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DESIGN AND SYNTHESIS OF A POTENTIAL CHEMOTHERAPEUTIC AGENT USING GONIOTHALAMIN AS A NATURAL PRODUCT TEMPLATE

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Abstract

Goniothalamin, a natural product extracted from tree bark of the *Goniothalamus* genus, has been shown to induce apoptosis in cancer cells. It is hypothesized that goniothalamin's biological activity is due to its ability to react with thiols. Goniothalamin has been shown to decrease levels of glutathione, a natural antioxidant, found in cancer cells. This causes a redox imbalance, which ultimately leads to cell death. Thiol-reactive compounds, like goniothalamin, have also been shown to inhibit nuclear factor-κB (NF-κB). NF-κB is a transcription factor that has been implicated in unregulated cell growth. Through a nine step sequence, a novel analogue of goniothalamin has been prepared that replaces the lactone core of the natural product with a cyclohexenone. The synthetic sequence features a unique enol ether protection of a beta-diketone which allows facile preparation of the desired analogue. It is anticipated that the novel goniothalamin derivative will demonstrate increased cytotoxicity against cancer cells.

Keywords: Cancer, Goniothalamin, Glutathione, Nuclear factor–κB, Cyclohexenone, Enol ether

Introduction

Goniothalamin **[1]** was first isolated from the dried stem bark of the plant *Goniothalamus sesquipedalis* (1). This natural product exhibits IC_{50} values in the low micromolar range against a variety of cancers (2). Due to its bioactivity and limited availability from natural sources, goniothalamin has received considerable attention as a synthetic target (1). The first synthesis of goniothalamin was performed in 1979 (3). Since then, numerous enantioselective syntheses of goniothalamin have been reported (2,4). A large number of synthetic analogues of goniothalamin have been prepared in an attempt to determine the structural features necessary for bioactivity (2,4,5). These studies have focused primarily on the manipulation of the styryl substituent. This paper discusses the design of a new analogue of goniothalamin that focuses on manipulating the lactone core of the molecule. In previous work, the lactone core of goniothalamin was replaced by a lactam ring system (6). This research involves synthesizing a cyclohexenone core (analogue **[2]**, Figure 1).

It is hypothesized that goniothalamin induces cytotoxicity in cancer cells via two pathways. Both of these mechanisms have one thing in common - goniothalamin's ability to react with intracellular thiols. Glutathione (GSH) is a natural antioxidant found in all cells. Literature precedence shows that goniothalamin induces rapid depletion of GSH levels within cancer cells (7). It is hypothesized that the α,β–unsaturated carbonyl found in goniothalamin can react with thiols through a 1,4-addition reaction (8). By forming a covalent, thioether bond with GSH, goniothalamin effectively removes GSH from the cell. This decrease in GSH is what triggers the build-up of oxygen free radicals and the initiation of apoptosis.

Goniothalamin can also inhibit nuclear factor-kappa B (NF-κB). NF-κB is a protein complex that controls the transcription of DNA during cell division. Over-activation of NF-κB has been found in a wide variety of tumor types (9). One method to prevent the activation of NF-κB is through inhibition of a kinase enzyme, IKK. Phosphorylation of two serine residues, Ser-177 and Ser-181, on IKK are what starts the NF-κB activation sequence (10). It has been reported that thiol-reactive agents can inhibit IKK by reacting with a cysteine residue, Cys-179, located between the two serine residues (11). This reaction prevents serine amino acid phosphorylation and therefore NF-κB activation, presumably due to sterics.

Goniothalamin's bioactivity is a direct result of its ability to react with thiols. Therefore, altering the structure of goniothalamin to further increase the positive character of the β-carbon should promote 1,4-addition reactions with thiols and increase bioactivity. The design of cyclohexenone **[2]** (Scheme 1) is based on the knowledge that ketones are better electrophiles than esters. By changing goniothalamin's core structure into an α,β-unsaturated cyclohexenone, it should make the compound more reactive towards thiols like GSH and Cys-179.

Experimental

Column chromatography was performed on 230-400 mesh silica gel. NMR spectroscopy was recorded on a Jeol 300 MHz NMR spectrometer. Gas chromatography/ mass spectrometry was carried out on an Agilent Technologies 7890A gas chromatograph. The GC column (30 m x 0.25 mm) had a 0.25 mm thick polydimethylsiloxane (PDMS) with 5% phenyl substitution stationary phase. The oven conditions were: injection port temperature = 250° C; oven starting temperature = 70° C for 5 minutes; ramp of 20° C / minute up to 250° C; the temperature was held for 20 minutes at 250° C. All starting chemicals were purchased from Sigma-Aldrich, Inc. (Atlanta, GA) and used as received.

