1,4- and 2,6-Disubstituted Amidoanthracene-9,10-dione Derivatives as Inhibitors of Human Telomerase

Philip J. Perry,[†] Sharon M. Gowan,[‡] Anthony P. Reszka,[†] Paolo Polucci,[†,§] Terence C. Jenkins,[†,||] Lloyd R. Kelland,[‡] and Stephen Neidle*[,†]

Cancer Research Campaign Biomolecular Structure Unit and Centre for Cancer Therapeutics, The Institute of Cancer Research, Sutton, Surrey SM2 5NG, U.K.

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A number of 1,4- and 2,6-difunctionalized amidoanthracene-9,10-diones have been prepared. We have examined their in vitro cytotoxicity in several tumor cell lines and their ability to inhibit the telomere-addition function of the human telomerase enzyme together with their inhibition of the *Taq* polymerase enzyme. Compounds with -$(CH_2)_2$- side chains terminating in basic groups such as piperidine show inhibition of telomerase at $^{tel}IC_{50}$ levels of 4−11 μM. These are thus among the most potent nonnucleoside telomerase inhibitors reported to date. Cytotoxicity levels in human tumor cell lines were at comparable levels for several compounds. Implications for amidoanthracene-9,10-dione telomerase inhibitors as potential anticancer agents are discussed.

Introduction

The ends of chromosomes, termed telomeres, consist of specialized tandem-repeating G-rich sequences,[1] which serve to protect the ends from recombination, end-to-end fusion, and degradation. The telomeric repeat in vertebrate chromosomes[2] is $(5'\text{-TTAGGG})_n$. DNA synthesis at chromosome ends results in progressive telomere shortening during successive rounds of cell replication; since DNA polymerase can only replicate these ends in an incomplete manner, DNA synthesis occurs solely in the 5′ to 3′ direction. Thus, in the absence of a mechanism to maintain or extend telomere length,[3−5] this end-replication problem means that normal cells can only undergo ca. 20 rounds of cell division before cellular senescence occurs. The enzyme telomerase is able to perform this function of length extension, since it is a specialized reverse transcriptase with an endogenous RNA template on which successive telomeric repeats are synthesized.[6−9]

It has been found that telomerase activity is detectable in 80−90% of human tumors, but largely absent in somatic cells (see, for example, refs 10−14). This correlates with the shortened but stable length of telomeres in tumors compared to the somewhat longer average telomere lengths found in somatic cells. Thus, tumor cells have an almost infinite capacity to divide, and hence can be immortalized, whereas normal cells can only undergo a finite number of rounds of replication (and hence telomere shortening) before senescence and cellular crisis. These findings have led to the concept of telomerase as a highly selective antitumor target.[15] Studies with antisense DNA and PNA sequences targeted to the RNA template sequence have shown inhibition of telomerase activity.[16−18] Nucleosides such as AZT,[19] which have reverse transcriptase activity, have also shown inhibition of telomerase activity.

We have adopted a distinct approach, with the aim of rationally designing molecules capable of recognizing guanine-quadruplex four-stranded DNA structures[20] and also of promoting their formation. It is well-established that the telomerase template requires a linear, nonfolded telomere DNA primer in order for telomere extension to take place and that the presence of a G-quadruplex results in inhibition of the enzyme.[21] We recently reported, on the basis of unequivocal evidence from UV and NMR titration experiments,[22] that a 2,6-bis(ω-aminopropionamido)anthracene-9,10-dione (anthraquinone) derivative (compound **1**, Figure 1), is able to form G-quadruplex complexes with telomeric DNA sequences. We also showed, by means of a telomerase primer extension assay, that the 2,6-diamidoanthraquinone **1** has telomerase inhibitory activity ($^{tel}IC_{50} = 23\ \mu$M), with evidence indicating that the most likely molecular target is the telomeric DNA intramolecular G-quadruplex formed by successive 5′-TTAGGG repeats. Subsequently, it has been found that the porphyrin tetra-(*N*-methyl-4-pyridyl) porphine binds to G-quadruplexes[23,24] and inhibits telomerase.[24]

Anthraquinone-based compounds currently occupy a prominent position in cancer chemotherapy, with the naturally occurring aminoglycoside anthracycline doxorubicin and the aminoanthraquinone mitoxantrone both being in clinical use.[25] These and other experimental anthraquinone derivatives (see, for example, refs 26 and 27) are believed to act at the duplex DNA level, probably through the stabilization of a ternary complex with DNA topoisomerase II.[28] These compounds tend to be highly cytotoxic and suffer from a lack of true selectivity to tumor cells. The present paper, by contrast, examines the ability of a range of isomeric 1,4- and 2,6-diami-

* Corresponding author: Professor Stephen Neidle, CRC Biomolecular Structure Unit, The Institute of Cancer Research, Cotswold Road, Sutton, Surrey SM2 5NG, U.K. Tel: (+044)-181-643-1675 (direct). Fax: (+044)-181-643-1675. E-mail: steve@iris5.icr.ac.uk.

[†] CRC Biomolecular Structure Unit.

[‡] CRC Centre for Cancer Therapeutics.

[§] Permanent address: Department of Chemistry, University of Camerino, Italy.

[||] Present address: School of Chemical and Life Sciences, University of Greenwich, Woolwich, London SE18 6PF, U.K.

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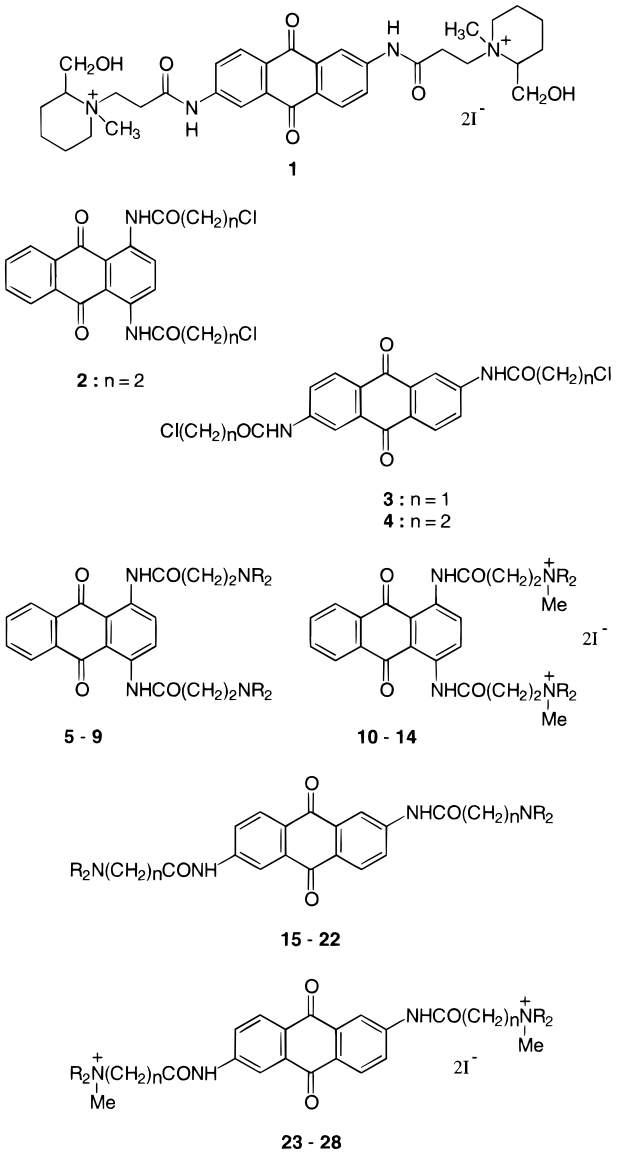

Figure 1. Anthracene-9,10-diones examined in this study. Substituents (NR_2) and side chain linker length (n) are shown in Table 1.

doanthraquinone compounds to inhibit the tumor-selective target enzyme telomerase and extends our previous anthraquinone study.[22] We report telomerase activity, as assessed by a modified telomerase repeat amplification protocol (TRAP) assay and conventional in vitro cytotoxicity in several human tumor cell lines. As a prerequisite to the evaluation of compounds in the TRAP assay, we also describe their ability to inhibit the *Taq* enzyme (a thermophilic DNA polymerase used in the PCR-based TRAP assay). This inhibitory ability also provides an indication of nonselective polymerase inhibition. A range of side chains has been examined to establish structure−activity relationships as a basis for subsequent rational drug design.

