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Tuberculosis: Finding a New Potential Antimycobacterium Derivative in a Aldehyde–Arylhydrazone–Oxoquinoline Series

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Abstract Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis*, which remains a serious public health problem. The emergence of resistant bacterial strains has continuously increased and new treatment options are currently in need. In this work, we identified a new potential aldehyde–arylhydrazone–oxoquinoline derivative (**4e**) with interesting chemical structural features that may be important for designing new anti-TB agents. This 1-ethyl-*N*′-[(1E)-(5-nitro-2-furyl)methylene]-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**4e**) presented an in vitro active profile against *M. tuberculosis* H37Rv strain (minimum inhibitory concentration, MIC = 6.25 μg/mL) better than other acylhydrazones described in the literature (MIC = 12.5 μg/mL) and close to other antitubercular agents currently on the market. The theoretical analysis showed the importance of several structural features that together with the 5-nitro-2-furyl group generated this active compound (**4e**). This new compound and the analysis of its molecular properties may be useful for designing new and more efficient antibacterial drugs.

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Introduction

Tuberculosis (TB) is a serious contagious disease caused by *Mycobacterium tuberculosis* also known as Koch bacillus. The disease usually affects the lungs, but occasionally may also occur in other body organs presenting no lung damage [1]. Today, TB remains a serious public health problem. The World Health Organization (WHO) estimates that about one-third of the population in the world is infected with the bacillus, often causing no symptoms. Among the new cases of TB reported, 80 % occur in developing countries where TB occupies a prominent position among the most important infectious diseases suffered with resistant strains [1, 5, 6, 13, 14].

Chemotherapy has been used in the treatment of TB since the 1940s. Since then, a few agents have been discovered including *p*-aminosalicylic acid (PAS), isoniazide (INH), pyrazinamide (PZA), cycloserine, ethionamide, rifampicin (RPM), and ethambutol (EMB) [13, 27]. Treatment of TB usually starts with a first-line drug that includes RPM, INH, EMB, and PZA. The second-line drugs (streptomycin, ethionamide, cycloserine, amikacin, PAS, thioacetazone, and terizidone) are used when the first treatment fails. Oxoquinoline derivatives such as ciprofloxacin, moxifloxacin, and levofloxacin are also included

in the strategy for treatment of multiple drug-resistant TB (MDR-TB) [2, 27, 30, 34].

The main problems in the treatment of TB include not only the emergence of multiresistant bacilli (MDR) caused by using such drugs, but also the length of treatment and the adverse effects (i.e., nausea, vomiting, jaundice, asthma, visual impairment, peripheral neuropathy, abdominal pain, and even blindness) [13]. In fact, the emergence of MDR-TB reinforced the urgent need for new anti-TB drugs.

The literature describes *N*-acylhydrazone derivatives 1–3 (*NAH*) with anti-TB activity [8–10, 12, 17, 19] (Fig. 1).

Aiming to identify some *NAH* structural features that may guide rational design for more effective anti-TB agents [8–10], this work explores the addition of different aromatic rings (e.g., phenyl, bromothienyl, pyridyl, furyl and nitrofuryl) to the imine moiety in the new aldehyde–arylhydrazone–oxoquinoline derivatives (**4a–i**). In addition, since oxoquinolines are described as important for the antimicrobial profile of some molecules (i.e., ciprofloxacin), in this work we also explored the stereoelectronic effect linked to the *NAH*–pharmacophoric group at C-3 position of the oxoquinoline ring of these new derivatives (Fig. 2).

Thus, we have synthesized and tested nine acylhydrazone derivatives (**4a–i**) against the *M. tuberculosis* strain. In addition, we have performed a molecular modeling study to gain insight into the stereoelectronic properties, which are important for their biological activity.

Materials and Methods

Chemistry

Melting points were determined through a Fisher–Johns apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer FT-IR 1600 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. NMR spectra were recorded on a Varian Unity Plus 300 spectrometer operating at 300.00 MHz (^1H) and 75.0 MHz (^{13}C), in the specified solvents. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Hydrogen and carbon NMR spectra were typically obtained at room temperature. The two-dimensional experiments were acquired by means of standard Varian Associates automated programs for data acquisition and processing. High Resolution Mass Spectra were obtained on a Waters-Micromass QTOF/Micro spectrometer (See Supplementary Material).

