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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel derivatives of 5-amino-1-cyclopropyl-7-[(3*R*,5*S*)3,5-dimethylpiperazine-1-yl]-6,8-difluoro-4-oxo-quinoline-3-carboxylic acid: their synthesis, antimicrobial, antifungal, and urease inhibitory studies

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Abstract Sparfloxacin (SPFX) or 5-amino-1-cyclopropyl-7-[(3*R*,5*S*)3,5-dimethylpiperazine-1-yl]-6,8-difluoro-4-oxo-quinoline-3-carboxylic acid is an orally active synthetic, broad spectrum third generation quinolone, with excellent activity against Gram-positive bacteria with selectivity against anaerobes and atypical pathogens. Three derivatives of SPFX (**2, 3,** and **4**) were synthesized by reacting different aromatic carboxylic acids with SPFX (**1**). Chemistry involved the formation of amide between reacting species through nucleophilic substitution reactions. The synthesized derivatives were then structurally characterized by IR, NMR, and mass spectroscopic techniques. The antimicrobial activities of these derivatives were evaluated against four Gram-positive, seven Gram-negative bacteria, and six fungi, using SPFX as a reference. Statistical analysis revealed these derivatives as active antimicrobial agents, and **2** was more potent antimicrobial agents than the parent drug as well other fluoroquinolones. Compounds **3** and **4** showed a significant activity against *Fusarium solani*. Moreover, these three derivatives were evaluated for inhibitory activities against enzyme urease, carbonic anhydrase II, and α-chymotrypsin. Results showed their selectivity against urease enzyme. Based on their nontoxic behavior, these derivatives may be potential agents for further studies.

Keywords Sparfloxacin · Derivatives · Antimicrobial activities · Urease · Enzyme inhibition

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Introduction

The quinolones class of synthetic origin has a broad antibacterial activity and used for the treatment of a wide variety of infections (Haroon *et al.*, 2012). Sparfloxacin (SPFX) (Haroon *et al.*, 2012) (Fig. 1) is an orally active broad spectrum third generation quinolone, characterized by good to excellent activity against Gram-positive cocci (notably *S. pneumoniae*) and selectivity against anaerobes, and atypical pathogens (Grady *et al.*, 1997; Andersson and MacGowan, 2003). Functional groups at C-7 in the quinolones are important for their antimicrobial activities (Foroumadi *et al.*, 2007; Sultana *et al.*, 2009a, b). Therefore, the substituents at C-7 position can alter the properties of quinolones, such as antibacterial and pharmacokinetic properties as well as adverse side effects (Domagala *et al.*, 1988; Chu *et al.*, 1985; Shen *et al.*, 1989). A five or six member cyclic amino moiety (e.g., pyrrolidine or piperazine ring) is the most commonly used substitution at C-7 position (De Sarro and De Sarro, 2001; Anderson and Osheroff, 2001).

In an effort to develop new analogs of quinolones as agents with better therapeutic profiles (Sultana *et al.*, 2009a, b, 2011a, b, 2012; Arayne *et al.*, 2009a, b, 2010) and analogs of known therapeutic agents with objectives to achieve enhanced therapeutic profiles and minimum adverse effects, we synthesized a series of SPFX derivatives with various aromatic carboxylic acids. In this

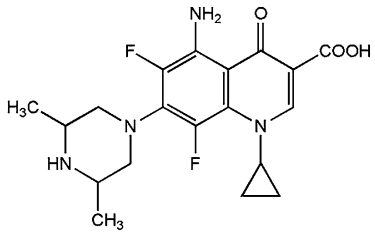

Fig. 1 Sparfloxacin (SPFX) (**1**)

process, our main focus was nucleophilic substitution of carboxylic acid group forming the amide of substrate. Carboxylic acids reacted with phosphorous pentachloride (PCl_5) or sulfonylchloride ($SOCl_2$) to form acid chloride. The amide formation proceeded via attack by the amino group of SPFX, which acted as nucleophile, on the carboxyl carbon of the acid chloride (Boteva and Krasnykh, 2009). The derivatives obtained in 57–78 % yields were subjected to biological activity evaluation. Urease (urea amidohydrolase EC 3.5.15), an enzyme responsible for an organism to use urea as nitrogen source (Mobley and Hausinger, 1989) catalyzed the hydrolysis of urea to ammonia and carbon dioxide (Olivera-Severo *et al.*, 2006).

In systemic nitrogen transport pathways of plants, it is also used as defensive protein (Polacco and Holland, 1993; Sirko and Brodzik, 2000). Urease produced by *Helicobacter pylori* is one of the major causes of pathologies, and thus, play an important role in the pathogenesis of gastric and peptic ulcers, apart from cancer (Smoot *et al.*, 1990). It is directly involved in the pathogenesis of urolithiasis, pyelonephritis, and hepatic encephalopathy, hepatic coma, and urinary catheter encrustation (Mobley and Hausinger, 1989; Smoot *et al.*, 1990; Mobley *et al.*, 1995).

In agriculture, by contrast, a hydrolysis of fertilizer urea by soil urease, if too rapid, results in unproductive volatilization of nitrogen and may cause ammonia toxicity or alkaline-induced plant damage (Krajewska, 2002). Due to the diverse functions of this enzyme, its inhibition by potent and specific compounds could provide an effective tool for the treatment of infections caused by urease-producing bacteria (Amtul *et al.*, 2004).

In this paper, we report synthesis, characterization, antimicrobial, and anti-enzymatic activities of three SPFX derivatives (compounds **2**, **3**, and **4**), which showed potent antibacterial and antifungal activities.

