Synthesis, characterization and antibacterial activity of some $N$-alkyl benzimidazol piperazine fluoroquinolines

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Abstract
A series of $N$-alkyl benzimidazol piperazine fluoroquinolines with remarkable improvement in antimicrobial activity as compared to the moxifloxacin were synthesized and characterized by $^1$HNMR, $^{13}$C NMR, IR, Mass and elemental analysis. These derivatives were evaluated for their invitro activity against Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae. The results showed that all the synthesized derivatives of novel fluoroquinolines possess antimicrobial activity. However, compound derivatives IV and V3 have antibacterial activities against Pseudomonas, Klebsiella and Staphylococcus epidermidis. Among all these derivatives, compound V3 exhibit potent inhibitory activity with MIC of 19 $\mu$g/mL.

Keywords: $N$-alkylbenzimidazolpiperazinefluoroquinolines, antimicrobial activity, MIC.

1. Introduction
Pathogenic bacteria can cause extensive damage to our bodies, including death.¹ Nowadays, about 70% of the bacteria that cause infections in hospitals are resistant to at least one of the drugs most commonly used for treatment. Increasing in resistance of bacteria that cause community acquired infections has also been documented especially in the Staphylococci and Pneumococci (Streptococcus pneumoniae), which are prevalent causes of disease and mortality. In a recent study, 25% of bacterial pneumonia cases were shown to be resistant to penicillin and an additional 25% of cases were resistant to more than one antibiotic.² Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic. Bacteria are constantly exposed to use and misuse of antibiotics leading to the emergence of antibiotic-resistant strains which make the existing drugs ineffective.³ This ability of bacteria to develop resistance to the antibiotics currently used, warrants novel research into new families of antimicrobials. Literature survey reveals that fluorinated quinolones,⁴ are extensively used in medicinal chemistry, most of them were using notable worldwide patented drugs for antibacterial, for example, norfloxacin, fleroxacin, ciprofloxacin, lomefloxacin, ofloxacin, pefloxacin, enoxacin, grepafloxacin, sparofloxacin, trovafloxacin, clinafloxacin, moxifloxacin and gatifloxacin.⁵ In view of these observation herein, we report $N$-alkylation of $1H-$
benzimidazolpiperazinefluoroquinoline scaffold with various analogues to create a new structural core with improved effectiveness to kill bacteria resistant to the previous generations.

2. Results and discussion

2.1. Chemistry

The focus of the present investigation is on the development of a few N-substituted 7-(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (IV1-12) starting from compound I, i.e., 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid as shown in scheme 1. This starting material has been synthesized by known protocol Muralidhar et al and the experimental results were matched with literature reference. Compound I is a β-keto acid and protected with boronic acid in presence of acetic anhydride and zinc chloride to yield cyclic boron complex (II). This compound is extremely unstable for a long time, so the next reaction taken without giving time interval.

Regioselective substitution of 7th fluorine of compound I by 2-(piperidine-4-yl)-1H-benzo[d]imidazole (III) in basic medium followed by hydrolysis of boron complex with HCl provided key intermediate compound IV in good yields. Typical aliphatic proton shift alignments of compound IV in 1H NMR was finally confirmed by COSY spectrum and compared with HMBC spectra. Nitrogen attached cyclopropyl hydrogen appears at around 4 ppm as a multiplet and most down field than all other aliphatic protons and this hydrogen have a strong interaction with a peak resonated at 1.15-1.05 ppm, this indicates these are the remaining cyclopropyl ring protons and similar interactions found for compound IV was depicted in Table 1 and Fig. 1 and showed COSY spectrum in Fig. 2. However, carboxylic acid proton resonates at 14.96 ppm and exchangeable with D2O in 1H NMR spectra. Enone carbon resonated downfield than all the carbons at 176.8 ppm where as carboxylic acid carbon resonating at 166.1 in 13C NMR spectra of compound IV. Fluorine attached carbon resonated at 146.6 ppm and O-methyl carbon appeared at 63.4 in 13C NMR spectra. IR spectra showed a peak at 3437 cm⁻¹ indicates for N-H stretching frequency. Molecular weight of compound IV has 477 [M+H]+ indicates that it is having even number of nitrogens. HMBC spectra interaction between 13C NMR and 1H NMR of compound IV was provided in Table 2 and spectra in Fig. 3.

