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Title: A Quantitative Structure Activity Relationships Study of Antiinfectives Based on the Nalidixic Acid Structure

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Nalidixic acid and its derivatives act through inhibition of bacterial DNA gyrase activity. Recently there have been a series of papers reporting the antibacterial activity against three different types of bacteria (S. aureus, E. coli and P. aeruginosa) by a series of 1,4-dihydro-4-oxoquinoline (or 1,8-naphthyridine) 3-carboxylic acids. Consistent biological data is available for a quantitative structure activity relationships (QSAR) study on three sets of compounds totaling over 120 potential antibacterial agents.

The two most frequently used models in quantitative structure activity relationship are the linear free energy relationships (LFER) regression model developed by Hansch and the additive substituent or de novo model developed by Free and Wilson. The object of this research is to apply the Hansch and Free-Wilson statistical models to a series of antibacterial analogues of pyridone carboxylic acids.

The Hansch model mostly uses physicochemical parameters as the independent variables to predict and explain the biological activity. In this project the partition coefficients, Log P calculated by the

fragment (f) method, molar refractivity (MR), and STERIMOL (L, B1 and B5) parameters were used in a LFER analysis.

The Free-Wilson model measures the contribution of a specific substituent to the biological activity. Both the standard de novo model and a mixed model using physicochemical parameters and Free-Wilson's indicator variables as independent variables were examined in this research.

There are three locations on the molecule in which substituents are varied. These are position 1, 6 and 7. Both the LFER and de novo models show position 7 to have the most variance. In other words, position 1 tends to be insensitive to changes in substituents. Position 6 requires a fluorine to maximize activity. Subsets of compounds in which the substituent were varied only at position 7 were examined. In general only specific substituents contribute significantly to antibacterial activity.

A Quantitative Structure Activity Relationships Study of Antiinfectives Based on the Nalidixic Acid Structure

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INTRODUCTION

I. Purpose

The purpose of this research project was to reevaluate an existing quantitative structure activity relationship (QSAR) model on a series of nalidixic acid analogues using newer physicochemical parameters. Potential QSAR relationships were then evaluated for a data set of newer nalidixic acid based structure.

II. Background

A. General

Since the introduction in 1963 of nalidixic acid (Fig. 1) (1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid) as a systemic Gram-negative antibacterial agent, a large number of analogues have been synthesized and evaluated some of which have come onto the international market. (1,2) A comprehensive review has outlined the synthetic methods, microbiology and structure activity relationships of those derivatives reported prior to 1977. (2)

After two decades the activity of the new 6-fluoroquinolones have far surpassed that of nalidixic acid. The most significant changes made in the quinolone nucleus, addition of 6-fluorine and 7-piperazine, have provided the fluoroquinolones with activity against Gram-negative bacteria comparable to that of the major classes of antibiotics. (3,4,7)

An evolution of structural modifications of nalidixic acid has resulted in increased potency/spectrum such that the newest agents

have excellent activity against Gram-negative bacteria (including P. aeruginosa), increased activity against Gram-positive bacteria, and in some instances, better activity against anaerobes. (5,7) This increased potency coupled with better biodistribution properties, has broadened the therapeutic potential of quinolones for parenteral and oral treatment of systemic infections other than urinary tract infections. (5) Relative to the first commercially introduced fluoroquinolone, norfloxacin (Fig. 1), subsequent analogues have shown greater oral absorption (pefloxacin, enoxacin) (Fig. 1), increase serum half-life (pefloxacin), overall increased in vitro potency (ciprofloxacin; Fig. 1) and an increase spectrum to include Gram-positive cocci (CI-934; Fig. 3) and anaerobic bacteria (difloxacin; Fig. 5). (6,7)

The structure of a 4-quinolone nucleus and a carboxylate substituent at position 3 are common to the six new and the two established compounds (nalidixic acid and oxolinic acid; Fig. 1). Substituents on the 1-nitrogen of the quinolone and the para position of the piperazine group vary from agent to agent. Both nalidixic acid and enoxacin are nitrogen substituted at position 8 of the quinolone, making them 1,8-naphthyridines. Oxolinic acid is further distinguished by a 6-7 methylenedioxy substituent, and ofloxacin (Fig. 1) is distinguished by a ring linkage of the 1-nitrogen and the 8 carbon of the quinolone nucleus. (4)

B. Mechanism of Action

Nalidixic acid and its analogues act by inhibition of bacterial

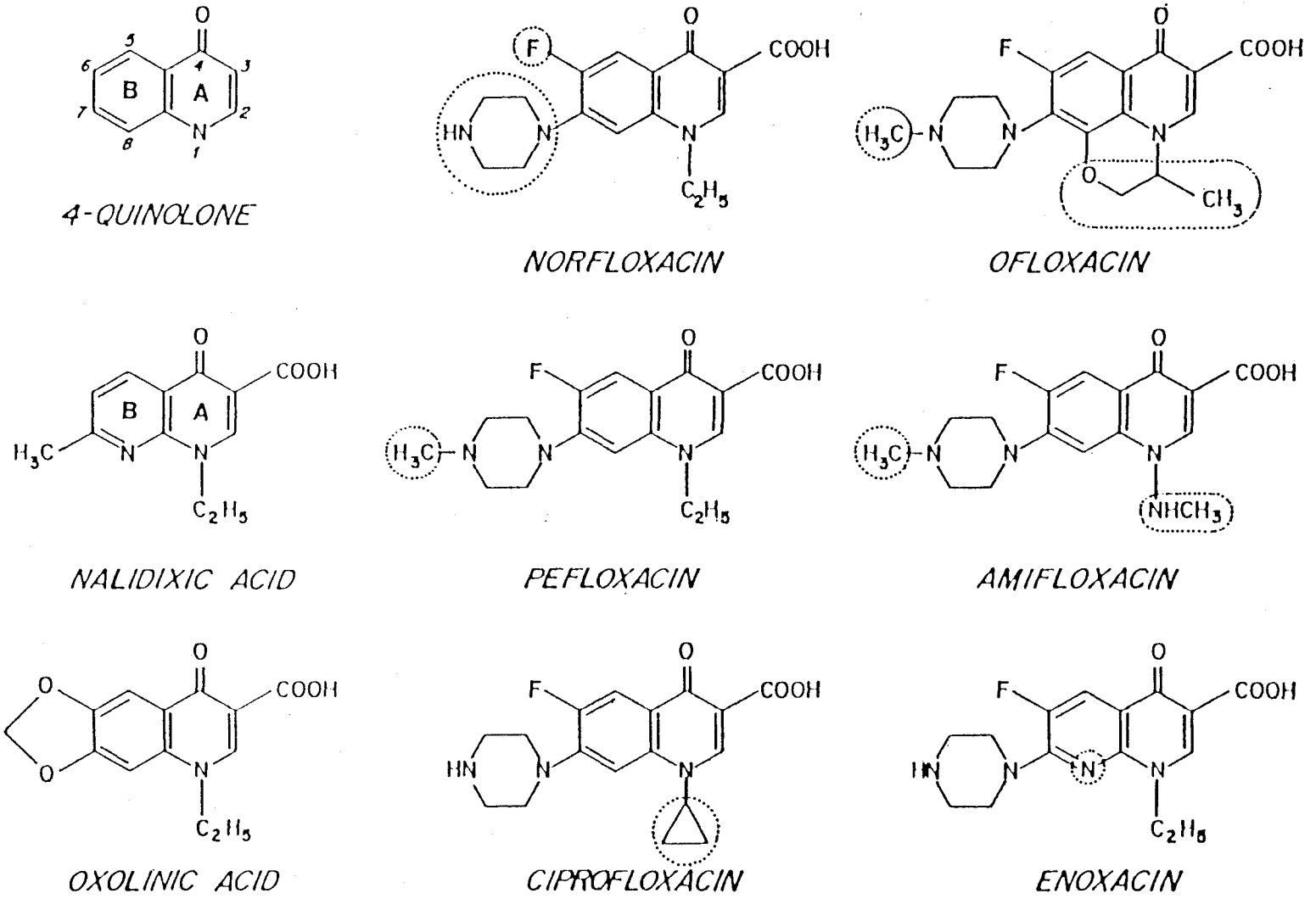


Figure 1. Structure of 4-Quinolone, Nalidixic Acid, Oxolinic Acid, and Six Fluoroquinolones (ref. 4)

DNA synthesis. (8) The biochemical target of quinolones is the bacterial DNA gyrase, a type II topoisomerase. (6) This bacterial enzyme maintains the topology of bacterial DNA through its unique supercoiling and relaxing activities. In an energy requiring process, bacterial DNA gyrase introduces negative supercoiling into circular duplex DNA. Negative supercoiling relieves the torsional stress of unwinding helical DNA that is essential for replication and transcription. (9)

DNA gyrase has been studied and found to consist of A and B subunits. Quinolones bind to the A subunit while the antibiotic novobiocin interacts with the B subunit. (10) There has been a suggestion that norfloxacin and other quinolones bind to purified DNA rather than to purified DNA gyrase. (4,10)

C. Resistance

Spontaneous mutations resulting in high level resistance to nalidixic acid produce cross resistance to the fluoroquinolones. (4) Purified enzyme that contains A subunits isolated from such a mutant is manyfold more resistant to inhibition by nalidixic acid and oxolinic acid. (4) Quinolone resistance can also occur from reduced cellular permeability. Mutants of this type also can show cross-resistance to beta-lactam antibiotics. (11) So far, resistance to the fluoroquinolone appears to be plasmid independent. (5)

D. Toxicology

Quinolone antibacterials generally are well tolerated. (7) The

most prominent toxic effect observed is erosion of cartilage in joints of immature animals. Clinical side effects can include dizziness, hemolytic anemia, visual disturbance, photosensitivity, and intracranial hypertension. (5)

E. Structure Activity Relationships (SAR)

In 1977 R. Albrecht indicated that a characteristic of nalidixic acid is the combination of the 1-ethyl-1,4-dihydro-4-oxo-3-pyridine carboxylic acid moiety A with a substituted pyridine ring B (Fig. 1). The methyl-substituted pyridine nucleus B can be replaced by other aromatic or heteroaromatic rings. (2)

This class of compounds all possess a 4-pyridone-3-carboxylic acid moiety as a common structure (Fig. 2, I). Analog of I ($R' = C_2H_5$, $R_2 = R_3 = -(CH_2)_n-$), which have an alicyclic system instead of the aromatic system B, are inactive. These findings point to the fact that the structural component A probably is responsible for the intrinsic effect. However, combination with a second aromatic or heteroaromatic ring is necessary. (2)

More closely related structural analogues of nalidixic acid containing the quinoline ring system can be represented by the general structure II (Fig. 2). The unsubstituted compound II ($R' = C_2H_5$, $R_5 = R_6 = R_7 = R_8 = H$), only has very slight activity. Substitution in the benzene nucleus is of decisive importance for the in vitro activity of quinolone carboxylic acids. (2)

The presence of substituents in the 1-position (N-substituent) is important. N-unsubstituted compounds ($R_1 = H$) whose quinolone

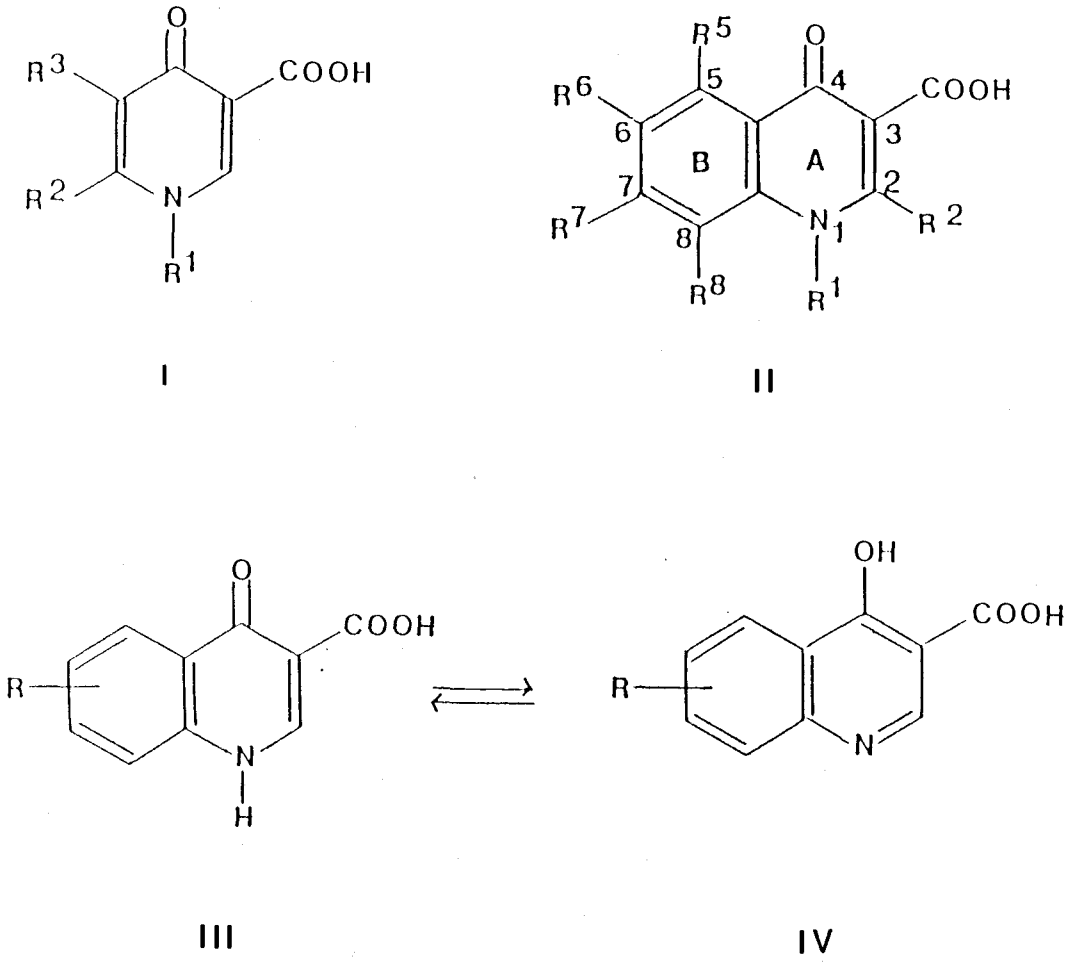


Figure 2. Structures of I, II, III and IV

structure is not fixed can form the tautomeric phenol and only exhibit a very weak antibactrial effect or no effect at all (Fig. 2, III-IV). In general the N-ethyl substituted quinolone carboxylic acids show the best activity. (2)

A series of N-alkoxy compounds has been synthesized. These compounds had anti-Gram negative activity comparable to that of the corresponding N-ethyl derivatives. (12) Compounds with N-vinyl were found to have the *in vitro* activity equivalent to that of the N-ethyl derivatives. (2)

Hogberg *et al*. investigated the N-1 atom itself as a possible contributor to the molecular mode of action. Their results indicated that the N-1 atom plays a significant role in enzymic and bacteriological inhibition. (13) Placing a substituent at C2 abolishes antibacterial activity. (14)

Santille *et al*. reported the synthesis and antibacterial screening results of several 1,2,3,4-tetrahydro-4-oxo-1,8 napthyridine-3-carboxylic acid esters (Z = CO_2R, R = alkyl), carbonitriles (Z = CN) and carboxamides (Z = CONH) in which the 2,3 double bond is fully saturated (Fig. 3, V-VI). Only two derivatives, the ethyl (FIg. 3, V-VI(a)) and butyl (Fig. 3, V-VI(b)) esters of 1-ethyl-1,2-dihydro-4-hydroxy-7-methyl-1,8-naphthyridines 3-carboxylic acid, protected the animals against *E. coli* and several Gram-negative pathogens. Since neither of those two compounds shows *in vitro* antibacterial activity but gives good protection *in vivo*, some type of biotransformation of these substance must take place. Whether or

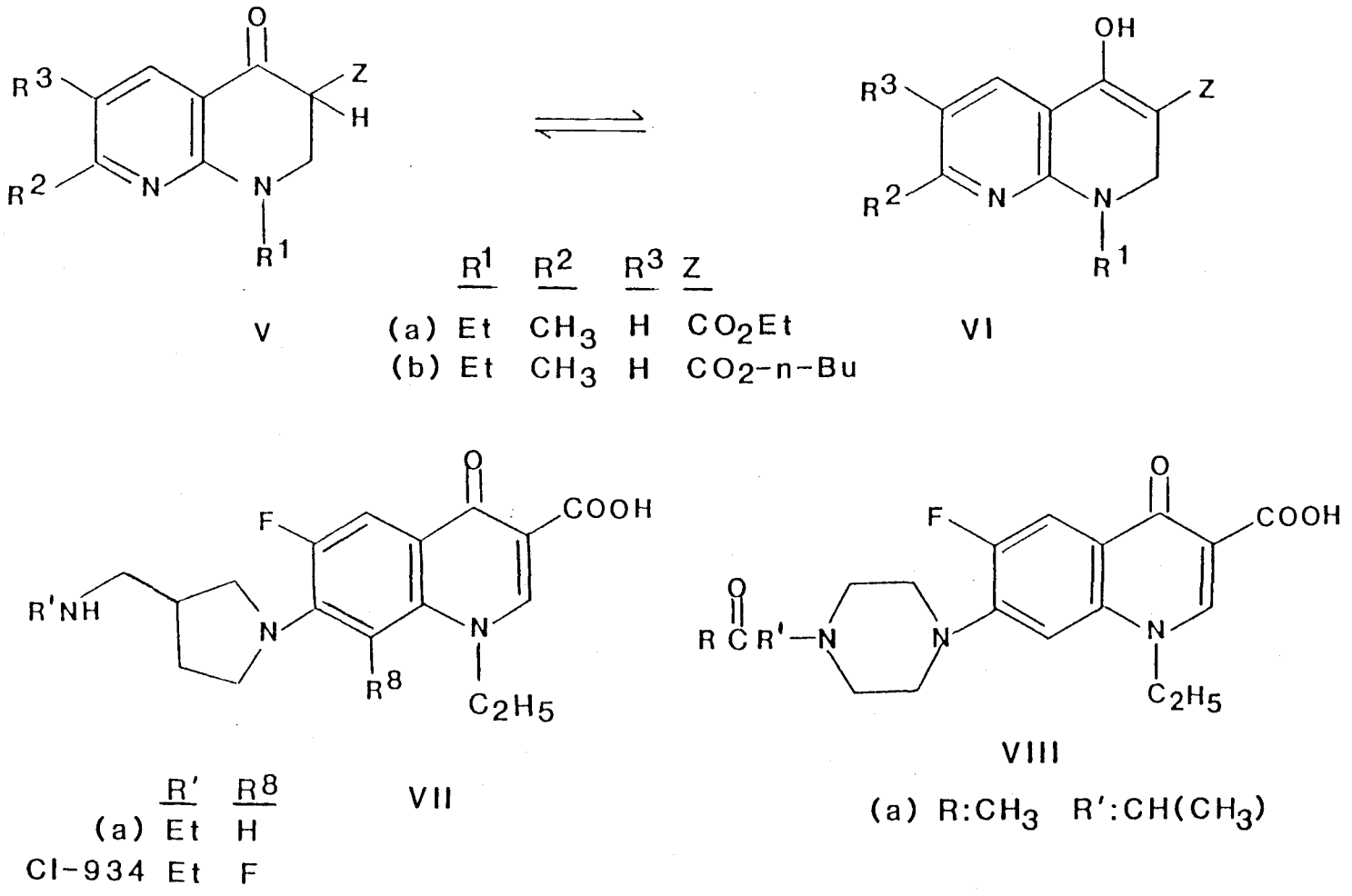


Figure 3. Structures of V, VI, VII and VIII

not this apparent pro-drug type of action produces a nalidixic acid type derivative or some other active moiety is not clear. (15)

Several N-(oxoalkyl)norfloxacin derivatives (Fig. 3, VIII) were synthesized and evaluated for antibacterial activity *in vitro* and *in vivo*. (16) Most of the compounds exhibited *in vitro* activity comparable to that of norfloxacin for Gram-positive bacteria, whereas their activity was lower than for Gram-negative bacteria.

N-(2-oxopropyl)norfloxacin (Fig. 3, VIII(a)) liberated norfloxacin in the blood after oral administration in mice, and the serum level of norfloxacin was about 3-fold higher than that of norfloxacin itself. N-(2-oxopropyl)norfloxacin showed high antibacterial activity *in vivo*. The increased activity of N-(2-oxopropyl)norfloxacin may be explained by the facts that it is absorbed better, gives an active metabolite, and is active by itself. Generally, it is suggested that both an increase of oral absorbability by N-masking norfloxacin and a production of some active species by metabolism make an important contribution to enhancing the *in vivo* activity. (16)

Removal or replacement of the carboxylic acid in position 3 shows a loss of activity. (2) The carboxyl replacement by methylsulfinyl and methylsulfonyl groups, (13) or sulfonamides and phosphoric acids, lead to inactive products. (17) The ester and amide derivatives of the carboxylic acid are active to the extent that they hydrolyze *in vivo* to the free carboxylic acid. (18) Replacement of the 4-oxo group of the quinolone carboxylic acid by a

sulfonyl group causes a loss of antibacterial properties. (2) Substitution at C5 generally results in inactivity with the amino function being a unique exception. (14)

Addition of a third ring system onto the quinolone system has been found to produce excellent activity. (2) One example, oxolinic acid (Fig. 1), a 6-7 dioxoquinololine derivative was the first nalidixic acid analog to demonstrate significantly better potency with a broadened spectrum.

Mitscher et al. synthesized methylenedioxy positional isomers in the 5,6 and 7,8 positions of quinoline system and compared the antimicrobial activity of these compounds with that of oxolinic acid. The study showed that the methylenedioxy aromatic substituent must reside at C6, C7 in the quinolone nucleus (i.e. oxolinic acid) for optimal antibacterial activity. (19)

Albrecht also noted the monosubstituent effect of 1-ethyl-1,4-dihydro-4-oxo-3-quinoline carboxylic acids in positions 6,7 and position 8. The 6-methyl or 6-methoxy substituent of quinolone carboxylic acids give the same minimum inhibitory concentration against E. Coli. The 7-methyl or 7-methoxy groups increase activity significantly. Compounds containing a 7-piperazinyl or a 7-methyl piperazinyl group exhibit vary good antibacterial activity. An 8-methoxy group reduces activity. (2)

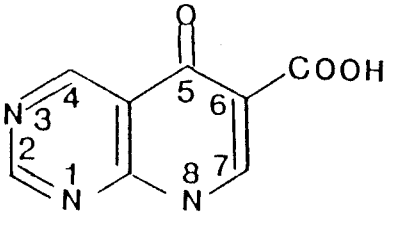
Those structures in which a ring closure has taken place from the N-atom to the 8-position of the quinolone system represent a special case in terms of the chosen classification of compounds

because substitution in the benzene nucleus can not be separated from substitution at the N-atom. (2) One example, ofloxacin (Fig. 1), exhibits potency similar or exceeding ciprofloxacin depending on the bacteria. (3)

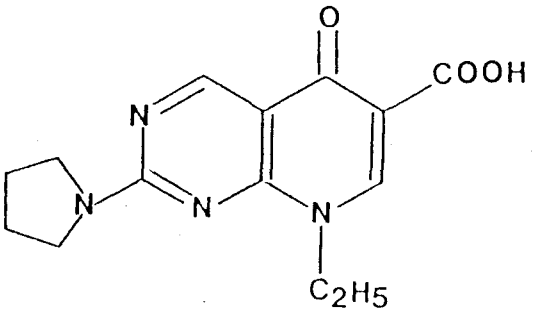
Many compounds having quinolone, 1,8-naphthyridine and pyrido [2,3-d]pyrimidine ring systems have been synthesized and their biological results reported. The 1,8-naphthyridine ring system shows very similar chemical properties as the quinolone ring system.

The pyrido[2,3-d]pyrimidine ring system (Fig. 4, IX) has been investigated. Different substituents in position 2 give inactive to active antibacterial activity. (2) Minami et al. point out that both of 5-oxo-6-carboxyl and 8-alkyl groups are essential for activity. The presence of an electron-releasing substituent at position 2 is important to the enhancement of the activity. (20) One example, piromidic acid (Fig. 4; 8-ethyl-5,8-dihydro-5-oxo-2-pyrrolidino-pyrido[2,3-d]pyrimidine-6-carboxylic acid), possesses good in vitro and in vivo activity against staphylococci and Gram-negative bacteria except P. aeruginosa. (21)

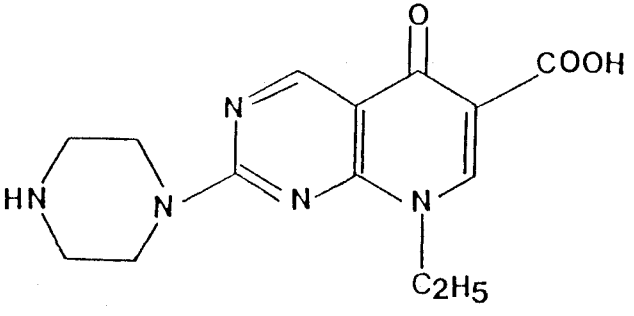
Based on the finding of piromidic acid, Matsumoto et al. extended their study on the derivatives of pyrido[2,3-d]pyrimidine The in vitro and in vivo data demonstrate that unsubstituted piperazinyl at position 2 and ethyl or vinyl at position 8 are the most favorable substituents in this series for activity against Gram-negative bacteria, in particular, the Pseudomonas species. Pipemedic acid (Fig. 4; 8-ethyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)-pyrido[2,3-d]pyrimidine-6-carboxylic acid) is superior to piromidic



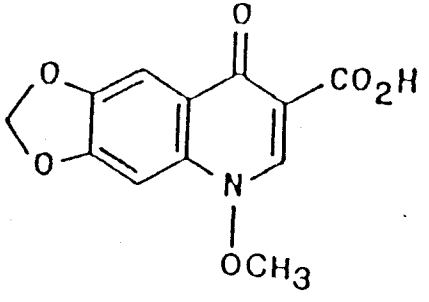
IX



Piromidic acid



Pipemidic acid



Miloxacin

Figure 4. Structures of IX, Piromidic Acid, Pipemidic Acid and Miloxacin

acid regarding the experimental infections caused by the Gram-negative bacteria in mice. (22)

Koga et al. began to develop compounds having not only more potent activity and broader spectrum, but also lower oral toxicity as well as higher resistance to metabolism that any other nalidixic acid analogues. The 4-quinolone-3-carboxylic acid (Fig. 2, II, $R^2 = R^5 =$ H) was selected as the reference compound. (23)

Analogues having substituents inserted at one or more of the 6,7,8 positions were then synthesized. It was found that among the substituents tested (nitro, acetyl, chloro, methyl, methoxy, dimethylamino, piperazinyl, and hydrogen), the piperazinyl group showed the most promise in position 7. The results for 6-substituted 7-piperazinyl derivatives showed that fluorine was preferable in position 6. Among these compounds, norfloxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)quinolone-3-carboxylic acid) was more potent in vitro against S. aureus, E. coli and P. aeruginosa than the other analogues with differing substitutions at these positions and position 1.

At the same time the quantitative structure activity relationship (QSAR) on a series of nalidixic acid analogues was first analyzed stepwise in terms of substituent effects at each position and the analyses were then extended to the multiply substituted analogues. (24) This QSAR analysis of quinoline derivatives was performed by Koga. (25) The physicochemical parameters such as STERIMOL L in position 1 [L(1)], B4 in position 8 [B4(8)], steric effect Es in position 6 [Es(6)], π-hydrophobic constant in position 7

$\pi(7)$], Log P (lipophilicity of whole molecule) and their squared terms ($L(1)^2$, $B4(8)^2$, $Es(6)^2$, $\pi(7)^2$ and Log P^2) were examined by regression analysis. The indicator variable at position 7, I(7) (I = 1 if substituted; I = 0 if hydrogen) and an indicator variable I(7N-CO) for carbonyl functions as a part of the 7-N-heterocyclic substituents such as N'-acylpiperazinyl and 4-carbamoylpiperidinyl also were included for analysis by Koga. The sum terms of the π-constant in positions 6,7 and 8 - $\Sigma\pi(6,7,8)$, its squared term $\Sigma\pi(6,7,8)^2$ and sum terms of field-inductive electronic effect in position 6, 7 and 8, $\Sigma F(6,7,8)$ also were included as independent variables in the derivation of the equations

The derived equations 1 to 6 are shown in Table 1. Eqs. 1-3 were developed by Koga to show the specific contribution of bulk (eq. 1), lipophilicity (eq. 2) and length of substituents (eq. 3). The 21 compounds reported in eq. 1 vary at position 6, 7 and 8. the 22 compounds studied in eq. 2 vary only at position 7 with a fluorine at position 6 and ethyl at N1. Finally eq. 3 was developed from eight compounds in which the substituent at position 1 were varied and fluorine fixed at C6 and unsubstituted piperazine at C7. Eqs. 4-6 show equivalent models based on 71 compounds. The underlined variables are unique to that particular model. As will be seen in the results and discussion, more than one statistically equivalent equation were obtained from some of the data sets analyzed in this thesis.

The r^2 of eq. 4 or eq. 6 was slightly less than that of eq. 5 using either $\pi(7)$ or Log P instead of $\Sigma\pi(6,7,8)$. The variances in

Table 1 Previously Published LFER Models on a Series of Nalidixic Acid Analogues (ref. 25)

(1) $\log(1/MIC) = -3.236(\pm 0.89)[Es(6)^2] - 4.210(\pm 1.26)Es(6) + 1.358(\pm 0.40)I(7)$
$-1.024(\pm 0.32)[B4(8)^2] + 3.770(\pm 1.43)B4(8) + 1.251$

$N = 21 \quad s = 0.205 \quad r = 0.978 \ (r^2 = 0.957) \quad F = 67.50$

(2) $\log(1/MIC) = -0.244(\pm 0.05)[\pi(7)^2] - 0.675(\pm 0.15)\pi(7) - 0.705(\pm 0.27)I(7N\text{-}CO)$
$+5.987$

$N = 22 \quad s = 0.242 \quad r = 0.943 \ (r^2 = 0.889) \quad F = 47.97$

(3) $\log(1/MIC) = -0.492(\pm 0.18)[L(1)^2] + 4.102(\pm 1.59)L(1)] - 1.999$

$N = 8 \quad s = 0.126 \quad r = 0.955 \ (r^2 = 0.912) \quad F = 25.78$

(4) $\log(1/MIC) = -0.423(\pm 0.26)[L(1)^2] + 3.532(\pm 2.32)L(1) - 2.499(\pm 0.55)[Es(6)^2]$
$-3.163(\pm 0.77)Es(6) + 0.223(\pm 0.06)[\pi(7)^2] - 0.633(\pm 0.13)\pi(7)$
$-1.036(\pm 0.26)I(7) - 0.774(\pm 0.28)I(7N\text{-}CO) - 0.868(\pm 0.25)[B4(8)^2]$
$+2.961(\pm 0.99)B4(8) - 0.686(\pm 0.40)\Sigma F(6,7,8) - 5.030$

$N = 71 \quad s = 0.285 \quad r = 0.961 \ (r^2 = 0.923) \quad F = 64.18$

(5) $\log(1/MIC) = -0.362(\pm 0.25)[L(1)^2] + 3.036(\pm 2.21)L(1) - 2.499(\pm 0.55)[Es(6)^2]$
$-3.345(\pm 0.73)Es(6) + 0.986(\pm 0.24)I(7) - 0.734(\pm 0.27)I(7N\text{-}CO)$
$-1.023(\pm 0.23)[B4(8)^2] + 3.724(\pm 0.92)B4(8) - 0.205(\pm 0.05)[\Sigma\pi(6,7,8)^2]$
$-0.485(\pm 0.10)\Sigma\pi(6,7,8) - 0.681(\pm 0.39)\Sigma F(6,7,8) - 4.571$

$N = 71 \quad s = 0.274 \quad r = 0.964 \ (r^2 = 0.929) \quad F = 70.22$

(6) $\log(1/MIC) = -0.294(\pm 0.27)[L(1)^2] + 2.528(\pm 2.54)L(1) - 2.497(\pm 0.57)[Es(6)^2]$
$-3.316(\pm 0.77)Es(6) + 0.956(\pm 0.25)I(7) - 0.792(\pm 0.28)(7N\text{-}CO)$
$-0.985(\pm 0.24)[B4(8)^2] + 3.557(\pm 0.96)B4(8) - 0.188(\pm 0.05)[\text{Log } P^2]$
$-0.370(\pm 0.09)\text{Log } P - 0.665(\pm 0.40)\Sigma F(6,7,8) - 3.343$

$N = 71 \quad s = 0.286 \quad r = 0.961 \ (r^2 = 0.923) \quad F = 64.07$

$\Sigma\pi(6,7,8)$ and Log P are mostly caused from $\pi(7)$. There is high collinearity between $\pi(7)$ and $\Sigma\pi(6,7,8)$ or Log P, 0.94 and 0.92 respectively, for these 71 compounds. Eq. 4 indicates that the hydrophobicity is significant only for the substituent in position 7. It also was pointed out that the hydrophobicity (Log P) of the whole molecule (eq. 6) seems to play an important role, possibly in the transport process to the active site. In eqs. 4, 5 and 6 the $\Sigma F(6,7,8)$ term is a negative contributor to activity.

