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European Journal of Medicinal Chemistry 40 (2005) 361–369
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Original article

Benzenesulfonamide analogs of fluoroquinolones. Antibacterial activity and QSAR studies

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Received 27 May 2004; received in revised form 24 November 2004; accepted 29 November 2004

Available online 24 February 2005

Abstract

The structure–activity relationships (SAR) of new antibacterial benzenesulfonamidefluoroquinolones (BSFQs), coming from derivatization of N^4-piperazinyl of ciprofloxacin (CIP) were studied. The behavior of the new BSFQ series was similar to the previously norfloxacin (NOR) analogs reported, making possible a quantitative structure–activity relationships (QSAR) analysis of the complete set of BSFQs. The presence of the benzenesulfonylamido (BS) groups shifted the activity of classic antimicrobial fluoroquinolones from being more active against Gram-negative to Gram-positive strains. QSAR studies through Hansch analysis showed a linear correlation of the activity with electronic and steric parameters. Small electron-donor groups would increase the in vitro activity against Gram-positive bacteria. Hydrophobic properties played a minor role when activity is measured as minimum inhibitory concentration (MIC). QSAR analysis also reinforces previous biological findings about the presence of new interactions with target topoisomerases.

Keywords: Antimicrobial; Fluoroquinolones; Ciprofloxacin; QSAR; SAR; Lipophilicity

1. Introduction

Ciprofloxacin (CIP) and norfloxacin (NOR) are fluoroquinolones antimicrobial agents (AMFQs) with highly chemotherapeutical relevance. As of yet, AMFQs are the only direct inhibitors of DNA synthesis and exert their action by inhibiting DNA gyrase and topoisomerase IV, both type II topoisomerases that human cells lack [1,2]. Genetic studies based on fluoroquinolone-resistant mutants and studies on purified topoisomerases indicated that the primary drug target enzyme often differs between Gram-positive and Gram-negative bacteria. Most AMFQs would have a preferential affinity for topoisomerase IV in Gram-positive bacteria such as *Staphylococcus aureus*, but DNA gyrase would be the primary drug target in Gram-negative bacteria such as *Escherichia coli* [2–4]. Exceptions to this rule have been reported, and it was identified that AMFQs appeared to act through both topoisomerase targets [2,5]. These "dual targeting" compounds may be a preferred objective in design new AMFQs since they would be less prompted to develop bacterial resistance.

Benzenesulfonylamidofluoroquinolones (BSFQs) are a new class of AMFQs reported previously by Manzo et al. [6,7]. Some of those BSFQs have exhibited high in vitro activity against *S. aureus* ATCC 29213 [8,9] and also against other Gram-positive clinical strains [10]. The new analogs has, as unique structural difference with CIP or NOR, the presence of *p*-substituted benzenesulfonylamido (BS) groups bound to piperazine. The new compounds would exert their biological action through a quinolone-like mechanism of action [8]. Although new BSFQs could be considered hybrid drugs from a structural point of view since they carry a sulfa and a quinolone portion, a sulfa-like mode of action would make little or no contribution to the in vitro activity of BSFQs [8,9]. It was demonstrated by structure–activity relationships (SAR) and microbiological studies that *p*-NH_2 substituent is not essential for keeping the anti-staphylococci in vitro activity in the BSFQs series. Instead, a *p*-NH_2-$C_6H_4SO_2$-Y is the pharmocophore of the sulfa drugs with antibacterial activity [11,12]. Moreover, microbiological studies such as PABA competition, TMP interaction and inhibitory activity of **1** on

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doi:10.1016/j.ejmech.2004.11.008

DHPS from *S. aureus* have demonstrated that the sulfa portion is not contributing to the activity because of its mechanism of action. On the other hand, quinolone-like mode of action was confirmed to be similar to other AMFQs in its kinetics of penetration of the bacterial envelope and its ability to inhibit the DNA gyrase catalyzed reaction [8]. It was also reported that BSFQs have displayed a more favorable kinetics of access to the bacterial cell in *S. aureus* ATCC 29213 [8]. Studies on *Streptococcus pneumoniae* and *S. aureus* have identified the BSFQs as "dual targeting" agents [13,14]. It was reported that the new analogs with a sulfa moiety on piperazinyl group inhibit *E. coli* DNA gyrase in similar way as CIP [8].

