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N1-Phenyl substituted 4-quinolones of tuberculostatic activity

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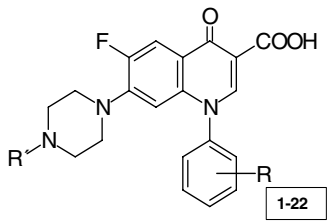
Various different substituted N1-phenyl-6-fluoro-7-piperazinyl-4-quinolone 3-carboxylates and the diazoniumtetrafluoroborate of sparfloxacin were tested for their ability to inhibit the growth of various *Mycobacterium* strains of different origin. The N1-*p*-hydroxylphenyl substituted compound **22** and the methoxyphenyl substituted compound missing the piperazine ring **12** were identified as promising candidates for the further development of an improved treatment of tuberculosis. In addition, compound **22** and the N1-*p*-fluorophenyl substituted analogue **4** were found to be active against *Mycobacterium avium*, which is often isolated from patients suffering from AIDS.

1. Introduction

Tuberculosis (TB) is still a widespread disease throughout the world [1] with increasing problems concerning the treatment. It kills more adult people than any other single infection. Due to inadequate treatment, e. g. in the post-perestroika period of eastern Europe and the Russian Federation, TB is becoming increasingly resistent to tuberculostatics in clinical practice [2]. In addition, Russian emigrants, especially coming to Germany, spread these multi-drug-resistant *Mycobacteria* (MDR-TB) throughout Europe. HIV infections further accelerate the onset of acute MDR-TB.

Since many years, in United Kingdom the Joint Tuberculosis Committee recommended a six month regimen consisting of the almost fifty years old rifamycin, isoniazid, pyrazinamide and ethambutol for the initial two month and rifamycin and isoniazid for a further four month period [3]. The increasing rate of MDR-TB does not only create problems for the treatment, but also the costs are exploding. Thus, new drugs and therapy regimen are necessary to overcome the current problems of therapy. Ofloxacin, ciprofloxacin as well as a couple of new quinolones, such as levofloxacin, gatifloxacin and HSR-903 [4] are reported to be effective in the therapy of TB. However, none of the new 4-quinolones is launched for treatment of TB.

As a part of a greater project a congeneric series of N1-phenyl substituted 6-fluoro-7-piperazinyl-4-quinolone 3-carboxylates **1**–**22** characterized by a systematic variation of the phenyl substitution and alkyl substituents of increasing size on the peripheral piperazine nitrogen were previously synthesised [5] and tested for their ability to inhibit the growth of a wide range of bacteria [5] and the extent of protein binding [6–8]. The purpose of this paper was to investigate the ability of these compounds to inhibit the growth of various strains of *Mycobacteria*. Within the frame of the "Tuberculosis Antimicrobial Acquisition & Coordinating Facility" (TAACF) in Birmingham, Alabama, USA, the compounds were subjected to *Mycobacterium tuberculosis* assays. In addition, a selected number of compounds were evaluated for their ability to inhibit the growth of *Mycobacterium avium* which is a common cause of bacteremia and disseminated disease in HIV infected patients [9].



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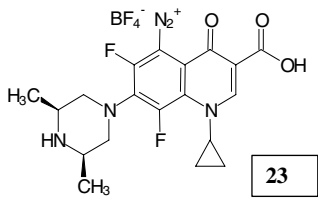

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2. Investigations, results and discussion

The inhibition of the growth of *Mycobacterium tuberculosis* strain $H_{37}Rv$ (percentage) at a concentration of 12.5 μg/ml is summarized in the Table. The comparison of the differently substituted N1-phenyl-4-quinolones re-

Table: Results of the initially screened *Mycobacterium tuberculosis* strain $H_{37}Rv$ at a concentration of 12.5 μg/ml

Compd.	R	R′	Inhibition* (%)
1	H	H	82
2	o-fluoro	H	71
3	m-fluoro	H	18
4	p-fluoro	H	99 (6.25)
5	o-CF_3	H	62
6	p-CF_3	H	0
7	o-CH_3	H	20
8	p-CH_3	H	49
9	o-OCH_3	H	0
10	m-OCH_3	H	44
11	m-OCH_3	CH_3	63
12	p-OCH_3	no piperazine**	99 (12.5)
13	p-OCH_3	H	34
14	p-OCH_3	C_2H_5	10
15	p-OCH_3	C_3H_7	1
16	o-CN	H	37
17	m-CN	H	0
18	p-CN	H	0
19	m-NO_2	H	0
20	p-NO_2	H	24
21	m-OH	H	100 (6.25)
22	p-OH	H	100 (6.25)

