

Article

Development of Novel Isatin-Tethered Quinolines as Anti-Tubercular Agents against Multi and Extensively Drug-Resistant *Mycobacterium tuberculosis*

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Abstract: We describe the design and synthesis of two isatin-tethered quinolines series (**Q6a–h** and **Q8a–h**), in connection with our research interest in developing novel isatin-bearing anti-tubercular candidates. In a previous study, a series of small molecules bearing a quinoline-3-carbohydrazone moiety was developed as anti-tubercular agents, and compound **IV** disclosed the highest potency with MIC value equal to 6.24 µg/mL. In the current work, we adopted the bioisosteric replacement approach to replace the 3,4,5-trimethoxy-benzylidene moiety in the lead compound **IV** with the isatin motif, a privileged scaffold in the TB drug discovery, to furnish the first series of target molecules **Q6a–h**. Thereafter, the isatin motif was *N*-substituted with either a methyl or benzyl group to furnish the second series **Q8a–h**. All of the designed quinoilne-isatin conjugates **Q6a–h** and **Q8a–h** were synthesized and then biologically assessed for anti-tubercular actions towards drug-susceptible, MDR, and XDR strains. Superiorly, the *N*-benzyl-bearing compound **Q8b** possessed the best activities against the examined *M. tuberculosis* strains with MICs equal 0.06, 0.24, and 1.95 µg/mL, respectively.

Keywords: quinolone; hydrazone; anti-mycobacterial activity; *Mycobacterium* resistance

1. Introduction

Tuberculosis (TB) remains one of the world's most lethal infectious illnesses, killing an estimated 1.5 million people each year [1]. In 2018, the WHO reported a range of estimated incident cases between 9.0 and 11.1 million patients in addition to notifying 7.0 million new cases of TB [2]. The gap between the estimated incident cases and the new ones is obvious, showing the large global range of TB patients lacking the access to health care [3,4]. Furthermore, the situation worsened as a result of multiple aggravating factors, including difficulty in obtaining therapy, as well as the poor tolerability and efficacy of therapeutic regimens due to the administration of long-term drug combinations consisting of 1st-line medications (isoniazid, pyrazinamide, rifampicin, and ethambutol) alongside the more toxic, expensive, and less efficacious second-line agents (fluoroquinolones, aminoglycosides, and clofazimine). These drug protocols cause anti-tuberculosis drug-induced hepatotoxicity (ATDH), which causes significant morbidity and mortality while decreasing treatment effectiveness. Finally, incomplete treatment reduces effectiveness and eventually

contributes to failure and relapse, which complicates disease resolution and results in serious TB drug resistance.

There is currently a substantial amount of information available regarding the spread of *M. tuberculosis* strains that are resistant to both first-line and second-line drugs for tuberculosis treatment. [5]. This resistance was studied and defined by the WHO into two types known as multi-drug-resistant tuberculosis (MDR-TB) that is considered to be resistant to rifampicin or isoniazid, and the extensively drug-resistant (XDR) tuberculosis that is able to resist also at least fluoroquinolone as well as either kanamycin or amikacin [6]. According to the World Health Organization (WHO), there were approximately 500,000 newly reported cases of rifampicin resistance (RR-TB) in 2018. Of these, 78% of the patients were MDR TB and 8.5% were XDR TB [2]. For patients suffering from XDR-TB, the efficiency of the standard treatments was quite limited. New hopes were provided through the approval of bedaquiline by the FDA. Rapidly, the failure of the clinically introduced drug by its first resistant *M. tuberculosis* isolate dramatically terminated the story [1]. As a result, the creation of novel anti-TB medications is urgently required. Novel drug candidates need to be able to shorten the therapeutic time period and simplify the actual treatment protocols, to enhance tolerance and to be effective towards resistant strains (MDR-TB and XDR-TB).