Biological Assays

In vitro anti-TB activities of compounds **4a–i** were assessed against *M. tuberculosis* H37Rv strain (ATCC 27294), susceptible to both RPM and INH. The tests were carried out by means of the Microplate Alamar Blue assay [7, 22, 31] and RPM was used as positive control and presented minimum inhibitory concentration (MIC) = 1 μg/mL. The MIC (μg/mL) was the lowest drug concentration that prevented growth (Fig. 3).

Molecular Modeling

All structure–activity relationship (SAR) computations were performed by means of SPARTAN'08 (Wavefunction Inc., Irvine, CA, 2000). The structures were optimized to a local minimum and the equilibrium geometry obtained in vacuum using the semiempirical RM1 module. Afterward, we calculated the electronic and chemical properties for all compounds' best conformation as described elsewhere [16, 20]. The theoretical study of toxicity, physical–chemical parameters, and druglikeness and drug-score were performed by means of the Osiris Property Explorer, whereas the Lipinski rule of five was determined by means of the Molinspiration programs as described elsewhere [16, 20, 29]. Druglikeness evaluates if the molecule contains the best fragments predominantly, commonly present in commercial drugs. The drug-score combines druglikeness, clog

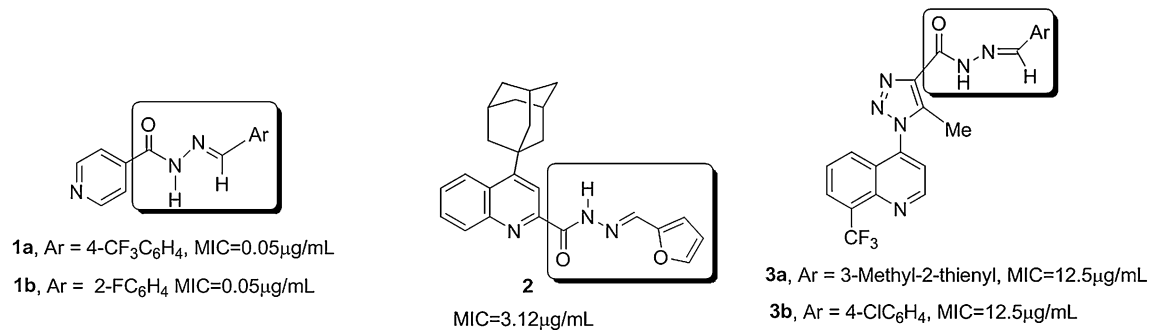


Fig. 1 *NAH* with antimycobacterial activity

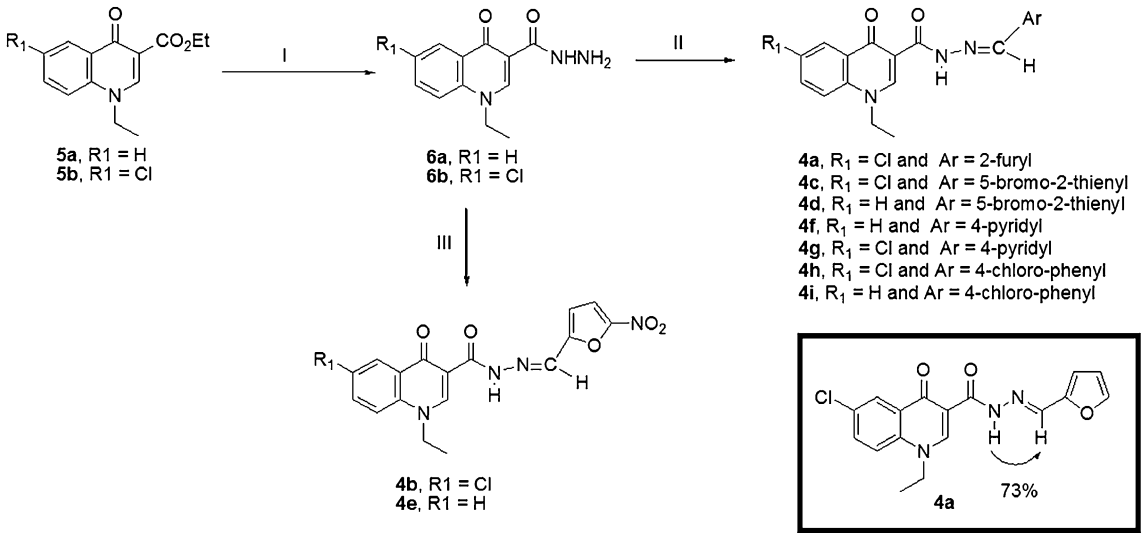
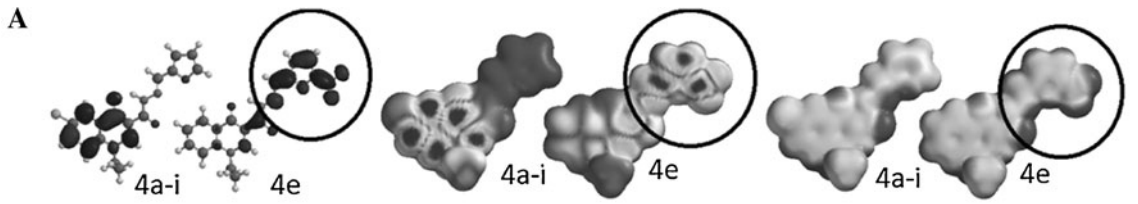


