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Protein binding in a congeneric series of antibacterial quinolone derivatives

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Abstract

The extent of plasma protein binding has been determined in a series of gyrase inhibitors characterised either by a varying substitution on the phenyl ring in position *N*1 or by an increasing alkyl chain at the nitrogen *N*4′ of the piperazine moiety. It was tried to derive quantitative structure activity relationships. Especially substituents at the *m*-position of the *N*1-phenyl ring were found to influence the extent of protein binding; an optimum of size could be determined. The increase of the alkyl group at the outer piperazine nitrogen which is combined with an augmented lipophilicity resulted in an increase in the degree of protein binding. So it can be concluded that the *N*1-phenyl ring as well as the piperazine ring take part in the interaction with the plasma protein. Both substituents can be used to regulate the extent of protein binding in new gyrase inhibitors.

Keywords: Plasma protein binding; *N*1-phenyl substituted quinolones; QSAR

1. Introduction

Drugs usually interact with macromolecules in body fluids and tissue. The portion of a drug bound to plasma protein is inactive in terms of receptor/enzyme activity, metabolism and elimination (Fichtl et al., 1991). Furthermore, drugs with a high affinity to plasma proteins are able to compete with other drugs for the protein binding site. For example, the antirheumatic phenylbutazone replaces phenprocoumon and nalidixic acid replaces warfarin; both replacements result in a strong increase in the anticoagulant concentration and, in line with this, an enhancement of haemorrhagic diathesis. In addition, drugs characterized by a high affinity to plasma proteins can also replace endogenous substances, e.g. bilirubin (Derendorf and Garrett, 1987). Taken together, the extent of protein binding of a drug may influence pharmacokinetic parameters, i.e. the clearance and the apparent volume of distribu-

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tion, and pharmacodynamic processes (Proost et al., 1996).

Acidic drugs are known to bind more or less specifically to albumin at least at two binding sites (Oberdisse, 1997). In order to characterize this binding processes, structurally diverse compounds are usually studied by replacement studies or by comparison of their extent of protein binding or affinity to the plasma protein (Olson and Christ, 1996). Recently, we reported on the protein binding behaviour of 19 gyrase inhibitors in clinical practice (Zlotos et al., 1998). Within this structurally heterogenous series of compounds, rough structure–activity relationships (SAR) could be derived only: especially the western part of the molecule, i.e. the piperazine ring and its neighbourhood, was found to be crucial for protein binding. In contrast, the contribution of *N*1-substituents and neighbouring groups to the protein binding could not clearly be extracted from this series of compounds.

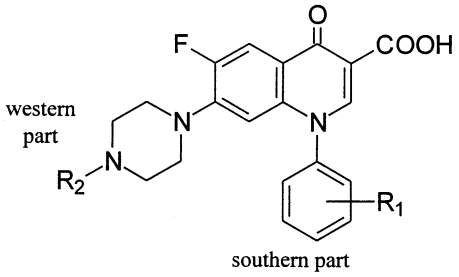
Thus, the purpose of this study was to gain more insight in the molecular mode of protein binding using a congeneric series of potent quinolone-like gyrase inhibitors, synthesized and microbiologically characterized in our group (Jürgens et al., 1996). Two structural variations were taken into account to derive SARs. Firstly, the benzene ring at the nitrogen *N*1 (see Fig. 1) was substituted by hydroxyl, nitro, cyano, fluoro, methyl, trifluoromethyl and methoxy groups in *ortho*, *meta* and *para* positions, respectively. Secondly, with the substituent at the outer nitrogen of the piperazine ring (*N*4′) having been found to strongly influence the extent of protein binding (Zlotos et al., 1998), it was aimed to lengthen the alkyl chain on *N*4′ step-by-step. For this purpose, *N*4′-alkyl-substituted derivatives were synthesized.

In order to find out the contribution of each substituent to the protein binding, the remaining part of the quinolone molecule was kept constant in either case. For sake of comparison, the already known, structurally related drugs temafloxacin and difloxacin as well as ciprofloxacin, *N*-methylciprofloxacin and enrofloxacin (Fig. 2) were taken into consideration for SAR. The technique of ultrafiltration was chosen to determine the extent of protein binding because this method has already proven to be worthwhile in case of gyrase inhibitors (Zlotos et al., 1998).

