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ORIGINAL ARTICLE

Design synthesis and antibacterial activity studies of new thiadiazoloquinolone compounds

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Abstract

New 9-(alkyl/aryl)-4-fluoro-6-oxo[1,2,5]thiadiazolo[3,4-*h*]quinoline-5-carboxylic acids and their esters were designed and synthesized. A detailed discussion of the reactions utilized in the preparation of the intermediate and target compounds is reported. All the newly synthesized compounds were fully characterized using all the physico-chemical means needed. All the intermediates and the final esters and acids were tested against bacterial and fungal strains. The acids **25a** and **25c** proved to be very active against Gram positive and Gram negative bacteria with MIC 0.15–3 μg/mL. The structure–activity relationship of antibacterial thiadiazoloquinolones shows that compounds **25a** and **25c** are twice less potent than the corresponding cyclopropyl derivative **16**. Therefore, the cyclopropyl moiety on *N*-9 seems to be the most suitable substituent.

Keywords

6-Fluoroquinolone, antibacterial activity, synthesis, thiadiazoloquinolone

History

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Introduction

Norfloxacin (**1**) and ciprofloxacin (**2**) were among the first quinolones marketed as chemotherapeutic agents for the treatment of systemic bacterial infections[1–3]. Several new members of the quinolone family have emerged with enhanced activities such as grepafloxacin (**3**), levofloxacin (**4**), rufloxacin (**5**), and compounds **6**–**9** (Figure 1). Much has been learned about how molecular modifications of the core quinolone structure affect the antibacterial profile. The structure–activity relationship of the quinolones has been the subject of extensive reviews[4–16].

In our continuous effort to develop new chemotherapeutics with enhanced activities against resistant bacterial strains[17,18], we introduced a new class of tricyclic compounds, namely thiadiazoloquinolones[19]. In our methodology, we took advantage of many known benzothiadiazoles (Figure 2) with diverse biopharmaceutical activities[20–26] and we hybridized the thiadiazole moiety with the fluoroquinolone structure to introduce the new compound 9-cyclopropyl-4-fluoro-6-oxo-6,9-dihydro [1,2,5]thiadiazolo[3,4-h]quinoline carboxylic acid (**16**). The latter compound was tested against several clinical isolate bacterial strains and did prove to be quite active compared to the drugs used in the market[19].

Several drugs used in the market show a variation at *N*-1, e.g. cyclopropyl, ethyl, 2,4-difluorophenyl and many others. Our strategy for the synthesis of compound **16**, banked on the introduction of the fluorine atom, for its crucial advantage, and also on the replacement of the ethyl with the cyclopropyl moiety, with respect to thiadiazoloquinolones **14** and **15**.

The result of that study was very interesting, the strategy proved its success via enhancement of the activity compared to the previous thiadiazoloquinolones **14** and **15**, and also the potency of compound **16** was comparable to the drug ciprofloxacin.[19]

Herein, we report on the synthesis and the antibacterial activities of new compounds based on the same strategy by fixing the fluorine atom and replacing the cyclopropyl with different moieties, namely ethyl, phenyl, *p*-fluorophenyl and *p*-methoxyphenyl, which are based on the structure of important fluoroquinolone agents.

Materials and methods

All the chemicals and solvents used in this study were purchased from Acros. Melting points were measured by Fischer-Johns Melting Point Apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker DPX-300 instrument. Chemical shifts (δ) are expressed in ppm with reference to TMS as internal standard. High resolution mass spectra (HRMS) were measured in positive ion mode by electrospray ionization (ESI) on a Bruker instrument APEX IV 2008. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and injected using a syringe pump with a flow rate of 2 μL/min. External calibration was conducted using Arginine cluster in a mass range *m*/*z* 175–871. For all HRMS data, mass error: 0.00–0.50.

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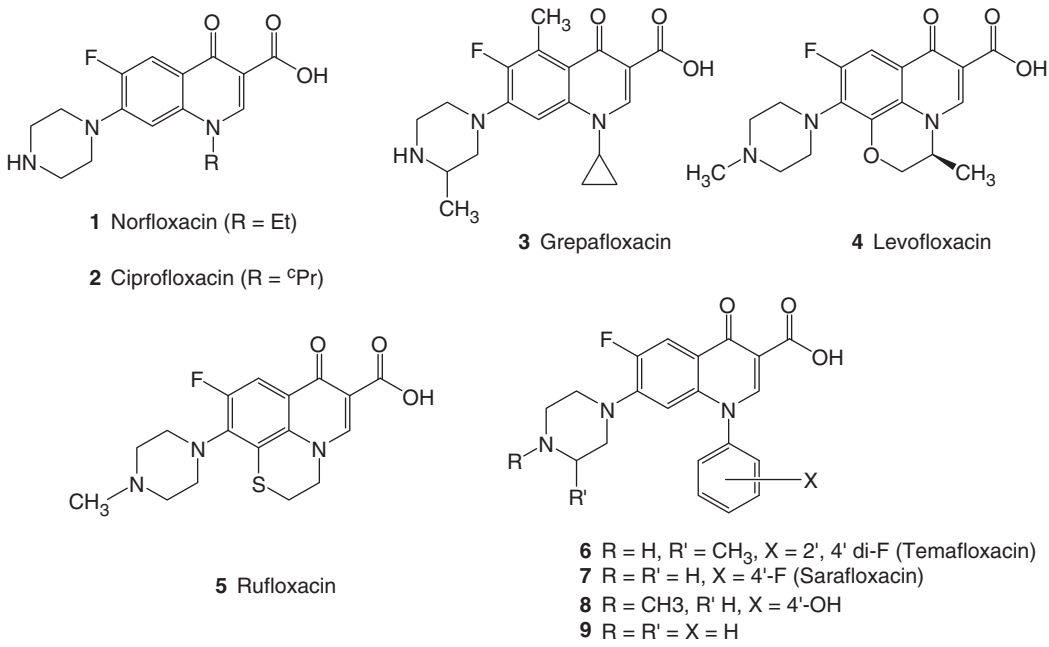
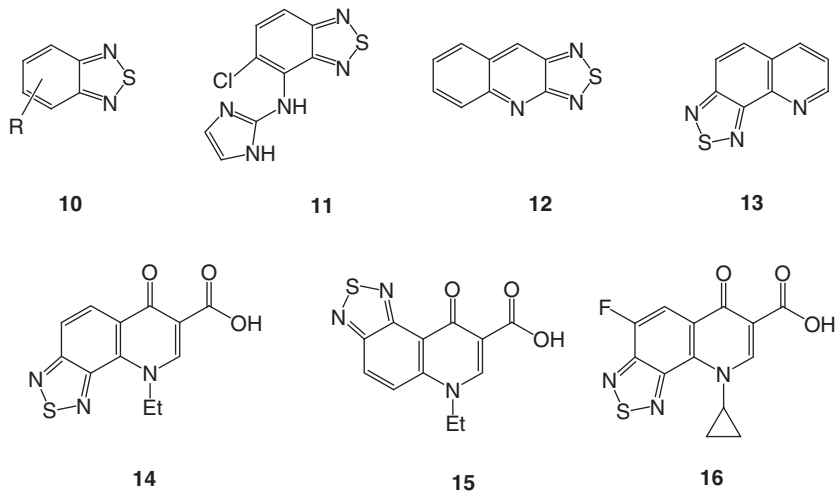


Figure 1. Some fluoroquinolones of which **1–6** are in clinical use.

Figure 2. Some compounds containing thiadiazolo moiety known in the literature.



Chemistry

General procedure for the preparation of compounds

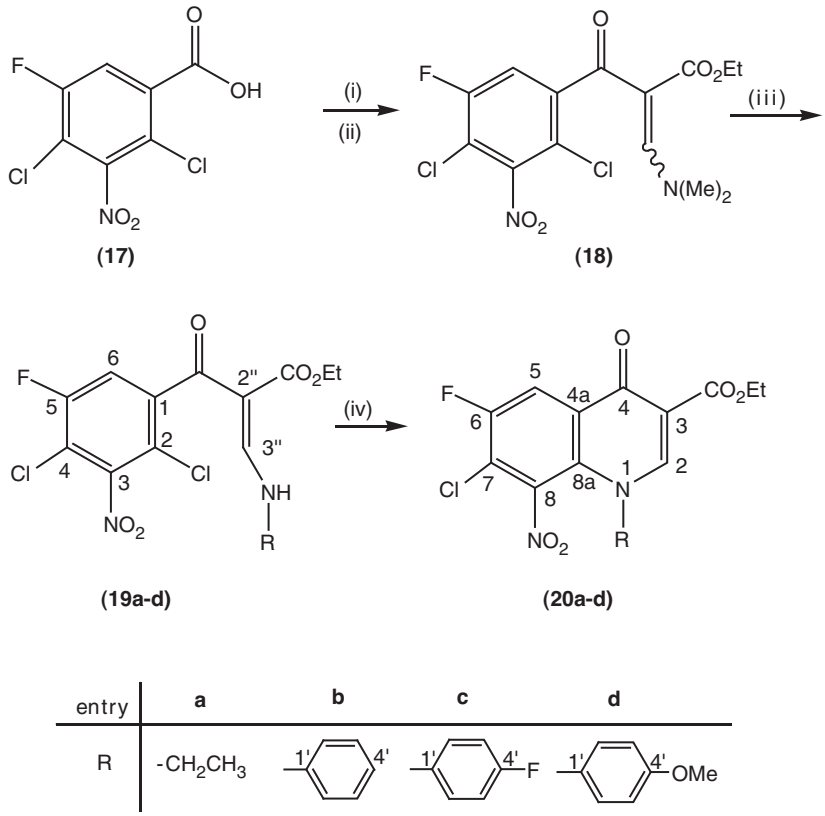
Ethyl 3-(N,N-dimethylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (**18**). It was prepared using reported methods starting from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid **17** (Scheme 1).[19]

