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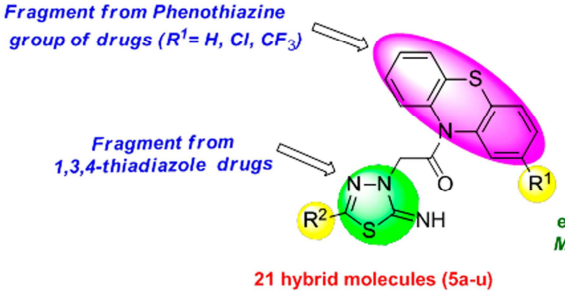

compound	MIC (µg/mL)	IC_{50} (Vero cells)	SI
5a	1.6	433.33	270.83
5b	1.6	470	293.75
5c	1.6	350	218.75
5e	1.6	756.67	472.95
5g	0.8	215	268.75
5l	1.6	733.33	458.33
5m	1.6	251.67	157.29
5n	0.8	245	306.25

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Design of new phenothiazine-thiadiazole hybrids via molecular hybridization approach for the development of potent antitubercular agents

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Abstract

A new library of phenothiazine and 1,3,4-thiadiazole hybrid derivatives (**5a-u**) was designed based on the molecular hybridization approach and the molecules were synthesized in excellent yields using a facile single-step chloro-amine coupling reaction between 2-chloro-1-(10*H*-phenothiazin-10-yl)ethanones and 2-amino-5-subsituted-1,3,4-thiadiazoles. The compounds were evaluated for their *in vitro* inhibition activity against *Mycobacterium tuberculosis* H_{37}Rv (*MTB*). Compounds **5g** and **5n** were emerged as the most active compounds of the series with MIC of 0.8 μg/mL (~1.9 μM). Also, compounds **5a**, **5b**, **5c**, **5e**, **5l** and **5m** (MIC = 1.6 μg/mL), and compounds **5j**, **5k** and **5o** (MIC = 3.125 μg/ml) showed significant inhibition activity. The structure-activity relationship demonstrated that an alkyl (methyl/n-propyl) or substituted (4-methyl/4-Cl/4-F) phenyl groups on the 1,3,4-thiadiazole ring enhance the inhibition activity of the compounds. The cytotoxicity study revealed that none of the active molecules are toxic to a normal Vero cell line thus proving the lack of general cellular toxicity. Further, the active molecules were subjected to molecular docking studies with target enzymes InhA and CYP121.

Keywords: *Mycobacterium tuberculosis*; phenothiazine; 1,3,4-thiadiazole; antimycobacterial activity; cytotoxicity; molecular docking

1. Introduction

Tuberculosis (TB) is a contagious disease caused by the infection with bacteria *Mycobacterium tuberculosis*. It causes a massive amount of human deaths despite the availability of more than 20 antiTB drugs and the Bacille Calmette Guerin (BCG) vaccine [1]. Further, the traditional anti-TB agents have limited efficacy against the new forms of TB, extensively drug-resistant TB (XDRTB) and multidrug resistant TB (MDRTB). Hence there is an emergent requirement for the development of newer fast acting antiTB drugs which have a safer toxic profile. In this direction, several molecular design strategies are being employed in order to indentify potent chemical entities [2]. The molecular hybridization approach which involves the hybridization of two active pharmacophores into a single molecular framework has become one of most promising approaches to develop potent antiTB agents because many such hybrid derivatives possessed improved efficiency and efficacy, when compared to the parent compounds [3-5]. In our previous studies on imidazo[2,1-*b*][1,3,4]thiadazole (ITD) based hybrid molecules, we have incorporated substituted benzimidazole or 1,2,3-triazole moiety at position-5 of the ITD ring and evaluated the antitubercular activity of the hybrid molecules. Interestingly, most of the hybrid compounds exhibited significant inhibition activity against *Mtb* H37Rv strain and a safe toxicity profile towards a normal cell line [6, 7]. These results and some literature reports on significant antitubercular activity of phenothiazine and 1,3,4-thiadiazole derivatives prompted us to design new phenothiazine-thiadiazole hybrids for the development of potent molecules. The *in vitro* antitubercular activity of phenothiazines is well-known for many years [8-10]. In clinical trials, thioridazine (I) (figure 1) is being used in combination with Linezolid and Moxifloxacin as front-line drug in combinatorial therapeutic approaches for the treatment of *Mtb* infection [11]. Chlorpromazine (II) is effective against virulent *Mtb* strain H37Rv in cultured human macrophage model of infection and it is described as synergistic with both INH and RIF [12]. A recent report demonstrated the significant antitubercular activity of a series of 4,5-dihydro-1*H*-phenothiazine containing pyrazolo[3,4-*d*]pyrimidines (III). One of the derivatives was more potent (with MIC_{MABA} value of 0.025µg/mL) than the standard drug Isoniazid [13]. Further, several *N*-substituted phenothiazine derivatives demonstrated promising antitubercular activity [14-18]. On the other hand, 1,3,4-thiadiazole derivatives are a important class of heterocyclic compounds in medicinal chemistry research [19, 20] which exhibit wide variety of biological effects including anti-tubercular [21], anticancer [22], antibacterial [23] and anti-fungal [24, 25] activities. Also, 1,3,4-thiadiazole ring is the core

structural unit in several marketed drugs such as Acetazolamide, Methazolamide, sulfamethizole, Cefazedone, Cefazolin, Ceftezole etc. In addition, a few literature reports revealed promising antitubercular activity of 1,3,4-thiadiazoles [26]. For example, 3-heteroarylthioquinoline derivatives [27] of 1,3,4-thiadiazole demonstrated MIC of ~3.5 μM against *Mtb* (IV) whereas a pyridinyl-thiadiazole derivative (V) exhibited MIC of 0.07 μM [21] (figure 1). In view of these facts on promising antimycobacterial activity of 1,3,4-thiadiazole and phenothiazine derivatives, we envisaged the incorporation of these two molecular units in to a single molecular framework and synthesized a new series of hybrid derivatives (**5a-u**).

(Figure 1 here)

2. Results and discussion

2.1. Chemistry

The target phenothiazine-thiadiazole hybrids were synthesized according to the synthetic route presented in Scheme 1. 2-Subsituted-1-(2-chloro-10*H*-phenothiazin-10-yl) ethanones (**2a-c)** were synthesized by the reaction of substituted phenothiazines (**1a-c)** with chloroacetyl chloride under reflux conditions [15, 28]. 5-Methyl-2-amino-1,3,4-thiadiazole (**4a**) was synthesized by reacting thiosemicarbazide with acetyl chloride (**3a**) using a reported procedure [6]. Other 5-aryl-1,3,4-thiadiazole-2-amines (**4b-g**) were synthesized by treating the corresponding aromatic acid (**3b-g**) with thiosemicarbazide in the presence of phosphorous oxychloride. The target compounds, 1-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-2-(10*H*-phenothiazin-10-yl)ethanone derivatives (**5a–u**) were synthesized by the reaction between compounds **4a-g** and **2a-c** in ethanol under reflux conditions. The plausible reaction mechanism for the formation of final derivatives (**5a-u**) is shown in the Scheme 2. The structure of the target molecules (**5a-u)** was confirmed by spectral (^{1}H NMR, ^{13}C NMR, ESI-MS and FTIR) and elemental analysis. For instance, the ^{1}H NMR spectrum of compound **5a** showed a broad singlet with one proton at δ 8.16 ppm due to the imine (C=NH) proton and another singlet at δ 2.20 ppm due to methyl protons of the 1,3,4- thiadiazole ring. The singlet at δ 4.74 ppm corresponds to the CH_2 group. The broad NH at δ 8.16 ppm was disappeared upon D_2O exchange. Also, its mass spectrum showed the molecular ion peak at m/z 355.1, which corresponds to M+1 peak of the molecule and is in agreement with its molecular formula $C_{17}H_{14}N_4OS_2$. The IR spectrum of compound **5a** showed an absorption peak at 3314

due to N-H stretching (C=NH). Further, the signals at 1696 and 1601cm^{-1} correspond to C=O and C=N stretching respectively. The spectral and elemental analysis data of all target compounds are given in the experimental part and some representative spectra are given the supplementary information. The physical data of compounds (**5a-u**) are tabulated in Table 1.

