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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antibacterial activity of novel 7-haloanilino-8-nitrofluoroquinolone derivatives

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Abstract The synthesis of new 7-(halophenylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid derivatives is described. Substitution of quinolonic compound **1a** with chloro and fluoro aniline derivatives at position 7 produced target compounds **2–9** with relatively low yields. Alternatively, the product of correspondent carboxylate ester **1b** subsequent with acid hydrolysis produced satisfactory yield. The prepared targets have shown interesting antibacterial properties against standard and resistant gram-positive strains. In particular, 2-chloro and 3-chloro aniline derivatives (**3** and **4**) depict MIC values of 6.7 and 0.9 μg/ml, respectively against standard *S. aureous*. Both compounds showed good activity against resistant strains of *S. aureous*.

Keywords 7-Anilinofluoroquinolone · 8-Nitrofluoroquinolone derivatives · Fluoroquinolone · Antibacterial activity

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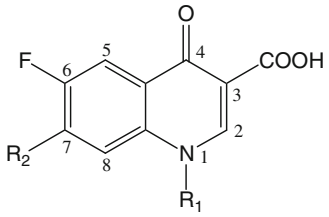
Introduction

Many fluoroquinolone antibacterial agents have been introduced into clinical use with significant improvement in antibacterial spectrum and activity. A vast array of fluoroquinolones presents antiinfective armory of the clinicians (for example, ciprofloxacin and norfloxacin, Chart 1), because of their therapeutic efficacy and tolerability (Baker *et al.*, 2004; Ball, 2000; Felmingham *et al.*, 1985; Grohe, 1993; Okada *et al.*, 1993; Wise *et al.*, 1983). However, continual prescribing of this class has led to the recent emergence of fluoroquinolone-resistant bacteria that has necessitated the search for innovative drugs efficacious against resistant strains (Baker *et al.*, 2004; Ball, 2000; Chen and Lo, 2003; Cohen, 2000). The present work aims to develop new 7-haloanilino-fluoroquinolones with potential activity, lower toxicity, lower resistance or all of these.

Results and discussion

The structure–activity relationship of different positions of the fluouroquinolone system has been thoroughly discussed (Ball, 2000; Cianchetta *et al.*, 2004; Emami *et al.*, 2005; Foroumadi *et al.*, 2003). It is well established that C-7 substituent is regarded as drug–enzyme interaction domain, where most chemical modifications led to major influence on activity. The inhibition of DNA gyrase and cell permeability of quinolones is greatly influenced by the nature of C-7 substitution, Chart 1. C-7 piperazinyl group in addition to C-6 fluorine substituent has antibacterial potency far superior to that of earlier classical quinolones against both gram-positive and gram-negative bacteria (Cianchetta *et al.*, 2004; Emami *et al.*, 2005; Foroumadi *et al.*, 2003).



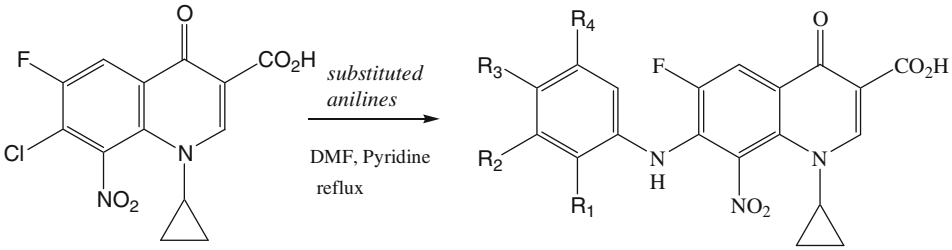
ciprofloxacin ($R_1 = c\text{-}C_3H_5$; $R_2 = N$-piperazinyl)

norfloxacin ($R_1 = C_2H_5$; $R_2 = N$-piperazinyl)

Chart 1 Fluoroquinolone nucleous

In the same vein, previous studies by our team for introducing more lipophilic groups at C-7 such as anilines revealed noticeable increase in gram-positive activity, especially against resistant strains with significant loss of gram-negative activity (Al-Hiari et al. 2007, 2011; Foroumadi *et al.*, 2003). As a continuation of previous research findings, this research aims at further investigating 7-haloanilino derivatives **2–9** of the main nucleus 6-fluoro-8-nitro-4-quinolone-3-carboxylic acid (**1**), Schemes 1 and 2 (Al-Hiari et al. 2007, 2011). Synthon **1** possesses an electron withdrawing C-8 nitro group to facilitate coupling of the substituted anilines.

