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Antitumour and antimalarial activity of artemisinin–acridine hybrids

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ABSTRACT

Artemisinin–acridine hybrids were prepared and evaluated for their in vitro activity against tumour cell lines and a chloroquine sensitive strain of *Plasmodium falciparum*. They showed a 2–4-fold increase in activity against HL60, MDA-MB-231 and MCF-7 cells in comparison with dihydroartemisinin (DHA) and moderate antimalarial activity. Strong evidence that the compounds induce apoptosis in HL60 cells was obtained by flow cytometry, which indicated accumulation of cells in the G1 phase of the cell cycle.

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Artemisinin **1** and its semisynthetic and synthetic analogues are effective antimalarial agents and are used to treat chloroquine resistant strains of the disease. Since the early 1990s they have also been shown to have antiproliferative and antitumour activity.^{1–5} Their activity is greatest against rapidly proliferating neoplastic cells with high iron content, since their mode of action almost certainly involves iron(II) catalysed reductive cleavage of the peroxide bond leading to the formation of C-centred radical or cationic intermediates able to alkylate biomolecules and induce cell death.^{6–10} We and others have postulated that a possible cellular target of these alkylating intermediates is DNA^{10,11} and hoped to enhance their antitumour properties by preparing DNA-targeted 1,2,4-trioxane–acridine hybrids. Acridines are known to intercalate with DNA and have been employed as antibacterial, antiparasitic and antitumour agents,^{12,13} moreover their fluorescent properties allow the use of confocal microscopy to examine the accumulation and cellular location of these drug hybrids.¹⁴

Hybrid drugs are formed by covalently linking two distinct chemical moieties with differing biological modes of action with the aim of creating bitherapies that have improved biological activ-

ity and are less susceptible to the development of drug resistance.^{15–17} We have synthesised a short series of artemisinin–acridine hybrids in which a 1,2,4-trioxane derived from artemisinin has been covalently linked to the 9-diaminoalkyl-6-chloro-2-methoxyacridines **2–5**, and evaluated these hybrids for their in vitro antitumour and antimalarial activity (Fig. 1).

The hybrids were designed to incorporate a metabolically stable C-10 carba linkage at the 1,2,4-trioxane moiety, therefore the carboxylic acid **6** was prepared from C-10 allyldeoxoartemisinin.¹⁸ Ozonolysis of the terminal double bond in methanol and reduction of the intermediate ozonide with triphenylphosphine afforded the aldehyde in 76 % yield, followed by oxidation with sodium chlorite to give the carboxylic acid in quantitative yield. Treatment of the

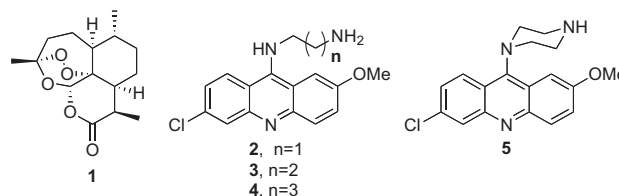


Figure 1.

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acid with oxalyl chloride followed by addition of the appropriate 9-(diaminoalkyl)acridine afforded the hybrids **7–10** in moderate to good yields (55–74 %), Scheme 1.¹⁹ The 9-(diaminoalkyl)acridines **2–5** were prepared from 6,9-dichloro-3-methoxyacridine and the corresponding diamines in the presence of phenol.

The hybrids were evaluated for their in vitro activity against four tumour cell lines (HL60, Colon HT29-AK and Breast MDA-MB-231 and MCF-7)²⁰ and against a chloroquine sensitive strain of *P. falciparum* (3D7)²¹ relative to DHA as the control (Tables 1 and 2).

The hybrids were cytotoxic in the cell lines evaluated, with the exception of **10**, towards HT29-AK (Table 1). The rank order of sensitivity of the cells to the hybrids were as follows: HL60 > MCF-7 > MDA-MB-231 > HT29-AK. The observed cytotoxicity of the hybrids and DHA against HL60 cells was anticipated since this cell line is characterised by its rapid proliferation and high iron content. In HL60, MDA-MB-231 and MCF-7 cells, the hybrids displayed activity 2–4-fold greater than DHA. In HT29-AK cells, only hybrid **8** was more active than DHA; the remaining hybrids displayed only moderate activity with the acridine apparently inhibiting the activity of the 1,2,4-trioxane. In HL60 cell lines the side-chain acridine **2** was also examined and shown to have activity of 12.5 μM. This indicates that the addition of the endoperoxide to this unit enhances cytotoxicity; in breast cancer cell lines HT29-AK and MDA-MB-231 this is clearly not the case since the hybrids are less potent than **2**. The hybrids were also evaluated for their in vitro antimalarial activity against chloroquine sensitive 3D7 strain of *P. falciparum* but none of them were more active than the positive controls (DHA and artemether). Indeed, only **7** had good activity, while **8** and **9** displayed moderate activity and **10** was more than a 100 times less active than DHA (Table 2).

