Understanding and targeting SKN-1, a master regulator of detoxification genes

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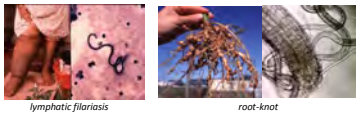
Background

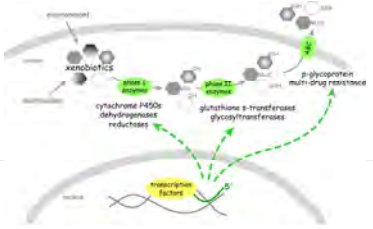
Parasitic nematodes have enormous medical and agricultural impacts



lymphatic filariasis | root-knot

- Parasitize agricultural animals and 25-30% of humans causing neglected diseases
- Cause ~$80 billion of crop loss per year
- Multidrug resistance is a growing problem that is poorly understood in nematodes

Xenobiotic detoxification promotes drug resistance

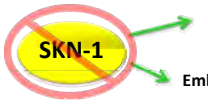


- Xenobiotic detoxification occurs in three phases that promote drug modification and export
- Phase II includes conjugation of glutathione by Glutathione S-Transferases (GSTs)

Aims

Develop inhibitors of SKN-1, a master regulator of detoxification genes

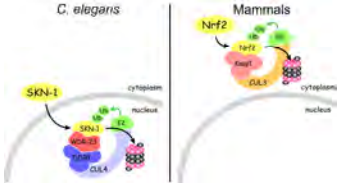
SKN-1, a promising target?



Detoxification (drug resistance)

Embryonic development

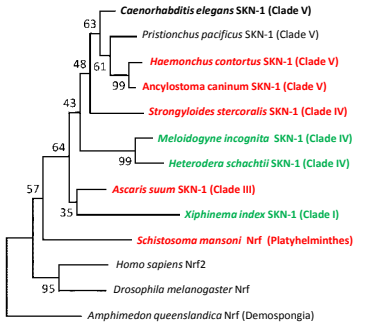
- **Activates ~100 detoxification / antioxidant genes**
- **Essential for embryonic development**
- **Regulated by a different pathway from the mammalian homologue Nrf2 (Choe et al., MCB 2009)**



- **Binds to DNA by a mechanism distinct from other transcription factors (Rupert et al., Nat Struc Biol 1998)**

Results

SKN-1 is present throughout the nematode phylum

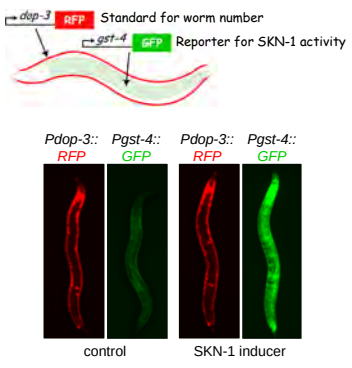


Red = animal parasite ***Green = plant parasite***

Neighbor-joining tree of SKN-1/Nrf DNA binding domains

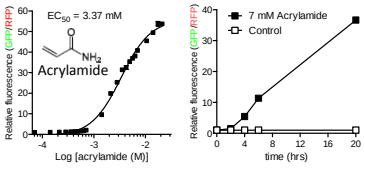
Nothing is know about SKN-1 regulation or function in nematodes other than *C. elegans*

A robust fluorescence assay of SKN-1 activity in *C. elegans*

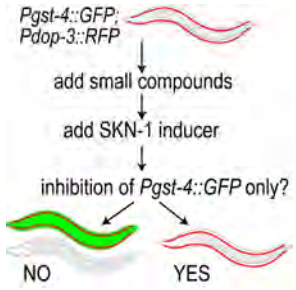


Compatible with 96, 384, and 1536 well plates

Liquid culture of 10^6 worms | Dispense into plates (30-35 worms/well) | Fluorescence reading



Primary screen for SKN-1 inhibitors



- ~364,000 compounds in NIH library
- 274 x 1536-well plates
- 50 plates screened per week
- Completed in 5 weeks (Leung et al., PLoS ONE 2013)

Screening and chemistry produced two SKN-1 inhibitors

MLPCN Library ~364,000 Compounds

PubChem (AID): 602249 | Primary screen @ 21.8 μM

1795 Hits (0.5%)
>35% Inhibition of GFP/RFP

Cheminformatic filtering of promiscuous compounds

1381 retested

364 Reconfirmed Hits
26% Confirmation rate

128 Compounds with IC_{50} ≤ 10 μM
GFP/RFP Fluorescence

Selectivity test

125 Compounds specific to SKN-1
vs. heat shock response: Phsp-16.2::GFP

Chemical analysis

56 compounds from 13 chemical scaffolds
Selected by chemists

Toxicity tests

8 compounds from 2 scaffolds non-cytotoxic
Best activity to toxicity relationships

Medicinal Chemistry

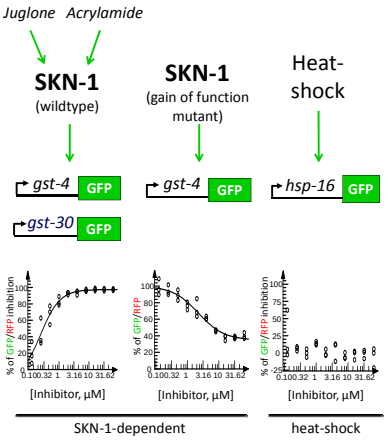
Scaffold A: 45 analogs
Scaffold B: 49 analogs
Structure-Activity Relationships (SAR)

