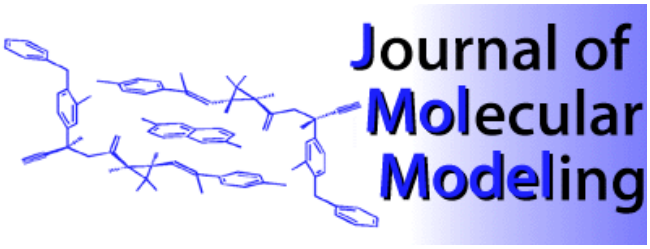
J. Mol. Model. **1998**, *4*, 250 – 258



FULL PAPER

A Molecular Electrostatic Potential Mapping Study of Some Fluoroquinolone Anti-Bacterial Agents

Santhosh Chidangil[1], Manoj K. Shukla[2], and Phool C. Mishra[2]

[1]International School of Photonics; Cochin University of Science and Technology; Cochin-682022, India

[2]Department of Physics; Banaras Hindu University; Varanasi-221 005, India. E-mail: pcmishra@banaras.ernet.in

Received: 30 December 1997 / Accepted: 11 February 1998 / Revised: 10 July 1998 / Published: 5 August 1998

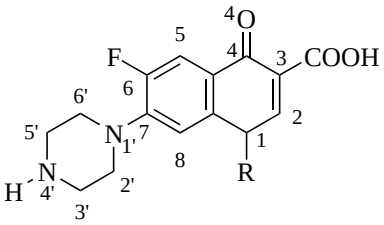
Abstract Molecular electrostatic potential (MEP) maps of some fluoroquinolones having varying degrees of activity against the bacterium Staphylococcus Aureus have been studied using the optimized hybridization displacement charges (HDC) combined with Löwdin charges obtained by the AM1 method. The roles of different substitutions at the N_1-position in the parent quinolone ring have been studied. The conformation of the carboxylic group attached to the quinolone ring was shown to be such that there is an intramolecular hydrogen bonding between the hydrogen atom of this group and the oxygen atom of the carbonyl group of the quinolone moiety. The carbonyl oxygen atom of the quinolone moiety, hydroxyl oxygen atom of the carboxylic group and the terminal nitrogen atom of the piperazin ring attached to the quinolone ring appear to be involved in the action of the drugs through electrostatic interactions while the N_1-alkyl substituents seem to be involved in the same through hydrophobic interactions.

Keywords Fluoroquinolone, Molecular electrostatic potential (MEP), Hybridization displacement charge (HDC), DNA gyrase enzyme

Introduction

Pharmaceutical research on fluoroquinolones has made a considerable progress in the recent years [1-10]. Several of these drugs possess a broad spectrum anti-bacterial activity and a high degree of potency against gram-positive and gram-negative bacteria and are, therefore, in common clinical use [1-10]. Quinolones are also active as anti-tumor, anti-malarial and anti-viral agents [11-15]. Quinolones inhibit the bacterial DNA gyrase enzyme, a type II topoisomerase [10]. The activity of the enzyme is essential for DNA replication [16]. The molecular mechanism of action of quinolones has been studied by Shen et. al [17-21]. According to the model due to these authours [17-21], quinolones bind to DNA gyrase-induced specific site on relaxed DNA. A detailed analysis of effects of structural variations on the biological activity of quinolones was given by Chu et. al [10,22,23]. They observed that modification of every position except the groups attached to the C_3 and C_4 sites (Figure 1) in the parent quinolone molecule by using different substitutions led to large changes in potency [10,22,23]. According to them, the foremost requirement for a high anti-bacterial activity of these molecules is the presence of a 3-carboxylic acid group and a 4-keto group [10,22,23]. Another neces-

Correspondence to: P. C. Mishra



R	Molecule
$-CH_3$	**1**
$-C_2H_5$	**2**
$-CH=CH_2$	**3**
$-CH_2CH_2F$	**4**
$-CH_2CH_2OH$	**5**
$-NHCH_3$	**6**
$-n\text{-}C_3H_7$	**7**
$-CH(CH_3)_2$	**8**
$-C(CH_3)_3$	**9**
– △	**10**

Figure 1 *Structures of fluoroquinolones and atomic numbering scheme*

sity for anti-bacterial activity of these molecules is that the substituent at the N_1-position should be lipophilic [1,10]. This study suggested that antibacterial potency of these molecules is strongly influenced by the steric bulk of the N_1-alkyl substituent (Figure 1), the size of an ethyl group being optimum [10]. Fujita [24] studied stucture-activity relationship (SAR) for a set of N_1-alkyl derivatives of quinolones quantitatively and suggested that an optimum length for the N_1-alkyl substituent is 4.2 A, that of an ethyl group being 4.1 A. Most of the marketed quinolones such as nalidixic acid, oxonilic acid, norfloxacin and pefloxacin have an ethyl group at the N_1-position each. However, one of the most active molecules of this group which is in common clinical use today is ciprofloxacin which has a cyclopropyl substitution at the N_1-position. It has been suggested that in addition to the steric bulk of the N_1-alkyl substituent, other factors such as electronic π-donation or conjugation ability, may also have influence on biological activity of the drugs [22,23,25]. It has been noted that presence of a fluorine substituent at the C_6 site and a basic substituent at the C_7 site (Figure 1) enhance transport of the drugs through the cell membrane, resulting in their enhanced anti-bacterial activity [10,26-28].

