Synthetic strategies to a telomere-targeted pentacyclic heteroaromatic salt†

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Three routes have been explored to synthesise the telomere-targeted agent 3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate **3**. Application of a 6-(2-azidophenyl)phenanthridine precursor **11** gave an entry to the indazolo[2,3-*f*]phenanthridine ring system **12** not the required quino[4,3,2-*kl*]acridine. A six step synthesis starting from 2,6-dibromo-4-methylbenzonitrile **13** *via* a 1-arylacridin-9(10*H*)-one intermediate, **19** or **21**, gave the required **3** in low overall yield (<10%). The most efficient route entailed the one-pot (five step) conversion of 1,2-dimethyl-6-fluoroquinolinium methosulfate **23** to **3** in 33% yield employing triethylamine as base and nitrobenzene as solvent.

Introduction

Prior to initiation of our own work, the tetracyclic pyrido[4,3,2-*kl*]acridine skeleton was represented in the literature only by the natural product necatorone **1**, elaborated by the fungus *Lactarius necator* and whose structure was deduced by Steglich and colleagues.[2] In previous papers in this series we have developed a range of synthetic approaches to tetra-, penta- and hexa-cyclic systems based on the necatorone scaffold. These methods have included thermal extrusion of nitrogen from triazolyl-substituted acridines,[3] radical cyclisations of bromo-substituted 9-anilinoacridines[4] and palladium(0)-mediated derivatisation of substituted acridin-9(10*H*)-ones.[5] From these endeavours we have identified two series of bioactive pentacyclic systems with an affinity for DNA: topoisomerase II-inhibitory compounds such as the indolizino[7,6,5-*kl*]acridinium chloride **2**[6] which intercalates into duplex DNA sequences;[7] and quaternary 8,13-dimethylquino[4,3,2-*kl*]acridinium salts exemplified by the methosulfate **3** (RHPS4)[8] (Fig. 1) which binds to, and stabilises, DNA G-quadruplex structures, for example the $(TTAGGG)_n$ sequences[9] which characterise the ends (telomeres[10]) of chromosomes.

The enzyme telomerase maintains telomere lengths, is upregulated in most human tumour types and is considered a relevant molecular target in the search for novel anticancer therapies.[11] The quinoacridinium salt **3** is a potent inhibitor of telomerase (IC_{50} 0.33 μM)[12] and, in our recent pharmacological investigations, has been shown to compromise telomeric integrity and rapidly provoke the emergence of a senescent phenotype in human breast[1] and melanoma tumour cells.[13] In an effort to enhance the ability of pentacyclic acridinium salts to stabilise G-quadruplex structures and increase their potency as telomere-targeted agents, we have exploited palladium(0) couplings to synthesise and biologically profile quinoacridinium salts related to **3** but bearing varied and multiple substituents at the 3-, 6- and 10-(or 11) positions.[5,14] (See Fig. 1 for numbering system). Of the many novel structures prepared compound **3** still retained the most desirable range of pharmacological[1,13] and pharmaceutical properties[14] and has emerged as the clinical candidate from this series. In this paper we assess different synthetic routes which might be adaptable to a large-scale synthesis of **3**.

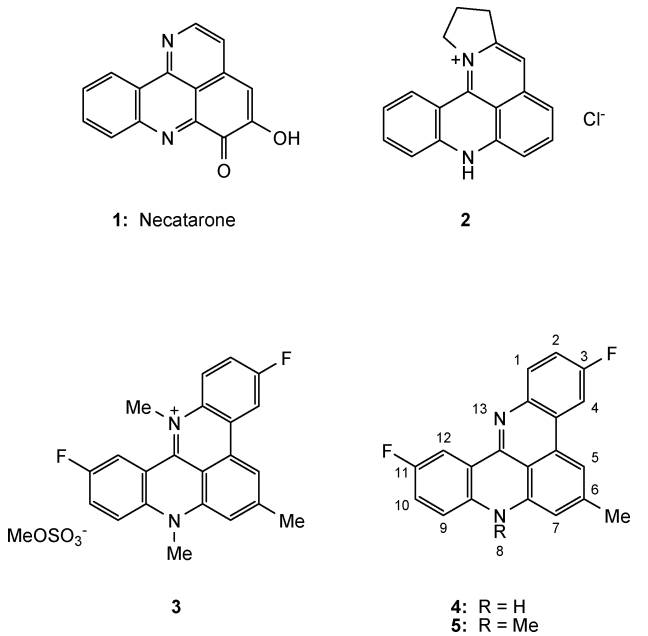

Fig. 1 Structures of bioactive polycyclic acridines and numbering of the quino[4,3,2-*kl*]acridine ring system.

Results and discussion

General synthetic considerations

In planning synthetic strategies to an appropriately-substituted quino[4,3,2-*kl*]acridine **4** or **5** which might be further processed by methylation to furnish salt **3**, we have investigated two potential disconnections which could be applied generally to synthesise molecules of this class bearing varied substituents (X,Y,Z) in

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the three homocyclic rings. These involve cyclisations of 6-arylphenanthridine (Fig. 2; Route A) and 1-arylacridin-9(10*H*)-one (Route B) precursors. These routes might provide alternatives to the unlikely processing of a 1,2-methylquinolinium salt directly to **3** (Route C, see Scheme 3),[8] an adaptation of a reaction reported by Ozczapowicz *et al.* in 1988.[15]

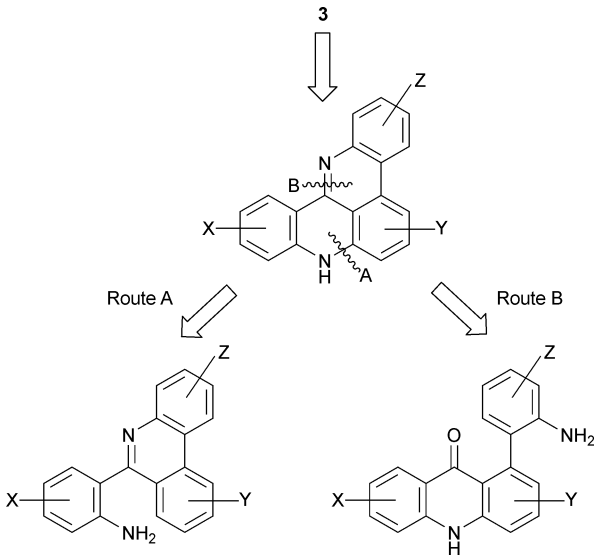


Fig. 2 Synthetic strategies to trisubstituted quino[4,3,2-*kl*]acridines.

