Syntheses and Biological Activities of New N_1-Aryl Substituted Quinolone Antibacterials

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Summary

A series of quinolones with a systematically varied substitution at the phenyl ring at N1 has been synthesized. Three lipophilicity descriptors (log *K*, log *P*, R_m) and the pK_a values have been determined as well as the microbiological activity: The MIC values for eight different strains of three Gram-positive and three Gram-negative species and the inhibitory concentrations of DNA supercoiling (IC_{90} and IC_{100}) were determined. From a principal component and a QSAR analysis relationships between the antibacterial activity concerning the whole-cell system and electronic properties as well as the length of the substituents at the phenyl rings could be derived. The activity in a cell-free system was governed by the lipophilicity and width of the substituents. It is speculated that the quinolones take a defined place in the DNA gyrase-DNA complex which is characterized by polar amino acids. This is in agreement with findings from studies of mutant gyrases.

Introduction

During the last thirty years, quinolones have been attracting interest as potent antibacterials. The initial agents, such as nalidixic acid and oxolinic acid, lacked activity on Gram-positive bacteria and had insufficient bioavailibility. With the synthesis of norfloxacin in 1980, the age of the fluoroquinolones began. The new compounds derived from this lead substance are characterized by broad-spectrum activity. In the meantime, thousands of new quinolones, all possessing a fluoro substituent at position 6 and nitrogen-containing heterocycle such as piperazine at position 7, have been synthesized and microbiologically tested. About 6 new substances have been introduced into clinical practice in Germany.

Current knowledge about structure-activity relationships (SAR) has been derived from several studies[1–8]. Domagala[2] and Klopman[3] stressed the importance of the nitrogen-containing substituent at C-7 and the 6-fluorine atom by means of qualitative SAR and a quantitative structure-activity relationship (QSAR) method called CASE (computer automated structure evaluation), respectively. Using the computer calculated hydrophobicity descriptor CLOG*P*, Okada et al.[4] found that the antibacterial activity of different 7-azetidine substituted derivatives is influenced by their lipophilicity: Gram-negative bacteria show a parabolic relationship whereas Gram-positive microorganism seem to correlate in a weak linear manner. For a heterogeneous series of compounds Bazile and coworkers[5] found a correlation between log *D* (logarithm of the coefficient of distribution) and the accumulation in *S. aureus* and an inverse correlation between log *D* and accumulation in *E. coli*. Chu et al.[6] synthesized 7-methylpiperazinyl substituted N_1-arylquinolones which had different substituents at the phenyl ring. But the series was too small to perform a QSAR study. Investigations of Domagala[7] as well as Ohta and Koga[8] focussed on the influence of the substituents at N_1 on the antibacterial activity: Within a series of heterogenous alkyl- and aryl-substituted derivatives Domagala et al.[7] found a strong dependence on the STERIMOL length and width as well as the level of unsaturation of the substituent (in position 1) and the biological activity. Ohta and Koga[8] used conformational analyses and receptor mapping to find the optimum volume of these substituents. It has to be stressed that the latter study was done retrospectively and therefore could use only a rough classification of the antibacterial activity instead of MIC values directly. Thus, for analyses of structure-activity relationships a systematic variation of compounds as well as consistent microbiological data produced in one laboratory are desirable.

Therefore, the aims of this study were, first, to synthesize systematically varied N_1-phenyl substituted quinolones (see Craig plot[9]) and, secondly, to investigate the relationships between the physicochemical properties and the antibacterial activities. To characterize the overall lipophilicities of the derivatives R_m, log *P*, and log *k'* values (at pH 7.4) were measured, and pK_a-values were determined UV spectroscopically. The microbiological activities were measured in a whole-cell system determined as the minimal inhibitory concentration (MIC) and in a cell-free system determined as the inhibition of the supercoiling activity of the DNA gyrase (IC_{90} and IC_{100} values). Finally, a QSAR analysis was performed.

Results

Chemistry. The compounds were synthesized using the cycloaracylation strategy first published by Grohe[10] and displayed in Scheme 1. Starting off with dichlorofluorobenzene the key compound, the 3-ethoxy-2-aroylacrylate **2**, can be built up in 6 steps in rather good yields. The ethoxy substituent of the acrylate was replaced with differently substituted anilino residues obtaining *E/Z* mixture of the isomeric enamines **3**. Both isomers could be identified and fully assigned in coupled ^{13}C NMR spectra. The C=O ester group of

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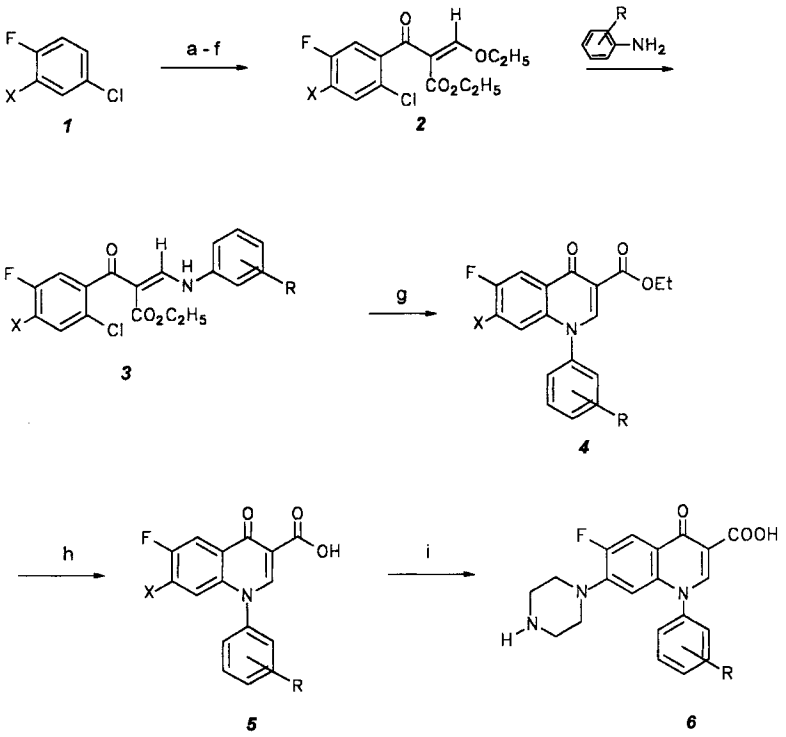

Scheme 1: Synthetic pathways. a) $CH_3COCl/AlCl_3$; b) NaOCl/Δ; c) $SOCl_2$; d) $(C_2H_5O)_2Mg/C_2H_5O_2C\text{-}CH_2\text{-}CO_2C_2H_5$; e) *p*-Tsa/EtOH; f) $HC(OC_2H_5)_3$/acetic anhydride; g) K_2CO_3/DMF or K-*tert*-butylate/dioxane or NaH/dioxane; h) NaOH/THF; i) piperazine/DMSO.

the *E*-isomer is characterized by a chemical shift of δ = 166 ppm and a coupling constant of J = 3.0 Hz and the C=O keto group by δ = 191 ppm and J = 8.0 Hz whereas the Z-isomers show δ = 168 ppm and I = 10.0 Hz attributed to the C=O ester group and δ = 189 ppm and J = 4.0 Hz to the C=O keto group, respectively. The integrals of corresponding signals reveal a surplus of the *E* isomer for all derivatives (65 to 100 per cent). However, both compounds were energetically comparable ($\Delta\Delta H_f$ (*E-Z*) ≈ 1 kcal/mol, calculated by means of AM1). Thus, an isomerization can occur easily at room temperature and the mixture is suitable for the subsequent cyclization reaction. In most cases this reaction is catalyzed by potassium carbonate in dimethylformamide. Enamines with electron-withdrawing groups in the phenyl ring (e.g. CN or nitro groups) need stronger bases such as potassium *tert*-butylate in dioxane for cyclization. The yields of **4** were in a range of 40 to 60 per cent depending on the chemical nature of the substituent. Although a broad spectrum of different reaction conditions were tested the *o*-nitrophenyl substituted quinolone was not obtainable. Finally, the ester function was hydrolysed using alkali and the chloro substituent (or fluoro substituent; see Experimental) at C-7 replaced with the piperazine in DMSO obtaining the quinolones **6**. Two compounds, the phenyl and the *p*-flourophenyl substituted derivatives **6a** and **6n** (Sarafloxacin), have been synthesized by Chu et al.[6].They were included in the set of compounds for a proper QSAR analysis.

