Bioorganic & Medicinal Chemistry 18 (2010) 5873–5884

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

# Synthesis and biological evaluation of tetracyclic fluoroquinolones as antibacterial and anticancer agents

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ARTICLE INFO

*Article history:*
Received 28 February 2010
Revised 25 June 2010
Accepted 29 June 2010
Available online 3 July 2010

*Keywords:*
6-Fluoroquinolones
4-Oxo-1,4-dihydropyrido[2,3-*a*]carbazoles
4-Oxothieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline
Antibacterial activity
Anticancer activity

ABSTRACT

A simple and efficient synthesis of 6-fluoro-4-oxopyrido[2,3-*a*]carbazole-3-carboxylic acids (**13a**–**e**) and a structurally related 6-fluoro-4-oxothieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline (**13f**) was achieved via Stille arylation of 7-chloro-6-fluoro-8-nitro-4-oxoquinoline-3-carboxylate and a subsequent microwave-assisted phosphite-mediated Cadogan reaction. The new compounds were tested for their in vitro antimicrobial and antiproliferative activity. The ability of **13a**–**f** to inhibit the activity of DNA gyrase and topoisomerase IV was also investigated. The thieno isostere (**13f**) emerged as the most active antibacterial, while the 9-fluoro derivative (**13e**) was the most potent against multidrug-resistant staphylococci. Compounds **13a**, **13c**–**f** displayed growth inhibition against MCF-7 breast tumor and A549 non-small cell lung cancer cells coupled with an absence of cytotoxicity toward normal human-derm fibroblasts (HuDe). Compound **13e** was the most active anticancer against MCF-7 cells, with greater potency than ellipticine ($IC_{50}$ 0.8 and 1.6 μM, respectively). The most active compounds in this series show promise as dual acting anticancer and antibacterial chemotherapeutics.



## 1. Introduction

After the discovery of the first fluoroquinolone, norfloxacin,[1] in 1980 as an antibacterial agent with potent and broad spectrum activity, several new members of this family (Fig. 1) have emerged with enhanced activity against Gram positive and anaerobic bacteria and improved pharmacokinetic profile. Much has been learned about how molecular modifications of the core quinolone structure affect the antibacterial profile. The structure–activity relationship of quinolones has been the subject of extensive review.[2] For most of the current agents, a hydrogen at position 2, a carboxyl group at position 3 and a keto group at position 4 in the bicyclic ring cannot be changed without a significant loss of activity. Furthermore it appears that a cyclopropyl group is optimal at C-1. The fluorine atom at position 6 imparts increased intracellular penetration and enhanced DNA gyrase activity and some efficacy against Gram positive bacteria. It can be considered a breakthrough for the enhanced Gram negative activity, which led to the group of the modern 6-fluoro compounds (or second generation quinolones). The substituent at position 7 greatly influences potency, spectrum and safety. A nitrogen heterocyclic moiety is optimal and piperazine, pyrrolidine and their substituted derivatives have been the most successfully employed side chains as evidenced by the compounds currently on the market. The substituent at C-8, and similarly the substituent at C-5, affects the overall steric configuration and the number of intracellular targets on bacterial type II topoisomerases.[2d] A fused ring with a bridge between C-8 and N-1 is found in levofloxacin and rufloxacin. Recently, last generation fluoroquinolones demonstrated the favorable influence of an $OCH_3$ substituent at position 8 on Gram positive and on anaerobic bacteria. Moreover, the optimal substituent placed on C-8, combined with a bulky addition at C-7 has been shown to markedly reduce the development of fluoroquinolone resistance in *Staphylococcus aureus*.[3]

From these observations, changes in substitution on the quinolone C-7 and C-8 positions appear to play a significant role in the target preference and may offer new insight into the structure–activity relationship of the fluoroquinolone antibacterials. To the best of our knowledge, fused rings with a bridge between the critical 7 and 8 positions has not yet been extensively investigated in this system.

As part of an ongoing program aimed at developing facile synthesis of novel heterocycles [*h*]fused onto 1-cylopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, we have recently explored the synthesis of some 9-cyclopropyl-4-fluoro-6-oxoimidazo[4,5-*h*]quinoline-7-carboxylic acids[4] and of 9-cyclopropyl-4-fluoro-6-oxo[1,2,5]thiadiazolo[3,4-*h*]quinoline-5-carboxylic acid, which is endowed with strong antibacterial activity.[5]

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doi:10.1016/j.bmc.2010.06.098

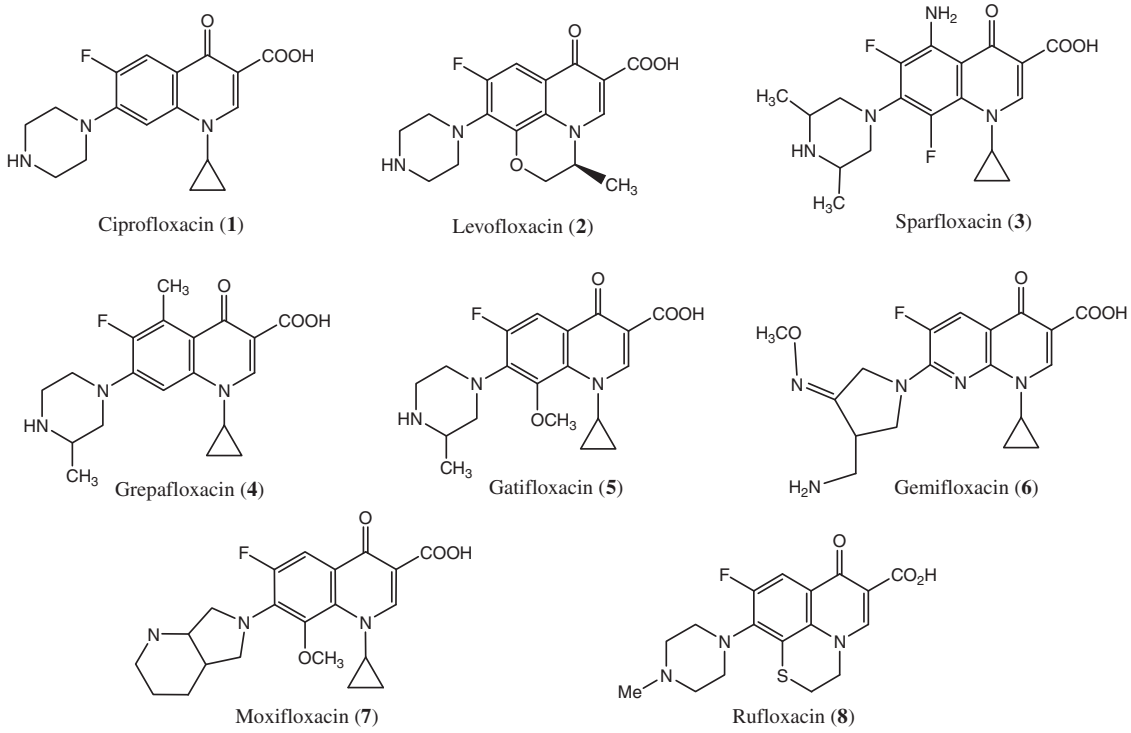
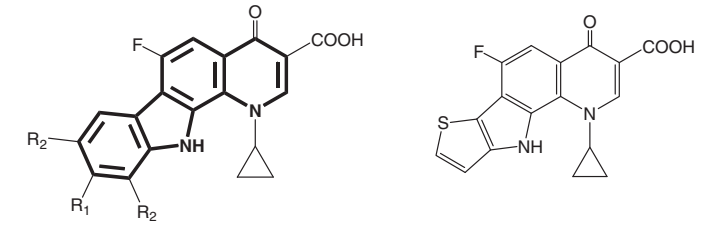


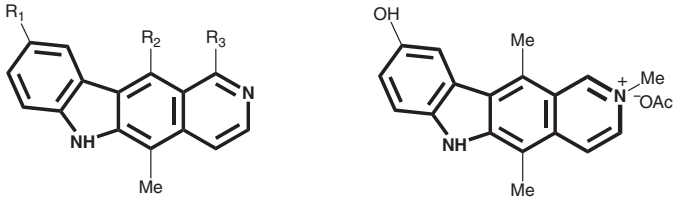

**Figure 1.** Structures of some fluoroquinolones in clinical use.

We report here the synthesis and in vitro evaluation of the antimicrobial and antitumor properties of novel tetracyclic fluoroquinolones (Fig. 2) such as the variously substituted 1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydropyrido[2,3-*a*]carbazole-3-carboxylic acids (**13a–e**) and the structurally related 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylic acid (**13f**), a thiophene isostere of **13a**.

The investigation of the antitumor activity of the tetracyclic fluoroquinolones **13a–f** was suggested by the use of the 4-quinolone-3-carboxylic acid motif as a multivalent scaffold in medicinal chemistry,[6] and the fact that the structure of **13a–e** closely resembles the pyrido[4,3-*b*]carbazole system that constitutes the skeleton of the naturally occurring anticancer ellipticine and its derivatives (Fig. 2: bold structures).[7] Ellipticine **14a**, an alkaloid




**13a** $R_1$ = H; $R_2$ = H;
**13b** $R_1$ = Me; $R_2$ = H
**13c** $R_1$ = OMe; $R_2$ = H
**13d** $R_1$ = OMe; $R_2$ = Me
**13e** $R_1$ = F; $R_2$ = H




**14a** $R_1$ = $R_3$ = H; $R_2$ = Me;
**14b** $R_1$ = OMe; $R_2$ = Me; $R_3$ = H
**14c** $R_1$ = OH; $R_2$ = Me; $R_3$ = H
**15** $R_1$ = $R_2$ = H; $R_3$ = Me

**Figure 2.** New synthesized fluoroquinolones (**13a–f**), ellipticines (**14a–c**, **16**), and olivacine (**15**).

isolated from *Apocynaceae* plants,[8,9] such as *Ochrosia elliptica* labil, and several of its derivatives (e.g., the natural analogs 9-methoxyellipticine **14b**, 9-hydroxyellipticine **14c**, and the isomeric olivacine **15**) show promise in the treatment of osteolytic breast cancer metastases, kidney sarcoma, brain tumors and myeloblastic leukemia.[10–17] A number of successful ellipticine analogues have been designed and synthesized with improved cytotoxicity and anticancer activities against a panel of cancer cell lines.[18–20] The derivative 9-hydroxy-2-methylellipticinium acetate **16** (elliptinium acetate), with a good solubility, has found application in the treatment of breast, kidney, and thyroid cancer.[21–23] The interest in ellipticine and its derivatives for clinical purposes is mainly due to their high efficiency against several types of cancer, limited toxic side effects, and complete lack of hematological toxicity.[24]

The antiproliferative activity of all compounds investigated in the present study was evaluated against MCF-7 (breast), A549 (non-small cell lung cancer) tumor cells, and normal human-derm fibroblasts (HuDe). Compound **13e**, with the best antiproliferative activity, was further assessed, in comparison to ellipticine, by flow cytometric analysis on cell cycle distributions. Moreover apoptosis induction and p53 expression were studied to obtain further insight, at the molecular level, into the mechanism of their action.

