Seth Amankrah Adu: MS 2022

Thesis: "SYNTHESIS AND CHARACTERIZATION OF ANTIMONY CYANOXIMATES FOR MICROBIOLOGICAL STUDIES"

When Seth Adu came to my research laboratory early in 2019 he was assigned modified project that had been initiated by his predecessor – Kevin Pinks – dealing with synthesis and characterization of a series of new organoantimony(V) compounds with the aim of investigations of their antimicrobial potential. Seth was asked to prepare a group of trimethylantimony(V) cyanoximates of $Sb(CH_3)_3L_2$ composition (L = specially selected ligands shown in Figure 1) and provide their detailed characterization. An abstract of his work gives more to his project.

ABSTRACT

Antibiotic-resistant pathogenic microorganisms (such as MRSA and alike) represent a real and significant threat to the health and wellbeing of the general population. The search for new compounds of non-antibiotic nature that act as antimicrobial agents is an important task with obvious practical applications in the biomedical field. Metal-based coordination and organometallic compounds that demonstrate antimicrobial activity was found to exhibit a completely different mode of action compared to antibiotics. That suggests the absence of the mechanism of developing tolerance to these types of compounds at least for 30 - 40 years. Thus, there are numerous organotin (IV), silver (I), copper (II) and arsenic (III) compounds that have been extensively studied with that goal in the past. However, most of them are toxic to biological species. On the contrary, organoantimony (III, V) compounds possess much lower general toxicity but demonstrate appreciable antimicrobial properties and better body clearance. Unfortunately, there is still an insufficient amount of information regarding the latter class of compounds. Our research group's previous experience clearly identified cyanoximes – compounds with the general formula NC-C(=NOH)-R, with R being an electron-withdrawing group – as potent biologically active compounds. These small molecules also act as very good ligands binding a variety of metal ions and metalloids with many compounds showing a good potential for biomedical applications. Except for one publication in 2000, there are no investigations of any kind of Sb-based compounds with oxime-bearing ligands. Therefore, the goal of the proposed research is to synthesize and characterize a series of antimony complexes with some specifically selected biologically active cyanoximes structures. All synthesized novel compounds were characterized by elemental analysis (C,H,N), IR & NMR spectroscopy, thermal analysis (TG/DSC), and X-ray crystallography. The biological activity of the synthesized compounds was tested against bacterial strains MRSA, PAO1, and *E. coli.*

Figure 1 shows two types of selected for investigations mono-cyanoximes: amides and aromatic compounds. The first group of ligands for Sb(V) was used in the past in Kevin Pinks' project and in previous research in the area of biomedical applications of cyanoximes. All of them were found to be active and potentially useful in the field of biomedical research. The second group has not been studied well, although one patent and several papers dealing with chemistry and application of halogenated arylcyanoximes were generated from my research laboratory in 2004-2007. We selected two mono-chlorinated and two di-chlorinated cyanoximes for this project with specific choices of these molecules stemming from our 2010 patent No 7,727,967 B2 (USA) "Cyanoxime Inhibitors of Carbonyl Reductase and methods of Using Said Inhibitors in Treatments Involving Antracyclines", where they showed incredible biological activity inhibiting enzyme that prevents efficient anticancer treatment.

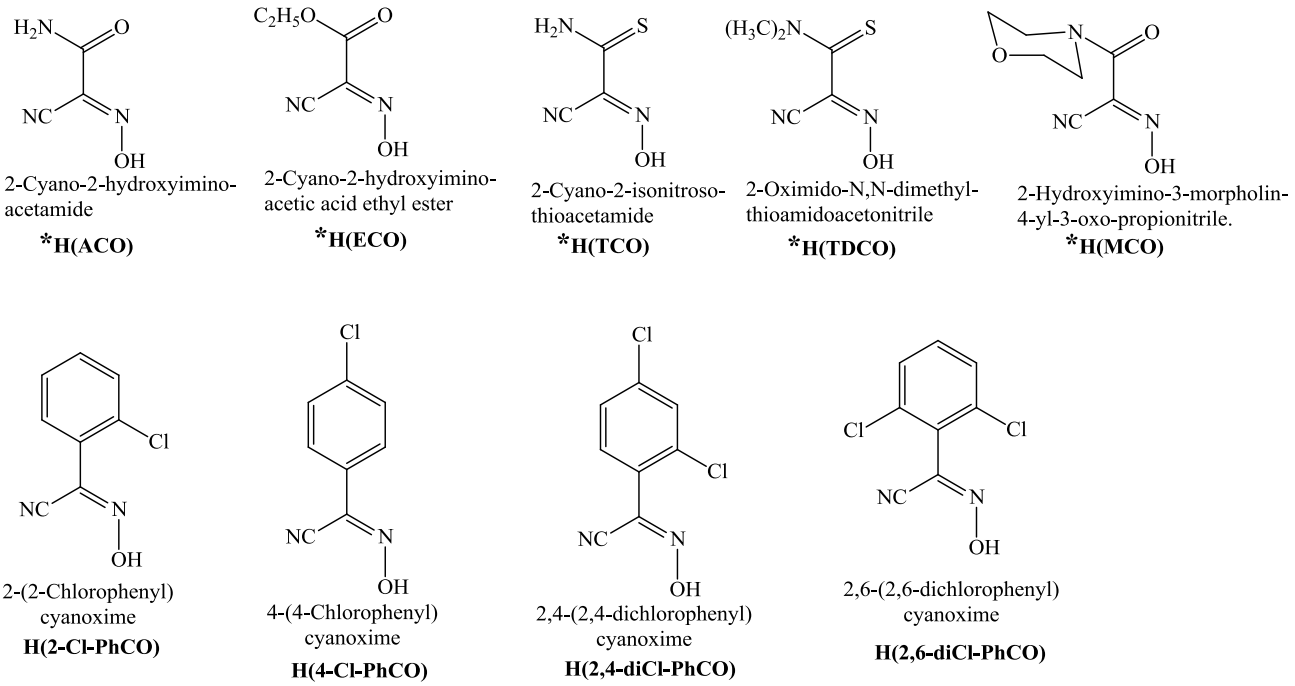

Figure 1. Choice of ligands selected for making new organoantimonials and their antimicrobial studies. An asterisk (*) indicates ligands used by Kevin Pinks in his pioneering work.

Project description and main results.

In order to make $Sb(CH_3)_3L_2$ compounds starting thimethylantimony(V) dibromide $Sb(CH_3)_3Br_2$ is necessary to have in sufficient for the whole work amount, which is ~10 g. The route to this compound was developed some decades ago displayed in Figure 2. It requires the use of the Grignard reagent for making trimethylantimony(III), which then can needs to be converted into the starting compound by bromination reaction. Final product represents nice white needle-shaped crystalline material that is soluble in common organic solvents but is not soluble in water. The metathesis reaction between that dibromide and silver(I) or thallium(I) derivatives of cyanoximes in polar aprotic solvents resulted in high yield preparation of desired $Sb(CH_3)_3L_2$ compounds as displayed in Figure 3. The beauty of such reaction is that both AgBr and TlBr are completely insoluble in organic solvents and can be easily removed from the reaction mixture via filtration (or, as in our case, centrifugation). Thus, Seth obtained 9 new organoantimony(V) compounds and along with them 5 salts of Tl(I) that were not previously made.

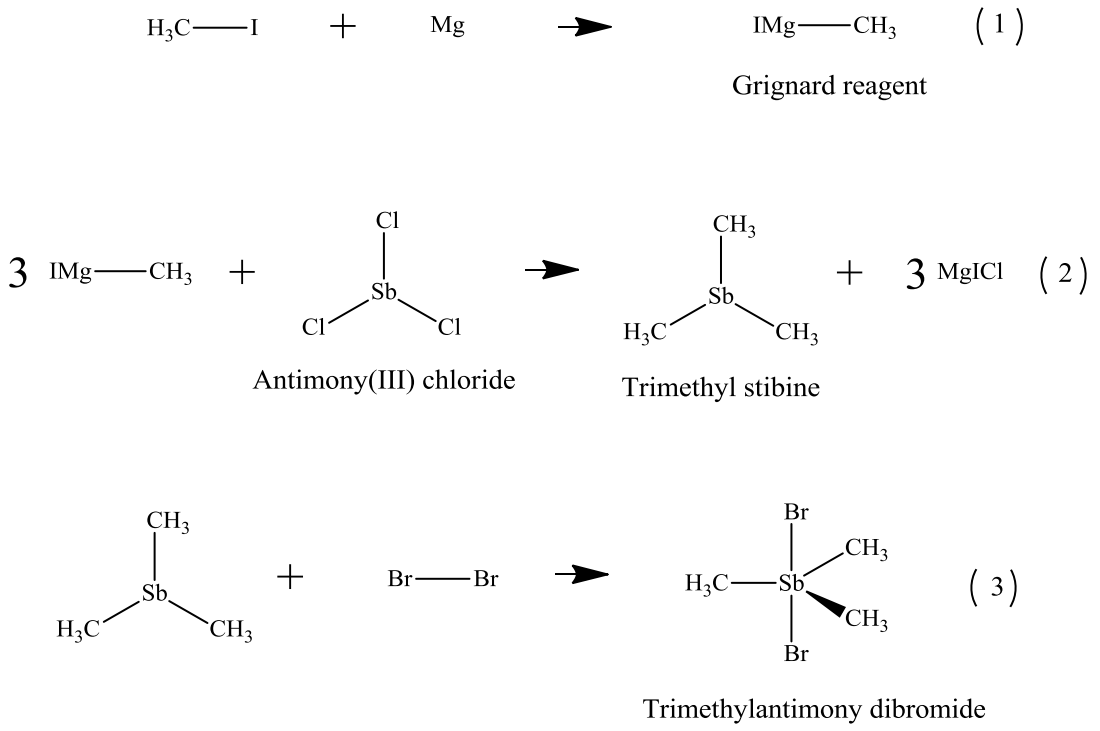

Figure 2. Synthesis of the initial for the project compound - trimethylantimony(V) dibromide.

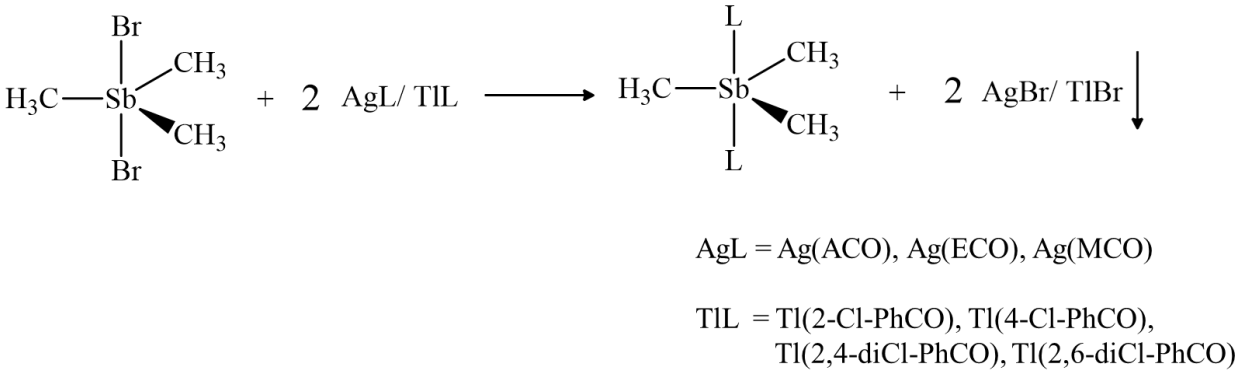

Figure 3. The most efficient route to trimethylantimony(V) bis-cyanoximates.

Below I present and summarize the most interesting fragments of Seth Adu MS thesis. These are: crystal data for all compounds he made, and tables of biological activity of his compounds.

Structures of the initial cyanoximes and their alkali metal salts: Figures 4-6.

Seth was extremely lucky to be able to crystallize one specific and rare *syn*-diastereomer of the di-chlorinated cyanoxime structure of which as *anti*-diastereomet was established in 2008.