Scheme 1. Synthesis of N-alkyl modified moxifloxacins. Reagents and conditions: a = Ac2O, ZnCl2, B(OH)3, 120-125°C; b = i). TEA, DMAP, CH3CN, DMF, 5h; ii). HCl, H2O, pH = 1-2; c = R-Br (1-11), SiO2, TEA, 100°C, 3 min, MW, 100W; d = i). NaOH, MeOH, H2O, reflux, 5h; ii). HCl, pH = 1-2.
COSY results

![Structure of compound IV](image)

**Figure 1:** Structure of compound IV and parenthesis for respective protons or carbons of aliphatic region for Table 1 and Table 2

**Table 1 COSY spectra interactions for aliphatic region of key intermediate IV**

| Sl. No. | Interation | Source of interaction |
|--------|------------|-----------------------|
| 1      | 4.18-4.20  | 1.15-1.05             |
| 2      | 3.66-3.52  | 2.27-2.07             |

Compound IV further subjected to N-alkylation with various alkyl halides (1-11) in basic medium under microwave irradiation in moderate to excellent yields, with shorter reaction time compared to the conventional thermal method gave title compounds V1-11. Compound V11 undergoes basic hydrolysis with aq. NaOH followed by acidification with HCl yielded respective acid V12. A doublet signal appeared for a proton of adjacent to the fluorine atom at around 7.8 ppm with the coupling constant of 12 Hz, due to this proton has strongly coupled with adjacent fluorine atom. Mass spectra molecular ions of title compounds (V1-12) matched with their respective molecular weights. Melting points of compounds V1-12 were also proved by DSC spectral analysis and every compound showed a sharp peak at their respective melting point range. Obtained peaks were V1-238.01°C, V2-247.85°C, V3-220.74°C, V4-223.67°C, V5-254.09°C, V6-194.19°C, V7-204.70°C, V8-188.38°C, V9-237.91°C, V10-156.33°C, V11-145.60°C, V12-279.38°C.

![COSY spectra of compound IV](image)

**Figure 2:** COSY spectra of compound IV
Figure 3 HMBC spectra of compound IV

HMBC results

Table 2 $^{13}$C and $^1$H NMR interactions for aliphatic region of key intermediate IV

| Sl. No. | Interaction | $^{13}$C NMR ppm | $^1$H NMR ppm | Source of interaction |
|---------|-------------|-------------------|---------------|----------------------|
| 1       |             | 9.46              | 1.15-1.05     | 3                    |
| 2       |             | 30.38             | 2.27-2.07     | 5                    |
| 3       |             | 34.60             | 3.66-3.52     | 1                    |
| 4       |             | 41.30             | 4.18-4.20     | 2                    |
| 5       |             | 50.52             | 3.66-3.52     | 4                    |
| 6       |             | 63.48             | 3.82          | 6                    |

2.2. Biological evaluation
2.2.1. Antimicrobial activity
2.2.1.1 Methods
All the compounds were tested for *invitro* antibacterial activity against Gram negative *Escherichia coli* MTCC-443 (*E. coli/E.c*), *Psedomonas aeruginosa* MTCC-441 (*P. aeruginosa/P.a*), *Kliebsiella pneumoniae* ATCC 27736 (*K. pneumonia/K.p*) and Gram positive bacteria *Staphylococcus aureus* ATCC 25923 (*S. aureus/S.a*) and *Staphylococcus epidermidis* MTCC 435 (*S. epidermidis/S.e*) and fungal strain *Aspergillus niger*, *Aspergillus flavus* and yeast *Candida albicans* (*C.a*) by the agar diffusion method. Microbial cultures were maintained on agar slant at 4°C and sub cultured on appropriate agar plates 24 h prior to any antimicrobial test. Nutrient agar and Sabouraud glucose agar and Czapek’s dox agar were used for the activation of bacteria. The Mueller Hinton Broth (MHB) was used for the MIC and MMC determinations.