(Scheme 1 here)

(Table 1 here)

2.2. *In vitro* antimycobacterial activity

All the target derivatives (**5a-u**) were screened against *Mtb* H37Rv (ATCC27294) using MABA method [29] and their antimycobacterial activity was evaluated in terms of minimum inhibitory concentration (MIC) values. The MIC values in μg/mL of **5a-u** along with those of standard drugs for comparison are presented in figure 2. The MIC values of the compounds are in the range of 0.8-50 μg/mL. Interestingly, eleven compounds of the series showed significant inhibitory activity (MIC ≤ 3.125 μg/mL) among which eight compounds exhibited MIC ≤ 1.6 μg/mL (figure 3). The MIC values of these compounds are comparable with those of the standard drugs ethambutol (EMB) and ciprofloxacin (INN). Compounds **5g** and **5n** which contain 4-methylphenyl and *n*-propyl groups respectively on the thiadiazole ring emerged as the most potent leads with a MIC of 0.8 μg/mL and are more potent than standard drugs EMB and INN. The nature of the substituents on the phenothiazine (R^1) and 1,3,4-thiadiazole (R^2) rings affected the activity of the molecules. The presence of a methyl (compounds **5a-c**) or *n*-propyl (compounds **5m-o**) group on the 1,3,4-thiadiazole ring substantially increased the antitubercular activity regardless of the nature of the substituent at R^1 (H, Cl or CF_3). Among derivatives which contain an unsubstituted phenothiazine ring (R^1=H), those with a 4-methylphenyl (**5g**) or 4-fluorophenyl (**5j**) substituent at R^2 displayed substantial activity. In the case of chloro substituted (R^1=Cl) derivatives, a 4-chlorophenyl (**5e**) or 4-fulorophenyl (**5k**) substituent at R^2 enhanced the activity. Among trifluoromethyl substituted analogs, only one compound (**5l**) with a 4-fluoro phenyl substituent at R^2 showed promising activity. The presence of electron rich groups such as 3,4-dimethoxy phenyl or thienyl at R^2 failed to enhance the inhibition activity of the molecules (**5p-u**). This is evident from the observation that except compound **5p** which showed a MIC of 12.5 μg/mL, all other molecules (**5q-u**) were only moderately active. Further, an alkyl/substituted (4-methyl/4-Cl/4-F) phenyl group on the 1,3,4-thiadiazole ring and a chlorosubstituted/unsubstituted phenothiazine ring serve to enhance the antitubercular activity of the hybrid derivatives. The

structure activity relationship suggest that the phenothiazine-thiadiazole core could be considered as a promising structural unit for the development of new antiTB agents.

(Figure 2 here)

(Figure 3 here)

2.3. Antibacterial activity

The *in vitro* antibacterial activity of synthesized compounds **5a-u** was tested using the disc diffusion method [30]. All the compounds were screened against three bacterial strains viz. *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* using ciprofloxacin as the standard drug. The compounds were dissolved in DMSO with two concentrations (75 μg/mL and 50 μg/mL). Compounds **5a**, **5g** and **5p** demonstrated significant inhibition activity (Table 2) against all three bacterial strains at both concentrations. It is interesting to note that all three derivatives contain an unsubstituted phenothiazine ring (R^1=H) and an electron rich substitution (R^2) on the 1,3,4-thiadiazole ring.

(Table 2 here)

2.4. *In vitro* cytotoxicity studies

The cytotoxicity of the potent compounds was evaluated against VERO (African Green Monkey Kidney) cell lines using the MTT assay [31]. The graphical representation of the IC_{50} value and selectivity index (SI) of the compounds is shown in figure 4. The high IC_{50} value (>215 μg/mL) indicates the nontoxicity of the compounds. Further, the compounds exhibited high selectivity index (SI = IC_{50} against Vero cells/MIC against *M. tuberculosis*) in the range 76 - 473. The compounds with SI values greater than 10 against Vero cells are considered as nontoxic [32]. Hence, these molecules which possess significant antiTB activity with a low toxicity profile are suitable for further development as new antiTB agents.

(Figure 4 here)

2.5. Molecular docking studies

In order to further understand the mechanism of action of the new phenothiazine-1,3,4-thiadiazole hybrids, the active molecules were subjected to molecular docking studies against two enzymes, enoyl-acyl carrier protein reductase (InhA) and cytochrome P450 monooxygenase (CYP121) of *M. tuberculosis,* which have been validated as effective antiTB targets. The molecules were docked with in the active sites of InhA (PDB code: 1P44) and

CYP121 (PDB code: 4KTF) using Glide 6.6 (Schrodinger, 2015-1) package. The ligands from the crystal structure of the enzyme-ligand complexes were rebuilt and redocked to validate the docking procedure. The RMSD values of the docking pose from the original orientation of the ligands were found to be 0.6106 and 1.1467 Å respectively for 1P44 and 4KTF. The docking poses of molecules **5g**, **5n**, **5l** and **5o** are shown in figure 5. The docking score of all the active molecules and details of interacting amino acid residues are given in the supplementary data. The target molecules, as they contain 4 or 5 aromatic rings, showed strong *pi-pi* stacking interactions in addition to hydrogen bonding interaction with the target enzymes. Most of the active compounds interacted with Phe 149 residue of 1P44 and Phe 168 residue of CYP121. For instance, both **5g** and **5n** (the most active compounds of the series) showed *pi-pi* stacking interaction with these amino acid residues. Compound **5g** with a docking score of -8.98 displayed additional interactions with 1P44 *viz* a *pi-pi* stacking interaction with Tyr 158 and an H-bonding interaction with Gly 104. It is interesting to note that the two phenyl rings of **5g** interacted simultaneously with both Phe 149 and Tyr 158 residues (figure 5a) which ensures the stronger binding of the compound with the target enzyme. The docking pose of **5g** with target enzyme 4KTF (figure 5d) reveals the additional *pi-pi* stacking interaction with Trp 182 residue. Compound **5l**, which showed an MIC of 1.6 μg/mL, displayed the highest docking scores with both target enzymes (-9.37 with 1P44 and -9.1 with 4KTF). The *pi-pi* stacking interaction of the molecule with the target enzymes is evident from the docking poses presented in figures 5b and 5e. These results suggest that the strong *pi-pi* stacking interaction of the active molecules with the target enzymes could be responsible for their significant inhibition activity against the MTB strain. The presence of a *pi-pi* stacking interaction was observed also in the case of isoniazid (INH), which is a first line antiTB drug. It is known that INH acts by inhibiting InhA and shows a *pi-pi* stacking interaction with Phe 149 residue of the enzyme. Hence, the MTB inhibition and docking studies signifies that the active phenothiazine-thiadiazole hybrids are suitable for the development new antiTB agents.

(Figure 5 here)

3. Conclusions

We have designed a series of 2-(2-imino-1,3,4-thiadiazol-3(2*H*)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone derivatives (**5a-u)** following the molecular hybridization

approach. The antiTB assay against *Mtb* H37Rv revealed the significant inhibition activity of the molecules. Interestingly, eleven compounds of the series showed remarkable inhibitory activity with MIC ≤ 3.125 μg/mL, among which derivatives **5g** and **5n** emerged as the most potent leads with MIC of 0.8 μg/mL which suggests that the phenothiazine-thiadiazole core could be considered as a new active structural unit for the development of antiTB leads. The structure-activity relationship revealed that alkyl (methyl/n-propyl) or substituted (4-methyl/4-Cl/4-F) phenyl groups on the 1,3,4-thiadiazole ring enhance the inhibition activity of the compounds. Also, compounds containing chloro/unsubstituted phenothiazine ring exhibited remarkable antiTB activity. Further, the cytotoxicity study revealed the nontoxic nature of the active molecules to a normal Vero cell line. The molecular docking study demonstrated that strong *pi-pi* stacking interaction of the active molecules with the target enzymes could be responsible for their significant inhibition activity against MTB strain. These results suggest that the molecules are promising leads for further studies and development of new antiTB agents.

4. Experimental part

4.1. Synthesis

*4.1.1. 2-chloro-1-(10H-phenothiazin-10-yl)ethanone (**2a**):* To the solution of 10*H*-phenothiazine 1 (5.0 g, 25.11 mmol) in toluene (75 mL) was cooled to 0 °C. To the above solution chloroacetyl chloride (3.0 mL, 37.6 mmol) was added and reaction mixture was heated at 80 °C for 12 h. The reaction mixture was allowed cool to room temperature, concentrated under reduced pressure (< 45 °C) and to the crude material water (50 mL) was added and extracted with dichloromethane (2 × 50 mL). Organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to get compound **2a** as off white solid. Yield: 6.56 g, 95 %; m.p: 114-115 °C; FTIR (ATR, cm^{-1}): 2998, 2950, 1670, 1586, 1476, 1459, 1442, 1401, 1339, 1275, 1248, 1193, 1170, 1123, 782, 751, 647, 615; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):7.52 (s, 2H, Ar-H), 7.40 (s, 2H, Ar-H), 7.29 (d, J = 5.7 Hz, 2H, Ar-H), 7.21 (d, J = 6.3 Hz, 2H, Ar-H), 4.12 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.5, 137.9, 133.1, 128.1, 127.4, 127.3, 126.5, 41.8; ESI-MS (*m/z*) = 276.1 (M + 1); calculated for $C_{14}H_{10}ClNOS$; C, 60.98; H, 3.66; N, 5.08; S, 11.63. Found: C, 60.87; H, 3.68; N, 5.10; S, 11.65.

*4.1.2 2-chloro-1-(2-chloro-10H-phenothiazin-10-yl)ethanone (**2b**):* White solid. Yield: 6.0 g, 90 %; m.p: 118-119 °C; FTIR (ATR, cm^{-1}): 3057, 2993, 2943, 1673, 1574, 1451, 1398,

1328, 1268, 1162, 1125, 1090, 801, 755, 732, 689, 654; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.58 (s, 1H, Ar-H), 7.46 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.31 (d, *J* = 3.4 Hz, 2H, Ar-H), 7.21 (d, *J* = 14.9 Hz, 2H, Ar-H), 4.16 (d, *J* = 12.5 Hz, 1H, CH), 4.08 (d, *J* = 12.5 Hz, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):165.4, 139.2, 137.5, 132.4, 132.3, 131.9, 129.7, 128.6, 128.3, 128.2, 127.9, 127.4, 127.3, 43.2; ESI-MS (*m/z*) = 310.1 (M + 1); calculated for $C_{14}H_9Cl_2NOS$; C, 54.21; H, 2.92; N, 4.52; S, 10.34. Found: C, 54.18; H, 2.93; N, 4.55; S, 10.35.