Chemistry. Synthesis of the 1,4- (**5**−**14**) and 2,6-difuctionalized anthraquinones (**15**−**28**) was accomplished using procedures somewhat modified from those described previously.[29,30] Briefly, acylation of the appropriately substituted diaminoanthraquinone with the appropriate (n = 2 for 1,4-isomers; n = 1 or 2 for 2,6-isomers) chloro-acyl chloride gave the intermediate bis-(ω-chloroalkanamides) **2**−**4** in essentially quantitative yield. Subsequent aminolysis by reflux treatment with the appropriate secondary amine in the presence of NaI catalyst gave the desired disubstituted anthraquinones **5**−**9** and **15**−**22** (Figure 1). Compounds were obtained in good yield and were adjudged pure by elemental analysis, mass spectrometry, and ^{1}H NMR. The free-base compounds were only sparingly soluble in water; hence, they were converted into their acid addition salts in order to improve their aqueous solubility. In addition, quaternary dimethiodide salts (**10**−**14** and **23**−**28**) were also prepared by treatment with iodomethane. The salt forms of all compounds were used for the cytotoxicity studies and the *Taq* polymerase, and human telomerase inhibition studies.

Biological Studies. The cytotoxic behavior of the compounds were evaluated against a panel of human ovarian carcinoma cell lines (A2780, CH1, and SKOV-3) using the sulforhodamine B (SRB) assay as described previously.[31] Results are presented in Table 1 as the concentrations required to inhibit cell growth by 50% (IC_{50} values). The established anthraquinone-based antitumor agents doxorubicin and mitoxantrone were included as controls.

Prior to the evaluation of compounds in the PCR-based telomerase assay, the agents were tested for their ability to inhibit *Taq* polymerase in order to examine the selectivity of polymerase versus telomerase inhibition. Compounds were tested at concentrations of 10 and 50 μM, and semiquantitative estimates of their ability to inhibit *Taq* polymerase are shown (Table 1) for each concentration. Agents that were found not to inhibit *Taq* polymerase at either concentration were subsequently evaluated for their ability to inhibit human telomerase in a modified cell-free TRAP assay.[28] Agents were tested at concentrations of 1, 5, 10, 20, and 50 μM; the concentrations required to inhibit telomerase activity by 50% ($^{tel}IC_{50}$ values) are reported in Table 2.

Results

Both the control agents, doxorubicin and mitoxantrone, are highly cytotoxic in the three cell lines used in this study. By comparison, the amidoanthraquinones reported here are, in general, less effective as cytotoxic agents, although two of the most active compounds, **5** and **8**, are approximately as equipotent as doxorubicin in the A2780 cell line. Members of the 2,6-difunctionalized series (compounds **1** and **15**−**28**) are generally less cytotoxic than their 1,4-isomers.

A typical gel pattern arising from a *Taq* polymerase experiment, showing the full range in inhibitory activities, is shown in Figure 2A. While compound **18** (lane 5) showed no inhibition at either 10 or 50 μM, both **25** and **19** (lanes 4 and 6, respectively) showed inhibition at 50 μM, and compound **7** (lane 7) showed inhibition at 50 μM together with some inhibitory activity at the lower concentration. Approximately half of the anthraquinones examined completely inhibit *Taq* polymerase activity at a concentration of 50 μM (Table 1), whereas only two compounds, **5** and **7**, show significant inhibitory effect at 10 μM. Furthermore, there does not appear to be any correlation between *Taq* polymerase inhibition and cytotoxicity. The two anthraquinone-based control drugs, doxorubicin and mitoxantrone, showed total inhibition of *Taq* polymerase activity at 50 μM and at least some inhibition at a 10 μM level.

Table 1. Side Chain Substituents, in Vitro Cytotoxicity, and *Taq* Inhibition Data for Disubstituted Anthracene-9,10-diones

				IC_{50}[c] (μM)			*Taq* inhibition[d]	
compd	substituent (NR_2)	n[a]	salt[b]	A2780	CH1	SKOV-3	10 μM	50 μM
1,4-derivatives								
5	NMe_2	2	HCl	0.016	0.04	0.36	+	+++
6	$N(CH_2CH_2CH_3)_2$	2	HCl	0.13	0.48	4.5	−	−
7	1-piperidinyl	2	HCl	0.29	0.26	2.4	+	+++
8	1-(2-hydroxymethyl)piperidinyl	2	HCl	0.02	0.064	0.27	−	+++
9	4-morpholinyl	2	HCl	3.7	1.95	21.0	−	−
10	NMe_2	2	MeI	2.15	2.0	11.5	−	++
11	$N(CH_2CH_2CH_3)_2$	2	MeI	1.4	4.45	16.0	−	+++
12	1-piperidinyl	2	MeI	1.75	4.3	32.5	−	−
13	1-(2-hydroxymethyl)piperidinyl	2	MeI	0.38	0.54	1.5	−	−
14	4-morpholinyl	2	MeI	0.76	1.65	8.5	−	−
2,6-derivatives								
1	1-(2-hydroxymethyl)piperidinyl	2	MeI	1.25	2.3	1.8	−	+++
15	NMe_2	2	AcOH	2.55	1.8	2.9	−	+++
16	$N(CH_2CH_2CH_3)_2$	2	AcOH	0.38	1.4	2.4	−	−
17	1-piperidinyl	1	AcOH	1.65	5.05	5.0	−	−
18	1-piperidinyl	2	AcOH	1.3	5.9	4.0	−	−
19	1-(2-hydroxymethyl)piperidinyl	2	AcOH	2.45	14.5	2.4	−	+++
20	4-morpholinyl	1	AcOH	5.9	4.15	4.5	−	−
21	4-morpholinyl	2	AcOH	4.8	20.0	9.0	−	−
22	1-(4-hydroxyethyl)piperazinyl	2	AcOH	11.0	7.9	7.0	−	−
23	NMe_2	2	MeI	2.25	1.9	4.4	−	−
24	$N(CH_2CH_2CH_3)_2$	2	MeI	0.5	0.68	2.0	−	+++
25	1-piperidinyl	1	MeI	6.5	4.3	9.0	−	+++
26	1-piperidinyl	2	MeI	<1	1.85	2.2	−	+++
27	4-morpholinyl	1	MeI	13.0	18.0	45.5	−	+++
28	4-morpholinyl	2	MeI	1.9	1.7	3.0	−	+++
controls								
doxorubicin				0.0096	0.0063	0.078	++	+++
mitoxantrone				0.00055	0.00265	0.0053	+	+++

[a] Number of methylenes in linker side chain (see Figure 1). [b] Salts used refer to dihydrochloride, diacetate, and dimethiodide, respectively. [c] Concentration required to inhibit cell growth by 50% relative to controls. [d] Key: (+++) total, (++) significant, (+) slight, or (−) no inhibition.

