Detection of 5-Fluoro-2-Oxindole and Products by HPLC after Reaction with Human Liver Enzymes

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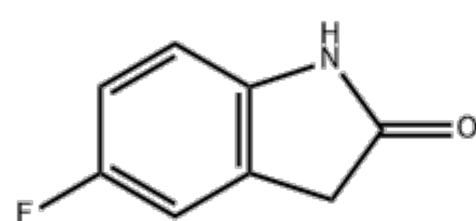
Introduction

The 5-fluoro-2-oxindole (5F2O) moiety is a common structural component of many new drugs in a variety of pharmaceutical classes, including anti-cancer and anti-inflammatory drugs. The reactivity of this moiety with oxidative enzymes in the human liver, namely CYP enzymes, is an important consideration when assessing the potential for toxic products of degradation or adverse side effects. These oxidative reactions are dependent on NADPH in the liver, and they are catalyzed by human S9 liver enzymes. Determining the structure of the major products of reactions catalyzed in the liver is necessary when evaluating the possible toxicity associated with drug candidates, such as Ciprofloxacin and Tylenol, that contain the 5F2O moiety.



In order to determine the structures of these products, high performance liquid chromatograph (HPLC) and liquid chromatography-mass spectrometry (LCMS) conditions must first be established such that a consistent peak can be viewed at a consistent retention time each run. After these conditions are established, reaction parameters must also be generated in such a way that degradation of the 5F2O peak can be viewed on the aforementioned instruments, and a new peak, the product peak, can be visualized. Using LCMS, the molecular weight of the product can be determined and used to identify the product formed by CYP2A6R degradation of 5F2O.

Research Questions

1. Which conditions are required to view 5F2O on an HPLC?
2. Which reaction conditions produce peak degradation when viewed on the HPLC?
3. Which conditions are required to view 5F2O on LCMS?
4. What is the structure of the metabolites produced by the reaction of CYP2A6R and 5F2O catalyzed by NADPH?

