Journal of Chromatography A, 655 (1993) 177–184
Elsevier Science Publishers B.V., Amsterdam

CHROM. 25 109

Influence of the chromatographic capacity factor (log k') as an index of lipophilicity in the antibacterial activity of a series of 6-fluoroquinolones

Relationship between physico-chemical and structural properties and their hydrophobicity☆

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(First received December 10th, 1992; revised manuscript received March 17th, 1993)

ABSTRACT

The aim of this study was to establish the influence of lipophilicity on the antibacterial activity (log $1/MIC_{50}$) of 22 fluoroquinolones and to assess the influence of their electronic, steric and topological properties on their hydrophobicity. The lipophilicity of the compounds, expressed as the chromatographic capacity factor (log k'), was determined by ion-pair reversed-phase HPLC. On the basis of the mathematical models developed, an attempt was made to confirm the mechanism of interaction of the quinolones with DNA-gyrase proposed previously.

INTRODUCTION

Fluoroquinolones are a family of antibacterial agents extensively used in both human and veterinary clinics. They are bactericides and act by inhibiting bacterial DNA-gyrase [1].

In 1962, Lesher *et al.* [2] isolated nalidixic acid (Fig. 1) as a by-product in the synthesis of chloroquine, and 2 years later it was introduced into general practice for the treatment of urinary infections [3]. Later, structural modifications were made to the basic skeleton of nalidixic acid and new derivatives were synthesized, *e.g.*, ox-

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☆ Presented at the *21st Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Granada, October 21–23, 1992.*

0021-9673/93/$06.00

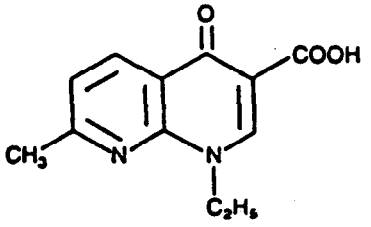


Fig. 1. Nalidixic acid.

olinic acid, piperamic acid and piromidic acid. All these quinolinecarboxylic acids constitute the first-generation quinolone series.

In 1973, Gerster synthesized flumequine [4]. For the first time the C-6 position was functionalized with a fluorine atom, thus constituting the first synthesis of a fluoroquinolone. Its antibacterial activity surpassed that of the first-generation quinolones, and this molecule formed a bridge between the first and third generations. Flumequine was included in the second generation of quinolones.

In 1980, Koga [5] synthesized norfloxacin, which was the first fluoroquinolone of the third generation. Two structural modifications were performed in this synthesis which are fundamental for the antibacterial activity of these drugs: the C-6 position was functionalized with a fluorine atom and the C-7 position was functionalized with an aliphatic cyclic amine (piperazine N-alkylpiperazine, etc.). It was soon realized that these compounds were much more active *in vitro* and that they showed a broader range of antibacterial activity. In contrast to quinolones of the first generation, the fluoroquinolones showed a systemic action *in vivo*. From the pharmacokinetic point of view, their absorption is excellent when taken orally and their metabolization is slight [6–11].

Since then, many pharmaceutical companies began to synthesize new fluoroquinolonic derivatives, carrying out functional and isosteric substitutions in the basic skeleton of 3-quinolinecarboxylic acid. Some of these derivatives are now commercially available in human and veterinary clinics, *e.g.*, norfloxacin, ciprofloxacin and enrofloxacin, and others are at an advanced clinical stage.

The aim of this work was to study the influence of the lipophilicity of these compounds, expressed as the chromatographic capacity factor (log k') (determined by ion-pair reversed phase HPLC) on their antibacterial activity (log $1/MIC_{50}$) by means of the establishment of the best simple linear relationship.

Budvári-Bárány and Szász [12] have correlated log k' with the logarithm of the partition coefficient at pH 7.40 for a series of fluoroquinolones. From the results they concluded that it is possible to use log k' as a descriptor of lipophilicity in quantitative structure–activity relationship (QSAR) studies for this series.

Numerous papers have described the use of reversed-phase HPLC to determine quinolones using ultraviolet and fluorescence detectors. The same authors Budvári-Bárány and Szász [12] indicated the need to use the ion-pair technique, determining the differences between cetrimide and sodium hexanesulphonate.

