First Time One-Step Synthesis of Bioactive Quinone Natural Products

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Natural Product Synthesis

Natural products are organic compounds that are generated by living organisms as secondary metabolites and may function as defense chemicals. A wide range of natural products have been identified to have significant inhibitory activity vs. certain enzymes, thus suggesting their therapeutic potentials. Paclitaxel (Taxol) and vinblastine (Velban), isolated from *Taxus brevifolia* (the Pacific Yew) and *Catharanthus roseus*, respectively, have played an important role in cancer treatment. In fact, the use of plants as sources of medicines has been employed by humans for ages with the earliest written record dating back thousands of years.

In modern medicinal chemistry, the traditional use of medicines is recognized as a way to identify drugs for future pharmaceutical development. Due to the challenge that bioactive natural products are only generated by organisms in small amount, the large-scale extraction of molecules from a natural source is infeasible. Thus, laboratory total synthesis of natural products in sufficient quantities allows for the further investigation into their biological activities and pharmacology.

Naturally Occurring Quinones

Quinones are a class of organic compounds characterized by their conjugated cyclic di-ketone structures. Some representative members of the class are 1,4-benzoquinone, 1,2-benzoquinone, and 1,4-naphthoquinone. As shown in **Figure 1**, allyl and isoprenoid quinones are often found as bioactive natural products and are important components of the cell membrane in living organisms[1]. Ubiquinones (coenzyme Q), examples of 1,4-benzoquinones, actively participate in the electron transport chain in eukaryotic mitochondria. Plastoquinone (PQ), an isoprenoid quinone molecule found within photosystem II, is involved in the light-dependent reactions in photosynthesis. Various forms of vitamin K, such as phylloquinone (vitamin K_1) and menaquinone (vitamin K_2), are essential in the synthesis and function of Vitamin K-dependent proteins.

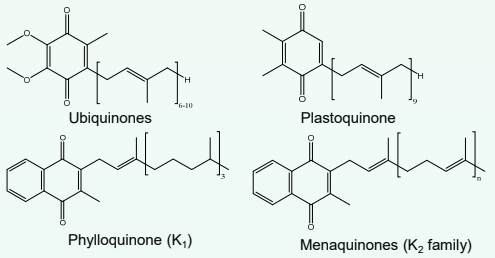

Figure 1. Molecules of bioactive quinones. Ubiquinones and Plastoquinone belong to the class of 1,4-benzoquinones. Phylloquinone and Menaquinones are examples of 1,4-naphthoquinones.

Quinones have also been demonstrated to have a wide range of biological activities, including but not limited to antibiotic, anticancer, antimalarial, and antioxidant properties[2-4]. Thus, our synthetic targets are naturally occurring quinones derivatives with therapeutic potentials.

As shown in **Figure 2**, our synthesized compound **1a** has been found in the stems and leaves of *Gunnera perpensa* and has shown significant antimicrobial activity against *Staphylococcus epidermidis*[5]; compound **1b** has been extracted from *Pyrola media*[6]; compound **2** has been shown to exist in *Pyrola media*[6], *Ligularia virgaurea*[7], and *Cystophora harveyi*, and it is a known constituent of Asian medicinal herbs[8]. Notably, for the first time, compounds **1b** and **2** were synthesized in the lab.

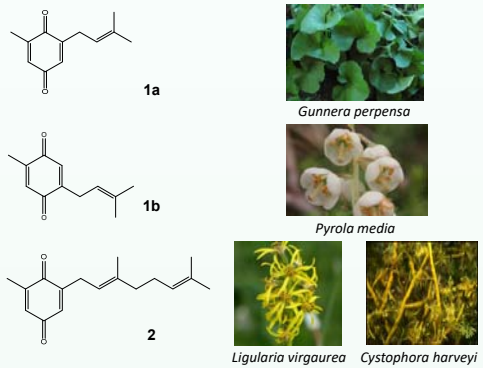

Figure 2. Naturally occurring 1,4-benzoquinones synthesized in our lab and their sources in nature. The molecules were sent off to Max Planck Institute in Germany for further biological analysis. Data is pending.

