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Synthesis and cytotoxicity of potential anticancer derivatives of pyrazolo[3,4,5-*kl*]acridine and indolo[2,3-*a*]acridine

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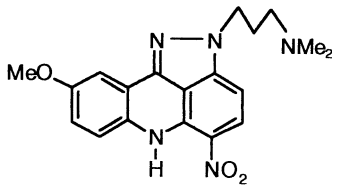
Abstract—Pyrazolo[3,4,5-*kl*]acridines were prepared by reaction of ethyl 1,9-dioxo-1,2,3,4,9,10-hexahydroacridine-4-carboxylate (**4**) with hydrazine and its methyl and 2-(dimethylamino)ethyl derivatives, followed by aromatization of the intermediate products with 1,4-benzoquinone. Conversion of the ester function to a carboxamide was also carried out and *N*-(2-(dimethylamino)ethyl)-1-(2-(dimethylamino)ethyl)-1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxamide (**13c**) was appreciably cytotoxic in a panel of cell lines. Reaction of **4** with 4-methoxyphenylhydrazine gave instead a novel indolo[2,3-*a*]acridine derivative.

1. Introduction

Many modifications have been made to the acridine skeleton in the search for anticancer compounds. Among these are the pyrazolo[3,4,5-*kl*]acridines, for example **1**,[1] a compound in phase II clinical trial.[2] The basic sidechain is essential for activity and the nitro group provided activation for the pyrazole ring forming reaction through displacement of a chlorine at the 1-position of a precursor acridine.

The acridine-4-carboxamides are another class of anticancer acridines, illustrated by **2**[3] (also in clinical trial[4]), where the basic side chain is linked through an amide function specifically located *peri* to the ring nitrogen. The related tetracyclic carboxamides **3**[5] are also effective cytotoxins.

We have previously synthesized **4**[6] (Scheme 1), which contains an ester precursor of the desired carboxamide in the key position, and 1,9-functionality which might allow a pyrazole ring to be constructed. We were therefore interested in this possibility and to make compounds which combined some features of **1** and **2**.



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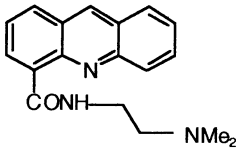

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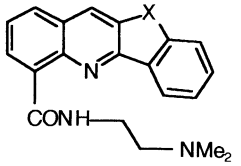

3 X = CO, O, S, NH

2. Results and discussion

2.1. Chemistry

Reaction of **4** with 1–2 mol equiv. of hydrazine or the methyl and 2-(dimethylamino)ethyl derivatives in hot ethanol gave the corresponding hydrazones (**5a–c**) (Schemes 1 and 2); the target cyclization did not occur under these conditions, but neither did any reaction of the ester group.

Keywords: hydrazone; cyclization; 1,4-benzoquinone; carboxamide.
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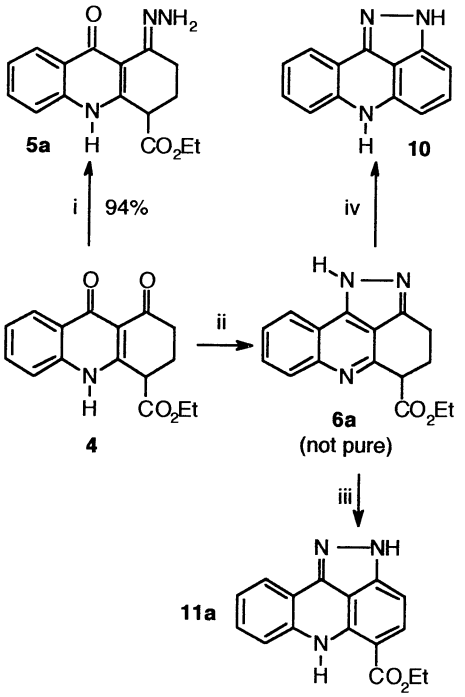


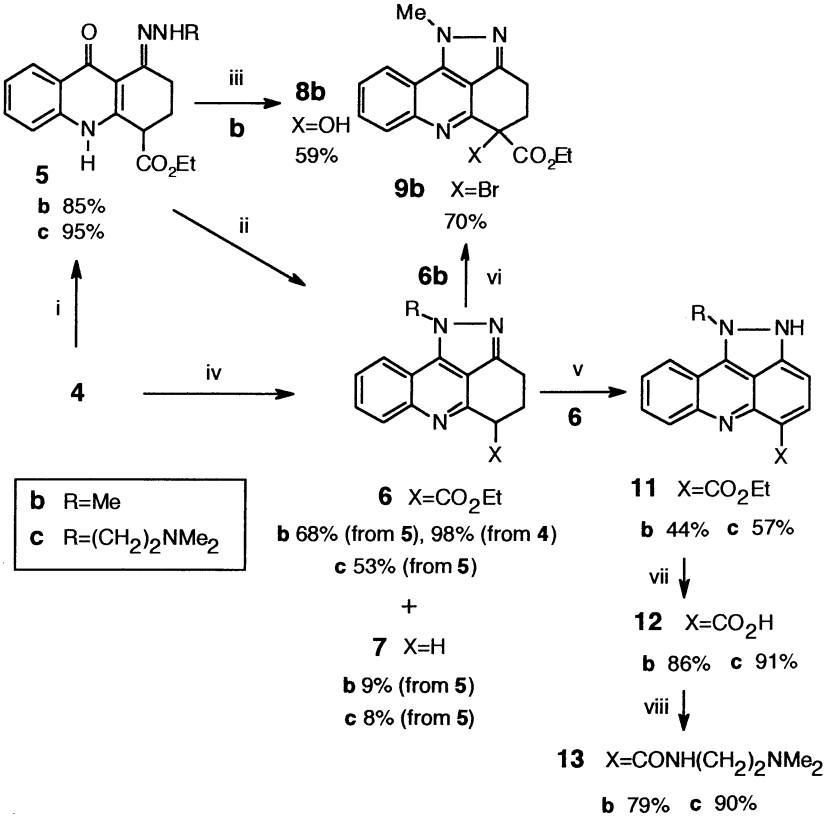
Scheme 1. (i) NH_2NH_2/EtOH/reflux, (ii) $NH_2NH_3^+$/NaOAc/EtOH/reflux, (iii) 1,4-benzoquinone/dioxane/reflux, (iv) Pd/C/Ph_2O/N_2/reflux.

