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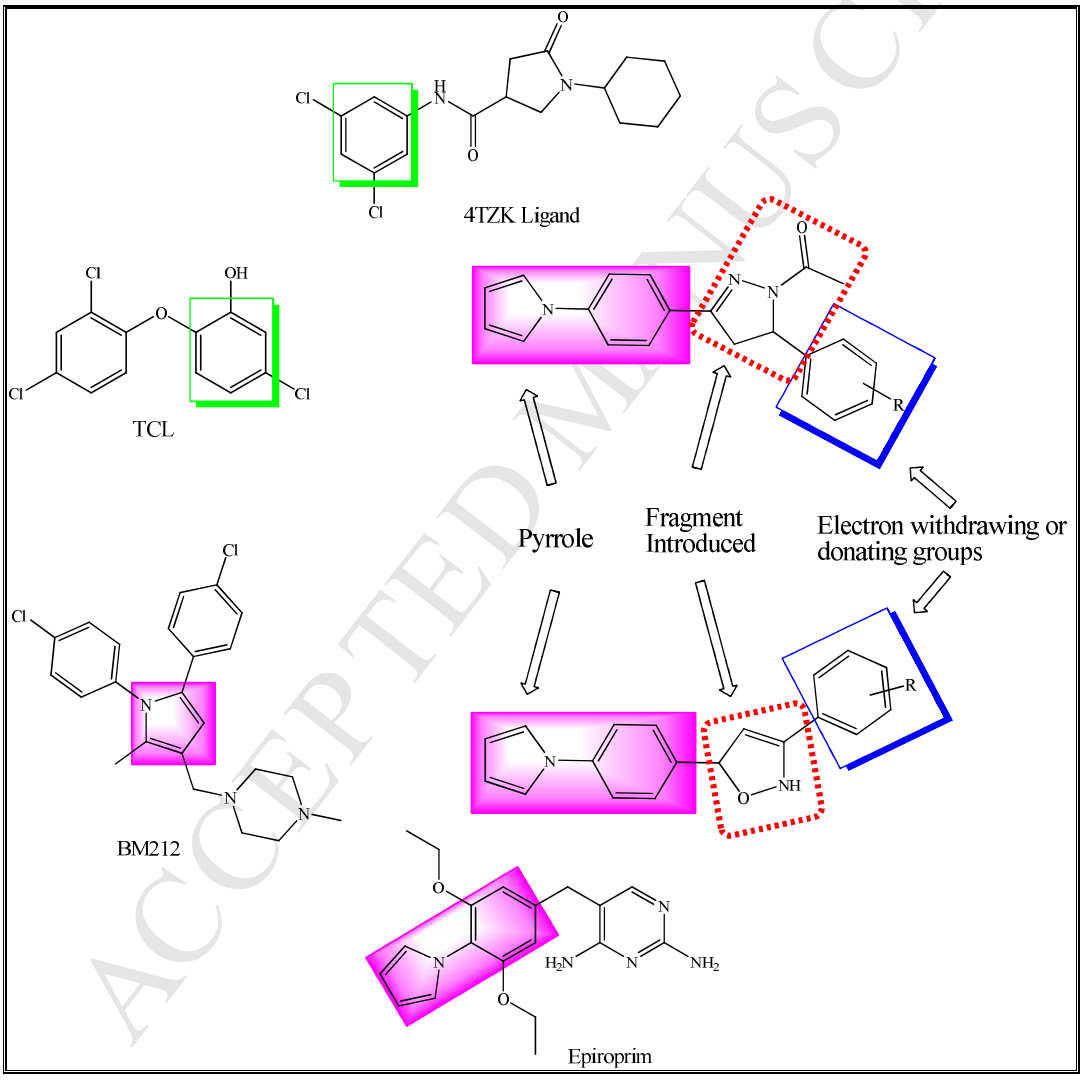
Antitubercular activity of novel series of pyrrolyl chalcone, pyrazline, isoxazole and phenyl urea derivatives was analyzed. Molecular modeling constructed using Surflex-Dock study using enoyl ACP reductase from *Mycobacterium tuberculosis.*



Synthesis, antimycobacterial screening and ligand-based molecular docking studies on novel pyrrole derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties

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** Authors dedicate this work on the eve of retirement of Dr. K. V. S. N. Raju after 37 years of service at IICT, Hyderabad

Abstract:

We report here the synthesis, antibacterial and antitubercular evaluation of 61 novel pyrrolyl derivatives bearing pyrazoline, isoxazole and phenyl thiourea moieties. Molecular docking was carried out on enoyl ACP reductase from *M. tuberculsosis* using Surflex-Dock, which is one of the key enzymes involved in type II fatty acid biosynthetic pathway of *M. tuberculosis*, an attractive target for designing novel antitubercular agents. Docking analysis of the crystal structure of ENR performed using Surflex-Dock in Sybyl-X 2.0 software indicates the occupation of substituted pyrrolyl derivatives into hydrophobic pocket of InhA enzyme. Compounds **9b** and **9d** exhibited the highest antitubercular activity almost close to isoniazid (0.4 µg/mL) with a MIC value of 0.8 µg/mL. All other compounds showed the good activity with a MIC value of 6.25-100 µg/mL. The compounds were further tested for mammalian cell toxicity using human lung cancer cell-line (A549) and were nontoxic. Some compounds exhibited inhibition activities against InhA.

Keywords: Surflex docking, Pyrrolyl chalcones, Pyrrolyl isoxazoles, Pyrrolyl pyrazolines, Anti-tubercular activity, Cytotoxicity activity, Enzyme inhibition studies.

1. Introduction

Tuberculosis (TB) is a chronic disorder caused by five closely related mycobacteria such as *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti and Mycobacterium canetti*. Among these, *Mycobacterium tuberculosis* (*M. tuberculosis*), is an infection caused by slow-growing bacteria in parts of the body having high level of blood and oxygen is often found in lungs, called pulmonary TB. The disease also spreads to other parts of the body, called as extra-pulmonary TB that may be latent or active. In other case, treatment of the active TB is more complex due to multi-drug resistance (MDR-TB), extensive-drug resistance (XRD-TB) and HIV infection. The MDR-TB is a type of TB which occurs once MTB strain turns resistant to the most efficient anti-TB drugs i.e. rifampin and isoniazid. In 2013, 0.45 million people developed MDR-TB worldwide and there were 0.21 million deaths resulting from MDR-TB. XDR-TB occurs when MTB strain is resistant to at least isoniazid and rifampin in addition to being resistant to one of the fluoroquinolones, as well as resistant to at least one of the second line injectable drugs i.e. amikacin, kanamycin or capreomycin. XDR-TB was found worldwide in 100 countries by the end of 2013. About, 9% of MDR-TB cases lead to XDR-TB which is related with higher mortality rate than MDR-TB. World Health Organization (WHO) estimated 9.0 million people with cases of TB in 2013 of which 1.5 million died and 360,000 of them were affected with HIV-positive. TB is a foremost public health problem in India. India accounts for one-fifth of the world TB incident cases. Every year about 2 million people in India develop TB, of which around 0.87 million are infectious cases. Furthermore, it is estimated that yearly around 330,000 Indians die due to TB. The MTB generally attacks the lungs, spine, kidney, and brain. Therefore, if TB is not treated properly, it can be severe and fatal.

Over the past decades, several anti-tubercular (anti-TB) drugs have been developed see Fig. 1, but drug-resistance issue has not been solved. There is thus a tremendous need to develop

new anti-TB drugs that are active against both acute and chronic growth phases of mycobacterium to stop all forms of drug resistant-TB [1, 2]. In this perspective, many studies have been made on targeting the cell wall of mycobacteria.

Mycolic acid biosynthesis has been carried out [2] by numerous successive enzymatic cycles equivalent to Fatty Acid Synthase (FAS) systems viz., FAS I and II. Mycolic acid is a unique signature fatty acid, which is a core constituent of the mycobacterial cell wall present in fatty acid synthase system of *M. tuberculosis.* InhA, the enoyl acyl carrier protein reductase (ENR) from *M. tuberculosis* is the key enzyme for type II fatty acid synthesis (FAS II), which catalyses NADH-dependent reduction of 2-trans-enoyl-ACP (acyl carrier protein) to yield NAD+ and reduced enoyl thioester-ACP substrate, which in turn, helps the synthesis of mycolic acid.

Chalcone is a central core for many important biological compounds that are synthesized by aldol condensation reaction between substituted aryl ketones and aromatic aldehydes in the presence of sodium hydroxide as a catalyst. These undergo variety of chemical reactions to produce innumerable heterocyclic compounds that are used as intermediates to prepare drugs with therapeutic value. Literature reveals that chalcone derivatives from natural and synthetic analogs exhibit diverse pharmacological activities such as anti-TB, anti-inflammatory, anti-cancer, anti-neoplastic, anti-bacterial, anti-fungal, anti-malarial, anti-viral, anti-allergic and estrogenic [3-9]. On the other hand, isoxazole derivatives constitute a class of nitrogen and oxygen containing five membered heterocyclic compounds that are the important class of heterocyclic pharmaceuticals due to their wide spectrum of biological activities, including potent and selective antagonism of NMDA receptor [10], anti-HIV activity [11], anti-tuberculosis, anti-bacterial, antibiotic, anti-fungal, anti-cancer, ulcerogenic activities and also used as COX-2 inhibitors and anti-inflammatory drugs [12-16].

Pyrazolines have been widely used as anti-tubercular [17], anti-bacterial [18] and anti-cancer [19] agents, as these have a broad spectrum antimicrobial activity and hence, can be explored further. The most prominent compounds featuring pyrazoline nucleus are econazole, ampyrone, phenazone and propylphenzone see Fig. 2a. Some of the reported pyrazoline skeletals which exhibit anti-tubercular activity are shown in Fig. 2b [20-23].

Pyrrole is an important heterocycle of the plant and animal kingdom as a subunit of chlorophyll in plant cells, hemin and vitamin B_{12} in animal cells. First isolated in 1857 from the products of bone pyrolysis, it showed biological activities that are characteristic of haemoglobin [24], Earlier, pyrrole derivatives have shown *in vitro* anti-tubercular activity [25, 26] and recently, much of the research was carried out on anti-TB drug design using pyrroles as templates for synthesis [27, 28], including molecular modeling along with the laboratory investigations [29-31]. Biava et al [32] reported several 1,5-diarylpyrrole derivatives with a very good activity against MTB (BM 212). Based on the work of Deidda et al. [33], Lupin developed a series of pyrrole compounds, of which LL3858 is currently in pre-clinical trials for the treatment of TB [34], suggesting the importance of pyrrole derivatives as the anti-TB agents. This prompted us to undertake detailed investigation on the design and synthesis of new pyrrole derivatives useful as anti-TB agents.

In our previous studies [35, 36], we have synthesized the potential inhibitors of InhA bearing pyrrole as a central core with different pharmacophores in a single molecular framework along with 2D and 3D-QSAR studies. In this work, we have undertaken to develop new chemical entities containing pyrrole as the core that inhibits enoyl ACP reductase enzyme along with their *in vitro* anti-bacterial and anti-TB activities. Fig. 2 represents some of the marketed drugs that were considered in the synthesis of new derivatives following the *Paal-Knorr* pyrrole synthesis. Fig. 3 indicates the design concept used to describe our framework by combining molecular docking and classification techniques to understand the structural

characteristics affecting the binding of pyrrolylchalcones, pyrazolines and isoxazoles with enoyl ACP reductase receptor. Molecular docking studies have been used to correlate in *silico* results with *in vitro* analysis to find the ENR as a potential target of pyrrolylchalcone, pyrazoline and isoxazole derivatives.

2. Molecular modeling/docking studies

The 3D structures were generated using SYBYL package (Tripos Associates, St. Louis, MO, USA) [37]. The geometry optimization was done with the help of standard Tripos force field [38] using a distance dependent-dielectric function, energy gradient of 0.001 kcal/ mol and MMFF94 as the electrostatics. Conformational analyses of all the 61 compounds were performed using a repeated molecular dynamics-based simulated annealing approach as implemented in Sybyl-X 2.0. The molecule was heated up to 1000 K within 2000 fs, held at this temperature for 2000 fs and annealed to 0 K for 10,000 fs using an exponential annealing function. By employing this procedure, 100 conformations were sampled out during 100 cycles to account the conformational flexibility to find the most likely conformations occurring most often in the resulting pool. All conformations were minimized with Tripos force field and atomic charges were calculated using MMFF94 (Merck Molecular Force Field) method.

2.1. Molecular docking using Surflex-Dock

Molecular docking was used to clarify the binding mode of the compounds to provide straightforward information for further structural optimization. Surflex-Dock that adopted an empirical scoring function and a patented searching engine [39, 40] was employed for molecular docking. The crystal structure of *M. tuberculosis* enoyl reductase (InhA) complexed with 1-cyclohexyl-*N*-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide (PDB ID 4TZK, 1.62 Å X-ray resolution) was extracted from the Brookhaven Protein Database (PDB http://www.rcsb.org/pdb). During the docking process, water molecules and all the ligands in the crystal structures were removed (except co-factor NAD^+). The polar hydrogens as well as united atom Amber7 FF99 were assigned for the protein PDB ID 4TZK. Then, ligand-based mode was adopted to generate the “protomol”, leaving the threshold and bloat

parameters at their default values of 0.50 and 0 Å; all the inhibitors were docked within the prepared protein.

The mode of interaction of the relative ligand in the crystal structure against 4TZK PDB was used as a standard docked model. The maximum number of poses per ligand was set to 20 with no constraints to perform the molecular docking. The docking complex was assumed to represent the ligand-receptor interactions, which was selected based on three criteria: (i) docking score of the pose possessed the highest docking score, (ii) its orientation of aromatic rings of the ligand oriented into the active site in a similar manner with the cocrystallized ligands orientation, and (iii) preservation of the two key interactions viz., H- bonds with Tyr158 and Co-factor NAD^+. For comparative analysis of the designed molecules, D_score [41], PMF_score [42], G_score [43] and Chem_score [44] were estimated using the C-Score module of the Sybyl-X 2.0.

3. Results and discussion

3.1. Chemistry

All the compounds were synthesized as per steps outlined in Schemes 1, 2 and 3. The *Paal-Knorr* reaction was performed to synthesize (4-pyrrol-1-yl)acetophenone (**2**) by condensing 4-amino acetophenone (**1**) with 2,5-dimethoxytetrahydrofuran. The required key intermediates viz., chalcones **(3a-r)** were obtained by Claisen-Schmidt condensation of (4-pyrrol-1-yl)acetophenone (**2**) with the substituted aldehydes in the presence of sodium hydroxide catalyst in ethanol. Chalcones **(3a-r)** were treated with hydrazine hydrate and glacial acetic acid in a solvent free condition to obtain the corresponding *N*-acetyl pyrazolines **(4a-s)**. The reaction of chalcones with hydroxylamine hydrochloride and sodium acetate in the presence of glacial acetic acid led to the synthesis of 5-(4-(1*H*-pyrrol-1yl)phenyl)-3-substituted phenylisoxazoles **(5a-r)** as per schemes 1 and 2.

o-Phenylenediamine (**6**) reacted with acetonyl acetone (**7**) to afford 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)aniline (**8**). Next, different phenylisothiocyanates were reacted with intermediate **8** in dry chloroform to get the final desired 1-(2-(2,5-dimethyl-1*H*-pyrrol-1-yl)phenyl)-3-(substituted phenyl)thioureas **(9a-k)** with good yields (Scheme 3).

All the synthesized compounds were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. FTIR spectrum of the compound **3e,** as a representative of chalcone series, showed an absorption band at 1657 cm^{-1} associated with carbonyl group from the α, β-unsaturated fragment. In the ^{1}H NMR spectrum of compound **3e**, a singlet at δ 3.74 ppm indicates the presence of methoxy group, two doublets at δ 7.98 and 8.00 ppm are assigned to H_α and H_β, respectively for vinylic system. Protons of pyrrole moiety resonated as two triplets at δ 6.29 and 7.07 ppm, respectively. The ^{13}C NMR spectrum of compound **3e** showed the signal at δ 188.92 ppm corresponding to carbonyl group of α, β-unsaturated fragment. The signal corresponding to methoxy group appears at δ 55.45 ppm. All the other aromatic

carbons resonated in the expected region of δ 111.55-144.74 ppm. The molecular ion peak at *m/z* 288.13 (100%) (EI-MS) confirmed the formation of the desired product.

FTIR spectrum of pyrazoline (Compound **4g**) showed absorption bands at 1661 (C=O) and 1609 cm^{-1} (C=N). Formation of pyrazoline was further confirmed by ^{1}H NMR, wherein CH_2 protons of pyrazoline ring resonated as a pair of doublets of doublets at δ 3.17 ppm (H_a) and δ 3.79 ppm (H_b) with J_{AB} = 22.2-29.5 Hz. The –CH(H_x) proton appeared as a doublet of doublets at δ 5.60 ppm due to vicinal coupling with two magnetically non-equivalent protons of methylene group at position 4 of the pyrazoline ring with a J_{AX} value of 16.44 Hz. A singlet at δ 2.42 ppm was assigned to methyl protons of acetyl group (-$COCH_3$). The ^{13}C NMR data of the compound **4g** supported the structure via $\underline{CO}CH_3$ and $CO\underline{CH_3}$ resonances appearing at δ 168.86 and 21.92 ppm, respectively. Furthermore, peaks observed at δ 152.94, 59.35 and 42.24 ppm are due to C_1, C_4 and C_5 of pyrazoline, respectively. The mass spectrum of **4g** showed a molecular ion peak at *m/z* 347 (80%), which confirmed its molecular weight.

The ^{1}H NMR spectrum of **5e** showed a doublet at δ 6.72 ppm (J = 16 Hz) due to C_4 proton of isoxazole. The structure of **5e** was further confirmed by ^{13}C NMR, which displayed signals at δ 159.29, 110.54 and 156.22 ppm due to C_3, C_4 and C_5 carbons of isoxazole ring, respectively. All the other carbons resonated in the expected regions. The mass spectrum of **5e** exhibited molecular ion peak at *m/z* 317 (100%) confirming its molecular weight.

FTIR spectrum of **9c** displayed stretching bands at 3304 and 3170 cm^{-1} due to NH group. The ^{1}H NMR spectrum of this sample displayed a singlet at δ 1.81 ppm due to methyl protons of pyrrole, while a singlet at δ 5.81 ppm was due to C_3 and C_4 protons of pyrrole. Two singlets at δ 8.56 and 8.28 ppm were assigned to two NH protons. The ^{13}C NMR data of **9c** also supported the structure, which displayed the peaks at δ 12.38 ppm due to two methyl carbons of pyrrole. The C=S resonance appeared at δ 178.45 ppm. The mass spectrum of **9c** showed a

molecular ion peak at *m/z* 339 (80%), which confirmed its molecular weight. Various compounds synthesized with their data are indicated in the experimental section.

