www.jmscr.igmpublication.org
Impact Factor (SJIF): 6.379
Index Copernicus Value: 71.58
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v6i5.91

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

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 µg/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 pneumonia*), 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, sparofl-oxacin, trovafloxacin, clinafloxacin, moxifloxacin and gatifloxacin.[5] In view of these observation herein, we report *N*-alkylation of 1*H*-

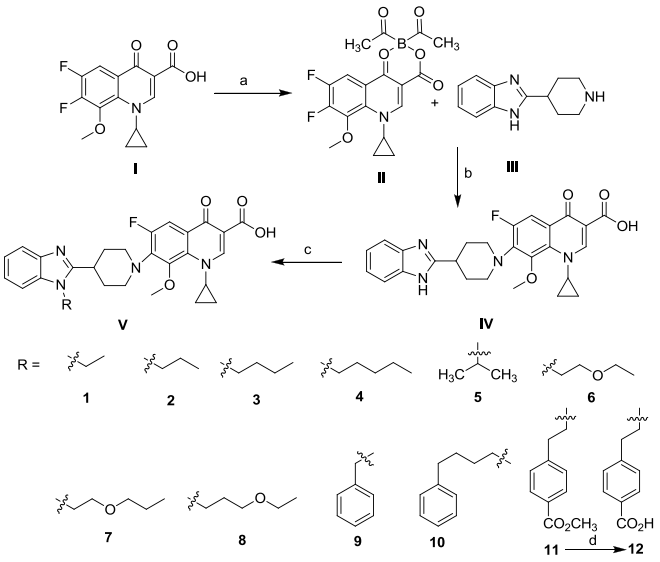
benzimidazolpiperazinefluoroquinoline scaffold with various analogues to create a new structural core with improved effectiveness to kill bacteria resistant to the previous generations.

2. Results and discussion

2.1. Chemistry

The focus of the present investigation is on the development of a few *N*-substituted 7-(4-(1*H*-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*[6] 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**).[7] 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)-1*H*-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 ^{1}H 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 D_2O in ^{1}H NMR spectra. Enone carbon resonated downfield than all the carbons at 176.8 ppm where as carboxylic acid carbon resonating at 166.1 in ^{13}C NMR spectra of compound **IV**. Fluorine attached carbon resonated at 146.6 ppm and O-methyl carbon appeared at 63.4 in ^{13}C 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 ^{13}C NMR and ^{1}H 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 = Ac_2O, $ZnCl_2$, $B(OH)_3$, 120-125°C; b = i). TEA, DMAP, CH_3CN, DMF, 5h; ii). HCl, H_2O, pH = 1-2; c = R-Br (**1-11**), SiO_2, TEA, 100°C, 3 min, MW, 100W; d = i). NaOH, MeOH, H_2O, reflux, 5h; ii). HCl, pH = 1-2.

COSY results

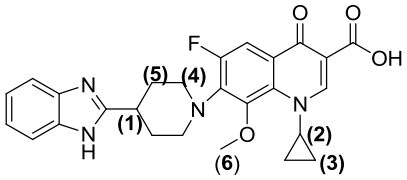


Figure 1: Structure of compound **IV** and parenthesis for respective protons or carbons of aliphatic region for Table 1 and Table 2

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

Sl. No.	Interaction		Source of interaction
	^{1}H NMR ppm	^{1}H NMR ppm	
1	4.18-4.20	1.15-1.05	2-3
2	3.66-3.52	2.27-2.07	4-5

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.