Experimental

Material and reagents

SPFX (**1**) was obtained as a gift by Abbott Pharmaceuticals (Karachi), while solvents and chemicals of analytical grade were purchased from various chemical suppliers. All the solutions were prepared fresh before work.

Instruments

Melting points were measured on a Gallenkamp apparatus, and are uncorrected. TLC spots were detected under UV light. Infrared spectra were recorded in KBr pellets on Shimadzu 470 instrument. ^{1}H and ^{13}C NMR spectra were obtained by using Bruker XWIN NMR spectrometer with TMS as an internal standard. Mass spectra were obtained by using MAT 312 mass spectrometer and Jeol MS instrument (range 50–800). Derivatives were dissolved in $CDCl_3$ or CD_3OD for NMR measurement.

General procedure for the preparation of derivatives

Acid chlorides of benzoic, salicylic, and anthranilic acids were reacted with SPFX. In first case, benzoyl chloride was used as starting material, while salicylic acid was first reacted with thionyl chloride in presence of DMF using benzene as a solvent. In order to obtain oxychloride of anthranilic acid, amino group was first protected by tert-butyloxycarbonylation of amino group (Sarkar *et al.*, 2011), and later removed by trifluoroacetic acid in dichloromethane (Isidro-Llobet *et al.*, 2009).

For the synthesis of derivatives of SPFX, equimolar solutions of both the drug and respective acid chloride were mixed individually in methanol (double distilled) and refluxed on water bath till the completion of reaction, monitored by TLC on pre-coated silica gel plates using methanol:ethyl acetate as eluting solvent. Compounds were recrystallized in methanol till constant melting point. Physicochemical and spectroscopic properties of these derivatives are as follows:

5-Amino-1-cyclopropyl-7-((3R,5S)-3,5-dimethylpiperazine-1-yl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (SPFX)

Yellow powder, m.p. 260 °C. IR (KBr) 3460, 2519, 1718, 1636, 1581, 1454, 1394, 1352, 1317, 1294, 1180, 1104, 1047, 1024. ^{1}H NMR (400 MHz-$CDCl_3$): 0.53–0.28 (3H-cyclopropyl), 3.56–3.31 (m, 4H, piperazinyl ring protons), 4.0 (NH_2), 7.96 (1H-phenyl), 8.51 (1H-quinolone), 11 (1H–OH, Carbonyl). ^{13}C NMR Shifts (ppm): CH 33.3, CH_2 7.7, C 136.3, C 137.5, C 177.5, C 126.1, C 136.0, CH 146.9, CH 47.7, CH_2 53, C 132.2, C 99.8. Mass *m/z* for $C_{19}H_{22}F_2N_4O_3$ = 393. EIMS: *m/z* (rel. abundance %) 393 (M^{+1} 10.5), 392 (base peak 100), 375 (26.4), 361 (8.8), 321 (7.5), 305 (2.4), 291 (42.8), 276, 234, 18 (5.7).

*5-Amino-7-((3S,5R)-4-(2-aminobenzoyl)-3,5-dimethylpiperazine-1-yl)-1-cyclopropyl-6,8-difluro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid (**2**)*

Yield: 0.39 g (78 %), shiny yellow powder, m.p. 228 °C. IR (KBr) 1,636 cm^{-1}(N–H bending), 1,715 cm^{-1}(C=O), 3,408 cm^{-1} (Symmetrical N–H stretching). ^{1}H NMR (400 MHz-$CDCl_3$): δ 1.050–1.23 (5H-cyclopropyl), 3.29-3.31 (m, 8H, piperazinyl), 1.18(3H, CH_3), 8.82 (NH_2), 6.45–7.24 (m, 4H, phenyl), 8.62(NH)0.14.48 (s, 1H of carboxylic acid); ^{13}C NMR Shifts (ppm): CH 35.2, CH_2 5.6 (cyclopropane), C 101.3, 118.9, 128.9, 131.2, 134.7, 136.5, 138.0, 149.5 (1-benzene), C189.9 (1-carbonyl), CH_2 45.2 (aliphatic), CH, 46.4 (cyclohexane), CH_2 50.3 (cyclohexane) CH 116.1, 118.5, 128.0, 130.5 (1-benzene), 59.4 (aliphatic), C 178.1 (1-carboxyl), C 168.3 (1-amide), CH_3 18.7 (aliphatic). Mass *m/z* for $C_{26}H_{29}F_2N_5O_4 = 511$.

*5-Amino-7-((3S,5R)-4-benzoyl-3,5-dimethylpiperazine-1-yl)-1-cyclopropyl-6,8-difluro-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylic acid (**3**)*

Yield: 0.365 g (73 %), yellow powder, mp. 222 °C, IR (KBr) 1,664 cm^{-1}(N–H bending), 1,718 cm^{-1}(C=O), 3,291–3,417 cm^{-1}(N–H stretching). ^{1}H NMR (400 MHz-$CDCl_3$): δ 0.97–1.09 (5H-cyclopropyl), 3.25–3.32 (m, 8H, piperazinyl), 2.89–2.90 (t, CH_3), 4.00–4.02 (NH_2), 7.45–7.91 (5H, phenyl), 8.49 (NH), 14.46 (s, 1H of carboxylic acid); ^{13}C NMR Shifts (ppm): CH 35.2, CH_2 56 (cyclopropane), 46.4 (cyclohexane), C 101.3, 127.2, 128.9, 131.2, 134.7, 135.2, 136.5, 138.0 (1-benzene), C189.9 (1-carbonyl), CH_2 45.2 (aliphatic), CH_2, 50.3 (cyclohexane), CH 59.4 (aliphatic), C 178.1 (1-carboxyl), C 171.7 (1-amide), CH3 18.7 (aliphatic). Mass *m/z* for $C_{26}H_{28}F_2N_4O_4 = 496$.

