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Original article

Synthesis, antibacterial evaluation and QSAR studies of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl] quinolone derivatives

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ABSTRACT

A series of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl] quinolones (**I-XXI**) were synthesized using an appropriate synthetic route and characterized by elemental and spectral analysis. The antibacterial activities of all the synthesized compounds were evaluated against identifiable bacterial strains. Compounds **III**, **IV**, **VII**, **VIII**, **IX**, **X**, **XI**, **XV**, & **XVIII** showed better activity than parent compound against all the selected strains. QSAR study on the synthesized molecules tested for their antibacterial activity was performed using multiple linear regression method. Generated models revealed a decrease in HOMO energy as favorable descriptor for determining and predicting the antibacterial activity of the synthesized compounds. Further, the developed models were cross validated using LOO method for their predictive nature.

1. Introduction

Fluoroquinolones are the most widely used antibacterial agents in modern therapy due to their broad spectrum and excellent oral bioavailability [1]. Most of the compounds introduced in last decade possess improved activity against Gram positive pathogens as compared to ciprofloxacin whereas some have potent activity against anaerobes and pathogens that are resistant to many other groups of antimicrobials [2].

The fluoroquinolones targets two related enzymes, DNA gyrase and DNA topoisomerase IV [3]. Gyrase is responsible for introducing negative supercoils in DNA and for relieving torsional stress expected to accumulate ahead of transcription and replication complexes. Topoisomerase IV provides a potent decatenating activity. Both gyrase and topoisomerase IV are essential enzymes, and therefore the agents that inhibit them are expected to block bacterial growth.

The SAR of fluoroquinolones has been subjected to extensive review [4–6]. β-keto carboxylic acid moiety is required for hydrogen bonding interaction with DNA bases in the single stranded region of double helix of DNA created by the action of the enzyme and therefore it is essential. The antibacterial activity is greatly influenced by the steric bulk of N-1 substituents and optimal groups are found to be cyclopropyl, ethyl, fluorophenyl and *t*-butyl, while C-8 substituents should be small in size to show optimum activity [5,7]. Groups at C-5 and C-6 have also been optimized in which an amino and fluoro substituents respectively at C-5 and C-6 appears to be the best. The structure activity relationship analysis of the fluoroquinolone antibiotics shows one of the most significant structural changes for the activity was increasing bulk at C-7 position of the main 6-fluoroquinolone scaffold. The steric bulk at C-7 position leads to reduced side effects, improved potency and *in vivo* efficacy against Gram positive species [8].

A number of QSAR studies have been reported for the fluoroquinolone nucleus. The recent study on three sets of structurally similar fluoroquinolones was performed [8] using comprehensive set of molecular descriptors with the help of Artificial Neural Network (ANN) technique. The report suggested role of topological & electrostatic descriptors to have high coding capabilities for the antibacterial activity of the compounds. Further study of relationship between antibacterial activity of fluoroquinolone derivatives against *Pseudomonas aeruginosa* and descriptors like steric, electronic and hydrophobic using quantum chemical and chemometric methods revealed importance of E_{LUMO} (energy of lowest unoccupied molecular orbital), ΔE_{HL} (energy difference between the highest occupied and lowest unoccupied molecular orbitals), Q_5 (charge at

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R5 position of fluoroquinolone nucleus), Q_6 (charge at R6 position of fluoroquinolone nucleus), MR (molar refractivity) and MP (molecular polarizability [9]. Keeping in view of all these studies with the aim to increase bulk at C-7 position in fluoroquinolone nucleus various derivatives were synthesized having substituted oxadiazole at C-7 position. The synthesized compounds were evaluated for their antibacterial activity. Further the synthesized compounds were subjected to QSAR investigations using MLR technique in order to determine the contribution of molecular descriptors in antibacterial potential using descriptors like (i) topological descriptors like first Zagreb index M1 (ZM1) [10], total structure connectivity index (xt) [11] Wiener index (W) [12], Balban distance connectivity index (J) [13] eccentric connectivity index (CSI) [14] (ii) connectivity indices [15] like connectivity index chi-0 (χ^0), Randic connectivity index chi-1(χ^1), connectivity index chi-2 (χ^2) connectivity index chi-3 (χ^3); (iii) quantum chemical descriptors like HOMO energy [16] LUMO energy [17] and Total energy [18] and (iv) physical properties like molar refractivity (MR) [19] and partition coefficient (clogP) [20] of newly synthesized titled derivatives.

