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Re-engineering nalidixic acid's chemical scaffold: A step towards the development of novel anti-tubercular and anti-bacterial leads for resistant pathogens

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ABSTRACT

Occurrence of antibacterial and antimycobacterial resistance stimulated a thrust to discover new drugs for infectious diseases. Herein we report the work on re-engineering nalidixic acid's chemical scaffold for newer leads. Stepwise clubbing of quinoxaline, 1,2,4-triazole/ 1,3,4-oxadiazole with nalidixic acid yielded better compounds. Compounds were screened against ciprofloxacin resistant bacteria and *Mycobacterium tuberculosis* $H_{37}Rv$ species. Results were obtained as minimum inhibitory concentration, it was evident that molecule with quinoxaline linked azide as side chain served as antitubercular lead (<6.25 μg/ml) whilst molecule with oxadiazole or triazole linked quinoxaline side chain served as antibacterial lead. Few compounds were significantly active against *Escherichia coli* and *Proteus vulgaris* with MIC less than 0.06 μg/ml and relatively potent than ciprofloxacin. No true compound was potentially active against *Salmonella* species as compared to amoxicillin.

Discovery of antibiotics and synthetic antibacterials revolutionized the treatment of infectious diseases and saved millions of lives. It seems that the battle was over. Now, pathogens are evolving and mutating in a bid to survive, and they successfully developed resistance to the existing antibiotics and synthetic antibacterials. In 2014, World Health Organization (WHO) report on global surveillance of antimicrobial resistance stated that the present antibiotic resistance is no longer a prediction for the future and the fight against antimicrobial resistance is not an optimistic. In 2013, there were 480,000 new cases of MDR-TB in the world and it was evident that 20.5% of previously treated TB cases are estimated to have MDR-TB (multidrug resistant tuberculosis). In Addition, extensively drug-resistant TB (XDR-TB, defined as MDR-TB with resistance to any fluoroquinolone and any second-line injectable drug) has been notified in 100 countries, of the globe.[1,2]

Infectious diseases caused by *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Salmonella paratyphi* are the most prevalent and fatal infectious diseases.[3] Now-a-days, upon infection by these organisms, patient shows wide range of symptoms and then become resistant to the current antibacterials/ antibiotics such as, beta-lactams, fluoroquinolones and macrolides.[4,5] Finding new efficacious antibiotic/antibacterial against bacterial resistance is possible but it's a complex and challenging area of research. Antitubercular research, is also an area that has not been a primary focus for the pharmaceutical industry. The reason may be due to a variety of factors such as, (a) misperception that new treatments were not needed, (b) the lack of financial incentive as antibiotics generally represent a relatively low return on investment, (c) the demanding regulations for drug development.[6]

WHO stated[7] that 'Owing to the ineffective remedy and risk in the treatment option in tuberculosis, by 2020, the global burden of tuberculosis is estimated to be 2.3 million, of which 99% will be in developing countries'. In the year 2000, the global alliance was established to accelerate the development of new antitubercular agents and to ensure their availability and affordability in high-epidemic countries.[8] For the first time in decade, under the guidance of global alliance and pharmaceutical companies, there were 20 new molecules developed with promising characteristics during in vitro and in vivo animal studies. However those molecules were not brought to the realization and precluded their future development. In connection with the above discussion, it was felt that there is an urgent demand for newer agents in the

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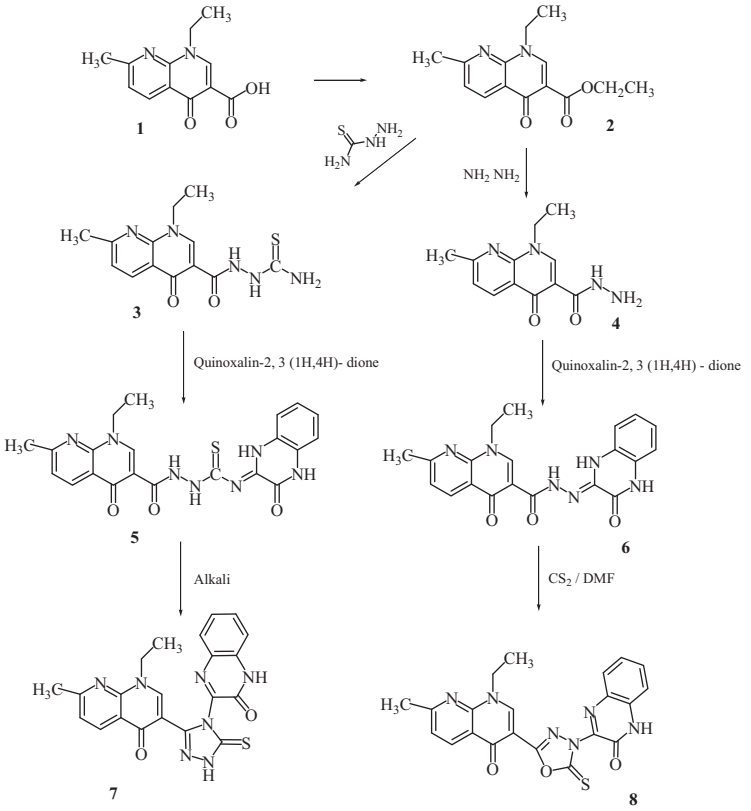
chemotherapy of tuberculosis and drug resistant bacterial infections and search for new entity is still continuing interest.