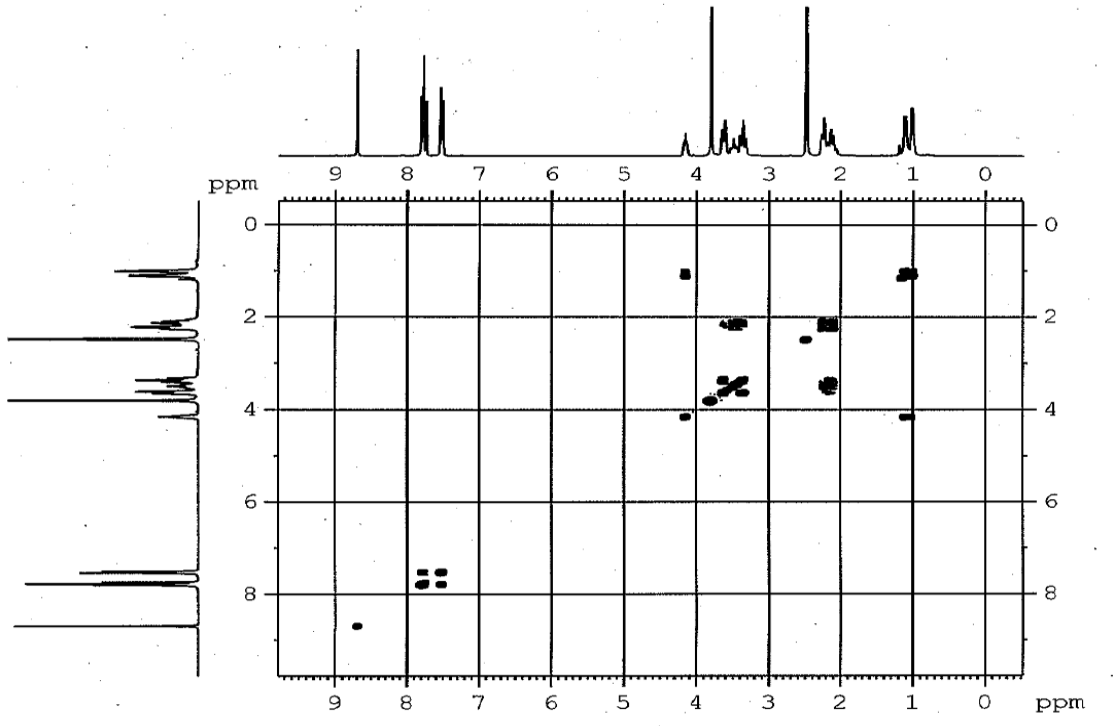


Figure 2: COSY spectra of compound **IV**

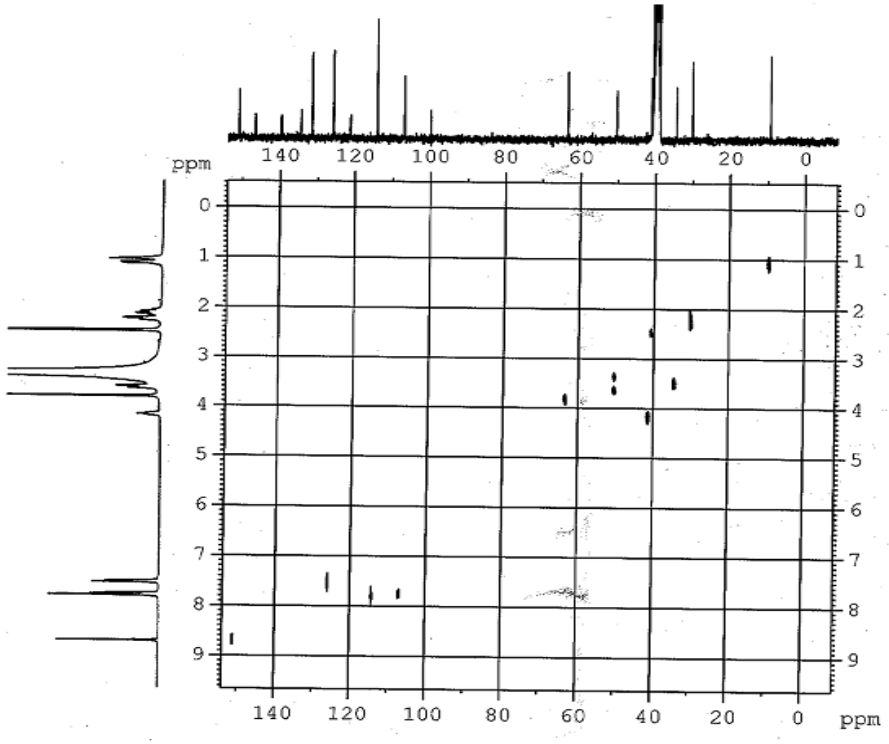


Figure 3 HMBC spectra of compound **IV**

HMBC results

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

Sl. No.	Interaction		Source of interaction
	^{13}C NMR ppm	^{1}H NMR ppm	
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.[8]