The ability of hybrid **8** to induce apoptosis was assessed in HL60 cells by flow cytometric measurement of mitochondrial membrane depolarisation,²² DNA degradation²³ and Western blot analysis of caspase-3 activation.²⁴ This compound was shown to induce mitochondrial membrane depolarisation in a concentration-dependent manner, reaching a significant level at 1 μM increasing until a

Table 1

Cytotoxicity results for compounds **7–10** and DHA against HL60, HT29-AK, MDA-MB-231 and MCF-7 tumour cell lines

| Compds | HL60 | HT29-AK | MDA-MB-231 | MCF-7 |
|---|--------------|-----------------|----------------|---------------|
| Cytotoxicity IC ₅₀ , μM ^a | | | | |
| 2 | 12.5 (±0.25) | 6.57 (±1.34) | 8.12 (±1.13) | ND |
| 7 | 1.17 (±0.35) | 193.55 (±22.88) | 48.98 (±7.72) | 13.69 (±1.78) |
| 8 | 3.08 (±0.13) | 9.91 (±1.85) | 11.64 (±0.23) | 11.85 (±0.19) |
| 9 | 1.58 (±0.72) | 247.27 (±44.13) | 43.30 (±1.39) | 11.70 (±0.22) |
| 10 | 0.56 (±0.17) | >750 | 21.14 (±0.86) | 3.51 (±0.42) |
| DHA | 2.41 (±0.71) | 16.12 (±1.10) | 99.76 (±10.96) | 45.23 (±3.54) |

^a Values are means of three experiments; standard deviation is given in parentheses. ND—not determined.

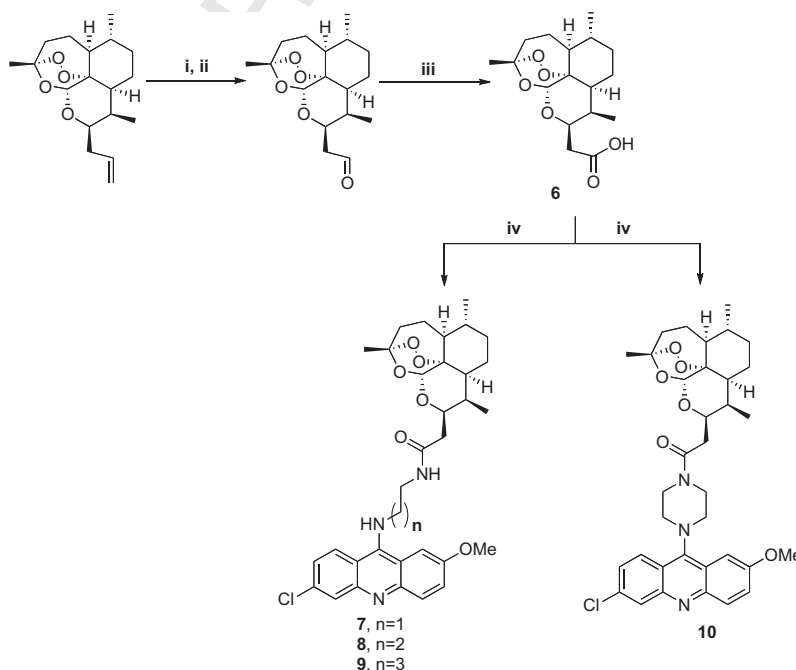
Table 2

Results for antimalarial activity of compounds **7–10** against chloroquine sensitive 3D7 *P. falciparum*

| Compds | IC ₅₀ , nM ^a |
|-------------------|------------------------------------|
| 7 | 5.96 (±1.50) |
| 8 | 22.42 (±1.97) |
| 9 | 20.34 (±3.11) |
| 10 | 289.52 (±10.52) |
| Artemether | 3.53 (±1.91) |
| DHA | 2.30 (±1.50) |

