Tetrahedron 66 (2010) 4977–4986

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

anti-Tuberculosis natural products: synthesis and biological evaluation of pyridoacridine alkaloids related to ascididemin

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ARTICLE INFO

Article history:
Received 8 February 2010
Received in revised form 22 April 2010
Accepted 10 May 2010
Available online 1 June 2010

Keywords:
Tuberculosis
Natural Products
Ascididemin
pyridoacridine

ABSTRACT

There is an urgent need for novel therapeutics possessing new modes of action to treat tuberculosis (TB) infections. In this study we report on the synthesis and biological evaluation of a series of pyrido[2,3,4-*kl*] acridin-6-one alkaloids related to the *anti*-TB (MIC 0.35 μM) but cytotoxic (IC_{50} <0.14 μM) marine natural product ascididemin (**1**). The most interesting compounds identified were **21** and **24**, which were found to inhibit the growth of *Mycobacterium tuberculosis* (Mtb) $H_{37}Rv$ with MIC 2.0 μM, but with negligible cytotoxicity towards Vero and P388 cells (IC_{50}>25 μM). Another analogue (**10**) was evaluated against a range of singly-drug-resistant strains of Mtb and was found to exhibit no cross-resistance. These results suggest that the pyrido[2,3,4-*kl*]acridin-6-one skeleton may provide a useful scaffold for future studies directed towards possible *anti*-TB drugs.

1. Introduction

It has been estimated that two billion people or one-third of the world's population are infected with *Mycobacterium tuberculosis* (Mtb), the microbe that causes tuberculosis (TB). Of those infected, 10% will become sick in their lifetime, making TB one of the leading causes of death in the world.[1] New drugs that provide shorter treatment regimes than those currently available with existing drugs and that can overcome the increasing emergence of drug resistant strains are urgently needed. Screening programmes are in place, e.g., Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF),[2] to discover new chemical entities that can act as scaffolds for future drug development. Whilst much recent attention has focused on purely synthetic leads, such as R207910[3] and PA-824,[4] there is continuing interest in the ability of natural products to act as new drug leads.[5]

As part of our search for new antituberculosis lead compounds, we have screened a purified compound library of marine natural products for activity against Mtb through The TAACF Program.[2] Among a number of hits identified as exhibiting a minimum inhibitory concentration (MIC) of <6.25 μg/mL was the pyridoacridone alkaloid ascididemin (**1**)[6] (Fig. 1).

Preliminary investigation of the mechanism of action of **1**, utilizing differential gene expression technology, identified an effect upon iron regulation in Mtb.[7] Ascididemin, like other marine-derived pyridoacridine alkaloids is known to exert cytotoxicity via inhibition of DNA topoisomerase II, leading to cleavage of DNA and cell death, with IC_{50}s against mammalian cell lines as low as 0.3 μM.[8] While these cytotoxic effects clearly limit the potential antibiotic utility of the natural product, we were interested in exploring modifications to the pentacyclic-core of ascididemin in an effort to decouple mammalian cell toxicity from *anti*-tuberculosis activity. Herein, we describe the synthesis, biological evaluation and structure/activity relationships (SAR) of a series of natural product and natural product-related pyridoacridine and pyridoacridone alkaloids.

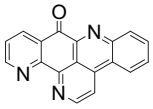

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Figure 1. Chemical structure of ascididemin.

2. Chemistry

Our ongoing interest in the secondary metabolites of New Zealand marine organisms from the Class Ascidiacea (ascidians) has afforded us a purified compound library comprised of a number of examples of pyridoacridine and pyridoacridone alkaloids. Previous investigation of extracts from a Northland, New Zealand collection of the ascidian *Lissoclinum notti* yielded the pyridoacridone alkaloid

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doi:10.1016/j.tet.2010.05.033

diplamine (**2**), which was first reported from a Fijian collection of the ascidian *Diplosoma* sp.,[9] along with previously unreported analogues isodiplamine (**3**) and lissoclinidine (**4**).[10] Specimens of the same organism species collected in the south of New Zealand provided samples of the previously reported alkaloids kuanoniamine D (**5**)[11] and shermilamine B (**6**).[12] The addition of two other structurally related marine natural products to our study was of interest: 11-hydroxyascididemin (**7**)[13] and kuanoniamine A (**8**),[11] were both prepared by literature methods[14,15] (Fig. 2).

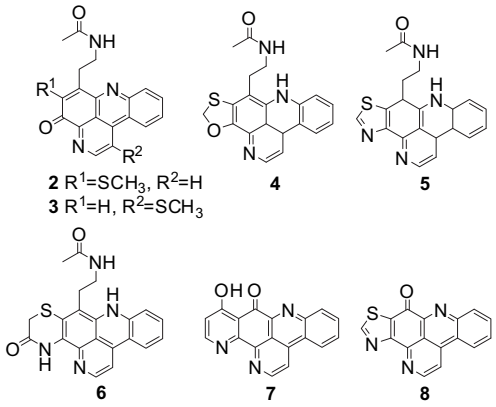


Figure 2. Chemical structures of other natural pyridoacridines investigated for *anti*-TB activity.

Preparation of alkaloids with variation in size of the pentacyclic-core of ascididemin was targeted as a priority, with the express desire of reducing DNA binding properties and inherent cytotoxicity. We have previously reported the syntheses of tetracyclic pyrido[2,3,4-*kl*]acridin-6-one analogues **9**, **10**, **11** and **12** as part of a structure/activity relationship study of 11-hydroxyascididemin[14] (Fig. 3).

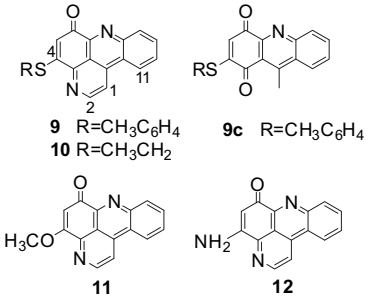
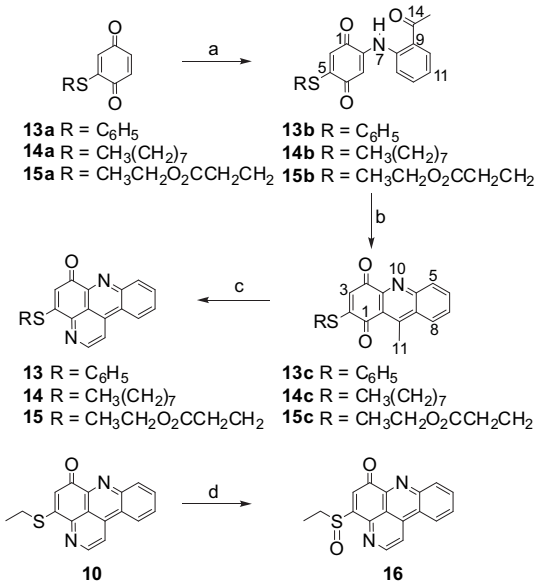


Figure 3. Chemical structures of tetracyclic pyrido[2,3,4-*kl*]acridin-6-ones initially investigated for *anti*-TB activity.

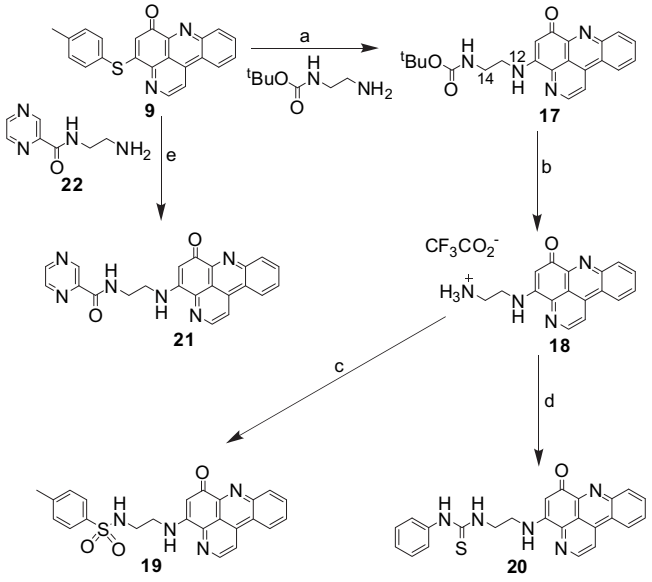
This series was initially extended by preparation of the thiophenyl (**13**), thio-*n*-octyl (**14**) and thioethylpropanate (**15**) analogues via a similar route (Scheme 1). Thus, the thio-substituted 1,4-benzoquinones **13a**,[16] **14a** and **15a** were prepared in good yield by reaction of the appropriate thiol (1.0 equiv) with benzoquinone (2.0 equiv) in ethanol at room temperature. Subsequent oxidative coupling of these quinones with 2′-aminoacetophenone yielded the desired 5-(2-acetylanilino)-2-thio substituted 1,4-benzoquinones **13b**, **14b** and **15b** (45–61%), which upon acid catalysed cyclisation led to acridinediones **13c**, **14c** and **15c** in yields of 79–92%. Application of a one pot annulation using paraformaldehyde and ammonium chloride in glacial acetic acid,[15] led to pyrido[2,3,4-*kl*]acridin-6-one analogues **13**, **14** and **15**. Oxidation of sulfide **10**[14] with *m*-chloroperoxybenzoic acid yielded the sulfoxide **16** in 73% yield.

The propensity of 4-(4-methylphenylthio)pyrido[2,3,4-*kl*]acridin-6-one (**9**) to undergo substitution with a variety of nucleophiles[14] was



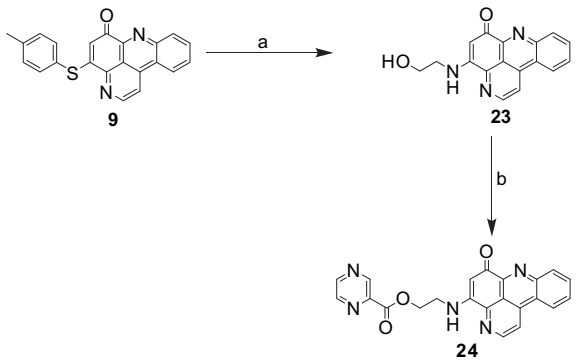
Scheme 1. Reagents and conditions: (a) $CeCl_3 \cdot 7H_2O$, 2′-aminoacetophenone, air, 45–61% yield; (b) AcOH, H_2SO_4, 79–92% yield; (c) NH_4Cl, $(CH_2O)_n$, AcOH, reflux, 50–83% yield; (d) *m*-CPBA, NaOAc, CH_2Cl_2, 73%.

taken advantage of in the preparation of compounds **11**, **12**, **17**, **21** and **23**. Thus the reaction of **9** with *tert*-butyl *N*-(2-aminoethyl)carbamate in the presence of a catalytic amount of sodium methoxide in MeOH afforded **17** in 53% yield (Scheme 2). Deprotection using TFA in CH_2Cl_2 provided the unstable ammonium salt **18**, which upon reaction with tosylchloride in MeCN afforded sulfonamide **19** in 61% yield, or reaction with phenylisothiocyanate also in MeCN provided thiourea **20** in 45% yield. Amide **21** was prepared by direct coupling of the monosubstituted pyrazine ethylene diamine **22** with sulfide **9**.



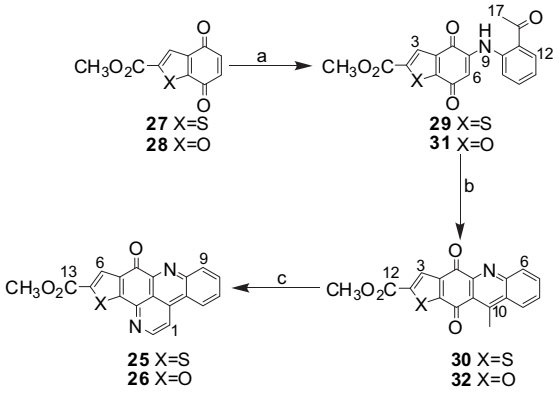
Scheme 2. Reagents and conditions: (a) *tert*-butyl *N*-(2-aminoethyl)carbamate, NaOMe, Et_3N, N_2, 53%; (b) TFA, 100%; (c) Tosylchloride, Et_3N, 61%; (d) Phenylisothiocyanate, Et_3N, 45%; (e) NaOMe, Et_3N, N_2, 36%.

