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Synthesis and antitubercular activity of 7-chloro-4-quinolinylhydrazones derivatives

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ABSTRACT

A series of twenty-one 7-chloro-4-quinolinylhydrazones (**3a**–**u**) have been synthesized and evaluated for their in vitro antibacterial activity against *Mycobacterium tuberculosis* $H_{37}Rv$. The compounds **3f**, **3i** and **3o** were non-cytotoxic and exhibited an important minimum inhibitory concentration (MIC) activity (2.5 μg/mL), which can be compared with that of the first line drugs, ethambutol (3.12 μg/mL) and rifampicin (2.0 μg/mL). These results can be considered an important start point for the rational design of new leads for anti-TB compounds.

Tuberculosis (TB) is still an important worldwide public health problem. According to statistics, more than two billion people, equal to one third of the world's total population, are infected with TB bacilli (*Mycobacterium tuberculosis*) and a total of 1.77 million people died from TB in 2007.[1]

The emergence of drug-resistant TB is an important fact that made the resurgence of TB especially alarming. According the World Health Organization (WHO), there are two types of resistant strains: multidrug-resistant TB (MDR-TB, resistant to isoniazid and rifampicin) and extensively drug-resistant TB (XDR-TB, resistant to all the most effective drugs). The spread of MDR-TB could cost between 100 and 1400 times the available treatment costs and further it threatens to make TB incurable. Currently, MDR-TB represents, on average, 5.3% of all TB cases. Furthermore, WHO estimates 490,000 MDR-TB cases emerge every year, with more than 110,000 deaths. The true scale of XDR-TB is unknown due to the lack of necessary equipment in many countries and capacity to accurately diagnose it. However, it is estimated that there are around 40,000 cases per year.[2]

Due to the high impact of MDR and recently XDR in TB treatment, there is an urgent need for new drugs to treat this disease efficiently. In this context, the quinoline nucleus is an important class of heterocyclic compounds found in many synthetic and natural products with a wide variety of pharmacological activities, such as antiviral, anticancer, antibacterial, antifungal, antiobesity and anti-inflammatory.[3]

Another important application of this nucleus in TB drug discovery is the diarylquinoline TMC207, which has a new mechanism of anti-TB activity, based on the inhibiting of mycobacterial adenosine triphosphate (ATP) synthase. This enzyme is responsible for energy source for the bacterium. The results of the phase 2 studies of this compound show that the adverse side effects of TMC207 are moderate and this drug may shorten the time of treatment for MDR-TB.[4]

Another reason to explore the potential antitubercular activity of quinoline derivatives is a recent study developed by our research group, which showed that some 7-chloro-4-amino-quinoline derivatives exhibited a significant activity (MIC = 12.5–3.12 μg/mL), when compared to first line drugs such ethambutol (MIC = 3.12 μg/mL).[5] In this study, we have observed that the chlorine atom at C-7 position in the quinoline nucleus is essential for the anti-TB activity. Because of that, we decided to synthesize different 7-chloro-quinoline compounds, which are described in this article.

In this context, in our continuous program in the search for new candidates to antitubercular agents, we proposed the synthesis of some hydrazones, containing the 7-chloro-quinoline moiety that was designed by molecular hybridization. Due to its synthetic and biological versatility, hydrazones are attractive target compounds

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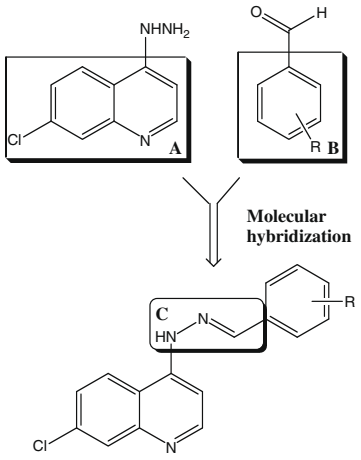