Physicochemical properties. pK_a value: The pK_a value of the carboxylic acid was measured by means of UV/Vis spectroscopy using buffers of different pH values, and are depicted in Table I. With exception of **6t** all values are approximately 5.5 to 5.9 as expected from the literature[11]. Because the phenyl residue takes a nearly perpendicular position to the plane of the quinolone (according to force field calculations) an influence of these substituents of this aromatic ring by mesomeric effects is unlikely. Therefore, the influence of the substituents on the pK_a is rather small. The poor correlation found between the σ and pK_a values proves this finding (Table VII). The pK_{a2} value of the piperazine residues has not been determined because the different phenyl substituents normally do not influence this pK_a value[11].

Lipophilicity: The overall lipophilicity of the compound was measured in three different ways: First, the partition coefficients between an aqueous buffer solution and an octanol phase were determined using UV spectroscopy and were denoted by log *P* value[12]. Second, the capacity values log *k'* were measured by means of RP-HPLC in a methanol/buffer system[12] and third, the R_m values were determined by TLC using paraffin coated silica gel plates and acetone/buffer system[13]. In each case a phosphate buffer (pH 7.4) was used. The values are displayed in Table I.

Microbiology. The major target of the quinolone antibacterials is the bacterial enzyme DNA gyrase. Quinolone-mediated inhibition of DNA gyrase blocks DNA replication. This blockage in turn triggers the onset of a stress response cascade which is assumed to ultimately lead to cell death. Therefore, the antibacterial activities of the different compounds were investigated in two ways: First, the minimal inhibitory concentrations of the different quinolone derivatives were determined according to NCCLS[14] (see Table II) for eight reference strains of different Gram-positive (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, ATCC 29213, *Streptococcus pyogenes* ATCC 10389) and Gram-negative species (*Escherichia coli* ATCC 25922,

Table I: Physicochemical parameters of quinolone derivatives **6.**

No.	R	log *P*	log *k'*	R_m	pK_{a1}	σ	*MR*	*L*	*B*-1	*B*-5	ℑ	ℜ
6 a	H	–0.987	1.995	1.23	5.81	0.00	1.03	2.06	1.00	1.00	0.000	0.000
6 b	*o*-OCH_3	–0.663	2.265	1.34	5.79	0.12	7.90	3.98	1.35	3.07	0.324	–0.440
6 c	*m*-OCH_3	–0.753	2.216	1.35	5.74	0.11	7.90	3.98	1.35	3.07	0.255	–0.177
6 d	*p*-OCH_3	–0.672	2.259	1.49	5.75	–0.14	7.90	3.98	1.35	3.07	0.260	–0.510
6 e	*o*-CH_3	–0.639	2.238	1.36	5.77	–0.004	5.70	2.87	1.52	2.04	–0.050	–0.112
6 f	*m*-CH_3	–0.374	2.427	1.49	5.87	–0.06	5.70	2.87	1.52	2.04	–0.039	–0.045
6 g	*p*-CH_3	–0.357	2.475	1.45	5.84	–0.17	5.70	2.87	1.52	2.04	–0.040	–0.130
6 h	*o*-CF_3	–0.541	2.029	1.52	5.32	0.45	5.00	3.30	1.99	2.61	0.474	0.164
6 i	*m*-CF_3	–0.463	2.181	1.62	5.59	0.46	5.00	3.30	1.99	2.61	0.372	0.066
6 l	*o*-F	–1.050	1.978	1.30	5.62	0.26	0.90	2.65	1.35	1.35	0.537	–0.293
6 m	*m*-F	–1.074	1.868	1.21	5.71	0.34	0.90	2.65	1.35	1.35	0.421	–0.118
6 n	*p*-F	–0.714	1.852	1.05	5.72	0.10	0.90	2.65	1.35	1.35	0.430	–0.340
6 o	*m*-NO_2	–1.487	1.388	1.21	5.27	0.71	7.40	3.44	1.70	2.44	0.657	0.056
6 p	*p*-NO_2	–0.986	1.593	0.95	5.49	0.77	7.40	3.44	1.70	2.44	0.670	0.160
6 r	*m*-OH	–0.969	1.725	1.07	5.71	0.12	2.80	2.74	1.35	1.93	0.280	–0.222
6 s	*p*-OH	–0.742	1.729	1.17	5.70	–0.37	2.80	2.74	1.35	1.93	0.290	–0.640
6 t	*o*-CN	–1.399	1.278	0.94	4.97	0.62	6.30	4.23	1.60	1.60	0.636	0.164
6 u	*m*-CN	–1.597	1.609	0.82	5.54	0.62	6.30	4.23	1.60	1.60	0.500	0.066
6 v	*p*-CN	–1.336	1.344	0.81	5.56	0.65	6.30	4.23	1.60	1.60	0.510	0.190

Table II: Minimal inhibitory concentrations (MIC), denoted by log 1/*c* (*c* in mol/L).

No.	R	*E. coli* ATCC 25922	*E. coli* ATCC 35218	*S. aureus* ATCC 25923	*S. aureus* ATCC 29213	*E. faecalis* ATCC 29212	*P. aerug.* ATCC 27853	*S. pyogenes* ATCC 10389	*K. pneum.* ATCC 10389
6 a	H	6.47	6.47	5.57	5.57	4.66	5.26	5.26	6.17
6 b	*o*-OCH_3	4.70	4.70	<4.09	<4.09	<4.09	<4.09	<4.09	4 .40
6 c	*m*-OCH_3	5.90	5.90	5.30	5.30	4.70	4.70	5.30	5.60
6 d	*p*-OCH_3	4.50	4.70	4.09	4.09	<4.094	<4.10	4.09	4.40
6 e	*o*-CH_3	6.18	5.88	4.68	4.68	<4.08	4.68	4.68	5.58
6 f	*m*-CH_3	6.18	5.88	5.28	5.28	4.38	4.98	5.28	5.88
6 g	*p*-CH_3	6.18	5.88	4.68	4.68	4.08	4.68	4.68	5.88
6 h	*o*-CF_3	5.64	5.64	5.04	4.74	4.43	4.43	4.74	5.04
6 i	*m*-CF_3	5.64	5.34	4.43	4.43	<4.13	4.13	4.13	5.34
6 l	*o*-F	6.49	6.49	5.29	5.59	4.68	5.29	5.29	6.19
6 m	*m*-F	6.19	5.89	4.98	4.98	4.08	4.98	4.98	5.89
6 n	*p*-F	6.49	6.49	5.59	5.89	4.98	5.59	5.59	6.49
6 o	*m*-NO_2	6.22	6.22	4.71	4.71	4.11	5.01	5.01	5.62
6 p	*p*-NO_2	6.52	6.52	4.71	4.71	4.41	5.31	5.01	5.92
6 r	*m*-OH	5.58	5.28	4.38	4.38	4.38	5.28	5.58	5.28
6 s	*p*-OH	5.58	5.58	4.98	4.38	4.68	5.28	5.89	5.28
6 t	*o*-CN	4.09	<4.09	<4.09	<4.09	<4.09	<4.09	<4.09	<4.09
6 u	*m*-CN	4.99	4.99	4.09	4.09	<4.09	4.69	4.09	4.69
6 v	*p*-CN	5.29	5.29	<4.09	<4.09	<4.09	4.39	4.09	4.99

ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 27736). Second, for DNA gyrase isolated from *E. coli* K-12 the quinolone concentrations necessary to achieve a 90 and 100% inhibition of the enzymatic supercoiling activity were recorded as IC_{90} and IC_{100} values, respectively, and denoted by log 1/*c* values in Table III. For sake of comparison ciprofloxacin was included in the investigations. In each lane the concentrations cover a range of about 2.5 log activity units.

Quantitative Structure-Activity Relationships. Three aspects were investigated quantitatively. First, intercorrelations were sought among the different lipophilicity parameters by means of regression analysis. Second, a search for correlations between the various MIC values and between these and the IC_{90} and IC_{100} values, respectively, was performed using principal component analysis (PCA). Third, by use of multiple regression analysis the relationships between the biological activities and various physicochemical descriptors were studied. For QSAR analysis, parameters were chosen to characterize the bulk, lipophilicity and electronic effects: The STERIMOL parameters *L*, B_1, and B_5 as well as MR (molar refractivity) were examined as descriptors of the steric bulk[12]. The Hammett constants σ (sigma values for *o*-, *m*-, *p*-position are taken from[15]), resonance (ℜ)[12] as well as field parameter (ℑ)[12], and the experimentally determined pK_a value were used as descriptors of the electronic properties and R_m, log *k'*, and log *P* were tested as descriptors of the hydrophobicity. The full set of parameters is depicted in Table I.

