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Innovative Synthesis of Diltiazem/Clentiazem Analogs

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Abstract

Cardiovascular disease defines disorders of the heart and blood vessels, and is the number one cause of death in America. Diltiazem and clentiazem are common calcium channel blockers incorporated into drugs used to treat various cardiovascular diseases. Methods to synthesize 5-[2-(dimethylamino)ethyl]-8-hydroxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one, an analog of the core of diltiazem and clentiazem, using cost-efficient starting materials allows for affordable treatment and increased availability to affected individuals. *N-(N,N*-dimethylethanamine)-4-aminophenol can be oxidized to form a quineoneimine, which can be further reacted with 3-mercaptopropionic acid via Michael addition. Subsequent addition of a coupling reagent, N,N'-dicyclocarbodiimide (DCC),[1] produces 5-[2-(dimethylamino)ethyl]-8-hydroxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one**,** analogous to the core structure of diltiazem and clentiazem. Final compounds can then be subjected to biological testing to determine successful inhibition of calcium channels leading to vasodilation. This four step total synthesis approach provides an innovative synthesis methodology.

Introduction

Cardiovascular disease is the leading cause of death in America.[1,2] This disease can affect people of all ages, sex, and color, and is often triggered by stress, unhealthy habits, and/or genetics. Cardiovascular disease is a simple term used to describe various disorders of the heart, including those involved with build-up of plaque in arteries, reducing blood flow, and increasing the risk of a heart attack or stroke. According to Centers for Disease Control and Prevention, about 600,000 people die of heart disease in the United States every year.[3,4] Of the different types of cardiovascular diseases, coronary heart disease is the most common. Lack of knowledge and late detection of symptoms have led to death rate increases per year.

Researchers have put forth effort to synthesize drugs to treat various cardiovascular diseases. Dongming Cai and his team of researchers have found a correlation between benzothiazepines, Ca^{2+} channels, and heart disease.[5] According to Cai, members of benzothiazepines are useful in treating disorders such as: angina, hypertension, and cardiac arrhythmias. Benzothiazepines act as a calcium channel blocker, relaxing blood vessels, allowing blood to flow regularly. Derivatives of benzothiazepine, diltiazem and clentiazem, are known calcium channel blockers incorporated into drugs used to treat cardiovascular diseases.

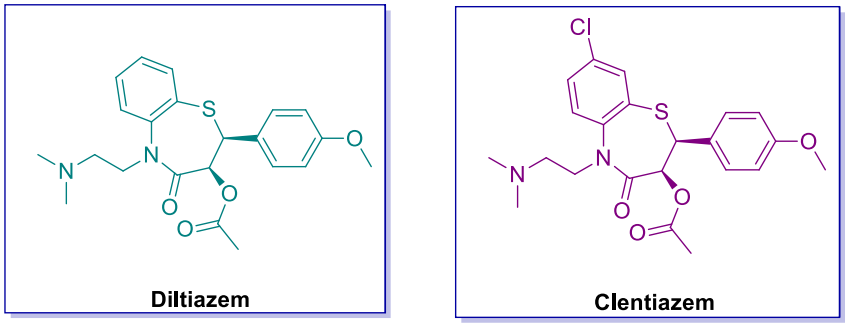
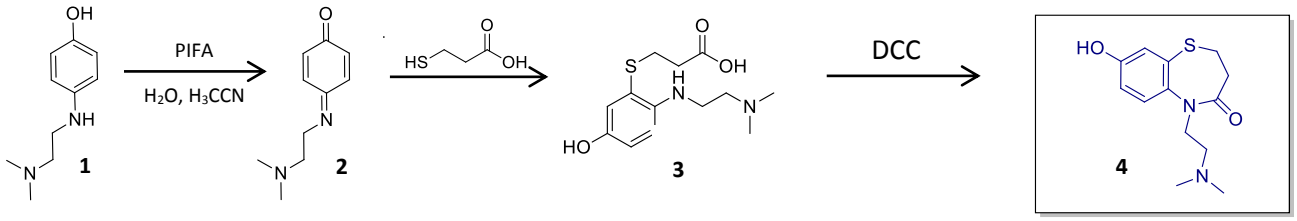


Figure 1. Structures of Diltiazem and Clentiazem

In this experiment, we will be synthesizing an analog of diltiazem and clentiazem, following the work of Alan Katritzky et al. on the synthesis of benzothiazepinones and benzothiazinones.[6] However, a different starting material will be used, *N-(N,N-*dimethylethanamine)-4-aminophenol, obtained from a previous research experiment performed by David Chang.

