ABSTRACT

The Formation of Sulfur Oxoacid Generators and the Effects of Dimedone on the Breakdown of Disulfiram

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Cardiovascular disease (CVD) is the largest cause of death in the United States. Drugs that generate the small gaseous molecule Nitric Oxide (NO) have been used for hundreds of years to treat cardiovascular disease. Recently, Hydrogen Sulfide (H_2S), another small gaseous molecule has been shown to have similar effects in the cardio vasculature; both NO and H_2S affect the cGMP pathway by inhibiting PDE, a cardiovascular signaling molecule that decyclizes cGMP. Multiple drugs have been developed to release H_2S in cells. The Farmer lab has identified small oxoacids of sulfur (SOS) as endogenously produced metabolites of H_2S, which may play a role in its activity. The lab has also identified several families of compounds which release these metabolites, and these compounds show unusual activity in models of CVD. In this work, attempts were made to expand and improve the syntheses of these compounds, which include N,N'-(1,2,4,5-tetrathiane-3,6-diylidene)bis(N-ethylethanamine) dication ($BITT^{2+}$) and diethyldithiocarbamate mono/di-oxide anions $(DeDTCO_{1/2})^-$. Additional experiments were performed to assess if the SOS generation were important in the known activity of disulfiram and $BITT^{2+}$ against melanoma in cell culture. Specifically, whether trapping SOS chemically inhibited generation of a toxic $Cu(DeDTC)_2$ complex. Only initial experiments on the formation of the $Cu(DeDTC)_2$ complex while trapping SOS could be completed.

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The Formation of Sulfur Oxoacid Generators and the Effects of Dimedone on the Breakdown of Disulfiram

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the
Honor Program

By

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Waco, TX

May 2020

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ACKNOWLEDGMENTS

I wish to express my sincere thanks to Dr. Farmer for his support, assistance, and providing me with necessary facilities and materials for this research. I would also like to that Dr. Kumar Murugaeson for his help in both in lab and planning experiments.

I would also like to thank my committee members, Dr. Pinney and Dr. Martin. Their willingness to serve on my committee and review my thesis is greatly appreciated.

Next, I would like to thank my parents and grandparents for their encouragement and support through the difficult times. I would also like to thank my friends who helped encourage me throughout this process.

Finally, I am grateful to God for providing me with the good health and wellbeing needed to complete this thesis.

CHAPTER ONE

The Scope of the H_2S and NO in Cardiovascular Health

Heart attacks, strokes, and other cardiovascular diseases are the leading causes of death in the United States (1). The world is in desperate need of more cardiovascular treatments. In the U.S. in 2017, someone died from cardiovascular disease every 37 seconds, and 365,914 people died from coronary heart disease alone (1), (2). Heart disease is responsible for 23.4% of deaths in the United States, and heart disease is only behind cancer in total deaths in the United States (1).

Nitric oxide (NO) is a small molecule signaling agent that is a critical part of vascular health and important for consideration in the treatment of cardiovascular disease (CVD) (3). Nitroglycerin and other related nitro-pharmaceuticals have been used to treat CVD for hundreds of years because of their conversion to NO in the body (4). NO plays an important role in controlling blood pressure by activating soluble guanylyl cyclase (sGC) by binding to a heme receptor, which induces changes the conformation and increases the production of cyclic GMP (cGMP) (3). This increase of cGMP is a critical downstream signal since cGMP targets multiple effector molecules such as protein kinases and gated ion channels (5). Another enzyme that regulates cGMP signaling is phosphodiesterase (PDE), which decyclizes cGMP, thus decreasing the down-stream signaling; Viagra and other similar drugs work by inhibiting PDE (6).

Most recently, the small gasses H_2S have been shown to have similar protective effects in CVD models, targeting similar vasodilative and anti-oxidant pathways (7). H_2S is produced constitutively in human cells, but the nature of its action is still uncertain.

The Farmer group has shown that biological oxidation of H_2S produces small oxoacids of sulfur (SOS), small and elusive compounds such as sulfenic (HSOH) and sulfinic acid (H_2SO_2) produced from H_2S, which may play a role in the vascular effects of H_2S (7).

The Farmer group has shown that cells generate and extrude SOS. These compounds, examples including HSOH, H_2SO_2, and thiosulfoxylic acid ($H_2S_2O_2$), are highly reactive under aqueous and biological conditions (7). Each SOS compound has some tautomeric forms, Figure 1 (7). These SOS compounds were detected through trapping by using a combination of nucleophilic and electrophilic trapping reagents and characterization by high resolution liquid chromatography mass spectrometer, HD LC MS (7). Sulfenyl was trapped with the nucleophilic reagent dimedone, Figures 2 and 3, and the electrophilic reagent iodoacetamide along with mono-bromobimane and di-bromobimane (7). Oxidation of H_2S in biological systems results in products that derive from the tautomeric forms of sulfenyl and sulfinyl, tautomers of sulfenic acid (7).

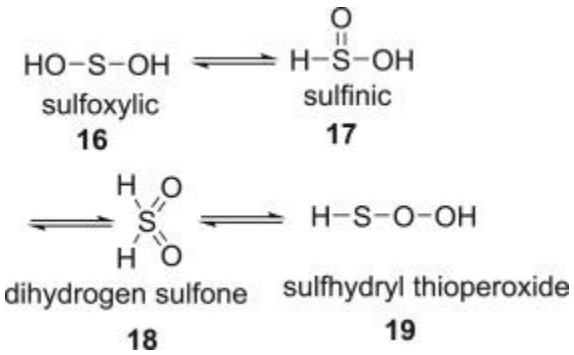


Figure 1: The four tautomers of sulfoxylic acid (7).

