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Original article

Investigation of Ugi-4CC derived 1*H*-tetrazol-5-yl-(aryl) methyl piperazinyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid: Synthesis, Biology and 3D-QSAR analysis

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ABSTRACT

Novel series of 7-piperazinylquinolones with tetrazole derivatives were synthesized and evaluated for their antibacterial activity against various strains of *Staphylococcus aureus*. All the synthesized compounds showed significant *in vitro* antibacterial activity against Gram-positive bacteria whereas some compounds displayed moderate activity against Gram-negative bacteria. Among all the synthesized compounds, compounds (**6a**–**c**, **6e**–**g**, **6i**–**k**, **6m**, **6′f** and **6′m**) were found to be more effective with MIC ranging from (0.78–3.12 μg/mL) against *S. aureus* (ATCC-29213) than the control; ciprofloxacin (MIC = 25 μg/mL). Moreover, these analogues displayed no toxicity up to MIC = 0.39 μg/mL against mammalian cell line L-929. Furthermore, to correlate the biological activities of synthesized compounds with their 3D conformation, we attempted 3D-QSAR study.

1. Introduction

Despite increasing focus and research in medicinal chemistry, Antibiotic resistance to presently available antibacterial agents is a growing health problem in the community and hospitals [1]. On a worldwide basis, nosocomial multidrug-resistant Gram-positive staphylococcal pathogen isolates and found to display resistance to frontline antibacterials such as methicillin, vancomycin and gentamicin, and the urgent need for safe and affordable antibacterial agents capable to overcome the resistance is required [2–4]. The numbers of solutions to remove the problem of bacterial resistance are possible and the most prevalent approach is to continue modifying the existing classes of antibacterial agents to provide new analogues [5,6].

An initial study reported that quinolones are the significant class of antibacterial, antitubercular [7] and antimalarial agents [8], which are extensively approved for the treatment of human infections [9]. The beginning of nalidixic acid in 1962 has shown the new avenue for the patients with the bacterial infections and also the dramatic impact in changing morbidity and mortality rate compared with the chemotherapy during 1940s [8]. But in the period of 1980s, the first fluoroquinolone norfloxacin [10] and other quinolone derivatives like ciprofloxacin [11], sparfloxacin [12] and trovafloxacin [13] (Fig. 1) have launched which changed the landscape of antibacterial chemotherapy and displayed activity against both Gram-negative and Gram-positive bacterial pathogens. The structure activity relationship (SAR) studies showed that, the fluorine atom and the 1-alkyl, 1,4-dihydro-4-oxoquinoline-3-carboxylic acid skeleton of fluoroquinolones is responsible for potency represented in binding with type-II topoisomerase enzymes, DNA gyrase and topoisomerase IV [14,15]. Moreover, it is believed that the 6-fluoro and 7-piperazinyl groups are responsible for the broad spectrum and antipseudomonal activities of fluoroquinolones. Furthermore, it is clear that chemical modifications at C-7 is suitable for controlling of the pharmacokinetic properties and hence changes in the cell permeability of these antibiotics [16]. Recently Emami et al. synthesized mannich bases of 7-piperazinylquinolones with kojic acid and chlorokojic acid as potent antibacterials [17].

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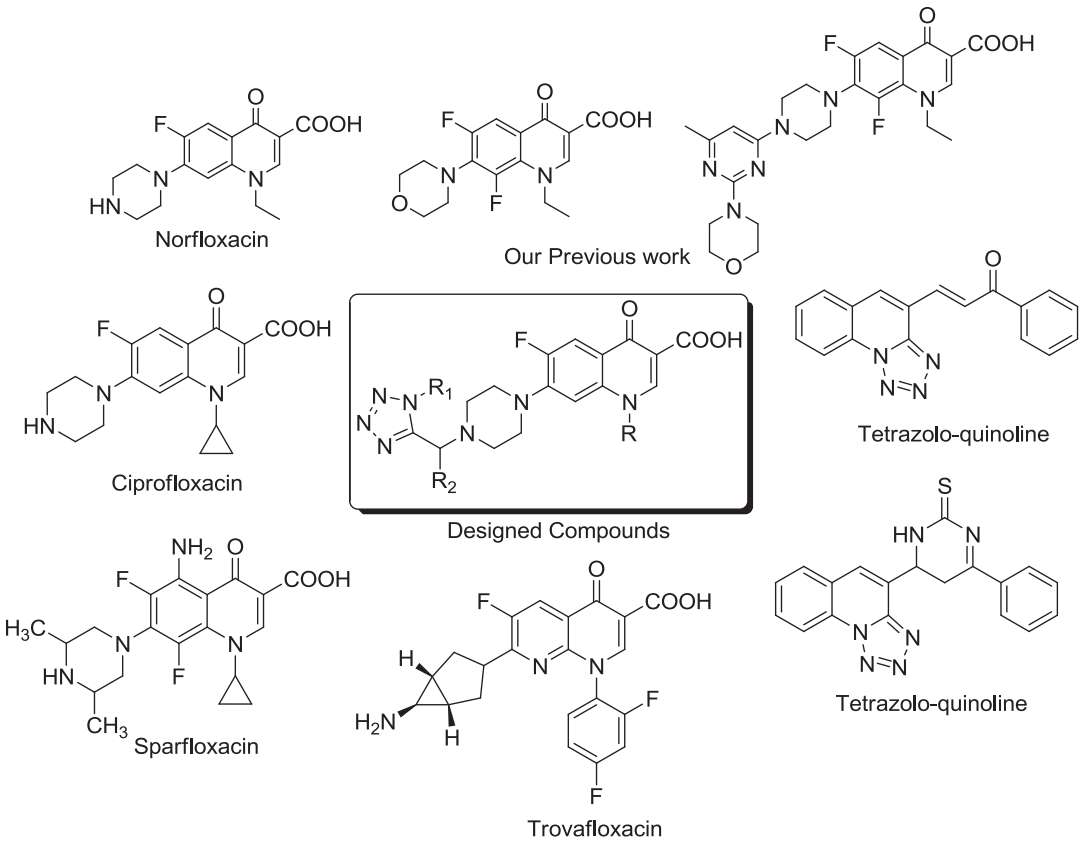


Fig. 1. Designing of quinolone, tetrazole-based derivatives showing antibacterial activity.

In this regards, tetrazole is a class of heterocycles with wide range of applications that is receiving considerable attention [18]. Tetrazole and their derivatives exhibited various biological applications such as anti-allergic, antibiotic, antihypertensive, antibacterial and anticonvulsant agents [19–22]. Previously we have synthesized 8-fluoro Norfloxacin derivatives and their hybrid with triazines and pyrimidine which showed potent activity against methicillin and vancomycin resistant strains of *Staphylococcus aureus* [23]. In our effort and continuation of our ongoing anti-infective research programme [24–28], considering our previous work. We envisaged the incorporation of tetrazole scaffolds with 7-piperazinylquinolones.

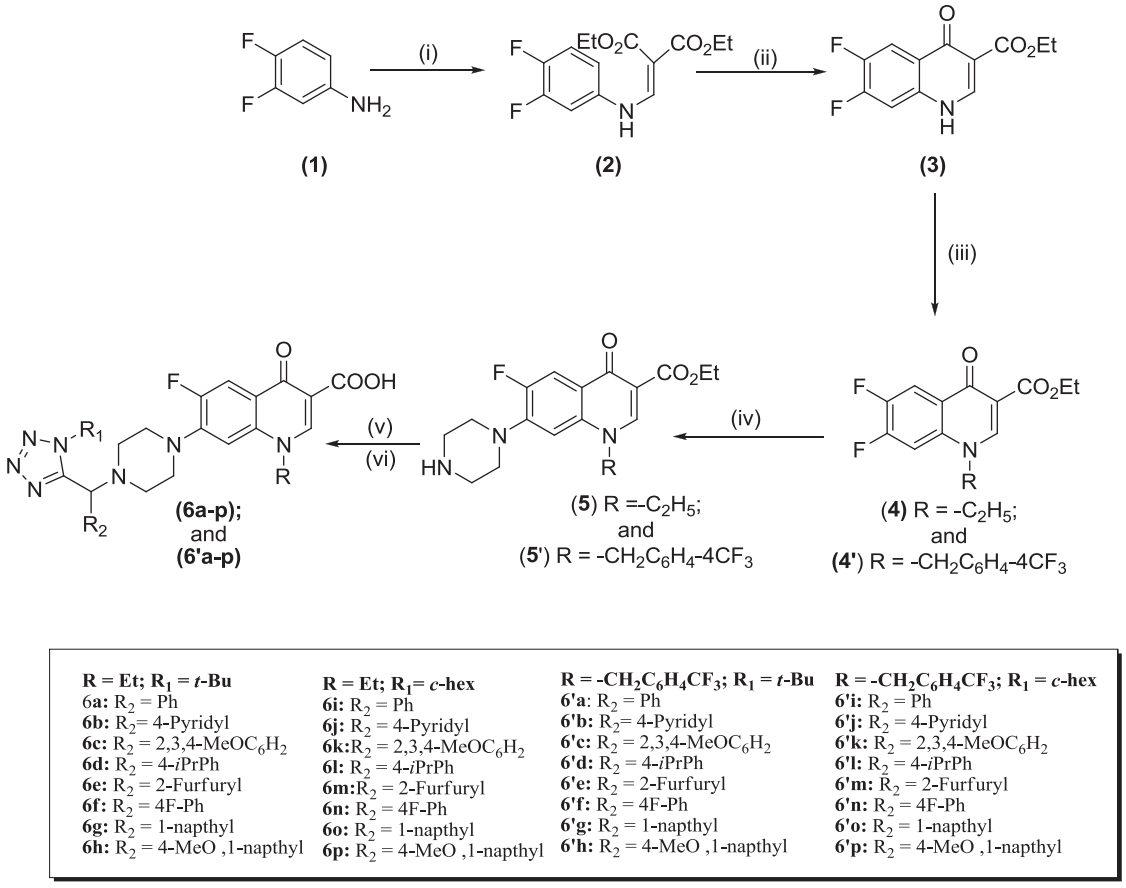
In order to identify molecular properties that have the largest impact on antibacterial activity of the synthesized compounds, we performed the QSAR (Quantitative structure activity relationship) analysis [29]. QSAR have been applied for decades in the improvement of relationships between physicochemical properties of chemical substances and their biological activities to achieve a reliable statistical model for prediction of the activities of new chemical entities [30]. In this contest 3D-QSAR has emerged as a natural extension to the classical Hansch and Free-Wilson approaches, which develops the three-dimensional properties of the ligands to predict their biological activities using robust chemometric techniques such as PLS, G/PLS, ANN etc. Several success stories of QSAR have attracted the medicinal chemists to investigate the relationship of structural properties with biological activity [31].