Attempted synthesis of pentacyclic salt 3 from a 2-arylphenanthridine (Route A)

The tolylboronic acid **6** was coupled with 2-bromo-4-fluoroaniline under Suzuki conditions[16] to afford biaryl **7** (68%) which reacted with 5-fluoro-2-nitrobenzoic acid under DCC coupling conditions to generate the anilide **8**. This was cyclised under Bischler–Napieralski conditions to the nitroaryl-substituted phenanthridine **9** which was further routinely transformed to the corresponding amino- and azido-phenylphenanthridines **10** and **11**, respectively. Thermolysis of **11** in boiling 1,2,4-trichlorobenzene at 214 °C gave a product with the molecular weight (by mass spectrometry: *m*/*z* 319 [M + 1]) expected for pentacycle **4**. However, its physical properties were inconsistent with this assignment: significantly, the product was a pale yellow colour with very poor solubility in methanol, whereas an orange–red colouration and methanol-soluble character would be expected for compound **4**. Moreover, in earlier work we have shown that the unsubstituted quino[4,3,2-*kl*]acridine ring system shows a long wavelength absorption at λ_{max} 443 nm in ethanol with a bathochromic shift to 488 nm on addition of HCl.[4] Significantly, the product of thermolysis of **11** showed a UV absorption (λ_{max}) at 380 nm with no bathochromic shift on the addition of HCl. We have assigned the indazolo[2,3-*f*]phenanthridine structure **12** to this compound based on analogous cyclisations of *o*-azidoarenes bearing suitably disposed heteroaromatic residues.[17] In the present example, nucleophilic displacement of nitrogen from the azido group by the phenanthridine nitrogen atom to form **12** is favoured over a nitrene insertion process which might have led to the required quino[4,3,2-*kl*]acridine **4** (Scheme 1).

Synthesis of pentacyclic salt 3 from a 1-arylacridin-9(10*H*)-one (Route B)

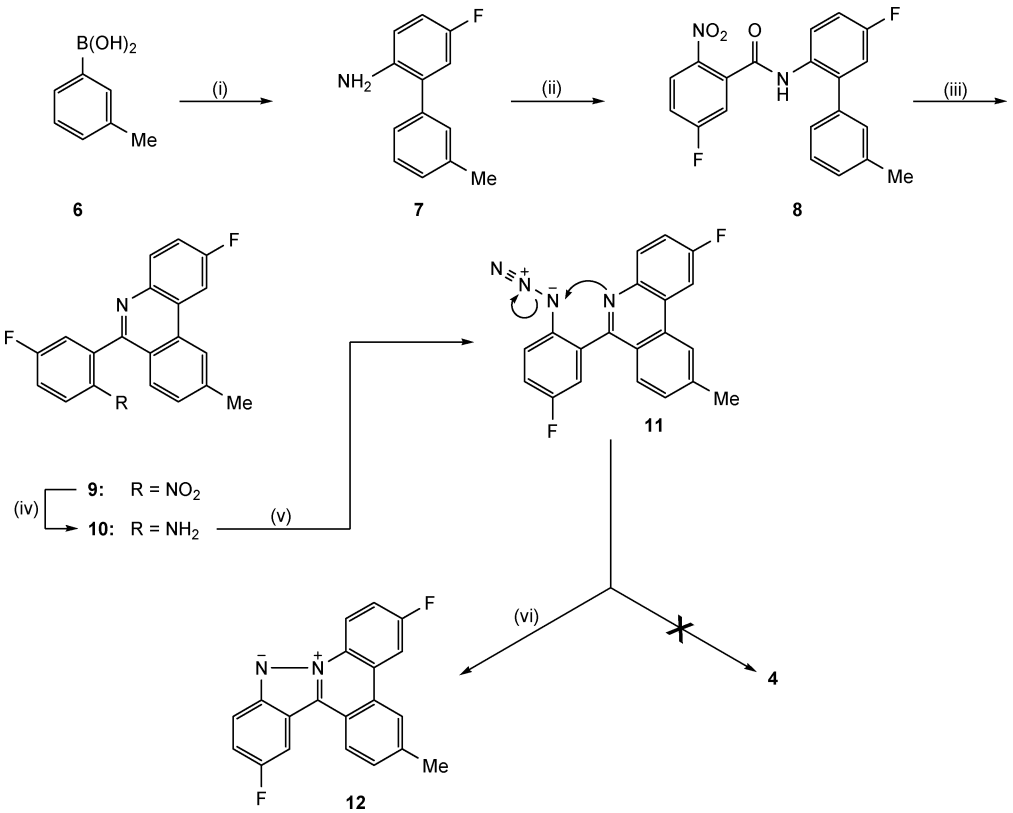
The starting point to prepare a substituted 1-arylacridinone suitable for cyclisation to the methylated pentacycle **5** was 2,6-dibromo-4-methylbenzonitrile **13**, available by a Sandmeyer reaction on diazotised 2,6-dibromo-4-methylaniline.[18] Coupling between **13** and 4-fluoroaniline was achieved under Buchwald conditions[19] mediated by $Pd(OAc)_2$–Cs_2CO_3–BINAP in refluxing toluene to give a mixture of the diarylamine **14** (65%), together with the disubstituted product **15** (20%) and unreacted starting material (15%). Cyclisation of **14** to the 1-bromoacridinone **16** was accomplished in 80% H_2SO_4 at 100 °C and methylation to the 10-methylacridinone **17** involved the use of NaH and dimethyl sulfate (DMS). On a medium scale it was possible to prepare 25 g of the methylacridinone **17** from 60 g of benzonitrile **13**.