Preparation of ethyl 3-N-(aryl/alkyl)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylates (**19a–d**). A stirred solution of (**18**) (~38 mmol) in chloroform (50 mL) and methanol (10 mL), cooled to 8–10 °C, was treated dropwise with the appropriate aryl or alkylamine (1.5 eq) dissolved in MeOH (3 mL). The resulting mixture was further stirred for additional 10–15 min at 5–10 °C. Methanol (90 mL) was then added and the reaction mixture was stirred at rt for 1–2 h. The precipitated white solid product was filtered, washed with cold ethanol (10–20 mL) and air dried. The resultant product was used for the next step without further purification. For identification purpose, a small amount was purified on TLC plates eluting with 2% MeOH/$CHCl_3$ (50:1, v/v).

Ethyl 3-(N-ethylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (**19a**). Yield 95%; m.p. 154 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.01 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.35 (t, $J = 7.1$ Hz, 3H, NCH_2CH_3), 3.52 (q, $J = 7.1$ Hz, 2H, NCH_2CH_3), 3.98 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.11 (d, $^3J_{H-F} = 8.0$ Hz, 1H, H-6), 8.19 (d, $J = 14.4$ Hz, 1H, N–C(3″)-H), 10.99 (br d, $J = 14.4$ Hz, 1H, exchangeable N–H); ^{13}C NMR δ ppm ($CDCl_3$): 13.9 (OCH_2CH_3), 15.6 (NCH_2CH_3), 40.5 (NCH_2CH_3), 60.1 (OCH_2CH_3), 99.8 (C-2″), 114.1 (d, $^2J_{C-F} = 23.3$ Hz, C-4), 115.8 (d, $^2J_{C-F} = 23.3$ Hz, C-6), 117.7 (d, $^3J_{C-F} = 4.4$ Hz, C-1), 144.0 (d, $^3J_{C-F} = 6.2$ Hz, C-3), 148.6 (C-2), 156.7 (d, $^1J_{C-F} = 253.4$ Hz, C-5), 161.2 (*N–C*-3″), 165.9 (CO_2Et), 188.3 (C=O ketone); HRMS (ESI): calcd. for $C_{14}H_{13}Cl_2FN_2O_5Na$ $[M + Na]^+$: 401.00832, found: 401.00778.

Ethyl 3-(N-phenylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (**19b**). Yield 95%; m.p. 150 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.07 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 4.07 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.19 (d, $^3J_{H-F} = 8.0$ Hz, 1H, H-6), 7.24 (d, $J = 7.6$ Hz, 1H, H-4′), 7.28 (dd, $J = 7.8$, 7.6, Hz, 2H, H-3′/H-5′), 7.44 (d, $J = 7.8$ Hz, 2H, H-2′/H-6′), 8.67 (d, $J = 13.8$ Hz, 1H,

Scheme 1. synthesis of ethyl 7-chloro-1-(ethyl/aryl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**20a–d**).



Reagents and conditions:

(i) $SOCl_2$, benzene / reflux;
(ii) $(EtO_2C)HC = CHN(Me)_2$, benzene, NEt_3;
(iii) RNH_2, methanol, chloroform;
(iv) DMF, K_2CO_3 / 60 °C, 1h.

N–C(3″)-H), 12.65 (br d, $J = 13.8$ Hz, 1H, exchangeable N–H); ^{13}C NMR δ ppm ($CDCl_3$): 13.9 (OCH_2CH_3), 60.7 (OCH_2CH_3), 102.2 (C-2″), 114.3 (d, $^2J_{C-F} = 23.3$ Hz, C-4), 115.9 (d, $^2J_{C-F} = 22.7$ Hz, C-6), 118.0 (C-1), 118.5 (C-3′/C-5′), 127.0 (C-4′), 130.1 (C-2′/C-6′), 138.1 (C-1′), 143.5 (C-3), 148.6 (C-2), 154.2 (*N–C*-3″), 156.7 (d, $^1J_{C-F} = 254$ Hz, C-5), 165.6 (CO_2Et), 189.3 (C=O ketone); HRMS (ESI): Calcd for $C_{18}H_{13}Cl_2FN_2O_5Na$ $[M + Na]^+$: 449.00832, found: 449.00778.

Ethyl 3-(N-4-fluorophenylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (**19c**). Yield 91%; m.p. 130 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.05 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 4.05 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.09 (d, $^3J_{H-F} = 8.0$ Hz, 1H, H-6), 7.15 (d, $J = 8$ Hz, 2H, H-2′/C-6′), 7.25 (dd, $J_{H-H} = 8$ Hz, $^3J_{H-F} = 8$ Hz, 2H, H-3′/H-5′), 8.57 (d, $J = 11.9$ Hz, 1H, N–C(3″)-H), 12.66 (br d, $J = 11.9$ Hz, 1H, exchangeable N–H); ^{13}C NMR δ ppm ($CDCl_3$): 13.8 (OCH_2CH_3), 60.6 (OCH_2CH_3), 102.2 (C-2″), 114.3 (d, $^2J_{C-F} = 23.3$ Hz, C-4), 115.9 (d, $^2J_{C-F} = 23.6$ Hz, C-6), 117.0 (d, $^2J_{C-F} = 23.3$ Hz, C-3′/C-5′), 117.8 (C-1), 120.3 (d, $^3J_{C-F} = 8.3$ Hz, C-2′/C-6′), 134.5 (C-1′), 143.4 (C-3), 148.6 (C-2), 154.6 (*N–C*-3″), 156.7 (d, $^1J_{C-F} = 254.1$ Hz, C-5), 161.2 (d, $^1J_{C-F} = 245.8$ Hz, C-4′), 165.5 (CO_2Et), 189.3 (C=O ketone); HRMS (ESI): Calcd for $C_{18}H_{12}Cl_2F_2N_2O_5Na$ $[M + Na]^+$: 466.99890, found: 466.99835.

Ethyl 3-(N-4-methoxyphenylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (**19d**). Yield 97%; m.p. 162 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.05 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 3.81 (s, 3H, OCH_3), 4.04 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 6.93 (d, $J = 8.9$ Hz, 2H, H-3′/H-5′), 7.15 (d, $^3J_{H-F} = 8.0$ Hz, 1H, H-6), 7.21 (d, $J = 8.9$ Hz, 2H, H-2′/H-6′), 8.56 (d, $J = 13.9$ Hz, 1H, N–C(3″)-H), 12.7 (br d, $J = 13.9$ Hz, 1H, exchangeable N–H); ^{13}C NMR δ ppm ($CDCl_3$): 13.9 (OCH_2CH_3), 55.7 (OCH_3), 60.5 (OCH_2CH_3), 101.5 (C-2″), 114.3 (d, $^2J_{C-F} = 23.3$ Hz, C-4), 115.2 (C-3′/C-5′), 115.9 (d, $^2J_{C-F} = 23.4$ Hz, C-6), 117.8 (C-1), 120.0 (C-2′/C-6′), 131.4 (C-1′), 143.6 (d, $^3J_{C-F} = 6.3$ Hz, C-3), 148.6 (C-2), 154.3 (C-3″), 156.7 (d, $^1J_{C-F} = 253.7$ Hz, C-5), 158.6 (C-4′), 165.6 (CO_2Et), 188.8 (C=O Ketone); HRMS (ESI): Calcd for $C_{19}H_{15}Cl_2FN_2O_6^+$ $[M + H]^+$: 457.03694, found: 457.03694.

Preparation of ethyl 7-chloro-1-(aryl/alkyl)-6 fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**20a–d**). A solution of the particular precursor **19a–d** (30 mmol) in DMF (50 mL) and potassium carbonate (90 mmol) was heated at 60 °C. The progress of the cyclization reaction was monitored by TLC (eluent: EtOAc: N–Hexane, 1:1/v:v). The reaction was complete within 80 to 90 min. The reaction mixture was then poured slowly onto crushed ice (100 g) under vigorous stirring, the precipitated pale yellow solid product was collected, washed with water, triturated with cold ethanol, and air dried to produce the pure product.

