Three-Dimensional Structure-Activity Relationships and Receptor Mapping of N_1-Substituents of Quinolone Antibacterials[1]

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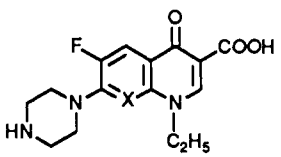
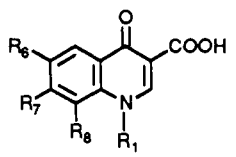
Quantitative structure-activity relationships for quinolone antibacterials have been previously examined and a steric parameter L for the N_1-substituents found to be important in QSAR equations. But some compounds for which previous QSAR equations could not predict the activity have appeared recently. In this study, conformations of a variety of N_1-substituents of quinolone antibacterials were analyzed by a molecular modeling method. An active conformation of each of the compounds was estimated with information of the energy profile calculated by molecular orbital methods and of its biological activity. A model of a receptor corresponding to the N_1-substituents was constructed by superposing van der Waals volumes of active conformer of highly active compounds. As a result of these conformational analyses and receptor mapping, it is proposed that there are two different optimum volumes for increasing the activity of quinolone antibacterials and two unfavorable regions for reducing the activity. It is suggested that the steric parameter L which appeared in previous QSAR equations corresponds to one of the optimum volumes of the proposed receptor model. With this receptor model, a relation between structure and activity of the compounds, including those mispredicted compounds in previous QSAR equations, is able to be rationalized qualitatively and elegantly. We believe that this receptor model is useful for a prediction of the activity of compounds not yet synthesized as well as for designing new quinolone antibacterials.

Norfloxacin (NFLX, 1), which was synthesized by one of the present authors (Koga[2]), opened a new age not only in quinolone antibacterials but also in oral antiinfectives. The appearance of NFLX expanded the clinical uses of quinolones greatly. NFLX is superior to the earlier quinolones in that it has a potent activity against Gram-positive bacteria as well as Gram-negative bacteria (including *Pseudomonas aeruginosa*), a low incidence of resistant bacteria, an incomplete cross-resistance against nalidixic acid resistant bacteria, an oral effectiveness against systemic infections, metabolic stability, and a low oral toxicity.[3] After NFLX was discovered, many related compounds including its bioisoster, enoxacin (2), have been developed and quite a few have been marketed[2,4] (Table I). These compounds are commonly called "new quinolones" or "fluoroquinolones" because they share a common 6-fluoro-7-aminoquinolone structure.



1: X = CH (norfloxacin, NFLX)
2: X = N (enoxacin)



3

Koga[2b,c] previously examined the quantitative structure-activity relationships (QSAR) for a set of 71 compounds shown as a generic structure 3. From the statistical point of view, one of the best correlations is shown as eq 1. In this equation, the minimum inhibitory concentration

$$\log (1/\mathrm{MIC}) = -0.362L(1)^2 + 3.036L(1) - 2.499E_s(6)^2 - 3.345E_s(6) - 0.205[\sum\pi(6,7,8)]^2 - 0.485\sum\pi(6,7,8) + 0.986I(7) - 0.734I(7\mathrm{N-CO}) - 1.023B_4(8)^2 + 3.724B_4(8) - 0.681\sum F(6,7,8) - 4.571 \quad (1)$$

$$(n = 71,\ s = 0.274,\ r = 0.964,\ r^2 = 0.929,\ F = 70.22)$$

in mol/L (MIC) against *Escherichia coli* NIHJ JC-2 is used for biological activity. L(1) is Verloop's STERIMOL parameter representing the length (L) of the substituent at the position 1. $E_s(6)$ represents the Taft-Kutter-Hansch steric parameter (E_s) of R_6. $\sum\pi(6,7,8)$ stands for the sum of the hydrophobic constant (π) of the R_6, R_7, and R_8 substituents. This hydrophobic parameter seems to be related mainly with the transport of compounds into the active site. $I(7)$ is an indicator variable equal to zero when the 7-substituent is hydrogen and expressed as unity when it is not. $I(7\mathrm{N-CO})$ is another indicator variable which is expressed as unity when there is a carbonyl group within the 7-amino substituent moiety, and zero when there is not. $B_4(8)$ is the STERIMOL parameter and represents the maximum width of R_8. It seems that the direction of the maximum width of the R_8 substituent ($B_4(8)$) corresponds to the side opposite to the R_1 substituent (R_7 side) because the steric interaction between the R_1 and R_8 substituent was able to occur. $\sum F(6,7,8)$ is the sum of the Swain-Lupton-Hansch inductive electronic parameter (F) of the 6-, 7-, and 8-substituents. Equations that were statistically almost equivalent or less significant were also formulated with either $\pi(7)$ for the hydrophobicity of only the R_7 substituent or log P for the whole molecule instead of the hydrophobic parameter $\sum\pi(6,7,8)$ in eq 1. Equation 1 indicates clearly that the steric factor of the R_1, R_6, and R_8 substituents are important for the activity of the quinolone antibacterials. It also suggests that compounds having an R_1 substituent with a length (L) of approximately 4.2 Å (e.g., ethyl, vinyl, methoxy, methylamino, fluoroethyl, cyclopropyl), an R_6 substituent with an E_s value of approximately -0.65 (e.g., fluoro, chloro, oxygen, nitrogen), an R_7 substituent with a π value of approximtely -1.4 [or a $\sum\pi(6,7,8)$ value of approximately -1.2 or a log P value of approximately -1.0] (e.g., piperazinyl, aminopiperidinyl, aminopyrrolidinyl as the R_7 substituent), and an R_8 substituent with a width (B_4) of approximately 1.8 Å (e.g., fluoro, chloro, bromo, methyl, methylene, oxygen)

(1) This work was presented at the 15th Symposium on Structure-Activity Relationships, Nov. 6-8, 1987, Tokyo: *Abstract of papers*; pp 338-341.
(2) (a) Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Irikura, T. *J. Med. Chem.* **1980**, *23*, 1358. (b) Koga, H. In *Structure-Activity Relationships—Quantitative Approaches; Applications to Drug Design and Mode-of-Action Studies*; Fujita, T., Ed.; Nankodo: Tokyo, 1982; pp 177-202. (c) Fujita, T. In *Drug Design: Fact or Fantasy*; Jolles, G., Wooldridge, K. R. H., Eds.; Academic Press: New York, 1984; p 19.
(3) Holmes, B.; Brogden, R. N.; Richards, D. M. *Drugs* **1985**, *30*, 482.
(4) Fernandes, P. B.; Chu, D. T. W. *Ann. Rep. Med. Chem.* **1988**, *23*, 133 and references therein.