2. Chemistry overview

The realization of a facile access towards 1*H*-tetrazol-5-yl-(aryl) methyl piperazinyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid from readily available substrates, a small library of 32 compounds were synthesized for the structure activity relationship (SAR). The synthesis of norfloxacin analogues possessing Ugi derived substructure (**6a**–**p** and **6′a**–**p**) is outlined in Scheme 1. The intermediate ethyl 6,7-difluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylate **3** was achieved *via* cyclization of diethyl 2-((3, 4-difluorophenylamino) methylene) malonate **2** in diphenyl ether under refluxing condition (~250 °C), which was obtained from 3, 4-difluoro aniline **1**. The treatment of **3** with ethyl iodide and K_2CO_3 in dry DMF at 90 °C produced ethyl 1-ethyl-6,7-difluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate **4** in good yield and with 1-(bromomethyl)-4-(trifluoromethyl)benzene NaH mediated N-alkylation in DMF at 90 °C gave ethyl 6,7-difluoro-4-oxo-1-(4-(trifluoromethyl)benzyl)-1,4-dihydroquinoline-3-carboxylate **4′** in quantitative yield. Further, the nucleophilic substitution at 7th position of **4** and **4′** with piperazino in the presence of K_2CO_3 using acetonitrile as solvent under refluxing condition offered their respective derivatives **5** and **5′** in 84 and 80% yield respectively. In this context, compound **5** and **5′** was allowed to react with different commercially available aldehydes, isocyanides and azidotrimethylsilane ($TMSN_3$) in anhydrous methanol to form the desired quinolone–tetrazole ester derivatives [32]. Esters were hydrolysed to their respective carboxylic acids **6a**–**p** and **6′a**–**p** by treating with aqueous NaOH in methanol (1:1) quantitatively.

3. *In vitro* antibacterial assay

All the synthesized compounds (**6a**–**p** and **6′a**–**p**) were screened for antibacterial activity against Gram-positive bacteria *S. aureus* (ATCC-25923), *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213) and *S. aureus* (ATCC-33592) and Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* using standard



R = Et; R_1 = *t*-Bu	**R = Et; R_1= *c*-hex**	**R = -$CH_2C_6H_4CF_3$; R_1 = *t*-Bu**	**R = -$CH_2C_6H_4CF_3$; R_1 = *c*-hex**
6a: R_2 = Ph	**6i:** R_2 = Ph	**6'a**: R_2 = Ph	**6'i:** R_2 = Ph
6b: R_2= 4-Pyridyl	**6j:** R_2 = 4-Pyridyl	**6'b:** R_2= 4-Pyridyl	**6'j:** R_2 = 4-Pyridyl
6c: R_2 = 2,3,4-$MeOC_6H_2$	**6k:**R_2 = 2,3,4-$MeOC_6H_2$	**6'c:** R_2 = 2,3,4-$MeOC_6H_2$	**6'k:** R_2 = 2,3,4-$MeOC_6H_2$
6d: R_2 = 4-*i*PrPh	**6l:** R_2 = 4-*i*PrPh	**6'd:** R_2 = 4-*i*PrPh	**6'l:** R_2 = 4-*i*PrPh
6e: R_2 = 2-Furfuryl	**6m:**R_2 = 2-Furfuryl	**6'e:** R_2 = 2-Furfuryl	**6'm:** R_2 = 2-Furfuryl
6f: R_2 = 4F-Ph	**6n:** R_2 = 4F-Ph	**6'f:** R_2 = 4F-Ph	**6'n:** R_2 = 4F-Ph
6g: R_2 = 1-napthyl	**6o:** R_2 = 1-napthyl	**6'g:** R_2 = 1-napthyl	**6'o:** R_2 = 1-napthyl
6h: R_2 = 4-MeO ,1-napthyl	**6p:** R_2 = 4-MeO ,1-napthyl	**6'h:** R_2 = 4-MeO ,1-napthyl	**6'p:** R_2 = 4-MeO ,1-napthyl

Scheme 1. Representative Scheme for the preparation of 1*H*-tetrazol-5-yl-(aryl) methyl piperazinyl-6-fluoro-Qs. Reagents and conditions: (i) Diethyl ethoxy methylene malonate, 120 °C; (ii) Diphenyl ether, reflux; (iii) Ethyl iodide, K_2CO_3, DMF, 90 °C (compound 4) and 4-Triflouromethylbenzyl-bromide, NaH, DMF, 90 °C (compound 4′); (iv) Piperazine, K_2CO_3, CH_3CN, 90 °C (compound 5 and 5′); (v) R2-CHO, R1-NC, TMSN3, MeOH, rt, (vi) KOH, MeOH/H_2O (1:1), 90 °C, (**6a-p** and **6′a–p**).

techniques and compared to the standard drugs ciprofloxacin and gentamicin. The bacterial strains were grown on nutrient agar at 37 °C. After 24 h of incubation, bacterial cells were suspended in normal saline containing Tween 20 at 0.05% at a concentration of approximately 1.0–2.0 × 10^7 cells/mL by matching with 0.5 McFarland standards. The activity of compounds was determined as per NCCLS protocol using Mueller Hinton broth (Becton Dickinson, USA) in 96-well tissue culture plates [33].

4. Structure activity relationship

The results of compounds **6a–p** and **6′a–p** were presented in Table 1. At a glance, compounds having N-ethyl substitution (**6a–p**) were found to be more potent in assessment with the compounds having 4-(trifluoromethyl) benzyl group as N-substituent (**6′a–p**) of quinolone ring. Compounds (**6a–h**) having t-butyl substituent at R_1, Compound **6a** which contained the benzylidene at R_2 position displayed significant MIC = 0.39 μg/mL against *S. aureus* (ATCC-25923), and MIC = 25, 0.78 and 0.39 μg/mL against M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592), respectively which is more potent than that of ciprofloxacin and gentamicin against *S. aureus* (ATCC-25923) and *S. aureus* (ATCC-29213). Compound **6b** (R_2 = 4-pyridine) exhibited potent MIC values (MIC = 1.56, >50, 1.56 and 1.56 μg/mL) against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). While compound **6c** having R_2 = 3, 4, 5-trimethoxy benzylidene showed MIC values (MIC = 1.56, 50, 3.12 and 3.12 μg/mL) against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). Surprisingly, in case of 4-isopropylbenzylidene compound **6d** activity diminished. Furthermore, compound **6e** with 2-furfuralehyde substituent found to be more potent than gentamicin and display equal potency with ciprofloxacin. Compound **6f,** having 4-fluorobenzyledene also showed good activity against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592) than controls; ciprofloxacin and gentamicin. Compounds **6g** exhibited potent activity against all the strains of *S. aureus* while Compounds **6h** showed remarkable activity. Similarly, in compounds (**6i–p**) having cyclohexyl substituent at R_1, compound **6i** R_2 = benzylidene showed potent activity MIC = 1.56 μg/mL against *S. aureus* (ATCC-25923) and MIC = 12.5, 3.12 and 1.56 μg/mL against M-R *S. aureus* ATCC 700699, *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592) respectively which is more potent than ciprofloxacin and gentamicin. Compound **6j** having R_2 = 4-pyridine showed MIC = 1.56 μg/mL against *S. aureus* (ATCC-25923) and MIC ≥ 50, 3.12 and 3.12 μg/mL M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592) respectively. While compound **6k** = 2, 3, 4 trimethoxy benzylidene activity enhance against *S. aureus* (ATCC-29213), and similar MIC values against G-R *S. aureus* (ATCC-33592). Surprisingly, the substitution with R_2 = 4-isopropylbenzylidene compound **6l** activity diminished in all strains of *S. aureus*. Furthermore, compound **6m** R_2 = 2-furfuraldehyde found to be more potent than gentamicin-with MIC values 0.78, 25, 1.56 and 0.78 μg/mL against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-

Table 1
Antibacterial activities of 1*H*-tetrazol-5-yl-(aryl) methyl piperazinyl-6-fluoro-Quinolones.

Entry	[a]ATCC 25923			[b]ATCC 700699			[c]ATCC 29213			[d]ATCC 33592		
	MIC	MIC[e]	IC_{50}[e]	MIC	MIC[e]	IC_{50}[e]	MIC	MIC[e]	IC_{50}[e]	MIC	MIC[e]	IC_{50}[e]
6a	0.39	0.38	0.37	25	8.3	3.8	0.78	0.50	0.20	0.39	0.38	0.34
6b	1.56	0.90	0.50	50	50	50	1.56	0.90	0.60	1.56	0.70	0.35
6c	1.56	0.81	0.40	50	50	50	3.12	1.40	0.80	3.12	0.87	0.75
6d	50	50	50	50	50	50	50	>50	50	>50	50	>50
6e	0.78	0.40	0.30	50	46.9	24.6	0.78	0.60	0.20	0.78	0.50	0.20
6f	0.78	0.90	0.30	12.5	12.5	10.0	0.78	0.70	0.20	0.78	0.50	0.20
6g	1.56	0.80	0.70	6.25	4.90	3.50	3.12	2.40	0.20	0.78	0.70	0.20
6h	12.5	18.0	7.00	12.5	6.90	7.50	25.0	50	50	12.5	50	50
6i	1.56	0.80	0.40	12.5	11.6	4.90	3.12	1.50	0.80	1.56	0.78	0.75
6j	1.56	1.09	0.37	50	>50	50	3.12	1.00	0.60	3.12	0.60	0.10
6k	1.56	1.35	0.37	25	28.9	14.6	1.56	0.80	0.70	1.56	0.80	0.70
6l	12.5	19.0	8.00	50	50	50	12.5	3.30	3.10	3.12	1.60	1.50
6m	0.78	0.45	0.22	25	8.20	3.70	1.56	1.10	0.60	0.78	0.88	0.40
6n	1.56	2.10	0.50	50	50	50	50	>50	50	50	50	50
6o	3.12	1.40	0.77	25	8.20	3.70	6.25	2.90	1.60	3.12	4.20	1.60
6p	6.25	4.40	1.70	25	9.00	4.06	12.5	6.90	3.60	6.25	4.70	3.60
6′a	50	50	50	25	50	50	25	50	50	12.5	50	50
6′b	50	50	50	50	50	50	50	50	50	50	50	50
6′c	50	50	50	50	50	50	50	50	50	50	50	50
6′d	3.12	3.12	1.80	3.12	1.60	1.40	6.25	3.10	1.50	3.12	0.87	0.80
6′e	50	50	50	50	50	50	25	50	50	12.5	50	50
6′f	6.25	3.10	1.00	25	19.8	8.09	3.12	1.60	1.40	6.25	2.50	0.50
6′g	50	50	50	50	50	50	50	50	>50	50	>50	>50
6′h	50	>50	50	>50	50	50	>50	>50	>50	>50	>50	>50
6′i	12.5	17.5	5.20	>50	>50	>50	6.25	4.40	2.20	25	40.5	21.0
6′j	50	50	>50	50	50	>50	50	50	>50	>50	>50	50
6′k	50	50	>50	>50	>50	>50	>50	>50	>50	>50	>50	50
6′l	50	50	>50	>50	50	>50	>50	>50	50	>50	>50	50
6′m	0.78	0.52	0.20	50	50	>50	1.56	0.82	0.75	0.78	0.78	0.40
6′n	12.5	12.5	5.20	>50	>50	>50	>50	>50	>50	>50	>50	>50
6′o	50	>50	50	>50	>50	>50	>50	>50	>50	>50	>50	>50
6′p	50	>50	50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Cipr[f]	0.78	0.9	0.3	25	20.2	16.7	25	20.2	16.7	0.19	0.4	0.2
Gent[g]	1.56	2.1	1.7	>50	>50	>50	>50	>50	>50	>50	>50	>50

[a] *S. aureus* (ATCC-25923).
[b] MRSA: methicillin-resistant *S. aureus* (ATCC-700699).
[c] *S. aureus* (ATCC-29213).
[d] GRSA: Gentamicin-resistant *S. aureus* (ATCC-33592).
[e] spectrophotometrically determined.
[f] Ciprofloxacin.
[g] Gentamicin.

