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Molecular hybridization approach for phenothiazine incorporated 1,2,3-triazole hybrids as promising antimicrobial agents: Design, synthesis, molecular docking and in silico ADME studies

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MOLECULAR HYBRIDIZATION APPROACH FOR PHENOTHIAZINE INCORPORATED 1,2,3-TRIAZOLE HYBRIDS AS PROMISING ANTIMICROBIAL AGENTS : DESIGN, SYNTHESIS, MOLECULAR DOCKING AND IN SILICO ADME STUDIES

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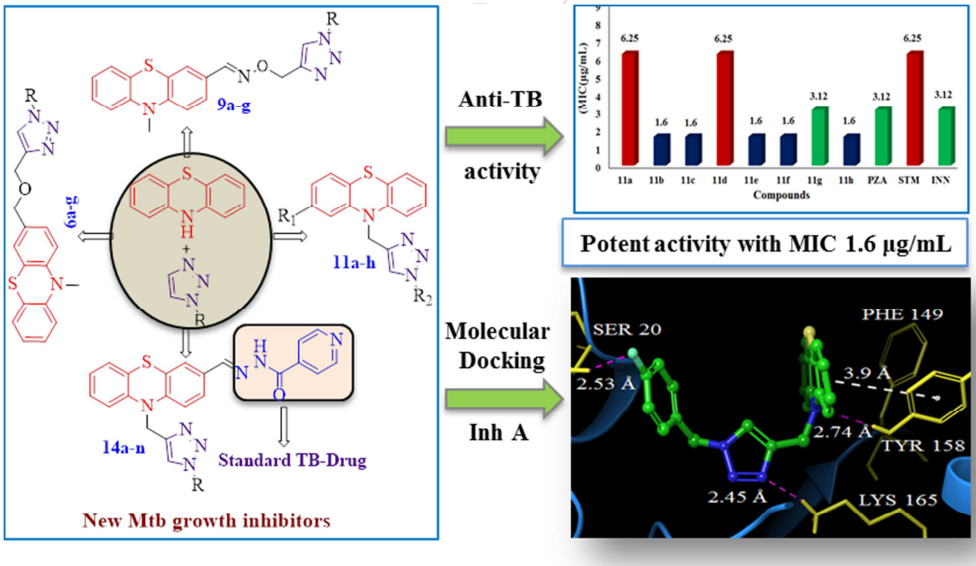
Graphical abstract



MOLECULAR HYBRIDIZATION APPROACH FOR PHENOTHIAZINE INCORPORATED 1,2,3-TRIAZOLE HYBRIDS AS PROMISING ANTIMICROBIAL AGENTS : DESIGN, SYNTHESIS, MOLECULAR DOCKING AND IN SILICO ADME STUDIES

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Abstract

The objective of the current study is to synthesize a library consisting of four sets of phenothiazine incorporated 1,2,3-triazole compounds using molecular hybridization approach. In total, 36 new hybrid molecules were synthesized and screened for *in vitro* growth inhibition activity against *Mycobacterium tuberculosis* H37Rv strain (ATCC-27294). Among the tested compounds, nineteen compounds exhibited significant activity with MIC value 1.6 μg/mL, which is twofold higher than the MIC value of standard first-line TB drug Pyrazinamide. In addition, all these compounds are proved to be non-toxic (with selective index > 40) against VERO cell lines. However, these compounds did not inhibit significantly the growth of *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains: the activity profile is similar to that observed for standard anti-TB drugs (isoniazid and pyrazinamide), indicating the specificity of these compounds towards the *Mycobacterium tuberculosis* strain. Also, we report the molecular docking studies against two target enzymes (Inh A and CYP121) to further validate the antitubercular potency of these molecules. Furthermore, prediction of in silico-ADME and pharmacokinetic parameters indicated that these compounds have good oral bioavailability. The results suggest that these phenothiazine incorporated 1,2,3-triazole compounds are a promising class of molecular entities for the development of new antitubercular leads.

Keywords: Molecular hybridization, Phenothiazine, 1,2,3-triazole, *Mycobacterium tuberculosis*, Molecular docking.

1. Introduction

Mycobacterium tuberculosis (*M.tb*) is an airborne pathogen and the causative agent of Tuberculosis (TB). TB is one of the ancient diseases but continues to be responsible for the mortality of about one billion people worldwide for the last two centuries. According to statistics of WHO 2017 [1] report, more than 10 million new TB cases noticed and 1.8 million people died, including 0.4 million co-infected HIV cases. The currently recommended treatment for TB employs a four-drug regimen includes isoniazid, rifampin, pyrazinamide and ethambutol for the first two months and isoniazid and rifampin for further four months. Further, the traditional anti-TB drugs have limited efficacy against the new forms of TB; MDR-TB (multidrug resistance tuberculosis) is resistant to two first-line TB drugs isoniazid and rifampin [2], XDR-TB (extensively drug-resistant tuberculosis) is additionally resistant to the second line TB drugs [3] and totally drug drug-resistant tuberculosis (TDR-TB) is resistance to broad range of first-line and second-line drugs [4]. Over the last 40 years only a few antitubercular drugs have either clinically approved or now in advanced clinical trials [5]. Bedaquiline [6] (TMC207), a diaryl quinoline-based molecule, has been approved by the US-FDA and EMA (European Medicines Agency) recently to treat pulmonary MDR-TB in adults [7]. Similarly, delamanid [8] (OPC-67683), a nitro dihydro imidazole derivative received approval by the EMA to treat the pulmonary MDR-TB in adults [9]. Diamine analogue [10] (SQ-109), Pretomanid [11] a nitroimidazole derivative (PA-8240), and salicylic acid-1,2,3-triazolyl acetamide analogue (I-A09) are other represents, which are under clinical trial. However, bedaquiline displayed toxic side-effects such as cardiac arrhythmias, which led to significant death rates in clinical testing [12]. Hence, there is an emergent need to address these challenges and to develop novel safe and fast acting TB drugs. In this direction, various design strategies are being conformed to identify potent chemical entities. Molecular hybridization is a powerful tool in drug design, which involves the hybridization of two active pharmacophores of either similar bioactive substances or different bioactive substances into a single molecular framework. It is being widely employed because of the fact that many such hybrid derivatives possessed improved efficiency and safer toxicity profile, when compared to the parent compounds [13]. In view of this, several

pharmacologically active structural units, mostly heterocyclic moieties are being explored to identify novel lead molecules.

Phenothiazines are well known over a hundred years for their *in vitro* antitubercular activity against *M.tb* [14]. Few of the phenothiazine derivatives such as chlorpromazine, thioridazine, methdilazine, and trifluoperazine (Figure 1) which are effective in treating neurodegenerative disorders, also possess effective inhibition activity against *M. tuberculosis* [15]. Example, thioridazine accelerated the chemotherapeutic activity against the *in vitro* latent *M.tb* bacilli, when it is used as supportive drug along with isoniazid or rifampicin (>1 log CFU reduction) [16]. Also it is being used as a front-line drug in combination therapy along with Moxifloxacin and Linezolid for the treatment of *M.tb* and XDR-TB [17]. One of the other derivatives, chlorpromazine is effective against replicating *M.tb* strain in cultured normal human macrophage model and it is found to act in synergy with *M.tb* susceptible to primary drugs isoniazid and rifampicin [18]. Interestingly, phenothiazines are known to have a specific mechanism of action, inhibits the type II NADH dehydrogenase of *M.tb* [19,20]. Type II NADH dehydrogenase is a key enzyme, which plays a vital role in the metabolic transition of *M.tb* to dormant state. Hence, phenothiazine analogs may be effective in preventing the transition of *M.tb* from latent stage to non-replicating stage. Additionally, a few recent reports demonstrated the promising antitubercular activity of phenothiazines (Figure 2). For instance, a series of novel pyrazolo[3,4-d]pyrimidine derivatives containing a 4,5-dihydro-1*H*-phenothiazine (I) exhibited significant antitubercular activity against *M.tb* H37Rv [21]. Ramprasad et al. synthesized a set of phenothiazine and 1,3,4-thiadiazole hybrid derivatives using the molecular hybridization approach and among these, compounds 1-(2-chloro-10*H*-phenothiazin-10-yl)-2-(2-imino-5-(4-propylphenyl)-1,3,4-thiadiazol-3(2*H*)-yl)ethanone(II) and 2-(2-imino-5-(p-tolyl)-1,3,4-thiadiazol-3(2H)-yl)-1-(10*H*-phenothiazin-10-yl)ethanone (III) exhibited excellent antitubercular activity with MIC of 0.8 μg/mL [22]. Further, several literatures demonstrated the promising antitubercular activity of N-substituted phenothiazine derivatives [23–25].

Over the past few decades, 1,2,3-triazoles have been an important class of nitrogen-based heterocyclic compounds because of their intriguing pharmacological and biological applications such as anti-cancer [26], anti-tubercular [27], antibacterial [28], anti-fungal [29], anti-HIV [30], and Src-kinase inhibition [31] activities. It is quite interesting that the triazole ring owns some favorable properties like modest dipole moment, the capability of hydrogen bond formation,

stability and rigidity, which might be responsible for their enhanced pharmacological activities [32]. Moreover, 1,2,3-triazole derivatives exhibit a promising antitubercular activity profile (Figure 2). For example, dibenzo[b,d]thiophene-1,2,3-triazole derivatives (IV) [33] and 4-substituted N-phenyl-1,2,3-triazole derivatives inhibit the growth of *M.tb* H37Rv. Among them, (E)-N-[(1-aryl)-1*H*-1,2,3-triazole-4-yl)methylene]isonicotinoyl hydrazides (V) displayed excellent activity with MIC values ranging from 2.5 to 0.62 μg/mL [34]. Further, [1,1'-biphenyl]-4-yloxy incorporated 1*H*-1,2,3-triazole analogs (VI) exhibited significant anti-tubercular activity [35]. Recently, benzofuran salicylic acid containing 1,2,3-triazolyl acetamide I-A09 (VII) has emerged as the promising antitubercular drug, which is under clinical trial [36]. In addition, a library of novel 2-(trifluoromethyl) phenothiazine-1,2,3-triazole hybrids (VIII) was developed through molecular hybridization approch by Addla et al and three of these compounds exhibited promising MIC value (6.25 μg/mL) against *M.tb* H37Rv strain [37]. In view of these literature reports on the promising antitubercular activity of phenothiazines and 1,2,3-triazole derivatives we designed and synthesized phenothiazine-1,2,3-triazole hybrids by incorporation of these two molecular entities in to a single framework, in which the 1,2,3-triazole fragment was attached to phenothiazine through different linkers as represented in Figure 3.

2. Results and discussion

2.1 Chemistry

The synthesis of phenothiazine incorporated 1,2,3-triazole-based compounds **6a-g, 9a-g, 11a-h** and **14a-n** was achieved through efficient and versatile synthetic routes from commercially available starting materials as summarized in schemes 1-4. The general synthetic strategy for 3-(((1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy)methyl)-10-methyl-10*H*-phenothiazine derivatives (**6a-g),** involves the Huisgen 1,3-dipolar cycloaddition reaction (click reaction) in which 10-methyl-3-((prop-2-yn-1-yloxy)methyl)-10*H*-phenothiazine intermediate (**5**) was treated with diversely substituted aryl bromides in presence of sodium azide, sodium ascorbate and copper sulphate as catalyst (Scheme 1). In the first step, commercially available phenothiazine was alkylated with methyl iodide using sodium hydride to afford 10-methyl-10*H*-phenothiazine (**2**). Intermediate, 10-methyl-10*H*-phenothiazine-3-carbaldehyde (**3**) was synthesized *via* Vilsmeier-Haack formylation of compound **2** with DMF and phosphorous oxychloride. Reduction of the phenothiazine aldehyde (**3**) was carried out by using $NaBH_4$ to yield (10-

methyl-10*H*-phenothiazin-3-yl)methanol (**4**). Then O-alkylation of compound **4** with propargyl bromide resulted the intermediate **5.**

In order to investigate the effect of replacement of the ether linker in **6a-g** by an oxime linker on the antitubercular activity of the compounds, we have synthesized the oxime analogs **9a-g** (Scheme 2). The key intermediate 10-methyl-10*H*-phenothiazine-3-carbaldehyde oxime (**7**) was synthesized by the condensation of compound **3** with hydroxylamine hydrochloride. The oxime derivative (**7**) was then propargylated and the propargyl derivative (**8**) was subjected to Huisgen 1,3-dipolar cycloaddition reaction with different aryl bromides to get the target oxime analogs **9a-g**. In the third series, 1,2,3-triazole unit is linked to core phenothiazine ring *via* the ring nitrogen of later and N-substituted phenothiazine-1,2,3-triazole hybrid derivatives (**11a-h**) were synthesized according to the synthetic route given in Scheme 3. The key intermediate 10-(prop-2-yn-1-yl)-10*H*-phenothiazine (**10**) was prepared in good yield by treating phenothiazine with propargyl bromide and sodium hydride. Huisgen 1,3-dipolar cycloaddition reaction of compound **10** with a series of benzyl bromides afforded the target molecules **11a-h**. The synthetic strategy developed for the synthesis of a final set of analogs **14a-n** is outlined in Scheme 4. These molecules are designed by the molecular hybridization of N-substituted phenothiazine-1,2,3-triazole skeleton with some of the anti-TB agents such as isoniazid, pyrazine-2-carbohydrazide and nicotinohydrazide etc. Synthesis of 4-((4-((3-formyl-10*H*-phenothiazin-10-yl) methyl)-1*H*-1,2,3-triazol-1-yl)methyl)benzonitrile derivatives **12** and **13** was achieved by the Vilsmeier-Haack formylation of compounds **11e** and **11h** respectively. Intermediates **12** and **13** were then condensed with differently substituted aromatic hydrazides to yield (E)-N'-((10-((1-(4-cyanobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-phenothiazin-3-yl)methylene) hydrazide derivatives (**14a-n**).

The chemical structure of all the synthesized compounds was confirmed by elemental analysis, mass and NMR (^{1}H and ^{13}C) spectral analysis. The ^{1}H NMR spectrum of compound **3** displayed a singlet at δ 9.81 ppm corresponding to the aldehyde hydrogen, which confirmed the formylation of compound **2**. The key intermediate **7** displayed two singlet signals at δ 11.34 and 8.45 ppm corresponding to the oxime proton (N=OH) and proton of CH=N- respectively in ^{1}H NMR spectrum. The ^{1}H NMR spectrum of final compounds (**6a-g, 9a-g**) exhibited distinct singlet signals at around δ 8.10-7.43 ppm due to triazole-CH proton, δ 5.80-5.51 ppm due to methylene (CH_2) protons attached to the triazole, δ 4.56-4.45 ppm due to bridge methylene(–O-

CH_2–) protons attached to the triazole, and δ 3.37-3.18 due to methyl (N-CH_3) protons. In addition, compounds **6a-g** displayed a singlet signal at around δ 4.74-4.61 ppm, for bridge methylene (-CH_2-O-) protons attached to the phenothiazine ring whereas compounds **9a-g** displayed a singlet signal at around δ 8.19-8.24 ppm, for oxime (CH=N-) proton. Further, these findings were confirmed by respective ^{13}C NMR spectra of all the compounds. For instance, the ^{13}C NMR spectrum of **11c** showed all the characteristic peaks according to its molecular structure: the two methylene carbons appeared at δ 47.7 and δ 45.0 ppm. The most prominent carbon signals observed at δ 161.5 and δ 123.9 ppm accounted for aromatic carbons carrying flouro group and triazole (CH) ring respectively. The mass spectrum of all the compounds showed their molecular ion peaks, corresponds to M+1 peak of the respective molecules and is in agreement with their molecular formula. For example, The ESI mass spectra of compounds **6d, 9b, 11b** and **14a** exhibited $[M+H]^+$ peaks at m/z 415.2, 473.3, 416.1 and 543.1 which are in agreement with their molecular formula $C_{24}H_{22}N_4OS$, $C_{24}H_{20}N_6O_3S$, $C_{22}H_{17}N_5O_2S$ and $C_{30}H_{22}N_8OS$ respectively. The three dimensional structure of final compound **9c** was confirmed by single crystal XRD study (Figure 4) and the crystal data is given in Table 1. The structural characterization data of all the synthesized compounds is presented in the experimental part and ESI.