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*-iodonitroterazoliun 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 micro-organisms namely, *P. aeruginosa*, *S. epidermidis*, *K. pneumonia* with MIC of 19 µg/mL with compound **V3**. *P. aeroginosa* 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. aeroginosa*, *K. pnemonia* 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.e*	*K.p*	*C.a*
1	Moxi	520	--	260	--	260	--
2	Cipro	130	38	38	65	38	65
3	Levo	65	38	38	38	19	38
4	Chlo	65	65	38	38	38	--
5	Nystatin	--	--	--	--	--	65
6	**IV**	65	38	19	19	19	
7	**V1**	130	--	260	65	65	130
8	**V2**	130	--	260	65	38	130
9	**V3**	65	38	19	19	19	38
10	**V4**	130	65	65	65	260	520
11	**V5**	65	38	19	38	19	65
12	**V6**	130	260	65	130	260	520
13	**V7**	130	--	260	260	65	130
14	**V8**	130	130	130	65	65	130
15	**V9**	260	--	260	260	130	260
16	**V10**	520	--	130	--	130	260
17	**V11**	260	--	260	--	130	260
18	**V12**	260	65	130	260	65	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.[15] 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*	*K.p*	*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-(1*H*-benzo[*d*]imidazol-2-yl) piperidin-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% H_2SO_4 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. ^{1}H NMR and ^{13}C 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-carboxyclic 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-carboxyclic 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 contents to rt and stirred for 1h, 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-(1*H*-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)-1*H*-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 5h 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, υ): 3437, 3026, 1730, 1672, 1509, 1456, 1318, 1232, 1056 cm^{-1}. ^{1}H 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); ^{13}C 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 $C_{26}H_{25}FN_4O_4$: C, 65.54; H, 5.29; N, 11.76%. Found: C, 65.88; H, 5.32; N, 11.95%.

4.3.1 General procedure for the synthesis of *N*-substituted 7-(4-(1*H*-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 SiO_2 gel column chromatography with DCM:methanol (95:5%) to afford title compounds **(V1-12).**

4.3.2 General procedure for the synthesis of *N*-substituted 7-(4-(1*H*-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 alkylbromides (**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 SiO_2 gel column chromatography with hexane:EtOAc (60:40%) to afford.

(^{c} = yield of conventional method; ^{m} = yield of microwave method).

4.3.3 1-Cyclopropyl-7-(4-(1-ethyl-1*H*-benzo [*d*]imidazol-2-yl) piperidin-1-yl)-6-fluoro-8-

methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V1): Color: White; Yield: 89%[m], (76%)[c]; M.p.: 238-40°C; IR (KBr, υ): 2934, 2834, 1731, 1621, 1511, 1457, 1384, 1315, 1236, 1184, 1116, 1060 cm^{-1}. ^1H NMR (DMSO): δ 14.83 (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). ^{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 $[M+H]^+$; Anal. Calcd. for $C_{28}H_{29}FN_4O_4$: 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-1*H*-benzo[*d*]imidazol-2-yl) piperidin-1-yl)- 1,4-dihydroquinoline-3-carboxylic acid (V2): Color: Pale yellow; Yield: 84%[m], (80%)[c]; M.p.: 247-49°C; IR (KBr, υ): 3053, 2840, 1730, 1615, 1598, 1534, 1502, 1441, 1384, 1314, 1276, 1236, 1187, 1116, 1091 cm^{-1}. ^1H NMR (DMSO): δ 14.82 (br. s, 1H), 8.82 (s, 1H), 7.88 (d, 1H, J = 12.0 Hz), 7.78-7.75 (m, 3H), 7.37-7.26 (m, 3H), 4.16 (t, 2H, J = 7.2 Hz), 4.09-4.05 (m, 1H), 3.90 (s, 3H), 3.75-3.70 (m, 2H), 3.45-3.37 (m, 2H), 3.09-3.03 (m, 1H), 2.42-2.29 (m, 2H), 2.09-1.94 (m, 2H), 1.83-1.92 (m, 2H), 1.25 (t, 3H, J = 7.2 Hz), 1.06-0.91 (m, 4H); ^{13}C NMR (DMSO): δ 177.0, 166.7, 156.9, 149.8, 146.0, 142.6, 139.8, 134.9, 133.7, 122.1, 121.9, 119.4, 109.5, 108.0, 107.6, 62.3, 51.3, 45.1, 40.6, 34.4, 31.9, 23.5, 11.4, 9.5; Mass (ES): *m/z* 519 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{31}FN_4O_4$: C, 67.17; H, 6.03; N, 10.80%. Found: C, 66.89; H, 6.25; N, 10.51%.

