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# Synthesis, characterization, antituberculosis activity and computational studies on novel Schiff bases of 1,3,4-thiadiazole derivatives

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**ABSTRACT**: A series of novel Schiff bases were designed and synthesized by the condensation of 1,3,4-thiadiazoles that contain aromatic primary amine and variously substituted benzaldehydes. The synthesized compounds were screened for their antituberculosis activity against *Mycobacterium tuberculosis H37Rv* using BACTEC 460 radiometric system. Among the tested compounds, 2-(4-nitrophenyl)amino-5-[4-(3-(4-phenoxy))benzylideneaminophenyl]-1,3,4-thiadiazole (3n) showed the highest inhibitory activity (80%). The activities of the newly synthesized Schiff bases were higher in comparison to those of intermediate products 2-(4-aminophenyl)-5-aryl/alkylamino-1,3,4-thiadiazoles (2a-l). The computational studies were also performed to estimate drug-like profile of the compounds by using QikProp analysis.

**KEYWORDS**: Schiff bases; 1,3,4-thiadiazoles; antituberculosis activity; *Mycobacterium tuberculosis* $H_{37}$Rv; ADME.

## 1. INTRODUCTION

Tuberculosis (TB) is known as an infectious disease, especially monitored on lungs, caused by a group of *Mycobacterium* species, mostly by *Mycobacterium tuberculosis* [1]. It is informed that among the world, every one of three people comes face to face with latent tuberculosis. And, one-tenth of latent tuberculosis turns into active TB disease [2]. With respect to the WHO's Global Tuberculosis Report 2019, TB is one of the important causes of death worldwide. Additionally, among the single infectious agents, it is more dangerous than HIV/AIDS. Furthermore, drug resistant TB continues to threaten public health, particularly in developing countries [3, 4]. Especially, for the treatment of multidrug resistant (MDR) TB, extremely drug resistant (XDR) TB and TB-HIV co-infections, the discovery of new antituberculosis agents is a necessity [5].

Schiff bases are important organic structures bearing C=N linkage, the nitrogen atom linked to an alkyl or aryl moiety. They were firstly synthesized by Hugo Schiff at 1864 and since then they are called as Schiff bases. They are obtained by the condensation of primary amines with aldehydes or ketones [6-8]. Although mostly being distinguished by their antibacterial and antimicrobial activities, they also have come to the forefront by their antitubercular, antifungal, antioxidant, anti-inflammatory, antiviral and anticonvulsant activities [9-14]. Among this group, Terizidone is an important example to bacteriostatic agents bearing Schiff base structure, used in multidrug-resistant tuberculosis [15].

1,3,4-Thiadiazole is also a potent scaffold taking part in many drugs with various activities such as acetazolamide, methazolamide as CAs inhibitor; cefazolin, cefazedone as antimicrobial; megazole as antimicrobial and tripanoside etc. Besides their different pharmacological activities, 1,3,4-thiadiazoles exhibit remarkable antitubercular activity [16-21].

In the view of such information, we have combined these two active moieties with the aim to obtain new antitubercular agents. Besides elemental analysis, the newly synthesized compounds were characterized by various spectroscopic methods.

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## 2. RESULTS AND DISCUSSION

### 2.1. Chemistry

The novel compounds **(3a-n)** were synthesized according to the sequence shown in Figure 1. Firstly, 1,3,4-thiadiazoles (**2a-n)** were obtained by a four step reaction, starting from benzocaine, according to our previous reports [18, 22]. Secondly, the Schiff bases (**3a-n)** were obtained by refluxing the (**2a-n)** compounds with various aldehydes, in methanolic medium over 45 minutes [23].

