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HUMAN TELOMERASE INHIBITION BY SUBSTITUTED ACRIDINE DERIVATIVES

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Abstract: A series of 3,6-disubstituted acridine derivatives have been rationally designed as telomerase inhibitors. They have been designed on the basis that inhibition of telomerase occurs by stabilising G-quadruplex structures formed by the folding of telomeric DNA. The most potent inhibitors have IC_{50} values against telomerase of between 1.3 and 8 μM, comparable to their cytotoxicity in ovarian cancer cell lines.

Telomeres are specialized DNA-protein complexes at ends of chromosomes which represent a potential target for novel anti-cancer agents.[1] They contain guanine rich regions of single stranded DNA and are essential for protection against DNA degradation and aberrant recombination. The specialized reverse transcriptase telomerase has the ability to maintain telomere length, by synthesising further telomere repeats and adding them on to the end of telomeres.[2,3] It is not activated in normal somatic cells, which progressively lose telomeric repeats during successive rounds of cell division, leading to the non-replicating state of senescence, and ultimately to cell crisis. In contrast, some 80–90% of tumor cells have activated telomerase, resulting in stabilization of telomere length and an ability for sustained cellular proliferation.[1,4] Telomerase is thus an essential factor in cellular immortalisation and consequently tumorigenesis.[5] There is currently much interest in the inhibition of telomerase as a novel anticancer target.[1,6] It has been proposed that inhibitors would require up to ca 20 rounds of cell division in target cells before their telomeres become critically shortened, when they would become non-replicating. Recent evidence suggests that telomerase activity in some cell types can be dissociated from telomere maintenance, suggestive of a regulatory function for telomerase involving telomere capping[7]. Consequently telomerase inhibition may result in anti-proliferative effects after just a small number of generations of cell growth.

Single stranded guanine-rich telomeric DNA sequences can associate to form guanine quadruplex structures.[8-11] Such structures when formed by the telomere primer, inhibit telomerase from synthesising further telomeric repeats. We have developed a strategy for telomerase inhibition by small molecules based on

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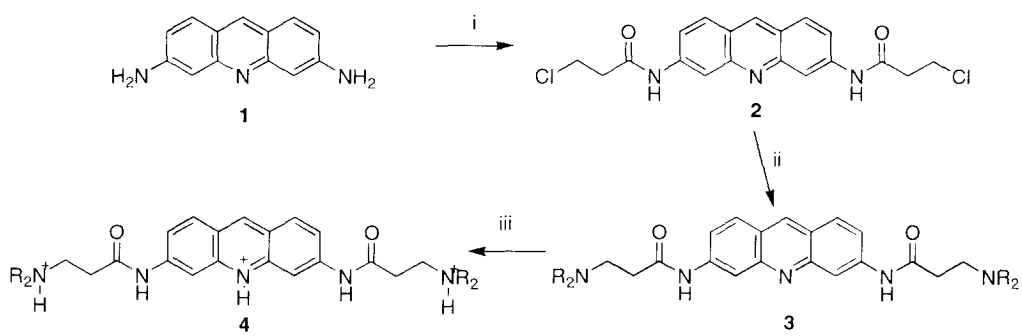
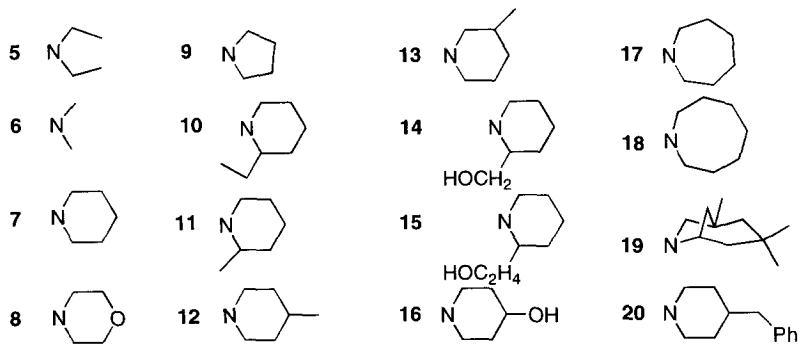
this effect, with the initial demonstration using amidoanthraquinones capable of binding to, and stabilising guanine-quadruplexes[12,13]. This has also been applied to porphyrins[14] and a perylene compound[15]. We have studied in detail[16,17] the synthesis and evaluation of several series of anthraquinone-based derivatives, as examples of small molecule inhibitors of the telomerase enzyme complex, and recently have shown that analogous fluorenone derivatives possess similar telomerase activity[18]. Structure-activity studies on these classes of compounds have shown the effect of chromophore, chain linker length and regioisomerism on telomerase activity.