We also aimed to study the influence of electronic, steric and topological descriptors on hydrophobicity by means of a multiple linear relationship between the representative parameter of lipophilicity (log k') and the electronic, steric and topological parameters studied.

The electronic descriptors used are normally the net charges associated with each atom of a molecule, dipolar moment and HOMO and LUMO molecular orbits. Molecular surface area and volume are the steric descriptors most frequently used. Of the topological descriptors, the most usual is the molecular connectivity index of order $i\ ^{i}\chi$, first introduced by Kier and Hall [13]. These indices allow the parameterization of a determinate chemical structure as a function of its chains, rings and branches. We thus aimed to confirm the mechanism of interaction of the quinolones with bacterial DNA-gyrase proposed by Shen and Mitsher [14].

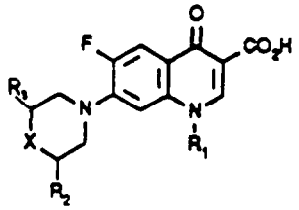
EXPERIMENTAL

Quinolones

Following the method described by Koga [5], 22 fluoroquinolones were synthesized in our laboratory. All intermediates used in the synthesis were of synthesis quality. All products were properly identified and analysed by IR and

TABLE I

CHEMICAL STRUCTURE OF THE 22 FLUOROQUINOLONES ASSAYED



Quinolone	R_1[a]	R_2	R_3	X
1	cC_3H_5	H	H	NH
2	cC_3H_5	H	H	NCH_3
3	cC_3H_5	H	H	NC_2H_5
4	cC_3H_5	H	H	CH_2
5	cC_3H_5	H	H	O
6	cC_3H_5	H	H	HCH_2CH_2OH
7	C_2H_5	H	H	NH
8	C_2H_5	H	H	NCH_3
9	C_2H_5	H	H	NC_2H_5
10	C_2H_5	H	H	O
11	C_2H_5	H	H	CH_2
12	cC_2H_5	CH_3	H	NH
13	C_2H_5	CH_3	H	NH
14	cC_3H_5	H	H	$CHCH_3$
15	cC_3H_5	CH_3	H	CH_2
16	cC_3H_5	H	CH_3	CH_2
17	cC_3H_5	H	H	S
18	C_2H_5	H	H	$CHCH_3$
19	C_2H_5	CH_3	H	CH_2
20	C_2H_5	H	CH_3	CH_2
21	C_2H_5	H	H	S
22	C_2H_5	H	H	NC_6H_5

[a] c = Cyclo.

NMR spectroscopy. Their structures are shown in Table I.

Materials

For the determination of MIC_{50}, Müller–Hinton agar was supplied by DIFCO (Detroit, MI, USA). HPLC-grade methanol was supplied by Merck (Darmstadt, Germany), analytical-reagent grade phosphoric acid 85% by Probus (Barcelona, Spain), HPLC-grade sodium heptanesulphonate by Scharlau (Barcelona, Spain) and analytical-reagent grade sodium hydroxide, methanol, potassium dihydrogenphosphate and potassium dichromate by Jansen Chimica (Geel, Belgium).

The analytical column (300 × 2 mm I.D.), filled with 10-μm C_{18}-functionalized silica particles, was μBondapak C_{18}, supplied by Waters (Milford, MA, USA).

Equipment

A Model PU 9700 IR spectrophotometer (Pye Unicam, Cambridge, UK) and a Model AW 80 NMR spectrometer (Bruker, Karlsruhe, Germany) were used for the identification of molecular structures. Petri plates for the MIC_{50} assay were grown in an oven (Selecta, Barcelona, Spain). Theoretical descriptors were calculated using a PC/AT computer (Olivetti, Ivrea, Italy), equipped with a math coprocessor. The HPLC equipment consisted of two Model 110B pumps, a Model 406 analogue interface, an injector with a 20-μl loop and a Model 167 variable-wavelength UV detector (Beckman Instruments, San Ramon, CA, USA). The chromatographic apparatus was controlled and the analogue signals were analysed using System Gold software (Beckman Instruments) installed in a PC/AT computer (Syswest, Barcelona, Spain).