2.2.1.2 Materials
All the microbial strains are pathogenic isolates procured from IM Tech, Chandigarh, India. Moxifloxacin (Moxi), ciprofloxacin (Cipro), levofloxacin (Levo) and chloramphenicol (Chlo) were used as reference antibiotics respectively, against bacteria. *P*-iodonitroterazolium chloride from Sigma-Aldrich was used as microbial growth indicator.
2.2.1.3 MIC and MMC determinations

The MIC determinations were conducted using rapid INT calorimetric assay according to described methods\(^9,10\) with some modifications. The test compounds were dissolved in 5mL of methanol/dichloromethane (2:1 v/v) to give a final concentration of 520µg/mL and serially diluted two fold to obtain concentration ranges. 100 µL of each concentration was added in a well (96 well microplate) containing 95 µL of MHB and 5 µL of inoculums (standardized at 1.5x10^6 CFU/mL by adjusting the optical density to 0.1 at 600 nm SH1MADZU UV-120-01 spectrophotometer).\(^11\) The negative control will consisted of 195 µL of MHB and 5µL of standard inoculums.\(^12\) The plates were covered with a sterile plate sealer then agitated to mix the contents of the wells using a plate shaker and incubated at 37°C for 24 h. The assay was repeated in triplicates. The MIC of synthesized derivatives was detected following addition (40 µL) of 0.2 mg/mL p-iodonitrotetrazolium chloride and incubation at 37°C for 30 min.\(^9,10\) Viable micro-organisms reduce the yellow dye to a pink color. MIC was defined as the lowest compound derivative concentration that prevented this change and exhibited complete inhibition of bacterial growth.

For the determination of MMC, a portion of liquid (5µL) from each well that showed no change in color was plated on MH agar and incubated at 37°C for 24 h. The lowest concentration that yielded no growth after the sub culturing was taken as the MMC.\(^13\)

The MIC results (Table 3) indicated that the N-alkyl derivatives of benzimidazolpiperazine fluoroquinolines IV, V3, V4, V5, V6, V8 and V12, inhibited the growth of all tested microbial species. All other compounds showed selective activity, their inhibitory effects being noted on 4 of the 5 tested organisms. The lowest MIC range of 19-65 µg/mL for compounds IV, V3 and V5 was recorded. However, significant inhibitory effect was shown on three of the tested microorganisms namely, P. aeruginosa, S. epidermidis, K. pneumonia with MIC of 19 µg/mL with compound V3. P. aeruginosa is an important nosocomial pathogen highly resistant to commonly used antibiotics, causing a wide spectrum of infectious and leading to substantial morbidity; and mortality.\(^14\) The lowest MIC value of 65µg/mL was recorded with compounds V4 and V6, on P. aeruginosa. Compounds IV and V3, on K. pneumonia (MIC of 19 µg/mL) showing medicinal potential of the compounds, as the activities on P. aeruginosa, K. pneumonia and S. epidermidis were better than that of chloramphenicol (MIC of 38 µg/mL).

Table 3: MIC (µg/mL) of synthesized compounds and reference antibiotics on the studied microbial species

| Sl. No | Test compounds | Micro-organisms, strains and MIC (µg/mL) |
|--------|----------------|----------------------------------------|
|        |                | S.a | E.c | P.a | S.a | K.P | C.a |
| 1      | Moxi           | 520 | --  | 260 | --  | 260 | --  |
| 2      | Cipro          | 130 | 38  | 38  | 65  | 38  | 65  |
| 3      | Levo           | 65  | 65  | 38  | 38  | 38  | 38  |
| 4      | Chlo           | 65  | 65  | 38  | 38  | 38  | 38  |
| 5      | Nystatin       | --  | --  | --  | --  | --  | 65  |
| 6      | IV             | 65  | 19  | 19  | 19  | 19  | --  |
| 7      | V3             | 130 | 260 | 65  | 65  | 130 |
| 8      | V2             | 130 | 260 | 65  | 65  | 130 |
| 9      | V4             | 65  | 38  | 19  | 19  | 19  | 19  | 38  |
| 10     | V5             | 65  | 65  | 38  | 38  | 19  | 19  | 38  |
| 11     | V6             | 130 | 260 | 65  | 130 | 260 | 130 |
| 12     | V7             | 130 | 260 | 65  | 130 | 260 | 130 |
| 13     | V8             | 130 | 130 | 65  | 65  | 65  | 65  |
| 14     | V9             | 260 | 260 | 65  | 130 | 260 | 130 |
| 15     | V10            | 520 | 130 | --  | --  | 130 | 260 |
| 16     | V11            | 260 | --  | 260 | --  | 130 | 260 |
| 17     | V12            | 260 | 130 | 260 | 65  | 130 | 130 |