Protected 2-aminobenzeneboronic acids required for Suzuki coupling to the 1-bromo-10-methylacridinone **17** have been prepared from *N*-pivaloylanilines *via* directed *ortho*-lithiation with *n*-BuLi and trimethyl borate followed by an acid quench.[5,20] Yields of the required amino-protected fluorobenzeneboronic acid **18** from this route were very low (<5%). But encouragingly, Suzuki coupling between **17** and **18** with $Pd(PPh_3)_4$ catalyst and $NaHCO_3$–aqueous DME afforded the coupled pivaloylamine **19** which could be deprotected and cyclised in EtOH–5 M HCl to pentacyclic acridine **5** in 65% yield. An alternative coupling partner to **17**, the boronic ester **20**, could be prepared more efficiently from 3-fluoro-6-nitrophenol[21,22] and afforded the 1-(3-fluoro-6-nitroaryl)acridinone **21** (47%) with dichloro[1,1-bis(diphenylphosphino)ferrocene]palladium(II) and Na_2CO_3 in dioxane. Reductive cyclisation of **21** to the pentacycle **5** (65%) was effected with tin(II) chloride in EtOH, followed by 5 M HCl. Finally, methylation of **5** to the required 8,13-dimethylquinoacridinium salt **3** was accomplished with DMS in nitromethane (48%). Thus the required compound **3** is available in six steps from the benzonitrile **13** (Scheme 2) but in <10% overall yield.

Synthesis of pentacyclic salt 3 from 6-fluoro-2-methylquinoline (Route C)

Without the serendipitous discovery by Ozczapowicz and co-workers of the mechanistically-intriguing first synthesis of a 8,13-diethylquino[4,3,2-*kl*]acridinium salt from the conversion of an *N*-ethylquinaldinium salt with base[15] it is unlikely that this approach would have been contemplated. We have earlier (Scheme 3) proposed a tentative reaction pathway for the Ozczapowicz synthesis initiated by addition of the carbanion **24**, generated from **23** and base, to the iminium bond of the cation of **23** to form adduct **25**.[8] This could undergo an electrocyclic ring-opening to **26**, thereby generating a conjugated system aligned to undergo cyclisation to the tetrahydro-quinoacridinium cation **27**. Oxidation, presumably *in situ*, would give the salt **3**.

We have now revisited this synthesis as we have been unable to replicate the high yields reported by the Polish workers.[15,23] Our efforts to secure a short route to **3** started with 6-fluoro-2-methylquinoline **22** which was methylated with DMS to afford the 1,2-dimethylquinolinium methosulfate **23**. The presence of the highly-fluorescent maroon salt **3** in reaction mixtures can be



Scheme 1 Attempted synthesis of quino[4,3,2-*kl*]acridine **4** from a 2-arylphenanthridine precursor (Route A). *Reagents and conditions*: (i) 2-bromo-4-fluoroaniline, tetrakis triphenylphosphine palladium (5 mol%), Na_2CO_3, DME–EtOH–H_2O under nitrogen, reflux; (ii) 2-nitro-5-fluorobenzoic acid, DCC in $CHCl_3$, 25 °C; (iii) $POCl_3$, 90 °C; (iv) tin(II) chloride dihydrate, EtOH, reflux, then NaOH; (v) $NaNO_2$, 1 M H_2SO_4, 5 °C, then NaN_3; (vi) 214 °C in 1,2,4-trichlorobenzene.

readily monitored by TLC and the product isolated preferably by column chromatography on silica gel employing butan-1-ol–AcOH–H_2O (0.65 : 0.15 : 0.25) as eluting solvents, or by recrystallisation. Salt **3** has unusual physical properties, being soluble in both water and (to some extent) organic solvents; it has a disconcerting habit of disappearing into plastic cuvettes and tubing. We have attributed this amphiphilic nature to the fact that the delocalised positive charge of the iminopyridinium core is shielded by a hydrophobic aryl cladding.[14]

Standardising on the use of ethanol as solvent, it is clear that the nature of the base is critical to the efficiency of the Ozczapowicz conversion **23** → **3**. Employing the primary aliphatic amine (*n*-butylamine), secondary aliphatic amines (pyrrolidine and morpholine), tertiary amines (triethylamine and Hünigs base) and heterocyclic bases pyridine and 4-methylpyridine as substitutes for piperidine, gave lower yields of the pentacyclic salt; whereas the use of DBU or the inorganic bases $NaHCO_3$, K_2CO_3, Cs_2CO_3 and even basic alumina gave only traces (TLC). The reaction is apparently sensitive to concentration effects and the stoichiometry of the base: the optimum conditions, determined after much experimentation, involved reacting **23** (5 g) and piperidine (3 mol. eq.) in boiling ethanol (150 mL) for 2 days which gave 0.6 g (15%) of product **3** when crystallised from acetone—and this on a good day! Increasing reaction time to 7 days did not increase the yield. Exploring a range of refluxing protic solvents gave yields of **3** in the order: ethanol > methanol > propan-2-ol > butan-2-ol ≫ trifluoroethanol; no product was detected (TLC) employing dioxane as solvent.

