Characterization of Selenazole Compounds as IDO Inhibitors

Timothy Foo[1,2], Tito S. Sempértegui Plaza[2], and Andrew C. Terentis[2]

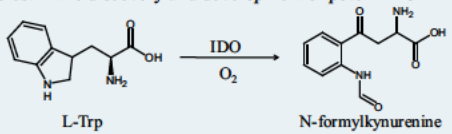
[1]NSF-Undergraduate Research & Mentoring Program

[2]Department of Chemistry & Biochemistry, Florida Atlantic University, Boca Raton, FL, USA

Introduction:

Indoleamine-2,3-dioxygenase (IDO) is a cytosolic, heme enzyme expressed by mammalian cells that performs regulatory roles within the immune system. IDO catalyzes the reaction between L-Trp and molecular oxygen to form N-formylkynurenine **(Figure 1).** Evidence suggests that diseases exploit IDO to gain tolerance against immune responses, as seen in cancer.[1] Inhibitors of IDO are emerging as a new target for cancer chemotherapeutics.[2] Thus, there is significant interest in the discovery and development of potent IDO inhibitors.

Figure 1:



A new class of IDO inhibitors has been discovered by the initial characterization of ebselen, a selenazole compound.[3] Ebselen binds to IDO's cysteine residues, which leads to structural changes within IDO that cause a decrease in catalytic activity.[3] Current stages in the development of selenazole inhibitors are to ascertain which chemical groups promote inhibition and what modifications of these chemical groups enhance inhibition. Four newly synthesized selenazole compounds have been chosen to undergo screening for IDO inhibitory effects.

The goals of this experiment are for each selenazole compound to:

1. **Obtain a dose-response curve**
2. **Obtain an apparent inhibition constant (K_i^{app})**
3. **Correlate a trend between selenazole structure and IDO inhibition**

This will allow the progress of identifying superlative IDO inhibitors that may lead to new disease therapeutics.

Methods:

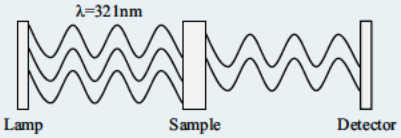
- To obtain IDO's catalytic activity (velocity of reaction) we spectroscopically measured the rate of formation of N-formylkynurenine from L-Trp, the reaction catalyzed by IDO, using a dual beam UV-visible spectrophotometer in time-scan mode set at a 321nm wavelength, the λ_{max} of N-formylkynurenine **(Figure 2)**
- This was conducted in the well-established *methylene blue ascorbate assay*, which consists of the following: 50nM of IDO, 25μM of methylene blue, 250μg/mL of catalase, 10mM of sodium ascorbate, 50μM of tryptophan and 100mM of potassium phosphate buffer (pH 7.4)[4]
- We chose 12 selenazole concentrations within the range of 8μM-4nM
- Experimental procedure:

IDO was pre-incubated with each selenazole at a specific ratio for 1 hour to reach completion of reaction → This solution was then added to the substrate (tryptophan) and the cofactors (methylene blue, catalase, ascorbate) → This solution was placed in a spectrophotometer and the activity was measured

- The fractional velocities (v_i/v_o) and selenazole concentrations, [EB], were fitted to the *Morrison equation* (eq. 1), which models tight binding inhibitors, to determine the apparent inhibition constant (K_i^{app}) of each selenazole[5]

$$\frac{v_i}{v_o} = 1 - \frac{[IDO] + [EB] + K_i^{app} - \sqrt{([IDO]+[EB]+K_i^{app})^2 - 4[IDO]\cdot[EB]}}{2[IDO]} \quad \text{eq. 1}$$

Experimental Schematic:



$$v = \frac{d[\text{N-formylkynurenine}]}{dt} \propto \frac{dA_{321nm}}{dt}$$

Figure 2: The spectrophotometric measurement of absorbance at 321nm (A_{321nm}) as a function of time to obtain the velocity of the IDO catalyzed reaction.

