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Novel Hybrid-Pyrrole Derivatives: Their Synthesis, Antitubercular Evaluation and Docking Studies

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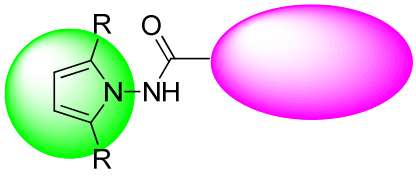

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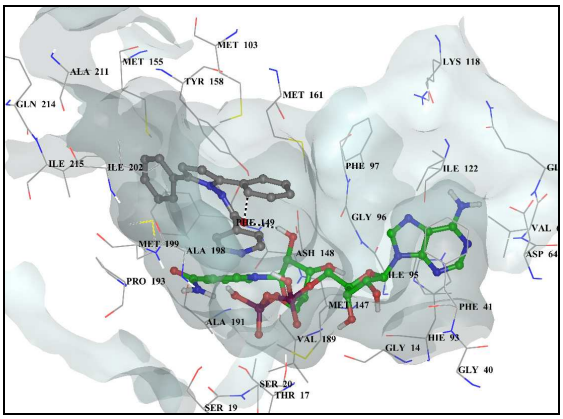

Docking on Mycobacterium InhA

Hybridization of the molecular fragments proved to be beneficial as revealed by the biological activity of the synthesized compounds.

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Hybrid new molecules for the treatment of tuberculosis are one of the latest approaches. Keeping this concept in mind, thirty two hybrid compounds were synthesized, with pyrrole as one of the moiety, clubbed to coumarin, ibuprofen, isoniazid etc. The compounds were evaluated against *Mycobacterium tuberculosis* $H_{37}Rv$ strain. Compounds **7e** and **8e** exhibited MIC of 3.7 and 5.10 μg/mL and growth inhibition of 95 and 92% respectively. These compounds were also active against single drug resistant bacterial strains. The compounds were devoid of cytotoxicity when tested against Vero African green monkey kidney cell line. Docking study was carried out on enoyl acyl carrier protein enzyme to provide some understanding into the mechanism of action of these compounds.

1. Introduction

Despite the enormous efforts have been made in the quest of new drugs, tuberculosis (TB) still is one of the rapidly emerging and deadly infections caused by the pathogen *Mycobacterium tuberculosis* (Mtb). In the year 2013, 9 million new cases were reported with 1.4 million fatal incidents[1]. The rampant bacterial resistances *viz.* multi-drug resistant (MDR) TB and extensively drug-resistant (XDR) TB to existing anti-TB agents has become a significant concern for the effective treatment of TB[2, 3].

Antitubercular agents can be divided into two classes: first-line drugs: isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), rifampin (RIF), and streptomycin given for 6 months. If the treatment fails due to bacterial drug resistance, second line drugs such as *p*-amino salicylic acid (PAS), kanamycin, fluoroquinolones, capreomycin, ethionamide, and cycloserine are used. These are mostly either less effective or more toxic with serious side-effects. Hence, it is quite essential to develop safe and cost-effective new antitubercular agents.

Molecular hybridization of potential pharmacophore scaffold is being used as valuable approach by medicinal chemists to design new prototypes. These molecules effectively address resistance problem and have an increased efficacy. The hybrid molecules are generated by covalent addition of two active subunits either by (a) a spacer or (b) fusion or (c) merging of compounds with desired activities[4].

Various hybrid derivatives have been synthesized and evaluated for antitubercular activity, for example isoniazid-pyrazinamide[5], isoniazid-fluoroquinolone[6] and pyrazole-quinazoline[7] (**Fig. 1**) and a synergistic activity profile has been observed.

Space for Fig. 1

Keywords: Antitubercular agents, hybrid molecule, *Mycobacterium tuberculosis*, Coumarin, Ibuprofen, microwave synthesis, pyrroles
Running title: Hybrid-pyrrole as antitubercular agents

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Pyrrole derivatives have been found to possess a wide spectrum of activities[8, 9] of which anti-TB is one of the prominent one[10-12]. Also, coumarin containing compounds have been widely explored for different activities like antibacterial, anticancer, antitubercular, etc.[13-15]. Calanolide A, a natural product with coumarin nucleus is reported to have antitubercular action at MIC of 3.13 μg/mL[16]. Similarly ibuprofen (MIC 75 μg/mL), naproxen (MIC 90 μg/mL) and other known anti-inflammatory agents are reported to possess good antitubercular activity against Mtb $H_{37}Rv$[17]. Since INH contains a pyridine nucleus, large numbers of scientists have developed novel agents bearing pyridine moiety for the treatment of TB[18].

Space for Fig. 2

Keeping in view the hybrid concept along with the antitubercular potential of different moieties it was planned to synthesize hybrid compounds (**Fig. 2**) that comprise the pyrrole nucleus with the aforementioned fragments and their evaluation of anti-TB activity.

All the compounds were synthesized by conventional as well as microwave method. Microwave reactions gave higher yields with reduced reaction times. Whole cell assay of these compounds was done by microplate alamar blue assay (MABA), against drug sensitive and resistant strains. Compounds with more than 60% growth inhibitory property were further selected for cytotoxicity studies to figure out whether those are non-toxic or not.
These compounds are thought to act by inhibiting enoyl acyl carrier protein enzyme (InhA target of INH). This was supported by docking studies of the synthesized pyrrole-hybrids using GLIDE module of Schrodinger. Since the N-(3,5-dichlorophenyl)-5-oxo-1-phenylpyrrolidine-3-carboxamide is direct inhibitor of InhA which forms a hydrogen bond with Tyr158, we focused to dock the most potent compound in a similar manner.

2. Results and discussion

Chemistry

The concept of hybridization is being widely used to synthesize novel agents to target different diseases specially related to development of resistance to existing drugs. Similar approach has been used to develop novel antitubercular agents containing different moieties linked together directly or through linkers. In this paper we report the hybrid of pyrrole moiety with different moieties like

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isobutyl benzene, pyridyl, naphthyl, coumarin to explore their potential as antitubercular agents. Thirty two different hybrid compounds (**7a-p** & **8a-p**) reported herein were prepared by following scheme 1. **Step 1a** describes the preparation of intermediate hydrazides (**3a-o**) from different acids (**1a-o**). Primarily, corresponding aromatic acid (**1a-o**) and catalytic amount of concentrated HCl was refluxed in methanol to produce corresponding ester (**2a-o**). The hydrazides (**3a-o**) were synthesized by refluxing ester (**2a-o**) with 99% hydrazine hydrate according to the reported procedure[19]. Step **1b** involves the preparation of coumarin based hydrazides (**3p**), where the ester obtained from 7-hydroxy-4-methyl-2*H*-chromen-2-onecorresponding ester (**2p**) by refluxing with ethyl 2-bromo acetate in presence of potassium carbonate as base. The starting material phenacyl bromide (**4**) was readily prepared from the acetophenone by treating with bromine at room temperature in diethyl ether. 1,4-Diphenylbutane-1,4-dione (**5**) was synthesized by refluxing bromo acetophenone **4** with zinc and pinch of iodine suspension in dry tetrahydrofuran. Following which the ester was refluxed with hydrazine hydrate leading to the formation of **3p**. The starting material, 1-phenylpentane-1,4-dione (**6**) was purchased from Sigma Aldrich. Finally, the title compounds (**7a-p** & **8a-p**) were produced by the nucleophilic attack of the most basic nitrogen of hydrazides (**3a-p**) to the carbonyl group of the γ-diketone (**5** & **6**) following Paal-Knorr cyclocondensation. The Paal-Knorr condensation was carried out by conventional heating as well as under microwave irradiation, however carrying the reaction by conventional method needs a catalyst (*p*-toluene sulfonic acid), and takes longer time with lesser yields. The neat reagents were irradiated for 7-15 minutes in the range of 100-130 ^{o}C, water droplets appeared as reaction proceeded. The details are provided in supplementary data.

The structure of all the synthesized compounds (**7a-p** & **8a-p**) was established by analytical data. In general, IR peaks at 3268-3182 cm^{-1} for NH and 1700-1640 cm^{-1} for C=O confirmed that all the derivatives contain amide bond. The analysis of ^{1}H NMR spectra (**7a-p** & **8a-p**) showed the signals of the corresponding protons, which were confirmed on the basis of their chemical shifts, multiplicities and coupling constants. These spectra showed two characteristic signals for CON**H** proton between 8.0 to 11.0 ppm and C**H**=C**H** pyrrole protons at 6.2-6.8 ppm singlet type in case of **7a-p**. But C**H**=C**H** pyrrole protons of second series (**8a-p**) appeared as doublet with different chemical shift values. The N**H** protons of all compounds were confirmed by D_2O exchange. In ^{13}C NMR, peaks were observed at 164-169 (C=O), 104-110 (pyrrole ring), further confirming the desired structures. Remaining resonances were also observed at their expected values. In MS (ESI) m/z was found at M+H showing 100% base peak, which corresponds with actual molecular weight of the compounds. The elemental analysis confirmed the purity of the compounds, experimental values were found within ± 0.4% of the theoretical values. The purification of all compounds (**7a-p** & **8a-p**) was achieved by chromatography using gradient elution method (hexane and 0-30% ethyl acetate mixture), and was confirmed by HPLC coupled with mass spectrometer. All the compounds are more than 90% pure.