Even though eq. 4, 5 or 6 are nearly equivalent using $\pi(7)$, $\Sigma\pi(6,7,8)$ or Log P, the π-constant is not really an additive principle when more than one substituent is present. (53)

Matsumoto et al. synthesized 1,6,7-trisubstituted 1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acid with hydrogen, nitro, amino, cyano, chloro or fluoro at C6 in order to investigate the antibacterial effect of the C6 substituent. (26)

A series of the 1-ethyl, 1-vinyl, 1-(2-fluoroethyl), or 1-(difluoromethyl) analogues of 7-substituted was prepared. The 1-pyrrolidinyl, 1-piperazinyl and N-methyl-1-piperazinyl groups were introduced at C7 on the basis of development of piromidic and pipemidic acids.

In this series, enoxacin (1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7(1-piperazinyl)-1,8-naphthyridine-3-carboxylic acid) was found to show the most broad and potent in vitro antibacterial activity, an excellent in vivo efficacy on systemic infections and a weak acute toxicity. (26) In 1984 Matsumoto et al. reported the synthesis and antibacterial activity of another series of 1,8-naphthyridine

analogues with amino or hydroxy substituted alicyclic amino groups such as 1-azetidinyl, 1-pyrrolidinyl, 1-piperidinyl at the C7 position, fluorine fixed at C6, and ethyl, vinyl or 2-fluoroethyl on the dihydropyridine nitrogen. (27)

This work was mainly directed at a search for analogues with a substituent that might cause a greater enhancement in activity than the piperazinyl group. It was thought that amino-substituted alicyclic amino groups such as 3-aminopyrrolidinyl or 3-aminoazetidinyl may be expected to offer such an enhancement of activity since the physicochemical properties of these groups seem to be generally similar to those of the piperazinyl group.

As a result, 1-ethyl and 1-vinyl-7-(3-amino-1-pyrrolidinyl)-6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids (see Table 10, D33A and D33B) and 1-vinyl-7-[3-(methylamino)-1-pyrrolidinyl analogue (Table 10, D34B) were found to be more active than enoxacin and to be worthy of further biological study. In conclusion, the 3-aminopyrrolidinyl group proved to be equivalent to or more effective than the piperazinyl group. (27)

The SAR studies indicated that the antibacterial potency is related closely to the steric bulk of the 1-substituent. (28) For the 1-alkyl naphthyridine/quinoline antibacterial agents, the ethyl analogues are generally more potent than those analogues having smaller or larger 1-alkyl substituents. Two other variants at position 1 are miloxacin (Fig. 4) (12) and ciprofloxacin (Fig. 1), which have 1-methoxy and 1-cyclopropyl substituents, respectively.

In some instance the vinyl analogues, which have similar steric bulk, showed potencies comparable to those of the ethyl derivatives. (28)

Using molar refractivity (MR) as a measure of bulk and the fact that the MR values of methylamino ($NHCH_3$) and ethyl, 10.33 and 10.30 respectively, are nearly identical. Wentland et al. prepared and evaluated a series of novel 3-quinoline-carboxylic acid derivatives characterized by fluorine at the 6-position and substituted amino groups at 1 and 7 positions. Amifloxacin (Fig. 1), the 1-methylamino analogue of perfloxacin, showed comparable in vitro and in vivo antimicrobial potency to this known agent. (28)

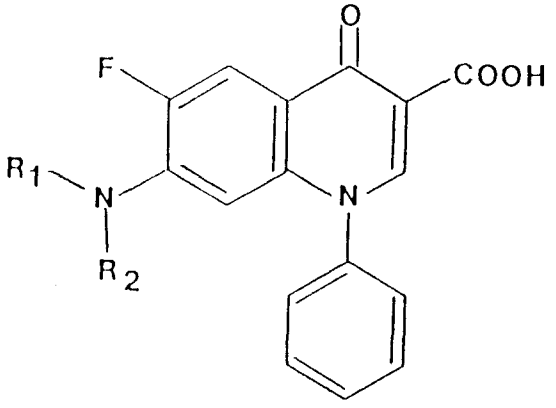
According to this work the correlation between the steric bulk of 1-alkylamino substituents and antibacterial activity, in vitro and in vivo, was in general agreement with published SAR studies involving 1-alkyl and 1-alkoxy naphthyridine/quinoline antiinfectives. These workers also indicated that in vivo antibacterial potencies of the 1-methyl amino derivatives are greater when the cyclic amine at position 7 has an additional basic nitrogen incorporated in it. Overall they concluded that, from the available data, it cannot be determined which parameters (steric bulk, electronic and/or hydrophobicity) best account for the observed activity of these 7-substituted quinolines. (28)

A series of novel arylfluoroquinlones have been synthesized by Chu et al. These derivatives are characterized by having a fluorine at the 6-position, substituted amino groups at the 7-position, and substituted phenyl groups at the 1-position. (29) At position 1, phenyl groups are bulkier than the N-ethyl group. In this series of

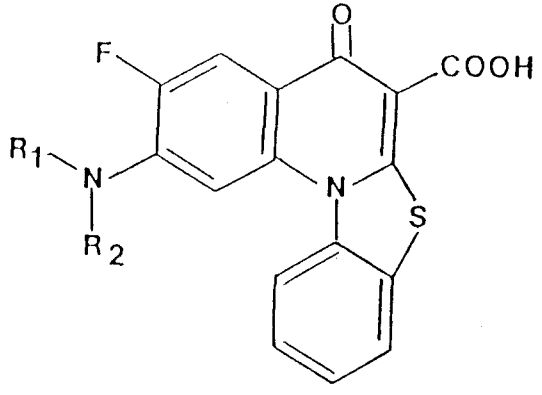
compounds norfloxacin is the reference compound for antibacterial activity. The replacement of basic nitrogen in the 4-piperazine in position 7 with a nonbasic atom resulted in improved activity against Gram-positive bacteria and slightly decreased activity against Gram-negative bacteria.

SAR studies indicated that the in vitro antibacterial potency is greatest when the 1-substituent is either p-fluorophenyl or p-hydroxyphenyl and the 7-substituent is either 1-piperazinyl, 4-methyl-1-piperazinyl, or 3-amino-1-pyrrolidinyl. The biological data are not in agreement with the generally accepted conventional notion that the antibacterial potency of this class of antibacterials is closely related to the steric bulk of the 1-substituent, with the ethyl group being most potent. Hence, steric bulk alone does not determine biological activity in this class of antibacterial compounds. It was suggested that the electronic and spatial properties of the 1-substituent, as well as the steric bulk, play important role in the antimicrobial potency in this class of antibacterials. (29) As a result of this study, compounds A-56619 (difloxacin) and A-56620 (Fig. 5) were found to possess excellent in vitro potency and in vivo efficacy.

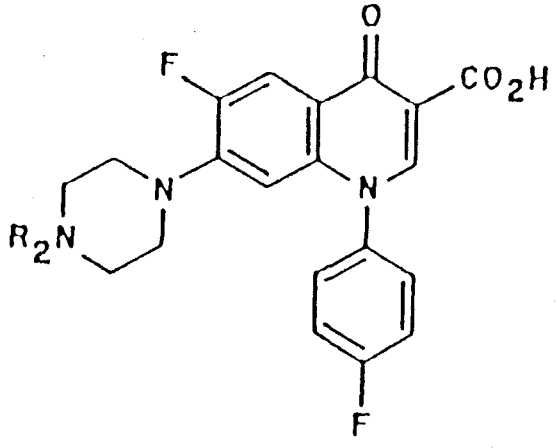
The synthesis and antibacterial activity of 2-substituted amino 3-fluoro-5,12-dihydro-5-oxobenzo-thiazolo[3,2a]quinoline-6-carboxylic acid (Fig. 5, XI) derivatives were reported. (30) The compounds are conformationally restricted analogues of 7-substituted amino-6-fluoro-1-aryl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids (Fig. 5, X). The purpose of this work was to determine the effect on



X



XI



Difloxacin R_2 : CH_3

A-56620 R_2 : H

Figure 5. Structures of X, XI, Difloxacin and A-56620

antibacterial activity of forcing the N-1 phenyl substituent into rigid planar conformation. It was hoped that these compounds would provide further insight into the importance of spatial characteristic of 1-phenyl substitution.

The fact that conformationally restricted benzothiazolo[3,2d] quinolones possess high antibacterial potency is of considerable interest because 2-alkyl-substituted 4-quinolone antibacterial agents are generally inactive. Since the 5,12-dihydro-5-oxobenthiazono [3,2-d]quinoline-6-carboxylic acid system has the phenyl ring nearly coplanar with the quinolone ring, the data indicate that the favorable conformation for the inhibitor during its inhibition of DNA gyrase may be that with the phenyl and quinolone rings close to coplanar and not perpendicular to each other. (30)

Domagala et al. observed that the piperazine group at position 7, although beneficial, was not essential for displaying low minimum inhibitory concentrations (MICs) against bacteria or against the target enzyme DNA gyrase. (31) It was suggested that the piperazine, possibly through the basic nitrogen, did confer proportionally good in vivo activity to those derivatives to which it was appended. A new side chain was sought in order to improve the spectrum of anti-bacterial activity without losing the obvious benefits of the piperazine moiety. With the aid of molecular modelling and computer graphics it appeared that the amino group in the 3-(aminomethyl) pyrrolidines might mimic the 4-piperazinyl nitrogen present in the known active drugs. Certainly the amino group in the 3-(aminomethyl) pyrrolidines would have several degrees of freedom relative to the

piperazinyl nitrogen and might possess properties unique to this feature.

The currently significant analogues (i.e. norfloxacin, pefloxacin, enoxacin, amifloxacin, ciprofloxacin) which have potent activity against Gram-negative organisms were examined. All of these compounds also possessed good anti-gyrase activity, displaying enzyme inhibition at concentration 2-20 times lower than that for oxolinic or nalidixic acids. (31)

The pyrrolidinylquinolines which represent the primary amino, methylamino, and 3-[(ethylamino)methyl]-1-pyrrolidinyl analogues of norfloxacin were prepared and tested. (31) The [3-(ethylamino) methyl-1-pyrrolidinyl]quinoline (Fig. 3, VII(a)) showed excellent MICs against Gram-positive organisms. It was concluded that replacement of the piperazine moiety with the 3-(aminomethyl)-1-pyrrolidinyl moiety did not compromise the gyrase inhibition, which is further proof that the piperazine group is not essential for antibacterial activity.

However, the *in vivo* activity of VII(a) was very poor. In order to increase the *in vivo* potency of VII(a) without sacrificing the MICs and gyrase activity, small molecular changes to increase solubility and possibly absorption were pursued. The result of this search led to the synthesis of the 6,8-difluoro analogues containing [3-(ethylamino)methyl-1-pyrrolidinyl]-6,8-difluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (CI-934; Fig. 3), a new quinolone with excellent Gram-positive activity. (31)

In another report Domagala questioned whether the biological activity of the quinolones might not be controlled by at lease two variables. (32) The first is the inhibition of DNA gyrase and its essential ability to supercoil relaxed bacterial DNA. The second variable involved the ability of these drugs to penetrate the bacterial cell and subsequently lead to the death of the cell. Lending credence to the importance of this second variable has been the discovery of the quinolone resistant factors in bacteria associated with permeability of the drug. (33) In order to develop a more meaningful structure activity relationships, the activity of certain quinolones has been compared side-by-side using DNA gyrase assays and MICs. (32)

Two values for DNA gyrase assay were used : (1) The gyrase cleavage value represents a "thermodynamic" value reflecting the amount of the drug-gyrase-DNA complex present at equilibrium. (2) The gyrase I_{50} values represent "kinetic" parameters and are related to how the drug actually inhibits the supercoiling process. (I_{50} is determined from the concentrations of drug that give initial inhibition and complete inhibition.)

For example, enoxacin has a cleavage value of 5 μg/ml but an I_{50} of 27.5 μg/ml, while ofloxacin, with an identical cleavage value, has an I_{50} of 6.3 μg/ml. Enoxacin, while showing a low concentration for initial inhibition, has difficulty inhibiting the supercoiling reaction completely.

One significant point involved the relationship between gyrase inhibition and MIC. Enoxacin, which is clearly a less potent gyrase

inhibitor than norfloxacin by either assay (gyrase cleavage : 5 μg/ml vs. 1 μg/ml ; I_{50} : 28 μg/ml vs. 5.5 μg/ml), must be able to penetrate the cell with greater efficacy in order to have MICs comparable to those of norfloxacin. This MIC leveling effect could be the result of different cell permeabilities or other penetration phenomena.

This study focused on the changes in DNA gyrase inhibition brought about by certain features of the molecules, namely, the fluorine at position 6 or the nature of the substituent at position 7. The effect of the 6-fluorine (norfloxacin) was investigated by comparison with the desfluoronorfloxacin compound. Norfloxacin is 18 times more potent in the gyrase cleavage assay than the desfluoronorfloxacin and 63 times more active in the MIC against E. coli H560. An 18 fold improvement in the drug-gyrase-DNA complex binding and 3.5 fold (63÷18) increase in cell penetration also is seen. The 6-fluorine has been shown to cause a simultaneous increase in enzyme inhibition and the "cell penetration variable".

After examination of the nature of the 7-substituent, the combined data strongly suggest that linear or small substituents and larger groups (ring with atom chains > three) possess moderate to weak gyrase inhibition and low MICs. In contrast five or six member rings by themselves or with small substituents have very good gyrase-DNA complex binding and have good to excellent MICs as well. The kind of substituent on the ring does not profoundly influence the activity if the size requirements are met. These substituent need not be basic as one might have suspected from all the published

derivatives containing piperazine. By using gyrase activity as a guide, there appears to be much more structural flexibility at position 7 than was otherwise suspected.

In conclusion, the data strongly suggest that the activity of the quinolones is determined not only by their intrinsic inhibition of DNA gyrase but also by the ability to penetrate the bacterial cell and/or inhibit cell growth through their action on DNA gyrase. (32)

III. Quantitative Structure Activity Relationships (QSAR) Models

The two most frequently used models in quantitative structure activity relationships are Hansch's linear free energy relationship (LFER) multiple regression model and Free-Wilson's additive substituent or de novo model. (34)

In 1963 Hansch and coworkers derived an equation using two experimentally based variables, σ and π or Log P for correlating the effect of a given substituent on the biological activity of a parent compound. (35)

Hansch derived equation (1) (equation 2 is an alternate form)

$$\text{Log}(1/C) = -K\pi^2 + K'\pi + \rho\sigma + K" \qquad (1)$$

$$\text{Log}(1/C) = -K(\text{Log P})^2 + K'(\text{Log P}) + \rho\sigma + K" \qquad (2)$$

C is the molar concentration that elicits a constant biological response (e.g. ED_{50}); σ is the substituent electronic effect of Hammett; π is an analogous constant representing the difference in the logarithms of the partition coefficients of the substituted compound and its unsubstituted reference compound; Log P is the

partition coefficient between 1-octanol and water. Log P is an additive and constitutive property and, in principle, is calculable from molecular structure. K, K', and ρ are the regression coefficients derived from the least squares statistical curve fitting. K" is the intercept term. The reciprocal of the concentration reflects the fact that higher potency is associated with lower dose. The negative sign for the π^2 or $(\text{Log P})^2$ term reflects the expectation of an optimum lipophilicity, designated π_o or Log P_o. The wide spread use of the Hansch model has provided an important stimulus for the review and extension of established scales of substituent effects.

About the same time that the Hansch model was proposed, Free and Wilson demonstrated a general mathematical method both for assessing the occurrence of additive substituent effects and for quantitatively estimating their magnitude. (36) According to their method, the molecules of a drug series are structurally decomposed into a common moiety or core that is variously substituted in multiple positions. A series of linear equations of the form

$$BA_i = \sum_j a_j X_{ij} + \mu \qquad (3)$$

are constructed where BA is the biological activity; X_j is the jth substituent with a value of 1 if present and 0 if not; a_j is the contribution of the jth substituent to BA; and μ is the overall average activity. The series of linear equations generated is solved by the method of least squares for the a_j and μ.

Originally, a set of restriction equations were used because the activity contributions at each position of substitution must sum to zero. Fujita and Ban showed that restriction equations not required. (38) Instead, the intercept term is the biological activity of the unsubstituted reference compound. This model is based on the assumption of activity additivity and each substituent's contribution to the biological activity is independent if the presence or absence of substituents at the other position on the molecule.

Purcell et al has discussed the requirements and constraints of the original Free-Wilson model. (37) They concluded that, when using the Free-Wilson model for quantitative structure-activity studies, it is advisable with any series of compounds, to check the stability of the system by randomly selecting subsets of compounds, solving the system of equations again, and comparing the two sets of solution values. The regression coefficients should remain constant.

There are three limitations of the Free-Wilson model: (1) A substantial number of compounds with varying substituent combinations is required for a meaningful analysis. This is represented by the equation,

$$N = 1 + \sum_{j}(n_i - 1) \qquad (4)$$

where N is the total number of compounds that must be synthesized, j is the number of position of substitution, and n_i is the number of substituents at position i.

(2) The derived substituent contributions give no reasonable

basis for extrapolating predictions outside of the substituent matrix analyzed.

(3) The model will break down if nonlinear dependence on a substituent property is important or if there are interactions between the substituents.

There are two advantages of the Free-Wilson model: (1) An experimental design model helps the synthetic medicinal chemist maximize the information of the substituent contribution from a small number of compounds.

(2) The model can point out the contributions of specific substituents on activity.

In 1971 Fujita and Ban suggested a modified Free-Wilson model. This model is given by equation (5). (38)

$$\text{Log } A = \Sigma G_i X_i + C \qquad (5)$$

Here, Log A is the log of activity, G_i is the log activity contribution or the log activity enhancement factor of the ith substituent relative to that of hydrogen, X_i is a parameter with a value of 1 or 0 according to the presence or absence of the ith substituent, C is the biological activity of the reference compound.

The Fujita-Ban modified model differs from the original Free-Wilson model in two aspects. First, in the original model the activity contributions of substituents including hydrogen have to be considered and restriction equations used where the group contributions at each position are summed to zero. In the Fujita-Ban

model the activity contribution of a substituent is relative to that of hydrogen at each position, and restriction or equations are not required. Secondly, the constant or intercept term in the original Free-Wilson model should be equal to the overall average of the activity values defined as the activity contribution from the parent "skeleton". In the Fujita-Ban model the constant term obtained by the least-squares method is the theoretically predicted activity value of the reference compound itself. The Fujita-Ban modification is the form of the Free-Wilson model in common use today.

In 1976 Hugo Kubinyi showed how to interpret and interrelate the Hansch and Free-Wilson models. (39) He assumed the Free-Wilson model is equivalent to a nonparabolic Hansch model which can be used to study additivity or nonadditivity of group contributions and to control and improve the fitting of Hansch equations. He showed that the goal of Free-Wilson analysis should be derivation of a significant Hansch equation which give us a better understanding of how drugs act at the molecular level.

Based on the theoretical and numerical equivalence of Hansch's linear multiple regression model and the modified Free-Wilson model, a mixed approach is developed. (equation 6)

$$\log(1/C) = K_1\pi^2 + \sum_i a_i + \sum_j K_j\phi_j + K' \qquad (6)$$

Σa_i is the Free-Wilson portion for parameters X_i, $\Sigma K_j\phi_j$ is the Hansch portion for parameters Y_i, and a term $K_1\pi^2$ is the parabolic dependence of Log(1/C) values on lipophilic character (note that π in $K_1\pi^2$ must be $\pi_x + \pi_y$).

In the mixed model the Free-Wilson now is applicable also in the case of parabolic dependence of biological activity on a particular physical property (e.g. Log P or π). The mixed approach is a combination of both models which makes use of the advantages of each model and widens the applicability of Hansch and Free-Wilson analysis.

In most cases the Hansch approach is the more general and useful model but there are also limitations to this model. For certain groups of compounds only the Free-Wilson model can give correlations between chemical structure and biological activity. If the correct LFER parameters are not available, then only the presence or a absence of a substituent can be used.

A further limitation of the Hansch model comes from little structural variation in a definite position of the molecule, There must be a meaningful range in the values of quantitative parameters in order to have a valid Hansch model.

The free energy model of Hansch and its elaborations has been by far the most widely used. This has been due not only to the many successful applications reported by the Hansch group, but also to its direct conceptual linkage to established physical organic chemical principles, and the ready availability of a database of substituent parameters.

In general the Hansch LFER model can explain how a substituent affects activity and can suggest other untested substituents. In contrast the Free-Wilson (or <u>de novo</u>) model can point out the contributions of specific substituents on activity and suggest which

combination of substituents from a design set will produce the most active compounds. It will not explain how these substituents affect activity nor can it be used to predict the contribution of untested substituents.

The purpose of this research is to apply the Hansch and Free-Wilson mathematical models to a series of antibacterial agents analogues of pyridone carboxylic acids. There have been a series of articles reporting the antibacterial activity against three different types of bacteria (S. aureus, E. coli and P. aeruginosa) by a series of 6,7-disubstituted quinoline and 1,8-naphthyridine 3-carboxylic acids derivatives. Consistent biological data is available for a quantitative structure activity relationships study on over 120 compounds. (23,26,27)

EXPERIMENTAL

In this research project log P calculated by the fragment (f) method, molar refractivity (MR) and STERIMOL (L, B1 and B5) parameters were used in a linear free energy relationship (LFER or Hansch) analysis. A mixed model using the physicochemical parameters and Free-Wilson's indicator variables as independent variables also were examined. The in vitro activity measured by minimum inhibitory concentration (μg/mL) against S. aureus, E. coli and P. aeruginosa was converted to molar concentration and used as the dependent variables.

I. Types of Descriptors

A. Hydrophobic Parameters (Log P, π)

Meyer and Overton, who showed that the relative potencies of drugs affect the nervous system correlated with their oil/water partition coefficient (P), initiated the use of such measurements as a means for defining relative hydrophobicity of biologically active organic compounds. (40) In the early 1950s, Collander generated new interest in oil/water partition coefficients by demonstrating that the rate of penetration by a wide variety of organic compounds into plant cell membranes was related to their partition coefficients. (40)

The partition coefficient, P is defined as the equilibrium concentration of the monomeric species of a compound in the nonaqueous phase, [D]o, divided by that of the neutral form in the

aqueous phase, [D]w :

$$P = \frac{[D]o}{[D]w}$$

Hence P is a pH dependent property. It usually is expressed as the logarithm of P. (41)

In 1964 an extensive study of the additive-constitutive nature of the partition coefficient was published by Fujita et al. (42) The use of the partition coefficient in structure-activity studies has been discussed by Hansch. (43) An important problem is the choice of the solvent pair used as the reference system. Collander defined (eq. 1) a linear relation between partition coefficients in different solvent systems .

$$\text{Log } P_1 = a\text{Log } P_2 + b \qquad (1)$$

In equation (1), a and b are constants and P_1 and P_2 are partition coefficients for a group of organic compounds between two different lipophilic solvents and water. (44) If the interaction of drugs (in water) with the biophase is regarded as a partitioning phenomenon, this equation becomes the basis for the use of the partition coefficient in octanol/water, expressed as P_2, as a model for the partitioning between biophase and water, expressed as P_1. (45)

Other studies have since confirmed that polar hydrogen bonding solvents are best suited to model lipophilic substances reacting with biosystems. (40) Hansch has chosen the system n-octanol/water as a reference system for partition coefficients. (43)

A number of more polar organic solvents have been used as the model for the nonaqueous phase, but octanol is the most widely used solvent in partition coefficient determination. Because of its hydroxy group and its ability to dissolve water, octanol is a rather good mimic of the lipid bilayer membrane model. (41) Nevertheless, it is by no means clear that this is the ideal solvent system for modeling all the interactions of organic compounds with biologic system. (40)

Although Log P can be used as a measure of the hydrophobicity of a whole molecule, it is more common to utilize the hydrophobic property of substituents. This is feasible when a large portion of the parent structure remains constant. In order to separate hydrophobic character from electronic and steric effects of substituents, the parameter π was defined. (42)

$$\pi_X = \text{Log } P_{YX} - \text{Log } P_{YH} \qquad (2)$$

π_X is the contribution of substituent X to the partition coefficient of the substituted compound. P_{YX} is the partition coefficient of the substituted compound and P_{YH} is the partition coefficient of the unsubstituted or reference compound. Fujita et al. also found that, although π varies continuously for a given function depending on its electronic environment, the range over which it varies is small. (42)

In the early work with π calculations, erroneous values for a few aliphatic hydrocarbons led to the conclusion that the intrinsic hydrophobicity of hydrogen atom in octanol/water system was close to

zero, and thus a fair approximation of Log P could be obtained by summing π constants. It is now realized that summing of π values can give misleading results. (40,53)

Nys and Rekker undertook a statistical survey of the partitioning data available in order to develop a set of fragment values which could be used in an additive fashion according to the following equation. (40)

$$\text{Log } P = \Sigma a_n f_n \qquad (3)$$

Where a is the number of occurrences of fragment f of the structural type n. This group also published values for a "proximity effect" in which Log P increases when two polar groups are on the same or adjacent carbon atoms. (41)

The relationship between the π-constant and fragment values can be shown by this equation. (40)

$$\pi_X = \text{Log } P_{YX} - \text{Log } P_{YH} = (f_Y + f_X) - (f_Y + f_H) = f_X - f_H \qquad (4)$$

By staring with Log P values for a large number of structures, Nys and Rekker used a reductionist approach to calculate $-CH_3$, $-CH_2$, -CH, etc. In contrast, Leo started with very few carefully measured coefficients for simple structures that could contained no "surprise" interactions.

Leo's method might be looked upon as constructionist. The fragment constants $f_H = 0.23$ and $f_C = 0.20$ become the only

fundamental ones needed in calculating all alkane structures. This method retains constant fragment values for the fundamental structural elements and then looks for other factors (F) that affect the partitioning equilibrium in more complex solutes where summation of fragments alone lead to spurious values. Using Leo's approach equation (3) can be expanded to

$$\text{Log } P = \Sigma a_n f_n + \Sigma b_m F_m \qquad (5)$$

In this project the $\sigma-\rho$ interaction (ortho effect) is an important correction factor. The value could be split evenly between the two ortho substituents and added to the calculated lipophilic parameters or it could be treated as a separate variable. Because summed lipophilic parameters were not significant and the purpose was to determine what was important for activity at each positions, the electronic or $\sigma-\rho$ interaction was treated separately.

B. Molar Refractivity (MR)

Molar refractivity is an additive constitutive property of a compound which is easily and unambiguously measurable. Experimentally, MR usually is obtained via the Lorentz-Lorenz equation : (46)

$$MR = \frac{n^2 - 1}{n^2 + 2} \cdot \frac{MW}{d} \qquad (6)$$

In this equation, n is the index of refraction, d is the density, and MW is the molecular weight of the compound. Pauling and Pressman (47) suggested that dispersion forces could be modeled by the molar refractivity of substituents. They point out that MR is related to London dispersion forces as follows :

$$E = \frac{-3\alpha_a\alpha_b}{2r^6} \cdot \frac{I_aI_b}{I_a + I_b} \qquad (7)$$

$$MR = \frac{4\pi N\alpha}{3} \qquad (8)$$

E in equation (7) is the cohesive energy between two atoms, a and b, whose polarizability is represented by α. The distance between a and b is represented by r, and I is the ionization potential. Equation (8) shows the relationship between MR and α and, therefore, how MR is related to E. In equation (8), N is Avogardo's number and π is 3.14. (not the "π" associated with the substituent contribution to the partition coefficient) Dunn has shown that there is considerable collinearity between MR and Es^c. (48) Here Es^c is a corrected steric parameter. Overall, molar refractivity is a complex term which measures both polarizability and steric contribution.

Complete tables of MR values of the common atoms found in organic molecules are available. It is relatively easy to calculate MR's for substituents without having to resort to many correction factors.

C. STERIMOL

The steric influence of substituents in the interaction of organic compounds with macromolecules or drug receptors is many orders of magnitude more complicated than the steric effects in simple homogenous organic reactions for which Taft's electronic steric parameter Es was designed. (40)

Verloop et al. (49) have undertaken a multiparameter approach to determine steric effects, and their ideas may lay the groundwork for a more detailed analysis. Five dimensions were selected for each substituent and a computer program developed using van der Waals radii, standard bond angles and length, and "reasonable" conformations to define the space requirements of a substituent.

The five dimensions were labeled L, B1, B2, B3 and B4. Fig. 6(a) and Fig. 6(b) illustrate the projections. The length parameter, L, is defined as the length of the substituent along the axis of the bond between the first atom of the substituent and the parent molecule. The four width parameters B1-B4 are determined by the distance at their maximum point perpendicular to this attachment bond axis and each other. B1 is the smallest and B4 is the largest width. (49)

After some applications of STERIMOL values by other investigators, discrepancies with respect to the values of some of the parameters appeared. The deviations occurred mainly with the parameters B2 and B3 and, to a lesser extent, with B4. It was indicated that the discrepancies arose form a certain ambiguity of the original formulation of the B1 parameters. The value of B1

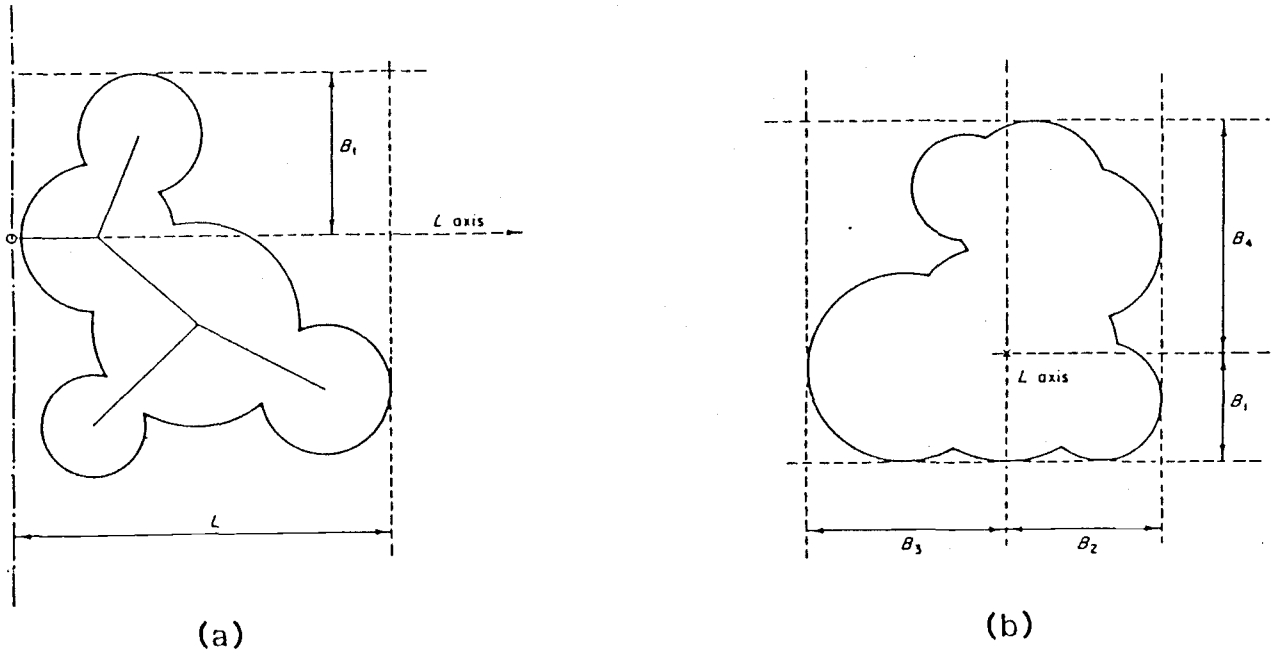


Figure 6. (a) Projection of a Substituent Along the L Axis Showing the Parameters L and B1
(b) Projection of a Substituent Perpendicular to the L Axis Showing the Four B Parameters (ref. 49)

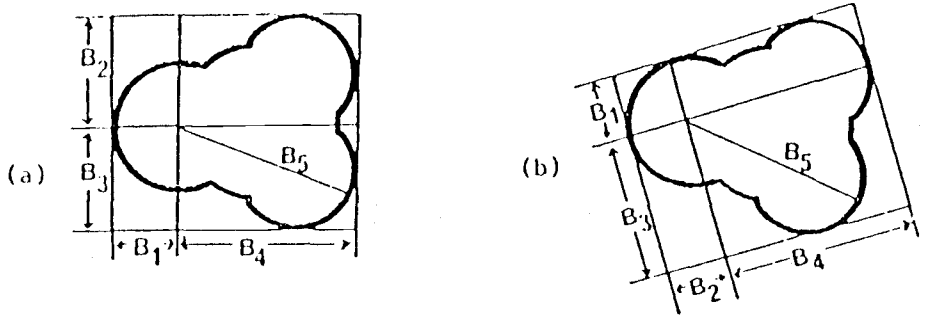


Figure 7. Different Possibilities for the Measurement of the Minimum Width Parameter B1 of the OCH_3 Substituent (ref. 50)

itself is uniquely defined, but not its position at the substituent. This is illustrated in Fig. 7 for the OCH_3 substituent where the B parameters are projected in a plane perpendicular to the L-axis. In Fig. 7(a) one possibility is presented, which results in a situation where the largest width parameter B4 lies in the opposite direction. Another possibility is showed in Fig. 7(b). In the cases where more B1 directions were possible, the choice was in general made in such a way that the resulting B4 value would be as close as possible to the maximum width. (50)

In the original approach five directions were chosen as a compromise between a reasonable description of the shape of the substituents and the avoidance of too many parameters. Still, it was felt by some workers that the number chosen absorbed too many degrees of freedom requiring any QSAR applications be restricted to large series. It also was indicated that the strongest intercorrelation is present between B2 and B3 and, in the about 35 studies applying the STERIMOL approach, the B2 and B3 constants hardly ever contributed significantly to the regression equations that were obtained. (50)

Later a second generation STERIMOL approach was developed. Its characteristics include: (1) The length parameter L is maintained; (2) The minimum width parameter B1 is maintained; (3) B2 and B3 are omitted; and (4) The new maximum width parameter B5 is introduced which replaces the B4 parameter. By these changes, the problem of the choice of the direction of B1 is no longer existent because B5 has no directional relationship with B1 as is illustrated in Fig. 7.

