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Quinolinyl Pyrimidines: Potent Inhibitors of NDH-2 as a Novel Class of Anti-TB Agents

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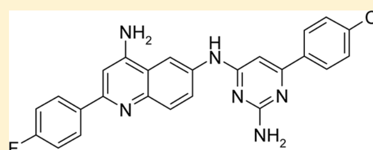
Supporting Information

ABSTRACT: NDH-2 is an essential respiratory enzyme in *Mycobacterium tuberculosis* (Mtb), which plays an important role in the physiology of Mtb. Herein, we present a target-based effort to identify a new structural class of inhibitors for NDH-2. High-throughput screening of the AstraZeneca corporate collection resulted in the identification of quinolinyl pyrimidines as the most promising class of NDH-2 inhibitors. Structure−activity relationship studies showed improved enzyme inhibition (IC_{50}) against the NDH-2 target, which in turn translated into cellular activity against Mtb. Thus, the compounds in this class show a good correlation between enzyme inhibition and cellular potency. Furthermore, early ADME profiling of the best compounds showed promising results and highlighted the quinolinyl pyrimidine class as a potential lead for further development.



KEYWORDS: *antituberculosis, respiratory chain, NDH-2, quinolinyl pyrimidines*

The contributory driving force of tuberculosis (TB) is *Mycobacterium tuberculosis* (Mtb), a leading killer worldwide that currently infects one-third of the human population.[1] The World Health Organization (WHO) reports 2 million deaths every year.[2−4] Only 2−23% of individuals infected with Mtb carry lifetime risk of developing an active disease.[2−4] The risk, however, radically increases if the carrier's immune system is suppressed. In spite of a 6 month long, four drug combination therapy (rifampicin, isoniazid, pyrazinamide, and ethambutol) for the treatment of TB, multiple drug resistant (MDR) TB with resistance to isoniazid and rifampicin is reported in 5−10% of cases.[2−4] Novel drugs for TB are essential to reduce the duration of treatment of drug-sensitive as well as drug-resistant TB, to impact the rate of transmission of this disease. Novel and shorter treatment regimens could improve compliance and reduce the emergence of drug resistance.

Because Mtb can exist in both actively replicating and nonreplicating phases, it is essential for a novel anti-TB agent to have activity against both of these populations. Respiration, being an essential pathway in any physiological state, could be a potentially high value target.[5] The well-known example is ATP synthase, which is the target of TMC-207, a clinical candidate that has comparative killing efficiency against replicating and nonreplicating Mtb.[5] TMC-207 is currently in phase II clinical studies in patients with multidrug-resistant TB.[6] Similarly, type II NADH-dehydrogenase (NDH-2) is an essential respiratory enzyme in Mtb having a significant role in the physiology of Mtb. The enzyme has been characterized in many species like *Saccharomyces cerevisiae*,[7] *Escherichia coli*,[8,9] *Bacillus subtilis*,[10] *Methyloccocus capsulatus*,[11] *Corynebacterium glutamicum*,[12,13] *Acidianus ambicalens*,[14,15] and *Sulfolobus metallicus*.[16] It is composed of a single polypeptide chain, comprising a flavin as an exclusive cofactor.[17] It is worth mentioning that this enzyme is not found in the mammalian mitochondria. The fundamental role of NDH-2 in Mtb respiration is supported by a wide range of biochemical[18] and transcriptional studies.[19] The Mtb genome contains two copies of *ndh* genes (*ndh* and *ndhA*). The Mtb NDH-2 and NDH-2A share 67% sequence identity. A strain of Mtb in which *ndh* has been disrupted by transposon mutagenesis is nonviable;[17,20] however, a *ndhA* deletion mutant of Mtb can be easily isolated.[17,21] The effect of *ndh* deletion mutant lacking NDH-2 on bacterial growth under various culture conditions and on animal infection has been reported.[18]

Phenothiazines (a class of antipsychotic drugs) are reported to be inhibitors of NDH-2.[17] This class of compounds is known to inhibit the growth of bacteria in vitro as well as bacteria inside macrophages. Thus, NDH-2 appears to be a druggable target.[17] However, phenothiazines cannot be used as antibacterial agents as they exhibit potent CNS activities at doses much lower than those required for antibacterial activity.[22]

