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Synthesis and antibacterial evaluation of novel 8-fluoro Norfloxacin derivatives as potential probes for methicillin and vancomycin-resistant *Staphylococcus aureus*

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ABSTRACT

A series of novel 8-fluoro Norfloxacin derivatives and the hybrids of its piperazinyl derivatives incorporated with 1,3,5-triazine and pyrimidine were synthesized. All the above compounds were evaluated for their antibacterial activity against *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* and methicillin & vancomycin-resistant *S. aureus*. Among all, compounds having Morpholine, *N*-methyl/phenyl/benzyl/pyrimidinyl piperazines and *n*-butylamine substitution at C-7 position, have shown increased potency in comparison to norfloxacin and ciprofloxacin.

1. Introduction

Introduction of nalidixic acid during 1962 has shown the new avenue for the patients with the bacterial infections and also the dramatic impact in altering morbidity and mortality rate compared with the chemotherapy during 1940s. The continuous effort to increase the efficacy against bacteria has led to identify new prototype quinolone which is active only against Gram-negative bacteria, similar to that of nalidixic acid. But during 1980s, the introduction of norfloxacin the first fluoroquinolone [1], and later on, the discovery of other fluoroquinolones like ciprofloxacin [2], sparfloxacin [3] and trovafloxacin [4] have changed the landscape of antibacterial chemotherapy, which were active against both Gram-negative and Gram-positive bacterial pathogens (Fig. 1).

However, for the past few years the emerging resistant bacteria has diminished the efficiency of antibiotics due to their excessive use and maltreatment [5–7]. This rapid emergence of drug resistance pathogens like, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococci*, methicillin-resistant *Staphylococcus aureus* and multi-resistant *Salmonellae* has now become a serious public health problem [8–10]. Among these, methicillin-resistant *S. aureus* (MRSA) has emerged as a predominant nosocomial Gram-positive pathogen [11]. In 2005, "The Surveillance Network–USA" (TSN), reported that the methicillin-resistant *S. aureus* (MRSA) rates of 55–59% for strains from inpatients and 48% from outpatients [12]. Moreover, the patients infected with MRSA tend to have more serious underlying diseases and other adverse prognostic factors compared with patients infected with methicillin-susceptible *S. aureus* (MSSA) [13–15]. Until recently, the treatment of infections caused by methicillin-resistant pathogens was effectively done with the glycopeptides-notably vancomycin. However, the overuse of this antibiotic in oral form for conditions such as pseudomembranous colitis has led to the emergence of vancomycin-intermediate and vancomycin-resistant MRSA (VISA and VRSA, respectively) [16–19]. On other hand, *Klebsiella pneumoniae* is the most common Gram-negative bacterium and is responsible for a significant proportion of hospital-acquired infections including septicemias, urinary tract infections, pneumonia and soft tissue infections [20,21]. The ability of this organism to spread rapidly through the hands of healthcare workers and by the gastrointestinal tract of hospitalized infants as they act as reservoirs for its transmission, has also increased the incidence of multiple hospital outbreaks in the last few years [22,23]. Not surprisingly, the spread of MRSA, coupled with the emergence of VISA and VRSA,

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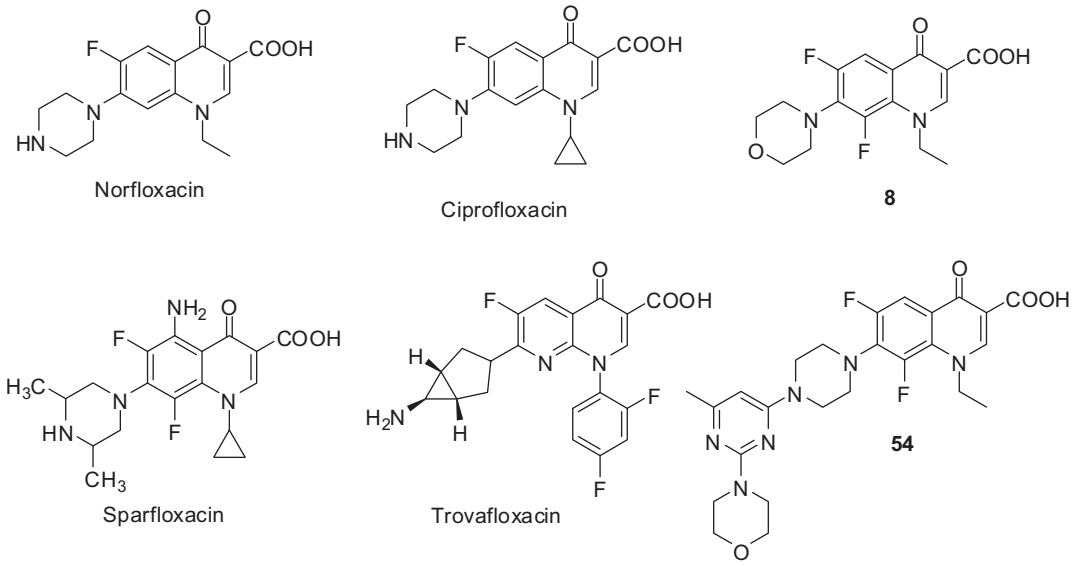


Fig. 1. Structure of fluoroquinolone drugs and most active compounds of the series.

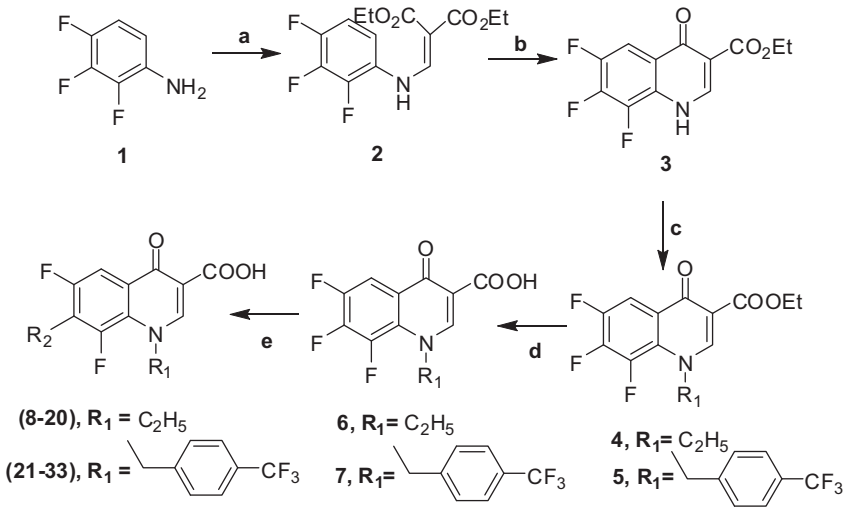
has now become a major cause of concern among clinicians and microbiologists and thus new classes of antimicrobial agents effective against MRSA, VRSA and *K. pneumoniae* infections are urgently required.

The main target of these antibacterial fluoroquinolones is DNA gyrase, an essential bacterial enzyme responsible for the maintenance of DNA topology within bacterial cell. Gyrase catalyzes the ATP-dependent introduction of negative supercoils into the DNA and by binding to the gyrase–DNA complex; the quinolones block the transcription and the DNA replication, which results in cell death [24]. The success of quinolones as antibacterial agents with relatively few side effects lies behind the difference between gyrase and its eukaryotic equivalent, topoisomerase (topo) II. That renders gyrase susceptible to the action of quinolones while topo II remains largely unaffected [25]. On the other hand, Dihydrofolate reductase (DHFR), an enzyme responsible for the NADPH-dependent reduction of 5,6-dihydrofolate to 5,6,7,8-tetrahydrofolate, an essential cofactor in the synthesis of purines, thymidylate, and methionine required for DNA synthesis by the bacteria, has gained much attention as a useful target for the discovery of novel antibacterial agents after the clinical success of diaminopyrimidines (DAPs) like trimethoprim (TMP) which binds bacterial DHFRs 10^5 times more than it does to vertebrate DHFRs [26]. It exhibits a broad spectrum of activity against a wide range of Gram-positive bacterial pathogens in vitro like *S. aureus*, both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA), *Streptococcus pyogenes*, *S. pneumoniae*, *Streptococcus viridans*, *Enterococcus faecalis* and also many Gram-negative bacteria including *Escherichia coli*, *K. pneumoniae*, *Proteus* spp., *Salmonella* spp. and *Haemophilus influenzae* have been found to be sensitive to TMP. Based on TMP clinical efficacy and excellent safety profile, another novel diaminopyrimidine named Iclaprim [27], was discovered. Iclaprim exhibit potent activity against Gram-positive pathogens resistant not only to TMP, but also to other clinically used antibiotics including methicillin and oxacillin (e.g. methicillin-resistant *S. aureus* or MRSA), macrolides, quinolones and glycopeptides (including activity against VISA and VRSA) [28] and is currently in phase III clinical development. In case of bacterial infections, the simultaneous inhibition of the two enzymes has been proven to be synergistic and highly effective. The most prominent and first example is the combination of sulfisoxazole, a Dihydropteroate synthetase (SYN, an enzyme which produces dihydropteroate, an intermediate useful in folate synthesis) inhibitor with TMP [29] (Bactrim, Eusaprim), a DHFR inhibitor, used mainly against infections with Gram-negative bacteria. More recently, combination of TMP with sulfamethoxazole (SMX) has been used in the treatment of community acquired MRSA infections [30–33].

Therefore, the aim of our present study was: (i) To evaluate the antibacterial activity of certain novel norfloxacin derivatives with an additional functional moiety like fluorine atom at C-8 position and ethyl-4-(trifluoromethyl)benzene at N-1 position (to provide extra hydrogen bonding capacities with the DNA gyrase) against methicillin-resistant *S. aureus* (MRSA), methicillin & vancomycin-resistant *S. aureus* (VRSA) and Gram-negative bacteria *K. pneumoniae*. (ii) To find new lead structures, derived from the combination of above norfloxacin derivatives with DHFR inhibitors like 1,3,5-triazines and pyrimidines at the N-4 position of the C-7 piperazin-1-yl group, that may work out in increasing the efficiency against resistant bacteria.

2. Chemistry

The synthesis of targeted fluoroquinolones, 1-ethyl-6,8-difluoro-4-oxo-7-(substituted)-1,4-dihydroquinoline-3-carboxylic acids (**8**–**20**) and 6,8-difluoro-4-oxo-7-(substituted)-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acids (**21**–**33**) is outlined in Scheme 1. The intermediate ethyl 6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **3** was achieved by the cyclization of diethyl 2-((2,3,4-trifluorophenylamino)methylene)malonate **2** in diphenyl ether at 250 °C, which was obtained from 2,3,4-trifluoro aniline **1** according to the previously reported procedure [34]. Reaction of **3** with iodoethane and K_2CO_3 in dry DMF at 90 °C produced ethyl 1-ethyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **4** and with 1-(bromomethyl)-4-(trifluoromethyl)benzene and NaH in DMF at 90 °C gave ethyl 6,7,8-trifluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylate **5** in good yield. Compounds **4** and **5** were further hydrolyzed with 2N HCl in acetic acid to obtain their acids **6** and **7** respectively in 75–80% yield. Finally, the nucleophilic substitution on **6** and **7** at 7th position with various cyclic/acyclic amines in the presence of K_2CO_3 in acetonitrile at 90 °C gave their respective derivatives **8**–**20** and **21**–**33** in 70–84% overall yield.

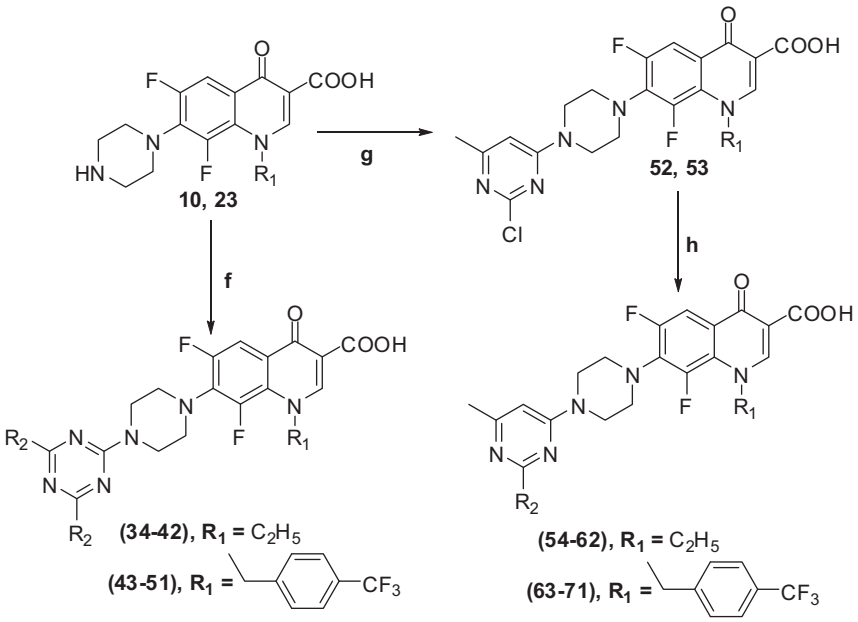


Scheme 1. Reagents and conditions: (a) Diethyl ethoxymethylenemalonate (DEMM), 120 °C, reflux; (b) Diphenyl ether, 250 °C, reflux; (c) (1) Iodoethane, K_2CO_3, DMF, 90 °C reflux; (2) 4-(trifluoromethylbenzyl)bromide, NaH, DMF, 90 °C reflux; (d) 2N HCl, AcOH, 110 °C, reflux; (e) Different amines, CH_3CN, 90 °C, reflux.

