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Synthesis, antibacterial and antitubercular activities of some 7-[4-(5-amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] fluoroquinolonic derivatives

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Abstract

In the present study, a series of 7-[4-(5-amino-1,3,4 thiadiazole-2-sulfonyl)]-1-piperazinyl fluoroquinolonic derivatives **VIIa–d** were synthesized in good yields and characterized by IR, ^{1}H-NMR, ^{13}C-NMR, FAB Mass spectral and elemental analyses. The compounds were evaluated for their preliminary in vitro antibacterial activity against some Gram-positive and Gram-negative bacteria and selected compounds **VIIa**, **b** were screened for antitubercular activity against *Mycobacterium tuberculosis* H_{37}Rv strain by broth dilution assay method. The antibacterial data of the tested N-sulfonylfluoroquinolones **VIIa–d** indicated that all the synthesized compounds showed better activity against Gram-positive bacteria *S. aureus*, *E. faecelis*, *Bacillus* sp. (MIC = 1–5 μg ml^{-1}, respectively) compared to reference drugs. The MIC values of tested derivatives connotes that the sparfloxacin and gatifloxacin derivatives **VIIc**, **d** were most active against the tested Gram-positive bacterial strains (MIC = 1–5 μg ml^{-1}). All the tested compounds **VIIa–d** showed poor activity against the Gram-negative bacteria. The in vitro antitubercular activity reports of selected compounds **VIIa**, **b** against *M. tuberculosis* strain H_{37}Rv showed moderate activity at MIC of 10 μg ml^{-1}.

Keywords: N-sulfonyl fluoroquinolones; 1,3,4-Thiadiazole; Antibacterial activity; Antitubercular activity; Broth dilution assay method

1. Introduction

Quinolone antibacterials are compounds of profound interest because of their broad antibacterial spectrum both to Gram-positive and Gram-negative bacteria and their in vivo chemotherapeutic efficacy [1,2]. To date many quinolone antibacterial agents have been introduced into clinical use and significant improvements in antibacterial spectrum and activity has been achieved [3]. Since the discovery of nalidixic and oxolinic acids [4,5], many modifications in the chemical structure of the quinolonic ring yielded new drugs with enhanced bioavailability and improved antibacterial efficacy against Gram-positive and anaerobic bacteria [6,7]. Further advances in the development of quinolones are likely to provide better compounds for clinical use [8]. Fluorine-containing nalidixic acid derivatives, the fluoroquinolones (FQs) were introduced into clinical practice in the 1980s. With broad-spectrum antimicrobial activity, FQs are widely recommended and employed for the treatment of various bacterial infections of the respiratory, gastrointestinal and urinary tracts, as well as sexually transmitted diseases and chronic osteomyelitis [9–12]. They accounted for approximately the 11% of all antibiotic sales in the USA in 2002 [13], their use is increasing in the USA and throughout the world.

FQs are presently used to treat tuberculosis primarily in cases involving resistance or intolerance to first-line anti-tuberculosis therapy [14] and are currently approved as second-line agents for the treatment of multidrug-resistant (MDR) tuberculosis by the WHO [15]. They are reported to penetrate into macrophages and possessing better bactericidal activity [16,17] and also have good in vivo activity against *Mycobacterium*

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tuberculosis [18,19]. FQs when combined with various first-line antituberculosis drugs have shown greater reductions in colony forming units (cfus) of intramacrophage *M. tuberculosis* than the individual drugs alone [20], indicating that their in vitro activity is a valuable analytic tool that may be useful in helping to predict in vivo efficacy.

The newer FQs, sparfloxacin, gatifloxacin exhibit lower minimum inhibitory concentrations (MICs) than levofloxacin, ciprofloxacin and ofloxacin [21–25] against rifampicin tolerant persistent organisms: *bacilli* that survive and persist despite chemotherapy in an in vitro model examining sterilizing activity, it was found that the gatifloxacin and moxifloxacin showed highest bactericidal activities of the FQs tested [26].

FQs are the only direct inhibitors of DNA synthesis by binding to the enzyme–DNA complex; they stabilize DNA strand breaks created by DNA gyrase and topoisomerase IV. Ternary complexes of drug, enzyme and DNA block progress of the replication fork [3]. The inhibition of DNA gyrase and cell permeability of quinolones is greatly influenced by the nature of C-7 substituent on the standard structure of 4-quinolone-3-carboxylic acid [27]. In addition, substitution of bulky functional groups is permitted at the C-7 position [28]. SAR of antibacterial FQs have been extensively investigated and the substituent at the C-7 position has a great impact modulating potency, spectrum, biopharmaceutics and pharmacokinetics [29].