Aminoalcohol **23** was synthesised by nucleophilic substitution of sulfide **9** with ethanolamine in the presence of sodium methoxide in MeOH in low yield (25%) (Scheme 3). Subsequent preparation of ester **24** was achieved by reaction of **23** with pyrazinecarboxylic acid in the presence of BOP-Cl and Et_3N.



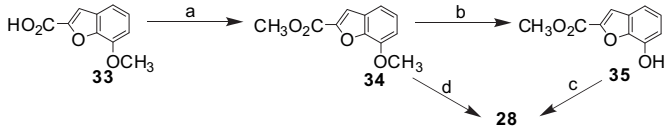
Scheme 3. Reagents and conditions: (a) Ethanolamine, NaOMe, N_2, 25%; (b) Pyrazine carboxylic acid, BOP-Cl, Et_3N, 38%.

The synthesis of the ring A modified analogues of ascididemin (**1**) possessing either methyl carboxylate thiophene (**25**) or furan (**26**) functionality was achieved using previously reported methodology starting from the appropriately functionalised quinones, **27**[17] and **28**, respectively. Reaction of thiophene quinone **27** with 2′-aminoacetophenone in the presence of cerium chloride and in situ oxidation using air yielded adduct **29** in high yield (92%) (Scheme 4). The purple adduct **29** was then subjected to dehydrative cyclisation in the presence of 10:1 glacial acetic acid/H_2SO_4 at 100 °C to yield the yellow acridinedione **30** in 94% yield. Final one pot annulation, by reaction with paraformaldehyde and ammonium chloride in the presence of acid[15] afforded the desired thiopheno-acridinone **25** in 83% yield.



Scheme 4. Reagents and conditions: (a) $CeCl_3{\cdot}7H_2O$, 2′-aminoacetophenone, air, 92% for X=S, 22% for X=O*; (b) AcOH, H_2SO_4, 81–94%; (c) NH_4Cl, $(CH_2O)_n$, AcOH, reflux, 76–83%. *X=O also yields the spontaneously cyclised compound (61%).

Furan quinone **28** was prepared from commercially available **33** after esterification (**34**) and either methyl ether cleavage with boron tribromide in CH_2Cl_2 to yield **35** followed by CAN oxidation to **28**, or direct formation of **28** from the methyl ether **34** using cerium ammonium sulfate in quantitative yield (Scheme 5). A subsequent reaction sequence of substitution, cyclisation and annulation using quinone **28**, carried out in similar fashion to that performed on quinone **27**, yielded the target furano-acridinone **26** (Scheme 4).



Scheme 5. Reagents and conditions: (a) MeOH, H_2SO_4, reflux, 100%; (b) BBr_3, 86%; (c) CAN, 83%; (d) $Ce(NH_4)_4(SO_4)_4$, H_2SO_4, 100%.

3. Results and discussion

Pyrido[2,3,4-*kl*]acridin-6-one analogues were prepared and evaluated for the ability to inhibit the in vitro growth of *M. tuberculosis* $H_{37}Rv$ at Southern Research Institute under the auspices of the TAACF program.[2] All of the active, and soluble, compounds were also assessed for in vitro cytotoxicity towards the Vero and murine leukaemia P388 cell-lines. Comparison of the in vitro *anti*-TB activity determined for ascididemin (**1**) to a series of structurally related natural pyridoacridines **2**–**8** revealed that **1** was by far the most potent and that the complete iminoquinone moiety, as seen in compounds **1**, **7** and **8**, was required for biological activity (Table 1). Interestingly, 11-hydroxyascididemin (**7**) exhibited weak activity against Mtb $H_{37}Rv$ with an MIC >42 μM despite still having the intact iminoquinone functionality. However, despite ascididemin (**1**) exhibiting extremely good potency (MIC 0.35 μM) against Mtb in vitro, the selectivity index was low, with similar cytotoxicity values being observed against both Vero (<0.14 μM) and P388 (0.4 μM) cell-lines.

Table 1
In vitro *anti*-TB activity and cytotoxicity of pyridoacridines and pyridoacridone natural products

Compound	$H_{37}Rv$ MIC (μM)	Vero cells IC_{50} (μM)	P388 cells IC_{50} (μM)
1	0.35	<0.14	0.4
2	>17	ND	1.9
3	>17	ND	2.1
4	>17	ND	4.6
5	>34	ND	ND
6	>32	ND	ND
7	>42	ND[a]	2.3
8	10.7	I[b]	0.3
RMP[c]	0.152	154	ND

[a] ND: not determined.
[b] I: insoluble in Vero cell line assay media.
[c] RMP: rifampin.

In order to investigate if it was possible to synthesise analogues that maintain or improve *anti*-TB activity while reducing cytotoxicity, we investigated a series of synthetic pyridoacridines possessing substitution at the 4-position of a pyrido[2,3,4-*kl*]acridin-6-one core. Previous work by our group had shown that the cytotoxicity of the pyridoacridine pharmacophore could be attenuated by altering the substitution at this position and synthetic routes were well established and high-yielding.[14] A variety of aryl- and alkyl-sulfides was prepared and assessed for in vitro *anti*-TB activity and cytotoxicity. The most potent analogue of this series was identified as 4-ethylthiopyrido[2,3,4-*kl*]acridin-6-one (**10**) with an MIC of 0.34 μM, comparable to ascididemin (**1**) (Table 2). Encouragingly, the level of cytotoxicity observed for **10** was 1–2 orders of magnitude less, yielding a selectivity index of 20. Interestingly, oxidation of the sulfide to sulfoxide **16** resulted in a significant reduction in the *anti*-TB activity. In general, for the sulfide series, the alkyl sulfides showed greater potency than the aryl sulfides and had comparable cytotoxicity values. Some activity was also observed for this series against the Gram-positive bacterium *Bacillus subtilis* and the fungus *Trichophyton mentagrophytes* in disc diffusion assays. The trend of antibacterial activity for the pyridoacridines correlated reasonably well with *anti*-TB activity while the antifungal activity seemed to correlate with cytotoxicity, with possibly some solubility effects for the relatively non-polar examples **9** and **14** explaining their low activity in the disc diffusion assays. Replacement of the thio-substituent with a methoxyl group (**11**) resulted in a slight reduction of *anti*-TB activity while increasing the cytotoxicity. The thiophene (**25**) and furan (**26**) analogues also exhibited similar potencies against Mtb $H_{37}Rv$ and cytotoxicity as **10** but were not investigated further due their poor solubilities and more complicated synthetic routes. The 2,3,4-trisubstituted pyridine ring present in **9**, **14** and **15** is

Table 2
In vitro biological activities of substituted thio- and oxo- acridinediones and pyrido[2,3,4-*kl*]acridin-6-ones

Compound	$H_{37}Rv$ MIC (μM)[a]	*B.sub*[b]	*T.ment*[c]	Vero cells IC_{50} (μM)	Selectivity index[d]	P388 cells IC_{50} (μM)
9c	9.0	1	0	23.8	2.6	28.0
9	2.20	0	0	I[e]		19.5
10	0.34	3	1	6.8	20	6.8
11	1.5	8	6	10.7	7.1	3.3
13	18.4	1	1	I		21.1
14c	17.0	1	0	4.9	0.3	3.3
14	1.0	0	0	14.1	14	27.1
15c	17.6	6	3	15.2	0.9	1.0
15	2.1	6	3	5.8	2.7	17.9
16	>20	ND[f]	ND	ND		ND
25	0.58	1 (20)	0 (20)	I		8.5
26	0.61	3 (20)	7 (20)	I		3.0
30	18.5	ND	ND	I		ND
32	19.5	ND	ND	I		ND
RMP[g]	0.152			154		

[a] MIC (μM) against *M. tuberculosis* $H_{37}Rv$.
[b] *B. subtilis* inhibition zone (mm) at 120 μg/disc unless indicated in brackets.
[c] *T. mentagrophytes* inhibition zone (mm) at 120 μg/disc unless indicated in brackets.
[d] Selectivity index: Vero cell line cytotoxicity/Mtb MIC.
[e] I: insoluble in Vero cell line media.
[f] ND: not determined.
[g] RMP: rifampin.

necessary for good selectivity indices: in general, the respective acridinediones **9c**, **14c**, **15c**, **30** and **32** were less potent against *M. tuberculosis* and were more cytotoxic that the corresponding pyridoacridines and therefore, were also not investigated further. It was evident from this initial study that separation of *anti*-TB activity and cytotoxicity could be achieved through the size-reduced analogues of ascididemin (**1**).

A series of 4-aminopyrido[2,3,4-*kl*]acridin-6-one analogues were then prepared and evaluated for *anti*-TB activity and cytotoxicity. While the 4-amino derivative **12** exhibited less potent *anti*-TB activity (MIC 3.2 μM) and a lower selectivity index of 7.3 (Table 3), it was found that analogues bearing a 2-carbon spacer and a terminal bulky group were significantly less cytotoxic while maintaining respectable *anti*-TB activity. Initial compounds prepared included the *tert*-butyloxycarbonyl derivative **17**, tosylate **19** and phenylthiourea **20**, all of which exhibited reasonable *anti*-TB activity and much reduced cytotoxicity against both Vero and P388 cells. The free terminal amine **18** or alcohol **23** was relatively more cytotoxic. Good antibacterial activity was also observed for these compounds against *B. subtilis*, and along with the low cytotoxicity, no antifungal activity was observed for any of the amino derivatives. Due to the observation of limited solubility for analogues **17**, **19** and **20**, it was considered desirable to incorporate a bulky but basic group. Pyrazinecarboxylic acid was chosen as it is known to be the active pharmacophore of the *anti*-TB drug pyrazinecarboxamide[18] after intracellular amide-bond cleavage, and should provide higher aqueous solubility along with the possibility of a hybrid drug interaction with Mtb. Amide (**21**) and ester (**24**) analogues were found to exhibit moderate *anti*-TB activity with MIC's of 2.0 μM against *M. tuberculosis* $H_{37}Rv$ while having no measurable cytotoxicity of doses up to 25 μM against both Vero and P388 cells. The amide **21** also exhibited the highest antibacterial activity of the series in the disc diffusion assay against *B. subtilis* with a radius of 10 mm at 120 μg/disc.

Table 3
In vitro Biological Activities of Substituted 4-aminopyrido[2,3,4-*kl*]acridin-6-ones **12**, **17**–**21**, **23** and **24**

Compound	$H_{37}Rv$ MIC (μM)[a]	*B.sub*[b]	*T.ment*[c]	Vero cells IC_{50} (μM)	Selectivity index[d]	P388 cells IC_{50} (μM)
12	3.2	ND[e]	ND	23.5	7.3	ND
17	2.0	5	0	>25	>12.5	41.0
18	5.5	8	0	2.1	0.4	8.6
19	3.5	5	0	I[f]	ND	51.6
20	3.7	5	0	I	ND	>59
21	2.0	10	0	>25	>12.5	>63
23	1.3	1	0	12.7	9.8	50.2
24	2.0	3	0	>25	>12.5	>63
RMP[g]	0.152			154		

[a] MIC (μM) against *M. tuberculosis* $H_{37}Rv$.
[b] *B. subtilis* inhibition zone (mm) at 120 μg/disc.
[c] *T. mentagrophytes* inhibition zone (mm) at 120 μg/disc.
[d] Selectivity index: Vero cell line cytotoxicity/Mtb MIC.
[e] ND: not determined.
[f] I: insoluble in Vero cell line media.
[g] RMP: rifampin.

In a preliminary effort directed towards determination of the mechanism of action, analogue **10** was profiled against a panel of singly-drug-resistant strains of *M. tuberculosis* and found to be extremely potent with MIC's 0.34 μM or less against all strains (Table 4). This suggests the mechanism of action of pyrido[2,3,4-*kl*]acridin-6-one alkaloids is different from those of current antituberculosis agents.

Table 4
In vitro *anti*-TB profiling of 4-ethylthiopyrido[2,3,4-*kl*]acridin-6-one (**10**) against singly-drug-resistant strains of Mtb

MIC (μM)					
$H_{37}Rv$	EMB-R[a]	INH-R[b]	RMP-R[c]	KM-R[d]	CIP-R[e]
0.34	<0.17	0.34	<0.17	<0.17	<0.17

[a] Ethambutol resistant.
[b] Isoniazid resistant.
[c] Rifampicin resistant.
[d] Kinamycin resistant.
[e] Ciprofloxacin resistant.