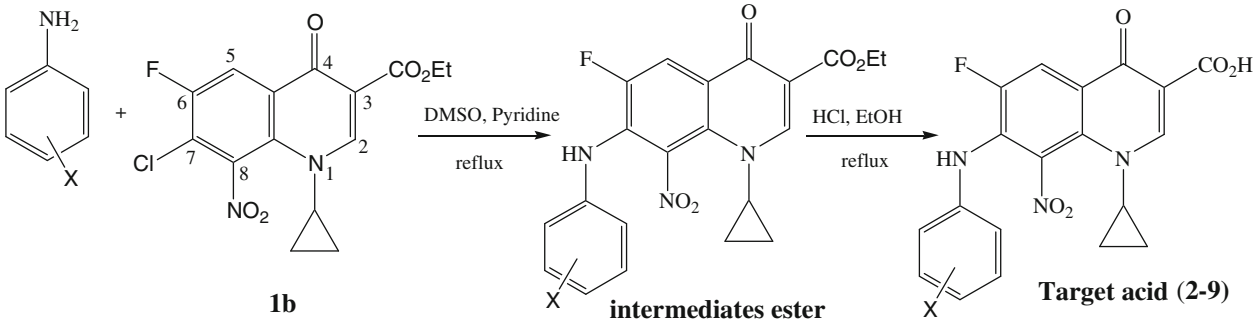
Scheme 1 Synthesis of target compounds by acid pathway **2–9**. *The values are calculated according to best method option of Chemdraw Ultra software



(1a)

Target Compounds **2-9**

Compound	R_1	R_2	R_3	R_4	Calculated LogP*
2	H	H	H	H	3.16
3	Cl	H	H	H	3.72
4	H	Cl	H	H	3.72
5	H	H	Cl	H	3.72
6	Cl	H	H	Cl	4.27
7	F	H	H	H	3.32
8	F	H	F	H	3.47
9	F	F	F	H	3.63



1b **intermediates ester** **Target acid (2-9)**

Scheme 2 Synthesis of target compounds by ester pathway **2–9**

Chemistry

Clinically used fluoroquinolone antibiotics have C-7 substitutions of secondary amino group derivatives. However, our research on primary amine substituents at C-7 position proved that primary amino substitution produces also active antibacterial compounds (Al-Hiari et al. 2007, 2011). From chemical point of view, primary amino groups are weak nucleophiles to undergo nucleophilic aromatic substitution reaction into C-7 position of quinolone nucleus. This makes such reactions hard, low yielding and involves many side products as proved in our initial attempts. Therefore, the addition of nitro group into C-8 position of quinolone nucleus in the above compound **1** was intended to increase the electropositivity of C-7 carbon atom (Al-Hiari *et al.*, 2007).

As depicted in Scheme 1, the synthesis of compounds **2–9** was carried out through direct interacting of 7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**1a**) with 2 molar excess of halogenated anilines in DMF/pyridine (7:3 V/V), followed by heating the mixture at 70–80°C for 2–3 days. On work up, the precipitate was purified over plate chromatography to produce the targets in low yields. Although different procedures and conditions were attempted to improve the yields, minimal amounts were obtained and this was because of many factors. The main reason was because of the weak nucleophilicity of halogenated anilines that permitted other stronger nucleophiles to attack at the same position leading to two major side products; **10** (7-hydroxy derivative when DMF was used as solvent) and **11** (in case of using ethanol as solvent) (Al-Hiari *et al.*, 2007). Other major side products isolated with DMF/pyridine procedure were the defluorinated side product **12** and the dicoupled **13**, Chart 2. De-fluorination of side product **12** is confirmed by disappearance of NMR coupling of flour atom with H_5. Typical H_5–F coupling with fluorine (12 Hz ca) has disappeared and substituted with a new doublet signal possess coupling constant around 8 Hz representing H_5–H_6 coupling. H_6 is also appeared at expected position as doublet peak with J-value = 8 Hz. Moreover, these findings are supported by MS and EA.