2. Material and methods

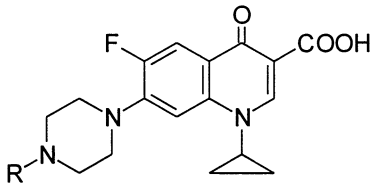
2.1. Materials

Melting points were determined with a Dr Tottoli melting point apparatus (Büchi) and were not corrected. ^{1}H and ^{13}C NMR spectra were recorded on a Varian EM 360 A (^{1}H 60 MHz), and Varian XL 300 (^{1}H, 299.956 MHz; ^{13}C, 75 MHz). Abbreviations for data quoted are: d, doublet; t, triplet; q, quintet; m, multiplet. IR spectra were obtained using a Perkin-Elmer 298 infrared

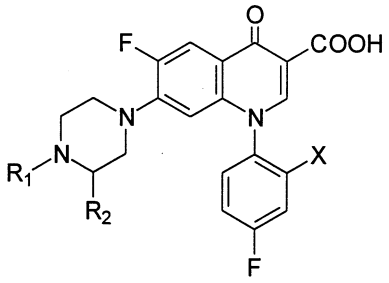


1	**R_1:**	-H	**R_2:**	-H
2a - 2c		o-, m-, p-F		-H
3a - 3c		o-, m-, p-CF_3		-H
4a - 4c		o-, m-, p-CH_3		-H
5a - 5c		o-, m-, p-OCH_3		-H
6b - 6c		o-, m-, p-CN		-H
6b - 6c		m-, p-NO_2		-H
7b - 7c		m-, p-OH		-H
5ba		m-OCH_3		-CH_3
5ca		p-OCH_3		-CH_3
5cb		p-OCH_3		-C_2H_5
5cc		p-OCH_3		n-C_3H_7
5cd		p-OCH_3		n-C_5H_{11}

Fig. 1. Structural formula of the quinolone compounds studied.



R=H ciprofloxacin
R=CH_3 N-methylciprofloxacin
R=C_2H_5 enrofloxacin



R_1=CH_3, R_2=X=H difloxacin
R_1=H, R_2=CH_3, X=F temafloxacin

Fig. 2. Structural formula of commercial available gyrase inhibitors discussed.

spectrometer (Bodensee, Germany). UV/Vis spectra were recorded on a Hewlett-Packard HP 8452A diode array spectrometer. Mass spectra were measured on a MS 50 (Kratos). TLC was 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). The chemicals were purchased from Aldrich (Milwaukee, WI). Dry solvents were used throughout. The 7-piperazinyl-6-fluoro-1,4-dihydro-1-phenyl-4-oxo-quinoline-3-carbonic acids of varying substitution on the phenyl rings **1**–**8** were taken from Jürgens et al. (1996).

2.2. Synthesis of 7-(4′-alkylpiperazinyl)-6-fluoro-1,4-dihydro-1-(m/p-methoxyphenyl)-4-oxo-quinoline-3-carbonic acids ***5ba***, ***5ca***, ***5cb***, ***5cc***

7-Chloro-6-fluoro-1,4-dihydro-1-(*m*/*p*-methoxyphenyl)-4-oxo-quinoline-3-carbonic acid (Jürgens et al., 1996) was dissolved in DMSO (75–100 ml), mixed with a five-fold excess of methyl-, ethyl- and propylpiperazine, respectively, and heated for 2 h at 140°C. The solvent was removed by distillation, the remaining oil suspended in water, heated at 90°C for 1 h and cooled down. The so obtained crystals were washed several times with H_2O and recrystallized from ethanol by means of activated charcoal.

The *N*4′-pentylpiperazine derivative **5cd** was obtained using a procedure reported by Merino et al. (1995): 1 mmol of 7-piperazinyl-6-fluoro-1,4-dihydro-1-(*p*-methoxyphenyl)

× -4-oxo-quinoline-3-carbonic acids was dissolved

in DMF and mixed with 1.5 mmol of 1-iodopentane and 150 mg triethylamine. The mixture was heated for 3 h at 120°C under N_2 atmosphere and afterwards evaporated to dryness. The residue was washed several times with diethylether and recrystallized from ethanol.

The analytical and spectroscopical data are in agreement with the structure assigned.

2.3. Determination of the water–octanol partition coefficient

The determination of the water–octanol partition coefficient log *D* was performed according to Zlotos et al. (1998).