Route 1



Route 2

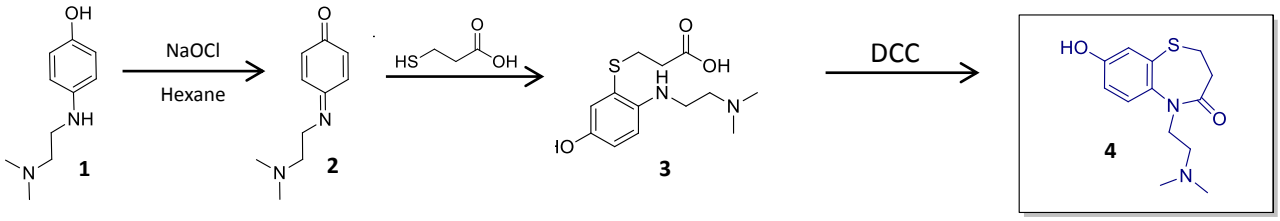


Figure 2. Experimental design

The goal of this project is to design an alternative method to synthesize 5-[2-(dimethylamino)ethyl]-8-hydroxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one, an analog of the core of diltiazem and clentiazem, using cost-efficient starting materials. The compound will be synthesized by oxidizing *N-(N,N-*dimethylethanamine)-4-aminophenol with an appropriate oxidizing agent, and adding 3-mercaptopropionic acid via Michael addition. Afterwards, a coupler will be introduced to synthesize our desired product. Biological testing will then be

performed on the compound to evaluate its reactivity in the human body and its success as a calcium channel blocker.

Methods

Experimental procedures were performed at Southern Adventist University Chemistry Department using standard laboratory glassware, and materials and chemicals manufactured by Acros, Alfa Aesar, AnalTech, Chemglass, and Fisher Scientific. Each reaction was carried out using Chemglass Pie-Blocks (CG-1991-P), Wilmad LabGlass Rotovapor (WG-EV311), RE11 Buchi Rotavapor (1024648), Chemglass Optimag Magnetic Hot Plate Stirrers (CG-1994), HP/Agilent 1100 MSD LC/MS, and a Spectroline Short Wave UV lamp (EF-140C, 254nm).

Reaction 1: Synthesis of Compound 2

Route 1. A solution was prepared by dissolving *N-(N,N*-dimethylethanamine)-4-aminophenol (0.052 g, 0.277 mmol) in acetonitrile (0.454 mL) and water (0.230 mL) in a reaction vial. While the solution was stirring, bis(trifluoroacetoxy)iodobenzene (PIFA) (0.2393, 0.277 mmol) was added. The reaction was run at 25˚C for one hour, and thin-layer chromatography (TLC) was performed to monitor formation of product from starting material. The reaction was left stirring for seven days and further analyzed via TLC.

Route 2. A mixture was prepared by dissolving *N-(N,N*-dimethylethanamine)-4-aminophenol (0.0503 g, 0.277 mmol) in hexane (0.9 mL) and three drops of methanol. While the mixture was stirring, NaOCl was added (6% conc., 0.400 mL, 0.0277 mmol). The reaction vial was covered in foil and left stirring at 25˚C for one hour. The reaction was monitored by TLC and analyzed using liquid chromatography mass spectrometry (LCMS). The vial was stored in a refrigerator at 4˚C for one week.

Reaction 2: Synthesis of compound 3.

Route 1. After compound **2** was synthesized**,** 3-mercaptopropionic acid (0.020 mL, 0.277 mmol) was injected into the reaction vial, and left running for seven days. The solution was monitored by TLC, and analyzed using LCMS.

Route 2. After compound **2** was synthesized, 3-mercaptopropionic acid (0.025 mL, 0.277 mmol) was injected into the reaction vial, and left stirring at 25˚C for one hour. The reaction was monitored by TLC, and stored in a refrigerator at 4˚C for one week.

Reaction 3: Synthesis of 5-[2-(dimethylamino)ethyl]-8-hydroxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one (compound 4)

Route 1. Sodium sulfate (3.628 g) was added into the vial to remove excess water. The solution was filtered using a 50 mL filtering flask, followed by subsequent washes with acetonitrile. The filtrate was transferred back into the reaction vial, and dicyclocarbodiimide (DCC) (0.577 g, 0.277 mmol) was added. Reaction was left stirring for one week at 25˚C. The reaction was monitored by TLC and further analyzed by LCMS. Once reaction reached completion, the solvent was evaporated under reduced pressure to achieve a dark brown solid.

