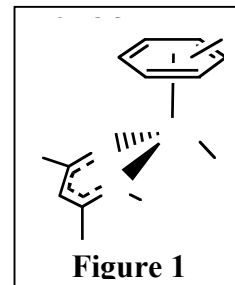


Cytotoxicity of Ruthenium Complexes Ruthenium(II) complexes of the type shown in

Figure 1 have been of increasing interest recently due to their cytotoxicity towards human cancer cells. These compounds have a “piano stool-type” structure in which the arene ligand forms the “seat” and the bidentate ligand and the chloride form the “legs”.

The electronic and steric properties of these compounds are remarkably easy to “tune”

because of the wide variety of arene and bidentate ligands that are synthetically accessible. The Sadler group has examined the ligand structure-cytotoxicity relationships for some of these compounds¹. Freedman has been studying similar Ru complexes, with the β -ketoiminate ligand shown in Fig. 1, for their ability to catalyze transfer hydrogenation (TH) reactions. As Freedman’s TH-Ru catalysts are structurally similar to Sadler’s cytotoxic compounds, Morrow performed preliminary analysis and determined the TH-Ru catalysts to be cytotoxic to a variety of cancer cell lines.



To further elucidate the structure- cytotoxicity relationships of the [(arene)Ru(β -ketoiminate)Cl] compounds, we propose to have a student study the cytotoxicity of a series of these compounds. As the β -ketoiminate ligands are readily synthesized with a wide variety of different substituents, we can readily examine the cytotoxicity of a variety of compounds to elucidate more subtle structural factors that contribute to the cytotoxicity. The student will first work with Morrow (Biology) to determine the cytotoxicity of the current collection of [(arene)Ru(β -ketoiminate)Cl] compounds by employing a standard SRB cytotoxicity assay² on the A549 ovarian cancer cell line. Based on the differences in cytotoxicity, the student will work with Freedman (Chemistry) to vary the R' group. The student will then study the effect of altered steric access to the metal center and hydrophobic or hydrophilic properties on cytotoxicity. The student will also work with Freedman to measure the equilibrium constant for chloride hydrolysis of the ruthenium complexes and the pKa of the resulting aqua complexes, as these reactions are likely to occur in the cell and can potentially be correlated with cytotoxicity. Additional cell lines will also be tested for sensitivity to these compounds.

1. Habtemariam, et al. (2006) *J. Med. Chem.* 49: 6858.

2. Vichai & Kirtikara (2006) *Nature Protocols* 1: 1112.

Optimization of α -Pinene derivative house fly attractancy The house fly (*Musca domestica*) is a widely distributed insect vector of a variety of devastating animal pathogens. Control strategies for this species utilize traps and/or poison stations baited with synthetic attractants. The high fecundity and short reproductive cycle of these insects rapidly lead to the development of resistance to poison baits; long-term control requires periodic reformulation of baits and insecticides³. This project will seek to develop and characterize new insect attractants and investigate the underlying effects of these substances on the behavior and physiology of *M. domestica*.

Certain terpenes are reported as strong insect attractants. α -Pinene is a rigid bicyclic monoterpene and is a major constituent of pine oil. Preliminary results using an olfactory repellometer developed by undergraduate students in Haselton's lab (Biology) have shown that both enantiomers of α -Pinene are attractive to *M. domestica*, the R enantiomer being a stronger attractant.

α -Pinene has a double bond that allows for a variety of electrophilic addition reactions and thus functional group transformations and skeletal changes⁴. α -Pinene may be modified to structurally resemble previously identified organic molecules with demonstrated effects on insect behavior. The student working in Dhar's lab (Chemistry) will synthesize a variety of α -Pinene derivatives. The insect attractancy of each of these molecules will be determined and compared by the student in Haselton's lab using repellometer assays. Furthermore, electrophysiological responses to synthesized volatile α -Pinene derivatives will be measured using electroantennogram assays in the Haselton lab. Information obtained from behavioral and physiological assays in the Haselton lab will be used to direct subsequent α -Pinene modifications in the Dhar lab. Assessing and optimizing the attractancy of α -Pinene derivatives will help lead to a better understanding of how the structure of α -pinene affects its attracting capacity, what effects these structures have on fly behavior and physiology, and possibly lead to the development of novel control tools.

