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Fluorine Walk: The Impact of Fluorine in Quinolone Amides on their Activity Against African Sleeping Sickness

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Abstract

Human African Trypanosomiasis, also known as African sleeping sickness, is caused by the parasitic protozoa of the genus *Trypanosoma*. If there is no pharmacological intervention, the parasites can cross the blood-brain barrier (BBB), inevitably leading to death of the patients. Previous investigation identified the quinolone amide **GHQ168** as a promising lead compound having a nanomolar activity against *T. b. brucei*. Here, the role of a fluorine substitution at different positions was investigated in regard to toxicity, pharmacokinetics, and antitrypanosomal activity. This 'fluorine walk' led to new compounds with improved metabolic stability and consistent activity against *T. b. brucei*. The ability of the new quinolone amides to cross the BBB was confirmed using an ^{18}F-labelled quinolone amide derivative by means of *ex vivo* autoradiography of a murine brain.

Keywords: quinolone amides, *Trypanosoma brucei brucei*, structure-activity relationship, fluorine walk, metabolism, blood-brain barrier, autoradiography

1 Introduction

Human African Trypanosomiasis (HAT) is a serious life threatening disease occurring in 36 African countries and is caused by the vector-borne parasites *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*) or *Trypanosoma brucei gambiense* (*T. b. gambiense),* respectively [1]. HAT can be differentiated into two clinically relevant stages: stage 1 is characterized by unspecific headache, fever, and pruritus [2]. In stage 2, parasites have crossed the blood-brain barrier (BBB) and invaded the central nervous system (CNS), causing confusion, sensory impairments, and the eponymous sleep disturbances. Untreated patients finally progress to coma, systemic organic failure, and inevitable to death [3]. Among available drugs, there are only three applicable compounds, i.e. melarsoprol, eflornithine, and nifurtimox which is also active against stage 2 of HAT [4]. Additionally, the current chemotherapy suffers from severe side effects, requires an intravenous or intramuscular administration, and has a limited efficacy targeting only one of the *Trypanosoma* subtypes. E.g., an intravenous infusion of melarsoprol being administered over a long time period is the only effective drug against *T. b. rhodesiense* being able to pass the BBB [5]. Moreover, the accompanying side effects of the toxic arsenic are severe, resulting in an encephalic reaction in approximately 10% of the treated patients and being lethal in 50% of the cases [6]. Taken together, there is an urgent need for novel and safe antitrypanosomal drug candidates with CNS permeability that allow application in both stages 1 and 2 of HAT. Hence, we recently established quinolone amides that are active against *T. b. brucei* in the nanomolar concentration range and possess *in vivo* efficacy [7, 8].

Generally, in medicinal chemistry the incorporation of a fluorine atom in molecules can influence conformation, pK_a value, intrinsic potency, membrane permeability, metabolic stability, as well as pharmacokinetics [9-11]. The systematic ‘walk’ of fluorine around the quinolone scaffold was already applied successfully for quinolones addressing the M_1 acetylcholine receptor [12]. Additionally, the ^{18}F isotope of fluorine can be deployed for labelling, and subsequent positron-emission tomography (PET) and autoradiography, respectively [9]. The impact of different substitution patterns, particularly of fluorine, on the quinolone amide skeleton is explored herein. The aim of this ‘fluorine walk’ was to enhance the antitrypanosomal potency and to expand the structure-activity relationship. As lipophilicity and metabolic stability are strongly affected by fluorine, these parameters were studied simultaneously. The most potent drug candidate **GHQ168** was used for optimization with regard to activity, cytotoxicity, metabolism, and lipophilicity and solubility.

The ability of the quinolone amide to pass the BBB was investigated by means of *ex vivo* autoradiography. Therefore, the quinolone amide **GHQ168** was radiofluorinated with ^{18}F and injected intravenously into mice for the respective studies.

2 Results and Discussions

2.1 Chemistry

Starting off with the corresponding aniline derivatives, the quinolone scaffolds **1a-h** were formed via Gould-Jacobs procedure (cf. Scheme 1) [13]. Subsequently, the *N*-1 position was deprotonated utilizing potassium carbonate followed by alkylation using alkyl halides (*n*-bromobutane (**A**) and benzyl-protected 3-bromopropan-1-ol (**B**)) in the presence of catalytic amounts of potassium iodine; compounds **2a-j** were obtained [14]. Compound **2j** was synthesized using non-freshly distilled *N*,*N*-dimethylformamide which contains residual amounts of dimethyl amine as a degradation product. The amination of position 5 via a nucleophilic aromatic substitution proceeded due to stabilisation effects of the C-4 oxo group [15, 16]. Without preceding characterisation of the ethyl esters **2a-j**, the products **2a-j** were hydrolysed to yield the carboxylic acid derivatives **3a-j**. Depending on the respective substitution pattern and leaving group, either 2 M HCl (compounds **2c-f**, **2h**, **2j**) or 3 M KOH (compounds **2a**, **2b**, **2g**, **2i**) was used for hydrolysis of the ethyl esters. Position 5 of compound **3d** was further utilized for introducing a methoxy group into the quinolone scaffold, obtaining compound **3k**.

Position 7 of the quinolone scaffold was substituted with morpholine, resulting in compounds **4a-k** [7]. In order to favour a substitution in position 7 over position 5, position 7 of compounds **3d** and **3f** was activated by means of a borate complex according to ref. [15, 17] (cf. Scheme 1). To this end, compounds **3d**, **3f**, and boron triflouride diethyl etherate were dissolved in CH_2Cl_2 and heated to 50 °C yielding the boron-chelated intermediate which was subsequently refluxed in morpholine for 4 h at 80 °C to give **C1** and **C2**. The ensuing hydrolysis of the boron ester with 2 M NaOH afforded compounds **4d** and **4f** with a regioselective substitution in position 7.

Finally, the amidation step was carried out after generating the anhydride derivatives from the corresponding carboxylic acids *in-situ* which were subsequently reacted with the benzyl amines to generate the quinolone amides **5-18** [7].

The benzyl protected alcohol group of **12** was cleaved using microwave irradiation and catalytical amounts of Pd/C suspended in $CHCl_3$ (cf. Scheme 2). The resulting compound **19**

was treated with *N*,*N*-diethylaminosulfur trifluoride (DAST) to give the monofluoroalkyl derivative **21** as a reference standard for the corresponding labelled [^{18}F]**21**. For the synthesis of the precursor **20**, compound **19** was treated with methanesulfonyl chloride, attaching a mesylate ester to the terminal alcohol residue. The mesylated precursor **20** was radiofluorinated via nucleophilic substitution. The crude labelled product was purified by means of radio-HPLC and a tC_{18} cartridge to give [^{18}F]**21** in 60 ± 5% radiochemical yield (RCY). The total synthesis time was 50 min including purification and formulation with 10% EtOH/saline solution. The identity and radiochemical purity of the radiotracer [^{18}F]**21** were confirmed by co-injection with the corresponding standard **21** using radio-HPLC (cf. Fig. S7).

2.2 Structure-activity relationship – the fluorine walk

The *in vitro* antitrypanosomal activities were demonstrated by means of the trypomastigote forms of *T. b. brucei* laboratory strain TC 221 using the AlarmarBlue® assay and photometric measurement of the viability [7, 18-20]. The cytotoxicity was evaluated on the viability of macrophage cell line J774.1 [8, 20, 21]. The *in vitro* results are summarized in Table 1.

The quinolone amide **GHQ168** carrying a fluorine in position 6 showed a high activity against *T. b. brucei* (IC_{50} = 47 nM), *T. b. rhodesiense* (IC_{50} = 9 nM), and a moderate cytotoxicity (CC_{50} = 57 µM) [7]. Here, the chemical strategy and the SAR analysis targeted the antitrypanosomal improvement particularly by varying the fluorine substitution pattern.

Quinolone Core. *Desfluoroquinolone*. The development of commercially available antibacterial quinolones started with non-fluorinated quinolones (e.g., nalidixic acid) and proceeded with 6-fluoro substituted compounds (e.g., ciprofloxacin), but none of them showed any antitrypanosomal activity [22]. Fluorine is generally used as a bioisosteric interchange for hydrogen, as demonstrated herein. However, size and electronic effects of the two atoms are fairly different. The fluorine atom is approximately 20% larger than hydrogen (van der Waals radius 1.20 Å vs 1.47 Å) and their electronegativities differ in 1.78 resulting in a highly polarised C-F bond [23]. Since the target site of the quinolone amides is not yet elucidated, the impact of fluorine towards this compound class is not understood to date. Hence, despite of the marked differences between hydrogen and fluorine, it was worthwhile to investigate a quinolone core that lacks this particular electron withdrawing element. The antitrypanosomal activity of the des-fluoroquinolone **5** was decreased by a factor of five (IC_{50} = 230 nM for **5**) indicating the considerable impact of fluorine on the activity. However, the CC_{50} was higher than 100 µM.

Shifting Fluorine. Relocation of the fluorine atom from position 6 to 8 (cf. compound **6**) resulted in a decreased antitrypanosomal activity (IC_{50} = 790 nM). Fluorine in position 8 apparently could not compensate the missing fluorine atom in position 6 and additionally negatively influenced the activity against trypanosomes comparing to the desfluorquinolone **5**. When shifting fluorine from position 6 to 5 (cf. compound **7**), the trypanocidal activity was comparable to **GHQ168** (IC_{50} = 50 nM for **7**) and interestingly, no cytotoxic effects were observed up to concentration levels of 100 µM. Thus, **7** surpassed the selectivity index (SI = CC_{50}/IC_{50}) of the lead compound and was 2000 times more selective towards *T. b. brucei* parasites. Hence, the 5-fluoro quinolone core was a superior scaffold possessing promising biological properties.

Additional Fluorine. Inserting an additional fluorine in position 8 of some approved fluoroquinolones results in a very high antibacterial activity, whereas the corresponding amide **8** did not benefit from a double fluorination with the antitrypanosomal activity remaining the same (IC_{50} = 60 nM). This supported the initially assertion that the fluorine in position 8 did not positively influence the antitrypanosomal activity. Introducing an extra fluorine in position 5 slightly affected the trypanocidal activity of compound **9** (IC_{50} = 40 nM). Thus, fluorine in position 5 was assigned a more important role since it was at least well-tolerated or even advantageous (cf. compounds **7, 9**).

Fluorine Replacement. The methoxy moiety demonstrated bioisosteric properties to fluorine in thrombin inhibitors [11, 24] and in the drug ezetimibe [25, 26]. Beside the difference in size, both groups (C-F and C-O) are hydrogen bond acceptors, even though fluorine possesses considerably less proton affinity [11]. Accordingly, we explored a methoxy group in position 6 (cf. compound **10**) leading to a loss in activity by a factor of nearly seven (IC_{50} = 310 nM for **10**), while the cytotoxicity remained in a range comparable to **GHQ168**. Thus, a more stronger hydrogen bond acceptor in position 6 was not beneficial. Afterwards, evaluating the influence of an even more voluminous residue the fluorine atom was replaced by a trifluoromethyl group (van der Waal radius 2.12 Å; cf. compound **11**) which is apparently similar to an isopropyl and an ethyl group, respectively [11, 23]. The antitrypanosomal activity of 6-CF_3-substituted **11** was diminished to an IC_{50} value of 540 nM and a CC_{50} value of 78.6 µM. Hence, this particular bulky and rather lipophilic substituent did not enhance the antitrypanosomal activity.

Since the 5-fluorine quinolone core (cf. compound **7**) exhibited high antitrypanosomal activity the compatibility of further substituents in position 5 was investigated. Compounds **13** and **14**, having a dimethylamine and a methoxy group in position 5, respectively, possessed a strongly

reduced antitrypanosomal activity (IC_{50} = 1.85 µM and 0.76 µM, respectively). The decreased biological activity might be attributed to the poor tolerance towards sterically demanding residues in position 5. Nevertheless, **14** was superior to **13** because of its slightly reduced cytotoxicity (CC_{50} = 51.8 µM vs. 44.0 µM), and thus higher selectivity (SI = 68 vs. 24).

Benzylamide Residue. Compound **15** having a *p*-fluorine substituent exhibited a high activity against *T. b. brucei* (IC_{50} = 50 nM). Additionally, compound **16** bearing a fluorine in ortho position exhibited only one-eighth of the antitrypanosomal activity of **GHQ168** (IC_{50} = 410 nM for **16**), and a moderate cytotoxicity (CC_{50} = 37.0 µM). When occupying both para and ortho positions with fluorine (cf. compound **17**), the activity could be restored again and was even slightly improved (IC_{50} = 30 nM, CC_{50} = 59.6 µM).

Compound **18** combined the superior fluorine substitution pattern of the quinolone core in position 5 and the fluorination of the benzylamide residue in para position, subsequently leading to the most active substance with an IC_{50} value of 20 nM and a CC_{50} value higher than 25 µM (SI = > 1250).

N-1 Alkyl Residue. Compound **19**, having a terminal alkyl hydroxyl group, merely possessed approximately one fifth of the trypanocidal activity of **GHQ168**. Neither the hydrogen bond acceptor nor the donor properties of the hydroxyl moiety positively affect the biological activity. Compound **21** carrying a terminal fluorine was only as half effective as the lead compound. The respective activities of 270 nM and 120 nM were still in the submicromolar concentration range. Additionally, both analogues exhibited a moderate cytotoxicity (CC_{50} = 43.1 µM and 42.4 µM, respectively).

2.3 Metabolism

2.3.1 Metabolites of **7, 15, 18**

For newly developed drugs the metabolism and the metabolites are important to know, because these compounds can be harmful to the organism, and may influence the pharmacokinetics. Therefore, the phase-I-metabolism of **GHQ168** was investigated [23] using microsomes from rat male liver (induced by phenobarbital and ß-naphthoflavon) and cytosol from rat male liver (induced by aroclor 1254). These studies were carried out as described by Gareis [23] and were applied here, accordingly.

For compound **7**, eight metabolites could be identified by means of LC/MSD ion trap (cf. Scheme 3 and Fig. S3). The m/z value of 454 hints to two different kinds of hydroxylation: the first one at the *N*-benzyl substituent, and the second one at the *N*-alkyl chain. The structures of both metabolites could be confirmed by LC-MS/MS fragmentation due to the

corresponding *N*-debenzylation resulting in m/z values of 348 and 364, respectively. The metabolite with m/z = 442 can be explained by a double hydroxylation of the benzyl moiety and an oxidative *N*-desalkylation, the biotransformation to m/z = 349 indicates an amide hydrolysis, and m/z = 306 hints at an oxidative *N*-desalkylation in combination with an amide hydrolysis. Finally, two more metabolites (m/z = 426 and 412) were identified where hydroxylation and desalkylation are very likely. In conclusion, the metabolites were mainly produced by aromatic and aliphatic hydroxylation as well as oxidative *N*-desalkylation and amide hydrolysis combined with hydroxylation. Mainly, metabolites by hydroxylation are formed.

The metabolites of compounds **15** and **18** are similar; they were predominantly metabolized via an *N*-desalkylation reaction. As expected, a hydroxylation of the benzyl substituent was not observed due to the fluorine at the phenyl ring, but a hydroxylation at the *N*-alkyl chain, *N*-desalkylation, amide hydrolysis, and a combination of hydroxylation and *N*-desalkylation were found for both compounds. The assignment of the metabolites was achieved in analogy to references [8][20]. Details can be found in Figures S4 and S5.

2.3.2 Metabolic turnover

Blocking metabolically reactive sites by fluorine substituents is a well-established method [1]. Since fluorination enhances the metabolic stability, the benzyl amide moiety of **GHQ168** ($t_{1/2}$ = 5.8 h [20]), which was prone to metabolism (hydroxylation), was fluorinated. The electron withdrawing effect of fluorine should inactivate the benzyl residue for oxidative metabolic processes and enhance the compound's stability. Indeed, **15** was less prone to metabolism ($t_{1/2}$ = 6.4 h). Interestingly, compound **7** ($t_{1/2}$ = 7.2 h) with fluorine in position 5 exhibited an even more metabolic stability than **15**. Consequently, the combined feature of fluorine in position 5 and a para fluorinated benzyl residue (**18**, $t_{1/2}$ = 7.7 h) is the most stabile substitution pattern.

The highest proportion of metabolites was formed in the cytosol, indicating that aldehyde dehydrogenases and monoamine oxidases might be mainly responsible for the metabolic transformation. Furthermore, in-depth investigations of the quinolone amides turnover in the cytosol revealed compound **18** (cf. Table 2) to exhibit the lowest turnover in this environment (3.89 ± 0.29 pmol×min^{-1}×mg×proteine^{-1}). These findings substantiated the outcome of an enhanced metabolic stability of the quinolone amides in the order **GHQ168** < **15** < **7** < **18**.

2.4 Lipophilicity and solubility

The lipophilicity, represented as logP value, was determined by means of an HPLC method. The logarithmized capacity factor of the calibration substances was correlated with the experimental octanol/water logP values (cf. Fig. S2) [7, 27]. Since the lipophilicity strongly influences permeation and solubility processes, it can be regarded as a surrogate parameter for predicting oral bioavailability; a logP value lower than 5 is considered more desirable [28].

In general, additional fluorine substituents at an aromatic ring system increase lipophilicity due to good overlapping orbitals which holds true for **8** and **15** (logP = 4.57 and 4.13) (cf. Table 1) [29]. Shifting the fluorine atom from position 6 to 5 of the quinolone scaffold (cf. compound **7**) significantly and unexpectedly reduced the lipophilicity (logP = 3.36). This effect could possibly emerged due to the polarization of the carbonyl oxygen atom in position 4 by the fluorine atom in close proximity [30]. Consequently, the surrounding water molecules might form more stronger hydrogen bonds with this oxygen atom. Additionally, the fluorine in vicinity to the oxygen could increase the overall polarity of the quinolone molecule [30]. Hence, in contrary to the general rule the double fluorinated compounds **9** and **18** possessed a lower logP value (logP = 3.97 and 3.41, respectively) than the mono fluorinated **GHQ168**. Adding a fluorine substituent to a saturated alkyl chain could rather lead to a reduced logP, consequently compound **21** has a lower logP value of 3.34 [29]. Additionally, compared to the lead compound **GHQ168** (logP = 4.10) the logP could be decreased to 3.04 by introducing a hydrophilic hydroxyl group at the *N*-1 residue (cf. compound **19**).

Compound **GHQ168** possessed a low thermodynamic solubility of 0.005 µg/mL in PBS buffer [8]. Therefore, the solubility of certain quinolone amides (**5-7**, **10**, **19**, **21**) was examined applying the shake flask method in accordance to reference [8]. In general, solubility could be moderately improved for compounds **5-7**, and **21** (S_w = 0.12-1.36 µg/mL; cf. Fig. S6). However, the solubility of compounds **10** and **19** was remarkably enhanced (S_w = 2.73 µg/mL and 18.34 µg/mL, respectively; cf. Fig. S6). As indicated by the logP value of **19**, water solubility could be substantially improved by the introduction of polar groups, e.g. a hydroxy group (cf. compound **19**, logP = 3.04).

2.5 Radiochemistry and Autoradiography

The positron emitter radionuclide F-18 exhibits good radionuclear properties with low positron energy ($E_{\beta max}$ = 0.635 MeV) and a suitable half-life time ($t_{1/2}$) of 109 min. Since most commercially available antibacterial quinolones originally possess a fluorine atom, a direct nucleophilic displacement of F-19 to F-18 for lemofloxacin and trovafloxacin via isotopic

exchange was reported [31, 32]. However, a direct nucleophilic exchange in position 6 through a nucleophilic aromatic substitution was described not to occur [33]. Thus, an appropriate leaving group, i.e., a mesylate group, was attached to the quinolone amide at the *N*-hydroxyalkyl substituent (**19**). F-18 labelling was conveniently applied as the very last step (cf. Scheme 2), resulting in compound **21**.

Since the delivery to the brain is crucial for the treatment of stage 2 of HAT, the permeation of the quinolone amide was evaluated by means of autoradiography. The labelled compound [^{18}F]**21** was injected into a mouse tail vein and the mouse was sacrificed 60 min afterwards. The murine brain was dissected and the ex vivo autoradiography illustrated the distribution of compound [^{18}F]**21** within the brain tissue (cf. Figure 1 and Fig S8). The compound accumulated within the entire brain in medium concentration levels (indicated by green), combined with areas of high concentration levels in the inner brain sections (indicated by red). The autographic images confirmed the uptake of the quinolone amides in healthy murine brain.

3 Conclusion

The systematic walk of fluorine around the scaffold of the lead compound **GHQ168** was successfully implemented. Compound **18** with a striking antitrypanosomal activity and moderate cytotoxicity (IC_{50} = 20 nM against *T. b. brucei*, CC_{50} = >25 µM) was revealed. Hence, the biological activity against trypanosomes was enhanced and the 5-fluoro-substituted quinolone is considered superior to the lead compound. Its logP value suggested a moderate water solubility, but the aqueous solubility was determined at 0.12 µg/mL, though. Additionally, the influence of fluorine substitution patterns on metabolic stability was examined. The most potent compound **18** ($t_{1/2}$ = 7.7 h) showed an extension of the metabolic half-live of 25% in comparison to **GHQ168** ($t_{1/2}$ = 5.8 h[20]), resulting in a longer drug exposure to the trypanosomes.

Moreover, the permeation of quinolone amides through the murine BBB could be proved using the [^{18}F]-labelled derivative [^{18}F]**21**. Therefore, this compound class could be suitable for treatment of HAT stage 2, when parasites have affected CNS.

4 Experimental Section

4.1 Chemistry

Microwave reactions were performed using synthWAVE and rotaPREP systems (both MLS, Leutkirch, Germany). Melting points were determined with a capillary melting point apparatus (Sanyo Gallenkamp, Leicestershire, UK) and were not corrected. IR spectra were recorded on a JASCO FT-IR-6100 spectrometer (Jasco, Groß-Umstadt, Germany). Thin layer chromatography (TLC) was performed on pre-coated silica gel (UV_{254}) glass plates (Macherey-Nagel, Düren, Germany). Column chromatography was performed using silica gel with a particle size of 0.063-0.200 mm (Merck, Darmstadt, Germany). ^{1}H (400.131 MHz) and ^{13}C (100.623 MHz) Nuclear magnetic resonance (NMR) spectra were recorded using a Bruker AV 400 NMR spectrometer (Bruker Biospin, Ettlingen, Germany). The signals of the deuterated solvents were used as an internal standard (DMSO-d_6: ^{1}H 2.50 ppm, ^{13}C 39.43; $CDCl_3$: ^{1}H 7.26 ppm, ^{13}C 77.00). ^{19}F NMR spectra were recorded on a Bruker 400 NMR spectrometer. NMR data are presented with chemicals shifts in ppm, the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; m, multiplet; dd, doublet of doublet), and coupling constants *J* given in Hz. The electron spray mass spectra were acquired on a Shimadzu LCMS 2020 instrument. Reagents were purchased with a minimum purity of 95% from conventional commercial suppliers and were used without further purification. The purity of target compounds was ≥95% and was confirmed by means of HPLC analysis using a Synergi 4µ fusion-RP column (150 mm × 4.6 mm) (Phenomenex, Aschaffenburg, Germany), a Shimadzu instrument (Kyoto, Japan) equipped with an SPD-20A UV/Vis detector (λ = 254 nm), and a mobile phase being a mixture of water (A) and methanol (B); gradient elution programme: 5% B → 90% B from 0 to 8 min; 90% B from 8 to 13 min; 90% B → 5% B from 13 to 15 min; 5% B from 15 to 18 min. The flow rate was adjusted to 1 mL/min. [^{18}F]Fluoride was produced on the PETtrace® cyclotron (GE Medical Systems, Uppsala, Finland) at the interdisciplinary PET centre of the University of Würzburg via an ^{18}O(p,n)^{18}F reaction by irradiating 3.0 mL of 95% enriched [^{18}O]water with 16.5 MeV protons.

4.1.1 *General synthesis of 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids* ***3a-i*** *according to [7, 14]*. The appropriate ethyl-4-hydroxyquinoline-3-carboxylate **1a-h** (1 eq) (cf. Fig. S1, synthesised according to ref [7, 14, 34-38]) and potassium carbonate (4 eq) were suspended in *N*,*N*-dimethylformamide under Ar atmosphere. The reaction was heated 30 min at 60 °C, followed by adding a catalytic amount of potassium iodide and the appropriate alkyl halide (1.5-5 eq). After 20-48 h of heating at 75-90 °C, the solvent was removed *in vacuo* and

water was added. The aqueous layer was extracted with EtOAc and the combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed *in vacuo* and the oily residue was subsequently purified by column chromatography utilizing silica gel. Without further characterization, the resulting products **2a-2i** were hydrolysed using either 2 M HCl or 3 M KOH at 100 °C. If necessary, the solution was acidified to pH 2 and the precipitated product was collected. Afterwards, the solid was washed with cold water and dried *in vacuo* to give compounds **3a-i**.

4.1.1.1 *1-Butyl-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3a**).* The compound was synthesised according to the general procedure described in 4.1.1. Spectroscopic data are in accordance with reference [7].

4.1.1.2 *7-Bromo-1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3b**).* According to the general procedure 4.1.1, a solution of compound **1b** (1 eq, 3.2 g, 10.8 mmol) and potassium carbonate (4 eq, 6.0 g, 43.2 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 5.8 mL, 54.0 mmol), and the reaction was heated at 85 °C for 24 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/*i*-PrOH = 75:1, R_f = 0.62) and then hydrolysed by refluxing compound **2b** under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield 2.38 g of **3b**. Yield: 68%; mp 224–225 °C. IR [cm^{-1}]: 3144, 3084, 2976, 2904, 1695, 1607, 1523, 1458, 1374, 1192, 1069. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 14.94 (s, 1H), 9.02 (s, 1H), 8.29 (m, 3H), 8.57 (dd, ^3J = 8.8, ^4J = 1.2, 1H), 4.57 (t, ^3J = 7.2, 2H), 1.74 (quint, ^3J = 7.2, 2H), 1.35 (sext, ^3J = 7.6, 2H), 0.93 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 177.7, 165.6, 150.0, 140.0, 129.4, 128.3, 127.8, 124.5, 120.5, 107.9, 53.6, 30.6, 18.9, 13.4.

4.1.1.3 *1-Butyl-7-chloro-8-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3c**).* According to the general procedure 4.1.1, a solution of compound **1c** (1 eq, 3.00 g, 11.1 mmol) and potassium carbonate (4 eq, 6.15 g, 44.5 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 6.0 mL, 55.6 mmol), and the reaction was heated at 80 °C for 48 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.72) and then hydrolysed by refluxing compound **2c** under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield 1.80 g of **3c**. Yield: 55%; mp 211-213 °C. IR [cm^{-1}]: 3091, 3048, 2930, 2858, 1713, 1620,

1602, 1541, 1440. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 14.85 (s, 1H), 9.01 (s, 1H), 8.20 (dd, ^{3}J = 7.2, ^{5}J = 1.6, 1H), 8.57 (dd, ^{3}J = 8.8, ^{4}J = 6.4, 1H), 4.60–4.56 (m, 2H), 1.82–1.77 (m, 2H), 1.38 –1.33 (m, 2H), 0.94 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 177.7 (d, $^{4}J_{C,F}$ = 2.4, 1C), 165.1, 152.1, 147.2 (d, $^{1}J_{C,F}$ = 251.4, 1C), 129.7 (d, $^{2}J_{C,F}$ = 7.1, 1C), 127.4, 126.8, 126.4 (d, $^{2}J_{C,F}$ = 18.9, 1C), 122.5 (d, $^{4}J_{C,F}$ = 4.9, 1C), 108.1, 58.0 (d, $^{4}J_{C,F}$ = 14.4, 1C), 31.9 (d, $^{5}J_{C,F}$ = 4.1, 1C), 18.9, 13.3.