Purification. The crude product was dissolved in ethyl acetate and dichloromethane and purified using column chromatography. A 40:1 ratio of silica gel to crude mass was calculated for the column. Silica gel was mixed with ethyl acetate and was poured into the column. After washing the column three times with ethyl acetate, the dissolved crude product was added. The solvent system used was a 9.7:0.3:0.03 ratio of ethyl acetate: methanol: acetic acid. The column was subject to pressurized nitrogen gas; twenty-five 20 mL fractions were obtained.

Fractions containing product were evaporated under reduced pressure to obtain a maroon solid.

A second column chromatography run was performed with the same solvent system, using a dry pack loading method prepared with crude product obtained from the previous chromatography run. Next, a 2:1 ratio of silica gel to crude mass was calculated. The calculated amount of silica gel was added to the crude product, mixed with the least polar solvent. The solvent was evaporated, leaving a dry powder to be added into the column. Twenty-five 20 mL fractions were collected and analyzed via TLC. Selected fractions containing product were evaporated under reduced pressure to obtain a dark brown solid. The solid was further analyzed using LCMS and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.

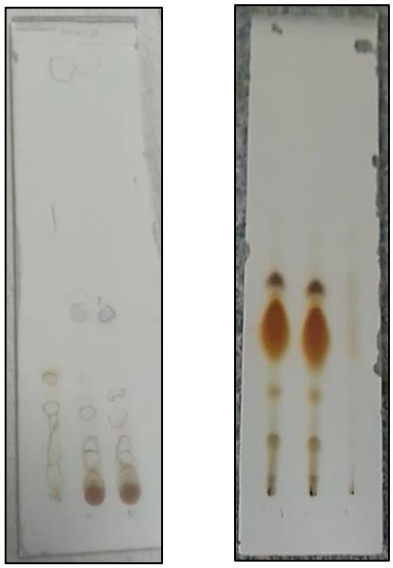
Route 2. Sodium sulfate (0.5073 g) was added to remove excess water, and the solution was filtered using a 50 mL filtering flask, followed by subsequent washes with acetonitrile. Filtrate was transferred into a new reaction vial and solvent was evaporated under reduced pressure to obtain a dark yellow solid. The solid was dissolved in 1 mL tetrahydrofuran (THF) followed by the addition of DCC (0.583 g, 0.277 mmol). The reaction was left stirring for one week at 25˚C, while being monitored via TLC. TLC analysis showed the presence of a new spot, believing to be compound **5.** Solvent was again removed under reduced pressure to obtain a dark yellow solid.

Purification. Column chromatography was performed using a solvent system composed of hexane and ethyl acetate in a 1:1 ratio. A 20:1 ratio of silica gel to crude mass was calculated for the column. Silica gel was poured into the column followed by the addition of hexane. Next, a 2:1 ratio of silica gel to crude mass was calculated. The calculated amount of silica gel

was added to the crude product and mixed with the least polar solvent. The solvent was evaporated, leaving a dry powder to be added into the column. Thirty 8 mL fractions were collected and analyzed via TLC. Selected fractions containing product were evaporated under reduced pressure to obtain a white solid. The solid was further analyzed using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy.

Results

Reaction 1



A B

Figure 3. TLC analysis of compound 2 synthesized via Route 1 (A) and Route 2 (B).

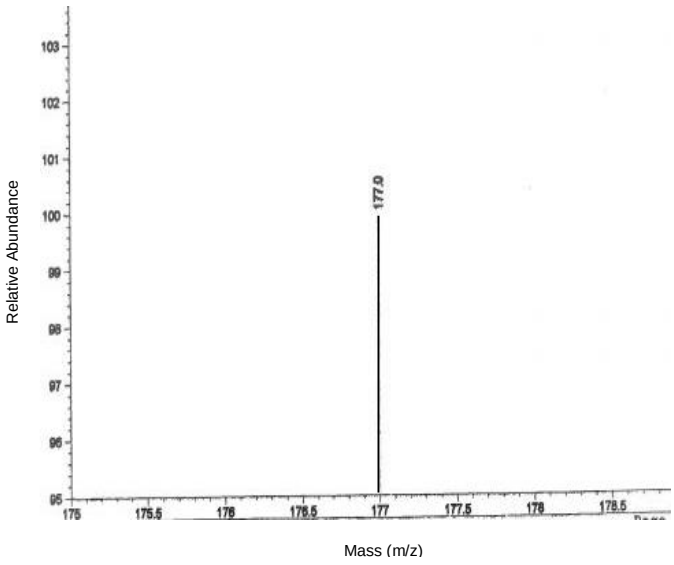


Figure 4. LCMS results for compound 2 synthesized using NaOCl

Reaction 2

Figure 5. TLC results of compound 3 (Route 2)

