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Novel fluoroquinolones: design, synthesis, and in vivo activity in mice against *Mycobacterium tuberculosis* $H_{37}Rv$

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Abstract—Novel 6,8-difluoro-1-alkyl-5-amino-1,4-dihydro-4-oxo-7-{4-substituted piperazin-1-yl}-quinoline-3-carboxylic acids, with the substituents at 4th position of piperazine being -[2(pyridine-4-carbonyl) hydrazono]propyl and -2 [(pyrazine-2-carbonyl) amino] ethyl, were synthesized and evaluated in vivo against *Mycobacterium tuberculosis* $H_{37}Rv$ in Swiss albino mice. Test compounds exhibited activity comparable to that of sparfloxacin (survival rate, reduction of splenomegaly and reduced tubercular lesions) at a dose of 200 mg/kg.
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Tuberculosis (TB) is a serious health problem, due to which three million people die every year worldwide.[1] The association of TB and HIV infection is so dramatic that in some cases, nearly two-thirds of the patients diagnosed with TB are also HIV seropositive. In addition to this, the increase in *M. tuberculosis* strains resistant to front-line anti-mycobacterial drugs such as rifampin (RIF) and isoniazid (INH) has further complicated the problem, which clearly indicates the need for more effective drugs for the efficient management of tuberculosis.[2]

Among the quinolone class of anti-bacterial agents, fluoroquinolones have shown promise in curing TB. Ofloxacin at a dose of 300–800 mg and Levofloxacin at a dose of 250–500 mg daily when used in combination with agents like *p*-aminosalicylic acid, ethionamide, and cycloserine is effective in the treatment of multi-drug resistant tuberculosis. These fluoroquinolones are found to be highly concentrated in the host cells, which further enhance their anti-mycobacterial action.

Fluoroquinolones act by interfering with the action of the bacterial DNA gyrase, which results in the degradation of chromosomal DNA and leads to termination of chromosomal replication and interference with cell division and gene expression.[3]

As mycobacteria have lipid rich cell walls, lipophilicity is an important consideration in the design and activity of novel molecules.[4] Similarly as C-7 substitution in the fluoroquinolones affect their pharmacokinetic profile and the spectrum of activity,[5] in the present work structural modifications were mainly centered at the C-7 position.

An intermediate 1-(*tert*-butyl)-7-(piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**vi**) is the intermediate for compounds **I** and **III**. It was synthesized as shown in Scheme 1.

Equimolar amounts (0.171 M) of 3-chloro-4-fluoroaniline (**i**) and diethylethoxymethylenemalonate (**ii**) were condensed at 145 °C to get 3-chloro-4-fluoroanilinomethylenemalonic acid diethyl ester (**iii**), which was then cyclized by heating at 250 °C in diphenyl ether to get ethyl 7-chloro-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylate (**iv**), which was then reacted with *tert*-butyl bromide in *N*,*N*-dimethylformamide (DMF) in presence of sodium hydride and the product formed was hydrolysed to get the free acid (**v**), which was then condensed with piperazine in DMF to get **vi** (mp: 204 °C).

Reacting compound **vi/x** (0.02 M) with bromoacetone (0.03 M) in presence of triethylamine in 120 ml of DMF at 80 °C for 2.5 h gave intermediates, which after removal of excess bromoacetone were then reacted with