Sulfonyl fluoroquinolones (NSFQs) are a new class of antibacterial FQs with especially high in vitro activity against Gram-positive bacteria [30]. From a structural point of view, NSFQs could be considered hybrid drugs, since they incorporate moieties of both sulfonamides and FQs. In fact the NSFQs would exert the principal biological action though a quinolone-like mechanism although carrying a sulfa portion [31]. During recent years a number of quinolones with substitution on piperazine ring at C-7 position of the basic structure of quinolones were synthesized and evaluated for antibacterial activities [32–35]. Inspired by the previous research results [36] and in continuation of our research [37,38], in this paper we report the synthesis, spectral studies, in vitro antibacterial and antituberculosis activity of a series of 7-[4-(5-amino-1,3,4 thiadiazole-2-sulfonyl)]-1-piperazinyl fluorquinolonic derivatives.

2. Chemistry

The synthesis of 7-[4-(5-amino- [1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] fluoroquinolonic derivatives **VIIa–d**, denominated as N-sulfonyl fluorquinolonic derivatives was achieved through the versatile and efficient synthetic route outlined in Scheme 1. The starting compound 2-amino-5-mercapto-1,3,4-thiadiazole **II** was prepared by direct cyclization of thiosemicarbazide **I** [39]. Compound **II** was converted to 2-acetylamino-5-mercapto-1,3,4-thiadiazole **III** by heating with acetic anhydride, which has been oxidized and chlorinated to 5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl chloride **IV** [40]. The N-sulfonyl fluorquinolonic derivatives **VIIa–d** were prepared from the 7-(1-piperazinyl) FQs **Va–d**, by reaction with N-acetylsulfonyl chloride **IV**, producing the intermediate compounds 7-[4-(5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] FQs **VIa–d**, which lead to final products **VIIa–d** by hydrolysis in good yields.

3. Biological activity

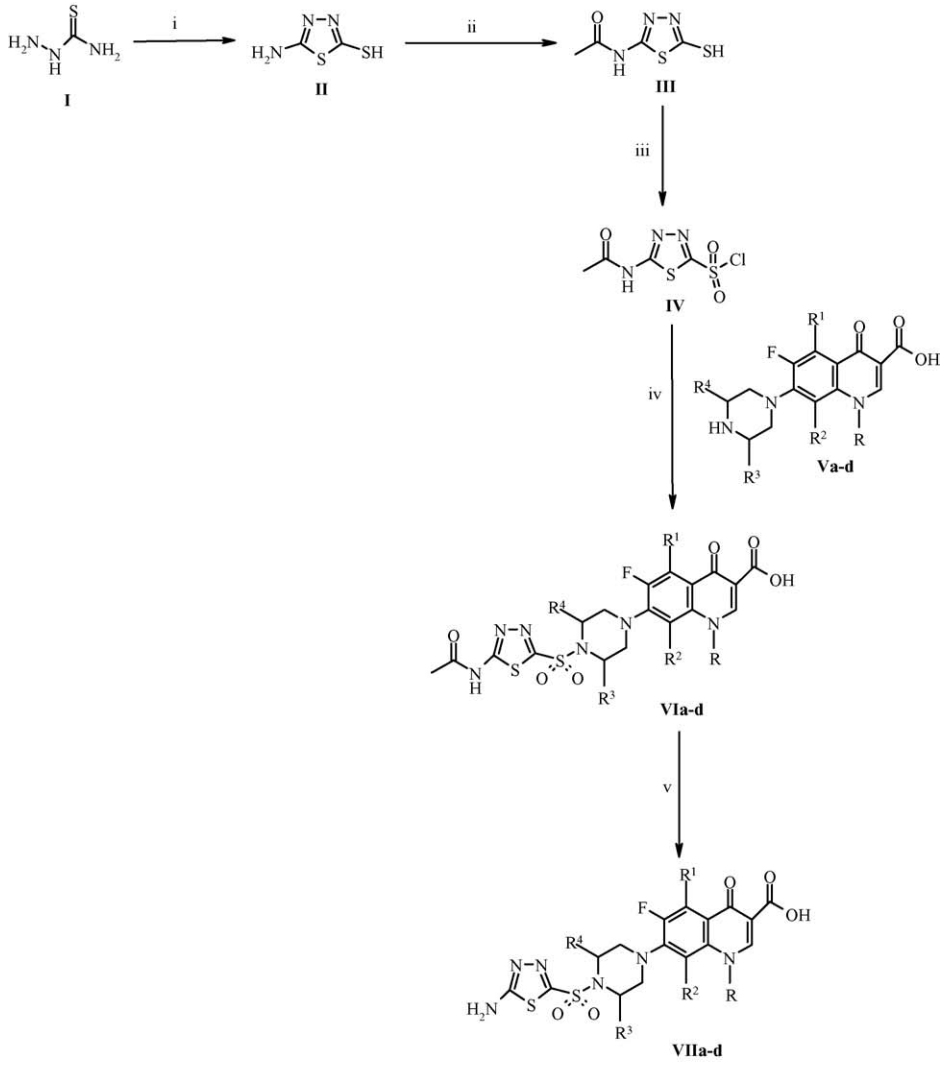
The standard strains were procured from the Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of synthesized compounds **VIIa–d** was performed by broth microdilution method [41,42] against the following standard bacterial strains: *S. aureus* (MTCC 3160), *E. faecelis* (MTCC 2729), *S. epidermidis* (MTCC 3382), *Bacillus* sp. (MTCC 297), *P. aeruginosa* (MTCC 1034), *E. coli* (MTCC 1089), *Klebsiella* sp. (MTCC 3384), *Citrobacter freundi* (MTCC 1658). The compounds were also screened against the following pathological strains *Proteus* sp., *Corynebacterium*.