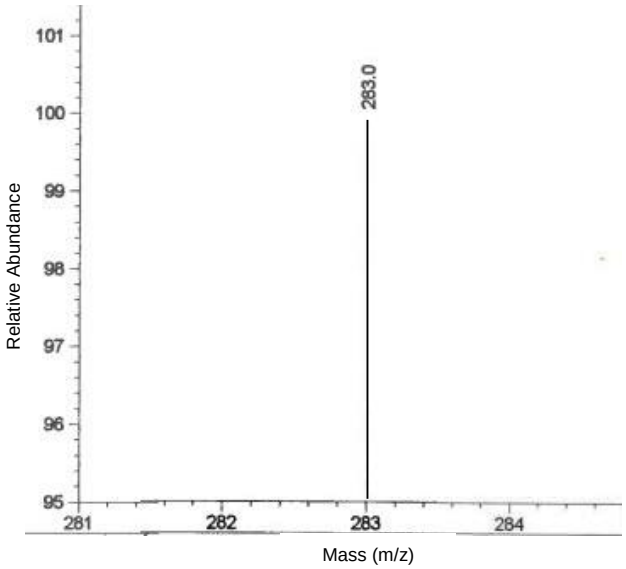


Figure 6. LCMS results for compound 3 (Route 1) under single ion mode (SIM).

Reaction 3

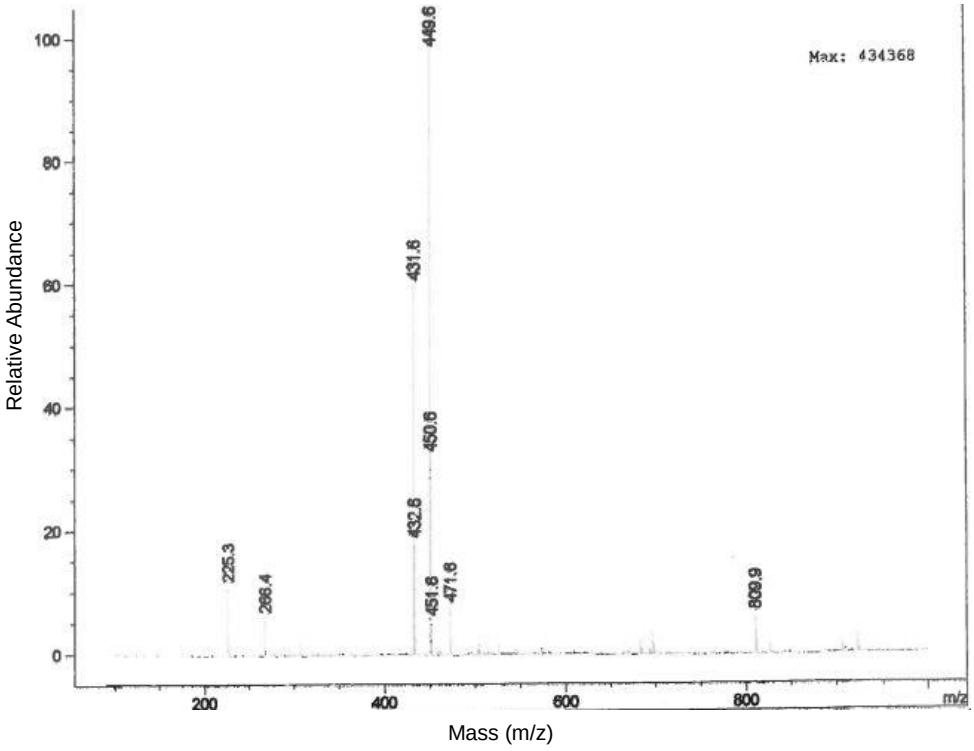


Figure 7. LCMS results for compound 4 (Route 1) after purification on scan mode.