Note: -- represents no zone inhibition
The result of Table 4 showed detectable MMC value for some of the studied compounds on the tested microbial strains. When analyzed carefully, the MIC and MMC results for the compounds IV, V3; it can be noted that MMC / MIC ratios lower than 4 were obtained with these compounds on the test microbial species, suggesting that the killing effects could be expected. However, all MMCs values obtained were greater than the MICs. It can also be noted that the reference antibiotics were in most of the case more active than all studied compound.

Table 4 MMC (µg/mL) of some synthesized compounds and reference antibiotics on the studied microbial species.

| Sl. No | Test compounds | Micro-organisms, strains and MMC (µg/mL) |
|-------|----------------|----------------------------------------|
|       |                | S.a | E.c | P.a | S.e | Kp | C.a |
| 1     | Cipro          | 260 | 65  | 65  | 130 | 65 | 130 |
| 2     | Levo           | 130 | 65  | 65  | 130 | 65 | 130 |
| 3     | Chlo           | 260 | 260 | 65  | 65  | 65 | --  |
| 4     | Nystatin       | --  | --  | --  | --  | -- | 130 |
| 5     | V3             | 130 | 65  | 38  | 65  | 38 | 65  |
| 6     | V5             | 130 | --  | 38  | 130 | 130| 130 |
| 7     | V11            | 520 | --  | 520 | --  | 260| 520 |
| 8     | V12            | 520 | 130 | 260 | 520 | 130| 260 |

3 Conclusions

In summary, we have synthesized a N-substituted 7-(4-(1H-benzo[d]imidazol-2-yl) piperdin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (IV, V 1-12) by conventional and also under microwave irradiation methods. Microwave heating can be quite effective in improving the yields and decreasing the reaction time. Antibacterial activities against various resistant Gram-positive and Gram-negative bacteria were evaluated for prepared compounds. It was interesting to note that compounds IV, V3, V4, V5 and V8, had high anti-bacterial activity than that of the standard drugs. The presence of alkyl donating groups like n-butyl, n-pentyl and i-propyl groups at N-1 position of benzimidazol piperazine fluoroquinolines increases the antibacterial activity whereas presence of phenyl group compounds (V9-V12) decreases the antibacterial activity. The present investigation provides supportive data for the use of potent compounds for the treatment of infectious associated with the studied micro-organisms. However, this will be confirmed with further pharmacological (invivo activity) and toxicological studies (acute and sub-acute toxicities) using animal models.

4 Experimental

The reagents and solvents were of analytical grade and were used without further purification unless otherwise mentioned. Thin layer chromatography (TLC) was carried out on aluminum sheets coated with silica gel 60 F254 (Merck). TLC plates were inspected under UV light or developed by charring after spraying with 5% H2SO4 in ethanol. Micro-analytical data were obtained by employing a Perkin-Elmer 240c analyzer. IR spectra (KBr pellets) were recorded with a Perkin-Elmer-1700 spectrophotometer. 1H NMR and 13C NMR spectra were measured on a Varian INOVA-500 spectrometer. The following abbreviations have been used to explain the observed multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; br. s, broad singlet. Coupling constants have been assigned and listed without duplication in the 1H NMR description of the synthesized compounds. GCMS was recorded on aVarian 300-MS and electron spray-mass spectra were recorded on an LCQ system (Finngan MAT, USA) using methanol as the mobile phase. Melting points
were recorded on a Polmon MP 96. Microwave reactions performed in MARS 240/50, model No. 907510.

