Daniel Edings: MS 2003

Thesis "The Synthesis, Characterization, Spectroscopic, and Biological Activity Studies of Pt(II) and Pd(II) Cyanoximates."

The justification for conducted by Daniel investigation follows. It has been found that several compounds of platinum (II, IV) as well as palladium(II) are well-established as inorganic anticancer pharmaceuticals (see description below). At the same time substantial biological activity (growth regulating, antimicrobial, anticancer, pesticide detoxifying) has also been demonstrated during the last two decades for the cyanoximes - compounds having general formula NC-C(R)=NOH, where R is an electron withdrawing group. Therefore, studies are underway that are directed towards combining the useful properties of both classes of compounds in order to create a new group of antitumor agents that may have lesser side effects and general toxicity and better solubility than cisplatin family of metal-based anticancer drugs.

Cisplatin: History and Mechanism of Action. Cis-$[PtCl_2(NH_3)_2]$ commonly known as cisplatin is a very successful antitumor drug. It is most commonly used in the

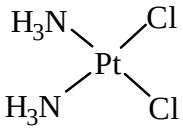
treatment of testicular and ovarian cancer, head and neck tumors, cervical, bladder and small cell lung cancer. Cisplatin was first synthesized by Michel Peyrone in 1847. Its powerful cytotoxicity against *E. Coli* was discovered accidentally by B. Rosenberg in 1964. $Pt(NH_3)_2Cl_2$ was adopted for clinical use in 1979. Cisplatin exerts its anticancer effects by binding to DNA inducing apoptosis (programmed cell death) described previously. The mechanism of cisplatin has been firmly established. Thus, after intravenous administration of cisplatin, the compound encountered a high concentration of chloride in the extra cellular fluid. This high concentration of chloride limits the replacement of chloride on cisplatin by water molecules. However, after cisplatin crosses the plasma membrane (due to passive diffusion) and enters the cell, the chloride ion of cisplatin is replaced by a water molecule as the result of the anation reaction. The monoaquated platinum compound then binds to the DNA. Cisplatin reacts with nucleophilic sites on the DNA forming monoadducts as well as intra- and interstrand crosslinks. The N7 atoms of the imidazole rings of the guanine and adenine located in the major grove of the double helix are the most accessible nucleophilic sites for platinum binding to the DNA. This binding causes distortion of the DNA helix, leading to a damage repair pathway or apoptosis.

Cisplatin: Drawbacks. Although cisplatin has become a successful anticancer drug, it also has several drawbacks, as was mentioned earlier. Cisplatin can cause severe nausea, vomiting, nephrotoxicity, ototoxicity, neurophathy and myelosuppression. Because of the toxicity of cisplatin, the dose administered is limited to a maximum of 200 mg/m^2 per course. However, the typical dose is 100 mg of cisplatin for five consecutive days. The adverse side affects of cisplatin treatment are noticeable with a single dose greater than 50 mg/m^2.

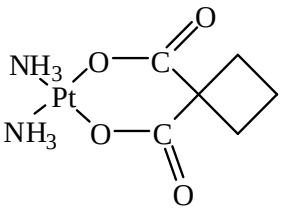
Tumor resistance is associated with the use of cisplatin. One possible cause of tumor resistance is that some tumors were intrinsically insensitive to cisplatin treatment. For those tumors that are at one point affected by cisplatin they can become cisplatin resistant due to three factors: 1) decreased platinum uptake; 2) increased intracellular detoxification; 3) enhanced DNA repair. It is known that only 5-15% of cisplatin is DNA bound where 75-85 % of the drug is protein bound. Cisplatin has a high affinity for proteins found in the blood plasma, with increased attention to plasmas containing a thiol

group such as human serum albumin and the amino acid cysteine. The most common repair pathway for DNA treated with cisplatin is nucleotide excision repair (NER). Increased NER in cisplatin-resistant cell lines has been shown to occur in both intra- and interstrand cisplatin – DNA adducts. This repair pathway is used to repair significant distortions of the DNA helix caused by cisplatin. NER is a complicated repair pathway that uses DNA endonuclease, which makes an incision in the DNA several nucleotides to each side of the DNA damage. This fragment is extracted and filled by DNA polymerase and covalently sealed by DNA ligase.