4.1.1.4 *1-Butyl-5,7-difluoro-4-oxo-1,4-diyhdroquinoline-3-carboxylic acid (**3d**)*. According to the general procedure 4.1.1, a solution of compound **1d** (1 eq, 10.0 g, 39.5 mmol) and potassium carbonate (4 eq, 16.7 g, 158.0 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 21.3 mL, 197.5 mmol), and the reaction was heated at 90 °C for 20 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 50:1, R_f = 0.80) and then hydrolysed by refluxing compound **2d** under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield 9.77 g of **3d**. Yield: 88%; mp 208–210 °C. IR [cm^{-1}]: 3368, 3118, 2965, 2871, 1708, 1614, 1512, 1437, 1343, 1283, 1168, 1127, 1014. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 14.98 (s, 1H), 8.98 (s, 1H), 7.78–7.75 (m, 1H), 7.51–7.46 (m, 1H), 4.47 (t, ^{3}J = 7.2, 2H), 1.76–1.68 (m, 2H), 1.34 (m, 2H), 0.89 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 176.8 (d, $^{3}J_{C,F}$ = 1.6, 1C), 165.43, 164.6 (dd, $^{1}J_{C,F}$ = 250.4, $^{3}J_{C,F}$ = 15.2, 1C), 156.7 (dd, $^{1}J_{C,F}$ = 248.7, $^{3}J_{C,F}$ = 15.6, 1C), 150.1, 144.3 (dd, $^{3}J_{C,F}$ = 15.0, $^{3}J_{C,F}$ = 14.2, 1C), 113.7 (dd, $^{2}J_{C,F}$ = 8.4, $^{4}J_{C,F}$ = 2.4, 1C), 108.8, 100.1 (dd, $^{2}J_{C,F}$ = 25.1, $^{2}J_{C,F}$ = 25.1, 1C), 92.6 (dd, $^{2}J_{C,F}$ = 26.7, $^{4}J_{C,F}$ = 4.5, 1C), 54.2, 30.3, 18.9, 13.5.

4.1.1.5 *1-Butyl-6,7,8-trifluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3e**)*. According to the general procedure 4.1.1, a solution of compound **1e** (1 eq, 2.40 g, 8.85 mmol) and potassium carbonate (4 eq, 4.90 g, 35.4 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 5.75 mL, 44.3 mmol), and the reaction was heated at 90 °C for 48 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/*i*-PrOH = 50:1, R_f = 0.49) and then hydrolysed by refluxing compound **2e** under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield 1.62 g of **3e**. Yield: 62%; mp 216–218 °C. IR [cm^{-1}]: 3056, 2963, 2934, 2875, 1713, 1614, 1560, 1519, 1482, 1455, 1412, 1390, 1284, 1110, 1056. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 14.48 (s, 1H), 9.04 (s, 1H), 8.24–8.19 (m, 1H), 4.62–4.57 (m, 2H), 1.84–1.80 (m, 2H), 1.38–1.33 (m, 2H), 0.93 (t, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 175.4 (d, $^3J_{C,F}$ = 1.2, 1C), 164.6, 152.6 149.2 (m, 1C), 146.9 (m, 1C), 142.5 (m, 1C), 127.1, 122.6 (d, $^3J_{C,F}$ = 6.8, 1C), 108.2 (m, 1C), 107.4, 58.1 (d, $^4J_{C,F}$ = 13.5, 1C), 32.4 (d, $^5J_{C,F}$ = 3.9, 1C), 19.4, 13.9. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -134.44 (dd, $J_{6,8}$ = 24.8, $J_{6,7}$ = 6.9), -140.68 (dd, $J_{6,8}$ = 6.9, $J_{7,8}$ = 20.0), -149.05 (dd, $J_{6,7}$ = 24.8, $J_{7,8}$ = 20.0).

4.1.1.6 *1-Butyl-5,6,7-trifluoro-4-oxo-1,4-diyhroquinoline-3-carboxylic acid (**3f**).* According to the general procedure 4.1.1, a solution of compound **1f** (1 eq, 9.12 g, 33.6 mmol) and potassium carbonate (4 eq, 18.60 g, 134.4 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 18.0 mL, 168.0 mmol), and the reaction was heated at 70 °C for 20 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.81) and then hydrolysed by refluxing compound **2f** under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield 2.13 g of **3f**. Yield: 28%; mp 233-236 °C. IR [cm^{-1}]: 3099, 2964, 2879, 1715, 1650, 1455, 1346, 1297, 1186. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 9.00 (s, 1H), 8.12–8.07 (m, 1H), 4.49 (t, ^3J = 7.2, 2H), 1.71 (quint, ^3J = 7.2, 2H), 1.34 (sext, ^3J = 7.2, 2H), 0.89 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 176.2, 165.3, 153.2 (ddd, $^1J_{C,F}$ = 251.9, $^2J_{C,F}$ = 11.0, $^3J_{C,F}$ = 4.6, 1C), 150.0, 149.8 (ddd, $^1J_{C,F}$ = 264.2, $^2J_{C,F}$ = 10.8, $^3J_{C,F}$ = 5.0, 1C), 136.9 (dt, $^1J_{C,F}$ = 248.8, $^2J_{C,F}$ = 15.3, 1C), 136.5 (d, $^3J_{C,F}$ = 12.9, 1C), 113.9 (d, $^2J_{C,F}$ = 5.3, 1C), 108.4, 102.4 (dd, $^2J_{C,F}$ = 22.5, $^3J_{C,F}$ = 4.5, 1C), 54.1, 30.3, 18.9, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -123.76 (m), -133.87 (m), -161.99 (m).

4.1.1.7 *1-Butyl-7-chloro-6-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3g**).* According to the general procedure 4.1.1, a solution of compound **1g** (1 eq, 5.5 g, 19.6 mmol) and potassium carbonate (4 eq, 10.83 g, 78.4 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 10.5 mL, 98.0 mmol), and the reaction was heated at 85 °C for 24 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/*i*PrOH = 150:1, R_f = 0.59) and then hydrolysed by refluxing compound **2g** under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield 4.13 g of **3g**. Yield: 68%; mp 232–233 °C. IR [cm^{-1}]: 3041, 2963, 2941, 2874, 1702, 1607, 1457, 1433, 1219, 1058. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 15.20 (s, 1H), 8.98, 8.27 (s, 1H), 7.86 (s, 1H), 4.61 (t, ^3J = 7.2, 2H), 1.74 (quint, ^3J = 7.6, 2H), 1.32 (sext, ^3J = 7.6, 2H), 0.98 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 176.3, 165.8, 152.8, 148.3, 133.6, 129.6, 125.8, 119.9, 107.7, 106.6, 56.7, 53.5, 30.8, 18.9, 13.4.

4.1.1.8 *1-Butyl-7-fluoro-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic acid (**3h**).* According to the general procedure 4.1.1, a solution of compound **1h** (1 eq, 5.20 g, 17.1 mmol) and potassium carbonate (4 eq, 9.45 g, 68.4 mmol) in *N*,*N*-dimethylformamide was treated with *n*-bromobutane (5 eq, 9.2 mL, 85.5 mmol), and the reaction was heated at 80 °C for 24 h. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 150:1, R_f = 0.43) and then hydrolysed by refluxing compound **2h** under acidic conditions (2 M HCl). The precipitates were collected and dried *in vacuo* to yield 3.11 g of **3h**. Yield: 55%; mp 198-200 °C. IR [cm^{-1}]: 3050, 2967, 2878, 1721, 1609 1455, 1388 1304, 1257, 1139. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 14.45 (s, 1H), 9.11 (s, 1H), 8.57 (d, ^4J = 8.0, 1H), 8.30 (d, ^3J = 12.8, 1H), 4.54 (t, ^3J = 7.6, 2H), 1.76 (quint, ^3J = 7.6 2H), 1.35 (sext, ^3J = 7.2, 2H), 0.91 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 177.4, 165.6, 161.2 (d, $^1J_{C,F}$ = 257.0, 1C), 152.0, 144.0 (d, $^3J_{C,F}$ = 6.8, 1C), 126.9 (m, 1C), 122.6 (d, $^4J_{C,F}$ = 1.8, 1C), 122.3 (q, $^1J_{C,F}$ = 269.9, 1C), 115.9–115.2 (m, 1C), 109.5, 107.6 (m, $^2J_{C,F}$ = 26.1, 1C), 54.3, 31.0, 19.4, 13.9. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -60.49 (d, *J* = 12.2, 3F), -107.92 (m).

4.1.1.9 *1-(3-(Benzyloxy)propyl)-7-chloro-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**3i**).* According to the general procedure 4.1.1, a solution of compound **1a** (1 eq, 5.80 g, 21.5 mmol) and potassium carbonate (4 eq, 10.6 g, 86.0 mmol) in *N*,*N*-dimethylformamide was treated with ((3-bromopropoxy)methyl)benzene (1.5 eq, 5.7 mL, 36.0 mmol), and the reaction was heated at 85 °C for 48 h. The crude product was purified by column

chromatography (eluent: $CHCl_3$/EtOAc = 20:1, R_f = 0.40) and then hydrolysed by refluxing compound **2i** under basic conditions (3 M KOH). After acidification with 2 M HCl under ice cooling, the precipitates were collected and dried *in vacuo* to yield 7.48 g of **3i**. Yield: 89%; mp 179–180 °C; IR [cm^{-1}]: 3046, 2864, 1715, 1613, 1557, 1508, 894. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 9.01 (s, 1H), 8.40 (d, ^4J = 6.0, 2H), 8.17 (d, ^3J = 9.2, 1H), 7.31–7.23 (m, 5H), 4.66 (t, ^3J = 6.0, 2H), 4.39 (s, 2H), 3.49 (t, ^3J = 6.0, 2H), 2.08 (quint, ^3J = 6.0, 2H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 176.4 (d, $^4J_{C,F}$ = 2.5, 1C), 165.4, 155.6 (d, $^1J_{C,F}$ = 247.8, 1C), 150.3, 137.9, 136.4 (d, $^4J_{C,F}$ = 1.7, 1C), 128.1 (2C), 127.4 (2C), 127.3, 127.1, 125.9 (d, $^2J_{C,F}$ = 6.5, 1C), 121.0, 111.8 (d, $^2J_{C,F}$ = 22.7, 1C), 107.6, 72.0, 66.2, 51.6, 28.3.

4.1.1.10 *1-Butyl-5-(dimethylamino)-7-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(3j)***. A solution of ethyl 5,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **1c** (1 eq, 7.27 g, 29.25 mmol) in non-freshly distilled *N*,*N*-dimethylformamide (40 mL, containing the degradation product dimethyl amine) was treated with potassium carbonate at 60 °C for 30 min. Afterwards, *n*-bromobutane (20.04 g, 146.25 mmol, 15.8 mL) and a catalytic amount of potassium iodide was added to the reaction and was heated at 90 °C for 24 h. The solvent was removed under reduced pressure and the crude product was mixed with water (60 mL). The aqueous layer was extracted with EtOAc (3 x 50 mL), the organic layers were combined, and the solvent was evaporated. The intermediate carboxylic ethyl ester **2c** was hydrolysed in 2 M HCl by refluxing for 6 h. The mixture was consequently extracted with $CHCl_3$ (3 x 25 mL) and the combined organic layers were dried over anhydrous sodium sulfate. After the evaporization of the solvent, the product was recrystallized from EtOH to give 1.6 g of **3j**. Yield: 18%; mp 205–208 °C. IR [cm^{-1}]: 3057, 2952, 2867, 1716, 1628, 1563, 1513, 1436, 1276, 1231, 1187, 1167, 1124, 1029. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 15.53 (s, 1H), 8.60 (s, 1H), 6.62–6.57 (m, 2H), 4.13 (t, ^3J = 7.2, 2H), 2.97 (s, 6H), 1.91–1.83 (m, 2H), 1.76 (sext, ^3J = 7.2, 2H), 1.00 (t, ^3J = 7.2, 3H). ^{13}C-NMR ($CDCl_3$ δ [ppm], *J* [Hz]): 177.1, 167.4, 165.4 (d, $^1J_{C,F}$ = 249.0, 1C), 156.7 (d, $^3J_{C,F}$ = 13.0, 1C), 147.4, 144.3 (d, $^3J_{C,F}$ = 15.0, 1C), 113.7 (d, $^4J_{C,F}$ = 1.2, 1C), 109.0, 100.1 (d, $^2J_{C,F}$ = 25.0, 1C), 92.6 (d, $^2J_{C,F}$ = 27.8, 1C), 55.3, 44.5 (2C), 30.4, 19.9, 13.5. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -102.56. Mass: $[M + H]^+$ 307.2 *m/z*, found 307.1 *m/z*.

4.1.1.11 *1-Butyl-7-fluoro-5-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(3k)***. A cold suspension of compound **3d** (1 eq, 1.25 g, 4.44 mmol) and methanol (30 mL) was treated with sodium hydride (10 eq, 107 mg, 44.4 mmol) under ice cooling. Afterwards, the reaction was heated at 90 °C for 10 h and then quenched with aqueous HCl (3 M). The reaction solution was extracted with $CHCl_3$ and the combined organic layers were dried over Na_2SO_4 with removing subsequently of the organic solvent under reduced pressure. Yield: 40%. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 15.19 (s, 1H), 8.60 (s, 1H), 6.66 (dd, ^4J = 2.0, ^2J = 8.0, 1H), 6.64 (dd, ^4J = 2.0, ^2J = 10.8, 1H), 4.12 (t, ^3J = 7.2, 2H), 3.97 (s, 3H), 1.82 (quint, ^3J = 7.2, 2H), 1.39 (sext, ^3J = 7.2, 2H), 0.95 (t, ^3J = 7.2, 3H). ^{13}C-NMR ($CDCl_3$ δ [ppm], *J* [Hz]): 176.4, 166.1, 165.8 (d, $^1J_{C,F}$ = 250.2, 1C), 159.7 (d, $^3J_{C,F}$ = 14.4, 1C), 148.2, 145.3 (d, $^3J_{C,F}$ = 14.6, 1C), 113.3 (d, $^4J_{C,F}$ = 1.2, 1C), 109.3, 96.3 (d, $^2J_{C,F}$ = 24.0, 1C), 92.4 (d, $^2J_{C,F}$ = 25.0, 1C), 55.9, 53.6, 30.3, 19.3, 13.5.

4.1.2 *General synthesis of 1-alkyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **4a**, **4f-i**, **4k** [7]*. 1-Alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **3a**, **3f-k** (1 eq), dissolved in 5-15 ml morpholine, was heated 4-10 h under microwave irradiation at 110 °C. The reaction was acidified with 2 M HCl at 0 °C (pH 2) and the precipitate was collected. The resulting yellow solid was dried subsequently *in vacuo* and recrystallized from EtOH/EtOAc/$CHCl_3$.

4.1.2.1 *1-Butyl-6-fluoro-7-morpholino-4-oxo-1,4-diyhdroquinoline-3-carboxylic acid **(4a)***. The compound was synthesised according to the general procedure described 4.1.2. Spectroscopic data are in accordance with reference [7].

4.1.2.2 *1-Butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(4b)***. According to the general procedure 4.1.2, compound **3b** (400 mg, 1.34 mmol) was dissolved in 10.0 mL morpholine and was heated 4.5 h under microwave irradiation at 110 °C. After acidification (pH 2) and drying *in vacuo*, the yellow solid was recrystallized from EtOH to yield 210 mg of **4b**. Yield: 47%; mp 229–230 °C; IR [cm^{-1}]: 3066, 2956, 2862, 1714, 1615, 1519, 1444, 1243, 1103. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 15.33 (s, 1H), 8.62 (s, 1H), 8.34 (d, ^3J = 9.4, 1H), 7.13 (dd, ^3J = 9.2, ^4J = 2.4, 1H), 6.66 (d, ^4J = 2.4, 1H), 4.22 (t, ^3J = 7.6, 2H), 3.92–3.90 (m, 4H), 3.40–3.38 (m, 4H), 1.91 (quint, ^3J = 7.6, 2H), 1.47 (sext, ^3J = 7.6, 2H), 1.02 (t, ^3J = 7.2, 3H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 177.48, 167.6, 154.9, 148.0, 141.3, 128.5, 118.4, 114.5, 107.9, 97.8, 66.4, 53.6, 47.6, 30.6, 18.9, 13.4.

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520 4.1.2.3 *1-Butyl-8-fluoro-7-morpholino-1,4-dihydroquinoline-3-carboxylic acid (**4c**).*
521 Compound **3c** (400 mg, 1.34 mmol) was dissolved in *N*,*N*-dimethylformamide (7.5 mL) and
522 the reaction was treated with morpholine (0.5 mL), and was heated for 20 h at 130 °C.
523 Afterwards, the solvent was reduced under reduced pressure, the mixture was acidified to
524 pH 2, and the yellow solid was collected. The crude product was recrystallized from EtOH to
525 yield 290 mg of **4c**. Yield: 62%; mp 272-273 °C; IR [cm^{-1}]: 3046, 2955, 2857, 1719, 1614,
526 1444, 1247, 1119, 926. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.58 (s, 1H), 8.26 (dd, ^{3}J = 8.8, ^{5}J
527 = 1.6, 1H), 7.19 (m, 1H), 4.41 (m, 2H), 3.92–3.91 (m, 4H,), 3.30–3.27 (m, 4H), 1.88 (quint, ^{3}J
528 = 7.2, 2H), 1.34 (sext, ^{3}J = 7.2, 2H), 0.92 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J*
529 [Hz]): 177.2 (d, $^{4}J_{C,F}$ = 2.5, 1C), 166.8, 150.9, 144.7 (d, $^{2}J_{C,F}$ = 8.5, 1C), 143.2 (d, $^{1}J_{C,F}$ =
530 246.9, 1C), 129.9 (d, $^{2}J_{C,F}$ = 6.3, 1C), 123.4 (d, $^{4}J_{C,F}$ = 3.9, 1C), 122.1, 117.3 (d, $^{3}J_{C,F}$ = 3.1,
531 1C), 108.1, 66.7 (2C), 59.4 (d, $^{4}J_{C,F}$ = 16.0, 1C), 50.7 (d, $^{4}J_{C,F}$ = 4.2, 2C), 32.8 (d, $^{5}J_{C,F}$ = 4.0,
532 1C), 19.7, 13.6.

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534 4.1.2.4 *1-Butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4d**).*
535 Compound **3d** (1 eq, 1.0 g, 3.78 mmol), triethylamine (1.5 eq, 750 µL, 5.36 mmol) and boron
536 trifluoride diethyl etherate (1.5 eq, 675 µL, 5.36 mmol) were dissolved in CH_2Cl_2 and
537 refluxed for 2 h. The solvent was removed under reduced pressure and the crude product was
538 washed with water/MeOH (1:1), and dried *in vacuo*. Afterwards, the borate complex (1 eq,
539 980 mg, 2.98 mmol) was dissolved in 25 mL ethanol and the reaction mixture was treated
540 with triethylamine (2 eq, 830 µL, 5.97 mmol) and morpholine (1 eq, 260 mL, 2.98 mmol),
541 and heated 4 h at 60 °C. The solvent was removed under reduced pressure and the
542 intermediate **C1** was refluxed in 2 M NaOH for 2 h. Finally, the compound **4d** precipitated
543 after the addition of 2 M HCl. Yield: 25%; mp 257–260 °C. IR [cm^{-1}]: 3389, 3049, 2975,
544 1701, 1630, 1539, 1519, 1448, 1365, 1265, 1216, 1160, 1118, 1051. ^{1}H-NMR ($CDCl_3$, δ
545 [ppm], *J* [Hz]): 8.54 (s, 1H), 6.71 (d, ^{3}J = 14.4, 1H), 6.45 (s, 1H), 4.18 (t, ^{3}J = 6.8, 2H), 3.92–
546 3.90 (m, 4H), 3.40–3.38 (m, 4H), 1.90–1.87 (m, 2H), 1.42 (sext, ^{3}J = 7.2, 2H), 1.09 (t, ^{3}J =
547 7.2, 3H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 177.1 (d, $^{3}J_{C,F}$ = 1.7, 1C), 167.2, 163.4 (d, $^{1}J_{C,F}$
548 = 260.8, 1C), 154.5 (d, $^{3}J_{C,F}$ = 12.9, 1C), 148.3, 142.6 (d, $^{3}J_{C,F}$ = 5.7, 1C), 108.6, 108.3 (d,
549 $^{2}J_{C,F}$ = 9.9), 100.4 (d, $^{2}J_{C,F}$ = 25.6, 1C), 94.0, 66.1 (2C), 55.0, 47.1 (2C), 30.3, 19.9, 13.6.

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551 4.1.2.5 *1-Butyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4e**).*
552 In accordance to 4.1.2.3, compound **3e** (600 mg, 1.81 mmol) was dissolved in *N*,*N*-

dimethylformamide (10.0 mL) and the reaction mixture was treated with morpholine (0.5 mL), and was heated 20 h at 130 °C. Afterwards, the solvent was removed under reduced pressure, the mixture was acidified, and the yellow precipitate was collected. The crude product was purified by means of column chromatography on silica gel (eluent: $CHCl_3$/MeOH/FA = 100:2:1, R_f = 0.31) and subsequent recrystallization from EtOH yielding 240 mg of **4d**. Yield: 36%; mp 210–212 °C. IR [cm^{-1}]: 3051, 2953, 2850, 1715, 1615, 1539, 1464, 1378, 1279, 1207, 1113, 1051, 1016. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 14.73 (s, 1H), 8.91 (s, 1H), 7.86 (dd, ^3J = 11.2, ^5J = 2.4, 1H), 4.59–4.55 (m, 2H), 3.75–3.72 (m, 4H), 3.34 (br, 4H), 1.80 (quint, ^3J = 7.2, 2H), 1.30 (sext, ^3J = 7.2, 2H), 0.91 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 175.6 (d, $^4J_{C,F}$ = 2.4, 1C), 165.4, 154.3 (dd, $^1J_{C,F}$ = 248.4, $^3J_{C,F}$ = 6.2, 1C), 151.3, 146.3 (dd, $^1J_{C,F}$ = 249.7, $^3J_{C,F}$ = 6.6, 1C), 133.4 (m, 1C), 127.2 (dd, $^2J_{C,F}$ = 7.1, $^4J_{C,F}$ = 2.0, 1C), 120.6 (m, 1C), 107.2 (dd, $^2J_{C,F}$ = 22.8, $^4J_{C,F}$ = 2.7, 1C), 106.7, 66.6 (2C), 57.9 (d, $^4J_{C,F}$ = 15.6, 1C), 47.1 (t, $^4J_{C,F}$ = 3.8, 1C), 31.9 (d, $^5J_{C,F}$ = 4.1, 1C), 18.9, 13.3. ^{19}F-NMR (DMSO-d_6, δ [ppm], J [Hz]): -119.37 (d, $J_{6,8}$ = 11.4), -129.13 (d, $J_{6,8}$ = 11.4).

4.1.2.6 *1-Butyl-5,6-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4f**).* In accordance to 4.1.2.4, compound **3f** (1 eq, 650 mg, 2.17 mmol), triethylamine (1.5 eq, 451 µL, 3.26 mmol) and boron trifluoride diethyl etherate (1.5 eq, 465 µL, 3.26 mmol) were dissolved in CH_2Cl_2 and refluxed for 2 h. The solvent was removed under reduced pressure and the product was washed with water/MeOH (1:1), and dried *in vacuo*. Afterwards, the borate complex (1 eq) was dissolved in 25 mL ethanol and was treated with triethylamine (2 eq), and morpholine (1 eq), and was heated 4 h at 60 °C. The solvent was removed under reduced pressure and the intermediate **C2** was refluxed in 2 M NaOH for 2 h. Finally, the compound **4f** precipitated after addition of 2 M HCl. Yield: 25%; ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 8.63 (s, 1H), 6.56 (d, ^4J = 5.6, 1H), 4.21 (t, ^3J = 7.2, 3H), 3.93-3.90 (m, 4H), 3.34-3.32 (m, 4H), 1.88 (quint, ^3J =7.2, 2H), 1.45 (sext, ^3J = 7.2, 2H), 1.02 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 175.0, 165.3, 153.2 (dd, $^1J_{C,F}$ = 249.1, $^2J_{C,F}$ = 12.4, 1C), 149.8, 144.8 (m, 1C), 139.8 (d, $^3J_{C,F}$ = 10.4, 1C), 138.6 (dd, $^1J_{C,F}$ = 250.0, $^2J_{C,F}$ = 14.5, 1C), 112.9 (d, $^2J_{C,F}$ = 6.8, 1C), 110.1, 103.4 (d, $^3J_{C,F}$ = 4.5, 1C), 66.3 (2C), 53.5, 48.9 (d, $^4J_{C,F}$ = 4.2, 2C), 29.3, 18.9, 13.4.

4.1.2.7 *1-Butyl-6-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4g**).* According to the general procedure 4.1.2, compound **3g** (330 mg, 1.07 mmol) was dissolved in 15.0 mL morpholine and was heated 9 h under microwave irradiation at 110 °C. After

acidification and drying *in vacuo*, the yellow solid was recrystallized from EtOH to yield 110 mg of **4d**. Yield: 29%; mp 248–250 °C. IR [cm^{-1}]: 3045, 2952, 1711, 1616, 1470, 1446, 1256, 1229, 1111, 1041, 1006. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 15.77 (s, 1H), 8.87 (s, 1H), 7.67 (s, 1H), 7.09 (s, 1H), 4.58 (t, ^{3}J = 7.2, 2H), 3.95 (s, 3H), 3.79–3.75 (m, 4H), 3.26–3.23 (m, 4H), 1.79 (quint, ^{3}J = 7.2, 2H), 1.33 (sext, ^{3}J = 7.2, 2H), 0.92 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 175.8, 166.5, 150.7, 147.2, 147.1, 135.0, 120.0, 106.5, 104.9**,** 104.8, 66.0 (2C), 55.8, 53.2, 49.9 (2C), 30.3, 19.0, 13.4.

4.1.2.8 *1-Butyl-morpholino-4-oxo-6-(trifluoromethyl)-1,4-diyhdroquinoline-3-carboxylic acid (**4h**).* According to the general procedure 4.1.2, compound **3h** (600 mg, 1.81 mmol) was dissolved in 10.0 mL morpholine and was heated 6 h under microwave irradiation at 110 °C. After acidification and drying *in vacuo*, the yellow solid was recrystallized from EtOH to yield 500 mg of **4h**. Yield: 69%; mp 182-185 °C. IR [cm^{-1}]: 3046, 2952, 2857, 1725, 1610, 1455, 1393, 1303, 1244, 1102. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 14.52 (s, 1H), 8.68 (s, 1H), 8.67 (s, 1H), 7.18 (s, 1H), 4.25 (t, ^{3}J = 7.6, 2H), 3.84-3.82 (m, 4H), 3.07-3.05 (m, 4H), 1.85 (quint, ^{3}J = 7.2, 2H), 1.40 (sext, ^{3}J = 7.6, 2H), 0.91 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 177.4, 165.4, 156.2, 149.4, 142.2, 128.6 (q, $^{3}J_{C,F}$ = 5.5, 1C), 124.6 (q, 1C, $^{2}J_{C,F}$ = 30.6, 1C), 123.2 (q, $^{1}J_{C,F}$ = 278.5, 1C), 121.7, 109.4, 109.2, 66.8 (2C), 54.3, 53.4 (2C), 30.7, 19.8, 13.5.