Ethyl 7-chloro-1-ethyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**20a**).Yield 97%; m.p. 150 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.39 (t, $J = 7.2$ Hz, 3H, OCH_2CH_3), 1.43 (t, $J = 7.2$ Hz, 3H, NCH_2CH_3), 4.04 (q, $J = 7.2$ Hz, 2H, NCH_2CH_3), 4.40 (q, $J = 7.2$ Hz, 2H, OCH_2CH_3), 8.43 (s, 1H, H-2), 8.47 (d, $^3J_{H-F} = 8.34$ Hz, 1H, H-5); ^{13}C NMR δ ppm ($CDCl_3$): 14.7 (OCH_2CH_3), 16.1 (NCH_2CH_3), 50.3 (NCH_2Me), 60.9 (OCH_2Me), 112.1 (C-3), 115.8 (d, $^2J_{C-F} = 22.8$ Hz, C-5), 120.7 (d, $^2J_{C-F} = 23.9$ Hz, C-7), 128.8 (C-4a), 130.9 (d, $^4J_{C-F} = 5.7$ Hz, C-8a), 140.5 (C-8), 152.6 (C-2), 154.0 (d, $^1J_{C-F} = 249$ Hz, C-6), 164.0

(CO_2Et), 170.4 (C-4) (C=O ketone); HRMS (ESI): calcd. for $C_{14}H_{12}ClFN_2O_5$ $[M+H]^+$: 343.04970, found: 343.04915.

Ethyl 7-chloro-6-fluoro-8-nitro-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxylate (**20b**). Yield 97%; m.p. 194 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.36 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 4.36 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 7.33 (d, $J=7.7$ Hz, 2H, H-2′/H-6′), 7.48 (dd, $J=7.7$, 7.3 Hz, 2H, H-3′/H-5′), 7.56 (d, $J=7.3$ Hz, 1H, H-4′), 8.37 (s, 1H, H-2), 8.44 (d, $^3J_{H-F}=8.3$ Hz, 1H, H-5); ^{13}C NMR δ ppm ($CDCl_3$): δ 14.4 (OCH_2CH_3), 61.6 (OCH_2Me), 112.0 (C-3), 115.4 (d, $^2J_{C-F}=22.9$ Hz, C-5), 121.8 (d, $^2J_{C-F}=23.9$ Hz, C-7), 127.6 (C-3′/C-5′), 128.8 (d, $^3J_{C-F}=3$ Hz, C-4a), 129.8 (C-8a), 129.9 (C-2′/C-6′), 131.0 (C-4′), 139.5 (C-1′), 141.5 (C-8), 152.0 (C-2), 154.8 (d, $^1J_{C-F}=253$ Hz, C-6), 164.1 (CO_2Et), 171.0 (C-4) (C=O ketone); HRMS (ESI): Calcd for $C_{18}H_{12}ClFN_2O_5Na$ $[M+Na]^+$: 413.03165, found: 413.03110.

Ethyl 7-chloro-6-fluoro-1-(4-fluorophenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**20c**). Yield 97%; m.p. 226 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.36 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 4.36 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 7.18 (dd, $J=8.9$ Hz, $^2J_{H-F}=7.9$ Hz, 2H, H-3′/H-5′), 7.35(dd, $J_{H-H}=8.9$ Hz, $^3J_{H-F}=4.5$ Hz, 2H, H-2′/H-6′), 8.31 (s, 1H, H-2), 8.43 (d, $^3J_{H-F}=8.2$ Hz, 1H, H-5); ^{13}C NMR δ ppm ($CDCl_3$): 14.4 (OCH_2CH_3), 61.7 (OCH_2Me), 112.2 (C-3), 115.4 (d, $^2J_{C-F}=22.8$ Hz, C-5), 117.1 = (d, $^2J_{C-F}=23.2$ Hz, C-3′/C-5′), 122.0 (d, $^2J_{C-F}=23.7$ Hz, C-7), 128.7 (C-4a), 129.7 (C-8a), 129.8 (d, $^3J_{C-F}=9.2$ Hz, C-2′/C-6′), 135.2 (d, $^3J_{C-F}=3.4$ Hz, C-8), 135.5 (C-1′), 151.9 (C-2), 154.8 (d, $^1J_{C-F}=253$ Hz, C-6), 163.5 (d, $^1J_{C-F}=253$ Hz, C-4′), 164 (CO_2Et), 170.9 (C-4)(C=O); HRMS (ESI): Calcd for $C_{18}H_{11}Cl_2F_2N_2O_5Na$ $[M+Na]^+$: 431.02223, found: 431.02168.

Ethyl 7-chloro-6-fluoro-1-(4-methoxyphenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3- carboxylate (**20d**). Yield 96%; m.p. 176 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.33 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 3.86 (s, 3H, OCH_3), 4.32 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 6.91 (d, $J=8.8$ Hz, 2H, H-2′/H-6′), 7.24 (d, $J=8.8$ Hz, 2H, H-3′/H-5′), 8.31 (s, 1H, H-2), 8.38 (d, $^3J_{H-F}=8.1$ Hz, 1H, H-5).^{13}C NMR δ ppm ($CDCl_3$): 14.4 (OCH_2CH_3), 55.8 (OCH_3), 61.5 (OCH_2CH_3), 111.7 (C-3), 114.7 (C-3′/C-5′), 115.2 (d, $^2J_{C-F}=23$ Hz, C-5), 121.7 (d, $^2J_{C-F}=23.5$ Hz, C-7),128.6 (C-4a), 129.1 (C-2′/C-6′), 129.7 (C-8a), 132.0 (d, $^3J_{C-F}=3.4$ Hz, C-8), 141.2 (C-1′), 152.4 (C-2), 154.6 (d, $^1J_{C-F}=252.8$ Hz, C-6), 161.1 (C-4′), 164.1 (CO_2Et), 171.0 (C-4)(C=O). HRMS (ESI): Calcd for $C_{19}H_{14}ClFN_2O_6Na$ $[M+Na]^+$: 421.06027, found: 421.05972.

Preparation of ethyl 7-azido-1-(aryl/alkyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**21a–d**). A warm (~35 to 40 °C) solution of soduim azide (7.8 g, 120 mmol) in dimethylsulfoxide (DMSO, 50 mL) was added to a solution of the appropriate ethyl 7-chloro-1-aryl(or alkyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoxaline-3-carboxylate (20 mmol) (**20a–d**) in DMSO (50 mL). The resulting mixture acquired yellow turbidity within few minutes, and was further stirred at (~35 to 40 °C) for 16–20 h. Thereafter, the reaction mixture was diluted with cold water (250 mL), and the precipitated solid product was collected under suction, washed with cold water and air dried.

Ethyl 7-azido-1-ethyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**21a**). Yield 90%; m.p. 150 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.39 (m, 6H, NCH_2CH_3, OCH_2CH_3), 3.98 (q, $J=7.2$ Hz, 2H, NCH_2CH_3), 4.38 (q, $J=7.2$ Hz, 2H, OCH_2CH_3), 8.37 (s, 1H, H-2), 8.39 (d, $^3J_{H-F}=11.2$ Hz, 1H, H-5);^{13}C NMR δ ppm ($CDCl_3$): 14.4 (OCH_2CH_3), 16.2 (NCH_2CH_3), 50.5 (NCH_2CH_3), 61.5 (OCH_2CH_3), 112.3 (C-3), 115.9 (d, $^2J_{C-F}=20.9$ Hz, C-5), 128.2 (C-4a), 128.8 (d, $^2J_{C-F}=11.1$ Hz, C-7), 127.7 (d, $^4J_{C-F}=5.7$ Hz, C-8a), 135.2 (C-8), 151.3 (C-2), 154.9 (d, $^1J_{C-F}=250.2$ Hz, C-6), 164.5 (CO_2Et), 171.0 (C-4)(C=O); HRMS (ESI): Calcd for $C_{14}H_{12}FN_5O_5Na$ $[M+Na]^+$: 372.07202, found: 372.07147.

Ethyl 7-azido-6-fluoro-8-nitro-4-oxo-1-phenyl-1,4-dihydroquinoline-3-carboxylate (**21b**). Yield 92%; m.p. 160 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.35 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 4.35 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 7.30 (d, $J=7.6$ Hz, 2H, H-2′/H-6′), 7.45 (dd, $J=7.6$, 7.3 Hz, 2H, H-3′/H-5′),7.55 (d, $J=7.3$ Hz, 1H, H-4′), 8.31 (d, $^3J_{H-F}=9.4$ Hz, 1H, H-5), 8.37 (s, 1H, H-2). ^{13}C NMR δ ppm ($CDCl_3$): 14.4 (OCH_2CH_3), 61.5 (OCH_2CH_3), 112.2 (C-3), 115.4 (d, $^2J_{C-F}=21.2$ Hz, C-5), 118.1 (C-4a), 127.5 = (C-3′/C-5′), 128.9 (C-8a), 129.8 (C-2′/C-6′), 130.5 (C-4′), 132.4 (d, ^2JC–F = 23.9 Hz, C-7), 141.5 (C-8), 139.6 (C-1′), 151.7 (C-2), 153.1 (d, $^1J_{C-F}=251$ Hz, C-6), 164.2 (CO_2Et), 171.0 (C-4)(C=O). HRMS (ESI): calcd. for $C_{18}H_{12}FN_5O_5Na$ $[M+Na]^+$: 420.07202, found: 420.07147.