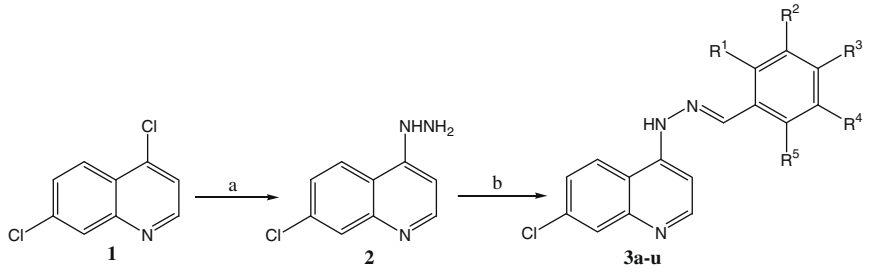
Figure 1. Design concept of 7-chloro-4-quinolinylhydrazones derivatives.

for new drug development. In literature, many pharmacological activities have been associated to hydrazones, such as antidepressant, anticonvulsant, anti-inflammatory, antimicrobial[6] and antitubercular activities.[7–12] For this reason, the design concept of these compounds explores the introduction of monosubstituted benzaldehydes moieties (**A**) into 7-chloro-quinoline core (**B**) to obtain hydrazones groups (**C**). This modification aims to investigate the influence of some substituents at the phenyl ring (**B**) on in vitro biological activity of these compounds (Fig. 1).

The synthetic route for the preparation of 7-chloro-4-quinolinylhydrazones derivatives **3a–u** is summarized in Scheme 1. Firstly, 7-chloro-4-hydrazinoquinoline **2** was prepared from 4,7-dichloroquinoline **1** using hydrazine hydrate (80%) in ethanol under reflux.[13] After that, the compounds **3a–u** were obtained through reaction between the compound **2** and appropriated benzaldehydes as it is described in the general procedure (Table 1).[14] In general, the ^{1}H NMR spectra showed the characteristic signal for the N=C*H* proton at 8.37–8.81 ppm. Furthermore, the IR spectra showed N–H and N=C stretching vibrations at 3197–3247 and 1570–1585 cm^{-1}, respectively.

The antimycobacterial activities of the derivatives **3a–u** were assessed against *M. tuberculosis* ATCC 27294[18] using the microplate Alamar Blue assay (MABA)[19] (Table 1). This methodology is nontoxic, uses a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[20,21] These results showed that compounds **3a–c**, **3e–g**, **3i–j**, **3m–o** and **3s** exhibited an antimycobacterial activity between 12.5 and 2.5 μg/mL. Therefore, these compounds were selected for evaluation of their cytotoxicities by Mosmanśs assay.

The cellular viability in the presence and absence of the test compounds **3a–c**, **3e–g**, **3i–j**, **3m–o** and **3s** was determined by Mosmanśs MTT (3-(4.5-demethylthylthiazol-2-yl)-2.5-dimethyl-



Scheme 1. Reagents and conditions: (a) $N_2H_4{\cdot}H_2O$ (80%), EtOH, 80 °C, 2 h, 80%; (b) corresponding benzaldehyde, EtOH, rt, 4–24 h, 64–91%.

Table 1
Antimycobacterial activities, melting points, *c*logP measurements, and yields of 7-chloro-4-quinolinylhydrazones derivatives **3a–u**