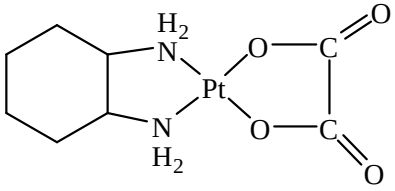
The structure and chemical reactivity pathway of cisplatin in the cell makes it a very effective compound in tumor treatment. The development of second generation platinum drugs utilizes similar structural elements that are defined by the following characteristics. 1) The compound should have two amine groups with cis- geometry as well as two leaving groups with cis- geometry. 2) The leaving groups should be only moderately prone to aquation. 3) The compound should also be neutral. 4) Lastly, when designing a new platinum drug for cancer treatment, a compound with fewer substituents on the amine ligand possesses greater activity and at least one substituent on the amine should be hydrogen. All these empirical observations guided *in vitro* cytotoxicity screening of thousands of novel Pd / Pt complexes.



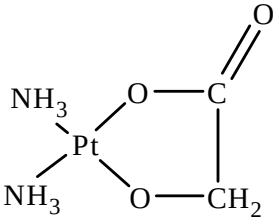
Cisplatin



Carboplatin



Oxaliplatin



Nedaplatin

The cisplatin family of anticancer drugs.

In Daniel Eddings work a series of five cyanoximes (compounds having the general formula NC-C(=NOH)-R, where R is an amide or carboxylic ester groups) have been synthesized, spectroscopically and structurally characterized. These are 2-cyano-2-isonitrosoacetamide (later **HACO**), 2-cyano-2-isonitrosothioacetamide (**HTCO**), 2-cyano-2-isonitrosoethylacetate (**HECO**), 2-cyano-2-isonitroso-N-piperidinylacetamide (**HPiPCO**) and 2-cyano-2-isonitroso-N-morpholinylacetamide (**HMCO**) (Figure 2). A high yield method of synthesis was developed for the last two previously unknown amidocyanoximes (Figure 1). Crystal structures of the latter two ligands were determined (Figure 3). Variable temperature ^{13}C NMR studies in dmso-d_6 solutions allowed the determination of rotational energy barriers for these two new cyanoximes. The **HPiPCO** and **HMCO** oxime molecules adopt *trans-anti* configuration in a solid state according to x-ray analysis. Reactions between aqueous solutions of K^+L^- (L = cyanoximate anions: **TCO**$^-$, **PiPCO**$^-$ and **MCO**$^-$) and $K_2[MCl_4]$ (M=Pd, Pt) resulted in the formation of ML_2 complexes. Crystal structure of $Pd(MCO)_2 \cdot DMSO$ was determined and showed the formation of a coplanar dimeric $[Pd(MCO)]_2$ units with 3.13 Å Pd---Pd separation (Figure 4). Complex adopts *cis*-geometry with anions being in *nitroso*- form. In the presence of bivalent Pd and Pt, **ACO**$^-$ and **ECO**$^-$ anions completely or partially hydrolyze in aqueous solutions to the dianion of 2-cyano-2-isonitrosoacetic acid (**AACO**$^{2-}$). The crystal structure of the product of the hydrolysis reaction, $K_2[Pd(AACO)_2] \cdot 4H_2O$ was determined. Data revealed planar and *cis*- geometry of the $[Pd(AACO)_2]^{2-}$ anion where cyanoximes are in the *nitroso*- form and adopt a *cis-anti* configuration (Figure 5). All synthesized cyanoxime ligands and nine their Pd(II) and Pt(II) complexes were tested *in vitro* on anti-proliferating activity using human cervical cancer HeLa cell lines, and cisplatin as a positive control substance. The two out of nine studied complexes, $Pd(MCO)_2$ and Pt(MCO), were found to be active compounds inflicting death on 28% and 16% of the cells respectively with 55% value for the cisplatin at the same conditions.

