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# Design and synthesis of 5-(5-nitrothiophen-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole derivatives with improved solubility and potential antituberculosis activity

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**We report the design-synthesis of several nitrothiophene containing molecules as antituberculosis agents. The molecules were designed on the basis of previously reported nitrofuran molecules in our laboratory and the α,β-unsaturated linker was modified to cyclized linker in order to overcome the challenge of low solubility and possible toxicity. The stereoelectronic properties such as HOMO, LUMO and HOMO-LUMO gap (HLG) along with other properties such as aqueous solvation energies and QPLogS values were studied. The designed molecules were synthesized and tested for *in vitro* antituberculosis activity and some molecules were found to be highly active comparable to standard drugs. Further, the aqueous solubility was determined using visual inspection method and the designed molecules were found to be more soluble than their chalcone counterparts. Cytotoxicity studies were performed and the molecules were found to be non-cytotoxic. Electroanalytical studies proved nitro reduction as the mechanism of action for these molecules. Thus, this study provides potential nitrothiophene containing hits with improved solubility and reduced chances of toxicity.**

**Keywords**: Nitrothiophene, Antituberculosis, Solubility, Cytotoxicity, Electroanalysis

*Mycobacterium tuberculosis* (*Mtb*), an obligate bacterial pathogen, is the causative agent of tuberculosis (TB). Despite elucidation of the genomic sequence of *Mtb* and the emergence of a multiplicity of targets, residence of *Mtb* within the macrophages coupled with its largely impermeable lipid cell wall barrier, remain key issues challenging the development of successful therapies. Additionally, development of resistance, polytherapy for prolonged duration, and serious side effects with some of the agents[1] pose barriers for the successful therapy. Hence, there is a need to develop new potent inhibitors against latent and resistant bacteria acting by novel mechanisms, which will have minimal chances of development of cross resistance with current drugs. Nitroheterocycles are an important class of drugs that have been well studied in modern chemotherapy. Various nitrofuran derivatives like nitrofurantoin, nitrofurazone, and furazolidone have been used for the treatment of burns and urinary tract infections. Delamanid, a nitrodihydroimidazooxazole derivative[2], has been approved for medical use in Europe, Japan and South Korea while Pretomanid, a nitroimidazopyran derivative[3], is currently in Phase III clinical trials.

Previously in our laboratory, a series of 4-(5-nitrofuran-2-yl)prop-2-en-1-one derivatives as potent antituberculosis agents were reported. In this work, monocyclic nitrofuran ring was explored and it was proposed that these compounds possessed characteristic localized negative potential regions close

to both the oxygen atoms of the nitro group. Screening of these compounds for their antimycobacterial activity led to identification of several derivatives with high antituberculosis activity[4]. Therefore, these nitrofuran derivatives were considered as starting point for this study (**Figure 1**).

Various nitrothiophene derivatives have been reported in literature as anti-infective agents[5–7]. Further, inclusion of the sulphur atom significantly changes the shape, size and lipophilicity of the five membered heterocycle. Considering this, the first part of the design involved the replacement of the furan ring with the thiophene ring.

The other design aspect involved the addition of 5-membered heterocyclic ring in the structure, which was based on various modifications carried out on several conformationally rigid biphenyl analogs of Pretomanid (PA-824) that suffered from poor solubility[8]. Using the concept of substituting the rigid aromatic ring with the heterocyclic ring by Sutherland et al[9] and combining it with the knowledge of pyrazoline containing antituberculosis compounds[10,11] The α, β-carbonyl bridge in the structures reported by our laboratory were replaced by cyclized pyrazoline ring along with nitrothiophene to yield a series of 5-(5-nitrothiophen-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole (Figure 2). Various mono, di, and tri substitutions were included to obtain the preliminary structure-activity relationships for the designed compounds.

Thereafter, these compounds were considered for synthesis and bioassay against *Mtb*. The compounds **3a–3g** were synthesized using acid catalyzed Claisen–Schmidt condensation. These intermediates were then converted into corresponding cyclized derivatives by microwave assisted organic synthesis using hydrazine hydrate with ethanol as solvent. The purity of the compounds was checked by chromatographic techniques and the representative structure were confirmed by spectral data (FT-IR, $^{1}$H NMR, $^{13}$C NMR, and MS).

The synthesized compounds (**4a–4g**) were screened against *Mtb* $H_{37}Rv$ using two-fold dilution to determine the actual minimum inhibitory concentration (MIC) by employing the Resazurin microtiter assay (REMA)[12]. MIC was determined by visual inspection (color change from blue to pink) (**Table 1**). Isoniazid (INH) and ethambutol (EMB) were used as the positive control.

