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Antituberculosis agents IV: *In vitro* antimycobacterial activity and cytotoxicity of *N*-piperazinyl quinolone derivatives containing 2-thienyl and 2-furyl moiety

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A series of *N*-[2-(2-furyl)-2-oxoethyl], *N*-[2-(2-furyl)-2-oxyiminoethyl], *N*-[2-oxo-2-(2-thienyl)ethyl] and *N*-[2-oxyimino-2-(2-thienyl)ethyl] piperazinyl quinolones (**1a**–**h**; **2a**–**h**) were evaluated for antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ using the BACTEC 460 radiometric system and BACTEC 12B medium. Our results indicated that compounds **1a**, **1e** and **1g** were efficient antimycobacterial agents showing MIC values ranging from 0.78 to 6.25 μg/ml. In general, ciprofloxacin derivatives were more active than norfloxacin derivatives and the oxime analogues were less active than corresponding ketones. Active compounds (**1a**, **1e** and **1g**) were also screened by serial dilution to assess toxicity to VERO cell line. The cytotoxicity of tested compounds indicated that compound **1a** was the less toxic compound ($IC_{50} > 62.5$ μg/ml). This compound was tested for efficacy *in vitro* in TB-infected macrophage model ($EC_{90} = 3.25$ μg/ml).

1. Introduction

Tuberculosis is the leading cause of death by infectious disease with one-third of the world population infected [1]. Due to multi-drug resistant strains of mycobacteria and to a high prevalence of tuberculosis in patients who have acquired human-immunodeficiency syndrome (AIDS), the number of patients infected with the disease is increasing world wide [2]. The resurgence of tuberculosis and the emergence of multi-drug resistant mycobacteria necessitate the development of new antituberculosis drugs [3]. No new antituberculosis agents have been developed since the introduction of rifampicin into clinical use, although fluoroquinolones have been investigated for potential efficacy in tuberculosis [4]. *In vitro* studies have shown that they have excellent bactericidal activity against many mycobacterial disease, especially tuberculosis [5].

As part of a study attempting to further optimize the quinolone antibacterials against *M. tuberculosis*, our research focused on the development of new potential therapeutic agents.

In an earlier paper, we reported the syntheses of *N*-[2-oxo-2-phenylethyl] piperazinyl quinolone derivatives, which had antibacterial activity against some gram-positive and gram-negative organisms [6]. These compounds showed significant activity against *M. tuberculosis* strain $H_{37}Rv$ [7]. Here, we report the antituberculosis activity and cytotoxicity of some *N*-[2-(2-furyl)-2-oxoethyl], *N*-[2-(2-furyl)-2-oxyimino ethyl], *N*-[2-oxo-2-(2-thienyl)ethyl] and *N*-[2-oxyimino-2-(2-thienyl)ethyl] piperazinyl quinolones (**1a**–**h**, **2a**–**h**) which had previously been shown antibacterial activity against some gram positive and gram-negative bacteria [8, 9].

2. Investigations, results and discussion

All compounds (**1a**–**h**, **2a**–**h**) were initially screened against *M. tuberculosis* strain $H_{37}Rv$ at the single concentration 6.25 μg/ml. Compounds were considered active in the primary screen if at this concentration inhibition was ≥90% (Table).

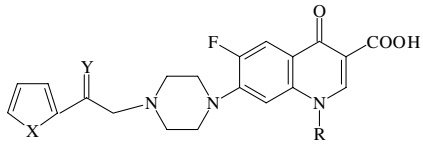
Active compounds were retested in order to determine the actual MIC against *M. tuberculosis* $H_{37}Rv$ (Table). Rifampin was used as reference drug. The antituberculosis results indicate that ciprofloxacin derivatives are more active against *M. tuberculosis* than norfloxacin derivatives. Ciprofloxacin derivatives bearing 2-(2-furyl)-2-oxoethyl (**1a**) and 2-(2-thienyl)-2-oxoethyl(**1e**) groups attached to the piperazine ring showed significant activity against *M. tuberculosis* (MIC = 0.78 and 1.56 μg/ml respectively) while the corresponding norfloxacin derivatives (**2a**, **2e**) were inactive.