3.2. Antitubercular and antibacterial activities

The anti-TB activity of the compounds was studied with *M. tuberculosis* (see Table 1). The preliminary anti-TB screening revealed that majority of compounds showed quite moderate to good activity. The activities of **3a-r**, **4a-q**, **5a-o** and **9a-k** are expressed in terms of minimum inhibitory concentration (MIC) values using ethambutol and rifampicin as the reference drugs. In the first series of compounds, **4d**, **4e**, **4m, 4p, 4q** and **5m** showed better activity with the inhibition of mycobacterium at MIC of 6.25 µg/mL, while those from the third series of compounds **9b** and **9d** showed the highest activity with MIC value of 0.8 µg/mL. A good anti-TB activity is due to the presence of pharmacologically active hetero-aryl groups such as pyrazoline, isoxazole and aromatic ring with -NH-CS-NH-linkage attached to pyrrole ring. The pyrazoline derivatives displayed relatively higher inhibitory activity compared to chalcones and isoxazoles. It is encouraging to observe that compounds **9b** and **9d** showed very good anti-TB activities against MTB strain (MIC=0.8 µg/mL), while **9c** showed the promising activity (MIC=1.6 µg/mL).

Antibacterial activity was also carried out for all the compounds against both Gram positive bacteria (*S. aureus*) and Gram negative bacteria (*E. Coli*). The antimicrobial screening data revealed that all the compounds showed moderate to significant microbial inhibition. Among all the compounds tested, compounds **9a-k** showed excellent activity with the MIC values of 0.2-1.6 µg/mL. Compounds **3a-r**, **4a-q**, **5a-o** exhibited good to moderate antimicrobial activity with the MIC values of 6.25-100 µg/mL.

Among the compounds tested (Table 1), compounds **9d** and **9b** bearing *para*-trifluoro and *ortho*-fluoro substitution respectively, on the aryl ring gave the best MIC. Clearly the fluoro substitution at the aryl ring, which is more electro-negative than other halogens like Cl and

Br, is important for activity compared to other molecules in the series. The presence of chloro or bromo substitution at 3rd or 4th position on the aryl ring shows comparatively less activity. The lack of inhibition for these compounds on InhA in contrast with low MIC suggests that the target is not InhA.

The presence of trifluoro substitution at 4th position of aryl ring resulted in low MIC values. Compound **9j** bearing chloro substitution exhibit lower MIC (6.25 µg/mL) by comparison with compound **9d**, which is a non-chloro derivative (MIC 0.8 µg/mL). Therefore we believe that some of these derivatives might have biological target other than InhA and a mode of action different than triclosan thereby making them relevant candidates for further drug design in MDR-TB research. In general, these compounds are not only active on InhA but also on *M. tuberculosis* H37Rv strain.

3.3.MTT-based cytotoxicity studies

Certain therapeutic properties are to be identified to show the antimycobacterial potential of a drug. Toxicity is one of these criteria. Hence, we have investigated the potential toxicity of eight selected pyrrolyl derivatives (**3d**, **3n**, **4m**, **4e**, **5m**, **5e**, **9b** and **9d**) towards A549 (lung adenocarcinoma) cell-lines up to concentrations of 62.5 µg/mL. These compounds showed a moderate cytotoxicity compared to cisplatin (Table 2). Specifically, the most potent compounds viz., **4m** and **5m** exhibited a good safety profile as their IC_{50} value was 39.6 µmol/L against the A549 cell-line.

3.4.Enzyme inhibition studies

By considering *in vitro* antimycobacterial studies, we have selected four compounds for *in vitro* enzyme inhibition activity against InhA from the *M. tuberculosis* at 50 µM by applying the commonly used approach. Triclosan was tested first at the same concentration and showed complete inhibition of InhA at 50 µM. The results are shown in Table 3.

The compounds bearing chlorine atom (compound **9h**) on the aromatic ring are very week (or not) inhibitors of InhA. In fact it doesn't showed any inhibition at 50 µM. but the 2,4-dichloro substituted analogue **9j** shows a 13% inhibition at 50 µM. Based on previous reported research's, we hypothesized that substitution of highly electro-negative groups mimic the InhA substrate might provide compounds with higher affinity. The introduction of lipophilic groups like bulkier groups or halogen might facilitate more hydrophobic interaction in the pocket. The result shows an increase in the inhibitory activity (Table 3). Compound **9d** bearing trifluoro substitution by in comparison with **9j** presents 100% inhibition at 50 µM.

3.5.Molecular docking studies

To investigate the mechanism of anti-TB activity and detailed intermolecular interactions between the synthesized compounds, molecular docking studies were performed on the crystal structure of *M. tuberculosis* enoyl reductase (InhA) complexed with 1-cyclohexyl-*N*-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide (PDB ID 4TZK, 1.62 Å X-ray resolution) using the surflex-dock programme of sybyl-X 2.0 software. On the basis of greater level of resistance associated with INH isolates against InhA, docking studies were performed on InhA complex with 1-cyclohexyl-*N*-(3,5-dichlorophenyl)-5-oxopyrrolidine-3-carboxamide, which indicates the presence of drug-receptor interactions. All the 61 inhibitors were docked into the active site of ENR as shown in Fig 4A and 4B. The predicted binding energies of the compounds are listed in Table 4.

The interaction of compound **4q** with the enzyme depicted in Fig. 6A and 6B shows that oxygen of methoxy group at the 4th position of phenyl ring attached to pyrazoline moiety of compound **4q** has one H-bonding interaction with the hydrogen of MET98 (-$\underline{O}CH_3$ ------ H-MET98). As depicted in Fig. 7A and 7B, the fluorine atom of CF_3 group of compound **9d** exhibited H-bonding interaction with the hydrogen of MET98 (C-F ---- H-MET98; 2.49 Å). In Fig. 8A and 8B, the oxygen and nitrogen of isoxazole ring (compound **5g**) makes two H-

bonding interactions with the hydrogen of MET98 (O ----- H-MET98; 2.49 Å and N ----- H-MET98; 1.90 Å), while fluorine atom makes an H-bonding interaction with GLN100 (F ------ HE-GLN100; 2.40 Å). On the other hand, hydrophobic (Ile105, Leu207, Met103, Trp160, Ala206, Met161, Met98, Pro99, Ala157, Ala211, Pro156, Ala201, Ile202, Val203, Ile215, Leu218, Leu217, Phe97, Ile122, Val65, Ile16, Ile95, Ala198, Leu197, Met199, Phe149, Pro193, Ile194, Ala191, Ile21, Met147, Trp222, Trp230) and hydrophilic (Asp148, Ser94, Gly192, Asp150, Gly14, Lys165, Ser20, Ser19, Thr196, Arg195, Glu219, Gly96, Thr162, Ser123, Gly119, Asp64, Tyr158, Asn159, Thr196, Arg195, Glu219, Gln216, Ser200, Gln214, Gly205, Gly104, Gln100, Gly102, Asn159, Tyr158, Gly119) amino acid residues are surrounded to the representative compound **4q** are depicted in the Figure 9A and 9B.

All the compounds showed consensus score in the range 7.51-1.60, indicating the summary of all forces of interaction between ligands and the InhA. Charge and van der Waals interactions between protein and ligands varied from -75.28 to -180.16. The Helmholtz free energies of interactions for protein ligands atom pairs range between -16.05 and 88.56 However, its H-bonding, complex (ligand-protein), and internal (ligandeligand) energies range from -113.69 to -323.98, while those values -26.14 to -49.79 indicate the ligands due to H-bonding, lipophilic contact, and rotational entropy, as well as intercept terms. These scores indicate that molecules preferentially bind to InhA in comparison to the reference 4TZK ligand (Table 4). In general, it was observed that -OCH_3 and C=O groups make the H-bond with a substrate binding site and presence of electron donating or withdrawing substitution on the aromatic ring attached to pyrazoline/isoxazole moiety may favours the activity, while those of pyrrole, pyrazoline, isoxazole and phenylthiourea moieties help to occupy or penetrate the molecule at the active sites.

4. Experimental section

Melting points were determined using Shital-digital programmable melting point apparatus and are uncorrected. FTIR spectra in KBr pellets were recorded on a Bruker FTIR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE II at 400 and 100/75 MHz, respectively; chemical shifts are expressed in parts per million (δ ppm) relative to TMS. The abbreviations used to describe the peak patterns are: (b) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet.

Mass spectra (MS) were recorded in a JEOL GCMATE II GC-Mass spectrometer and Schimadzu QP 20105 GC-Mass spectrometer. Elemental analysis data (performed on Leco Tru Spec CHNS Analyzer) for C, H, and N were all within $\pm$ 0.4% of the theoretical values. Analytical thin-layer chromatography (TLC) was performed on the precoated TLC sheets of silica gel 60 F_{254} (Merck, Darmstadt, Germany) visualized by long- and short-wavelength UV lamps. Chromatographic purifications were performed on Merck aluminium oxide (70-230 mesh) and Merck silica gel (70-230 mesh).

*4.1. General procedure for the synthesis of 4-(1-H-pyrrol-1-yl)-acetophenone (**2**)* [45]

A mixture of 2,5-dimethoxytetrahydrofuran (4.23 g, 0.032 mol) and 4-aminoacetophenone (4.05 g, 0.030 mol) taken in glacial acetic acid (12 mL) was refluxed for 1 h, poured into ice cold water and basified with $NaHCO_3$ solution. The solid separated was washed with water, dried and recrystallized from ethanol.

*4.2.General procedure for the synthesis of 1-(4-(1H-pyrrol-1yl)phenyl)-3-substitutedprop-2-en-1-ones (**3a-r**)*

To a mixture of 4-(1-*H*-pyrrol-1-yl)-acetophenone (0.01 mol) and substituted aldehydes (0.01 mol) in ethanol (20 mL), a solution of sodium hydroxide (40%, 8 mL) was added slowly. The mixture was stirred for 24-30 h, poured into ice-cold water, and neutralized with hydrochloric acid. The solid separated was filtered off, washed with water, dried and purified

by column chromatography on silica gel with ethyl acetate/petroleum ether (6:4) as the eluent.

*4.2.1. 1-(4-(1H-pyrrol-1yl)phenyl)-3-phenylprop-2-en-1-one (**3a**)*

(Yield 70%). mp 255-257 ^{o}C; FTIR (KBr): 1653.95 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.40 (t, 2H, pyrrole-C_3, C_4-H), 7.19 (t, 2H, pyrrole-C_2, C_5-H), 7.40-7.44 (m, 3H, phenyl-C_3, C_4, C_5-H), 7.49-7.53 (m, 2H, phenyl-C_2, C_6-H), 7.67 (q, 2H, bridging phenyl-C_2, C_6-H), 7.86 (d, 2H, *J* =16 Hz, bridging phenyl-C_3, C_5-H), 8.09-8.13 (m, 2H, -CH=CH-); MS (ESI): *m/z* = found 273.12 [M^{+}]; calcd. 273.12. Anal. Calcd. For $C_{19}H_{15}NO$: C, 83.49; H, 5.53; N, 5.12. Found: C, 84.60; H, 6.32; N, 6.33.

*4.2.2. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(4-chlorophenyl)prop-2-en-1-one (**3b**)*

(Yield 70%). mp 208-210 ^{o}C; FTIR (KBr): 1655.83 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.34 (dd, 2H, pyrrole-C_3, C_4-H), 7.39 (dd, 2H, pyrrole-C_2, C_5-H), 7.42-7.95 (m, 8H, 4-chlorophenyl-C_2, C_3, C_4, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.23 (d, 2H, *J* =8 Hz, -CH=CH-); MS (ESI): *m/z* = found 308.08 [M^{+} + 1]; calcd. 307.08. Anal. Calcd. For $C_{19}H_{14}ClNO$: C, 74.15; H, 4.58; N, 4.55. Found: C, 75.75; H, 5.32; N, 5.33.

*4.2.3. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2-chlorophenyl)prop-2-en-1-one (**3c**)*

(Yield 65%). mp 103-105 ^{o}C; FTIR (KBr): 1656.02 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.41 (dd, 2H, pyrrole-C_3, C_4-H), 7.16-7.20 (m, 2H, pyrrole-C_2, C_5-H), 7.30-7.37 (m, 2H, 2-chlorophenyl-C_4, C_5-H), 7.43-8.13 (m, 6H, 2-chlorophenyl-C_3, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.22 (d, 2H, *J* =16 Hz, -CH=CH-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm:111.62, 119, 119.44, 124.40, 127.07, 127.79, 130.31, 130.49, 131.20, 133.21, 134.65, 135.50, 140.62, 143.94, 188.80; MS (ESI): *m/z* = found 308.08 [M^{+} + 1]; calcd. 307.08. Anal. Calcd. For $C_{19}H_{14}ClNO$: C, 74.15; H, 4.58; N, 4.55. Found: C, 75.75; H, 5.32; N, 5.33.

*4.2.4. 1-(4-(1H-pyrrol-1yl)phenyl)-3-p-tolylprop-2-en-1-one (**3d**)*

(Yield 70%). mp 188-190 ^{o}C; FTIR (KBr): 1656.63 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.39 (s, 3H, -C<u>H</u>$_3$), 6.40 (t, 2H, pyrrole-C_3, C_4-H), 7.19 (t, 2H, 4-methylphenyl-C_3, C_5-H), 7.25 (t, 2H, pyrrole-C_2, C_5-H), 7.48-7.84 (m, 6H, 4-methylphenyl-C_2, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.09-8.12 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.58, 111.59, 119.07, 119.48, 120.62, 128.55, 129.76, 130.35, 132.14, 135.16, 141.21, 143.81, 144.99, 188.99; MS (ESI): *m/z* = found 288.13 [M^+ + 1]; calcd. 287.13. Anal. Calcd. For $C_{20}H_{17}NO$: C, 83.59; H, 5.96; N, 4.87. Found: C, 84.75; H, 6.32; N, 6.33.

*4.2.5. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (**3e**)*

(Yield 80%). mp 168-170 ^{o}C; FTIR (KBr): 1657.47 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.74 (s, 3H, -OC<u>H</u>$_3$), 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.84 (d, 2H, *J* =8 Hz, 4-methoxyphenyl-C_3, C_5-H), 7.07 (t, 2H, pyrrole-C_2, C_5-H), 7.30-7.72 (m, 6H, 4-methoxyphenyl-C_2, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.00 (dd, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.45, 111.55, 111.47, 119.47, 127.61, 130.28, 130.30, 135.31, 143.72, 144.74, 161.76, 188.92; MS (ESI): *m/z* = found 304.13 [M^+ + 1]; calcd. 303.13. Anal. Calcd. For $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 80.75; H, 6.32; N, 6.33.

*4.2.6. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(3-bromophenyl)prop-2-en-1-one (**3f**)*

(Yield 60%). mp 158-160 ^{o}C; FTIR (KBr): 1657.46 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.41-6.44 (m, 2H, pyrrole-C_3, C_4-H), 7.18-7.84 (m, 10H, pyrrole-C_2, C_5-H; bridging phenyl-C_2, C_3, C_5, C_6 and 3-bromophenyl-C_2, C_4, C_5, C_6-H), 8.12-8.16 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.66, 119.44, 122.74, 123.08, 127.29, 130.40, 130.47, 130.78, 133.27, 134.59, 136.94, 142.93, 144, 188.35; MS (ESI): *m/z* = found 352.03 [M^+ + 1]; calcd. 351.03. Anal. Calcd. For $C_{19}H_{14}BrNO$: C, 64.79; H, 4.01; N, 3.98. Found: C, 66.15; H, 5.32; N, 5.33.

*4.2.7. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(4-fluorophenyl)prop-2-en-1-one (**3g**)*

(Yield 70%). mp 198-200 ^{o}C; FTIR (KBr): 1654.24 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.31 (t, 2H, pyrrole-C_3, C_4-H), 7.22 (q, 2H, 4-fluorophenyl-C_3, C_5-H), 7.43 (t, 2H, pyrrole-C_2, C_5-H), 7.71 (t, 2H, 4-fluorophenyl-C_2, C_6-H), 7.74-7.91 (m, 4H, bridging phenyl-C_2, C_3, C_5, C_6-H), 8.22 (d, 2H, *J*=8Hz, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.62, 116.01, 116.30, 119.01, 119.45, 121.28, 130.31, 130.34, 130.43, 131.11, 134.84, 143.55, 143.91, 162.42, 188.68; MS (ESI): *m/z* = found 292.11 [M^+ + 1]; calcd. 291.11. Anal. Calcd. For $C_{19}H_{14}FNO$: C, 78.33; H, 4.84; N, 4.81. Found: C, 79.75; H, 5.32; N, 5.33.

*4.2.8. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2-bromophenyl)prop-2-en-1-one (**3h**)*

(Yield 60%). mp 214-216 ^{o}C; FTIR (KBr): 1656.29 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.38-6.41 (m, 2H, pyrrole-C_3, C_4-H), 7.15-7.20 (m, 2H, pyrrole-C_2, C_5-H), 7.24-8.17 (m, 10H, 2-bromophenyl C_3, C_4, C_5, C_6-H; bridging phenyl-C_2, C_3, C_5, C_6-H and -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.62, 119, 119.42, 124.66, 125.89, 127.70, 127.87, 130.51, 131.35, 133.56, 134.60, 135.01, 143.19, 143.94, 188.82; MS (ESI): *m/z* = found 352.03 [M^+ + 1]; calcd. 351.03. Anal. Calcd. For $C_{19}H_{14}BrNO$: C, 64.79; H, 4.01; N, 3.98. Found: C, 66.15; H, 5.32; N, 5.33.

*4.2.9. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(3,4-dimethoxyphenyl)prop-2-en-1-one (**3i**)*

(Yield 78%). mp 183-185 ^{o}C; FTIR (KBr): 1657.02 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 3.94 (s, 3H, -OC<u>H</u>$_3$), 3.96 (s, 3H, -OC<u>H</u>$_3$), 6.41 (t, 2H, pyrrole-C_3, C_4-H), 6.92 (d, 1H, *J*= 8 Hz, methoxyphenyl-C_5-H), 7.17-7.20 (m, 2H, methoxyphenyl-C_2, C_6-H), 7.24-7.27 (m, 2H, pyrrole-C_2, C_5-H), 7.50-7.53 (d, 2H, bridging phenyl-C_2, C_6-H), 7.82 (d, 2H, *J*= 16 Hz, phenyl-C_3, C_5-H), 8.09-8.13 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.93, 55.96, 110.04, 111.07, 111.50, 119.38, 119.48, 123.18, 127.76, 130.23, 135.18, 143.65, 144.98, 149.20, 151.44, 188.86; MS (ESI): *m/z* = found 334.14 [M^+

+ 1]; calcd. 333.14. Anal. Calcd. For $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 76.75; H, 6.32; N, 5.33.