The designed hybrids of fluoroquinolone-1,3,5-triazine/pyrimidine were synthesized from the precursors **10** and **23** as shown in Scheme 2. 1,3,5-triazine derivatives (**34**–**51**) were synthesized in one step by monosubstituting **10**, **23** initially at 0 °C on cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) at 2-position, followed by different amines at 4,6-positions *in situ* in the presence of K_2CO_3 in dry THF at 90 °C, yielded the targeted fluoroquinole-1,3,5-triazine hybrids **34**–**42** and **43**–**51** respectively. While pyrimidine derivatives (**54**–**71**) were achieved in two steps. Initially the intermediates **52**, **53** were obtained by reacting **10** and **23** respectively with 2,4-dichloro-6-methylpyrimidine and diisopropylethylamine (DIPEA) in DMF at room temperature [35] and these intermediates were further subjected to nucleophilic substitution with different amines in the presence of K_2CO_3 in EtOAc at 90 °C, to obtain respective fluoroquinolone-pyrimidine hybrids **54**–**62** and **63**–**71**. All the synthesized compounds were well characterized by IR, Mass, NMR and their purity was established with elemental analysis.

3. Biological assay method

The targeted fluoroquinolones (**8**–**33**) and hybrids of fluoroquinolone-1,3,5-triazine/pyrimidine (**34**–**71**) were screened against *K. pneumoniae*, methicillin-resistant *S. aureus* and methicillin-resistant and vancomycin-resistant *S. aureus*, where Norfloxacin and Ciprofloxacin were used as a standard drug. The bacterial strains were grown on nutrient agar at 37 °C. After 24 h of incubation, bacterial cells were suspended in normal saline containing Tween 20 at 0.05% at a concentration of approximately 1.0 F02Dnbsp;2.0 F0B4 10^7 cells/mL by matching with 0.5 Mc Farland standards. The activity of compounds was determined as per NCCLS protocol using Mueller Hinton broth (Becton Dickinson, USA) in 96-well tissue culture plates. Proper growth control, drug control and the negative control were adjusted onto the plate. Compounds were dissolved in DMSO at a concentration of 1 mg/mL and 20 μL of this was added to each well of 96-well tissue culture plate having 180 μL Mueller Hinton broth. From here the solution



Scheme 2. Reagents and conditions: (f) Cyanuric chloride, THF, rt, 1 h, *in situ* Different amines, reflux; (g) 2,4-dichloro-6-methylpyrimidine, DIPEA, DMF, rt; (h) Different amines, EtOAc, K_2CO_3, reflux.

was serially diluted resulting in twofold dilution of the test compounds in subsequent wells. 100 μL of Mc Farland matched bacterial suspension was diluted in 10 ml of media and then 100 μL of it was added in each well and kept for incubation. The maximum concentration of compounds tested was 50 μg/mL. The micro-titer plates were incubated at 35 °C in a moist, dark chamber and MICs were recorded spectrophotometrically after 24 h using SOFT max Pro 4.3 Software (Molecular Devices, Sunnyvale, USA) [36,37].

4. Results and discussion

The in vitro antibacterial activity of targeted fluoroquinolones (**8**–**33**) and hybrids of fluoroquinolone-1,3,5-triazine/pyrimidine (**34**–**71**) against *K. pneumoniae*, methicillin-resistant (M-R) *S. aureus* and methicillin-resistant and vancomycin-resistant (M-R, V-R) *S. aureus*, are summarized in Table 1 and Table 2 along with the Norfloxacin and Ciprofloxacin used as a standard drugs. In general, compounds having *N*-ethyl substitution (**8**–**20**) were more active in comparison to the compounds having 4-(trifluoromethyl)benzyl group as *N*-substituent (**21**–**33**) of quinolone ring, with the exception of compound **26** having MIC of 0.39, 0.78 and 1.56 μg/ml against *K. pneumoniae*, (M-R) *S. aureus* and (M-R,V-R) *S. aureus* respectively, which is equal to that of norfloxacin. Compound with an ethyl substituent at N-1 position and morpholine at C-7 position (**8**) found to exhibited the most potent inhibitory activity against *K. pneumoniae*, (M-R) *S. aureus* and (M-R, V-R) *S. aureus* bacteria with a MIC of 0.022 μg/ml and 0.045 μg/ml respectively and is 18–35 fold more potent than that of norfloxacin and 4–8 fold than the ciprofloxacin. On replacing morpholine with piperidine (**9**), activity decreases but still found 2 fold more active against *K. pneumoniae* bacteria and 4 fold more against both (M-R) and (M-R,V-R) *S. aureus* in comparison to norfloxacin. While substitution with piperazine (**10**) activity against *K. pneumoniae* decreases to 0.045 μg/ml from 0.022 μg/ml. Surprisingly, in case of substituted piperazines, compounds **11** and **14** having *N*-methylpiperazine, *N*-benzylpiperazine as $\mathbf{R_2}$ respectively, found 35 fold more potent against *K. pneumoniae* than norfloxacin and 18 fold more than the ciprofloxacin. And against (M-R) and (M-R, V-R) *S. aureus* they showed 4–8 fold more inhibition than the norfloxacin. Whereas compound **13**, having *N*-phenylpiperazine as $\mathbf{R_2}$ showed 18 and 4 fold more potency against (M-R, V-R) *S. aureus* than of norfloxacin and ciprofloxacin respectively. In case of acyclic amines, compound **17** with $\mathbf{R_2}$ = *n*-butylamine found to be the most promising candidate with 2–18 fold more inhibitory activity than that of norfloxacin against *K. pneumoniae*, M-R *S. aureus* and M-R and V-R *S. aureus*. With the above SAR studies it is evident that the presence of fluorine atom at C-8 position enhanced the potency against resistant bacteria and substituents like morpholine, *N*-methyl/phenyl/benzyl piperazines and *n*-butylamine has remarkably increased the antibacterial activity in comparison to standard drugs.

Similarly, in fluoroquinolone-1,3,5-triazine derivatives (**34**–**51**), compounds having ethyl group as N-1 substituent were more potent than that of the molecules having 4-(trifluoromethyl)benzyl group as N-1 substituent. Compound **41** having *n*-propylamine as $\mathbf{R_2}$ and ethyl group as N-1 substituent is 2 fold more active against *K. pneumoniae* and (M-R,V-R) *S. aureus* than the norfloxacin, while its 4-(trifluoromethyl)benzyl derivative (**50**) showed decreased potency. Similarly, compounds **36**, **39** having *N*-methylpiperazine and *n*-butylamine as $\mathbf{R_2}$ respectively, were much active in comparison to their 4-(trifluoromethyl)benzyl derivatives (**45**, **48**)

Table 1
In vitro Antibacterial activity of new fluoroquinolones [MIC μg/mL].

Comp	$\mathbf{R_2}$	*K. pneumoniae*	*S. aureus* (M-R)	*S. aureus* (M-R,V-R)
8	morpholine	0.022	0.022	0.045
9	piperidine	0.19	0.19	0.39
10	piperazine	0.045	0.78	0.39
11	*N*-methylpiperazine	0.011	0.19	0.19
12	*N*-ethylpiperazine	0.19	0.78	0.39
13	*N*-phenylpiperazine	0.09	0.39	0.09
14	*N*-benzylpiperazine	0.011	0.19	0.19
15	aminoehtylmorpholine	1.56	3.12	ND
16	aminopropylmorpholine	1.56	3.12	ND
17	*n*-butylamine	0.022	0.39	0.39
18	*t*-butylamine	0.09	12.5	ND
19	*n*-propylamine	0.78	12.5	ND
20	*iso*-propylamine	0.09	0.78	0.78
21	morpholine	12.5	12.5	ND
22	piperidine	12.5	>50	ND
23	piperazine	12.5	>50	ND
24	*N*-methylpiperazine	>50	>50	ND
25	*N*-ethylpiperazine	>50	>50	ND
26	*N*-phenylpiperazine	0.39	0.78	1.56
27	*N*-benzylpiperazine	1.56	1.56	ND
28	aminoehtylmorpholine	12.5	>50	ND
29	aminopropylmorpholine	25	>50	ND
30	*n*-butylamine	>50	>50	ND
31	*t*-butylamine	>50	>50	ND
32	*n*-propylamine	3.12	1.56	ND
33	*iso*-propylamine	1.56	3.12	ND
Norfloxacin		0.39	0.78	1.56
Ciprofloxacin		0.19	0.09	0.38

Table 2
In vitro Antibacterial activity of hybrids of fluoroquinolone-1,3,5-triazines/pyrimidines [MIC μg/mL][a].

Comp	$\mathbf{R_2}$	K. pneumoniae	*S. aureus* (M-R)	*S. aureus* (M-R,V-R)
34	morpholine	3.12	3.12	ND
35	piperidine	>50	>50	ND
36	*N*-methylpiperazine	0.39	0.39	0.39
37	aminoehtylmorpholine	0.78	0.78	1.56
38	aminopropylmorpholine	12.5	12.5	ND
39	*n*-butylamine	0.39	0.39	0.39
40	*t*-butylamine	0.39	3.12	ND
41	*n*-propylamine	0.19	0.78	0.78
42	*iso*-propylamine	0.39	0.39	0.78
43	morpholine	1.56	3.12	1.56
44	piperidine	50	50	ND
45	*N*-methylpiperazine	12.5	25	ND
46	aminoehtylmorpholine	6.25	12.5	ND
47	aminopropylmorpholine	50	>50	ND
48	*n*-butylamine	0.39	3.12	0.39
49	*t*-butylamine	1.56	3.12	6.25
50	*n*-propylamine	0.78	1.56	1.56
51	*iso*-propylamine	0.39	0.78	0.78
54	morpholine	0.045	0.39	0.045
55	piperidine	0.045	0.39	0.39
56	*N*-methylpiperazine	>50	>50	ND
57	aminoehtylmorpholine	0.22	0.22	0.19
58	aminopropylmorpholine	0.22	0.22	0.19
59	*n*-butylamine	0.78	0.78	0.09
60	*t*-butylamine	0.78	1.56	0.78
61	*n*-propylamine	0.09	0.39	0.39
62	*iso*-propylamine	0.011	0.22	0.19
63	morpholine	0.78	1.56	1.56
64	piperidine	0.39	0.78	0.39
65	*N*-methylpiperazine	6.25	6.25	12.5
66	aminoehtylmorpholine	1.56	0.78	1.56
67	aminopropylmorpholine	6.25	25	ND
68	*n*-butylamine	6.25	6.25	1.56
69	*t*-butylamine	0.19	1.56	1.56
70	*n*-propylamine	1.56	1.56	3.12
71	*iso*-propylamine	0.19	0.78	0.78
Norfloxacin		0.39	0.78	1.56
Ciprofloxacin		0.19	0.09	0.38

[a] Organisms selected: *K. pneumoniae* (ATCC 27736); *S. aureus* (ATCC 25923): Methicillin-resistant (M-R); *S. aureus* (ATCC 29213): Methicillin-resistant and Vancomycin-resistant (M-R and V-R).

with 2–4 fold more potency against (M-R) and (M-R,V-R) *S. aureus* than that of norfloxacin. Contrarily, compounds **42**, **51** having *iso*-propylamine as $\mathbf{R_2}$ and ethyl, 4-(trifluoromethyl)benzyl groups respectively as N-1 substituent, showed same potency against both *K. pneumoniae* and (M-R,V-R) *S. aureus*.

Surprisingly, fluoroquinolone-pyrimidine derivatives (**54**–**71**) have shown better activity profile in comparison to 1,3,5-triazine derivatives irrespective of N-1 substitution. Moreover, compounds having ethyl group as N-1 substituent shown promising activity against three bacteria irrespective of substitution of $\mathbf{R_2}$ as compared to their 4-(trifluoromethyl)benzyl analogues. Compound **62** having *iso*-propylamine as $\mathbf{R_2}$ showed excellent 35 & 18 fold potency against *K. pneumoniae*, while compounds **54** and **55** having morpholine and piperidine as $\mathbf{R_2}$ showed 8 & 4 fold more inhibition than that of norfloxacin and ciprofloxacin respectively. And also compound **54** was found to exhibit remarkable 35 & 8 fold more potency against (M-R, V-R) *S. aureus* in comparison to standard drugs. Similarly, rest of the compounds **57**–**61** have also shown above moderate potency against three bacteria. The above investigation of SAR clearly shows that the pyrimidine has played a significant role in increasing the potency against resistant bacteria. Thus, the excellent activity profile of these fluoroquinolone-pyrimidines has proved them as new lead molecules in the development of effective antibacterial agents.

5. Conclusions

In conclusion, the concept of developing new effective antibacterials by the introduction of new substituents on quinolone pharmacophore, design and synthesis of new hybrids with a multiple targets is a successful approach to conquer the problem of resistance. In this perception, we have chosen to explore Norfloxacin moiety by introducing fluorine atom at C-8 position, variation of N-1 position and cyclic/acyclic amines at C-7 position. Additionally, DHFR inhibitors like 1,3,5-triazines and pyrimidines were incorporated at the N-4 position of the C-7 piperazin-1-yl group of newly developed norfloxacin entity. The outcome of our approach was successful in identifying the new substitutes for piperazine, like Morpholine, *N*-methyl/phenyl/benzyl/pyrimidinyl piperazines and *n*-butylamine at C-7 position of fluoroquinolones for the development of future antibacterial agents.