An alternative strategy using the ester derivative **1b** was used instead of the acid **1a**. Coupling **1b** with haloaniline followed by hydrolysis of the ester was a successful alternative approach to obtain the target compounds (**2–9**). The haloaniline was reacted with the correspondent ester **1b** to produce the target acids in satisfactory yield on hydrolysis of the ester intermediate, Scheme 2. The target compounds were produced in higher yields and minimal side products. This might be related to better solubility of ester derivatives in reaction solvent (DMSO) in comparison with acids.

IR, MS, EA and NMR spectral data of **2–9** are in accordance with the assigned structures and are given in the experimental part. MS spectra display molecular weight ions [M^+] in good agreement with the calculated values of their molecular formulae. ^{1}H NMR signals and their coupling constants were informative enough for assigning each hydrogen in target structures. Moreover, EA results were correct and within calculated values.

Antimicrobial screening

In vitro antibacterial activity of a series of 7-(chloro and fluoro phenyl-amino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**2–9**) were evaluated against standard and resistant isolates of *S. aureus* and *E. coli*. Target compounds were all tested using broth dilution method to determine the minimum inhibitory concentrations (MICs). Results of active antibacterial compounds were reported in Table 1.

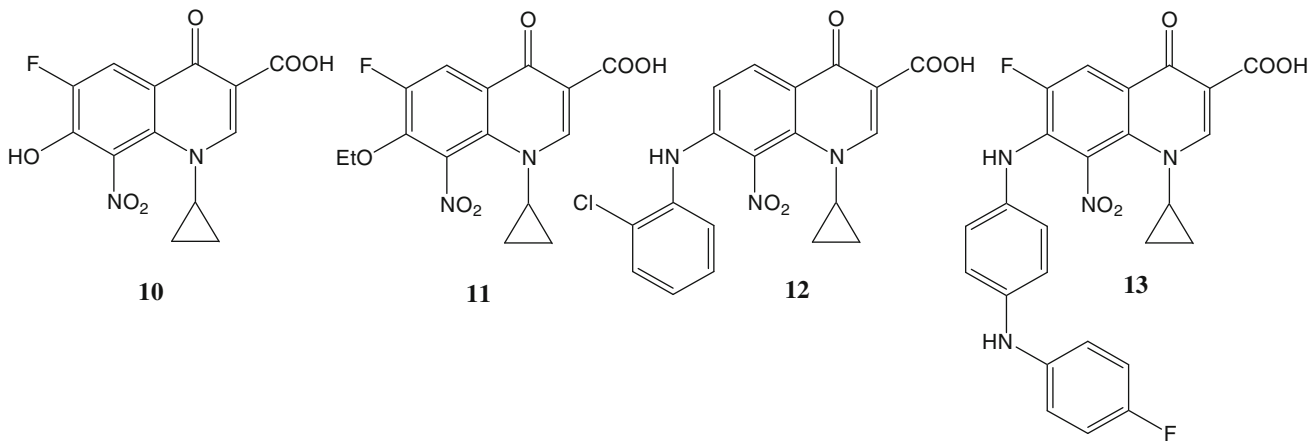


Chart 2 Major side products isolated

Table 1 Minimum inhibitory concentrations (*MIC*, μg/ml) for active compounds against standard and resistant isolates of *S. aureus* compared with ciprofloxacin