According to the proposed mechanisms of action of AMFQs, substituents at 7-position of quinolone ring would be involved in the interaction with the enzyme through electrostatic forces [1,15]. SAR of antibacterial fluoroquinolones have been extensively investigated and the substituent at the C-7 position has a great impact modulating potency, spectrum, biopharmaceutics and pharmacokinetics [1]. In our previous report, we have informed the quantitative structure–activity relationships (QSAR) of *p*-substituted-benzenesulfonamide derivatives of NOR [9]. The stimulating results obtained prompted us to develop a new series of derivatives using a different scaffold, the CIP, to compare the model obtained for NOR derivatives and to evaluate the possibility that the two series were alike. In order to get consistent results we design a series of CIP benzenesulfonamide derivatives, using the same approach as in the previous work.

In the present report, we describe a QSAR analysis for the complete series of BSFQs (NOR and CIP derivatives) as well. Hansch analysis correlates biological activity values with electronic, steric, and hydrophobic influences of substituents through linear multiple regression analysis. Therefore, the structural homogeneity of the present series has allowed a classical Hansch aproach [16,17]. The changes in lipophilic, electronic or steric characteristics induced by substituents were correlated with the antibacterial activity using appropriate descriptors.

2. Chemistry

Analogs of CIP, **1**–**10**, used in the present study are listed in Table 1 along with yields, melting point (m.p.) and in vitro microbiological activities. Compounds **1**–**10** were prepared as described previously [6,18].

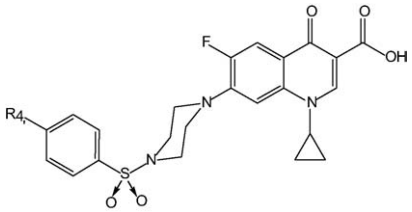
3. Biological activity

In vitro assessments of BSFQs antibacterial activities against *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were performed by using standard methodologies [19]. The lowest concentration of drug that completely inhibited visible bacteria growth after 16–20 h at 35 °C was considered to be the minimum inhibitory concentration (MIC). These values are reported in Table 1 as –log MIC (when expressed in μg/ml) and –log MIC_M, being MIC_M the minimum molar inhibitory concentration.

4. QSAR analysis

The selection of parameters is the first step in any QSAR study. If the association between the parameter(s) selected

Table 1
Structure and biological activity of BSFQs **1**–**10**



Coumpound	R4′	Yield (%)	m.p. (°C)	*S. aureus* ATCC 29213		*E. coli* ATCC 25922	
				$\log \frac{1}{MIC}$	$\log \frac{1}{MIC_M}$	$\log \frac{1}{MIC}$	$\log \frac{1}{MIC_M}$
1	NH_2	Ref. [13]	Ref. [13]	1.52	7.21	0.90	6.59
2	$NHCOCH_3$	Ref. [13]	Ref. [13]	0.30	6.02	0.30	6.02
3	$N(CH_3)COCH_3$	83	289	0.00	5.73	0.00	5.73
4	$NHCH_3$	61	274	1.52	7.22	0.30	6.00
5	$N(CH_3)_2$	50	284	0.60	6.31	–	–
6	H	50	306	1.22	6.90	–0.60	5.07
7	CH_3	47	282	0.60	6.29	0.60	6.29
8	OCH_3	45	232	n.d.	n.d.	n.d.	n.d.
9	Cl	46	240	n.d.	n.d.	n.d.	n.d.
10	NO_2	45	274	1.52	7.24	0.60	6.31
CIP				0.60	6.12	2.12	10.64

and activity is strong, then activity predictions and mechanistic interpretation will be possible. In the present study, parameters that were considered relevant to the activity of BSFQs series (electronic, hydrophilic and steric) were selected and considered as consistent [16,17].

4.1. Hydrophobic properties and parameters

The hydrophobic properties of new BSFQs were investigated and depicted in the correlation analysis by parameters coming from the calculated partition coefficient (*C*log*P* and CLOGP [20]), π of Hansch and experimental chromatographic parameters (logk' and R_{Mw}) [16,17]. The *C*log*P* was obtained by Eq. 1 as previously described for BSFQs derivatives of NOR [9,21]:

$$C\log P = \log P_{CIP} - f_H + \log P_{Sulfa} - f_{NH_2} + \Delta F_\sigma \quad (1)$$

where $\log P_{CIP} = 0.447$ [22]; f_H is the fragment value for hydrogen; $\log P_{Sulfa}$ is an experimental value for corresponding *p*-substituted benzene sulfanilamide; f_{NH_2} is the fragment value for NH_2 in SO_2NH_2 and $\Delta F_\sigma = F_\sigma(SO_2NR_2, R) - F_\sigma(SO_2NR_2, R)$. The values of *f* and *F* were taken from literature [23].