The proposed mechanism (Scheme 3) requires a final oxidation step to remove, formally, 4 atoms of hydrogen from cation **27**. If the reaction is conducted in degassed or air-gassed ethanol, or under argon, formation of compound **3** is still observed. Evidently molecular oxygen is not the oxidant in this case, implying that reaction intermediates must be consumed as oxidants, thus accounting for the poor yield. Again, standardising on ethanol as solvent, incorporation of external oxidants MnO_2, SeO_2, *p*-chloroanil, benzoquinone or iodine gave no improvement in yields. In order to avoid potential premature oxidation of reaction intermediates by oxidants, aliquots of DDQ (0.1–0.2 mol. eq.) were titrated into the reaction mixture every 45, 90 or 120 min. Yields of the required salt **3** remained stubbornly in the 5–15% range. Mindful of the role of nitrobenzene as solvent and oxidant in the Scraup reaction, its use as external oxidant was explored. Application of nitrobenzene as solvent at 120 °C with either piperidine, pyridine, K_2CO_3 or Cs_2CO_3 as bases either gave no reaction (pyridine), or led to the formation of black mixtures where only traces of **3** could be detected (TLC). Finally, on the point of conceding defeat we discovered that reaction of **23** with triethylamine as a base in nitrobenzene at 120 °C for 15 h gave the required salt in 33% yield making this adaptation a feasible process for large-scale synthesis. Microwave irradiation (100 W) at 180 °C for 13 min gave a reduced yield.

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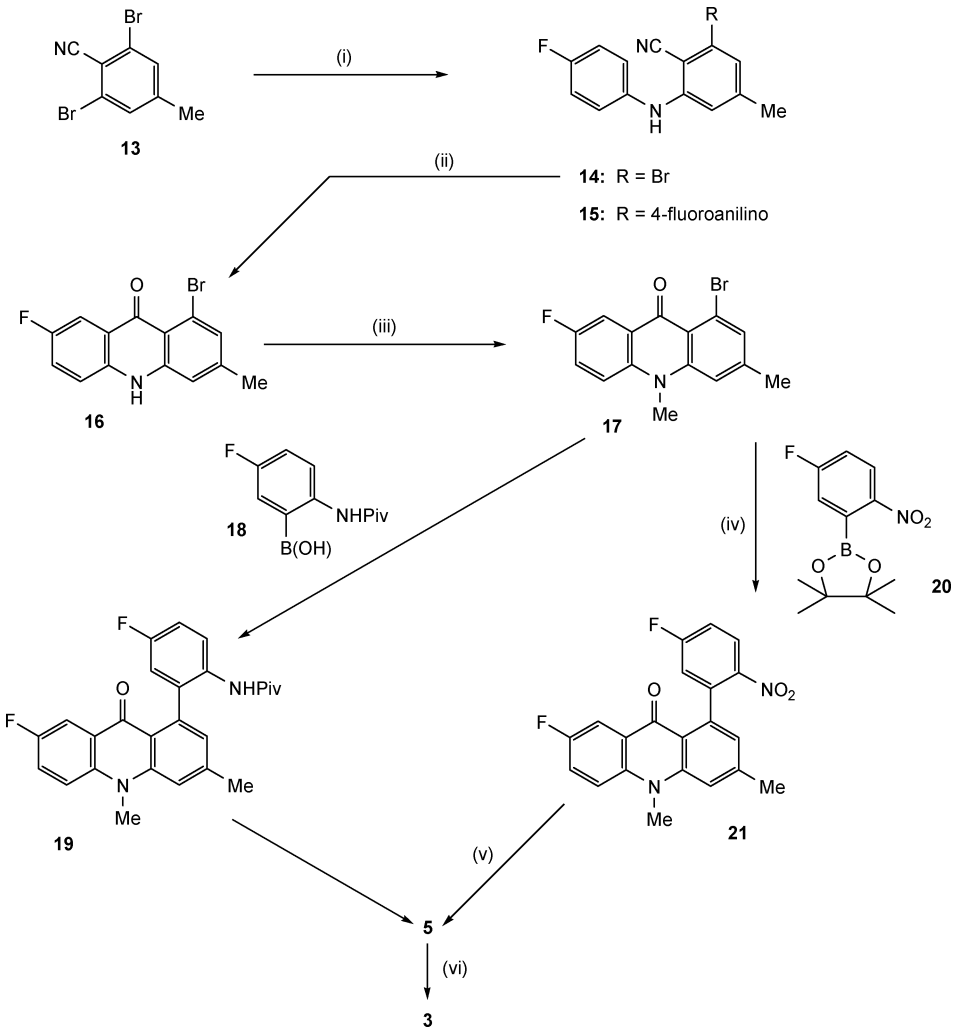


Scheme 2 Synthesis of quino[4,3,2-*kl*]acridine **3** from a 1-arylacridin-9(10*H*)-one precursor (Route B). *Reagents and conditions*: (i) 4-fluoroaniline, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 100 °C; (ii) 80% H_2SO_4, 100 °C; (iii) NaH in DMF, Me_2SO_4; (iv) **17** and **20** in dioxan, $PdCl_2$(dppf) (10 mol%), Na_2CO_3, 80 °C; (v) tin(II) chloride dihydrate, EtOH, reflux; (vi) Me_2SO_4 in $MeNO_2$, reflux.