Antitubercular activity

Preliminary anti-TB screening of all the synthesized compounds (**7a-p** & **8a-p**) was done against drug sensitive strain of Mtb H_{37}Rv (ATCC 27294) by microplate alamar blue assay (MABA) as reported by Collins and Franzblau[20]. This methodology is simple, non-toxic, uses a thermally stable reagent and shows good correlations with the proportional and BACTEC methods[21]. The minimum inhibitory concentration (MIC) *i.e.* concentration of compounds required to completely inhibit the Mtb growth were recorded. The MIC was calculated from dose response curve. The compounds with more than 90 % inhibition of initial primary screening were further assayed for determination of MIC against different Mtb clinical isolates (drug sensitive and resistant). The pyrrole scaffold was used as the basis for the development of structural analogues, which yielded compounds **7a-p** & **8a-p** and their activity data is presented in **Table 1**. The activity results indicate that thirteen compounds (**7a**, **7e**, **7j**, **7l**, **7n**, **7p**, **8a**, **8e**, **8h**, **8j**, **8l**, **8n** and **8p**) which have different fragments show moderate to good activity in the range of 60 to 95% inhibition at 50 μg/mL concentrations, while rest of the compounds show either a decreased inhibition or are devoid of inhibition. The MIC values of the 2, 5-di phenyl pyrrole analogs (**7a-p**) were found to be better as compared to their 2-methyl 5-phenyl analogues (**8a-p**) as the overall hydrophobicity increased. Gratifyingly, compounds **7e** and **8e** with 4-pyridoyl fragments were found to be most active with MIC value of 3.7 and 5.10 μg/mL and 95% and 92% growth inhibition respectively comparable or better than other existing drugs e.g. pyrazinamide 50 μg/mL; cycloserine 50 μg/mL[22].

Space for Table 1

The compound **7e** and **8e** has also shown good activity against rifampin (RMP-R1 & RMP-R2) and fluoroquinolone resistant (FQ-R) Mtb strains; whereas it exhibited moderate activity against isoniazid resistant (INH-R) strains with MIC value of >67.8 μg/mL (**Table 2**). As **7e** and **8e** both have activity against resistant strain, these molecules can serve as lead for further generation of more active compounds. The intracellular (macrophage) drug screening assay evaluates intracellular drug effectiveness. The assay results depicts that reduction in cell viability exhibited by compound **7e** is 2.4 log reduction of CFU at 3.7 μg/mL concentration; which is comparable with the control drug INH. This is important because Mtb can survive inside macrophages, which contributes to treatment failure and disease relapse.

Space for Table 2 and Fig. 3

The cytotoxicity of compounds was assessed in Vero African green monkey kidney cell line. Selectivity index is defined as TC_{50}/MIC. The compounds **7e** and **8e** are non-toxic with high selectivity index (>27 and >19 respectively). The MBC is determined subsequent to MIC testing by sub-culturing diluted aliquots from wells that fail to exhibit macroscopic growth. Since, the MBC value is close to MIC, the compound **7e** and **8e** is bactericidal in nature (**Table 1** and **Fig. 3**).

It is believed that compounds with an MIC ≤6.25 μg/mL and an SI>10 are interesting compounds, and are considered as excellent leads[23]. These features make **7e** and **8e** as very promising anti-TB agent.

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Structure activity relationship (SAR)

Based on the above observation following structure activity relationship could be established.

1. Replacement of one of the phenyl group with methyl group at 5-position in pyrrole ring resulted in decrease in activity as can be seen from the differences in MIC of **7a-p** and **8a-p**, eg. MIC of **7e** is 3.7 μg/mL whereas **8e** is 5.10 μg/mL

2. In general, group with electropositive character attached to amide link resulted in increase in activity, like change of phenyl group to pyridyl group in **7e** and **8e** gave the most active compounds with low MIC, GI of 95 and 92% & cell viability 100%. On the other hand, when the phenyl group is attached with steric (+π) group (**7b-d** and **8b-d**), the growth inhibitory activity decreases. Moreover, introduction of OCH_3 group in phenyl ring (+σ and –π) favors the inhibitory property

3. Introduction of bulky groups like *p*-isobutyl benzene, or coumarin or naphthyl group also increased the activity as indicated by MIC of 6.25 μg/mL for **7l**, **7p** and **8p**, 12.5 μg/mL for **7j**, **7n** and **8l**

4. Moreover, when tested against a panel of single-drug resistant Mtb strains, derivatives **7e** and **8e** maintained the activity as for the wild type, indicating that these derivatives may act with a different mechanism of action when compared to the existing drugs

Docking study

Since the most potent molecules share fragment of INH thus it was assumed that the active compounds may act via a similar mechanism as isoniazid but directly inhibiting the enzyme, therefore docking studies of all the synthesized molecules was carried out on InhA. The crystal structure of enoyl acyl carrier protein (2H7M) was used for docking studies to get a preliminary idea about the interaction of ligands with target. It was observed that some of the compounds having docking scores more than -6.0 Kcal/mol were not good in *in vitro* assay, which may be attributed to their high logP value. These results suggest that these inhibitors do not yet have optimal membrane permeability or are actively pumped out of the bacterial cells by efflux pumps[24]. The visual inspection of the target ligand interactions made it clear that the hydrogen bonding interaction with Tyr 158 and NAD^+ is important for activity as supported by the *in vitro* results, compounds (**7f**, **7h**, **7o** and **8o**) are inactive because they do not show this interaction. Additionally extra interaction with Phe 149 has proven to be important for potency. Compounds lacking π stacking with Phe 149 were found less active in *in vitro* studies comparative to active ones. There may be some other factor for their reduced activity in addition to this.

Space for Fig. 4(A), (B), (C) and (D)

A complete overview of receptor-inhibitor binding interactions of **7e** is illustrated in **Fig. 4**. The inhibitor fits the binding pocket of InhA in the same manner as co-crystalized ligand. The most active compound **7e** binds with the enzyme tightly to form a complex. The oxygen of amide group and pyridyl nitrogen are connected through hydrogen bond to 2'-hydroxyl moiety of the nicotinamide ribose and hydroxyl group of **Tyr158**, one of the catalytic residues in the InhA active site. This hydrogen-bonding network is most important feature among all the InhA-inhibitors identified to date. The hydrogen bonds formed between **Tyr158** and compound **7e** clearly supports that this compound might be acting through this mechanism. **7e** also shows van der waals interactions with the hydrophobic residues Gly96, Met103, **Phe149**, Met155, Pro156, Ala157, Met161, Pro193, Ala198, Ile215, and Leu218.

***In silico* pharmacokinetic property**

Many drugs, at a late later stage of development, as well as lead compounds fail due to adverse pharmacokinetic properties. It is therefore important to incorporate ADME (adsorption, distribution, metabolism and excretion) properties of above derivatives to continue the further research. Most of the existing anti-TB drugs are hydrophilic molecules, hence their low cellular penetration may be contributing to the development of Mtb drug resistance [25]. Therefore, the development of hydrophobic molecules could increase the cellular penetration of target tissues as it was hypothesize that they are likely to pass easily through Mtb's cell envelope [26]. In addition to their promising *in vitro* bactericidal activity against Mtb, all the fragment based pyrrole derivatives have good physicochemical properties that indicate great potential of these agents as orally available compounds. They fulfill at least four of the five physicochemical properties defined by the Lipinski 'rule-of-five', which predicts aqueous solubility and intestinal permeability. All compounds of the both series have <10 hydrogen bond acceptor, <5 hydrogen bond donor and molecular weights <500 $gmol^{-1}$. Details are provided in supplementary data. In terms of lipophilicity, compounds **7e**, **8a-k** and **8m-p** show miLogP value of <5, while rest of the compounds display miLogP values just above 5. The ease of synthesis coupled with the promising physicochemical properties signify that these compounds can be attractive leads for further development as novel anti-TB agents.

3. Conclusions

The hybrid design of compounds to develop potent antitubercular agent was achieved successfully. Thirty two compounds were synthesized and screened for anti-TB activity. Two compounds **7e** and **8e** were active against Mtb at low MIC and also against rifampin and fluoroquinoline resistance strains. The biological evaluation of the synthesized compounds helped in identifying a hybrid of 4-pyridyl with pyrrole moiety, as a lead molecule.

4. Experimental

4.1. Analysis and Instruments

All the chemicals were purchased from Sigma Aldrich, Spectrochem Pvt. Ltd. and S.D. Fine Chemicals (India) and were used without further purification. Tetrahydrofuran was dried over sodium/benzophenone prior to use. Anhydrous reactions were performed under a positive pressure of inert nitrogen gas. Melting points were determined on a digital melting point apparatus by the open tube capillary method and are uncorrected. The thin layer chromatography (TLC) plates (silica gel G) were used to check the purity of commercial reagents used, desired product purity and to monitor the reaction progress. Various solvent systems (ethyl acetate: hexane (3:7) and methanol: chloroform (1:9)) were used to run the TLC and spots were located under iodine vapors/UV light.

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An infrared spectrum (IR) was recorded on a Bruker FT-IR spectrometer using KBr as pellet. Elemental analyses were carried out on a Perkin–Elmer 2400 analyzer (USA) and were found within ±0.4 % of the theoretical values. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker spectrometer.

HPLC and MS analysis

The purity of the compounds was measured by reversed-phase liquid chromatography and a mass spectrometer (Agilent 1100 LC/MSD) with a UV detector at λ = 214 and 254 nm and an electron spray ionization (ESI) source. MS data were recorded using an Agilent 1100LC/MSD VL system (Phenomenex Gemini C18 column, 3×50 mm; 0.5 mL/min flow rate, acetonitrile/water binary solvent (95:5)). UV detection was monitored at 214 and 254 nm. MS data was acquired in positive mode scanning over the mass range of 50-1000 (attached in supporting information).

Microwave irradiation experiments

A mono mode CEM-Discover microwave reactor (CEM Corporation, P.O. Box 200, Matthews, NC 28105) was used in the standard configuration, including proprietary software. All the experiments were carried out in sealed microwave process vials (10 mL) at the maximum power and designated temperature (100-130 ^{o}C). After completion of the reaction, the vial was cooled to 50 ^{o}C via air-jet cooling before it was opened.

