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A novel quinoline derivative that inhibits mycobacterial FtsZ

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Abstract

High throughput phenotypic screening of large commercially available libraries through two NIH programs has produced thousands of potentially interesting hits for further development as antitubercular agents. Unfortunately, these screens do not supply target information, and further follow up target identification is required to allow optimal rational design and development of highly active and selective clinical candidates. Cheminformatic analysis of the quinoline and quinazoline hits from these HTS screens suggested a hypothesis that certain compounds in these two classes may target the mycobacterial tubulin homolog, FtsZ. In this brief communication, activity of a lead quinoline against the target FtsZ from *M. tuberculosis (Mtb)* is confirmed as well as good *in vitro* whole cell antibacterial activity against Mtb H₃₇Rv. The identification of a putative target of this highly tractable pharmacophore should help medicinal chemists interested in targeting FtsZ and cell division develop a rational design program to optimize this activity towards a novel drug candidate.

Keywords

tuberculosis; FtsZ; quinoline

1. Introduction

There is an urgent demand for new antitubercular drugs possessing novel mechanisms of action with the goal of developing more effective combination treatments against drug resistant forms of *M. tuberculosis*.^{1–2} As part of the NIH strategy to encourage the discovery and development of new drugs against Mtb, the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) and the Molecular Libraries Screening Center Network (MLSCN) have made high throughput screening data against Mtb H₃₇Rv for over 300,000 drug-like small molecules available via the PubChem database.^{3–5} Through this effort, over one thousand interesting compounds appear to show excellent lead antibacterial activity as well as selectivity relative to a mammalian cell toxicity counter screen. Unfortunately,

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continued progression of these leads is hindered by a lack of target information that would greatly facilitate a modern rational drug design program to produce highly potent and selective second generation analogs for clinical development. As part of our ongoing effort to advance leads from the TAACF and MLSCN programs, we have continued to mine the active samples from these whole cell phenotypic screens as they are an excellent basis set for the development of new antitubercular drugs due to the fact that these samples may serve as privileged scaffolds active versus whole bacteria for further target identification and chemical optimization. Such an approach may circumvent reported issues from pharmaceutical target-based infectious disease HTS campaigns.⁶ In this regard, we have had notable success pursuing whole cell active samples for target identification and chemical optimization through cheminformatic approaches as exemplified by our work with mycobacterial DHFR, IMPDH, and FtsZ.⁷⁻⁹

Among the reported actives from the government HTS programs, a small number of interesting quinolines and quinazolines were reported to have significant antitubercular activity, and one sample (**1**) showed excellent potency and good selectivity as measured relative to growth inhibition of a mammalian cell, Vero cells.

A further cheminformatic search of highly related quinoline scaffolds noted significant and diverse antibacterial activity of this class, and small molecule quinoline-based compounds show a variety of interesting target activities including inhibition of *Mtb*.¹⁰⁻¹³ In fact a diarylquinoline, Bedaquiline (TMC207 or R207910), is a recently approved FDA antituberculosis drug.¹³ Beyond the broad antitubercular activity of the two classes, however, there was a large body of useful data concerning the mechanism of action of highly related compounds in the area of anticancer drug discovery. Published work from Sirisoma *et al.* demonstrated potent activity of structurally related quinazolines (see Figure 1 structure **2**) against mammalian tubulin along with a thorough structure-activity analysis for the putative colchicine binding site in tubulin.¹⁴⁻¹⁶ Our work with FtsZ and the reported activity of colchicine against *Mtb* FtsZ suggested a hypothesis that at least one target of these quinolines that are highly homologous to the quinazolines reported by Sirisoma *et al.* may be a potential colchicine like binding site in *Mtb* FtsZ.⁹ The tubulin homolog FtsZ (Filamenting temperature-sensitive mutant Z) is essential for cell division in most bacteria, including *Mtb*, and it is an ideal target for novel antimicrobials that has recently received considerable attention in the discovery of novel anti-TB drugs.¹⁷⁻²⁸

Herein we report further target-based studies relating to the initially reported quinoline (**1**) as well as the preparation and screening of a new analog (**3**) with the goal of identifying a potential target in *Mtb* that will help researchers in the area of *Mtb* drug discovery advance this excellent and synthetically tractable pharmacophore.