Ethyl 7-azido-6-fluoro-1-(4-fluorophenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**21c**). Yield 91%; m.p. 172 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.36(t, $J=7.1$ Hz, 3H, OCH_2CH_3), 4.36 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 7.14 (dd, $J=8.9$ Hz, $^4J_{H-F}=2.2$ Hz, 2H, H-2′/H-6′), 7.31 (dd, $J=8.9$ Hz, $^3J_{H-F}=4.5$ Hz, 2H, H-3′/H-5′), 8.34 (d, $^3J_{H-F}=11.2$ Hz, 1H, H-5), 8.38 (s, 1H, H-2); ^{13}C NMR δ ppm ($CDCl_3$): 14.3 (OCH_2CH_3), 61.6 (OCH_2CH_3), 112.2 (C-3), 116.8 (d, $^2J_{C-F}=22.8$ Hz, C-5), 117.1 = (d, $^2J_{C-F}=22.5$ Hz, C-3′/C-5′), 126.5 (C-4a), 129.7 (d, $^3J_{C-F}=7.2$ Hz, C-2′/C-6′), 129.9 (d, $^2J_{C-F}=23.7$ Hz, C-7), 132.6 (C-8a), 135.2 (d, $^3J_{C-F}=3.2$ Hz, C-8), 135.5 (C-1′), 151.7 (C-2), 154.7 (d, $^1J_{C-F}=247$ Hz, C-6), 163.5 (d, $^1J_{C-F}=252.2$ Hz, C-4′), 164 (CO_2Et), 171.0 (C-4)(C=O). HRMS (ESI): Calcd for $C_{18}H_{11}F_2N_5O_5Na$ $[M+Na]^+$: 438.06259, found: 438.06205.

Ethyl 7-azido-6-fluoro-1-(4-methoxyphenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**21d**). Yield 94 %; m.p. 170 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.35 (t, $J=7.1$ Hz, 3H, OCH_2CH_3), 3.86 (s, 3H, OCH_3), 4.34 (q, $J=7.1$ Hz, 2H, OCH_2CH_3), 6.90 (d, $J=8.9$ Hz, 2H, H-2′/H-6′), 7.21 (d, $J=8.9$ Hz, 2H, H-3′/H-5′), 8.28 (d, $^3J_{H-F}=6.5$ Hz, 1H, H-5) 8.38 (s, 1H, H-2); ^{13}C NMR δ ppm ($CDCl_3$): 14.3 (OCH_2CH_3), 55.7 (OCH_3), 61.4 (OCH_2CH_3), 112.0 (C-3), 114.6 (d, $^2J_{C-F}=21.5$ Hz, C-5), 117.9 (C-3′/C-5′), 126.6 (d, $^2J_{C-F}=23.5$ Hz, C-7),128.6 (C-4a), 129.0 (C-2′/C-6′), 129.3 (C-8a), 132.1 (d, $^3J_{C-F}=3.4$ Hz, C-8), 132.3 (C-1′), 152.2 (C-2), 154.6 (d, $^1J_{C-F}=252.8$ Hz, C-6), 161.0 (C-4′), 164.4 (CO_2Et), 171.1 (C-4)(C=O). HRMS (ESI): Calcd for $C_{19}H_{14}FN_5O_6$ $[M+H]^+$: 428.10064, found: 428.10009.

Preparation of ethyl 7,8-diamino-1-(aryl/alkyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**22a–d**). Anhydrous stannous chloride (5 g, 26 mmol) was added portionwise to a stirred ice-cooled (4–8 °C) solution of the appropriate ethyl 7-azido-1-alkyl or 1-aryl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**21a–d**) (Scheme 2) (6.3 mmol) in concentrated hydrochloric acid (36%, 50 mL). The reaction mixture was further stirred at room temperature (24 h), and the reaction progress was monitored by TLC. Upon completion of the reaction, the reaction mixture was diluted with ice-cold water (50 mL), basified with 40% cold aqueous sodium hydroxide solution to pH ~ 13 and set aside for a few minutes. The precipitated solid product was collected by suction filtration and recrystallized from 90% ethanol.

Ethyl 7,8-diamino-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**22a**). Yield 85%; m.p. (dec) 347 °C; ^{1}H NMR δ ppm ($CDCl_3$): 1.08 (t, $J=7.0$ Hz, 3H, OCH_2CH_3), 1.23 (t, $J=7.0$ Hz, 3H, NCH_2CH_3), 4.16 (q, $J=7.0$ Hz, 2H, NCH_2CH_3), 4.63 (q, $J=7.0$ Hz, 2H, OCH_2CH_3), 4.73 (br s, 2H, C(8)–NH_2), 5.53 (br s, 2H, C(7)–NH_2),7.32 (d, $^3J_{H-F}=11.3$ Hz, 1H, H-5), 8.38 (s, 1H, H-2).^{13}C NMR δ ppm ($CDCl_3$):14.8 (OCH_2CH_3), 16.0 (NCH_2CH_3), 50.8 (NCH_2CH_3), 60.0 (OCH_2CH_3), 101.9 (d, $^2J_{C-F}=20.6$ Hz, C-5), 108.7 (C-3), 120.7 (C-4a), 124.7 (d, $^3J_{C-F}=5.1$ Hz, C-8), 127.7 (C-8a), 131.2 (d, $^3J_{C-F}=15.1$ Hz, C-7),

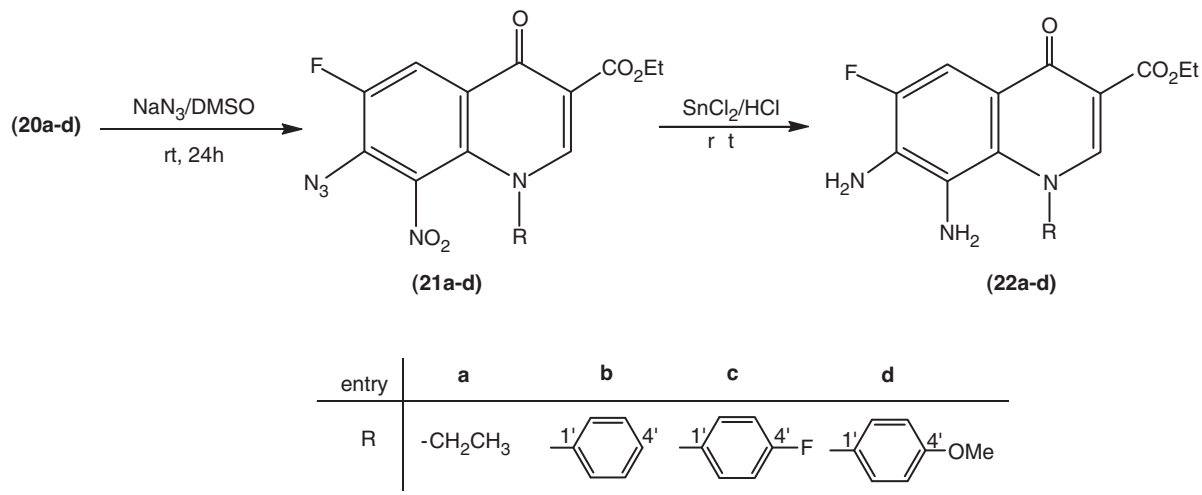
150.0 (d, $^{1}J_{C-F} = 236.6$ Hz, C-6), 151.8 (C-2), 165.4 (CO_2Et), 173.1 (C-4)(C=O); IR (KBr) v: br 3472.33, 3387.38, 1710.58, 1682.94, 1611.55, 1555.40, 1460.96, 1202.94 cm^{-1}; HRMS (ESI): calcd. for $C_{14}H_{16}FN_3O_3$ $[M+H]^+$: 294.12539, found: 294.12485.