Fig. 2 Synthetic pathways used for **4a–i**. Route *I* hydrazine monohydrate 80 %, ethanol, reflux 2 h; Route *II* ArCHO, HCl (ca.), ethanol, 1 h; Route *III* (5-nitro-2-furyl)methylene diacetate, EtOH/H_2SO_4 50 %, 1 h. *Inset* nOe observed for compound **4a**



B

#	MIC	Dipole	TPSA[c]	PSA[c]	N+O[c]	Nrotb	Log S[b]	Lipinski Rule of Five			
								cLogP[a]	MW	nHBA	nHBD
4a-4i	>100	7.04-11.12	61.77-120.74	44.01-91.15	6-10	4-6	-3.87 - -6.26	1.99-3.87	322.37-440.75	5-9	1
4e	6.25	12.59	122.43	92.11	10	6	-5.33	1.91	368.35	9	1

Fig. 3 Structural (**a**), biological and theoretical (**b**) comparison of the active compound **4e** with the inactive aldehyde–arylhydrazone–oxoquinoline derivatives (**4a–i**). **a** LUMO distribution (*left*), LUMO density maps (*center*), and electrostatic potential map (*dark gray* negative region) (*right*) at same orientation with the main structural differences marked with a *circle*. **b** MIC (μg/mL) against *M. tuberculosis* strain compared to different theoretical parameters including dipole moment (dipole, Debye), PSA (Å^2), TPSA (Å^2), lipophilicity (log *P* and clog *P*), water solubility (log *S*), Nrotb, sum of H-bond acceptors and donors (N + O), MW (g/mol), nHBA and nHBD. [a]Metoprolol (clog $P = 1.48$, log $P = 1.72$) is known to be 95 % absorbed from human gastro-intestinal tract. Thus, compounds with log $P > 1.72$ and clog $P > 1.48$ are categorized as high-permeability. [b]All compounds tested have lower water solubility (log *S*) values than metoprolol (−2.09). Thus, according to Dahan et al. [4], using the Provisional BCS classification based on in silico calculations, we may classify **4e** as a Class II drug (high-permeability with low solubility). [c]The compounds with TPSA <140 Å^2 and sum of H-bond acceptors and donors (N + O) <12 present bioavailability >20 % [32]. To compounds with bioavailability (*F*) >10 %, if PSA ≤75, 75–150, or ≥150 Å^2, $F = 85$ %, 56 %, or 11 %, respectively, according to Martin [18] (Color figure online)

P, log *S*, molecular weight (MW), and toxicity risks in one value to judge the compounds' overall theoretical potential to qualify for a drug.

Results and Discussion

The synthesis of the new aldehyde–arylhydrazone–oxoquinoline derivatives **4a–i** is described in Fig. 2. The ethyl 1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**5a**) and ethyl 6-chloro-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (**5b**) were prepared by standard procedures [24, 26]. Carbohydrazides **6a** and **6b** were prepared in moderated yields by the condensation of **5a–b** with hydrazine monohydrate in refluxing ethanol [11].

The new aldehyde–arylhydrazone–oxoquinoline derivatives **4a–i** were prepared by reacting carbohydrazides **6a–b** and suitable aromatic aldehydes in ethanol using hydrochloric acid as catalyst. 5-nitrofuran derivatives **4c** and **4e** were obtained by the condensation of compounds

6a–b with commercially available (5-nitro-2-furyl)-methylene diacetate in a mixture of ethanol/sulfuric acid 50 %.