## 2. Results and discussion

### 2.1. Synthesis

A generally applicable method for the synthesis of substituted carbazoles is based on the phosphite-mediated Cadogan reductive cyclization of the corresponding 2-nitrobiaryls.[25–27] However, this process demands drastic conditions and long reaction times, making it a less-favored synthetic route. Recently, microwave irradiation[28–30] has emerged as a valuable tool in organic synthesis whereby rate enhancements and improved yields are often attainable for a wide variety of reaction types performed under focused conditions. In this context, microwave-enhanced Cadogan cyclization of nitro compounds, in combination with phosphite reagent, has provided easy access to substituted carbazoles and other fused heterocyclic systems.[31] The requisite 2-nitrobiaryls and related congeners are accessible via palladium-catalyzed cross-coupling reactions of *ortho*-halonitroarenes with the appropriate organometal partners, exemplified by aryl(trialkyl) stannanes[32–34] of the Stille reaction.[35–43]

Based on the above methodologies, the synthesis of the desired 6-fluoro-4-oxo-1,4-dihydropyrido[2,3-*a*]carbazole-3-carboxylic acids (**13a–e**) and their ethyl esters (**12a–e**) was achieved by utilizing ethyl 7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**9**)[5,44,45] as the starting material, and constructing the indole nucleus via a two-step procedure as illustrated in Scheme 1. The first key-step involves palladium-catalyzed carbon–carbon cross coupling between **9** and the appropriate aryltrimethyl stannane (**10a–e**) (Stille reaction) with ultimate production of the respective ethyl 7-aryl-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**11a–e**). In a subsequent step, the latter compounds undergo phosphite-mediated reductive cyclization (Cadogan reaction) assisted by microwave irradiation to furnish the respective ethyl 4-oxo-4,11-dihydropyrido[2,3-*a*]carbazole-3-carboxylates **12a–e**. Compound **12f** is obtained through the same procedure, using 2-thienyltrimethyl stannane (**10f**) (Scheme 2).[34] This reaction entails the generation of a nitrene intermediate that undergoes insertion into a 7-aryl C–H bond to form a fused 'pyrrole' ring (C) embedded in the tetracyclic product. It is of note that in absence of microwave irradiation, the phosphite-mediated nitro group-deoxygenation step, leading to **12**, does not proceed to any detectable extent at 160 °C for 96 h. Acid-catalyzed hydrolysis of the esters **12a–f** produced the corresponding target 4-oxo-1,4-dihydropyrido[2,3-*a*]carbazole-3-carboxylic acids **13a–e** and 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]-pyrrolo[3,2-*h*]quinoline-3-carboxylic acid **13f**.

The new compounds **11–13** were characterized by elemental analyses, IR, MS and NMR spectral data. These data, detailed in Section 4, are consistent with the suggested structures. 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. DEPT and 2D (COSY, HMQC, HMBC) experiments showed correlations that helped in the $^{1}H$- and $^{13}C$-signal assignments to the different carbons and their attached, and/or neighboring hydrogens. For compounds **11a–f**, long-range correlations are observed between H-5 and each of C-8a, C-4 and C-7; likewise, H-2 is correlated with C-8a, C-4, C-1′, and $CO_2Et$. For compounds **12a–e** and **13a–e**, similar long-range correlations are also observed between H-5 and each of C-11b, C-4, and C-6a, as well as between H-2 and each of C-11b, C-4, C-1′, and $CO_2Et/CO_2H$. For compounds **12f** and **13f** similar correlations are likewise observed between each of H-2, H-5, H-8, H-9, and the neighboring carbons. For compounds **11–13**, the carbons of the benzo-fused ring B are readily identified by their doublet signals (with varying *J*-values) originating from coupling with the nearby fluorine atom at C-6.

### 2.2. Biology

#### 2.2.1. Antimicrobial activity

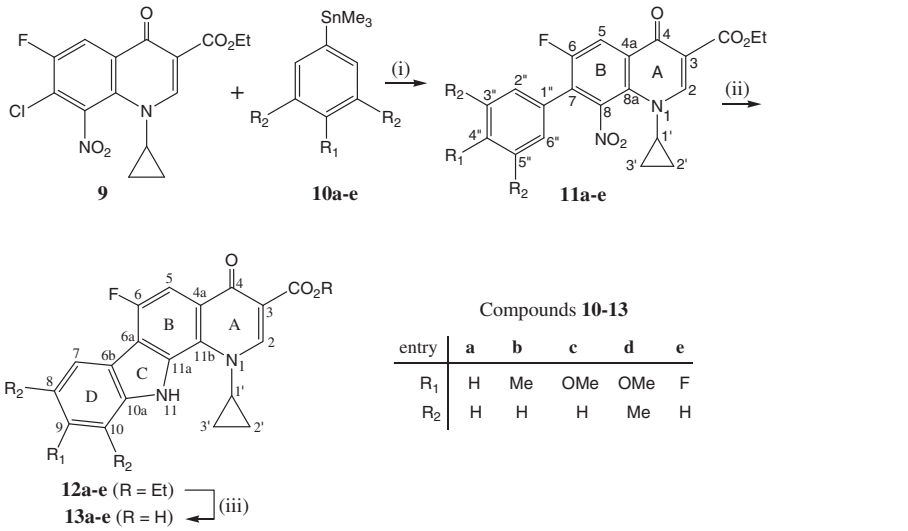
The antimicrobial activity of the novel heterocyclic compounds **13a–f** was assayed against 16 standard microorganisms representative of Gram positive and Gram negative bacteria, yeasts, and molds. Bearing in mind that the spread of resistant strains reduces the number of available chemotherapeutic agents, multidrug-resistant bacterial species, such as clinical isolates of quinolone- and penicillin-resistant *S. aureus*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were included in the investigation.

The inhibitory activity of **13a–f** against bacteria is summarized in Table 1, together with the results obtained for commercial quinolones ciprofloxacin, levofloxacin, moxifloxacin, and gemifloxacin, which were used as reference drugs.

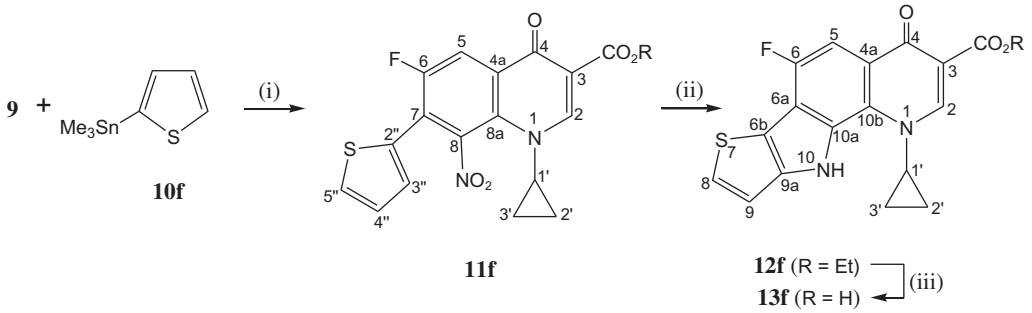
All new compounds exhibited strong activity against *Bacillus subtilis*, the most sensitive microorganism to the tested substances. The minimum inhibitory concentrations (MIC) of **13a–d** ranged from 0.015 to 0.07 μg/mL and were close to those of standard quinolones, while a higher effect was exerted by **13e** and **13f** (MIC 0.003 μg/mL). In addition, **13a** and, above all, **13e–f** showed the best activity against wild staphylococci at the same concentrations observed for the reference drugs. These bacteria were also significantly inhibited by compounds **13c** and **13d**.

Of particular relevance is that compounds **13e** and **13f** had effective antibacterial properties against both multidrug-resistant strains of *S. aureus* (SAR 72) and *S. epidermidis* with MIC values 1.5–12 μg/mL and 3–50 μg/mL, respectively. Compound **13a** was also a moderate inhibitor of SAR 72 at 12 μg/mL concentration. It is worth noting that, against the above mentioned staphylococci, **13a** and **13f** exhibited greater inhibitory activity than that of ciprofloxacin and levofloxacin and comparable to that of moxifloxacin and gemifloxacin, whereas **13e** (MIC 1.5–3 μg/mL) was found to be more potent than all the reference quinolones (MIC from 6 to >100 μg/mL).

MIC values in Table 1 show that all the tested compounds exhibited relatively better inhibition of Gram positive bacteria than Gram negative ones. Among the latter, *Haemophilus influenzae* was found to be the most sensitive (MIC 0.015–6 μg/mL) and it was inhibited by **13a** and **13e–f** at the same concentrations of standard quinolones. A lower potency was exhibited by the above mentioned compounds, relative to the standard quinolones, for the inhibition of *A. baumannii* and *E. coli* and by **13f** towards *P. aeruginosa*, while




**Scheme 1.** Reagents and conditions: (i) $Pd(OAc)_2$, CsF, DMF, 70–100 °C; (ii) $P(OEt)_3$, 200 °C (MW); (iii) 10% aq HCl, EtOH, reflux.




**Scheme 2.** Reagents and conditions: (i) $Pd(OAc)_2$, CsF, DMF, 90 °C; (ii) $P(OEt)_3$, 200 °C (MW); (iii) 10% aq HCl, EtOH, reflux.

no activity was noted against multidrug-resistant Gram negative strains. Thus, **13f** was the most active quinolone against all tested microorganisms, with the exception of multidrug-resistant staphylococci that were more significantly inhibited by **13e**.

In order to reveal potential antifungal properties, the new compounds were tested on *Aspergillus niger*, *Candida tropicalis*, and *Saccharomyces cerevisiae*, but no activity was observed at, or below, the concentration of 100 μg/mL (data not shown).

Analysis of the structure–activity relationships shows that the different substituents at ring (D), fused onto the pyrroloquinolone scaffold, affect the antibacterial properties of the studied substances. Concerning the benzo-fused derivatives, bulky substituents on the benzene ring, such as a lipophilic methyl group or a hydrophilic methoxy group, did not improve the antibacterial activity of the parent analogue **13a**. In fact, MIC values of compound **13a** were always lower than those of compounds **13b–d**. Among these, the methyl derivative **13b** was active only against *B. subtilis*. Better activity was noted for the methoxy derivative **13c**, which showed excellent inhibition of all wild type Gram positive bacteria tested and Gram negative *H. influenzae* and also a moderate effect against *A. baumannii*. As expected, the introduction of two methyl groups in **13c**, that produces **13d**, led to decreased antibacterial activity. It is worth noting that the most effective compounds contained a fluorine atom. For instance, compound **13e**, bearing fluorine in the *para* position of the benzene ring, was the most active carbazole derivative. The electronic influence on the antibacterial activity of the selected substituents in the series **13a–e**, can be seen from the favorable effect of the electron-withdrawing fluorine (**13e**) and, conversely, the detrimental action of the electron-donating methyl and methoxy groups (**13b–d**).

The structure–activity relationships analysis of the data reported in Table 1 further shows that the thiophene isosteric replacement of the D benzene moiety played a positive, crucial role in the antibacterial effectiveness of the tested compounds. Hence, **13f** possessed the highest inhibitory properties as compared to the corresponding benzofused derivatives **13a–e**. However, it is noteworthy that, in the case of multidrug-resistant staphylococci, the presence of a fluoro substituted benzene ring (**13e**) contributed to an activity enhancement, with respect to the thiophene analogue **13f**.

### 2.2.2. DNA gyrase and topoisomerase IV inhibition

To elucidate the mechanism by which the novel tetracyclic fluoroquinolones induce antibacterial activity, the inhibitory activities of all the compounds **13a–f** were examined against DNA gyrase and topoisomerase IV isolated from *E. coli* (Table 2). Antibacterial quinolones exert their activity by targeting Gram negative bacteria DNA gyrase and Gram positive bacteria topoisomerase IV, and inhibiting DNA replication process.[2] We hypothesized that fluoroquinolones **13a–f** could afford favorable binding to the DNA–enzyme complex, but surprisingly we found in most cases moderate inhibition of DNA gyrase (compounds **13a–c** and **13e–f** $IC_{50}$ 1.1–4.8 μg/mL) and a weak inhibition of topoisomerase IV (compounds **13a** and **13c–e** $IC_{50}$ 28–48 μg/mL). All of the tested compounds displayed higher $IC_{50}$ values than the reference ciprofloxacin and moxifloxacin, nevertheless most of them, as reported below (Table 1), exhibit against Gram positive

