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7-((4-Substituted)piperazin-1-yl) derivatives of ciprofloxacin: Synthesis and in vitro biological evaluation as potential antitumor agents

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ABSTRACT

Ciprofloxacin (CP), an antibiotic has been shown to have antiproliferative and apoptotic activities in several cancer cell lines. Moreover, several reports have highlighted the interest of increasing the lipophilicity to improve the antitumor efficacy. These studies have led us to synthesize new CP derivatives of various lipophilicities and to evaluate their activity in five human cancer cell lines. With an easy and cost-efficient procedure, 31 7-((4-substituted)piperazin-1-yl) derivatives of CP were prepared that displayed IC_{50} values ranging from μM to mM concentrations and are non-toxic in vivo in healthy mice as shown by their maximal tolerated dose (MTD) indices >80 mg/kg. Several derivatives displayed higher in vitro antitumor activity than parent CP however this was not dependent on the lipophilicity of the substituent. Among all synthesized derivatives, the most potent were **2** and **6h** whose IC_{50} values were $\leqslant$10 μM in three (derivative **2**) or four (derivative **6h**) cancer cell lines.

1. Introduction

Ciprofloxacin (CP, **1**; Fig. 1), a commonly used broad-spectrum fluoroquinolone (FQ) antibiotic with low side effects, has been shown to have antiproliferative and apoptotic activities in several cancer cell lines.[1–6] Several other FQ derivatives including ofloxacin, levofloxacin, and fleroxacin have also been shown to inhibit the growth of transitional cell bladder carcinoma cell lines.[4,5,7] All FQs induced nearly similar types of morphological alterations, that is, some cells became rounded, detached and showed cell membrane blebbing, a typical morphological change indicating initiation of apoptotic processes.[6] Induction of apoptosis also resulted from CP treatment of human transitional cells of bladder, colorectal, or prostate carcinoma.[1–3] In a cell free system, Hussy et al.[8] demonstrated the inhibitory activity of several FQs against mammalian DNA topoisomerases I and II and DNA polymerase and reported that CP was the most potent FQ inhibitor of these enzymes. The mechanism by which FQs exert their growth inhibitory effect and lead to cell death is not fully understood but it has been suggested that these events may be mediated through intrinsic apoptotic pathway[9] or through induction of cell cycle arrest by involvement of different cell cycle molecules.[10] The fact that derivatives of the FQ class of drugs showed topoisomerase II inhibitory activity has provided a rationale for quinolone-based drug design in the search for novel anticancer agents.

Moreover, several reports have provided evidence in support of increasing the lipophilicity of compounds to improve their antitumor efficacy. Increasing lipophilic substituents at C-7 of camptothecin led to the discovery of gimatecan that is currently in a Phase II clinical trial.[11] Sodium butyrate and the more lipophilic valproic acid are being investigated in Phase I and II clinical trials as histone deacetylase inhibitors.[12] A positive correlation between lipophilicity, in vitro antitumor activity and rate of drug uptake was observed with platinum(II) complexes tested on lung carcinoma and leukemia cell lines.[13] The antiproliferative activity of bis-quinolinium choline kinase inhibitors against the HT-29 colon cancer cell line has also been improved with a higher lipophilicity of the substituents.[14]

We thus initiated a screening program to search for new FQ derivatives as potential antitumor agents. In the current study, we report the synthesis of new derivatives of CP and their antiproliferative properties on five human cancer cell lines. First, 7-(substituted-piperazin-1-yl) derivatives of CP with non bulky substituents were synthesized, that is, the 7-(4-(2-chloroacetyl)piperazin-1-yl) **2** and the 7-(4-(*tert*-butoxycarbonyl)piperazin-1-yl) **3** derivatives of CP (Scheme 1). As these modifications led to enhanced in vitro antitumor activity compared to CP, we chose

Abbreviations: CP, ciprofloxacine; FQ, fluoroquinolone; MTD, maximum tolerated dose.

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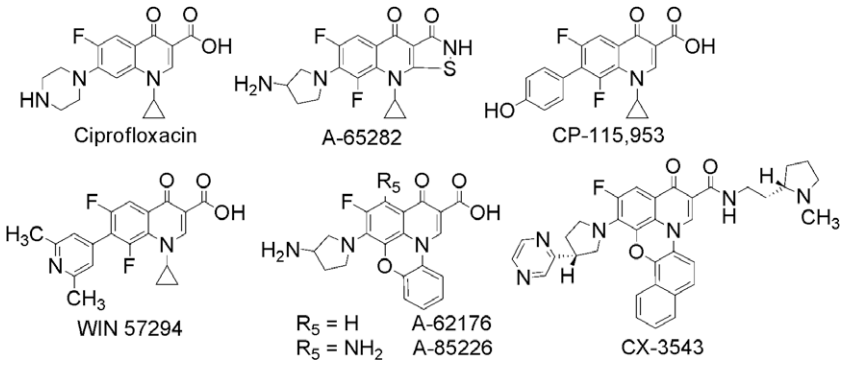
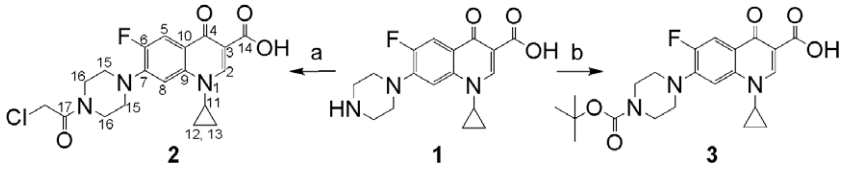


Figure 1. Chemical structures of ciprofloxacine, 6,8-difluoroquinolones (A-65282,5; CP-115,953,6 and WIN572947), quinobenzoxazines (A-621768 and A-852269), and cytotoxic quinolone CX-3543.

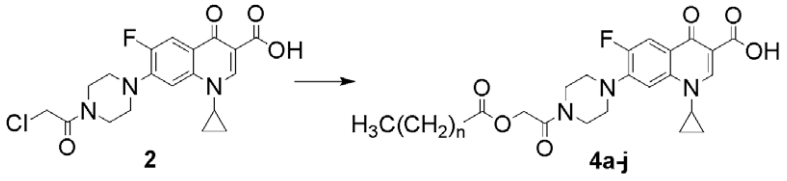
to extend the synthesis to create more lipophilic derivatives. So, the 7-(4-(2-chloroacetyl)piperazin-1-yl) derivative **2** of CP was used as a precursor of a 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) homologous series **4** (Scheme 2). We also synthesized two other homologous series of CP, that is, a 7-(4-(alkoxycarbonyl)piperazin-1-yl) series **5** and a 7-(4-(alkanoyl)piperazin-1-yl) series **6** (Schemes 3 and 4).

2. Chemistry

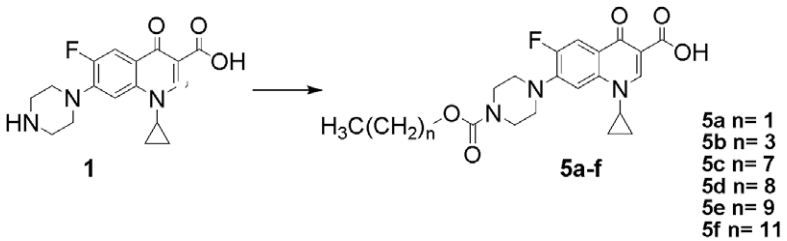
Starting from commercially available CP **1**, the 7-(4-(2-chloroacetyl)piperazin-1-yl) **2** and the 7-(4-(*tert*-butoxycarbonyl)piperazin-1-yl) **3** derivatives were easily prepared in one step. Acylation of **1** with chloroacetyl chloride or reaction of **1** with Boc_2O in dioxane/NaOH aqueous solution (2 M) led to compounds **2** or **3** in 73% and 74% yields after purification, respectively (Scheme 1, Table 1). The homologous series of 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) derivatives of CP was prepared by a subsequent condensation of commercially available carboxylic acids with **2** in dimethylformamide to produce the corresponding compounds **4a–j** in yields ranging from 27% to 75% (Scheme 2, Table 1). The 7-(4-(alkoxycarbonyl)piperazin-1-yl) derivatives were prepared in methylene chloride by condensation of **1** with commercially available alkylchloroformates to produce compounds **5a–f** in yields ranging from



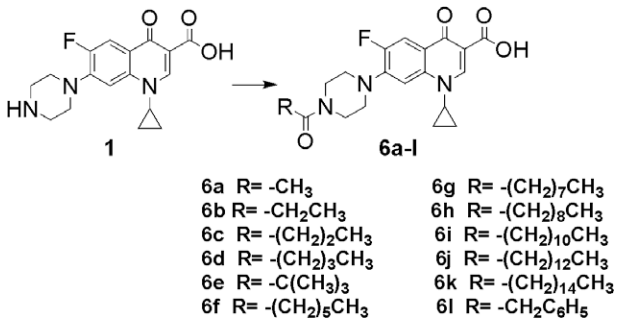
Scheme 1. Preparation of the 7-(4-(2-chloroacetyl)piperazin-1-yl) 2 and 7-(4-(*tert*-butoxy-carbonyl)piperazin-1-yl) **3** derivatives of ciprofloxacin. Reagents and conditions: (a) Et_3N, $ClCH_2COCl$, CH_2Cl_2, 0 °C–rt; (b) Boc_2O, dioxane/NaOH aq solution 2 M, 0 °C–rt.



Scheme 2. Preparation of 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) derivatives **4a–j** from 7-(4-(2-chloroacetyl)piperazin-1-yl) derivative **2** of ciprofloxacin. Reagents and conditions: $CH_3(CH_2)_nCOOH$, K_2CO_3, DMF, 100 °C.



Scheme 3. Preparation of 7-(4-(alkoxycarbonyl)piperazin-1-yl) derivatives **5a–f** of ciprofloxacin. Reagents and conditions: ROCOCl, CH_2Cl_2, 0 °C–rt.



Scheme 4. Preparation of 7-(4-(alkanoyl)piperazin-1-yl) derivatives **6a–l** of ciprofloxacin. Reagents and conditions: RCOCl, Et_3N, CH_2Cl_2, 0 °C–rt.

33% to 66% (Scheme 3, Table 1). The 7-(4-(alkanoyl)piperazin-1-yl) derivatives **6a–l** were obtained by acylation of **1** with commercially available acylchlorides in good yields (45–90%) (Scheme 4, Table 1). Some derivatives of the same series were difficult to purify, which explains the differences among yields.

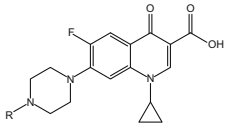
Hydrolysis of the synthesized CP derivatives could lead to CP or to the 7-(4-(2-hydroxyacetyl)piperazin-1-yl) derivative **8**. So, with the aim to study stabilities of synthesized derivatives, compound **8** was prepared in two steps. First, acylation of **1** with benzyloxyacetyl chloride results in a 46% yield the 7-(4-(2-benzyloxyacetyl)piperazin-1-yl) derivative **7** that was submitted to a catalytic hydrogenation to give **8** in a 60% yield after purification (Scheme 5). The stability of compounds **2**, **3**, **4b–d**, **4h**, **5a–c**, **6f**, **6h**, and **6k** was determined using HPLC after the incubation of each compound in phosphate buffer (pH 7.4) at 37 °C for six days.