2. Results and Discussion

The TAACF active 6-Chloro-2-methyl-N-(4-propoxyphenyl)quinolin-4-amine (**1**) was repurchased as a dry powder from ChemBridge as its hydrochloride salt and was used as supplied without further analysis. Based on the reported SAR work of Sirisoma *et al.* suggesting that the 4-NMe group reduced free rotation of the 4-substituent and possible off target kinase binding, we decided to prepare and screen the related derivative **3**. The synthetic route for compound **3** is outlined in Scheme 1 following reported methods.^{16,29,30}

Both compounds **1** and **3** were screened for inhibition of: 1) polymerization of *Mtb* FtsZ, and its mammalian homolog, tubulin, by reported methods⁹; 2) *in vitro* growth of *Mtb* H₃₇Rv to determine the IC₉₀ (the concentration that inhibits 90% of growth) by reported methods⁴; and 3) *in vitro* growth of Vero cells for cytotoxicity (CC₅₀).⁴ Screening results

are given in Table 1. Compound **1** was only modestly less active than its N-methyl analog **3** against H₃₇Rv growth *in vitro* but showed good activity for FtsZ. The N-Me compound was inactive against FtsZ and showed good activity in the tubulin polymerization assay.

In summary, through a thorough cheminformatics analysis of active quinolines and quinazolines reported under the auspices of the TAACF and MLSCN programs, a hypothesis was developed that the active quinoline **1** may target the putative colchicine binding site in Mtb FtsZ. It is notable that this hypothesis derived from related quinazolines reported as highly active anticancer compounds that were shown to bind in the colchicine binding site of tubulin. Mammalian target data were also extremely useful in identifying potential targets of the N,N'-diphenylurea class in our earlier Mtb IMPDH work, and it is highly recommended that other researchers assessing antitubercular HTS activity data not ignore this vast body of important data as appropriate as it not only helps address potential toxic effects, but it may also help elucidate possible antibacterial targets for screening and medicinal chemistry development. Beyond pinpointing a potential target of this class in Mtb, the vast body of anticancer/tubulin information and the resulting SAR clearly suggested optimal approaches that would help clarify and separate the anticancer/tubulin and antibacterial/FtsZ activity. Among these, Sirisoma *et al.* clearly demonstrated the value of N-methylation to enhance target anticancer activity and selectivity. Interestingly, the -NMe (**3**) analog of the lead antitubercular agent **1**, while it retained strong antitubercular activity *in vitro*, was inactive versus FtsZ, was highly toxic to Vero cells, and was a good inhibitor of tubulin polymerization. The fact that compound **3** still retained activity versus Mtb H₃₇Rv *in vitro* while losing FtsZ activity suggests that these agents may target more than FtsZ in Mtb H₃₇Rv. Surprisingly, the finding that a methylamino substitution is required for activity against tubulin and completely abolishes activity against FtsZ is a useful finding for selectivity against mycobacterial FtsZ. Furthermore, the toxicity of **3** is possibly due to inhibition of tubulin polymerization.

While the numerous actives from the NIH phenotypic Mtb screens are an excellent resource, it has historically been extremely difficult to develop potent and selective compounds without specific target information. Based on our analysis and screens, we would propose that one possible target of the lead quinoline **1** is the crucial cell division protein FtsZ. Furthermore, while there is significant interest in this target in bacteria and Mtb, there are very few compounds targeting mycobacterial FtsZ, and several of the reported scaffolds suffer from issues of ease of synthesis as well as problems with toxicity and medicinal chemistry profiles. We would suggest that the quinolines are an established "privileged" scaffold with broad based activities that include bioavailability and antitubercular activity. As such, with the information provided herein, the class and target is an excellent starting point for the development of a rational medicinal chemistry program to develop new antitubercular agents with a novel mechanism of action. Overall, these data as well as the ease of preparing new analogs suggest that these drug-like 4-substituted quinolines may be a good lead series for further scaffold expansion in order to develop new antitubercular agents targeting a novel and essential protein in *Mtb*, FtsZ.

