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Synthesis, Antimicrobial and Anticancer Studies of Isatin Derivatives of Sparfloxacin

Sirajunisa Talath*[1], Bhoomendra A. Bhongade[1]

1.Department of Pharmaceutical Chemistry, RAK College of Pharmaceutical Sciences, RAKMHSU, POB 11172, Ras Al Khaimah, UAE

ABSTRACT

Mannich bases of sparfloxacin were synthesized from the reaction of 5-acetylamino-1-cyclopropyl-7-(3´,5´-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo1,4-dihydro-quinoline-3-carboxylic acid with formaldehyde and several isatin derivatives. The structures of the synthesized compounds were elucidated from the IR, ^{1}H NMR, ^{13}C NMR and FAB Mass data. The synthesized compounds were evaluated for the antibacterial, antitubercular and anticancer activity. The compounds showed good activity against Gram-positive bacteria and moderate activity against tested Gram-negative bacteria. The MIC for the compounds against *M. tuberculosis* H_{37}Rv strain was <6.25 µg/mL while the IC_{50} values against cancer cell lines SW480, HeLa, A549 and HepG2 was in the range of 18.31- >50 µg/mL. The most potent compound in this series **3c** exhibited enhanced antibacterial activity than sparfloxacin against *S. aureus, Bacillus* Sp while the anticancer activity was in the range of 18.31- 23.61 µg/mL.

Keywords : Sparfloxacin, Mannich bases, Isatin, Antibacterial, Antitubercular, Anticancer.

*Corresponding Author Email: sirajunisa@rakmhsu.ac.ae; restalath@gmail.com

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INTRODUCTION

The fluoroquinolones (FQs) comprises of a relatively large, growing and most fascinating class of antibacterial drugs which have made a major impact in the field of antimicrobial chemotherapy.[1,2] They potentially offer many of the attributes of an ideal antibiotic combining high potency, broad spectrum of activity, good pharmacokinetic and pharmacodynamic properties and potentially low incidence of side-effects. In addition, they are also being explored in the treatment of tuberculosis (TB) and HIV infection.[3,4] FQs act by interfering with the bacterial DNA gyrase resulting in the degradation of chromosomal DNA and interference with cell division and gene expression.[5] During the last two decades a large number of FQs have been introduced in the clinical use whilst very few of them have been withdrawn from the market owing to their toxicities.[6] Ciprofloxacin, ofloxacin and sparfloxacin were recommended by the World Health Organization as second-line agents for the treatment of tuberculosis (TB).[7] A large number of syntheses of FQ derivatives have been reported alongwith their corresponding structure-activity relationship (SAR) studies.[8,9] The SAR studies of FQs reveals that the C-7 position is the major site where the bulky lipophilic substitution influences their antibacterial, anti-MTB activity, potency and toxicity.[10-14]

Isatin (1*H*-indole-2,3-dione) and its derivatives are privileged scaffold endowed with wide spectrum of biological activities, including antitumor, antiangiogenic, antiviral, antibacterial, antitubercular, antifungal, anticonvulsant and antimalarial activities.[15-20] Introduction of heteroaryl ring at C-7 position of quinolone transforms its selectivity from bacterial to human topoisomerase II.[1,21] Novel classes of quinolones possessing considerable inhibitory activity of mammalian topoisomerase II have been reported. FQs that displayed potent activity towards the eukaryotic type II enzyme were CP-115, 953, WIN572946, A-621767 and A-852268.[22,23]

By considering these facts and in continuation of our search for various biologically active molecules and encouraging antibacterial, antimycobacterial and in-vitro cytotoxicity activities of isatin Mannich bases of quinolones[24-26] has promoted us to synthesize some new *N*-substituted piperazinyl quinolones. In the present study, we have introduced various isatinyl moieties into antibacterial sparfloxacin at its C-7 position utilizing Mannich reaction and evaluated for their preliminary in vitro antibacterial, antitubercular and anticancer activity.

MATERIALS AND METHOD

The pure drug sample of sparfloxacin was procured as gift sample 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 column chromatography on Silica gel G (mesh 230-400, E. Merck). TLC was performed on precoated Silica gel G plates (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 and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively on a Bruker AM spectrometer and their chemical shifts are reported in δ ppm units with respect to TMS as internal standard. The FAB MASS spectrum 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.

