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Synthesis and Antiplasmodial and Antimycobacterial Evaluation of New Nitroimidazole and Nitroimidazooxazine Derivatives

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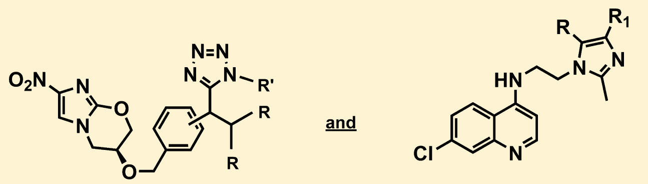
Supporting Information

ABSTRACT: The synthesis and antiplasmodial and antimycobacterial evaluation of two new series of nitroimidazole and nitroimidazooxazine derivatives is described. The majority of these compounds, especially hybrids **9d**, **9f**, and **14b**, exhibited potent activity against the chloroquine-resistant K1 strain of *Plasmodium falciparum*. Furthermore, a notable number from the tetrazole series were significantly more active against *M. tuberculosis* than kanamycin, a standard TB drug.



KEYWORDS: *Nitroimidazoles, nitroimidazooxazines, antiplasmodial, antimycobacterial activity*

According to the World Health Organization (WHO), nearly one-third of the world's population harbors *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis (TB).[1] TB is the second leading cause of death due to an infectious organism:[2] current WHO estimates stand at 9.2 million new cases and 1.8 million deaths annually.[1,3] Malaria, on the other hand, infects about 255 million people worldwide, resulting in 781 000 deaths, mostly to children under 5 years and pregnant women.[4] Currently, there are no effective vaccines against these pathogens and treatment success in some areas remains low due to poor management and patient noncompliance.[5] This situation is further exacerbated by the increasing prevalence of multi- (MDR) and extensive-drug resistant (XDR) strains of *Mtb*.[6] Therefore, there is an urgent need for new, fast acting, and more efficacious antimalarial and anti-TB therapies to replace the existing drug regimens.

Although there has been no introduction of a new anti-TB drug over the last 40 years, a number of different classes of compounds are undergoing clinical development,[2] namely nitroimidazooxazines (PA-824, **1**),[7] diarylquinolines (TMC207, **2**),[8] oxazolidinones (eperesol, **3**, and linezolid, **4**),[9] and ethylenediamines (SQ109, **5**)[10] (Figure 1). The use of the antibiotic metronidazole in the management of anaerobic bacterial and protozoan infections has reinvigorated interest in the nitroimidazole scaffold over the past decade.[11,12] Nitroimidazoles (e.g., **1**) are pro-drugs that are highly effective against both the replicating and nonreplicating persistent forms of *Mtb*, and their activity is believed to arise as a result of metabolic activation of the nitro group, which leads to the generation of nitric oxide as an active species.[13] The main drawbacks of **1** are its poor aqueous solubility and its propensity to bind to proteins in human plasma.[3,14] Various measures have been undertaken in addressing these shortcomings, such as the synthesis of biphenyl analogues of **1**,[15] the use of urea, carbamate, and amide linkers in the place of the benzyl ether,[16] and the hybridization of **1** with oxazolidinone,[17] among others. The most promising results from these studies were achieved when the benzyl group of **1** was replaced with various (hetero)biaryl side-chains and amide groups. It was noted that compounds that contain these scaffolds exhibited better *in vitro* and *in vivo* potencies and improved absorption, distribution, metabolism, and excretion (ADME) properties compared to those of **1**.[18−21]

In this context, we desired to investigate the antiplasmodial and antimycobacterial properties of new analogs that contain the key nitroimidazole pharmacophore. The first series was designed to contain a tetrazole moiety in the place of the lipophilic trifluoromethoxy group of **1**, as it was hypothesized that the inclusion of this moiety will significantly aid in improving the physicochemical properties. These tetrazole-containing compounds were synthesized in three-steps; the first involved the synthesis of aralkyl halides **7a−c** from known literature methods.[22] These aralkyl halides were then reacted