Nalidixic acid is the first quinolone based drug to be marketed in 1963 as an antibacterial agent, reserved for urinary tract infection, having α,β unsaturated carboxylic acid at 2, 3 positions of 1,8 naphthyridine moiety.[9,10] The antibacterial spectrum of nalidixic acid is widely varied, known to exhibit resistance towards most of the common pathogens like *Salmonella*, *Staphylococcus*, *Pseudomonas*, *Proteus* and *Escherichia* species. It is a bactericidal and acts by topoisomerase enzyme inhibition.[11] Frequent use of fluoroquinolones in bacterial infections has resulted in declined susceptibility for above pathogens. There are reports of decreased susceptibility to ciprofloxacin in common pathogens[12] indicating that the need for newer quinolone in future. A study indicated that nalidixic acid resistant pathogens with decreased susceptibility to fluoroquinolones are now endemic in many developing countries.[13] Owing to the toxicity of newer synthetic agents, chemimanipulation of proven leads or drug candidate, would help us in better molecule with lower toxicity. In this communication, the mechanism of nalidixic acid that inhibit nucleic acid synthesis was considered, and it was chosen as a precursor to perform reengineering of molecule by chemical scaffold. In the modification process, –COOH group was targeted to link potent heterocyclic moieties.

The antimicrobial and antitubercular study of various quinoxaline derivatives has become of much interest in recent years.[14–17] The potent antimicrobial spectrum of quinoxaline towards pathogens may be due to its wide range of mode of action such as binding to guanine residue of DNA and function as bifunctional intercalating agent,[18] inhibition of RNA synthesis,[19] and HIV-1-reverse transcriptase inhibition.[20] In continuation with our earlier Letters on molecular modification of nalidixic acid[21] and antitubercular potential of quinoxaline-2,3-diones[22,23] the present work was designed to exploit –COOH group of nalidixic acid for a new class of antimicrobial and antitubercular agents.

In the present communication, nalidixic acid **1**, was subjected to the esterification reaction to afford carboxylate **2** (Scheme 1). The compound **2** was refluxed with thiosemicarbazide and hydrazine hydrate to give thiosemicarbazide **3** and carbohydrazide **4**, respectively. Compounds **3** and **4** were treated in equimolar with quinoxaline-2,3(1*H*,4*H*)-dione in refluxing to give the corresponding carbohydrazide **6**. The *J* values in ^{1}H NMR data of compounds **5**, **6** were found to be in between 9 and 13 Hz for multiplet for Ar–H of quinoxaline and the shift value of $-CH_3$ (1.5 ppm) in compounds **3** and **4** was protected their quinoxaline conjugates **5** and **6**.

The reaction was confirmed by proton NMR signal at 6.9–7.1 ppm as a multiplet with *J* value of 2 Hz and 4 Hz for quinoxaline protons and ^{13}C NMR signals at 184.3 (C=S), 168.9 (C=O amide), 165.5 (C=O quinone), 159.2 (C=O, quinox). Further the compound **5** was subjected to cyclization in alkali by reflux and afforded compound **7** and in the same manner compound **6** was converted into compound **8** by treating with carbon disulfide in DMF. Respective conversion of compound **5** and compound **6** to 1,2,4-triazole and 1,3,4-oxadiazole side chains were established by disappearance of coupled vibration of C=O functional group in IR-spectra around 1680 cm^{-1} and bathochromic shift in λ_{max} to visible region. Further, it was established by the disappearance of ^{1}H NMR Signals around 7.3 ppm and ≈11 ppm NH proton. It was confirmed by the disappearance of ^{13}C NMR Signals for –CONH– at ≈169 ppm. However, there is a probability for the formation 1,2,4-thiadiazole from compound **5**. But, spectral data of compound **7** showed the presences of deshielded ^{13}C NMR signal at 189 ppm for C=S and thus confirms the formation of 1,2,4-triazole. Reaction progress and homogeneity were confirmed by TLC technique using ethyl acetate/methanol/water at the ratio of



Scheme 1. Synthesis of nalidixic acid derivatives.

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