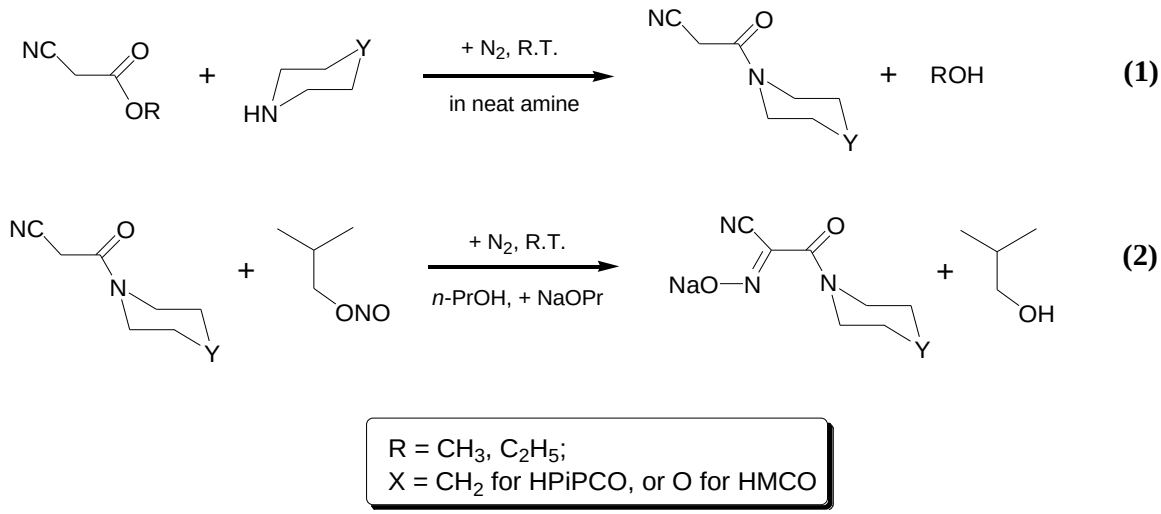
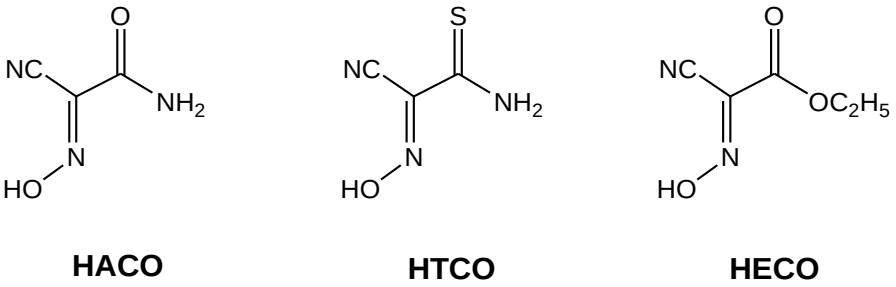


Figure 1. **Route to the 2-cyano-2-isonitroso acetamides: 1 – nucleophilic substitution in cyanoacetic esters, and 2 – nitrosation of substituted cyanacetamides at basic conditions.**



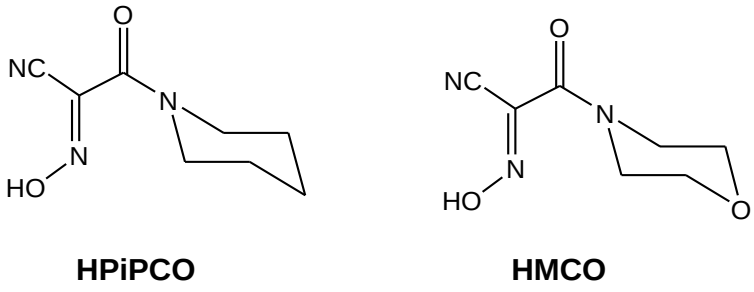


Figure 2. **The list of cyanoxime ligands that were synthesized by Daniel Eddings in his research.**