Therefore, we embarked upon finding a new scaffold through screening of a library of 100K compounds selected from the AZ corporate collection using Mtb NDH-2 enzyme assay in high-throughput screening (HTS) format. The enzyme assay mix contained 1 nM purified Mtb NDH-2 protein (Supporting Information), 100 mM NaCl, 0.008% Brij-35, and 300 μM NADH in 50 mM Hepes-NaOH buffer, pH 7.5. The reaction was started by addition of 50 μM menadione, and the reaction mix was incubated at 37 °C for 1 h. A decrease in the absorbance at 340 nm as a result of NADH oxidation during the assay was monitored using an UV spectrophotometer. During screening,

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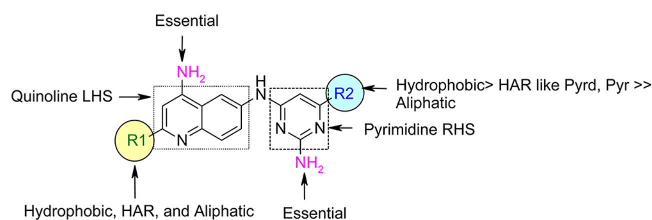


Figure 1. SAR for quinolinyl pyrimidines (HAR, heteroaryl; Pyrd, pyridine; and Pyr, pyrimidine).

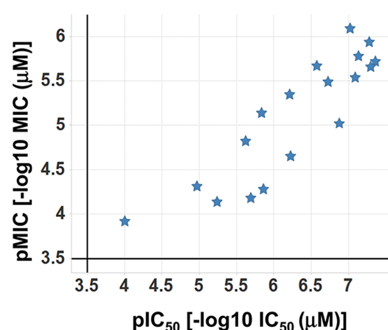
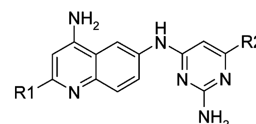


Figure 2. Scatter plot of pIC_{50} vs pMIC.

compounds were incubated with the enzyme in the reaction mixture prior to the addition of menadione. Thus, the screening of the 100K library at a concentration of 20 μM resulted in 1685 hits, which had >50% inhibition that were selected for concentration−response studies. After the structures were removed with undesirable features, frequent hitters, and reactive clusters, hit evaluation analysis gave five clusters as inhibitors of NDH-2. These five clusters had IC_{50} values ranging from 0.6 to 50 μM and a minimum inhibitory concentration (MIC) between 8 and >32 μg/mL. Compounds that had the best activity belonged to the quinolinyl-pyrimidine class. In this report, we described our efforts to understand the structure−activity relationship (SAR) of this scaffold and improve the biochemical potency against the target enzyme, which in turn translates into good cellular potency. We have also built the in vitro DMPK properties of this scaffold to identify molecules suitable for efficacy studies in an animal model of TB.

Our initial SAR strategy was to identify the key pharmacophore required for activity. The scaffold has two main areas of opportunity, namely, the quinoline left-hand side (LHS), and the pyrimidine right-hand side (RHS) to explore the SAR and structure−property relationship (SPR) (Figure 1). The results of our studies indicate that either one or both of the primary amines of quinoline and pyrimidine ring are critical for NDH-2 enzyme activity as shown in Table 1, compounds **13a**−**j** (IC_{50} = 0.04−0.6 μM). However, when one or both amine functions were removed, the enzyme potency was weaker or completely lost (compounds **14**−**16**, IC_{50} = 2 to >100 μM). These results suggest that quinolinyl pyrimidine with two primary amines is a key pharmacophore, which may have critical hydrogen-bonding interactions with NDH-2 target protein. To reduce the hydrophobic requirement of the scaffold, we introduced a number of different substituents at various positions of the scaffold as summarized in Table 1. On the pyrimidine ring, a monosubstituted phenyl ring (R2) was much better (compounds **13a**−**i**) than hetero aryl groups like pyridine (**13j**) or pyrazole (**13o**), which in turn are better than simple aliphatic groups such as ethyl or isopropyl group (**13k**−**m**). On the quinoline ring, a

