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Synthesis and Anti-tubercular Evaluation of 4-Quinolylhydrazones

Luisa Savini,* Luisa Chiasserini, Alessandra Gaeta and Cesare Pellerano

Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy

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Abstract—A series of 4-quinolylhydrazones were synthesized and tested against *Mycobacterium tuberculosis* H37Rv. Preparation of the title compounds was achieved by reaction of 4-quinolylhydrazine and aryl- or heteroaryl-carboxaldehyde. For the most of derivatives interesting antitubercular properties were showed; two compounds ($\mathbf{3}_2$ and $\mathbf{3}_{25}$), identified as the most active, were tested also against *Mycobacterium avium*.

Introduction

Tuberculosis (TB), an infection of *Mycobacterium tuberculosis* still remains the leading cause of worldwide death among infectious diseases;[1,2] one-third of the population is infected with *M. tuberculosis* and the World Health Organization (WHO) estimates that within the next 20 years about 30 million people will catch tuberculosis.[3–7]

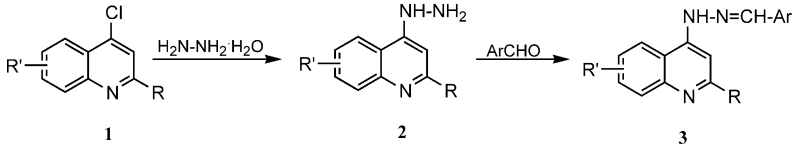
The resurgence of TB is associated with the emergence of HIV/AIDS epidemic[8] and the fast development of multidrug resistant TB bacterial strains.[9,10] In the developing world, probably 50% of HIV seropositive individuals are co-infected with TB. Therefore taking into account what is reported above there is a pressing need to develop new and more effective anti-tubercular agents.

Among the new structures recently reported as potential anti-tubercular of particular interest seems to be some 2,6-bis(alkylthio)4-pyridinecarboxamides,[11] *N*-alkyl-1,2-dihydro-2-thioxo-3-pyridinecarbothioamides,[12] 6-chloro-3-phenyl-4-thioxo-2H-1,3-benzoxazine-2(3H)-ones, -dithiones[13] and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin, sparfloxacin, gatifloxacin and moxifloxacin).[14]

Within a program aimed at developing new quinoline derivatives, we previously synthesized numerous quinolylhydrazones, some of which resulted were endowed with anti-mycoplasmal, anti-bacterial, anti-cestode, anti-viral, anti-tumoral and anti-tubercular properties.[15–24]

Therefore, as a consequence of previous researches and on the basis of the obtained results, a series of 4-quinolylhydrazones **3**, with various substituents on quinoline nucleus and with aryl- or heteroaryl-hydrazonic moiety, were synthesized as potential anti-tubercular agents.

The new synthesized quinolylhydrazones **3** and the starting quinolylhydrazines **2** were tested against *M. tuberculosis* H37Rv and interesting anti-tubercular properties resulted for most of the title compounds.



R,R', Ar as defined in Table 1

Scheme 1. R,R′, Ar as defined in Table 1.

*Corresponding author. Tel.: +39-0577-234304; fax: +39-0577-234333; e-mail: savini@unisi.it

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Chemistry

The synthesis of 4-quinolylhydrazones **3** was carried out, according to the previously described procedure,[18] by reaction of equimolar amounts of 4-hydrazinoquinoline **2** and the appropriate carboxaldehyde in EtOH, as illustrated in Scheme 1.

The 4-quinolylhydrazine **2** necessary for preparation of 4-quinolylhydrazones **3** were obtained from the corresponding 4-chloroquinoline **1** and hydrazine hydrate as we previously described.[24]

The structures assigned to the synthesized compounds are in good agreement with elemental analyses, IR and

Table 1. Physicochemical data and molecular formula of derivatives **2** and **3**