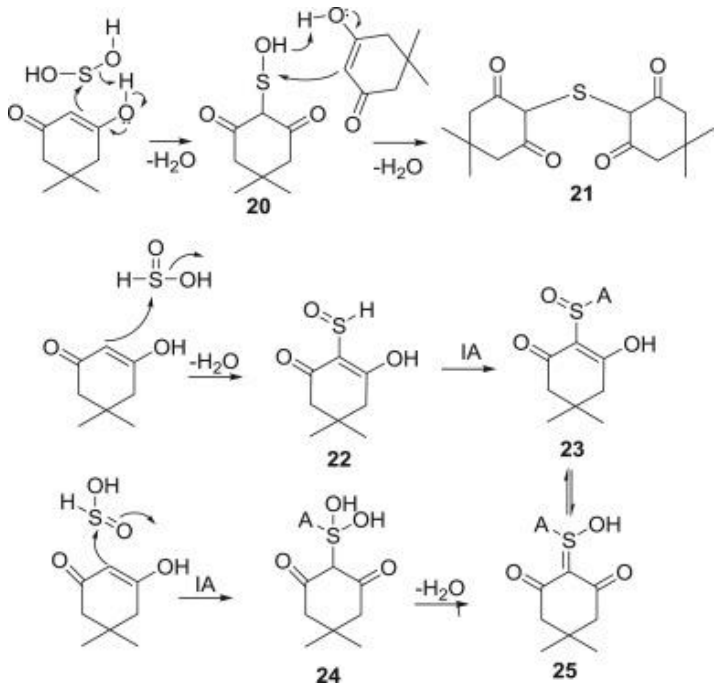
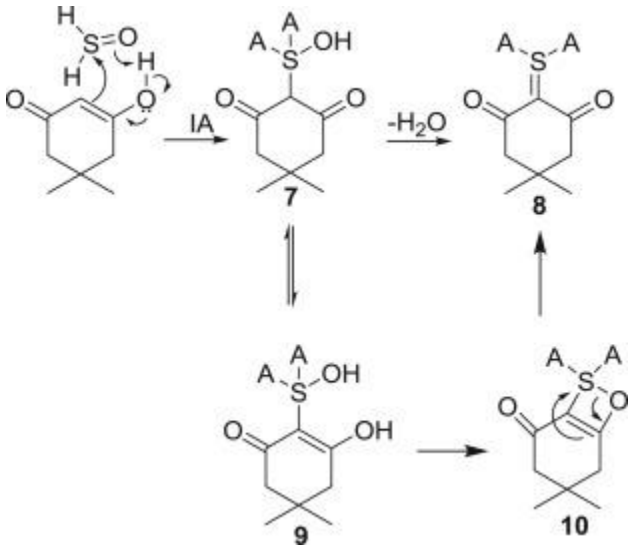


Figure 2: The trapping mechanism for sulfoxylic acid (7).



Scheme 3: The mechanism for trapping sulfinyl (7).

A current goal in the Farmer group is to synthesize compounds which release SOS, in order to test their effects in CVD model systems. These compounds have been collaboratively tested across numerous universities. Certain groups of pro-SOS

compounds have been found to activate sGC in a dose dependent fashion by Emil Martin at UT Medical (8). Interestingly, this activation of sGC differed from activation by other sGC activators or initiators, thus the mechanism of action is unknown (8).

Sulfenic acid (HSOH) is the first oxoacid that arises from H_2S oxidation, Figure 4 (7). Sulfenic acid demonstrates both electrophilic and nucleophilic reactivity (7). A second SOS is sulfoxylic acid (H_2SO_2), which is formed from the dioxygenation of H_2S. This SOS can exist in one of four different forms: sulfoxylic acid, sulfinic acid, dihydrogen sulfone, or sulfhydryl peroxide. Only sulfoxylic acid and sulfinic acid were the only tautomers observed from trapping (7).

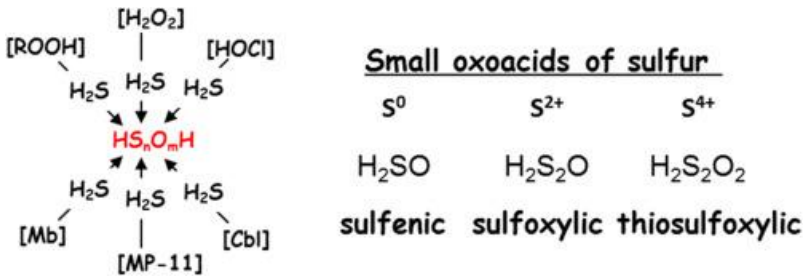


Figure 4: This figure shows the different reactions that turn H_2S into SOS compounds (7).

This thesis concerns the generation of SOS-releasing compounds; DeDTC-O_2 a dioxygenated form of diethyldithiocarbamate, and $BITT^{2+}$, a bisimine tetrathiolate derived from oxidation of diethyldithiocarbamate disulfide (DSF), an aldehyde dehydrogenase inhibitor used in alcohol aversion therapy (7).

Disulfiram breaks down into diethyldithiocarbamate, Figure 5 (9), under biotic conditions. Dithiocarbamates have been used in lubricants, pesticides, and vulcanization acceleration as a metal chelator (10), (11), (12). One metal complex of diethyldithiocarbamate is $Cu(DeDTC)_2$. The Farmer group has shown that disulfiram is

converted to $Cu(DeDTC)_2$ in the presence of copper salts in cell culture, inducing apoptotic cell death in metastatic melanoma cells (9). The Farmer group has also shown that Zn forms complexes with pre-oxygenated DTCs, which are proto-typical SOS-releasing compounds (9).

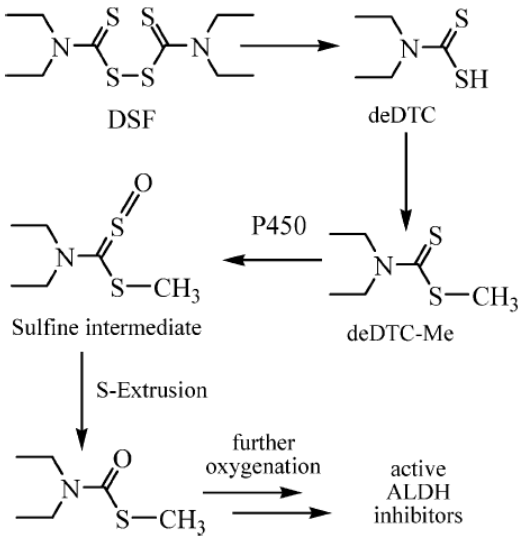


Figure 5: This is the pathway for the breakdown of DSF into DeDTC and other compounds in oxidizing conditions (9).