Starting materials 4-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-*N*-pyrimidin-2-yl-benzenesulfonamide, *N*-(5,6-dimethoxy-pyrimidin-4-yl)-4-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-benzenesulfonamide, *N*-(4,6-dimethyl-pyrimidin-2-yl)-4-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-benzenesulfonamide, *N*-(5-methyl-isoxazol-3-yl)-4-(2-oxo-1,2-dihydro-indol-3-ylideneamino)-benzenesulfonamides were prepared as described in literature by reacting isatin with sulfadiazine, sulfadoxine, sulfadimidine, and sulfamethoxazole in the presence of glacial acetic acid and absolute ethanol as solvent.[27,28] 5-acetylamino-1-cyclopropyl-7-(3′,5′-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **2** was prepared as described in the literature.[26] Mannich bases of sparfloxacin 3a-e were prepared by condensing the active hydrogen atom of isatin derivatives with formaldehyde and the secondary amino function (piperazino moiety) of compound 2 (Figure 1).

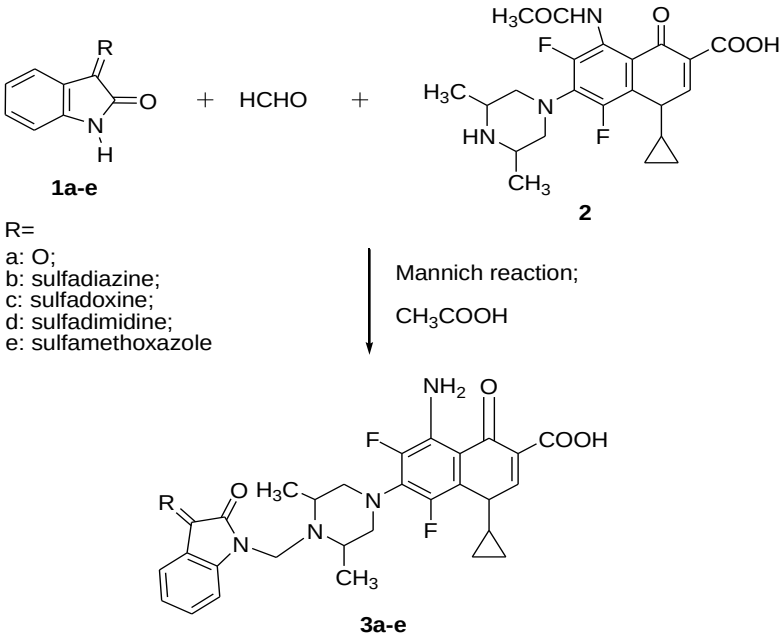

Figure 1: Synthesis of sparfloxacin derivatives 3a-e

General procedure for the preparation of Mannich bases of sparfloxacin (3a-e)

To a solution of 5-acetylamino-1-cyclopropyl-7-(3′,5′-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (**2**, 0.02 mol) in ethanol:dioxane (1:1, 50 mL), isatin derivatives (**1a-e**, 0.02 mol), 37% formalin (1 mL) and one drop of conc. H_2SO_4 were added. The reaction mixture was heated under reflux for 24 h. On cooling, the precipitate was collected, washed with cold ethanol and recrystallized from a mixture of chloroform-methanol to give **3a-e**.

5-Amino-1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(3,5-dimethyl-4-((2,3-dioxoindolin-1-yl)methyl)piperazin-1-yl)-4-oxoquinoline-3-carboxylic acid (3a)

(Yield. 79%) Mp 227-229 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.10 (s, 1H, COOH), 8.75 (s, 1H, H2-quinoline), 8.12-7.42 (m, 4H, Ar-H), 5.25 (s, 2H, -NCH_2N-), 4.50 (s, 2H, NH_2, C5-quinoline), 3.80 (m, 6H, 3′,5′-dimethyl of piperazine), 3.60-3.25 (m, 6H, piperazine and 1H cyclopropyl), 1.42-1.21 ppm (m, 4H, cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 181.54, 178.03, 175.99, 165.85, 163.09, 160.86, 153.88, 151.40, 148.31, 144.73, 136.98, 128.66, 128.32, 119.49, 111.23, 107.07, 106.14, 105.83, 68.02, 50.19, 48.93, 37.81, 14.23, 7.52 ppm. IR (KBr) ν_{max} 3438, 3178, 2916, 2865, 2778, 1718, 1650, 1620, 1561, 1499, 1330, 1252, 1153, 1129, 1040, 756 cm^{-1}. MS (FAB) m/z: 551. Anal $C_{28}H_{27}F_2N_5O_5$.