5-Amino-1-cyclopropyl-6,8-difluoro-7-((*3S,5R*)-4-(2-hydroxybenzoyl)-3,5 dimethylpiperazine-1-yl)-4-oxo-1,2,3,4-tetrahydroquinoline-3-carboxylicacid (**4**)

Yield: 0.285 g (57 %), green crystalline powder, mp 232 °C. IR (KBr) 1,647 cm^{-1}(N–H bending), 1,717 cm^{-1}(C=O), 3,339–3,447 cm^{-1}(N–H stretching). ^{1}H NMR (400 MHz-$CDCl_3$): δ 1.08–1.22 (5H-cyclopropyl), 2.48–3.16 (m, 8H, piperazinyl), 3.99–4.01 (NH_2), 6.64–7.66 (4H, phenyl), 8.52 (NH), 14.64 (s, 1H of carboxylic acid); ^{13}C NMR Shifts (ppm): CH 35.2, CH_2 5.6 (cyclopropane), C 101.3, 120.8, 128.9, 131.2, 134.7, 135.2, 136.5, 138.0, 159.1 (1-benzene), C189.9 (1-carbonyl), CH_2 45.2 (aliphatic), CH 46.4, (cyclohexane), CH_2 50.3 (cyclohexane), CH 117.5,128.6, 121.1, 131.1 (1-benzene), CH 59.4 (aliphatic), C 178.1 (1-carboxyl), C 168.3 (1-amide), CH_3 18.7 (aliphatic). Mass *m/z* for $C_{26}H_{28}F_2N_4O_5 = 512$.

Antibacterial activity

The synthesized derivatives of SPFX (**1**) were screened for their antimicrobial activity against a series of Gram-positive (*Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus,* and *Streptococcus features*), and Gram-negative (*Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis, Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter*, and *Shigella flexneri)* organisms by the conventional cylinder-plate method (Remington, 2006). The solutions for soaking disks were made of different 5, 10, 20, and 40 ppm dilutions, using water:methanol (9:1 v/v) as solvent. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with methanol at the same dilutions as used in the experiment. Nutrient agar was prepared, autoclaved at 121 °C for 15 min, cooled and then poured in Petri dishes. Streaking was done with the help of sterile cotton swab, soaked disks of complex solutions were placed in them and the dishes were incubated for 24 h at 37 °C. Finally, the zones of inhibition were carefully measured with the help of Vernier's caliper.

Antifungal activity

Same procedure as for antibacterial activity was carried out against a number of fungi (*Candida albicans*, *Fusarium solani*, *Trichophyton rubrum*, *Aspergillus parasiticus*, and *A. effusus*). Sabraoud dextrose agar instead of agar was used and the dishes were incubated for 48 h.

Urease inhibition assay

In each well of 96-well plates, 25 µL of enzyme urease (jack bean) solution and 5 µL of test compounds (0.5 mM) were incubated with 55 µL of buffer containing 100 mM urea for 15 min at 37 °C. Ammonia production was determined as a urease activity through indophenol method, as described by Weatherburn (1967). Final volume was maintained as 200 µL by addition of 45 µL phenol reagent (1 % w/v phenol and 0.005 % w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5 % w/v NaOH and 0.1 % NaOCl) to each well. Using a microplate reader (Molecular Devices, CA, USA), the increase in absorbance was measured at 630 nm after 50 min at pH 6.8. By using softMax Pro software (Molecular Devices), the result (change in absorbance per min) was collected. Thiourea was used as the standard inhibitor of urease. Percentage inhibitions were calculated from the formula $100 - (OD_{testwell}/OD_{control}) \times 100$ (Arfan *et al.*, 2010).

Results and discussion

Derivatives of SPFX (**1**) were prepared by refluxing aromatic carboxylic acids with SPFX. Chemistry involves the formation of amide between reacting species and parent compound by nucleophilic substitution reactions. Carboxylic acids react with phosphorous pentachloride (PCl_5) and sulfonylchloride ($SOCl_2$) to form acid chlorides. The amide formation proceeds via attack by the amino group of SPFX, which acts as a nucleophile, on the carboxyl carbon of the acid chloride. In case of reaction of anthranilic acid, 1-alkyl-3-methylimidazolium cation-based ionic liquid (IL) was used to catalyze *N*-tert-butyloxycarbonylation of amine nitrogen with excellent chemoselectivity. This IL is envisaged as "electrophilic activation" of di-tert-butyl dicarbonate $(Boc)_2O$ through bifurcated hydrogen bond formation with the C-2 hydrogen of IL, and has been supported by a downfield shift of the imidazolium C-2 hydrogen of 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide from δ 8.39 to 8.66 in the presence of $(Boc)_2O$ in the 1H NMR, and a drastic reduction of the catalytic efficiency with 1-butyl-2,3-dimethylimidazolium ionic liquids that are devoid of the C-2 hydrogen. The synthesis of derivatives is shown in Fig. 2. All derivatives were synthesized in good yields, and characterized by spectroscopic techniques.

Antibacterial activity

As shown in Tables 1, 2, and 3 (Figs. 3, 4), the in vitro antibacterial activities of these derivatives were evaluated against a series of Gram-positive and Gram-negative bacteria, and compared with the parent drug as well as with other relatively recently developed fluoroquinolones.