**Table 1**
Inhibitory activity against Gram positive and Gram negative bacteria, expressed as MIC (μg/mL)

| Bacteria[a] | Compound[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **13a** | **13b** | **13c** | **13d** | **13e** | **13f** | CIP | LEV | MOX | GEM |
| *Gram positive* | | | | | | | | | | |
| BS | 0.015 | 0.07 | 0.07 | 0.07 | 0.003 | 0.003 | 0.03 | 0.03 | 0.015 | 0.007 |
| SA | 0.3 | >100 | 3 | 6 | 0.07 | 0.03 | 0.3 | 0.07 | 0.03 | 0.015 |
| SAR 72 | 12 | —[c] | >100 | >100 | 1.5 | 12 | 100 | 25 | 12 | 6 |
| SAR 4790 | >100 | — | >100 | >100 | >100 | >100 | >100 | 100 | 50 | 50 |
| SE | 0.15 | >100 | 3 | 25 | 0.07 | 0.03 | 0.07 | 0.15 | 0.07 | 0.015 |
| SER | >100 | — | >100 | >100 | 3 | 50 | 100 | >100 | 50 | 50 |
| *Gram negative* | | | | | | | | | | |
| AB | 12 | >100 | 50 | >100 | 12 | 3 | 0.7 | 0.15 | 0.15 | 0.3 |
| ABR | >100 | — | >100 | — | >100 | >100 | >100 | >100 | 50 | 100 |
| EC | 50 | >100 | >100 | >100 | 25 | 1.5 | 0.015 | 0.03 | 0.03 | 0.015 |
| ECR | >100 | — | — | — | >100 | >100 | 100 | 25 | 50 | 50 |
| HI | 0.03 | >100 | 1.5 | 6 | 0.15 | 0.015 | 0.15 | 0.15 | 0.03 | 0.015 |
| PA | >100 | >100 | >100 | >100 | >100 | 50 | 0.07 | 0.3 | 0.7 | 0.07 |
| PAR | — | — | — | — | — | >100 | 25 | 50 | >100 | >100 |

[a] BS, *Bacillus subtilis* ATCC 6633; SA, *Staphylococcus aureus* ATCC 6538; SAR 72 and SAR 4790, *Staphylococcus aureus* quinolone- and penicillin-resistant clinical isolates; SE, *Staphylococcus epidermidis* ATCC 12228; SER, *Staphylococcus epidermidis* quinolone- and penicillin-resistant clinical isolate; AB, *Acinetobacter baumannii* ATCC 19606; ABR, *Acinetobacter baumannii* quinolone- and penicillin-resistant clinical isolate; EC, *Escherichia coli* ATCC 8739; ECR, *Escherichia coli* quinolone- and penicillin-resistant clinical isolate; HI, *Haemophilus influenzae* ATCC 19418; PA, *Pseudomonas aeruginosa* ATCC 9027; PAR, *Pseudomonas aeruginosa* quinolone- and penicillin-resistant clinical isolate.
[b] CIP, ciprofloxacin; LEV, levofloxacin; MOX, moxifloxacin; GEM, gemifloxacin.
[c] Not tested because inactive against the corresponding quinolone-sensitive microorganism.

**Table 2**
Inhibitory activity against DNA gyrase and topoisomerase IV of *E. coli*, expressed as 50% inhibitory concentration (μg/mL)

| Compound | $IC_{50}$ | |
|---|---|---|
| | Gyrase[a] | Topoisomerase IV[b] |
| **13**° | 1.7 | 35 |
| **13b** | 3.0 | >300 |
| **13c** | 4.8 | 48 |
| **13d** | >30 | 31 |
| **13e** | 1.8 | 28 |
| **13f** | 1.1 | 100 |
| Ciprofloxacin | 0.34 | 4.6 |
| Moxifloxacin | 0.85 | 5.0 |

Representative results of at least three independent experiments are reported.
[a] *E. coli* DNA gyrase supercoiling assay.
[b] *E. coli* topoisomerase IV decatenation assay.

**Table 3**
Effects of compounds **13a–f** and ellipticine on MCF-7, A549, and HuDE cell viability

| Compound | $IC_{50}$ MCF-7 (μM) | $IC_{50}$ A549 (μM) | $IC_{50}$ HuDe (μM) |
|---|---|---|---|
| **13a** | 3.6 ± 1.09 | 6.4 ± 1.02 | >10 |
| **13b** | >10 | >10 | >10 |
| **13c** | 2.4 ± 1.05 | 4.9 ± 1.01 | >10 |
| **13d** | 1.7 ± 1.04 | 3 ± 1.03 | >10 |
| **13e** | 0.8 ± 1.04 | 3.5 ± 1.05 | >10 |
| **13f** | 4.4 ± 1.09 | 6.4 ± 1.07 | >10 |
| Ellipticine | 1.6 ± 1.16 | 3.4 ± 1.04 | >10 |

Cells were treated with indicated compounds at concentrations ranging from 0.1 to 20 μM for 72 h and then viability was determined by MTT assay. Concentration that inhibits 50% ($IC_{50}$) (e.g., the point at which viability is 50%) was extrapolated from the dose–response curves. Representative results of at least three independent experiments are reported.

bacteria lower or comparable MICs in respect to that of the reference quinolones. Lack of correlation between the MICs and the $IC_{50}$s indicates that inhibition of bacterial cell growth, when detected for compounds **13a–f**, was not mainly caused by inhibition of the topoisomerase IV and suggests that other different mechanisms could be involved in the antibacterial effect.

### 2.2.3. Antitumor activity

The antitumor activity of compounds **13a–f** was assayed with respect to ellipticine by evaluating cell proliferation in MCF-7 breast cancer and in A549 non-small cell lung cancer (NSCLC) cell lines and cell cycle distribution, apoptosis induction and p53 expression in MCF-7 cell line.

After 72 h, all the tested compounds showed significant inhibition of cell proliferation in a dose-dependent manner (Table 3). Ellipticine showed $IC_{50}$ of 1.6 μM. The most interesting compounds (**13c–e**) had comparable $IC_{50}$ to the reference substance, ranging between 0.8 and 2.4 μM against MCF-7 cells and between 3 and 4.9 μM against A549 cells. In contrast, compound **13b** showed a reduced effect on cell proliferation against both cell lines ($IC_{50}$ >10 μM).

The structure–activity relationships analysis of the data reported in Table 3 shows that among carbazole analogues **13a–e**, a lipophilic and electron-donor substituent, such as a methyl group (**13b**) at the *para* position of the D benzene ring, significantly decreased the antiproliferative activity on both cell lines ($IC_{50}$ >10 μM). By contrast, substitution of the parent compound **13a** with a hydrophilic and electron-donor methoxy group (to give **13c**), resulted in increased potency ($IC_{50}$ 2.4 and 4.9 μM against MCF-7 and A549 cells, respectively), compared to **13a** ($IC_{50}$ 3.6 and 6.4 μM against MCF-7 and A549 cells, respectively). Compound **13e**, bearing a fluoro substituent with electron-withdrawing properties, had a fourfold increased potency in breast cells ($IC_{50}$ 0.8 μM) compared to the unsubstituted analogue **13a** ($IC_{50}$ 3.6 μM) and was even more active than the reference ellipticine ($IC_{50}$ 1.6 μM). A significantly increased activity was also observed for **13e** against A549 cells ($IC_{50}$ 3.5 vs 6.4 μM). Interestingly, the 4-methoxy-3,5-dimethyl derivative **13d** was more potent than the 4-methoxy substituted carbazole **13c**, showing that the presence of two small lipophilic and electron-donor methyl groups in the 3 and 5 positions was beneficial. This led us to speculate that the biological target responsible for the antiproliferative activity of the fluoroquinolones/carbazoles under study has two additional drug-binding sites, with two small hydrophobic pockets at the 3 to 5 benzene positions distance.

Replacement of the D benzene ring of compound **13a** with its heterocyclic bioisoster thiophene (**13f**) did not significantly modify the antiproliferative activity, further evidence for the bioisosteric equivalence between benzene and thiophene rings.

To examine the mechanism responsible for cell growth inhibition, cell cycle distribution was evaluated using flow cytometric

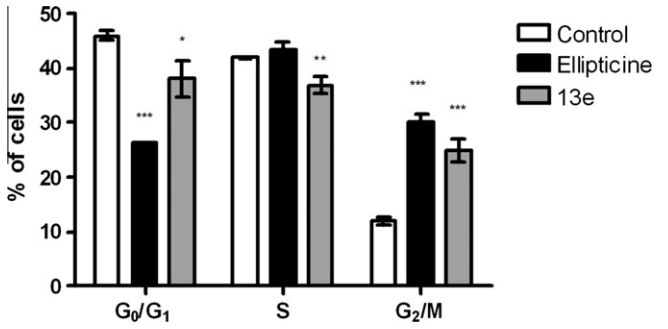



**Figure 3.** Determination of cell cycle distribution. MCF-7 cells were incubated in the absence or in the presence of 5 μM of ellipticine and compound **13e**, and after 24 h cells were stained with propidium iodide and analyzed by flow cytometry for cell cycle–phase distribution. $N = 3$. Values are means ± SD $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

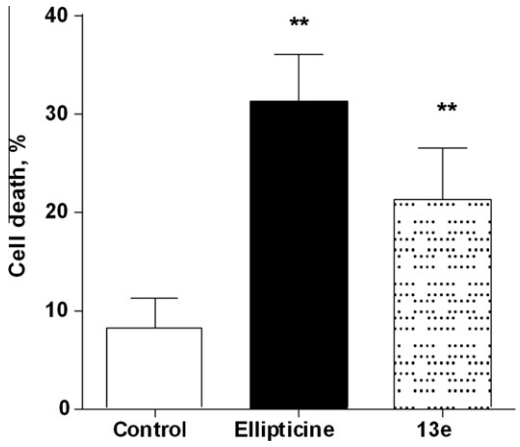



**Figure 4.** Determination of cell death after treatment with ellipticine and compound **13e**. MCF-7 cells were treated with ellipticine and compound **13e** at 5 μM. Cell death was quantitated after 48 h by fluorescence microscopy on Hoechst 33342/PI stained cells. Data are expressed as percent values. Columns show means of three separate experiments, bars SD. $^{**}P < 0.01$.

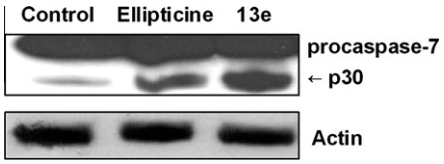



**Figure 5.** Cleavage of pro-caspase-7 after treatment with ellipticine and compound **13e**. MCF-7 cells were incubated with each compound at 5 μM for 24 h. Cells lysates were analyzed by Western blotting to assess the expression of pro-caspase-7, the p30 fragment and actin proteins. Representative blot of three independent experiments is reported.

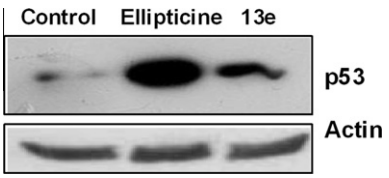



**Figure 6.** Modulation of p53 protein expression during treatment with ellipticine and compound **13e**. MCF-7 cells were incubated with each compound at 5 μM for 24 h. Cells lysates were analyzed by Western blotting to assess the expression of p53 and actin proteins. Representative blot of three independent experiments is reported.

analysis in MCF-7 cells. To determine the effect of ellipticine and the most active compound **13e** on MCF-7 cell cycle distribution, cells treated with 5 μM for 24 h were analyzed by flow cytometry. As shown in Figure 3, both ellipticine and **13e** caused a significant increase in the proportion of cells in $G_2$/M phase with a decrease in $G_0/G_1$ phase.

Using fluorescence microscopy analysis with Hoechst 33342/PI staining (Fig. 4), we demonstrated that treatment of MCF-7 cells with ellipticine and with compound **13e** at 5 μM for 48 h was associated with the induction of cell death. Western blotting analysis revealed that programmed cell death by apoptosis was involved in the loss of viability. As shown in Figure 5, the activation and cleavage of caspase 7 (MCF-7 cells are caspase 3 null) was detected in MCF-7 cells treated with ellipticine and with **13e**.

It has been reported that in MCF-7 ellipticine treatment resulted in an increase of p53 and KIP1/p27.[12] As shown by Western blot analysis in Figure 6, ellipticine treatment induced p53 accumulation in MCF-7 cells expressing wild type p53 protein. In contrast, compound **13e** induced only a modest increase of p53 protein, suggesting that other mechanisms were involved in its antiproliferative effect.

The compounds were also evaluated against normal human-derm fibroblasts (HuDe) and, as shown in the Table 3, no effect on cell proliferation and viability was detected until 10 μM indicating a selectivity of action, toward tumor cells coupled with a lack of cytotoxicity towards normal cells.

In summary, the selective antiproliferative effect observed for the fluoroquinolone **13e** as a representative of the novel fluoroquinolones with a carbazole core (**13a**–**e**) or with a bioisosterically thieno related structure (**13f**) seems to be due to multiple mechanisms.

