Synthesis and antibacterial activity of 9-cyclopropyl-4-fluoro-6-oxo-6,9-dihydro-[1,2,5]thiadiazolo[3,4-*h*]quinoline-7-carboxylic acid and its ethyl ester

Raed A. Al-Qawasmeh,[a] Jalal A. Zahra,[a] Franca Zani,[b] Paola Vicini,[b,*] Roland Boese,[c] and Mustafa M. El-Abadelah[a,*]

[a] *Chemistry Department, Faculty of Science, The University of Jordan, Amman 11942, Jordan*
[b] *Dipartimento Farmaceutico, Università degli Studi di Parma, Viale G. P. Usberti 27/A, Parma 43100, Italy*
[c] *Institut für Anorganische Chemie, Universität Duisburg-Essen, Campus Essen, Universität Strasse 3-5, D-45117 Essen, Germany*

E-mails: paola.vicini@unipr.it, mustelab@ju.edu.jo

Abstract

We report on the synthesis and the antimicrobial activity of 9-cyclopropyl-4-fluoro-6-oxo[1,2,5]thiadiazolo[3,4-*h*]quinoline-5-carboxylic acid (**16**), representative of a new class of antibacterial agents structurally related to the clinically successful fluoroquinolones. The novel 6-fluoroquinolone (**16**) is herein prepared, in good yield, by thermal cyclocondensation of ethyl 7,8-diamino-9-cyclopropyl-4-fluoro-6-oxoquinoxaline-7-carboxylate with thionyl chloride, followed by acid-catalysed hydrolysis of the ester intermediate (**15**). Their structures were characterized by IR, MS, ^{1}H- and ^{13}C NMR spectra and X-ray crystal structure of **16** is presented. The antimicrobial evaluation against a broad panel of wild and resistant Gram-positive and Gram-negative bacteria and fungal species demonstrated the high antibacterial activity of **16**, even at the concentration of 0.015 μg mL^{-1}.

Keywords: 6-Fluoroquinolone, synthesis, 4-fluorothiadiazolo[3,4-*h*]quinolone, antibacterial activity, X-ray crystal data

Introduction

Resistance to currently available antibacterial drugs is bringing alarming threat to public health and causing growing concern among people across the globe. At the same time as the old antibiotics are losing their effectiveness, the supply of new drugs is drying up. Our best weapon

against this threat is to continually develop new antibiotics and new synthetic antibacterials against which bacteria have not yet developed resistance.

Several substituted benzo[*c*][1,2,5]thiadiazoles **1** (Figure 1) were reported to exhibit diverse bio-pharmacological properties such as insecticidal and acaricidal,[1] fungicidal and nematocidal,[2-4] antimicrobial[5] or antiviral[6] activities. Quite recently, some derivatives of **1** have been shown to be active as ubiquitin ligase inhibitors.[7] In this respect, it is worth mentioning that benzo[*c*][1,2,5]thiadiazole ring system constitutes the skeleton of Tizanidine **2**, a muscle relaxant used as antispastic agent for treatment of central nervous system disorders,[8] and for spasticity in multiple sclerosis, stroke, and spinal cord injury.[9-11] On the other hand, fluoroquinolone-based drugs (e.g. ciprofloxacin[12-14]) represent some of the most effective antiinfectious drugs currently in clinical use.[15-21] Various types of synthetic thiadiazoloquinolines, e.g. **3**[22] and **4**,[23-26] have been reported and recently, synthetic tricyclic system **5,** having the thiadiazole moiety [*h*]-fused to 4-oxoquinoline-3-carboxylic acid, has been shown to be active against several Gram-negative and Gram-positive bacterial strains.[27,28] Compound **5** was prepared *via* the traditional Gould-Jacobs reaction[29-31] by condensation of 4-aminobenzo[*c*][1,2,5]thiadiazole with diethyl ethoxymethylene malonate, followed by thermal cyclization of the resulting enamino ester, subsequent *N*(1)-ethylation and hydrolysis of the ester group.[27,28] The isomeric [1,2,5]thiadiazolo[3,4-*f*]quinolone-8-carboxylic acid (**6**) was similarly prepared, utilizing 5-aminobenzo[*c*][1,2,5]thiadiazole and diethyl ethoxymethylenemalonate, and was patented as antibacterial agent.[32]