Reversed-phase chromatographic parameters logk_w or R_{Mw} were determined by RP-HPLC and RP-TLC methods. The validity of these parameters as a measure of lipophilicity has been well established and their reliability as lipophilicity indices for QSAR analysis has been previously reported [17,24–26]. The chromatographic indices logk'_w or R_{Mw} were obtained by a polycratic approach [25,26]. There is a limited range of ϕ concentration where the linear relationship expressed by those equations is valid. Capacity factors (k') were determined at six to seven different concentration of methanol in water (50%, 55%, 60%, 65%, 70%, 75% and 80%) and expressed as their logarithms. Lower methanol levels led to so great retention times that can not be measured. The extrapolated logk_w values are used in order to suppress the effect of the organic modifier and to obtain lipophilicity values independent of the eluent conditions. The R_M values were calculated by means of the Eq. 2.

$$R_M = \log\left[\left(\frac{1}{R_f}\right) - 1\right] \quad (2)$$

logk' and R_M were plotted against the percentage of organic modifiers. Eqs. 3 and 4 for RP-HPLC and RP-TLC, respectively, were used to determine the values of logk_w and R_{Mw} by extrapolating to 100% water [24–26].

$$\log k' = a_{kw}\phi + \log k_w \quad (3)$$

$$R_M = a_{RM}\phi + R_{Mw} \quad (4)$$

4.2. Electronic parameters

The electronic distribution of the BS group in the BSFQs is modulated by R4′ substituent through inductive and resonance effects described, in the present study, by empirical (Linear Free Energy Relationships) and spectroscopic parameters. Both, empirical and experimental descriptors are extremely valuable in outlining the electronic influence of substituents [16,17]. Therefore, Hammet σ_p, σ^0, σ^+, σ^- and Taft's ℞ and parameters, coming from LFER, are included in QSAR studies when electronic properties should be analyzed [12,16,17]. Finally, IR or NMR data has been previously used in QSAR studies with great success [16c,27]. So, parameters such as logSO and and logδ_C were tested. logSO, being SO the ν_{asym} of S–O bond, were obtained from IR spectroscopy. logδ_C, where δ_C is the chemical shifts of the carbons of the phenyl ring, were obtained from ^{13}C NMR spectroscopic measurements.

4.3. Steric parameters

Steric substituent constants (E_s) derived form Taft's model, and Sterimol parameters (B_1, B_5 and L) were used in the present analyses. These parameters were taken from Ref. [28].

4.4. Indicator variables

To combine CIP and NOR series, an indicator variable, *I*, was used. For CIP derivatives $I = 1$, and for the NOR derivatives $I = 0$ [16].

4.5. Correlation analyses

A classical Hansch multivariate regression analysis using the least-square method was chosen to derive QSAR equations for the data set [16,17,23b]. The level of significance of each coefficient is judged by statistical procedures such as the Student's *t* and *F* tests [16a,29]. Statistic analysis was carried out using the program STAT 5.0. For each equation several indices of goodness of fit are given: the regression coefficient *r*, the standard deviation (S.D.), the measure of the level of statistical significance *F*, the predicted sum of square PRESS, the bootstrapping coefficient (BSr^2). The probability value of confidence *P* was also considered, although not informed [29].

To obtain suitable equations two factors were taken into account. First, a ratio of compounds to variables greater than 5, and second, an intercorrelation among the independent variables smaller than 0.6 [16a]. So, for the CIP series only correlation with one parameter was possible, but when it dealt with the whole series of BSFQs three parameters could be considered.

5. Results

Table 1 shows the structure and yield of the benzenesulfonyl derivatives of CIP **1**–**10** utilized in the present study along with their m.p. and antimicrobial in vitro activity.

As can be appreciated in Table 1, the antibacterial properties of compounds **1**–**10** were very similar to that of BSFQs

analogs of NOR previously reported [9]. The evaluated derivatives **1**, **4**, **6** and **10** were more active against Gram-positive strains than Gram-negative, following the trends of every BSFQs reported so far [7–9]. Also, when compared in vitro activity against *S. aureus* ATCC 29213, compound **1**, **4**, **6** and **10** had a greater inhibitory effect (lower MIC) than CIP. Instead, compounds **5** and **7** had similar MIC, and **2** and **3** were less active than the reference AMFQ. Compounds **1**, **4**, **6** and **10** had shown similar behavior against clinical isolates [10]. Derivatives **8** and **9** could not be evaluated due to solubility limitations.

5.1. Hydrophobicity

Table 2 shows hydrophobic properties of BSFQs **1**–**10**. *C*log*P* calculated through Eq. 1 and chromatographic parameters obtained after applied Eqs. 3 and 4 to experimental data.