29213), and G-R *S. aureus* (ATCC-33592). Compound **6n**, having 4-fluorobenzyledene at R_2 displayed good activity against *S. aureus* (ATCC-25923) while activity reduced in resistant strains of *S. aureus*. In case of compounds **6o** and **6p** R_2 = naphthyledene and 4-methoxynaphthyledene, showed good activity against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). Compounds (**6′a**–**p**) were having 4-(trifluoromethyl) benzyl group as N-substituent and in this sequence compounds (**6′a**–**h**) were having t-butyl substituent at R_1. Compounds **6′a**, **6′b** and **6′c** which contained the R_2 = benzylidene, 4-pyridine and 3, 4, 5-trimethoxy benzylidene respectively exhibited less potent activity against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). In case of compound **6′d** R_2 = 4-isopropylbenzylidene showed potent activity with MIC values 3.12, 3.12, 6.25 and 3.12 μg/mL against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). In case of compounds **6′e**, **6′g** and **6′h** having R_2 = 2-furfuraldehyde, naphthyledene and 4-methoxynaphthyledene, showed nil activity against all the strains of *S. aureus*. Interestingly compound **6′f** having R_2 = 4-fluorobenzyledene, displayed potent MIC values (MIC = 6.25, 25, 3.12 and 6.25 μg/mL) against *S. aureus* (ATCC-25923), M-R *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213), and G-R *S. aureus* (ATCC-33592). Compounds (**6′i**–**p**) were having cyclohexyl substituent at R_1 position showed no potent activity against all the strains of *S. aureus* except compound **6′m**, which showed good activity (MIC = 0.78, 50, 1.56 and 0.78 μg/mL) against (MIC = 6.25, 25, 3.12 and 6.25 μg/mL).

Some compounds of this series showed moderate to good activity against Gram negative bacteria *E. coli* and *K. pneumoniae*. Compounds **6e**, **6j** and **6′m** exhibited potent activity against *E. coli* and compounds **6j**, **6m** and **6′m** showed significant activity against *K. pneumoniae* which are presented in Table 2.

Table 2
Antibacterial activities of Gram negative bacteria *Escherichia coli* and *Klebsiella pneumoniae*.

Entry	*Escherichia coli*			*Klebsiella pneumoniae*		
	MIC	MIC[a]	IC_{50}[a]	MIC	MIC[a]	IC_{50}
6b	25	27.7	20.5	25	26	24
6c	50	46	29	>50	>50	>50
6e	6.25	4.2	2.9	12.5	10.1	5.3
6j	6.25	4.2	2.1	6.25	6.3	3.5
6m	12.5	11.6	4.9	6.25	5.2	2.4
6′m	6.25	4.2	2.9	6.25	3.4	1.7
Cipro[b]	0.012	0.015	0.007	0.012	0.049	0.020
Genta[c]	1.56	2.1	1.7	1.56	2.1	1.7

[a] spectrophotometrically determined.
[b] Ciprofloxacin.
[c] Gentamicin.

5. Cytotoxicity evaluation

The cytotoxicity effect of the selected compounds of 7-piperazinylquinolones–tetrazole derivatives were evaluated against mammalian cells, mouse fibroblast cell line L-929. Compounds **6e** did not exhibit any toxicity up to MIC 0.78 μg/mL also showed in Fig. 2. However, a decline in cell survival of compounds **6e**, **6m** and **6k** was observed at 50 μg/mL, 25 μg/mL and 12.5 μg/mL concentration for the compounds as shown in Fig. 3.

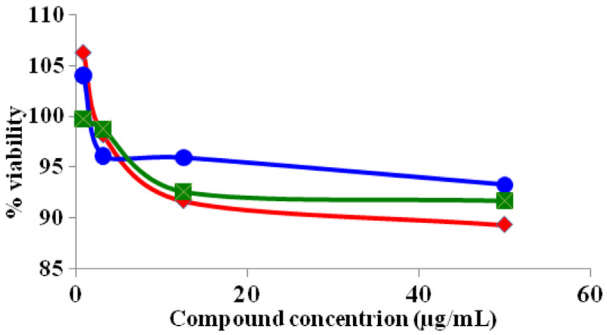


Fig. 3. Cytotoxicity representation of compounds **6e** (Red), **6m** (Blue) and **6k** (Green). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

6. Development of 3D-QSAR models

In order to correlate the biological activities of synthesized compounds with their 3D conformation, we attempted 3D-QSAR study Fig. 4. CoMFA and CoMSIA studies on thirty compounds 1*H*-tetrazol-5-yl-(aryl)methyl piperazinyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid are resulted in acceptable statistical model. Initially total thirty two molecules were selected for 3D-QSAR study, however during model development we found compound **6e** and **6′e** as outliers, and they were not included in final 3D-QSAR models. Partial least square (PLS) analysis in case of CoMFA showed cross validated r^2 (r^2_{cv}) of 0.501 at 3 component. Non cross validated r^2 (r^2_{ncv}) and standard error of estimate (SEE) showed value of 0.831 and 0.315 respectively. Field contribution depicted that steric contribution and electrostatic contribution explains 76.6% and 23.4% of variance in activity. We also obtained predictive r^2 (r^2_{pred}) greater than 0.5, which validates CoMFA model. For predictive r^2, we used 7 compounds belonging to test set (Fig. 5 and Fig. 6). Similarly PLS analysis in case of CoMSIA showed r^2_{cv} of 0.542, r^2_{ncv} of 0.821 and SEE of 0.324 at 3 components. Field contribution details are given in Table 3. Predictive r^2 value (0.710) was better than CoMFA, indicating that the model was very good in predicting activities of test set compounds (Table 4).

Contour maps obtained after 3D-QSAR analysis were mapped on compounds under study. In CoMFA green and yellow (in the web version) contours represents sterically favoured and disfavoured region respectively (Fig. 7). Figs. 8 and 9) Electrostatic favourable regions are represented by red (in the web version) contour and disfavoured region by blue (in the web version) contour. Large green (in the web version) contour near piperazinyl attached carbon showed that bulky group is favourable in compound **6′c** (MIC = 66.37 μM) and **6′g** (MIC = 35.05 μM) when compare to compound **6′a** (MIC = 75.38 μM). Similarly at position-N^1 presence of green contour indicates that bulkier is favourable near this region, that's why **7′d** (MIC = 70.89 μM) is more active than 7d (MIC = 86.91 μM). During analysis of electrostatic contour map we found blue contour near para position of piperazinyl attached benzene ring, which suggests greater activity of **6c** (MIC = 2.50 μM) than **6f** (MIC = 2.83 μM). At N^1-position red contour map display that electronegative group is favourable near this region, which explain why **7′d** (MIC = 70.89 μM) is better active than **7d** (MIC = 86.91 μM). Similarly we also analysed CoMSIA contour maps for steric, electrostatic, hydrophobic, H-bond acceptor and H-bond donor. Steric and electrostatic contour maps were almost similar to CoMFA. Favourable hydrophobic contour maps (magenta) explained that at N^1-position substitution by more hydrophobic group will increase the activity of compounds, for example compound **6′d** (MIC = 70.89 μM) is more active than compound **6d** (MIC = 86.9 μM). In case of H-bond acceptor map, we see a large green contour near para position of piperazinyl attached benzene ring, which indicates that an acceptor group is favourable here and explains the higher activity of compound **6′c** (MIC = 66.37 μM) than compound **6′a** (MIC = 75.38 μM).

7. Conclusion

In conclusion, the concept of developing new effective antibacterials by the introduction of new substituents on quinolone pharmacophore is a successful approach to conquer the problem of resistance. In this observation, we have chosen to explore norfloxacin moiety by incorporated tetrazole scaffold at the N-4 position of the C-7 piperazin-1-yl group of newly developed norfloxacin entity. Among all, compounds (**6a–c**, **6e–g**, **6i–k**, **6m**, **6′f** and **6′m**) were found to be more effective with MIC ranging from (0.78–3.12) μg/mL against *S. aureus* (ATCC-29213) than the control; ciprofloxacin (MIC = 25 μg/mL). Moreover, these analogues displayed no toxicity up to MIC = 0.39 μg/mL against mammalian cell line L-929. Furthermore thirty two compounds were scrutinized by CoMFA and CoMSIA techniques of 3D-QSAR. Good predictive r^2_{pred} values for CoMFA and CoMSIA models reflected the robustness of the predictive ability. Through CoMFA and CoMSIA studies, we were able to explain the effect of 3D conformation on biological activities of compounds. These promising and safe results of 7-piperazinylquinolones–tetrazole derivatives against various strains of *S. aureus* make them possible drug candidate for the treatment of bacterial infection.

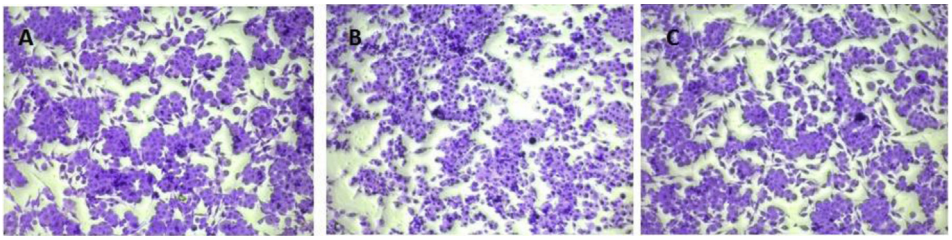


Fig. 2. Morphological changes of L-929 cells exposed to Compound **6e** for 24 h. (A) Control; these fibroblast cells (L-929) are incubated without any compound treatment, (B) compounds were two fold serially diluted (50, 25, and 12.5.) and cells were treated at higher concentrations 50 μg/mL, (C) morphology of fibroblast cells (L-929) up to MIC of compound **6e**. According to the above morphological figure, all the tested compounds are nontoxic.