*4.1.3 2-chloro-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl)ethanone (**2c**):* White solid. Yield: 6.16 g, 96 %; m.p: 110-111 °C; FTIR (ATR, cm^{-1}): 3002, 2947, 1683, 1607, 1465, 1324, 1298, 1274, 1242, 1163, 1128, 1083, 823, 745, 639.63; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.84 (s, 1H, Ar-H), 7.54 – 7.38 (m, 4H, Ar-H), 7.33 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 4.18 (d, *J* = 12.5 Hz, 1H, CH), 4.06 (d, *J* = 12.5 Hz, 1H, CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.4, 138.0, 137.1, 128.9, 128.3, 128.2, 127.9, 126.1, 125.2, 124.1, 123.9, 41.5; ESI-MS (*m/z*) = 344.1 (M + 1); calculated for $C_{15}H_9ClF_3NOS$; C, 52.41; H, 2.64; N, 4.07; S, 9.33. Found: C, 52.38; H, 2.63; N, 4.05; S, 9.35.

*4.2.1. 5-amino-2-methyl-1, 3, 4-thiadiazole (**4a**):* To the thiosemicarbazide (7.5 g, 82.29 mmol), acetyl chloride (14.27 mL, 164.58 mmol) was added slowly and the mixture was stirred for 4 h at RT. To this reaction mixture, ice cold water was added and the solid obtained was filtered off. The solid was then taken in ice cold water and to this slurry, a solution of 50% NaOH was added till the pH of the solution becomes basic. The solid obtained was filtered off and dried under vacuum to get the compound (**4a**) as white solid. Yield: 7.2 g, 76 %; m.p: 269–270 °C; FTIR (ATR, cm^{-1}): 3288, 3105, 2925, 1614, 689; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.05 (br s, 2H, NH_2), 2.18 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 148.8, 10.9; ESI-MS (*m/z*) = 116.02 (M + 1); calculated for $C_3H_5N_3S$; C, 31.9; H, 4.38; N, 36.49; S, 27.84. Found: C, 31.89; H, 4.41; N, 36.42; S, 27.44.

4.2.2. General procedure for the synthesis of 5-subsituted-1,3,4-thiadiazole-2-yl amine (**4b-g):** A mixture of appropriate aromatic carboxylic acid (5.0 g, 1 eq), thiosemicarbazide (1 eq) and $POCl_3$ (1.85 eq) was heated to 75 °C and maintained at the same temperature for 30 min under stirring. The reaction mixture was then cooled to room temperature, water (11 v) was added and the mixture was refluxed for 4 h. After cooling, the mixture was basified with 50%

NaOH to pH 8 by the drop wise addition under stirring. The obtained solid was filtered and recrystallized from ethanol to give the target compounds **4b-g**.

*4.2.2.1. 5-(4-Chlorophenyl)-1,3,4-thiadiazol-2-amine (**4b**):* White solid. Yield: 5.20 g, 77 %; m.p: 210–211 °C; FTIR (ATR, cm^{-1}): 3258, 3093, 1633, 751, 684; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.47 (s, 2H), 7.52–7.54 (d, J = 6.55 Hz, 2H, Ar-H), 7.76–7.78 (d, J = 6.24 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):128.38, 129.63, 130.34, 134.43, 155.59, 168.30; ESI-MS (*m/z*) = 212.6 (M + 1); calculated for $C_8H_6ClN_3S$; C, 45.39; H, 2.86; N, 19.85; S, 15.15. Found: C, 45.30; H, 2.88; N, 19.90; S, 15.14.

*4.2.2.2. 5-(4-Methylphenyl)-1,3,4-thiadiazol-2-amine (**4c**):* White solid; yield: 6.31 g, 90 %; m.p: 213–214 °C; FTIR (ATR, cm^{-1}): 3278, 3103, 2955, 1611, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H), 7.25–7.27 (d, J = 8.00 Hz, 2H, Ar-H), 7.36 (s, 2H), 7.63–7.65 (d, J = 8.12 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.35, 126.72, 128.80, 130.11, 139.76, 156.95, 168.65; ESI-MS (*m/z*) = 192.2 (M + 1); Calculated for $C_9H_9N_3S$; C, 56.22; H, 4.74; N, 21.97; S, 16.77. Found: C, 56.20; H, 4.74; N, 21.90; S, 16.74.

*4.2.2.3. 5-(4-Fluorophenyl)-1,3,4-thiadiazol-2-amine (**4d**):* Colorless solid. Yield: 5.69 g, 82 %; m.p: 230-231 °C; FTIR (ATR, cm^{-1}): 3346, 3251, 2934, 1633, 1063, 683; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.29–7.33 (t, J = 8.77 Hz, 2H, Ar-H), 7.41 (s, 2H), 7.79–7.83 (dd, J = 5.46 Hz, 8.59 Hz, 2H, Ar-H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 118.13, 129.43, 130.21, 154.32, 157.28, 168.10; ESI-MS (*m/z*) = 196.4 (M + 1); calculated for $C_8H_6FN_3S$; C, 49.22; H, 3.10; N, 21.52; S, 16.43. Found: C, 49.20; H, 3.11; N, 21.52; S, 16.44.

*4.2.2.4. 5-Propyl-1,3,4-thiadiazol-2-amine (**4e**):* White solid. Yield: 6.33 g, 78 %; m.p: 209-210 °C; FTIR (ATR, cm^{-1}): 3228, 3075, 2956, 2928, 1634, 1520, 1497, 1459, 1327, 1187, 1039, 686; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.82 (br s, 2H), 2.61 (t, J = 7.6 Hz, 2H), 1.61 (sext, J = 7.6 Hz, 2H), 0.92 (t, J = 7.6 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 148.8, 31.4, 21.7, 13.3; ESI-MS (*m/z*) = 144.1 (M + 1); calculated for $C_5H_9N_3S$; C, 41.93; H, 6.33; N, 29.34; S, 22.39. Found: C, 41.89; H, 6.35; N, 29.22; S, 22.44.

*4.2.2.5. 5-(3,4-Dimethoxyphenyl)-1,3,4-thiadiazol-2-amine (**4f**):* White solid. Yield: 5.53 g, 85 %; m.p: 213-214 °C; FTIR (ATR, cm^{-1}): 3251, 3081, 3004, 2949, 1619, 1589,

1517, 1468, 1419, 1248, 1058, 1044, 1022, 861, 797, 758, 704, 662; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.65–8.69 (m, 3H, Ar-H), 7.61 (br s, 2H), 3.71 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.7, 154.1, 144.1, 132.8, 125.5, 118.4, 115.8, 108.1, 55.8, 55.2; ESI-MS (*m/z*) = 238.1 (M + 1); calculated for $C_{10}H_{11}N_3O_2S$; C, 50.62; H, 4.67; N, 17.71; S, 13.51. Found: C, 50.72; H, 4.66; N, 17.75; S, 13.54.

*4.2.2.6. 5-(Thiophen-2-yl)-1,3,4-thiadiazol-2-amine (**4g**):* White solid. Yield: 6.57 g, 92 %; m.p: 175-176 °C; FTIR (ATR, cm^{-1}): 3271, 3097, 1618, 1510, 1463, 1419, 1328, 1257, 1229, 1134, 1068, 1044, 914, 844, 775, 703; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.70 – 7.64 (m, 2H, Ar-H), 7.63 – 7.57 (m, 1H, Ar-H), 7.34 (br s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.3, 154.3, 134.9, 131.6, 133.7, 127.4; ESI-MS (*m/z*) = 183.9 (M + 1); calculated for $C_6H_5N_3S_2$; C, 39.32; H, 2.75; N, 22.93; S, 35.00. Found: C, 39.29; H, 2.74; N, 22.89; S, 35.03.

4.2.3. General Procedure for the Synthesis of derivatives (**5a-u)**: A mixture of 5-subsituted 2-amino-1,3,4-thiadiazole (**4a-g**) (0.2 g, 1.0 mmol) and 2-chloro-1-(2-substituted)-10*H*-phenothiazin-10-yl)ethanone (**2a-c**) (1.0 mmol) in ethanol was stirred at 80-85 °C for 17 h. After the completion of reaction (as confirmed by TLC), the reaction mass was cooled to room temperature and then concentrated under reduced pressure. The obtained crude product was basified with aqueous 10% Na_2CO_3 solution. To the above solution ethyl acetate (20 mL) was added, the organic layer was separated, washed with brine solution (2 × 10 mL), dried over anhydrous $Na_2S_2O_4$ and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography (100-200 mesh) eluted with methanol and dichloromethane (0.5 : 9.5) to give pure products **5a-u**.

*4.2.3.1. 2-(2-imino-5-methyl-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl)ethanone (**5a**):* White solid. Yield: 0.647 g; FTIR (ATR, cm^{-1}): 3314, 1696, 1601, 1460, 1259, 1157, 948, 751, 704; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.16 (br s, 1H, NH, D_2O exchangeable proton), 7.68 (s, 2H, Ar-H), 7.56 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.40 (t, *J* = 7.2 Hz, 2H, Ar-H), 7.32 (t, *J* = 7.3 Hz, 2H, Ar-H), 4.74 (s, 2H), 2.20 (s, 3H, CH_3); ^{1}H NMR (400 MHz, D_2O) δ 7.61 (s, 2H), 7.53 (d, *J* = 7.4 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 4.68 (s, 2H), 2.15 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.9, 137.9, 132.5, 128.4, 127.9, 127.8, 127.5, 124.7, 49.3, 17.08; ESI-MS (*m/z*) = 355.1 (M + 1); anal.

calcd (%) for $C_{17}H_{14}N_4OS_2$: C, 57.61; H, 3.98; N, 15.81; S, 18.09. Found: C, 57.42; H, 4.02; N, 15.78; S, 18.12.