The less significant B2 and B3 parameters are omitted, which reduces the number of STERIMOL parameters to three. (50)

D. Indicator (dummy) Variables

Frequently, when calculating the structure activity relationships of series of compounds which were not planned for such analysis, there are discontinuities in the structural features of the molecules which are not easily accounted for by the usual physical properties. Such features may be accounted for by the use of indicator variables. These variables are arbitrarily assigned a value to indicate the presence of a particular substitunet and another value to indicate its absence. Usually 1.0 and 0.0 are used as these values, respectively. The importance of the substituent is easily estimated from the regression equation.

The ultimate in use of indicator variables is the Free-Wilson technique which is a from of experimental design using only indicator variables as the independent variables in a regression model. (42) This model has been described in the introductory chapter of this thesis.

II. Statistical Approach

Certainly one of the most important considerations in QSAR is the statistical analysis of the correlation of the observed biological activity with structural parameters, either the extrathermodynamic (Hansch) or the indicator variables (Free-Wilson). The coefficients of the structural parameters that establish the

correlation with the biological activity are usually obtained by the least squares procedure in a regression analysis.

The multiple linear regression analyses (51) were performed on the Oregon State University CYBER 170 using the statistical interactive programming system (SIPS). (52) Two approaches were used in developing the models. A forward stepwise in which the next most significant variable would enter the model and any variable already in the model that becomes insignificant (usually $p > 0.05$) would be dropped. The final model would be checked by adding all variables and dropping the insignificant ones. A second approach was to force in a variable initially and build a model. In each case, the final models were checked for consistency by omitting five or six randomly selected compounds and examine the consistency of the regression coefficients. This was repeated three times. A number of statistics are derived in conjunction with such a calculation including s, the standard error, r, the correlation coefficient, r^2, the percentage of data variance accounted for by the model, F, a statistic for assessing the overall significance of the derived equation, and t values and p values for the individual regression coefficients in the equation. The comparison of calculated antimicrobial activity with observed biologic activity was included.

Log P and MR were obtained from CLOG P v. 3.3 written by Dr. AL. Leo and calculated by Dr. James King. STERIMOL values were calculated by Dr. Verloop.

DESCRIPTION OF THE DATA SET

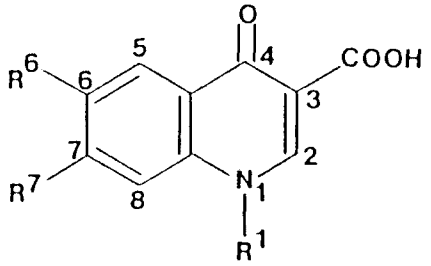
I. Set A

The structures of the 6,7-disubstituted 1-alkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids and their in vitro antibacterial activity against Gram-positive (S. aureus 209P) and Gram-negative bacteria (E. coli NIHJ JC-2 and P. aeruginosa V-1) are shown in Table 2. (23) The data of monosubstituted A3 is included for comparison. The biological results of the 1-ethyl-7-piperazinyl compounds A34, A37, A39 and A41-45 which vary only at position 6 indicated that fluorine was preferable for the 6-substituent of A3. The substitution of the hydrogen of the piperazine NH group in A34 by an alkyl or acyl group reduced the activity against Gram-negative bacteria, particularly P. aeruginosa. The replacement of the 1-ethyl group in A34 by 2-fluoroethyl and vinyl groups (A49 and A67) resulted in almost equal activity against Gram-negative bacteria while substitution by more or less sterically hindered groups (A48 and A51-54) decreased activity. Esters A81 and A82 did not show any significant activity indicating that a free carboxyl group is required.

The data matrices of lipophilicity (F), molar refractivity (MR), STERIMOL (L, B1, B5) parameters and Free-Wilson indicator variables for each substituent in positions 1, 6 and 7 are shown in Tables 3, 4 and 5. All of these variables were used as independent variables in the least squares statistical analysis.

The contribution to the partition coefficient by the

Table 2 6,7-Disubstituted 1-Alkyl-1,4-dihydro-4-oxo-quinoline-3-carboxylic Acids and Their *in vitro* Antibacterial Activity (Set A)



No.	R^1	R^6	R^7	min inhibitory concn μg/mL (ref. 23) *S. aureus* 209P	*E. coli* NIHJ JC-2	*P. aeruginosa* V-1
A18	C_2H_5	F	Cl	12.5	1.56	100
A32	C_2H_5	F	CH_3	6.25	0.39	50
A33	C_2H_5	F	H_2N	>100	3.13	>100
A34	C_2H_5	F	$HN(CH_2CH_2)_2N-$	0.39	0.05	0.39
A36	C_2H_5	F	$CH_3N(CH_2CH_2)_2N-$	0.39	0.10	1.56
A37	C_2H_5	Cl	$HN(CH_2CH_2)_2N-$	1.56	0.20	3.13
A38	C_2H_5	Cl	$H_3N(CH_2CH_2)_2N-$	1.56	0.78	25
A39	C_2H_5	Br	$HN(CH_2CH_2)_2N-$	3.13	0.39	12.5
A40	C_2H_5	Br	$CH_3N(CH_2CH_2)_2N-$	1.56	0.39	100
A41	C_2H_5	CH_3	$HN(CH_2CH_2)_2N-$	3.13	0.39	6.25
A42	C_2H_5	CH_3S	$HN(CH_2CH_2)_2N-$	25	0.78	12.5
A43	C_2H_5	CH_3CO	$HN(CH_2CH_2)_2N-$	100	100	>100
A44	C_2H_5	CN	$HN(CH_2CH_2)_2N-$	12.5	0.39	6.25
A45	C_2H_5	O_2N	$HN(CH_2CH_2)_2N-$	25	0.78	12.5
A46[a]	C_2H_5	H	$HN(CH_2CH_2)_2N-$	3.13	0.39	6.25
A47[b]	C_2H_5	H	$HN(CH_2CH_2)_2N-$	12.5	0.78	1.56
A48	CH_3	F	$HN(CH_2CH_2)_2N-$	6.25	0.39	1.56
A49	FCH_2CH_2	F	$HN(CH_2CH_2)_2N-$	1.56	0.10	0.78
A50	FCH_2CH_2	F	$CH_3N(CH_2CH_2)_2N-$	0.39	0.10	3.13
A51	$OHCH_2CH_2$	F	$HN(CH_2CH_2)_2N-$	1.56	0.39	3.13
A52	$n-C_3H_7$	F	$HN(CH_2CH_2)_2N-$	1.56	0.20	3.13

Table 2 continued on next page.

Table 2 continued

A53	$CH_2CH=CH_2$	F	$HN(CH_2CH_2)_2N-$	3.13	0.20	1.56
A54	$C_6H_5CH_2$	F	$HN(CH_2CH_2)_2N-$	1.56	0.78	1.56
A55	C_2H_5	F	$(CH_3)_2N-$	0.78	0.39	50
A56	C_2H_5	F	$(CH_2)_4N-$	0.20	0.39	12.5
A57	C_2H_5	F	$(CH_2)_5N-$	0.78	1.56	50
A58	C_2H_5	F	$O(CH_2CH_2)_2N-$	0.78	0.20	12.5
A59	C_2H_5	F	$(HO)CH(CH_2CH_2)_2N-$	0.39	0.20	12.5
A60	C_2H_5	F	$(H_2NCO)CH(CH_2CH_2)_2N-$	1.56	1.56	100
A61	C_2H_5	F	$[(CH_3)_2N]CH(CH_2CH_2)_2N-$	0.39	0.10	3.13
A62	C_2H_5	F	3-oxo-1-piperazinyl	3.13	0.39	12.5
A63	C_2H_5	F	$(H_2NCH_2CH_2)NH-$	>100	6.25	50
A64	C_2H_5	$HN(CH_2CH_2)_2N-$	Cl	>100	>100	>100
A67	$CH_2=CH$	F	$HN(CH_2CH_2)_2N-$	3.13	0.10	0.39
A68	C_2H_5	F	$C_2H_5N(CH_2CH_2)_2N-$	0.39	0.10	3.13
A69	C_2H_5	F	$(HOCH_2CH_2)N(CH_2CH_2)_2N-$	0.78	0.10	6.25
A70	C_2H_5	F	$(CH_2=CHCH_2)N(CH_2CH_2)_2N-$	0.39	0.39	6.25
A71	C_2H_5	F	$(C_6H_5)N(CH_2CH_2)_2N-$	0.39	0.78	50
A72	C_2H_5	F	$(O_2N\text{-}p\text{-}C_6H_4CH_2)N(CH_2CH_2)_2N-$	1.56	6.25	>100
A73	C_2H_5	F	$(OHC)N(CH_2CH_2)_2N-$	1.56	0.39	6.25
A74	C_2H_5	F	$(CH_3CO)N(CH_2CH_2)_2N-$	0.78	1.56	25
A75	C_2H_5	F	$(C_6H_5CO)N(CH_2CH_2)_2N-$	1.56	3.13	25
A76	$CH_2=CH$	F	$CH_3N(CH_2CH_2)_2N-$	1.56	0.10	3.13
A78	C_2H_5	F	$(CH_3CO)NH(CH_2)_2NH-$	>100	25	>100
A79	C_2H_5	F	$(H_2N\text{-}p\text{-}C_6H_4CH_2)N(CH_2CH_2)_2N-$	0.39	0.39	12.5
A80[c]	C_2H_5	F	$HN(CH_2CH_2)_2N-$	>100	>100	>100
A81[d]	C_2H_5	F	$HN(CH_2CH_2)_2N-$	100	12.5	50
A82[e]	C_2H_5	F	$HN(CH_2CH_2)_2N-$	50	12.5	50
A1	Nalidixic acid			>100	3.13	100
A3	C_2H_5	H	$HN(CH_2CH_2)_2N-$	12.5	0.78	3.13
A83	Pipemidic acid			25	1.56	12.5
A84	Oxolinic acid			3.13	0.10	25

[a]Chlorine in position 8; [b]Fluorine in position 8; [c]H in position 3 instead of carboxylic acid
[d]methyl ester of A34; [e]ethyl ester of A34

Table 3 Physicochemical Parameters and Indicator Variables in Position 1 of Set A

NO.	F(1)[a]	MR(1)	L(1)	B1(1)	B5(1)	IE(1)[b]	IEF(1)[c]	IEO(1)[d]	IA(1)[e]	IBM(1)[f]	IV(1)[g]	IB(1)[h]	IM(1)[i]
A18	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A32	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A33	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A34	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A36	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A37	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A38	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A39	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A40	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A41	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A42	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A43	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A44	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A45	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A48	.876	.5525	2.87	1.52	2.04	0	0	0	0	0	0	0	0
A49	1.128	1.0318	4.70	1.52	3.17	0	1	0	0	0	0	0	1
A50	1.128	1.0318	4.70	1.52	3.17	0	1	0	0	0	0	0	0
A51	.245	1.1694	4.79	1.52	3.38	0	0	1	0	0	0	0	0
A52	1.934	1.4801	4.92	1.52	3.49	0	0	0	0	0	0	0	0
A53	1.390	1.4547	5.11	1.52	3.78	0	0	0	1	0	0	1	0
A54	2.444	3.0637	4.62	1.52	6.02	0	0	0	0	1	0	0	0
A55	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A56	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A57	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A58	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A59	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A60	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A61	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A62	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A63	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A67	.861	.9909	4.29	1.60	3.09	0	0	0	0	0	1	0	0
A68	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A69	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A70	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A71	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A72	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A73	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A74	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A75	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A76	.861	.9909	4.29	1.60	3.09	0	0	0	0	0	1	0	0
A78	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A79	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0
A3	1.405	1.0163	4.11	1.52	3.17	1	0	0	0	0	0	0	0

[a]Calculated lipophilicity of the substituent; [b]C_2H_5; [c]CH_2CH_2F; [d]CH_2CH_2OH; [e]$CH_2CH_2{=}CH_2$; [f]$CH_2C_6H_5$; [g]$CH{=}CH_2$; [h]$n\text{-}CH_3$; [i]CH_3

Table 4 Physicochemical Parameters and Indicator Variables in Position 6 of Set A

NO.	F(6)[a]	MR(6)	L(6)	B1(6)	B5(6)	IF(6)[b]	IBR(6)[c]	IS(6)[d]	ICO(6)[e]	ICN(6)[f]	INO(6)[g]	ICL(6)[h]	IM(6)[i]	API[j]
A18	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.000
A32	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.000
A33	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.302
A34	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A36	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A37	.940	.5801	3.52	1.80	1.80	0	0	0	0	0	0	1	0	.171
A38	.940	.5801	3.52	1.80	1.80	0	0	0	0	0	0	1	0	.171
A39	1.090	.0657	3.82	1.95	1.95	0	1	0	0	0	0	0	0	.171
A40	1.090	.0657	3.82	1.95	1.95	0	1	0	0	0	0	0	0	.171
A41	.876	.5525	2.87	1.52	2.04	0	0	0	0	0	0	0	1	.000
A42	.786	1.3500	4.30	1.70	3.26	0	0	1	0	0	0	0	0	.000
A43	-.334	1.0520	4.06	1.60	3.13	0	0	0	1	0	0	0	0	.311
A44	-.340	.5664	4.23	1.60	1.60	0	0	0	0	1	0	0	0	.397
A45	-.030	.8142	3.44	1.70	2.44	0	0	0	0	0	1	0	0	.366
A48	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A49	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A50	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A51	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A52	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A53	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A54	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A55	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A56	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A57	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A58	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A59	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A60	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A61	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A62	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A63	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.302
A67	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A68	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A69	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A70	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A71	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A72	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A73	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A74	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A75	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A76	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A78	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.302
A79	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	0	0	0	.171
A3	.227	.0887	2.06	1.00	1.00	0	0	0	0	0	0	0	0	.000

[a]Calculated lipophilicity of the substituent; [b]Fluorine; [c]Bromine; [d]SCH_3; [e]$COCH_3$; [f]CN; [g]NO_2; [h]Chlorine; [i]CH_3;
[j]σ-ρ electronic potential interactions between Position 6 and 7

Table 5 Physicochemical Parameters and Indicator Variables in Position 7 of Set A

NO.	F(7)[a]	MR(7)	R11(7)[b]	R12(7)[c]	R13(7)[d]	R14(7)[e]	INCO(7)[f]
A18	.940	.5801	0	0	0	0	0
A32	.876	.5525	0	0	0	0	0
A33	-1.000	.4574	0	0	0	0	0
A34	-.100	2.5039	1	0	0	0	0
A36	.756	2.9677	1	0	0	0	0
A37	-.100	2.5039	1	0	0	0	0
A38	.756	2.9677	1	0	0	0	0
A39	-.100	2.5039	1	0	0	0	0
A40	.756	2.9677	1	0	0	0	0
A41	-.100	2.5039	1	0	0	0	0
A42	-.100	2.5039	1	0	0	0	0
A43	-.100	2.5039	1	0	0	0	0
A44	-.100	2.5039	1	0	0	0	0
A45	-.100	2.5039	1	0	0	0	0
A48	-.100	2.5039	1	0	0	0	0
A49	-.100	2.5039	1	0	0	0	0
A50	.756	2.9677	1	0	0	0	0
A51	-.100	2.5039	1	0	0	0	0
A52	-.100	2.5039	1	0	0	0	0
A53	-.100	2.5039	1	0	0	0	0
A54	-.100	2.5039	1	0	0	0	0
A55	.422	1.3850	0	0	0	0	0
A56	1.216	2.1352	0	1	0	0	0
A57	1.775	2.5990	0	0	1	0	0
A58	.132	2.2883	0	0	0	1	0
A59	-.312	2.7521	0	0	1	0	0
A60	-.782	3.4672	0	0	1	0	1
A61	.500	3.0953	0	0	1	0	0
A62	-.432	2.5396	0	0	0	0	1
A63	-.964	1.7537	0	0	0	0	0
A67	-.100	2.5039	1	0	0	0	0
A68	1.205	3.4315	1	0	0	0	0
A69	.097	3.5846	1	0	0	0	0
A70	1.110	3.8699	1	0	0	0	0
A71	2.698	4.3333	1	0	0	0	0
A72	2.341	6.2044	1	0	0	0	0
A73	-.464	3.0034	1	0	0	0	1
A74	.048	2.9072	1	0	0	0	1
A75	1.347	3.9235	1	0	0	0	1
A76	.756	2.9677	1	0	0	0	0
A78	-1.074	2.7170	0	0	0	0	0
A79	1.310	5.8476	1	0	0	0	0
A3	-.100	2.5039	1	0	0	0	0

[a]Calculated lipophilicity of the substituent; ring indicator of [b]$HN(CH_2CH_2)_2N$-; [c]$(CH_2)_4N$-; [d]$(CH_2)_5N$-; [e]$O(CH_2CH_2)_2N$-; [f]An indicator of an amide nitrogen for 7-N-heterocyclic substituents

substituents at positions 6 and 7 were summed [ΣF(6,7)], as were the molar refractivity contributions [ΣMR(6,7)]. These two sets of summed variables paralleled a similar approach used by Koga in his QSAR analysis of a set of quinoline derivatives. (25) In order to check for parabolic relationships, squared terms for Log P, MR (in position 6 and 7, individually and summed), L, B1, B5 (in position 6) and API ($\sigma-\rho$ electronic potential interactions between position 6 and 7) were included as independent variables.

II. Set B

The structures and the in vitro antibacterial activity of 1,6,7-trisubstituted-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids against Gram-positive (S. aureus 209 PJC-1) and Gram-negative bacteria (E. coli NIHJ JC-2 and P. aeruginosa Tsuchijima) are shown in Table 6. (26) Minimum inhibitory concentrations of B3A-C and BNA (nalidixic acid) are included for comparsion. The 6-substituent represented by the B15 series, B18 series, B22-24 series were compared with B3A-C. In a series of pyrrolidinyl compounds (B15A, B18A, B22A, B23A, B24A) the fluoro and cyano groups cause an increase in activity against all the bacteria tested, whereas other substituents at C6 result in a loss of activity, particularly against the Gram-negative bacteria. With respect to the piperazinyl and N-methyl-piperazinyl derivatives (series B and C of compounds B15, B18 and B22-24), introduction of a substituent at position 6 tends to enhance the activity against both Gram-positive and Gram-negative organisms, with a few exceptions. In both series of compounds, the

activity against S. aureus increases in the order $NH_2 \leq H < NO_2 = CN < Cl < F$, whereas the Gram-negative activity (C series against E. coli and P. aeruginosa) follows the sequence $NO_2 \leq H < NH_2 = CN = Cl < F$. In the "B" series of set B, the activity against E. coli increases in the order $H = NO_2 < NH_2 < CN < F$. The replacement of hydrogen by halogen, especially fluorine, at position 6 in the 1,8-naphthyridine system results in significant enhancement of the activity against both Gram-positive and Gram-negative organisms.

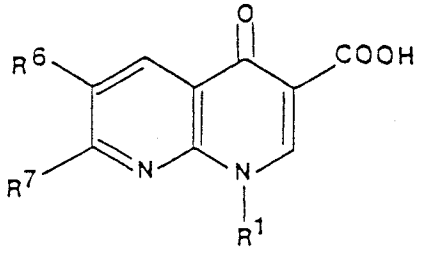
Modification of the cyclic amino moiety at position 7 resulted in a significant decrease in activity as observed in B24A and B27C-I as compared with B24B. Only compound B24A with the 1-pyrrolidinyl group is more active than B24B against S. aureus, whereas B24B and B24C have better activity against Gram-negative organisms.

A comparison of the activity between B24B and B27D, as well as between B27G and B27H, indicates that the presence of a basic NH group in the cyclic amino function is a prerequisite to optimal activity. However, if the NH group is an amide, such as in the 3-oxo-1-piperazinyl group of B27C, activity is decreased. Introduction of an alkyl, aralkyl or aryl group at the piperazinyl N-4 of B24B (i.e. B24C, B27J-L and B28A-C) causes a decrease in activity.

Comparisons between B24B and B36, as well as between B24C and B37, indicates that replacement of the ethyl group by a vinyl group increases effectiveness against Gram-negative organism but is less effective against Gram-positive organisms.

The physicochemical parameters and indicator variables of this set of compounds for each substituents in position 1, 6 and 7 are

Table 6 1,6,7-Trisubstituted 1,4-Dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids and Their in vitro Antibacterial Activity (Set B)



No.	R^1	R^6	R^7	min inhibitory concn μg/mL (ref. 26) *S. aureus* 209P JC-1	*E. coli* NIHI JC-2	*P. aeruginosa* Tsuchijima
B3A	C_2H_5	H	$(CH_3)_4N$-	12.5	25	>100
B3B	C_2H_5	H	$HN(CH_2CH_2)_2N$-	25	6.25	25
B3C	C_2H_5	H	$CH_3N(CH_2CH_2)_2N$-	25	6.25	25
B15A	C_2H_5	Cl	$(CH_3)_4N$-	12.5	>100	>100
B15B	C_2H_5	Cl	$HN(CH_2CH_2)_2N$-	3.13	0.78	6.25
B15C	C_2H_5	Cl	$CH_3N(CH_2CH_2)_2N$-	6.25	1.56	12.5
B18A	C_2H_5	CN	$(CH_3)_4N$-	3.13	12.5	25
B18B	C_2H_5	CN	$HN(CH_2CH_2)_2N$-	6.25	1.56	6.25
B18C	C_2H_5	CN	$CH_3N(CH_2CH_2)_2N$-	12.5	1.56	12.5
B22A	C_2H_5	O_2N	$(CH_3)4N$-	25	100	>100
B22B	C_2H_5	O_2N	$HN(CH_2CH_2)2N$-	6.25	6.25	25
B22C	C_2H_5	O_2N	$CH_3N(CH2CH_2)_2N$-	12.5	3.13	50
B23A	C_2H_5	H2N	$(CH_3)_4N$-	>100	>100	>100
B23B	C_2H_5	H_2N	$HN(CH_2CH_2)_2N$-	>100	3.13	6.25
B23C	C_2H_5	H_2N	$CH_3N(CH_2CH_2)_2N$-	25	1.56	12.5
B24A	C_2H_5	F	$(CH_3)_4N$-	0.39	1.56	3.13
B24B	C_2H_5	F	$HN(CH_2CH2)_2N$-	0.78	0.2	0.78
B24C	C_2H_5	F	$CH_3N(CH_2CH_2)_2N$-	1.56	0.39	1.56
B27A	C_2H_5	F	H_2N-	>100	1.56	50
B27B	C_2H_5	F	$H_2NCH_2CH_2NH$-	25	6.25	50
B27C	C_2H_5	F	3-oxo-1-piperazinyl	6.25	0.78	12.5
B27D	C_2H_5	F	$(CH_2)_5N$-	0.78	6.25	12.5
B27E	C_2H_5	F	$O(CH2CH_2)_2N$-	1.56	3.13	6.25
B27F	C_2H_5	F	$S(CH_2CH_2)_2N$-	1.56	3.13	12.5
B27G	C_2H_5	F	homopiperazinyl	1.56	0.78	1.56
B27H	C_2H_5	F	1-azepinyl	3.13	6.25	50
B27I	C_2H_5	F	$(CH_2)_7N$-	12.5	25	>100
B27J	C_2H_5	F	$Ph\text{-}N(CH_2CH_2)_2N$-	6.25	12.5	>100
B27K	C_2H_5	F	$PhCH_2N(CH_2CH_2)_2N$-	0.78	6.25	12.5
B27L	C_2H_5	F	$Et\text{-}N(CH_2CH_2)_2N$-	0.78	0.78	3.13
B28A	C_2H_5	F	$n\text{-}Pr\text{-}N(CH_2CH_2)_2N$-	1.56	1.56	12.5
B28B	C_2H_5	F	$n\text{-}Bu\text{-}N(CH_2CH_2)_2N$-	3.13	3.13	25
B28C	C_2H_5	F	$s\text{-}Bu\text{-}N(CH_2CH_2)_2N$-	1.56	3.13	25
B29	C_2H_5	F	$OHC\text{-}N(CH_2CH_2)_2N$-	3.13	1.56	12.5
B30	C_2H_5	F	$CH_3CON(CH_2CH_2)_2N$-	3.13	3.13	50
B36	CH_2=CH	F	$HN(CH_2CH_2)_2N$-	1.56	0.1	0.2
B37	CH_2=CH	F	$CH_3N(CH_2CH_2)_2N$-	3.13	0.2	0.78
B38	FCH_2CH_2	F	$HN(CH_2CH_2)_2N$-	0.39	0.2	0.78
B39	FCH_2CH_2	F	$CH_3N(CH_2CH_2)_2N$-	0.78	0.2	1.56
B40	F_2CH	F	$HN(CH_2CH_2)_2N$-	6.25	0.78	6.25
BNA	C_2H_5	H	CH_3	50	1.56	50
PPA	Pipemidic acid			6.25	1.56	6.25

Table 7 Physicochemical Parameters and Indicator Variables in Position 1 of Set B

NO.	F(1)[a]	MR(1)	L(1)	B1(1)	B5(1)	IE(1)[b]	IV(1)[c]	IEF(1)[d]	ICF(1)[e]
B3A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B3B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B3C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B15A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B15B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B15C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B18A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B18B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B18C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B22A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B22B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B22C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B23A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B23B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B23C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B24A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B24B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B24C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27D	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27E	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27F	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27G	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27H	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27I	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27J	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27K	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B27L	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B28A	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B28B	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B28C	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B29	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B30	1.405	1.0163	4.11	1.52	3.17	1	0	0	0
B36	.861	.9909	4.29	1.60	3.09	0	1	0	0
B37	.861	.9909	4.29	1.60	3.09	0	1	0	0
B38	1.128	1.0310	4.70	1.52	3.17	0	0	1	0
B39	1.128	1.0310	4.70	1.52	3.17	0	0	1	0
B40	1.322	.5035	3.30	1.71	2.61	0	0	0	1
BNA	1.405	1.0163	4.11	1.52	3.17	1	0	0	0

[a]Calculated lipophilicity of the substituent; [b]C_2H_5; [c]$CH{=}CH_2$; [d]CH_2CH_2F; [e]CHF_2

Table 8 Physicochemical Parameters and Indicator Variables in Position 6 of Set B

NO.	F(6)[a]	MR(6)	L(6)	B1(6)	B5(6)	IF(6)[b]	ICN(6)[c]	INO(6)[d]	ICL(6)[e]	INH(6)[f]	BPJ[g]
B3A	.227	.0887	2.06	1.00	1.00	0	0	0	0	0	.512
B3B	.227	.0887	2.06	1.00	1.00	0	0	0	0	0	.512
B3C	.227	.0887	2.06	1.00	1.00	0	0	0	0	0	.512
B15A	.940	.5801	3.52	1.80	1.80	0	0	0	1	0	.627
B15B	.940	.5801	3.52	1.80	1.80	0	0	0	1	0	.627
B15C	.940	.5801	3.52	1.80	1.80	0	0	0	1	0	.627
B18A	-.340	.5664	4.23	1.60	1.60	0	1	0	0	0	.779
B18B	-.340	.5664	4.23	1.60	1.60	0	1	0	0	0	.779
B18C	-.340	.5664	4.23	1.60	1.60	0	1	0	0	0	.779
B22A	-.030	.8142	3.44	1.70	2.44	0	0	1	0	0	.758
B22B	-.030	.8142	3.44	1.70	2.44	0	0	1	0	0	.758
B22C	-.030	.8142	3.44	1.70	2.44	0	0	1	0	0	.758
B23A	-1.000	.4574	2.78	1.35	1.97	0	0	0	0	1	1.420
B23B	-1.000	.4574	2.78	1.35	1.97	0	0	0	0	1	1.420
B23C	-1.000	.4574	2.78	1.35	1.97	0	0	0	0	1	1.420
B24A	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B24B	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B24C	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27A	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	1.088
B27B	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	1.088
B27C	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27D	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27E	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27F	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27G	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27H	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27I	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27J	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27K	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B27L	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B28A	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B28B	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B28C	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B29	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B30	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B36	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B37	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B38	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B39	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
B40	.370	.1042	2.65	1.35	1.35	1	0	0	0	0	.627
BNA	.227	.0887	2.06	1.00	1.00	0	0	0	0	0	.000

[a]Calculated lipophilicity of the substituent; [b]Fluorine; [c]CN; [d]NO_2; [e]Chlorine; [f]NH_2;

[g]σ-ρ electronic potentical interactions between position 6 and 7

Table 9 Physicochemical Parameters and Indicator Variables in Position 7 of Set B

NO.	F(7)[a]	MR(7)	RI1(7)[b]	RI2(7)[c]	RI3(7)[d]	RI4(7)[e]	IHCO(7)[f]	ICH3(7)[g]	IRH1(7)[h]
B3A	1.216	2.1352	0	1	0	0	0	0	0
B3B	-.100	2.5039	1	0	0	0	0	0	1
B3C	.756	2.9677	1	0	0	0	0	1	0
B15A	1.216	2.1352	0	1	0	0	0	0	0
B15B	-.100	2.5039	1	0	0	0	0	0	1
B15C	.756	2.9677	1	0	0	0	0	1	0
B18A	1.216	2.1352	0	1	0	0	0	0	0
B18B	-.100	2.5039	1	0	0	0	0	0	1
B18C	.756	2.9677	1	0	0	0	0	1	0
B22A	1.216	2.1352	0	1	0	0	0	0	0
B22B	-.100	2.5039	1	0	0	0	0	0	1
B22C	.756	2.9677	1	0	0	0	0	1	0
B23A	1.216	2.1352	0	1	0	0	0	0	0
B23B	-.100	2.5039	1	0	0	0	0	0	1
B23C	.756	2.9677	1	0	0	0	0	1	0
B24A	1.216	2.1352	0	1	0	0	0	0	0
B24B	-.100	2.5039	1	0	0	0	0	0	1
B24C	.756	2.9677	1	0	0	0	0	1	0
B27A	-1.000	.4574	0	0	0	0	0	0	0
B27B	-.964	1.7537	0	0	0	0	0	0	0
B27C	-.432	2.5396	0	0	0	0	1	0	0
B27D	1.775	2.5990	0	0	1	0	0	0	0
B27E	.132	2.2883	0	0	0	1	0	0	0
B27F	.852	2.9415	0	0	0	0	0	0	0
B27G	-.003	2.9677	0	0	0	0	0	0	1
B27H	1.902	2.9866	0	0	0	0	0	0	0
B27I	2.893	3.5266	0	0	0	0	0	0	0
B27J	2.669	5.0151	1	0	0	0	0	0	0
B27K	2.538	5.4789	1	0	0	0	0	0	0
B27L	1.205	3.4315	1	0	0	0	0	0	0
B28A	1.654	3.8953	1	0	0	0	0	0	0
B28B	2.103	4.3591	1	0	0	0	0	0	0
B28C	1.883	4.3591	1	0	0	0	0	0	0
B29	-.464	3.0034	1	0	0	0	1	0	0
B30	.040	3.4672	1	0	0	0	1	0	0
B36	-.100	2.5039	1	0	0	0	0	0	1
B37	.756	2.9677	1	0	0	0	0	1	0
B38	-.100	2.5039	1	0	0	0	0	0	1
B39	.756	2.9677	1	0	0	0	0	1	0
B40	-.100	2.5039	1	0	0	0	0	0	1
BNA	.876	.5525	0	0	0	0	0	0	0

[a]Calculated lipophilicity of the substituent; ring indicator of [b]$HN(CH_2CH_2)_2N$-; [c]$(CH_2)_4N$-; [d]$(CH_2)_5N$-; [e]$O(CH_2CH_2)_2N$-;
[f]An indicator of an amide nitrogen for 7-N-heterocyclic substituents;
[g]An indicator of methyl group in piperazinyl ring; [h]An indicator of hydrogen in piperazinyl ring

shown in Tables 7-9. The sum terms and square terms of this set included in statistical analysis are the same as described in first set of quinoline derivatives.