3. Pharmacological evaluation

3.1. In vitro determination of the drug-induced inhibition of human cancer cell line growth

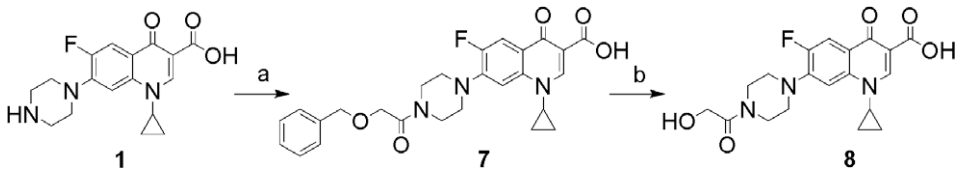
The in vitro antitumor activity of compounds **2**, **3**, **4a–j**, **5a–f**, **6a–l**, **8**, and CP **1** have been determined for prostate (PC-3), glioblastoma (U373-MG), colorectal (LoVo), NSCLC (A549), and breast (MCF-7) human cancer cell lines. The results are summarized in Table 1. For each compound, nine concentrations were tested for five days on each cancer cell line. We made use of the MTT colorimetric assay, which indirectly assesses the effect of the potentially anticancer compounds on the overall growth of

Table 1
Synthesized compounds: structures, yields of reaction, lipophilicity, stability, and IC_{50}



R	Compound number	Yields[b] (%)	Clog *P*[c]	Stability[d] (%)	IC_{50}[a] (μM)				
					PC-3	U373-MG	LoVo	A549	MCF-7
H	**1**[e]		−1.14		143 ± 1	96 ± 4	89 ± 2	280 ± 11	476 ± 13
$COCH_2Cl$	**2**	73	0.78	>99	8 ± 0.1	4 ± 0.3	17 ± 0.3	10 ± 0.2	23 ± 0.2
$C(O)OC(CH_3)_3$	**3**	74	3.06	>99	26 ± 0.6	35 ± 0.9	40 ± 0.4	18 ± 0.2	13 ± 0.3
$COCH_2OCOCH_3$	**4a**	60	0.74		176 ± 2	99 ± 2	268 ± 4	373 ± 8	279 ± 6
$COCH_2OCO(CH_2)_2CH_3$	**4b**	63	1.80	100	715 ± 10	586 ± 6	613 ± 6	456 ± 5	1000 ± 11
$COCH_2OCO(CH_2)_4CH_3$	**4c**	75	2.86	>99	14 ± 0.1	28 ± 0.7	27 ± 0.8	20 ± 0.3	72 ± 2
$COCH_2OCO(CH_2)_6CH_3$	**4d**	45	3.92	>97	23 ± 0.3	53 ± 0.7	29 ± 0.4	16 ± 0.2	818 ± 17
$COCH_2OCO(CH_2)_7CH_3$	**4e**	27	4.44		257 ± 4	272 ± 7	267 ± 2	216 ± 1	255 ± 3
$COCH_2OCO(CH_2)_8CH_3$	**4f**	47	4.97		416 ± 8	291 ± 5	317 ± 2	273 ± 4	353 ± 4
$COCH_2OCO(CH_2)_9CH_3$	**4g**	43	5.50		nd	>1000	828 ± 13	>1000	816 ± 17
$COCH_2OCO(CH_2)_{10}CH_3$	**4h**	46	6.03	>98	3 ± 0.1	49 ± 2	39 ± 0.7	15 ± 0.2	621 ± 12
$COCH_2OCO(CH_2)_{12}CH_3$	**4i**	41	7.09		745 ± 14	748 ± 23	766 ± 12	742 ± 18	828 ± 14
$COCH_2OCO(CH_2)_{14}CH_3$	**4j**	46	8.15		30 ± 0.4	119 ± 0.6	75 ± 2	112 ± 1	56 ± 0.8
$C(O)OCH_2CH_3$	**5a**	66	2.34	100	385 ± 14	228 ± 3	272 ± 8	>1000	455 ± 12
$C(O)O(CH_2)_3CH_3$	**5b**	42	3.40	100	9 ± 0.3	31 ± 0.5	52±1	42 ± 1.3	14 ± 0.2
$C(O)O(CH_2)_7CH_3$	**5c**	37	5.52	100	24 ± 0.6	79 ± 0.7	43 ± 1	21 ± 0.5	23 ± 1
$C(O)O(CH_2)_8CH_3$	**5d**	33	6.05		30 ± 0.5	283 ± 9	30 ± 0.1	38 ± 2	35+2
$C(O)O(CH_2)_9CH_3$	**5e**	41	6.58		24 ± 0.7	401 ± 44	32 ± 0.1	44 ± 5	20 ± 1
$C(O)O(CH_2)_{11}CH_3$	**5f**	38	7.63		217 ± 2	94 ± 7	347 ± 6	273 ± 7	200 ± 3
$COCH_3$	**6a**	88	0.17		680 ± 33	535 ± 13	323 ± 8	584 ± 14	803 ± 15
$COCH_2CH_3$	**6b**	75	0.69		352 ± 7	279 ± 6	306 ± 6	402 ± 21	727 ± 17
$CO(CH_2)_2CH_3$	**6c**	76	1.22		85 ± 2	104 ± 2	148 ± 2	697 ± 18	172 ± 9
$CO(CH_2)_3CH_3$	**6d**	64	1.75		73 ± 0.7	86 ± 2	87 ± 2	64 ± 0.8	316 ± 11
$COC(CH_3)_3$	**6e**	90	1.40		246 ± 4	67±3	93 ± 5	509 ± 11	85 ± 1
$CO(CH_2)_5CH_3$	**6f**	50	2.81	100	779 ± 21	729 ± 9	793 ± 14	808 ± 12	754 ± 12
$CO(CH_2)_7CH_3$	**6g**	61	3.87		7 ± 0.1	24 ± 0.3	30 ± 0.4	6 ± 0.1	26 ± 0.3
$CO(CH_2)_8CH_3$	**6h**	45	4.40	100	4 ± 0.1	5 ± 0.2	7 ± 0.2	3 ± 0.1	20 ± 0.4
$CO(CH_2)_{10}CH_3$	**6i**	66	5.45		4 ± 0.1	18 ± 0.2	45 ± 0.9	7 ± 0.1	108 ± 2
$CO(CH_2)_{12}CH_3$	**6j**	65	6.51		94 ± 1	205 ± 3	101 ± 0.9	56 ± 0.4	96 ± 1
$CO(CH_2)_{14}CH_3$	**6k**	58	7.57	100	114 ± 0.6	16 ± 0.1	290 ± 4	65 ± 0.7	145 ± 1
$-COCH_2C_6H_5$	**6l**	50	3.90		243 ± 2	34 ± 0.7	41 ± 0.6	29 ± 0.3	32 ± 0.5
$COCH_2OH$	**8**	26	0.38		433 ± 19	294 ± 4	408 ± 12	456 ± 8	985 ± 26

[a] IC_{50} values were determined using the MTT colorimetric assay on five human cancer cell lines (PC-3: prostate cancer, U373-MG: glioblastoma, LoVo: colon cancer, A549: lung cancer and MCF-7: breast cancer) treated for 5 days.
[b] Yields after preparative chromatography on silica (see Section 6).
[c] Lipophilicity was evaluated by in silico calculation. Software-predicted lipophilicity was estimated by means of the software Clog *P* (ChemDraw Ultra 8.0). The Clog *P* predictor is based on topological structure descriptors and was developed with artificial neural networks.
[d] Stability studies were carried out using HPLC analysis after incubation of each compound in phosphate buffer (pH 7.4) at 37 °C for 6 days.
[e] Commercially available from Fluka.



Scheme 5. Preparation of 7-(4-(2-hydroxyacetyl)piperazin-1-yl) derivative **8** of ciprofloxacin. Reagents and conditions: (a) Et_3N, $C_6H_5CH_2OCH_2COCl$, CH_2Cl_2, 0 °C–rt; (b) H_2, Pd/C, DMF, rt.

adherent cell lines.[15,16] The IC_{50} values, which are the concentrations that reduce the mean growth value of the cells by 50%, were determined for each drug and compared to the mean control growth value, and are listed in Table 1.

3.2. In vivo maximum tolerated dose (MTD) in healthy mice

The in vivo tolerance of compounds **1**, **2**, and **3** as well as that of those derivatives from series **5** and **6** with the lowest or highest in vitro activity (**5a–c**, **6f**, **6h**, **6k**) was determined as the MTD index, which represents the highest single dose of the compound that can be administered ip to experimental groups of three healthy mice per group over a maximum period of 28 days without causing their death.[17]

4. Results

The 31 synthesized 7-(4-(substituted)piperazin-1-yl) derivatives and the native pharmacophore CP **1**, evaluated in vitro for their antitumor activity, displayed IC_{50} values ranging from μM to mM concentrations.

Compounds **2** and **3** showed higher antitumor activity in vitro than the native pharmacophore CP with mean IC_{50} values of 9 and 48 μM, respectively, compared to 250 μM for **1**. Compounds **2** and **3** exhibited IC_{50} values 5–28-fold and 2–37-fold lower than that of CP, respectively, depending on human cancer cell lines (Table 1).

Starting from compound **2**, 10 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) derivatives (**4a–j**) with increasing lipophilicity of the alkyl chain bearing one to 15 carbon atoms (Clog *P* ranging from 0.74 to 8.15) have been synthesized. No correlation can be observed between in vitro antitumor activity and chain length (Table 1). Among these 10 compounds, only three derivatives (**4c** (mean IC_{50}: 26 μM); **4d** (32 μM); **4h** (37 μM)) showed higher activities than **1** and **3** but none of them showed higher activity when compared to **2**. The most interesting compound of this series was **4h** whose IC_{50} value on the prostate cancer cell line (3 μM) was 47-fold lower than that of **1**. Stability tests of compounds **4c**, **4d**, and **4h** in phosphate buffer at pH 7.4 have shown no degradation into **8**. As **8** has a very weak in vitro antitumor activity (mean IC_{50}: 484 μM), the observed antitumor activities recorded for **4c**, **4d**, and **4h** are thus not related to a degradation of these compounds.

Due to the higher antitumor activity observed in vitro for compound **3** as compared to CP, six 7-(4-(alkoxycarbonyl)piperazin-1-yl) derivatives (**5a–f**) with different length chains from two to 12 carbon atoms (Clog *P* ranging from 2.34 to 7.63) have been prepared. No correlation between the activity and the chain length could again be established (Table 1). Indeed, compounds **5a** and **5f** were not active against human cancer cells (Table 1). In addition, compared with **3**, no pharmacological benefit (in terms of in vitro antitumor activity) was observed for compounds **5b–e** (Table 1). Compounds **5b** and **5c** present in vitro antitumor activity against the five cancer cell lines that is similar to **3**. Moreover, a significant loss of activity against the glioblastoma cancer cell line was observed for compounds **5d** and **5e** when compared to **3** (Table 1).

Twelve 7-(4-(alkanoyl)piperazin-1-yl) derivatives (**6a–l**) with different chain lengths from one to 15 carbon atoms (Clog *P* ranging from 0.17 to 7.57) have been synthesized and evaluated for their in vitro antitumor activities. Only three compounds (**6g**, **6h**, and **6i**) showed $ICs_{50} \leqslant 15$ μM (Table 1). The most potent compound against all cancer cell lines was **6h**, which exhibited IC_{50} values from 13- to 93-fold lower than that of CP (Table 1).

In vivo tolerance was determined for compounds **1**, **2**, **3**, **5a**, **5b**, **5c**, **6f**, **6h**, and **6k**, all of which displayed MTD indices >80 mg/kg.

5. Discussion

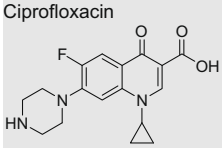
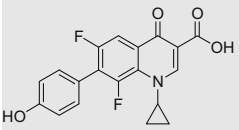
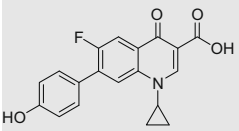
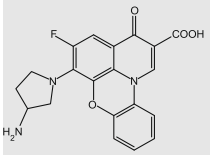
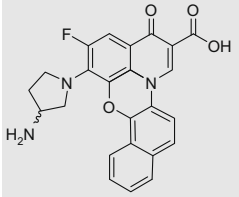
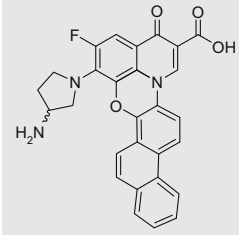
Among all the compounds tested in the current study, **2** and **6h** were the most potent antitumor agents. However, their antitumor activity is weak relative to natural compounds such as taxol,[16,18] vincristine,[19] or sodium pump inhibitors,[18,20] whose IC_{50} values in vitro are in the nM range. In addition, all the compounds that have been tested in vivo for tolerance displayed MTD indices >80 mg/kg.