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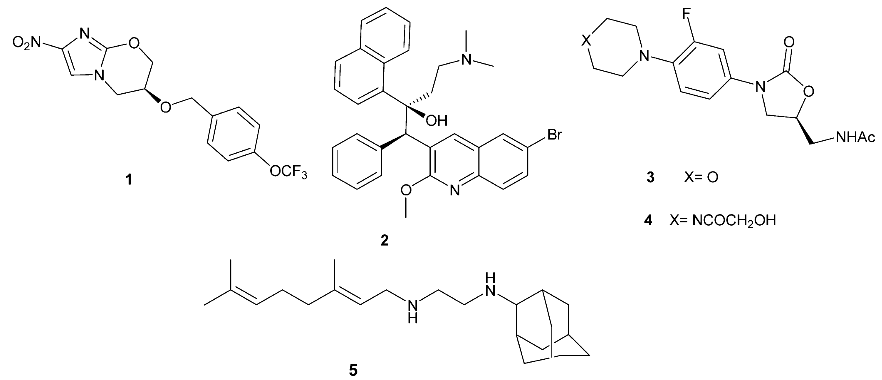
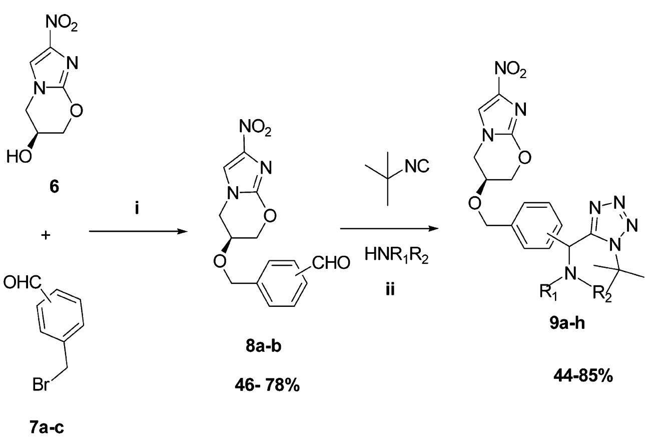


Figure 1. Chemical structures of lead TB compounds in clinical development.

with the commercially available nitroimidazooxazine-alcohol (**6**) in the presence of sodium hydride[7,23] to afford aldehydes **8a–b** (*meta* and *para*) (Scheme 1). The reaction failed in the case of the *ortho*-aldehyde, presumably due to steric hindrance. Aldehydes **8a–b** were then subjected to the modified $TMSN_3$–Ugi multicomponent reaction (MCR)[24] involving amines and the convertible *tert*-butyl isocyanide. Amine inputs included primaquine and 4-aminoquinoline diamines. Target compounds **9a–h** were obtained in moderate to excellent yields (Figure 2) and diastereoselectivity (based on the ^{1}H NMR); compounds **9a–f** were obtained exclusively as single diastereomers while **9g** and **9h** were obtained as 1:1 diastereomeric mixtures, owing to the fact that the commercial primaquine salt used in this study is racemic.

Scheme 1[a]



[a]Reagents and conditions: (i) NaH, DMF, N_2, 0 °C to rt, 12 h; (ii) $TMSN_3$, MeOH, 40 °C, 12 h.

The synthesis of the second series of compounds, **14a–b**, **15a–b**, and **16**, began with the synthesis of intermediates **10a** and **10b** as described by Chauhan and co-workers[25] (Scheme 2). These intermediates were then mesylated to the corresponding methanesulfonic acid esters **11a** and **11b**, which in turn underwent nucleophilic substitution reaction with 2-methyl-4-nitro-1*H*-imidazole to yield hybrids **15a–b** in reasonable yields. Hybrid **16** was obtained *via* the sodium hydride mediated reaction of **11b** with alcohol **6**. In addition, the methanesulfonic acid esters **11a–b** were also reacted with *N*-(methyl)ethanolamine to furnish intermediates **12a–b**, which on chlorination using thionyl chloride yielded **13a–b**. These chloride derivatives were subsequently reacted with 2-methyl-4-nitro-1*H*-imidazole in the presence of anhydrous potassium carbonate to afford the isomeric mixture of **14a** and **14b** (Scheme 2).