We have studied molecular electrostatic potential (MEP) maps of some 6-fluoro, C_7-piperazin substituted quinolones with varying substitutions at the N_1-position and examined the correlation between the computed MEP values and activities of the drugs against a gram-positive bacterium called Staphylococcus Aureus which produces a toxin and thereby causes one of the most common types of bacterial food poisoning [29]. MEP mapping is a valuable tool to study structure-activity relationships of molecules in particular where electrostatic interactions play the dominant role [30-38]. It is proposed that the mechanism of action of the quinolones involves electrostatic interactions i.e. hydrogen bonding, with the bacterial DNA nucleotides [17-21], and therefore, a MEP mapping study of the problem appears to be desirable. We used the Hybridization Displacement Charge (HDC)-based method, the reliability of which to study MEP maps has been established earlier [39-45]. MEP maps can also be computed to varying degrees of accuracy using certain other approximate methods, e.g. (a) the asymptotic density model [46], (b) the bond increment method [47], and (c) the cumulative atomic multipole moments method [48]. Several review articles dealing with properties, computational methodologies and applications of MEP maps have been published [49]. The areas in which MEP mapping has been shown to be useful include quantitative structure-activity relationship (QSAR), hydrogen bonding, biomolecular recognition, chemical reactions and catalysis [44,49-55].

Method of calculation

The molecular electrostatic potential V(**r**) at a point **r** is defined as

$$V(\mathbf{r}) = \sum_i \frac{Z_i}{|\mathbf{R_i} - \mathbf{r}|} - \int \left(\frac{\rho(\mathbf{r}')}{|\mathbf{r}' - \mathbf{r}|} \right) d\mathbf{r}' \qquad (1)$$

where Z_i is the charge on nucleus i located at **R** and ρ(**r**´) is the molecular electron density at a point **r**´ near the given molecule. To improve the accuracy of MEP calculations with respect to the commonly used point charge model, the electronic charges are considered to be distributed spherically symmetrically in three dimension on each atomic and HDC site according to the form of the square of the corresponding valence Slater ns (n=principal quantum number 1,2,3 etc.) atomic orbital [39-45]. The hybridization displacement charge (HDC) Q associated with a non-hydrogen atom is defined as

$$Q = \mu_h / R \qquad (2)$$

where μ_h is the total hybridization dipole moment of the atom under consideration arising due to displacement of the charge Q to a distance **R** from the atom. We know

$$\mu_h = \left[\left(\mu_x^h\right)^2 + \left(\mu_y^h\right)^2 + \left(\mu_z^h\right)^2 \right]^{\frac{1}{2}} \qquad (3)$$

where μ_a^h (a = x,y,z) are the hybridization dipole moment components arising due to mixing of the 2s and $2p_a$ atomic orbitals, and are given by

$$\mu_a^h = D_a Q_i$$
(a = x, y, z and i = 1,2,3 respectively)
where $D_a = \left(2s|a|2p_a\right)$ (a = x, y, z) (4)

and Q_i is given by the ($2s,2p_a$) density matrix element, with a - sign, i.e.

$$Q_i = -2P_{2s2p_a}$$

We also have

$$R = \left(D_x^2 + D_y^2 + D_z^2\right)^{\frac{1}{2}} \quad (5)$$

From the eqs. (2-5), we get

$$Q = \left[\left(Q_1^2 + Q_2^2 + Q_3^2\right)\Big/3\right]^{\frac{1}{2}} \quad (6)$$

The displacement of the hybridization displacement charge Q with respect to the atom under consideration would take place along the direction given by

$$\theta = \tan^{-1}\left(\frac{\mu_y^h}{\mu_x^h}\right) \quad (7a)$$

$$\varphi = \tan^{-1}\left(\frac{\mu_z^h}{\mu_h}\right) \quad (7b)$$

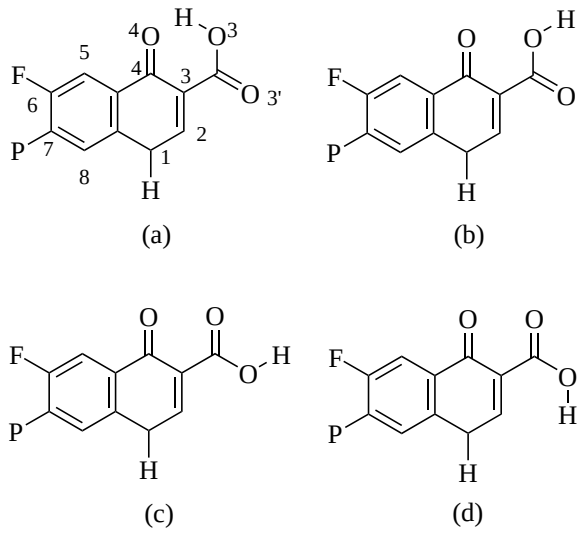


Figure 2 *Four conformers of fluoroquinolone obtained by rotation of the COOH group attached to the C_3 atom, denoted as (a), (b), (c) and (d). P stands for piperazin moiety*

Equation (4) can also be written as

$$\mu_a^h = \left(KD_a\right)\left(Q_i\Big/K\right) = D_a' Q_i' \quad (8)$$
(a = x, y, z and i = 1,2,3 respectively)

Where K is an adjustable parameter which controls the amount of the charge (Q'_i) and the distance (D'_a) of HDC from the atom under consideration. K and Slater exponents (ζ) of HDC have been treated as adjustable parameters in the HDC-based method to compute MEP maps as discussed earlier [39-45]. Further, these parameters (K and ζ) have been optimized for different types of atoms so as to reproduce *ab inito* MEP features using charge distributions obtained by the semi-empirical AM1 and MNDO methods [39-45]. Here, we have used the AM1 method as it gives somewhat better results than the MNDO method [45]. The constants K and ζ are available in the literature [42-45].