4. Conclusion

The marine natural product ascididemin (**1**) exhibits potent in vitro growth inhibition of *M. tuberculosis* $H_{37}Rv$ (MIC 0.35 μM) but also significant levels of cytotoxicity (IC_{50}<0.14 μM). This investigation of a series of 4-substituted pyrido[2,3,4-*kl*]acridin-6-ones led to the identification of several compounds found to possess *M. tuberculosis* MICs in the low micromolar range while exhibiting significantly reduced cytotoxicity, and exemplifies the value of screening natural products libraries as a starting point for further lead development programmes. In addition, the observation of growth inhibition by thioethyl analogue **10** towards a range of single drug resistant strains of Mtb is particularly encouraging, and suggests that size-reduced analogues of ascididemin may provide a useful scaffold for future studies.

5. Experimental section

5.1. Chemistry: general methods

All solvents used were analytical grade or better and/or purified according to standard procedures. Chemical reagents used were either purchased from standard chemical suppliers and used as purchased or prepared according to literature procedures and purified to match the reported physical and spectral data. Analytical thin layer chromatography was carried out on Merck DC-plastikfolien Kieselgel 60 F_{254} plates and products were visualised by UV fluorescence. Flash column chromatography was performed using Merck 40e63 mm silica gel. Mass spectra were recorded on a VG-7070 mass spectrometer operating at a nominal accelerating voltage of 70 eV. All spectra were obtained using EI, FAB or CI ionisation techniques using perfluorokerosene, 3-nitrobenzylalcohol or polyethylene glycol as the internal standards. Infrared spectra were recorded as dry films on sodium chloride with

a Spectrum One Fourier Transform infrared spectrometer, with the 1603 cm^{-1} absorption band of polystyrene being used as a reference. Melting points were recorded on a Reichert/Kofler block and are uncorrected. NMR spectra were recorded at 298 K at either 300.13 or 400.13 MHz for ^1H and 75.47 or 100.62 MHz for ^{13}C on Bruker Avance 300 or 400 spectrometers. Chemical shifts are given in parts per million on the δ scale referenced to the residual solvent peaks ($CHCl_3$: ^1H 7.25, ^{13}C 77.0 ppm; DMSO-d_6: ^1H 2.50, ^{13}C 39.4 ppm; MeOH-d_4: ^1H 3.30, ^{13}C 49.0 ppm). Assignments were aided by DEPT135, HSQC and HMBC experiments. Microanalyses were carried out by the Campbell Laboratory, University of Otago, Dunedin, New Zealand. Target compounds ascididemin (**1**), 11-hydroxyascididemin (**7**), kuanoniamine A (**8**) and pyridoacridines **9**, **10**, **11** and **12** were prepared as previously described,[14,15] as were the quinone starting materials **13a**[16] and **27**.[17]

*5.1.1. 2-(Phenylthio)-5-(2-acetylanilino)-1,4-benzoquinone (**13b**).* 2′-Aminoacetophenone (0.44 g, 3.3 mmol) in methanol (10 mL) was added to a solution of 2-phenythio-l,4-benzoquinone (**13a**)[16] (0.8 g, 3.7 mmol) and cerium trichloride heptahydrate (0.56 g, 1.5 mmol) in ethanol (60 mL). The solution was stirred vigorously for 24 h, yielding a dark red precipitate that was filtered and recrystallised from methanol to afford **13b** (0.8 g, 61% yield) as a dark red crystalline solid. Mp 164–168 °C; R_f (100% CH_2Cl_2) 0.71; IR ν_{max}, (film) 1658, 1651, 1614, 1558, 1620, 1452, 1300, 1250, 1210, 997, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.02 (1H, s, NH), 7.92 (1H br d, J=7.8 Hz, ArH), 7.55 (2H, br d, J=5.0 Hz, ArH), 7.50 (5H, m, ArH), 7.17 (1H, m, ArH), 6.47 (1H, s), 5.84 (1H, s), 2.63 (3H, s, $COCH_3$); ^{13}C NMR ($CDC1_3$, 75 MHz) δ 201.2, 183.2, 179.7, 158.6, 143.2, 139.8, 135.5, 134.1, 132.3, 130.4, 130.2, 127.5, 125.6, 123.2, 122.0, 120.7, 102.4, 28.4; MS *m*/*z* (%) 349, 331 (M^+-H_2O, 48), 307 (M^+–CHCO, 100), 186 (M^+–163, 60); HREIMS calcd for $C_{20}H_{15}NO_3S$ 349.0773, found 349.0773.

*5.1.2. 2-(Phenylthio)-9-methyl-l,4-acridinedione (**13c**).* 2-(Phenylthio)-5-(2-acetylanilino)-1,4-benzoquinone (**13b**) (1.1 g, 3.3 mmol) was dissolved in a premixed solution of glacial acetic acid (36 mL) and concd H_2SO_4 (4 mL) and heated at 80 °C for 10 min. Addition of water (100 mL) afforded a precipitate, which was filtered, washed with water (5×20 mL) and cold methanol (2×30 mL). Recrystallisation from chloroform/methanol yielded **13c** (0.78 g (79% yield)) as yellow needle-like crystals. Mp 207–210 °C; R_f (100% CH_2Cl_2) 0.34; IR ν_{max} (film) 1660sh, 1576, 1560, 1376, 1337, 1250, 1184, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (1H, dm, J=9.0 Hz, H-5), 8.33 (1H, dm, J=8.6 Hz, H-8), 7.89 (1H, ddd, J=8.4, 6.8, 1.8 Hz, H-6), 7.75 (1H, ddd, J=8.4, 6.9, 1.8 Hz, H-7), 7.52 (5H, m, H-13,14,15,16,17), 6.36 (1H, s, H-3), 3.21 (3H, s, H_3-11); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 183.6 (C-1), 180.2 (C-4), 160.2 (C-2), 151.8 (C-9), 148.4 (C-10a), 147.2 (C-4a), 135.7 (C-14,16), 132.6 (C-6), 132.2 (C-5), 130.7 (C-15), 130.5 (C-13,17), 129.5 (C-7), 129.2 (C-8a), 128.4 (C-3), 127.2 (C-12), 125.5 (C-8), 123.2 (C-9a), 16.2 (C-11); MS *m*/*z* (%) 331 (M^+, 100), 302 (M^+–CHO, 20), 275 (23), 274 (50), 254 (41); HREIMS calcd for $C_{20}H_{13}NO_2S$ 331.0667, found 331.0667.

*5.1.3. 4-Phenylthiopyrido[2,3,4-kl]acridin-6-one (**13**).* 2-Phenylthio-9-methyl-1,4-acridione (**13c**, 33 mg, 0.10 mmol) and ammonium chloride (380 mg, 7.10 mmol) in glacial acetic acid (1.0 mL) were stirred at 100 °C. Paraformaldehyde (24 mg, 0.8 mmol) in glacial acetic acid (10 mL) was added and heating continued at 94 °C for 5 min under nitrogen. The dark purple reaction mixture was cooled, poured onto ice, made basic with concd ammonia and extracted with chloroform (3×100 mL). The combined organic extracts were washed with water/concd ammonia (4:1, three times), then brine (100 mL), dried over magnesium sulfate, and the solvent removed in vacuo. The yellow/orange residue was purified by flash column chromatography (silica gel, chloroform), with recrystallisation from dichloromethane/methanol yielding **13** (28 mg, 83% yield) as yellow/brown crystals. Mp 210–211 °C; R_f (100% CH_2Cl_2) 0.23; IR ν_{max} (film) 1650, 1644, 1599, 1552, 1469, 1433, 1358, 1242, 1168, 974, 858, 759 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.05 (1H, d, J=5.7 Hz, H-2), 8.61 (1H, dd, J=8.4, 1.4 Hz, H-11), 8.57 (1H, dd, J=8.4, 1.7 Hz, H-8), 8.49 (1H, d, J=5.7 Hz, H-1), 7.97 (1H, ddd, J=8.4, 7.0, 1.4 Hz, H-9), 7.90 (1H, ddd, J=8.4, 7.1, 1.4 Hz, H-10), 7.67 (2H, m, H-13/17), 7.53 (3H, m, H-14/15/16), 6.29 (1H, s, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 180.3 (C-6), 160.5 (C-4), 149.5 (C-3a), 148.2 (C-2), 146.6 (C-6a), 145.9 (C-7a), 137.0 (C-11b), 136.1 (C-13/17), 133.1 (C-8), 131.8 (C-9), 130.6 (C-15), 130.6 (C-10), 130.3 (C-14/16), 127.9 (C-12), 124.9 (C-5), 122.9 (C-11), 122.8 (C-11a), 117.4 (C-1), 116.3 (C-3b); MS *m*/*z* (%) 340 (M^+, 100), 311 (M^+–CHO, 79); HREIMS calcd for $C_{21}H_{12}N_2OS$ 340.0670, found 340.0668.

*5.1.4. 2-(Octylthio)benzo[l,4]quinone (**14a**).* A solution of octanethiol (2.98 g, 20.4 mmol) in MeOH (5 mL) was added to a suspension of 1,4-benzoquinone (4.4 g, 40.7 mmol) in MeOH (30 mL). The dark orange solution was swirled for 5 min, poured into ice-cold water (60 mL), then cooled to yield the bright orange crude product, which was filtered and dried to give **14a** (3.42 g, 67%). Mp 96–97 °C; R_f (100% CH_2Cl_2) 0.8; IR ν_{max} (KBr) 1656, 1637, 1622 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.78 (1H, d, J=10.1 Hz, H-6), 6.69 (1H, dd, J=10.1, 2.3 Hz, H-5), 6.35 (1H, d, J=2.3 Hz, H-3), 2.74 (2H, t, J=7.3 Hz, H_2-7), 1.71 (2H, m), 1.43 (2H, m), 1.26 (8H, m), 0.86 (3H, t, J=7.0 Hz, H_3-14); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.9 (C-1 or C-4), 183.8 (C-4 or C-1), 153.2 (C-2), 137.4 (C-5), 136.1 (C-6), 124.7 (C-3), 31.6 (C-12), 30.4 (C-7), 29.0 (C-9, C-10 and C-11), 27.2 (C-8), 22.5 (C-13), 14.0 (C-14); EIMS *m*/*z* (%) 254 (M^++2H, 22), 252 (M^+, 28), 142 (100); HREIMS calcd for $C_{14}H_{20}O_2S$ 252.1184, found 252.1180. Anal. Calcd for $C_{14}H_{20}O_2S$: C, 66.63; H, 7.99. Found: C, 66.42; H, 8.28.

*5.1.5. 2-(2-Acetylanilino)-5-(octylthio)benzo[l,4]quinone (**14b**).* A mixture of 2′-aminoacetophenone (1.95 g, 14.4 mmol), quinone **14a** (3.32 g, 13.1 mmol), cerium trichloride heptahydrate (2.34 g, 6.57 mmol) and EtOH (100 mL) was stirred at room temperature with air bubbling through it for 4 days. The dark red crude product was removed by filtration every day and dried to afford **14b** (2.56 g, 51%). Mp 115–117 °C; R_f (100% CH_2Cl_2) 0.72; IR ν_{max} (KBr) 2926, 1644, 1614, 1581, 1557, 1532, 1454 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.13 (1H, s, NH-7), 7.94 (1H, d, J=8.4 Hz, H-10), 7.56 (2H, m, H-12, 13), 7.18 (1H, m, H-11), 6.47 (1H, s, H-3), 6.36 (1H, s, H-6), 2.78 (2H, t, J=7.2 Hz, H_2-16), 2.67 (3H, s, H_3-15), 1.75 (2H, m, H_2-17), 1.48 (2H, m, H_2-18), 1.29 (8H, m), 0.89 (3H, t, J=6.9 Hz, H_3-23); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.2 (C-14), 183.1 (C-4), 179.3 (C-1), 157.4 (C-5), 143.1 (C-2), 139.9 (C-8), 134.1 (C-12), 132.3 (C-10), 125.7 (C-9), 123.1 (C-11), 120.9 (C-13 or C-6), 120.6 (C-6 or C-13), 102.9 (C-3), 31.7, 30.6 (C-16), 29.0 (3×C), 28.4 (C-15), 27.3 (C-17), 22.6, 14.0 (C-23); EIMS *m*/*z* (%) 387 (M^++2H, 100), 385 (M^+, 10), 352 (70), 274 (35), 256 (48); HREIMS calcd for $C_{22}H_{27}NO_3S$ 385.1712, found 385.1712. Anal. Calcd for $C_{22}H_{27}NO_3S$: C, 68.54; H, 7.06; N, 3.63. Found: C, 68.44; H, 6.95; N, 3.51.