*4.2.3.2. 1-(2-chloro-10H-phenothiazin-10-yl) -2- (2-imino-5-methyl-1, 3, 4-thiadiazol -3(2H)yl)ethanone (**5b**):* Off-white solid. Yield: 0.573 g; FTIR (ATR, cm^{-1}): 3313, 2919, 1694, 1598, 1456, 1242, 1172, 1131, 1092, 948, 808, 744, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.95 (br s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.70 (d, J = 7.5 Hz, 1H, Ar-H), 7.57 (d, J = 8.3 Hz, 2H, Ar-H), 7.45 – 7.37 (m, 2H, Ar-H), 7.34 (t, J = 7.6 Hz, 1H, Ar-H), 4.84 (s, 1H, CH), 4.62 (s, 1H, CH), 2.18 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 141.6, 140.3, 138.2, 137.7, 134.5, 129.3, 128.3, 127.6, 125.7, 125.1, 124.4, 122.2, 121.2, 49.7, 17.1; ESI-MS (*m/z*) = 389.1 (M + 1); anal. calcd (%) for $C_{17}H_{13}ClN_4OS_2$: C, 52.50; H, 3.37; N, 14.41; S, 16.49. Found: C, 52.48; H, 3.40; N, 14.38; S, 16.52.

*4.2.3.3. 2-(2-imino-5-methyl-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl) ethanone (**5c**):* Light brown solid. Yield: 0.645 g, FTIR (ATR, cm^{-1}): 3341, 2893, 1692, 1594, 1467, 1414, 1362, 1326, 1296, 1245, 1166, 1122, 1085, 1029, 984, 951, 885, 814, 744, 695, 656; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.98 (br s, 1H, NH), 7.84 (d, J = 8.0 Hz, 1H, Ar-H), 7.68 (dd, J = 22.2, 7.5 Hz, 3H, Ar-H), 7.52 – 7.35 (m, 3H, Ar-H), 4.74 (s, 2H, CH_2), 2.46 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.4, 143.9, 141.81, 138.87, 137.73, 129.9, 128.33, 127.6, 127.04, 125.77, 125.12, 124.84, 122.29, 116.02, 52.79, 17.6; ESI-MS (*m/z*) = 423.1 (M + 1); anal. calcd (%) for $C_{18}H_{13}F_3N_4OS_2$: C, 51.18; H, 3.10; N, 13.26; S, 15.18. Found: C, 51.22; H, 3.12; N, 13.28; S, 15.22.

*4.2.3.4. 2-(5-(4-chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl)ethanone (**5d**):* White solid. Yield: 0.375 g, FTIR (ATR, cm^{-1}): 3314, 3056, 1690, 1459, 1343, 1262, 1236, , 1182, 1129, 827, 757, 659; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.01 (br s, 1H, NH), 7.78 (d, J = 7.5 Hz, 4H, Ar-H), 7.61 (d, J = 7.7 Hz, 4H, Ar-H), 7.46 (s, 2H, Ar-H), 7.38 (s, 2H, Ar-H), 5.27 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.1, 137.4, 137.3, 137.0, 136.9, 130.0, 129.6, 128.6, 128.1, 127.3, 52.8; ESI-MS (*m/z*) = 451.1 (M + 1); anal. calcd (%) for $C_{22}H_{15}ClN_4OS_2$: C, 58.59; H, 3.35; N, 12.42; S, 14.22. Found: C, 58.52; H, 3.32; N, 12.48; S, 14.25.

*4.2.3.5. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(5-(4-chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl) ethanone (**5e**):* Light brown solid. Yield: 0.412 g; FTIR (ATR, cm^{-1}):

3303, 2976, 1689, 1634, 1572, 1458, 1401, 1366, 1287, 1245, 1177, 1091, 996, 903, 821, 749, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.02 (br s, 1NH), 7.82-7.75 (m, 4H), 7.60 (d, J = 7.8 Hz, 4H), 7.45 (m, 3H), 5.28 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 163.5, 146.8, 146.7, 145.8, 144.7, 142.3, 141.5, 137.6, 137.5, 137.2, 130.1, 129.0, 126.5, 53.9; ESI-MS (*m/z*) = 484.9 (M + 1); anal. calcd (%) for $C_{22}H_{14}Cl_2N_4OS_2$: C, 54.44; H, 2.91; N, 11.54; S, 13.21. Found: C, 54.48; H, 2.91; N, 11.56; S, 13.23.

*4.2.3.6. 2-(5-(4-chlorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl)ethanone (**5f**):* Light brown solid. Yield: 0.451 g; FTIR (ATR, cm^{-1}): 3340, 1690, 1604, 1418, 1400, 1370, 1327, 1299, 1248, 1169, 1126, 1087, 1014, 985, 955, 934, 905, 886, 827,745, 652; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.08 (br s, 1NH), 7.79 (s, 1H), 7.68 (d, J = 7.8 Hz, 2H), 7.60-7.54 (m, 5H), 7.45 (d, J = 7.4 Hz, 2H), 7.40 (s, 1H, Ar-H), 4.83 (s, 1H), 4.70 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.15, 138.2, 135.1, 129.7, 129.4, 129.3, 128.7 128.5, 128.3, 127.5, 124.4, 124.3, 124.2, 122.9, 49.84; ESI-MS (*m/z*) = 519.1 (M + 1); anal. calcd (%) for $C_{23}H_{14}ClF_3N_4OS_2$: C, 53.23; H, 2.72; N, 10.80; S, 12.36. Found: C, 53.33; H, 2.71; N, 10.79; S, 12.40.

*4.2.3.7. 2-(2-imino-5-p-tolyl-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl) ethanone (**5g**):* Off-white solid. Yield: 0.382 g; FTIR (ATR, cm^{-1}): 3330, 2949, 1690, 1593, 1458, 1362, 1256, 1178, 1146, 979.76, 750, 724, 696; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.78 (s, 1H), 7.73 (s, 2H), 7.58 (d, J = 6.5 Hz, 2H), 7.48 (d, J = 6.7 Hz, 2H), 7.41 (s, 2H), 7.33 (s, 2H), 7.28 (d, J = 6.4 Hz, 2H), 4.96 (s, 2H), 2.33 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.5, 141.0, 137.8, 132.5, 130.2, 128.5, 127.9, 127.5, 127.3, 126.07, 50.50, 21.42; ESI-MS (*m/z*) = 431.1 (M + 1); anal. calcd (%) for $C_{23}H_{18}N_4OS_2$: C, 64.16; H, 4.21; N, 13.01; S, 14.90. Found: C, 64.20; H, 4.19; N, 13.10; S, 14.88.

*4.2.3.8. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(2-imino-5-(p-tolyl)-1,3,4-thiadiazol-3(2H) yl) ethanone (**5h**):* Light yellow solid. Yield: 0.426 g; FTIR (ATR, cm^{-1}): 3316, 2920, 1694, 1586, 1503, 1455, 1395, 1356, 1277, 1242, 1206, 1175, 1129, 1093, 1026, 981, 949, 901, 812, 743, 693; ^{1}H NMR (400 MHz, DMSO) δ 8.45 (br s, NH), 7.82 (s, 1H, Ar-H), 7.73-7.69 (m, 2H, Ar-H), 7.60 (d, J = 7.4 Hz, 2H), 7.48 (d, J = 7.3 Hz, 2H), 7.41 (s, 2H), 7.25 – 7.17 (m, 2H 96 (s, 2H), 2.38 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.7, 141.6, 139.1, 136.8, 135.4, 136.7, 135.5, 130.0, 129.3, 129.0, 128.4, 127.9, 126.3, 125.8, 125.4, 124.6, 122.6, 121.2, 51.2, 21.33; ESI-MS (*m/z*) = 464.1 (M + 1); anal. Calcd (%) for

$C_{23}H_{17}ClN_4OS_2$: C, 59.41; H, 3.69; N, 12.05; S, 13.79. Found: C, 59.43; H, 3.70; N, 12.08; S, 13.80.

*4.2.3.9. 2-(2-imino-5-(p-tolyl)-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl) ethanone (**5i**):* Yellow solid. Yield: 0.474 g; FTIR (ATR, cm^{-1}): 3339, 3021, 2893, 1693, 1592, 1466, 1414, 1362, 1326, 1296, 1245, 1166, 1122, 1085, 1029, 984, 951, 886, 816, 746, 694, 656; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.38 (br s, NH, 1H), 7.92 (s, 1H, Ar-H), 7.71 (t, J = 6.1 Hz, 2H), 7.66 – 7.58 (m, 4H, Ar-H), 7.40 (d, J = 7.4 Hz, 2H, Ar-H), 7.31 (dd, J = 14.23, 7.4 Hz, 2H, Ar-H), 4.83 (s, 1H), 4.70 (s, 1H), 2.35 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.7, 143.9, 142.81, 140.1, 137.7, 136.4, 131.0, 129.9, 129.0, 128.3, 127.6, 127.0, 126.2, 125.7, 125.1 , 124.8, 122.2, 116.0, 52.7, 21.3; ESI-MS (*m/z*) = 499.1 (M + 1); anal. calcd (%) for $C_{24}H_{17}F_3N_4OS_2$: C, 57.82; H, 3.44; N, 11.24; S, 12.86. Found: C, 57.78; H, 3.48; N, 11.22; S, 12.90.