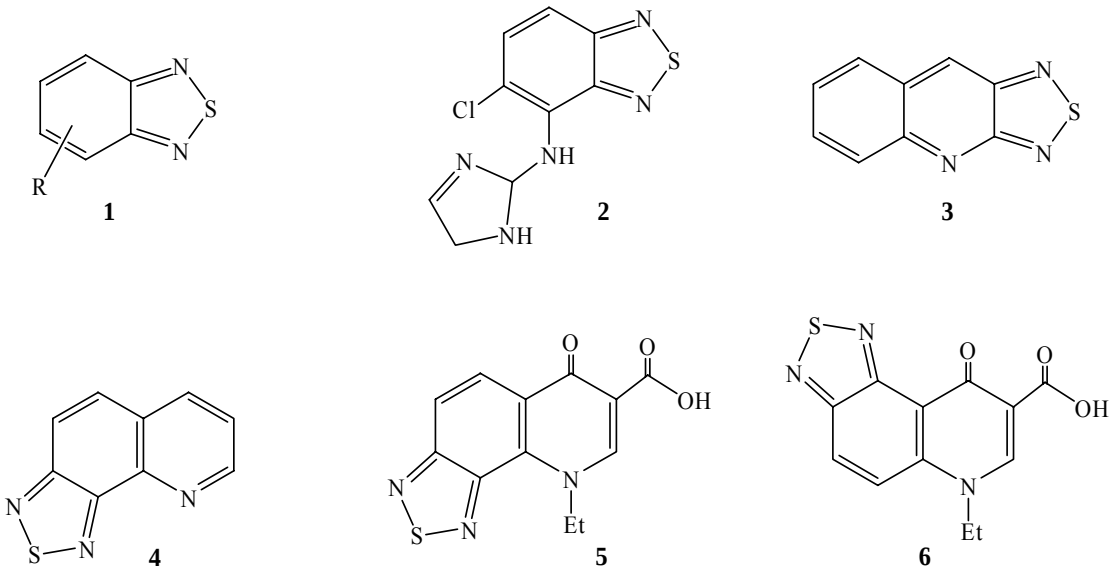


Figure 1. Structures of some benzo[*c*][1,2,5]thiadiazoles, thiadiazoloquinolines and thiadiazolo-quinolone carboxylic acids.

Structure-activity relationship studies for a large number of quinolone derivatives have shown that the vast majority of clinically useful quinolone antimicrobial agents are fluorinated in

the C-6 position[33,34] because the fluorine atom increases not only the penetration of the drug into the bacterial cell, but also the inhibitory activity against DNA gyrase.[35] A cyclopropyl group, appended at the *N*(1)-position of fluoroquinolones, has also been used extensively as it imparts better activity than the *N*(1)-ethyl group; examples of successful *N*(1)-cyclopropyl-6-fluoroquinolones include ciprofloxacin **7**, gatifloxacin **8**, grepafloxacin **9**, sparfloxacin **10**, clinafloxacin **11** and moxifloxacin **12** (Figure 2).

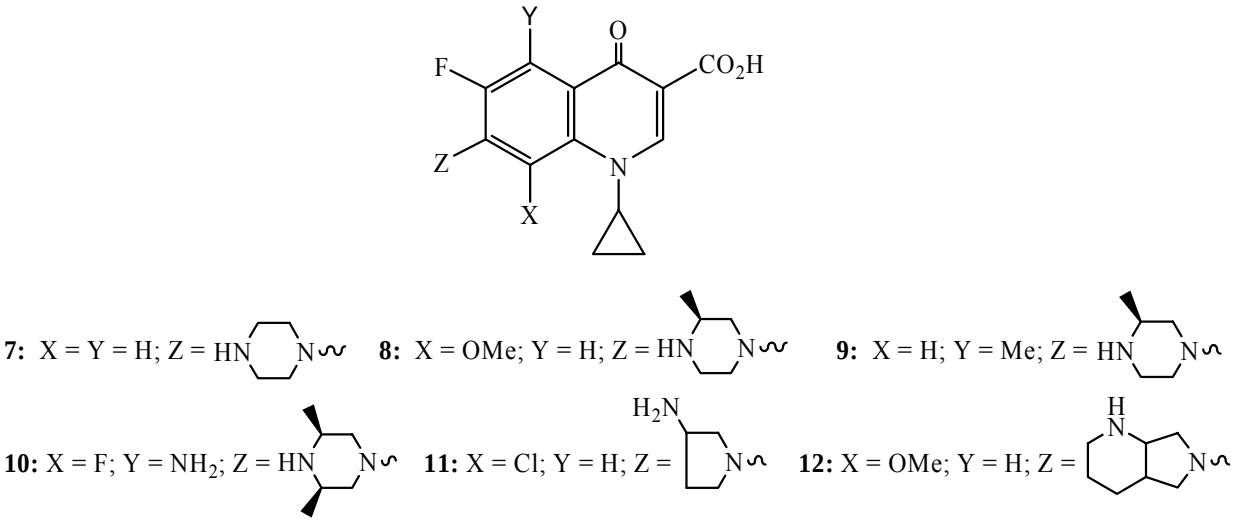


Figure 2. Structures of some 1-cyclopropyl-6-fluoroquinolone drugs in clinical use.