Ethyl 7,8-diamino-6-fluoro-1-phenyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**22b**). Yield 80%; m.p. 240 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.19 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 4.14 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 3.87 (br s, 2H, C(8)-NH_2), 5.39 (br s, 2H, C(7)-NH_2),7.32 (dd, $J_{H-H} = 8.6$ Hz, $J_{H-F} = 2.3$ Hz, 2H, H-2′/H-6′), 7.38 (dd, $^{3}J_{H-H} = 8.6$ Hz, $J_{H-F} = 4.3$ Hz, 2H, H-3′/H-5′), 7.56 (d, $^{3}J_{H-F} = 8.3$ Hz, 1H, H-5), 8.15 (s, 1H, H-2); ^{13}C NMR, δ ppm (DMSO-d_6): 14.7 (OCH_2CH_3), 60.3 (OCH_2Me), 112.2 (C-3), 115.4 (d, $^{3}J_{C-F} = 22.8$ Hz, C-5), 117.1 = (d, $^{2}J_{C-F} = 23.2$ Hz, C-3′/C-5′), 122.0 (d, $^{2}J_{C-F} = 23.7$ Hz, C-7),128.6 (C-4a), 129.8 (d, $^{3}J_{C-F} = 7.2$ Hz, C-2′/C-6′), 129.9 (C-8a), 135.2 (d, $^{3}J_{C-F} = 3.4$ Hz, C-8), 135.5 (C-1′), 151.9 (C-2), 154.8 (d, 1JC–F = 253 Hz, C-6), 163.5 (d, $^{1}J_{C-F} = 253$ Hz, C-4′), 164 (CO2Et), 170.9 (C-4); HRMS (ESI): Calcd for $C_{15}H_{17}FN_3O_3^+$ $[M+H]^+$: 306.12540, found: 306.12475.

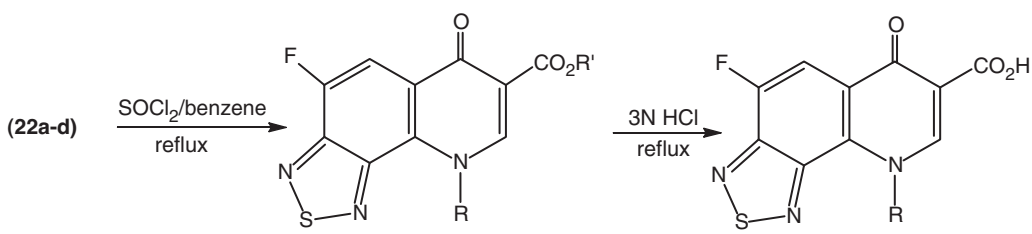
Ethyl 7,8-diamino-6-fluoro-1-(4-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (**22c**). Yield 83%; m.p. 274 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.19 (t, $J = 7.0$ Hz, 3H, OCH_2CH_3), 4.14 (q, $J = 7.0$ Hz, 2H, OCH_2CH_3), 3.87 (br s, 2H, C(8)-NH_2), 5.39 (br s, 2H, C(7)-NH_2),7.32 (dd, $J = 8.6$ Hz, $^{4}J_{H-F} = 2.3$ Hz, 2H, H-2′/H-6′), 7.38 (dd, $J = 8.6$ Hz, $^{3}J_{H-F} = 4.3$ Hz, 2H, H-3′/H-5′), 7.56 (d, $^{3}J_{H-F} = 8.3$ Hz, 1H, H-5), 8.15 (s, 1H, H-2); ^{13}C NMR, δ ppm (DMSO-d_6): 14.7 (OCH_2CH_3), 60.3 (OCH_2CH_3), 101.1 (d, $^{2}J_{C-F} = 21$ Hz, C-5), 109.0 (C-3), 116.8 (d, $^{2}J_{C-F} = 23.0$ Hz, C-3′/C-5′), 120.9 (d, $^{3}J_{C-F} = 8.3$ Hz, C-4a), 124.4 (d, $^{3}J_{C-F} = 6.1$ Hz, C-8), 125.8 (C-8a), 128.6 (d, $^{3}J_{C-F} = 8.9$ Hz, C-2′/C-6′), 129.7 (d, $^{2}J_{C-F} = 16.1$ Hz, C-7), 140.2 (d, $^{4}J_{C-F} = 2.9$ Hz, C-1′), 150.0 (d, $^{1}J_{C-F} = 236$ Hz, C-6), 151.2 (C-2), 162 (d, $^{1}J_{C-F} = 244$ Hz, C-4′), 164.9 (CO_2Et), 172.7 (C-4)(C=O); HRMS (ESI): calcd. for $C_{18}H_{15}F_2N_3O_3Na$ $[M+Na]^+$: 382.09792, found: 382.09737.

Ethyl 7,8-diamino-6-fluoro-1-(4-methoxyphenyl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (**22d**). Yield 89%; m.p. 274 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.20 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 3.75 (br s, 2H, C(8)-NH_2), 3.81 (s, 3H, OCH_3), 4.14 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 5.29 (br s, 2H, C(7)-NH_2), 7.09 (d, $J = 8.9$ Hz, 2H, H-2′/H-6′), 7.32 (d, $^{3}J_{H-F} = 11.3$ Hz, 1H, H-5), 7.48 (d, $J = 8.9$ Hz, 2H, H-3′/H-5′), 8.11 (s, 1H, H-2). ^{13}C NMR, δ ppm (DMSO-d_6): 14.8 (OCH_2CH_3), 56.1 (OCH_3), 60.1 (OCH_2CH_3), 100.9 (d, $^{2}J_{C-F} = 20.9$ Hz, C-5), 108.5 (C-3), 115.4 (C-3′/C-5′), 120.1 (d, $^{3}J_{C-F} = 7.2$ Hz, C-4a), 124.5 (d, $^{3}J_{C-F} = 6.1$ Hz, C-8), 125.7 (C-8a), 127.8 (C-2′/C-6′), 129.3 (d, $^{2}J_{C-F} = 16.2$ Hz, C-7), 136.6 (C-1′), 150.0 (d, $^{1}J_{C-F} = 235.5$ Hz, C-6), 151.2 (C-2), 159.8 (C-4′), 164.9 (CO_2Et), 172.7 (C-4). IR (cm^{-1}, KBr): br 3294.77, 2980.30, 1716.58, 1674.64, 1616.90, 1508.57, 1461.32 cm^{-1}; HRMS (ESI): Calcd for $C_{19}H_{18}FN_3O_4$ $[M+H]^+$: 372.13596, found: 372.13541.

Preparation of ethyl(methyl) 9-(aryl/alkyl)-4 fluoro-6-oxo-6,9-dihydro-[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylates (**23a**,**b**,**d/24b–d**). To a stirred suspension of the particular diamine compound (**22a–d**) (Scheme 3) (3 mmol) in dry benzene (25 mL), was added purified thionylchloride (6 mL) and the resulting mixture was heated at 80–85 °C for 8 h. Benzene and excess



Scheme 2. Synthesis of ethyl 7,8-diamino-1-(aryl/alkyl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylates (**22a–d**).



23a R = Et, R' = Me
23b R = Ph, R' = Me
23d R = 4-OMe-ph, R' = Me
24b R = Ph R' = Et
24c R = 4-F-ph, R' = Et
24d R = 4-OMe-ph, R' = Et

Scheme 3. Synthesis of 9-(aryl/alkyl)-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-*h*]quinoline-7-carboxylic acids **25a–d**.

thionyl chloride were distilled off in vacuum and the residue was cooled, dissolved in $CHCl_3$ and washed with water (2×30 mL). The organic layer was separated, dried (anhydrous $MgSO_4$) and the solvent $CHCl_3$ was then evaporated to dryness under reduced pressure. The residual product was recrystallized from dichloromethane/methanol. In some cases, methanol was used to destroy the excess thionyl chloride within the reaction. Upon using this workup process transesterfication occurs and the resultant product was either methyl ester instead of ethyl ester or a mixture of both esters.

Methyl 9-ethyl-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo [3,4-h]quinoline-7-carboxylate (**23a**). Yield 80%, m.p. 325 °C. ^{1}H NMR, δ ppm (D_2O): 1.42 (t, $J = 6.9$ Hz, 3H, NCH_2CH_3), 3.76 (s, 3H, CO_2CH_3), 5.05 (q, $J = 6.9$ Hz, 2H, NCH_2CH_3), 8.49 (d, $^3J_{H-F} = 10.2$ Hz, 1H, H-5), 8.86 (s, 1H, H-2); ^{13}C NMR, δ ppm (D_2O): 16.5 (NCH_2CH_3), 52.1 (OCH_3), 52.1 (NCH_2CH_3), 109.1 (d, $^2J_{C-F} = 19.7$ Hz, C-5), 114.1 (C-7), 127.5 (d, $^3J_{C-F} = 5.8$ Hz, C-5a), 130.4 (Hz, C-9a), 148.4 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 148.8 (d, $^3J_{C-F} = 1.6$ Hz, C-9b), 149.5 (C-8), 150.0 (d, $^1J_{C-F} = 256$ Hz, C-4), 165.1 (CO_2Me), 171.3 (C-6)(C=O); HRMS (ESI): Calcd for $C_{14}H_{12}FN_3O_3S$ $[M + H]^+$: 308.05052, found: 308.04982.