Table III: 90 and 100% inhibition of the enzymatic supercoilling reaction.

No.	R	Conc. (μg/mL) 90% Inhib.	log 1/*c* (*c* in mol/L)	Conc. (μg/mL) 100% Inhib.	log 1/*c* (*c* in mol/L)	MIC *E. coli* 25922 log 1/*c*
6 a	H	4	4.963	8	4.662	6.47
6 b	*o*-OCH_3	100	3.599	120	3.520	4.70
6 c	*m*-OCH_3	32	4.094	40	3.997	5.90
6 d	*p*-OCH_3	160	3.395	200	3.298	5.00
6 e	*o*-CH_3	8	4.678	16	4.377	6.18
6 f	*m*-CH_3	2	5.280	4	4.979	6.18
6 g	*p*-CH_3	8	4.678	16	4.377	6.18
6 h	*o*-CF_3	60	3.860	80	3.736	5.64
6 i	*m*-CF_3	80	3.736	100	3.639	5.64
6 l	*o*-F	1	5.586	2	5.285	6.49
6 m	*m*-F	4	4.984	8	4.683	6.19
6 n	*p*-F	1	5.586	2	5.285	6.49
6 o	*m*-NO_2	4	5.013	8	4.712	6.22
6 p	*p*-NO_2	2	5.314	4	5.013	6.52
6 r	*m*-OH	0.5	5.885	1	5.584	5.58
6 s	*p*-OH	0.4	5.982	0.5	5.885	5.58
6 t	*o*-CN	45	3.940	50	3.895	4.09
6 u	*m*-CN	2	5.292	4	4.991	5.29
6v	*p*-CN	>40	<3.991	60	3.815	5.29
ciprofloxacin		1	5.520	2	5.219	7.34

Discussion

Concerning the influence of lipophilicity on the biological activity conflicting statements have been published from different groups[4, 5]. On the other hand, from different methods slightly different values of lipophilicity can be obtained. Therefore, in this study the three classical procedures have been carried out. Normally, log *k'*, log *P* and R_m are closely related[12]. Interestingly, in this set of 20 compounds the lipophilicity descriptors do not correlate properly as can be seen from the equations (1) to (3):

$$\log k' = 1.28\ (0.19)\ R_m + 0.32\ (.24) \quad (1)$$
$$n = 20 \quad r^2 = 0.71 \quad F = 43$$

$$\log P = 1.32\ (.20)\ R_m + 2.51\ (.26) \quad (2)$$
$$n = 20 \quad r^2 = 0.70 \quad F = 41$$

$$\log k' = .83\ (.12)\ \log P + 2.63\ (.11) \quad (3)$$
$$n = 20 \quad r^2 = 0.73 \quad F = 51$$

(*n* is the number of derivatives, *F* the number of explained to unexplained variance, and r^2 the regression coefficient, the number in brackets is the standard deviation of the regression coefficients). These results show clearly that the lipophilicity descriptors are sensitively dependent on methods, in which different lipophilic phases were used such as octanol, paraffin, and reversed phase (C18), respectively. At this point it is difficult to decide which descriptor represents the lipophilicity of the quinolones best. Additionally, it is surprising that the different lipophilicity descriptors are differently correlating with the pK_a value (Table VII).

Qualitative examination of the results of the MIC determination and the DNA gyrase supercoiling inhibition (Table III) exhibits a slightly different rank order of antibacterial potency: Whereas the most active compounds in the cell-free enzym system are among the N_1-phenol substituted derivatives, more active than ciprofloxacin, the fluorophenyl and *p*-nitrophenyl substituted derivatives are superior in the MIC test system which includes both, the inhibition of the DNA gyrase and the penetration through the bacterial envelope.

For a quantitative examination a principal component analysis (PCA) of the microbiological data was performed to distinguish between high specific information and redundancies[16]. The correlation matrix of all biological data is displayed in Table IV. Significant relationships between the MIC values of *E. coli* and *K. pneumoniae* as well as the MIC values of *P. aeruginosa* and *S. pyogenes*, and the 90% enzyme inhibition can be gathered from the correlation matrix. This data matrix was analysed for multiple intercorrelations in terms of the PCA (resulting values of the principal components (PC) see Table V). Two significant PCs (PC1-PC2) were obtained which together describe 89% of the variance of the biological data (Table Va). PC1 is loaded by the MIC values of *E. coli, K. pneumoniae* and *S. aureus* and PC2 by the MIC values of *P. aeruginosa, S. pyogenes* and the 90% and 100% enzyme inhibition. The MIC's against *E. faecalis* have been omitted from PCA because of too many data points are missing. Remarkably, the data obtained for *E. coli* in the

Table IV: Correlation matrix for MIC-values and %-inhibition of gyrase (l/log).

		1 *E. coli* 25922	2 *E. coli* 35218	3 *P. aerug*	4 *K. pneum.*	5 *S. pyog*	6 *S. aureus*	7 *S. aureus* 29213	8 90% inhib.	9 100% inhib.
1	*E. coli* 25922	1.000								
2	*E. coli* 35218	0.959	1.000							
3	*P. aerug.*	0.644	0.702	1.000						
4	*K. pneum.*	0.879	0.888	0.703	1.000					
5	*S. pyog.*	0.434	0.520	0.878	0.497	1.000				
6	*S. aureus*	0.688	0.750	0.599	0.700	0.661	1.000			
7	*S. aureus* 29213	0.785	0.811	0.564	0.801	0.528	0.923	1.000		
8	90% inhib	0.395	0.433	0.891	0.495	0.780	0.320	0.232	1.000	
9	100% inhib	0.341	0.392	0.880	0.451	0.803	0.316	0.208	0.994	1.000

Table Va: PCA statistics.

	Multiple cor. coeff.	Variance	PC	Eigenvalue	Σ –%	*F*-value	Significance
E. coli 25922	0.9730	0.2223	1	6.1104	67.89	53.57	99.98
E. coli 35218	0.9767	0.2792	2	1.9189	89.21	38.75	99.93
P. aerug.	0.9814	0.1921	3	0.6138	96.03	25.06	99.70
K. pneum.	0.9365	0.2631	4	0.1594	97.80	8.28	96.56
S. pyog.	0.9572	0.2701	5	0.0824	98.72	4.77	90.62
S. aureus	0.9623	0.2288	6	0.0550	99.34	3.56	84.46
S. aur. 29213	0.9725	0.2820	7	0.0383	99.76	3.13	78.01
90% inhib.	0.9975	0.6017	8	0.0188	99.97	3.36	67.67
100% inhib.	0.9975	0.5233	9	0.0025	100.0	0.00	0.00

Table Vb: Loadings after varimax rotation. Total data set left, *m*-OCH_3 omitted left.

	Total data set		*m*-OCH_3 omitted	
	PC1	PC2	PC1	PC2
E. coli 25922	0.8990	0.2270	0.9216	0.2006
E. coli 35218	0.9076	0.2856	0.9143	0.2783
P. aerug.	0.4953	0.8488	0.5109	0.8390
K. pneum.	0.8569	0.3351	0.8662	0.3241
S. pyog	0.3863	0.8151	0.3456	0.8755
S. aureus	0.8535	0.2385	0.8337	0.2988
S. aur. 29213	0.9440	0.1136	0.9358	0.1651
90% inhib.	0.1527	0.9713	0.1933	0.9649
100% inhib.	0.1137	0.9845	0.1430	0.9799

whole-cell system (MIC) and in the cell-free system (IC_{90}) were found to be in different PCs (Table Vb).

The scores of every PC after varimax rotation are depicted in Table VI. In Table VII the correlation matrix of the PCs and physicochemical parameters is displayed (containing 16 compounds). The matrix reveals significant relationships between PC1, the Sterimol parameter *L* as well as the indicator variable I_{OH} and between PC2 and the lipophilicity R_m as well as the Sterimol parameter *B5*.

An indicator, I_{OH}, has been introduced to indicate the absence or presence of a phenolic OH group. This is the only substituent within the data set which is partially ionized under experimental conditions. The pK_a is, however, not known and thus an indicator variable being 1 in the presence and zero in the absence of this substituent is used.