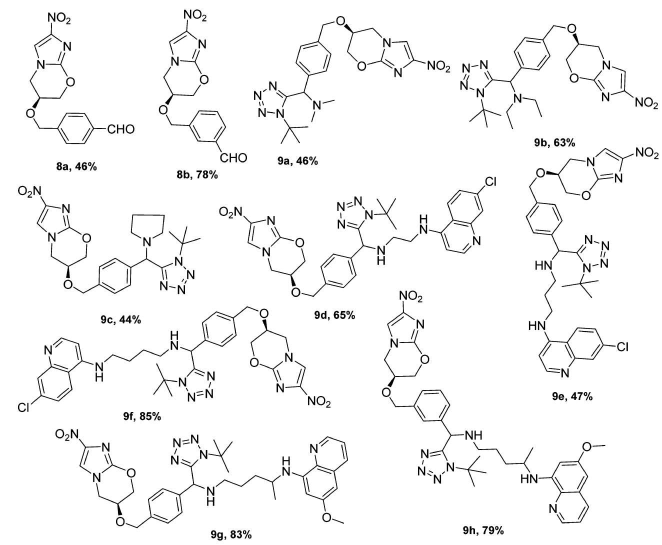
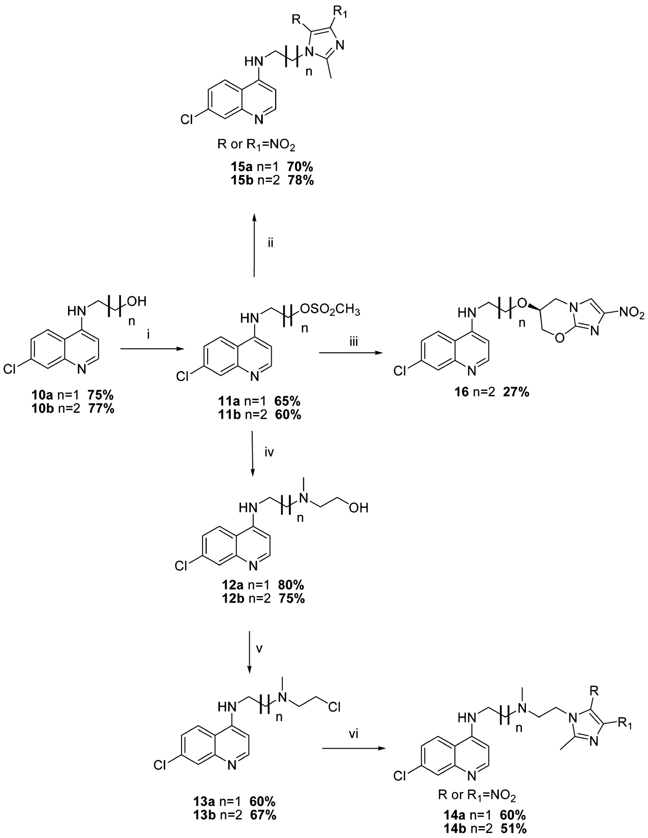


Figure 2. Structures and yields of MCR intermediates and target compounds.

All synthesized compounds were evaluated *in vitro* for their antiplasmodial (against the multidrug-resistant K1 strain) and antimycobacterial activity (against the drug-sensitive $H_{37}Rv$ *Mtb* strain). Chloroquine, primaquine, kanamycin, and streptomycin were used as positive controls, and the results are tabulated in Table 1.

Hybrid compounds **9d–h** and **14a–16** showed antiplasmodial IC_{50} values in the low micromolar range. More specifically, the PA-824–chloroquinoline hybrids **9d** (IC_{50} = 0.100 μM) and **9f** (IC_{50} = 0.164 μM) and the methylnitroimidazole–chloroquinoline hybrid **14b** (IC_{50} = 0.094 μM) were the most active, endowing superior activity than both chloroquine (IC_{50} = 0.213 μM) and primaquine (IC_{50} = 0.643 μM). Moreover, hybrids **9d**, **9f**, and **14b** demonstrated an improved activity over their intermediates, exemplified by **8b** and quinoline diamine **17a**, and the equimolar combination of the individual components, an indication of a synergistic effect. Additionally, the PA-824–chloroquinoline hybrids were found to be more efficacious than the PA-824–primaquine hybrids [**9g** (IC_{50} = 2.042 μM) and **9h** (IC_{50} = 0.985 μM)], and the latter hybrids showed an antagonistic effect. The covalent attachment of methylnitroimidazole to intermediates **11a** and **11b** induced a significant reduction in antiplasmodial activity, as seen in **15a**

Scheme 2[a]



[a]Reagents and conditions: (i) MsCl, TEA, THF, 0 °C, N_2, 1 h; (ii) 2-methyl-4-nitro-1*H*-imidazole, K_2CO_3, DMF, 80 °C, 6 h; (iii) 2-nitroimidazo[2,1-*b*][1,3]oxazine, NaH, DMF, −50 °C to rt, 12 h; (iv) *N*-(methyl)ethanol amine, TEA, 0−60 °C, 3 h; (v) $SOCl_2$, DMF, toluene, 0 to rt, 14 h; (vi) 2-methyl-4(5)-nitroimidazole, K_2CO_3, DMF, 100−110 °C, 6 h.