Step 1a: *General procedure for the preparation of substituted hydrazide* (**3a-o**): To a solution of appropriate acids in ethanol, 2 drops of concentrated sulfuric acid was added and refluxed at 80 ^{o}C for 8-14 h. The reaction progress was monitored by TLC. After the completion of ester formation, hydrazine hydrate (1.5 times, 90%) was added and refluxed for 4-5 h. The residual solvent was evaporated under reduced pressure; solid was filtered and washed with ice-cold water. The crude mass was purified by recrystallization from methanol. The purity was checked by TLC. After recrystallization by methanol, the desired compounds **3a-o**, were isolated as solid (60-80%).

Step 1b: *Typical procedure for the preparation of 2-((4-Methyl-2-oxo-2H-chromen-7-yl)oxy)acetohydrazide* (**3p**): 7-Hydroxy-4-methyl-2*H*-chromen-2-one or 7-hydroxy-2*H*-chromen-2-one (10 mmol) was dissolved in 100 mL dry acetone. To this, anhydrous potassium carbonate (15 mmol), followed by ethyl 2-bromoacetate (15 mmol) was added and refluxed for 12 h. After cooling, the organic layer was filtered and concentrated *in vacuo*. The mixture was diluted with water (40 mL), extracted with $CHCl_3$ (15 mL×3), dried over anhydrous Na_2SO_4, concentrated under reduced pressure and crystallized from methanol to give **2p**.

To the solution of ester (**2p**) in absolute ethanol (100 mL), hydrazine hydrate (15 mmol, 90%) was added and refluxed for 8-10 h. On cooling, the precipitate obtained was filtered and washed with ice-cold water, dried and recrystallized from methanol as solid crystal **3p** (70% yield).

Step 2: *Synthesis of phenacyl bromide*: phenacyl bromide was prepared according to the reported procedure and used for the preparation of 1,4-diphenylbutane-1,4-dione[27]

Synthesis of 1,4-diphenylbutane-1,4-dione (**5**): To a solution of phenacyl bromide (3 g, 15 mmol) in dry tetrahydrofuran (30 mL), zinc (1.04 g, 16.5 mmol) and pinch of iodine were added and refluxed at 80 ^{o}C for 3 h. After completion of reaction, zinc was removed by filtration over celite. The organic filtrate was extracted from water layer, dried over anhydrous sodium sulfate, concentrated under reduced pressure and purified by column chromatography. Yield 40%, FT-IR (KBr pellet) cm^{-1}: 1674 (C=O str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.03 (d, 4H, *J* = 7.6 Hz, aromatic-H), 7.57 (t, 2H, *J* = 6.8 Hz, aromatic-H), 7.47 (d, 4H, *J* = 7.6 Hz, aromatic-H), 3.46 (s, 4H, CH_2).

Step 3: General procedure for the preparation of pyrrole derivatives (**7a-p & 8a-p**):

Method I (Conventional method): An equimolar mixture of 1,4-diphenylbutane-1,4-dione (**5**, 10 mmol) or 1-phenylpentane-1,4-dione (**6**, 10 mmol), catalytic amount of *p*-toluene sulfonic acid and hydrazide derivatives (10 mmol) (**3a-p**) in absolute ethanol (30 mL) were refluxed for 10-24 h. After completion of reaction, the residual ethanol was evaporated to dryness and then purified by column chromatography to yield **3a-p**.

Method II (Microwave irradiation method): To a dry 10 mL microwave vial equipped with a magnetic stir bar, 1,4-diphenylbutane-1,4-dione (**5**, 2 mmol) or 1-phenylpentane-1,4-dione (**6**, 2 mmol) and hydrazide derivative (**3a-p**) (2 mmol) were added and the vial was capped. The vial was shaken to mix the contents and then heated in the microwave at 100-130 ^{o}C for 7–15 min (CEM Discover reactor). Silica gel column chromatography (10 cm × 2 cm; hexanes/ethyl acetate, 0-30%) gave **7a–p** & **8a-p** as solids in satisfactory yield.

Spectral data

N-(2,5-Diphenyl-1H-pyrrol-1-yl)benzamide (**7a**). FT-IR (KBr pellet) cm^{-1}: 3200 (N–H str.), 1648 (C=O str.), 1483 (C–O–N str.), 1294 (C-N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.61 (bs, 1H, N**H**, D_2O exchangeable), 8.49 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.96-8.01 (m, 3H, aromatic-**H**), 7.72-7.77 (m, 2H, aromatic-**H**), 7.45-7.47 (m, 4H, *J* = 7.2 Hz, aromatic-**H**), 7.37-7.42 (m, 4H, aromatic-**H**), 6.34 (s, 2H, C**H**=C**H**-pyrrole); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.80 (>C=O), 138.86, 135.26, 131.25, 129.94, 128.64, 128.07, 127.97, 127.50, 126.12, 126.66, 126.45, 107.35 (CH=CH-pyrrole); MS-EI: m/z 339.2 (M+1); Anal Calcd for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36; N, 8.29, Found C, 81.65; H, 5.40; N, 8.29.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-methylbenzamide (**7b**). FT-IR (KBr pellet) cm^{-1}: 3208 (amide N–H), 1688 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.01 (bs, 1H, N**H**, D_2O exchangeable), 7.92 (d, 1H, *J* = 7.2 Hz, aromatic-**H**), 7.65-768 (m, 6H, aromatic-**H**), 7.35-7.39 (m, 5H, aromatic-**H**), 7.20-7.22 (m, 2H, aromatic-**H**), 6.42 (s, 2H, C**H**=C**H**-pyrrole), 2.36 (s, 2H, C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 165.61(>C=O), 138.26, 135.27, 131.25, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.47, 107.48 (CH=CH-pyrrole), 20.02 (CH_3); MS-EI: m/z 353.2 (M+1); Anal. Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72, Found C, 81.80, H, 5.75; N, 7.95.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-3-methylbenzamide (**7c**). FT-IR (KBr pellet) cm^{-1}: 3209 (amide N–H), 1656 (C=O str.), 1584 (C-N str.), 1477 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.36 (bs, 1H, N**H**, D_2O exchangeable), 7.51-7.55 (m, 6H, aromatic-

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H), 7.31-7.35 (m, 6H, aromatic-**H**), 7.19-7.22 (m, 2H, aromatic-**H**), 6.43 (s, 2H, C**H**=C**H**-pyrrole), 2.28 (s, 2H, C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 165.90 (>C=O), 138.08, 135.36, 132.81, 131.79, 131.64, 128.54, 128.38, 127.85, 127.15, 126.84, 124.25, 107.46 (C$\underline{H}$=C$\underline{H}$-pyrrole), 20.84 (CH_3); MS-EI: m/z 353.2 (M+1); Anal Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.96, found C, 81.82, H, 5.77; N, 7.96.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-4-methylbenzamide (**7d**). FT-IR (KBr pellet) cm^{-1}: 3209 (aromatic C–H str.), 1656 (C=O str.), 1584 (C-N str.), 1477 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.89 (bs, 1H, N**H**, D_2O exchangeable), 7.82 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.59-7.51 (m, 6H, aromatic-**H**), 7.33 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 6.95 (d, 4H, *J* = 6.8 Hz, aromatic-**H**), 6.43 (s, 2H, C**H**=C**H**-pyrrole), 2.36 (s, 3H, C$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.08 (>C=O), 140.14, 135.26, 131.25, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.47, 107.38 (C$\underline{H}$=C$\underline{H}$-pyrrole), 21.2 (CH_3); MS-EI: m/z 353.2 (M+1); Anal. Calcd for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.76, Found C, 81.82, H, 5.87; N, 7.96.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)isonicotinamide (**7e**). FT-IR (KBr pellet) cm^{-1}: 3254 (amide N-H), 1675 (C=O str.), 1287 (C–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.75 (bs, 1H, NH, D_2O exchangeable), 8.58 (d, 2H, *J* = 2.8 Hz, aromatic-**H**), 7.99-8.07 (m, 1H, aromatic-C**H**), 7.51 (d, 2H, *J* = 2.8 Hz, aromatic-C**H**), 7.45 (d, 4H, *J* = 7.2 Hz, aromatic-C**H**), 7.24 (t, 3H, *J* = 7.2 Hz, aromatic-**H**), 7.14 (t, 2H, *J* = 7.2 Hz, aromatic-C**H**), 6.33 (s, 2H, C**H**=C**H**-pyrrole); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 164.68 (>C=O), 149.94, 138.86, 135.26, 131.25, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.47, 120.93, 107.38 (C$\underline{H}$=C$\underline{H}$-pyrrole); MS-EI: m/z 340.2 (M+1); Anal. Calcd for $C_{22}H_{17}N_3O$: C, 77.86; H, 5.05; N, 12.38, Found C, 77.84; H, 5.45; N, 12.40.

2-Bromo-N-(2,5-diphenyl-1H-pyrrol-1-yl)benzamide (**7f**). FT-IR (KBr pellet) cm^{-1}: 2947 (amide N–H), 1675 (C=O str.), 1511 (C-N str.), 1291 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 11.42 (bs, 1H, N**H**, D_2O exchangeable), 7.56 (d, 4H, *J* = 7.6 Hz, aromatic-**H**), 7.50 (t, 1H, *J* = 7.6 Hz, aromatic-**H**), 7.35 (t, 4H, *J* = 7.6 Hz, aromatic-**H**), 7.22-7.27 (m, 4H, aromatic-**H**), 6.92 (t, 1H, *J* = 7.6 Hz, aromatic-**H**), 6.33 (s, 2H, C**H**=C**H**-pyrrole); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 166.19 (>C=O), 135.71, 133.00, 131.49, 131.26, 128.71, 128.01, 127.83, 126.81, 119.42 (C-Br), 107.19 (C$\underline{H}$=C$\underline{H}$-pyrrole); MS-EI: m/z 417.2 (M+1), 419.1 (M+2); Anal Calcd for $C_{23}H_{17}BrN_2O$: C, 66.20; H, 4.11; N, 6.71, Found C, 66.26, H, 4.17; N, 6.77.