4.3.5 1-Cyclopropyl-7-(4-(1-butyl-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V3): Color: White; Yield: 86%[m], (62%)[c]; M.p.: 220-21°C; IR (KBr, υ): 3049, 2954, 2853, 1729, 1618, 1600, 1506, 1440, 1382, 1329, 1314, 1277, 1146, 1062, 1029 cm^{-1}; ^1H NMR (DMSO): δ 14.82 (br. s, 1H), 8.82 (s, 1H), 7.88 (d, 1H, J = 12.1 Hz), 7.79-7.64 (m, 1H), 7.37-7.34 (m,1H), 7.29-7.25 (m, 1H), 4.19 (t, 2H, J = 6.6 Hz), 4.10-4.08 (m, 1H), 3.91 (s, 3H), 3.71 (t, 2H, J = 7.6 Hz), 3.41 (t, 2H, J = 7.6 Hz), 3.06 (t, 1H, J = 7.6 Hz), 2.42-2.31 (m, 2H), 2.09-1.90 (m, 2H), 1.87-1.80 (m, 2H), 4.17-4.12 (m, 2H), 1.22 (t, 3H), 1.04-0.92 (m, 4H); ^{13}C NMR (DMSO): δ 177.0, 166.7, 158.3, 156.8, 155.0, 149.9, 146.0, 142.6, 139.9, 134.8, 133.7, 122.2, 122.1, 121.9, 119.4, 109.4, 108.4, 107.6, 62.3, 51.3, 43.3, 40.6, 34.5, 32.3, 31.9, 20.2, 13.7, 9.54. Mass (ES): *m/z* 533 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{33}FN_4O_4$: C, 67.65; H, 6.25; N, 10.52%. Found: C, 68.02; H, 6.55; N, 10.11%.

4.3.6 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-(1-pentyl-1*H*-benzo[*d*]imidazol-2-yl) piperidin-1-yl)- 1,4-dihydroquinoline-3-carboxylic acid (V4): Color: Pale yellow; Yield: 73%[m], (74%)[c]; M.p.: 222-24°C; IR (KBr, υ): 2955, 2934, 2854, 1718, 1614, 1463, 1323, 1242, 1171, 1113, 1056 cm^{-1}; ^1H 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); ^{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 $[M+H]^+$; Anal. Calcd. for $C_{31}H_{35}FN_4O_4$: C, 68.11; 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-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl)-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V5): Color: Pale yellow; Yield: 75%[m], (51%)[c]; M.p.: 254-55°C; IR (KBr, υ): 3076, 2954, 1724, 1621, 1507, 1454, 1435, 1385, 1315, 1274, 1147, 1059 cm^{-1}; ^1H 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), 7.56-753 (m, 1H),