## 3. Conclusion

Novel 1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydropyrido[2,3-*a*]-carbazole-3-carboxylic acids (**13a**–**e**) and the structurally related 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylic acid (**13f**) were synthesized by efficient synthetic routes and their antibacterial and antiproliferative activity assessed. The six tetracyclic fluoroquinolones exhibited high antibacterial activity against Gram positive strains, including a few resistant ones. Furthermore, most of them had high antiproliferative activity against breast MCF-7 and lung A549 tumor cell lines, and were devoid of cytoxicity against normal cells. **13e** emerged as the most active antibacterial compound against multidrug-resistant staphylococci and the most potent antiproliferative compound against MCF-7 cells.

While the investigation at the molecular level identified only in part the targets and the binding modes of these new molecules need to be further elucidated, the combination of potent activity against Gram positive bacteria and cancer cell lines, the absence of cytoxicity against normal cells, make these agents of interest in the search for potential cancer chemotherapeutics. These compounds, possessing both anticancer and antibacterial activity, have a promising therapeutic potential due to their selective cytoxicity coupled with the ability to reduce the danger of bacterial infections in the frequently immunocompromised cancer patient. This is supported by recent clinical data,[49] demonstrating a positive effect of quinolone antimicrobials on cancer patients' survival, and makes the multiple targeted approach, as here, a promising avenue for drug development.

## 4. Experimental

### 4.1. Chemistry

2,4-Dichloro-5-fluoro-3-nitrobenzoic acid, ethyl 3-(*N*,*N*-dimethylamino)acrylate, cyclopropylamine, palladium(II) acetate, cesium fluoride, trimethyltin chloride, and triethyl phosphite were

purchased from Acros. Bromobenzene, 4-bromotoluene, 4-bromoanisole, 4-bromofluorobenzene and 4-bromo-2,6-dimethylanisole were purchased from Aldrich. Silica gel for column chromatography was purchased from Macherey-Nagel GmbH & Co (Germany).

Melting points (uncorrected) were determined on a Stuart scientific melting point apparatus in open capillary tubes. $^1$H and $^{13}$C NMR spectra were recorded on a 300 MHz spectrometer (Bruker DPX-300) in DMSO-$d_6$ or $CDCl_3$ with *TMS* as the internal standard. Chemical shifts are expressed in $\delta$ units; $^1$H–$^1$H, H–F, and C–F coupling constants are given in Hertz. Electron-impact mass spectra (EIMS) were obtained using a Finnegan MAT TSQ-70 spectrometer at 70 eV; ion source temperature: 200 °C. High resolution mass spectra (HRMS) were measured by electrospray ionization (ESI) technique on a Bruker APEX-IV instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v mixed with 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. Microwave irradiation experiments, conducted for the Cadogan-nitrene insertion reactions, were carried out with a Biotage Initiator 2.0 Microwave Synthesizer instrument. Elemental analyses were performed on a Euro Vector Elemental Analyzer (EA 3000 A).

#### 4.1.1. Synthesis of ethyl 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (9)

This compound was prepared from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid and ethyl 3-(*N*,*N*-dimethylamino)acrylate, according to the literature procedure.[5,44,45]

#### 4.1.2. General procedure for the synthesis of ethyl 7-aryl-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylates (11a–e)

The appropriate aryltrimethyl stannane (**10a–e**)[32,33] (3.2 mmol), $Pd(OAc)_2$ (0.065 g, 0.29 mmol), and CsF (0.44 g, 2.9 mmol) were successively added to a stirred solution of **9** (2.8 mmol) in DMF (4 mL). The reaction mixture was heated at 70–100 °C under nitrogen atmosphere for 8–16 h. The resulting solution was then cooled to rt and quenched with water (10 mL), producing a black gummy precipitate. After filtration, the latter crude product was dissolved in $CHCl_3$, filtered, dried over anhydrous $Na_2SO_4$, and the solvent was evaporated under reduced pressure to give a brown residue which was purified by column chromatography using silica gel and eluting with chloroform/ethyl acetate. The final product was crystallized from dichloromethane/*n*-hexane.

**4.1.2.1. Ethyl 1-cyclopropyl-6-fluoro-8-nitro-4-oxo-7-phenyl-1,4-dihydroquinoline-3-carboxylate (11a).** Yellow solid (reaction temperature: 100 °C; reaction time: 16 h; ratio of the eluting mixture: 1:2, v/v). Yield 0.50 g (45%); mp 205–206 °C. Anal. Calcd for $C_{21}H_{17}FN_2O_5$ (396.37): C, 63.63; H, 4.32; N, 7.07. Found: C, 63.21; H, 4.04; N, 6.83. IR (KBr) $\nu_{max}$: 3098, 2993, 1729, 1638, 1614, 1541, 1460, 1441, 1367, 1337, 1311, 1271, 1228, 1195, 1173, 1138, 1105, 1030, 855, 801, 760, and 698 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): 1.01–1.11 (m, 4H, 2 H-2′ 2 H-3′), 1.39 (t, 3H, *J* = 7.1, C*H*$_3$CH$_2$O–), 3.59–3.66 (m, 1H, H-1′), 4.38 (q, *J* = 7.1 Hz, 2H, –OC*H*$_2$Me), 7.28 (dd, *J* = 7.0, 1.4 Hz, 2H, H-2″/H-6″), 7.44 (dd, *J* = 6.0, 1.4 Hz, 1H, H-4″), 7.46 (dd, *J* = 6.0, 7.0 Hz, 2H, H-3″/H-5″), 8.34 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-5), 8.64 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 10.9 (C-2′/C-3′), 14.4 (*C*H$_3$CH$_2$O–), 37.9 (C-1′), 61.4 (–OCH$_2$Me), 111.7 (C-3), 115.6 (d, $^2J_{C-F}$ = 24.8 Hz, C-5), 128.6 (C-4″), 128.7 (C-2″/C-6″), 129.0 (d, $^3J_{C-F}$ = 6.5 Hz, C-4a), 129.7 (C-3″/C-5″), 130.0 (C-1″), 130.2 (C-8a), 131.4 (d, $^2J_{C-F}$ = 22.5 Hz, C-7), 141.6 (C-8), 151.7 (C-2), 156.0 (d, $^1J_{C-F}$ = 249 Hz, C-6), 164.4 (*C*O$_2$Et), 171.3 (d, $^4J_{C-F}$ = 2 Hz, C-4); HRMS (ESI) *m*/*z*: calcd for $C_{21}H_{18}FN_2O_5$ $[M+H]^+$: 397.11997. Found 397.11945; EIMS *m*/*z* (%): 396 ($M^+$, 34), 379 (70), 351 (42), 324 (72), 307 (78), 279 (96), 277 (100%), 264 (62), 251 (50), 222 (57), 208 (38), 203 (36), 176 (26), 133 (18), 120 (13).

**4.1.2.2. Ethyl 1-cyclopropyl-6-fluoro-7-(4-methylphenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11b).** Yellow solid (reaction temperature: 90 °C; reaction time: 10 h; ratio of the eluting mixture: 1:5, v/v). Yield 0.78 g (68%); mp 217–219 °C. Anal. Calcd for $C_{22}H_{19}FN_2O_5$ (410.40): C, 64.39; H, 4.67; N, 6.83. Found: C, 64.29; H, 4.29; N, 6.46. IR (KBr) $\nu_{max}$: 3114, 3079, 2986, 1728, 1635, 1613, 1542, 1459, 1384, 1366, 1340, 1308, 1277, 1193, 1169, 1135, 1105, 1037, 1022, 951, 803, and 768 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.99–1.11 (m, 4H, 2 H-2′/2 H-3′), 1.40 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$O–), 2.40 (s, 3H, C(4″)–CH$_3$), 3.58–3.67 (m, 1H, H-1′), 4.40 (q, *J* = 7.1 Hz, 2H, –OC*H*$_2$Me), 7.18 (d, *J* = 8.1 Hz, 2H, H-2″/H-6″), 7.27 (d, *J* = 8.1 Hz, 2H, H-3″/H-5″), 8.38 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-5), 8.65 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 10.9 (C-2′/C-3′), 14.4 (*C*H$_3$CH$_2$O–), 21.4 (C(4″)–*C*H$_3$), 37.8 (C-1′), 61.4 (–OCH$_2$Me), 111.7 (C-3), 115.6 (d, $^2J_{C-F}$ = 24.8 Hz, C-5), 125.9 (C-1″), 128.5 (C-2″/C-6″), 129.5 (C-8a), 129.6 (C-3″/C-5″), 130.2 (d, $^2J_{C-F}$ = 23.2 Hz, C-7), 131.2 (d, $^3J_{C-F}$ = 6.8 Hz, C-4a), 139.9 (C-4″), 141.6 (C-8), 151.7 (C-2), 156.1 (d, $^1J_{C-F}$ = 248 Hz, C-6), 164.6 (*C*O$_2$Et), 171.4 (d, $^4J_{C-F}$ = 2.0 Hz, C-4); HRMS (ESI) *m*/*z*: calcd for $C_{22}H_{20}FN_2O_5$ $[M+H]^+$: 411.13562. Found 411.13501; EIMS *m*/*z* (%): 410 ($M^+$, 31), 393 (70), 365 (42), 338 (66), 321 (89), 308 (62), 291 (100%), 278 (75), 265 (65), 236 (49), 222 (38), 216 (23), 183 (21), 133 (15), 118 (12).

**4.1.2.3. Ethyl 1-cyclopropyl-6-fluoro-7-(4-methoxyphenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11c).** Yellow solid (reaction temperature: 75 °C; reaction time: 10 h; ratio of the eluting mixture: 1:5, v/v). Yield 0.75 g (62%); mp 207–208 °C. Anal. Calcd for $C_{22}H_{19}FN_2O_6$ (426.40): C, 61.97; H, 4.49; N, 6.57. Found: C, 61.57; H, 4.36; N, 6.20. IR (KBr) $\nu_{max}$: 3092, 3012, 2989, 2935, 1733, 1611, 1539, 1523, 1458, 1344, 1298, 1248, 1176, 1111, 1027, 836, 806, and 695 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.00–1.12 (m, 4H, 2 H-2′/2 H-3′), 1.40 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$O–), 3.59–3.67 (m, 1H, H-1′), 3.84 (s, 3H, C(4″)–OCH$_3$), 4.39 (q, *J* = 7.1 Hz, 2H, –OC*H*$_2$Me), 6.98 (d, *J* = 8.6 Hz, 2H, H-3″/H-5″), 7.23 (d, *J* = 8.6 Hz, 2H, H-2″/H-6″), 8.37 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-5), 8.65 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 11.0 (C-2′/C-3′), 14.4 (*C*H$_3$CH$_2$O–), 37.8 (C-1′), 55.4 (C(4″)–OCH$_3$), 61.4 (–OCH$_2$Me), 111.6 (C-3), 114.3 (C-3″/C-5″), 115.5 (d, $^2J_{C-F}$ = 25.3 Hz, C-5), 120.7 (C-1″), 129.9 (d, $^2J_{C-F}$ = 22.8 Hz, C-7), 130.1 (C-2″/C-6″), 131.1 (d, $^3J_{C-F}$ = 6.6 Hz C-4a), 131.2 (C-8a), 141.7 (d, $^3J_{C-F}$ = 1.4 Hz, C-8), 151.7 (C-2), 156.3 (d, $^1J_{C-F}$ = 248 Hz, C-6), 160.7 (C-4″), 164.6 (*C*O$_2$Et), 171.4 (d, $^4J_{C-F}$ = 1.8 Hz, C-4); HRMS (ESI) *m*/*z*: calcd for $C_{22}H_{20}FN_2O_6$ $[M+H]^+$: 427.13054. Found 427.12997; EIMS *m*/*z* (%): 426 ($M^+$, 42), 409 (76), 368 (29), 354 (54), 337 (98), 308 (100%), 294 (80), 281 (63), 253 (48), 239 (30), 195 (24), 169 (18), 132 (16), 111 (13).