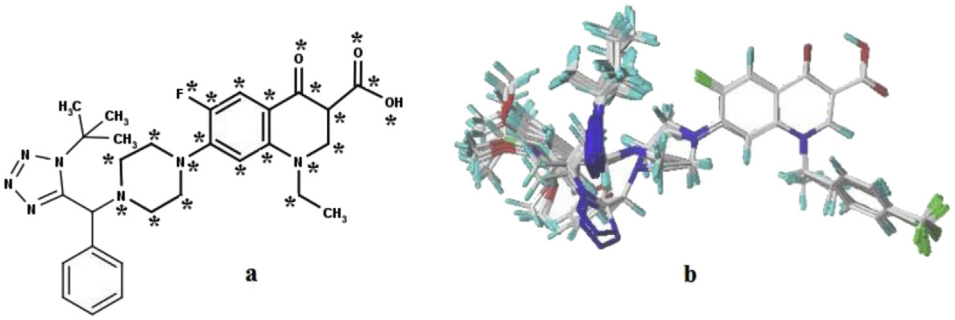


Fig. 4. (a) 2D-sketch of most active compound showing common core region (highlighted with bold bond and aestric) and (b) aligned compounds.

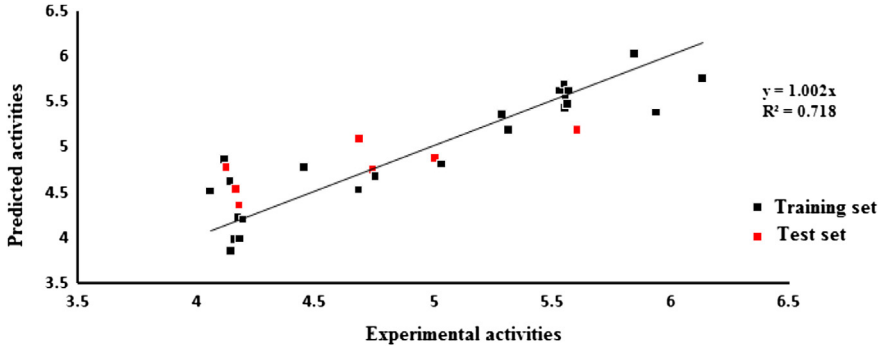


Fig. 5. Graph showing relationship between experimental activities and CoMFA predicted activities.

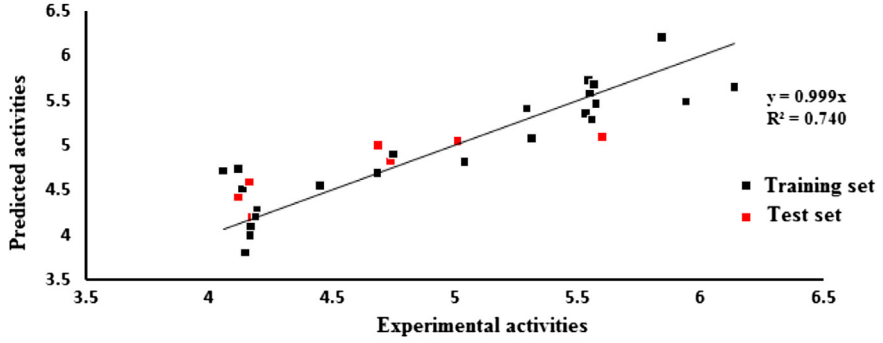


Fig. 6. Graph showing relationship between experimental activities and CoMSIA predicted activities.

Table 3
Statistical parameters of CoMFA and CoMSIA models.

Statistical parameters	CoMFA	CoMSIA
r^2_{cv}(LOO)	0.501	0.542
No of components	3	3
r^2_{ncv}	0.831	0.821
r^2_{pred}	0.578	0.710
SEE	0.315	0.324
F value	31.250	28.999
Field contribution (%)		
S	76.6	21.5
E	23.4	30.4
H	–	42.2
D	–	0.5
A	–	5.4

8. Experimental section

8.1. General information

All reagents and solvents were commercially available and were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance DRX-300 or DPX-200 FT spectrometers using TMS as an internal reference and the samples were dissolved in suitable deuterated solvents (Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hz). IR spectra were recorded on a FTIR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). Electro Spray Ionisation Mass spectra (ESI-MS) were recorded by micromass quattro II instrument. HR-DART MS were recorded on JEOL, JMS T100LC Accu TOF. Column chromatography purifications were performed in flash using 60–120 or 100–200 Mesh silica gel. Thin-layer chromatography (TLC) was carried out with silica gel plates (silica gel 60 F254), that were

Table 4
Compounds considered for 3D-QSAR studies with their experimental MIC, predicted MIC and residual values.

Entry	MIC (μg/mL)	MIC (μM)	PMIC	CoMFA	Residual	CoMSIA	Residual
6a	0.39	0.73	6.136	5.746	0.390	5.629	0.507
6b	1.56	2.92	5.534	5.625	−0.090	5.338	0.196
6c*	1.56	2.5	5.602	5.176	0.426	5.078	0.523
6d	50	86.91	4.06	4.516	−0.455	4.712	−0.650
6f	0.78	2.83	5.548	5.694	−0.145	5.71	−0.162
6g	1.56	2.67	5.573	5.625	−0.051	5.453	0.120
6h*	12.5	20.38	4.69	5.074	−0.383	4.982	−0.291
6i	1.56	2.79	5.554	5.521	0.033	5.581	−0.026
6j	1.56	2.78	5.555	5.420	0.136	5.286	0.270
6k	1.56	4.8	5.318	5.181	0.138	5.059	0.259
6l	12.5	20.79	4.682	4.529	0.152	4.674	0.007
6m	0.78	1.42	5.847	6.012	−0.163	6.195	−0.347
6n	1.56	2.7	5.568	5.465	0.103	5.661	−0.092
6o	3.12	5.12	5.29	5.35	−0.059	5.41	−0.118
6p*	6.25	9.77	5.01	4.861	0.149	5.027	−0.017
6′a	50	75.38	4.122	4.849	−0.726	4.722	−0.599
6′b*	50	75.27	4.123	4.761	−0.637	4.421	−0.297
6′c	50	66.37	4.178	4.359	−0.181	4.183	−0.004
6′d	3.12	70.89	4.149	3.838	0.310	3.803	0.346
6′f	6.25	9.17	5.037	4.815	0.222	4.804	0.233
6′g	50	35.05	4.455	4.761	−0.305	4.538	−0.083
6′h	50	67.27	4.172	4.212	−0.039	4.076	0.096
6′i*	12.5	18.13	4.741	4.736	0.005	4.822	−0.080
6′j	50	72.43	4.14	4.613	−0.472	4.512	−0.372
6′k	50	64.1	4.193	4.205	−0.011	4.257	−0.063
6′l	50	68.37	4.165	3.972	0.193	3.975	0.190
6′m	0.78	1.15	5.939	5.384	0.555	5.487	0.452
6′n	12.5	17.67	4.752	4.663	0.089	4.901	−0.148
6′o*	50	67.63	4.169	4.527	−0.357	4.589	−0.419
6′p	50	64.99	4.187	3.990	0.197	4.204	−0.016

Star (*) labelled compounds belong to test set.

visualized by exposure to ultraviolet light. Purity of all tested compounds was ascertained on the basis of their elemental analysis and was carried out on Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

*8.2. Representative procedure for the synthesis of 7-(4-((1-tert-butyl-1H-tetrazol-5-yl) (phenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6a**)*

A solution of compound **5** (300 mg, 0.864 mmol), benzaldehyde (0.08 mL, 0.864 mmol) and *tert-butyl* isocyanide (0.06 mL, 0.864 mmol) were stirred in anhydrous methanol (5 mL) at room temperature for 10 min. Subsequently, trimethylsilyl azide (0.13 mL, 1.03 mmol) was added and the resulting mixture was further stirred for 8–12 h. On completion of the reaction (checked by TLC analysis), the methanol was removed *in vacuo* and further these reaction mixture was reflux in NaOH solution in 50% methanol/water after completion the reaction the methanol was removed *in vacuo* and the crude product was purified by chromatography on silica gel (eluting with 2% methanol in chloroform) to afford the target product **6a** as White solid, Yield = 82%, mp = 228–230 °C. IR (KBr): 3439, 2989, 1703, 1629, 1218, 1141 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.08 (s, 1H), 8.65 (s, 1H), 8.05 (d, J = 13.0 Hz, 1H), 7.38–7.36 (m, 5H), 6.81 (d, J = 5.7 Hz, 1H), 5.33 (s, 1H), 4.30 (bs, 2H), 3.32 (s, 4H), 3.00–2.96 (m, 2H), 2.69–2.65 (m, 2H), 1.69 (s, 9H), 1.55 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.9, 167.2, 154.0, 147.1, 137.0, 134.7, 129.7, 128.8, 120.6, 112.8, 112.5, 108.3, 103.9, 65.1, 61.5, 50.1, 41.0, 30.3, 14.4. ESI-MS $C_{28}H_{32}FN_7O_3$ (m/z): 534.0 $(M + H)^+$; HRMS: calc.: 534.2623 (MH^+); Found: 534.2653 (MH^+).

The following compounds **6b**–**p** and **6′a**–**p** were prepared using a procedure similar to that described for compound **6a**.

*8.3. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (pyridin-4-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6b**)*

White solid, Yield = 85%, mp = 220–222 °C. IR (KBr): 3426, 2996, 1721, 1630, 1262, 1133 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.02 (s, 1H), 8.66 (d, J = 4.8 Hz, 3H), 8.04 (d, J = 12.9 Hz, 1H), 7.41 (d, J = 5.7 Hz, 2H), 6.80 (d, J = 6.7 Hz, 1H), 5.40 (s, 1H), 4.30 (bs, 2H), 3.31 (s, 4H), 3.03–2.99 (m, 2H), 2.69–2.65 (m, 2H), 1.74 (s, 9H), 1.57 (t, J = 6.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 176.5, 166.6, 159.6, 158.1, 153.8, 151.6, 151.4, 148.6, 145.5, 145.4, 142.5, 137.4,

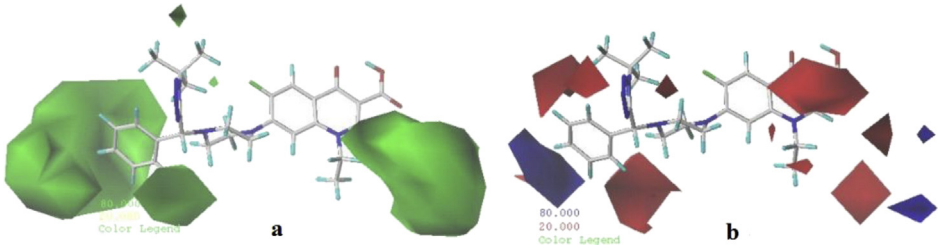


Fig. 7. (a) CoMFA steric contour map and (b) CoMFA electrostatic contour map.

