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Identification of heteroarylenamines as a new class of antituberculosis lead molecules

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Abstract—Enamine-containing analogues of heteroarylquinones were prepared based on initial screening data observed against *Mycobacterium tuberculosis* $H_{37}Rv$ (Mtb). Several analogues showed moderate to good inhibitory activity, with one analogue (**7**) also demonstrating acceptable toxic selectivity (MIC 0.39 μg/mL, SI 15). Activity towards a range of single-drug-resistant strains of Mtb was suggestive of a novel mechanism of action for **7**.
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Current epidemiological evidence indicates that one-third of the world's population are infected with *Mycobacterium tuberculosis*, that 8 million new cases emerge annually and that 3 million deaths per year are directly attributable to infection with the bacillus.[1] Active disease following new infection, as well as reactivation of latent tuberculosis, is particularly prevalent in individuals with compromised immune systems, such as those that are HIV positive. In addition, the emergence of drug-resistant strains of *M. tuberculosis* has led to increased pressure on current chemotherapy regimes.[2]

As part of a program to discover new classes of antimycobacterial agents, the UoA Group has screened libraries of purified marine natural products and synthetically derived compounds for growth inhibitory activity towards *M. tuberculosis* $H_{37}Rv$ (Mtb) through the Tuberculosis Antimicrobial Acquisition and Coordination Facility (TAACF).[3] Two compounds identified as exhibiting activity (Table 1) were the marine alkaloid ascididemin (**1**)[4–6] and the synthetic precursor enamine **2**[7] (Fig. 1). In an effort to assess the potential of enamine **2** to act as a template for the development of new antituberculosis agents, a number of analogues of **2** were prepared and evaluated in a range of Mtb assays, both in vitro and in vivo.

Table 1. Antimycobacterial activity against *M. tuberculosis* and cytotoxicity of compounds **1**–**10**

Compound	MIC[a] *M. tuberculosis* $H_{37}Rv$	IC_{50}[b] VERO cells	Selectivity index, SI[c]
1	0.1	<0.04	<0.4
2	3.13	<0.26	<0.08
3	6.25	[d]	[d]
4	3.13	1.4	0.45
5	1.56	9.1	5.8
6	21.0[e]	n.d.[f]	n.d.
7	0.39	5.9	15.1
8	0.78	3.5	4.5
9	0.2	[d]	[d]
10	1.56	[d]	[d]
Rifampin	0.125–0.25	100	400

[a] MIC in μg/mL.
[b] IC_{50} in μg/mL.
[c] SI = VERO IC_{50}/MIC.
[d] Poor solubility prevented determination of IC_{50} and SI values.
[e] MIC against *M. tuberculosis* $H_{37}Rv$ pFPCA1 in a green fluorescent protein microplate assay (GFPMA).[13]
[f] Not determined.

The aim of the present study was to prepare a number of analogues of **2** in order to explore preliminary aspects of the structure–antituberculosis activity relationship. Analogues **3**,[8] **4**,[7] **5**,[7] and **6**[9] were prepared by literature procedures to assess the influence of the enamine moiety, steric size, and quinone functionality on biological activity (Chart 1). The preparations of **7**, **8**, and **9**, to explore requirements for nitrogen and sidechain functionality, are presented in Scheme 1.[10] Reaction of dione

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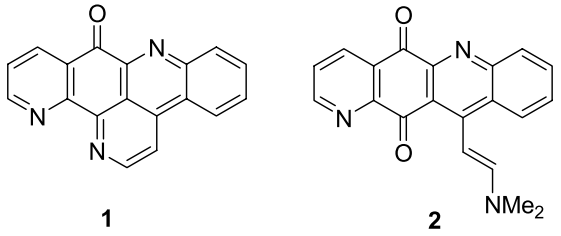


Figure 1. Structures of hit compounds resulting from library screening.

