Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 4347–4351
Bioorganic & Medicinal Chemistry Letters

Synthesis, biophysical and biological evaluation of 3,6-bis-amidoacridines with extended 9-anilino substituents as potent G-quadruplex-binding telomerase inhibitors

Christoph M. Schultes, Bérangère Guyen, Javier Cuesta[†] and Stephen Neidle[*]

Cancer Research UK Biomolecular Structure Group, University of London School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX, UK

Received 26 March 2004; revised 30 April 2004; accepted 18 May 2004

Abstract—Telomerase and telomere maintenance are emerging targets for the treatment of human cancers. We report here on the targeting of the telomere–telomerase complex with a series of small molecules based on an acridine platform. A series of 3,6-bis-amidoacridines with extended 9-anilino sidechains were designed and synthesised as potential telomeric G-quadruplex DNA (G4) interacting compounds. G4-stabilisation was assessed using a high-throughput FRET (fluorescence resonance energy transfer) assay and telomerase inhibition quantified by a modified TRAP (telomerase repeat amplification protocol) method. Within the series, the compounds showed significant G4-stabilising ability (ΔT_m values of 25–36 °C at 1 μM concentration) and telomerase inhibition in the nanomolar region ($^{tel}EC_{50}$ values of 80–318 nM). Furthermore, a direct correlation between the FRET and TRAP assays was observed, supporting the use of the rapid screening FRET assay for early assessment of potential G4-stabilising telomerase inhibitors.

1. Introduction

The telomerase enzyme complex is increasingly regarded as playing a key role in telomere maintenance and tumourigenesis, and is thus an important target for the development of novel cancer therapeutic agents.[1,2] Various strategies have been proposed to induce telomerase inhibition[3] and a promising method has been to focus on the disruption of the telomere–telomerase interaction via small molecules capable of interacting with the enzyme substrate, thereby preventing telomere elongation by the telomerase complex. A number of structural studies have shown[4–6] that single-stranded DNA present at the 3′-ends of telomeres can fold into higher-order DNA quadruplex structures. The design of small molecules capable of binding selectively to, and stabilising, these structures thus holds promise as a means of influencing telomere maintenance.[1,7]

The rapid screening of the DNA-binding abilities of various compound series by fluorescence techniques is a swift, semi-quantitative way of selecting promising new candidates for further in-depth biological testing.[8–10] Demonstration of a correlation between the results obtained from this type of biophysical screen and the TRAP method[10,11] lends support to the proposed mechanism of action for a particular series of ligands, and would also speed up the process of comparing and evaluating telomerase inhibitors that function in this way.

Previous studies from this laboratory have focused on generating acridine-based molecules with varying patterns of substitution around the aromatic core.[12,13] Coupled to the acridine scaffold, the basic 3,6-bis-(3-pyrrolidin-1-yl-propionamido) substitution pattern has been shown to be an effective starting point for further chemical studies[12] and recent studies in this laboratory have thus focused on refining the structure–activity relationships (SAR) of functional groups at the 9-position (Fig. 1). The nature of this substitution is expected to play a critical role both in enhancing ligand–DNA interactions and in increasing the selectivity of these molecules for quadruplex over duplex DNA (dsDNA),

Keywords: Telomerase; Telomere; Quadruplex DNA; FRET; TRAP; Acridines.
* Corresponding author. Tel.: +44-207-753-5969; fax: +44-207-753-5970; e-mail: stephen.neidle@ulsop.ac.uk
† Present address: Max-Planck-Institut für Molekulare Physiologie, Otto-Hahn-Strasse 11, 44227 Dortmund, Germany.