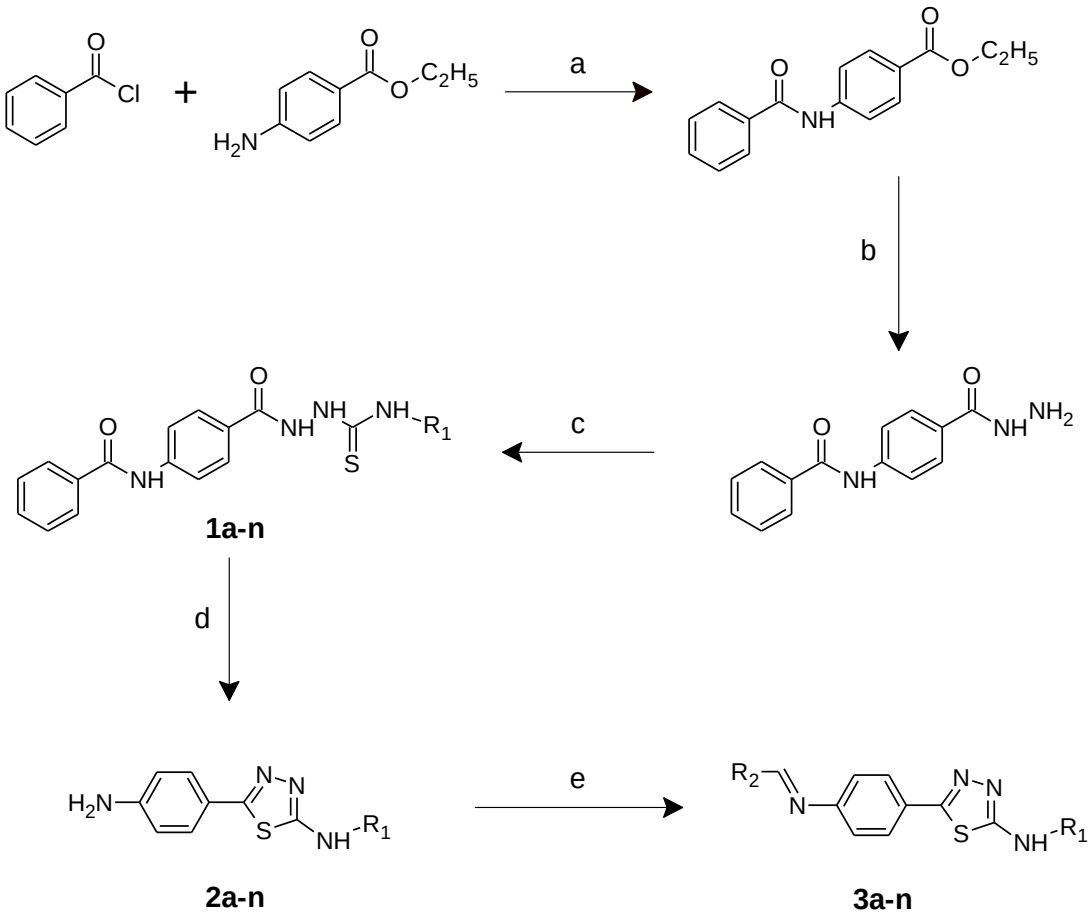


**Figure 1.** Synthetic pathway for compounds **3a-n**. Reagents and conditions: **a**: Diethyl ether; **b**: $NH_2NH_2.H_2O$, $C_2H_5OH$; **c**: $R_1$-NCS, $C_2H_5OH$; **d**: 50% $H_2SO_4$, NaOH; **e**: $R_2$-CHO, $CH_3OH$.

The structures of the newly synthesized **3a-n** compounds were confirmed by FT-IR, $^1$H-NMR and mass spectroscopic methods, besides elemental analysis. The spectral data was in agreement with the proposed structures. According to the FT-IR spectra; the O-H stretching bands were screened at 3300-3390 $cm^{-1}$ while the N-H stretching bands were screened at 3150-3259 $cm^{-1}$. Also, C=N and C-Cl stretching bands were screened between 1614-1643 $cm^{-1}$ and 1072-1099 $cm^{-1}$, respectively.

According to the $^1$H-NMR spectra; the CH=N protons resonated at 8.69-10.53 ppm. In comparison with our previous study, the newly synthesized **3a-n** compounds were thought to be in *E*-isomer form [24]. Although, the phenolic protons of compounds **3a-c, 3e, 3h,** and **3k** were detected at 14.11-14.40 ppm, as similar to the literature [22, 25], the phenolic proton of compound **3l** couldn't been determined at the spectrum. The thiadiazole -NH- protons were detected as singlets at 10.39-11.32 ppm, and also the aromatic protons were observed at the expected regions.

According to the mass spectroscopic fragmentation of the compounds, which were studied under Atmospheric Pressure Chemical Ionization (APCI) positive polarity, the $(M+H)^+$ peaks were identified in agreement with their molecular structure. According to the mass spectra, in consequence of the cleavage of imine group, amine and substituted methylene fragments were occurred.