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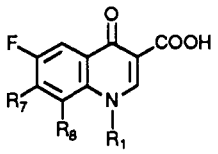
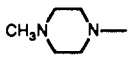
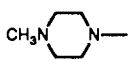
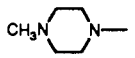
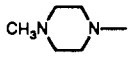
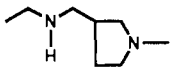
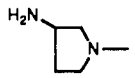
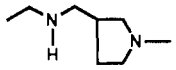
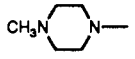
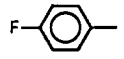
Table I. Fluoroquinolones



compd (name)	R_7	R_8	R_1
4 (AM-833)		F	FCH_2CH_2
5 (ofloxacin)		$-OCH_2CH(CH_3)-$	
6 (amifloxacin)		H	CH_3NH
7 (ciprofloxacin)		H	
8 (CI-934)		F	C_2H_5
9 (AM-1091)		Cl	
10 (PD117558)		F	
11 (difloxacin)		H	

could exhibit an activity comparable to or more potent than NFLX. These predictions have been proved by further syntheses and the biological evaluations of such compounds as, e.g., AM-833 (4), ofloxacin (5), amifloxacin (6), ciprofloxacin (7), CI-934 (8), AM-1091 (9), and PD117558 (10) (Table I).[4]

Recently, Chu et al.[5a] and Narita et al.[5b] reported the synthesis of 1-(*p*-fluorophenyl)fluoroquinolone 11, which possesses a potent activity comparable to that of NFLX [original value of log (1/MIC) (mol/L) of NFLX against *E. coli* NIHJ JC-2 is 6.629].[2] Equation 1 predicts a much lower activity for this compound (11) and the calculated value of log (1/MIC) (mol/L) against *E. coli* NIHJ JC-2 for compound 11 is 3.82. Simple aromatic groups as the N_1-substituent of the quinolones were already cited in the earlier report.[6] Equation 1 was derived from only simple alkyl groups, not including any aryl groups, with small variations in R_1. This appears to be the reason for the misprediction of the activity of N_1-arylquinolones like compound 11. If the structure and activity of these N_1-arylquinolones are included in the analysis, the steric effect of the N_1-substituent seems to be more complex than the case of simple alkyl groups where only the length factor of the N_1-substituent appeared in eq 1. In this report, we analyzed the conformation of the compounds, including the N_1-arylquinolones, by using a systematic search method, and reexamined the steric effect of the N_1-substituents of the quinolone antibacterials precisely. We attempted to rationalize the relationship between biological activity and the three-dimensional structure of the N_1-substituent and create a receptor map corresponding to the N_1-substituent of quinolones 3. We also tried to explain the relationship between eq 1 and a receptor mapping for the N_1-substituent.

Compounds and Biological Activity

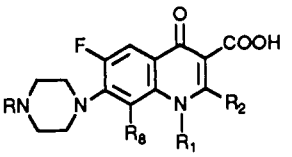
The compounds and biological activity analyzed are listed in Table II. The MIC against *E. coli* was chosen as the biological activity because it is one of the representative Gram-negative bacteria and, in the case of the quinolone antibacterials, the activity against *E. coli* roughly parallels the activity against other Gram-negative bacteria.[2,4] The relative activity of each compound compared to the activity of the standard drug, NFLX (1), was calculated because all of these biological assays were not always tested under the same conditions. The compounds were classified into five groups according to their relative activity as shown in Table II. In this data set, the class 1 compounds have the highest biological activity and the range of the relative activity of the class 1 compounds is from 4 to 0.5. The class 5 compound has the lowest activity and the relative activity of this class is less than or equal to $^1/_{2000}$. The range of the overall activity variation covers a range almost 8000-fold.

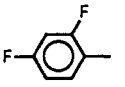
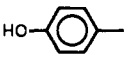
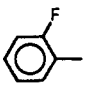
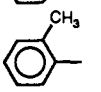
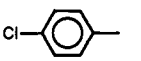
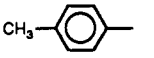
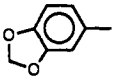
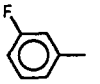
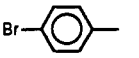
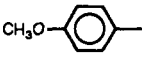
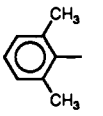
Molecular Modeling

A systematic search method was used for the analysis of conformational aspects of these compounds. The three-dimensional structure of the quinolone ring was constructed from the X-ray data of oxolinic acid (3: $R_1 = C_2H_5$, $R_6,R_7 = -OCH_2O-$, $R_8 = H$).[12] The structure of the substituents of each compound was taken from the fragment library of a SYBYL system.[13] Original models of each compound were built with standard bond lengths and angles. The energy of each compound was then minimized with molecular mechanics with a Tripos force field.[13] These structures were used as original coordinates for further energy optimization using quantum mechanics. The 7-unsubstituted 6-fluoroquinolones (3: $R_6 = F$, $R_7 = H$) were used as the model structure for the conformational analysis because the conformation of the N_1-substituents was not affected by the 7-substituents within compounds analyzed here, and the energy calculation was easier when the 7-position was unsubstituted. Conformations were preliminarily examined by the SEARCH routine in the SYBYL system. Conditions for preliminary search were defined so that all rotatable bonds within N_1-substituents were rotated by an increment of 5°. Conformations were then analyzed by using such molecular orbital (MO) methods as CNDO/2[14] and AM1.[15] The Gaussian 82 (STO-3G)[16]