**Table 1:** Anti-TB activity (MIC), cytotoxicity (CC50), HOMO and LUMO for the designed compounds 4a-4g.

| Code | R | Activity (μM) | CC50 (μM) | HOMO (eV) | LUMO (eV) |
|---|---|---|---|---|---|
| 4a | -H | 5.71 | >292 | -0.21 | -0.09 |
| 4b | 4-F | 2.09 | 254.89 | -0.21 | -0.09 |
| 4c | 4-$CH_3$ | 2.71 | >278.41 | -0.22 | -0.09 |
| 4d | 2-Cl | 5.06 | 209.26 | -0.22 | -0.09 |
| 4e | 3-Cl | 10.15 | 221.28 | -0.21 | -0.09 |
| 4f | 3,4-dimethoxy | 4.67 | 235.78 | -0.20 | -0.09 |
| 4g | 3,4,5-trimethoxy | 2.14 | 208.59 | -0.20 | -0.09 |
| INH | - | 0.73 | ND | ND | ND |
| EMB | - | 7.64 | ND | ND | ND |

ND: Not determined

All the synthesized compounds of this series showed potential activity around or below 10 μM. The unsubstituted derivative, 4a showed an activity of 5.71 μM. Substitution by an electron withdrawing group such as 4-fluoro at the para position led to increased activity as observed in compound 4b. The ortho-chloro substituted compound retained the activity whereas the meta-chloro substituted derivative 4d showed reduction in activity. The 3,4,5-trimethoxy derivative showed an excellent activity which was higher as compared to that of the dimethoxy derivative. Earlier work carried out in our laboratory[4,13] using the Density Functional Theory (DFT) postulates indicated that the presence of characteristic localized negative potential regions near both the oxygen atoms of the nitro group. Further, higher negative values of LUMO energy distribution were observed for the most active compounds. It was indicative of the electron accepting capacity of these compounds, suggesting that these compounds are prodrugs and must be activated by TB-nitroreductase. Taking this into consideration, the stereoelectronic properties like HOMO, LUMO, and HLG were calculated for the designed nitrothiophene derivatives. HOMO and LUMO values were found to be ranging from -0.20 to -0.21 and from -0.11 to -0.09 eV respectively. Therefore, this observation corroborated the above mentioned postulates proposed by our research group.

The nitroheterocycles suffer from the disadvantage of mutagenicity, which was responsible for arresting the development of well-known nitroheterocyclic lead CGI-17341[14]. Earlier QSAR studies[15–17]indicated that the important features responsible for mutagenicity were high log P values, large fused planar hydrophobic rings and presence of 2 or more nitro groups among others. Additionally, the analysis of the MIC, $CC_{50}$, and HOMO and LUMO values of earlier derivatives indicated that the HLG of −0.09 to −0.13 eV is sufficient to retain activity without causing cytotoxicity. Thus, taking into consideration all these structural findings, it was postulated that the designed molecules were less likely to be cytotoxic. To confirm this hypothesis, $CC_{50}$ values were evaluated using HEPG2 cell lines, and it was found that the designed compounds showed low cytotoxicity values.

For a compound to be orally active, aqueous solubility is one of the most important properties. A solubility value of 50 µg/mL is considered as the acceptable limit for most of the preclinical drug development programs. The aqueous solubility was calculated for the designed molecules along with their respective chalcone intermediates using the standard Poisson–Boltzmann solver in Jaguar[18].The solvation free energies for the molecules under study varied from −10.787 to −12.387 kcal/mol. Solvation energy gives a measure of compound solubility. Higher negative values of solvation free energy are indicative of higher aqueous solubility. The free energy of solvation for cyclized molecules was found to be higher than that of the intermediate chalcones, which indicates that the designed cyclized derivatives have more aqueous solubility. QikProp[19] predictions for the molecules under study revealed that QPlogS for the molecules was −3.53 to −5.13 (**supporting information**). The higher the negative values, higher the solubility, and therefore, cyclized molecules were found to be more soluble than the non-cyclized chalcone intermediates. Experimentally, the aqueous solubility was determined using visual inspection and a similar trend was observed, showing up to 2 to 3 times increase in solubility.