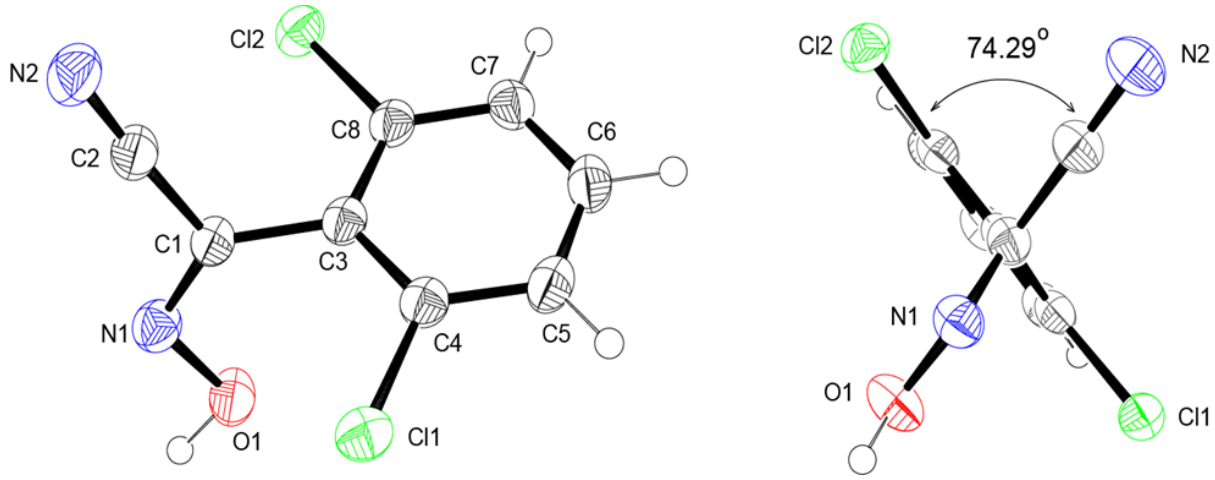

Figure 4. Molecular structure and numbering scheme for *syn*-H(2,6-diCl-PhCO) (in ORTEP representation drawn at 50% thermal ellipsoids probability level) showing two orthogonal views and a large dihedral angle between two planar fragments.

Sulfur-containing H(TDCO) cyanoxime was obtained in 1992, but its crystal structure was not known until 2019, when it was determined after ~30 years of crystallization in a deep freezer. There is remarkable are also rare H-bonding detected between OH-group and S-atom of neighboring molecule.

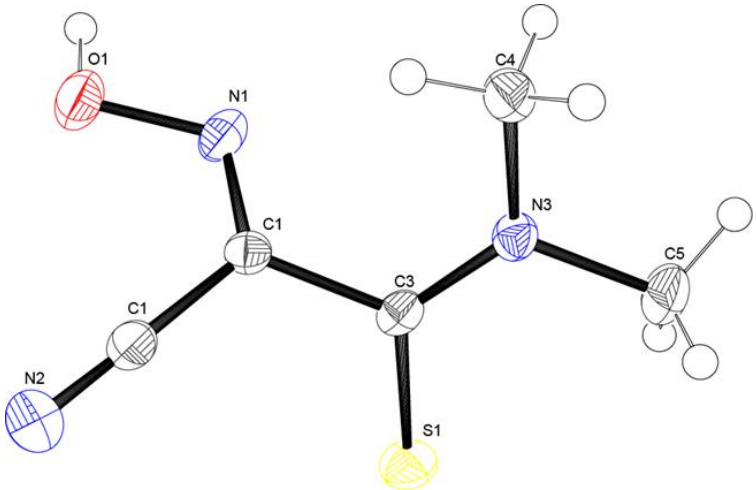

Figure 5. The asymmetric unit (ASU) in the structure of one of the initial compounds H(TDCO) showing atomic numbering scheme.

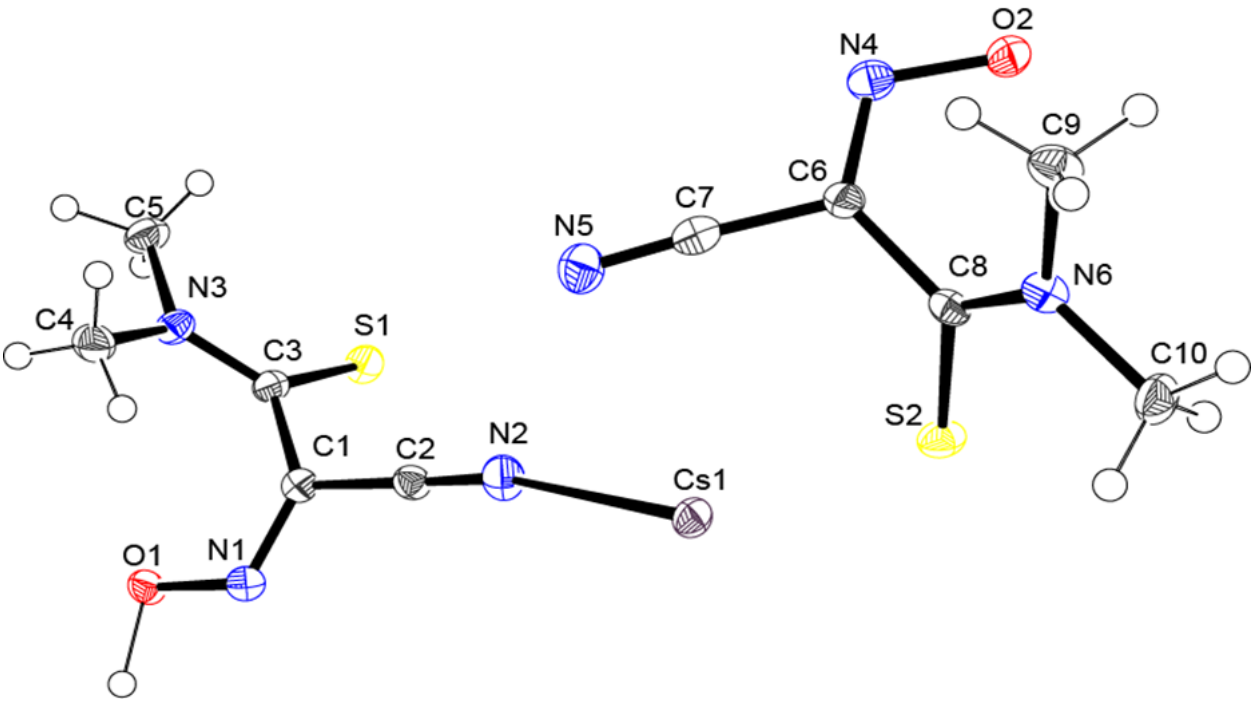

Figure 6. The ASU in the structure of Cs(TDCO)×H(TDCO) depicting the numbering scheme.

The H(TDCO) was used to make alkali metal (Cs) salt and Tl salt for the metathesis reaction shown in Figure 3. We were lucky to isolate suitable for the X-ray analysis crystals of the "acid salt" $CsH(TDCO)_2$ structure of which is shown in Figure 6.

Structures of Tl-salts of cyanoximes.

We found that the use of thallium(I) salts of cyanoximes for the metathesis reaction was completely justified for making organometallic derivatives of Sn, Te and Sb because of the following circumstances:

1) The Ag(I) complexes of the thioamide-cyanoximes TCO^- and $TDCO^-$ do not exist: quickly decompose to insoluble black Ag_2S and ligands are decomposed.
2) To the contrary, Tl-salts of Tl(TCO) and Tl(TDCO) are light stable, readily available and formed with high yield.
3) TlX (X = Cl, Br) are insoluble in organic solvents and water, easily removable by centrifugation; their formation is a driving force for the metathesis reaction.
4) Tl-compounds are not light sensitive as many their Ag(I) counterparts which rapidly decompose upon exposure to light even during quick synthetic procedures. That is precisely the case for all mono- and di-halogenated aryl-cyanoximes.
5) Tl-compounds do not act as oxidizers contrary to silver(I) compounds frequently used in organic syntheses as mild oxidizers.

Therefore, counting aforementioned provisions **1** and **4** we prepared appropriate Tl(I) cyanoximates, while for other syntheses we used light stable Ag(ACO), Ag(ECO), Ag(MCO) compounds.

Below I show in Figures 7-9 structures of Tl(I) cyanoximates that were used in Seth project of making $Sb(CH_3)_3L_2$ compounds.

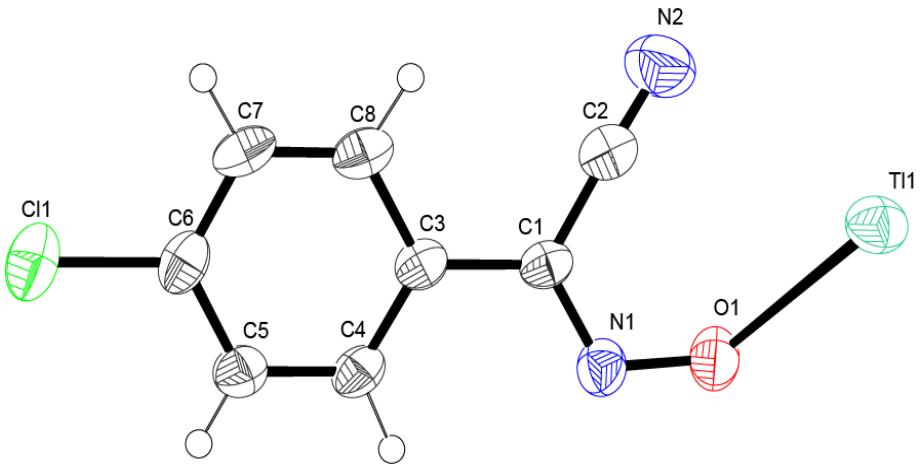

Figure 1. The ASU in the structure of one of the initial compounds Tl(4-Cl-PhCO) drawn in ORTEP representation at 50% ellipsoid probability level showing atomic numbering.

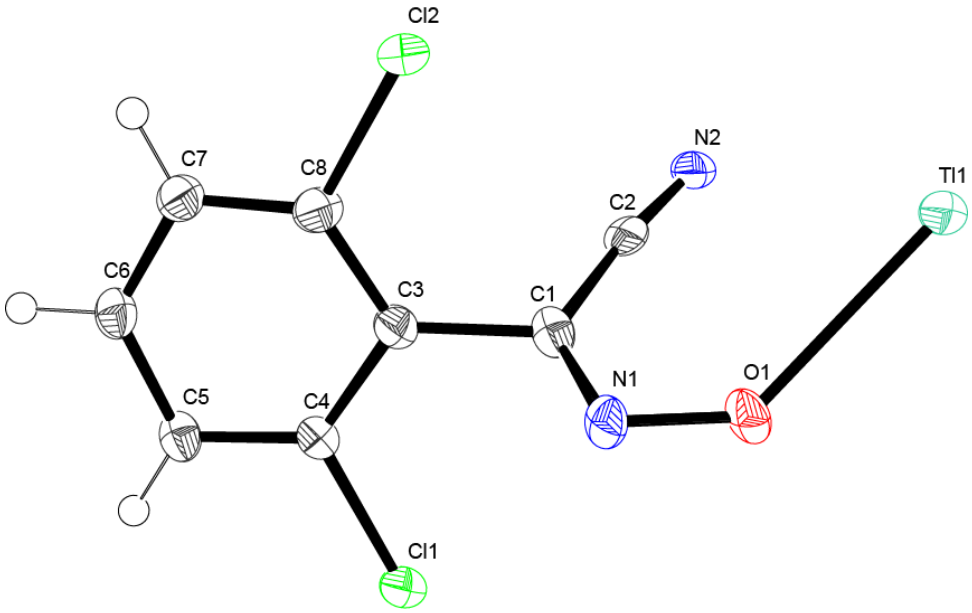

Figure 8. The ASU in the structure of one of the initial compounds Tl(2,6-diCl-PhCO) drawn in ORTEP representation showing atomic numbering.