MIC of compounds **VIIa** and **VIIb** was determined against *M. tuberculosis* strain $H_{37}Rv$ by using broth dilution assay method [43,44].

4. Results and discussion

A series of 7-[4-(5-amino-1,3,4 thiadiazole-2-sulfonyl)]-1-piperazinyl fluorquinolonic derivatives **VIIa–d** were synthesized in good yields using the synthetic route outlined in Scheme 1. The starting material 5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl chloride **IV** was synthesized by oxidative chlorination of 2-acylamino-5-mercapto-1,3,4-thiadiazole **III** in an aqueous acid solution and passing in the chlorine gas. Compound **IV** was later refluxed with various 7-(1-piperazinyl) FQs **Va–d** producing the intermediates 7-[4-(5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] FQs **VIa–d** in good yields, which were later hydrolyzed to title compounds **VIIa–d**. Structures of the synthesized compounds were established on the basis of IR, ^{1}H-, ^{13}C-NMR, FAB Mass spectral data and elemental analyses.

The IR spectrum of intermediates **VIa–d** showed broad absorption bands around 3410–3450 cm^{-1} for OH (COOH), 3250–3281 cm^{-1} for NH (CONH), while the distinguishing broad absorption peaks C=O for acid was observed in the range 1711–1732 cm^{-1} and C=O for amide was observed in the range 1682–1702 cm^{-1}. The title compounds **VIIa–d** displayed absorption bands ranging from 3468 to 3530 cm^{-1} for NH_2, 3400–3420 cm^{-1} for OH. Strong absorption band ranging from 1697 to 1730 cm^{-1} was observed for C=O acid and between 1620 and 1651 cm^{-1} for C=O (4-oxo-quinolone), in their respective IR spectra. These compounds also exhibited appropriate peaks at corresponding δ ppm (see spectral data) in their ^{1}H-NMR, ^{13}C-NMR spectra which were in conformity with the assigned structures. ^{1}H-NMR spectrum showed the characteristic singlets around 14.84–15.09 δ ppm for carboxylic acid protons and around 8.98–10.15 δ ppm for the NH_2 protons, in particular compound **VIIc** displayed the NH_2



	R	R^1	R^2	R^3	R^4
V-VII a		H	H	H	H
V-VII b	$-CH_2CH_3$	H	H	H	H
V c		$NHCOCH_3$	F	CH_3	CH_3
VI c		$NHCOCH_3$	F	CH_3	CH_3
VII c		NH_2	F	CH_3	CH_3
V-VII d		H	OCH_3	H	CH_3

Scheme 1. *Reagents and conditions*: (i) EtOH, CS_2, reflux, 4 h; (ii) acetic acid anhydride, reflux, 2 h; (iii) 33% acetic acid, gaseous chlorine (iv) dried acetone, reflux, 2 h; (v) 2 N NaOH reflux, 4 h; 10% acetic acid.

protons (at C5 quinolone) around 4.25 δ ppm, aryl protons between δ 8.76 and 7.25 ppm and the other alkyl protons appeared as multiplets between δ 3.88 and 1.05 ppm confirming the reaction of 7-(1-piperazinyl) FQs **Va**–**d** with N-acetylsulfonyl chloride **IV**. In ^{13}C-NMR for the representative **VIIa**–**d**, we have observed most characteristic signals appeared at around δ 207–201 ppm for carbonyl carbon peak, δ 177–176 ppm for carboxylic acid carbon peak, aromatic carbons around δ 167–105 ppm and aliphatic carbons around δ 51–7.0 ppm. The FAB Mass spectra showed an accurate molecular ion peak data at *m*/*z* 494 for compound **VIIa** and at *m*/*z* 554 [M + 1] for compound **VIIc**. All the compounds gave satisfactory chemical analysis (± 0.4%).