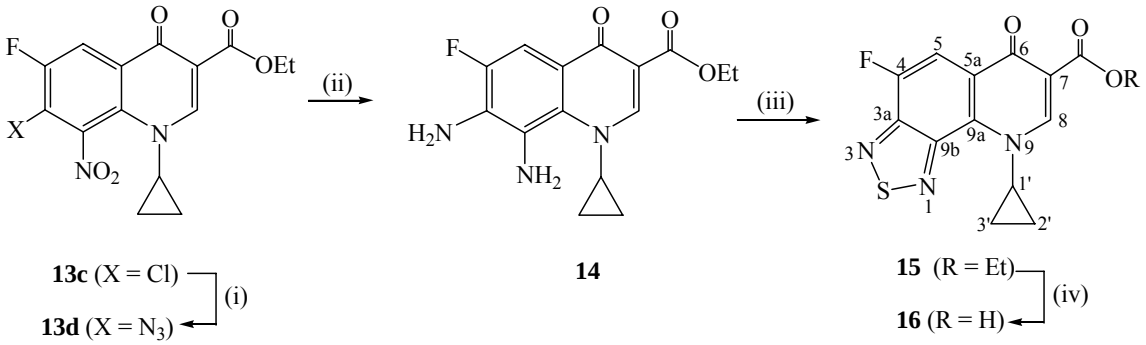
In light of these facts, we thought it would be worthwhile to incorporate, into compound **5**, a fluorine atom and a cyclopropyl group at C(4)- and C(9)-positions, respectively. Both substituents are expected to give an impetus to the antibacterial potency of **5**. Accordingly, we report herein on the synthesis of 9-cyclopropyl-4-fluoro-6-oxo[1,2,5]thiadiazolo[3,4-*h*]quinoline-7-carboxylic acid (**16**) and its ethyl ester (**15**) utilizing the diamino compound **14** as outlined in Scheme 1, together with X-ray crystal data of **16** and the results of a detailed antibacterial evaluation of **15** and **16**.

Results and Discussion

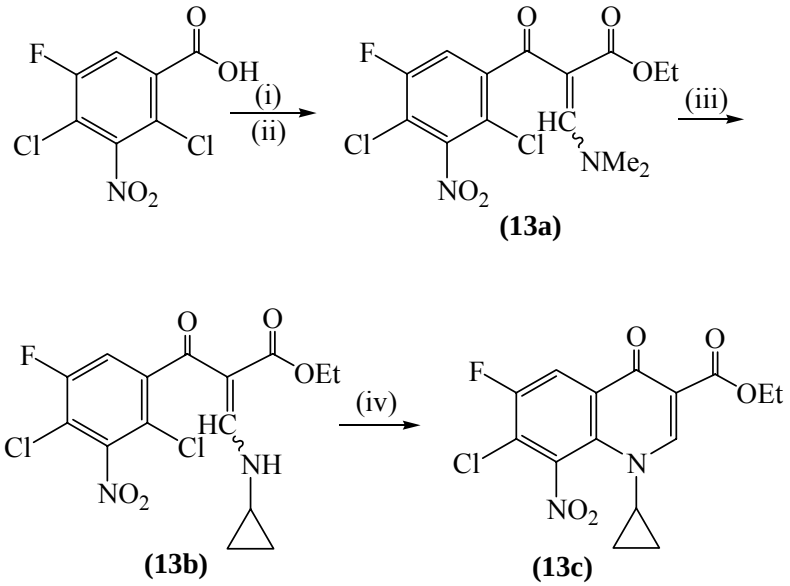
Chemistry

Condensed 1,2,5-thiadiazoles are readily prepared *via* direct interaction of the appropriate *ortho*-diaminoarene/heteroarene with thionyl chloride.[22,23,36] Following this versatile and general route, ethyl 6-oxothiadiazolo[3,4-*h*]quinoline-7-carboxylate (**15**) is herein prepared by thermal cyclocondensation of ethyl 7,8-diamino-9-cyclopropyl-4-fluoro-6-oxoquinoxaline-7-carboxylate (**14**)[37] with thionyl chloride (Scheme 1). Compound **14** was obtained by reduction of ethyl 7-

azido-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**13d**),[38] using $SnCl_2$ and concentrated HCl at rt, according to a literature procedure.[37] The required 7-chloro intermediate **13c** was prepared from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid *via* a multistep procedure depicted in Scheme 2.[39-42] Acid-catalysed hydrolysis of **15** produced the respective thiadiazolo[3,4-*h*]quinoline-7-carboxylic acid (**16**).



Scheme 1. Synthesis of thiadiazolo[3,4-*h*]quinolones **15** and **16**. Reagents and conditions: (i) NaN_3/DMSO; (ii) $SnCl_2$/HCl; (iii) $SOCl_2$, toluene, 80-85 ºC, 8 h; (iv) 6N HCl, EtOH, reflux, 20-24 h.