Compd	R	R′	Ar	Mp (°C)	Cryst. solvent	Molecular formula
2$_1$[a]	H	7-OCH_3				
2$_2$[a]	CH_3	7-OCH_3				
2$_3$[a]	CH_3	8-OCH_3				
2$_4$[a]	C_6H_5	6-OCH_3				
2$_5$	H	7-OC_2H_5		250–253	EtOH	$C_{11}H_{13}N_3O$·HCl
2$_6$[a]	CH_3	7-OC_2H_5				
2$_7$[b]	H	6-*n*.C_4H_9				
2$_8$[c]	CH_3	6-*n*.C_4H_9				
2$_9$[b]	H	6-*n*.OC_4H_9				
2$_{10}$[c]	CH_3	6-*n*.OC_4H_9				
2$_{11}$	H	6-Cyclohexyl		287–291	EtOH anhyd.	$C_{15}H_{19}N_3$·HCl
2$_{12}$[c]	CH_3	6-Cyclohexyl				
2$_{13}$[a]	H	7-Cl				
2$_{14}$[b]	CH_3	7-Cl				
2$_{15}$	H	5,7-Cl		184–187	EtOH	$C_9H_7Cl_2N_3$
2$_{16}$	CH_3	5,7-Cl		202–205	EtOH	$C_{10}H_9Cl_2N_3$
2$_{17}$[a]	CH_3	6-F				
3$_1$	H	H	4-OCH_3–naphthyl	195 (dec.)	Benzene	$C_{21}H_{17}N_3O$
3$_2$	H	7-OCH_3	2-OCH_3–naphthyl	118–121	EtOH	$C_{22}H_{19}N_3O_2$·1/4H_2O
3$_3$	H	7-OCH_3	4-OCH_3–naphthyl	235–238	EtOH	$C_{22}H_{19}N_3O_2$
3$_4$	H	7-OCH_3	4-N(C_2H_5)$_2$–C_6H_4	201 (dec.)	EtOH-H_2O	$C_{21}H_{24}N_4O$·1/4H_2O
3$_5$	CH_3	7-OCH_3	2-OCH_3–naphthyl	202 (dec.)	EtOH	$C_{23}H_{21}N_3O_2$·H_2O
3$_6$	CH_3	7-OCH_3	4-OCH_3–naphthyl	286–289	EtOH	$C_{23}H_{21}N_3O_2$·HCl·H_2O
3$_7$	CH_3	7-OCH_3	4-N(C_2H_5)$_2$–C_6H_4	128–132	EtOH-H_2O	$C_{22}H_{26}N_4O$·H_2O
3$_8$	CH_3	7-OCH_3	3,4-(OCH_2O)–C_6H_3	215 (dec.)	EtOH	$C_{19}H_{17}N_3O_3$
3$_9$	CH_3	8-OCH_3	4-OCH_3–naphthyl	261–262	EtOH-H_2O	$C_{23}H_{21}N_3O_2$
3$_{10}$	H	7-OC_2H_5	2-OCH_3–naphthyl	95 (dec.)	EtOH	$C_{23}H_{21}N_3O_2$·1/2H_2O
3$_{11}$	H	7-OC_2H_5	4-OCH_3–naphthyl	200–202	EtOH	$C_{23}H_{21}N_3O_2$
3$_{12}$	H	7-OC_2H_5	4-N(C_2H_5)$_2$–C_6H_4	119–122	EtOH	$C_{22}H_{26}N_4O$·1/2H_2O
3$_{13}$	CH_3	7-OC_2H_5	2-OCH_3–naphthyl	232–235	EtOH	$C_{24}H_{23}N_3O_2$
3$_{14}$	CH_3	7-OC_2H_5	4-OCH_3–naphthyl	231–234	EtOH	$C_{24}H_{23}N_3O_2$·3/4H_2O
3$_{15}$	CH_3	7-OC_2H_5	4-N(C_2H_5)$_2$–C_6H_4	115–118	EtOH	$C_{23}H_{28}N_4O$·H_2O