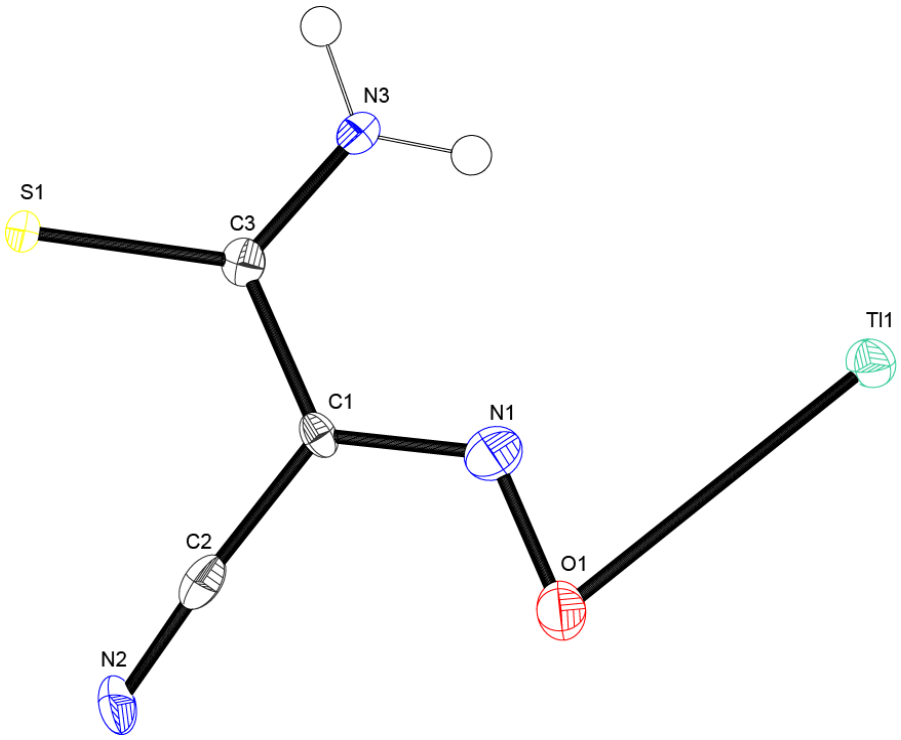

Figure 9. The ASU in the structure of one of the initial compounds Tl(TCO) drawn in ORTEP representation at 50% ellipsoid probability level.

All used in preparations Tl-cyanoximates represent stable crystalline compounds that easily can be dried in desiccator, ground to powdery state and, using necessary precautionary measures, safely used in syntheses.

Structures of target $Sb(CH_3)_3L_2$ compounds.

All synthesized organoantimony(V) cyanoximates represent crystalline compounds and were fortunate to grow suitable for the X-ray analysis single crystals for their structural characterization. Below in Figures 10-17 are shown determined molecular structures of all characterized that way compounds, which all turned out to be monomeric, molecular substances of expected $Sb(CH_3)_3L_2$ composition. However, there are rather interesting peculiarities in cyanoximes' geometry were observed and I comment on them along the way.

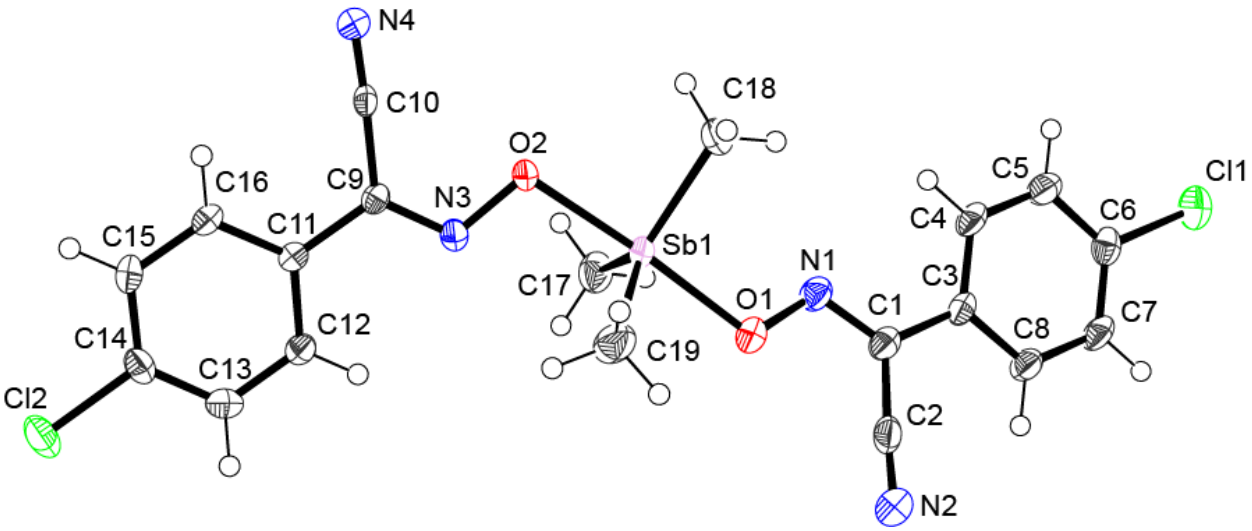

Figure 10. Molecular structure and atomic numbering of $SbMe_3(4Cl\text{-}PhCO)_2$ which represents only *anti*-diastereomer.

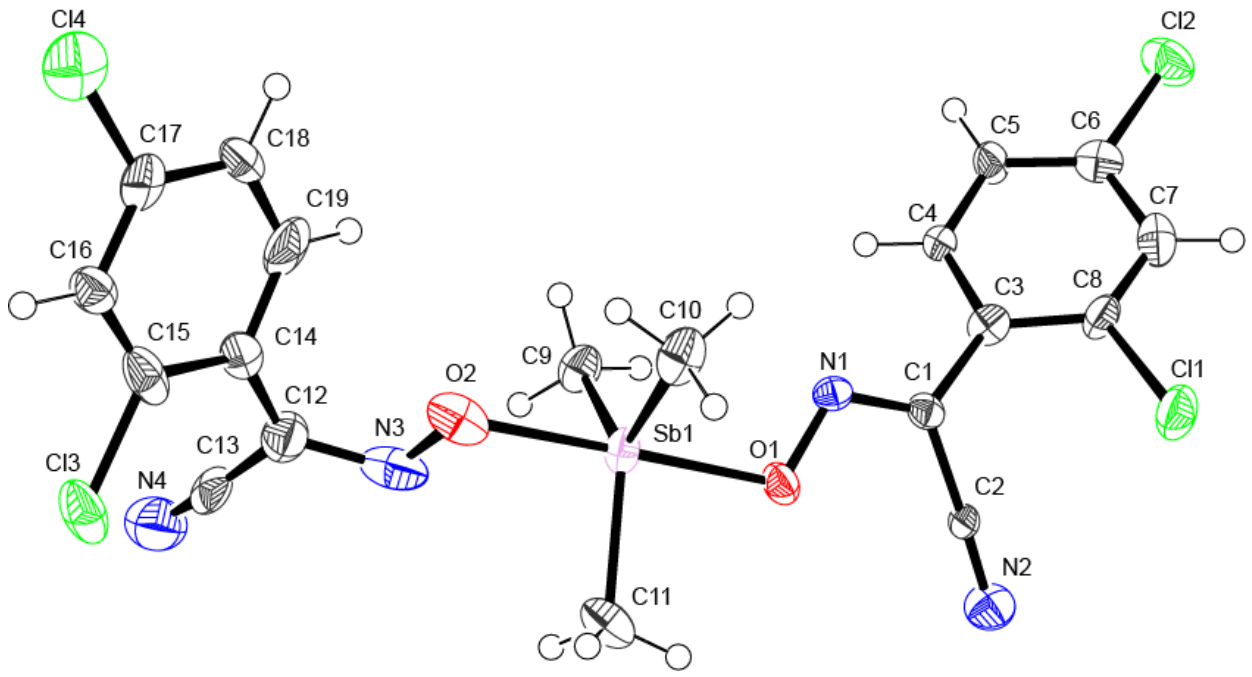

Figure 11. Molecular structure and atomic numbering of $SbMe_3(2,4\text{-}diCl\text{-}PhCO)_2$ which represents quite unique mixture of *syn*- and *anti*- diastereomers present at exactly 1:1 ratio in the structure (shown by arrows).

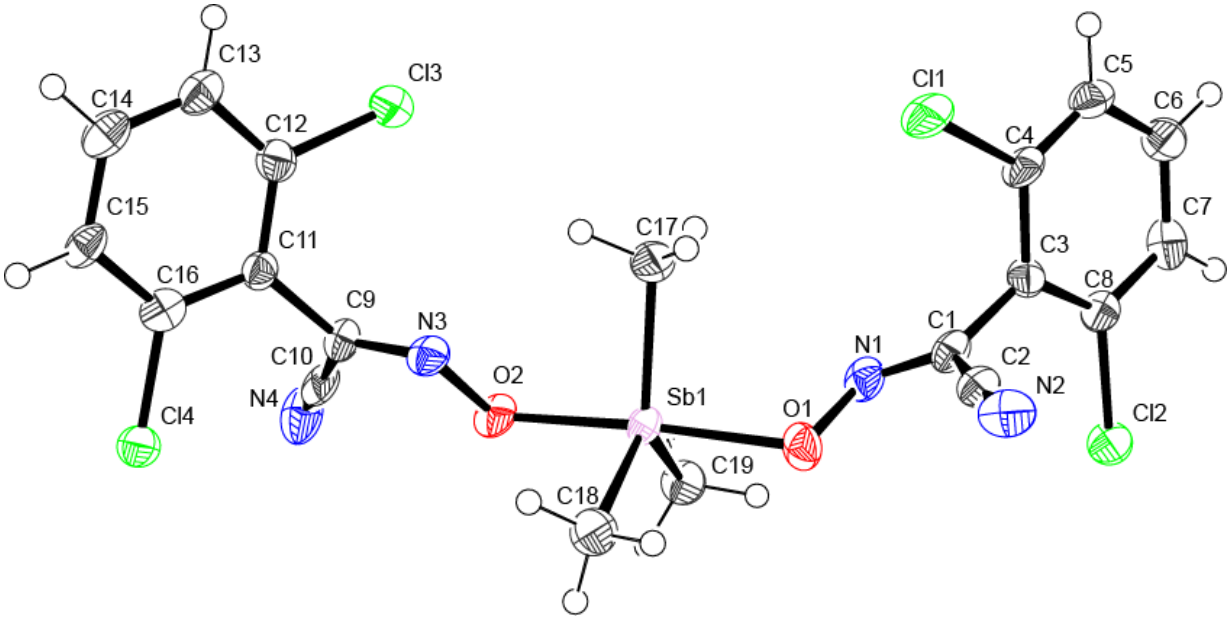

Figure 12. Molecular structure of $SbMe_3(2,6\text{-diCl-PhCO})_2$ in which only one anti-diastereomer is present.

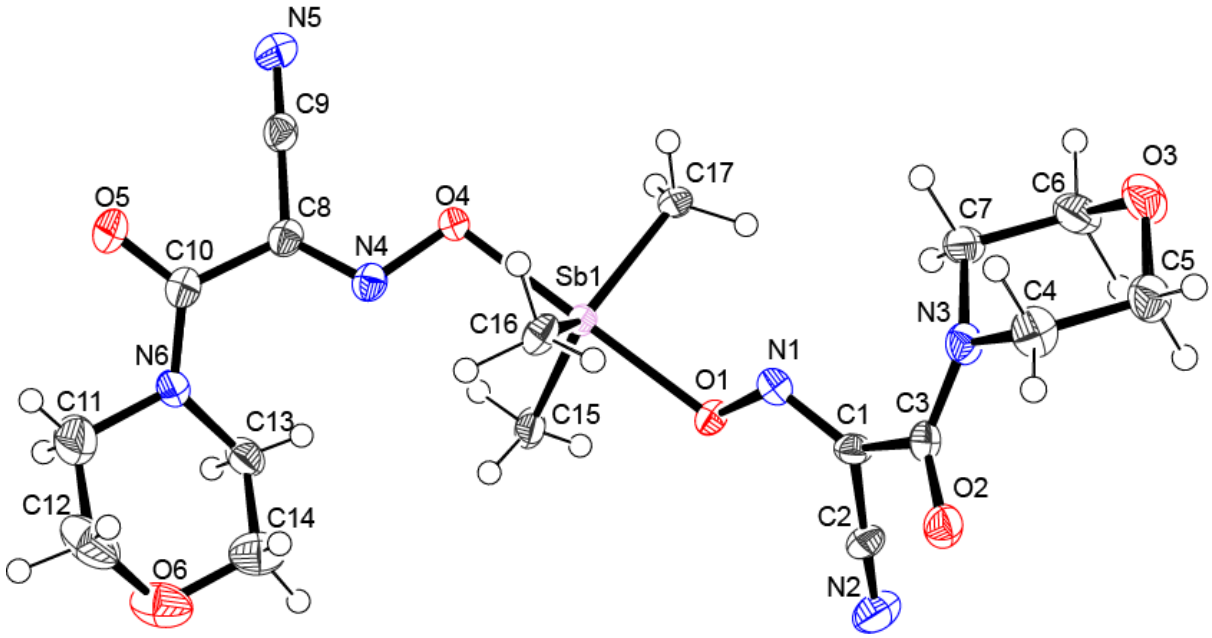

Figure 13. Molecular structure and atomic numbering for $SbMe_3(MCO)_2$ in which only one *anti*-diastereomer is present.