4.1.2.9 *1-(3-(Benzyloxy)propyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4i**).* According to the general procedure 4.1.2, compound **3i** (4.00 g, 10.3 mmol) was dissolved in 10.0 mL morpholine and was heated 6 h under microwave irradiation at 110 °C. After acidification and drying *in vacuo*, the yellow solid was recrystallized from EtOAc/$CHCl_3$ (10:1) to yield 2.61 g of **4i**. Yield: 58%; mp 191–192 °C. IR [cm^{-1}]: 2858, 1713, 1626, 1508, 1453, 1406, 1354, 1302, 1265, 1206, 1101, 1025. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 14.7 (s, 1H), 8.91 (s, 1H), 7.91 (d, ^{3}J = 13.6, 2H), 7.33–7.28 (m, 5H), 7.19 (d, ^{3}J = 7.2, 1H), 4.64 (t, ^{3}J = 6.8, 2H), 4.44 (s, 2H), 3.72–3.70 (m, 4H), 3.49 (t, ^{3}J = 5.6, 2H), 3.23-3.22 (m, 4H), 2.11 (quint, ^{3}J = 6.0, 2H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 176.1 (d, $^{4}J_{C,F}$ = 2.5, 1C), 166.0, 152.4 (d, $^{1}J_{C,F}$ = 247.8, 1C), 149.1, 145.1 (d, $^{2}J_{C,F}$ = 10.4, 1C), 138.1, 128.2 (2C), 128.1 (2C), 127.5, 119.3 (d, $^{3}J_{C,F}$ = 10.4, 1C), 111.1 (d, $^{2}J_{C,F}$ = 23.2, 1C), 106.8, 105.7 (d, $^{3}J_{C,F}$ = 4.8, 1C), 107.6, 72.1, 66.4, 65.7 (2C), 51.6, 49.6 (d, $^{4}J_{C,F}$ = 4.7, 2C), 28.1.

4.1.2.10 *1-Butyl-5-(dimethylamino)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**4j**).* In accordance to 4.1.2.3, compound **3j** (500 mg, 1.78 mmol) was dissolved in *N*,*N*-dimethylformamide (20 mL) and was treated with morpholine (0.5 mL), and the mixture was heated 2 h at 130 °C. The solvent was removed under reduced pressure and the crude product was purified by means of column chromatography (eluent: $CHCl_3$/MeOH/FA, R_f = 0.61). Recrystallization from EtOAc yielded 160 mg of **4j**. Yield 56%; mp 179–180 °C. IR [cm^{-1}]: 2945, 2833, 1696, 1595, 1526, 1434, 1359, 1233, 1191, 1110, 1009. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 8.67 (s, 1H), 6.44–6.42 (m, 2H), 4.39 (t, ^3J = 7.2, 2H), 3.77–3.74 (m, 4H), 3.41–3.38 (m, 4H), 2.80 (s, 6H), 1.76–1.71 (m, 2H), 1.32 (sext, ^3J = 7.2, 2H), 0.92 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 175.9, 167.0, 154.7, 153.9, 147.6, 143.9, 108.7, 106.5, 99.7, 91.4, 65.8 (2C), 53.5, 46.6 (2C), 44.1 (2C), 29.7, 19.0, 13.4. Mass: $[M + H]^+$ 374.2 *m/z*, found 374.5 *m/z*.

4.1.2.11 *1-Butyl-5-methoxy-7-morpholino-4-oxo-1,3-dihydroquinoline-3-carboxylic acid (**4k**).* According to the general procedure 4.1.2, compound **3k** (4.00 g, 10.3 mmol) was dissolved in 10.0 mL morpholine and was heated 6 h under microwave irradiation at 110 °C. After acidification and drying *in vacuo*, the yellow solid was recrystallized from EtOAc/$CHCl_3$ (10:1) to yield 2.61 g of **4k**. Yield: 34%; mp 258-259 °C. IR [cm^{-1}]: 2961, 2863, 1704, 1624, 1600, 1545, 1421, 1375, 1263, 1115. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 8.54 (s, 1H), 6.44 (d, ^4J = 1.6, 1H), 6.43 (d, ^3J = 1.6, 1H), 4.15 (t, ^3J = 7.6, 2H), 4.00 (s, 3H), 3.93–3.91 (m, 4H), 3.41–3.39 (m, 4H), 1.87 (quint, ^3J = 7.2, 2H), 1.44 (sext, ^3J = 7.2, 2H), 0.99 (t, ^3J = 7.2 3H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 177.8, 167.9, 162.6, 154.8, 147.6, 143.5, 109.9, 108.7, 95.3, 91.6, 65.8 (2C), 56.3, 55.1, 47.6 (2C), 30.3, 19.9, 13.6.

4.1.3 *General synthesis of N-benzyl-1-alkyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide **GHQ168**, **5-18** [7].* 1-Alkyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **4a-k** (1 eq) and *N*-methylmorpholine (NMM, 5 eq) were dissolved in *N*,*N*-dimethylformamide under Ar atmosphere and the reaction was stirred 1 h at 0 °C. Then, *i*-butyl chloroformate (4 eq) was added and stirred for 1 h at 0 °C, until the benzylamine derivative (4 eq) was added. After 45 min of stirring at room temperature, the solvent was removed *in vacuo* and residue was purified by column chromatography on silica gel. The crude solid was recrystallized to give **GHQ168**, and **5-18**, respectively as white crystals.

4.1.3.1 *N-Benzyl-1-butyl-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide **(GHQ168)**.* The compound was synthesised according to the general procedure described in 4.1.3. Spectroscopic data are in accordance with reference [7].

4.1.3.2 *N-Benzyl-1-butyl-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide **(5)**.* A solution of compound **4b** (460 mg, 1.39 mmol) in *N*,*N*-dimethylformamide was treated with NMM (764 µL, 6.95 mmol), *i*-butyl chloroformate (723 µL, 5.56 mmol), and benzylamine (608 µL, 5.56 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.57) and recrystallized from EtOAc to produce 99 mg of **5**. Yield: 17%; mp 172 °C. IR [cm^{-1}]: 3171, 3039, 2965, 2937, 2877, 2840, 1652, 1597, 1542, 1529, 1466, 1451, 1237, 1126. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.5 (t, ^3J = 6.0, 1H), 8.71 (s, 1H), 8.12 (d, ^3J = 9.2, 1H), 7.35–7.33 (m, 5H), 7.20 (dd, ^3J = 9.2, ^4J = 0.8, 1H), 6.90 (d, 1H, ^4J = 0.8), 4.53 (d, ^3J = 5.6, 2H), 4.42 (t, ^3J = 7.2, 2H), 3.77-3.79 (m, 4H), 3.34 (m, 4H), 1.75 (quint, ^3J = 7.2, 2H), 1.32 (sext, ^3J = 7.6, 2H), 0.91 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.7, 164.4, 153.9, 147.8, 140.5, 139.4, 128.3 (2C), 127.3 (3C), 126.8, 119.0, 113.4, 110.0, 98.4, 65.8 (2C), 52.4, 47.0 (2C), 42.0, 30.2, 19.1, 13.5. Mass: $[M + H]^+$ 420.2 *m/z*, found 420.3 *m/z*. HPLC purity 97%.

4.1.3.3 *N-Benzyl-1-butyl-8-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide **(6)**.* A solution of compound **4c** (230 mg, 0.66 mmol) in *N*,*N*-dimethylformamide was treated with NMM (362 µL, 3.3 mmol), *i*-butyl chloroformate (343 µL, 2.64 mmol), and benzylamine (289 µL, 2.64 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:3, R_f = 0.28) and recrystallized from EtOH to produce 180 mg of **6**. Yield: 62%; mp 170 °C; IR [cm^{-1}]: 3039, 2951, 2854, 1654, 1590, 1555, 1549, 1447, 1243, 1121. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.25 (t, ^3J = 6.0, 1H), 8.72 (s, 1H), 8.08 (dd, ^3J = 8.8, ^5J = 0.8, 1H), 7.34–7.31 (m, 4H), 7.27–7.24 (m, 2H), 4.54 (d, ^3J = 6.0, 2H), 4.48-4.46 (m, ^3J = 7.2, 2H), 3.78–3.76 (m, 4H), 3.20–3.17 (m, 4H), 1.75 (quint, ^3J = 7.2, 2H), 1.31 (sext, ^3J = 7.2, 2H), 0.90 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.2 (d, $^4J_{C,F}$ = 1.7, 1C), 163.8, 150.5, 143.5 (d, $^2J_{C,F}$ = 8.4, 1C), 143.1 (d, $^1J_{C,F}$ = 246.1, 1C), 139.3, 129.1 (d, $^2J_{C,F}$ = 5.9, 1C), 128.3 (2C), 127.3 (2C), 126.8, 122.9, 122.3 (d, $^4J_{C,F}$ = 3.6, 1C), 116.6 (d, $^2J_{C,F}$ = 2.4, 1C), 110.1, 66.0 (2C), 57.5 (d, $^4J_{C,F}$ = 15.8), 50.3 (d, $^4J_{C,F}$ = 4.0, 2C), 42.1, 32.0 (d, $^5J_{C,F}$ = 3.8, 1C), 18.9, 13.4.

^{19}F-NMR (DMSO-d_6, δ [ppm], J [Hz]): -134.80. Mass: $[M + H]^+$ 438.2 *m/z*, found 438.2 *m/z*. HPLC purity 96%.

4.1.3.4 *N-Benzyl-1-butyl-5-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (7).* A solution of compound **4d** (260 mg, 0.75 mmol) in *N,N*-dimethylformamide was treated with NMM (412 µL, 3.75 mmol), *i*-butyl chloroformate (390 µL, 3.00 mmol), and benzylamine (330 µL, 3.00 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.35) and recrystallized from EtOAc to produce 130 mg of **7**. Yield: 40%; mp 200 °C. IR [cm^{-1}]: 3030, 2964, 2945, 2983, 2856, 1662, 1624, 1573, 1530, 1497, 1467, 1264, 1215, 1117, 1003. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.39 (t, ^3J = 6.0, 1H), 8.65 (s, 1H), 7.34–7.31 (m, 4H), 7.27–7.24 (m, 1H), 6.91 (dd, ^3J = 15.6, ^4J = 1.6, 1H), 6.65 (d, ^4J = 1.6, 1H), 4.52 (d, ^3J = 6.0, 2H), 4.37 (t, ^3J = 7.2, 2H), 3.76–3.73 (m, 4H), 3.39–3.37 (m, 4H), 1.72 (quint, ^3J = 7.2, 2H), 1.31 (sext, ^3J = 7.2, 2H), 0.90 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.3 (d, $^3J_{C,F}$ = 1.7, 1C), 164.1, 162.7 (d, $^1J_{C,F}$ = 256.1, 1C), 153.5 (d, $^3J_{C,F}$ = 13.1, 1C), 147.9, 141.9 (d, $^3J_{C,F}$ = 5.7, 1C), 139.5, 128.3 (2C), 127.3 (2C), 126.8, 110.7, 108.6 (d, $^3J_{C,F}$ = 8.5, 1C), 99.2 (d, $^2J_{C,F}$ = 25.6, 1C), 94.3, 65.6 (2C), 52.9, 46.5 (2C), 42.5, 29.8, 19.1, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm]): -109.862. Mass: $[M + H]^+$ 438.2 *m/z*, found 438.2 *m/z*. HPLC purity: 97%.

4.1.3.5 *N-Benzyl-1-butyl-6,8-difluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (8).* A solution of compound **4e** (190 mg, 0.52 mmol) in *N,N*-dimethylformamide was treated with NMM (316 µL, 2.6 mmol), *i*-butyl chloroformate (270 µL, 2.1 mmol), and benzylamine (227 µL, 2.1 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 50:1, R_f = 0.77) and recrystallized from EtOAc to produce 60 mg of **8**. Yield: 25%; mp 165–167 °C. IR [cm^{-1}]: 3176, 3064, 3033, 2861, 1651, 1596, 1536, 1472, 1450, 1377, 1281, 1213, 1109, 1026. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.16 (t, ^3J = 6.0, 1H), 8.74 (s, 1H), 7.80 (dd, ^3J = 10.8, ^5J = 1.6, 1H), 7.35–7.32 (m, 4H), 7.27–7.24 (m, 1H), 4.55 (d, ^3J = 6.0, 2H), 4.48–4.44 (br, 2H), 3.73–3.71 (m, 4H), 3.29 (br, 4H), 1.80–1.73 (m, 2H), 1.38–1.31 (m, 2H), 0.91 (t, ^3J = 7.2, 3H). ^{13}C NMR (DMSO-d_6, δ [ppm], J [Hz]): 173.1 (d, $^4J_{C,F}$ = 1.4, 1C), 163.5, 153.6 (dd, $^1J_{C,F}$ = 246.0, $^3J_{C,F}$ = 6.0, 1C), 150.3, 146.3 (dd, $^1J_{C,F}$ = 249.0, $^3J_{C,F}$ = 7.0, 1C), 139.2, 132.3 (t, $^2J_{C,F}$ = 14.0, 1C), 128.3 (2C), 127.3 (2C), 126.8, 126.6 (m, 1C), 122.7 (d, $^3J_{C,F}$ = 8.0, 1C), 109.7, 107.0 (dd, $^2J_{C,F}$ = 19.0, $^4J_{C,F}$ = 2.0, 1C), 66.6 (2C), 57.7 (d, $^4J_{C,F}$ = 15.0,

1C), 50.7 (t, $^{4}J_{C,F}$ = 6.0, 2C), 42.1, 32.0 (d, $^{5}J_{C,F}$ = 4.0, 1C), 18.9, 13.4. ^{19}F-NMR (DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -121.44 (d, $J_{6,8}$ = 9.6), -130.03 (d, $J_{6,8}$ = 9.6). Mass: $[M + H]^+$ 456.2 *m/z*, found 456.2 *m/z*. HPLC purity: 99%.

4.1.3.6 *N-Benzyl-1-butyl-5,6-difluoro-7-morpholino-4-oxo-1,4-diyhdroquinoline-3-carboxamide (**9**)*. A solution of compound **4f** (200 mg, 0.55 mmol) in *N,N*-dimethylformamide was treated with NMM (300 µL, 2.75 mmol), *i*-butyl chloroformate (286 µL, 2.20 mmol), and benzylamine (240 µL, 2.20 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.33) and recrystallized from EtOAc to produce 110 mg of **9**. Yield: 44%; mp 179-180 °C. IR [cm^{-1}]: 3035, 2958, 2867, 1655, 1629, 1602, 1484 1377, 1274, 1115, 1009. ^{1}H-NMR (DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 10.26 (t, 1H, ^{3}J = 6.0), 8.74 (s, 1H), 7.35–7.32 (m, 4H), 7.27–7.23 (m, 1H), 6.82 (d, ^{4}J = 6.4, 1H), 4.54 (d, ^{3}J = 6.0, 2H), 4.43 (t, ^{3}J = 7.2, 2H), 3.79–3.77 (m, 4H), 3.30–3.27 (m, 4H), 1.74 (quint, ^{3}J = 7.2, 2H), 1.31 (sext, ^{3}J = 7.2, 2H), 0.91 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-*d*$_6$, δ [ppm], *J* [Hz]): 174.2, 163.7, 149.4 (dd, $^{1}J_{C,F}$ = 257.7, $^{2}J_{C,F}$ = 13.2, 1C), 147.0, 144.1 (m, 1C), 139.5 (dd, $^{1}J_{C,F}$ = 245.6, $^{2}J_{C,F}$ = 13.7, 1C), 139.4, 136.3 (d, $^{3}J_{C,F}$ = 3.9, 1C), 128.3 (2C), 127.3 (2C), 126.7, 111.5 (d, $^{2}J_{C,F}$ = 5.4, 1C), 110.9, 99.5, 65.7 (2C), 53.0, 49.3 (d, $^{4}J_{C,F}$ = 4.4, 2C) 42.0, 29.8, 19.1, 13.4. ^{19}F-NMR (DMSO-*d*$_6$, δ [ppm], *J* [Hz]): -140.03 (d, $J_{5,6}$ = 18.0), -151.62 (dd, $J_{5,6}$ = 18.0, $J_{6,8}$ = 8.0). Mass: $[M + H]^+$ 456.2 *m/z*, found 456.1 *m/z*. HPLC purity: 99%.

4.1.3.7 *N-Benzyl-1-butyl-6-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**10**).* A solution of compound **4g** (230 mg, 0.63 mmol) in *N,N*-dimethylformamide was treated with NMM (346 µL, 3.2 mmol), *i*-butyl chloroformate (327 µL, 2.52 mmol), and benzylamine (275 µL, 2.52 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 50:1, R_f = 0.73) and recrystallized from acetonitrile to produce 100 mg of **10**. Yield: 34%; mp 156 °C. IR [cm^{-1}]: 3045, 2952, 1711, 1616, 1470, 1446, 1256, 1229, 1111, 1041, 1006. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 10.57 (t, ^3J = 5.2, 1H), 8.70 (s, 1H), 7.85 (s, 1H), 7.42–7.21 (m, 5H), 6.82 (s, 1H), 4.71 (d, ^3J = 5.6, 2H), 4.23 (t, ^3J = 7.6, 2H), 3.99 (s, 3H), 3.94–3.92 (m, 4H), 3.24–3.22 (m, 4H), 1.90 (quint, ^3J = 7.2, 2H), 1.4 (sext, ^3J = 7.2, 2H), 1.0 (t, ^3J = 7.2, 3H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 175.4, 165.4, 150.4, 146.6, 146.2, 138.9, 134.5, 128.8 (2C), 127.7 (2C), 127.0, 123.42, 111.0, 106.6, 103.7, 66.8 (2C), 55.5, 54.0, 50.7 (2C), 43.3, 31.0, 20.0, 13.6. Mass: $[M + H]^+$ 450.2 *m/z*, found 450.3 *m/z*. HPLC purity: 96%.

4.1.3.8 *N-Benzyl-1-butyl-7-morpholino-4-oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxamide (**11**).* A solution of compound **4h** (220 mg, 0.56 mmol) in *N,N*-dimethylformamide was treated with NMM (307 µL, 2.8 mmol), *i*-butyl chloroformate (231 µL, 2.24 mmol), and benzylamine (214 µL, 2.56 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.67) and recrystallized from EtOAc to produce 40 mg of **11**. Yield: 15%; mp 168 °C. IR [cm^{-1}]: 3232, 2958, 2935, 1659, 1626, 1598, 1486, 1452, 1267 1240 1108. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.18 (t, ^3J = 7.6, 1H), 8.90 (s, 1H), 8.54 (s, 1H), 7.68 (s, 1H), 7.35-7.33 (m, 4H), 7.28-7.25 (m, 1H), 4.57-4.54 (m, 4H), 3.77-3.75 (m, 4H), 3.09-3.05 (m, 4H), 1.75 (quint, ^3J = 7.2, 2H), 1.35 (sext, ^3J = 7.6, 2H), 0.92 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.6, 163.6, 154.9, 149.5, 142.0, 139.1, 128.4 (2C), 127.3 (2C), 126.6, 126.5 (m, 1C), 124.5 (m, 1C), 122.7, 121.8 (m, 1C), 112.0, 111.5, 66.3 (2C), 52.9 (2C), 52.7, 42.1, 30.4, 19.0, 13.4. Mass: $[M + H]^+$ 488.2 *m/z*, found 488.1 *m/z*. HPLC purity: 97%.

4.1.3.9 *N-Benzyl-1-(3-(benzyloxy)propyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**12**)*. A solution of compound **4i** (400 mg, 0.90 mmol) in *N,N*-dimethylformamide was treated with NMM (463 µL, 4.50 mmol), *i*-butyl chloroformate (468 µL, 3.60 mmol), and benzylamine (393 µL, 3.60 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH =

100:3, R_f = 0.56) and recrystallized from EtOAc to produce 360 mg of **12**. Yield: 76%; mp 174–175 °C. IR [cm^{-1}]: 3181, 3038, 2939, 2856, 1652, 1536, 1586, 1358, 1266. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.37 (t, ^3J = 6.8, 1H), 8.81 (s, 1H), 7.87 (d, ^3J = 13.6, 2H), 7.36–7.25 (m, 10H), 7.13 (d, ^4J = 7.2, 1H), 4.58–4.55 (m, 5H), 4.44 (s, ^3J = 6.0, 2H), 3.75–3.70 (m, 4H), 3.49 (t, ^3J = 5.6, 2H), 3.20–3.18 (m, 4H), 2.10 (quint, ^3J = 6.0, 2H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.7 (d, $^4J_{C,F}$ = 2.5), 164.6, 153.2 (d, $^1J_{C,F}$ = 247.8), 148.6, 144.8 (d, $^2J_{C,F}$ = 10.4), 139.9, 138.1, 137.2, 128.9 (2C), 128.7 (2C), 128.7, 128.1 (2C), 127.8, 127.9 (2C), 122.0 (d, $^4J_{C,F}$ = 7.0), 112.1 (d, $^2J_{C,F}$ = 22.9, 1C), 110.6, 106.0 (d, $^3J_{C,F}$ = 4.8, 1C), 72.1, 66.8, 66.7 (2C), 51.6, 50.3 (d, $^4J_{C,F}$ = 4.7, 2C), 42.6, 28.8. $[M + H]^+$ 530.3 *m/z*, found 530.1 *m/z*. HPLC purity: 100%.

4.1.3.10 *N-Benzyl-1-butyl-5-(dimethylamino)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**13**).* A solution of compound **4j** (350 mg, 0.94 mmol) in *N*,*N*-dimethylformamide was treated with NMM (517 µL, 4.70 mmol), *i*-butyl chloroformate (490 µL, 3.78 mmol), and benzylamine (410 µL, 3.78 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:3, R_f = 0.82) and recrystallized from EtOH to produce 180 mg of **13**. Yield: 42%; mp 168–170 °C. IR [cm^{-1}]: 3154, 3030, 2954, 2861, 2823, 1653, 1594, 1564, 1526, 1485, 1452, 1373, 1284, 1230, 1120, 1013, 741. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.69 (t, ^3J = 6.0, 1H), 8.54 (s, 1H), 7.34–7.33 (m, 4H), 7.27–7.25 (m, 1H), 6.36–6.34 (m, 2H), 4.52 (d, ^3J = 6.0, 2H), 4.29 (t, ^3J = 7.2, 2H), 3.76–3.74 (m, 4H), 3.34–3.31 (m, 4H), 2.75 (s, 6H), 1.74–1.70 (m, 2H), 1.33 (sext, 2H, ^3J = 7.2), 0.91 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.8, 164.7, 154.5, 153.2, 146.3, 143.5, 139.6, 128.3 (2C), 127.2 (2C), 126.7, 111.1, 110.5, 99.1, 91.2, 65.9 (2C), 53.0, 47.0 (2C), 42.0 (2C), 40.1, 29.9, 19.1, 13.5. Mass: $[M + H]^+$ 463.3 *m/z*, found 463.3 *m/z*. HPLC purity: 99%.

4.1.3.11 *N-Benzyl-1-butyl-5-methoxy-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**14**).* A solution of compound **4k** (220 mg, 0.61 mmol) in *N*,*N*-dimethylformamide was treated with NMM (335 µL, 3.05 mmol), *i*-butyl chloroformate (317 µL, 2.44 mmol), and benzylamine (305 µL, 2.44 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 50:1, R_f = 0.63) and recrystallized from EtOH to produce 140 mg of **14**. Yield: 51%; mp 159-161 °C. IR [cm^{-1}]: 2987, 2954, 2924, 2894, 1661, 1611, 1523, 1470, 1356, 1258, 1237. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.67 (t, ^3J = 6.0, 1H), 8.57 (s, 1H), 7.35–7.32 (m, 4H),

7.28-7.25 (m, 1H), 6.53 (d, ^4J = 1.6, 1H), 6.43 (d, ^4J = 1.6, 1H), 4.50 (d, ^3J = 6.0, 2H), 4.32 (t, ^3J = 7.6, 2H), 3.82 (s, 3H), 3.78–3.76 (m, 4H), 3.39–3.37 (m, 4H), 1.72 (quint, ^3J = 7.2, 2H), 1.31 (sext, ^3J = 7.2, 2H), 0.91 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 175.25, 164.5, 161.8, 153.8, 146.8, 142.6, 139.5, 128.3 (2C), 127.3 (2C), 126.7, 110.9, 110.2, 95.0, 91.3, 65.8 (2C), 55.8, 53.0, 46.9 (2C), 42.0, 29.8, 19.1, 13.5. Mass: $[M + H]^+$ 450.2 *m/z*, found 450.1 *m/z*. HPLC purity: 98%.

4.1.3.12 *1-Butyl-6-fluoro-N-(4-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**15**).* A solution of compound **4a** (90 mg, 0.26 mmol) in *N*,*N*-dimethylformamide was treated with NMM (141 µL, 1.29 mmol), *i*-butyl chloroformate (134 µL, 1.02 mmol), and (4-fluorophenyl)methanamine (118 µL, 1.02 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.75) and recrystallized from EtOAc to produce 70 mg of **15**. Yield: 58%; mp 177–179 °C; IR [cm^{-1}]: 3032, 2954, 2871, 1654, 1625, 1603, 1536, 1485, 1468, 1478, 1257, 1213, 1113. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.36 (t, ^3J = 6.0, 1H), 8.78 (s, 1H), 7.87 (d, ^3J = 13.6, 1H), 7.40–7.36 (m, 2H), 7.18–7.09 (m, 2H), 7.09 (d, ^4J = 7.6, 1H), 4.53 (d, ^3J = 6.0, 2H), 4.48 (t, ^3J = 7.2, 2H), 3.80–3.78 (m, 4H), 3.26–3.24 (m, 4H), 1.77 (quint, ^3J = 7.6, 2H), 1.32 (sext, ^3J = 7.6, 2H), 0.92 (t, ^3J = 7.2, 2H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.6 (d, $^4J_{C,F}$ = 2.5, 1C), 164.0, 160.2 (d, $^1J_{C,F}$ = 240.4, 1C), 152.4 (d, $^1J_{C,F}$ = 245.6, 1C), 147.7, 144.3 (d, $^2J_{C,F}$ = 10.3, 1C), 136.6, 135.6 (d, $^4J_{C,F}$ = 3.0, 1C), 129.3 (d, $^3J_{C,F}$ = 8.0, 2C), 121.4 (d, $^3J_{C,F}$ = 6.9, 1C), 115.1 (d, $^2J_{C,F}$ = 21.1, 2C), 111.0 (d, $^2J_{C,F}$ = 22.4, 1C), 109.9, 105.5 (d, $^3J_{C,F}$ = 3.3, 1C), 65.8 (2C), 52.8, 49.8 (d, $^4J_{C,F}$ = 4.4, 2C), 41.3, 30.2, 19.1, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -116.04, -123.79. Mass: $[M + H]^+$ 456.2 *m/z*, found 456.2 *m/z*. HPLC purity 98%.