*4.2.10. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2,4-dichlorophenyl)prop-2-en-1-one (**3j**)*

(Yield 70%). mp 192-194 ^{o}C; FTIR (KBr): 1655.78 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.41 (dd, 2H, pyrrole-C_3, C_4-H), 7.20 (dd, 2H, pyrrole-C_2, C_5-H), 7.32 (dd, 1H, chlorophenyl-C_5-H), 7.47-7.53 (m, 4H, chlorophenyl-C_3, C_6-H and bridging phenyl-C_2, C_6-H), 7.71 (d, 2H, J= 8 Hz, phenyl-C_3, C_5-H), 8.08-8.12 (m, 2H, -CH=CH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.69, 118.97, 119.42, 124.56, 127.54, 128.48, 130.14, 130.47, 131.78, 134.46, 136.07, 136.49, 139.29, 144.03, 188.42; MS (ESI): *m/z* = found 342.04 [M^+ + 1]; calcd. 341.04. Anal. Calcd. For $C_{19}H_{13}Cl_2NO$: C, 66.68; H, 3.83; N, 4.09. Found: C, 67.75; H, 4.32; N, 5.33.

*4.2.11. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2,3-dichlorophenyl)prop-2-en-1-one (**3k**)*

(Yield 67%). mp 157-159 ^{o}C; FTIR (KBr): 1651.62 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.39-6.41 (m, 2H, pyrrole-C_3, C_4-H), 7.20 (t, 2H, pyrrole-C_2, C_5-H), 7.29 (t, 1H, chlorophenyl-C_5-H), 7.46-7.54 (m, 3H, chlorophenyl-C_6-H and bridging phenyl-C_2, C_6-H), 7.67 (dd, 1H, chlorophenyl-C_4-H), 8.09-8.21 (m, 4H, bridging phenyl-C_3, C_5-H and -CH=CH-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.70, 118.99, 119.44, 125.47, 125.89, 127.38, 130.02, 130.52, 131.65, 134.40, 135.60, 140.49, 144.06, 188.48; MS (ESI): *m/z* = found 342.04 [M^+ + 1]; calcd. 341.04. Anal. Calcd. For $C_{19}H_{13}Cl_2NO$: C, 66.68; H, 3.83; N, 4.09. Found: C, 67.75; H, 4.32; N, 5.33.

*4.2.12. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2,6-dichlorophenyl)prop-2-en-1-one (**3l**)*

(Yield 67%). mp 136-140 ^{o}C; FTIR (KBr): 1662.12 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.41 (t, 2H, pyrrole-C_3, C_4-H), 7.20 (t, 2H, pyrrole-C_2, C_5-H), 7.24 (d, 1H, J=8 Hz, chlorophenyl-C_4-H), 7.41 (d, 2H, J= 8 Hz, chlorophenyl-C_3, C_5-H), 7.50-7.53 (m, 2H, bridging phenyl-C_2, C_6-H), 7.71 (d, J=16 Hz, 1H, bridging phenyl-C_3-H), 7.91 (d, 1H, J=16

Hz, bridging phenyl-C_5-H), 8.13 (m, 2H, -C<u>H</u>=C<u>H</u>); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.67, 118.99, 119.45, 128.86, 130.61, 134.40, 135.19, 137.80, 144.06, 188.57; MS (ESI): *m/z* = found 342.04 [M^+ + 1]; calcd. 341.04. Anal. Calcd. For $C_{19}H_{13}Cl_2NO$: C, 66.68; H, 3.83; N, 4.09. Found: C, 67.75; H, 4.32; N, 5.33.

*4.2.13. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(4-isopropylphenyl)prop-2-en-1-one (**3m**)*

(Yield 70%). mp 196-198 ^{o}C; FTIR (KBr): 1655.40 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 1.28 (t, 6H, -CH(C<u>H</u>$_3$)$_2$), 2.97 (t, 1H, -C<u>H</u>$(CH_3)_2$), 6.40 (t, 2H, pyrrole-C_3, C_4-H), 7.19 (t, 2H, pyrrole-C_2, C_5-H), 7.30 (d, 2H, *J*= 8 Hz, 4-isopropylphenyl-C_3, C_5-H), 7.49-7.85 (m, 6H, 4-isopropylphenyl-C_2, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.09-8.12 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 23.75, 34.12, 111.53, 119.01, 119.43, 120.66, 127.10, 128.62, 130.91, 132.47, 135.11, 143.75, 144.96, 152.05, 188.98; MS (ESI): *m/z* = found 316.17 [M^+ + 1]; calcd. 315.41. Anal. Calcd. For $C_{22}H_{21}NO$: C, 83.78; H, 6.71; N, 4.44. Found: C, 84.75; H, 7.32; N, 5.33.

*4.2.14. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2,4-dimethoxyphenyl)prop-2-en-1-one (**3n**)*

(Yield 75%). mp 108-110 ^{o}C; FTIR (KBr): 1655.59 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 3.86 (s, 3H, -OC<u>H</u>$_3$), 3.91 (s, 3H, -OC<u>H</u>$_3$), 6.40 (t, 2H, pyrrole-C_3, C_4-H), 6.49 (d, 1H, *J*= 4 Hz, methoxyphenyl-C_3-H), 6.56 (dd, 1H, methoxyphenyl-C_5-H), 7.19 (t, 2H, pyrrole-C_2, C_5-H), 7.48-7.59 (m, 4H, bridging phenyl-C_2, C_3, C_5, C_6-H), 8.05-8.11 (m, 3H, methoxyphenyl-C_6-H and -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.48, 55.54, 98.43, 105.40, 111.37, 117.07, 119.03, 119.39, 119.94, 130.21, 131.05, 135.65, 140.58, 143.46, 160.43, 163.06, 189.56; MS (ESI): *m/z* = found 333 [M^+]; calcd. 333.14. Anal. Calcd. For $C_{21}H_{19}NO_3$: C, 75.66; H, 5.74; N, 4.20. Found: C, 76.75; H, 6.32; N, 5.33.

*4.2.15. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(3-methoxyphenyl)prop-2-en-1-one (**3o**)*

(Yield 85%). mp 130-132 ^{o}C; FTIR (KBr): 1660.54 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 3.85 (s, 3H, -OC<u>H</u>$_3$), 6.40 (dd, 2H, pyrrole-C_3, C_4-H), 6.95-6.98 (m, 1H, 3-

methoxyphenyl-C_4-H), 7.15-7.81 (m, 9H, pyrrole-C_2, C_5-H; 3-methoxyphenyl-C_2, C_5, C_6-H and bridging phenyl-C_2, C_3, C_5, C_6-H), 8.08-8.12 (m, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 55.32, 111.57, 113.48, 119, 119.42, 121.07, 121.87, 129.95, 130.35, 134.89, 136.19, 143.84, 144.73, 159.92, 188.84; MS (ESI): *m/z* = found 303 [M^+]; calcd. 303.13. Anal. Calcd. For $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 80.75; H, 6.32; N, 6.33.

*4.2.16. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(3-phenoxyphenyl)prop-2-en-1-one (**3p**)*

(Yield 70%). mp 126-128 ^{o}C; FTIR (KBr): 1655.59 (C=O), 1249.88 (C-O-C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 6.40 (t, 2H, pyrrole-C_3, C_4-H), 6.94-7.52 (m, 13H, pyrrole-C_2, C_5-H; phenoxyphenyl-C_2, C_4, C_5, C_6, C_9, C_{10}, C_{11}, C_{12}, C_{13}-H and bridging phenyl-C_2, C_6-H), 7.79 (d, 2H, *J*= 12 Hz, phenyl-C_3, C_5-H), 8.01-8.10 (q, 2H, -C<u>H</u>=C<u>H</u>-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.61, 117.07, 118.10, 119.02, 119.43, 120.82, 122.24, 123.53, 123.68, 129.67, 129.90, 130.90, 134.76, 136.66, 143.91, 144.10, 157.60, 157.82, 188.76; MS (ESI): *m/z* = found 365 [M^+]; calcd. 365.14. Anal. Calcd. For $C_{25}H_{19}NO_2$: C, 82.17; H, 5.24; N, 3.83. Found: C, 83.75; H, 6.32; N, 5.33.

*4.2.17. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(2-hydroxyphenyl)prop-2-en-1-one (**3q**)*

(Yield 50%). mp 174-176 ^{o}C; FTIR (KBr): 3425.61 (-OH), 1645.39 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.83 (t, 1H, hydroxyphenyl-C_3-H), 6.91 (t, 1H, hydroxyphenyl-C_5-H), 7.15-7.19 (m, 1H, hydroxyphenyl-C_4-H), 7.28 (t, 2H, pyrrole-C_2, C_5-H), 7.56 -7.58 (m, 2H, hydroxyphenyl-C_6-H; phenyl-C<u>H</u>=CH-), 7.61-7.63 (m, 1H, phenyl-CH=C<u>H</u>-), 7.75 (d, 1H, *J*= 16 Hz, bridging phenyl-C_2-H), 8.03 (d, 1H, *J*= 16 Hz, bridging phenyl-C_6-H), 8.10 (q, 2H, bridging phenyl-C_3, C_5-H), 9.99 (s, 1H, -OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.55, 116.65, 119.01, 119.39, 120.86, 122.28, 127.84, 129.63, 130.48, 131.83, 131.97, 140.91, 143.80, 155.88, 190.10; MS (ESI): *m/z* = found 289 [M^+]; calcd. 289.11. Anal. Calcd. For $C_{25}H_{19}NO_2$: C, 78.87; H, 5.23; N, 4.84. Found: C, 80.75; H, 6.32; N, 6.33.

*4.2.18. 1-(4-(1H-pyrrol-1yl)phenyl)-3-(3-furan-2-yl)prop-2-en-1-one (**3r**)*

(Yield 80%). mp 177-179 ^{o}C; FTIR (KBr): 1656.40 (C=O) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 6.40 (t, 2H, pyrrole-C_3, C_4-H), 6.53 (q, 1H, furan-C_4-H), 6.74 (d, 2H, IJ= *4* Hz, furan-C_3-H), 7.18 (t, 2H, pyrrole-C_2, C_5-H), 7.45-7.54 (m, 4H, bridging phenyl-C_2, C_3, C_5, C_6-H), 7.64 (d, 1H, *J*= 16 Hz, furan-C_5-H), 8.10-8.14 (m, 2H, -CH=CH-); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 111.53, 112.70, 119, 119.42, 130.25, 130.63, 134.91, 143.80, 144.95, 151.63, 188.16; MS (ESI): *m/z* = found 264.10 [M^+ + 1]; calcd. 263.09. Anal. Calcd. For $C_{17}H_{13}NO_2$: C, 77.55; H, 4.98; N, 5.32. Found: C, 79.75; H, 5.32; N, 6.33.

*4.3.General procedure for the synthesis of 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-substitutedphenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanones (**4a-p**)*

A mixture of chalcones (**3a-r**) (0.67 mmol), hydrazine hydrate 99% (0.87 mmol) and dried acetic acid (2 mL) was heated under reflux for 7 h. The reaction was monitored using TLC. After cooling to ambient temperature, the reaction mixture was neutralized with strong ammonia solution. The separated solid was filtered, washed with water, dried and purified by column chromatography on silica gel with ethyl acetate/petroleum ether (6:4) as eluent to afford the corresponding pyrazole derivatives in high purity with good yields.

*4.3.1. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4a**)*

(Yield 70%). mp 156-158 ^{o}C; FTIR (KBr): 1677.81 (C=O), 1564.80 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.43 (s, 3H, -$COCH_3$), 3.19 (dd, 1H, pyrazoline-C_4-H_a), 3.79 (dd, 1H, pyrazoline-C_4-H_b), 5.63 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.14 (t, 2H, pyrrole-C_2, C_5-H), 7.22-7.27 (m, 3H, phenyl-C_2, C_4, C_6-H), 7.34 (d, 2H, *J*= 8 Hz, phenyl-C_3, C_5-H), 7.42-7.45 (m, 2H, bridging phenyl-C_2, C_6-H), 7.77-7.80 (m, 2H, bridging phenyl-C_3, C_5-H); MS (ESI): *m/z* = found 330.16 [M^+ + 1]; calcd. 329.15. Anal. Calcd. For $C_{21}H_{19}N_3O$: C, 76.57; H, 5.81; N, 12.76. Found: C, 77.75; H, 7.32; N, 14.33.

*4.3.2. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4b**)*

(Yield 60%). mp 162-164 ^{o}C; FTIR (KBr): 1657.95 (C=O), 1606.57 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, -COC$\underline{H}_3$), 3.16 (dd, 1H, pyrazoline-C_4-H_a), 3.80 (dd, 1H, pyrazoline-C_4-H_b), 5.59 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.13-7.30 (m, 6H, chlorophenyl-C_2, C_3, C_5, C_6-H and pyrrole-C_2, C_5-H), 7.46 (d, 2H, *J*= 12 Hz, bridging phenyl-C_2, C_6-H), 7.79 (d, 2H, *J*= 8 Hz, bridging phenyl-C_3, C_5-H).

*4.3.3. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4c**)*

(Yield 60%). mp 166-168 ^{o}C; FTIR (KBr): 1661.98 (C=O), 1608.24 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.49 (s, 3H, -COC$\underline{H}_3$), 3.09 (dd, 1H, pyrazoline-C_4-H_a), 3.89 (dd, 1H, pyrazoline-C_4-H_b), 5.95 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 7.05-7.08 (m, 1H, chlorophenyl-C_4-H), 7.13 (t, 2H, pyrrole-C_2, C_5-H), 7.18-7.23 (m, 2H, chlorophenyl-C_5, C_6-H), 7.39-7.44 (m, 3H, bridging phenyl-C_2, C_6-H and chlorophenyl-C_3-H), 7.76-7.79 (m, 2H, phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.87, 41.39, 57.77, 111.17, 119.97, 125.82, 127.25, 127.96, 128.28, 128.79, 130.02, 131.68, 138.32, 141.94, 153.45, 168.87; MS (ESI): *m/z* = found 363 [M^+]; calcd. 363.11. Anal. Calcd. For $C_{21}H_{18}ClN_3O$: C, 69.32; H, 4.99; N, 11.55. Found: C, 70.75; H, 5.32; N, 13.33.

*4.3.4. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4d**)*

(Yield 60%). mp 128-130 ^{o}C; FTIR (KBr): 1678.83 (C=O), 1564.33 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.30 (s, 3H, -$COCH_3$), 2.42 (s, 3H, -CH_3), 3.18 (dd, 1H, pyrazoline-C_4-H_a), 3.78 (dd, 1H, pyrazoline-C_4-H_b), 5.59 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.11-7.14 (m, 4H, methylphenyl-C_2, C_3, C_5, C_6-H), 7.26 (s, 2H, pyrrole-C_2, C_5-H), 7.45 (t, 2H, bridging phenyl-C_2, C_6-H), 7.77-7.80 (m, 2H, bridging

phenyl-C_3, C_5-H); MS (ESI): *m/z* = found 344.17 [M^+ + 1]; calcd. 343.17. Anal. Calcd. For $C_{22}H_{21}N_3O$: C, 76.94; H, 6.61; N, 12.24. Found: C, 78.75; H, 7.32; N, 14.33.

*4.3.5. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4e**)*

(Yield 60%). mp 124-125 ^oC; FTIR (KBr): 1658.31 (C=O), 1608.60 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 2.41 (s, 3H, -COC$\underline{H_3}$), 3.19 (dd, 1H, pyrazoline-C_4-H_a), 3.74 (t, 1H, pyrazoline-C_4-H_b), 3.77 (s, 3H, -OC$\underline{H_3}$), 5.58 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 6.83-6.86 (m, 2H, methoxyphenyl-C_3, C_5-H), 7.13-7.19 (m, 4H, pyrrole-C_2, C_5-H and methoxyphenyl-C_2, C_6-H), 7.45 (dd, 2H, bridging phenyl-C_2, C_6-H), 7.78-7.80 (m, 2H, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.95, 42.22, 55.24, 59.48, 111.14, 114.20, 120, 126.87, 127.92, 133.94, 141.83, 153.03, 158.99, 168.78; MS (ESI): *m/z* = found 359 [M^+]; calcd. 359.16. Anal. Calcd. For $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69. Found: C, 71.75; H, 6.32; N, 12.33.

*4.3.6. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(3-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4f**)*

(Yield 75%). mp 120-122 ^oC; FTIR (KBr): 1660.58 (C=O), 1570.62 (C=N) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, -COC$\underline{H_3}$), 3.17 (dd, 1H, pyrazoline-C_4-H_a), 3.80 (dd, 1H, pyrazoline-C_4-H_b), 5.58 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.13-7.48 (m, 8H, pyrrole-C_2, C_5-H, bridging phenyl-C_2, C_6-H and bromophenyl-C_2, C_4, C_5, C_6-H), 7.77-7.79 (m, 2H, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.30, 42.24, 59.46, 111.22, 120.03, 123, 124.31, 128.01, 128.18, 128.57, 130.51, 130.87, 142, 143.95, 152.92, 168.93; MS (ESI): *m/z* = found 407 [M^+], 409 [M^+ + 2]; calcd. 407.06. Anal. Calcd. For $C_{21}H_{18}BrN_3O$: C, 61.78; H, 4.44; N, 10.29. Found: C, 62.75; H, 5.02; N, 12.33.

*4.3.7. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4g**)*

(Yield 75%). mp 172-174 ^{o}C; FTIR (KBr): 1661.21 (C=O), 1568.89 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.42 (s, 3H, -COC$\underline{H_3}$), 3.17 (dd, 1H, pyrazoline-C_4-H_a), 3.79 (m, 1H, pyrazoline-C_4-H_b), 5.60 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 6.98-7.03 (m, 2H, fluorophenyl-C_3, C_5-H), 7.14 (t, 4H, pyrrole-C_2, C_5-H), 7.19-7.23 (m, 2H, fluorophenyl-C_2, C_6-H), 7.43-7.46 (m, 2H, bridging phenyl-C_2, C_6-H), 7.80 (dd, 2H, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.92, 42.24, 59.35, 111.21, 115.62, 115.91, 120.02, 127.29, 127.41, 127.96, 137.52, 137.56, 141.94, 152.94, 160.50, 168.86; MS (ESI): *m/z* = found 347 [M^+]; calcd. 347.14. Anal. Calcd. For $C_{21}H_{18}FN_3O$: C, 72.61; H, 5.22; N, 12.10. Found: C, 73.75; H, 6.32; N, 13.33.

*4.3.8. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4h**)*

(Yield 75%). mp 160-162 ^{o}C; FTIR (KBr): 1671.47 (C=O), 1604.39 (C=N) cm^{-1}.