Table 2. Human Telomerase Inhibition Data for Disubstituted Anthracene-9,10-diones

compd	isomer	n[a]	salt[b]	$^{tel}IC_{50}$[c] (μM)
6	1,4	2	HCl	50
9	1,4	2	HCl	33.5
12	1,4	2	MeI	11.1
13	1,4	2	MeI	9.4
14	1,4	2	MeI	34.5
16	2,6	2	AcOH	>50
17	2,6	1	AcOH	>>50
18	2,6	2	AcOH	4.5
20	2,6	1	AcOH	>>50
21	2,6	2	AcOH	>50
22	2,6	2	AcOH	16.5
23	2,6	2	MeI	17.3
ddGTP				8.6

[a] Number of methylenes in linker side chain (see Figure 1). [b] Salts used refer to dihydrochloride, diacetate, and dimethiodide, respectively. [c] Concentration required to inhibit telomerase activity by 50% relative to controls.

Compounds which showed no inhibitory activity at either 10 or 50 μM in the *Taq* polymerase assay were subsequently evaluated for their ability to inhibit telomerase activity, using the TRAP method. A typical result is shown in Figure 2B. Telomeric repeat ladders are seen in the 0.02- and 0.04-μg protein positive control (lanes 2 and 3, respectively), while ladders were not produced either in the absence of telomerase (lane 1) or when heat inactivation of the protein extract was performed (lane 4). Compound **18** was shown to completely inhibit the reaction at a concentration of 25 μM (lane 5), and compounds **9** (lane 7) and especially **16** (lane 6) showed less inhibitory activity at 25 μM. 2′,3′-Dideoxyguanosine 5′-triphosphate (ddGTP), which showed no inhibition in the *Taq* polymerase assay at either 10 or 50 μM, was used as a positive control in the TRAP assay since it has been described previously to show telomerase inhibition.[19] A summary of results obtained from the TRAP assay ($^{tel}IC_{50}$ values) for all

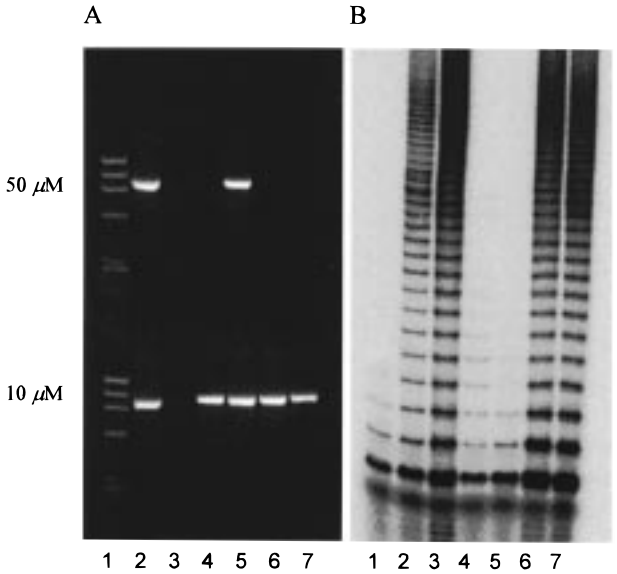

Figure 2. (A) Inhibition of *Taq* polymerase at either 50 μM (upper band) or 10 μM (lower band) of added compound. Lane 1, molecular weight markers; lane 2, water PCR control (900-bp product); lane 3, doxorubicin; lane 4, **25**; lane 5, **18**; lane 6, **19**; lane 7, **7**. (B) Typical result of a TRAP experiment. Lane 1, lysis buffer negative control; lane 2, positive control 0.02 μg of telomerase protein; lane 3, positive control 0.04 μg of protein; lane 4, heat-inactivated negative control; lane 5, **18** (25 μM); lane 6, **16** (25 μM); lane 7, **9** (25 μM).

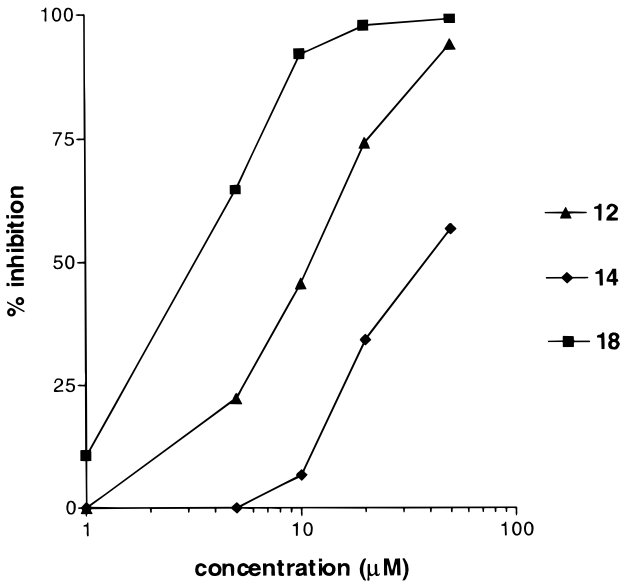

Figure 3. Dose−response plots for compounds **12**, **14**, and **18**, with telomerase inhibition measured by the TRAP assay.

"*Taq*-negative" compounds is given in Table 2. Figure 3 shows, for three representative compounds, the dose−response plots for variation of telomerase inhibition with respect to concentration. In all three cases a broadly log-linear increase in inhibition was observed with increasing concentration, with **18** being more potent than either **12** or **14**. We were unable to determine $^{tel}IC_{50}$ values for either doxorubicin or mitoxantrone due to inhibition of *Taq* polymerase at concentrations above 1 μM. However, both drugs showed only minimal activity against telomerase at concentrations below this level.

Discussion

We have previously developed series of isomeric 1,4- and 2,6-diamidoanthraquinones as duplex DNA-interactive agents.[29,30] These compounds have the ability to differentiate between duplex and triplex DNA, as subsequently shown by the triplex-stabilizing effects of the 2,6-isomers compared to the triplex-disrupting properties of compounds in the 1,4-isomeric series.[33] Molecular modeling studies suggested that appropriate members of the 2,6-isomeric series in particular could also interact with four-stranded DNA. This hypothesis has been supported by biophysical studies[22−24] on short-length telomeric sequences, which have shown that discrete complexes are formed with G-quadruplex structures. These, together with the present studies demonstrating activity against the telomerase enzyme for a range of 1,4- and 2,6-diamidoanthraquinones, are consistent with our proposal[22] that a drug−G-quadruplex binary complex is formed by these compounds. This may be the necessary initial event that results in telomerase inhibition and secession of further telomere extension. Such a mechanism, if it is to be therapeutically useful, requires compounds to have the ability to effectively discriminate between duplex and quadruplex DNA. *Taq* polymerase requires duplex DNA for activity. Thus, amidoanthraquinones which favor duplex binding would be expected to inhibit *Taq*; such compounds (Table 1) have been assumed to be nonselective and have not been studied further.