3$_{16}$	C_6H_5	6-OCH_3	4-$NHCOCH_3$–C_6H_4	138 (dec.)	EtOH-H_2O	$C_{25}H_{22}N_4O_2$
3$_{17}$[d]	C_6H_5	6-OCH_3	5-NO_2–2–furyl			$C_{23}H_{21}N_3O_2$
3$_{18}$	H	6-*n*.C_4H_9	4-OCH_3–naphthyl	195–196	EtOH-H_2O	$C_{25}H_{25}N_3O$
3$_{19}$	CH_3	6-*n*.C_4H_9	4-OCH_3–naphthyl	237–239	EtOH	$C_{26}H_{27}N_3O$
3$_{20}$	CH_3	6-*n*.C_4H_9	3,4-(OCH_2O)–C_6H_3	202–204	EtOH	$C_{22}H_{23}N_3O_2$
3$_{21}$	H	6-*n*.OC_4H_9	4-OCH_3–naphthyl	204–205	EtOH	$C_{25}H_{25}N_3O_2$
3$_{22}$	CH_3	6-*n*.OC_4H_9	4-OCH_3–naphthyl	209–210	EtOH-H_2O	$C_{26}H_{27}N_3O_2$
3$_{23}$	H	6-Cyclohexyl	4-OCH_3–naphthyl	182–184	EtOH	$C_{27}H_{17}N_3O$
3$_{24}$[b]	H	6-Cyclohexyl	1-C_6H_5–2,5–CH_3–3–pyrrolyl			$C_{26}H_{27}N_3O$
3$_{25}$	CH_3	6-Cyclohexyl	C_6H_5	252–254	EtOH-H_2O	$C_{23}H_{25}N_3$
3$_{26}$	CH_3	6-Cyclohexyl	4-N(C_2H_5)$_2$–C_6H_4	163–166	EtOH-H_2O	$C_{27}H_{34}N_4$
3$_{27}$	CH_3	6-Cyclohexyl	4-OCH_3–naphthyl	290–292	DMF	$C_{28}H_{29}N_3O$
3$_{28}$[b]	CH_3	6-Cyclohexyl	1-C_6H_5–2,5–CH_3–3–pyrrolyl			
3$_{29}$	H	7-Cl	2-OCH_3–naphthyl	237–239	EtOH	$C_{21}H_{16}ClN_3O$
3$_{30}$	H	7-Cl	4-OCH_3–naphthyl	132–136	EtOH	$C_{21}H_{16}ClN_3O$·3/4H_2O
3$_{31}$[e]	H	7-Cl	4-N(C_2H_5)$_2$–C_6H_4	245–248	EtOH	$C_{20}H_{21}ClN_4$
3$_{32}$	CH_3	7-Cl	2-OCH_3–naphthyl	259–262	EtOH	$C_{22}H_{18}ClN_3O$
3$_{33}$	CH_3	7-Cl	4-OCH_3–naphthyl	224–226	EtOH	$C_{22}H_{18}ClN_3O$
3$_{34}$	CH_3	7-Cl	4-N(C_2H_5)$_2$–C_6H_4	249–252	EtOH	$C_{21}H_{23}ClN_4$
3$_{35}$	H	5,7-Cl	3,4-(OCH_2O)–C_6H_3	223–225	EtOH	$C_{17}H_{11}Cl_2N_3O_2$
3$_{36}$	H	5,7-Cl	4-OCH_3–naphthyl	164–167	EtOH	$C_{21}H_{15}Cl_2N_3O$·1/4H_2O
3$_{37}$	CH_3	5,7-Cl	3,4-(OCH_2O)–C_6H_3	218–220	EtOH	$C_{18}H_{13}Cl_2N_3O_2$
3$_{38}$	CH_3	5,7-Cl	4-OCH_3–naphthyl	158–160	EtOH	$C_{22}H_{17}Cl_2N_3O$
3$_{39}$	CH_3	6-F	4-OCH_3–naphthyl	269–271	EtOH	$C_{22}H_{18}FN_3O$