As indicated in the Introduction, the mechanisms of action by which some FQ antibiotics, including CP, exert antitumor effects on various human cancer cell lines were determined to be through pro-apoptotic effects.[1–3,6,8] However, it must be emphasized that these time- and dose-dependent pro-apoptotic effects were observed when CP was evaluated within a 600–1500 μM concentration range, while in vitro growth inhibition values often occur at lower, or even much lower, doses as we detail in Table 2. The possibility thus remains that some FQ derivatives exert their antitumor activity through non-apoptotic-dependent mechanisms. We will investigate in the near future whether or not the most active compounds we synthesized in the current study induce sustained pro-autophagic- and/or lysosomal membrane permeabilization-related cell death instead of a pro-apoptotic-related cell death, such as we recently demonstrated for various compounds including for example a CXC chemokine pan-antagonist,[21,22] sodium pump antagonists,[19,23] and temozolomide in the context of galectin-1 deficiency.[24]

In addition, flow cytometric analysis of bladder cancer cells treated with CP showed that these cells were arrested in the S/G2-M phases of the cell cycle, suggesting CP-mediated effects on cancer cell cycle kinetics.[1] Evaluation of the antiproliferative activity of some FQ antibiotics, including CP, on a human non-small-cell lung carcinoma (NSCLC) cell line in culture has demonstrated that the treatment induced morphological alterations, inhibited cellular proliferation, increased population doubling time, and reduced saturation density.[6]

Usually, we employ computer-assisted phase contrast microscopy to grossly decipher the mechanisms of action of novel antitumor drugs.[21–23,25] However, CP was reported early on to be photounstable.[26] Mass-spectrometry evidence has suggested that a photodimer of unknown structure is formed,[27] while further studies have led to the isolation of products resulting from the degradation of the piperazinyl side chain.[28] Later on, defluorination was observed together with the photolytic replacement of the C-6 fluo-

Table 2
In vitro cell proliferation and postulated mechanisms of action of ciprofloxacin and cytotoxic quinolones (data from literature)

Compound	Cell lines and in vitro IC_{50} (μM) growth inhibitory values	Postulated mechanism of action
Ciprofloxacin 	• HeLa epithelial (300,[42] 800[43]) • MG63 osteosarcoma (480[43]) • Chinese Hamster V79 (>1000[44]) • K-562 leukemia (>150[45]) • NCI-H460 NSC lung (60[6])	Inhibitor of the catalytic activity of the topoisomerase II • Activity against topoisomerase II (inhibition of DNA relaxation) = 104 μM[42] • 120 μM of ciprofloxacin increases the percentage of apoptotic cells from 17% to 35%[6]
	• CHO ovary (9[38]) • Vpm^R-5 mutant CHO ovary (12[38])	Enhance topoisomerase II-mediated DNA cleavage • Activity against topoisomerase II (inhibition of DNA relaxation): 60 μM[38]
	• CHO ovary (20[38]) • Vpm^R-5 mutant CHO ovary (40[38])	Enhance topoisomerase II-mediated DNA cleavage • Activity against topoisomerase II (inhibition of DNA relaxation): 130 μM[38]
	• A-549 lung (1.3[46]) • HT-29 colon (0.5,[46] 0.2,[34] 0.3,[47] 0.5[35]) • HCT-8 colon (0.3[46]) • MCF-7 breast (0.8,[46] 0.7[35]) • A546 breast (0.7[47]) • DU-145 prostate (0.2,[34] 0.5[35]) • P-388 leukemia (0.05[47]) • 8226/S myeloma (0.3[35])	Both a topoisomerase II poison and a catalytic inhibitor • Conversion of catenated to decatenated kDNA by human topoisomerase II: 0.5 μM,[34] 0.5 μM,[47] 3 μM[48] • Concentration of DNA unwinding: 50 μM[34]
 Stereoisomers (S) and (R)	• B16 Melanoma (0.2 (*S*), 0.02 (*R*))[34] • MDA-231 Breast (0.08 (*S*), 0.005 (*R*))[34] • H226 non-small cell lung (0.03 (*S*), 0.01 (*R*))[34] • HT-29 colon (0.05 (*S*), 0.03 (*R*))[34] • DU-145 prostate (0.06 (*S*), 0.03 (*R*))[34]	Unwind DNA and catalytic inhibitor of topoisomerase II • Conversion of catenated to decatenated kDNA by human topoisomerase II: 0.5 μM (*S*), 0.2 μM (*R*)[34] • Concentration of DNA unwinding: 10 μM (*S*), 3 μM (*R*)[34]
 Stereoisomers (S) and (R)	• MCF-7 breast (1 (*S*), 0.5 (*R*))[48] • 8226/S myeloma (0.7 (*S*), 0.3 (*R*))[45]	Major mechanism of action for stereoisomer (*S*): Topoisomerase II poisonMajor mechanism of action for stereoisomer (*R*): G-quadruplex interaction. • G-quadruplex interaction polymerase stop assay: IC_{50} = 0.7 μM (*S*); 0.06 μM (*R*)[48]

rine atom by a hydroxyl group.[29] This photounstability thus precluded the use of quantitative videomicroscopy to investigate the mechanisms of action of the compounds associated with the highest anti-tumor activity in the current study. We will proceed with a whole genomic approach as we did previously with respect to other types of anti-cancer agents.[22,30] However, before proceeding with such experiments, we must still select a lead compound through the characterization of in vivo activity on a broad panel of biologically aggressive human xenograft models.[19,21–23]

From initial approaches to define structure–activity relationships for cytotoxic quinolones derived from structural modifications of native CP, it became evident that no single position has a controlling effect on the observed in vitro antitumor activity.[31,32] A combination of various structural modifications on several positions of the native FQ, as shown by the structures of cytotoxic quinolones depicted in Figure 1, contributes to enhance cytotoxicity (IC_{50} values on several human and murine cell lines ranged from nM to μM concentrations).[33–37] For example, it has been shown that the introduction of

Table 3
In vitro cell proliferation inhibition of ciprofloxacin and some other fluoroquinolones (data from literature)

FQ	In vitro IC_{50} (μM) growth inhibitory values[a]										
	HeLa	MG63	NCI-H460	MCF-7	A431	EJ	SW480	KB	SKMEL	MC3T3-E1	NIH 3T3
Ciprofloxacin	800,[43] 270[51]	480[43]	60[6]	199,[40] 76[(this study)]	202,[40] 135[52]	202[40]	128[40]	160,[40] 177[52]	196[40]	120[49]	>1000[50]
Norfloxacin	390[51]	—	86[6]	238[40]	191,[40] 70[52]	210[40]	168[40]	163,[40] 150[52]	194[40]	—	>1000[50]
Enoxacin	170[51]		36[6]	193[40]	175,[40] 150[52]	178[40]	159[40]	137,[40] 154[52]	196[40]		
Levofloxacin			152[6]							220[49]	
Lomefloxacin	410[51]										483[50]
Moxifloxacin	320[43]	230[43]									
Trovafloxacin										<1.2[49]	
Sparfloxacin	265[51]										122[50]
Gatifloxacin	400[51]										
Tosufloxacin	32[51]										

[a] HeLa: epithelial, MG63: osteosarcoma, NCI-H460: NSC lung, MCF-7: breast carcinoma, A431: epidermo carcinoma, EJ: bladder carcinoma, W480: colon carcinoma, KB: cervical carcinoma, SKMEL: melanoma, MC3T3-E1: osteoblastic, NIH 3T3: fibroblast.

a C-8 fluorine in CP-115,953 enhanced the cytotoxicity by twofold against wild type CHO cells.[38] Nevertheless, we chose to synthesize compounds with single modification on C-7 of CP as it has been observed a more efficient in vitro growth inhibition in a panel of cancer cell lines treated with norfloxacin or CP thus structurally modified (IC_{50} ranging from 3 to >100 μM).[39,40] It can be seen from the data presented in Table 1 that the IC_{50} values measured for our compounds are in the same order of magnitude as those previously reported. Moreover, the breakage of the zwitterionic properties of quinolones by modifying the C-7 basic substituent could change the electron distribution and thus the protonation equilibria,[31] and eventually the transmembrane passage. Finally, QSAR analysis of 7-(pyrrolidin-1-yl)-4-oxo-1-(thiazol-2-yl)-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid derivatives, structurally similar to FQ, has demonstrated that bulky substituents with low electron density (e.g., alkyl groups) on the C-7 position enhanced the antitumor activity.[41]

Several of the novel FQ derivatives of this current study displayed higher in vitro antitumor activity than parent CP or other more recent FQ (Table 3). By increasing lipophilicity of CP, we could expect an increase in the affinity of the compounds for the cell membrane and thus an easier penetration into the cell. However, if substitutions on the piperazinyl group of CP can lead to an increase in antitumor activity, this is not dependent on the lipophilicity of the substituent since the most potent compounds **2** and **6h** have Clog *P* values of 0.78 and 4.40, respectively. In comparing each homologous series or each cancer cell line independently, no correlation between Clog *P* and IC_{50} values can be observed. However, in comparing the same chain length but with a different functional linker to CP, the alkanoyl derivatives **6** seem to be more potent than the alkoxycarbonyl **5** or oxoethylalcanoate **4** derivatives. For example, the most potent antiproliferative derivative **6h**, with a chain length of nine carbon atoms, is more active than the corresponding alkoxycarbonyl **5d** or oxyethylalcanoate **4f** derivatives.

In conclusion, we have synthesized, with an easy and cost-efficient procedure, 31 7-((4-substitued)piperazin-1-yl) derivatives of CP that are non-toxic as shown by their MTD values. Among all these derivatives, two of them, that is, compounds **2** and **6h**, showed potent in vitro antitumor activity. This study thus demonstrates that the cytotoxicity of CP can be positively modulated through the introduction of simple substituents on the piperazinyl group.

6. Experimental

6.1. Chemistry

All reagents were purchased from commercial sources and used as supplied without further purification. CP was purchased from Fluka (>98%). Chromatography was performed on silica gel (<70 μm) by means of the solvent systems indicated. Melting points were determined using a Kofler type system and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer. Data are reported in the following order: chemical shift δ in ppm, signal multiplicity, number of protons and value(s) of coupling constant(s). ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer. The methylene groups of alkyl chains are reported in Greek alphabetical order starting from CH_3. The other attributions are reported according to the numbering in Scheme 1. Elemental analyses were carried out by the 'Service Commun de Microanalyse élémentaire UPS-INP' in Toulouse. Mass spectra were recorded on a Perkin Elmer SCIEX API 100 operating in chemical ionization (NH_3) mode or on a Nermag R10-10 operating in Fast Atom Bombardment (MNBA) mode.