Table 1 summarizes the in vitro antibacterial activity of synthesized compounds **VIIa**–**d** against some Gram-positive (*Corynebacterium*, *S. aureus*, *E. faecelis*, *S. epidermidis*, *Bacillus* sp.) and Gram-negative (*P. aeruginosa*, *E. coli*, *Klebsiella* sp., *C. freundi*, *Proteus* sp.) bacteria using broth microdilution

Table 1
In vitro antibacterial activity of 7-[4-(5-amino-1,3,4 thiadiazole-2-sulfonyl)]-1-piperazinyl fluorquinolonic derivatives **VIIa–d**

Compound	MICs (μg ml^{-1})									
	Gram-positive organisms[a]					Gram-negative organisms[b]				
	Cb	Sa	Ef	Se	B	Pa	Ec	Ks	Cf	Ps
VIIa	1	< 1	1	10	5	> 50	5	5	≥ 20	5
VIIb	1	< 1	1	20	5	> 50	5	5	5	5
VIIc	< 1	< 5	< 5	1	1	> 50	<5	<5	< 5	< 5
VIId	1	1	1	< 1	1	> 50	≥ 20	≥ 20	5	5
CIP[c]	< 1	< 5	< 1	1	≤ 1	> 5	≤ 1	≤ 1	≤ 1	≤ 1
NOR[d]	< 1	< 5	5	1	≤ 1	> 5	≤ 1	≤ 1	≤ 1	≤ 1
SPR[e]	< 1	< 5	< 5	> 1	≤ 1	< 10	≤ 1	≤ 1	≤ 1	≤ 1
GTZ[f]	< 1	5	> 5	> 1	≤ 1	> 10	≤ 1	≤ 1	≤ 1	≤ 1

The screening organisms.
[a] Gram-positive bacteria: *Corynebacterium* (Ca), *S. aureus* MTCC 3160 (Sa), *E. faecelis* MTCC 2729 (Ef), *S. epidermidis* MTCC 3382 (Se), *Bacillus* sp. MTCC 297 (B).
[b] Gram-negative bacteria: *P. aeruginosa* MTCC 1034(Pa), *E. coli* MTCC 1089 (Ec), *Klebsiella* sp. MTCC 3384 (Ks), *C. freundi* MTCC 1658 (Cf), *Proteus* sp. Reference drugs.
[c] ciprofloxacin.
[d] norfloxacin.
[e] sparfloxacin.
[f] gatifloxacin.

method. The antibacterial activity of test compounds was assessed in side-by-side comparison with reference drugs. The antibacterial data indicated that the N-sulfonyl fluorquinolonic derivatives **VIIa–d** showed better activity against Gram-positive bacteria *S. aureus*, *E. faecelis*, *Bacillus* sp. (MIC = 1–5 μg ml^{-1}, respectively) compared to the respective reference drugs. Compounds **VIIa–b** showed lesser spectrum of activity against *S. epidermidis* (MIC = 10, 20 μg ml^{-1}, respectively) and all derivatives exhibited almost similar spectrum of activity as reference drugs against *Corynebacterium*. The MIC values of tested derivatives **VIIa–d** indicated that the sparfloxacin and gatifloxacin derivatives **VIIc–d** were active compounds in this series against all the tested Gram-positive strains of bacteria (MIC = 1–5 μg ml^{-1}). The tested compounds **VIIa–d** showed poor activity against the Gram-negative bacteria.

Selective antibacterial spectrum of derivatives **VIIa–d** against Gram-positive bacteria is in contrast to the good activity of unsubstituted piperazinyl quinolones against both Gram-positive and Gram-negative organisms. These findings are in accordance with the earlier reports, where substitution at C-7 of FQs is not only responsible for antibacterial activity but also for distinguishing between Gram-positive and Gram-negative bacteria [46]. The earlier reports on aryl/heteroaryl substitution at C-7 of ciprofloxacin and norfloxacin showed enhanced activity against Gram-positive bacteria and decreased activity against Gram-negative bacteria [34–36,47,48]. In the present study, it was observed that the synthesized 7-[4-(5-amino-1,3,4-thiadiazole-2-sulfonyl)] derivatives **VIIa–d** exhibited better antibacterial activity, which may be due to the presence of free NH_2 group similar to the 7-(4-aminophenyl-sulfonyl) piperazinyl fluorquinolonic derivatives [36].