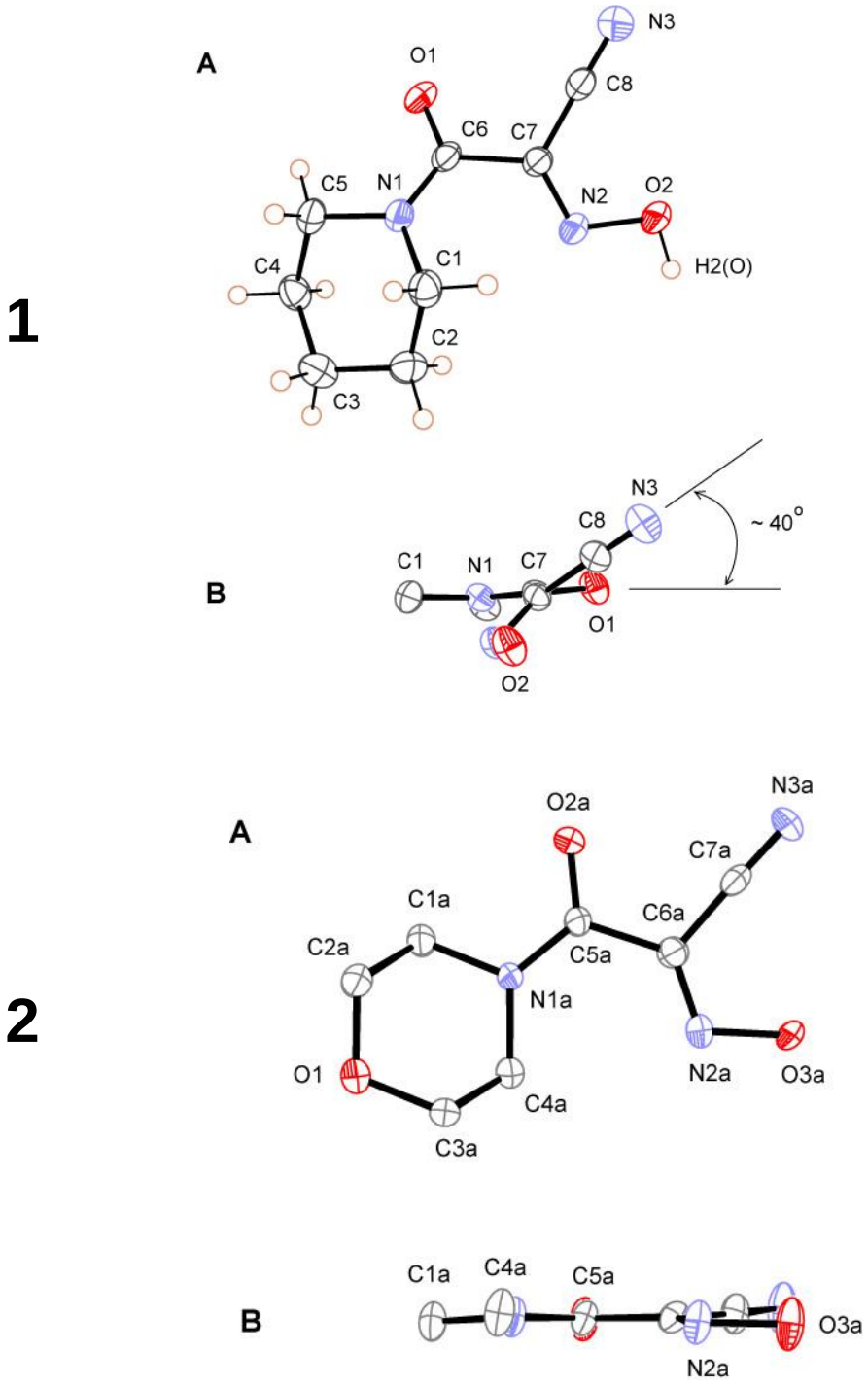


Figure 3. **Crystal structures of two new cyanoximes HPiPCO (1, top and side views) and HMCO (2, top and side views)**