Results and discussion

Ground state geometry

Ground state geometries of the fluoroquinolones (**1-10**) (Figure 1) were optimized using the AM1 method [56]. Further, the geometries of the four conformers (a), (b), (c) and (d) (Figure 2) obtained by considering the different orientations of the C=0 and OH bonds of the carboxylic group attached to the C_3 atom of the quinolone ring were fully optimized in each case. The relative total energies of the four conformers in the different cases as obtained by the AM1 calculations are presented in Table 1. The AM1 method predicts the quinolone conformer (a) to be most stable in all the cases (Table 1). The intramolecular hydrogen bonding between the hydrogen atom of the OH group attached to the C_3 atom and the O_4 oxygen atom (Figure 2) appears to be mainly responsible for the higher stability of the conformer (a) over those of other three conformers in the different cases. In order to further examine the relative stabilities of the four conformers (a) to (d) of the fluoroquinolones (Figure 2), we carried out *ab initio* gas phase and water-solvation calculations on molecules obtained by replacing the N_1- alkyl substituent and the C_7-substituent (i.e. the piperazin ring) by a hydrogen atom each but keeping the fluorine substituent at C_6, using their AM1 optimized geometries, polarized continuum model [57,58], 3-21G basis set and the Gaussian 94 (Windows version) program [59]. These calculations yielded the following information: (i) The relative total energies of the conformers (a) to (d) (Figure 2) in gas phase as obtained by the *ab initio* calculations were found to be 0.0, 0.22, 0.19 and 0.98 eV respectively, and (ii) Among the four conformers, the conformer (a) was stabilised most and the conformers (b), (c) and (d) were less stabilised than the conformer (a) by 0.138, 0.126 and 0.104 eV respectively on solvation in water. Thus the relative total energies of the water-solvated conformers

Table 1 *Relative total energies (kJ·mol^{-1}) of the four conformers of the quinolones obtained by full geometry optimization using the AM1 method*

Molecule [e]	N_1-alkyl substituent	Conformer [f] (a)	(b)	(c)	(d)
1	CH_3	0.0	7.40	9.42	48.73
2	C_2H_5	0.0	7.37	9.62	49.16
3	$CH{=}CH_2$	0.0	7.00	8.70	47.44
4	CH_2CH_2F	0.0	8.80	9.29	48.40
5	CH_2CH_2OH	0.0	7.23	9.29	48.40
6	NHCH	0.0	7.71	10.56	26.26
7	n-C_3H_7	0.0	6.97	9.50	49.60
8	$CH(CH_3)_2$	0.0	7.87	10.07	49.60
9	$C(CH_3)_3$	0.0	8.03	11.81	55.07
10	△	0.0	9.62	14.93	48.39

[e] For structures, see Figure 1
[f] The atomic arrangements in the four conformers are shown in Figure 2

(a) to (d) were found to be 0.0, 0.358, 0.316 and 1.084 eV respectively. These results suggest that the conformer (a) of the fluoroquinolones is most stable both in gas phase and in the solution environment and we should preferably consider it to explain the antibacterial activities of the molecules. Shen et. al [17-20] considered the conformer (c) of the molecules to explain their antibacterial activity. It may be mentioned that in some active 2-3 bridged quinolones, an intramolecular hydrogen bonding as that in conformer (a) has been suggested [60].

Molecular electrostatic potential maps

In order to examine the possible importance of electrostatic interactions in the mechanism of action of quinolones in general, we considered certain quinolone ring analogues present in several popular antibacterial agents, which are shown in Figure 3. The computed minimum MEP values near the oxygen atom O_4 of the carbonyl group of these molecules are presented in Table 2. The following information is available in the literature [61-65] regarding the relative activities of the drugs containing these ring systems (Figure3): (i) When the N_1H_1 group in the norfloxacin ring (Figure 3(b)) is replaced by a CH_2 group resulting in the molecule 1-oxo, 1,4-dihydronaphthalene (Figure 3(d)), the molecule becomes inactive [61], (ii) Quinolone drugs with additional 1,8 bridged six-membered rings e.g. ofloxacin, flumequine and rufloxacin are usually more active than those without this additional ring, (iii) Rufloxacin analogues appear to have higher activity than those of ofloxacin [62,63], and (iv) A significant reduction in activity occurs in going from norfloxacin to cinoxacin [64,65], this change in activity occurring due to the presence of the nitrogen atom at the 2-position in the latter molecule (Figure 3(c)) [21]. The above variation in activities of the drugs is qualitatively similar to that in the magnitudes of the minimum MEP values near the oxygen atom of the carbonyl group (O_4). Thus we find that the oxygen atom of the carbonyl group is likely to be involved in electrostatic interactions relating to the DNA gyraze inhibitory activity of the drugs derived from these ring systems (Figure 3).

In order to evaluate the roles of the different alkyl groups substituted at the N_1-position, we calculated MEP values near the O_4 atom of the different N_1-alkyl substituted molecules after replacing the carboxyl group at C_3, the flourine atom at C_6 and the piperazin ring at C_7 by a hydrogen atom each and full geometry optimization (Figure 1). The minimum MEP values so obtained near the O_4 atom are presented in Table 3. We find that as the size of the alkyl chain changes, the MEP value near the O_4 atom also changes, but by a small amount in each case. Potencies of the drugs have been measured in terms of molar inhibition concentration (MIC) in μg/ml. It has been reported that inhibitory concentrations for 50% in-

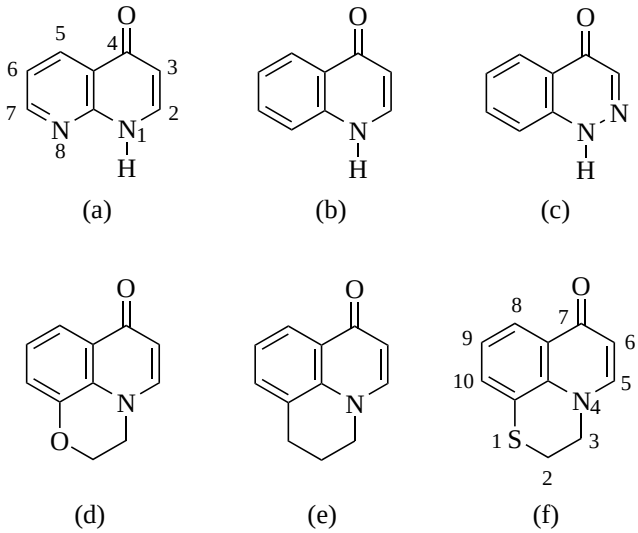


Figure 3 *Basic ring systems of the quinolones. The rings occur in (a) Nalidixic acid, (b) Norfloxacin, (c) Cinoxacin, (d) Ofloxacin, (e) Flumequine and (f) Rufloxacin*