6.1.1. 7-(4-(2-Chloroacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (2)

CP **1** (3.0 g, 9 mmol) and triethylamine (1.3 mL, 9 mmol) were stirred in 40 mL of dry methylene chloride at 0 °C for 15 min. Chloroacetyl chloride (1.1 mL, 14 mmol) was added dropwise. After stirring at 0 °C for 15 min and at room temperature for 1 h, the mixture was filtered and the resulting solids were washed with water and methylene chloride. The aqueous layer was extracted with 3 × 30 mL of CH_2Cl_2. The organic layers were collected, dried over $MgSO_4$, and concentrated. The remaining residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 2%) to yield the desired product (2.68 g, 73%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (t, 2H, ^3J = 7.2 Hz, CH_2 (12)), 1.32 (t, 2H, ^3J = 6.9 Hz, CH_2 (13)), 3.37 (m, 4H, CH_2 (16)), 3.70 (m, 4H, CH_2 (15)), 3.82 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.9 Hz, C*H* (11)), 4.45 (s, 2H, CH_2–Cl), 7.58 (d, 1H, $^4J_{H-F}$ = 7.6 Hz, C*H* (8)), 7.92 (d, 1H, $^3J_{H-F}$ = 13.2 Hz, C*H* (5)), 8.66 (s, 1H, C*H* (2)), 14.7 (s, 1H, –COO*H*). ^{13}C NMR (DMSO-d_6) δ 176.2 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 165.7 (*C*14), 166.7 (*C*17), 152.8 (d, $^1J_{C-F}$ = 249.3 Hz, *C*6), 147.9 (*C*2), 144.6 (d, $^2J_{C-F}$ = 10.2 Hz, *C*7), 138.9 (*C*9), 118.7 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 110.8 (d, $^2J_{C-F}$ = 23.1 Hz, *C*5), 106.6 (*C*3), 106.4 (d, $^3J_{C-F}$ = 3.1 Hz, *C*8), 49.3 (*C*15), 48.9 (*C*15), 44.8 (*C*16), 41.6 (*C*H_2-Cl), 41.2 (*C*16), 35.8 (*C*11), 7.4 (*C*12,13). FAB-MS *m*/*z* 408 [MH^+]. Anal. Calcd for $C_{19}H_{19}ClFN_3O_4 \cdot 0.4H_2O$: C, 54.99; H, 4.81; N, 10.12. Found: C, 54.90; H, 4.65; N, 10.15.

6.1.2. 7-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (3)

To a solution of CP **1** (0.5 g, 1.5 mmol) in 10 mL of dioxane, sodium hydroxide (0.9 g, 22 mmol) in 10 mL of water was added at 0 °C. A solution of di-*tert*-butyl dicarbonate (0.36 g, 1.65 mmol) in 10 mL of dioxane was then added dropwise and the reaction mixture was stirred at 0 °C for 1 h and at room temperature for 24 h. Dioxane was concentrated and 50 mL of water were added. The pH of the aqueous solution was adjusted to 3–4 with a 20%

aqueous solution of monohydrate citric acid. The obtained precipitate was filtered and washed twice with ethanol and diethyl ether. The remaining residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 2%) to yield the desired product (0.49 g, 74%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR ($CDCl_3$) δ 1.14 (m, 2H, CH_2 (12)), 1.33 (m, 2H, CH_2 (13)), 1.49 (s, 9H, CH_3), 3.22 (m, 4H, CH_2 (16)), 3.50 (m, 1H, C*H* (11)), 3.60 (m, 4H, CH_2 (15)), 7.29 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.93 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.67 (s, 1H, C*H* (2)), 14.88 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.5 Hz, *C*4), 166.6 (*C*14), 154.9 (*C*17), 153.4 (d, $^1J_{C-F}$ = 249.1 Hz, *C*6), 148.5 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.2 Hz, *C*7), 139.7 (*C*9), 119.4 (d, $^3J_{C-F}$ = 7.2 Hz, *C*10), 111.6 (d, $^2J_{C-F}$ = 23.5 Hz, *C*5), 107.6 (*C*3), 107.1 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 80.3 (*C*(CH$_3$)$_3$), 49.9 (*C*15), 49.5 (*C*15), 45.5 (*C*16), 41.9 (*C*16), 35.4 (*C*11), 28.5 (CH_3), 8.1 (*C*12,13). FAB-MS *m*/*z* 432 [MH$^+$]. Anal. Calcd for $C_{22}H_{26}FN_3O_5{\cdot}0.5H_2O$: C, 59.99; H, 6.17; N, 9.54. Found: C, 59.93; H, 6.01; N, 9.52.

6.1.3. General procedure for the preparation of 7-(4-(2-oxoethylalcanoate)piperazin-1-yl) derivatives of CP (4a–j)

To a solution of carboxylic acid (0.74 mmol) in dry dimethylformamide (10 mL), potassium carbonate (117 mg, 0.85 mmol) was added at room temperature and the reaction mixture was stirred for 1 h at 110 °C, after which compound **2** (0.3 g, 0.74 mmol) was added. The mixture was stirred for 4 h at 110 °C. DMF was evaporated in vacuo. For compounds **4a–e**, the residue was diluted with 20 mL of water. The aqueous layer was extracted with 4 × 20 mL of ethyl acetate. The organic layers were collected, dried over $MgSO_4$, and evaporated. The remaining residue was purified by silica gel chromatography. For compounds **4f–j**, purification was done directly after evaporation in vacuo of DMF.

6.1.3.1. 7-(4-(2-Oxoethylethanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4a).

A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–2%) provided the desired compound (194 mg, 60%) as a white solid. Mp >260 °C. ^{1}H NMR (DMSO-d_6) δ 1.18 (m, 2H, CH_2 (12)), 1.30 (m, 2H, CH_2 (13)), 2.09 (s, 3H, CH_3), 3.33 (m, 4H, CH_2 (16)), 3.66 (m, 4H, CH_2 (15)), 3.81 (m, 1H, C*H* (11)), 4.84 (s, 2H, –O–CH_2–CO–), 7.58 (d, 1H, $^4J_{H-F}$ = 7.5 Hz, C*H* (8)), 7.91 (d, 1H, $^3J_{H-F}$ = 13.2 Hz, C*H* (5)), 8.66 (s, 1H, C*H* (2)), 15.10 (s, 1H, –COO*H*). ^{13}C NMR (DMSO-d_6) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.2 (CH$_3$CO–), 166.6 (*C*14), 165.2 (*C*17), 153.6 (d, $^1J_{C-F}$ = 250.2 Hz, *C*6), 147.5 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.8 (*C*9), 120.1 (d, $^3J_{C-F}$ = 7.5 Hz, *C*10), 112.5 (d, $^2J_{C-F}$ = 23.5 Hz, *C*5), 108.1 (*C*3), 105.3 (d, $^3J_{C-F}$ = 2.9 Hz, *C*8), 60.9 (–O–CH$_2$–CO–), 49.9 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.5 (*C*11), 19.1 (CH_3), 8.1 (*C*12,13). CI/NH$_3$-MS (positive mode) *m*/*z* 431.1 [M], 432.1 [MH$^+$]. Anal. Calcd for $C_{21}H_{22}FN_3O_6{\cdot}0.2H_2O$: C, 57.98; H, 5.19; N, 9.65. Found: C, 58.01; H, 5.18; N, 9.67.

6.1.3.2. 7-(4-(2-Oxoethylbutanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4b).

A purification by silica gel chromatography (AcOEt–MeOH 1%) provided the desired compound (213 mg, 63%) as a pale yellow solid. Mp 211 °C. ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, ^3J = 7.5 Hz, CH_3), 1.20 (m, 2H, CH_2 (12)), 1.39 (m, 2H, CH_2 (13)), 1.69 (sext, 2H, ^3J = 7.5 Hz, CH_2 α), 2.42 (t, 2H, ^3J = 7.5 Hz, CH_2 β), 3.35 (m, 4H, CH_2 (16)), 3.55 (m, 1H, C*H* (11)), 3.66 (m, 2H, CH_2 (16)), 3.85 (m, 2H, CH_2 (15)), 4.78 (s, 2H, –O–CH_2–CO–), 7.34 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.90 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.65 (s, 1H, C*H* (2)), 14.82 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 3.0 Hz, *C*4), 173.3 (CH$_{2\beta}$CO–), 166.7 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 249.7 Hz, *C*6), 147.5 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.5 Hz, *C*10), 112.4 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 108.0 (*C*3), 105.3 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.9 (–O–CH$_2$–CO–), 49.9 (*C*15), 49.3 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.8 (*C*11), 35.4 (CH_2 β), 18.4 (CH_2 α), 13.6 (CH_3), 8.2 (*C*12,13). CI/NH$_3$-MS (positive mode) *m*/*z* 459.2 [M], 460.2 [MH$^+$]. Anal. Calcd for $C_{23}H_{26}FN_3O_6{\cdot}0.5H_2O$: C, 58.97; H, 5.81; N, 8.97. Found: C, 59.02; H, 5.83; N, 9.01.

6.1.3.3. 7-(4-(2-Oxoethylhexanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4c).

A purification by silica gel chromatography (AcOEt–MeOH 1%) provided the desired compound (268 mg, 75%) as a pale yellow solid. Mp 167 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.19 (m, 2H, CH_2 (12)), 1.35 (m, 6H, CH_2 (13,α,β)), 1.67 (qt, 2H, ^3J = 6.9 Hz, ^3J = 7.5 Hz, CH_2 γ), 2.43 (t, 2H, ^3J = 7.5 Hz, CH_2 δ), 3.35 (m, 4H, CH_2 (16)), 3.55 (m, 1H, C*H* (11)), 3.66 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 4.77 (s, 2H, –O–CH_2–CO–), 7.34 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.90 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.65 (s, 1H, C*H* (2)), 14.82 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.9 (d,$^4J_{C-F}$ = 2.3 Hz, *C*4), 173.5 (CH$_{2\delta}$CO–), 166.7 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 249.7 Hz, *C*6), 147.5 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.5 Hz, *C*10), 112.4 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 108.0 (*C*3), 105.3 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.9 (*C*15), 49.3 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.4 (*C*11), 33.9 (CH_2 δ), 31.2 (CH_2 γ), 24.5 (CH_2 β), 22.3 (CH_2 α), 13.9 (CH_3), 8.2 (*C*12,13). CI/NH$_3$-MS (positive mode) *m*/*z* 487.2 [M], 488.2 [MH$^+$]. Anal. Calcd for $C_{25}H_{30}FN_3O_6{\cdot}0.4H_2O$: C, 60.69; H, 6.27; N, 8.49. Found: C, 60.8; H, 6.30; N, 8.52.

6.1.3.4. 7-(4-(2-Oxoethyloctanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4d).

A purification by silica gel chromatography (AcOEt–MeOH 1%) provided the desired compound (171 mg, 45%) as a white solid. Mp 176 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.30 (m, 12H, CH_2 (12,13,α–δ)), 1.67 (tt, 2H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, CH_2 ε), 2.45 (t, 2H, ^3J = 7.5 Hz, CH_2 ζ), 3.35 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.9 Hz, C*H* (11)), 3.66 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 4.78 (s, 2H, –O–CH_2–CO–), 7.35 (d, 1H,$^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.94 (d, 1H, $^3J_{H-F}$ = 12.6 Hz, C*H* (5)), 8.68 (s, 1H, C*H* (2)), 14.84 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.2 Hz, *C*4), 173.4 (CH$_{2\zeta}$CO–), 166.6 (*C*14), 165.3 (*C*17), 153.8 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.6 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 6.8 Hz, *C*10), 112.0 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.7 (*C*3), 105.3 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.7 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.4 (*C*11), 33.9 (CH_2 ζ), 31.6 (CH_2 ε), 29.0 (CH_2 δ), 28.9 (CH_2 γ), 24.8 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH$_3$-MS (positive mode) *m*/*z* 515.3 [M], 516.3 [MH$^+$]. Anal. Calcd for $C_{27}H_{34}FN_3O_6{\cdot}0.5H_2O$: C, 61.82; H, 6.72; N, 8.01. Found: C, 61.81; H, 6.70; N, 8.12.

6.1.3.5. 7-(4-(2-Oxoethylnonanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4e).