^a Values are means of three experiments; standard deviation is given in parentheses.

maximum effect, 98 ± 3% of cells depolarised, was observed at 10 μM after 24 h. Analysis of cellular DNA content with PI staining showed concentration-dependent formation of a sub-G₀/G₁ population with a maximum effect of 57 ± 2% of cells at 10 μM after 24 h (Fig. 2A). Western blot analysis of caspase-3 activity also showed concentration-dependent appearance of the catalytically active subunit of processed caspase-3 with a concomitant decrease of the inactive 32 kDa precursor (Fig. 2B). Together these results are strongly indicative of the ability of hybrid **8** to induce cell death by apoptosis.



Scheme 1. Reagents and conditions: (i) O₃, MeOH, −78 °C, 1 h; (ii) PPh₃, MeOH, −78 °C to rt; (iii) NaClO₂, 2-methyl-2-butene, NaH₂PO₄, *t*-BuOH/H₂O, rt, 2 h; (iv) (COCl)₂, CH₂Cl₂, 0 °C → rt, 90 min then diaminoacridine (**2–5**), NEt₃, CH₂Cl₂, 0 °C → rt, 16 h.

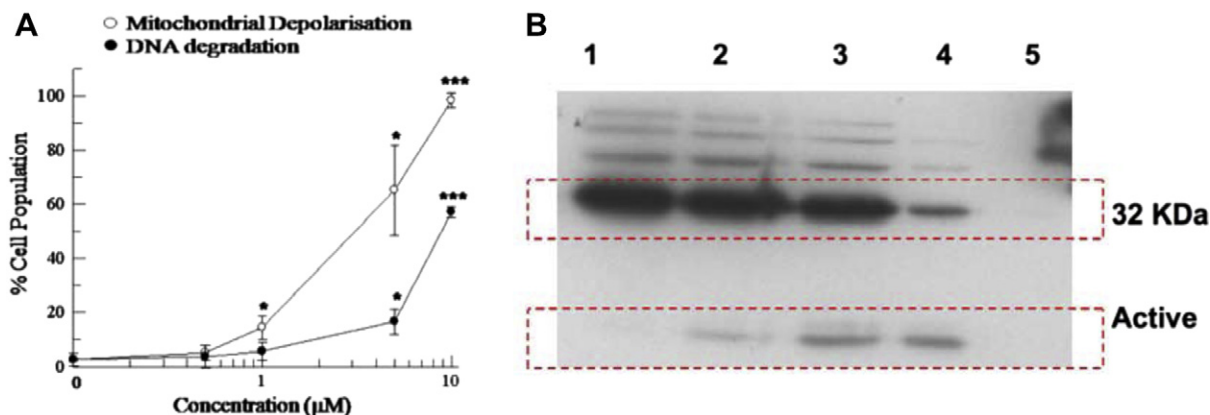


Figure 2. (A) Concentration-dependent induction of mitochondrial depolarisation and DNA degradation by compound **8** in HL-60 cells (24 h). Key- Open circle: percentage of cell population with depolarised mitochondria. Filled circles: percentage of cell population with degraded DNA (sub G_0/G_1 population). Results are the mean \pm SD of three independent sets of experiments. *** $p < 0.001$ and $p < 0.05$ significance of data compared with drug blank as tested by the Mann-Whitney U test for nonparametric data. (B) Concentration-dependent processing of caspase-3 induced by compound **8** in HL60 cells (24 h). Lane 1: control without **8**, Lanes 2-5 increasing concentrations of **8**: Lane 2, 0.5 μM , Lane 3: 1.0 μM , Lane 4: 5 μM and Lane 5: 10 μM .

Confocal microscopy was used to determine the cellular location of both hybrid **7** and 9-(1,2-diaminoethyl)-6-chloro-2-methoxyacridine **2** in treated parasite infected erythrocytes and MRC-50 human lung fibroblast cells (**7** was selected based on the fact that this hybrid was the most potent antimalarial in the series). Both hybrid **7** and 9-aminoacridine **2** were shown to accumulate selectively in infected erythrocytes and on the nuclear membrane of MRC-5 cells (Fig. 3A-C). In malaria parasites acridine **2** was easily removed by washing with a buffer solution (Fig. 3B) whereas the endoperoxide hybrid **7** remained, implying covalent binding of the hybrid (and not the acridine) to intraparasitic cellular biomol-

ecules (Fig. 3 C). Similar experiments were performed in the presence of a ferric iron chelator (desferrioxamine (DFO), and under these conditions the hybrid was removed from the cells on washing with the buffer (data not shown).¹⁴ This result implies that chelatable iron catalysed reductive cleavage of the peroxide bond is required for covalent binding of the hybrid to the cellular targets. (Since DFO is selective for ferric iron it is apparent that intraparasitic reduction of iron to its ferrous form must be achieved to facilitate endoperoxide bond cleavage.)