Some indications of structure−activity relationships for telomerase inhibition are evident from the results presented here. Compounds containing piperidine or functionalized piperidine substituents were consistently found to be the more potent inhibitors. For example, the most active compound from the TRAP assay, with a $^{tel}IC_{50}$ of 4.5 μM, is the piperidine 2,6-anthraquinone derivative **18**. The piperidine and 2-(hydroxymethyl)-piperidine quaternary dimethiodide salts (**12** and **13**, respectively) also show good activity (with $^{tel}IC_{50}$ values of 11.1 and 9.4 μM, respectively). The activities of the two morpholino 1,4-anthraquinone derivatives (**9** and **14**) are lower, with $^{tel}IC_{50}$ values of 33.5 and 34.5 μM, respectively. This suggests that for these derivatives, with weak basic end groups, reduced cationic charge results in reduced telomerase inhibition. Both the dipropylamino 1,4- and 2,6-anthraquinone derivatives (**6** and **16**) exhibit only moderate activity whereas the two compounds (**17** and **20**) containing a shorter ($n = 1$) side chain linker were essentially found to be inactive at concentrations up to 50 μM. In general, a bulky cationic substituent at the terminus of the side chain appears to produce optimum activity against telomerase, at least in the amidoanthraquinone series examined. The level of activity of compound **18** (4.5 μM) is very comparable with that recently reported[24] for a potent porphyrin-based inhibitor of telomerase, with a $^{tel}IC_{50}$ of 6.5 μM. These represent some of the most potent nonnucleoside telomerase inhibitors reported to date.

We have previously shown[22] that compound **1** has a $^{tel}IC_{50}$ of 23 μM and an almost complete inhibition of telomerase activity at 100 μM, using a non-PCR based telomerase primer extension assay. However, the present study shows that this compound significantly inhibits *Taq* polymerase activity at 50 μM. In common with other similarly behaving compounds in Table 1, it was not evaluated in the TRAP assay in view of its potential nonselective affinity for DNA polymerases. The recent crystal structure of *Taq* polymerase[34] indicates that its active site shares at least some common features with other DNA polymerases.[35]

Comparison of Tables 1 and 2 shows that certain amidoanthraquinones, notably compounds **18** and **22**, have in vitro cytotoxic potency which is at a level comparable to that of their inhibitory activity against telomerase, with $IC_{50} \approx {}^{tel}IC_{50}$. If such compounds are to have application as antitumor agents, with telomerase inhibition in tumor cells leading to the attrition of telomere length and consequent senescence, then conventional cytotoxicity should be minimal (i.e., $IC_{50} \gg {}^{tel}IC_{50}$), since long-term administration of such agents would be required. By contrast the established anthraquinone-based anticancer drugs doxorubicin and mitoxantrone, even though they show telomerase activity at levels similar to those exhibited by the compounds presented here, are typically several orders of magnitude more cytotoxic (Table 1). It is striking that the data in Tables 1 and 2 indicate little, if any, correlation between cytotoxicity and telomerase inhibition, indicating that the two effects are unrelated.

The precise role of telomerase in tumorigenesis has yet to be fully established, and thus its relevance as a selective target for chemotherapy remains to be proven.

There is now abundant evidence for selective telomerase expression in tumor cells and tissues (see for example, refs 10−14), with increased levels being associated with disease progression and decreased disease-free survival.[36] The provocative finding that telomerase is not required for oncogenic transformation in mice,[37] even though telomere shortening was observed in these telomerase knockout animals, has led to suggestions that the enzyme may not be playing an essential role in tumor formation, at least in mice. However, apart from the well-documented large differences in length between murine and human telomeres, it is apparent that a minority of human tumors maintain telomere length by a non-telomerase mechanism,[38] and it is conceivable that murine tumors operate by an analogous control process. A longer-term study[39] of telomerase knockout mice has very recently found chromosomal and other abnormalities consistent with the hypothesis that telomerase is essential for cells with high proliferation rates. The role of telomerase in human cells has been clarified by the demonstration[40] that transfection of normal human cells with telomerase results in increased telomere lengths and the bypassing of cellular senescence. The strong implication is that the lack of the senescence stage in tumor cells and their consequent immortalization can be reversed by the inhibition of telomerase, using agents such as those described here. The results of such studies will be reported elsewhere.

Experimental Section

Synthetic Chemistry. Melting points (mp) are uncorrected. IR and UV−visible spectra were recorded using Perkin-Elmer 1720X-FT and Shimadzu UV-2101 PC instruments, respectively. NMR spectra were recorded at 250 MHz on a Bruker AC250 spectrometer in d_6-Me_2SO solution at 293 ± 1 K using Me_4Si as internal standard. Electron impact mass spectra were obtained on a VG7070H spectrometer (115−150 °C source temperature) at an ionization energy of 70 eV. Elemental analyses were carried out by Medac Ltd. (Brunel Science Centre, Egham, Surrey, U.K.); results for elements indicated by symbols were within ±0.4% of theoretical values. TLC was carried out using silica gel (Merck 60F-254), with visualization at 254 and 366 nm. *N*,*N*-Dimethylformamide (DMF) was distilled under reduced pressure from molecular sieves (4 Å) before use. Ether refers to sodium-dried diethyl ether. 1,4-Bischloro-propionamide **2** and derivatives **7** and **12** were prepared as described previously.[29] 2,6-Bischloro-acetamide **3** and -propionamide **4**, together with the 2,6-disubstituted derivatives **1**, **17**−**22**, and **25**−**28** were prepared as described previously.[30]

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (5). General Aminolysis Procedure for the 1,4-Derivatives. Dimethylamine (5.4 mL of 33% w/w solution in EtOH, 30 mmol) was added during 15 min to a stirred, refluxing suspension of **3** (1.0 g, 2.4 mmol) in EtOH (50 mL). After reflux for 40 min, at which time a TLC (EtOH containing 0.1% v/v aqueous NH_4OH, sp gr 0.88) indicated completion of reaction, the mixture was concentrated and then chilled in ice. The solid that separated was removed by filtration and washed with dry ether. Recrystallization from EtOH afforded aminoamide **5** (1.02 g, 91%) as a brown powder: mp 167−168 °C; IR (KBr) 3435 (NH), 3247, 2944, 1697 (C═O), 1636 (quinone C═O), 1592, and 1582 cm^{-1}; NMR δ 2.24 (s, 12H, NCH_3), 2.64 (br m, 8H, COCH_2CH_2N), 7.93 (dd, J = 5.7, 3.3 Hz, 2H, H-6,7), 8.18 (dd, J = 5.7, 3.3 Hz, 2H, H-5,8), 8.89 (s, 2H, H-2,3), and 12.27 (s, D_2O removes, 2H, CONH); MS (rel intensity) *m*/*z* 436 ([M]$^{\bullet+}$, 6), 391 ([M − C_2H_7N]$^{\bullet+}$, 7), 346 ([M − $C_4H_{14}N_2$]$^{\bullet+}$, 56), 292 ([M − $C_7H_{16}N_2O$]$^{\bullet+}$, 36), 238 ([M − $C_{10}H_{18}N_2O_2$]$^{\bullet+}$, 50), and 56 ([C_3H_4O]$^{\bullet+}$, 100); UV−vis [CH_3OH, λ (log ϵ)], 254 (5.77), 310 (5.05), and 463 (4.84) nm. Anal. ($C_{24}H_{28}N_4O_4\cdot0.25H_2O$) C, H, N. Dihydrochloride salt: mp >280 °C dec.