2.4. Determination of the extent of plasma protein binding

The ultrafiltration was performed with the micropartition system MPS-1 Amicon (Lexington, MA, No. 4104) equipped with YMT-membranes (cut-off choice, 30000), and a Sorvall centrifuge RC-5B with a SM-24 rotor. The HPLC system consists of a Kontron Instruments HPLC Pump 420, a Rheodyne injection valve, a RP18 column (Spherisorb ODS-2 endcapped, 5 μm, 250 × 4.6 mm, Grom, Herrenberg, Germany), a UV diode array detector (Perkin-Elmer LC-480 Autoscan) and a software system (LC-DESplus, LabControl GmbH); the column temperature was adapted to 37°C using a Haake Thermostat D1. The pH-

value was determined using 3560 Digital pH-meter (Beckman, München, Germany).

Commercially available acetonitrile of HPLC grade and twice-distilled methanol was used throughout. All other reagents were commercially available and of analytical grade. The plasma was a grateful gift from the Institut für Exp. Hämatologie und Transfusionsmedizin, Blutbank, Universität Bonn, Germany.

2.4.1. Binding experiment

A stock solution of the gyrase inhibitors was prepared by dissolution of 80 μmol of a corresponding drug in 0.1 ml 1 M NaOH and dilution to 2 ml with water. Aqueous solutions of different concentrations of the drugs (0.2–2.0 μmol/ml) were prepared using the stock solution and added to a constant volume of human plasma (v/v, 1:19) to obtain a drug concentration ranging from 0.01 to 0.1 μmol/ml. The mixture was incubated for 30 min at 37°C. An aliquot of 1 ml was ultrafiltrated using a micropartition system and then centrifuged at 2000 × *g* for 20 min at 10°C. The total volume collected as filtrate was 25% of the starting volume. Since the adsorption of the drug to the membrane was less than 3%, no corrections were made.

The concentration of the free gyrase inhibitors in the ultrafiltrate was determined by HPLC using an internal standard method.

HPLC conditions (see Table 1). The mobile phase was composed of varying amounts of acetonitrile and buffer (0.1 mol citric acid, 0.02 mol NH_4ClO_4). To each mixture 0.005 mol TBAS was added, and pH 2.5 adjusted using 1 M NaOH. A Spherisorb ODS-2 endcapped solid phase was applied in each case. The flow rate was 1.2 ml/min for **5cc** and 1.0 ml/min for the other compounds.

UV data for each compound were taken from Jürgens (1994). The concentration of the analyte was calculated from the absorbance at the λ_{max} of the drug. The calibration solutions were prepared using the ultrafiltrate of plasma and different concentrations (in a range of 0.005–0.1 μmol/ml) of gyrase inhibitors. The quality of calibration in the whole range was checked by three SQC (spiked quality control) concentrations.

The concentration of the bound gyrase inhibitor was calculated from the difference of the concentration of the free drug measured in the ultrafiltrate and the total concentration in plasma used. The analysis of data was performed by means of Excel 5.0 (Microsoft Corporation, Redmond, WA) and Inplot software 4.0 (Graphpad, San Diego, CA). In order to perform QSAR analyses, the percentage of bound drug was logit-transformed: $\text{logit}(\%) = \log((100 - [\%]\text{bound})/[\%]\text{bound}) = \log([\%]\text{free}/\log[\%]\text{bound})$. The QSAR analysis was performed using the program package BILIN, developed by H. Kubinyi, BASF 1997 (Ludwigshafen, Germany).

3. Results

The method of ultrafiltration by means of the micropartition system MPS-1 was chosen because the protocol had been optimized in a previous study with commercially available gyrase inhibitors (Zlotos et al., 1998). Herein, the already optimized conditions concerning pH range and amount of ultrafiltrate have been applied. The mobile phase of the HPLC system was adapted for each compound. The composition is displayed in Table 1. The concentration range of the quinolones employed in this study was reduced to 0.01–0.1 μmol/ml in comparison with the previous study, because the differences which were found over the larger range were almost negligible. Additionally, it was checked for each compound and each concentration whether they adsorb to the ultrafiltration membrane. Whereas the recovery rate of the compounds with an unsubstituted piperazine ring (**1**–**8c**) and *N*-methyl- and *N*-ethyl-substituted piperazine (**5ba**, **5ca** and **5cb**) were found to be within the accuracy of the method, the *N*-propyl- and *N*-pentyl-substituted derivatives **5cc** and **5cd** adsorbed substantially to the membrane (**5cc** about 15% and **5cd** 30%). In the case of **5cc**, the protein binding could be corrected and led to a reproducible value. In the case of **5cd**, it was impossible to obtain a valid extent of protein binding. Thus, **5cd** was not considered in the subsequent QSAR analysis.