6. Experimental

IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Shimadzu spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker Advance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μl per min. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. The spectra were collected in 6s scans and the print outs are averaged spectra of 6–8 scans. In case of multiplets the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet and bs, broad singlet. The electron spray mass spectra were recorded on triple quadrupole mass spectrometer. EI mass spectra were recorded on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass on Quantro-II, micro mass. Purity of all tested compounds was ascertained on the basis of their elemental analysis and was carried out on Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

6.1. General procedure for the synthesis of compound **2**

A mixture of 2,3,4-trifluoro aniline **1** (1 equiv.) and diethyl ethoxymethylenemalonate (1.008 equiv.) was heated at 110–120 °C for 2 h, the resulting EtOH was eliminated under pressure. Then, the mixture was cooled and the residue was recrystallized from *n*-hexane to yield **2** as colorless needles.

6.1.1. Diethyl 2-((2,3,4-trifluorophenylamino)methylene)malonate (**2**)

Yield: 74%; mp 94–95 °C; ESMS: 318 (M + 1); IR (KBr): 3255, 3170, 2986, 2940, 2875, 3082, 1651, 1609, 1505, 1689, 1251, 1228 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 11.01 (bs, 1H), 8.36 (d, 1H, J = 6.94 Hz), 7.07 (m, 2H), 4.31 (m, 2H), 4.25 (m, 2H), 1.39–1.31 (m, 6H). ^{13}C (50 MHz, $CDCl_3$): 168.63, 165.48, 151.14, 96.21, 126.03, 125.97, 112.63, 112.26, 110.49, 110.25, 60.85, 60.51, 14.48, 14.36. Anal. Calc for $C_{14}H_{14}F_3NO_4$: C: 53.0, H: 4.45, N: 4.41. Found: C: 53.15, H: 4.43, N: 4.32.

6.2. General procedure for the synthesis of compound **3**

Compound **2** (5.30 g, 16.70 mmol) was added to diphenyl ether (35 mL) and refluxed at 250 °C for 8 h. After the solution was cooled, the resulting precipitate was filtered off, washed with hexane and recrystallized from EtOH to give **3** as a white solid.

6.2.1. Ethyl 6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**3**)

Yield: 77%; mp >250 °C; ESMS: 272 (M + 1); IR (KBr): 3002, 2986, 3082, 1572, 1479, 1717, 1298, 1251, 1628 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 9.10 (s, 1H), 7.99 (m, 1H), 4.43 (m, 2H), 1.30 (t, 3H, J = 7.18 Hz). ^{13}C (50 MHz, $CDCl_3$): 171.67, 166.54, 153.06, 108.28, 152.31, 141.17, 130.35, 125.25, 119.57, 113.82, 60.34, 17.19. Anal. Calc for $C_{12}H_8F_3NO_3$: C: 53.15, H: 2.97, N: 5.16. Found: C: 53.23, H: 2.88, N: 5.12.

6.3. General procedure for the synthesis of compound **4**

A mixture of **3** (1 equiv.), K_2CO_3 (2 equiv.) and EtI (5 equiv.) in DMF was heated at 95 °C with stirring for 10 h. The precipitate of K_2CO_3 and $KHCO_3$ was filtered off and the filtrate was evaporated to dryness. The solid residue was dissolved in water and extracted with DCM, which was dried over anhyd Na_2SO_4. The solution was concentrated to solid residue which was purified by flash chromatography using ($CHCl_3$/MeOH = 5:1) to obtain compound **4**.

6.3.1. Ethyl 1-ethyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate (**4**)

Yield: 76%; mp 201–202 °C; ESMS: 300 (M + 1); IR (KBr): 2986, 3082, 1680, 1323, 1611, 1490, 1106, 1100 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.40 (s, 1H), 8.20 (m, 1H), 4.43 (m, 4H), 1.60 (t, 3H, J = 6.78 Hz), 1.28 (t, 3H, J = 7.18 Hz). ^{13}C (50 MHz, $CDCl_3$): 171.45, 165.17, 151.24, 109.76, 110.28, 61.35, 53.49, 16.18, 14.33. Anal. Calc for $C_{14}H_{12}F_3NO_3$: C: 56.19, H: 4.04, N: 4.68. Found: C: 56.04, H: 4.01, N: 4.57.

6.4. General procedure for the synthesis of compound **5**

To a suspension of sodium hydride (1.5 equiv.) in dry DMF, compound **3** (1 equiv.) was added and allow to stir at room temperature. After 1 h, 4-(triflouromethylbenzyl) bromide (1.5 equiv.) was added to the reaction mixture and refluxed for 4 h. The solvent was evaporated to dryness and water was added to solid residue. The compound was extracted with DCM, dried over anhyd Na_2SO_4, and concentrated under vacuum. The residue was

purified by flash chromatography using ($CHCl_3$/MeOH = 10:1) to afford compound **5**.

*6.4.1. Ethyl-6,7,8-trifluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylate (**5**)*

Yield: 72%; mp 243–245 °C; ESMS: 430 (M + 1); IR (KBr): 3466, 3070, 2925, 2854, 1729, 1656, 1620, 1488, 1391, 1327, 1278, 1121, 1066 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.87 (d, 1H, $J = 7.08$ Hz), 8.04 (t, 1H, $J = 8.08$ Hz), 7.73 (d, 2H, $J = 7.44$ Hz), 7.41 (d, 2H, J = 6.96 Hz), 5.82 (s, 2H), 4.26–4.23 (m, 2H), 1.29 (t, 3H, J = 7.12 Hz). ^{13}C (50 MHz, $CDCl_3$,): 171.13, 164.37, 153.28, 141.91, 128.63, 126.37, 126.95, 123.27, 120.45, 115.28, 110.94, 109.26, 60.73, 52.14, 14.68. Anal. Calc for $C_{20}H_{13}F_6NO_3$: C: 55.95, H: 3.05, N: 3.26. Found: C: 55.87, H: 3.08, N: 3.17.

*6.5. General procedure for the synthesis of compounds **6** and **7***

Compound **4** (2.0 gm, 0.0066 mol) was refluxed in acetic acid (100 mL) in the presence of 25 mL of 2N HCl for 2 h. Reaction mixture was evaporated to dryness and water was added to it. Precipitate occurred was filtered and washed with water and dried over $CaCl_2$ to obtain the compound **6**. Same method was applied to get compound **7** from **5**.

*6.5.1. 1-Ethyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6**)*

Yield: 78%; mp 236–238 °C; ESMS: 272 (M + 1); IR (KBr): 3431, 3050, 2994, 2862, 1724, 1621, 1569, 1492, 1346, 1253, 1114, 1055 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.49 (s, 1H), 9.05 (s, 1H), 8.17 (t, 1H, $J = 8.90$ Hz), 4.61 (m, 2H), 1.44 (t, 3H, $J = 6.70$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.65, 167.48, 149.82, 148.26, 142.31, 132.28, 127.65, 116.94, 108.46, 107.81, 54.76, 16.63. Anal. Calc for $C_{12}H_8F_3NO_3$: C: 53.15, H: 2.97, N: 5.16. Found: C: 53.07, H: 2.95, N: 5.11.

*6.5.2. 6,7,8-Trifluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**7**)*

Yield: 80%; mp >250 °C; ESMS: 402 (M + 1); IR (KBr): 3439, 3054, 2924, 2739, 1723, 1621, 1569, 1494, 1330, 1293, 1117, 1065 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.37 (bs, 1H), 9.26 (s, 1H), 8.26–8.20 (m, 1H), 7.73 (d, 2H, J = 5.44 Hz), 7.44 (d, 2H, $J = 5.40$ Hz), 5.99 (d, 2H, $J = 1.76$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.72, 166.65, 151.14, 150.82, 139.47, 138.84, 132.58, 131.53, 126.75, 126.62, 126.17, 115.35, 109.63, 108.64, 61.72. Anal. Calc for $C_{18}H_9F_6NO_3$: C: 53.88, H: 2.26, N: 3.49. Found: C: 53.75, H: 2.21, N: 3.41.

*6.6. General procedure for the synthesis of targeted compounds **8**–**33***

A mixture of compound **6** (1 equiv.) and respective amine (Table 1) (1.2 equiv.) in acetonitrile was refluxed for 7 h at 90 °C. Reaction mixture was evaporated to dryness and the solid residue obtained was purified with flash column chromatography using chloroform to methanol gradient elution to afford final targeted compounds **8**–**20**. The same procedure was followed to obtain compounds **21**–**33** from the intermediate **7**.

*6.6.1. 1-Ethyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**8**)*

Yield: 71%; mp 246–248 °C; ESMS: 339 (M + 1); IR (KBr): 3429, 3040, 2978, 2813, 1706, 1632, 1549, 1474, 1336, 1244, 1097 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.66 (bs, 1H), 8.62 (s, 1H), 7.97 (d, 1H, $J = 11.76$), 4.49–4.47 (m, 2H), 3.86 (m, 4H), 3.41 (m, 4H), 1.57 (t, 3H, $J = 6.88$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.47, 167.35, 149.82, 143.25, 133.86, 127.54, 116.57, 108.36, 107.95, 67.61, 54.96, 51.52, 16.38. Anal. Calc for $C_{16}H_{16}F_2N_2O_4$: C: 56.80, H: 4.77, N: 8.28. Found: C: 56.74, H: 4.79, N: 8.25.

*6.6.2. 1-Ethyl-6,8-difluoro-4-oxo-7-(piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**9**)*

Yield: 76%; mp 201–203 °C; ESMS: 337 (M + 1); IR (KBr): 3342, 3034, 2915, 2868, 1715, 1619, 1550, 1464, 1327, 1258, 1152 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.65 (bs, 1H), 8.59 (s, 1H), 7.93 (d, 1H, $J = 12.08$ Hz), 4.48–4.45 (m, 2H), 3.33 (m, 4H), 1.71 (m, 6H), 1.56 (t, 3H, J = 6.55 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.65, 167.27, 150.45, 148.32, 142.57, 134.85, 127.61, 121.45, 108.82, 108.57, 54.71, 50.56, 26.38, 25.32, 16.54. Anal. Calc for $C_{17}H_{18}F_2N_2O_3$: C: 60.71, H: 5.39, N: 8.33. Found: C: 60.63, H: 5.41, N: 8.30.

*6.6.3. 1-Ethyl-6,8-difluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**10**)*

Yield: 71%; mp 228–230 °C; ESMS: 338 (M + 1); IR (KBr): 3411, 3078, 2940, 2871, 1722, 1617, 1547, 1466, 1337, 1264, 1162 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.66 (bs, 1H), 8.93 (s, 1H), 7.85 (d, 1H, $J = 11.92$ Hz), 4.61 (m, 2H), 3.62 (m, 4H), 2.91 (m, 4H), 1.48 (t, 3H, J = k6.17 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.25, 167.18, 150.34, 148.26, 142.31, 134.86, 127.64, 121.53, 108.91, 108.44, 54.98, 48.95, 46.63, 16.51. Anal. Calc for $C_{16}H_{17}F_2N_3O_3$: C: 56.97, H: 5.08, N: 12.46. Found: C: 56.91, H: 5.09, N: 12.42.

*6.6.4. 1-Ethyl-6,8-difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**11**)*

Yield: 74%; mp 221–223 °C; MS: 352 (M + 1); IR (KBr): 3353, 3048, 2926, 2847, 1721, 1635, 1542, 1466, 1355, 1221, 1110 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.69 (bs, 1H), 8.60 (s, 1H), 7.96 (d, 1H, $J = 11.66$ Hz), 4.47–4.45 (m, 2H), 3.46 (m, 4H), 2.65 (m, 4H), 2.42 (s, 3H), 1.56 (t, 3H, $J = 6.38$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.62, 167.18, 150.43, 148.27, 142.35, 134.86, 127.61, 121.59, 108.94, 108.47, 55.82, 54.85, 51.31, 46.65, 16.86. Anal. Calc for $C_{17}H_{19}F_2N_3O_3$: C: 58.11, H: 5.45, N: 11.96. Found: C: 58.07, H: 5.42,. N: 11.89.

*6.6.5. 1-Ethyl-7-(4-ethylpiperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**12**)*

Yield: 70%; mp 213–215 °C; MS: 366 (M + 1); IR (KBr): 3428, 3015, 2927, 1718, 1622, 1549, 1485, 1343, 1219, 1107 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.59 (s, 1H), 7.95 (dd, 1H, J = 11.79, 1.55 Hz), 4.49–4.44(m, 2H), 3.48–3.38 (m, 4H), 2.68–2.47 (m, 6H), 1.56 (t, 3H, $J = 6.80$ Hz), 1.21 (t, 3H, $J = 7.29$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.64, 167.18, 150.42, 148.57, 142.54, 134.82, 127.56, 121.49, 108.91, 108.40, 55.28, 53.62, 52.85, 51.26, 16.83, 13.71. Anal. Calc for $C_{18}H_{21}F_2N_3O_3$: C: 59.17, H: 5.79, N: 11.50. Found: C: 59.14, H: 5.75, N: 11.48.