Generally, the oximes were less active than the corresponding ketones. Replacement of the hydrogen of the oxime with a methyl group resulted in variable percent of inhibition (Inh%, Table). Among the oximes, only the ciprofloxacin derivative with 2-methoxyimino-2-(2-thienyl)ethyl group (**1g**) showed a moderate activity against *M. tuberculosis* (MIC = 6.25 μg/ml) and the others were inactive.

If the hydrogen of oxime is replaced with a benzyl group, percent of inhibition (% Inh, Table) increased, however, their antituberculosis activity was not significant (MIC > 6.25 μg/ml).

The most active compounds (**1a**, **1e**) were tested for cytotoxicity (IC_{50}) in VERO cells and the results are reported in the Table. The selectivity index (SI) is defined as the

Table: ***In vitro*** **antituberculosis activity, cytotoxicity (IC_{50}) and selectivity index(SI) of *N*-piperazinyl quinolone derivatives***



Comp.	R	X	Y	MIC	% Inh	Activity	MIC (μg/ml)	IC_{50}	SI
1a	Cyclopropyl	O	O	<6.25	103	+	0.78	>62.5	>80
1b	Cyclopropyl	O	NOH	>6.25	26	−			
1c	Cyclopropyl	O	$NOCH_3$	>6.25	45	−			
1d	Cyclopropyl	O	$NOCH_2C_6H_5$	>6.25	74	−			
1e	Cyclopropyl	S	O	<6.25	96	+	1.56	>10	>6.4
1f	Cyclopropyl	S	NOH	>6.25	37	−			
1g	Cyclopropyl	S	$NOCH_3$	<6.25	99	+	6.25		
1h	Cyclopropyl	S	$NOCH_2C_6H_5$	>6.25	69	−			
2a	Ethyl	O	O	>6.25	33	−			
2b	Ethyl	O	NOH	>6.25	29	−			
2c	Ethyl	O	$NOCH_3$	>6.25	23	−			
2d	Ethyl	O	$NOCH_2C_6H_5$	>6.25	74	−			
2e	Ethyl	S	O	>6.25	41	−			
2f	Ethyl	S	NOH	>6.25	1	−			
2g	Ethyl	S	$NOCH_3$	>6.25	4	−			
2h	Ethyl	S	$NOCH_2C_6H_5$	>6.25	48	−			

* Rifampin MIC 0.015–0.125 μg/ml, IC_{50} >100 μg/ml, SI > 800

ratio of the measured IC_{50} in VERO cells to the MIC against *M. tuberculosis* $H_{37}Rv$ (Table). The cytotoxicity data of tested compounds indicated that compound **1a** was the less toxic compound ($IC_{50} > 62.5$ μg/ml, $SI > 80$). The most promising compound **1a** was tested for efficacy *in vitro* in a TB-infected macrophage model. The result of macrophage assays showed $EC_{90} = 3.25$ and $EC_{99} > 12.5$ μg/ml. This compound with $EC_{90}/MIC = 4.17$ was found active in this model.

3. Experimental

3.1. Synthesis of products

The products were synthesized according to procedures previously described [8, 9].

3.2. Biological assay

All of the compounds were evaluated for *in vitro* antituberculosis activity against Mycobacterium tuberculosis as part of TAACF TB screening program under direction of the U.S. National Institute of Health, NIAID division. Primary screening was conducted at the single concentration of 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [10].
Compounds effecting <90% inhibition in the primary screen (MIC >6.25 μg/ml) were not evaluated further. The active compounds (Inh.% ≥ 90, MIC ≤ 6.25 μg/ml) were retested by serial dilution beginning at a concentration of 6.25 μg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460 radiometric system and BACTEC 12B medium. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

3.3. Cytotoxicity assay

Compounds were screened by serial dilution to assess toxicity to a VERO cell line(IC_{50}), beginning at 10 × MIC if sample solubility in culture media was permitted. The selectivity index (SI) is defined as the ratio of the measured IC_{50} in VERO cells to the MIC described above.

3.4. Macrophage assay

Selective compounds as determined in cytotoxicity assays were tested for efficacy in vitro in a TB-infected macrophage model and the concentration effecting 90% (EC_{90})and 99%(EC_{99}) reduction in residual mycobacterial growth after seven days, compared to untreated controls. Compounds with $EC_{90} \leq 16 \times MIC$ were considered active in this model.

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