### 2.2. Antituberculosis activity

Results of antituberculosis activity of the compounds are given in Table 1 and Table 2. The inhibition values of the synthesized compounds were found between 59-80% compared to rifampicin. These values could be considered as a significant increase with reference to the intermediate products, **2a-l**. For example, the **3e-g** compounds derived from **2e** (having 4-fluorophenyl), showed 63-65% inhibition. However, the **2e** compound revealed only 3% inhibition. Similarly, **2k** (bearing 4-methoxyphenyl), the precursor of the **3k** compound, showed only 7% inhibition. Whereas the **3k** presented 69% inhibitory activity.

**Table 1.** Antituberculosis activity results of compounds **2a-l** [17].

| Compounds | $R_1$ | MIC (μg $ml^{-1}$) | Inhibition% |
|---|---|---|---|
| **2a** | methyl | >6.25 | 29 |
| **2b** | ethyl | >6.25 | 34 |
| **2c** | cyclohexyl | >6.25 | 41 |
| **2d** | benzyl | >6.25 | 37 |
| **2e** | 4-fluorophenyl | >6.25 | 3 |
| **2h** | 4-methylphenyl | >6.25 | 22 |
| **2k** | 4-methoxyphenyl | >6.25 | 7 |
| **2l** | 4-nitrophenyl | >6.25 | 37 |
| **Rifampicin** | - | 0.25 | 98 |

As a result, the antituberculosis activities of the newly synthesized Schiff bases seemed to be higher than the intermediate 2-substitutedamino-5-(4-aminophenyl)-1,3,4-thiadiazoles. The biological activity results indicated that compound **3n** having phenoxy substituent attached to the 4-nitrophenylthiadiazole ring showed the highest inhibition with 80% inhibition value against *Mycobacterium tuberculosis H37Rv* at concentration of 6.25 μg $ml^{-1}$.

### 2.3. Drug-likeliness analysis

With exception to Lipinski RO5, predicted values for total solvent accessible surface area (SASA), cell permeability (QPPMDCK), percentage of human absorption (HOA%), blood brain barrier coefficient (QlogP BB) and polar surface area (PSA) values calculated for 14 molecules understudy are satisfying the drug-likeness criteria (Table 3). For SASA, recommended value for a molecule with good drug likeliness property should be in the range of 300.0 – 1000.0 while recommended values for Predicted brain/blood partition (QlogP BB) coefficient should be within the range of -3.0 – 1.2 [26]. In case of QikProp predicted permeability through the monolayers of Madin−Darby Canine Kidney cells (QPPMDCK), past studies reported that ligands having <25 nm/sec score have poor MDCK cell permeability whereas ligands bearing >500 nm/sec QPPMDCK value shows great potential of being a drug-like molecule. Similarly, human oral absorption (HOA%) count in term of percentage should be preferably higher than 25% and to assess the bioavailability, polar absorption area of drug like compounds with ≤ 140$Å^2$ value are considered good for drug optimization [27].

All the predicted drug likeliness parameters for 14 compounds given in Table 3 were within the recommended ranges/values. This indicate that these molecules are theoretically strong druggable candidates. Except compound **3a** and compound **3b,** all compounds are non-complaint to Lipinski RO5. Although it is a drug-likeness guideline but there are so many Food and Drug Administration (FDA) approved drugs that don't follow Lipinski RO5 [28]. Drug-likliness has now thought to be linked with the dynamic nature of the chemical compound in which each possible conformation exhibits a distinct pattern of hydprophobicity, lipophilicity and molecular electrostatic potential (MEF).

## 3. CONCLUSION

Novel derivatives of Schiff bases were obtained from substituted 1,3,4-thiadiazoles. All of the final compounds were evaluated for their *in vitro* antituberculosis activity against *Mycobacterium tuberculosis H37Rv.* The activity results revealed that 2-(4-nitrophenyl)amino-5-[4-(3-(4-phenoxy))benzylideneaminophenyl]-1,3,4-thiadiazole **3n** was the most active compound by 80% inhibition value. According to the *in silico* calculations, all the synthesized compounds exhibit promising physicochemical properties, thus regarded as potential drug-like compounds for the discovery of therapeutic solutions against *Mycobacterium tuberculosis* infections. In this context, compound **3n** could be used as a lead compound for further studies.