Both the BITT and DTC-$O_{1/2}$ families produce SOS when hydrolyzed. Importantly, the assays show that both induce relaxation at ca 100-fold lower concentrations than H_2S under the same conditions. Different pro-SOS compounds produced slightly different results, Figure 6 (13). $BITT^{2+}$ produced the most profound increase in relaxation in the shortest time. DeDTCO was the second fastest, but $DeDTCO_2$ produced a more pronounced change after a few minutes. The best pro-SOS drug tested was BITT-4, which has $CuCl_2^-$ as counterion, problematic as Cu salts are often toxic (14). Other published $BITT^{2+}$ compounds have I_3^- and $CoCl_4^-$ counterions, both are problematic. The triiodide (I_3^-) anion is not very stable and exists in equilibrium

with iodine (I_2) (15). Further, cobalt chloride $CoCl_4^{2-}$ is cytotoxic to cells (16). Another potential counterion that could be used to form $BITT^{2+}$ is tetrafluoroborate, BF_4^-. Tetrafluoroborate is stable and less toxic than $CoCl_4^{2-}$ or $CuCl_2^-$ (17), (18), (19). As such, the first set of experiments were attempts to generate $BITT(BF_4)_2$ and to generate and purify DeDTC-O_2, Chapter 3.

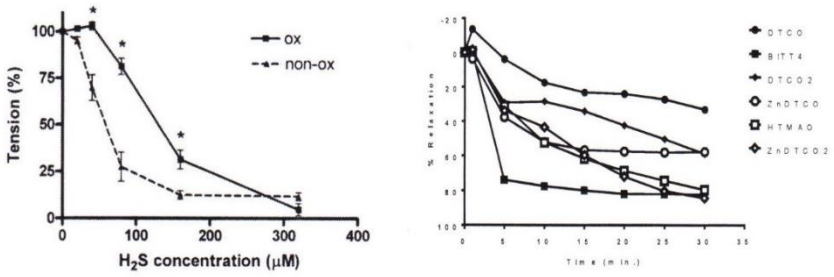


Figure 6: Left: Comparison of response in percent relaxation of rat thoracic aortic rings under oxygenated (95% O_2) and unoxygenated conditions (13). Right: Comparison of response in percent relaxation of murine aortic rings when exposed to 10 µM of various SOS prodrugs.

CHAPTER TWO

Methods and Procedures for the Formation of $BITT^{2+}$ and Oxidation of DeDTC

Introduction

The primary goal of the experiments described in this chapter was to generate new SOS releasing compounds based on other compounds previously used in the Farmer lab. One of the most active SOS precursors in biological testing was N,N'-(1,2,4,5-tetrathiane-3,6-diylidene)bis(N-ethylethanaminium), $BITT^{2+}$, Figure 7.

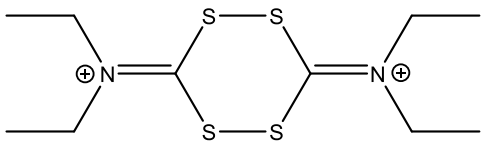


Figure 7: N,N'-(1,2,4,5-tetrathiane-3,6-diylidene)bis(N-ethylethanaminium), $BITT^{2+}$

It had first been synthesized with the counterions $CuCl_2^-$, by Victoriano et al, 1995 (20), I_3^-, Ainscough et al, 1977 (21), and $CoCl_4^{2-}$, Khitrich et al, 2006 (22). In these reactions the metal ions oxidized DSF to form $BITT^{2+}$ (21), and the reduced metal ion complexes were found as counterions. Since these metal-based counterions were toxic and I_3^- is unstable, we attempted to form $BITT^{2+}$ using various mild oxidants in the presence of BF_4^-, a less toxic counterion (18), (19), (15), (17).

Other experiments were conducted forming thione-oxides, (R=S-O), which also release SOS in aqueous solution and had shown good bioactivity. The diethyldithiocarbamate oxides (DeDTC-O and DeDTC-O_2) had been synthesized in the Farmer group as Zn^{2+} complexes, using peroxides as O-atom donors (9). A mild and

selective oxidation of thiones had been reported using MTO as a catalyst (23). Therefore, I undertook experiments to see if DeDTC oxides could be formed using MTO as the catalyst and remain stable without being complexed to a metal ion such as Zn(II).

Generation of $BITT^{2+}$ compounds

The first set of experiments attempted to form the dication $BITT^{2+}$ with BF_4^- as the counterion. These reactions were performed under nitrogen to limit the exposure to water vapor because $BITT^{2+}$ breaks down into DeDTC complexes in aqueous environments (24).

Reactions of DSF with $NOBF_4$

The first experiments $NOBF_4$ as oxidant. NO^+ was used as the oxidant due to its high reduction potential of 1.50V (25). While commercially available, this compound is extremely hydroscopic and needs to be stored in a glove box (26). In order to use the $NOBF_4$ in the experiment, a dram vial was weighed, tared, and cycled into the glove box containing $NOBF_4$. Next, a sample of $NOBF_4$ was added to the vial, and the vial was cycled out of the glove box. The vial was then weighed to determine the grams of $NOBF_4$. Disulfiram was weighed in a 1:2 DSF:$NOBF_4$ ratio. Dry acetonitrile was then added to the vial with $NOBF_4$ and the DSF was dissolved in minimum dry acetonitrile in a round bottom flask with a magnetic stir bar under nitrogen. The DSF solution turned a dull light yellow after the DSF dissolved. The $NOBF_4$ solution was added to the flask via pipette to limit the possible exposure to air. More dry acetonitrile was added until the $NOBF_4$ was completely dissolved, and the reaction was allowed to stir for two hours. The solution turned a brighter yellow after the reaction. This reaction proved unsuccessful in

forming $BITT^{2+}$, Figure 8. The sextuplet is probably two quadruplets superimposed on top of each other. This is most likely because the reaction pathway should not provide an opportunity for a sextuplet to form and many byproducts of the reaction have quadruplets. The quadruplet around 4 and the triplet around 1.25 are the starting material.