The susceptibility of bacterial strains toward SPFX, and its derivatives were evaluated by measuring the zones of

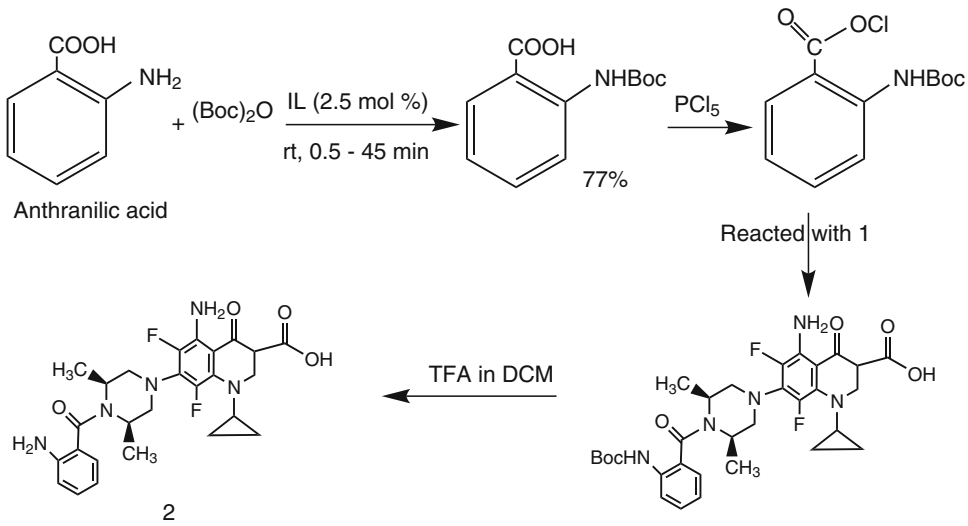

Fig. 2 Synthesis scheme for SPFX derivatives (**2–4**)

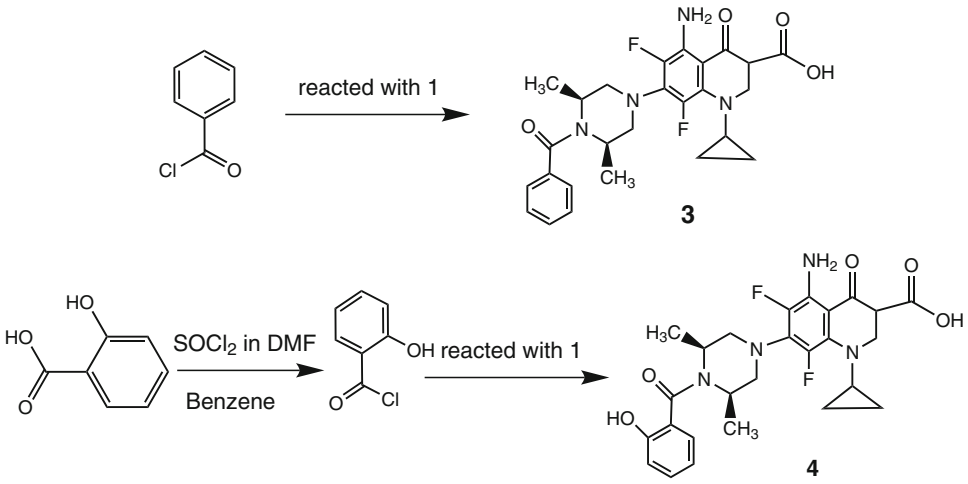

Table 1 Antibacterial activity of SPFX derivatives and their comparisons with other fluoroquinolones against Gram-positive organisms

Derivative	Zone of inhibition (mm)															
	B. subtilis				*M. luteus*				*S. aureus*				*S. features*			
Concentration (ppm)	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
2	7	13	14	21	10	15	17	21	11	14	16	18	6	12	13	18
3	10	12	14	15	9	10	11	15	8	9	11	12	9	10	12	14
4	10	12	13	15	9	11	12	14	7	9	10	11	7	10	11	12
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sparfloxacin	12	13	15	16	11	15	17	19	9	10	14	15	7	9	11	13
Gatifloxacin	7	8	11	13	8	9	11	12	7	16	19	21	7	9	11	12
Gemifloxacin	–	–	8	9	10	11	11	12	11	12	13	15	7	8	9	11

Table 2 Antibacterial activity of SPFX derivatives and their comparisons with other fluoroquinolones against Gram-negative organisms

Species	Zone of inhibition (mm)																											
	Citrobacter				*K. pneumoniae*				*P. mirabilis*				*P. aeruginosa*				*E. coli*				*S. flexneri*				*S. typhi*			
Conc (ppm)	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40	5	10	20	40
2	8	12	16	17	6	11	14	17	5	9	14	17	7	12	17	19	11	14	16	19	8	11	17	18	12	13	16	19
3	8	10	11	13	10	11	12	14	9	10	13	15	10	11	14	15	10	12	13	15	10	12	13	18	10	11	13	15
4	9	10	11	13	8	11	12	14	9	10	11	14	9	11	13	15	10	12	14	16	10	12	13	15	7	11	12	13
Control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sparfloxacin	7	9	11	13	10	13	16	17	10	12	15	16	11	13	17	18	9	11	14	15	9	11	13	15	9	10	15	16
Gatifloxacin	–	20	25	26	11	12	25	26	9	10	12	13	12	12	14	15	14	23	23	24	19	20	23	24	–	7	8	9
Gemifloxacin	8	8	9	10	–	–	12	13	10	11	12	14	11	12	12	13	7	9	10	12	7	8	13	15	8	12	25	26