2-Chloro-N-(2,5-diphenyl-1H-pyrrol-1-yl)benzamide (**7g**). FT-IR (KBr pellet) cm^{-1}: 3208 (amide N-H), 1698 (C=O str.), 1618 (C-N str.), 1392 (C–O–N str.), 1020 (C-Cl str.); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 8.23 (bs, 1H, N**H**, D_2O exchangeable), 7.58 (d, 4H, *J* = 6.9 Hz, aromatic-**H**), 7.38-7.44 (m, 6H, aromatic-**H**), 7.35 (d, 2H, *J* = 3 Hz, aromatic-**H**), 7.20 (d, 2H, *J* = 3 Hz, aromatic-**H**), 6.45 (s, 2H, C**H**=C**H**-pyrrole); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 167.08 (>C=O), 134.16 (C-Cl), 133.76, 131.25, 129.64, 128.27, 127.41, 127.22, 126.03, 125.99, 125.47, 107.18 (C$\underline{H}$=C$\underline{H}$-pyrrole); MS-EI: m/z 373.1 (M+1); Anal Calcd for $C_{23}H_{17}ClN_2O$: C, 74.30; H, 4.62; N, 7.51, Found C, 74.80; H, 4.71; N, 7.53.

2,4-Dichloro-N-(2,5-diphenyl-1H-pyrrol-1-yl)benzamide (**7h**). FT-IR (KBr pellet) cm^{-1}: 3208 (amide N–H), 1688 (C=O str.), 1628 (C-N str.), 1392 (C–O–N str.), 1025 (C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.68 (bs, 1H, N**H**, D_2O exchangeable), 7.86-7.88 (m, 4H, aromatic-**H**), 7.44-7.47 (m, 6H, aromatic-**H**), 7.41-7.42 (m, 2H, aromatic-**H**), 6.60 (s, 2H, C**H**=C**H**-pyrrole); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 168.23 (>C=O), 135.74 (C-Cl), 131.25, 129.04, 128.64, 128.55, 127.42, 127.00, 108.21 (C$\underline{H}$=C$\underline{H}$-pyrrole); MS-EI: m/z 406.1 (M+1); Anal Calcd for $C_{27}H_{20}N_2O_4$: C, 67.83; H,3.96; N, 6.88, Found C, 67.89, H, 3.96; N, 6.89.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-phenoxyacetamide (**7i**). FT-IR (KBr pellet) cm^{-1}: 3254 (amide N–H), 1693 (C=O str.), 1598 (C-N str.), 1491 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.78 (bs, 1H, N**H**, D_2O exchangeable), 7.46 (d, 4H, *J* = 8.0 Hz, aromatic-**H**), 7.32 (t, 4H, *J* = 8.0 Hz, aromatic-**H**), 7.25-7.29 (m, 2H, aromatic-**H**), 7.20 (t, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.96-7.00 (m, 1H, aromatic-**H**), 6.70 (d, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.40 (s, 2H, C**H**=C**H**-pyrrole), 4.45 (s, 2H, OC$\underline{H}_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 167.88 (>C=O), 156.38, 133.62, 131.15, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.27, 122.83, 106.99 (C$\underline{H}$=C$\underline{H}$-pyrrole), 66.72 (OC$\underline{H}_2$); MS-EI: m/z 369.2 (M+1); Anal. Calcd. for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60, Found C, 78.26; H, 5.71; N, 7.61.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-4-methoxybenzamide (**7j**). FT-IR (KBr pellet) cm^{-1}: 3182 (amide N-H), 1678 (C=O str.), 1300 (C-N str.); ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 8.27 (bs, 1H, N**H**, D_2O exchangeable), 7.82 (d, 2H, *J* = 8.7 Hz, aromatic-**H**), 7.59-7.51 (m, 6H, aromatic-**H**), 7.33 (t, 4H, *J* = 7.5 Hz, aromatic-**H**), 6.95 (d, 2H, *J* = 8.7 Hz, aromatic-**H**), 6.42 (s, 2H, C**H**=C**H**-pyrrole), 3.80 (s, 3H, OC$\underline{H}_3$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 167.68 (>C=O), 133.98, 131.25, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.89, 126.38, 116.83, 107.22 (C$\underline{H}$=C$\underline{H}$-pyrrole), 58.7; MS-EI: m/z 369.2 (M+1); Anal Calcd for $C_{24}H_{20}N_2O_2$: C, 78.24; H, 5.47; N, 7.60, Found C, 78.46; H, 5.77; N, 7.60.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-phenylacetamide (**7k**). FT-IR (KBr pellet) cm^{-1}: 3271 (amide N-H), 1676 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.17 (bs, 1H, N**H**, D_2O exchangeable), 7.89-7.90 (m, 2H, aromatic-**H**), 7.92-7.94 (m, 5H, aromatic-**H**), 7.56-7.52 (m, 4H, aromatic-**H**), 7.28-7.31 (m, 4H, aromatic-**H**), 6.47 (s, 2H, C**H**=C**H**-pyrrole), 3.52 (s, 2H, C$\underline{H}_2$); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 171.21 (>C=O), 136.22, 133.62, 131.15, 129.64, 128.64, 128.07, 127.55, 127.05, 126.61, 107.57 (C$\underline{H}$=C$\underline{H}$-pyrrole), 41.07 (OC$\underline{H}_3$); MS-EI: m/z 353.2 (M+1); Anal Calcd for $C_{28}H_{22}N_2O_2$: C, 81.79; H, 5.72; N, 7.95, Found C, 81.76; H, 5.78; N, 7.96.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-(4-isobutylphenyl)propanamide (**7l**). FT-IR (KBr pellet) cm^{-1}: 3233 (aromatic C–H str.), 1669 (C=O str.), 1601 (C-N str.), 1354 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 7.49 (bs, 1H, N**H**, D_2O exchangeable), 7.29 (d, 4H, *J* = 2.7 Hz, aromatic-**H**), 7.17-7.24 (m, 6H, aromatic-**H**), 6.82 (d, 2H, *J* = 7.8 Hz, aromatic-**H**), 6.71 (d, 2H, *J* = 7.8 Hz, aromatic-**H**), 6.25 (s, 2H, C**H**=C**H**-pyrrole), 3.35 (q, 1H, *J* = 7.2 Hz, C$\underline{H}$-CH_3), 2.36 (d, 2H, *J* = 6.9 Hz, C$\underline{H}_2$-CH), 1.77 (sep, 1H, *J* = 6.9 Hz, C**H**-$(CH_3)_2$), 1.28 (d, 3H, *J* = 7.2 Hz, CH-C$\underline{H}_3$), 0.82 (d, 6H, *J* = 6.9

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Hz, CH-(C**H**$_3$)$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 171.68 (>C=O), 140.12, 134.41, 132.51, 129.42, 128.64, 127.47, 126.03, 126.47, 125.19, 107.8 (C**H**=C**H**-pyrrole), 45.41 (C**H**-CH$_3$), 44.84 (C**H**$_2$-CH), 29.05 {C**H**-(CH$_3$)$_2$}, 22.18 {CH-(C**H**$_3$)$_2$}, 15.65 (CH-C**H**$_3$); MS-EI: m/z 423.2 (M+1); Anal. Calcd. for $C_{29}H_{30}N_2O$: C, 82.43; H, 7.16; N, 6.63, Found C, 82.49; H, 7.23; N, 6.63.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-(naphthalen-1-yl)acetamide (**7m**). FT-IR (KBr pellet) cm^{-1}: 3218 (amide N-H), 1686 (C=O str.), 1628 (C-N str.), 1390 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 10.96 (bs, 1H, N**H**, D$_2$O exchangeable), 8.16 (m, 2H, aromatic-**H**), 7.92 (m, 5H, aromatic-**H**), 7.83-7.87 (m, 5H, aromatic-**H**), 7.54-7.48 (m, 4H, aromatic-**H**), 7.18 (d, 1H, aromatic-**H**), 6.38 (s, 2H, C**H**=C**H**-pyrrole), 4.13 (s, 2H, C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 168.81 (>C=O), 134.86, 133.15, 129.14, 128.34, 127.47, 126.11, 126.29, 125.22, 124.89, 107.10 (C**H**=C**H**-pyrrole), 41.56 (OC**H**$_2$); MS-EI: m/z 403.1 (M+1); Anal Calcd for $C_{28}H_{22}N_2O$: C, 83.56; H, 5.51; N, 6.96, Found C, 83.59; H, 5.59; N, 6.97.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-(naphthalen-2-yloxy)acetamide (**7n**). FT-IR (KBr pellet) cm^{-1}: 3228 (amide N-H), 1690 (C=O str.), 1612 (C-N str.), 1389 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 10.82 (bs, 1H, N**H**, D$_2$O exchangeable), 8.16 (m, 3H, aromatic-**H**), 7.92 (m, 4H, aromatic-**H**), 7.83 (m, 3H, aromatic-**H**), 7.57-7.44 (m, 5H, aromatic-**H**), 7.36 (m, 2H, aromatic-**H**), 6.40 (s, 2H, C**H**=C**H**-pyrrole), 4.65 (s, 2H, C**H**$_2$); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 168.08 (>C=O), 157.10, 133.26, 131.25, 132.04, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.47, 120.93, 118.29, 107.68 (C**H**=C**H**-pyrrole), 105.11, 67.23 (OC**H**$_2$); MS-EI: m/z 419.2 (M+1); Anal Calcd for $C_{28}H_{22}N_2O_2$: C, 80.36; H, 5.30; N, 6.69, Found C, 80.45; H, 5.76, N, 6.69.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-(o-tolyl)acetamide (**7o**). FT-IR (KBr pellet) cm^{-1}: 3260 (amide N–H), 1675 (C=O str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 11.27 (bs, 1H, N**H**, D$_2$O exchangeable), 7.46 (d, 4H, *J* = 7.2 Hz, aromatic-**H**), 7.33-7.35 (m, 4H, aromatic-**H**), 7.06-7.16 (m, 5H, aromatic-**H**), 6.95-6.99 (m, 1H, aromatic-**H**), 6.34 (s, 2H, C**H**=C**H**-pyrrole), 3.45 (s, 2H, C**H**$_2$), 2.25(s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 169.07 (>C=O), 138.86, 135.26, 133.25, 129.64, 128.64, 128.07, 127.47, 127.05, 126.03, 126.69, 126.47, 106.48 (C**H**=C**H**-pyrrole), 39.5 (C**H**$_2$), 18.94 (C**H**$_3$); MS-EI: m/z 367.2 (M+1); Anal. Calcd. for $C_{25}H_{22}N_2O$: C, 81.94; H, 6.05; N, 7.64, Found C, 81.95; H, 6.11; N, 7.66.