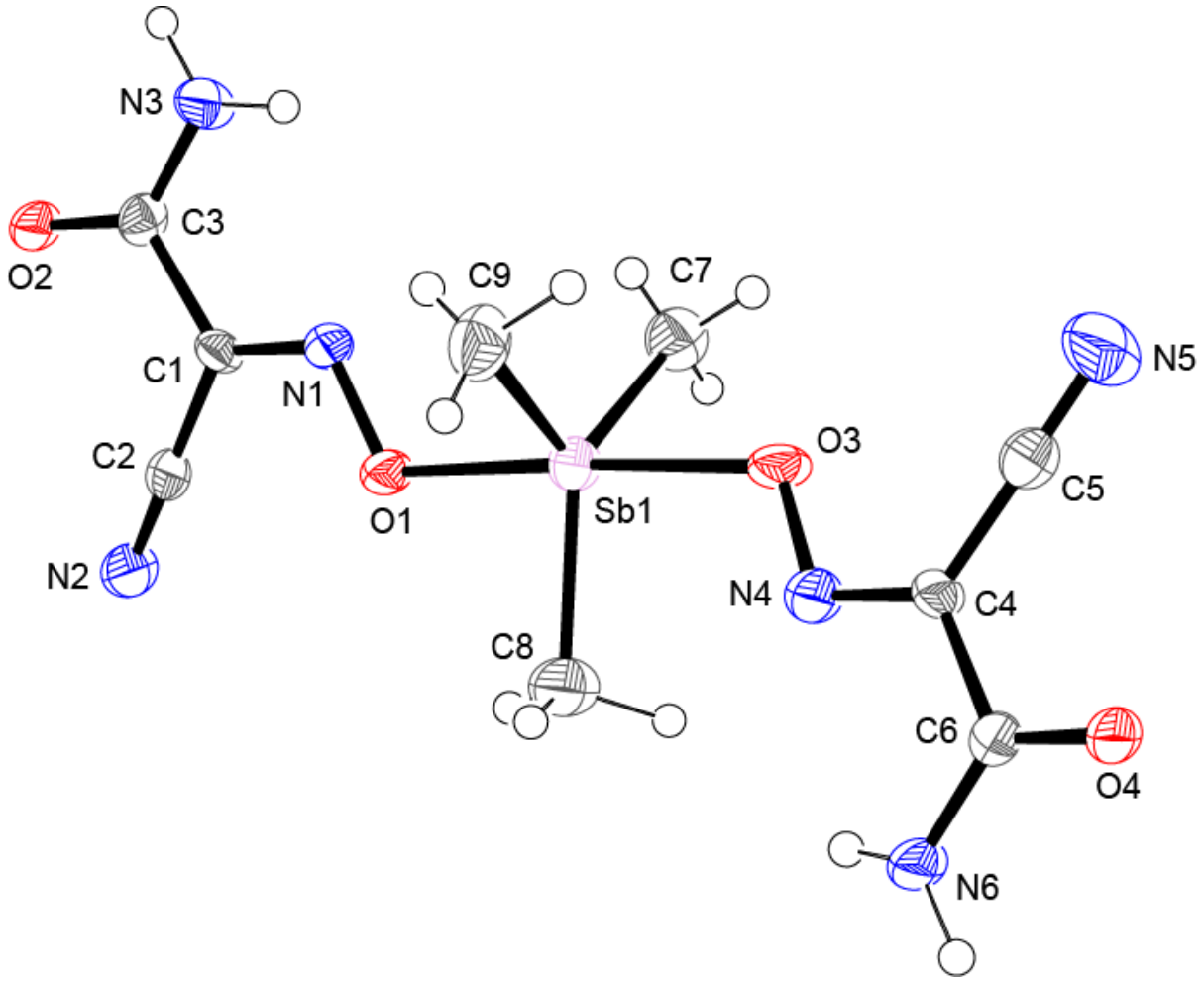

Figure 14. Molecular structure and numbering for $SbMe_3(ACO)_2$, where *anti*-diastereomer of the cyanoxime is present.

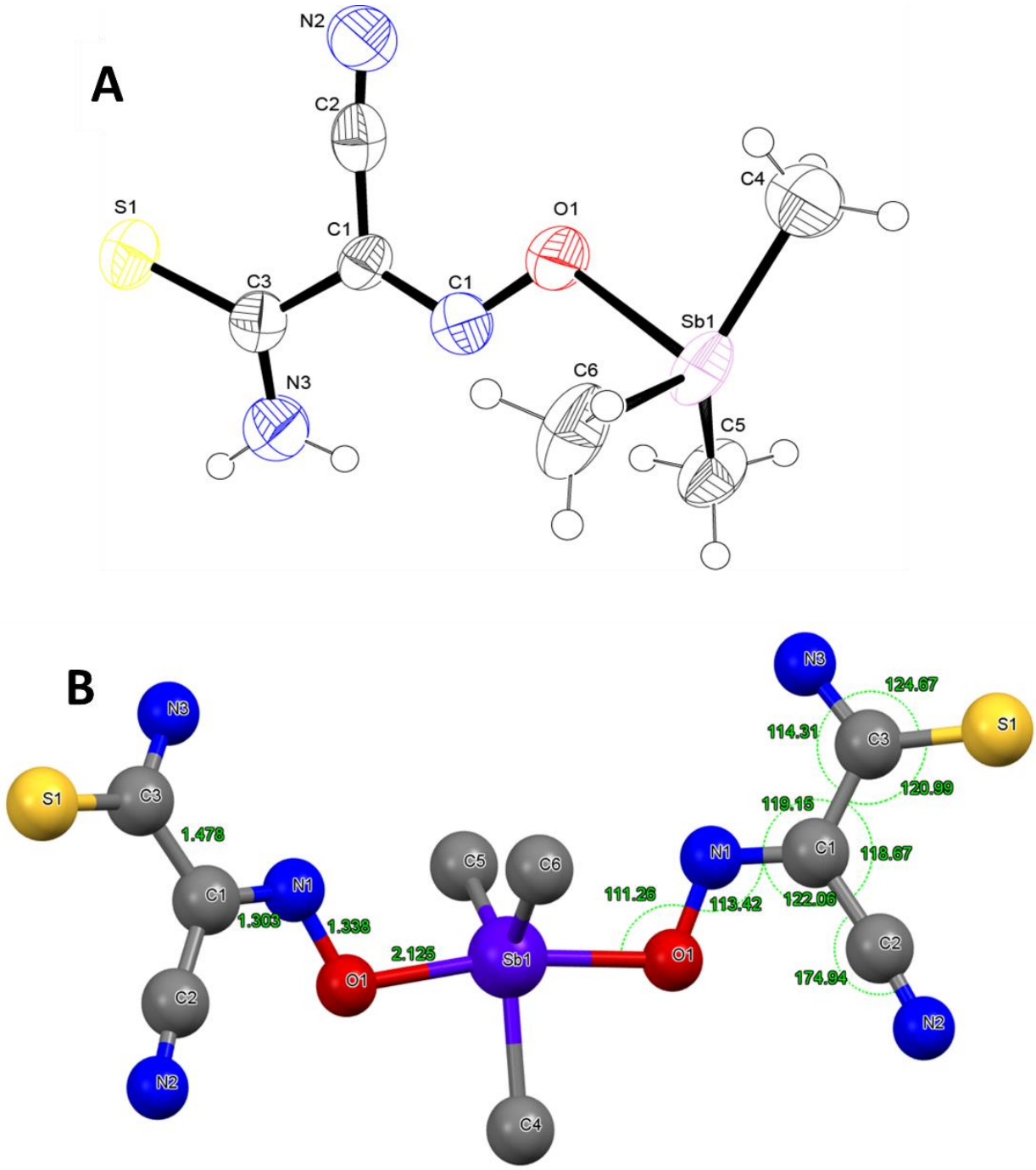

Figure 15. Crystal structure of $SbMe_3(TCO)_2$ compound: **A** – the ASU in ORTEP representation with atomic numbering; **B** – GROW fragment showing full structure with the most important bond lengths and valence angles (H-atoms are not shown for clarity).

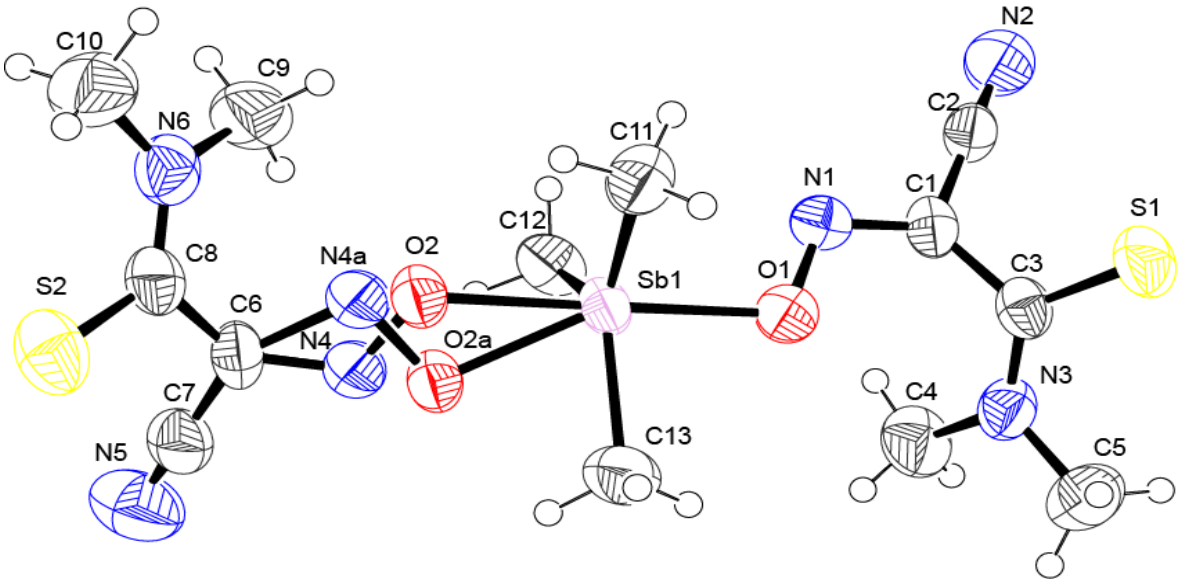

Figure 16. Molecular structure of $SbMe_3(TDCO)_2$ drawn in ORTEP showing the presence of co-crystallized both *syn*- and *anti*- diastereomers within the structure (indicated with arrow).

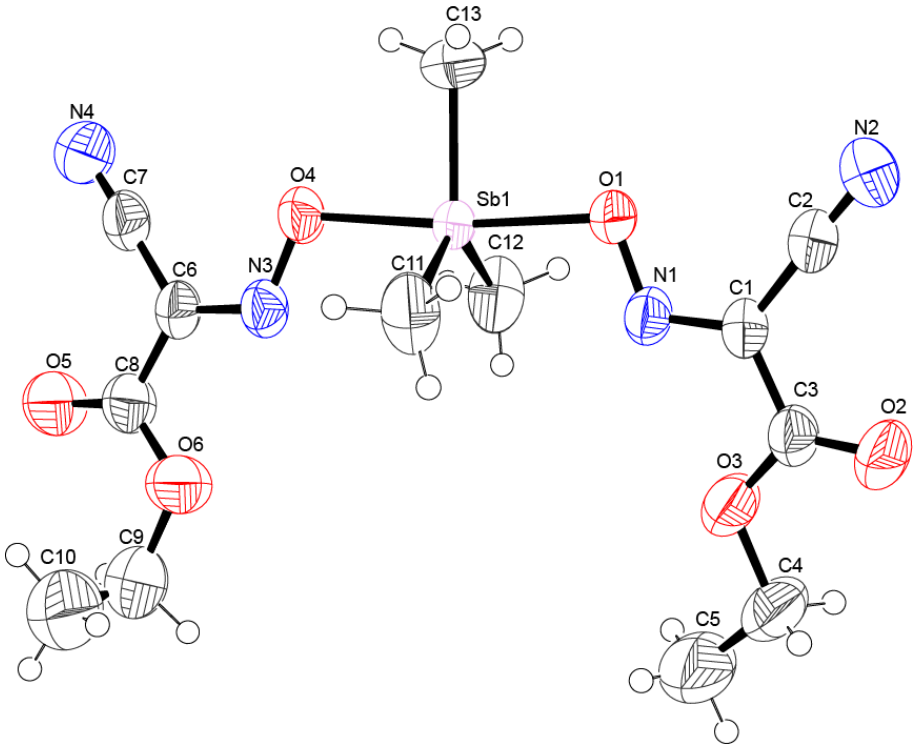

Figure 17. Molecular structure and atomic numbering for $SbMe_3(ECO)_2$.showing only one anti-diastereomer in the structure.