2.2 *In vitro* antitubercular activity

The *in vitro* antimycobacterial screening of target compounds **6a-g**, **9a-g**, **11a-h** and **14a-n** at the minimum inhibitory concentration (MIC) was carried out using MABA method against the *M.tb* H37Rv (ATCC No- 27294) strain [38]. Pyrazinamide, Ciprofloxacin and Streptomycin were used as the reference drugs. Initially, we designed, synthesized, and evaluated various phenothiazine-1,2,3-triazole hybrids **6a-g** bearing ether linker to probe the SAR exploration of the 1,2,3-triazole region. It is noteworthy that the presence of lipophilic groups such as fluorine in compounds **6c** and **6g** or the CN in compound **6e,** increases the inhibition activity against *M.tb* H37Rv, with the MIC of 12.5 μg/mL (Table 2). Replacement of the ether linker with oxime linker resulted in no notable enhancement in the activity of compounds (**9a-g**). In fact, the introduction of oxime linker led to a loss of activity in compounds **9c** and **9g** (MIC of 25 μg/mL) in comparison with the activity of their ether analogs **6c** and **6g (**MIC of 12.5 μg/mL). In order to further explore SAR of these hybrid derivatives, we have designed another set of molecules (**11a-h**) in which the 1,2,3-triazole residue is attached to the N-atom of the phenothiazine ring.

Interestingly, these compounds **11a-g** exhibited superior activity compared to their ether and oxime analogs **6a-g** and **9a-g**. Remarkably, four compounds namely **11b**, **11c**, **11e** and **11f** showed potent anti-tubercular activity with MIC of 1.6 μg/mL (Figure 5) whereas compound **11g** exhibited moderate anti-tubercular activity with MIC of 3.12 μg/mL. It is evident from figure 5 that the nature of the substituent on 1,2,3-triazole (R^2) rings affect the activity of the compounds. The presence of electron-withdrawing groups (such as NO_2, F and CN) as well as electron donating groups (-OCH_3), enhanced the activity of these compounds. At this point, it was encouraging to synthesize an analog compound **11h** with a triflouromethyl group at position-2 of the phenothiazine ring and to compare its anti-TB activity with the unsubstituted analog **11e.** However, no change in the activity was observed by the introduction of a triflouromethyl group; both **11e** and **11h** exhibited similar activity profile (MIC = 1.6 μg/mL) which makes them interesting lead compounds for further structural optimization. Finally, different hydrazine derivatives (including Isoniazid, a standard drug for TB) were hybridized with the most potent molecules (**11e** and **11h**) to obtain compounds **14a-n**. Surprisingly, we observed that such a molecular hybridization did not change the activity of the molecules; instead all the compounds (**14a-n**) retained the activity level at a MIC of 1.6 μg/mL(Table 3). Comparative study of the antitubercular activity of the final compounds and the reference drugs indicated that among 36 compounds, nineteen compounds showed significant inhibition activity against *M.tb* H_{37}Rv strain (MIC of 1.6 μg/mL) which is twofold and fourfold superior to the activity of standard first-line TB drug Pyrazinamide (MIC of 3.125 μg/mL) and the second-line TB drugs (Ciprofloxacin and Streptomycin) respectively.

2.3 *In vitro* antibacterial activity

All the final compounds were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains using the agar dilution method by measuring the minimum inhibitory concentration [39]. Ciprofloxacin, the commercial antibiotic was taken as the standard drug for comparison. Most of the tested compounds exhibited better inhibition activity against *Staphylococcus aureus* strain than the other tested strains (Table 4). Three compounds **11e**, **14i**, and **14m** showed significant activity with MIC of 6.25 μg/mL whereas other eight compounds **6e**, **9c**, **9e**, **9g**, **11c**, **11g**, **14c** and **14h** exhibited moderate activity with MIC of 12.5 μg/mL. It is noteworthy that all these active

molecules (except **14c** and **14h**) carry an electron withdrawing substitution. Interestingly, all the fluoro substituted analogs showed excellent inhibition activity which reveals the significant contribution of the fluoro substituent towards the antibacterial activity of these compounds. However, it can be seen from the MIC values that some of the molecules show superior inhibitory activity against *M.tb* H37Rv in comparison to their activity against the other tested bacterial strains.

2.4 Cytotoxicity

Compounds with MIC of 1.6 μg/mL against *M.tb* were further evaluated for their *in vitro* cytotoxicity using MTT assay [40] by measuring the concentration of compounds resulting in 50% inhibition (IC_{50}) of a VERO cell line. The graphical representation of IC_{50} values of the compounds is represented in Figure 6. Among the tested compounds **11c**, **11f, 11h, 14c, 14d, 14f, 14g** and **14n** exhibited very less growth inhibition with 3.3, 7.57, 5.0, 5.13, 7.32, 7.14, 7.57 and 6.28% respectively. Selectivity Index (SI = IC_{50} against VERO cell / MIC against *M.tb*) of all the potent molecules is > 40 (Table 5) which indicate that none of these compounds are toxic to the normal cells and hence their anti-TB activity was not due to some general cytotoxicity.

2.5 Molecular docking studies

Molecular docking studies are generally employed to investigate the binding energy and to validate molecular mechanisms for ligands at the active site of a protein. To understand the binding mode of the phenothiazine-1,2,3-triazole hybrids, all the title compounds were subjected to molecular docking studies against selected enzymes viz. InhA and CYP 121 of *M.tb* using Auto-dock Vina 1.1.2 software [41]. InhA is an NADH-dependent enoyl-acyl carrier protein reductase, essential for cell viability of the bacteria and is endorsed as an effective anti-TB target [42,43]. Further, isoniazid and ethionamide (anti-TB drugs) are predicted as the Inh A inhibitors. Another target, CYP 121 enzyme also was selected for the docking studies based on the recent literature on some triazole derivatives effectively binding with this target enzyme [44]. The crystal structures of enzymes, Inh A in complex with 641 (PDB entry: 4TZK) and CYP 121 (PDB entry: 4KTF) in complex with 1TM was retrieved from the PDB (protein data bank) and used as the template in molecular docking calculations.

Docking study with the enoyl acyl carrier protein reductase (Inh A) of *M. tuberculosis* H37Rv

The calculated binding energies for the studied 36 molecules are substantially high and are in the range of −9.2 to −12.2 kcal/mol owing to the fact that the target compounds have formed one or more hydrogen bonds with amino acid residues in the active pocket of Inh A (Figure 7). Tyr 158 is the crucial amino acid residue, which interacts with the long chain fatty acyl substrates that are required for mycolic acid synthesis in mycobacteria [45,46]. Almost all the active compounds showed hydrogen bond formation and pi-pi interaction with hydrophobic residue Tyr 158, which were the key binding interactions of the original ligand as well with enzyme Inh A (4TZK). In addition, few of them showed hydrogen bond formation with Lys 165 residue as well as key pi-pi stacking interaction with Phe 149 residue. One of the active compounds **11g** with the docking score (-9.9 kcal/mol) demonstrated substantial specific binding as illustrated in Figure 8, where the nitrogen atom (N_3) of triazole ring is hydrogen bonded to the Lys 165 residue with a bond distance of 2.45 Å and nitrogen atom of the phenothiazine ring is bonded with the Tyr 158 (2.74 Å) residue. Also, prominent pi-pi stacking interactions with the Tyr 158 (3.9 Å) residue and additional van der Waals interactions with Phe 149, Met 147, Asp 148, Ala 191, Pro 193, Ile 195 and Thr 196 residues are the other binding modes observed for the molecule. Whereas its oxime derivative **9g** (which was moderately active against *M.tb* MIC 25 µg/mL) didn't exhibit any interaction with Tyr 158 and Phe 149 residues which could be the reason for the higher activity of compounds **11a-g** than their oxime analogs. In the best docked pose of **14c**, three crucial hydrogen bonds are formed: the first between nitrogen of isoniazid ring and Gln 100 (2.33 Å) residue; the second hydrogen bonding interaction was observed between amide hydrogen and Met 91 (1.98 Å) residue and the third between nitrogen (N_3) of triazole ring and Tyr 158 (2.24 Å) residue. Additionally, a pi-pi interaction is seen between triazole ring and Phe 149 residue. These interactions resulted a docking score of -11.7 kcal/mol for the molecule.

Docking study with the cytochrome P450 monooxygenase (CYP 121) of *M. tuberculosis* H37Rv

The calculated binding energies for the target molecules are in the range of −9.6 to −11.7 kcal/mol. The binding sites of CYP 121 involved in these interactions are Gln 385, Arg 386, Phe 280, Gly 232, Val 228, Trp 182, Phe 168, Phe 161, Asn 85, Val 83, Leu 76, Asn 74, Leu 73, Arg

72, Thr 65, and Met 62 residues which seemed to play important roles in the drug binding through hydrogen bond formation. As shown in Figure 9, inhibitor **11b,** with a binding energy of -10.3 kcal/mol forms three hydrogen bonds with Thr 65 (2.6 Å), Arg 72 (2.3Å) and Gln 385(2.4 Å), among which two bonds originate through the nitro group. Compounds **14a** and **14c** exhibited binding energies of -11.2 and -11.4 kcal/mol, respectively, with two hydrogen bonding interactions with Leu 73 and Val 228 (Figure 9). Compound **14d** which exhibited the highest docking score (-11.7 kcal/mol) interacted with Val 228 (2.54 Å) whereas compound **14f** interacted with Asn 74 (2.2 Å), Val 228 (2.64 Å) and Asp 281 (2.09 Å) with a binding energy of -11.0 kcal/mol. Hence, the docking study further validates the activity profile observed in the *in vitro* studies of active analogs.

2.6 In silico ADME studies

In silico ADME properties (adsorption, distribution, metabolism and excretion), and another ten pharmacokinetics properties viz. partition coefficient of n-octanol and water, aqua solubility, polar surface area, number of rotatable bonds, number of hydrogen bond donors/acceptors, caco-2 cell permeability, human serum albumin binding, blood/brain partition co-efficient and human oral absorption of the 36 compounds were computed by using the QikProp program. The results of these computations are summarized in Table 6. Estimation of octanol-water partition coefficient (lipophilicity) is examined by QPlogPo/w and higher QPlogPo/w value was related to poor absorption. It is evident from the Table 6 that the most potent compounds **11b**, **11c**, **11e, 11f** and **11h** follow the Lipinski's rule and display favorable pharmacokinetic profiles for all descriptors expect QPlogPo/w value. Polar surface area (PSA), which should not be >140 Å, is another key property linked to drug bioavailability and the values for the target molecules are in the range of 34.649 – 80.821 Å. A noteworthy difference was observed in the Caco-2 cell permeability parameter (QPPcaco**)** and the aqua solubility parameter (QPlog S) of three different sets of molecules. The most potent compounds **11b**, **11c**, **11e, 11f** and **11h** displayed higher permeability and aqua solubility values than the respective ether and oxime linked analogs. Blood/Brain partition co-efficient (QPlogBB) value which is a measure of the ability of a drug to cross the blood-brain barrier, is in the acceptable range for these target molecules. Further, Human serum albumin binding co-efficient (QPlogKhsa) also is one of the key factors and predicted values for the compounds are in the range of 0.788 – 1.071. The

predicted values of human oral absorption are in the range of 91-100 %, which indicates the possibility of higher oral bioavailability of the active compounds. It is worthwhile reveling that the library of most active compounds (**14a-n)** falls into low QPlog S aqua solubility, QPPcaco permeability and oral bioavailability. In summary, these in silico ADME prediction of the active compounds may help in further development of new drug candidates with favorable oral bioavailability.

3. Conclusions

In conclusion, we have designed and synthesized four series of phenothiazine incorporated 1,2,3-triazole hybrids through molecular hybridization method by hybridizing two active pharmacophores, phenothiazine and 1,2,3-triazole. All the newly synthesized compounds **6a-g, 9a-g, 11a-h** and **14a-n** were evaluated for *in vitro* growth inhibition activity against *M.tb* H37Rv strain (ATCC-27294). Three compounds bearing ether linker (**6c**, **6e** and **6g)** showed moderate antitubercular activity with MIC 12.5 µg /mL. Modification of the ether linker by an oxime linker in compounds **6a-g** did not affect significantly the activity profile of the oxime analogs (**9a**-**g**). All the phenothiazine-N-substituted 1,2,3-triazole derivatives **11a-g** exhibited superior activity compared to their respective ether (**6a-g**) and oxime (**9a-g**) analogs. Four compounds **11b**, **11c**, **11e** and **11f** showed potent anti-tubercular activity with MIC of 1.6 µg/mL, which is twofold higher than the activity of standard first-line TB drug Pyrazinamide (MIC of 3.125 µg/mL) and one of the other compound **11g** showed significant activity with MIC of 3.12 µg/mL. Introduction of a triflouromethyl substituent at position-2 of the phenothiazine ring in **11e** did not alter the activity profile of the molecule. Hybridization of TB-active pharmacophores with active compounds **11e** and **11h** caused no further enhancement in the anti-TB activity of hybrid derivatives. However, the molecular hybridization resulted a library of potent molecules; all the 14 compounds (**14a-n)** exhibited MIC of 1.6 µg/mL. The cytotoxicity studies of the active compounds (MIC= 1.6 µg/mL) revealed that these compounds are non-toxic to the normal VERO cell lines. Further, compounds **11e**, **14i**, and **14m** showed significant antibacterial activity against all the tested bacterial strains. The SAR studies revealed that the electron withdrawing groups such as NO_2, F and CN on phenyl ring attached to 1,2,3-triazole nucleus play a vital role

in enhancing the pharmacological activities of the final compounds. These results were further supported by the molecular docking studies; the potent molecules displayed stronger binding affinity towards the target enzymes through multiple hydrogen bonds and pi-pi stacking interactions. In-silico ADME studies predicts these molecules to exhibit good oral bioavailability. Hence, the phenothiazine incorporated 1,2,3-triazole hybrids described here could be promising lead compounds for the identification and development of novel anti-TB drugs.

4. Experimental section

4.1 Synthesis

4.1.1. 10-Methyl-10H-phenothiazine (2).

Phenothiazine **1** (5 g, 25.09 mmol) was taken in anhydrous N, N-dimethyl formamide (DMF) (50 mL) in a dry round bottom flask. Sodium hydride (2 g, 50.18 mmol) was added under nitrogen condition at 0 °C and stirred for 30 minutes at room temperature. Methyl iodide (3.4 mL, 50.18 mmol) was added and continued the stirring for 8h. After that, the reaction mixture (monitored by TLC) was quenched with ice cold water (150 mL). The separated solid was filtered off, dried and used as such for the next step. White solid. Yield: 4.8 g, 89%; m.p: 105-106 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.262-7.160 (m, 3H, Ar-H), 7.141-6.822 (m, 5H, Ar-H), 3.495 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 151.2, 129.3, 128.1, 124.9, 123.3, 115.8, 37.2; ESI-MS (*m/z*) = 214.3 $(M+H)^+$; calculated for $C_{13}H_{11}NS$; C, 73.20; H, 5.20; N, 6.57; S, 15.03. Found: C, 73.18; H, 5.21; N, 6.57; S, 15.04.

4.1.2. 10-Methyl-10H-phenothiazine-3-carbaldehyde (3).

Phosphorous oxychloride ($POCl_3$) (3.5 mL, 37.50 mmol) was added drop wise to DMF (2.9 mL, 37.50 mmol) in RB flask under the inert condition at 0-5 °C. After the formation of the Vilsmeier-Haack salt, a solution of **2** (4 g, 18.75 mmol) in DMF was added and continued the stirring at 60 °C for about 4h. The reaction mixture was cooled to RT and poured into ice water slowly. The separated solid was filtered off, dried and washed with an excess of cold water and then purified by column chromatography with pet ether/ethyl acetate (9:1) system to give compound **3.** Yellow crystalline solid. Yield: 3.8g, 83.9%; m.p: 110-111 °C; ^{1}H NMR ($CDCl_3$,

400 MHz, δ in ppm): 9.812 (s, 1H, -CHO), 7.681-7.656 (m, 1H, Ar-H), 7.615-7.610 (m, 1H, Ar-H), 7.196-7.145 (m, 2H, Ar-H), 7.129-6.974 (m, 1H, Ar-H), 6.878-6.843 (m, 2H, Ar-H), 3.440 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 191.4, 151.6, 145.3, 131.2, 130.7, 129.8, 128.5, 127.5, 124.6, 123.9, 122.7, 116.4, 114.1, 37.5; ESI-MS (*m/z*) = 242.3 $(M+H)^+$; calculated for $C_{14}H_{11}NOS$; C, 69.68; H, 4.59; N, 5.80; S, 13.29. Found: C, 69.66; H, 4.59; N, 5.73; S, 13.26.