Initially, the dehydrative cyclization was attempted with polyphosphoric acid but complex product mixtures were the result. Various attempts at other acid catalyzed cyclizations were also unsuccessful for the parent **5a**, but reflux of the substituted hydrazones in toluene containing trifluoroacetic acid gave a satisfactory formation of the pyrazolo compounds **6b**,**c**, along with small amounts of the des-ethoxycarbonyl analogues **7b**,**c** (Scheme 2). It was essential that this reaction was done under nitrogen; without this, **5b** gave the unexpected and surprisingly stable tertiary alcohol **8b**.

It was subsequently found that the condensation and cyclization could be achieved for all hydrazines in one process, by refluxing **4** and a salt of the hydrazine with sodium acetate in ethanol, though the yield from the two-step sequence was better for the aminoalkyl derivative **6c**.

Compounds **6** had complex ^{1}H NMR spectra, especially in the aliphatic region. The simplest was **6b** where two *N*-methyl peaks (δ 4.17, 4.38 ppm) and two sets of ethyl signals in a 2:1 ratio could be distinguished. The explanation is probably that **6** exist as a mixture of isomers, the form drawn and some tautomeric form involving relocation of H-5 (there is more than one possibility). Other results support the lability of H-5; the hydroxylation of this position (**8b**) has been referred to, while bromination of **6b** gave **9b**. In contrast to **6**, these derivatives gave NMR spectra compatible with single species and standard DEPT and HETCOR NMR experiments showed that C-5 had no hydrogen attached. The ^{1}H NMR spectrum of **6a** from the one-step reaction was very complex and this crude sample was used in the next aromatization step.

We have reported previously[6] that palladium/charcoal in boiling diphenyl ether was successful in dehydrogenation

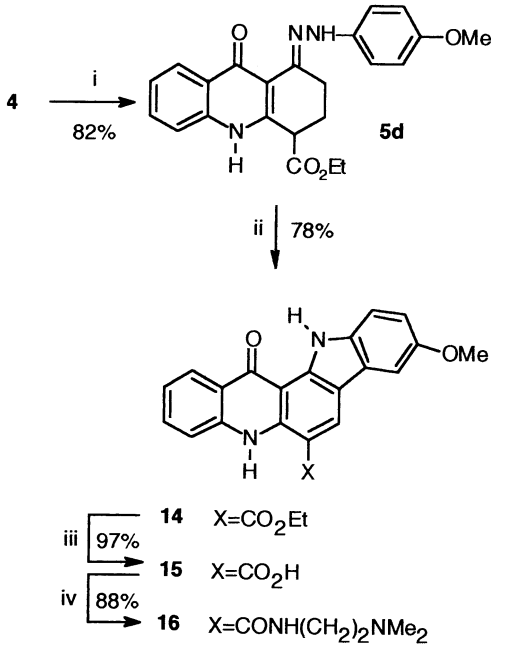


Scheme 2. (i) $RNHNH_2$/EtOH/reflux, (ii) CF_3CO_2H/toluene/N_2/reflux, (iii) CF_3CO_2H/toluene/reflux, (iv) $RNHNH_3^+$/NaOAc/EtOH/reflux, (v) 1,4-benzoquinone/dioxane/reflux, (vi) Br_2/HOAc, (vii) NaOH/H_2O/MeOH, (viii) CDI/dioxane, then $NH_2(CH_2)_2NMe_2$.

of **4** to 1-hydroxyacridones. However, aromatization of **6** proved to be a frustrating exercise.

When compounds **6** were subjected to the boiling diphenyl ether conditions, the product NMR spectra from the 1-substituted examples **b** and **c** were complex and no identifiable compounds were isolated. Reaction of **6a** was surprisingly successful, given the impure nature of the starting sample, except that the ester function was also removed and **10** resulted (Scheme 1). This occurred within 10 min at 260°C and also in 2 h at 200°C. Unchanged **6a** was present after shorter reaction times at both temperatures and in neither case was there any evidence for the target **11a**. Structure **10** is the preferred form, on the basis that the two NH peaks in the ^{1}H NMR spectrum had substantially different chemical shifts, and follows from the structure for **1** proposed from a crystal structure determination.[1] Much effort was spent on variations to the above Pd/C conditions, but with limited success, and quinone aromatization of **6** was then investigated as an alternative.

There are few references to the use of, for example, dichlorodicyanobenzoquinone in the aromatization of hydrazone related species.[7] With the present compounds, this reagent appeared to be too powerful and gave complex unidentified mixtures. However, the less reactive 1,4-benzoquinone ultimately gave general success and in boiling dioxane did produce the target **11**. The parent **11a** (Scheme 1) was quite readily isolated; the yield was only 29% but, again, this was reasonable considering that **6a** was impure. However, **11a** was difficult to work with, being insoluble in most solvents, and was not completely purified. It also proved to be very unstable in base conditions and further chemistry was abandoned. The *N*-substituted analogues were more tractable (Scheme 2); the methyl compound (**11b**) (44%), and the aminoalkyl compound (**11c**) (57%) were each obtained after chromatography. A characteristic



Scheme 3. (i) 4-$MeOC_6H_4NHNH_2$/EtOH/reflux, (ii) CF_3CO_2H/toluene/N_2/reflux, (iii) NaOH/H_2O/MeOH, (iv) CDI/dioxane, then $NH_2(CH_2)_2NMe_2$.

of successful dehydrogenation was the loss of the complex aliphatic signal pattern and appearance of the upfield aromatic doublet at δ *c* 6.6 ppm for H-3. The stable **11c** is interesting since a des-ethoxycarbonyl analogue (prepared by a different route) was reported to be quite unstable.[8]

Alkaline hydrolysis of esters **11** gave the intermediate acids **12**, and these were converted to the carboxamides **13** by reaction with 1,1′-carbonyldiimidazole to generate an intermediate imidazolide in situ, followed by amination with *N*,*N*-dimethylethylenediamine (Scheme 2).

