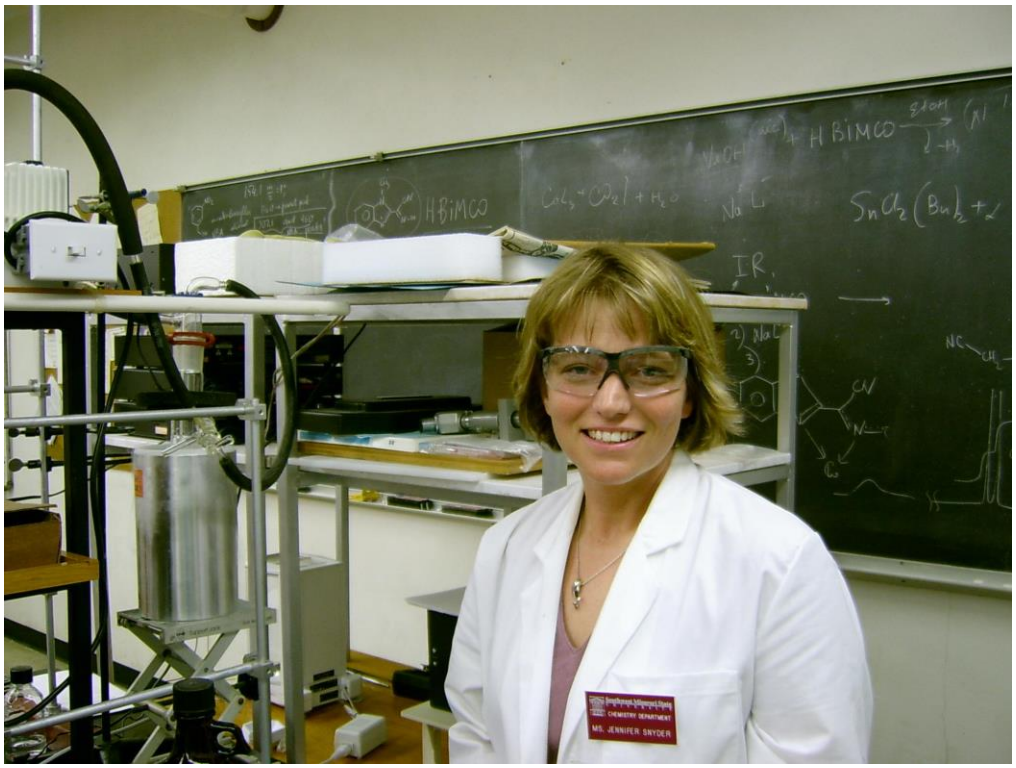


Jennifer Snyder: MS 2007

Thesis "Synthesis and Investigations of Several Dibutyltin(IV) Cyanoximates"

Jennifer's research project was dedicated to continuation of studies of *in vitro* cytotoxicity of several earlier reported by Tiffany Maher organotin(IV) cyanoximates (see Tiffany Maher section of this web page). She found in her work that compounds containing the acetamidecyanoxime anion, ACO-, showed very potent cytotoxicity. Jennyfer have seleceted 2 related cyanoximes that contain -NH_2 group and possess hydrophilic properties and able to form H-bonds, and 2 molecules that contain –$N(CH_3)_2$ hydrophobic group. Moreover, two out of four molecules were thioanalogs of the oxo-ligands (see below). Jennyfer Snyder stoichiometric Bu_2SnL_2 compounds (Bu = *n*-C_4H_9; L = deprotonated ACO^- , DCO^- and TCO^- , $TDCO^-$ anions shown below) were tested *in vitro* against HeLa (cervical) and colon (WiDR) human cancer cell lines using cisplatin as positive control. Studied complexes are comparable or surpass in several cases cytotoxicity of cisplatin, which warrants their further investigation.