*5.1.6. 2-(Octylthio)-9-methyl-acridine[l,4]dione (**14c**).* A solution of **14b** (209 mg, 0.54 mmol) in glacial acetic acid-H_2SO_4 (10:1, 5 mL) was heated at 100 °C for 5 min. The dark red reaction mixture was cooled, diluted with water (30 mL) and neutralised with concd ammonia. The mixture was filtered, the residue was washed with water then cold methanol and recrystallised from MeOH to afford a dark yellow product **14c** (170 mg, 85%). Mp 136–137 °C; R_f (100% CH_2Cl_2) 0.31; IR ν_{max} (KBr) 2925, 1664, 1574, 1562 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.33 (1H, d, J=8.4 Hz, H-5), 8.25 (1H, d, J=7.5 Hz, H-8), 7.83 (1H, t, J=7.5 Hz, H-6), 7.68 (1H, t, J=7.7 Hz, H-7), 6.80 (1H, s, H-3), 3.11 (3H, s, H-11), 2.81 (2H, t, J=7.4 Hz, H-12), 1.73 (2H, m, H-13), 1.45 (2H, m, H-14), 1.25 (8H, m), 0.85 (3H, t, J=6.8 Hz, H-19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.5 (C-1), 179.6 (C-4), 158.8 (C-2), 151.6 (C-9), 148.3 (C-10a), 147.1 (C- 4a), 132.5 (C-6), 132.1 (C-5), 129.4 (C-7), 129.1 (C-8a), 127.1 (C-3), 125.4 (C-8), 123.4 (C-9a), 31.7, 30.9 (C-

12), 29.0 (3×C), 27.3 (C-13), 22.5, 16.1 (C-11), 14.0 (C-19); EIMS *m*/*z* (%) 369 (M^++2H, 35), 367 (M^+, 40), 256 (100); HREIMS calcd for $C_{22}H_{25}NO_2S$ 367.1606, found 367.1606. Anal. Calcd for $C_{22}H_{25}NO_2S$: C, 71.90; H, 6.86; N, 3.81. Found: C, 71.88; H, 6.82; N, 4.06.

*5.1.7. 4-Octylthiopyrido[2,3,4-kl]acridin-6-one (**14**).* A mixture of **14c** (170 mg, 0.464 mmol), ammonium chloride (750 mg, 14 mmol) and paraformaldehyde (70 mg, 2.32 mmol) were heated in glacial acetic acid (40 mL) at 100 °C for 10 min. The purple solution was then made alkaline with 2 M NaOH and extracted with CH_2Cl_2 (3×100 mL). The extract was washed with brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed (silica), eluting with CH_2C1_2/MeOH (100:1) to afford **14** as a yellow, amorphous solid (88 mg, 50%). Mp 119–120 °C; R_f (100% CH_2Cl_2) 0.21; IR ν_{max} (KBr) 1651, 1644, 1597, 1551 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.91 (1H, d, *J*=5.8 Hz, H-2), 8.50 (1H, dd, *J*=8.1, 1.2 Hz, H-11), 8.46 (1H, dd, *J*=8.1, 1.2 Hz, H-8), 8.35 (1H, d, *J*=5.8 Hz, H-1), 7.89 (1H, ddd, *J*=8.1, 8.1, 1.2 Hz, H-9), 7.81 (1H, ddd, *J*=8.1, 8.1, 1.2 Hz, H-10), 6.72 (1H, s, H-5), 2.96 (2H, t, *J*=7.4 Hz, H_2-12), 1.83 (2H, m, H_2-13), 1.52 (2H, m, H_2-14), 1.32–1.26 (8H, m), 0.86 (3H, t, *J*=7.0 Hz, H_3-19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.6 (C-6), 159.0 (C-4), 149.5 (C-3a), 148.1 (C-2), 146.3 (C-6a), 145.7 (C-7a), 136.7 (C-11b), 132.9 (C-8), 131.6 (C-9), 130.4 (C-10), 123.5 (C-5), 122.7 (C-11), 122.6 (C-11a), 117.0 (C-1), 116.2 (C-11c), 31.7, 31.1 (C-12), 29.1 (3×C), 27.5 (C-13), 22.5, 14.0 (C-19); FABMS *m*/*z* (%) 379 (M^++2H+H, 34), 307 (27), 289 (14), 154 (100), 136 (66); HRFABMS calcd for $C_{23}H_{27}N_2OS$ 379.1844 $[M+2H+H]^+$, found 379.1841.

*5.1.8. 2-(3-Ethylpropanoatethio)benzo[l,4]quinone (**15a**).* Ethyl-3-mercaptopropionate (2.48 g, 18.5 mmol) in MeOH (5 mL) was added dropwise to a solution of 1,4-benzoquinone (4.00 g, 37.0 mmol) in MeOH (40 mL) and the dark orange solution was stirred for 10 min. Pouring into water (40 mL) and cooling produced crude product, which was filtered and recrystallised from MeOH to yield **15a** (3.86 g, 87%) as orange-red prisms. Mp 98–99 °C; R_f (100% CH_2Cl_2) 0.63; IR ν_{max} (KBr) 1732, 1655, 1632, 1373, 1212, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.76 (1H, d, *J*=10.1 Hz, H-6), 6.68 (1H, dd, *J*=10.1, 2.2 Hz, H-5), 6.38 (1H, d, *J*=2.2 Hz, H-3), 4.13 (2H, q, *J*=7.2 Hz, H_2-10), 3.02 (2H, t, *J*=7.3 Hz, H_2-7), 2.67 (2H, t, *J*=7.3 Hz, H_2-8), 1.22 (3H, t, *J*=7.2 Hz, H_3-11); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.7 (C-1 or C-4), 183.5 (C-4 or C-1), 170.5 (C-9), 151.8 (C-2), 137.3 (C-5), 136.0 (C-6), 124.8 (C-3), 61.0 (C-10), 32.1 (C-8), 24.9 (C-7), 14.0 (C-11); EIMS *m*/*z* (%) 240 (M^+, 75), 153 (70), 55 (100); HREIMS calcd for $C_{11}H_{12}O_4S$ 240.0456, found 240.0453. Anal. Calcd for $C_{11}H_{12}O_4S$: C, 54.99; H, 5.03. Found: C, 54.86; H, 4.81.

*5.1.9. 2-(2-Acetylanilino)-5-(3-ethylpropanoatethio)benzo[l,4]quinone (**15b**).* A mixture of 2′-aminoacetophenone (2.35 g, 17.4 mmol), **15a** (3.80 g, 15.8 mmol) and cerium trichloride heptahydrate (2.95 g, 7.9 mmol) were dissolved in MeOH (100 mL) and stirred for 7 days. The dark red precipitate was filtered and washed to yield **15b** (2.68 g, 45%). Mp 137–138 °C; R_f (100% CH_2Cl_2) 0.45; IR ν_{max} (KBr) 3434, 1732, 1651, 1614, 1557, 1529, 1451, 1303, 1251 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 11.14 (1H, s), 7.95 (1H, d, *J*=8.1 Hz), 7.55 (2H, m), 7.17 (1H, q, *J*=6.1 Hz), 6.46 (1H, s), 6.40 (1H, s), 4.19 (2H, q, *J*=7.1 Hz), 3.08 (2H, t, *J*=7.1 Hz), 2.74 (2H, t, *J*=7.3 Hz), 2.67 (3H, s), 1.29 (3H, t, *J*=4.8 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 201.2, 182.7, 179.1, 170.6, 156.1, 142.9, 139.7, 134.0, 132.2, 125.6, 123.1, 120.9, 120.5, 102.8, 61.0, 32.1, 28.3, 25.1, 14.0; EIMS *m*/*z* (%) 375 (M^++2H, 100), 373 (M^+, 35), 274 (60), 256 (45), 55(40); HREIMS calcd for $C_{19}H_{19}NO_5S$ 373.0984, found 373.0973. Anal. Calcd for $C_{19}H_{19}NO_5S$: C, 61.11; H, 5.13; N, 3.75. Found: C, 61.04; H, 4.90; N, 3.74.

*5.1.10. 3-(9-Methyl-1,4-acridinedion-2-ylthio)propanoate (**15c**).* Quinone adduct **15b** (200 mg, 0.54 mmol) was dissolved in glacial acetic acid/H_2SO_4 (10:1, 5 mL) and heated to 100 °C for 5 min. The cooled reaction mixture was diluted with ice and then neutralised with concd ammonia. The mixture was extracted with CH_2Cl_2 (3×100 mL), washed with brine and the solvent removed in vacuo. The residue was subjected to silica flash chromatography eluting with CH_2Cl_2 to afford **15c** (175 mg, 92%) as yellow needles from CH_2Cl_2. Mp 174–176 °C; R_f (100% CH_2Cl_2) 0.21; IR ν_{max} (KBr) 1729, 1662, 1573, 1561, 1377, 1341, 1252, 1200 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.31 (1H, d, *J*=8.4, H-5), 8.23 (1H, d, *J*=8.5 Hz, H-8), 7.83 (1H, t, *J*=7.6 Hz, H-6), 7.68 (1H, t, *J*=7.7 Hz, H-7), 6.82 (1H, s, H-3), 4.16 (2H, q, *J*=7.1 Hz, H_2-15), 3.12 (2H, t, *J*=7.1 Hz, H_2-12), 3.09 (3H, S, H_3-11), 2.75 (2H, t, *J*=7.3 Hz, H_2-13), 1.23 (3H, t, *J*=7.1 Hz, H_3-16); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 183.3 (C-1), 179.5 (C-4), 170.6 (C-14), 157.5 (C-2), 151.7 (C-9), 148.2 (C-10a), 146.9 (C-4a), 132.6 (C-6), 132.1 (C-5), 129.4 (C-7), 129.0 (C-8a), 127.1 (C-3), 125.4 (C-8), 123.2 (C-9a), 61.1 (C-15), 32.2 (C-13), 25.4 (C-12), 16.0 (C-11), 14.1 (C-16); EIMS *m*/*z* (%) 355 (M^+, 75), 256 (100), 254 (60), 55 (75); HREIMS calcd for $C_{19}H_{17}NO_4S$ 355.0878, found 355.0881.

*5.1.11. Ethyl 3-(4-thiopyrido[2,3,4-kl]acridin-6-one)propanoate (**15**).* A mixture of dione **15c** (89 mg, 0.251 mmol), ammonium chloride (402 mg, 7.52 mmol) and paraformaldehyde (38 mg, 1.25 mmol) in glacial acetic acid (25 mL) was heated at 100 °C for 10 min. Using 2 M NaOH, the cooled purple reaction mixture was then neutralised. The mixture was extracted with CH_2Cl_2 (3×100 mL), washed with brine (2×100 mL) and evaporated. The residue was subjected to silica flash chromatography eluting with CH_2Cl_2/MeOH (95:5) to yield **15** (60 mg, 66%). Mp 173–174 °C; R_f (100% CH_2Cl_2) 0.10; IR ν_{max} (KBr) 2923, 1730, 1645, 1598, 1551, 1429, 1357, 1242, 1183 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.97 (1H, d, *J*=5.5 Hz, H-2), 8.58 (1H, dd, *J*=8.3, 1.1 Hz, H-11), 8.55 (1H, dd, *J*=8.4, 1.0 Hz, H-8), 8.43 (1H, d, *J*=5.5 Hz, H-1), 7.96 (1H, ddd, *J*=7.0, 7.0, 1.5 Hz, H-9), 7.88 (1H, ddd, *J*=7.0, 7.0, 1.5 Hz, H-10), 6.80 (1H, s, H-5), 4.21 (2H, q, *J*=7.2 Hz, H_2-15), 3.29 (2H, t, *J*=7.4 Hz, H_2-12), 2.85 (2H, t, *J*=7.3 Hz, H_2-13), 1.29 (3H, t, *J*=7.2 Hz, H_3-16); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 179.8 (C-6), 170.8 (C-14), 158.0 (C-4), 149.3 (C-3a), 148.2 (C-2), 146.3 (C-6a), 145.9 (C-7a), 136.9 (C-11b), 133.1 (C-8), 131.8 (C-9), 130.6 (C-10), 123.5 (C-5), 122.8 (C-11a), 122.8 (C-11), 117.3 (C-1), 116.3 (C-11c), 61.2 (C-15), 32.5 (C-13), 25.7 (C-12), 14.2 (C-16); EIMS *m*/*z* (%) 367 (M^++2H+H, 15), 307 (30), 289 (15), 154 (100), 136 (65), 120 (10), 107 (20), 89 (20), 77 (20); HREIMS calcd for $C_{20}H_{19}N_2O_3S$ 367.1111 $[M+2H+H]^+$, found 367.1112.