4.1 Preparation of boron complex of 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (II)

To a mixture of acetic anhydride (50 mL) and zinc chloride (4 mmol) added boric acid slowly (4 mmol) at rt. Charged 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (I) (3 mmol) and heated the contents to 120-125°C for 5 h. The progress of the reaction was monitored by TLC for the absence of starting material. Cool the reaction mixture to 80-90°C and distilled off acetic anhydride under vacuum at the same temperature charged toluene (5.0 mL) and co-distilled under vacuum. Added fresh toluene (30 mL) again and cooled the compound to rt and stirred for 1 h, filtered and wash the wet compound with toluene followed by n-heptane, dry the compound at below 50°C. Yield: 10.2 g (86.7%).

4.2 Preparation of 7-(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (IV)

To a stirred solution of acetonitrile (5 mL), N,N-dimethyl formamide (5 mL), DMAP (50 mg) was added compound II (19.6 g, 5.0 mmol) and 2-(piperidin-4-yl)-1H-benzo[d]imidazole (III) (11.0 g, 5.5 mmol). Stirred for 30 minutes at rt added triethyl amine (4 mL) slowly, then stirred for 5 h at same temperature. The progress of the reaction is monitored by TLC until the absence of compound II. Added water to the reaction mixture and adjusted the pH 1.0 to 2.0 with aq. HCl and the isolated compound was filtered and washed with acetonitrile. Taken the wet compound and recrystallized from methanol. Color: off white; Yield: 17 g (71.4%); M.p.: 266-268°C; IR (KBr, v): 3437, 3026, 1730, 1672, 1509, 1456, 1318, 1232, 1056 cm⁻¹; ¹H NMR (DMSO): δ 14.96 (br. s, 1H), 8.71 (s, 1H), 7.81-7.76 (m, 3H), 7.54-7.51 (m, 2H), 4.18-4.20 (m, 1H), 3.82 (s, 3H), 3.66-3.62 (m, 3H), 3.58-3.52 (m, 2H), 2.27-2.18 (m, 2H), 2.15-2.07 (m, 2H), 2.15-2.07 (m, 2H), 1.14-1.05 (m, 4H), ¹³C NMR (DMSO): δ 176.8, 166.1, 156.6, 151.0, 146.6, 139.9, 139.7, 134.5, 131.5, 125.9, 121.5, 114.3, 107.2, 106.9, 63.4, 50.5, 41.3, 34.6, 30.3, 9.46; Mass (ES): m/z 477 [M+H]+, 499 [M+Na]+; Anal. Calcd. for C₂₀H₁₃FN₃O₄: C, 65.88; H, 5.32; N, 11.95%.

4.3 General procedure for the synthesis of N-substituted 7-(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (IV1-11) in microwave

Compound IV (5 mmol), alkyl halide (1-11) (5.5 mmol) and triethyl amine (8 mmol) was adsorbed on silicagel (200-400 mesh) in to a microwave vial. The vial was sealed and placed in microwave. The reaction was run at 100°C for 3 min. For the entire experiment, the power setting was held at 100 W. The reaction mixture was then cooled to room temperature and purified by SiO₂ gel column chromatography with DCM:methanol (95:5%) to afford title compounds (V1-12).

4.4 General procedure for the synthesis of N-substituted 7-(4-(1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (IV1-11) in conventional

To the stirred solution of compound IV (1 mmol) in dimethylformamide (20 mL) added sodium hydride (2.2 mmol) for l-8/potassium carbonate (2.2 mmol) for 9-11 heated the contents to 40-45°C and maintained for 30 min. Then added a mixture of alkyl bromides (1-11) (1.2 mmol) in 4 mL of DMF and monitored the reaction by TLC for the absence of compound IV, quenched the reaction mass with ice and stirred for 1 h, filtered the isolated compound. Purified by SiO₂ gel column chromatography with hexane:EtOAc (60:40%) to afford.