A purification by silica gel chromatography (AcOEt–MeOH 0.05%) provided the desired compound (100 mg, 27%) as a white solid. Mp 136 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.5 Hz, CH_3), 1.25 (m, 14H, CH_2 (12,13,α–ε)), 1.65 (tt, 2H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, CH_2 ζ), 2.44 (t, 2H, ^3J = 7.5 Hz, CH_2 η), 3.35 (m, 4H, CH_2 (16)), 3.55 (m, 1H, C*H* (11)), 3.66 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 4.78 (s, 2H, –O–CH_2–CO–), 7.34 (d, 1H, $^4J_{H-F}$ = 6.9 Hz, C*H* (8)), 7.91 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.67 (s, 1H, C*H* (2)), 14.88 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.5 Hz, *C*4), 173.4 (CH$_{2\eta}$CO–), 166.7 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.5 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 120.1 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.3 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.9 (*C*3), 105.2 (d, $^3J_{C-F}$ = 2.9 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.8 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.3 (*C*11), 33.8 (CH_2 η), 31.7 (CH_2 ζ), 29.1 (CH_2 ε), 29.0 (CH_2 γ,δ), 24.8 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH$_3$-MS (positive mode) *m*/*z* 529.3 [M], 530.3 [MH$^+$]. Anal. Calcd for $C_{28}H_{36}FN_3O_6{\cdot}0.5H_2O$: C, 62.44; H, 6.92; N, 7.80. Found: C, 62.49; H, 6.95; N, 7.83.

6.1.3.6. 7-(4-(2-Oxoethyldecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4f). A purification by silica gel chromatography (AcOEt–MeOH 0.05%) provided the desired compound (190 mg, 47%) as a white solid. Mp 162 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.30 (m, 16H, CH_2 (12,13,α–ζ), 1.66 (tt, 2H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, CH_2 η), 2.44 (t, 2H, ^3J = 7.5 Hz, CH_2 θ), 3.34 (m, 4H, CH_2 16), 3.39 (m, 1H, CH 11), 3.66 (m, 2H, CH_2 15), 3.86 (m, 2H, CH_2 15), 4.78 (s, 2H, –O–CH_2–CO–), 7.35 (d, 1H, $^4J_{H-F}$ = 6.6 Hz, CH 8), 7.94 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH 5), 8.69 (s, 1H, CH 2), 14.86 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.4 ($CH_{2\theta}CO$–), 166.7 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.2 Hz, *C*6), 147.5 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.4 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 108.0 (*C*3), 105.2 (d, $^3J_{C-F}$ = 2.9 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.9 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.3 (*C*11), 33.9 (CH_2 θ), 31.8 (CH_2 η), 29.3 (CH_2 ζ), 29.2 (CH_2 δ,ε), 29.1 (CH_2 γ), 24.8 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 543.3 [M], 544.3 [MH$^+$]. Anal. Calcd for $C_{29}H_{38}FN_3O_6{\cdot}0.25H_2O$: C, 63.54; H, 7.08; N, 7.67. Found: C, 63.58; H, 7.10; N, 7.69.

6.1.3.7. 7-(4-(2-Oxoethylundecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4g). A purification by silica gel chromatography (AcOEt–MeOH 0.05%) provided the desired compound (178 mg, 43%) as a white solid. Mp 166 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.3 Hz, CH_3), 1.25 (m, 18H, CH_2 (12,13,α–η), 1.69 (m, 2H, CH_2 θ), 2.45 (t, 2H, ^3J = 7.5 Hz, CH_2 ι), 3.38 (m, 4H, CH_2 (16)), 3.55 (m, 1H, CH (11)), 3.66 (m, 2H, CH_2 (15)), 3.87 (m, 2H, CH_2 (15)), 4.78 (s, 2H, –O–CH_2–CO–), 7.35 (d, 1H, $^4J_{H-F}$ = 6.6 Hz, CH (8)), 7.97 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.71 (s, 1H, CH (2)), 14.85 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.5 ($CH_{2\iota}CO$–), 166.7 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.2 Hz, *C*6), 147.5 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.6 Hz, *C*7), 138.9 (*C*9), 120.3 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.5 (d, $^2J_{C-F}$ = 23.3 Hz, *C*5), 108.1 (*C*3), 105.2 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.9 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.3 (*C*11), 33.9 (CH_2 ι), 31.8 (CH_2 θ), 29.5 (CH_2 η), 29.4 (CH_2 ζ), 29.3 (CH_2 ε), 29.2 (CH_2 δ), 29.1 (CH_2 γ), 24.8 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 557.3 [M], 558.3 [MH$^+$]. Anal. Calcd for $C_{30}H_{40}FN_3O_6{\cdot}0.5H_2O$: C, 63.59; H, 7.29; N, 7.41. Found: C, 63.61; H, 7.30; N, 7.43.

6.1.3.8. 7-(4-(2-Oxoethyldodecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4h). A purification by silica gel chromatography (AcOEt–MeOH 0.5%) provided the desired compound (193 mg, 46%) as a pale yellow solid. Mp 169 °C. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.22 (m, 20H, CH_2 (12,13,α–θ), 1.65 (tt, 2H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, CH_2 ι), 2.42 (t, 2H, ^3J = 7.5 Hz, CH_2 κ), 3.35 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.9 Hz, CH (11)), 3.66 (m, 2H, CH_2 (15)), 3.85 (m, 2H, CH_2 (15)), 4.77 (s, 2H, –O–CH_2–CO–), 7.33 (d, 1H, $^4J_{H-F}$ = 6.9 Hz, CH (8)), 7.84 (d, 1H, $^3J_{H-F}$ = 12.6 Hz, CH (5)), 8.61 (s, 1H, CH (2)), 14.82 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.3 ($CH_{2\kappa}CO$–), 166.5 (*C*14), 165.2 (*C*17), 153.4 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.3 (*C*2), 145.1 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.8 (*C*9), 119.8 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 112.0 (d, $^2J_{C-F}$ = 23.3 Hz, *C*5), 107.8 (*C*3), 105.2 (d, $^3J_{C-F}$ = 2.9 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.8 (*C*15), 49.2 (*C*15), 44.3 (*C*16), 41.4 (*C*16), 35.3 (*C*11), 33.8 (CH_2 κ), 31.8 (CH_2 ι), 29.5 (CH_2 θ,η), 29.3 (CH_2 ζ), 29.2 (CH_2 ε), 29.1 (CH_2 δ), 29.0 (CH_2 γ), 24.7 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 571.3 [M], 572.3 [MH$^+$]. Anal. Calcd for $C_{31}H_{42}FN_3O_6{\cdot}0.25H_2O$: C, 64.66; H, 7.44; N, 7.30. Found: C, 64.67; H, 7.58; N, 7.32.

6.1.3.9. 7-(4-(2-Oxoethyltetradecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4i). A purification by silica gel chromatography (AcOEt) provided the desired compound (180 mg, 41%) as a pale yellow solid. Mp 170 °C. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.22 (m, 24H, CH_2 (12,13,α–κ), 1.65 (tt, 2H, ^3J = 6.9 Hz, ^3J = 7.5 Hz, CH_2 λ), 2.42 (t, 2H, ^3J = 7.5 Hz, CH_2 μ), 3.35 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.6 Hz, CH (11)), 3.66 (m, 2H, CH_2 (15)), 3.85 (m, 2H, CH_2 (15)), 4.77 (s, 2H, –O–CH_2–CO–), 7.33 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 7.84 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.61 (s, 1H, CH (2)), 14.82 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.4 ($CH_{2\mu}CO$–), 166.6 (*C*14), 165.3 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.0 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.3 Hz, *C*5), 107.9 (*C*3), 105.3 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.8 (*C*15), 49.2 (*C*15), 44.4 (*C*16), 41.5 (*C*16), 35.4 (*C*11), 33.9 (CH_2 μ), 31.9 (CH_2 λ), 29.7 (CH_2 κ), 29.65 (CH_2 ι,θ), 29.6 (CH_2 η), 29.4 (CH_2 ζ), 29.3 (CH_2 ε), 29.2 (CH_2 δ), 29.1 (CH_2 γ), 24.8 (CH_2 β), 22.7 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 599.3 [M], 600.3 [MH$^+$]. Anal. Calcd for $C_{33}H_{46}FN_3O_6{\cdot}0.5H_2O$: C, 65.15; H, 7.79; N, 6.91. Found: C, 65.06; H, 7.78; N, 7.04.

6.1.3.10. 7-(4-(2-Oxoethylhexadecanoate)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4j). A purification by silica gel chromatography (AcOEt) provided the desired compound (213 mg, 46%) as a pale yellow solid. Mp 164 °C. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.22 (m, 28H, CH_2 (12,13,α–μ), 1.63 (tt, 2H, ^3J = 6.9 Hz, ^3J = 7.5 Hz, CH_2 ν), 2.42 (t, 2H, ^3J = 7.5 Hz, CH_2 ξ), 3.35 (m, 4H, CH_2 (16)), 3.55 (m, 1H, CH (11)), 3.66 (m, 2H, CH_2 (15)), 3.85 (m, 2H, CH_2 (15)), 4.77 (s, 2H, –O–CH_2–CO–), 7.33 (d, 1H, $^4J_{H-F}$ = 7.0 Hz, CH (8)), 7.85 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.61 (s, 1H, CH (2)), 14.86 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 173.3 ($CH_{2\xi}CO$–), 166.5 (*C*14), 165.2 (*C*17), 153.4 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.3 (*C*2), 145.1 (d, $^2J_{C-F}$ = 10.6 Hz, *C*7), 138.7 (*C*9), 119.9 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.3 Hz, *C*5), 107.8 (*C*3), 105.2 (d, $^3J_{C-F}$ = 2.9 Hz, *C*8), 60.9 (–O–CH_2–CO–), 49.8 (*C*15), 49.2 (*C*15), 44.3 (*C*16), 41.4 (*C*16), 35.3 (*C*11), 33.8 (CH_2 ξ), 31.8 (CH_2 ν), 29.6 (CH_2 κ,λ,μ), 29.55 (CH_2 θ,ι), 29.5 (CH_2 η), 29.3 (CH_2 ζ), 29.2 (CH_2 ε), 29.1 (CH_2 δ), 29.0 (CH_2 γ), 24.7 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 627.4 [M], 628.4 [MH$^+$]. Anal. Calcd for $C_{35}H_{50}FN_3O_6{\cdot}0.4H_2O$: C, 66.24; H, 8.07; N, 6.62. Found: C, 66.23; H, 8.12; N, 6.83.

6.1.4. General procedure for the preparation of 7-(4-(alkoxycarbonyl)piperazin-1-yl) derivatives of CP (5a–f)

To a solution of CP **1** (0.5 g, 1.5 mmol) in methylene chloride (20 mL) at 0 °C was added dropwise alkyl chloroformate (3.0 mmol) over a 15 min period. The reaction was stirred at 0 °C for 15 min and then 1 h at room temperature. The mixture was quenched by an aqueous solution of 1 M HCl (50 mL). The aqueous layer was extracted once with 30 mL of CH_2Cl_2. The organic layers were collected, dried over Na_2SO_4, and concentrated. The remaining residue was purified by silica gel chromatography.

6.1.4.1. 7-[4-(Ethoxycarbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5a). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1%) provided the desired compound (401 mg, 66%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR ($CDCl_3$) δ 1.20 (m, 2H, CH_2 (12)), 1.29 (t, 3H, ^3J = 7.0 Hz, CH_3), 1.39 (m, 2H, CH_2 (13)), 3.28 (m, 4H, CH_2 (16)), 3.54 (m, 1H, CH_2 (11)), 3.72 (m, 4H, CH_2 (15)), 4.18 (q, 2H, ^3J = 7.0 Hz, CH_2 α), 7.37 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 8.04 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.78 (s, 1H, CH (2)), 15.02 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 3.0 Hz, *C*4), 166.7 (*C*14), 155.4 (*C*17), 153.5 (d, $^1J_{C-F}$ = 249.5 Hz, *C*6), 147.5 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 120.1 (d, $^3J_{C-F}$ = 7.5 Hz, *C*10), 112.4 (d, $^2J_{C-F}$ = 23.0 Hz, *C*5), 108.1 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 65.6 (CH_2 α), 49.6 (*C*15), 43.4 (*C*16), 29.9 (*C*11), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 403.2 [M], 404.2 [MH$^+$].