Microorganisms and assay of antibacterial activity (MIC_{50})

Log $1/MIC_{50}$ was used as a descriptive parameter of antibacterial activity, where MIC_{50} is the minimum concentration that inhibits 50% of the bacterial strains analysed. We used 100 strains of *Escherichia coli* (kindly supplied by the Department of Microbiology at the Faculty of Medicine, Universitat Autònoma de Barcelona, Spain) to determined the value of MIC_{50}, and the assay was validated using the following typified strains: *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 2592, *Streptococcus faecalis* ATCC 29212 and *Staphylococcus aereus* ATCC 219213.

Antibacterial activity was determined using the method of serial dilutions in agar, following the specifications of the National Committee for Clinical Laboratory Standards [15]. The inoculum was applied to Müller–Hinton agar plates containing serial quantities of quinolone, using a

Steers replicator [16]. The plates were incubated at 37°C for 20 h and inspected immediately.

Chromatography and calculation of lipophilicity

The chromatographic capacity factor (log k') obtained by reversed-phase HPLC was used as an index of lipophilicity. It is defined as $k' = (t_r - t_0)/t_0$, where t_0 is the retention time of a control substance, which theoretically is not retained in the column, and t_r is the retention time of the analyte substance.

The isocratic mobile phase was composed of methanol–water (1:1), 25.5 m*M* potassium dihydrogenphosphate and 1 m*M* sodium heptanesulphonate, with the pH adjusted to 2.75 with 85% phosphoric acid. The flow-rate was 1 ml/min and the injection volume 20 μl. Absorption was monitored at 270 nm.

Approximately 0.3 m*M* of each quinolone was dissolved in 10 ml of 0.1 *M* NaOH, 10 ml of methanol were added and the volume was made up to 100 ml with water. A 1-ml aliquot was taken and diluted to 50 ml with water. Ten injections were made under the analytical conditions stated, with the aim of obtaining a mean value. In order to obtain the value of t_0, a 0.3 m*M* solution of potassium dichromate was used as a control. The deviation of the results was no greater than 8% for either t_r or t_0.

Calculation of electronic descriptors

The net charges associated with each atom of the molecules, the values of the dipolar moment and the energies of the HOMO and LUMO molecular orbits were calculated using the AM1 semi-empirical method [17], once the molecular geometry had been optimized. These calculations were performed on an IBM 3090-170 computer in the Physico-Chemical Section of the Faculty of Chemistry at Tarragona (Spain).

Calculation of steric descriptors

Molecular surface area and volume were calculated with the MOLSV program [18] in a version for PC-compatible computer, adapted by K.J. Tupper at the University of Indiana from the program developed by G.H. Smith. The program was run on a PC/AT computer. The cartesian coordinates introduced in the program corresponded to those structures with optimized geometry, based on the data obtained from calculations with the AM1 semi-empirical method.

Calculation of topological descriptors

The molecular connectivity indices of order i $^i\chi$ were calculated with the INDICES program, and the molecular connectivity indices of valency of order $^i\chi^v$ were calculated with the CONIND program, both running on a PC/AT-compatible computer.

RESULTS

A representative chromatogram, obtained from quinolone **12**, is shown in Fig. 2.

In order to study the influence of lipophilicity on the antibacterial activity of fluoroquinolones, a simple linear correlation was performed, in which antibacterial activity represented by the logarithm of $1/MIC_{50}$ was considered as the dependent variable and lipophilicity, as represented by log k', was considered as the independent variable. The calculation was performed using the linear regression program LREGR.

The values of log $1/MIC_{50}$ *vs.* the values of log k' are shown in Fig. 3. The linear correlation which best fits the experimental results corresponded to the following mathematical expression:

$$\log \frac{1}{MIC_{50}} = -0.8161\,(\text{S.D.} = \pm 0.1074) \cdot \log k' + 0.3700\,(\text{S.D.} = \pm 0.0900)$$

$$n = 22\,; \quad r = -0.8616\,; \quad F \text{ ratio} = 0.000\%$$

The theoretical and experimental values of log $1/MIC_{50}$ for the series of quinolones studied are shown in Table II, together with the residual values.

