Binding of Telomeric DNA G-Quadruplexes by Abietane Diterpene Natural Products

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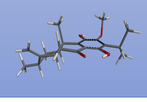

Introduction

G-quadruplexes are non-canonical higher order DNA structures formed from guanine rich sequences, made up of stacked G-tetrads stabilized by Hoogsteen base pairing and K^+ ions. G-quadruplexes are overrepresented in the promoter regions of oncogenes and the 5'UTR of mRNA[1]. As a result, G-Quadruplexes have been implicated as targets for possible anti-cancer therapeutic agents to treat previously "undruggable" targets like the c-myc and ras oncogenes[1].

The human telomeric repeat, $[5'G_3(T_2AG_3)_3]$, is a repeating, single stranded DNA sequence that can form G-quadruplexes. Telomerase, an enzyme expressed in ~90% of all cancers, is responsible for extending telomeric repeats, making cancer cells immortal. It has been shown that stabilization of telomeric G-quadruplexes can inhibit telomerase activity and therefore block the survival of cancer cells[2].

The compounds used in the study are a group of abietane diterpene natural products from *Hyptis verticillata*, a plant native to the Caribbean and central America that has been used traditionally as an ethnomedicine that has been shown to have a range of therapeutic effects. Some of these effects include anti-microbial, anti-inflammatory, and even anti-cancer activities[3]. Using several biophysical techniques, we have investigated the binding characteristics of these compounds to G-quadruplex DNA as a possible rationale for their observed anti-cancer therapeutic effects.

Experimental

Sample Preparation

DNA G-Quadruplexes were formed in solution using a 10 mM phosphate buffer, pH 7, with100mM K^+ added. Characteristic CD spectra confirmed the presence of quadruplexes in solution.

Circular dichroism

CD measurements were performed using a Jasco J-815 CD Spectrophotometer. For each run, the abietane solution was added to a solution of G-quadruplex DNA and the spectra was measured from 200-600nm.

Thermal Denaturation

Tm measurements were performed using a CARY-5000 UV-Vis-NIR Spectrophotometer. Absorption spectra of G-quadruplex-abietane complex were recorded at 295 nm as the temperature was increased from 25.00°C to 95.00°C.

Thioflavin-T Fluorescence Displacement

Fluorescent studies were performed using a PTI Quantamaster™ 40 UV-Vis Spectrofluorometer from 400-600nm. A Fluorogenic dye that binds specifically to DNA G-quadruplexes, Thioflavin-T (Tht), was used as a fluorescent indicator for the quadruplexes. A solution of Tht bound G-quadruplexes was prepared and the abietane solution was added. The solution was excited at 425 nm and fluorescence intensity was measured.

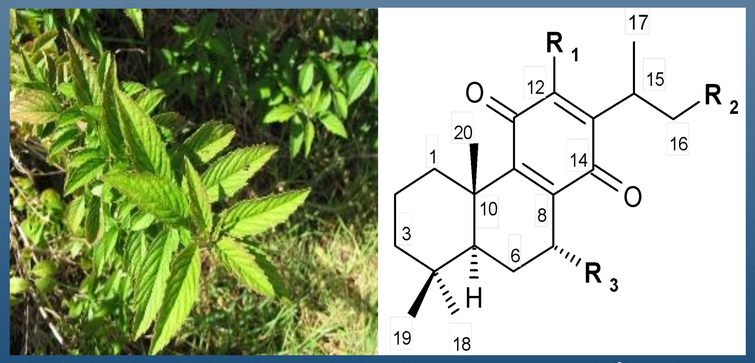

Figure 1. Left: A picture of *Hyptis verticillate*, the plant from which these compounds are derived[5]. Right: A generalized structure of the abietane diterpenes used.