Table 3 Antifungal activity of SPFX derivatives and their comparisons with other fluoroquinolones against Fungi

Derivatives species	Zone of inhibition (mm)											
	F. solani				*T. rubrub*				*C. albican*			
2	0	0	14	22	10	17	20	28	9	11	14	16
3	15	18	21	25	15	22	23	26	8	9	11	12
4	15	16	25	28	17	18	21	27	8	9	10	11
Control	–	–	–	–	–	–	–	–	–	–	–	–
Sparfloxacin	–	–	10	11	–	20	23	24	7	15	16	17
Gatifloxacin	–	7	12	18	–	8	12	14	–	–	8	9
Gemifloxacin	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5

inhibition. The drug and the derivatives showed inhibition diameters larger than 20 mm being recorded as highly sensitive (Livermore *et al.*, 2001; Simor *et al.*, 1989).

These derivatives exhibited moderate to good activity against a wide variety of Gram-positive and Gram-negative bacteria. Comparison of antibacterial activity data suggested that **2** is more potent antimicrobial agent, than the parent drug, as well as other fluoroquinolones, against Gram-positive and Gram-negative species, while **3** and **4** were found to be less active than the parent compound.

Derivative **2** was found to be most active against *E. coli, M. luteus, P. aeruginosa, S. typhi*, and *B. subtilis,* and displayed mild to moderate activity against *S. aureus, S. flexneri,* and *S. features*. Derivatives **3** and **4** did not show any significant activity with respect to parent drug.

Antifungal activity

SPFX (**1**) itself is not used as fungicidal agent (Sultana *et al.*, 2010), but its synthetic derivatives (**2–4**) showed very good

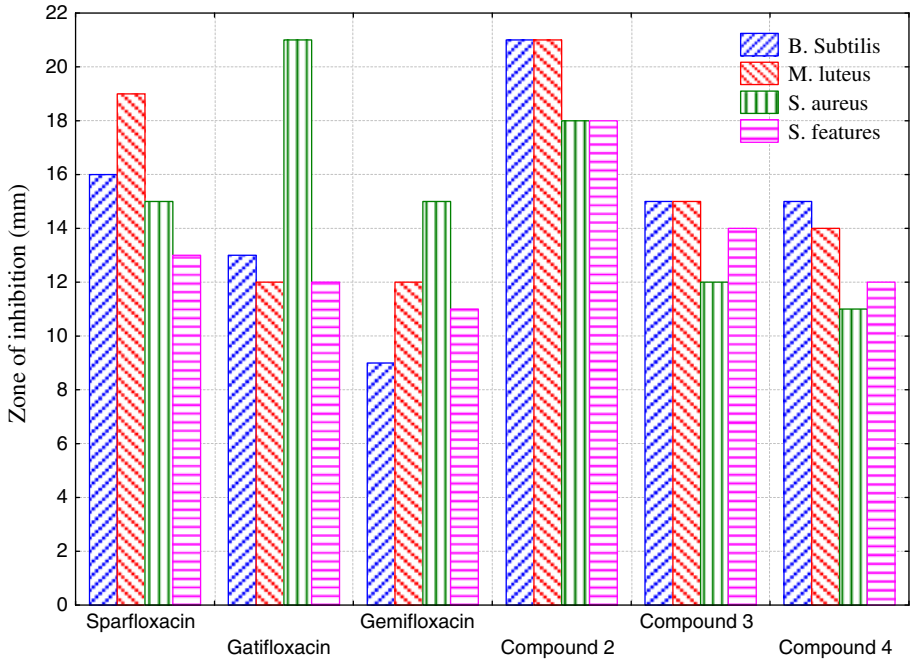

Fig. 3 Antibacterial activity of SPFX-derivatives against Gram-positive bacteria

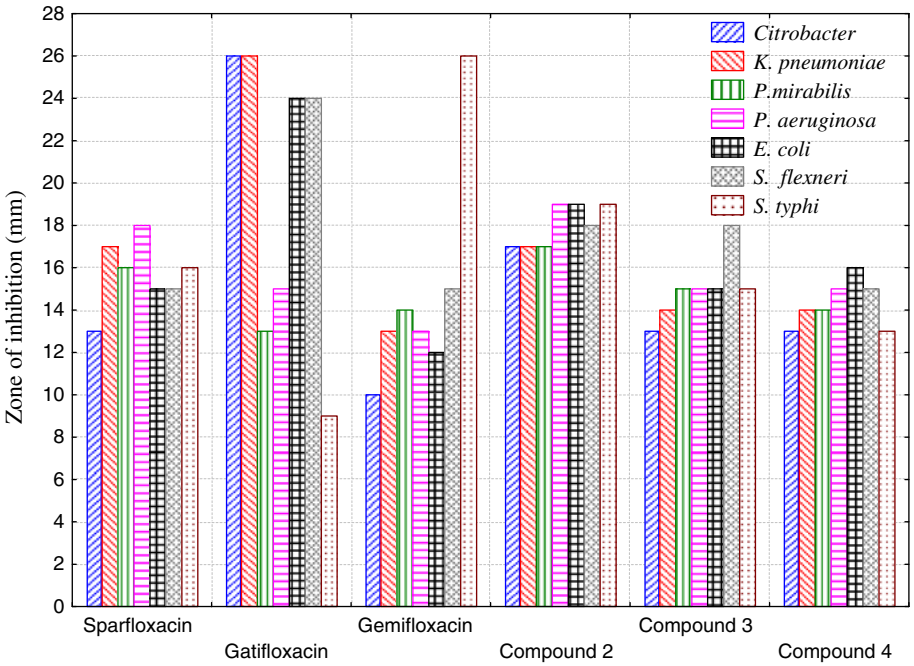

Fig. 4 Antibacterial activity of SPFX-derivatives against Gram-negative bacteria

activity against *F. solani* and *T. rubrum* (Table 4; Fig. 5). None of them, including SPFX, showed activity against *A. parasiticus* and *A. effusus*. In general, these derivatives can also be studied as potent antifungal agents.