N-(2,5-Diphenyl-1H-pyrrol-1-yl)-2-((4-methyl-2-oxo-2H-chromen-7-yl)oxy)acetamide (**7p**). FT-IR (KBr pellet) cm^{-1}: 3216 (amide N–H), 1727 (C=O str.), 1689 (C=O str.), 1613 (C-N str.), 1391 (C–O–N str.); ^{1}H NMR (400 MHz, DMSO-d$_6$): δ (ppm) 11.54 (bs, 1H, N**H**, D$_2$O exchangeable), 7.57 (d, 4H, *J* = 8.4 Hz, coumarin-C**H**), 7.47 (d, 4H, *J* = 6.8 Hz, aromatic-**H**), 7.30 (t, 4H, *J* = 6.8 Hz, aromatic-**H**), 7.21 (d, 2H, *J* = 6.8 Hz, aromatic-**H**), 6.83 (d, 1H, *J* = 8.4 Hz, coumarin-C**H**), 6.75 (s, 1H, coumarin-C**H**), 6.37 (s, 2H, C**H**=C**H**-pyrrole), 6.21 (s, 1H, coumarin-C**H**), 4.62 (s, 2H, OC**H**$_2$), 2.37 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ (ppm) 166.77 (>C=O), 160.22 (>C=O), 154.35, 153.19, 135.22, 131.33, 128.86, 126.78, 126.38, 113.85, 112.26, 111.59, 107.51 (C**H**=C**H**-pyrrole), 101.61, 66.32 (OC**H**$_2$), 18.09 (coumarin C**H**$_3$); MS-EI: m/z 451.2 (M+1); Anal Calcd for $C_{27}H_{20}N_2O_4$: C, 74.30; H, 4.62; N, 6.22, Found C, 74.36; H, 4.70; N, 6.23.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8a**). FT-IR (KBr pellet) cm^{-1}: 3284 (amide N–H), 1662 (C=O str.), 1600 (C-N str.), 1278 (C–O–N str.); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 11.12 (bs, 1H, N**H**), 7.86 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.48 (t, 1H, *J* = 7.6 Hz, aromatic-**H**), 7.37-7.42 (m, 4H, aromatic-**H**), 7.22 (t, 1H, *J* = 7.6 Hz, aromatic-**H**), 7.11 (t, 1H, *J* = 7.2 Hz, aromatic-**H**), 6.18 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.93 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 2.14 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 166.50 (>C=O), 132.28, 131.94, 131.72, 130.73, 128.13, 127.95, 127.31, 126.62, 125.94, 105.86, 104.88, 102.99, 10.80; ESIMS: m/z 277.2 (M+1); Anal. Calcd for $C_{18}H_{16}N_2O$: C, 78.24; H, 5.84; N, 10.14, Found C, 78.26; H, 5.94; N, 10.15.

2-Methyl-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8b**). FT-IR (KBr pellet) cm^{-1}: 3246 (amide N–H), 1658 (C=O str.), 1597 (C-N str.), 1392 (C–O–N str.); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 9.10 (bs, 1H, N**H**), 8.18-8.20 (m, 2H, aromatic-**H**), 7.87-7.89 (m, 3H, aromatic-**H**), 7.23-7.27 (m, 2H, aromatic-**H**), 7.15-7.16 (m, 2H, aromatic-**H**), 6.23 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.97 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 2.38 (s, 3H, C**H**$_3$), 2.16 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 166.61 (>C=O), 142.67, 132.71, 131.61, 131.0, 129.04, 128.31, 127.94, 127.08, 126.94, 126.17, 106.26, 105.13, 21.09, 10.81; ESIMS: m/z 291.2 (M+1); Anal Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65, Found C, 78.62; H, 6.29; N, 9.64.

3-Methyl-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8c**). FT-IR (KBr pellet) cm^{-1}: 3246 (amide N–H), 1657 (C=O str.), 1599 (C-N str.), 1285 (C–O–N str.); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.72 (bs, 1H, N**H**), 7.89-7.90 (m, 2H, aromatic-**H**), 7.83 (s, 1H, aromatic-**H**), 7.74-7.79 (m, 4H, aromatic-**H**), 7.20-7.21 (m, 2H, aromatic-**H**), 6.23 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.97 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 2.38 (s, 3H, C**H**$_3$), 2.14 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 166.80 (>C=O), 140.66, 133.21, 131.13, 130.9, 129.00, 128.01, 127.99, 127.00, 126.49, 126.17, 106.60, 105.30, 21.07, 10.79; ESIMS: m/z 291.14 (M+1); Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65, Found C, 78.54; H, 6.28; N, 9.64.

4-Methyl-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8d**). FT-IR (KBr pellet) cm^{-1}: 3236 (amide N–H), 1660 (C=O str.), 1605 (C-N str.), 1297 (C–O–N str.); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm) 8.72 (bs, 1H, N**H**), 7.58 (d, *J* = 8.0 Hz, 2H, aromatic-**H**), 7.36 (d, 2H, *J* = 8.0 Hz, aromatic-**H**), 7.23-7.27 (m, 2H, aromatic-**H**), 7.16-7.20 (m, 3H, aromatic-**H**), 6.23 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.97 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 2.37 (s, 3H, C**H**$_3$), 2.12 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, CDCl$_3$): δ (ppm) 166.58 (>C=O), 142.67, 132.71, 131.61, 131.0, 129.04, 128.31, 127.94, 127.08, 126.94, 126.17, 106.26, 105.13, 21.09, 10.81; ESIMS: m/z 291.2 (M+1); Anal Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 6.25; N, 9.65, Found C, 78.55; H, 6.27; N, 9.65.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)isonicotinamide (**8e**). FT-IR (KBr pellet) cm^{-1}: 3226 (amide N–H), 1665 (C=O str.), 1590 (C-N str.), 1276 (C–O–N str.); ^{1}H NMR (400 MHz, CDCl$_3$): δ (ppm)

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10.25 (bs, 1H, N**H**), 8.44 (2H, d, *J* = 5.6 Hz, aromatic-**H**), 7.40 (2H, d, *J* = 5.6Hz, aromatic-**H**), 6.14 (d, 1H, *J* = 3.2 Hz, pyrrole-**H**), 5.94 (d, 1H, *J* = 3.2 Hz, pyrrole-**H**), 2.02 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.12 (>C=O), 149.92, 138.54, 132.52, 131.34, 130.46, 128.03, 126.79, 126.41, 120.80, 106.60, 105.55, 10.68; ESIMS: m/z 278.2 (M+1); Anal Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15, Found C, 73.66; H, 5.65; N, 15.20.

2-Bromo-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8f**). FT-IR (KBr pellet) cm^{-1}: 3218 (amide N–H), 1672 (C=O str.), 1596 (C-N str.), 1292 (C–O–N str.), 748 (C-Br str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.19 (bs, 1H, N**H**), 7.56-7.59 (m, 1H, aromatic-**H**), 7.38 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.24-7.35 (m, 6H, aromatic-**H**), 6.19 (d, 1H, *J* = 4.0 Hz, pyrrole-**H**), 5.99 (d, 1H, *J* = 3.2 Hz, pyrrole-**H**), 2.28 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 166.2 (>C=O), 134.41, 133.22, 131.69, 131.42, 130.89, 129.14, 127.96, 127.72, 127.11, 126.59, 119.25, 106.5, 105.39, 10.2; ESIMS: m/z 355.2, 357 (M+1, M+2); Anal Calcd for $C_{18}H_{15}BrN_2O$: C, 60.86; H, 4.26; N, 7.89, Found C, 60.88; H, 4.31; N, 7.89.

2-Chloro-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8g**). FT-IR (KBr pellet) cm^{-1}: 3210 (amide N–H), 1651 (C=O str.), 1603 (C-N str.), 1282 (C–O–N str.), 1027 (C-Cl str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 11.01 (bs, 1H, N**H**), 7.86 (d, *J* = 7.2 Hz, 2H, aromatic-**H**), 7.42 (d, 2H, *J* = 6.4 Hz, aromatic-**H**), 7.22 (s, 2H, aromatic-**H**), 7.12 (d, 1H, *J* = 6.0 Hz, aromatic-**H**), 6.88 (d, 2H, *J*=7.2 Hz, aromatic-**H**), 6.17 (s, 1H, pyrrole-**H**), 5.92 (s, 1H, pyrrole-H), 2.12 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.92 (>C=O), 162.23, 132.27, 131.99, 130.83, 129.26, 127.95, 126.56, 125.87, 123.85, 113.35, 105.78, 104.80, 10.98; ESIMS: m/z 311 (M+1), 312 (M+2); Anal. Calcd for $C_{19}H_{18}N_2O$: C, 69.57; H, 4.86; N, 9.01, Found C, 69.58; H, 4.84; N, 9.00.