Finally, the in vitro affinity of hybrid **7** and acridine **2** for calf thymus (ct)-DNA and poly A-poly U was investigated spectrophotometrically.

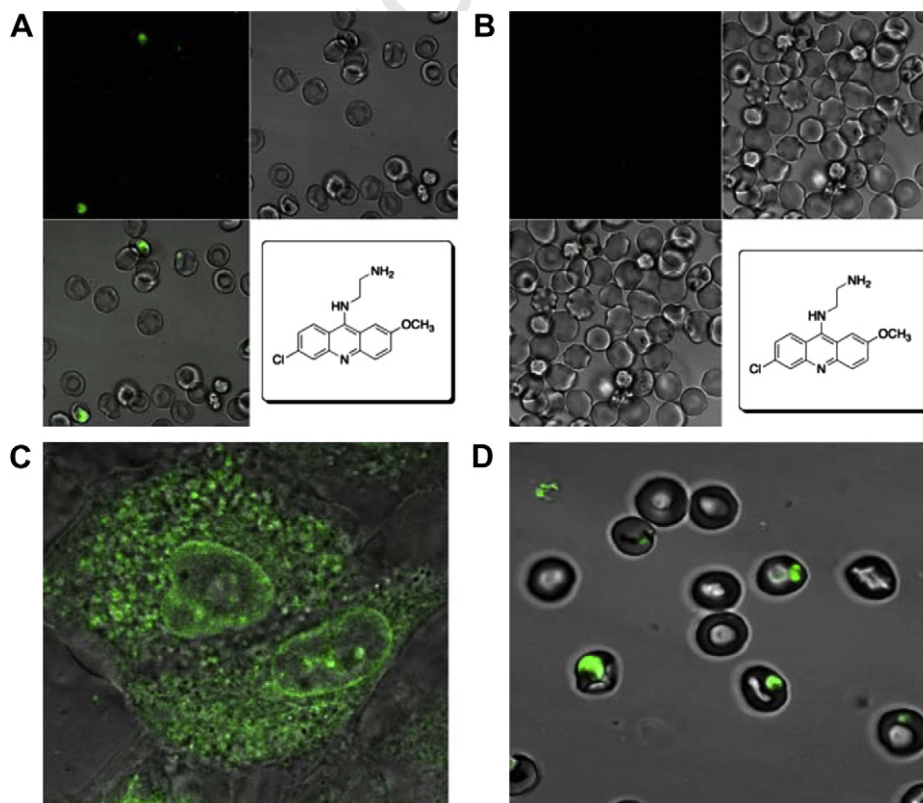


Figure 3. Confocal microscopy studies. (A) Accumulation of **2** in infected erythrocytes; (B) following perfusion of cells with buffer. (C) Accumulation of **7** in MRC-5 human lung fibroblasts. (D) Accumulation of **7** in infected erythrocytes. Attempts to remove **7** by cell perfusion failed in line with previous studies.

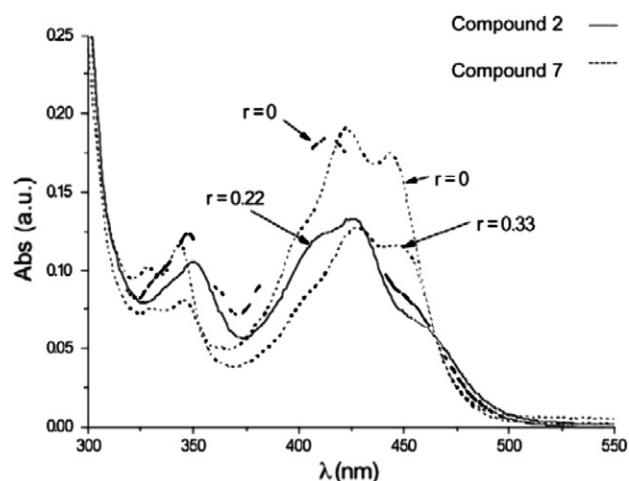


Figure 4. UV/vis spectra of **2** and **7** ($c = 5 \times 10^{-5} \text{ mol dm}^{-3}$) and complexes **2**/ctDNA and **7**/ctDNA at different ratios $r = [\mathbf{2} \text{ or } \mathbf{7}]/[\text{ctDNA}]$, pH 7, Na cacodylate buffer, $I = 0.05 \text{ M}$.