III. Set C

The structures and *in vitro* antibacterial activities for a series of 1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids with substituted azetidinyl, pyrrolidinyl and piperidinyl rings at position 7, fluorine at position 6, and ethyl, vinyl or 2-fluoroethyl on the dihydro pyridine nitrogen (position 1) against Gram-positive (*S. aureus* 209P JC-1) and Gram-negative bacteria (*E. coli* NIHJ JC-2 and *P. aeruginosa* Tsuchijima) are summarized in Table 10. (27) The data for enoxacin (D2) are included for comparison.

The replacement of the piperazinyl group at position 7 of D2 by the 3-aminopyrrolidinyl group (D33A) causes an enhancement in activity against all the bacteria tested. The replacement of 3-amino pyrrolidinyl ring by a larger member ring, such as 3- and 4-amino piperidine (D49A and D50A), results in a retention, or increase in activity against *S. aureus*, whereas it causes a decrease in activity against *P. aeruginosa*. The replacement by a smaller ring such as 3-aminoazetidine (D28A) shows the same level of activity as that of D2 against all the organisms.

Introduction of an alkyl group such as a methyl, ethyl, trifluoroethyl or propyl group to the amino nitrogen atom on the pyrrolidinyl ring of D33A (giving D34A-D37A) generally reduces the activity against the organisms in the same order. Acylation of the

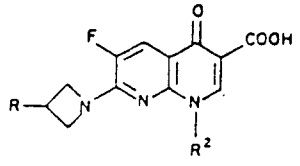
amino group on the pyrrolidinyl ring, giving D39A-42A, results in a decrease in activity.

The replacement of the amino group of D28A, D33A, D49A and D50A by a hydroxyl group (giving D30A, D46A, D55A and D56A respectively causes a significant decrease against Gram-negative activity compared with the corresponding amino-substituted compounds. Alkylation or formylation of the hydroxyl group (giving D31A, D32A and D47A) reduces further the activity against Gram-negative bacteria. Among 3-aminopiperidines (D49A-D56A), compound D50A is more active than that of the other compounds.

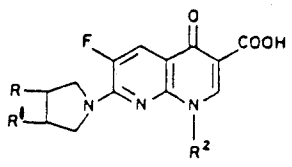
The effect of varying the N-substituent at position 1 can be seen when the 7-substituent is kept constant using the most active substituent, 3-aminopyrrolidinyl. Introduction of a vinyl group (D33B) enhances Gram-negative activity without a decrease of Gram-positive activity. Introduction of a fluoroethyl group (D33C) reduces Gram-positive activity, whereas Gram-negative activity remains unchanged. Either alkylation or acylation of compounds D33B-C (giving D34B-C and D42B-C) causes a decrease in activity. In each comparison between the ethyl compounds (series A of set C) and their vinyl analogues (series B of set C) of D28, D33, D34, D36, D38-40 and D42, the vinyl group enhances Gram-negative activity, whereas it reduces Gram-positive activity.

The physicochemical parameters and indicator variables for positions 1 and 7 which were included in the analysis are shown in Tables 11-12. The squared terms of log P [$F(7)^2$], molar refractivity [$MR(7)^2$] were used for analysis. The square terms of lipophilicity

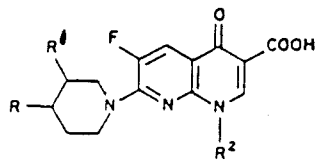
Table 10 1,7-Disubstituted 6-Fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic Acids and Their in vitro Antibacterial Activity (Set C)



D28-32



D33-48



D49-56

min inhibitory concn μg/mL (ref. 27)

No.	R	R′	R^2	*S. aureus* 209P JC-1	*E. coli* NIHJ JC-2	*P. aeruginosa* Tsuchijima
D2	Enoxacin			0.78	0.2	0.78
D28A	H_2N		C_2H_5	0.78	0.1	0.78
D28B	H_2N		CH_2=CH	1.56	0.1	0.39
D29A	$O(CH_2CH_2)_2N$		C_2H_5	1.56	6.25	50
D30A	HO		C_2H_5	0.78	0.78	3.13
D31A	CH_3O		C_2H_5	0.78	3.13	6.25
D32A	C_2H_5O		C_2H_5	0.78	3.13	25
D33A	H_2N	H	C_2H_5	0.2	0.1	0.39
D33B	H_2N	H	CH_2=CH	0.2	0.025	0.2
D33C	H_2N	H	FCH_2CH_2	0.39	0.1	0.39
D34A	CH_3NH	H	C_2H_5	0.39	0.2	1.56
D34B	CH_3NH	H	CH_2=CH	0.78	0.1	0.78
D34C	CH_3NH	H	FCH_2CH_2	0.78	0.2	0.78
D35A	C_2H_5NH	H	C_2H_5	0.78	0.78	3.13
D36A	CF_3CH_2NH	H	C_2H_5	0.78	1.56	50
D36B	CF_3CH_2NH	H	CH_2=CH	1.56	6.25	100
D37A	C_3H_7NH	H	C_2H_5	25	6.25	>100
D38A	$(CH_3)_2N$	H	C_2H_5	1.56	0.78	6.25
D38B	$(CH_3)_2N$	H	CH_2=CH	3.13	0.39	3.13
D38C	$(CH_3)_2N$	H	FCH_2CH_2	0.78	0.2	6.25
D39A	OHCNH	H	C_2H_5	0.78	0.78	3.13
D39B	OHCNH	H	CH_2=CH	0.78	0.39	3.13
D40A	CH_3CONH	H	C_2H_5	0.78	6.25	6.25
D40B	CH_3CONH	H	CH_2=CH	1.56	0.78	12.5
D40C	CH3CONH	H	FCH_2CH_2	1.56	1.56	25
D41A	CF_3CONH	H	C_2H_5	0.78	1.56	6.25
D42A	$CH_3CON(CH_3)$	H	C_2H_5	0.78	6.25	25
D42B	$CH_3CON(CH_3)$	H	CH_2=CH	1.56	3.13	25
D42C	$CH_3CON(CH_3)$	H	FCH_2CH_2	0.39	1.56	25
D43A	H_2NNH	H	C_2H_5	0.39	1.56	12.5
D44A	H_2NCONH	H	C_2H_5	6.25	3.13	25
D45A	H_2N	HO	C_2H_5	1.56	1.56	3.13
D46A	HO	H	C_2H_5	0.39	0.78	0.78
D47A	OHCO	H	C_2H_5	0.39	1.56	3.13
D48A	Cl	H	C_2H_5	0.2	0.39	12.5
D49A	H	H_2N	C_2H_5	0.78	0.78	6.25
D50A	H_2N	H	C_2H_5	0.2	0.2	1.56
D51A	C_6H_5CONH	H	C_2H_5	1.56	12.5	>100
D52A	H_2NCH_2	H	C_2H_5	0.39	1.56	12.5
D53A	$CH_3CONHCH_2$	H	C_2H_5	0.78	1.56	25
D54A	H	H_2NCO	C_2H_5	6.25	12.5	50
D55A	H	HO	C_2H_5	1.56	3.13	25
D56A	HO	H	C_2H_5	0.78	3.13	6.25

Table 11 Physicochemical Parameters and Indicator Variables in Position 1 of Set C

NO.	F(1)[a]	MR(1)	L(1)	B1(1)	B5(1)	IE(1)[b]	IEF(1)[c]	IV(1)[d]
D28A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D28B	.861	.9909	4.29	1.60	3.09	0	0	1
D29A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D30A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D31A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D32A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D33A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D33B	.861	.9909	4.29	1.60	3.09	0	0	1
D33C	1.120	1.0318	4.70	1.52	3.17	0	1	0
D34A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D34B	.861	.9909	4.29	1.60	3.09	0	0	1
D34C	1.128	1.0318	4.70	1.52	3.17	0	1	0
D35A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D36A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D36B	.861	.9909	4.29	1.60	3.09	0	0	1
D37A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D38A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D38B	.861	.9909	4.29	1.60	3.09	0	0	1
D38C	1.120	1.0318	4.70	1.52	3.17	0	1	0
D39A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D39B	.861	.9909	4.29	1.60	3.09	0	0	1
D40A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D40B	.861	.9909	4.29	1.60	3.09	0	0	1
D40C	1.128	1.0318	4.70	1.52	3.17	0	1	0
D41A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D42A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D42B	.861	.9909	4.29	1.60	3.09	0	0	1
D42C	1.128	1.0318	4.70	1.52	3.17	0	1	0
D43A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D44A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D45A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D46A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D47A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D48A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D49A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D50A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D51A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D52A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D53A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D54A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D55A	1.405	1.0163	4.11	1.52	3.17	1	0	0
D56A	1.405	1.0163	4.11	1.52	3.17	1	0	0

[a]Calculated lipophilicity of the substituents; [b]C_2H_5; [c]CH_2CH_2F; [d]$CH{=}CH_2$

Table 12 Physicochemical Parameters and Indicator Variables in Position 7 of Set C

NO.	F(7)[a]	MR(7)	FR(7)[b]	MRR(7)[c]	LR(7)[d]	B1R(7)[e]	B5R(7)[f]	R11(7)[g]	R12(7)[h]	R13(7)[i]	PROX1(7)[j]	INCO(7)[k]
D28A	-.194	1.7601	-1.7600	.4574	2.78	1.35	1.97	0	1	0	1.136	0
D28B	-.194	1.7601	-1.7600	.4574	2.78	1.35	1.97	0	1	0	1.136	0
D29A	.310	3.8710	.2060	2.2883	5.29	1.35	3.42	0	1	0	.934	0
D30A	-.248	1.8245	-1.8600	.2418	2.74	1.35	1.93	0	1	0	1.182	0
D31A	.410	2.2883	-1.2040	.7056	3.98	1.35	3.07	0	1	0	1.264	0
D32A	.939	2.7521	-.7550	1.1694	4.80	1.35	3.36	0	1	0	1.264	0
D33A	-.203	2.5039	-1.7600	.4574	2.78	1.35	1.97	1	0	0	.568	0
D33B	-.203	2.5039	-1.7600	.4574	2.78	1.35	1.97	1	0	0	.568	0
D33C	-.203	2.5039	-1.7600	.4574	2.78	1.35	1.97	1	0	0	.568	0
D34A	.003	2.9677	-1.6140	.9212	3.53	1.35	3.08	1	0	0	.708	0
D34B	.003	2.9677	-1.6140	.9212	3.53	1.35	3.08	1	0	0	.708	0
D34C	.003	2.9677	-1.6140	.9212	3.53	1.35	3.08	1	0	0	.708	0
D35A	.612	3.4315	-1.0050	1.3050	4.03	1.35	3.42	1	0	0	.708	0
D36A	1.371	2.0460	-.3260	1.0464	5.26	1.35	4.00	1	0	0	.708	0
D36B	1.371	2.0460	-.3260	1.0464	5.26	1.35	4.00	1	0	0	.708	0
D37A	1.141	3.0953	-.5560	1.0400	6.07	1.35	4.47	1	0	0	.708	0
D38A	.656	3.4315	-1.0480	1.3850	3.53	1.35	3.08	1	0	0	.715	0
D38B	.656	3.4315	-1.0480	1.3850	3.53	1.35	3.08	1	0	0	.715	0
D38C	.656	3.4315	-1.0480	1.3850	3.53	1.35	3.08	1	0	0	.715	0
D39A	-.172	3.0034	-1.7200	.9569	4.22	1.35	3.61	1	0	0	.559	1
D39B	-.172	3.0034	-1.7200	.9569	4.22	1.35	3.61	1	0	0	.559	1
D40A	-.348	3.4672	-2.5889	1.4207	5.09	1.35	3.61	1	0	0	.837	1
D40B	-.348	3.4672	-2.5889	1.4207	5.09	1.35	3.61	1	0	0	.837	1
D40C	-.348	3.4672	-2.5889	1.4207	5.09	1.35	3.61	1	0	0	.837	1
D41A	.761	3.5137	-1.0650	1.4672	5.62	1.79	3.61	1	0	0	.837	1
D42A	-.121	3.9310	-2.0580	1.8845	4.77	1.35	3.71	1	0	0	.940	1
D42B	-.121	3.9310	-2.0580	1.8845	4.77	1.35	3.71	1	0	0	.940	1
D42C	-.121	3.9310	-2.0580	1.8845	4.77	1.35	3.71	1	0	0	.940	1
D43A	-.511	2.8726	-2.1600	.8261	3.47	1.35	2.97	1	0	0	.660	0
D44A	-.696	3.3721	-2.4000	1.3256	5.06	1.35	3.61	1	0	0	.715	1
D45A	-.872	2.6570	-1.7600	.4574	2.78	1.35	1.97	1	0	0	.981	0
D46A	-.280	2.2883	-1.8600	.2418	2.74	1.35	1.93	1	0	0	.591	0
D47A	.105	2.7870	-1.3600	.7413	3.53	1.60	2.36	1	0	0	.476	1
D48A	1.399	2.6266	.0600	.5801	3.52	1.80	1.80	1	0	0	.350	0
D49A	.356	2.9677	.2270	.0887	2.06	1.00	1.00	0	0	1	.000	0
D50A	-.212	2.9677	-1.7600	.4574	2.78	1.35	1.97	0	0	1	.000	0
D51A	1.313	6.2445	-.2350	3.4601	8.30	1.53	3.84	0	0	1	.000	1
D52A	.407	3.4315	-1.1410	.9212	4.02	1.52	3.05	0	0	1	.000	0
D53A	-.007	4.3940	-1.5550	1.8845	5.67	1.52	4.75	0	0	1	.000	1
D54A	-.083	3.4672	.2270	.0887	2.06	1.00	1.00	0	0	1	.000	1
D55A	.279	2.7521	.2270	.0887	2.06	1.00	1.00	0	0	1	.000	0
D56A	-.312	2.7521	-1.8600	.2418	2.74	1.35	1.93	0	0	1	.000	0

[a]Calculated lipophilicity of the substituents; [b]Calculated lipophilicity, [c]MR, [d]L, [e]B1, [f]B5 of R-substituents; ring indicator of [g]$(CH_2)_4N$-; [h]$(CH_2)_3N$-; [i]$(CH_2)_5N$-; [j]Proximity effect between ring and R-substituent; [k]An indicator of an amide nitrogen for 7-N-heterocyclic substituents

[$FR(7)^2$], molar refractivity [$MRR(7)^2$], L [$LR(7)^2$], B1 [$B1R(7)^2$], and B5 [$B5R(7)^2$] of the R-substituent in position 7 were included as independent variables.

The minimum inhibitory concentration (MIC) (μg/mL) of these three sets of compounds was determined by means of a standard twofold dilution method using agar media (pH = 7.4). Based on the twofold dilution method the MIC greater than 100 (μg/mL) was replaced by using 200 (μg/mL) in the statistical analysis in order to force the model to include inactive compounds.

RESULTS AND DISCUSSION

I. Set A

For the first set of 6,7-disubstituted 1-alkyl-1,4-dihydro 4-oxoquinoline-3-carboxylic acids (23), the regression analysis was performed on a modified data set for S. aureus, E. coli and P. aeruginosa. (In Table 2, nine compounds were not included: A46 and A47 because they are the only two compounds having substituents in position 8; A64 and A80 because they are inactive in the three bacterial systems; A81 and A82 because they are ester derivatives; A1, A83 and A84 because they do not belong to the quinoline ring system.) The development of the LFER models for S. aureus and P. aeruginosa are shown in Table 13 and Table 15. (Table 15, n = 41. Two compounds were deleted; A34 because it was too active relative to the other compounds and A43 because ICO(6) which entered into an earlier model occurs only in one compound.) A significant LFER model for the E. coli could not be obtained ($r^2 = 0.558$, $F_{6,35} = 7.340$).

The observed activity, calculated activity, residuals and standardized residuals for S. aureus and P. aeruginosa based on the statistically acceptable models are shown in Table 14 (eq. 6) and Table 16 (eq. 7), respectively. The correlation matrix of the entire data set is shown in Table 17.

The regression was repeated for 41 compounds (A59 and A78 dropped). The same independent variables appeared in the model that was derived as eq. 6 (Table 13), but another two outliers (A63 and A3) (standardized residual >2.000) appeared. Because it is difficult

to rationalize dropping the initial outliers A59 and A78, it was decided to stay with eq. 6 (Table 13).

Three outliers appeared from eq. 7 (Table 15). The regression was repeated for 38 compounds (A37, A74 and A40 dropped). The same independent variables appeared in the model that was derived as eq. 7 (Table 15). The r^2 and F value are more significant ($n = 38$, $r^2 = 0.829$, $F_{7,30} = 20.796$), but there were another two outliers (A61 and A67). As before, there was no valid reason for dropping these outliers. Therefore it was decided to stay with eq. 7 (Table 15).

For S. aureus eq. 6 (Table 13) indicates that lipophilicity and molar refractivity of the substituents at position 6 are important determinants of activity. For the substituents at position 7, there is a parabolic relationship seen with these same descriptors. Comparsion of eq. 6 for S. aureus with eq. 7 for P. aeruginosa (Table 15) indicates a different QSAR. An ethyl substituent in position 1, minimum width, B1, in position 6 and the presence of a piperazinyl ring in position 7 appear in eq. 7 (Table 15). The parabolic relationship of lipophilicity and MR in eq. 7 (Table 15) is similar to that seen with in eq. 6 (Table 13). The statistically most significant model for this set of compounds was seen in the S. aureus test system.

A subset of 24 compounds (A18, A32-34, A36, A55-63, A68-75, A78-79) containing only a fluorine at position 6 and ethyl at position 1 was selected in order to better understand just what descriptors were important for activity at position 7. LFER models for three

bacterial test systems were derived and are shown in Tables 18, 20, and 23.

Eq. 9 (Table 18) for S. aureus indicates that only the presence of an amide nitrogen and $\sigma-\rho$ electronic interactions are important determinants. An amide nitrogen (INCO(7)) in position 7 reduces activity, and there is a parabolic relationship of $\sigma-\rho$ electronic interactions between position 6 and position 7 (API) in eq. 9. This nonlinear results for the latter could be due to the distribution of the $\sigma-\rho$ terms in this subset. Two compounds (A18 and A32) have a $\sigma-\rho$ interaction equal to 0.0, 19 compounds have an interaction term equal to 0.171 and three an interaction term equal to 0.302.

This same subset in the E. coli test system (Table 20, eq. 9) indicates that an amide nitrogen and lipophilicity in position 7 are negative factors, but that the presence of a piperazinyl ring enhances activity. But the significance of the latter coefficient is slightly greater than 0.05 (P = 0.0578). Dropping this indicator variable gives a model (eq. 10) that is statistically less significant as measured by r^2 and standard error s.

The parabolic relationship for $\sigma-\rho$ electronic interaction is significant for both S. aureus and E. coli. In contrast the LFER model for P. aeruginosa (Table 23, eq. 4, n = 23) indicates that only lipophilicity and molar refractivity are important determinants of activity. In the initial analysis for this subset using the P. aeruginosa test system, A34 (norfloxacin) was an outlier. Because it is the only compound showing such high activity, it was deleted.

The observed activity, calculated activity, residuals and

standardized residuals of the subset are shown in Table 19 (eq. 9), Table 21 (eq. 9), Table 22 (eq. 10) and Table 24 (eq. 4). The correlation matrix for this subset is shown in Table 25.

The stability of the regression coefficient found in eq. 6 (Table 13), eq. 7 (Table 15), and eq. 10 (Table 20) was checked by omitting compounds selected by a random number generator. For the models derived form 43 observations, six randomly selected compounds were omitted three times giving eqs. 1-3 (Table 26) which should be compared to eq. 6 (Table 13). For the models derived from 41 observations, five randomly selected compounds were omitted three times giving eqs. 4-6 (Table 26) which should be compared to eq. 7 (Table 15). A similar procedure except three compounds were deleted each time was done for the E. coli data on the subset of 24 compounds giving eqs. 7-9 for eq. 10 (Table 20). Similar results were obtained in each set, although there was some noise in the coefficient.

Many of the compounds listed in Table 2 were the same as those evaluated by Koga (25) on his QSAR study. This provided a means of comparing the two QSAR. Thus 36 compounds (Table 2, A3, A18, A32, A34, A36-42, A44-45, A48, A51-54, A56-62, A67-76, A79) active against E. coli were selected for regression analysis in order to compare the LFER model using the independent variables chose in this project with those of Koga (25).

No statistically valid model could be obtained on this set of 36 compounds using the independent variables in this study. When using Koga's variables (Es(6), $\Sigma\pi(6,7,8)$, $Es(6)^2$, $\Sigma\pi(6,7,8)^2$ and I(7N-CO)) on the these 36 compounds, r^2 only explained 66% of the variation.

Table 13 LFER Model Development for Set A Against S. aureus

Eq. No.	Log SA = Intercept	F(6)	MR(6)	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	r^2	F	d.f.
1.	1.977 (±0.111)			0.546 (±0.127)				0.312	18.581	(1,41)
2.	2.081 (±0.110)			0.916 (±0.178)	-0.284 (±0.103)			0.422	14.581	(2,40)
3.	1.344 (±0.261)			0.806 (±0.166)	-0.347 (±0.096)	0.296 (±0.097)		0.534	14.879	(3,39)
4.	1.577 (±0.241)		-0.880 (±0.258)	0.822 (±0.147)	-0.398 (±0.086)	0.304 (±0.086)		0.643	17.127	(4,38)
5.	0.766 (±0.338)		-0.966 (±0.234)	0.846 (±0.133)	-0.323 (±0.082)	0.918 (±0.212)	-0.106 (±0.034)	0.717	18.754	(5,37)
6.	0.549 (±0.319)	0.699 (±0.242)	-1.116 (±0.220)	0.812 (±0.119)	-0.314 (±0.073)	0.899 (±0.194)	-0.103 (±0.031)	0.771	20.131	(6,26)

N = 43 s = 0.419

Table 14 Comparison of Observed and Calculated MIC's from Eq. 6 (Table 13)

No.	Observed	Calculated	Residual	Std. residual[a]
A18	1.333	1.663	-.330	-.849
A32	1.600	1.626	-.026	-.066
A33	.097	-.045	.142	.366
A34	2.914	2.212	.701	1.807
A36	2.932	2.887	.045	.116
A37	2.333	2.080	.253	.651
A38	2.351	2.754	-.403	-1.039
A39	2.084	1.866	.218	.562
A40	2.402	2.540	-.138	-.356
A41	2.003	2.066	-.063	-.163
A42	1.143	1.103	.039	.101
A43	.535	.663	-.128	-.331
A44	1.417	1.201	.216	.557
A45	1.141	1.141	.000	.000
A48	1.688	2.212	-.524	-1.350
A49	2.334	2.212	.122	.314
A50	2.955	2.887	.068	.175
A51	2.332	2.212	.119	.307
A52	2.330	2.212	.117	.302
A53	2.024	2.212	-.188	-.485
A54	2.388	2.212	.176	.453
A55	2.551	2.025	.526	1.356
A56	3.182	2.664	.518	1.335
A57	2.611	2.783	-.173	-.445
A58	2.613	2.311	.302	.778
A59	2.932	2.102	.830	2.138
A60	2.365	1.745	.620	1.597
A61	2.967	2.959	.007	.019
A62	2.027	1.901	.126	.324
A63	.166	.877	-.711	-1.832
A67	2.006	2.212	-.207	-.533
A68	2.951	3.086	-.136	-.349
A69	2.668	2.667	.000	.000
A70	2.963	3.143	-.181	-.466
A71	3.020	2.559	.461	1.189
A72	2.463	2.490	-.026	-.068
A73	2.347	2.019	.328	.845
A74	2.666	2.473	.192	.496
A75	2.433	3.158	-.725	-1.868
A76	2.327	2.887	-.560	-1.442
A78	.224	1.140	-.916	-2.360
A79	3.011	2.956	.055	.141
A3	1.382	2.130	-.748	-1.927

[a]Standardized residual

Table 15 LFER Model Development for Set A Against P. aeruginosa

Eq. No.	Log PA = Intercept	IE(1)	B1(6)	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	RI1(7)	r^2	F	d.f.
1.	1.521 (±0.117)			-0.096 (±0.131)					0.014	0.543	(1,39)
2.	1.658 (±0.109)			0.373 (±0.172)	-0.362 (±0.100)				0.268	6.965	(2,38)
3.	2.306 (±0.163)	-0.895 (±0.191)		0.338 (±0.138)	-0.284 (±0.082)				0.542	14.586	(3,37)
4.	1.759 (±0.243)	-0.879 (±0.175)		0.259 (±0.130)	-0.332 (±0.077)	0.215 (±0.075)			0.627	15.115	(4,36)
5.	1.053 (±0.341)	-0.818 (±0.162)		0.279 (±0.119)	-0.274 (±0.073)	0.702 (±0.191)	-0.084 (±0.031)		0.693	15.729	(5,35)
6.	1.004 (±0.333)	-0.705 (±0.171)		0.232 (±0.120)	-0.244 (±0.074)	0.580 (±0.199)	-0.074 (±0.030)	0.303 (±0.178)	0.717	14.323	(6,34)
7.	2.082 (±0.176)	-0.524 (±0.176)	-0.904 (±0.371)	0.239 (±0.112)	-0.259 (±0.069)	0.584 (±0.186)	-0.081 (±0.028)	0.479 (±0.181)	0.760	14.964	(7,33)

N = 41 s = 0.375

Table 16 Comparison of Observed and Calculated MIC's from Eq. 7 (Table 15)

No.	Observed	Calculated	Residual	Std. residual[a]
A18	.431	.646	-.215	-.632
A32	.697	.647	.050	.147
A33	.097	.090	.007	.019
A36	2.330	1.871	.459	1.348
A37	2.030	1.339	.691	2.029
A38	1.146	1.464	-.318	-.935
A39	1.483	1.204	.279	.820
A40	.595	1.329	-.733	-2.155
A41	1.703	1.592	.111	.326
A42	1.444	1.430	.014	.041
A44	1.717	1.520	.197	.578
A45	1.442	1.430	.013	.038
A48	2.292	2.270	.021	.062
A49	2.636	2.270	.366	1.075
A50	2.050	2.395	-.346	-1.015
A51	2.030	2.270	-.241	-.707
A52	2.027	2.270	-.244	-.715
A53	2.327	2.270	.057	.166
A54	2.388	2.270	.118	.346
A55	.745	1.047	-.302	-.887
A56	1.386	1.124	.262	.769
A57	.804	.919	-.115	-.337
A58	1.408	1.278	.130	.382
A59	1.427	1.233	.194	.571
A60	.558	1.045	-.487	-1.432
A61	2.062	1.440	.622	1.828
A62	1.426	1.148	.278	.817
A63	.767	.643	.124	.365
A67	2.910	2.270	.640	1.879
A68	2.045	1.781	.264	.775
A69	1.764	1.892	-.127	-.374
A70	1.759	1.812	-.053	-.154
A71	.914	.590	.323	.950
A72	.356	.466	-.111	-.325
A73	1.745	1.675	.070	.205
A74	1.159	1.842	-.683	-2.006
A75	1.228	1.715	-.487	-1.431
A76	2.024	2.395	-.371	-1.090
A78	.224	.772	-.548	-1.611
A79	1.504	1.333	.171	.503
A3	1.983	2.062	-.079	-.233

[a]Standardized residual

Table 17 Correlation Matrix of the Variables Used in the Analyses of the S. aureus and P. aeruginosa Test Systems (Tables 13, 15)

	IE(1)	IF(6)	F(6)	MR(6)	B1(6)	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	RI1(7)	SA	EC	PA
IE(1)	1.000												
IF(6)	-0.283	1.000											
F(6)	0.067	-0.238	1.000										
MR(6)	0.244	-0.861	0.243	1.000									
B1(6)	0.205	-0.725	0.491	0.834	1.000								
F(7)	0.13	0.158	0.049	-0.137	-0.058	1.000							
$F(7)^2$	0.229	0.250	-0.265	-0.215	-0.159	0.752	1.000						
MR(7)	0.079	0.089	0.015	-0.078	-0.041	0.497	0.497	1.000					
$MR(7)^2$	0.142	0.156	-0.008	-0.135	-0.094	0.565	0.595	0.947	1.000				
RI1(7)	-0.339	-0.362	0.086	0.312	0.263	0.166	-0.027	0.421	0.307	1.000			
SA	-0.077	0.320	0.216	-0.334	-0.119	0.558	0.202	0.500	0.407	0.203	1.000		
EC	-0.328	0.162	0.258	-0.222	-0.041	0.111	-0.247	0.087	-0.011	0.260	0.676	1.000	
PA	-0.449	0.103	0.014	-0.173	-0.152	-0.119	-0.354	0.069	-0.071	0.465	0.414	0.733	1.000

Table 18 LFER Model Development for a Subset (Ethyl at Position 1; Fluorine at Position 6) Against S. aureus

Eq. No.	Log SA = Intercept	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	INCO(7)	API	API^2	r^2	F	d.f.
1.	2.050 (±0.179)	0.500 (±0.159)							0.310	9.875	(1,22)
2.	2.275 (±0.176)	0.928 (±0.206)	-0.340 (±0.121)						0.498	10.404	(2,21)
3.	1.530 (±0.281)	0.818 (±0.177)	-0.412 (±0.105)	0.306 (±0.098)					0.622	13.067	(3,20)
4.	0.676 (±0.358)	0.866 (±0.148)	-0.351 (±0.089)	0.983 (±0.230)	-0.116 (±0.037)				0.778	16.613	(4,19)
5.	0.906 (±0.418)	0.698 (±0.216)	-0.283 (±0.109)	1.079 (±0.246)	-0.126 (±0.038)		-2.357 (±2.228)		0.791	13.631	(5,18)
6.	1.158 (±0.229)	0.338 (±0.129)	-0.114 (±0.064)	0.185 (±0.188)	-0.026 (±0.025)		1.158 (±3.442)	-71.952 (±10.741)	0.943	46.519	(6,17)
7.	1.272 (±0.194)	0.317 (±0.123)	-0.113 (±0.060)				1.272 (±1.962)	-71.715 (±6.844)	0.939	73.199	(4,19)
8.	1.397 (±0.167)	0.126 (±0.120)	-0.054 (±0.054)			-0.406 (±0.133)	23.611 (±1.771)	-89.709 (±6.751)	0.960	86.027	(5.18)
9.	1.467 (±0.146)					-0.474 (±0.108)	24.167 (±1.618)	-94.327 (±4.822)	0.957	149.696	(3,20)

N = 24 s = 0.207

Table 19 Comparison of Observed and Calculated MIC's from Eq. 9 (Table 18)