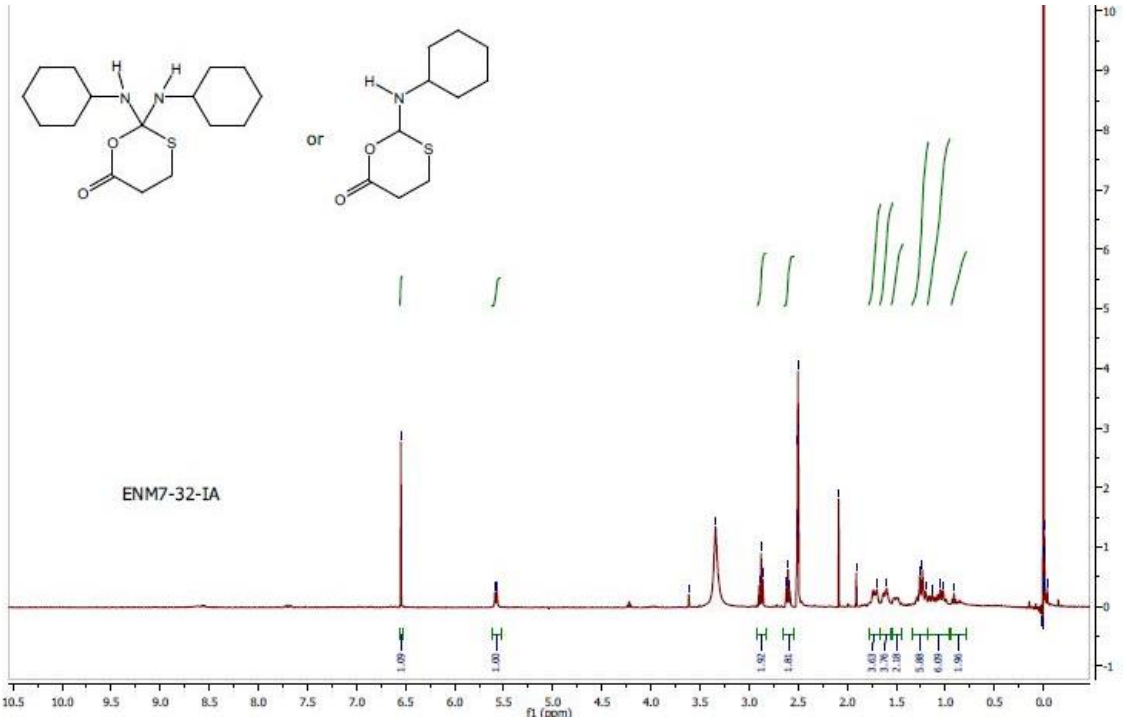


Figure 8. ^{1}H NMR results of compound 4 (Route 1) after purification.