Quinoline, a fused benzo[*b*]pyridine heterocycle, stands out as one of the most useful and versatile scaffolds endowed with different antibacterial activities. Interestingly, several quinoline-based anti-infectives are currently in clinical use as the antibacterial drug ciprofloxacin I (Figure 1), in addition to the recently identified anti-tubercular medications bedaquiline II and mefloquine III (Figure 1) [7–10]. Moreover, several research teams have reported a different series of small molecules tethered with the quinoline scaffold as promising anti-tubercular candidates [11–19]. In a recent study, novel small molecules bearing a quinoline-3-carbohydrazone moiety were synthesized and examined for the anti-tubercular impact on the drug-susceptible *M. tuberculosis* strain. The developed molecules exhibited moderate anti-tubercular activity and a disclosed MIC range of 6.24–99.84 $\mu\text{g}/\text{mL}$. Within this series, quinoline-derivative IV (Figure 1) disclosed the highest activity with a MIC value equal to 6.24 $\mu\text{g}/\text{mL}$ [20].

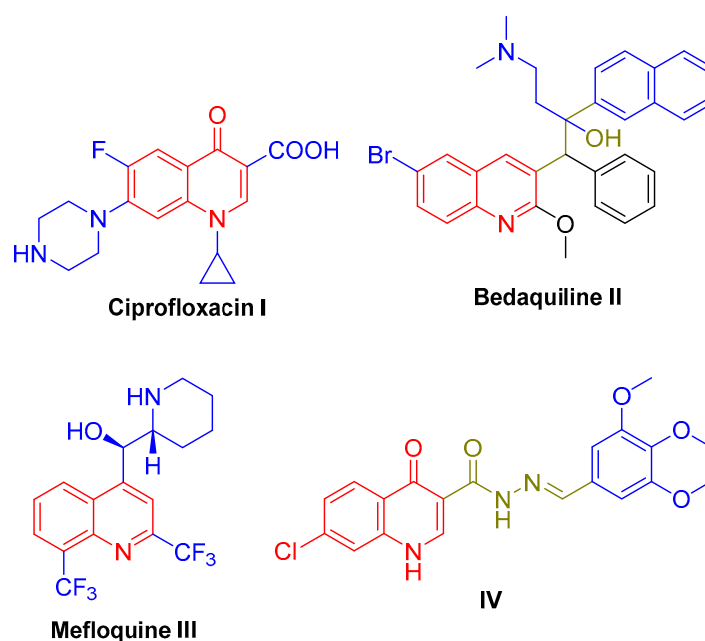


Figure 1. Structure for certain quinoline-based anti-tubercular drugs I–III, as well as the lead anti-tubercular compound IV.

Isatin is an endogenous motif that has been identified in many organisms. Isatin is found in numerous naturally occurring substances, including marine natural products, alkaloids, and fungal metabolites, in addition to being endogenously present in mammalian tissues and fluids, including those of humans and other mammals [21]. Isatin nucleus represents a versatile scaffold for chemical modifications, where its derivatives disclosed diverse pharmacological actions for the treatment of diverse diseases and disorders [21–27]. Isatin-tethered molecules such as nintedanib, sunitinib, semaxanib, and orantinib have either received clinical approval (nintedanib and sunitinib) or are currently the subject of clinical trials (semaxanib and orantinib) [28,29]. Such successful clinical applications, as well as their broad-spectrum pharmacological activities and the ease of the structural modifications, inspired and paved the way for the researchers to create a lot of isatin derivatives with significant structural diversity. In the last two decades, the isatin motif has attracted the researchers' attention as a promising privileged skeleton that could be exploited for the discovery and development of tuberculosis medications. Accordingly, a large number of isatin-based series were developed and biologically explored for their potential anti-tubercular actions towards drug-susceptible strains as well as towards drug-resistant TB strains [30–36].

In light of the aforementioned findings, and following up on our earlier efforts to identify promising anti-tubercular candidates [35–41], we decided to broaden our investigation by bioisosterically replacing the 3,4,5-trimethoxy benzylidene moiety in lead compound IV with the privileged isatin motif to afford the first series of targeted molecules Q6a–h (Figure 2). Furthermore, the isatin motif in series Q6 was *N*-substituted with either a methyl or benzyl group to furnish the second series of target molecules Q8a–h (Figure 2). All of the designed quinoline isatin conjugates Q6a–h and Q8a–h were synthesized and then biologically assessed for their anti-tubercular actions towards drug-susceptible MDR and XDR T.B. strains.