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.05.090

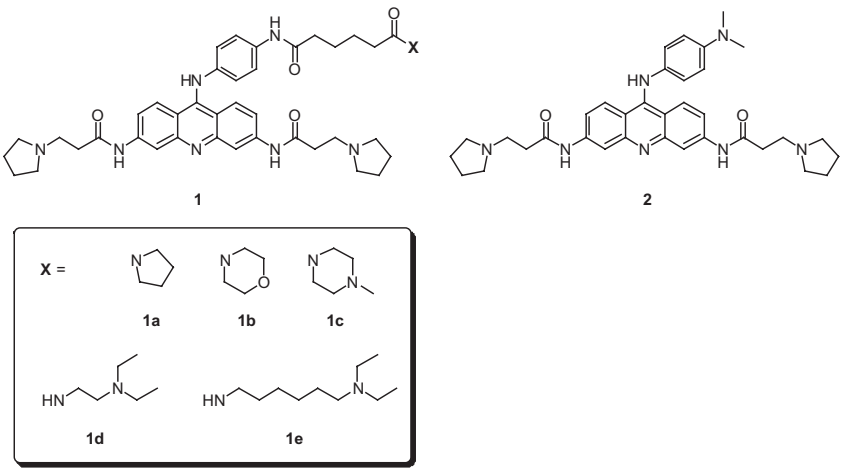


Figure 1. Structures of the trisubstituted acridines examined in this study (**1a**–**e**). The related compound **2** (BRACO-19)[12,14] is included as a reference.

and a number of potent low-nanomolar inhibitors have already been described.[13,14]

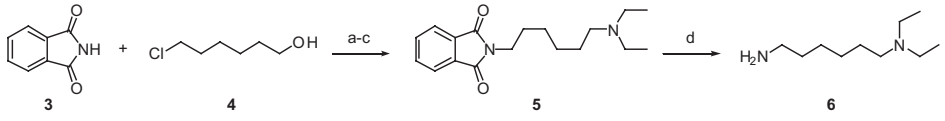
2. Synthesis

Preparation of the 9-anilino side-chains was carried out by reacting *p*-nitroaniline with adipic anhydride,[15] to afford the carbamoyl acid **9** in 86% yield. The latter was then reacted with suitable primary or secondary amines using the carbonic–carboxylic mixed anhydride method, and subsequent reduction of the nitro group using Pd on charcoal afforded the desired anilines **10a**–**e** in a 60 to 83% yield for the three steps (Scheme 2). The synthesis of the only noncommercially available amine **6** was based on the Gabriel synthesis, as has been described[16] (Scheme 1). Each of these anilines was then finally coupled with the 9-chloro-3,6-bis-(3-pyrrolidin-1-yl-propionamido) acridine **11**[14] to yield **1a**–**e** in 38–57% yield (Scheme 3). The final compounds were subsequently purified by flash chromatography using various mixtures of methanol (10–40%), dichloromethane (60–99%) and triethylamine (1–10%).

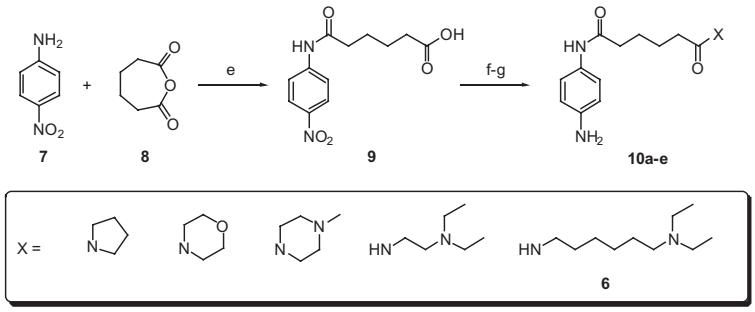
3. Biophysical and biological assays

The compounds were examined in two assays, a FRET (fluorescence resonance energy transfer) assay to assess their G4-stabilising ability and a TRAP (telomerase repeat amplification protocol) method to quantify their telomerase inhibitory effect ($^{tel}EC_{50}$).

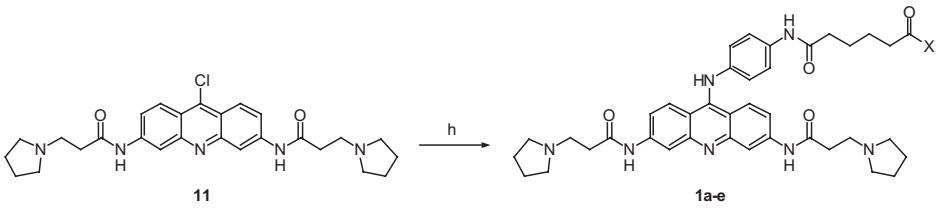
The FRET assay was conducted using the previously-reported protocol,[10] which enables the measurement of relative G4-stabilisation values in a 96-well plate format.