Methyl 4-fluoro-6-oxo-9-phenyl-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinol-ine-7-carboxylate (**23b**). Yield 40% m.p. 300 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 3.74 (s, 3H, CO_2CH_3), 7.61 (m, 5H, H-2′/H-3′/H-4′/H-5′/H-6′), 8.09 (d, $^3J_{H-F} = 10.8$ Hz, H-5), 8.43 (s, 1H, H-8); ^{13}C NMR, δ ppm (DMSO-d_6): 52.2 (CO_2CH_3), 108.9 (d, $^2J_{C-F} = 18.7$ Hz, C-5), 113.9 (C-7), 127.6 (C-5a), 127.7 (C-3′/C-5′), 130.1 (C-2′/C-6′), 130.2 (C-4′), 130.9 (C-9a), 143.6 (C-1′), 148.5 (C-3a), 148.9 (C-9b), 149.3 (C-8), 150.0 (d, $^1J_{C-F} = 214$ Hz, C-4), 164.7 (CO_2Et), 171.7 (C-6); HRMS (ESI): Calcd for $C_{17}H_{10}FN_3O_3SNa$ $[M + Na]^+$: 378.03246, found: 378.03209.

Methyl 4-fluoro-9-(4-methoxyphenyl)-6-oxo-6,9-dihydro [1,2,5]thiadiazolo [3, 4-h]quinoline-7-carboxylate (**23d**). Yield 40%; m.p. 281 °C; ^{1}H NMR, δ ppm ($CDCl_3$):δ 3.93 (s, 6H, OCH_3, CO_2CH_3), 7.04 (d, $J = 8.9$ Hz, 2H, H-2′/H-6′), 7.29 (d, $J = 8.9$ Hz, 2H, H-3′/H-5′), 8.32 (d, $^3J_{H-F} = 10.3$ Hz, 1H, H-5), 8.52 (s, 1H, H-8); ^{13}C NMR, δ ppm ($CDCl_3$): 52.5 (CO_2CH_3), 55.8 (OCH_3), 109.7 (d, $^2J_{C-F} = 19.6$ Hz, C-5), 113.8 (C-7), 114.9 (C-3′/C-5′), 128.2 (C-2′/C-6′), 128.7 (d, $^3J_{C-F} = 5.7$ Hz, C-5a), 130.5 (C-9a), 135.9 (C-1′), 148.5 (C-3a), 148.8 (C-9b), 149.6 (C-8), 150.5 (d, $^1J_{C-F} = 261$ Hz, C-4), 160.5 (C-4′), 165.5 (CO_2Et), 172.3 (C-6); HRMS (ESI): Calcd for $C_{18}H_{12}FN_3O_4SNa$ $[M + Na]^+$: 408.04302, found: 408.04225.

Ethyl 4-fluoro-6-oxo-9-phenyl-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylate (**24b**). Yield 40%, m.p. 285 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.25 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 4.22 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.60 (m, 5H, H-2′/H-3′/H-4′/H-5′/H-6′), 8.11 (d, $^3J_{H-F} = 10.8$ Hz, H-5), 8.41 (s, 1H, H-2); ^{13}C NMR, δ ppm (DMSO-d_6): 14.7 (OCH_2CH_3), 60.9 (OCH_2CH_3), 108.9 (d, $^2J_{C-F} = 19.2$ Hz, C-5), 114.4 (C-7), 127.8 (C-3′/C-5′),128.7 (d, $^3J_{C-F} = 5.8$ Hz, C-5a), 130.1 (C-2′/C-6′), 130.2 (C-4′), 130.9 (d, $^4J_{C-F} = 3$ Hz, C-9a), 139.0 (C-1′), 148.4 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 148.7 (d, $^3J_{C-F} = 1.6$ Hz, C-9b), 148.9 (C-8), 150.5 (d, $^1J_{C-F} = 214$ Hz, C-4), 164.2 (CO_2Et), 171.7 (C-6)(C=O); HRMS (ESI): Calcd for $C_{18}H_{12}FN_3O_3SNa$ $[M + Na]^+$: 392.04811, found: 392.04934.

Ethyl 4-fluoro-9-(4-fluorophenyl)-6-oxo-6,9-dihydro[1,2,5] thiadiazolo[3,4-h]quinoline-7-carboxylate (**24c**). Yield 79%, m.p. 274 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.38 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 4.38 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.26 (d, $J = 8.3$ Hz, 2H, H-2′/H-6′), 7.41 (dd, $J = 8.3$ Hz, $^4J_{H-F} = 4.6$ Hz, 2H, H-3′/H-5′), 8.28 (d, $^3J_{H-F} = 10.2$ Hz, 1H, H-5), 8.46 (s, 1H, H-2); ^{13}C NMR, δ ppm (DMSO-d_6): 14.4 (OCH_2CH_3), 61.5 (OCH_2Me), 109.6 (d, $^2J_{C-F} = 19.6$ Hz, C-5), 114.3 (C-7), 117.0 (d, $^2J_{C-F} = 23.0$ Hz, C-3′/C-5′),128.7 (d, $^3J_{C-F} = 5.8$; Hz, C-5a), 129.2 (d, $^3J_{C-F} = 9.0$ Hz, C-2′/C-6′), 130.1 (d, $^4J_{C-F} = 3$ Hz, C-9a), 139.0 (C-1′), 148.4 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 148.7 (d, $^3J_{C-F} = 1.6$ Hz, C-9b), 148.9 (C-8), 150.5 (d, $^1J_{C-F} = 261$ Hz, C-4), 163.3 (d, $^1J_{C-F} = 249$ Hz, C-4′), 164.7 (CO_2Et), 172.3 (C-6); HRMS (ESI): Calcd for $C_{18}H_{11}F_2N_3O_3SNa$ $[M + Na]^+$: 410.03869, found: 410.04116.

Ethyl 4-fluoro-9-(4-methoxyphenyl)-6-oxo-6,9-dihydro[1,2,5] thiadiazolo[3,4-h]quinoline-7-carboxylate (**24d**). Yield 40%; m.p. 237 °C; ^{1}H NMR, δ ppm (DMSO-d_6): 1.24 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 3.85 (s, 3H, OCH_3), 4.20 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.09 (d, $J = 8.8$ Hz, 2H, H-2′/H-6′), 7.54 (d, $J = 8.8$ Hz, 2H, H-3′/H-5′), 8.04 (d, $^3J_{H-F} = 10.8$ Hz, 1H, H-5), 8.37 (s, 1H, H-2); ^{13}C NMR, δ ppm (DMSO-d_6): 14.7 (OCH_2CH_3), 56.1 (OCH_3), 61.5 (OCH_2CH_3), 108.9 (d, $^2J_{C-F} = 19.1$ Hz, C-5), 114.1 (C-7), 115.1 (C-3′/C-5′),127.5 (d, $^3J_{C-F} = 5.4$ Hz, C-5a), 129.0 (C-2′/C-6′), 131.3 (C-9a), 136.6 (C-1′), 148.3 (C-3a), 148.9 (C-9b), 149.4 (C-8), 150.1 (d, $^1J_{C-F} = 249$ Hz, C-4), 160.4 (C-4′), 164.2 (CO_2Et), 171.7 (C-6); HRMS (ESI): Calcd for $C_{19}H_{14}FN_3O_4SNa$ $[M + Na]^+$: 422.05867, found: 422.05809.

Preparation of 9-(aryl/alkyl)-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylic acids (**25a–d**). A vigorously stirred suspension of the ester (**23a**,**b**,**d**/**24b–d**). (1.5 mmol) in 6 N HCl (15 mL) and ethanol (6 mL) was heated at 80–85 °C under reflux conditions. Progress of the ester hydrolysis was monitored by TLC and was completed within 20–24 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice (30 g) and the resulting heavy pale yellow precipitate was collected, washed with cold water, dried and recrystallized from *N,N*-dimethylformamide (DMF) to produce a pure compound.

9-Ethyl-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylic acid (**25a**). Yield 95%; m.p. 315 °C; ^{1}H NMR δ ppm (D_2O): 1.48 (t, J = 7.0 Hz, 3H, NCH_2CH_3), 5.26 (q, J = 7.0 Hz, 2H, NCH_2CH_3), 8.23 (d, $^3J_{H-F} = 10.4$ Hz, 1H, H-5), 8.68 (s, 1H, H-8), 15.14 (br s, 1H, CO_2H); ^{13}C NMR δ ppm (D_2O): 16.4 (NCH_2CH_3), 53.5 (NCH_2CH_3), 108.1 (d, $^2J_{C-F} = 19.6$ Hz, C-5), 111.9 (C-7), 125.7 (d, $^3J_{C-F} = 6.5$ Hz, C-5a), 132.2 (C-9a), 148.7 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 149.1 (d, $^3J_{C-F} = 3$ Hz, C-9b), 149.7 (C-8), 154.4 (d, $^1J_{C-F} = 259$ Hz, C-4), 165.7 (CO_2H), 176.3 (C-6); HRMS (ESI): Calcd for $C_{12}H_8FN_3O_3S$ $[M - H]^-$: 292.01921, found: 292.01976.