metrically using thermal melting analysis, electronic absorption, fluorimetric binding titrations and circular dichroism (CD) analysis. Addition of ct-DNA at concentrations close to equimolar in respect of studied compounds yielded significant bathochromic shifts in the electronic absorption spectra of **7** and **2** (Fig. 4) at wavelengths characteristic for acridine moiety.²⁵ However, at higher excess of ct-DNA over **2**, **7** precipitation occurred, hampering collection of enough data points for accurate processing by Scatchard equation.²⁶

Table 3

ΔT_m values^a ($^{\circ}\text{C}$) of studied ds-polynucleotides upon addition of **2** and **7** at ratio $r^b = 0.3$ at pH 7, Na cacodylate buffer, 0.02 M ($I = 0.005 \text{ M}$)^a

| Polynucleotide | ΔT_m w/ 2 ($^{\circ}\text{C}$) | ΔT_m w/ 7 ($^{\circ}\text{C}$) |
|----------------------------|---|---|
| ctDNA | 9.3 | 3.1 |
| Poly A–poly U | 2.2 | 2.4 |
| Poly A–poly T ^c | 2.9 | 0.6 |

^a Error in ΔT_m : $\pm 0.5 \text{ }^{\circ}\text{C}$.

^b $r = [\text{compound}]/[\text{polynucleotide}]$.

^c With the exception of this experiment (where $I = 0.05 \text{ M}$) melting studies were performed at $I = 0.005 \text{ M}$ due to the very weak effects at $I = 0.05 \text{ M}$.

At variance to UV/vis titrations, strong fluorescence of **2** and **7** allowed fluorimetric titrations with polynucleotides at significantly lower concentrations, at which no precipitation was observed. Emission of both **2** and **7** was strongly quenched by addition of ct-DNA and poly A–poly U and processing of the fluorimetric titration data by Scatchard equation²⁶ gave similar binding constants (in the range $K_s \approx 10^6 \text{ M}^{-1}$) and ratio binding $n_{[\text{compound}]/[\text{polynucleotide}]}$ of about 0.2. However, in thermal melting studies addition of **2** generally yielded a significantly stronger stabilisation effect on double stranded polynucleotides than its hybrid analogue **7** (Table 3).

However, CD studies were the most elucidating. CD analysis of ct-DNA (Fig. 5) and poly A–poly U in the presence of **2** showed an increase in the CD of DNA/RNA (220–300 nm range) and a weak negative induced CD at about 430 nm (absorption attributed to the acridine), characteristic of intercalation.²⁷ However, there was no change in the CD spectra of either ct-DNA or poly A–poly U upon addition of hybrid **7**, indicating that the acridine moiety of the hybrid is not intercalated into the double helix. That would imply that linking the acridine moiety to the trioxane prevents intercalation in double stranded polynucleotide or at least diminishes its role in binding, presumably due to the intramolecular aromatic stacking interaction between acridine and trioxane moiety (visible from comparison of the UV/vis spectrum of **2** and **7**). Consequently, changes in the electronic emission and absorption spectra of **7** upon addition of studied polynucleotides and the increase in melting temperature are caused either by counterion stabilisation or by mixed binding modes including to some electrostatic, hydrophobic and van der Waals interactions as well as intercalation of the acridine as a minor contribution.²⁸

In summary, artemisinin–acridine hybrids display promising antitumour activity in HL60, MDA-MB-231 and MCF-7 cells. They have been shown to induce cell death by apoptosis and to covalently bind to their intraparasitic cellular targets in the presence of iron(II). Linking an acridine to an artemisinin derivative was shown to enhance antitumour activity in the HL60 leukaemia cell line (comparing **2** and **7**), while it had a largely inhibitory effect in HT29-AK and MDA-MB-231 cells. Compared to DHA the hybrids had decreased in vitro antimalarial activity. Although hybrid **7** was shown to have high affinity toward DNA/RNA, intercalation of hybrid **7** into DNA/RNA in vitro was not dominant binding mode, presumably due to the competitive intramolecular aromatic stacking interaction between acridine and trioxane moiety. Future work will involve the synthesis and evaluation of redesigned hybrids in which longer linkers between the two moieties and a variety