We describe here a series of analogous 3,6-disubstituted acridine derivatives for use as such inhibitors developed with the specific aim of increasing telomerase activity by rational design. Molecular modelling studies (Read *et al*, to be published) have predicted that the acridine chromophore is at least comparable to the anthraquinone moiety in terms of G-quadruplex binding affinity. The acridine moiety is an inherently planar chromophore, but contains a heterocyclic nitrogen atom with the ability to be protonated at physiological pH. Protonation of this nitrogen heteroatom would increase electron deficiency through the chromophore, enabling enhanced G-quadruplex interactions. It was also predicted that the charged nitrogen heteroatom would improve water solubility compared to anthraquinones,[16,17] and incorporation of the preferential 3,6-diamide substitution, would be equivalent to the anthraquinone 2,7-disubstitution pattern previously examined.[16,17] Other categories of acridine derivatives with different patterns of ring substitution have previously been shown to possess potent cytotoxic activity, with high affinity for duplex DNA. For example recent interest has been shown in novel mono-substituted acridines (DACA and analogues) as potent anti-cancer agents.[19,20]

We have previously reported [16-18] the use of molecular modelling in studies with the folded structure formed by the four-repeat sequence d[$AG_3(T_2AG_3)_3$] of the human telomere.[21] Ligands were modelled into the intercalation site at the 5'-AG step, in accord with NMR data based upon other ligands binding to this sequence[15]. These studies (Read *et al*, to be published) have found that quantitative predictions of binding energies to the folded human telomere accord remarkably well with observed telomerase activity.



Scheme 1 Reagents and conditions (i) chloropropionyl chloride, 80 °C, 3-4 hours (ii) R_2NH, EtOH, NaI, reflux, 2-4 hours (iii) HCl, $CHCl_3$.



Groups (NR_2) attached to the amidoalkyl substituents

The synthesis of the 3,6-disubstituted acridine derivatives was carried out according to scheme 1, in a short synthetic procedure starting from proflavine (**1**). The procedure is similar to that previously described in the synthesis of difunctionalised anthraquinones and fluorenones.[16,17] Initial acylation of proflavine (freshly prepared from its hemisulphate salt) with chloropropionyl chloride at reflux provided the ω-chloroalkanamide **2**. Subsequent aminolysis by reflux treatment with the appropriate secondary amine in ethanol, in the presence of NaI catalyst produced the target disubstituted acridine derivatives, all steps provided yields above 80%. Purification in all cases was achieved by recrystalisation from ethanol / DMF and all compounds were judged pure by elemental analysis, ^{1}H NMR and high resolution MS.[23] To improve water solubility the compounds were converted to their hydrochloride addition salts, which were used in the biochemical and biological evaluations.

Compounds **5-20** were evaluated for *in vitro* cytotoxicity against three human ovarian carcinoma cell lines (A2780, CH1, and SKOV-3) using the sulforhodamine B (SRB) assay as described previously.[22] Results are presented in Table 1 as the concentrations required to inhibit cell growth by 50% (IC_{50} values). Prior to the evaluation of compounds in a modified PCR-based telomerase assay, the agents were examined for their ability to inhibit *Taq* polymerase. Compounds were tested at concentrations of 10, 20 and 50 μM; *Taq* polymerase inhibition results are given (Table 1) for each concentration. The agents were subsequently evaluated for their ability to inhibit human telomerase in a modified cell-free TRAP assay using extracts from the A2780 cell line.[16-18] Compounds were tested at concentrations of 0.5, 1, 5, 10, 20, 50 μM and up to the concentration where *Taq* polymerase inhibition was first observed. The concentrations required to inhibit telomerase activity by 50% ($^{tel}IC_{50}$ values) are reported.

Table 1. *In vitro* cytotoxicity, Telomerase and *Taq* polymerase inhibition data for 3,6-disubstituted acridine derivatives

Compound	$^{Tel}IC_{50}$ (μM)[a]	IC_{50} (μM)[b]			*Taq* Inhibition[c]		
		A2780	CH1	SKOV-3	10 μM	20 μM	50 μM
5	5.8	0.57	2.45	2.25	–	–	–
6	8.2	0.75	9.8	2.5	–	–	–
7	2.8	1.7	2.25	0.54	–	–	–
8	>50	3.1	15	13	–	–	–
9	5.2	2.65	8.2	2.6	–	–	–
10	2.7	2.2	2.2	3.9	–	–	–
11	2.6	1.75	2.3	3.4	–	–	–
12	1.35	2.15	2.05	2.4	–	–	–
13	4.4	2.1	2.2	2.85	–	–	–
14	5.4	2.3	10.5	7	–	–	–
15	4.1	3.2	11	10.5	–	–	–
16	8	6.2	15	13	–	–	–
17	3.1	1.1	1.85	2.5	–	–	–
18	>50	1.3	2.2	2.3	–	–	–
19	>50	0.5	0.54	2.2	–	–	–
20	>50	0.31	0.11	0.88	–	–	–

[a] Concentration required to inhibit telomerase activity by 50% relative to controls. [b] Concentration required to inhibit cell growth by 50% relative to controls. [c] Key:(–) no inhibition.