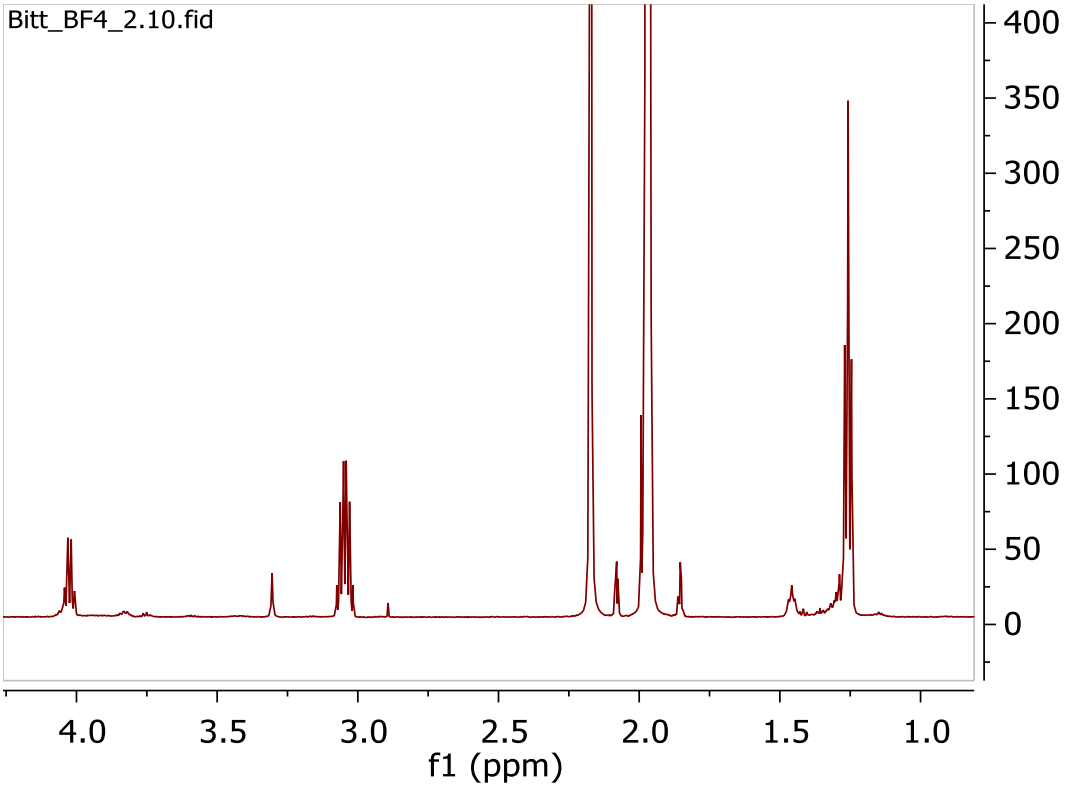


Figure 8: NMR spectrum of the product from the $NOBF_4$. The quadruplet around 4 and the triplet around 1.25 are the starting material. The sextuplet is probably two superimposed quadruplets due to the hydrogens and carbons not participating in the redox reaction.

Attempts to oxidize DSF using $AgBF_4$

A graduate student in the Farmer group had used Ag^+ reagents as a mild oxidant of anthroquinones and other N-heterocycles. One of these reagents was silver tetrafluoroborate. Since Ag^+ has a reduction potential of 0.8 eV (26), and $AgBF^4$ was available, Ag^+ was then next oxidant tested. A 263 mg sample of $AgBF_4$ was dissolved in 20 mL of toluene. Next, a 200 mg sample of DSF was dissolved in 20 mL of toluene in a

separate vial. The vial with DSF turned a dull light yellow after the DSF was dissolved. A pipette was then used to transfer the $AgBF_4$ solution into the disulfiram solution. A reaction occurred instantly at room temperature, when a bright yellow precipitate fell out from the mixture. The precipitate turned brown within 24 hours on the benchtop, however, when covered in acetonitrile or toluene, the precipitate remained yellow for two weeks. The compound formed was primarily a disulfiram bonded to silver, Figures 9 and 10 (27).

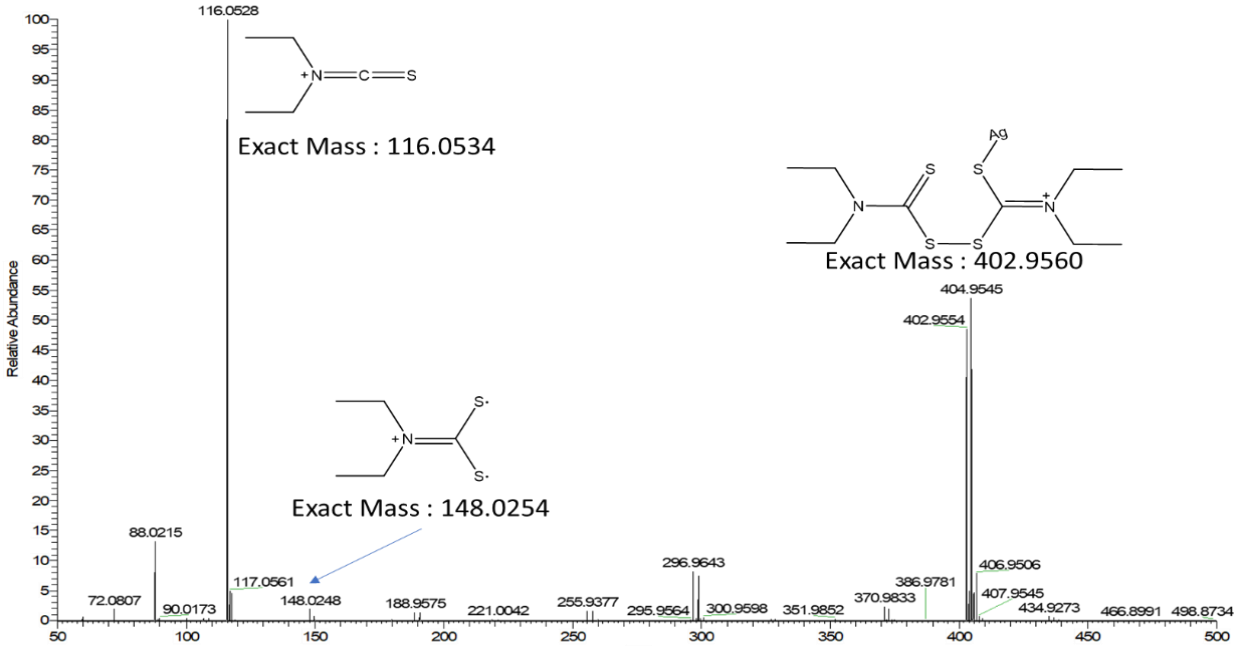


Figure 9: Mass spectrum of product mixture of reaction of DSF with $AgBF_4$ along with possible structures of the products (27).