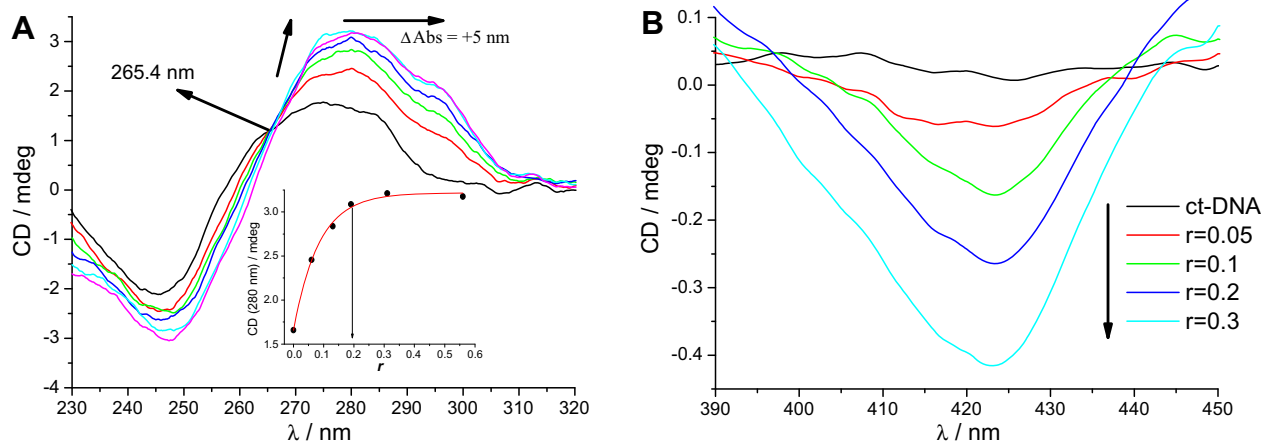


Figure 5. (A) Changes in the CD spectrum of ct-DNA ($c = 1 \times 10^{-5} \text{ mol dm}^{-3}$) upon addition of **2** (inset: changes at $\lambda = 280 \text{ nm}$ at various ratios $r = [\mathbf{2}]/[\text{ctDNA}]$); (B) induced CD band of acridine moiety at various ratios r ($c(\text{ct-DNA}) = 1 \times 10^{-4} \text{ mol dm}^{-3}$). Carried out at pH 7, Na cacodylate buffer, $I = 0.05 \text{ M}$.

of DNA targeting groups will be used in the hope of achieving greater DNA affinity and enhanced antitumour activity.

