Cancer is a term used for a class of diseases in which abnormal cells divide without control and are able to invade other tissues. It is caused by errors in the cell replication process, when the genetic material (DNA) is damaged or changed. These changes produce mutations that affect cell growth and division. In 2012 alone it is estimated that over half a million people died of cancer in the United States, making it an important subject of study for scientists.

There are various platinum-based anticancer compounds being used in a clinical setting against testicular, ovarian, and colorectal cancers. These platinum drugs are able to form platinum-DNA covalent bonds, which lead to the apoptosis, or programmed cell death, of cancer cells. However, increasing resistance to the drugs and harmful side effects necessitate new anticancer drug development. These new drugs would need to have different mechanisms of action, and the mechanism by which a drug acts is ultimately dependent on its chemical structure and properties.

The purpose of this research was to synthesize novel phenanthroline derivatives with the intent of later measuring their toxicity against various cancer cells. 1,10-Phenanthroline is a nitrogen-containing polycyclic aromatic hydrocarbon. The nitrogen atoms at positions 1 and 10 have a natural affinity for certain metal atoms, such as platinum and ruthenium. This affinity can be utilized to construct organometallic compounds with potential anticancer properties. Because the proposed targets are novel compounds, they may eventually result in the development of a drug with increased cytotoxicity compared to commercially available drugs.
Compound (1) contains an inotilone group bound to the phenanthroline scaffold via the platinum metal center. Inotilone is a natural compound found in the *Inontus species* mushroom. Extracts of these mushrooms have been shown to have anti-inflammatory, anti-hyperglycemic and anti-lipid peroxidative, anti-cancer, and anti-tumor activities. Inotilone, a 5-methyl-3(2H)-furanone derivative, has been shown to be an inflammatory inhibitor as well as an anti-cancer agent.

**Figure 1:** Structure of the first target molecule (1).

**Figure 2:** Structure of the second target molecule (2).
Compound (2) contains a biotin molecule attached to the phenanthroline scaffold via an amide linkage. Biotin is a B vitamin and an important component of enzymes in the body that are involved in metabolism, and therefore necessary for growth and development. Biotin is water soluble, which aids in the absorption of the drug into the body. Because biotin is found in higher concentrations in some types of cancer cells than in normal cells, the addition of a biotin group to an anticancer agent can allow for increased delivery and selectivity toward cancer cells.\(^5\)

![Figure 3: The structure of the third target molecule (3)](image)

Compound (3) is an already known compound. However, this molecule has yet to be bound to platinum. The addition of platinum could potentially increase the cytotoxicity of this drug.
Procedure

Figure 4: Reaction scheme to make (1)
Synthesis of 4-carbethoxy-5-methyl-3(2H)-furanone

A solution of ethyl diacetoacetate (4.0g, 23mmol) in ether (40mL) was placed on ice for 10 minutes and purged with argon. Bromine (8.0g, 48mmol) was added, and the reaction was left to sit overnight. The solution was separated with 20mL cold water, then extracted with ether and dried with magnesium sulfate. This was filtered and the excess solvent evaporated under reduced pressure. (7.75g, 102% yield)

Sodium iodide (8.1g, 54mmol) was dissolved in acetone (81mL) and poured into the dibromide. The solution was stirred for 20 minutes. A solution of sodium thiosulfate (17.5g, 70.5mmol) in water (54mL) was added to the flask and stirred at 25°C for 1h. Excess solvent was evaporated under reduced pressure. The remaining solution was separated with 4x30mL portions of ether, dried with magnesium sulfate, and the excess solvent was evaporated under reduced pressure. (7.41g, 97.6% yield)

The product was then purified using a fritted funnel packed half full with silica gel, dissolving the product in methylene chloride. The silica was rinsed with 50mL methylene chloride and then 50mL ethyl acetate until product was collected. Purity was determined by TLC. Fractions were collected and the excess solvent was evaporated under reduced pressure. (1.6g, 40.9% yield)

Synthesis of 4-carbethoxy-2-(m,p-dihydroxybenzylidene)-5-methyl-3(2H)-furanone

Furanone ester (1.6g, 9.37mmol), glacial acetic acid (30mL), and 3,4-dihydroxybenzaldehyde (0.66g, 4.69mmol) were stirred at 25°C for 4h. The reaction mixture was added to 5% HCl (65mL) and vacuum filtration was performed, washing with cold distilled water (40mL). (0.49g, 35.8% yield). The product was recrystallized in ethyl acetate and filtered using a fritted funnel. (0.26g, 19% yield)
Synthesis of Pt(1,10-phenanthroline-5,6-dione)Cl₂

A solution of 1,10-Phenanthroline-5,6-dione (0.0952g, 0.45mmol) in ethanol (25mL) was added dropwise to a solution of potassium tetrachloroplatinate (0.188g, 0.45mmol) in water (20mL) in the dark at 50°C and stirred for 24h. The solution was filtered and the precipitate was washed with 3x2mL portions of cold ethanol and diethyl ether (3x5mL) and dried under vacuum in the dark. (0.16g, 78.8% yield)

Synthesis of (1)

Platinated dione (0.16g, 0.36mmol) and furanone (0.104g, 0.36mmol) were combined with sodium bicarbonate (0.05998g) and purged with argon in the dark. A sparged mixture of ethanol (10mL) and DMF (2mL) was added to the reaction mixture and refluxed for 24h at 95°C. Reaction mixture was allowed to cool to RT, then diluted with 50mL ether to give a black precipitate, which was filtered and washed with 3x10mL portions of ether. The excess solvent in the filtrate was evaporated under reduced pressure to give the product. See Figure 9.