5-Amino- 1-cyclopropyl -7-(3′,5′- dimethyl-4- {2-oxo-3- [4-(pyrimidin- 2-yl sulfamoyl)-phenylimino]- 2,3-dihydro- indol- 1-ylmethyl}- piperazin- 1-yl)-6, 8-di fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3b)

(Yield 69%), Mp 188-190 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.20 (s, 1H, COOH), 10.54 (s, 1H, SO_2NH), 8.85 (s, 1H, H2-quinoline), 8.24- 7.01 (m, 7H, Ar-H), 5.20 (s, 2H, -NCH_2N-), 4.55 (s, 2H, NH_2, C5-quinoline), 3.89 (m, 6H, 3′,5′-dimethyl of piperazine), 3.66-3.33 (m, 6H, piperazine and 1H cyclopropyl), 1.64-1.25 ppm (m, 4H, cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 180.55, 176.04, 169.22, 165.92, 163.16, 157.17, 150.75, 149.27, 148.97, 138.22, 137.98, 136.78, 127.93, 127.78, 126.79, 125.19, 124.48, 123.52, 122.76, 117.95, 112.02, 111.77, 110.96, 106.78, 105.95, 62.99, 53.24, 51.02, 35.42, 14.15, 8.94 ppm. IR (KBr) ν_{max} 3443, 3057, 2911, 2841, 2778, 1720, 1660, 1620, 1560, 1464, 1331, 1250, 1160, 1120, 1014, 748 cm^{-1}. MS (FAB) m/z found 784 [M^{+1}]; calcd. 783. Anal $C_{38}H_{35}F_2N_9O_6S$.

5-Amino-1-cyclopropyl-7-(4-{3-[4-(5,6-dimethoxy-pyrimidin-4-yl sulfamoyl)-phenylimino]-2-oxo-2, 3-dihydro-indol- 1-ylmethyl}-3′, 5′-dimethyl-piperazin-1-yl)-6,8-difluoro -4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3c)

(Yield. 65%), Mp 179-181°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.22 (s, 1H, COOH), 10.45 (s, 1H, SO_2NH), 8.78 (s, 1H, H2-quinoline), 8.34-7.10 (m, 7H, Ar-H), 5.12 (s, 2H, -NCH_2N-), 4.88 (s, 2H, NH_2, C5-quinoline), 4.20 (s, 6H, 5,6-dimethoxy-pyrimidine), 4.01 (m, 6H, 3′,5′- dimethyl of piperazine), 3.81-3.31 (m, 6H, piperazine and 1H cyclopropyl), 1.71-1.30 ppm (m, 4H, cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 184.42, 177.99, 169.29, 165.99, 159.29, 157.95, 153.12, 152.92, 152.28, 150.82, 149.50, 140.59, 138.38, 124.85, 124.11, 123.98, 123.52, 122.75, 117.78, 112.26, 112.02, 111.71, 109.74, 106.45, 106.34, 61.21, 60.30, 55.97, 29.70, 24.28, 14.28, 8.78 ppm. IR (KBr)ν_{max} 3436, 2916, 2852, 2789, 1722, 1662, 1610, 1566, 1466, 1329, 1242, 1170, 1130, 1015, 746 cm^{-1}.