Anal. Calcd for $C_{20}H_{22}FN_3O_5$: C, 59.55; H, 5.50; N, 10.42. Found: C, 59.60; H, 5.41; N, 10.45.

6.1.4.2. 7-[4-(Butoxycarbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5b). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (290 mg, 42%) as a pale yellow solid. Mp 240 °C. ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H, ^3J = 7.5 Hz, CH_3), 1.20 (m, 2H, CH_2 (12)), 1.40 (m, 4H, CH_2 (13,α)), 1.65 (m, 2H, CH_2 β), 3.30 (m, 4H, CH_2 16), 3.55 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.2 Hz, CH (11)), 3.72 (m, 4H, CH_2 15), 4.13 (t, 2H, ^3J = 6.6 Hz, CH_2 γ), 7.37 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 8.00 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.73 (s, 1H, CH (2)), 14.91 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 166.7 (*C*14), 155.4 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.6 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.0 (d, $^3J_{C-F}$ = 7.9 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.9 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 65.6 (CH_2 γ), 49.6 (*C*15), 43.4 (*C*16), 35.3 (*C*11), 31.0 (CH_2 β), 19.1 (CH_2 α), 13.7 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 431.4 [M], 432.4 [MH$^+$]. Anal. Calcd for $C_{22}H_{26}FN_3O_5$: C, 61.24; H, 6.07; N, 9.74. Found: C, 61.50; H, 6.42; N, 9.51.

6.1.4.3. 7-[4-((Octyloxy)carbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5c). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1%) provided the desired compound (270 mg, 37%) as a pale yellow solid. Mp 154 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.27 (m, 14H, CH_2 (12,13,α–ε), 1.64 (m, 2H, CH_2 ζ), 3.30 (m, 4H, CH_2 (16)), 3.54 (m, 1H, CH (11)), 3.71 (m, 4H, CH_2 (15)), 4.12 (t, 2H, ^3J = 6.6 Hz, CH_2 η), 7.34 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 7.99 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.73 (s, 1H, CH (2)), 14.90 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 177.0 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 166.8 (*C*14), 155.4 (*C*17), 153.6 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.5 (*C*2), 145.7 (d, $^2J_{C-F}$ = 10.3 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.9 Hz, *C*10), 112.5 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 108.2 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 66.0 (CH_2 η), 49.7 (*C*15), 49.6 (*C*15), 43.4 (*C*16), 43.3 (*C*16), 35.3 (*C*11), 31.8 (CH_2 ζ), 29.2 (CH_2 ε), 29.1 (CH_2 δ), 28.9 (CH_2 γ), 25.9 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 487.3 [M], 488.3 [MH$^+$]. Anal. Calcd for $C_{26}H_{34}FN_3O_5$: C, 64.05; H, 7.03; N, 8.62. Found: C, 63.99; H, 7.09; N, 8.66.

6.1.4.4. 7-[4-((Nonyloxy)carbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5d). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1%) provided the desired compound (253 mg, 33%) as a pale yellow solid. Mp 157 °C. ^{1}H NMR ($CDCl_3$) δ 0.84 (m, 3H, CH_3), 1.25 (m, 16H, CH_2 (12,13,α–ζ), 1.63 (m, 2H, CH_2 η), 3.29 (m, 4H, CH_2 (16)), 3.54 (m, 1H, CH (11)), 3.69 (m, 4H, CH_2 (15)), 4.09 (t, 2H, ^3J = 6.6 Hz, CH_2 θ), 7.32 (d, 1H, $^4J_{H-F}$ = 6.7 Hz, CH (8)), 7.85 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.62 (s, 1H, CH (2)), 14.85 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 166.6 (*C*14), 155.4 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.4 Hz, *C*6), 147.3 (*C*2), 145.6 (d, $^2J_{C-F}$ = 10.3 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.1 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.8 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.1 Hz, *C*8), 65.9 (CH_2 θ), 49.6 (*C*15), 49.5 (*C*15), 43.3 (*C*16), 43.2 (*C*16), 35.3 (*C*11), 31.8 (CH_2 η), 29.4 (CH_2 ζ), 29.2 (CH_2 ε), 29.1 (CH_2 δ), 28.9 (CH_2 γ), 25.9 (CH_2 β), 22.6 (CH_2 α), 14.0 (CH_3), 8.1 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 501.3 [M], 502.3 [MH$^+$]. Anal. Calcd for $C_{27}H_{36}FN_3O_5$: C, 64.65; H, 7.23; N, 8.38. Found: C, 64.69; H, 7.20; N, 8.41.

6.1.4.5. 7-[4-((Decyloxy)carbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5e). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1%) provided the desired compound (319 mg, 41%) as a pale yellow solid. Mp 152 °C. ^{1}H NMR ($CDCl_3$) δ 0.82 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.24 (m, 18H, CH_2 (12,13,α–η), 1.57 (m, 2H, ^3J = 6.6 Hz, CH_2 θ), 3.23 (m, 4H, CH_2 (16)), 3.48 (m, 1H, CH (11)), 3.63 (m, 4H, CH_2 (15)), 4.02 (t, 2H, ^3J = 6.6 Hz, CH_2 ι), 7.25 (d, 1H, $^4J_{H-F}$ = 6.9 Hz, CH (8)), 7.78 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.54 (s, 1H, CH (2)), 14.80 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 166.7 (*C*14), 155.4 (*C*17), 153.5 (d, $^1J_{C-F}$ = 251.2 Hz, *C*6), 147.3 (*C*2), 145.7 (d, $^2J_{C-F}$ = 10.2 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 8.0 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.9 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 65.9 (CH_2 ι), 49.6 (*C*15), 49.5 (*C*15), 43.4 (*C*16), 43.3 (*C*16), 35.3 (*C*11), 31.8 (CH_2 θ), 29.5 (CH_2 η), 29.4 (CH_2 ζ), 29.3 (CH_2 ε), 29.2 (CH_2 δ), 28.9 (CH_2 γ), 25.9 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 515.3 [M], 516.3 [MH$^+$]. Anal. Calcd for $C_{28}H_{38}FN_3O_5$: C, 65.22; H, 7.43; N, 8.15. Found: C, 65.29; H, 7.40; N, 8.21.

6.1.4.6. 7-[4-((Dodecyloxy)carbonyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (5f). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1%) provided the desired compound (310 mg, 38%) as a pale yellow solid. Mp 140 °C. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.19 (m, 22H, CH_2 (12,13,α–ι), 1.59 (m, 2H, ^3J = 6.9 Hz, CH_2 κ), 3.24 (m, 4H, CH_2 (16)), 3.48 (m, 1H, CH (11)), 3.66 (m, 4H, CH_2 (15)), 4.06 (t, 2H, ^3J = 6.9 Hz, CH_2 λ), 7.29 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 7.94 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.67 (s, 1H, CH (2)), 14.83 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.2 Hz, *C*4), 166.7 (*C*14), 155.4 (*C*17), 153.5 (d, $^1J_{C-F}$ = 250.2 Hz, *C*6), 147.3 (*C*2), 145.7 (d, $^2J_{C-F}$ = 9.7 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 8.1 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.9 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 65.9 (CH_2 λ), 49.6 (*C*15), 43.4 (*C*16), 35.3 (*C*11), 31.8 (CH_2 κ), 29.7 (CH_2 ι), 29.6 (CH_2 θ), 29.5 (CH_2 η), 29.3 (CH_2 ζ), 29.2 (CH_2 ε), 28.9 (CH_2 γ,δ), 25.9 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 543.4 [M], 544.3 [MH$^+$]. Anal. Calcd for $C_{30}H_{42}FN_3O_5$: C, 66.28; H, 7.79; N, 7.73. Found: C, 66.25; H, 7.82; N, 7.77.

6.1.5. General procedure for the preparation of 7-(4-(alkanoyl)piperazin-1-yl) derivatives of ciprofloxacin (6a–j)

CP **1** (0.5 g, 1.5 mmol) and triethylamine (0.23 mL, 1.65 mmol) were stirred in 20 mL of methylene chloride at 0 °C for 15 min. Acylchloride (3.75 mmol) was added dropwise. After stirring at 0 °C for 15 min and then at room temperature for 1 h, 20 mL of water were added to the mixture. The aqueous layer was extracted once with CH_2Cl_2, and the organic phase was washed with water (20 mL). The organic layer was dried over Na_2SO_4 and concentrated. The remaining residue was purified by silica gel chromatography.

6.1.5.1. 7-(4-Acetylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6a). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (500 mg, 88%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (m, 2H, CH_2 (12)), 1.32 (m, 2H, CH_2 (13)), 2.07 (s, 3H, CH_3), 3.30 (m, 2H, CH_2 (16)), 3.36 (m, 2H, CH_2 (16)), 3.67 (m, 4H, CH_2 (15)), 3.82 (tt, 1H, ^3J = 7.5 Hz, ^3J = 6.9 Hz, CH (11)), 7.57 (t, 1H, $^4J_{H-F}$ = 7.5 Hz, CH (8)), 7.93 (d, 1H, $^3J_{H-F}$ = 13.5 Hz, CH (5)), 8.66 (s, 1H, CH (2)), 15.09 (s, 1H, –COOH). ^{13}C NMR (DMSO-d_6) δ 177.6 (d, $^4J_{C-F}$ = 2.5 Hz, *C*4), 174.8 (*C*17), 169.1 (*C*14), 152.3 (d, $^1J_{C-F}$ = 249.5 Hz, *C*6), 147.5 (*C*2), 143.3 (d, $^2J_{C-F}$ = 10.0 Hz, *C*7), 138.2 (*C*9), 122.2 (d, $^3J_{C-F}$ = 7.2 Hz, *C*10), 111.5 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 109.1 (*C*3), 105.8 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 50.1 (*C*15), 49.7 (*C*15), 45.7 (*C*16), 40.9 (*C*16), 34.3 (*C*11), 13.8 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 373.3 [M], 374.3 [MH$^+$]. Anal. Calcd for $C_{19}H_{20}FN_3O_4$: C, 61.12; H, 5.40; N, 11.25. Found: C, 61.15; H, 5.42; N, 11.27.

6.1.5.2. 7-(4-Propionylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6b). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (435 mg, 75%) as a pale yellow solid. Mp 258 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 5H, ^3J = 7.5 Hz, CH_3, CH_2 (12)),

1.40 (m, 2H, CH_2 (13)), 2.41 (q, 2H, ^3J = 7.5 Hz, CH_2 α), 3.33 (m, 4H, CH_2 (16)), 3.54 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.9 Hz, C*H* (11)), 3.80 (m, 4H, CH_2 (15)), 7.37 (d, 1H, $^4J_{H-F}$ = 6.9 Hz, C*H* (8)), 8.00 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.75 (s, 1H, C*H* (2)), 14.95 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 172.4 (*C*17), 166.8 (*C*14), 153.6 (d, $^1J_{C-F}$ = 251.4 Hz, *C*6), 147.5 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.5 (d, $^2J_{C-F}$ = 23.3 Hz, *C*5), 108.2 (*C*3), 105.2 (d, $^3J_{C-F}$ = 3.1 Hz, *C*8), 49.6 (*C*15), 41.1 (*C*16), 35.3 (*C*11), 26.4 (CH_2 α), 9.4 (CH_3), 8.3 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 387.3 [M], 388.3 [MH^+]. Anal. Calcd for $C_{20}H_{22}FN_3O_4 \cdot 0.2H_2O$: C, 61.43; H, 5.77; N, 10.74. Found: C, 61.33; H, 5.80; N, 10.76.