Step 1: (12)

Commercially available *trans*-cinnamaldehyde **[3]** (5.00 mL, 39.7 mmol) was dissolved in acetone **[4]** (60.0 mL, 817 mmol). A 10% NaOH solution (16.0 mL, 40.0 mmol) was added to the reaction mixture. The reaction was stirred at room temperature overnight. After the reaction was complete, 3M HCl was added until a pH of 2 was achieved (15 mL). An ether extraction was performed on the acidic solution (2 x 75 mL). The combined organic layers were dried over sodium sulfate and concentrated under vacuum to provide a red-brown

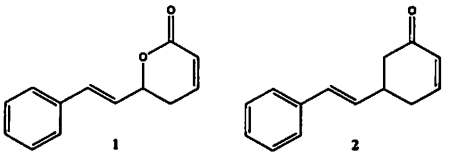
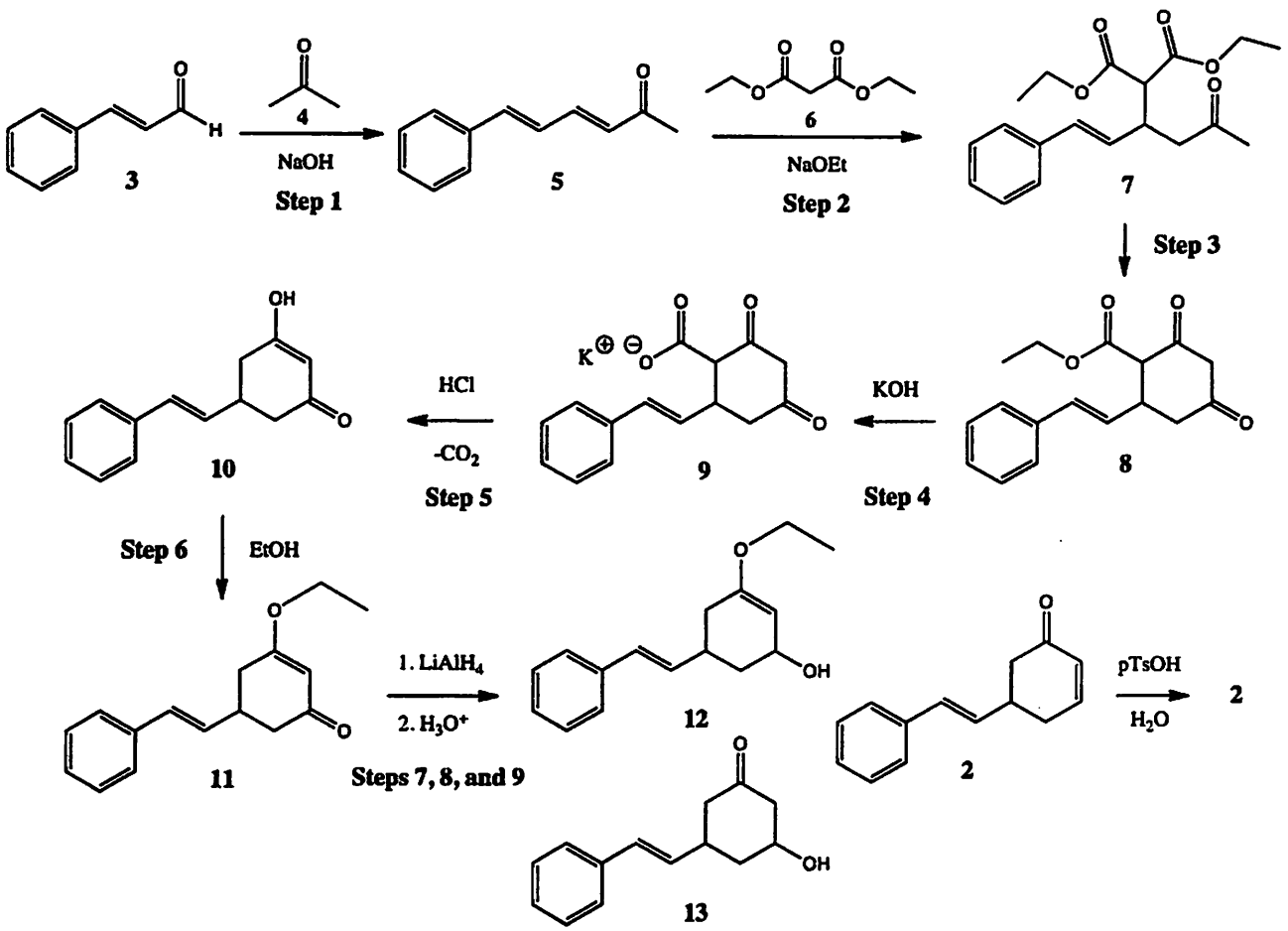