No.	Observed	Calculated	Residual	Std. residual[a]
A18	1.333	1.467	-.133	-.691
A32	1.600	1.467	.133	.691
A33	.097	.162	-.065	-.338
A34	2.914	2.841	.072	.376
A36	2.932	2.841	.091	.470
A55	2.551	2.841	-.290	-1.502
A56	3.182	2.841	.341	1.765
A57	2.611	2.841	-.230	-1.193
A58	2.613	2.841	-.229	-1.184
A59	2.932	2.841	.091	.470
A60	2.365	2.367	-.003	-.015
A61	2.967	2.841	.125	.650
A62	2.027	2.367	-.340	-1.764
A63	.166	.162	.003	.018
A68	2.951	2.841	.110	.568
A69	2.668	2.841	-.174	-.900
A70	2.963	2.841	.121	.629
A71	3.020	2.841	.179	.929
A72	2.463	2.841	-.378	-1.957
A73	2.347	2.367	-.021	-.106
A74	2.666	2.367	.298	1.545
A75	2.433	2.367	.066	.340
A78	.224	.162	.062	.321
A79	3.011	2.841	.170	.880

[a]Standardized residual

Table 20 LFER Model Development for a Subset (Ethyl at Position 1; Fluorine at Position 6) Against *E. coli*

Eq. No.	Log EC = Intercept	F(7)	$F(7)^2$	INCO(7)	API	API^2	RI1(7)	RI3(7)	r^2	F	d.f.
1.	2.683 (±0.156)	0.096 (±0.139)							0.021	6.954	(1.22)
2.	2.916 (±0.141)	0.538 (±0.165)	-0.352 (±0.097)						0.398	6.954	(2,21)
3.	2.916 (±0.164)	0.460 (±0.170)	-0.347 (±0.095)				0.355 (±0.243)		0.456	5.586	(3,20)
4.	2.650 (±0.188)	0.441 (±0.167)	-0.345 (±0.093)				0.504 (±0.262)	0.434 (±0.319)	0.505	4.836	(4,19)
5.	2.949 (±0.335)	0.222 (±0.263)	-0.252 (±0.119)			-8.391 (±7.812)	0.489 (±0.261)	0.353 (±0.328)	0.535	4.141	(5,18)
6.	0.333 (±0.337)	-0.120 (±0.272)	-0.156 (±0.119)	-0.705 (±0.291)		-16.959 (±7.779)	0.699 (±0.247)	0.398 (±0.291)	0.654	5.367	(6,17)
7.	2.887 (±0.287)	-0.341 (±0.218)	-0.068 (±0.095)	-0.947 (±0.233)	13.180 (±3.676)	-61.119 (±13.687)	0.296 (±0.219)	-0.082 (±0.261)	0.808	9.605	(7,16)
8.	2.879 (±0.279)	-0.326 (±0.207)	-0.075 (±0.090)	-0.940 (±0.226)	12.584 (±3.068)	-58.824 (±11.283)	0.336 (±0.177)		0.807	11.828	(6,17)
9.	2.954 (±0.262)	-0.477 (±0.102)		-1.010 (±0.208)	13.014 (±3.001)	-63.601 (±9.670)	0.353 (±0.174)		0.799	14.283	(5,18)
	N = 24	s = 0.346									
10.	2.882 (±0.280)	-0.398 (±0.102)		-0.921 (±0.219)	15.041 (±3.052)	-68.647 (±10.079)			0.753	14.532	(4,19)
	N = 24	s = 0.373									

Table 21 Comparison of Observed and Calculated MIC's from Eq. 9 (Table 20)

No.	Observed	Calculated	Residual	Std. residual[a]
A18	2.237	2.505	-.268	-.876
A32	2.804	2.536	.268	.876
A33	1.903	1.561	.342	1.117
A34	3.804	3.720	.084	.274
A36	3.523	3.312	.211	.690
A55	2.854	3.118	-.264	-.864
A56	2.893	2.739	.154	.502
A57	2.309	2.472	-.163	-.534
A58	3.204	3.257	-.053	-.172
A59	3.223	3.469	-.246	-.804
A60	2.365	2.683	-.319	-1.041
A61	3.558	3.081	.476	1.556
A62	2.932	2.516	.416	1.358
A63	1.672	1.544	.128	.417
A68	3.541	3.097	.443	1.448
A69	3.561	3.626	-.066	-.214
A70	2.963	3.143	-.180	-.588
A71	2.719	2.384	.335	1.093
A72	1.860	2.555	-.695	-2.269
A73	2.951	2.884	.067	.218
A74	2.365	2.640	-.275	-.899
A75	2.131	2.019	.111	.364
A78	1.127	1.597	-.469	-1.533
A79	3.011	3.047	-.036	-.118

[a]Standardized residual

Table 22 Comparison of Observed and Calculated MIC's from Eq. 10 (Table 20)

No.	Observed	Calculated	Residaul	Std. residual[a]
A18	2.237	2.508	-.271	-.798
A32	2.804	2.533	.271	.798
A33	1.903	1.562	.341	1.005
A34	3.804	3.487	.317	.935
A36	3.523	3.146	.377	1.111
A55	2.854	3.279	-.425	-1.253
A56	2.893	2.963	-.070	-.206
A57	2.309	2.740	-.431	-1.270
A58	3.204	3.395	-.190	-.561
A59	3.223	3.571	-.349	-1.028
A60	2.365	2.837	-.473	-1.394
A61	3.558	3.248	.310	.913
A62	2.932	2.698	.234	.689
A63	1.672	1.548	.124	.365
A68	3.541	2.967	.574	1.691
A69	3.561	3.408	.152	.449
A70	2.963	3.005	-.042	-.125
A71	2.719	2.372	.347	1.022
A72	1.860	2.514	-.654	-1.929
A73	2.951	2.711	.240	.707
A74	2.365	2.507	-.142	-.419
A75	2.131	1.989	.141	.417
A78	1.127	1.592	-.464	-1.369
A79	3.011	2.925	.086	.253

[a]Standardized residual

Table 23 LFER Model Developemnt for a Subset (Ethyl at Position 1; Fluorine at Position 6) Against P. aeruginosa

Eq. No.	Log PA = Intercept	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	r^2	F	d.f.
1.	0.766 (±0.288)			0.137 (±0.088)		0.103	2.407	(1.21)
2.	-0.081 (±0.375)			0.828 (±0.243)	-0.110 (±0.037)	0.380	6.130	(2,20)
3.	-0.073 (±0.353)		-0.149 (±0.069)	0.743 (±0.227)	-0.082 (±0.036)	0.501	6.355	(3,19)
4.	0.131 (±0.277)	0.760 (±0.114)	-0.302 (±0.069)	0.760 (±0.178)	-0.094 (±0.029)	0.709	10.971	(4,18)

N = 23 s = 0.375

Table 24 Comparison of Observed and Calculated MIC's from Eq. 4 (Table 23)

No.	Observed	Calculated	Residual	Std. residual[a]
A18	.431	.660	-.229	-.676
A32	.697	.650	.046	.137
A33	.097	-.255	.352	1.037
A36	2.330	1.698	.631	1.863
A55	.745	1.123	-.378	-1.117
A56	1.386	1.379	.007	.022
A57	.804	1.250	-.446	-1.315
A58	1.408	1.428	-.020	-.059
A59	1.427	1.354	.073	.215
A60	.558	1.132	-.575	-1.695
A61	2.062	1.798	.264	.778
A62	1.426	1.222	.204	.602
A63	.767	.498	.269	.795
A68	2.045	1.691	.354	1.044
A69	1.764	1.687	.077	.228
A70	1.759	1.751	.008	.024
A71	.914	.572	.342	1.009
A72	.356	.543	-.187	-.552
A73	1.745	1.311	.433	1.279
A74	1.159	1.567	-.407	-1.202
A75	1.228	1.674	-.446	-1.316
A78	.224	.713	-.489	-1.443
A79	1.504	1.389	.116	.341

[a]Standardized residual

Table 25 Correlation Matrix for the Subset (Ethyl at Position 1; Fluorine at Position 6; Tables 18, 20, 23)

	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	INCO(7)	RI1(7)	API	API^2	SA	EC	PA
F(7)	1.000										
$F(7)^2$	0.739	1.000									
MR(7)	0.471	0.478	1.000								
$MR(7)^2$	0.533	0.553	0.950	1.000							
INCO(7)	-0.273	-0.209	0.096	-0.008	1.000						
RI1(7)	0.411	-0.202	0.631	0.580	0.146	1.000					
API	-0.444	0.020	0.133	0.050	-0.016	-0.029	1.000				
API^2	-0.543	-0.201	-0.126	-0.134	-0.119	-0.214	0.918	1.000			
SA	0.557	0.119	0.528	0.404	0.042	0.470	-0.374	-0.683	1.000		
EC	0.146	-0.306	0.119	0.010	-0.139	0.301	-0.355	-0.555	0.736	1.000	
PA	-0.012	-0.302	0.154	0.016	-0.053	0.450	-0.075	-0.254	0.527	0.761	1.000

Table 26 Results from Random Sample Analyses (See Eq. 6, Table 13; Eq. 7, Table 15; Eq. 10, Table 20)

Eq. No.	Log SA = Intercept	F(6)	MR(6)	F(7)	$F(7))^2$	MR(7)	$MR(7)^2$	r^2	F	d.f.
1.	0.531	0.676	-1.019	0.812	-0.311	0.901	-0.103	0.751	15.108	(6,30)
	(±0.353)	(±0.345)	(±0.295)	(±0.133)	(±0.081)	(±0.209)	(±0.034)			
	N = 37		s = 0.452							
2.	0.779	0.655	-1.237	0.821	-0.307	0.850	-0.099	0.820	22.795	(6,30)
	(±0.328)	(±0.210)	(±0.194)	(±0.121)	(±0.070)	(±0.206)	(±0.034)			
	N = 37		s = 0.363							
3.	0.197	0.640	-1.155	0.860	-0.329	1.145	-0.136	0.775	17.175	(6,30)
	(±0.479)	(±0.236)	(±0.217)	(±0.138)	(±0.078)	(±0.275)	(±0.039)			
	N = 37		s = 0.406							

Eq. No.	Log PA = Intercept	IE(1)	B1(6)	F(7)	$F(7)^2$	MR(7)	$MR(7)^2$	RI1(7)	r^2	F	d.f.
4.	1.463	-0.490	-0.501	0.276	-0.266	0.607	-0.084	0.448	0.772	13.602	(7,28)
	(±0.583)	(±0.186)	(±0.400)	(±0.111)	(±0.068)	(±0.183)	(±0.028)	(±0.185)			
	N = 36		s = 0.365								
5.	2.270	-0.558	-0.951	0.315	-0.227	0.517	-0.069	0.495	0.745	11.688	(7,28)
	(±0.580)	(±0.189)	(±0.382)	(±0.128)	(±0.075)	(±0.226)	(±0.036)	(±0.185)			
	N = 36		s = 0.379								
6.	2.214	-0.564	-0.956	0.298	-0.273	0.614	-0.088	0.432	0.725	10.578	(7,28)
	(±0.695)	(±0.217)	(±0.478)	(±0.140)	(±0.078)	(±0.243)	(±0.035)	(±0.229)			
	N = 36		s = 0.392								

Table 26 continued on next page.

Table 26 continued

Eq. No.	Log EC = Intercept	F(7)	INCO(7)	API	API^2	r^2	F	d.f.
7.	2.862	-0.376	-0.889	14.720	-67.102	0.730	10.842	(4,16)
	(±0.290)	(±0.116)	(±0.250)	(±3.360)	(±11.536)			
	N = 21		s = 0.382					
8.	2.880	-0.396	-0.889	15.361	-70.337	0.723	10.463	(4,16)
	(±0.302)	(±0.110)	(±0.255)	(±3.387)	(±11.707)			
	N = 21		s = 0.402					
9.	2.864	-0.378	-0.941	14.587	-66.708	0.751	12.078	(4,16)
	(±0.285)	(±0.109)	(±0.248)	(±3.273)	(±10.855)			
	N = 21		s = 0.392					

Thus the inability to obtain a valid model using the E. coli system holds for both the subset of 36 compounds using two different sets of independent variables and the larger set of 43 compounds.

II. Set B

A second QSAR analysis was performed on a set of 1,6,7-trisubstituted-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids (Table 6) (26) which were analyzed by both the LFER and de novo models.

The LFER model development for the S. aureus, E. coli and P. aeruginosa test system is shown in Table 27, 29, 35 and 37 respectively. Originally 41 compounds (all compounds in Table 6 except pipemidic acid whose ring is different from the other 41 compounds) were included for statistical analysis. The initial analysis for S. aureus gave eq. 8 (Table 27) which had two outliers (B24A and B27I, Table 28). Deletion of B24A and B27I produced an interesting set of statistically equivalent equations, eq. 8, 12, 15, 16 (Table 29). In addition eq. 14 (Table 29) has the same independent variables as eq. 8 (Table 27) with essentially identical regression coefficients. Thus dropping compounds B24A and B27I has no measurable effect on the model other than increasing the statistical validity.

The observed activity, calculated activity, residuals and standardized residuals for Table 29 eqs. 8, 12, 14, 15 and 16 are shown in Tables 30-34, respectively. The r^2's for eq. 8, 12, 15, 16 in Table 29 are very similar, because there are some highly

correlated independent variables. For example in eq. 8, INH(6) correlate with BPI (r = 0.790) and INH(6) with BPI^2 (r = 0.901). This occurs because when there is an amine present at position 6 (INH(6) = 1) BPI = 1.420 and when there is no amine (INH(6) = 0) the BPI values cluster around 0.63. This leads to a line connection two clusters of points. The correlation matrix of this set for S. aureus is shown in Table 40. Because the correlation coefficient of $MR(6)^2$ with INO(6) is 0.790, two statistically equivalent equations (eq. 15 and 16) were obtained. The result of this collinearity in independent variables is four equations (eqs. 8, 12, 15, 16) nearly equivalent. The questions that must be explored is whether these four equations contain equivalent information.

All four equations show fluoroethyl (IEF(1)) enhance activity against S. aureus by the same amount. A similar statement can be made for the presence of a fluorine at position 6 (IF(6)). There is a parabolic relationship seen for BPI in eq. 8 and MR(7) in eq. 12. In eq. 15 there are parabolic relationships for MR(6) and MR(7). The STERIMOL L length of substituent in position 6 is an important determinant of activity (eq. 8 and eq. 12) and can replace MR(6) because these two steric parameters are highly correlated in this data set. For this data set eqs. 12 and 14 provide the same information and revolve around the question as to whether the STERIMOL length term (L) or molar refractivity (MR) provide the best estimate of size information. One can argue that length is a more precise description of size for substituents at position 6. On the other hand molar refractivity is easier to calculate. The acceptable

way to measure the relative merits of these two descriptors is to design a test set in which L(6) and MR(6) terms are less correlated with each other. When BPI^2 doesn't appear in the model, BPI, by itself, is a negative contributor to activity (eqs. 12, 15 and 16). What is interesting to note is that no lipophilicity term are statistically significant for activity against S. aureus. The $\sigma-\rho$ interaction term seems to contain all the necessary information. Overall fluorine in position 6, steric effects in position 6 and 7 and electronic interactions between position 6 and 7 are important for activity.

For E. coli, eq. 16 (Table 35) indicates that fluorine and amine groups in position 6 are important contributors to activity. It also indicates that lipophilicity in position 7 and BPI are negative contributors to activity. The methyl group (ICH3(7)) and hydrogen (IRH1(7)) in the piperazinyl ring also are important contributors. All p-values of each independent variables are <0.05 and no outliers (standardized residuals >2.000) appear in eq. 16.

In the development of eq. 16, it should be noted that there were earlier equations (eq. 13 and 14) that are statistically significant. In formulation eq. 13 from eq. 12, both BPI^2 and INH(6) are significant, but there is high correlation ($r = 0.901$) between these two variables. Addition of BPI^2 gives eq. 13 which has one outlier (B15B). With inclusion of BPI^2, the ring indicator variable (RI1(7)) becomes significant. Addition of this term gives eq. 14 which still has an outlier (B18A). Then BPI^2 was deleted in order to check the contributing by INH(6) (eq. 16). The latter gave a good equation

(eq. 16) with no outliers and removes the parabolic dependence on BPI. The observed activity, calculated activity, residuals and standardized residuals is shown in Table 36 (eq. 16).

For P. aeruginosa eq. 10 (Table 37) was developed for 41 compounds. It contains two outliers (B27K and B30). The regression analysis was repeated for 39 compounds (B27K and B30 dropped) producing a statistically more significant equation (eq. 11). Because the indicator variable for CHF_2 (ICF(1)) which entered at eq. 11 occurs only in one compound (B40), the regression was repeated for 38 compounds (B40 dropped). Eq. 12 (n = 38) was derived with same r^2 as eq. 11 and but a more significant F-value. In this model (eq. 12) no outliers appeared. The observed activity, calculated activity, residuals and standardized residuals are show in Table 38 (eq. 10) and 39 (eq. 12).

Eq. 12 indicates that vinyl in position 1, fluorine and cyanide in position 6 and methyl and hydrogen on the N-substituent of the piperazinyl ring in position 7 are important for activity. A parabolic relationship for lipophilicity in position 7 also is seen in eq. 12. The correlation matrix of the set for E. coli and P. aeruginosa is shown in Table 41.

It is interesting that in this set of 1,8-naphthyridine derivatives the presence of fluorine is significant for all three bacterial systems. The electronic effect (BPI) is important for activity against S. aureus and E. coli but not P. aeruginosa. The presence of methyl and hydrogen on the nitrogen of the piperazinyl

Table 27 LFER Model Development for Set B Against S. aureus

Eq. No.	Log SA = Intercept	IEF(1)	IF(6)	F(6)	MR(6)	MR(7)	$MR(7)^2$	BPI	r^2	F	d.f.
1.	1.628 (±0.112)			0.814 (±0.231)					0.242	12.456	(1,39)
2.	0.718 (±0.281)			0.731 (±0.205)		0.331 (±0.096)			0.432	13.953	(2,38)
3.	0.672 (±0.263)		0.512 (±0.199)	0.495 (±0.212)		0.255 (±0.094)			0.511	12.882	(3,37)
4.	-0.410 (±0.437)		0.558 (±0.181)	0.524 (±0.193)		1.047 (±0.282)	-0.135 (±0.046)		0.606	13.848	(4,36)
5.	0.318 (±0.493)		0.641 (±0.171)	0.081 (±0.248)		1.088 (±0.262)	-0.144 (±0.043)	-1.013 (±0.390)	0.670	14.186	(5,35)
6.	0.387 (±0.442)		0.665 (±0.153)			1.088 (±0.259)	-0.145 (±0.042)	-1.102 (±0.279)	0.669	18.191	(4,36)
7.	0.245 (±0.422)		1.045 (±0.217)		1.053 (±0.451)	0.961 (±0.250)	-0.128 (±0.040)	-1.306 (±0.278)	0.714	17.410	(5,35)
8.	0.282 (±0.404)	0.613 (±0.297)	0.987 (±0.210)		1.053 (±0.431)	0.909 (±0.241)	-0.117 (±0.039)	-1.280 (±0.266)	0.746	16.595	(6,34)

N = 41 s = 0.397

Table 28 Comparison of Observed and Calculated MIC's from Eq. 8 (Table 27)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.129	.231	.631
B3B	1.082	1.265	-.183	-.499
B3C	1.102	1.390	-.288	-.787
B15A	1.410	1.500	-.090	-.245
B15B	2.032	1.635	.396	1.082
B15C	1.750	1.760	-.011	-.030
B18A	2.000	1.291	.709	1.936
B18B	1.719	1.426	.293	.799
B18C	1.435	1.551	-.116	-.317
B22A	1.123	1.579	-.455	-1.243
B22B	1.745	1.714	.031	.084
B22C	1.461	1.839	-.378	-1.033
B23A	.179	.356	-.176	-.482
B23B	.200	.491	-.291	-.794
B23C	1.122	.616	.506	1.380
B24A	2.893	1.985	.908	2.477
B24B	2.613	2.121	.492	1.343
B24C	2.331	2.246	.085	.231
B27A	.099	.378	-.279	-.762
B27B	1.071	1.222	-.151	-.412
B27C	1.728	2.132	-.404	-1.102
B27D	2.611	2.150	.460	1.257
B27E	2.313	2.045	.268	.732
B27F	2.334	2.240	.094	.257
B27G	2.331	2.246	.085	.231
B27H	2.019	2.250	-.231	-.630
B27I	1.444	2.330	-.886	-2.419
B27J	1.801	2.198	-.397	-1.083
B27K	2.721	2.051	.670	1.828
B27L	2.650	2.321	.329	.898
B28A	2.366	2.346	.020	.054
B28B	2.080	2.320	-.240	-.656
B28C	2.382	2.320	.062	.169
B29	2.046	2.253	-.207	-.565
B30	2.063	2.325	-.262	-.714
B36	2.309	2.121	.188	.514
B37	2.025	2.246	-.220	-.601
B38	2.939	2.734	.205	.561
B39	2.654	2.859	-.205	-.561
B40	1.738	2.121	-.383	-1.045
BNA	.666	.843	-.177	-.483

[a] Standardized residual

Table 29 LFER Model Development for Set B Against S. aureus

Eq. No.	Log SA = Inercept	IEF(1)	IF(6)	INH(6)	L(6)	MR(6)	$MR(6)^2$	MR(7)	$MR(7)^2$	BPI	BPI^2	r^2	F	d.f.
1.	1.274		0.866									0.358	20.633	(1,37)
	(±0.146)		(±0.191)											
2.	2.067		0.762							-1.033		0.493	17,543	(2,36)
	(±0.288)		(±0.175)							(±0.333)				
3.	0.873		0.575							2.472	-2.072	0.618	18.861	(3,35)
	(±0.435)		(±0.164)							(±1.077)	(±0.613)			
4.	0.442		0.625	2.616						4.852	-4.686	0.736	23.617	(4,34)
	(±0.383)		(±0.139)	(±0.672)						(±1.096)	(±0.848)			
5.	0.501		1.066	2.773		1.058				3.538	-4.107	0.771	22.079	(5,33)
	(±0.363)		(±0.237)	(±0.640)		(±0.473)				(±1.192)	(±0.843)			
6.	0.505	0.570	1.066	2.662		1.035				3.483	-4.010	0.800	21.439	(6,32)
	(±0.344)	(±0.261)	(±0.226)	(±0.609)		(±0.449)				(±0.130)	(±0.800)			
7.	-0.296	0.562	0.940	2.938	0.461	0.114				2.954	-3.804	0.832	21.750	(7,31)
	(±0.463)	(±0.244)	(±0.213)	(±0.579)	(±0.192)	(±0.568)				(±0.077)	(±0.751)			
8.	-0.344	0.563	0.911	2.944	0.487					3.001	-3.826	0.831	26.336	(6,32)
	(±0.389)	(±0.240)	(±0.151)	(±0.570)	(±0.139)					(±1.035)	(±0.732)			

N = 39 s = 0.322

Table 29 continued on next page.

Table 29 continued

9.	0.417 (±0.483)	0.632 (±0.321)	1.126 (±0.195)	0.932 (±0.563)	0.700 (±0.179)					-1.968 (±0.550)	0.687	14.529	(5,33)
10.	0.539 (±0.489)	0.667 (±0.329)	0.943 (±0.164)		0.524 (±0.148)					-1.183 (±0.285)	0.661	16.589	(4,34)
11.	0.067 (±0.433)	0.764 (±0.279)	0.737 (±0.149)		0.460 (±0.126)			0.263 (±0.069)		-1.129 (±0.241)	0.764	21.439	(5,33)
12.	-0.614 (±0.409)	0.633 (±0.239)	0.765 (±0.126)		0.392 (±0.108)			0.951 (±0.193)	-0.117 (±0.031)	-1.189 (±0.205)	0.836	27.277	(6,32)
	N = 39	s = 0.318											
13.	0.302 (±0.376)	0.628 (±0.280)	0.578 (±0.135)					1.090 (±0.221)	-0.136 (±0.036)	-1.071 (±0.237)	0.769	22.036	(5,33)
14.	0.169 (±0.347)	0.627 (±0.256)	0.942 (±0.182)			1.008 (±0.370)		0.968 (±0.207)	-0.120 (±0.033)	-1.267 (±0.228)	0.813	23.086	(6,32)
15.	0.010 (±0.337)	0.615 (±0.243)	1.108 (±0.189)			4.118 (±1.492)	-3.491 (±1.628)	0.913 (±0.198)	-0.113 (±0.032)	-1.552 (±0.254)	0.837	22.731	(7,31)
	N = 39	s = 0.322											
16.	Intercept 0.131 (±0.329)	IEF(1) 0.622 (±0.243)	IF(6) 1.086 (±0.185)			MR(6) 1.734 (±0.487)	INO(6) -0.618 (±0.288)	MR(7) 0.923 (±0.197)	$MR(7)^2$ -0.114 (±0.032)	BPI -1.419 (±0.227)	0.837	22.731	(7,31)
	N = 39	s = 0.322											

Table 30 Comparison of Observed and Calculated MIC's from Eq. 8 (Table 29)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.192	.169	.569
B3B	1.082	1.192	-.110	-.371
B3C	1.102	1.192	-.090	-.304
B15A	1.410	1.746	-.336	-1.135
B15B	2.032	1.746	.285	.962
B15C	1.750	1.746	.003	.011
B18A	2.000	1.730	.270	.910
B18B	1.719	1.730	-.012	-.039
B18C	1.435	1.730	-.295	-.996
B22A	1.123	1.407	-.283	-.956
B22B	1.745	1.407	.338	1.141
B22C	1.461	1.407	.054	.184
B23A	.179	.500	-.321	-1.084
B23B	.200	.500	-.300	-1.014
B23C	1.122	.500	.622	2.098
B24B	2.613	2.234	.379	1.278
B24C	2.331	2.234	.097	.327
B27A	.099	.593	-.494	-1.668
B27B	1.071	.593	.478	1.612
B27C	1.728	2.234	-.506	-1.707
B27D	2.611	2.234	.377	1.272
B27E	2.313	2.234	.079	.268
B27F	2.334	2.234	.101	.339
B27G	2.331	2.234	.097	.327
B27H	2.019	2.234	-.215	-.725
B27J	1.801	2.234	-.433	-1.460
B27K	2.721	2.234	.487	1.645
B27L	2.650	2.234	.416	1.403
B28A	2.366	2.234	.132	.444
B28B	2.080	2.234	-.154	-.520
B28C	2.382	2.234	.148	.500
B29	2.046	2.234	-.188	-.633
B30	2.063	2.234	-.171	-.577
B36	2.309	2.234	.075	.253
B37	2.025	2.234	-.208	-.703
B38	2.939	2.796	.143	.482
B39	2.654	2.796	-.143	-.482
B40	1.738	2.234	-.496	-1.675
BNA	.666	.658	.007	.025

[a]Standardized residual

Table 31 Comparison of Observed and Calculated MIC's from Eq. 12 (Table 29)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.081	.279	.956
B3B	1.082	1.232	-.150	-.514
B3C	1.102	1.377	-.275	-.941
B15A	1.410	1.516	-.106	-.364
B15B	2.032	1.667	.364	1.247
B15C	1.750	1.812	-.062	-.213
B18A	2.000	1.614	.386	1.322
B18B	1.719	1.764	-.046	-.156
B18C	1.435	1.909	-.474	-1.621
B22A	1.123	1.329	-.206	-.705
B22B	1.745	1.480	.265	.906
B22C	1.461	1.625	-.164	-.560
B23A	.179	.283	-.104	-.357
B23B	.200	.434	-.234	-.802
B23C	1.122	.579	.543	1.860
B24B	2.613	2.091	.522	1.786
B24C	2.331	2.236	.095	.326
B27A	.099	.305	-.206	-.706
B27B	1.071	1.202	-.132	-.451
B27C	1.728	2.104	-.376	-1.286
B27D	2.611	2.125	.486	1.664
B27E	2.313	2.007	.307	1.050
B27F	2.334	2.229	.106	.362
B27G	2.331	2.236	.095	.326
B27H	2.019	2.240	-.221	-.757
B27J	1.801	2.274	-.472	-1.616
B27K	2.721	2.146	.575	1.968
B27L	2.650	2.330	.320	1.095
B28A	2.366	2.374	-.008	-.029
B28B	2.080	2.368	-.288	-.986
B28C	2.382	2.368	.014	.048
B29	2.046	2.245	-.198	-.679
B30	2.063	2.335	-.272	-.931
B36	2.309	2.091	.218	.746
B37	2.025	2.236	-.210	-.719
B38	2.939	2.724	.215	.736
B39	2.654	2.869	-.215	-.736
B40	1.738	2.091	-.353	-1.209
BNA	.666	.682	-.017	-.058

[a]Standardized residual

Table 32 Comparison of Observed and Calculated MIC's from Eq. 14 (Table 29)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.132	.229	.733
B3B	1.082	1.284	-.202	-.647
B3C	1.102	1.430	-.328	-1.050
B15A	1.410	1.481	-.071	-.228
B15B	2.032	1.634	.398	1.274
B15C	1.750	1.779	-.030	-.096
B18A	2.000	1.275	.725	2.322
B18B	1.719	1.427	.292	.934
B18C	1.435	1.573	-.138	-.441
B22A	1.123	1.551	-.428	-1.371
B22B	1.745	1.704	.041	.131
B22C	1.461	1.849	-.389	-1.244
B23A	.179	.353	-.174	-.556
B23B	.200	.505	-.305	-.978
B23C	1.122	.651	.471	1.508
B24B	2.613	2.096	.516	1.653
B24C	2.331	2.242	.089	.284
B27A	.099	.256	-.157	-.503
B27B	1.071	1.168	-.097	-.312
B27C	1.728	2.109	-.381	-1.221
B27D	2.611	2.131	.480	1.538
B27E	2.313	2.011	.302	.967
B27F	2.334	2.235	.099	.317
B27G	2.331	2.242	.089	.284
B27H	2.019	2.247	-.228	-.730
B27J	1.801	2.270	-.469	-1.502
B27K	2.721	2.138	.584	1.869
B27L	2.650	2.336	.313	1.003
B28A	2.366	2.379	-.014	-.044
B28B	2.080	2.370	-.291	-.931
B28C	2.382	2.370	.011	.037
B29	2.046	2.251	-.205	-.656
B30	2.063	2.341	-.278	-.892
B36	2.309	2.096	.212	.680
B37	2.025	2.242	-.217	-.694
B38	2.939	2.724	.216	.690
B39	2.654	2.869	-.216	-.690
B40	1.738	2.096	-.359	-1.149
BNA	.666	.756	-.091	-.291

[a]Standardized residual

Table 33 Comparison of Observed and Calculated MIC's from Eq. 15 (Table 29)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	.987	.374	1.282
B3B	1.082	1.130	-.048	-.165
B3C	1.102	1.266	-.165	-.565
B15A	1.410	1.685	-.275	-.943
B15B	2.032	1.828	.204	.698
B15C	1.750	1.964	-.215	-.737
B18A	2.000	1.447	.553	1.896
B18B	1.719	1.590	.129	.441
B18C	1.435	1.727	-.291	-1.000
B22A	1.123	1.306	-.183	-.627
B22B	1.745	1.449	.296	1.014
B22C	1.461	1.586	-.125	-.427
B23A	.179	.393	-.214	-.734
B23B	.200	.536	-.336	-1.154
B23C	1.122	.673	.449	1.542
B24B	2.613	2.113	.500	1.714
B24C	2.331	2.249	.081	.279
B27A	.099	.214	-.116	-.397
B27B	1.071	1.074	-.003	-.011
B27C	1.728	2.125	-.397	-1.362
B27D	2.611	2.145	.466	1.599
B27E	2.313	2.033	.280	.962
B27F	2.334	2.243	.092	.314
B27G	2.331	2.249	.081	.279
B27H	2.019	2.254	-.235	-.805
B27J	1.801	2.269	-.468	-1.605
B27K	2.721	2.142	.579	1.988
B27L	2.650	2.337	.313	1.073
B28A	2.366	2.376	-.010	-.036
B28B	2.080	2.366	-.286	-.982
B28C	2.382	2.366	.016	.054
B29	2.046	2.258	-.212	-.726
B30	2.063	2.342	-.279	-.956
B36	2.309	2.113	.196	.672
B37	2.025	2.249	-.224	-.768
B38	2.939	2.728	.211	.724
B39	2.654	2.865	-.211	-.724
B40	1.738	2.113	-.375	-1.288
BNA	.666	.818	-.152	-.522