Table 1. In Vitro Evaluation of Compounds[a]



No.	R1	R2	IC_{50} (μM)	MIC (μM)
13a	4-F-phenyl	4-Cl-phenyl	0.043	1.91
13b	4-F-phenyl	3-Me-phenyl	0.096	<0.81
13c	4-F-phenyl	4-CF_3-phenyl	0.081	2.88
13d	4-F-phenyl	4-F-phenyl	0.053	1.14
13e	4-F-phenyl	3-Cl-phenyl	0.050	2.19
13f	4-F-phenyl	phenyl	0.073	1.67
13g	4-F-phenyl	2-F-phenyl	0.187	3.21
13h	4-F-phenyl	2-OMe-phenyl	<0.62	4.42
13i	3-OMe-phenyl	4-Cl-phenyl	<0.26	2.13
13j	4-F-phenyl	2-pyridyl	0.133	9.45
13k	4-F-phenyl	isopropyl	1.467	7.28
13l	4-F-phenyl	ethyl	2.397	15.11
13m	4-F-phenyl	methyl	0.601	22.20
13n	H	3-Me-phenyl	10.79	48.87
13o	4-F-phenyl	1-Me-1H-pyrazol-3-yl	1.38	53.06
14			5.77	>73.02
15			2.05	66.60
16			>100	>121.06

[a] < and >, end points not determined.

phenyl ring (R1) with a 4-fluoro (**13a**) or 2-methoxy substituent (**13i**) showed better potency than the unsubstituted quinoline group (**13n**). This indicated the possibility of strong hydrophobic interactions with the protein. The potent enzyme activity

Table 2. Representative Compounds[a]

compd	13a	13b	13c	13d	13e
AZ log *D*	3.0	3.18	2.16	3.06	3.06
Mtb NDH-2 IC_{50} (μM)	0.043	0.096	0.081	0.053	0.050
Mtb MIC (μM)	1.91	<0.81	2.88	1.14	2.19
solubility (μM)	6	70	2450	12	15
PPB (% free)	0.8	1.44	ND	2.49	ND
Cl_{int} (μL/min/mg)	13.4	ND	<4	ND	ND
Sce MIC (μg/mL)	>32	>32	>32	>32	>32
RBC MLC (μg/mL)	>32	>32	>32	>32	>32

[a]ND, not determined; < and >, end points not determined.

(40−600 nM) of these compounds was translated into cellular potency (<0.35−4 μg/mL), while compounds with weak enzyme activity showed poor cellular potency. The relationship between the NDH-2 IC_{50} and the MIC against Mtb is shown in Figure 2. This excellent correlation between IC_{50} and MIC suggests that these compounds inhibit the NDH-2 target in Mtb.

The potent compounds in the series were profiled for physicochemical properties, in vitro plasma protein binding, and mouse microsomal intrinsic clearance (Cl_{int}). Solubility in the presence of 2% (v/v) DMSO was in the range of 6 μM and 2 mM. The significant improvement of solubility for compound **13c** may be due to lower log *D*. The protein binding of the series was generally on the higher side with percent free fraction between 1 and 3. The mouse Cl_{int} was normally <4 and 13.4 μL/min/mg. The MIC data against *S. cerevisiae* (Sce) suggest that the compounds in the series are selective for bacteria and inactive on eukaryotes. Furthermore, none of these compounds showed any membrane disruption activity [minimum lysis concentration (MLC)] in the red blood cell (RBC) hemolysis assay (Table 2). Representative advanced compounds **13a−e** are shown in Table 2 and Figure 3.