Scheme 1. Reagents and conditions: (a) potassium phthalimide, DMF, 80 °C; (b) CBr_4, PPH_3, CH_3CN, rt, (c) $NHEt_2$, K_2CO_3, 1,4-dioxane, rt; (d) H_2NNH_2, MeOH, rt.



Scheme 2. Reagents and conditions: (a) 1,4-dioxane, reflux; (b) H_2NR or HNR_2, ethyl chloroformate, Et_3N, CH_2Cl_2, 0 °C to rt; (c) 10% Pd/C, ammonium formate, MeOH, rt.



Scheme 3. Reagents and conditions: (a) **10a**–**e**, MeOH, 60 °C.

In brief, changes in the FRET signal upon melting of a DNA probe (telomeric sequence d[GGG(TTAGGG)$_3$] dual-labelled with 6-FAM and TAMRA at the 5′- and 3′-ends, respectively) were observed under conditions approximating those used in the crystallisation of the published structure[5] (50 mM cacodylate buffer, pH 7.4). All five compounds **1a**–**e** showed high levels of G4-stabilisation, with changes in the melting temperature of the native DNA sequence (ΔT_m values) upon ligand addition in the range 25–36 °C (at 1 μM ligand concentration; Table 1). Curves fitted to the data using the Origin package of software (OriginPro, OriginLab Corp.) showed dose–response behaviour when plotting ΔT_m against concentration (Fig. 2), and enabled the calculation of ΔT_m values over a range of concentrations tested. This provides a useful alternative for measuring the stabilising ability (e.g., $[concn]_{\Delta T_m=20\,°C}$: concentration required to achieve a ΔT_m value of 20 °C; Fig. 2 and Table 1), which is irrespective of the curve shape and allows the comparative testing of compounds with large differences in potency over a wide concentration range.

The modified TRAP assay was performed as previously described,[10,11] using 1 μg total protein extract from the A2780 ovarian carcinoma cell line. Compounds **1a**–**e** were examined in this way to a concentration of 1 μM (Fig. 3), and concomitant controls (PCR inhibition assay results, not shown) were carried out to verify that there was no interference with the correct functioning of *Taq* polymerase at these concentrations. Only **1d** and **1e** showed significant PCR inhibition (40–50%) at 1 μM, although this concentration is an order of magnitude higher than the calculated $^{tel}EC_{50}$ values (see below and Table 1).

The two compounds with diethyl amine side chains (**1d** and **1e**) showed the best results in both assays, with G4 stabilisation of over 30 °C at a 1 M ligand concentration and $^{tel}EC_{50}$ values below 100 nM (98 and 80 nM, respectively). The flexibility of the 9-substituted aliphatic amine chains are likely to be an important factor in G4 binding, as this would allow the chains to probe the quadruplex surface in order to optimise charge–charge contacts with the phosphate backbone. However, increased G4–DNA binding in a cellular environment is likely to be offset by the exposed nature of the terminal amide on the 9-position, which might be subject to intracellular amide bond cleavage.

Compounds **1a**–**c** performed less well in the assays, perhaps due to the greater rigidity and bulkiness of the