Figure 1. Goniothalamin [1] and Synthetic Analogue [2].



Scheme 1. Synthesis of α,β-unsaturated cyclohexenone [2].

solid. The solid was purified using recrystallization in absolute ethanol to provide pure ketone **[5]** (4.874 g, 71.30% two batches) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (dd, J = 8.0, 1.6 Hz, 2H), 7.40-7.23 (m, 4H), 6.95 (d, J = 15.4 Hz, 1H), 6.87 (dd, J = 15.5, 9.5 Hz, 1H), 6.25 (d, J = 15.4 Hz, 1H), 2.31 (s, 3H). GC/MS: RT=12.256 min, M^+=172.

Steps 2 and 3: (13)

A solution of sodium ethoxide was prepared by dissolving sodium metal (324 mg, 14.1 mmol) in absolute ethanol (10.0 mL). Diethyl malonate **[6]** (2.05 mL, 13.5 mmol) was then added to the solution. Through the use of a cannula, the solution was transferred to the flask containing ketone **[5]** (2.207 g, 12.83 mmol). The reaction mixture was allowed to reflux overnight. The resulting mixture was concentrated under vacuum, resulting in a dark red/brown solid, which was crude ester **[8]**. The crude product was carried directly into the next step.

Step 4: (13)

Ester **[8]** was placed in distilled water (14.0 mL). In a separate flask, KOH (1.39 g, 24.8 mmol) was dissolved in water (2.50 mL). The resulting 9.9M KOH solution was added to ester **[8]**. The solution was heated at reflux for 45 minutes. While refluxing, a small amount of brown solid was formed. The solid was removed and discarded using vacuum filtration. The filtrate, an aqueous solution of crude carboxylic acid salt **[9]**, was carried directly into the next step.

Step 5: (13)

Concentrated HCl (4.42 mL, 53.0 mmol) was added to the aqueous solution of crude carboxylic acid salt **[9]** and heated at reflux for 30 minutes. The flask was cooled to room temperature and then placed in an ice bath for 30 minutes. The resulting yellow-orange solid, enol **[10]**, was collected by vacuum filtration and carried directly into the next step. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.17 (br s, 1H), 7.40 (dd, J = 7.8, 1.4 Hz, 2H), 7.31 (dd, J = 7.3, 7.3 Hz, 2H), 7.22 (tt, J = 7.1, 1.4 Hz, 1H), 6.46 (d, J = 16.2 Hz, 1H), 6.31 (dd, J = 16.1, 6.6 Hz, 1H), 5.25 (s, 1H), 2.96-2.83 (m, 1H), 2.48-2.23 (m, 4H). GC/MS: RT=14.479 min, M^+=214.

Step 6:

Crude enol **[10]** was dissolved in absolute ethanol (25.0 mL, 428 mmol) and heated at reflux for three hours. Concentration under vacuum provided enol ether **[11]**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.23 (m, 4H), 7.23-7.14 (m, 1H), 6.40 (d, J = 15.9 Hz, 1H), 6.12 (dd, J = 15.9, 6.9 Hz, 1H), 5.38 (s, 1H), 3.87 (q, J = 7.0 Hz, 2H), 3.01-2.84 (m, 1H), 2.59-2.47 (m, 2H), 2.45-2.22 (m, 2H), 1.33 (t, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.3, 177.4, 136.9, 131.3, 130.2, 128.7 (2C), 127.7, 126.3 (2C), 102.5, 64.7, 42.7, 37.0, 35.3, 14.2. GC/MS: RT=15.826 min, M^+=242.

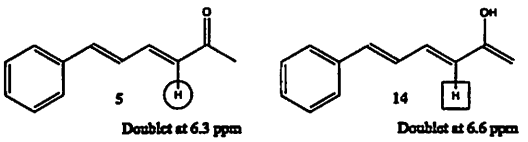


Figure 2. Keto/Enol Tautomers.