The results obtained in the present study have demonstrated that CIP series of derivatives has followed the same chromatographic behavior as NOR series had [21]. It means:

1) Linear relationship between the R_M of BSFQs and acetone concentration, ϕ, in the mobile phase ranged between 65% and 85% for more lipophilic compounds, and between 50% and 75% for more hydrophilic ones. The linearity between logk' and methanol content was similar for HPLC determinations.
2) The more hydrophilic the compounds were the less sensitive to the variation of solvent polarity, and the opposite also holds true. This can be appreciated in Table 3, where to a smaller logk_w (or R_{Mw}) corresponds a less negative slope, a.
3) When trying to correlate slopes and intercepts of the TLC (Eq. 4) or HPLC equations (Eq. 3), a linear relationship appeared indicating that derivatives **1**–**10** behave as congeners from a chromatographic point of view.
4) logk_w values were more sensitive than R_{Mw} values. Thus, at the experimental condition that can be used for CIP series, a difference in logk_w units of 1.96 between the most lipophilic and the most hydrophilic compounds could be appreciated. Otherwise, the difference was only of 1.11 for R_{Mw} values (Table 2). This could be a reason to prefer logk_w as a lipophilic index for BSFQ series.
5) Although there was a somewhat higher logk_w as compared with R_{Mw}, and both are much higher than *C*log*P* (Table 2), the highly significant linear relationship between these parameters, and between Δlogk_w or ΔR_{Mw} with π, strengthens the applicability of R_{Mw} and logk_w as a reliable lipophilicity index [24].

Table 2
Hydrophilic parameters of BSFQs **1**–**10**

Entry	logk_w [a] (a_{kw})[a]	Δlogk_w [d]	R_{Mw} [b] (a_{RM})[b]	ΔR_{Mw} [e]	*C*log*P*[c]
1	3.03 (–0.051)	–1.02	2.25 (–0.032)	–0.35	–0.55[a]
2	3.61 (–0.053)	–0.44	2.35 (–0.034)	–0.25	–0.22
3	3.49 (–0.055)	–0.56	2.31 (–0.031)	–0.29	–0.40
4	4.03 (–0.059)	–0.02	2.79 (–0.039)	0.19	0.34
5	4.86 (–0.066)	0.81	3.10 (–0.041)	0.50	1.08
6	4.05 (–0.058)	0.00	2.60 (–0.035)	0.00	0.18
7	4.82 (–0.066)	0.77	3.23 (–0.043)	0.63	0.68
8	4.68 (–0.064)	0.63	3.33 (–0.041)	0.73	0.47
9	4.99 (–0.067)	0.94	3.36 (–0.044)	0.76	0.89
10	3.79 (–0.054)	–0.26	2.62 (–0.034)	0.02	–0.08
Δ	1.96		1.11		1.63

[a] From Eq. 3.
[b] From Eq. 4.
[c] From Eq. 1.
[d] Δlogk_w is the difference between logk_w of a substituted BSFQ and logk_w of **6**, the unsubstituted compound.
[e] ΔR_{Mw} is the difference between R_{Mw} of a substituted BSFQ and R_{Mw} of **6**.

Table 3
Relationships between experimental and calculated lipophilic descriptors for whole series of BSFQs (n = 10)

Eq. #		r	S.D.	F
5	$ClogP = 0.68(\pm0.06)\log k_w - 2.77(\pm0.25)$	0.94	0.16	126.76
6	$ClogP = -75.34(\pm10.11)a - 4.47(\pm0.60)$	0.87	0.42	54.55
7	$\pi = 1.05(\pm0.10)\log k'_w - 0.25(\pm0.12)I - 4.26(\pm0.41)$	0.92	0.25	50.16
8	$\pi = -106.14(\pm13.67)a - 0.12(\pm0.16)I - 6.42(\pm0.80)$	0.88	0.32	30.12
9	$ClogP = 1.11(\pm0.14)R_{Mw} - 3.07(\pm0.39)$	0.88	0.23	59.73
10	$ClogP = -75.34(\pm10.11)a_{R_{Mw}} - 4.47(\pm0.60)$	0.87	0.24	54.55
11	$\pi = 1.61(\pm0.19)R_{Mw} - 0.26(\pm0.14)I - 4.49(\pm0.51)$	0.90	0.30	35.44
12	$\pi = -138.84(\pm23.51)a_{R_{Mw}} - 0.26(\pm0.18)I - 5.22(\pm0.85)$	0.82	0.40	17.44
13	$\log k_w = 1.55(\pm0.10)R_{Mw} - 0.18(\pm0.28)$	0.96	0.17	233.86
14	$\pi = 1.02(\pm0.10)\Delta\log k_w - 0.32(\pm0.06)$	0.92	0.25	104.68
15	$\pi = 1.21(\pm0.23)\Delta R_{Mw} - 0.34(\pm0.10)$	0.78	0.42	27.82

