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Antimycobacterial natural products: synthesis and preliminary biological evaluation of the oxazole-containing alkaloid texaline

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Abstract—Texaline, an antimycobacterial oxazole-containing alkaloid previously isolated from *Amyris texana* and *A. elemifera*, and related compounds have been synthesized in order to explore aspects of the structure–antituberculosis activity relationship. While texaline was found to be inactive in our assays, simpler diaryloxazoles were more active whilst also exhibiting modest toxic selectivity, leading to their identification as potential lead compounds.

With over one-third of the world's population infected and three million deaths per year directly attributable to the infection, tuberculosis remains one of the primary causes of death or suffering worldwide.[1] Increasing examples of multiple drug resistant strains of tuberculosis (MDR-TB) and the now intimate relationship between HIV/AIDS and tuberculosis has meant that there is increasing strain on the current range of therapies available.[2]

Introduced in the 1950s–1960s, natural products or their derivatives have had a remarkable impact on the treatment of tuberculosis, with current examples including rifampin, kanamycin and cycloserine. In the search for new classes of antituberculosis agents, investigations of natural products continue to provide useful new hits.[3] One such natural product is the oxazole alkaloid texaline (**1**) (Fig. 1),[4] isolated from the plants *Amyris texana* and *A. elemifera* and subsequently reported to inhibit the growth of *M. tuberculosis*, *M. avium* and *M. kansasii* with a minimum inhibitory concentration (MIC) of 25 μg ml^{-1}.[5]

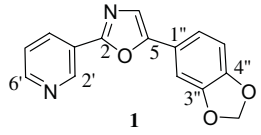

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Figure 1. Structure of texaline.

In an effort to explore new antimycobacterial chemotypes, we have undertaken the first reported synthesis of texaline, from commercially available 3,4-(methylenedioxy)acetophenone **2**, that is amenable to the preparation of a range of analogues.

α-Bromoketone **3**, prepared from 3,4-(methylenedioxy)acetophenone (**2**),[6] was converted to α-azidoacetophenone **4** by reaction with sodium azide in DMSO at room temperature (Scheme 1).[7]

Catalytic reduction of azide **4** with 10% palladium on activated carbon in glacial acetic acid, under an

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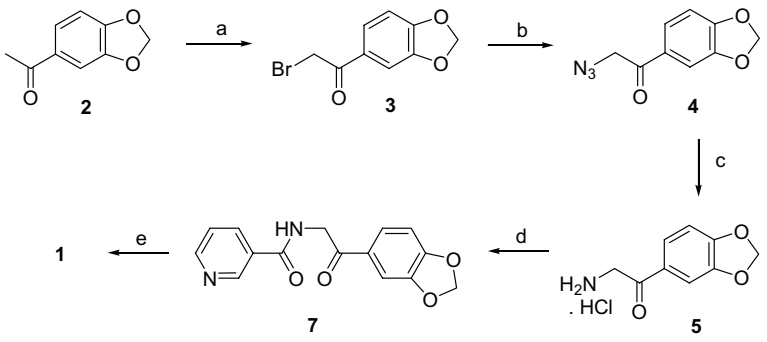

Scheme 1. Reagents and conditions: (a) *p*-TsOH·H_2O, NBS, MeCN, reflux (84%); (b) NaN_3, DMSO, 25 °C (73%); (c) H_2, 10% Pd/C, acetic acid, 25 °C (81%); (d) Nicotinoyl chloride hydrochloride (**6**), $NaHCO_3$, EtOAc/H_2O, 0–25 °C (16%); (e) Conc. H_2SO_4, acetic anhydride, 90 °C (46%).

atmosphere of hydrogen, followed by product precipitation with ethereal hydrogen chloride yielded amine **5** as the hydrochloride salt.[8] Nicotinoyl chloride hydrochloride **6** was then coupled with amine **5** using Schotten–Baumann conditions[9] to give key amide **7**. The low yield (16%) of this step is believed to be a result of the poor solubility of acid chloride **6**.[10] Subsequent cyclization of amide **7** using acidic conditions[11] yielded texaline (**1**), the spectroscopic data for which were in excellent agreement with reported data.[4,12]

Several analogues of texaline, namely 2-(3′-pyridyl)-5-phenyloxazole (**8**), 2,5-diphenyloxazole (**9**) and the natural product texamine (**10**)[4] were also prepared utilizing similar methodology (Fig. 2).[13]

The antimycobacterial activity and cytotoxicity of texaline (**1**), oxazole analogues **8**, **9** and **10** and *N*-phenacylnicotinamide (**11**) were determined against *M. tuberculosis* $H_{37}Rv$ in a number of different assay formats including the fluorescence readout Microplate Alamar Blue Assay (MABA)[14] and the non-fluorescent readout microbroth dilution assay (Table 1). Texaline (**1**) and **11**, the precursor amide to oxazole **8**, were inactive in all assays. Interestingly, the size reduced 2,5-disubstituted oxazole examples **8** and **9** exhibited modest activity, with some degree of toxic selectivity (SI (IC_{50}/MIC) > 3). In this limited series of compounds, the presence of a methylenedioxy moiety (e.g., **10**) appears to be detrimental to antimycobacterial activity.

