Investigations into the Pharmacological Impact of Mono- or Di-methoxy Substitutions on Chalcones in the Model Organism *Caenorhabditis elegans*

Curtis Plug[a], Dr. Nabyl Merbouh[b], Dr. Nathan Bialas[a]

a - Department of Biology, University of the Fraser Valley
b - Department of Chemistry, Simon Fraser University

Introduction

- Chalcones are a class of organic compounds, many of which can be derived from plants[1]
- Chalcones can have varied biological effects, ranging from lifespan extension to cytotoxicity[2,3]
- Multiple chalcones have been shown to possess antibacterial and antimalarial activities[4]
- Some activate the SKN-1 transcription factor[5]
  - Activation of human Nrf2 ortholog may have therapeutic applications[6]
- Literature on specific chalcones is limited and inconsistent
- Three different methoxy-chalcones were tested, shown below (Fig. 1)
  - A – 4'-methoxychalcone
  - B – 4,4'-dimethoxychalcone
  - C – 4-methoxychalcone

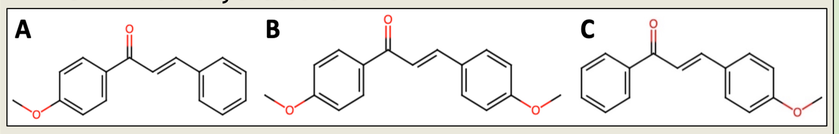


Figure 1. Structure of chalcones used in research study

Research Goal

- The goal of this research is to investigate the effects of chalcones on the lifespan of *Caenorhabditis elegans* and whether altering the position of methoxy groups on the chalcone backbone alters these effects

Model Organism

- *C. elegans* is an approximately 1mm long nematode
- Easily cultured, short lifespan, highly characterized, and many available mutants
- High proteomic homology with humans

Lifespan Assay

- Assay performed in triplicate
- Synchronized population of *C. elegans* N2 strain prepared with a controlled egg lay
- 60 hours later, 10 L4 nematodes were placed onto each treatment and control plate (10 plates each)
  - Treatment plates contained chalcone at a concentration of 42 μM (the optimal concentration in a study that demonstrated lifespan-extending effects of a chalcone)[2]
  - Control plates contained equal volume of the solvent DMSO
- Nematodes were recorded as dead or alive every day until all were dead
  - Counted as alive if any physical movement can be seen
  - Censored if desiccated or missing

Lifespan Assay - Results

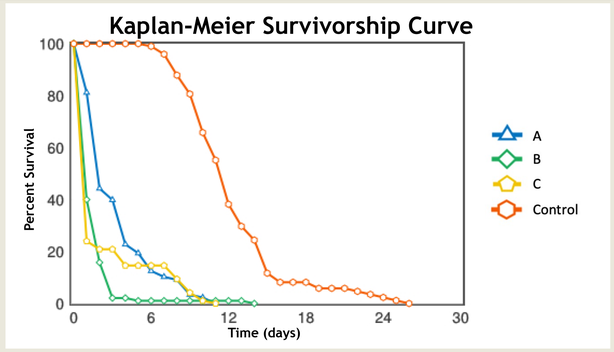


Figure 2. Kaplan-Meier Survivorship Curve. Percent survival of all treatments.

All treatment groups experienced lower average lifespan versus control

Treatment	N	Mean Lifespan (days)	95% Confidence Interval	P-Value			
				vs A	vs B	vs C	vs Control
A	101	3.45	2.93 - 3.97	-	0.000	0.001	0.000
B	100	1.69	1.40 - 1.99	0.000	-	0.275	0.000
C	100	2.39	1.83 - 2.95	0.001	0.275	-	0.000
Control	100	12.47	11.65 - 13.29	0.000	0.000	0.000	-

Table 1. Mean lifespan of all treatments. Note statistical significance of all chalcone treatments versus control as well as chalcone A versus B and C. Statistics calculated using Excel 2-tail p-test.