Results of microbiological studies (conducted at Oklahoma State University, Stillwater).

The biological activity studies were performed by our collaborators at Oklahoma State University in the Department of Microbiology and Molecular Genetics. These collaborators are Dr. Marianna Patrauchan and her research group (antibacterial studies), and Dr. Karen Wozniak and her research group (antifungal studies). Due to limited quantity of synthesized compounds and other technical difficulties, the biological activity data was obtained only for some of my synthesized compounds, and they will be described below. All results of conducted studies are presented in Charts 1 – 15 below.

Minimum Inhibition Concentration (MIC) Tests. Minimum Inhibition Concentration (MIC) assays determine the lowest concentration of an antimicrobial agent that prevents the visible growth of a microorganism.[92] A dilution gradient of the synthesized compound is made and tested against microbial strains. The cell growth at each concentration is measured to show the effectiveness of the compound. A total of nine newly synthesized compounds were submitted to OSU for biological testing. $SbMe_3Br_2$ and the cyanoxime ligands accompanied these compounds to OSU for biological activity testing. For these tests, the compounds had to be dissolved in DMSO. DMSO has inhibition effects at concentrations of 200 µg/mL and so that was taken into consideration when determining the effects of my newly synthesized compounds on bacteria and fungi. Antibacterial activity was assessed by testing my synthesized compounds against *P. aeruginosa, E. coli,* and *S. aureus.* The control used in all the testing was my initial compound $SbMe_3Br_2$. Unbound, free cyanoximes, HL, were also used as controls to see if there were any synergistic effect when Sb(V) source was combined with the cyanoxime. The independent variable was the concentrations of the compounds used in the MIC tests and the dependent variable was cell growth measured.

MIC Tests for the Most Effective Compounds. The $SbMe_3(MCO)_2$ was the most effective compound against *P. aeruginosa.* At ~ 50 µg/mL, there was barely any cell growth and at subsequent concentrations afterwards indicating significant antimicrobial effect. Its ligand, H(MCO) that is in the structure, did not have the same effect when tested against the same bacterial strain. Chart 1 shows the comparison between the effects of the $SbMe_3Br_2$ (which was our control substance), H(MCO), and $SbMe_3(MCO)_2$ on *P. aeruginosa.* The control also had significant activity at ~ 50 µg/mL but was less than that of $SbMe_3(MCO)_2$. All the other tested compounds on this strain on bacteria were not significantly effective. My compounds were also tested against *E. coli* and the cell growth analysis showed that $SbMe_3$(2,6-diCl-PhCO)$_2$ was the most effective compound at stopping the growth of *E. coli* at a concentration of ~ 50 µg/mL, like $SbMe_3(MCO)_2$ on *P. aeruginosa.* Chart 2 shows results of the MIC tests against *E. coli* where $SbMe_3$(2,6-diCl-PhCO)$_2$ was more effective than the control used. My antimony cyanoximates were not very effective against MRSA, with only the control being the compound that was able to completely stop bacterial cell growth at ~ 100 µg/mL, with significant effect being seen at ~ 50 µg/mL. Only the control and the ligand H(2,4-diCl-PhCO) were effective against the *S. aureus*

(MRSA) strain used in the biological testing. Significant MRSA cell growth inhibition was seen at~100 µg/mL for H(2,4-diCl-PhCO), with no cell growth recorded at~150 µg/mL (Chart 3).

MIC Tests for Remaining Compounds. The graphical results for the MIC tests of compounds that were not very effective against the strains of bacteria they were tested on will be presented in this section. The MIC results for *P. aeruginosa* are shown in Chart 4 to Chart 8. Charts 9 to Chart 13 illustrate the MIC results for compounds tested against *E. coli*, and Figure 66 shows the results acquired from testing the free cyanoxime, H(2Cl-PhCO), and $SbMe_3(2Cl\text{-}PhCO)_2$ against *MRSA*. Some of these graphs do not include the MIC test result for the free cyanoxime, which will be presented later. My research goals included assessing the effectiveness of my synthesized compounds against the most effective compounds in Kevin Pinks' research work. On comparison of the controls, ($SbMe_3Br_2$ vs $SbPh_4Br$), Chart 15 shows that $SbMe_3Br_2$ was more effective at inhibiting bacterial growth its bulkier counterpart, $SbPh_4Br$. The $SbMe_3Br_2$ had the lower MIC average of the two showing that a lower concentration of $SbMe_3Br_2$ is needed to inhibit bacterial cell growth.

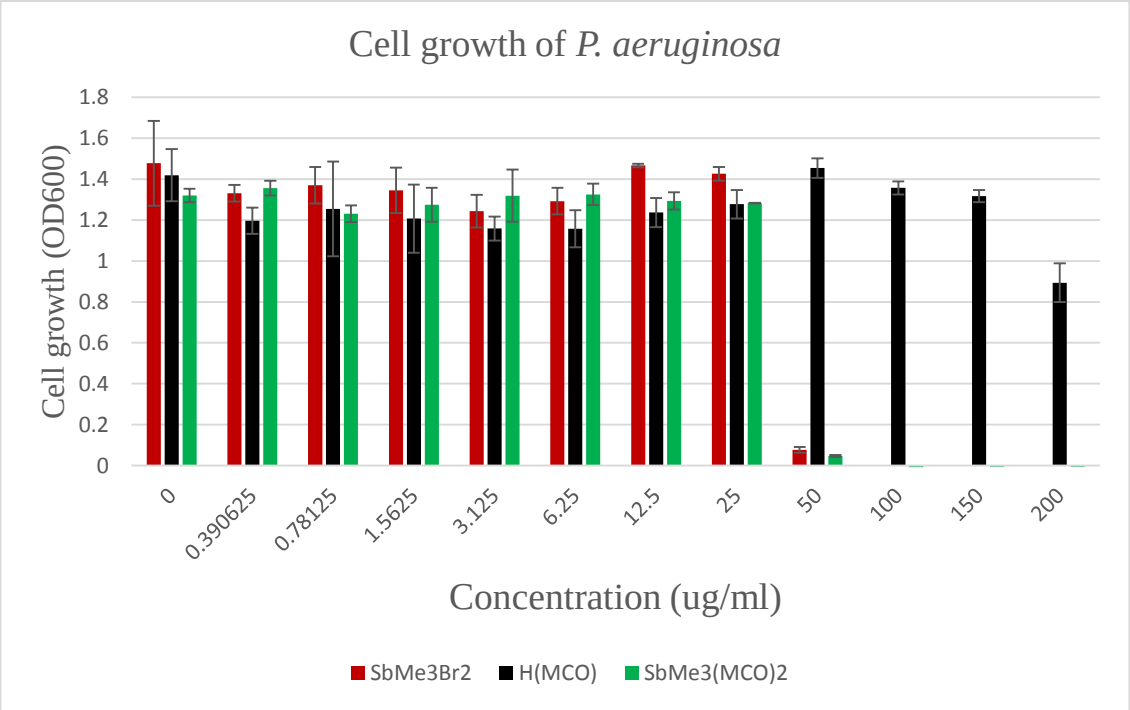

Chart 1. Results of MIC tests of $SbMe_3Br_2$, H(MCO) and $SbMe_3(MCO)_2$ against *P. aeruginosa*.

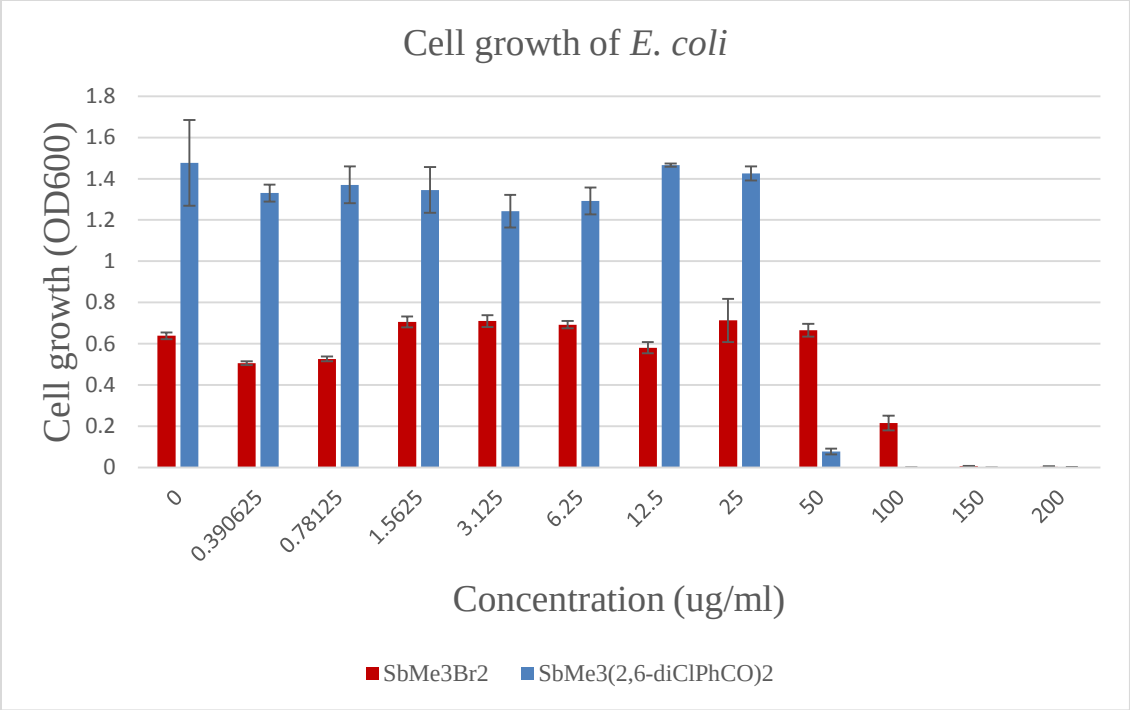

Chart 2. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3$(2,6-diCl-$PhCO)_2$ against *E. coli*.

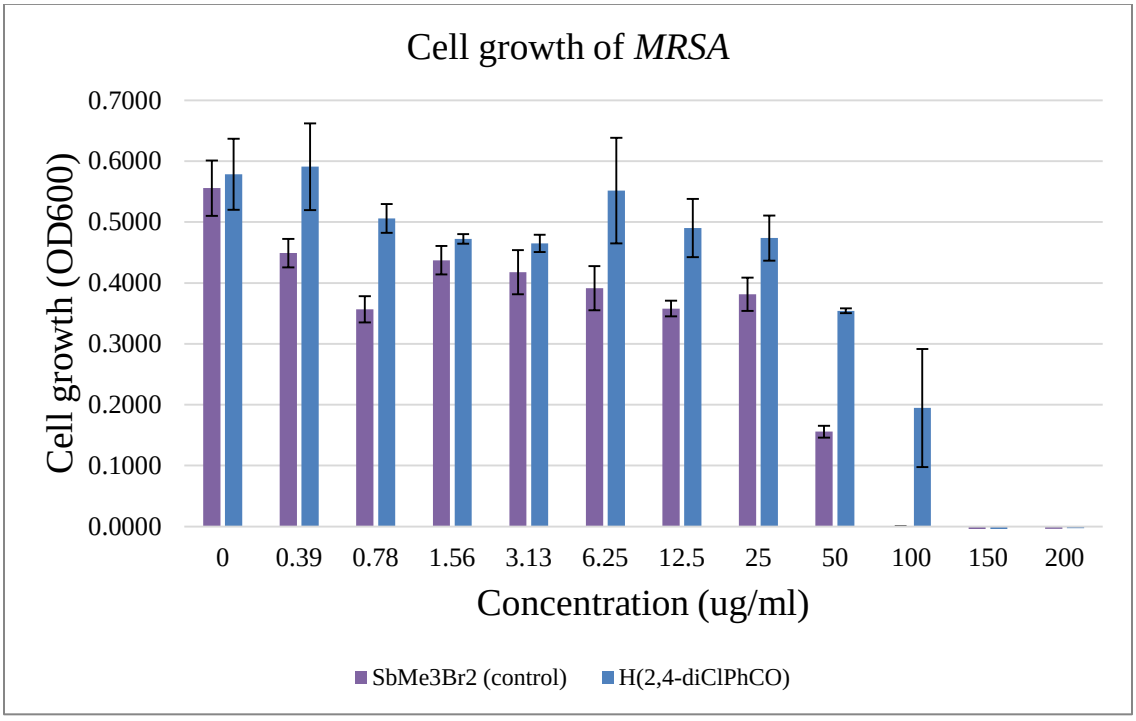

Chart 3. Results of H(2,4-diCl-PhCO) testing against MRSA, compared to $SbMe_3Br_2$ showing a downward trend for the free cyanoxime from 25 µg/mL to complete inhibition at 150 µg/mL.