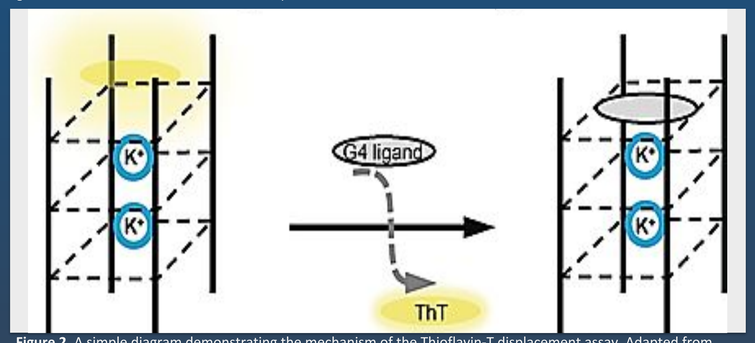

Figure 2. A simple diagram demonstrating the mechanism of the Thioflavin-T displacement assay, Adapted from Jamroskovic et al[7].

Results

Thioflavin Displacement

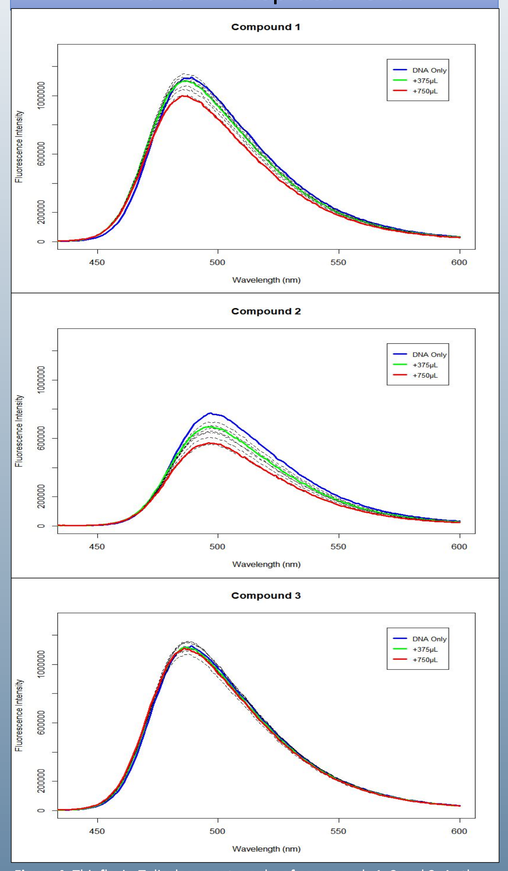

Figure 4. Thioflavin-T displacement graphs of compounds 1, 2 and 3. As the abietanes were bound, the fluorescence intensity changed base on whether or no the abietanes were able to displace Thioflavin-T from its binding site on the quadruplexes.

Circular Dichroism

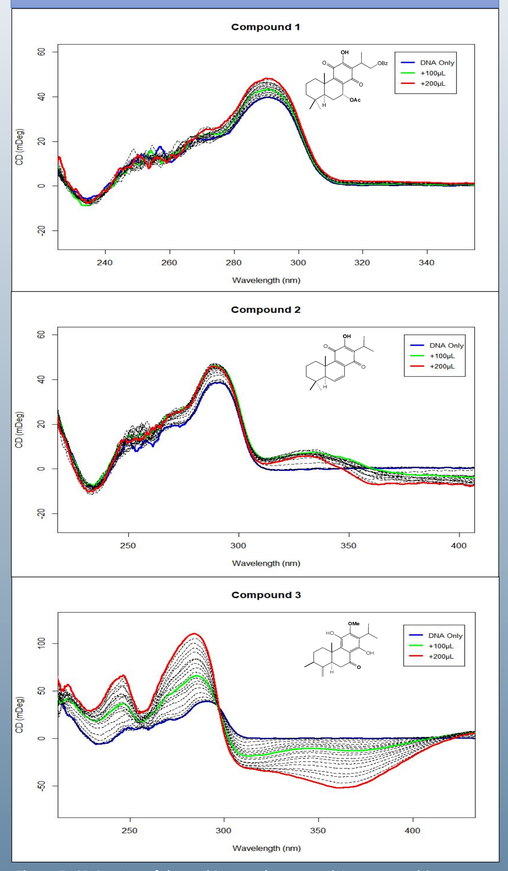

Figure 5. CD Spectra of three abietanes (compound 1, compound 2, compound 3) after being titrated into G-quadruplex solution. Structures of compounds 2 and 3 are shown.