In order to study the influence of electronic, steric and topological descriptors on the lipophilicity of fluoroquinolones, a multiple linear correlation was performed in which lipophilicity represented by log k' was considered as the dependant variable and the following were considered as independent variables: the net charges

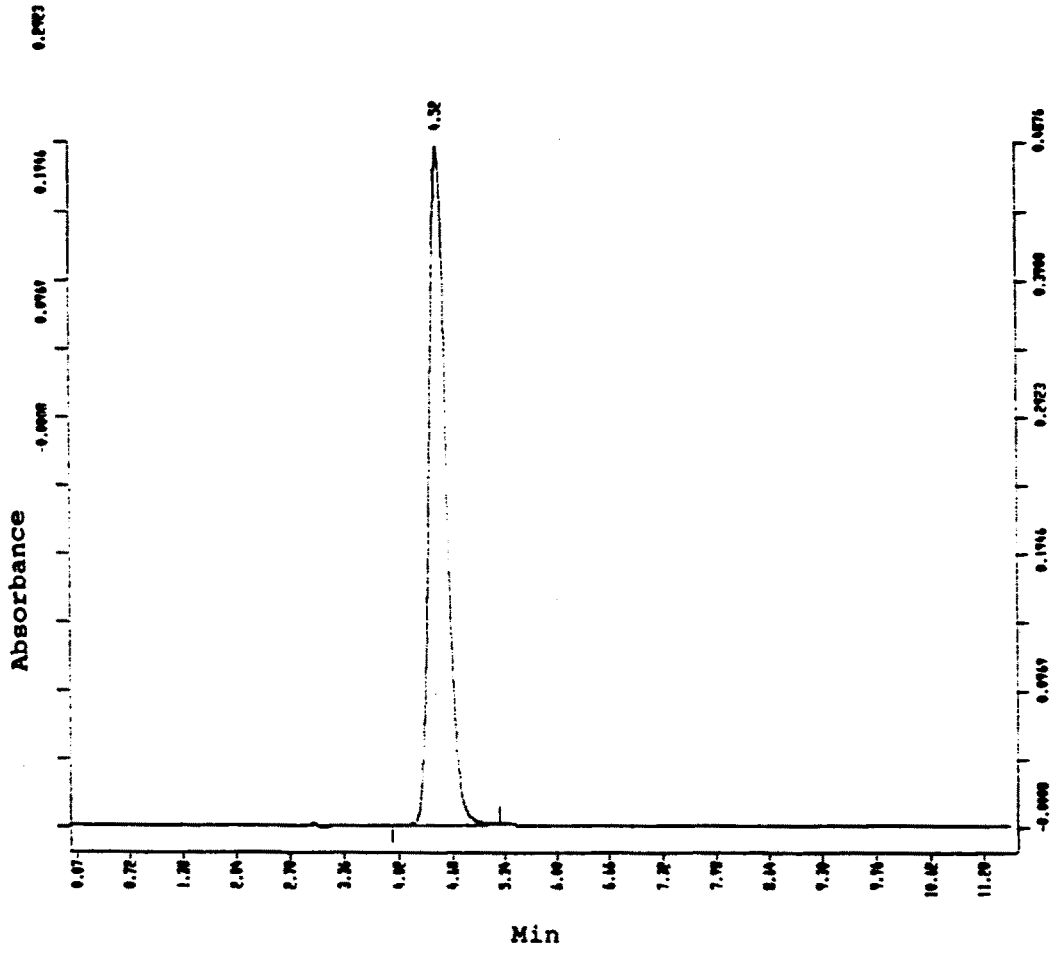


Fig. 2. Representative chromatogram of quinolone 12.