(_yield of conventional method; \( m \) = yield of microwave method).
methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V1): Color: White; Yield: 89%\textsuperscript{m}, (76%)\textsuperscript{f}; M.p.: 238-40°C; IR (KBr, v): 2934, 2834, 1731, 1621, 1511, 1457, 1384, 1315, 1236, 1184, 1116, 1060 cm\textsuperscript{-1}. \textsuperscript{1}H NMR (DMSO): δ 14.82 (br, s, 1H), 8.81 (s, 1H), 7.86 (d, 1H, J = 12.0 Hz), 7.78-7.76 (m, 1H), 7.35-7.27 (m, 3H), 4.28 (q, 2H, J = 6.8 Hz), 4.08-4.05 (m, 1H), 3.90 (s, 3H), 3.71 (t, 2H, J = 6.8 Hz), 3.42 (t, 2H, J = 6.8 Hz), 3.07-3.04 (m, 1H), 2.37-2.30 (m, 2H), 2.10-2.07 (m, 2H), 1.49 (t, 3H, J = 6.8 Hz), 1.24-1.14 (m, 2H), 1.02-0.87 (m, 2H). \textsuperscript{13}C NMR (DMSO): 177.0, 166.7, 158.3, 156.5, 155.0, 149.8, 145.9, 142.6, 139.8, 134.5, 133.7, 122.2, 122.0, 119.4, 109.3, 108.0, 107.6, 62.3, 51.3, 45.1, 40.6, 34.4, 31.8, 15.5, 9.53. Mass (ES): m/z 505 [M+H]\textsuperscript{+}; Anal. Calcd. for C\textsubscript{28}H\textsubscript{35}FN\textsubscript{4}O\textsubscript{4}: C, 66.65; H, 5.79; N, 11.10%. Found: C, 66.89; H, 6.02; N, 10.89%.

4.3.4 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-(1-propyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (V2): Color: Pale yellow; Yield: 73%\textsuperscript{m}, (74%)\textsuperscript{f}; M.p.: 224-24°C; IR (KBr, v): 2955, 2934, 2854, 1718, 1614, 1463, 1322, 1171, 1113, 1056 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO): δ 12.82 (br, s, 1H), 8.46 (s, 1H), 7.62 (d, 1H), 7.53-7.44 (m, 2H), 7.12-7.10 (m, 2H), 4.14 (t, 2H, J = 6.5 Hz), 4.09-4.01 (m, 1H), 3.80 (s, 3H), 3.21-3.01 (m, 5H) 2.13-1.99 (m, 4H), 1.66-1.61 (m, 2H), 1.34-1.32 (m, 2H), 1.05 (t, 3H, J = 6.8 Hz), 0.94-0.84 (m, 7H); \textsuperscript{13}C NMR (DMSO): δ 175.4, 163.9, 157.1, 152.2, 147.5, 140.3, 139.6, 133.8, 130.2, 127.2, 121.0, 115.2, 108.2, 106.4, 63.4, 51.3, 49.3, 41.2, 34.0, 31.4, 29.6, 27.4, 21.0, 14.3, 9.52. Mass (ES): m/z 547 [M+H]\textsuperscript{+}; Anal. Calcd. for C\textsubscript{30}H\textsubscript{35}FN\textsubscript{4}O\textsubscript{4}: C, 68.61; H, 6.45; N, 10.25%. Found: C, 68.48; H, 6.75; N, 9.91%.

4.3.7 1-Cyclopropyl-6-fluoro-7-(4-(1-isopropyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V5): Color: Pale yellow; Yield: 75%\textsuperscript{m}, (51%)\textsuperscript{f}; M.p.: 254-55°C; IR (KBr, v): 3076, 2954, 1724, 1621, 1507, 1454, 1435, 1385, 1315, 1274, 1147, 1059 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMSO): δ 14.85 (br, s, 1H), 8.78 (s, 1H), 8.80 (d, 1H, J =12.2 Hz), 7.80-7.74 (m, 1H), 7.56-7.53 (m, 1H),