*4.3.9. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4i**)*

(Yield 60%). mp 180-182 ^{o}C; FTIR (KBr): 1660.91 (C=O), 1608.20 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.43 (s, 3H, -COC$\underline{H_3}$), 3.20 (dd, 1H, pyrazoline-C_4-H_a), 3.78 (dd, 1H, pyrazoline-C_4-H_b), 3.87 (d, 6H, -2OC$\underline{H_3}$), 5.58 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 6.77-6.82 (m, 3H, methoxyphenyl-C_2, C_5, C_6-H), 7.15 (t, 2H, pyrrole-C_2, C_5-H) 7.46 (d, 2H, *J*= 8 Hz, bridging phenyl-C_2, C_6-H), 7.81 (d, 2H, *J*= 8 Hz, bridging phenyl-C_3, C_5-H); MS (ESI): *m/z* = found 389 [M^+]; calcd. 389.17. Anal. Calcd. For $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 72.75; H, 7.32; N, 12.33.

*4.3.10. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2,4-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4j**)*

(Yield 75%). mp 118-120 ^{o}C; FTIR (KBr): 1662.25 (C=O), 1561.83 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.48 (s, 3H, -COC$\underline{H_3}$), 3.06 (dd, 1H, pyrazoline-C_4-H_a), 3.88 (dd,

1H, pyrazoline-C_4-H_b), 5.88 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 7.02 (d, 1H, *J*= 8 Hz, chlorophenyl-C_6-H), 7.13 (t, 4H, pyrrole-C_2, C_5-H), 7.20 (dd, 1H, chlorophenyl-C_5-H), 7.42-7.78 (m, 5H, chlorophenyl-C_3-H and bridging phenyl-C_2, C_3, C_5, C_6-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.85, 41.23, 57.40, 111.23, 118.95, 119.97, 126.96, 127.97, 129.82, 132.39, 133.89, 137.06, 142.02, 153.34, 168.91; MS (ESI): *m/z* = found 398 [M^+ + 1]; calcd. 397.07. Anal. Calcd. For $C_{21}H_{17}Cl_2N_3O$: C, 63.33; H, 4.30; N, 10.55. Found: C, 65.15; H, 5.32; N, 11.33.

*4.3.11. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2,3-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4k**)*

(Yield 60%). mp 144-146 ^{o}C; FTIR (KBr): 1662.17 (C=O), 1607.52 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 2.49 (s, 3H, -COC$\underline{H_3}$), 3.07 (dd, 1H, pyrazoline-C_4-H_a), 3.91 (dd, 1H, pyrazoline-C_4-H_b), 5.95 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 6.99 (d, 1H, *J*= 8 Hz, chlorophenyl-C_6-H), 7.12-7.39 (m, 4H, pyrrole-C_2, C_5-H and chlorophenyl-C_4, C_5-H), 7.44 (d, 2H, *J*= 8 Hz, bridging phenyl-C_2, C_6-H), 7.78 (d, 2H, *J*= 8 Hz, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.87, 41.39, 57.77, 111.24, 119.98, 123.93, 127.72, 127.99, 128.09, 129.54, 129.99, 133.74, 140.65, 142.03, 153.40, 168.90; MS (ESI): *m/z* = found 397 [M^+]; calcd. 397.07. Anal. Calcd. For $C_{21}H_{17}Cl_2N_3O$: C, 63.33; H, 4.30; N, 10.55. Found: C, 64.75; H, 5.32; N, 12.33.

*4.3.12. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2,6-dichlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4l**)*

(Yield 60%). mp 148-150 ^{o}C; FTIR (KBr): 1664.34 (C=O), 1608.92 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) *δ* ppm: 2.37 (s, 3H, -COC$\underline{H_3}$), 3.34 (dd, 1H, pyrazoline-C_4-H_a), 3.74 (dd, 1H, pyrazoline-C_4-H_b), 6.27 (dd, 1H, pyrazoline-C_5-H_x), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.12-7.37 (m, 5H, pyrrole-C_2, C_5-H, bridging phenyl-C_2, C_6-H and chlorophenyl-C_4-H), 7.44-7.46 (m, 2H, chlorophenyl-C_3, C_5-H), 7.79-7.81 (m, 2H, bridging phenyl-C_3, C_5-H).

*4.3.13. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(4-isopropylphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4m**)*

(Yield 60%). mp 170-172 ^{o}C; FTIR (KBr): 1662.05 (C=O), 1526.30 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.22 (dd, 6H, -CH($C\underline{H}_3$)$_2$), 2.43 (s, 3H, -COC$\underline{H}_3$), 2.83-2.89 (p, 1H, -C$\underline{H}$(CH_3)$_2$), 3.20 (dd, 1H, pyrazoline-C_4-H_a), 3.77 (dd, 1H, pyrazoline-C_4-H_b), 5.61 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 7.13-7.19 (m, 6H, pyrrole-C_2, C_5-H and isopropylphenyl-C_2, C_3, C_5, C_6-H), 7.46 (dd, 2H, bridging phenyl-C_2, C_6-H), 7.80 (t, 2H, phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.94, 23.88, 23.90, 33.73, 42.28, 59.75, 111.14, 120, 125.44, 126.93, 127.94, 128.53, 139, 141.84, 148.22, 153.17, 168.85; MS (ESI): *m/z* = found 371 [M^+]; calcd. 371.20. Anal. Calcd. For $C_{22}H_{21}N_3O_2$: C, 77.60; H, 6.78; N, 11.31. Found: C, 79.75; H, 8.32; N, 13.33.

*4.3.14. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(3-phenoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4n**)*

(Yield 60%). mp 194-196 ^{o}C; FTIR (KBr): 1671.47 (C=O), 1604.39 (C=N) cm^{-1}.

*4.3.15. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(furan-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4o**)*

(Yield 60%). mp 194-196 ^{o}C; FTIR (KBr): 1675.54 (C=O), 1608.08 (C-O), 1591.43 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.40 (s, 3H, -COC$\underline{H}_3$), 3.48 (dd, 1H, pyrazoline-C_4-H_a), 3.63 (dd, 1H, pyrazoline-C_4-H_b), 5.73 (dd, 1H, pyrazoline-C_5-H_x), 6.31-6.34 (m, 2H, furan-C_3, C_4-H), 6.39 (t, 2H, pyrrole-C_3, C_4-H), 7.15 (t, 2H, pyrrole-C_2, C_5-H) 7.30 (d, 1H, furan-C_5-H), 7.46 (d, 2H, *J*= 8 Hz, bridging phenyl-C_2, C_6-H), 7.82 (d, 2H, *J*= 8 Hz, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.95, 38.18, 53.37, 107.61, 110.53, 111.16, 120.02, 127.96, 128.39, 141.90, 141.96, 151.96, 153.20, 168.94; MS (ESI): *m/z* = found 319 [M^+]; calcd. 319.13. Anal. Calcd. For $C_{22}H_{21}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 72.75; H, 6.32; N, 15.33.

*4.3.16. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(m-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4p**)*

(Yield 60%). mp 80-82 ^{o}C; FTIR (KBr): 1660.54 (C=O), 1526.24 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.43 (s, 3H, -COC$\underline{H}_3$), 3.18 (dd, 1H, pyrazoline-C_4-H_a), 3.75 (t, 1H, pyrazoline-C_4-H_b), 3.78 (s, 3H, -OC$\underline{H}_3$), 5.60 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 6.76-6.83 (m, 4H, methoxyphenyl-C_2, C_4, C_5, C_6-H), 7.14 (t, 4H, pyrrole-C_2, C_5-H), 7.44 (d, 2H, *J*= 8 Hz, bridging phenyl-C_2, C_6-H), 7.79 (d, 2H, *J*= 8 Hz, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.91, 42.32, 55.18, 59.91, 111.14, 111.46, 112.66, 118.97, 119.98, 127.93, 128.43, 130.02, 141.84, 143.35, 153.02, 159.84, 168.83; MS (ESI): *m/z* = found 359 [M^{+}]; calcd. 359.16. Anal. Calcd. For $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69. Found: C, 75.75; H, 7.32; N, 13.33.

*4.3.17. 1-(3-(4-(1H-pyrrol-1-yl)phenyl)-5-(2,4-dimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (**4q**)*

(Yield 60%). mp 90-92 ^{o}C; FTIR (KBr): 1659.87 (C=O), 1526.53 (C=N) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 2.45 (s, 3H, -COC$\underline{H}_3$), 3.06 (dd, 1H, pyrazoline-C_4-H_a), 3.71 (dd, 1H, pyrazoline-C_4-H_b), 3.76 (s, 3H, -OC$\underline{H}_3$), 3.82 (s, 3H, -OC$\underline{H}_3$), 5.79 (dd, 1H, pyrazoline-C_5-H_x), 6.38 (t, 2H, pyrrole-C_3, C_4-H), 6.42 (dd, 1H, methoxyphenyl-C_5-H), 6.46 (d, 1H, methoxyphenyl-C_3-H), 6.95 (d, 1H, *J*= 8 Hz, methoxyphenyl-C_6-H), 7.13 (t, 2H, pyrrole-C_2, C_5-H) 7.42 (t, 2H, bridging phenyl-C_2, C_6-H), 7.78 (dd, 2H, bridging phenyl-C_3, C_5-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm: 21.94, 41.37, 55.33, 55.42, 55.74, 99.03, 103.94, 111.05, 118.97, 119.94, 127.86, 128.82, 141.68, 154.02, 157.04, 160.28, 168.77; MS (ESI): *m/z* = found 389 [M^{+}]; calcd. 389.17. Anal. Calcd. For $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 72.75; H, 7.32; N, 12.33.

4.4.General procedure for the synthesis of 5-(4-(1*H*-pyrrol-1yl)phenyl)-3-substituted phenyl isoxazoles ***(5a-o)***

Hydroxylamine hydrochloride (0.69g, 0.01 mol) was dissolved in ethanol (25 mL). To this, anhydrous sodium acetate (0.82g, 0.01 mol) dissolved in minimum quantity of hot acetic acid was added. To the above mixture, 1-(4-(1*H*-pyrrol-1yl)phenyl)-3-substitutedprop-2-en-1-ones (**3a-r**) (0.01 mol) was added and heated under reflux for 16 h. The reaction was monitored by TLC to check for completion. The solvent was removed under reduced pressure, the residue was neutralized with 0.1% sodium hydroxide solution. The separated product was filtered, washed with water, dried and purified by column chromatography on silica gel with ethyl acetate/petroleum ether (6:4) as the eluent to offer the title compounds (**5a-o**).

*4.4.1. 5-(4-(1H-pyrrol-1yl)phenyl)-3-phenylisoxazole (**5a**)*

(Yield 70%). mp 180-187 ^{o}C; FTIR (KBr): 3022.60 (Ar-H), 1608.38 (C-O), 1574.99 (C=N), 1524.02 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.30 (t, 2H, pyrrole-C_3, C_4-H), 6.80 (d, 1H, isoxazole-C_4-H), 7.27 (t, 2H, pyrrole-C_2, C_5-H), 7.28 - 7.65 (m, 9H, pyrrolylphenyl-C_2, C_3, C_5, C_6 - H and phenyl-C_2, C_3, C_4, C_5, C_6 - H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 110.69, 110.74, 117.46, 118.90, 118.97, 126.72, 127.22, 128.74, 128.85, 128.98, 129.97, 130.06, 132.13, 135.99, 136.82, 140.07, 154.60.

*4.4.2. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(4-chlorophenyl)isoxazole (**5b**)*

(Yield 70%). mp 250-252 ^{o}C; FTIR (KBr): 2917.84 (Ar-H), 1606.31 (C-O), 1522.89 (C=N), 1475.10 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.78 (d, 1H, isoxazole-C_4-H), 7.30 - 7.75 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and chlorophenyl-C_2, C_3, C_5, C_6-H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 98.02, 110.78, 118.72, 118.90, 120.29, 126.89, 127.35, 127.77, 129.75, 129.89, 130.07, 130.42, 131.83, 132.20, 132.87, 133.79, 140.15, 154.25; MS (ESI): *m/z* = found 322 [M^+ + 2]; calcd. 320.77. Anal. Calcd. For $C_{19}H_{13}ClN_2O$: C, 71.14; H, 4.08; N, 8.73. Found: C, 72.75; H, 5.32; N, 10.33.

*4.4.3. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(2-chlorophenyl)isoxazole (**5c**)*

(Yield 70%). mp 220-222 ^{o}C; FTIR (KBr): 2921.56 (Ar-H), 1605.78 (C-O), 1523.55 (C=N), 1471.12 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.28 (t, 2H, pyrrole-C_3, C_4-H), 6.80 (d, 1H, isoxazole-C_4-H), 7.12 - 7.86 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and chlorophenyl-C_3, C_4, C_5, C_6-H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 98.02, 110.78, 118.72, 118.90, 120.29, 126.89, 127.35, 127.77, 129.75, 129.89, 130.07, 130.42, 131.83, 132.20, 132.87, 133.79, 140.15, 154.25; MS (ESI): *m/z* = found 323.09 [M^+ + 3]; calcd. 320.77. Anal. Calcd. For $C_{19}H_{13}ClN_2O$: C, 71.14; H, 4.08; N, 8.73. Found: C, 72.75; H, 5.32; N, 10.33.

*4.4.4. 5-(4-(1H-pyrrol-1yl)phenyl)-3-p-tolylisoxazole (**5d**)*

(Yield 70%). mp 218-220 ^{o}C; FTIR (KBr): 2918.63 (Ar-H), 1607.67 (C-O), 1523 (C=N), 1477.10 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 2.33 (s, 3H, -C$\underline{H_3}$), 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.75 (d, 1H, isoxazole-C_4-H), 7.16 - 7.61 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and methylphenyl-C_3, C_4, C_5, C_6-H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 20.90, 110.59, 111.32, 118.99, 126.46, 127, 128.73, 129.10, 129.40, 129.93, 132.30, 133.82, 139.64, 140.05, 154.68; MS (ESI): *m/z* = found 301 [M^+ + 1]; calcd. 300.35. Anal. Calcd. For $C_{20}H_{16}N_2O$: C, 79.98; H, 5.37; N, 9.33. Found: C, 81.75; H, 6.32; N, 10.33.

*4.4.5. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(4-methoxyphenyl)isoxazole (**5e**)*

(Yield 70%). mp 212-214 ^{o}C; FTIR (KBr): 2958.87 (Ar-H), 1604.67 (C-O), 1522.09 (C=N), 1477.08 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 3.85 (s, 3H, -OC$\underline{H_3}$), 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.73 (d, 1H, isoxazole-C_4-H), 6.93 (t, 2H, methoxyphenyl-C_3, C_5-H), 7.29 - 7.58 (m, 8H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and methoxyphenyl-C_4, C_6-H); ^{13}C NMR (100 MHz, DMSO) δ ppm: 54.99, 110.82, 119.11, 124.43, 127.42, 128.72, 130.17, 136.39, 140.02, 154.84, 159.88; MS (ESI): *m/z* = found 317 [M^+ + 1]; calcd. 316.35. Anal. Calcd. For $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86. Found: C, 77.75; H, 6.32; N, 10.33.

*4.4.6. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(3-bromophenyl)isoxazole (**5f**)*

(Yield 70%). mp 112-114 ^{o}C; FTIR (KBr): 2918.36 (Ar-H), 1606.38 (C-O), 1522.16 (C=N), 1473.20 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.26 (t, 2H, pyrrole-C_3, C_4-H), 6.71 (d, 1H, isoxazole-C_4-H), 7.14 - 7.76 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and bromophenyl-C_3, C_4, C_5, C_6-H); Anal. Calcd. For $C_{19}H_{13}BrN_2O$: C, 62.48; H, 3.59; N, 7.67. Found: C, 64.75; H, 4.32; N, 9.33.

*4.4.7. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(4-fluorophenyl)isoxazole (**5g**)*

(Yield 70%). mp 122-124 ^{o}C; FTIR (KBr): 2919.64 (Ar-H), 1606.49 (C-O), 1524.67 (C=N), 1473.69 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.27 (t, 2H, pyrrole-C_3, C_4-H), 6.77 (d, 1H, isoxazole-C_4-H), 7.33 - 7.93 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and fluorophenyl-C_3, C_4, C_5, C_6-H); MS (ESI): *m/z* = found 306 [M^+ + 2]; calcd. 304.32. Anal. Calcd. For $C_{19}H_{13}FN_2O$: C, 74.99; H, 4.31; N, 9.21. Found: C, 76.75; H, 5.32; N, 10.33.

*4.4.8. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(2-bromophenyl)isoxazole (**5h**)*

(Yield 70%). mp 224-226 ^{o}C; FTIR (KBr): 2918.26 (Ar-H), 1607.61 (C-O), 1523.59 (C=N), 1474.93 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 6.28 (t, 2H, pyrrole-C_3, C_4-H), 6.78 (d, 1H, isoxazole-C_4-H), 7.06 - 7.83 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and bromophenyl-C_3, C_4, C_5, C_6-H); MS (ESI): *m/z* = found 366 [M^+ + 1]; Anal. Calcd. 365.22. For $C_{19}H_{13}BrN_2O$: C, 62.48; H, 3.59; N, 7.67. Found: C, 64.75; H, 4.32; N, 9.33.

*4.4.9. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(3,4-dimethoxyphenyl)isoxazole (**5i**)*

(Yield 70%). mp 186-188 ^{o}C; FTIR (KBr): 2956.54 (Ar-H), 1606.74 (C-O), 1514.65 (C=N), 1464.41 (C=C, isoxazole) cm^{-1}; ^{1}H NMR (400 MHz, DMSO) δ ppm: 3.78 (s, 6H, -OC$\underline{H_3}$), 6.27 (t, 2H, pyrrole-C_3, C_4-H), 6.71 (d, 1H, isoxazole-C_4-H), 6.84 - 7.75 (m, 9H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and methoxyphenyl-C_3, C_4, C_5, C_6-H); ^{13}C NMR (100 MHz,

DMSO) δ ppm: 55.49, 82.38, 109.944, 110.76, 111.69, 115.41, 118.93, 120.78, 124.78, 128.09, 130.08, 140.03, 149.75, 154.95; MS (ESI): *m/z* = found 348 [M^+ + 2]; Anal. Calcd. 346.38. For $C_{21}H_{18}N_2O_3$: C, 77.82; H, 5.24; N, 8.09. Found: C, 79.75; H, 6.32; N, 9.33.

*4.4.10. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(2,4-chlorophenyl)isoxazole (**5j**)*

(Yield 70%). mp 194-196 ^oC; FTIR (KBr): 2919.68 (Ar-H), 1607.44 (C-O), 1521.94 (C=N), 1472.99 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 6.26 (t, 2H, pyrrole-C_3, C_4-H), 6.74 (d, 1H, isoxazole-C_4-H), 7.31 - 7.99 (m, 9H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and chlorophenyl-C_3, C_4, C_5, C_6-H); For $C_{19}H_{12}Cl_2N_2O$: C, 64.24; H, 3.41; N, 7.89. Found: C, 65.75; H, 4.32; N, 9.33.