4.1.3. (10-Methyl-10H-phenothiazin-3-yl)methanol (4).

Intermediate 3 (3 g, 12.43 mmol) was taken in methanol (30 ml) to which $NaBH_4$ (0.705 g, 18.64 mmol) was added in portions at 0 $^{\circ}$C under inert condition. Then the reaction was stirred for 4h. After that, the reaction mixture (Monitored by TLC) was quenched with ice cold water. The solid obtained was filtered under suction. The residue was washed with cold methanol, dried and recrystallized from ethanol to get desired product **4** as white solid. Yield: 2.6 g, 89 %; m.p: 143-144 ^{o}C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.415-7.341 (m, 2H, Ar-H), 7.072-7.011 (m, 2H, Ar-H), 6.714-6.598 (m, 3H, Ar-H), 4.914 (s, 2H, CH_2), 3.845 (s, 1H, OH), 3.438 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.1, 146.3, 135.1, 129.8, 128.7, 128.2, 127.6, 125.2, 124.6, 122.2, 116.4, 115.3, 65.8, 37.3; ESI-MS (*m/z*) = 244.3 $(M+H)^+$; calculated for $C_{14}H_{13}NOS$; C, 69.11; H, 5.39; N, 5.76; S, 13.18. Found: C, 69.02; H, 5.41; N, 5.81; S, 13.07.

4.1.4. 10-Methyl-3-((prop-2-yn-1-yloxy)methyl)-10H-phenothiazine (5).

The above procedure (synthesis of **2**) was adopted to synthesize the intermediate **5** by alkylation of compound **4** (2 g, 8.2 mmol) with propargyl bromide(1.24 ml, 16.43 mmol) in presence of sodium hydride (0.657 g , 16.43 mmol). Brown solid. Yield: 1.9 g, 82%; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.193-7.128 (m, 4H, Ar-H), 6.951-6.911 (m, 1H, Ar-H), 6.826-6.773 (m, 2H, Ar-H), 4.507 (s, 2H, CH_2), 4.129-4.122 (d, 2H, J = 2.8 Hz, CH_2), 3.374 (s, 3H, CH_3), 2.469-2.457 (t, 1H, J = 4.8, 2.0 Hz, Acetylene CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.3, 146.4, 131.4, 129.7, 128.8, 128.2, 127.9, 125.1, 124.7, 123.1, 116.7, 114.8, 79.7, 77.4, 74.5, 61.2, 37.4; ESI-MS (*m/z*) = 282.1 $(M+H)^+$; calculated for $C_{17}H_{15}NOS$; C, 72.57; H, 5.37; N, 4.98; S, 11.40. Found: C, 72.49; H, 5.40; N, 4.98; S, 11.41.

4.1.5. General Procedure for synthesis of 3-(((1-benzyl-1H-1,2,3-triazol-4-yl)methoxy) methyl)-10-methyl-10H-phenothiazine derivatives (6a-g): To a mixture of compound **5** (1.0 mmol), substituted benzyl bromide (1.0 mmol) and NaN_3 (1.0 mmol) in 1:1 mixture of t-BuOH and water (4 mL), sodium ascorbate (10 mol %) and copper (II) sulfate (5 mol %) were added

consecutively and the reaction mixture was stirred for 24 h. The reaction mixture was diluted with ice cold water (15 mL) once the starting material consumed completely (Monitored by TLC) and extracted with ethyl acetate. The solvent was removed under vacuum and the residue was purified by column chromatography with pet ether/ethyl acetate system to give compounds **6a-g**.

4.1.5.1. 10-Methyl-3-(((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10H-phenothiazine (6a). Brown solid. Yield: 83%, m.p: 145-146 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.902-7.882 (d, J = 8.0 Hz, 2H, Ar-H), 7.697 (s, 1H, Traizole-CH), 7.341-7.321 (d, J = 8.0 Hz, 2H, Ar-H), 7.174-7.116 (m, 4H, Ar-H), 6.934-6.915 (t, J = 7.6 Hz, 1H, Ar-H), 6.812-6.766 (t, J = 18.4 Hz, 8.0Hz, 2H, Ar-H), 5.799 (s, 2H, CH_2), 4.696 (s, 2H, CH_2), 4.505 (s, 2H, CH_2), 3.367 (s, 3H, CH_3), 2.449 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.4, 146.5, 143.7, 136.8, 134.8, 130.1, 129.6, 129.1, 128.5, 127.9, 127.6, 127.3, 125.2, 124.8, 123.6, 122.9, 116.8, 114.9, 73.7, 70.4, 58.5, 37.6, 22.8; ESI-MS (*m/z*) = 429.1 $(M+H)^+$; calculated for $C_{25}H_{24}N_4OS$; C, 70.07; H, 5.64; N, 13.07; S, 7.48. Found: C, 70.06; H, 5.57; N, 13.12; S, 7.49.

4.1.5.2. 10-Methyl-3-(((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10H-phenothiazine (6b). Pale yellow solid. Yield: 86%; m.p: 176-177 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.238-8.216 (d, J = 8.8 Hz, 2H, Ar-H), 7.503 (s, 1H, Traizole-CH), 7.407-7.385 (d, J = 8.8 Hz, 2H, Ar-H), 7.170-7.117 (m, 4H, Ar-H), 6.949-6.910 (m, 1H, Ar-H), 6.820-6.753 (m, 2H, Ar-H), 5.619 (s, 2H, CH_2), 4.638 (s, 2H, CH_2), 4.491 (s, 2H, CH_2), 3.363 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.7, 147.8, 146.6, 144.4, 143.8, 130.2, 129.7, 129.3, 128.6, 127.8, 127.4, 125.4, 124.9, 124.1, 123.7, 122.5, 116.9, 114.8, 73.7, 70.5, 58.6, 37.6; ESI-MS (*m/z*) = 460.1 $(M+H)^+$; calculated for $C_{24}H_{21}N_5O_3S$; C, 62.73; H, 4.61; N, 15.24; S, 6.98. Found: C, 62.80; H, 4.66; N, 15.29; S, 6.82.

4.1.5.3. 3-(((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10-methyl-10H-phenothiazine (6c). Grey solid. Yield: 78%; m.p: 118-119 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.154-8.134 (m, 2H, Ar-H), 7.512 (s, 1H, traizole-CH), 7.425-7.391 (m, 3H, Ar-H), 7.051-7.015 (m, 3H, Ar-H), 6.912-6.895 (m, 1H, Ar-H), 6.789-6.545 (m, 2H, Ar-H), 5.626 (s, 2H, CH_2), 4.647 (s, 2H, CH_2), 4.454 (s, 2H, CH_2), 3.362 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.7, 149.6, 147.9, 144.5, 132.5, 130.1, 129.9, 129.1, 128.7, 127.9, 127.6, 126.1, 125.5, 125.1, 124.7, 123.8, 122.4, 118.4, 117.1, 114.9, 73.8, 70.5, 50.2, 37.7; ESI-MS (*m/z*) =

433.51 $(M+H)^+$; calculated for $C_{24}H_{21}FN_4OS$; C, 66.65; H, 4.89; N, 12.95; S, 7.41 Found: C, 66.53; H, 4.94; N, 12.98; S, 7.46.

4.1.5.4. 3-(((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10-methyl-10H-phenothiazine (6d). Grey solid. Yield: 82%; m.p: 124-125 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.433 (s, 1H, Triazole-CH), 7.377-7.360 (m, 3H, Ar-H), 7.280-7.274 (d, J = 2.4 Hz, 2H, Ar-H), 7.186-7.117 (m, 4H, Ar-H), 6.944-6.907 (t, J = 14.8 Hz, 7.2 Hz, 1H, Ar-H), 6.816-6.749 (m, 2H, Ar-H), 5.507 (s, 2H, CH_2), 4.608 (s, 2H, CH_2), 4.471 (s, 2H, CH_2), 3.361 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.4, 146.7, 143.6, 135.5, 130.2, 129.7, 129.0, 128.6, 128.2, 127.8, 127.4, 126.7, 125.3, 124.7, 123.7, 122.9, 116.9, 114.8, 73.8, 70.7, 58.7, 37.5; ESI-MS (*m/z*) = 415.20 $(M+H)^+$; calculated for $C_{24}H_{22}N_4OS$; C, 69.54; H, 5.35; N, 13.52; S, 7.74 Found: C, 69.64; H, 5.43; N, 13.51; S, 7.81.

4.1.5.5. 4-((4-(((10-Methyl-10H-phenothiazin-3-yl)methoxy)methyl)-1H-1,2,3-triazol-1-yl) methyl)benzonitrile (6e). Pale green solid. Yield: 91%; m.p: 125-126 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.187-8.167 (d, J = 8.0 Hz, 2H, Ar-H), 7.509 (s, 1H, Traizole-CH), 7.414-7.393 (d, J = 8.4 Hz, 2H, Ar-H), 7.154-7.102 (m, 4H, Ar-H), 6.927-6.889 (m, 1H, Ar-H), 6.802-6.734 (m, 2H, Ar-H), 5.604 (s, 2H, CH_2), 4.622 (s, 2H, CH_2), 4.47 (s, 2H, CH_2), 3.358 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.6, 147.8, 144.5, 141.7, 134.8, 131.5, 130.1, 129.8, 128.7, 127.9, 127.5, 125.3, 124.9, 123.8, 122.6, 119.7, 116.9, 114.7, 110.8, 73.8, 70.6, 58.5, 37.7; ESI-MS (*m/z*) = 440.20 $(M+H)^+$; calculated for $C_{25}H_{21}N_5OS$; C, 68.32; H, 4.82; N, 15.93; S, 7.30 Found: C, 68.60; H, 4.87; N, 15.86; S, 7.33.

4.1.5.6. 3-(((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10-methyl-10H-phenothiazine (6f). Pale yellow solid. Yield: 88%; m.p: 121-122 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.990-7.968 (m, 2H, Ar-H), 7.708 (s, 1H, Traizole-CH), 7.184-7.122 (m, 4H, Ar-H), 7.011-6.989 (d, J = 8.8 Hz, 2H, Ar-H), 6.940-6.818 (m, 1H, Ar-H), 6.797-6.770 (m, 2H, Ar-H), 5.782 (s, 2H, CH_2), 4.700 (s, 2H, CH_2), 4.508 (s, 2H, CH_2), 3.904 (s, 3H, O-CH_3), 3.369 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 158.7, 149.6, 146.7, 143.8, 131.9, 131.2, 130.3, 129.4, 129.0, 128.6, 127.9, 125.1, 124.9, 123.7, 122.9, 116.7, 115.6, 114.9, 73.8, 70.5, 58.6, 57.6, 37.5; ESI-MS (*m/z*) = 445.40 $(M+H)^+$; calculated for $C_{25}H_{24}N_4O_2S$; C, 67.54; H, 5.44; N, 12.60; S, 7.21 Found: C, 67.52; H, 5.48; N, 12.55; S, 7.23.

4.1.5.7 3-(((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxy)methyl)-10-methyl-10H-phenothiazine (6g). Yellow solid. Yield: 80%; m.p: 119-120 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.014-7.993 (m, 2H, Ar-H), 7.561 (s, 1H, Traizole-CH), 7.345-7.284 (m, 4H, Ar-H), 7.181-7.160 (d, *J* = 8.4 Hz, 2H, Ar-H), 6.994-6.872 (m, 1H, Ar-H), 6.810-6.785 (m, 2H, Ar-H), 5.764 (s, 2H, CH_2), 4.714 (s, 2H, CH_2), 4.491 (s, 2H, CH_2), 3.357 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.4, 149.8, 147.8, 144.3, 133.1, 130.4, 129.8, 129.2, 128.6, 127.5, 126.4, 125.6, 124.8, 123.8, 122.3, 118.5, 117.2, 114.9, 73.9, 70.6, 58.4, 37.5; ESI-MS (*m/z*) = 433.1 $(M+H)^+$; calculated for $C_{24}H_{21}FN_4OS$; C, 66.65; H, 4.89; N, 12.95; S, 7.41 Found: C, 66.53; H, 4.94; N, 12.98; S, 7.46.

4.1.6. N-[(E)-(10-Methyl-10H-phenothiazin-3-yl)methylidene]hydroxylamine (7). To a solution of **3** (2 g, 8.26 mmol) in a minimum amount of anhydrous ethanol in a dry RB, hydroxylamine hydrochloride (684 mg, 9.92 mmol) and concentrated sulphuric acid (catalytic amount) were added and refluxed at 80 °C for 1 hour. After that, the reaction mixture (monitored by TLC) was quenched with ice cold water (150 mL). The separated solid was filtered off, dried, weighed and used as such for the next step. Pale yellow solid. Yield: 1.65 g, 78%; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.341 (s, 1H, N-OH), 8.451 (s, 1H, -CH=N), 7.673-7.612 (m, 1H, Ar-H), 7.505-7.410 (m, 1H, Ar-H), 7.269-7.155 (m, 2H, Ar-H), 6.960-6.921 (m, 1H, Ar-H), 6.857-6.814 (m, 2H, Ar-H), 3.41 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 149.3, 146.1, 144.5, 135.6, 129.1, 127.9, 127.5, 126.4, 124.9, 122.3, 120.7, 115.8, 113.5, 37.5; ESI-MS (*m/z*) = 257.3 $(M+H)^+$; calculated for $C_{14}H_{12}N_2OS$; C, 65.60; H, 4.72; N, 10.93; S, 12.51. Found: C, 65.50; H, 4.75; N, 10.96; S, 12.47.

4.1.7. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(prop-2-yn-1-yl)oxy]methanimine (8). The above procedure (synthesis of **2**) was adopted to synthesize the intermediate **8** by O-alkylation of compound **7** (1 g, 3.9 mmol) with propargyl bromide (0.59 ml, 7.81 mmol) in presence of sodium hydride (1.07 g, 7.81 mmol). Brown solid. Yield: 0.98 g, 85%; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 8.405 (s, 1H, -CH=N), 7.994-7.101 (m, 4H, Ar-H), 6.985-6.919 (m, 1H, Ar-H), 6.870-6.791 (m, 2H, Ar-H), 4.289 (d, *J* = 2.4 Hz, 2H, CH_2), 3.394 (s, 3H, CH_3), 3.186 (t, 1H, *J* = 4.4, 2.0 Hz, 1H, Acetylene-CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 150.8, 146.2, 144.8, 135.3, 129.2, 128.1, 127.4, 126.9, 124.9, 122.4, 122.2, 115.7, 112.6, 89.2, 79.1, 61.1, 37.5; ESI-

MS (*m/z*) = 295.2 $(M+H)^+$; calculated for $C_{17}H_{14}N_2OS$; C, 69.36; H, 4.79; N, 9.52; S, 10.89. Found: C, 69.35; H, 4.81; N, 9.46; S, 10.88.

4.1.8. General procedure for the synthesis of (E)-1-(10-methyl-10H-phenothiazin-3-yl)-N-[(1-benzyl-1H-1,2,3-triazol-4-yl)methoxy]methanimine derivatives (9a-g). The above procedure (synthesis of **6a-g**) was adopted to synthesize the compounds **9a-g** by click reaction between substituted benzyl bromides (1 mmol) and Intermediate **8** (1 mmol) in presence of sodium azide (1 mmol), sodium ascorbate (10 mol %) and copper (II) sulfate (5 mol %).

4.1.8.1. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(4-methylbenzyl-1H-1,2,3-triazol-4-yl) methoxy]methanimine (9a). Pale brown solid. Yield: 79%; m.p: 207-208 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.210 (s, 1H, -CH=N), 7.926-7.891 (m, 2H, Ar-H), 7.729 (s, 1H, Triazole-H), 7.392-7.280 (m, 2H, Ar-H), 7.208-7.164 (m, 4H, Ar-H), 6.987-6.862 (m, 1H, Ar-H), 6.821-6.745 (m, 2H, Ar-H), 5.714 (s, 2H, CH_2), 4.558 (s, 2H, CH_2), 3.282 (s, 3H, N-CH_3), 2.412 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.5, 150.3, 146.4, 144.8, 137.1, 135.3, 134.5, 131.9, 129.8, 129.3, 128.2, 127.8, 127.2, 126.0, 125.4, 125.2, 123.8, 123.1, 117.9, 68.2, 58.3, 37.3, 22.4; ESI-MS (*m/z*) = 442.51 $(M+H)^+$; calculated for $C_{25}H_{23}N_5OS$; C, 68.00; H, 5.25; N, 15.86; S, 7.26 Found: C, 68.03; H, 5.25; N, 15.90; S, 7.21.

