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Design, Synthesis, Characterisation and Biological Evaluation of some Novel 1, 3, 4 Thiadiazole Derivatives as Anti-tubercular agents Targeting Decaprenyl Phosphoryl Beta-D-Ribose 2ʾEpimerase-1

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ABSTRACT

Tuberculosis (Mtb) is a bacterial infectious disease that causes 1.8 million deaths every year worldwide. This point, to neediness for developing drug candidates to combat the associated drug resistance and control the disease. A latest research study reveals that Schiff bases are to produce, anti-tubercular, anticancer and anti-inflammatory activity. In the present research work a series of 5[Substituted] phenyl –N-[1E]-[Substituted] phenyl methylene]1,3,4 thiadiazol-2-amine based Schiff bases were designed and docked against Mtb enzyme target Decaprenyl phosphoryl Beta-D-ribose 2-epimerase-1. The molecules were screened based on the novelty, good docking-score and multiple interactions. The selected molecules were synthesized and repeatedly recrystallized to attain the expected purity. Characterization of purified products was carried out by various characterization techniques. Later, the evaluation for anti-tubercular activity against H37RV strain was performed by Microplate Alamar Blue Assay (MABA) *in vitro* assay method. The results shown that SDK3, and SDK5 compounds possess anti-tubercular activity in the range of 3.12 mcg/mL while Compound SDK1 and SDK2 show MIC value of 6.25mcg/mL.

KEY WORDS: Thiadiazole, Docking, Schiff base, Synthesis, MABA, Anti-tubercular.

1. INTRODUCTION

According to WHO (World Health Organization) statistics for 2014 there is an estimated incidence of 2.2 million cases of TB in India out of the overall incidence of 9 million. The WHO estimates that 36 million people will die of tuberculosis by 2020 if it is not controlled. This indicates the importance to develop newer anti-tubercular drugs. Resistance against available anti-tubercular therapeutics undermines the efforts to contain the global tuberculosis epidemic.

Mtb target enzyme Decaprenyl phosphoryl Beta –D- ribose 2epimerase-1 is a critical is enzyme for the lipid biosynthetic pathway in mtb. Mtb DPRE1, which is essential for cell wall biosynthesis and in turn for its viability. This led us to focuses DPRE as interest of drug target Thiadiazole contains the five-membered unsaturated ring structure having Molecular formula $C_2H_2N_2S$ containing two carbon atoms, two hydrogen, two nitrogen and one sulphur atoms.

Due to a broad spectrum of biological activities, molecules of Schiff bases have gained significance in medicinal chemistry and in research. Studies reveal the significance of Schiff bases in possessing activities like antifungal, anti-cancer and anti-tubercular.

Schiff base are formed by the reaction of an Aromatic aldehyde with an amine. In this reaction, amine is nucleophile. Amine reacts with aldehyde to give carbinolamine which is unstable. In acid/base catalyzed reactions carbinolamine loses water. Carbinolamine undergoes acid catalyzed dehydration as it is an alcohol. Rate determining step of Schiff bases is the dehydration of carbinolamine.

2. MATERIALS AND METHODS

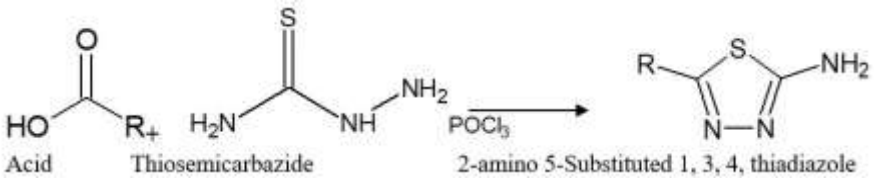
Docking: Docking program is used to fit the ligand molecules into the target structure in most favorable energetically pose. Docking mode is known as pose. A process of design and discovery of the new chemical entities using an automated docking program GLIDE®, Auto Dock® and Argus Lab® is called Docking (Sarah, 2012). Docking searches for molecules (ligands) which have maximum favorable interactions with receptors usually a protein. Docking is done by using Argus Lab® software. Argus Lab 4.0 software distributed freely available for windows platforms by plannaria software (Kitchen, 2004).

Insilco Screening of Drug Likeness: Drug likeness is a term used in drug design. Estimation of bioavailability is confirmed from the molecular structure by determining of drug likeness. A drug like molecule has properties such as: hydrophobicity, hydrogen bonding characteristics and presence of various pharmacophoric features that influence of the behavior of molecule in a living organism, including bioavailability (Ajujoy, 2006).

