Compound 7b

Yield: 84.8%, **m.p. (^{0}C):**246-248, Solid and Marigold orange, **IR (KBr) cm^{-1}:**3326, 3046, 2854, 1618, 1573, 1483, 1432, 1374, 1103; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.83 (s, 1H), 12.32 (s, 1H), 11.57 (s, 1H), 7.93 (t, *J* = 1.7 Hz, 1H), 7.79 (t, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.48 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.42 (s, 1H), 7.34 – 7.25 (m, 2H), 7.06 (dd, *J* = 4.0, 1.6 Hz, 1H), 6.99 – 6.88 (m, 2H), 6.18 (dd, *J* = 8.2, 1.8

Hz, 1H), 6.13 (d, *J* = 1.9 Hz, 1H), 5.49 (d, *J* = 3.8 Hz, 2H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.63, 160.41, 157.82, 149.89, 146.55, 135.51, 132.65, 131.05, 130.29 (d, *J* = 19.4 Hz), 120.98, 117.79, 117.11, 115.14, 112.69, 104.70, 100.01, 57.30; **Mass:** (M+1) – 352.42

Compound 7c

Yield:79.2%, **m.p. (^{0}C):**239-241, Solid and Honey yellow, **IR (KBr) cm^{-1}:**3373, 3047, 2943, 1623, 1587, 1485, 1428, 1378, 1088, 813; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 11.57 (s, 1H), 7.93 (t, *J* = 1.7 Hz, 1H), 7.79 (t, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.69 – 7.63 (m, 2H), 7.55 – 7.47 (m, 3H), 7.30 (dd, *J* = 4.0, 1.8 Hz, 1H), 7.06 (dd, *J* = 4.0, 1.6 Hz, 1H), 6.18 (dd, *J* = 8.2, 1.8 Hz, 1H), 6.13 (d, *J* = 1.9 Hz, 1H), 5.49 (d, *J* = 3.8 Hz, 2H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.64, 160.41, 149.89, 147.59, 136.63, 135.51, 131.79, 131.05, 130.19, 129.06, 128.65, 117.79, 112.31, 104.70, 100.01, 57.30; **Mass:** (M+2) – 371.52

Compound 7d

Yield: 82.7%, **m.p. (^{0}C):**241-243, Solid and Butterscotch yellow, **IR (KBr) cm^{-1}:**3370, 3113, 2968, 2840, 1622, 1602, 1508, 1440, 1379, 1251, 1109, 1024; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 11.57 (s, 1H), 7.93 (t, *J* = 1.7 Hz, 1H), 7.79 (t, *J* = 3.9 Hz, 1H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.53 – 7.45 (m, 3H), 7.30 (dd, *J* = 4.0, 1.8 Hz, 1H), 7.09 – 7.03 (m, 1H), 7.03 – 6.98 (m, 2H), 6.18 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.13 (d, *J* = 1.9 Hz, 1H), 5.49 (d, *J* = 3.8 Hz, 2H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.64, 161.35, 160.41, 149.89, 147.70, 135.51, 131.05, 130.19, 128.59, 126.93, 117.79, 114.32, 112.31, 104.70, 100.01, 57.30, 55.33; **Mass:** (M+1) – 366.22