In an attempt to extend the synthesis to arylhydrazines, **4** was reacted with 4-methoxyphenylhydrazine by the procedure described above for condensation/cyclization. However, unlike with the alkylhydrazines, this reaction only went as far as the hydrazone **5d** (Scheme 3) (which was better prepared as for the other **5**). When subjected to the TFA/toluene cyclization conditions, a preferential Fischer indole synthesis occurred instead and **5d** gave the novel indolo[2,3-*a*]acridine system **14** in 78% yield. This ester was converted by way of acid **15** to carboxamide **16** as described earlier for **11** to **13**.

2.2. Cytotoxicity results

Compounds **11c**, **13b**, **13c** and **16** were evaluated for growth inhibitory properties (measured as IC_{50} values) in a series of cell lines (Table 1). P388 is a murine leukemia, LLTC is a late-passage murine Lewis lung carcinoma, and the Jurkat lines are human leukemias.[5,9] JL_C is the wild-type (sensitive) line, while JL_A is 85-fold resistant to the topoisomerase II poison amsacrine and similar agents by virtue of a reduced level of topo II enzyme, and JL_D is a doxorubicin-resistant line, primarily by virtue of altered levels of topo II, but probably also by additional mechanisms.[10,11] IC_{50} values are given for the P388, LLTC and JL_C lines, together with ratios of IC_{50} values against JL_C and the other two Jurkat lines (JL_AJL_C and JL_D/JL_C). Values of these ratios of less than about 2-fold suggest a non-topo II mediated mechanism of action.

The three pyrazolo[3,4,5-*kl*]acridine derivatives (**11c**, **13b**, **13c**) showed significant activity across the various cell lines.

Table 1. Growth inhibitory properties

Cpd	IC_{50} (nM)[a]			IC_{50} ratio[b]	
	P388[c]	LLTC[d]	JL_C[e]	JL_A[f]/JL_C	JL_D[g]/JL_C
11c	14	181	357	0.6	0.8
13b	1700	254	415	0.8	0.9
13c	170	26	48	0.7	0.9
16	2100	980	1190	0.7	1.3
2[h]	98	189	580	1.9	2.3

[a] IC_{50}; concentration of drug (nM) to reduce cell number to 50% of control cultures (see text). The value is the average of at least two independent determinations; the coefficient of variation was 13–18%.
[b] Ratios of IC_{50}s in the cell lines shown.
[c] Murine P388 leukemia.
[d] Murine Lewis lung carcinoma.
[e] JL_C: wild-type human Jurkat leukemia.
[f] JL_A: amsacrine-resistant Jurkat.
[g] JL_D: doxorubicin-resistant Jurkat.
[h] Data from Ref. 9.

Analogues containing only the carboxamide (**11c**) or only the N-1 sidechain amine (**13b**) had similar cytotoxicity to DACA (**2**) while **13c**, which contained both functions and therefore combined structural features of **1** and **2**, had generally enhanced activity. The novel **16** was clearly much less active in all lines.

3. Experimental

3.1. General

NMR spectra were recorded, in $CDCl_3$ unless stated otherwise, on a Bruker AM-300 spectrometer operating at 300.13 MHz (^{1}H) and 75.47 MHz (^{13}C). Various standard techniques were used to identify proton-bound carbons in ^{13}C NMR spectra. The electrospray mass spectra were obtained on a VG Bio-Q triple quadrupole mass spectrometer using a water/methanol/acetic acid (50:50:1) mobile phase. LSI (3-nitrobenzyl alcohol as liquid matrix) mode high-resolution mass spectra (obtained by Dr N. Davies, University of Tasmania) or microanalyses (Campbell Microanalytical Laboratory, University of Otago, New Zealand) are reported for the compounds submitted for biological testing and for representative precursors. Electrospray mass spectra, and NMR spectra indicative of homogeneous samples were the criteria used for analogues.

3.2. Reaction of acridinediones with hydrazines in ethanol

A solution of acridinedione **4**[6] (0.25 g) and the appropriate hydrazine (2 mol equiv.) in ethanol (10 mL) was heated under reflux for 2 h (except **5a**, 1 h), with stirring, and then evaporated to dryness under reduced pressure. The residue was dissolved in chloroform, washed with water, and the chloroform was evaporated. The product in this form was generally sufficiently pure for further reaction. Representative examples were purified further and identified. In this way the following compounds were prepared:

3.2.1. Ethyl 1-hydrazono-9-oxo-1,2,3,4,9,10-hexahydroacridine-4-carboxylate (5a). The crude solid (94%) was recrystallized from ethanol to give the product as a yellow solid, mp 254–256°C. ^{1}H NMR δ 1.23 (t, J=7.1 Hz, 3H, CH_3), 2.20–2.74 (m, 4H, 2×CH_2), 4.11 (t, J=4.7 Hz, H-4), 4.17 (q, J=7.1 Hz, 2H, OCH_2), 5.23 (br s, 3H, NH+NH_2), 7.43 (t, J=7.3 Hz, 1H), 7.63 (t, J=8.1 Hz, 1H), 7.87 (d, J=8.6 Hz, 1H), 8.24 (d, J=8.6 Hz, 1H), 14.58 (s, 1H, NH-10). ^{13}C NMR δ 14.2 (CH_3), 21.1 (CH_2), 23.5 (CH_2), 48.4 (CH), 61.2 (CH_2), 106.7 (C), 120.6 (C), 122.8 (CH), 125.1 (CH), 128.4 (CH), 130.5 (CH), 147.8 (C), 153.5 (C), 155.3 (C), 163.4 (C), 172.3 (C). ESMS: m/z 300 (M+1). Anal. calcd for $C_{16}H_{17}N_3O_3$: C, 64.2; H, 5.7; N, 14.0. Found: C, 64.0; H, 5.6; N, 13.9%.

3.2.2. Ethyl 1-methylhydrazono-9-oxo-1,2,3,4,9,10-hexahydroacridine-4-carboxylate (5b). The crude material (85%) could be used in the next step, but a sample was purified by column chromatography (silica; ethyl acetate) to give an oil, R_f 0.51. ^{1}H NMR δ 1.19 (t, J=7.1 Hz, CH_3), 2.10–2.60 (m, 4H), 2.99 (s, NCH_3), 4.05 (t, J=4.7 Hz, H-4), 4.13 (q, J=7.1 Hz, OCH_2), 4.72 (br s, NH), 7.39 (t, J=7.4 Hz, 1H), 7.58 (t, J=7.8 Hz, 1H), 7.83 (d, J=8.4 Hz, 1 H), 8.19 (d, J=8.2 Hz, 1H), 14.67 (br s, NH). ^{13}C NMR δ 14.1 (CH_3), 21.6 (CH_2), 23.5 (CH_2), 38.2 (CH), 48.4 (NCH_3), 61.0 (OCH_2), 106.8 (C), 120.9 (C), 122.6 (CH), 125.0 (CH), 128.2 (CH), 130.2 (CH), 147.5 (C), 150.0 (C), 155.1 (C), 163.1 (C), 172.3 (C). ESMS: m/z 314 (M+1).

3.2.3. Ethyl 1-(2-(dimethylamino)ethyl)hydrazono-9-oxo-1,2,3,4,9,10-hexahydroacridine-4-carboxylate (5c). Reaction of **4** with 2-(dimethylamino)ethylhydrazine[12] was for 2 h. The crude oil (95%) was used in this state in further reaction. ^{1}H NMR δ 1.07 (t, J=7.1 Hz, CH_3), 2.12 (s, $N(CH_3)_2$), 2.24–2.53 (m, 6H), 3.18 (br s, NCH_2), 3.95 (t, J=4.9 Hz, H-4), 4.03 (q, J=7.1 Hz, OCH_2), 5.31 (br s, NH), 7.27 (t, J=7.2 Hz, 1H), 7.46 (t, J=7.0 Hz, 1H), 7.73 (d, J=8.5 Hz, 1H), 8.09 (d, J=7.9 Hz, 1H). ^{13}C NMR δ 13.8 (CH_3), 21.5 (CH_2), 23.2 (CH_2), 45.1 (2×CH_3), 48.0 (CH_2), 48.2 (CH), 57.3 (CH_2), 60.6 (CH_2), 106.6 (C), 120.6 (C), 122.3 (CH), 124.6 (CH), 128.0 (CH), 129.8 (CH), 147.2 (C), 150.1 (C), 154.9 (C), 162.8 (C), 171.9 (C). ESMS: m/z 371 (M+1).

3.3. Pyrazole ring formation

3.3.1. Ethyl 1-methyl-1,3,4,5-tetrahydropyrazolo[3,4,5-*kl*]acridine-5-carboxylate (6b). Method A. Trifluoroacetic acid (100 mg) was added to a solution of hydrazone **5b** (0.31 g, 1 mmol) in toluene (70 mL). The apparatus was flushed with nitrogen and the mixture was refluxed under Dean–Stark conditions for 16 h, also under nitrogen. The cooled solution was washed with water (30 mL×2), dried ($MgSO_4$), and the toluene was removed under reduced pressure. The residue was purified by column chromatography (silica; ethyl acetate) to give an inseparable mixture of isomers (c 2:1) (0.2 g, 68%) as an oil, R_f 0.51. ^{1}H NMR δ 1.22 (t, J=6.0 Hz, CH_3), 1.31 (t, J=6.0 Hz, CH_3), 2.3–3.3 (multiple complex signals), 4.1–4.25 (m, OCH_2×2), 4.17 (s, NCH_3), 4.38 (s, NCH_3), 7.03–7.12 (m), 7.24–7.32 (m), 7.54 (t, J=7.9 Hz), 7.64–7.71 (m), 8.21 (d, J=8.4 Hz). ESMS: m/z 296 (M+1). This sample was used directly in the next reaction.

The chromatography also gave a 9% yield of *1-methyl-1,3,4,5-tetrahydropyrazolo[3,4,5-kl]acridine* (**7b**) as an oil, R_f 0.14. ^{1}H NMR δ 2.30 (m, J=6.1 Hz, CH_2), 3.01 (t, J=6.1 Hz, CH_2), 3.08 (t, J=6.1 Hz, CH_2), 4.38 (s, NCH_3), 7.53 (t, J=7.4 Hz, 1H), 7.66 (t, J=7.7 Hz, 1H), 8.16 (d, J=8.4 Hz, 1H), 8.20 (d, J=8.1 Hz, 1H). ^{13}C NMR δ 22.7 (CH_2), 25.0 (CH_2), 30.4 (CH_2), 38.9 (CH_3), 116.7 (C), 117.0 (C), 121.6 (CH), 125.4 (CH), 128.5 (CH), 130.0 (CH), 137.7 (C), 147.3 (C), 147.6 (C), 158.5 (C). ESMS: m/z 224 (M+1).