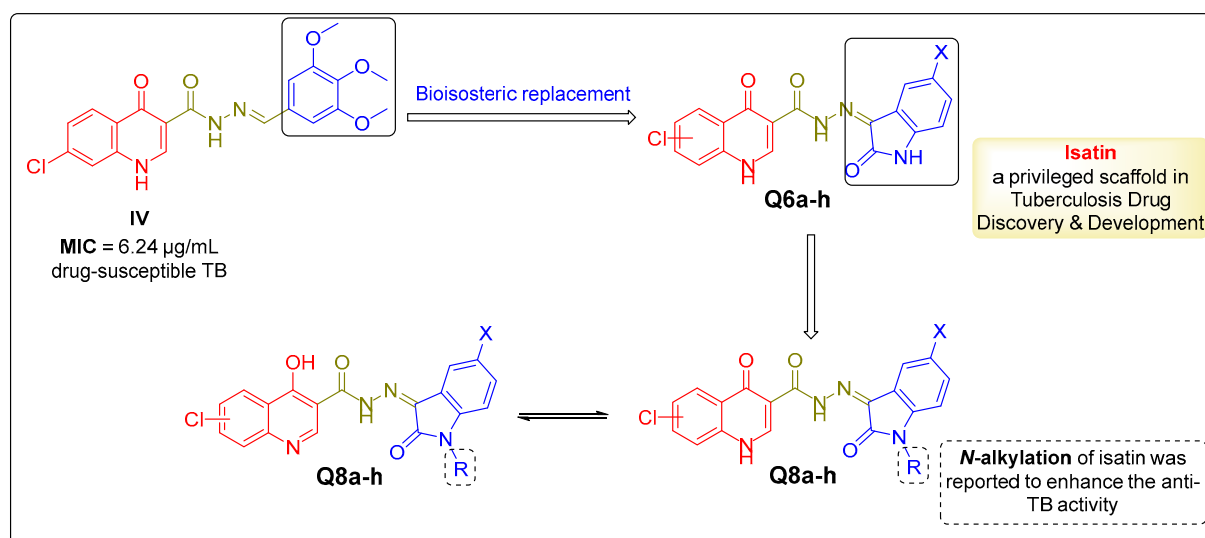


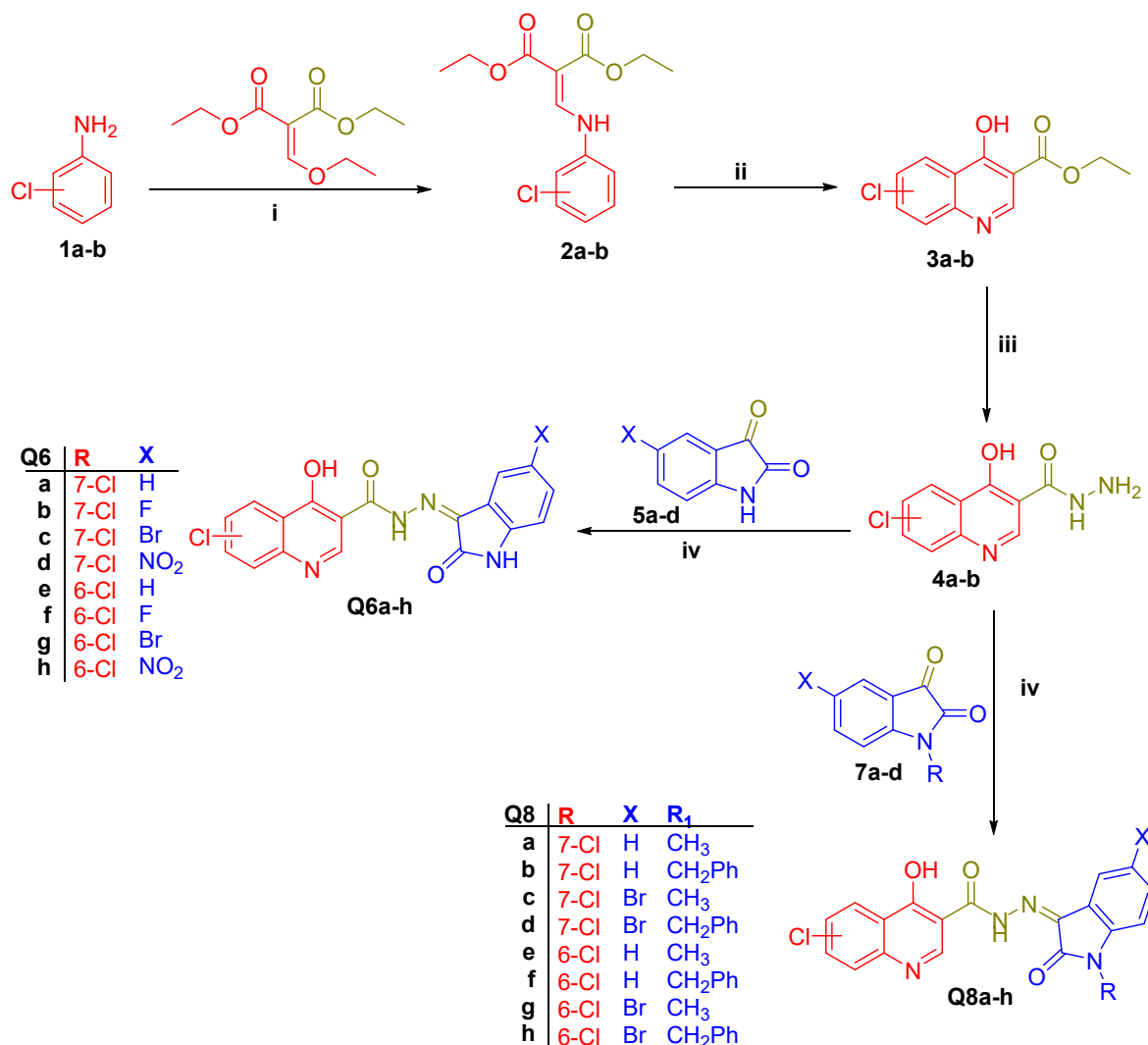
Figure 2. Design for the target quinoline-based molecules Q6a–h and Q8a–h.

2. Results and Discussion

2.1. Synthetic Chemistry

The presented Scheme 1 illustrates the synthetic approach used to create the novel desired quinoline-isatin conjugates Q6a–h and Q8a–h. Ethyl anilinomethylene malonate 2a–b was prepared through the condensation of 3-chloroaniline 1a and 4-chloroaniline 1b with diethyl ethoxymethylenemalonate. Then, the cyclization of 2a–b in refluxing diphenyl ether produced the corresponding ethyl esters of 4-hydroxyquinoline-3-carboxylic acids 3a–b, which then interacted with hydrazine hydrate (NH₂NH₂·H₂O, 99%) in refluxing ethanol to produce the acid hydrazides 4a–b. Finally, the target quinoline-isatin conjugates

Q6a–h and **Q8a–h** were obtained by the condensation reaction of hydrazides **4a–b** with diverse 5-substituted isatins (**5a–d**) or *N*-substituted isatins (**7a–d**), respectively, in glacial acetic acid under refluxing temperature.



Scheme 1. Synthetic route for target quinoline-isatin conjugates **Q6a–h** and **Q8a–h**; i: reflux 3 hours; ii: phenyl ether, reflux, 2 hours; iii: NH₂NH₂·H₂O, reflux, 12 hours; and iv: glacial acetic acid, reflux, 4 hours.