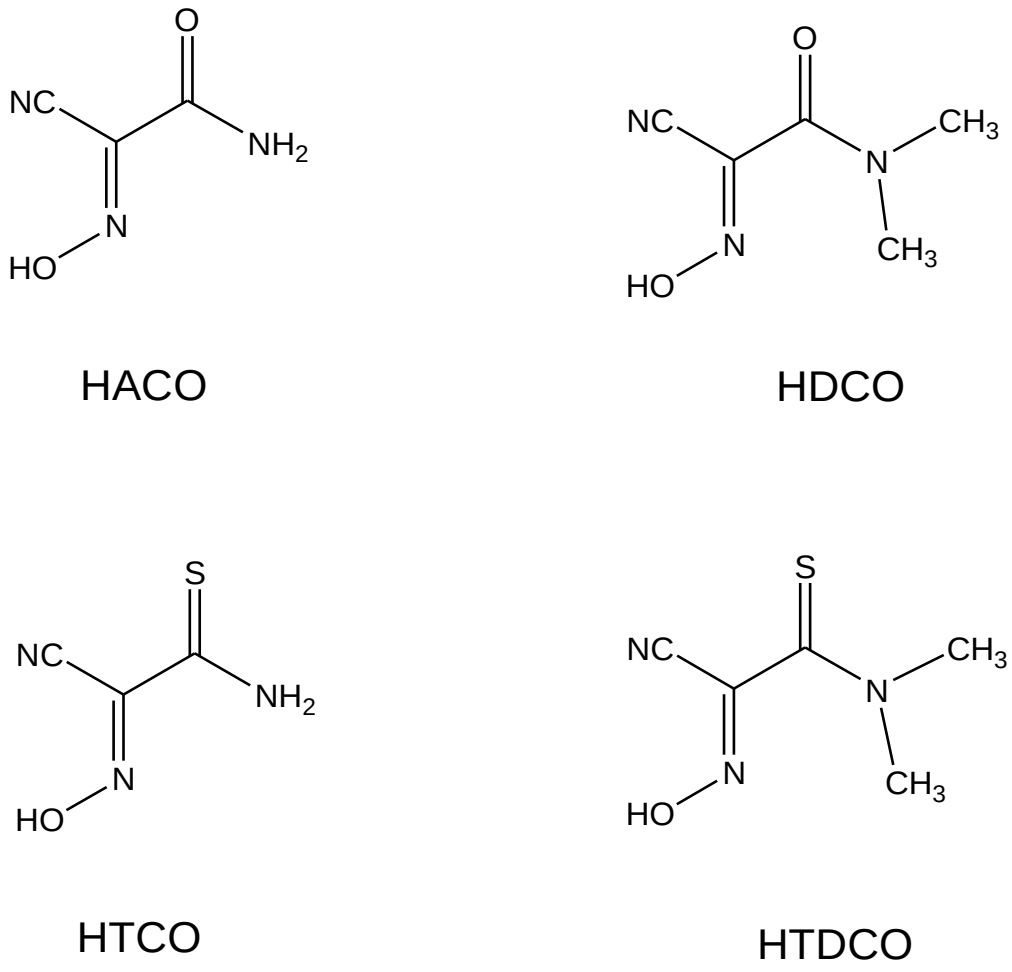


Figure 1. **Acetamide cyanoximes used in Jennifer Snyder research.**

Summary of known "structure – activity" relationship in a family of the organotin(IV) compounds is displayed below:

In organotin(IV) compounds toxicity changes with the alkyl chain:

$R_4Sn > R_3SnX > R_2SnX_2 > RSnX_3$

R = CH_3 very toxic; R = C_8H_{17} non-toxic;

X – halogen, or other groups directly bound to the metal center

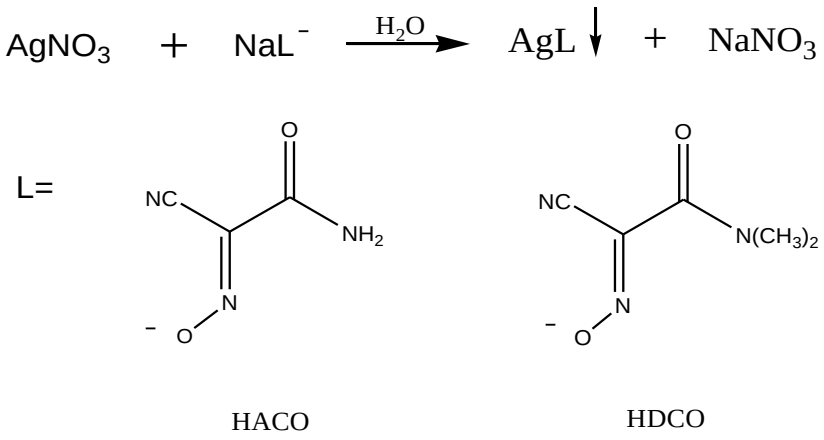
Several of these compounds were successfully used as paint additives to prevent attachment of ocean plants and animals to ships.

Over the past 50 years, various metal compounds have proved effective at combating cancer. However, many of the current products in use today for anti-tumor activity produce some severe side effects. Inorganic chemists have been evaluating various classes of compounds to improve upon these current treatments. Several publications indicate that tin (IV) compounds may be beneficial at reducing side effects and provide even greater activity against different types of cancer. This project was a continuation of research activity in the area of bio-inorganic chemistry of cyanoxime ligands and their metal complexes. Similarities exist between this class of compounds and other compounds that have been investigated for anti-tumor activity. This project examined four dibutyltin (IV) cyanoximates containing several different amidecyanoximes and investigated the effect that changing substituents will have upon anti-tumor activity.