4.1.3.13 *1-Butyl-6-fluoro-N-(2-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**16**).* A solution of compound **4a** (210 mg, 0.60 mmol) in *N*,*N*-dimethylformamide was treated with NMM (329 µL, 3.00 mmol), *i*-butyl chloroformate (328 µL, 2.40 mmol), and (2-fluorophenyl)methanamine (300 mg, 2.40 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.34) and recrystallized from EtOAc to produce 70 mg of **16**. Yield: 25%; mp 154-156 °C. IR [cm^{-1}]: 3053, 2963, 2848, 1651, 1604, 1482, 1452, 1305, 1267, 1247, 1122. ^{1}H-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.37 (t, ^3J = 6.0, 1H), 8.77 (s, 1H), 7.87 (d, ^3J = 13.6, 1H), 7.41–7.32 (m, 2H), 7.22–7.16 (m, 2H), 7.10 (d, ^4J = 7.2, 1H),

4.58 (d, ^{3}J = 6.0, 2H), 4.47 (t, ^{3}J = 7.2, 2H), 3.80–3.78 (m, 4H), 3.26–3.24 (m, 4H), 1.76 (quint, ^{3}J = 7.6, 2H), 1.31 (sext, ^{3}J = 7.6, 2H), 0.92 (t, ^{3}J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.1 (d, $^{4}J_{C,F}$ = 2.5, 1C), 164.2, 160.1 (d, $^{1}J_{C,F}$ = 242.9, 1C), 152.4 (d, $^{1}J_{C,F}$ = 246.1, 1C), 147.8, 144.3 (d, $^{2}J_{C,F}$ = 10.3), 136.6, 129.7 (d, $^{3}J_{C,F}$ = 4.5, 1C), 129.0 (d, $^{3}J_{C,F}$ = 8.0, 1C), 125.9 (d, $^{2}J_{C,F}$ = 14.9, 1C), 124.4 (d, $^{4}J_{C,F}$ = 3.4, 1C), 121.4 (d, $^{3}J_{C,F}$ = 6.9, 1C), 115.1 (d, $^{2}J_{C,F}$ = 21.0, 1C), 111.4 (d, $^{2}J_{C,F}$ = 22.4, 1C), 109.9, 105.5 (d, $^{3}J_{C,F}$ = 3.3, 1C), 65.9 (2C), 52.8, 49.8 (d, $^{2}J_{C,F}$ = 4.4, 2C), 36.1 (d, $^{3}J_{C,F}$ = 4.2, 1C), 30.2, 19.1, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -118.89 (m), -123.70 (dd, $J_{5,6}$ = 14.4, $J_{6,8}$ = 7.7). Mass: $[M + H]^+$ 456.2 *m/z*, found 456.1 *m/z*. HPLC purity: 95%.

4.1.3.14 *1-Butyl-N-(2,4-difluorobenzyl)-6-fluoro-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**17**).* A solution of compound **4a** (200 mg, 0.57 mmol) in *N*,*N*-dimethylformamide was treated with NMM (313 µL, 3.00 mmol), *i*-butyl chloroformate (297 µL, 2.28 mmol), and (2-fluorophenyl)methanamine (326 mg, 2.28 mmol) as depicted in the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.41) and recrystallized from EtOH to produce 80 mg of **17**. Yield: 30%; mp 160-161 °C. IR [cm^{-1}]: 3031, 2959, 2871, 1659, 1625, 1601, 1485, 1449, 1256, 1210, 1102. ^{1}H NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 10.36 (t, ^{3}J = 6.0, 1H), 8.76 (s, 1H), 7.88 (d, ^{3}J = 13.6, 1H), 7.46–7.40 (m, 1H), 7.26–7.21 (m, 1H), 7.10 (m, 2H), 4.56 (d, ^{3}J = 6.0, 2H), 4.46 (t, ^{3}J = 7.2, 2H), 3.80–3.78 (m, 4H), 3.26–3.24 (m, 4H), 1.76 (quint, ^{3}J = 7.6, 2H), 1.32 (sext, ^{3}J = 7.6, 2H), 0.91 (t, ^{3}J = 7.2, 3H). ^{13}C NMR (DMSO-d_6, δ [ppm], *J* [Hz]): 174.0 (d, $^{4}J_{C,F}$ = 2.5, 1C), 164.2, 161.4 (dd, $^{1}J_{C,F}$ = 243.4, $^{3}J_{C,F}$ = 12.1, 1C), 160.2 (dd, $^{1}J_{C,F}$ = 245.8, $^{3}J_{C,F}$ = 12.1, 1C), 152.4 (d, $^{1}J_{C,F}$ = 246.1, 1C), 147.8, 144.3 (d, $^{2}J_{C,F}$ = 10.3, 1C), 136.6, 130.8 (dd, $^{3}J_{C,F}$ = 9.8, $^{3}J_{C,F}$ = 6.8, 1C), 122.4 (dd, $^{2}J_{C,F}$ = 14.9, $^{4}J_{C,F}$ = 3.6, 1C), 124.4 (d, $^{4}J_{C,F}$ = 3.4, 1C), 121.4 (d, $^{3}J_{C,F}$ = 6.9, 1C), 111.4 (d, $^{2}J_{C,F}$ = 22.4, 1C), 111.3 (dd, $^{2}J_{C,F}$ = 20.9, $^{4}J_{C,F}$ = 3.5, 1C), 109.9, 105.5 (d, $^{3}J_{C,F}$ = 3.3, 1C), 103.7 (t, $^{2}J_{C,F}$ = 25.6, 1C), 65.8 (2C), 52.8, 49.8 (d, 2C, $^{2}J_{C,F}$ = 4.4, 1C), 36.1 (d, $^{3}J_{C,F}$ = 4.2, 1C), 30.2, 19.1, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -111.89 (m), -114.45 (m), -123.72 (dd, $J_{5,6}$ = 14.4, $J_{6,8}$ = 7.7). Mass: $[M + H]^+$ 474.2 *m/z*, found 474.1 *m/z*. HPLC purity: 98%.

4.1.3.15 *1-Butyl-5-fluoro-N-(4-fluorobenzyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**18**).* A solution of compound **4d** (100 mg, 0.29 mmol) in *N*,*N*-dimethylformamide was treated with NMM (160 µL, 1.45 mmol), *i*-butyl chloroformate (150 µL, 1.16 mmol), and (4-fluorophenyl)methanamine (145 mg, 1.16 mmol) as depicted in

the general procedure 4.1.3. The crude product was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:1, R_f = 0.53) and recrystallized from EtOH to produce 40 mg of **18**. Yield: 31%; mp 191 °C. IR [cm^{-1}]: 3063, 2958, 2860, 1662, 1629, 1603, 1583, 1550, 1534, 1508. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.38 (t, ^3J = 6.0, 1H), 8.65 (s, 1H), 7.39–7.35 (m, 2H), 7.18– 7.13 (m, 2H), 6.95 (dd, ^3J = 15.6, ^4J = 1.6, 1H), 6.65 (d, ^4J = 1.6, 1H), 4.50 (d, ^3J = 6.0, 2H), 4.37 (t, ^3J = 7.2, 2H), 3.76–3.73 (m, 4H), 3.39–3.37 (m, 4H), 1.72 (quint, ^3J = 7.2, 2H), 1.31 (sext, ^3J = 7.2, 2H), 0.90 (t, ^3J = 7.2, 3H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.2 (d, $^3J_{C,F}$ = 1.7, 1C), 164.1, 163.2 (d, $^1J_{C,F}$ = 256.1, 1C), 160.8 (d, $^1J_{C,F}$ = 240.8, 1C), 153.5 (d, $^3J_{C,F}$ = 13.1, 1C), 147.9, 142. (d, $^3J_{C,F}$ = 5.7, 1C), 135.7 (d, $^3J_{C,F}$ = 3.0, 1C), 129.2 (d, $^3J_{C,F}$ = 8.1, 2C), 115.0 (d, $^2J_{C,F}$ = 21.1, 2C), 110.7, 108.7 (d, $^3J_{C,F}$ = 8.5, 1C), 99.2 (d, ^2J = 25.6, 1C), 94.3, 65.6 (2C), 52.9, 46.5 (2C), 42.5, 29.8, 19.1, 13.4. ^{19}F-NMR (DMSO-d_6, δ [ppm], J [Hz]): -109.89 (d, $J_{5,6}$ = 16.6), -116.08 (m). Mass: $[M + H]^+$ 456.2 *m/z*, found 456.0 *m/z*. HPLC purity: 97%.

4.1.4 *N-benzyl-6-fluoro-1-(3-hydroxypropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**19**).* Compound **12** (0.20 g, 0.377 mmol) and a catalytic amount of Pd/C were suspended in chloroform (15 mL). The mixture was pressurized with hydrogen (25 bar) and was heated under microwave irradiation (100 °C/ 500 W) for 12 h. The solvent was then removed *in vacuo* and the residue was purified via column chromatography (eluent: $CHCl_3$/MeOH = 20:1, R_f = 0.22). The white residue was recrystallized from EtOAc to give 110 mg of **19** as pale-white crystals. Yield: 66%; mp 182 °C. IR [cm^{-1}]: 3384, 3070, 2858, 1651, 1628, 1600, 1536, 1488, 1448, 1364, 1259, 1170, 1036. ^{1}H-NMR (DMSO-d_6, δ [ppm], J [Hz]): 10.36 (t, ^3J = 6.8, 1H), 8.78 (s, 1H), 7.87 (d, ^3J = 13.6, 1H), 7.34–7.33 (m, 4H), 7.28–7.24 (m, 1H), 7.20 (d, ^4J = 7.2, 1H), 4.79 (t, ^3J = 4.8, 1H), 4.44–4.49 (m, 4H), 3.79–3.77 (m, 4H), 3.49–3.42 (m, 2H), 3.26–3.23 (m, 4H), 1.94 (quint, ^3J = 7.2, 2H). ^{13}C-NMR (DMSO-d_6, δ [ppm], J [Hz]): 174.0 (d, $^4J_{C,F}$ = 2.5, 1C), 164.0, 153.3 (d, $^1J_{C,F}$ = 247.8, 1C), 147.9, 144.1 (d, $^2J_{C,F}$ = 10.4, 1C), 139.2, 136.6, 128.3 (2C), 127.1 (2C), 126.7, 121.3 (d, $^3J_{C,F}$ = 7.0, 1C), 111.2 (d, $^2J_{C,F}$ = 22.9, 1C), 109.9, 105.5 (d, $^3J_{C,F}$ = 4.8, 1C), 65.7 (2C), 57.0, 50.2, 49.7 (d, $^4J_{C,F}$ = 4.7, 2C) 41.9, 31.1. Mass: $[M + H]^+$ 440.2 *m/z*, found 440.1 *m/z*. HPLC purity: 99%.

4.1.5 *Synthesis of 3-(3-(benzylcarbamoyl)-6-fluoro-7-morpholino-4-oxoquinolin-1(4H)-yl)propyl methanesulfonate (**20**).* Compound **19** (0.230 mg, 0.523 mmol), triethylamine (507 µL, 3.66 mmol) and methanesulfonyl chlorid (80 µL, 1.05 mmol) were dissolved in CH_2Cl_2 under Ar atmosphere at 0 °C. After 12 h of stirring at room temperature, the solvent

was removed under reduced pressure to give the crude product. The purification was carried out by subsequent column chromatography (eluent: $CHCl_3$/MeOH = 100:3, R_r = 0.49) yielded 141 mg of **20** as a white solid. Yield: 52%; mp 232–234 °C. IR [cm^{-1}]: 3168, 3045, 2935, 2830, 1654, 1625, 1601, 1541, 1487, 1352, 1263, 1250, 1169. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 10.37 (t, ^3J = 6.0, 1H), 8.72 (s, 1H), 8.06 (d, ^2J = 13.2, 1H), 7.38–7.32 (m, 4H), 7.28–7.22 (m, 1H), 6.82 (d, ^3J = 7.2, 1H), 4.66 (d, ^3J = 6.0, 2H), 4.40 (t, ^3J = 7.2, 2H), 4.33 (t, ^3J = 5.2, 2H), 3.91–3.89 (m, 4H), 3.29–3.27 (m, 4H), 3.08 (s, 3H), 2.38–2.32 (m, 2H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 175.3 (d, $^4J_{C,F}$ = 2.2, 1C), 164.8, 153.1 (d, $^1J_{C,F}$ = 248.1, 1C), 147.1, 145.2 (d, $^3J_{C,F}$ = 10.5, 1C), 138.7, 136.5, 128.6 (2C), 127.6 (2C), 127.1, 122.4 (d, $^3J_{C,F}$ = 7.4, 1C), 113.2 (d, $^2J_{C,F}$ = 22.9, 1C), 111.7, 103.2 (d, $^4J_{C,F}$ = 2.9, 1C), 66.6 (2C), 66.3, 50.3, 50.2 (d, $^4J_{C,F}$ = 4.2, 2C), 43.3, 37.6, 28.8.

4.1.6 *N-benzyl-6-fluoro-1-(3-fluoropropyl)-7-morpholino-4-oxo-1,4-dihydroquinoline-3-carboxamide (**21**) [39]*. A solution of *N,N*-diethylaminosulfur trifluoride (123 µl, 0.932 mmol) in CH_2Cl_2 (3 mL) was transferred to a solution of **19** (0.205 mg, 0.466 mmol) in CH_2Cl_2 (15 mL) at 0 °C. The reaction was stirred for 20 h at room temperature and followed by quenching by means of 5% $NaHCO_3$ (5 mL). The aqueous layer was extracted with CH_2Cl_2 (4 x 30 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The solid residue was purified by column chromatography (eluent: $CHCl_3$/MeOH = 100:3, R_f = 0.59) to give 110 mg of **21** as white solid. Yield: 53% mp 152 °C. IR [cm^{-1}]: 3196, 3059, 2965, 2906, 2855, 1654, 1627, 1604, 1537, 1485, 1449, 1377, 1359, 1303, 1254, 1206, 1172. ^{1}H-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 10.32 (t, ^3J = 6.0, 1H), 8.67 (s, 1H), 8.01 (d, ^3J = 13.2, 1H), 7.32–7.24 (m, 4H), 7.17–7.15 (m, 1H), 6.81 (d, ^4J = 7.2, 1H), 4.60 (d, ^3J = 7.2, 2H), 4.49 (dt, ^2J = 46.8, ^3J = 5.2, 2H), 4.32 (t, ^3J = 5.2, 2H), 3.85–3.82 (m, 4H), 3.21–3.18 (m, 4H), 2.28–2.16 (m, 2H). ^{13}C-NMR ($CDCl_3$, δ [ppm], *J* [Hz]): 175.3 (d, $^4J_{C,F}$ = 2.2, 1C), 164.8, 153.3 (d, $^1J_{C,F}$ = 248.1, 1C), 147.1, 145.1 (d, $^2J_{C,F}$ = 10.6, 1C), 138.8, 136.7, 128.6 (2C), 127.6 (2C), 127.1, 122.5 (d, $^3J_{C,F}$ = 7.3, 1C), 113.1 (d, $^2J_{C,F}$ = 22.9, 1C), 111.8, 103.2 (m, 1C), 80.2.3 (d, $^1J_{C,F}$ = 165.4, 1C), 66.6 (2C), 50.2 (d, $^4J_{C,F}$ = 4.2, 2C), 49.8 (d, $^3J_{C,F}$ = 3.1, 1C), 43.3, 37.6 (d, $^2J_{C,F}$ = 19.9, 1C). ^{19}F-NMR (DMSO-d_6, δ [ppm], *J* [Hz]): -123.80, -219.87. Mass: $[M + H]^+$ 442.2 *m/z*, found 442.2 *m/z*. HPLC purity: 97%.

4.1.7 *Radiosynthesis of [^{18}F]**21***. [^{18}F]Fluoride was separated from enriched water by SPE using an anion exchange cartridge (Sep-Pak Accell QMA light). It was then eluted with a solution of potassium carbonate (10 mg/mL, 400 µL) into a 5 mL conical vial containing a

solution of kryptofix (12 mg) in dry ACN 0.7 mL. The reaction mixture was dried with the addition of dry ACN (2 x 700 µL) under argon flow at 90 °C. The precursor **20** (5 mg, 9.0 µmol) in DMF (400 µL) was added to the dried [^{18}F]fluoride and the reaction mixture was heated at 120 °C for 5 min in sealed vial. The reaction mixture was cooled for 2 min and diluted with water (400 µL). Purification was carried out by radio-HPLC using a Nucleosil 100-10 C_{18}, 10 x 250 mm column (Macherey-Nagel) and a mobile phase of 50% (v/v) ACN/water at 5 mL/min. The radioactive fraction was collected and diluted with water (50 mL) and passed through a tC_{18} cartridge (Waters Sep-Pak Accell Light tC_{18} cartridge, prepared by washing with 10 mL of ethanol and then rinsing with 10 mL of water). The cartridge was washed with additional 10 mL of water and the product was eluted with ethanol (1 mL) and formulated with saline solution (RCY 60 ± 5%). The identity and radiochemical purity of the radiotracer were confirmed by co-injection with corresponding standard **21** using RP-HPLC with a Nucleosil 100-7 C_{18}, 4.6 x 250 mm column (Macherey-Nagel) and a mobile phase of 60% (v/v) ACN/water at 1 mL/min (t_R = 9.4 min) (cf. Fig. S7).

4.2 Biological assays

4.2.1 *Antitrypanosomal Assay.* Trypomastigote forms of *T. brucei brucei* laboratory strain TC 221 were cultured in Baltz medium according to standard conditions [18]. The AlarmarBlue® assay was realized according to previously reported procedure [7, 8, 19, 20]. Briefly, a defined number of parasites (10^4 trypanosomes per mL) were exposed in test chambers of 96 well plates to various concentration levels of the test substances in a final volume of 200 µL. Positive (trypanosomes in culture medium) and negative controls (test substance without trypanosomes) were run with each plate. The plates were then incubated at 37 °C in an atmosphere of 5% CO_2 for a total time period of 72 h. The effect of test substances was quantified in IC_{50} values by linear interpolation of two different measurements. The activity of the test substances was measured by light absorption in a MR 700 microplate reader at a wavelength of 550 nm with a reference wavelength of 630 nm, using the AlamarBlue®. The tests were performed in duplicate and IC_{50} values are presented as mean values of two independent experiments against the parasites.

4.2.2 *Macrophage Assay*. The macrophage cell line J774.1 was cultured in RPMI-1640 medium, supplemented with 10% FCS, 10 U/mL penicillin G, 10 µg/mL streptomycin, and 50 µM 2-mercaptoethanol in an atmosphere of 37 °C, 5% CO_2, 95% humidity. For the experimental procedure, previously reported protocol was applied [7, 8]. Briefly, the cells

were detached from the flasks with a cell scraper and cell densities were adjusted. J774.1 macrophages were seeded into the chambers of the 96 well plate and were incubated overnight to allow attachment and recovery. Then, the compounds were diluted in DMSO and incubated for 24 h with the cells. Following the addition of AlamarBlue® (20 µL) the plates were further incubated. The absorbance was read at a wavelength of 550 nm (reference wavelength 630 nm) indicating the viability. The CC_{50} values are presented as mean values of two independent experiments against the macrophages.

4.3 Metabolism

For investigating the phase I metabolism of **7**, **15**, and **18**, incubations were carried out with human liver microsomes, human and rat S9, and human cytosol at a protein concentration of 1 mg/mL, respectively. The incubations contained the compounds **7**, **15**, and **18** (100 µM), protein (1 mg/mL), phosphate buffer (0.1 M, pH 7.4), and $NADPH/H^+$ as a cofactor. Solutions for elucidating the metabolism were stored in a water bath at 37 °C for 0, 30, 60, and 90 min. The reactions were terminated by adding 500 µL of EtOAc. 7-(4-acetylpiperazin-1-yl)-*N*-(2,4-dichlorobenzyl)-6-fluoro-1-(2-fluorophenyl)-4-oxo-1,4-dihydroquinoline-3-carboxamide was added as internal standard and the reaction mixture was extracted three times with EtOAc. After evaporating the solvent, the residues were dissolved in 25 µL ACN and 20 µL were subjected to HPLC with UV detection at λ = 282 nm (Hewlett Packard Agilent Series 1100) to determine rate of decrease of the compounds **7**, **15** and **18**. For identifying the metabolites, an Agilent 1100 LC/MSD Trap SL was used using an electrospray ionization technique operating in positive-ion mode. HPLC separation was carried out using a Zorbax SB-C_{18} column (100 x 3 mm, 3.5 µm particle size) (Agilent Technologies); mobile phase A: water 0.5% FA, mobile phase B: ACN 0.5% FA: 5% B for 0 min, gradually increasing to 95% B within 25 min; flow rate: 0.5 ml/min; injection volume: 5 µL; temperature: 25 °C. Three independent incubations were performed.

The electrospray ionization interface parameters of the Agilent 1100 LC/MSD Trap SL were set as follows: capillary voltage 3.5 kV, source temperature 350°C, nebulizer gas 700 psi, dry gas 12 $Lmin^{-1}$, and fullscan modus 100-600 m/z. The fragmentation conditions are as follows: Manual MS(n); MS/MS: m/z to the respective metabolite; width: 4; amplitude: 1; amplitude by smart fragmentation: 30-200%; isolation and fragmentation activated at the system; time per fragmentation: 40 ms; cut off: m/z 430→ 116; m/z: 472 → 127; m/z: 454 → 123; m/z: 375 → 101.

4.4 logP Determination

The logarithmized capacity factor of the calibration substances was correlated with the experimental octanol/water logP values, and the resulting linear equation was utilized to calculate the logP value of the tested compounds [7, 27]. The following substances were used for calibration: acetanilide, 2-phenylethanol, benzene, toluene, chlorobenzene, ethylbenzene, biphenyl, and anthracene. A linear regression was performed for the log *k′*/logP data of the reference compounds ($y = 2{,}1393x + 1{,}6278$; $R^2 = 0{,}97551$). The regression equation was used to calculate the logP of the compounds (cf. Fig. S2).

4.5 Solubility

For assessing the thermodynamic solubility of compounds **5-7**, **10**, **19**, and **21**, the continuous shake flask protocol according to reference [8] was applied. The substance was dosed in excess into reaction tubes and dissolved in PBS buffer (pH 7.4). Samples were taken throughout a period of 24 h of continuous shaking (800 rpm) and constant warming (37 °C). After centrifugation (13.000 rpm, 1 min), the supernatant was analysed by HPLC-UV (detection wavelength λ = 280 nm) with a Eurosphere II 100-5, $C_{18}H$ column (Knauer, Berlin, Germany) and a mobile phase of ACN/water (72/28 v/v) at a flow rate of 1 mL/min.

4.6 Ex vivo autoradiographical study

[^{18}F]**21** (19.3±2.3 MBq) was administered intravenously into B6/J mice (n = 2) under 1.5% isoflurane anesthesia. The mice were sacrificed 60 min after injection. Brains were dissected and cut in 3 mm sections of brain tissue and exposed on a phosphor image plate (Biostep, Jahnsdorf, Germany) over night. The image plate was read on an image plate reader (Dürr Medical, Bietigheim-Bissingen, Germany) and data analysis was performed using the software AMIDE Medical Image Data Examiner (Version 1.0.4).

Animal investigation was approved by the local district government (Regierung von Unterfranken), 55.2-2531.01-23/11.

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6 Author Information

The authors declare no competing financial interest. MB did the synthesis, determined the lipophilicity and wrote the main part of the manuscript, CE studied the metabolism, AF and JS performed the trypanosoma testing, EAM and II performed the autoradiograhy and synthesized the labelled compound, MR and PG determined the solubility, SS supervised the autoradiography and participate in writing the paper, UH initiated and supervised the study and wrote parts of the manuscript.

7 Appendix: supplementary material

Supplementary data associated with this article can be found in the online version, at https://doi.... These data include synthesis of compound **1a-h**, logP calibration curve, metabolism schemes of compound **7**, **15**, and **18**, thermodynamic solubility, [^{18}F]-Labelling, autoradiography, NMR spectra of compound **5-11**, **13-19**, **21**.

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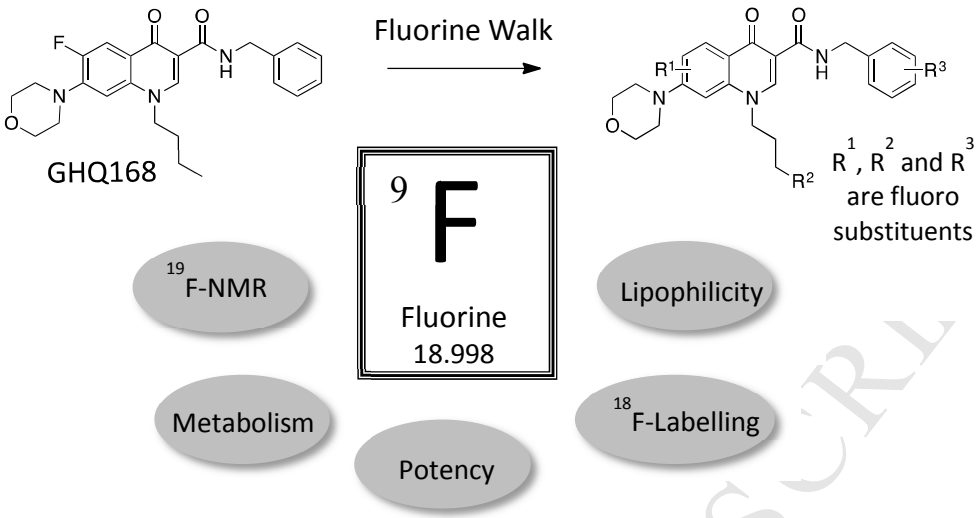
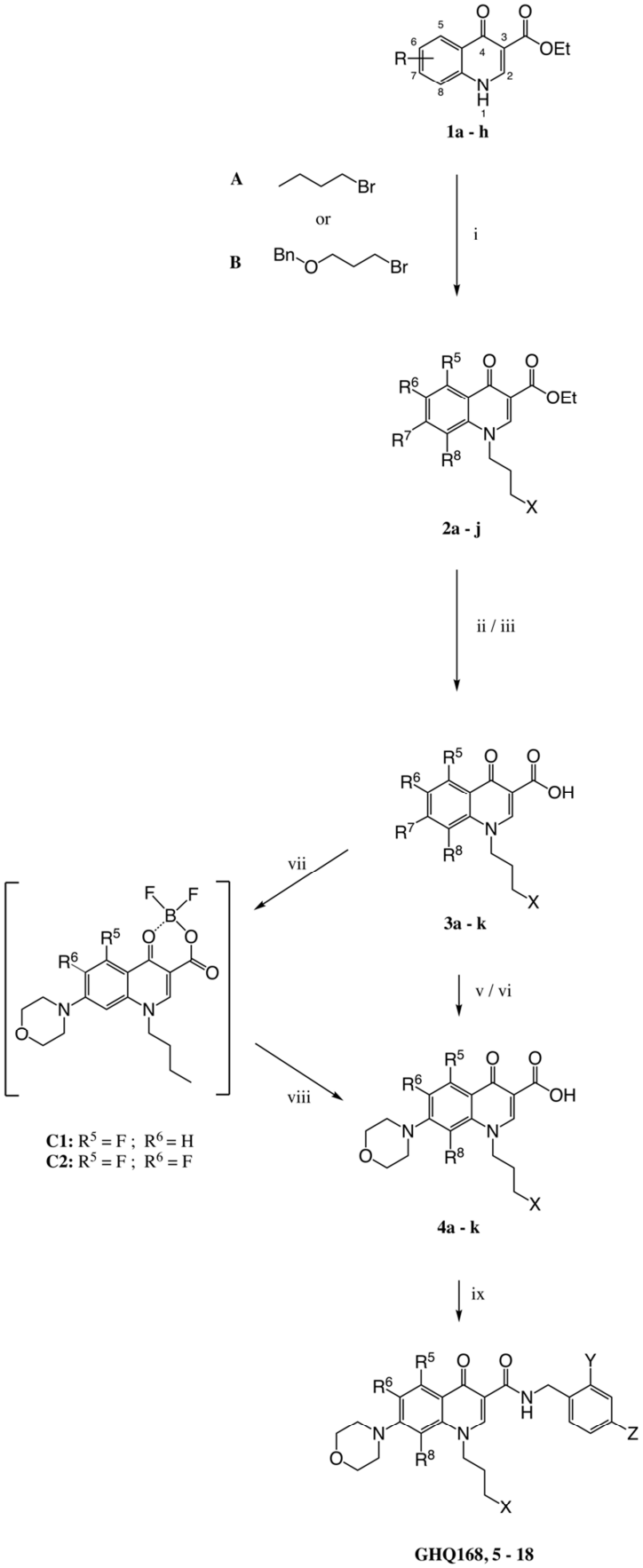
1181 Graphical Abstract