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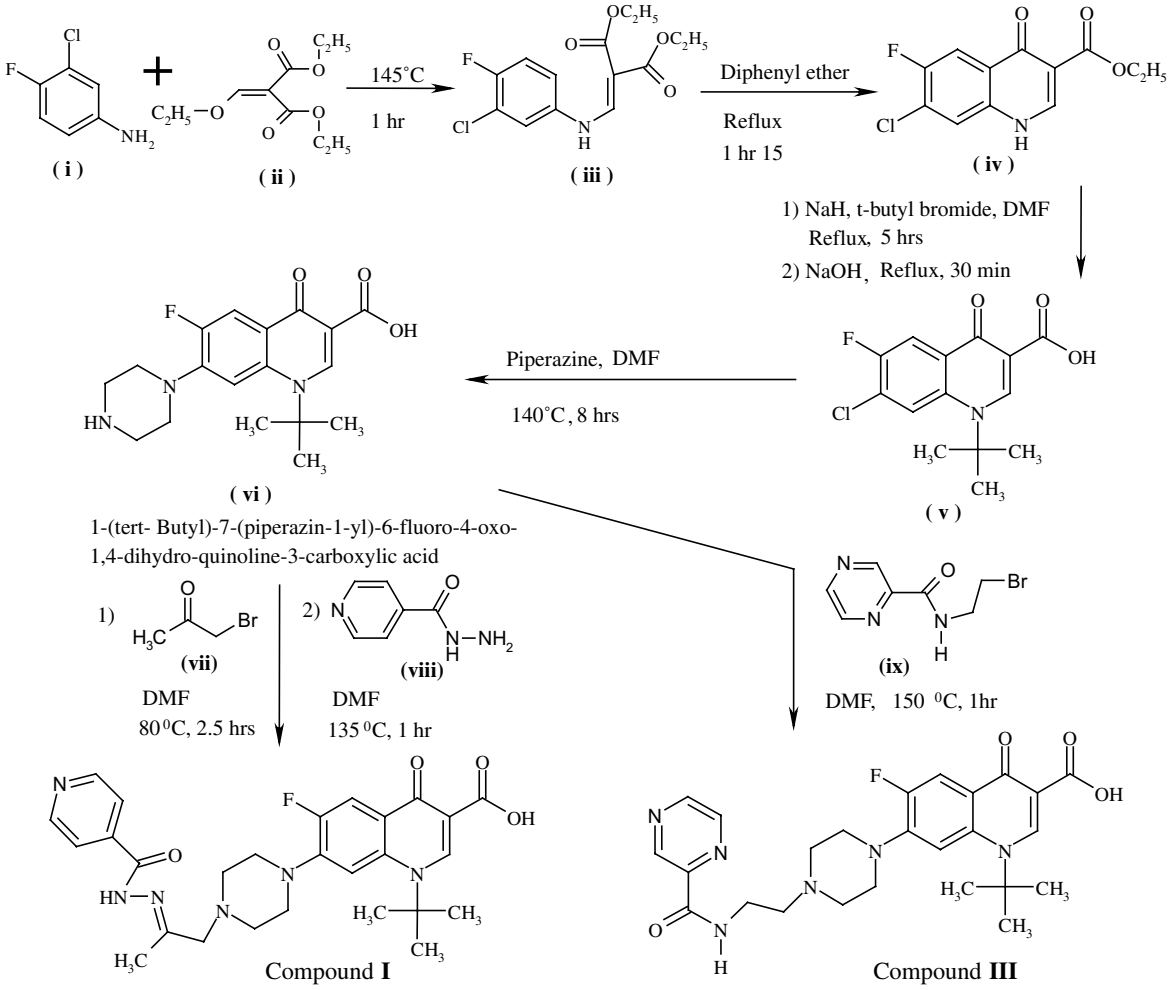
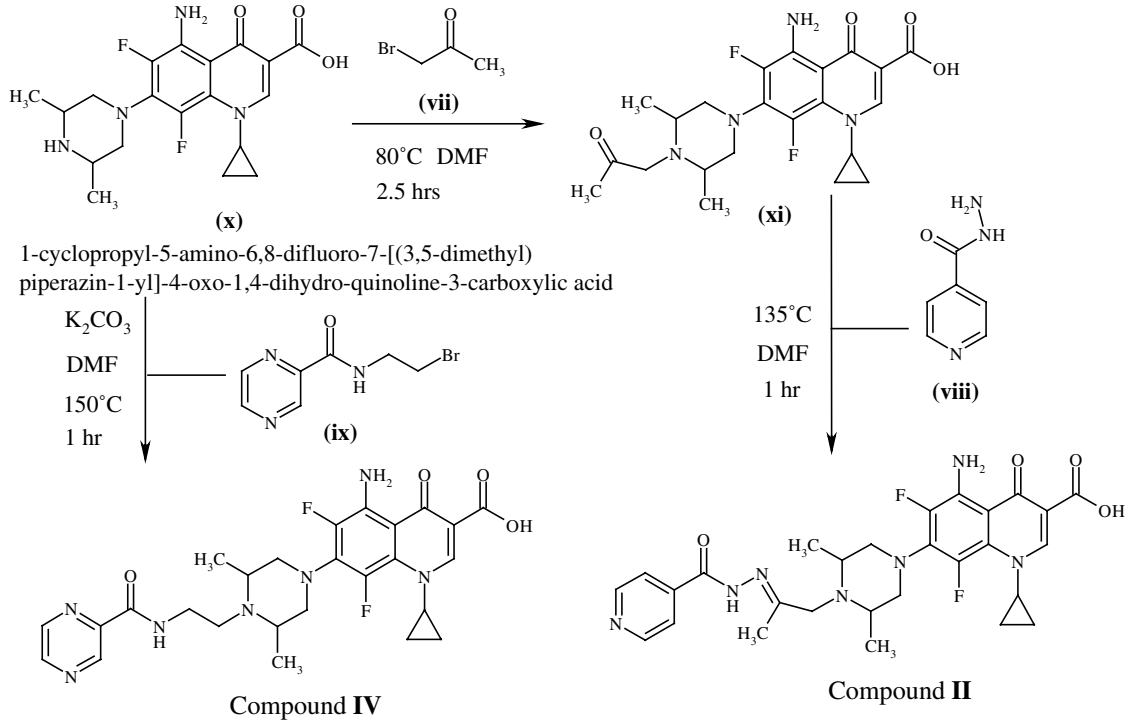