1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (6). Chloroamide **3** was treated with dipropylamine, according to the general aminolysis procedure, to give aminoamide **6** (89%) as a red-brown solid: mp 97−98 °C; NMR δ 0.81 (t, J = 7.3 Hz, 12H, CH_3), 1.39 (m, J = 7.3 Hz, 8H, $CH_2CH_2CH_3$), 2.39 (t, J = 7.3 Hz, 8H, $CH_2CH_2CH_3$), 2.58 (t, J = 7.0 Hz, 4H, $COCH_2CH_2N$), 2.81 (t, J = 7.0 Hz, 4H, $COCH_2$-CH_2N), 7.95 (dd, J = 5.7, 3.3 Hz, 2H, H-6,7), 8.19 (dd, J = 5.7, 3.3 Hz, 2H, H-5,8), 8.91 (s, 2H, H-2,3), and 12.21 (s, D_2O removes, 2H, CONH). Anal. ($C_{32}H_{44}N_4O_4\cdot0.25H_2O$) C, H, N. Dihydrochloride salt: mp 212−213 °C.

1,4-Bis[3-[2-(2-hydroxymethyl)piperidino]propionamido]anthracene-9,10-dione (8). Chloroamide **3** was treated with 2-piperidinemethanol, according to the general aminolysis procedure, to give aminoamide **8** (70%) as a red powder: mp 125−126 °C; NMR δ 1.28−1.73 (m, 12H, NCH_2-$(CH_2)_3$), 2.33 (m, 4H, $NCH_2(CH_2)_3$), 2.64−2.91 (m, 8H, CO-CH_2CH_2N), 3.14 (t, J = 6.4 Hz, 2H, C$H$$CH_2$), 3.55 (br s, 4H, C$H_2$OH), 4.46 (br s, D_2O removes, 2H, OH), 7.95 (dd, J = 5.7, 3.3 Hz, 2H, H-6,7), 8.18 (dd, J = 5.7, 3.3 Hz, 2H, H-5,8), 8.87 (s, 2H, H-2,3), and 12.17 (s, D_2O removes, 2H, CONH). Anal. ($C_{32}H_{40}N_4O_6\cdot H_2O$) C, H, N.

1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,-10-dione (9). Chloroamide **3** was treated with morpholine (3 h reflux) to give aminoamide **9** (93%) as a red-brown powder: mp 186−187 °C; NMR δ 2.50 (t, J = 4.6 Hz, 8H, N(CH_2-CH_2)$_2$O), 2.69 (m, 8H, $COCH_2CH_2N$), 3.61 (t, J = 4.6 Hz, 8H, N(CH_2CH_2)$_2$O), 7.96 (dd, J = 5.7, 2.4 Hz, 2H, H-6,7), 8.20 (dd, J = 5.7, 2.4 Hz, 2H, H-5,8), 8.88 (s, 2H, H-2,3), and 12.21 (s, D_2O removes, 2H, CONH). Anal. ($C_{28}H_{32}N_4O_6$) C, H, N. Dihydrochloride salt: mp 274−275 °C dec.

Preparation of Hydrochloric Acid Addition Salts. General Procedure. HCl gas was passed into a stirred solution or suspension of the aminoamide (1−2 mmol) in acetone at 0−5 °C until saturation. The solid that separated was filtered, washed with dry ether (3 × 20 mL), and dried in vacuo at 25 °C. The acid addition salts were typically formed as amorphous powders in 85−95% yield.

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione (15). General Aminolysis Procedure for the 2,6-Derivatives. Dimethylamine (20 mL of 33% w/w solution in EtOH, 0.112 mol) was added during 15 min to a stirred, refluxing suspension of **5** (4.0 g, 9.5 mmol) in EtOH (100 mL). After 4 h of reflux, at which time TLC (EtOH containing 0.1% v/v NH_4OH, sp gr 0.88) indicated completion of reaction, the reaction mixture was chilled in ice. The solid that separated was removed by filtration and washed with dry ether. Recrystallization from EtOH afforded the aminoamide **15** (3.84 g, 92%) as a brown powder: mp >300 °C; IR (KBr) 3343 (NH), 2979, 2944, 2822, 2767, 1704 (C═O), 1659 (quinone C═O), 1576, and 1527 cm^{-1}; NMR δ 2.19 (s, 12H, NCH_3), 2.51 (t, J = 5.2 Hz, 4H, $COCH_2CH_2N$), 2.58 (t, J = 5.2 Hz, 4H, $COCH_2$-CH_2N), 8.04 (dd, J = 8.6, 1.9 Hz, 2H, H-3,7), 8.14 (d, J = 8.6 Hz, 2H, H-4,8), 8.41 (d, J = 1.9 Hz, 2H, H-1,5), and 10.67 (s, D_2O removes, 2H, CONH); MS (rel intensity) *m*/*z* 437 ([M + 1]$^{\bullet+}$, 2), 436 ([M]$^{\bullet+}$, 4), 391 ([M − C_2H_7N]$^{\bullet+}$, 13), 346 ([M − $C_4H_{14}N_2$]$^{\bullet+}$, 75), 292 ([M − $C_7H_{16}N_2O$]$^{\bullet+}$, 45), 238 ([M − $C_{10}H_{18}N_2O_2$]$^{\bullet+}$, 80), 56 ([C_3H_4O]$^{\bullet+}$, 100), and 45 ([C_2H_7N]$^{\bullet+}$, 100); UV−vis [CH_3OH, λ (log ϵ)], 233 (5.63), 277 (5.83), 305 (5.65), and 352 (5.18) nm. Anal. ($C_{24}H_{28}N_4O_4\cdot0.25H_2O$) C, H; N: calcd, 12.70; found, 12.29%. Diacetate salt: mp >300 °C.

2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione (16). Refluxing chloroamide **5** with dipropylamine for 9 h and using the general aminolysis procedure for 2,6-isomers gave the aminoamide **16** (84%) as a brown powder: mp 204−205 °C dec; NMR δ 0.83 (t, J = 7.2 Hz, 12H, CH_3), 1.41 (m, J = 7.2 Hz, 8H, $CH_2CH_2CH_3$), 2.36 (t, J = 7.2 Hz, 8H, $CH_2CH_2CH_3$), 2.50 (m, 4H, $COCH_2CH_2N$), 2.76 (t, J = 6.6 Hz, 4H, $COCH_2CH_2N$), 8.06 (dd, J = 8.5, 2.0 Hz, 2H, H-3,7), 8.16 (d, J = 8.5 Hz, 2H, H-4,8), 8.42 (d, J = 2.0 Hz, 2H, H-1,5),

and 10.65 (s, D_2O removes, 2H, CON*H*). Anal. ($C_{32}H_{44}N_4O_4$) C, H, N. Diacetate salt: mp >200 °C dec.

Preparation of Acetic Acid Addition Salts. General Procedure. A solution of the aminoamide (1−2 mmol) in glacial HOAc (15 mL) is heated to 50−60 °C, treated with decolorizing charcoal (250 mg), and filtered. Trituration of the clear filtrate with ether gives a hygroscopic precipitate which is repeatedly digested with dry ether (3 × 50 mL), filtered, and then washed further with dry ether and dried in vacuo at 25 °C. The acid addition salts were typically formed as amorphous powders in 75−90% yield.

1,4-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N′*-Dimethiodide (10). General Procedure for the Preparation of Quaternary Dimethiodide Salts. A mixture containing diamine **5** (1 g, 2.56 mmol), iodomethane (10 mL, 0.16 mol), and acetone (40 mL) was stirred at 25 °C for 24 h. The solid which separates was filtered, washed with dry ether (3 × 50 mL), and dried in vacuo at 25 °C to give the bis(methylammonium) quaternary methiodide salt **10** (1.63 g, 99%) as an amorphous powder: mp 231−232 °C; NMR δ 3.15 (s, 18H, NC*H*$_3$), 3.23 (t, J = 7.4 Hz, 4H, COCH$_2$C*H*$_2$N), 3.70 (t, J = 7.4 Hz, 4H, COC*H*$_2$CH$_2$N), 8.00 (dd, J = 5.8, 3.3 Hz, 2H, H-6,7), 8.22 (dd, J = 5.8, 3.3 Hz, 2H, H-5,8), 8.87 (s, 2H, H-2,3), and 12.19 (s, D_2O removes, 2H, CON*H*); UV−vis [CH_3-OH, λ (log ϵ)], 221 (5.66), 256 (5.85), 318 (5.09), and 462 (4.85) nm. Anal. ($C_{26}H_{34}N_4O_4I_2$) C, H, N, I.

