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Anthracenyl isoxazole amides (AIMs) stabilize quadruplex DNA structures in telomeric and c-MYC promotor sequences

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Approximately 23,000 people are affected by malignant brain and CNS tumors in the United States each year and those afflicted have a median survival rate of only 12–15 months due to the limited treatment options available. The anthracenyl isoxazole amides (AIMs) are a novel class of compounds that have been shown to possess significant anti tumor activity in the NCI 60 cell line panel and to inhibit growth of SNB-19 glioblastoma cells at low micromolar and nanomolar concentrations. The goal of our current research is to characterize the mechanism underlying the anti tumor activity of the AIMs. We hypothesize the mechanism of growth inhibition to involve binding and stabilization of a DNA tertiary structure known as a guanine quadruplex. Various regulatory regions of DNA, such the c-MYC oncogene promoter sequence and repeating sequences formed at the end of telomeres, adopt the quadruplex conformation. Stabilization of quadruplex structures by small-molecule binding ligands has been reported to modulate the expression of genes and inhibit telomerase activity. Down-regulation of certain oncogenes or the inhibition of telomerase can cause tumor cells to undergo apoptosis or become unable to efficiently replicate. To establish whether interactions between the AIMs and quadruplex-forming sequences act to stabilize the quadruplex tertiary structure, circular dichroism spectroscopy (CD) was employed. CD is a method that utilizes the differential absorbance of left and right circularly polarized light to examine the chiral structure of molecules. CD thermal melting studies were conducted to determine whether the AIMs would increase the melting temperature of quadruplex forming sequences as an indication of increased stability. Our results demonstrated the AIMs, at two equivalents, increase the melting temperature (T_m) of both the c-MYC promoter and telomeric sequences by approximately 2–3 °C with strong statistical significance and reproducibility. Utilizing CD allows the use of low micromolar concentrations of DNA and this method will be used in the future to rapidly develop additional structure-activity relationships between novel AIMs and quadruplex forming sequences. Our laboratory has also shown chemical shifts in the imino region upon treatment with the AIMs for both the c-MYC promotor and telomeric sequence by NMR, providing additional evidence of the AIMs interaction with quadruplex structures. Interestingly, fluorescence microscopy of SNB-19 cells treated with AIMs show their localization is primarily in the mitochondria, and mitochondrial DNA contains several other important quadruplex forming sequences. Mitochondrial-dependent apoptosis has been suggested for other quadruplex binding ligands and therefore our future work will examine the potential stabilization of mitochondrial quadruplexes by these and other novel AIMs.