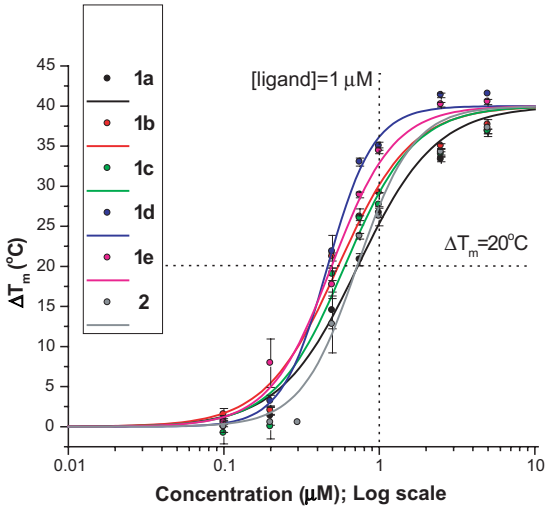


Figure 2. Dose–response curves fitted for the G4-stabilisation (FRET) experiments carried out on compounds **1a**–**e** and **2**. The baseline ($\Delta T_m = 0$) was set at the melting temperature of the native structure (53.2 °C). Dotted lines are intended to serve as visual references for the $concn_{(\Delta T_m=20\,°C)}$ and ΔT_m (at 1 μM) values reported in Table 1 (horizontal and vertical lines, respectively).

Table 1. G4-stabilisation (FRET) and telomerase inhibition (TRAP) results for the acridine derivatives **1a**–**e** and the reference compound **2**

Compound	FRET		TRAP	
	ΔT_m (°C)[a]	$Concn_{(\Delta T_m=20\,°C)}$ (μM)[b]	$^{tel}EC_{50}$ (nM)	Error (nM)
1a	25.4	0.728	318	37
1b	30.2	0.544	267	1
1c	29.2	0.599	165	32
1d	36.2	0.465	98	24
1e	32.9	0.493	80	2
2[c]	27.5	0.720	113	1

[a] ΔT_m values reported at 1 μM compound concentration.
[b] $Concn_{(\Delta T_m=20\,°C)}$ is equivalent to the turning point of the depicted dose–response curves (Fig. 2).
[c] The previously published[12,14] acridine **2** (BRACO-19) is included as a reference (see text).

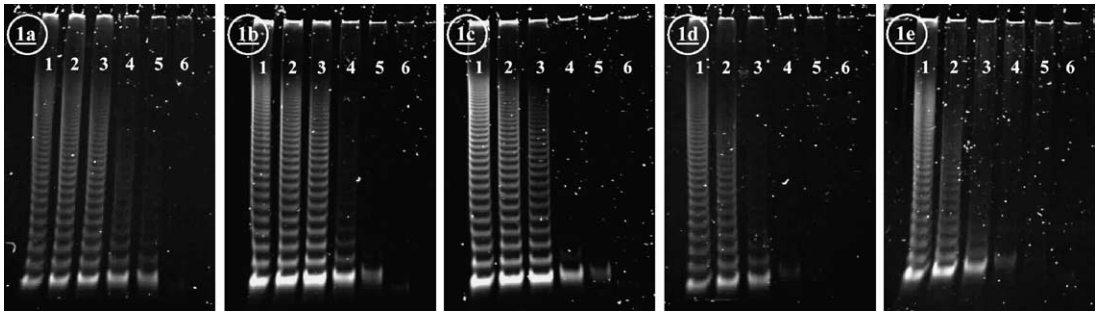


Figure 3. TRAP gels for compounds **1a**–**e**. In all cases, lanes 1–6 correspond to telomerase-containing cell extract incubated with the following compound concentrations: lane 1, 0 (positive control); lane 2, 0.1 μM; lane 3, 0.2 μM; lane 4, 0.5 μM; lane 5, 1.0 μM; lane 6, 0 (negative control; no protein). The last lane (negative control) containing no protein extract is intended as verification that there are no PCR artefacts present.

terminal functional groups on the 9-position. Although **1c**, with an extra positive charge on the piperazine ring, might have been expected to bind best to G4–DNA, it gave a slightly worse FRET result than the closely related **1b**, the added steric presence of the *N*-methyl group possibly offsetting any gain due to the additional charge. However, in this case G4-stabilising ability and telomerase inhibition were not correlated, with **1c** being a significantly more potent telomerase inhibitor, having a $^{tel}EC_{50}$ value of 165 nM compared to 267 nM for **1b**.

