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Identification and optimization of a new series of anti-tubercular quinazolinones

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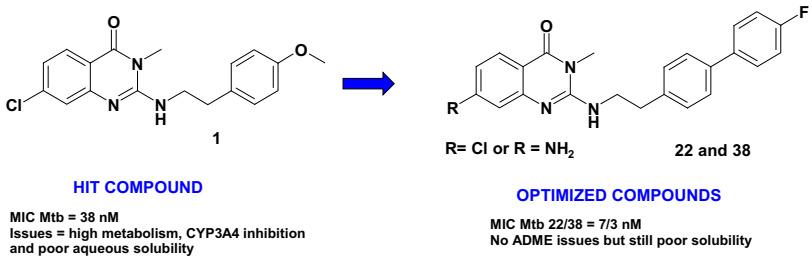
ABSTRACT

A high throughput phenotypic screening against *Mycobacterium smegmatis* led us to the discovery of a new class of bacteriostatic, highly hydrophobic antitubercular quinazolinones that potently inhibited the in vitro growth of either extracellular or intramacrophagic *M. tuberculosis* (*Mtb*), via modulation of an unidentified but yet novel target. Optimization of the initial hit compound culminated in the identification of potent but poorly soluble *Mtb* growth inhibitors, three of which were progressed to in vivo efficacy studies. Despite nanomolar in vitro potency and attractive PK properties, none of these compounds was convincingly potent in our in vivo mouse tuberculosis models. This lack of efficacy may be linked to the poor drug-likeness of the test molecules and/or to the properties of the target.



Tuberculosis (TB) is an airborne infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*) and that mostly affects the lungs. It is a complex disease caused by different bacterial populations that are located within the host either intracellularly in macrophages or extracellularly within cavities or hypoxic lesions. TB represents one of the top public health concerns worldwide. One-third of the world's population is infected with *Mtb* causing an estimated 9 million new cases and 1.5 million deaths in 2013.[1] The multidrug treatment established in the 1970s and still recommended today by the World Health Organization (WHO) has not been sufficient to eliminate TB due to the advent of HIV/AIDS, failure of treatment programs and emergence of drug-resistant TB.[2] In addition, poor compliance and deficient health care systems have often resulted in treatment interruptions, thereby causing or exacerbating the emergence and spread of drug resistance.[2] The currently recommended combination therapy for drug-sensitive TB is lengthy and complex: 2 months, associating Rifampicin, Isoniazid, Pyrazinamide and Ethambutol, followed by 4 months with Rifampicin and Isoniazid.[2] In the case of multi- and extensive-drug resistant (MDR/XDR) TB, treatments may even require daily medication by multiple and unsafe drugs, for up to

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24 months.[2] As a consequence, there is an urgent need for the discovery and development of new antitubercular agents that would act by new mechanisms of action and hence would be able to treat both the drug-sensitive and the resistant forms of the disease. With this objective, several paths in the TB drug discovery field have been followed in recent years such as preparing new analogues of TB drugs,[3] revisiting the targets of known anti-TB compounds,[4] repositioning non-TB drugs,[5] tapping into the natural product pool,[6] harnessing target-based approaches[7] and conducting traditional growth inhibition screenings.[8]

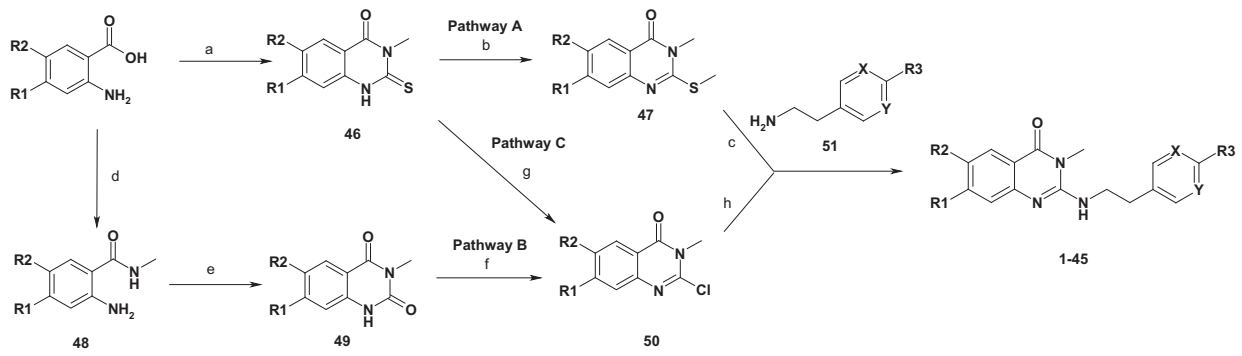
However, for various reasons, most of these strategies have so far met with limited success, with the notable exception of high-throughput (HTS) growth inhibition screenings of small-molecule libraries against *Mtb* (or more frequently against *Mtb* surrogates such as *Mycobacterium smegmatis* or *Mycobacterium bovis*). Indeed, this latter approach has delivered in recent years a variety of new anti-TB scaffolds.[9] Along these lines, we conducted a few years ago such a whole cell-based HTS, testing the Sanofi chemical library (800 K) against *M. smegmatis*.