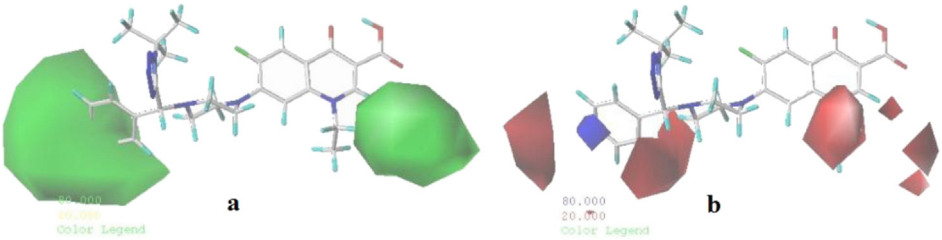


Fig. 8. (a) CoMSIA steric contour map and (b) CoMSIA electrostatic contour map.

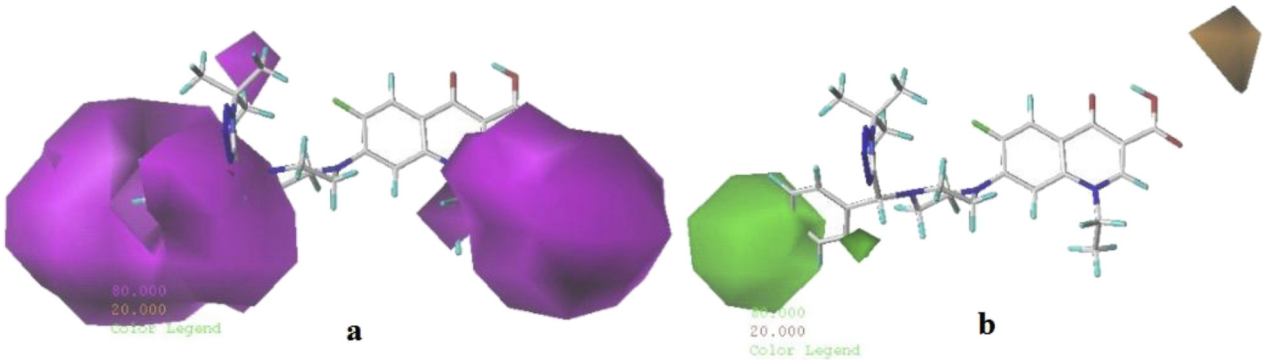


Fig. 9. (a) CoMSIA hydrophobic contour map and (b) CoMSIA H-bond acceptor and donor contour map.

128.3, 121.1, 120.0, 117.3, 113.5, 111.7, 111.3, 109.6, 107.6, 106.2, 62.8, 61.3, 49.9, 49.4, 49.1, 29.7, 14.3. ESI-MS $C_{27}H_{31}FN_8O_3$ (*m/z*): 535.0 $(M + H)^+$; HRMS: calc.: 535.2575 (MH^+); Found: 535.2578 (MH^+).

*8.4. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (3, 4, 5-trimethoxyphenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydro-quinoline-3-carboxylic acid (**6c**)*

White solid, Yield = 88%, mp = 232–234 °C. IR (KBr): 3406, 3015, 1714, 1624, 1218, 1125 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.06 (s, 1H), 8.65 (s, 1H), 8.04 (d, J = 13.0 Hz, 1H), 7.41 (d, J = 5.7 Hz, 2H), 6.81 (d, J = 6.7 Hz, 1H), 5.19 (s, 1H), 4.30 (bs, 2H), 3.85 (s, 9H), 3.33 (s, 2H), 2.94 (s, 2H), 2.94 (s, 2H), 2.67 (s, 2H), 1.74 (s, 9H), 1.58 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.0, 167.2, 154.2, 153.4, 147.1, 138.4, 130.2, 112.9, 107.0, 103.9, 65.5, 61.5, 60.8, 56.3, 50.4, 50.0, 30.3, 14.4. ESI-MS $C_{31}H_{38}FN_7O_6$ (*m/z*): 624.0 $(M + H)^+$; HRMS: calc.: 624.2940 (MH^+); Found: 624.2947 (MH^+).

*8.5. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-isopropylphenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6d**)*

Cream solid, Yield = 91%, mp = 218–220 °C. IR (KBr): 3436, 2962, 1724, 1628, 1218, 1138 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.07 (s, 1H), 8.64 (s, 1H), 8.03 (d, J = 9.7 Hz, 1H), 7.36 (d, J = 5.9 Hz, 2H), 7.22 (d, J = 5.9 Hz, 2H), 6.80 (d, J = 4.9 Hz, 1H), 5.28 (s, 1H), 4.29–4.26 (m, 2H), 4.09 (bs, 1H) 3.32 (s, 4H), 2.98–2.95 (m, 4H), 2.91–2.88 (m, 3H), 1.81 (s, 3H), 1.70 (s, 9H), 1.56 (t, J = 5.1 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.9, 167.2, 154.3, 149.6, 147.1, 146.0, 145.9, 137.0, 131.9, 129.6, 126.8, 120.6, 112.8, 108.3, 103.9, 65.0, 61.5, 50.1, 49.7, 33.7, 30.3, 23.8, 23.8, 14.4. ESI-MS $C_{31}H_{38}FN_7O_3$ (*m/z*): 576.0 $(M + H)^+$; HRMS: calc.: 576.3093 (MH^+); Found: 576.3085 (MH^+).

*8.6. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (furan-2-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6e**)*

Light brown solid, Yield = 89%, mp = 196–198 °C. IR (KBr): 3440, 2987, 1717, 1625, 1220, 1131 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.02 (s, 1H), 8.65 (s, 1H), 8.06 (d, J = 13.0 Hz, 1H), 7.45 (s, 1H), 6.80 (d, J = 6.3 Hz, 1H), 6.50 (s, 2H), 5.53 (s, 1H), 4.30 (bs, 2H), 3.32 (s, 4H), 3.10 (s, 2H), 2.76 (s, 2H), 1.75 (s, 9H), 1.62 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.9, 167.2, 152.0, 148.0, 147.1, 145.9, 143.0, 137.1, 120.6, 112.8, 112.5, 110.8, 108.3, 104.0, 61.8, 58.0, 50.0, 49.8, 30.0, 14.4. ESI-MS $C_{26}H_{30}FN_7O_4$ (*m/z*): 524.0 $(M + H)^+$; HRMS: calc.: 524.2416 (MH^+); Found: 524.2446 (MH^+).

*8.7. 7(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-fluorophenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6f**)*

White solid, Yield = 82%, mp = 217–219 °C. IR (KBr): 3421, 3020, 1722, 1626, 1217, 1137 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.06 (s, 1H), 8.63 (s, 1H), 8.01 (d, J = 12.8 Hz, 1H), 7.45 (s, 2H), 7.09 (t, J = 8.1 Hz, 2H), 6.79 (s, 1H), 5.32 (s, 1H), 4.29 (s, 2H), 3.31 (s, 4H), 2.93 (s, 2H), 2.65 (s, 2H), 1.70 (s, 9H), 1.54 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 176.6, 166.5, 165.4, 159.6, 159.1, 158.6, 158.1, 154.8, 151.5, 150.6, 150.6, 149.0, 144.2, 137.5, 133.4, 125.8, 121.2, 120.5, 117.4, 117.2, 116.9, 113.6, 111.7, 109.7, 107.7, 106.7, 63.3, 63.0, 51.1, 29.5, 14.6. ESI-MS $C_{28}H_{31}F_2N_7O_3$ (*m/z*): 552.0 $(M + H)^+$; HRMS: calc.: 552.2529 (MH^+); Found: 552.2550 (MH^+).

*8.8. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (naphthalen-1-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6g**)*

White solid, Yield = 90%, mp = 231–233 °C. IR (KBr): 3431, 2987, 1709, 1625, 1218, 1133 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.1 (s, 1H), 8.63 (s, 1H), 8.45 (d, J = 8.7 Hz, 1H), 8.03 (d, J = 12.9 Hz, 1H), 7.94–7.84 (m, 3H), 7.38 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 7.1 Hz, 1H), 6.75 (d, J = 6.2 Hz, 1H), 6.39 (s, 2H), 4.25 (bs, 2H), 3.26 (s, 4H), 2.85–2.83 (m, 4H), 1.90 (s, 3H), 1.54 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.2, 168.1, 153.3, 147.6, 145.4, 137.5, 131.9, 128.0, 127.7, 126.6, 125.9, 124.1, 111.9, 113.4, 107.2, 105.0, 102.8, 61.9, 60.2, 51.2, 49.3, 48.1, 31.0, 14.2. ESI-MS $C_{32}H_{34}FN_7O_3$ (*m/z*): 584.0 $(M + H)^+$; HRMS: calc.: 584.2779 (MH^+); Found: 584.2796 (MH^+).

*8.9. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-methoxynaphthalen-1-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6h**)*

Off white solid, Yield = 85%, mp = 198–200 °C IR (KBr): 3428, 2982, 1705, 1634, 1208, 1141 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.09 (s, 1H), 8.62 (s, 1H), 8.38 (t, J = 6.8 Hz, 2H), 8.03 (d, J = 13.1 Hz, 1H), 7.68 (t, J = 6.4 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.74 (d, J = 6.3 Hz, 1H), 6.67 (d, J = 8.2 Hz, 1H), 4.24 (bs, 2H), 3.99 (s, 3H), 3.24 (s, 6H), 2.85 (s, 4H), 2.69–2.65 (m, 2H), 1.55 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.9, 167.3, 156.2, 153.7, 147.0, 146.1, 146.0, 137.1, 132.5, 128.6, 127.4, 126.3, 125.5, 123.3, 123.0, 112.7, 112.4, 108.2, 103.9, 102.6, 61.4, 60.6, 55.6, 50.5, 49.7, 48.6, 30.0, 14.4. ESI-MS $C_{33}H_{36}FN_7O_4$ (*m/z*): 614.0 $(M + H)^+$; HRMS: calc.: 614.2885 (MH^+); Found: 614.2898 (MH^+).

*8.10. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (phenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6i**)*

White solid, Yield = 74%, mp = 233–235 °C. IR (KBr): 3442, 2951, 1716, 1668, 1231, 1142 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.07 (s, 1H), 8.65 (s, 1H), 8.05 (d, J = 12.8 Hz, 1H), 7.41–7.36 (m, 5H), 6.83 (s, 1H), 4.99 (s, 1H), 4.31 (s, 2H), 3.38 (s, 4H), 2.90 (s, 2H), 2.68 (s, 2H), 2.08 (s, 4H), 1.92 (s, 3H), 1.73 (s, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.0, 166.8, 153.6, 153.2, 148.5, 138.4, 137.6, 130.9, 128.8, 126.5, 105.7, 65.3, 60.9, 58.2, 56.3, 50.9, 49.4, 32.8, 30.5, 25.4, 13.7. ESI-MS $C_{30}H_{34}FN_7O_3$ (m/z): 560.0 (M^+ + H); HRMS: calc.: 560.2779 (MH^+); Found: 560.2786 (MH^+).