Figure 5: Reaction scheme to create (2). Attempted and unsuccessful reaction is on top, proposed reactions are below.
Synthesis of aldehyde

Phenanthroline (2g, 10.3mmol) and SeO₂ (2.45g, 22.0mmol) were suspended in 1,4-dioxane and water (192mL, 8mL) and the contents refluxed for 4h. After cooling, the solution was filtered through celite, then concentrated under vacuum. Alumina (basic, EMD) column purification gave an off-white powder. (1.04g)

Reductive Amination of Aldehyde

A 10% solution of the aldehyde in glacial acetic acid was stirred at 15°C. Ammonia (0.076g, 4.5mmol) was added dropwise, then NaBH₄ (0.169g, 4.5mmol) was added slowly over the hour. The acetic acid was evaporated under reduced pressure. Water was added, then a 20% HCl solution until the solution was a pH of 2-3. The acid solution was washed with ether, neutralized with 30% NaOH, and extracted with ether. The ether extracts were dried with magnesium sulfate, then the excess solvent was evaporated under reduced pressure. This yielded no product.

Aldehyde (0.1g, 4.47mmol) and ammonia (8.2g) were added to methanol (7.2ml) and stirred for 3h under reflux at 64°C. NaBH₄ (0.018g, 0.48mmol) was added in 3 installments over 15 minutes after removing the flask from the heat source. The residue was mixed with 25mL water, then extracted with 2x20mL DCM. This also yielded no product.

Figure 6: Reaction steps that have been taken to make (3).
**Synthesis of 5-nitro-1,10-phenanthroline**

Nitric acid (7.5mL) was added to a solution of 1,10-phenanthroline (2.5g, 13.9mmol) in concentrated sulfuric acid (15mL) dropwise at 160°C. The reaction mixture was refluxed for 3h and subsequently poured into ice water. Concentrated NaOH was added to bring the pH to 3, and the resulting yellow precipitate was filtered and washed with water to give a white solid. (5.0g)

**Synthesis of 5-amino-1,10-phenanthroline**

5-nitro-1,10-phenanthroline (5g) was dissolved in 100mL absolute ethanol, then 10% Pd/C catalyst (1g) was added. The reaction mixture was purged with argon, then 13mL hydrazine monohydrate was added dropwise over 30 minutes. The mixture was stirred at 70°C for 10h, then filtered. The filtrate was concentrated under reduced pressure then dried under vacuum. This yielded no product.

A solution of LAH (0.0855g) was dropped into a refluxing solution of 5-nitro-1,10-phenanthroline (0.29g) and ether (50mL) and stirred for 2h. Excess solution was destroyed with 100mL cold water and treated with saturated ammonium chloride solution (10mL). The product was extracted with ether and concentrated under reduced pressure. (0.1g, 31.03% yield)

**Synthesis of (3)**

Biotin (0.112g) was dissolved in SOCl₂ (4mL) and stirred for 20 minutes under argon. The excess solvent was distilled away, then 5-amino-1,10-phenanthroline (0.1g) was added with dried DCM (8mL) and DMAP (17mg) and stirred for 24h.
Results

Figure 7: $^1$H NMR of 4-carbethoxy-5-methyl-3(2H)-furanone in chloroform-d
Figure 8: $^{1}$NMR 4-carboxy-2-(m,p-dihydroxybenzylidene)-5-methyl-3(2H)-furanone in DMSO
Figure 9: $^1$H NMR of the compound made when attempting to produce (1).
Figure 10: $^1$H NMR of the aldehyde in chloroform-d
Figure 11: $^1$H NMR of amine in chloroform-d
Conclusions

The synthesis of (1) was unsuccessful, however, the NMR data suggests that the novel compound shown in Figure 12 may have been produced. This structure has been hypothesized due to the lack of phenanthroline protons present in Figure 9. If the structure of this compound can determined in the future and if it can be purified, biological studies may be conducted.

![Figure 12: Compound that may have been produced in the attempt to make (1)](image)

Compound (2) was not able to be produced successfully due to lack of time. Additional attempts to synthesize 2 will be carried out during the upcoming academic year, following the scheme showed in Figure 5.

The synthesis of (3) is ongoing. If successful, the reaction scheme will be optimized in order to maximize yield of the product. (3) along with the platinated product will be tested against cancer cells in order to determine their cytotoxicity.
References