*6.6.6. 1-Ethyl-6,8-difluoro-4-oxo-7-(4-phenylpiperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**13**)*

Yield: 78%; mp 195–197 °C; MS: 414 (M + 1); IR (KBr): 3435, 3028, 2951, 2756, 1715, 1630, 1575, 1459, 1324, 1249, 1109 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.69 (bs, 1H), 8.62 (s, 1H), 7.99 (d, 1H, J = 11.74 Hz), 7.36–7.28 (m, 2H), 7.03–6.94 (m, 3H), 4.51 (m, 2H), 3.69–3.58 (m, 4H), 3.35–3.19 (m, 4H), 1.58 (t, 3H, $J = 6.09$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.35, 167.42, 151.28, 150.54, 148.37, 143.18, 138.13, 132.25, 129.61, 120.96, 117.25, 116.73, 109.12, 108.57, 55.29, 51.33, 50.61, 16.78. Anal. Calc for $C_{22}H_{21}F_2N_3O_3$: C: 63.91, H: 5.12, N: 10.16. Found: C: 63.94, H: 5.06, N: 10.15.

*6.6.7. 7-(4-Benzylpiperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**14**)*

Yield: 72%; mp 196–198 °C; MS: 428 (M + 1); IR (KBr): 3426, 3044, 2929, 2812, 1721, 1625, 1525, 1474, 1325, 1288, 1124 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.68 (bs, 1H), 8.59 (s, 1H), 7.95

(dd, 1H, J = 11.93, 1.91 Hz), 7.38–7.29 (m, 5H), 4.55–4.42 (m, 2H), 3.66 (s, 2H), 3.46–3.09 (m, 4H), 2.69–2.48 (m, 4H), 1.58 (t, 3H, J = 6.12 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.62, 167.17, 150.95, 147.93, 146.54, 135.62, 132.36, 129.71, 129.57, 128.83, 127.87, 116.75, 109.02, 108.46, 63.41, 55.28, 53.84, 51.27, 16.82. Anal. Calc for $C_{23}H_{23}F_2N_3O_3$: C: 64.63, H: 5.42, N: 9.83. Found: C: 64.56, H: 5.41, N: 9.85.

*6.6.8. 1-Ethyl-6,8-difluoro-7-(2-morpholinoethylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**15**)*

Yield: 78%; mp 199–201 °C; ESMS: 382 (M + 1); IR (KBr): 3332, 3042, 2929, 2817, 1716, 1628, 1551, 1470, 1336, 1247, 1117 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.96 (bs, 1H), 8.54 (s, 1H), 7.91 (dd, 1H, J = 12.04, 1.58 Hz), 5.41 (bs, 1H), 4.47–4.43 (m, 2H), 3.77–3.67 (m, 6H), 2.72–2.56 (m, 6H), 1.56 (t, 3H, J = 6.78 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.52, 167.37, 149.83, 148.35, 142.27, 133.34, 127.43, 116.51, 108.36, 107.94, 67.36, 57.44, 54.96, 54.53, 53.61, 16.78. Anal. Calc for $C_{18}H_{21}F_2N_3O_4$: C: 56.69, H: 5.55, N: 11.02. Found: C: 56.61, H: 5.42, N: 11.05.

*6.6.9. 1-Ethyl-6,8-difluoro-7-(3-morpholinopropylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**16**)*

Yield: 71%; mp 218–220 °C; ESMS: 396 (M + 1); IR (KBr): 3347, 3042, 2948, 2806, 1711, 1632, 1549, 1478, 1340, 1245, 1098 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 15.03 (bs, 1H), 8.52 (s, 1H), 7.91–7.85 (m, 1H), 6.74 (bs, 1H), 4.46–4.41 (m, 2H), 4.01–3.66 (m, 6H), 2.63–2.39 (m, 6H), 2.25–2.16 (m, 2H), 1.55 (t, 3H, J = 6.77 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.64, 167.57, 149.83, 148.54, 142.21, 133.27, 127.53, 116.72, 108.49, 107.86, 66.73, 61.65, 54.92, 54.16, 30.12, 24.95, 16.52. Anal. Calc for $C_{19}H_{23}F_2N_3O_4$: C: 57.71, H: 5.86, N: 10.63. Found: C: 57.74, H: 5.72, N: 10.58.

*6.6.10. 7-(Butylamino)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**17**)*

Yield: 79%; mp 78–80 °C; MS: 325 (M + 1); IR (KBr): 3366, 3021, 2935, 1720, 1621, 1551, 1458, 1334, 1216, 1118 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.58 (bs, 1H), 8.58 (s, 1H), 7.91 (dd, 1H, J = 12.10, 1.80 Hz), 4.51–4.39 (m, 2H), 3.60–3.48 (m, 2H), 1.73–1.35 (m, 7H), 0.98 (t, 3H, J = 7.18 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.82, 167.57, 149.54, 147.91, 142.43, 132.85, 127.36, 116.62, 108.38, 108.16, 54.81, 45.85, 33.23, 20.75, 16.72, 14.26. Anal. Calc for $C_{16}H_{18}F_2N_2O_3$: C: 59.25, H: 5.59, N: 8.64. Found: C: 59.24, H: 5.55, N: 8.58.

*6.6.11. 7-(Tert-butylamino)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**18**)*

Yield: 75%; mp 160–162 °C; ESMS: 325 (M + 1); IR (KBr): 3358, 3014, 2943, 1731, 1617, 1562, 1446, 1342, 1210, 1103 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.60 (bs, 1H), 8.59 (s, 1H), 7.97 (d, 1H, J = 11.26 Hz), 4.49 (m, 3H), 1.58 (t, 3H, J = 6.84 Hz), 1.42 (s, 9H). ^{13}C (50 MHz, $CDCl_3$): 176.81, 167.53, 149.86, 147.84, 142.65, 132.83, 127.37, 116.65, 108.39, 108.15, 54.53, 51.19, 29.80, 16.73. Anal. Calc for $C_{16}H_{18}F_2N_2O_3$: C: 59.25, H: 5.59, N: 8.64. Found: C: 59.27, H: 5.46, N: 8.57.

*6.6.12. 1-Ethyl-6,8-difluoro-4-oxo-7-(propylamino)-1,4-dihydroquinoline-3-carboxylic acid (**19**)*

Yield: 76%; mp 178–180 °C; ESMS: 311 (M + 1); IR (KBr): 3353, 3067, 2972, 2882, 1715, 1629, 1562, 1412, 1322, 1264, 1110 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.53 (s, 1H), 7.84 (dd, 1H, J = 12.11, 1.71 Hz), 4.50–4.38 (m, 2H), 3.55–3.47 (m, 2H), 1.73–1.66 (m, 2H), 1.54 (t, 3H, J = 6.78 Hz), 1.32 (t, 3H, J = 7.15 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.54, 167.47, 149.83, 148.21, 142.25, 132.86, 127.59, 116.46, 108.72, 107.95, 54.65, 47.81, 24.59, 16.68. Anal. Calc for $C_{15}H_{16}F_2N_2O_3$: C: 58.06, H: 5.20, N: 9.03. Found: C: 58.04, H: 5.12, N: 8.97.

*6.6.13. 1-Ethyl-6,8-difluoro-7-(isopropylamino)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**20**)*

Yield: 77%; mp 196–198 °C; MS: 311 (M + 1); IR (KBr): 3401, 3066, 2977, 2937, 1721, 1618, 1548, 1461, 1333, 1261 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 8.56 (s, 1H), 7.91 (dd, 1H, J = 12.07, 1.77 Hz), 4.51–4.39 (m, 2H), 4.23–4.10 (m, 1H), 1.55 (t, 3H, J = 6.66 Hz), 1.26 (d, 6H, J = 6.90 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.54, 167.68, 149.64, 148.26, 142.25, 132.22, 127.57, 116.91, 108.43, 107.95, 54.87, 47.33, 24.52, 16.64. Anal. Calc for $C_{15}H_{16}F_2N_2O_3$: C: 58.06, H: 5.20, N: 9.03. Found: C: 58.02, H: 5.24, N: 9.01.

*6.6.14. 6,8-Difluoro-7-morpholino-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**21**)*

Yield: 72%; mp 220–222 °C; ESMS: 469 (M + 1); IR (KBr): 3428, 3042, 2987, 2820, 1715, 1631, 1545, 1475, 1332, 1249, 1120, 1087 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.52 (s, 1H), 8.73 (s, 1H), 7.98 (d, 1H, J = 11.58 Hz), 7.63 (d, 2H, J = 7.86 Hz), 7.26 (m, 2H), 5.66 (s, 2H), 3.79–3.75 (m, 4H), 3.26 (m, 4H). ^{13}C (50 MHz, $CDCl_3$): 176.85, 166.67, 164.91, 151.62, 150.98, 139.44, 138.87, 132.54, 131.41, 126.75, 126.26, 114.23, 109.29, 108.94, 67.62, 61.54, 51.46. Anal. Calc for $C_{22}H_{17}F_5N_2O_4$: C: 56.42, H: 3.66, N: 5.98. Found: C: 56.44, H: 3.58, N: 5.91.

*6.6.15. 6,8-Difluoro-4-oxo-7-(piperidin-1-yl)-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**22**)*

Yield: 70%; mp 247–249 °C; ESMS: 467 (M + 1); IR (KBr): 3398, 3027, 2906, 2872, 1719, 1621, 1548, 1467, 1328, 1254, 1125, 1071 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.56 (bs, 1H), 8.71 (s, 1H), 7.93 (d, 1H, J = 11.76 Hz), 7.63 (d, 2H, J = 7.34 Hz), 7.26 (m, 2H), 5.65 (s, 2H), 3.18 (m, 4H), 1.63 (m, 6H). ^{13}C (50 MHz, $CDCl_3$): 176.93, 166.65, 151.17, 150.61, 139.54, 138.62, 132.47, 131.58, 126.74, 126.63, 126.17, 114.49, 109.26, 108.83, 61.67, 50.49, 26.32, 25.35. Anal. Calc for $C_{23}H_{19}F_5N_2O_3$: C: 59.23, H: 4.11, N: 6.01. Found: C: 59.14, H: 4.15, N: 6.05.

*6.6.16. 6,8-Difluoro-4-oxo-7-(piperazin-1-yl)-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**23**)*

Yield: 72%; mp >250 °C; ESMS: 468 (M + 1); IR (KBr): 3362, 3035, 2924, 2862, 1726, 1621, 1532, 1442, 1326, 1129, 1078 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.81 (bs, 1H), 8.69 (s, 1H), 7.45 (d, 1H, J = 12.40 Hz), 7.29 (d, 2H, J = 7.68 Hz), 6.96 (d, 2H, J = 7.98 Hz), 5.52 (s, 2H), 2.78 (m, 4H), 2.33 (m, 4H), 1.11 (bs, 1H). ^{13}C (50 MHz, $CDCl_3$): 176.97, 166.63, 151.18, 150.82, 139.39, 138.91, 132.44, 131.57, 126.73, 126.25, 126.19, 114.46, 109.13, 108.87, 61.62, 46.74, 48.81. Anal. Calc for $C_{22}H_{18}F_5N_3O_3$: C: 56.51, H: 3.78, N: 8.92. Found: C: 56.54, H: 3.85, N: 8.96.

*6.6.17. 6,8-Difluoro-7-(4-methylpiperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**24**)*

Yield: 79%; mp 233–235 °C; ESMS: 482 (M + 1); IR (KBr): 3431, 3045, 2923, 2855, 1727, 1620, 1532, 1442, 1325, 1128, 1065 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.58 (s, 1H), 8.74 (s, 1H), 7.95 (d, 1H, J = 10.56 Hz), 7.63 (d, 2H, J = 7.54 Hz), 7.25 (m, 2H), 5.66 (s, 2H), 3.32 (m, 4H), 2.57 (m, 4H), 2.38 (s, 3H). ^{13}C (50 MHz, $CDCl_3$): 176.98, 166.53, 151.27, 150.95, 139.54, 138.61, 132.59, 131.56, 126.83, 126.72, 126.18, 115.56, 109.23, 108.86, 61.52, 55.82, 51.27, 46.85. Anal. Calc for $C_{23}H_{20}F_5N_3O_3$: C: 57.38, H: 4.19, N: 8.73. Found: C: 57.34, H: 4.09, N: 8.76.

*6.6.18. 7-(4-Ethylpiperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**25**)*

Yield: 76%; mp 220–222 °C; ESMS: 496 (M + 1); IR (KBr): 3430, 3021, 2932, 1716, 1624, 1548, 1481, 1342, 1220, 1131, 1056 cm^{-1}; ^{1}H

NMR (200 MHz, $CDCl_3$): δ (ppm) 14.22 (s, 1H), 8.74 (s, 1H), 7.95 (d, 1H, $J = 11.78$ Hz), 7.63 (d, 2H, $J = 7.80$ Hz), 7.25 (m, 2H), 5.66 (s, 2H), 3.35 (m, 4H), 2.62–2.45 (m, 6H), 1.20 (t, 3H, $J = 7.30$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.83, 166.61, 151.58, 150.45, 139.63, 139.37, 132.45, 131.52, 126.76, 126.68, 121.63, 114.51, 109.26, 108.84, 61.75, 53.49, 52.85, 50.91, 12.24. Anal. Calc for $C_{24}H_{22}F_5N_3O_3$: C: 58.18, H: 4.48, N: 8.48. Found: C: 58.14, H: 4.39, N: 8.51.