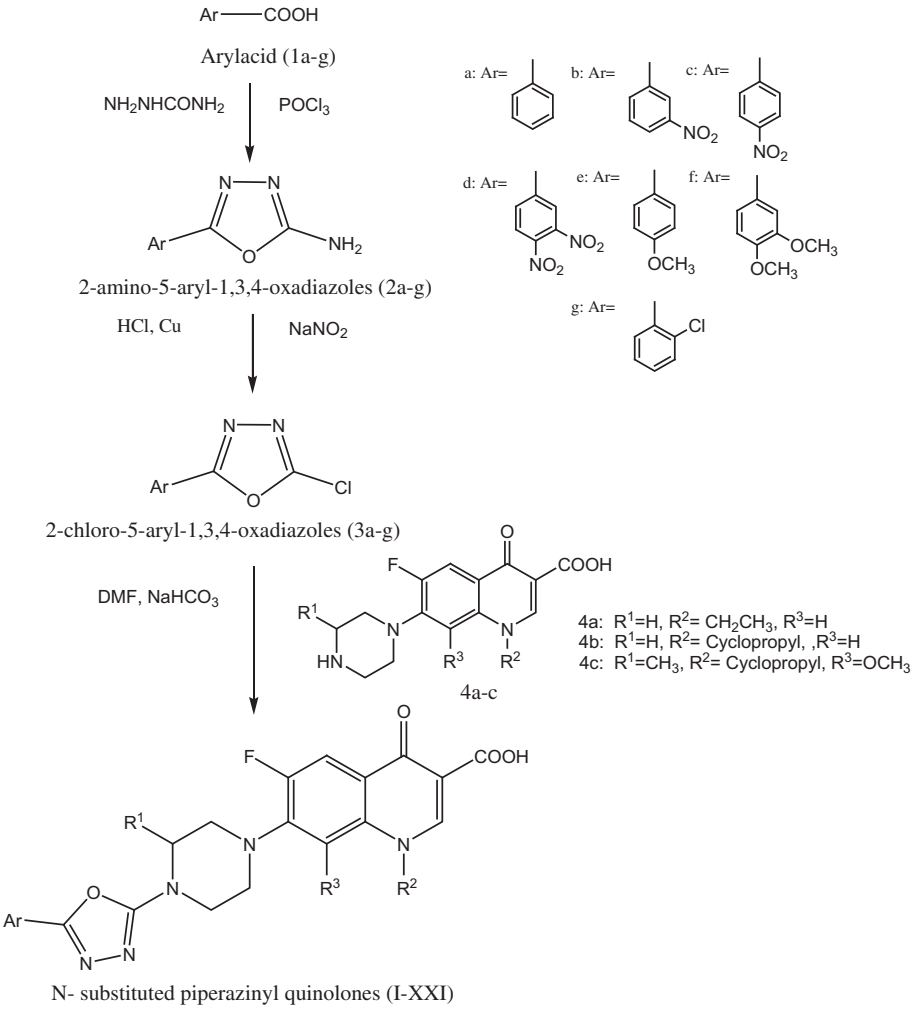
2. Results & discussion

2.1. Chemistry

The synthesis of compounds (**I-XXI**) was achieved with a synthetic route depicted in Scheme 1. Different arylcarboxylic acids (**1a**–**g**) and semicarbazide undergoes cyclodehydration in the presence of phosphorous oxychloride, which acts as a dehydrating agent to form 2-amino-5-aryl-1,3,4-oxadiazoles [21]. Diazotization of 2-amino-5-aryl-1,3,4-oxadiazoles in presence of nitrous acid, leads to formation of diazonium chloride salt, to which, further addition of finely divided copper powder having catalytic action at room temperature, leads to formation of 2-chloro-5-aryl-1,3,4-oxadiazoles along with evolution of nitrogen gas. Reaction of the latter with piperazinyl group of quinolones in DMF in the presence of a base (sodium bicarbonate) resulted in the synthesis of the final compounds (Scheme 1). The physicochemical characteristics of the synthesized compounds are presented in Table 1.

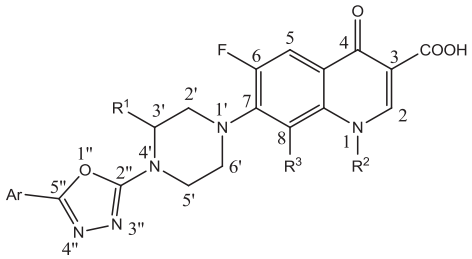
2.2. Antibacterial evaluation

All the final synthesized compounds were evaluated for their *in vitro* antibacterial activities against human pathogens. The *in vitro* antibacterial activity were performed against aerobic Gram positive bacterial strains including *Staphylococcus aureus* subsp. *aureus* (***MTCC 1430*** equivalent to ***ATCC 12600***), *Bacillus subtilis* (***MTCC 2423*** equivalent to ***ATCC 21332***) and Gram negative bacterium *Escherichia coli* (***MTCC 739*** equivalent to ***ATCC 10536***) using serial dilution method [22,23]. As all are derivatives of marketed drugs, that is why initial preliminary screening were not performed and directly, the synthesized compounds were evaluated for the MIC (minimun inhibitory concentration), where DMSO was used as



Scheme 1. Scheme for the syntheses 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl] quinolone derivatives.

Table 1
Physicochemical characteristics of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl) piperazinyl] quinolone derivatives.



Compound	Ar	R_1	R_2	R_3	Mol. formula (MW)	Mp[a] (°C)	Yield (%)	Rf[b]
I	Phenyl	H	Ethyl	H	$C_{24}H_{22}FN_5O_4$ (463.47)	258–260	61	0.45
II	3-nitrophenyl	H	Cyclopropyl	H	$C_{25}H_{21}FN_6O_6$ (520.48)	279–281	68	0.34
III	4-nitrophenyl	CH_3	Cyclopropyl	OCH_3	$C_{27}H_{25}FN_6O_7$ (564.53)	175–179	56	0.46
IV	3,4-dinitrophenyl	H	Ethyl	H	$C_{24}H_{20}FN_7O_8$ (553.47)	263–265	65	0.48
V	4-methoxyphenyl	H	Cyclopropyl	H	$C_{26}H_{24}FN_5O_5$ (505.51)	289–291	43	0.59
VI	3,4-dimethoxyphenyl	CH_3	Cyclopropyl	OCH_3	$C_{29}H_{30}FN_5O_7$ (579.59)	171–173	48	0.28
VII	2-chlorophenyl	H	Ethyl	H	$C_{24}H_{21}ClFN_5O_4$ (497.92)	269–272	50	0.46
VIII	Phenyl	H	Cyclopropyl	H	$C_{25}H_{22}FN_5O_4$ (475.48)	271–274	59	0.52
IX	3-nitrophenyl	CH_3	Cyclopropyl	OCH_3	$C_{27}H_{25}FN_6O_7$ (564.53)	161–163	57	0.45
X	4-nitrophenyl	H	Ethyl	H	$C_{24}H_{21}FN_6O_6$ (508.47)	256–258	71	0.68
XI	3,4-dinitrophenyl	H	Cyclopropyl	H	$C_{25}H_{20}FN_7O_8$ (565.48)	283–285	58	0.57
XII	4-methoxyphenyl	CH_3	Cyclopropyl	OCH_3	$C_{28}H_{28}FN_5O_6$ (549.56)	187–189	69	0.64
XIII	3,4-dimethoxyphenyl	H	Ethyl	H	$C_{26}H_{26}FN_5O_6$ (523.53)	263–264	52	0.39
XIV	2-chlorophenyl	H	Cyclopropyl	H	$C_{25}H_{21}ClFN_5O_4$ (509.93)	298–301	63	0.31
XV	Phenyl	CH_3	Cyclopropyl	OCH_3	$C_{27}H_{26}FN_5O_5$ (519.54)	168–171	67	0.63
XVI	3-nitrophenyl	H	Ethyl	H	$C_{24}H_{21}FN_6O_6$ (508.47)	245–246	48	0.50
XVII	4-nitrophenyl	H	Cyclopropyl	H	$C_{25}H_{21}FN_6O_6$ (520.48)	286–287	52	0.35
XVIII	3,4-dinitrophenyl	CH_3	Cyclopropyl	OCH_3	$C_{27}H_{24}FN_7O_9$ (609.53)	191–195	53	0.36
XIX	4-methoxyphenyl	H	Ethyl	H	$C_{25}H_{24}FN_5O_5$ (493.50)	253–256	62	0.31
XX	3,4-dimethoxyphenyl	H	Cyclopropyl	H	$C_{27}H_{26}FN_5O_6$ (535.54)	287–288	57	0.37
XXI	2-chlorophenyl	CH_3	Cyclopropyl	OCH_3	$C_{27}H_{25}ClFN_5O_5$ (553.98)	164–167	39	0.36

[a] Melting point of compounds at their decomposition.
[b] TLC mobile phase – Methanol:25% aqueous ammonia:ethyl acetate:acetonitrile:: 1:1:2:1.

negative control while ciprofloxacin was used as a positive control showing inhibition of growth of microbes. According to the values of control, the results were evaluated. The results of antibacterial activity are presented in Table 2.