6.1.5.3. 7-(4-Butyrylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6c). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–2%) provided the desired compound (460 mg, 76%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.20 (m, 2H, CH_2 (12)), 1.39 (m, 2H, CH_2 (13)), 1.71 (tq, 2H, ^3J = 7.2 Hz, ^3J = 7.8 Hz, CH_2 α), 2.37 (t, 2H, ^3J = 7.8 Hz, CH_2 β), 3.33 (m, 4H, CH_2 (16)), 3.55 (m, 1H, C*H* (11)), 3.80 (m, 4H, CH_2 (15)), 7.37 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.99 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.73 (s, 1H, C*H* (2)), 14.88 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 177.0 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 171.7 (*C*17), 166.8 (*C*14), 153.6 (d, $^1J_{C-F}$ = 251.2 Hz, *C*6), 147.5 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 139.0 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 112.6 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 108.2 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 50.3 (*C*15), 49.5 (*C*15), 45.4 (*C*16), 41.1 (*C*16), 35.3 (*C*11), 35.1 (CH_2 β), 18.7 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 401.3 [M], 402.3 [MH^+]. Anal. Calcd for $C_{21}H_{24}FN_3O_4 \cdot 0.5H_2O$: C, 61.45; H, 6.13; N, 10.23. Found: C, 61.38; H, 6.04; N, 10.28.

6.1.5.4. 7-(4-Pentanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6d). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (500 mg, 64%) as a pale yellow solid. Mp 258 °C. ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H, ^3J = 7.2 Hz, CH_3), 1.21 (m, 2H, CH_2 (12)), 1.38 (m, 4H, CH_2 (13,α)), 1.65 (tt, 2H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, CH_2 β), 2.39 (t, 2H, ^3J = 7.5 Hz, CH_2 γ), 3.33 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.2 Hz, C*H* (11)), 3.80 (m, 4H, CH_2 (15)), 7.36 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.98 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.72 (s, 1H, C*H* (2)), 14.95 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 171.8 (*C*17), 166.6 (*C*14), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 119.9 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.9 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 50.1 (*C*15), 49.4 (*C*15), 45.4 (*C*16), 41.1 (*C*16), 35.3 (*C*11), 32.9 (CH_2 γ), 27.3 (CH_2 β), 22.5 (CH_2 α), 13.8 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 415.4 [M], 416.4 [MH^+]. Anal. Calcd for $C_{22}H_{26}FN_3O_4$: C, 63.60; H, 6.31; N, 10.11. Found: C, 63.60; H, 6.82; N, 9.53.

6.1.5.5. 7-[4-(Pivaloyl)piperazin-1-yl]-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6e). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (560 mg, 90%) as a pale yellow solid. Mp >260 °C. ^{1}H NMR ($CDCl_3$) δ 1.21 (m, 2H, CH_2 (12)), 1.33 (s, 9H, CH_3), 1.40 (m, 2H, CH_2 (13)), 3.32 (m, 4H, CH_2 (16)), 3.54 (tt, 1H, ^3J = 7.2 Hz, ^3J = 7.5 Hz, C*H* (11)), 3.90 (m, 4H, CH_2 (15)), 7.37 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 8.01 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.74 (s, 1H, C*H* (2)), 14.90 (s, 1H, COO*H*). ^{13}C NMR ($CDCl_3$) δ 177.0 (d, $^4J_{C-F}$ = 2.5 Hz, *C*4), 176.7 (*C*17), 166.8 (*C*14), 153.6 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.5 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.2 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 112.5 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 108.2 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 49.9 (*C*15), 49.8 (*C*15), 44.8 (*C*16), 38.7 (*C*(CH_3)$_3$), 35.3 (*C*11), 28.4 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 415.4 [M], 416.4 [MH^+]. Anal. Calcd for $C_{22}H_{26}FN_3O_4 \cdot 0.2H_2O$: C, 63.05; H, 6.35; N, 10.03. Found: C, 62.95; H, 6.40; N, 10.03.

6.1.5.6. 7-(4-Heptanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6f). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (320 mg, 47%) as a pale yellow solid. Mp 175 °C. ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.32 (m, 10H, CH_2 (12,13,α−γ)), 1.65 (tt, 2H, ^3J = 6.9 Hz, ^3J = 7.5 Hz, CH_2 δ), 2.38 (t, 2H, ^3J = 7.5 Hz, CH_2 ε), 3.33 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.2 Hz, C*H* (11)), 3.80 (m, 4H, CH_2 (15)), 7.37 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.96 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.70 (s, 1H, C*H* (2)), 14.97 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.8 (d, $^4J_{C-F}$ = 2.3 Hz, *C*4), 172.0 (*C*17), 166.8 (*C*14), 153.4 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 8.0 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.8 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 50.1 (*C*15), 49.3 (*C*15), 45.3 (*C*16), 41.1 (*C*16), 35.3 (*C*11), 33.2 (CH_2 ε), 31.5 (CH_2 δ), 29.0 (CH_2 γ), 25.2 (CH_2 β), 22.4 (CH_2 α), 14.0 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 443.4 [M], 444.4 [MH^+]. Anal. Calcd for $C_{24}H_{30}FN_3O_4$: C, 64.99; H, 6.82; N, 9.47. Found: C, 65.17; H, 7.34; N, 8.98.

6.1.5.7. 7-(4-Octanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6g). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (420 mg, 61%) as a pale yellow solid. Mp 148 °C. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.26 (m, 14H, CH_2 (12,13,α−ε)), 1.59 (m, 2H, ^3J = 7.5 Hz, CH_2 ζ), 2.32 (t, 2H, ^3J = 7.5 Hz, CH_2 η), 3.27 (m, 4H, CH_2 (16)), 3.49 (m, 1H, C*H* (11)), 3.67 (m, 2H, CH_2 (15)), 3.80 (m, 2H, CH_2 (15)), 7.30 (d, 1H, $^4J_{H-F}$ = 6.0 Hz, C*H* (8)), 7.90 (d, 1H, $^3J_{H-F}$ = 15.0 Hz, C*H* (5)), 8.64 (s, 1H, C*H* (2)), 14.90 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.2 Hz, *C*4), 171.9 (*C*17), 166.7 (*C*14), 153.5 (d, $^1J_{C-F}$ = 249.7 Hz, *C*6), 147.3 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 119.7 (d, $^3J_{C-F}$ = 8.2 Hz, *C*10), 112.1 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.8 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 50.1 (*C*15), 49.4 (*C*15), 45.3 (*C*16), 41.1 (*C*16), 35.4 (*C*11), 33.2 (CH_2 η), 31.6 (CH_2 ζ), 29.4 (CH_2 ε), 29.1 (CH_2 γ,δ), 25.2 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 471.3 [M], 472.3 [MH^+]. Anal. Calcd for $C_{26}H_{34}FN_3O_4$: C, 66.22; H, 7.27; N, 8.91. Found: C, 66.19; H, 7.44; N, 8.98.

6.1.5.8. 7-(4-Decanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6h). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–2%) provided the desired compound (330 mg, 45%) as a pale yellow solid. Mp 146 °C. ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3H, ^3J = 6.9 Hz, CH_3), 1.30 (m, 16H, CH_2 (12,13,α−ζ)), 1.62 (tq, 2H, ^3J = 7.8 Hz, ^3J = 6.9 Hz, CH_2 η), 2.37 (t, 2H, ^3J = 7.8 Hz, CH_2 θ), 3.28 (m, 2H, CH_2 (16)), 3.35 (m, 2H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 7.2 Hz, ^3J = 6.9 Hz, C*H* (11)), 3.71 (m, 2H, CH_2 (15)), 3.85 (m, 2H, CH_2 (15)), 7.33 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, C*H* (8)), 7.86 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, C*H* (5)), 8.64 (s, 1H, C*H* (2)), 14.95 (s, 1H, –COO*H*). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 171.9 (*C*17), 166.7 (*C*14), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 119.9 (d, $^3J_{C-F}$ = 7.9 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.9 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 50.2 (*C*15), 49.4 (*C*15), 45.3 (*C*16), 41.1 (*C*16), 35.3 (*C*11), 33.2 (CH_2 θ), 31.8 (CH_2 η), 29.4 (CH_2 δ,ε,ζ), 29.2 (CH_2 γ), 25.3 (CH_2 β), 22.6 (CH_2 α), 14.1 (CH_3), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 485.6 [M], 486.6 [MH^+]. Anal. Calcd for $C_{27}H_{36}FN_3O_4$: C, 66.78; H, 7.47; N, 8.65. Found: C, 67.39; H, 8.01; N, 8.31.

6.1.5.9. 7-(4-Dodecanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6i). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–1.5%) provided the desired compound (508 mg, 66%) as a pale yellow solid. Mp 149 °C. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, 3H, ^3J = 6.3 Hz, CH_3), 1.20 (m, 20H, CH_2 (12,13,α−θ)), 1.60 (m, 2H, ^3J = 7.5 Hz, CH_2 ι), 2.32 (t, 2H, ^3J = 7.5 Hz, CH_2 κ), 3.28 (m, 4H, CH_2 (16)), 3.49 (m, 1H, CH (11)), 3.67 (m, 2H, CH_2 (15)), 3.80 (m, 2H, CH_2 (15)), 7.30 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 7.90 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.65 (s, 1H, CH (2)), 14.80 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.2 Hz, *C*4), 171.9 (*C*17), 166.7 (*C*14), 153.5 (d, $^1J_{C-F}$ = 249.7 Hz, *C*6), 147.4 (*C*2), 145.3 (d, $^2J_{C-F}$ = 10.5 Hz, *C*7), 138.9 (*C*9), 119.8 (d, $^3J_{C-F}$ = 7.5 Hz, *C*10), 112.2 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 107.8 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.7 Hz, *C*(8)), 50.2 (*C*15), 49.4 (*C*15), 45.3 (*C*16), 41.1 (*C*16), 35.4 (*C*11), 33.2 (*C*H$_2$ κ), 31.8 (*C*H$_2$ ι), 29.7 (*C*H$_2$ θ), 29.6 (*C*H$_2$ η), 29.5 (*C*H$_2$ ζ), 29.4 (*C*H$_2$ ε), 29.3 (*C*H$_2$ δ), 29.2 (*C*H$_2$ γ), 25.2 (*C*H$_2$ β), 22.6 (*C*H$_2$ α), 14.1 (*C*H$_3$) 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 513.4 [M], 514.4 [MH$^+$]. Anal. Calcd for $C_{29}H_{40}FN_3O_4{\cdot}0.5H_2O$: C, 66.64; H, 7.90; N, 8.04. Found: C, 66.67; H, 7.84; N, 8.08.

6.1.5.10. 7-(4-Tetradecanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6j). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–2%) provided the desired compound (490 mg, 65%) as a pale yellow solid. Mp 148 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.25 (m, 24H, CH_2 (12,13,α−κ)), 1.67 (tt, 2H, ^3J = 7.5 Hz, ^3J = 6.9 Hz, CH_2 λ), 2.37 (t, 2H, ^3J = 7.5 Hz, CH_2 μ), 3.32 (m, 4H, CH_2 (16)), 3.55 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.2 Hz, CH (11)), 3.72 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 7.34 (d, 1H, $^4J_{H-F}$ = 6.9 Hz, CH (8)), 7.95 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.70 (s, 1H, CH (2)), 14.88 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 171.9 (*C*17), 166.7 (*C*14), 153.5 (d, $^1J_{C-F}$ = 251.2 Hz, *C*6), 147.4 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.1 (d, $^3J_{C-F}$ = 7.9 Hz, *C*10), 112.4 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 108.1 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.1 Hz, *C*8), 50.2 (*C*15), 49.4 (*C*15), 45.3 (*C*16), 41.0 (*C*16), 35.3 (*C*11), 33.2 (*C*H$_2$ μ), 31.9 (*C*H$_2$ λ), 29.6 (*C*H$_2$ κ,ι,θ,η), 29.5 (*C*H$_2$ ζ,ε), 29.4 (*C*H$_2$ δ), 29.3 (*C*H$_2$ γ), 25.3 (*C*H$_2$ β), 22.6 (*C*H$_2$ α), 14.1 (*C*H$_3$), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 541.4 [M], 542.4 [MH$^+$]. Anal. Calcd for $C_{31}H_{44}FN_3O_4$: C, 68.73; H, 8.19; N, 7.76. Found: C, 68.69; H, 8.21; N, 7.73.