Enzyme inhibitory activity

These derivatives (**2–4**) were evaluated for enzyme inhibitory activity against urease, carbonic anhydrase II, and

Table 4 Urease inhibitory activity of SPFX derivatives

Compound	$IC_{50} \pm SEM$
2	421.76 ± 0.58
3	418.43 ± 2.88
4	393.9 ± 0.99
Thiourea[a]	21 ± 0.011

SEM standard error of the mean

[a] The standard inhibitors of the enzyme thiourea

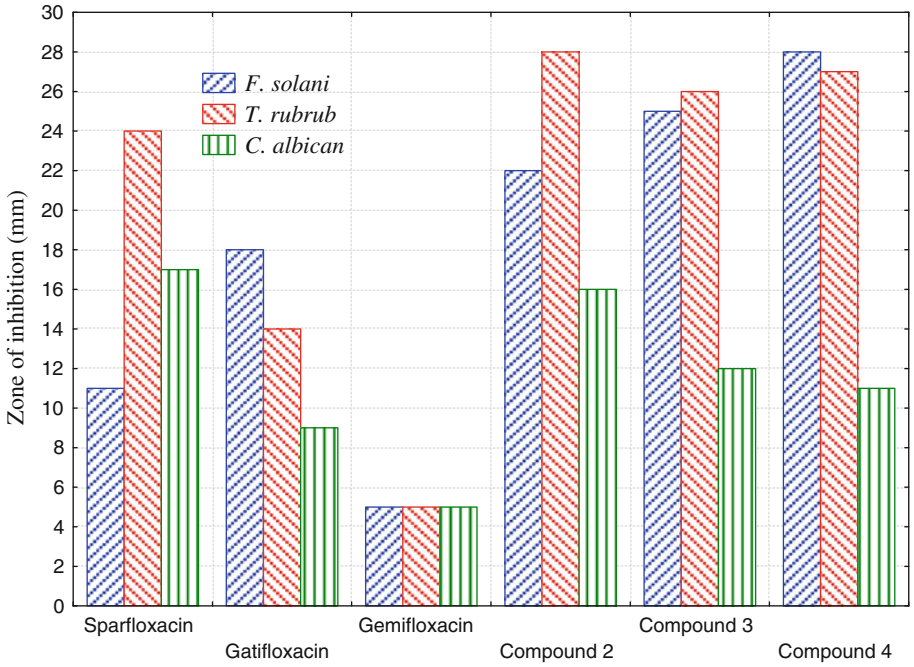

Fig. 5 Antifungal activity of SPFX-derivatives against fungi

α-chymotrypsin. In case of urease, all derivatives showed a moderate activity, while against carbonic anhydrase II and α-chymotrypsin they were inactive. From these studies, it is concluded that these derivatives are selective against urease. The results are presented in Table 4, where in thiourea was used as reference.

IR spectra

The characteristic absorption in the IR spectra of the derivatives is listed above. In general, IR spectra of original drug indicates the presence of functional groups at 1,300 cm^{-1}(C=O, ketone), 1,752–1,742 cm^{-1}(C=O, –COOH), 3,400 cm^{-1}(stretching bends of $–NH_2$), etc. (Dyer, 1974; Nakamoto, 2009; Silverstein *et al.*, 1981). Spectra of all derivatives showed new peaks at 1,664–1,647 cm^{-1} in addition of original peaks, confirming the formations of amide which indicated that the reaction took place between –NH group of parent drug and CO–OH of reacting species. It was further confirmed by shifting of peaks at 3,460–3,400 cm^{-1} which indicated N–H stretching of amide. These facts were further aided by nuclear magnetic resonance (NMR) spectroscopy and mass spectroscopy.

NMR spectra

These facts were further established by nuclear magnetic resonance (NMR) spectroscopy. In the reported spectra of SPFX, the peaks due to 1 β-N-cyclopropyl ring protons were at 0.53 and 0.28 ppm, while peaks of piperazinyl protons were found at 3.56 and 3.31 ppm. Protons of rings adjacent to pyridone ring were at 4.0 ppm, and carboxylic protons at 11 ppm. Comparing the main peaks of derivatives with that of SPFX, proton NMR spectrum showed a set of signals which were almost identical to those of SPFX, except for an up field of signals where new peaks are found at the range of 6.45–7.91 ppm indicated presence of new phenyl rings, and shifting of peaks particularly at carboxylic protons as well as protons of aromatic C–NH indicating that these targeted moieties took part in the synthesis of derivatives.

Mass spectra

Mass spectra of these SPFX derivatives showed molecular ion peaks along with isotopic peaks at *m*/*z* values, consistent with their respective molecular formulas. All derivatives showed easily distinguishable peaks at *m*/*z* values of 70, 278, and 322 along with characteristics fragmentation pattern before carboxylic acid moiety and aromatic C–NH moiety in their respective structure, indicating that the changes are made at these sites of reacting species.

Furthermore, peaks appearing at *m*/*z* values at 103, 304, and 75 in mass spectra of synthesized derivatives indicated the presence of new moieties with different fragmentation patterns but these fragmentations were unstable and showed a molecular ion peak at 392.

