2014

A New Methodology for the Synthesis of 2-Alkyl-5,6-bis(alkylthio)benzo[d]thiazole-4,7-dione

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Winter 2014

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CHEM 497 Introduction to Research

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Abstract:

Cancer is a rapidly growing fatal disease and with the various thiazole compounds being scientifically generated, possible treatment options can be implemented. Proper synthesis of 2,3-dimethoxy-1,4-benzoquinone allows for a variety of compounds to be made with further treatment of alkylthio reagents. Both thioacetamide and thiobenzamide are used to react with 2,3-dimethoxy-1,4-benzoquinone in order to build a library of thiazoles. Final compounds can be tested for the ability to inhibit recombinant enzyme activity and the capability to kill tumor cells. A basic oxidation procedure along with nucleophilic attack was used to create target products.

Introduction:

Cancer is a worldwide epidemic that is quickly growing and spreading. Special attention must be put on cancer research in order to put up an honest fight against its fatal qualities. With over one hundred different types, cancer is the disease characterized by exaggerated cell growth.[1] According to the World Cancer Research Fund International, in the year 2008, over 12.7 million new cases of cancer were diagnosed.[2] The most common type being lung cancer for men and breast cancer for women. The majority of cancers have a very low survival rate and are highly dependent on early detection and treatment. Andrei Gartel and his group of researchers found that synthesized thiazole antibiotics efficiently inhibited the growth and induced potent apoptosis in human cancer cell lines of different origin.[3] There seems to be a link between thiazole compounds and cancer treatment and with this thought in mind, this project is dedicated to the building of different thiazole compounds in order to find a similar inhibition of cancer cells.

Various chemists have devoted years of research to tap into this disease and figure out how it can be treated. Hans-René Bjørsvik and his team have tested switchable oxidation processes leading to various pharmaceutical intermediates.[4] Their approach was to design and implement a new process for the preparation of methyl- and methoxy- substituted arenes which would be the building blocks for the antioxidants, pharmaceutical chemicals, and compounds used in nutraceuticals. Using the most activated substrate, 1,2,3-trimethoxy-5-methylbenzene, Bjørsvik and his team achieved the highest yield.[3] In our experiment, we employed a similar procedure for the synthesis of 2,3-dimethoxy-5-methyl-1,4-benzoquinone, but instead use 1,2,3-

trimethoxybenzene **(1)** as our starting material. Yan-Ping Shi worked with synthesizing flavonoids and from his work we studied the procedure, which similarly to Bjørsvik, used **(1)** as a substrate to make 2, 3-dimethoxy-1,4-benzoquinone **(2)**[1]. However, our reaction conditions will be different to achieve this compound.

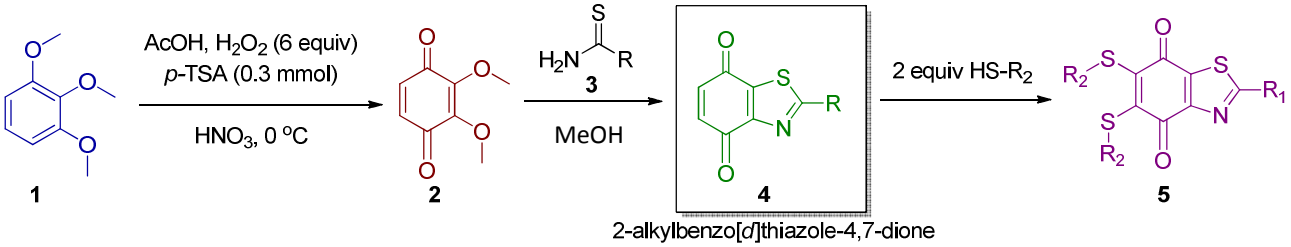

Figure 1. Experimental Design.

Following the work of Alan R. Katritzky and Wei-Qiang Fan on quinones containing fused ring systems, we used a similar procedure designed to synthesize 2-methylnaphtho[2,3-d]thiazole-4,9-dione from the thioacetamide and 2-mercapto-3-amino-1,4-naphthoquinone.[5] However, our project had some notable differences. Firstly, since our reaction would not produce HCl, thus we would not need to use 4-diazabicyclo [2.2.2]octane (DABCO) as a base or dimethyl sulfoxide (DMSO) as a solvent. Instead, methanol was used as a solvent since the reaction will generate it. Secondly, our backbone compound, 2,3-dimethoxy-1,4-benzoquinone was treated with a thioalkylamide **(3)** in hopes of reaching our goal of synthesizing 2-alkylbenzo[d]thiazole-4,7-dione **(4)**.