The synthetic strategy involved a convergent approach in which the two main fragments, namely, aminoquinoline and amino pyrimidines, were separately synthesized and condensed. Substituted quinoline-4,6-diamine (**8**) was synthesized from commercially available starting materials as described in Scheme 1. Condensation of substituted ketone (**1**) with diethyl carbonate (**2**) gave the corresponding oxopropanoates (**3**), which on further treatment with 4-nitroaniline gave the uncyclised products (**4**). These were cyclized in Dowtherm A to the substituted 6-nitroquinolin-4-ol derivatives (**5**). This was followed by conversion to the 4-chloro (**6**) and 4-azido (**7**) derivatives by treating with $POCl_3$ and sodium azide, respectively. The 4-azido derivative was reduced to give the quinoline-4,6-diamines (**8**).[23] The key hydroxy-pyrimidine (**11**) derivatives were synthesized using a modified literature procedure by treating guanidine hydrochloride (**9**) with various β-ketoesters (**10**) in the presence of sodium hydride. The resulting hydroxy-pyrimidines were converted to their corresponding triflates, tosylates, mesylates, or chlorides (**12**). Triflates were prepared by treating the hydroxyl derivative with trifluoromethanesulfonic anhydride, trifluoromethanesulfonyl chloride, or *N*-phenylbis (trifluoromethanesulfonimide). Chloro derivatives were prepared by treatment with $POCl_3$, PCl_5, or other chlorinating agents. Pyrimidin-4-yl-quinoline-diamine (**13**) was synthesized by nucleophilic substitution or coupling reaction of the activated pyrimidines and quinolinediamines in presence of catalytic HCl.

In conclusion, we have discovered a new class of compounds that target the NDH-2 enzyme with promising attributes of synthetic accessibility, good SAR, and antitubercular activity. This class has good in vitro DMPK properties, which can be further improved through further analogue generation and systematic medicinal chemistry evaluation. With new antiTB agents desperately needed, we believe that the quinolinyl pyrimidine class provides interesting potential for further optimization. Further improvements to this series will be communicated in due course.