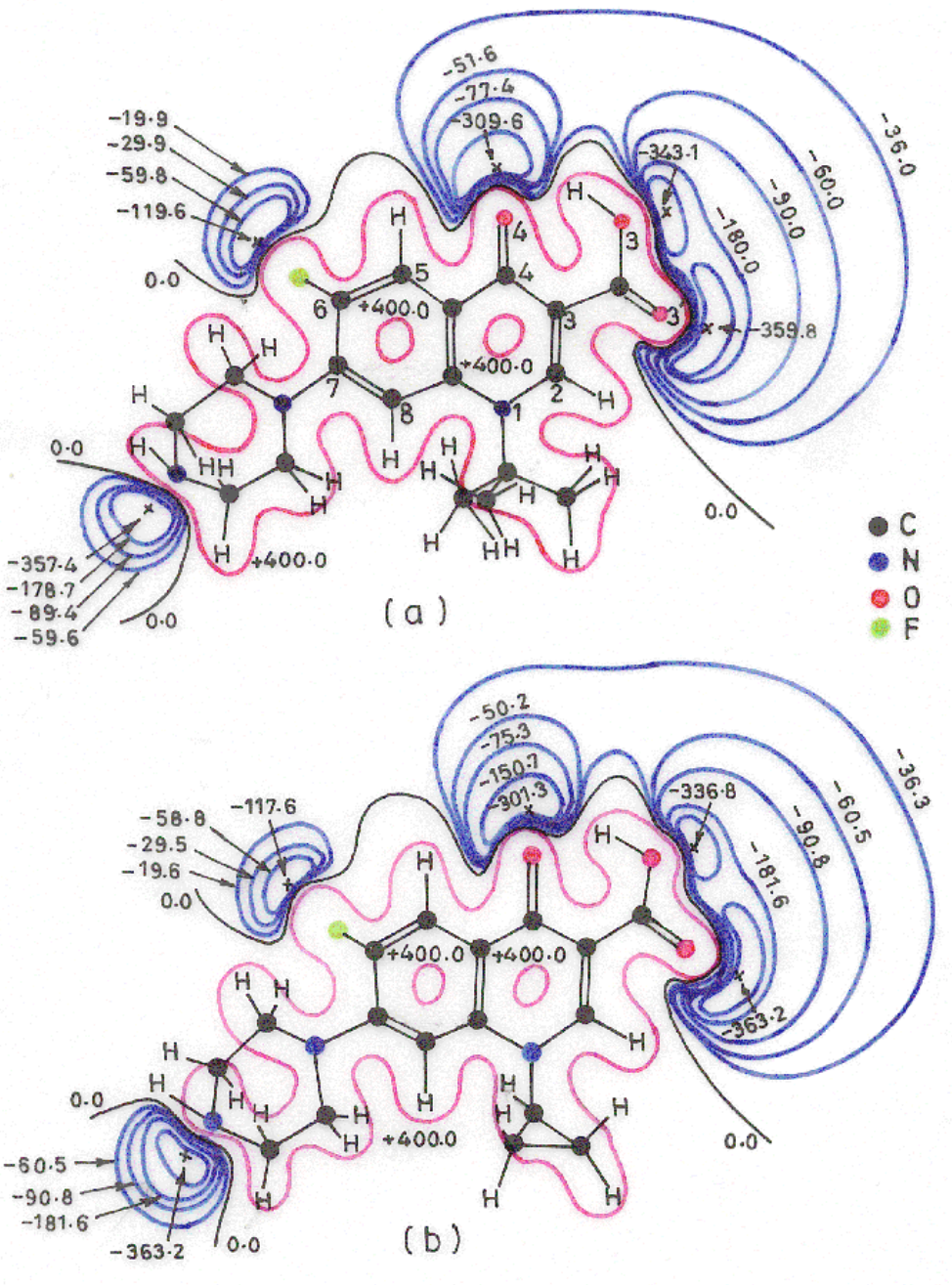


Figure 4 *(a) Molecular electrostatic potential map (kJ·mol^{-1}) obtained in the ring plane of molecule* ***9*** *and (b) Molecular electrostatic potential map (kJ·mol^{-1}) obtained in the ring plane of molecule* ***10***

hibition (IC_{50}) of the activity of Staphylococcus Aureus correlate well with the MIC values [66]. The activities of the drugs expressed as -log (MIC x 10^{-6}) (Tables 3,4) vary with the N_1-alkyl substituent as follows: $C(CH_3)_3$ > △ > C_2H_5 > $CH(CH_3)_2$ > n-C_3H_7 > CH_3. The variation of MEP values near O_4 is not paralleled quantitatively by this variation in the activities of the drugs against the gram-positive bacterium Staphylococcus Aureus. Therefore, it appears that the inhibitory activities of the drugs against Staphylococcus Aureus involve hydrophobic interactions of the alkyl groups as suggested earlier [10]. Further, in this context, the optimum size of the N_1-alkyl substutents appears to be around that of $C(CH_3)_3$, the sizes of C_2H_5 and cyclopropyl groups also being similar. The methyl group seems to fall short of the appropriate size drastically as the methyl substituted molecule has a poor activity (Table 3).