4.1.8.2. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(4-nitrobenzyl-1H-1,2,3-triazol-4-yl) methoxy]methanimine (9b). Brick red solid. Yield: 81%; m.p. 235-236 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.191 (s, 1H, -CH=N), 8.109-7.968 (m, 2H, Ar-H), 7.712-7.652 (s, 2H, Ar-H), 7.538-7.593 (m, 2H, Ar-H), 7.202-7.146 (m, 3H, Ar-H), 6.928-6.876 (m, 1H, Ar-H), 6.812-6.774 (m, 2H, Ar-H), 5.697 (s, 2H, CH_2), 4.495 (s, 2H, CH_2), 3.291 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.3, 150.2, 146.2, 145.9, 144.7, 144.6, 135.2, 131.8, 129.8, 129.2, 128.2, 127.1, 126.0, 125.2, 125.1, 124.9, 123.7, 123.0, 117.9, 68.4, 58.4, 37.4; ESI-MS (*m/z*) = 473.30 $(M+H)^+$; calculated for $C_{24}H_{20}N_6O_3S$; C, 61.00; H, 4.27; N, 17.79; S, 6.79 Found: C, 60.99; H, 4.28; N, 17.81; S, 6.80.

4.1.8.3. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(2-flourobenzyl-1H-1,2,3-triazol-4-yl) methoxy]methanimine (9c). Brown solid. Yield: 74%; m.p. 198-199 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.189 (s, 1H, -CH=N), 8.104 -7.956 (m, 2H, Ar-H), 7.703-7.645 (s, 2H, Ar-H), 7.581-7.523 (m, 2H, Ar-H), 7.209-7.134 (m, 3H, Ar-H), 6.912-6.857 (m, 1H, Ar-H), 6.801-6.767 (m, 2H, Ar-H), 5.669 (s, 2H, CH_2), 4.449 (s, 2H, CH_2), 3.289 (s, 3H, N-CH_3); ^{13}C NMR (100

MHz, $CDCl_3$) δ (ppm): 162.7, 155.6, 150.4, 146.6, 144.9, 135.6, 132.3, 131.2, 129.4, 128.4, 127.9, 127.1, 126.4, 126.0, 125.7, 125.3, 125.3, 123.7, 123.1, 117.8, 116.7, 68.5, 50.4, 37.6; ESI-MS (*m/z*) = 446.10 $(M+H)^+$; calculated for $C_{24}H_{20}FN_5OS$; C, 64.70; H, 4.52; N, 15.72; S, 7.20. Found: C, 64.72; H, 4.54; N, 15.71; S, 7.19.

4.1.8.4. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(1-benzyl-1H-1,2,3-triazol-4-yl) methoxy]methanimine (9d). Grey solid. Yield: 79%; m.p. 214-215 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.221 (s, 1H, -CH=N), 7.912-7.872 (m, 3H, Ar-H), 7.732 (s, 1H, Triazole-H), 7.389-7.278 (m, 2H, Ar-H), 7.198-7.152 (m, 4H, Ar-H), 6.978-6.856 (m, 1H, Ar-H), 6.811-6.734 (m, 2H, Ar-H), 5.701 (s, 2H, CH_2), 4.545 (s, 2H, CH_2), 3.278 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.1, 150.0, 146.3, 144.9, 135.8, 135.4, 131.9, 129.5, 129.4, 128.7, 127.7, 127.1, 126.8, 125.9, 125.2, 125.0, 123.9, 123.2, 117.9, 68.1, 58.2, 37.1; ESI-MS (*m/z*) = 428.51 $(M+H)^+$; calculated for $C_{24}H_{21}N_5OS$; C, 67.43; H, 4.95; N, 16.38; S, 7.50. Found: C, 67.45; H, 4.93; N, 16.43; S, 7.47.

4.1.8.5. (E)-4-((4-(((((10-Methyl-10H-phenothiazin-3-yl)methylene)amino)oxy)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (9e). Pale yellow solid. Yield: 85%; m.p. 217-218 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.235 (s, 1H, -CH=N), 8.191-7.976 (m, 2H, Ar-H), 7.724-7.665 (s, 2H, Ar-H), 7.210- 7.154 (m, 3H, Ar-H), 7.601-7.543 (m, 2H, Ar-H), 6.932-6.884 (m, 1H, Ar-H), 6.821-6.787 (m, 2H, Ar-H), 5.715 (s, 2H, CH_2), 4.512 (s, 2H, CH_2), 3.297 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 155.7, 150.5, 146.7, 144.9, 141.7, 135.8, 133.4, 132.0, 130.6, 129.5, 127.9, 127.3, 126.2, 125.5, 125.3, 123.9, 123.2, 119.9, 117.8, 111.0, 68.1, 58.1, 37.4; ESI-MS (*m/z*) = 453.10 $(M+H)^+$; calculated for $C_{25}H_{20}N_6OS$; C, 66.35; H, 4.45; N, 18.57; S, 7.09. Found: C, 66.31; H, 4.43; N, 18.59; S, 7.01.

4.1.8.6. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(1-methoxybenzyl-1H-1,2,3-triazol-4-yl)methoxy]methanimine (9f). Brown solid. Yield: 83%; m.p. 222-223 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.196 (s, 1H, -CH=N), 7.951-7.915 (m, 2H, Ar-H), 7.179-7.072 (m, 3H, Ar-H), 7.514 (s, 1H, Triazole-H), 6.987-6.817 (m, 6H, Ar-H), 5.724 (s, 2H, CH_2), 4.508 (s, 2H, CH_2), 3.912 (s, 3H, O-CH_3), 3.314 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 159.1, 155.4, 150.2, 146.4, 144.9, 135.2, 131.9, 131.1, 130.2, 129.5, 127.7, 127.3, 126.1, 125.3, 125.3, 123.9, 123.1, 117.7, 115.3, 68.5, 58.6, 57.0, 37.6; ESI-MS (*m/z*) = 458.40 $(M+H)^+$; calculated for $C_{25}H_{23}N_5O_2S$; C, 65.63; H, 5.07; N, 15.31; S, 7.01. Found: C, 65.61; H, 5.07; N, 15.33; S, 7.00.

4.1.8.7. (E)-1-(10-Methyl-10H-phenothiazin-3-yl)-N-[(4-methoxybenzyl-1H-1,2,3-triazol-4-yl)methoxy]methanimine (9g). Brown solid. Yield: 80%; m.p. 200-201 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.214 (s, 1H, -CH=N), 8.182-7.965 (m, 2H, Ar-H), 7.692-7.634 (s, 2H, Ar-H), 7.588-7.529 (m, 2H, Ar-H), 7.187-7.133 (m, 3H, Ar-H), 6.897-6.849 (m, 1H, Ar-H), 6.801-6.754 (m, 2H, Ar-H), 5.704 (s, 2H, CH_2), 4.498 (s, 2H, CH_2), 3.321 (s, 3H, N-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.2, 155.7, 150.4, 146.5, 144.8, 135.4, 133.0, 132.4, 129.7, 129.4, 127.9, 127.2, 126.1, 125.5, 125.2, 123.9, 123.0, 118.1, 116.8, 68.1, 58.2, 37.3; ESI-MS (*m/z*) = 446.10 $(M+H)^+$; calculated for $C_{24}H_{20}FN_5OS$; C, 64.70; H, 4.52; N, 15.72; S, 7.20. Found: C, 64.67; H, 4.50; N, 15.70; S, 7.21.

4.1.9. General procedure for synthesis of 10-(prop-2-yn-1-yl)-10H-phenothiazine derivatives (10 and 10a). The similar procedure (synthesis of **2**) was adopted to synthesize the intermediates (**10** and **10a**) by N-alkylation of Phenothiazine derivatives (**1** and **1a**) (1 mmol) with propargyl bromide (1.5 mmol) and sodium hydride (2 mmol).

4.1.9.1 10-(Prop-2-yn-1-yl)-10H-phenothiazine (10). Brown solid. Yield: 89%; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.193-7.154 (m, 4H, Ar-H), 6.867-6.752 (m, 2H, Ar-H), 6.801-6.747 (m, 2H, Ar-H), 4.749-4.743 (d, J = 2.4 Hz, 2H, CH_2), 2.783-2.771 (t, J = 4.8 Hz, 2.4 Hz, 1H, Acetylene-CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.3, 130.2, 129.4, 125.1, 122.8, 117.7, 99.7, 75.4, 39.1; ESI-MS (*m/z*) = 238.21 $(M+H)^+$; calculated for $C_{15}H_{11}NS$; C, 75.91; H, 4.67; N, 5.90; S, 13.51. Found: C, 75.88; H, 4.65; N, 5.92; S, 13.53.

4.1.9.2. 10-(Prop-2-yn-1-yl)-2-(trifluoromethyl)-10H-phenothiazine (10a). Pale brown solid. Yield: 88%; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.181-7.145 (m, 2H, Ar-H), 6.815-6.645 (m, 3H, Ar-H), 6.514 (s, 1H, Ar-H), 6.491-6.464 (m, 1H, Ar-H), 4.757-4.750 (d, J = 2.8 Hz, 2H, CH_2), 2.794-2.781 (t, J = 5.2 Hz, 2.8 Hz, 1H, Acetylene-CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 147.5, 145.4, 132.7, 130.4, 129.2, 128.4, 125.3, 125.1, 124.5, 123.6, 122.8, 117.6, 111.2, 99.8, 75.5, 39.2; ESI-MS (*m/z*) = 306.4 $(M+H)^+$; calculated for $C_{16}H_{10}F_3NS$; C, 62.94; H, 3.30; N, 4.59; S, 10.50. Found: C, 62.98; H, 3.25; N, 4.58; S, 10.56.

4.1.10. General procedure for synthesis of 10-((1-benzyl-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazine derivatives (11a-h). The above procedure (synthesis of **6a-g**) was adopted to synthesize the compounds **11a-h** by click reaction between substituted benzyl bromides (1 mmol) and respective 10-(prop-2-yn-1-yl)-10H-phenothiazine derivatives(**10** and **10a**) (1 mmol)

in presence of sodium azide(1mmol), sodium ascorbate (10 mol %) and copper (II) sulfate (5 mol %).

4.1.10.1. 10-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazine (11a). Pale yellow solid. Yield: 85%; m.p. 148-149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.836-7.816 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.477 (s, 1H, Triazole-H), 7.311-7.292 (d, *J* = 7.6 Hz, 2H, Ar-H), 7.121-7.051 (m, 4H, Ar-H), 6.915-6.878 (m, 2H, Ar-H), 6.837-6.816 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.734 (s, 2H, CH_2), 5.263 (s, 2H, CH_2), 2.436 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.4, 139.3, 137.6, 134.5, 130.7, 130.4, 130.2, 129.1, 128.6, 125.9, 122.8, 117.6, 51.6, 44.7, 22.3; ESI-MS (*m/z*) = 385.40 $(M+H)^+$; calculated for $C_{23}H_{20}N_4S$; C, 71.85; H, 5.24; N, 14.57; S, 8.34. Found: C, 71.89; H, 5.21; N, 14.57; S, 8.33.

4.1.10.2. 10-((1-(4-Nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazine (11b). Yellow solid. Yield: 91%; m.p. 174-175 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 8.149-8.127 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.344 (s, 1H, Triazole-H), 7.195-7.173 (d, *J* = 8.8 Hz, 2H, ArH), 7.137-7.040 (m, 4H, Ar-H), 6.936-6.915 (m, 2H, ArH), 6.899-6.759 (m, 2H, ArH), 5.560 (s, 2H, CH_2), 5.239 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 147.9, 144.2, 141.7, 128.3, 128.0, 127.3, 124.3, 124.2, 122.9, 115.4, 53.1, 44.7; ESI-MS (*m/z*) = 416.10 $(M+H)^+$; calculated for $C_{22}H_{17}N_5O_2S$; C, 63.60; H, 4.12; N, 16.86; S, 7.72. Found: C, 63.59, H, 4.10; N, 16.85; S, 7.71.

4.1.10.3. 10-((1-(2-Fluorobenzyl)-1H-1,2,3-triazol-4-yl) methyl)-10H-phenothiazine (11c). Grey solid. Yield: 80%; m.p. 134-135 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.351 (s, 1H, Triazole-H), 7.310-7.292 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.125-7.103 (m, 2H, ArH), 7.082-7.024 (m, 5H, Ar-H), 6.915-6.876 (m, 2H, Ar-H), 6.774-6.753 (m, 2H, Ar-H), 5.510 (s, 2H, CH_2), 5.207 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.6, 145.3, 144.2, 130.7, 130.6, 129.9, 127.3, 124.7, 124.0, 122.8, 122.0, 121.9, 115.6, 115.3, 47.7, 45.0; ESI-MS (*m/z*) = 389.10 $(M+H)^+$; calculated for $C_{22}H_{17}FN_4S$; C, 68.02; H, 4.41; N, 14.42; S, 8.25. Found: C, 68.09; H, 4.40; N, 14.39; S, 8.25.

4.1.10.4. 10-((1-Benzyl-1H-1,2,3-triazol-4-yl) methyl)-10H-phenothiazine (11d). Grey solid. Yield: 84%; m.p. 148-149 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.113-7.013 (m, 6H, Ar-H), 6.907-6.867 (m, 2H, Ar-H), 6.767-6.747 (m, 2H, Ar-H), 7.296-7.273 (m, 4H, Ar-H), 5.450 (s, 2H, CH_2), 5.199 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.6, 139.4, 137.4, 130.8, 130.9, 129.7, 129.4, 128.6, 128.2, 125.6, 123.1, 117.9, 51.7, 44.6; ESI-MS (*m/z*) = 371.10

$(M+H)^+$; calculated for $C_{22}H_{18}N_4S$; C, 71.32; H, 4.90; N, 15.12; S, 8.66. Found: C, 71.30; H, 4.93; N, 15.17; S, 8.67.

4.1.10.5. 4-((4-((10H-Phenothiazin-10-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (11e). Colourless solid. Yield: 98%; m.p. 184-185 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.581-7.560 (d, J = 8.4 Hz, 2H, Ar-H), 7.259 (s, 1H, Triazole-H), 7.136-7.117 (m, 4H, Ar-H), 7.113-7.031 (m, 2H, Ar-H), 6.932-6.891 (m, 2H, Ar-H), 6.771-6.752 (d, J = 7.6 Hz, 2H, Ar-H), 5.508 (s, 2H, CH_2), 5.234 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 145.6, 142.8, 139.4, 133.7, 131.9, 130.2, 129.4, 128.7, 125.1, 122.9, 120.2, 117.6, 113.5, 51.3, 44.7; ESI-MS (*m/z*) = 396.10 $(M+H)^+$; calculated for $C_{23}H_{17}N_5S$; C, 69.85; H, 4.33; N, 17.71; S, 8.11. Found: C, 69.89; H, 4.32; N, 17.63; S, 8.13.

4.1.10.6. 10-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl) methyl)-10H-phenothiazine (11f). Pale grey solid. Yield: 89%; m.p. 144-145 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.843-7.821 (m, 2H, ArH), 7.497 (s, 1H, Triazole-H), 7.324-7.276 (m, 2H, ArH), 7.132-7.107 (m, 4H, ArH), 6.924-6.891 (m, 2H, ArH), 6.843-6.821 (m, 2H, ArH), 5.285 (s, 2H, CH_2), 5.792 (s, 2H, CH_2), 3.914 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.1, 145.5, 139.0, 130.5, 130.1, 129.2, 128.6, 127.5, 125.0, 122.8, 117.4, 115.6, 57.5, 51.2, 43.6; ESI-MS (*m/z*) = 401.40 $(M+H)^+$; calculated for $C_{23}H_{20}N_4OS$; C, 68.98; H, 5.03; N, 13.99; S, 8.01. Found: C, 69.02; H, 5.04; N, 13.97; S, 7.95.

4.1.10.7. 10-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazine (11g). Grey solid. Yield: 84%; m.p. 136-137 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.568-7.546 (d, J = 8.8 Hz, 2H, Ar-H), 7.245 (s, 1H, Triazole-H), 7.148-7.108 (m, 5H, Ar-H), 7.098-7.012 (m, 2H, Ar-H), 6.915-6.876 (m, 2H, Ar-H), 6.764-6.745 (d, J = 7.6 Hz, 1H, Ar-H), 5.527 (s, 2H, CH_2), 5.289 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 161.8, 145.3, 139.3, 131.7, 130.2, 129.8, 128.6, 125.0, 122.9, 119.0, 117.7, 116.5, 51.8, 44.6; ESI-MS (*m/z*) = 389.10 $(M+H)^+$; calculated for $C_{22}H_{17}FN_4S$; C, 68.02; H, 4.41; N, 14.42; S, 8.25. Found: C, 67.93; H, 4.43; N, 14.51; S, 8.26.