The goal of this project is to design, synthesize and evaluate 2-alkyl-5, 6-bis(alkylthio)benzo[d]thiazole-4,7-dione derivatives as thioesterase (TE1) inhibitors to block TE1 activity or as cyclin-dependant kinase (CDK) inhibitors. The compounds will be synthesized by building a 2-alkylbenzo[d]thiazole-4, 7-dione library of compounds

which can then be treated with alkylthiol reagents and be converted into multiple final targets for a quick library of compounds. These compounds will then be tested to (i) inhibit recombinant enzyme activity, (ii) inhibit cellular fatty acid synthesis or CDK inhibition, and (iii) specifically kill tumor cells.

Methods:

Experimental procedures were completed at Southern Adventist University using standard laboratory glassware from ChemGlass or Kimble Kontes, and various reagents obtained commercially from Alfa Aesar, Sigma-Aldrich, Acros, Anal Tech and Fisher. All the reactions were carried out using CG-1994-Chemglass Optimag Magnetic Hot Plate Stirrers with Safety Controls with CG-1991-P Pie Blocks, Reaction Blocks, and Safety Holders. All solvents were evaporated using a Wilmad Lab Glass rotovap WG-EV311 or Buchi RE 111 rotovapor. When needed, products were run on a Jeol 400 mHz NMR Spectrometer used at the University of Tennessee Chattanooga.

Reaction 1

Synthesis of 2, 3-Dimethoxy-[1, 4]benzoquinone (2). A solution of 1,2,3-trimethoxybenzene (3.20 g, 19 mmol) and acetic acid (19 mL) was added to a three neck round bottom flask and stirred using a reflux condenser. A bubbler was set up using nitrogen gas and mineral oil to keep an open system for the exothermic reaction. Then, *p*-toluene sulfonic acid monohydrate (0.362 g, 1.9 mmol) and hydrogen peroxide (30%, 3.24 mL, 38 mmol) were added to flask. The reaction mixture was stirred and heated at 75℃ for 30 minutes and monitored using thin-layer chromatography (TLC) by checking the consumption of starting material and the formation of product **2**. Once the reaction was verified it was then cooled to 0℃. Nitric acid (90%, 0.41 mL, 8.7 mmol) was added dropwise. The cold reaction mixture was poured into water and extracted

with dichloromethane. The organic layers were combined and washed with water to remove residues of acetic acid, dried over anhydrous sodium sulfate and filtered. Finally, the solvent was evaporated under reduced pressure to achieve the crude as dark red oil.

Purification. The crude product was dissolved in boiling hexane and filtered. Solvent was removed under reduced pressure to provide the purer compound **2**. Column chromatography was used with dry packing to further purify the product. A 20:1 ratio of silica gel versus crude mass was calculated for the column. Silica gel was mixed with dichloromethane and poured into the column. A 2:1 ratio of silica gel versus crude mass was calculated for the dry pack. The gel was dissolved in the least polar solvent, hexane, mixed with crude and evaporated under reduced pressure to obtain powder to pack in column. The solvent system used was hexane:ethyl acetate, obtaining five 25 mL fractions of 9:1, 8:2, and 7:3 ratios, respectively. This solvent system was used throughout all subsequent reactions. Selected pool containing product evaporated under reduced pressure to obtain a maroon solid (0.250 g, 7.81% yield).

Reaction 2

Synthesis of 2-methylbenzo[d]thiazole-4,7-dione (4a, R=Me). Once product **2** (0.07 g, 0.42 mmol) was synthesized, it was treated with thioacetamide (0.086 g, 0.63 mmol) using methanol (1 mL) as the solvent. The mixture was stirred using Pie blocks and the reaction went to completion. The reaction was monitored using TLC. The mixture was also further purified by means of column chromatography. Fifteen fractions of 8 mL each were obtained from mixture and the selected pool was evaporated under reduced pressure (0.034 g, 63.89% yield).