Now let us consider the activities of the ten molecules studied here with all the relevant substitutions shown in Figure1. These molecules possess five regions of MEP minima, one each near O_4, O_3, $O_{3'}$, $N_{4'}$ and F (Figure 1). We computed linear correlation coefficients between the minimum MEP values in these five regions of all the molecules studied here and their potencies. Since the minimum MEP values near $O_{3'}$

Table 2 *Minimum MEP values near the O_4 site of ring systems of some quinolone analogues*

S.N.	Ring system [a]	Present in drug	MEP (kJ·mol^{-1})
1	4-oxo-1,4-dihydro [1,8] naphthyridine	Nalidixic acid	-241.4
2	4-oxo-1,4-dihydroquinolone	Norfloxacin	-297.1
3	4-oxo-1,4-dihydroclinoline	Cinoxacin	-255.2
4	7-oxo-2,3-dihydro-7H -pyrido-[1,2,3-de],4-benzoxazine	Ofloxacin	-292.9
5	6,7-dihydro-4-oxo-1H, 5H, benzo-[i,j]quinolizine	Flumequine	-304.2
6	7-oxo-2,3-dihydro-7H-pyrido-[1,2,3-de]benzothiozine	Rufloxacin	-376.6
7	1-oxo-1,4-dihydro-Naphthalene	–	-251.0

[a] For structures, see Figure 3

and F did not show any correlation with the activities of the drugs, they are not presented here and only the MEP values near O_4, O_3 and $N_{4'}$ are presented in Table 4. As discussed earlier, the hydrophobic interactions of the different N_1-alkyl substituents are very important in controlling activities of these drugs [10]. Therefore, although the O_4 site is likely to be involved in electrostatic interactions as discussed earlier, we do not expect any strong correlation between the minimum MEP values in this region and the activities of the drugs having different N_1-alkyl substitutions. If we consider the four similar N_1-alkyl substitutions C_2H_5, $CH(CH_3)_2$, $C(CH_3)_3$ and △ (cyclopropyl group) and the corresponding minimum MEP values near their O_3 sites, the correlation coefficient between the MEP values and the activities of these four molecules (**2**, **8**, **9**, **10**) is found to be 0.96. Further, use of the t-test [67] showed that this correlation is significant at the level of 5%. The correlation coefficient between the MEP values near the O_4 sites of the same four molecules (**2**, **8**, **9**, **10**) and their activities was found to be only 0.75 which is in accordance with the above mentioned expectation for this site. These results support the possibility that the O_3 and O_4 atoms of this class of molecules (Figure 2) would serve as hydrogen bond accepting sites. In the earlier work [17-21], O_4 and the oxygen atom of the C=O group of the carboxylic group were suggested to be involved in hydrogen bonding. Thus the present work suggests a modification in that model in the sense of involvement of the oxygen atom of the OH group rather than the oxygen atom of the carbonyl group of the carboxylic group in hydrogen bonding. The MEP maps of the molecules **9** and **10** in the ring plane are shown in Figure 4.

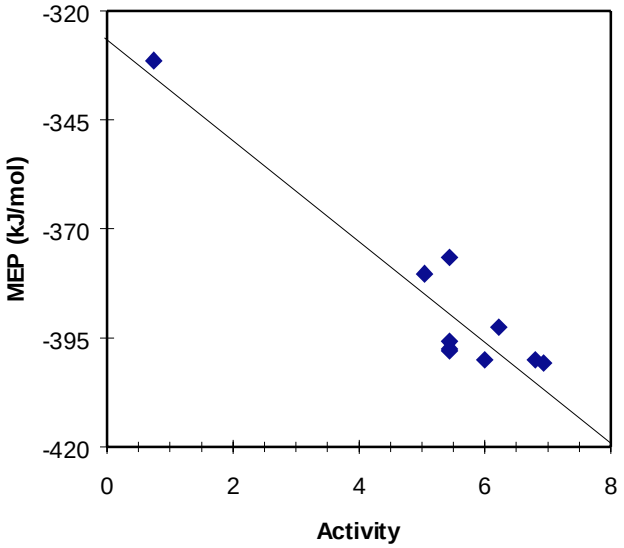


Figure 5 *Variation of magnitudes of minimum MEP near the $N_{4'}$ atom with the anti-bacterial activities of flouroquinolones (**1-10**) against Staphylococcus Aureus*

Table 3 *The minimum MEP values near the O_4 site of the quinolone moeity in molecules with different N_1-alkyl substitutions*

N_1-alkyl substituent [a]	Activity [b]	MEP (kJmol^{-1})
CH_3	0.75	-365.7
C_2H_5	6.22	-364.1
C_3H_7	5.44	-379.1
$C(CH_3)_3$	6.94	-374.9
△	6.80	-367.4

[a] For structures, see Figure 1.
[b] Activity against the bacterium Staphylococcus Aureus is given in MIC (Molar Inhibition Concentration) which is given in μg/ml of the drugs [10]. Here activity is expressed as $-\log_{10}(MIC\times10^{-6})$