Scheme 2. Synthesis of ethyl 1-cyclopropyl-8-nitro-4-oxoquinoline-3-carboxylate **13c**. Reagents and conditions: (i) $SOCl_2$, benzene, reflux, 3-4 h; (ii) $EtO_2C–CH=CH\sim NMe_2$ (iii) cyclopropylamine, CH_2Cl_2/MeOH; (iv) DMF, K_2CO_3, 85 ^oC, 1.5 h.

The IR, MS, NMR spectral data for the new compounds **15** and **16** are in accordance with the assigned structures; details are given in the experimental part. Thus, the mass spectra display the correct molecular ion peaks for which the measured high resolution (HRMS) data are in good agreement with the calculated values. Based on ^{1}H NMR, proton-decoupled ^{13}C NMR spectra, DEPT 135 and DEPT 90 experiments, the ester derivative **15** displayed 1CH_3, 2CH_2, 3CH and 8C- quaternary carbons, whereas the parent carboxylic acid **16** showed, as expected, 1CH_2 in addition to 3CH and 8C- quaternary carbons. 2D (COSY, HMQC, HMBC) experiments showed correlations that helped in the ^{1}H- and ^{13}C-signal assignments to the different carbons and the neighboring hydrogens. In HMBC experiments, distinct 'three-bond' (^{1}H, ^{13}C) correlations are observed between H-5 and each of C-3a, C-9a and C-6, and between H-8 and each of C-9a, C-1', C-6 and *C*O_2Et/*C*O_2H.

Crystallography

The structure of **16** was confirmed by means of the X-ray diffractive analysis of single-crystal. A summary of data collection and refinement parameters is given in Table 1. Selected bond angles are listed in Table 2. The molecular structure of **16**, based on crystallographic data, is displayed in Figure 3.

Table 1. Crystal data and structure refinement parameters for compound **16**

Empirical formula	C_{13} H_8 F N_3 O_3 S
Formula weight	305.28 Da
Temperature (K)	173(2)
Wavelength (Å)	0.71073
Crystal system	monoclinic
Space group	*P* $2_1/n$
Unit cell dimensions	
a (Å)	7.797(6)
b (Å)	8.126(7)
c (Å)	18.685(10)
β (^{o})	93.04(4)
Volume (Å^3)	1182.1(15)
Z	4
D_{calcd} (g/cm^3)	1.715
Absorption coefficient (mm^{-1})	0.303
F (000)	624

Table 1. Continued

Θ Range for data collection (°)	2.18 - 30.28
Index range	-11 ≤ h ≤ 10, -11 ≤ k ≤ 11, -26 ≤ l ≤ 26
Reflections collected	12896
Independent reflections	3324 [R(int) = 0.0490]
Completeness to Θ = 32.17	94.1 %
Data / restraints / parameters	2899 / 0 / 191
Goodness-of-fit on F^2	1.048
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0387, wR_2 = 0.1049
R indices (all data)	R_1 = 0.0443, wR_2 = 0.1094
Largest difference peak and hole (e. Å^{-3})	0.460 and -0.358

Table 2. Selected bond lengths (Å) and angles (°) for compound **16**

bond lengths		bond angles	
S(1)-N(3)	1.6078(17)	N(3)-S(1)-N(1)	101.18(7)
S(1)-N(1)	1.6110(13)	C(9B)-N(1)-S(1)	107.07(9)
N(1)-C(9B)	1.3358(19)	C(3A)-N(3)-S(1)	105.48(10)
N(3)-C(3A)	1.3383(17)	N(3)-C(3A)-C(4)	125.43(12)
C(3A)-C(4)	1.408(2)	N(3)-C(3A)-C(9B)	114.49(12)
C(3A)-C(9B)	1.4363(18)	F(1)-C(4)-C(3A)	118.35(12)
C(4)-C(5)	1.3432(19)	C(9A)-N(9)-C(10)	121.19(10)
C(5)-C(5A)	1.4318(18)	N(9)-C(9A)-C(9B)	123.27(11)
C(9A)-C(9B)	1.4444(17)	N(1)-C(9B)-C(3A)	111.78(11)
N(9)-C(9A)	1.3872(16)	N(1)-C(9B)-C(9A)	128.53(11)

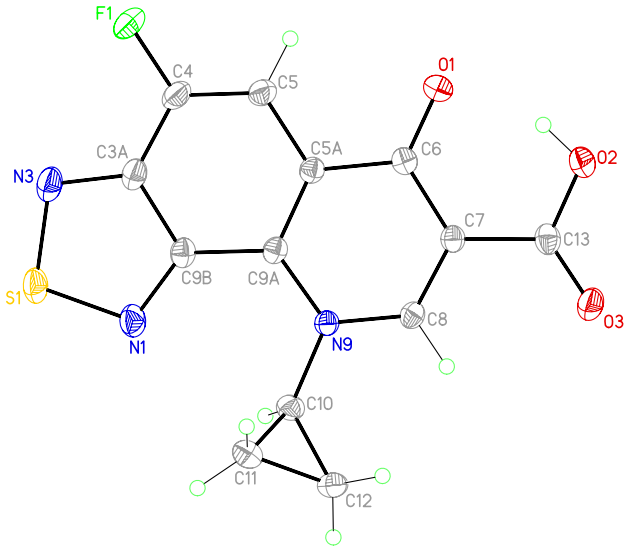


Figure 3. ORTEP plot of the molecular structure of **16**. Displacement ellipsoids are drawn at the 50 % probability level.