Steps 7,8, and 9: (14)

Enol ether **[11]** was dissolved in anhydrous diethyl ether (15.0 mL) and placed in an ice bath. Lithium aluminum hydride (0.360 g, 9.49 mmol) was added to the flask. The resulting suspension was heated at reflux for one hour. The mixture was quenched with 1M H_2SO_4 (20 mL) and a diethyl ether extraction (2 x 50 mL) was performed. The combined organic layers were dried over sodium sulfate, filtered, and concentrated under vacuum to afford a crude, red oil. GC-MS of the resulting product varied. Sometimes, complete conversion to the desired α,β-unsaturated cyclohexenone **[2]** was observed. Other times, significant amounts of alcohol **[12]** or β-hydroxy ketone **[13]** were observed. In these cases, an additional step was performed. The alcohol **[12]**/ β-hydroxy ketone **[13]**/ analogue **[2]** mixture was dissolved in acetone and treated with water (24 equivalents compared to moles of crude **[11]**) and *p*-toluenesulfonic acid (0.8 equivalents compared to crude **[11]**). The resulting solution was heated at reflux for 3 hours and then extracted with diethyl ether (2 x 75 mL) and 1M H_2SO_4 (30 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated to afford crude cyclohexenone **[2]**. Ketone **[2]** was purified by column chromatography (85:15 hexanes:ethyl acetate) to afford pure α,β-unsaturated cyclohexenone **[2]** (0.902 g, 35.5% based on the moles of ketone **[5]** used in Step 2) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.20 (m, 4H), 7.20-7.12 (m, 1H), 6.86 (ddd, J = 10.4, 5.6, 2.7 Hz, 1H), 6.34 (d, J = 15.8 Hz, 1H), 6.09 (dd, J = 15.8, 7.0 Hz, 1H), 6.00 (br d, J = 10.4 Hz, 1H), 2.90-2.75 (m, 1H), 2.53 (dd, J = 16.1, 4.0 Hz, 1H), 2.41 (ddd, J = 18.4, 5.1, 5.1 Hz, 1H), 2.29 (dd, J = 16.1, 12.1 Hz, 1H), 2.18 (dddd, J = 18.4, 9.8, 2.7, 2.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 198.7, 149.4, 137.0, 131.9, 129.9, 129.8, 128.7 (2C), 127.6, 126.3 (2C), 43.9, 38.4, 32.0. GC/MS: RT=13.524 min, M^+=198.

Results and Discussion

Analogue **[2]** was prepared in nine linear steps. The first step in the sequence was an aldol condensation reaction between *trans*-cinnamaldehyde **[3]** and acetone **[4]** (12). The product of this reaction, ketone **[5]**, exists in equilibrium with its tautomer, enol **[14]** (Figure 2). The ratio of keto/enol tautomers was found to be dependent on the reaction solvent. When a 50:50 solution of ethanol:water was used (15), the ratio of keto/enol tautomers was 3:97, favoring the enol; when just water was used, the ratio was 100:0, favoring the ketone. This phenomenon has been observed in the literature with acyclic β–diketones (16,17). Meyer's rule states that there is "a shift in the tautomeric equilibrium toward the keto tautomer with increasing solvent polarity (16)." This is presumably due to the keto tautomer being more polar than the enol tautomer and therefore more stable in polar solvents. The ratio of keto/enol tautomers was determined using proton NMR. The keto tautomer **[5]** has a large singlet at 2.3 ppm representing the methyl group adjacent to the ketone. The keto tautomer also has a doublet at 6.3 ppm from the vinyl hydrogen located alpha to the carbonyl. In the enol tautomer **[14]**, there is no peak at 2.3 ppm and the vinyl hydrogen located adjacent to the enol is shifted to 6.6 ppm. By integrating the doublet from the keto tautomer (6.3 ppm) and comparing it to the integration of the doublet from the enol tautomer (6.6 ppm), the ratio of keto/enol tautomers can be determined.