*4.4.11. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(2,3-chlorophenyl)isoxazole (**5k**)*

(Yield 70%). mp 202-204 ^oC; FTIR (KBr): 2918.50 (Ar-H), 1608.09 (C-O), 1521.76 (C=N), 1478.50 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 6.14 (m, 2H, pyrrole-C_3, C_4-H), 6.72 (d, 1H, isoxazole-C_4-H), 7.18 - 7.65 (m, 9H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and chlorophenyl-C_3, C_4, C_5, C_6-H); For $C_{19}H_{12}Cl_2N_2O$: C, 64.24; H, 3.41; N, 7.89. Found: C, 65.75; H, 4.32; N, 9.33.

*4.4.12. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(2,6-chlorophenyl)isoxazole (**5l**)*

(Yield 70%). mp 88-90 ^oC; FTIR (KBr): 2920.28 (Ar-H), 1605.87 (C-O), 1522.91 (C=N), 1475.83 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.82 (d, 1H, isoxazole-C_4-H), 7.30 -7.77 (m, 9H, pyrrole-C_2, C_5-H; phenyl-C_2, C_3, C_5, C_6 - H and chlorophenyl-C_3, C_4, C_5 -H); For $C_{19}H_{12}Cl_2N_2O$: C, 64.24; H, 3.41; N, 7.89. Found: C, 65.75; H, 4.32; N, 9.33.

*4.4.13. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(4-isopropylphenyl)isoxazole (**5m**)*

(Yield 70%). mp 204-206 ^oC; FTIR (KBr): 2959.29 (Ar-H), 1606.11 (C-O), 1521.39 (C=N), 1477.76 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 1.16 - 1.27 (m, 6H, 2C$\underline{H_3}$), 2.85 - 2.96 (m, 1H, -C$\underline{H}$), 6.29 (s, 2H, pyrrole-C_3, C_4-H), 6.77 (d, 1H, isoxazole-C_4-

H), 7.21 - 7.84 (m, 10H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and isopropylphenyl-C_3, C_4, C_5 -H); For $C_{22}H_{20}N_2O$: C, 80.46; H, 6.14; N, 8.53. Found: C, 81.75; H, 7.32; N, 10.33.

*4.4.14. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(3-phenoxyphenyl)isoxazole (**5n**)*

(Yield 70%). mp 98-100 ^oC; FTIR (KBr): 2917.62 (Ar-H), 1606.40 (C-O), 1522.03 (C=N), 1485.41 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.74 - 7.63 (m, 16H, isoxazole-C_4-H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and phenoxyphenyl-C_3, C_4, C_5 -H); For $C_{25}H_{18}N_2O_2$: C, 79.35; H, 4.79; N, 7.40. Found: C, 80.75; H, 5.32; N, 9.33.

*4.4.15. 5-(4-(1H-pyrrol-1yl)phenyl)-3-(3-furan-2-yl)isoxazole (**5o**)*

(Yield 70%). mp 206-208 ^oC; FTIR (KBr): 2917.62 (Ar-H), 1606.40 (C-O), 1522.03 (C=N), 1485.41 (C=C, isoxazole) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 6.29 (t, 2H, pyrrole-C_3, C_4-H), 6.92 (d, 1H, isoxazole-C_4-H,), 7.03 (d, 1H, furan-C_4-H), 7.33 - 7.72 (m, 8H, pyrrole-C_2, C_5-H, phenyl-C_2, C_3, C_5, C_6 - H and furan C_3, C_5 -H); For $C_{17}H_{12}N_2O_2$: C, 73.90; H, 4.38; N, 10.14. Found: C, 75.75; H, 5.32; N, 12.33.

*4.5.General procedure for the synthesis of 2-(2,5-dimethyl-1H-pyrrol-1yl)aniline (**8**)*

To a suspension of *o*-phenylenediamine **6** (0.32 g, 0.003 mol) in ethanol (10 mL) were added acetonyl acetone (**7**) (0.684 g, 0.06 mol) and glacial acetic acid (1 mL). The mixture was heated on boiling water bath for 3 h. it was further concentrated to half of its original volume, poured into crushed ice (50 g) and neutralized with sodium bicarbonate solution. The separated solid was filtered, washed with water, dried and purified by column chromatography on silica gel with ethyl acetate/petroleum ether (1:9) as a eluent. (Yield 70%). mp 160-162 ^oC; FTIR (KBr): 3238.32 (NH_2), 3022.60 (Ar-H) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ ppm: 1.81 (s, 6H, $2CH_3$), 5.80 (s, 2H, pyrrole-C_3, C_4-H), 6.35 (s, 2H, NH_2), 6.82-7.45 (m, 4H, phenyl C_3, C_4, C_5, C_6-H); MS (ESI): *m/z* = found 186.12 [M^+ + 1]; Calcd.

185.26. Anal. Calcd. For $C_{13}H_{15}N$: C, 84.28; H, 8.16; N, 7.56. Found: C, 85.25; H, 9.12; N, 8.53.

*4.6.General procedure for the synthesis of 1-(2-(2,5-dimethyl-1H-pyrrol-1yl)phenyl)-3-(substituted phenyl)thioureas (**9a-k**)*

A mixture of 2-(2,5-dimethyl-1*H*-pyrrol-1-yl)aniline (0.01 mol) and different substituted phenyl isothiocyanates in dry chloroform (20 mL) was refluxed for 22-24 h. The reaction was monitored with the help of TLC to check its completion. Upon cooling the reaction mixture to room temperature, the mixture was concentrated *in vacuo* to afford the crude product. Purification of crude products by column chromatography by eluting with petroleum ether-ethyl acetate (9:1) mixture to afford the compounds **9a-k**.

*4.6.1. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-phenylthiourea (**9a**)*

(Yield 85%). mp 172-174 ^{o}C; FTIR (KBr): 3308.39 & 3165.87 (N-H), 2978.35 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.81 (s, 6H, $2CH_3$), 5.80 (s, 2H, pyrrole-C_3, C_4-H), 6.92 (q, 2H, phenyl-C_6-H and thiophenyl-C_4-H), 7.18 (dd, 1H, J = 1.52, 1.6 Hz, phenyl-C_4-H), 7.24-7.48 (m, 6H, phenyl-C_3, C_5-H and thiophenyl-C_2, C_3, C_5, C_6-H), 8.14 & 8.52 (s, 2H, 2NH); MS (ESI): *m/z* = found 317 [M^+ + 1]; Calcd. 321.44. Anal. Calcd. For $C_{19}H_{19}N_3S$: C, 70.99; H, 5.96; N, 13.07. Found: C, 71.75; H, 6.32; N, 14.53.

*4.6.2. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(2-fluorophenyl)thiourea (**9b**)*

(Yield 90%). mp 165-167 ^{o}C; FTIR (KBr): 3301.91 & 3164.32 (N-H), 2923.78 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.83 (s, 6H, $2CH_3$), 5.77 (s, 2H, pyrrole-C_3, C_4-H), 7.06-7.49 (m, 8H, phenyl-C_3, C_4, C_5, C_6-H and 2-fluorophenyl-C_3, C_4, C_5, C_6-H), 7.88 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.56 (d, 1H, J = 7.8 Hz, -N<u>H</u>-CSNH-Ar); MS (ESI): *m/z* = found 339 [M^+]; Calcd. 339.43. Anal. Calcd. For $C_{19}H_{18}FN_3S$: C, 67.23; H, 5.35; N, 12.38. Found: C, 68.15; H, 6.12; N, 13.53.

*4.6.3. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(4-fluorophenyl)thiourea (**9c**)*

(Yield 95%). mp 190-192 ^{o}C; FTIR (KBr): 3332.57 & 3170.12 (N-H), 2990.67 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.81 (s, 6H, $2CH_3$), 5.81 (s, 2H, pyrrole-C_3, C_4-H), 6.91-7.18 (m, 6H, phenyl-C_4, C_6-H and 4-fluorophenyl-C_2, C_3, C_5, C_6-H), 7.24-7.28 (m, 1H, phenyl-C_5-H), 7.44-7.48 (m, 1H, phenyl-C_3-H), 8.28 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.56 (t, 1H, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.34, 106.98, 125.15, 125.58, 125.92, 127.61, 128.19, 128.58, 128.91, 130.30, 131.11, 135.33, 136.26, 178.69; MS (ESI): *m/z* = found 339 [M^{+}]; Calcd. 339.43. Anal. Calcd. For $C_{19}H_{18}FN_3S$: C, 67.23; H, 5.35; N, 12.38. Found: C, 68.15; H, 6.12; N, 13.53.

*4.6.4. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(4-(trifluoromethyl)phenyl)thiourea (**9d**)*

(Yield 95%). mp 178-180 ^{o}C; FTIR (KBr): 3308.64 & 3156.11 (N-H), 2984.17 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.88 (s, 6H, $2CH_3$), 5.81 (s, 2H, pyrrole-C_3, C_4-H), 7.07 (d, 2H, *J* = 8.36 Hz, triflurophenyl-C_2, C_6-H), 7.19-7.32 (m, 3H, phenyl-C_4, C_5, C_6-H), 7.46-7.59 (m, 3H, phenyl-C_3-H and trifluorophenyl-C_3, C_5-H), 8.25 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.56 (t, 1H, -N<u>H</u>-CS-NH-Ar); MS (ESI): *m/z* = found 389 [M^{+}]; Calcd. 389.44. Anal. Calcd. For $C_{20}H_{18}F_3N_3S$: C, 61.68; H, 4.66; N, 10.79. Found: C, 62.75; H, 5.12; N, 11.53.

*4.6.5. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(2-bromophenyl)thiourea (**9e**)*

(Yield 95%). mp 198-202 ^{o}C; FTIR (KBr): 3308.66 & 3169.51 (N-H), 2922.97 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.82 (s, 6H, $2CH_3$), 5.75 (s, 2H, pyrrole-C_3, C_4-H), 7.04 (s, 1H, phenyl-C_6-H), 7.10-7.19 (m, 3H, phenyl-C_4-H and brmomophenyl-C_4, C_6-H), 7.24-7.29 (m, 2H, phenyl-C_5-H and bromophenyl-C_5-H), 7.45-7.49 (m, 1H, phenyl-C_3-H), 7.61 (dd, 1H, *J* = 1.4, 1.36 Hz, bromoaniline-C_3-H), 7.78 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.59 (t, 1H, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.34, 106.98, 125.15, 125.58, 125.92, 127.61, 128.19, 128.58, 128.91, 130.30, 131.11, 135.33, 136.26, 178.69; MS (ESI): *m/z* = found 400 [M^{+}]; Calcd. 400.34. Anal. Calcd. For $C_{19}H_{18}BrN_3S$: C, 57.00; H, 4.53; N, 10.50. Found: C, 59.15; H, 5.12; N, 11.53.

*4.6.6. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(3-bromophenyl)thiourea (**9f**)*

(Yield 96%). mp 180-182 ^{o}C; FTIR (KBr): 3349.04 & 3169.03 (N-H), 2922.67 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.83 (s, 6H, $2CH_3$), 5.79 (s, 2H, pyrrole-C_3, C_4-H), 6.88 (dd, 1H, J = 1.56, 1.6 Hz, bromophenyl-C_6-H), 7.06 (s, 1H, phenyl-C_6-H), 7.17-7.30 (m, 4H, phenyl-C_4-H and brmomophenyl-C_2, C_4, C_5-H), 7.41-7.50 (m, 2H, phenyl-C_3, C_5-H), 8.06 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.54 (dd, 1H, J = 0.88, 0.8 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.37, 107.12, 123.59, 124.04, 128.18, 128.46, 128.73, 128.93, 130.89, 131.39, 136.14, 136.60, 178.69; MS (ESI): *m/z* = found 400 [M^+], 401 [M^+ + 1], 402 [M^+ + 2]; Calcd. 400.34 Anal. Calcd. For $C_{19}H_{18}BrN_3S$: C, 57.00; H, 4.53; N, 10.50. Found: C, 59.15; H, 5.12; N, 11.53.

*4.6.7. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(4-nitrophenyl)thiourea (**9g**)*

(Yield 97%). mp 188-190 ^{o}C; FTIR (KBr): 3282.66 & 3198.90 (N-H), 2915.32 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.90 (s, 6H, $2CH_3$), 5.90 (s, 2H, pyrrole-C_3, C_4-H), 7.17 (dd, 2H, J = 1.96, 2.00 Hz, nitrophenyl-C_2, C_6-H), 7.26 (t, 1H, phenyl-C_6-H), 7.32-7.53 (m, 3H, phenyl-C_3, C_4, C_5-H), 8.19 (dd, 2H, J = 2.04, 2.04 Hz, nitrophenyl-C_3, C_5-H), 8.31 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.46 (dd, 1H, J = 1.00, 0.96 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.48, 107.20, 123.22, 125.52, 125.62, 128.58, 128.93, 129.32, 131.33, 135.44, 142.05, 145.00, 178.03; MS (ESI): *m/z* = found 366 [M^+]; Calcd. 366.44. Anal. Calcd. For $C_{19}H_{18}N_4O_2S$: C, 62.28; H, 4.95; N, 15.29. Found: C, 63.15; H, 5.12; N, 16.53.

*4.6.8. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(4-chlorophenyl)thiourea (**9h**)*

(Yield 87%). mp 202-205 ^{o}C; FTIR (KBr): 3355.57 & 3136.23 (N-H), 2962.06 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.82 (s, 6H, $2CH_3$), 5.82 (s, 2H, pyrrole-C_3, C_4-H), 6.85-6.88 (m, 2H, chlorophenyl-C_2, C_6-H), 7.15-7.19 (m, 2H, phenyl-C_4, C_6-H), 7.25-7.30 (m, 3H, phenyl-C_5-H and chlorophenyl-C_3, C_5-H), 7.44-7.48 (m, 1H, phenyl-C_3-H), 8.22 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.56 (dd, 1H, J = 0.92, 0.84 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz,

DMSO) δ ppm: 12.48, 107.20, 123.22, 125.52, 125.62, 128.58, 128.93, 129.32, 131.33, 135.44, 142.05, 145.00, 178.03; MS (ESI): *m/z* = found 366 [M^+]; calcd. 355.88. Anal. Calcd. For $C_{19}H_{18}ClN_3S$: C, 64.12; H, 5.10; N, 11.81. Found: C, 65.25; H, 5.82; N, 13.53.

*4.6.9. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(3-chlorophenyl)thiourea (**9i**)*

(Yield 89%). mp 182-185 ^oC; FTIR (KBr): 3306.59 & 3164.11 (N-H), 2982.26 (Ar-H) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* ppm: 1.83 (s, 6H, $2CH_3$), 5.79 (s, 2H, pyrrole-C_3, C_4-H), 6.80-6.83 (m, 1H, chlorophenyl-C_6-H), 7.08 (t, 2H, phenyl-C_6-H and chlorophenyl-C_2-H), 7.19-7.30 (m, 4H, phenyl-C_4, C_5-H and chlorophenyl-C_4, C_5-H), 7.45-7.49 (m, 1H, phenyl-C_3-H), 8.23 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.54 (dd, 1H, *J* = 1.12, 1.00 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm:12.35, 107.10, 123.48, 126.01, 127.93, 128.18, 128.72, 128.93, 130.94, 131.17, 135.71, 136.13, 136.49, 178.62; MS (ESI): *m/z* = found 356 [M^+ + 1]; calcd. 355.88. Anal. Calcd. For $C_{19}H_{18}ClN_3S$: C, 64.12; H, 5.10; N, 11.81. Found: C, 65.25; H, 5.82; N, 13.53.

*4.6.10. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-(3,4-dichlorophenyl)thiourea (**9j**)*

(Yield 98%). mp 194-196 ^oC; FTIR (KBr): 3305.68 & 3170.18 (N-H), 2979.53 (Ar-H) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) *δ* ppm: 1.83 (s, 6H, $2CH_3$), 5.79 (s, 2H, pyrrole-C_3, C_4-H), 6.83 (dd, 1H, *J* = 2.48 Hz, chlorophenyl-C_6-H), 7.05 (s, 1H, phenyl-C_6-H), 7.17-7.21 (m, 2H, phenyl-C_4-H and chlorophenyl-C_2-H), 7.26-7.30 (m, 1H, phenyl-C_5-H), 7.39 (d, 1H, *J* = 8.52 Hz, chlorophenyl-C_5-H), 7.45-7.49 (m, 1H, phenyl-C_3-H), 8.22 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.60 (dd, 1H, *J* = 0.84 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.35, 107.07, 124.34, 127.46, 128.84, 128.99, 130.65, 131.67, 131.98, 133.99, 134.71, 135.98, 178.56; MS (ESI): *m/z* = found 390 [M^+]; calcd. 390.33. Anal. Calcd. For $C_{19}H_{17}Cl_2N_3S$: C, 58.46; H, 4.39; N, 10.77. Found: C, 59.58; H, 5.12; N, 12.15.

*4.6.11. 1-(2-(2,5-dimethyl-1H-pyrrol-1-yl)phenyl)-3-p-tolylthiourea (**9k**)*

(Yield 87%). mp 210-212 ^{o}C; FTIR (KBr): 3349.54 & 3142.46 (N-H), 2966.09 (Ar-H) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm: 1.81 (s, 6H, $2CH_3$), 2.36 (s, 3H, methylphenyl-CH_3), 5.78 (s, 2H, pyrrole-C_3, C_4-H), 6.81 (d, 2H, J = 8 Hz, methylphenyl-C_2, C_6-H), 7.10-7.27 (m, 5H, phenyl-C_4, C_5, C_6-H and methylphenyl-C_3, C_5-H), 7.43-7.48 (m, 1H, phenyl-C_3-H), 7.93 (s, 1H, -NHCS-N<u>H</u>-Ar), 8.59 (dd, 1H, J = 0.64, 0.60 Hz, -N<u>H</u>-CS-NH-Ar); ^{13}C NMR (100 MHz, DMSO) δ ppm: 12.34, 21.11, 106.86, 125.29, 125.72, 128.17, 128.59, 128.88, 130.85, 132.52, 136.35, 137.84, 178.82; MS (ESI): *m/z* = found 336 [M^+ + 1]; calcd. 335.47. Anal. Calcd. For $C_{20}H_{21}N_3S$: C, 71.61; H, 6.31; N, 12.53. Found: C, 73.15; H, 7.12; N, 13.15.

5. Biological activity

5.1. In vitro evaluation of antitubercular studies

All the compounds were tested for inhibition of *M. tuberculosis* strain H37RV using Microplate Alamar Blue Assay (MABA) as described earlier [46]. The 96 wells plate received 100 mL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with the drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25,12.5, 25, 50 and 100 mg/mL. Plates were covered and sealed with parafilm and incubated at 37^{o}C for 5 days. Then, 25 mL of freshly prepared 1:1 mixture of almar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, while pink color was scored as the growth. The MIC was defined as the lowest drug concentration, which prevented the color change from blue to pink. Table 1 reveals the anti-TB activity data, expressed in MIC.