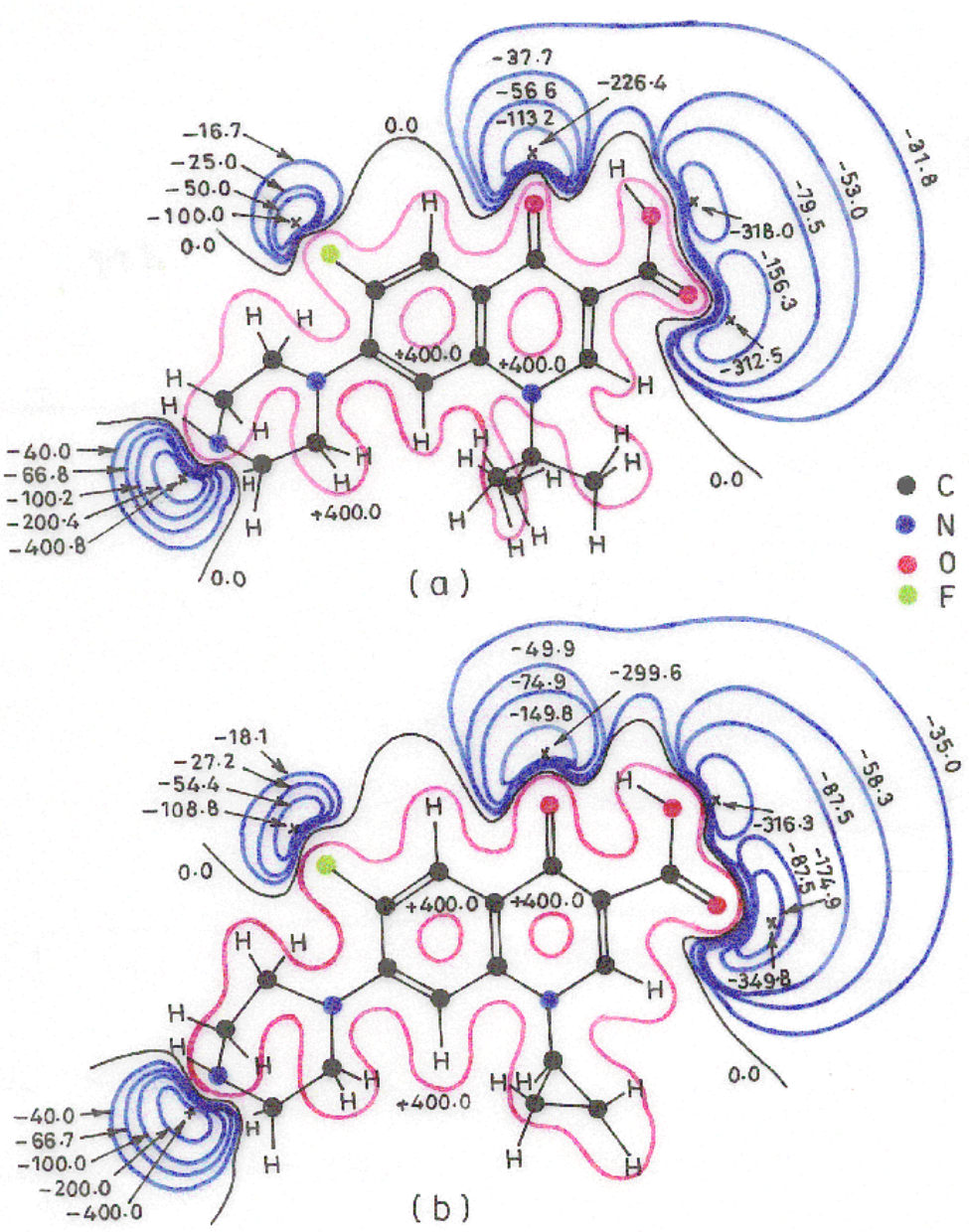


Figure 6 *(a) Molecular electrostatic potential map (kJ·mol^{-1}) drawn 0.6 Å above the ring plane of molecule* ***9****. (b) Molecular electrostatic potential map (kJ·mol^{-1}) drawn 0.25 Å above the ring plane of molecule (****10****)*

The linear correlation coefficient between the minimum MEP values near the $N_{4'}$ site of all the ten molecules and the activities of the drugs is found to be 0.94. The straight line obtained by a least squares fitting of the minimum MEP values near the $N_{4'}$ site and the activities of the drugs is presented in Figure 5. The MEP maps of the molecules **9** and **10** drawn in planes above the quinolone ring plane which contain the respective MEP minima, are shown in Figure 6. Use of the t-test [67] showed that the above mentioned correlation for the $N_{4'}$ site is significant at the level of 0.1%. However, it is noted from Figure 5 that this correlation is strongly influenced by one of the data points, namely that corresponding to the molecule **1** which has the lowest activity. Thus it appears that the $N_{4'}$ site is involved electrostatically in the action of the drugs against Staphylococcus Aureus. However, N_1-alkyl substitutions also seem to partly affect activity at the $N_{4'}$ site. This is suggested by the fact that if the molecule **1** is excluded and only the molecules **2** to **10** which have different N_1-alkyl substituents are considered, the variation of MEP at the $N_{4'}$ site with molecular activity does not remain strongly linear. It has been suggested earlier that the role of the $N_{4'}$ site is to enhance the penetration of the drugs into the bacterial cell wall [10]. In combination with that proposal, our present results imply that the interaction of the $N_{4'}$ site with the bacterial cell wall would be largely electrostatic in nature, e.g. one involving hydrogen bonding.

Table 4 *Minimum MEP values near the O_4 and $N_{4'}$ sites of fluoroquinolones and their anti-bacterial activities*

Molecule [a]	N_1-alkyl substitutent	Activity [b]	MEP (kJmol^{-1}) [c]		
			O_4	O_3	$N_{4'}$
1	CH_3	0.75	-297.9	-335.6	-331.4
2	C_2H_5	6.22	-300.4	-325.5	-392.5
3	$CH{=}CH_2$	5.44	-292.9	-334.7	-397.5
4	CH_2CH_2F	5.44	-287.4	-295.8	-395.8
5	CH_2CH_2OH	5.44	-297.1	-322.2	-397.9
6	NHCH	5.05	-303.8	-333.1	-380.3
7	n-C_3H_7	5.44	-297.1	-334.7	-376.6
8	$CH(CH_3)_3$	6.00	-300.0	-308.4	-400.0
9	$C(CH_3)_3$	6.94	-309.6	-343.1	-400.8
10		6.80	-301.3	-336.8	-400.0

[a] For structures, see Figure 1
[b] Activity against Staphylococcus Aureus is given in MIC (Molar Inhibition Concentration) which is given in µg/ml of the drugs [10]. Here activity is expressed as -log$_{10}$ (MIC × 10^{-6})
[c] For MEP maps, see Figures 4 and 5

Conclusion

The conclusions of this study are as follows: (i) The conformation of the carboxylic group attached to the quinolone ring at the C_3 site is such that there is an intramolecular hydrogen bonding between the hydrogen atom of this group and the O_4 atom of the quinolone moiety, (ii) The O_3 and O_4 sites of the drugs appear to be involved in electrostatic interactions e.g. hydrogen bonding with the receptor, (iii) The N_1-alkyl substituents appear to be involved in hydrophobic interactions with the receptor, and (iv) The $N_{4'}$ site of the molecules is involved electrostatically in their drug action which according to an earlier suggestion is related to their penetration through the bacterial cell wall.