The keto tautomer **[5]** was treated with sodium ethoxide and diethyl malonate **[6]**. A Michael addition occurred producing intermediate **[7]**, which subsequently underwent a Dieckmann condensation to provide β-ketoester **[8]**. Without purification, a saponification reaction was used to hyrolized ester **[8]** to prepare the carboxylic acid salt **[9]**. Carboxylate **[9]** was acidified to prepare the carboxylic acid, which upon heating performed a decarboxylation reaction (13). As with ketone **[5]**, ketone **[10]** could potentially exist in two forms - as a diketone or as a conjugated keto-enol. Based on proton NMR evidence, it appears that the compound exists exclusively as keto-enol **[10]**. The key peak in this identification was the 1H singlet at 5.25 ppm representing the vinyl enol hydrogen.

An attempt to purify ketone **[10]** using recrystallization in absolute ethanol led to the most unique reaction in the synthetic sequence. Ketone **[10]** was dissolved in a minimal amount of hot ethanol. Upon sitting, no crystals were observed. The resulting oil was analyzed by GC-MS and surprisingly a new peak was observed. The molecular ion peak observed for this new peak was 242 as compared to 214 observed for ketone **[10]**. The new peak was formed by addition of 28 mass units, which was concluded to be the result of replacement of an –OH with a $-OCH_2CH_3$. The attempt at recrystallization had converted the enol **[10]** into

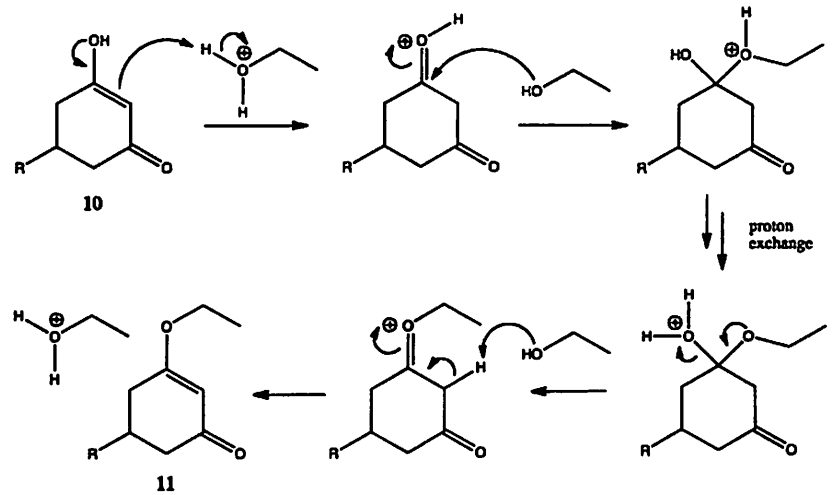


Figure 3. Mechanism of Ethyl Enol Ether [11] Formation.

an ethyl enol ether **[11]** (Figure 3). The acid catalyst needed to perform this transformation came from trace amounts of hydrochloric acid remaining from the previous decarboxylation step.

The significance of this step was that the ethyl enol ether group prepared on compound **[11]** could serve as a protecting group. With one of the carbonyls protected, all that remained to complete the synthesis of analogue **[2]** was to reduce the remaining ketone to an alcohol, remove the enol ether protecting group, and then eliminate the alcohol to make the desired α,β-unsaturated carbonyl system. Based on literature precedence, it was anticipated that all of these reactions could be completed in one step (14). The ketone was reduced with lithium aluminum hydride. The resulting alcohol was then treated with dilute sulfuric acid and extracted with diethyl ether. The dilute sulfuric acid, in a previously reported example, was sufficient to remove the enol ether protecting group and cause dehydration of the alcohol (14). In some trials, this same result was observed for ethyl enol ether **[11]**; complete conversion to analogue **[2]** was observed by GC-MS analysis. In other cases, however, significant amounts of alcohol **[12]** and ketone **[13]** were observed. When this occurred, the mixture of compounds **[12]**, **[13]**, and **[2]** were placed in water with acid catalyst and compounds **[12]** and **[13]** were transformed into the desired cyclohexenone **[2]**.

Conclusion

Cyclohexenone **[2]** was prepared in nine steps with an overall yield of 25.3%. This corresponds to an 85.8% yield per step. The elegance of this synthetic sequence arises from its use of only two purification steps. The first being the recrystallization of ketone **[5]** made in step 1 and the second being column chromatography performed on analogue **[2]** after the last step. The simplicity of this methodology allows for facile production of a large amount of the desired cyclohexenone **[2]** product. Future work involves analyzing analogue **[2]** for bioactivity against cancer cell lines. The methodology used to make analogue **[2]** will also be further explored to see if it has a more general use in making other substituted cyclohexenones and cyclohexanes.

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