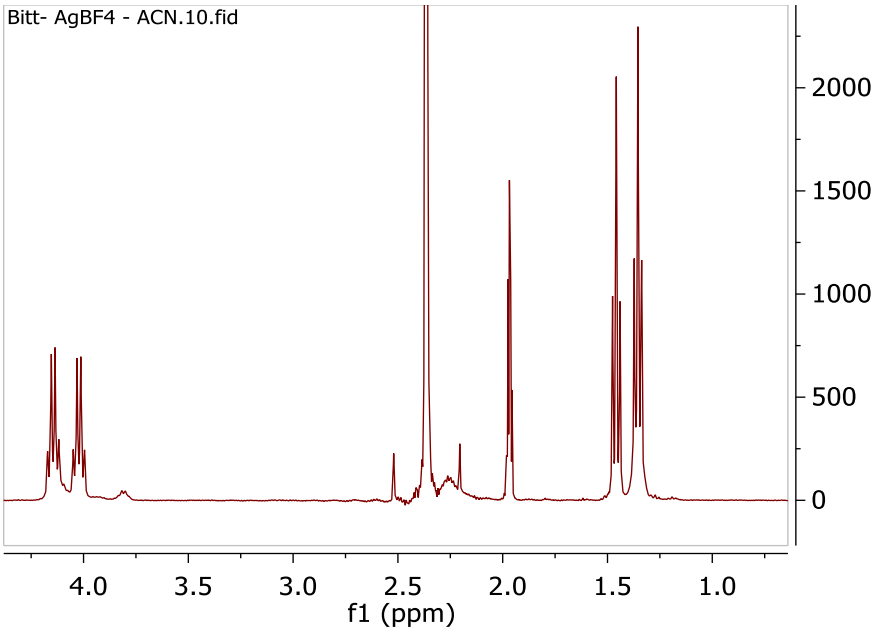


Figure 10: NMR spectra of the product of the reaction of DSF with $AgBF_4$. One side of the DSF was bonded with Ag^+ causing the duplicate sets of peaks around 4.1 and 1.35.

Formation of the $(DeDTC)_2CoCl_4$ complex

While working with $Ag(I)BF_4$, a DeDTC complex was formed with $CoCl_4^{2-}$ as the counterion. This was accomplished through the procedure for forming $Me_4DittCoBr_4$ from Khitrich et al, 2006 (22). A 370 mg sample of disulfiram (0.00125 mol) was dissolved in chloroform (20 mL) and a 99 mg sample of $CoCl_2^-$ (0.01 mol) dissolved in acetone (35 mL), the original experiment with MeTds used acetonitrile. The goal was to form $BITTCoCl_4$, but this decomposed into the DeDTC complex, $(DeDTC)_2CoCl_4$, almost immediately. This was due to using acetone as a solvent in the experiment instead of acetonitrile; acetone is a wet solvent and $BITT^{2+}$ breaks down into DeDTC complexes in aqueous environments (24).

Attempts to form $BITT^{2-}$ using Ferrocenium tetrafluoroborate ($C_{10}H_{10}BF_4Fe$)

Another commercial oxidizer with a BF_4^- counterion is Fc^+, ferrocenium, the cationic version of Fc, ferrocene, which can be easily separated from ionic products such as $BITT^{2+}$. In this reaction, a 200 mg sample of DSF was dissolved into 25 mL of acetonitrile. The solution turned a dull light yellow upon the addition of DSF. Next, a 368 mg sample of $Fc[BF_4]$ was added. The solution then turned a dark greenish blue, Figure 11. The solution was allowed to react for 2 hours.

Figure 11: The reaction mixture of ferrocenium tetrafluoroborate and disulfiram in acetonitrile.

This experiment was also unsuccessful, Figure 12, as complexes formed between iron and the disulfiram, the 351 peak in Figure 12, and occasionally with a cyclopentadienide from the ferrocenium remaining coordinated to the iron, the 417 peak in Figure 12. These products were determined by analyzation of the masses from the mass spectrum and Fe and S have been found to form a double bond (28).

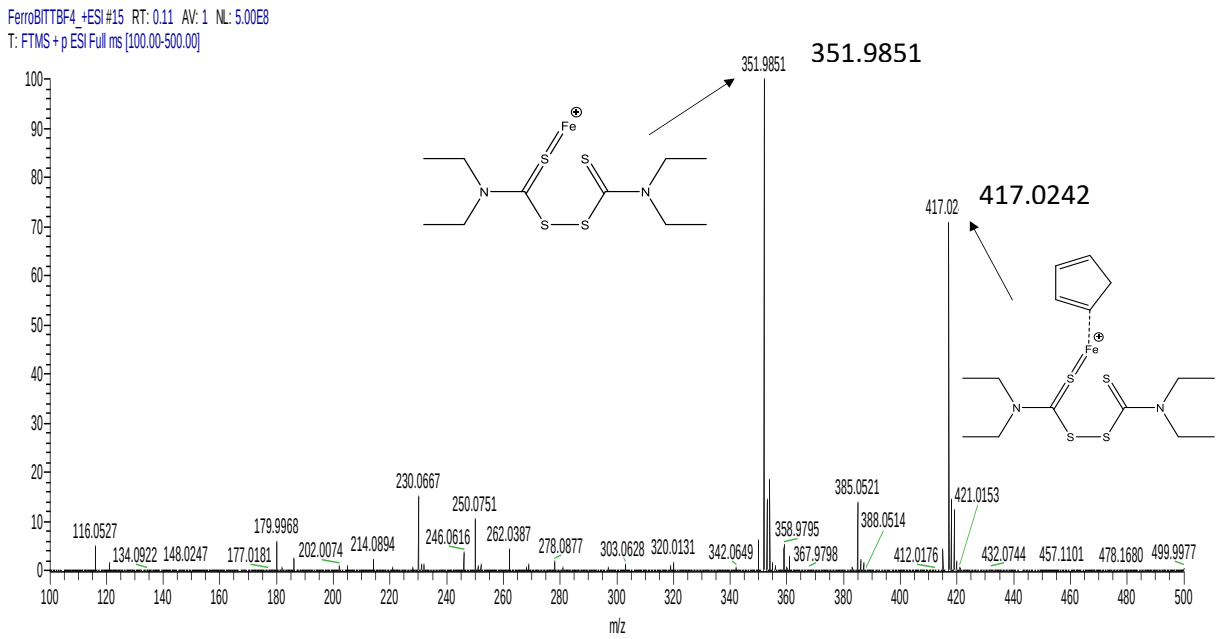
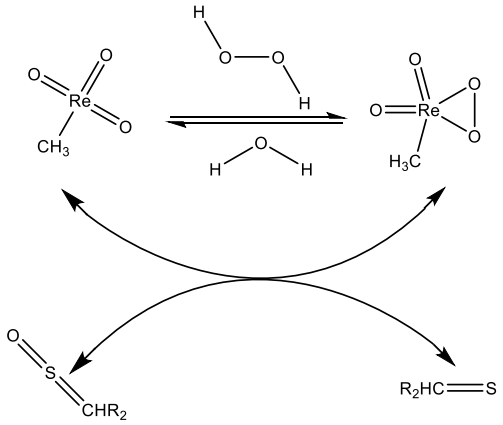


Figure 12: This mass spectrum is from the ferrocenium tetrafluoroborate $BITT^{2+}$ experiment. No product was observed. It is believed that these complexes were formed because the masses match and Fe and S can form a double bond (28).