6.1.5.11. 7-(4-Hexadecanoylpiperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6k). A purification by silica gel chromatography (CH_2Cl_2–MeOH 1–2%) provided the desired compound (298 mg, 58%) as a pale yellow solid. Mp 147 °C. ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, ^3J = 6.6 Hz, CH_3), 1.32 (m, 28H, CH_2 (12,13,α−μ)), 1.65 (tt, 2H, ^3J = 7.5 Hz, ^3J = 7.2 Hz, CH_2 ν), 2.37 (t, 2H, ^3J = 7.5 Hz, CH_2 ξ), 3.32 (m, 4H, CH_2 (16)), 3.55 (m, 1H, CH (11)), 3.71 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 7.34 (d, 1H, $^4J_{H-F}$ = 7.2 Hz, CH (8)), 7.94 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.68 (s, 1H, CH (2)), 14.86 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.9 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 171.9 (*C*17), 166.7 (*C*14), 153.5 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.4 (*C*2), 145.4 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.9 (*C*9), 120.0 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.3 (d, $^2J_{C-F}$ = 23.2 Hz, *C*5), 108.0 (*C*3), 105.0 (d, $^3J_{C-F}$ = 3.1 Hz, *C*8), 50.2 (*C*15), 49.3 (*C*15), 45.3 (*C*16), 41.0 (*C*16), 35.3 (*C*11), 33.2 (*C*H$_2$ ξ), 31.9 (*C*H$_2$ ν), 29.6 (*C*H$_2$ μ,λ,κ,ι,θ,η), 29.5 (*C*H$_2$ ζ,ε), 29.4 (*C*H$_2$ δ), 29.3 (*C*H$_2$ γ), 25.3 (*C*H$_2$ β), 22.6 (*C*H$_2$ α), 14.0 (*C*H$_3$), 8.2 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 569.4 [M], 570.4 [MH$^+$]. Anal. Calcd for $C_{33}H_{48}FN_3O_4$: C, 69.57; H, 8.49; N, 7.38. Found: C, 69.61; H, 8.41; N, 7.43.

6.1.5.12. 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-(2-phenylacetyl)piperazin-1-yl] quinoline-3-carboxylic acid (6l). A purification by silica gel chromatography (CH_2Cl_2–MeOH 2%) provided the desired compound (340 mg, 50%) as a pale yellow solid. Mp 252 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (m, 2H, CH_2 (12)), 1.38 (m, 2H, CH_2 (13)), 3.12 (m, 2H, CH_2 (16)), 3.27 (m, 2H, CH_2 (16)), 3.69 (s, 1H, CH_2 (11)), 3.69 (m, 2H, CH_2 (15)), 3.81 (s, 2H, CH_2Ph), 3.90 (s, 2H, CH_2 (15)), 7.29 (m, 6H, CH (8, ar)), 7.99 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.75 (s, 1H, CH (2)), 14.88 (s, 1H, –COOH). No ^{13}C NMR was available for this product due to its lack of solubility. CI/NH_3-MS (positive mode) *m*/*z* 449.4 [M], 450.4 [MH$^+$]. Anal. Calcd for $C_{25}H_{24}FN_3O_4{\cdot}0.2H_2O$: C, 66.27; H, 5.42; N, 9.27. Found: C, 66.39; H, 5.77; N, 8.61.

6.1.6. 7-(4-(2-Benzyloxyacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (7)

CP **1** (1.2 g, 3.6 mmol) and triethylamine (0.51 mL, 5.42 mmol) were stirred in 20 mL of dry methylene chloride at 0 °C for 15 min. Benzyloxyacetyl chloride (1 g, 5.4 mmol) was added dropwise. After stirring at 0 °C for 15 min and then at room temperature for 2 h, the mixture was filtered and the resulting solids were washed with water and methylene chloride. The aqueous layer was extracted with 3 × 20 mL of CH_2Cl_2. After extraction, the organic layers were dried over $MgSO_4$ and concentrated. The remaining residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 2%) to yield the desired product (0.73 g, 43%) as a pale yellow solid. Mp 210 °C. ^{1}H NMR ($CDCl_3$) δ 1.19 (m, 2H, CH_2 (12)), 1.37 (m, 2H, CH_2 (13)), 3.30 (m, 4H, CH_2 (16)), 3.52 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.2 Hz, CH (11)), 3.76 (m, 2H, CH_2 (15)), 3.86 (m, 2H, CH_2 (15)), 4.25 (s, 2H, Ph-CH_2–O–), 4.62 (s, 2H, –O–CH_2–CO–), 7.35 (m, 6H, CH (8,ar)), 8.00 (d, 1H, $^3J_{H-F}$ = 12.9 Hz, CH (5)), 8.73 (s, 1H, CH (2)), 14.90 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$) δ 176.7 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 167.7 (*C*17), 166.6 (*C*14), 153.4 (d, $^1J_{C-F}$ = 251.3 Hz, *C*6), 147.3 (*C*2), 145.2 (d, $^2J_{C-F}$ = 10.4 Hz, *C*7), 138.8 (*C*9), 136.9 (*C*q ar), 128.4 (*C* ar), 128.0 (*C* ar), 127.9 (*C* ar), 119.8 (d, $^3J_{C-F}$ = 7.8 Hz, *C*10), 112.1 (d, $^2J_{C-F}$ = 23.4 Hz, *C*5), 107.7 (*C*3), 105.1 (d, $^3J_{C-F}$ = 3.2 Hz, *C*8), 73.2 (–O–*C*H$_2$–CO), 69.5 (–*C*H$_2$-Ph), 50.0 (*C*15), 49.3 (*C*15), 44.8 (*C*16), 41.3 (*C*16), 35.3 (*C*11), 8.1 (*C*12,13). CI/NH_3-MS (positive mode) *m*/*z* 479.3 [M], 480.3 [MH$^+$]. Anal. Calcd for $C_{26}H_{26}FN_3O_5{\cdot}0.2H_2O$: C, 64.65; H, 5.51; N, 8.70. Found: C, 64.67; H, 5.50; N, 8.64.

6.1.7. 7-(4-(2-Hydroxyacetyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (8)

Compound **7** (0.52 g, 1.09 mmol) was dissolved in hot dimethylformamide. After cooling at room temperature, palladium on charcoal (10%, 116 mg, 0.1 mmol) was added. After two evacuations and fillings with hydrogen, the mixture was hydrogenated for 96 h at room temperature under atmospheric pressure. The catalyst was filtered through a pad of Celite. Celite was washed with methylene chloride and solvents were evaporated in vacuo. The remaining residue was purified by silica gel chromatography (CH_2Cl_2–MeOH 1%) to yield the desired product (175 mg, 60%) as a white solid. Mp >260 °C. ^{1}H NMR (DMSO-d_6) δ 1.19 (m, 2H, CH_2 (12)), 1.32 (m, 2H, CH_2 (13)), 3.30 (m, 4H, CH_2 (16)), 3.70 (m, 4H, CH_2 (15)), 3.82 (tt, 1H, ^3J = 6.9 Hz, ^3J = 7.5 Hz, CH (11)), 4.16 (d, 2H, ^3J = 6.0 Hz, –CH_2OH), 4.70 (t, 1H, ^3J = 6.0 Hz, –OH), 7.57 (d, 1H, $^4J_{H-F}$ = 7.5 Hz, CH (8)), 7.92 (d, 1H, $^3J_{H-F}$ = 13.2 Hz, CH (5)), 8.60 (s, 1H, CH (2)), 15.20 (s, 1H, –COOH). ^{13}C NMR (DMSO-d_6) δ 176.2 (d, $^4J_{C-F}$ = 2.6 Hz, *C*4), 165.7 (*C*17), 164.7 (*C*14), 152.8 (d, $^1J_{C-F}$ = 249.5 Hz, *C*6), 147.9 (*C*2), 144.6 (d, $^2J_{C-F}$ = 10.2 Hz, *C*7), 138.9 (*C*9), 118.7 (d, $^3J_{C-F}$ = 7.7 Hz, *C*10), 110.8 (d, $^2J_{C-F}$ = 23.1 Hz, *C*5), 106.7 (*C*3), 106.5 (d, $^3J_{C-F}$ = 3.0 Hz, *C*8), 60.0 (–*C*H$_2$OH), 49.3 (*C*15), 48.9 (*C*15), 44.8 (*C*16), 41.6 (*C*16), 35.7 (*C*11), 7.4 (*C*12,13). CI/NH_3-MS (negative mode) *m*/*z* 389.2 [M]. Anal. Calcd for $C_{19}H_{20}FN_3O_5{\cdot}1H_2O$: C, 56.02; H, 5.44; N, 10.31. Found: C, 56.12; H, 5.62; N, 10.34.

6.2. Stability tests

HPLC analysis was performed on a Waters system (Waters Associates Inc., Milford, MA, USA) consisting of a 600 controller pump, a PDA996 diode array detector, a 717 plus autosampler,

and a Lisa 30 Ecrosas (ICS) oven at 30 °C. The instrument was controlled by the Empower software. The experiments were carried out on a C18 Luna (Phenomenex®) reverse-phase column (length 100 mm × 3 mm i.d., 3 μm particle size) eluted with a mobile phase consisting of various linear gradients of acetonitrile (Solvent A)/water (+0.1% TFA) (Solvent B) at a flow rate of 0.6 mL/min. The eluting solvents were prepared daily and degassed with helium during analyses. The detection was set at 280 nm. All injections (10 μL) were made in duplicate. In a typical experiment, 100 μL of a 1 mM solution of tested compound in DMSO was diluted with phosphate buffer (pH 7.4) to a final volume of 1 mL. An aliquot was discarded and kept at 4 °C (t_0). The remaining solution placed in a sealed tube was heated in a water bath at 37 °C for 6 days (t_{6d}). To evaluate the rate of degradation of the tested compounds, CP **1** (degradation product expected for **3**, **5a–c**, **6f**, **6h**, and **6k**) and compound **8** (degradation product expected for **2**, **4b–d**, and **4h**) were injected. Their respective retention times were 2.6 and 1.6 min. For compounds whose stability was less than 100%, the ratio [compound peak area/(compound peak + degradation product peak) area] was calculated.

6.3. Biology

6.3.1. Evaluation of in vitro cell proliferation by means of the MTT colorimetric assay

The assessment of cell population growth is based on the capability of living cells to reduce the yellow product MTT (3-[4,5-dimethylthiazol-2yl]-diphenyl tetrazolium bromide; Sigma, Bornem, Belgium) to a blue product, formazan, by a reduction reaction occurring in the mitochondria.[15,16] The five cell lines were incubated for 24 h in 96-microwell plates (at a concentration of 10^4 to 3×10^4 cells/mL culture medium depending on the cell type) to ensure adequate plating prior to cell growth determination. The number of living cells after 120 h of culture in the presence or absence (control) of the various drugs is directly proportional to the intensity of the blue color, measured by spectrophotometry using a 680XR microplate reader (Bio-Rad Laboratories Inc, Hercules, CA) at a wavelength of 570 nm (with a reference at 630 nm). Each experiment was carried out in hexaplicate. Nine concentrations ranging from 10^{-3} to 10^{-7} M (with semi-log decrease in concentration) were tested for each of the compounds under study.

Supplementary data

HPLC analytical conditions for stability tests and retention times for compounds **1**, **2**, **3**, **4b–d**, **4h**, **5a–c**, **6f**, **6h**, **6k**, and **8**. In vivo testing maximum tolerated dose procedure. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.053.

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