**4.1.2.4. Ethyl 1-cyclopropyl-6-fluoro-7-(4-methoxy-3,5-dimethylphenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11d).** Yellow solid (reaction temperature: 70 °C; reaction time: 12 h; ratio of the eluting mixture: 1:3, v/v). Yield 0.9 g (70%); mp 243–245 °C. Anal. Calcd for $C_{24}H_{23}FN_2O_6$ (454.45): C, 63.43; H, 5.10; N, 6.16. Found: C, 63.09; H, 4.96; N, 5.96. IR (KBr) $\nu_{max}$: 3073, 3008, 2970, 1731, 1613, 1599, 1536, 1487, 1453, 1365, 1341, 1311, 1258, 1231, 1198, 1175, 1134, 1110, 1050, 1028, 1010, 807, and 769 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.01–1.10 (m, 4H, 2 H-2′/2 H-3′), 1.39 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$O–), 2.28 (s, 6H, C (3″)–CH$_3$/C(5″)–CH$_3$), 3.58–3.66 (m, 1H, H-1′), 3.74 (s, 3H,

C(4″)–$OCH_3$), 4.38 (q, $J$ = 7.1 Hz, 2H, –$OCH_2$Me), 6.92 (s, 2H, H-2″/H-6″), 8.32 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-5), 8.63 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 1.0 (C-2′/C-3′), 14.4 ($CH_3CH_2O$–), 16.2 (s, C (3″)–$CH_3$/C(5″)–$CH_3$), 37.9 (C-1′), 59.8 (C(4″)–$OCH_3$), 61.4 (–$OCH_2$Me), 111.6 (C-3), 114.7 (C-3″/C-5″), 115.4 (d, $^2J_{C-F}$ = 25.1 Hz, C-5), 124.0 (C-1″), 129.1 (C-2″/C-6″), 129.9 (d, $^3J_{C-F}$ = 2.8 Hz, C-8a), 130.1 (d, $^2J_{C-F}$ = 23.3 Hz, C-7), 131.1 (d, $^3J_{C-F}$ = 6.8 Hz, C-4a), 141.5 (C-8), 151.7 (C-2), 156.1 d, $^1J_{C-F}$ = 248 Hz, C-6), 158.2 (C-4″), 164.5 ($CO_2$Et), 171.4 (C-4); HRMS (ESI) $m/z$: calcd for $C_{24}H_{24}FN_2O_6$ $[M+H]^+$: 455.16184. Found 455.16130; EIMS $m/z$ (%): 454 ($M^+$, 34), 437 (50), 396 (22), 382 (40), 365 (60), 334 (46), 322 (64), 289 (52), 262 (80), 245 (94), 233 (86), 216 (100%), 203 (92), 176 (71), 162 (53), 133 (55), 120 (38), 107 (34).

**4.1.2.5. Ethyl 1-cyclopropyl-6-fluoro-7-(4-fluorophenyl)-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (11e).** Yellow solid (reaction temperature: 80 °C; reaction time: 16 h; ratio of the eluting mixture: 1:2, v/v). Yield 0.4 g (34%); mp 224–226 °C. Anal. Calcd for $C_{21}H_{16}F_2N_2O_5$ (414.36): C, 60.87; H, 3.89; N, 6.76. Found: C, 60.35; H, 3.72; N, 6.53. IR (KBr) $\nu_{max}$: 3096, 3050, 3015, 2977, 1735, 1638, 1616, 1534, 1512, 1451, 1387, 1341, 1308, 1284, 1270, 1230, 1174, 1158, 1136, 1103, 1035, 832, 804, and 790 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.01–1.10 (m, 4H, 2 H-2′/2 H-3′), 1.40 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 3.57–3.66 (m, 1H, H-1′), 4.39 (q, $J$ = 7.1 Hz, 2H, –$OCH_2$Me), 7.16 (dd, $^3J_{H-F}$ = 8.5 Hz, $J$ = 8.7 Hz, 2H, H-3″/H-5″), 7.28 (dd, $^4J_{H-F}$ = 3 Hz, $J$ = 8.7 Hz, 2H, H-2″/H-6″), 8.38 (d, $^3J_{H-F}$ = 8.5 Hz, 1H, H-5), 8.65 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 11.0 (C-2′/C-3′), 14.4 ($CH_3CH_2O$–), 37.9 (C-1′), 61.5 (–$OCH_2$Me), 111.8 (C-3), 115.7 (d, $^2J_{C-F}$ = 24.8 Hz, C-5), 116.1 (d, $^2J_{C-F}$ = 22 Hz, C-3″/C-5″), 124.7 (d, $^4J_{C-F}$ = 2.6 Hz, C-1″), 129.0 (d, $^2J_{C-F}$ = 23.2 Hz, C-7), 129.9 (d, $^4J_{C-F}$ = 2.7 Hz, C-8a), 130.7 (d, $^3J_{C-F}$ = 8.5 Hz, C-2″/C-6″), 131.5 (d, $^3J_{C-F}$ = 6.8 Hz, C-4a), 141.7 (C-8), 151.7 (C-2), 156.0 (d, $^1J_{C-F}$ = 249 Hz, C-6), 163.6 (d, $^1J_{C-F}$ = 249 Hz, C-4″), 164.4 ($CO_2$Et), 171.2 (d, $^4J_{C-F}$ 2.1 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{21}H_{17}F_2N_2O_5$ $[M+H]^+$: 415.11055. Found 415.11001; EIMS $m/z$ (%): 414 ($M^+$, 28), 397 (62), 369 (42), 342 (78), 325 (89), 313 (86), 296 (100%), 282 (69), 269 (60), 240 (67), 226 (52), 201 (42), 188 (33), 175 (25), 133 (18), 120 (13).

**4.1.2.6. Ethyl 1-cyclopropyl-6-fluoro-8-nitro-4-oxo-7-(thien-2-yl)-1,4-dihydroquinoline-3-carboxylate (11f).** This compound was prepared from 2-thienyltrimethyl stannane (**10f**)[34] (0.8 g, 3.24 mmol) and **9** (1.0 g, 2.82 mmol) by following similar procedure described above for compounds **11a**–**e**. Yellow solid (reaction temperature: 90 °C; reaction time: 8 h; ratio of the eluting mixture 1:1, v/v). Yield 0.45 g (40%); mp: 193–195 °C. Anal. Calcd for $C_{19}H_{15}FN_2O_5S$ (402.40): C, 56.71; H, 3.76; N, 6.96. Found: C, 56.55; H, 3.52; N, 6.57. IR (KBr) $\nu_{max}$: 3107, 2989, 1728, 1638, 1612, 1542, 1459, 1386, 1366, 1332, 1314, 1267, 1241, 1173, 1135, 1099, 1046, 1032, 820, 801, 715, 693 cm$^{-1}$; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.04–1.13 (m, 4H, 2 H-2′/2 H-3′), 1.39 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 3.58–3.67 (m, 1H, H-1′), 4.38 (q, $J$ = 7.1 Hz, 2H, –$OCH_2$Me), 7.13 (m, 2H, H-3″ + H-4″), 7.56 (m, 1H, H-5″), 8.36 (d, $^3J_{H-F}$ = 8.6 Hz, 1H, H-5), 8.64 (s, 1H, H-2); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 11.1 (C-2′/C-3′), 14.4 ($CH_3CH_2O$–), 37.8 (C-1′), 61.5 (–$OCH_2$Me), 111.6 (C-3), 115.5 (d, $^2J_{C-F}$ = 24.8 Hz, C-5), 123.5 (d, $^2J_{C-F}$ = 29 Hz, C-7), 127.0 (C-2″), 127.4, 130.1 (C-3″/C-4″), 129.4 (C-5″), 130.0 (d, $^4J_{C-F}$ = 2.9 Hz, C-8a), 131.8 (d, $^3J_{C-F}$ = 6.9 Hz, C-4a), 142.0 (C-8), 151.8 (C-2), 156.2 (d, $^1J_{C-F}$ = 250 Hz, C-6), 164.4 ($CO_2$Et), 171.2 (C-4); HRMS (ESI) $m/z$: calcd for $C_{19}H_{16}FN_2O_5S$ $[M+H]^+$: 403.0764. Found 403.07586; EIMS $m/z$ (%): 402 ($M^+$, 70), 385 (79), 357 (51), 344 (57), 330 (100%), 313 (91), 284 (81), 272 (54), 246 (51), 214 (47), 202 (24), 189 (17), 145 (14), 132 (13), 105 (8).

**4.1.3. General procedure for synthesis of ethyl 1-cyclopropyl-6-fluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylates (12a–e) and ethyl 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylate (12f)**

The appropriate ethyl 7-aryl-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**11a**–**f**) (0.55–0.85 mmol) and $P(OEt)_3$ (3–4 mL) were placed in a 10 mL biotage reactor glass vial. The vial was sealed tightly with an aluminum Teflon crimp® top and the mixture irradiated with microwaves (initial power level, 400 W) for 1.5–2 h at a preselected temperature of 200 °C. The vial was then cooled to 20 °C in a gas jet and excess $P(OEt)_3$ was evaporated in vacuo; the residual solid was treated with $CHCl_3$ ($3 \times 4$ mL). The respective title products **12a**–**f** were collected by suction filtration and crystallized from DMF as light yellow solids.

**4.1.3.1. Ethyl 1-cyclopropyl-6-fluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylate (12a).** Prepared from **11a** (0.30 g, 0.75 mmol); irradiation time: 1.75 h. Yield 0.10 g (36%); mp 278–280 °C. Anal. Calcd for $C_{21}H_{17}FN_2O_3$ (364.37): C, 69.22; H, 4.70; N, 7.69. Found: C, 69.05; H, 4.62; N, 7.53. IR (KBr) $\nu_{max}$: 3481, 3072, 2970, 1738, 1718, 1606, 1568, 1486, 1448, 1422, 1366, 1347, 1257, 1227, 1171, 1111, 1037, 802, 743 and 657 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.19–1.22 (m, 2H) and 1.40–1.47 (m, 2H) (2 H-2′/2 H-3′), 1.27 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 4.21 (q, $J$ = 7.1 Hz, 2H, –$CH_2$Me), 4.44–4.53 (m, 1H, H-1′), 7.32 (ddd, $J$ = 7.7, 7.5, 1.1 Hz, 1H, H-8), 7.55 (ddd, $J$ = 8.2, 7.7, 1.0 Hz, 1H, H-9), 7.66 (d, $^3J_{H-F}$ = 10.8 Hz, 1H, H-5), 7.80 (dd, $J$ = 8.2, 1.1 Hz, 1H, H-10), 8.15 (dd, $J$ = 7.5, 1.0 Hz, 1H, H-7), 8.55 (s, 1H, H-2), 11.65 (s, 1H, N(11)–H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 14.8 ($CH_3CH_2O$–), 38.2 (C-1′), 60.3 (–$CH_2$Me), 100.6 (d, $^2J_{C-F}$ = 20.9 Hz, C-5), 109.8 (C-3), 113.0 (C-10), 115.3 (d, $^2J_{C-F}$ = 23.1 Hz, C-6a), 118.8 (d, $^3J_{C-F}$ = 2.3 Hz, C-6b), 121.2 (C-8), 122.4 (d, $J$ = 3.9 Hz, C-7), 126.7 (d, $^4J_{C-F}$ = 1.7 Hz, C-11b), 127.5 (C-9), 127.7 (d, $^3J_{C-F}$ = 6.9 Hz, C-4a), 130.8 (d, $^3J_{C-F}$ = 10.0 Hz, C-11a), 140.5 (C-10a), 148.7 (C-2), 155.7 (d, $^1J_{C-F}$ = 244 Hz, C-6), 164.9 ($CO_2$Et), 172.3 (d, $^4J_{C-F}$ = 2.1 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{21}H_{18}FN_2O_3$ $[M+H]^+$): 365.13015. Found: 365.12949; EIMS $m/z$ (%): 364 ($M^+$, 30), 319 (7), 292 (100%), 291 (30), 263 (21), 236 (14), 221 (13), 131 (6).