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4.3.9 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V7): Color: Pale yellow; Yield: 85% m, (71%)\textsuperscript{m}; M.p.: 204-06°C; IR (KBr, v): 3078, 2960, 2933, 2854, 1720, 1620, 1505, 1457, 1382, 1330, 1278, 1235, 1116, 1063, 1037 cm\textsuperscript{-1}; \textsuperscript{13}C NMR (DMF): \(\delta\) 148.3 (s, 1H), 88.3 (s, 1H), 7.91-7.83 (m, 2H), 7.37-7.27 (m, 3H), 4.45 (t, 2H, \(J = 7.2\) Hz), 4.10-4.08 (m, 1H), 3.92 (s, 3H), 3.80-3.69 (m, 4H), 3.49-3.31 (m, 5H) 2.43-2.34 (m, 2H), 2.11-2.09 (m, 2H) 1.57-1.50 (m, 2H), 1.31 (t, 3H, \(J = 6.8\) Hz), 0.99-0.83 (m 4H); \textsuperscript{13}C NMR (DMF): \(\delta\) 177.4, 165.2, 155.6, 151.3, 147.2, 140.3, 139.0, 133.7, 132.6, 126.7, 124.5, 116.3, 107.0, 106.2, 72.6, 70.4, 63.0, 50.8, 48.7, 41.0, 33.7, 32.0, 26.5, 10.5, 9.32; Mass (ES): \(m/z\) 563 [M+H]\textsuperscript{+}; Anal. Calcd. for C\textsubscript{33}H\textsubscript{32}F\textsubscript{3}N\textsubscript{4}O\textsubscript{2}: C, 66.18; H, 6.27; N, 9.96%. Found: C, 66.01; H, 5.97; N, 10.09%.

4.3.10 1-Cyclopropyl-7-(4-(1-(3-ethoxypropyl)-1H-benzo[d]imidazol-2-yl)-piperidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V8): Color: White; Yield: 83% m, (76%)\textsuperscript{m}; M.p.: 188-90°C; IR (KBr, v): 3068, 2960, 2934, 2850, 1718, 1618, 1552, 1538, 1506, 1454, 1382, 1331, 1277, 1151, 1063 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMF): \(\delta\) 14.80 (br, s, 1H), 8.72 (s, 1H), 7.96 (d, 1H, \(J = 12.2\) Hz), 7.72-7.64 (m, 1H), 7.45-7.32 (m, 3H), 4.51 (t, 2H, \(J = 7.2\) Hz), 4.11-4.09 (m, 1H), 3.96 (s, 3H), 3.79-3.66 (m, 4H) 3.51-3.32 (m, 5H), 2.89-2.67 (m, 2H), 2.48-2.30 (m, 3H), 2.26-2.17 (m, 2H) 1.49 (t, 3H, \(J = 7.6\) Hz), 0.99-0.76 (m 4H); \textsuperscript{13}C NMR (DMF): \(\delta\) 175.7, 165.6, 159.3, 154.7, 148.9, 146.0, 142.3, 139.6, 133.2, 132.1, 129.1, 126.4, 122.5, 117.8, 110.0, 107.8, 107.0, 70.2, 69.4, 63.1, 51.3, 47.2, 41.0, 34.0, 31.9, 30.1, 16.7, 9.80; Mass (ES): \(m/z\) 563 [M+H]\textsuperscript{+}; Anal. Calcd. for C\textsubscript{33}H\textsubscript{32}F\textsubscript{3}N\textsubscript{4}O\textsubscript{2}: C, 66.18; H, 6.27; N, 9.96%. Found: C, 66.01; H, 6.69; N, 10.09%.