**Table 2.** Antituberculosis activity results of compounds **3a-n.**

| Compounds | $R_1$ | $R_2$ | MIC (μg ml$^{-1}$) | Inhibition% |
|---|---|---|---|---|
| **3a** | methyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 69 |
| **3b** | ethyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 68 |
| **3c** | cyclohexyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 72 |
| **3d** | benzyl | 2,4-dichlorophenyl | >6.25 | 61 |
| **3e** | 4-fluorophenyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 65 |
| **3f** | 4-fluorophenyl | 2,4-dichlorophenyl | >6.25 | 63 |
| **3g** | 4-fluorophenyl | 3,4-dichlorophenyl | >6.25 | 63 |
| **3h** | 4-methylphenyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 72 |
| **3i** | 4-methylphenyl | 2,4-dichlorophenyl | >6.25 | 62 |
| **3j** | 4-methylphenyl | 3-phenoxyphenyl | >6.25 | 73 |
| **3k** | 4-methoxyphenyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 69 |
| **3l** | 4-nitrophenyl | 2-hydroxy-3,5-dichlorophenyl | >6.25 | 64 |
| **3m** | 4-nitrophenyl | 3,4-dichlorophenyl | >6.25 | 59 |
| **3n** | 4-nitrophenyl | 3-phenoxyphenyl | >6.25 | 80 |
| **Rifampicin** | - | - | 0.25 | 98 |

**Table 3.** QikProp estimated drug-likeness parameters of compounds **3a-n**.

| Molecule | SASA | QPlogBB | QPPMDCK | HOA% | PSA | RO5 |
|---|---|---|---|---|---|---|
| **3a** | 646.79 | -0.47 | 4137.51 | 100 | 69.43 | 0 |
| **3b** | 682.19 | -0.50 | 4758.81 | 100 | 69.78 | 0 |
| **3c** | 767.89 | -0.44 | 6026.98 | 100 | 68.64 | 1 |
| **3d** | 737.19 | -0.01 | 10000 | 100 | 48.68 | 1 |
| **3e** | 750.03 | -0.35 | 10000 | 100 | 66.75 | 1 |
| **3f** | 735.48 | 0.10 | 10000 | 100 | 47.58 | 1 |
| **3g** | 739.18 | 0.13 | 10000 | 100 | 46.97 | 1 |
| **3h** | 773.05 | -0.47 | 5846.08 | 100 | 66.16 | 1 |
| **3i** | 852.83 | -0.46 | 2461.06 | 100 | 54.40 | 1 |
| **3j** | 614.58 | 0.01 | 1941.58 | 97.28 | 35.47 | 1 |
| **3k** | 778.90 | -0.53 | 5834.65 | 100 | 74.37 | 1 |
| **3l** | 780.69 | -1.69 | 564.20 | 82.61 | 111.84 | 1 |
| **3m** | 770.34 | -1.18 | 1273.97 | 93.04 | 91.97 | 1 |
| **3n** | 861.75 | -1.73 | 247.07 | 96.99 | 99.44 | 1 |

## 4. MATERIALS AND METHODS

### 4.1. Chemistry

All chemicals were purchased locally from Aldrich, Fluka and Merck. Melting points were determined by SMP II melting point apparatus and uncorrected. FT-IR spectra were obtained on a Schimadzu FTIR-8400S spectrophotometer. $^1$H-NMR spectra were run on a Bruker Avance DPX-400 spectrometer in DMSO-$d_6$ with tetramethylsilane as the internal standard and all chemical shifts were reported as δ (ppm) values. Mass spectra were obtained with an Agilent 1100 MSD spectrometer. Elemental analyses were performed with Leco CHNS 932.

In light of our previous studies, ethyl 4-(benzoylamino)benzoate, 4-(benzoylamino)benzoyl hydrazide, 1-aroyl-4-alkyl/arylthiosemicarbazides and 2-alkyl/arylamino-5-(4-aminophenyl)-1,3,4-thiadiazoles were prepared [18, 29].

*4.1.2. General procedure for the preparation of 2-substitutedamino-5-[4-substitutedbenzylideneaminophenyl]-1,3,4-thiadiazoles (3a-n)*

Compounds **2** (0.015 mol) were treated with equimolar amount of aromatic aldehydes in anhydrous methanol. The resulting mixture was heated under reflux for 45 min. After cooling to room temperature, the resulting products were filtered and washed with warm ethanol [22].