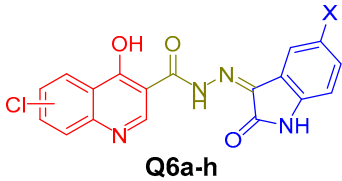
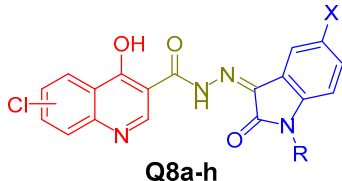
The IR spectra for quinoline-isatin conjugates **Q6a–h** and **Q8a–h** revealed absorption bands for the carbonyl functionality within the region 1629–1715 cm⁻¹, beside the absorption bands that assigned to the amino (NH) functionalities in the 3249–3413 cm⁻¹ region. The ¹H NMR spectra for conjugates **Q6a–h** and **Q8a–h** exhibited two D₂O-exchangeable singlets corresponding to the –CONH proton of Z and E isomers within the region δ 10.55–11.74 ppm, whereas another two D₂O exchangeable singlet signals at δ 13.71–15.19 ppm were assigned for the Z and E isomers of OH quinoline. In addition, the NH protons of the isatin motif for derivatives **Q6a–h** were identified around δ 8.88–9.39 ppm as a singlet signals. The ¹³C NMR spectrum for conjugate **Q6b** disclosed characteristic signals resonating around δ 161.51–176.14 ppm that were attributable to the Z and E isomers of carbonyl groups carbons, while compounds **Q6f** and **Q6g** showed one isomer only, and the carbons of carbonyl (C=O) functionality appeared in the region δ 161.54–175.46 ppm.

2.2. Biological Evaluation

2.2.1. Anti-Tubercular Effect for Quinolines Q6a–h and Q8a–h

The anti-tubercular activities for quinoline derivatives **Q6a–h** and **Q8a–h** towards RCMB 010126 *M. tuberculosis* were examined exploiting the microplate Alamar blue (MABA) protocol [42]. The used reference anti-tubercular drug was isoniazid. Table 1 summarises the anti-mycobacterial results, which are expressed as minimum inhibitory concentrations (MICs).

Table 1. Anti-tubercular activity for the synthesized quinolines (**Q6a–h**) and (**Q8a–h**) against *M. tuberculosis* (MIC).

Comp.	 Q6a-h		X	 Q8a-h		MIC
	Cl	R				
Q6a	7-Cl	H	–	0.48		
Q6b	7-Cl	F	–	0.24		
Q6c	7-Cl	Br	–	31.25		
Q6d	7-Cl	NO ₂	–	15.63		
Q6e	6-Cl	H	–	3.9		
Q6f	6-Cl	F	–	3.9		
Q6g	6-Cl	Br	–	7.81		
Q6h	6-Cl	NO ₂	–	7.81		
Q8a	7-Cl	H	–CH ₃	0.24		
Q8b	7-Cl	H	–CH ₂ –C ₆ H ₅	0.06		
Q8c	7-Cl	Br	–CH ₃	0.98		
Q8d	7-Cl	Br	–CH ₂ –C ₆ H ₅	15.63		
Q8e	6-Cl	H	–CH ₃	1.95		
Q8f	6-Cl	H	–CH ₂ –C ₆ H ₅	0.98		
Q8g	6-Cl	Br	–CH ₃	1.95		
Q8h	6-Cl	Br	–CH ₂ –C ₆ H ₅	0.12		
Isoniazide				0.12		

The outcomes demonstrated that the majority of the quinolines reported here had potent to moderate anti-*M. tuberculosis* activity, with the MICs range between 0.06 and 7.81 µg/mL, with an exception for quinolines **Q6c** and **Q6d** (MIC = 31.25 and 15.63 µg/mL, respectively). Isatin-tethered derivative **Q8b** outperformed the lead compound **IV** in terms of growth inhibition towards *M. tuberculosis*, displaying a MIC of 0.06 µg/mL, representing a 100-fold increase in activity (MIC for **IV** = 6.24 µg/mL). Moreover, compound **Q8h** also has potent anti-mycobacterial action with a MIC value of 0.12 µg/mL that equals the MIC for the standard isoniazide drug. In addition, quinolines **Q6a**, **Q6b**, **Q8a**, and **Q8f** exhibited potent anti-tubercular activity with MIC values in the range of 0.24–0.98 µg/mL, whereas quinolines **Q6e–Q6h**, **Q8c**, **Q8e**, and **Q8g** displayed moderate action with a range for the MIC values between 1.95 and 7.81 µg/mL.