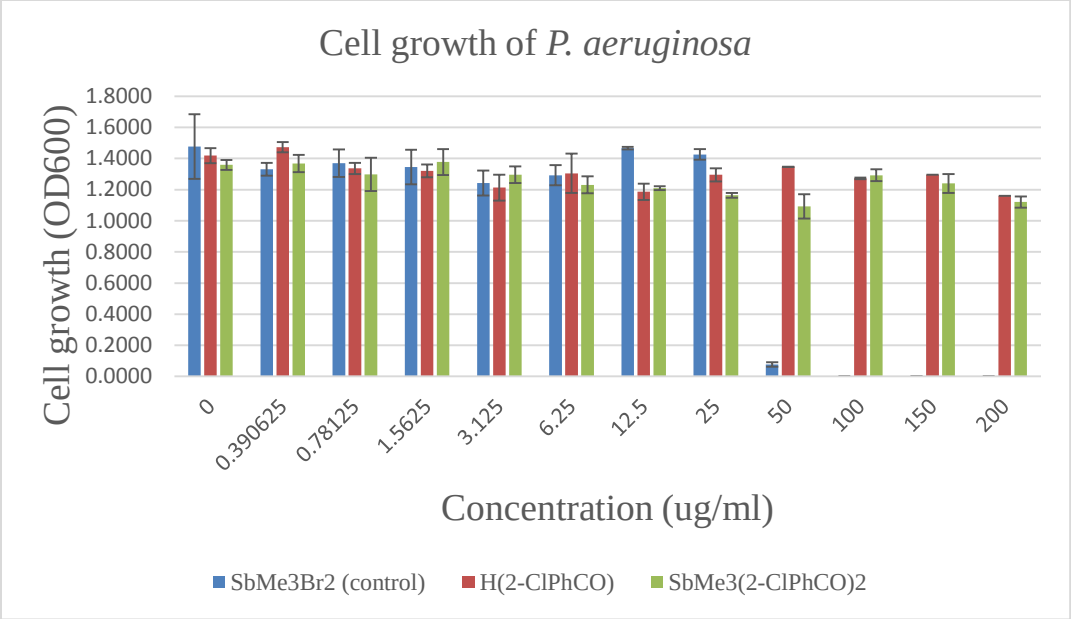

Chart 4. Results of MIC tests of $SbMe_3Br_2$, H(2-Cl-PhCO) and $SbMe_3(2\text{-Cl-PhCO})_2$ against *P. aeruginosa*.

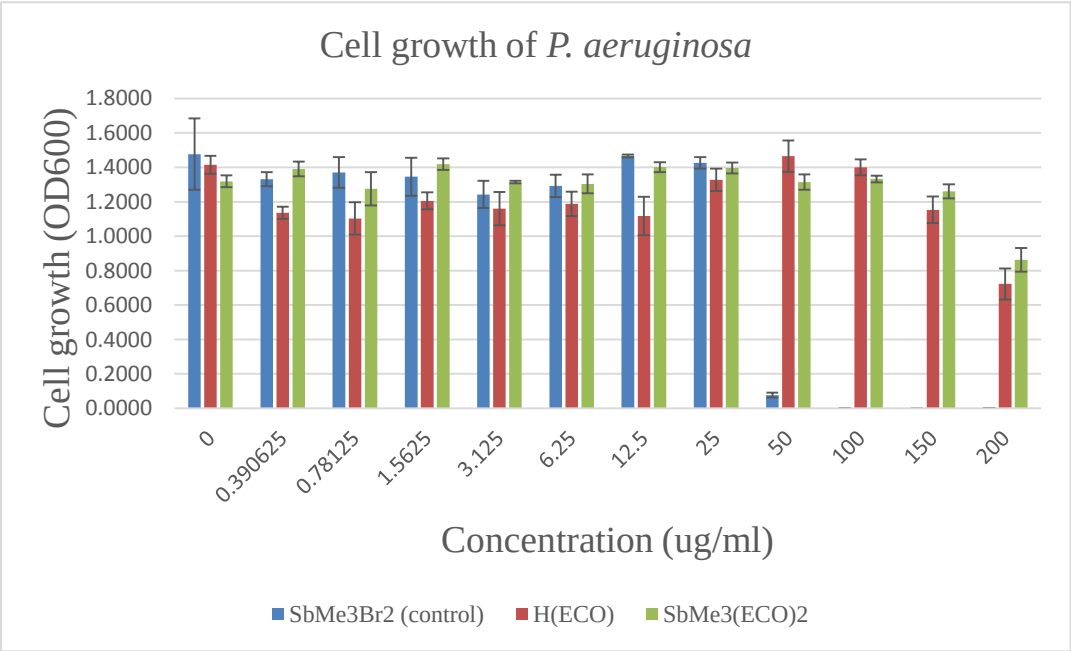

Chart 5. Results of MIC tests of $SbMe_3Br_2$, H(ECO) and $SbMe_3(ECO)_2$ against *P. aeruginosa*.

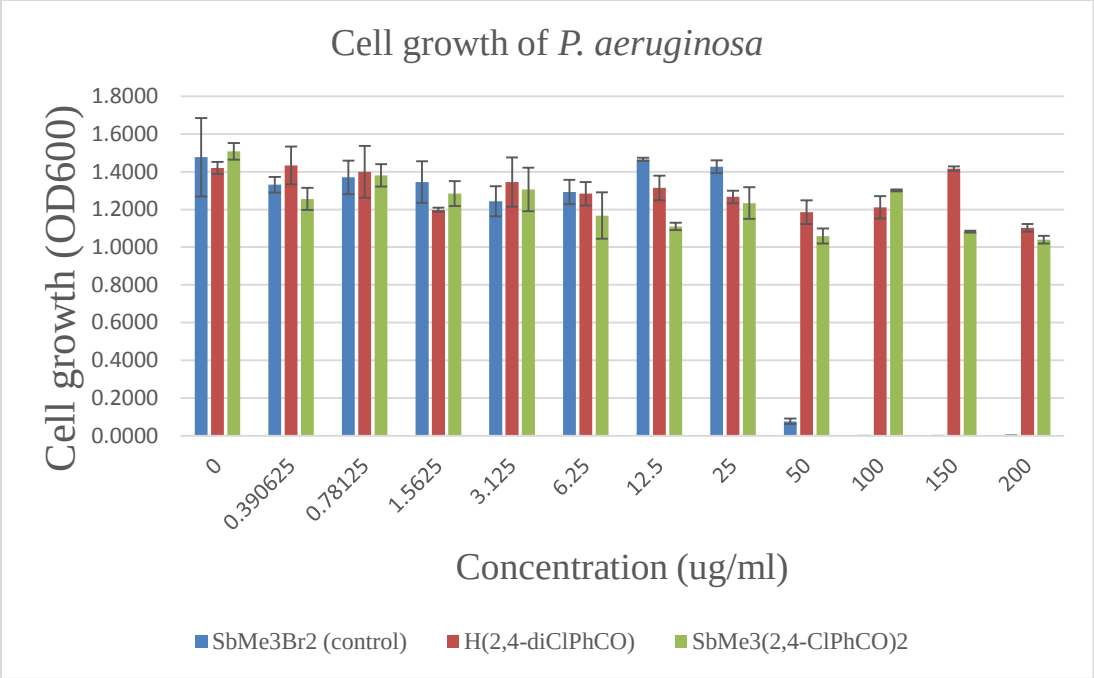

Chart 6. Results of MIC tests of $SbMe_3Br_2$, H(2,4-diCl-PhCO) and $SbMe_3$(2,4-diCl-PhCO)$_2$ against *P. aeruginosa*.

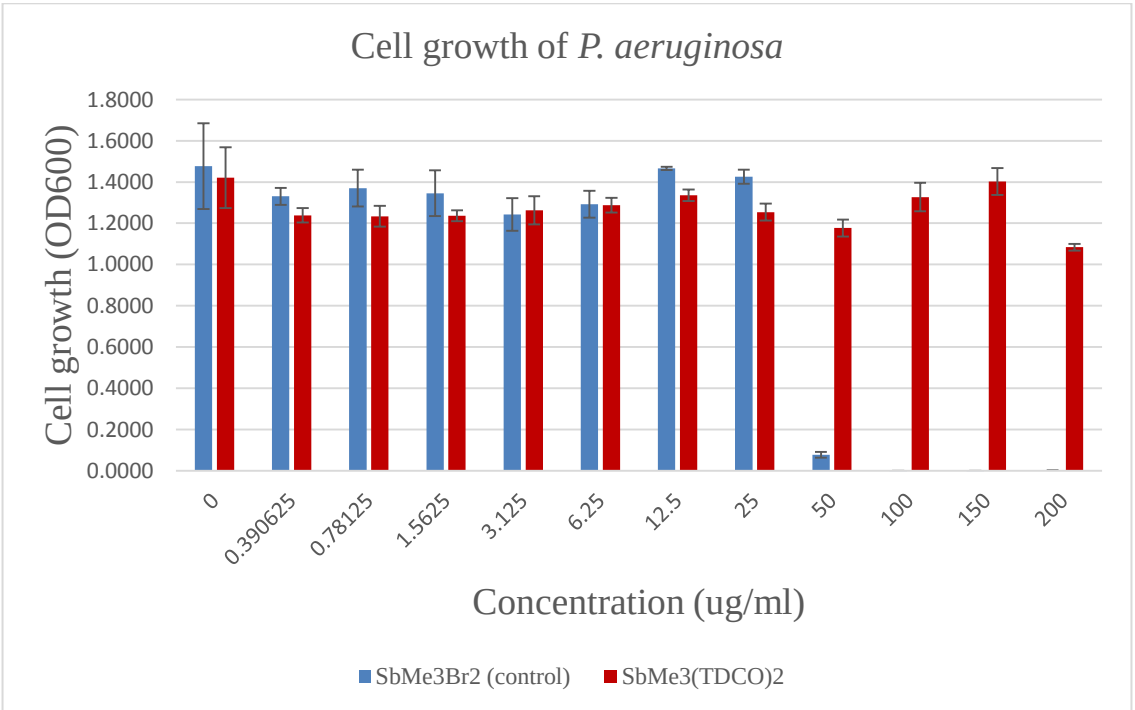

Chart 7. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3(TDCO)_2$ against *P. aeruginosa*.