**4.1.3.2. Ethyl 1-cyclopropyl-6-fluoro-9-methyl-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*] carbazole-3-carboxylate (12b).** Prepared from **11b** (0.25 g, 0.61 mmol); irradiation time: 1.5 h. Yield 0.14 g (61%); mp 293–295 °C. Anal. Calcd for $C_{22}H_{19}FN_2O_3$ (378.40): C, 69.83; H, 5.06; N, 7.40. Found: C, 69.58; H, 4.93; N, 7.24. IR (KBr) $\nu_{max}$: 3210, 2970, 1738, 1721, 1633, 1597, 1563, 1486, 1453, 1366, 1347, 1323, 1233, 1173, 1114, 1039, 861, 801, and 633 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18–1.22 (m, 2H) and 1.40–1.46 (m, 2H) (2 H-2′/2 H-3′), 1.27 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 2.51 (s, 3H, C(9)–$CH_3$), 4.21 (q, $J$ = 7.1 Hz, 2H, –$OCH_2$Me), 4.41–4.51 (m, 1H, H-1′), 7.14 (d, $J$ = 8 Hz, 1H, H-8), 7.58 (s, 1H, H-10), 7.64 (d, $^3J_{H-F}$ = 10.8 Hz, 1H, H-5), 8.01 (d, $J$ = 8 Hz, 1H, H-7), 8.53 (s, 1H, H-2), 11.46 (s, 1H, N(11)–H); $^{13}$C NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 14.8 ($CH_3CH_2O$–), 22.3 (C(9)–$CH_3$), 38.2 (C-1′), 60.3 (–$OCH_2$Me), 100.6 (d, $^2J_{C-F}$ = 20.9 Hz, C-5), 109.8 (C-3), 112.6 (C-10), 115.5 (d, $^2J_{C-F}$ = 22.1 Hz, C-6a), 116.7 (d, $^3J_{C-F}$ = 2.3 Hz, C-6b), 122.1 (d, $J$ = 2.4 Hz, C-7), 122.9 (C-8), 126.6 (d, $^4J_{C-F}$ = 1.6 Hz, C-11b), 127.4 (d, $^3J_{C-F}$ = 8 Hz, C-4a), 130.7 (d, $^3J_{C-F}$ = 10.2 Hz, C-11a), 137.3 (C-9), 141.0 (C-10a), 148.5 (C-2), 155.5 (d, $^1J_{C-F}$ = 243 Hz, C-6), 165.0 ($CO_2$Et), 172.3 (d, $^4J_{C-F}$ = 2.1 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{22}H_{20}FN_2O_3$ $[M+H]^+$: 379.14581. Found: 379.14524; EIMS $m/z$ (%): 378 ($M^+$, 37), 333 (9), 307 (22), 306 (100%), 277 (20), 250 (12), 221 (8).

**4.1.3.3. Ethyl 1-cyclopropyl-6-fluoro-9-methoxy-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylate (12c).** Prepared from **11c** (0.30 g, 0.70 mmol); irradiation time: 1 h. Yield 0.13 g (47%); mp 286–288 °C. Anal. Calcd for $C_{22}H_{19}FN_2O_4$ (394.40): C, 67.00; H, 4.86; N, 7.10. Found: 66.76; H, 4.57; N, 6.82. IR (KBr) $\nu_{max}$; 3471, 3076, 2989, 2934, 2836, 1709, 1636, 1607, 1571, 1488, 1451, 1422, 1342, 1322, 1274, 1233, 1196, 1165, 1140, 1109, 1066, 1037, 801 and 636 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18–1.25 (m, 2H) and 1.41–1.48 (m, 2H) (2 H-2′/2 H-3′), 1.26 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 3.86 (s, 3H, C(9)–$OCH_3$), 4.18 (q, $J$ = 7.1 Hz, 2H, –$OCH_2Me$), 4.36–4.46 (m, 1H, H-1′), 6.91 (dd, $J$ = 8.6, 2 Hz, 1H, H-8), 7.25 (d, $J$ = 2.1 Hz, 1H, H-10), 7.58 (d, $^3J_{H-F}$ = 10.8 Hz, 1H, H-5), 7.95 (d, $J$ = 8.6 Hz, 1H, H-7), 8.48 (s, 1H, H-2), 11.47 (s, 1H, N(11)–H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 14.8 ($CH_3CH_2O$–), 38.2 (C-1′), 55.9 (C(9)–$OCH_3$), 60.3 (–$OCH_2Me$), 95.5 (C-10), 100.8 (d, $^2J_{C-F}$ = 20.9 Hz, C-5), 109.6 (C-3), 111.4 (C-8), 112.7 (d, $^3J_{C-F}$ = 2.0 Hz, C-6b), 115.7 (d, $^2J_{C-F}$ = 23.4 Hz, C-6a), 123.3 (d, $J$ = 3.9 Hz, C-7), 126.4 (d, $^4J_{C-F}$ = 1.6 Hz, C-11b), 126.7 (d, $^3J_{C-F}$ = 6.2 Hz, C-4a), 130.4 (d, $^3J_{C-F}$ = 10.4 Hz, C-11a), 142.1 (C-10a), 148.6 (C-2), 155.2 (d, $^1J_{C-F}$ = 242 Hz, C-6), 159.9 (C-9), 165.0 ($CO_2Et$), 172.4 (d, $^4J_{C-F}$ = 2.1 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{22}H_{20}FN_2O_4$ $[M+H]^+$: 395.14071. Found: 395.13998; EIMS $m/z$ (%): 394 ($M^+$, 26), 349 (9), 323 (22), 322 (100%), 293 (16), 279 (12), 209 (6).

**4.1.3.4. Ethyl 1-cyclopropyl-6-fluoro-9-methoxy-8,10-dimethyl-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylate (12d).** Prepared from **11d** (0.25 g, 0.55 mmol); irradiation time: 2 h. Yield 0.12 g (52%); mp 233–235 °C. Anal. Calcd for $C_{24}H_{23}FN_2O_4$ (422.45): C, 68.24; H, 5.49; N, 6.63. Found: C, 68.22; H, 5.37; N, 6.56. IR (KBr) $\nu_{max}$; 3555, 3478, 3092, 2970, 2932, 1723, 1632, 1614, 1571, 1484, 1447, 1423, 1407, 1366, 1338, 1227, 1170, 1109, 1054, 1031, 1004 and 802 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.21–1.27 (m, 2H) and 1.31–1.41 (m, 2H) (2 H-2′/2 H-3′), 1.26 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 2.37 (s, 3H, C(8)–$CH_3$), 2.56 (s, 3H, C(10)–$CH_3$), 3.74 (s, 3H, C(9)–$OCH_3$), 4.19 (q, $J$ = 7.1 Hz, 2H, –$OCH_2Me$), 4.70–4.80 (m, 1H, H-1′), 7.60 (d, $^3J_{H-F}$ = 10.7 Hz, 1H, H-5), 7.76 (s, 1H, H-7), 8.53 (s, 1H, H-2), 10.45 (s, 1H, N(11)–H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.4 (C-2′/C-3′), 11.2 (C(10)–$CH_3$), 14.8 ($CH_3CH_2O$–), 17.0 (C(8)–$CH_3$), 37.8 (C-1′), 60.3 (–$OCH_2Me$), 60.6 (C(9)–$OCH_3$), 101.1 (d, $^2J_{C-F}$ = 20.6 Hz, C-5), 109.7 (C-3), 114.4 (C-10), 115.2 (d, $^3J_{C-F}$ = 1.8 Hz, C-6b), 116.1 (d, $^2J_{C-F}$ = 23.4 Hz, C-6a), 121.0 (d, $J$ = 3.8 Hz, C-7), 125.2 (C-8), 126.6 (d, $^4J_{C-F}$ = 1.5 Hz, C-11b), 127.2 (d, $^3J_{C-F}$ = 6.2 Hz, C-4a), 131.2 (d, $^3J_{C-F}$ = 10.4 Hz, C-11a), 139.6 (C-10a), 148.8 (C-2), 155.4 (d, $^1J_{C-F}$ = 244 Hz, C-6), 156.7 (C-9), 165.0 ($CO_2Et$), 172.3 (d, $^4J_{C-F}$ = 2 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{24}H_{24}FN_2O_4$ [M + H]$^+$: 423.17201. Found: 423.17146; HRMS (ESI): found $[M+H]^+$, requires; EIMS $m/z$ (%): 422 ($M^+$, 33), 396 (10), 382 (20), 365 (30), 350 (100%), 335 (41), 321 (40), 307 (26), 294 (21), 279 (18), 237 (12), 210 (8), 175 (10), 146 (8).

**4.1.3.5. Ethyl 1-cyclopropyl-6,9-difluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylate (12e).** Prepared from **11e** (0.35 g, 0.85 mmol); irradiation time: 2 h. Yield 0.13 g (40%); mp 291–293 °C. Anal. Calcd for $C_{21}H_{16}F_2N_2O_3$ (382.36): C, 65.97; H, 4.22; N, 7.33. Found: C, 65.69; H, 4.03; N, 7.12. IR (KBr) $\nu_{max}$; 3549, 3473, 3401, 3073, 2985, 2922, 1706, 1634, 1606, 1569, 1485, 1449, 1423, 1341, 1227, 1172, 1107, 1034, 869, 802 and 636 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18–1.25 (m, 2H) and 1.39–1.48 (m, 2H) (2 H-2′/2 H-3′), 1.27 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 4.21 (q, $J$ = 7.1 Hz, 2H, –$OCH_2Me$), 4.40–4.48 (m, 1H, H-1′), 7.16 (ddd, $^3J_{H-F}$ = 10 Hz, $J$ = 8.6, 2.1 Hz, 1H, H-8), 7.51 (dd, $^3J_{H-F}$ = 10 Hz, $J$ = 2.1 Hz, 1H, H-10), 7.66 (d, $^3J_{H-F}$ = 10.7 Hz, 1H, H-5), 8.13 (dd, $J$ = 8.6 Hz, $^4J_{H-F}$ = 5.4 Hz, 1H, H-7), 8.53 (s, 1H, H-2), 11.72 (s, 1H, N(11)–H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 14.8 ($CH_3CH_2O$–), 38.2 (C-1′), 60.3 (–$OCH_2Me$), 99.2 (d, $^2J_{C-F}$ = 26.4 Hz, C-10), 101.1 (d, $^2J_{C-F}$ = 20.7 Hz, C-5), 109.7 (d, $^2J_{C-F}$ = 24.5 Hz, C-8), 109.8 (C-3), 115.1 (d, $^2J_{C-F}$ = 25.9 Hz, C-6a), 115.7 (d, $^3J_{C-F}$ = 2.9 Hz, C-6b), 124.1 (dd, $^3J_{C-F}$ = 10.2 Hz, $J$ = 3.8 Hz, C-7), 126.6 (d, $^4J_{C-F}$ = 1.6 Hz, C-11b), 127.5 (d, $^3J_{C-F}$ = 7.0 Hz, C-4a), 131.6 (d, $^3J_{C-F}$ = 8.9 Hz, C-11a), 141.3 (d, $^3J_{C-F}$ = 11.4 Hz, C-10a), 148.7 (C-2), 155.3 (d, $^1J_{C-F}$ = 244 Hz, C-6), 162.1 (d, $^1J_{C-F}$ = 240 Hz, C-9), 164.9 ($CO_2Et$), 172.3 (d, $^4J_{C-F}$ = 2 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{21}H_{17}F_2N_2O_3$ $[M+H]^+$: 383.12072. Found: 383.12023; EIMS $m/z$ (%): 382 ($M^+$, 26), 316 (11), 310 (100%), 281 (25), 205 (17), 149 (38), 85 (32), 71 (62).