[a]Ref 24.
[b]Ref 25.
[c]Ref 26.
[d]Ref 15.
[e]Ref 27.

^{1}H NMR spectral data. The IR spectra of quinolylhydrazones **3** have a weak band between 3350 and 3170 cm^{-1} due to the stretching vibration of the NH group. In the ^{1}H NMR spectra a singlet appears between 8.3–9.2 δ, which is typical of the aldehyde proton. Physicochemical properties are summarized in Table 1.

Table 2. In vitro evaluation of antimicrobial activity versus *Mycobacterium tuberculosis* H37Rv [minimum inhibitory concentration (MIC) in μg/mL]

Compd	*M. tuberculosis*				
	MIC (μg/mL)	% Inhibition	MIC (μg/mL)	IC_{50} (μg/mL)	SI
$\mathbf{2}_1$[b]	<12.5	97	12.5		
$\mathbf{2}_2$	>12.5	94	12.5		
$\mathbf{2}_3$	>12.5	54			
$\mathbf{2}_4$	<12.5	99	6.25	3.78	0.60
$\mathbf{2}_5$	>12.5	88			
$\mathbf{2}_6$	<12.5	98	12.5	25.2	2.02
$\mathbf{2}_7$[b]	<12.5	99	6.25		
$\mathbf{2}_8$	<12.5	99	6.25	13.40	2.14
$\mathbf{2}_9$	<12.5	99	12.5	7.77	0.62
$\mathbf{2}_{10}$	<12.5	99	6.25	5.94	0.95
$\mathbf{2}_{11}$	<12.5	99	6.25	10.28	1.64
$\mathbf{2}_{12}$	<12.5	100	6.25	5.31	0.85
$\mathbf{2}_{13}$	<12.5	98	12.5	53.54	4.28
$\mathbf{2}_{14}$	<12.5	97	12.5	76.97	6.16
$\mathbf{2}_{15}$	>12.5	82			
$\mathbf{2}_{16}$	>12.5	87			
$\mathbf{2}_{17}$	>12.5	69			
$\mathbf{3}_1$	<12.5	100	3.13	3.50	1.10
$\mathbf{3}_2$[a]	<12.5	100	6.25	73	11.68
$\mathbf{3}_3$	<12.5	100	0.78	7.20	9.23
$\mathbf{3}_4$	<12.5	100	12.5	7.20	0.58
$\mathbf{3}_5$	<12.5	100	3.13	23	7.00
$\mathbf{3}_6$[b]	<12.5	99	1.56		
$\mathbf{3}_7$[b]	<12.5	100	12.5		
$\mathbf{3}_8$	<12.5	100	12.5	5.40	0.43
$\mathbf{3}_9$	<12.5	100	1.56	2.70	1.73
$\mathbf{3}_{10}$	<12.5	100	3.13		
$\mathbf{3}_{11}$	<12.5	100	6.25	<4.20	<0.70
$\mathbf{3}_{12}$	<12.5	100	6.25	6.40	1.02
$\mathbf{3}_{13}$	<12.5	100	6.25	6.90	1.10
$\mathbf{3}_{14}$	<12.5	100	1.56	3.60	2.31
$\mathbf{3}_{15}$	<12.5	100	12.5	6.20	0.50
$\mathbf{3}_{16}$[b]	<12.5	95	12.5		
$\mathbf{3}_{17}$[b]	>12.5	72	12.5		
$\mathbf{3}_{18}$	<12.5	100	6.25	5.70	0.91
$\mathbf{3}_{19}$	<12.5	100	6.25	9.80	1.57
$\mathbf{3}_{20}$	<12.5	100	6.25	6.80	1.09
$\mathbf{3}_{21}$	<12.5	100	6.25	3.40	0.54
$\mathbf{3}_{22}$	<12.5	100	0.78	5.20	6.67
$\mathbf{3}_{23}$	<12.5	100	3.13	13.20	4.22
$\mathbf{3}_{24}$	<12.5	100	6.25	12.60	2.02
$\mathbf{3}_{25}$[a]	<12.5	100	6.25	64	10.24
$\mathbf{3}_{26}$	<12.5	100	1.56	12.1	7.76
$\mathbf{3}_{27}$	<12.5	100	1.56	13.1	8.4
$\mathbf{3}_{28}$	<12.5	100	1.56	6.60	4.23
$\mathbf{3}_{29}$[b]	<12.5	99	6.25		
$\mathbf{3}_{30}$	<12.5	99	3.13	3.40	1.09
$\mathbf{3}_{31}$	<12.5	100	3.13		
$\mathbf{3}_{32}$	<12.5	100	6.25	3.30	0.56
$\mathbf{3}_{33}$	<12.5	100	0.78	3.40	4.36
$\mathbf{3}_{34}$	<12.5	100	6.25	11.90	1.9
$\mathbf{3}_{35}$[b]	>12.5	4			
$\mathbf{3}_{36}$[b]	>12.5	10			
$\mathbf{3}_{37}$[b]	>12.5	4			
$\mathbf{3}_{38}$[b]	>12.5	53			
$\mathbf{3}_{39}$[b]	<12.5	100	3.13	1.90	0.61
Rifampin (RMP)	0.031	90			

[a]MIC against *M. tuberculosis* Erdman was 3.13.
[b]Insoluble in DMSO, unable to test.