Antimicrobial assay

The new fluoroquinolone **16** and its ester parent compound **15** were tested *in vitro* against a wide spectrum of Gram-positive (Table 3) and Gram-negative (Table 4) bacteria, yeasts and moulds. The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC), both expressed in μg mL^{-1}, were determined and compared to those of ciprofloxacin **7** as reference drug (Tables 3 and 4). Since the development of new antimicrobial agents is increasingly important due to the continual emergence of microbial strains that demonstrate multidrug resistance, both methicillin- and ciprofloxacin-resistant bacteria were assayed simultaneously.

Compound **16** shows a very strong activity against Gram-positive bacilli and staphylococci (MICs 0.015-1.5 μg mL^{-1}), including methicillin-resistant *Staphylococcus aureus*, and against most of the Gram-negative bacteria tested (MICs 0.07-3 μg mL^{-1}). A limited effect is detected for the ester parent compound **15** against the same microorganisms (MICs 3-200 μg mL^{-1} for Gram-positive bacteria and 12-400 μg mL^{-1} for Gram-negative ones), confirming that the carboxylic group on 7-position is an optimal requirement for the antibacterial potency of these compounds.[27] The spectrum of activity and the degree of efficacy of compound **16** against the different strains of bacteria is similar to that of ciprofloxacin **7**, with MIC values identical or higher than those of the reference compound. The only exception is *Staphylococcus haemolyticus* that is more sensitive to compound **16** than ciprofloxacin. Both **15** and **16** act as bacteriostatic agents, being MBC values always higher than the corresponding MICs. As expected, compound **16**, bearing a fluorine atom and a cyclopropyl group at C(4)- and C(9)-

positions, respectively, showed increased antibacterial activity when compared with compound **5** (Tables 3 and 4).[28]

Table 3. Antibacterial activity against Gram-positive bacteria expressed as MIC (μg mL^{-1}) and, in brackets, as MBC (μg mL^{-1})

Microorganism	Compound			
	15	**16**	**5**[a]	**7**
Bacillus cereus[b]	12 (100)	0.15 (1.5)		0.15 (0.7)
Bacillus megaterium BGSC 7A2	3 (50)	0.015 (0.15)		0.007 (0.07)
Bacillus subtilis ATCC 6633	3 (12)	0.07 (0.7)		0.03 (0.3)
Bacillus thuringiensis var. *kurstaki* BGSC 4D1	12 (200)	0.07 (1.5)		0.03 (0.3)
Sarcina lutea ATCC 9341	>400	25 (100)		3 (12)
Staphylococcus aureus ATCC 6538	50 (>400)	0.7 (12)	6.25	0.3 (6)
Staphylococcus aureus methicillin-resistant[b]	200 (400)	1.5 (6)		0.7 (6)
Staphylococcus aureus methicillin- and cipro-resistant[b]	>400	100 (>400)		100 (>400)
Staphylococcus epidermidis ATCC 12228	100 (400)	0.7 (12)	25	0.07 (1.5)
Staphylococcus epidermidis methicillin- and cipro-resistant[b]	>400	100 (>400)		100 (200)
Staphylococcus haemolyticus[b]	>400	100 (>400)		400 (>400)
Streptococcus agalactiae[b]	>400	100 (>400)		0.7 (6)
Streptococcus faecalis cipro-resistant[b]	>400	400 (>400)		400 (>400)
Streptococcus faecium cipro-resistant[b]	>400	400 (>400)		400 (>400)
Streptococcus pyogenes cipro-resistant[b]	>400	>400		100 (>400)

[a] Values previously reported[28]; [b] Clinical isolate

Table 4. Antibacterial activity against Gram-negative bacteria expressed as MIC (μg mL^{-1}) and, in brackets, as MBC (μg mL^{-1})