Thermal Denaturation

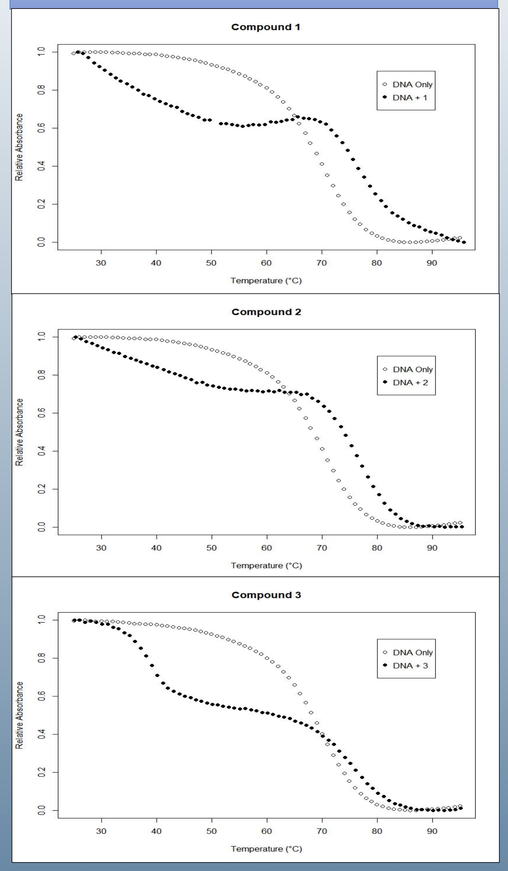

Figure 6. Thermal Denaturation plots of compounds 1, 2, and 3. Each compound was run alongside a DNA only control.

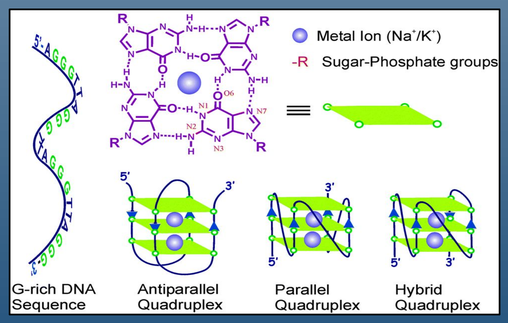

Figure 3. A simplified diagram of DNA G-quadruplexes formed from human telomeric DNA[6].

Table 1. ΔTm values of each compound determined from the thermal denaturation studies. Apparent Stern-Volmer constants were determined for each compound via the Thioflavin-T displacement studies.

Compound	ΔTm (°C)	Stern-Volmer Constant (mM^{-1})
1	5.7	0.0075
2	5.4	0.0169
3	-17.86	-
4	8.1	0.0151
5	8.4	0.0118
6	8.46	0.0136
7	-	-
8	2.2	-

Conclusions

- Displacement studies showed that 5 of the 8 abietane diterpenes interacted with the DNA-ThT complex, quenching the fluorescence.
- CD Spectra indicated that of the 8 compounds tested, compounds 1, 2 , and 3 showed the most significant interactions with the telomeric G-quadruplexes.
- The unique spectra of compound 3 indicates a shift from antiparallel quadruplexes to a different form of quadruplex, although it is unclear what that form may be. The positive peaks at 295nm and negative peaks at 240nm shown by the spectra for compounds 1 and 2 indicate an increase in stabilization of the antiparallel form of G-quadruplex[8].
- Thermal denaturation studies showed that the binding of all the compounds resulted in an increase in thermal stability (Tm) at higher temperatures, after an initial structural transition. An early transition/species was most evident for **3**.
- The spectra we have recorded for all compounds, specifically compound 3, is very interesting and warrants further investigation.

References

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Acknowledgments

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