

## Diverse Plant-Parasitic Nematodes are Selectively Killed by Oxadiazole Thioether Pro-Nematicides

Jessica Knox<sup>1,2</sup>, Andrew R. Burns<sup>1,2</sup>, Brittany Cooke<sup>1,2</sup>, Savina R. Cammalleri<sup>1,2</sup>, Megan Kitner<sup>3</sup>, Justin Ching<sup>4</sup>, Jack M.P. Castelli<sup>1,2</sup>, Emily Puumala<sup>1</sup>, Jamie Snider<sup>2</sup>, Emily Koury<sup>5</sup>, J.B. Collins<sup>5</sup>, Erik C. Andersen<sup>6</sup>, Igor Stagljar<sup>2</sup>, Leah E. Cowen<sup>1</sup>, Mark Lautens<sup>4</sup>, Inga Zasada<sup>3</sup> and Peter J. Roy<sup>1,2,7\*</sup>

1. Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada
2. Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada
3. United States Department of Agriculture – Agricultural Research Service, Horticultural Crops Disease and Pest Management Research Unit, Corvallis, OR, USA
4. Davenport Research Laboratories, Department of Chemistry, University of Toronto, Toronto, ON, Canada
5. Molecular Biosciences, Northwestern University, Evanston, IL, USA
6. Biology Department, Johns Hopkins University, Baltimore, MD, USA
7. Department of Pharmacology and Toxicology, University of Toronto, Toronto, ON, Canada

\* Corresponding author. Email: [peter.roy@utoronto.ca](mailto:peter.roy@utoronto.ca)

**One Sentence Summary:** A 1,3,4-oxadiazole thioether scaffold is bioactivated by nematode cytochrome P450s to attain selective lethality.

## **ABSTRACT**

Left unchecked, plant-parasitic nematodes have the potential to devastate crops globally. Highly effective but non-selective nematicides are justifiably being phased-out, leaving farmers with limited options for managing nematode infestation. Here, we report our discovery of a 1,3,4-oxadiazole thioether scaffold called Cyprocide that selectively kills diverse plant-parasitic nematodes. Cyprocide is bioactivated into a lethal reactive electrophilic metabolite by specific nematode cytochrome P450 enzymes. Because Cyprocide fails to kill non-target organisms, we infer that the targeted lethality of this pro-nematicide derives from P450 substrate selectivity. Our findings demonstrate that Cyprocide is a selective nematicidal scaffold with broad-spectrum activity that holds the potential to help safeguard our global food supply.

## INTRODUCTION

The expanding human population, increasing demand for high quality protein, decreasing arable land, and impacts of climate change constitute significant challenges that jeopardize global food security (1, 2). Compounding these issues, more than 4000 species of plant-parasitic nematodes (PPNs) reduce global crop yield by over 12% annually (3, 4). The phasing out of effective but non-selective chemical nematicides adds additional pressure to our food production pipeline (5–7). Consequently, there is a pressing need for novel nematicides with improved selectivity.

Our group recently discovered a novel class of imidazothiazole compounds called the Selectivins that selectively kill nematodes via cytochrome P450-mediated bioactivation (8). Xenobiotic-metabolizing cytochrome P450 enzymes (P450s) typically detoxify their substrates, catalyzing monooxygenation reactions that enhance substrate hydrophilicity and promote metabolite excretion via drug efflux pumps (9). However, some substrate-P450 pairs transform a relatively inert compound into a metabolite that exhibits novel or heightened biological activity (10–12). This bioactivation can be phylum or species-selective due to the vast phylogenetic diversity of P450s (8, 13). Indeed, we have shown that the Selectivins are selectively bioactivated by specific nematode P450s into reactive electrophilic products that kill nematodes but not non-target species from diverse phyla (8). Our discovery of the Selectivins established P450-mediated bioactivation as a novel approach to achieve nematode selectivity.

Here, we have re-screened our collection of worm-active molecules (14) for P450-dependent lethality and found multiple disubstituted oxadiazole (DODA) molecules with nematode-selective activity. We consequently assembled an expanded library of DODA compounds and screened it against multiple nematodes, including the free-living *Caenorhabditis elegans* and three species of PPNs from distinct genera. This pipeline yielded a broad-acting P450-dependent 1,3,4-oxadiazole thioether with nematode selectivity that we call cyproicide-B. We identified the nematode P450s responsible for bioactivating cyproicide-B into an oxidized electrophile. The bioactivated product reacts with low molecular weight (LMW) thiols such as glutathione and likely kills the cell via

LMW thiol depletion. Importantly, this bioactivation mechanism is observed across diverse nematodes but not in the non-target organisms examined. Thus, the Cyprocide scaffold is a phylum-selective nematicide with the potential to improve food security.