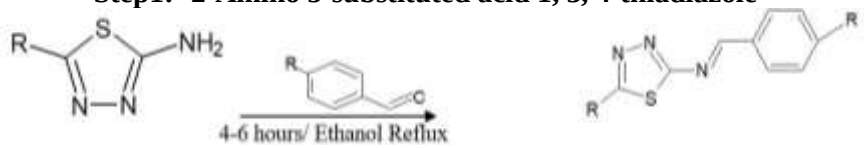
Toxicity Risk Assessment: Toxicity is one of the major criteria to be considered for a molecule to be a successful clinical candidate in pharmaceutical research. About 20-40% of drug failure comes under this category Toxicity screening is done *in silico* using OSIRIS® Property Explorer. The OSIRIS® Property Explorer help us to checks various properties of drugs. Prediction results are valued and color coded. Undesired Properties are shown in red.

Whereas a green color indicates drug-conform behavior. (Lin, 2003) Such of those molecules which show good drug like properties, favorable docking score, and favorable interactions with no toxicity were taken up for synthesis.

Experimental design:



Step1:- 2-Amino 5-substituted acid 1, 3, 4-thiadiazole



Step2: 5[substituted] phenyl-N-[1E]-[substituted] phenyl methylene]-1, 3, 4 thiadiazol-2-amine

Synthetic procedure:

Step1: 2-Amino 5-substituted acid 1, 3, 4-thiadiazole: An equimolar mixture of Aromatic carboxylic acid (0.1mole) and Thiosemicarbazide (0.1mole), in Phosphorus oxy chloride ($POCl_3$) (excess), was refluxed for one hour, crushed ice (90ml) poured to the mixture and again refluxed for another 4 hours. The reaction was monitored by TLC. On completion the reaction, the mixture was cooled to room temperature and filtered. The filtrate was neutralized by saturated potassium hydroxide solution, filtered, dried and re-crystallized from suitable solvent

Step 2: 5[substituted] phenyl-N-[1E]-[substituted] phenyl methylene]-1, 3, 4 thiadiazol-2-amine: (Shankar Gaddeppa, 2011; Maria, 2007). An equimolar quantity of 2-amino 5-substituted 1, 3, 4-thiadiazole (0.01mole) was added to various aldehydes [0.01mole] and dissolved in absolute ethanol. The reaction mixture was refluxed for 4-6 hours. Completion of reaction was monitored by TLC, and then cooled and poured over ice when ten products were formed. It was filtered dried and re-crystallized using ethanol.

Acids used [R]: Benzoic Acid (Intermediate for II-Step synthesis); Phenoxy Acetic Acid; Hippuric Acid.

Aldehyde used [R1]: 2-Hydroxyl Benzaldehyde; 4- Hydroxyl Benzaldehyde; 2,4-dichloro Benzaldehyde; 3-Nitro Benzaldehyde.

Justification of Purity:

Melting Point: The melting point was determined by one end open capillary method. The melting points were sharp and are presented corrected.

Thin Layer Chromatography: Precoated aluminium TLC plates were used. Solutions of the reactants and products were prepared by dissolving them in methanol. A single spot not corresponding to the parent compound was noticed; hence synthesized compounds purity is justified.

Biological Evaluation:

Method: Micro plate Alamar Blue Assay [MABA]

Preparation of inoculums: hundred micro liters 100µl of the Middle brook 7H9 broth.

Requirements: 96 wells plate, Para film (all are sterilized by dry heat).

Nutrient medium: 1:1 mixture of Alamar Blue reagent (25µl) and ten percentage tween 80.

Working procedure: Stock solutions of synthesized compounds and standard drug prepared and taken in the concentration of 0.1 to 100µl/ml. During incubation there will be evaporation of medium. To avoid that, Two hundred micro liters (200µl) of sterile deionized water added to outer perimeter wells of sterile 96 wells plate. Middle-brook 7H9 broth (100µl) and serial dilutions of compounds were also added to the 96 wells plate. About 100 to 0.2µg/ml concentrations of final drug were prepared to test. Covered and sealed plates incubated at 37°c for five days. After this, 25µl of 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate. Then incubated for 24hrs. Interpretation of blue color and pink color indicates as no bacterial growth and growth respectively. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink (Solak, 2006; Brian, 2008; Vanitha, 2004).