1,4-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione *N,N′*-Dimethiodide (11). The general procedure was used to give **11** (96%) as an amorphous powder: mp 153−154 °C; NMR δ 0.93 (t, J = 7.1 Hz, 12H, CH$_2$CH$_2$C*H*$_3$), 1.72 (m, J = 7.1 Hz, 8H, CH$_2$C*H*$_2$CH$_3$), 3.05 (s, 6H, N$^+$C*H*$_3$), 3.15−3.45 (m, 12H, C*H*$_2$CH$_2$CH$_3$ and COCH$_2$C*H*$_2$N), 3.63 (t, J = 7.2 Hz, 4H, COC*H*$_2$CH$_2$N), 8.00 (dd, J = 5.8, 3.3 Hz, 2H, H-6,7), 8.23 (dd, J = 5.8, 3.3 Hz, 2H, H-5,8), 8.88 (s, 2H, H-2,3), and 12.19 (s, D_2O removes, 2H, CON*H*). Anal. ($C_{34}H_{50}N_4O_4I_2$·0.5H_2O) C, H, N, I.

1,4-Bis[3-[2-(2-hydroxymethyl)piperidino]propionamido]anthracene-9,10-dione *N,N′*-Dimethiodide (13). The general procedure was used to give **13** (97%) as an amorphous powder: mp 175−176 °C; NMR δ 1.45−1.90 (m, 12H, NCH$_2$(C*H*$_2$)$_3$), 3.08 (s, 6H, N$^+$C*H*$_3$), 3.30−3.60 (m, 12H, NC*H*$_2$(CH$_2$)$_3$ and COC*H*$_2$C*H*$_2$N), 3.70−3.90 (m, 6H, C*H*CH$_2$ and C*H*$_2$OH), 5.38 (br s, D_2O removes, 2H, O*H*), 8.01 (dd, J = 5.8, 3.2 Hz, 2H, H-6,7), 8.21 (dd, J = 5.8, 3.2 Hz, 2H, H-5,8), 8.90 (s, 2H, H-2,3), and 12.23 (s, D_2O removes, 2H, CON*H*). Anal. ($C_{34}H_{46}N_4O_6I_2$·0.25H_2O) C, H, N, I.

1,4-Bis[3-(4-morpholino)propionamido]anthracene-9,-10-dione *N,N′*-Dimethiodide (14). The general procedure was used to give **14** (98%) as an amorphous powder: mp 234−235 °C; NMR δ 3.22 (s, 6H, C*H*$_3$), 3.29 (m, 4H, COCH$_2$C*H*$_2$N), 3.53 (m, 8H, N(C*H*$_2$CH$_2$)$_2$O), 3.85 (m, 4H, COC*H*$_2$CH$_2$N), 4.15 (m, 8H, N(CH$_2$C*H*$_2$)$_2$O), 8.00 (dd, J = 5.8, 2.2 Hz, 2H, H-6,7), 8.23 (dd, J = 5.8, 2.2 Hz, 2H, H-5,8), 8.90 (s, 2H, H-2,3), and 12.22 (s, D_2O removes, 2H, CON*H*). Anal. ($C_{30}H_{38}N_4O_6I_2$) C, H, N, I.

2,6-Bis[3-(dimethylamino)propionamido]anthracene-9,10-dione *N,N′*-Dimethiodide (23). The general procedure was used to give **23** (99%) as an amorphous powder: mp 266−267 °C dec; IR (KBr) 3440 (br, NH), 3098, 3050, 1700 (C=O), 1666 (quinone C=O), 1590, and 1533 cm^{-1}; NMR δ 3.01 (t, J = 7.3 Hz, 4H, COCH$_2$C*H*$_2$N), 3.12 (s, 18H, N$^+$(C*H*$_3$)$_3$), 3.70 (t, J = 7.3 Hz, 4H, COC*H*$_2$CH$_2$N), 8.04 (dd, J = 8.5, 1.4 Hz, 2H, H-3,7), 8.19 (d, J = 8.5 Hz, 2H, H-4,8), 8.47 (d, J = 1.4 Hz, 2H, H-1,5), and 10.87 (s, D_2O removes, 2H, CON*H*); UV−vis [CH_3OH, λ (log ϵ)], 220 (5.82), 277 (5.84), 303 (5.67), and 349 (5.14) nm. Anal. ($C_{26}H_{34}N_4O_4I_2$) H, N, I; C: calcd, 43.35; found, 43.81%.

2,6-Bis[3-(dipropylamino)propionamido]anthracene-9,10-dione *N,N′*-Dimethiodide (24). The general procedure was used to give **24** (96%) as an amorphous powder: mp 203−204 °C dec; NMR δ 0.92 (t, J = 7.1 Hz, 12H, CH$_2$CH$_2$C*H*$_3$), 1.71 (m, J = 7.1 Hz, 8H, CH$_2$C*H*$_2$CH$_3$), 2.97 (t, J = 7.2 Hz, 4H, COCH$_2$C*H*$_2$N), 3.03 (s, 6H, N$^+$C*H*$_3$), 3.20−3.27 (m, 4H, C*H*$_2$CH$_2$CH$_3$), 3.61 (t, J = 7.2 Hz, 4H, COC*H*$_2$CH$_2$N), 8.05 (dd, J = 8.4, 1.9 Hz, 2H, H-3,7), 8.21 (d, J = 8.4 Hz, 2H, H-4,8), 8.47 (d, J = 1.9 Hz, 2H, H-1,5), and 10.82 (s, D_2O removes, 2H, CON*H*). Anal. ($C_{34}H_{50}N_4O_4I_2$) C, H, N; I: calcd, 30.48; found, 29.98%.

Biological Studies. *Taq* Polymerase Assay. Prior to the evaluation of compounds in the PCR-based telomerase assay (see below) the agents were tested for their ability to inhibit *Taq* polymerase in order to address the selectivity of polymerase/telomerase inhibition. Compounds (acid addition and quaternary dimethiodide salts) were included at both 10 and 50 μM final concentrations in a PCR 50-μL master mix comprising 10 ng of pCI-neo mammalian expression vector (Promega, Southampton, U.K.), forward (GGAGTTCCGCGTTACATAAC) and reverse (GTCTGCTCGAAGCATTAACC) primers (200 nmol), reaction buffer (75 mM Tris-HCl, pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% v/v Tween 20), 2.5 mM $MgCl_2$, 200 μM of each deoxynucleotide triphosphate, and thermostable DNA polymerase ("red hot", Advanced Biotechnologies) 1.25 units. A reaction mix containing water and no drug was used as a positive control, producing a product of approximately 1 kb. Amplification (30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2.5 min) were performed using a thermal cycler (Hybaid, U.K.). PCR products were then separated by electrophoresis on a 2% w/w agarose gel and visualized using ethidium bromide.