[a]Standardized residual

Table 34 Comparison of Observed and Calculated MIC's from Eq. 16 (Table 29)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.009	.351	1.205
B3B	1.082	1.155	-.073	-.249
B3C	1.102	1.294	-.192	-.658
B15A	1.410	1.698	-.288	-.988
B15B	2.032	1.843	.188	.646
B15C	1.750	1.982	-.233	-.798
B18A	2.000	1.459	.541	1.858
B18B	1.719	1.604	.115	.395
B18C	1.435	1.743	-.307	-1.055
B22A	1.123	1.300	-.177	-.606
B22B	1.745	1.445	.300	1.028
B22C	1.461	1.584	-.123	-.422
B23A	.179	.360	-.181	-.621
B23B	.200	.506	-.306	-1.049
B23C	1.122	.644	.478	1.639
B24B	2.613	2.105	.508	1.743
B24C	2.331	2.244	.087	.299
B27A	.099	.253	-.154	-.529
B27B	1.071	1.122	-.052	-.177
B27C	1.728	2.117	-.389	-1.335
B27D	2.611	2.137	.474	1.625
B27E	2.313	2.023	.290	.995
B27F	2.334	2.237	.097	.334
B27G	2.331	2.244	.087	.299
B27H	2.019	2.248	-.229	-.786
B27J	1.801	2.271	-.470	-1.612
B27K	2.721	2.145	.576	1.978
B27L	2.650	2.333	.316	1.085
B28A	2.366	2.374	-.009	-.030
B28B	2.080	2.366	-.286	-.983
B28C	2.382	2.366	.016	.054
B29	2.046	2.252	-.206	-.707
B30	2.063	2.338	-.275	-.945
B36	2.309	2.105	.204	.701
B37	2.025	2.244	-.218	-.748
B38	2.939	2.727	.212	.728
B39	2.654	2.866	-.212	-.728
B40	1.738	2.105	-.367	-1.260
BNA	.666	.760	-.094	-.324

[a]Standardized residual

Table 35 LFER Model Development for Set B Against *E. coli*

Eq. No.	Log EC = Intercept	IE(1)	IF(6)	INH(6)	F(7)	$F(7)^2$	RI1(7)	RI2(7)	ICH3(7)	IRH1(7)	BPI	BPI^2	r^2	F	d.f.
1.	3.167 (±0.293)	-1.254 (±0.313)											0.292	16.105	(1,39)
2.	2.619 (±0.340)	-1.011 (±0.304)	0.549 (±0.204)										0.405	12.951	(2,38)
3.	2.891 (±0.288)	-0.959 (±0.252)	0.277 (±0.180)					-1.036 (±0.240)					0.604	18.830	(3,37)
4.	2.847 (±0.276)	-0.838 (±0.247)	0.366 (±0.177)		-0.184 (±0.085)			-0.903 (±0.237)					0.650	16.700	(4,36)
5.	2.550 (±0.251)	-0.607 (±0.222)	0.614 (±0.167)		0.270 (±0.144)	-0.261 (±0.071)		-1.046 (±0.208)					0.746	20.649	(5,35)
6.	2.377 (±0.277)	-0.535 (±0.225)	0.687 (±0.173)		0.173 (±0.159)	-0.218 (±0.077)		-0.901 (±0.230)	0.283 (±0.205)				0.760	17.940	(6,34)
7.	1.752 (±0.408)	-0.261 (±0.255)	0.899 (±0.196)		0.197 (±0.152)	-0.200 (±0.074)		-0.641 (±0.256)	0.595 (±0.249)	0.463 (±0.230)			0.786	17.366	(7,33)
8.	1.386 (±0.197)		1.017 (±0.159)		0.205 (±0.152)	-0.206 (±0.074)		-0.558 (±0.242)	0.718 (±0.218)	0.589 (±0.194)			0.780	20.088	(6,34)
9.	1.109 (±0.200)		0.962 (±0.179)					-0.327 (±0.252)	1.053 (±0.216)	0.872 (±0.194)			0.700	21.005	(4,36)

Table 35 continued on next page

Table 35 continued

10.	0.930 (±0.146)	1.090 (±0.151)					1.184 (±0.192)	0.988 (±0.174)	0.686 26.900 (3,37)
11.	1.119 (±0.168)	1.082 (±0.145)		-0.169 (±0.084)			1.126 (±0.187)	0.787 (±0.194)	0.718 22.946 (4,36)
12.	1.626 (±0.268)	1.021 (±0.139)		-0.209 (±0.081)			1.131 (±0.176)	0.752 (±0.184)	-0.615 (±0.262) 0.756 21.679 (5,35)
13.	2.259 (±0.376)	1.143 (±0.142)		-0.200 (±0.076)			1.188 (±0.168)	0.809 (±0.175)	-2.597 (±0.904) 1.173 (±0.514) 0.788 21.150 (6,34)
14.	2.276 (±0.330)	1.091 (±0.138)		-0.235 (±0.075)	0.322 (±0.157)		0.948 (±0.199)	0.579 (±0.202)	-2.713 (±0.866) 1.242 (±0.493) 0.812 20.493 (7,33)
15.	1.599 (±0.261)	0.967 (±0.139)		-0.241 (±0.081)	0.294 (±0.168)		0.908 (±0.213)	0.538 (±0.216)	-0.615 (±0.255) 0.776 19.622 (6,34)
16.	2.074 (±0.330)	1.114 (±0.139)	0.906 (±0.422)	-0.258 (±0.080)			1.123 (±0.168)	0.696 (±0.177)	-1.353 (±0.425) 0.785 20.779 (6,34)

N = 41 s = 0.386

Table 36 Comparison of Observed and Calculated MIC's form Eq. 16 (Table 35)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.060	1.068	-.008	-.023
B3B	1.684	2.103	-.419	-1.176
B3C	1.703	2.310	-.607	-1.702
B15A	.206	.913	-.706	-1.982
B15B	2.635	1.948	.687	1.927
B15C	2.352	2.154	.197	.554
B18A	1.397	.707	.690	1.936
B18B	1.350	1.742	-.392	-1.100
B18C	2.340	1.949	.391	1.098
B22A	.521	.735	-.214	-.600
B22B	1.745	1.770	-.026	-.072
B22C	2.062	1.977	.085	.238
B23A	.179	.745	-.566	-1.588
B23B	2.006	1.780	.226	.633
B23C	2.327	1.987	.340	.955
B24A	2.292	2.027	.265	.743
B24B	3.204	3.062	.142	.400
B24C	2.932	3.268	-.337	-.944
B27A	2.206	1.974	.233	.653
B27B	1.728	1.964	-.236	-.663
B27C	2.631	2.451	.180	.504
B27D	1.708	1.883	-.175	-.491
B27E	2.011	2.306	-.295	-.828
B27F	2.032	2.120	-.088	-.247
B27G	2.631	3.037	-.406	-1.139
B27H	1.719	1.850	-.131	-.368
B27I	1.143	1.595	-.452	-1.268
B27J	1.500	1.652	-.152	-.427
B27K	1.818	1.686	.132	.370
B27L	2.650	2.030	.620	1.740
B28A	2.366	1.914	.452	1.267
B28B	2.080	1.798	.282	.790
B28C	2.080	1.855	.225	.631
B29	2.349	2.459	-.111	-.311
B30	2.063	2.328	-.265	-.742
B36	3.503	3.062	.441	1.239
B37	3.220	3.268	-.048	-.135
B38	3.228	3.062	.166	.466
B39	3.246	3.268	-.023	-.064
B40	2.642	3.062	-.420	-1.177
BNA	2.171	1.849	.322	.903

[a]Standardized residual

Table 37 LFER Model Development for Set B Against P. aeruginosa

Eq. No.	Log PA = Intercept	F(1)	MR(1)	IV(1)	IF(6)	ICN(6)	F(7)	$F(7)^2$	ICH3(7)	IRH1(7)	r^2	F	d.f.
1.	6.034 (±1.028)	-3.417 (±0.751)									0.347	20.670	(1,39)
2.	5.282 (±0.974)	-2.976 (±0.703)								0.620 (±0.211)	0.468	16.682	(2,38)
3.	4.126 (±0.968)	-2.381 (±0.670)			0.520 (±0.174)					0.733 (±0.196)	0.571	16.442	(3,37)
4.	2.163 (±0.924)	-1.250 (±0.615)			0.801 (±0.159)				0.861 (±0.203)	1.069 (±0.180)	0.714	22.462	(4,36)
5.	2.151 (±0.852)	-1.118 (±0.569)			0.881 (±0.149)			-0.100 (±0.037)	0.728 (±0.193)	0.869 (±0.182)	0.764	22.708	(5,35)
6.	1.639 (±0.078)	-0.872 (±0.515)			1.034 (±0.142)		0.401 (±0.128)	-0.264 (±0.062)	0.653 (±0.173)	1.019 (±0.170)	0.816	25.121	(6,34)
7.	0.339 (±0.141)				1.143 (±0.130)		0.435 (±0.130)	-0.282 (±0.063)	0.762 (±0.167)	1.120 (±0.163)	0.801	28.208	(5,35)
8.	-1.781 (±0.877)		2.046 (±0.837)		1.204 (±0.124)		0.452 (±0.122)	-0.292 (±0.059)	0.774 (±0.156)	1.223 (±0.158)	0.831	27.798	(6,34)

Table 37 continued on next page.

Table 37 continued

9.	-1.852	2.037		1.288	0.456	0.455	-0.294	0.766	1.216	0.853	27.343	(7,33)
	(±0.831)	(±0.792)		(±0.124)	(±0.205)	(±0.116)	(±0.056)	(±0.148)	(±0.150)			
10.	-1.714	1.951	0.505	1.213	0.460	0.431	-0.281	0.689	1.146	0.871	27.087	(8,32)
	(±0.793)	(±0.754)	(±0.240)	(±0.123)	(±0.195)	(±0.111)	(±0.053)	(±0.145)	(±0.146)			
	N = 41	s = 0.304										

Eq. No.	Log PA = Intercept	ICF(1)	IV(1)	IF(6)	ICN(6)	F(7)	$F(7)^2$	ICH3(7)	IRH1(7)	r^2	F	d.f.
11.	0.342	-0.874	0.424	1.256	0.463	0.450	-0.338	0.626	1.062	0.922	44.471	(8,30)
	(±0.137)	(±0.263)	(±0.193)	(±0.099)	(±0.155)	(±0.088)	(±0.044)	(±0.117)	(±0.119)			
	N = 39	s = 0.242										
12.	0.342		0.424	1.256	0.463	0.450	-0.338	0.626	1.062	0.922	50.512	(7,30)
	(±0.103)		(±0.193)	(±0.099)	(±0.155)	(±0.088)	(±0.044)	(±0.117)	(±0.119)			
	N = 38	s = 0.242										

Table 38 Comparison of Observed and Calculated MIC's from Eq. 10 (Table 37)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	.157	.378	-.222	-.815
B3B	1.082	1.369	-.287	-1.055
B3C	1.102	1.124	-.022	-.082
B15A	.206	.378	-.172	-.633
B15B	1.730	1.369	.362	1.330
B15C	1.447	1.124	.323	1.189
B18A	1.096	.838	.258	.949
B18B	1.719	1.829	-.110	-.403
B18C	1.435	1.584	-.149	-.546
B22A	.220	.378	-.158	-.581
B22B	1.143	1.369	-.226	-.832
B22C	.857	1.124	-.267	-.982
B23A	.179	.378	-.199	-.733
B23B	1.706	1.369	.337	1.238
B23C	1.423	1.124	.298	1.097
B24A	1.987	1.591	.396	1.456
B24B	2.613	2.582	.031	.114
B24C	2.331	2.337	-.006	-.023
B27A	.701	.770	-.068	-.252
B27B	.770	.805	-.035	-.130
B27C	1.427	1.243	.184	.677
B27D	1.407	1.363	.044	.161
B27E	1.710	1.534	.176	.648
B27F	1.431	1.645	-.215	-.790
B27G	2.331	2.626	-.296	-1.087
B27H	.815	1.287	-.471	-1.733
B27I	.240	.380	-.140	-.516
B27J	.297	.633	-.336	-1.237
B27K	1.516	.768	.748	2.750
B27L	2.046	1.594	.452	1.663
B28A	1.462	1.427	.035	.129
B28B	1.177	1.147	.030	.110
B28C	1.177	1.299	-.121	-.446
B29	1.445	1.221	.224	.823
B30	.860	1.502	-.642	-2.360
B36	3.201	3.037	.164	.603
B37	2.629	2.793	-.164	-.603
B38	2.636	2.612	.025	.090
B39	2.354	2.367	-.013	-.050
B40	1.738	1.737	.000	.001
BNA	.666	.432	.234	.861

[a]Standardized residual

Table 39 Comparison of Observed and Calculated MIC's from Eq. 12 (Table 37)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	.157	.389	-.232	-1.065
B3B	1.082	1.356	-.274	-1.256
B3C	1.102	1.115	-.013	-.062
B15A	.206	.389	-.183	-.839
B15B	1.730	1.356	.375	1.718
B15C	1.447	1.115	.332	1.523
B18A	1.096	.853	.244	1.119
B18B	1.719	1.819	-.100	-.461
B18C	1.435	1.579	-.143	-.658
B22A	.220	.389	-.169	-.774
B22B	1.143	1.356	-.213	-.978
B22C	.857	1.115	-.258	-1.185
B23A	.179	.389	-.210	-.963
B23B	1.706	1.356	.350	1.604
B23C	1.423	1.115	.307	1.409
B24A	1.987	1.645	.342	1.570
B24B	2.613	2.612	.001	.004
B24C	2.331	2.371	-.041	-.186
B27A	.701	.810	-.109	-.499
B27B	.770	.850	-.081	-.370
B27C	1.427	1.340	.087	.397
B27D	1.407	1.331	.076	.347
B27E	1.710	1.651	.059	.269
B27F	1.431	1.736	-.305	-1.399
B27G	2.331	2.659	-.328	-1.505
B27H	.815	1.230	-.415	-1.904
B27I	.240	.070	.170	.780
B27J	.297	.390	-.093	-.428
B27L	2.046	1.649	.397	1.822
B28A	1.462	1.417	.045	.207
B28B	1.177	1.049	.129	.590
B28C	1.177	1.246	-.069	-.316
B29	1.445	1.316	.129	.590
B36	3.201	3.035	.166	.761
B37	2.629	2.795	-.166	-.761
B38	2.636	2.612	.025	.113
B39	2.354	2.371	-.018	-.081
BNA	.666	.477	.189	.867

[a]Standardized residual

Table 40 Correlation Matrix of the Variables Used in the Analyses of the S. aureus Test System (Table 29)

	IEF(1)	IF(6)	INH(6)	INO(6)	F(6)	L(6)	MR(6)	$MR(6)^2$	F(7)	MR(7)	$MR(7)^2$	BPI	BPI^2	SA	EC	PA
IEF(1)	1.000															
IF(6)	0.181	1.000														
INH(6)	-0.064	-0.351	1.000													
INO(6)	-0.064	-0.351	-0.079	1.000												
F(6)	0.080	0.443	-0.788	-0.160	1.000											
L(6)	-0.080	-0.444	-0.031	0.317	-0.194	1.000										
MR(6)	-0.137	-0.757	0.245	0.663	-0.395	0.798	1.000									
$MR(6)^2$	-0.128	-0.705	0.131	0.794	-0.297	0.740	0.980	1.000								
F(7)	-0.098	0.081	-0.033	-0.033	0.040	-0.049	-0.072	-0.066	1.000							
MR(7)	-0.015	0.333	-0.079	-0.079	0.117	-0.043	-0.168	-0.158	0.604	1.000						
$MR(7)^2$	-0.045	0.351	-0.104	-0.104	0.140	-0.103	-0.223	-0.209	0.636	0.958	1.000					
BPI	-0.069	-0.201	0.795	0.059	-0.693	0.238	0.357	0.265	-0.205	-0.094	-0.124	1.000				
BPI^2	-0.083	-0.286	0.901	0.009	-0.765	0.126	0.319	0.218	-0.194	-0.190	-0.186	0.961	1.000			
SA	0.313	0.590	-0.510	-0.141	0.492	-0.012	-0.330	-0.287	0.215	0.480	0.392	-0.468	-0.586	1.000		
EC	0.349	0.482	-0.208	-0.231	0.260	-0.203	-0.388	-0.371	-0.368	0.071	0.031	-0.217	-0.232	0.500	1.000	
PA	0.339	0.393	-0.103	-0.240	0.131	-0.037	-0.261	-0.273	-0.348	0.079	-0.014	-0.107	-0.155	0.601	0.859	1.000

Table 41 Correlation Matrix of the Variables Used in the Analyses of the E. coli and P. aeruginosa Test Systems (Tables 35, 37)

	IV(1)	MR(1)	IF(6)	ICN(6)	INH(6)	L(6)	F(7)	$F(7)^2$	INCO(7)	RI1(7)	ICH3(7)	IRH1(7)	BPI	BPI^2	SA	EC	PA
IV(1)	1.000																
MR(1)	-0.049	1.000															
IF(6)	0.181	-0.132	1.000														
ICN(6)	-0.064	0.046	-0.351	1.000													
INH(6)	-0.064	0.046	-0.351	-0.079	1.000												
L(6)	-0.080	0.059	-0.444	0.734	-0.031	1.000											
F(7)	-0.098	0.143	0.081	-0.033	-0.033	-0.049	1.000										
$F(7)^2$	-0.128	0.115	0.292	-0.103	-0.103	-0.132	0.847	1.000									
INCO(7)	-0.064	0.046	0.225	-0.079	-0.079	-0.099	-0.304	-0.181	1.000								
RI1(7)	0.181	-0.132	-0.025	0.033	0.033	0.078	-0.084	-0.123	0.033	1.000							
ICH3(7)	0.174	0.072	-0.237	0.098	0.098	0.147	0.011	-0.210	-0.138	0.394	1.000						
IRH1(7)	0.135	-0.282	-0.128	0.059	0.059	0.095	-0.499	-0.402	-0.160	0.338	-0.280	1.000					
BPI	-0.069	0.051	-0.201	0.083	0.795	0.238	-0.205	-0.103	-0.086	-0.003	0.083	0.042	1.000				
BPI^2	-0.083	-0.061	-0.286	-0.029	0.901	0.126	-0.194	-0.124	-0.103	-0.044	0.075	0.027	0.961	1.000			
SA	0.115	0.021	0.590	-0.033	-0.510	-0.012	0.215	0.108	0.056	0.228	-0.047	0.053	-0.468	-0.586	1.000		
EC	0.386	-0.134	0.482	-0.137	-0.208	-0.203	0.368	-0.351	0.104	0.489	0.296	0.296	-0.217	-0.232	0.500	1.000	
PA	0.466	-0.097	0.393	0.149	-0.103	-0.348	-0.416	-0.050	0.448	0.211	0.466	0.601	-0.106	-0.155	0.601	0.859	1.000

ring enhances activity in both E. coli and P. aeruginosa but has no significant effect S. aureus.

A subset of 1,8-naphthyridine derivatives containing 25 compounds (Table 6, B24A-C, B27A-L, B28A-C, B29-30, B36-B40) in which position 6 is fixed with fluorine were analyzed in order to investigate what is the effect of substituents on positions 1 and 7. The LFER models of the three bacterial systems for this subset is shown in Tables 42, 45, 47 and 49. For S. aureus the regression analysis was performed on 24 compounds. (Compound B27D was dropped because the $(CH_2)_5N$-RI3(7) variable which appeared in the initial run is found only on the one compound.) Eq. 7 and eq. 8 (Table 42) indicate either vinyl or lipophilicity along with length L of the substituent are important at position 1. The presence of a vinyl group (IV(1)) is inversely correlated with lipophilicity (F(1)) ($r = -0.880$; Table 51). Eqs. 7 and 8 (Table 42) also indicate that molar refractivity and lipophilicity in position 7 are important for activity. The presence of an amide nitrogen INCO(7) and BPI reduces activity, but the pyrrolidinyl ring (RI2(7)) enhances activity in the S. aureus system.

Two outliers appeared from eq. 7 (B27J, B27L) and eq. 8 (B27J, B27G) (see Table 43-44). The regression was repeated deleting the two outliers seen in each of the equations (eq. 7, eq. 8). The equations were derived using the same independent variables found in eq. 7 or eq. 8, but another one to three outliers appeared. It was decided to stay with eq. 7 and eq. 8 (Table 42). These latter

equations contain different variables from the models obtained from the parent data set (eqs. 8, 12, 15, 16; Table 29).

For E. coli eq. 14 (Table 45) was derived. Eq. 14 indicates that lipophilicity, methyl and hydrogen substituents on the piperazinyl ring and piperazinyl ring itself in position 7 are important determinants of activity. In this model (eq. 14) there are no outliers (see Table 46). Eq. 14 (Table 45) is very similar to eq. 16 (Table 35), because the BPI term is related to a variety of substituents at position 6.

Eq. 7 in Table 47 is another model of the E. coli test system for this same subset of 25 compounds. The lipophilicity term at position 7 was forced in as the first variable. This model (eq. 7) includes a lipophilicity term for position 1 and indicates that $\sigma-\rho$ electronic interactions (BPI) and an amide nitrogen (INCO(7)) are negative factors but that the piperazinyl ring RI1(7) increases activity. Eq. 7 (Table 47) is different from eq. 14 of Table 45. The calculated values and residuals are listed in Table 48.

Eq. 10 (Table 49) for P. aeruginosa indicates that lipophilicity in position 1, a parabolic relationship of lipophilicity and an unsubstituted piperazinyl ring (IRH1(7)) are important for activity. There were two outliers (B27K and B30). The observed activity, calculated activity, residuals and standardized residuals for eq. 10 are shown in Table 50. The regression was repeated without the two outliers using the same independent variables as eq. 10, but another outlier (B40) appeared. It was decided to stay with eq. 10 (Table 49). The correlation matrix of this subset is shown in Table 51.

Table 42 LFER Model Development for a Subset (Fluorine at Position 6) Against S. aureus

Eq. No.	Log SA = Intercept	IV(1)	L(1)	F(1)	F(7)	MR(7)	RI2(7)	INCO(7)	BPI	r^2	F	d.f.
1.	1.357 (±0.366)					0.250 (±0.113)				0.182	4.891	(1,22)
2.	4.592 (±0.767)					-0.008 (±0.100)			-3.675 (±0.817)	0.584	14.670	(2,21)
3.	1.475 (±1.550)		0.733 (±0.325)			0.0003 (±0.092)			-3.587 (±0.748)	0.668	13.401	(3,20)
4.	1.785 (±1.521)		0.706 (±0.316)			-0.014 (±0.090)		-0.344 (±0.233)	-3.758 (±0.736)	0.702	11.173	(4,19)
5.	1.517 (±1.384)		0.697 (±0.287)		-0.278 (±0.123)	0.210 (±0.129)		-0.666 (±0.255)	-3.758 (±0.673)	0.768	11.921	(5,18)
6.	0.509 (±1.017)		0.737 (±0.205)		-0.444 (±0.096)	0.426 (±0.105)	1.211 (±0.283)	-0.772 (±0.184)	-3.547 (±0.490)	0.888	22.509	(6,17)
7.	0.440 (±0.924)	-0.372 (±0.173)	0.807 (±0.189)		-0.471 (±0.088)	0.423 (±0.095)	1.171 (±0.258)	-0.848 (±0.171)	-3.768 (±0.457)	0.913	24.063	(7,16)
	N = 24	s = 0.219										
8.	-1.118 (±1.137)		0.952 (±0.204)	0.808 (±0.343)	-0.497 (±0.088)	0.422 (±0.093)	1.144 (±0.253)	-0.915 (±0.174)	-3.953 (±0.468)	0.917	25.251	(7,16)
	N = 24	s = 0.214										

Table 43 Comparison of Observed and Calculated MIC's from Eq. 7 (Table 42)

No.	Observed	Calculated	Residual	Std. residual[a]
B24A	2.893	2.893	.000	.000
B24B	2.613	2.497	.115	.632
B24C	2.331	2.290	.041	.223
B27A	.099	.319	-.221	-1.207
B27B	1.071	.850	.221	1.207
B27C	1.728	1.820	-.092	-.503
B27E	2.313	2.297	.017	.091
B27F	2.334	2.234	.101	.551
B27G	2.331	2.647	-.317	-1.733
B27H	2.019	1.758	.261	1.427
B27I	1.444	1.520	-.076	-.415
B27J	1.801	2.254	-.453	-2.476
B27K	2.721	2.512	.210	1.146
B27L	2.650	2.274	.375	2.053
B28A	2.366	2.259	.107	.583
B28B	2.080	2.243	-.164	-.895
B28C	2.382	2.347	.035	.191
B29	2.046	2.031	.015	.082
B30	2.063	1.986	.077	.421
B36	2.309	2.271	.038	.209
B37	2.025	2.064	-.038	-.209
B38	2.939	2.973	-.034	-.184
B39	2.654	2.766	-.112	-.614
B40	1.738	1.844	-.106	-.581

[a]Standardized residual

Table 44 Comparison of Observed and Calculated MIC's from Eq. 8 (Table 42)

No.	Observed	Calculated	Residual	Std. residual[a]
B24A	2.893	2.893	.000	.000
B24B	2.613	2.559	.054	.301
B24C	2.331	2.329	.002	.009
B27A	.099	.320	-.221	-1.238
B27B	1.071	.849	.221	1.238
B27C	1.728	1.824	-.096	-.537
B27E	2.313	2.353	-.039	-.219
B27F	2.334	2.270	.064	.358
B27G	2.331	2.706	-.376	-2.101
B27H	2.019	1.768	.252	1.407
B27I	1.444	1.503	-.059	-.331
B27J	1.801	2.243	-.441	-2.468
B27K	2.721	2.503	.218	1.219
B27L	2.650	2.302	.348	1.947
B28A	2.366	2.274	.091	.510
B28B	2.080	2.247	-.167	-.934
B28C	2.382	2.356	.026	.144
B29	2.046	2.036	.010	.058
B30	2.063	1.977	.086	.480
B36	2.309	2.291	.018	.102
B37	2.025	2.061	-.036	-.199
B38	2.939	2.897	.042	.237
B39	2.654	2.667	-.014	-.076
B40	1.738	1.720	.017	.096

[a]Standardized residual

Table 45 Stepwise LFER Model Development for a Subset (Fluorine at Position 6) Against E. coli

Eq. No.	Log EC = Intercept	F(1)	B5(1)	F(7)	$F(7)^2$	MR(7)	RI1(7)	ICH3(7)	IRH1(7)	r^2	F	d.f.
1.	2.696 (±0.114)				-0.178 (±0.037)					0.497	22.726	(1,23)
2.	5.174 (±0.583)	-1.907 (±0.444)			-0.141 (±0.029)					0.727	29.281	(2,22)
3.	4.319 (±0.554)	-1.437 (±0.398)			-0.149 (±0.025)		0.402 (±0.124)			0.818	31.470	(3,21)
4.	4.068 (±0.550)	-1.297 (±0.390)			-0.135 (±0.025)		0.376 (±0.119)		0.262 (±0.154)	0.841	26.466	(4,20)
5.	3.328 (±0.585)	-0.806 (±0.407)			-0.118 (±0.024)		0.305 (±0.112)	0.480 (±0.202)	0.459 (±0.161)	0.878	27.293	(5,19)
6.	3.139 (±0.867)	-0.860 (±0.604)					0.195 (±0.162)	0.772 (±0.287)	0.800 (±0.217)	0.716	12.646	(4,20)
7.	3.188 (±0.724)	-0.792 (±0.505)		-0.190 (±0.061)			0.314 (±0.141)	0.670 (±0.241)	0.556 (±0.197)	0.812	16.414	(5,19)
8.	3.360 (±0.590)	-0.821 (±0.410)		0.082 (±0.097)	-0.152 (±0.046)		0.285 (±0.115)	0.441 (±0.208)	0.466 (±0.163)	0.882	22.517	(6,18)

Table 45 continued on next page.

Table 45 continued

9.	0.504 (±1.608)	-0.797 (±0.384)	0.888 (±0.469)	0.076 (±0.090)	-0.150 (±0.043)		0.311 (±0.108)	0.459 (±0.195)	0.572 (±0.162)	0.903	22.571	(7,17)
10.	0.429 (±2.510)	-0.840 (±0.599)	0.843 (±0.733)				0.218 (±0.162)	0.788 (±0.233)	0.904 (±0.285)	0.734	10.539	(5,19)
11.	-0.109 (±2.083)	-0.598 (±0.502)	1.100 (±0.612)			-0.248 (±0.079)	0.583 (±0.178)	0.612 (±0.242)	0.713 (±0.203)	0.828	14.414	(6,18)
12.	-1.039 (±1.953)		1.137 (±0.618)			-0.263 (±0.079)	0.626 (±0.176)	0.751 (±0.215)	0.803 (±0.190)	0.814	16.659	(5,19)
13.	2.531 (±0.233)					-0.244 (±0.083)	0.571 (±0.184)	0.754 (±0.227)	0.687 (±0.190)	0.781	17.762	(4,20)
14.	2.065 (±0.111)			-0.194 (±0.063)			0.345 (±0.144)	0.684 (±0.213)	0.869 (±0.186)	0.788	18.535	(4,20)

N = 25 s = 0.312

Table 46 Comparison of Observed and Calculated MIC's from Eq. 14 (Table 45)

No.	Observed	Calculated	Residual	Std. residual[a]
B24A	2.292	1.829	.462	1.622
B24B	3.204	3.114	.090	.315
B24C	2.932	3.133	-.201	-.704
B27A	2.206	2.260	-.053	-.187
B27B	1.728	2.253	-.524	-1.839
B27C	2.631	2.149	.482	1.689
B27D	1.708	1.721	-.013	-.045
B27E	2.011	2.040	-.029	-.101
B27F	2.032	1.900	.133	.465
B27G	2.631	2.750	-.119	-.419
B27H	1.719	1.696	.023	.081
B27I	1.143	1.503	-.361	-1.265
B27J	1.500	1.892	-.392	-1.374
B27K	1.818	1.918	-.100	-.349
B27L	2.650	2.177	.473	1.660
B28A	2.366	2.089	.276	.969
B28B	2.080	2.002	.078	.273
B28C	2.080	2.045	.035	.123
B29	2.349	2.501	-.152	-.533
B30	2.063	2.401	-.338	-1.186
B36	3.503	3.114	.389	1.363
B37	3.220	3.133	.088	.308
B38	3.228	3.114	.113	.397
B39	3.246	3.133	.113	.396
B40	2.642	3.114	-.472	-1.656

[a]Standardized residual

Table 47 LFER Model Development for a Subset (Fluorine at Position 6) Against *E. coli* by Forcing F(7) in as the Initial Variable

Eq. No.	Log EC = Intercept	F(1)	F(7)	$F(7)^2$	INCO(7)	RI1(7)	BPI	r^2	F	d.f.
1.	2.578 (±0.133)		-0.276 (±0.097)					0.259	8.047	(1,23)
2.	2.689 (±0.111)		0.234 (±0.159)	-0.273 (±0.074)				0.542	13.042	(2,22)
3.	5.063 (±0.578)	-1.825 (±0.440)	0.164 (±0.121)	-0.210 (±0.058)				0.749	20.845	(3,21)
4.	5.525 (±0.701)	-2.241 (±0.527)	-0.215 (±0.075)					0.593	16.029	(2,22)
5.	6.471 (±0.674)	-1.890 (±0.465)	-0.329 (±0.074)				-1.996 (±0.657)	0.718	17.718	(3,21)
6.	5.536 (±0.732)	-1.547 (±0.447)	-0.334 (±0.067)			0.346 (±0.148)	-1.583 (±0.622)	0.778	17.510	(4,20)
7.	5.495 (±0.628)	-1.103 (±0.414)	-0.442 (±0.069)		-0.591 (±0.207)	0.387 (±0.128)	-2.218 (±0.578)	0.845	20.666	(5,19)

N = 25 s = 0.274

Table 48 Comparison of Observed and Calculated MIC's from Eq. 7 (Table 47)

No.	Observed	Calculated	Residual	Std. residual[a]
B24A	2.292	2.018	.274	1.124
B24B	3.204	2.986	.218	.894
B24C	2.932	2.608	.324	1.329
B27A	2.206	1.975	.231	.948
B27B	1.728	1.959	-.231	-.948
B27C	2.631	2.156	.475	1.949
B27D	1.708	1.770	-.063	-.257
B27E	2.011	2.497	-.486	-1.993
B27F	2.032	2.179	-.146	-.599
B27G	2.631	2.557	.074	.304
B27H	1.719	1.714	.005	.019
B27I	1.143	1.276	-.133	-.547
B27J	1.500	1.762	-.262	-1.073
B27K	1.818	1.820	-.002	-.007
B27L	2.650	2.409	.241	.987
B28A	2.366	2.211	.155	.635
B28B	2.080	2.012	.068	.278
B28C	2.080	2.109	-.030	-.121
B29	2.349	2.557	-.208	-.853
B30	2.063	2.330	-.267	-1.096
B36	3.503	3.586	-.083	-.341
B37	3.220	3.208	.013	.053
B38	3.228	3.292	-.064	-.263
B39	3.246	2.913	.332	1.364
B40	2.642	3.078	-.436	-1.787