4.1.10.8. 4-((4-((2-(Trifluoromethyl)-10H-phenothiazin-10-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (11h). Grey solid. Yield: 80%; m.p: 142-143 °C; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 7.580-7.559 (d, J = 8.4 Hz, 2H, Ar-H), 7.424-7.7.404 (d, J = 8.0 Hz, 2H, Ar-H), 7.254 (s, 1H, Triazole-H), 7.091-7.214 (m, 4H, Ar-H), 6.973-6.871 (m, 2H, Ar-H), 6.771-6.752

(d, *J* = 7.6 Hz, 1H, Ar-H), 5.514 (s, 2H, CH_2), 5.238 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 147.5, 145.3, 142.8, 139.1, 133.2, 132.8, 131.9, 130.2, 129.1, 128.5, 127.6, 125.2, 124.6, 123.6, 122.8, 120.3, 118.7, 117.6, 113.4, 111.3, 51.5, 44.7; ESI-MS (*m/z*) = 464.10 $(M+H)^+$; calculated for $C_{24}H_{16}F_3N_5S$; C, 62.19; H, 3.48; N, 15.11; S, 6.92. Found: C, 62.21; H, 3.50; N, 15.09; S, 7.01.

4.1.11. General procedure for synthesis of 4-((4-((3-formyl-10H-phenothiazin-10-yl) methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile derivatives (12 and 13). The above procedure (synthesis of **2**) was adopted to synthesize the compounds **12** and **13** by formylation of compounds **11e** and **11h** respectively.

4.1.11.1. 4-((4-((3-Formyl-10H-phenothiazin-10-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl) benzonitrile (12). Yellow solid. Yield: 74%; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.816 (s, 1H, Aldehyde-CH), 7.585-7.542 (m, 3H, Ar-H), 7.252 (s, 1H, Triazole-H), 7.187-7.134 (m, 4H, Ar-H), 7.114-7.081 (m, 3H, Ar-H), 6.965-6.947 (m, 1H, Ar-H), 5.524 (s, 2H, CH_2), 5.282 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 191.4, 146.4, 142.9, 139.4, 137.9, 133.0, 132.1, 130.5, 130.2, 129.2, 128.6, 125.3, 122.8, 121.7, 120.4, 117.6, 113.9, 113.4, 51.5, 44.6; ESI-MS (*m/z*) = 424.4 $(M+H)^+$; calculated for $C_{24}H_{17}N_5OS$; C, 68.07; H, 4.05; N, 16.54; S, 7.57. Found: C, 68.05; H, 4.07; N, 16.52; S, 7.55.

4.1.11.2. 4-((4-((7-Formyl-2-(trifluoromethyl)-10H-phenothiazin-10-yl)methyl)-1H-1,2,3-triazol-1-yl)methyl)benzonitrile (13). Yellow solid. Yield: 76%; ^{1}H NMR ($CDCl_3$, 400 MHz, δ in ppm): 9.813 (s, 1H, Aldehyde-CH), 7.579-7.546 (m, 3H, Ar-H), 7.256 (s, 1H, Triazole-H), 7.182-7.139 (m, 4H, Ar-H), 7.107-7.092 (d, *J* = 6.0 Hz, 1H, Ar-H), 6.976-6.932 (m, 2H, Ar-H), 5.527 (s, 2H, CH_2), 5.289 (s, 2H, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 191.6, 147.5, 144.2, 142.9, 137.6, 133.2, 131.0, 130.3, 130.1, 128.7, 125.3, 124.5, 123.6, 121.7, 120.0, 114.2, 113.5, 111.4, 51.6, 44.7; ESI-MS (*m/z*) = 492.1 $(M+H)^+$; calculated for $C_{25}H_{16}F_3N_5OS$; C, 61.09; H, 3.28; N, 14.25; S, 6.52. Found: C, 60.9; H, 3.28; N, 14.19; S, 6.41.

4.1.12 General procedure for synthesis of N'-((10-((1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoromethyl)-10H-phenothiazin-3-yl)methylene)hydrazide derivatives (14a-n). A mixture of compound **12/13** (1 mmol) and hydrazine derivative (1 mmol) was taken in ethanol (10 mL) in a dry 50 mL RB flask. A catalytic amount of conc. H_2SO_4 was added and the mixture was stirred for 10-15 minutes at RT. After the complete consumption of both starting materials

(monitored by TLC), the reaction mixture was poured into ice cold water (10 mL). The solid separated was filtered off under suction and washed with ethanol and water.

4.1.12.1. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)nicotinohydrazide (14a). Pale yellow solid. Yield: 89%; m.p: 250-251 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.945 (s, 1H, Amide-NH), 9.029 (s, 1H, Ar-H), 8.741-8.733 (d, *J* = 3.2 Hz, 1H, Ar-H), 8.282 (s, 1H, C=N-H), 8.230-8.211(m, 1H, Ar-H), 8.107 (s, 1H, Ar-H), 7.803-7.782 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.564-7.442 (m, 3H, Ar-H), 7.303-7.282 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.151-7.100 (m, 2H, Ar-H), 6.971-6.911 (m, 3H, Ar-H), 5.671 (s, 2H, -CH_2), 5.192 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.7, 150.4, 149.6, 148.7, 144.2, 141.8, 141.7, 138.2, 137.8, 134.1, 132.0, 131.8, 130.7, 129.1, 128.0, 127.5, 124.9, 124.5, 124.0, 122.8, 121.7, 119.1, 116.6, 116.3, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 543.10 $(M+H)^+$; calculated for $C_{30}H_{22}N_8OS$; C, 66.40; H, 4.09; N, 20.65; S, 5.91. Found: C, 66.28; H, 4.14; N, 20.58; S, 5.94.

4.1.12.2. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)pyrazine-2-carbohydrazide (14b). Yellow solid. Yield: 97%; m.p: 112-113 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.220 (s, 1H, Amide-NH), 9.239 (s, 1H, Ar-H), 8.906-8.899 (d, *J* = 2.8 Hz, 1H, Ar-H), 8.771-8.761 (m, 1H, Ar-H), 8.489 (s, 1H, C=N-H), 8.112 (s, 1H, Ar-H), 7.807-7.786 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.464-7.412 (m, 2H, Ar-H), 7.309-7.289 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.155-7.104 (m, 2H, Ar-H), 6.990-6.915 (m, 3H, Ar-H), 5.675 (s, 2H, CH_2), 5.197 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 157.7, 148.7, 146.2, 145.7, 141.8, 141.7, 138.2, 134.1, 132.0, 131.8, 130.7, 129.1, 128.0, 127.5, 124.9, 124.5, 124.0, 121.7, 119.1, 118.2, 116.6, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 544.10 $(M+H)^+$; calculated for $C_{29}H_{21}N_9OS$; C, 64.07; H, 3.89; N, 23.19; S, 5.90. Found: C, 64.05; H, 3.93; N, 23.14; S, 5.92.

4.1.12.3. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)isonicotinohydrazide (14c). Brown solid. Yield: 98%; m.p: 166-167 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.194 (s, 1H, Amide-NH), 8.975-8.961 (d, *J* = 5.6 Hz, 2H, Ar-H), 8.475 (s, 1H, C=N-H), 8.105 (s, 1H, Ar-H), 7.815-7.793 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.516-7.466 (m, 2H, Ar-H), 7.318-7.274 (m, 4H, Ar-H), 7.091-7.029 (m, 3H, Ar-H), 6.991-6.935 (s, 2H, Ar-H), 5.682 (s, 2H, -CH_2), 5.192 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm) 164.7, 151.4, 148.7, 144.2, 141.8, 141.7, 139.2, 138.2, 134.1, 132.0, 130.7, 129.1, 128.0, 127.5,

124.9, 124.5, 124.0, 123.5, 121.7, 119.1, 118.2, 116.6, 116.3, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 543.10 $(M+H)^+$; calculated for $C_{30}H_{22}N_8OS$; C, 66.40; H, 4.09; N, 20.65; S, 5.91. Found: C, 66.30; H, 4.12; N, 20.68; S, 5.86.

4.1.12.4. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoromethyl)-10H-phenothiazin-3-yl)methylene)nicotinohydrazide (14d). Brown solid. Yield: 92%; m.p: 208-209 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.949 (s, 1H, Amide-NH), 9.036 (s, 1H, Ar-H), 8.745-8.737 (d, *J* = 3.2 Hz, 1H, Ar-H), 8.286 (s, 1H, C=N-H), 8.235-8.217 (m, 1H, Ar-H), 8.116 (s, 1H, Ar-H), 7.807-7.785 (d, *J* = 8.8 Hz, 2H, Ar-H), 7.576-7.485 (m, 3H, Ar-H), 7.342-7.301 (m, 4H, Ar-H), 7.119 (s, 1H, Ar-H), 7.071-7.052 (m, 1H, Ar-H), 5.664 (s, 2H, CH_2), 5.185 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.7, 150.4, 149.6, 148.7, 146.3, 141.8, 141.7, 138.2, 137.8, 134.1, 132.0, 131.8, 131.6, 130.7, 129.3, 127.5, 124.9, 124.5, 124.1, 123.4, 122.8, 122.5, 119.1, 116.3, 112.4, 110.9, 50.2, 43.5; ESI-MS (*m/z*) = 611.40 $(M+H)^+$; calculated for $C_{31}H_{21}F_3N_8OS$; C, 60.98; H, 3.47; N, 18.35; O, 2.62; S, 5.25. Found: C, 60.96; H, 3.47; N, 18.37; S, 5.23.

4.1.12.5. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoromethyl)-10H-phenothiazin-3-yl)methylene)pyrazine-2-carbohydrazide (14e). Pale yellow solid. Yield: 85%; m.p: 232-233 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.252 (s, 1H, Amide-NH), 9.241 (s, 1H, Ar-H), 8.909-8.904 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.774-8.764 (m, 1H, Ar-H), 8.503 (s, 1H, C=N-H), 8.161 (s, 1H, Ar-H), 7.781-7.760 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.489-7.464 (m, 2H, Ar-H), 7.365-7.250 (m, 4H, Ar-H), 7.115 (s, 1H, Ar-H), 7.057-7.036 (m, 1H, Ar-H), 5.688 (s, 2H, -CH_2), 5.266 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 157.7, 148.7, 146.3, 145.7, 141.8, 141.7, 138.2, 134.1, 132.0, 131.8, 131.6, 130.7, 129.3, 127.5, 124.9, 124.5, 124.1, 123.4, 122.5, 119.1, 118.2, 112.4, 110.1, 50.2, 43.5; ESI-MS (*m/z*) = 612.1 $(M+H)^+$; calculated for $C_{30}H_{20}F_3N_9OS$; C, 58.91; H, 3.30; N, 20.61; O, 2.62; S, 5.24. Found: C, 58.88; H, 3.25; N, 20.59; S, 5.25.

4.1.12.6. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoromethyl)-10H-phenothiazin-3-yl)methylene)isonicotinohydrazide (14f). Reddish brown solid. Yield: 96%; m.p: 218-219 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.967 (s, 1H, Amide-NH), 9.058 (s, 1H, Ar-H), 8.754-8.745 (d, *J* = 3.6 Hz, 1H, Ar-H), 8.301 (s, 1H, C=N-H), 8.254-8.237 (m, 1H, Ar-H), 8.128 (s, 1H, Ar-H), 7.842-7.821 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.597-7.458 (m, 3H, Ar-

H), 7.386-7.796 (m, 4H, Ar-H), 7.128 (s, 1H, Ar-H), 7.094-7.081 (m, 1H, Ar-H), 5.668 (s, 2H, CH_2), 5.187 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.7, 151.4, 148.7, 146.3, 141.8, 141.7, 139.2, 138.2, 134.1, 132.0, 131.6, 130.7, 129.3, 127.5, 124.9, 124.5, 124.1, 123.5, 123.4, 122.5, 119.1, 118.2, 116.3, 112.4, 110.1, 50.2, 43.5; ESI-MS (*m/z*) = 611.1 $(M+H)^+$; calculated for $C_{31}H_{21}F_3N_8OS$; C, 60.98; H, 3.47; N, 18.35; S, 5.25. Found: C, 60.97; H, 3.45; N, 18.34; S, 5.24.

4.1.12.7. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)-4-nitrobenzohydrazide (14g). Reddish brown solid. Yield: 98%; m.p: 132-133 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.071 (s, 1H, Amide-NH), 8.836-8.823 (d, *J* = 5.2 Hz, 2H, Ar-H), 8.316 (s, 1H, Ar-H), 8.116 (S, 1H, C=N-H), 7.916-7.868 (m, 2H, Ar-H), 7.807-7.763 (m, 2H, Ar-H), 7.490-7.454 (m, 2H, Ar-H), 7.309-7.223 (m, 2H, Ar-H), 7.155-7.106 (m, 2H, Ar-H), 6.989-6.898 (m, 3H, Ar-H), 5.677 (s, 2H, -CH_2), 5.199 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 149.2, 148.7, 144.1, 141.8, 141.7, 138.2, 138.1, 134.1, 132.0, 130.7, 129.1, 129.0, 128.0, 127.5, 124.9, 124.4, 124.1, 124.0, 121.7, 119.1, 118.2, 116.5, 116.3, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 587.10 $(M+H)^+$; calculated for $C_{31}H_{22}N_8O_3S$; C, 63.47; H, 3.78; N, 19.10; S, 5.47. Found: C, 63.55; H, 3.76; N, 19.07; S, 5.47.

4.1.13.8. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)benzohydrazide (14h). Pale green solid. Yield: 97; m.p: 152-153 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.052 (s, 1H, Amide-NH), 8.312 (s, 1H, C=N-H), 8.106 (S, 1H, Ar-H), 8.074- 8.063 (d, *J* = 4.4 Hz, 2H, Ar-H), 7.812-7.716 (m, 4H, Ar-H), 7.691-7.625 (m, 3H, Ar-H), 7.523-7.458 (m, 2H, Ar-H), 7.314-7.272 (m, 1H, Ar-H), 7.102-7.029 (m, 2H, Ar-H), 6.957-6.868 (m, 2H, Ar-H), 5.681 (s, 2H, -CH_2), 5.210 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 148.7, 144.1, 141.8, 141.7, 138.2, 134.1, 132.7, 132.0, 131.9, 130.7, 129.1, 128.5, 128.3, 128.0, 127.5, 124.9, 124.4, 121.7, 119.1, 118.2, 116.5, 116.3, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 542.1 $(M+H)^+$; calculated for $C_{31}H_{23}N_7OS$; C, 68.74; H, 4.28; N, 18.10; S, 5.92. Found: C, 68.72; H, 4.31; N, 18.15; S, 5.93.

4.1.12.9. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)-4-fluorobenzohydrazide (14i). Pale yellow solid. Yield: 89%; m.p: 107-108 °C; ^{1}H NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.065 (s, 1H, Amide-NH), 8.311 (s, 1H, C=N-H), 8.112 (S, 1H, Ar-H), 8.085- 8.073 (d, *J* = 4.8 Hz, 2H, Ar-H), 7.899-7.852 (m, 2H, Ar-H), 7.791-7.746

(m, 2H, Ar-H), 7.412-7.369 (m, 2H, Ar-H), 7.294-7.209 (m, 2H, Ar-H), 7.098-7.051 (m, 2H, Ar-H), 6.943-6.854 (m, 3H, Ar-H), 5.686 (s, 2H, -CH_2), 5.216 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.0, 164.3, 148.7, 144.1, 141.8, 141.7, 138.2, 134.1, 132.0, 130.7, 130.5, 129.1, 129.0, 128.0, 127.5, 124.9, 124.4, 124.0, 121.7, 119.1, 118.2, 116.5, 116.3, 115.9, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 560.1 $(M+H)^+$; calculated for $C_{31}H_{22}FN_7OS$; **C,** 66.53; H, 3.96; N, 17.52; S, 5.73. Found: C, 66.57; H, 3.95; N, 17.45; S, 5.75.