Entry	Substituents	Yield (%)	Mp (°C)	MIC [a] (μg/mL)	*c*Log *P* [b]
3a	R^1 = Cl; R^2 = R^3 = R^4 = R^5 = H	91	192–194[15]	6.25	6.28
3b	R^2 = Cl; R^1 = R^3 = R^4 = R^5 = H	64	240[15]	3.12	6.31
3c	R^3 = Cl; R^1 = R^2 = R^4 = R^5 = H	82	225–226[15]	12.5	6.33
3d	R^1 = Br; R^2 = R^3 = R^4 = R^5 = H	81	273–275	>100.0	6.41
3e	R^2 = Br; R^1 = R^3 = R^4 = R^5 = H	77	189–191	3.12	6.44
3f	R^3 = Br; R^1 = R^2 = R^4 = R^5 = H	84	198–199	2.50	6.46
3g	R^1 = F; R^2 = R^3 = R^4 = R^5 = H	74	234–236[15]	3.12	5.77
3h	R^2 = F; R^1 = R^3 = R^4 = R^5 = H	80	225[15]	>100.0	5.79
3i	R^3 = F; R^1 = R^2 = R^4 = R^5 = H	74	245–246[15]	2.50	5.82
3j	R^1 = OH; R^2 = R^3 = R^4 = R^5 = H	82	233–235[16]	2.50	5.59
3k	R^2 = OH; R^1 = R^3 = R^4 = R^5 = H	79	270	>100.0	5.15
3l	R^3 = OH; R^1 = R^2 = R^4 = R^5 = H	80	219–220	6.25	5.17
3m	R^1 = OMe; R^2 = R^3 = R^4 = R^5 = H	82	186–188	3.25	5.66
3n	R^2 = OMe; R^1 = R^3 = R^4 = R^5 = H	77	117–119	3.25	6.87
3o	R^3 = OMe; R^1 = R^2 = R^4 = R^5 = H	85	144–145	2.5	5.71
3p	R^1 = NO_2; R^2 = R^3 = R^4 = R^5 = H	72	253	>100.0	5.56
3q	R^2 = NO_2; R^1 = R^3 = R^4 = R^5 = H	76	281[17]	>100.0	5.59
3r	R^3 = NO_2; R^1 = R^2 = R^4 = R^5 = H	70	188–190	>100.0	5.61
3s	R^2 = CN; R^1 = R^3 = R^4 = R^5 = H	77	212–214	6.25	5.38
3t	R^3 = CN l; R^1 = R^2 = R^4 = R^5 = H	82	230–231	>100.0	5.41
3u	R^1 = R^2 = R^3 = R^4 = R^5 = H	70	223–225[13]	>100.0	5.65
Ethambutol	—	—	—	3.25	−0.72

[a] Minimum inhibitory concentration.
[b] Calculated using www.molinspiration.com.

Table 2
Data of the cellular viability for a macrophage cell line J774 (ATCC TIB-67™) by Mosmanśs assay

Compound	% Cell viability/dose (μg/mL)		
	1	**10**	**100**
3a	83	7	3
3b	100	5	6
3c	100	100	96
3e	88	60	3
3f	100	100	91
3g	77	5	3
3i	100	100	97
3j	100	97	88
3l	86	5	3
3m	100	4	3
3n	87	4	2
3o	100	100	100
3s	89	4	4
Ethambutol	**100**	**93**	**82**

Table 3
Data of the cellular viability for a macrophage cell line J774 infected (ATCC TIB-67™) with BCG by Mosmans's assay

Compound	% Cell viability/dose (μg/mL)		
	1	**10**	**100**
3c	100	100	90
3f	100	100	85
3i	100	100	93
3o	100	100	88
Ethambutol	**92**	**88**	**85**

tetrazolium bromide; Merck) microcultured tetrazolium assay.[22,23] The results were represented as percentage cell viability (Table 2).

This table shows that the compounds **3b, 3c, 3d, 3i** and **3o** did not kill more than 5% of the host cells in the minimum concentration tested. Hence, these compounds were selected to be tested on macrophages infected with *Mycobaterium bovis* Bacillus Calmette-Guerin (BCG) (Table 3).

The purpose of this test is to evaluate the action of these compounds against macrophages that show their metabolism changed after infection. Then, all the derivatives **3c**, **3d**, **3i** and **3o** were not cytotoxic once they did not kill more than 5% of the cells at the minimum concentration tested. It is important be mentioned that theses compounds are less cytotoxic towards normal cells than the first line drug ethambutol in 1 and 10 μg/mL.

In conclusion, the synthesis of twenty-one 7-chloro-4-quinolinylhydrazones derivatives **3a–u** was performed in good yields (64–91%). Among them eleven are new compounds (**3d–f**, **3k–n**, **3p** and **3r–t**). All these compounds were submitted to antimycobacterial activity evaluation and twelve derivatives (**3a–c**, **3e–g**, **3i–j**, **3m–o** and **3s**) exhibited MIC between 12.5 and 2.5 μg/mL. Therefore, these compounds were selected to evaluation of their cytotoxicities by Mosmansś assay with non-infected and BCG-infected macrophages. Among these derivatives, only **3c**, **3f**, **3i** and **3o** were not cytotoxic to host cells in the effective concentrations to inhibit the growth *M. tuberculosis*. Furthermore, the compounds **3f**, **3i** and **3o** exhibited a significant activity (2.5 μg/mL) when compared with first line drugs such as ethambutol (MIC = 3.12 μg/mL) and could be considered a good start point to find new lead compounds in the fight against multidrug-resistant tuberculosis.

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