2,4-Dichloro-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8h**). FT-IR (KBr pellet) cm^{-1}: 3216 (amide N–H), 1672 (C=O str.), 1584 (C-N str.), 1290 (C–O–N str.), 1048 (C-Cl str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.47 (s, 1H, N**H**), 7.30-7.35 (s, 1H, aromatic-**H**), 7.20-7.25 (m, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.19 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.88 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 2.20 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 164.58 (>C=O), 137.30, 132.87, 131.62, 131.39, 130.63, 130.46, 130.40, 129.79, 127.88, 127.50, 127.09, 126.54, 106.53, 105.45, 11.02; ESIMS: m/z 345.0, 346 (M+1, M+2); Anal. Calcd for $C_{18}H_{14}Cl_2N_2O$: C, 62.62; H, 4.09; N, 8.11, Found C, 62.71; H, 4.10; N, 8.11.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)-2-phenoxyacetamide (**8i**). FT-IR (KBr pellet) cm^{-1}: 3187 (amide N–H), 1685 (C=O str.), 1596 (C-N str.), 1233 (C–O–N str.), 1079 (C-O-C str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.85 (bs, 1H, N**H**), 7.25-7.36 (m, 7H, aromatic-**H**), 7.04 (t, 1H, *J* = 7.2 Hz, aromatic-**H**), 6.85 (d, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.24 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 6.02 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 4.60 (s, 2H, OC**H**$_2$), 2.14 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 167.23(>C=O), 156.37, 132.81, 131.31, 130.56, 129.41, 128.05, 127.09, 126.46, 122.06, 114.16, 106.68, 105.51, 66.51, 10.88; ESIMS: m/z 306.2 (M+1); Anal Calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14, Found C, 74.59; H, 5.97; N, 9.15.

4-Methoxy-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)benzamide (**8j**). FT-IR (KBr pellet) cm^{-1}: 3211 (amide N–H), 1652 (C=O str.), 1604 (C-N str.), 1283 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.91 (bs, 1H, N**H**), 7.84 (d, 2H, *J* = 8.4 Hz, aromatic-**H**), 7.39 (d, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.19 (t, 2H, *J* = 7.2 Hz, aromatic-**H**), 7.08 (d, 1H, *J* = 7.2 Hz, aromatic-**H**), 6.85 (d, 2H, *J* = 8.4 Hz, aromatic-**H**), 6.15 (d, 1H, *J* = 3.2 Hz, pyrrole-**H**), 5.90 (d, 1H, *J* = 3.2 Hz, pyrrole-**H**), 3.76 (s, 3H, OC**H**$_3$), 2.11 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 160.38 (>C=O), 157.21, 127.31, 126.39, 125.86, 124.25, 122.92, 121.60, 120.85, 118.89, 108.32, 100.79, 99.80, 49.98, 5.97; ESIMS: m/z 307.2 (M+1); Anal Calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14, Found C, 74.49; H, 5.98; N, 9.15.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)-2-phenylacetamide (**8k**). FT-IR (KBr pellet) cm^{-1}: 3244 (amide N–H), 1670 (C=O str.), 1600 (C-N str.), 1348 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.17 (bs, 1H, N**H**), 7.24-7.38 (m, 6H, aromatic-**H**), 7.12-7.19 (m, 4H, aromatic-**H**), 6.14 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.89 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 3.52 (s, 2H, C**H**$_2$), 2.01 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 169.97 (>C=O), 132.99, 130.47, 128.92, 128.85, 127.86, 126.13, 126.5, 106.79, 105.6, 41.0, 10.1; ESIMS: m/z 291.1 (M+1); Anal. Calcd for $C_{19}H_{18}N_2O$: C, 78.59; H, 5.92; N, 9.65, Found C, 78.62; H, 5.92; N, 9.65.

2-(4-Isobutylphenyl)-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)propanamide (**8l**). FT-IR (KBr pellet) cm^{-1}: 3227 (amide N–H), 1666 (C=O str.), 1591 (C-N str.), 1281 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.61 (bs, 1H, N**H**), 7.83-7.84 (m, 2H, aromatic-**H**), 7.34-7.36 (m, 3H, aromatic-**H**), 7.28 (d, 2H, *J* = 8.0 Hz, aromatic-**H**), 7.11 (d, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.05 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.90 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 3.58 (q, 1H, *J* = 6.0 Hz, C**H**-CH$_3$), 2.44 (d, 2H, *J* = 6.8 Hz, CH-C**H**$_2$-Ph), 2.12 (s, 3H, C**H**$_3$), 1.85 (d, 1H, *J* = 6.0 Hz, C**H**), 1.48 (d, 3H, *J* = 9.6 Hz, C**H**$_3$), 0.90 (d, 6H, *J* = 6.0 Hz, CH-(C**H**$_3$)$_2$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 176.11 (>C=O), 141.71, 134.69, 132.3, 131.22, 130.16, 129.60, 127.08, 107.13 (CH near the Ph of pyrrole), 106.72 (CH near the methyl of pyrrole), 45.40 (CH-CH$_3$), 39.98 (C**H**$_2$-CH), 29.15 [C**H**-(CH$_3$)$_2$], 22.12 [CH-(C**H**$_3$)$_2$], 17.41 (CH-C**H**$_3$), 10.72 (CH$_3$); ESIMS: m/z 360.3 (M+1); Anal. Calcd for $C_{24}H_{28}N_2O$: C, 79.96; H, 7.83; N, 7.77, Found C, 79.98, H, 7.87; N, 7.78.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)-2-(naphthalen-1-yl)acetamide (**8m**). FT-IR (KBr pellet) cm^{-1}: 3211 (amide N–H), 1665 (C=O str.), 1596 (C-N str.), 1317 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.19 (bs, 1H, N**H**), 7.57–7.45 (m, 4H, aromatic-**H**), 7.31-7.32 (m, 2H, aromatic-**H**), 7.06-7.20 (m, 6H, aromatic-**H**), 6.13 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.90 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 4.11 (s, 2H, C**H**$_2$), 2.11 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 169.12 (>C=O), 136.76, 132.69, 131.22, 130.6, 130.3, 129.16, 127.53, 126.84, 106.09, 105.21, 41.5, 10.8; ESIMS: m/z 341.2 (M+1); Anal. Calcd for $C_{23}H_{20}N_2O$: C, 81.15; H, 5.92; N, 8.23, Found C, 81.19, H, 5.96; N, 8.23.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)-2-(naphthalen-2-yloxy)acetamide (**8n**). FT-IR (KBr pellet) cm^{-1}: 3245 (amide N–H), 1692 (C=O str.), 1628 (C-N str.), 1394 (C–O–N str.), 1063 (C-O-C str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 11.02 (bs, 1H, N**H**),

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7.29-7.36 (m, 6H, aromatic-**H**), 7.24-7.25 (m, 4H, aromatic-**H**), 7.03-7.08 (m, 2H, aromatic-**H**), 6.45 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.88 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 4.66 (s, 2H, OC**H**$_2$), 2.12 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 170.11 (>C=O), 156.09, 136.12, 131.22, 130.43, 129.29, 127.13, 126.64, 118.19, 106.3 (CH near the methyl of phenyl), 105.12 (CH near the methyl of pyrrole), 70.01 (O<u>C</u>H$_2$), 10.31 (<u>C</u>H$_3$); ESIMS: m/z 357.2 (M+1); Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86, Found C, 77.55, H, 5.68; N, 7.85.

N-(2-Methyl-5-phenyl-1H-pyrrol-1-yl)-2-(o-tolyl)acetamide (**8o**). FT-IR (KBr pellet) cm^{-1}: 3249 (amide N–H), 1674 (C=O str.), 1601 (C-N str.), 1348 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.54 (bs, 1H, N**H**), 7.06-7.36 (m, 7H, aromatic-**H**), 7.02 (t, 2H, *J* = 8.0 Hz, aromatic-**H**), 6.14 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 5.92 (d, 1H, *J* = 3.6 Hz, pyrrole-**H**), 3.66 (s, 2H, OC**H**$_2$), 3.16 (s, 3H, C**H**$_3$), 2.09 (s, 3H, C**H**$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 169.07 (>C=O), 136.76, 132.69, 131.3, 131.22, 130.61, 130.37, 129.94, 129.61, 127.3, 126.41, 106.34, 105.2, 39.5 (<u>C</u>H$_2$), 18.94 (<u>C</u>H$_3$), 10.78 (<u>C</u>H$_3$); ESIMS: m/z 305.2 (M+1); Anal. Calcd for $C_{20}H_{20}N_2O$: C, 78.92; H, 6.62; N, 9.20, Found C, 78.95; H, 6.68; N, 9.20.

2-((4-Methyl-2-oxo-2H-chromen-7-yl)oxy)-N-(2-methyl-5-phenyl-1H-pyrrol-1-yl)acetamide (**8p**). FT-IR (KBr pellet) cm^{-1}: 3208 (amide N–H), 1711(C=O str.), 1682 (C=O str.), 1612 (C-N str.), 1391 (C–O–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.87 (bs, 1H, NH), 7.76 (1H, d, *J* = 8.4 Hz, aromatic-H), 7.06-7.36 (m, 7H, aromatic-H), 7.02 (t, 2H, *J* = 8.4 Hz, aromatic-H), 6.99 (1H, dd, *J* = 8.4 Hz, *J* = 2.0 Hz, aromatic-H), 6.08 (d, 1H, *J* = 3.6 Hz, pyrrole-H), 6.08 (s, 1H, coumarin-H), 5.93 (d, 1H, *J* = 3.6 Hz, pyrrole-H), 4.70 (s, 2H, OCH_2), 2.28 (s, 3H, CH_3), 2.10 (s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 170.21 (>C=O), 160.89 (C=O of coumarin), 159.09 (C-O), 131.76, 130.46, 129.09, 127.78, 117.44, 108.03 (CH near the Ph of pyrrole), 106.21 (CH near the methyl of pyrrole), 104.73, 68.20 (O<u>C</u>H$_2$), 19.53 (coumarin <u>C</u>H$_3$), 10.18 (pyrrole <u>C</u>H$_3$); ESIMS: m/z 389.2 (M+1); Anal. Calcd for $C_{23}H_{20}N_2O_4$: C, 71.12; H, 5.19; N, 7.21, Found C, 71.16; H, 5.25; N, 7.22.