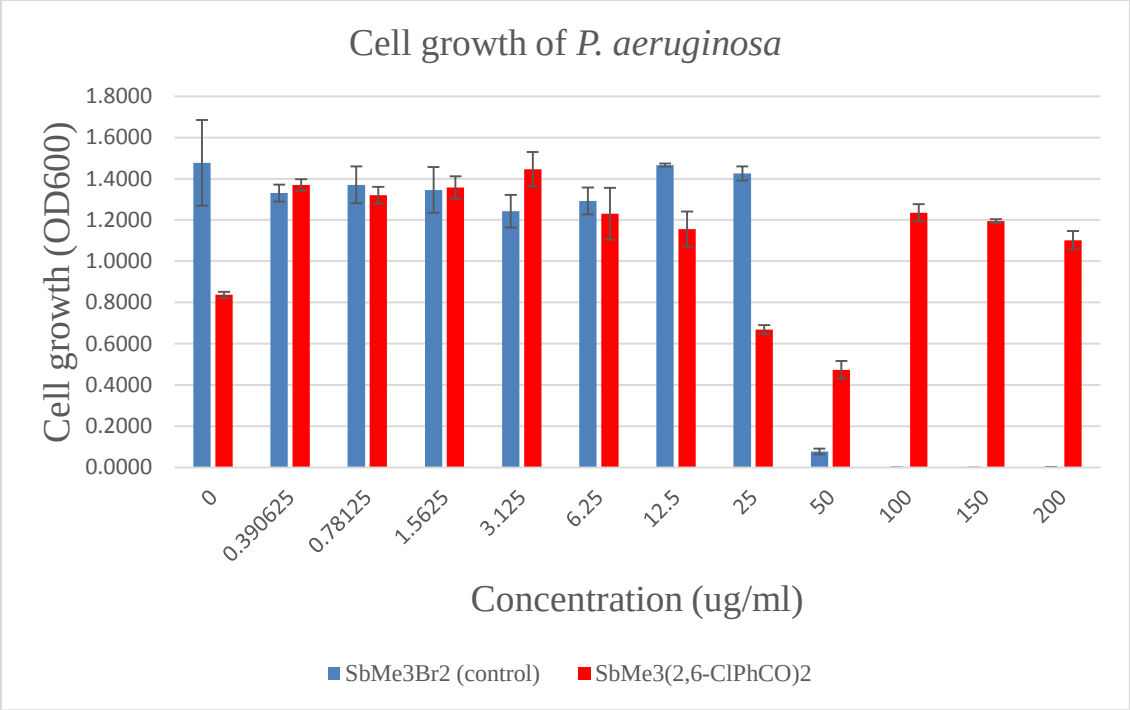

Chart 8. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3(2,6\text{-diCl-PhCO})_2$ against *P. aeruginosa*.

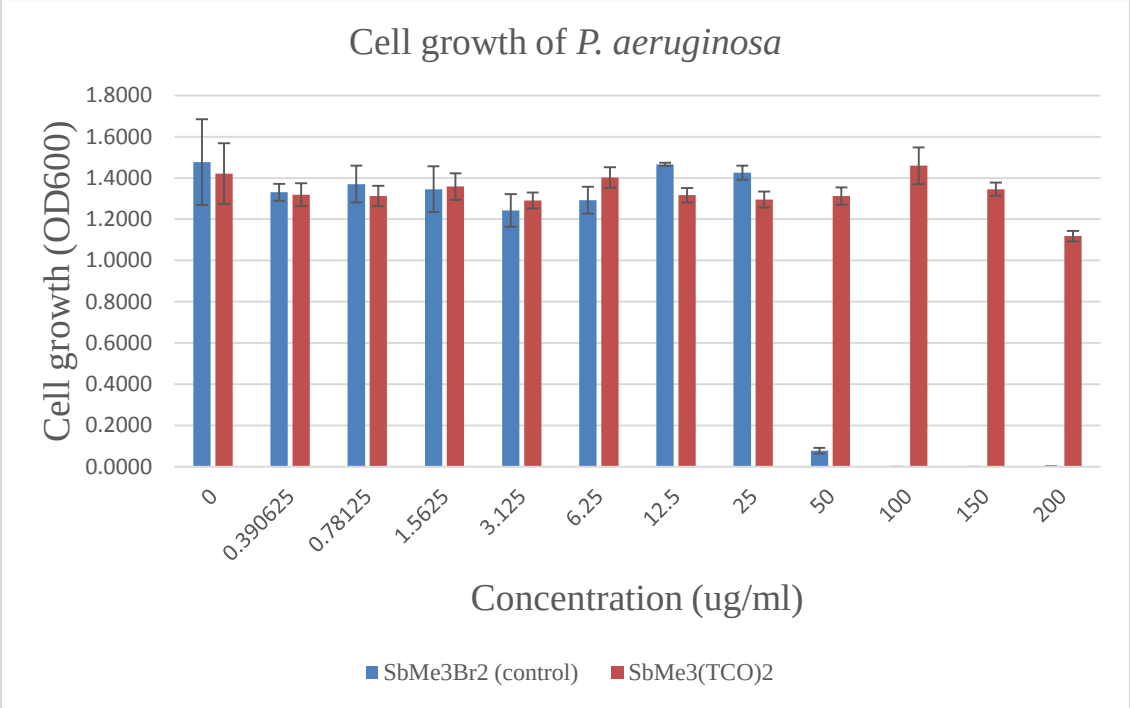

Chart 9. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3(TCO)_2$ against *P. aeruginosa*.

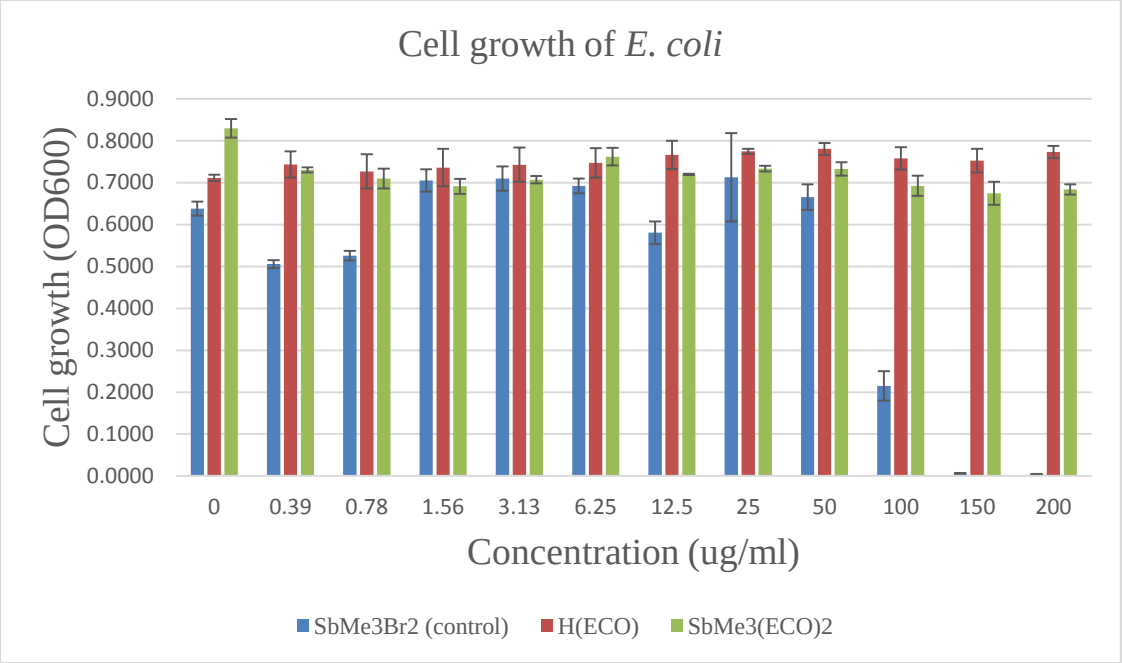

Chart 10. Results of MIC tests of $SbMe_3Br_2$, H(ECO) and $SbMe_3(ECO)_2$ against *E. coli*.

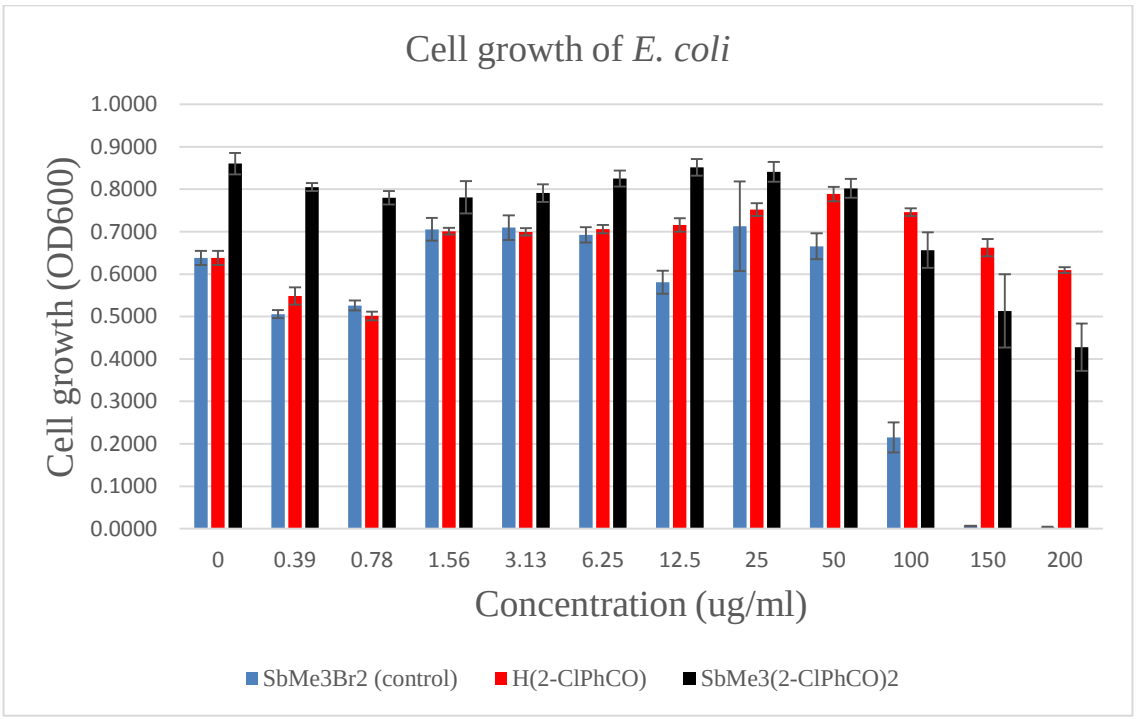

Chart 11. Results of MIC tests of $SbMe_3Br_2$, H(2-ClPhCO) and $SbMe_3(2\text{-}ClPhCO)_2$ against *E. coli*.

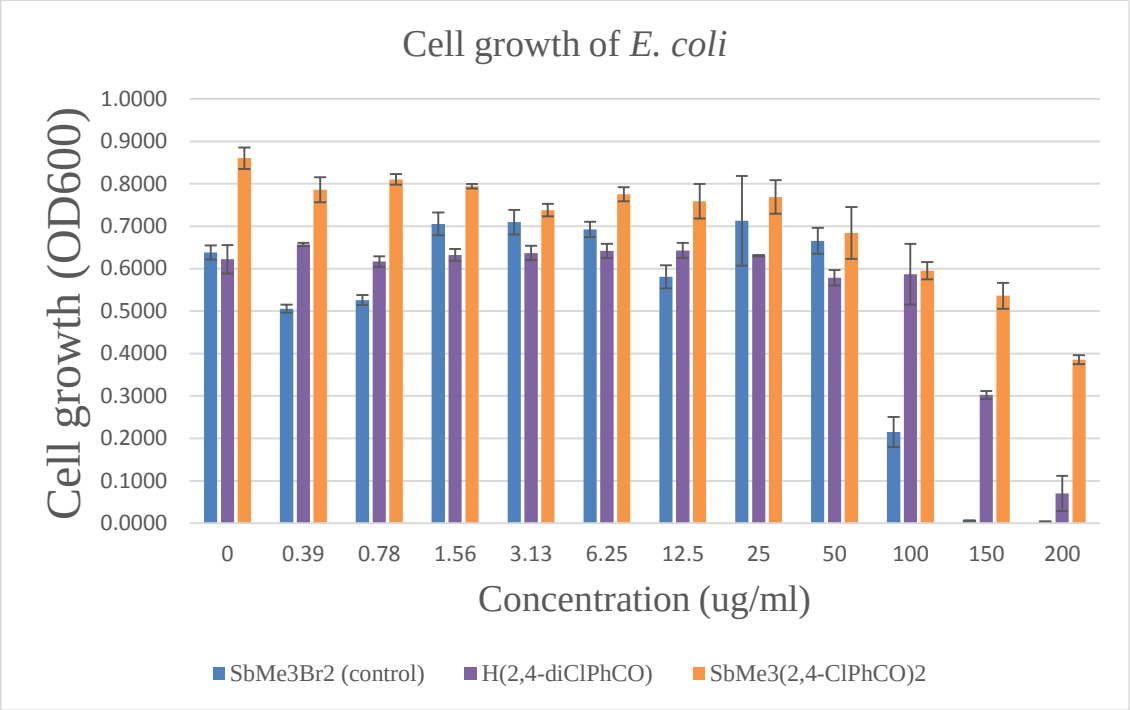

Chart 12. Results of MIC tests of $SbMe_3Br_2$, H(2,4-diCl-PhCO) and $SbMe_3$(2,4-diCl-PhCO)$_2$ against *E. coli*.