Experimental

General details

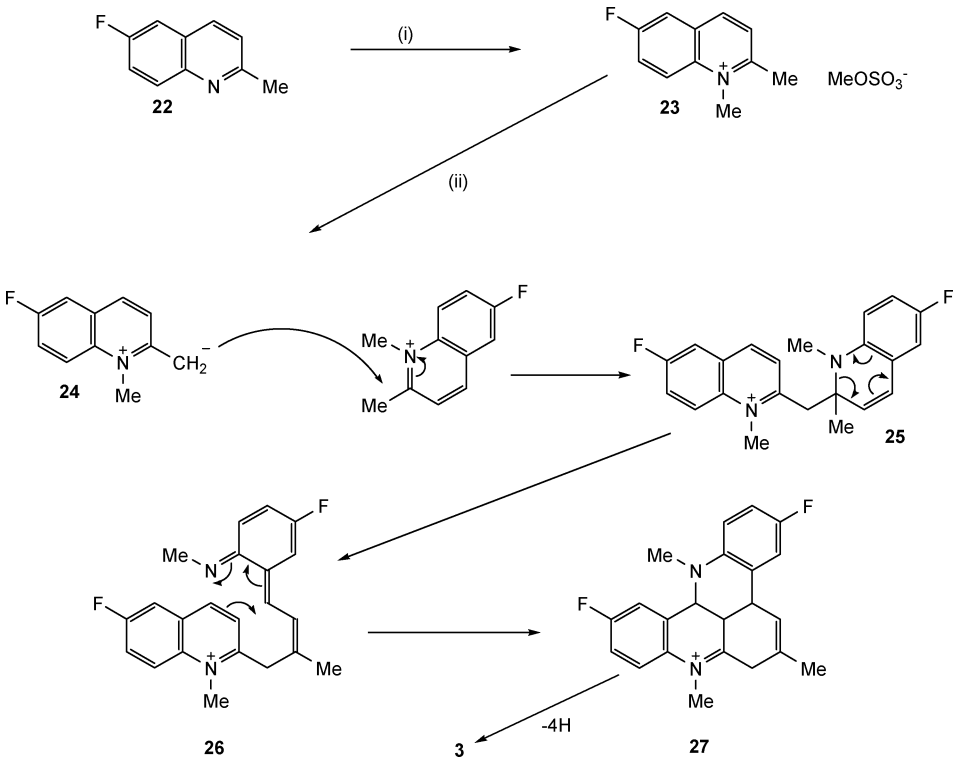
Melting points were measured on a Galenkamp apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer as KBr discs and UV spectra on a Pharmacia Biotech Ultraspec 2000 UV/Visible spectrophotometer. Mass spectra were recorded on a Micromass LCT spectrometer. NMR spectra were recorded on a Bruker Avance 400 instrument at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C) in [$^2H_6$]DMSO or $CDCl_3$; coupling constants are in Hz. Merck silica gel 60 (40–60 μm) was used for column chromatography.

2-Amino-5-fluoro-3′-methylbiphenyl 7

2-Bromo-4-fluoroaniline (14 g, 0.073 mol) and *m*-tolylboronic acid (10 g, 0.073 mol) were dissolved in a solution of 1,2-dimethoxyethane (400 mL), ethanol (40 mL) and water (20 mL). Nitrogen gas was bubbled through the resulting solution for 10 min, after which, sodium carbonate (17 g, 0.16 mol) and tetrakis triphenylphosphine palladium (4.2 g, 5 mol%) were added. The resulting mixture was heated under reflux for 15 h, cooled, and solvent removed under vacuum. The residue was dissolved in chloroform (500 mL), washed with water (250 mL), dried (sodium sulfate) and evaporated to leave a yellow oil. The product was purified by vacuum distillation to leave **7** as a colourless oil (14.6 g, 98%), bp 130–132 °C (1.4 mbar) (Found: C, 77.3; H, 6.0; N, 6.9%. $C_{13}H_2FN$ requires C, 77.6; H, 6.0; N, 7.0%); ν_{max}/cm^{-1} 3356, 3370 (NH), 1606, 1500, 1272, 1201, 1163, 812; ^{1}H NMR δ_H ($CDCl_3$) 7.37 (1 H, t, *J* 7.6, ArH), 7.27–7.19 (3 H, m, ArH), 6.92–6.86 (2 H, m, ArH), 6.71 (1 H, dd, *J* 4.8, 9.2, ArH), 3.66 (2 H, br s, NH_2), 2.43 (3 H, s, CH_3); *m*/*z* (ES) 202 (M + 1).

5-Fluoro-*N*-(5-fluoro-3′-methylbiphenyl-2-yl)-2-nitrobenzamide 8

Dicyclohexylcarbodiimide (12.25 g, 0.054 mol) was added to a solution of biphenyl **7** (10.86 g, 0.054 mol) and 2-nitro-5-fluorobenzoic acid (10 g, 0.054 mol) in chloroform (200 mL) at room temperature. After stirring for 15 h, the precipitate was filtered from solution and the filtrate evaporated under vacuum. The residue was recrystallised from ethyl acetate–heptane to give **8**



Scheme 3 Synthesis of quino[4,3,2-*kl*]acridine **3** from 6-fluoro-2-methylquinoline (Route C). *Reagents and conditions*: (i) Me_2SO_4 at 100 °C; (ii) NEt_3 in nitrobenzene, 120 °C, 24 h.

as a white crystalline solid (18.4 g, 93%), mp 151–153 °C (Found: C, 64.8; H, 3.8; N, 7.5%. $C_{20}H_{14}F_2N_2O_3$ requires C, 65.2; H, 3.8; N, 7.6%); ν_{max}/cm^{-1} 3310 (NH), 1654 (CO), 1587, 1530, 1354 (NO_2), 1209, 812; ^{1}H NMR δ_H ([$^2H_6$]DMSO) 10.15 (1 H, br s, NH), 8.25 (1 H, dd, *J* 4.8, 9.2, ArH), 7.63 (1 H, dd, *J* 5.6, 8.8, ArH), 7.57 (1 H, ddd, *J* 2.8, 6.4, 9.2, ArH), 7.38–7.20 (7 H, m, ArH), 2.35 (3 H, s, CH_3); *m*/*z* (ES-HRMS) 369.1040 (M + 1). Calcd for $C_{20}H_{15}F_2N_2O_3$ (M + 1) 369.1050.