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References and notes

1. Woerdenbag, H. J.; Moskal, T. A.; Pras, N.; Malingre, T. M.; Elferaly, F. S.; Kampinga, H. H.; Konings, A. W. T. *J. Nat. Prod.* **1993**, *56*, 849.
2. Jung, M. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1091.
3. Posner, G. H.; Ploypradith, P.; Hapangama, W.; Wang, D. S.; Cumming, J. N.; Dolan, P.; Kensler, T. W.; Klinedinst, D.; Shapiro, T. A.; Zheng, Q. Y.; Murray, C. K.; Pilkington, L. G.; Jayasinghe, L. R.; Bray, J. F.; Daughenbaugh, R. *Bioorg. Med. Chem.* **1997**, *5*, 1257.
4. Moore, J. C.; Lai, H.; Li, J. R.; Ren, R. L.; McDougall, J. A.; Singh, N. P.; Chou, C. K. *Cancer Lett.* **1995**, *98*, 83.
5. Efferth, T.; Dunstan, H.; Sauerbrey, A.; Miyachi, H.; Chitambar, C. R. *Int. J. Oncol.* **2001**, *18*, 767.
6. Efferth, T.; Benakis, A.; Romero, M. R.; Tomicic, M.; Rauh, R.; Steinbach, D.; Hafer, R.; Stamminger, T.; Oesch, F.; Kaina, B.; Marschall, M. *Free Radical Biol. Med.* **2004**, *37*, 998.
7. Efferth, T.; Oesch, F. *Biochem. Pharmacol.* **2004**, *68*, 3.
8. O'Neill, P. M.; Posner, G. H. *J. Med. Chem.* **2004**, *47*, 2945.
9. Efferth, T. *Drug Res. Updates* **2005**, *8*, 85.
10. Li, P. C. H.; Lam, E.; Roos, W. P.; Zdzienicka, M. Z.; Kaina, B.; Efferth, T. *Cancer Res.* **2008**, *68*, 4347.
11. Jeyadevan, J. P.; Bray, P. G.; Chadwick, J.; Mercer, A. E.; Byrne, A.; Ward, S. A.; Park, B. K.; Williams, D. P.; Costick, R.; Davies, J.; Higson, A. P.; Irving, E.; Posner, G. H.; O'Neill, P. M. *J. Med. Chem.* **2004**, *47*, 1290.
12. Albert, A.; Goldacre, R. *Nature* **1948**, *161*, 95.
13. Denny, W. A. *Curr. Med. Chem.* **2002**, *9*, 1655.
14. (a) Eckstein-Ludwig, U.; Webb, R. J.; van Goethem, I. D. A.; East, J. M.; Lee, A. G.; Kimura, M.; O'Neill, P. M.; Bray, P. G.; Ward, S. A.; Krishna, S. *Nature* **2003**, *424*, 957; (b) Stocks, P. A.; Bray, P. G.; Barton, V. E.; Al-Helal, M.; Jones, M.; Araujo, N. C.; Gibbons, P.; Ward, S. A.; Hughes, R. H.; Biagini, G. A.; Davies, J.; Amewu, R.; Mercer, A. E.; Ellis, G.; O'Neill, P. M. *Angew. Chem., Int. Ed.* **2007**, *46*, 6278.
15. Takahashi, T.; Tanaka, H.; Matsuda, A.; Doi, T.; Yamada, H.; Matsumoto, T.; Sasaki, D.; Sugiura, Y. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3303.
16. Perez, J. M.; Lopez-Solera, I.; Montero, E. I.; Brana, M. F.; Alonso, C.; Robinson, S. P.; Navarro-Ranninger, C. *J. Med. Chem.* **1999**, *42*, 5482.
17. Meunier, B. *Acc. Chem. Res.* **2008**, *41*, 69.
18. O'Neill, P. M.; Miller, A.; Bishop, L. P. D.; Hindley, S.; Maggs, J. L.; Ward, S. A.; Roberts, S. M.; Scheinmann, F.; Stachulski, A. V.; Posner, G. H.; Park, B. K. *J. Med. Chem.* **2001**, *44*, 58.
19. *Synthesis of hybrid 7*: To a stirred solution of artemisinin acid chloride (1 equiv) and appropriate alkylaminoacridine (1 equiv) in anhydrous CH₂Cl₂ at 0 °C was added anhydrous triethylamine (1.1 equiv). The reaction mixture was stirred at low temperature (0 °C) for 18 h and then allowed to warm to ambient temperature. The volatiles were removed in vacuo and the resultant yellow oil was purified by flash column chromatography on silica gel to give a yellow solid (20:80 = MeOH/CH₂Cl₂). *Analysis for 7*: mp 125 °C ¹H NMR: (CDCl₃,