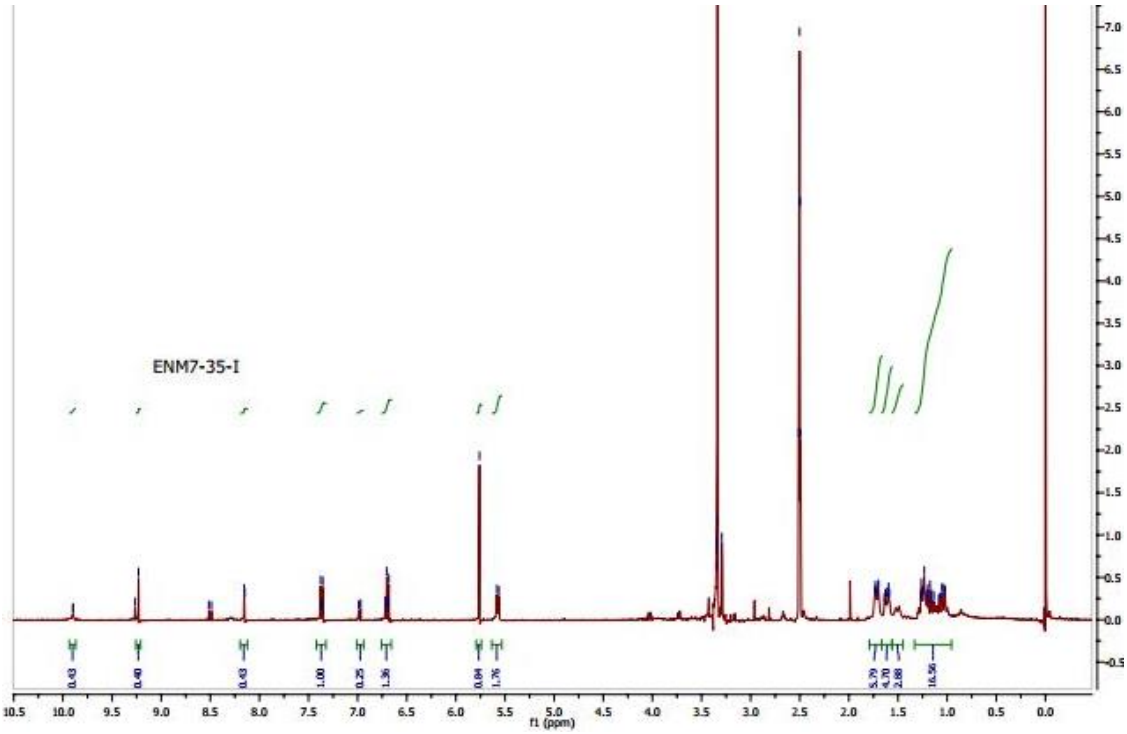


Figure 9. ^{1}H NMR results of compound 4 (Route 2) after purification

Discussion

Various approaches were made to promote reaction completion. For *reaction 1*, under ***route 1*** and ***route 2***, reaction vials were covered in foil to protect the oxidized product from light, and was kept at 25˚C to prevent heat degradation.

Route 1

As can be seen from Figure 3A, a new spot with an R_f value of 0.583 appeared during TLC, suggesting the oxidation reaction had occurred. TLC results were unclear for *reaction 2,* but when performing LCMS on single-ion mode (SIM), data showed a signal at the molecular weight of our deprotonated product (Figure 6). This gave a strong indication that the reaction proceeded to completion.