*8.11. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (pyridin-4-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6j**)*

White solid, Yield = 88%, mp = 213–215 °C. IR (KBr): 3436, 3019, 1716, 1628, 1218, 1136 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ_H 8.69 (d, J = 17.0 Hz, 3H), 8.03 (d, J = 12.2 Hz, 1H), 7.50 (s, 2H), 7.35 (s, 1H), 6.89 (s, 1H), 5.13 (s, 1H), 4.43–4.31 (m, 4H), 3.48–3.38 (m, 10H), 2.83 (s, 2H), 2.67 (s, 2H), 2.00 (s, 5H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 176.5, 166.6, 153.7, 150.8, 148.5, 145.5, 142.4, 137.4, 128.0, 121.1, 117.3, 113.4, 109.6, 107.6, 106.0, 60.1, 57.9, 49.9, 49.4, 49.0, 33.2, 25.0, 24.7, 14.2. ESI-MS $C_{29}H_{33}FN_8O_3$ (m/z): 561.0 (M + H)$^+$; HRMS: calc.: 561.2732 (MH^+); Found: 561.2749 (MH^+).

*8.12. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (3, 4, 5-trimethoxyphenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6k**)*

White solid, Yield = 89%, mp = 186–188 °C. IR (KBr): 3442, 2986, 1709, 1632, 1241, 1123 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.03 (s, 1H), 8.64 (s, 1H), 8.03 (d, J = 12.9 Hz, 1H), 6.82 (s, 1H), 6.66 (s, 2H), 4.85 (s, 1H), 4.36 (s, 2H), 3.84 (s, 9H), 3.39 (s, 4H), 2.84 (s, 2H), 2.69 (s, 2H), 2.03–1.88 (m, 9H), 1.75–1.56 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 160.5, 159.9, 154.3, 140.3, 138.1, 121.5, 116.7, 112.9, 107.6, 105.4, 63.7, 61.0, 59.7, 56.3, 51.9, 50.9, 46.6, 32.9, 31.9, 24.9, 24.6, 24.3, 13.8. ESI-MS $C_{33}H_{40}FN_7O_6$ (m/z): 650.0 (M^+ + H); HRMS: calc.: 650.3096 (MH^+); Found: 650.3119 (MH^+).

*8.13. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-isopropylphenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6l**)*

Light yellow solid, Yield = 93%, mp = 214–216 °C. IR (KBr): 3437, 2939, 1719, 1623, 1218, 1127 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.04 (s, 1H), 8.66 (s, 1H), 8.06 (d, J = 13.0 Hz, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.24 (d, J = 7.8 Hz, 2H), 6.83 (d, J = 6.6 Hz, 1H), 5.28 (s, 1H), 4.95 (s, 2H), 4.38 (s, 2H), 4.29 (s, 1H), 3.37 (s, 4H), 2.91–2.87 (m, 3H), 2.68 (s, 2H), 2.08–2.00 (m, 4H), 1.92 (t, J = 8.7 Hz, 6H), 1.73 (s, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.3, 167.1, 155.4, 153.0, 148.2, 139.8, 131.6, 129.5, 112.9, 108.2, 105.1, 66.0, 61.5, 60.8, 56.8, 51.5, 50.2, 31.8, 23.8, 14.5. ESI-MS $C_{33}H_{40}FN_7O_3$ (m/z): 602.0 (M^+ + H); HRMS: calc.: 602.3249 (MH^+); Found: 602.3274 (MH^+).

*8.14. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (furan-2-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6m**)*

White solid, Yield = 84%, mp = 196–198 °C IR (KBr): 3430, 2967, 1712, 1632, 1241, 1132 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.04 (s, 1H), 8.66 (s, 1H), 8.06 (d, J = 13.0 Hz, 1H), 7.47 (s, 1H), 6.82 (s, 1H), 6.58 (s, 1H), 6.44 (s, 1H), 5.27 (s, 1H), 4.33 (s, 2H), 3.35 (s, 4H), 2.84 (s, 2H), 2.67 (s, 2H), 2.05–1.99 (m, 4H), 1.88–1.81 (m, 3H), 1.58–1.41 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.9, 167.1, 151.3, 147.3, 143.3, 137.0, 120.6, 112.8, 112.5, 111.9, 108.2, 103.9, 58.6, 58.2, 50.3, 49.8, 33.1, 33.0, 25.5, 24.8, 14.4. ESI-MS $C_{28}H_{32}FN_7O_4$ (m/z): 550.0 (M + H)$^+$; HRMS: calc.: 550.2572 (MH^+); Found: 550.2596 (MH^+).

*8.15. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-fluorophenyl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6n**)*

White solid, Yield = 90%, mp = 205–207 °C. IR (KBr): 3430, 2988, 1718, 1646, 1231, 1143 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.02 (s, 1H), 8.06 (d, J = 13.4 Hz, 2H), 7.44 (s, 2H), 7.11 (t, J = 7.8 Hz, 2H), 6.82 (s, 1H), 5.20 (s, 1H), 4.30 (s, 4H), 3.30 (s, 2H), 2.83 (s, 2H), 2.65 (s, 2H), 2.06–1.97 (m, 4H), 1.88–1.79 (m, 3H), 1.61–1.53 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 178.1, 167.5, 160.2, 154.3, 153.4, 147.6, 137.8, 130.1, 113.0, 107.2, 103.8, 66.2, 61.7, 60.2, 56.1, 50.8, 50.2, 31.0, 14.5. ESI-MS $C_{30}H_{33}F_2N_7O_3$ (m/z): 578.0 (M + H)$^+$; HRMS: calc.: 578.2685 (MH^+); Found: 578.2694 (MH^+).

*8.16. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (naphthalen-1-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6o**)*

White solid, Yield = 81%, mp = 208–210 °C. IR (KBr): 3436, 3019, 1720, 1627, 1218, 930 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.8 (s, 1H), 8.54 (s, 1H), 8.42 (d, J = 8.6 Hz, 1H), 8.00 (d, J = 13.0 Hz, 1H), 7.92–7.81 (m, 2H), 7.71–7.62 (m, 2H), 7.41 (t, J = 7.7 Hz, 1H), 7.04 (d, J = 7.3 Hz, 1H), 6.78 (d, J = 6.4 Hz, 1H), 6.42 (s, 1H), 4.26–4.22 (m, 2H), 3.38 (s, 4H), 2.86 (s, 2H), 2.66 (s, 2H), 2.03–1.96 (m, 4H), 1.86–1.78 (m, 3H), 1.60–1.52 (m, 7H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.0, 168.3, 153.2, 147.9, 145.8, 137.9, 131.4, 128.2, 127.8, 126.1, 125.7, 124.5, 111.2, 113.7, 107.0, 105.4, 102.6, 61.8, 61.2, 51.3, 49.6, 48.3, 31.0, 30.6, 25.8, 24.6, 14.3. ESI-MS $C_{34}H_{36}FN_7O_3$ (m/z): 610.0 (M + H)$^+$; HRMS: calc.: 610.2936 (MH^+); Found: 610.2961 (MH^+).

*8.17. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-methoxynaphthalen-1-yl) methyl) piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid (**6p**)*

White solid, Yield = 86%, mp ≥ 250 °C IR (KBr): 3438, 2982, 1713, 1634, 1232, 1131 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.0 (s, 1H), 8.63 (s, 1H), 8.37 (t, J = 6.9 Hz, 2H), 8.11 (d, J = 13.0 Hz, 1H), 7.71 (t, J = 6.5 Hz, 2H), 7.62 (t, J = 7.6 Hz, 1H), 6.96 (d, J = 8.2 Hz, 1H), 6.64 (d, J = 8.2 Hz, 1H), 6.31 (s, 1H), 4.94 (s, 1H), 4.32 (s, 3H), 3.34 (s, 4H), 3.01–2.95 (m, 2H), 2.72 (s, 2H), 2.65–2.61 (m, 2H), 1.83–1.64 (m, 7H), 1.58 (t, J = 6.5 Hz, 3H), 1.28–1.17 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.3, 167.4, 156.6, 152.6, 148.2, 147.0, 137.0, 133.6, 129.0, 127.1, 126.8, 125.3, 121.0, 113.0, 112.6, 108.4, 103.4, 102.8, 61.9, 60.7, 55.9, 51.9, 49.5, 48.8, 32.7, 31.0, 26.9, 25.5, 24.0, 14.6. ESI-MS $C_{35}H_{38}FN_7O_4$ (m/z): 640.0 (M + H)$^+$; HRMS: calc.: 640.3042 (MH^+); Found: 640.3056 (MH^+).

*8.18. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (phenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′a**)*

White solid, Yield = 86%, mp = 198–200 °C. IR (KBr): 3430, 2978, 1709, 1641, 1220, 1131 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.9 (s, 1H), 8.82 (s, 1H), 8.00 (s, 2H), 7.64 (s, 4H), 7.48 (s, 5H), 5.26 (s, 1H), 4.41 (s, 2H), 3.10 (s, 3H), 2.86 (s, 2H), 2.56 (s, 3H), 1.66 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.2, 168.1, 155.1, 154.0, 149.2, 138.8, 131.3, 128.3, 113.2, 108.6, 104.2, 66.1, 62.0, 60.9, 57.2, 51.6, 51.2, 31.0. ESI-MS $C_{34}H_{33}F_4N_7O_3$ (m/z): 664.0 (M^+ + H); HRMS: calc.: 664.2653 (MH^+); Found: 664.2682 (MH^+).

*8.19. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (pyridin-4-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′b**)*

Yellow solid, Yield = 90%, mp = 227–229 °C IR (KBr): 3439, 2989, 1732, 1641, 1209, 1142 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.0 (s, 1H), 8.77 (s, 1H), 8.64 (s, 2H), 8.00 (d, J = 9.6 Hz, 1H), 7.67 (s, 4H), 7.34 (s, 2H), 6.52 (s, 1H), 5.63 (s, 1H), 4.96 (s, 2H), 3.35 (s, 4H), 2.78 (s, 2H), 2.56 (s, 2H), 1.69 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 171.1, 157.9, 154.9, 148.9, 142.6, 132.9, 131.0, 129.8, 67.1, 66.5, 61.1, 56.3, 50.4, 50.0, 34.7. ESI-MS $C_{33}H_{32}F_4N_8O_3$ (m/z): 665.0 (M^+ + H); HRMS: calc.: 665.2606 (MH^+); Found: 665.2637 (MH^+).