The selected synthesized compounds **VIIa** and **VIIb** were evaluated for their in vitro antituberculosis activity against *M. tuberculosis* strain $H_{37}Rv$ by broth dilution assay. The compounds showed moderate antitubercular activity at MIC of 10 μg ml^{-1} compared to isoniazid standard.

5. Experimental procedures

5.1. Chemistry protocols

The pure FQ drugs used in the synthesis were procured as gift samples from Emcure Pharmaceuticals, Pune, India. Melting points were determined using open capillary tube method and are uncorrected. The purification of synthesized compounds was achieved by passage through column chromatography on Silica Gel 60 (mesh 230–400, E. Merck) with the indicated solvent system. Thin layer chromatography was performed on precoated Silica Gel 60 F_{254} plates from E. Merck and visualized by exposure to iodine vapors. Spectra were obtained as follows: Infra red (IR) spectra was recorded using KBr disk on a Nicolet MX-1 FTIR spectrophotometer, ^{1}H -NMR spectra were recorded at 400 MHz and ^{13}C-NMR spectra were recorded at 100 MHz on a Bruker AM spectrometer and their chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Microanalyses for C, H, N was performed in a Heraeus CHN Rapid Analyzer. The FAB Mass spectra was recorded on Autospec Mass spectrometer. All new compounds yielded spectral data consistent with the proposed structure and microanalysis within ± 0.4% of the theoretical values. ClogP, the measure of hydrophobicity/lipophilicity was calculated using ChemDraw Ultra 6.0 software [45] integrated with CambridgeSoft Software Development Kit (CambridgeSoft Corporation).

5.1.1. Synthesis of 2-amino-5-mercapto-1,3,4-thiadiazole *(II)*

Compound **II** was synthesized employing the synthetic procedure stated in literature [39] by refluxing a mixture of thio-

semicarbazide with carbon disulfide and anhydrous sodium carbonate in absolute ethanol as yellow crystals in 85% yield.

M.p. 236–238 °C (dec.) [Ref. [39] 231–232 °C (dec.)]. IR (KBr) ν_{max}: 3327, 3264, 2768, 2543, 1552 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 7.99 (br, s, 2H, NH_2), 3.45 ppm (s, 1H, SH). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 181.14, 161.43 ppm.

5.1.2. Synthesis of 2-acetylamino-5-mercapto-1,3,4-thiadiazole (III)

Compound **III** was synthesized using the literature procedure [40] by heating 2-amino-5-mercapto-1,3,4-thiadiazole (**II**, 0.01 mol) with 10 ml of acetic acid anhydride on a water bath for 10–15 min, cooled, the crystalline mass was recrystallized from ethanol and obtained as yellow crystals in 94% yield. M.p. 208–210 °C (dec.) [Ref. [40] 210 °C (dec.)]. IR (KBr) ν_{max}: 3402, 3155, 2899, 2613, 1659, 1571 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 10.02 (s, 1H, NH), 3.33 (s, 1H, SH), 2.1 ppm (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 183.68, 169.39, 152.19, 22.23 ppm.

5.1.3. Synthesis of 5-acetylamino-1,3,4-thiadiazole-2-sulfonyl chloride (IV)

2-Acylamino-5-mercapto-1,3,4-thiadiazole **III** (1.75 g, 0.01 mol) was suspended in 10 ml of 33% acetic acid and while vigorously stirring at 0–2 °C, gaseous chlorine was introduced during 2 h. The excess of chlorine gas in the reaction mixture was indicated by green color, sulfonyl chloride obtained was filtered, washed with 50 ml of ice-cold water till the last washing water shows neutral to pH. The crude sulfonyl chloride was dried over sulfuric acid, recrystallized from ethylene dichloride and was obtained as white crystals in 80% yield. M.p. 192–194 °C (dec.) [Ref. [40] 195 °C (dec.)]. IR (KBr) ν_{max}: 3412, 3153, 2911, 2777, 1694, 1555, 1376, 1235, 1113 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.94 (s, 1H, NH), 2.21 ppm (s, 3H, CH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 169.30, 159.60, 147.87, 144.27, 22.16 ppm.