For the larger data set (*E. coli* MIC) it was first tested if the influence of the STERIMOL parameter, *L*, varies in different loci of substitution.

$$\log 1/MIC_{E.\ coli} = -.788(.26)\, I_{OH} - 1.13\,(.15)\, L_o - .672(.15)\, L_m - .729(.15)\, L_p + 12.06(.92) \quad (4)$$

$$n = 19 \quad r^2 = .81 \quad s = .33 \quad F = 14.97 \quad Q^2 = .67 \quad \text{Press}/N = .38$$

where Q^2 is the cross validated r^2 by using the leave-one-out procedure. Q^2 can adopt values between 1 and less than zero. Press = Predictive Residual Sum of Squares.

As expected the influence in ortho-position seems to be a little larger compared to meta- and para-position and one looses significance of the regression equation by using, *L*, independent of the locus of substitution (see Eq. 5).

$$\log 1/MIC_{E.\ coli} = -.702(.34)\ I_{OH} - .834(.16)\ L + 8.58(.54) \quad (5)$$

$n = 19\ r^2 = .64\ s = .43\ F = 14\ Q^2 = .52$

For comparison with PC1 we have, however, to use only L, as we have only 16 data points in the PC analysis.

$$PC1 = -1.93(.51)\ I_{OH} - 1.17(.29)\ L + 3.89(.95) \quad (6)$$

$n = 16\ r^2 = .65\ s = .66\ F = 12\ Q^2 = .48$ Press/N =.72

The regression equations are comparable. Close inspection of Eq. (6) shows, however, that the *m*-OCH_3 derivative deviates more than two standard deviations from the regression. The reason for this is probably the deviation of *m*-OCH_3 from the general trend in the data set where no significant differences in MIC, especially between *m*- and *p*-position of the same substituents is observed for the studied compounds. The *m*-OCH_3, however, is about ten times more active than its *p*-isomer. This singularity cannot be considered in the general regression equation. This fact is also disturbing the PC analysis. If the PCA is performed omitting the *m*-OCH_3 derivative, the separation (orthogonality) between PC1 and PC2 becomes even better (see Table Vb).

Omitting the *m*-OCH_3 derivative, the following equation is obtained:

$$PC1_{-OCH3} = -1.95(.37)\ I_{OH} - 1.48(.24)\ L + 4.72(.76) \quad (7)$$

$n = 15\ r^2 = .81\ s = .49\ F = 25.8\ Q^2 = .74$ Press/N = .51

The QSAR analysis for PC1 and log $1/MIC_{E.\ coli}$ are in agreement and show that the antibacterial activity of the studied derivatives against the strains loading PC1 is negatively influenced by a steric effect (L) of the substituents and the presence of partially ionized OH groups. Both effects may be connected with the transport of the derivatives into the bacterial cell, which proceeds probably through the pores. A similar observation has been made previously from a QSAR derived for sulfanilamido-1-penylpyrazoles substituted at the phenyl ring[16b].

The correlation matrix in Table VII shows also correlations between PC2 and the concentration for 90% inhibition of *E. coli* derived gyrase, which loads PC2 together with the MIC's against *P. aeruginosa* and *S. pyogenes*, with R_m, $B5$ and I_{OH}.

Stepwise regression analysis results in the following equations:

$$PC2 = -1.87(.46)\ I_{OH} + 1.17(.39)\ B5_o + .817(.28)\ B5_m + .839(.29)\ B5_p - 3.53(1.06) \quad (8)$$

$n = 16\ r^2 = .76\ s = .60\ F = 8.5\ Q^2 = .496$ Press/N = .71

The regression coefficient with $B5_o$ is larger than the regression coefficients with $B5_m$ and $B5_p$, but they do overlap considering the standard deviation so that in the further analysis only $B5$ is used.

$$PC2 = -1.67(.35)\ I_{OH} + .573(.21)\ B5 + 1.84(.60)\ R_m - 3.31(.71) \quad (9)$$

$n = 16\ r^2 = .85\ s = .45\ F = 22.6\ Q^2 = .74$ Press/N = .51

Table VI: Scores after varimax rotation.

No.	R	PC1	PC2
6a	H	1.4528	–0.0883
6c	*m*-OCH_3	0.6847	0.8384
6d	*p*-OCH_3	–1.7272	1.6409
6e	*o*-CH_3	0.1088	0.5361
6f	*m*-CH_3	0.1308	–0.3868
6g	*p*-CH_3	0.1767	0.5175
6h	*o*-CF_3	0.1072	1.2231
6i	*m*-CF_3	–0.4990	1.6134
6l	*o*-F	1.1515	–0.5492
6m	*m*-F	0.3687	0.0268
6n	*p*-F	1.7103	–0.6931
6o	*m*-NO_2	0.1346	–0.1059
6p	*p*-NO_2	0.4445	–0.4652
6r	*m*-OH	–1.1423	–1.6888
6s	*p*-OH	–1.0903	–1.9183
6u	*m*-CN	–1.5164	–0.5007

This regression equation is highly significant with high predictive power. It shows the importance of the OH group, the bulkiness, $B5$, and the lipophilicity, R_m, of the substituents for the loading (scores) of PC2. The intercorrelation between the used parameters is negligible (Tab. VII). If the sterimol parameter, L, is used instead of B5, the regression is of lower statistical significance ($r^2 = .76$, $s = .60$).

PC2 is mainly loaded by the concentrations causing 90% and 100% inhibition, respectively, of *E. coli* derived gyrase and should therefore be compared to the regression obtained for 90 % inhibition.

$$\log 1/C_{90\ \%\ inhib.} = .879(.31)\ I_{OH} - .589(.19)\ B5 - 1.32(.54)\ R_m + 7.67(.64) \quad (10)$$

$n = 16\ r^2 = .78\ s = .40\ F = 14.6\ Q^2 = .63$ Press/N = .46

The equation predicts the not included *o*-OCH_3 derivative quite well (log $1/C_{obs}$ = 3.6, log $1/C_{pred}$ = 4.08) but not the *o*-CN derivative. This compound deviates more than 3 standard deviations from the regression and is therefore to be considered as an outlier. The reason for this is not known. It is the only derivative studied which shows no difference in its activity in the whole-cell and cell-free system and its whole-cell activity is extremely low (may be due to solubility problems).

Finally we tried to explain the observed difference between whole-cell and cell-free activities against *E. coli*:

$$\log 1/MIC_{E.Coli} - \log 1/C_{90\ \%\ inhib.} = -1.37(.27)\ I_{OH} + 1.37(.46)R_m + .198(.15)\ B5 - .899(.54) \quad (11)$$

$n = 16\ r^2 = .83\ s = .34\ F = 19\ Q^2 = .65$ Press/N = .42

In this equation the contribution of $B5$ is only significant at the 77% level. This is probably due to partially cancelling off of the steric bulk effect (L and $B5$) on whole-cell and cell-free activities, respectively ($B5/L$, $r = .71$). If the sterimol parame-

Table VII: Correlation matrix of $MIC_{E.\ coli}$, 90 % inhibition, principal component scores and physicochemical parameters.