*6.6.19. 6,8-Difluoro-4-oxo-7-(4-phenylpiperazin-1-yl)-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**26**)*

Yield: 83%; mp 232–234 °C; MS: 544 (M + 1); IR (KBr): 3428, 3017, 2941, 2766, 1718, 1636, 1570, 1443, 1325, 1242, 1137, 1061 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.52 (s, 1H), 7.99 (d, 1H, $J = 11.68$ Hz), 7.64 (d, 2H, $J = 7.40$ Hz), 7.32–7.26 (m, 6H), 6.98 (m, 1H), 5.68 (s, 2H), 3.30–3.19 (m, 8H). ^{13}C (50 MHz, $CDCl_3$): 176.94, 166.76, 151.31, 150.95, 148.36, 139.54, 138.55, 132.42, 131.64, 129.75, 126.78, 126.62, 126.11, 120.93, 116.72, 115.36, 109.64, 108.78, 61.92, 51.35, 50.46. Anal. Calc for $C_{28}H_{22}F_5N_3O_3$: C: 61.88, H: 4.08, N: 7.73. Found: C: 61.84, H: 4.05, N: 7.71.

*6.6.20. 7-(4-Benzylpiperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**27**)*

Yield: 78%; mp >250 °C; ESMS: 558 (M + 1); IR (KBr): 3425, 3045, 2926, 2817, 1722, 1624, 1526, 1467, 1332, 1298, 1126, 1058. cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.53 (s, 1H), 8.73 (s, 1H), 7.96 (d, 1H, $J = 11.58$ Hz), 7.62 (d, 2H, $J = 7.36$ Hz), 7.35–7.26 (m, 5H), 7.19 (m, 2H), 5.64 (s, 2H), 3.56 (m, 2H), 3.34 (m, 4H), 2.63 (m, 4H). ^{13}C (50 MHz, $CDCl_3$): 176.78, 167.27, 151.43, 150.95, 147.46, 139.51, 135.62, 132.67, 131.26, 129.75, 129.52, 128.84, 126.71, 126.59, 121.72, 116.75, 109.81, 108.96, 63.44, 61.78, 53.74, 51.19. Anal. Calc for $C_{29}H_{24}F_5N_3O_3$: C: 62.48, H: 4.34, N: 7.54. Found: C: 62.41, H: 4.36, N: 7.48.

*6.6.21. 7-(Butylamino)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**28**)*

Yield: 72%; mp 208–210 °C; MS: 455 (M + 1); IR (KBr): 3372, 3018, 2927, 1724, 1622, 1549, 1436, 1335, 1217, 1136, 1064 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 15.16 (s, 1H), 9.05 (s, 1H), 7.81–7.67 (m, 3H), 7.32 (d, 2H, $J = 8.12$ Hz), 6.57 (bs, 1H), 5.91 (d, 2H, $J = 3.24$ Hz), 3.24–3.21 (m, 2H), 1.25–1.06 (m, 4H), 0.73 (t, 3H, $J = 7.02$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.56, 166.52, 151.67, 140.18, 139.41, 132.53, 131.87, 126.65, 126.49, 121.32, 116.85, 109.94, 108.59, 61.78, 45.82, 33.23, 20.61, 14.25. Anal. Calc for $C_{22}H_{19}F_5N_2O_3$: C: 58.15, H: 4.21, N: 6.17. Found: C: 58.11, H: 4.16, N: 6.15.

*6.6.22. 7-(Tert-butylamino)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**29**)*

Yield: 76%; mp 155–157 °C; ESMS: 455 (M + 1); IR (KBr): 3343, 3017, 2942, 1729, 1618, 1560, 1456, 1324, 1215, 1173, 1102 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.72 (s, 1H), 8.86 (s, 1H), 7.97 (d, 1H, $J = 10.86$ Hz), 7.62 (d, 2H, $J = 7.52$ Hz), 7.26 (m, 2H), 5.67 (s, 2H), 1.24 (s, 9H). ^{13}C (50 MHz, $CDCl_3$): 176.54, 167.47, 150.93, 141.27, 139.54, 132.51, 131.95, 126.62, 126.17, 121.35, 116.86, 109.97, 108.51, 61.6, 51.16, 29.85. Anal. Calc for $C_{22}H_{19}F_5N_2O_3$: C: 58.09, H: 4.21, N: 6.17. Found: C: 58.16, H: 4.24, N: 6.13.

*6.6.23. 6,8-Difluoro-4-oxo-7-(propylamino)-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**30**)*

Yield: 75%; mp 203–205 °C; ESMS: 441 (M + 1); IR (KBr): 3347, 3071, 2975, 2880, 1717, 1632, 1565, 1421, 1323, 1268, 1111, 1055 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.71 (s, 1H), 8.68 (s, 1H), 7.93 (d, 1H, $J = 11.60$ Hz), 7.62 (d, 2H, $J = 6.78$ Hz), 7.26 (m, 2H), 5.64 (s, 2H), 3.39 (m, 2H), 1.59–1.52 (m, 2H), 0.92 (t, 3H, $J = 6.90$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.63, 167.87, 151.65, 141.39, 139.54, 132.32, 131.58, 126.72, 126.49, 121.36, 117.75, 109.81, 108.54, 61.57, 47.73, 24.52, 11.57. Anal. Calc for $C_{21}H_{17}F_5N_2O_3$: C: 57.24, H: 3.79, N: 6.36. Found: C: 57.29, H: 3.85, N: 6.38.

*6.6.24. 6,8-Difluoro-7-(isopropylamino)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**31**)*

Yield: 76%; mp 213–215 °C; ESMS: 441 (M + 1); IR (KBr): 3387, 3056, 2967, 2935, 1722, 1623, 1544, 1465, 1334, 1260, 1167, 1049 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.70 (s, 1H), 8.68 (s, 1H), 7.93 (d, 1H, $J = 11.84$ Hz), 7.62 (d, 2H, $J = 7.76$ Hz), 7.26 (m, 2H), 5.65 (s, 2H), 4.02 (m, 1H), 1.17 (d, 6H, $J = 5.98$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.65, 167.72, 151.54, 140.27, 139.43, 132.38, 131.64, 126.71, 126.65, 121.37, 116.82, 109.95, 108.57, 61.64, 47.28, 24.35. Anal. Calc for $C_{21}H_{17}F_5N_2O_3$: C: 57.28, H: 3.89, N: 6.36. Found: C: 57.26, H: 3.84, N: 6.27.

*6.6.25. 6,8-Difluoro-7-(2-morpholinoethylamino)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**32**)*

Yield: 75%; mp 169–171 °C; ESMS: 512 (M + 1); IR (KBr): 3345, 3049, 2927, 2871, 1717, 1632, 1560, 1485, 1321, 1110, 1079 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.84 (s, 1H), 8.65 (s, 1H), 7.93 (d, 1H, $J = 12.02$ Hz), 7.63 (d, 2H, $J = 7.52$ Hz), 7.26 (m, 2H), 5.64 (s, 2H), 5.25 (bs, 1H), 3.68 (m, 4H), 3.52 (m, 2H), 2.59 (m, 2H), 2.45 (m, 4H). ^{13}C (50 MHz, $CDCl_3$): 176.75, 166.92, 151.54, 150.94, 139.57, 132.43, 131.58, 126.75, 126.52, 126.27, 121.76, 116.51, 108.56, 108.18, 67.34, 61.73, 57.39, 53.46, 41.25. Anal. Calc for $C_{24}H_{22}F_5N_3O_4$: C: 56.36, H: 4.34, N: 8.22. Found: C: 56.32, H: 4.29, N: 8.26.

*6.6.26. 6,8-Difluoro-7-(3-morpholinopropylamino)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**33**)*

Yield: 71%; mp 188–190 °C; ESMS: 526 (M + 1); IR (KBr): 3356, 3052, 2925, 2855, 1720, 1629, 1561, 1481, 1327, 1119, 1065 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.86 (s, 1H), 8.64 (s, 1H), 7.89 (d, 1H, $J = 12.3$ Hz), 7.62 (d, 2H, $J = 7.72$ Hz), 7.26 (m, 3H), 5.63 (s, 2H), 3.66 (m, 6H), 2.56 (m, 6H), 1.65 (m, 2H). ^{13}C (50 MHz, $CDCl_3$): 176.75, 167.17, 150.82, 147.45, 139.63, 132.65, 131.72, 126.68, 126.54, 126.47, 121.85, 115.39, 108.61, 108.26, 66.84, 61.62, 61.36, 54.06, 30.12, 24.65. Anal. Calc for $C_{25}H_{24}F_5N_3O_4$: C: 57.14, H: 4.60, N: 8.00, Found: C: 57.06, H: 4.57, N: 8.04.

*6.7. General procedure for the synthesis of targeted compounds **34**–**51***

To the solution of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) in dry THF, Compound **10** (1 equiv.) was added in portion during 30 min. After stirring for 1 h at room temperature, respective amines (2.2 equiv.) (Table 2) were added to the reaction mixture and refluxed for 7 more hours. When the reaction was completed, the reaction mixture was evaporated to dryness under vacuum. The solid residue obtained was purified with flash column chromatography using chloroform to methanol gradient elution to obtain the final compounds **34**–**42**. The same procedure was carried out to synthesize targeted compounds **43**–**51** from the compound **23**.

*6.7.1. 7-(4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**34**)*

Yield: 64%; mp >250 °C; ESMS: 587 (M + 1); IR (KBr): 3058, 2927, 2853, 1727, 1563, 1458, 1267, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.59 (bs, 1H), 8.63 (s, 1H), 7.99 (d, 1H, $J = 11.48$ Hz), 4.48 (m, 2H), 4.02–3.79 (m, 12H), 3.61 (m, 8H), 3.31 (m, 4H), 1.58 (t, 3H, $J = 6.83$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 176.93, 167.41, 166.57, 150.13, 138.84, 115.24, 108.72, 67.27, 55.17, 51.54, 45.18, 16.11. Anal. Calc for $C_{27}H_{32}F_2N_8O_5$: C: 55.28, H: 5.50, N: 19.10. Found: C: 55.27, H: 5.42, N: 19.07.

*6.7.2. 7-(4-(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**35**)*

Yield: 62%; mp >250 °C; ESMS: 583 (M + 1); IR (KBr): 3053, 2927, 2856, 1721, 1586, 1443, 1274, 1126 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.53 (bs, 1H), 8.61 (s, 1H), 7.98 (d, 1H, *J* = 11.54 Hz), 4.47 (m, 2H), 3.93 (m, 4H), 3.72 (m, 8H), 3.39 (m, 4H), 1.68–1.57 (m, 15H). ^{13}C ($CDCl_3$, 50 MHz): 176.72, 167.14, 166.11, 165.66, 150.44, 139.69, 114.49, 108.49, 55.48, 51.43, 44.54, 44.24, 26.25, 25.44, 16.81. Anal. Calc for $C_{29}H_{36}F_2N_8O_3$: C: 59.78, H: 6.23, N: 19.23. Found: C: 59.75, H: 6.18, N: 19.21.

*6.7.3. 7-(4-(4,6-Bis(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl) piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**36**)*

Yield: 66%; mp 205–207 °C; ESMS: 613 (M + 1); IR (KBr): 3067, 2929, 2849, 1722, 1563, 1449, 1239, 1122 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.58 (bs, 1H), 8.73 (s, 1H), 8.04 (d, 1H, *J* = 11.24 Hz), 4.49 (m, 2H), 4.06 (m, 4H), 3.33 (m, 4H), 3.24 (m, 8H), 2.82 (m, 8H), 2.44 (s, 6H), 1.58 (t, 3H, *J* = 6.53 Hz). ^{13}C (50 MHz, $CDCl_3$): 178.44, 176.27, 166.52, 151.63, 138.07, 115.89, 108.41, 55.56, 49.29, 48.31, 47.26, 16.17. Anal. Calc for $C_{29}H_{38}F_2N_{10}O_3$: C: 56.85, H: 6.25, N: 22.86. Found: C: 56.81, H: 6.28, N: 22.77.

*6.7.4. 7-(4-(4,6-Bis(2-morpholinoethylamino)-1,3,5-triazin-2-yl) piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**37**)*

Yield: 61%; mp 202–204 °C; MS: 673 (M + 1); IR (KBr): 3343, 3057, 2928, 2853, 1728, 1598, 1432, 1251, 1122 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.59 (bs, 1H), 8.61 (s, 1H), 8.01 (d, 1H, *J* = 11.37 Hz), 5.98 (bs, 2H), 4.49 (m, 2H), 4.01–3.67 (m, 12H), 3.37 (m, 8H), 2.56–2.41 (m, 12H), 1.57 (t, 3H, *J* = 6.73 Hz). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 178.18, 168.27, 166.38, 153.87, 141.27, 116.94, 111.28, 69.23, 57.44, 53.18, 52.74, 48.69, 19.38. Anal. Calc for $C_{31}H_{42}F_2N_{10}O_5$: C: 55.35, H: 6.29, N: 20.82. Found: C: 55.28, H: 6.22, N: 20.74.