2-Fluoro-6-(5-fluoro-2-nitrophenyl)-9-methylphenanthridine 9

2-Nitrobenzamide **8** (18 g, 0.048 mol) was dissolved in phosphorous oxychloride (100 mL) and heated at 90 °C for 2 h. After cooling, the reaction mixture was quenched with ice (500 g), the precipitate filtered from solution and washed with water (200 mL). The white solid was recrystallised from ethanol to give **9** as a white powder (16.1 g, 94%), mp 198–201 °C (Found: C, 68.6; H, 3.4; N, 7.9%. $C_{20}H_{12}F_2N_2O_2$ requires C, 68.6; H, 3.4; N, 8.0%); ν_{max}/cm^{-1} 1619 (C=N), 1583, 1352 (NO_2), 1210, 815; ^{1}H NMR δ_H ([$^2H_6$]DMSO) 8.77 (1 H, s, ArH), 8.67 (1 H, dd, *J* 5.6, 8.0, ArH), 8.08 (1 H, dd, *J* 6.0, 9.2, ArH), 7.75–7.57 (5 H, m, ArH), 2.62 (3 H, s, CH_3); *m*/*z* (ES-HRMS) 351.0822 (M + 1). Calcd for $C_{20}H_{13}F_2N_2O_2$ (M + 1) 351.0945.

6-(2-Amino-5-fluorophenyl)-2-fluoro-9-methylphenanthridine 10

Tin(II) chloride dihydrate (22.5 g, 0.1 mol) was added to a solution of phenanthridine **9** (14 g, 0.04 mol) in ethanol (250 mL). The resulting mixture was heated under reflux for 2 h, cooled and the solvent removed under vacuum. The residue was dissolved in ethyl acetate (400 mL), washed with 5 M sodium hydroxide solution (2 × 200 mL), followed by water (100 mL), and dried (sodium sulfate). The concentrated solution gave a yellow solid which was recrystallised from ethanol to give **10** as a white powder (10.5 g, 82%), mp 155–157 °C (Found: C, 74.7; H, 4.3; N, 8.7%. $C_{20}H_{14}F_2N_2$ requires C, 75.0; H, 4.4; N, 8.7%); ν_{max}/cm^{-1} 3423, 3316, 3226 (NH), 1643, 1619, 1570, 1497, 1442, 1234, 829; ^{1}H NMR δ_H ([$^2H_6$]DMSO) 8.72 (1 H, s, ArH), 8.63 (1 H, dd, *J* 2.8, 10.8, ArH), 8.12 (1 H, dd, *J* 5.6, 8.8, ArH), 7.75 (1 H, d, *J* 8.4, ArH), 7.65 (1 H, ddd, *J* 2.6, 8.8, 11.6, ArH), 7.58 (1 H, dd, *J* 1.2, 8.8, ArH), 7.10 (1 H, ddd, *J* 3.2, 8.8, 12.0, ArH), 7.00 (1 H, dd, *J* 3.2, 9.6, ArH), 6.87 (1 H, dd, *J* 4.8, 8.8, ArH), 4.86 (2 H, br s, NH_2), 2.61 (3 H, s, CH_3); *m*/*z* (ES) 321 (M + 1).

6-(2-Azido-5-fluorophenyl)-2-fluoro-9-methylphenanthridine 11

The aminophenylphenanthridine **10** (5 g, 0.016 mol) was stirred in 1 M sulfuric acid (200 mL) at room temperature for 3 h, then cooled to 5 °C in an ice bath. A solution of sodium nitrite (1.3 g, 0.019 mol) in water (10 mL) was added dropwise, and stirring continued for a further 60 min. A solution of sodium azide (1.22 g, 0.019 mol) in water (10 mL) was then added over a 15 min period. After stirring at 5 °C for a further 1 h, the resulting precipitate was filtered from solution and washed with water (100 mL) to leave **11** as an off-white solid (4.95 g, 92%), mp 189 °C (decomp.) (Found: C, 69.1; H, 3.5; N, 15.9%. $C_{20}H_{12}F_2N_4$ requires C, 69.4; H, 3.5; N, 16.2%); ν_{max}/cm^{-1} 2126 (N_3), 1620 (C = N), 1489, 1204, 815; ^{1}H NMR δ_H ([$^2H_6$]DMSO) 8.76 (1 H, s, ArH), 8.67 (1 H, dd, *J* 2.8, 10.4, ArH), 8.14 (1 H, dd, *J* 6.0, 9.2, ArH), 7.69 (1 H, ddd, *J* 2.8, 8.4, 11.2, ArH), 7.62–7.52 (4 H, m, ArH), 7.42 (1 H, dd, *J* 2.4, 8.8, ArH), 2.63 (3 H, s, CH_3); *m*/*z* (ES-HRMS) 347.1118 (M + 1). Calcd for $C_{20}H_{13}F_2N_4$ (M + 1) 347.1108.

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6,13-Difluoro-3-methylindazolo[2,3-*f*]phenanthridine 12

6-(2-Azido-5-fluorophenyl)-2-fluoro-9-methylphenanthridine **11** (4.0 g, 0.011 mol) was suspended in 1,2,4-trichlorobenzene (100 mL) and heated at 214 °C for 3 h. The solvent was removed by vacuum distillation and the residue triturated with diethyl ether to precipitate the product, which was filtered from solution and recrystallised from chloroform to give **12** as a pale yellow solid (3.2 g, 87%), mp 247–249 °C (Found: C, 75.1; H, 3.8; N, 8.7%. $C_{20}H_{12}F_2N_2$ requires C, 75.2; H, 4.1; N, 8.8%); ^{1}H NMR δ_H ($CDCl_3$) 8.89 (1 H, dd, *J* 5.6, 9.2, ArH), 8.33 (1 H, d, *J* 8.0, ArH), 8.15 (1 H, s, ArH), 8.11 (1 H, dd, *J* 2.4, 10.0, ArH), 7.94–7.91 (2 H, m, ArH), 7.56 (1 H, dd, *J* 1.2, 8.0, ArH), 7.47 (1 H, dt, *J* 2.8, 7.6, ArH), 7.34 (1 H, dt, *J* 2.8, 8.8, ArH), 2.62 (3 H, s, CH_3); ^{13}C NMR δ_C ($CDCl_3$) 161.2 (CF, *J* 244), 158.7 (CF, *J* 239), 146.4 (C), 137.9 (C), 130.7 (CH), 130.2 (CH), 129.1 (C, *J* 8), 125.5 (C, *J* 3), 124.5 (C, *J* 8), 123.3 (CH), 123.2 (CH), 120.2 (CH, *J* 9), 119.3 (CH, *J* 9), 118.5 (CH, *J* 28), 117.2 (CH, *J* 24), 116.4 (C, *J* 11), 109.1 (CH, *J* 24), 104.5 (CH, *J* 25), 30.0 (C), 22.0 (CH_3); *m/z* (ES-HRMS) 319.1006 (M + 1). Calcd for $C_{20}H_{13}F_2N_2$ (M + 1) 319.1047.