	PC1	PC2	*E. coli* 25922	90% inhib.	log *k'*	log *P*	R_m	pK_a	*MR*	*L*	*B5*	*B1*	*M*	ℑ	ℜ	I_{OH}	σ
PC1	1.000																
PC2	–0.000	1.000															
E. coli 25922	0.899	–0.227	1.000														
90% inhib.	0.153	–0.971	0.395	1.000													
log *k'*	0.002	0.565	–0.098	–0.542	1.000												
log *P*	0.002	0.412	–0.041	–0.423	0.833	1.000											
R_m	0.013	0.698	–0.121	–0.695	0.794	0.785	1.000										
pK_a	0.101	–0.095	0.088	0.103	0.603	0.467	0.172	1.000									
MR	–0.411	0.462	–0.378	–0.513	0.189	0.122	0.176	–0.260	1.000								
L	–0.514	0.412	–0.612	–0.480	–0.044	–0.194	–0.053	–0.384	0.814	1.000							
B5	–0.413	0.569	–0.479	–0.676	0.273	0.360	0.454	–0.272	0.832	0.707	1.000						
B1	–0.253	0.413	–0.216	–0.387	–0.016	0.179	0.282	–0.660	0.470	0.440	0.515	1.000					
M	–0.202	0.523	–0.284	–0.551	–0.112	0.083	0.304	–0.750	0.444	0.512	0.643	0.883	1.000				
ℑ	–0.028	–0.122	–0.020	0.122	–0.755	–0.646	–0.438	–0.810	0.041	0.367	0.102	0.389	0.562	1.000			
ℜ	0.196	0.307	0.246	–0.212	–0.073	–0.132	–0.016	–0.481	0.276	0.160	0.058	0.568	0.472	0.197	1.000		
I_{OH}	–0.475	–0.682	–0.319	0.522	–0.273	–0.009	–0.260	0.117	–0.263	–0.245	–0.078	–0.228	–0.226	–0.046	–0.512	1.000	
σ	0.063	0.126	0.121	–0.055	–0.549	–0.567	–0.339	–0.793	0.237	0.390	0.098	0.579	0.618	0.768	0.749	–0.380	1.000

ter, *B5*, is omitted, a regression with the identical high statistical significance is obtained:

$$\log 1/MIC_{E.\ Coli} - \log 1/C_{90\ \%\ inhib.} = \quad (12)$$
$$-1.35(.27)\, I_{OH} + 1.63(.42)\, R_m - .822(.55)$$

$$n = 16\ \ r^2 = .81\ \ s = .35\ \ F = 26.9\ \ Q^2 = .67\ \ \text{Press}/N = .40$$

This means the difference in the inhibitory activities in the two test systems depends on the presence of the phenolic hydroxy groups which favour the activity against gyrase and on the lipohilicity which decreases the activity in the cell-free system.

Taken together, the smaller the lipopholicity, the smaller the substituent at the N_1-phenyl residue and presence of phenolic OH groups the higher is the inhibitory potency of a given quinolone on DNA gyrase. The biological activity in the whole-cell system is negatively influenced by both the size of the substituent and the presence of OH substituent at N_1-phenyl moiety. Even though the predictive power of these QSAR analyses is not in all derived equations satisfying, some conclusions can be drawn.

The differences between the cell-free and the whole-cell systems obtained from PCA and QSAR analysis may indicate that beside the DNA gyrase other components of the bacterial cell may function as targets for the drugs: – topoisomerase IV believed to be the primary target of quinolone action in *S. aureus*[17] and secondary target in *E. coli*[17a, 17b], – outer membrane protein F, which is a channel-forming pore in the outer membrane of *E. coli* assumed to mediate passive influx of hydrophilic quinolones into the cell[18],– lipopolysaccharide (LPS) of the outer membrane involved in the passage of hydrophobic quinolones through the outer membrane[19]. These alternative targets may play an additional role for the bactericidal action of the compounds.

Considering the IC_{90} values for E. coli DNA gyrase, the molecular interaction of quinolones with the target requires small hydrophilic substituents at the N_1-benzene ring for enhanced inhibitory potency. This seems to argue against the model of Shen et al.[20]. Based on their finding that the binding of quinolones to the DNA gyrase-DNA complex is highly specific and saturatable they proposed the interaction of quinolone molecules with a short single-stranded DNA pocket and hydrophobic interactions between N_1-residues of inversely orientated quinolone molecules. Another hypothesis, however, suggested by Reece and Maxwell[21] seems to be compatible with the data presented here: Based on the findings made by several investigators [17c, 22–27] that nearly all *gyr*A or *gyr*B mutations associated with quinolone resistance lead to the loss of hydrogen bond donor or acceptor residues, they assume the direct involvement of DNA gyrase in quinolone binding. This view is supported by the results of two studies showing a severalfold reduction in quinolone binding to a complex of DNA and DNA gyrase carrying a tryptophan[28] or a leucine residue[29] instead of a highly conserved serine at position 83 of the DNA gyrase A subunit. Assuming that this serine-83 plays a central role in the interaction between DNA-bound gyrase and quinolone a small hydrophilic substituent at the N_1-benzene ring of the quinolones studied is an appropriate candidate for the interaction with this serine.

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Table XIII: Analytical data of derivatives **6**.

No.	R	mmol	Formula mol. weight	Yield %	Mp (°C) recryst. solvent
6 a*	H	1.3	$C_{20}H_{18}FN_3O_3$ 367.4	210 mg 44	236–238 (dec.) EtOH
6 b	*o*-OCH_3	2.3	$C_{21}H_{20}FN_3O_4$ 397.4	273 mg 30	>200 (dec.) EtOH/DMF
6 c	*m*-OCH_3	2.0	$C_{21}H_{20}FN_3O_4$ 397.4	140 mg 18	>200 (dec.) EtOH
6 d	*p*-OCH_3	2.9	$C_{21}H_{20}FN_3O_4$ 397.4	700 mg 61	208–210 (dec.) *i*-Prop./ H_2O
6 e	*o*-CH_3	3.0	$C_{21}H_{20}FN_3O_3$ 381.2	920 mg 81	>200 (dec.) EtOH/ DMF
6 f	*m*-CH_3	2.5	$C_{21}H_{20}FN_3O_3$ 381.2	460 mg 48	>200 (dec.) EtOH
6 g	*p*-CH_3	1.5	$C_{21}H_{20}FN_3O_3$ 381.2	245 mg 43	235–238 (dec.) *i*-Prop./H_2O
6 h	*o*-CF_3	1.5	$C_{21}H_{17}F_4N_3O_3$ 435.1	380 mg 58	>200 (dec.) EtOH
6 i	*m*-CF_3	1.5	$C_{21}H_{17}F_4N_3O_3$ 435.1	312 mg 48	>200 (dec.) EtOH
6 k	*p*-CF_3	2.6	$C_{21}H_{17}F_4N_3O_3$ 435.1	388 mg 34	233 (dec.) EtOH/ DMF
6 l	*o*-F	2.5	$C_{20}H_{17}F_2N_3O_3$ 385.2	590 mg 61	>200 (dec.) EtOH
6 m	*m*-F	3.6	$C_{20}H_{17}F_2N_3O_3$ 385.2	600 mg 44	>240 (dec.) EtOH
6 n*	*p*-F	2.9	$C_{20}H_{17}F_2N_3O_3$ 385.2	395 mg 36	>240 (dec.) EtOH
6 o	*m*-NO_2	1.7	$C_{20}H_{17}FN_4O_5$ 412.2	253 mg 36	>250 (dec.) EtOH
6 p	*p*-NO_2	1.4	$C_{20}H_{17}FN_4O_5$ 412.2	312 mg 54	>250 (dec.) EtOH
6 r	*m*-OH	9.0	$C_{20}H_{18}FN_3O_4$ 383.4	770 mg 22	>240 (dec.) EtOH/ DMF
6 s	*p*-OH	2.7	$C_{20}H_{18}FN_3O_4$ 383.4	660 mg 64	>240 (dec.) EtOH/ DMF
6 t	*o*-CN	1.7	$C_{21}H_{17}FN_4O_3$ 392.2	285 mg 43	238 (dec.) EtOH/ DMF
6 u	*m*-CN	1.2	$C_{21}H_{17}FN_4O_3$ 392.2	160 mg 34	>240 (dec.) EtOH/ DMF
6 v	*p*-CN	1.8	$C_{21}H_{17}FN_4O_3$ 392.2	124 mg 18	>240 (dec.) EtOH/ DMF

* Hydrochloride [6]

Experimental

Melting points were determined with a Dr. Tottoli melting point apparatur (Büchi) and were not corrected. ^1H and ^{13}C NMR spectra were recorded on a Varian EM 360 A (^1H 60 MHz), Varian XL 300 (^1H 299.956 MHz, ^{13}C 75 MHz) and a Bruker AMX 500 (^1H 500.138 MHz, ^{13}C 125 MHz). Abbreviations for data quoted are:d, doublet; t, triplett; q, quintet; m, multiplet. IR spectra were obtained using a Perkin Elmer 298 infrared spectrometer. UV/Vis spectra were recorded on a Hewlett-Packard HP 8452A diode array spectrometer. Mass spectra were measured on a MS 50 (Kratos). TLC were carried out using silica gel 60 F_{254} (Merck No. 5554) and RP-18 254 S (Merck No. 15685), column chromatography silica gel 70–230 mesh (Merck No. 7734). For HPLC a Kontron Instruments Pump 420 equipped with a Rheodyne-outlet (10µl), a Merck Hitachi D-2000 Chromato-Integrator and a Spherisorb ODS-2 endcapped column (250 × 4.6 mm, 5µm) was used at temperature of 40 °C. Dry solvents were used throughout.