5.2. In vitro evaluation of Antibacterial activity

MIC determination of the tested compounds was investigated by a side-by-side comparison with norfloxacin and ciprofloxacin against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*) by the broth microdilution method [47, 48]. Serial dilutions of the test compounds and reference drugs were prepared in Mueller-Hinton agar. Drugs (10 mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL). Further progressive dilutions with the molten Mueller-Hinton agar were performed to obtain the required concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL. The tubes were inoculated with 105 cfu mL^{-1} (colony forming unit/mL) and incubated at 37^{o}C for 18 h. MIC was the lowest concentration of the tested compound that yielded no visible growth on the plate. To ensure that solvent had no effect on bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments and DMSO had no effect on the

microorganisms in the concentrations studied. Table 1 reveals the antibacterial activity (MIC values) data.

5.3. MTT-based cytotoxicity activity

Cellular conversion of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] into a formazan product [49] was used to evaluate cytotoxic activity (IC_{50}) of some of the compounds against A549 (lung adenocarcinoma) cell-line up to concentrations of 50 mg/mL using Promega Cell Titer 96 non-radioactive cell proliferation assay [50] with cisplatin as the positive control. The IC_{50} values are the averages ± SEM of three independent experiments, which are presented in Table 2.

5.4. Enzyme inhibition studies

5.4.1. InhA expression and purification

The production and purification of InHA-6xHis protein from a protease-deficient strain of *E. coli* (BL21) transformed with the pHAT5/InhA plasmid were performed as follows. 1 mL of bacteria was grown in 100 mL of Lysogeny broth (LB) medium containing ampicillin (100 µg/mL) and 2% glucose at 37°C. After 4 h, the solution was re-diluted in 1 L of the same medium and re-grown at 37°C. When the proper concentration (OD_{595} = 0.6 - 0.8) was reached, the culture was centrifuged at 3300 g for 10 min at 4^{o}C and the bacteria were suspended in LB medium containing ampicillin (100 µg/mL). Protein expression was induced for overnight incubation in 1 mM Isopropyl-β-D-galactopyranoside (IPTG) at 20°C. Cells were harvested by centrifugation at 6000 g for 30 min at 4°C. The dry pellet was kept at -80°C for several months and purification was performed with Ni-NTA Agarose from QIAGEN as described by the manufacturer's protocol. The purified recombinant protein was applied to PD-10 desalting columns (GE Healthcare, Piscataway, NJ) equilibrated with PIPES 30 mM pH 6.8, 150 mM NaCl to remove imidazole. Samples were analyzed using

SDS-PAGE and Coomassie blue staining and then stored at 4°C for short-term storage at -80 °C with 20% glycerin for long-term storage [51].

5.4.2. InhA activity inhibition

Triclosan and NADH were obtained from Sigma-Aldrich. Stock solutions of the selected compounds were prepared in DMSO such that the final concentration of this co-solvent was constant at 5% (v/v) in the final volume of 1 mL for all kinetic reactions. Kinetic assays were performed using *trans*-2-dodecenoyl-coenzyme A (DDCoA) and wild type InhA as previously described [52]. Briefly, reactions were performed at 25°C in an aqueous buffer (30 mM PIPES and 150 mM NaCl pH 6.8) containing 250 µM cofactor (NADH), 50 µM substrate (DDCoA) and the test compound (at 50 µM). Reactions were initiated by the addition of InhA (100 nM final) and NADH oxidation was followed at a fixed 340 nm wavelength. Inhibitory activity of each derivative was expressed as % inhibition of InhA activity (initial velocity of the reaction) with respect to control reaction without inhibitor. These results are shown in Table 3.

Conclusion

In this study, novel compounds viz., 1-(4-(1*H*-pyrrol-1yl)phenyl)-3-substitutedprop-2-en-1-ones (**3a-s**), 1-(4-(1*H*-pyrrol-1yl)phenyl)-3-substitutedprop-2-en-1-ones (**4a-s**), 5-(4-(1*H*-pyrrol-1yl)phenyl)-3-substitutedphenylisoxazoles (**5a-r**) and 1-(2-(2,5-dimethyl-1*H*-pyrrol-1yl)phenyl)-3-(substituted phenyl)thioureas (**9a-k**) have been synthesized and identified as the potent InhA inhibitors. These pyrrole derivatives were further explored in search of novel antitubercular and antibacterial agents, identifying several derivatives with reasonable inhibitory activities against *M. tuberculosis.* Of all the compounds tested, **9a-k** displayed better activities against both Gram positive and Gram negative bacteria with the MIC value of 0.2 - 1.6 µg/mL. Compounds **3d, 3e, 3g, 3m, 3o, 4d, 4e, 4i, 4m, 4p, 4q** and **5m** displayed asignificant activities (6.25 µg/mL) against *M. tuberculosis* H37Rv strain. The two compounds viz., **9b** and **9d** exhibited interesting anti-TB activities with the MIC of 0.8 µg/mL and no apparent cytotoxicites towards human lung cancer cell-line (A549). This outcome indicates that novel chemophores are not toxic and might be considered for further structural modification. Furthermore, compounds **9b** and **9d** displayed good inhibition activities InhA.

Molecular docking of the compounds was carried out for better understanding of the drug-receptor interaction. Docking simulation studies have shown that these compounds are bound mainly with the substrate binding site of InhA and the scoring function for most of the compounds is similar to that of the reference inhibitor. The anti-TB activity of these compounds was fully supported by in *silico* molecular docking calculations. The synthesized compounds will be quite useful as the lead compounds for developing InhA inhibitors.

Our future perspective is to identify the mechanism of action and explore pyrrole analogues which might serve as new template for further investigations in this field amid selective, less

toxic anti-TB agents to merit cost effective and reduced treatment time. More studies are underway to improve their efficiency against InhA and *M. tuberculosis*

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Figure captions:

Figure 1. Milestone in TB drug research.

Figure 2a. Commercially available drugs containing chalcones, isoxazole and pyrazolines.

Figure 2b. Some of the pyrazoline based antitubercular agents.

Figure 3. Design concept for the synthesis of titled compounds.

Figure 4. (A & B). Alignment of docked compound in the active site of the enzyme (PDB ID 4TZK).

Figure 5. Superimposition of X-ray crystal structures of A) ligand (redorange) with B) compound **4p** (yellow), C) compound **5d** (magenta) D) compound **3b** (blue) at the InhA binding pocket.

Figure 6 (A & B). Docking confirmation of compound **4q** and H-bonds are indicated by a dashed yellow line.

Figure 7 (A & B). Docking confirmation of compound **9d** and H-bond is indicated by a dashed yellow line.

Figure 8 (A & B). Docking confirmation of compound **5g** and H-bonds are indicated by a dashed yellow line.

Figure 9. A) Hydrophobic and B) hydrophilic amino acid residues of active site surrounded to **4q.**

Table 1: *In vitro* evaluation of antitubercular and antibacterial activity

Comp.	MIC values (µg/mL)	Gram Positive	Gram Negative	Comp.	MIC values (µg/mL)	Gram Positive	Gram negative
	M. tuberculosis H37Rv	*S. aureus*	*E. Coli*		*M. tuberculosis* H37Rv	*S. aureus* (µg/mL)	*E. Coli* (µg/mL)
3a	50	25	25	**4n**	12.5	100	50
3b	50	50	50	**4o**	25	50	100
3c	25	25	50	**4p**	6.25	25	50
3d	12.5	12.5	12.5	**4q**	6.25	25	12.5
3e	12.5	25	12.5	**5a**	100	50	25
3f	100	50	25	**5b**	50	25	25
3g	12.5	25	12.5	**5c**	25	50	50
3h	50	50	25	**5d**	12.5	25	12.5
3i	25	12.5	12.5	**5e**	12.5	25	25
3j	25	50	50	**5f**	50	25	25
3k	25	25	50	**5g**	12.5	25	12.5
3l	25	25	50	**5h**	100	50	50
3m	12.5	12.5	12.5	**5i**	12.5	25	25
3n	50	12.5	12.5	**5j**	50	25	50
3o	25	25	12.5	**5k**	25	50	25
3p	25	50	50	**5l**	100	25	50
3q	25	50	50	**5m**	6.25	25	12.5
3r	50	25	25	**5n**	12.5	50	50
4a	25	50	50	**5o**	25	50	25
4b	25	25	50	**9a**	50	0.8	0.2
4c	25	50	25	**9b**	0.8	0.2	0.2
4d	6.25	25	12.5	**9c**	1.6	0.8	0.8
4e	6.25	25	12.5	**9d**	0.8	0.4	0.2
4f	50	50	25	**9e**	50	1.6	0.8
4g	12.5	12.5	12.5	**9f**	50	0.2	0.2
4h	100	100	50	**9g**	50	0.2	0.4
4i	12.5	25	12.5	**9h**	50	0.2	0.2
4j	25	50	25	**9i**	50	0.2	0.4
4k	25	25	50	**9j**	6.25	0.2	0.2
4l	25	25	50	**9k**	50	0.4	6.25
4m	6.25	12.5	25				
		Ethambutol			0.5	--	--
		Rifampicin			0.4	--	--
		Norfloxacin			--	2.02	4
		Ciprofloxacin			--	0.15	0.25

Table 2: MTT-based cytotoxicity activity of selected compounds against human lung cancer cell line A549

Compound	R	IC_{50} (µM)
3d	4-CH_3	198
3n	2,4-OCH_3	99
4m	4-$CH(CH_3)_2$	39.6
4e	4-OCH_3	247.5
5m	4-$CH(CH_3)_2$	39.6
5e	4-OCH_3	79.2
9b	2-F	198
9d	4-CF_3	99
Cisplatin	--	9.90

IC_{50} - is half maximal inhibitory concentration- it is the half maximal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC_{50})

Table 3: Enzyme inhibition values. Results are expressed as % InhA inhibition.

Compound	% Inhibition at 50 µM
9a	ND
9b	29
9c	ND
9d	100
9e	ND
9f	ND
9g	ND
9h	NI
9i	ND
9j	13
9k	ND
Triclosan	>99

ND - not determined, NI - no inhibition at the given concentration

Table 4: Surflex dock scores (kcal/mol) of pyrrolyl chalcones, pyrazolines, isoxazoles and thiourea derivatives.

Comp.	C score[a]	Crash score[b]	Polar score[c]	D score[d]	PMF score[e]	G score[f]	Chem score[g]
4TZK ligand	8.73	-1.39	1.18	-168.11	-49.19	-285.29	-37.47
3a	5.80	-1.00	1.61	-102.00	-67.74	-192.04	-42.65
3b	6.05	-1.09	1.71	-131.14	-58.88	-208.11	-42.39
3c	4.94	-1.06	0.00	-109.79	-39.02	-221.85	-40.75
3d	1.65	-2.20	0.38	-174.48	88.56	-261.85	-49.79
3e	4.79	-1.23	0.82	-126.58	-59.57	-203.34	-37.65
3f	3.70	-1.86	1.15	-108.42	-61.57	-187.67	-43.68
3g	5.06	-0.78	0.00	-101.19	-40.81	-190.30	-37.33
3h	4.55	-1.50	0.00	-116.54	-50.39	-213.85	-40.84
3i	5.20	-1.15	0.00	-142.18	-61.77	-205.51	-39.58
3j	4.41	-1.89	0.95	-93.58	-41.37	-191.63	-34.14
3k	3..42	-1.33	0.96	-79.29	-47.83	-170.84	-32.62
3l	5.12	-1.23	1.56	-115.86	-62.80	-186.28	-44.87
3m	3.99	-1.86	1.13	-93.44	-52.66	-188.23	-33.90
3n	5.78	-1.36	0.00	-140.35	-35.78	-203.00	-38.39
3o	6.61	-1.41	0.00	-125.15	-54.73	-240.52	-41.96
3p	**7.01**	-1.34	0.93	-118.38	-76.89	-218.97	-41.81
3q	6.54	-1.17	2.66	-106.43	-72.33	-196.33	-43.50
3r	4.53	-1.04	1.13	-80.70	-46.66	-180.28	-34.20
4a	4.08	-2.73	0.00	-118.01	-49.95	-229.73	-35.99
4b	4.40	-1.04	0.00	-116.97	-31.11	-197.54	-31.39
4c	3.95	-1.65	0.00	-125.00	-31.09	-214.20	-32.38
4d	4.52	-1.24	0.00	-116.78	-28.18	-205.00	-30.89
4e	6.80	-2.03	1.13	-154.81	-44.41	-287.71	-43.23
4f	4.11	-1.10	0.00	-118.84	-35.65	-199.77	-31.79
4g	1.60	-3.79	0.56	-180.16	57.04	-282.42	-47.64
4h	5.86	-3.16	0.00	-157.86	-34.35	-287.55	-37.51
4i	**6.90**	-2.82	1.13	-170.15	-35.30	-314.27	-45.18
4j	4.53	-1.10	0.00	-121.75	-29.52	-215.85	-32.17
4k	4.85	-1.09	0.01	-145.64	-41.19	-247.42	-36.43
4l	1.97	-5.39	0.00	-157.45	16.26	-323.98	-36.72
4m	4.87	-2.75	0.00	-151.61	-35.78	-292.48	-42.14
4n	6.03	-2.22	0.00	-154.08	-22.72	-262.82	-38.42
4o	2.88	-0.69	0.00	-75.28	-51.69	-172.25	-30.03
4p	6.84	-0.94	0.00	-136.82	-39.23	-229.22	-32.45
4q	**7.51**	-2.37	1.20	-169.32	-44.52	-312.90	-44.52
5a	4.54	-0.91	0.95	-81.10	-33.14	-196.08	-37.21
5b	4.30	-0.45	0.05	-93.94	-41.78	-206.15	-36.53
5c	3.90	-0.32	0.79	-75.51	-34.98	-159.19	-35.56
5d	5.26	-0.83	0.00	-98.78	-31.47	-223.46	-38.13
5e	5.07	-1.00	0.00	-104.54	-43.91	-226.27	-35.36
5f	3.85	-2.38	0.00	-128.59	-16.05	-240.05	-37.67
5g	4.84	-0.82	0.91	-85.35	-35.36	-201.72	-37.78

5h	5.13	-1.55	0.00	-132.36	-22.17	-247.07	-39.18
5i	4.84	-0.47	0.00	-117.96	-52.44	-219.58	-35.51
5j	4.30	-0.87	0.04	-104.69	-45.67	-231.13	-38.17
5k	5.28	-1.70	0.01	-142.16	-21.78	-261.78	-39.61
5l	3.89	-1.59	0.00	-107.26	-41.88	-220.67	-39.62
5m	4.73	-0.77	0.00	-121.57	-43.82	-236.66	-36.77
5n	6.21	-0.89	0.00	-133.84	-42.92	-251.75	-39.53
5o	5.08	-0.62	0.00	-106.44	-51.49	-233.16	-37.89
9a	4.09	-0.81	0.00	-94.29	-33.82	-121.67	-30.39
9b	4.65	-1.09	0.00	-99.41	-27.43	-126.33	-30.54
9c	3.24	-4.25	0.01	-151.61	-51.35	-223.51	-39.91
9d	5.40	-0.85	0.00	-109.10	-29.25	-150.10	-32.73
9e	3.25	-4.80	0.36	-150.16	-56.42	-193.21	-41.33
9f	2.10	-4.72	0.26	-142.14	-55.21	-211.83	-39.26
9g	3.02	-1.64	0.67	-85.14	-18.52	-106.16	-25.81
9h	3.53	-3.38	0.00	-132.27	-41.31	-163.28	-40.16
9i	3.99	-2.20	0.00	-110.73	-32.54	-160.55	-34.46
9j	6.38	-0.70	0.00	-110.73	-33.25	-155.99	-35.03
9k	6.49	-0.74	0.00	-110.49	-31.31	-160.99	-33.03

[a] CScore (Consensus Score) integrates a number of popular scoring functions for ranking the affinity of ligands bound to the active site of a receptor and reports the output of total score.

[b] Crash-score revealing the inappropriate penetration into the binding site. Crash scores close to 0 are favorable. Negative numbers indicate penetration.

[c] Polar indicating the contribution of polar interactions to the total score.

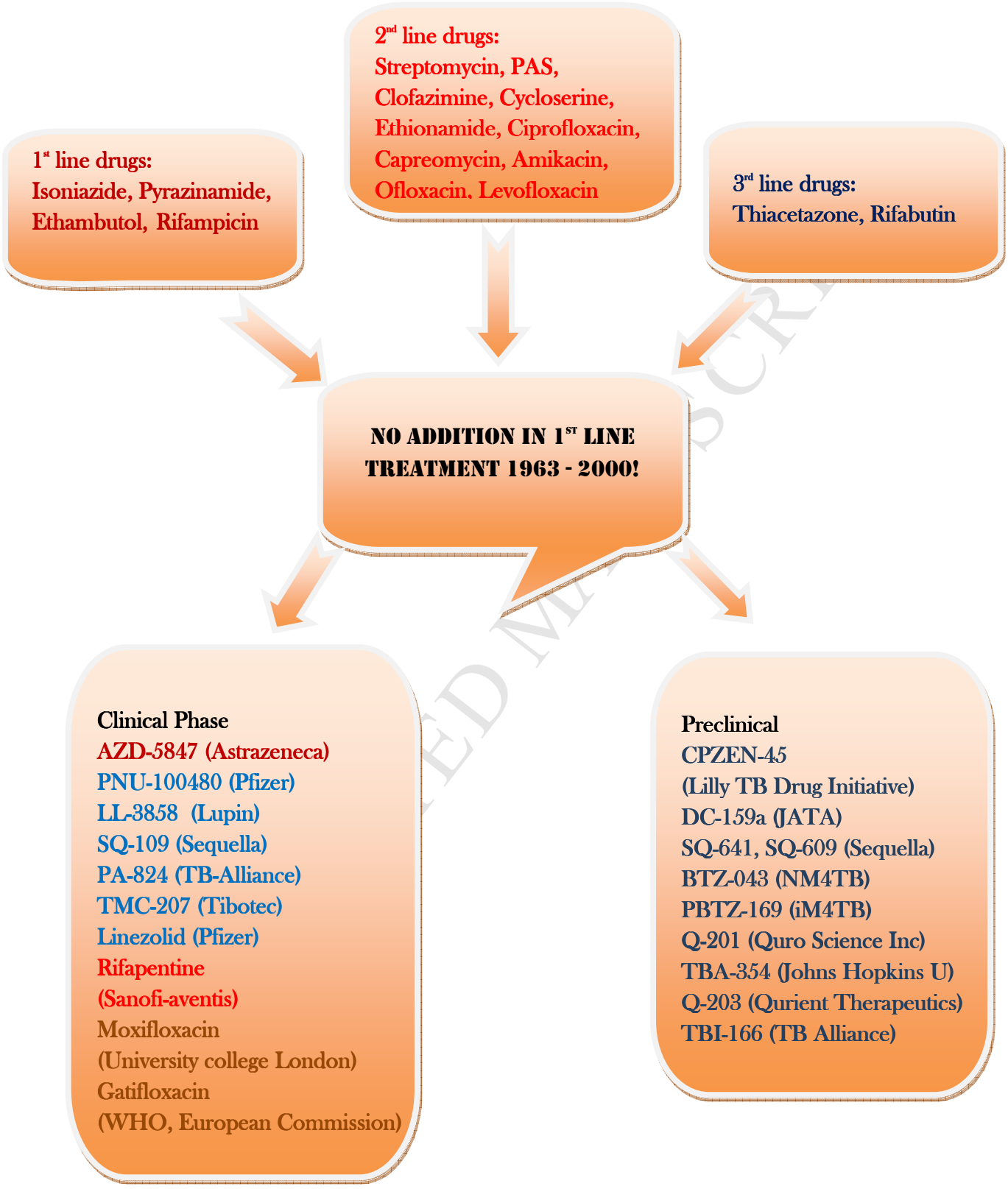
[d] D-score for charge and van der Waals interactions between the protein and the ligand (work of Kuntz) [32].