Modified Telomeric Repeat Amplification Protocol (TRAP) Assay. The ability of agents to inhibit telomerase in a cell-free assay was assessed using modifications of the previously described TRAP assay.[32] Initially, a protein extract was prepared from exponentially growing A2780 human ovarian carcinoma cells by lysing them for 30 min on ice in a CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate)-based buffer (0.5% w/w CHAPS, 10 mM Tris-HCl (pH 7.5), 1 mM $MgCl_2$, 1 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′*,N′-tetraacetic acid (EGTA), 5 mM 2-mercaptoethanol, and 10% v/v glycerol) with 0.1 mM AEBSF (4-(2-aminoethyl)benzenesulfonyl fluoride) freshly added. The lysate was then centrifuged at 12 000 rpm for 30 min at 4 °C and the supernatant collected and stored in aliquots at −80 °C for up to 3 months. Total cellular protein was then determined (BioRad Laboratories). The TRAP assay was then performed in 2 steps (a) telomerase-mediated extension of a nontelomeric oligonucleotide forward primer (TS, 5′-AATCCGTCGAGCAGAGTT, Oswel Ltd., Southampton, U.K.) contained in a 40 μL reaction mix comprising TRAP buffer (20 mM Tris-HCl (pH 8.3), 68 mM KCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 0.05% v/v Tween 20), 0.05 μg of bovine serum albumin, 50 μM of each deoxynucleotide triphosphate, 0.1 μg of TS primer, and 3 μCi of [α-^{32}P]dCTP (Amersham plc, U.K.). Protein (0.04 μg) was then incubated with the reaction mix ± agent (acid addition and quaternary dimethiodide salts) at final concentrations of up to 50 μM for 20 min at 25 °C. A lysis buffer (no protein) control, heat-inactivated protein control, and 50% protein (0.02 μg) control were included in each assay. (b) While the mixture was being heated at 80 °C in a PCR block of a thermal cycler (Hybaid, U.K.) for 5 min to inactivate telomerase activity, 0.1 μg of reverse CX primer (3′-AATCCCATTCCCATTCCCATTCCC-5′) and 2 units of *Taq* DNA polymerase (red hot, Advanced Biotechnologies) were added. A 3-step PCR was then performed 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min for 31 cycles. Telomerase-extended PCR products in the presence or absence of compounds were then determined either by electrophoretic separation using 8% w/w acrylamide denaturing gels and analysis by phosphorimaging or autoradiography or by harvesting on Whatman filters (25 mm glass microfiber). PCR products were precipitated on ice for 60 min, using 5% trichloroacetic acid with 20 mM tetrasodium pyrophosphate, and harvested onto filters using a Millipore 1225 sampling manifold (Millipore, Bedford, MA). Quantitation was then achieved by liquid scintillation counting (Ultima Gold, Packard, Wallac 1410 counter).

Growth Inhibition Assay. Growth inhibition was measured against a panel of human ovarian carcinoma cell lines

(A2780, CH1, and SKOV-3), using the sulforhodamine B (SRB) assay as described previously.[31] Cell lines were maintained free of Mycoplasma and were grown as monolayers in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (Imperial Laboratories, Andover, U.K.), 2 mM glutamine and 0.5 μg mL^{-1} hydrocortisone in a humidified 6% CO_2/94% air atmosphere. Between 3000 and 6000 cells, dependent on the doubling time of the cell line, were seeded into the wells of 96-well microtiter plates and allowed to attach overnight. Agents (acid addition and quaternary dimethiodide salts) were dissolved at 500 μM in water and immediately added to wells in quadruplicate at final concentrations of 0.05, 0.25, 1, 5, and 25 μM. Following an incubation period of 96 h, the number of attached cells per well were assessed by fixing with ice-cold 10% w/v trichloroacetic acid (30 min) and staining with 0.4% SRB in 1% v/v acetic acid (15 min). After washing and air-drying overnight, protein-bound SRB was solubilized in 10 mM Tris, and the optical absorbance per well was determined at 540 nm (Titertek Multiscan MCC/340 MkII plate reader, Flow Laboratories). Mean absorbance for each drug concentration was expressed as a percentage of the control untreated well absorbance, and IC_{50} values (concentration required to inhibit cell growth by 50%) were determined for each agent.