3. RESULTS

The designed molecules were docked against the selected target Decaprenyl phosphoryl Beta-D-ribose 2 epimerase-1. The best docked pose and score was selected and the multiple interactions.

Docking view:

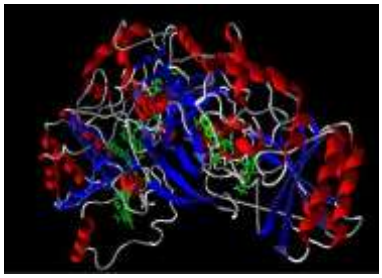


Figure.1. SDK3

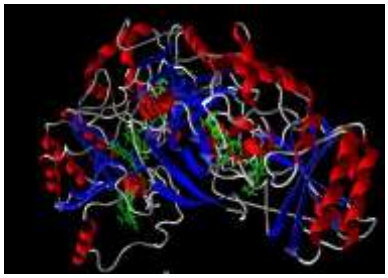
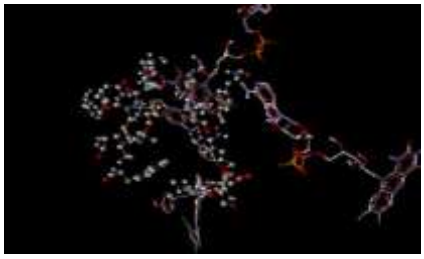


Figure.2. SDK5

Figure.1, 2: Compounds SDK3, SDK5 Docked against the Protein Decaprenyl phosphoryl Beta-D- ribose 2 epimerase-1 (Docking view).

Interactions:



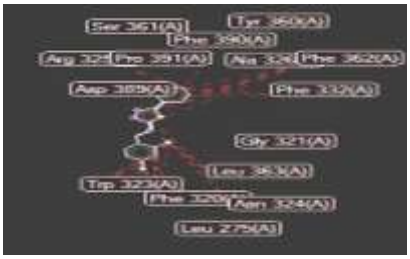
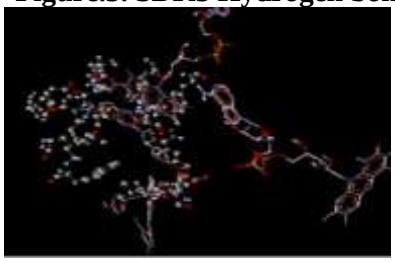


Figure.3. SDK3 Hydrogen bond interactions and their interaction with amino acids



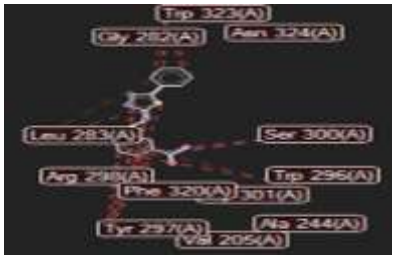


Figure.4. Hydrogen bond interactions and their interactions with amino acids

Toxicity prediction: Prediction results are color coded. Red colour express high risks with undesired effects, such as mutagenicity, tumarogenic, irritant and reproductive effects or a poor intestinal absorption. Yellow colour shows moderate risks with undesired effects and green color indicates drug-conform behavior.

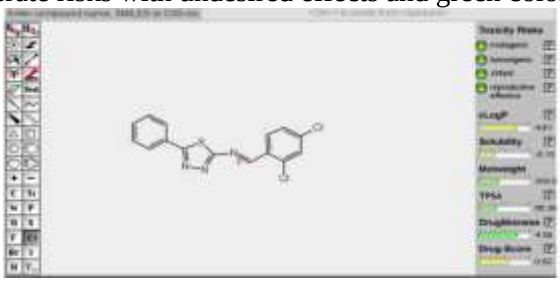


Figure.5. SDK3

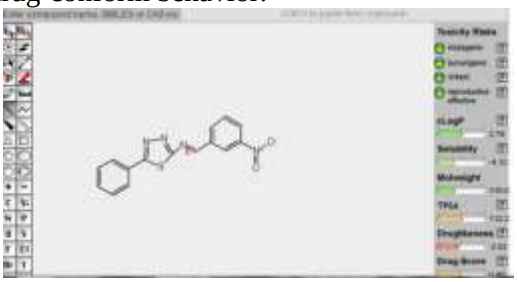


Figure.6. SDK5

Spectral Analysis:

Compound: SDK1: 2-{(*E*) - [(5-phenyl-1, 3, 4-thiadiazol-2-yl) imino] methyl} phenol IR (cm^{-1}):3085.88 [Ar- CHStret], 3440.78 [OH Stre], 1627.80 [C=N Stre], 756.04 [C-S-C] H1NMR: 6-9.2 (11H, m, Ar-H), 2.51 (2H, s, $-CH_2$), 1.23[1H, s, OH); MASS (g/Mol): Actual Mass: 281.32 g/Mol, Expected Mass: 281.32g/Mol.