References and Acknowledgments

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Results and Discussion

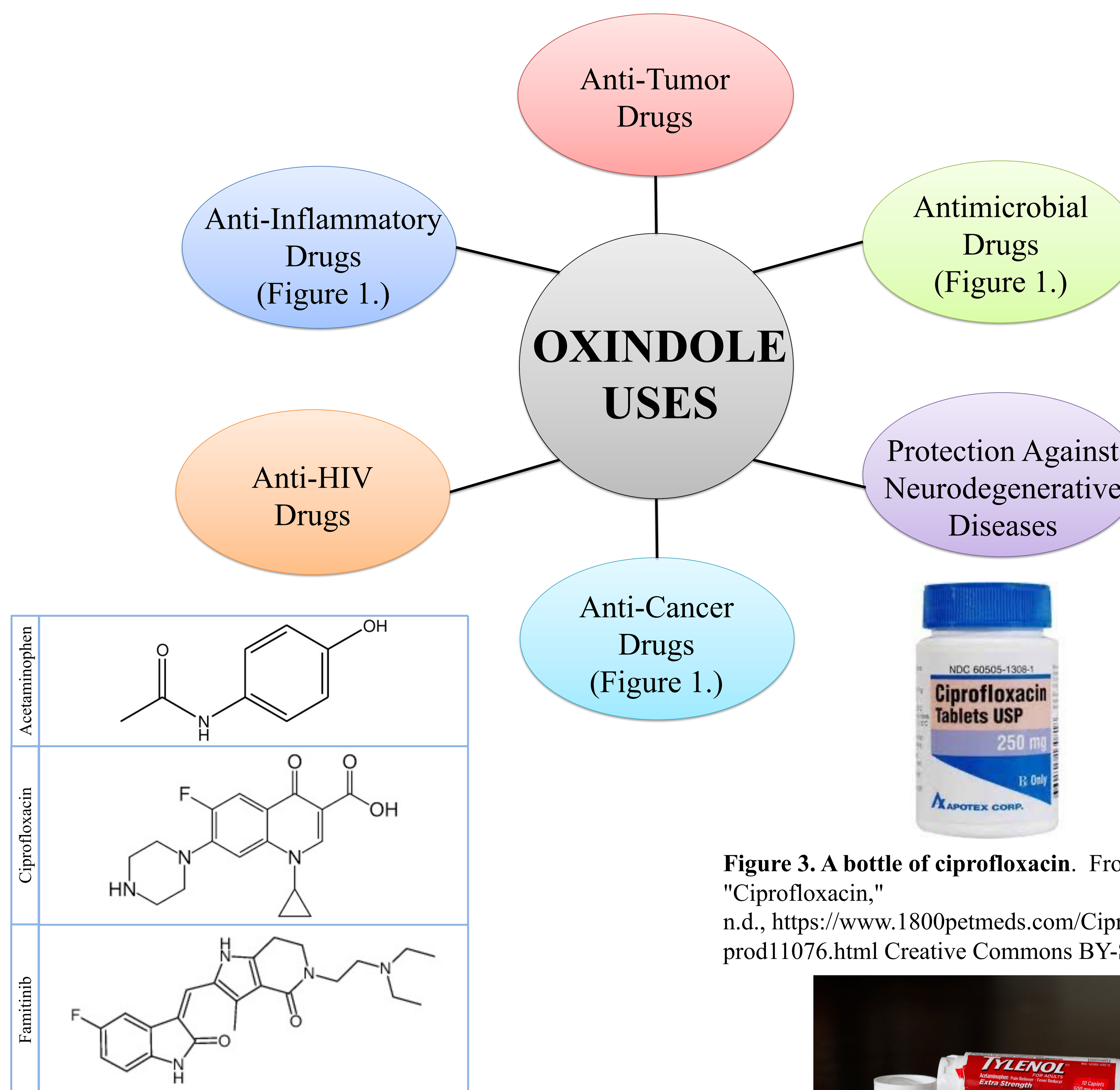

Figure 1. Chemical structures for oxindole-derived compounds. Current therapies for anti-inflammatory, antibiotics, and anti-cancer, such as Acetaminophen, Ciprofloxacin, and Famitinib, contain oxindole moieties.

Figure 3. A bottle of ciprofloxacin. From PetMeds: "Ciprofloxacin," n.d., https://www.1800petmeds.com/Ciprofloxacin-prod11076.html Creative Commons BY-SA 4.0

Figure 4. A bottle of Tylenol. Photo by Alexa Deckert.

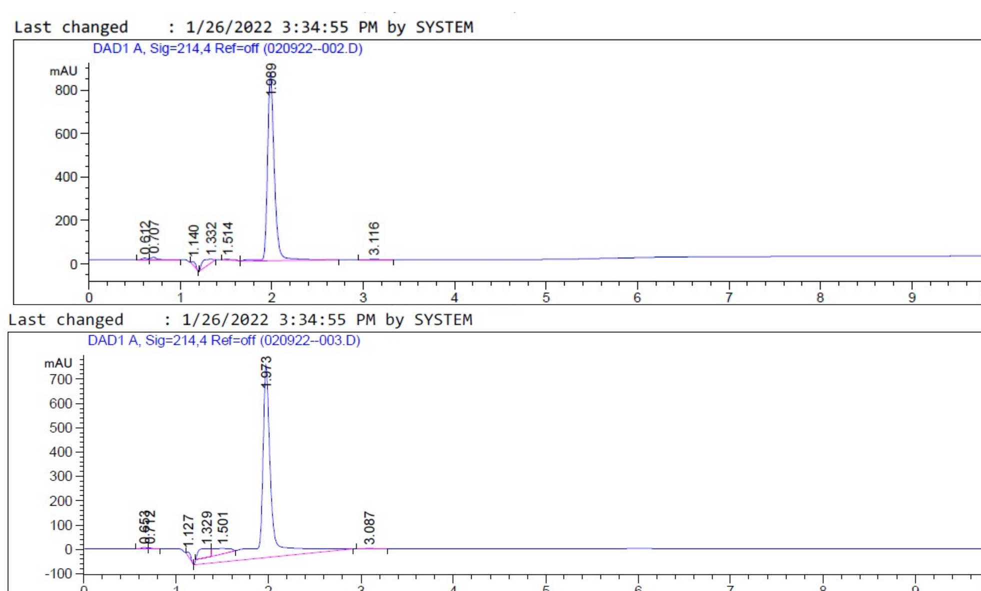

Figure 2. Consistency with OxindoleTrial2.M. 20mM 5F2O was run using HPLC OxindoleTrial2.M which consisted of 65:35 diH_2O:ACN. This method produced consistent peaks amongst various trials over a period of weeks. This method was implicated in further experimentation with human liver enzymes.