When the above reaction was carried out for 20 h without the nitrogen atmosphere, chromatography of the crude product (silica; ethyl acetate/hexane, 4:1) gave a 59% yield of *ethyl 5-hydroxy-1-methyl-1,3,4,5-tetrahydropyrazolo[3,4,5-kl]acridine-5-carboxylate* (**8b**) as an oil, R_f 0.37. ^{1}H NMR δ 1.19 (t, J=7.1 Hz, CH_3), 2.48 (m, 1H, H-4a), 2.69 (m, 1H, H-4b), 3.23 (m, 2H, H-3), 4.17–4.30 (m, OCH_2), 4.40 (s, NCH_3), 4.53 (br s, OH), 7.57 (t, J=7.9 Hz, 1H), 7.65 (t, J=7.2 Hz, 1H), 8.20 (d, J=7.9 Hz,

1H), 8.23 (d, J=7.8 Hz, 1H). ^{13}C NMR δ 14.1 (CH_3), 20.3 (CH_2), 36.2 (CH_2), 39.0 (NCH_3), 62.3 (OCH_2), 74.9 (C, C-5), 116.0 (C), 117.3 (C), 121.6 (CH), 126.3 (CH), 128.5 (CH), 131.0 (CH), 138.3 (C), 146.2 (C), 146.9 (C), 155.5 (C), 173.4 (C). ESMS: m/z 312 (M+1).

Method B. A mixture of dione **4** (0.57 g, 2.0 mmol), methyl hydrazine sulfate (0.57 g, 4.0 mmol) and sodium acetate (2.0 g) in ethanol (50 mL) was heated under reflux for 24 h. The ethanol was removed under reduced pressure and the residue was extracted with chloroform (30 mL×2). The combined extracts were washed with water, dried (Na_2SO_4).and the solvent was removed to give the crude **6b** as an orange oil (0.59 g, 98%), which was sufficiently pure for use in the next step.

3.3.2. Ethyl 1-(2-(dimethylamino)ethyl)-1,3,4,5-tetrahydropyrazolo[3,4,5-*kl*]-acridine-5-carboxylate (6c). From **5c** by method A. Reaction was for 16 h and the crude product was purified by column chromatography (silica; chloroform/methanol, 4:1) to give a mixture of isomers (*c* 3:1, 53%) as an oil, R_f 0.65. ^{1}H NMR δ 1.2 (t, J=6.0 Hz, CH_3), 1.3 (t, J=6.0 Hz, CH_2), 2.33 (s, $N(CH_3)_2$), 2.36 (s, $N(CH_3)_2$), 2.4–3.2 (multiple complex signals), 4.1–4.25 (m, OCH_2×2), 4.6 (t, J=7.4 Hz, NCH_2), 4.8 (t, J=7.7 Hz, NCH_2), 7.1–8.22 (m×5). ESMS: m/z 353 (M+1).

The chromatography also gave 8% of *1-(2-(dimethylamino)ethyl)-1,3,4,5-tetrahydropyrazolo[3,4,5-kl]acridine* (**7c**) as an oil, R_f 0.46. ^{1}H NMR δ 2.32 (m, CH_2), 2.37 (s, $N(CH_3)_2$), 2.91 (t, J=7.6 Hz, CH_2), 3.05 (t, J=6.3 Hz, CH_2), 3.11 (t, J=6.3 Hz, CH_2), 4.83 (t, J=7.6 Hz, CH_2), 7.58 (t, J=7.0 Hz, 1H), 7.70 (t, J=8.4 Hz, 1H), 8.18 (d, J=8.6 Hz, 1H), 8.21 (d, J=7.7 Hz, 1H). ^{13}C NMR δ 22.8 (CH_2), 24.9 (CH_2), 30.4 (CH_2), 45.8 (CH_3), 50.1 (CH_2), 58.8 (CH_2), 116.8 (2×C), 121.6 (CH), 125.6 (CH), 128.5 (CH), 130.2 (CH), 137.4 (C), 147.8 (C), 148.0 (C), 158.6 (C). ESMS: m/z 281 (M+1).

3.3.3. Ethyl 1,3,4,5-tetrahydropyrazolo[3,4,5-kl]acridine-5-carboxylate (6a). Method B. Reaction was as for **6b**. After the ethanol was removed the residue was boiled with water (50 mL), cooled to room temperature and the solid was collected by filtration, washed with water and dried. The yellow solid (0.30 g from 0.57 g **4**), mp 248–249°C, had a very complex ^{1}H NMR spectrum. ESMS m/z 282 (78%; M+1, **6a**), 300 (30%; M+1, **5a**), 354 (35%; unknown), 595 (30%, unknown).