Reaction 3

Synthesis of 2-phenyl-4,7-benzothiazoledine (4b, R=Ph). Using product **2** (0.05 g, 0.30 mmol), the reaction was run using thiobenzamide (0.045 g, 0.33 mmol) and methanol (1 mL) as a solvent. The mixture was stirred using Pie blocks and the reaction went to completion. The reaction was monitored using TLC and was further purified by means of column chromatography. Twenty fractions of 5 mL each were obtained from mixture and the selected pool was evaporated under reduced pressure to obtain yellow solid (0.067 g, 92.5% yield).

Reaction 4

Treatment of 2-alkylbenzo[d]thiazole-4, 7-dione using N-Acetylcysteamine. 2-phenyl-4, 7-benzothiazoledine (0.067 g, 0.278 mmol) **(4b)** was dissolved in methanol (0.70 mL) and treated with *N*-acetylcysteamine (31 µL, 0.278 mmol). The reaction mixture was allowed to stir for two days at a constant temperature of 60℃. Methanol was evaporated under reduced pressure and later purified by column chromatography.[7] HNMR indicated that starting material was recovered. Another attempt to synthesize the desired targets is underway.

Results:

Reaction 1

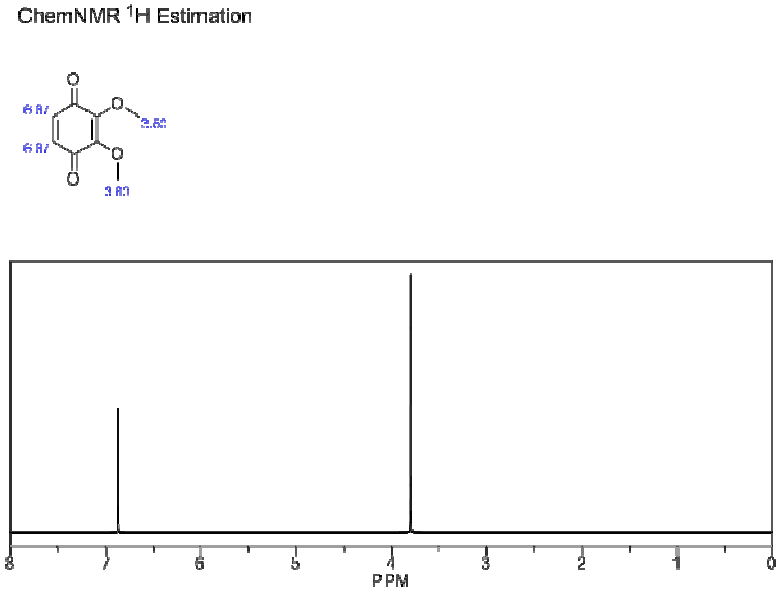

Figure 2. NMR estimation for product **2.**

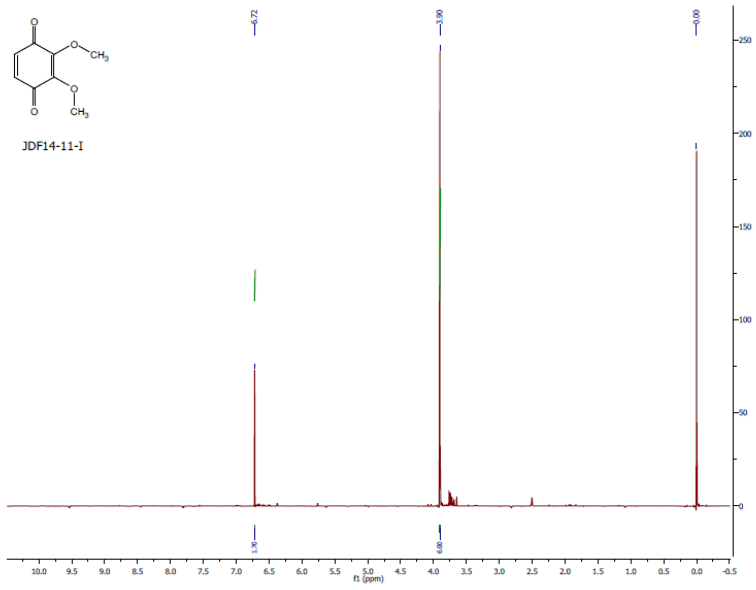

Figure 3. NMR for product **2**.