Generation of DTC-O compounds

The second set of experiments focused on forming oxygenated forms of DeDTC using a catalyst. MTO, Methyltrioxorhenium(VII), Scheme 1, is a powerful O-atom transfer catalyst using hydrogen peroxide as the oxidizing agent.(29)



Scheme 1: Catalyzed oxidation of $R_2HC{=}S$ compounds with MTO (23).

MTO produces two active catalytic species when catalyzing hydrogen peroxide reactions, monoperoxo and diperoxo η^2 metal complexes (23). Huang and Espenson were the first to use MTO to form $R_2C=S=O$ compounds with hydrogen peroxide as the oxidizing agent (23). We used an analogous procedure, but with DeDTC as the substrate. The reproduction of the method was initially successful.

Oxygenation of DeDTC using MTO as a catalyst

A 200 mg sample of sodium diethyldithiocarbamate NaDeDTC was added to a mixture of 4 mL acetonitrile and 1 mL of DI water. Next, 17.66 µL trifluoromethanesulfonic acid, triflic acid, CF_3SO_3H, was added to stabilize the MTO. A 5.6 mg sample of MTO was then added, turning the solution a dark cloudy blue for a moment before turning to a darker yellow. Hydrogen peroxide (90 µL) was then added to the solution as the oxidant. The reaction was characterized by mass spectrometry. Immediately after the reaction, the solution was pure DeDTCO; however, after 3—4 hours, the solution was a mixture of DeDTCO, $DeDTCO_2$, and $DeDTCO_3$.

Reaction of NaDeDTC with MTO as a catalyst with the addition of Zn(II)

This experiment merged the use of MTO as a catalyst with the procedure from Brayton et al, 2006 (9). A 201.5 mg sample of NaDeDTC was dissolved in 5 mL MeOH with a stir bar creating a light-yellow mixture. Triflic acid (17.66 µL) was then added to this solution to stabilize the MTO, and a 6.2 mg sample of MTO was added immediately after. The reaction turns a dark blue initially after the MTO is added, eventually to a darker yellow with brownish clouds that move through the solution. Hydrogen peroxide

(95 µL) is then added to the solution. An excess of zinc chloride $ZnCl_2$ (207 mg) was then added. The solution was allowed to stir for 24 hours before being filtered by vacuum filtration. The white powder was washed with cold methanol, Figure 13. This produced 38.3 mg of a mixed solution of ZnDeDTC and ZnDeDTCO for a 28.9% yield of the mixture.

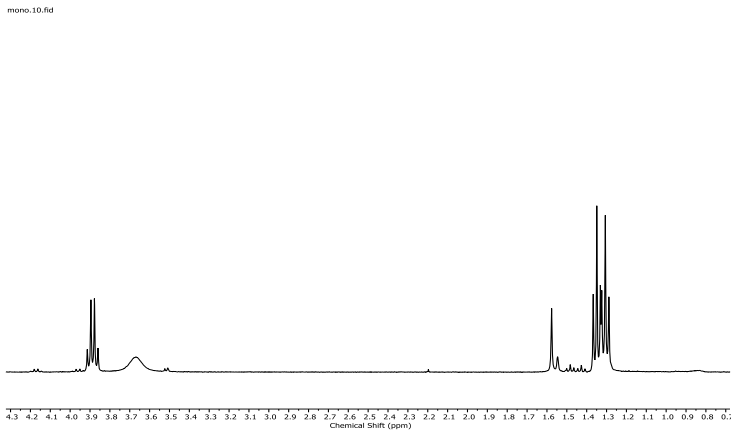


Figure 13: NMR spectra of the DeDTCO. It shows a mixture of [Zn]DeDTC and [Zn]DeDTCO. This analysis was based on the analysis from Brayton et al, 2006 (9).

The next version of the reaction was going to use urea hydrogen peroxide (UHP) instead of liquid hydrogen peroxide as per the procedure in Brayton et al, 2006 (9). A 200 mg sample of NaDeDTC should be dissolved in 10 mL of methanol. Next, 35.32 µL of triflic acid would be added along with 5.6 mg MTO. Then a 127 mg sample of UHP should then be added. Once the UHP has been completely dissolved, a 200 mg sample of $ZnCl_2$ should be added.

Attempts to form DeDTC-O_2 using MTO as a catalyst

After following the procedure from the above paper to form DeDTC=O, experiments were performed to create the compound DeDTC-O_2. Initially, MTO was

used as the catalyst to produce the di-oxidation of DeDTC. This was attempted by doubling the amount of MTO, triflic acid, and H_2O_2, but these changes did not produce the desired product. It was then speculated that MTO experienced difficulty catalyzing the second addition of oxygen. As a result, the next method used MCPBA and urea hydrogen peroxide, UHP, instead of hydrogen peroxide and did not use MTO. For this reaction, 200 mg of DeDTC, 313 mg of UHP, and 574 mg of MCPBA was dissolved into 20mL of acetonitrile. The dissolved disulfiram appeared yellow, and a dark blue color change occurred briefly after all of the reactants were added. The color returned to yellow almost immediately. The solution still contained starting material along with the mono-oxidized version, DeDTC=O, Figure 14.

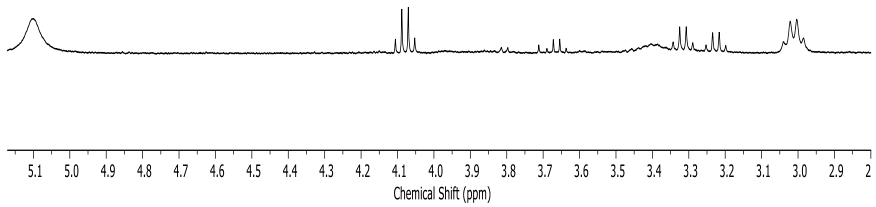


Figure 14: This is an NMR of the $DeDTCO_2$. This NMR spectrum shows a myriad of products including DeDTC, DeDTCO, and $DeDTCO_2$ This analysis was based on the analysis from Brayton et al, 2006 (9).