The bicyclic nitroimidazole Pretomanid is a pro-drug with a very complex mechanism of action active against both replicating and hypoxic, non-replicating *Mycobacterium tuberculosis*. It is a pro-drug activated by deazaflavin (cofactor F420) dependent nitroreductase (Ddn). Consequently, the reduction of the nitro group in the PA-824 and metronidazole is considered to be a key step in the metabolic activation. There is a marked similarity between the electrochemical reactions at the electrode/solution interface, and the enzymatic redox reactions. Thus, knowledge of the electrochemical mechanism of reduction of such type of compounds would be very useful in providing additional information on the mechanism of action. Taking into consideration these studies, nitrothiophene molecule **4c** and metronidazole, as a standard, were selected for electroanalytical studies. Cyclic voltammogram of metronidazole showed a peak at -500 mV indicating reduction of nitro group and another peak around -1400 mV corresponding to diazole moiety (Figure 3). Nitrothiophene derivatives with pyrazoline spacer showed a similar pattern. Therefore it can be concluded that these derivatives act via reduction of nitro group and the reduction potential indicates possible involvement of Ddn.

Thus, in the present work, 5-(5-nitrothiophen-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole were designed as antituberculosis agents considering the previously reported nitrofuran derivatives. The quantum properties such as HOMO, LUMO, and HLG were calculated and were correlated with the activity and mutagenicity of the designed derivatives. The free energy of solvation for the intermediate chalcones and the designed derivatives was calculated, and it was observed that the designed derivatives exhibit more aqueous solubility than the intermediates. Further, these molecules were successfully synthesized and characterized using spectroscopic techniques. *In vitro* activity testing was performed against *Mtb* $H_{37}$Rv using REMA method. The derivatives **4b** and **4g** were found to possess activity comparable with that of INH and more than that of EMB. Cytotoxicity studies were performed and the molecules were found to be non-cytotoxic. This study has emphasized the potential of combining DFT calculations with synthetic approaches and biological evaluation in developing improved nitroaromatics as antituberculosis agents. Mechanistic investigations carried out using electroanalytical method showed that nitro reduction is responsible for the antituberculosis activity of these designed molecules. Further detailed structure-activity relationship and in-depth mechanistic studies are under progress in our laboratory.

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**Conflict of Interest**

Authors declare no conflicts of interest from a financial or commercial standpoint.

**Figure captions:**

**Figure 1.** Design of molecules

**Figure 2.** Designed derivatives to explore preliminary SAR

**Scheme 1.** Synthetic scheme for the designed compounds

**Figure 3.** Cyclic voltammogram for (a) Metronidazole (b) molecule **4c**

## References


(1) Ma, Z.; Lienhardt, C.; McIlleron, H.; Nunn, A. J.; Wang, X. Global Tuberculosis Drug Development Pipeline: The Need and the Reality. *Lancet* **2010**, *375* (9731), 2100–2109.

(2) Matsumoto, M.; Hashizume, H.; Tomishige, T.; Kawasaki, M.; Tsubouchi, H.; Sasaki, H.; Shimokawa, Y.; Komatsu, M. OPC-67683, a Nitro-Dihydro-Imidazooxazole Derivative with Promising Action against Tuberculosis in Vitro and in Mice. *PLoS Med.* **2006**, *3* (11), 2131–2144.

(3) Stover, C. K.; Warrener, P.; VanDevanter, D. R.; Sherman, D. R.; Arain, T. M.; Langhorne, M. H.; Anderson, S. W.; Towell, J. a; Yuan, Y.; McMurray, D. N.; Kreiswirth, B. N.; Barry, C. E.; Baker, W. R. A Small-Molecule Nitroimidazopyran Drug Candidate for the Treatment of Tuberculosis. *Nature* **2000**, *405* (6789), 962–966.

(4) Tawari, N. R.; Bairwa, R.; Ray, M. K.; Rajan, M. G. R.; Degani, M. S. Design, Synthesis, and Biological Evaluation of 4-(5-Nitrofuran-2-Yl)prop-2-En-1-One Derivatives as Potent Antitubercular Agents. *Bioorg. Med. Chem. Lett.* **2010**, *20* (21), 6175–6178.

(5) Rando, D. G.; Sato, D. N.; Siqueira, L.; Malvezzi, A.; Leite, C. Q. F.; Do-Amaral, A. T.; Ferreira, E. I.; Tavares, L. C. Potential Tuberculostatic Agents. Topliss Application on Benzoic Acid [(5-Nitro-Thiophen-2-Yl)-Methylene]-Hydrazide Series. *Bioorganic Med. Chem.* **2002**, *10* (3), 557–560.