The compounds **III**, **X** and **XV** were found to be the most effective compounds against *S. aureus* with pMIC value of 3.61, 3.63, and 3.62 respectively. For activity against *B. subtilis* the compounds **IV**, **VIII**, **IX**, **X** and **XV** yielded better activity in comparison to other

Table 2
Observed and calculated antimicrobial activity of 7-[4-(5-aryl-1,3,4-oxadiazole-2-yl)piperazinyl] quinolone derivatives using the best QSAR models.

Compounds	pMIC sa (eq. (1))			pMIC bs (eq. (2))			pMIC bs (eq. (3))			pMIC ec (eq. (4))			pMIC ec (eq. (5))		
	Obs.	Calc.	Res.	Obs.	Calc.	Res.	Obs.	Calc.	Res.	Obs.	Calc.	Res.	Obs.	Calc.	Res.
I	2.58	2.818	−0.238	2.58	2.784	−0.204	2.58	2.742	−0.162	2.65	2.821	−0.171	2.65	2.758	−0.108
II	2.24	2.664	−0.424	2.28	2.592	−0.312	2.28	2.537	−0.257	2.17	2.642	−0.472	2.17	2.559	−0.389
III	3.61	3.466	0.144	3.11	2.967	0.143	3.11	2.938	0.172	3.24	2.993	0.247	3.24	2.948	0.292
IV	3.24	3.179	0.061	3.87	3.733	0.137	3.87	3.722	0.148	3.63	3.241	0.389	3.63	3.224	0.406
V	3.12	2.944	0.176	3.09	2.941	0.149	3.09	2.909	0.181	3.20	2.968	0.232	3.20	2.921	0.279
VI	3.36	3.204	0.156	3.27	3.263	0.007	3.27	3.254	0.016	3.27	3.269	0.001	3.27	3.256	0.014
VII	3.21	3.344	−0.134	3.21	3.437	−0.227	3.21	3.440	−0.230	3.60	3.432	0.168	3.60	3.436	0.164
VIII	3.26	3.142	0.118	3.87	3.687	0.183	3.87	3.673	0.197	3.22	3.198	0.022	3.22	3.176	0.044
IX	3.25	3.369	−0.119	3.55	3.469	0.081	3.55	3.474	0.076	3.55	3.462	0.088	3.55	3.469	0.081
X	3.63	3.526	0.104	3.63	3.664	−0.034	3.63	3.683	−0.053	3.24	3.644	−0.404	3.24	3.671	−0.431
XI	2.95	3.229	−0.279	3.25	3.295	−0.045	3.25	3.288	−0.038	3.65	3.999	−0.349	3.65	3.988	−0.338
XII	3.17	2.975	0.195	3.09	3.101	−0.011	3.09	3.115	−0.025	3.07	2.985	0.085	3.07	3.006	0.064
XIII	2.43	2.862	−0.432	2.43	2.838	−0.408	2.43	2.800	−0.370	2.68	2.872	−0.192	2.68	2.814	−0.134
XIV	2.43	2.709	−0.279	2.38	2.648	−0.268	2.38	2.597	−0.217	2.43	2.695	−0.265	2.43	2.617	−0.187
XV	3.62	3.509	0.111	3.54	3.620	−0.08	3.54	3.595	−0.055	2.94	3.042	−0.102	2.94	3.003	−0.063
XVI	2.84	2.691	0.149	2.84	2.625	0.215	2.84	2.572	0.268	2.69	2.673	0.017	2.69	2.593	0.097
XVII	2.63	2.628	0.002	2.32	2.547	−0.227	2.32	2.489	−0.169	2.32	2.600	−0.280	2.32	2.513	−0.193
XVIII	2.64	3.073	−0.433	2.96	3.101	−0.141	2.96	3.081	−0.121	3.66	3.818	−0.158	3.66	3.787	−0.127
XIX	3.20	2.748	0.452	3.20	2.697	0.503	–	–	–	3.50	2.740	0.760	–	–	–
XX	2.91	2.580	0.330	2.67	2.487	0.183	2.67	2.425	0.245	2.67	2.544	0.126	2.67	2.450	0.220
XXI	3.24	2.898	0.342	3.24	2.882	0.358	3.24	2.847	0.393	3.17	2.913	0.257	3.17	2.860	0.310
Ciprofloxacin[a]	3.16	–	–	3.16	–	–	3.16	–	–	3.52	–	–	3.52	–	–

[a] Not included in dataset for QSAR analysis.

Table 3
Values of selected molecular descriptors used in the regression analysis.

