Analysis of Interactions Between Caffeine and Berberine Anti-Cancer Drug in Aqueous Solution Using NMR Spectroscopy

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Background

• Cancer is a disease of uncontrolled cell proliferation. DNA replication occurs more frequently due to loss of cell cycle control mechanisms, leading to tumor propagation.

• Berberine is a planar, aromatic compound under investigation as a treatment for certain cancers. Berberine can induce cancer cell apoptosis by intercalating into the bases of the cell’s DNA, thus inhibiting replication and suppressing tumor growth.

• NMR Spectroscopy was used to analyze the chemical shifts of aqueous samples of caffeine and berberine molecules individually and in mixture to determine association constants (K_a) and derive points of interaction between the two molecules. Understanding these interactions will contribute to understanding effectiveness of Berberine as an anticancer drug and caffeine's role in inhibiting the effects of berberine in fighting cancer.

Figure 1: Structure of Berberine. Number labels represent peak positions on berberine's 1HNMR spectrum.

Figure 2: Structure of Caffeine. Number labels represent peak positions on caffeine's 1HNMR spectrum.

• Caffeine is also a planar molecule which may interfere with the effectiveness of anti-cancer intercalating agents such as berberine by directly binding with the drug and preventing it from targeting the DNA of cancer cells.

Methods

• Samples were prepared using serial dilutions of caffeine and berberine in D_2O with 4,4-Dimethyl-4-silapentane-1-sulfonic acid (DSS) reference standard, providing a range of concentrations for each sample set.

• Caffeine samples and Berberine samples were each tested at 25 C and 35 C and compared to mixtures of the two compounds together at the same temperatures.

• 1H NMR experiments were run on a Varian Unity INOVA® 500MHz NMR.

• An NMR stack of chemical shift behavior was prepared for each sample using MestreNova analysis software. Peak chemical shifts were analyzed for potential interaction using K_a calculations on MestreNova and Microsoft Excel.

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• In higher concentrations, berberine peaks tend to increase in K_a value, indicating that berberine may be more likely to self-associate at body temperature than to interact with caffeine.

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• At lower temperatures, increased K_a values indicate that berberine peak 1 as well as peaks 5, 6, 7, and 11 may be involved in stacking with caffeine, while at higher temperatures berberine peaks 1, 6, 7, 8, and 10 all show the opposite trend and instead peaks 3, 5, and especially 9 may become more involved in stacking behavior, whether with caffeine or in self-stacking.

• Overall, it might be inferred that at lower temperatures, multiple berberine peaks may play a role in associating with all caffeine peaks - especially caffeine’s peak 4 – and in self-association. However, as temperatures increase to conditions similar to body temperature, berberine may dissociate from caffeine and increase in self-association at peaks 3, 5, and 9 while caffeine may increase in self-association at peak 4.

• Further studies may benefit from measurements using different reference standards for comparison, as there was some indication that the molecules being studied may have had some level of interaction with the DSS reference standard.

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References