*6.7.5. 7-(4-(4,6-Bis(3-morpholinopropylamino)-1,3,5-triazin-2-yl) piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**38**)*

Yield: 61%; mp 211–213 °C; ESMS: 701 (M + 1); IR (KBr): 3393, 3051, 2925, 2855, 1719, 1569, 1475, 1251, 1118 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.69 (bs, 1H), 8.71 (s, 1H), 8.02 (d, 1H, *J* = 11.53 Hz), 5.83 (bs, 2H), 4.49 (m, 2H), 4.04 (m, 12H), 3.77 (m, 4H), 3.37 (m, 4H), 2.61 (m, 12H), 1.72 (m, 4H), 1.58 (t, 3H, *J* = 6.83 Hz). ^{13}C ($CDCl_3$, 50 MHz): 176.47, 166.38, 166.11, 164.18, 151.32, 139.87, 114.72, 109.13, 66.43, 55.23, 51.16, 45.38, 43.09, 26.19, 15.89. Anal. Calc for $C_{33}H_{46}F_2N_{10}O_5$: C: 56.56, H: 6.62, N: 19.99. Found: C: 56.53, H: 6.57, N: 19.94.

*6.7.6. 7-(4-(4,6-Bis(butylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**39**)*

Yield: 63%; mp 194–196 °C; ESMS: 559 (M + 1); IR (KBr): 3445, 3020, 2971, 1721, 1561, 1443, 1216, 1045 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.41 (bs, 1H), 8.62 (s, 1H), 7.99 (d, 1H, *J* = 11.02 Hz), 5.92 (bs, 2H), 4.46 (m, 2H), 3.94 (m, 4H), 3.38 (m, 8H), 1.74 (m, 8H), 1.57 (t, 3H, *J* = 6.34 Hz), 0.97 (t, 6H, *J* = 6.87 Hz). ^{13}C (50 MHz, $CDCl_3$): 177.03, 167.08, 166.43, 164.98, 150.51, 138.70, 113.89, 109.11, 108.58, 55.23, 51.33, 44.21, 40.81, 30.11, 20.48, 16.83, 14.26. Anal. Calc for $C_{27}H_{36}F_2N_8O_3$: C: 58.05, H: 6.50, N: 20.06. Found: C: 58.02, H: 6.44, N: 20.09.

*6.7.7. 7-(4-(4,6-Bis(tert-butylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**40**)*

Yield: 60%; mp >250 °C; ESMS: 559 (M + 1); IR (KBr): 3374, 3047, 2941, 2862, 1724, 1579, 1463, 1227, 1112 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz): δ (ppm) 14.59 (bs, 1H), 8.94 (s, 1H), 7.92 (d, 1H, *J* = 11.13 Hz), 5.58 (bs, 2H), 4.59 (m, 2H), 4.09 (m, 4H), 3.32 (m, 4H), 1.61 (t, 3H, *J* = 6.84 Hz), 1.42 (s, 18H). ^{13}C (DMSO-d_6, 50 MHz): 176.31, 166.72, 164.58, 151.37, 137.23, 116.02, 109.31, 56.62, 54.72, 51.76, 45.84, 31.61, 16.28. Anal. Calc for $C_{27}H_{36}F_2N_8O_3$: C: 58.05, H: 6.50, N: 20.06. Found: C: 58.09, H: 6.43, N: 20.01.

*6.7.8. 7-(4-(4,6-Bis(propylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**41**)*

Yield: 63%; mp 225–227 °C; ESMS: 531 (M + 1); IR (KBr): 3436, 3020, 2964, 2864, 1721, 1549, 1443, 1283, 1216 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.47 (bs, 1H), 8.62 (s, 1H), 7.98 (d, 1H, *J* = 11.54 Hz), 4.97 (bs, 2H), 4.47 (m, 2H), 3.96 (m, 4H), 3.39–3.32 (m, 8H), 1.64–1.54 (m, 7H), 0.97 (t, 6H, *J* = 7.27 Hz). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 180.53, 171.74, 170.21, 168.83, 154.71, 139.14, 121.37, 112.31, 59.16, 55.12, 48.30, 46.73, 27.05, 20.61, 15.65. Anal. Calc for $C_{25}H_{32}F_2N_8O_3$: C: 56.59, H: 6.08, N: 21.12. Found: C: 56.61, H: 6.03, N: 21.06.

*6.7.9. 7-(4-(4,6-Bis(isopropylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**42**)*

Yield: 67%; mp 227–229 °C; ESMS: 531 (M + 1); IR (KBr): 3383, 3061, 2931, 2861, 1723, 1572, 1476, 1271, 1140 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.61 (bs, 1H), 8.63 (s, 1H), 8.01 (d, 1H, *J* = 10.78 Hz), 5.58 (bs, 2H), 4.48 (m, 2H), 4.12–4.01 (m, 6H), 3.41 (m, 4H), 1.57 (t, 3H, *J* = 6.74 Hz), 1.25 (m, 12H). ^{13}C (50 MHz, $CDCl_3$): 176.58, 167.28, 166.53, 151.17, 139.52, 114.17, 109.32, 55.67, 52.11, 45.27, 44.92, 25.51, 16.72. Anal. Calc for $C_{25}H_{32}F_2N_8O_3$: C: 56.59, H: 6.08, N: 21.12. Found: C: 56.53, H: 6.11, N: 21.09.

*6.7.10. 7-(4-(4,6-Dimorpholino-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**43**)*

Yield: 67%; mp >250 °C; ESMS: 717 (M + 1); IR (KBr): 3047, 2921, 2857, 1729, 1614, 1548, 1442, 1255, 1115 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+DMSO-d_6): δ (ppm) 14.93 (bs, 1H), 8.87 (s, 1H), 7.84 (d, 1H, *J* = 11.74 Hz), 7.55 (d, 2H, *J* = 7.58 Hz), 7.21 (d, 2H, *J* = 7.54 Hz), 5.72 (s, 2H), 3.77 (m, 4H), 3.63 (m, 16H), 3.17 (m, 4H). ^{13}C (75 MHz, $CDCl_3$+DMSO-d_6): 181.15, 171.02, 170.18, 168.17, 156.55, 144.41, 139.19, 135.24, 132.16, 131.13, 125.82, 113.13, 71.46, 55.44, 48.33. Anal. Calc for $C_{33}H_{33}F_5N_8O_5$: C: 55.31, H: 4.64, N: 15.64. Found: C: 55.27, H: 4.59, N: 15.63.

*6.7.11. 7-(4-(4,6-Di(piperidin-1-yl)-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**44**)*

Yield: 68%; mp 242–244 °C; ESMS: 713 (M + 1); IR (KBr): 3049, 2932, 2855, 1721, 1621, 1537, 1440, 1230, 1125 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.54 (bs, 1H), 8.73 (s, 1H), 7.98 (d, 1H, *J* = 11.22 Hz), 7.64 (d, 2H, *J* = 7.82 Hz), 7.24 (d, 2H, *J* = 7.87 Hz), 5.65 (s, 2H), 3.89 (m, 4H), 3.71 (m, 8H), 3.25 (m, 4H), 1.59 (m, 12H). ^{13}C ($CDCl_3$, 50 MHz): 177.81, 176.54, 166.45, 165.82, 165.66, 151.27, 139.46, 127.13, 126.82, 120.89, 109.12, 56.03, 51.43, 47.18, 46.27, 25.73, 23.82. Anal. Calc for $C_{35}H_{37}F_5N_8O_3$: C: 58.98, H: 5.23, N: 15.72. Found: C: 58.97, H: 5.18, N: 15.73.

*6.7.12. 7-(4-(4,6-Bis(4-methylpiperazin-1-yl)-1,3,5-triazin-2-yl) piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**45**)*

Yield: 65%; mp 180–182 °C; ESMS: 743 (M + 1); IR (KBr): 3051, 2933, 2853, 1721, 1620, 1540, 1441, 1276, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.67 (bs, 1H), 8.73 (s, 1H), 7.97 (d, 1H, *J* = 10.71 Hz), 7.63 (d, 2H, *J* = 7.38 Hz), 7.23 (d, 2H, *J* = 7.44 Hz), 5.66

(s, 2H), 3.78 (m, 4H), 3.24 (m, 12H), 2.42 (m, 8H), 2.33 (s, 6H). ^{13}C ($CDCl_3$, 50 MHz): 176.82, 166.76, 165.85, 165.70, 151.55, 139.24, 126.67, 121.87, 108.92, 55.35, 51.23, 46.60, 44.08, 43.38. Anal. Calc for $C_{35}H_{39}F_5N_{10}O_3$: C: 56.60, H: 5.29, N: 18.86. Found: C: 56.52, H: 5.27, N: 18.84.

*6.7.13. 7-(4-(4,6-Bis(2-morpholinoethylamino)-1,3,5-triazin-2-yl) piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**46**)*

Yield: 62%; mp 121–123 °C; ESMS: 803 (M + 1); IR (KBr): 3389, 3051, 2956, 2857, 1727, 1622, 1570, 1479, 1275, 1116 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD, 200 MHz): δ (ppm) 14.47 (bs, 1H), 8.68 (s, 1H), 7.77 (d, 1H, J = 11.32 Hz), 7.42 (d, 2H, J = 7.58 Hz), 7.06 (d, 2H, J = 7.52 Hz), 5.63 (s, 2H), 4.17 (m, 12H), 3.51 (m, 4H), 3.15 (m, 4H), 2.79 (m, 4H), 2.31 (m, 8H). ^{13}C ($CDCl_3$+CD_3OD, 50 MHz): 179.16, 169.32, 166.78, 165.86, 154.27, 140.58, 128.13, 125.76, 112.47, 70.83, 58.63, 57.37, 53.74, 49.17. Anal. Calc for $C_{37}H_{43}F_5N_{10}O_5$: C: 55.36, H: 5.40, N: 17.45. Found: C: 55.34, H: 5.32, N: 17.41.

*6.7.14. 7-(4-(4,6-Bis(3-morpholinopropylamino)-1,3,5-triazin-2-yl) piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**47**)*

Yield: 61%; mp 178–180 °C; ESMS: 831 (M + 1); IR (KBr): 3380, 3054, 2930, 2856, 1726, 1624, 1573, 1477, 1326, 1117 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD, 200 MHz): δ (ppm) 14.76 (bs, 1H), 8.83 (s, 1H), 7.86 (d, 1H, J = 11.58 Hz), 7.54 (d, 2H, J = 7.58 Hz), 7.19 (d, 2H, J = 7.52 Hz), 5.62 (s, 2H), 3.77 (m, 4H), 3.67 (m, 8H), 3.34 (m, 4H), 3.15 (m, 4H), 2.42 (m, 12H), 1.84–1.66 (m, 4H). ^{13}C ($CDCl_3$+CD_3OD, 50 MHz): 179.13, 169.37, 166.73, 166.33, 154.17, 140.21, 126.58, 126.35, 114.54, 67.22, 57.29, 54.03, 53.86, 51.15, 43.99, 26.49. Anal. Calc for $C_{39}H_{47}F_5N_{10}O_5$: C: 56.38, H: 5.70, N: 16.86. Found: C: 56.36, H: 5.65, N: 16.81.

*6.7.15. 7-(4-(4,6-Bis(butylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**48**)*

Yield: 64%; mp 191–193 °C; MS: 689 (M + 1); IR (KBr): 3352, 3049, 2929, 2860, 1728, 1619, 1574, 1480, 1282, 1129 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.63 (bs, 1H), 8.75 (s, 1H), 8.01 (d, 1H, J = 11.48 Hz), 7.64 (d, 2H, J = 7.56 Hz), 7.23 (d, 2H, J = 7.49 Hz), 5.71 (bs, 2H), 5.66 (s, 2H), 3.91 (m, 4H), 3.35 (m, 4H), 3.26 (m, 4H), 1.59–1.52 (m, 4H), 1.25 (m, 4H), 0.98 (t, 6H, J = 6.92 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.73, 166.81, 165.83, 165.65, 151.54, 139.53, 126.89, 126.41, 121.36, 109.16, 56.02, 51.26, 46.39, 42.52, 29.16, 20.43, 14.18. Anal. Calc for $C_{33}H_{37}F_5N_8O_3$: C: 57.55, H: 5.42, N: 16.27. Found: C: 57.51, H: 5.39, N: 16.23.

*6.7.16. 7-(4-(4,6-Bis(tert-butylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**49**)*

Yield: 61%; mp >250 °C; ESMS: 689 (M + 1); IR (KBr): 3358, 3047, 2926, 2858, 1723, 1623, 1581, 1480, 1224, 1123 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.59 (bs, 1H), 8.72 (s, 1H), 7.98 (d, 1H, J = 11.43 Hz), 7.59 (d, 2H, J = 7.49 Hz), 7.21 (d, 2H, J = 7.46 Hz), 5.89 (bs, 2H), 5.68 (s, 2H), 3.82 (m, 4H), 3.31 (m, 4H), 1.34 (s, 18H). ^{13}C (50 MHz, $CDCl_3$): 176.72, 166.78, 165.47, 165.01, 151.57, 139.32, 126.13, 121.62, 108.73, 56.12, 54.29, 51.76, 46.18, 31.84. Anal. Calc for $C_{33}H_{37}F_5N_8O_3$: C: 57.55, H: 5.42, N: 16.27. Found: C: 57.49, H: 5.37, N: 16.26.