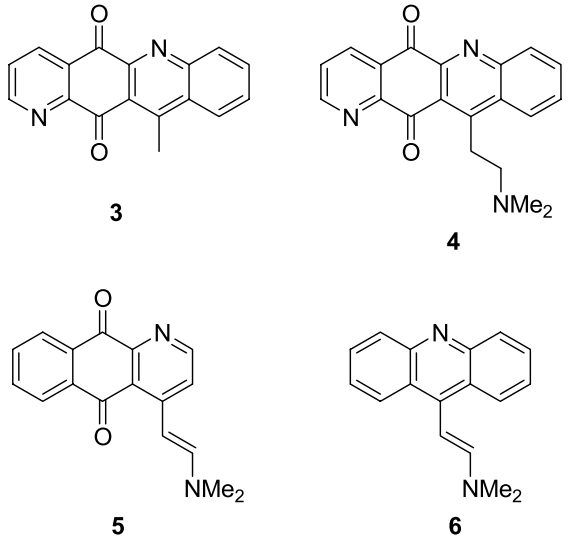
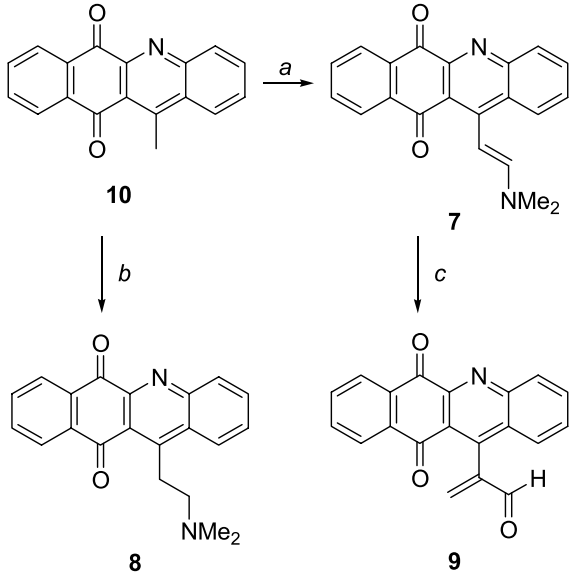


Chart 1.



Scheme 1. Reagents and conditions: (a) DMF–DEA, DMF, N_2, 120 °C, 30 min; (b) Eschenmoser's salt, DMF, N_2, 115 °C, 25 min; (c) Eschenmoser's salt, DMF, N_2, 105 °C, 10 min.

10[11] with DMF–DEA in DMF at 120 °C for 30 min yielded enamine **7**. Dimethylaminoethyl analogue **8** was prepared by reaction of dione **10** with Eschenmoser's salt in DMF at 115 °C (98%), while reaction of enamine **7** with Eschenmoser's salt afforded enal **9** (83%).

All compounds were initially evaluated for MIC against *M. tuberculosis* $H_{37}Rv$ in BACTEC 12B media using the microplate alamar blue assay (MABA).[12] The results, summarized in Table 1, indicate that the antimycobacterial activity of enamine **2** varies only slightly with the absence of the enamine moiety (vs **3**) or with sidechain saturation (vs **4**) but that these changes modify in vitro cytotoxicity and aqueous solubility. Preparation of the corresponding benzene analogues (**7**, **8**, and **10**) highlighted a similar antimycobacterial trend, with **7** exhibiting less pronounced in vitro cytotoxicity towards the VERO cell line. Sidechain modification, removal of ring-D, or complete removal of quinone functionality resulted in either poor solubility (**9**) or lowered potency (**5**, **6**).

Enamine **7** was the only compound in the series to satisfy the criterion of selectivity index $\geqslant 10$, where SI is defined as VERO IC_{50}/MIC, so its in vitro spectrum of activity was further investigated. Evaluation against a panel of single-drug-resistant strains of *M. tuberculosis* indicated no cross-resistance to any of the antitubercular agents ethambutol (EMB), isoniazid (INH), rifampin (RMP), kanamycin (KM), and ciprofloxacin (CIP) (all MIC <0.2 μg/mL), suggestive of a novel mechanism of action. Activity was also observed towards a number of clinical isolates of the nontubercular mycobacterium *Mycobacterium avium* (MIC 1–2 μg/mL), an organism which is responsible for frequent lethal infections in terminal AIDS patients.

It is now widely accepted that a physiological state of nonreplicating persistence of the tubercle bacillus (NRP-TB) is responsible for the long treatment duration for tuberculosis and that the key to shorten the 6-month regimen lies in targeting this subpopulation. In an in vitro low oxygen recovery assay that models NRP-TB,[14] **7** exhibited an MIC of 3.7 μg/mL (MIC RMP 7.9 μg/mL). The structurally simpler acridine enamine **6** exhibited an MIC of 54 μg/mL in the same assay, suggesting that enamines could be useful in targeting NRP-TB. Further evaluation of **7** against *M. tuberculosis* Erdman in monolayers of mouse bone marrow macrophages[16] indicated that the concentration effecting 90% reduction in the viable cell count after 7 days, compared to untreated controls (EC_{90}), was 2.1 μg/mL suggestive of reduced macrophage penetration.