Acknowledgements The authors are thankful to C.S.I.R. (New Delhi) and U.G.C. (New Delhi) for financial support.

References

1. Albretch, R. *Prog. Res.* **1977**, *21*, 9.
2. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
3. Wolfson, J.S.; Hooper, D.C. *Antimicrob. Agents Chemother.* **1985**, *28*, 581.
4. Hooper, D.C.; Wolfson, J.S. *Antimicrob. Agents Chemother.* **1985**, *28*, 716.
5. Hoiby, N. *Eur. J. Clin. Microb.* **1986**, *5*, 138.
6. Janknegt, R. *Pharm. Week Sci. Ed.* **1986**, *8*, 1.
7. Nue, H.C. *Pharmacol. Ther.* **1989**, *41*, 204.
8. Fernandes, P.B. *J. Clin.Pharmacol.* **1988**, *28*, 156.
9. Paton, J.H.; Reeves, D.S. *Drugs* **1988**, *36*, 193.
10. Chu, D.T.W.; Fernandes, P.B. In *Advances in Drug Research*; Bernard Testa; Ed., Academic Press: New York, **1991**; Vol. 21, p. 39.
11. Wang, J.C. In *First Conference on DNA Topoisomerase in Cancer Chemotherapy*; NIH publication no. 87-2943, *4*, **1987**, p. 3.
12. Barrett, J.F.; Gootz, T.D.; McGuirk, P.R.; Farrell, C.A.; Sokolowski, S.A. *Antimicrob. Agents Chemother.* **1989**, *33*, 1697.
13. Wyler, D.J. *Ann. Intern. Med*. **1989**, *111*, 269.
14. Sarma, P.S. *Ann. Intern. Med.* **1989**, *111*, 336.
15. Crumplin, G.C.; Ed., In *The 4-Quinolones: Antibacterial Agents In Vitro*; Springer Verlag: Berlin, **1990**, p. 53.
16. Carty, M.; Menzel, R. *Proc. Natl Acad. Sci. (USA)* **1989**, *86*, 8882.
17. Shen, L.L. *Biochem. Pharmacol.* **1989**, *38*, 2042.
18. Shen, L.L.; Pernet, A.G. *Proc. Natl. Acad. Sci. (USA)* **1985**, *82*, 307.
19. Shen, L.L.; Baranowski, J.; Pernet, A.G. *Biochem.* **1989**, *28*, 3879.
20. Shen, L.L.; Kohlrenner, W.E.; Weigl, W.; Baronowski, J. *J. Biol. Chem.* **1989**, *264*, 2973.
21. Shen, L.L.; Mitscher, L.A.; Sharma, P.N.; O'Donnell, T.J.; Chu, D.T.W.; Cooper, C.S.; Rosen, T.; Pernet, A.G. *Biochem.* **1989**, *28*, 3886.
22. Chu, D.T.W.; Fernandes, P.B.; Claiborne, A.K.; Pihuleac, E.; Nordeen, C.W.; Maleczka, R.E.; Pernet, A.G. *J. Med. Chem.* **1985**, *28*, 1558.
23. Chu, D.T.W.; Fernandes, P.B.; Pernet, A.G. *J. Med. Chem.* **1986**, *29*, 1531.
24. Fujita, T. In *Drug Design: Factory Fantasy*; G. Jolles and K.R.H. Woolride; Eds. Academic Press: London., **1984**, p.19.
25. Domagala, J.M.; Heifetz, C.L.; Huff, M.P.; Mich, T.F.; Nichols, J.B.; Solomon, M.; Worth, D.F. *J. Med. Chem.* **1988**, *31*, 991.
26. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
27. Domagala, J.M.; Hanna, L.D.; Heifetz, C.L.; Huff, M.P.; Mich, T.F.; Sanchez, J.P.; Solomon, M. *J. Med. Chem.* **1986**, *29*, 394.