7.27-7.19 (m, 2H), 4.81-4.76 (m, 1H), 4.08-4.02 (m, 1H), 3.82 (s, 3H), 3.77-3.69 (m, 2H), 3.45-3.34 (m, 2H), 3.10-3.06 (m, 1H), 2.38-2.25 (m, 1H), 2.11-2.00 (m, 2H), 1.70 (d, 6H, *J* = 6.6 Hz), 1.26-1.20 (m, 2H), 1.04-0.98 (m, 2H); ^{13}C NMR (DMSO): *δ* 177.0, 166.8, 156.2, 149.8, 145.9, 143.1, 139.8, 133.8, 133.2, 121.5, 121.4, 119.6, 111.7, 107.9, 107.3, 62.3, 51.1, 47.4, 40.6, 34.8, 31.5, 21.5, 9.46; Mass (ES): *m/z* 519 $[M+H]^+$; Anal. Calcd. for $C_{29}H_{31}FN_4O_4$: C, 67.17; H, 6.03; N, 10.80%. Found: C, 66.89; H, 5.59; N, 10.51%.

4.3.8 1-Cyclopropyl-7-(4-(1-(2-ethoxyethyl)-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V6): Color: Pale yellow; Yield: 75%[m], (74%)[c]; M.p.: 194-96°C; IR (KBr, υ): 2932, 2855, 1719, 1618, 1506, 1455, 1383, 1317, 1277, 1115, 1035 cm^{-1}; ^{1}H NMR (DMSO): *δ* 14.84 (br. s, 1H), 8.83 (s, 1H), 7.90 (d, 1H, *J* = 12.4 Hz), 7.80-7.77 (m, 1H), 7.37-7.25 (m, 3H), 4.44 (t, 2H, *J* = 7.8 Hz), 4.09-4.05 (m, 1H), 3.90 (s, 3H), 3.78-3.69 (m, 5H) 3.49-3.39 (m, 4H), 2.11-2.01 (m, 4H), 1.13 (t, 3H, *J* = 7.6 Hz), 1.05-0.95 (m, 4H); ^{13}C NMR (DMSO): *δ* 175.7, 167.6, 154.8, 151.3, 147.2, 139.6, 139.2, 134.0, 132.6, 125.0, 120.8, 113.4, 108.3, 106.1, 71.0, 66.3, 63.4, 51.3, 49.6, 41.0, 34.7, 29.7, 15.9, 9.62; Mass (ES): *m/z* 549 $[M+H]^+$; Anal. Calcd. for $C_{30}H_{33}FN_4O_5$: C, 65.68; H, 6.06; N, 10.21%. Found: C, 65.29; H, 6.41; N, 10.51%.

4.3.9 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(4-(1-(2-propoxyethyl)-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl) -1,4-dihydroquinoline-3-carboxylic acid (V7): Color: Pale yellow; Yield: 85%[m], (71%)[c]; M.p.: 204-06°C; IR (KBr, υ): 3078, 2960, 2933, 2854, 1720, 1620, 1505, 1457, 1382, 1330, 1278, 1235, 1116, 1063, 1037 cm^{-1}; ^{1}H NMR (DMSO): *δ* 14.83 (s, 1H), 8.83 (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); ^{13}C NMR (DMSO): *δ* 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, 64.3, 50.8. 48.7, 41.0, 33.7, 30.2, 26.5, 10.5, 9.32; Mass (ES): *m/z* 563 $[M+H]^+$; Anal. Calcd. for $C_{31}H_{35}FN_4O_5$: 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)-1*H*-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%)[c]; M.p.: 188-90°C; IR (KBr, υ): 3068, 2960, 2934, 2850, 1718, 1618, 1552, 1538, 1506, 1454, 1382, 1331, 1277, 1151, 1063 cm^{-1}; ^{1}H NMR (DMSO): *δ* 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); ^{13}C NMR (DMSO): *δ* 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 $[M+H]^+$; Anal. Calcd. for $C_{31}H_{35}FN_4O_5$: 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-1*H*-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%)[c]; M.p.: 236-38°C; IR (KBr, υ): 3198, 3072, 2916, 1716, 1685, 1614, 1583, 1544, 1495, 1463, 1375, 1325, 1243, 1171, 1114, 1056 cm^{-1}; ^{1}H NMR (DMSO): *δ* 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); ^{13}C NMR (DMSO): *δ* 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 $[M+H]^+$; Anal. Calcd. for $C_{33}H_{31}FN_4O_4$: 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)-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (V10): Color: off White; Yield: 82%[m], (69%)[c]; M.p.: 155-57°C; IR (KBr, υ): 2927, 2853, 1727, 1619, 1508, 1452, 1384, 1316, 1278, 1235, 1117, 1090 cm^{-1}; ^1H NMR (DMSO): δ 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), 1.26-120 (m, 4H), 1.03-1.00 (m, 2H); ^{13}C NMR (DMSO): δ 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 $C_{36}H_{37}FN_4O_4$: C, 71.03; H, 6.13; N, 9.20%. Found: C, 70.87; H, 6.29; N, 9.03%.