*5.1.12. 4-Ethylsulfoxopyrido[2,3,4-kl]acridin-6-one (**16**).* *m*-Chloroperoxybenzoic acid (85%) (31 mg, 0.15 mmol) was added to a solution of 4-ethylthiopyrido[2,3,4-*kl*]acridin-6-one (**10**) (40 mg, 0.137 mmol) and sodium acetate (50 mg) in CH_2Cl_2 (40 mL). The solution was stirred at room temperature for 30 min, then washed with water, dried over anhydrous magnesium sulfate and the solvent removed in vacuo. Purification was achieved by flash chromatography eluting with CH_2Cl_2/MeOH (200:1) yielding **16** (31 mg, 73%) and starting material **10** (10%). IR ν_{max} (film) 1654, 1577, 1430, 1359, 1232, 1062, 1038, 896, 846, 773, 762 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.96 (1H, d, *J*=5.8 Hz, H-2), 8.65 (1H, dd, *J*=8.4, 1.5 Hz, H-8), 8.62 (1H, dd, *J*=8.3, 1.4 Hz, H-11), 8.49 (1H, d, *J*=5.8 Hz, H-1), 8.04 (1H, ddd, *J*=8.4, 7.0, 1.4 Hz, H-9), 7.97 (1H, ddd, *J*=8.3, 7.0, 1.5 Hz, H-10), 7.55 (1H, s, H-5), 3.52 (1H, m, H-13A), 3.24 (1H, m, H-13B), 1.38 (3H, t, *J*=7.4 Hz, H_2-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.1 (C-6), 158.8 (C-4), 148.3 (C-2), 148.1 (C-3a), 146.3 (C-6a), 146.2 (C-7a), 137.4 (C-11b), 133.5 (C-8), 132.3 (C-5), 131.5 (C-9), 131.3 (C-10), 123.0 (C-11a), 122.9 (C-11), 117.4 (C-1), 116.8 (C-11c), 47.5 (C-13), 6.0 (C-14); EIMS *m*/*z* (%) 292 (M^+–O, 20), 260 (50), 259 (41), 204 (100), 177 (22); HRFABMS calcd for $C_{17}H_{15}N_2O_2S$ 311.0849 $[M+2H+H]^+$, found 311.0850.

*5.1.13. tert-Butyl 2-(6-oxo-6H-pyrido[2,3,4-kl]acridin-4-ylamino) ethylcarbamate (**17**).* To a solution of 4-(4-methylphenylthio)pyrido[2,3,4-*kl*]acridin-6-one (**9**) (130 mg, 0.37 mmol), *tert*-butyl 2-aminoethylcarbamate (296 mg, 1.84 mmol), Et_3N (619 μL, 4.44 mmol) in dry CH_2Cl_2/MeOH (1:1, 6 mL), a solution of Na (17 mg, 0.74 mmol) in dry methanol (4 mL) was added. The reaction

mixture was then heated to 65 °C under N_2 for 7 h. H_2O (100 mL) was then added and the resulting mixture was neutralised with 1 M H_2SO_4, and extracted with CH_2Cl_2 (3×60 mL). The combined organic extracts were washed with brine (2×60 mL), dried over anhydrous magnesium sulfate and the solvents removed in vacuo. Silica flash column chromatography eluting with CH_2Cl_2/MeOH (98:2) yielded **17** as an orange solid (76 mg, 53%). Mp 187–190 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.61; IR ν_{max} (film) 3358, 2928, 1693, 1610, 1566, 1519, 1162 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$ (1:1), 400 MHz) δ 8.81 (1H, d, J=5.4 Hz, H-2), 8.50 (1H, d, J=7.9 Hz, H-11), 8.38 (1H, d, J=5.2 Hz, H-1), 8.34 (1H, d, J=8.2 Hz, H-8), 7.89 (1H, t, J=7.7 Hz, H-9), 7.80 (1H, t, J=7.5 Hz, H-10), 5.91 (1H, s, H-5), 3.53 (4H, br s, H_2-13, H_2-14), 1.41 (9H, s, ^{t}Bu); ^{13}C NMR ($CDCl_3/CD_3OD$ (1:1), 100 MHz) δ 179.7 (C-6), 156.9 (C-16), 153.4 (C-4), 147.1 (C-6a), 146.9 (C-2), 145.1 (C-3a), 144.7 (C-7a), 136.2 (C-11b), 131.2 (C-8), 131.0 (C-9), 129.3 (C-10), 122.5 (C-11), 122.0 (C-11a), 117.3 (C-1), 115.7 (C-11c), 98.0 (C-5), 79.1 (C-16), 42.9 (C-13)*, 38.1 (C-14)*, 27.5 (C-18), (*these assignments may be interchanged); FABMS m/z 391 $[M+H]^+$; HRFABMS calcd for $C_{22}H_{23}N_4O_3$ 391.1770, found 391.1777.

*5.1.14. 4-(2-Aminoethylamino)pyrido[2,3,4-kl]acridin-6-one (**18**).* A solution of **17** (56 mg, 0.14 mmol) in CH_2Cl_2/TFA (20:1, 20 mL) was stirred at room temperature for 2 h while shielded from light with Al foil. Removal of the solvents in vacuo yielded **18** as the trifluoroacetate salt (58 mg, 100%), a light sensitive orange oil. R_f (10% MeOH/CH_2Cl_2) 0.09; IR ν_{max} (film) 3428, 1682, 1569, 1439, 1207, 1137 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 8.48 (2H, m, H-2, H-11), 8.23 (2H, m, H-1, H-8), 7.92 (1H, t, J=7.3 Hz, H-9), 7.83 (1H, t, J=7.3 Hz, H-10), 5.83 (1H, s, H-5), 3.79 (2H, t, J=6.0 Hz, H_2-13)*, 3.44 (2H, t, J=6.0 Hz, H_2-14)*, (*these assignments may be interchanged); ^{13}C NMR (CD_3OD, 75 MHz) δ 180.7 (C-6), 162.0 ($CF_3CO_2^-$), 154.5 (C-4), 148.3 (C-2), 147.5 (C-6a), 145.4 (C-3a and C-7a), 136.6 (C-11b), 133.0 (C-9), 132.2 (C-8), 131.4 (C-10), 124.4 (C-11), 123.2 (C-11a), 118.5 (C-1), 115.9 (C-11c), 100.3 (C-5), 41.0 (C-13)*, 39.2 (C-14)*, (*these assignments may be interchanged), resonance for $CF_3CO_2^-$ was obscured; FABMS m/z 291 $[M+H]^+$; HRFABMS calcd for $C_{17}H_{15}N_4O$ 291.1246, found 291.1233.

*5.1.15. 4-Methyl-N-(2-(6-Oxo-6H-pyrido[2,3,4-kl]acridin-4-ylamino) ethyl)benzenesulfonamide (**19**).* To a solution of amine **18** (44 mg, 0.11 mmol) and Et_3N (48 μL, 0.34 mmol) in MeCN (20 mL), *p*-tosylchloride (42 mg, 0.22 mmol) was added. The reaction mixture was stirred under N_2 for 1 h. CH_2Cl_2 (150 mL) was added and the organic layer then washed with 5% satd $NaHCO_3$ (80 mL), H_2O (80 mL), 0.2 M HCl (80 mL) and brine (80 mL) then dried over anhydrous magnesium sulfate. Silica flash column chromatography eluting with CH_2Cl_2/MeOH (100:0 to 97:3) yielded **19** as an orange solid (30 mg, 61%). Mp 228–231 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.53; IR ν_{max} (film) 3353, 2918, 1615, 1567, 1519, 1156 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$ (1:1), 400 MHz) δ 8.91 (1H, d, J=5.6 Hz, H-2), 8.61 (1H, dd, J=8.0, 1.1 Hz, H-11), 8.49 (1H, d, J=5.7 Hz, H-1), 8.48 (1H, dd, J=8.0, 0.9 Hz, H-8), 7.97 (1H, td, J=8.3, 1.3 Hz, H-9), 7.88 (1H, td, J=8.2, 1.1 Hz, H-10), 7.75 (2H, d, J=8.3 Hz, H-17/21), 7.21 (2H, d, J=8.5 Hz, H-18/20), 5.91 (1H, s, H-5), 3.60 (2H, t, J=6.0 Hz, H_2-13)*, 3.31 (2H, t, J=6.0 Hz, H_2-14)*, 2.27 (3H, s, H_3-22), (*these assignments may be interchanged); ^{13}C NMR ($CDCl_3$:CD_3OD (1:1), 100 MHz) δ 180.2 (C-6), 153.1 (C-4), 147.4 (C-6a), 147.2 (C-2), 145.5 (C-3a), 145.2 (C-7a), 143.2 (C-18), 136.7 (C-11b and C-16), 131.9 (C-8), 131.5 (C-9), 129.8 (C-10), 129.3 (C-18/20), 126.6 (C-17/21), 122.7 (C-11), 122.3 (C-11a), 117.7 (C-1), 116.2 (C-11c), 98.6 (C-5), 42.0 (C-13)*, 40.7 (C-14)*, 20.8 (C-22), (*these assignments may be interchanged); FABMS m/z 445 $[M+H]^+$; HRFABMS calcd for $C_{24}H_{21}N_4O_3S$ 445.1334, found 445.1327.

*5.1.16. 1-(2-(6-Oxo-6H-pyrido[2,3,4-kl]acridin-4-ylamino)ethyl)-3-phenylthiourea (**20**).* To a solution of amine **18** (58 mg, 0.14 mmol) and Et_3N (29 μL, 0.21 mmol) in dry MeCN (20 mL), phenylisothiocyanate (33 μL, 0.28 mmol) was added. The reaction mixture was stirred under N_2 for 1 h. H_2O (100 mL) was added and extracted with CH_2Cl_2 (3×60 mL) then washed with 0.2 M HCl (50 mL), H_2O (50 mL) and brine (50 mL) then dried over anhydrous magnesium sulfate. Silica flash column chromatography eluting with CH_2Cl_2/MeOH (100:0 to 97:3) yielded **20** as an orange solid (27 mg, 45%). Mp >300 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.57; IR ν_{max} (film) 3390, 1557, 1494, 1248 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$ (1:1), 300 MHz) δ 8.84 (1H, d, J=5.7 Hz, H-2), 8.57 (1H, d, J=7.9 Hz, H-11), 8.41 (2H, m, H-1, H-8), 7.93 (1H, td, J=8.3, 1.4 Hz, H-9), 7.86 (1H, td, J=8.2, 1.4 Hz, H-10), 7.33 (4H, m, H-19/20/22/23), 7.20 (1H, m, H-21), 6.00 (1H, s, H-5), 4.09 (2H, t, J=6.0 Hz, H_2-14), 3.73 (2H, t, J=6.0 Hz, H_2-13); ^{13}C NMR ($CDCl_3/CD_3OD$ (1:1), 75 MHz), δ 181.6 (C-16), 180.0 (C-6), 153.6 (C-4), 147.3 (C-6a), 147.1 (C-2), 145.3 (C-3a), 145.0 (C-7a), 137.0 (C-18), 136.5 (C-11b), 131.6 (C-8), 131.4 (C-9), 129.6 (C-10), 128.9 (C-20/22), 125.9 (C-21), 124.5 (C-19/23), 122.6 (C-11), 122.2 (C-11a), 117.5 (C-1), 115.9 (C-11c), 98.4 (C-5), 42.4 (C-14), 42.1 (C-13). FABMS m/z 426 $[M+H]^+$; HRFABMS calcd for $C_{24}H_{20}N_5OS$ 426.1389, found 426.1386.