Compound no.	MIC (μg/ml)	
	S. aureus (standard) ATCC6538	*S. aureus* (resistant isolates)
Ciprofloxacin	2.9	1.4
2	11.7	ND
3	6.7	6.7
4	0.9	7.0
5	6.7	9.8
7	106.0	106.0
9	39.0	9.8

Interestingly, chlorinated targets have shown stronger activity against both standard and resistant isolates of *S. aureus* gram-positive bacteria compared with fluorinated ones. On the other hand, chlorinated aniline derivatives **3–5** and some fluorinated targets **9** have shown good activity towards both standard and resistant isolates of *S. aureus* bacteria with MIC values less than 10 μg/ml. MIC values of 3-chloroaniline derivative **4** were excellent against both standard *S. aureus* and resistant isolates of *S. aureus*, with values of 0.9 and 7.0 μg/ml, respectively. The fluorinated derivative **9** has shown better activity against resistant isolates of *S. aureus*.

The most interesting phenomenon of some tested compounds is their significant activity against resistant gram-positive strains. This might be because of the increase of lipophilicity of these compounds (Table 1) in comparison with ciprofloxacin (log$P$ = 1.32) and norfloxacin (log$P$ = 1.37).

Experimental

General

Melting points were determined on a Stuart scientific electro-thermal apparatus, whereas infra red (IR) spectra were recorded using Shimadzu 8400F-FT-IR spectrophotometer as KBr or NaCl discs. ^{1}H- and ^{13}C NMR spectra were recorded on Varian Oxford-300 (300 MHz) spectrometer and Bruker Avance-400 (400 MHz) Ultrashield spectrometer. Chemical shifts are expressed in ppm related to tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded on Agilent 1100 series LC–MS, under electro spray ionization (ESI) condition with negative-ion mode. Elemental analysis (CHN) were performed on Euro Vector Elemental Model (EA 3000 A) and the results were in good agreement with the calculated values (±0.4%).

Chemicals involved in the preparation of synthone **1** are 2,4-dichloro-5-fluoro-3-nitrobenzoic acid, ethyl 3-(*N*,*N*-dimethyl-amino) acrylate and cyclopropyl amine. They were all purchased from Acros (Belgium). Primary halogenated anilines used are bought from the following: Aniline from Merck (Dermstadt, Germany). 2-Bromoaniline, 4-bromoaniline, 2,4-dibromoaniline, 4-fluoroaniline and 2,3,4-trifluorooaniline from Acros (Belgium). 3-Chloroaniline from Aldrich Chemicals (England). 4-Chloroaniline, 2,5-dichloroaniline, 2-fluoroaniline and 2,4-difluoroaniline from Fluka (Switzerland).

Mobile-phase mixtures for TLC were adjusted to be 94:5:1 chloroform–methanol–formic acid ($CHCl_3$–MeOH–FA) (system 1) and 90:10:1 $CHCl_3$–MeOH–FA (system 2).

General procedure for the synthesis

The title synthon **1**, 7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, required in this work, is prepared from 2,4-dichloro-5-fluoro-3- nitrobenzoic acid, ethyl 3-(*N*,*N*-dimethylamino) acrylate and cyclopropyl amine by following the stepwise synthetic procedures reported in previous work (Al-Hiari *et al.*, 2007).

Acid pathway

The target compounds were synthesized using synthone **1a** to produce the targeted **2–9** using the following general procedure.

Two molar equivalents of the halogenated aniline derivative was gradually added into a solution of 7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**1a**, 0.5 g) in DMF/pyridine (7:3 V/V) and heated at 70–80°C. At the end of the reaction (2–3 days), cold water was added and the resulting precipitate was filtered, washed with water and left to dry at room temperature. The resulting solid was then dissolved in acetone and separated on TLC plates using system 1 as eluent followed by second elution step using system 2. The band of interest was collected from the plate and the silica was grounded and washed with acetone. The organic layer was then evaporated and the target was left to dry at room temperature (yields 10–20%).