2-Bromo-6-(4-fluoroanilino)-4-methylbenzonitrile 14

2,6-Dibromo-4-methylbenzonitrile **13**[18] (60 g, 0.22 mol), 4-fluoroaniline (27.2 g, 0.24 mol), cesium carbonate (100 g, 0.28 mol), BINAP (2.7 g, 4.3 mmol) and palladium acetate (0.47 g, 2.1 mmol) were dissolved in toluene (800 mL) under nitrogen and heated at 100 °C for 12 h. The solvent was then removed under vacuum and the residue purified by column chromatography, initially eluting with 20% chloroform–hexane to remove unreacted **13**, increasing to 50% chloroform–hexane to elute **14**, and finally neat chloroform to elute **15**. Benzonitrile **14** was obtained as white needles (43 g, 65%), mp 175–176 °C (Found: C, 54.9; H, 3.3; N, 9.1%. $C_{14}H_{10}BrFN_2$ requires C, 55.1; H, 3.3; N, 9.2%); ν_{max}/cm^{-1} 3315 (NH), 2223 (C≡N), 1608, 1568, 1506, 1210, 820; ^{1}H NMR δ_H ($CDCl_3$) 7.21–7.10 (4 H, m, ArH), 6.89 (1 H, d, *J* 0.4, H-3), 6.67 (1 H, d, *J* 0.4, H-5), 6.28 (1 H, br s, NH), 2.25 (3 H, s, CH_3); *m/z* (APCI) 305.0/307.0 (M + 1). The di(4-fluoroanilino)benzonitrile **15** was obtained as a white solid (14.6 g, 20%), mp 170–171 °C (Found: C, 71.5; H, 4.5; N, 12.5%. $C_{20}H_{15}F_2N_3$ requires C, 71.6; H, 4.5; N, 12.5%); ν_{max}/cm^{-1} 3321 (NH), 2201 (C≡N), 1607, 1578, 1511, 1463, 1219, 819; ^{1}H NMR δ_H ($CDCl_3$) 7.17 (4 H, dd, *J* 2.4, 6.8, ArH), 7.06 (4 H, dd, *J* 2.4, 6.8, ArH), 6.18 (2 H, d, *J* 0.2, ArH), 6.08 (1 H, br s, NH), 2.11 (3 H, s, CH_3); *m/z* (ES-HRMS) 336.1298 (M + 1). Calcd for $C_{20}H_{16}F_2N_3$ (M + 1) 336.1312.

1-Bromo-7-fluoro-3-methylacridin-9(10*H*)-one 16

Benzonitrile **14** (40 g, 0.13 mol) was suspended in 80% sulfuric acid (200 mL) and heated at 100 °C for 15 h. After cooling, the reaction mixture was quenched on ice (800 g). The resulting precipitate was collected, washed with water (200 mL) and allowed to dry. The yellow solid **16** (34 g, 85%) was used in the next step without further purification. Physical characteristics of **16**: ν_{max}/cm^{-1} 3394 (NH), 1629 (CO), 1517, 1205, 838; ^{1}H NMR δ_H ([$^2H_6$]DMSO) 11.81 (1 H, br s, NH), 7.81 (1 H, dd, *J* 4.0, 12.0, ArH), 7.64 (1 H, ddd, *J* 4.0, 8.0, 12.0, ArH), 7.55 (1 H, dd, *J* 4.0, 8.0, ArH), 7.33 (1 H, d, *J*, ArH), 7.28 (1 H, d, *J*, ArH), 2.41 (3 H, s, CH_3); *m/z* (ES) 305.0/307.0 (M + 1).

1-Bromo-7-fluoro-3,10-dimethylacridin-9(10*H*)-one 17

1-Bromo-7-fluoro-3-methylacridin-9(10*H*)-one **16** (30 g, 0.1 mol) was dissolved in DMF (300 mL) and added dropwise over 30 min to a suspension of sodium hydride (2.6 g, 0.11 mol) in DMF (150 mL). After stirring at room temperature for 60 min, dimethyl sulfate (18.5 g, 0.147 mol) was added and stirring continued for a further 1 h. The reaction mixture was poured carefully into water (1 L) and the precipitate collected and dried. The resulting yellow solid was purified by column chromatography (chloroform) to give **17** as a bright yellow powder (25 g, 80%), mp 262–265 °C (Found: C, 56.3; H, 3.4; N, 4.4%. $C_{15}H_{11}BrFNO$ requires C, 56.3; H, 3.5; N, 4.4%); ν_{max}/cm^{-1} 1637, 1604, 1501, 1271, 815; ^{1}H NMR δ_H [$^2H_6$]DMSO 7.83–7.88 (2 H, m, H-5, H-8), 7.69 (1 H, dt, *J* 9.6, 3.2, H-6), 7.62 (1 H, d, *J* 0.8, H-2), 7.40 (1 H, d, *J* 0.8, H-4), 3.87 (3 H, s, NCH_3), 2.45 (3 H, s, CH_3); *m/z* (ES) 319.7/321.7 (M + 1).