[e] PMF-score indicating Helmholtz free energies of interactions for proteineligand atom pairs (Potential of Mean Force, PMF) (work of Muegge and Martin) [33].

[f] G-score showing hydrogen bonding, complex (ligand-protein), and internal (ligandeligand) energies (work of Willett's group) [34].

[g] Chem-score points for H-bonding, lipophilic contact, and rotational entropy, along with an intercept term (work of Eldridge, Murray, Auton, Paolini, and Mee) [35]

Fig. 1. Milestones in TB drug research



Abbreviations: TB, Tuberculosis; MDR, Multi-Drug resistant; JATA, Japan Anti-Tuberculosis Association; NM4TB, New Medicines for Tuberculosis; iM4TB, Innovative Medicines for Tuberculosis.

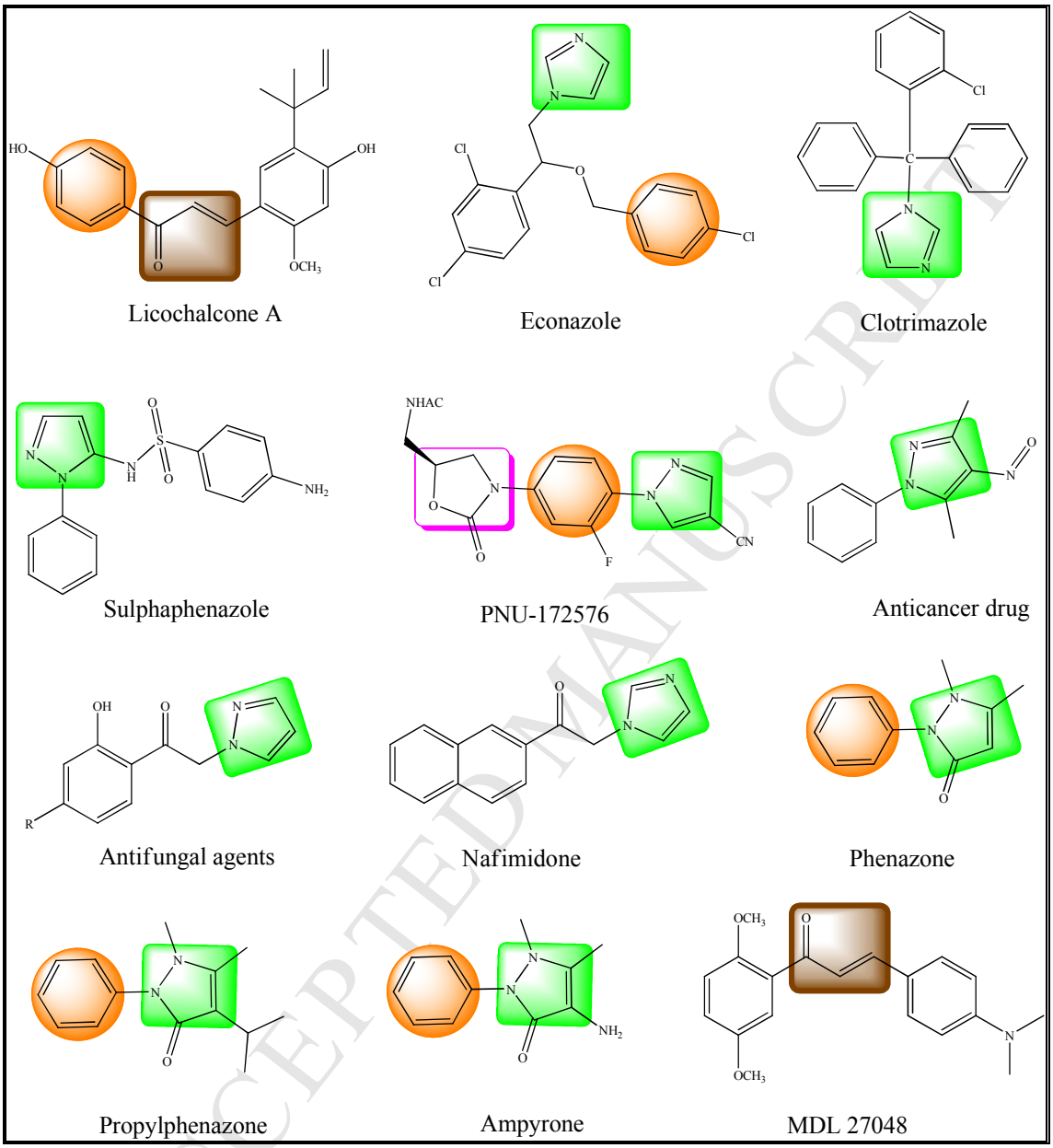


Fig. 2a. Commercially available drugs containing chalcones, isoxazole and pyrazolines.

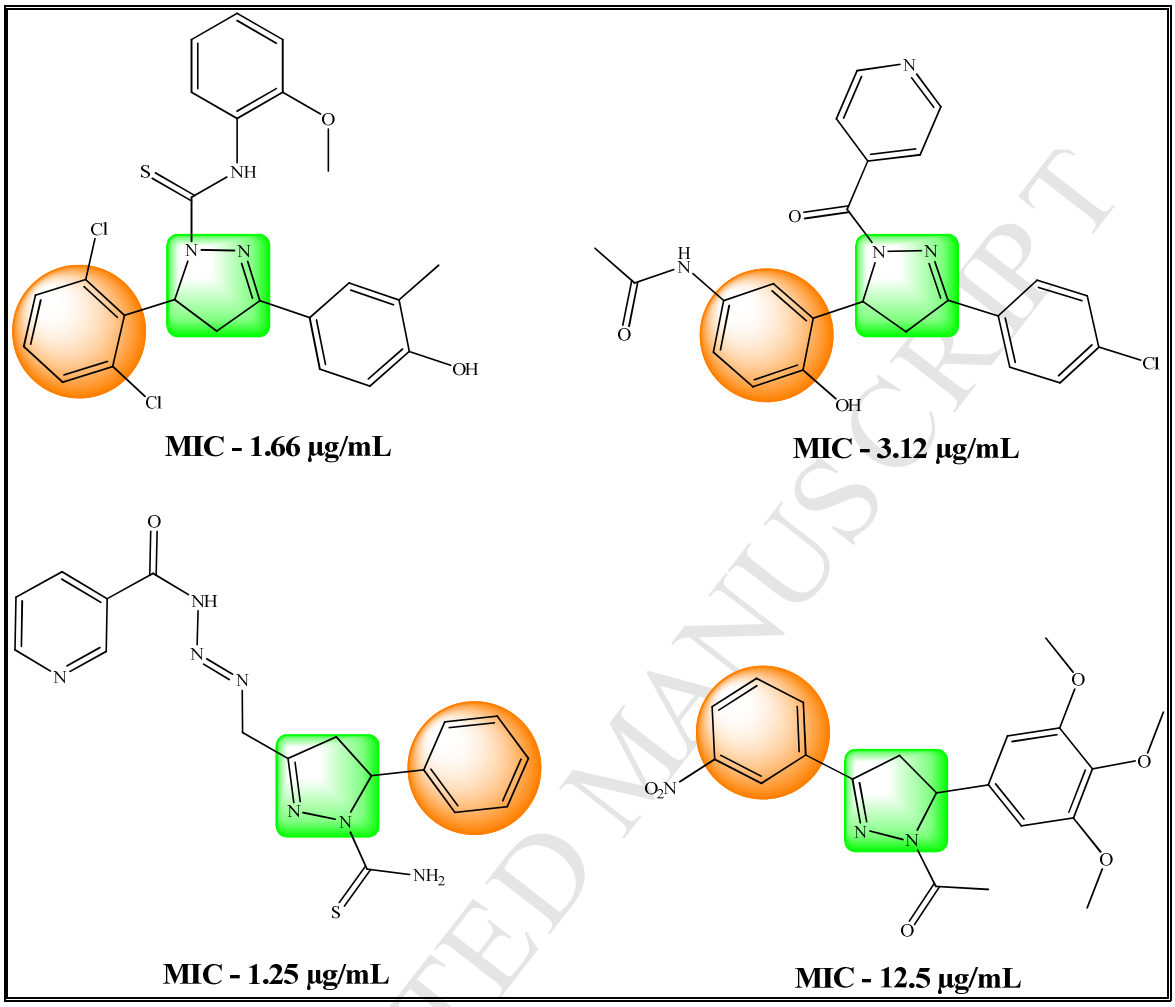


Fig. 2b. Some of the pyrazoline based antitubercular agents.

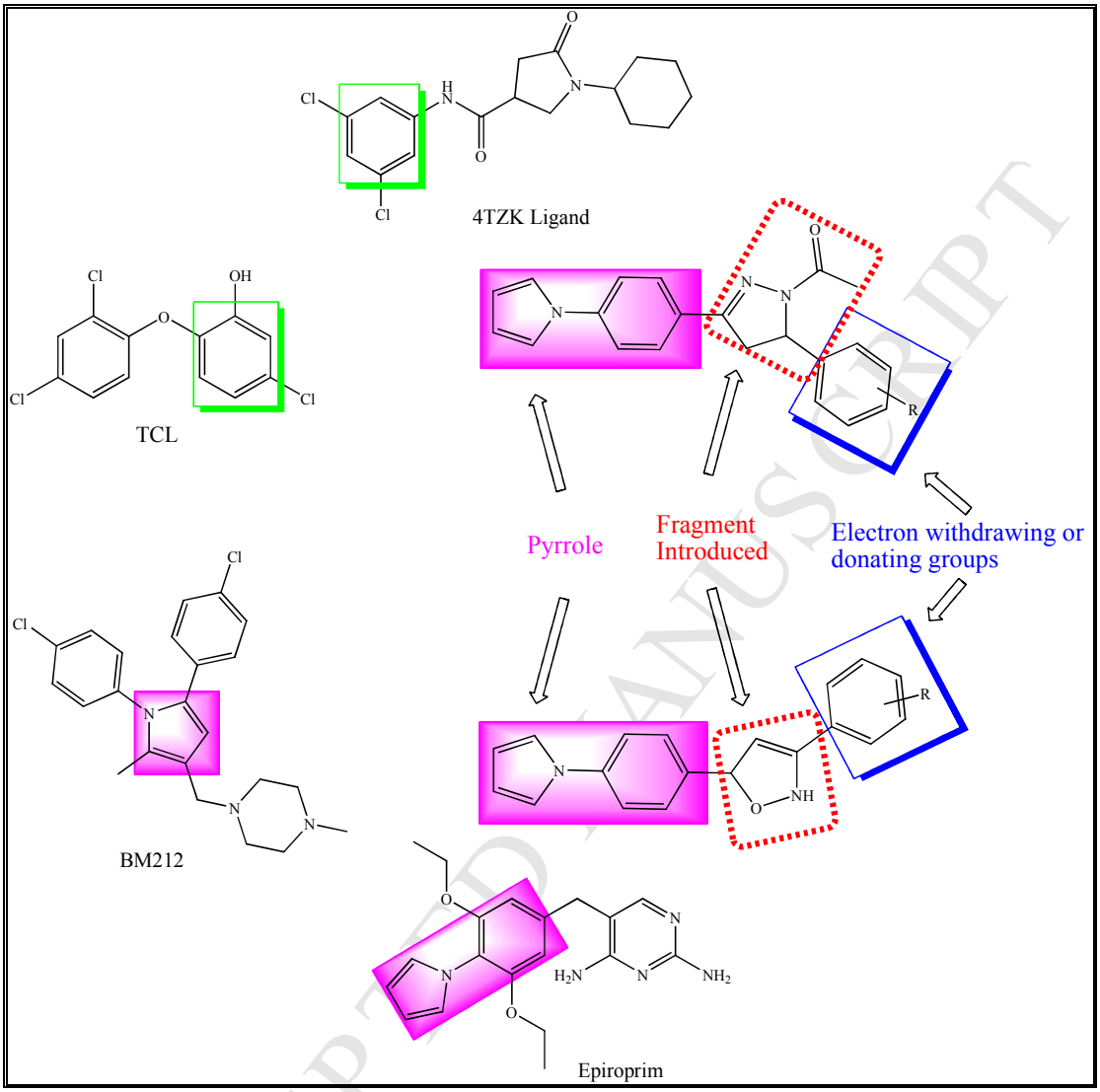


Fig. 3. Design concept for the synthesis of titled compounds.

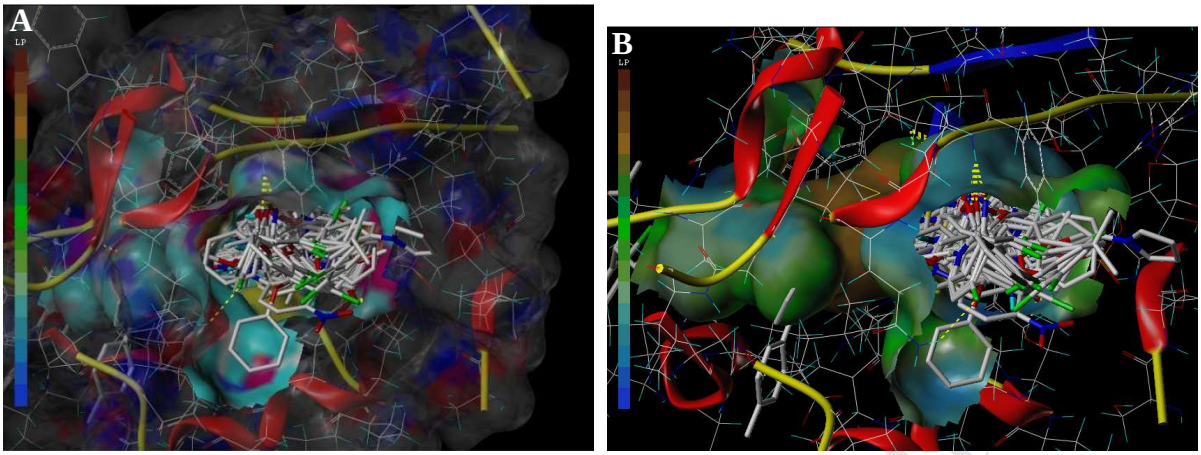


Fig. 4 (A & B). Alignment of docked compound in the active site of the enzyme (PDB ID 4TZK).

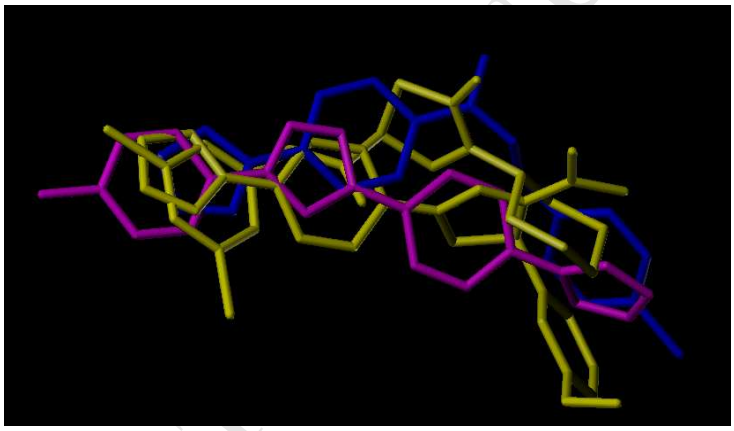


Fig. 5. Superimposition of X-ray crystal structures of ligand (redorange), compound **4p** (yellow), compound **5d** (magenta) and compound **3b** (blue) in the InhA binding pocket.

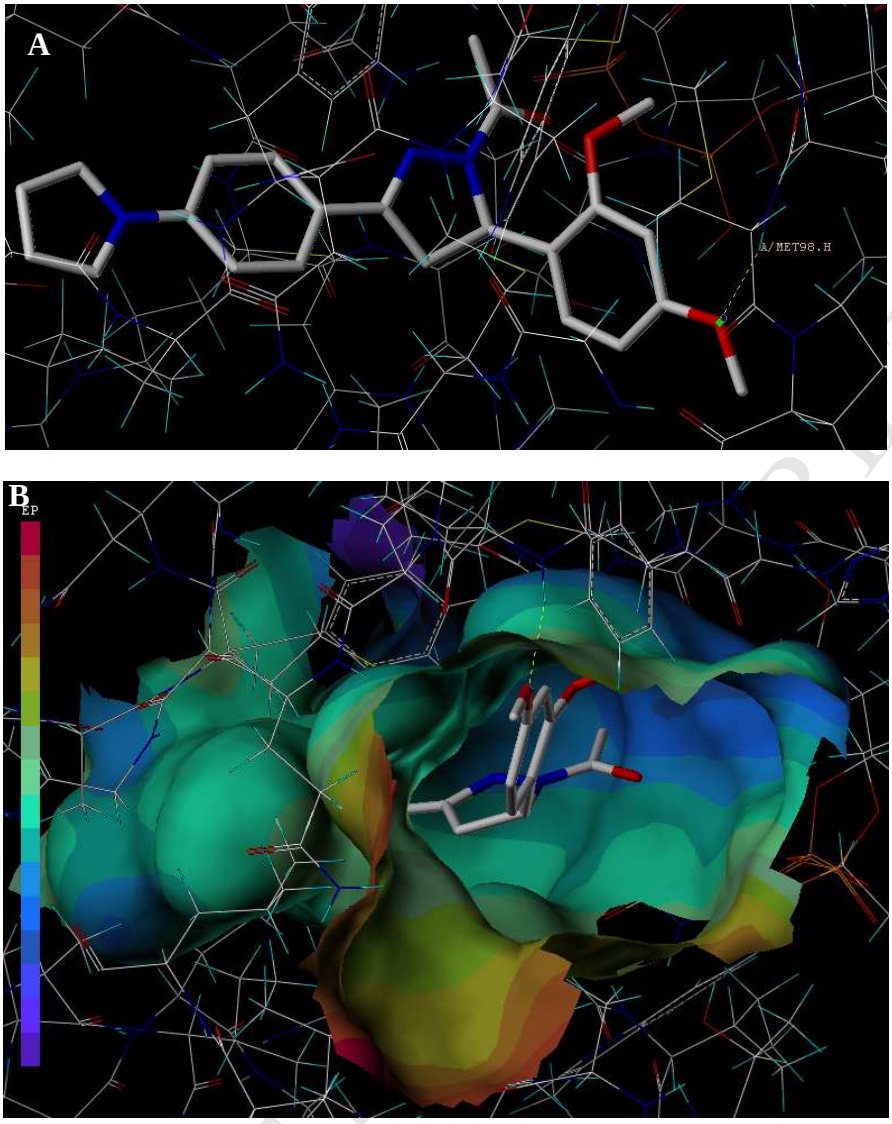


Fig. 6 (A & B). Docking confirmation of compound **4q** at the active site.