(5) (a) Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. E., Jr.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558. (b) Narita, H.; Konishi, Y.; Nitta, J.; Nagaki, H.; Kobayashi, Y.; Watanabe, Y.; Minami, S.; Saikawa, I. *Yakugaku Zasshi* **1986**, *106*, 795.
(6) Albrecht, R. *Prog. Drug Res.* **1977**, *21*, 9.
(7) Atarashi, S.; Yokohama, S.; Yamazaki, K.; Sakano, K.; Imamura, M.; Hayakawa, I. *Chem. Pharm. Bull.* **1987**, *35*, 1896.
(8) Wentland, M. P.; Bailey, D. M.; Cornet, J. B.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. *J. Med. Chem.* **1984**, *27*, 1103.
(9) Wolfson, J. S.; Hooper, D. C. *Antimicrob. Agents Chemother.* **1985**, *28*, 581.
(10) Enomoto, H.; Kise, M.; Ozaki, M.; Kitano, M.; Morita, I. Japanese Patent Kokai 103393, 1983; *Chem. Abstr.* **1983**, *98*, 53877*w*.
(11) Matsumura, S.; Kise, M.; Ozaki, M.; Tada, S.; Kazuno, K.; Watanabe, H.; Kunimoto, K.; Tsuda, M. Japanese Patent Kokai 136588, 1982; *Chem. Abstr.* **1983**, *98*, 53877*w*.
(12) Cygler, M.; Huber, C. P. *Acta Crystallogr.* **1985**, *C41*, 1052.
(13) SYBYL Molecular Modeling System; Tripos Associates: St. Louis.
(14) Pople, J. A.; Segal, G. A. *J. Chem. Phys.* **1966**, *44*, 3289.
(15) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902.
(16) Brinkley, J. S.; Frish, M. J.; DeFrees, D. J.; Raghavachari, K.; Whiteside, R. A.; Schlegel, H. B.; Fluder, E. M.; Pople, J. A. *Gaussian 82*; Carnegie Mellon University, Pittsburgh, PA, 1983.

Table II. Relative Activity of Fluoroquinolones



no.	R	R_8	R_1	R_2	ref
			Class 1 ($4-{}^1/_2$)[a]		
1	H	H	C_2H_5	H	2
5 (*S*)	CH_3		$-OCH_2CH(CH_3)-$	H	7
6	CH_3	H	CH_3NH	H	8, 9
7	H	H		H	9
11	CH_3	H		H	5
12	H	H	FCH_2CH_2-	H	2
13	H	H	$CH_2=CH-$	H	2
14	CH_3	H		H	5
15	CH_3	H		H	5
16	H	H	$-CH=CH-S-$		10
17	H	H	$-C(CH_3)=CH-S-$		10
18	CH_3	H	$-CH_2CH_2S-$		11
			Class 2 ($^1/_4-{}^1/_{16}$)[a]		
19	H	H	$CH_3CH_2CH_2-$	H	2
20	H	H	$CH_2=CHCH_2-$	H	2
21	H	H	$HOCH_2CH_2-$	H	2
22	H	H	$C_6H_5CH_2-$	H	2
23	H	H	CH_3	H	2
5 (*R*)	CH_3			H	7
24	CH_3	H	C_6H_5	H	5
25	CH_3	H		H	5
26	CH_3	H		H	5
27	CH_3	H		H	5
28	CH_3	H		H	5
29	CH_3	H		H	5
			Class 3 ($^1/_{32}-{}^1/_{128}$)[a]		
30	H	H	$(CH_3)_2NCH_2CH_2-$	H	2
31	CH_3	H		H	5
32	CH_3	H		H	5
			Class 4 ($^1/_{250}-{}^1/_{1000}$)[a]		
33	CH_3	H		H	5
			Class 5 ($\leq {}^1/_{2000}$)[a]		
34	CH_3	H		H	5

[a] Relative activities compared to that of norfloxacin (1) are in parentheses.

program was also used for analyzing compounds having ethyl, cyclopropyl, and phenyl as the N_1-substituent. Three MO methods were used since we wanted to know the energy profiles for various conformations as far as possible to examine the differences between the MO procedures in finding the energy minimum. In the MO

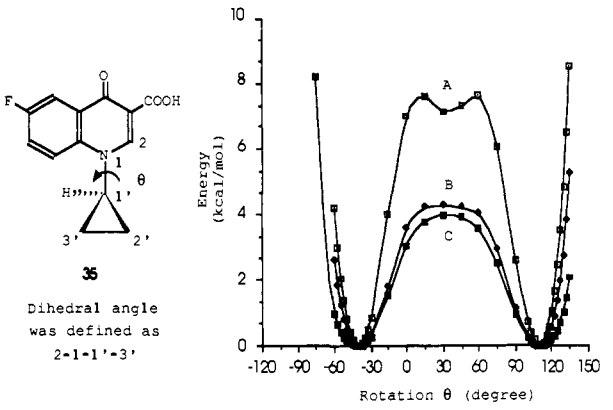


Figure 1. Rotational energy map of N_1–R_1 bond of the 1-cyclopropyl compound **35**: (A) energy curve calculated by Gaussian 82 (□), (B) energy curve calculated by AM1 (◆), (C) energy curve calculated by CNDO/2 (■).

analysis, all rotatable bonds in N_1-substituents were rotated by a 15° increment and then scanned with a 5° increment around the local minimum. After the determination of the minimum-energy conformation of each of the N_1-substituents, a piperazinyl or an *N*-methylpiperazinyl group was attached at position 7 of the model compounds and their conformation was optimized by using the AM1 program.

The "total" occupied volume of the N_1-substituents for each of the compound classes was calculated by superposing the van der Waals volumes of the active conformer of the N_1-substituents of each of the compound classes using the MVOLUME routine in the SYBYL system. The difference in the total volume between the different classes was calculated by subtracting the total volume for all of the more active compound class from that of the less active compound class.

Results and Discussion

The procedure in defining the "active" conformation essential for the activity is very important in discussing the steric effect of the substituents.