As a prelude to animal testing, the maximum tolerated dose (MTD) of **7** was determined by using an escalating dose of drug (100, 300, and 500 mg/kg) given to mice by oral gavage. No adverse effects or reactions were observed, though solubility limited the 500 mg/kg dose. Subsequent in vivo evaluation of **7** was made at a dose of 300 mg/kg in infected C57BL/6 interferon-γ gene-depleted mice.[17] Although control treatment with INH (25 mg/kg/day) afforded reductions of 2.6 and 4.0 log CFU in lung and spleen tissues, respectively, enamine **7** reduced bacterial load in the spleen by 0.5 log CFU, which was not considered significant.[18,19] Poor bioavailability or rapid hydrolysis of the enamine under acidic conditions in the stomach may account for the lack of observed efficacy of **7** in animals.[20]

In conclusion, we have shown that the potency and activity towards a range of drug-resistant strains of *M. tuberculosis*, *M. avium*, and nonreplicating persistent forms of *M. tuberculosis* exhibited by these compounds make them valuable leads for synthesizing new analogues in the search of improved treatments for tuberculosis.

Acknowledgments

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10. All new compounds exhibited satisfactory spectroscopic and analytical data. Enamine **7**. Dark red solid, mp 194–196 °C, HREIMS 328.1213, $C_{21}H_{16}N_2O_2$ requires 328.1212, ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.25 (2H, m), 8.18 (2H, d, J = 8.6), 7.73 (1H, td, J = 7.5, 1.4), 7.67 (2H, td, J = 7.4, 1.2), 7.41 (1H, td, J = 7.6, 1.2), 7.39 (1H, d, J = 13.0, H-14), 7.06 (1H, d, J = 13.0, H-13), 3.10 (6H, s, NMe_2). Anal. Calcd for $C_{21}H_{16}N_2O_2 \cdot H_2O$: C, 73.1; H, 4.8; N, 7.9. Found: C, 72.8; H, 5.2; N, 8.1. Compound **8**: mp 197 °C (dec.), HREIMS 330.1370, $C_{21}H_{18}N_2O_2$ requires 330.1367, ^{1}H NMR (200 MHz, $CDCl_3$) δ: 8.37–8.21 (4H, m), 7.85–7.65 (4H, m), 3.96 (2H, t, J = 7.9, 2H-14), 2.80 (2H, t, J = 9, 2H-13), 2.54 (6H, s, NMe_2). Anal. Calcd for $C_{21}H_{18}N_2O_2 \cdot 1.25CHCl_3$: C, 55.7; H, 4.1; N, 5.8. Found: C, 55.9; H, 4.3; N, 5.9. Compound **9**: mp >220 °C, HREIMS *m/z* 313.0740 ($C_{20}H_{11}NO_3$ requires 313.0739), ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.97 (1H, s, H-1′), 8.44 (1H, d, J = 8.5, H-7), 8.38 (1H, m, H-1 or H-4), 8.18 (1H, m, H-1 or H-4), 7.98 (1H, d, J = 8.5, H-10), 7.89 (1H, ddd, J = 8.3, 7.0, 1.3, H-8), 7.80 (2H, m, H-2 and H-3), 7.68 (1H, ddd, J = 8.3, 7.0, 1.3, H-9), 6.76 (1H, s, H-3′B), 6.38 (1H, s, H-3′A). Anal. Calcd for $C_{20}H_{11}NO_3 \cdot CH_3OH$ C, 73.0; H, 4.4; N, 4.1. Found: C, 72.9; H, 4.2; N, 4.4.
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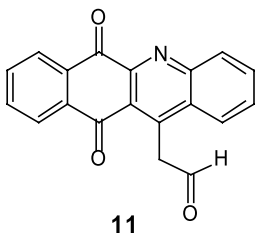
20. Preliminary studies suggest that the enamine functionality in **7** is relatively resistant to hydrolysis and that the hydrolytic product (**11**) also exhibits anti-*M. tuberculosis* activity. Dissolving **7** in 50% HCl (0.6 mg/mL) for 2 h afforded a 4% yield of the hydrolysis product **11** with the balance of material being unreacted **7**. Stirring **7** for 6 h in H_2O (0.08 mg/mL) yielded 9% of **11**. Aldehyde **11** was found to exhibit an MIC of 0.1 μg/mL and an SI = 18, comparable to enamine **7**. HREIMS *m/z* 301.0740 ($C_{19}H_{11}NO_3$ requires 301.0739), ^{1}H NMR δ: 10.04 (1H, s), 4.93 (2H, s).



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