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A Novel Inhibitor of Human Telomerase Derived from 10*H*-Indolo[3,2-*b*]quinoline

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Abstract—The bis-dimethylaminoethyl derivative of quindoline (10*H*-indolo[3,2-*b*]quinoline), an alkaloid from the West African shrub *Cryptolepis sanguinolenta*, has been synthesised. This has been shown to have modest cytotoxicity, as well as inhibitory activity against the telomerase enzyme. It is hypothesised that the latter activity is due to stabilisation of an intermediate guanine-quadruplex complex, in accordance with computer modelling. © 2000 Elsevier Science Ltd. All rights reserved.

The specialised DNA sequences at the ends of chromosomes comprise tandem repeats of simple oligonucleotide sequences. The telomeric repeat in vertebrates, including humans, is 5′-TTAGGG.[1,2] Telomeres have a number of functions including protection of the ends of chromosomes from degradation and recombination, and an involvement in control of senescence, replication and the cell cycle clock.[3] In normal cells, successive rounds of cell division are accompanied by telomere shortening, by around 50–200 nucleotides per division, due to the inability of DNA polymerase to fully replicate the ends. In cancer cells, although the telomeres are typically shorter than those of normal (or of germ-line) cells, their length is maintained by addition of telomere repeat sequences, a process catalysed by the enzyme telomerase.[4] This enzyme is also directly implicated in the immortalisation of cancer cells, and it is significant that around 85–90% of human tumours possess telomerase activity while somatic cells invariably lack the enzyme[5] (although stem cells, which tend to have long telomeres, do have significant telomerase activity).

The possibility of designing drugs with inhibitory activity against telomerase is emerging as an attractive strategy for cancer chemotherapy.[6] It is proposed that telomerase inhibition would result in progressive telomere shortening in tumour cells over a number of generations, leading to selective inhibition of tumour cell growth. This has now been demonstrated using (i) PNA antisense molecules targeted against the RNA template of telomerase,[7] and (ii) with telomerase dominant negative mutants.[8]

We have developed an approach to the rational design of telomerase inhibitors which has involved the folding of the single-stranded telomere primer into four-stranded quadruplex structures;[9] these cannot be substrates for the enzyme. Folding has been achieved with a range of small molecules, sharing the structural features of a planar electron-deficient chromophore and basic side chain(s), including derivatives of amido-substituted tricyclic anthraquinones, fluorenones and acridines.[10] Molecular modelling[11] and other biophysical studies[12] have shown that these molecules bind to G-quadruplexes, and that strength of binding correlates with extent of telomerase inhibition.

The original discovery that forms the basis for the work described here, arises from studies on the West African shrub *Cryptolepis sanguinolenta*. This has a long association with Ghanaian folk medicine, and is prized for its use in the treatment of fevers, including malaria, and for the control of infections of the urinary and upper respiratory tracts.[13] During the past few years, the major alkaloidal constituent, cryptolepine **1** (Fig. 1) has been the object of numerous biological studies, and the medicinal value of the plant has been fully substantiated.

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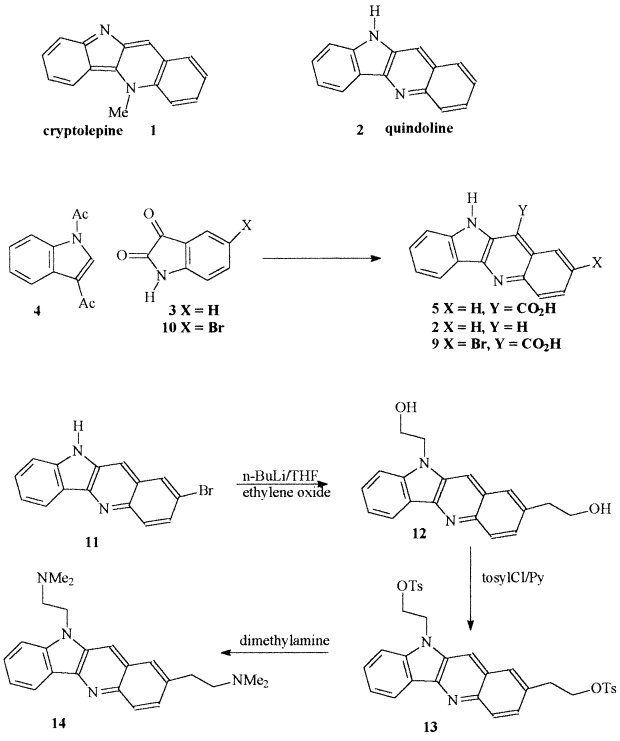


Figure 1.