and **15b**. Interestingly, the methylnitroimidazole derivative **14b** displayed a 4-fold increase in potency compared to intermediate **12b** whereas the opposite was observed for hybrid **14a**, which had a 10-fold reduction in potency compared to **12a**. The 2- and 4-carbon spacer appeared to be more favored in PA-824-chloroquinoline hybrids whereas the 3-carbon spacer was favorable in the methylnitroimidazole−chloroquinoline hybrids. The selective indices of the most active compounds were closer to or greater than 100, suggesting that these compounds are more selective toward the chloroquine-resistant *Plasmodium falciparum* parasite.

Against replicating H37Rv *M. tuberculosis*, the MCR series intermediates and target tetrazoles (**8** and **9**) exhibited MIC_{99} values ranging between 0.25 and 1.25 μM, except for **9e** and **9h**, while from the second series only hybrid **16** showed potent antimycobacterial activity. Also, compounds **9a−d**, **9f−g**, and **16** were more active than the standard TB drug, kanamycin (MIC_{99} = 5.40 μM), and the two antimalarial drugs, chloroquine (MIC_{99} > 160 μM) and primaquine (MIC_{99} = 80 μM). Interestingly, hybridization was found to be less beneficial for antimycobacterial activity, as evidenced by the antagonistic effect in both the PA-824-aminoquinoline and PA-824-primaquine hybrids. Notably, an increase in the length of the alkyl side-chain of the PA-824-chloroquinoline hybrids resulted in reduced activity.

Table 1. *In Vitro* Antimycobacterial and Antiplasmodial Activity of the Synthesized Compounds

entry	*P. falciparum* IC_{50} (μM) K1	*M. tuberculosis* MIC_{99} (μM) $H_{37}Rv$	cytotoxicity IC_{50} (μM) L6	SI[a]
6	>270	40	330.6	
8a	nd[b]	0.25	nd	
8b	62.62	0.625	34.3	0.54
9a	15.79	0.625	176.9	11.2
9b	7.95	0.313	112.7	14.2
9c	8.70	0.313	75.23	8.65
9d	0.100	0.313	24.6	246
9e	0.485	nd	41.52	85
9f	0.164	0.625	29.3	175
9g	2.042	0.625	75.9	37
9h	0.985	5	142.8	145
10a	1.468	>160	233.17	159
10b	1.086	>160	178.3	164
12a	0.078	>160	77.57	995
12b	0.391	>160	24.75	63
14a	0.812	>160	33.69	41.5
14b	0.0943	>160	17.6	186.6
15a	11.063	>160	140.8	13
15b	4.25	>160	136.8	32.2
16	10.52	1.25	134.5	12.8
17a[c]	0.298	>160	21.22	71.2
8a/17a[d]	0.303	0.25	20.41	67.4
8a/17b[d]	1.874	0.25	21.89	11.7
8a/17c[d]	2.514	0.25	11.81	4.69
8a/primaquine[c]	0.900	0.5	29.00	32.2
8b/primaquine[c]	0.721	0.625	22.94	31.8
chloroquine	0.213	>160		
primaquine	0.643	80		
podophyllotoxin			0.0193	
kanamycin		5.40		
streptomycin		0.27		

[a]Selective indices [(IC_{50} L6 cell-line)/IC_{50} (K1)]. [b]nd: not determined. [c]Structures of quinoline diamines **17a−c** can be found in the Supporting Information (section 1.10). [d]Equimolar combination of individual components.

In summary, we have designed and synthesized new nitroimidazole and nitroimidazooxazine derivatives, and these were screened for antiplasmodial and antimycobacterial activity. The majority of these compounds, especially hybrids **9d**, **9f**, and **14b**, exhibited potent activity against the K1 strain of *P. falciparum*, with IC_{50} values in the low micromolar range. Furthermore, compounds from the MCR series possessed superior antimycobacterial activity, with MIC_{99} values in the region of 0.25−125 μM while only one compound, **16**, from the second series showed an appreciable activity. Furthermore, the majority of the active compounds were more efficacious than kanamycin, a standard TB drug, in these assays

■ ASSOCIATED CONTENT

Supporting Information

Synthetic experimental procedures, characterization of final compounds, and details regarding biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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