4.1.12.10. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-10H-phenothiazin-3-yl)methylene)-4-methylbenzohydrazide (14j). Brown solid. Yield: 89%; m.p: 175-176 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 12.054 (s, 1H, Amide-NH), 8.304 (s, 1H, C=N-H), 8.104 (S, 1H, Ar-H), 8.067- 8.055 (d, *J* = 4.8 Hz, 2H, Ar-H), 7.862-7.814 (m, 2H, Ar-H), 7.756-7.712 (m, 2H, Ar-H), 7.401-7.357 (m, 2H, Ar-H), 7.276-7.192 (m, 2H, Ar-H), 7.044-6.996 (m, 2H, Ar-H), 6.912-6.824 (m, 3H, Ar-H), 5.681 (s, 2H, -CH_2), 5.212 (s, 2H, CH_2), 2.335 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 148.7, 144.1, 142.8, 141.8, 141.7, 138.2, 134.1, 132.0, 131.2, 130.7, 129.1, 128.8, 128.3, 128.0, 127.5, 124.9, 124.4, 124.0, 121.7, 119.1, 118.2, 116.5, 116.3, 112.4, 50.2, 43.5, 21.1; ESI-MS (*m/z*) = 556.1 $(M+H)^+$; calculated for $C_{32}H_{25}N_7OS$; C, 69.17; H, 4.53; N, 17.65; S, 5.77. Found: C, 69.21; H, 4.54; N, 17.63; S, 5.71.

4.1.12.11. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoro methyl)-10H-phenothiazin-3-yl)methylene)-4-nitrobenzohydrazide (14k). Yellow solid. Yield: 84%; m.p: 190-191 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.257 (s, 1H, Amide-NH), 8.149-8.093 (m, 3H, Ar-H), 7.913 (S, 1H, Ar-H), 7.777-7.536 (m, 2H, Ar-H), 7.496-7.469 (m, 2H, Ar-H), 7.360-7.244 (m, 4H, Ar-H), 7.153-7.112 (m, 3H, Ar-H), 7.012-6.991 (d, *J* = 8.4 Hz, 1H, Ar-H), 5.689 (s, 2H, -CH_2), 5.256 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 149.2, 148.7, 146.3, 141.9, 141.7, 138.2, 138.1, 134.1, 132.0, 130.7, 129.3, 129.0, 127.5, 124.9, 124.4, 124.1, 124.0, 123.4, 122.5, 119.1, 118.2, 116.3, 110.1, 112.4, 50.2, 43.5; ESI-MS (*m/z*) = 655.1 $(M+H)^+$; calculated for $C_{32}H_{21}F_3N_8O_3S$; C, 58.71; H, 3.23; N, 17.12; S, 4.90. Found: C, 58.62; H, 3.25; N, 17.21; S, 4.89.

4.1.12.12. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoro methyl)-10H-phenothiazin-3-yl)methylene)benzohydrazide (14l). Colourless solid. Yield: 98%; m.p: 167-168 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.246 (s, 1H, Amide-NH), 8.105-8.049 (m, 3H, Ar-H), 7.914 (S, 1H, Ar-H), 7.735-7.548 (m, 4H, Ar-H), 7.489-7.465 (m, 2H, Ar-H), 7.326-7.245 (m, 3H, Ar-H), 7.107-7.061 (m, 3H, Ar-H), 6.985-6.966 (d, *J* = 7.6 Hz, 1H, Ar-H), 5.685

(s, 2H, CH_2), 5.245 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 148.7, 146.3, 141.8, 141.7, 138.2, 134.1, 132.7, 132.0, 131.9, 131.6, 130.7, 129.3, 128.5, 128.3, 127.5, 124.9, 123.4, 122.5, 119.1, 118.2, 116.3, 112.4, 110.1, 50.2, 43.5; ESI-MS (*m/z*) = 610.1 $(M+H)^+$; calculated for $C_{32}H_{22}F_3N_7OS$; C, 63.05; H, 3.64; N, 16.08; S, 5.26. Found: C, 63.04; H, 3.65; N, 16.06; S, 5.27.

4.1.12.13. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoro methyl)-10H-phenothiazin-3-yl)methylene)-4-fluorobenzohydrazide (14m). Yellow solid. Yield: 90%; m.p: 141-142 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.251 (s, 1H, Amide-NH), 8.094-8.039 (m, 3H, Ar-H), 7.907 (S, 1H, Ar-H), 7.741-7.514 (m, 2H, Ar-H), 7.472-7.446 (m, 2H, Ar-H), 7.331-7.216 (m, 4H, Ar-H), 7.104-7.064 (m, 3H, Ar-H), 6.997-6.976 (d, *J* = 8.4 Hz, 1H, Ar-H), 5.701 (s, 2H, CH_2), 5.267 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.0, 164.3, 148.7, 146.3, 141.8, 141.7, 138.2, 131.6, 134.1, 132.0, 130.7, 130.5, 129.3, 129.0, 127.5, 124.9, 124.4, 123.4, 122.5, 119.1, 118.2, 116.3, 115.9, 112.4, 110.1, 50.2, 43.5; ESI-MS (*m/z*) = 628.1 $(M+H)^+$; calculated for $C_{32}H_{21}F_4N_7OS$; C, 61.24; H, 3.37; N, 15.62; S, 5.11. Found: C, 61.26; H, 3.35; N, 15.61; S, 5.10.

4.1.12.14. (E)-N'-((10-((1-(4-Cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-8-(trifluoro methyl)-10H-phenothiazin-3-yl)methylene)-4-methylbenzohydrazide (14n). Brown solid. Yield: 94%; m.p: 168-169 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz, δ in ppm): 11.245 (s, 1H, Amide-NH), 8.073-8.017 (m, 3H, Ar-H), 7.902 (S, 1H, Ar-H), 7.724-7.498 (m, 2H, Ar-H), 7.456-7.432 (m, 2H, Ar-H), 7.298-7.184 (m, 4H, Ar-H), 7.095-7.056 (m, 3H, Ar-H), 6.974-6.954 (d, *J* = 8.0 Hz, 1H, Ar-H), 5.694 (s, 2H, CH_2), 5.256 (s, 2H, CH_2), 2.344 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 164.3, 148.7, 146.3, 142.8, 141.8, 141.7, 138.2, 134.1, 132.0, 131.6, 131.2, 130.7, 129.3, 128.8, 128.3, 127.5, 124.9, 124.4, 123.4, 122.5, 119.1, 118.2, 116.3, 112.4, 110.1, 50.2, 43.5, 21.1; ESI-MS (*m/z*) = 624.1 $(M+H)^+$; calculated for $C_{33}H_{24}F_3N_7OS$; C, 63.55; H, 3.88; N, 15.72; S, 5.14. Found: C, 63.57; H, 3.86; N, 15.77; S, 5.11.

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Table 5. % Growth inhibition and Selectivity index (SI) of the potent compounds (at 62.5 mg/mL concentration).

Table 6. *In-silico* ADME predictions of target compounds.

Figure 1. Clinically using phenothiazine based drugs.

Figure 2. Reported phenothiazine and 1,2,3-triazole based anti-tubercular drugs.

Figure 3. Structural design strategy through molecular hybridization.

Figure 4. ORTEP diagram showing the X-ray crystal structure of compound **11c** with displacement ellipsoids at 50% probability level.

Figure 5. *In vitro* anti-tubercular activity of the title compounds (**11a-h**) against *M.tb* H37Rv (ATCC No- 27294).

Figure 6. *In vitro* cytotoxicity (IC_{50}) of potent compounds against VERO cell line.

Figure 7. Illustrations of structural interactions of compound **14d** within Inh A active pocket.

Figure 8. Binding modes of lead compounds a) **9g,** b) **11g,** c) **14c** and d) **14k** within Inh A active pocket.

Figure 9. Binding modes of lead compounds a) **11b**, b) **14c**, c) **14f** and f) **14k** within CYP 121 active pocket.

Scheme 1. Synthesis of the final compounds (**6a-g**) with ether linker**.**

Reagents and conditions : a) NaH, MeI, DMF, rt, 8h, yield: 89%; b) DMF-$POCl_3$, 60-70 ^oC, 16h, yield: 83.9% ; c) $NaBH_4$, MeOH, 0 ^oC to rt, 2h, yield: 89%; d) proprgyl bromide, NaH, THF, 0 ^oC to rt, 4h, yield: 82%; e) R-Br, NaN_3, sodium ascorbate, copper(II)sulfate, t-BuOH/H_2O, rt, 16h, yield: 78-91%.

Scheme 2. Synthesis of the final compounds (**9a-g**) with oxime linker**.**

Reagents and conditions: a) $NH_2OH.HCl$, cat. H_2SO_4, EtOH, 80 °C, 2h, yield: 78% ; b) proprgyl bromide, NaH, THF, 0 ^oC to rt, 8h, yield: 85%; c) R-Br, NaN_3, sodium ascorbate, copper(II)sulfate, t-BuOH/H2O, rt, 16h, yield: 74-85%.

Scheme 3. Synthesis of N-substituted phenothiazine-1,2,3-triazole derivatives (**11a-h**).

Reagents and conditions : a) Propargyl bromide, NaH, THF, 0 ^oC to rt, 6h, yield: 88-89%; b) R-Br, NaN_3, sodium ascorbate, copper(II)sulfate, t-BuOH/H_2O, rt, 16h, yield: 80-94%;

Scheme 4. Synthesis of (E)-N'-((10-((1-(4-Cyanobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)-10*H*-phenothiazin-3-yl)methylene) derivatives (**14a-n**).

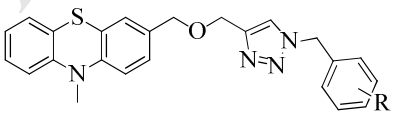
Reagents and conditions: a) DMF-$POCl_3$, 60-70 ^oC, 16h, yield: 74-76%; b) Substituted hydrazides, Cat H_2SO_4, EtOH, 1h, yield: 84-98%.

Table 1. Single crystal x-ray data of compound **11c.**

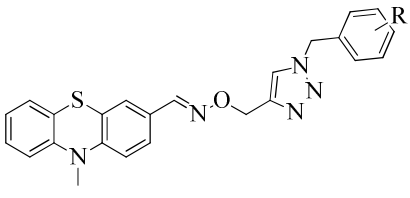
Parameters	**Crystal Data**
Empirical formula	$2(C_{22}H_{17}FN_4S)$
Formula weight	776.91
Temperature (K)	293(2)
Wavelength (Kα, Å)	0.71073
Crystal system	Orthorhombic

Space group	*Pca2₁*
Unit cell dimensions (Å,°)	*a* = 25.8300(9)
	b = 5.51265(16)
	c = 26.8968(9)
Volume ($Å^3$)	3829.9(2)
Calculated density (Mg/m^3)	1.347
Absorption coefficient (mm^{-1})	0.193
Crystal dimension	0.10 x 0.10 x 0.12
Angle α, β, γ (°)	90, 90, 90
Z	4
$F_{(000)}$	1616
CCDC number	1872610
R [$I > 2\sigma(I)$][a]	0.0678
Reflections collected	29646
Reflections unique	6726
R_{int}	0.050
Theta range	1.6 to 25.0

Table 2. *In vitro* anti-tubercular activity of the title compounds (**6a-g** and **9a-g**) against *M. tuberculosis* H37Rv (ATCC No- 27294).



6a-g

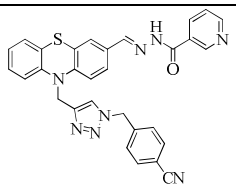
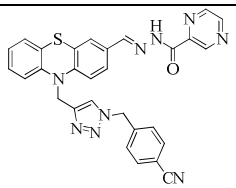
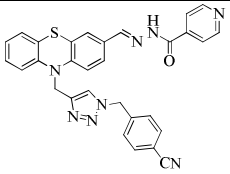
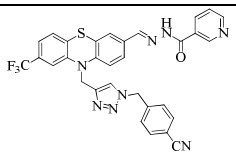
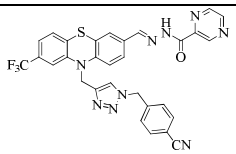
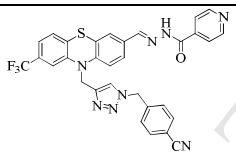
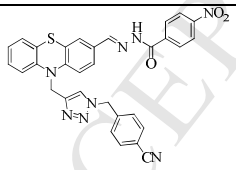
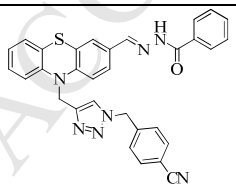
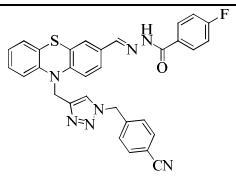


9a-g

Compound	R	*In vitro* Mtb activity (MIC)		Yield(%)	Melting Point(^{0}C)
		µg/mL	µM		
6a	4-methyl	25	58.3	83	148-149
6b	4-nitro	25	54.4	86	174-175
6c	2-flouro	12.5	28.9	78	134-135
6d	4-hydro	25	60.3	82	148-149
6e	4-cyano	12.5	28.4	91	184-185
6f	4-methoxy	25	56.2	88	144-145
6g	4-flouro	12.5	28.9	80	141-142
9a	4-methyl	25	56.6	79	207-208
9b	4-nitro	25	52.9	81	235-236
9c	2-flouro	25	56.1	74	198-199
9d	4-hydro	25	58.4	79	214-215
9e	4-cyano	12.5	27.6	85	217-218
9f	4-methoxy	25	54.6	83	222-223
9g	4-flouro	25	56.1	80	209-210

Table 3. *In vitro* anti-tubercular activity of the title compounds (**14a-n)** against *M.tb* H37Rv (ATCC No- 27294).

Compound	Structure	*In vitro* Mtb activity (MIC)		Yield (%)	Chemical Formula
		µg/mL	µM		

14a		1.6	2.94	89	$C_{30}H_{22}N_8OS$
14b		1.6	2.94	97	$C_{29}H_{21}N_9OS$
14c		1.6	2.94	98	$C_{30}H_{22}N_8OS$
14d		1.6	2.62	92	$C_{31}H_{21}F_3N_8OS$
14e		1.6	2.61	85	$C_{30}H_{20}F_3N_9OS$
14f		1.6	2.62	96	$C_{31}H_{21}F_3N_8OS$
14g		1.6	2.72	98	$C_{31}H_{22}N_8O_3S$
14h		1.6	2.95	97	$C_{31}H_{23}N_7OS$
14i		1.6	2.85	94	$C_{31}H_{22}FN_7OS$

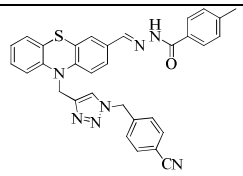
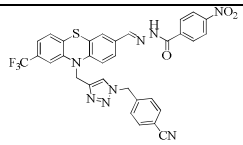
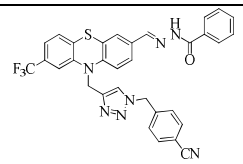
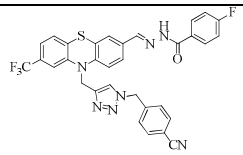
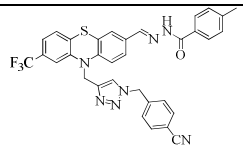
14j		1.6	2.87	89	$C_{32}H_{25}N_7OS$
14k		1.6	2.44	84	$C_{32}H_{21}F_3N_8O_3S$
14l		1.6	2.62	98	$C_{32}H_{22}F_3N_7OS$
14m		1.6	2.55	90	$C_{32}H_{21}F_4N_7OS$
14n		1.6	2.56	94	$C_{33}H_{24}F_3N_7OS$

Table 4. *In vitro* antibacterial activity of the title compounds (**6a-g**, **9a-g**, **11a-h**, and **14a-n**) against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Compound	***E. Coli*** **MIC(µg/mL)**	***S.aureus*** **MIC(µg/mL)**	***P.aeruginosa*** **MIC(µg/mL)**
6a	100	50	100
6b	50	25	50
6c	25	25	50
6d	50	50	100

6e	25	12.5	25
6f	100	50	50
6g	25	25	50
9a	50	25	100
9b	50	25	100
9c	25	12.5	25
9d	100	50	50
9e	25	12.5	25
9f	100	50	100
9g	25	12.5	25
11a	100	50	100
11b	50	50	100
11c	12.5	12.5	25
11d	50	50	100
11e	12.5	6.25	12.5
11f	25	25	50
11g	12.5	12.5	12.5
14a	25	12.5	50
14b	50	50	100
14c	25	12.5	25
14d	100	50	100
14e	50	50	100
14f	50	50	100
14g	50	25	50
14h	25	12.5	25
14i	12.5	6.25	12.5
14j	50	25	100
14k	50	25	50
14l	50	50	50
14m	12.5	6.25	12.5
14n	50	25	50
Ciprofloxacin	2	2	4

Table 5. % Growth inhibition and Selectivity index (SI) of the potent compounds (at 62.5 mg/mL concentration).