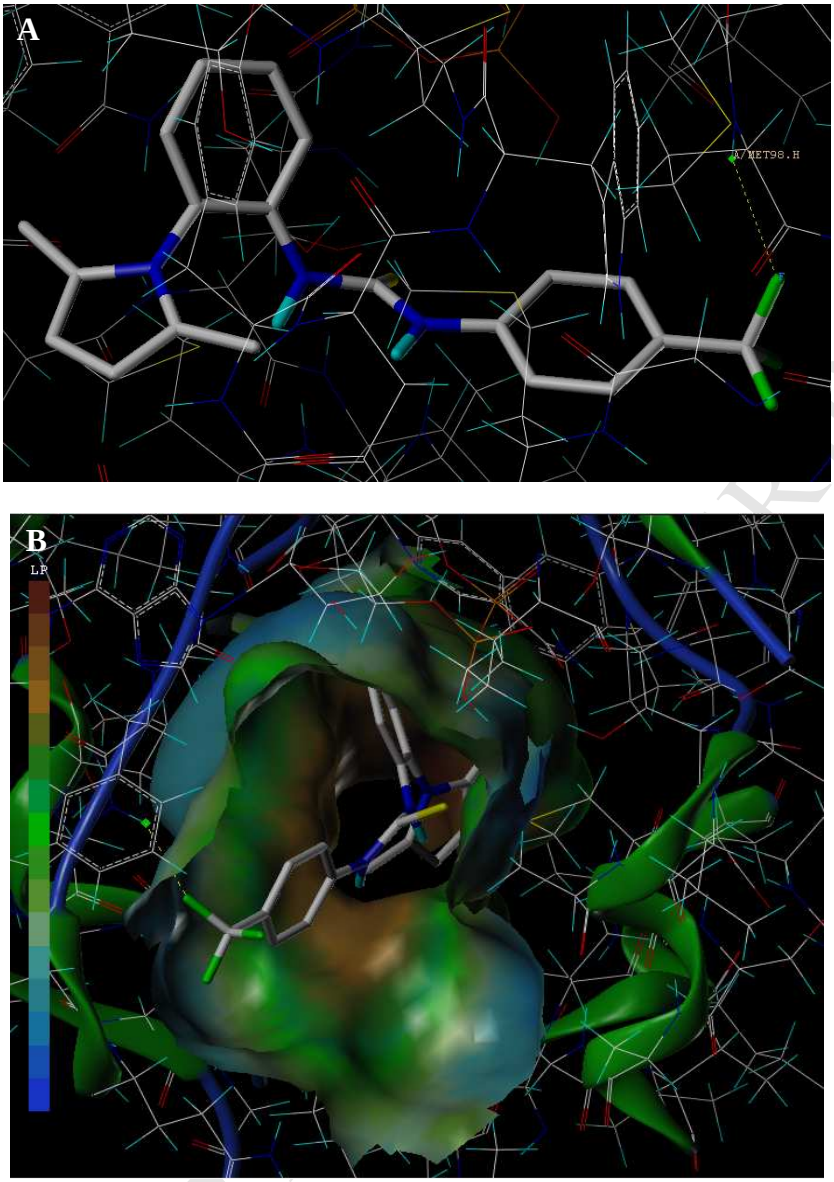


Fig. 7(A & B). Docking confirmation of compound **9d** at the active site

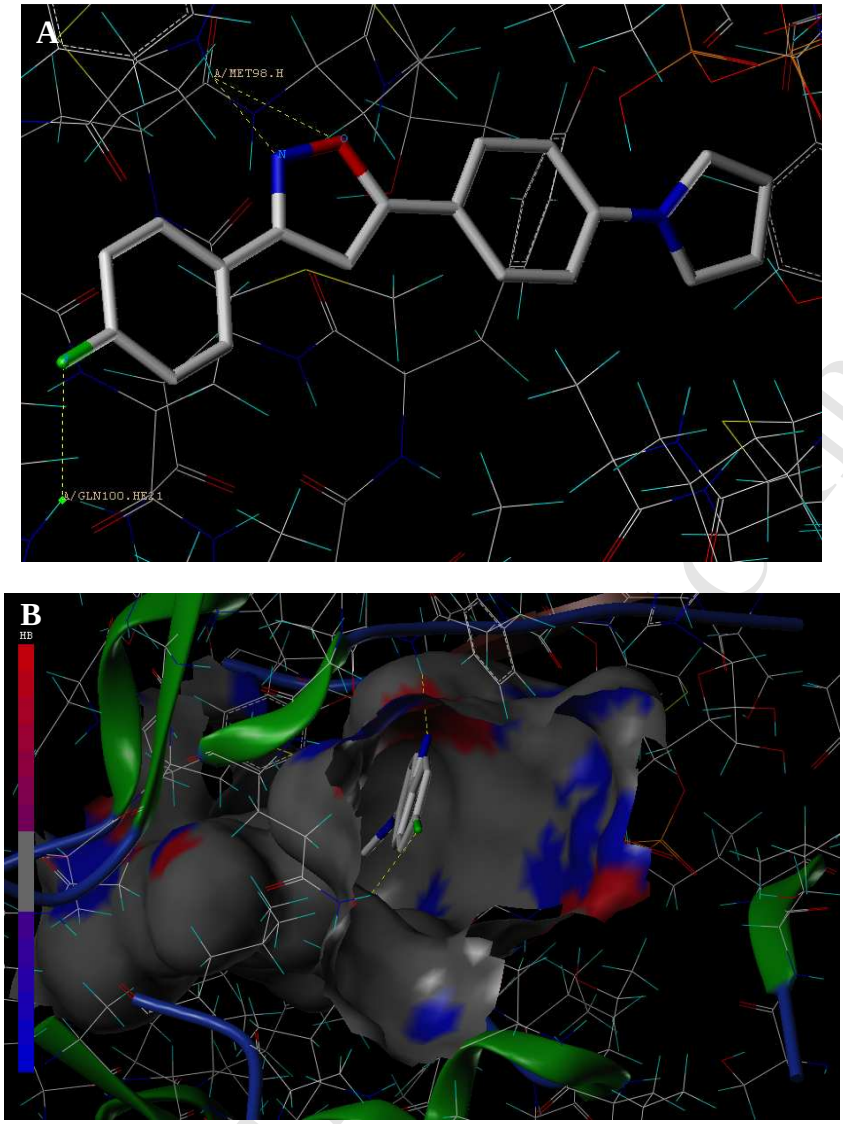


Fig. 8 (A & B). Docking confirmation of compound **5g** at the active site.

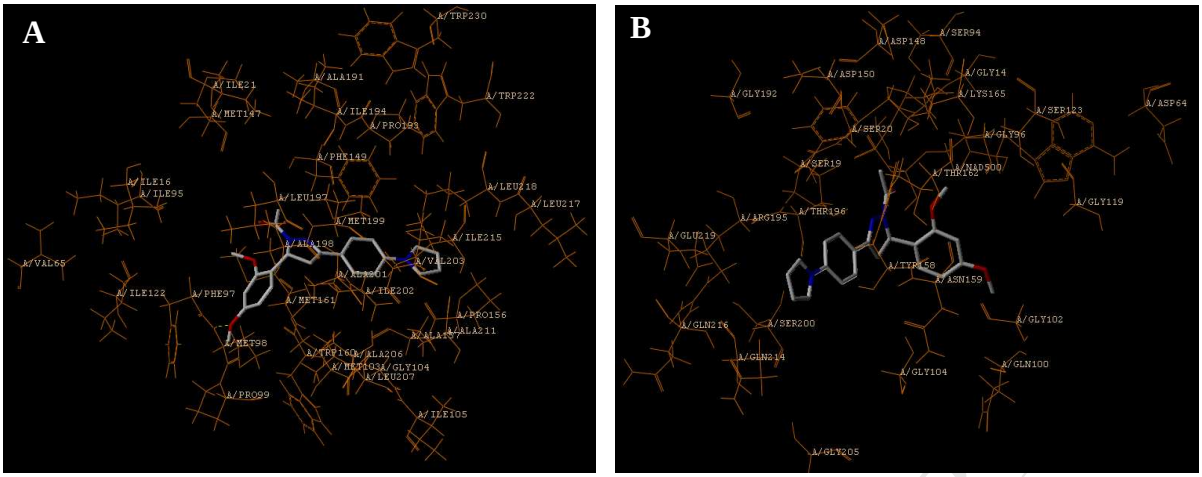
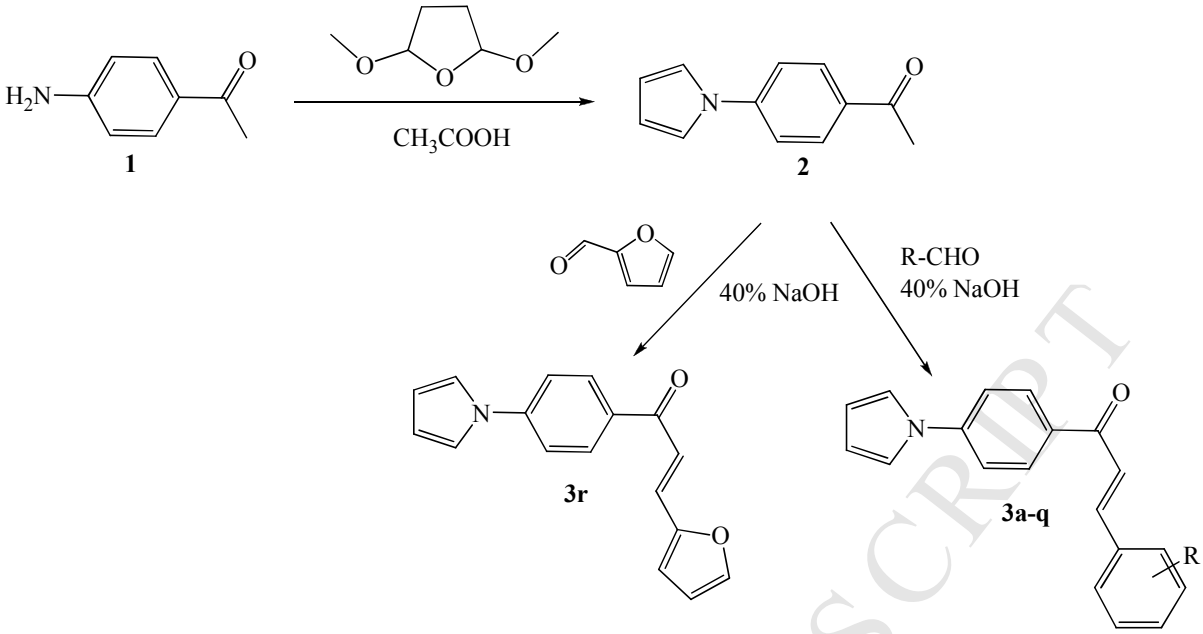
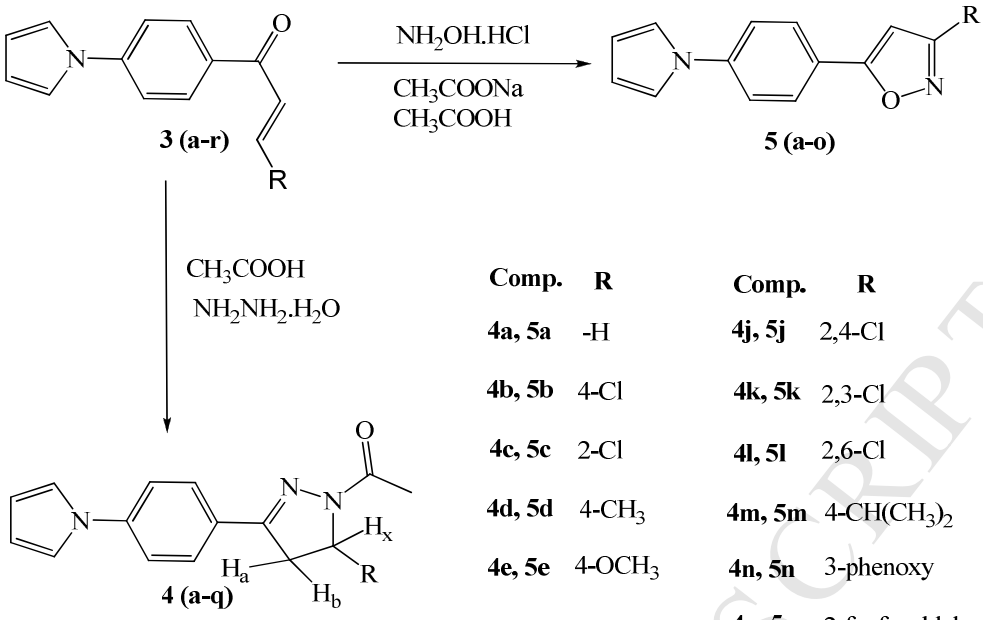


Fig. 9. A) Hydrophobic and B) hydrophilic amino acid residues of active site surrounded to **4q.**



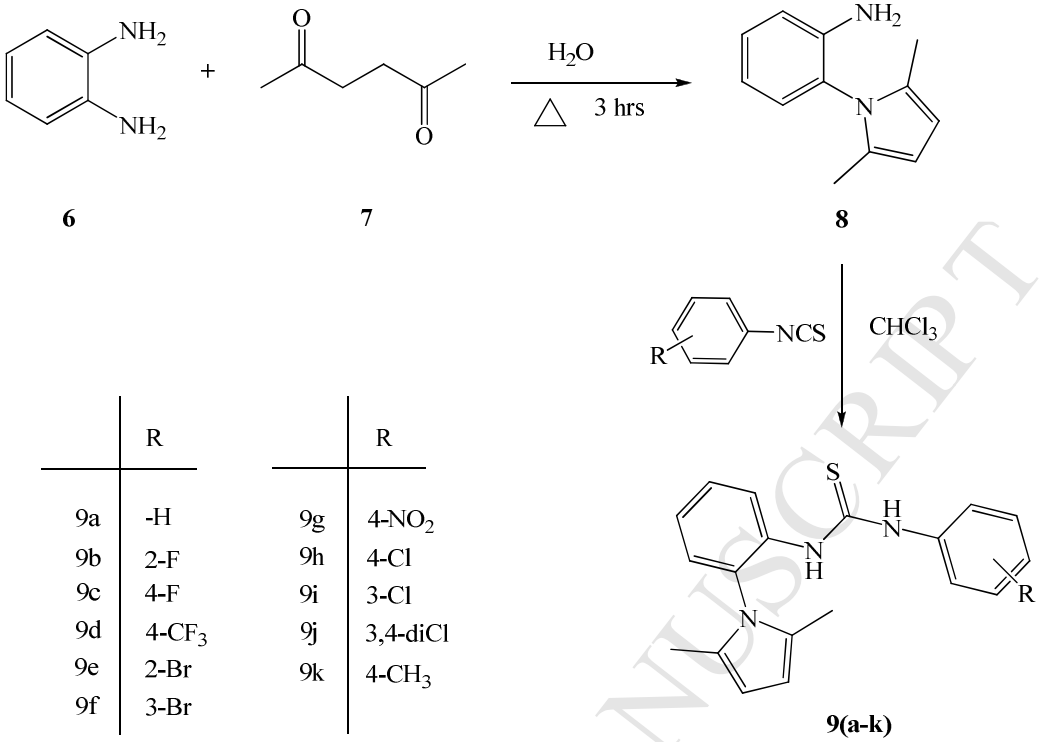
Comp.	R	Comp.	R
3a	-H	**3j**	2,4-Cl
3b	4-Cl	**3k**	2,3-Cl
3c	2-Cl	**3l**	2,6-Cl
3d	4-CH_3	**3m**	4-$CH(CH_3)_2$
3e	4-OCH_3	**3n**	2,4-OCH_3
3f	3-Br	**3o**	3-OCH_3
3g	4-F	**3p**	3-phenoxy
3h	2-Br	**3q**	2-OH
3i	3,4-OCH_3		

Scheme 1: Synthetic route of a novel series of pyrrole chalcone derivatives.



Comp.	R	Comp.	R
4a, 5a	-H	**4j, 5j**	2,4-Cl
4b, 5b	4-Cl	**4k, 5k**	2,3-Cl
4c, 5c	2-Cl	**4l, 5l**	2,6-Cl
4d, 5d	4-CH_3	**4m, 5m**	4-$CH(CH_3)_2$
4e, 5e	4-OCH_3	**4n, 5n**	3-phenoxy
4f, 5f	3-Br	**4o, 5o**	2-furfuraldehyde
4g, 5g	4-F	**4p**	3-OCH_3
4h, 5h	2-Br	**4q**	2,4-OCH_3
4i, 5i	3,4-OCH_3		

Scheme 2: Synthetic route of a novel series of pyrrole isoxazole and pyrazoline derivatives.



	R
9a	-H
9b	2-F
9c	4-F
9d	4-CF_3
9e	2-Br
9f	3-Br

	R
9g	4-NO_2
9h	4-Cl
9i	3-Cl
9j	3,4-diCl
9k	4-CH_3

Scheme 3: Synthetic route of a novel series of pyrrolyl phenyl thioureas.

Research Highlights

- Inhibitors of mycobacterial Enoyl ACP reductase were designed using in *silico* approach.
- Synthesis of a range of these pyrrolyl chalcones, pyrazoles, isoxazoles and phenyl thiourea derivatives is described.
- Surflex docking studies were carried out to understand the binding affinity of the compounds
- Inhibitors were active against *Mycobacterium tuberculosis, Staphylococcus aureus, Eschrichia coli, Cell-line (A549)* and *InhA*.