Telomerase and cytotoxic activity for the 3,6-disubstituted acridines **5-20** are given in Table 1. The acridine derivatives examined show consistent telomerase inhibitory activity over several determinations for each compound, with the most potent inhibitor having a $^{tel}IC_{50}$ value of 1.35 μM. DNA polymerase selectivity was also suggested from these results, with no inhibition of *Taq* DNA polymerase being observed at concentrations up to 50μM. Compound **8**, containing a morpholine substituent, was found to be totally inactive at concentrations up to 50 μM and follows a trend previously observed for the analogous anthraquinone and fluorenone derivatives.[16-18] In addition, compounds containing bulky end-groups (**18-20**) were also found to be inactive. Modelling studies have shown that these derivatives are unable to interact effectively with the human G-quadruplex structure due to steric restrictions, and we therefore suggest that their lack of activity against telomerase is directly attributable, at least in part, to this effect. It is striking that compounds **18-20** still however, show levels of cytotoxic potency comparable to the active compounds, in all three cell lines tested. Direct comparisons of these acridine compounds with analogous 2,7-disubstituted anthraquinone and fluorenone derivatives reveals that they show a general increase in levels of telomerase inhibition coupled with comparable (and sometimes lower) levels of cytotoxicity. A surprising effect among all the acridines **5-20** is the lack of *Taq* polymerase inhibition, suggesting that they have selectivity at the

polymerase level. Many acridine antitumour agents (eg DACA) have potent cytotoxicity, with IC_{50} levels typically in the nM range. We suggest that the 3,6 pattern of acridine disubstitution results in compounds **5-20** having steric features sub-optimal for binding to duplex DNA yet selective for G-quadruplexes.

These new acridine derivatives as a class are the most active intercalating G- quadruplex stabilisers reported as telomerase inhibitors. This, together with their moderate cytotoxicity, suggest that they may be suitable leads for finding candidates for long-term cellular studies of the effects of telomerase inhibition, when compounds with $^{tel}IC_{50} < IC_{50}$ are required. The 4-methyl piperidine derivative with a $^{tel}IC_{50}$ of 1.35 μM and cytotoxicity in the cell lines tested between 2-2.5 μM, is a first step in this direction.

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(23) Analysis of selected compounds:

3,6-Bis[(3-diethylaminopropionamido)]acridine, (5), mp 224-225 °C dec.; δ_H 1.18 (12H, t, *J* 7.1, NCH_2CH_3), 2.57 (4H, t, *J* 5.1, $COCH_2CH_2N$), 2.72 (8H, q, *J* 7.1, NCH_2CH_3), 2.82 (4H, t, *J* 5.1, $COCH_2CH_2N$), 7.89 (2H, s, H-4,5), 8.00 (2H, d, *J* 1.9, H-2,7) 8.05 (2H, d, *J* 1.9, H-1,8), 8.59 (1H, s br, H-9), 11.80 (2H, s, NHCO) *m/z* (EI) 464.3040 ($C_{27}H_{38}N_5O_2$ requires 464.3026); Fnd: C; 69.84, H; 8.08, N; 15.08, Calcd: C; 69.95, H; 8.04, N; 15.10.

3,6-Bis[(3-dimethylaminopropionamido)]acridine, (6), mp 217-218 °C dec.; δ_H 2.21 (12H, s, NCH_3), 2.61 (4H, t, *J* 5.2, $COCH_2CH_2N$), 2.78 (4H, t, *J* 5.2, $COCH_2CH_2N$), 7.65 (2H, s, H-4,5), 8.03 (2H, d, *J* 1.9, H-2,7) 8.12 (2H, d, *J* 1.9, H-1,8), 8.56 (1H, s, H-9), 12.30 (2H, s br, NHCO), *m/z* (EI) 408.2420 ($C_{23}H_{30}N_5O_2$ requires 408.2400); Fnd: C; 66.45 H; 7.03 N; 16.90, Calcd: C; 67.79, H; 7.17, N; 17.18

3,6-Bis[(3-morpholinopropionamido)]acridine, (8), mp 270-272 °C dec.; δ_H 2.63 (4H, t, *J* 6.1, $COCH_2CH_2N$), 2.66 (8H, t, *J* 4.7, $N(CH_2CH_2)_2O$), 2.81 (4H, t, *J* 6.1, $COCH_2CH_2N$), 3.90 (8H, t, 4.7, $N(CH_2CH_2)_2O$) 7.9 (4H, m, H-1,2,7,8) 8.14 (2H, s, H-4,5), 8.61 (1H, s, H-9), 11.17 (2H, s br, NHCO), *m/z* (EI) 492.2630 ($C_{27}H_{34}N_5O_4$ requires 492.2611); Fnd: C; 65.67, H; 6.81, N; 13.99, Calcd: C; 65.97, H; 6.77, N; 14.24.