Ester pathway

Two molar equivalent of the haloaniline was gradually added into a solution of ethyl 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**1b**, 0.5 g ≈ 1.41 mmol) and 10 ml of DMSO and drops of pyridine. The mixture was heated at 70–80°C under anhydrous conditions. The reaction mixture was left to crystallize, then filtered and the product was left to dry in dark place. The resulting solid was re-crystallized from methanol/chloroform (3×) to give

fairly pure ester intermediate (25–65%). The intermediate was further hydrolyzed on dissolving the solid in ethanol and adding concentrated HCl (10 ml). The mixture was refluxed at 70–80°C for 5 h, then target compound was filtered and collected on drying as yellow solid (80–90%).

7-(2-Chloro-phenylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(3)***

Colour: bright yellow. Yield: 6% acid pathway,; mp 279–281°C (decomp); R_f value in system (**2**) = 0.60; IR (KBr): *v* 3,456, 3,435, 2,951, 2,630, 2,734, 2,510, 1,731, 1,676, 1,663, 1,605, 1,543, 1,506, 1,473, 1,440, 1,355, 1,232, 799, 773 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.90–0.96 (m, 4H, H_2-2′/H_2-3′), 3.61 (m, 1H, H-1′), 7.10 (d,d,d, J = 1.2, 6.6, 7.5, 1H, Ar–H: H-4″), 7.34 (d,d,d, J = 2.0, 7.5, 8.4, 1H, Ar–H: H-5″), 7.52 (d,d, J = 1.0, 7.2 Hz, 1H, Ar–H: H-6″), 7.64 (d, $^3J_{H-F}$ = 12.3 Hz, 1H, H-5), 8.48 (s, 1H, H-2), 8.55 (d,d, J = 1.0, 5.7 Hz, 1H, Ar–H: H-3″), 8.66 (br s, 1H, NH, D_2O exchangeable), 13.01 (br s, 1H, CO_2H); MS (CI/ESI –ve): *m/z* (%): calcd. for $C_{19}H_{13}ClFN_3O_5$ (417.8): 420(2), 719(8), 418 (37), 417 (27), 416 (100), 397(1), 381(2), 360(3), 359 (16), 325 (3), 306(6), 265(35), 217(1), 197(2),161 (2); EA: Calculated for $C_{19}H_{13}ClFN_3O_5$, C, 54.62; H, 3.14; N, 10.06. Found: C, 54.65; H, 3.15; N, 9.99.

7-(3-Chloro-phenylamino)-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(4)***

Colour: orange. Yield: 8% acid pathway; mp 240–243°C (decomp); R_f value in system (2) = 0.45; IR (KBr): *v* 3,434, 3,390, 2,942, 1,735, 1,640, 1,535, 1,467, 1,365, 1,210, 1,225 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.95, 1.02 (2 m, 4H, H_2-2′/H_2-3′), 3.71 (m, 1H, H-1′), 7.16 (d,d, J = 1.0, 7.8 Hz, 1H, Ar–H: H-6″), 7.39 (d,d, J = 7.8, 8.1 Hz, 1H, Ar–H: : H-5″), 7.49 (d, J = 8.4 Hz, 1H, Ar–H: H-4″), 7.98 (m, J = 11.2 Hz, 2H, Ar–H: H-2″ and H-5), 8.50 (br s, 1H, NH, D_2O exchangeable), 8.76 (s, 1H, H-2), 12.95 (br s, 1H, CO_2H); MS (CI/ESI −ve): *m/z* (%): calcd. for $C_{19}H_{13}ClFN_3O_5$ (417.8): 419 (5), 418 (34), 417 (21), 416 (78), 383 (4), 360 (5), 359 (35), 339 (7), 325 (6), 311 (8), 293 (12), 265 (100), 191 (6), 161 (6); EA: Calculated for $C_{19}H_{13}ClFN_3O_5$, C, 54.62; H, 3.14; N, 10.06. Found: C, 54.61; H, 3.20; N, 9.97.