5.2. General procedure for the synthesis of 7-[4-(5-amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] fluorquinolonic derivatives (VIIa–d)

In an erlenmeyer supported on a heating pad provided with a magnetic stirrer, 7-(1-piperazinyl) FQs **Va–d** (0.032 mol) were suspended in 5 ml of distilled and dried acetone, then N-acetylsulfonyl chloride **IV** (0.035 mol) was added. After supporting a refrigerator on the nozzle of the erlenmeyer, the mixture was heated to boiling for 2 h, cooled and the solid residues of the 7-[4-(5-acetylamino- [1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] FQs **VIa–d** were isolated by filtration, washed with cold acetone and dried. The residues were incorporated into an appropriate vessel (RBF), 10 ml of 2 N aqueous NaOH was added and refluxed for 4 h. After cooling, the solution was neutralized with 10% acetic acid solution. The title compounds 7-[4-(5-amino- [1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl] FQs **VIIa–d** were filtered, washed with ice-cold water. Purification of the crude products was achieved by passing through column chromatography on silica gel (mesh 230–400) using $CHCl_3/CH_3OH$ (9:1) solvent system and visualization by exposing to iodine vapors or aqueous potassium permanganate and recrystallized from appropriate solvents.

5.2.1. 7-[4-(5-Amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (VIIa)

The compound **VIIa** was synthesized by refluxing a mixture of ciprofloxacin **Va** (1.059 g, 32 mmol) and 5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl chloride **IV** (0.845 g, 35 mmol) using the general procedure as described above. The intermediate 7-[4-(5-acetyl amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **VIa** was collected by filtration, washed with cold acetone and obtained as white crystals in 78% yield. M.p. 246–248 °C (dec.).

IR (KBr) ν_{max}: 3442, 3276, 3155, 2925, 2750, 1732, 1702, 1626, 1465, 1311, 1178, 1034, 966, 745 cm^{-1}. Hydrolysis of **VIa** yielded compound **VIIa**, which was purified from column chromatography, recrystallized from a mixture of DMF, ethanol and water (5:5:1) and obtained as colorless solid crystals in 82% yield. M.p. 262–264 °C (dec.); IR (KBr) ν_{max}: 3529, 3415, 3092, 2922, 2843, 1708, 1626, 1457, 1313, 1144, 1033, 937, 741 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 15.09 (s, 1H, COOH), 9.45 (br, s, 2H, NH_2), 8.67 (s, 1H, H2-quinoline), 7.96 (d, 1H, H5-quinoline, J = 13.08 Hz), 7.61 (d, 1H, H8-quinoline, J = 7.42 Hz), 3.88–3.82 (m, 4H, piperazine), 3.63–3.51 (m, 4H, piperazine and 1H, CH, cyclopropyl), 1.32–1.18 ppm (m, 4H, cyclopropyl). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 207.22, 176.41, 165.76, 154.13, 151.13, 148.15, 144.16, 139.12, 119.45, 111.32, 111.10, 106.89, 46.34, 42.49, 35.94, 7.61 ppm. MS (FAB) m/z: 494. Anal. $C_{19}H_{19}FN_6O_5S_2$ (C, H, N). (ClogP: **Va** = –1.1462; **VIIa** = –0.01823).

5.2.2. 7-[4-(5-Amino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (VIIb)

The compound **VIIb** was synthesized refluxing a mixture of norfloxacin **Vb** (1.02 g, 32 mmol) and 5-acetylamino-[1,3,4] thiadiazole-2-sulfonyl chloride **IV** (0.845 g, 35 mmol) using the general procedure described above. The intermediate 7-[4-(5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl)-piperazin-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **VIb** was collected by filtration, washed with cold acetone and obtained as white crystals in 70% yield. M.p. 228–230 °C (dec.); IR (KBr) ν_{max}: 3429, 3281, 3035, 2980, 2857, 2756, 1730, 1685, 1622, 1486, 1308, 1259, 1198, 1040, 943, 749 cm^{-1}. Hydrolysis of **VIb** yielded the title compound **VIIb**, which was purified from column chromatography, recrystallized from a mixture of DMF, ethanol and water (5:5:1) and obtained as yellowish white crystals in 84% yield. M.p. 160–164 °C (dec.); IR (KBr) ν_{max}: 3478, 3408, 3124, 2978, 2893, 1720, 1620, 1461, 1321, 1146, 1063, 943, 745 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 15.09 (s, 1H, COOH),

8.98 (br, s, 2H, NH_2), 8.13 (s, 1H, H2-quinoline), 7.99 (d, 1H, H5-quinoline, $J = 13.17$ Hz), 7.25 (d, 1H, H8-quinoline, $J = 7.31$ Hz), 4.55 (q, 2H, NCH_2CH_3, $J = 6.96$ Hz), 3.61–3.60 (m, 4H, piperazine), 3.33–3.00 (m, 4H, piperazine), 1.41 ppm (t, 3H, NCH_2CH_3, $J = 6.95$ Hz). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 205.22, 176.18, 165.96, 161.01, 148.52, 141.16, 138.20, 118.51, 111.34, 111.17, 106.48, 105.65, 50.38, 49.92, 44.81, 14.31 ppm. Anal. $C_{18}H_{19}FN_6O_5S_2$ (C, H, N). (ClogP: **Vb** = –0.9712; **VIIb** = 0.1567).