5-Amino-1-cyclopropyl-7-(4-{3-[4-(4,6-dimethyl-pyrimidin-2-ylsulfamoyl)-phenylimino]-2-oxo-2, 3-dihydro-indol- 1-ylmethyl}-3′, 5′-dimethyl- piperazin-1-yl)- 6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3d)

(Yield. 69%), Mp. 202-204 °C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 15.20 (s, 1H, COOH), 10.65 (s, 1H, SO_2NH), 8.82 (s, 1H, H2-quinoline), 8.24-7.12 (m, 7H, Ar-H), 5.30 (s, 2H, -NCH_2N-), 4.45 (s, 2H, NH_2, C5-quinoline), 3.98-3.88 (m, 6H, 3′,5′-dimethyl of piperazine), 3.85-3.15 (m, 6H, piperazine and 1H cyclopropyl), 2.98 (s, 6H, 4,6-dimethyl-pyrimidine), 1.71-1.30 ppm (m, 4H, cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 179.68, 177.80, 168.91, 165.09, 165.34, 157.22, 150.86, 148.26, 146.10, 143.03, 138.57, 134.46, 134.02, 129.25, 128.93, 124.66, 123.63, 122.66, 121.72, 115.53, 114.85, 112.20, 111.50, 107.64, 105.81, 63.66, 60.15, 58.90, 53.34, 21.31, 16.21, 8.52 ppm. IR (KBr) ν_{max} 3437, 3057, 2910, 2855, 2773, 1722, 1665, 1606, 1567, 1465, 1336, 1249, 1154, 1128, 1012, 747 cm^{-1}.

5-Amino -1- cyclopropyl-7- (3′,5′-dimethyl-4- {3-[4-(5-methyl-isoxazol-3-yl sulfamoyl)-phenylimino]-2-oxo-2, 3-dihydro-indol-1-ylmethyl}-piperazin-1-yl)-6, 8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (3e)

(Yield: 65%); Mp. 174-75°C. ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 15.10 (s, 1H, COOH), 10.42 (s, 1H, SO_2NH), 8.92 (s, 1H, H2-quinoline), 8.32-6.99 (m, 7H, Ar-H), 5.30 (s, 2H, -NCH_2N-), 4.90 (s, 2H, NH_2, C5-quinoline), 4.31 (s, 3H, 5-methylisoxazol), 3.98-3.84 (m, 6H, 3′,5′-dimethyl of piperazine), 3.82-3.15 (m, 6H, piperazine and 1H cyclopropyl), 1.54-1.20 ppm (m, 4H, cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 181.60, 176.02, 171.22, 169.36, 165.96, 163.02, 160.97, 153.93, 151.46, 148.31, 145.16, 145.06, 144.89, 137.02, 129.28, 128.52, 128.04, 119.50, 111.26, 111.04, 107.12, 106.26, 106.13, 105.02, 98.50, 50.27, 49.02, 48.41, 28.94, 21.23, 14.33, 8.41 ppm. IR (KBr) ν_{max}:3444, 3059, 2918, 2860, 2761, 1726, 1664, 1618, 1456, 1561, 1328, 1251, 1172, 1120, 1012, 748 cm^{-1}.

BIOLOGICAL EVALUATION

The standard bacterial strains and *M. tuberculosis* $H_{37}Rv$ were procured from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, India. The antibacterial activity of synthesized compounds **3a-e** was performed by broth microdilution method[29,30] against standard bacterial strains: Gram-positive bacteria [*S. aureus* (MTCC 3160), *S. epidermidis* (MTCC 3382), *Bacillus* Sp. (MTCC 297)] and Gram-negative bacteria [*P. aeruginosa* (MTCC 1034), *E. coli* (MTCC 1089), *Citrobacter freundi* (MTCC 1658)]. The antitubercular activity of compounds **3a-e** was carried out by measuring the minimum inhibitory concentration (MIC) against *M. tuberculosis* virulent strain $H_{37}Rv$ using broth dilution assay method.[31,32] The anticancer activity of compounds **3a-e** was performed by MTT assay against human cancer cell lines: SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), HepG2 (human hepatic carcinoma cells).[33-34]

Antibacterial activity[29,30]

Two fold serial dilutions of the test compounds **3a-e** and reference drug (sparfloxacin) were prepared in Muller-Hinton agar. The compounds (6.4mg) were dissolved in dimethylsulfoxide (DMSO, 1 mL) and the solution was diluted with distilled water (9 mL). Further, progressive double dilutions with melted Muller-Hinton agar were performed to obtain the required concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0.13, 0.06, 0.03, 0.015, 0.008 and 0.004 mg/mL. Petri dishes were inoculated with 1-/5 x 10^4 colony forming units and incubated at 37 ºC 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.