Compound	HOMO energy	LUMO Energy	Total Energy	MR	ClogP	CSI	W	J	X0	X1	X2	X3	Xt	ZM1
I	−8.6262	−0.8885	128.22	12.538	2.91225	1100	4060	1.048	24.104	16.991	15.850	13.628	0.192	198
II	−8.5843	−0.8626	183.15	12.667	3.61625	1037	3799	1.149	23.819	16.457	15.017	13.345	0.199	186
III	−8.6662	−0.8811	2958.48	13.614	3.37516	1173	4750	1.131	26.551	18.367	17.053	14.696	0.187	214
IV	−8.7241	−1.5047	118.78	13.141	2.68331	1257	5202	1.027	26.551	18.296	17.383	14.676	0.187	214
V	−8.6604	−1.5474	173.79	13.278	3.38731	1190	4891	1.131	26.267	17.761	16.575	14.348	0.193	202
VI	−8.7308	−1.5021	2949.12	14.222	3.14267	1334	6006	1.105	28.999	19.672	18.586	15.745	0.182	230
VII	−8.7687	−1.6811	118.77	13.141	2.68331	1311	5289	1.012	26.551	18.296	17.371	14.738	0.187	214
VIII	−8.7141	−1.7212	173.78	13.278	3.38731	1240	4975	1.115	26.267	17.761	16.538	14.409	0.193	202
IX	−8.7757	−1.6771	2949.06	14.222	3.14266	1392	6102	1.097	28.999	19.672	18.574	15.806	0.182	230
X	−8.8182	−2.4895	160.93	13.753	2.43179	1366	6392	1.036	28.999	19.655	18.928	15.684	0.182	230
XI	−8.7377	−2.5277	222.57	13.879	3.13579	1299	6031	1.141	28.714	19.066	18.095	15.392	0.188	218
XII	−8.8044	−2.4834	2991.21	14.833	2.89114	1443	7310	1.103	31.747	20.976	20.133	16.749	0.178	246
XIII	−8.6382	−0.8986	141.19	13.146	2.97425	1272	4866	1.022	25.681	17.923	16.641	14.747	0.188	208
XIV	−8.5966	−0.8740	196.13	13.284	3.67825	1201	4570	1.124	25.397	17.389	15.808	14.119	0.194	196
XV	−8.6778	−0.8917	2971.42	14.227	3.43355	1353	5637	1.101	28.129	19.299	17.843	15.515	0.183	224
XVI	−8.5916	−0.8829	3062.78	13.763	2.69393	1344	5630	1.022	27.258	18.872	17.362	15.207	0.185	218
XVII	−8.5746	−0.8537	3118.90	13.901	3.39793	1273	5301	1.126	26.974	18.337	16.529	14.879	0.199	206
XVIII	−8.6954	−8.9584	2988.51	14.844	3.50328	1425	6476	1.096	29.706	20.248	18.565	16.272	0.186	234
XIX	−8.6072	−0.8773	130.92	13.021	3.38835	1133	4387	1.047	24.974	17.402	16.378	14.095	0.198	204
XX	−8.5615	−0.8419	185.72	13.158	4.09235	1070	4111	1.156	24.649	16.867	15.545	13.767	0.197	192
XXI	−8.6477	−0.8647	2960.49	14.102	3.84775	1206	5110	1.129	27.422	18.778	17.581	15.163	0.185	220

synthesized compounds. The antimicrobial spectrum of quinolones derivatives against *E. coli* demonstrated that compounds **IV**, **VII**, **IX**, **XI** & **XVIII** were the most active ones.

2.3. QSAR analysis

An attempt was made to establish the relationship between structure and antibacterial activity. The MIC values obtained from the experiment were expressed in terms of μg/ml concentration. For the purpose of correlation, the MIC values were converted to their molar units and subsequently to free energy related negative logarithmic state, which is log (1/MIC) and further used as dependent variable in developing the QSAR models. The structures of the molecules were drawn using HYPERCHEM 8.0.5 [24] and later subjected to minimization of energy using the MM+ method. The energy minimized molecules were subjected to molecular descriptor calculation. Topological descriptors and connectivity indices were calculated using Dragon 3.0 web version [25], quantum chemical descriptors and physical properties were calculated with the help of CS Chemoffice [26]. The values of the selected descriptors used in the regression analysis are presented in Table 3. The models were built using the multiple linear regression (MLR) method as employed in the BuildQsar software [27]. The developed models (eq. (1)–(5)) were cross validated using Leave one out (LOO) method.

Preliminary analysis was carried out in terms of a correlation analysis between pMIC and various molecular descriptors and the results are presented in Table 4. This was done to remove the chances of intercorrelation among the molecular descriptors that may lead to false predicting models.

The data depicted in Table 4, indicated that antibacterial activity against all three microbes were showing good correlation with HOMO energy ($r = 0.734$ for pMIC sa, 0.882 for pMIC bs, 0.840 for pMIC ec). The correlation with HOMO energy as an independent variable with pMIC sa, pMIC bs and pMIC ec were significant as shown in the equations below.

2.3.1. QSAR model for antibacterial activity against S. aureus

$$\text{pMIC sa} = -3.6847(\pm 1.6354)\ \text{HOMO energy} - 28.9669(\pm 14.1897) \quad (1)$$

($n = 21$; $r = 0.734$; $R^2 = 0.538$; $s = 0.272$; $F = 22.238$; $p < 0.0002$; $Q2 = 0.444$; SPress = 0.299; SDEP = 0.291).

Table 4
Correlation matrix for pMIC with molecular descriptors.

	pMIC sa	pMIC bs	pMIC ec	HOMO energy	LUMO energy	Total energy	MR	ClogP	CSI	W	J	X0	X1	X2	X3	Xt	ZM1
pMIC sa	1.000																
pMIC bs	0.926	1.000															
pMIC ec	0.859	0.907	1.000														
HOMO energy	0.734	0.839	0.750	1.000													
LUMO energy	0.007	0.215	0.145	0.341	1.000												
Total energy	0.198	0.148	0.089	0.085	0.205	1.000											
MR	0.418	0.440	0.302	0.462	0.540	0.794	1.000										
ClogP	0.355	0.408	0.404	0.595	0.032	0.092	0.010	1.000									
CSI	0.458	0.525	0.436	0.694	0.504	0.533	0.815	0.501	1.000								
W	0.575	0.631	0.484	0.753	0.526	0.530	0.871	0.448	0.946	1.000							
J	0.155	0.188	0.326	0.261	0.000	0.193	0.192	0.791	0.298	0.136	1.000						
X0	0.599	0.644	0.494	0.729	0.511	0.611	0.925	0.322	0.904	0.984	0.000	1.000					
X1	0.575	0.625	0.503	0.710	0.521	0.663	0.924	0.372	0.927	0.974	0.109	0.985	1.000				
X2	0.694	0.747	0.629	0.818	0.455	0.531	0.837	0.471	0.891	0.964	0.186	0.968	0.975	1.000			
X3	0.544	0.594	0.475	0.683	0.524	0.657	0.932	0.345	0.938	0.973	0.106	0.982	0.995	0.963	1.000		
Xt	0.579	0.624	0.571	0.683	0.447	0.648	0.823	0.474	0.888	0.888	0.334	0.889	0.950	0.942	0.941	1.000	
ZM1	0.635	0.684	0.592	0.745	0.477	0.631	0.870	0.432	0.897	0.943	0.211	0.954	0.985	0.985	0.974	0.981	1.000

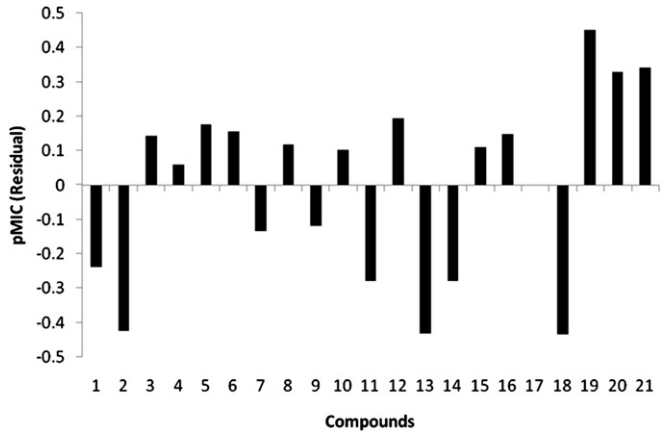


Fig. 1. Histogram of residual pMIC sa value of the compounds calculated using eq. (1).