Pharmacology

The in vitro evaluation of antituberculosis activity was carried out at the GWL Hansen's Disease Center within the Tuberculosis Antimicrobial Acquisition Coordinating Facility (TAACF) screening program for the discovery of novel drugs for treatment of tuberculosis. Under the direction of the US National Institute of Allergy and Infectious Diseases (NIAID), Southern Research Institute coordinates the overall program. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capacity to inhibit the growth of virulent *M. tuberculosis*.

Biological tests have been performed according to the method described in refs 28–30 and the results are reported in Tables 2–4.

Results and Discussion

From the primary screening against *M. tuberculosis* H37Rv the maiority of the tested compounds showed an inhibitory activity between 95 and 100%, therefore these compounds were submitted to a second level assay to evaluate the actual minimum inhibitory concentration (MIC) and to assess cytotoxicity towards the VERO cell line and selectivity index (SI), defined as the ratio of the measured IC_{50} in VERO cells to the MIC.

On the basis of the obtained data we can remark the interesting anti-tubercular properties showed by some quinolylhydrazones **3**. The activity is significantly affected by substituents both on quinoline nucleus and hydrazonic moiety. On quinoline nucleus the most effective substituents resulted 6-cyclohexyl, 7-methoxy, 7-ethoxy and 7-chloro, while, as for the hydrazonic moiety, greater effectiveness resulted for *para*- and

Table 3. Evaluation of anti-*Mycobacterium avium* activity

Compd	MIC (μg/mL)[a]	SI
$\mathbf{3}_8$	3.13	23.32
$\mathbf{3}_9$	25	0.29
$\mathbf{3}_{25}$	3.13	20.45

[a]MICs are determined in the MABA against a strain of *M. avium* (ATCC 25291).

Table 4. Macrofage assay

Compd	MIC (μg/mL)	SI	EC_{90}	EC_{99}	EC_{90}/MIC
$\mathbf{3}_2$	6.25	11.68	0.65	2.00	0.10
$\mathbf{3}_9$	0.78	9.23	>1	>1	>1.28
$\mathbf{3}_{25}$	6.25	10.24	0.80	1.61	0.13

The columns labeled EC_{90} and EC_{99} list the concentrations effecting 90 and 99% reduction in residual mycobacterial growth after 7 days compared to untreated controls. Compounds with $EC_{90} > 16 \times$MIC (as reported in the column labeled EC_{90}/MIC) are considered inactive.

ortho-methoxynaphtyl substituents. MIC values for those compounds ranged from 0.78 to 3.13 μg/mL (Table 2). The chlorodisubstitution led to inactive derivatives ($\mathbf{3}_{35–39}$). As far as the substitution in position 2 of quinoline nucleus is concerned the methyl appears to be most favourable group. Quinolylhydrazines **2**, even if endowed with appreciable inhibiting action, showed a SI generally below 4, with the only exception of $\mathbf{2}_{13}$ and $\mathbf{2}_{14}$ with a SI = 4.28 and 6.16 respectively, while for some quinolylhydrazones ($\mathbf{3}_{2,3,5,22,25,26,27}$) a significantly higher SI was found (Table 2).

Three compounds ($\mathbf{3}_2$, $\mathbf{3}_3$ and $\mathbf{3}_{25}$), with a high SI value (11.68, 9.23, and 10.24, respectively), were then tested for efficacy in vitro in a TB-infected macrophage model, showing good values of EC_{90} and EC_{99}. Furthemore, these compounds were evaluated for their inhibitory activity against a single strain of *M. avium*, an opportunistic pathogen which has been associated with tuberculosis in patients infected by HIV (Table 3). As for compounds $\mathbf{3}_2$ and $\mathbf{3}_{25}$, which showed a SI of 23.32 and 20.45 respectively, further evaluation in additional *M. avium* assays is in progress.

It is important to point out the low toxicity particularly shown by quinolylhydrazones $\mathbf{3}_2$ and $\mathbf{3}_{25}$ active against *M. tuberculosis* H37Rv and *M. avium*.