Research involved the preparation and spectroscopic characterization of organic ligands and the synthesis of silver and thallium salts of the ligands. Dibutyltin (IV) compounds were synthesized via a metathesis reaction with the silver/thallium salts. The compounds were studied and characterized using modern spectroscopic and structural methods. Dibutyltin (IV) cyanoximates were investigated *in vitro* against WiDr and HeLa cell lines. All four complexes were found to be active at a 1μM concentration. Further, the activity appeared to be a function of whether the ligand contained a carbonyl or a thiocarbonyl, as opposed to its hydrophobicity.

Once again, shown in Figure 1 the four cyanoxime ligands share structural similarities, while varying in some of their other properties, such as hypophilicity and polarity. Two of the cyanoximes contain an amide functional group, while the other two contain thioamide groups. This should help determine whether the carbonyl group has a direct impact on the activity of the complex. It might also confirm whether sulfur is a biologically significant element that enhances the activity of the whole complex. The other major structural comparison involves the hydrophilicity of the ligands. Two of the ligands, HACO and HTCO, contain the primary amide-NH_2 groups in contrast to the tertiary hydrophobic moiety-$N(CH_3)_2$ in HDCO and HTDCO. This structural change should affect the ability to form H-bonds and the ability to transport the complex through the cell membrane.

A.



B.

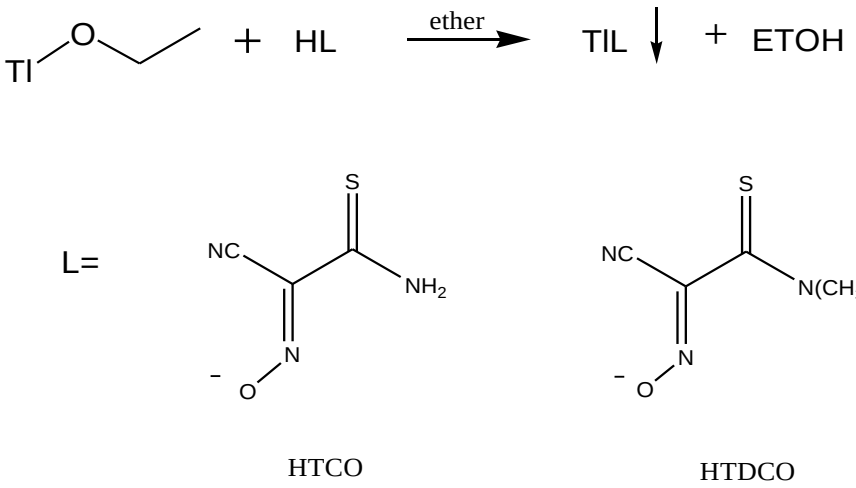


Figure 2. **Preparation of monovalent Ag (A) and Tl (B) compounds used in preparation of organotin(IV) complexes.**