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30. 6-Chloro-N,2-dimethyl-N-(4-propoxyphenyl)quinolin-4-amine(3) Concentrated HCl (1 drop) was added to a solution of 4,6-dichloro-2-methylquinoline **4** (175 mg, 0.825 mmol) and N-methyl-4-propoxyaniline²⁹ (150 mg, 0.91 mmol) in 10 mL isopropanol while stirring at room temperature. The reaction mixture was stirred at room temperature under argon atmosphere for 18 hours. The reaction mixture was diluted with CH₂Cl₂ and washed with aq. NaHCO₃. The aqueous layer was washed with CH₂Cl₂ (2 × 20 mL). The combined organic layer was dried over anhydrous Na₂SO₄ and the solvent was removed under vacuum. The product was purified by flash column chromatography to afford the title compound **3** (86 mg, 31%, LCMS Purity: 100%) as a white solid. mp 83–84 °C. ¹H NMR (CDCl₃, 300 MHz): δ 7.86 (d, 1H, J = 9.0 Hz, 8-H), 7.56 (d, 1H, J = 2.4 Hz, 5-H), 7.46 (dd, 1H, J = 2.4 Hz, 9.0 Hz, 7-H), 6.90–6.80 (m, 5H, 2'-H, 3'-H, 5'-H, 6'-H, 3-H), 3.91 (t, 2H, J = 6.6 Hz, -OCH₂), 3.38 (s, 3H, -NCH₃), 2.67 (s, 3H, 2-CH₃), 1.85–1.73 (m, 2H, -CH₂-CH₃), 1.05 (t, 3H, J = 7.5 Hz, -CH₂-CH₃). HRMS calcd for [C₂₀H₂₁ClN₂O+H]⁺: 341.14152, Found: 341.14182.

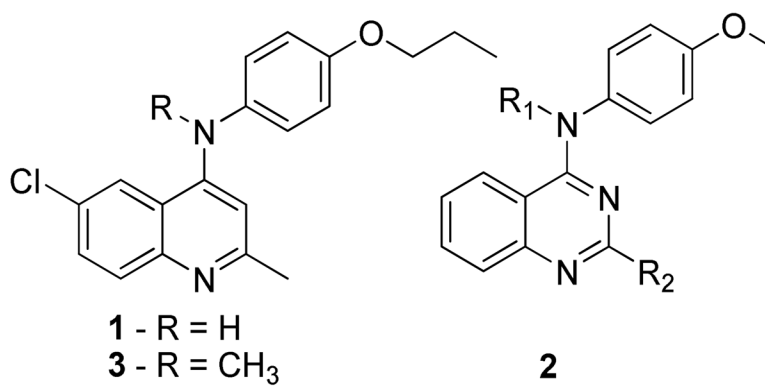
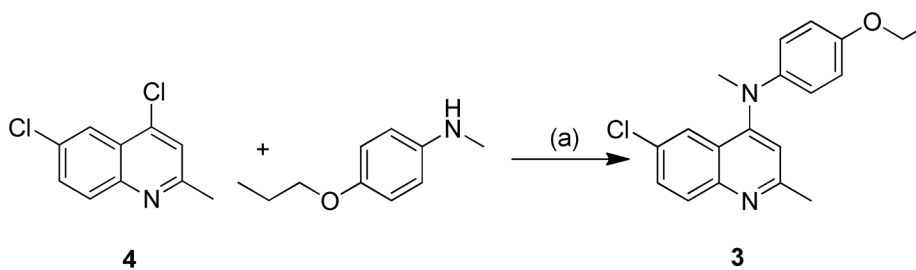


Figure 1. Structures of quinoline compounds **1** and **3** and generalized quinazolines (**2**) reported by Sirisoma *et al.* where R₁ = H or Me and R₂ = Cl or Me.

**Scheme 1.**

Synthetic pathway to compound 3. N-methyl-4-propoxyaniline is available via reported methods.²⁹ *Reagents and conditions:* a) Conc. HCl, *iso*-propanol, rt.³⁰

Table 1Biological data for compounds **1** and **3**.^a

Compounds	Mtb FtsZ Polymerization ^b ID ₅₀ (μ M)	Tubulin Polymerization ^b ID ₅₀ (μ M)	Mtb H ₃₇ Rv IC ₉₀ (μ M)	Vero Cytotoxicity CC ₅₀ (μ M)
1	22.7 \pm 0.4	>100 μ M	20.4	>120
3	>100 μ M	5.4 \pm 0.1	15.7	<0.2

^aMethods are reported in references 4 and 9.^bEach compound was analyzed at least three times per assay. For the polymerization assays, the mean \pm the standard deviations are reported.