Compound	%Inhibition	SI (Selectivity Index)	Compound	%Inhibition	SI (Selectivity Index)
11b	11.36	42.5	**14e**	11.55	182.9
11c	3.33	277.5	**14f**	7.14	207.0
11e	11.06	119.3	**14g**	7.57	91.49
11f	7.57	796.99	**14h**	13.43	32.34

11g	8.34	456.23	**14i**	11.42	66.35
11h	5.0	416.8	**14j**	10.64	42.87
14a	8.42	191.69	**14k**	8.91	126.49
14b	12.33	194.73	**14l**	12.34	44.70
14c	5.13	252.63	**14m**	8.42	76.79
14d	7.32	115.46	**14n**	6.28	249.68

Table 6. *In-silico* ADME predictions of target compounds.

Comp	**Mol. Wt**	**Don[a]-HB (<5)**	**Acp[b]-HB (<10)**	**QPlogP[c] (o/w) (<5)**	**n-rot[d] (0-15)**	**QPlogS[e] (< 0.5)**	**PSA[f] (<140 Å)**	**QPPcaco[g] (<25 poor; >500 high)**	**QPlog Khsa[h] (-1.5 – 1.5)**	**QPlog BB[i] (-3.0-1.2)**	**%OA[j] (>80% is high;<25% is poor)**
6a	428.551	0	4.7	6.327	6	-7.718	42.831	3235.747	1.132	-1.175	100
6b	459.522	0	5.7	5.254	7	-7.222	87.587	389.464	0.860	-0.351	91
6c	432.514	0	4.7	6.102	6	-7.327	43.142	2984.618	0.979	-0.157	100
6d	414.524	0	4.7	5.967	6	-7.070	41.960	3214.502	0.947	-2.602	100

6e	439.534	0	6.2	5.159	7	-7.940	68.493	676.071	0.702	-2.847	94
6f	444.550	0	5.45	6.081	7	-7.346	51.055	3242.123	0.939	-2.246	100
6g	432.514	0	4.7	6.626	6	-7.447	42.763	3252.488	0.999	-3.627	100
9a	441.550	0	6.2	5.787	7	-7.474	56.899	2196.481	0.811	-1.511	100
9b	472.520	0	7.2	4.678	8	-6.801	101.233	265.211	0.528	-0.642	100
9c	445.513	0	6.2	5.613	7	-7.017	56.283	2227.930	0.661	-0.461	100
9d	427.523	0	6.2	5.461	7	-6.864	57.040	2180.915	0.638	-0.280	100
9e	452.533	0	7.7	4.610	8	-7.611	82.751	454.802	0.380	-1.455	100
9f	457.549	0	6.95	5.515	8	-6.194	65.217	2201.999	0.609	-0.240	100
9g	445.513	0	6.20	5.695	7	-7.229	56.967	2188.091	0.682	-0.273	100
11a	384.498	0	3.4	6.219	4	-7.334	36.031	3680.068	1.217	-0.889	100
11b	415.469	0	4.0	5.145	5	-6.781	80.821	440.453	0.946	-0.119	91
11c	388.461	0	3.0	6.094	4	-6.914	34.649	4084.866	1.071	-0.059	100
11d	370.471	0	3.0	5.912	4	-6.688	34.883	4026.061	1.037	-0.586	100
11e	395.481	0	4.5	5.050	5	-7.493	61.725	765.115	0.788	-1.778	95.1
11f	400.497	0	3.75	5.957	5	-6.852	44.440	3688.854	1.020	-0.468	100
11g	388.461	0	3.0	6.130	4	-7.095	36.008	3676.488	1.089	-0.571	100
11h	463.477	0	4.5	6.365	5	-8.243	61.612	795.645	1.012	-1.231	100
14a	542.617	1	8.5	5.418	9	-9.589	125.636	88.865	0.988	-2.148	67.6
14b	543.605	1	9.5	4.754	9	-9.110	137.674	58.007	0.741	-2.523	73.4
14c	542.617	1	8.5	5.436	9	-9.519	124.334	99.122	0.981	-3.284	68.6
14d	610.615	1	8.5	6.425	9	-11.037	125.556	90.140	1.256	-1.926	73.6
14e	611.603	1	9.5	5.751	9	-10.542	137.772	58.150	1.005	-2.393	66.3
14f	610.615	1	8.5	6.423	9	-11.035	125.577	90.005	1.256	-3.165	73.6
14g	586.627	1	8.0	5.687	10	-10.470	157.419	19.878	1.291	-2.650	44.6
14h	541.629	1	7.0	6.399	9	-10.325	112.570	166.763	1.354	-2.391	78.3
14i	559.619	1	7.0	6.631	9	-10.688	112.561	166.824	1.396	-2.029	79.6
14j	555.647	1	7.0	6.278	9	-10.144	113.124	166.612	1.325	-2.445	77.4
14k	654.625	1	8.0	6.470	10	-11.126	156.604	19.545	1.522	-2.415	49.1
14l	609.627	1	7.0	7.413	9	-11.793	112.536	168.419	1.628	-2.184	84.3

14m	627.618	1	7.0	7.631	9	-12.125	112.434	168.386	1.666	-1.889	85.5
14n	623.646	1	7.0	7.275	9	-11.643	112.761	168.584	1.612	-2.276	83.2

[a] Number of hydrogen bond donors (Don-HB).

[b] Number of hydrogen bond acceptors (Acp-HB).

[c] Logarithm of partition coefficient between n-octanol and water (QPlog P).

[d] Number of rotatable bonds (n-rot).

e Aqua solubility parameter (QPlog S).

[f] Polar Surface Area (PSA).

[g] Caco-2 Cell permeability in nm/s.

[h] Human serum albumin binding co-efficient (QPlogKhsa).

[i] Blood/Brain partition co-efficient (QPlogBB).

[j] Percentage of human oral absorption (%OA).

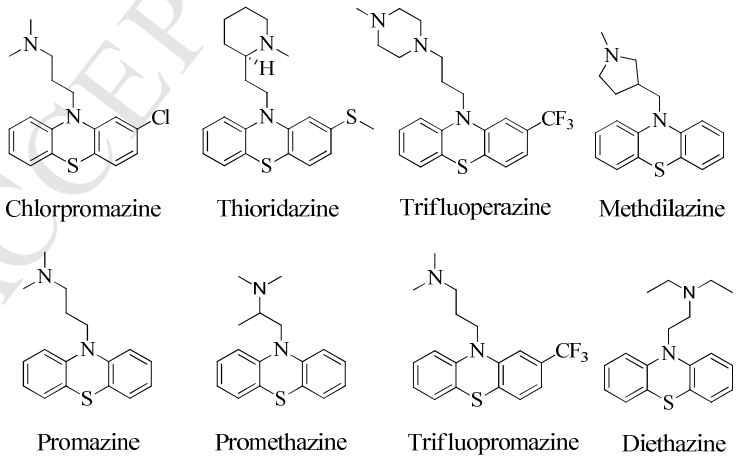


Figure 1

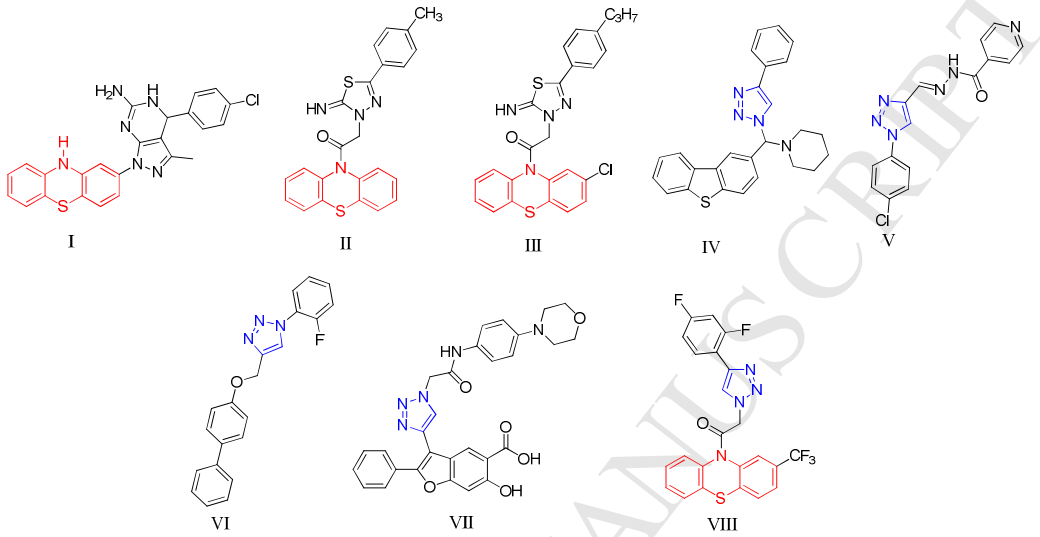


Figure 2

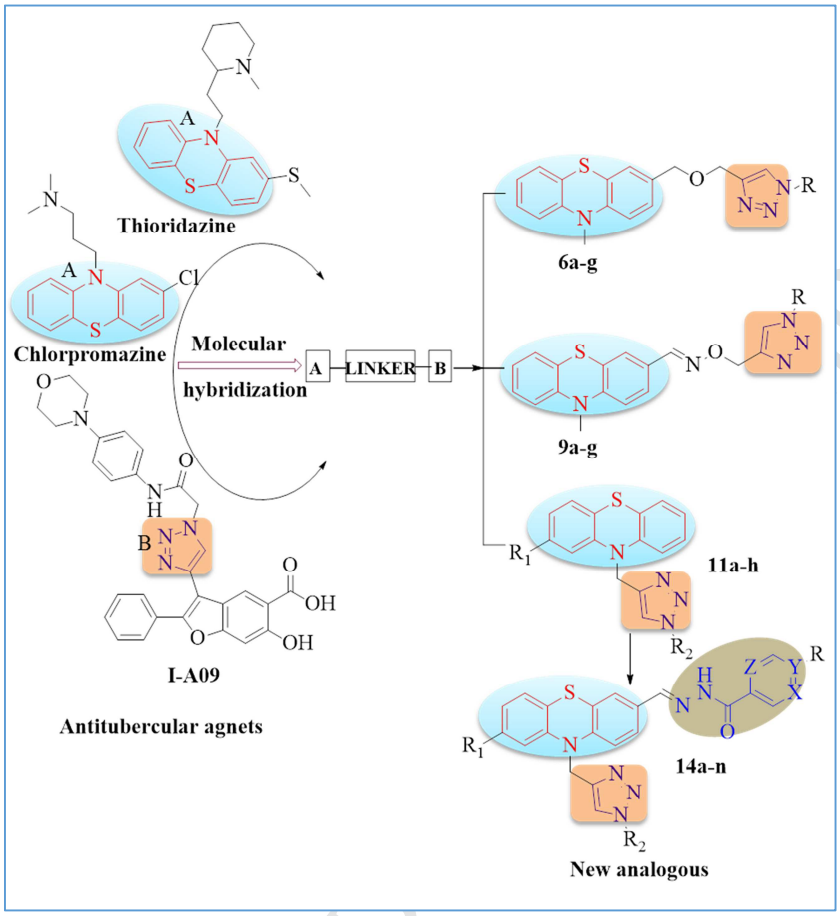
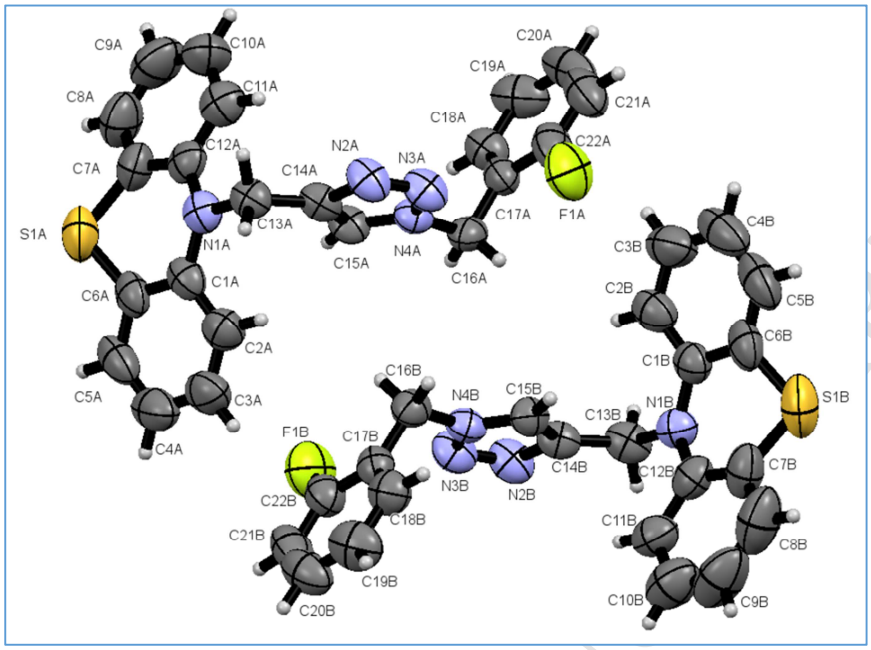


Figure 3



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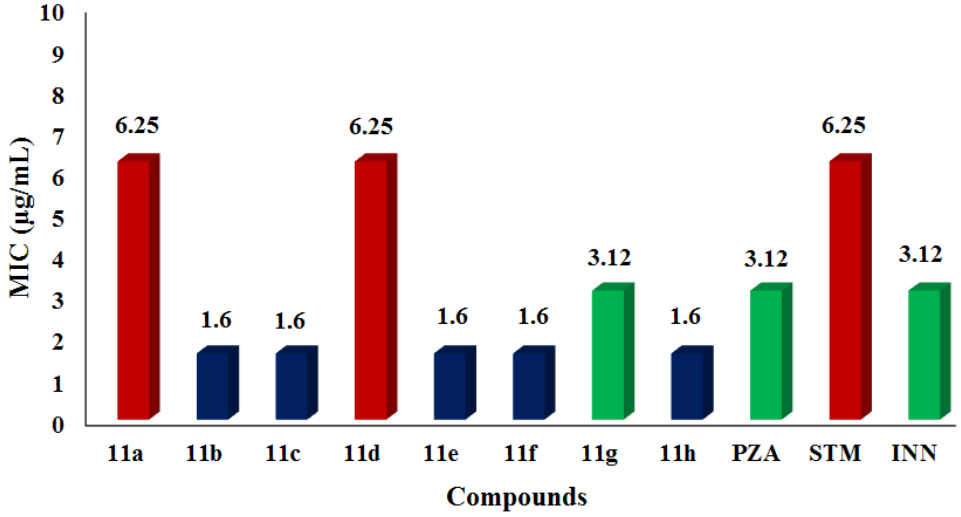