28. Klopman, G.; Macina, O.T.; Levinson, M.E.; Rosenkranz, H.S. *Antimicrob. Agents Chemother.* **1987**, *31*, 1831.
29. Stanier, R.Y.; Ingraham, J.L.; Wheelis, M.L.; Painter, P.R. In *General Microbiology*, 5th Edition, Macmillan: London, **1995**, p.626.
30. Scrocco, E.; Tomasi, J. In *Advances in Quantum Chemistry*, P.O. Löwdin, Ed., Academic press: New York, **1978**, V.11, p. 116.
31. Politzer, P.; Truhlar, D.G.; Eds., *Chemical Applications of Atomic and Molecular Electrostatic Potentials*, Plenum Press: New York, **1981**.
32. Politzer, P.; Hedges, W.L. *Int. J. Quant. Chem. Bio. Symp.* **1982**, *9*, 307.
33. Kumar, A.; Mishra, P.C. *J. Mol. Struct.(Theochem)* **1992**, *277*, 299.
34. Kumar, A.; Mishra, P.C. *J. Mol. Struct. (Theochem)* **1993**, *288*, 151.
35. Mohan, C.G.; Kumar, A.; Mishra, P.C. *Int. J. Quant. Chem.* **1993**, *48*, 233.
36. Santhosh, C.; Mishra, P.C. *Int. J. Quant. Chem.* **1994**, *51*, 335.
37. Santhosh, C.; Mishra, P.C. *J. Mol. Struct. (Theochem)* **1994**, *306*, 77.
38. Mohan, C.G.; Kumar, A.; Mishra, P.C. *J. Mol. Struct. (Theochem)* **1995**, *32*, 171.
39. Kumar, A.; Mohan, C.G.; Mishra, P.C. *Int. J. Quant. Chem.* **1995**, *55*, 53.
40. Kumar, A.; Mohan, C.G.; Mishra, P.C. *J. Mol. Struct. (Theochem)* **1996**, *361*, 135.
41. Mohan, C.G.; Kumar, A.; Mishra, P.C. *Int. J. Quant. Chem.* **1996**, *60*, 699.
42. Santhosh, C.; Mishra, P.C. *J. Mol. Mod.* **1997**, *3*, 172.
43. Santhosh, C.; Mishra, P.C. *Indian J. Biochem. Biophys* **1996**, *33*, 158.
44. Mishra, P.C.; Kumar, A. In *Molecular Electrostatic Potentials: Concepts and Applications*, J.S. Murray, K.D. Sen, Eds., Elsevier: Amsterdam, **1996**, p. 257.
45. Mohan, C.G.; Mishra, P.C. *Int. J. Quant. Chem.* **1997**, *62*, 67.
46. Krack, M.; Jug, K. In *Molecular Electrostatic Potentials: Concepts and Applications*, J.S. Murray, K.D. Sen, Eds., Elsevier: Amsterdam, **1996**, p. 330 and references cited therein.
47. Angyan, J.G.; Náray-Szabó, G. In *Theoretical Models for Chemical Bonding, Part IV*, Z.B. Maksic, Ed., Springer-Verlag: Berlin, **1991**, p.1.
48. Sokalski, W.A.; Maruszewski, K.; Hariharan, P.C.; Kaufman, J.J. *Int. J. Quant. Chem. Quantum Biol. Symp.* **1989**, *16*, 119.
49. *Molecular Electrostatic Potentials: Concepts and Applications*, J.S. Murray, K.D. Sen, Eds., Elsevier: Amsterdam, **1996**.
50. Tan, R.C.; Truong, T.N.; McCammon, J.A. *Biochemistry* **1993**, *32*, 401.
51. Sung, S.S. *Biophys. J.* **1995**, *68*, 826.
52. Ehrlich, J.I.; Schramm, V.L. *Biochemistry* **1994**, *33*, 8890.
53. Karla, M.; Cuomo, M.; Arcangeli, A.; Olivotto, M. *Biophys. J.* **1995**, *68*, 2615.
54. Nicholas, J.B.; Hess, A.C. *J. Am. Chem. Soc.* **1994**, *116*, 5428.
55. Brenner, D.W.; Robertson, D.H.; Alert, M.L.; White, C.T. *Phys. Rev. Lett.* **1993**, *70*, 2174.
56. Dewar, M.J.S.; Zoebisch, E.G.; Healy, E.F.; Stewart, J.J.P. *J. Am. Chem. Soc.* **1985**, *102*, 3902.
57. Miertus, S.; Tomasi, J. *Chem. Phys.* **1982**, *65*, 239.
58. Miertus, S.; Scrocco, E.; Tomasi, J. *Chem. Phys.* **1981**, *55*, 117.
59. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Gill, P.M.W.; Johnson, B.G.; Robb, M.A.; Cheeseman, J.R.; Keith, T.; Petersson, G.A.; Montgomery, J.A.; Raghavachari, K.; Al-Laham, M.A.; Zakrzewski, V.G.; Ortiz, J.V.; Foresman, J.W.; Peng, C.Y.; Ayala, P.Y.; Chen, W.; Wong, M.W.; Andres, J.L.; Replogle, E.S.; Gomperts, R.; Martin, R.L.; Fox, D.J.; Binkley, J.S.; Defrees, D.J.; Baker, J.; Stewart, J.P.; Head-Gordon, M.; Gonzalez, C.; Pople, J.A. *Guassian 94, Revision B.2*, Gaussian Inc.: Pittsburgh PA, **1995**.
60. Chu, D.T.W.; Fernandes, F.B.; Claiborne, A.K.; Shen, L.L.; Pernet, A.G. *Drug. Exptl. Clin. Res.* **1988**, *14*, 379.
61. Hoyberg, T.; Khanna, I.; Drake, S.D.; Mitscher, L.A.; Shen, L.L. *J. Med. Chem.* **1984**, *27*, 306.
62. Morrisey, I.; Hoshino, K.; Sato, K.; Yoshida, A.; Hayakawa, I.; Bures, M.G.; Shen, L.L. *Antimicrob. Chem. Ther.* **1996**, *40*, 1775.
63. Vanzoest, W.J.; Marx, A.F.; Koger, H.S. *Eur. Pat. Appl. EP-A-O368410* (16 May) **1990**.
64. Miyamoto, T.; Matsumoto, J. *Chem. Pharm. Bull.* **1988**, *36*, 1321.
65. Matsumoto, J.; Miyamoto, T. In *International Telesymposium on Quinolones*; P.B. Fernandes, Ed., J.R. Prous: Barcelona, **1989**, p.109.
66. Tanaka, T.M.; Sato, K.; Kimura, Y.; Une, T.; Osada, Y.; Nishino, T. *29th International Conference on Anti-microbial Agents and Chemotherapy.*, Houston, 23-26 October, **1989**, Abst. 190.
67. Downie, N.M.; Heath, R.W. *Basic Statistical Methods*, 3rd Edition, Harper and Row: New York, **1970**, p. 230.