*4.2.3.10. 2-(5-(4-fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl) ethanone (**5j**):* Light brown solid. Yield: 0.409 g; FTIR (ATR, cm^{-1}): 3305**,** 3055**,** 2355**,** 1687**,** 1593**,** 1501**,** 1459**,** 1395**,** 1357**,** 1300**,** 1255**,** 1228**,** 1177**,** 1157**,** 1127**,** 1030**,** 987**,** 948**,** 835**,** 811**,** 756**,** 692**,** 660**,** 630; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.35 (br s, 1H, NH), 7.71 (s, 2H, Ar-H), 7.63 – 7.54 (m, 4H, Ar-H), 7.40 (t, J = 7.6 Hz, 2H, Ar-H), 7.31 (dd, J = 16.3, 7.8 Hz, 4H, Ar-H), 4.88 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.1, 162.8, 156.4, 137.7, 129.3, 128.4, 128.2, 127.6, 125.86, 122.3, 114.6, 50.21; ESI-MS (*m/z*):= 435.1 (M + 1); anal. calcd (%) for $C_{22}H_{15}FN_4OS_2$: C, 60.81; H, 3.48; N, 12.89; S, 14.76. Found: C, 60.77; H, 3.50; N, 12.88; S, 14.70.

*4.2.3.11. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(5-(4-fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)ethanone (**5k**):* Brown solid. Yield: 0.456 g; FTIR (ATR, cm^{-1}): 3313**,** 3066**,** 1691**,** 1592**,** 1501**,** 1456**,** 1396**,** 1356**,** 1322**,** 1279**,** 1232**,** 1175**,** 1158**,** 1131**,** 1094**,** 987**,** 950**,** 835**,** 811**,** 744**,** 694; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.35 (br s, 1H, NH), 7.82 (s, 1H, Ar-H), 7.73 (s, 1H, Ar-H), 7.64 – 7.53 (m, 3H), 7.46-7.35 (m , 3H, Ar-H), 7.31 (dd, J = 15.7, 7.0 Hz, 3H), 5.01 (s, 1H), 4.77 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.0, 164.67, 159.91, 159.24, 158.90, 149.25, 139.1, 132.2, 129.57, 128.65, 127.9, 127.5, 127.46, 117.3, 116.7, 49.8; ESI-MS (*m/z*) = 469.1 (M + 1); anal. calcd (%) for $C_{22}H_{14}ClFN_4OS_2$: C, 56.35; H, 3.01; N, 11.95; S, 13.68. Found: C, 56.28; H, 3.00; N, 11.93; S, 13.70.

*4.2.3.12. 2-(5-(4-fluorophenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl) ethanone (**5l**):* Yellow solid. Yield: 0.453 g; FTIR (ATR, cm^{-1}): 3328**,** 2921**,** 1694**,** 1594**,** 1501**,** 1466**,** 1414**,** 1359**,** 1326**,** 1296**,** 1239**,** 1165**,** 1122**,** 1086**,** 1029**,** 987**,** 950**,** 889**,** 833**,** 751**,** 694**,** 629; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.33 (br s, 1H, NH), 8.03 (s, 1H, Ar-H), 7.78 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.65 (d, *J* = 6.9 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.9, 5.3 Hz, 3H, Ar-H), 7.45 (dd, *J* = 14.2, 7.6 Hz, 1H, Ar-H), 7.38 (dd, *J* = 13.0, 6.3 Hz, 1H), 7.34 – 7.26 (m, 2H, Ar-H), 5.06 (s, 1H), 4.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.1, 162.22, 138.3, 137.1, 129.3, 128.76, 128.5, 128.3, 128.2, 128.15, 124.3, 116.8, 116.6, 49.8; ESI-MS (*m/z*) = 503.1 (M + 1); anal. calcd (%) for $C_{23}H_{14}F_4N_4OS_2$: C, 54.97; H, 2.81; N, 11.15; S, 12.76. Found: C, 54.88; H, 2.81; N, 11.16; S, 12.66.

*4.2.3.13. 2-(2-imino-5-propyl-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl) ethanone (**5m**):* Off-white solid. Yield: 0.432 g; FTIR (ATR, cm^{-1}): 3370**,** 2992**,** 2925**,** 2871**,** 2784**,** 1687**,** 1636**,** 1577**,** 1550**,** 1480**,** 1460**,** 1416**,** 1363**,** 1302**,** 1283**,** 1262**,** 1240**,** 1182**,** 1163**,** 1128**,** 1077**,** 1026**,** 974**,** 766**,** 656; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.68 (s, 2H, Ar-H), 7.63 (d, *J* = 6.9 Hz, 2H, Ar-H), 7.45 (s, 2H, Ar-H), 7.38 (s, 2H, Ar-H), 5.84 (s, 1H), 4.92 (s, 1H), 2.80 (t, *J* = 7.2 Hz, 2H), 1.61 (sextet, *J* = 7.2 Hz, 2H), 0.88 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm):169.01, 163.5, 158.3, 158.1, 128.7, 128.6, 128.5, 128.3, 128.2, 53.3, 31.5, 21.7, 13.4; ESI-MS (*m/z*) = 383.1 (M + 1); anal. calcd (%) for $C_{20}H_{14}N_4OS_3$: C, 56.85; H, 3.34; N, 13.26; S, 22.77. Found: C, 56.94; H, 3.36; N, 13.24; S, 22.69.

*4.2.3.14. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(2-imino-5-propyl-1,3,4-thiadiazol-3(2H)yl)ethanone (**5n**):* Light yellow solid. Yield: 0.504 g; FTIR (ATR, cm^{-1}): 3321**,** 2957**,** 2926**,** 2869**,** 1690**,** 1589**,** 1561**,** 1478**,** 1455**,** 1391**,** 1277**,** 1231**,** 1174**,** 1122**,** 1091**,** 1028**,** 916**,** 794**,** 739; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.21 (br s, 1H, NH), 7.79 (s, 1H, Ar-H), 7.70 (d, *J* = 7.6 Hz, 2H), 7.63 – 7.54 (m, 2H, Ar-H), 7.19 (d, *J* = 7.6 Hz, 2H), 5.08 (s, 1H), 4.85 (s, 1H), 2.77 (t, *J* = 7.8 Hz, 2H), 1.54 (sextet, *J* = 7.8 Hz, 2H), 0.89 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.2, 163.8, 159.3, 141.6, 138.5, 134.5, 130.2, 128.3, 127.9, 126.7, 125.4, 124.5, 122.31, 121.23, 49.5, 31.3, 21.4, 13.2; ESI-MS (*m/z*) = 417.1 (M + 1); anal. calcd (%) for $C_{19}H_{17}ClN_4OS_2$: C, 54.73; H, 4.11; N, 13.44; S, 15.38. Found: C, 54.62; H, 4.14; N, 13.39; S, 15.44. ESI-MS (*m/z*) = 417.1 (M + 1);

*4.2.3.15. 2-(2-imino-5-propyl-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl)ethanone (**5o**):* Light brown solid. Yield: 0.560 g; FTIR (ATR, cm^{-1}): 3310**,** 2961**,** 2932**,** 2873**,** 1693**,** 1603**,** 1553**,** 1464**,** 1433**,** 1390**,** 1326**,** 1299**,** 1229**,** 1162**,** 1113**,** 1084**,** 1029**,** 952**,** 929**,** 869**,** 810**,** 743**,** 688**,** 649; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.21 (s, 1H, Ar-H), 7.89 – 7.73 (m, 2H, Ar-H), 7.73 – 7.54 (m, 3H, Ar-H), 7.52 – 7.29 (m, 2H, Ar-H), 4.80 (s, 2H, CH_2), 2.79 (t, J = 7.3 Hz, 2H), 1.57 (sextet, J = 7.2 Hz, 2H), 0.89 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 168.3, 163.4, 159.4, 144.9, 138.5, 137.7, 136.82, 133.5, 132.3, 125.5, 125.0, 124.3, 122.2, 116.0, 50.8, 31.2, 22.1, 13.5; ESI-MS (*m/z*) = 451.1 (M + 1); anal. calcd (%) for $C_{20}H_{17}F_3N_4OS_2$: C, 53.32; H, 3.80; N, 12.44; S, 14.24. Found: C, 53.38; H, 3.81; N, 12.45; S, 14.26.

*4.2.3.16. 2-(5-(3,4-dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl) ethanone (**5p**):* White solid. Yield: 0.317 g; FTIR (ATR, cm^{-1}): 3319**,** 2932**,** 2834**,** 1689**,** 1587**,** 1505**,** 1459**,** 1417**,** 1356**,** 1301**,** 1248**,** 1173**,** 1131**,** 1019**,** 947**,** 859**,** 836**,** 805**,** 759**,** 735**,** 696**,** 660**,** 634; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.20 (s, 1H), 7.69 (d, J = 17.4 Hz, 2H, Ar-H), 7.60 – 7.53 (m, 2H, Ar-H), 7.40 (t, J = 7.0 Hz, 2H, Ar-H), 7.32 (t, J = 7.1 Hz, 2H, Ar-H), 7.12 (s, 1H, Ar-H), 7.06 – 6.97 (m, 2H, Ar-H), 4.86 (s, 2H), 3.78 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.2, 159.0, 158.7, 149.4, 145.3, 141.9, 136.7, 132.7, 132.6, 128.4, 127.8, 127.5, 119.7, 112.2, 108.0, 56.1, 56.0, 49.8; ESI-MS (*m/z*) = 477.2 (M + 1); anal. calcd (%) for $C_{24}H_{20}N_4O_3S_2$: C, 60.49; H, 4.23; N, 11.76; S, 13.46. Found: C, 60.52; H, 4.23; N, 11.78; S, 13.50.