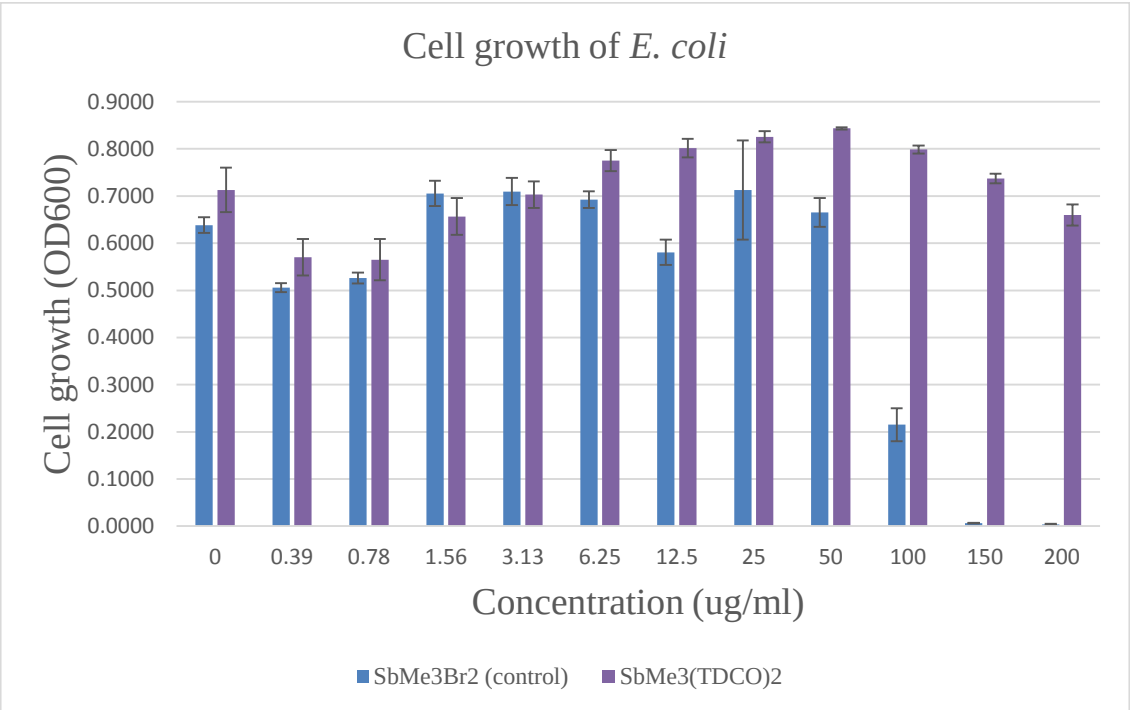

Chart 13. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3$(TDCO)$_2$ against *E. coli*.

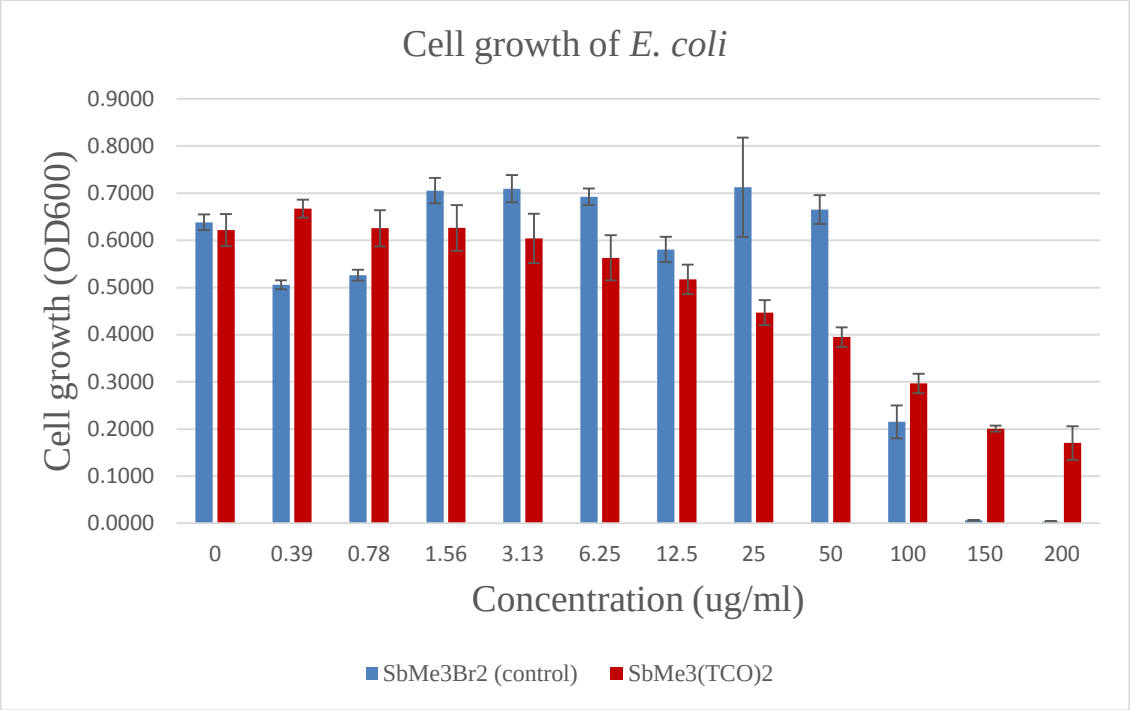

Chart 14. Results of MIC tests of $SbMe_3Br_2$, and $SbMe_3(TCO)_2$ against *E. coli*.

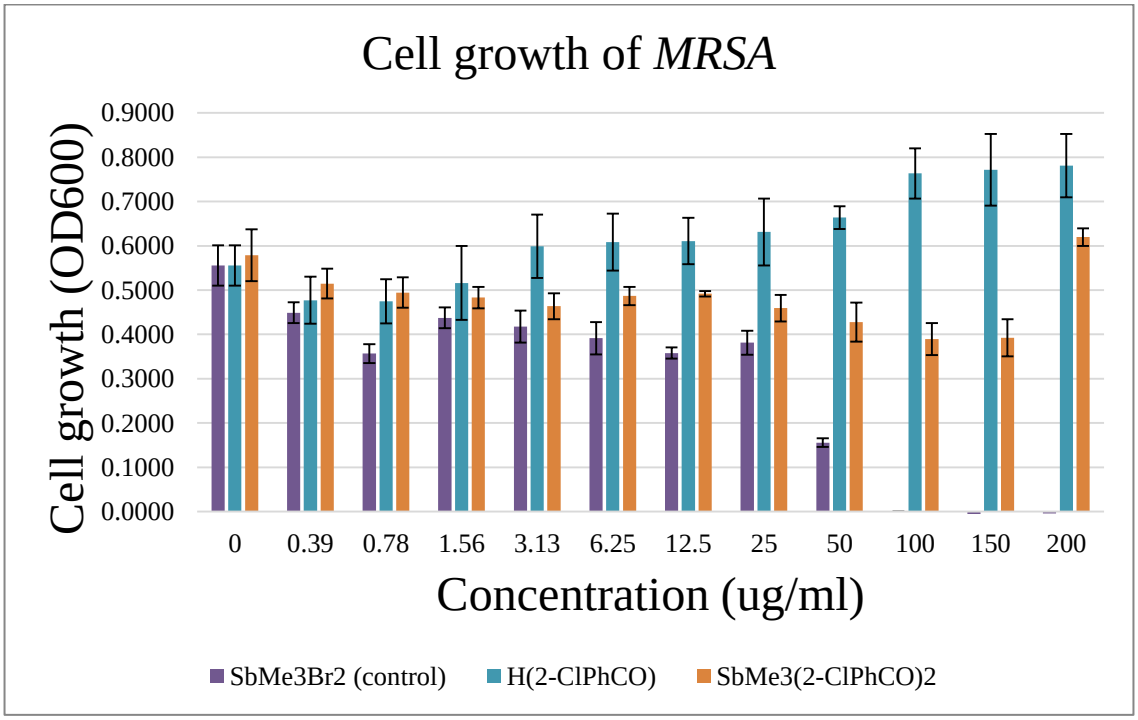

Chart 15. Results of MIC tests of $SbMe_3Br_2$, H(2-ClPhCO) and $SbMe_3(2\text{-}ClPhCO)_2$ against *MRSA*.

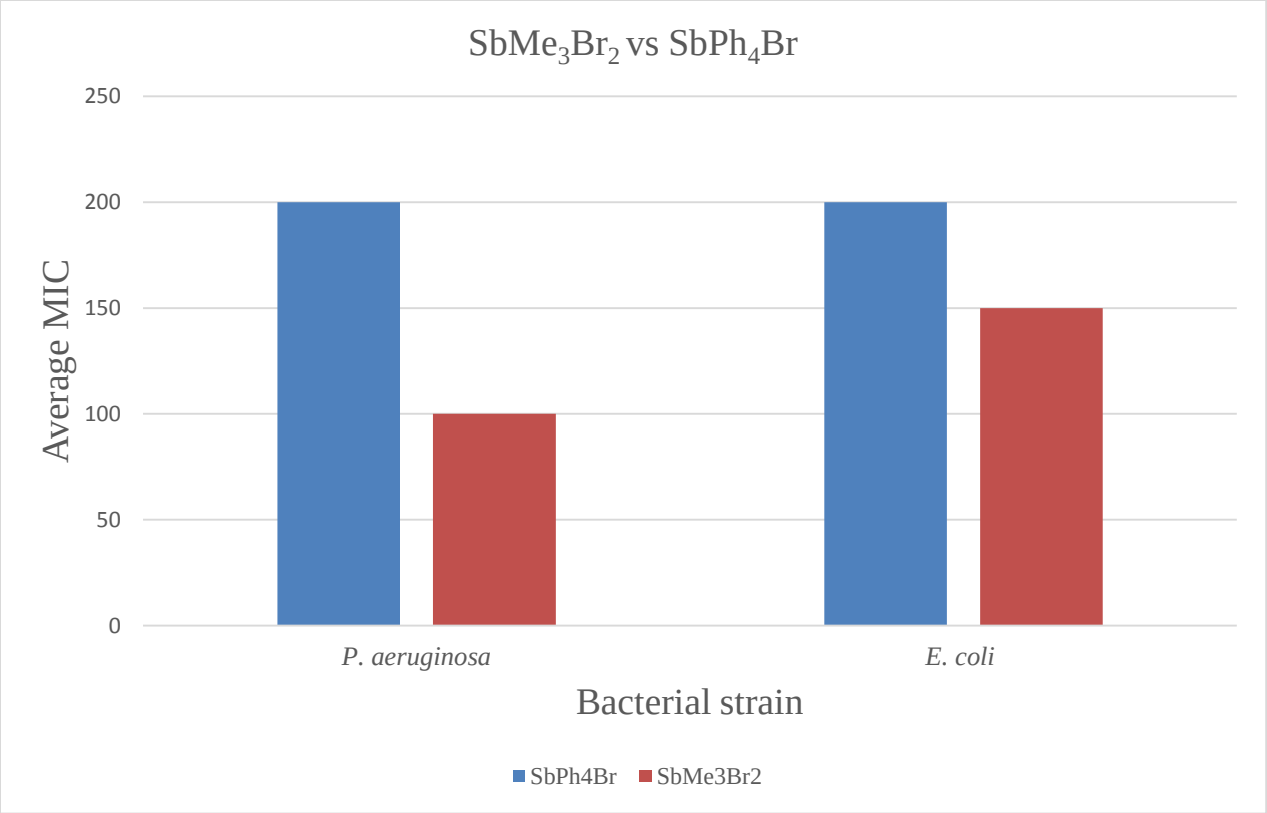

Chart 16. Comparison of the average MIC for both $SbMe_3Br_2$ and $SbPh_4Br$ against *E. coli and P. aeruginosa.*

Other activities: trips!

In October of 2019 we went as a group of graduate students to Bridal Cave and Ha-Ha-Tonka state parks.

January 2020: Oklahoma State University.

My graduate students working on organoantimony(V) project during trip to Oklahoma State University campus.

Group photo of MSU chemists and collaborative OSU microbiologists in Stillwater, January of 2020.

THE DAY! – Graduation!

My 14th graduate Master of Science student with his parents in Temple Hall on his graduation day in May of 2022.

Touring my research laboratory with Seth Adu family.