(i) (ii) (iii) (iv) (v) (vi) (vii) (viii) (ix)

1-(tert- Butyl)-7-(piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid

Scheme 1. Synthesis of compounds **I** and **III**.



(x) (xi) (ix) (viii)

1-cyclopropyl-5-amino-6,8-difluoro-7-[(3,5-dimethyl) piperazin-1-yl]-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid

Scheme 2. Synthesis of compounds **II** and **IV**.

Table 1. Characterization data of test compounds

Compound	Mp (°C)	Yield (%)	Spectral analysis
I	295–297	62.08	FT-IR (KBr): 3522 (O–H), 3067 (C–H, Ar), 1697 (C=O, acids), 1626 (C=O, amide), 1539 (C=N, imine), 1253 ($(CH_3)_3$–C) H^1 NMR (DMSO-d_6): 11.5 (s, 1H, O–H), 8.86 (s, 1H, C-2 of quinolone), 8.44, 8.12 (d, 2H, Ar), 5.32 (s, 1H, –CONH–N=), 4.14, 2.50 (m, 8H, piperazine), 1.41 (s, 9H, *t*-butyl)
II	304–308	62.31	FT-IR (KBr): 3481 (O–H), 3283 (N–H), 2980 (C–H aromatic), 2851 (C–H aliphatic), 1728 (C=O acids), 1635 (C=O amide), 1026 (C–H, cyclopropyl) H^1 NMR (DMSO-d_6): 8.76 (s, 1H, C-2 of quinolone), 8.46, 7.80 (m, 4H, Ar) 7.20 (s, 2H, NH_2), 3.96 (m, 1H, cyclopropyl), 3.33 (m, 6H, 3,5-dimethyl of piperazine), 2.83–2.49 (m, 6H, piperazine), 1.05 (m, 4H, cyclopropyl)
III	210–212	74.33	FT-IR (KBr): 3499 (O–H), 3329 (N–H), 2930 (C–H, Ar), 2892 (C–H aliphatic), 1630 (C=O amide), 1572 (N–H amide) H^1 NMR (DMSO-d_6): 8.62 (s, 1H, C-2 of quinolone), 8.1, 8.0 (d, 2H, Ar), 7.7, 7.6, 7.4 (m, 2H, pyrazine), 5.6 (m, 1H, –CONH–), 4.08 (m, 2H, –CH2), 3.6, 2.5 (m, 8H, piperazine), 2.6 (m, 2H, –CH_2), 1.23 (s, 9H, *t*-butyl)
IV	226–228	72.61	FT-IR (KBr): 3545 (O–H), 3323 (N–H), 2930 (C–H, Ar), 2852 (C–H, –CH_2–), 1631 (C=O, amide) H^1 NMR (DMSO-d_6): 9.1 (s, 1H, O–H), 8.83 (s, 1H, C-2 of quinolone), 8.71, 8.58, 7.82 (m, 3H, pyrazine), 7.3 (m, 2H, NH_2), 5.6 (m, 3H, pyrazine), 4.06 (m, 1H, cyclopropyl), 4.03, 3.73 (m, 4H, –CH_2–), 3.46 (m, 6H, 3,5-dimethyl of piperazine), 2.91–2.50 (m, 6H, piperazine), 1.63 (m, 4H, cyclopropyl)

isonicotinic acid hydrazide (0.013 M) at 135 °C for 1 h. Finally removal of DMF and recrystallization from ethanol–DMF (70:30) gave compounds **I** (Scheme 1) and **II** (Scheme 2), respectively.