Table 1
HPLC conditions and protein binding data of quinolone derivatives studied (number of experiments at each concentration = 3)

Quinolones	HPLC conditions					Percentage and standard deviation of bound drug		
	λ_{max} (nm)	Internal standard	Retention time		Mobile phase buffer/acetonitrile v/v (%)	0.01 μmol/ml	0.05 μmol/ml	0.1 μmol/ml
			Analytical (min)	Int. standard (min)				
1 (-H)	280	Fleroxacin	10.96	6.31	82:18	41 ± 2	40 ± 2	37 ± 4
2a (*o*-F)	281	Fleroxacin	7.54	4.46	80:20	33 ± 3	31 ± 5	30 ± 4
2b (*m*-F)	281	Fleroxacin	8.41	4.71	80:20	53 ± 3	49 ± 4	50 ± 2
2c (*p*-F)	280	Fleroxacin	9.12	4.88	80:20	32 ± 3	30 ± 5	30 ± 3
3a (*o*-CF_3)	282	**1**	10.55	4.62	75:25	39 ± 4	38 ± 5	38 ± 2
3b (*m*-CF_3)	282	**1**	10.20	4.57	75:25	66 ± 2	62 ± 2	59 ± 1
3c (*p*-CF_3)	281	**1**	9.94	4.65	75:25	40 ± 7	40 ± 2	45 ± 2
4a (*o*-CH_3)	282	Fleroxacin	8.75	4.31	78:22	36 ± 4	38 ± 3	38 ± 1
4b (*m*-CH_3)	280	Fleroxacin	10.56	4.36	78:22	69 ± 4	61 ± 3	65 ± 2
4c (*p*-CH_3)	281	Fleroxacin	8.74	4.39	78:22	47 ± 3	41 ± 4	39 ± 1
5a (*o*-OCH_3)	280	Fleroxacin	10.85	4.67	78:22	30 ± 4	29 ± 5	29 ± 4
5b (*m*-OCH_3)	281	Fleroxacin	9.10	4.44	78:22	37 ± 9	34 ± 3	34 ± 2
5c (*p*-OCH_3)	281	Fleroxacin	8.38	4.35	78:22	33 ± 4	30 ± 1	30 ± 3
7b (*m*-NO_2)	279	Fleroxacin	9.21	4.43	78:22	69 ± 4	—	63 ± 1
7c (*p*-NO_2)	284	Fleroxacin	8.15	4.19	78:22	29 ± 7	31 ± 3	29 ± 5
8b (*m*-OH)	280	diflo xacin	6.66	9.73	80:20	34 ± 4	37 ± 2	34 ± 2
8c (*p*-OH)	280	diflo xacin	6.29	9.93	80:20	35 ± 2	38 ± 5	33 ± 7
5ba (*m*-OCH_3, *N*-CH_3)	280	Fleroxacin	8.54	4.03	78:22	51 ± 2	55 ± 1	55 ± 3
5ca (*p*-OCH_3, *N*-CH_3)	281	Fleroxacin	8.95	4.09	78:22	43 ± 2	43 ± 7	44 ± 3
5cb (*p*-OCH_3, *N*-C_2H_5)	281	Fleroxacin	11.54	4.16	78:22	51 ± 1	52 ± 2	51 ± 3
5cc (*p*-OCH_3, *N*-C_3H_7)	281	**1**	14.39	5.07	77:23	54 ± 5	55 ± 2	52 ± 10

With the exception of the cyanophenyl-substituted compounds (**6a**–**6c**), which hydrolyzed during the course of the experiment, the extent of protein binding could be determined with rather good precision. For all compounds, the extent of protein binding is displayed in Table 1 and visualized in Fig. 3. The percentage ranged from 25 to 70.