**2-Methylamino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3a).** Yield: 37%; mp: 250 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3377 (O-H), 3150 (N-H), 1614 (C=N), 1072 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 2.85 (s, 3H, $CH_3$), 7.39-7.88 (m, 6H, Ar-H), 8.98 (s, 1H, -N=C-H), 14.11 (s, 1H, -OH); APCI Pos *m/z* 380 ($M+H^+$, 8%), 79.1 (100%). Anal. Calcd for $C_{16}H_{12}Cl_2FN_4OS$ (379.26): C, 50.67; H, 3.19; N, 14.77; S, 8.45. Found: C, 50.89; H, 3.70; N, 14.88; S, 8.52.

**2-Ethylamino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3b).** Yield: 79%; mp: 255 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3390 (O-H), 3170 (N-H), 1614 (C=N), 1074 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 1.21 (t, 3H, $CH_3$), 3.37 (q, 2H, $CH_2$), 7.58-7.89 (m, 6H, Ar-H), 8.01 (t, 1H, -NH of thiadiazole), 9.09 (s, 1H, -N=C-H), 14.24 (s, 1H, -OH); APCI Pos *m/z* 394 ($M+H^+$, 4%), 221.0 (100%). Anal. Calcd for $C_{17}H_{14}Cl_2N_4OS$ (393.29): C, 51.92; H, 3.59; N, 14.25; S, 8.15. Found: C, 52.51; H, 4.00; N, 14.53; S, 8.21.

**2-Cyclohexylamino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3c).** Yield: 72%; mp: 241-243 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3300 (O-H), 3179 (N-H), 1614 (C=N), 1082 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 1.11-2.09 (m, 10H, cyclohexyl $CH_2$), 3.57 (s, 1H, CH), 7.57-7.99 (m, 7H, Ar-H and NH), 9.09 (s, 1H, -N=C-H), 14.40 (s, 1H, -OH); APCI Pos *m/z* 448 ($M+H^+$, 29%), 275.1 (100%); Anal. Calcd for $C_{21}H_{20}Cl_2N_4OS$ (447.38): C, 56.38; H, 4.51; N, 12.52; S, 7.17. Found: C, 56.78; H, 4.62; N, 12.79; S, 7.15.

**2-Benzylamino-5-[4-(2,4-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3d).** Yield: 64%; mp: 260-263 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3194 (N-H), 1614 (C=N), 1099 (C-CI); APCI Pos *m/z* 440 ($M+H^+$, 6%), 79.1 (100%); Anal. Calcd for $C_{22}H_{16}CI_2N_4S$ (439.36): C, 60.14; H, 3.67; N, 12.75; S, 7.30. Found: C, 60.00; H, 3.84; N, 12.74; S, 7.18.

**2-(4-Fluorophenyl)amino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3e).** Yield: 41%; mp: 319-321°C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3370 (O-H), 3150 (N-H), 1620 (C=N), 1229 (C-F), 1099 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 7.23 (t, 2H, *ortho*-protons to F), 7.41-8.09 (m, 8H, Ar-H), 9.11 (s, 1H, -N=C-H), 10.62 (s, 1H, -NH of thiadiazole), 14.26 (s, 1H, -OH); APCI Pos *m/z* 460 ($M+H^+$, 5%), 79.1 (100%); Anal. Calcd for $C_{21}H_{13}Cl_2FN_4OS$ (459.32): C, 54.91; H, 2.85; N, 12.20; S, 6.98. Found: C, 54.92; H, 3.71; N, 12.43; S, 7.09.

**2-(4-Fluorophenyl)amino-5-[4-(2,4-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3f).** Yield: 86%; mp: 265-267°C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3170 (N-H), 1622 (C=N), 1227 (C-F), 1095 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 7.22 (t, 2H, *ortho*-protons to F), 7.44 (d, 2H, *ortho*-protons to imine, *J*= 8.5 Hz ), 7.58 (dd, 1H, $C_6$ proton of 2,4-dichlorophenyl ring, *J*= 2.0 Hz, *J*= 8.5 Hz), 7.64-7.77 (dd, 2H, *meta*-protons to F, *J*= 4.7 Hz, *J*= 9.0 Hz), 7.82 (d, 1H, $C_3$ proton of 2,4-dichlorophenyl ring, *J*= 2.0 Hz), 7.95 (d, 2H, *meta*-protons to imine, *J*= 8.4 Hz), 8.22 (d, 1H, $C_5$ proton of 2,4-dichlorophenyl ring, *J*= 8.50 Hz), 8.87 (s, 1H, -N=C-H), 10.59 (s, 1H, -NH of thiadiazole); APCI Pos *m/z* 444 ($M+H^+$, 13%), 474.9 (100%); Anal. Calcd for $C_{21}H_{13}C_{l2}FN_4S$ (443.32): C, 56.89; H, 2.96; N, 12.64; S, 7.23. Found: C, 57.33; H, 2.74; N, 12.97; S, 7.29.