*8.20. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (3, 4, 5-trimethoxyphenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′c**)*

Off white solid, Yield = 78%, mp = 225–227 °C. IR (KBr): 3439, 3013, 1719, 1624, 1219, 1126 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.02 (s, 1H), 8.80 (s, 1H), 7.96 (d, J = 12.6 Hz, 2H), 7.63 (s, 2H), 7.26 (s, 2H), 6.64 (s, 2H), 5.53 (s, 2H), 5.12 (s, 1H), 3.81 (s, 9H), 3.11 (s, 4H), 2.81 (s, 2H), 2.55 (s, 2H), 1.69 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.2, 166.8, 154.2, 153.3, 148.4, 138.4, 137.6, 130.1, 126.5, 112.5, 108.5, 107.0, 104.7, 65.5, 61.5, 60.8, 56.3, 50.3, 49.6, 30.3. ESI-MS $C_{37}H_{39}F_4N_7O_6$ (m/z): 754.0 (M^+ + H); HRMS: calc.: 754.2970 (MH^+); Found: 754.2962 (MH^+).

*8.21. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-isopropylphenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′d**)*

White solid, Yield = 93%, mp = 242–244 °C. IR (KBr): 3446, 2988, 1729, 1632, 1221, 1143 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ_H 15.1 (s, 1H), 9.23 (s, 1H), 7.86 (d, J = 13.0 Hz, 1H), 7.73 (s, 2H), 7.52 (s, 2H), 7.44 (d, J = 7.8 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.04 (s, 1H), 5.96 (s, 1H), 5.41 (s, 2H), 4.68 (s, 2H), 3.12 (s, 4H), 2.89 (s, 2H), 2.87–2.82 (m, 1H), 1.87 (m, 6H), 1.72 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 177.0, 166.4, 160.2, 152.8, 148.0, 131.8, 129.8, 128.6, 126.5, 126.0, 64.1, 62.0, 60.6, 57.1, 49.9, 30.3, 24.0. ESI-MS $C_{37}H_{39}F_4N_7O_3$ (m/z): 706.0 (M^+ + H); HRMS: calc.: 706.3123 (MH^+); Found: 706.3146 (MH^+).

*8.22. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (furan-2-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′e**)*

White solid, Yield = 89%, mp = 242–244 °C. IR (KBr): 3436, 3019, 1626, 1218, 1128 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.9 (s, 1H), 8.78 (s, 1H), 8.06 (d, J = 13.0 Hz, 1H), 7.65 (d, J = 7.3 Hz, 2H), 7.42 (s, 1H), 7.28 (s, 3H), 6.59–6.53 (m, 1H), 6.43 (s, 1H), 5.53 (s, 2H), 5.16 (s, 1H), 3.12 (s, 4H), 2.76 (s, 2H), 2.58 (s, 2H), 1.75 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.2, 167.1, 155.2, 154.0, 148.2, 138.4, 130.2, 110.9, 107.0, 103.9, 68.3, 61.5, 60.8, 52.3, 50.4, 50.0, 30.1. ESI-MS $C_{32}H_{31}F_4N_7O_4$ (m/z): 654.0 (M^+ + H); HRMS: calc.: 654.2446 (MH^+); Found: 654.2472 (MH^+).

*8.23. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-fluorophenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′f**)*

White solid, Yield = 86%, mp ≥ 250 °C. IR (KBr): 3451, 2998, 1729, 1685, 1231, 1153 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.0 (s, 1H), 8.74 (s, 1H), 8.04 (d, J = 13.0 Hz, 2H), 7.53 (d, J = 6.7 Hz, 2H), 6.83–6.76 (m, 4H), 6.54 (s, 2H), 5.18 (s, 1H), 4.31 (s, 2H), 3.27 (s, 4H), 2.95 (s, 2H), 2.83 (s, 2H), 1.78 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.4, 167.3, 154.4, 153.6, 147.3, 138.2, 130.1, 113.0, 107.2, 105.2, 65.4, 61.8, 59.3, 56.1, 52.0, 51.3, 30.2. ESI-MS $C_{34}H_{32}F_5N_7O_3$ (m/z): 682.0 (M^+ + H); HRMS: calc.: 682.2559 (MH^+); Found: 682.2566 (MH^+).

*8.24. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (naphthalen-1-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′g**)*

White solid, Yield = 91%, mp = 196–198 °C. IR (KBr): 3423, 2981, 1702, 1626, 1219, 1134 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.2 (s, 1H), 8.56 (s, 3H), 8.43 (d, J = 8.6 Hz, 1H), 8.02 (d, J = 13.0 Hz, 1H), 7.93–7.88 (m, 3H), 7.72–7.63 (m, 3H), 7.42 (t, J = 7.6 Hz, 1H), 7.02 (d, J = 7.2 Hz, 1H), 6.76 (d, J = 6.2 Hz, 1H), 5.19 (s, 1H), 4.30 (s, 2H), 3.35 (s, 4H), 2.96 (s, 2H), 2.66 (s, 2H), 1.78 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.2, 166.3, 155.4, 154.7, 147.4, 138.5, 130.2, 114.0, 108.2, 104.3, 66.0, 62.5, 61.6, 52.5, 51.6, 30.5. ESI-MS $C_{38}H_{35}F_4N_7O_3$ (m/z): 714.0 (M^+ + H); HRMS: calc.: 714.2810 (MH^+); Found: 714.2826 (MH^+).

*8.25. 7-(4-((1-Tert-butyl-1H-tetrazol-5-yl) (4-methoxynaphthalen-1-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′h**)*

White solid, Yield = 79%, mp = 210–212 °C. IR (KBr): 3436, 2898, 1719, 1628, 1218, 1153 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.1 (s, 1H), 8.68 (s, 3H), 8.03 (d, J = 12.8 Hz, 1H), 7.74–7.65 (m, 3H), 7.42 (d, J = 5.8 Hz, 2H), 6.81 (d, J = 6.7 Hz, 2H), 6.69 (s, 2H), 5.19 (s, 1H), 4.36 (s, 2H), 3.83 (s, 3H), 3.31 (s, 2H), 2.98 (s, 2H), 2.93 (s, 2H), 2.68 (s, 2H), 1.73 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.3, 166.8, 155.0, 154.5, 147.3, 138.8, 131.0, 113.2, 107.6, 103.8, 66.4, 62.8, 61.9, 56.2, 52.4, 51.3, 30.3. ESI-MS $C_{39}H_{37}F_4N_7O_4$ (m/z): 744.0 (M^+ + H); HRMS: calc.: 744.2915 (MH^+); Found: 744.2936 (MH^+).

*8.26. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (phenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′i**)*

White solid, Yield = 90%, mp = 186–188 °C. IR (KBr): 3451, 2989, 1703, 1635, 1221, 1142 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.91 (s, 1H), 8.80 (s, 1H), 8.04 (d, J = 12.8 Hz, 1H), 7.66 (d, J = 7.4 Hz, 2H), 7.37 (s, 5H), 7.25 (s, 2H), 6.59 (d, J = 5.3 Hz, 1H), 5.49 (s, 2H), 4.90 (s, 1H), 3.16 (s, 4H), 2.76 (s, 2H), 2.59 (s, 2H), 1.92–1.78 (m, 11H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.1, 166.9, 154.6, 153.1, 152.1, 148.5, 145.6, 145.5, 137.7, 137.3, 129.0, 128.7, 126.5, 112.7, 112.5, 108.4, 104.8, 64.9, 58.1, 57.8, 50.6, 49.4, 32.8, 32.6, 25.3, 24.6. ESI-MS $C_{36}H_{35}F_4N_7O_3$ (m/z): 690.0 (M^+ + H); HRMS: calc.: 690.2810 (MH^+); Found: 690.2842 (MH^+).

*8.27. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (pyridin-4-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′j**)*

White solid, Yield = 85%, mp = 244–246 °C. IR (KBr): 3422, 2992, 1710, 1628, 1219, 1132 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.8 (s, 1H), 8.78 (s, 1H), 8.65 (s, 3H), 8.02 (d, J = 9.6 Hz, 2H), 7.65 (s, 2H), 7.37 (s, 2H), 6.57 (s, 1H), 5.51 (s, 1H), 4.98 (s, 2H), 3.14 (s, 4H), 2.74 (s, 2H), 2.55 (s, 2H), 1.99–1.92 (m, 5H), 1.75–1.68 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 176.9, 166.4, 154.4, 152.4, 151.9, 150.2, 145.4, 143.3, 140.5, 137.8, 128.1, 126.2, 125.8, 124.7, 120.0, 111.8, 107.9, 106.9, 60.8, 57.4, 56.4, 33.1, 25.1. ESI-MS $C_{35}H_{34}F_4N_8O_3$ (m/z): 691.0 (M^+ + H); HRMS: calc.: 691.2762 (MH^+); Found: 691.2789 (MH^+).

*8.28. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (3, 4, 5-trimethoxyphenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′k**)*

White solid, Yield = 79%, mp ≥ 250 °C. IR (KBr): 3440, 2978, 1702, 1622, 1217, 1133 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.92 (s, 1H), 8.82 (s, 1H), 8.07 (d, J = 12.8 Hz, 1H), 7.69 (d, J = 5.8 Hz, 3H), 6.63 (s, 4H), 5.49 (s, 1H), 4.75 (s, 2H), 4.40–4.28 (m, 2H), 3.82 (s, 9H), 3.17 (s, 4H), 2.73 (s, 4H), 1.93–1.73 (m, 5H), 1.70–1.65 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.2, 167.1, 155.8, 152.6, 147.3, 138.2, 131.0, 112.6, 108.0, 103.7, 66.1, 62.0, 61.6, 55.8, 52.6, 51.2, 31.2. ESI-MS $C_{39}H_{41}F_4N_7O_6$ (m/z): 780.0 (M^+ + H); HRMS: calc.: 780.3127 (MH^+); Found: 780.3141 (MH^+).

*8.29. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-isopropylphenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′l**)*

White solid, Yield = 92%, mp = 193–195 °C. IR (KBr): 3440, 2988, 1721, 1639, 1238, 1141 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ_H 15.1 (s, 1H), 9.21 (s, 1H), 7.88 (d, J = 12.7 Hz, 1H), 7.71 (s, 2H), 7.51 (s, 2H), 7.41 (d, J = 7.7 Hz, 2H), 7.28 (d, J = 7.7 Hz, 2H), 7.03 (s, 1H), 5.96 (s, 1H), 5.41 (s, 2H), 4.68 (s, 2H), 4.42–4.29 (m, 2H), 3.12 (s, 4H), 2.89–2.85 (m, 1H), 1.92–1.71 (m, 5H), 1.87–1.66 (m, 6H), 1.21–1.16 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ_C 178.0, 167.1, 153.9, 150.3, 149.0, 132.5, 129.6, 128.1, 126.8, 126.2, 106.2, 103.8, 62.2, 57.2, 49.8, 33.5, 33.0, 30.3, 25.1. ESI-MS $C_{39}H_{41}F_4N_7O_3$ (m/z): 732.0 (M^+ + H); HRMS: calc.: 732.3279 (MH^+); Found: 732.3315 (MH^+).