4-Fluoro-6-oxo-9-phenyl-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylic acid (**25b**). Yield 90%; m.p. 301 °C; ^{1}H NMR δ ppm (D_2O): 7.64 (m, 5H, H-1′/H-2′/H-3′/H-4′/H-5′), 8.23 (d, $^3J_{H-F} = 10.7$ Hz, 1H, H-5), 8.68 (s, 1H, H-8), 14.91 (br s,1H, CO_2H); ^{13}C NMR δ ppm (D_2O): 107.9 (d, $^2J_{C-F} = 19.0$ Hz, C-5), 111.4 (C-7), 125.7 (Hz, C-5a), 127.5 (C-3′/C-5′), 130.2 (C-2′/C-6′), 130.7 (C-4′), 131.2 (C-9a), 143.4 (C-1′), 148.6 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 149.0 (C-9b), 149.7 (C-8), 154.3 (d, $^1J_{C-F} = 259$ Hz, C-4), 165.5 (CO_2H), 176.8 (C-6); HRMS (ESI): Calcd for $C_{16}H_8FN_3O_3S$ $[M - H]^-$: 340.01921, found: 340.01976.

4-Fluoro-9-(4-fluorophenyl)-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylic acid (**25c**). Yield 93%; m.p. 278 °C; ^{1}H NMR δ ppm (DMSO-d_6); 7.44 (d, $J = 8.4$ Hz, 2H, H-2′/H-6′), 7.72 (dd, $J = 8.4$ Hz, $^3J_{H-F} = 4.4$ Hz, 2H, H-3′/H-5′), 8.19 (d, $^3J_{H-F} = 10.4$ Hz, 1H, H-5), 8.71 (s, 1H, H-8), 14.9 (br s,1H, CO_2H); ^{13}C NMR δ ppm (DMSO-d_6); 107.9 (d, $^2J_{C-F} = 19.3$ Hz, C-5), 114.3 (C-7), 117.0 (d, $^2J_{C-F} = 23.0$ Hz, C-3′/C-5′),125.7 (d, $^3J_{C-F} = 6.5$ Hz, C-5a), 130.1 (d, $^3J_{C-F} = 9.0$ Hz, C-2′/C-6′), 132.2 (C-9a), 139.6 (C-1′), 148.6 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 149.0 (d, $^3J_{C-F} = 3$ Hz, C-9b), 149.7 (C-8), 151.3 (d, $^1J_{C-F} = 259$ Hz, C-4), 163.1 (d, $^1J_{C-F} = 245$ Hz, C-4′), 165.6 (CO_2H), 176.6 (C-6); HRMS (ESI): Calcd for $C_{16}H_7F_2N_3O_3SNa$ $[M + Na]^+$: 382.00739, found: 382.00684.

4-Fluoro-9-(4-methoxyphenyl)-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-h]quinoline-7-carboxylic acid (**25d**). Yield 92%; m.p. 300 °C; ^{1}H NMR δ ppm (DMSO-d_6); 3.86 (OCH_3), 7.12 (d, $J = 8.9$ Hz, 2H, H-2′/H-6′), 7.57 (d, $J = 8.9$ Hz, H-3′/H-5′), 8.20 (d, $^3J_{H-F} = 10.4$ Hz, 1H, H-5), 8.64 (s, 1H, H-8), 14.86 (br s,1H, CO_2H); ^{13}C NMR δ ppm (DMSO-d_6); 56.1 (OCH_3), 107.9 (d, $^2J_{C-F} = 19.5$ Hz, C-5), 111.4 (C-7), 115.2 (C-3′/C-5′), 125.7 (d, $^3J_{C-F} = 6.5$ Hz, C-5a), 128.8 (C-2′/C-6′), 132.2 (C-9a), 136.4 (C-1′), 148.6 (d, $^2J_{C-F} = 10.5$ Hz, C-3a), 149.0 (d, $^3J_{C-F} = 3$ Hz, C-9b), 150.0 (C-8), 150.9 (d, $^1J_{C-F} = 259$ Hz, C-4), 160.7 (C-4′), 165.5 (CO_2H), 176.8 (C-6).; IR (KBr) v: 3061.36, 1732.53, 1611.62, 1504.53, 1474.49, 1253.78 cm^{-1}; HRMS (ESI): Calcd for $C_{17}H_{10}FN_3O_4S$ [M − H]$^-$: 370.02978, found: 370.03033.

Antimicrobial activity

The biological screening of compounds **19–22a–d**, **23a**, **24b–d** and **25a–d** was performed *in vitro* by the two fold broth dilution method[27]. The antibacterial activity was tested against *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538 Gram positive strains, *Escherichia coli* ATCC 8739 and *Haemophilus influenzae* ATCC 19418 Gram negative ones using Mueller Hinton medium. The antifungal properties were evaluated against *Candida tropicalis* ATCC 1369 and *Saccharomyces cerevisiae* ATCC 9763 yeasts and *Aspergillus niger* ATCC 6275 mould in Sabouraud dextrose broth. The compounds at the concentration range of 0.0015–400 μg/mL in 1% DMSO were used in this study, with ciprofloxacin as reference antibacterial substance and miconazole as reference antifungal drug. DMSO was loaded as negative control. Suspensions of the microorganisms were prepared to inoculate 5×10^5 bacteria/mL and 1×10^3 fungi/mL. The minimum inhibitory concentration (MIC, μg/mL) value was taken as the lowest concentration of compound that showed inhibition of microbial growth after 24 h of incubation at 37 °C for bacteria and after 48 h of incubation at 30 °C for fungi. Each experiment has been done in triplicate and repeated at least three times.

Results and discussion

Chemistry

Our synthesis banks on the reported strategy used by us and others in building the quinolone skeleton. Scheme 1 shows the synthesis of our *N*-aryl/alkyl substituted quinolones, in which the benzoic acid derivative **17** was converted to the benzoyl chloride via interaction with thionyl chloride under reflux conditions. The resulting benzoyl chloride was reacted with ethyl 3-(*N*,*N*-dimethylamine)acrylate in benzene to produce the intermediate **18** which was reacted with the desired amino compound to get the respective **19a–d** as the predominant Z isomers.

The structures of compounds **19a–d** are supported by spectral (NMR and MS) data. The various ^{1}H and ^{13}C NMR signals were assigned based on DEPT and 2D NMR (HMQC) experiments. The ^{1}H NMR spectra show the methylene protons (O–CH_2CH$_3$) as quartet in the range δ 3.98–4.07 ppm, while the methyl protons (O–CH$_2$CH_3) resonate as triplet in the range δ 1.01–1.07 ppm. The H-3″ proton resonates as a doublet signal in the range δ 8.19–8.67 ppm (with $J = 11$–14 Hz) indicating that the products are the Z-isomers. The H-6 proton of this series is well characterized as a sharp doublet in the range δ 7.09–7.19 ppm, (with $^3J_{H-F} = 8.0$–8.2 Hz) due to spin–spin coupling interaction with the vicinal fluorine atom.

The ^{13}C NMR spectra of compounds **19a–d** reveal clearly the presence of the fluorine atom and its effect on the adjacent carbons; thus the following carbons showed through-bonds coupling with the fluorine atom and appear as doublets (C-5, $^1J = 253$–254 Hz), (C-6, $^2J = 22.5$–22.7 Hz). The ^{13}C NMR spectra also indicate the presence of the keto group at 189.3–189.8 ppm, while the carbonyl carbon of the ester group resonates in the range 164.1–165.9 ppm. The C-3″ carbon resonates in the range 154.2–161.2 ppm, while C-2″ in the range 99.8–102.2 ppm.

The quinolone ring in **20a–d** was constructed through intramolecular cyclization of (**19a–d**) in which the chlorine atom at position 2 was replaced by the amino group *via* nucleophilic aromatic substitution (S_NAr) assisted by the neighboring nitro group and fluorine atom[28,29].

In this group of compounds, ^{1}H NMR showed the disappearance of N–H broad peak at 11–12.7 ppm present in their acyclic precursors **19a–d**; the spectrum showed a change in the doublet peak of CH to a singlet one. Both H-5 and H-2 in **20a–d** are more deshielded than the corresponding protons in the **19a–d** precursors. These changes constitute diagnostic criteria for ring closure (**19a–d** → **20a–d**).

The ^{13}C NMR spectra of compounds **20a–d** reveal that most of the carbons are deshielded except for C-2, C-4 and C-8a.

In the ^{13}C NMR spectrum, the carbon of the keto group [=O on C-4] for **20a–d** resonates in the range 170.4–170.9 ppm, which is more shielded than the corresponding C-4 in compounds **19a–d** (188–189 ppm); similarly, C-2 resonates around 152 ppm and C-8a resonates around 130 ppm and are more shielded than the corresponding carbons in **19a–d** (154 ppm) and (148 ppm), respectively.

Having the essential quinolone compounds in hand, we could easily convert them to the diamino compounds **22a–d** as shown in Scheme 2, in which the chlorine atom was replaced with the azide moiety via the standard nucleophilic aromatic substitution. The structure of compounds **21a–d** is supported by spectral (NMR and MS) data which are in agreement with the literature data. In the ^{1}H NMR spectra, there is not any difference in the chemical shift compared to the compounds **20a–d**. In the ^{13}C NMR spectra there is a small difference for C-7, which appears downfield in compounds **21a–d** (∼128 ppm) compared to the corresponding C-7 in **20a–d** due to the replacement of –Cl by the azide group.