Furthermore, when compared with the reference compound **2** the FRET–TRAP correlation is not straightforward. This compound, which has a considerably shorter and uncharged 9-anilino-substituent (Fig. 1), shows a comparatively low ΔT_m value at 1 μM, yet also has high telomerase activity, with a very low $^{tel}EC_{50}$ value (113 nM). This can be rationalised in terms of side-chain bulk and charge. The presence of the 9-anilino group in **2** is clearly advantageous for the mechanism of telomerase inhibition while the lack of a charge on this 9-substituent (compared to **1c**–**e**, for instance) would explain the decreased ability of **2** to stabilise the G-quadruplex (see Fig. 4 for a direct comparison of the FRET and TRAP data obtained for compounds **1a**–**e** and **2**). Both FRET and TRAP values are still within the general range observed for other 3,6,9-acridines.

We take these results to indicate that although comparing the FRET and TRAP data for compounds within a structurally related set can yield meaningful structure–activity relationships, as has been previously shown for a small set of nonacridine based molecules[10] and is observed here for compounds **1a**–**e**, a lower correlation is found when comparing compounds that have greater chemical diversity but are still closely related structurally. Thus whilst a broad, overall trend can be found for the data with compounds that have major structural variations (such as a different number of charges), there may be significant deviations from this trend within yet larger sets of molecules possessing for example the same number of charges or types of terminal functional groups, but differing in their other chemical features. This perhaps reflects the inherent difficulty in selectively targeting compounds to specific types of DNA structure or sequence when the ligand–DNA interactions are

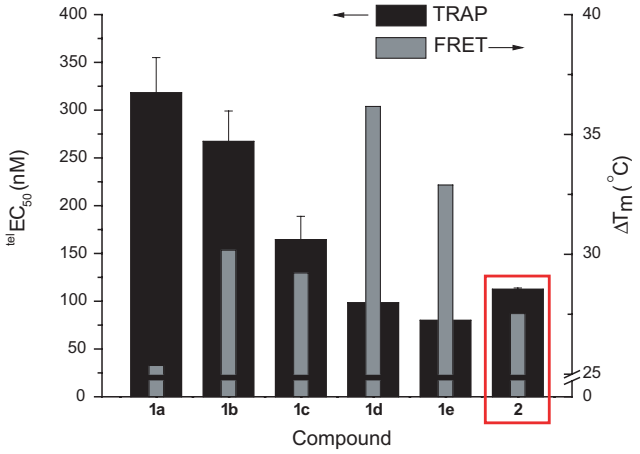


Figure 4. Correlation between values obtained from the FRET and TRAP experiments for compounds **1a**–**e** and **2**. Compounds increase in telomerase inhibition as the $^{tel}EC_{50}$ value (left hand *y* axis) decreases, while G4-stabilising ability increases with increasing ΔT_m value (right hand *y* axis). The overall trend seen shows the compounds increasing in their ability to stabilise G4–DNA and inhibit telomerase processivity going from **1a** to **1e** (left to right); the red box indicates the reference **2**, which shows high comparative telomerase activity but relatively poor FRET stabilising ability (see text).

dominated by charge–charge contacts. It thus seems possible to make broad conclusions about telomerase inhibition based on preliminary FRET results, but detailed predictions are not always going to be reliable. Some of our future work will focus on extending the FRET–TRAP correlation studies to a larger set of compounds, in order to understand this further.

Although no significant PCR inhibition was observed at 1 μM for compounds **1a**–**c**, the observed *Taq* interference of **1d** and **1e** at the same concentration raises the question of compound selectivity for G4–DNA over dsDNA. Testing for *Taq* inhibition is not directly indicative of the strength of duplex binding but this assay nevertheless yields important information as to whether interference of other cellular DNA maintenance processes can be expected due to unintended interactions of these compounds with dsDNA, and whether telomeres and telomerase can be pinpointed as the sole

targets in the event of a cellular reaction to longer-term treatment. Binding studies using surface plasmon resonance (SPR) have however shown[12,13] that there is a significant difference in the affinity of related 3,6,9-trisubstituted acridines for the two types of DNA, thus leaving open an exploitable window for telomerase inhibition. In the case of compounds **1d** and **1e**, this window stretches over an order of magnitude in concentration, as the $^{tel}EC_{50}$ values lie just under 100 nM and 50% *Taq* inhibition is not observed until over 1 μM. Furthermore, the generally low acute cytotoxicity of the 9-anilino-3,6-bis-amidoacridines supports this premise,[14] and future work will attempt to address these issues in more detail.