4.3.11 7-(4-(1-Benzyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V9): Color: White; Yield: 96% m, (72%)\textsuperscript{m}; M.p.: 236-38°C; IR (KBr, v): 3198, 3072, 2916, 1716, 1685, 1614, 1583, 1544, 1495, 1463, 1375, 1325, 1243, 1171, 1114, 1056 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (DMF): \(\delta\) 12.29 (br, s, 1H), 8.52 (s, 1H), 7.65-7.61 (m, 1H), 7.48-7.31 (m, 7H), 7.13-7.10 (m, 2H), 5.26 (s, 2H) 4.01-3.93 (m, 1H), 3.79 (s, 3H), 3.49-3.36 (m, 5H), 2.13-1.99 (m, 4H), 1.07-0.94 (m, 4H); \textsuperscript{13}C NMR (DMF): \(\delta\) 175.1, 165.0, 157.2, 153.4, 144.7, 143.6, 141.3, 139.7, 138.1, 135.7, 129.6, 127.5, 126.1, 123.2, 115.4, 107.5, 106.0, 64.1, 52.5, 48.7, 43.3, 35.2, 30.2, 9.72; Mass (ES): \(m/z\) 567 [M+H]\textsuperscript{+}; Anal. Calcd. for C\textsubscript{33}H\textsubscript{32}F\textsubscript{3}N\textsubscript{4}O\textsubscript{2}: C, 69.95; H, 5.51; N, 9.89%. Found: C, 70.21; H, 5.32; N, 10.09%.
4.3.12 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-(1-(4-phenylbutyl)-1H-benzo[d]imidazol-2-yl)piperdin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (V10): Color: Off White; Yield: 82%\(^m\), (69%)\(^s\); M.p.: 155-57°C; IR (KBr, v): 2927, 2853, 1727, 1619, 1508, 1452, 1384, 1316, 1278, 1235, 1117, 1090 cm\(^{-1}\); \(^1\)H NMR (DMSO): \(\delta\) 14.82 (br, s, 1H), 8.48 (s, 1H), 7.97-7.88 (m, 1H), 7.40-7.15 (m, 9H), 4.27-4.22 (m, 1H), 4.03 (s, 3H), 3.70 (t, 2H, \(J = 7.2\) Hz), 3.38-3.30 (m, 2H), 3.07-3.02 (m, 1H), 2.72-2.56 (m, 4H); 1.97-1.93 (m, 4H), 1.79-1.70 (m, 2H), 1.26-1.20 (m, 4H), 1.03-1.00 (m, 2H); \(^13\)C NMR (DMSO): \(\delta\) 177.0, 166.8, 158.4, 155.1, 149.9, 146.4, 140.8, 139.6, 133.7, 132.9, 128.6, 126.3, 122.4, 117.7, 110.3, 108.2, 107.5, 62.8, 51.0, 44.3, 40.7, 35.0, 34.3, 31.4, 29.2, 28.1, 9.54; Mass (ES): \(m/z\) 611 [M+H]\(^+\); Anal. Calcd. for C\(_{38}\)H\(_{37}\)F\(_4\)N\(_4\): C, 70.87; H, 6.29; N, 9.03%.

4.3.13 1-Cyclopropyl-7-(4-(1-(4-methoxy carbonyl)phenethyl)-1H-benzo[d]imidazol-2-yl)piperdin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V11): Color: Off White; Yield: 82%\(^m\), (69%)\(^s\); M.p.: 144-46°C; IR (KBr, v): 3063, 2927, 2848, 1720, 1617, 1509, 1442, 1385, 1315, 1276, 1253, 1178, 1089 cm\(^{-1}\); \(^1\)H NMR (DMSO): \(\delta\) 14.86 (s, 1H), 12.28 (br, s, 1H), 8.55 (s, 1H), 7.98-7.95 (m, 2H), 7.66-7.60 (m, 3H), 7.55-7.53 (m, 1H), 7.44-7.41 (m, 1H), 7.16-7.10 (m, 2H), 5.35 (s, 2H), 4.04 (m, 1H), 3.84 (s, 3H), 3.77-3.68 (m, 3H), 3.59-3.50 (m, 2H), 2.13-1.99 (m, 4H), 1.08-0.95 (m, 4H); \(^13\)C NMR (DMSO): \(\delta\) 177.9, 168.3, 165.4, 156.9, 152.3, 146.0, 144.5, 140.7, 139.6, 137.2, 135.4, 129.6, 128.7, 126.6, 122.4, 115.7, 107.9, 106.3, 65.2, 51.2, 48.3, 43.2, 34.6, 31.2, 9.27; Mass (ES): \(m/z\) 611 [M+H]\(^+\), 633 [M+Na]\(^+\); Anal. Calcd. for C\(_{35}\)H\(_{31}\)F\(_4\)N\(_4\): C, 66.88; H, 5.12; N, 9.18% . Found: C, 66.58; H, 5.39; N, 9.23%.

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