Reaction 2

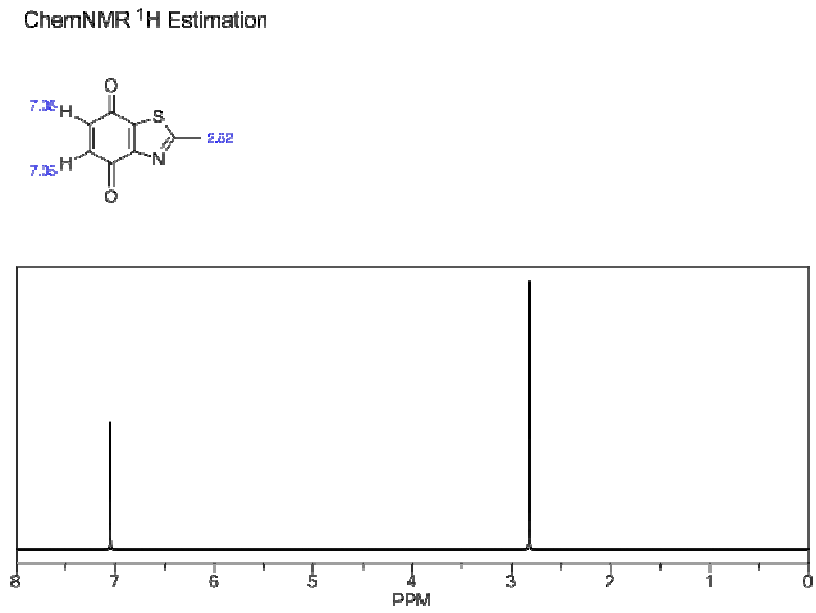

Figure 4. NMR estimation for product **4** with R=Me.

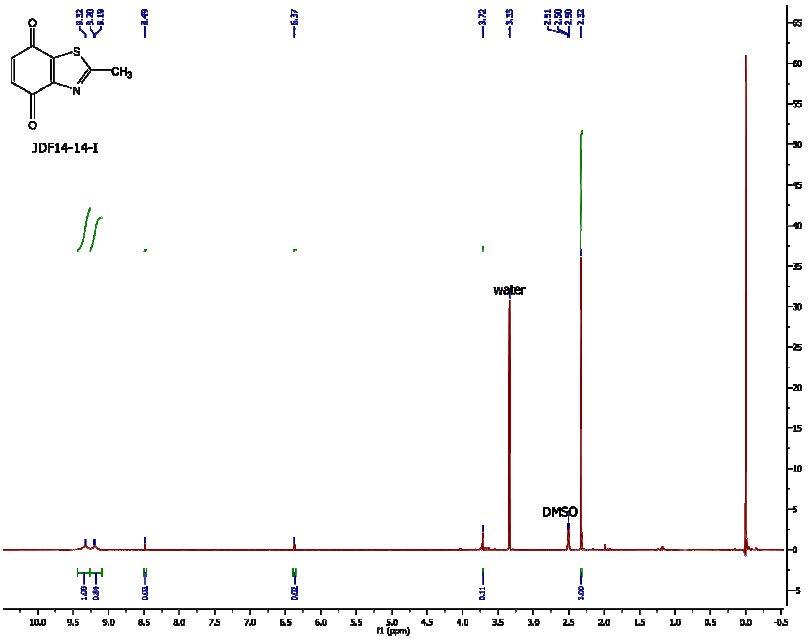

Figure 5. NMR results for product **4** with R=Me.