## RESULTS

### Cyprocide Selectively Kills Nematodes

To identify bioactivated compounds within our custom set of uncharacterized nematicidal small molecules (14), we asked whether the small molecule's lethality in the free-living nematode *C. elegans* could be suppressed by disrupting P450 metabolism. To do this, we genetically reduced the activity of the *C. elegans* P450 oxidoreductase (POR) EMB-8, which transfers electrons to all microsomal P450s (see methods for details) (15–19). We found that 14 of the 87 compounds assayed required POR to exert maximal lethal activity in *C. elegans* (Fig. 1A, Data S1). Among these 14 compounds is selectivin-A, which we have previously shown to be P450-bioactivated (8). By contrast, the succinate dehydrogenase inhibitor wact-11, which does not require bioactivation for activity (14), was found to kill worms in a POR-independent manner (Fig. 1A). These results suggest that the remaining 13 POR-dependent molecules are metabolized by P450s into a toxic product.

Three of the 13 remaining POR-dependent nematicides (wact-4, wact-191, and wact-476) exhibit selective lethality towards nematodes (14) and share a common disubstituted oxadiazole (DODA) core scaffold (Fig. 1B). Given the structural similarity and nematicidal properties of these hits, we expanded our exploration of DODA molecules to identify those that might have improved potency and/or broad-spectrum activity against PPNs. We assembled a library of 846 commercially available small molecules with a DODA core that comprised 151 unique scaffolds (Data S2). We screened this library at 60  $\mu$ M for nematicidal activity against *C. elegans* and three economically significant PPN species from distinct genera: the stem and bulb nematode *Ditylenchus dipsaci*, the root lesion nematode *Pratylenchus penetrans*, and the root knot nematode *Meloidogyne hapla* (Fig. 1C, Data S2) (20).

These screens revealed that the 2,5-disubstituted-1,3,4-oxadiazole thioether scaffold, which we have named Cyprocide, has multiple analogs that exhibit activity against diverse nematode species (Fig. 1C-D). While wact-4 is a Cyprocide (cyprocide-A), we have focused our characterization efforts on cyprocide-B (2-(4-chlorophenyl)-5-

(ethylthio)-1,3,4-oxadiazole) because it more potently kills plant-parasitic nematodes (Fig. 1D, 1E). Notably, cyprocide-B is relatively inactive against non-target organisms that include human cells, fungi, and plant beneficial rhizobacteria at concentrations that kill nematodes *in vitro*, performing similarly in these assays to the commercial nematicide tioxazafen (Fig. 1E).

### **Cyprocide is Bioactivated to a Reactive Electrophile**

Using HPLC (21), we found that cyprocide-B is metabolized into five products (M1-M5, Fig. S1A) in wild-type *C. elegans*. In POR-disrupted *C. elegans*, we found significantly less of the cyprocide-B metabolites were produced ( $p < 0.01$ ) (Fig. S1A-B) and cyprocide-B's lethality was coincidentally suppressed (Fig. S1C). This POR disruption-mediated suppression of lethality extended to other Cyprocide family members but not the wact-11 control (14) (Fig. S1C). These findings indicate that cyprocide-B is metabolically converted to a lethal product in *C. elegans*.

LC-MS analysis of cyprocide-B-treated wild-type *C. elegans* lysates revealed that the parent compound likely undergoes an initial P450-catalyzed S-oxidation to generate an electrophilic sulfoxide metabolite (Fig. 2A-D). Similar phenyloxadiazole sulfoxides have been shown to react with cysteine thiols in the cell by nucleophilic aromatic substitution, whereas their corresponding thioethers are virtually nonreactive (22). Soft electrophiles are known to interact with soft nucleophiles such as low-molecular-weight (LMW) thiols within cells (23). Indeed, we found abundant masses in the *C. elegans* lysates that corresponded to glutathione,  $\gamma$ -glutamylcysteine, cysteinylglycine and cysteine LMW thiol conjugates of the electrophilic cyprocide-B sulfoxide metabolite (Fig. 2E-H).