2-(5-Fluoro-2-nitrophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane 20

Potassium acetate (6 g, 0.06 mol), bis(pinacolato)diborane (5.8 g, 0.022 mol) and dichloro[1,1′-bis(diphenylphosphino)-ferrocene]palladium(II) (0.9 g, 3 mol%) were added to a solution of 5-fluoro-2-nitrophenyltrifluoromethane sulfonate[21] (5.9 g, 0.02 mol) in dioxane (60 mL) under a nitrogen atmosphere. The resulting solution was stirred at 80 °C for 20 h. After cooling, the solvent was removed under reduced pressure and the residue purified by column chromatography (dichloromethane) to give **20** as a pale cream solid (3.8 g, 70%), mp 79–80 °C (from MeOH) (Found: C, 53.9; H, 5.6; N, 5.1%. $C_{12}H_{15}BFNO_4$ requires C, 53.9; H, 5.6; N, 5.2%); ν_{max}/cm^{-1} 1578, 1526, 1411, 1346; ^{1}H NMR δ_H ($CDCl_3$) 8.21 (1 H, dd, *J* 4.8, 10.0, H-3), 7.17–7.28 (2 H, m, H-4, H-6), 1.43 (12 H, s, 4 × CH_3).

7-Fluoro-1-(5-fluoro-2-nitrophenyl)-3,10-dimethylacridin-9(10*H*)-one 21

Dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium(II) (90 mg, 10 mol%) was added to a solution of 1-bromo-7-fluoro-3,10-dimethylacridin-9(10*H*)-one **17** (0.36 g, 1.1 mmol) and dioxaborolane **20** (0.45 g, 1.7 mmol) in dioxane (10 mL) under nitrogen. 2 N sodium carbonate (2 mL) was added and the resulting solution heated at 80 °C for 24 h. The solvent was removed under vacuum and the residue purified by column chromatography (chloroform followed by dichloromethane) to give **21** as a yellow solid (0.2 g, 47%), mp 238–241 °C (Found: C, 66.0; H, 3.7; N, 7.1%. $C_{21}H_{14}F_2N_2O_3$ requires C, 66.3; H, 3.7; N, 7.4%); ν_{max}/cm^{-1} 1607, 1582, 1507, 1358, 1262, 1209; ^{1}H NMR δ_H ($CDCl_3$) 8.29 (1 H, dd, *J* 4.8, 8.8, ArH), 7.99 (1 H, dd, *J* 3.2, 9.2, ArH), 7.52–7.55 (3 H, m, ArH), 7.21 (1 H, dt, *J* 2.8, 7.2, 9.2, ArH), 7.00 (1 H, dd, *J* 2.8, 8.4, ArH), 6.84 (1 H, d, *J* 1.2, ArH), 3.98 (3 H, s, NCH_3), 2.58 (3 H, s, CH_3); *m/z* (ES) 380.9 (M + 1).

3,11-Difluoro-6,8-dimethyl-8*H*-quino[4,3,2-*kl*]acridine 5

Tin(II) chloride dihydrate (1.6 g, 7.1 mmol) was added to a solution of acridinone **21** (0.92 g, 2.3 mmol) in ethanol (10 mL), and the resulting solution heated under reflux for 2 h. 5 M HCl (10 mL) was then added, and refluxing continued for a further 15 h. After cooling, the ethanol was removed under vacuum, and

the aqueous phase basified with 5 M sodium hydroxide (to pH 12). The resulting precipitate was collected from solution and purified by column chromatography (1% methanol–chloroform) to leave **5** as an orange solid (0.59 g, 75%), mp 256–258 °C (Found: C, 76.2; H, 4.2; N, 8.1%. $C_{21}H_{14}F_2N_2$ requires C, 75.9; H, 4.3; N, 8.4%); ν_{max}/cm^{-1} 1619, 1511, 1473, 1236, 1184, 796; ^{1}H NMR δ_H [$^2H_6$]DMSO 8.35 (1 H, dd, *J* 3.2, 10.0, ArH), 8.30 (1 H, dd, *J* 2.8, 10.8, ArH), 7.93 (1 H, s, ArH), 7.90 (1 H, dd, *J* 6.0, 9.2, ArH), 7.58 (1 H, dd, *J* 4.4, 9.2, ArH), 7.54–7.44 (2 H, m, ArH), 7.21 (1 H, s, ArH), 3.66 (3 H, s, NCH_3), 2.67 (3 H, s, CH_3); *m*/*z* (ES-HRMS) 333.1189 (M + 1). Calcd for $C_{21}H_{14}F_2N_2$ (M + 1) 333.1203.

3,11-Difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]acridinium methosulfate 3 by methylation of 5

Dimethyl sulfate (0.28 g, 2.26 mmol) was added to a solution of 3,11-difluoro-6,8-dimethyl-8*H*-quino[4,3,2-*kl*]acridine **5** (0.15 g, 0.45 mmol) in nitromethane (5 mL) and heated under reflux for 15 h. After cooling, the solvent was removed under vacuum and the residue triturated with ice cold acetone. The precipitated solid was filtered from solution and washed with a small amount of ice cold acetone to leave **3** as a dark red solid (0.1 g, 48%), identical (TLC, ^{1}H NMR and MS spectrum) to an authentic sample.[8]

One-pot synthesis of 3,11-difluoro-6,8,13-trimethyl-8*H*-quino[4,3,2-*kl*]-acridinium methosulfate 3 (Route C) from 1,2-dimethyl-6-fluoroquinolinium methosulfate 23

Triethylamine (10 g, 0.1 mol) was added to a solution of **23** (10 g, 0.035 mol) in nitrobenzene (100 mL) and heated at 120 °C for 24 h. After cooling, the solvent was removed by vacuum distillation and the residue purified by column chromatography (1-butanol–acetic acid–water: 0.6 : 0.15 : 0.25) to leave a dark red solid (2.63 g, 33%), identical (TLC, ^{1}H NMR and MS spectrum) to an authentic sample.[8]

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