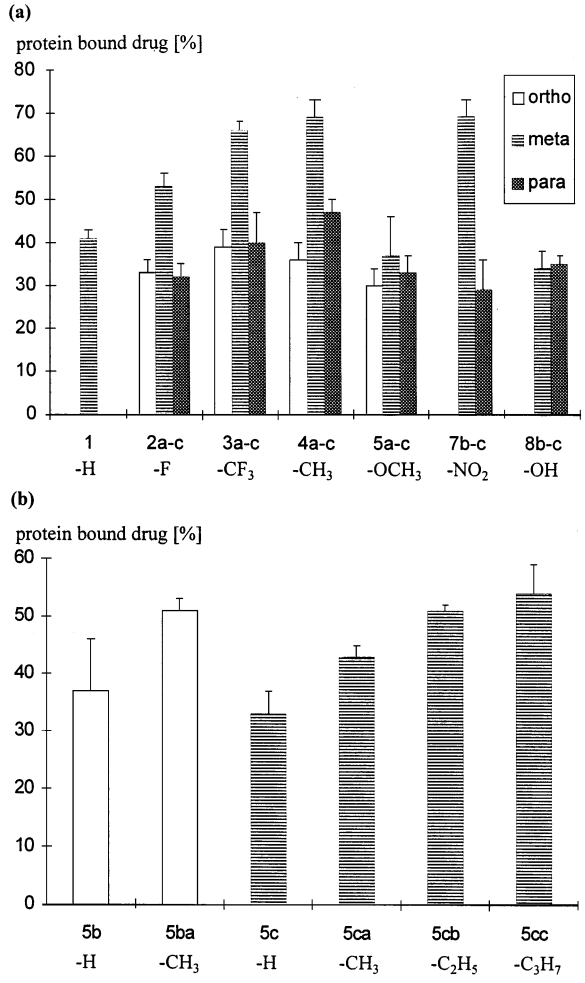


Fig. 3. Extent of protein binding (a) for compounds **1**–**8c** with varying substituents on the *N*1 phenyl ring and (b) for compounds with increasing alkyl chains on the piperazine ring (concentration 0.01 μM).

4. Discussion

The study presented was carried out to derive SARs. A rough comparison within the series of varying *N*1-phenyl substitution clearly shows that the locus of substitution determines the extent of protein binding in a higher degree than the type of substitution: none 39%; *ortho* 29–38% (mean 34%); *meta* 35–66% (mean 52%); *para* 30–37% (mean 35%). In case of *ortho* and *para* substitution the dissemination of the extent of protein binding is less than 10% indicating an almost negligible influence of the substituents on the affinity to the plasma protein. This is in accordance with the degree of protein binding of difloxacin and temafloxacin characterized by a 2,4-difluorosubstitution on the *N*1-phenyl ring, which we have already reported to amount to 34 and 25%, respectively (Zlotos et al., 1998). In contrast, the range of protein binding for *m*-phenyl substituted compounds is some 30% indicating that substituents in this position can considerably enhance the affinity to the plasma protein. Especially substituents with the negative inductive effect, such as fluoro, trifluoromethyl and nitro groups (**2b**, **3b** and **7b**), respectively, induce a high degree of protein binding. In addition, the more lipophilic methylbenzene-substituted compound **4b** binds strongly to the plasma protein, too. Thus, it was checked whether the extent of protein binding can quantitatively be described by lipophilic and/or electronic parameters, such as π and σ values (see Table 2), respectively. Therefore, the percentage of protein binding was transformed into logit values. The lipophilicity was characterized by $\log D$ and π (Kubinyi, 1993), the electronic properties by σ (Kubinyi, 1993) and pK_a values, the steric bulk by STERIMOL parameters *L*, *B*1 and *B*5 (Kubinyi, 1993) (see Table 2). The multiple regression analysis including all compounds resulted in one correlation which is rather weak:

$$\text{logit}(\%) = -0.66(\pm 0.26)B1_m + 0.92(\pm 0.31)$$
$$n = 17;\ r^2 = 0.67;\ s = 0.15;\ F = 31 \qquad (1)$$

with n = number of compounds, r^2 = regression coefficient, s = standard deviation and F = number of explained to unexplained variance. Since

Table 2
Physicochemical parameters of quinolone derivatives studied

Compound	logit (%)	π	Log D	pK_a	σ	MR	L	$B1$	$B5$
1	0.158	0.00	−0.987	5.81	0.00	0.103	2.06	1.00	1.00
2a	0.308	0.00	−1.050	5.62	0.54	0.092	2.65	1.35	1.35
2b	−0.052	0.22	−1.074	5.71	0.34	0.092	2.65	1.35	1.35
2c	0.327	0.15	−0.840	5.72	0.06	0.092	2.65	1.35	1.35
3a	0.194	1.04	−0.541	5.32	0.40	0.502	3.30	1.98	2.61
3b	−0.288	1.10	−0.463	5.59	0.43	0.502	3.30	1.98	2.61
3c	0.176	1.04	−0.449	5.55	0.54	0.502	3.30	1.98	2.61
4a	0.250	0.84	−0.515	5.77	−0.01	0.565	2.87	1.52	2.04
4b	−0.347	0.52	−0.374	5.87	−0.07	0.565	2.87	1.52	2.04
4c	0.052	0.60	−0.357	5.84	−0.17	0.565	2.87	1.52	2.04
5a	0.368	−0.33	−0.663	5.79	0.30	0.787	3.98	1.35	3.07
5b	0.231	0.12	−0.754	5.74	0.12	0.787	3.98	1.35	3.07
5c	0.308	−0.03	−0.672	5.75	−0.27	0.787	3.98	1.35	3.07
7b	−0.347	0.11	−1.487	5.27	0.71	0.736	3.44	1.70	2.44
7c	0.389	0.22	−0.986	5.49	0.78	0.763	3.44	1.70	2.44
8b	0.288	−0.50	−0.969	5.71	0.12	0.285	2.74	1.35	1.93
8c	0.269	−0.61	−0.742	5.70	−0.37	0.285	2.74	1.35	1.93