We hypothesized that the cyprocide-B sulfoxide metabolite induces lethality by depletion of the aforementioned anti-oxidant LMW thiols (11). To test this hypothesis, we asked whether exogenously supplied N-acetylcysteine ethyl ester (NACET), which is converted by the cell into cysteine,  $\gamma$ -glutamylcysteine and glutathione (24), can suppress the lethality conferred by cyprocide-B. Indeed, addition of 5 mM NACET to *C. elegans* larvae robustly suppressed cyprocide-B-induced lethality but not that of POR-

independent control nematicides wact-11 and wact-55 (Fig 1A, Fig. 2I). Together, our results indicate that cyproicide-B is bioactivated by P450(s) into an electrophilic metabolite that likely depletes LMW thiols to confer nematicidal activity *in vivo*.

### **Cyproicide is Bioactivated by *C. elegans* CYP-35D1**

To identify the P450 responsible for cyproicide-B bioactivation in *C. elegans*, we asked whether the RNAi-knockdown of any of 69 of the 76 *C. elegans* P450 (“cyp”) genes (25) suppressed cyproicide-B lethality. We found that RNAi-knockdown of *cyp-35D1* conferred significant resistance to cyproicide-B ( $p < 0.0001$ ) (Fig. 3A). This result was confirmed using two distinct deletion mutants of *cyp-35D1* (Fig. 3B, Fig. S2). Hence, CYP-35D1 is necessary for cyproicide-B bioactivity.

We next asked whether CYP-35D1 is sufficient for metabolizing cyproicide-B into a lethal metabolite. We expressed CYP-35D1 in *Saccharomyces cerevisiae* yeast and tested its ability to metabolize cyproicide-B and coincidentally induce lethality. Indeed, heterologous CYP-35D1 expression hypersensitized yeast to cyproicide-B (Fig. 3C) and induced the production of the LMW thiol conjugates of cyproicide-B (Fig 3D). Together, these results demonstrate that CYP-35D1 is both necessary and sufficient for cyproicide-B metabolism into a lethal product.

### **Cyproicide is Bioactivated by P450s in Diverse PPN Species**

We profiled cyproicide-B metabolite production in lysates of PPN species *Ditylenchus dipsaci*, *Pratylenchus penetrans*, and *Meloidogyne hapla* after exposure to 100  $\mu$ M cyproicide-B using LC-MS. Cyproicide-B-LMW thiol conjugates were produced in all PPNs tested, indicating a P450-catalyzed metabolic bioactivation had occurred similar to what was observed in *C. elegans* (Fig. S3). The reactive sulfoxide metabolite of cyproicide-B was either not detected or observed in low abundance, suggesting that it was efficiently consumed by the LMW thiols in the PPNs, which is consistent with the efficient conjugation of xenobiotic electrophiles seen in other species (8, 24).

Four lines of evidence indicate that Cyprocide's lethality in PPNs is P450-dependent. First, we tested whether the small molecule pan-P450 inhibitor 1-aminobenzotriazole (1-ABT) (26) can suppress cyprocide-B-induced lethality in *C. elegans* and four PPN species and found significant suppression in all species tested ( $p < 0.0001$ ) (Fig. S4). Second, we examined the impact of 1-ABT on metabolite production in the PPN *D. dipsaci*. We found 1-ABT exposure caused an increased abundance of unmodified cyprocide-B parent and a corresponding reduction in metabolite abundance ( $p < 0.05$ ) (Fig. 4A-D). Third, we found that preincubation of *D. dipsaci* with NACET significantly suppressed lethality induced by cyprocide-B ( $p < 1E-6$ ) but not that conferred by the fluopyram nematicide control (Fig. 4E). Finally, we sought to identify a P450 in PPNs that can carry out Cyprocide bioactivation using the notorious root knot nematode *Meloidogyne incognita* as a representative species (20). To do this experiment, we heterologously expressed 19 representative *M. incognita* P450s in yeast and measured the impact of cyprocide-B on strain growth (Fig. 4F). Yeast expressing CYP4731A3 grew significantly slower in response to cyprocide-B relative to the control ( $p < 0.001$ ) (Fig. 4F-G). LC-MS analysis revealed that *M. incognita* CYP4731A3-expressing yeast robustly metabolized cyprocide-B, producing the same LMW thiol conjugates that were found in the CYP-35D1-expressing yeast strain (Fig. 4H-I). Together, these results indicate that P450-mediated bioactivation of Cyprocide is necessary and sufficient to generate reactive lethal metabolites in diverse impactful plant-parasitic nematode species.