*4.2.3.17. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(5-(3,4-dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)ethanone (**5q**):* Off-white solid. Yield: 0.348 g; FTIR (ATR, cm^{-1}): 3318**,** 2934**,** 2835**,** 1687**,** 1586**,** 1503**,** 1456**,** 1414**,** 1354**,** 1301**,** 1248**,** 1173**,** 1131**,** 1019**,** 947**,** 859**,** 836**,** 811**,** 759**,** 735**,** 696**,** 660**,** 634; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.19 (s, 1H), 7.98 (s, 1H, Ar-H), 7.74 (d, J = 7.4 Hz, 2H, Ar-H), 7.64 (d, J = 7.2 Hz, 1H, Ar-H), 7.57 – 7.51 (m, 1H, Ar-H), 7.46 (t, J = 7.6 Hz, 1H, Ar-H), 7.32 (t, J = 7.3 Hz, 1H, Ar-H), 7.21 (s, 1H, Ar-H), 7.04 – 6.95 (m, 2H, Ar-H), 4.98 (s, 1H), 4.69 (s, 1H), 3.74 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.6, 161.8, 155.8, 153.1, 150.6, 141.6, 137.7, 134.5, 129.3, 128.2, 127.6, 125.7, 125.1, 124.4, 122.2, 121.2, 118.4, 115.8, 107.9, 56.8, 56.5, 50.3; ESI-MS (*m/z*) = 511.1 (M + 1); anal. Calcd (%) for $C_{24}H_{19}ClN_4O_3S_2$: C, 56.41; H, 3.75; N, 10.96; S, 12.55. Found: C, 56.38; H, 3.74; N, 10.97; S, 12.49.

*4.2.3.18. 2-(5-(3,4-dimethoxyphenyl)-2-imino-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl)-10H-phenothiazin-10-yl)ethanone (**5r**):* Brown solid. Yield: 0.390 g; FTIR (ATR, cm^{-1}): 3302**,** 2933**,** 1693**,** 1590**,** 1505**,** 1462**,** 1414**,** 1358**,** 1326**,** 1244**,** 1166**,** 1121**,** 1084**,** 1019**,** 949**,** 887**,** 831**,** 756**,** 701**,** 634; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.25 (br s, 1H, NH), 8.05 (s, 1H, Ar-H), 7.79 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.66 (d, *J* = 7.1 Hz, 1H, Ar-H), 7.64 – 7.57 (m, 1H, Ar-H), 7.46 (t, *J* = 7.0 Hz, 1H, Ar-H), 7.37 (t, *J* = 7.3 Hz, 1H, Ar-H), 7.15 – 7.08 (m, 1H, Ar-H), 7.04 – 6.97 (m, 2H, Ar-H), 5.07 (s, 1H, CH), 4.71 (s, 1H, CH), 3.78 (s, 6H, dimethoxy); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.7, 159.8, 155.84 (s), 153.1, 150.6, 143.9, 137.7, 137.7, 133.8, 131.0, 125.9, 125.5, 125.03, 124.04, 122.2, 118.4, 116.0, 115.8, 107.9, 56.2, 55.9, 49.9; ESI-MS (*m/z*) = 545.1 (M + 1); anal. calcd (%) for $C_{25}H_{19}F_3N_4O_3S_2$: C, 55.14; H, 3.52; N, 10.29; S, 11.78. Found: C, 55.10; H, 3.56; N, 10.22; S, 11.80.

*4.2.3.19. 2-(2-imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2H)-yl)-1-(10H-phenothiazin-10-yl) ethanone (**5s**):* Light brown solid. Yield: 0.405 g; FTIR (ATR, cm^{-1}): 3298**,** 3061**,** 2933**,** 1688**,** 1588**,** 1477, 1459, 1427, 1392, 1357, 1300, 1279, 1254, 1177, 1126, 1080, 1051, 1030, 946, 897, 840, 757, 700; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.33 (br s, 1H, NH), 7.72 (s, 2H, Ar-H), 7.68 (dd, *J* = 5.1, 1.1 Hz, 1H, Ar-H), 7.57 (d, *J* = 7.7 Hz, 2H, Ar-H), 7.39 (dd, *J* = 10.9, 4.4 Hz, 2H, Ar-H), 7.32 (dd, *J* = 8.4, 4.6 Hz, 3H, Ar-H), 7.11 (dd, *J* = 5.1, 3.7 Hz, 1H, Ar-H), 4.84 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 165.9, 137.9, 129.0, 128.9, 128.7, 128.5, 128.0, 127.9, 127.8, 127.7, 127.6, 127.5,127.4, 49.7; ESI-MS (*m/z*) = 423.1 (M + 1); anal. calcd (%) for $C_{20}H_{14}N_4OS_3$: C, 56.85; H, 3.34; N, 13.26; S, 22.77. Found: C, 56.94; H, 3.36; N, 13.24; S, 22.69.

*4.2.3.20. 1-(2-chloro-10H-phenothiazin-10-yl)-2-(2-imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2H)-yl) ethanone (**5t**):* Off-white solid. Yield: 0.443 g; FTIR (ATR, cm^{-1}): 3317**,** 3066**,** 1692**,** 1588**,** 1456**,** 1428**,** 1395**,** 1357**,** 1279**,** 1243**,** 1175**,** 1129**,** 1092**,** 1052**,** 947**,** 917**,** 876**,** 841**,** 811**,** 765**,** 742**,** 701; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (br s, 1H, NH), 7.82 (s, 1H, Ar-H), 7.79 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.68 – 7.64 (m, 2H, Ar-H), 7.62 – 7.56 (m, 2H, Ar-H), 7.33 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.16 (t, *J* = 5.4 Hz, 2H), 5.03 (s, 1H, CH), 4.82 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 166.2, 148.3, 145.8, 141.6, 137.7, 134.5, 133.5, 130.0, 129.3, 128.3, 127.6, 127.4, 127.2, 125.7, 125.1, 124.4, 122.2, 121.2, 50.7; ESI-MS (*m/z*) = 456.9 (M + 1); anal. calcd (%) for $C_{20}H_{13}ClN_4OS_3$: C, 52.56; H, 2.87; N, 12.26; S, 21.05. Found: C, 52.63; H, 2.88; N, 12.30; S, 20.98.

*4.2.3.21. 2-(2-imino-5-(thiophen-2-yl)-1,3,4-thiadiazol-3(2H)-yl)-1-(2-(trifluoromethyl) -10H-phenothiazin-10-yl)ethanone (**5u**):* Off-white solid. Yield: 0.465 g, 87 %; m.p: 108-109 °C; FTIR (ATR, cm^{-1}): 3321, 3075, 2936, 1693, 1591, 1486, 1465, 1417, 1358, 1326, 1298, 1245, 1166, 1120, 1084, 980, 947, 922, 880, 841, 825, 754, 738, 702, 634, 618; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.31 (br s, 1H, NH), 8.04 (s, 1H, Ar-H), 7.79 (d, J = 8.2 Hz, 2H, Ar-H), 7.70 – 7.64 (m, 2H, Ar-H), 7.63 – 7.57 (m, 1H, Ar-H), 7.45 (dd, J = 11.5, 3.8 Hz, 1H, Ar-H), 7.37 (t, J = 7.1 Hz, 1H, Ar-H), 7.31 (d, J = 2.7 Hz, 1H, Ar-H), 7.11 (dd, J = 5.0, 3.7 Hz, 1H, Ar-H), 5.02 (s, 1H), 4.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.1, 148.3, 146.8, 143.9, 137.7, 133.4, 130.9, 128.4, 127.6, 127.4, 127.1, 125.7, 125.1, 124.8, 122.2, 116.02, 50.7; ESI-MS (*m/z*) = 491.1 (M + 1); anal. calcd (%) for $C_{21}H_{13}F_3N_4OS_3$: C, 51.42; H, 2.67; N, 11.42; S, 19.61. Found: C, 51.50; H, 2.63; N, 11.42; S, 19.62.

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List of figures and tables

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Table 1. Physical properties and structural features of target compounds (**5a-u**).