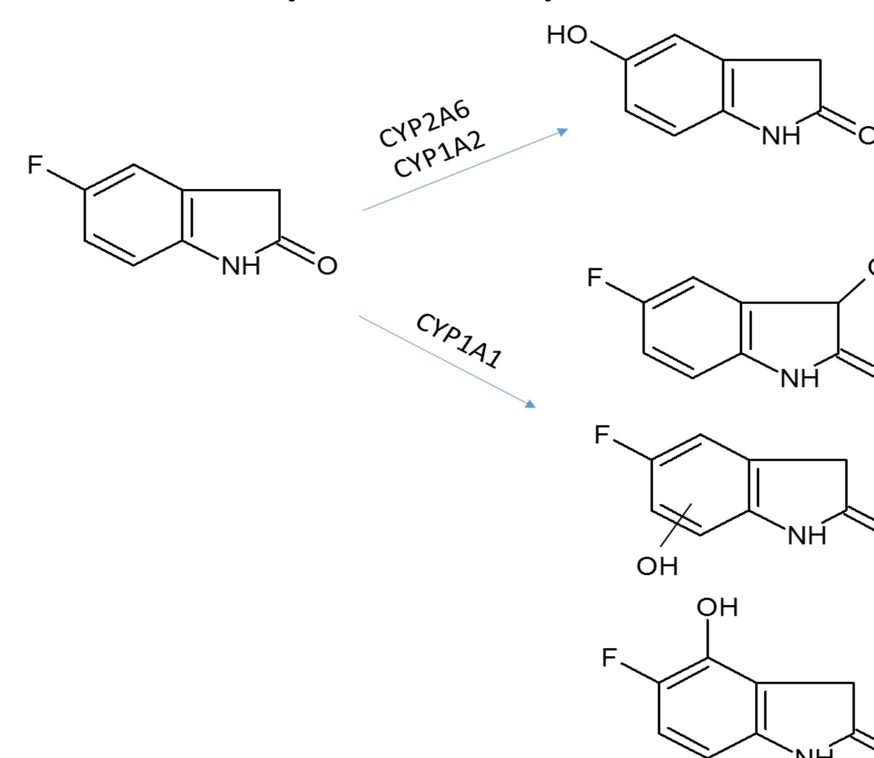

Figure 5. Metabolites of the reaction between CYP enzymes and 5F2O. Several products were hypothesized based on LC/MS and HPLC data from other previous research.

Methods

General HPLC Protocols

1. 5F2O diluted in methanol to 200mM stock.
2. Diluted to 1-20mM in reaction.
3. Transferred 500uL of reaction to HPLC vial.
4. Inject 25uL on C18 reverse phase column with a flow rate of 1mL/min for 10 minutes.
5. Viewed at 214nm.
6. Different ratios based on individual method trials, but consistent solvents of diH_2O (0.1% TFA) and acetonitrile (0.1% TFA).
7. Increased proportion of diH_2O:ACN to shift peak further right (will come off column later).

Individual Methods Used

1. Oxindole.M was a gradient method running from 100% diH_2O to 0% and 0% ACN to 100% over 20 minutes.
2. OxindoleTrial1.M was an isocratic (non-gradient) method with 80:20 diH_2O:ACN.
3. OxindoleTrial2.M was an isocratic method with 65:35 diH_2O:ACN.

Reaction With Human Liver Enzymes

1. A reaction with final volume of 1mL contained the following reagents: 1mM 5F2O, 1mM NADPH, 50mM phosphate buffer, pH 7.5, 1uL CYP2A6R, diH_2O.
2. Incubated at 35 °C for 10 minutes.
3. Quenched with 1mM methanol.
4. Negative control generated by omitting NADPH
5. 500uL of each reaction transferred to HPLC vial.

Conclusion

5-fluoro-2-oxindole can be detected by HPLC at 214nm using a non-gradient method. This detection is optimized at a 65:35 diH_2O:ACN isocratic method to consistently separate the peak from the solvent peaks.

Future Work

Future work will begin with running additional reactions with human liver enzymes to find which conditions will optimize a product peak. Further work will feature the development of a protocol for LCMS analysis of both product and substrate peaks. The products of the reaction will then be analyzed to determine their structure and relative toxicity.