### **Cyprocide Controls Root Infestation by *M. incognita***

To further explore Cyprocide's utility against PPNs, we asked whether the nematicide can maintain its activity in soil where many candidate nematicides lose efficacy because of rapid degradation or poor mobility (27). We pre-drenched 90 grams of soil with a 60  $\mu$ M aqueous solution of small molecule to assay the ability of the Cyprocides to protect a tomato host plant from 500 *M. incognita* infective juveniles in a standard root infection assay (28). The Cyprocides were tested alongside two next-generation soil-applied commercial nematicide controls, fluopyram and tioxazafen. After six weeks of infection, the number of eggs per milligram of root tissue was measured. We found that

cyprocide-E reduced root egg burden by an average of 73% relative to untreated controls ( $p < 0.05$ ) (Fig. 4J), outperforming tiozazafen (53% reduction in eggs/mg root tissue) but not fluopyram. The presence of LMW thiol conjugates of cyprocide-E in *M. incognita* CYP4731A3-expressing yeast lysates suggests that cyprocide-E is bioactivated similarly to cyprocide-B (Fig. S5). The Cyprocides did not negatively impact tomato root growth relative to solvent controls ( $p > 0.05$ ) (Fig. 4K). Hence, the Cyprocides have potential utility in preventing root infestation by PPNs in a soil environment.

### **A Structure-Activity Analysis Reveals Cyprocide Analogs with Increased Potency**

Our DODA screen revealed six Cyprocide analogs (Fig. 1D, cyprocide-A-F) that yielded a serendipitous set of structure-activity relationships (SAR). In the hopes of identifying molecules with increased potency, the SAR was expanded to an additional 23 rationalized analogs that vary in structure on either side of the 1,3,4-oxadiazole thioether core (Fig. S6). The molecules were either procured from commercial sources or synthesized in-house and screened against three nematode species (Fig. S6). Because Cyprocide's mode-of-action necessitates testing analogs in living systems, differences in analog activity in the different species likely reflect not only P450 engagement but also absorption, distribution, metabolism (by other enzymes), and drug-export (ADME). We found that the trifluoromethylated cyprocide-I analog exhibited eight-fold greater potency *in vitro* against *M. hapla* compared to cyprocide-B. Cyprocide-I's increased potency was consistent with Cyprocide's mode-of-action whereby sulfoxidation increased the electrophilicity of the oxadiazole C5. The trifluoromethyl leaving group's increased electron withdrawing capability should further increase the electrophilicity of the oxadiazole's C5 upon P450-mediated sulfoxidation, enhancing reactivity of the metabolite. Molecules with the trifluoromethylated R<sup>2</sup> substituent maintained high potency when combined with distinct R<sup>1</sup> electron withdrawing groups (cyprocide-N and -N-2, cyprocide-U and -U-2), suggesting that many potent cyprocide analogs can be generated with different physicochemical properties and perhaps biological ones as well.

## DISCUSSION

Here we report our discovery and characterization of the Cyprocide scaffold of selective nematicides. The otherwise inert Cyprocides are bioactivated by P450s across three genera of plant-parasitic nematode species yet have limited activity in the non-target organisms tested. This suggests that nematode-selectivity is derived from the potentially unique ability of nematode P450s to metabolize the Cyprocides into electrophilic products. This mode-of-action (MOA) is shared with the recently discovered Selectivin class of nematicides (8) and provides evidence that P450 bioactivation is a viable mechanism through which nematode-selectivity can be achieved with distinct chemical structures.

Both Cyprocide and the newly discovered Selectivin (8) are bioactivated by *M. incognita*'s CYP4731A3. This P450 has high basal expression in infective juveniles (29), making it an ideal bioactivating enzyme to target. Given the structural distinctiveness of Cyprocide and Selectivin, CYP4731A3 is likely capable of metabolizing additional structurally diverse substrates. Yeast heterologously expressing CYP4731A3 are a convenient system with which to identify such molecules, which is an especially important in our search for control agents against pests that are difficult to culture in the laboratory.

The difference in agricultural output between fields infested with PPNs and those managed with nematicides is stark (3, 4). Given the phase-out of non-selective nematicides (5–7) and the potential for the development of resistance to some of the next-generation nematicides (30, 31), additional selective nematicides are needed. The Cyprocides have the potential to meet this need and help secure global food production.

## METHODS

### ***Caenorhabditis elegans* Strains and Culture Methods**

The *Caenorhabditis elegans* wild-type (N2), CB1370 *daf-2(e1370)* and MJ69 *emb-8(hc69)* strains were obtained from the *Caenorhabditis* Genetics Center (CGC, University of Minnesota). The *cyp-35D1(ean221)* and *cyp-35D1(ean222)* deletion mutants were generated in the lab of Erik Andersen (Johns Hopkins University). *C. elegans* N2 and the *cyp-35D1* mutants were cultured on MYOB media using standard methods at 20°C (32). The temperature sensitive *daf-2(e1370)* and *emb-8(hc9)* mutants were cultured on MYOB at the permissive temperature of 16°C.