**2-(4-Fluorophenyl)amino-5-[4-(3,4-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3g).** Yield: 86%; mp: 272-275 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3150 (N-H), 1620 (C=N), 1227 (C-F), 1095 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 7.22 (t, 2H, *ortho*-protons to F), 7.43 (d, 2H, *ortho*-protons to imine, *J*= 8.51 Hz), 7.58-7.77 (m, 2H, *meta*-protons to F), 7.82 (d, 1H, $C_6$ proton of 3,4-dichlorophenyl ring), 7.86-8.04 (m, 3H, *meta*-protons to imine and $C_5$ proton of 2,4-dichlorophenyl ring), 8.18 (s, 1H, $C_2$ proton of 3,4-dichlorophenyl ring), 8.72 (s, 1H, -N=C-H), 10.58 (s, 1H, -NH of thiadiazole); APCI Pos *m/z* 444 ($M+H^+$, 7%), 287.0 (100%); Anal. Calcd for $C_{21}H_{13}C_{l2}FN_4S$ (443.32): C, 56.89; H, 2.96; N, 12.64; S, 7.23. Found: C, 57.16; H, 3.24; N, 13.11; S, 7.25.

**2-(4-Methylphenyl)amino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3h).** Yield: 58%; mp 301-303 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3300 (O-H), 3190 (N-H), 1614 (C=N), 1098 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 2.24 (s, 3H, $CH_3$), 6.63-7.99 (m, 10H, Ar-H), 9.81 (s, 1H, -N=C-H), 10.52 (s, 1H, -NH of thiadiazole), 14.22 (s, 1H, -OH); APCI Pos *m/z* 456 ($M+H^+$, 2%), 283.0 (100%); Anal. Calcd for $C_{22}H_{16}Cl_2N_4OS$ (455.35): C, 58.16; H, 3.33; N, 12.33; S, 7.06. Found: C, 58.00; H, 3.23; N, 11.55; S, 7.05.

**2-(4-Methylphenyl)amino-5-[4-(2,4-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3i).** Yield: 78%; mp 247-250 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3159 (N-H), 1620 (C=N), 1095 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ

(ppm): 2.31 (s, 3H, $CH_3$), 7.02-8.21 (m, 11H, Ar-H), 8.87, 8.89 (2s, 1H, -N=C-H), 10.46 (s, 1H, -NH of thiadiazole); APCI Pos *m/z* 440 ($M+H^+$, 21%), 283.0 (100%); Anal. Calcd for $C_{22}H_{16}Cl_2N_4S$ (439.36): C, 60.14; H, 3.67; N, 12.75; S, 7.30. Found: C, 60.31; H, 3.47; N, 13.09; S, 7.39.

**2-(4-Methylphenyl)amino-5-[4-(3-(4-phenoxy))benzylideneaminophenyl]-1,3,4-thiadiazole (3j).** Yield: 47 %; mp 218-220 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3178 (N-H), 1617 (C=N); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 2.29 (s, 3H, -$CH_3$), 7.07-7.99 (m, 17H, Ar-H), 8.69 (s, 1H, -N=C-H), 10.44 (s, 1H, -NH of thiadiazole); APCI Pos *m/z* 463 ($M+H^+$, 4 %), 283.0 (100%); Anal. Calcd for $C_{28}H_{22}N_4OS$ (462.56): C, 72.70; H, 4.79; N, 12.11; S, 6.93. Found: C, 72.46; H, 4.62; N, 12.51; S, 6.93.