1-Cyclopropyl-7-(2,5-dichloro-phenylamino)-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ***(6)***

Colour: greenish-yellow. Yield 12%; mp 265–267°C (decomp); R_f value in system (**2**) = 0.60; IR (KBr): *v* 3,720, 3,402, 2,905, 1,713, 1,605, 1,587, 1,539, 1,462, 1,340, 1,295, 1,220 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.88–0.98 (m, 4H, H_2-2′/H_2-3′), 3.62 (m, 1H, H-1′), 7.17 (d,d, J = 4, 8 Hz, 1H, Ar–H: H-4″), 7.56 (d, J = 8 Hz, 1H, Ar–H: H-3″), 7.63 (d, $^3J_{H-F}$ = 12.0 Hz, 1H, H-5), 8.39 (br s, 1H, NH, D_2O exchangeable), 8.57 (s, 1H, H-2), 8.68 (d, J = 4 Hz, 1H, Ar–H: H-6″), 13.25 (s, 1H, CO_2H); MS (CI/ESI −ve): *m/z* (%): calc. for $C_{19}H_{12}Cl_2FN_3O_5$ (452.22): 454 (8), 453 (12), 452 (57), 451 (19), 450 (100), 437 (48), 436 (83), 401 (3), 400 (7), 265 (7); EA: Calculated for $C_{19}H_{12}Cl_2FN_3O_5$, C, 50.46; H, 2.67; N, 9.29. Found: C, 50.55; H, 2.74; N, 9.32.

1-Cyclopropyl-6-fluoro-7-(2-fluoro-phenylamino)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(7)***

Colour: green. Yield 9%; mp 292–294°C (decomp); R_f value in system (2) = 0.60; IR (KBr): *v* 3,420, 3,064, 2,920, 2,856, 1,675, 1,624, 1,601, 1,538, 1,506, 1,491, 1,462, 1,368, 1,319, 1,227, 1,095 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.84–0.96 (m, 4H, H_2-2′/H_2-3′), 3.61 (m, 1H, H-1′), 7.10 (d,d,d, J = 4, 8, 16 Hz, 1H, Ar–H: H-6″), 7.19 (d,d, J = 8, 16 Hz, 1H, Ar–H: H-4″), 7.30 (d,d,d, J = 4, 8, 16 Hz, 1H, Ar–H: H-3″), 7.64 (d, $^3J_{H-F}$ = 12.0 Hz, 1H, H-5), 7.94 (br s, 1H, NH, D_2O exchangeable), 8.49 (d,d, J = 8, 16 Hz, 1H, Ar–H: H-5″), 8.56 (s, 1H, H-2), 12.97 (br s, 1H, CO_2H); MS (CI/ESI −ve): *m/z* (%):calcd. for $C_{19}H_{13}F_2N_3O_5$ (401.32): 402 (4), 401 (22), 400.0 (100), 247 (4), 212 (4), 157 (6); EA: Calculated for $C_{19}H_{13}F_2N_3O_5$, C, 56.86; H, 3.27; N, 10.47. Found: C, 56.80; H, 3.30; N, 10.41.

1-Cyclopropyl-7-(2,4-difluoro-phenylamino)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(8)***

Colour: dark green. Yield: 80 mg,13%; mp 260–262°C (decomp); R_f value in system (2) = 0.85; IR (KBr): *v* 3,501, 3,381, 2,942, 2,490, 1,679, 1,640, 1,604, 1,550, 1,532, 1,467, 1,356, 1,317, 1,225, 1,042, 957, 852, 803, 771 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.92, 0.97 (2 m, 4H, H_2-2′/H_2-3′), 3.71 (m, 1H, H-1′), 7.10 (d,d, J = 8.5, 8.9 Hz, 1H, Ar–H: H-6″), 7.39 (dd, J = 8.8, 11.0 Hz, 1H, Ar–H: H-3″), 7.57 (br s, 1H, NH, exchangeable), 8.0 (d, $^3J_{H-F}$ = 11.5 Hz, 1H, H-5), 8.44 (dd, J = 9.1, 15.5 Hz, 1H, Ar–H: H-5″), 8.76 (s, 1H, H-2), 12.40 (br s, 1H, CO_2H); MS (CI/ESI −ve): *m/z* (%): calcd. for $C_{19}H_{12}F_3N_3O_5$ (419.31): 419 (5), 418 (18), 417 (100), 339 (3), 293 (8), 256 (8), 265 (51), 113 (7); EA: Calculated for $C_{19}H_{12}F_3N_3O_5$, C, 54.42; H, 2.88; N, 10.02. Found: C, 54.40; H, 2.81; N, 10.11.