Conformation analysis was started from N_1-cyclopropyl compound **35**. Ciprofloxacin (**7**), the 7-piperazinyl derivative of compound **35**, has the highest activity among the N_1-substituted quinolones in this analysis (Table II) and the activity relative to NFLX (**1**) is equal to 4. The N_1–R_1 bond of the N_1-cyclopropyl derivative **35** was rotated from 0 ° to 360° and the energy of each conformer was calculated as described above (Figure 1). After this analysis two energy minima were found. There was no substantial differences between the results of the three MO methods as shown in Figure 1. One of the energy minima corresponds to the conformation where the cyclopropyl moiety is above the plane of the quinolone ring (dihedral angle 2–1–1′–3′ is equal to 110°), and the other to that below the plane (dihedral angle 2–1–1′–3′ = −40°). There was no energy difference between the two. With only this information, it was impossible to determine which is the active conformation. Therefore a conformation analysis of ofloxacin (**5**), which has a fairly rigid structure and a potent activity, was carried out to obtain more insight into the active conformation of the N_1-cyclopropyl. Ofloxacin (**5**) has two optical isomers and it has been reported that the activity of the *S* isomer [**5**(*S*)] is higher than that of the *R* isomer [**5**(*R*)].[7] The fact that the *S* isomer is more active than the *R* isomer is also reported for the structurally similar compound S-25930 (**3**: R_6 = F, R_7 = CH_3, R_8,R_1 = $-CH_2CH_2CH(CH_3)-$).[17] The conformation analysis of the model compound [**3**: R_6 = F, R_7 = H, R_8,R_1 = $-OCH_2CH(CH_3)-$] taken from compound **5** showed that the *S* isomer has two stable conformations without significant energy difference (the energy difference is 0.03 kcal/mol with the AM1 method). One of the stable conformers is that where the branched methyl moiety is perpendicular to the plane of the quinolone ring (dihedral angle 2–1–1′–2′ = 80°) and the other is that where it is fairly inclined to the plane of the ring (dihedral angle 2–1–1′–2′ = 32°). It seemed that, in the overall shape of the N_1-substituent of these two compounds, the latter conformer of **5**(***S***) resembles the conformer of the N_1-cyclopropyl compound **35** well if the N_1-cyclopropyl group locates above the plane of the quinolone ring. Consequently, the matched conformers of compounds **5**(***S***) and **7** were selected as active conformers for the N_1-substituents (Figure 2). This selected active conformer of **5**(***S***) was also the lowest energy conformer.

Next, the active conformer of the model compound (**3**: R_6 = F, R_7 = R_8 = H, R_1 = CH_2CH_3) taken from norfloxacin **1**, was examined. There were three energy minima which correspond to the states in which the N_1-ethyl locates above (dihedral angle 2–1–1′–2′ is equal to 100°), below (2–1–1′–2′ = −100°), and parallel (2–1–1′–2′ = 0°) to the quinolone ring. The energy difference between the "above" conformer (2–1–1′–2′ = 100°) and the "parallel" conformer (2–1–1′–2′ = 0°) is 0.47 kcal/mol with the AM1 method, and there was no energy difference between the "above" and "below" conformers. The conformation in which the N_1-ethyl was above the quinolone ring was selected as the active conformer because, among three conformers, it resembled the active conformation of the N_1-substituents of **5**(***S***) and **7** well. This selected active conformer of NFLX (**1**) was also the lowest energy conformer.

In the case of the N_1-phenyl derivative (**3**: R_1 = C_6H_5, R_6 = F, R_7 = R_8 = H), the conformational search showed that there were two energy minima which correspond to the angle between the quinolone and the phenyl ring are 70° and 110° and there was no difference in energy between the two conformers. The latter conformer was selected as the active conformer of compound **24** by a comparison of the shapes of the N_1-substituent of the two conformers with the active conformations of compounds **1**, **5**(***S***), and **7** (Figures 2 and 6). The active conformation of the other compounds was defined in a similar way so that, among the low-energy conformers, the conformer which resembles the active conformer of the N_1-substituents of highly active compounds (**1**, **5**(***S***), and **7**) was selected (Figures 2–4 and 6–11). The torsion angles of the active conformer of each of the N_1-substituents are shown in Table III.

For the N_1-benzyl derivative (**22**), the active conformation was selected as shown in Figure 6. There were a number of low-energy conformers for the N_1-benzyl substituent. Among these low-energy conformers, there was a conformer which occupies a region close to the meta position of the N_1-phenyl in compound **24**. As described later, the region close to the meta position of the N_1-phenyl in compound **24** resulted in a detrimental effect on the activity (Figure 6). Since the N_1-benzyl compound (**22**) is a fairly active class 2 compound, the active conformation of the N_1-benzyl was selected so that this substituent did not occupy the detrimental region. This selected con-

(17) Gerster, J. I.; Rolfing, S. R.; Pecore, R. M.; Winandy, R. M.; Stern, R. M.; Landmesser, J. E.; Olsen, R. A.; Gleason, W. B. *J. Med. Chem.* **1987**, *30*, 839.

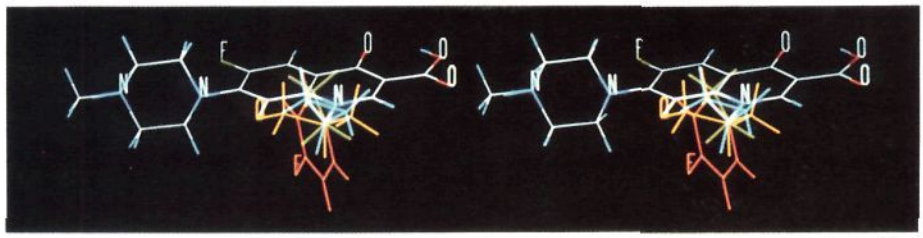


Figure 2. Stereoview of the superposition of the proposed active conformers of **1** (green), **5(*S*)** (yellow), **7** (blue), and **11** (orange).

