Proposal: Enzyme Inhibitory Properties of Acetylpyrazine Thiosemicarbazones and their Metal Complexes

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II. Abstract

We need funds to investigate the biological activity of 2-acetylpyrazine thiosemicarbazones and their copper (II) and palladium (II) complexes. The antimicrobial activity of these compounds have been tested by determining the minimum inhibitory concentrations against four bacteria (*Bacillus subtilis, Saphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*), two yeast (*Canida albicans and Sacchromyces cervisiae*), and a mold (*Aspergillus niger*). We have found that two of our compounds the APZ-tertButylTSC and the APZ-dimethylTSC have high antiproliferative activity, and interestingly, the copper (II) and the palladium (II) complexes have even higher activity, but we have not tested all of the compounds in our series. We want to use the enzyme assay kit for Topoisomerase II to determine actual inhibition of this crucial enzyme with our copper and palladium complexes.

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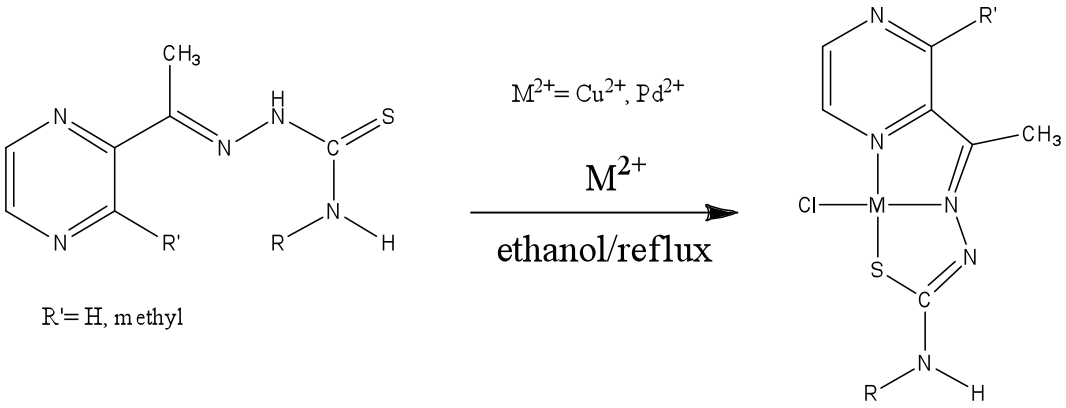
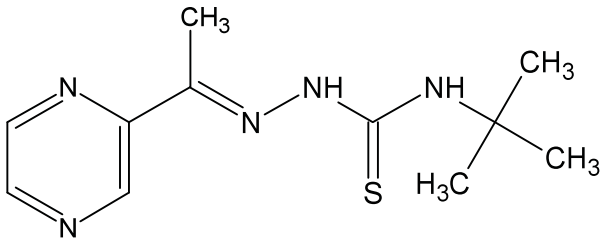
IV. Research Plan

Introduction and Background with previous results

Thiosemicarbazone compounds are a class of chemicals that have been reported to have many biologically active properties; including antitumor and antimicrobial properties.[1] We have created the compound 2-acetylpyrazine thiosemicarbazone (APZ-TSC), shown in figure 1, for this reason. These compounds have a high affinity for iron and other transition metals. This property has been suggested as the reason for some of the biological properieties.[3] In recent experiments it has been shown that these 2-acetylpyrazine thiosemicarbazones react readily with copper and palladium.



The palladium complex formation reaction is depicted in figure 2 with 2-acetylpyrazine tert-butyl thiosemicarbazone (APZ-tBTSC). The APZ-tBTSC compound is one of the best bacteria killing compounds we have made.



Furthermore, research has shown that the Cu^{2+} metal complexes of acetylpyridine thiosemicarbazones can inhibit Topoiosomerase II2α.[9] Therefore, we want to test our good compounds with this Topoiosomerase II2α Kit to confirm our hypothesis that our compounds likely inhibit the same enzyme as do the acetylpyridine thiosemicarbazone complexes.