(6) Behrouzi-Fardmoghadam, M.; Poorrajab, F.; Ardestani, S. K.; Emami, S.; Shafiee, A.; Foroumadi, A. Synthesis and in Vitro Anti-Leishmanial Activity of 1-[5-(5-Nitrofuran-2-Yl)-1,3,4-Thiadiazol-2-Yl]- and 1-[5-(5-Nitrothiophen-2-Yl)-1,3,4-Thiadiazol-2-Yl]-4-Aroylpiperazines. *Bioorganic Med. Chem.* **2008**, *16* (8), 4509–4515.

(7) Masunari, A.; Tavares, L. C. A New Class of Nifuroxazide Analogues: Synthesis of 5-Nitrothiophene Derivatives with Antimicrobial Activity against Multidrug-Resistant Staphylococcus Aureus. *Bioorganic Med. Chem.* **2007**, *15* (12), 4229–4236.

(8) Palmer, B. D.; Thompson, A. M.; Sutherland, H. S.; Blaser, A.; Kmentova, I.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Denny, W. a. Synthesis and Structure−Activity Studies of Biphenyl Analogues of the Tuberculosis Drug (6 *S* )-2-Nitro-6-{[4-(Trifluoromethoxy)benzyl]oxy}-6,7-Dihydro-5 *H* -imidazo[2,1- *B* ][1,3]oxazine (PA-824). *J. Med. Chem.* **2010**, *53* (1), 282–294.

(9) Sutherland, J. J.; O'Brien, L. a.; Weaver, D. F. A Comparison of Methods for Modeling Quantitative Structure-Activity Relationships. *J. Med. Chem.* **2004**, *47* (22), 5541–5554.

(10) Ali, M. A.; Yar, M. S.; Kumar, M.; Pandian, G. S. Synthesis and Antitubercular Activity of Substituted Novel Pyrazoline Derivatives. *Nat. Prod. Res.* **2007**, *21* (7), 575–579.

(11) Ahmad, A.; Husain, A.; Khan, S. A.; Mujeeb, M.; Bhandari, A. Synthesis, Antimicrobial and Antitubercular Activities of Some Novel Pyrazoline Derivatives. *J. Saudi Chem. Soc.* **2016**, *20* (5), 577–584.

(12) Palomino, J.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Resazurin Microtiter Assay Plate : Simple and Inexpensive Method for Detection of Drug Resistance in Mycobacterium Tuberculosis Resazurin Microtiter Assay Plate : Simple and Inexpensive Method for Detection of Drug Resistance in Mycobacterium Tuberculosis. *Antimicrobail Agents Chemother.* **2002**, *46* (8), 2720–2722.

(13) Tawari, N.; Lele, A.; Khambete, M.; Degani, M. Original Article Mutagenicity prediction for nitroaromatic compounds using qstr modeling. **2014**, *6* (7).

(14) Nagarajan, Kuppuswamy, Radhakrushnan Gowri Shankar, SrinivasachariRajappa, S. J. S. and R. C.-P. Nitroimidazoles XXI 2,3-Dihydro-6-nitroimidazo[2,1-B]oxazoles with Antitubercular Activity. *Eur. J. Med. Chem.* **1989**, *24* (848), 631–633.

(15) Debnath, A. K.; Lopez de Compadre, R. L.; Debnath, G.; Shusterman, a J.; Hansch, C. Structure-Activity Relationship of Mutagenic Aromatic and Heteroaromatic Nitro Compounds. Correlation with Molecular Orbital Energies and Hydrophobicity. *J. Med. Chem.* **1991**, *34* (2), 786–797.

(16) Glende, C.; Klein, M.; Schmitt, H.; Erdinger, L.; Boche, G. Transformation of Mutagenic Aromatic Amines into Non-Mutagenic Species by Alkyl Substituents: Part II: Alkylation Far Away from the Amino Function. *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.* **2002**, *515* (1–2), 15–38.

(17) Klein, M.; Erdinger, L.; Boche, G. From Mutagenic to Non-Mutagenic Nitroarenes: Effect of Bulky Alkyl Substituents on the Mutagenic Activity of Nitroaromatics in

Salmonella Typhimurium. Part II. Substituents Far Away from the Nitro Group. *Mutat. Res. - Genet. Toxicol. Environ. Mutagen.* **2000**, *467* (1), 69–82.

(18) Jaguar, Schrödinger, LLC, New York, NY, 2016.

(19) QikProp, Schrödinger, LLC, New York, NY, 2016.