**4.1.3.6. Ethyl 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylate (12f).** Prepared from **11f** (0.25 g, 0.62 mmol); irradiation time: 1.5 h. Yield 0.11 g (48%); mp: 301–303 °C. Anal. Calcd for $C_{19}H_{15}FN_2O_3S$ (370.40): C, 61.61; H, 4.08; N, 7.56. Found: C, 61.52; H, 4.23; N, 7.36. IR (KBr) $\nu_{max}$; 3337, 3086, 2971, 2941, 1714, 1629, 1597, 1570, 1521, 1486, 1448, 1366, 1333, 1234, 1195, 1175, 1102, 1039, 801, 741 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.18–1.22 (m, 2H) and 1.38–1.45 (m, 2H) (2 H-2′/2 H-3′), 1.26 (t, $J$ = 7.1 Hz, 3H, $CH_3CH_2O$–), 4.20 (q, $J$ = 7.1 Hz, 2H, –$OCH_2Me$), 4.32–4.42 (m, 1H, H-1′), 7.32 (d, $J$ = 5.2 Hz, 1H, H-9), 7.62 (d, $^3J_{H-F}$ = 10.5 Hz, 1H, H-5), 7.82 (d, $J$ = 5.2 Hz, 1H, H-8), 8.50 (s, 1H, H-2), 11.89 (s, 1H, N(10)–H); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 14.8 ($CH_3CH_2O$–), 38.1 (C-1′), 60.3 (–$OCH_2Me$), 100.3 (d, $^2J_{C-F}$ = 20.3 Hz, C-5), 109.9 (C-3), 113.2 (C-9), 115.1 (d, $^2J_{C-F}$ = 26.1 Hz, C-6a), 125.2 (d, $^3J_{C-F}$ = 6.0 Hz, C-4a), 127.7 (C-10b), 129.1 (C-10a), 131.2 (d, $^3J_{C-F}$ = 10.4 Hz, C-6b), 131.9 (C-8), 146.4 (C-9a), 148.2 (C-2), 152.7 (d, $^1J_{C-F}$ = 242 Hz, C-6), 165.0 ($CO_2Et$), 172.3 (C-4); HRMS (ESI) $m/z$: calcd for $C_{19}H_{16}FN_2O_3S$ $[M+H]^+$: 371.08657. Found: 371.08598; EIMS $m/z$ (%): 370 ($M^+$, 27), 356 (8), 325 (16), 298 (100%), 269 (19), 242 (12), 228 (14).

### 4.1.4. General procedure for synthesis of 1-cyclopropyl-6-fluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acids (13a–e) and 1-cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylic acid (13f)

A suspension of the appropriate ester **12a**–**f** (1 mmol) in 10% aq HCl (8 mL) and ethanol (10 mL) was refluxed for 20–24 h. The solvents were evaporated in vacuo from the reaction mixture and the residual solid product was washed with methanol (3–5 mL), collected by in vacuo filtration, dried and crystallized from DMSO.

**4.1.4.1. 1-Cyclopropyl-6-fluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acid (13a).** Yield 0.32 g (95%); mp: 341–342 °C (dec). Anal. Calcd for $C_{19}H_{13}FN_2O_3$ (336.32): C, 67.86; H, 3.90; N, 8.33. Found: C, 67.62; H, 3.75; N, 8.21. IR (KBr) $\nu_{max}$; 3335, 1703, 1637, 1597, 1572, 1514, 1481, 1455, 1339, 812, 750, 721 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.28–1.32 (m, 2H) and 1.46–1.52 (m, 2H) (2 H-2′/2 H-3′), 4.59–4.69 (m, 1H, H-1′), 7.36 (dd, $J$ = 7.6, 7.3 Hz, 1H, H-8), 7.60 (dd, $J$ = 7.8, 7.3 Hz, 1H, H-9), 7.75 (d, $^3J_{H-F}$ = 10.3 Hz, 1H, H-5), 7.86 (d, $J$ = 7.8 Hz, 1H, H-10), 8.18 (d, $J$ = 7.6 Hz, 1H, H-7), 8.79 (s, 1H, H-2), 11.97 (s, 1H, N(11)–H), 15.37 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 39.5 (C-1′), 99.7 (d, $^2J_{C-F}$ = 21.4 Hz, C-5), 107.3 (C-3), 113.3 (C-10), 116.3 (d, $^2J_{C-F}$ = 22.5 Hz, C-6a), 118.6 (d, $^3J_{C-F}$ = 4.6 Hz, C-6b), 121.6 (C-8), 122.6 (d, $J$ = 3.9 Hz, C-7), 124.6 (d, $^3J_{C-F}$ = 8.4 Hz, C-4a), 127.9 (d, $^4J_{C-F}$ = 1.4 Hz, C-11b), 128.2 (C-9), 130.6 (d, $^3J_{C-F}$ = 10.7 Hz, C-11a), 140.5 (C-10a), 148.4 (C-2), 156.3 (d, $^1J_{C-F}$ = 247 Hz, C-6), 166.4 ($CO_2H$), 177.3 (d, $^4J_{C-F}$ = 3 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{19}H_{12}FN_2O_3$ $[M-H]^+$: 335.08374. Found: 335.08417; EIMS $m/z$ (%): 336 ($M^+$, 18), 292 (100%), 263 (31), 236 (24), 223 (28), 222 (12), 196 (9), 130 (6).

**4.1.4.2. 1-Cyclopropyl-6-fluoro-9-methyl-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acid (13b).** Yield 0.33 g (94%); mp: 340–342 °C. Anal. Calcd for $C_{20}H_{15}FN_2O_3$ (350.35): C, 68.57; H, 4.32; N, 7.99. Found: C, 68.41; H, 4.24; N, 7.86. IR (KBr) $\nu_{max}$; 3339, 3016, 2970, 1738, 1634, 1594, 1566, 1519, 1441, 1367, 1338, 1229, 1217, 1124, 1034, 855, 799, 767, 740, 643 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.25–1.29 (m, 2H) and 1.47–1.53 (m, 2H) (2 H-2′/2 H-3′), 2.52 (s, 3H, C(9)–$CH_3$), 4.63–4.73 (m, 1H, H-1′), 7.19 (d, $J$ = 7.9 Hz, 1H, H-8), 7.67 (s, 1H, H-10), 7.73 (d, $^3J_{H-F}$ = 10.3 Hz, 1H, H-5), 8.05 (d, $J$ = 7.9 Hz, 1H, H-7), 8.77 (s, 1H, H-2), 11.85 (s, 1H, N(11)–H), 15.34 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 22.4 (C(9)–$CH_3$), 39.3 (C-1′), 99.7 (d, $^2J_{C-F}$ = 21.2 Hz, C-5), 107.4 (C-3), 112.9 (C-10), 116.4 (d, $^2J_{C-F}$ = 18.5 Hz, C-6a), 116.6 (C-6b), 122.2 (d, $J$ = 3.5 Hz, C-7), 123.4 (C-8), 124.3 (d, $^3J_{C-F}$ = 8.4 Hz, C-4a), 127.8 (C-11b), 130.5 (d, $^3J_{C-F}$ = 10.6 Hz, C-11a), 138.1 (C-9), 141.2 (C-10a), 148.3 (C-2), 156.1 (d, $^1J_{C-F}$ = 247 Hz, C-6), 166.4 ($CO_2H$), 177.3 (d, $^4J_{C-F}$ = 3 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{20}H_{16}FN_2O_3$ $[M+H]^+$: 351.11450. Found: 351.11399; EIMS $m/z$ (%): 350 ($M^+$, 22), 306 (100%), 291 (38), 277 (36), 237 (30), 210 (10), 145 (9).

**4.1.4.3. 1-Cyclopropyl-6-fluoro-9-methoxy-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acid (13c).** Yield 0.35 g (96%); mp: 325–326 °C (dec). Anal. Calcd for $C_{20}H_{15}FN_2O_4$ (366.35): C, 65.57; H, 4.13; N, 7.64. Found: C, 65.41; H, 4.24; N, 7.86. IR (KBr) $\nu_{max}$: 3324, 3106, 3088, 3000, 2953, 2829, 1703, 1637, 1597, 1568, 1525, 1452, 1426, 1343, 1314, 1270, 1230, 1205, 1172, 1110, 1034, 834, 801, 743 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.29–1.34 (m, 2H) and 1.47–1.54 (m, 2H) (2 H-2′/2 H-3′), 3.88 (s, 3H, –$OCH_3$), 4.61–4.71 (m, 1H, H-1′), 6.99 (d, $J$ = 8.6 Hz, 1H, H-8), 7.31 (s, 1H, H-10), 7.75 (d, $^3J_{H-F}$ = 10.4 Hz, 1H, H-5), 8.05 (d, $J$ = 8.6 Hz, 1H, H-7), 8.79 (s, 1H, H-2), 11.77 (s, 1H, N(11)–H), 15.38 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 39.4 (C-1′), 55.9 (C(9)–$OCH_3$), 95.5 (C-10), 100.0 (d, $^2J_{C-F}$ = 21.5 Hz, C-5), 107.2 (C-3), 112.1 (C-8), 112.5 (d, $^3J_{C-F}$ = 1.5 Hz, C-6b), 116.9 (d, $^2J_{C-F}$ = 23.4 Hz, C-6a), 123.5 (d, $J$ = 3.9 Hz, C-7), 123.6 (d, $^3J_{C-F}$ = 8.3 Hz, C-4a), 127.7 (C-11b), 130.3 (d, $^3J_{C-F}$ = 10.7 Hz, C-11a), 142.3 (C-10a), 148.3 (C-2), 155.8 (d, $^1J_{C-F}$ = 246 Hz, C-6), 160.3 (C-9), 166.4 ($CO_2H$), 177.3 (d, $^4J_{C-F}$ = 2.9 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{20}H_{14}FN_2O_4$ $[M-H]^+$: 365.09431. Found: 365.09482; EIMS $m/z$ (%): 366 ($M^+$, 2), 323 (29), 322 (100%), 293 (28), 279 (23), 253 (20), 223 (10), 210 (24), 147 (6), 139 (5).

**4.1.4.4. 1-Cyclopropyl-6-fluoro-9-methoxy-8,10-dimethyl-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acid (13d).** Yield 0.37 g (94%); mp: 310–312 °C (dec). Anal. Calcd for $C_{22}H_{19}FN_2O_4$ (394.40): C, 67.00; H, 4.86; N, 7.10. Found: C, 67.12; H, 4.63; N, 7.02. IR (KBr) $\nu_{max}$: 3422, 3098, 2920, 1710, 1630, 1601, 1572, 1510, 1445, 1328, 1219, 1194, 1121, 965, 863, 808 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.30–1.35 (m, 2H) and 1.39–1.46 (m, 2H) (2 H-2′/2 H-3′), 2.40 (s, 3H, C(8)–$CH_3$), 2.59 (s, 3H, C(10)–$CH_3$), 3.76 (s, 3H, C(9)–$OCH_3$), 4.92–5.02 (m, 1H, H-1′), 7.75 (d, $^3J_{H-F}$ = 10.3 Hz, 1H, H-5), 7.85 (s, 1H, H-7), 8.81 (s, 1H, H-2), 10.71 (s, 1H, N(11)–H), 15.34 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.5 (C-2′/C-3′), 11.3 (C(10)–$CH_3$), 17.0 (C(8)–$CH_3$), 39.1 (C-1′), 60.7 (C(9)–$OCH_3$), 100.2 (d, $^2J_{C-F}$ = 21.2 Hz, C-5), 107.4 (C-3), 114.7 (C-10), 115.1 (d, $^3J_{C-F}$ = 2.5 Hz, C-6b), 117.0 (d, $^2J_{C-F}$ = 22.8 Hz, C-6a), 121.1 (d, $J$ = 3.8 Hz, C-7), 124.2 (d, $^3J_{C-F}$ = 8.1 Hz, C-4a), 125.8 (C-8), 127.8 (C-11b), 131.0 (d, $^3J_{C-F}$ = 10.4 Hz, C-11a), 139.8 (C-10a), 148.7 (C-2), 156.0 (d, $^1J_{C-F}$ = 247 Hz, C-6), 157.2 (C-9), 166.4 ($CO_2H$), 177.3 (d, $^4J_{C-F}$ = 2.5 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{22}H_{18}FN_2O_4$ $[M-H]^+$: 393.12561. Found: 393.12606; EIMS $m/z$ (%): 394 ($M^+$, 27), 350 (100%), 335 (46), 320 (25), 307 (24), 279 (14), 266 (10), 237 (12), 223 (7), 175 (10), 125 (5).