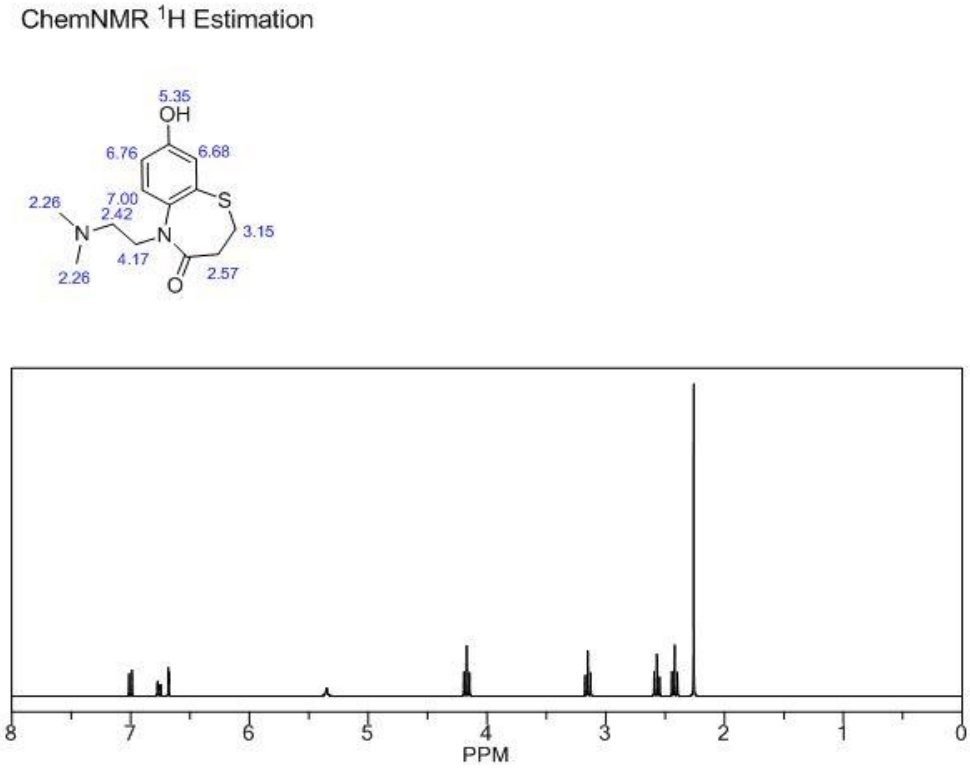


Figure 10. Predicted ^{1}H NMR results of compound 4

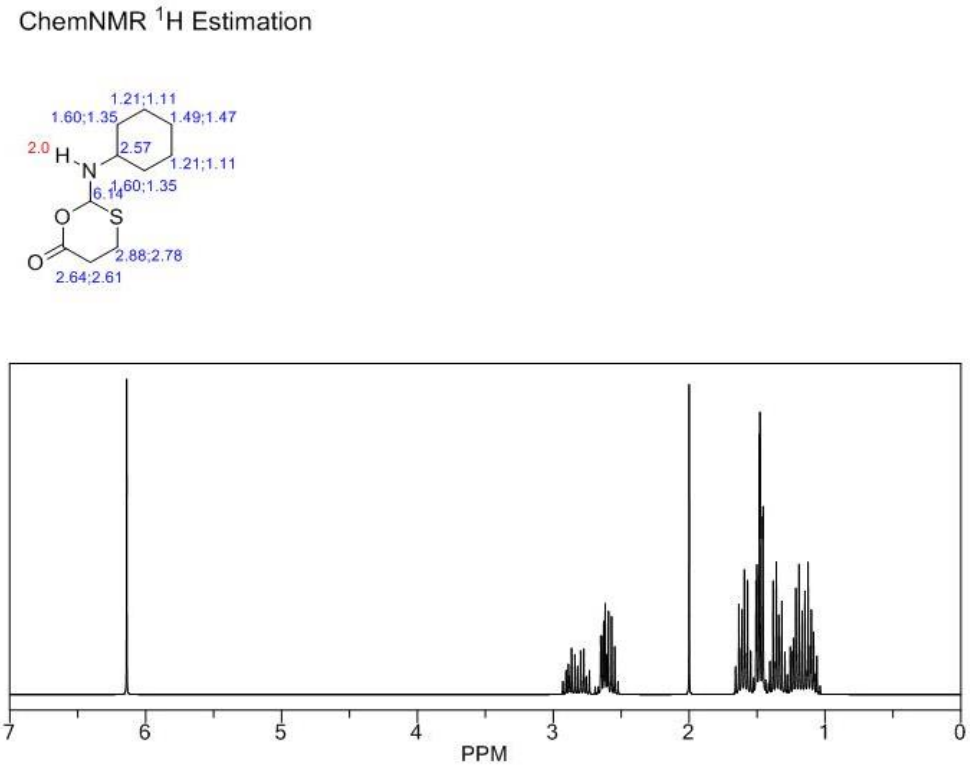


Figure 11. HNMR prediction of possible compound ENM7-32-IA (Route 1)