Table 4
Spectroscopic descriptors of BSFQs

Entry	logSO[a]	log$\delta_{C1'}$[b]	log$\delta_{C2'}$[c]	log$\delta_{C3'}$[d]	log$\delta_{C4'}$[e]
1	3.0631	2.1283	2.1196	2.0553	2.1779
2	3.0643	2.1130	2.1075	2.0733	2.1476
3	3.0663	2.1335	2.1041	2.1089	2.1717
4	3.0630	2.1222	2.1094	2.1044	2.1875
5	3.0626	2.1123	2.1125	2.0793	2.1875
6	3.0674	2.1427	2.1051	2.1108	2.1248
7	3.0667	2.1201	2.1051	2.1136	2.1591
8	3.0678	2.1858	2.1096	2.1048	2.2121
9	3.0637	2.1271	2.1108	2.1137	2.1423
10	3.0626	2.1474	2.1126	2.0744	2.1858

[a] logSO: the logarithm of the SO symmetrical stretch frequency.
[b] log$\delta_{C1'}$: the logarithm of the C1′ chemical shift in ^{13}C-RMN.
[c] log$\delta_{C2'}$: the logarithm of the C2′ chemical shift in ^{13}C-RMN.
[d] log$\delta_{C3'}$: the logarithm of the C3′ chemical shift in ^{13}C-RMN.
[e] log$\delta_{C4'}$: the logarithm of the C4′ chemical shift in ^{13}C-RMN.

Since both NOR and CIP benzenesulfonyl derivatives have shown similar chromatographic behavior, it was considered worthwhile to explore the relationships between hydrophobic parameters on the whole series of BSFQs, i.e. by including NOR and CIP analogs. Table 3 shows the correlations obtained between hydrophobic parameters in the whole series of BSFQs.

A very good statistical significance of correlation has confirmed the anticipated theoretical agreement between both chromatographic parameters (Eq. 13). Table 3 also shows a high interrelation when $\Delta\log k_w$ or ΔR_{Mw} was compared with π (Eqs. 14 and 15). Furthermore, the correlation between chromatographic parameters and *C*log*P* was good (Eqs. 5 and 9). Eqs. 7 and 11 have confirmed a good relationship between π and each chromatographic index.

Data shown in Table 2 for the CIP derivatives **1**–**10** and those previously reported for the NOR series [21] allows us to establish the following ranking of lipophilicity from *C*log*P* and logk_w for the whole series of BSFQs:

1 < 3 < 2 < 10 < 6 < 4 < 8 < 7 < 5 < 9

The ranking taking in account R_{Mw} values is:

1 < 2 < 3 < 10 < 6 < 4 < 8 < 7 < 5 < 9

The only transposition observed could be due to the close values in the R_{Mw} and to a low discrimination power of the RP-TLC method for the BSFQ series.

Therefore, the results of this study have confirmed that is possible to describe the hydrophobicity of BSFQs using *C*log*P* or chromatographic indexes. Also, it could be possible to predict the lipophilicity of new BSFQs compounds by using Eq. 1.

5.2. Electronic descriptors

Table 4 shows the spectroscopic descriptors utilized as electronic parameters. The relationship among semiempirical parameters and the spectroscopic ones for compounds **1**–**10** validate the use of the last one as electronic descriptors not only for the CIP series but also for the whole BSFQs series. Eqs. 16–19 show the correlations found between the electronic parameters of NOR and CIP benzenesulfonyl analogs.

$$\log SO = 0.0046(\pm 0.0003)\mathfrak{R} + 3.0670(\pm 0.0002) \quad (16)$$

$r = 0.95$; S.D. = 0.00; $F = 180.22$; $n = 20$

$$\log SO = 0.0069(\pm 0.0007)\sigma_R^0 + 3.0670(\pm 0.0002) \quad (17)$$

$r = 0.92$; S.D. = 0.00; $F = 102.57$; $n = 20$

$$\log SO = 0.0030(\pm 0.0004)\sigma_p + 3.0657(\pm 0.0002) \quad (18)$$

$r = 0.86$; S.D. = 0.00; $F = 50.06$; $n = 20$

$$\log\delta_1 = 0.023(\pm 0.002)\sigma_p + 0.013(\pm 0.002)\ \mathrm{HA} + 2.129(\pm 0.001) \quad (19)$$

$r = 0.94$; S.D. = 0.00; $F = 65.85$; $n = 20$

From Eqs. 16–19, some considerations can be drawn about the influence of the R4′ substituents on the spectroscopic indexes of BSFQs:

1. The variation of ν_s(SO), within the BSFQs series follows that of the electronic changes (σ_p). So, electron-donor substituents decrease the value of ν_s, and the opposite holds for electron-acceptor ones.
2. The variation of the $\delta_{C\text{-}1'}$ (^{13}C NMR chemical shifts) shows that *p*-phenyl substituent especially influenced C-1′ of BSFQs which δ increases with the corresponding σ_p increasing.