3. J. Keiding (1999) *Bull. Entomol. Res.* 89:S7–S67.

4. Z. Szakonyi, T. et al. (2000) *Tetrahedron: Asymmetry* 11:4571-4579.

Determination of the binding constant from a nuclear receptor-ligand pair using fluorescence

Nuclear receptors (NRs) are a family of metazoan transcription factors that control an array of systemic processes via regulation of gene expression, and in turn are controlled via interactions with small lipophilic effector molecules referred to as ligands making them ideal targets for pharmaceutical intervention. This project will be part of a broader effort to identify and quantify the ligand interaction with proteins derived from non-human model organisms. Here we will probe the binding affinity between the *Ciona intestinalis* NR ciem834j15 (the closest *Ciona* homologue to the human estrogen receptor) and fluorescein labeled estradiol and subsequently probe the details of the interaction using site-directed mutagenesis. Binding affinity will indicate the physiological relevance and some details of the interaction between receptor and ligand. These studies will be critical for homology comparisons and structure-based drug design with this model organism.

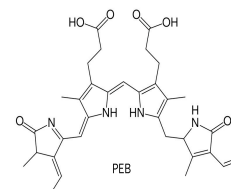
Using an existing expression vector containing the ciem834j15 ligand binding domain (LBD), the student will express, harvest and purify the protein to homogeneity in Reinking's lab (Biology) using standard molecular biology, affinity chromatography and other chromatographic techniques⁵. With purified protein in hand, the student will label estradiol with a fluorescent dye in St. John's lab (Chemistry). Briefly, this will be accomplished by selectively attaching fluorescein to one of the existing hydroxyl groups through an amide linkage⁶. The student will then measure the extent of binding between the labeled estradiol and protein by either monitoring the change in fluorescence intensity or the change in fluorescence polarization as a function of ligand concentration using a SPEX Fluorolog-3. From the binding curves, the baseline affinity between ciem834j15 LBD and fluorescein-estradiol will be obtained. Reinking and St. John will discuss these results with the student so as to design a plan for generating mutations within the putative ligand-binding pocket of ciem834j15 LBD that will be expected to change the binding affinity. The student will perform site-directed mutagenesis in Reinking's lab and determine the effect the mutations will have on the binding affinity in St. John's lab. Based on these results, additional mutations will be generated to determine the characteristics of the receptor-ligand interaction. This methodology will be later extended to other *Ciona* NRs as well.

5. Reinking, et al. (2005) *Cell* 122:195-207.

6. Parker, et al. (2000) *J. Biomol Screening* 5:77-88. Yu, et al. (2002) *Bioorg and Med Chem Letters* 12:1283.

Chemical characterization and activity of *Aplysia* tetrapyrroles on crustacean chemoreceptors.

Most species of marine snails in the genus *Aplysia* secrete a purple ink that acts as an active defense against predatory sea anemones, fish, crabs and lobsters.⁷ About 65% of the ink (by weight) is composed of r-phycoerythrin (PEB), which is derived from the photosynthetic biliprotein, r-phycoerythrin found in their red algal diet. Current work in Nolen's lab has demonstrated that low molecular weight ink fractions as well as commercially available, purified r-phycoerythrin,



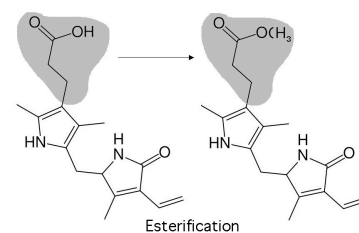
activate the amino acid chemoreceptors of sympatric predatory crabs. Other ink components, including amino acids and intrinsic proteins also activate chemoreceptors in lobsters.⁷

This project will address: (1) To what extent does purified PEB activate the dactyl chemoreceptors of crabs? (2) What is the nature of the active chemical components/structures of PEB? The student will work with both Nolen (Biology) and Andersen (Chemistry) to extract and purify PEB from the red seaweed *Gracillaria* sp.⁸ The student will work with Nolen to determine concentration activity curves and cross adaptation

interactions (with common amino-acid stimulants) of purified PEB using electrophysiological recording from the dactyl chemoreceptor axons of the crab, *Cancer antennarius*. The student will work with Andersen to modify

the tetrapyrrole structure of PEB and these products will be analyzed with NMR and/or spectrometry. Initially, we will examine whether a substitution of a

methyl ester on a carboxylic acid (shaded) will disrupt its activity as a stimulant, as it does for the natural stimulant glutamate. Nolen, Andersen and the student



will discuss the assay results and based on these discussions, the student will further modify the PEB or will synthesize structural/chemical analogs of the PEB tetrapyrrole. These new compounds will be assessed for electrophysiological activity on crab chemoreceptors Overall, we will begin to identify the chemical groups responsible for chemoreceptive "recognition" of *Aplysia* ink by crustaceans predators.

7. Kicklighter et al. (2005), *Current Biology* 15: 549-554.

8. Jian-Feng Niua, et al. (2006). *Protein Expression and Purification*. 49(1): 23-31.