[a]Standardized residual

Table 49 LFER Model Development for a Subset (Fluorine at Position 6) Against *P. aeruginosa*

Eq. No.	Log PA = Intercept	IE(1)	F(1)	MR(1)	F(7)	$F(7)^2$	ICH3(7)	IRH1(7)	r^2	F	d.f.
1.	1.800 (±0.182)				-0.236 (±0.133)				0.121	3.168	(1,23)
2.	2.547 (±0.284)	-1.024 (±0.326)			-0.146 (±0.116)				0.394	7.143	(2,22)
3.	2.478 (±0.218)	-0.737 (±0.259)			0.473 (±0.176)	-0.346 (±0.085)			0.661	13.594	(3,21)
4.	2.059 (±0.254)	-0.447 (±0.257)			0.545 (±0.159)	-0.348 (±0.076)		0.671 (±0.262)	0.744	14.529	(4,20)
5.	4.923 (±0.996)	0.560 (±0.406)	-2.770 (±0.940)		0.528 (±0.136)	-0.338 (±0.064)		0.793 (±0.226)	0.825	17.917	(5,19)
6.	4.302 (±0.986)	0.734 (±0.392)	-2.529 (±0.889)		0.460 (±0.132)	-0.294 (±0.064)	0.566 (±0.294)	1.036 (±0.246)	0.855	17.589	(6,18)
7.	2.308 (±0.860)		-1.214 (±0.605)		0.463 (±0.141)	-0.295 (±0.069)	0.422 (±0.303)	0.821 (±0.235)	0.825	17.917	(5,19)
8.	1.141 (±1.262)		-1.074 (±0.561)	1.840 (±0.874)	0.460 (±0.129)	-0.294 (±0.063)	0.468 (±0.280)	1.010 (±0.234)	0.859	18.309	(6,18)
9.	2.063 (±1.187)		-1.592 (±0.489)	1.726 (±0.912)	0.520 (±0.130)	-0.332 (±0.062)		0.833 (±0.218)	0.838	19.516	(5,19)
10.	3.928 (±0.703)		-1.676 (±0.517)		0.517 (±0.138)	-0.330 (±0.066)		0.672 (±0.213)	0.807	20.971	(4,20)

N = 25 s = 0.373

Table 50 Comparison of Observed and Calculated MIC's from Eq. 10 (Table 49)

No.	Observed	Calculated	Residual	Std. residual[a]
B24A	1.987	1.714	.273	.801
B24B	2.613	2.190	.423	1.239
B24C	2.331	1.776	.555	1.628
B27A	.701	.727	-.025	-.075
B27B	.770	.769	.001	.003
B27C	1.427	1.288	.139	.407
B27D	1.407	1.452	-.045	-.132
B27E	1.710	1.636	.074	.218
B27F	1.431	1.774	-.344	-1.008
B27G	2.331	2.243	.087	.256
B27H	.815	1.363	-.548	-1.608
B27I	.240	.309	-.069	-.202
B27J	.297	.604	-.307	-.900
B27K	1.516	.761	.755	2.214
B27L	2.046	1.717	.329	.965
B28A	1.462	1.526	-.064	-.187
B28B	1.177	1.202	-.025	-.072
B28C	1.177	1.377	-.200	-.587
B29	1.445	1.262	.182	.535
B30	.860	1.597	-.737	-2.162
B36	3.201	3.102	.100	.292
B37	2.629	2.687	-.058	-.171
B38	2.636	2.654	-.018	-.052
B39	2.354	2.240	.114	.334
B40	1.738	2.329	-.592	-1.735

[a]Standardized residual

Table 51 Correlation Matrix of the Variables Used in the Analyses of the Subset (Fluorine at Position 6; Tables 42, 45, 47, 49)

	IV(1)	L(1)	F(1)	MR(1)	F(7)	$F(7)^2$	MR(7)	INCO(7)	RI1(7)	RI2(7)	ICH3(7)	IRH1(7)	BPI	SA	EC	PA
IV(1)	1.000															
L(1)	0.188	1.000														
F(1)	-0.880	-0.431	1.000													
MR(1)	-0.025	0.742	0.070	1.000												
F(7)	-0.123	0.009	0.189	0.166	1.000											
$F(7)^2$	-0.196	-0.066	0.291	0.166	0.870	1.000										
MR(7)	-0.089	-0.006	0.136	0.111	0.766	0.655	1.000									
INCO(7)	-0.109	-0.046	0.160	0.079	-0.355	-0.270	-0.015	1.000								
RI1(7)	0.241	0.101	-0.354	-0.174	0.115	-0.021	0.501	0.050	1.000							
RI2(7)	-0.060	-0.025	0.089	0.043	0.077	-0.035	-0.182	-0.075	-0.250	1.000						
ICH3(7)	0.345	0.355	-0.475	0.064	-0.013	-0.203	-0.028	-0.136	0.301	-0.075	1.000					
IRH1(7)	0.221	-0.079	0.351	-0.414	-0.391	-0.392	-0.220	-0.185	0.204	-0.102	-0.185	1.000				
BPI	-0.087	-0.036	0.128	0.063	-0.468	-0.113	-0.561	-0.109	0.361	-0.060	-0.109	-0.147	1.000			
SA	0.012	0.311	-0.148	0.152	0.306	-0.087	0.405	-0.120	0.350	0.254	0.119	0.202	-0.762	1.000		
EC	0.488	0.326	-0.663	-0.113	-0.509	-0.705	-0.290	-0.007	0.470	-0.023	0.471	0.563	-0.191	0.340	1.000	
PA	0.505	0.314	-0.634	-0.058	-0.348	-0.618	-0.201	-0.179	0.355	0.101	0.401	0.587	-0.340	0.582	0.906	1.000

A second subset of 22 compounds (Table 6, B3A-C, B15A-C, B18A-C, B22A-C, B23A-C, B24A-C, B36-39) were selected using ethyl group in position 1, hydrogen in position 6, and an unsubstituted piperazinyl ring in position 7 as the reference compound (B3B) for a Free-Wilson analysis. The indicator variables vinyl and fluoroethyl in position 1; fluorine, cyanide, nitro, chlorine and amine group in position 6; pyrrolidinyl ring and methyl group on the piperazinyl ring were included in the analysis. Equations for the three bacterial system were derived by using stepwise and dropworst procedures.

The de novo models of this subset is shown in Table 52. For all three bacterial systems, fluorine in position 6 is very significant. For S. aureus eq. 2 (Table 52) indicates that NH_2 group in position 6 is a negative contributor to activity. Eq. 4 of E. coli and eq. 8 of P. aeruginosa shows that the pyrrolidinyl ring RI2(7) reduces activity. Eq. 8 of P. aeruginosa also indicates that cyanide in position 6 is a positive factor, but the methyl group of piperazinyl ring ICH3(7) reduces activity. The observed activity, calculated activity, residuals and standardized residuals of each model is shown in Table 53 (eq. 2), 54 (eq. 4), 55 (eq. 8).

The LFER models based on the same 22 compounds as in the Free-Wilson's analyses were derived. The model development for the three bacterial systems is shown in Tables 56, 59 and 61. The LFER model of this subset indicates that the fluorine is very significant in all three bacterial systems. For S. aureus, length L or BPI^2 gave eq. 3 or eq. 4 (Table 56), respectively. The r^2 and F value of eq. 3 are more significant than that of eq. 4, but there is one outlier (B23C)

for eq. 3. Eq. 3 indicates that STERIMOL L and electronic effect are important for activity, whereas eq. 4 indicates that a parabolic relationship of BPI alone with fluorine is important contributor to activity. L(6) and BPI are orthogonal having a correlation coefficient of 0.113 (see Table 63).

For E. coli eqs. 2-4 (Table 59) were derived. In the development of this model, F(7) and $MR(7)^2$ have equal entering t-values. Eq. 3 or 4 containing F(7) or $MR(7)^2$, respectively, have the same r^2 and F-values. Eq. 3 indicates that lipophilicity and molar refractivity in position 7 are important determinants for activity. Eq. 4 indicates that a parabolic relationship of molar refractivity in position 7 alone with fluorine is important for activity. F(7) and $MR(7)^2$ are orthogonal having a correlation coefficient of -0.141 (see Table 63).

For P. aeruginosa eqs. 3-5 (Table 61) were derived. The presence of ICN(6) or L(6) in the model gave eq. 4 or eq. 5, respectively. The r^2 and F-value of eq. 4 are the same as that of eq. 8 (Table 52). Note the regression coefficients for IF(6) and ICN(6) are identical in both equations, but the intercept terms differ by a factor of 10. The Free-Wilson model (Table 52) contain two additional indicator variables, ICH3(7) and RI2(7) (the pyrrolidinyl ring) whereas eq. 4 (Table 61) has fragment and molar refractivity variables at position 7. This indicates that lipophilicity and bulk are important determinants for activity against P. aeruginosa in this subset of 22 compounds. To make the model in Table 61 more LFER in style, ICN(6) was deleted and length

L(6) added giving eq. 5. The calculated values and residuals for each of the models are listed in Tables 53-55, 57-58, 60 and 62.

Another 18 compounds (B3A-C, B15A-C, B18A-C, B22A-C, B23A-C, B24A-C) were selected using as the reference compound (B3B) ethyl fixed in position 1, hydrogen in position 6 and an unsubstituted piperazinyl ring in position 7 for the Free-Wilson analysis. The derived equations for the three bacterial systems (Table 64) are very similar as Table 52 except the methyl substituent on the piperazinyl ring [ICH3(7)] is not in the model (eq. 3 in Table 61) for P. aeruginosa. The residual analysis was essential identical to that in Tables 53-55.

In order to determine the contribution of substituents on position 1 and the effect of N-substitution on the piperazinyl ring at position 7, six compounds (B24B-C, B36-39) were selected using position 6 fixed with fluorine, ethyl in position 1 and unsubstituted piperazinyl ring in position 7 as the reference compound (B24B) for a Free-Wilson analysis. Only 3 independent variables [IV(1), IEF(1) and ICH3(7)] were used in the regression.

For S. aureus and E. coli no significant model could be obtained. The de novo model of P. aeruginosa is shown in Table 65. Eq. 2 indicates that vinyl in position 1 and methyl of piperazinyl ring in position 7 are important for activity, It is implied that fluoroethyl in position 1 is not important for activity.

The stability of the regression coefficients found in eq. 14 (Table 29), eq. 16 (Table 35), and eq. 12 (Table 37) was checked by omitting five compounds selected by a random number generator giving

Table 52 de novo Model Development for Three Bacterial Systems on a Subset (Reference Compound: B3B)

Eq. No.	Log SA[a] = Intercept	IF(6)	INH(6)	r^2	F	d.f.
1.	1.315 (±0.128)	1.223 (±0.227)		0.591	28.903	(1,20)
2.	1.518 (±0.104)	1.019 (±0.171)	-1.018 (±0.232)	0.797	37.302	(2,19)

N = 22 s = 0.359

Eq. No.	Log EC[b] = Intercept	IF(6)	RI2(7)	r^2	F	d.f.
3.	1.571 (±0.178)	1.518 (±0.316)		0.536	23.059	(1,20)
4.	1.992 (±0.119)	1.278 (±0.181)	-1.262 (±0.190)	0.861	58.530	(2,19)

N = 22 s = 0.389

Table 52 continued on next page.

Table 52 continued

Eq. No.	Log PA[c] = Intercept	IF(6)	ICN(6)	ICH3(7)	RI2(7)	r^2	F	d.f.
5.	1.034 (±0.137)	1.502 (±0.243)				0.657	38.199	(1,20)
6.	1.340 (±0.101)	1.327 (±0.155)			-0.920 (±0.162)	0.873	65.100	(2,19)
7.	1.244 (±0.097)	1.423 (±0.141)	0.479 (±0.188)		-0.920 (±0.143)	0.907	58.261	(3,18)
8.	1.385 (±0.111)	1.423 (±0.130)	0.479 (±0.173)	-0.282 (±0.134)	-1.016 (±0.147)	0.926	53.193	(4,17)

N = 22 s = 0.267

[a]S. aureus ; [b]E. coli ; [c]P. aeruginosa

Table 53 Comparison of Observed and Calculated MIC's (S. aureus) from Eq. 2 (Table 52)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.518	-.158	-.462
B3B	1.082	1.518	-.436	-1.277
B3C	1.102	1.518	-.416	-1.219
B15A	1.410	1.518	-.108	-.317
B15B	2.032	1.518	.513	1.502
B15C	1.750	1.518	.231	.677
B18A	2.000	1.518	.482	1.410
B18B	1.719	1.518	.201	.588
B18C	1.435	1.518	-.083	-.243
B22A	1.123	1.518	-.395	-1.156
B22B	1.745	1.518	.227	.663
B22C	1.461	1.518	-.057	-.168
B23A	.179	.500	-.321	-.940
B23B	.200	.500	-.300	-.879
B23C	1.122	.500	.622	1.820
B24A	2.893	2.538	.355	1.040
B24B	2.613	2.538	.075	.219
B24C	2.331	2.538	-.207	-.606
B36	2.309	2.538	-.229	-.669
B37	2.025	2.538	-.512	-1.499
B38	2.939	2.538	.402	1.176
B39	2.654	2.538	.116	.340

[a]Standardized residual

Table 54 Comparison of Observed and Calculated MIC's (*E. coli*) from Eq. 4 (Table 52)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.060	.730	.330	.894
B3B	1.684	1.992	-.308	-.833
B3C	1.703	1.992	-.288	-.781
B15A	.206	.730	-.523	-1.417
B15B	2.635	1.992	.643	1.740
B15C	2.352	1.992	.360	.974
B18A	1.397	.730	.667	1.806
B18B	1.350	1.992	-.642	-1.738
B18C	2.340	1.992	.348	.943
B22A	.521	.730	-.208	-.563
B22B	1.745	1.992	-.247	-.669
B22C	2.062	1.992	.070	.190
B23A	.179	.730	-.550	-1.490
B23B	2.006	1.992	.014	.037
B23C	2.327	1.992	.335	.907
B24A	2.292	2.007	.284	.770
B24B	3.204	3.270	-.065	-.177
B24C	2.932	3.270	-.338	-.914
B36	3.503	3.270	.234	.632
B37	3.220	3.270	-.049	-.133
B38	3.228	3.270	-.042	-.113
B39	3.246	3.270	-.024	-.065

[a]Standardized residual

Table 55 Comparison of Observed and Calculated MIC's (P. aeruginosa) from Eq. 8 (Table 52)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	.157	.324	-.167	-.695
B3B	1.082	1.385	-.303	-1.261
B3C	1.102	1.104	-.002	-.008
B15A	.206	.324	-.118	-.490
B15B	1.730	1.385	.345	1.435
B15C	1.447	1.104	.344	1.429
B18A	1.096	.803	.293	1.219
B18B	1.719	1.865	-.146	-.605
B18C	1.435	1.583	-.148	-.614
B22A	.220	.324	-.104	-.431
B22B	1.143	1.385	-.243	-1.009
B22C	.857	1.104	-.247	-1.026
B23A	.179	.324	-.145	-.602
B23B	1.706	1.385	.320	1.332
B23C	1.423	1.104	.319	1.326
B24A	1.987	1.747	.240	.999
B24B	2.613	2.808	-.196	-.813
B24C	2.331	2.527	-.196	-.814
B36	3.201	2.808	.393	1.635
B37	2.629	2.527	.102	.426
B38	2.636	2.808	-.172	-.714
B39	2.354	2.527	-.173	-.719

[a]Standardized residual

Table 56 LFER Model Development for the Free-Wilson Subset Against S. aureus (See Table 52)

Eq. No.	Log SA = Intercept	L(6)	IF(6)	BPI	BPI^2	r^2	F	d.f.
1.	1.315 (±0.128)		1.223 (±0.227)			0.591	28.903	(1,20)
2.	2.224 (±0.290)		1.010 (±0.195)	-1.110 (±0.331)		0.743	27.540	(2,19)
3.	0.961 (±0.434)	0.390 (±0.113)	1.229 (±0.169)	-1.094 (±0.264)		0.845	32.712	(3,18)
	N = 22	s = 0.322						
4.	-0.821 (±0.113)		1.035 (±0.168)	5.903 (±2.515)	-3.505 (±1.245)	0.822	27.558	(3,18)
	N = 22	s = 0.346						

Table 57 Comparison of Observed and Calculated MIC's from Eq. 3 (Table 56)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.204	.157	.525
B3B	1.082	1.204	-.122	-.409
B3C	1.102	1.204	-.102	-.342
B15A	1.410	1.647	-.237	-.796
B15B	2.032	1.647	.384	1.288
B15C	1.750	1.647	.102	.342
B18A	2.000	1.758	.242	.811
B18B	1.719	1.758	-.039	-.131
B18C	1.435	1.758	-.323	-1.082
B22A	1.123	1.473	-.350	-1.172
B22B	1.745	1.473	.272	.911
B22C	1.461	1.473	-.012	-.040
B23A	.179	.491	-.312	-1.046
B23B	.200	.491	-.291	-.976
B23C	1.122	.491	.631	2.115
B24A	2.893	2.538	.355	1.191
B24B	2.613	2.538	.075	.251
B24C	2.331	2.538	-.207	-.694
B36	2.309	2.538	-.229	-.767
B37	2.025	2.538	-.512	-1.717
B38	2.939	2.538	.402	1.346
B39	2.654	2.538	.116	.389

[a]Standardized residual

Table 58 Comparison of Observed and Calculated MIC's from Eq. 4 (Table 56)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.361	1.283	.077	.241
B3B	1.082	1.283	-.201	-.628
B3C	1.102	1.283	-.181	-.566
B15A	1.410	1.503	-.093	-.290
B15B	2.032	1.503	.529	1.650
B15C	1.750	1.503	.247	.770
B18A	2.000	1.651	.349	1.088
B18B	1.719	1.651	.068	.211
B18C	1.435	1.651	-.216	-.674
B22A	1.123	1.640	-.517	-1.615
B22B	1.745	1.640	.104	.325
B22C	1.461	1.640	-.180	-.560
B23A	.179	.495	-.316	-.987
B23B	.200	.495	-.295	-.922
B23C	1.122	.495	.627	1.956
B24A	2.893	2.538	.355	1.109
B24B	2.613	2.538	.075	.234
B24C	2.331	2.538	-.207	-.646
B36	2.309	2.538	-.229	-.714
B37	2.025	2.538	-.512	-1.599
B38	2.939	2.538	.402	1.254
B39	2.654	2.538	.116	.362

[a] Standardized residual

Table 59 LFER Model Development for the Free-Wilson Subset Against *E. coli* (See Table 52)

Eq. No.	Log EC = Intercept	IF(6)	F(7)	MR(7)	$MR(7)^2$	r^2	F	d.f.
1.	1.571 (±0.178)	1.518 (±0.316)				0.536	23.059	(1,20)
2.	-2.030 (±0.834)	1.355 (±0.232)		1.420 (±0.325)		0.768	31.568	(2,19)
3.	-1.287 (±0.693)	1.277 (±0.185)	-0.565 (±0.161)	1.266 (±0.261)		0.863	37.618	(3,18)
	N = 22	s = 0.396						
4.	-25.931 (±6.837)	1.278 (±0.185)		20.334 (±5.391)	-3.675 (±1.046)	0.863	37.618	(3,18)
	N = 22	s = 0.396						

Table 60 Comparison of Observed and Calculated MIC's from Equivalent Eqs 3 and 4 (Table 59)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	1.060	.730	.330	.901
B3B	1.684	1.940	-.256	-.698
B3C	1.703	2.044	-.340	-.928
B15A	.206	.730	-.523	-1.428
B15B	2.635	1.940	.694	1.894
B15C	2.352	2.044	.308	.840
B18A	1.397	.730	.667	1.820
B18B	1.350	1.940	-.590	-1.610
B18C	2.340	2.044	.296	.809
B22A	.521	.730	-.208	-.568
B22B	1.745	1.940	-.195	-.533
B22C	2.062	2.044	.018	.050
B23A	.179	.730	-.550	-1.501
B23B	2.006	1.940	.066	.179
B23C	2.327	2.044	.283	.773
B24A	2.292	2.007	.284	.775
B24B	3.204	3.218	-.014	-.037
B24C	2.932	3.321	-.389	-1.062
B36	3.503	3.218	.285	.778
B37	3.220	3.321	-.101	-.275
B38	3.228	3.218	.010	.027
B39	3.246	3.321	-.076	-.206

[a]Standardized residual

Table 61 LFER Model Development for the Free-Wilson Subset Against P. aeruginosa (See Table 52)

Eq. No.	Log PA = Intercept	L(6)	IF(6)	ICN(6)	F(7)	MR(7)	r^2	F	d.f.
1.	1.034 (±0.137)		1.502 (±0.243)				0.657	38.199	(1,20)
2.	1.473 (±0.131)		1.383 (±0.169)		-0.704 (±0.147)		0.845	51.724	(2,19)
3.	-0.038 (±0.548)		1.327 (±0.146)		-0.644 (±0.127)	0.581 (±0.176)	0.892	49.745	(3,18)
4.	-0.134 (±0.469)		1.423 (±0.130)	0.479 (±0.173)	-0.644 (±0.109)	0.581 (±0.176)	0.926	53.193	(4,17)
	N = 22	s = 0.267							
5.	-0.798 (±0.586)	0.228 (±0.100)	1.454 (±0.142)		-0.644 (±0.114)	0.581 (±0.185)	0.918	47.538	(4,17)
	N = 22	s = 0.281							

Table 62 Comparison of Observed and Calculated MIC's from Eq. 5 (Table 61)

No.	Observed	Calculated	Residual	Std. residual[a]
B3A	.157	.159	-.002	-.008
B3B	1.082	1.220	-.138	-.545
B3C	1.102	.938	.163	.646
B15A	.206	.491	-.285	-1.127
B15B	1.730	1.553	.178	.702
B15C	1.447	1.271	.176	.696
B18A	1.096	.653	.443	1.751
B18B	1.719	1.714	.004	.018
B18C	1.435	1.433	.002	.010
B22A	.220	.473	-.253	-.999
B22B	1.143	1.534	-.392	-1.548
B22C	.857	1.253	-.396	-1.564
B23A	.179	.323	-.144	-.567
B23B	1.706	1.384	.321	1.270
B23C	1.423	1.102	.320	1.264
B24A	1.987	1.747	.240	.949
B24B	2.613	2.808	-.196	-.772
B24C	2.331	2.527	-.196	-.774
B36	3.201	2.808	.393	1.553
B37	2.629	2.527	.102	.405
B38	2.636	2.808	-.172	-.678
B39	2.354	2.527	-.173	-.683

[a]Standardized residual

Table 63 Correlation Matrix for the LFER Models (Tables 56, 59, 61) Development from Free-Wilson Subset

	L(6)	IF(6)	ICN(6)	F(7)	MR(7)	$MR(7)^2$	BPI	BPI^2	SA	EC	PA
L(6)	1.000										
IF(6)	-0.393	1.000									
ICN(6)	0.724	-0.271	1.000								
F(7)	0.057	-0.146	0.040	1.000							
MR(7)	-0.063	0.160	-0.043	-0.188	1.000						
$MR(7)^2$	-0.061	0.154	-0.042	-0.141	0.998	1.000					
BPI	0.113	-0.324	0.030	0.048	-0.052	-0.050	1.000				
BPI^2	0.020	-0.317	-0.031	0.047	-0.051	-0.049	0.994	1.000			
SA	-0.002	0.769	0.008	-0.164	0.107	0.100	-0.619	-0.642	1.000		
EC	-0.228	0.732	-0.147	-0.487	0.594	0.575	-0.259	-0.257	0.673	1.000	
PA	-0.171	0.810	-0.044	-0.548	0.414	0.391	-0.204	-0.208	0.687	0.926	1.000

Table 64 de novo Model Development for Three Bacterial Systems on a Subset (Reference Compound: B3B)

Eq. No.	Log SA[a] = Intercept	IF(6)	INH(6)	r^2	F	d.f.
1.	1.315 (±0.136)	1.297 (±0.333)		0.487	15.246	(1,16)
2.	1.518 (±0.104)	1.094 (±0.232)	-1.018 (±0.232)	0.775	25.746	(2,15)
	N = 18	s = 0.361				

Eq. No.	Log EC[b] = Intercept	IF(6)	RI2(7)	r^2	F	d.f.
3.	2.195 (±0.186)		-1.252 (±0.322)	0.486	15.110	(1,16)
4.	1.989 (±0.133)	1.238 (±0.273)	-1.252 (±0.216)	0.783	27.021	(2,15)
	N = 18	s = 0.432				

Table 64 continued on next page.

Table 64 continued

Eq. No.	Log PA^c = Intercept	IF(6)	ICN(6)	RI2(7)	r^2	F	d.f.
5.	1.034 (±0.144)	1.277 (±0.353)			0.450	13.061	(1,16)
6.	1.336 (±0.103)	1.277 (±0.212)		-0.908 (±0.168)	0.814	32.619	(2,15)
7.	1.240 (±0.095)	1.373 (±0.184)	0.479 (±0.184)	-0.908 (±0.143)	0.875	32.605	(3,14)

N = 18 s = 0.285

[a] S. aureus ; [b] E. coli ; [c] P. aeurginosa

Table 65 *de novo* Model Development for a Subset (Reference: Compound: B24B) Against *P. aeruginosa*

Eq. No.	Log PA = Intercept	IV(1)	ICH3(7)	r^2	F	d.f.
1.	2.483 (±0.124)	0.432 (±0.214)		0.505	4.082	(1,4)
2.	2.673 (±0.063)	0.432 (±0.085)	-0.379 (±0.080)	0.942	24.344	(2,3)

N = 6 s = 0.098

Table 66 Results from Random Sample Analyses (See Eq. 14, Table 29; Eq. 16, Table 35; Eq. 12, Table 37)

Eq. No.	Log SA = Intercept	IEF(1)	IF(6)	MR(6)	MR(7)	$MR(7)^2$	BPI	r^2	F	d.f.
1.	0.236	0.716	0.973	1.546	0.842	-0.100	-1.341	0.803	19.814	(6,29)
	(±0.360)	(±0.267)	(±0.197)	(±0.457)	(±0.217)	(±0.035)	(±0.242)			
	N = 36	s = 0.352								
2.	0.235	0.827	1.045	1.173	0.886	-0.114	-1.269	0.743	13.924	(6,29)
	(±0.431)	(±0.428)	(±0.241)	(±0.483)	(±0.253)	(±0.041)	(±0.277)			
	N = 36	s = 0.411								
3.	0.406	0.617	0.771	0.675	0.987	-0.127	-1.298	0.750	14.515	(6,29)
	(±0.425)	(±0.307)	(±0.284)	(±0.553)	(±0.261)	(±0.042)	(±0.273)			
	N = 36	s = 0.406								

Eq. No.	Log EC = Intercept	IF(6)	INH(6)	F(7)	ICH3(7)	IRH1(7)	BPI	r^2	F	d.f.
4.	2.057	1.121	0.882	-0.250	1.110	0.746	-1.336	0.771	16.356	(6,29)
	(±0.347)	(±0.160)	(±0.463)	(±0.085)	(±0.185)	(±0.193)	(±0.459)			
	N = 36	s = 0.404								
5.	2.050	1.091	0.887	-0.259	1.144	0.712	-1.331	0.765	15.770	(6,29)
	(±0.339)	(±0.154)	(±0.421)	(±0.080)	(±0.183)	(±0.193)	(±0.427)			
	N = 36	s = 0.385								
6.	2.267	1.077	0.968	-0.268	1.197	0.779	-1.565	0.820	22.063	(6,29)
	(±0.334)	(±0.143)	(±0.412)	(±0.079)	(±0.171)	(±0.183)	(±0.423)			
	N = 36	s = 0.377								

Table 66 continued on next page.

Table 66 continued

Eq. No.	Log PA = Intercept	IV(1)	IF(6)	ICN(6)	F(7)	$F(7)^2$	ICH3(7)	IRH1(7)	r^2	F	d.f.
7.	0.339	0.538	1.089	0.417	0.379	-0.253	0.733	1.063	0.840	21.053	(7,28)
	(±0.149)	(±0.271)	(±0.147)	(±0.220)	(±0.129)	(±0.062)	(±0.173)	(±0.166)			
	N = 36	s = 0.338									
8.	0.291	0.302	1.162	0.441	0.403	-0.265	0.722	1.126	0.839	20.862	(7,28)
	(±0.137)	(±0.348)	(±0.136)	(±0.198)	(±0.115)	(±0.056)	(±0.165)	(±0.153)			
	N = 36	s = 0.307									
9.	0.289	0.428	1.207	0.430	0.439	-0.287	0.685	1.176	0.876	28.270	(7,28)
	(±0.135)	(±0.254)	(±0.136)	(±0.206)	(±0.120)	(±0.058)	(±0.160)	(±0.157)			
	N = 36	s = 0.316									

eqs. 1-3 (Table 66) for comparison with eq. 14 (Table 29), eqs. 4-6 for comparison with eq. 16 (Table 35), eqs. 7-9 for comparison with eq. 12 (Table 37). Similar results were obtained in each set, although there was some noise in the coefficients.

III. Set C

A third QSAR analysis was performed on a set of 1,7-di substituted 6-fluoro-1,4-dihydro-4-oxo-1,8-naphthyridine 3-carboxylic acids (Table 10). (27) Forty two compounds were analyzed. Enoxacin (D2) was deleted because it was the only example with a piperazinyl ring at position 7. For the S. aureus and E. coli test systems, statistically valid models could not be obtained using the LFER approach.

The LFER models for P. aeruginosa are listed in Table 67. They were developed by the stepwise procedure up to eq. 5 (Table 67). At eq. 5 there were three nearly equivalent variables, IE(1), F(1) and RI3(7). Addition of each of these variables individually to eq. 5 gave eqs. 6, 7 and 8 respectively. All three of these equations are nearly equivalent and give nearly identical results in terms of predicting activity. Any combination of two of the three variables causes one of the included variables to lose significance, especially IE(1) and F(1) each lose significance when RI3(7) is present. It also should be noted that the maximum width of the R-substituent at position 7 (B5R(7)) and a parabolic relationship for lipophilicity of the R-substituent are important reducer of activity. Indeed, all substituent terms weaken activity.

The observed activity, calculated activity, residuals and standardized residuals are shown in Table 68 (eq. 6), 69 (eq. 7), and 70 (eq. 8). The correlation matrix for P. aeruginosa is shown in Table 71.

A structured subset (see Free-Wilson discussion below) of 25 compounds (Table 10, D28A-B, D30A, D33A-C, D34A-B, D36A-B, D38A-C, D39A-B, D40A-C, D42A-C, D46A, D50A, D56A) were selected in order to carry out a LFER and Free-Wilson analyses on the same set of compounds. For S. aureus no LFER model on the 25 compounds could be obtained.

The LFER model of E. coli and P. aeruginosa is shown in Tables 72 and 74. For E. coli eq. 4 (Table 72) indicates that an ethyl group in position 1, STERIMOL L (length) and MR of the R-substituent in position 7 are important determinants of activity. The calculated values and residuals are listed in Table 73.

For P. aeruginosa eq. 3 (Table 74) indicates that STERIMOL L and MR of the R-substituent at position 7 are important as also was found in the E. coli system. It also indicates that molar refractivity of the R-substituent is a negative factor, but that the pyrrolidinyl ring RI1(7) enhances activity. In eq. 3, L and MR of the R-substituent is highly correlated (correlation matrix, Table 76). The calculated values and residuals for eq. 3 (Table 74) are shown in Table 75. The correlation matrix for the LFER models (Tables 72, 74) is shown in Table 76.