*5.2.3. 5-Amino-7-[4-(5-amino-[1,3,4]-thiadiazole-2-sulfonyl)-3′,5′-dimethylpiperazin-1 -yl]-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**VIIc**)*

The starting material 5-acetylamino-1-cyclopropyl-7-(3′,5′-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **Vc** used in the synthesis of title compound **VIIc** was prepared by refluxing a mixture of sparfloxacin (3.92, 0.01 mol), acetic anhydride (10 ml) and pyridine (5 ml) on a steam bath for 4 h, left over night, the crystalline mass separated was filtered, recrystallized from aqueous DMF and obtained as yellow crystals in 70% yield. M.p. 212–214 °C (dec.); IR (KBr) ν_{max}: 3420, 3312, 2977, 2845, 1721, 1659, 1618, 1444, 1320, 1234, 1046, 927, 773 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 14.71 (s, 1H, COOH), 9.70 (d, 1H, NH, piperazine and 1H, NH of $NHCOCH_3$), 7.48 (s, 1H, H2-quinoline), 3.97 (s, 3H, CH_3 of $NHCOCH_3$), 3.24 (m, 6H, 3′,5′-dimethyl of piperazine), 3.22–2.68 (m, 6H, piperazine and 1H cyclopropyl), 1.35–1.01 ppm (m, 4H, cyclopropyl). EIMS *m*/*z*: found 434.24 [M + 1]; calcd. 434.17. Anal $C_{21}H_{24}F_2N_4O_4$.

The compound **VIIc** was synthesized by reacting a mixture of compound **Vc** (1.38 g, 32 mmol) and 5-acetylamino-[1,3,4] thiadiazole-2-sulfonyl chloride **IV** (0.845 g, 35 mmol) using the general procedure. The intermediate 5-acetylamino-7-[4-(5-acetylamino-[1,3,4] thiadiazole-2-sulfonyl)-3′,5′-dimethyl-piperazin-1-yl]-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **VIc** was obtained as yellow crystals in 63% yield. M.p. 231–232 °C (dec.); IR (KBr) ν_{max}: 3410, 3250, 3018, 2948, 2856, 1724, 1680, 1620, 1458, 1334, 1230, 1163, 1060, 940, 739 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 14.60 (s, 1H, COOH), 9.82, 3.81 (d, 2H, NH, NH; d, 6H, CH_3, CH_3 of $NHCOCH_3$ and $NHCOCH_3$), 8.42 (s, 1H, H2-quinoline), 3.35 (m, 6H, 3′,5′-dimethyl piperazine), 3.14–2.18 (m, 6H, piperazine and 1H cyclopropyl), 1.43–1.01 ppm (m, 4H, cyclopropyl). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 181.60, 176.02, 165.96, 163.20, 160.97, 153.93, 151.46, 148.31, 145.16, 137.03, 129.28, 128.52, 119.50, 111.26, 48.41, 47.71, 35.72, 28.94, 21.23, 9.13 ppm. Anal. $C_{25}H_{27}F_2N_7O_6S_2$ (C, H, N). Hydrolysis of **VIc** yielded the title compound **VIIc**, which was purified from column chromatography, recrystallized from aqueous DMF and obtained as yellow crystals in 70% yield. M. p. 208–210 °C (dec.); IR (KBr) ν_{max}: 3468, 3401, 3122, 2950, 2868, 1730, 1619, 1461, 1334, 1158, 1060, 972, 740 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 14.84 (s, 1H, COOH), 10.15 (s, 2H, NH_2), 8.82 (s, 1H, H2-quinoline), 4.25 (br, s, 2H, NH_2, C5-quinoline), 3.63 (m, 6H, 3′,5′-dimethyl of piperazine), 3.34–2.12 (m, 6H, piperazine and 1H cyclopropyl), 1.45–1.16 ppm (m, 4H, cyclopropyl). MS (FAB) *m*/*z*: 554 [M + 1]. Anal. $C_{21}H_{23}F_2N_7O_5S_2$ (C, H, N). (ClogP: sparfloxacin = –1.0256; **Vc** = –1.3413; **VIIc** = 0.1023).