Entry	Product	R^1	R^2	Yield (%)	m.p (°C)
1	5a	H	CH_3	82	184-185
2	5b	Cl	CH_3	85	167-168
3	5c	CF_3	CH_3	88	203-204
4	5d	H	4-Cl C_6H_4	88	219-220
5	5e	Cl	4-Cl C_6H_4	90	180-181
6	5f	CF_3	4-Cl C_6H_4	92	150-151
7	5g	H	4-CH_3 C_6H_4	85	189-190
8	5h	Cl	4-CH_3 C_6H_4	88	160-161
9	5i	CF_3	4-CH_3 C_6H_4	91	175-176
10	5j	H	4-F C_6H_4	92	161-162
11	5k	Cl	4-F C_6H_4	95	120-121
12	5l	CF_3	4-F C_6H_4	88	118-119
13	5m	H	$CH_3(CH_2)_2$	82	210-211
14	5n	Cl	$CH_3(CH_2)_2$	88	150-151
15	5o	CF_3	$CH_3(CH_2)_2$	89	135-136
16	5p	H	3,4-diOMe C_6H_3	79	105-106
17	5q	Cl	3,4-diOMe C_6H_3	81	110-111
18	5r	CF_3	3,4-diOMe C_6H_3	85	106-107
19	5s	H	2-Thienyl	88	110-111
20	5t	Cl	2-Thienyl	89	109-110
21	5u	CF_3	2-Thienyl	87	108-109

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Table 2. Antibacterial activity of target compounds (**5a-u**) against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Compounds	Zone of inhibition in mm; Mean±SD					
	Escherichia Coli		***Staphylococcus aureus***		***Pseudomonas Aeruginosa***	
Cocn. in µg/ml	**75**	**50**	**75**	**50**	**75**	**50**
5a	25±0.1	20±0.4	26±0.2	22±0.3	25±0.4	20±0.1
5b	16±0.2	14±0.3	13±0.3	10±0.3	13±0.2	09±0.4
5c	20±0.1	15±0.1	17±0.1	14±0.2	15±0.1	12±0.1
5d	10±0.2	08±0.2	09±0.3	07±0.3	10±0.2	07±0.4
5e	15±0.4	12±0.4	17±0.2	15±0.1	20±0.3	15±0.1
5f	12±0.1	11±0.2	13±0.1	11±0.2	14±0.2	09±0.2
5g	27±0.3	24±0.2	21±0.2	16±0.2	11±0.1	09±0.2
5h	09±0.3	07±0.4	12±0.3	10±0.1	07±0.2	04±0.1
5i	13±0.4	11±0.4	11±0.1	09±0.2	09±0.1	06±0.2
5j	11±0.1	08±0.2	08±0.2	05±0.3	12±0.2	08±0.2
5k	12±0.4	07±0.2	09±0.1	05±0.3	10±0.1	07±0.1
5l	11±0.1	09±0.4	10±0.4	07±0.2	13±0.2	07±0.3
5m	14±0.4	11±0.2	16±0.1	12±0.1	15±0.3	11±0.1
5n	12±0.1	08±0.4	10±0.3	08±0.2	12±0.2	10±0.2
5o	09±0.4	06±0.3	11±0.4	07±0.2	07±0.3	03±0.1
5p	16±0.3	13±0.2	21±0.1	18±0.3	25±0.1	20±0.4
5q	08±0.1	04±0.2	08±0.2	06±0.2	06±0.1	04±0.2
5r	10±0.2	08±0.1	07±0.1	04±0.1	09±0.2	06±0.1
5s	07±0.3	06±0.2	−	−	04±0.2	02±0.2
5t	08±0.1	04±0.2	−	−	11±0.2	07±0.1
5u	10±0.2	08±0.1	08±0.1	05±0.1	12±0.1	08±0.3
Control	00	00	00	00	00	00
INN	32±0.2	27±0.2	26±0.1	21±0.2	21±0.2	18±0.1

INN: Ciprofloxacin (anti-bacterial standard); −: inhibition not detected; control: DMSO

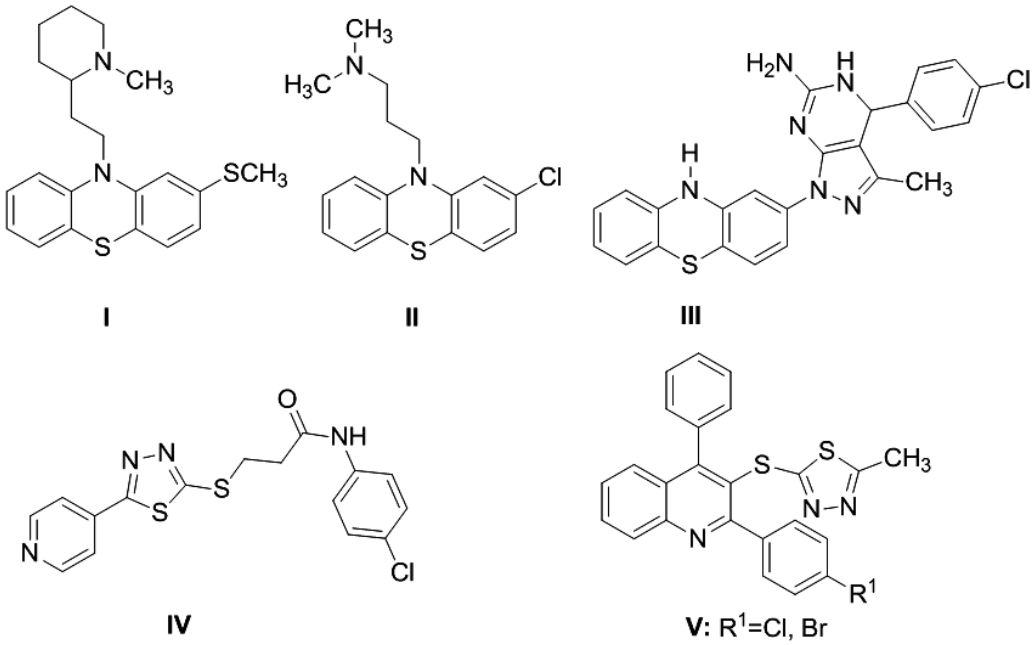


Figure 1.

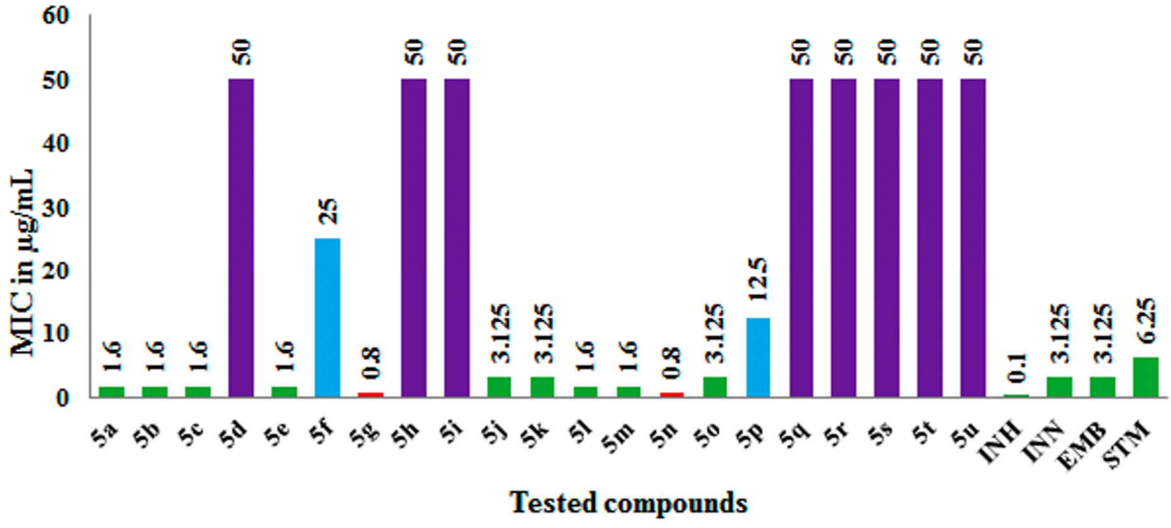


Figure 2.

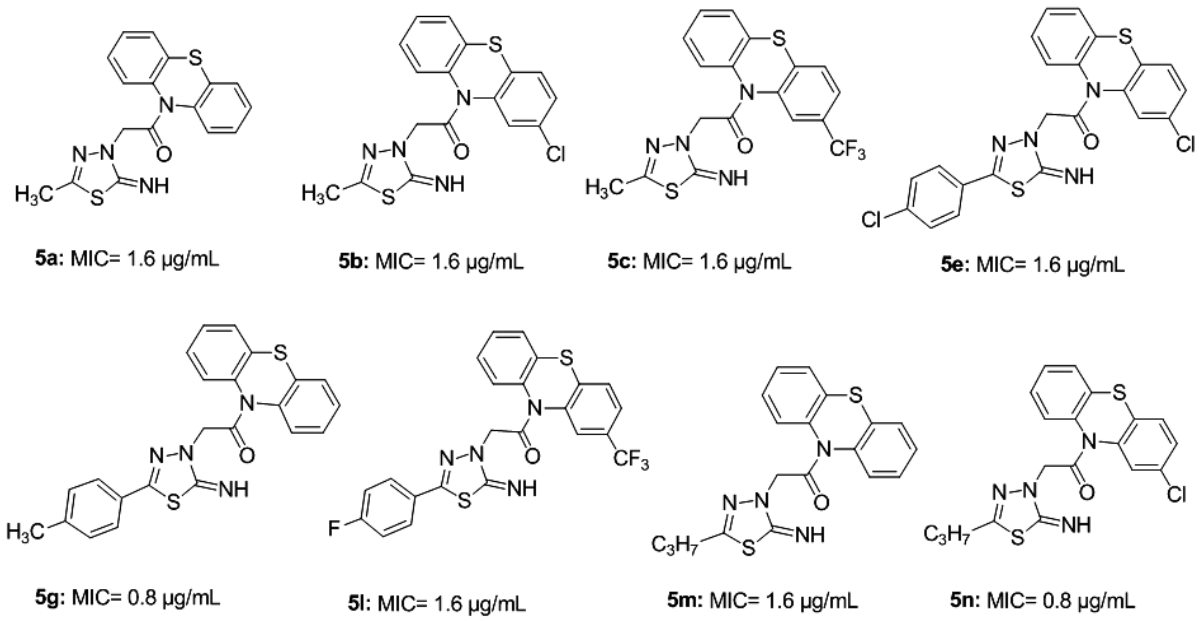


Figure 3.