Microorganism	Compound			
	15	**16**	**5**[a]	**7**
Acinetobacter baumannii methicillin- and cipro-resistant[b]	>400	>400		>400
Enterobacter cloacae ATCC 23355	>400	6 (12)	6.25	0.07 (0.15)
Escherichia coli ATCC 8739	100 (>400)	0.7 (1.5)	6.25	0.015 (0.07)
Escherichia coli methicillin- and cipro-resistant[b]	>400	100 (>400)		100 (>400)
Haemophilus influenzae ATCC 19418	25 (200)	0.3 (25)		0.15 (6)
Haemophilus influenzae[b]	25 (200)	0.3 (0.7)		0.15 (3)
Klebsiella pneumoniae[b]	100 (>400)	3 (12)	6.25	0.07 (0.3)
Proteus mirabilis methicillin- and cipro-resistant[b]	>400	>400		100 (>400)
Proteus vulgaris[b]	12 (25)	0.07 (0.7)	>25	0.007 (0.03)
Pseudomonas aeruginosa ATCC 9027	100 (>400)	12 (25)	>25	0.07 (0.3)
Pseudomonas aeruginosa methicillin- and cipro-resistant[b]	>400	>400		25 (200)
Salmonella typhimurium ATCC 14028	50 (400)	1.5 (3)	12.5	0.03 (0.3)
Serratia marcescens ATCC 8100	400 (>400)	3 (12)	3.13	0.3 (1.5)

[a] Values previously reported;[28] [b] Clinical isolate

No antifungal activity is exhibited against *Saccharomyces cerevisiae* ATCC 9763, *Candida tropicalis* ATCC 1369 and *Aspergillus niger* ATCC 6275 up to the concentration of 400 μg mL^{-1} (data not shown).

Conclusions

The 9-cyclopropyl-4-fluoro-6-oxothiadiazolo[3,4-*h*]quinoline-7-carboxylic acid **16** described herein represents a potential antibacterial agent for treatment of serious Gram-positive and Gram-negative infections. However, this compound still lacks effectiveness against methicillin- and quinolone-resistant strains. These results encourage further modifications of the fluorothiadiazoloquinoline scaffold to provide novel compounds more active than the existing quinolones.

Experimental Section

General. Pure grade thionyl chloride, benzene, methanol and dichloromethane were purchased from Acros Organics (Geel, Belgium). Ciprofloxacin used as standard quinolone was obtained from Sigma (Milano, Italy). Melting points (uncorrected) were determined on a Gallenkamp electrothermal melting-temperature apparatus. ^{1}H- and ^{13}C NMR spectra were measured on a Bruker DPX-300 instrument. Chemical shifts are expressed in ppm with reference to *TMS* as internal standard. Electron impact mass spectra (EIMS) were obtained using a Finnigan MAT TSQ-70 spectrometer at 70 eV and at an ion source temperature of 200ºC. High-resolution mass spectra (HRMS) were measured in positive ion mode using electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid) and infused 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. IR spectra were recorded as KBr discs on a Nicolet Impact-400 FT-IR spectrophotometer (abbreviations: vs = very strong, s = strong, m = medium, w = weak, br = broad). Elemental analyses (C, H, N, S) were performed at the Microanalytical Laboratory of the Hashemite University, Zarqa-Jordan, and the results were found to be in good agreement (± 0.4%) with the calculated values.

Ethyl 3-(*N*,*N*-dimethylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate 13a. A mixture of 2,4-dichloro-5-fluoro-3-nitrobenzoic acid (10.2 g, 40 mmol) and thionyl chloride (19.0 g, 160 mmol) in dry benzene (120 mL) was refluxed for 3-4 h under anhydrous conditions. The solvent and excess thionyl chloride were then distilled off under reduced pressure, and dry benzene (20 mL) was then introduced into the reaction vessel and re-distilled so as to remove traces of thionyl chloride. The resulting 2,4-dichloro-5-fluoro-3-nitrobenzoyl chloride, formed as thick oil, was used as such for the next step without further purification.

To a stirred and cooled (5-10 °C) solution of ethyl 3-(*N*,*N*-dimethylamino)acrylate (6.3 g, 44 mmol) and triethylamine (8.1 g, 80 mmol) in dry benzene (50 mL) was added dropwise a solution of the crude acid chloride (prepared above) in dry benzene (25 mL). The resulting mixture was refluxed for 2 h, then cooled to rt and washed with water (2 × 30 mL). The organic

layer was separated, dried (anhydrous $MgSO_4$) and the solvent benzene was then evaporated to dryness under reduced pressure. The residual product was soaked in methanol (10 mL) whereby the title compound was produced as yellowish powder which was collected by suction filtration and dried. Yield 13.8 g (91%), mp 140-141°C (Lit.[42] 139-141 °C).

Ethyl 3-(*N*-cyclopropylamino)-2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)acrylate (13b). A stirred solution of **13a** (14.4 g, 38 mmol) in dichloromethane (50 mL) and methanol (10 mL), cooled to 8-10 °C, was treated dropwise with cyclopropylamine (3.2 g, 56 mmol) in MeOH (3 mL), and the resulting mixture was further stirred for additional 10-15 min at 8-10 °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 (95%, 10 mL) and dried. Yield 11.0 g; a second crop of **13b** (1.7 g) was obtained upon concentration of the mother liquor. Total yield 12.7 g (93%), mp 143-145 °C (Lit.[42] 142-145 °C).