Statistical analysis

Statistical package for the Social Sciences (SPSS Inc. Chicago, IL, USA) was used for antimicrobial data examination. Statistical analysis was appraised by one-way

analysis of variance (ANOVA) with the level of significance chosen at $p < 0.05$ or $p < 0.01$ (any values lesser than 0.05 or 0.01 were measured as significant). Multiple comparisons of data were done by Dunnett's test.

Conclusion

Present study shows that revisiting some of the well known and making certain changes in their structures can lead to better therapeutic profile and safer activity. We synthesized three SPFX derivatives in good yields. Gram-positive bacteria and fungi (*F. solani,* and *T. rubrum*) proved to be more sensitive toward newly synthesized derivatives (**2–4**). The result of statistical analysis reveals that the synthetic derivatives are active antimicrobial agents, whereas **2** is more potent than the parent drug (**1**) as well as other fluoroquinolones against Gram-positive and Gram-negative species. Compounds **3** and **4** showed significant activity against *F. solani*. Anti-enzymatic assay showed that these derivatives to be selective inhibitors of urease enzyme. These derivatives thus are good candidates for further study.

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References

Amtul Z, Rasheed M, Choudhary MI, Rosanna S, Khan KM, Atta-Ur-Rahman (2004) Kinetics of novel competitive inhibitors of urease enzymes by a focused library of oxadiazoles/thiadiazoles and triazoles. Biochem Biophys Res Commun 319:1053–1063. http://www.sciencedirect.com/science/article/pii/S0006291X04010125

Anderson VE, Osheroff N (2001) Type II topoisomerases as targets for quinolone antibacterials: turning Dr. Jekyll into Mr. Hyde. Curr Pharm Des 7:337–353

Andersson MI, MacGowan AP (2003) Development of the quinolones. J Antimicrob Chemother 51(Suppl S2):1–11. doi:10.1093/jac/dkg212

Arayne MS, Sultana N, Haroon U, Rizvi SBS (2009a) Synthesis, characterization, antibacterial and anti-inflammatory activities of enoxacin metal complexes. Bioinorg Chem Appl. doi:10.1155/2009/914105, http://www.hindawi.com/journals/bca/2009/914105/ref/

Arayne MS, Sultana N, Haroon U, Ahmed Mesaik M, Asif M (2009b) Synthesis and biological evaluation of enoxain carboxamide derivatives. Arch Pharm Res 32(7):967–974. http://www.ncbi.nlm.nih.gov/pubmed/19641876

Arayne MS, Sultana N, Haroon U, Zuberi MH, Rizvi BS (2010) Synthesis, characterization and biological activity of a series of carboxamide derivatives of ofloxacin. Arch Pharm Res 33(12):1901–1909. doi:10.1007/s12272-010-1203-4, http://www.springerlink.com/content/711t150286152321/

Arfan M, Ali M, Ahmad H, Anis I, Khan A, Choudhary MI, Shah MR (2010) Urease inhibitors from *Hypericum oblongifolium* WALL. J Enzyme Inhib Med Chem 25:296–299. http://informahealthcare.com/doi/abs/10.3109/14756360903179385

Boteva AA, Krasnykh OP (2009) The methods of synthesis, modification, and biological activity of 4-quinolones. Chem Heterocycl Compd 45(7):757–785

Chu DT, Fernandes PB, Claiborne AK, Pihuleac E, Nordeen CW, Maleczka RE Jr, Pernet AG (1985) Synthesis and structure-activity relationships of novel arylfluoroquinolone antibacterial agents. J Med Chem 28(11):1558–1564

De Sarro A, De Sarro G (2001) Adverse reactions to fluoroquinolones: an overview on mechanistic aspects. Curr Med Chem 8:371–374

Domagala JM, Heifetz CL, Hutt MP, Mich TF, Nichols JB, Solomon M, Worth DF (1988) 1-Substituted 7-[3-[(ethylamino)methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids. New quantitative structure-activity relationships at N1 for the quinolone antibacterials. J Med Chem 31(5):991–1001

Dyer JR (1974) Application of absorption spectroscopy of organic compounds. Prentice-Hall of India Pvt. Ltd, New Delhi, p 35

Foroumadi A, Firoozpour L, Emami S, Mansouri S, Ebrahimabadi AH, Asadipour A, Amini M, Saeid-Adeli N, Shafiee A (2007) Synthesis and antibacterial activity of *N*-[5-(chlorobenzylthio)-1,3,4-thiadiazol-2-yl] piperazinyl quinolone derivatives. Arch Pharm Res 30(2):138–145. http://link.springer.com/content/pdf/10.1007%2FBF02977685

Grady FO, Harold PL, Roger GF, David GW (1997) Antibiotic and chemotherapy anti-infective agent and their use in therapy, 7th edn. Churchill Livingstone Inc., New York, pp 419, 451

Haroon U, Zuberi MH, Arayne MS, Sultana N (2012) New improved quinolone derivatives against infection. In: Bobbarala V (ed) Biochemistry, genetics and molecular biology—"a search for antibacterial agents", pp 235–248. ISBN 978-953-51-0724-8. doi:10.5772/46048

Isidro-Llobet A, lvarez MA, Albericio F (2009) Amino acid-protecting groups. Chem Rev 109(6):2455–2504. http://pubs.acs.org/doi/abs/10.1021/cr800323s

Krajewska B (2002) Ureases: roles, properties and catalysis. Wiad Chem 56:223–253. http://www.chemia.uj.edu.pl/zespol_en.php?id=10112