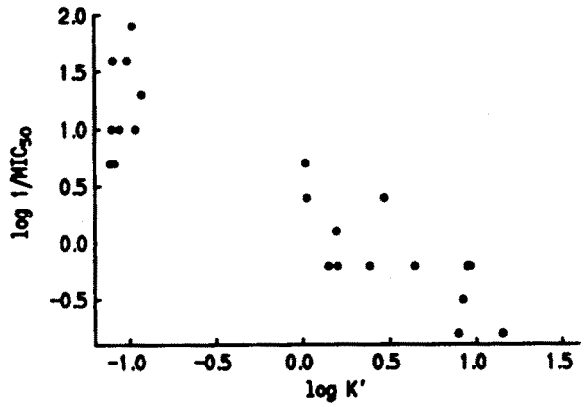


Fig. 3. Value of log $1/MIC_{50}$ *vs.* the value of log *k'*.

associated with the atom (QX_i), where X_i is the atom situated at position *i*, the dipolar moment (*DM*), the HOMO and LUMO molecular orbits, the molecular surface area (*MS*) and volume (*MV*) and the valence and non-valence molecular connectivity indices and their difference ($^{i}\chi^{v}$, $^{i}\chi$, DIF $^{i}\chi$).

Of all the electronic, steric and topological descriptors estimated for all the quinolones assayed, the values of those descriptors which, on the basis of the matrix correlation, are linearly independent are *MV*, QN_1, QC_7, QN_{16}, QX_{24}, *DM* and DIF $^{3}\chi_c$.

With the aim of obtaining this multiple linear correlation, the calculation was performed using the stepwise linear regression analysis program

TABLE II

THEORETICAL AND EXPERIMENTAL VALUES OF LOG $1/MIC_{50}$ FOR THE SERIES OF QUINOLONES STUDIED

Quinolone	Log $1/MIC_{50}$		Difference (%)[a]
	Experimental	Theoretical	
1	1.6020	1.2396	23
2	1.6020	1.1730	27
3	1.3010	1.1042	15
4	0.0969	0.1694	−75*
5	0.6980	0.3158	55
6	0.6980	1.2323	−77*
7	1.0000	1.2089	−21
8	1.0000	1.1336	−13
9	0.6980	1.2549	−80*
10	0.3979	0.3109	22
11	−0.2040	−0.2021	−1
12	1.9030	1.1486	40
13	1.0000	1.2465	−25
14	−0.2040	−0.4557	−123*
15	−0.2040	−0.4694	−130*
16	−0.2040	0.1638	20
17	0.3979	−0.0563	114*
18	−0.5050	−0.4337	14
19	−0.8060	−0.4112	49
20	−0.2040	0.2072	202*
21	−0.2040	0.0150	107*
22	−0.8060	−0.6140	24

[a] For explanation of the values marked with asteriks, see Discussion.

TABLE III

DESCRIPTORS CORRESPONDING TO THE MATHEMATICAL EXPRESSION RESULTING FROM THE MULTIPLE LINEAR CORRELATION

QX_{24} = net charge associated with the heteroatom X situated at position 4 of the heterocyclic aliphatic ring which functionalizes position C-7; *MD* = dipolar moment; DIF $^3\chi_c$ = difference between the molecular connectivity index of valency and non-valency of order 3 cluster.

Quinolone	QX_{24}	*DM*	DIF $^3\chi_c$
1	−0.2895	10.769	0.354
2	−0.2635	9.715	0.452
3	−0.2633	9.543	0.434
4	−0.1605	9.775	0.388
5	−0.2526	9.020	0.388
6	−0.2408	7.901	0.434
7	−0.2927	10.721	0.355
8	−0.2271	10.786	0.420
9	−0.2536	10.877	0.401
10	−0.2572	9.170	0.355
11	−0.1591	9.782	0.355
12	−0.2866	10.769	0.548
13	−0.2950	10.328	0.439
14	−0.0933	9.785	0.387
15	−0.1492	9.770	0.387
16	−0.1523	10.043	0.435
17	−0.0318	9.037	0.463
18	−0.0990	8.543	0.355
19	−0.1488	10.286	0.355
20	−0.1510	9.942	0.467
21	−0.0477	9.498	0.355
22	−0.2240	5.810	0.375