The ethyl 2-(2,4-dichloro-5-fluorobenzoyl)-3-ethoxyacrylate **2** was synthesized according to Grohe[30] and Chu[6] , the ethyl 2-(2-chloro-4,5-difluorobenzoyl)-3-ethoxyacrylate was a generous gift of D.T.W. Chu, Abbott, USA.

Synthesis of Differently Substituted Ethyl 3-Aryl-2-(chloro/fluorobenzoyl)-acrylates **3**

Equimolar amounts of **2**, dissolved in ethanol (75 to 100 ml), and differently substituted aniline derivatives were stirred overnight and afterwards refluxed for 1 h. The reaction was observed by means of TLC using silica gel and CH_2Cl_2/MeOH (9+1). For crystallization the solution was allowed to stand at −10 °C. The resulting crystals were filtered, washed with cold ethanol and recrystallized from ethanol or i-propanol. For analytical and spectroscopical data see Tables VIII, IX [31].

General Cyclization Procedure

Arylamino-2-benzoyl-acrylates **3** were dissolved in the solvent (100 ml), equimolar amounts of base, each given in Table X, were added, and the solution refluxed for 3 h. The reaction was observed using TLC (silica gel with the eluent methanol/CH_2Cl_2). After cooling the solvent was evaporated *in vacuo*, the obtained oil diluted with water (75 ml) and heated for 1 h at 90 °C. For crystallization the solution was allowed to stand at −5 °C overnight. The crystals were filtered and recrystallized from ethanol or a mixture of ethanol/DMF. For analytical and spectroscopic data of **4** see Tables X and XI [31].

General Hydrolysation Procedure

Equimolar amounts of the quinolone acid ester **4** and NaOH (0.1 M aqueous solution) were mixed, an equal amount of tetrahydrofuran was added and the obtained solution was refluxed for 2 h. The reaction was observed by means of TLC using silica gel and CH_2Cl_2/methanol (9+1). After cooling the solvent was evaporated *in vacuo*, the remaining oil diluted with water (100 ml), adjusted at pH 1 with HCl (dil), and allowed to stand overnight at −5 °C. The obtained crystals were filtered, washed several times with water and recrystallized from ethanol or ethanol/DMF mixtures. For analytical data of **5** see Table XII [31].

Synthesis of 1-Aryl-6-fluoro-7-piperazinyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid **6**, *General Procedure*

The quinolone-3-carboxylic acids **5** were dissolved in DMSO (75–100 ml) and a fivefold surplus of dry piperazine added. After heating at 140 °C for 2h (reaction control with RP 18 TLC plates using methanol/H_2O/NH_3 = 60 + 20 + 1), DMSO was removed *in vacuo*, the obtained oil diluted with water (50 ml) and heated at 90 °C for 1h. The solution was allowed to stand overnight, the crystal filtered and washed several times with water. The crystals were recrystallized from ethanol or ethanol/DMF mixtures using activated charcoal. For analytical and spectroscopic data of **6** see Tables XIII, XIV, XV, and XVI.

Determination of Lipophilicity, log k'

The procedure was carried out according to ref.[12] using a Spherisorb ODS-2 column. The following eluent systems were used: Methanol/phosphate buffer (pH 7.4, DAB9), flow in brackets (ml/min): 80 + 20 (1.0), 70 + 30 (1.0), 60 + 40 (1.0), 50 + 50 (1.0), 40 + 60 (2.5), and 30 + 70 (3.0). Quinolone solution: 5 mg **6** was dissolved in NaOH (0.1 M, 1.0 ml), diluted to 50.0 ml with phosphate buffer, and again 1:10 diluted with the eluent. t_o was determined using thiourea in the corresponding eluent. $\log k' = \log ((t_{ret} - t_o)/t_o)$. The $\log k'$ for 0% methanol was extrapolated by means of linear regression analysis using InPlot 4.0 (GraphPad Software, San Diego) and diplayed in Table I.

log P

About 1.0 mg quinolone was dissolve in NaOH (0.1 M, 1 ml) and diluted to 100 ml in phosphate buffer (pH 7.4, DAB 9) saturated with octanol and the absorbance (A_o) measured at 270 and 272 nm, respectively. A 1.0 ml

Table XIV: ^{1}H NMR (DMSO-d_6) and IR spectroscopic data of derivatives **6**.

Compd. (DMSO-d_6)	R	H-2	H-5	H-8	phenyl ring	R	piperazine	IR cm^{-1} (KBr)
6 a	H	8.58 s	7.92 d, 13.0	6.37 d, 7.5	7.7 s		2.74–2.89 (m, 4H), 2.90–2.95 (m, 4H)	3400 br., 1720, 1620, 1480, 1260
6 b	*o*-OCH_3	8.55 s	7.93 d, 13.5	6.25 d, 7.0	7.23 (td, 8.0;1,5; 1H, H-4′), 7.39 (dd, 8.0;1.5; 1H, H-6′), 7.61-7.66 (m, 1H, H-5′), 7.69 (dd, 8.0; 2.0; 1H, H-3′)	3.76 s	2.73–2.79 (m, 4H), 2.87–2.95 (m, 4H)	3400 br., 2840, 1725, 1630, 1480, 1250
6 c	*m*-OCH_3	8.59 s	7.95 d, 13.5	6.45 d, 7.5	7.22–7.26 (m, 2H, H-4′, H-6′), 7.34 (t, 2.0; 1H, H-2′), 7.59 (t, 8.5; 1H, H-5′)	3.72 s	2.78–2.83 (m, 4H) 2.94–2.99 (m, 4H)	3400 br., 2830, 1720, 1630, 1480, 1290
6 d	*p*-OCH_3	8.54 s	7.93 d, 13.0	6.42 d, 7.5	7.27 (AB, 9,0; H-3′, H-5′), 7.65 (AB,9.0; H-2′, H-6′)	3.88 s	2.75–2.80 (m, 4H), 2.92–2.97 (m, 4H)	3450 br., 3840,1725, 1630, 1500, 1470, 1260
6 e*	*o*-CH_3	8.59 s	8.00 d, 13.0	6.2 d, 7.0	7.49–7.60 (m, 4H,arom.)	1.99 s	3.19 (s, br.)	3400 br., 1720, 1620, 1460, 1250
6 f*	*m*-CH_3	8.60 s	8.01 d, 13.0	6.50 d, 7.5	7.47–7.61 (m, 4H,arom.)	2.4 s	3.3 (s, br.)	3410 br., 1725, 1630, 1580, 1500, 1290
6 g	*p*-CH_3	8.54 s	7.93 d, 13.0	6.41 d, 7.5	7.49 (AB, 8.5; H-3′, H-5′), 7.57 (AB, 9.0; H-2′, H-6′)	2.45 s	2.74–2.79 (m, 4H), 2.90–2.95 (m, 4H)	3400 br., 1625, 1580, 1400, 1290
6 h	*o*-CF_3	8.78 s	7.95 d, 13.0	6.02 d, 7.0	7.90–7.97 (m, H-4′, H-5), 8.01 (qd, 7.5;2.0; H-3′), 8.1 (dd, 7.5;2.0; H-6′)		2.71–2.76 (m, 4H), 2.87–2.93 (m, 4H)	3420 br., 1725, 1630, 1460, 1310, 1260,
6 i	*m*-CF_3	8.73 s	7.95 d, 13.0	6.30 d, 7.5	7.9–8.24 (m, 4H, arom.)		2.73–2.91 (m,4H), 2.90–2.99 (m, 4H)	3400 br., 1725, 1620, 1460, 1320, 1260
6 k*	*p*-CF_3	8.68 s	8.00 d, 13.0	6.45 d; 7.0	7.92 (AB, 7.5; H-3′, H-5′), 8.00 (AB, 7.5; H-2′, H-6′)		3.23 (s, br.)	3400 br., 1720, 1610, 1460, 1320, 1270
6 l	*o*-F	8.70 s	7.91 d, 13.0	6.45 d, 7.0	7.53 (dt, 9.0;2.0; 1H, H-6′), 7.59 (dt, 9.0; 2.0; 1H, H-3′), 7.75 (qm, H-4′) 7.79 (dt, 9.0; 2.0; 1H, H-5′)		2.74–2.77 (m, 4H), 2.93–2.97 (m, 4H)	3400 br., 1725, 1600, 1500, 1260, 1190
6 m	*m*-F	8.65 s	7.93 d, 13.0	6.38 d, 7.0	7.52–7.59 (m, 2H, arom.), 7.70–7.8 (2H, arom.)		2.75–2.81 (m, 4H), 2.93–3.00 (m, 4H)	3400 br., 1725, 1610, 1490, 1260, 1190
6 n	*p*-F	8.61 s	7.94 d, 13.0	6.35 d, 7.0	7.53 (t, $^3J_{H\text{-}F}$ = 9.0; 2H, H-3′, H-5′), 7.78 (dd, $^3J_{H\text{-}F}$ = 9.0; $^4J_{H\text{-}F}$ =5.0, 2H, H-2′, H-6′)		2.78–2.83 (m, 4H), 2.30–2.95 (m, 4H)	3420 br., 1725, 1620, 1500, 1260, 1160
6 o*	*m*-NO_2	8.77 s	8.01 d, 13.5	6.42 d, 7.0	7.93–8.6 (m, 4H,arom.)		3.23 (s, br.)	3400 br., 1720, 1630, 1530, 1480, 1350, 1260
6 p*	*p*-NO_2	8.71 s	7.98 d, 13.5	6.40 d, 7.2	8.08 (AB, 8.7; 2H, H-3′, H-5′), 8.51 (AB, 8.7; 2H, H-2′, H-6′)		2.84–2.87 (m, 4H) 3.02–3.11 (m, 4H)	3400 br., 1725, 1630, 1590, 1530, 1350, 1260
6 r*	*m*-OH	8.56 s	7.96 d, 12.9	6.53 d, 7.2	7.02–7.09 (m, 4H, arom.)		3.22, s br.	3400 br., 1710, 1630, 1450, 1270
6 s*	*p*-OH	8.56 s	7.99 d, 12.9	6.51 d, 7.5	7.01 (AB, 9.0; 2H, H-3′, H-6′), 7.47 (AB, 9.0; 2H, H-2′, H-6′)		3.24, s, br .	3400 br., 1720, 1630, 1580, 1510, 1270
6 t*	*o*-CN	8.94 s	8.05 d, 12.9	6.25 d, 6.9	7.90–8.21 (m, 4H, arom.)		3.23, s, br.	3400 br., 2220, 1720, 1630, 1500, 1290
6 u*	*m*-CN	8.74 s	8.03 d, 13.2	6.39 d, 7.2	8.03 (t, 8.1; 1H, H-5′), 8.05–8.16 (m, 2H, H-4′, H-6′), 8.28 (t, 1.8, 1H, H-2′)		3.21, s, br.	3400 br., 2220, 1720, 1620, 1260
6 v*	*p*-CN	8.70	8.03 d, 13.2	6.43 d, 7.5	7.95 (AB, 8.7; 2H, H-3′, H-5′), 8.17 (AB, 8.7; 2H, H-2′, H-6′)		3.27, s, br.	3400 br., 2220, 1720, 1620, 1460, 1260