4. Conclusion

Within this series of 3,6,9-trisubstituted acridines, a direct correlation has been observed between G4-stabilisation, as measured in the FRET assay, and telomerase inhibition (Fig. 4). This supports the use of the FRET assay as a screening tool for the rapid identification of novel G4-stabilising ligands so that promising candidates can be chosen for more detailed biological, biophysical and structural studies in order to further elucidate their interactions with G4–DNA.

Acknowledgements

This work has been supported by project and programme grants to S.N. from Cancer Research UK, by a project grant from The International Association for Cancer Research and by a Cancer Research UK Studentship to C.M.S.

References and notes

1. Neidle, S.; Parkinson, G. *Nat. Rev. Drug Discov.* **2002**, *1*, 383.
2. Rezler, E. M.; Bearss, D. J.; Hurley, L. H. *Curr. Opin. Pharmacol.* **2002**, *2*, 415.
3. Mergny, J. L.; Riou, J. F.; Mailliet, P.; Teulade-Fichou, M. P.; Gilson, E. *Nucleic Acids Res.* **2002**, *30*, 839.
4. Wang, Y.; Patel, D. J. *Structure* **1993**, *1*, 263; Phan, A. T.; Patel, D. J. *J. Am. Chem. Soc.* **2003**, *125*, 15021; Ying, L.; Green, J. J.; Li, H.; Klenerman, D.; Balasubramanian, S. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 14629.
5. Parkinson, G. N.; Lee, M. P.; Neidle, S. *Nature* **2002**, *417*, 876.
6. Neidle, S.; Parkinson, G. N. *Curr. Opin. Struct. Biol.* **2003**, *13*, 275.
7. Cuesta, J.; Read, M. A.; Neidle, S. *Mini. Rev. Med. Chem.* **2003**, *3*, 11.
8. Mergny, J. L.; Maurizot, J. C. *Chembiochemistry* **2001**, *2*, 124.
9. Mergny, J. L.; Lacroix, L.; Teulade-Fichou, M. P.; Hounsou, C.; Guittat, L.; Hoarau, M.; Arimondo, P. B.; Vigneron, J. P.; Lehn, J. M.; Riou, J. F.; Garestier, T.; Hélène, C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *98*, 3062.
10. Guyen, B.; Schultes, C. M.; Hazel, P.; Mann, J.; Neidle, S. *Org. Biomol. Chem.* **2004**, *2*, 981.
11. Burger, A. M. In *Methods in Molecular Biology*; Double, J. A., Thompson, M. J., Eds.; Humana: Totowa, NJ, 2002; Vol. 191, pp 109–124.
12. Read, M.; Harrison, R. J.; Romagnoli, B.; Tanious, F. A.; Gowan, S. H.; Reszka, A. P.; Wilson, W. D.; Kelland, L. R.; Neidle, S. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 4844.
13. Harrison, R. J.; Cuesta, J.; Chessari, G.; Read, M. A.; Basra, S. K.; Reszka, A. P.; Morrell, J.; Gowan, S. M.; Incles, C. M.; Tanious, F. A.; Wilson, W. D.; Kelland, L. R.; Neidle, S. *J. Med. Chem.* **2003**, *46*, 4463.
14. Gowan, S. M.; Harrison, J. R.; Patterson, L.; Valenti, M.; Read, M. A.; Neidle, S.; Kelland, L. R. *Mol. Pharmacol.* **2002**, *61*, 1154.
15. Hill, J. W. *J. Am. Chem. Soc.* **1930**, *52*, 4110.
16. Aviñó, A.; Garcia, R. G.; Albericio, F.; Mann, M.; Wilm, M.; Neubauer, G.; Eritja, R. *Bioorg. Med. Chem.* **1996**, *4*, 1649.