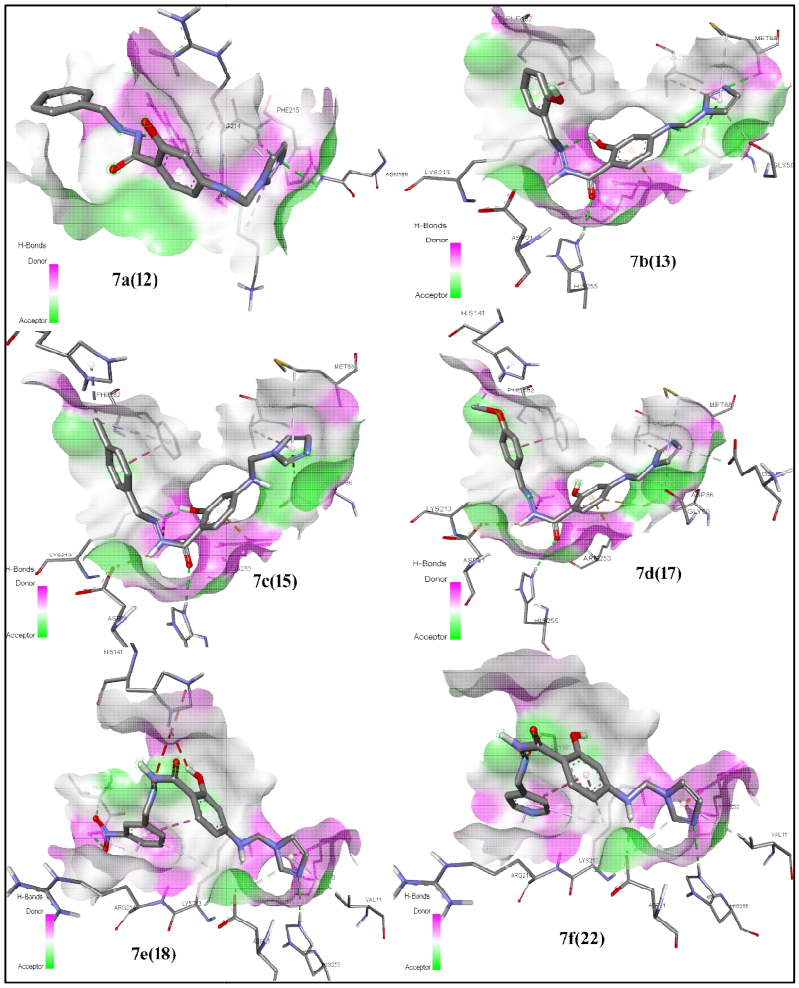
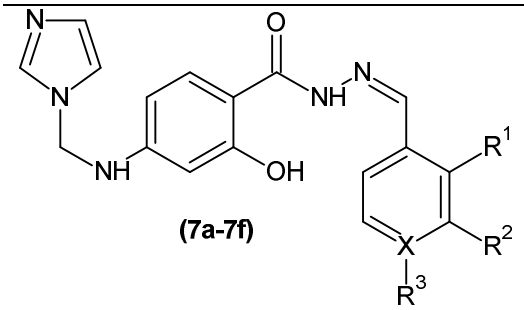


Fig. 4. 3D binding poses of 7a-7f, structural analogs of PAS along with bound amino acids

Table 5. Drug-likeness properties of designed structural analogs of PAS



(7a-7f)

Compound	X	R^1	R^2	R^3	MIC Values (µg/ml)
PAS					6.25
7a	C	-H	-H	-H	12.5
7b	C	-OH	-H	-H	3.125
7c	C	-H	-H	-Cl	3.125
7d	C	-H	-H	$-OCH_3$	3.125
7e	C	-H	$-NO_2$	-H	1.6
7f	N	-H	-H	----	3.125
Streptomycin					6.25
Ciprofloxacin					3.125
Pyrazinamide					3.125

Compound 7e

Yield: 80.1%, **m.p. (^{0}C):**256-258, Solid and Canary yellow, **IR (KBr) cm^{-1}:**3369, 3160, 3085, 2970, 1625, 1587, 1526, 1435, 1377, 1350, 1078; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.51 (s, 3H), 11.57 (s, 3H), 8.41 (t, *J* = 1.9 Hz, 3H), 8.16 – 8.09 (m, 3H), 7.93 (s, 1H), 7.93 – 7.86 (m, 5H), 7.79 (t, *J* = 3.9 Hz, 3H), 7.76 – 7.69 (m, 6H), 7.65 (dd, *J* = 8.4, 7.4 Hz, 3H), 7.30 (dd, *J* = 4.0, 1.8 Hz, 3H), 7.06 (dd, *J* = 4.0, 1.6 Hz, 3H), 6.18 (dd, *J* = 8.2, 1.8 Hz, 3H), 6.13 (d, *J* = 1.9 Hz, 3H), 5.49 (d, *J* = 3.8 Hz, 6H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.64, 160.41, 149.89, 147.40, 144.62, 135.51, 134.47, 131.93, 131.05, 130.19, 129.39, 124.10, 121.82, 117.79, 112.31, 104.70, 100.01, 57.30; **Mass:** (M+1) – 381.35