Prior Results

Here are some of the results that we have obtained from our bacteria studies. The picture below is a jpg image of an excel spreadsheet that shows the anti-proliferative behavior of the metal complexes. We tested these against several different microorganisms. We see that the gram-positive bacteria (Bacillus Subtilus and Staphylococcus Aureus), which have an overall thinner cell wall, have more inhibition than the gram-negative bacteria (E. Coli, and Pseudomonas Aeruginosa).

The copper complexes all show high activity is extremely encouraging!

Conc	1	2	3	4	5	6	7	8	9	10	Bact control
ug/mL	250.0	125.0	62.5	31.25	15.625	7.8125	3.906	1.953	0.977	0.4883	
Microrg											
Escherichia coli	-	-	-	-	+	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	+	+
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	-	-	-	+	+	+	+	+	+
Candida albicans	-	-	-	+	+	+	+	+	+	+	+
Sacchromyces cerevisiae	-	-	-	-	+	+	+	+	+	+	+

[Cu(APZ-PTSC)Cl]

Chemical 2

Conc	1	2	3	4	5	6	7	8	9	10	Bact control
ug/mL	500.0	250.0	125.0	62.50	31.25	15.625	7.813	3.906	1.953	0.9766	
Microrg											
Escherichia coli	-	-	-	-	-	-	-	-	-	+	+
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	+
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	-	+	+	+	+	+	+	+	+
Candida albicans	-	-	-	+	+	+	+	+	+	+	+
Sacchromyces cerevisiae	-	-	-	-	-	+	+	+	+	+	+
Chemical Control	1000 ug/mL										

[Cu(APZ-TbTSC)Cl]

Conc	1	2	3	4	5	6	7	8	9	10	Bact control
ug/mL	500.0	250.0	125.0	62.50	31.25	15.625	7.813	3.906	1.953	0.9766	
Microrg											
Escherichia coli	-	-	-	-	-	-	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	+
Pseudomonas aeruginosa	-	-	-	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	-	-	+	+	+	+	+	+	+
Candida albicans	-	-	-	+	+	+	+	+	+	+	+
Sacchromyces cerevisiae	-	-	-	-	-	-	-	+	+	+	+
Chemical Control	1000 ug/mL										

[Cu(APZ-dMTSC)Cl]

Conc	1	2	3	4	5	6	7	8	9	10	Bact control
ug/mL	500.0	250.0	125.0	62.50	31.25	15.625	7.813	3.906	1.953	0.9766	
Microrg											
Escherichia coli	-	-	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	+
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	-	+	+	+	+	+	+	+	+
Candida albicans	-	-	-	-	+	+	+	+	+	+	+
Sacchromyces cerevisiae	-	-	-	-	-	+	+	+	+	+	+
Chemical Control	1000 ug/mL										

[Pd(APZ-tbTSC)Cl]

Conc	1	2	3	4	5	6	7	8	9	10	Bact control
ug/mL	500.0	250.0	125.0	62.50	31.25	15.625	7.813	3.906	1.953	0.9766	
Microrg											
Escherichia coli	-	-	+	+	+	+	+	+	+	+	+
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+	+
Bacillus subtilis	-	-	-	-	-	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	-	+	+	+	+	+	+	+	+
Candida albicans	-	-	-	-	+	+	+	+	+	+	+
Sacchromyces cerevisiae	-	-	-	-	-	+	+	+	+	+	+
Chemical Control	1000 ug/mL										

[Pd(APZ-DMTSC)Cl]

Methodology

The proposed research of the copper and palladium complexes and their ligands for microbial studies requires:

(a) Creating the 2-acetylpyrazine thiosemicarbazone with copper or palladium,

(b) Culturing the microorganisms from stock plates,

(c) Sterilization of the compounds,

(d) Determining the MIC of each of the compounds, and

(e) Using the Topoiosomerase II2α Kit.