Selectivity over Nrf2
IC50 Nrf2 >> IC50 SKN-1

In progress

Mode of action studies
Drug target(s) identification
Bioassays
Testing in parasites

SKN-1 inhibitors are specific in *C. elegans*



Results are representative for inhibitor A

Inhibitors suppress many detoxification genes in *C. elegans*

Gene	Control mean	Control SE	Acrylamide mean	Acrylamide SE	Acrylamide p	Acrylamide & Inhibitor B mean	Acrylamide & Inhibitor B SE	Acrylamide & Inhibitor B p	Acrylamide & Inhibitor B %inhib.	Acrylamide and Inhibitor A mean	Acrylamide and Inhibitor A SE	Acrylamide and Inhibitor A p	Acrylamide and Inhibitor A %inhib.
gst-30	1.0	0.1	140.2	7.8	0.001	33.4	1.2	0.001	**76.2**	40.0	1.8	0.001	**71.5**
gst-12	1.0	0.1	49.0	1.5	0.001	17.4	0.9	0.001	**64.4**	11.9	0.5	0.001	**75.8**
gst-14	1.0	0.0	23.8	0.7	0.001	3.5	0.5	0.001	**85.2**	5.7	0.3	0.001	**76.2**
gst-10	1.0	0.0	7.6	0.2	0.001	4.1	0.1	0.001	**45.9**	4.7	0.1	0.001	**37.8**
gst-4	1.0	0.1	7.4	0.3	0.001	1.6	0.2	0.001	**78.3**	1.1	0.1	0.001	**85.2**
F56D5.3	1.0	0.0	6.7	0.4	0.001	0.5	0.1	0.001	**92.0**	0.3	0.1	0.001	**95.0**
gst-38	1.0	0.1	0.9	0.0	ns	0.3	0.0	0.001	**66.3**	0.1	0.0	0.001	**93.3**
gst-32	1.0	0.0	0.8	0.0	ns	0.5	0.0	0.010	**36.8**	0.3	0.1	0.001	**65.5**
skn-1	1.0	0.2	1.0	0.1	ns	0.3	0.0	0.001	**74.6**	0.4	0.0	0.001	**72.9**

Data are from quantitative PCR in adults

Inhibitors suppress inducible detoxification genes in *Haemunchus contortus*

H. contortus is a major gastrointestinal parasite of ruminants that is commonly drug resistant.

L1 larvae Gene	Control mean	Control SE	Acrylamide mean	Acrylamide SE	Acrylamide p	Acrylamide & Inhibitor B mean	Acrylamide & Inhibitor B SE	Acrylamide & Inhibitor B p	Acrylamide & Inhibitor B %inhib.	Acrylamide and Inhibitor A mean	Acrylamide and Inhibitor A SE	Acrylamide and Inhibitor A p	Acrylamide and Inhibitor A %inhib.
HCC02208	1.0	0.1	14.0	0.5	0.001	6.9	0.1	0.001	**50.8**	4.3	0.2	0.001	**69.4**
HCC00159	1.0	0.1	13.1	0.8	0.001	11.6	0.5	ns	11.4	14.3	0.5	ns	-9.5
HCC06690	1.0	0.1	3.2	0.2	0.001	1.3	0.1	0.001	**59.5**	1.5	0.2	0.001	**54.0**
HCC08509	1.0	0.1	0.9	0.1	ns	0.9	0.1	ns	-7.6	1.0	0.1	ns	-15.5
HCC01446	1.0	0.2	0.5	0.1	ns	0.4	0.1	ns	6.5	0.4	0.1	ns	17.4
Hc skn-1	1.0	0.1	0.7	0.1	ns	1.1	0.1	ns	-66.5	0.8	0.1	ns	-21.8

Adults Gene	Control mean	Control SE	Acrylamide mean	Acrylamide SE	Acrylamide p	Acrylamide & Inhibitor B mean	Acrylamide & Inhibitor B SE	Acrylamide & Inhibitor B p	Acrylamide & Inhibitor B %inhib.	Acrylamide & Inhibitor A mean	Acrylamide & Inhibitor A SE	Acrylamide & Inhibitor A p	Acrylamide & Inhibitor A %inhib.
HCC00159	1.0	0.0	9.7	0.1	0.001	3.0	0.2	0.001	**69.2**	3.6	0.2	0.001	**62.4**
HCC01446	1.0	0.0	2.5	0.1	0.001	0.7	0.0	0.001	**70.7**	1.6	0.1	0.001	**35.9**
HCC08509	1.0	0.0	1.9	0.1	0.001	0.9	0.0	0.001	**51.0**	2.4	0.2	ns	-27.7
HCC06690	1.0	0.0	1.4	0.0	ns	1.0	0.0	ns	27.4	1.5	0.2	ns	-9.5
HCC02208	1.0	0.1	1.1	0.0	ns	0.7	0.0	0.050	**29.9**	1.3	0.1	ns	-22.3
Hc skn-1	1.0	0.0	2.7	0.1	0.001	1.2	0.1	0.001	**57.1**	1.5	0.1	0.001	**43.1**

Larvae and adults were exposed in vitro
Data are from quantitative PCR

Ongoing Projects

1. Identify drug targets and mode-of-action
2. Test for bioactivity in infection models
3. Further medicinal chemistry

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