Formation of $DeDTCO_2$ without MTO

This experiment was planned, but it was never performed. NaDeDTC (200 mg) would be dissolved in 5 mL acetone. Hydrogen peroxide (190 mL) would add to this solution and the mixture should be allowed to react overnight. This solution should then be filtered and washed with 5 mL cold acetone. The filtered product should be pure.

CHAPTER THREE

Effects of Dimedone on the Breakdown of the $Cu(DeDTC)_2$ Complex

Background

Disulfiram, DSF, has been shown to selectively cause apoptosis in melanoma cell culture and has been used in several clinical trials over the years (30). The Farmer group showed that the addition of Cu significantly increased the apoptotic response (24). Likewise, an increase in Cu concentration within the melanoma cells was observed after DSF treatment; analogous treatment of melanocytes showed little increase in Cu uptake in culture (24). The Farmer group also showed that DSF and Cu salts react to form a $Cu(diethyldithiocarbamate)_2$ complex, Figure 15 (24). This complex redox reaction represents the oxidative catabolism of DSF, via proposed decomposition in Figure 16, to generate reduced dithiocarbamate byproducts that then complex with Cu to form the toxic $Cu(DeDTC)_2$ species (24).

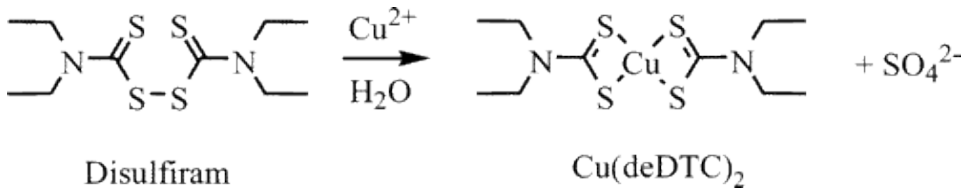


Figure 15: This is a scheme for the reaction of DSF with Cu^{2+} in water. It shows the formation of the $Cu(DeDTC)_2$ complex and SO_4^{2-} as a side product (24).

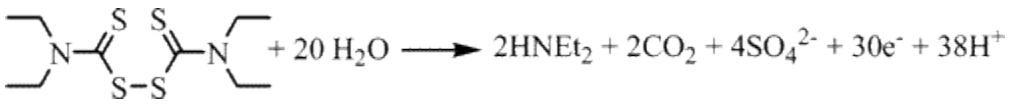


Figure 16: This scheme shows the decomposition of DSF during reaction with Cu^{2+} (24).

Melanoma cells struggle to manage oxidative stress (via reactive oxygen species, ROS) as compared to melanocytes(33). When exposed to reactive oxygen species (ROS) (7), melanoma cells have a higher concentration of super oxides (31). The Farmer lab showed that Cu(II) salts react with melanin to form ROS compounds (33). They also showed that Cu and Zn dithiocarbamate complexes are toxic to melanoma cell culture at micromolar concentrations, mimicking the induction of apoptosis shown for $Cu(DeDTC)_2$.

We hypothesize that SOS compounds might be generated during the oxidative decomposition of DSF and thus have a similar effect as ROS. This may indicate that the decomposition of the $Cu(DeDTC)_2$ complex could be partly responsible for the observed toxicity of DSF/Cu (30). Therefore, we expected that dimedone trapping of SOS from the breakdown of the DSF, thereby halting the decomposition, would decrease observed toxicity.

Purpose behind the experiments

The initial reports of Brayton et.al, 2004 (24), demonstrated the loss of DSF and formation of the $Cu(DeDTC)_2$ under a variety of conditions. However, the effects of trapping with dimedone and HPE-IAM (β-(4-hydroxyphenyl)ethyl iodoacetamide), Figure 17, on the breakdown of the DSF has not been analyzed. These experiments seek to determine how these traps might affect the generation of the $Cu(DeDTC)_2$ complex from these reactions.

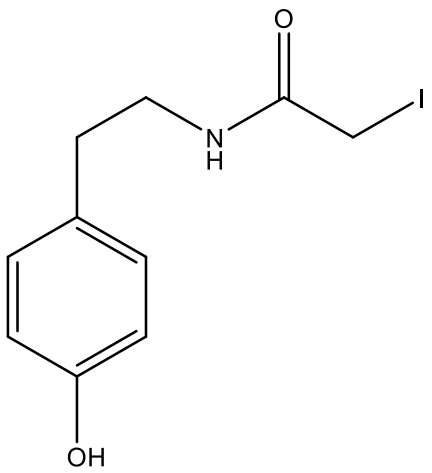


Figure 17: HPE-IAM

Two traps were used in these experiments, dimedone and HPE-IAM. Dimedone is a nucleophilic trap for sulfenic moieties (S-OH) in SOS (34); it selectively alkylates the Sulfur generating stable S-dimedone adducts that are observable products in a high definition liquid chromatography mass spectrometer, HD LC MS. HPE-IAM is an electrophilic trap for thiols moieties (RS-H) (35), which generates corresponding thioethers (RS-T) that are easily seen in the MS. These were used to trap intermediate oxidized products of the reaction between Cu(II) and DSF.