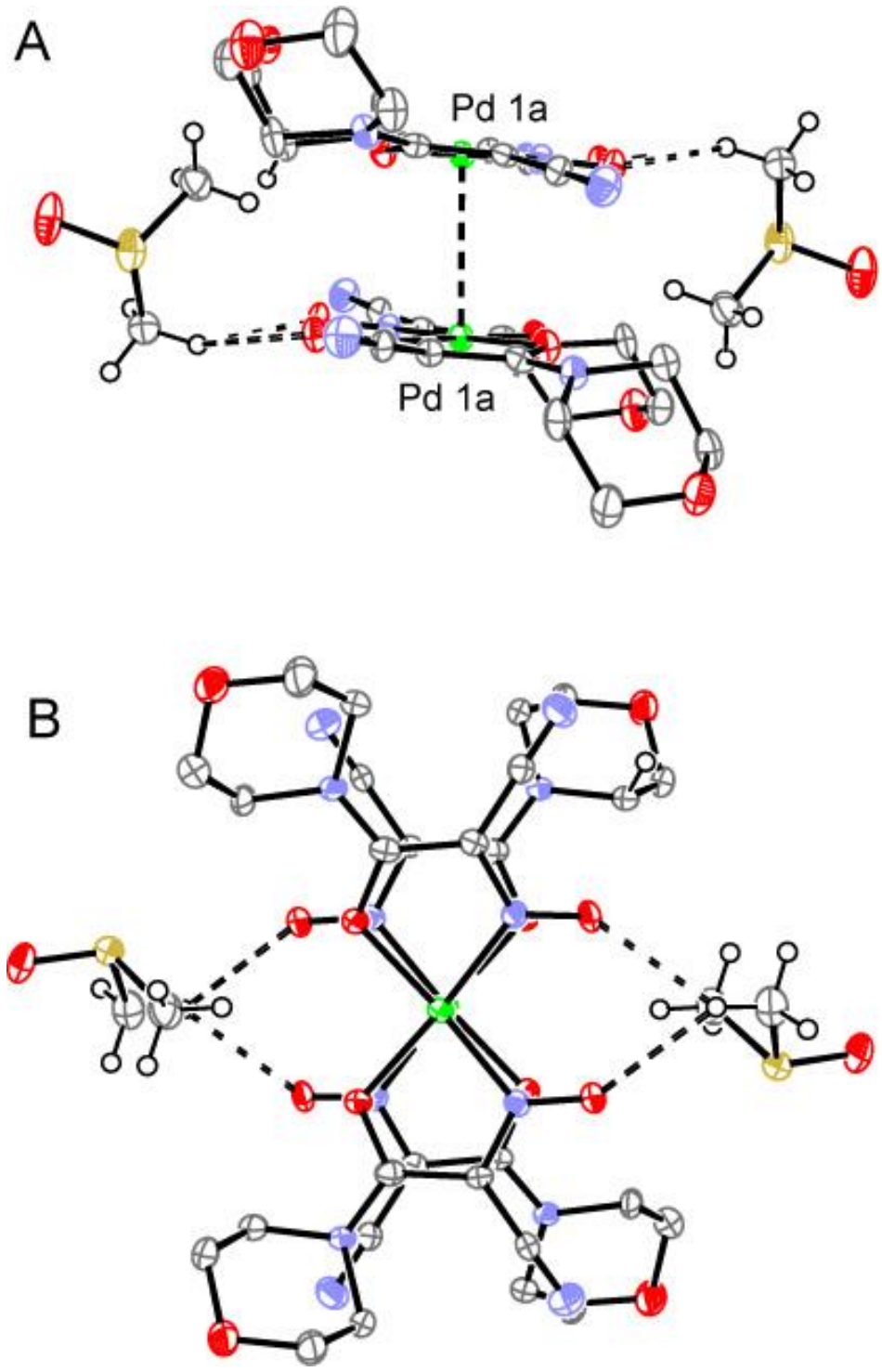