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References

(1) Blackburn, E. H. Structure and Function of Telomeres. *Nature* **1991**, *350*, 569−573.
(2) Meyne, J.; Ratliff, R. L.; Moyzis, R. K. Conservation of the Human Telomere Sequence $(TTAGGG)_n$ among Vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 7049−7053.
(3) Harley, C. B.; Futcher, A. B.; Greider, C. W. Telomeres Shorten during Ageing of Human Fibroblasts. *Nature* **1990**, *345*, 458−460.
(4) Allsopp, R. C.; Harley, C. B. Evidence for a Critical Telomere Length in Senescent Human Fibroblasts. *Exp. Cell Res.* **1995**, *219*, 130−136.
(5) Ishikawa, F. Telomere Crisis, the Driving Force in Cancer Cell Evolution. *Biochem. Biophys. Res. Commun.* **1997**, *230*, 1−6.
(6) Morin, G. B. The Human Telomere Terminal Transferase Activity is a Ribonucleoprotein that Synthesises TTAGGG Repeats. *Cell* **1989**, *59*, 521−529.
(7) Feng, J.; Funk, W.; Wang, S.-S.; Weinrich, S. L.; Avilion, A. A.; Chiu, C.-P.; Adams, R. R.; Chang, E.; Allsopp, R. C.; Yu, J.; Le, S.; West, M. D.; Harley, C. B.; Andrews, W. H.; Greider, C. W.; Villeponteau, B. The RNA Component of Human Telomerase. *Science* **1995**, *269*, 1236−1241.
(8) Meyerson, M.; Counter, C. M.; Eaton, E. N.; Ellison, L. W.; Steiner, P.; Caddle, S. D.; Ziaugra, L.; Beijersbergen, R. L.; Davidoff, M. J.; Liu, Q.; Bacchetti, S.; Haber, D. A.; Weinberg, R. A. hEST2, the Putative Human Telomerase Catalytic Subunit Gene, is Up-regulated in Tumor Cells and during Immortalization. *Cell* **1997**, *90*, 785−795.
(9) Nakamura, T. M.; Morin, G. B.; Chapman, K. B.; Wenrich, S. L.; Andrews, W. H.; Lingner, J.; Harley, C. B.; Cech, T. R. Telomerase Catalytic Subunit Homologs from Fission Yeast and Human. *Science* **1997**, *277*, 955−959.
(10) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Coviello, G. M.; Wright, W. E.; Weinrich, R. L.; Shay, J. W. Specific Association of Human Telomerase Activity with Immortal Cells and Cancer. *Science* **1994**, *266*, 2011−2015.
(11) Counter, C. M.; Hirte, H. W.; Bacchetti, S.; Harley, C. B. Telomerase Activity in Human Ovarian Carcinoma. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 2900−2904.
(12) Sommerfeld, H. J.; Meeker, A. K.; Piatyszek, M. A.; Bova, G. S.; Shay, J. W.; Coffey, D. S. Telomerase Activity: a Prevalent Marker of Malignant Human Prostate Tissue. *Cancer Res.* **1996**, *56*, 218−222.
(13) Tatematsu, K.; Nakayama, J.; Danbara, M.; Shionoya, S.; Sato, H.; Omine, M.; Ishikawa, F. A Novel Quantitative 'Stretch PCR Assay' that Detects a Dramatic Increase in Telomerase Activity during the Progression of Myeloid Leukemias. *Oncogene* **1996**, *13*, 2265−2274.
(14) Landberg, G.; Nielsen, N. H.; Nilsson, P.; Emdin, S. O.; Cajander, J.; Roos, G. Telomerase Activity is Associated with Cell Cycle Deregulation in Human Breast Cancer. *Cancer Res.* **1997**, *57*, 549−554.
(15) (a) Raymond, E.; Sun, D.; Chen, S.-F.; Windle, B.; von Hoff, D. D. Agents that Target Telomerase and Telomeres. *Curr. Opin. Biotechnol.* **1996**, *7*, 583−591. (b) Hamilton, S. E.; Corey, D. R. Telomerase: Anti-cancer Target or just a Fascinating Enzyme? *Chem. Biol.* **1996**, *3*, 863−867. (c) Parkinson, E. K. Do Telomerase Antagonists Represent a Novel Anti-cancer Strategy? *Brit. J. Cancer* **1996**, *73*, 1−4.
(16) Norton, J. C.; Piatyszek, M. A.; Wright, W. E.; Shay, J. W.; Corey, D. R. Inhibition of Human Telomerase Activity by Peptide Nucleic Acids. *Nature Biotechnol.* **1996**, *14*, 615−619.
(17) Fletcher, T. M.; Salazar, M.; Chen, S.-F. Human Telomerase Inhibition by 7-Deaza-2′-deoxypurine Nucleoside Triphosphates. *Biochemistry* **1996**, *35*, 15611−15617.
(18) Hamilton, S.E.; Pitts, A. E.; Katipally, R. R.; Jia, X.; Rutter, J. P.; Davies, B. A.; Shay, J. W.; Wright, W. E.; Corey, D. R. Identification of Determinants for Inhibitor Binding within the RNA Active Site of Human Telomerase using PNA Scanning. *Biochemistry* **1997**, *36*, 11873−11880.
(19) Strahl, C.; Blackburn, E. H. The Effects of Nucleoside Analogs on Telomerase and Telomeres in *Tetrahymena*. *Nucleic Acids Res.* **1994**, *22*, 893−900.
(20) Wang, Y.; Patel, D. J. Solution Structure of the Human Telomeric Repeat $d[AG_3(T_2AG_3)_3]$ G-tetraplex. *Structure* **1993**, *1*, 263−282.
(21) Zahler, A. M.; Williamson, J. R.; Cech, T. R.; Prescott, D. M. Inhibition of Telomerase by G-quartet DNA Structures. *Nature* **1991**, *350*, 718−720.
(22) Sun, D.; Thompson, B.; Cathers, B. E.; Salazar, M.; Kerwin, S. M.; Tent, J. O.; Jenkins, T. C.; Neidle, S.; Hurley, L. H. Inhibition of Human Telomerase by a G-Quadruplex-interactive Compound. *J. Med. Chem.* **1997**, *40*, 2113-2116.
(23) Anantha, N. V.; Azam.; M.; Sheardy, R. D. Porphyrin Binding to Quadruplexed T_4G_4. *Biochemistry* **1998**, *37*, 2709−2714.
(24) Wheelhouse, R.; Sun, D.; Han, H.; Han, F. X.; Hurley, L. H. Cationic Porphyrins as Telomerase Inhibitors: the Interaction of Tetra-(*N*-methyl-4-pyridyl)porphine with Quadruplex DNA. *J. Am. Chem. Soc.* **1998**, *120*, 3261−3262.
(25) *Anthracycline and Anthracenedione-Based Anticancer Agents*; Lown, J. W., Ed.; Elsevier: Amsterdam, 1988.
(26) Palumbo, M.; Palù, G.; Gia, O.; Antonello, C.; Meloni, G. A. Bis-substituted Hydroxy-anthraquinones: DNA-Binding and Biological Activity. *Anti-Cancer Drug Des.* **1987**, *1*, 337−346.
(27) Gatto, B.; Zagotto, G.; Sissi, C.; Cera, C.; Uriarte, E.; Palù, G.; Capranico, G.; Palumbo, M. Peptidyl Anthraquinones as Potential Antineoplastic Drugs: Synthesis, DNA Binding, Redox Cycling, and Biological Activity. *J. Med. Chem.* **1996**, *39*, 3114−3122.
(28) Zunino, F.; Capranico, G. DNA Topoisomerase II as the Primary Target of Anti-Tumor Anthracyclines. *Anti-Cancer Drug Des.* **1990**, *5*, 307−317.
(29) Collier, D. A.; Neidle, S. Molecular Modeling, DNA Binding and Antitumour Properties of Some Substituted Amidoanthraquinones. *J. Med. Chem.* **1988**, *31*, 847−857.
(30) Agbandje, M.; Jenkins, T. C.; McKenna, R.; Reszka, A. P.; Neidle, S. Anthracene-9,10-diones as Potential Anticancer Agents. Synthesis, DNA Binding and Biological Studies on a Series of 2,6-Disubstituted Derivatives. *J. Med. Chem.* **1992**, *35*, 1418−1429.
(31) Kelland, L. R.; Abel, G.; McKeage, M. J.; Jones, M.; Goddard, P. M.; Valenti, M.; Murrer, B. A.; Harrap, K. R. Preclinical Antitumor Evaluation of Bis-acetato-ammine-dichloro-cyclohexylamine Platinum(IV): an Orally Active Platinum Drug. *Cancer Res.* **1993**, *53*, 2581−2586.
(32) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L. C.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. Specific Association of Human Telomerase Activity with Immortal Cells and Cancer. *Science* **1994**, *266*, 2011−2015.
(33) Fox, K. R.; Polucci, P.; Jenkins, T. C.; Neidle, S. A Molecular Anchor for Stabilising Triple-helical DNA. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7887−7891.
(34) Eom, S. H.; Wang, J.; Steitz, T. A. Structure of *Taq* Polymerase with DNA at the Polymerase Active Site. *Nature* **1996**, *382*, 278−281.
(35) Doublié, S.; Tabor, S.; Long, A. M.; Richardson, C. C.; Ellenberger, T. Crystal Structure of a Bacteriophage T7 DNA Replication Complex at 2.2Å Resolution. *Nature* **1998**, *391*, 251−258.
(36) Clark, G. M.; Osborne, C. K.; Levitt, D.; Wu, F.; Kim, N. W. Telomerase Activity and Survival of Patients with Node-positive Breast Cancer. *J. Natl. Cancer Inst.* **1997**, *89*, 1874−1881.
(37) Blasco, M. A., Lee, H.-W.; Hande, M. P.; Samper, E.; Lansdorp, P. M.; DePinho, R. A.; Greider, C. W. Telomere Shortening and Tumor Formation by Mouse Cells Lacking Telomerase RNA. *Cell* **1997**, *91*, 25−34.

(38) Bryan, T. M.; Englezou, A.; Dalla-Pozza, L.; Dunham, M. A.; Reddel, R. R. Evidence for an Alternative Mechanism for Lengthening Telomeres in Human Tumors and Tumor-derived Cell Lines. *Nature Med.* **1997**, *3*, 1271−1274.
(39) Lee, H.-W.; Blasco, M. A.; Gottlieb, G. J.; Horner, J. W.; Greider, C. W.; DePinho, R. A. Essential Role of Mouse Telomerase in Highly Proliferative Organs. *Nature* **1998**, *392*, 569−574.
(40) Bodnar, A. G.; Ouellette, M.; Frolkis, M.; Holt, S. E.; Chiu, C.-P.; Morin, G. B.; Harley, C. B.; Shay, J. W.; Lichtsteiner, S.; Wright, W. E. Extension of Life-span by Introduction of Telomerase into Normal Human Tumour Cells. *Science* **1998**, *279*, 349−352.

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