Hydrolysis Studies of Vitamin K Analogues

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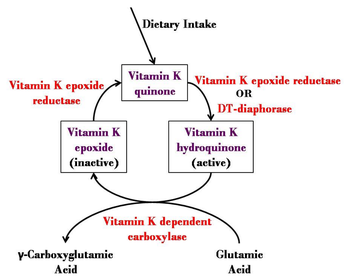
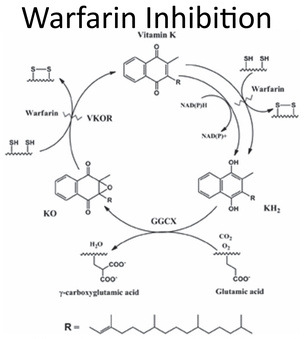
Abstract

Our goal is to find an analog of Vitamin K that is a better and safer anticoagulant than the current drug, warfarin (Coumadin®). This is important because it is difficult to find a safe, stable and effective dose of warfarin. We have reported the synthesis of two esters with the potential to act as vitamin K analogues, and inhibit the vitamin K-dependent clotting cascade. One of these esters has shown unexpected activity when co-administered with warfarin to rats. The activity of the one highly active compound is confusing because at the beginning of the trial, it acts as a potent antagonist of warfarin anticoagulation, while later in the trial, using the same protocols, it becomes a potent enhancer of warfarin anticoagulation. As part of our work aimed at identifying the compounds responsible for both the anti-anticoagulant activity and the super-anticoagulant activity, we are carrying out hydrolysis of the esters under conditions that mimic the gastrointestinal environment in the animal.



showing the normal action of the Vitamin K cycle in creating clotting factors to allow ts. The γ-Carboxyglutamic Acid is the active clotting factor.

Warfarin Inhibition

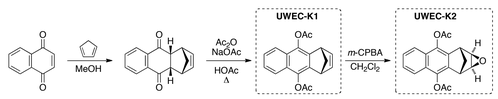


Warfarin inhibits the transition back to the active form, Vitamin K hydroquinone (KH_2 in this figure) by inhibiting Vitamin K epoxide reductase.

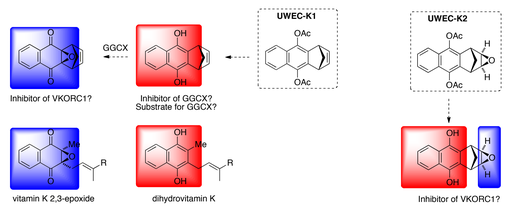
Creation of Vitamin K analogues

- The synthesis of UWEC-K1 and UWEC-K2 [*Bioorg. Med. Chem. Lett*. **2010**, *20*, 1928-1932] is now well established in our laboratory, and these molecules are the precursors used in the different reactions to attempt to find the actual compound responsible for the anticoagulant activity.

Synthesis of UWEC-K1 and UWEC-K2

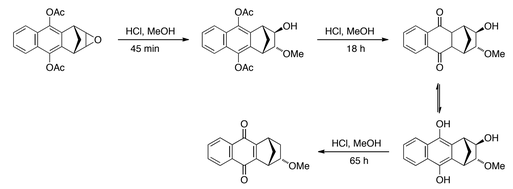


Structural Similarities of UWEC-K compounds and vitamin K derivatives



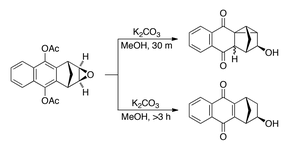
Methanolysis of UWEC-K2 in acid

- Another attempt to mimic how compound is changed in stomach



- Epoxide methanolysis occurs much more rapidly than ester cleavage
- *trans*-ring opening of epoxide is observed exclusively
- Slower methanolysis of the ester groups leads to the diketone with the center ring not aromatic
- longer exposure to acid gives the quinone by dehydration (presumably through the aromatic intermediate)

Methanolysis of UWEC-K2 in base



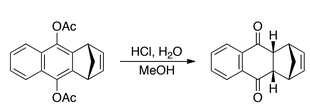
- Initial product formed is a cyclopropyl ketone
- Slow isomerization gives the quinone

Hydrolysis of UWEC-K2 in aqueous solution

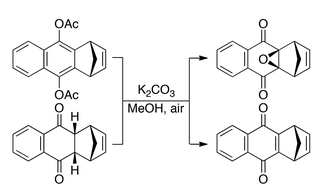
- Stomach acid is aqueous, not alcoholic.
- We needed to hydrolyze UWEC-K2 under aqueous conditions.
- Acid hydrolysis reactions on UWEC-K2 were run using 0.1 *M* HCl in mixed aqueous-organic (THF, acetone, MeOH) solution.
- The residence time in the rat stomach is less than 2 hours, so this was the maximum time of each hydrolysis experiment
- Every reaction yielded compounds that are insoluble in common organic solvents, so they could not be isolated by extraction.
- The observed yields decreased with increasing reaction time.
- The simplest explanation is that the products are water soluble.
- We are still working on the isolation and structure determination of these products.

Acid-catalyzed Methanolysis of UWEC-K1

- To mimic how the molecule would be changed in the stomach, UWEC-K1 was treated with concentrated HCl in methanol.
- We observed both hydrolysis of the ester groups, and loss of aromaticity in the center ring



Methanolysis of UWEC-K1 in base



Acknowledgements

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