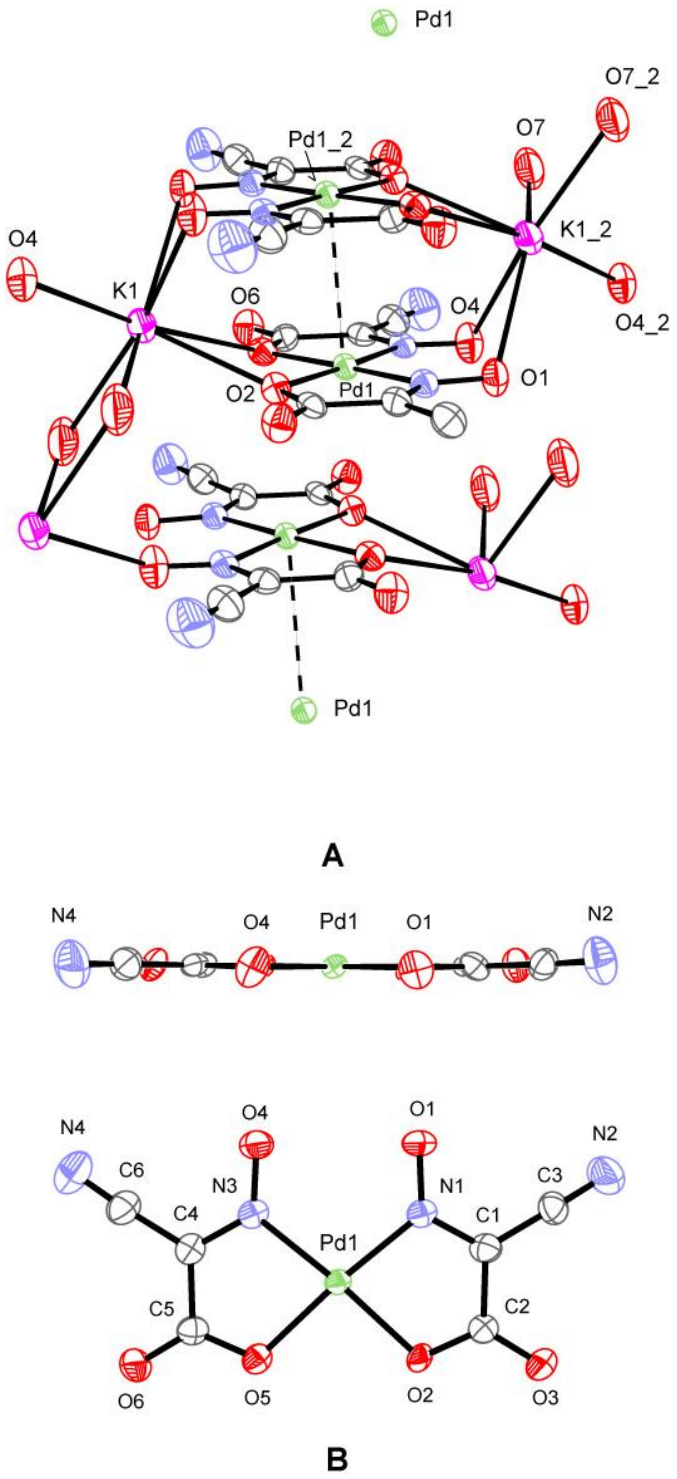