3.4. Bromination

3.4.1. Ethyl 5-Bromo-1-methyl-1,3,4,5-tetrahydropyrazolo[3,4,5-*kl*]acridine-5-carboxylate (9b). A solution of bromine in acetic acid (3 mL×0.1 M, 0.3 mmol) was added dropwise, with stirring, to a solution of **6b** (90 mg, 0.3 mmol) in acetic acid (5 mL). The solution became green. After the addition was complete, water (25 mL) was added immediately and the mixture was extracted with chloroform (20 mL×3). The combined chloroform extracts were washed with saturated sodium bicarbonate (10 mL), water (15 mL×2), dried ($MgSO_4$) and the chloroform was removed under reduced pressure to give an oil (80 mg), the predominant component of which was assigned as **9b**. ^{1}H NMR δ 1.28 (t, J=7.1 Hz, CH_3), 2.76–2.82 (m, 1 H, H-4a), 3.05–3.13 (m, 1 H, H-4b), 3.22 (m, 2 H, H-3), 4.33 (q, J=7.1 Hz, OCH_2), 4.38 (s, NCH_3), 7.49 (t, J=8.1 Hz, 1H), 7.63 (t, J=8.3 Hz, 1H), 8.13 (d, J=7.9 Hz, 1H), 8.22 (d, J=8.4 Hz, 1H). ^{13}C NMR δ 14.0 (CH_3), 21.5 (CH_2), 39.0 (NCH_3), 39.5 (CH_2), 61.1 (C), 63.1 (OCH_2), 114.3 (C), 117.1 (C), 121.3 (CH), 126.6 (CH), 128.5 (CH), 131.3 (CH), 138.5 (C), 144.5 (C), 146.9 (C), 153.8 (C), 168.4 (C). ESMS: m/z 374 (100%), 375 (20), 376 (100), 377 (22), all (M+1) for $C_{17}H_{16}BrN_3O_2$.

3.5. Aromatization

3.5.1. 2,6-Dihydropyrazolo[3,4,5-*kl*]acridine (10). A mixture of crude **6a** (0.1 g) and 10% Pd/C (0.05 g) in diphenyl ether (5 mL) was refluxed for 10 min, then passed through a Celite bed and hexane was added to the filtrate. The solid which separated (0.06 g) was filtered off and recrystallized from toluene to give the product as a pale yellow solid (0.04 g), mp>300°C. ^{1}H NMR (DMSO-d_6) δ 6.02 (d, J=7.2 Hz, 1 H), 6.49 (d, J=8.2 Hz, 1H), 6.93 (t, J=7.5 Hz, 1H), 7.00–7.05 (m, 2H), 7.22 (t, J=7.3 Hz, 1H), 7.67 (d, J=7.9 Hz, 1H), 9.86 (s, NH), 12.2 (s, NH). ^{13}C NMR (DMSO-d_6) δ 95.7 (CH), 97.3 (CH), 116.1 (CH), 116.9 (C), 117.4 (C), 120.2 (CH), 122.5 (CH), 128.7 (CH), 129.9 (CH), 137.3 (C), 141.1 (C), 141.2 (C), 141.9 (C). ESMS: m/z 208 (M+1). Anal. calcd for $C_{13}H_9N_3{\cdot}0.25H_2O$: C 73.8, H, 4.5, N 19.9. Found: C 73.9; H 4.5; N 19.8%.

3.5.2. Ethyl 1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxylate (11a). A mixture of crude **6a** (0.28 g) and 1,4-benzoquinone (0.43 g) in 1,4-dioxan (20 mL) was heated under reflux for 2 h. The solvent was evaporated and the residue was extracted with hot ethanol (2×15 mL), then with hot ethyl acetate (2×15 mL). The insoluble material was extracted into chloroform (80 mL) and the solvent was evaporated to give the product (0.08 g) as a dark brown solid, which still contained minor impurities. ^{1}H NMR δ 1.38 (t, J=7.2 Hz, CH_3), 4.36 (q, J=7.2 Hz, OCH_2), 6.63 (d, J=9.0 Hz, H-3), 7.30 (t, J=7.5 Hz, 1H), 7.39 (d, J=8.3 Hz, 1H), 7.66 (t, J=7.3 Hz, 1H), 8.12 (d, J=9.0 Hz, H-4), 8.38 (d, J=7.3 Hz, 1H), 12.32 (s, NH), 12.51 (br s, NH).

3.5.3. Ethyl 1-methyl-1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxylate (11b). A mixture of **6b** (0.30 g, 1.0 mmol) and 1,4-benzoquinone (0.43 g, 4.0 mmol) in dioxane (30 mL) was heated under reflux for 16 h. The solvent was removed under reduced pressure and the residue was extracted with chloroform (3×20 mL). The combined organic extracts were washed with 2% sodium hydroxide (2×15 mL), water, dried, and the chloroform was removed. Chromatography of the residue (0.21 g) (alumina; ethyl acetate containing 2% diethylamine) gave the the product (0.13 g, 44%) as a dull yellow, blue-tinged solid, R_f 0.7, mp 169–171°C. ^{1}H NMR δ 1.39 (t, J=7.1 Hz, CH_3), 4.28 (s, NCH_3), 4.32 (q, J=7.1 Hz, OCH_2), 6.61 (d, J=9.2 Hz, H-3), 7.08–7.14 (m, 2H), 7.33 (t, J=8.4 Hz, 1H), 7.52 (d, J=9.2 Hz, H-4), 7.71 (d, J=7.9 Hz, 1 H), 10.10 (br s, NH). ^{13}C NMR δ 14.5 (CH_3), 39.3 (NCH_3), 59.8 (OCH_2), 94.6 (C), 104.0 (CH), 114.7 (C), 118.0 (CH), 122.4 (CH), 122.5 (CH), 129.6 (CH), 129.8 (CH), 134.0 (C), 139.1

(2×C), 141.8 (C), 148.5 (C), 168.3 (C). ESMS: *m/z* 294 (M+1). Anal. calcd for $C_{17}H_{15}N_3O_2$: C, 69.6; H, 5.1; N, 14.3. Found: C, 69.7; H, 5.2; N, 14.5%.