2.3.2. QSAR model for antibacterial activity against B. subtilis

$$\text{pMIC bs} = -4.5854(\pm 1.4281)\ \text{HOMO energy} - 36.7704(\pm 12.3913) \quad (2)$$

($n = 21$; $r = 0.839$; $R^2 = 0.703$; $s = 0.238$; $F = 45.159$; $p < 0.0001$; $Q2 = 0.645$; SPress = 0.260; SDEP = 0.254).

$$\text{pMIC bs} = -4.8993(\pm 1.2952)\ \text{HOMO energy} - 39.5206(\pm 11.2421) \quad (3)$$

($n = 20$; $r = 0.882$; $R^2 = 0.777$; $s = 0.210$; $F = 63.165$; $p < 0.0001$; $Q2 = 0.730$; SPress = 0.232; SDEP = 0.226) [Compound **XIX** outlier].

2.3.3. QSAR model for antibacterial activity against E. coli

$$\text{pMIC ec} = -4.2824(\pm 1.8127)\ \text{HOMO energy} - 34.1193(\pm 15.7283) \quad (4)$$

($n = 21$; $r = 0.750$; $R^2 = 0.562$; $s = 0.302$; $F = 24.448$; $p < 0.0001$; $Q2 = 0.457$; SPress = 0.336; SDEP = 0.328).

$$\text{pMIC ec} = -4.7565(\pm 1.5202)\ \text{HOMO energy} - 38.2724(\pm 13.1950) \quad (5)$$

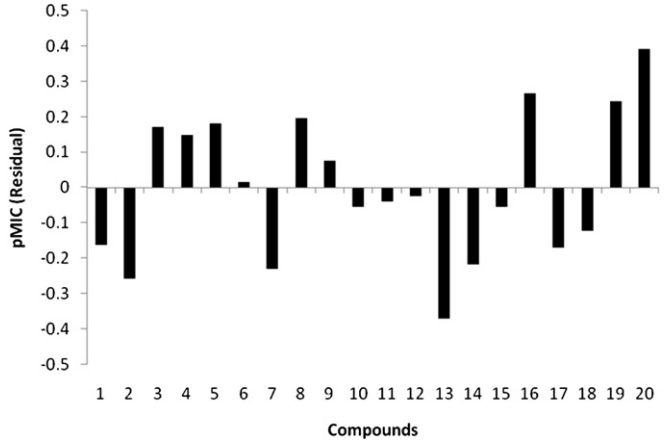


Fig. 2. Histogram of residual pMIC bs value of the compounds calculated using eq. (3).

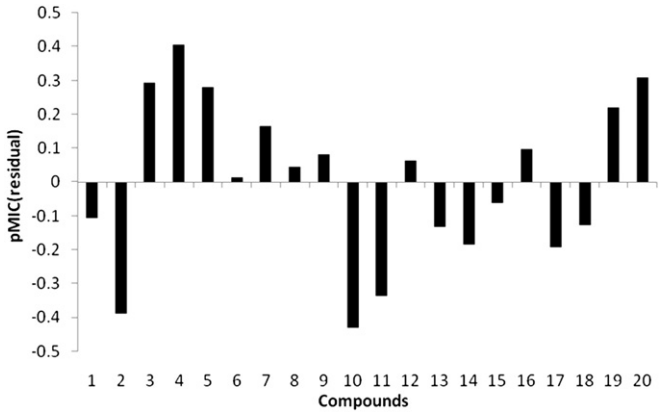


Fig. 3. Histogram of residual pMIC ec value of the compounds calculated using eq. (5).

($n = 20$; $r = 0.840$; $R^2 = 0.705$; $s = 0.247$; $F = 43.217$; $p < 0.0001$; $Q2 = 0.624$; SPress = 0.279; SDEP = 0.272) [Compound **XIX** outlier].

The quality of the model is indicated by following parameters. r, correlation coefficient; R^2, squared multiple correlation coefficient; F, Fischer's significance statistics; s, standard error of estimate; $Q2$, cross validated r^2; SPress, predicted residual sum of square; SDEP, standard deviation of prediction of errors; n, number of data points.

The treatment of data for developing QSAR models in the case of pMIC bs (eq. (2)) and pMIC ec (eq. (4)) has shown compound XIX as an outlier because of higher leverage value of 0.503 & 0.760 respectively as shown in Table 2. When outliers are present, regression analysis may produce results that strongly reflect a small number of atypical cases rather than the general relationship observed in the rest of data. The outliers often have dramatic effect on the fitted least square regression function, therefore it is important to study the outlying case carefully and decide whether they should be retained or eliminated [28]. Therefore, the analysis was done using method of deleted residuals [29]. The compound XIX was deleted from the dataset and the regression equation was calculated using other n − 1 = 20 cases. The results of the analysis are presented in eq. (3) (for pMIC bs) & in eq. (5) (for pMIC ec). Using these new regression equations with compound **XIX** deleted from the dataset, we calculated the predicted value for compound **XIX**. Further, the deleted residual was calculated using the difference between the actual observed value and the estimated expected value from the developed model. The value of deleted residual for the compound **XIX** for eq. (3) was found to be 0.56 and for eq. (5) was found to be 0.84. The greater magnitude of the deleted residual than of the ordinary residual further put forward the compound XIX as an

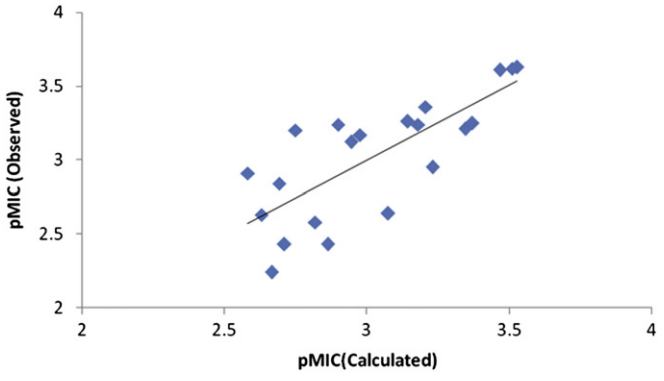


Fig. 4. Plot of calculated pMIC sa activity values against the observed pMIC sa values for the linear regression developed model by eq. (1).