The techniques to be used in this study are documented in the literature. [3,4,6,7,8]

Each of the microorganisms will be taken from stock culture plates provided by the TTU Biology department and replated on Tryptic Soy Agar plates. After the microorganisms have been incubated for 24 hours at 35^{o} C, the ligands and their metal complexes will be dissolved in dimethylformamide (DMF). Once dissolved, the compounds will be sterilized by sterilizing syringe filters (0.45 micrometer) under a laminar flow hood. These compounds will then be diluted 10 times with 1mL of each dilution concentration in a sterile test tube. This will result in final concentrations of 500 ug/mL all the way down to 0.906 ug/mL before the microorganism is added. Each microorganism will have a concentration of $1.5 \text{ X } 10^{8}$ cfu/mL as determined by using a 0.5 McFarland standard. The microorganisms be diluted to $1.5 \text{ X } 10^{6}$ cfu/mL and then added to the diluted compounds. The test tubes containing both the chemicals and the microorganisms will be incubated at 35^{o} C. After 24 hours, the results will be analyzed. This will be repeated many times to ensure accuracy. The assay kits will use the instructions provided.

Work Plan

The proposed work continues throughout this semester and will continue next semester. I work every week doing synthesis and characterization on Thursdays, and on Tuesdays at 5:00 P.M. we do the bacteria studies.

Management Plan

The tasks required to complete this project will be carried out mainly by Christine Beck with assistance by Amanda Koch, Lizzie Monroe, and Kathleen Mansur. Dr. Edward Lisic will oversee the project and ensure that all tasks are being carried out on schedule and that all safety procedures are being followed.

V. Anticipated Results

The investigation of potential anti-cancer substances is very important due to the growing number of women with breast cancer. The chemicals being tested in this proposed study could very well be chemotherapy agents. These compounds also may be tested on actual cancer patients. I will present my research at the American Chemical Society meeting in New Orleans in April. The results of this study have the potential to bring attention to not only the chemistry and biology departments of

Tennessee Technological University, but also the university as a whole. We intend to publish the results of this project in a peer-reviewed journal such as the *Journal of Inorganic Biochemistry*.

VI. Cooperative Features

The Chemistry and Biology departments at Tennessee Technological University will be providing us with facilities and some supplies. Dr. Edward Lisic will be providing use of his laboratory and materials, which are needed for preparing the ligands and the copper and palladium complexes. The microbiology tests will be conducted in James Ventrice's laboratory and will be using his supplies such as the incubator, the TSA broth and the TSA plates. The Biology department is providing the use of a sterile laminar flow hood.

VII. Personnel

Qualifications

Christine Beck is a Bachelor of Science in Chemistry with a focus in Biochemistry student in her junior year at Tennessee Tech University. She has performed research for Dr. Edward Lisic for two years and has presented posters on her research at the 241st National Meeting of the American Chemical Society, the 243rd National Meeting of the American Chemical Society, at the 2011 Tennessee Technological University Student Research Day and at the 2012 Tennessee Technological University Student Research Day. Relevant course work includes BIOL 3230 (Health Science Microbiology), CHEM 3010 (Organic Chemistry I), and CHEM 3020 (Organic Chemistry II). Additionally, she is the President of the American Chemical Society and the Treasurer for American Society for Biochemistry and Molecular Biology Undergraduate Affiliate Network and a student member of the Chemical-Medical Sciences club.

Faculty Advisors

Dr. Edward Lisic will be overseeing the chemistry side of this project and James Ventrice will be overseeing the microbiology side of this project.

VIII. Facilities

Equipment and facilities in the Chemistry Department (Dr. Lisic's lab), and in the Biology Department (James Ventrice's lab).

IX. Budget

Item	Quantity	Cost
Wheaton 20 mL PET Liquid Scintillation Vials, PE Cap, No Liner, Screw Caps Attached to Vials (fishersci.com)	500	$202.57
Copper (II) Bromide (strem)	50 grams	$28.00
2-Acetylpyrazine (sigma aldrich)	100 grams	$105.00
Fisherbrand* Powder-Free Nitrile Exam Gloves (fishersci.com)	2 boxes of 100	$57.46
SLLG025SS Hydrophilic PTFE Millex-LG Filter Unit, Sterile, 25mm Diameter, .20 Micron, 100 mL Process Volume (amazon.com)	50	$178.88
Human Topoisomerase II Assay Kit (topogen.com)	250 Assays	$379.00
Human Topoisomerase II Enzyme (topogen.com)	250 Units	$249.00
	total	$1199.91

X. Appendix

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