3.5.4. Ethyl 1-(2-(dimethylamino)ethyl)-1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxylate (11c). This was prepared from **6c**, as for **11b**, and obtained after column chromatography (alumina; ethyl acetate), R_f 0.63, as a bluish yellow oil (57%) which gradually solidified on standing, mp 92–93°C. ^{1}H NMR δ 1.39 (t, *J*=6.9 Hz, CH_3), 2.34 (s, $N(CH_3)_2$), 2.89 (t, *J*=7.6 Hz, CH_2), 4.33 (q, *J*=7.0 Hz, OCH_2), 4.70 (t, *J*=7.5 Hz, CH_2), 6.64 (d, *J*=9.2 Hz, 1H), 7.12–7.21 (m, 2H), 7.36 (t, *J*=7.5 Hz, 1H), 7.55 (d, *J*=9.2 Hz, 1H), 7.75 (d, *J*=7.9 Hz, 1H), 10.23 (s, NH). ^{13}C NMR δ 14.53 (CH_3), 45.8 ($N(CH_3)_2$), 50.7 (CH_2), 58.6 (CH_2), 59.8 (CH_2), 94.5 (C), 104.2 (CH), 114.6 (C), 118.0 (C), 118.2 (CH), 122.5 (CH), 122.7 (CH),129.6 (CH), 129.8 (CH), 133.7 (C), 139.2 (C), 142.0 (C), 148.8 (C), 168.3 (C). LSIMS: Found 351.1825 ($M+H^+$). $C_{20}H_{23}N_4O_2$ requires 351.1816.

3.6. Indoloacridine formation

3.6.1. Ethyl 4-methoxy-13-oxo-8,13-dihydro-1*H*-indolo-[2,3-*a*]acridine-7-carboxylate (14). A mixture of **4** (0.18 g, 0.63 mmol) and 4-methoxyphenylhydrazine hydrochloride (0.13 g, 0.74 mmol) in ethanol (15 mL) was heated under reflux for 16 h, then cooled on ice. The yellow hydrazone **5d** which separated (0.21 g, 82%) was filtered off and washed with a little ethanol. ^{1}H NMR δ 1.22 (t, *J*=7.0 Hz, CH_3), 2.1–3.0 (m, 4H), 3.77 (s, OCH_3), 4.16 (q, *J*=7.0 Hz, CH_2), 4.35 (br t, 1H), 6.87 (d, *J*=8.5 Hz, 2H), 7.04 (d, *J*=8.5 Hz, 2H), 7.45 (t, *J*=7.3 Hz, 1H), 7.65 (t, *J*=7.6 Hz, 1H), 8.01 (d, *J*=8.4 Hz, 1H), 8.26 (d, *J*=8.2 Hz, 1H). ESMS: *m/z* 406 (M+1).

This was dissolved in toluene (15 mL), trifluoroacetic acid (40 mg) was added and the mixture was heated under reflux for 16 h, then evaporated to dryness at reduced pressure to give the product as a yellow solid (0.19 g, 78%), mp 272°C (from ethanol). ^{1}H NMR (assigned from additional HMQC, HMBC and ^{1}H–^{1}H COSY experiments) δ 1.53 (t, *J*=7.1 Hz, CH_3), 3.94 (s, OCH_3), 4.50 (q, *J*=7.1 Hz CH_2), 7.07 (dd, *J*=8.6, 2.3 Hz, H-3), 7.35 (t, *J*=7.5 Hz, H-11), 7.47 (d, *J*=8.7 Hz, H-2), 7.49 (d, *J*=8.0 Hz, H-9), 7.54 (d, *J*=2.0 Hz, H-5), 7.70 (t, *J*=7.6 Hz, H-10), 8.48 (d, *J*=8.0 Hz, H-12), 9.02 (s, H-6), 11.46 (s, NH), 12.45 (s, NH). ^{13}C NMR δ 14.5 (CH_3), 56.0 (CH_3), 61.2 (CH_2), 102.7 (CH), 104.0 (C), 106.8 (C), 112.5 (CH), 114.7 (CH), 115.3 (C), 117.9 (CH), 121.8 (C), 122.4 (CH), 123.6 (C), 126.0 (CH), 130.2 (CH), 133.3 (CH), 134.2 (C), 139.4 (C), 141.6 (C), 143.0 (C), 155.2 (C), 168.9 (C), 178.9 (C). Anal. calcd for $C_{23}H_{18}N_2O_4$: C 71.5; H 4.7; N 7.3. Found: C 71.3; H 4.6; N 7.1%.

3.7. Preparation of amides

3.7.1. *N*-(2-(Dimethylamino)ethyl)-4-methoxy-13-oxo-8,13-dihydro-1*H*-indole[2,3-*a*]acridine-7-carboxamide (16). A mixture of ester **14** (0.20 g, 0.52 mmol), methanol (8 mL) and 10% NaOH (10 mL) was heated under reflux for 24 h, then cooled and acidified with concentrated HCl. The precipitate which formed was filtered off, washed with water and dried to give the intermediate acid **15** as a yellow solid (0.18 g, 97%), mp>300°C. (some sublimes >254°C).

This acid (0.18 g, 0.50 mmol), with 1,1′-carbonyldiimidazole (0.12 g, 0.75 mmol) in dioxane (30 mL) was refluxed with stirring for 6 h. *N*,*N*-Dimethylethylenediamine (70 mg, 0.75 mmol) was added and the solution was refluxed for 24 h. The solvent was removed at reduced pressure and the residue was dissolved in chloroform, washed with 10% sodium carbonate, then with warm water, and dried ($MgSO_4$). Evaporation of the solvent gave the amide as a yellow solid (0.19 g, 88%), mp 239–240°C (from acetonitrile). ^{1}H NMR δ 2.42 (s, $N(CH_3)_2$), 2.76 (t, *J*=5.7 Hz, CH_2), 3.70 (q, *J*=5.7 Hz, CH_2), 3.83 (s, OCH_3), 7.01 (dd, *J*=8.7, 2.3 Hz), 7.26 (s, CONH), 7.30–7.37 (m, 2H), 7.42–7.46 (m, 2 H), 7.65 (t, *J*=7.2 Hz, 1H), 8.42 (s), 8.48 (d, *J*=7.9 Hz, 1H), 11.38 (s, NH), 12.76 (s, NH). ^{13}C NMR δ 37.4 (CH_2), 45.5 ($N(CH_3)_2$), 55.8 (OCH_3), 58.0 (CH_2), 102.1 (CH), 108.1 (C), 112.3 (CH), 114.1 (CH), 114.4 (C), 118.1 (CH), 121.5 (C), 121.9 (CH), 123.1 (C), 125.3 (CH), 125.7 (CH), 133.0 (CH), 134.0 (C), 139.5 (C), 140.6 (C), 141.3 (C), 154.8 (C), 169.6 (C), 178.9 (C). ESMS: *m/z* 429 (M+1). Anal. calcd for $C_{25}H_{24}N_4O_3\cdot H_2O$: C 67.3; H 5.9; N 12.6. Found: C 67.6; H 5.7; N 12.2%.