It is now widely accepted that a physiological state of non-replicating persistence of the tubercle bacillus (NRP-TB) is responsible for the long treatment duration for tuberculosis and that the key to shortening the 6-month regimen lies in targeting this subpopulation. In an in vitro low oxygen recovery assay that models NRP-TB,[15] **9** exhibited an MIC of 62.5 μg/mL, suggestive that simple oxazoles could be useful in targeting NRP-TB.

Table 1. Comparative activity of texaline and analogues **8**–**11** against *M. tuberculosis* $H_{37}Rv$ and VERO cell-line cytotoxicity

Compound	MIC (μg ml^{-1}) for *M. tuberculosis* $H_{37}Rv$			IC_{50} (μg ml^{-1})
	MABA[a]	Microbroth[b]	LORA[c]	VERO cell-line[d]
1	>128	>125	>128	>128
8	30.1	31.25	>128	>102
9	29.0	31.25	62.5	nt[e]
10	>128	>125	>128	nt
11	>128	>125	>128	>102
Rifampin	0.11	nt	7.9	104

[a] MIC = 90% growth inhibition in Microplate Alamar Blue Assay.[14]
[b] MIC = 100% growth inhibition in microbroth dilution assay.
[c] MIC = 90% growth inhibition in the Low Oxygen Response Assay.[15]
[d] IC_{50} = 50% growth inhibition.
[e] nt: not tested.

In summary, we have synthesized texaline (**1**) following a synthetic pathway that also allowed for the preparation of several analogues. While texaline, previously reported to exhibit antimycobacterial activity, was inactive in our hands, two simpler analogues were found to be active against *M. tuberculosis*. This work suggests that simple oxazole derivatives may be viable leads in the search for new classes of antituberculosis agents and that further structure–activity studies are clearly warranted.

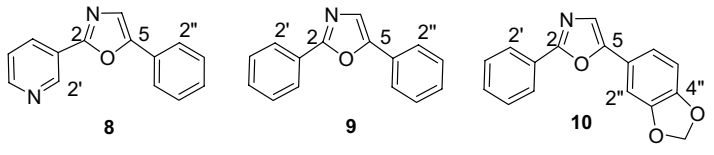

Figure 2. Structures of texaline analogues.

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10. When stronger bases (e.g. NaOH) were utilized in an attempt to increase the solubility of nicotinoylchloride hydrochloride (**6**), complex mixtures were produced. Subsequent use, under identical reaction conditions, of benzoyl chloride in the preparation of analogue **10** afforded the corresponding amide in 52% yield further supporting poor substrate solubility as the cause of the low yield of **7**.
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12. Spectral data for Texaline (**1**): Mp 167–169 °C (lit.[4] 171–174 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.31 (1H, d, $J = 1.8$ Hz, H-2′), 8.68 (1H, dd, $J = 4.9$, 1.6 Hz, H-6′), 8.33 (1H, dt, $J = 8.0$, 1.9 Hz, H-4′), 7.41 (1H, dd, $J = 7.7$, 5.1 Hz, H-5′), 7.34 (1H, s, H-4), 7.24 (1H, dd, $J = 8.1$, 1.7 Hz, H-6″), 7.17 (1H, d, $J = 1.6$ Hz, H-2″), 6.90 (1H, d, $J = 8.2$ Hz, H-5″), 6.03 (2H, s, OCH_2O); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 158.21 (C-2), 151.93 (C-5), 150.81 (C-6′), 148.31 (C-4″), 148.23 (C-3″), 147.46 (C-2′), 133.23 (C-4′), 123.72 (C-3′), 123.58 (C-5′), 122.55 (C-4), 121.76 (C-1″), 118.62 (C-6″), 108.92 (C-5″), 104.91 (C-2″), 101.47 (OCH_2O); HREIMS m/z 266.0694 (calcd for $C_{15}H_{10}N_2O_3$: 266.0691).
13. Spectral data for some key compounds: Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.35 (1H, d, $J = 1.7$ Hz, H-2′), 8.70 (1H, dd, $J = 4.9$, 1.6 Hz, H-6′), 8.37 (1H, dt, $J = 8.1$, 1.8 Hz, H-4′), 7.73 (2H, m, H-2″, H-6″), 7.49–7.36 (5H, m, H-4, H-5′, H-3″, H-4″, H-5″); HREIMS m/z 222.0794 (calcd for $C_{14}H_{10}N_{20}$: 2220793). Compound **9**: ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.11–8.08 (2H, m, H-2′, H-6′), 7.71–7.67 (2H, m, H-2″, H-6″), 7.51–7.38 (6H, m, H-4, H-3′, H-4′, H-5′, H-3″, H-5″), 7.30 (1H, m, H-4″); HREIMS m/z 221.0843 (calcd for $C_{15}H_{11}NO$: 221.0841). Texamine **10**: Mp 134–136.5 °C (lit.[4] 134–137 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.08–8.05 (2H, m, H-2′, H-6′), 7.49–7.41 (3H, m, H-3′, H-4′, H-5′), 7.28 (1H, s, H-4), 7.20 (1H, dd, $J = 8.1$, 1.7 Hz, H-6″), 7.14 (1H, d, $J = 1.7$ Hz, H-2″), 6.85 (1H, d, $J = 8.1$ Hz, H-5″), 5.97 (2H, s, OCH_2O); HREIMS m/z 265.0738 (calcd for $C_{16}H_{11}NO_3$: 265.0739).
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