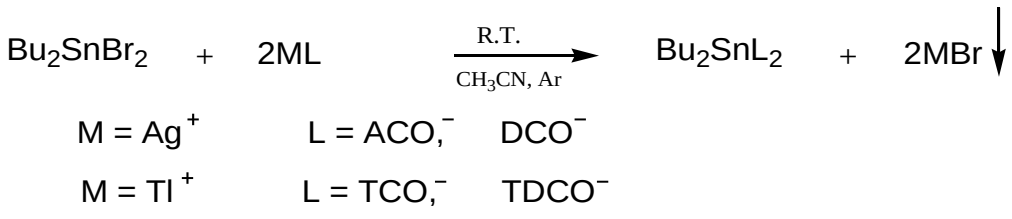
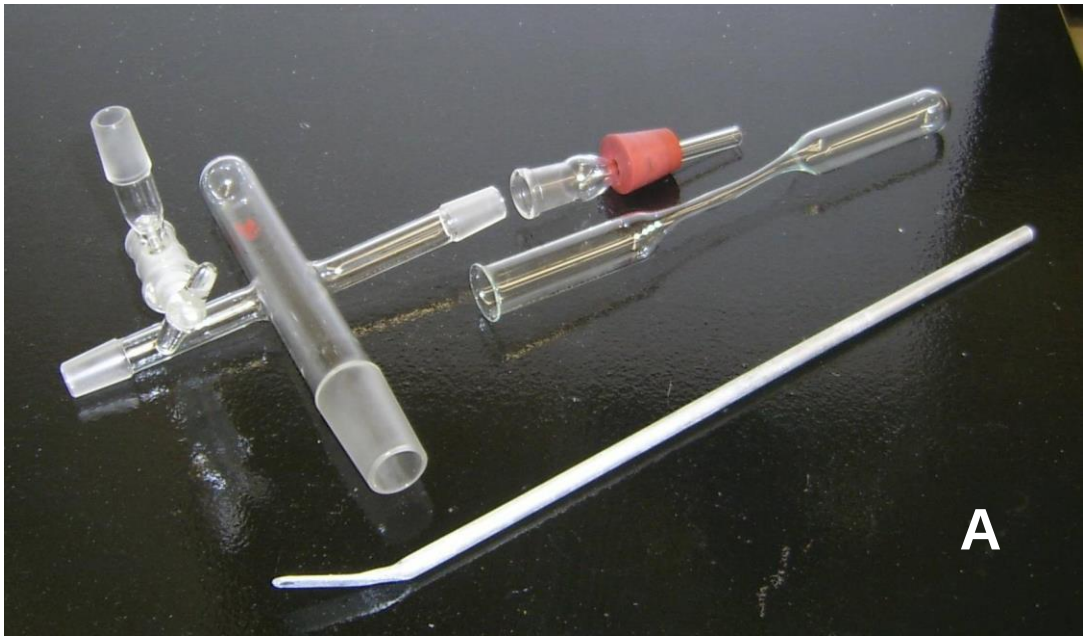


Figure 3. **Route to dibutyltin(IV) acetamidecyanoximes.**



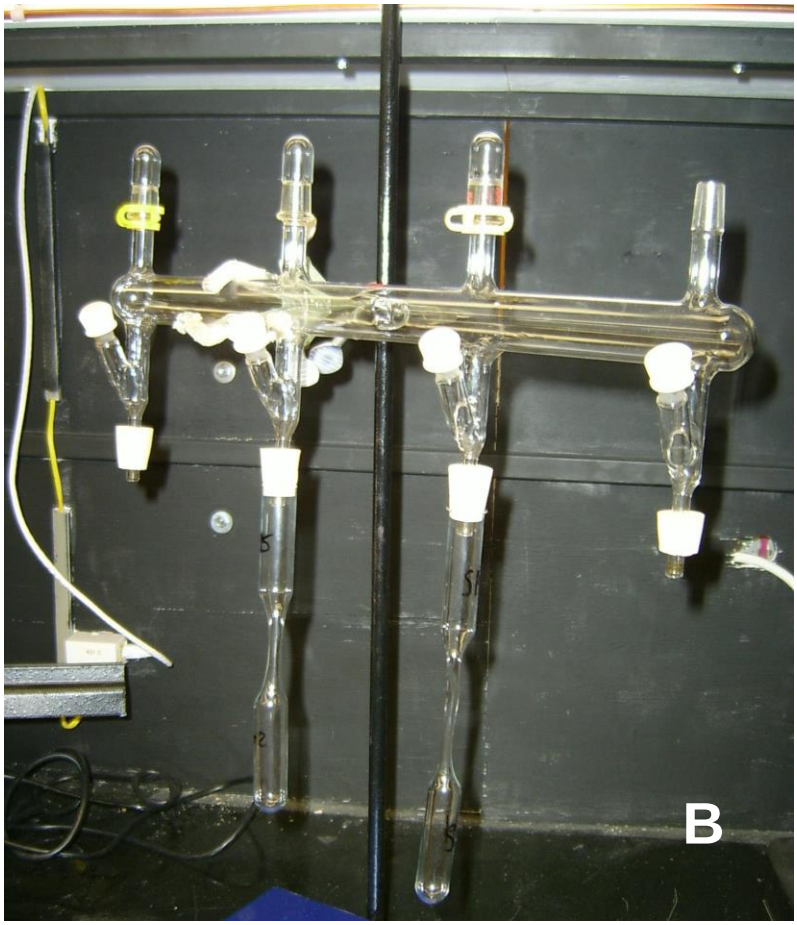


Figure 4. **(A) glassware for packing air-sensitive solids and (B) specially designed manifold for packing oily compounds into ampoules for further studies.**