4.2. Antitubercular activity

All the synthesized compounds were tested through *in vitro* against *Mycobacterium tuberculosis* $H_{37}Rv$ (Mtb $H_{37}Rv$) in a high throughput screen using procedure adapted from the microplate alamar blue assay. The experiment was carried out in 96-well microplates 7H9-Tween-OADC medium by measuring bacterial growth after 5 days in the presence of test compounds. Test compounds were prepared as ten-fold serial dilutions in DMSO. Compounds **7a–p** & **8a-p** and reference drugs were dissolved in DMSO at a concentration of 5 mg/mL and refrigerated until used. Plates were inoculated with Mtb $H_{37}Rv$ ATCC 27294, covered and sealed with parafilm and incubated at 37 ^oC for five days. Testing was conducted in duplicate and the following controls were taken i) medium only (sterility control); ii) organism in medium (negative control); and iii) reference compounds (positive control). The growth was measured by OD_{590} and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy 4 plate reader. To calculate the MIC, the 10-point dose response curve was plotted as % growth and fitted to the Gompertz model using GraphPad Prism 5. The MIC was defined as the minimum concentration at which growth was completely inhibited and was calculated from the dose response curve at zero growth.

% inhibition = [1 - (growth index of test sample/growth index of control)] × 100.

Intracellular activity assay

Murine J774 macrophages were infected with a luminescent strain of $H_{37}Rv$ (which constitutively expresses luxABCDE) at a multiplicity of infection of 1. After 18 h, extracellular bacteria were removed by washing and subsequently test and positive control (INH) were added. Infected macrophages were incubated in the presence of compound for 4 days at 1 and 10 times of MIC (as determined in aerobic culture in liquid medium). Bacteria were harvested from macrophages by lysis with 0.1% SDS, inoculated into growth media and allowed to grow aerobically for 5 days, where the amount of bacteria present was determined by luminescence. All assays were conducted in triplicate; each assay included a positive control (INH) and a negative control (DMSO). The baseline of infection was determined by harvesting bacteria from macrophages at 0 day before compound addition and plating for CFU determination in triplicate.

The intracellular activity = [Log CFU Day 4 Compound] - [Log CFU Day 4 DMSO]

Activity against resistant isolates

The activity of compounds against five resistant isolates of Mtb strains under aerobic conditions was assessed by determining the minimum inhibitory concentration (MIC) of compounds. Strains tested were two isoniazid resistant strains (INH-R1 and INH-R2), two rifampicin resistant strains (RIF-R1 and RIF-R2) and a fluoroquinolone resistant strain (FQ-R1). The assay is based on measurement of growth in liquid medium of each strain where the readout is optical density (OD).

INH-R1 was derived from $H_{37}Rv$ and is a katG mutant (Y155* = truncation). INH-R2 is strain ATCC35822. RIF-R1 was derived from $H_{37}Rv$ and is an rpoB mutant (S522L). RIF-R2 is strain ATCC35828. FQ-R1 is a fluoroquinolone-resistant strain derived from $H_{37}Rv$ and has an unidentified mutation.

The MIC of compound was determined by measuring bacterial growth after 5 days in the presence of compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration. Plates were inoculated with Mtb and incubated for 5 days; growth was measured by OD_{590}. To calculate the MIC, the 10-point dose response curve was plotted as % growth and fitted to the Gompertz model using GraphPad Prism 5. In addition dose response curves were generated using the Levenberg-Marquardt algorithm and the concentrations that resulted in 50% and 90% inhibition of growth were determined (IC_{50} and IC_{90} respectively).

4.3. Evaluation of Cytotoxicity

A drug cytotoxicity control plate assay (MTT cell proliferation) was conducted using Vero African green monkey kidney cell line to confirm that concentrations utilized for testing were not toxic to that cell line. The cells were plated in flat-bottom 96-well plates cultured for 72 h in a controlled atmosphere and non-adherent cells were washed by gentle flushing with RPMI 1640 supplemented with 10% fetal bovine serum. Compounds were prepared as 10-point three-fold

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serial dilutions in DMSO. The highest concentration of compound tested was 100 μg/mL where compounds were soluble in DMSO. The plates were incubated for additional 2 days after addition of test compounds and MTT reagent was added. The cell viability was measured on the basis of absorbance in each well and using positive control drug and medium as blank. Each plate included staurosporine as a control.

4.4. Computational work

The protein crystal structure of Mtb ENR (InhA) (PDB ID 2H7M) with co-crystallized ligand was downloaded from protein data bank. The protein was prepared using the "protein preparation tool" of Schrödinger. After adding hydrogen atoms, bond orders and formal charges were checked following which minimization was performed with the root-mean-square-displacement (RMSD) cutoff of 0.30Å. A grid file was created surrounding the area in the cavity that contains co-crystallized ligand [N-(3,5-dichlorophenyl)-5-oxo-1-phenylpyrrolidine-3-carboxamide] and the grid was defined as an enclosing box with 15Å in all three dimensions. Three dimensional coordinates of the all the ligand (**7a-p and 8a-p**), their isomeric, ionization and tautomeric states were generated using the LigPrep module of Maestro 9.4 (LigPrep with default setting, Schrödinger). The poses were selected based on hydrophobic, hydrogen bond and charge-charge interactions. Special care was also taken to identify poses that may involve binding modes of the molecules in the hydrophobic cavity. The cutoff distance for hydrogen bond, van der waals interaction, and electrostatic interaction is 3.20 Å (angle >90-180°), 4.20 Å, and 4.00 Å respectively.

Prediction of pharmacokinetic property (ADME)

Pharmacokinetic properties of title compounds **(7a-p** and **8a-p)** were calculated using Molinspiration online property calculation program. Polar surface area (TPSA), miLogP, number of rotatable bonds (n-ROTB), molecular volume (MV), number of hydrogen donor (n-OH and NH) and acceptor atoms (n-O and N atom) and violations of Lipinski's rule of five of titled compounds (**7a-p** and **8a-p**) are presented in **Table 4**.

Software used: (a) Schrödinger Suite 2013 Protein Preparation Wizard; LLC, New York, NY, Glide 5.9 Schrödinger, LLC; New York, NY, 2013, LigPrep, version 2.6, Schrödinger, LLC, New York, NY, 2013. (b) Molinspiron property calculation program

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Conflict of interest

The authors report no declarations of interest.

† **Supplementary data:** Physicochemical properties, calculated ADMET properties, ^{1}H-, ^{13}C-NMR and purity data of the compounds can be found online.

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Scheme 1 Synthetic route of hybrid-pyrrole analogues **7a-p** & **8a-p**

Figure legends

Figure 1. Structures of different hybrid drugs showing antitubercular activity

Figure 2. Compounds represented with antitubercular activity and portions highlighted have been used to design new hybrid compounds

Figure 3. Dose response plot for minimum bactericidal concentration (MBC) determination

Figure 4. (A) Reference ligand binding pose (B) Surface of the binding pose between reference ligand and InhA (C) Docked conformation of the most potent inhibitor **7e** with the crystal structure of a cofactor NAD^+ and **Tyr 158** in InhA (D) Surface of the binding pose which shows the interactions between InhA site and compound 7**e**

Table captions

Table 1 Preliminary antitubercular activity of **7a-p** & **8a-p** against Mtb $H_{37}Rv$

Table 2 Cytotoxicity, minimum bactericidal concentration (MBC), drug sensitive and single drug-resistant (SDR) antitubercular activities of **7e** & **8e**

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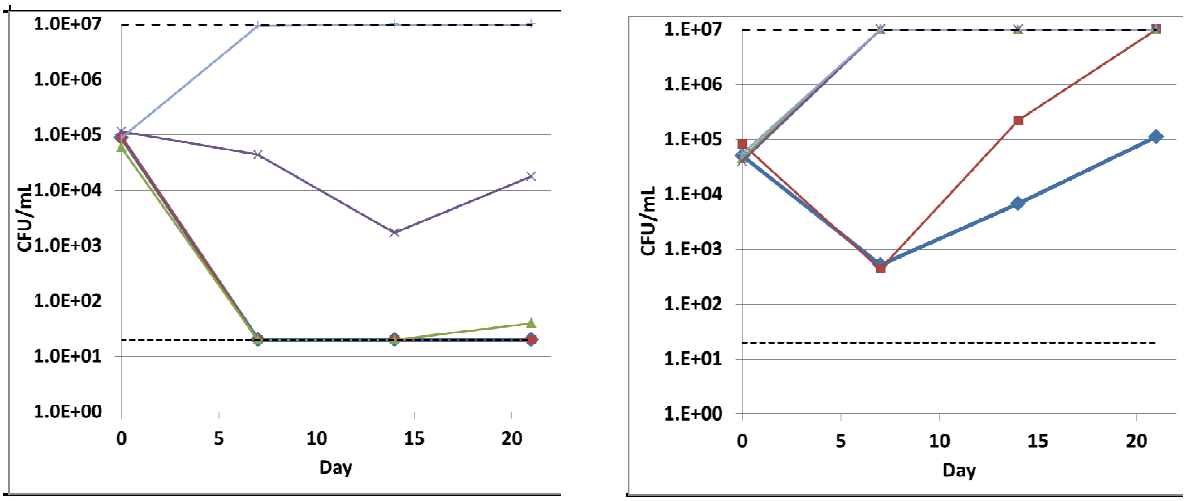

Fig. 3 Dose response plot for minimum bactericidal concentration (MBC) determination (**7e** & **8e**); ___130 µg/mL; ____65 µg/mL; ____4.4 µg/mL; ___3.25 µg/mL; ___ DMSO; **….**Lower limit of detection; _ _ _upper limit of detection; MBC of rifampin is 0.78 µg/mL