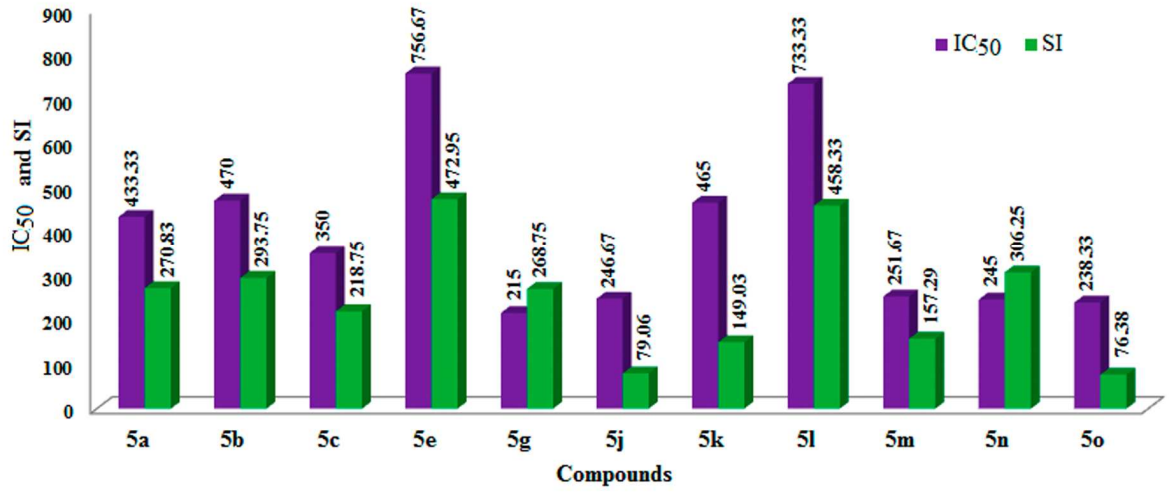


Figure 4.

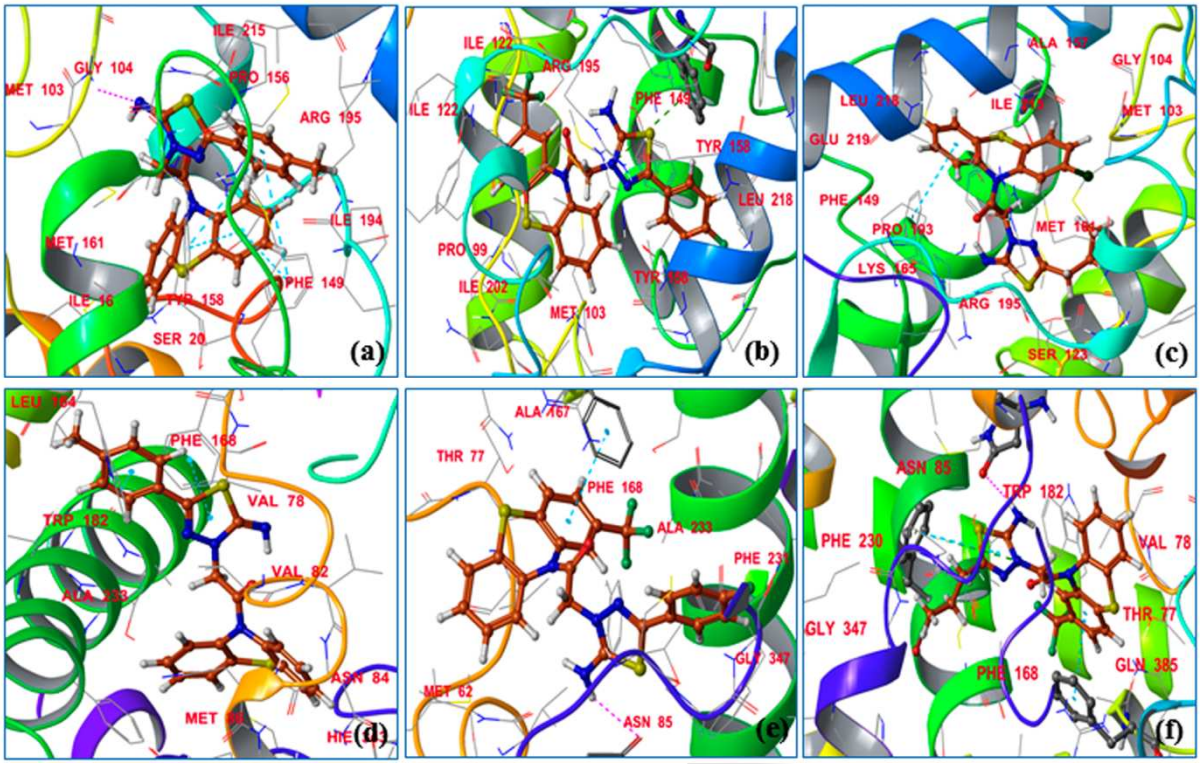
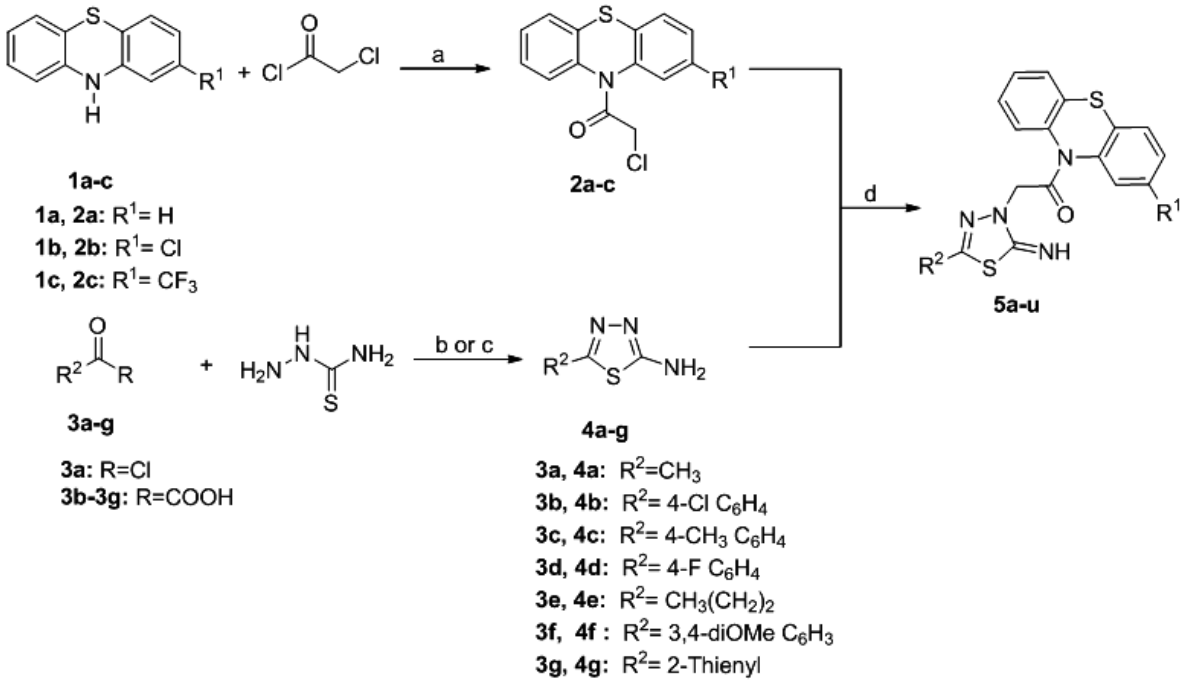
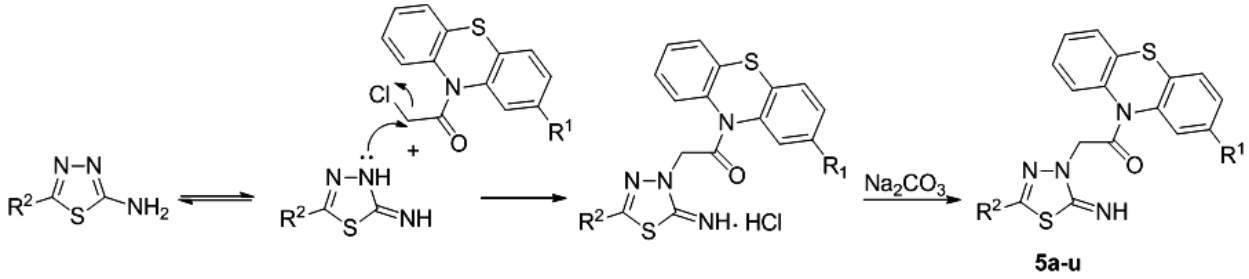


Figure 5.



Scheme 1



Scheme 2.

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Highlights

- 21 New phenothiazine and 1,3,4-thiadiazole hybrid derivatives were synthesized.
- Molecular hybridization of two pharmacophores yielded potent antiTB agents.
- 11 Compounds showed significant inhibitory activity with MIC ≤ 3.125 μg/mL.
- The most potent compounds showed MIC of 0.8 μg/mL.
- All the potent compounds are nontoxic to a Vero normal cell line.

ACCEPTED MANUSCRIPT