*DMSO-d_6 + CF_3COOD

Table XV: ^{13}C NMR data of the derivatives **6** (DMSO-d_6; J_{C-F} values are given in each second row).

No. (DMSO -d_6)	C-2	C-3	C-4	C-4a	C-5	C-6	C-7	C-8	C-8a	-COOH	Piperazine	*N*-1-phenyl
6 a	147.8	107.2	176.2 d; 2.6	118.1 d; 7.9	110.7 d; 23.1	152.6 d; 248	145.4 d; 10.1	105.9 d; 3.6	138.8	165.4	44.98; 50.20; 50.26	126.9; 130.07; 130.08; 130.1; 130.2; 139.7
6 b	148.6	107.3	176.2	118.2 d; 7.7	110.6 d; 23.5	152.4 d; 248	145.6 d; 10.2	105.4	138.7	165.3	45.0; 50.31; 50.38	56.02; 113.1; 121.2; 128.4; 132.0
6 c	147.9	107.2	176.2 d; 2.6	118.2 d; 7.7	110.7 d; 23.4	152.7 d; 248	145.3 d; 10.7	106.1	138.8	165.4	44.89; 50.0; 50.06	55.6; 112.5; 116.3; 118.9; 131.8; 140.7; 160.2
6 d	148.2	107.1	176.2 d; 2.6	118.2 d; 7.8	110.7 d; 23.5	152.6 d; 248	145.4 d; 9.8	106.1 d; 3.4	139.2	165.4	45.0; 50.24; 50.30	55.5; 115.1; 128.3; 132.3; 159.9
6 e*	148.7	108.4	177.1 d; 2.6	120.2 d; 7.8	112.0 d; 23.2	152.5 d; 248	145.0 d; 10.7	106.7 d; 3.2	138.6	165.9	42.91; 46.40; 46.46	16.7; 121.0; 128.1; 128.4; 132.1; 139.0; 159.4
6 f*	148.2	107.3	176.2 d; 2.5	119.3 d; 8.0	111.1 d; 22.9	152.6 d; 248	144.0 d; 10.6	107.1 d; 2.3	138.9	165.3	42.41; 42.46; 45.99; 46.04	19.7; 123.9; 127.3; 129.7; 130.8; 140.2; 158.3
6 g	147.9	107.2	176.2 d; 2.7	118.2	110.7 d; 23.3	152.6 d;248.3	145.4 d; 10.1	106.1 d; 3.4	138.9	165.4	45.03; 50.28 50.35	20.7; 126.6; 130.5; 139.8; 151.0
6 h	148.4	107.4	176.4 d; 2.4	117.8 d; 7.8	110.9 d; 23.6	152.6 d; 248	145.5 d; 10.3	105.5 d; 3.8	139.2	165.1	44.9; 50.2; 50.29	122.54 (q, 271.7); 125.83 (q.,31.0); 127.81 (q, 0.9); 127.9 (q, 3.5)
6 i	148.4	107.4	176.4	118.2	110.8 d; 23.6	152.5 d; 248	145.3 d; 9.8	105.7	138.7	165.3	44.87;44.91; 50.10; 50.2	124.61; 126.87 (q, 1.65); 123.23 (q, 271.0);130.65 (q, 32.0)
6 k	148.8	107.3	176.9 d; 2.3	hidden	111.7 d; 23.0	153.2 d;247.9	144.5 d; 10.6	105.6	138.7	165.6	42.90;46.0; 48.2	115.2 (q, 285); 127.6 (q, 3.5); 128.8; 158.4 (q, 38.9)
6 l	149.3	108.8	177.1	118.8 d; 7.8	111.8 d; 23.0	153.5 d 248.5	146.5 d; 10.5	105.6	139.2	166.1	45.00; 50.23; 50.30	117.8 (18.7) ; 126.8 (3.6); 127.4 (12.6); 130.2 ; 133.6 (7.9) ; 157.2 (249.6)
6 m	148.0	107.3	176.3	118.6 d; 7.8	110.8 d; 23.2	152.1 d; 248	145.4	105.8 d; 3.2	138.7	165.3	44.99; 50.16; 50.22	115.1 (24.5); 117.3 (21.0); 123.5 (2.3); 131.7 (9.1); 156.4 (249.8)
6 n	148.2	107.2	176.4	118.1 d; 7.9	110.8 d; 23.5	152.3 d; 248	145.3 d; 10.1	105.9 d. 3.5	139.0	165.3	44.85; 49.96; 50.02	117.0 (23.3); 129.6 (9.1); 135.9 (2.3);158.1 (249.8)
6 o*	148.9	106.8	176.6	119.3 d; 8.0	111.3 d; 23.6	152.7 d; 247.8	144.0	hidden d; 10.0	138.6	165.2	42.46; 42.53; 46.14	123.3; 125.2; 131.4; 134.2; 148.0; 157.3
6 p*	148.4	107.7	176.4 d; 2.5	119.3 d; 7.5	111.3 d; 23.6	152.7 d; 248	144.0 d; 10.5	106.7 d; 3.9	138.2	165.1	42.43; 42.47; 46.07; 46.09	125.4; 129.1; 148.0; 158.7
6 r*	148.4	107.8	176.8 d; 2.5	120.0 d; 7.1	111.5 d; 23	153.1 d;247.8	144.4 d; 10.5	107.4	138.9	165.8	42.8; 46.5	114.2; 117.5; 117.7; 131.3; 141.0; 159.0
6 s	148.7	107.3	176.3	119.5 d; 7.5	111.1 d; 23.0	152.7 d;247.8	143.9 d; 10.6	107.2 d; 2.6	139.1	165.4	42.48; 42.5; 46.11	116.4; 128.2; 131.0; 158.5
6 t*	148.7	108.2	176.5 d; 2.0	119.1 d; 8.0	111.6 d; 23.0	152.8 d;248.3	144.5 d; 11.0	106.1 d; 2.5	138.4	165.1	42.58; 46.07; 46.13	111.2; 115.0; 129.4; 131.4; 134.6; 135.3; 158.5
6 u*	148.8	107.7	176.5 d; 2.0	119.4 d; 7.5	111.3 d; 23.0	152.7 d;247.8	144.0 d; 10.5	106.9 d; 2.5	138.5	165.2	42.44; 42.51 46.08	116.8; 131.3; 131.5; 132.5; 134.1; 151.0
6 v	148.5	107.7	176.4 d;1.0	119.4 d; 7.5	111.3 d; 23.0	152.7 d;247.8	144.0 d; 10.1	106.8	138.3	165.2	42.42; 42.49; 46.09	116.8; 128.6; 134.2