Results of *in Vitro* cytotoxicity studies

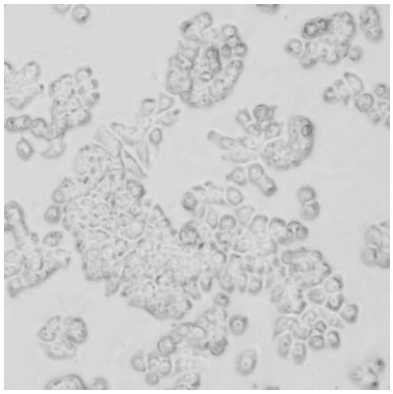
Four dibutyltin (IV) cyanoximates were synthesized and tested for anti-proliferative activity against WiDr and HeLa human cancer cell lines as described in III.5. This part of our work was carried out in direct cooperation with Mrs. J. Ratcliff. One objective of the study was to determine if there is a correlation between the structure of diorganotin (IV) cyanoximates and its activity. All four cyanoxime ligands have different degrees of lypophylicity and ability to form H-bonds that are important for the intracellular intake. This should affect the compound's transport into the cell and influence activity. With these limited structural differences, a relationship between efficacy and structure might be apparent.

In order to evaluate dibutyltin (IV) cyanoximate's stability towards hydrolysis in aqueous solutions, ampoules with compounds were sent to the University of Heidelberg Chemistry Department for studies in mass- spectrometric laboratory. Professor Andrew A. Mokhir and his group helped to work on this part of the project. Thus, ampoules with dibutyltin (IV) cyanoximates were opened and content (~ 15 – 30 mg of complexes) were dissolved in anhydrous CH_3CN. An electro-spray mass spectra was recorded from these solutions and were used as a baseline for further observations. Aqueous saline buffer with pH = 7.4 was added to the acetonitrile solutions and spectra were recorded again within 2 minutes after mixing, and 1 hour later. Results have indicated absence of hydrolysis of organotin cyanoximates within this period of time at T = 293 K. This finding was surprising since we did not expect complexes to be that stable in H_2O solutions. Therefore, all our conclusions regarding compound's integrity and stability during solution's preparation for biological studies remain correct. Complexes did not decompose within the time of their administration into the wells with living cancerous cells. Consequently, results of compound's cytotoxicity can be associated with their formulas. More detailed analysis of the obtained data will follow in a forthcoming publication.

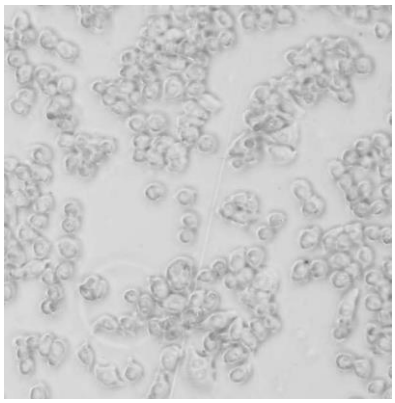
The cell studies were carried out in triplicate. The cells were plated one day and 24 hours later compounds were added while healthy cells were in an active growth phase. Cisplatin was used as a positive control, while untreated cells were also a control. The complex compounds were dissolved in DMSO to generate a stock solution to allow for a

variety of concentrations to be administered to the cells. The dibutyltin (IV) cyanoximates are moisture sensitive and were stored under argon in individual ampoules. Each ampoule was opened and used for one experiment, and then discarded. To make these solutions the ampoules were opened and content was mixed immediately, so compounds could be administered within 30-40 minutes of opening. The cells were incubated for another 24 hrs and then fixed, stained, washed and photographed. Representative pictures for both cell lines are shown below.

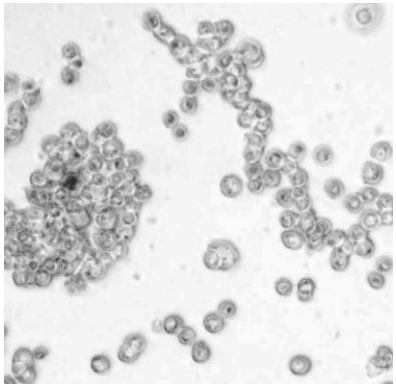
The results demonstrate a strong activity against both WiDr and HeLa cell lines at 1 μM concentrations for all of the dibutyltin (IV) cyanoximates as seen in Tables and Figures below. At higher concentrations, cell viability falls off steadily with 100% cell death occurring for three of the complex compounds at a 6 μM concentration.