SLREG (part of Labsware computational package; Compudrug, Budapest, Hungary). The multiple linear correlation which best fits the experimental data was that corresponding to the following expression:

$$\log k' = 6.8448\ (\text{S.D.} = \pm 1.3184) \cdot QX_{24} - 0.2249\ (\text{S.D.} = \pm 0.0933) \cdot DM - 4.0366\ (\text{S.D.} = \pm 2.1135) \cdot \text{DIF}\ ^3\chi_c + 5.0421$$

$$n = 22; \quad r = 0.8543; \quad F \text{ ratio} = 0.002\%$$

where QX_{24} is the negative net charge associated with the heteroatom X situated at position 4 of the heterocyclic aliphatic ring which functionalizes position C-7, *DM* is the dipolar moment and DIF $^3\chi_c$ is the difference between the molecular connectivity index of valency and non-valency of order 3 cluster (Table III).

Table IV shows the theoretical and experimental values of log k' for the quinolone series studied, together with the residual values.

DISCUSSION

The antibacterial activity of the fluoroquinolones is related to lipophilicity, although differences are noted in some members of the series (marked with asterisks in Table II). Under the analytical conditions used there may be some kind of interaction of these compounds with the

TABLE IV

THEORETICAL AND EXPERIMENTAL VALUES OF LOG k' FOR THE SERIES OF QUINOLONES SERIES STUDIED TOGETHER WITH THE RESIDUAL VALUES

Quinolone	Log k'		Difference (%)[a]
	Experimental	Theoretical	
1	−1.0936	−0.7830	28
2	−1.0132	−0.8108	101
3	−0.9303	−0.6836	26
4	0.1972	0.2770	−40
5	0.0206	−0.2608	1366
6	−1.0847	−0.1340	88
7	−1.0565	−0.8015	24
8	−0.9657	−0.6217	36
9	−1.1120	−0.7560	32
10	0.0265	−0.1724	750
11	0.6453	0.4444	31
12	−0.9838	1.6905	272
13	−1.1019	−1.1319	−3
14	0.9512	0.7981	12
15	0.9677	0.3243	66
16	0.2040	0.0536	74
17	0.4695	1.0796	−130
18	0.9247	1.1918	−29
19	0.8975	0.4085	54
20	0.1516	−0.0665	144
21	0.3871	1.3780	256
22	1.1530	0.7640	34

residual silanol groups on the column, which might explain these differences.

According to Shen and Mitsher [14], the fluoroquinolones have three domains of action (Fig. 4): domain of hydrogen bridges with the bases of DNA; domain of hydrophobic interactions between N-alkyl groups and quinolones joined to each of the two strands of DNA; and domain of interaction with DNA-gyrase. The heterocycle that functionalizes position C-7 belongs to the last domain. An increase in the net negative charge associated with the heteroatom X_{24} produces a decrease in the lipophilicity of the fluoroquinolones and consistently an increase in the antibacterial activity. This increase in net negative charge follows the order, $S < C < O < N$. On the other hand, the

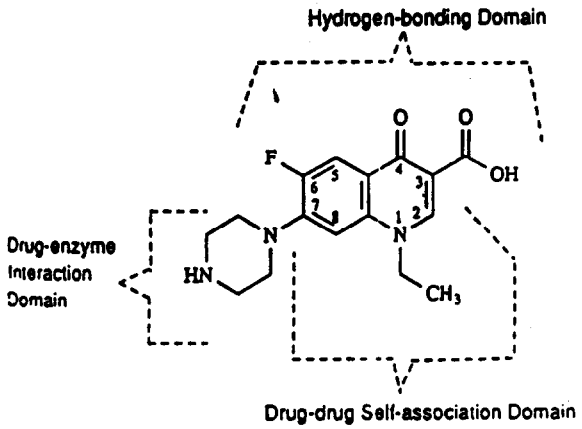


Fig. 4. Domains of action of the fluoroquinolones [14].

increase in antibacterial activity follows the order $C < S < O < N$. The increase in the value of the dipolar moment (DM) of the fluoroquinolones leads to a decrease in lipophilicity and also a consequent rise in antibacterial activity.

It thus appears that an increase in net negative charge associated with the heteroatom X_{24} favours the interaction of fluoroquinolones with DNA-gyrase, in such a way that the increase in the density of the negative charge of the heteroatom X_{24} would stabilize the interaction with DNA-gyrase.

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