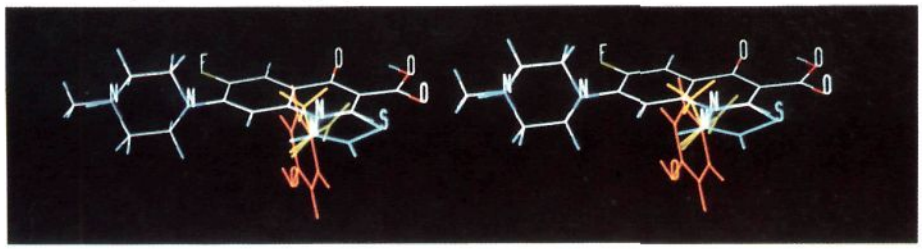


Figure 3. Stereoview of the superposition of the proposed active conformers of **6** (yellow), **13** (green), **15** (orange), and **16** (cyan).

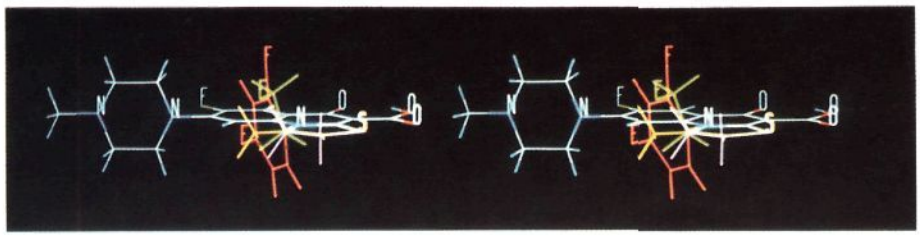


Figure 4. Stereoview of the superposition of the proposed active conformers of **12** (green), **14** (red), **17** (yellow), and **18** (violet).

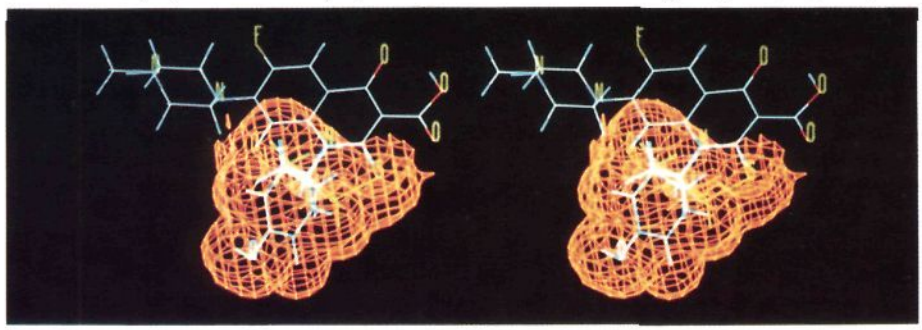


Figure 5. Stereoview of the total volume (orange) of the N_1-substituents of class 1 compounds.

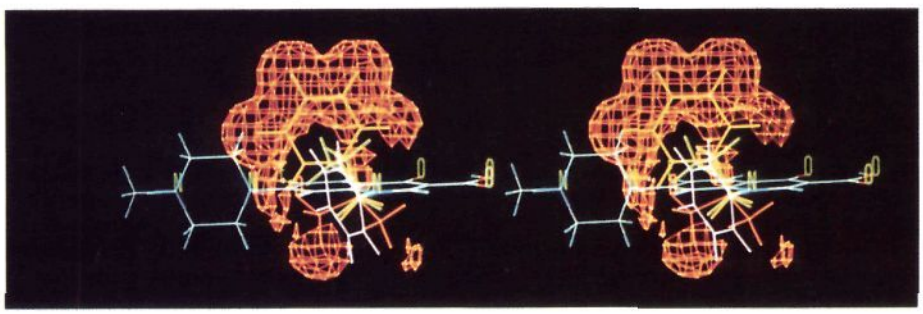


Figure 6. Stereoview of the superposition of the proposed active conformers of **19** (green), **22** (yellow), **5(*R*)** (orange), and **24** (violet) and the difference (orange) in the total volume between the set of **19**, **22**, **5(*R*)**, and **24** and class 1 compounds.

former of compound **22** was also the lowest energy conformer.

For the N_1-propyl, -allyl, -(hydroxyethyl), and -[(dimethylamino)ethyl] substituents in compounds **19–21** and **30**, there were two plausible, low-energy conformers: one is the extended form and the other the bent form. The former was selected as the active conformation in this analysis because the most significant factor for the effect of N_1-substituents was represented by the quadratic function of the length L in eq 1, and this suggested that these substituents interact with the receptor in their extended form (Figures 6–9). These selected conformers of compound **19–21** and **30** were also the lowest energy conformers.

The active conformers of the class 1 compounds were superposed by matching the corresponding atoms in the quinolone ring of each molecule. The total occupied volume for the N_1-substituents of superposed "active"

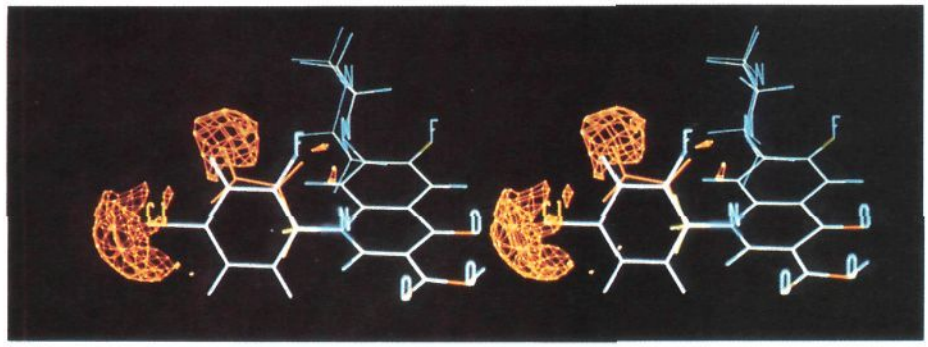


Figure 7. Stereoview of the superposition of the proposed active conformers of **20** (orange), **23** (green), **25** (blue), and **27** (yellow) and the difference (orange) in the total volume between the set of **20**, **23**, **25**, and **27** and class 1 compounds. Since phenyl rings of the N_1-substituents of compound **25** and **27** overlap, it looks white. N_1-Methyl of compound **23** and N_1-allyl of compound **20** also overlap and the *N*-methyl looks white or yellow-green.