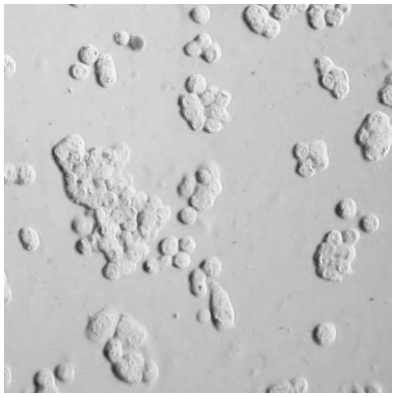
WiDr untreated cells



1µM cisplatin

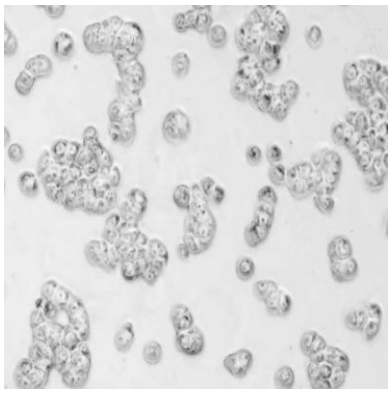


1µM $Bu_2Sn(ACO)_2$



1µM $Bu_2Sn(DCO)_2$

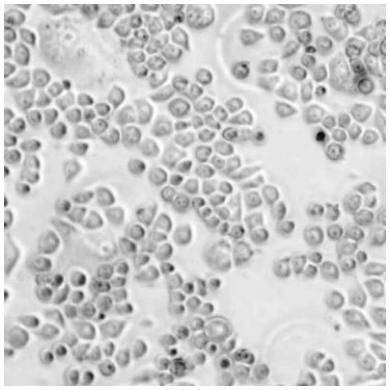
1µM $Bu_2Sn(TCO)_2$



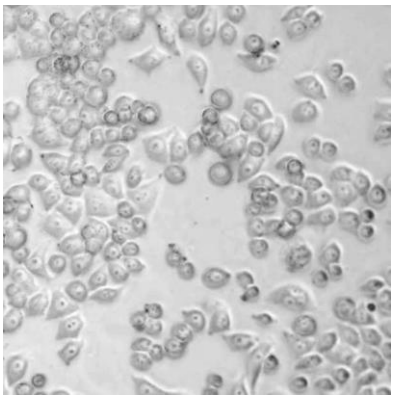
1µM $Bu_2Sn(TDCO)_2$

Figure 5 Representative pictures of WiDr cells

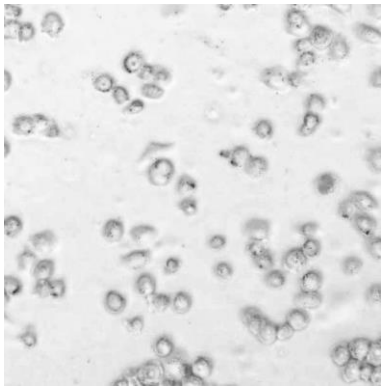
Cisplatin was used as a positive control.



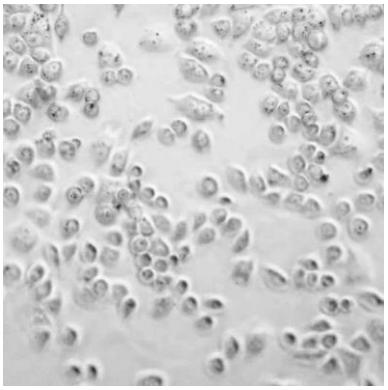
Hela untreated cells



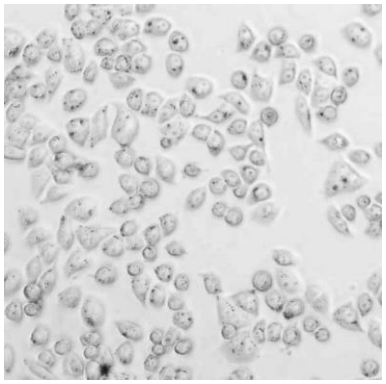
1 μM Cisplatin



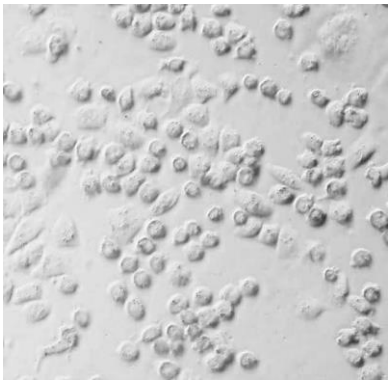
1μM $Bu_2Sn(ACO)_2$



1μM $Bu_2Sn(DCO)_2$



1μM $Bu_2Sn(TCO)_2$



1μM $Bu_2Sn(TDCO)_2$

Figure 6 Representative pictures of Hela cells

Cisplatin was used as a positive control.

