

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* H₃₇Rv 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 (IC₅₀ > 62.5 µg/ml). This compound was tested for efficacy *in vitro* in TB-infected macrophage model (EC₉₀ = 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 H₃₇Rv [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

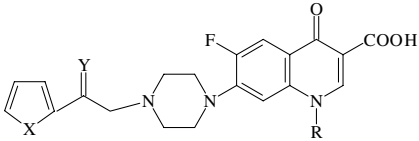
All compounds (**1a–h**, **2a–h**) were initially screened against *M. tuberculosis* strain H₃₇Rv at the single concentration 6.25 µg/ml. Compounds were considered active in the primary screen if at this concentration inhibition was ≥90% (Table).

Active compounds were retested in order to determine the actual MIC against *M. tuberculosis* H₃₇Rv (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 (IC₅₀) 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 (IC₅₀) 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 ₂ C ₆ H 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 ₂ C ₆ H 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 ₂ C ₆ H 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 ₂ C ₆ H ₅	>6.25	48	—			

* Rifampin MIC 0.015–0.125 μg/ml, IC₅₀ >100 μg/ml, SI > 800

ratio of the measured IC₅₀ in VERO cells to the MIC against *M. tuberculosis* H₃₇Rv (Table). The cytotoxicity data of tested compounds indicated that compound **1a** was the less toxic compound (IC₅₀ > 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 EC₉₀ = 3.25 and EC₉₉ > 12.5 μg/ml. This compound with EC₉₀/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* H₃₇Rv (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* H₃₇Rv 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(IC₅₀), beginning at 10 × MIC if sample solubility in culture media was permitted. The selectivity index (SI) is defined as the ratio of the measured IC₅₀ 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% (EC₉₀) and 99%(EC₉₉) reduction in residual mycobacterial growth after seven days, compared to untreated controls. Compounds with EC₉₀ ≤ 16 × MIC were considered active in this model.

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