### **Commercial Chemical Sources**

The POR-dependency screen compounds were purchased from ChemBridge Corporation (Data S1 for details). The DODA library compounds were purchased from ChemBridge Corporation and MolPort (Data S2 for details). Cyprocide-A, -C, -D, -E, and -F analogs and tioxazafen were purchased from ChemBridge Corporation. Cyprocide-B was purchased from ChemBridge Corporation and Vitas-M. Cyprocide-H, -M, -N, -Q, -W, -Y, and -M-2 were purchased from MolPort. Fluopyram and 1-Aminobenzotriazole (1-ABT) were purchased from MilliporeSigma. N-acetyl-L-Cysteine ethyl ester (NACET) was purchased from Cayman Chemical.

### ***C. elegans* POR-Dependency Screen**

For the wild-type chemical screens, synchronized first larval stage (L1) N2 worms were plated on nematode growth media (NGM) agar plates (33) supplemented with 1 mM IPTG and 100 µg/mL carbenicillin and seeded with HT115(DE3) *E. coli* carrying the empty L4440 RNAi feeding control vector. The bacteria used to seed the NGM plates were grown overnight in LB media supplemented with 100 µg/mL ampicillin at 25°C in 50 mL conical tubes, without aeration. Once the cultures achieved an OD<sub>600</sub> of 0.6, 1 mM of IPTG was added and the cultures were incubated on a nutating shaker at 20°C for one hour. After IPTG induction, the cultures were concentrated five-fold with double-distilled water (ddH<sub>2</sub>O), and then seeded onto the solid NGM plates. The following day

the plates were dried for 45 minutes in a laminar flow hood. After drying, synchronized L1s that were obtained from an alkaline bleach treatment of gravid adults ('embryo preparation') (34) were deposited onto the plates. The worms were grown for 48 hours at 25°C until they reached the late L4 stage. The L4 worms were then washed off of the plates with M9 buffer (33), and worm density was adjusted to 2.5 worms per microliter. Screening plates were prepared by adding 40 µL of HT115(DE3) + L4440 bacteria suspended in liquid NGM to each well of several 96-well flat-bottom culture plates, pinning 0.3 µL of each library chemical (at 5 mM) into the corresponding wells, and then adding 10 µL of the worm suspension (~25 worms) to each well. The final chemical concentration in the wells was 30 µM. The bacteria for the liquid-based screening plates were grown and prepared the same way as for the solid NGM plates (see above), except after IPTG induction the cultures were concentrated with liquid NGM media instead of water. The screening plates were sealed with parafilm and stored in a box containing several paper towels soaked with water. The plates were incubated for one day at 25°C with shaking at 200 rpm. After incubation, worm viability was assessed. Relatively clear worms, with poorly defined internal structures, that failed to move after vigorous jostling of the plate were scored as dead. The percent of living worms in each well was calculated. The *emb-8* knockdown screen was performed identically to the wild-type screen, except the MJ69 strain was used in place of the wild-type strain, and worms were fed HT115(DE3) *E. coli* harbouring an RNAi feeding vector expressing dsRNA targeting the *emb-8* gene.

As our POR knockdown protocol necessitated screening in young adult worms, we selected a subset of our uncharacterized nematicide collection (14) that killed ≥ 20% of young adult *C. elegans* at 30 mM to include in the POR-dependency screen. This step left us with a focused library of 87 compounds, comprising 67 distinct structural scaffolds which we assayed in both wild-type and POR knockdown conditions to assess the impact of P450-catalyzed metabolism on nematicidal activity in the worm. From 68 untreated control wells we found that the average survival rate for EMB-8 knockdown worms is 98.6%. Since our focused library of nematicides was constructed such that at most 80% of nematicide-treated worms will survive, we reasoned that an increase in

survival of 18.6 percentage points or more for *emb-8* knockdown worms, relative to wild-type, is indicative of EMB-8-dependent killing. These nematicides require functional EMB-8 for activity and are putatively bioactivated by P450 enzymes in the worms. Conversely, a decrease in survival by 18.6 percentage points or more is indicative of EMB-8-dependent resistance, *i.e.*, worms require EMB-8 to resist the nematicidal activity of the compounds. These nematicides are putatively detoxified by worm P450s.