Table 1 **Summary of WiDr cell viability**

Treatment	Live Cells	Trypan Blue	% Viable Cells	% dead cells
Untreated	1230	5	100%	0%
Cisplatin	815	415	69%	31%
$Bu_2Sn(ACO)_2$	795	435	59%	41%
$Bu_2Sn(DCO)_2$	764	466	56%	44%
$Bu_2Sn(TCO)_2$	563	667	46%	54%
Bu2Sn(TDCO)2	666	564	57%	43%

Table 2 **Summary of Hela cell viability**

Treatment	Live Cells	Trypan Blue	% Viable Cells	% dead cells
Untreated	1073	5	100%	0%
Cisplatin	845	228	79%	21%
$Bu_2Sn(ACO)_2$	405	668	38%	62%
$Bu_2Sn(DCO)_2$	578	495	54%	46%
$Bu_2Sn(TCO)_2$	632	441	59%	41%
$Bu_2Sn(TDCO)_2$	686	387	64%	36%

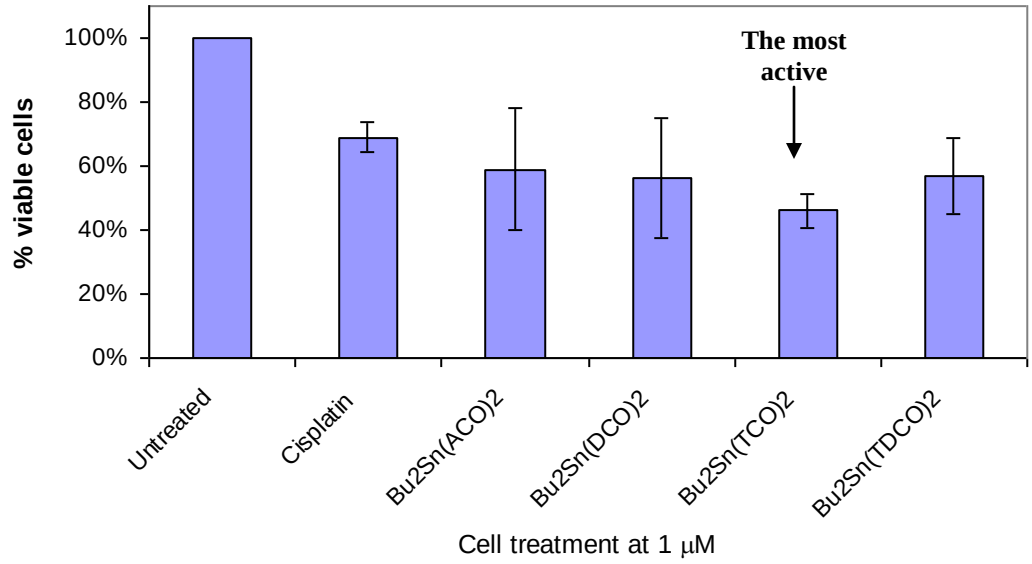


Figure 7 Results of *in vitro* studies using WiDr cell line. Data is reported as mean +/- standard error of the mean for three independent experiments performed in triplicate.

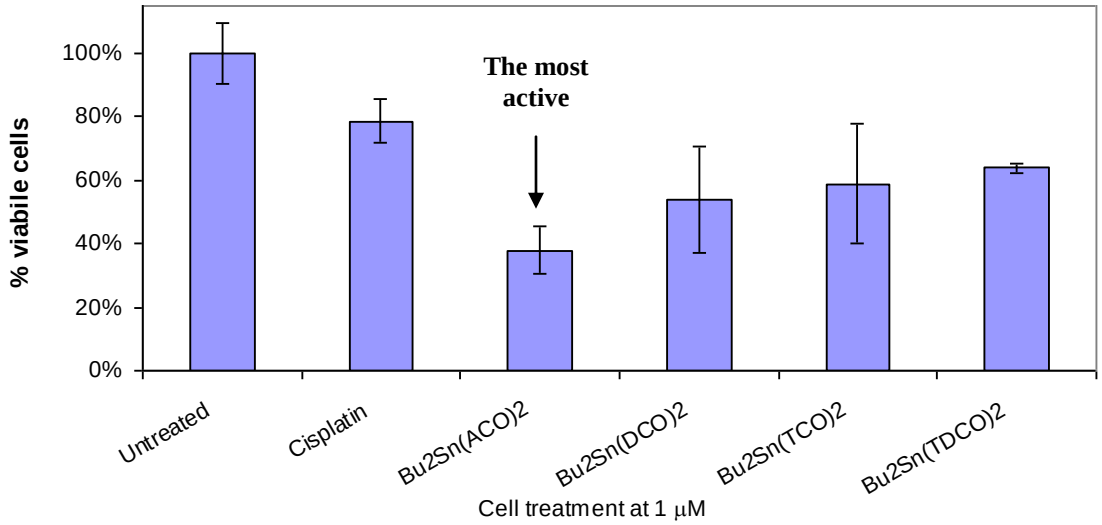


Figure 8 Results of *in vitro* cytotoxicity studies using HeLa cell line. Data is reported as mean +/- standard error of the mean for three independent experiments performed in triplicate.