*5.1.17. tert-Butyl 2-(pyrazine-2-carboxamido)ethylcarbamate (**22a**).* A mixture of pyrazinecarboxylic acid (138 mg, 1.11 mmol), *tert*-butyl 2-aminoethylcarbamate (178 mg, 1.11 mmol), Et_3N (0.34 mL, 2.44 mmol) and BOP-Cl (540 mg, 1.22 mmol) in dry CH_2Cl_2 (10 mL) was stirred under N_2 at room temperature for 3 h. CH_2Cl_2 (30 mL) was then added and washed with 0.2 M HCl (30 mL), H_2O (30 mL), 5% satd $NaHCO_3$ (30 mL) and brine (30 mL) then dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to yield **22a** (295 mg, 100%) as a white solid. Mp 110–112 °C; R_f (10% MeOH/CH_2Cl_2) 0.86; IR ν_{max} (film) 3374, 2987, 2936, 1688, 1660, 1531, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 9.39 (1H, d, J=1.2 Hz), 8.75 (1H, d, J=2.4 Hz), 8.54 (1H, dd, J=2.3, 1.5 Hz), 8.30 (1H, br s, N*H*CO), 5.38 (1H, br s, N*H*CO_2), 3.62 (2H, m), 3.42 (2H, m), 1.41 (9H, s, (CH_3)$_3$C); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.6 (*C*O), 156.4 (O*C*O), 147.1, 144.3, 144.1, 142.5, 79.3 ((CH_3)$_3$*C*O), 40.0×2, 28.1 ((CH$_3$)$_3$C); EIMS m/z 266 $[M]^+$; HREIMS calcd for $C_{12}H_{18}N_4O_3$ 266.1379, found 266.1378.

*5.1.18. N-(2-Aminoethyl)pyrazine-2-carboxamide trifluoroacetate salt (**22**).* Compound **22a** (295 mg, 1.11 mmol) in CH_2Cl_2/TFA (9:1, 10 mL) was stirred at room temperature for 1.5 h. Solvents were removed in vacuo to yield **22** (320 mg, 100%) as a colourless oil. R_f (10% MeOH/CH_2Cl_2) 0.18; IR ν_{max} (film) 3344, 2938, 1676, 1537, 1202, 1136 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 9.23 (1H, d, J=1.4 Hz), 8.78 (1H, d, J=2.5 Hz), 8.66 (1H, dd, J=2.5, 1.5 Hz), 3.77 (2H, t, J=5.8 Hz), 3.26 (2H, t, obsc.); ^{13}C NMR (CD_3OD, 75 MHz) δ 166.5, 148.6, 145.9, 144.8, 144.6, 40.9, 38.3; CIMS m/z 167 $[M+H]^+$; HRCIMS calcd for $C_7H_{11}N_4O$ 167.0933, found 167.0934.

*5.1.19. N-(2-(6-Oxo-6H-pyrido[2,3,4-kl]acridin-4-ylamino)ethyl)pyrazine-2-carboxamide (**21**).* To a solution of 4-(4-methylphenylthio) pyrido[2,3,4-*kl*]acridin-6-one (**9**) (40 mg, 0.11 mmol), **22** (73 mg, 0.44 mmol), Et_3N (153 μL, 1.1 mmol) in dry CH_2Cl_2/MeOH (1:1, 5 mL), a solution of Na (10 mg, 0.44 mmol) in dry methanol (5 mL) was added. The reaction mixture was then heated to 65 °C under N_2 for 7 h. H_2O (150 mL) was then added and the resulting mixture was neutralised with 1 M H_2SO_4, and extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed with H_2O (2×100 mL) and brine (100 mL), dried over anhydrous magnesium sulfate and the solvents removed in vacuo. Silica flash column chromatography eluting with $CHCl_3$/MeOH (95:5) yielded **21** as an orange solid (16 mg, 36%). Mp >300 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.31; IR ν_{max} (film) 3283, 2924, 1665, 1529, 1166, 984 cm^{-1}; ^1H NMR ($CDCl_3/CD_3OD$ (1:1), 300 MHz) δ 9.34 (1H, d, J=1.4 Hz, H-18), 8.90 (1H, d, J=5.7 Hz, H-2), 8.75 (1H, d, J=2.5 Hz, H-21), 8.64

(1H, dd, *J*=2.4, 1.5 Hz, H-20), 8.58 (1H, d, *J*=8.1 Hz, H-11), 8.48 (1H, d, *J*=5.8 Hz, H-1), 8.41 (1H, d, *J*=8.1 Hz, H-8), 7.93 (1H, td, *J*=8.3, 1.3 Hz, H-9), 7.84 (1H, td, *J*=8.2, 1.3 Hz, H-10), 6.04 (1H, s, H-5), 3.93 (2H, t, *J*=6.0 Hz, H-14), 3.75 (2H, t, *J*=6.0 Hz, H-13); ^{13}C NMR ($CDCl_3$/CD_3OD (1:1), 75 MHz) δ 180.2 (C-6), 164.3 (C-16), 153.4 (C-4), 147.4 (C-6a), 147.3 (C-2), 146.9 (C-21), 145.5 (C-3a), 145.2 (C-7a), 144.2 (C-17), 143.6 (C-18), 142.9 (C-20), 136.6 (C-11b), 131.9 (C-8), 131.4 (C-9), 129.7 (C-10), 122.6 (C-11), 122.3 (C-11a), 117.6 (C-1), 116.2 (C-11c), 98.7 (C-5), 42.7 (C-13), 37.7 (C-14); FABMS *m*/*z* 397 $[M+H]^+$; HRFABMS calcd for $C_{22}H_{17}N_6O_2$ 397.1413, found 397.1417.

*5.1.20. 4-(2-Hydroxyethylamino)pyrido[2,3,4-kl]acridin-6-one (**23**).* To a solution of 4-(4-methylphenylthio)pyrido[2,3,4-*kl*]acridin-6-one (**9**) (131 mg, 0.37 mmol), ethanolamine (112 μL, 1.85 mmol) in dry CH_2Cl_2/MeOH (1:1, 6 mL), a solution of Na (17 mg, 0.74 mmol) in dry methanol (4 mL) was added. The reaction mixture was then heated to 65 °C under N_2 for 7 h. Water (100 mL) was then added and the resulting mixture was neutralised with 1 M H_2SO_4, and extracted with CH_2Cl_2 (3×60 mL). The combined organic extracts were washed with brine (2×60 mL), dried over anhydrous magnesium sulfate and the solvents removed in vacuo. Silica flash column chromatography eluting with CH_2Cl_2/MeOH (100:0 to 92:8) yielded **23** as an orange solid (27 mg, 25%). Mp 278–280 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.44; IR ν_{max} (film) 3337, 2920, 1609, 1557, 1433, 1253, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD (1:1), 300 MHz) δ 8.96 (1H, d, *J*=5.7 Hz, H-2), 8.66 (1H, dd, *J*=8.1, 1.2 Hz, H-11), 8.58 (1H, d, *J*=5.8 Hz, H-1), 8.47 (1H, dd, *J*=8.3, 1.0 Hz, H-8), 7.97 (1H, td, *J*=8.3, 1.3 Hz, H-9), 7.89 (1H, td, *J*=7.0, 1.3 Hz, H-10), 6.04 (1H, s, H-5), 3.97 (2H, t, *J*=5.5 Hz, H_2-14), 3.62 (2H, t, *J*=5.5 Hz, H_2-13); ^{13}C NMR ($CDCl_3$/CD_3OD (1:1), 75 MHz) δ 180.0 (C-6), 153.5 (C-4), 147.4 (C-6a), 147.1 (C-2), 145.4 (C-3a), 145.0 (C-7a), 136.6 (C-11b), 131.5 (C-8), 131.2 (C-9), 129.5 (C-10), 122.7 (C-11), 122.3 (C-11a), 117.7 (C-1), 116.1 (C-11c), 98.2 (C-5), 58.9 (C-14), 44.4 (C-13); FABMS *m*/*z* 292 $[M+H]^+$; HRFABMS calcd for $C_{17}H_{14}N_3O_2$ 292.1086, found 292.1091.

*5.1.21. 2-(6-Oxo-6H-pyrido[2,3,4-kl]acridin-4-ylamino)ethyl pyrazine-2-carboxylate (**24**).* To a solution of alcohol **23** (24 mg, 0.08 mmol) in dry CH_2Cl_2 (15 mL), pyrazinecarboxylic acid (15 mg, 0.12 mmol), Et_3N (40 μL, 0.29 mmol) and BOP-Cl (65 mg, 0.15 mmol) in dry CH_2Cl_2 (5 mL) was added. The reaction mixture was stirred at room temperature under N_2 for 3 h, at which time further quantities of pyrazinecarboxylic acid (15 mg, 0.12 mmol), Et_3N (40 μL) and BOP (65 mg) were added and reaction stirred for another 2 h. CH_2Cl_2 (100 mL) was added then washed with 0.2 N HCl (60 mL), H_2O (60 mL), 5% satd $NaHCO_3$ (60 mL) and brine (60 mL), dried over anhydrous magnesium sulfate and the solvents removed in vacuo. Silica flash column chromatography eluting with CH_2Cl_2/MeOH (100:0 to 97:3) yielded **24** as an orange solid (12 mg, 38%). Mp 190 °C (decomp.); R_f (10% MeOH/CH_2Cl_2) 0.67; IR ν_{max} (film) 3339, 2922, 1724, 1613, 1563, 1299, 1141, 840 cm^{-1}; ^{1}H NMR ($CDCl_3$/CD_3OD (1:1), 400 MHz) δ 9.35 (1H, d, *J*=1.4 Hz, H-18), 8.96 (1H, d, *J*=5.7 Hz, H-2), 8.84 (1H, d, *J*=2.5 Hz, H-21), 8.78 (1H, dd, *J*=2.4, 1.5 Hz, H-20), 8.67 (1H, dd, *J*=8.1, 1.1 Hz, H-11), 8.57 (1H, d, *J*=5.8 Hz, H-1), 8.47 (1H, d, *J*=8.6 Hz, H-8), 7.97 (1H, td, *J*=7.6, 1.2 Hz, H-9), 7.89 (1H, td, *J*=8.1, 1.1 Hz, H-10), 6.12 (1H, s, H-5), 4.83 (2H, t, *J*=5.3 Hz, H_2-14), 3.99 (2H, t, *J*=5.3 Hz, H_2-13); ^{13}C NMR ($CDCl_3$/CD_3OD (1:1), 100 MHz) δ 180.3 (C-6), 163.4 (C-16), 153.2 (C-4), 147.7 (C-21), 147.6 (C-6a), 147.2 (C-2), 145.8 (C-18), 145.4 (C-3a), 145.1 (C-7a), 144.2 (C-20), 142.4 (C-17), 136.7 (C-11b), 131.6 (C-8), 131.3 (C-9), 129.7 (C-10), 122.7 (C-11), 122.3 (C-11a), 117.7 (C-1), 116.2 (C-11c), 98.8 (C-5), 63.3 (C-14), 41.2 (C-13); FABMS *m*/*z* 398 $[M+H]^+$; HRFABMS calcd for $C_{22}H_{16}N_5O_3$ 398.1253, found 398.1258.

*5.1.22. 7-Oxo-7H-4-thia-3,8-diaza-benzo[de]cyclopenta[b]anthracene-5-carboxylic acid methyl ester (**25**).* To a stirred solution of 10-methyl-4,11-dioxo-4,11-dihydro-1-thia-5-aza-cyclopenta[*b*]anthracene-2-carboxylic acid methyl ester (**30**) (100 mg, 0.30 mmol) and ammonium chloride (472 mg, 8.9 mmol) in glacial acetic acid (50 mL) was added paraformaldehyde (45 mg, 1.48 mmol). The mixture was heated to reflux for 45 min, then cooled, poured onto ice and made basic with concd ammonia, then extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were washed with brine (3×100 mL), then water (2×100 mL), and dried over anhydrous magnesium sulfate. The solvent was then removed in vacuo. Purification by flash column chromatography (silica, CH_2Cl_2/MeOH, 95:5) yielded **25**, as a yellow solid (77 mg, 83%). Recrystallisation from acetone afforded fine yellow needles. Mp 340–343 °C (decomp.); IR ν_{max} (film) 3010, 1710, 1665, 1589, 1418, 1281, 1261, 937, 879, 845, 763, 743 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.98 (1H, d, *J*=6.0 Hz, H-2), 8.66 (1H, d, *J*=7.1 Hz, H-12), 8.63 (1H, d, *J*=7.1 Hz, H-9), 8.46 (1H, s, H-6), 8.44 (1H, d, *J*=6.0 Hz, H-1), 8.02 (1H, ddd, *J*=8.2, 8.2, 1.2 Hz, H-10), 7.94 (1H, ddd, *J*=8.2, 8.2, 1.2 Hz, H-11), 3.98 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.0 (C-7), 162.0 (C-13), 154.0 (C-3b), 149.2 (C-2), 147.8 (C-3a), 145.8 (C-8a), 139.2 (C-5), 137.6 (C-12b), 136.5 (C-6a), 133.3 (C-9), 132.5 (C-6), 132.0 (C-10), 130.9 (C-11), 123.0 (C-12a), 122.9 (C-12), 116.7 (C-12c), 116.6 (C-1), 52.9 (OCH_3), C-7a not observed; EIMS *m*/*z* (%) 346 (M^+, 30), 315 (28), 259 (6), 243 (7), 215 (6), 83 (9), 69 (7), 55 (11); HREIMS calcd for $C_{19}H_{10}N_2O_3S$ 346.0412, found 346.0403.