*5.2.4. 7-[4-(5-Amino-1,3,4 thiadiazole-2-sulfonyl)]-3′-methylpiperazinyl-1 cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**VIId**)*

The compound **VIId** was synthesized by reacting a mixture of gatifloxacin **Vd** (1.20 g, 32 mmol) and 5-acetylamino-[1,3,4] thiadiazole-2-sulfonyl chloride **IV** (0.845 g, 35 mmol) using the general procedure as described above. The intermediate 7-[4-(5-acetylamino-[1,3,4]thiadiazole-2-sulfonyl)-3′-methylpiperazin-1-yl]-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **VId** was collected, washed with cold acetone, recrystallized from aqueous DMF and obtained as pale yellow solid in 72% yield. M.p. 204–207 °C (dec.); IR (KBr) ν_{max}: 3450, 3265, 3168, 2940, 2794, 1721, 1683, 1617, 1515, 1448, 1303, 1173, 1035, 947, 743 cm^{-1}. Hydrolysis of later yielded title compound **VIId**, which was purified from column chromatography, recrystallized from aqueous DMF and obtained as colorless crystals in 82% yield. M.p. 170–172 °C (dec.). IR (KBr) ν_{max}: 3435, 3258, 3158, 2914, 2779, 1697, 1629, 1550, 1430, 1370, 1228, 1102, 1063, 962, 741 cm^{-1}. ^{1}H -NMR (DMSO-d_6, 400 MHz) δ: 14.84 (s, 1H, COOH), 8.99 (br, s, 2H, NH_2), 8.74 (s, 1H, H2-quinoline), 7.84 (d, 1H, H5-quinoline, $J = 12.01$ Hz), 3.81 (s, 3H, OCH_3), 3.45–3.26 (m, 7H, piperazine and 1H, CH, cyclopropyl), 1.28 (m, 3H, 3′-methylpiperazine), 1.26–1.05 ppm (m, 4H, cyclopropyl). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 201.33, 177.43, 167.06, 155.28, 151.06, 146.65, 141.77, 137.45, 118.77, 111.32, 109.10, 105.69, 46.34, 42.49, 35.94, 22.98, 16.22, 9.61 ppm. Anal. $C_{21}H_{23}FN_6O_6S_2$ (C, H, N). (ClogP: **Vd** = 0.6868; **VIId** = 0.4411).

5.3. Biological assay

5.3.1. In vitro evaluation of antibacterial activity

The MIC determination of the tested compounds was investigated in side-by-side comparison with ciprofloxacin, norfloxacin, sparfloxacin and gatifloxacin against Gram-positive (*Corynebacterium*, *S. aureus*, *E. faecelis*, *S. epidermidis*, *Bacillus* sp.) and Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *Klebsiella* sp., *C. freundi*, *Proteus* sp.) by broth microdilution method [41,42]. Serial dilutions of the test compounds and reference drugs were prepared in Mueller–Hinton agar. Drugs (6.4 mg) were dissolved in dimethylsulfoxide (DMSO, 1 ml) and the solution was diluted with distilled water (9 ml). Further progressive dilutions with melted Mueller–Hinton agar were performed to obtain the required concentrations of 1, 5, 10, 20 and 50 μg ml^{-1}. The petri dishes were inoculated with 1–5 × 10^4 cfu ml^{-1} and incubated at 37 °C for 18 h. The **MIC** was the lowest concentration of the tested compound that yield no visible growth on the plate. To ensure that the solvent had no effect on the bacterial growth, a control was performed with the test medium supplemented with DMSO at the same dilutions as used in the experiments.

5.3.2. In vitro evaluation of antitubercular activity

The preliminary antitubercular screening for test compounds **VIIa**, **b** was obtained for *M. tuberculosis* $H_{37}Rv$, the MIC of each drug was determined by broth dilution assay [43,44] and is defined as the lowest concentration of drug, which inhibits ≥ 99% of bacterial population present at the beginning of the assay. A frozen culture in Middlebrook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 2×10^5 cfu ml^{-1} for *M. tuberculosis* and used as the inoculum. In the assay U-tubes were used to accommodate compounds in 1–50 μg ml^{-1} dilutions. Each test compound was dissolved in DMSO then diluted in broth at twice the desired concentration. The final concentration of DMSO in the assay medium was 1.3%. Each U-tube was then inoculated with 0.05 ml of standardized culture and then incubated at 37 °C for 21 days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard isonaizid.

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