Other pertinent data of biological activity studies can be found in Jennifer Snyder MS thesis located in the Meyer Library of Missouri State University.

CONCLUSIONS

As a result of these investigations, the following have been achieved:

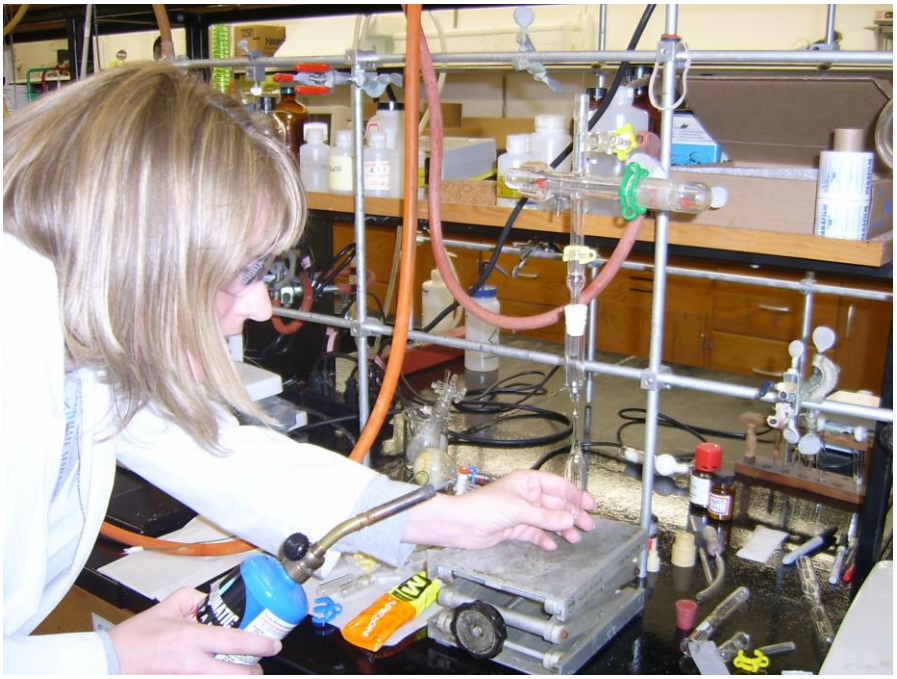
1. Four cyanoxime ligands were synthesized using the corresponding acetamide and acidic conditions. Spectroscopic methods, including IR, NMR, and UV-visible, were employed to characterize ligands.
2. Variable temperature NMR was used to determine if HTDCO would exhibit free rotation around the C-N amide bond. It was found that HTDCO doesn't exhibit free rotation, but the presence of anti- and syn- isomers were established for the first time at room temperature.
3. Four dibutyltin (IV) cyanoximates were synthesized and tested *in vitro* against WiDr and HeLa human cell lines. $Bu_2Sn(ACO)_2$ was previously tested and found to have promising *in vitro* activity. This study confirmed those results and further investigated the relationship between activity and structure. It appears toxicity is more a function of whether the ligand is a thiolcarbonyl or carbonyl regardless of whether it contains the $–NH_2$ or $–N(CH_3)_2$ amide. Two of the dibutyltin(IV) cyanoximates, $Bu_2Sn(TCO)_2$ and $Bu_2Sn(ACO)_2$, should be further studied to determine what role the thiolcarbonyl and carbonyl have in the activity.

Work of Jennifer Snyder in my research group has resulted in a presentation at the national meetings of the American Chemical Society:

1. Maher, T., Snyder, J., Durham, P., Gerasimchuk, N.N. "New anticancer active bis-{organotin (IV)-cyanoximates}". Inorganic chemistry section, poster presentation (719); Spring 229 ACS Meeting, March 13-17th, 2005, San Diego, CA.

Graduation DAY!

Pictures from research laboratory, 2004-2006.



Work with air-/moisture sensitive compounds requires a lot of patience and a blow torch…

Pictures from trips to Missouri Inorganic Day at MU, Columbia, 2004 (above), and Regional Midwest ACS Meeting, Manhattan, KS, 2004 (below).