Compound: SDK2: 4-{(*E*) - [(5-phenyl-1, 3, 4-thiadiazol-2-yl) imino] methyl} phenol IR (cm^{-1}):3085.88 [Ar-CHStret], 3440.78 [OH Stre], 1627.80 [C=N Stre], 756.04 [C-S-C] H^1NMR: 6-9.2 (11H, m, Ar-H), 2.51 (2H, s, $-CH_2$), 1.23[1H, s, OH); MASS (g/Mol**):** Actual Mass: 281.32 g/Mol, Expected Mass: 281.32g/Mol.

Compound: SDK3: (*E*)-1-(2, 4-dichlorophenyl)-*N*-(5-phenyl-1, 3, 4-thiadiazol-2-yl) methanimine IR (cm^{-1}): 3085.88 [Ar-CHStre], 2962.44[Alip-CHStre], 1635.52[C=N Stre], 756.04[C-S-C], H^1NMR: 6-9(9H, m, Ar-H), 2.51(2H, s, $-CH_2$); Mass(g/mol): Actual Mass: 334.42g/mol, Expected Mass: 334.42.

Compound: SDK5: (*E*)-1-(3-nitrophenyl)-*N*-(5-phenyl-1, 3, 4-thiadiazol-2-yl) methanimine IR (cm^{-1}): 3085.88[Ar CH-Stre], 2962.44[Alip CH Stre], 1635.52[C=NStre], 756.04[C-S-C], 1512.08[N=O Stre], H^1NMR: 6-9(10H, m, Ar-H), 2.51(2H, s, $-CH_2$); MASS (g/mol): Actual Mass: 310.08g/mol, Expected Mass: 309.24g/mol.

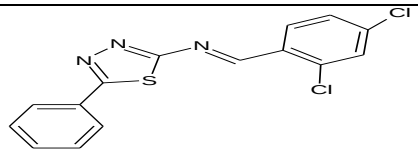
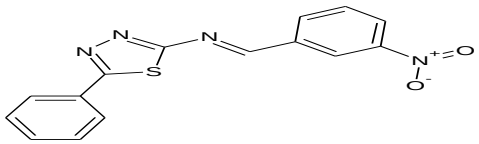
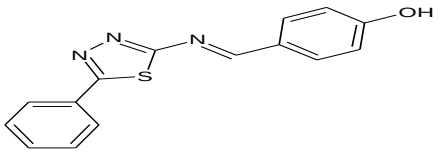
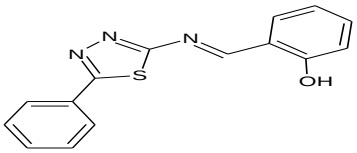
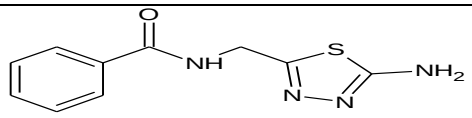
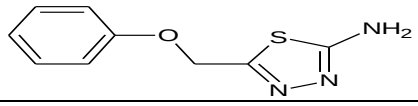
Compound: PAA: 5-(phenoxy methyl)-1, 3, 4-thiadiazol-2-amine IR (cm^{-1}): 3105.88[Ar CH Stre], 2707.86[Alip CH Stre], 3271.03[N-H Stre], 624.04[C- S-C] H^1NMR: 6-9(9H, m, Ar-H), 2.51(2H, s, -CH2) 12.5[2H, s, $-NH_2$]; Mass (g/mol**):** Actual Mass: 207.08g/mol, Expected Mass: 207.24g/mol.

Compound: HA: *N*-[(5-amino-1, 3, 4-thiadiazol-2-yl) methyl] benzamide IR (cm^{-1}): 3070.88[Ar CH Stre], 2831.86[Alip CH Stre], 3247.03[N-H Stre], 924.04[C- S-C] H^1NMR: 6-9 (9H, m, Ar-H), 2.51(2H, s, $-CH_2$) 12.5[2H, s, $-NH_2$]; Mass (g/mol): Actual Mass: 219.28g/mol, Expected Mass: 219.24g/mol.