*5.1.23. 7-Oxo-7H-4-oxa-3,8-diaza-benzo[de]cyclopenta[b]anthracene-5-carboxylic acid methyl ester (**26**).* Paraformaldehyde (42 mg, 1.4 mmol) was added to a stirred solution of 10-methyl-4,11-dioxo-4,11-dihydro-1-oxa-5-aza-cyclopenta[*b*]anthracene-2-carboxylic acid methyl ester (**32**) (90 mg, 0.28 mmol) and ammonium chloride (446 mg, 8.4 mmol) in glacial acetic acid (50 mL). The solution was stirred under nitrogen at 80 °C for 35 min. After cooling, the dark blue solution was poured into water (200 mL), made basic with concd ammonia and extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed with brine (3×100 mL), then water (2×100 mL) and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo yielding 70 mg (76%) of **26**. Recrystallisation from $CHCl_3$/MeOH yielded a green microcrystalline solid. Mp 340–345 °C (decomp.); R_f (100% CH_2Cl_2) 0.17; IR ν_{max} (film) 3433, 1720, 1675, 1605, 1570, 1415, 1306, 1226, 1199, 1127, 1051, 763 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.08 (1H, d, *J*=5.8 Hz, H-2), 8.64 (1H, dd, *J*=8.2, 1.3 Hz, H-12), 8.61 (1H, dd, *J*=8.3, 1.3 Hz, H-9), 8.45 (1H, d, *J*=5.8 Hz, H-1), 8.01 (1H, ddd, *J*=8.3, 8.3, 1.5 Hz, H-10), 7.94 (1H, ddd, *J*=8.2, 8.2, 1.3 Hz, H-11), 7.82 (1H, s, H-6), 4.01 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.6 (C-7), 158.5 (C-3b), 158.3 (C-13), 149.3 (C-2), 147.5 (C-5), 147.1 (C-7a), 145.7 (C-8a), 143.2 (C-3a), 137.7 (C-12b), 133.4 (C-9), 132.1 (C-10), 131.1 (C-11), 126.2 (C-6a), 123.0 (C-12a), 122.8 (C-12), 117.0 (C-1), 116.6 (C-12c), 115.4 (C-6), 52.6 (OCH_3); EIMS *m*/*z* (%) 330 (M^+, 100), 299 (67), 272 (11), 243 (8), 215 (45), 199 (23), 188 (12), 129 (10), 97 (14), 83 (20), 69 (28), 55 (43), 41 (48); HREIMS calcd for $C_{19}H_{10}N_2O_4$ 330.0641, found 330.0643.

*5.1.24. 5-(2-Acetylanilino)-4,7-dioxo-4,7-dihydro-benzo[b]thiophene-2-carboxylic acid methyl ester (**29**).* To a stirred suspension of methyl 4,7-dihydro-4,7-dioxobenzo[*b*]thiophene-2-carboxylate (**27**)[17] (0.58 g, 2.59 mmol) and cerium trichloride heptahydrate (0.48 g, 1.30 mmol) in MeOH (100 mL) was added 2′-aminoacetophenone (0.70 g, 5.21 mmol). Air was continuously bubbled through the mixture, which was stirred for two weeks, during which time it turned purple. The solvent was removed in vacuo, and the residue was extracted with CH_2Cl_2 (3×100 mL). The combined organic extracts were washed with brine (3×100 mL) followed by water (2×100 mL) and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo and the purple solid was purified by silica flash column chromatography eluting with CH_2Cl_2 to yield **29** (0.85 g, 92%). Recrystallisation from MeOH afforded dark red hair-like needles. Mp 286–290 °C (decomp.); R_f (100% CH_2Cl_2) 0.41; IR

ν_{max} (film) 3422, 1712, 1685, 1653, 1620, 1582, 1536, 1452, 1302, 1288, 1261, 1181, 1078, 749 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.26 (1H, br s, NH-9), 8.16 (1H, s, H-3), 7.95 (1H, dd, J=8.5, 1.1 Hz, H-12), 7.60 (1H, d, J=5.9 Hz, H-15), 7.57 (1H, m, H-14), 7.20 (1H, ddd, J=8.1, 6.0, 1.5 Hz, H-13), 6.59 (1H, s, H-6), 3.94 (3H, s, OCH_3), 2.67 (3H, s, H_3-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.4 (*C*OCH_3). 179.5 (C-7), 176.6 (C-4), 161.6 (C-16), 150.4 (C-7a), 144.4 (C-5), 139.9 (C-10), 138.1 (C-2), 137.4 (C-3a), 134.2 (C-14), 132.4 (C-12), 130.8 (C-3), 125.9 (C-11), 123.5 (C-13), 120.8 (C-15), 104.7 (C-6), 52.9 (OCH_3), 28.5 (C-17); EIMS m/z (%) 355 (M^+, 27), 337 (63), 313 (26), 306 (21), 278 (37), 206 (45), 164 (52), 149 (35), 120 (11), 77 (11), 70 (11), 57 (29), 41 (29); HREIMS calcd for $C_{18}H_{13}NO_5S$ 355.0515, found 355.0510. Anal. Calcd for $C_{18}H_{13}NO_5S$: C, 60.84; H, 3.69; N, 3.94. Found: C, 61.07; H, 3.67; N 3.88.

*5.1.25. 10-Methyl-4,11-dioxo-4,11-dihydro-1-thia-5-aza-cyclopenta [b]anthracene-2-carboxylic acid methyl ester (**30**).* To a premixed solution of glacial acetic acid (14 mL) and concd H_2SO_4 (1.4 mL) was added 5-(2′-acetylanilino)-4,7-dioxo-4,7-dihydro-benzo[*b*]thiophene-2-carboxylic acid methyl ester (**29**) (0.5 g, 1.42 mmol). The mixture was stirred at 80 °C for 35 min then cooled and poured onto ice before being neutralised with solid $NaHCO_3$. The product was extracted with CH_2Cl_2 (3×50 mL), and the combined organic layers were dried over anhydrous magnesium sulfate. The solvent was removed in vacuo yielding a yellow solid, which was washed with CH_2Cl_2/MeOH (1:1) to yield **30** (0.45 g, 94%). Mp 332–340 °C (decomp.); R_f (100% CH_2Cl_2) 0.17; IR ν_{max} (film) 2954, 1721, 1686, 1655, 1538, 1435, 1375, 1285, 1245, 1078, 880, 752 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.44 (1H, d, J=8.3 Hz, H-6 or H-9), 8.37 (1H, s, H-3), 8.36 (1H, d, J=8.2 Hz, H-6 or H-9), 7.92 (1H, ddd, J=8.2, 8.2, 1.1 Hz, H-7 or H-8), 7.78 (1H, ddd, J=8.2, 8.2, 1.1 Hz, H-7 or H-8), 4.00 (3H, s, OCH_3), 3.30 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 179.9, 176.9, 161.4, 152.5, 152.1, 148.5, 148.3, 141.7, 140.9, 132.8, 132.5, 131.5, 129.9, 129.3, 125.5, 124.7, 53.1 (OCH_3), 16.4 (CH_3); EIMS m/z (%) 337 (M^+, 56), 322 (8), 306 (37), 250 (8), 222 (9), 206 (8), 196 (10), 178 (12), 147 (100), 120 (12), 71 (21), 58 (43), 43 (18), 41 (22); HREIMS calcd for $C_{18}H_{11}NO_4S$ 337.0409, found 337.0405. Anal. Calcd for $C_{18}H_{11}NO_4S$: C, 64.09; H, 3.29; N, 4.15. Found: C, 63.87; H, 3.20; N, 4.20.

*5.1.26. 5-(2-Acetylanilino)-4,7-dioxo-4,7-dihydro-benzofuran-2-carboxylic acid methyl ester (**31**).* To a stirred suspension of 4,7-dioxo-4,7-dihydro-benzofuran-2-carboxylic acid methyl ester (**28**) (0.44 g, 2.14 mmol) and cerium trichloride heptahydrate (0.80 g, 2.14 mmol) in MeOH (100 mL) was added 2′-aminoacetophenone (0.32 g, 2.39 mmol). Air was continuously bubbled through the mixture for 5 days during, which time the solution turned bright red. The mixture was evaporated to dryness under reduced pressure. The residue was extracted with CH_2Cl_2 (3×100 mL) and the combined organic layers were washed with brine (3×100 mL), then with water (3×100 mL). After drying over anhydrous magnesium sulfate, the solvent was removed in vacuo to yield a purple solid, which was purified by silica flash column chromatography eluting with CH_2Cl_2. The red adduct, 5-(2′-acetylanilino)-4,7-dioxo-4,7-dihydro-benzofuran-2-carboxylic acid methyl ester (**31**) (0.59 g, 22%) and the spontaneously cyclised yellow solid, 10-methyl-4,11-dioxo-4,11-dihydro-1-oxa-5-aza-cyclopenta[*b*]anthracene-2-carboxylic acid methyl ester (**32**) (0.41 g, 61%) were obtained. Recrystallisation of **31** from methanol yielded dark red, fine hair-like needles. Mp 278–280 °C; R_f (100% CH_2Cl_2) 0.29; IR ν_{max} (film) 3579, 3130, 1722, 1692, 1642, 1568, 1528, 1453, 1434, 1370, 1292, 1260, 1196, 1169, 979, 753 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 11.23 (1H, br s, NH-9), 7.95 (1H, dd, J=7.6, 1.2 Hz, H-12), 7.61 (1H, d, J=5.6 Hz, H-15), 7.58 (1H, m, H-14), 7.53 (1H, s, H-3), 7.21 (1H, ddd, J=8.1, 8.1, 2.8 Hz, H-13), 6.48 (1H, s, H-6), 3.95 (3H, s, OCH_3), 2.68 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 201.4 (C-16), 177.9 (C-4), 175.5 (C-7), 158.1 (C-8), 154.0 (C-7a), 147.4 (C-2), 144.3 (C-5), 139.7 (C-10), 134.2 (C-14), 132.4 (C-12), 126.0 (C-11), 124.8 (C-3a), 123.7 (C-13), 121.0 (C-15), 113.8 (C-3), 102.8 (C-6), 52.7 (OCH_3), 28.5 (C-17); EIMS m/z (%) 339 (M^+, 13), 321 (100), 310 (3), 306 (13), 297 (7), 293 (20), 262 (37), 235 (14), 206 (5), 178 (14), 151 (7), 140 (5), 89 (7), 77 (4), 43 (7); HREIMS calcd for $C_{18}H_{13}NO_6$ 339.0743, found 339.0748. Anal. Calcd for $C_{18}H_{13}NO_6$: C, 63.72; H, 3.86; N, 4.13. Found: C, 63.69; H, 3.77; N, 4.06.