In conclusion, for the development of our research, it seems advisable to synthesize new derivatives with suitably chosen substituents both on the quinoline nucleus and hydrazonic moiety. Additional tests are required in order to obtain more definitive and clear answers from structure–activity relationships.

Experimental

Chemistry

Melting points were determined on a Köfler blok or on a Büchi 510 apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer 240 C Elemental Analyzer by Laboratories of Dipartimento Farmaco Chimico Tecnologico, Università di Siena (Italy) and the data for C, H, and N are within ±0.4% of the theoretical values. IR spectra were determined in Nujol mull on a Perkin-Elmer FT-IR 1600 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian XL-200 or AC200 Bruker instruments. The chemical shifts (δ) are relative to Me_4Si used as internal standard; the following abbreviations were used: s = singlet, t = triplet, dd = double doublet, q = quartet, qt = quintet, st = sextet, m = multiplet, u = unresolved, b = broad. The coupling constants *J* were in Hertz. TLC on silica-gel plates (Merck, 60, F_{254}) was used for purity check and reaction monitoring. Column chromatography on silica gel (Merck, 70–230 mesh and 230–400 mesh ASTH for flash chromatography) was applied when necessary, to isolate and purify the different reactions products.

All the reagents were of the best available commercial quality and were used without further purification. The preparation of the unknown 4-quinolylhydrazines ($\mathbf{2}_5$, $\mathbf{2}_{11}$, $\mathbf{2}_{15}$, $\mathbf{2}_{16}$) are also described in this section; analytical and physicochemical data are reported in Table 1. Spectroscopic data (IR and ^{1}H NMR) are fully consistent with the proposed structures.

7-Ethoxy-4-hydrazinoquinoline hydrochloride ($\mathbf{2}_5$). Equimolar amounts of 7-ethoxy-4-chloroquinoline and hydrazine hydrate (85%) in anhydrous ethanol were heated under reflux for 7–8 h. The corresponding hydrazinoquinoline hydrochloride precipitated already in the course of refluxing and completely after cooling. The obtained precipitate was purified by recrystallization (65% yield).

6-Cyclohexyl-4-hydrazinoquinoline hydrochloride ($\mathbf{2}_{11}$). The compound was obtained, according to the method described for $\mathbf{2}_5$, by refluxing equimolar amounts of 6-cyclohexyl-4-chloroquinoline and hydrazine hydrate (85%) for 8 h (61% yield).

4-Hydrazino-5,7-dichloroquinoline ($\mathbf{2}_{15}$). 4,5,7,-Trichloroquinoline (21.5 mmol, 5 g) and hydrazine hydrate (85%) (25 mL) in anhydrous ethanol were refluxed for 9–10 h. After cooling and dilution with water the 4-hydrazino-5,7-dichloroquinoline ($\mathbf{2}_{15}$) precipitated, it was collected, and recrystallized (60% yield).

2-Methyl-4-hydrazino-5,7-dichloroquinoline ($\mathbf{2}_{16}$). The compound was obtained by refluxing, for 3–4 h, the 2-methyl-4,5,7-trichloroquinoline (20.29 mmol, 5 g) and hydrazine hydrate (85%) (25 mL) under the same conditions described above for compound $\mathbf{2}_{15}$ (80% yield).

General procedure for the preparation of 4-quinolylhydrazones (3)

The title compounds were prepared from 4-quinolylhydrazine or its HCl salt and the appropriate carboxaldehyde in EtOH for 1–2 h. After cooling and diluting with H_2O the quinolylhydrazone **3** precipitated from the reaction mixture; it was collected and purified by crystallization from the suitable solvent (Table 1) (70–90% yields). In those instances where 4-hydrazinoquinoline hydrochloride was employed, an equimolar amount of NaOAc was added to the mixture to liberate the free base for reaction. The ^{1}H NMR spectral data of some representative 4-quinolylhydrazones are here described.