## **DODA Library Small Molecule Screening Methods**

### *C. elegans* L1 Survival and Reproduction Screen

The DODA library screening method was adapted from previously described liquid-based screening protocols (14). *Escherichia coli* strain HB101 grown overnight in LB was resuspended in an equal volume of liquid NGM. 40  $\mu$ L of bacterial suspension was dispensed into each well of a flat-bottomed 96-well culture plate, and 0.3  $\mu$ L of the small molecules or dimethyl sulfoxide (DMSO) solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Approximately 20 synchronized first-stage larvae (L1) wild-type N2 worms obtained from an embryo preparation on the previous day were then added to each well in 10  $\mu$ L M9. The final concentration of library compound in the wells was 60  $\mu$ M (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 6 days at 20°C while shaking at 200 rpm (New Brunswick I26/I26R shaker, Eppendorf). On day six the plates were observed under a dissection microscope and the survival and reproduction phenotypes were assessed in each condition. In solvent exposure conditions we observe that by day 6 the deposited parental larvae have reached adulthood and produced numerous (>>50) next generation larvae in the well. Two biological replicates of the screen were performed in a single technical replicate. A scoring system was used to summarize these phenotypes where a score of 0 indicates 0-10 worms were living in the well, 1 indicates 11-20 worms, 2 indicates 21-50, 3 is greater than 50 worms and 4 indicates the well is overgrown and starved of bacterial food with many more than 50 worms in the well. Hits were defined as any compound for which the average score of the two replicates was 1 or lower.

### *C. elegans* Dauer Mobility Screen

*C. elegans* strain CB1370 *daf-2(e1370)* synchronized L1s were obtained from an embryo preparation that was left to hatch at 15°C overnight. The L1s were plated on 10 cm MYOB media plates (5000 L1s per plate) seeded with *E. coli* OP50 and transferred to 25°C for 48 hours to progress into dauer stage (100% dauer formation is expected at 25°C). 40 µL of NGM was dispensed into each well of a 96-well plate, and 300 nL of the small molecules or DMSO solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Dauer larvae were washed off of the MYOB plates using M9 and approximately 20 dauer larvae were added to each well of the 96-well plates in 10 µL M9. The final concentration of library compound in the wells was 60 µM (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 5 days at 20°C while shaking at 200 rpm. On day five the plates were observed under a dissection microscope and proportion of mobile (living) worms was quantified in each condition. Two biological replicates of the screen were performed in a single technical replicate and the mobility scores for these two replicates were averaged. The mean proportion mobile (relative to DMSO controls) and standard deviation amongst all library compounds was calculated and used to generate Z-scores for each compound screened. Hits were defined as any compound with a Z-score of -2 or lower, which correlated to a proportion mobile of 0.63 or lower.

#### *Ditylenchus dipsaci* Mobility Screen

The *D. dipsaci* strain G-137 used in this work was collected from garlic in Prince Edward County, Ontario, Canada and was provided to us by Qing Yu (Agriculture and Agri-Food Canada). *D. dipsaci* was cultured and collected for screening as previously described (35). Briefly, *D. dipsaci* was maintained on pea plants in Gamborg B-5 agar media. Six-8 weeks after inoculation, the *D. dipsaci* were extracted from the plates for use in small molecule screens. The agar plate and pea plant tissue were cut into small cubes and placed in a coffee filter lined funnel. The funnel was placed in a beaker filled with distilled water, allowing the worms to travel through the filter and into the collection beaker. The next day, the *D. dipsaci* worms in the collection beaker were quantified and were ready for use in the chemical screen. To prepare the screen plates, 40 µL of distilled water was dispensed into each well of a 96-well plate, and 0.3 µL of the small

molecules or DMSO solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Approximately 20 *D. dipsaci* worms were added to each well of the 96-well plates in 10  $\mu$ L distilled water. The final concentration of library compound in the wells was 60  $\mu$ M (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 5 days at 20°C while shaking at 200 rpm. On day five, the plates were observed under a dissection microscope and the proportion of mobile (living) worms was quantified in each condition after adding 2  $\mu$ L of 1 M NaOH to each well to stimulate movement. Two biological replicates of the screen were performed in a single technical replicate and the mobility scores for these two replicates were averaged. The mean proportion mobile (relative to DMSO controls) and standard deviation amongst all library compounds was calculated and used to generate Z-scores for each compound screened. Hits were defined as any compound with a Z-score of -2 or lower, which correlated to a proportion mobile of 0.86 or lower.