Results:

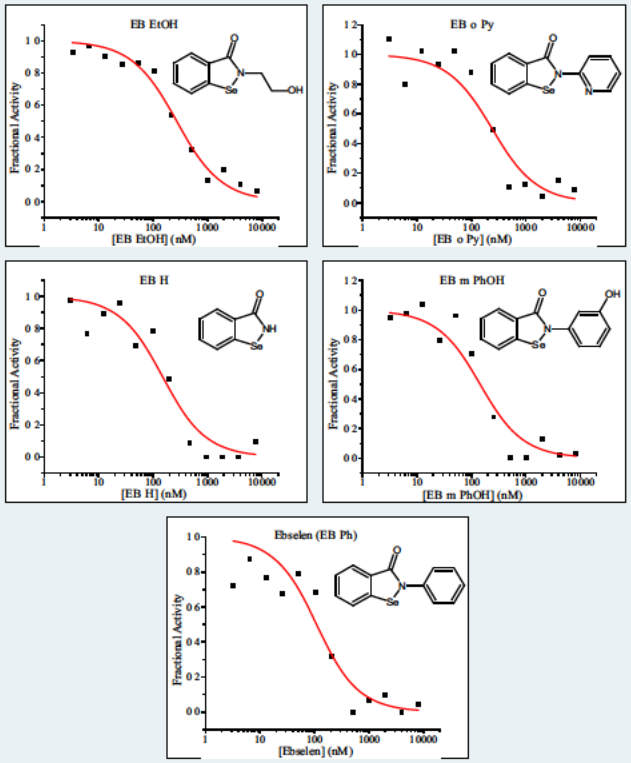


Figure 3: A dose-response plot of IDO fractional activity (v_i/v_o) was obtained for each selenazole compound (inset in top right of graph). The rate of formation of N-formylkynurenine was measured spectroscopically following a 1 hour pretreatment with varying concentrations of each selenazole. The fitted curve (red) was generated by a nonlinear least-squares fitting routine using the Morrison equation and was performed using OriginPro 8.1 graphing software.

Results:

Compound	Inhibition Constant K_i^{app} (nM)	Standard Error (nM)	Substituent
EB-EtOH	246	32	
EB-o-Py	215	63	
EB-H	125	33	-H
EB-m-PhOH	120	33	
Ebselen (EB-Ph)	85	28	

Table 1: For each selenazole compound, the inhibition constant and the respective standard error was obtained by a nonlinear least-squares fitting routine using the Morrison equation (n 1). The differential substituent of each compound is shown. Key of abbreviations: EtOH ethanol, o-Py ortho-pyridine, H hydrogen, m-PhOH meta-phenol and Ph phenyl.

Discussion:

- All of the selenazole compounds showed inhibitory effects on IDO by decreasing IDO's catalytic activity
- Ebselen had the most inhibitory effects on IDO, the observed K_i^{app} in this experiment was within the range of our previously reported value of 94±17 nM, which validates our current experimental protocol[3]
- Although the observed trend in K_i^{app} does not attest to correlate with specific structural characteristics within each selenazole, this experiment establishes the foundation for future IDO-selenazole kinetic studies
- Development of identifying superlative IDO inhibitors from our current selenazole compounds will be addressed in further studies, which include UV-visible spectroscopy, kinetic assays, circular dichroism, mass spectrometry and resonance Raman spectroscopy
- IDO inhibitors that show significant potential can be screened in vivo to develop novel disease therapeutics

References:

1. A. J. Muller, J. B. DuHadaway, P. S. Donover, E. Sutanto-Ward, G. C. Prendergast, *Nat Med* **2005**, *11*, 312-319.
2. A. J. Muller, G. C. Prendergast, *Curr Cancer Drug Targets* **2007**, *7*, 31-40.
3. A. C. Terentis, M. Freewan, T. S. Sempertegui Plaza, M. J. Raftery, R. Stocker, S. R. Thomas, *Biochemistry* **2010**, *49*, 591-600.
4. M. Sono, T. Taniguchi, Y. Watanabe, O. Hayaishi, *J Biol Chem* **1980**, *255*, 1339-1345.
5. J. F. Morrison, *Biochimica et Biophysica Acta (BBA) - Enzymology* **1969**, *185*, 269.

Acknowledgements:

- FAU Office of the Dean of Undergraduate Studies – Undergraduate Research Grant
- National Science Foundation – NSF-URM Research Fund
- American Chemical Society – Petroleum Research Fund
- Dr. Shane R. Thomas • Dr. Govindasamy Mugesh • Dr. Krishna P. Bhabak