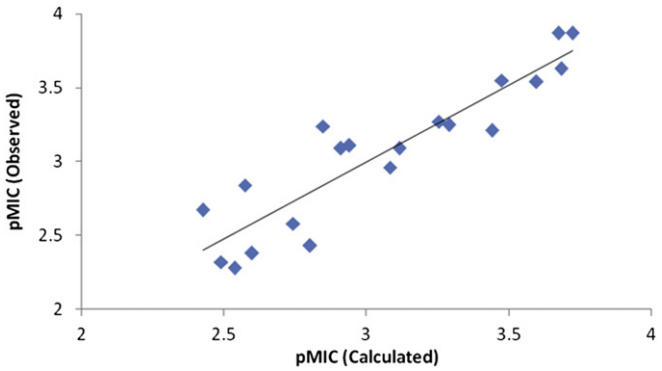


Fig. 5. Plot of calculated pMIC bs activity values against the observed pMIC bs values for the linear regression developed model by eq. (3).

outlier. Therefore, an improvement in statistical parameters was carried out by treating it as an outlier from the dataset.

The QSAR equations derived showed a very good *r* (correlation coefficient) value of 0.734, 0.882 and 0.840 for three different models respectively. All three are associated with low value of standard error of estimate and found to be highly statistically significant with high *F* test value of 22.238, 63.165 and 43.217 respectively. All three models also showed high confidence level of more than 99.98%. All the developed models were cross validated with LOO method for their predicting power every time an equation is developed by eliminating one compound from the series and the activity of the eliminated compound is determined using the developed equation. The histogram plot of residual values of QSAR model of equations (1), (3) and (5) are depicted in Figs. 1–3 are showing distribution toward both positive and negative axis indicating good predictive power of the developed models. The observed antibacterial activity is plotted against predicted activity using the developed models. The fit line is shown in the Figs. 4–6. Perusal of above equation indicated that HOMO energy is having detrimental effect on the development of molecules, so during pharmacophore development, we have to avoid the nucleophilic group substitutions on phenyl ring of the oxadiazole.

HOMO is the outer most orbital containing the electrons. The generated QSAR models indicated the higher value of HOMO energy contributing negatively to the antibacterial activity. An electron donating substituent such as hydroxyl or methyl or methoxy group on the ring increases the HOMO energy, similarly electron withdrawing groups such as halogens, lowers the HOMO energy. Thus, the design of molecules with electron withdrawing substituents would have been suggested to have better antibacterial activity.

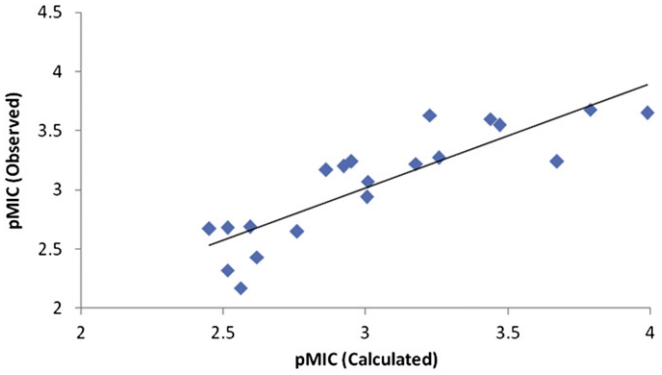


Fig. 6. Plot of calculated pMIC ec activity values against the observed pMIC ec values for the linear regression developed model by eq. (5).

3. Conclusion

Summarizingly, a series of substituted fluoroquinolone derivatives have been synthesized successfully in appreciable yields and screened for their *in vitro* antibacterial activity against aerobic bacterial strains of *E. coli, S. aureus,* and *B. subtilis*. QSAR analysis carried out to investigate the role of molecular descriptors in attributing the antibacterial activity of the synthesized derivatives indicated the importance of the HOMO energy in predicting the antibacterial activity. Further, the QSAR models are statistically, chemically and significantly sound and explain more than 99% of confidence level; finally it can be concluded from the developed models, that this work shows substantial promise in the prediction of the antibacterial activity of other novel fluoroquinolone derivatives prior to their synthesis.

4. Experimental

Melting points were determined in an open end capillary tube on Elico melting point apparatus and are uncorrected. Reaction progress was monitored by ascending thin layer chromatography on silica gel sheets (Merck silica gel-G), visualized by iodine vapors and the purity of the compounds was ascertained by single spot on TLC plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in Bruker Avance II 400 NMR spectrometer using deuterated DMSO and are reported in parts per million (δ) relative to tetramethylsilane (TMS) as an internal standard. Infrared (IR) spectra were recorded on Thermo FTIR spectrometer (KBr disks). The MS-ESI spectra were recorded on Waters Micromass Q-TOF micro. Elemental analysis (CHN) was performed on Vario ELIII CHNS analyzer using sulphanilic acid as a standard and all the values were within ±0.4% of the theoretical composition.

*4.1. General procedure for the synthesis of 2-amino-5-aryl-1,3,4-oxadiazoles (**2a**–**g**)*

A mixture of substituted aromatic acid (**1a**–**g**) (0.1 mmol) with semicarbazide (0.01 mmol) in phosphorous oxychloride (15 ml) was refluxed over a steam bath for 5–6 h. The progress of reaction was monitored by TLC using ethyl acetate: acetone (9:1) as eluent. The reaction mixture was cooled and poured on to crushed ice (200 g) with continuous stirring. The solid mass separated was neutralized with sodium bicarbonate solution (10% W/V). The resulted solid thus obtained was collected by filtration, washed well with cold water, dried in vacuum and recrystallized from ethanol. The compounds are already reported previously [30,31].