Past Synthetic Approaches

Traditional synthesis of bioactive prenylated benzoquinones involves phenol starting materials. A schematic review is shown in **Figure 3**, in which a carbon side chain is introduced onto *ortho*-cresol and the final quinone product is obtained through oxidation. However, the reaction scheme involves multistep synthesis, oxygen-prenylated byproducts, and low reaction yields. A novel synthetic pathway has to be designed.

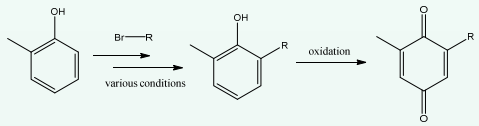

Figure 3. Generalized reaction scheme for traditional synthesis of benzoquinone structural analogs.

A Novel Synthetic Approach Towards Bioactive 1,4-Benzoquinone Derivatives

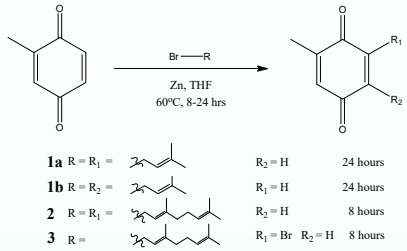

Figure 4. Our reaction scheme in development. Products **1a-2** are yielded with varying conditions shown. Brominated 1,4-benzoquinone (compound **3**) is an undesired byproduct. Prenyl side chain = C_5H_9, geranyl side chain = $C_{10}H_{17}$.

Applying the synthetic methods by de Oliveira and coworkers[9], we successfully introduced the prenyl and geranyl side chain onto 1,4-benzoquinone in one step. The reactions were carried out in ambient pressure, moderate temperature, relative short reaction time, and economic starting material (methyl-*p*-benzoquinone, $1.36/gram from Sigma-Aldrich). We have also found that the use of anhydrous reaction solvent is unnecessary. Thus, our work provides a new synthetic pathway towards the synthesis of naturally occurring quinone derivatives.

Synthetic Challenges

A possible mechanism proposed by Sugihara and coworkers[10] currently may explain our reaction mechanisms. However, failed synthesis attempts have occurred when some side chains were introduced. Commonly these side chains are missing double bonds between carbon 2 and 3 position, as labelled in **Figure 5**. This may undermine the application of our methods in reactions with all types of side chains. Thus, we envision further investigation on the reaction mechanisms and the improvement of reaction conditions to accommodate chain sides as such.

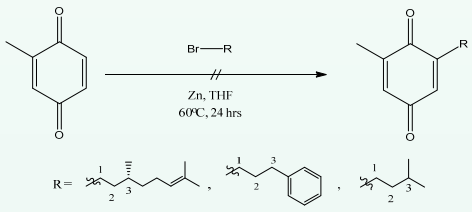

Figure 5. Unsuccessful synthesis attempts with different side chains that lack double bond between carbon 2 and 3, as labelled. These side chains are unable to form radicals in carbon-alkylation and less likely to be attached to the quinone ring.

Experimental

General Procedure: 1.4 equivalent methyl-p-benzoquinone, 3.2 equivalent Zinc granules, and ~50 mL Tetrahydrofuran were added to a 100 mL round bottle flask. Over the course of 5 mins, prenyl or gerany bromide (1 equivalent) was added slowly via syringe to the flask with stirring. The reaction mix was refluxed at 60 ºC for 8-24 hours and the solvent was removed from the filtrate *in vacuo*. The products were dissolved in diethyl ether, and extracted with deionized water three times. The organic layers were dried over Na_2SO_4 for one hour and the solvent was removed *in vacuo*.

Identification and Analysis: Quinone products were eluded via column chromatography in 20:1 Hexane to Ethyl Acetate solvent system. The final products were analyzed via 1D and 2D NMR, and GC/MS. Two regioisomers **1a** and **1b**, and compound **2** were obtained in yellow oil. Biological screening for anticancer properties of the molecules was performed via NAD+/NADH assay and enzymological studies.

Future Works

Quinone Natural Product Libraries: More side chains will be introduced to the quinone ring through our reaction schemes. We are actively developing novel and convenient synthetic methodologies to naturally occurring quinone molecules in search of their bioactivities.

Reaction Optimization: In an effort to maximize yield of our desired products, we will carry out several optimization reactions under varied reaction conditions. This will minimize the formation of byproducts, alleviate the challenges during purification processes, and give our novel synthetic methods a competitive edge over other multi-step pathways.

Acknowledgments

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