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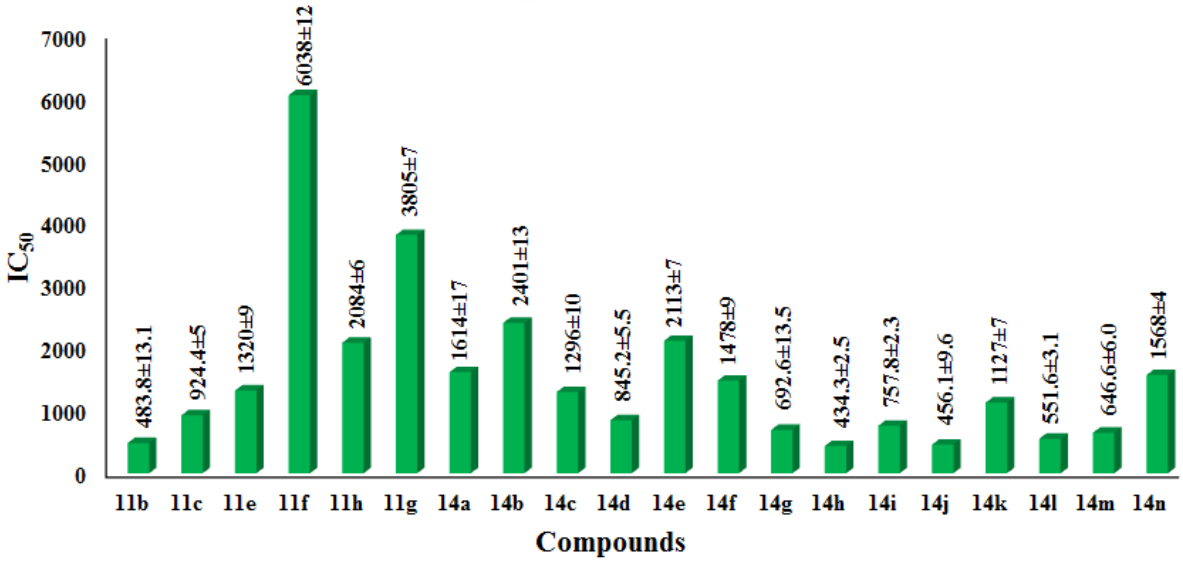
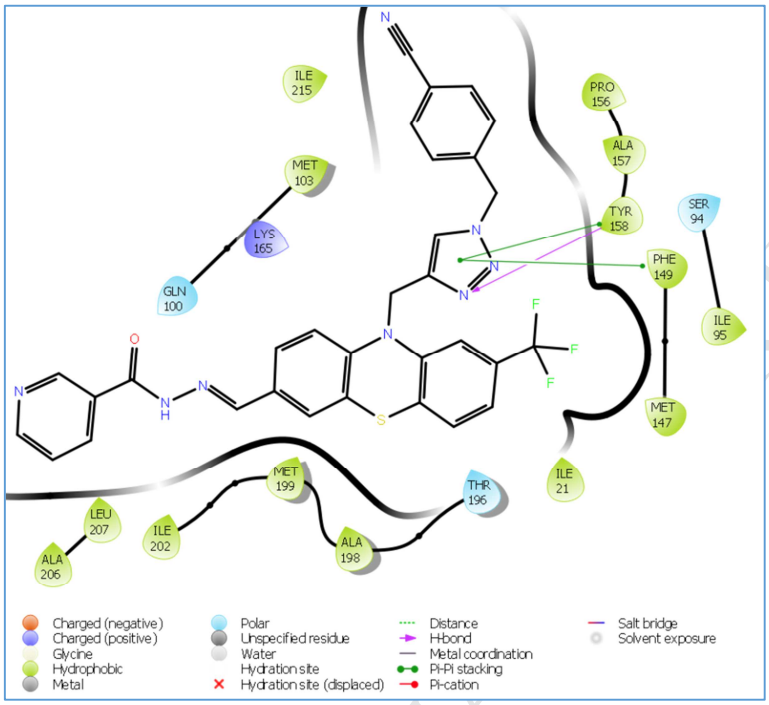


Figure 6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ALA 198
ILE 202
LEU 207
ALA 206
Charged (negative)
Charged (positive)
Glycine
Hydrophobic
Metal
Polar
Unspecified residue
Water
Hydration site
Hydration site (displaced)
Distance
H-bond
Metal coordination
Pi-Pi stacking
Pi-cation
Salt bridge
Solvent exposure

Figure 7

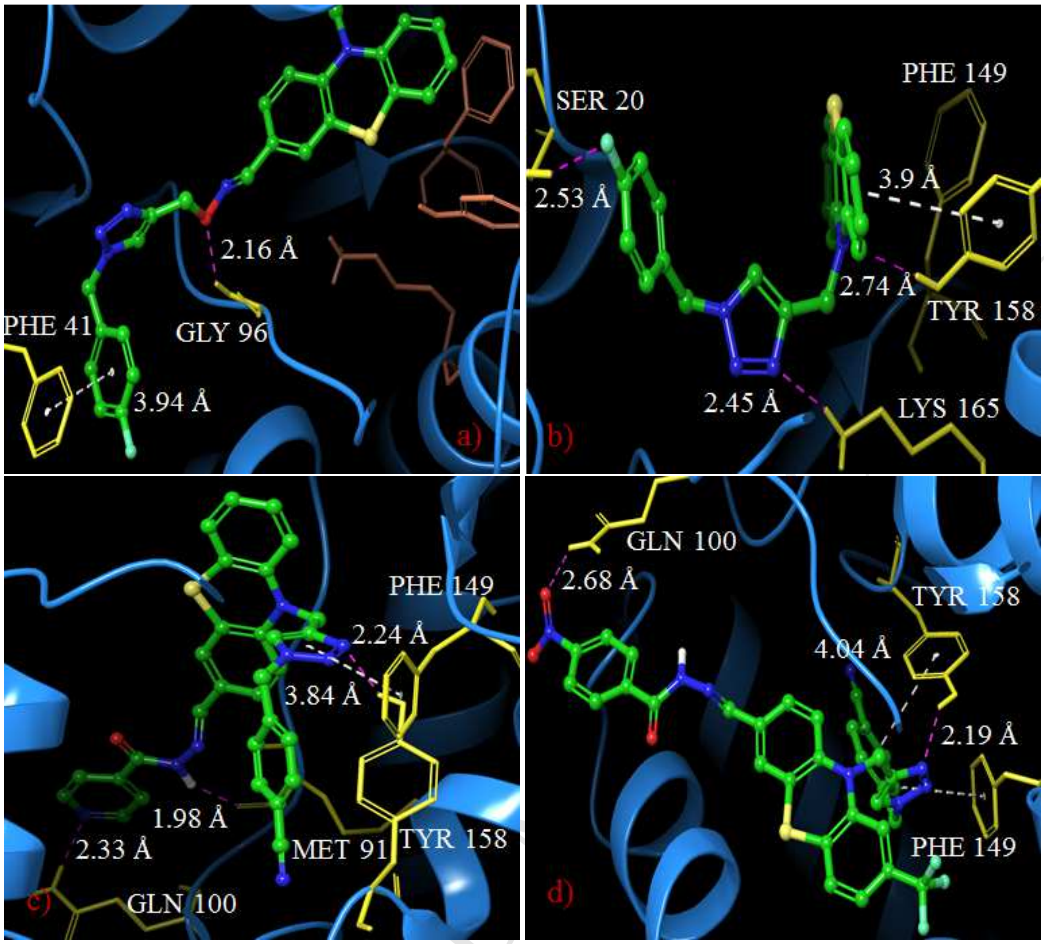


Figure 8

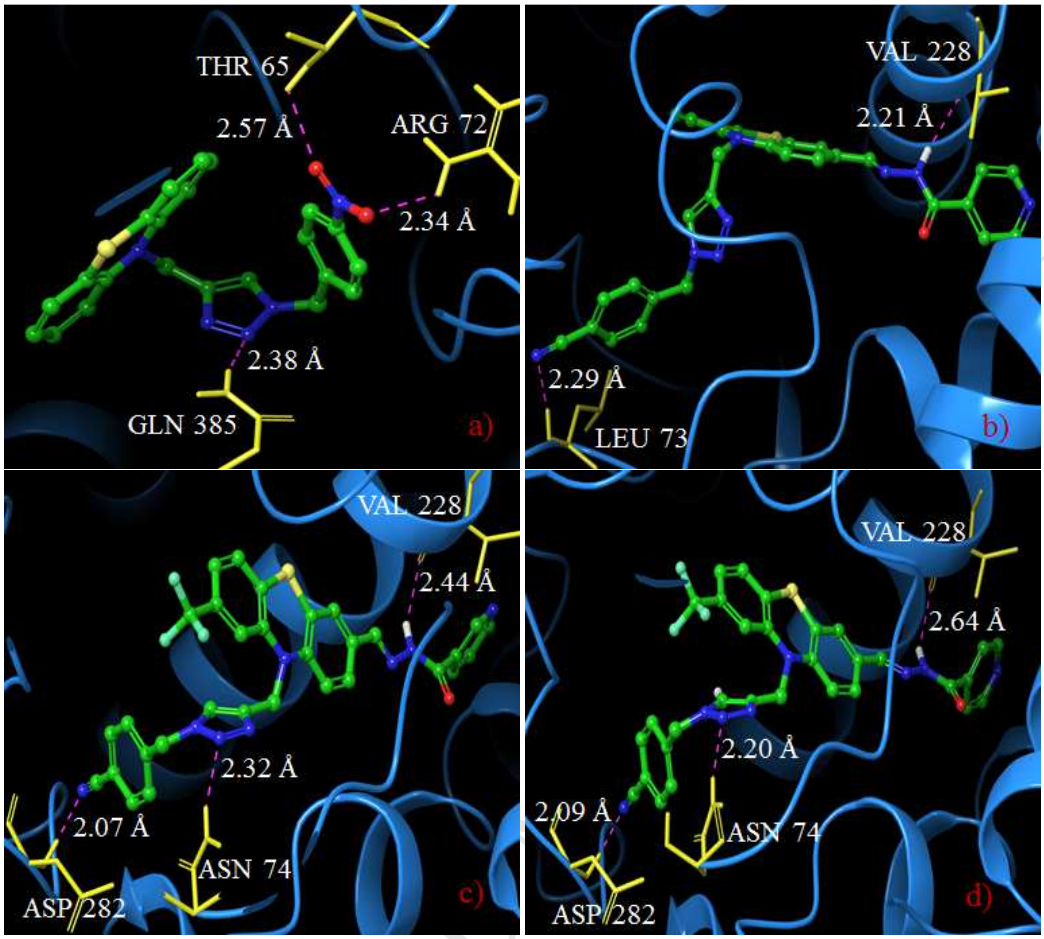
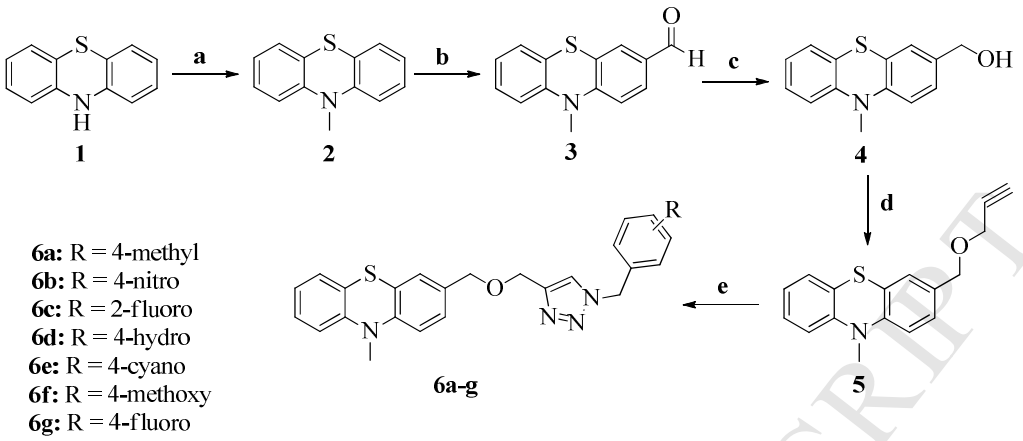
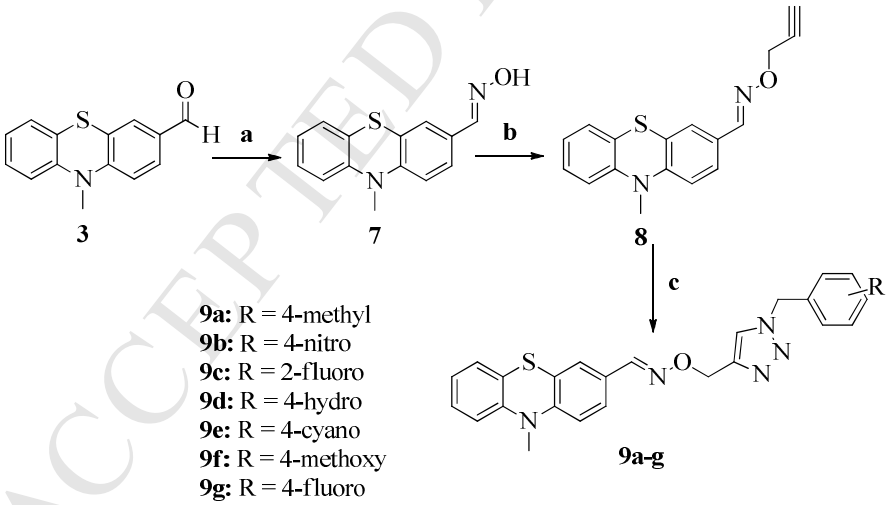


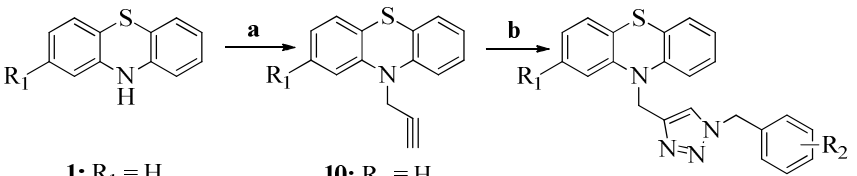
Figure 9



Scheme 1



Scheme 2



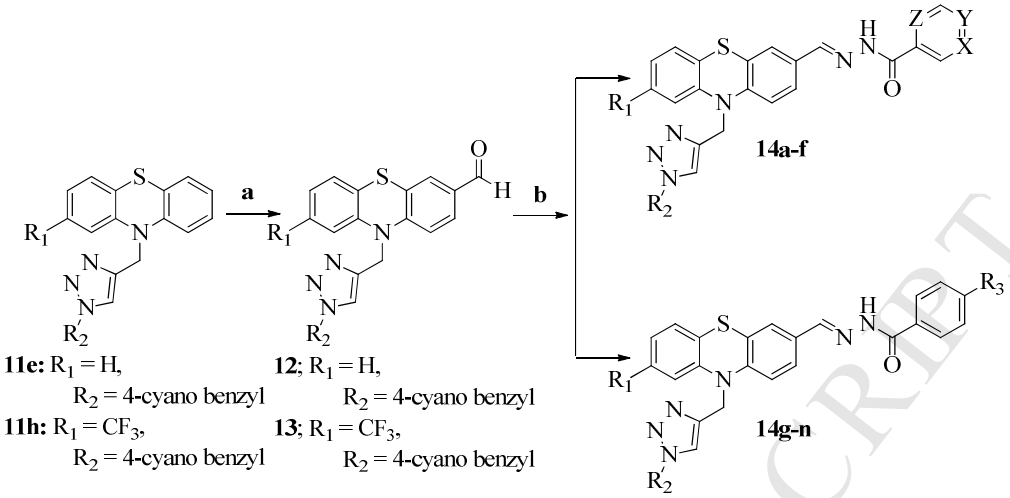
1: $R_1 = H$
1a: $R_1 = CF_3$

10: $R_1 = H$
10a: $R_1 = CF_3$

11a-h

11a: $R_1 = H, R_2 = 4$-methyl
11b: $R_1 = H, R_2 = 4$-nitro
11c: $R_1 = H, R_2 = 2$-fluoro
11d: $R_1 = H, R_2 = 4$-hydro
11e: $R_1 = H, R_2 = 4$-cyano
11f: $R_1 = H, R_2 = 4$-methoxy
11g: $R_1 = H, R_2 = 4$-fluoro
11h: $R_1 = CF_3, R_2 = 4$-cyano

Scheme 3



14a: R_1 = H, R_2 = 4-cyano benzyl, X = N, Y = CH, Z = CH
14b: R_1 = H, R_2 = 4-cyano benzyl, X = N, Y = CH, Z = N
14c: R_1 = H, R_2 = 4-cyano benzyl, X = CH, Y = N, Z = CH
14d: R_1 = CF_3, R_2 = 4-cyano benzyl, X = N, Y = CH, Z = CH
14e: R_1 = CF_3, R_2 = 4-cyano benzyl, X = N, Y = CH, Z = N
14f: R_1 = CF_3, R_2 = 4-cyano benzyl, X = CH, Y = N, Z = CH
14g: R_1 = H, R_2 = 4-cyano benzyl, R_3 = NO_2
14h: R_1 = H, R_2 = 4-cyano benzyl, R_3 = H
14i: R_1 = H, R_2 = 4-cyano benzyl, R_3 = F
14j: R_1 = H, R_2 = 4-cyano benzyl, R_3 = methyl
14k: R_1 = CF_3, R_2 = 4-cyano benzyl, R_3 = NO_2
14l: R_1 = CF_3, R_2 = 4-cyano benzyl, R_3 = H
14m: R_1 = CF_3, R_2 = 4-cyano benzyl, R_3 = F
14n: R_1 = CF_3, R_2 = 4-cyano benzyl, R_3 = methyl

Scheme 4

Highlights

- 36 New Phenothiazine incorporated 1,2,3-triazole hybrids are synthesized.
- Click chemistry protocol is followed for the synthesis.
- 19 Compounds exhibit potent antiTB activity (MIC = 1.6 μg/mL).
- Compounds are non-toxic to normal cells.
- Compounds exhibit strong binding interactions with target enzymes, InhA and CYP121.