1182



1183

1184 Scheme 1[a]



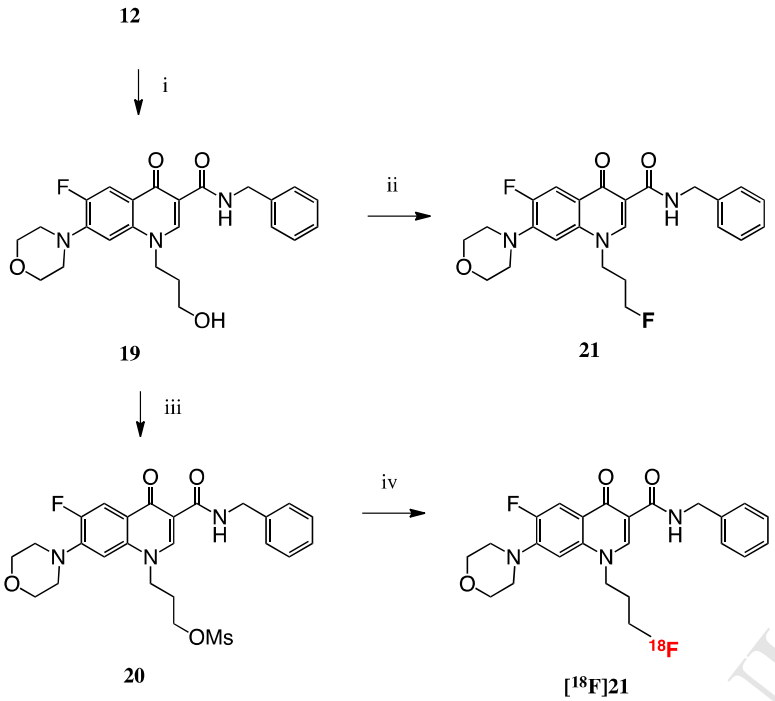
compd	R^5	R^6	R^7	R^8	X
a	H	F	Cl	H	CH_3
b	H	F	Br	H	CH_3
c	H	H	Cl	F	CH_3
d	F	H	F	H	CH_3
e	H	F	F	F	CH_3
f	F	F	F	H	CH_3
g	H	OCH_3	Cl	H	CH_3
h	H	CF_3	F	H	CH_3
i	H	F	Cl	H	OBn
j	$N(CH_3)_2$	H	H	H	CH_3
3k	OCH_3	H	F	H	CH_3

compd	R^5	R^6	R^8	X	Y	Z
GHQ168	H	F	H	CH_3	H	H
5	H	H	H	CH_3	H	H
6	H	H	F	CH_3	H	H
7	F	H	H	CH_3	H	H
8	H	F	F	CH_3	H	H
9	F	F	H	CH_3	H	H
10	H	OCH_3	H	CH_3	H	H
11	H	CF_3	H	CH_3	H	H
12	H	F	H	OBn	H	H
13	$N(CH_3)_2$	H	H	CH_3	H	H
14	OCH_3	H	H	CH_3	H	H
15	H	F	H	CH_3	H	F
16	H	F	H	CH_3	F	H
17	H	F	H	CH_3	F	F
18	F	H	H	CH_3	H	F

1185

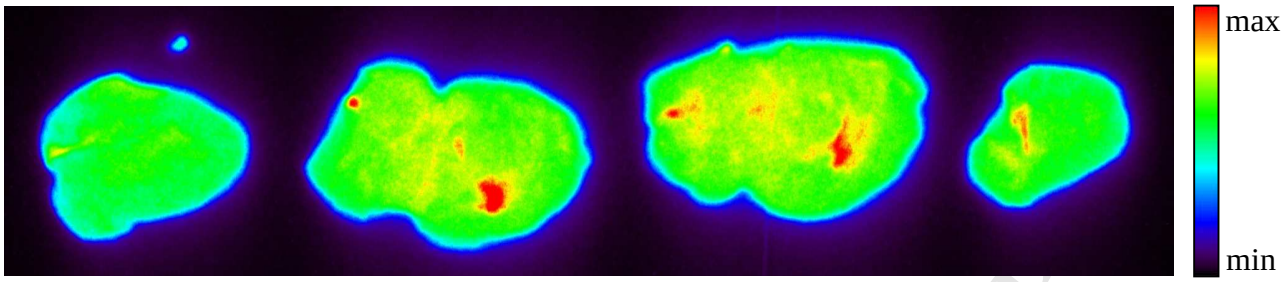
[a]Reagents and conditions: (i) alkyl bromide, K_2CO_3, KI, $DMF_{abs.}$, 80 °C (ii) 2 M HCl, reflux; (iii) 3 M KOH, reflux; (iv) NaH, $MeOH_{abs.}$, 90 °C (v) morpholine, MW (500 W), 110 °C; (vi) morpholine, DMF, 130 °C (vii) 1) $BF_3 \bullet OEt_2$, $DCM_{abs.}$, reflux 2) morpholine, TEA, EtOH, 80 °C; (viii) 2 M NaOH, reflux; (ix) benzyl amine derivative, NMM, *i*-butyl chloroformate, $DMF_{abs.}$, 0 °C/rt

Scheme 2[a]



[a]Reagents and conditions: (i) Pd/C, $CHCl_3$, MW (500 W), 100 °C, H_2 (25 bar); (ii) DAST, $DCM_{abs.}$, 0 °C/rt; (iii) methanesulfonyl chloride, TEA, $DCM_{abs.}$, 0 °C/rt; (iv) $K^{18}F$, Kryptofix, ACN, 120 °C

Figure 2: Ex-vivo autoradiography of brain sections 60 min after [^{18}F]**21** injection. Murine brain was cryosectioned in sagittal direction in four parts. Red areas determine a high accumulation of [^{18}F]**21**.



1208 Table 1: LogP value, antitrypanosomal activity, cytotoxicity and selectivity index of selected
1209 quinolone amide derivatives.

Compound	LogP	IC_{50}[a] [µM]		CC_{50}[b] [µM]	Selectivity index (SI)
		48h	72h		
GHQ168[7]	4.10	0.047 ± 0.00	0.05 ± 0.01	57	1212
5	3.51	0.23 ± 0.01	0.20 ± 0.02	>100	>435
6	4.09	0.79 ± 0.06	0.86 ± 0.00	>25[d]	>32
7	3.36	0.05 ± 0.01	0.08 ± 0.05	>100	>2000
8	4.57	0.06 ± 0.00	0.20 ± 0.01	>20[d]	>333
9	3.97	0.04 ± 0.02	0.03 ± 0.00	>25[d]	>625
10	3.73	0.31 ± 0.16	0.24 ± 0.05	42.0 ± 2.8	135
11	4.18	0.54 ± 0.01	0.59 ± 0.00	78.6 ± 1.9	146
12	3.90	ND[c]	ND	>100	ND
13	ND	1.85 ± 0.26	4.80 ± 1.07	44.0 ± 0.4	24
14	ND	0.76 ± 0.01	0.65 ± 0.00	51.8 ± 0.8	68
15	4.13	0.05 ± 0.01	0.09 ± 0.02	>20[d]	>400
16	ND	0.41 ± 0.07	0.46 ± 0.00	37.0 ± 1.6	90
17	ND	0.03 ± 0.00	0.03 ± 0.00	59.6 ± 0.4	1987
18	3.41	0.02 ± 0.00	0.02 ± 0.00	>25[d]	>1250
19	3.04	0.27 ± 0.07	0.55 ± 0.05	43.1 ± 2.0	160
21	3.34	0.12 ± 0.06	0.17 ± 0.11	42.4 ± 1.8	353

1210

1211 [a] IC_{50}: growth inhibition of *T. b. brucei* strain TC 221. Values represent the mean of two
1212 experiments.

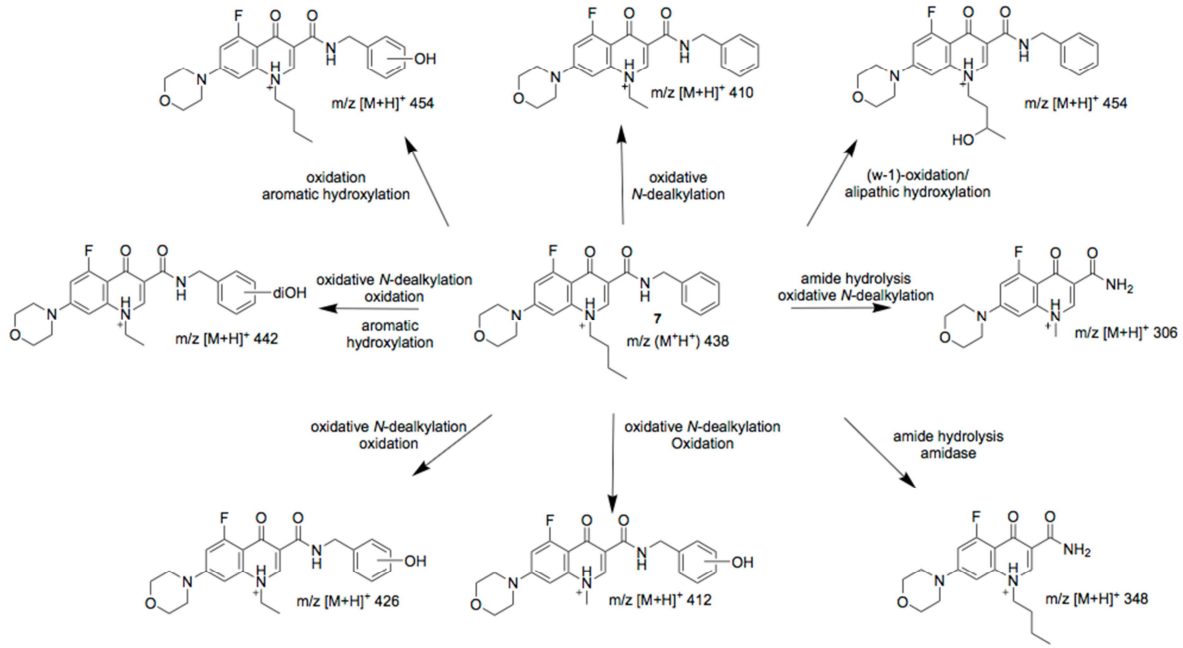
1213 [b] CC_{50}: growth inhibition of the macrophage cell line J774.1. Values represent the mean of
1214 two experiments.

1215 [c] ND, not determined.

1216 [d] Precipitation occurred at this concentration level, thus no further statements about
1217 cytotoxicity can be made.

1218

1219 Scheme 3: Metabolic pathways of compound **7**



1220

1221

1222 Table 2: Cytosolic turnover of selected compounds.

Substrate	**Turnover** (pmol×min^{-1}×mg×protein^{-1})
7	4.30 ± 0.23
15	5.94 ± 1.65
18	3.89 ± 0.29
GHQ168	47.11 ± 20.84 [20]

1223

Highlights:

- Quinolone amides against *Trypanosoma brucei*
- Fluorine walk to improve trypanocidal activity, cytotoxicity and metabolic stability
- Autoradiography studies to check the passage of the blood-brain barrier.

Fluorine Walk: The Impact of Fluorine in Quinolone Amides on their Activity against African Sleeping Sickness

Berninger, Michael[1]; Erk, Christine[1]; Fuß, Antje[2]; Skaf, Joseph[1]; Al-Momani, Ehab[3]; Israel, Ina[3]; Raschig, Martina[1]; Güntzel, Paul[1]; Samnick, Samuel[3]; Holzgrabe, Ulrike[1*]

[1] Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

[2] Medical Mission Institute Würzburg, Hermann-Schell-St. 7, 97074 Würzburg, Germany

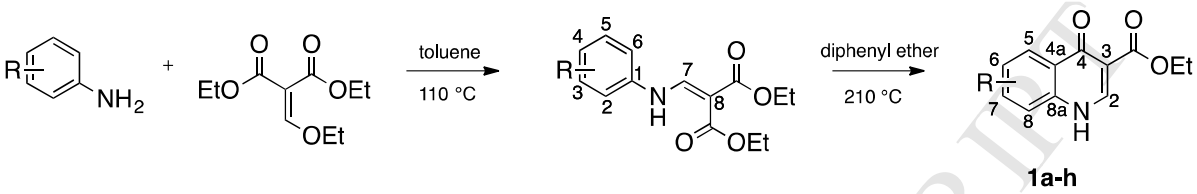
[3] Department of Nuclear Medicine, University Hospital of Würzburg, Oberduerrbacher-Str. 6, 97080 Würzburg, Germany

Supplementary material

Content:

Synthesis and spectroscopic data

Fig S1: Gould Jacobs Synthesis



	1a[1]	1b[2]	1c[3]	1d[4]	1e[5]	1f[6]	1g[7]	1h[8]
R^5	H	H	H	F	H	F	H	H
R^6	F	H	H	H	F	F	OCH_3	CF_3
R^7	Cl	Br	Cl	F	F	F	Cl	F
R^8	H	H	F	H	F	H	H	H

General synthesis of the ethyl-4-hydroxyquinoline-3-carboxylate 1a-h.

A solution of the appropriate aniline derivative (1 eq) in toluene (20-50 mL) was treated with diethyl 2-(ethoxymethylene)malonate (1.2 eq) and was refluxed for 15-20 h. The solvent was removed under reduced pressure and the crude product was recrystallized from *n*-hexane at -20 °C. After that, the resulting diethyl (2-(amino)methylene)malonate was dissolved in 5-10 mL diphenyl ether and was reacted for 20-60 min at 210 °C under microwave irradiation.

Fig S2: Calibration curve for determination of logP values

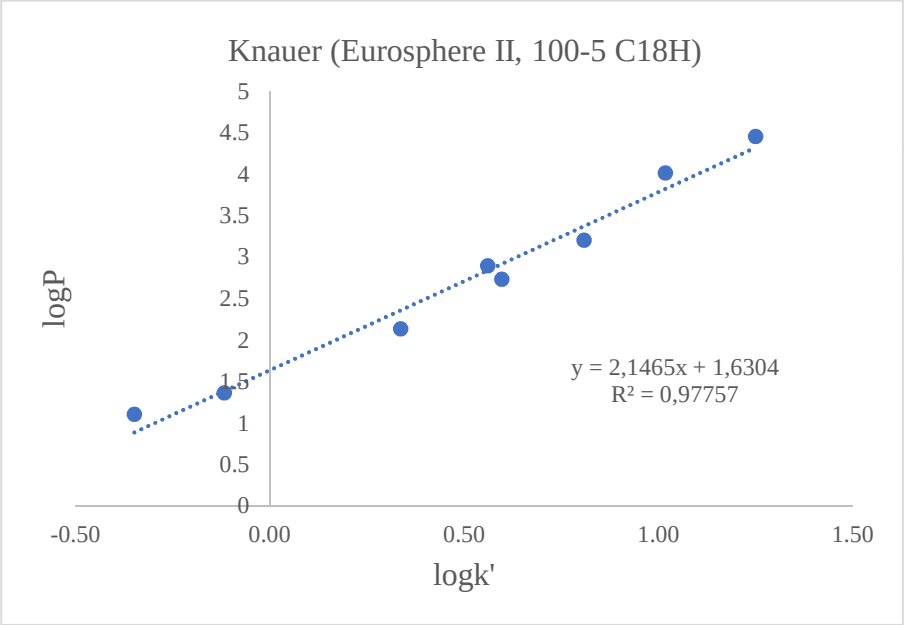


Fig S3: Fragmentation of compound **7**

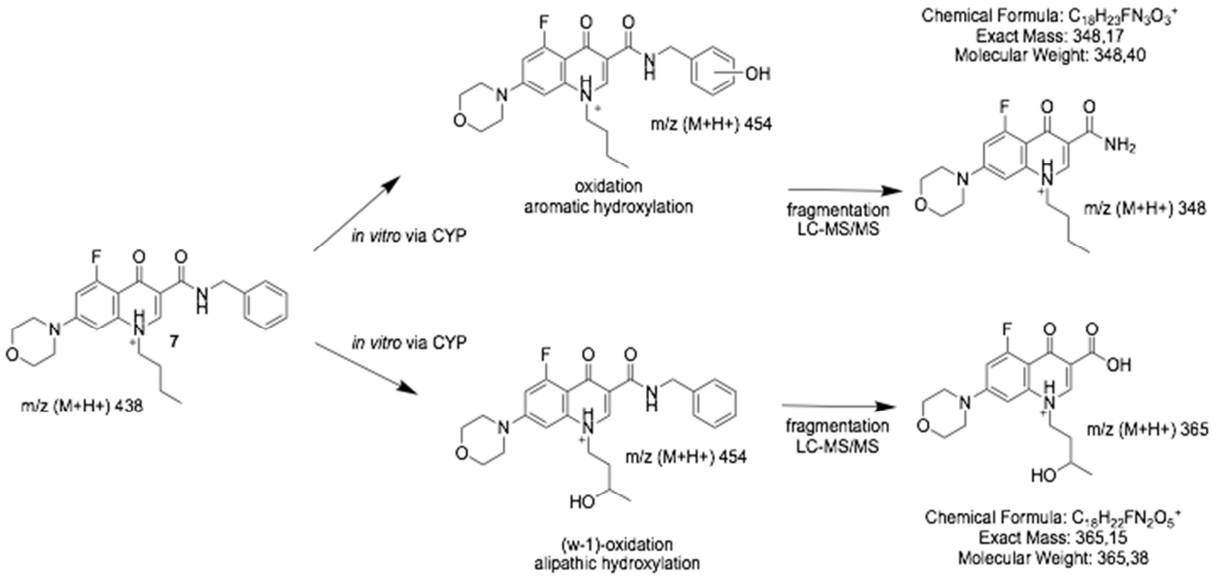


Fig S4: Metabolites of compound **15**

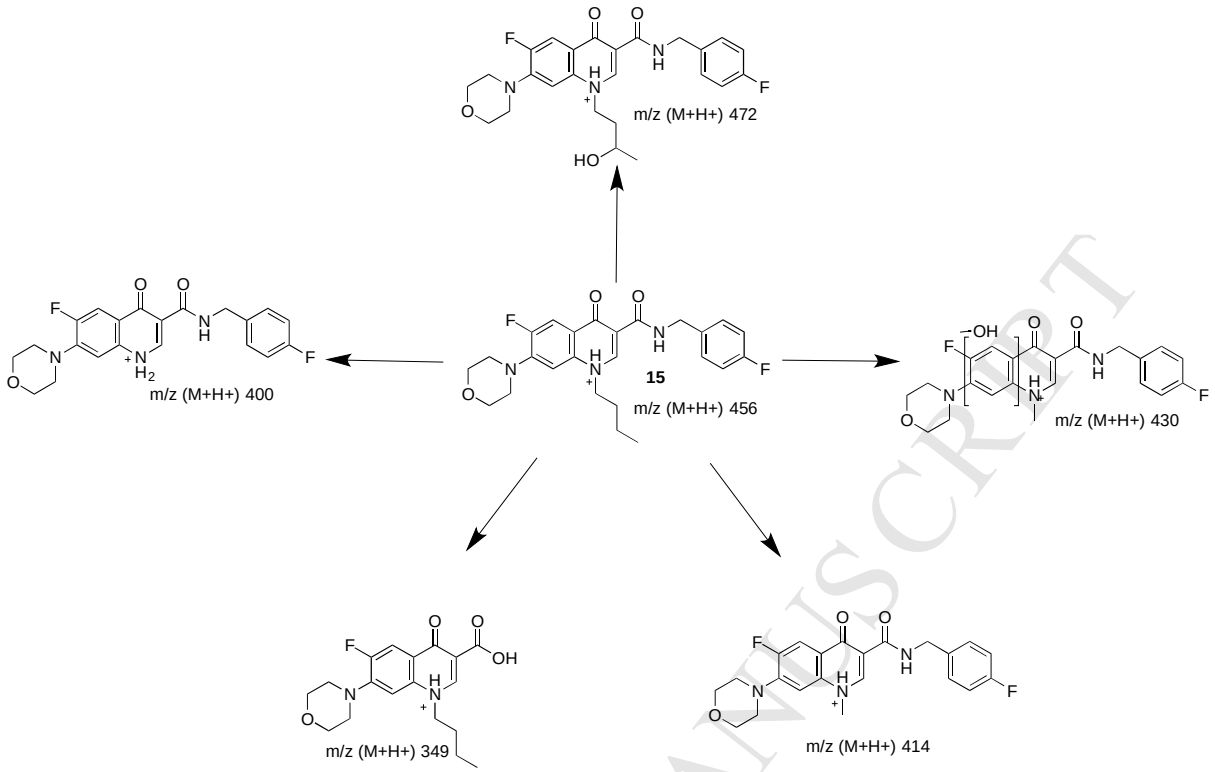


Fig S5: Metabolites of compound **18**

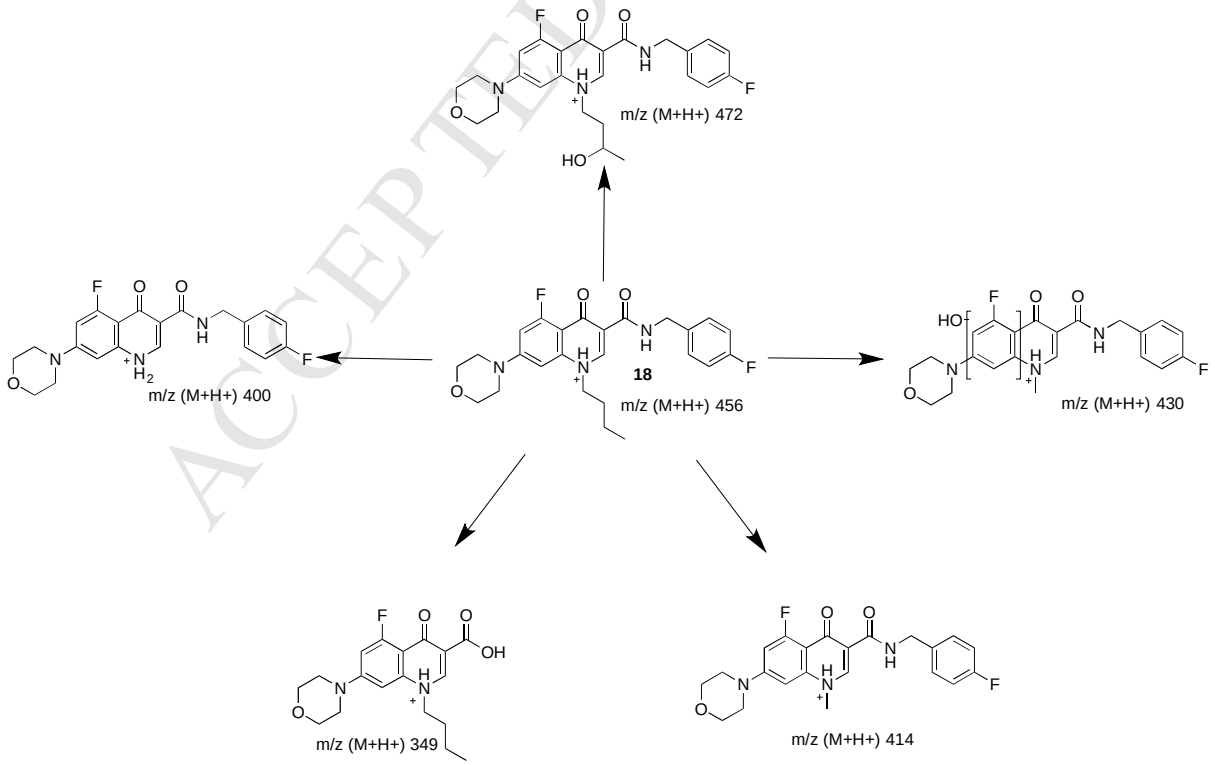


Fig S6: Thermodynamic solubility of the quinolone amides.

compd	mp	logP	S_w [µg/mL]
GHQ168	171	4.10	0.005 ± 0.01[9]
5	172	3.15	1.36 ± 0.00
6	170	4.09	0.23 ± 0.12
7	200	3.38	0.12 ± 0.03
10	156	3.68	2.73 ± 0.42
19	182	3.04	18.70 ± 0.90
21	152	3.36	1.41 ± 0.01

S_w: Measured equilibrium solubility in PBS 7.4 according ref [9].