1-Cyclopropyl-6-fluoro-8-nitro-4-oxo-7-(2,3,4-trifluoro-phenylamino)-1,4-dihydroquinoline-3-carboxylic acid ***(9)***

Colour: green. Yield 11%; mp 330–332°C (decomp); R_f value in system (2) = 0.60; IR (KBr): *v* 3,449, 3,278, 3,196, 3,063, 1,680, 1,623, 1,600, 1,541, 1,509, 1,462,

1,317, 1,231, 1,051, 944, 779 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 0.92, 0.95 (2 m, 4H, H_2-2′/H_2-3′), 3.63 (m, 1H, H-1′), 7.32 (d,d, $J = 8$, 16 Hz, 1H, H-6″), 7.64 (d, $J = 12$ Hz, 1H, H-5″), 8.26 (d, $^3J_{H-F} = 8.0$ Hz, 1H, H-5), 8.48 (br s, 1H, NH, D_2O exchangeable), 8.55 (s, 1H, H-2), 13.14 (s, 1H, CO_2H); MS (CI/ESI −ve): *m/z* (%): calc. for $C_{19}H_{11}F_4N_3O_5$ (437.30); 436 (100), 435 (2), 419 (2), 418 (5), 417 (3), 416 (6), 402 (4), 401 (13), 400 (23), 359 (9), 255 (3); EA: Calculated for $C_{19}H_{11}F_4N_3O_5$, C, 52.18; H, 2.54; N, 9.61. Found: C, 52.40; H, 2.55; N, 9.58.

*Compound **2** and **5** were prepared using acid procedure in this research and their data were previously reported (Al-Hiari *et al.*, 2007) using different procedure.

*Compounds **10** and **11** were also cited in our previous research (Al-Hiari *et al.*, 2007).

7-(2-Chlorophenylamino)-1-cyclopropyl-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(12)***

This side product was obtained during the workup of target compound **3** from acid pathway. Data for **12** are reported as model, although all defluorinated derivatives of targets **2–9** were also isolated and identified.

Colour: yellow; yield: 20%; mp = 278–280°C (decomposition); R_f value in system (2) = 0.88; IR (KBr): *v* 3,466, 3,444, 2,950, 2,627, 2,710, 1,738, 1,655, 1,660, 1,611, 1,545, 1,512, 1,482, 1,454, 1,341, 1,233, 805, 782 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.93, 1.01 (2 m, 4H, H_2-2′/H_2-3′), 3.62 (m, 1H, H-1′), 7.18 (m, 1H, Ar–H: H-6″), 7.39 (m, 1H, Ar–H: H-4″), 7.57 (m, 2H, Ar–H: H-3″/5″), 8.08 (d, $J = 8.0$ Hz, 1H, H-6), 8.28 (br s, 1H, NH, D_2O exchangeable), 8.55 (d, $J = 8.0$ Hz, 1H, H-5), 8.82 (s, 1H, H-2), 12.55 (br s, 1H, CO_2H). MS (CI/ESI −ve): *m/z* (%): 398 (12), 343 (10), 331 (29), 325 (8), 324 (30), 317 (15), 312 (88), 309 (11), 293 (20), 290 (37), 281 (38), 259 (25), 247 (16), 243 (21), 231 (30), 212 (100), 193 (17), calcd. for $C_{19}H_{14}ClN_3O_5$ (399.78).