Reaction 3

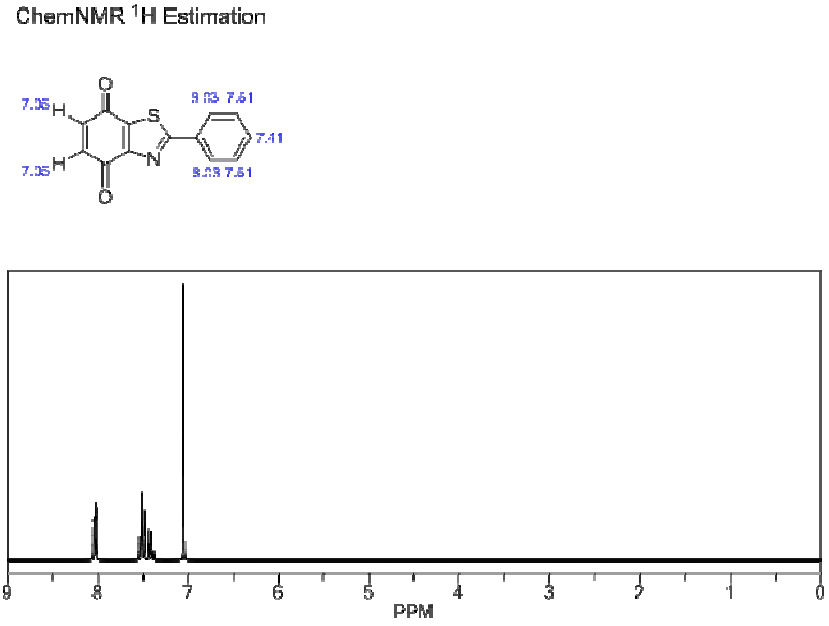

Figure 6. NMR estimation for product **4** with R=Ph.

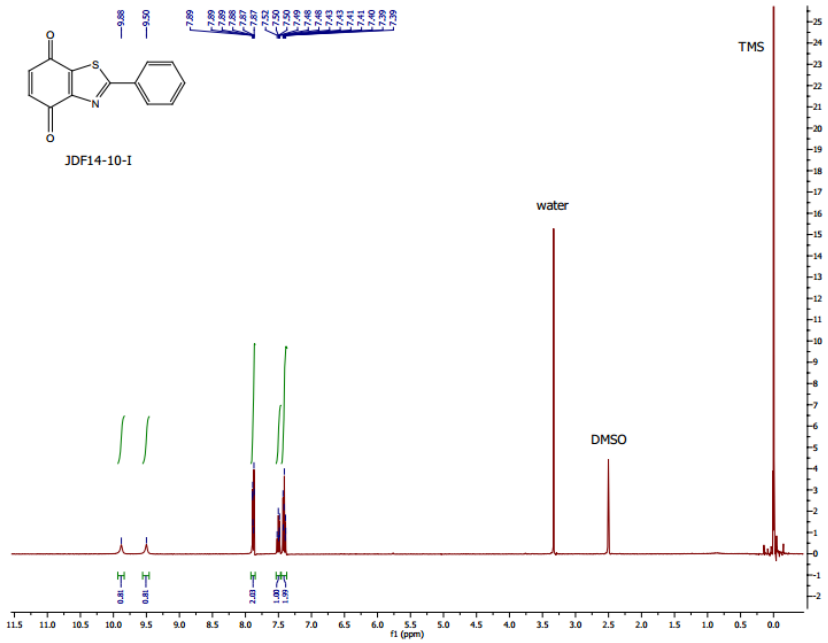

Figure 7. NMR data received for purified sample of product **4** with R=Ph.

Discussion:

Various attempts were made in the refining of the methods for reaction 1. Pie blocks were implemented to control the temperature of the reaction but after many tries, an open bubbler system was found to achieve better results as the exothermic activity could be monitored via bubbling the mineral oil. Dicholomethane was used as the final solvent during the evaporation process to adhere cleaner NMR readings. The lower yield shown in Reaction 1 is a result of maintaining inaccurate temperatures due to the lack of a Pie block system, possibly causing starting material to have been burnt. However, NMR results show a very pure product was obtained therefore the lower yield is more readily accepted.

Reaction 3 shows successful product purity and yield. The products purity relied heavily on the fact that pure product from Reaction 1 was used and had no impurities. Both reactions were also purified using column chromatography. Successful production of this product allows for further nucleophilic attack on the quinone side of the molecule. The NMR estimation is assuming that the hydrogen atoms on the quinone side are the same, but they are not because the molecule is not symmetrical. Another reason for purity would be that this was a short reaction and the proper conditions were implemented for optimal results.

The results of Reaction 4 were verified by means of NMR and resulted in recovery of starting material. This means either the reaction did not go to completion, or simply did not work. Further oxidation reactions might have to be implemented to obtain proper product.

Conclusions:

According to Figure 1, the synthesis of two derivatives (R=Ph and Me) have been achieved in this research project. Larger scale syntheses of either product will be necessary to continue the building of the thiazole products. In order to continue this research, further nucleophilic attack would have to be completed on either product, resulting in the final product **5**. With successful methods now developed for products **2,** and **4a** with R=Me and with **4b** with R=Ph, continuation of this project would expand the library of viable heterocyclic compounds to test for cancer. Various alkylthio nucleophiles can be used to make different versions of product **5** and once these products have been properly synthesized; further testing can be done to assess their chances against fighting cancer.

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