Fig S7: Co-injection of the [^{18}F]**21** (Red) with the non-radioactive reference **21** (Blue). (Nucleosil 100-7 C18, 4.6 x 250 mm column (Macherey-Nagel) and ACN/water 60% (v/v) as mobile phase at 1 mL/min)

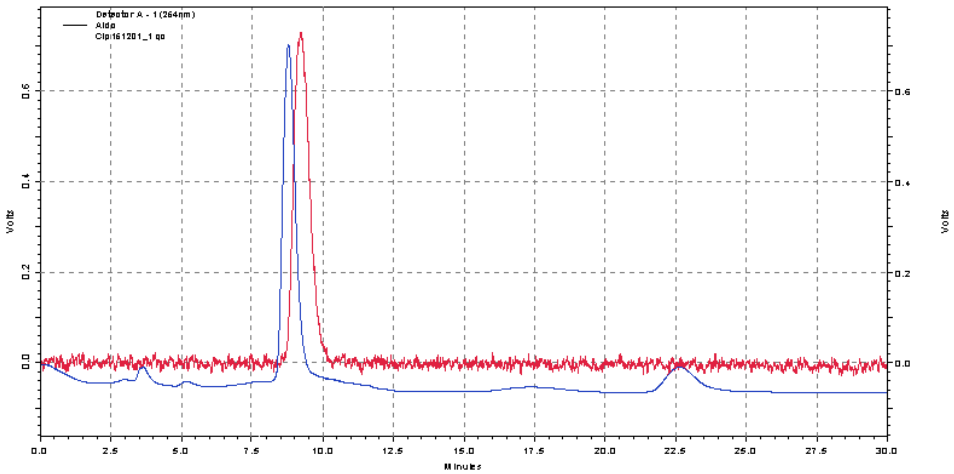
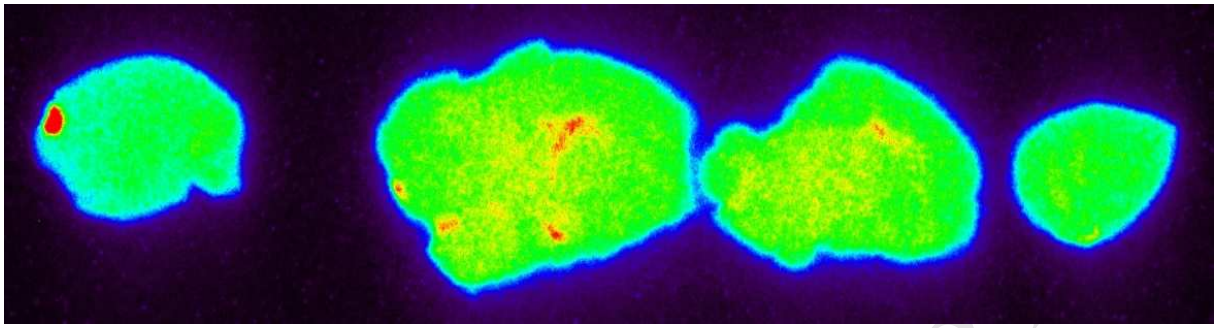
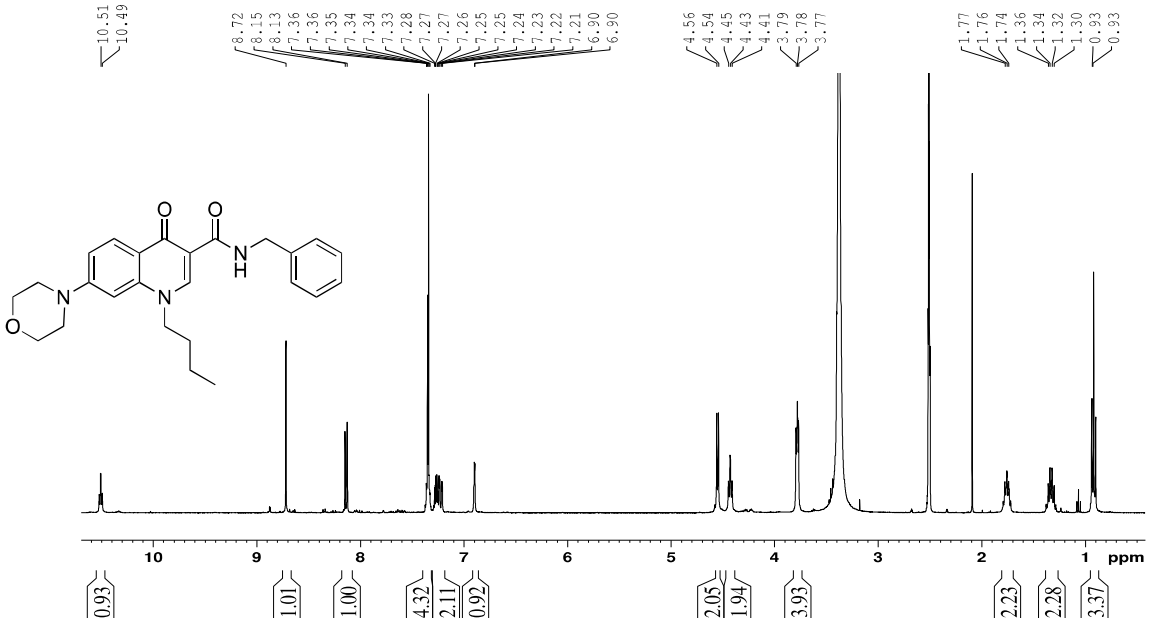
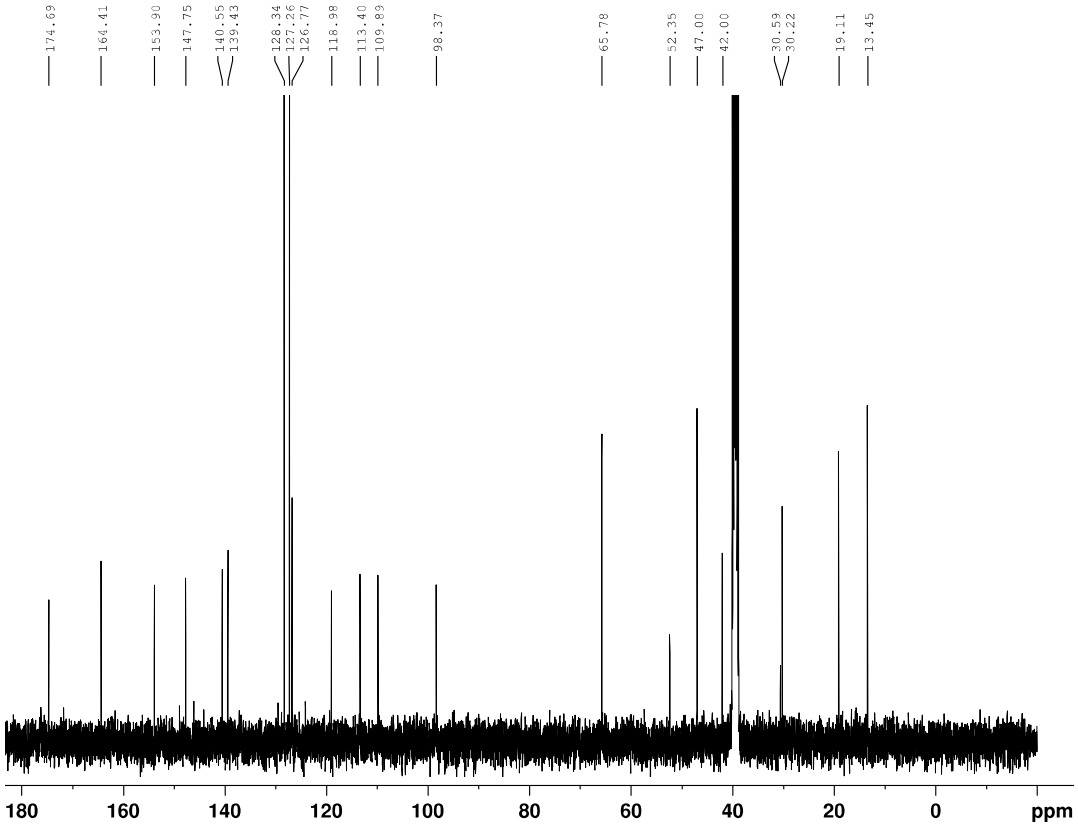


Fig S8: Ex-vivo autoradiography of brain sections from mouse #2 sacrificed and cryosectioned at 60 min after [^{18}F]**21** injection. The autoradiographic images confirming the uptake of [^{18}F]**21** in healthy brain after intravenous injection.

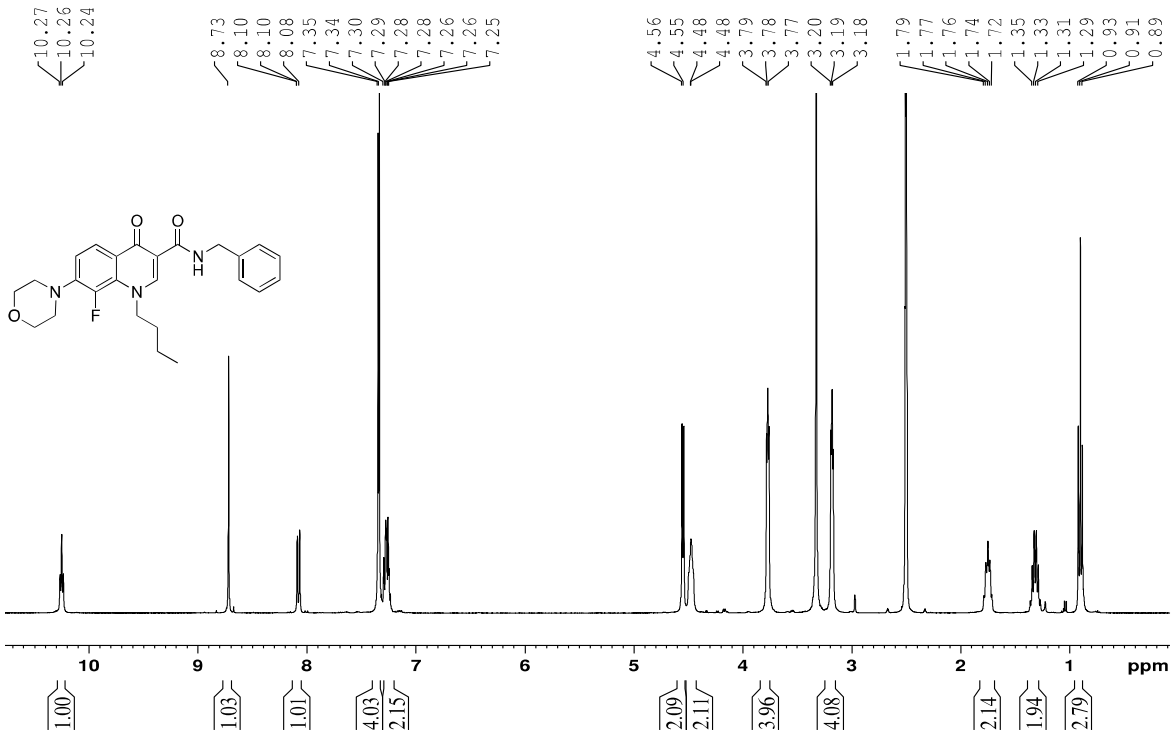




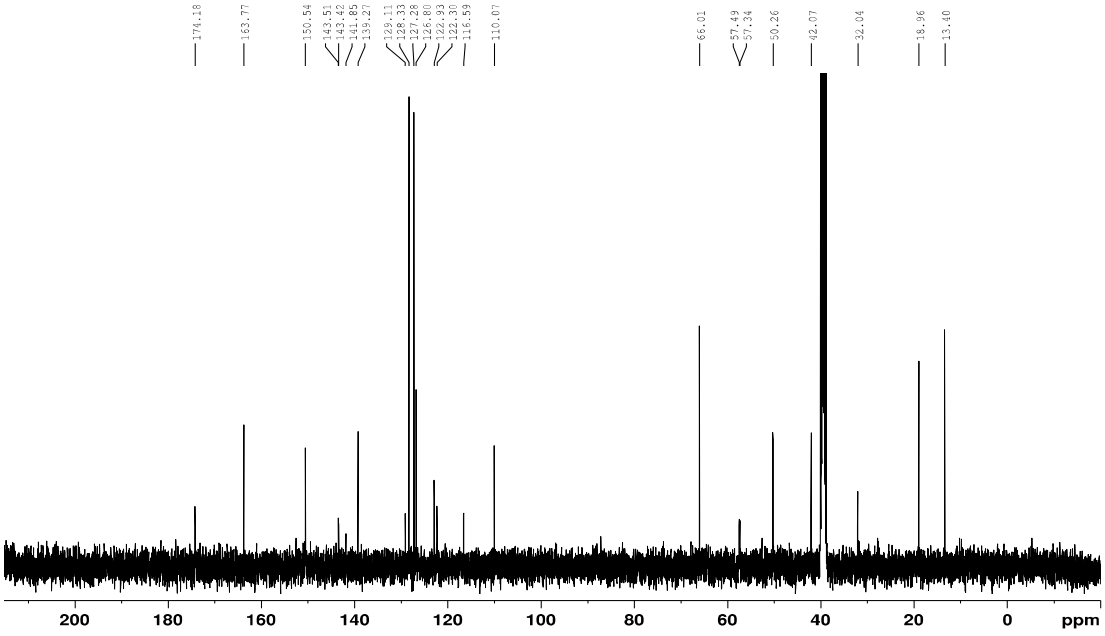
1 H NMR spectra of compound **5**



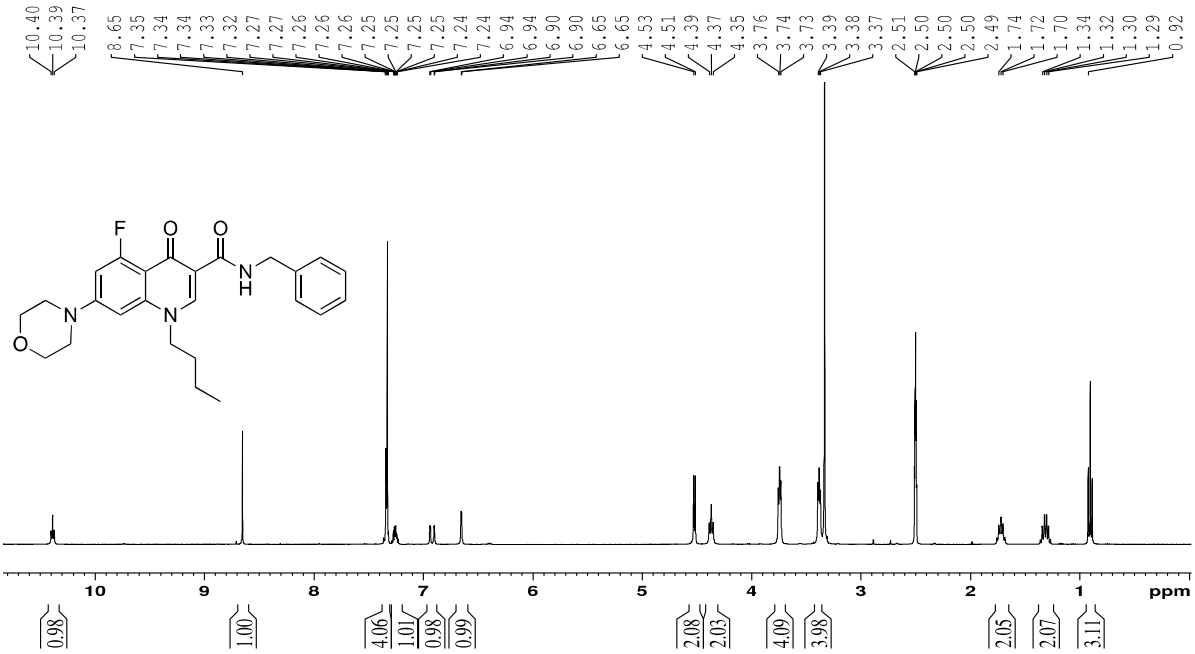
13 C NMR spectra of compound **5**



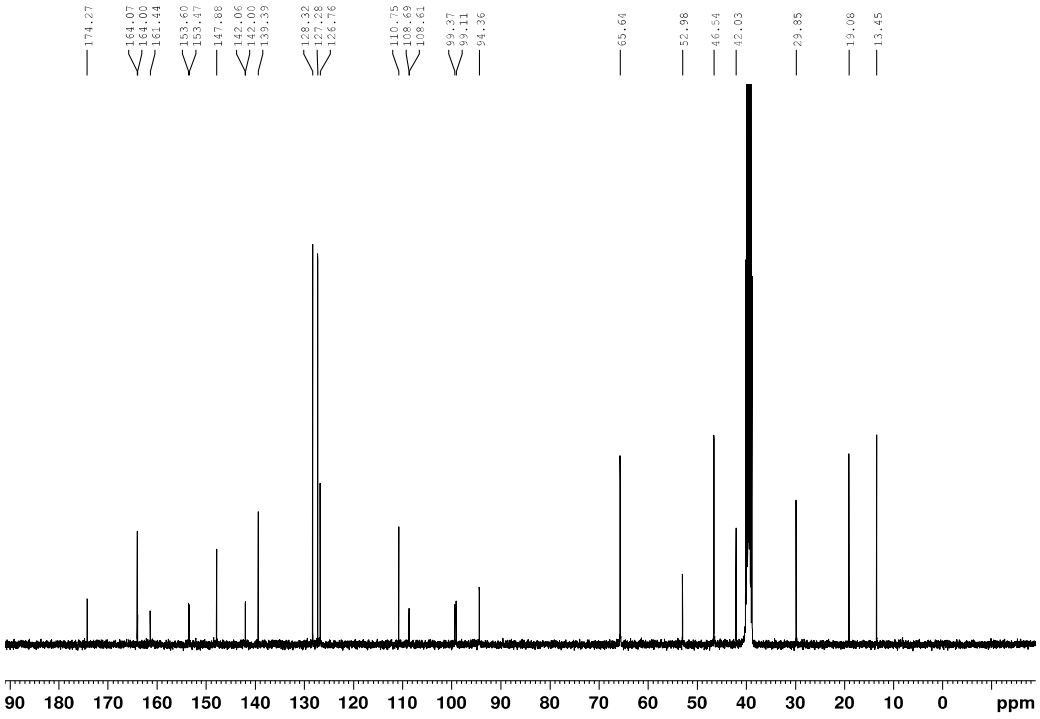
1 H NMR spectra of compound **6**



13 C NMR spectra of compound **6**



1 H NMR spectra of compound **7**



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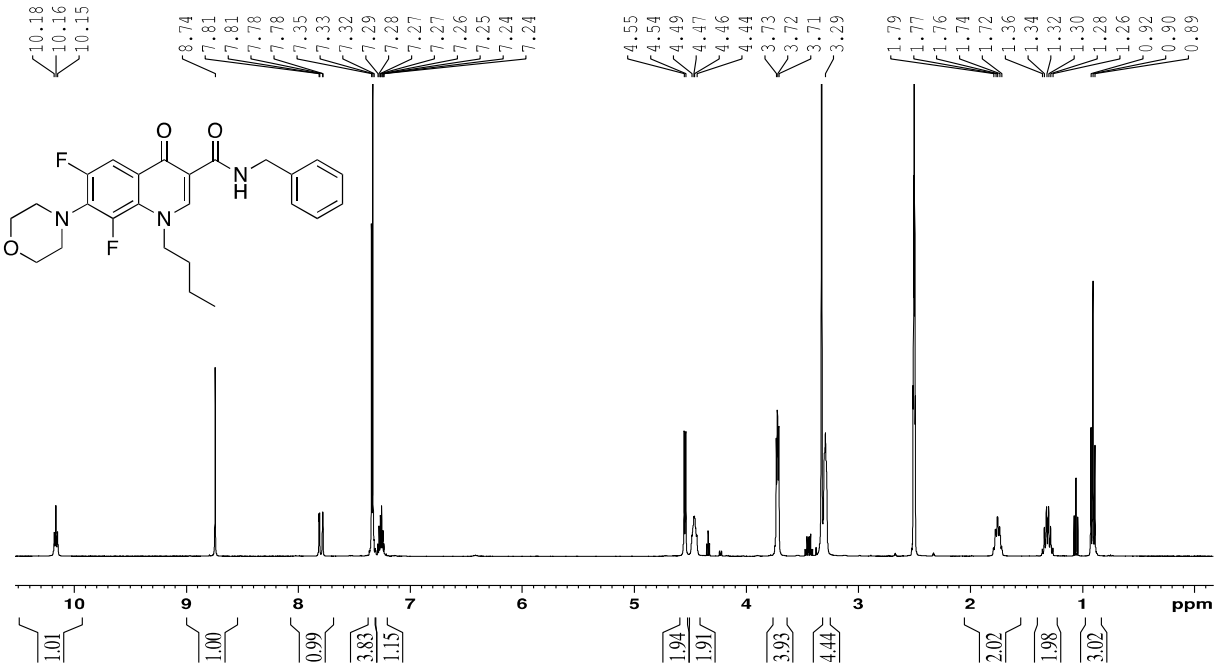
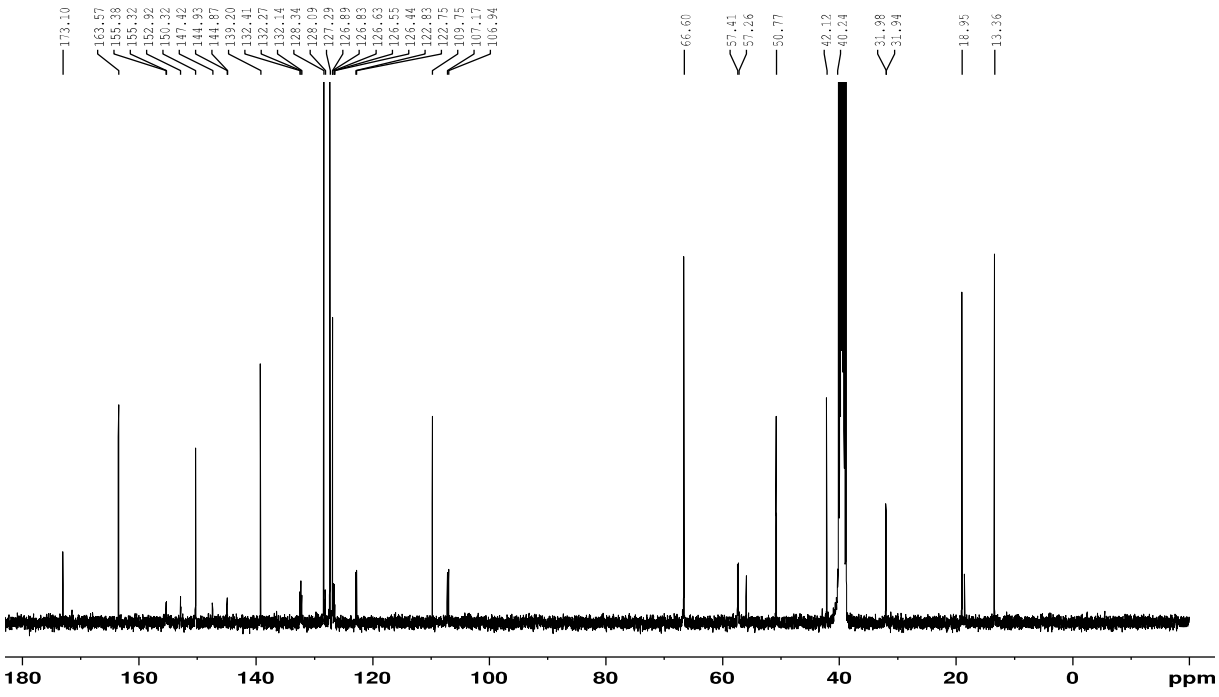
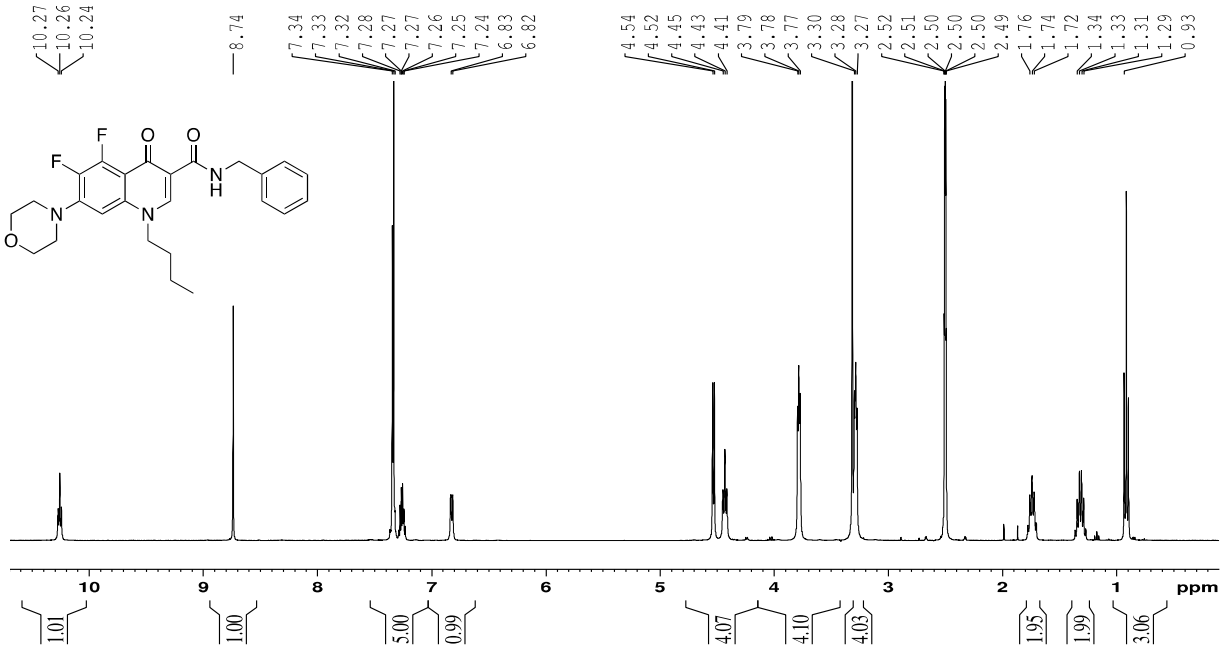


1 H NMR spectra of compound **8**



13 C NMR spectra of compound **8**



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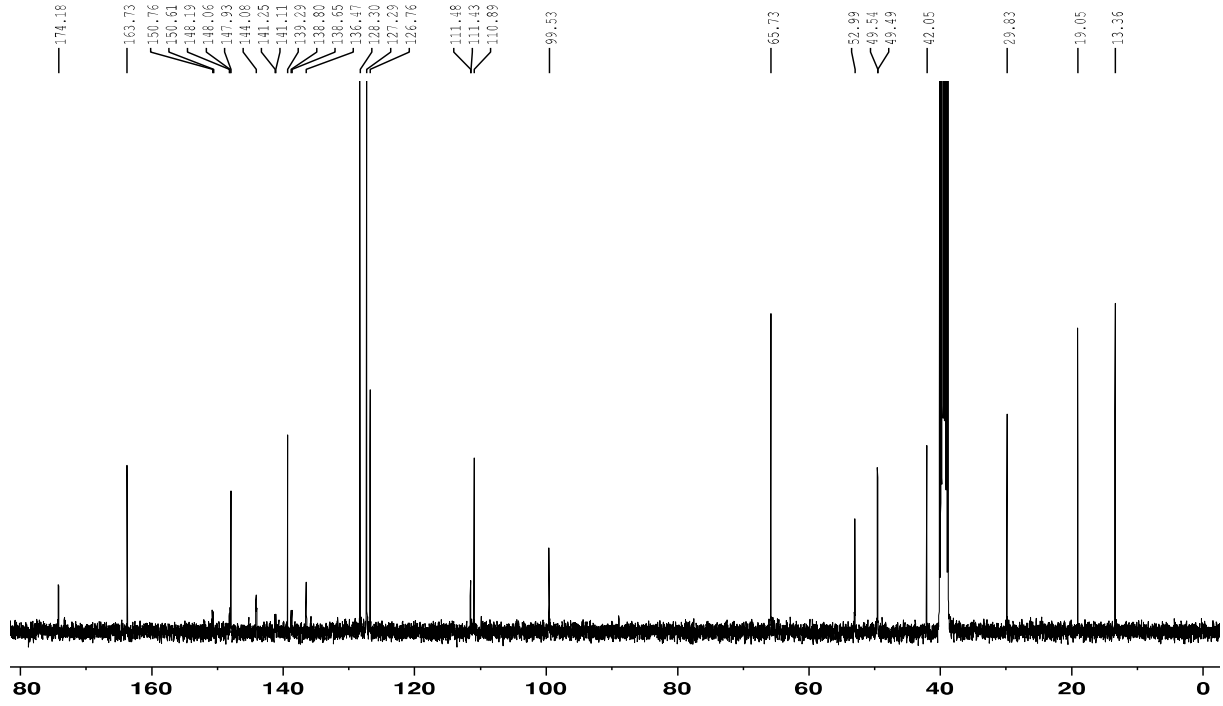

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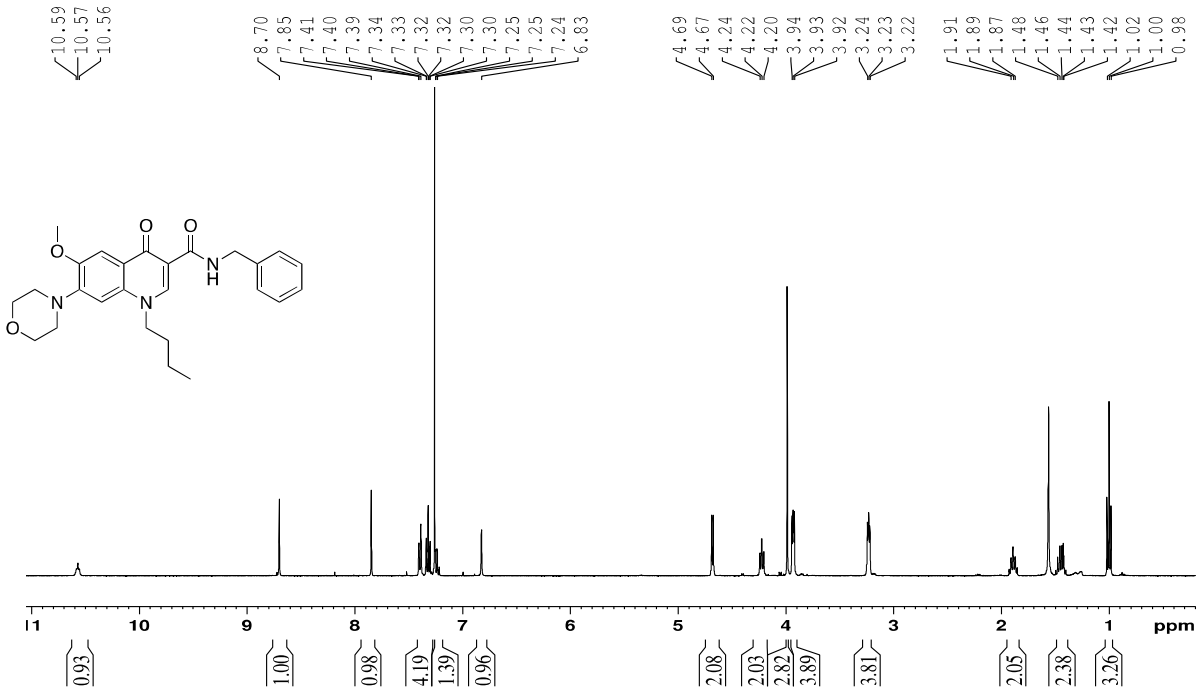