Compound 7f

Yield:78.6%, **m.p. (^{0}C):**233-235, Solid and Honey orange, **IR (KBr) cm^{-1}:**3378, 3088, 2854, 1697, 1583, 1488, 1425, 1367, 1089, 1055; **^{1}H NMR** (400 MHz, DMSO-d_6) δ 13.42 (s, 1H), 11.57 (s, 1H), 8.69 – 8.63 (m, 2H), 7.93 (t, *J* = 1.7 Hz, 1H), 7.83 – 7.76 (m, 2H), 7.72 (d, *J* = 8.2 Hz, 1H), 7.61 – 7.56 (m, 2H), 7.30 (dd, *J* = 4.0, 1.8 Hz, 1H), 7.06 (dd, *J* = 4.0, 1.6 Hz, 1H), 6.18 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.13 (d, *J* = 1.9 Hz, 1H), 5.49 (d, *J* = 3.8 Hz, 2H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ 163.64, 160.41, 151.69, 149.89, 145.88, 135.51, 131.50, 131.05, 130.19, 122.32, 117.79, 112.31, 104.70, 100.01, 57.30; **Mass:** (M+1) – 337.31

3.6 *In-vitro* Antitubercular Activity (MABA)

The *In-vitro* antitubercular assay was performed on *MTb* non-virulent strain for the estimation of MIC values with streptomycin, ciprofloxacin, and pyrazinamide as standard drugs [33,34]. Based on the results of MABA, the MIC of PAS was found to be 6.25 µg/ml. The MIC values of synthesized structural analogs of PAS, 7a-f, were found to fall between 1.6 – 12.5 µg/ml (Table 5). Out of all synthesized analogs, 7e was effective at 1.6 µg/ml; 7b, 7c, 7d, and 7f were effective at 3.125 µg/ml; 7a was effective at 12.5 µg/ml, hence the structural analogs, 7b-f were found to have improved MIC values than PAS. MIC values of standards were also compared with the structural analogs to understand the potency of antitubercular activity. Streptomycin (MIC: 6.25 µg/ml), ciprofloxacin (MIC: 3.125 µg/ml), and pyrazinamide (MIC: 3.125 µg/ml) as standard drugs were compared with that of the structural analogs. Based on these results, except 7a, remaining all structural analogs were potent enough when compared with the standard drugs.

4. CONCLUSION

Using functional group modification technique, the free carboxylic acid and free amino groups were modified into Mannich base and Schiff's base respectively to improve the antitubercular activity of PAS by improving drug-target interactions. Based on this, 22 analogs were designed and screened for their activity, drug-likeness, and toxicity using computational drug discovery tools, which resulted in the selection of 6 analogs to go with molecular docking, synthesis, characterization, and activity screening. Molecular docking results showed an improved binding affinity for the structural analogs than PAS. *In-vitro* antitubercular assay showed 7e analog is more potent than the standard drugs and PAS with MIC of 1.6 µg/ml; 7b, 7c, 7d, and 7f analogs are equipotent as standard drugs with MIC of 3.125 µg/ml and more potent than PAS; 7a is less potent than standard drugs and PAS. Based on the results, it can be concluded that the application of computational tools in the drug discovery and design process in the field of Medicinal Chemistry proved to be effective in the screening and selection of promising structural analogs.

CONSENT AND ETHICAL APPROVAL

Not applicable for this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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