4.3.13 1-Cyclopropyl-7-(4-(1-(4-(methoxycarbonyl) phenethyl) -1*H*-benzo[*d*]imidazol-2-yl)piperidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V11): Color: White; Yield: 90%[m], (79%)[c]; M.p.: 144-46°C; IR (KBr, υ): 3071, 2924, 1722, 1618, 1509, 1443, 1384, 1314, 1278, 1179, 1110 cm^{-1}; ^1H NMR (DMSO): δ 14.99 (br. s, 1H), 8.68 (s, 1H), 7.93-7.91 (m, 2H), 7.64-7.61(m, 1H), 7.41-7.40(m, 1H), 7.23-7.13(m, 4H), 5.69 (s, 2H), 4.17-4.15 (m, 1H), 4.02-4.01 (m, 1H), 3.82 (s, 6H), 3.42-3.30 (m, 4H), 2.07-1.90 (m, 4H), 1.21-1.02 (m, 4H); ^{13}C NMR (DMSO): δ 177.6, 167.2, 164.5, 154.0, 151.6, 144.9, 143.9, 141.2, 139.0, 137.6, 135.0, 130.2, 129.1, 127.1, 125.4, 122.1, 116.9, 107.9, 65.0, 51.6, 50.5, 48.6, 42.9, 34.7, 31.8, 9.21; Mass (ES): *m/z* 625 $[M+H]^+$; Anal. Calcd. for $C_{35}H_{33}FN_4O_6$: C, 67.30; H, 5.32; N, 8.97%. Found: C, 66.98; H, 5.00, N, 9.12%.

4.4 Synthesis of 1-Cyclopropyl-7-(4-(1-(4-(methoxycarbonyl)phenethyl)-1*H*-benzo[*d*]imidazol-2-yl) piperidin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (V12)

Saponification of 7-(4-(1-(4-carboxyphenethyl)-1*H*-benzo[*d*] imidazol-2-yl)piperidin-1-yl)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**V11**) (6.24 g, 1 mmol) by 10% aq. sodium hydroxide solution (10 mL) in methanol (10 mL), heated to reflux and maintained for 5 h at the same temperature, cooled to rt, pH of the reaction mixture was adjusted to 1 to 2 by using concentrated HCl, filtered and washed with water followed by chilled methanol. Yield: 4.88 g (80%); Color: Pale yellow; Yield: 80%[c]; M.p.: 278-80°C; IR (KBr, υ): 3063, 2927, 2848, 1720, 1617, 1509, 1442, 1385, 1315, 1276, 1253, 1178, 1089 cm^{-1}; ^1H NMR (DMSO): δ 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): δ 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, 42.3, 34.6, 31.2, 9.27; Mass (ES): *m/z* 611 $[M+H]^+$, 633 $[M+Na]^+$; Anal. Calcd. for $C_{34}H_{31}FN_4O_6$: C, 66.88; H, 5.12; N, 9.18% . Found: C, 66.58; H, 5.39; N, 9.23%.

Acknowledgments

The authors express their sincere thanks to Neuland laboratories Limited, Hyderabad, India for supporting for this research work. Authors are also thankful to authorities of Jawaharlal Nehru Technological University, Hyderabad and NES Science College, Nanded.

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