The new compounds **4a–i** were purified by recrystallization from ethanol and fully characterized by spectroscopic analysis. Regarding their ^{1}H NMR spectra, it is important to highlight the following: The singlet between 9.01 and 8.96 ppm refers to H-2 oxoquinolinic hydrogen; the singlet at 8.64–8.46 ppm was attributed to hydrogen H-1′ of the acylhydrazone moiety.

The signals of oxoquinolinic hydrogens were observed between 8.54 and 7.74 ppm. The quartet in 4.58–4.56 ppm ($J = 6.9$ Hz) and triplet in 1.44–1.42 ppm ($J = 6.9$ Hz) were attributed to the *N*-ethyl group hydrogens. The ^{1}H NMR spectra of these derivatives also detected the presence of only one of the diastereoisomers, showing only one singlet between 8.64 and 8.46 ppm referred to the N–H signal of the imine double bound. It is known that the (*E*)-diastereoisomers of acylhydrazone derivatives are the most stable ones [3].

In order to assure the stereochemistry of the imine double bound, the nuclear Overhauser effect (nOe) experiment was performed, irradiating the N–H hydrogen signal at 12.93 ppm of compound **4a**, resulting in a nOe-enhancement of N=CH hydrogen singlet at 8.46 ppm (73 %). X-ray data for the derivative **4f** [25] also confirmed the (*E*)-stereochemistry for the imine double bound of the acylhydrazone derivatives **4a–i**. Despite the possibility of formation of two diastereoisomers, only the (*E*)-isomer was isolated from the reactions (Fig. 2).

Aldehyde–arylhydrazone–oxoquinoline derivatives **4a–i** were initially screened against *M. tuberculosis* H37Rv (ATCC 27294) at 100 μg/mL, but **4a–d** and **4f–i** presented no significant anti-TB activity. Only 1-ethyl-*N*′-[(1E)-(5-nitro-2-furyl)methylene]-4-oxo-1,4-dihydroquinoline-3-carbohydrazide (**4e**) was effective against *M. tuberculosis* (Fig. 3). The determination of the MIC of **4e** showed a value (6.25 μg/mL) better than some described in the literature and similar to others current on the market (Figs. 1, 3). Our compound has as much potential as some molecules reported in the latest literature including acetyl and propionyl group substituted thiadiazole derivatives (6.25 μg/mL) and arylideneamino triazolethiones (4 and 8 μg/mL) [21, 23, 28]. Thus, **4e** structure points to a new antitubercular design that may be further explored together with other active groups to create new active molecules and treatment options against resistant strains.

In the effort to identify some structural features important to anti-TB activity of **4e** (SAR), we initially evaluated some stereoelectronic and physical–chemical properties such as clog *P*, MW, molecular volume, topological polar surface area (TPSA), number of hydrogen bond acceptors (nHBAs) and donors (nHBDs), HOMO and LUMO energy, dipole moment, and number of rotatable bonds (Nrotb) (Fig. 3). The overall analysis suggested that the anti-TB activity of **4e** is not directly related to only one stereoelectronic parameter (Fig. 3). Apparently, different physical–chemical features (i.e., dipole, TPSA, log *P*, polar surface area [PSA], and Nrotb) seem to contribute all together to the active structure of **4e** since this compound presents some of the highest or lowest values of the series depending on the parameter evaluated (Fig. 3).

In this work, we analyzed R1 and Ar positions and the effect of the nitrogen atom of the quinoline nucleus to evaluate their role as pharmacophoric groups (Fig. 3). The comparison among four series, 1, 2, and 3 pointed out the insertion of the ethyl substituent in the nitrogen atom of quinoline nucleus as possibly involved in the lack of antitubercular activity of most compounds probably due to steric hindrance effects. Interestingly, the ethyl-quinoline ring without R1 substitution and the 5-nitro-2-furyl group at Ar in the active compound (**4e**) seem to be important for the antitubercular activity. Apparently these structural features allow not only the LUMO density distribution concentrated in the ethyl group, but also a higher dipole moment, TPSA, and PSA that could help **4e** in interacting with the bacterial target (Fig. 3) [33]. These **4e** features are structural parameters that should be considered when designing new antitubercular molecules. In addition, a further exploring of other substituents for R1 of the quinoline nucleus may help to perform new SAR studies in the future.