Livermore DM, Carter MW, Bagel S, Wiedemann B, Baquero F, Loza E, Endtz HP, van Den Braak N, Fernandes CJ, Fernandes L, Frimodt-Moller N, Rasmussen LS, Giamarellou H, Giamarellos-Bourboulis E, Jarlier V, Nguyen J, Nord CE, Struelens MJ, Nonhoff C, Turnidge J, Bell J, Zbinden R, Pfister S, Mixson L, Shungu DL (2001) In vitro activities of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. Antimicrob Agents Chemother 45(6):1860–1867. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC90558/

Mobley HLT, Hausinger RP (1989) Microbial ureases: significance, regulation, and molecular characterization. Microbiol Rev 53:85–108

Mobley HLT, Island MD, Hausinger RP (1995) Molecular biology of microbial ureases. Microbiol Rev 59:451–480. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC239369/

Nakamoto K (2009) Infrared and Raman spectra of inorganic and coordination compounds, applications in coordination, organometallic, and bioinorganic chemistry, 6th edn. Wiley, New York, pp 279–280

Olivera-Severo D, Wassermann GE, Carlini CR (2006) Ureases display biological effects independent of enzymatic activity. Is there a connection to diseases caused by urease-producing bacteria? Braz J Med Biol Res 39:851–861. http://www.scielo.br/scielo.php?pid=S0100-879X2006000700002&script=sci_arttext

Polacco JC, Holland MA (1993) Roles of urease in plant cells. In: Jeon KW, Jarvik J (eds) International review of cytology, vol 145. Academic Press, Inc., San Diego, pp 65–103

Remington (2006) The science and practice of pharmacy, 21th edn, vol 1. Lippincott Williams & Wilkins, Baltimore, p 561. ISBN

0-7817-4673-6. http://books.google.com.pk/books?id=NFGSSSbaWjwC&printsec=frontcover&source=gbs_atb#v=onepage&q&f=false

Sarkar A, Roy SR, Parikh N, Chakraborti AK (2011) Nonsolvent application of ionic liquids: organo-catalysis by 1-alkyl-3-methylimidazolium cation based room-temperature ionic liquids for chemoselective *N*-tert-butyloxycarbonylation of amines and the influence of the C-2 hydrogen on catalytic efficiency. J Org Chem 76:7132–7140. http://www.organic-chemistry.org/abstracts/lit3/374.shtm

Shen LL, Mitscher LA, Sharma PN, O'Donnell TJ, Chu DW, Cooper CS, Rosen T, Pernet AG (1989) Mechanism of inhibition of DNA gyrase by quinolone antibacterials: a cooperative drug—DNA binding model. Biochemistry 28(9):3886–3894

Silverstein RM, Bassler GC, Morrill TC (1981) Spectrometric identification of organic compounds, 4th edn. Wiley, New York, QD272.S6 S55

Simor AE, Ferro S, Low DE (1989) Comparative in vitro activities of six new fluoroquinolones and other oral antimicrobial agents against *Campylobacter pylori*. Antimicrob Agents Chemother 33(1):108–109

Sirko A, Brodzik R (2000) Plant ureases: roles and regulations. Acta Biochim Pol 47(4):1189–1195. http://www.actabp.pl/pdf/4_2000/1189-1195s.pdf

Smoot DT, Mobley HLT, Chippendale GR, Lewison JF, Resau JH (1990) *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. Infect Immun 58:1992–1994

Sultana N, Naz A, Khan B, Arayne MS and Mesaik MA (2009a) Synthesis, characterization, antibacterial, antifungal and immunomodulating activities of gatifloxacin derivatives. Med Chem Res **19**(9):1210–1221. doi:10.1007/500044-009-9264-y, http://www.springerlink.com/content/727644w025665076/

Sultana N, Arayne MS, Rizvi B, Mesaik MA (2009b) Synthesis, characterization and biological evaluation of a series of levofloxacin carboxamide analogues Bull Korean Chem Soc 30(10):2294–2298. http://journal.kcsnet.or.kr/main/j_search/j_abstract_view.htm?code=B091024&qpage=j_search&spage=b_bkcs&dpage=ar

Sultana N, Arayne MS, Gul S, Shamim S (2010) Sparfloxacin–metal complexes as antifungal agents—their synthesis, characterization and antimicrobial activities. J Mol Struct **975**(1–3):285–291. http://www.sciencedirect.com/science/article/pii/S0022286010003911

Sultana N, Arayne MS, Rizvi SBS, Haroon U (2011a) Synthesis, characterization and biological evaluations of ciprofloxacin carboxamide analogues. Bull Korean Chem Soc 32(2):483–488. doi:10.5012/bkcs.2011.32.2.483, http://journal.kcsnet.or.kr/main/j_search/j_abstract_view.htm?code=B110221&qpage=j_search&spage=b_bkcs&dpage=ar

Sultana N, Hamza E, Arayne MS, Haroon U (2011b) Effect of metal ions on the in vitro availability of enoxacin, its in vivo implications, kinetic and antibacterial studies. Quim Nova 34(2):186–189. http://www.scielo.br/scielo.php?pid=S0100-40422011000200003&script=sci_abstract

Sultana N, Arayne MS, Rizvi SBS, Haroon U, Mesaik MA (2012) Synthesis, spectroscopic and biological evaluation of some levofloxacin metal complexes. Med Chem Res. doi:10.1007/s00044-012-0132-9

Weatherburn MW (1967) Urea colorimetric endpoint determination urease—berthelot reaction. Anal Chem 39:971. http://www.multi-quimicos.com/plasmatecpdfs/UREACOL125.pdf