5.3. QSAR

Due to the fact that only seven compounds, **1**, **6** and **9**, can be included in this analysis, equations with only one descriptor are accepted. Compounds **7** and **8** have precipitated during the bioassay and led to no trustable results. As in previous QSAR study for BSFQs analogs of NOR [9], compound **10** was excluded due to consideration as an outlier with the standardized residual bigger than 3-sigma [29]. Eqs. 20–22 show the best correlation for this series when one parameter was used and it was evident for the poor statistical significance that simple correlations could not describe appropriately the relationship between activity and physicochemical descriptors.

$$\log\frac{1}{MIC_M} = -0.37(\pm 0.14)L + 7.84(\pm 0.64) \quad (20)$$

$r = 0.69$; S.D. = 0.46; $F = 4.63$; $n = 16$

$$\log\frac{1}{MIC_M} = -3.18(\pm 1.18) + 6.94(\pm 0.22) \quad (21)$$

$r = 0.77$; S.D. = 0.41; $F = 7.21$; $n = 16$

$$\log\frac{1}{MIC_M} = -0.85(\pm 0.45)\sigma_p + 6.26(\pm 0.23) \quad (22)$$

$r = 0.65$; S.D. = 0.49; $F = 3.59$; $n = 16$

Since CIP and NOR analogs had shown similar REA and to go further in QSAR analysis a study including both series of BSFQs was performed. Therefore, the new study was carried out with 16 compounds (nine from NOR and seven from CIP series). Stepwise regression analyses with two or three independent variables were performed and using the indicator variable I when semiempirical parameters are considered ($I = 0$ for NOR and $I = 1$ for CIP derivatives). To avoid spurious relationships, we only have considered descriptors with a $r < 0.4$ in the intervariable correlation matrix (See Section 9). So, more than 50 equations were developed and analyzed. Among those with $r > 0.8$, Eqs. 23–27 have described the best relationships between structure and activity for the whole series of BSFQs.

$$\log \frac{1}{\mathrm{MIC_M}} = -1.36(\pm 0.14)\sigma_p - 1.02(\pm 0.13)B_1 + 0.50(\pm 0.10)I + 7.15(\pm 0.19) \quad (23)$$

$n = 16$; $r = 0.96$; S.D. = 0.20; $F = 46.48$; PRESS = 0.812; $\mathrm{BSr^2} = 0.921$

$$\log \frac{1}{\mathrm{MIC}_M} = -1.48(\pm 0.32)\text{ꟾ} - 1.15(\pm 0.12)B_1 + 0.58(\pm 0.17)I + 7.05(\pm 0.33) \quad (24)$$

$n = 16$; $r = 0.87$; S.D. = 0.34; $F = 12.64$; PRESS = 2.403; $\mathrm{BSr^2} = 0.761$

$$\log \frac{1}{\mathrm{MIC}_M} = -1.48(\pm 0.25)\sigma_p - 1.21(\pm 0.27)B_1 + 0.17(\pm 0.18)\log\ k_w + 6.26(\pm 0.27) \quad (25)$$

$n = 16$; $r = 0.88$; DE = 0.33; $F = 13.48$; PRESS = 2.486 ; $\mathrm{BSr^2} = 0.774$

$$\log \frac{1}{\mathrm{MIC}_M} = -1.48(\pm 0.25)\sigma_p - 1.20(\pm 0.27)B_1 + 0.19(\pm 0.22)C\log\ P + 7.62(\pm 0.40) \quad (26)$$

$n = 16$; $r = 0.88$; DE = 0.34; $F = 13.36$; PRESS = 2.562; $\mathrm{BSr^2} = 0.767$

$$\log \frac{1}{\mathrm{MIC}_M} = -1.44(\pm 0.25)\sigma_p - 1.14(\pm 0.26)B_1 + 0.18(\pm 0.31)R_{\mathrm{Mw}} + 7.04(\pm 0.69) \quad (27)$$

$n = 16$; $r = 0.87$; DE = 0.34; $F = 12.86$; PRESS = 2.524; $\mathrm{BSr^2} = 0.776$

$$\log \frac{1}{\mathrm{MIC}_M} = -0.81(\pm 0.31)\sigma_p - 0.17(\pm 0.18)\pi + 0.52(\pm 0.24)I + 5.69(\pm 0.18) \quad (28)$$