Cryptolepine is reported to have useful biological activity as an antimicrobial agent,[14] an anti-imflammatory agent,[15] as a vasodilator,[16] and most recently, as an antihyperglycaemic agent.[17] Its value as an antimalarial agent appears so promising[18] that several African countries have proceeded with large-scale therapeutic programmes. However, the potential of cryptolepine or others constituents of the plant as anti-tumour agents, has not, to our knowledge, been investigated. Our synthetic, molecular modelling and biological studies with a derivative of the parent indoloquinoline compound, quindoline (**2**) are reported here.

Quindoline has been synthesised by a number of groups, and we initially used the method of Petrow et al.[19] which involves the reaction between isatin **3** and *O*,*N*-diacetylindoxyl **4**, in the presence of aqueous KOH, to produce quindoline-10-carboxylic acid **5**. This reaction is not without problems, because any unreacted indoxyl must be oxidised (in a stream of air) to produce indigo, which must then be removed before isolation of the acid. The isolation procedures are thus plagued by the presence of highly coloured material. The decarboxylation of **5** was then achieved by heating the acid in liquid paraffin at around 280 °C, and in this way quindoline could be produced on the multigram scale in a maximum overall yield of around 50%.

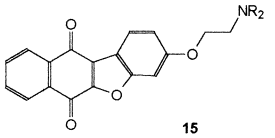
The cytotoxicity of quindoline was evaluated[20] using five human ovarian cancer cell lines: three parent lines SKOV-3, A2780 and CH1, and two drug resistant ones. IC_{50} values are given in Table 1, which show that quindoline has only moderate activity, although it has comparable activity against the resistant cell lines. These results have prompted the synthesis of a range of analogues. Our route to analogues of quindoline bearing one or more such side-chains is shown in the scheme.

Table 1. Cytotoxicity, telomerase inhibition and molecular modelling data for three tetracyclic compounds compared to the analogous 2,6-dimethylamino anthraquinone.[10] E_{total} is the sum of van der Waals and electrostatic interaction energy between ligand and quadruplex, in kcal mol^{-1}

	A2780	A2780^R	CH1	CH1^R	SKOV-3	$^{tel}IC_{50}$	E_{total}
Quindoline **2**	21.5	24.5	15.5	30	66	n.d.	n.d.
Comp. **14**	12.5	10.5	8.4	12.5	6.3	16	−145
Comp. **15**[21]	0.15	n.d.	0.18	n.d.	3.6	7.0	−168
2,6-AQ	2.6	n.d.	1.8	n.d.	2.9	4.1	−181

The synthesis of 2-bromo-10*H*-indolo[3,2-*b*]quinoline-11-carboxylic acid **9** was accomplished by the same general route used for the synthesis of quindoline-10-carboxylic acid, that is by treatment of 5-bromoisatin **10** and *O*,*N*-diacetylindoxyl **4** with aqueous KOH solution. As before, we had great difficulty separating the side-products (mainly indigo) from the product, and the yield of **9** was rarely above 50% on the 60 mmol scale. Decarboxylation to provide 2-bromo-10*H*-indolo[3,2-*b*]quinoline **11** could be effected (as before) by heating in liquid paraffin at 250–300 °C with yields in excess of 80% on the 10 gram scale. Reaction of this bromoquindoline with *n*-butyl lithium in THF, followed by addition of excess ethylene oxide produced the anticipated bis-hydroxyethyl derivative **12** (61%), which was converted into the bis-tosylate **13** (tosylCl/pyridine; 41%), and thence into the bis-dimethylaminoethyl derivative **14** (Me_2NH/DCM; 64%). Preliminary biological evaluation shows that this compound has about twice the cell growth inhibitory activity of quindoline.

Compound **14** was also evaluated in a modified TRAP assay[11,12] for its ability to inhibit the extension of telomeres by human telomerase, using partially-purified enzyme extracted from human ovarian carcinoma A2780 cells. Experiments at a range of concentrations gave a value of 16 μM for $^{tel}IC_{50}$, the concentration required to produce 50% inhibition of telomerase. We have previously studied another tetracyclic system[21] based on benzo[*b*]naphtho[2,3-*d*]furan (**15**), with a single -O-$(CH_2)_2$-NR_2 substituent (where R = Et or Me). The $^{tel}IC_{50}$ value for the Et compound (as methiodide derivative), is given in Table 1.