Antitubercular activity[31,32]

The minimum inhibitory concentration of the test compounds **3a-e** and sparfloxacin that inhibits the colony forming ability of *M. tb* was determined by incorporating decreasing concentration of test compounds and standard drug in the Middlebrook 7H9 broth supplemented with ADC enrichment and 0.2% glycerol. A frozen culture in Middlebrook 7H9 broth supplemented with 10% ADC and 0.2% glycerol was thawed and diluted in broth to 2 x 10^5 cfu/mL and used as the inoculum. In the assay, U-tubes were used to accommodate compounds in 100-5 µg/mL 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 inoculated with 0.05 mL of standardized culture and incubated at 37 ºC for 21days. The growth in the U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and standard sparfloxacin.

Anticancer activity[33,34]

The anticancer activity of compounds **3a-e** against human cancer cell lines SW480, HeLa, A549 and HepG2 by MTT assay according to known protocol.[33,34] The exponentially growing cells were harvested and plated in 96-well plates at a concentration of 1x 10^4 cells/well. After 24 h incubation at 37 °C under a humidified 5% CO_2 to allow cell attachment, the cells in the wells were treated with test compounds **3a-e** at concentrations 10, 50, 100, 200 µg/mL in DMEM/MEM with 10% FBS medium for 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to the cells. A solution of 3-(4,5-dimethyl thizao1-2-y1)-2,5-diphenyltetrazolium bromide (MTT), was prepared at 5 mg/ml in phosphate buffered saline (PBS; 1.5 mM KH_2PO_4, 6.5 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl; pH 7.4). 20 µl of this solution were added to each well. After incubation for 4 h at 37°C in a humidified incubator with 5% CO_2, the medium/MTT mixtures were removed and the formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in 100 µl of DMSO per well. Absorbance was recorded at 570 nm using the microplate spectrophotometer system (Spectra max190-Molecular Devices). IC_{50} was determined from plot of % inhibition (from control) versus concentration. Effects of the drug cell viability were calculated using cell treated with DMSO as control.

RESULTS AND DISCUSSION

Chemistry

The synthetic route involved in the preparation of target compounds **3a-e** is described in the synthetic scheme (Figure 1). Isatin derivatives **1a-e** were reacted with formaldehyde and secondary amino (piperazino) function of 5-acetylamino-1-cyclopropyl-7-(3′,5′-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo 1,4-dihydro-quinoline-3-carboxylic acid **2** to form the isatin Mannich bases of sparfloxacin **3a-e** in 65-79% yields. The purity of the synthesized compounds was checked by TLC and the structures were identified by spectral data.

Infrared spectra (IR) showed C=N (azomethine) peak around 1650-1665 cm^{-1} and CH_2 (Mannich methylene) peak around 2841-2865 cm^{-1}. In the ^1H NMR spectra, the signals of the respective protons of the prepared sparfloxacin derivatives **3a-e** showed a characteristic singlets around δ 5.12-5.25 ppm corresponding to $–NCH_2N–$group, δ 4.45-4.88 ppm corresponding to

NH_2 of C5 quinolone and δ 8.75-8.89 ppm for C2-H. The remaining aromatic and aliphatic protons appeared as multiplets at their respective δ ppm. The compounds also exhibited appropriate peaks for carbon at the corresponding δ ppm in their respective ^{13}C-NMR spectrum. The FAB MASS spectra showed an accurate molecular ion peak at m/z 551 and 784 [M+1] for compounds **3a** and **3b** respectively.

Antimicrobial activity

The MIC values (Table 1) for the isatinyl Mannich bases **3a-e** illustrated good activity (MIC=0.25-4 µg/mL) against Gram-positive bacteria and moderate to poor activity (MIC=1-64 µg/mL) against Gram-negative pathogens. From the MIC data of antibacterial activity it is observed that compound **3c** (MIC=0.25 µg/mL) followed by **3b, 3d** (MIC=0.5 µg/mL) were better in inhibiting the growth of *S. aureus* and *S. epidermis*, while the compounds **3e** and **3a** (MIC=0.5-2.0 µg/mL) were equivalent in their antibacterial spectrum. The antibacterial activity of compounds **3a-e** against *Bacillus* Sp. showed comparable activity (MIC=0.5-2.0 µg/mL) with reference to standard drug (MIC=0.5 µg/mL). Among the synthesized compounds, sulfadoxine derivative **3c** was found to be the most potent in the series against Gram-positive microorganisms (MIC=0.25-0.5 µg/mL) and rest of the compounds had respectable (MIC=0.5-2.0 µg/mL) activity, but were less active than reference drug (MIC=0.03-0.5 µg/mL). The compounds also showed moderate activity (MIC=1-32 µg/mL) against Gram-negative bacteria *E. coli* and *citrobacter freundi* with an exception of antibacterial activity against *P. aeruginosa*.