- 400 MHz) δ 8.40 ppm (1H, br s), 8.15 (1H, d, J = 9.2 Hz), 8.10 (1H, d, J = 2.5 Hz), 7.97 (1H, d, J = 9.1 Hz), 7.40 (1H, dd, J = 2.4 Hz, 9.3 Hz), 7.38 (1H, d, J = 2.1 Hz), 7.20 (1H, dd, J = 2.1 Hz, 9.3 Hz), 5.02 (1H, s), 4.0 (1H, s), 3.91 (1H, m), 3.70 (3H, s), 3.43, (2H, m), 3.28 (2H, m), 2.48 (1H, m) 2.45–2.17 (3H, m) 2.07–1.20 (10H, m) including 1.41 (3H, s) 0.96 (3H, d, J = 5.9 Hz) and 0.89 (3H, d, J = 7.67 Hz); ¹³C NMR: (CDCl₃, 400 MHz) δ 174.7, 156.8, 148.6, 148.0, 134.9, 131.0, 128.9, 128.0, 127.3, 121.9, 118.6, 104.6, 98.0, 96.2, 92.8, 84.5, 67.5, 56.2, 54.0, 44.6, 37.3, 36.3, 30.8, 30.4, 29.5, 25.3, 23.7, 22.8, 19.0, 18.4, 11.8, 11.4; HRMS (CI): C₃₃H₄₀ClN₃O₆ [M+H]⁺ requires 611.1479 found: 611.1499; Anal. Calcd for C₃₃H₄₀ClN₃O₆: C, 64.96; H, 6.61; N, 6.89. Found: C, 65.00; H, 6.78; N, 6.93.
20. *Measurement of cytotoxicity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay*: HL-60 Cells (2.5 × 10⁴/well) were plated in triplicate, in flat bottom 96-well plates and were exposed to 0.01–100 μM of each compound for 72 h (5% CO₂ at 37 °C). Following incubation, cell viability measurements using the MTT assay were carried out by the addition of 20 μL of MTT solution (5 mg/mL in HBSS) to each well and incubating for 3 h at 37 °C. Thereafter, 100 μL of a lysing solution (20% w/v sodium dodecylsulfate, 50% w/v N,N-dimethyl formamide) was added to each well to dissolve the formazan crystals and incubated for an additional 4 hrs. The absorbances of the samples were measured at a test wavelength of 570 nm and a reference wavelength of 590 nm with a plate reader (MRX, Dynatech Laboratories). All results were expressed as a percentage of vehicle only cells. The IC₅₀ values were calculated from individual inhibition curves plotted by Grafit software. The same method was used to determine cytotoxicity against HT29-AK and MDA-MB-231 cells at drug concentrations up to 750 μM.
 21. For in vitro antimalarial assessment versus the 3D7 strain of *Plasmodium falciparum* the following protocol was employed. Parasites were maintained in continuous culture using the method of Jensen and Trager: (a) Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673–675; Antimalarial activity was assessed with an adaption of the 48 hr sensitivity assay of Desjardins et al. using [3H]-hypoxanthine incorporation as an assessment of parasite growth: (b) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
 22. Mitochondrial membrane potential (MMP) was measured using tetramethylrhodamine ethyl ester (TMRE) to quantify HL-60 cells with a high MMP. Drug treated cells (5 × 10⁵ cells) were washed in HBSS and the resultant cell pellet was resuspended in 500 μL of TMRE solution (50 nM in HBSS) and incubated for 10 min at 37 °C. A minimum of 500 cells were measured by flow cytometry on fluorescence channel FL-2 (Coulter Epics, XL Software). The data was analysed using WinMDI, v2.8 software (Scripps Institute, California, USA).
 23. The DNA major groove binder PI was used to quantify cellular DNA content to measure the formation of a sub-G₀/G₁ population of HL-60 cells. Drug treated cells (1 × 10⁶) were washed twice in HBSS, fixed in 1 mL of ice-cold 70% ethanol, and frozen at –20 °C. After 2 h the 70% ethanol was removed and the cell pellet was resuspended in 1 mL of PI stock solution (phosphate-buffered saline containing 40 μg/mL PI, 0.1 mg/mL RNase, and 3.8 mM sodium citrate) and incubated at 37 °C (30 min). A minimum of 5000 cells were analysed by flow cytometry. PI fluorescence was measured on fluorescence channel FL-2. The proportion of cells in each stage of the cell cycle was calculated from the DNA content of the cell using WinMDI, v2.8 software.
 24. Cell lysate samples were prepared, cells (3 × 10⁶) were washed before sonication with a sonic probe, (2 × 5 s cycles). The samples were then assayed for protein content using the Bradford assay. Lysates, with equal amounts of protein (20 μg per lane), were mixed with SDS–PAGE loading buffer and denatured at 95 °C for 3 min prior to being resolved on 14% SDS–PAGE. Proteins were transferred to nitrocellulose membrane for Western blotting analysis as described previously.²⁹ Zimmermann, Physicochemical and cytochemical investigations on the binding of ethidium and acridine-dyes to DNA and to organelles in living cells.
 25. Zimmermann, H. W. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 115.
 26. McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *103*, 679.
 27. Eriksson, M.; Norden, B. In *Drug-Nuc. Acid Int.*, **2001**; Vol. 340, p-68.
 28. Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 271.