*6.7.17. 7-(4-(4,6-Bis(propylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**50**)*

Yield: 63%; mp 224–226 °C; ESMS: 661 (M + 1); IR (KBr): 3374, 3058, 2963, 2851, 1726, 1620, 1534, 1474, 1274, 1123 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.63 (bs, 1H), 8.72 (s, 1H), 7.99 (d, 1H, J = 11.33 Hz), 7.63 (d, 2H, J = 7.42 Hz), 7.24 (d, 2H, J = 7.51 Hz), 5.65 (s, 2H), 4.95 (bs, 2H), 3.85 (m, 4H), 3.28 (m, 8H), 1.57 (m, 4H), 0.93 (t, 6H, J = 7.01 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.74, 166.87, 165.32, 164.97, 151.62, 139.29, 126.68, 121.83, 109.11, 55.89, 51.18, 44.08, 42.93, 23.41, 11.90. Anal. Calc for $C_{31}H_{33}F_5N_8O_3$: C: 56.36, H: 5.03, N: 16.93. Found: C: 56.35, H: 5.01, N: 16.88.

*6.7.18. 7-(4-(4,6-Bis(isopropylamino)-1,3,5-triazin-2-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**51**)*

Yield: 62%; mp 181–183 °C; MS: 661 (M + 1); IR (KBr): 3332, 3057, 2950, 2853, 1719, 1653, 1561, 1457, 1255, 1136 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.61 (bs, 1H), 8.74 (s, 1H), 7.97 (d, 1H, J = 11.66 Hz), 7.63 (d, 2H, J = 7.64 Hz), 7.23 (d, 2H, J = 7.58 Hz), 5.66 (s, 2H), 4.74 (bs, 2H), 4.13–4.09 (m, 2H), 3.84 (m, 4H), 3.23 (m, 4H), 1.18 (d, 12H, J = 5.97 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.14, 166.47, 165.27, 151.53, 139.87, 126.93, 126.45, 121.63, 109.13, 55.27, 51.26, 46.67, 43.02, 23.41. Anal. Calc for $C_{31}H_{33}F_5N_8O_3$: C: 56.36, H: 5.03, N: 16.96. Found: C: 56.33, H: 5.06, N: 16.91.

*6.8. General procedure for the synthesis of compounds **52** and **53***

To a stirred solution compound **10** (1 equiv.) and diisopropylethylamine (DIPEA) (1.5 equiv.) in dry DMF, 2,4-dichloro-6-methylpyrimidine (1 equiv.) was added and allowed to stir at room temperature for 4 h. Reaction mixture was evaporated to dryness, mixed with H_2O and extracted with DCM. The concentrated residue was purified with flash column chromatography using chloroform to methanol gradient elution to afford the intermediate **52**. The same procedure was carried out to obtain the intermediate **53** from the compound **23**.

*6.8.1. 7-(4-(2-Chloro-6-methylpyrimidin-4-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**52**)*

Yield: 68%; mp 238–240 °C; MS: 464 (M + 1); IR (KBr): 3052, 2922, 2850, 1720, 1589, 1479, 1237, 807 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.59 (bs, 1H), 8.62 (s, 1H), 8.01 (d, 1H, J = 11.62 Hz), 6.32 (s, 1H), 4.47 (m, 2H), 3.84 (m, 4H), 3.45 (m, 4H), 2.37 (s, 3H), 1.57 (t, 3H, J = 6.71 Hz). ^{13}C ($CDCl_3$, 50 MHz): 176.72, 168.82, 166.91, 163.92, 161.21, 150.85, 139.14, 108.64, 100.75, 55.28, 50.65, 45.20, 24.51, 16.88. Anal. Calc for $C_{21}H_{20}ClF_2N_5O_3$: C: 54.37, H: 4.35, N: 15.10. Found: C: 54.35, H: 4.28, N: 15.07.

*6.8.2. 7-(4-(2-Chloro-6-methylpyrimidin-4-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**53**)*

Yield: 68%; mp 185–187 °C; ESMS: 594 (M + 1); IR (KBr): 3049, 2924, 2855, 1721, 1592, 1446, 1326, 1127, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.46 (bs, 1H), 8.75 (s, 1H), 8.01 (d, 1H, J = 11.28 Hz), 7.64 (d, 2H, J = 7.81 Hz), 7.23 (d, 1H, J = 7.74 Hz), 6.28 (s, 1H), 5.66 (s, 2H), 3.76 (m, 4H), 3.31 (m, 4H), 2.35 (s, 3H); ^{13}C ($CDCl_3$, 50 MHz): 176.82, 168.70, 166.59, 163.63, 160.78, 151.72, 139.23, 127.72, 126.63, 114.48, 108.93, 100.26, 61.07, 50.71, 45.06, 24.46; Anal. Calc for $C_{27}H_{21}ClF_5N_5O_3$: C: 54.60, H: 3.56, N: 11.79. Found: C: 54.57, H: 3.51, N: 11.72.

*6.9. General procedure for the synthesis of targeted compounds **54–71***

The mixture of compound **52** (1 equiv), respective amines (1.2 equiv) (Table 2) and K_2CO_3 (1.2 equiv) in EtOAc was refluxed for 8 h. The reaction mixture was evaporated to dryness under vacuum. The obtained solid residue was purified with flash column

chromatography using chloroform to methanol gradient elution to obtain the targeted compounds **54**–**62**. The same procedure was carried out to synthesize targeted compounds **63**–**71** from the intermediate **53**.

*6.9.1. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-morpholinopyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**54**)*

Yield: 65%; mp >250 °C; MS: 515 (M + 1); IR (KBr): 3055, 2921, 2851, 1720, 1580, 1474, 1242, 1116 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.66 (bs, 1H), 8.61 (s, 1H), 8.01 (d, 1H, $J = 11.62$ Hz), 5.85 (s, 1H), 4.49 (m, 2H), 4.01–3.77 (m, 8H), 3.58–3.45 (m, 8H), 2.37 (s, 3H), 1.57 (t, 3H, $J = 6.85$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.61, 167.04, 166.92, 163.70, 150.51, 139.47, 114.32, 109.05, 108.53, 92.53, 67.38, 55.26, 51.40, 45.06, 24.51, 16.83. Anal. Calc for $C_{25}H_{28}F_2N_6O_4$: C: 58.36, H: 5.49, N: 16.33. Found: C: 58.29, H: 5.43, N: 16.27.

*6.9.2. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-(piperidin-1-yl) pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**55**)*

Yield: 67%; mp 228–230 °C; MS: 513 (M + 1); IR (KBr): 3055, 2925, 2852, 1721, 1576, 1475, 1276, 1145 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.68 (bs, 1H), 8.61 (s, 1H), 7.99 (dd, 1H, $J = 1.87$, 11.23 Hz), 5.88 (s, 1H), 4.48 (m, 2H), 4.01 (m, 4H), 3.58 (m, 4H), 3.45 (m, 4H), 2.37 (s, 3H), 1.63–1.53 (m, 9H). ^{13}C (50 MHz, $CDCl_3$): 176.64, 167.10, 166.98, 163.44, 150.50, 139.69, 114.48, 108.53, 92.62, 55.26, 51.42, 45.56, 45.08, 26.28, 25.99, 25.15, 16.83. Anal. Calc for $C_{26}H_{30}F_2N_6O_3$: C: 60.93, H: 5.90, N: 16.40. Found: C: 60.87, H: 5.92, N: 16.34.

*6.9.3. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-(4-methylpiperazin-1-yl)pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**56**)*

Yield: 65%; mp 182–184 °C; MS: 528 (M + 1); IR (KBr): 3058, 2942, 2863, 1723, 1568, 1441, 1257, 1128 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 14.68 (bs, 1H), 8.62 (s, 1H), 7.98 (dd, 1H, $J = 1.83$, 11.75 Hz), 5.82 (s, 1H), 4.48 (m, 2H), 3.98 (m, 4H), 3.76 (m, 4H), 3.44 (m, 4H), 2.67 (m, 4H), 2.47 (m, 3H), 2.37 (s, 3H), 1.56 (t, 3H, $J = 6.61$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 176.47, 167.17, 166.98, 163.21, 150.59, 139.84, 114.51, 108.59, 92.65, 55.23, 51.33, 45.99, 45.10, 43.63, 24.42, 16.66. Anal. Calc for $C_{26}H_{31}F_2N_7O_3$: C: 59.19, H: 5.92, N: 18.58. Found: C: 59.21, H: 5.83, N: 18.57.

*6.9.4. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-(2-morpholinoethylamino)pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**57**)*

Yield: 62%; mp 124–126 °C; MS: 558 (M + 1); IR (KBr): 3410, 3052, 2925, 2847, 1726, 1593, 1443, 1246, 1116 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$+CD_3OD): δ (ppm) 14.61 (bs, 1H), 8.64 (s, 1H), 7.84 (d, 1H, $J = 11.23$ Hz), 6.28 (s, 1H), 5.93 (bs, 1H), 4.41 (m, 2H), 3.75–3.64 (m, 8H), 3.35 (m, 6H), 2.53–2.43 (m, 6H), 2.38 (s, 3H), 1.46 (t, 3H, $J = 6.84$ Hz). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 178.13, 171.07, 168.34, 163.58, 160.52, 152.23, 140.72, 132.80, 127.06, 124.57, 108.38, 100.33, 67.07, 55.95, 53.56, 50.81, 45.16, 24.04, 17.82. Anal. Calc for $C_{27}H_{33}F_2N_7O_4$: C: 58.16, H: 5.97, N: 17.58. Found: C: 58.14, H: 5.92, N: 17.53.

*6.9.5. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-(3-morpholinopropylamino)pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**58**)*

Yield: 61%; mp 123–125 °C; MS: 572 (M + 1); IR (KBr): 3439, 3049, 2927, 2851, 1728, 1621, 1590, 1479, 1216, 1118 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.52 (bs, 1H), 8.72 (s, 1H), 7.78 (d, 1H, $J = 11.27$ Hz), 6.29 (s, 1H), 6.13 (bs, 1H), 4.42 (m, 2H), 3.78–3.69 (m, 8H), 3.36 (m, 4H), 3.07 (m, 2H), 2.42 (m, 6H), 2.35 (s, 3H), 1.83 (m, 2H), 1.58 (t, 3H, $J = 6.79$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.39, 168.76, 166.14, 163.28, 151.29, 139.33, 114.47, 109.51, 92.53, 66.91, 56.86, 54.83, 51.27, 46.63, 39.33, 26.19, 24.35, 16.13. Anal. Calc for $C_{28}H_{35}F_2N_7O_4$: C: 58.83, H: 6.17, N: 17.15. Found: C: 58.77, H: 6.13, N: 17.11.

*6.9.6. 7-(4-(2-(Butylamino)-6-methylpyrimidin-4-yl)piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**59**)*

Yield: 68%; mp 231–233 °C; ESMS: 501 (M + 1); IR (KBr): 3405, 3054, 2924, 2852, 1720, 1586, 1476, 1270, 1146 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.68 (bs, 1H), 8.62 (s, 1H), 7.98 (dd, 1H, $J = 1.81$, 11.74 Hz), 6.32 (s, 1H), 5.77 (bs, 1H), 4.48 (m, 2H), 4.02 (m, 4H), 3.45–3.29 (m, 6H), 2.37 (s, 3H), 1.60–1.53 (m, 5H), 1.33–1.25 (m, 2H), 1.01 (t, 3H, $J = 6.72$ Hz); ^{13}C (50 MHz, $CDCl_3$): 176.81, 167.11, 166.87, 163.84, 150.41, 139.72, 114.54, 108.48, 92.30, 55.24, 51.45, 45.10, 30.11, 24.50, 20.55, 16.82, 14.25. Anal. Calc for $C_{25}H_{30}F_2N_6O_3$: C: 59.99, H: 6.04, N: 16.79. Found: C: 59.97, H: 6.02, N: 16.71.

*6.9.7. 7-(4-(2-(Tert-butylamino)-6-methylpyrimidin-4-yl) piperazin-1-yl)-1-ethyl-6,8-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**60**)*

Yield: 61%; mp 219–221 °C; MS: 501 (M + 1); IR (KBr): 3354, 3054, 2927, 2857, 1728, 1573, 1454, 1231, 1117 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.61 (bs, 1H), 8.63 (s, 1H), 7.98 (d, 1H, $J = 11.34$ Hz), 5.89 (s, 1H), 5.77 (bs, 1H), 4.49 (m, 2H), 4.01 (m, 4H), 3.47 (m, 4H), 2.36 (s, 3H), 1.58 (t, 3H, $J = 6.88$ Hz), 1.32 (s, 9H). ^{13}C (50 MHz, $CDCl_3$): 176.57, 166.84, 166.63, 163.51, 151.25, 139.76, 114.52, 109.02, 92.51, 56.89, 55.91, 46.72, 31.44, 24.77, 16.47. Anal. Calc for $C_{25}H_{30}F_2N_6O_3$: C: 59.99, H: 6.04, N: 16.79. Found: C: 59.94, H: 5.96, N: 16.72.