*4.2. General procedure for the synthesis of 2-chloro-5-aryl-1,3,4-oxadiazoles (**3a**–**g**)*

Compound **2a**–**g** (10 mmol) were ground with an excess of $NaNO_2$ (1.59 g, 30 mmol) and the mixture was introduced in small portions and with stirring into a ice cooled solution of conc. HCl (30 ml) and water (13 ml), containing Cu powder (0.45 g). The reaction mixture was allowed to reach room temperature and heated to 55 °C for 20 min. The reaction mixture was cooled and extracted with 30 ml $CHCl_3$ (three times). The combined extracts were washed with $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated to give crude. The product was crystallized from ethanol. The compounds were characterized by IR (cm^{-1}, KBr) 1624–1590 (C=N stretch), 1274–1229 (aryl–C–O–C–assym. stretch), 1065–1025 (aryl–C–O–C–sym. stretch), 690–671 (C–Cl). Mass spectra of compound **3a** exhibited molecular ion peak at *m/z* 180 (M^+), other important fragment were observed at 182 ($M^+ + 2$). Compound **3a** is already reported previously [32].

4.3. General procedure for synthesis of N-substituted piperazinyl quinolones (**I-XXI**)

A mixture of equimolar quantity of compound **3a–g** (0.05 mmol), piperazinyl quinolone (0.05 mmol) and $NaHCO_3$ (0.05 mmol) in DMF (10 ml), was heated under reflux at 85–90 °C for 12 h. After cooling, water was added (10 ml) and the precipitate was filtered off, washed with water and recrystallized from the mixture of DMF and H_2O to give the final product.

4.3.1. 1-Ethyl-6-fluoro-4-oxo-7-(4-(5-phenyl-1,3,4-oxadiazol-2-yl) piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**I**)

IR (cm^{-1}, KBr): 3050 (aromatic C–H stretch), 1724 (carboxylic acid C=O str), 1629 (pyridinone C=O str), 1590 (pyridinone ring C=C str), 1219 (C–O str), 1176 (piperazine C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.31–1.33 (t, 3H, C_1–CH_3), 3.29–3.48 (m, 8H, C_{2-6}'), 4.58 (q, 2H, C_1–CH_2), 7.09–7.11 (d, 1H, C_8 $J = 7$ Hz), 7.42–7.53 (m, 3H, Ar–H), 8.01–8.05(m, 3H, C_5 & 2Ar–H), 9.12 (s, 1H, C_2), 14.86 (s, 1H, –COOH); MS-ESI: 463.17 (M^+), 464.17 ($M^+ + 1$), 465.17 ($M^+ + 2$); Anal. Cal%: C, 62.20; H, 4.78; N, 15.11. Found%: C, 62.86; H, 4.96; N, 14.99.

4.3.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**III**)

IR (cm^{-1}, KBr): 3048 (aromatic C–H stretch), 1728 (carboxylic acid C=O str), 1632 (pyridinone C=O str), 1595 (pyridinone ring C=C str), 1211 (C–O str), 1160 (piperazine C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.10–1.15 (m, 3H, C_3'–CH_3), 1.19–1.39 (m, 4H, C_1–CH_2–), 3.08–3.12 (m, 1H, C_3'), 3.38–3.65 (m, 6H, $C_{2,5-6}'$), 3.73 (s, 3H, C_8–OCH_3), 3.78–3.81 (m, 1H, C_1–CH–), 7.48–7.50 (d, 2H, Ar–H), 7.85–7.89 (d, 1H, C_5), 8.31–8.33 (d, 2H, Ar–H), 8.73 (s, 1H, C_2), 14.83 (s, 1H, –COOH).

4.3.3. 1-Cyclopropyl-6-fluoro-7-(4-(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**V**)

IR (cm^{-1}, KBr): 3041 (aromatic C–H stretch), 1719 (carboxylic C=O str), 1627 (pyridinone C=O str), 1582 (pyridinone ring C=C str), 1256 (C–O str), 1133 (C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.17–1.37 (m, 4H, C_1–CH_2–), 3.32–3.45 (m, 8H, C_{2-6}'), 3.71 (s, 3H, –OCH_3), 3.78–3.84 (m, 1H, C_1–CH–), 6.91–6.93 (m, 2H, Ar–H), 7.14–7.16 (d, 1H, C_8), 7.76–7.93 (m, 3H, C_5 & 2Ar–H), 8.64 (s, 1H, C_2), 14.82 (s, 1H, –COOH); MS-ESI: 505.18 (M^+), 506.08 ($M^+ + 1$), 507.08 ($M^+ + 2$); Anal. Cal%: C, 61.78; H, 4.79; N, 13.85. Found%: C, 61.33; H, 4.63; N, 13.81.

4.3.4. 7-(4-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**VII**)

IR (cm^{-1}, KBr): 3058 (aromatic C–H stretch), 1728 (carboxylic C=O str), 1632 (pyridinone C=O str), 1578 (pyridinone ring C=C str), 1243 (C–O str), 1156 (C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.21–1.24 (t, 3H, C_1–CH_3), 3.28–3.56 (m, 8H, C_{2-6}'), 4.55–4.62 (m, 2H, C_1–CH_2–), 7.18–7.20 (d, 1H, C_8), 7.38–7.74 (m, 4H, Ar–H), 7.84–7.88 (d, 1H, C_5), 8.74 (s, 1H, C_2), 14.76 (s, 1H, –COOH).

4.3.5. 1-Cyclopropyl-6-fluoro-4-oxo-7-(4-(5-phenyl-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (**VIII**)

IR (cm^{-1}, KBr): 3052 (aromatic C–H stretch), 1721 (carboxylic C=O str), 1635 (pyridinone C=O str), 1572 (pyridinone ring C=C str), 1243 (C–O str), 1133 (C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.21–1.37 (m, 4H, C_1–CH_2–), 3.29–3.58 (m, 8H, C_{2-6}'), 3.74–3.79 (m, 1H, C_1–CH–), 7.18–7.20 (d, 1H, C_8), 7.44–7.76 (m, 5H, Ar–H), 7.84–7.88 (d, 1H, C_5), 8.62 (s, 1H, C_2), 14.64 (s, 1H, –COOH).

4.3.6. 1-Ethyl-6-fluoro-7-(4-(5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**X**)

IR (cm^{-1}, KBr): 3047 (aromatic C–H stretch), 1720 (carboxylic C=O str), 1629 (pyridinone C=O str), 1588 (pyridinone ring C=C str), 1257 (C–O str), 1148 (piperazine C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.23–1.27 (t, 3H, C_1–CH_3), 3.35–3.58 (m, 8H, C_{2-6}'), 4.52 (q, 2H, C_1–CH_2–), 7.12–7.14 (d, 1H, C_8, $J = 7.2$), 7.85–8.05 (m, 3H, C_5 & 2Ar–H), 8.32–8.34 (m, 2H, Ar–H), 8.88 (s, 1H, C_2), 14.89 (s, 1H, –COOH); Anal. Cal%: C, 56.69; H, 4.16; N, 16.53. Found%: C, 56.31; H, 4.21; N, 16.48.