the degree of protein binding was found to run through an optimum of lipophilicity for some xanthone compounds (Hersey et al., 1991), it was checked whether such an optimum could be found for the quinolones studied here:

$$\text{logit}(\%) = -1.73(\pm 0.80)\log D^2 - 2.94(\pm 1.38)\log D + 0.95(\pm 0.55)$$
$$n = 17;\ r^2 = 0.61;\ s = 0.21;\ F = 11;$$
$$\log D_{\text{optimum}} = -0.85 \pm 0.09 \qquad (2)$$

This weak correlation is also unable to describe the protein binding properly. Fig. 4 clearly shows that especially the *m*-substituted compounds **2b**, **3b** and **4b** do not meet the parabolic function. Thus, it was decided to evaluate a QSAR analysis using the *m*-substituted compounds only, which additionally showed the largest differences in the extent of protein binding. Taking **1**, **2b**, **3b**, **4b**, **5b**, **7b**, and **8b** into consideration, two non-significant correlations with σ and π could be derived only. Interestingly, in the case of the correlation with σ, the residue of the *m*-CH_3-substituted compound **4b** was found to be too large and, in the case of the correlation with π, the residue of the *m*-NO_2-substituted compound **7b** was too large. Omitting those compounds from either correlation, the following equations can be derived:

$$\text{logit}(\%) = -0.95(\pm 0.53)\sigma + 0.27(\pm 0.20)$$
$$n = 6;\ r^2 = 0.87;\ s = 0.11;\ F = 25 \quad \text{without } \mathbf{4b} \qquad (3)$$

$$\text{logit}(\%) = -0.43(\pm 0.36)\pi + 0.10(\pm 0.20)$$
$$n = 6;\ r^2 = 0.74;\ s = 0.16;\ F = 11; \quad \text{without } \mathbf{7b} \qquad (4)$$

Since no reason for the outliers can be given from the data examined here, the significance of these

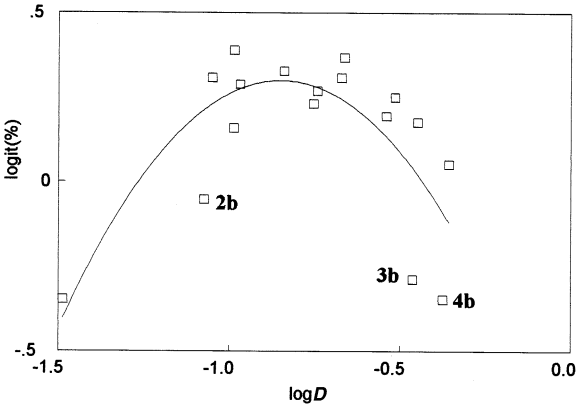


Fig. 4. Correlation between logit (%) and log D of **1**–**8c**.

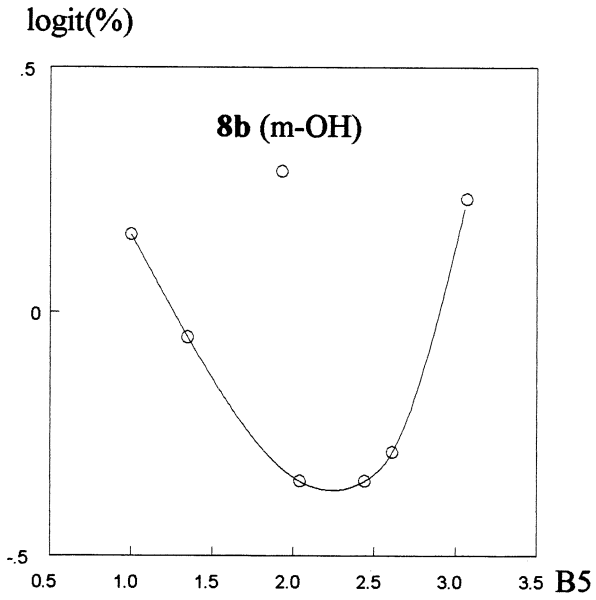


Fig. 5. Correlation between logit (%) and $B5$ of the *meta*-substituted compounds.