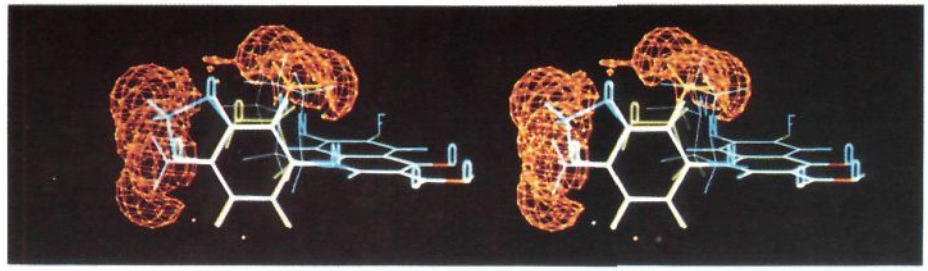


Figure 8. Stereoview of the superposition of the proposed active conformers of **21** (green), **26** (yellow), **28** (violet), and **29** (blue) and the difference (orange) in the total volume between the set of **21**, **26**, **28**, and **29** and class 1 compounds.

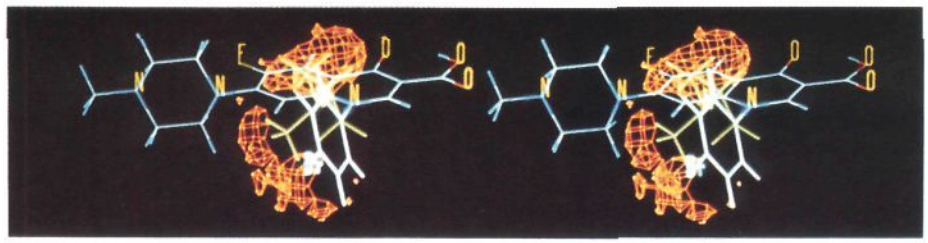


Figure 9. Stereoview of the superposition of the proposed active conformers of **30** (green), **31** (yellow), and **32** (cyan) and the difference (orange) in the total volume between the union of compound **30**, **31**, and **32** and the union of class 1 and class 2 compounds.

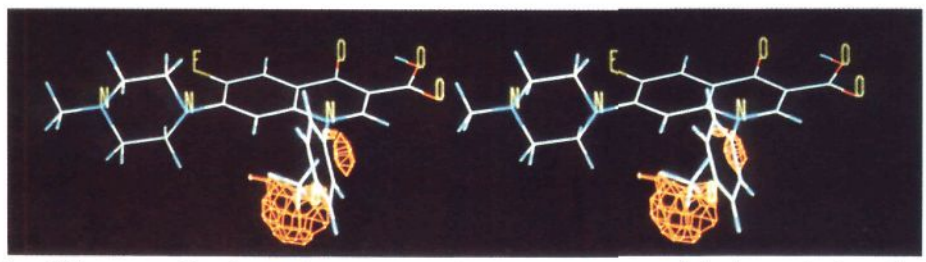


Figure 10. Stereoview of the proposed active conformer of **33** and the difference (orange) in the total volume between compound **33** and the union of class 1, 2, and 3 compounds.

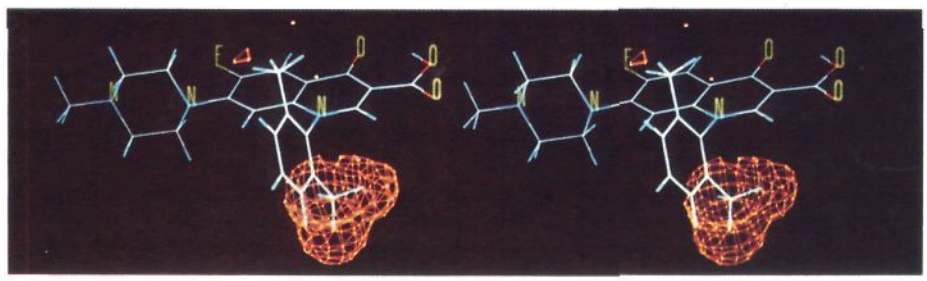


Figure 11. Stereoview of the proposed active conformer of **34** and the difference volume (orange) between compound **34** and the total of class 1, 2, 3, and 4 compounds.

molecules was then calculated to estimate the receptor model (active volume) of quinolone antibacterials (Figure 5). This model represents an estimated optimum volume for the N_1-substituent of quinolone antibacterials when it interacts with its receptor and can be used for verification of the biological activity of quinolone antibacterials and, further, for prediction of the activity of compounds not yet synthesized. If a compound fits well with this receptor model, it is expected to have high activity. This receptor model should be corrected whenever new information about the quinolone receptor or novel N_1-substituents and their biological activity is gained.

In the next step, the difference in occupied volume of the N_1-substituents between class 1 and the less active class 2 compounds was calculated by subtracting the total occupied volume of class 1 from that of class 2 (Figures 6–8). The total occupied volume of the class 2 compounds was calculated by procedures similar to the those for the class 1 compounds. The increase in occupied volume compared to the class 1 receptor model was thought to make the activity of the class 2 compounds lower. The region representing the difference between two occupied volumes seems to be the region where a steric repulsion occurs between the receptor wall and the N_1-substituent of the class 2 compounds. Figures 6–8 show that the *o*-methyl (**26**), *m*-(methyleneoxy) (**29**), *p*-methyl (**28**), and *p*-chloro (**27**) substituents of the N_1-phenyl and N_1-benzyl (**22**) compounds occupy extra regions not occupied by the class 1 compounds.