Studying the structural activity relationships of these quinoline-based hybrids revealed that their anti-tubercular actions have been influenced by two essential factors: the incorporation of a substituent at C-5 and N-1 of the oxindole motif, and the position of the chloro substituent (C-7 vs. C-6) at the quinoline moiety. With regard to the effect of the C-5 substitution within the oxindole motif within series **Q6**, it was found that grafting a 5-fluoro substituent resulted in an improved (for compound **Q6b**; MIC = 0.24 µg/mL) or maintained (for compound **Q6f**; MIC = 3.9 µg/mL) antitubercular impact in comparison to their unsubstituted analogues **Q6a** and **Q6e** (MICs = 0.48 and 3.9 µg/mL, respectively). The incorporation of 5-bromo (compounds **Q6c** and **Q6g**; MICs = 31.25 µg/mL and 7.81 µg/mL, respectively) or 5-nitro substituents (compounds **Q6d** and **Q6h**; MICs = 15.63 and 7.81 µg/mL,

respectively), on the other hand, has resulted in a decreased anti-tubercular action in comparison to the unsubstituted analogues **Q6a** and **Q6e**.

Furthermore, upon investigation of the impact of the decoration of the oxindole motif with a 5-bromo substituent within series **8**, we found that such substitution has led to a decreased activity for 7-chloroquinoline-bearing derivatives **Q8c** and **Q8d** (MICs = 0.98 and 15.63 µg/mL, respectively) with regard to their unsubstituted counterparts **Q8a** and **Q8b** (MICs = 0.24 and 0.06 µg/mL, respectively), whereas this substitution maintained or enhanced the anti-tubercular activity for the 6-chloroquinoline-bearing derivatives **Q8g** and **Q8h** (MICs = 1.95 and 0.12 µg/mL, respectively) in comparison to their unsubstituted analogues **Q8e** and **Q8f** (MICs = 1.95 and 0.98 µg/mL, respectively).

Thereafter, we explored the obtained results to assess the effect of the *N*-methylation and *N*-benzylation of the oxindole motif on the anti-tubercular activity of series **8**. The results revealed that *N*-methylation enhanced the activity of both 7-chloroquinoline-bearing derivatives **Q8a** and **Q8c** (MICs = 0.24 and 0.98 µg/mL, respectively) and 6-chloroquinoline-bearing derivatives **Q8e** and **Q8g** (MICs = 1.95 µg/mL and 1.95 µg/mL, respectively). Similarly, the *N*-benzylation of the oxindole motif improved the activity of both the 7-chloroquinoline-bearing molecules **Q8b** and **Q8d** (MICs = 0.06 and 15.63 µg/mL, respectively) as well as the 6-chloroquinoline-bearing derivatives **Q8f** and **Q8h** (MICs = 0.98 µg/mL and 0.12 µg/mL, respectively). It is worth mentioning that the *N*-benzylated derivatives **Q8b**, **Q8f**, and **Q8h** (MICs = 0.06 µg/mL, 0.98 µg/mL, and 0.12 µg/mL) displayed better anti-tubercular activity than their corresponding *N*-methylated counterparts **Q8a**, **Q8e**, and **Q8g** (MICs = 0.24, 1.95, and 1.95 µg/mL).

Finally, upon comparing the anti-tubercular effect of 7-chloroquinoline-bearing derivatives **Q6a–Q6d** against their 7-chloroquinoline-bearing counterparts **Q6e–Q6h**, it was found that the introduction of the chloro substituent at C-7 is more beneficial for the activity of hybrids containing the un- and 5-F substituted oxindole motif (compounds **Q6a** and **Q6b**; MIC = 0.24 and 0.48 µg/mL, respectively), whereas grafting the chloro substituent at C-6 was found to be more advantageous than C-7 for hybrids endowed with the larger 5-Br and 5-NO₂ substituents (compounds **Q6g** and **Q6h**; MIC = 7.81 µg/mL for both).

2.2.2. Anti-Tubercular Activities toward MDR and XDR T.B

The activity for the target quinolines **Q6a–h** and **Q8a–h** was assessed against the MDR strain (FJ05120) and the XDR strain (FJ05195). In Table 2, the assay outcomes are shown as minimum inhibitory concentration (MIC) values.

Table 2. Anti-tubercular activities for the target quinolines (**Q6a–h**) and (**Q8a–h**) against MDR and XDR *M. tuberculosis*.