$\mathbf{3}_7$. (DMSO-d_6) δ 1.09 (t, 6H, 2 CH_3CH_2N); 2.44 (s, 3H, CH_3); 3.35 (q, 4H, 2 CH_3CH_2N); 3.84 (s, 3H, OCH_3); 6.68 (d, 2H, PhH, $J_{orto} = 8.6$); 6.97–7.08 (m, 3H, ArH and H-3 quinoline); 7.53 (d, 2H, PhH, $J_{orto} = 8.6$); 8.12 (d, 1H, H-5, $J_{5–6} = 9.2$); 8.19 (s, 1H, CH=N); 10.55 (bs, 1H, NH, D_2O exchangeable).

$\mathbf{3}_{13}$. (DMSO-d_6) δ 1.38 (s, 3H, CH_3CH_2O); 2.48 (s, 3H, CH_3); 4.00 (s, 3H, OCH_3); 4.14 (q, 2H, CH_3CH_2O); 7.01–7.11 (m, 3H, H-3 quinoline and 2 ArH); 7.38–7.52 (m, 2H, ArH); 7.63 (t, 1H, ArH); 7.88–8.00 (m, 2H, ArH); 8.24 (d, 1H, ArH, $J_{orto} = 9.06$); 9.07 (s, 1H, CH=N); 9.30 (d, 1H, ArH, $J_{orto} = 8.6$); 10.98 (bs, 1H, NH, D_2O exchangeable).

3_{18}. (DMSO-d_6) δ 0.96 (t, 3H, $CH_3CH_2CH_2CH_2$–); 1.39 (st, 2H, $CH_3CH_2CH_2CH_2$–); 1.72 (qt, 2H, $CH_3CH_2CH_2CH_2$–); 2.81 (t, 2H, $CH_3CH_2CH_2CH_2$–); 4.06 (s, 3H, OCH_3); 7.13 (d, 1H, H-3 quinoline, $J_{3-2}=8.2$); 7.31 (bs, 1H, ArH); 7.52–7.79 (m, 5H, ArH); 7.93 (d, 1H, ArH, $J_{orto}=8.1$); 8.16 (s, 1H, ArH); 8.29 (d, 1H, H-2 quinoline, $J_{2-3}=8.2$); 8.98 (ud, 2H, 1ArH and CH=N); 10.95 (bs, 1H, NH, D_2O exchangeable).

3_{23}. (DMSO-d_6) δ 1.39–1.63 and 1.92–2.27 (2m, 10H, cyclohexyl); 2.51–2.72 (m, 1H cyclohexyl); 4.06 (s, 3H, OCH_3); 7.13 (d, 1H, H-3 quinoline $J_{3-2}=8.4$); 7.62–7.80 (m, 6H, ArH); 7.94 (d, 1H, ArH); 8.17 (ud, 1H, H-5 quinoline); 8.30 (d, 1H, H-2 quinoline, $J_{2-3}=8.4$); 9.01 (bs, 2H, ArH and CH=N); 11.70 (bs, 1H, NH, D_2O exchangeable).

3_{37}. (DMSO-d_6) δ 2.56 (s, 3H, CH_3); 6.10 (s, 2H, OCH_2O); 7.36 (dd, 2H, H-3 quinoline and 2 PhH); 7.46–7.79 (m, 4H ArH); 8.32 (s, 1H, CH=N); 10.51 (bs, 1H, NH, D_2O exchangeable).

Biological evaluation

In vitro evaluation of anti-tuberculosis activity. Primary screening was conducted at 12.5 μg/mL against *M. tuberculosis* H37Rv in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay.[28] Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system too.[29] Compounds showing $\geq$90% inhibition in the primary screening were considered active and then retested at lower concentration against *M. tuberculosis* H37Rv to determine the actual minimum inhibitory concentration (MIC), using MABA.

The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to the controls. Compounds were tested also for cytotoxicity (IC_{50}) in a VERO cell line at concentration equal to and greater than the MIC for *M. tuberculosis* H37Rv. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 Non-radioactive Cell Proliferation assay. The selectivity index (SI) was also determined, it is considered significant when >4. Rifampin (RMP) was used as reference compound.

Macrophage assay. Compounds with a MIC $\leq$6.25 μg/mL and a SI >10 were then tested to evaluate efficacy in vitro in a TB-infected macrophage model.[30] The EC_{90} and EC_{99} are defined as the concentrations effecting 90 and 99% reduction in residual mycobacterial growth after 7 days, compared to untreated controls. Compounds with $EC_{90}>16\times$MIC are considered inactive.

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