In the Free-Wilson analysis of the 25 compounds the reference compound (D50A) contained ethyl at position 1, amine for the R-

Table 67 LFER Model Development for Set C Against *P. aeruginosa*

Eq. No.	Log PA = Intercept	IE(1)	F(1)	B5R(7)	LR(7)	FR(7)	$FR(7)^2$	RI3(7)	r^2	F	d.f.
1.	3.11 (±0.287)				-0.348 (±0.068)				0.394	25.944	(1,40)
2.	2.608 (±0.280)				-0.344 (±0.059)	-0.365 (±0.094)			0.562	25.000	(2,39)
3.	2.383 (±0.251)				-0.335 (±0.051)	-1.165 (±0.233)	-0.361 (±0.098)		0.677	26.473	(3,38)
4.	2.338 (±0.242)			-0.331 (±0.165)	-0.122 (±0.117)	-1.431 (±0.260)	-0.443 (±0.103)		0.708	22.518	(4,37)
5.	2.228 (±0.219)			-0.487 (±0.070)		-1.561 (±0.229)	-0.483 (±0.096)		0.700	29.479	(3,28)
6.	2.534 (±0.253)	-0.296 (±0.137)		-0.502 (±0.067)		-1.518 (±0.219)	-0.484 (±0.091)		0.733	25.429	(4,37)
	N = 42	s = 0.392									
7.	3.134 (±0.460)		-0.639 (±0.289)	-0.502 (±0.067)		-1.518 (±0.219)	-0.481 (±0.091)		0.735	25.647	(4,37)
	N = 42	s = 0.391									
8.	2.523 (±0.239)			-0.526 (±0.067)		-1.429 (±0.222)	-0.453 (±0.091)	-0.415 (±0.171)	0.741	26.564	(4,37)
	N = 42	s = 0.386									

Table 68 Comparison of Observed and Calculated MIC's from Eq. 6 (Table 67)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	2.593	2.422	.172	.461
D28B	2.893	2.718	.175	.470
D29A	.876	.189	.687	1.842
D30A	1.991	2.418	-.427	-1.145
D31A	1.710	1.849	-.139	-.373
D32A	1.127	1.423	-.295	-.792
D33A	2.914	2.422	.492	1.320
D33B	3.201	2.718	.484	1.298
D33C	2.939	2.718	.222	.595
D34A	2.331	1.882	.449	1.204
D34B	2.629	2.178	.451	1.210
D34C	2.654	2.178	.476	1.277
D35A	2.046	1.599	.447	1.198
D36A	.907	.675	.232	.621
D36B	.602	.971	-.369	-.989
D37A	.258	.690	-.432	-1.159
D38A	1.745	1.752	-.007	-.020
D38B	2.043	2.048	-.005	-.012
D38C	1.767	2.048	-.281	-.754
D39A	2.046	1.606	.440	1.182
D39B	2.043	1.902	.142	.380
D40A	1.762	1.112	.650	1.743
D40B	1.460	1.408	.052	.138
D40C	1.182	1.408	-.226	-.607
D41A	1.824	1.495	.329	.883
D42A	1.177	1.450	-.273	-.733
D42B	1.175	1.746	-.571	-1.532
D42C	1.197	1.746	-.549	-1.473
D43A	1.428	1.768	-.340	-.912
D44A	1.162	1.282	-.120	-.322
D45A	2.031	2.422	-.391	-1.049
D46A	2.614	2.418	.196	.526
D47A	2.047	2.223	-.176	-.472
D48A	1.434	1.242	.192	.514
D49A	1.728	1.367	.361	.969
D50A	2.331	2.422	-.091	-.244
D51A	.340	.642	-.302	-.810
D52A	1.445	1.810	-.365	-.979
D53A	1.194	1.045	.149	.399
D54A	.860	1.367	-.507	-1.360
D55A	1.127	1.367	-.240	-.643
D56A	1.728	2.418	-.690	-1.851

[a]Standardized residual

Table 69 Comparison of Observed and Calculated MIC's from Eq. 7 (Table 67)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	2.593	2.428	.165	.445
D28B	2.893	2.776	.117	.315
D29A	.876	.187	.690	1.855
D30A	1.991	2.426	-.434	-1.169
D31A	1.710	1.851	-.141	-.379
D32A	1.127	1.421	-.294	-.791
D33A	2.914	2.428	.486	1.306
D33B	3.201	2.776	.426	1.145
D33C	2.939	2.605	.334	.899
D34A	2.331	1.886	.444	1.195
D34B	2.629	2.234	.395	1.062
D34C	2.654	2.063	.590	1.588
D35A	2.046	1.600	.446	1.201
D36A	.907	.672	.234	.630
D36B	.602	1.020	-.418	-1.124
D37A	.258	.688	-.430	-1.156
D38A	1.745	1.752	-.008	-.021
D38B	2.043	2.100	-.057	-.153
D38C	1.767	1.929	-.162	-.437
D39A	2.046	1.611	.435	1.170
D39B	2.043	1.959	.084	.227
D40A	1.762	1.128	.634	1.704
D40B	1.460	1.476	-.016	-.044
D40C	1.182	1.305	-.124	-.333
D41A	1.824	1.495	.329	.885
D42A	1.177	1.460	-.282	-.760
D42B	1.175	1.807	-.632	-1.700
D42C	1.197	1.637	-.439	-1.182
D43A	1.428	1.779	-.350	-.943
D44A	1.162	1.295	-.133	-.359
D45A	2.031	2.428	-.398	-1.069
D46A	2.614	2.426	.189	.507
D47A	2.047	2.226	-.179	-.480
D48A	1.434	1.240	.194	.522
D49A	1.728	1.365	.363	.977
D50A	2.331	2.428	-.097	-.262
D51A	.340	.639	-.299	-.804
D52A	1.445	1.811	-.366	-.984
D53A	1.194	1.049	.145	.391
D54A	.860	1.365	-.505	-1.358
D55A	1.127	1.365	-.238	-.640
D56A	1.728	2.426	-.698	-1.877

[a]Standardized residual

Table 70 Comparison of Observed and Calculated MIC's from Eq. 8 (Table 67)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	2.593	2.598	-.004	-.012
D28B	2.893	2.598	.295	.803
D29A	.876	.411	.465	1.265
D30A	1.991	2.598	-.606	-1.651
D31A	1.710	1.996	-.286	-.779
D32A	1.127	1.577	-.450	-1.224
D33A	2.914	2.598	.316	.860
D33B	3.201	2.598	.604	1.643
D33C	2.939	2.598	.341	.930
D34A	2.331	2.029	.302	.822
D34B	2.629	2.029	.600	1.634
D34C	2.654	2.029	.625	1.701
D35A	2.046	1.742	.305	.830
D36A	.907	.838	.069	.188
D36B	.602	.838	-.236	-.641
D37A	.258	.827	-.569	-1.549
D38A	1.745	1.903	-.159	-.432
D38B	2.043	1.903	.140	.382
D38C	1.767	1.903	-.136	-.371
D39A	2.046	1.741	.305	.830
D39B	2.043	1.741	.302	.822
D40A	1.762	1.286	.476	1.297
D40B	1.460	1.286	.174	.474
D40C	1.182	1.286	-.104	-.282
D41A	1.824	1.633	.191	.521
D42A	1.177	1.593	-.416	-1.132
D42B	1.175	1.593	-.418	-1.137
D42C	1.197	1.593	-.396	-1.077
D43A	1.428	1.933	-.504	-1.373
D44A	1.162	1.443	-.281	-.765
D45A	2.031	2.598	-.567	-1.545
D46A	2.614	2.598	.017	.046
D47A	2.047	2.387	-.340	-.925
D48A	1.434	1.489	-.055	-.151
D49A	1.728	1.235	.493	1.342
D50A	2.331	2.183	.148	.402
D51A	.340	.400	-.060	-.164
D52A	1.445	1.545	-.100	-.272
D53A	1.194	.736	.457	1.245
D54A	.860	1.235	-.375	-1.021
D55A	1.127	1.235	-.108	-.294
D56A	1.728	2.183	-.455	-1.238

[a]Standardized residual

Table 71 Correlation Matrix of the Variables Used in the Analyses of the P. aeruginosa Test System (Table 67)

	IE(1)	F(1)	LR(7)	B5R(7)	FR(7)	$FR(7)^2$	MRR(7)	$MRR(7)^2$	RI3(7)	PROX1(7)	SA	EC	PA
IE(1)	1.000												
F(1)	0.943	1.000											
LR(7)	0.010	0.006	1.000										
B5R(7)	-0.150	-0.145	0.868	1.000									
FR(7)	0.285	0.245	0.019	-0.203	1.000								
$FR(7)^2$	-0.263	-0.221	-0.001	0.143	-0.936	1.000							
MRR(7)	-0.102	-0.090	0.929	0.837	0.079	-0.068	1.000						
$MRR(7)^2$	-0.001	0.002	0.857	0.622	0.216	-0.174	0.930	1.000					
RI3(7)	0.325	0.306	-0.106	-0.304	0.371	-0.293	-0.118	-0.099	1.000				
PROX1(7)	-0.232	-0.223	0.123	0.279	-0.367	0.299	0.131	-0.045	-0.842	1.000			
SA	0.015	0.067	-0.250	-0.221	-0.201	0.112	-0.240	-0.226	-0.012	-0.118	1.000		
EC	-0.423	-0.360	-0.488	-0.315	-0.284	0.135	-0.431	-0.468	-0.271	0.880	0.484	1.000	
PA	-0.240	-0.234	-0.627	-0.468	-0.422	0.265	-0.613	-0.516	-0.261	0.139	0.505	0.855	1.000

Table 72 LFER Model Development for the Free-Wilson Subset (Reference Compound: D50A) Against *E. coli*

Eq. No.	Log EC = Intercept	IE(1)	LR(7)	MRR(7)	MRR(7)^2	r^2	F	d.f.
1.	3.475 (±0.219)			-0.640 (±0.185)		0.343	11.989	(1,23)
2.	3.855 (±0.240)	-0.527 (±0.195)		-0.763 (±0.170)		0.506	11.258	(2,22)
3.	4.881 (±0.495)	-0.496 (±0.179)	-0.464 (±0.201)	-0.072 (±0.337)		0.606	10.735	(3,21)
4.	4.215 (±0.485)	-0.384 (±0.159)	-0.514 (±0.174)	1.866 (±0.731)	-0.861 (±0.298)	0.722	12.956	(4,20)

N = 25 s = 0.370

Table 73 Comparison of Observed and Calculated MIC's from Eq. 4 (Table 72)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	3.485	3.076	.409	1.212
D28B	3.483	3.461	.022	.065
D30A	2.595	2.824	-.229	-.679
D33A	3.504	3.076	.428	1.268
D33B	4.105	3.461	.644	1.907
D33C	3.529	3.461	.068	.201
D34A	3.223	3.006	.217	.642
D34B	3.521	3.390	.131	.388
D34C	3.246	3.390	-.145	-.429
D36A	2.411	1.639	.772	2.288
D36B	1.807	2.023	-.217	-.641
D38A	2.650	2.950	-.301	-.891
D38B	2.947	3.335	-.388	-1.149
D38C	3.263	3.335	-.072	-.213
D39A	2.650	2.660	-.011	-.032
D39B	2.947	3.045	-.098	-.290
D40A	1.762	2.129	-.367	-1.089
D40B	2.664	2.514	.150	.444
D40C	2.386	2.514	-.128	-.378
D42A	1.780	1.839	-.060	-.176
D42B	2.077	2.224	-.147	-.434
D42C	2.402	2.224	.178	.529
D46A	2.614	2.824	-.210	-.622
D50A	3.223	3.076	.146	.433
D56A	2.030	2.824	-.795	-2.354

[a]Standardized residual

Table 74 LFER Model Development for the Free-Wilson Subset Against *P. aeruginosa* (See Table 72)

Eq. No.	Log PA = Intercept	LR(7)	MRR(7)	RI1(7)	r^2	F	d.f.
1.	4.300 (±0.333)	-0.608 (±0.085)			0.688	50.739	(1,23)
2.	4.332 (±0.300)	-0.733 (±0.091)		0.547 (±0.216)	0.758	34.474	(2,22)
3.	3.653 (±0.370)	-0.395 (±0.151)	-0.683 (±0.258)	0.684 (±0.198)	0.819	31.740	(3,21)

N = 25 s = 0.322

Table 75 Comparison of Observed and Calculated MIC's from Eq. 3 (Table 74)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	2.593	2.242	.351	1.162
D28B	2.893	2.242	.651	2.152
D30A	1.991	2.405	-.414	-1.369
D33A	2.914	2.926	-.013	-.042
D33B	3.201	2.926	.275	.910
D33C	2.939	2.926	.013	.043
D34A	2.331	2.313	.017	.058
D34B	2.629	2.313	.316	1.044
D34C	2.654	2.313	.340	1.126
D36A	.907	.998	-.091	-.302
D36B	.602	.998	-.396	-1.309
D38A	1.745	1.996	-.252	-.832
D38B	2.043	1.996	.047	.156
D38C	1.767	1.996	-.229	-.758
D39A	2.046	2.016	.030	.099
D39B	2.043	2.016	.027	.090
D40A	1.762	1.356	.406	1.343
D40B	1.460	1.356	.104	.344
D40C	1.182	1.356	-.174	-.576
D42A	1.177	1.165	.012	.039
D42B	1.175	1.165	.010	.033
D42C	1.197	1.165	.032	.106
D46A	2.614	3.089	-.475	-1.571
D50A	2.331	2.242	.089	.293
D56A	1.728	2.405	-.677	-2.239

[a]Standardized residual

Table 76 Correlation Matrix for the LFER Models (Tables 72, 74) Development from Free-Wilson Subset

	IE(1)	LR(7)	MRR(7)	$MRR(7)^2$	RI1(7)	SA	EC	PA
IE(1)	1.000							
LR(7)	-0.207	1.000						
MRR(7)	-0.268	0.892	1.000					
$MRR(7)^2$	-0.216	0.866	0.978	1.000				
RI1(7)	-0.320	0.538	0.580	0.501	1.000			
SA	0.300	-0.350	-0.388	-0.341	0.007	1.000		
EC	-0.232	-0.678	-0.585	-0.654	-0.120	0.364	1.000	
PA	0.020	-0.829	-0.816	-0.858	-0.223	0.413	0.843	1.000

substituent on the ring at position 7 and the piperidinyl ring at position 7. In this subset the indicator variables for CH_2=CH, CH_2CH_2F at position 1, OH, CH_3NH, CF_3CH_2NH, $(CH_3)_2N$, OHCNH, CH_3CONH, $CH_3CON(CH_3)$ substituents on the rings on position 7 and ring indicator variables for azetidinyl ring, pyrrolidinyl ring were all included in a Free-Wilson analysis.

For S. aureus, a de novo model could not be obtained just as was the case for the LFER model. The de novo models of E. coli and P. aeruginosa are shown in Table 77 and Table 79.

Eq. 6 (Table 77) indicates that no other substituent at position 1 other than the reference ethyl substituent is an important contributors to activity. Several of the R-substituents (OH, CF_3CH_2NH, $(CH_3)_2N$, OHCNH, CH_3CONH and $CH_3CON(CH_3)$ are negative contributor to activity relative to the reference R-substituent (NH_2). Eq. 3 (Table 77) would indicate that it is the steric influences of these R-substituents that are important.

The situation with P. aeruginosa is more complex (eq. 9, Table 79). As with E. coli, the position 1 substituent appears to be not an important determinant of activity. Note that in eq. 6 (Table 74) there are no terms relative to position 1. On the other hand P. aeruginosa seems sensitive to the presence of a methylamine (CH_3NH) on the R-substituent and more importantly, the azetidinyl (RIA(7)) and pyrrolidinyl (RIP(7)) rings are positive contributors to activity. The calculated values and residuals of 25 compounds are shown in Table 78 (eq. 6) and 80 (eq. 9).

One drawback to the this Free-Wilson analysis in the fact that 20 of the 25 compounds have the pyrrolidinyl ring at position 7. Therefore the analysis was repeated on these 20 compounds (Table 10, D33A-C, D34A-C, D36A-B, D38A-C, D39A-B, D40A-C, D42A-C, D46A). The reference compound (D33A) was ethyl in position 1 and the amine for the R-substituent on the pyrrolidinyl ring on position 7.

As before, a statistically valid model could not be obtained for S. aureus. The de novo model for E. coli and P. aeruginosa is shown in Tables 81 and 83. Eq. 4 (Table 81) and eq. 6 (Table 83) indicate that every indicator variable in the model is a negative contributor to activity. The difference between eq. 4 (Table 81) and eq. 6 (Table 83) is that CH_3NH appears in eq. 6 and the OH group appears in eq. 4.

Eq. 4 (Table 81) and eq. 6 (Table 83) are similar to eq. 6 (Table 77) for E. coli and eq. 9 (Table 79) for P. aeruginosa respectively. Only one more indicator variable, OH on the R-substituents appears in eq. 9 (Table 79) as compared to eq. 6 (Table 83) for P. aeruginosa. The calculated values and residuals of these 20 compounds are shown in Table 82 and 84.

The stability of the regression coefficients found in eq. 8 (Table 67) and eq. 4 (Table 72) was checked by omitting compounds selected by a random number generator. For the models derived from 42 observations, six randomly selected compounds were omitted three times giving eqs. 1-3 (Table 85) for comparison with eq. 8 (Table 67). A similar procedure was done for the E. coli data on the subset of 25 compounds giving eqs. 4-6 for comparison with eq. 4 (Table 72)

except four compounds were deleted each time. Similar results were obtained in each set, although there was some noise in the coefficients.

Table 77 de novo Model Development for a Subset (Reference Compound: D50A) Against E. coli

Eq. No.	Log EC = Intercept	IOH(7)[a]	ICFCN(7)[b]	IC2N(7)[c]	IOHCN(7)[d]	ICON(7)[e]	ICCNC(7)[f]	r^2	F	d.f.
1.	2.911 (±0.126)						-0.825 (±0.364)	0.183	5.129	(1,23)
2.	3.012 (±0.126)					-0.742 (±0.241)	-0.926 (±0.341)	0.327	5.345	(2,22)
3.	3.118 (±0.116)		-1.009 (±0.357)			-0.848 (±0.299)	-1.032 (±0.299)	0.513	7.373	(3,21)
4.	3.270 (±0.103)	-0.857 (±0.246)	-1.161 (±0.292)			-0.999 (±0.246)	-1.183 (±0.246)	0.697	11.503	(4,20)
5.	3.348 (±0.104)	-0.935 (±0.232)	-1.239 (±0.275)		-0.550 (±0.275)	-1.078 (±0.232)	-1.262 (±0.232)	0.750	11.346	(5,19)
6.	3.480 (±0.107)	-1.067 (±0.213)	-1.371 (±0.250)	-0.527 (±0.213)	-0.681 (±0.250)	-1.209 (±0.213)	-1.393 (±0.213)	0.813	13.069	(6,18)

N = 25 s = 0.319

Indicator of [a]OH ; [b]CF_3CH_2NH ; [c]$(CH_3)_2N$; [d]OHCNH ; [e]CH_3CONH ; [f]$CH_3CON(CH_3)$

Table 78 Comparison of Observed and Calculated MIC's from Eq. 6 (Table 77)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	3.485	3.480	.006	.020
D28B	3.483	3.480	.003	.011
D30A	2.595	2.413	.182	.658
D33A	3.504	3.480	.025	.089
D33B	4.105	3.480	.625	2.258
D33C	3.529	3.480	.049	.177
D34A	3.223	3.480	-.257	-.930
D34B	3.521	3.480	.042	.150
D34C	3.246	3.480	-.234	-.846
D36A	2.411	2.109	.302	1.092
D36B	1.807	2.109	-.302	-1.092
D38A	2.650	2.953	-.303	-1.096
D38B	2.947	2.953	-.006	-.023
D38C	3.263	2.953	.310	1.119
D39A	2.650	2.798	-.149	-.537
D39B	2.947	2.798	.149	.537
D40A	1.762	2.271	-.509	-1.838
D40B	2.664	2.271	.393	1.420
D40C	2.386	2.271	.116	.418
D42A	1.780	2.086	-.307	-1.108
D42B	2.077	2.086	-.009	-.033
D42C	2.402	2.086	.316	1.141
D46A	2.614	2.413	.201	.727
D50A	3.223	3.480	-.257	-.930
D56A	2.030	2.413	-.383	-1.385

[a]Standardized residual

Table 79 de novo Model Development of a Subset Against P. aeruginosa (See Table 77)

Eq. No.	Log PA = Intercept	IOH(7)[a]	ICNH(7)[i]	ICFCN(7)[b]	IC2N(7)[c]	IOHCN(7)[d]	ICON(7)[e]	ICCNC(7)[f]	RIA(7)[g]	RIP(7)[h]	r^2	F	d.f.
1.	2.105			-1.351							0.278	8.834	(1,23)
	(±0.129)			(±0.454)									
2.	2.243			1.489				-1.060			0.520	11.909	(2,22)
	(±0.115)			(±0.381)				(±0.318)					
3.	2.380			-1.626			-0.912	-1.197			0.695	15.932	(3,21)
	(±0.102)			(±0.313)			(±0.262)	(±0.262)					
4.	2.493			-1.739	-0.642		-1.026	-1.310			0.779	17.722	(4,20)
	(±0.098)			(±0.276)	(±0.232)		(±0.232)	(±0.232)					
5.	2.598	-0.486		-1.843	-0.746		-1.130	-1.414			0.826	17.722	(5,19)
	(±0.101)	(±0.217)		(±0.256)	(±0.217)		(±0.217)	(±0.217)					
6.	2.721	-0.609		-1.966	-0.869	-0.676	-1.253	-1.537			0.887	23.664	(6,18)
	(±0.092)	(±0.184)		(±0.215)	(±0.184)	(±0.215)	(±0.184)	(±0.184)					
7.	2.509	-0.503		-2.072	-0.975	-0.782	-1.359	-1.643		0.318	0.910	24.410	(7,17)
	(±0.134)	(±0.177)		(±0.205)	(±0.177)	(±0.205)	(±0.177)	(±0.177)		(±0.155)			
8.	2.553	-0.614	-0.533	-2.316	-1.219	-1.026	-1.603	-1.888		0.518	0.948	36.278	(8,16)
	(±0.106)	(±0.142)	(±0.156)	(±0.176)	(±0.156)	(±0.176)	(±0.156)	(±0.156)		(±0.135)			
9.	2.318	-0.577	-0.524	-2.307	-1.210	-1.017	-1.594	-1.878	0.367	0.744	0.961	40.886	(9,15)
	(±0.141)	(±0.128)	(±0.139)	(±0.157)	(±0.139)	(±0.157)	(±0.139)	(±0.139)	(±0.164)	(±0.157)			

N = 25 s = 0.178

See Table 77 for footnotes a - f; [g]Azetidinyl ring; [h]Pyrrolidinyl ring; [i]CH_3NH

Table 80 Comparison of Observed and Calculated MIC's from Eq. 9 (Table 79)

No.	Observed	Calculated	Residual	Std. residual[a]
D28A	2.593	2.685	-.091	-.650
D28B	2.893	2.685	.208	1.481
D30A	1.991	2.108	-.117	-.831
D33A	2.914	3.061	-.148	-1.051
D33B	3.201	3.061	.140	.997
D33C	2.939	3.061	-.122	-.869
D34A	2.331	2.538	-.207	-1.474
D34B	2.629	2.538	.091	.649
D34C	2.654	2.538	.116	.825
D36A	.907	.754	.152	1.084
D36B	.602	.754	-.152	-1.084
D38A	1.745	1.852	-.107	-.761
D38B	2.043	1.852	.192	1.364
D38C	1.767	1.852	-.085	-.603
D39A	2.046	2.045	.001	.010
D39B	2.043	2.045	-.001	-.010
D40A	1.762	1.468	.294	2.094
D40B	1.460	1.468	-.008	-.058
D40C	1.182	1.468	-.286	-2.036
D42A	1.177	1.183	-.006	-.043
D42B	1.175	1.183	-.008	-.057
D42C	1.197	1.183	.014	.100
D46A	2.614	2.485	.130	.923
D50A	2.331	2.318	.013	.092
D56A	1.728	1.741	-.013	-.092

[a]Standardized residual

Table 81 de novo Model Development for a Subset (Reference Compound: D33A) Against E. coli

Eq. No.	Log EC = Intercept	IV(1)[g]	1EF(1)[h]	1OH(7)[a]	ICNH(7)[i]	ICFCN(7)[b]	IC2N(7)[c]	IOHCN(7)[d]	ICON(7)[e]	ICCNC(7)[f]	r^2	F	d.f.
1.	3.516 (±0.203)	0.298 (±0.164)	0.291 (±0.187)	-0.902 (±0.368)	-0.383 (±0.251)	-1.556 (±0.286)	-0.759 (±0.251)	-0.867 (±0.286)	-1.442 (±0.251)	-1.629 (±0.251)	0.883	8.409	(9,10)
2.	3.325 (±0.169)	0.298 (±0.174)	0.291 (±0.198)	-0.710 (±0.367)		-1.365 (±0.272)	-0.568 (±0.230)	-0.676 (±0.272)	-1.251 (±0.230)	-1.435 (±0.230)	0.856	8.229	(8,11)
3.	3.459 (±0.149)	0.186 (±0.164)		-0.845 (±0.372)		-1.443 (±0.279)	-0.568 (±0.241)	-0.734 (±0.279)	-1.251 (±0.241)	-1.435 (±0.241)	0.828	8.233	(7,12)
4.	3.521 (±0.141)			-0.907 (±0.372)		-1.412 (±0.281)	-0.568 (±0.243)	-0.723 (±0.281)	-1.251 (±0.243)	-1.435 (±0.243)	0.809	9.151	(6,13)

N = 20 s = 0.345

See Table 77 for footnotes a - f; [g] CH_2=CH; [h] FCH_2CH_2; see Table 79 for footnote i

Table 82 Comparison of Observed and Calculated MIC's from Eq. 4 (Table 81)

No.	Observed	Calculated	Residual	Std. residual[a]
D33A	3.504	3.521	-.017	-.059
D33B	4.105	3.521	.583	2.048
D33C	3.529	3.521	.007	.026
D34A	3.223	3.521	-.299	-1.049
D34B	3.521	3.521	.000	.001
D34C	3.246	3.521	-.276	-.968
D36A	2.411	2.109	.302	1.061
D36B	1.807	2.109	-.302	-1.061
D38A	2.650	2.953	-.303	-1.065
D38B	2.947	2.953	-.006	-.022
D38C	3.263	2.953	.310	1.087
D39A	2.650	2.798	-.149	-.522
D39B	2.947	2.798	.149	.522
D40A	1.762	2.271	-.509	-1.786
D40B	2.664	2.271	.393	1.380
D40C	2.386	2.271	.116	.406
D42A	1.780	2.086	-.307	-1.077
D42B	2.077	2.086	-.009	-.032
D42C	2.402	2.086	.316	1.109
D46A	2.614	2.614	.000	.000

[a]Standardized residual

Table 83 de novo Model Development for a Subset Against P. aeruginosa (See Table 81)

Eq. No.	Log PA = Intercept	ICNH(7)[i]	ICFCN(7)[b]	IC2N(7)[c]	IOHCN(7)[d]	ICON(7)[e]	ICCNC(7)[f]	r^2	F	d.f.
1.	2.049 (±0.153)		-1.295 (±0.485)					0.284	7.130	(1,18)
2.	2.222 (±0.139)		-1.468 (±0.405)				-1.039 (±0.340)	0.537	9.886	(2,17)
3.	2.411 (±0.121)		-1.656 (±0.319)			-0.943 (±0.269)	-1.227 (±0.269)	0.738	15.029	(3,16)
4.	2.597 (±0.107)		-1.843 (±0.250)	-0.745 (±0.214)		-1.129 (±0.214)	-1.414 (±0.214)	0.855	22.078	(4,15)
5.	2.755 (±0.088)		-2.000 (±0.186)	-0.903 (±0.160)	-0.710 (±0.186)	-1.287 (±0.160)	-1.571 (±0.160)	0.929	36.660	(5,14)
6.	2.917 (±0.099)	-0.379 (±0.151)	-2.163 (±0.172)	-1.065 (±0.151)	-0.872 (±0.171)	-1.449 (±0.151)	-1.734 (±0.151)	0.952	43.171	(6,13)

N = 20 s = 0.198

See Table 77 for footnotes b - f; see Table 79 for footnote i

Table 84 Comparison of Observed and Calculated MIC's from Eq. 6 (Table 83)

No.	Observed	Calculated	Residual	Std. residual[a]
D33A	2.914	2.917	-.004	-.022
D33B	3.201	2.917	.284	1.738
D33C	2.939	2.917	.022	.135
D34A	2.331	2.538	-.207	-1.266
D34B	2.629	2.538	.091	.558
D34C	2.654	2.538	.116	.709
D36A	.907	.754	.152	.931
D36B	.602	.754	-.152	-.931
D38A	1.745	1.852	-.107	-.654
D38B	2.043	1.852	.192	1.172
D38C	1.767	1.852	-.085	-.518
D39A	2.046	2.045	.001	.009
D39B	2.043	2.045	-.001	-.009
D40A	1.762	1.468	.294	1.799
D40B	1.460	1.468	-.008	-.050
D40C	1.182	1.468	-.286	-1.749
D42A	1.177	1.183	-.006	-.037
D42B	1.175	1.183	-.008	-.049
D42C	1.197	1.183	.014	.086
D46A	2.614	2.917	-.303	-1.852

[a]Standardized residual

Table 85 Results from Random Sample Analyses (See Eq. 8, Table 67; Eq. 4, Table 72)

Eq. No.	Log PA = Intercept	B5R(7)	FR(7)	$FR(7)^2$	RI3(7)	r^2	F	d.f.
1.	2.456	-0.491	-1.307	-0.417	-0.387	0.733	21.379	(4,31)
	(±0.236)	(±0.070)	(±0.220)	(±0.091)	(±0.167)			
	N = 36		s = 0.374					
2.	2.563	-0.568	-1.646	-0.554	-0.334	0.748	23.041	(4,31)
	(±0.265)	(±0.073)	(±0.285)	(±0.119)	(±0.176)			
	N = 36		s = 0.383					
3.	2.779	-0.572	-1.214	-0.378	-0.571	0.725	20.432	(4,31)
	(±0.302)	(±0.082)	(±0.251)	(±0.100)	(±0.224)			
	N = 36		s = 0.394					

Eq. No.	Log EC = Intercept	IE(1)	LR(7)	MRR(7)	$MRR(7)^2$	r^2	F	d.f.
4.	4.620	-0.322	-0.464	0.812	-0.469	0.778	14.067	(4,16)
	(±0.460)	(±0.152)	(±0.154)	(±0.738)	(±0.297)			
	N = 21		s = 0.322					
5.	4.222	-0.246	-0.686	2.960	-1.202	0.753	12.214	(4,16)
	(±0.556)	(±0.194)	(±0.227)	(±0.996)	(±0.368)			
	N = 21		s = 0.362					
6.	4.318	-0.456	-0.518	1.647	-0.741	0.703	9.481	(4,16)
	(±0.568)	(±0.190)	(±0.209)	(±0.907)	(±0.369)			
	N = 21		s = 0.402					

SUMMARY AND CONCLUSION

From the derived LFER models differences between the three bacterial systems can be seen. in some cases a model could not be derived, possible due to the "cell penetration variable" as indicated by Domagala et al. (32) The "cell penetration variable" is related to the bacteria cell wall and cytoplasmic membrane. Both Gram-positive (S. aureus) and Gram-negative (E. coli and P. aeruginosa) have the cytoplasmic membrane (In Gram-negative it is called "inner membrane".) in which one of the functions is responsible for selective permeability and transport of solutes. (54) The components of the Gram-positive cell wall contain peptidoglycan, teichoic acid, teichuronic acids and polysaccharides. In Gram-negative bacteria, the cell wall includes peptidoglycan, lipoprotein, outer membrane and lipopolysaccharide layers. The cell wall is, in general, non-selectively permeable. One layer of the Gram-negative wall (the outer membrane) hinders the passage of relatively large molecules. The presence of proteinacetous pores in the outer membrane makes it permeable to low molecule weight solutes. Large antibiotic molecules penetrate it relatively slowly, which accounts for the relatively high antibiotic resistance of Gram-negative bacteria. The permeability of the outer membrane varies widely from one Gram-negative species to another. In P. aeruginosa, which is extremely resistant to antibacterial agents, the outer membrane is considerably less permeable than that of E. coli. (54) Thus measurement of minimum inhibitory concentration (MIC) includes drug penetration into

the cell, inhibition of the bacterial DNA gyrase and possible bio-transformation by the bacteria.

The conclusion of these results is as follows : (1)In general, indicator variables for substituents at position 1 tend to be more significant than the common LFER parameters. (2)Fluorine is the most active substituent at position 6. It seems to be related to the electronic (σ–ρ) interaction between position 6 and 7. Fluorine is approximately the same size as a hydrogen, but does alter the lipophilicity at position 7. (3)Based on the Free-Wilson analysis only a small number of substituents at position 7 are important for activity. This may explain why there tends to be poor results using LFER parameters. The fact that the indicator variable for amide nitrogen (INCO(7)) is negative and the test were run at pH 7.4, indicates that positive charged aliphatic amines are important for activity. Additional work needs to be done to determine if this is because there is an anionic binding site on the enzyme or if the charged nitrogen simply reduces lipophilicity.

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