3,6-Bis[3-(2-ethylpiperidino)propionamido]acridine, (10), mp 215-216 °C dec.; δ_H 0.95 (6H, t, *J* 7.5, CH_2CH_3), 1.31 (4H, m, CH_2CH_3), 1.65 (12H, m, $N(CH_2CH_2)_2CH_2$), 2.60 (4H, t, *J* 6.2, $COCH_2CH_2N$) 2.68 (6H, m, $N(CH_2CH_2)_2CH_2$), 2.91 (4H, t, *J* 6.2, $COCH_2CH_2N$), 7.9 (4H, m, H-1,2,7,8) 8.01 (2H, s, H-4,5), 8.58 (1H, s, H-9), 11.68 (2H, s br, NHCO), *m/z* (EI) 544.3652 ($C_{33}H_{46}N_5O_2$ requires 544.3623); Fnd: C; 72.73, H; 8.41, N; 12.86, Calcd: C; 72.89, H; 8.34, N; 12.87

3,6-Bis[3-(4-methylpiperidino)propionamido]acridine, (12), mp 224-225 °C dec.; δ_H 1.06 (6H, d, *J* 5.58, CH_3), 1.22 (2H, m, $N(CH_2CH_2)CH$), 1.35 (4H, m, $N(CH_2CH_{2a})_2CH$), 1.68 (4H, m, $N(CH_2CH_{2b})_2CH$), 2.13 (4H, t, *J* 10.4, $N(CH_{2a}CH_2)_2CH$), 2.60 (4H, t, *J* 5.0, $COCH_2CH_2N$), 2.74 (4H, t, *J* 5.0, $COCH_2CH_2N$), 3.11 (4H, t, *J* 10.4, $N(CH_{2b}CH_2)_2CH$) 7.8 (4H, m, H-1,2,7,8) 8.22 (2H, s, H-4,5), 8.60 (1H, s, H-9), 11.62 (2H, s br, NHCO), *m/z* (EI) 516.3303 ($C_{31}H_{42}N_5O_2$ requires 516.3339); Fnd: C; 71.96, H; 8.05 N; 13.48, Calcd: C; 72.20. 95, H; 8.01, N; 13.57

3,6-Bis[3-(2-methylpiperidino)propionamido]acridine, (11), mp 223-225 °C dec.; δ_H 1.19 (6H, d, *J* 6.3, CH_3), 1.6 (12H, m, $N(CH_2CH_2)CH_2$), 2.45 (4H, t, *J* 6.4, $COCH_2CH_2N$) 2.68 (6H, m, $N(CH_2CH_2)CH_2$), 2.71 (4H, t, *J* 6.4, $COCH_2CH_2N$), 7.9 (4H, m, H-1,2,7,8) 8.18 (2H, s, H-4,5), 8.60 (1H, s, H-9), 11.79 (2H, s br, NHCO), *m/z* (EI) 516.3303 ($C_{31}H_{42}N_5O_2$ requires 516.3339); Fnd: C; 72.05, H; 8.09, N; 13.40, Calcd: C; 72.20, H; 8.01, N; 13.57

3,6-Bis[(4-hydroxypiperidinopropionamido)]acridine, (16), mp 237-238 °C dec.NMR δ 1.38 (4 H, m, $(CH_2CH_aH_b)_2CHOH$, 1.68 (4 H, m, $(CH_2CH_aH_b)_2CHOH$, 2.05 (4H, m, $(CH_aH_bCH_2)_2CHOH$), 2.49 (4 H, m, $COCH_2CH_2$), 2.59 (4 H, m, $COCH_2CH_2$), 2.73 (4 H, m, $(CH_aH_bCH_2)_2CHOH$), 3.35 (2 H, m, $(CH_2CH_2)_2CHOH$ 4.56 (2 H, d, CHOH), 7.9 (4H, m, H-1,2,7,8) 8.01 (2H, s, H-4,5), 8.58 (1H, s, H-9), 11.68 (2H, s br, NHCO), *m/z* (EI) 534.2934 ($C_{29}H_{38}N_5O_4$ requires 534.2956); Fnd: C; 64.70, H; 7.19, N; 15.59, Calcd: C; 65.15, H; 7.16, N; 15.72.