#### *Meloidogyne hapla* Egg Hatching Screen

The *M. hapla* strain used in this work was collected from a muck soil sample in Ste-Clotilde, Quebec, Canada and was provided to us by Benjamin Mimee (Agriculture and Agri-Food Canada). *M. hapla* was maintained on tomato (*Solanum lycopersicum* ‘Rutgers’) plants and eggs were collected from tomato roots as previously described (36). To prepare the screen plates, 40  $\mu$ L of distilled water was dispensed into each well of a 96-well plate, and 0.3  $\mu$ L of the small molecules or DMSO solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Approximately 75 *M. hapla* eggs were added to each well of the 96-well plates in 10  $\mu$ L distilled water. The final concentration of library compound in the wells was 60  $\mu$ M (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 12 days at 25°C. On day 12, the plates were observed under a dissection microscope and the number of hatched J2s was quantified in each condition. The relative egg hatching score for each library compound was calculated by dividing the number of eggs hatched in the compound well by the average of the number of eggs hatched in the eight DMSO solvent control wells on the same 96-well plate. Two biological replicates of the screen were performed in a single technical replicate and the egg hatching scores for these two replicates were averaged.

The mean egg hatching score and standard deviation amongst all library compounds was calculated and used to generate Z-scores for each compound screened. Hits were defined as any compound with a Z-score of -2 or lower, which correlated to an egg hatching score relative to DMSO of 0.21 or lower.

#### *Meloidogyne hapla* J2 Mobility Screen

Infective second-stage juveniles (J2) of *M. hapla* were collected as previously described (36). To prepare the screen plates, 40  $\mu$ L of distilled water was dispensed into each well of a 96-well plate, and 0.3  $\mu$ L of the small molecules or DMSO solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Approximately 25 *M. hapla* J2s were added to each well of the 96-well plates in 10  $\mu$ L distilled water. The final concentration of library compound in the wells was 60  $\mu$ M (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 5 days at 20°C while shaking at 200 rpm. On day five, the plates were observed under a dissection microscope and the proportion of mobile (living) worms was quantified in each condition after adding 2  $\mu$ L of 1 M NaOH to each well to stimulate movement. The relative proportion mobile for each library compound was calculated by dividing the mobility score in the compound exposure by the average of the mobility scores in the eight DMSO control wells on the same 96-well plate. Two biological replicates of the screen were performed in a single technical replicate and the mobility scores for these two replicates were averaged. The mean relative proportion mobile and standard deviation amongst all library compounds was calculated and used to generate Z-scores for each compound screened. Hits were defined as any compound with a Z-score of -2 or lower, which correlated to a proportion mobile of 0.70 or lower.

#### *Pratylenchus penetrans* Mobility Screen

The *P. penetrans* strain used in this work was provided to us by Benjamin Mimee (Agriculture and Agri-Food Canada) and was cultured and collected for screening as previously described for *D. dipsaci* (35) with modifications: *P. penetrans* cultures were maintained on excised Golden Jubilee corn roots (OSC Seeds) in Gamborg B-5 agar media (8g/L agar). Six-8 weeks after inoculation, the *P. penetrans* were extracted from the plates for use in small molecule screens. The agar plate and corn root tissue were

cut into small cubes and placed in a coffee filter lined funnel. The funnel was placed in a beaker filled with distilled water, allowing the worms to travel through the filter and into the collection beaker. The next day, the *P. penetrans* worms in the collection beaker were quantified and were ready for use in the chemical screen. To prepare the screen plates, 40  $\mu\text{L}$  of distilled water was dispensed into each well of a 96-well plate, and 0.3  $\mu\text{L}$  of the small molecules or DMSO solvent control was pinned into the wells using a 96-well pinning tool (V&P Scientific). Approximately 20 *P. penetrans* worms were added to each well of the 96-well plates in 10  $\mu\text{L}$  distilled water. The final concentration of library compound in the wells was 60  $\mu\text{M}$  (0.6% v/v DMSO). Plates were sealed with Parafilm and incubated for 5 days at 20°C while shaking at 200 rpm. On day five, the plates were observed under a dissection microscope and proportion of mobile (living) worms was quantified in each condition. The relative proportion mobile for each library compound was calculated by dividing the mobility score in the compound exposure by the average of the mobility scores in the eight DMSO control wells on the same 96-well plate. Two biological replicates of the screen were performed in a single technical replicate and the mobility scores for these two replicates were averaged. The mean relative proportion mobile and standard deviation amongst all library compounds was calculated and used to generate Z-scores for each compound screened. Hits were defined as any compound with a Z-score of -2 or lower, which correlated to a proportion mobile of 0.74 or lower.