*5.1.27. 10-Methyl-4,11-dioxo-4,11-dihydro-1-oxa-5-aza-cyclopenta [b]anthracene-2-carboxylic acid methyl ester (**32**).* To a premixed solution of glacial acetic acid (4.5 mL) and concd H_2SO_4 (0.45 mL) was added 5-(2′-acetylanilino)-4,7-dioxo-4,7-dihydro-benzofuran-2-carboxylic acid methyl ester (**31**), (0.16 g, 0.47 mmol). The solution was stirred at 80 °C for 35 min, then cooled and poured into ice water (30 mL). It was then neutralised with solid $NaHCO_3$, extracted into CH_2Cl_2 (3×50 mL) and the combined organic layers were dried over anhydrous magnesium sulfate. Removal of the solvent in vacuo yielded 0.12 g (81%) of a yellow solid, 10-methyl-4,11-dioxo-4,11-dihydro-1-oxa-5-aza-cyclopenta[*b*]anthracene-2-carboxylic acid methyl ester (**32**). Recrystallisation from $CHCl_3$/MeOH yielded fine yellow needles. Mp 275–280 °C (decomp.); R_f (100% CH_2Cl_2) 0.2; IR ν_{max} (film) 3609, 3449, 3123, 1735, 1697, 1669, 1591, 1546, 1437, 1380, 1289, 1273, 1229, 1187, 1007, 952, 770 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.42 (1H, d, J=8.3 Hz), 8.35 (1H, d, J=8.1 Hz), 7.92 (1H, ddd, J=8.3, 8.3, 1.2 Hz), 7.78 (1H, ddd, J=8.3, 8.3, 1.1 Hz), 7.71 (1H, s, H-3), 4.00 (3H, s, OCH_3), 3.29 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.5, 175.0, 157.9, 154.8, 152.8, 148.8, 148.4, 147.9, 132.9, 132.5, 130.0, 129.7, 129.3, 125.5, 123.8, 114.3, 52.9 (OCH_3), 16.3 (CH_3); EIMS m/z (%) 321 (M^+, 100), 306, (17), 293 (22), 262 (39), 235 (27), 206 (7), 178 (17), 151 (10), 140 (7), 89 (9), 75 (7), 55 (10), 41 (12); HREIMS calcd for $C_{18}H_{11}NO_5$ 321.0637, found 321.0641. Anal. Calcd for $C_{18}H_{11}NO_5$: C, 67.29; H, 3.45; N, 4.36. Found: C, 67.11; H, 3.66; N, 4.56.

*5.1.28. 7-Methoxy-benzofuran-2-carboxylic acid methyl ester (**34**).* A stirred solution of 7-methoxy-benzofuran-2-carboxylic acid (**33**) (0.5 g, 2.6 mmol) in CH_2Cl_2 (8 mL), MeOH (1.5 mL), and concd H_2SO_4 (150 μL) was heated to reflux for 17 h. The solution was cooled, poured into water, neutralised with solid $NaHCO_3$ and extracted into CH_2Cl_2 (3×50 mL). The combined organic layers were dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to yield **34** (0.57 g, 100%) of a white crystalline solid. Mp 102–104 °C; R_f (100% CH_2Cl_2) 0.74; IR ν_{max} (film) 3006, 2953, 1732, 1622, 1585, 1579, 1493, 1433, 1428, 1363, 1329, 1307, 1272, 1231, 1201, 1094, 974, 915, 851, 800, 732 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.51 (1H, s, H-3), 7.23 (1H, dd, J=7.9, 1.5 Hz, H-4 or H-6), 7.19 (1H, dd, J=7.5, 7.5 Hz, H-5), 6.90 (1H, dd, J=7.5, 1.3 Hz, H-4 or H-6), 4.00 (3H, s, OCH_3), 3.96 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 159.6, 145.8, 145.4, 145.2, 128.4, 124.3, 114.4, 114.1, 108.8, 55.8, 52.1; EIMS m/z (%) 206 (M^+, 100), 191 (10), 175 (45), 163 (12), 119 (9), 117 (5), 89 (6), 76 (9), 65 (6), 50 (7); HREIMS calcd for $C_{11}H_{10}O_4$ 206.0579, found 206.0580.

*5.1.29. 7-Hydroxy-benzofuran-2-carboxylic acid methyl ester (**35**).* A solution of 7-methoxy-benzofuran-2-carboxylic acid methyl ester (**34**) (100 mg, 0.49 mmol) in dry CH_2Cl_2 (20 mL) was stirred at −20 °C in an ethylene glycol/dry ice bath under nitrogen. Boron tribromide (0.7 mL, 7.28 mmol) was added slowly and the mixture was stirred for 1 h after which time it was allowed to warm to room temperature. After stirring for further 2 h, aqueous sodium hydroxide (10%, 1 mL) was added to quench the reaction. The mixture was made acidic with aqueous hydrochloric acid (10%), extracted into CH_2Cl_2 (3×50 mL). The combined organic layers were dried over anhydrous magnesium sulfate, and the solvent was removed in vacuo to yield **35** as a white solid (80 mg, 86%). R_f (100% CH_2Cl_2) 0.17; IR ν_{max} (film) 3375, 1717, 1597, 1566, 1490, 1437, 1346, 1315, 1290, 1219, 1196 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.51 (1H, s, H-3), 7.22 (1H, dd, J=7.7, 1.3 Hz, H-4 or H-6), 7.17 (1H, dd, J=7.7, 7.7 Hz, H-5), 6.99 (1H, dd, J=7.7, 1.3 Hz, H-4 or H-6), 5.4 (1H, br s, OH), 3.97

(3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 160.1, 145.3, 144.5, 141.5, 128.4, 124.9, 114.7, 114.6, 113.6, 52.5 (OCH_3); EIMS m/z (%) 192 (M^+, 100), 161 (99), 134 (14), 105 (48), 77 (21), 57 (14), 51 (33); HREIMS calcd for $C_{10}H_8O_4$ 192.0420, found 192.0423.

*5.1.30. 4,7-Dioxo-4,7-dihydro-benzofuran-2-carboxylic acid methyl ester (**28**). 5.1.30.1. A: from 7-hydroxy-benzofuran-2-carboxylic acid methyl ester (**35**).* To a stirred solution of 7-hydroxy-benzofuran-2-carboxylic acid methyl ester (**35**) (60 mg, 0.31 mmol) in MeCN/water (1 mL/0.5 mL) was added CAN (0.69 g, 1.25 mmol). The mixture was stirred for 30 min. Water (20 mL) was added and it was extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solvent removed in vacuo to yield **28** as a yellow solid (52 mg, 83%). R_f (100% CH_2Cl_2) 0.59; IR ν_{max} (film) 2956, 1734, 1679, 1572, 1437, 1285, 1225, 1202, 1094, 1048, 1022, 974, 921, 840, 806, 762, 668 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.46 (1H, s, H-3), 6.80 (2H, s, H-5, H-6), 3.95 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 181.6 (C-4), 174.7 (C-7), 157.9 (C-8), 151.5 (C-7a), 148.2 (C-2), 137.4 (C5 or C-6), 136.3 (C-5 or C-6), 128.3 (C-3a), 113.5 (C-3), 52.9 (OCH_3); EIMS m/z (%) 206 (M^+, 64), 175 (58), 149 (36), 127 (10), 113 (14), 99 (18), 85 (47), 71 (69), 57 (100), 43 (63); HREIMS calcd for $C_{10}H_6O_5$ 206.0215, found 206.0210.

*5.1.30.2. B: from 7-methoxy-benzofuran-2-carboxylic acid methyl ester (**34**).* To a stirred solution of 7-methoxy-benzofuran-2-carboxylic acid methyl ester (**34**) (100 mg, 0.49 mmol) in MeCN/2 M H_2SO_4 (20 mL/5 mL) at room temperature was added ceric ammonium sulfate (1.84 g, 2.9 mmol) in 2 M H_2SO_4 (25 mL) in one portion. The orange mixture was heated to 60 °C for 90 min during which time it turned to yellow with a precipitate of cerous ions. The solution was cooled, decanted from the precipitated cerous ions into water (25 mL) and extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were washed with brine (3×50 mL), then water (2×50 mL), and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to yield **28** as a yellow crystalline solid (102 mg, 100%). All spectra were identical to product prepared via method A.

5.2. Biological assays

Details of the antituberculosis,[2] P388[19] and disc diffusion antimicrobial[19] assays have been presented elsewhere.

Acknowledgements

We would like to acknowledge Dr. J. Maddry of TAACF for facilitating access to Mtb assays and Mrs. G. Barns for P388 and antimicrobial disc diffusion assays. DRA acknowledges a University of Auckland doctoral scholarship and we thank Ming Wu and Jaime Yim for preliminary synthetic studies.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.05.033. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. WHO. World Health Organization. Global Tuberculosis Control 2008. Surveillance, Planning, Financing. WHO, 2008.
2. (a) .www.taacf.org; (b) Ananthan, S.; Faaleolea, E. R.; Goldman, R. C.; Hobrath, J. V.; Kwong, C. D.; Laughon, B. E.; Maddry, J. A.; Mehta, A.; Rasmussen, L.; Reynolds, R. C.; Secrist, J. A.; Shindo, N.; Showe, D. N.; Sosa, M. I.; Suling, W. J.; White, E. L. *Tuberculosis* **2009**, *89*, 334–353.
3. Andries, K.; Verhasselt, P.; Guillemont, J.; Göhlmann, H. W. H.; Neefs, J.-M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; de Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. *Science* **2005**, *207*, 223–227.
4. Stover, C. K.; Warrener, P.; VanDevanter, D. R.; Sherman, D. R.; Arain, T. M.; Langhorne, M. H.; Anderson, S. W.; Towell, J. A.; Yuan, Y.; McMurray, D. N.; Krelswirth, B. N.; Barry, C. E.; Baker, W. R. *Nature* **2000**, *405*, 962–966.
5. (a) Copp, B. R.; Pearce, A. N. *Nat. Prod. Rep.* **2007**, *24*, 278–297; (b) Copp, B. R. *Nat. Prod. Rep.* **2003**, *20*, 535–557.
6. (a) Kobayashi, J.; Cheng, J. F.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1988**, *29*, 1177–1180; (b) Chung, G. A. C.; Aktar, Z.; Jackson, S.; Duncan, K. *Antimicrob. Agents Chemother.* **1995**, *39*, 2235–2238.
7. Boshoff, H. I. M.; Myers, T. G.; Copp, B. R.; McNeil, M. R.; Wilson, M. A.; Barry, C. E. *J. Biol. Chem.* **2004**, *279*, 30174–40184.
8. (a) Lindsay, B. S.; Barrows, L. R.; Copp, B. R. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 739–742; (b) Bonnard, I.; Bontemps, N.; Lahmy, S.; Banaigs, B.; Combaut, G.; Francisco, C.; Colson, P.; Houssier, C.; Waring, M. J.; Bailly, C. *Anti-Cancer Drug Des.* **1995**, *10*, 333–346; (c) Matsumoto, S. S.; Sidford, M. H.; Holden, J. A.; Barrows, L. R.; Copp, B. R. *Tetrahedron Lett.* **2000**, *41*, 1667–1670; (d) Marshall, K. M.; Barrows, L. R. *Nat. Prod. Rep.* **2004**, *21*, 731–751.
9. Charyulu, G. A.; McKee, T. C.; Ireland, C. M. *Tetrahedron Lett.* **1989**, *30*, 4201–4202.
10. Appleton, D. R.; Pearce, A. N.; Lambert, G.; Babcock, R. C.; Copp, B. R. *Tetrahedron* **2002**, *58*, 9779–9783.
11. Carroll, A. R.; Scheuer, P. J. *J. Org. Chem.* **1990**, *55*, 4426–4431.
12. Carroll, A. R.; Cooray, N. M.; Poiner, A.; Scheuer, P. J. *J. Org. Chem.* **1989**, *54*, 4231–4232.
13. Schmitz, F. J.; DeGuzman, F. S.; Hossain, M. B.; Van der Helm, D. *J. Org. Chem.* **1991**, *56*, 804–808.
14. Copp, B. R.; Hansen, R. P.; Appleton, D. R.; Lindsay, B. S.; Squire, C. J.; Clark, G. R.; Rickard, C. E. F. *Synth. Commun.* **1999**, *29*, 2665–2676.
15. Lindsay, B. S.; Pearce, A. N.; Copp, B. R. *Synth. Commun.* **1997**, *27*, 2587–2592.
16. Snell, J. M.; Weissberger, A. *J. Am. Chem. Soc.* **1939**, *61*, 450–453.
17. Ruiz, V. M.; Tapia, R.; Valderrama, J.; Vega, J. C. *J. Heterocycl. Chem.* **1981**, *18*, 1161–1164.
18. Zimhony, O.; Cox, J. S.; Welch, J. T.; Vilcheze, C.; Jacobs, W. R. *Nat. Med.* **2000**, *6*, 1043–1047.
19. van Klink, J. W.; Larsen, L.; Perry, N. B.; Weavers, R. T.; Cook, G. M.; Bremer, P. J.; MacKenzie, A. D.; Kirikae, T. *Bioorg. Med. Chem.* **2005**, *13*, 6651–6662.