Reaction of Cu(II) with DSF in the presence of SOS traps for MS and EPR analyses

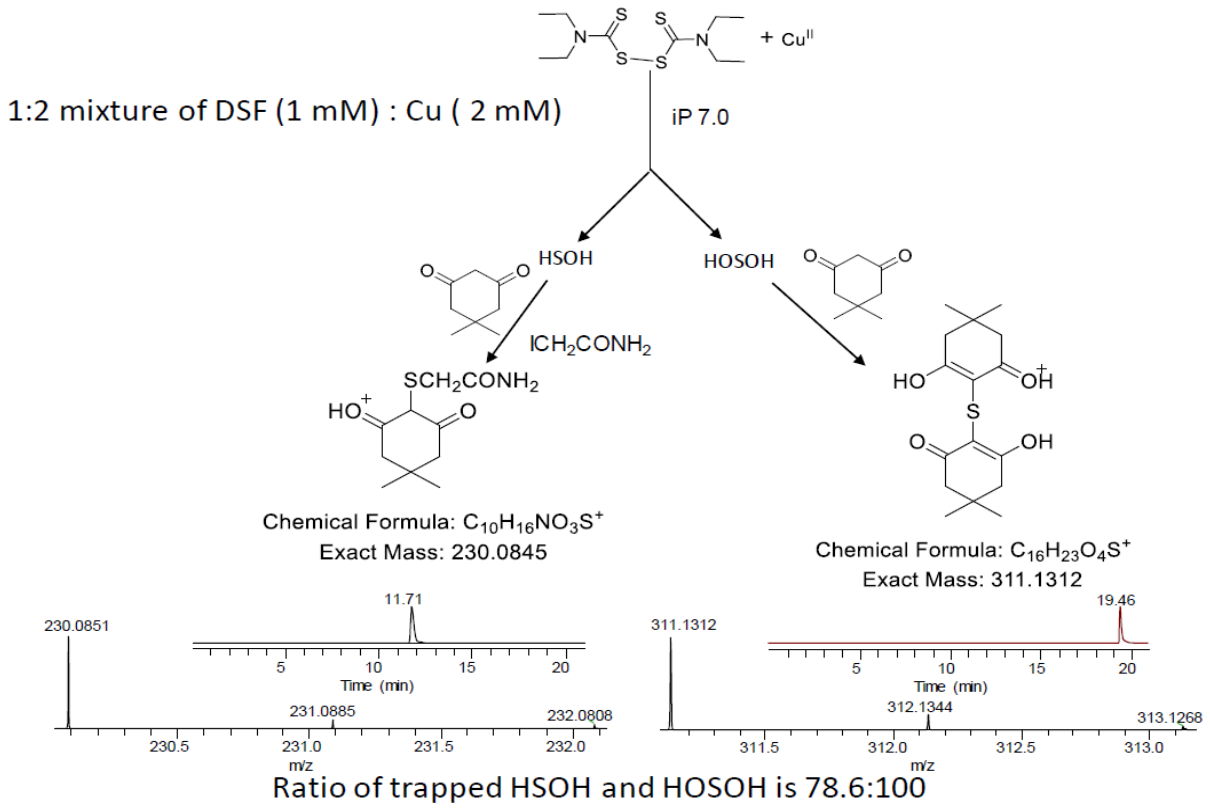
Four stock solutions were made for these reactions. The first solution was $CuSO_4$ dissolved in a pH 7 phosphate buffer in a 10 mM concentration. The other three solutions contained DSF, dimedone, and HPE-IAM respectively in 10 mM concentrations in dimethyl sulfoxide (DMSO).

Six experiments were conducted in two sets of three using these stock solutions. All six reaction mixtures consisted of 10 µL of the DSF and 10 µL of the $CuSO_4$ solution with the addition of 10 µL of the trapping species dimedone, HPE-IAM with 10 µL of

both the dimedone and HPE-IAM standards combined in a 960 µL of pH 7 phosphate buffer. This resulted in a 100 µM concentration for each reaction. In the first set, the two trapping standards were added before the reactants (Cu/DSF), and in the second set, they were added 45 minutes after the reactants.

Mass Spectrometry analysis

The samples were given to Dr. Kumar Murugaeson for analysis in the high definition liquid chromatography mass spectrometer. The results showed that the dimedone had trapped HSOH (Sulfenic acid) and HOSOH (sulfinic acid), Scheme 2. This indicates that trapping SOS does not inhibit the formation of the $Cu(DeDTC)_2$ complex.



Scheme 2: This diagram, made by Dr. Kumar Murugaeson, shows the mass spectrums from the reactions involving $CuSO_4$, DSF, dimedone, and HPE-IAM. The dimedone did not inhibit the breakdown of the $Cu(DeDTC)_2$ complex into SOS compounds.

EPR analysis

The original solutions from the experiments for mass spectrometry were used for the two experiments conducted for electron paramagnetic resonance spectroscopy (EPR). EPR is primarily used to study radicals (36). This was important to our experiments because $Cu(DeDTC)_2$ ROS compounds in vivo (37), (38). This environment was roughly imitated with the 7-pH phosphate buffer. This method has previously been used by other labs such as Chen et al, 2011 (39). Reactive oxygen species are radicals and should show up in the EPR (36). Two experiments were made from those stock solutions. The first experiment was 10 µL of each solution added to 960 µL of pH 7 buffer. The second experiment was 10 µL of the DSF and $CuSO_4$ solutions added to 980 µL of pH 7 buffer. The samples were given to Dr. Kumar Murugaeson to run an EPR spectrum. However, the concentration was too low for the EPR machine to register, so no results were produced.

Original Reaction used by Brayton et al, 2004

In this paper, an 87 mg sample of DSF and a 50 mg sample of $CuCl_2^-$ 100 mL DI water. The resulting solution was stirred for 24 hours. Three 100 mL partitions of chloroform were used to purify the solution. The desired product was found in the chloroform portion. This resulted in a 91.4% yield of $Cu(DeDTC)_2$ (24).

Anaerobic reaction of DSF and $CuCl_2^-$ replicated

The first reaction completed was to replicate the results of experiment from Brayton et al, 2004. This reaction was replicated to 87 mg sample of DSF and a 50 mg sample of $CuCl_2^-$ were dissolved into 100 mL DI water under nitrogenous conditions. The

resulting mixture was then allowed to stir for 24 hours. The solution turned a dark brownish black. Three portions of 25 mL $CHCl_3$ were used to remove any organic products. The remaining solution was a light bluish green.

Anaerobic reaction of DSF and $CuCl_2^-$ with dimedone

This reaction was planned to test how dimedone affected the breakdown of the $Cu(DeDTC)_2$ The plan was to take an 87 mg sample of DSF and a 33 mg sample of dimedone and dissolve them into 100 mL DI water under nitrogenous conditions. A 50 mg sample of $CuCl_2$ was then going to be added to the solution. The solution would then be allowed to stir for 24 hours. Three portions of $CHCl_3$ would be used to remove the organic products. The desired product would be found in the chloroform fraction.

Results

The mass spectrum showed SO_4 was trapped by dimedone, and the complex was observed, Scheme 1. This indicates that the dimedone did not prevent the formation of the $Cu(DeDTC)_2$ complex. Because the $Cu(DeDTC)_2$ complex responsible for the toxicity, the toxicity of DSF and $CuCl_2^-$ should not be affected (30). Unfortunately, the last reaction was not able to be completed, preventing the attempt to replicate this result and conclusion.

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