correlations is limited. However, a highly significant bilinear correlation can be found with the steric bulk characterized by $B5$:

$$\begin{aligned}\text{logit}(\%) = &-0.64(\pm 0.03)B5 \\ &-4.59(\pm 0.22)\log(\beta B5 + 1) \\ &+0.78(\pm 0.55) \\ &n = 6;\ r^2 = 1.00;\ s = 0.01;\ F = 2727; \\ &B5_{\text{optimum}} = 2.05 \\ &\log \beta = -3.05 \end{aligned} \quad (5)$$

As can be seen from Fig. 5, the extent of protein binding is small in the case of small and large *m*-substituents. The width of the *m*-substituent result in an optimum: in this case the extent of protein binding achieves the maximum. The correlation exhibited one outlier, the *m*-hydroxy-substituted compound **8b**. Since the acidic, phenolic OH-group will be partially deprotonated in physiological medium, the $B5$ value for OH does not correctly mirror the width of the substituent. Thus, it is allowed to omit the outlier.

Taking this part of the QSAR analysis together, it can be stated that the phenyl substituent definitely takes part in the interaction with the plasma protein. Especially the steric volume of the substituents in the *m*-position of the phenyl ring determines the extent of protein binding. With small or large substituents the protein binding can be reduced to a minimum. The contributions of electronic and lipophilic properties could not be extracted from the data set, because the data set is too small and, additionally, the plasma protein is characterized by a multitude of different proteins of varying structure. Thus, we see an average of interactions only.

In the second part of the study, the hydrogen at the outer piperazine nitrogen $N4'$ was replaced by an alkyl chain of increasing size for two substitution patterns: in case of a *m*-methoxyphenyl substituted compound the hydrogen was replaced by a methyl group, in case of the *p*-methoxysubstituted compound the hydrogen was replaced by methyl, ethyl, and propyl groups. In both series of compounds, the extent of protein binding is augmented with increasing length of the substituent, which is combined with increasing lipophilicity (see Table 1). The contribution of the alkane substituents at the piperazine to the protein binding can be quantified after logit transformation:

$$\begin{aligned}\text{logit}(\%) = &-0.13(\pm 0.09)L + 0.54(\pm 0.33) \\ &n = 4;\ r^2 = 0.94;\ s = 0.05;\ F = 37 \end{aligned} \quad (6)$$

$$\begin{aligned}\text{logit}(\%) = &-0.15(\pm 0.04)B5 + 0.45(\pm 0.11) \\ &n = 4;\ r^2 = 0.98;\ s = 0.02;\ F = 235 \end{aligned} \quad (7)$$

In addition, the longer the alkyl chain the higher the lipophilicity of the compounds. Thus, the following equation can be obtained:

$$\begin{aligned}\text{logit}(\%) = &-0.25(\pm 0.17)\pi + 0.28(\pm 0.17) \\ &n = 4;\ r^2 = 0.92;\ s = 0.05;\ F = 39 \end{aligned} \quad (8)$$

The equations clearly demonstrate that increasing size and lipophilicity of the alkyl substituent at the piperazine increase the degree of protein binding. This is in good agreement with results previously obtained for ciprofloxacin, and the *N*-methyl and *N*-ethyl compound (ciprofloxacin 23% ($\log D$ −1.1); *N*-methylciprofloxacin 29% ($\log D$ 0.17); enrofloxacin 35% ($\log D$ 0.27)) (Zlotos et al., 1998). Though it is impossible to deduce

from these data whether the protein binding is governed by size or lipophilicity it seems likely that the enlargement of the substituent will result in an optimum of the extent of protein binding. Since compounds with longer *N*4′ alkyl chains bind substantially to the ultrafiltration membrane, it is impossible to determine the maximum of protein binding.

However, the question is raised whether the extent of protein binding depends on the pK_a values of the piperazine nitrogen *N*4′. Even though the pK_a values of the substituted piperazine nitrogens were not determined (Jürgens et al., 1996), the comparison of corresponding derivatives (e.g. ciprofloxacin 8.74; *N*-methylciprofloxacin, 7.86; enrofloxacin, 7.68; Montero et al., 1997) and other quinolone derivatives (Takacs-Novak et al., 1990; Ross and Riley, 1992; Sörgel and Kinzig, 1993) revealed no correlation between the extent of protein binding and the pK_a values. This is in accordance with the findings in the series of gyrase inhibitors in clinical practice (Zlotos et al., 1998).