Following the screening step at 10 μM, dose–responses of compounds inhibiting more than 50% of the bacterial growth were determined. Compounds displaying MIC < 20 μM were then screened against *M. tuberculosis* H37Rv. Confirmed actives were counter-screened versus the human HepG2 cells to discard compounds with unspecific antimycobacterial activities: only compounds with a selectivity index (SI = TC_{50}/MIC) > 10 were selected for further analysis. Sorting and prioritization of the resulting selective actives led to several series, of which quinazolinone **1** emerged as a potential attractive starting point due to its high potency (MIC = 38 nM against H37Rv *Mtb*), an attractive SI (TC_{50} HepG2 = 13.4 μM), good activity against the growth of *Mtb* in mouse macrophages (MIC = 163 nM) and promising activity against drug-resistant *Mtb*. Indeed, when tested on *Mtb* strains mono-resistant to known TB drugs, no-cross-resistance was seen, suggesting a new mechanism of action. Compound **1** was also shown to exhibit bacteriostatic properties against *Mtb* while antimycobacterial potency significantly decreased in the presence of mouse, fetal calf or human sera (for instance MIC shifted from 13 to 429 nM in the presence of 25% mouse serum) suggestive of high protein binding, consistent with high hydrophobicity (see below).

There was no report (in the literature) of anti-Mtb activity or anti-bacterial activity on the analogues of compound **1** which exhibited a high experimental log*P* (log*P* = 4.08), significantly different from the calculated value provided by the ACD9 software (*c*log*P* = 3). In line with this high log*P* value, equilibrium aqueous solubility was found to be low at pH 7.4 or 1 (respectively < 1 and 11 μg/ml). High hydrophobicity, low polarity (PSA = 54) and the polyaromatic nature of compound **1** all pointed to poor overall drug-like properties, somewhat compensated by a low molecular weight (MW = 344). ADMET profiling showed that compound **1** had strong potential for oral absorption as suggested by its high permeability though Caco2 cells (data not shown), moderately inhibited the hERG channel (35% inhibition at 10 μM in an automatic patch clamp assay, at 20 °C) and was Ames-negative. Furthermore, while being a weak inductor of CYP3A4, **1** potently inhibited cytochrome CYP3A4 (IC_{50} < 1 μM for the testosterone probe) and also displayed high metabolism in the presence of mouse microsomes, incompatible with in vivo efficacy in mouse models of TB. Metabolism was also found high in the presence of human microsomes (see Table 4) or human hepatocytes (data not shown). Demethylation of the methoxy group and hydroxylations of the distal aryl ring were identified as the main metabolism pathways.

At this point, we decided to embark on a program aimed at exploring structure–activity relationship around compound **1** while striving to solve the issues identified during the profiling phase, namely high metabolism, CYP inhibition, poor solubility and strong serum shifts. Herein, we report the results of this optimization program that led us to advanced analogues that were tested in mouse infection models. We will discuss the in vitro activities as well as the ADMET and drug-like properties across the series. We will also present the in vivo antitubercular activities of the best analogues and comment on the reasons for their lack of efficacy.

Synthesis of compounds **1**–**45** followed the routes described in Scheme 1. Three different routes (A, B and C) were used in order to synthesize the targeted compounds. The main difference between these different routes was the nature of the leaving group (thiomethyl or chloro) in intermediates **47** and **50** that was designed to allow the installation of the amino side chain by aromatic nucleophilic substitution. Starting from variously substituted 2-aminobenzoic acids, the thioethers **47** were obtained in two steps following cyclization with methylthioisocyanate and subsequent methylation of the thiono sulfur atom of **46**. Intermediates **46** were alternatively transformed into compounds **50** upon reaction with sulfuryl chloride. In the cases of substituents such as methoxy or nitro on the aryl ring, compounds **49** were obtained in a three-step sequence from 2-amino benzoic acids: conversion into 2-amino N-methyl amino benzamides **48**, cyclization into compounds **49** in the presence of carbonyl di-imidazole and treatment with phosphoryl chloride. The targeted compounds **1**–**45** were finally obtained by condensation of compounds **47** or **50** with various primary amines **51**. In pathway A, intermediates **46** were heated at 160 °C with di-isopropyl ethyl amine as the base, in *N*-methyl pyrrolidinone, to afford compounds **1**–**8** and **12**–**45**. Of note, these conditions sometimes proved too drastic, resulting in the degradation of the reaction mixtures and in side products. In these cases,



Scheme 1. (a) MeNCS, AcOH, 150 °C, 24 h (b) Me_2SO_4, NaOH, MeOH, rt, 5 h (c) DIEA, NMP, 160 °C, 24 h (d) $MeNH_2$·HCl, HOBt, DCI, DIEA, DMF, rt, 16 h (e) Im_2CO, DBU, THF, rt, 20 h (f) $POCl_3$, DIEA, 90 °C; 20 h (g) SO_2Cl_2, $CHCl_3$, 60 °C, 6 h (h) Et_3N, EtOH μW, 130 °C, 30 min.