*6.9.8. 1-Ethyl-6,8-difluoro-7-(4-(6-methyl-2-(propylamino) pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**61**)*

Yield: 64%; mp 179–181 °C; MS: 487 (M + 1); IR (KBr): 3427, 3054, 2920, 2851, 1720, 1576, 1476, 1240, 1112 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.69 (bs, 1H), 8.61 (s, 1H), 7.99 (dd, 1H, $J = 1.94$, 11.72 Hz), 5.78 (s, 1H), 5.62 (bs, 1H), 4.48 (m, 2H), 3.97 (m, 4H), 3.79 (m, 2H), 3.44 (m, 4H), 2.37 (s, 3H), 1.68–1.57 (m, 5H), 1.01 (t, 3H, $J = 6.83$ Hz). ^{13}C (50 MHz, $CDCl_3$): 176.65, 166.97, 166.81, 163.49, 150.48, 138.97, 114.73, 109.06, 91.17, 55.23, 51.45, 51.11, 45.03, 24.96, 23.23, 16.83, 11.96. Anal. Calc for $C_{24}H_{28}F_2N_6O_3$: C: 59.25, H: 5.80, N: 17.27. Found: C: 59.21, H: 5.77, N: 17.21.

*6.9.9. 1-Ethyl-6,8-difluoro-7-(4-(2-(isopropylamino)-6-methylpyrimidin-4-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**62**)*

Yield: 64%; mp 176–178 °C; ESMS: 487 (M + 1); IR (KBr): 3438, 3054, 2923, 2853, 1720, 1595, 1476, 1242, 1152 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.46 (bs, 1H), 8.63 (s, 1H), 8.02 (d, 1H, $J = 11.41$ Hz), 6.31 (s, 1H), 5.84 (bs, 1H), 4.49 (m, 2H), 3.97–3.83 (m, 5H), 3.45 (m, 4H), 2.38 (s, 3H), 1.57 (t, 3H, $J = 6.93$ Hz), 1.26 (d, 6H, $J = 5.83$ Hz). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 180.17, 170.63, 166.11, 162.83, 155.17, 141.07, 116.78, 111.23, 103.01, 57.36, 53.31, 47.67, 26.15, 25.50, 16.83. Anal. Calc for $C_{24}H_{28}F_2N_6O_3$: C: 59.25, H: 5.80, N: 17.27. Found: C: 59.17, H: 5.77, N: 17.19.

*6.9.10. 6,8-Difluoro-7-(4-(6-methyl-2-morpholinopyrimidin-4-yl) piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**63**)*

Yield: 66%; mp 149–151 °C; MS: 645 (M + 1); IR (KBr): 3051, 2921, 2853, 1727, 1621, 1581, 1415, 1274, 1118 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.58 (bs, 1H), 8.74 (s, 1H), 8.02 (d, 1H, $J = 11.61$ Hz), 7.63 (d, 2H, $J = 7.37$ Hz), 7.24 (d, 2H, $J = 7.34$ Hz), 5.79

(s, 1H), 5.66 (s, 2H), 3.85–3.57 (m, 8H), 3.29 (m, 4H), 3.18 (m, 4H), 2.25 (s, 3H). ^{13}C (50 MHz, $CDCl_3$): 176.84, 166.58, 162.93, 161.16, 151.47, 139.23, 129.56, 126.20, 108.73, 92.07, 66.93, 61.59, 50.48, 44.46, 24.25. Anal. Calc for $C_{31}H_{29}F_5N_6O_4$: C: 57.76, H: 4.53, N: 13.04. Found: C: 57.75, H: 4.48, N: 12.96.

*6.9.11. 6,8-Difluoro-7-(4-(6-methyl-2-(piperidin-1-yl)pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**64**)*

Yield: 67%; mp 162–164 °C; MS: 643 (M + 1); IR (KBr): 3045, 2928, 2853, 1728, 1619, 1574, 1443, 1240, 1127 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.57 (bs, 1H), 8.74 (s, 1H), 8.01 (d, 1H, J = 11.37 Hz), 7.64 (d, 2H, J = 7.18 Hz), 7.23 (d, 2H, J = 7.29 Hz), 5.79 (s, 1H), 5.66 (s, 2H), 3.86–3.73 (m, 8H), 3.31 (m, 4H), 2.23 (s, 3H), 1.61 (m, 6H). ^{13}C (50 MHz, $CDCl_3$): 176.96, 166.66, 163.54, 161.98, 151.59, 139.20, 126.67, 108.90, 91.42, 61.73, 50.96, 45.47, 44.91, 26.28, 25.38, 24.91. Anal. Calc for $C_{32}H_{31}F_5N_6O_3$: C: 59.81, H: 4.86, N: 13.08. Found: C: 59.74, H: 4.83, N: 13.02.

*6.9.12. 6,8-Difluoro-7-(4-(6-methyl-2-(4-methylpiperazin-1-yl) pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**65**)*

Yield: 64%; mp 168–170 °C; ESMS: 658 (M + 1); IR (KBr): 3059, 2928, 2852, 1725, 1622, 1582, 1474, 1276, 1121 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD): δ (ppm) 14.61 (bs, 1H), 8.79 (s, 1H), 8.03 (d, 1H, J = 11.26 Hz), 7.51 (d, 2H, J = 7.19 Hz), 7.18 (d, 2H, J = 7.22 Hz), 5.73 (s, 1H), 5.62 (s, 2H), 3.99 (m, 4H), 3.28 (m, 4H), 3.18 (m, 4H), 2.56 (m, 4H), 2.36 (s, 3H), 2.29 (s, 3H). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 181.07, 169.40, 166.52, 164.60, 154.16, 143.31, 130.15, 129.82, 112.13, 94.67, 58.14, 55.60, 48.61, 48.36, 46.67, 27.54. Anal. Calc for $C_{32}H_{32}F_5N_7O_3$: C: 58.44, H: 4.90, N: 14.91. Found: C: 58.37, H: 4.84, N: 14.87.

*6.9.13. 6,8-Difluoro-7-(4-(6-methyl-2-(2-morpholinoethylamino) pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**66**)*

Yield: 61%; mp 132–134 °C; ESMS: 688 (M + 1); IR (KBr): 3424, 3020, 2931, 2851, 1729, 1622, 1590, 1478, 1216, 1122 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD): δ (ppm) 14.46 (bs, 1H), 8.78 (s, 1H), 7.84 (d, 1H, J = 11.23 Hz), 7.48 (d, 2H, J = 7.11 Hz), 7.13 (d, 2H, J = 7.23 Hz), 6.22 (s, 1H), 5.83 (bs, 1H), 5.59 (s, 2H), 3.99 (m, 8H), 3.20 (m, 6H), 2.44–2.38 (m, 6H), 2.33 (s, 3H). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 180.14, 171.46, 167.08, 163.61, 161.54, 154.17, 141.71, 130.15, 129.76, 112.17, 104.07, 70.51, 58.74, 57.20, 54.14, 26.72. Anal. Calc for $C_{33}H_{34}F_5N_7O_4$: C: 57.64, H: 4.98, N: 14.26. Found: C: 57.62, H: 4.93, N: 14.21.

*6.9.14. 6,8-Difluoro-7-(4-(6-methyl-2-(3-morpholinopropylamino) pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**67**)*

Yield: 61%; mp 135–137 °C; ESMS: 702 (M + 1); IR (KBr): 3404, 3019, 2959, 2847, 1725, 1662, 1588, 1477, 1217, 1119 cm^{-1}; ^{1}H NMR ($CDCl_3$+CD_3OD, 200 MHz): δ (ppm) 14.21 (bs, 1H), 8.75 (s, 1H), 7.85 (d, 1H, J = 11.27 Hz), 7.48 (d, 2H, J = 7.28 Hz), 7.12 (d, 2H, J = 7.29 Hz), 6.23 (bs, 1H), 5.68 (s, 1H), 5.58 (s, 2H), 3.86 (m, 8H), 3.14 (m, 6H), 2.39–2.27 (m, 9H), 1.74–1.68 (m, 2H). ^{13}C ($CDCl_3$+CD_3OD, 50 MHz): 180.73, 169.17, 166.34, 164.52, 154.76, 143.17, 129.37, 128.96, 125.03, 113.62, 94.86, 70.26, 58.34, 55.72, 53.17, 50.43, 43.23, 29.83, 27.96. Anal. Calc for $C_{34}H_{36}F_5N_7O_4$: C: 58.20, H: 5.17, N: 13.97. Found: C: 58.11, H:5.15, N: 13.89.

*6.9.15. 7-(4-(2-(Butylamino)-6-methylpyrimidin-4-yl)piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**68**)*

Yield: 66%; mp 148–150 °C; MS: 631 (M + 1); IR (KBr): 3412, 3059, 2921, 2857, 1727, 1619, 1595, 1449, 1236, 1122 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.42 (bs, 1H), 8.75 (s, 1H), 8.02 (d, 1H, J = 11.32 Hz), 7.64 (d, 2H, J = 7.26 Hz), 7.24 (d, 2H, J = 7.31 Hz), 6.28 (s, 1H), 5.83 (bs, 1H), 5.67 (s, 2H), 3.76 (m, 4H), 3.31 (m, 6H), 2.25 (s, 3H), 1.67 (m, 2H), 1.25 (m, 2H), 0.89 (t, 3H, J = 7.21 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.68, 168.59, 166.39, 163.54, 160.67, 151.59, 139.01, 127.58, 126.64, 121.23, 109.19, 100.14, 61.68, 50.60, 44.88, 29.98, 24.47, 14.04. Anal. Calc for $C_{31}H_{31}F_5N_6O_3$: C: 59.04, H: 4.95, N: 13.33. Found: C: 59.01, H: 4.88, N: 13.21.

*6.9.16. 7-(4-(2-(Tert-butylamino)-6-methylpyrimidin-4-yl) piperazin-1-yl)-6,8-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**69**)*

Yield: 60%; mp 191–193 °C; ESMS: 631 (M + 1); IR (KBr): 3424, 3051, 2922, 2853, 1723, 1627, 1594, 1448, 1235, 1121 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.58 (bs, 1H), 8.77 (s, 1H), 8.02 (d, 1H, J = 11.27 Hz), 7.61 (d, 2H, J = 7.22 Hz), 7.23 (d, 2H, J = 7.18 Hz), 6.28 (s, 1H), 5.98 (bs, 1H), 5.68 (s, 2H), 3.75 (m, 4H), 3.30 (m, 4H), 2.27 (s, 3H), 1.32 (s, 9H). ^{13}C (50 MHz, $CDCl_3$): 176.54, 168.69, 166.27, 163.62, 160.76, 151.57, 139.24, 126.64, 108.97, 100.26, 61.53, 54.19, 50.71, 44.99, 32.87, 24.48. Anal. Calc for $C_{31}H_{31}F_5N_6O_3$: C: 59.04, H: 4.95, N: 13.33. Found: C: 58.98, H: 4.91, N: 13.27.

*6.9.17. 6,8-Difluoro-7-(4-(6-methyl-2-(propylamino)pyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**70**)*

Yield: 63%; mp 188–190 °C; ESMS: 617 (M + 1); IR (KBr): 3380, 3053, 2923, 2857, 1723, 1594, 1447, 1235, 1122 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 14.45 (bs, 1H), 8.75 (s, 1H), 8.01 (d, 1H, J = 11.35 Hz), 7.63 (d, 2H, J = 7.26 Hz), 7.23 (d, 2H, J = 7.32 Hz), 6.28 (s, 1H), 5.97 (bs, 1H), 5.67 (s, 2H), 3.92 (m, 4H), 3.31 (m, 6H), 2.35 (s, 3H), 1.96 (m, 2H), 0.88 (t, 3H, J = 6.86 Hz). ^{13}C (50 MHz, $CDCl_3$): 176.62, 168.72, 166.53, 163.64, 160.81, 151.71, 139.27, 126.62, 108.96, 100.24, 61.28, 50.71, 45.03, 30.10, 24.46, 14.17. Anal. Calc for $C_{30}H_{29}F_5N_6O_3$: C: 58.44, H: 4.74, N: 13.63. Found: C: 58.41, H: 4.68, N: 13.56.

*6.9.18. 6,8-Difluoro-7-(4-(2-(isopropylamino)-6-methylpyrimidin-4-yl)piperazin-1-yl)-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylic acid (**71**)*

Yield: 65%; mp >250 °C; ESMS: 617 (M + 1); IR (KBr): 3378, 3053, 2922, 2856, 1729, 1592, 1479, 1275, 1122 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD): δ (ppm) 14.89 (bs, 1H), 9.08 (s, 1H), 7.89 (d, 1H, J = 11.13 Hz), 7.38 (d, 2H, J = 7.36 Hz), 7.11 (d, 2H, J = 7.32 Hz), 6.23 (s, 1H), 5.64 (s, 2H), 3.65 (m, 5H), 3.16 (m, 4H), 2.25 (s, 3H), 1.17 (d, 6H, J = 6.01 Hz). ^{13}C (50 MHz, $CDCl_3$+CD_3OD): 178.18, 167.46, 164.30, 163.93, 152.16, 140.24, 130.61, 130.07, 116.92, 104.31, 64.36, 54.53, 48.93, 44.03, 27.70. Anal. Calc for $C_{30}H_{29}F_5N_6O_3$: C: 58.44, H: 4.74, N: 13.63. Found: C: 58.37, H: 4.68, N: 13.61.

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