Our theoretical evaluation regarding **4e** toxicity and drug profile similarities revealed a low theoretical toxicity profile and positive druglikeness and drug-score with values similar or better than commonly used drugs for treating TB (e.g., RPM, EMB, PZA, and INH) (Fig. 4).

The Lipinski et al. [15] rule of five is a widely used filter which may indicate if a compound present good theoretical oral bioavailability. Other different parameters were also used (i.e., TPSA, Nrotb, and PSA) to help in predicting oral absorption and bioavailability [18, 20, 32]. The theoretical Biopharmaceutics Classification System (BCS) was described and incorporates models for both solubility and permeability. These data may help the strategy for drug development, for instance, regarding choice of pharmaceutical dosage form [4]. The theoretical analysis of the active compound **4e** revealed that: (a) it fulfills the Lipinski rule of five (clog $P \leq 5$, MW ≤ 500, nHBAs ≤ 10, and nHBDs ≤ 5), (b) it belongs to the BCS Class II drugs (high-permeability and low solubility compared to metoprolol), and (c) it presents theoretical bioavailability >20 % (Fig. 3). All these theoretical data together reinforced the potential profile of this molecule as a feasible antitubercular prototype.

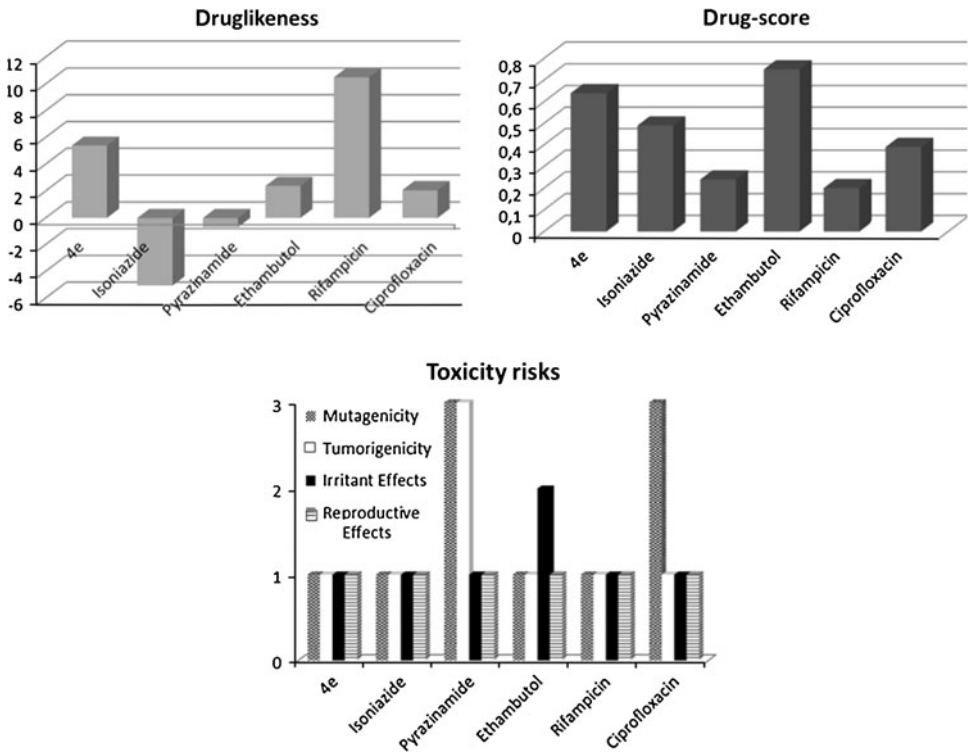


Fig. 4 ADMET parameters (druglikeness, drug-score and toxicity risks) evaluation of **4e** oxoquinoline derivative compared to antitubercular drugs currently on the market including INH, PZA, EMB, RPM, and ciprofloxacin

Conclusion

A new series of aldehyde–arylhydrazone–oxoquinoline derivatives **4a–i** has been synthesized and evaluated for their activity against *M. tuberculosis H37Rv* strain. The compound **4e** showed a significant in vitro biological profile against *M. tuberculosis.* The theoretical analysis pointed to the presence of the nitro group attached to the furan ring; the high values of Dipole, TPSA, log *P*, PSA, and Nrotb; and LUMO and electrostatic distribution as some of the important structural features to be considered for further exploring in designing new biological active structures. In summary, according to the biological assay data and the SAR study, **4e** is a potential antitubercular prototype for further experimental investigation.

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