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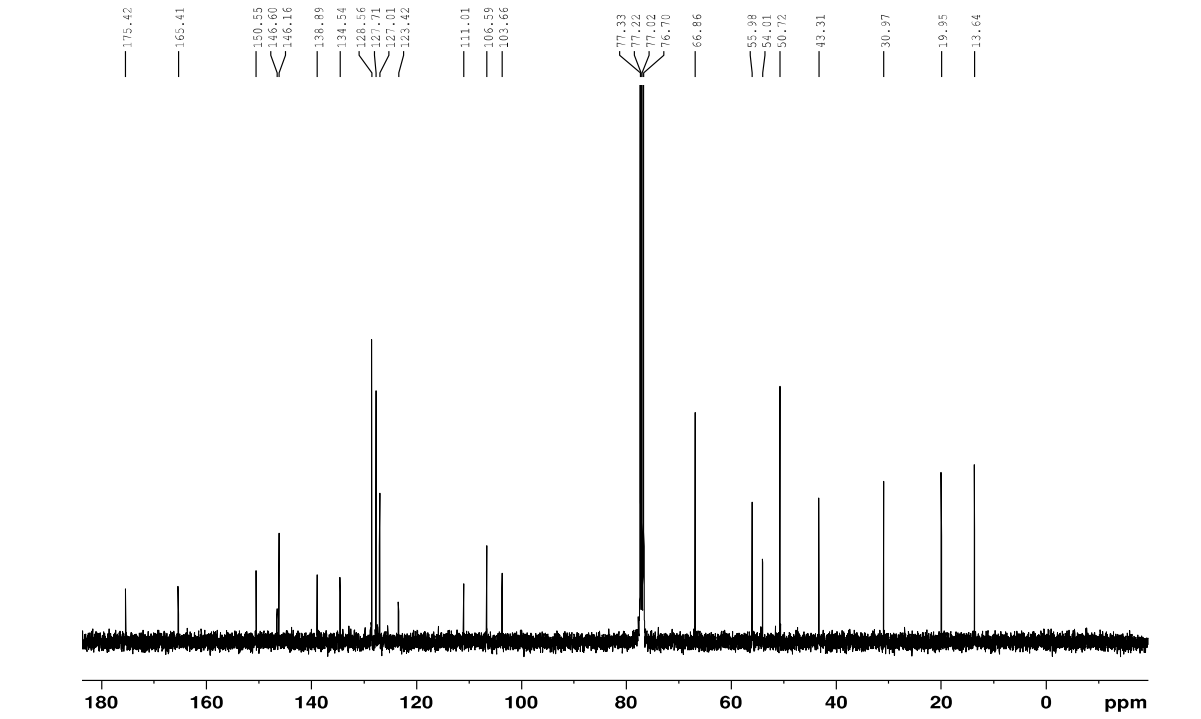

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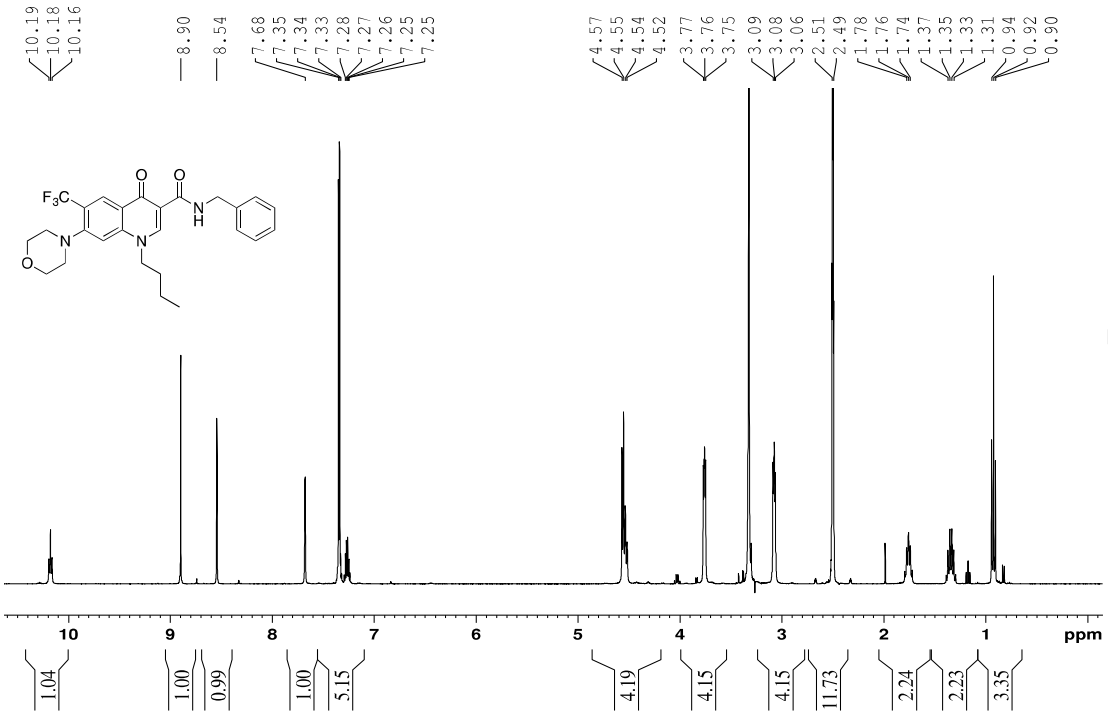
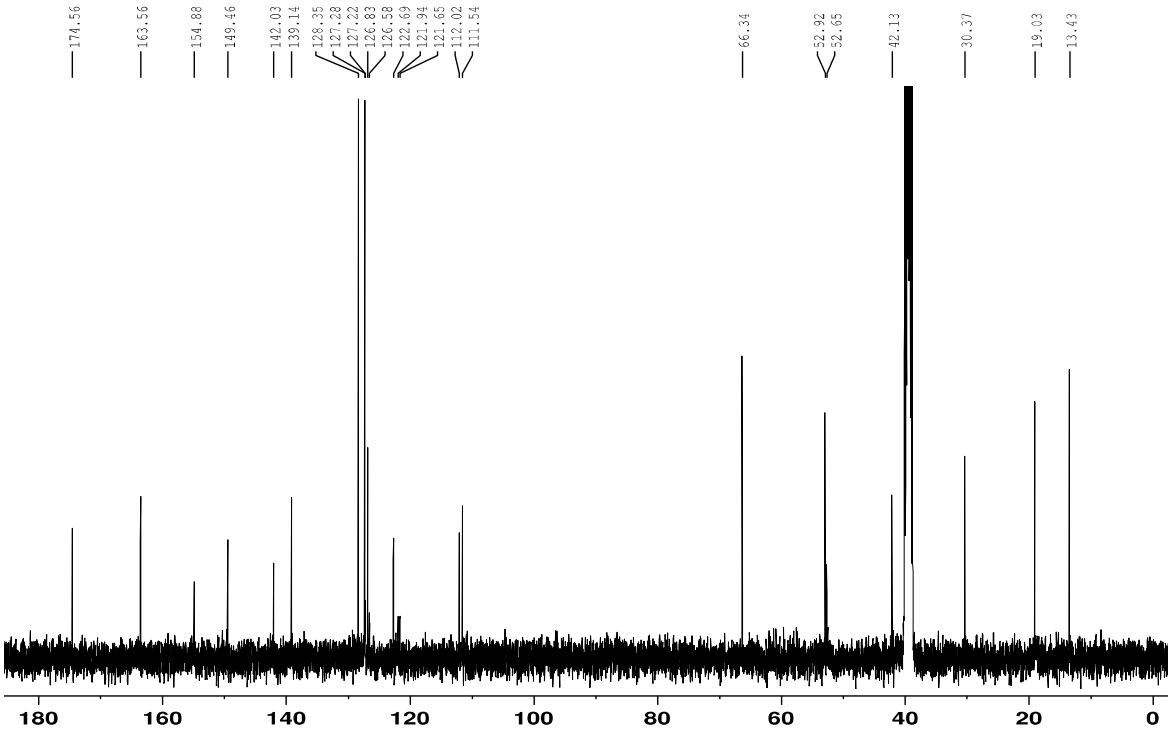


1 H NMR spectra of compound **11**



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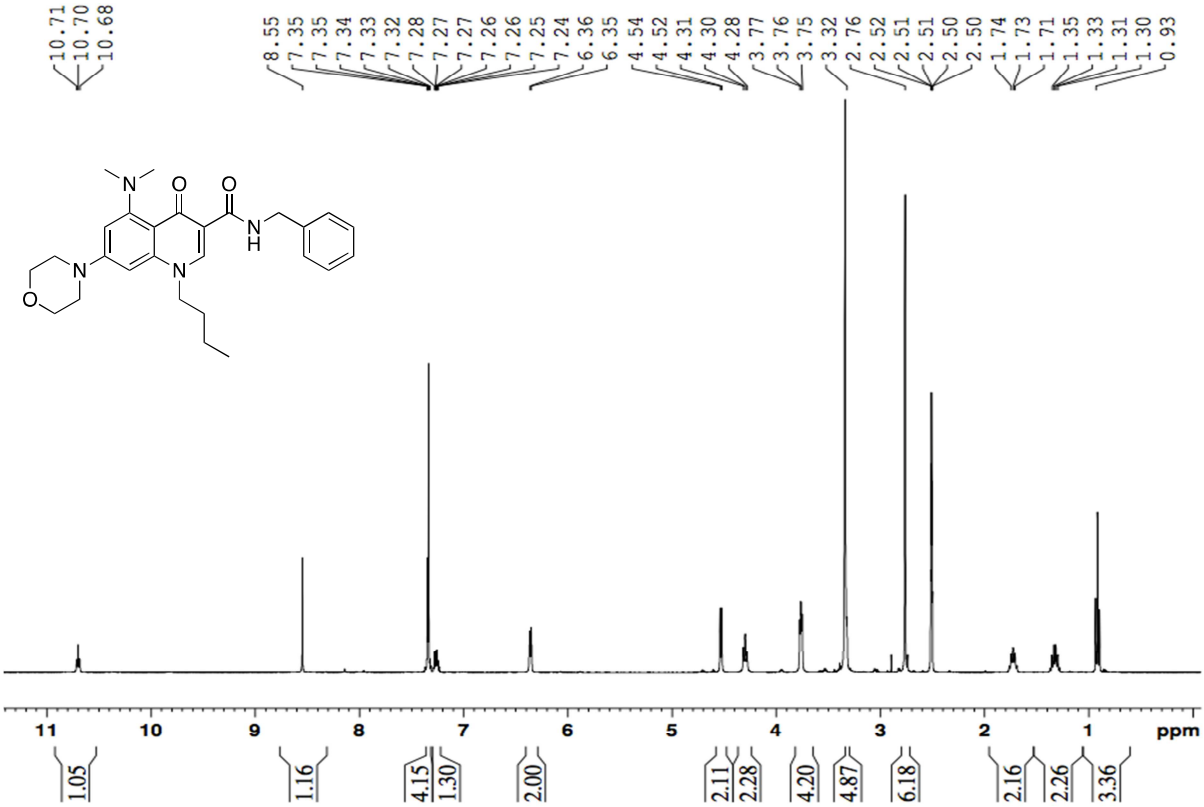
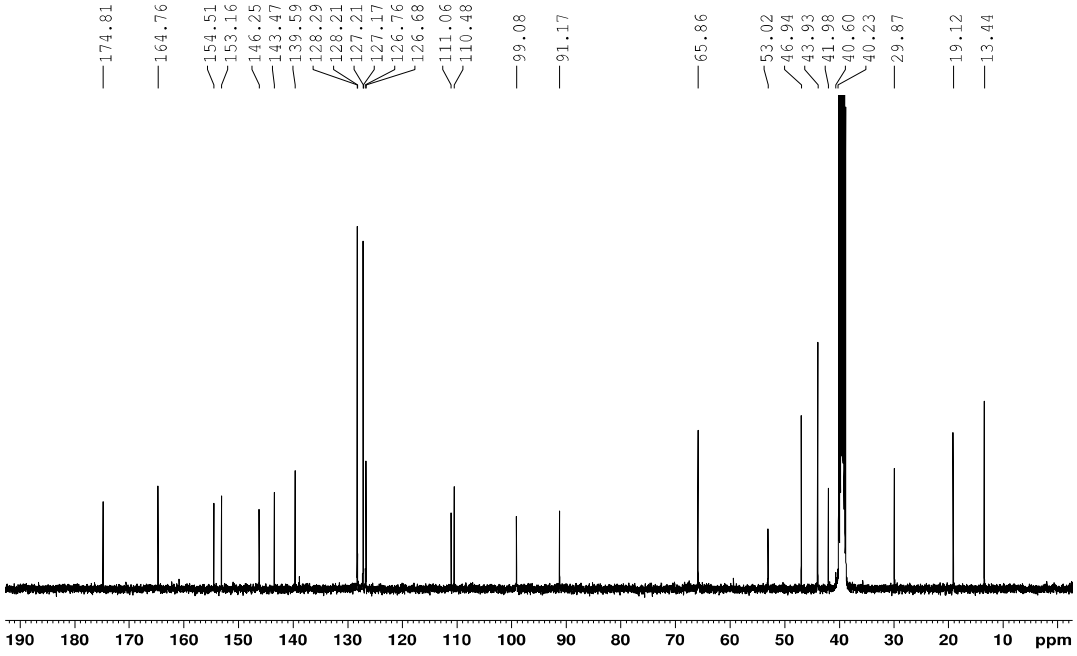
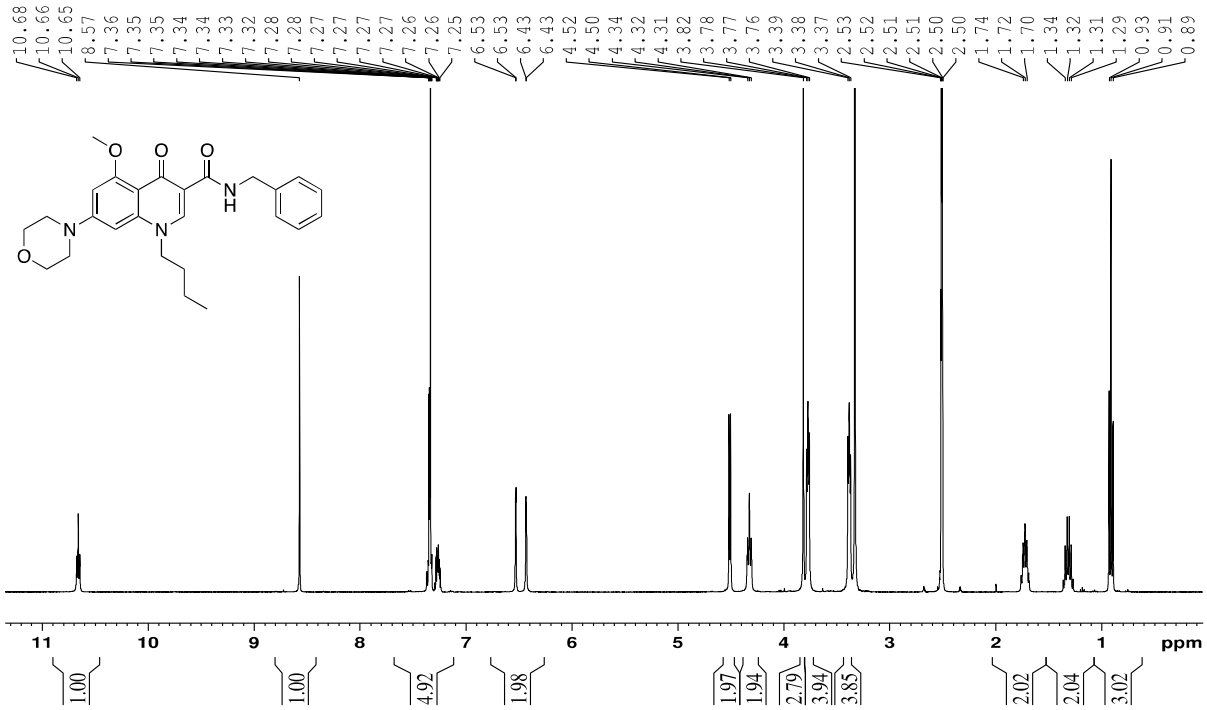
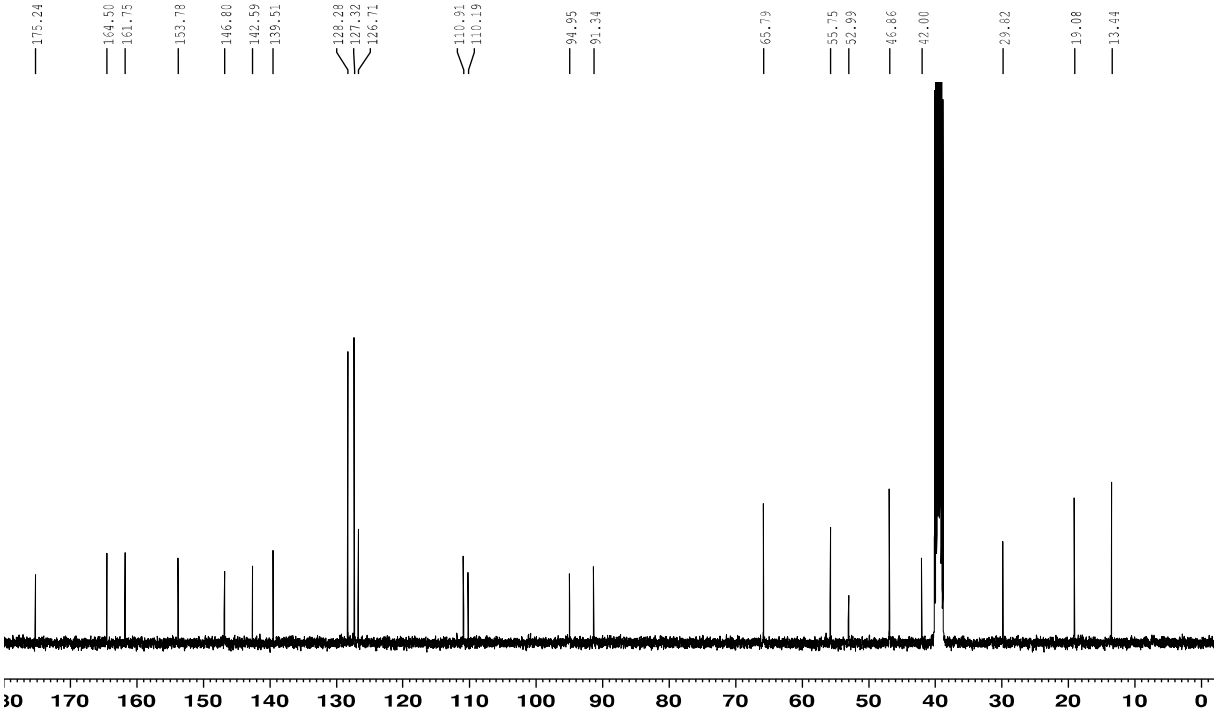