Reduction of the particular ethyl 7-azido-1-(alkyl/aryl)-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**21a–d**) with anhydrous stannous chloride in concentrated hydrochloric acid afforded the respective 7,8-diamino compounds (**22a–d**) in good yield (78–85%). The effective reduction of **21a–d** to afford **22a–d** was deduced from the presence of NH band at 3462–3295 cm^{-1} in the IR spectra. The IR spectra of **22a–d** also show absorption bands (1672, 1632 cm^{-1}), which indicate the presence of a conjugated keto group, while the carboxy group absorbs at 1718 cm^{-1}. The structure of **22a–d** is further supported by NMR spectral data. The ^{1}H NMR spectra of **22a–d** indicate the appearance of the amine protons signals at 5.05 (NH_2 on C-8) and 5.43 (NH_2 on C-7), while H-5 appears as a doublet at 7.19 ppm, and is thus shielded compared to its position in **21a–d**. The ^{13}C NMR spectra show the C-8 and C-5 resonating around 125.7 and 100.6 ppm, respectively, which are shielded compared to their positions in **21a–d** (133.1 and 115.8 ppm, respectively).

Cyclocondensation of the synthesized diamine **22a–d** using thionyl chloride produces the targeted bicyclic benzo[*c*][1,2,5]thiadiazoles system **23/24** (Scheme 3). The driving force of formation is due to: (i) strong electrophilicity of thionyl chloride, and (ii) stability of the tricyclic product due to aromaticity. It is worth mentioning that upon the workup procedure, where methanol was used to destroy the excess thionyl chloride, trans-esterification of the ethyl ester produced completely the methyl ester **23a**, while a 1:1 ratio of methyl and ethyl esters were isolated for the product **23b/24b** and **23d/24d**. Compound **24c** was isolated as the ethyl ester only.

The following changes constitute diagnostic criteria for ring closure: the ^{1}H NMR spectra of compounds (**23/24**) indicate the disappearance of the amine protons signals at 3.75–4.80 (NH_2 on C-8) and 5.29–5.61 (NH_2 on C-7), while H-5 appears as a doublet at 8.09–8.32 ppm, and is thus deshielded compared to its position in (**22a–d**). The ^{13}C-NMR spectra show the C-3a and C-9b around 148.4 and 148.9 ppm, respectively, which are deshielded compared to their positions in **22a–d**.

The hydrolysis of ethyl(methyl) 9-(aryl/alkyl)-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo- [3,4-*h*]quinoline-7-carboxylate (**23/24a–d**) gave the corresponding acid products (**25a–d**) in ~90 % yield by using 12% aqueous HCl under reflux conditions.

The structures of **25a–d** are supported by spectral (^{1}H NMR and MS) data. The ^{1}H NMR spectra show the disappearance of ethyl or the methyl protons signals (in previous esters **23/24b–d**) from the spectra and the appearance of a broad signal at 14.9 (exchangeable CO_2H proton). ^{13}C-NMR spectra show the disappearance of ethyl or the methyl carbons signals (in previous esters **23/24b–d**) from the spectra.

Antimicrobial activity

The *in vitro* antimicrobial properties of the acids **25a–d**, their esters **23a** and **24b–d** and of the starting compounds **19/22a–d** were detected against Gram positive and Gram negative bacteria, yeasts and mould.

Table 1 summarizes the results obtained when compounds **23a**, **24b–d** and **25a–d** were assayed against *B. subtilis* and *S. aureus* Gram positive bacteria and *E. coli* and *H. influenzae* Gram negative ones. The antibacterial activity of desfluorinated *N*-ethylquinolone acid **14** and of the 6-fluoro-*N*–Cyclopropyl analogous **16** together with its ethyl ester is also reported for an useful comparison.

The thiadiazoloquinolone acids **25a–d** are the most active compounds. *N*-ethyl derivative **25a** and *N*-4-fluorophenyl derivative **25c** had strong inhibitory effect on the growth of both Gram positive and Gram negative tested strains (MIC 0.15–3 μg/mL). Significant inhibition was also exhibited by phenyl derivative **25b** with MIC values in the 1.5–50 μg/mL range. A lower activity was observed for 4-methoxyphenyl derivative **25d** against *B. subtilis* and *S. aureus* (MIC 100–200 μg/mL). Gram positive *B. subtilis* was the most sensitive microorganism. Among thiadiazoloquinolone esters, *N*-ethyl derivative **23a** and *N*-4-fluorophenyl derivative **24c** showed good antimicrobial properties against *B. subtilis* at 12 μg/mL, whereas phenyl derivative **24b** had some activity against the same strain at the concentration of 100 μg/mL. Compound **24c** was the only ester that inhibited the growth of *H. influenzae* and *S. aureus* (MIC 200–400 μg/mL, respectively).

All the newly synthesized compounds exhibited antibacterial activity lower than that of ciprofloxacin used as reference substance. Furthermore, no inhibition was exerted against *S. cerevisiae*, *C. tropicalis* and *A. niger* fungal strains up to the concentration of 400 μg/mL (data not shown; MICs of miconazole 12 μg/mL, 6 μg/mL and 3 μg/mL, respectively).

Structure–activity relationship shows that, among the newly synthesized thiadiazoloquinolone acids and among their corresponding esters, the *N*-4-fluorophenyl derivatives **24c** and **25c** exhibited the highest activity, followed, in the order, by *N*-ethyl- (**23a** and **25a**), *N*-phenyl- (**24–25b**) and *N*-4-methoxyphenyl- (**24–25d**) quinolones. However, these new thiadiazoloquinolones exhibited MIC values higher than those of the *N*–Cyclopropyl acid **16** and its ethyl ester, confirming the current knowledge that the replacement of the cyclopropyl at the *N*-1 position of the quinolone ring with ethyl or substituted and unsubstituted phenyl moiety plays a negative role on the antibacterial properties. Likewise, the introduction of fluorine at position C-6, in compound **25a**, enhanced, by a factor four or two, the activity of **14** against *S. aureus* and *E. coli*, respectively.

As concerns the intermediates **19/22a–d**, only *B. subtilis*, the most sensitive microorganism, was inhibited by **22c** at 100 μg/mL and by 7-chloro-8-nitroquinolines **20a** and **20c** at 200 μg/mL. A low antibacterial activity appeared when compounds **19** cyclize to quinolone structure **20**. This inhibition disappeared in the derivatives **21**, probably owing to the loss of chlorine substituent. Replacing the azido fragment with the amino group for substances **22** led to an increase in antibacterial properties of 4-fluorophenyl derivative **22c** against *B. subtilis*. The subsequent cyclization of the two amino groups at 7 and 8 positions on the fused thiadiazole ring (compounds **23a** and **24b-d**) enhanced the activity. Compound **24c** showed a MIC value of 12 μg/mL towards *B. subtilis* and broadened the range of its inhibition against *S. aureus* and *H. influenzae*. This change in the structure resulted also in increased activity of **23a** and **24b** towards *B. subtilis* (MIC 12 and 100 μg/mL, respectively).

As expected, the sizeable increase of activity was observed in the target compounds **25a–d**, whose activity is discussed above, by replacing the C-3 ester group with the carboxylate that is considered critical and not yet improved by any change.

Table 1. *In vitro* antibacterial activity of thiadiazoloquinolones (minimum inhibitory concentration in μg/mL).

	Microorganism			
Compound	*Bacillus subtilis* ATCC 6633	*Staphylococcus aureus* ATCC 6538	*Escherichia coli* ATCC 8739	*Haemophilus influenzae* ATCC 19418
23a	12	>400	>400	>400
24b	100	>400	>400	>400
24c	12	400	>400	200
24d	>400	>400	>400	>400
25a	0.15	1.5	3	1.5
25b	1.5	12	50	6
25c	0.15	1.5	3	0.7
25d	100	200	>400	>400
14*	nt†	6	6	nt
16 ethyl ester*	3	50	100	25
16*	0.07	0.7	0.7	0.3
Ciprofloxacin	0.03	0.3	0.015	0.15

*Results reported in previous article.[19]
†Not tested.

Conclusion

The aim of this study is to investigate whether the thiadiazolo fused ring, as a bridge between the critical 7 and 8 positions of the fluoroquinolone scaffold, offers new insight into the structural–activity relationship of the class of quinolones antibacterials. In this study, 26 new compounds were successfully synthesized and fully characterized. The synthesized thiadiazolofluoroquinolones show broad spectrum of activity. The fluoroquinolone **25c** was the most active compound in this study. The activities of this class of compounds were expected as a consequence of the modifications at the positions 7 and 8 of the fluoroquinolone ring, and are in agreement with the analogous previous generations of fluoroquinolones. This modification is expected to increase the intracellular targets on the type II topoisomerase and to impair the action of efflux pump, thus lowering the development of quinolone resistance in Gram positive pathogens. Extensive mechanism of action for this class of compounds will be investigated.

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Declaration of interest

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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