SKN-1::GFP Localization Assay

- Assay performed in triplicate
- Utilized *C. elegans* LD1 strain
  - Has SKN-1::GFP expression construct
- Synchronized population prepared with a controlled egg lay
- 60 hours later, 30 L4 nematodes placed onto treatment and control plates
- 24 hours after treatment start, nematodes were anaesthetized, and photos were taken using Revolve microscope
- SKN-1 nuclear localization of each nematode was scored as low intermediate, or high, as shown below (Fig. 3)

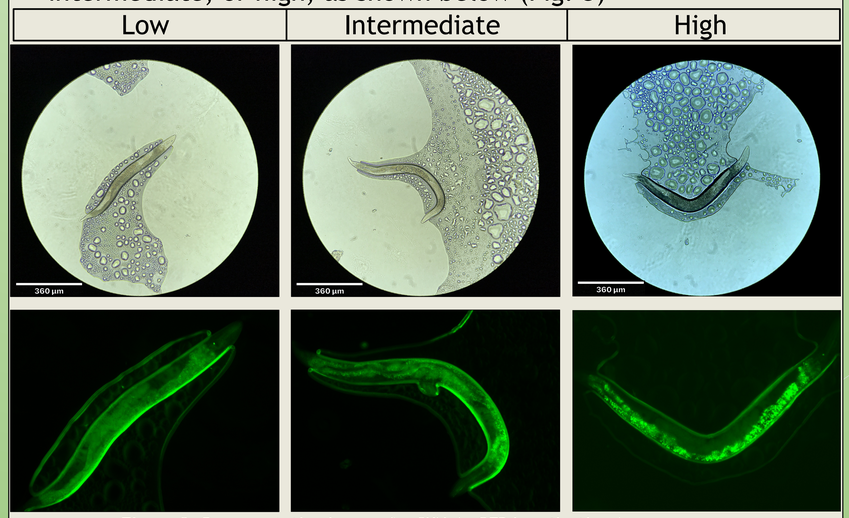
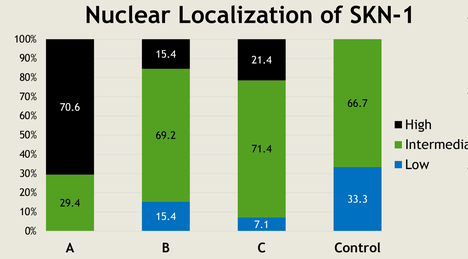


Figure 3. Representative images for SKN-1::GFP Localization Assay.

SKN-1::GFP Localization Assay - Results



- Low, intermediate, and high nuclear localization were assigned scores of 0, 5, and 10
- All chalcones resulted in significant nuclear localization of SKN-1 vs the control
- Chalcone A resulted in significantly higher localization than chalcones B and C

Figure 4. Distribution of scores across all four conditions

Treatment	Mean Score	P-Value			
		vs A	vs B	vs C	vs Control
A	8.53	-	0.000	0.006	0.000
B	4.62	0.000	-	0.279	0.180
C	5.71	0.006	0.279	-	0.018
Control	3.33	0.000	0.180	0.018	-

Table 2. Quantified mean localization scores of all conditions. Note statistical significance of chalcones A and C vs control, as well as A vs B and C. Statistics calculated using Excel 2-tail p-test

Discussion & Future Research

- All three chalcones resulted in a significantly reduced lifespan versus the control (Fig. 2)
  - Methoxy substitution at the 4 position (the right side as shown) may result in increased toxicity
- We observed high mortality in first few days
- Chalcone A resulted in highest nuclear localization of SKN-1 (Fig. 4)
  - This does not directly correlate to toxicity
  - Lack of methoxy substitution at 4 position may correlate with increased nuclear localization of SKN-1
- Future research could further investigate expression of SKN-1 and downstream products through quantitative blots or qPCR
- Investigations into the initial exposure hours of each chalcone could be performed to obtain a clearer picture of the acute toxicity

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