$n = 16$; $r = 0.74$; S.D. = 0.47; $F = 4.71$

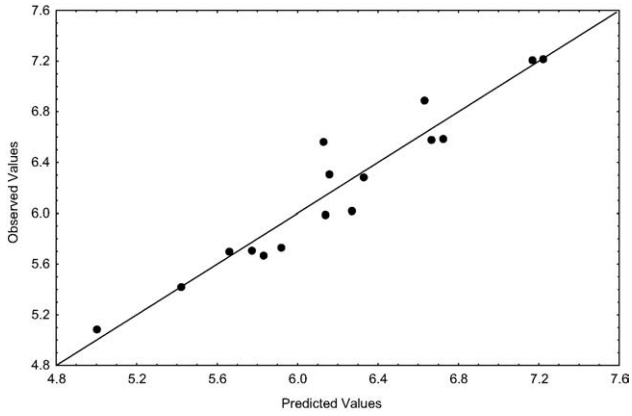


Fig. 1. Relationship between experimental and calculated antibacterial activity using Eq. 23.

$$\log \frac{1}{\mathrm{MIC}_M} = -0.65(\pm 0.23)\pi - 0.40(\pm 0.35)B_1 + 0.58(\pm 0.29)I + 6.52(\pm 0.59) \quad (29)$$

$n = 16$; $r = 0.59$; S.D. = 0.57; $F = 2.13$

The best correlations are displayed in Eqs. 23 and 24 and they confirm our previous findings related to physicochemical properties best describing the biological activity: σ_p and B_1. The correlation is poor when hydrophobic parameters are considered (Eqs. 25–29).

The following conclusions can be drawn:

1) When attempting a single regression analysis, a good correlation was not found between the antibacterial activity and the physicochemical parameters, being that the electronic distribution had the higher r.
2) In the multiple regression analysis, a very good correlation was obtained when the steric and the electronic parameters were considered (Eq. 23). Also, both parameters are significant and have similar weights in the equation.
3) The negativity of both terms, B_1 and σ_p, in Eq. 23, indicates that the substituents that increased the activity are small electron-donors.
4) The nitro derivatives are outliers. The unexpected high in vitro antibacterial activity can be explained if we consider the reduction of the nitro group in the bacterial cell, as it was observed in other antibacterials [30].

Fig. 1 shows the good correlation between experimental and calculated antibacterial activity obtained from Eq. 23.

6. Discussion

BSFQs **1**–**10** (CIP derivatives) are congeners of the previously reported NOR derivatives as was demonstrated by the SAR through a QSAR study described here. In this new class of AMFQs, when a BS moiety is bound to the N of the piperazine ring of CIP or NOR, the improved in vitro Gram-positive antibacterial activity originated should be structurally related to the new substituent on the 7-position of the

AMFQ. Also, the decrease in antibacterial activity against Gram-negative strain should be related to the same modification on the structure. These findings are in agreement with previous reports of Llorente et al. [31]. They have identified the substituent at C-7 of AMFQs as being important not only for the activity, but also for distinguishing between Gram-positive and negative bacteria. Our results are also coherent with results informed by Ohta and Koga [32] showing that the presence of a *N*-carbonyl substituent, bioisoster of sulfonyl group, contribute negatively to the *E. coli* antimicrobial activity. Domagala [33] has reported that the alkylation of the heterocycle at C-7 enhanced activity against Gram-positive bacteria. The addition of a pyridone moiety to the 7-pyrrolidinyl ring resulted in an improvement of activity against Staphylococci and a decreasing of the activity against Gram-negative bacteria when compared to CIP [34].

It is known that at least two factors determine the potency of AMFQs against bacteria: the transport of the drug into the cells and the inhibition of the target enzyme, DNA gyrase or topoisomerase IV. A substituent on the 7-position would play a key role in both events [34].

BSFQs carry on only one ionizable group in the biological pH range while CIP and NOR are zwitterionic molecules. This factor was considered of great importance to explain the higher uptake of **1** as compared with CIP [8]. Later reports by Peterson [34,35] also indicated a reduced capacity to cause efflux in staphylococci by bulkier and hydrophobic quinolones. So, the better intracellular accumulation of **1** could be rationalized by both phenomena.