On the contrary, it seemed that the *p*-hydrogen of the N_1-phenyl (**24–26**) is somewhat too small to fit the important region, the *p*-hydroxy or *p*-fluoro region of the N_1-phenyl group, of the receptor model. The *p*-hydroxy or *p*-fluoro region of the N_1-phenyl group is considered to be important for the activity because *p*-hydroxyphenyl (**15**) and *p*-fluorophenyl (**11**) compounds have the highest activity (class 1) among the substituted N_1-phenyl derivatives, and the compounds which do not have an optimum volume around this region (e.g. compounds **27** and **28** are too large and compounds **24–26** are too small) have lower activity.

It seems that the N_1-methyl (**23**) is also somewhat too small to fit the other important region, which corresponds to the N_1-cyclopropyl region of **7**, of the receptor model because compound **7**, having N_1-cyclopropyl, has the highest activity among the compounds analyzed here (as described above) and this region seems to correspond to the optimum value (4.2 Å) of $L(1)$ in eq 1.

The terminal groups of the N_1-propyl (**19**), -allyl (**20**), and -(hydroxyethyl) (**21**) substituents occupy regions corresponding to the meta positions of the N_1-phenyl group as shown in Figures 6–8. These regions seem to reduce the activity of compounds **19–21**. Thus, the meta groups on the N_1-phenyl substituents could make the activity deteriorate. The methylene and branched methyl in the R_8,R_1 cyclic moiety of **5(*R*)** are fixed below the plane of a quinolone ring as shown in Figure 6. This region below the quinolone ring seems to interfere with the binding of compound to the receptor and seems to cause a reduction of activity.

The difference of the total occupied volume of the N_1-substituents of the class 3 compounds from that of both the class 1 and 2 compounds is shown in Figure 9. This volume was calculated by subtracting the total occupied volume of both class 1 and class 2 from that of class 3. A still extra region is indicated to be occupied by the two *N*-methyls of the N_1-[(dimethylamino)ethyl] (**30**) and the bromine at the para position of the N_1-phenyl substituent (**32**). These regions were thought to cause a further reduction of activity for compounds **30** and **32** compared to those of class 1 and 2 compounds. For the N_1-(*m*-fluorophenyl) derivative (**31**), the volume of the hydrogen at the para position is too small to fit the receptor wall as described in the case of compounds **24–26**. The region occupied by the *m*-fluorine brings a reduction of activity as described in the case of compounds **19–21**. These two factors seem to work synergistically and lower the activity of compound **31** to class 3.

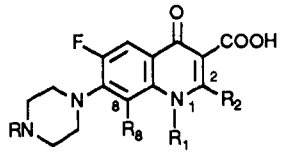
Figure 10 shows the difference in the total occupied volume between the class 4 and class 1–3 compounds. This volume was calculated by subtracting the total occupied volume of all of the class 1–3 compounds from that of the class 4 compound. The new region occupied by the *p*-methoxy group of the N_1-phenyl (**33**) would probably be unfavorable to the relevant binding with the receptor more significantly than N_1-substituents of class 1–3 compounds are.

Finally, the difference in the total volume between the least active class 5 compound, N_1-(2,6-dimethylphenyl) derivative **34**, and the class 1–4 compounds was examined (Figure 11). As you can see from Figure 11, the region occupied by one of the *o*-methyl groups below the quinolone ring was thought to exert a fatal effect upon the activity.

These analyses bring new and important insights into three-dimensional structure–activity relationships for N_1-substituents of quinolone antibacterials. We propose that there are two optimum regions for increasing the activity. One of the optimum regions corresponds to the region occupied by the N_1-cyclopropyl group above the plane of the quinolone ring. The other corresponds to the region occupied by fluorine and hydroxyl at the para position of the N_1-phenyl.

We also propose that there are two regions unfavorable to proper receptor binding and the occupancy of these regions leads to reduction in the activity. One of the unfavorable regions corresponds to the region below the plane of the quinolone ring and the other corresponds to the meta position of the N_1-phenyl group above the quinolone ring plane. The reasons the region corresponding to the meta position of the N_1-phenyl group above the quinolone ring plane causes a bad effect on the activity are as follows. For the N_1-alkyl series, for example, N_1-ethyl compound NFLX (**1**) is a class 1 compound and N_1-propyl (**19**), -allyl (**20**), and -(hydroxyethyl) (**21**), which occupy the position corresponding to the meta position of the N_1-phenyl substituent, are class 2 compounds. For the N_1-phenyl series, N_1-phenyl derivative **24** is class 2 and N_1-(*m*-fluorophenyl) derivative **31**, which also has a fluorine at the meta position, is a class 3 compound. This reduction of activity arises from the occupancy of the unfavorable region. In these two different series of the N_1-substituent, the reduction of the activity occurred by occupancy of the same region. We conclude the region below the plane of the quinolone ring reduces the activity because, for example, for the N_1-phenyl series, N_1-(2-methylphenyl) derivative **26** belongs to class 2 and the N_1-(2,6-dimethylphenyl) derivative **34**, of which the one of the methyl group occupies the region under the quinolone ring (Figure 11), has almost no activity. Additionally, compound **5(*R*)** is a class 2 compound and occupies an extra region, which is not occupied by class 1 compounds, below the quinolone ring (Figure 6). A modified receptor model based on these insights is shown in Figure 12. This model is created with the total volume occupied by the N_1-cyclopropyl group and the hydroxy group of the N_1-(*p*-hydroxyphenyl, which