Ethyl 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (13c). A solution of **13b** (11.7 g, 30 mmol) in DMF (50 mL) and potassium carbonate (12.4 g, 90 mmol) was heated at 85 °C. Progress of the cyclisation reaction was monitored by TLC (eluent: AcOEt + *n*-hexane, 1:1 v/v) and was completed within 90-100 min. The reaction mixture was then poured slowly onto crushed ice (500 g) under vigorous stirring, the precipitated pale yellow solid product was collected, washed with water, triturated with cold ethanol and dried. Yield 9.5 g (89%), mp 165-166 °C (Lit.[42] 165-167 °C).

Ethyl 7-azido-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (13d). Sodium azide (7.8 g, 120 mmol) was added to a solution of **13c** (7.1 g, 20 mmol) in dimethylsulfoxide (100 mL). The resulting mixture acquired white turbidity within few minutes, and was further stirred at rt for 6-8 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 dried. Yield 5.6 g (78 %), mp 184-185 °C (dec.) (Lit.[38] 183-184 °C).

Ethyl 7,8-diamino-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (14). Anhydrous stannous chloride (5.3 g, 28 mmol) was added portionwise to a stirred and ice-cooled (4-8 °C) solution of **13d** (2.2 g, 6.3 mmol) in 36% aqueous HCl (50 mL). The reaction mixture was further stirred at rt for 24 h, then diluted with ice-cooled water (50 mL), basified with 40% cold aqueous NaOH solution to pH 11-12 and set aside for 10-20 min. The precipitated solid product was collected by suction filtration, washed with cold water, dried and purified by column chromatography on silica gel, using chloroform followed by chloroform / methanol (95:5, v/v) as the eluent. Yield: 1.4 g (73 %), mp 284-286 °C (dec.) (Lit.[37] 282-284 °C (dec.)).

Ethyl 9-cyclopropyl-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-*h*]quinoline-7-carboxylate (15). To a stirred suspension of **14** (0.92 g, 3 mmol) in dry toluene (25 mL), was added purified thionyl chloride (6 mL) and the resulting mixture was heated at 80-85 ºC for 8 h. Toluene and excess thionyl chloride were distilled off *in vacuo* and the residue was cooled, treated with methanol (4 mL); cold water (50 mL) was then added to afford a precipitate, which was collected, dried and purified by recrystallization from dichloromethane/methanol. Yield 0.72 g (72 %), mp 205- 207 °C (yellow needles). IR (KBr, cm^{-1}) *v*: 3101(w), 2980(w), 1728(vs),

1622(s), 1586(m), 1541(m), 1481(vs), 1412(m), 1331(m), 1298(m), 1231(s), 1170(s), 1074(m). ^{1}H NMR (300 MHz, DMSO-d$_6$), δ (ppm): 1.18 (m, 2H) and 1.30 (m, 2H) (H_2-2' + H_2-3'), 1.27 (t, J = 7 Hz, 3H, CH_3CH$_2$), 4.22 (q, J = 7 Hz, 2H, CH_2Me), 4.32 (m, 1H, H-1'), 7.96 (d, $J_{H\text{-}F}$ = 10.8 Hz, 1H, H-5), 8.55 (s, 1H, H-8). ^{13}C NMR (75 MHz, DMSO-d$_6$), δ (ppm): 10.6 (C-2' + C-3'), 14.7 (*C*H$_3$CH$_2$-), 40.6 (C-1'), 60.8 (*C*H$_2$Me), 108.9 (d, $^2J_{C\text{-}F}$ = 18.9 Hz, C-5), 113.9 (C-7), 127.9 (d, $^3J_{C\text{-}F}$ = 4.9 Hz, C-5a), 132.3 (C-9a), 148.3 (C-8), 148.4 (d, $^2J_{C\text{-}F}$ = 10.5 Hz, C-3a), 149.3 (d, $^3J_{C\text{-}F}$ = 1.5 Hz, C-9b), 150.1 (d, $^1J_{C\text{-}F}$ = 242 Hz, C-4), 164.4 *C*O$_2$Et), 171.4 (d, $J_{C\text{-}F}$ = 1.5 Hz, C-6). MS-EI: *m/z* (% rel int): 333 (M^+, 11), 319 (7), 289 (10), 288 (11), 287 (11), 286 (12), 261 (100), 260 (59), 246 (18), 232(32), 205 (7), 192 (8), 191 (7), 106 (6). HRMS-ESI m/z (+): calculated mass for $C_{15}H_{13}FN_3O_3S^+$ $[M+H]^+$: 334.06562, observed mass: 334.06558; calculated mass for $C_{15}H_{12}FN_3O_3SNa^+$ $[M+Na]^+$: 356.04756, observed mass: 356.04751. Anal. calcd. for $C_{15}H_{12}FN_3O_3S$ (333.34): C 54.05, H 3.63, N 12.61, S 9.62; found: C 53.82, H 3.46, N 12.58, S 9.54.