Comp.	MIC	
	MDR (FJ05120)	XDR (FJ05195)
Q6a	3.9	15.63
Q6b	3.9	7.81
Q6c	NA	N.A.
Q6d	62.5	N.A.
Q6e	15.63	125
Q6f	31.25	N.A.
Q6g	62.5	N.A.
Q6h	>125	N.A.
Q8a	7.81	31.25
Q8b	0.24	1.95
Q8c	3.9	15.63
Q8d	>125	N.A.
Q8e	7.81	31.25
Q8f	7.81	15.63
Q8g	15.63	>125
Q8h	0.98	3.9
Isoniazid	7.19	27.22

Compounds **Q8b** and **Q8h** showed superior activities against both MDR and XDR *M. tuberculosis* strains with MIC values equal to (0.24 µg/mL and 0.98 µg/mL) for MDR and (1.95 µg/mL, 3.9 µg/mL) for XDR, respectively. Additionally, quinolines **Q6a**, **Q6b**, **Q8a**, **Q8c**, **Q8e**, and **Q8f** exerted good activity against the MDR strain, with MIC values of 3.9, 3.9, 7.81, 3.9, 7.81, and 7.81 µg/mL, respectively (Table 2), whereas compounds **Q6a**, **Q6b**, **Q8c**, and **Q8f** displayed moderate anti-tubercular activity against the XDR strain, with a MIC range from 7.81 to 15.63 µg/mL.

2.2.3. Cytotoxicity against Non-Tumorous Human Cells

In this study, T. Mosmann's MTT colorimetric test was used to evaluate the cytotoxic potential of isatin-tethered quinoline derivatives **Q8b** and **Q8h** towards the human non-tumorigenic WI-38 lung fibroblast cell line [43] (Table 3). Notably, both examined quinolines **Q8b** and **Q8h** had no cytotoxic effect on WI-38 cells, with IC₅₀ values of 36.6 ± 0.9 and 42.8 ± 1.12 µM, respectively.

Table 3. Impact of quinolines **Q8b** and **Q8h** toward the human non-tumorigenic lung fibroblast WI-38 cell line.

Comp.	IC ₅₀ (µM)
	WI-38 Cells
Q8b	36.6 ± 0.96
Q8h	42.8 ± 1.12
Staurosporine	17.5 ± 0.46

3. Conclusions

Briefly, two sets of chemically synthesized isatin-tethered quinolines **Q6a–h** and **Q8a–h** were assessed for their anti-tubercular activities towards a drug-susceptible strain, as well as towards the MDR and XDR strains. The design of these molecules is based on the bioisosteric replacement for the 3,4,5-trimethoxy-benzylidene motif in the lead compound **IV** with the privileged isatin motif to furnish series **Q6**. Then, the isatin motif was *N*-substituted with either a methyl or benzyl group to furnish the second series **Q8**. The results demonstrated that the majority of the quinolines that were evaluated had powerful to moderate potency against drug-susceptible *M. tuberculosis*, with MICs ranging from 0.06 to 7.81 g/mL. Among the investigated molecules, compound **Q8b** is the most potent counterpart with MIC = 0.06 µg/mL; it represents about 100-fold enhanced activity compared to the lead compound **IV** (MIC = 6.24 µg/mL). Furthermore, the assessment of the activities against the MDR and XDR strains revealed that quinolines **Q8b** and **Q8h** showed superior activities against both MDR and XDR strains with MIC values equal to 0.24 µg/mL and 0.98 µg/mL for MDR and 1.95 µg/mL and 3.9 µg/mL for XDR, respectively. In the cytotoxicity MTT assay, quinolines **Q8b** and **Q8h** disclosed non-significant activity (IC₅₀ = 36.6 ± 0.96 µM and 42.8 ± 1.12 µM, respectively) toward non-tumorigenic human WI-38 lung fibroblast cells.

4. Experimental

4.1. Chemistry

4.1.1. General

Melting points (m.p.) were measured by a Stuart melting point apparatus and were uncorrected. The NMR spectra were recorded utilizing a Bruker 400 MHz FT-NMR spectrometer (Bruker, Billerica, MA, USA). Both ¹H and ¹³C spectra were run at 400 MHz and 101 MHz, respectively, in DMSO-*d*₆. Moreover, a Bruker FT-IR spectrophotometer was used to record the IR spectra.