Table III. Calculated Dihedgral Angles of Fluoroquinolones



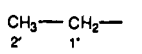
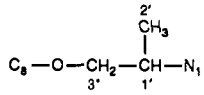
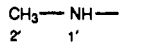
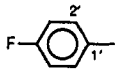
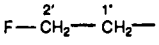
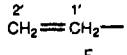
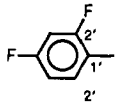
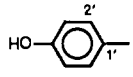
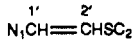
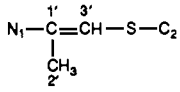
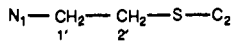
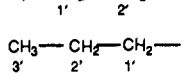
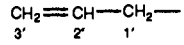
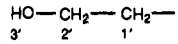
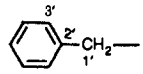
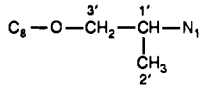
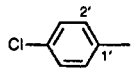
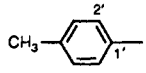
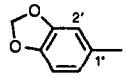
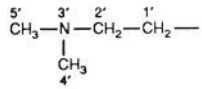
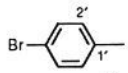
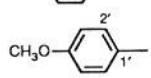
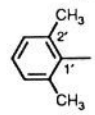
no.	dihedral angle, deg	definition of dihedral angle	R_1
1	100	2-1-1′-2′	
5 (*S*)	155 32	2-1-1′-3′ 2-1-1′-2′	
6	100	2-1-1′-2′	
7	110 40	2-1-1′-3′ 2-1-1′-2′	
11	110	2-1-1′-2′	
12	100	2-1-1′-2′	
13	46	2-1-1′-2′	
14	109	2-1-1′-2′	
15	110	2-1-1′-2′	
16	0	2-1-1′-2′	
17	0 180	2-1-1′-3′ 2-1-1′-2′	
18	0	2-1-1′-2′	
19	100 180[b]	2-1-1′-2′ 1-1′-2′-3′	
20	100 180[b]	2-1-1′-2′ 1-1′-2′-3′	
21	100 180[b]	2-1-1′-2′ 1-1′-2′-3′	
22	110 −61[b]	2-1-1′-2′ 1-1′-2′-3′	
23			
5 (*R*)	−157 −35	2-1-1′-3′ 2-1-1′-2′	
24	110	2-1-1′-2′	
25	109	2-1-1′-2′	
26	107	2-1-1′-2′	
27	110	2-1-1′-2′	
28	110	2-1-1′-2′	
29	110	2-1-1′-2′	

Table III (Continued)

no.	dihedral angle, deg	definition of dihedral angle	R_1
30	100 180[b] 180[b] −60[b]	2−1−1′−2′ 1−1′−2′−3′ 1′−2′−3′−4′ 1′−2′−3′−5′	
31	110	2−1−1′−2′	
32	110	2−1−1′−2′	
33	110	2−1−1′−2′	
34	107	2−1−1′−2′	

[a] Dihedral angle of this compound is defined in Figure 1. [b] Cis = 0.

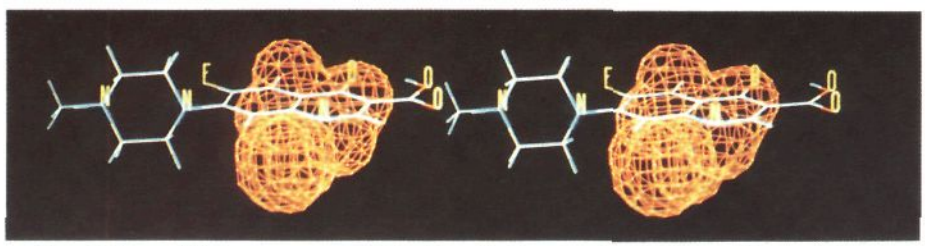


Figure 12. Stereoview of the modified receptor model for N_1-substituents of quinolone antibacterials.

correspond to two optimum regions as described above.

From the present receptor model, it is possible to rationalize why L (length) worked best as the steric parameter for N_1-substituents in the QSAR eq 1 and why eq 1 was unable to predict the activity of N_1-phenyl derivatives. The $L(1)$ parameter in eq 1 has an optimum at 4.2 Å, corresponding to the length of cyclopropyl, ethyl, etc. Equation 1 shows that the activity of the compounds without N_1-phenyl substituents decreases as the L value changes toward either side of the optimum value. The situation does, in fact, explain the activity variations in these compounds in terms of the one-dimensional projection of the receptor model onto the L axis, and the optimum value of L corresponds to the important N_1-cyclopropyl region of the receptor model. For example, methyl was too small to fit the receptor wall corresponding to N_1-cyclopropyl, but the n-propyl, allyl, hydroxyethyl, and benzyl extrude to the region unfavorable to proper receptor binding, which corresponds to the meta position of the N_1-phenyl. The terminal methyls of (dimethylamino)ethyl penetrate further into the unfavorable region. The receptor model in Figure 12 has two optimum volumes for increasing the activity as described above. The unfavorable region corresponding to the meta position of the N_1-phenyl exists above the plane of the quinolone ring. The N_1-substituents of the compounds which were used to derive eq 1 could be accommodated to various extents only in the optimum volume corresponding to N_1-cyclopropyl, and they could not reach the other optimum region corresponding to the para position of the N_1-phenyl. But N_1-phenyl derivatives can reach the other optimum region, the para position of N_1-phenyl, and no information of this important region was included in eq 1. These are the reasons eq 1 could not predict the activity of N_1-phenyl derivatives. These things indicate, if one uses the steric parameter in the QSAR equation, that one must be careful with regard to the relation between a steric parameter and an active conformation.

Recently, Domagala et al.[18] reported a QSAR analysis for N_1-substituents of 1-substituted 7-[3-[(ethylamino)-methyl]-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids and that the electronic parameter (unsaturation parameter) was significant at a ≥90% level along with the STERIMOL parameters, different from our conclusions. However, the validity of the unsaturation factor remains to be elucidated. We are currently examining the three-dimensional QSAR for quinolone antibacterials based on our analysis, and the details will be discussed elsewhere.

Experimental Section

The molecular models were built with the program SYBYL and a SYBYL standard fragment library.[13] Original coordinates of the models were taken from the crystallographic data of oxolinic acid.[12] *The calculations of molecular mechanics, CNDO/2*[14] *and AM1,*[15] *were* made on a VAX. The Gaussian 82[16] data were calculated on a Cray XMP/216. The conformations were examined on an Evans & Sutherland PS330 computer terminal using the program SYBYL. The figures were photographed directly from the screen with Kodak Ektachrome 100 HC film.

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(18) Domagala, J. M.; Heifetz, C. L.; Hutt, M. P.; Mich, T. F.; Nichols, J. B.; Solomon, M.; Worth, D. F. *J. Med. Chem.* **1988**, *31*, 991.