1-Cyclopropyl-6-fluoro-7-[4-(4-fluorophenylamino)-phenylamino]-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ***(13)***

This side product was obtained during the preparation of 1-cyclopropyl-6-fluoro-7-(4-fluoro-phenylamino)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid that was not successful using this acid pathway.

Colour: orange; mp: 282–283°C (decomposition); R_f value in system (2) = 0.0.92. IR (KBr): *v* 3,356, 3,056, 1,678, 1,605, 1,588, 1,560, 1,506, 1,461, 1,407, 1,368, 1,334, 1,289, 1,207, 1,156 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.60 (m, 4H, H_2-2′/H_2-3′), 3.32 (m, 1H, H-1′), 6.60–6.71 (m, 4H, Ar–H: H-2″/H-3″/H-5″/H-6″), 6.80 (m, 2H, Ar–H: H-2‴/H-6‴), 7.30 (m, 2H, Ar–H: H-3‴/H-5‴), 7.75 (d, $^3J_{H-F} = 11.0$ Hz, 1H, H-5), 8.42 (s, 1H, H-2), 8.55 (br s, 1H, NH, exchangeable), 11.55 (s, 1H, CO_2H); MSEI *m/z* (%): 493 (M^+, 100), 463, 401.

In vitro antibacterial activity testing

Materials and instruments

Nutrient agar and nutrient broth were obtained from Himedia (Mumbai, India); 0.5 McFarland suspension (prepared by adding 0.5 ml of $BaCl_2$ (1.175% w/v $BaCl_2{\cdot}2H_2O$) to 99.5 ml of 0.36 N H_2SO_4 (1.0% v/v). Microbiology samples were incubated at 37°C using WTC binder incubator, Tuttlingen (Germany). The 96-flat bottom microplates were used in the conduction of broth dilution test. ELx 800UV universal microplate reader, Biotek instrument was used to determine the turbidity in the wells. The in vitro antibacterial activity for all targets (**2–9**) using broth dilution method was carried out on both standard and resistant isolates of *Staphylococcus aureus* and *Escherichia coli*. The used standard strains were gram-positive bacteria (*S. aureus* ATCC 6538) and gram-negative (*E. coli* ATCC-8739). The minimal inhibitory concentration (MICs) of the chemical compounds assays was carried out as described by Foroumadi *et al.* (2003), with minor modification. Ciprofloxacin was used as standard antibacterial agent.

Bacterial suspensions were prepared in sterilized distilled water, in a concentration around 1×10^7 cfu/ml, which was standardized according to 0.5 McFarland suspension as described by the Clinical and Laboratories Standards Institute (CLSI) 2007.

Determination of minimum inhibitory concentration

The MICs (μg/ml) of targets were determined by the broth dilution method using the two serial dilution techniques with concentrations in the range 250–0.244 μg/ml. The MIC is defined as the average of two successive concentrations of the tested compound showing growth and no growth.

Tested compounds (1,000 μg) were first dissolved in 200 μl DMSO, and then 800 μl of sterilized distilled water was added to end up with a final concentration of the stock solution of 1,000 μg/ml.

The MIC test was performed in 96-flat bottom microtiter plates, 100 μl of previously prepared and sterilized broth was added in each well followed by 100 μl of tested compounds stock solution (1,000 μg/ml) that was added to the first well, mixed with the broth, followed by twofold serial dilution on to successive wells across the plate to end up with 11 successive twofold dilutions for each tested compound. Then, 10 μl of bacterial suspension was used to inoculate each well.

Control tests for each experiment were performed. Positive growth control was performed by adding one drop of each micro-organism suspensions to four wells in each plate of the culture medium without the test compound. Negative growth control was also performed using four uninoculated wells of medium without the test compound. Plates were incubated at 37°C for 24 h and were checked for turbidity.

Twofold serial dilutions were carried out in a similar manner for DMSO (20% v/v in water) to test its antibacterial activity. Ciprofloxacin standard was also tested as reference compound. The turbidity was determined visually and using microplate reader. The results are presented in the Table 1.

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