3.7.2. *N*-(2-(Dimethylamino)ethyl)-1-methyl-1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxamide (13b). A mixture of ester **11b** (0.18 g, 0.61 mmol), 10% NaOH (10 mL) and methanol (5 mL) was heated under reflux for 1 h. After being cooled, most of the methanol was removed at reduced pressure, the remainder was filtered and the filtrate was taken to pH 2 with concentrated HCl. The precipitate which separated was filtered off, washed with water and dried to give the intermediate acid **12b** as a yellow solid (0.14 g, 86%).

Amidation as for the preparation of **16** gave the crude product (79%). A sample was purified by column chromatography (alumina; ethyl acetate with 4% diethylamine) followed by recrystallization from benzene/light petroleum (bp 60–90°C) to give a pale yellow solid, mp 157–159°C, which gained a blue tinge as it gradually absorbed water. ^{1}H NMR δ 2.34 (s, $N(CH_3)_2$), 2.61 (t, *J*=5.7 Hz, CH_2), 3.53 (q, *J*=5.7 Hz, CH_2), 4.31 (s, NCH_3), 6.65 (d, *J*=9.0 Hz, 1H), 6.78 (br t, NH), 7.08 (t, *J*=7.6 Hz, 1H), 7.13–7.20 (m, 3H), 7.31 (t, *J*=7.4 Hz, 1H), 7.73 (d, *J*=7.9 Hz, 1H), 10.92 (s, NH). ^{13}C NMR δ 36.3 (CH_2), 39.4 (CH_3), 45.0 (2×CH_3), 58.1 (CH_2), 96.7 (C), 103.8 (CH), 114.7 (C), 118.0 (CH), 118.5 (C), 122.0 (CH), 122.3 (CH), 126.7 (CH), 129.8 (CH), 134.1 (C), 139.6 (C), 141.0 (C), 148.0 (C), 166.9 (C). ESMS: *m/z* 336(M+1). Anal. calcd for $C_{19}H_{21}N_5O\cdot 0.5H_2O$: C, 66.3; H, 6.4; N, 20.3. Found: C, 66.0; H, 6.4; N, 20.0%.

3.7.3. *N*-(2-(Dimethylamino)ethyl)-1-(2-(dimethylamino)-ethyl)-1,2-dihydropyrazolo[3,4,5-*kl*]acridine-5-carboxamide (13c). To a solution of ester **11c** (0.25 g, 0.71 mmol) in methanol (10 mL) was added 10% NaOH (8 mL) and the mixture was refluxed with stirring for 2 h. Most of the methanol was removed, water (10 mL) was added and the solution was washed with chloroform (2×10 mL), then carefully acidified with concentrated HCl to pH 3–4. The solution was saturated with potassium chloride and a blue solid

separated on standing. This was filtered off and dried overnight in a vacuum oven at 50°C to give the intermediate acid **12c** (0.21 g, 91%), mp 177°C (dec.). ^{1}H NMR (DMSO-d_6) δ 2.86 (s, $N(CH_3)_2$), 3.69 (t, J=6.2 Hz, CH_2), 5.13 (t, J=6.2 Hz, CH_2), 6.58 (d, J=9.1 Hz, 1H), 7.24 (t, J=7.7 Hz, 1H), 7.46–7.50 (m, 2H), 7.79 (d, J=8.2 Hz, 1H), 8.12 (d, J=7.9 Hz, 1H), 10.50 (s, NH), 10.83 (br s, CO_2H).

Amidation as for the preparation of **16** gave the amide **13c** as a blue semi-solid (90%).^{1}H NMR δ 2.26 (s, $N(CH_3)_2$), 2.34 (s, $N(CH_3)_2$), 2.51 (t, J=5.9 Hz, CH_2), 2.87 (t, J=7.6 Hz, CH_2), 3.47 (q, J=5.4 Hz, CH_2), 4.69 (t, J=7.6 Hz, CH_2), 6.63–6.66 (m, 2H, H-3 and CONH), 7.06–7.17 (m, 3 H), 7.31 (t, J=7.6 Hz, 1H), 7.71 (d, J=8.0 Hz, 1H), 10.99 (s, NH). ^{13}C NMR δ 36.6 (CH_2), 45.2 ($N(CH_3)_2$), 45.8 ($N(CH_3)_2$), 50.6 (CH_2), 57.9 (CH_2), 58.6 (CH_2), 96.7 (C), 103.8 (CH), 114.4 (C), 118.1 (CH), 118.5 (C), 122.1 (CH), 122.3 (CH), 126.7 (CH), 129.8 (CH), 133.8 (C), 139.6 (C), 141.1(C), 148.2 (C), 168.9 (C). LSIMS: Found 393.2399 ($M+H^+$). $C_{22}H_{29}N_6O$ requires 393.2397.

3.8. In vitro growth delay assays

These were carried out as reported previously.[10,11] Independent assays were performed in duplicate, and coefficients of variation were from 13 to 18%.

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