Condensation of equimolar quantities (0.0086 M) of compound **vi/x** with 2(pyrazinamido)-1-bromoethane in presence of anhydrous potassium carbonate in 20 ml of DMF at 150 °C for 1 h followed by removal of DMF, washing with water and recrystallization from DMF–water (60:40) gave compounds **III** (Scheme 1) and **IV** (Scheme 2), respectively.

Compounds **ix** and **x** were synthesized as per literature methods[6,7] and were characterized by their melting points and spectral analysis. Characterization data for test compounds **I**–**IV** is given in Table 1.

The test compounds were evaluated for anti-tubercular activity in Swiss albino mice[8] using sparfloxacin (SPFX) as a standard (dose equivalents correlating anti-tubercular activity in mice: SPFX: 60 mg/kg≈INH: 25 mg/kg≈RIF: 20 mg/kg).[9,10] A group of five mice were used for testing each compound. The mice were infected intravenously with *M. tuberculosis* $H_{37}Rv$ (≈10^6 organisms) and after 24 h, compounds **I**–**IV** were administered orally six times a week for a period of four weeks. The parameters studied were survival rate, spleen weights, gross lung lesions, and colony forming units (CFUs) in the spleen. Groups treated with **III** and sparfloxacin exhibited 100% survival rate while those treated with **I**, **II**, and **IV** gave a value of 80% (Fig. 1). All treated groups exhibited reduction of splenomegaly (Fig. 2). Groups treated with **III**, **IV**, and sparfloxacin showed absence of lung lesions, while those with **I** and **II** showed few lung lesions. Groups treated with **II**, **III**, and sparfloxacin showed 55%, 75%, and 56% inhibition of CFUs, respectively, at a dose of 200 mg/kg (Fig. 1).

Results of the study indicate potent anti-tubercular activity for test compounds **I**–**IV**. Compound **III** with

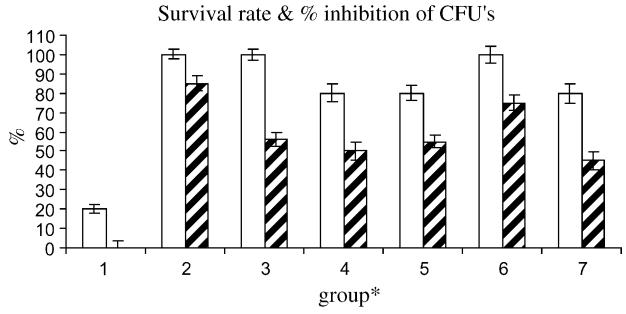


Figure 1. Effect of test compounds **I**–**IV** and sparfloxacin on the survival rate (determined on 30th day) and inhibition of CFUs observed on Lowenstein–Jensen medium expressed as percentage. Open columns represent survival rate and striped columns represent %inhibition of CFUs in spleen in mice.
*Group 1-Infected mice without any drug treatment (−ve control); 2-Sparfloxacin treated (300 mg/kg) (+ve control); 3-Sparfloxacin treated (200 mg/kg) (+ve control); 4-Compound **I** treated (200 mg/kg); 5-Compound **II** treated (200 mg/kg); 6-Compound **III** treated (200 mg/kg); 7-Compound **IV** treated (200 mg/kg).

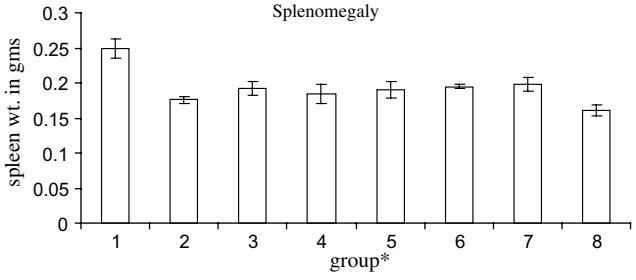


Figure 2. Effect of test compounds **I**–**IV** and sparfloxacin on spleen enlargement observed during tuberculosis infection in mice. *Group 1-Infected mice without any drug treatment (−ve control); 2-Sparfloxacin treated (300 mg/kg) (+ve control); 3-Sparfloxacin treated (200 mg/kg) (+ve control); 4-Compound **I** treated (200 mg/kg); 5-Compound **II** treated (200 mg/kg); 6-Compound **III** treated (200 mg/kg); 7-Compound **IV** treated (200 mg/kg); 8-Non-infected mice.

100% survival rate, absence of lung lesions, and 75% inhibition of CFUs was most potent.

Testing against clinical isolates of *M. tuberculosis* and *M. avium* complex would establish their clinical efficacy and potential for use in immunocompromised systems.

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