9-Cyclopropyl-4-fluoro-6-oxo-6,9-dihydro[1,2,5]thiadiazolo[3,4-*h*]quinoline-7-carboxylic acid (16). A vigorously stirred suspension of the ester **15** (0.5 g, 1.5 mmol) in 6 N HCl (15 mL) and ethanol (6 mL) was heated at 80-85 ^{o}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 faint yellow precipitate was collected, washed with cold water, dried and recrystallized from *N*,*N*-dimethylformamide (DMF), or from DMF + DMSO (1 : 1, v/v). Yield 0.42 g (92 %). mp 299-301 ^{o}C (yellow blocks). IR (KBr, cm^{-1}) *v*: 3342 (br s, O-H), 3096(w), 2955(w), 1733(s), 1635(s), 1607(vs), 1573(vs), 1494(vs), 1398(m), 1356(m), 1311(s), 1138(m), 1033(m). ^{1}H NMR (300 MHz, DMSO-d$_6$), δ (ppm): 1.26 (m, 2H) and 1.38 (m, 2H) (H_2-2' + H_2-3'), 4.50 (m, 1H, H-1'), 8.13 (d, $^3J_{H\text{-}F}$ = 9.3 Hz, 1H, H-5), 8.87 (s, 1H, H-8), 14.95 (s, 1H, CO_2H). ^{1}H NMR (300 MHz, 3% NaOD in D_2O), δ (ppm): 1.13 (m, 2H) and 1.42 (m, 2H) (H_2-2' + H_2-3'), 4.31 (m, 1H, H-1'), 7.83 (d, $^3J_{H\text{-}F}$ = 11.0 Hz, 1H, H-5), 8.61 (s, 1H, H-8) . ^{13}C NMR (75 MHz, 3% NaOD in D_2O), δ (ppm): 9.4 (C-2' + C-3'), 39.8 (C-1'), 107.8 (d, $^2J_{C\text{-}F}$ = 19.4 Hz, C-5), 120.8 (C-7), 125.9 (d, $^3J_{C\text{-}F}$ = 6.0 Hz, C-5a), 131.6 (C-9a), 145.6 (C-8), 147.4 (d, $^2J_{C\text{-}F}$ = 17.4 Hz, C-3a), 147.7 (d, $^3J_{C\text{-}F}$ = 2.9 Hz, C-9b), 148.9 (d, $^1J_{C\text{-}F}$ = 256 Hz, C-4), 171.2 (*C*O_2H), 173.7 (d,$^4J_{C\text{-}F}$ = 2.7 Hz, C-6). HRMS-ESI m/z (+): calculated mass for $C_{13}H_9FN_3O_3S^+$ $[M+H]^+$: 306.03432, observed mass: 306.03437; calculated mass for $C_{13}H_8FN_3O_3SNa^+$ $[M+Na]^+$: 328.01626, observed mass: 328.01626. Anal. calcd. for $C_{13}H_8FN_3O_3S$ (305.28): C 51.15, H 2.64, N 13.76, S 10.50; found: C 50.88, H 2.53, N 13.62, S 10.41.

X-ray diffraction

Yellow block crystals, suitable for X-ray crystallography, were grown slowly from a solution of **16** in DMF. Crystal size (mm^3): 0.34 x 0.26 x 0.22. Crystal data collection was made with a Siemens SMART three axis goniometer with APEX II area detector system. The data were reduced with Bruker AXS APEX 2 Vers. 2.0-2 2006, and the structure was solved by the direct method using AXS SHELXTL programs Vers. 2008 /4/(c) 2008.

All non-hydrogen atoms were refined anisotropically by full-matrix, least-squares procedure based on F^2 using all unique data. The hydrogen atoms were placed in calculated positions and treated as riding groups, with the 1.2 fold (1.5 fold for methyl groups) isotropic displacement parameters of the equivalent Uij of the corresponding carbon atom.
Crystallographic data (excluding structure factors) of **16** have been deposited at the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 721031. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Antimicrobial assay
The *in vitro* antimicrobial activity was performed by the broth dilution technique[43] against Gram-positive and Gram-negative bacteria, yeasts and moulds. The compounds were tested at concentrations ranging from 400 to 0.0007 μg mL^{-1}. Ciprofloxacin was used as a reference standard. The inoculum size was $5x10^5$ bacteria/mL and $1x10^3$ fungi/mL. After incubation at 37°C for 24 h (bacteria) or at 30°C for 48 h (fungi), the minimum inhibitory concentration (μg mL^{-1}) at which no growth was observed was taken as the MIC value. The minimum bactericidal concentration (MBC, μg mL^{-1}) was determined by subculturing in fresh medium 100 μL of liquid from each suspension remaining clear and incubating the sample at 37°C for 24 h.
All experiments were performed in triplicate and repeated at least three times.

Acknowledgements

We wish to thank the Deanship of Scientific Research (University of Jordan, Amman- Jordan) and DFG (Germany) for financial support.

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