Table.1. Physical Data of the Synthesized Compounds

Compound code	Molecular weight(g/mol)	Melting point (^{o}c)	Molecular Formula	Solubility	Yield
SDK1	281.33	222	$C_{15}H_{11}N_3OS$	Ethanol/Methanol	81%
SDK2	281.33	222	$C_{15}H_{11}N_3OS$	Ethanol/Methanol	83%
SDK3	334.22	230	$C_{15}H_{11}Cl_2N_3S$	Ethanol/Methanol	80%
SDK5	310.33	228	$C_{15}H_{10}N_4O_2S$	Ethanol/Methanol	77%
PAA	207.25	148	$C_9H_9N_3OS$	Ethanol/Methanol	70%
HA	219.25	151	$C_9H_8N_4OS$	Ethanol/Methanol	68%

Table.2. Docking score with mic value

Compound	Synthesized Compounds	Docking Score (kcal/mol)	MIC Value Mcg/ml
SDK3		--11.73	3.12
SDK5		-11.32	3.12
SDK2		-10.82	6.25
SDK1		-10.82	6.25
HA		-9.146	12.5
PAA		-8.537	12.5

Anti-Tubercular Activity: All the compounds showed good and moderate activity against mycobacterium tuberculosis.

Table.3. Maba Report of the Synthesized Compounds

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
SDK1	S	S	S	S	**S**	R	R	R
SDK2	S	S	S	S	**S**	R	R	R
SDK3	S	S	S	S	S	**S**	R	R
SDK5	S	S	S	S	S	**S**	R	R
HA	S	S	S	**S**	R	R	R	R
PAA	S	S	S	**S**	R	R	R	R

Note: S- Sensitive, R- Resistant, Strain used: - M. tuberculosis [H37RV]: ATCC NO-27294.

Toxicity prediction reports- The cyto toxicity study: Toxicity predictions of synthesized compounds were performed by Osiris property explorer and its toxicity characteristics were observed.

Table.4. Toxicity Prediction

Samples	SDK1	SDK2	SDK3	SDK5	PAA	HA
Mutagenic	+	+	+	+	+	+
Tumorigenic	+	+	+	+	+	+
Irritant	+	+	+	+	+	+
Reproductive effect	+	+	+	+	+	+

[-] indicates Presence of toxicity; [+] indicates absence of toxicity.

DISCUSSION

- The compounds with best docking score against the specific target were synthesized. Synthesized compounds purity determined, they exhibited sharp melting point and a single spot was obtained in the TLC.
- Of the six compounds, five of them were obtained at 98% purity. This was confirmed by GC-MS analysis (obtaining a single peak) and molecular weight also obtained at ± 1 variation. Then the functional group determination was done using FT-IR. It was confirmed by obtaining the specific absorption band in the spectra.
- The Anti-Tubercular activity of the compounds is denoted that the specific organism was sensitive at 3.12 and 3.12mcg/ml and showed better activity compared to standard drugs.
- Cyto toxicity test was performed for compounds that showed the least MIC of 3.12µg/mL, i.e. for compounds SDK3 and SDK5. These compounds found to be nontoxic.

4. CONCLUSION

A series of Schiff bases of 5[Substituted] phenyl –N-[1E]-[Substituted] phenyl methylene]1,3,4 thiadiazol-2-amine derivatives were designed and docked against Mtb enzyme target, which Decaprenyl phosphoryl Beta –D-ribose 2 epimerase-1. This enzyme is a critical is enzyme of the organism. The molecules screened against novelty, good docking-score and multiple interactions. The selected molecules were synthesized and repeatedly re-crystallized to attain the expected purity. Characterization of purified compounds by IR, NMR, and GCMS spectral analysis. Anti- mycobacterial evaluation was carried out against tuberculosis H37RV strain by Microplate Alamar Blue Assay (MABA) method. The test results show that compound SDK3 and SDK5have anti tubercular activity with an MIC value of 3.12mcg/ml while Compound SDK1 and SDK2 showed anti tubercular activity with an MIC Value 6.25mcg/ml. Compounds such as PAA and HA, possesses moderate activity with MIC of 12.5 mcg/ml.

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