All findings are in good agreement with the extent of protein binding, which we have previously found for temafloxacin and difloxacin, which are characterized by a 2,4-difluoro- and 4-fluorophenyl substitution, and a hydrogen and methyl group at the piperazine ring, respectively (Zlotos et al., 1998). In both cases the *m*-position of the phenyl ring is not substituted. Thus, some 30% of protein binding should be expected; 25% were measured for temafloxacin which has a hydrogen at the piperazine. The extent of protein binding for difloxacin is higher (34%), because the piperazine ring is methylated.

5. Conclusion

From a congeneric series of antibacterial quinolone compounds with systematically varying substituents at the *N*1 benzene ring and the outer nitrogen of the piperazine in position 7, a consistent picture of structure–protein binding relationships could be derived: substituents in the *m*-position of the *N*1 benzene ring contribute significantly to the affinity of the compounds to the plasma protein, especially substituents with a defined width. In addition, the extent of protein binding can be augmented by alkyl groups at the outer nitrogen of the piperazine ring. This indicates, on the one hand, that these groups participate in the interaction with the protein and, on the other hand, that all compounds may bind to the same protein of the plasma, likely the albumin protein.

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References

Derendorf, H., Garrett, E.R., 1987. Pharmakokinetik, 1st ed. Wiss. Verlagsges., Stuttgart, 102 pp.

Fichtl, B., Nieciecki, A., Walter, K., 1991. Tissue binding versus plasma binding of drugs: General principles and pharmacokinetic consequences. Adv. Drug Res. 20, 118–166.

Hersey, A., Hyde, R.M., Livingstone, D.J., Rahr, E., 1991. A quantitative structure-activity relationship approach to the minimization of albumin binding. J. Pharm. Sci. 80, 333–337.

Jürgens, J., 1994. Thesis, Bonn, Germany.

Jürgens, J., Schedletsky, H., Heisig, P., Seydel, J.K., Wiedemann, B., Holzgrabe, U., 1996. Synthesis and biological activity of new *N1*-aryl substituted quinolone antibacterials. Arch. Pharm. Pharm. Med. Chem. 329, 179–190.

Kubinyi, H., 1993. Hansch Analysis and Related Approaches in QSAR. VCH, Weinheim.

Merino, V., Freixas, J., del Val Bermejo, M., Garrigues, T.M., Moreno, J., Pla-Delfina, J.M., 1995. Biophysical models as an approach to study passive adsorption in drug development: 6-fluoroquinolones. J. Pharm. Sci. 84, 777–782.

Montero, M.T., Freixas, J., Hernandez-Borrell, J., 1997. Expression of the partition coefficients of a homologous series of 6-fluoroquinolones. Int. J. Pharm. 149, 161–170.

Oberdisse, E., 1997. In: Oberdisse, E., Hackenthal, E., Kuschinsky, K. (Eds.), Pharmakologie und Toxikologie, 1st ed. Springer Verlag, Heidelberg, p. 15.

Olson, R.E., Christ, D.D., 1996. Plasma protein binding of drugs. Annu. Rep. Med. Chem. 31, 327–336.

Proost, J.H., Wierda, J.M.K.H., Meijer, D.K.F., 1996. An extended pharmacokinetic/pharmacodynamic model describing quantitatively the influence of plasma protein binding, tissue binding and receptor binding on the potency and time course of action of drugs. J. Pharmacokin. Biopharm. 24, 45–77.

Ross, D.L., Riley, C.M., 1992. Physicochemical properties of the fluoroquinolone antimicrobials. II acid ionization constants and their relationship to structure. Int. J. Pharm. 83, 267–272.

Sörgel, F., Kinzig, M., 1993. Pharmacokinetics of gyrase inhibitors, Part 1: Basic chemistry and gastrointestinal disposition. Am. J. Med. 94 (3A), 44–55.

Takacs-Novak, K., Noszal, B., Hermecz, I., Kereszturi, G., Podanyi, B., Szasz, G., 1990. Protonation equilibria of quinolone antibacterials. J. Pharm. Sci. 79, 1023–1028.

Zlotos, G., Bücker, A., Kinzig-Schippers, M., Sörgel, F., Holzgrabe, U., 1998. Plasma protein binding of gyrase inhibitors. J. Pharm. Sci. 87, 215–220.