* DMSO-d_6 + CF_3COOD

portion of this solution was shaken for 4h with 1.0 ml octanol saturated with buffer. The emulsion was allowed to stand overnight, centrifuged for 5 min (3000 U/min.), the water layer separated and again the absorption (A_1) measured. $\log P = \log ((A_0 - A_1)/A_1)$ is displayed in Table I.

R_m

Silica gel TLC plates were coated with paraffin. Varying mixtures of eluent were used: 5%, 7.5%, 10%, 15%, 20%, 25%, 30% acetone in phosphate buffer (pH 7.4, BP 93). 5 µl solution of each derivative (about 1 mg/ml) were developed in the different systems and the R_f value determined using UV detection. Each value was measured in triplicate, the $R_m = \log (1/R_f - 1)$ were calculated for each mixture, the R_m (0% acetone) extrapolated by linear regression analysis using the InPlot 4.0 and displayed in Table I.

Determination of the pK_a Value

10 mg quinolone **6** were dissolved in DMSO (2.5 ml) and diluted to 100.0 ml with bidest. water (*c* about 3×10^{-4} M). 5.0 ml of this solution was diluted with 0.01 M HCl and 0.01 M NaOH, respectively, to 50 ml. The absorbance of each solution was measured (284 to 286 nm). 5.0 ml of the stock solution were diluted with buffer pH 5.0 (Fluka) to 50.0, again the absorbance measured, the pH determined electrometrically and the first pK_a calculated (pK_a = pH + log $[(A - A_i)/(A_m - A)]$; A = absorption of the solution; A_i = absorption of the ionized molecule; A_m = absorption of the neutral molecule. Then, a set of 7 buffers consisting of the pK_a, $pK_a \pm 0.2$, $pK_a \pm 0.4$, and $pK_a \pm 0.6$ was prepared. 5.0 ml of the stock solution were diluted to 50.0 ml with each buffer, the absorbance and pH measured, pK_a value calculated and the mean *pKa* formed. These value are displayed in Table I.

Table XVI: MS data of derivatives **6**.

No.	R	*m/z* (intensity)	$M^{+\cdot}$ (calc.)	$M^{+\cdot}$ (found)
6 a	H	367 $M^{+\cdot}$ (59); 325 (70); 323 (72); 281 (100); 266 (12) 56 (10)	367.1333	367.1337
6 b	*o*-OCH_3	397 $M^{+\cdot}$(57); 355 (55); 353 (86); 311 (100); 296 (11); 269 (8); 56 (10)	397.1438	397.1437
6 c	*m*-OCH_3	397 $M^{+\cdot}$(46); 355 (55); 353 (72); 311 (100); 296 (6); 269 (10); 56 (7)	397.1438	397.1432
6 d	*p*-OCH_3	397 $M^{+\cdot}$(46); 355 (55); 353 (92); 311 (100); 296 (12); 269 (6); 56 (8)	397.1438	397.1441
6 e	*o*-CH_3	381 $M^{+\cdot}$(52); 339 (52); 337 (91); 295 (100); 280 (12); 56 (4)	381.1489	381.1490
6 f	*m*-CH_3	381 $M^{+\cdot}$(38); 339 (48); 337 (72); 295 (100); 280 (10); 56 (8)	381.1489	381.1493
6 g	*p*-CH_3	381 $M^{+\cdot}$(38); 339 (48); 337 (72); 295 (100); 280 (10); 56 (8)	381.1489	381.1491
6 h	*o*-CF_3	435 $M^{+\cdot}$(54); 393 (82); 391 (59); 349 (100); 334 (6); 56 (12)	435.1206	435.1205
6 i	*m*-CF_3	435 $M^{+\cdot}$(58); 393 (97); 391 (50); 349 (100); 334 (6); 56 (14)	435.1206	435.1205
6 k	*p*-CF_3	435 $M^{+\cdot}$(58); 393 (88); 391 (66); 349 (100); 334 (15); 56 (8)	435.1206	435.1205
6 l	*o*-F	385 $M^{+\cdot}$(65); 343 (78); 341 (38); 299 (100)	385.1238	385.1241
6 m	*m*-F	385 $M^{+\cdot}$(41); 343 (67); 341 (48); 299 (100); 284 (8); 256 (5); 56 (11)	385.1238	385.1239
6 n	*p*-F	385 $M^{+\cdot}$(56); 343 (76); 341 (55); 299 (100); 284 (8); 256 (6); 56 (16)	385.1238	385.1243
6 o	*m*-NO_2	412 $M^{+\cdot}$(45); 370 (94); 368 (45); 326 (100); 296 (38); 280 (50); 56 (59)	412.1183	412.1179
6 p	*p*-NO_2	412 $M^{+\cdot}$(36); 370 (85); 368 (50); 326 (100); 296 (45); 280 (46); 56 (24)	412.1183	412.1183
6 r	*m*-OH	383 $M^{+\cdot}$(35); 341 (59); 339 (63); 297 (100); 69 (58); 56 (16)	383.1281	383.1285
6 s	*p*-OH	383 $M^{+\cdot}$(47); 341 (68); 339 (58); 297 (100); 56 (15); 44 (28)	383.1281	383.1280
6 t	*o*-CN	392 $M^{+\cdot}$(47); 350 (100); 348 (26); 306 (16); 304 (30); 44 (48)	392.1285	392.1289
6 u	*m*-CN	392 $M^{+\cdot}$(61); 350 (100); 348 (55); 306 (92); 291 (14); 56 (18)	392.1285	392.1287
6 v	*p*-CN	392 M^{+}(15); 350 (46); 348 (35); 306 (100); 291 (7); 56 (19)	392.1285	392.1285

Microbiology: Determination of the Minimal Inhibitory Concentration (MIC)

Twofold serial dilutions of a compound ranging from 0.125 mg/l to 1,024 mg/l were inoculated with 10^4 colony forming units (CFU) in 100 µl unsupplemented Mueller-Hinton broth. The MIC of a compound was the lowest concentration which was able to inhibit visible growth of the bacteria after 18 hours incubation at 37 °C according to the procedure of the National Committee for Clinical Laboratory Standards (NCCLS, Ref.[14]).

Determination of the Inhibitory Concentrations of Supercoiling (IC_{90} and IC_{100})

Subunit A and B of DNA gyrase from *E. coli* K-12 were isolated separately from *E.coli* K-12 strains JM83 [pGP1-2/pBP7614] (overproducing subunit A) and JM83[pGP1-2/pBP7647] (overproducing subunit B) as described previously[25]. One unit of DNA gyrase activity was defined as the amount of enzyme that catalyzes the conversion of 500 ng plasmid DNA (pBR322) from the relaxed to the supercoiled form in 30 min. at 30 °C as discerned by agarose gel electrophoresis. For the determination of the IC_{90} and IC_{100} values one unit DNA gyrase was incubated in a final volume of 20 µl under standard conditions in the presence of different concentrations of a quinolone. Reactions were stopped by proteinase K treatment and the DNA topoisomers were separated in a 1% agarose gel. The concentration that inhibits the supercoiling reaction by 90 (IC_{90}) or 100% (IC_{100}), respectively, was determined visually.

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