* MIC (μg/ml) in brackets;
** the piperazine ring is replaced with a chloro atom

vealed the phenol substituted substances **21** and **22** to be superior to all other analogues. The MIC values were found to be 6.25 μg/ml. The *ortho*- and *para*-fluoro substituted compounds **2** and **4** also showed considerable activity which is in accordance with corresponding studies performed by Domagala et al. [10, 11] with *Mycobacterium fortuitum* and *Mycobacterium smegmatis*. In addition, the *para*-methoxyphenyl substituted compound **12** missing a piperazine ring in 7-position, which is an intermediate of the quinolone synthesis, was tested. Surprisingly, this compound was as active as the *p*-fluorophenyl-4-quinolone **4**. Moreover, the replacement of the chlorine atome with the piperazine ring diminished the activity substantially and alkylation of the peripheral nitrogen of the piperazine further decreased the activity with increasing length of alkane chain (cf. compounds **12**–**15**). Interestingly, the 5-diazonium salt of sparfloxacin **23** was found to be active in the same range of concentration.

The most active compounds mentioned above were subjected to the next level of testing. The cytotoxicity of the compounds (IC_{50} values) were checked in VERO cells at concentrations 10 times the MIC of *M. tuberculosis* $H_{37}Rv$. In the case of the *p*-hydroxy- and *p*-fluorophenyl substituted compounds **4** and **22** the IC_{50} values were measured to be higher than 100 and 50 μg/ml resulting in an selectivity index (IC_{50}/MIC) higher than 16 and 8 which indicates a low cytotoxicity. In contrast, the cytotoxicity of the N1-*m*-hydroxy- and N1-*p*-methoxyphenyl substituted compounds **12** and **21**, the latter having no piperazine ring, and the 5-diazonium substituted compound **23** were found to be too high for the treatment of tuberculosis.

The N1-*p*-hydroxyphenyl substituted compound **22** was forwarded to tests with *Mycobacterium tuberculosis* strains resistant to isoniazid, rifamycin, and ethambutol using the Alamar blue assay [12]. Since the ratios of MICs in the resistant and non-resistant strains were found to be between 0.5 and 1, a cross-resistance with these drugs is unlikely. However, a cross-resistance with ciprofloxacin was observed (ratio MIC(Cip)/MIC = 4).

Only a few compounds such as advanced-generation macrolides, ethambutol, and rifabutin have activity against *M. avium in vivo* [13]. Furthermore the emergence of macrolide resistance and drug interactions between rifamycins and protease inhibitors emphasise the need for additional compounds. Therefore, we tested some of the most active compounds of this study (**4, 8, 12** and **22**) against *Mycobacterium avium* isolate from an AIDS patient. The inhibition zones in agar disk diffusion assays revealed considerable activity for compounds **4** and **22** at a concentration of 12.5 μg/ml. The inhibition zones were 3.3 cm and 4 cm, respectively. In comparison, the inhibition zones of azithromycin and rifabutin which were used as internal standards were 2.5 cm and 7.5 cm.

Since the compounds tested in this study were not synthesized for tuberculostatic purposes, the evaluation could be seen as a random screeening. However, with respect to tuberculosis it can be stated that two hits were identified, the N1-*p*-hydroxylphenyl substituted compound **22** and the methoxyphenyl substituted compound missing the piperazine ring **12.** Both are promising candidates for further structural variation which are necessary to enhance the antimycobacterial activity. Corresponding studies are in progress. In comparison, whereas compound **22** was also found to be active against *Mycobacterium avium*, compound **12** did not show any activity against the *Mycobacterium* isolated from AIDS patients which demonstrates the different structure-activity relationships and, along with this, a selectivity of action.

3. Experimental

The N1-phenyl substituted compounds were synthesized as reported [5]. The sparfloxacin-5-diazonium tetrafluoroborate **23** was synthesised starting with sparfloxacin using the standard procedure of diazotation with tetrafluoroborate and $NaNO_2$ [14]. IR (Nujol): 2120, 2300–2200 cm^{-1} (broad). ^1H NMR (300 MHz, DMSO-d_6): 8.56 (s, 1 H, =CH), 4.09 (m, 1 H, cycloprop.), 3.98–3.08 (m, 6 H, pip), 1.24–1.21 (m, 10 H, pip, cycloprop.).

The microbiological testing with *Mycobacterium tuberculosis* was performed using the Alamar method [12]. Strain used: *Mycobacterium tuberculosis* $H_{37}Rv$ ATCC 27294; *Mycobacterium tuberculosis* strains resistant to isoniacid ATCC 35822, rifampicin ATCC 35838. The microbial testing with *Mycobacterium avium* (ATCC 35713) was performed using the agar disk diffusion assay [15].

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