4.1.2. Preparation of 4-Hydroxyquinoline-3-Carboxylic Acid Ethyl Esters **3a–b**

A mixture of 3/4-chloroanilines **1a–b** (1.9 g, 15 mmol) and diethyl ethoxymethylmalonate (4.3 g, 20 mmol) was heated at 130 °C for three hours, where the produced ethyl alcohol was evaporated off. The crude mixture was filtered by a sintered funnel, washed with petroleum ether, and dried. The malonates **2a–b** (3.6 g, 12 mol) were refluxed in diphenylether (40 mL) for 2 h. Upon cooling, the resultant solid was filtered off, dried, and recrystallized from acetonitrile to yield the corresponding hydroxyquinoline ethyl esters **3a–b** [44].

4.1.3. Preparation of Quinoline-3-Carbohydrazide Derivatives **4a–b**

After adding hydrazine hydrate 99% (1 mL, 20 mmol) to a solution of quinoline ethyl esters **3a–b** (1 g, 4 mmol) in absolute ethanol (12 mL), the mixture was refluxed for 12 h. Before the mixture was added to the crushed ice, the excess solvent was removed. The solid that had precipitated was filtered through, washed with water, and then recrystallized from a mixture of DMF and water and dried to produce the corresponding hydroxyquinoline-3-carbohydrazides **4a–b** [44].

4.1.4. Preparation of Target Quinoline-Isatin Conjugates **Q6a–h** and **Q8a–h**

The appropriate isatin derivative **5a–d** or **7a–b** (1.2 mmol) was added to a solution of quinoline-3-carbohydrazide derivatives **4a–b** (0.35 g, 1.5 mmol) in acetic acid (15 mL). This mixture was reflux-heated for 4 h. The target isatin-tethered quinolines **Q6a–h** and **Q8a–h** were obtained in 72–87% yield by recrystallizing the precipitate from DMF/ethanol after it had been filtered off while still hot and washed with hot ethanol.

4.2. Biological Evaluation

4.2.1. Anti-Mycobacterial Activity

The MICs for the target isatin-tethered quinolines **Q6a–h** and **Q8a–h** against drug-susceptible, MDR, and XDR *M. tuberculosis* strains have been evaluated by the microplate alamar blue (MABA) assay, as described previously [36,45]. The detailed methodology was provided in the Supplementary Materials.

4.2.2. MTT Colorimetric Assay

The cytotoxic activity for isatin-tethered quinolines **Q8b** and **Q8h** against lung WI-38 cells was assessed using T. Mosmann's MTT colorimetric assay [43,46]. The detailed methodology was provided in the Supplementary Materials.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27248807/s1>, Chapter S1. Characterisation details (NMR, IR and elemental analysis) for the target quinolines (**6a–h** and **8a–h**). Chapter S2. Microplate Alamar Blue Anti-Tubercular Assay. Chapter S3. MTT Cytotoxicity Assay. Figures S1–S19: The ¹H, ¹³C NMR spectra for conjugates **Q6a–h** and **Q8a–h**. Figures S20–S23: The IR spectra for quinoline-isatin conjugates **6a,6d,6g,8h**.

Author Contributions: Conceptualization, M.A.A., W.M.E. and M.A.S.; methodology, M.A.A., H.A., T.A.-W., M.M.A.-A. and T.A.M.; validation, M.A.A., H.A., T.A.-W. and T.A.M.; formal analysis, M.M.A.-A. and H.A.; investigation, T.A.-W., W.M.E. and M.A.S.; resources, M.M.A.-A. and M.A.S.; data curation, H.A. and M.A.S.; writing—original draft preparation, M.A.A., M.M.A.-A., W.M.E. and M.A.S.; writing—review and editing, M.A.A., T.A.M. and W.M.E.; supervision, W.M.E. and M.A.S.; project administration, W.M.E.; funding acquisition, T.A.-W. and T.A.M. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of the compounds are not available from the authors.

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