Molecular modelling was used to examine possible interactions of compound **14** with a guanine quadruplex. Coordinates from the solution NMR structure of the human telomeric repeat d[$AG_3(T_2AG_3)_3$] G-quadruplex[22] were used to give an initial low energy starting model. This was optimised by molecular mechanics energy minimization followed by molecular dynamics and a final set of molecular mechanics minimisations.[11] An intercalation site was introduced

between the diagonal T_2A loop and the G-quartet segment of the structure (the 5′-AG step) by breaking the phosphate backbones and separating the structure whilst monitoring the distance between the segments. The sugar-phosphate chains were reconnected and molecular mechanics energy minimization was used to relieve any resulting steric distortion while retaining the G-quartet and loop motifs with positional restraints.

A model for compound **14** was created, minimised and docked into the intercalation site using the DOCKING module within the INSIGHTII package. This enables molecular orientation to be explored whilst monitoring electrostatic and van der Waals interactions between ligand and quadruplex. Both ligand geometry and that of the intercalation site were allowed to vary during the search. Possible starting orientations were chosen by means of a Monte Carlo algorithm. Individual bases were constrained at this initial docking stage, although the backbone around the intercalation site was unconstrained. The best chromophore positions were subjected to 100 steps of unconstrained molecular mechanics minimisation, to give the final structure shown in Figure 2, with its total interaction energy given in Table 1.

The quindoline derivative **14** has significant activity against human telomerase, at a level comparable to several nucleoside inhibitors of the reverse transcriptase activity of telomerase.[6] It is approximately 10-fold less active than the best quadruplex-mediated inhibitors based on tricyclic chromophores[10] (which all have two aminoalkyl substituents), and is 2-fold less active than the benzo[*b*]naphtho[2,3-*d*]furan derivative **15**. The computed energies of interaction with the guanine-quadruplex are in accord with this ranking order. We take this as suggestive evidence that the mechanism of telomerase inhibition by the quindoline **14** does involve quadruplex stabilisation. Structure–activity studies on tricyclic inhibitors has previously demonstrated the importance of two protonated side-chains; the relatively poor activity of **14** compared to what might have been expected for a tetracyclic chromophore (having superior stacking interactions with the guanine quartet) is due to its side-chains being of insufficient length to avoid some steric clashes with the bases (Fig. 2). It is notable that both quindoline and **14** have relatively low acute cytotoxicities compared to the other telomerase inhibitors in the Table, and thus greater selectivity. We have recently developed a more convergent route that is not only more efficient but should also allow access to a wide range of second-generation analogues with varying side-chains and thus potentially superior selectivity against telomerase.

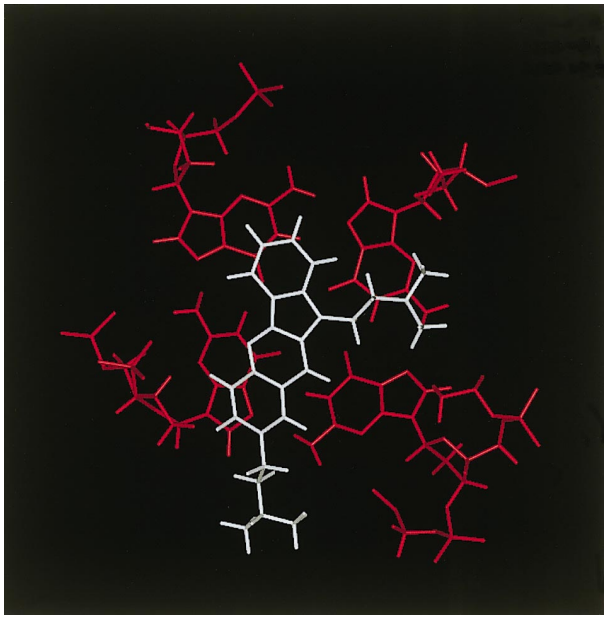


Figure 2. Plot of compound **14** bound in the global minimum-energy position in the human G-quadruplex structure, viewed onto the plane of the chromophore.

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