As the mycobacterium has rich lipid cell walls, lipophilicity is an important consideration in the design and activity of novel molecules. Similarly as C-7 substitution in the FQs affect their pharmacokinetic and spectrum of activity, hence in the present work structural modification were centered mainly at the C-7 position.[35] The antitubercular MICs of the compounds **3a-e** and sparfloxacin against *M. tb* $H_{37}Rv$ strain is shown in table 1. From activity results, it was found that all the compounds inhibited the growth at concentration <6.25 µg/mL. The compounds were less active than reference drug (MIC=0.5 µg/mL).

Selective antibacterial spectrum of some of the isatinyl Mannich bases of sparfloxacin against Gram-positive bacteria is in comparison with the activity exhibited by sparfloxacin against both Gram-positive organisms. These findings are in accordance with the earlier reports on the aryl substitution at C-7 of piperazinyl quinolones that displayed enhanced activity against Gram-positive and decreased activity against Gram-negative bacteria.[11,12] The present studies supports that the sulfonamide derivatives of isatin are well tolerated at N-4 position of piperazine ring and enhance antibacterial potency especially against Gram-positive bacteria**.**

Table 1. In vitro antibacterial, antitubercular and anticancer activities of compounds 3a-e

Comp	Anti-bacterial Activity (µg/mL)						Anti-tubercular Activity MIC (µg/mL)	IC_{50} values (µg/mL) of anticancer activity			
	Gram-positive bacteria			Gram-negative bacteria							
	Sa	*Se*	*B.sp.*	*Pa*	*Ec*	*Cf*	*M.tb* $H_{37}Rv$	SW480	A549	HepG2	HeLa
3a	4	2	2	>64	32	16	<6.25	>50	43.71	46.53	>50
3b	0.5	0.5	1.0	>64	2	1	<6.25	>50	31.71	26.53	>50
3c	0.25	0.25	0.5	>64	1	1	<6.25	27.62	18.31	23.52	33.61
3d	0.5	0.5	1.0	>64	4	2	<6.25	>50	>50	42.16	38.91
3e	1	0.5	1.0	>64	4	2	<6.25	>50	>50	44.16	40.91
SPR	0.5	0.03	0.5	>64	0.03	0.03	0.5	ND	ND	ND	ND

ND= Not determined; *Sa=S. aureus, Se= S. epidermidis, B.sp.= Bacillus Sp., Pa= P. aeruginosa, Ec=E. coli, Cf=Citrobacter freund;* SW480 (human colon adenocarcinoma cells), HeLa (human cervical cancer cells), A549 (human lung carcinoma cells), HepG2 (human hepatic carcinoma cells). SPR= Sparfloxacin

Anticancer activity

The tested compounds **3a-e** showed IC_{50} (µg/mL) values ranging from 27.62- >50 against SW480 (human colon adenocarcinoma cells), 33.61- >50 against HeLa (human cervical cancer cells), 18.31- >50 against A549 (human lung carcinoma cells) and 23.52- 46.53 against HepG2 (human hepatic carcinoma cells).

Amongst the test compounds, it was found that the compound **3c** exhibited highest anticancer activity against the cancer cell lines used in the study with the IC_{50} (µg/mL) values in the range 18.31-33.61.

CONCLUSION

The isatin Mannich bases of sparfloxacin resulted in enhanced antibacterial activity as compared sparfloxacin against some Gram positive microbes. However, the Mannich bases exhibited loss of activity against Gram negative microbes and *M. tuberculosis* $H_{37}Rv$ and good anticancer activity against the tested panel of cancer cell lines. In summary, we have developed a series of new series of isatin Mannich bases of sparfloxacin with improved Gram-positive activity and can be considered as hybrid drugs containing quinolone and isatinyl moiety.

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