4.3.7. 1-Cyclopropyl-7-(4-(5-(3,4-dinitrophenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**XI**)

IR (cm^{-1}, KBr): 3048 (aromatic C–H stretch), 1724 (carboxylic C=O str), 1633 (pyridinone C=O str), 1582 (pyridinone ring C=C str), 1248 (C–O str), 1133 (C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.19–1.23 (m, 4H, C_1–CH_2–), 3.32–3.56 (m, 8H, C_{2-6}'), 3.73–3.76 (m, 1H, C_1–CH–), 7.18–7.20 (d, 1H, C_8), 7.85–7.89 (d, 1H, C_5), 8.12–8.14 (d, 1H, Ar–H), 8.52–8.54 (d, 1H, Ar–H), 8.63 (s, 1H, Ar–H), 8.69 (s, 1H, C_2), 14.69 (s, 1H, –COOH).

4.3.8. 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methyl-4-(5-phenyl-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**XV**)

IR (cm^{-1}, KBr): 3040 (aromatic C–H stretch), 1720 (carboxylic C=O str), 1617 (pyridinone C=O str), 1555 (pyridinone ring C=C str), 1278 (C–O str), 1112 (C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.10–1.14 (m, 3H, C_3'–CH_3), 1.17–1.38 (m, 4H, C_1–CH_2–), 3.08–3.12 (m, 1H, C_3'), 3.32–3.51 (m, 6H, $C_{2,5-6}'$), 3.76 (s, 3H, C_8–OCH_3), 3.73–3.78 (m, 1H, C_1–CH–), 7.32–7.48 (m, 3H, Ar–H), 7.56–7.58 (d, 2H, Ar–H), 7.63–7.67 (d, 1H, C_5), 8.52 (s, 1H, C_2), 14.74 (s, 1H, –COOH); Anal. Cal%: C, 56.69; H, 4.16; N, 16.53. Found%: C, 56.58; H, 4.24; N, 16.51.

4.3.9. 1-Cyclopropyl-7-(4-(5-(3,4-dinitrophenyl)-1,3,4-oxadiazol-2-yl)-3-methylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**XVIII**)

IR (cm^{-1}, KBr): 3052 (aromatic C–H stretch), 1724 (carboxylic acid C=O str), 1629 (pyridinone C=O str), 1590 (pyridinone ring C=C str), 1239 (C–O str), 1146 (piperazine C–N str); ^1H NMR, δ ppm (DMSO-d_6): 1.13–1.17 (m, 3H, C_3'–CH_3), 1.19–1.29 (m, 4H, C_1–CH_2–), 3.03–3.08 (m, 1H, C_3'), 3.28–3.53 (m, 6H, $C_{2,5-6}'$), 3.76 (s, 3H, C_8–OCH_3), 3.77–3.84 (m, 1H, C_1–CH–), 7.56–7.59 (d, 1H, C_5), 8.13–8.15 (d, 1H, Ar–H), 8.49 (s, 1H, C_2), 8.51–8.53 (d, 1H, Ar–H), 8.68 (s, 1H, Ar–H), 14.76 (s, 1H, –COOH).

4.3.10. 1-Cyclopropyl-7-(4-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**XX**)

IR (cm^{-1}, KBr): 3055 (aromatic C–H stretch), 1718 (C=O str, carboxylic acid), 1628 (pyridinone C=O str), 1581 (pyridinone ring C=C str), 1259 (C–O, str), 1137 (C–N, str, piperazine); ^1H NMR, δ ppm (DMSO-d_6): 1.19–1.28 (m, 4H, C_1–CH_2–), 3.29–3.47 (m, 8H, C_{2-6}'), 3.76 (s, 6H, Ar–OCH_3), 3.82–3.87 (m, 1H, C_1–CH–), 6.93–6.99 (m, 2H, Ar–H), 7.14–7.16 (d, 1H, C_8), 7.48–7.50 (d, 1H, Ar–H), 7.82–7.86 (d, 1H, C_5), 8.62 (s, 1H, C_2), 14.82 (s, 1H, –COOH); MS-ESI: 535.19 (M^+), 536.19 ($M^+ + 1$), 537.19 ($M^+ + 2$); Anal Cal%: C, 60.56; H, 4.89; N, 13.08. Found%: C, 60.28; H, 4.76; N, 12.97.

4.4. Antibacterial activity

All the synthesized compounds were evaluated for their *in vitro* antibacterial activities against aerobic Gram positive bacteria – *S. aureus* subsp. aureus (***MTCC 1430***) i.e. Strain type Serovar 3, *B.*

subtilis (***MTCC 2423***) and Gram negative bacterium – *E. coli* (***MTCC 739***) by two fold tube dilution method [22,23]. DMSO was used as negative control while ciprofloxacin was used as a positive control showing inhibition of growth of microbes. According to the values of control, the results were evaluated.

4.4.1. Minimum inhibitory concentration measurement

Two fold dilution techniques [33] were followed to determine the minimum inhibitory concentration of the synthesized compounds. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium at the required final concentration starting from 10 μg/ml till MIC was achieved. A tube containing only the culture medium and DMSO in same dilution was used as a negative control. The MIC values were recorded after incubation at 30 °C for a period of 24 h. The lowest concentration of test substance that completely inhibited the growth of microorganism was reported as pMIC which is the negative logarithm of molar minimum inhibitory concentration. Ciprofloxacin was used as a positive control. All experiments were performed in triplicate.

4.5. QSAR studies

The antibacterial activities of the synthesized derivatives were obtained in the terms of MIC that is the minimum concentration of the antimicrobial that inhibits the visible growth of microbes against the three representative strains. These MIC values were expressed in terms of μg/ml concentration. For the purpose of correlation, the MIC values were converted to their molar units and subsequently to free energy related negative logarithmic state, i.e. log (1/MIC). The structure of the molecules was drawn using CS Chemoffice. All the molecules were subjected to energy minimization using molecular mechanics (MM2) method, until the root mean square gradient value reaches a value smaller than 0.001 Kcal/mol Å using HyperChem.

The calculation of the molecular descriptors for the synthesized fluoroquinolone derivatives was done using Dragon web version software package for windows. Regression analysis was performed using the BuildQsar software package. The predictive powers of the equation were validated by the determination of various statistical parameters using the leave one out (LOO) cross-validation method.

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