On the other hand, Shen et al. [15] have suggested, along with his cooperative drug–enzyme-DNA-binding model, that the 7-position was related with drug–enzyme interactions. These conclusions were also confirmed by SAR studies reviewed by Domagala [33]. Genetic studies conducted by Fisher et al. [13,14] provided the first direct evidence that C-7 substituent in BSFQs, i.e. benzenesulfonyl group, makes critical enzyme contacts that determine their particular bacterial target recognition. Indeed, compound **1** behaves as a "dual targeting" drug with gyrase as its primary target in *S. pneumoniae* and *S. aureus* as opposite to CIP behavior which primary target is topoisomerase IV in Gram-positive bacteria [13,14].

We have demonstrated here through a QSAR study that a benzenesulfonyl group by itself could be necessary but by no means sufficient to convert or reconvert topoisomerase IV primary target of CIP in DNA gyrase primary target in **1**. Indeed, only a BS moiety with proper stereoelectronic characteristic met the structural requirements to fulfill the biological action described above. Among the congeneric series of BSFQs coming from NOR and CIP, only those *p*-substituted derivatives with small electron-donor properties would contribute positively to enhance activity against *S. aureus*.

In a strict sense, it could not be stated if the enhanced *S. aureus* antibacterial activity of the BSFQs is the result of a complex influence of BS group on target selection and on intracellular accumulation. However, a BS substituent on the 7-position of CIP or NOR is indeed enough to enhance the Gram-positive and decrease Gram-negative activity. The BS substituent would also switch the primacy toward target enzymes. While NOR and CIP have preference to topoisomerase IV in Gram-positive bacteria, BSFQs prefer DNA gyrase [1,13,14]. The particular structural characteristics of the different *p*-substituted benzenesulfonyl group are exclusively responsible of the SAR observed. Since stereoelectronic properties play a key role in determining activity in BSFQs, it could be suggested that dipolar interactions are present in molecular recognition. On the other hand, it could be hypothesized that the major influence of the substituents should be centered in the interactions with the enzyme since hydrophobic properties would play a minor role in discriminating the activity of BSFQs.

7. Conclusions

A series of new BSFQs analogs of CIP have been synthesized, characterized, and tested. The presence of the BS groups with different *p*-substituents on the phenyl ring shifted the activity of classic antimicrobial fluoroquinolones from being more active against Gram-negative to Gram-positive bacteria. These CIP analogs and their congeneric NOR derivatives show a similar trend in their antimicrobial properties.

All of the lipophilic parameters studied are useful for QSAR studies being those obtained from HPLC the more reliable ones. The QSAR study of all the compounds prepared indicates that the electronic and the steric parameters best describe the antibacterial activity. The lipophilic properties do not improve the correlation, when is included in the multiple regression analysis. In agreement with previous findings, these results show that the hydrophilicity plays a minor role in the antibacterial activity. Finally, according to this QSAR study the amino and the methyl amino derivatives are the most active analogs within these series.

8. Experimental

8.1. General

All chemicals and solvents were analytical reagent grade and were used without further purification. Reagent grade water was generated by a Millipore Milli-Q Water purification system.

8.2. Chromatography

HPLC chromatography was performed with a KONIK model 500G, with an UV-V-KNK-029-757 absorbance detector with the wavelength set at 272 nM, a Rheodyne 7125 injector, a Spectra Physics 4600 Data Jet integrator, and a 250 × 4.6 mm Lichrosorb RP-18 5 μm HPLC column (Merk). The mobile phase composition ranged from 50% to 80% (v/v)

methanol/water, with the pH adjusted to 2.80 with phosphoric acid 85%. The flow rate was 1.25 ml/min and the injection volume 20 μl.

Approximately 5 mg of each quinolone was dissolved in 1 ml of DMSO, 15 ml of water was added, and then 1 ml of NaOH 1 M, and the volume was made up to 25 ml with water. A 1-ml aliquot was taken and diluted to 50 ml with methanol. Four injections were made under the analytical condition stated, with the aim of obtaining a mean value of the retention time for each compound.

R_M values were measured in an acetone–water mixture as mobile phases in a pH range of 7.0–7.5. For logk' determinations a pH 2.8 was chosen, due to the best separation that can be obtained under these conditions.

8.3. Biological

MICs were determined by broth macrodilution techniques (Mueller–Hinton broth; Merck) [19]. The inoculum size was approximately 5×10^5 CFU/ml. The MIC was defined as the lowest concentration of a drug that completely inhibited visible growth of the organisms after 16–20 h at 35 °C.

9. Supporting material

Correlation Matrix, a complete set of equations that were considered during the QSAR study, and the spectroscopic characteristics of the new compounds are available upon request.

Acknowledgments

Financial support was granted by CONICOR, CONICET and SECyT-UNC. The authors thank Bioq. Beatriz Bottini for helpful discussion in statistical analysis.

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