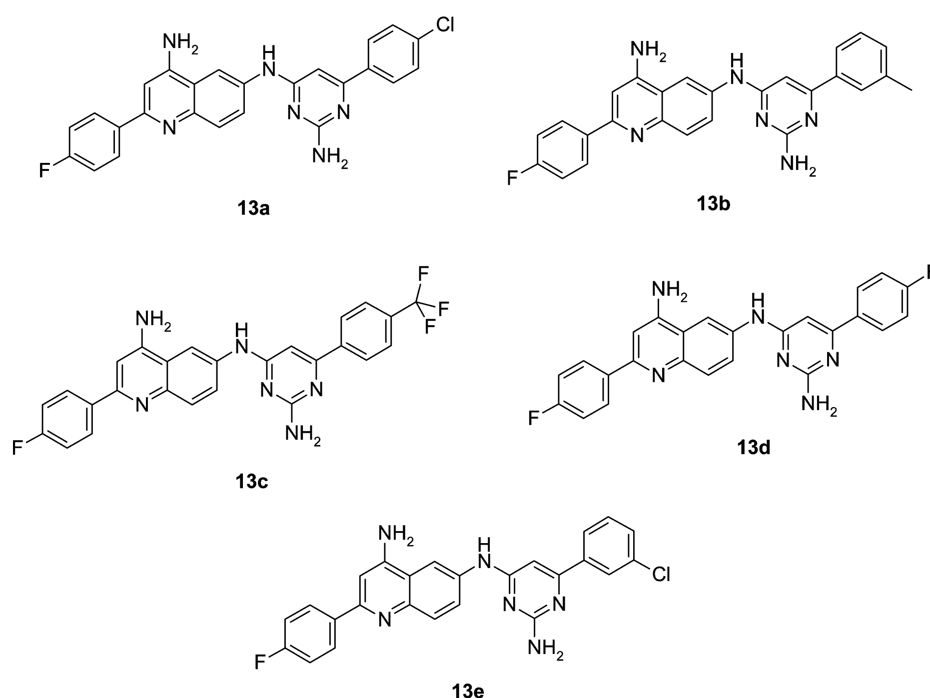
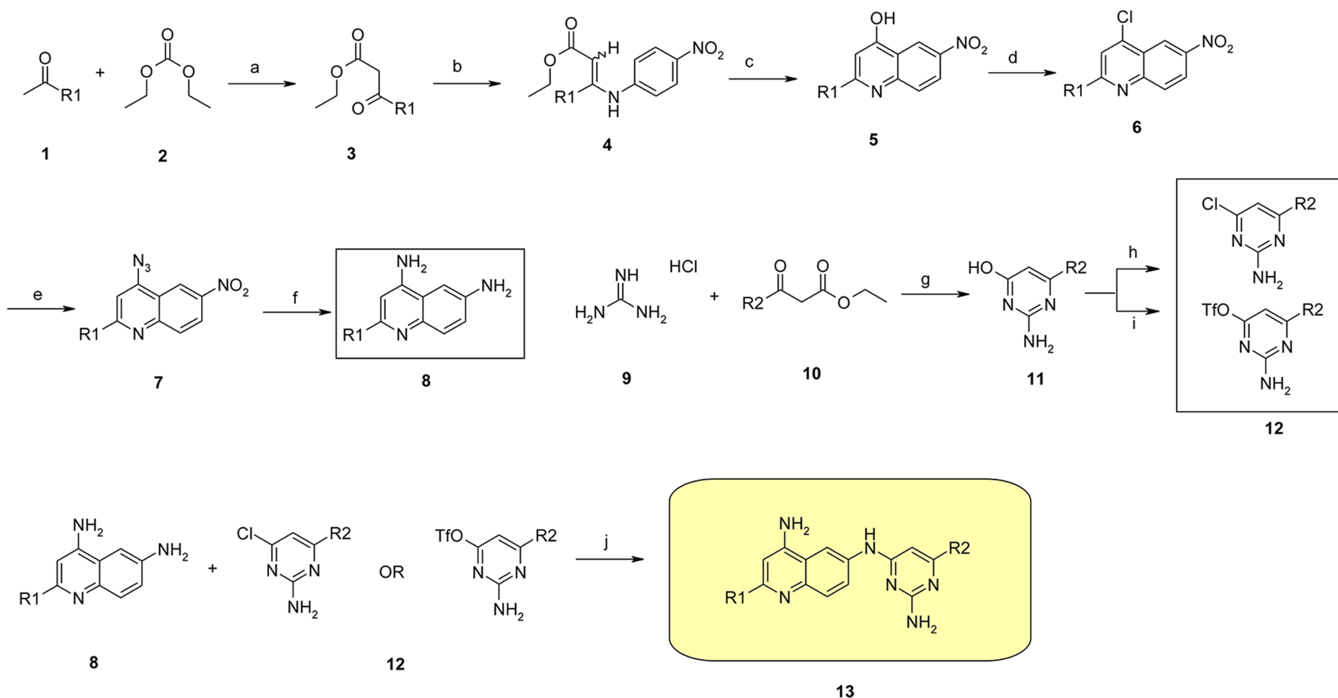


Figure 3. Representative compounds.

Scheme 1. Synthesis of Quinolinyl Pyrimidines[a]



[a]Reagents: (a) NaH, DMF, reflux, 16 h. (b) 4-Nitroaniline, *n*-butanol, 4 Å sieves, conc., HCl reflux, 48 h. (c) Dowtherm, 250 °C, 2 h. (d) $POCl_3$, 110 °C, 4 h. (e) NaN_3, NMP, 60 °C, 18 h. (f) $SnCl_2$, ethylacetate−ethanol (5:1) reflux, 2 h. (g) NaH, DMF, reflux, 16 h. (h) PCl_5, $POCl_3$, reflux, 16 h. (i) *N*-Phenyltrifluoromethanesulphonamide, triethylamine, 1 h. (j) 4 mol equiv of HCl (4 M in 1,4-dioxane), NMP, 80 °C, 10 h.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental details for compounds synthesized and descriptions of biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

P.S.S. drafted the manuscript and participated in the design and execution of this study. B.P. performed and interpreted enzyme data. N.R.C. and C.K. performed the chemical syntheses. B.B. and B.G.U. contributed in scientific discussions.

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Notes

The authors declare no competing financial interest.

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