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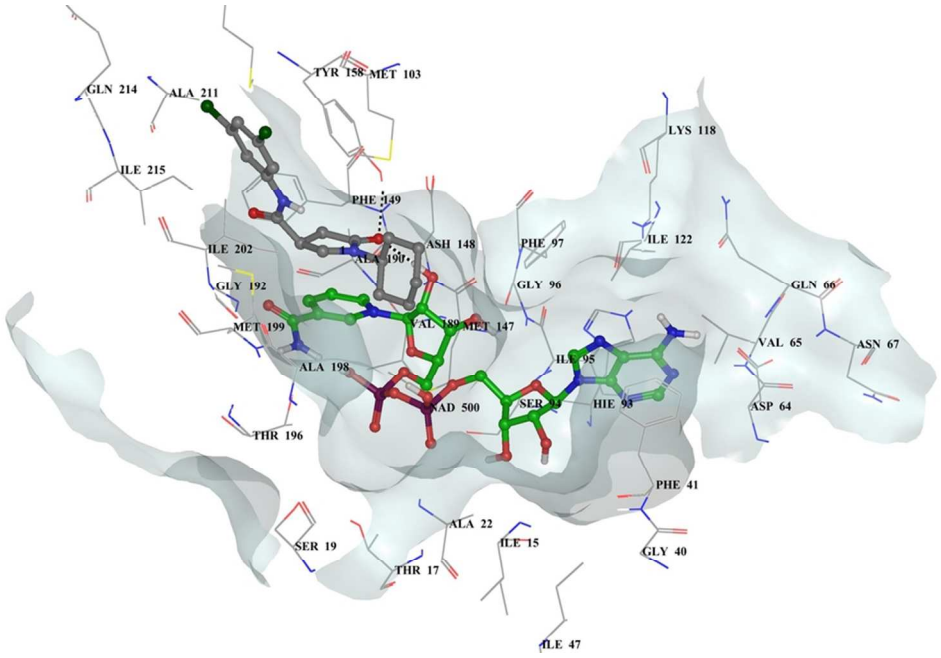

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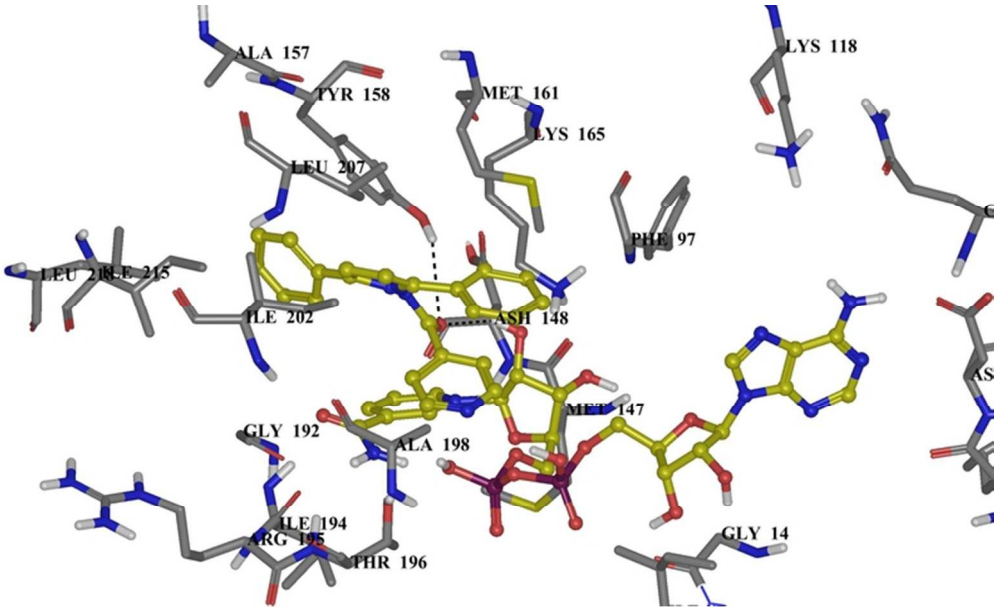

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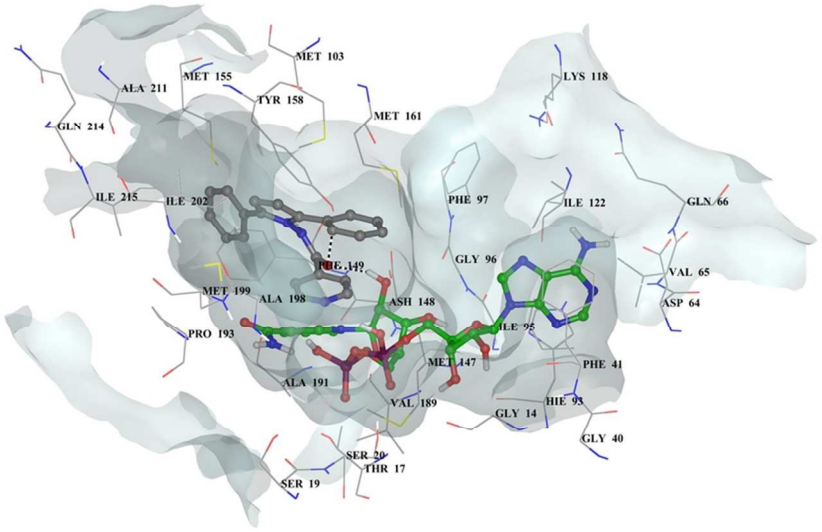

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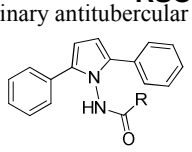
Table 1 Preliminary antitubercular activity of **7a-p** & **8a-p** against Mtb $H_{37}Rv$



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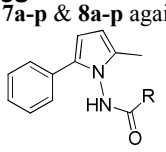
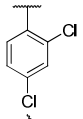
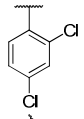
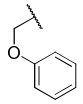
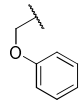
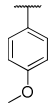
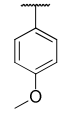
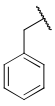
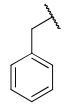
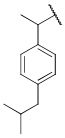
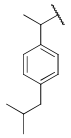
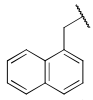
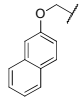
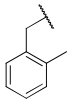
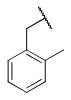
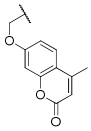
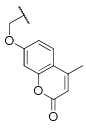


8

ID	R	MIC[a, b]	GI[c]	% Cell viability[d]	Docking Score	ID	R	MIC[a,b]	GI[c]	% Cell viability[d]	Docking Score
7a		25	70	100	-5.017	8a		50	70	100	-6.412
7b		>100	26	ND	-4.585	8b		>100	26	ND	-5.956
7c		>100	NS	ND	-5.393	8c		>100	NS	ND	-6.496
7d		>100	22	ND	-7.164	8d		>100	22	ND	-6.045
7e		**3.70**	**95**	**100**	**-5.873**	**8e**		**5.10**	**92**	**100**	**-6.832**
7f		>100	NS	ND	-5.741	8f		>100	NS	ND	-6.150
7g		62.5	52	ND	-6.164	8g		>100	42	ND	-6.337
7h		>100	NS	ND	-5.469	8h		12.5	62	95	-6.463
7i		>100	28	ND	-6.697	8i		>100	28	ND	-6.305
7j		12.5	75	90	-6.102	8j		25	65	100	-7.034
7k		>100	NS	ND	-4.653	8k		>100	NS	ND	-4.369
7l		6.25	70	100	-4.528	8l		12.5	60	90	-4.565
7m		>100	40	ND	-4.355	8m		>100	40	ND	-5.181
7n		12.5	80	97	-6.502	8n		25	75	100	6.514
7o		>100	NS	ND	-3.058	8o		>100	NS	ND	-3.840
7p		6.25	80	97	-6.076	8p		6.25	85	100	-6.097
RIF		0.06	98	ND	ND	INH		0.4	94	ND	ND
PYZ		50	85	ND	ND	PC		~125	-	ND	-8.907

a, minimum inhibitory concentrations (MIC) are the highest concentration which prevent the complete growth of inoculum; b, µg/mL; c, growth inhibition (GI) of Mtb $H_{37}Rv$ at 50 µg/mL; d, percent viability of cells at 100 µg/mL; Docking score calculated as –Kcal/mol; ND, not determined; NS, Not significant; PC, N-(3,5-dichlorophenyl)-5-oxo-1-phenylpyrrolidine-3-carboxamide which is used as reference molecule in the docking study

Table 2 Cytotoxicity, minimum bactericidal concentration (MBC), drug sensitive and single drug-resistant (SDR) antitubercular activities of **7e** & **8e**

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	TC_{50}	Strain	MIC	IC_{50}	IC_{90}	MBC*
7e	> 100	$H_{37}Rv$	3.7	2.71	3.72	4.4
		INH-R1	>67.8	>67.8	>67.8	
		INH-R2	>67.8	>67.8	>67.8	
		RMP-R1	1.25	0.78	1.12	
		RMP-R2	2.44	2.84	4.06	
		FQ-R1	2.40	1.62	2.61	
		LORA	>67.8	>67.8	>67.8	
		Intracellular macrophage (3.7 and 37.0 μg/mL)	2.4 (Log reduction of CFU)			
8e	>100	$H_{37}Rv$	5.10	4.92	5.16	7.8
		INH-R1	>55.4	>55.4	>55.4	
		INH-R2	>55.4	>55.4	>55.4	
		RMP-R1	14.9	14.4	19.9	
		RMP-R2	30.4	21.32	30.4	
		FQ-R1	28.5	20.77	27.7	
		LORA	1.94	0.86	1.27	
		Intracellular macrophage (5.10 and 51.0 μg/mL)	2.4 (Log reduction of CFU)			
INH		$H_{37}Rv$	0.4-0.6	0.12	0.21	
		INH-R1	>200	110	130	
		INH-R2	>200	95	110	

All concentration are in μg/mL; selectivity index (SI) = TC_{50}/MIC; *, MBC of Rifampin is 0.78 μg/mL