**4.1.4.5. 1-Cyclopropyl-6,9-difluoro-4-oxo-4,11-dihydro-1*H*-pyrido[2,3-*a*]carbazole-3-carboxylic acid (13e).** Yield 0.33 g (93%); mp: 320–322 °C (dec). Anal. Calcd for $C_{19}H_{12}F_2N_2O_3$ (354.31): C, 64.41; H, 3.41; N, 7.90. Found: C, 64.22; H, 3.35; N, 7.78. IR (KBr) $\nu_{max}$: 3317, 1695, 1641, 1597, 1575, 1510, 1477, 1441, 1423, 1332, 1310, 1219, 1107, 1034, 801 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.27–1.32 (m, 2H) and 1.47–1.54 (m, 2H) (2 H-2′/2 H-3′), 4.73–4.84 (m, 1H, H-1′), 7.22 (dd, $^3J_{H-F}$ = 9 Hz, $J$ = 8.3 Hz, 1H, H-8), 7.55 (d, $^3J_{H-F}$ = 10 Hz, 1H, H-10), 7.73 (d, $^3J_{H-F}$ = 10.2 Hz, 1H, H-5), 8.16 (dd, $J$ = 8.3 Hz, $^4J_{H-F}$ = 5.5 Hz, 1H, H-7), 8.78 (s, 1H, H-2), 11.98 (br s, 1H, N(11)–H), 15.28 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 39.4 (C-1′), 99.3 (d, $^2J_{C-F}$ = 26.2 Hz, C-10), 100.3 (d, $^2J_{C-F}$ = 21.2 Hz, C-5), 107.4 (C-3), 110.4 (d, $^2J_{C-F}$ = 24.8 Hz, C-8), 115.5 (C-6b), 116.1 (d, $^2J_{C-F}$ = 23.6 Hz, C-6a), 124.4 (d, $^3J_{C-F}$ = 8.4 Hz, C-4a), 124.5 (dd, $^3J_{C-F}$ = 8.5 Hz, $J$ = 3 Hz, C-7), 127.8 (C-11b), 131.2 (d, $^3J_{C-F}$ = 11.2 Hz, C-11a), 141.2 (d, $^3J_{C-F}$ = 13.2 Hz, C-10a), 148.6 (C-2), 155.9 (d, $^1J_{C-F}$ = 247 Hz, C-6), 162.4 (d, $^1J_{C-F}$ = 241 Hz, C-9), 166.3 ($CO_2H$), 177.3 (d, $^4J_{C-F}$ = 2.3 Hz, C-4); HRMS (ESI) $m/z$: calcd for $C_{19}H_{11}F_2N_2O_3$ $[M-H]^+$: 353.07432. Found: 353.07485; EIMS $m/z$ (%): 354 ($M^+$, 10), 310 (100%), 295 (25), 281 (39), 254 (32), 241 (39), 214 (13), 200 (9), 141 (6).

**4.1.4.6. 1-Cyclopropyl-6-fluoro-4-oxo-4,10-dihydro-1*H*-thieno-[2′,3′:4,5]pyrrolo[3,2-*h*]quinoline-3-carboxylic acid (13f).** Yield 0.31 g (91%); mp: 356–357 °C. Anal. Calcd for $C_{17}H_{11}FN_2O_3S$ (342.35): C, 59.64; H, 3.24; N, 8.18. Found: C, 59.45; H, 3.11; N, 7.89. IR (KBr) $\nu_{max}$: 3346, 3106, 3091, 3011, 1724, 1630, 1550, 1517, 1470, 1445, 1354, 1328, 1245, 1216, 1186, 1092, 1041, 863, 710, 667 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.25–1.30 (m, 2H) and 1.47–1.53 (m, 2H) (2 H-2′/2 H-3′), 4.51–4.61 (m, 1H, H-1′), 7.38 (d, $J$ = 5.2 Hz, 1H, H-9), 7.71 (d, $^3J_{H-F}$ = 10.1 Hz, 1H, H-5), 7.89 (d, $J$ = 5.2 Hz, 1H, H-8), 8.74 (s, 1H, H-2), 12.25 (s, 1H, N(10)–H), 15.50 (s, 1H, $CO_2H$); $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 10.2 (C-2′/C-3′), 39.3 (C-1′), 99.4 (d, $^2J_{C-F}$ = 20.5 Hz, C-5), 107.4 (C-3), 113.4 (C-9), 116.0 (d, $^2J_{C-F}$ = 25.4 Hz, C-6a), 122.2 (d, $^3J_{C-F}$ = 8.0 Hz, C-4a), 129.0 (C-10b), 113.7 (C-10a), 130.9 (d, $^3J_{C-F}$ = 11.0 Hz, C-6b), 133.0 (C-8), 147.1 (C-9a), 147.9 (C-2), 153.4 (d, $^1J_{C-F}$ = 245 Hz, C-6), 166.4 ($CO_2H$), 177.2 (C–4); HRMS (ESI) $m/z$: calcd for $C_{17}H_{10}FN_2O_3S$ $[M-H]^+$: 341.04016. Found: 341.04059; EIMS $m/z$ (%): 342 ($M^+$, 23), 298 (100%), 283 (13), 269 (27), 242 (20), 229 (21), 228 (12), 202 (9), 158 (5), 148 (5).

## 4.2. Biology

### 4.2.1. Antimicrobial activity

The newly synthesized compounds were tested for their in vitro antimicrobial properties against the following microorganisms: Gram positive bacteria (*B. subtilis* ATCC 6633, *S. aureus* ATCC 6538, *S. epidermidis* ATCC 12228, quinolone- and penicillin-resistant clinical isolates of both *S. aureus* and *S. epidermidis*), Gram negative bacteria (*A. baumannii* ATCC 19606, *E. coli* ATCC 8739, *H. influenzae* ATCC 19418, *P. aeruginosa* ATCC 9027, quinolone- and penicillin-resistant clinical isolates of all *A. baumannii*, *E. coli* and *P. aeruginosa* strains), yeasts (*C. tropicalis* ATCC 1369, *S. cerevisiae* ATCC 9763), and mold (*A. niger* ATCC 6275). The screening was performed according to the broth dilution method.[46] Three types of specific culture media were used in this study: Haemophilus Test Medium for *H. influenzae*, Mueller Hinton Broth for the other bacteria, and Sabouraud Dextrose Broth for fungi. Test compounds were dissolved in dimethyl sulfoxide and diluted in the growth media to give the final concentration which ranged from 100 μg/mL to 0.00015 μg/mL. The final size of inocula was $5 \times 10^5$ CFU/mL and $1 \times 10^3$ CFU/mL for bacteria and for fungi, respectively. The minimum inhibitory concentrations (MIC, μg/mL) were determined after 24 h incubation at 37 °C for antibacterial activity and

after 48 h at 30 °C for antifungal activity. MICs were considered to be the lowest concentrations of the tested compounds which inhibited the reproduction of the tested microorganisms.

Drug-free tubes and organism-free tubes were kept as growth controls and purity controls, respectively. Controls containing only solvent showed that dimethyl sulfoxide, at the concentrations studied, had no effect on the microorganisms. Ciprofloxacin, levofloxacin, moxifloxacin, and gemifloxacin were used as standard antibacterial drugs.

All compounds were tested in triplicate and the experiments were repeated at least three times.

#### 4.2.2. DNA gyrase and topoisomerase IV inhibition

The inhibitory activity of new fluoroquinolones against target enzymes was detected by using *E. coli* DNA gyrase and topoisomerase IV (Inspiralis, Norwich, UK).

DNA gyrase activity was measured in a supercoiling assay with relaxed pBR322 DNA as a substrate. The reaction mixtures contained 35 mM Tris–HCl pH 7.5, 24 mM KCl, 4 mM $MgCl_2$, 2 mM dithiothreitol, 1.8 mM spermidine, 1 mM ATP, 6.5% w/v glycerol, 0.1 mg/mL albumin, 16 ng/μL relaxed pBR322 DNA, 0.026 U/μL of gyrase protein, and different amounts of each fluoroquinolone. All mixtures were incubated for 30 min at 37 °C and then the reactions were stopped by adding chloroform/isoamyl alcohol 24:1 solution and bromophenol blue loading dye. After a brief vortex, the blue aqua phase was analyzed by electrophoresis in 1.0% w/v agarose gel.

Topoisomerase IV activity was measured by decatenation assay using kinetoplast DNA as a substrate. The reaction mixtures contained 40 mM HEPES–KOH pH 7.6, 100 mM potassium glutamate, 10 mM $Mg(OAc)_2$, 10 mM dithiothreitol, 1 mM ATP, 50 μg/mL albumin, 6.6 ng/μL kDNA, 0.016 U/μL topoisomerase IV protein, and different amounts of each fluoroquinolone. All mixtures were incubated for 30 min at 37 °C and then the reactions were stopped by adding chloroform/isoamyl alcohol 24:1 solution and bromophenol blue loading dye. After a brief vortex, the blue aqua phase was analyzed by electrophoresis in 1.0% w/v agarose gel.

The products of the reactions were quantitated using a Bio-Rad gel documentation system and the 50% inhibitory concentration ($IC_{50}$, μg/mL) was determined as the drug concentration that reduced by 50% the supercoiling and the decatenation activity of the cited enzymes observed with drug-free controls. Ciprofloxacin and moxifloxacin were used as reference fluoroquinolones.

Results were confirmed in at least three independent experiments.

#### 4.2.3. Antibodies and reagents

Caspase-7 antibody was obtained from Cell Signaling Technology (Beverly, MA, USA); monoclonal anti-p53 antibody (DO-1) was obtained from Santa Cruz Biotechnology (CA, USA); monoclonal anti-actin antibody was obtained from Sigma (Dorset, UK).

Horseradish peroxidase-conjugated (HRP) secondary antibodies and the enhanced chemiluminescence system (ECL) were from Millipore (Millipore, MA, USA). Reagents for electrophoresis and blotting analysis were obtained from Bio-Rad Laboratories. Ellipticine was from Sigma (Dorset, UK).

#### 4.2.4. Cell proliferation assay

MCF-7 breast cancer cells and A549 NSCLC cell lines were from ATCC and were cultured in RPMI; human-derm fibroblasts (HuDe) were obtained from Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy, and were cultured in DMEM. All media were supplemented with 2 mM glutamine and 10% Fetal Bovine Serum (FBS, Gibco Life Technologies) and cells were maintained under standard cell culture conditions at 37 °C in a water-saturated atmosphere of 5% $CO_2$ in air.

Cell viability was assessed, after 3 days of treatment, with tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), obtained from Sigma (Dorset, UK), and assayed as previously described.[47] Representative results of at least three independent experiments were used for evaluation of dose–response curves, calculated from experimental points using single or double Hill functions (Graph Pad Prism Software 5, San Diego California USA, www.graphpad.com). $IC_{50}$ concentrations were obtained from the dose–response curves by extrapolation.

In all assays, the drugs were dissolved in DMSO immediately before the addition to cell cultures. The final concentration of DMSO never exceeded 0.1% (v/v), and equal amounts of the solvent were added to control cells.

#### 4.2.5. Cell cycle analysis

Distribution of the cells in the cell cycle was determined by propidium iodide staining and flow cytometry analysis (EPICS XL-MCL cytometer; Beckman Coulter). Cells ($5 \times 10^5$) were harvested, washed in PBS, fixed in cold 70% ethanol, and then stained with 40 μg/mL propidium iodide while treating with RNase. Analysis was done with a Beckman Coulter EPICS XL-MCL cytometer (Beckman Coulter). Cell cycle distributions were analyzed by Multi-Cycle DNA Content and Cell Cycle Analysis Software (Phoenix Flow Systems, Inc.) as previously described.[48]

#### 4.2.6. Western blot analysis

Procedures for protein extraction, solubilization, and protein analysis by 1D PAGE are described elsewhere.[48] Briefly, 30–50 μg proteins from lysates were resolved by 5–15% SDS–PAGE and transferred to PDVF membranes. The membranes were then incubated with primary antibody, washed and then incubated with HRP-anti-mouse or HRP-anti-rabbit antibodies. Immunoreactive bands were visualized using an enhanced chemiluminescence system.

#### 4.2.7. Detection of apoptosis

Apoptosis was assessed by: (a) morphology on stained (Hoechst 33342, PI) or unstained cells using light-, phase contrast- and fluorescence microscopy; (b) activation of caspase-7 (detection of cleavage products) by Western Blotting procedure as previously described.[48]

## Acknowledgment

We wish to thank the Higher Council for Science and Technology (Amman, Jordan) for financial support.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.098. These data include MOL files and InChiKeys of the most important compounds described in this article.

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