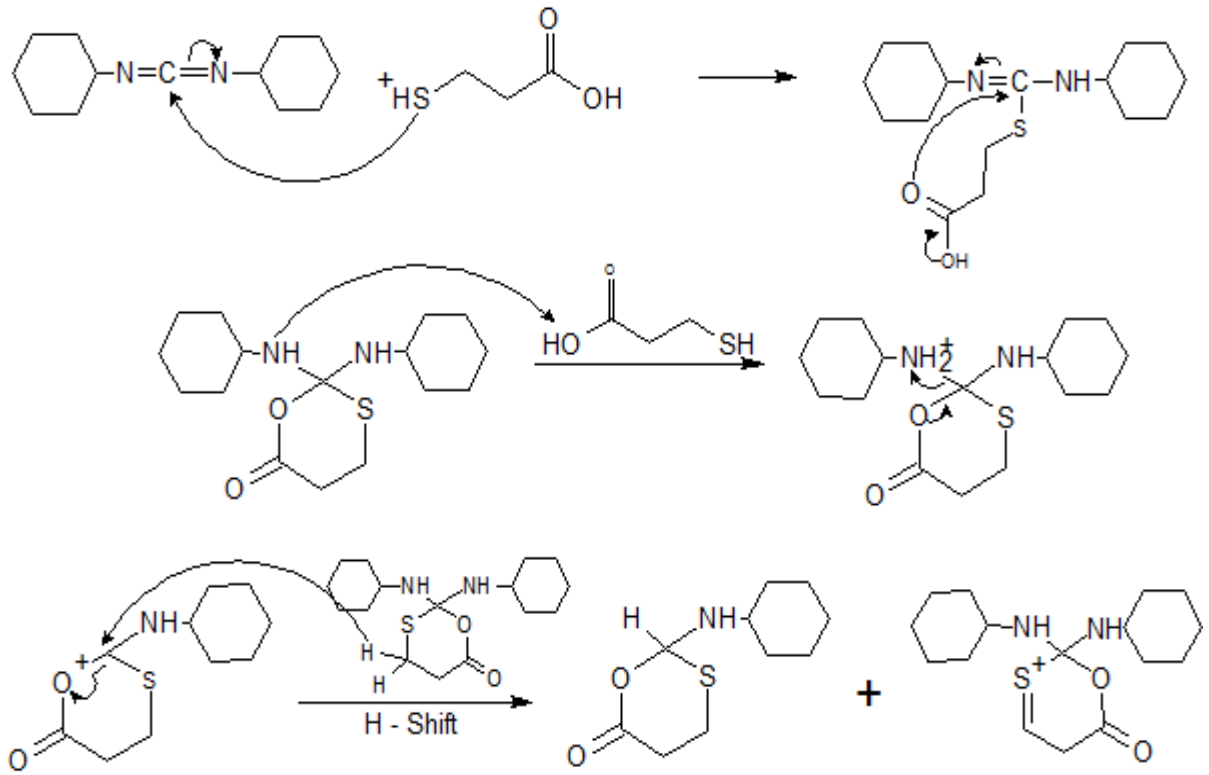


Figure 12. Proposed mechanism for possible products formed in reaction 3 following Route 1

Before beginning *reaction 3*, sodium sulfate was introduced to compound **3** to remove water introduced from *reaction 1* that may interfere with DCC. During filtration, small amounts of product may have been lost, decreasing our overall yield. In Figure 7, LCMS analysis on scan mode of our newly purified product detected other compounds present in our final reaction. A peak at the target product molecular weight (266.354) can be seen among various peaks, most likely belonging to impurities. ^{1}HNMR spectroscopy was performed to confirm the synthesis of compound **4**, followed by comparison to our ^{1}HNMR prediction in Figure 10. From the data presented in Figure 8, peaks at 6.7 – 7.0 ppm were absent, and peaks at 1.0-2.2 ppm were detected, suggesting a different compound was synthesized. We hypothesize that a reaction occurred between DCC and 3-mercaptopropionic acid forming a cyclized structure of 3-mercaptopropionic acid attached to DCC (compound **5**). Compound **5** can be further reacted with excess 3-mercaptopropionic acid and undergo a hydride shift to produce compound **6**. The predicted ^{1}HNMR results in Figure 8 reveals peaks corresponding to hydrogens surrounding cyclohexane, providing an explanation for signals detected at 1.0-2.2 ppm in Figure 8. A proposed mechanism for the synthesis of reaction is shown in Figure 12.

Route 2.

In Figure 3B, the disappearance of a spot in the product lane (R_f = 0.337) suggests that the oxidation using NaOCl was successful during *reaction 1*. LCMS analysis was also performed to confirm our prediction; results show a signal at the expected deprotonated mass (177.0), shown in Figure 4. After completing *reaction 2,* TLC analysis gave a positive indication that the thionucleophile (3-mercaptopropionic acid) reacted with the quinoneimine (compound **2**), showing the appearance of a new spot with an R_f of 0.50 (Figure 5).

Before proceeding with *reaction 3*, sodium sulfate was added to compound **3** to remove additional water incorporated with NaOCl in *reaction 1* that may interfere with DCC. During filtration, small amounts of product may have been lost, decreasing our overall yield. After a crude solid was obtained, there was difficulty in dissolving solid in a solvent such as dichloromethane (DCM); THF was chosen as the most suitable solvent. Insoluble solids found in the solution may have been remaining sodium ions from sodium sulfate. ^{1}HNMR spectroscopy was performed to confirm the synthesis of compound **4**, and was compared to our ^{1}HNMR prediction in Figure 10. From the data presented in Figure 9, peaks at 6.5 – 7.5 ppm were present, suggesting the presence of a chromophore. However, peaks at 1.0-2.2 ppm, 5.5-6.0 ppm, and 8.0-10.0 ppm were detected, suggesting a different compound was synthesized. Possible structures synthesized from this reaction are yet to be determined.

Conclusion

According to Figure 6 and 7, the target product was not synthesized in this experiment, therefore determination of effective oxidizing and coupling agents is essential for the production of the target compound. Future research can be performed using a combination of various oxidizing methods and different coupling agents in an attempt to synthesize and isolate 5-[2-(dimethylamino)ethyl]-8-hydroxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one (**5**). Possible methods include isolating compound **2** to ensure successful synthesis before proceeding with subsequent steps. A good reaction procedure for adding the thionucleophile to the quinoneimine must be established before continuing on to produce compound **5**. Once compound **5** is successfully synthesized, reaction with chiral thiol nucleophiles can be performed to develop a library of chiral 1,5-benzothiazepinones and complete the synthesis of

diltiazem and clentiazem analogs. Biological testing can then be done to further assess their usefulness in treating cardiovascular diseases.

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