1 H NMR spectra of compound **13**

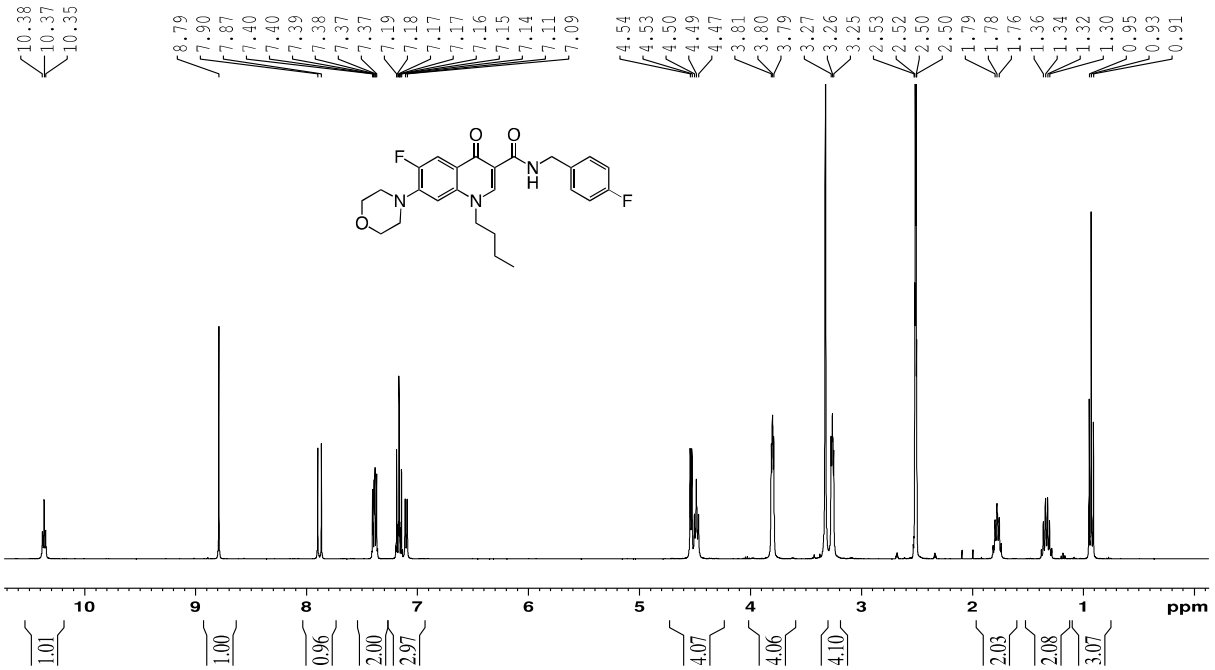




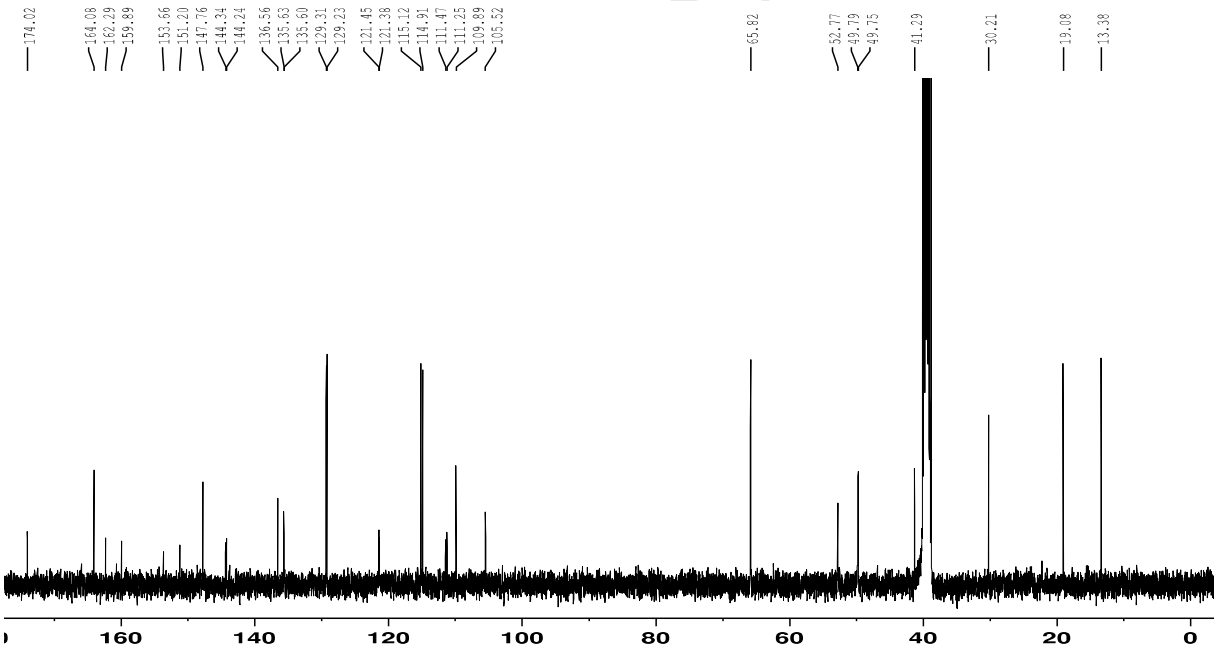
1 H NMR spectra of compound **14**



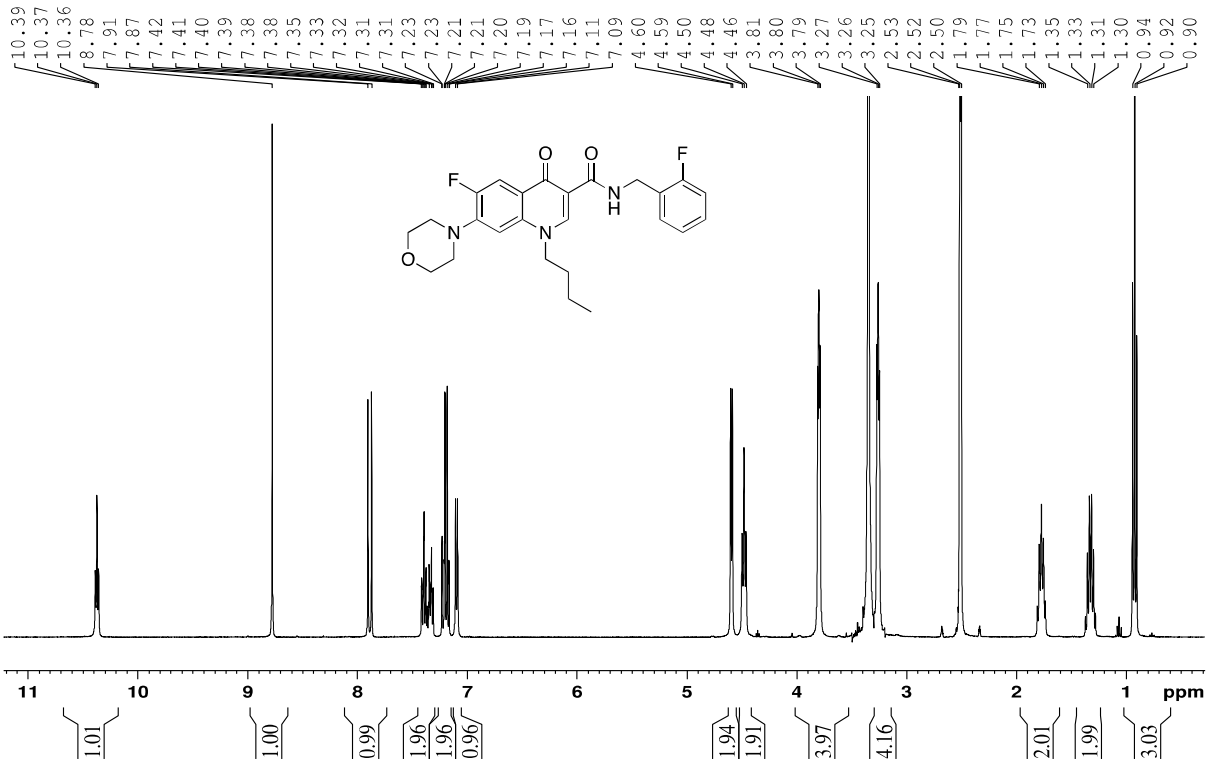
13 C NMR spectra of compound **14**



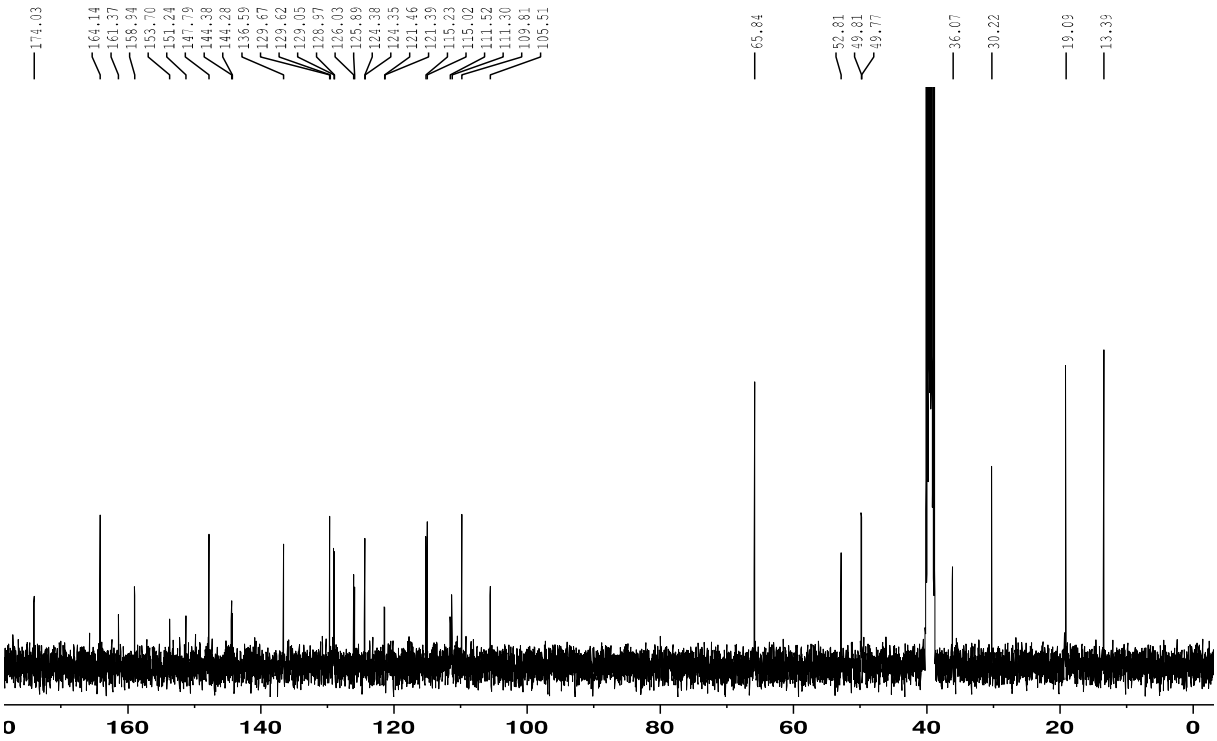
1 H NMR spectra of compound **15**



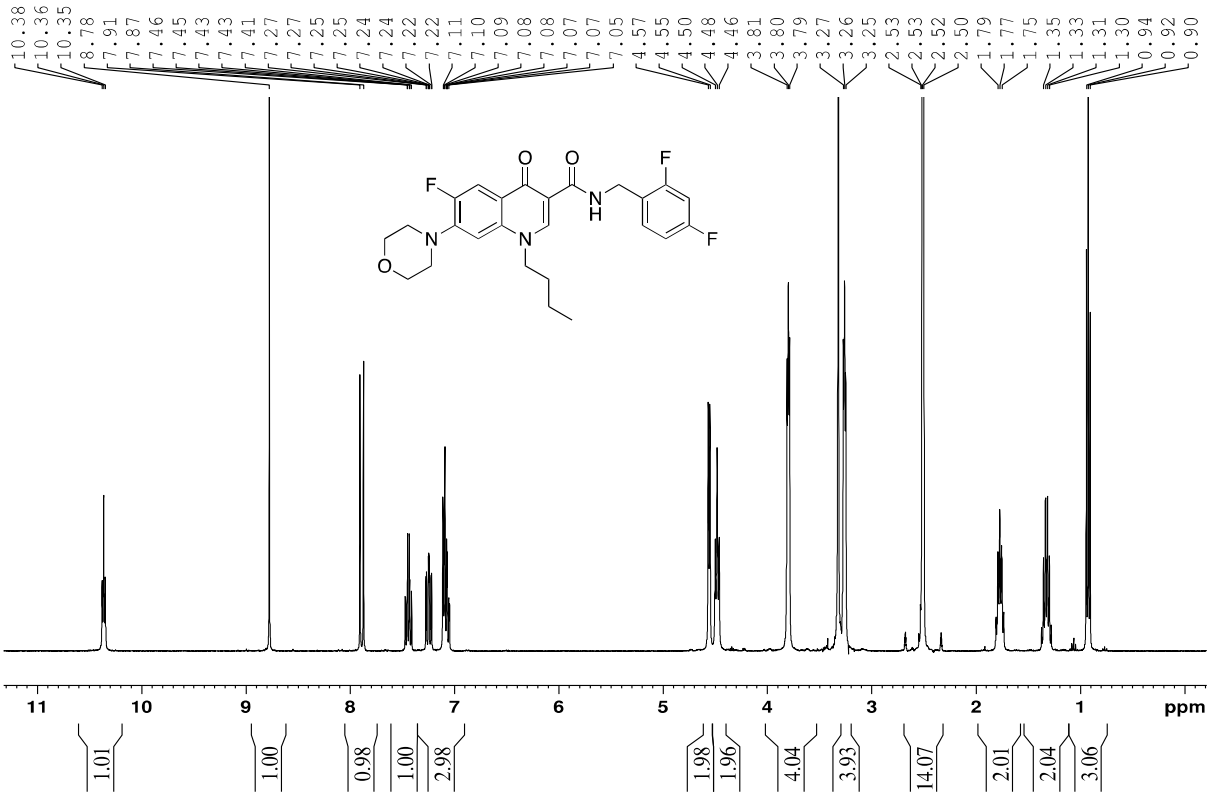
13 C NMR spectra of compound **15**



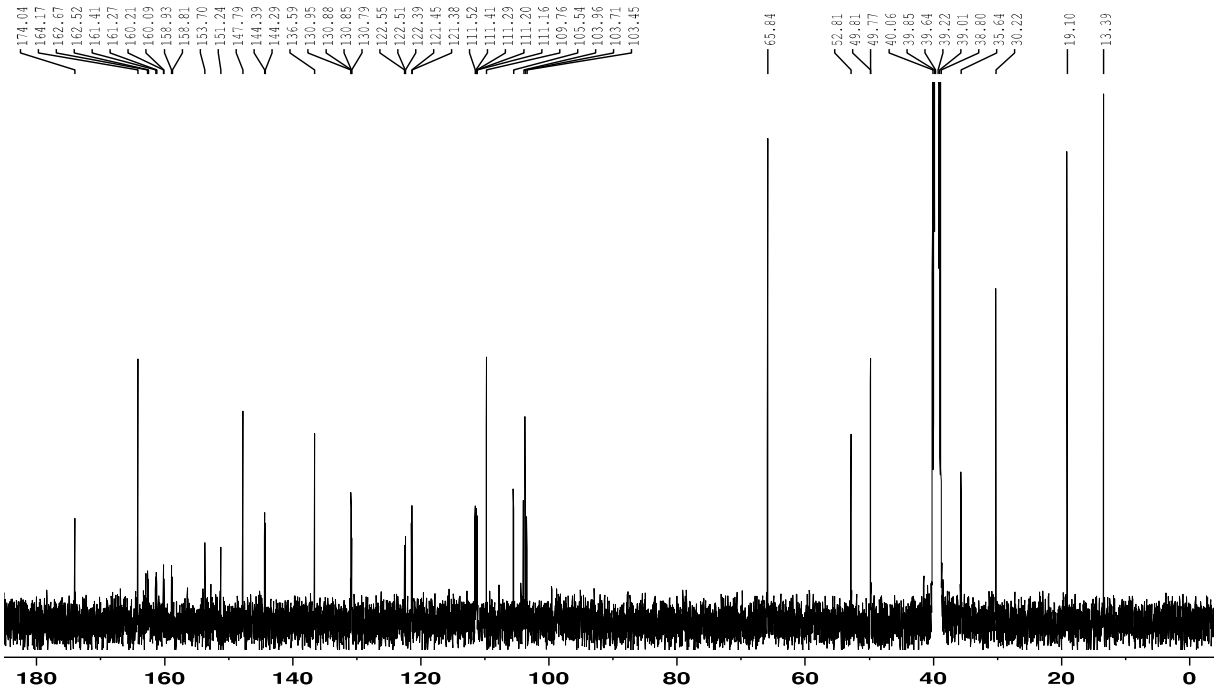
1 H NMR spectra of compound **16**



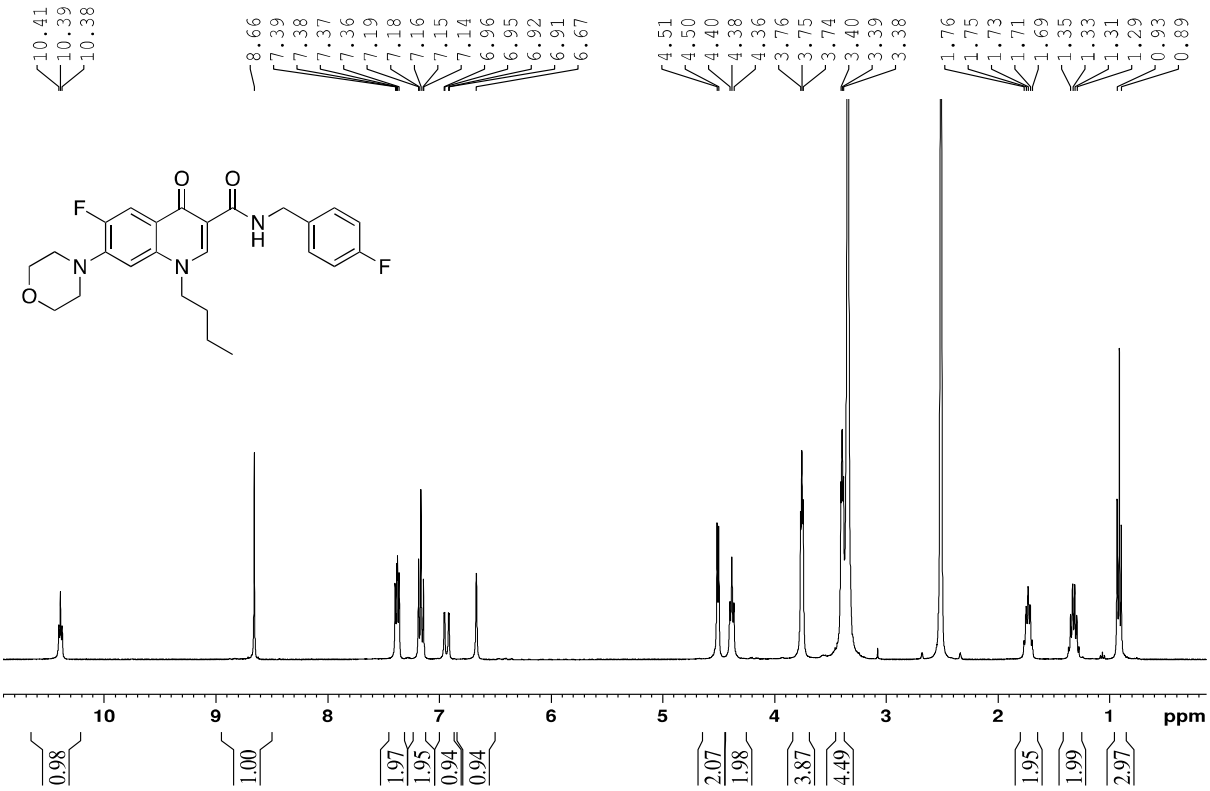
13 C NMR spectra of compound **16**



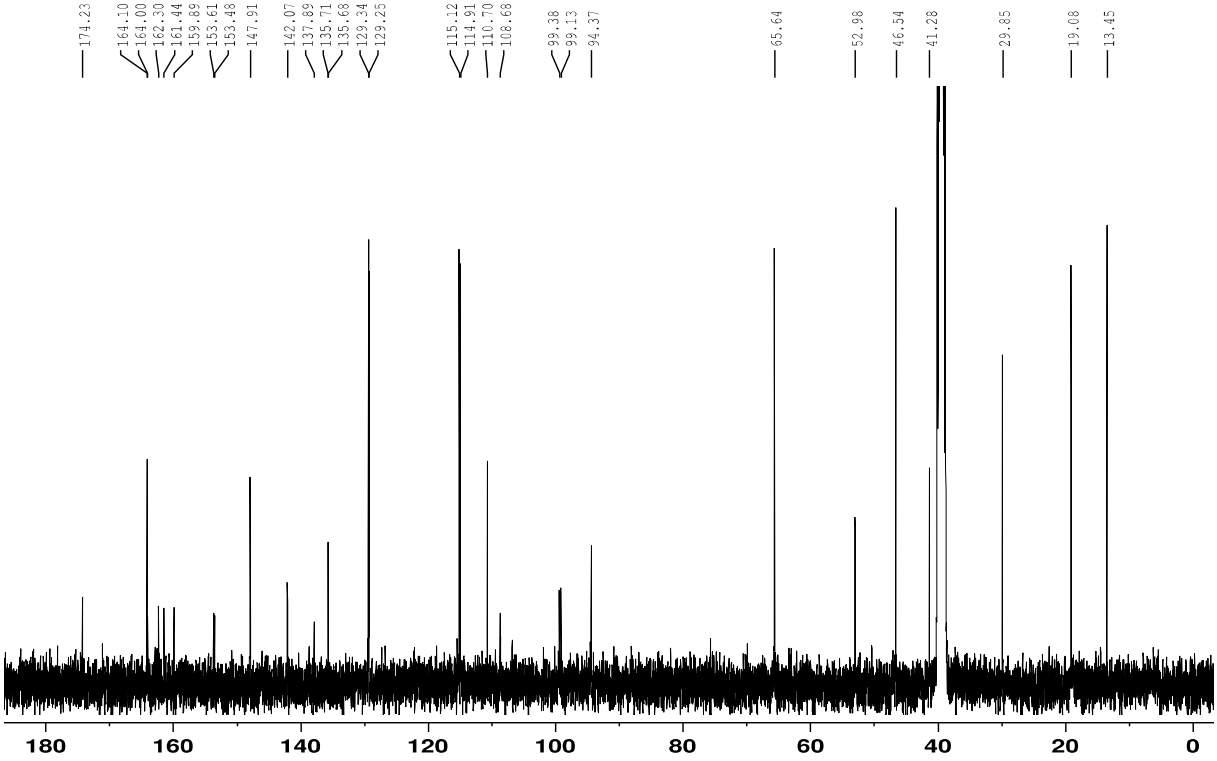
1 H NMR spectra of compound **17**



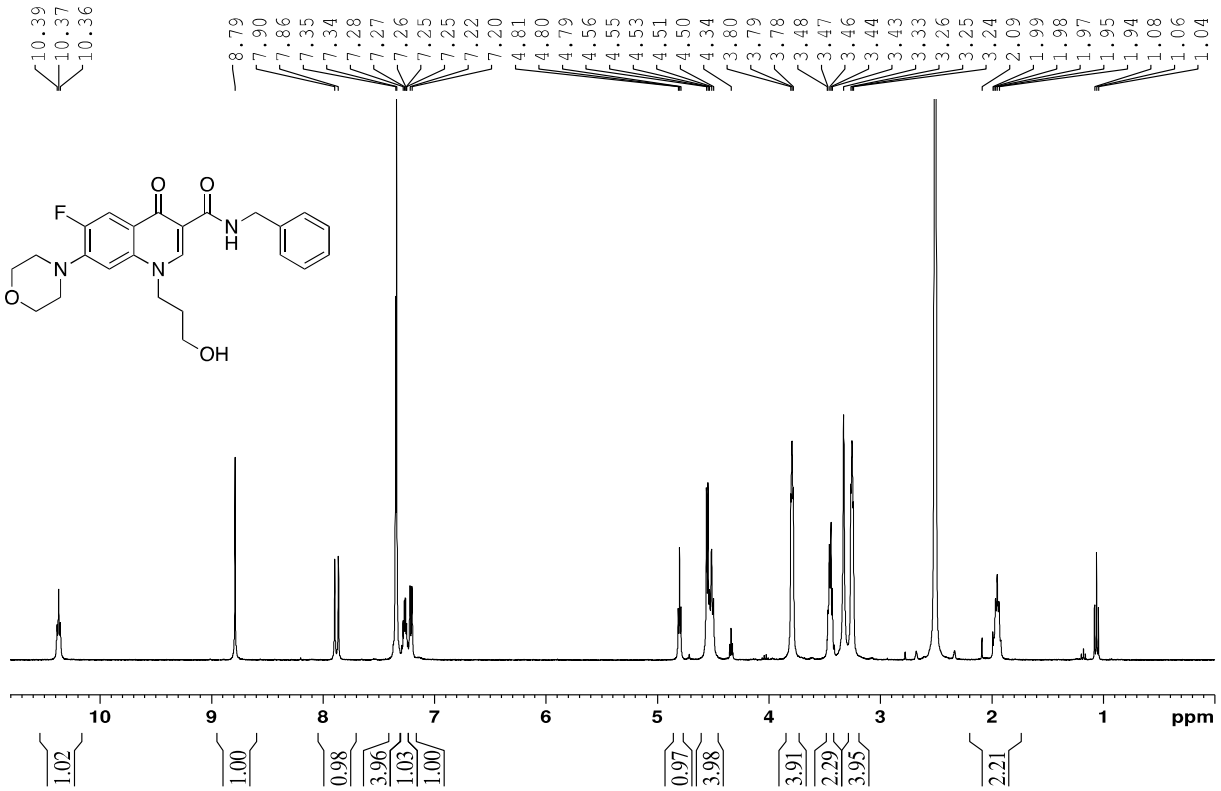
13 C NMR spectra of compound **17**



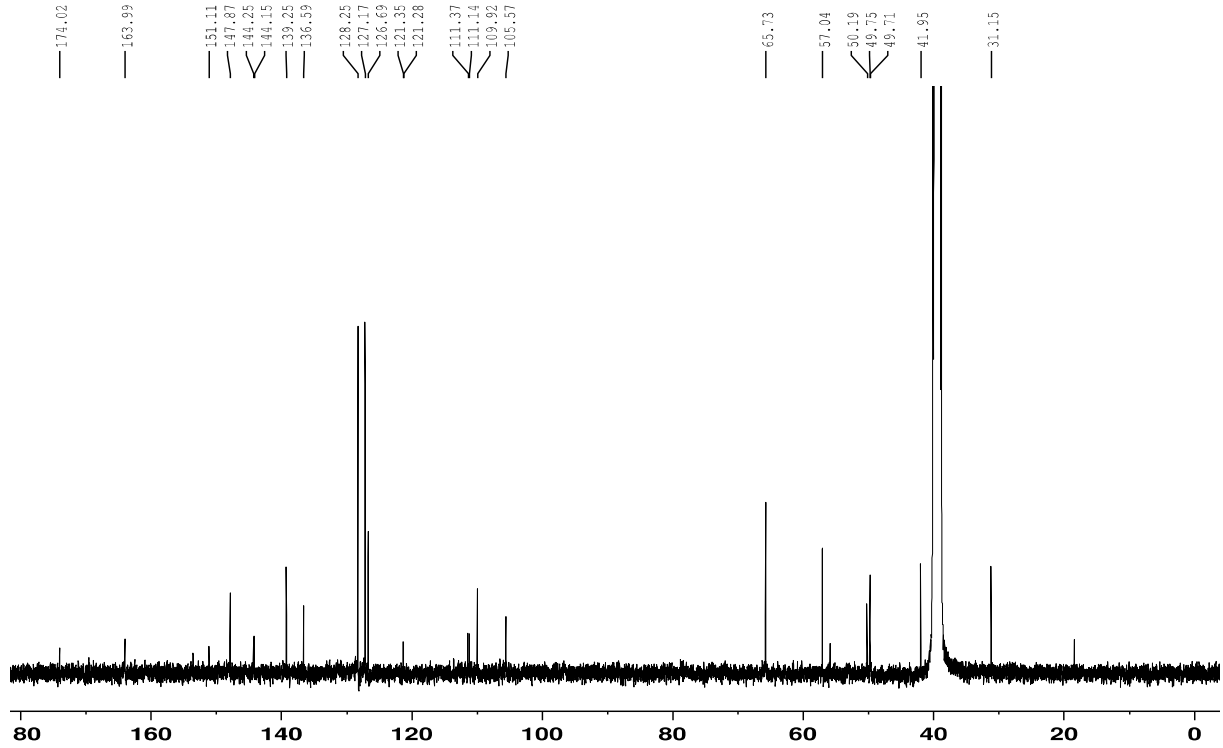
1 H NMR spectra of compound **18**



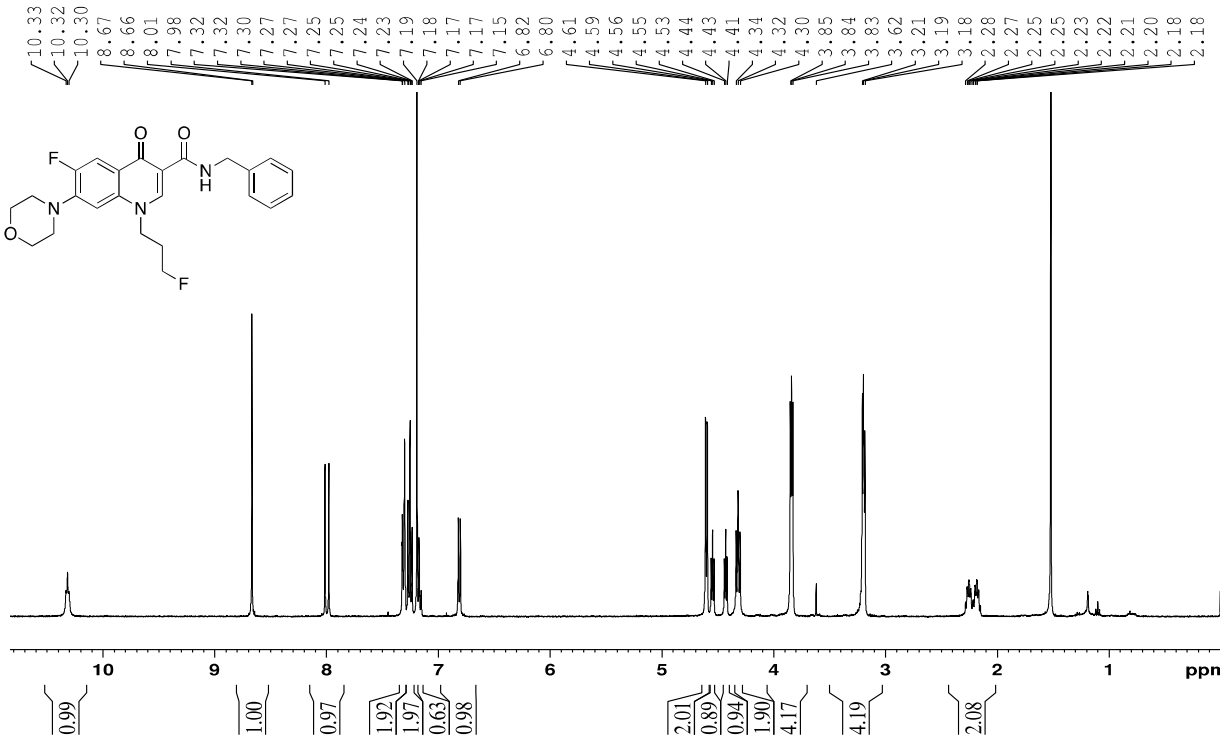
13 C NMR spectra of compound **18**



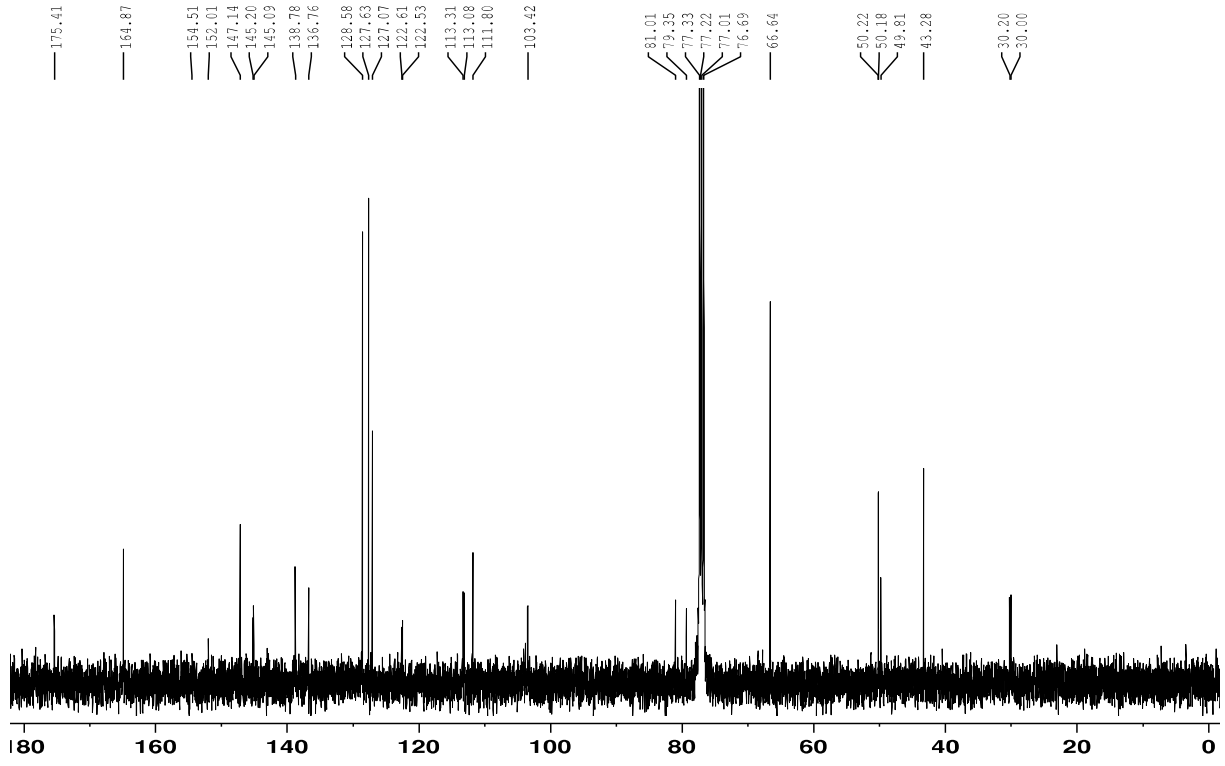
1 H NMR spectra of compound **19**



13 C NMR spectra of compound **19**



1 H NMR spectra of compound **21**



13 C NMR spectra of compound **21**

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