*8.30. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (furan-2-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′m**)*

White solid, Yield = 87%, mp = 216–218 °C. IR (KBr): 3445, 2937, 1719, 1625, 1219, 1138 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 8.79 (s, 1H), 8.04 (d, J = 12.8 Hz, 1H), 7.65 (d, J = 7.2 Hz, 2H), 7.43 (s, 1H), 7.29 (s, 3H), 6.58–6.52 (m, 2H), 6.40 (s, 1H), 5.50 (s, 2H), 5.18 (s, 1H), 3.11 (s, 5H), 2.74 (s, 2H), 2.55 (s, 2H), 1.98 (s, 6H), 1.76 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 177.1, 166.8, 154.5, 152.0, 151.3, 148.5, 147.2, 145.5, 145.4, 143.3, 143.2, 137.6, 137.3, 126.5, 112.8, 112.6, 111.8, 110.8, 108.5, 104.8, 58.6, 58.1, 57.8, 50.1, 49.5, 33.0, 29.6, 25.4, 25.3, 24.7. ESI-MS $C_{34}H_{33}F_4N_7O_4$ (m/z): 680.0 (M^+ + H); HRMS: calc.: 680.2602 (MH^+); Found: 680.2616 (MH^+).

*8.31. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-fluorophenyl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′n**)*

White solid, Yield = 83%, mp = 228–230 °C. IR (KBr): 3452, 2991, 1718, 1631, 1208, 1152 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.1 (s, 1H), 8.73 (s, 1H), 8.04 (d, J = 12.8 Hz, 2H), 7.52 (d, J = 6.7 Hz, 2H), 6.81–6.73 (m, 4H), 6.52 (s, 2H), 5.17 (s, 1H), 4.32 (s, 2H), 3.24 (s, 4H), 2.95 (s, 2H), 2.82 (s, 2H), 1.93 (s, 6H), 1.76 (s, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.5, 167.2, 154.6, 152.3, 151.0, 148.6, 147.3, 145.6, 143.2, 137.0, 126.7, 112.9, 111.4, 110.1, 108.6, 105.2, 58.7, 58.2, 57.6, 52.0, 50.1, 33.2, 29.8, 25.6. ESI-MS $C_{36}H_{34}F_5N_7O_3$ (m/z): 708.0 (M^+ + H); HRMS: calc.: 708.2716 (MH^+); Found: 708.2751 (MH^+).

*8.32. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (naphthalen-1-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1, 4-dihydroquinoline-3-carboxylic acid (**6′o**)*

White solid, Yield = 78%, mp = 219–221 °C. IR (KBr): 3442, 2988, 1710, 1648, 1228, 1141 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 14.9 (s, 1H), 8.79 (s, 1H), 8.49 (d, J = 7.8 Hz, 2H), 7.97 (d, J = 13.0 Hz, 2H), 7.89 (t, J = 7.5 Hz, 2H), 7.60–7.43 (m, 4H), 6.61 (s, 1H), 5.72 (s, 1H), 5.53 (s, 2H), 3.17 (s, 4H), 2.86 (s, 2H), 2.72 (s, 2H), 1.82–1.62 (m, 7H), 1.24–1.17 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ_C 176.8, 166.9, 152.8, 148.4, 134.1, 131.3, 130.6, 129.3, 129.0, 127.0, 126.5, 124.9, 123.2, 112.9, 112.5, 108.5, 61.4, 58.1, 50.6, 49.7, 32.7, 32.4, 25.1, 24.5. ESI-MS $C_{40}H_{37}F_4N_7O_3$ (m/z): 740.0 (M^+ + H). HRMS: calc.: 740.2966 (MH^+); Found: 740.3002 (MH^+).

*8.33. 7-(4-((1-Cyclohexyl-1H-tetrazol-5-yl) (4-methoxynaphthalen-1-yl) methyl) piperazin-1-yl)-6-fluoro-4-oxo-1-(4-(trifluoromethyl) benzyl)-1,4-dihydroquinoline-3-carboxylicacid (**6′p**)*

White solid, Yield = 89%, mp = 223–225 °C. IR (KBr): 3441, 3011, 1721, 1642, 1209, 1132 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ_H 15.2 (s, 1H), 8.92 (s, 1H), 8.48 (d, J = 7.7 Hz, 2H), 8.01 (d, J = 12.6 Hz, 2H), 7.88–7.83 (m, 2H), 7.62–7.52 (m, 4H), 6.70 (s, 2H), 5.85 (s, 2H), 5.74 (s, 1H), 4.26 (s, 3H), 3.21 (s, 4H), 2.88 (s, 2H), 2.76 (s, 2H), 1.82–1.73 (m, 7H), 1.22–1.15 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ_C 177.0, 166.8, 156.1, 153.2, 148.6, 135.0, 131.6, 130.9, 128.8, 128.2, 127.0, 126.6, 125.0, 124.2, 112.7, 112.3, 109.5, 62.5, 58.0, 56.4, 51.3, 50.1, 32.6, 32.2, 25.0, 24.7. ESI-MS $C_{41}H_{39}F_4N_7O_4$ (m/z): 770.0 (M^+ + H); HRMS: calc.: 770.3072 (MH^+); Found: 770.3087 (MH^+).

8.34. Biological methods

8.34.1. In vitro antibacterial assay

The targeted 7-piperazinyl quinolone–tetrazole derivatives (**6a-p** and **6′a–p**) were screened against Gram-positive bacteria *S. aureus* (ATCC-25923), *S. aureus* (ATCC-700699), *S. aureus* (ATCC-29213) and *S. aureus* (ATCC-33592) and Gram-negative bacteria *E. coli* and *K. pneumoniae* where Ciprofloxacin and Gentamicin were used as a reference drugs. The bacterial strains were grown on nutrient agar at 37 °C. After 24 h of incubation, bacterial cells were suspended in normal saline containing Tween 20 at 0.05% at a concentration of approximately 1.0–2.0 × 10^7 cells/mL by matching with 0.5 McFarland standards. The activity of compounds was determined as per NCCLS protocol using Mueller Hinton broth (Becton Dickinson, USA) in 96-well tissue culture plates. Proper growth control, drug control and the negative control were adjusted onto the plate. Compounds were dissolved in DMSO at a concentration of 1 mg/mL and 20 μL of this was added to each well of 96-well tissue culture plate having 180 μL Mueller Hinton broth. From here the solution was serially diluted resulting in two fold dilution of the test compounds in subsequent wells. 100 μL of McFarland matched bacterial suspension was diluted in 10 mL of media and then 100 μL of it was added in each well and kept for incubation. The maximum concentration of compounds tested was 50 μg/mL. The micro-titer plates were incubated at 35 °C in a moist, dark chamber and MICs were recorded spectrophotometrically after 24 h using SOFT max Pro 4.3 Software (Molecular Devices, Sunnyvale, USA).

8.34.2. In vitro cytotoxicity evaluation assay

The toxicity evaluation of lead compounds **6e**, **6m** and **6k** against murine fibroblast cell line L-929 was used. All test compounds were solubilised in DMSO (1 mg/mL). The cell line L-929 was grown in RPMI 1640 medium supplemented with 10% FBS and 1× antimycotic and antibacterial solution (sigma, USA) at 37 °C in incubator having 5% CO_2. The confluent fibroblast cells are collected from tissue culture flask and maintained 1 × 10^5 cells per millilitre in RPMI serum free medium. Hundred microlitres of cell suspension prepared in RPMI serum free medium was dispensed in 96 well tissue culture plates and incubated for 5 h to adhere the cells properly. In the other replica plate test compounds were diluted in 2 fold manner (25, 12.5,

6.25, 3.12, 1.56, 0.78, 0.39, 0.19, 0.09 μg/mL) were added to the growing cells along with control (with no compounds) and incubated for 24 h in incubator at 37 °C. After incubation wells were washed with PBS and 200 μL of MTT solution (0.5 mg of MTT in RPMI 1640 medium) was added to each well and incubated for 4 h at 37 °C. After incubation 100 μL DMSO was added to each well and incubated for 30 min at room temperature O. D was determined spectrophotometrically at 570 nm and 630 nm. The morphology of the cells was observed using Giemsa stain under Phase contrast microscope. After fixation of the cells in 96 well tissue culture plates, Giemsa stain was added to each well and incubated for 30 min at room temperature. The excess stain was removed by thorough washing with phosphate buffer saline and the culture plates were air dried and observed under a phase contrast microscope [34,35].

8.35. Material and methods of 3D-QSAR analysis

Thirty two molecules belonging 1H-tetrazol-5-yl-(aryl) methyl piperazinyl-6-fluoro-4-oxo-1, 4-dihydroquinoline-3-carboxylic acid were selected for CoMFA [36] and CoMSIA [37] studies. MIC values of reported molecules were converted into respective PIC_{50} (-Log IC_{50}). All molecules were drawn using sketch option in Sybyl 7.1 [38]. These compounds were subjected to energy minimization by Powell gradient method after assigning Gasteiger Huckel charges. Minimization was performed till convergence. Data set was randomly divided into training set of twenty five molecules and test set of seven molecules. Alignment is considered crucial step in developing 3D-QSAR model [25]. Molecules in data set were aligned by automatic database alignment on common substructure.

CoMFA (comparative molecular field alignment) method uses previously aligned molecules to generate steric and electrostatic fields. For this purpose all molecules were placed automatically in defined cubic GRID with grid spacing 2A°. Steric (Lennard-jones 6–12 potential) and electrostatic field (columbic potential) for each molecule were calculated using +1.0 charged SP^3 carbon atom. Minimum Column filtering was set to 2.0 kcal/mol to improve the signal to noise ratio by omitting those lattice points, whose energy variation was below this threshold. Steric and electrostatic fields generated were scaled by the CoMFA-Standard method in SYBYL with default cut-off energy (30 kcal/mol). The calculated descriptors were used to develop CoMFA model. CoMSIA method has advantage over CoMFA in way that it calculates also field created by Hydrogen acceptor, Hydrogen donor and Hydrophobic groups. SP^3 C atom with +1 charge was used to calculate steric and electrostatic field. Hydrogen and hydrophobic fields were calculated using.

Partial least square is a statistical method to calculate linear relationship between a dependent variable and set of predictor variable. CoMFA and CoMSIA models were developed by considering PIC_{50} values as dependent variable and field values as independent variables. Both models were subjected to LOO (Leave one out) cross-validation. After getting optimal no of components, no validation was performed to get r^2_{ncv}. Predictive r^2 was calculated using test set molecules in order to further verify the models.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.069.

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