Figure 4. **Molecular structure of biologically active centrosymmetric dimer $Pd(MCO)_2]_2$, DMSO solvate. Coloring scheme: Pd – green, O – red, N – blue, C –grey. A – side view emphasizing record short Pd---Pd distance of 3.13 Å; B – top view.**



1

2

Figure 5. Structure of non-cytotoxic $K_2[Pd(AACO)_2]$ $2H_2O$. Fragment of crystal packing (1). The ligand AACO is a product of hydrolysis of ACO^- anion: the $–C(O)NH_2$ amide group was transformed into the carboxylic group $–C(O)O^-$ Coloring scheme: Pd – green, O – red, N – blue, C –grey; Molecular structure of the transition metal part (2): A – side view, B – top view.

Work of Daniel Eddings in my research group has resulted in one major publication:

Eddings, D., Barnes, C., Durham, P., Gerasimchuk, N.N., Domasevich, K.V. "First bivalent palladuim and platinum cyanoximates: synthesis, characterization and biological activity." *Inorganic Chemistry*, **2004**, *43*, N°13, pp. 3894-3909.

and in 5 presentations at the regional, national meetings of the American Chemical Society and International Conference on Coordination Chemistry (ICCC-36):

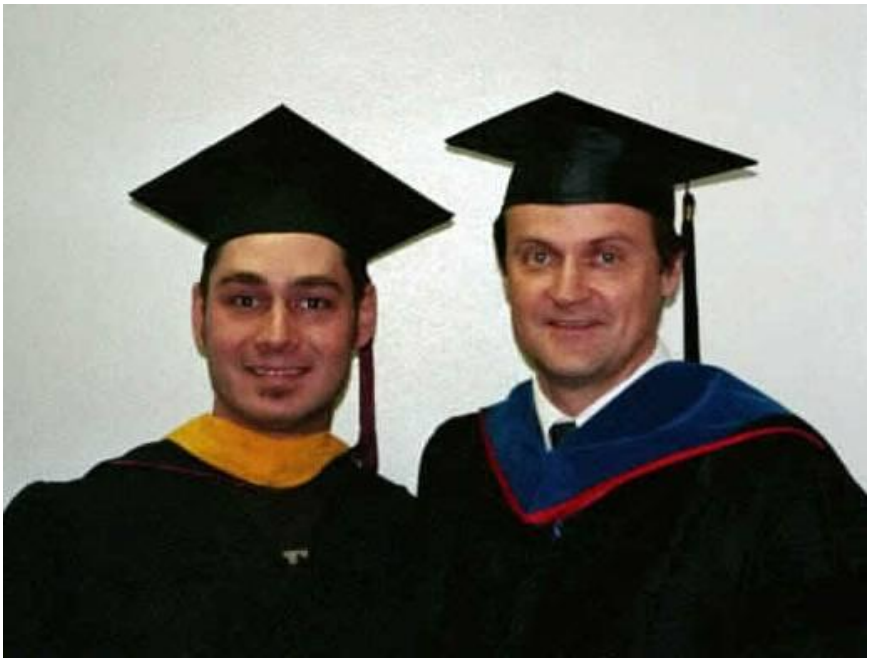
1. Gerasimchuk, N., Durham, P., Eddings, D. "First biologically active Pd(II) and Pt(II) cyanoximates." Proceedings of 39th Midwest Regional Meeting of the ACS; p. 291.

2. Gerasimchuk, N.; Durham, P.; Goeden, L.*; Abbey, M.; Bowen, E.; Eddings, D. "Synthesis, characterization and anticancer activity studies of several Pd(II) and Pt(II) cyanoximates." Proceedings of 40th Midwest Regional Meeting of the ACS; p.52.

3. Eddings, D., Gerasimchuk, N.N. "Synthesis, spectroscopic, structural studies and activity of novel Pd(II) and Pt(II) cyanoximates". Bioinorganic chemistry section, presentation 132 (poster). Spring 226 ACS Meeting, March 23-29, 2003, New Orleans, LA.

4. Gerasimchuk, N.N., Durham, P., Eddings, D.* "Synthesis, spectroscopic characterization and biological activity of the first Pd(II) and Pt(II) cyanoximates". Inorganic chemistry section, poster presentation. Fall 228 ACS Meeting, August, 22-26; 2004, Philadelphia, PA.

5. Gerasimchuk, N., Durham, P., Eddings, D. "First Pd(II) and Pt(II) cyanoximates: synthesis, structures and biological activity". P. 475 in Proceedings of 36th International Coordination Chemistry Conference (ICCC-36); Merida, July 18-24th 2004; Mexico.



Graduation!