**2-(4-Methoxyphenyl)amino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3k).** Yield 55%; mp 277°C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3370 (O-H), 3150 (N-H), 1614 (C=N), 1080 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm) 3.77 (s, 3H, $CH_3$), 6.63-8.08 (m, 10H, Ar-H), 9.10 (s, 1H, -N=C-H), 10.39 (s, 1H, -NH of thiadiazole), 14.21 (s, 1H, -OH); APCI Pos *m/z* 472 ($M+H^+$, 1%), 299 (100%); Anal. Calcd for $C_{22}H_{16}Cl_2N_4O_2S$ (471.36): C, 56.06; H, 3.42; N, 11.89; S, 6.80. Found: C, 55.73; H, 3.20; N, 11.90; S, 6.55.

**2-(4-Nitrophenyl)amino-5-[4-(2-hydroxy-3,5-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3l).** Yield 80%; mp 343-345 °C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3340 (O-H), 3200 (N-H), 1643 (C=N), 1077 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 7.23-8.44 (m, 10H, Ar-H), 10.53 (s, 1H, -N=C-H), 11.29 (br.s, 1H, -NH of thiadiazole); APCI Pos *m/z* 487 ($M+H^+$, 2%), 417.9 (100%); Anal. Calcd for $C_{21}H_{13}Cl_2N_5O_3S$ (486.33): C, 51.86; H, 2.69; N, 14.40; S, 9.87. Found: C, 51.78; H, 2.70; N, 14.37; S, 9.87.

**2-(4-Nitrophenyl)amino-5-[4-(3,4-dichlorobenzylideneamino)phenyl]-1,3,4-thiadiazole (3m).** Yield 61%; mp 345°C; IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3390 (O-H), 3259 (N-H), 1643 (C=N), 1074 (C-CI); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 7.29-8.50 (m, 11H, Ar-H), 10.53 (s, 1H, -N=C-H), 11.18 (br.s, 1H, -NH of thiadiazole); APCI Pos *m/z* 471 ($M+H^+$, 1%), 418.0 (100%). Anal. Calcd for $C_{21}H_{13}Cl_2N_5O_2S.3H_2O$ (524.37): C, 48.10; H, 3.65; N, 13.36; S, 6.11. Found: C, 47.75; H, 3.93; N, 13.73; S, 6.75.

**2-(4-Nitrophenyl)amino-5-[4-(3-(4-phenoxy))benzylideneaminophenyl]-1,3,4-thiadiazole (3n).** Yield: 60%; mp 243-245 °C. IR (FTIR) ($\upsilon_{max}$, $cm^{-1}$): 3350 (O-H), 3150 (N-H), 1614 (C=N); $^1$H-NMR (DMSO-$d_6$, 400 MHz) δ (ppm): 6.98-8.38 (m, 17H, Ar-H), 8.69 (s, 1H, -N=C-H), 11.32 (br.s, 1H, -NH of thiadiazole); APCI Pos *m/z* 495 ($M+H^+$, 36%), 314.0 (100%); Anal. Calcd for $C_{27}H_{19}N_5O_3S.4H_2O$ (565.59): C, 57.34; H, 4.81; N, 12.38; S, 5.67. Found: C, 57.59; H, 4.15; N, 12.48; S, 5.93.

### 4.2. Antituberculosis activity

All of the compounds were evaluated for their *in vitro* antituberculosis activity against *Mycobacterium tuberculosis H37Rv* by Tuberculosis Activity Antimicrobial Acquisition and Coordinating Facility (TAACF) of Southern Research. Primary screening was conducted at 6.25µg $ml^{-1}$ against *Mycobacterium tuberculosis H37Rv* in BACTEC 12B medium using broth microdilution assay [30].

### 4.3. Computational analysis

*4.3.1. QikProp analysis*

Three-dimensional (3D) structure of all aforementioned ligands were sketched in Chem Draw ultra 12.0.2.1076 and imported to LigPrep module of Schrödinger [31]. In LigPrep, ionization state of each compound was maintained at pH 7.0±2 and for each ligand, tautomer's were also generated [32]. Computational quantitative assessment of drug likeliness for each compound was also performed which includes multiple parameters related to Lipinski's Rule of five (RO5) criteria, absorption, distribution, metabolism and excretion (ADME) were calculated using QikProp. Molecular descriptors calculated in the current study are SASA, QPPMDCK, HOA%, QPlogBB and PSA to provide insight into hydrophobicity, lipophilicity and metabolic properties of the compounds under study [33, 34].

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