### **Cheminformatic Analysis**

Molecule scaffolds were generated from chemical SMILES provided by vendors for all DODA library molecules using the Strip-it command line tool version 1.0.2 (RINGS\_WITH\_LINKERS\_1 scaffold definition). The ChemmineR Cheminformatics Toolkit for R package (37) was used to create an atom pair distance matrix of these scaffolds and perform hierarchical clustering to create a dendrogram display of the library organized by structural similarity. The full chemical structures of the Cyproside scaffold molecules in the DODA library were analyzed for pairwise structural similarity. Similarity scores were calculated as the Tanimoto coefficient of shared FP2 fingerprints using OpenBabel (<http://openbabel.org>). The visualization of scaffold similarity in Fig.

1D was generated using Cytoscape version 3.9.1 (38) with individual Cyprocide compounds (nodes) connected by an edge if they share a structural similarity (Tanimoto coefficient) of  $> 0.725$ .

## **Dose-Response Experiments**

### *C. elegans* L1 Dose-Response Assays

The dose-response experiments on *C. elegans* L1s were set up using the same methodology as described for the DODA library screen with the exception that the cyprocide-B and tioxazafen assays displayed in Fig. 1E the proportion of viable worms in each condition was quantified after 3 days of chemical exposure. The relative proportion of viable worms in each chemical exposure condition was calculated by dividing the proportion viable worms in that condition to the proportion mobile worms in the DMSO controls. At least two biological replicates were performed with three technical replicates on each day and the relative proportion viable worms was averaged across the replicates.

### *C. elegans* Dauer Dose-Response Assays

The dose-response experiments on *C. elegans* dauers were set up using the same methodology as described for the DODA library screen with the exception that the dose-response assays were quantified on day three of chemical exposure after adding 1  $\mu$ L of 1 M NaOH to each well to stimulate movement. The relative proportion mobile (living) worms in each chemical exposure condition was calculated by dividing the proportion mobile worms in that condition to the proportion mobile worms in the DMSO controls. Three replicates were performed, and the relative proportion mobile was averaged across the replicates.

### *D. dipsaci* Dose-Response Assays

The dose-response experiments on *D. dipsaci* were set up using the same methodology as described for the DODA library screen with the exception that for the cyprocide-B and tioxazafen assays displayed in Fig. 1E the proportion of mobile (living) worms in each condition was quantified after 3 days of chemical exposure. The relative viability

for each compound exposure condition was calculated by dividing the proportion mobile in that condition by the proportion mobile worms in the DMSO solvent controls. At least two biological replicates were performed with three technical replicates on each day and the relative proportion mobile was averaged across the replicates.

### *M. hapla* Egg Dose-Response Assays

The dose-response experiments on *M. hapla* eggs were set up using the same methodology as described for the DODA library screen with the following modifications: the final volume of distilled water in each well of the 96-well plate was 100  $\mu$ L and the compounds were added to the wells using a multichannel pipette to a final DMSO concentration of 0.6% (v/v). The dose-response assays were quantified after 12 days of chemical exposure and the data is presented as the number of eggs that hatched in each condition relative to the number that hatched in DMSO controls. Three biological replicates were performed with at least two technical replicates on each day and the relative egg hatching was averaged across the replicates.

### *M. hapla* J2 Dose-Response Assays

The dose-response experiments on *M. hapla* J2s were set up using the same methodology as described for the DODA library screen with the exception that for the cyprodicide-B and tioxazafen assays displayed in Fig. 1E the proportion of mobile (living) worms in each condition was quantified after 3 days of chemical exposure. The relative viability for each compound exposure condition was calculated by dividing the proportion mobile in that condition by the proportion mobile worms in the DMSO solvent controls. At least two biological replicates were performed with three technical replicates on each day and the relative proportion mobile was averaged across the replicates.

### *P. penetrans* Dose-Response Assays

The dose-response experiments on *P. penetrans* were set up using the same methodology as described for the DODA library screen with the exception that the dose-response assays were quantified after 3 days of chemical exposure. The relative viability for each compound exposure condition was calculated by dividing the

proportion mobile in that condition by the proportion mobile worms in the DMSO solvent controls. Three biological replicates were performed with at least three technical replicates on each day and the relative proportion mobile was averaged across the replicates.

#### Wild-type *Saccharomyces cerevisiae* Dose-Response Assays

An overnight saturated culture of *S. cerevisiae* BY4741 was diluted in YPD to an OD<sub>600</sub> of 0.015 and 100  $\mu$ L of culture was dispensed into each well of 96-well flat-bottom culture plates. Plates were incubated for 4 hours at 30°C with shaking at 140 rpm. Compounds were added to the wells using a multichannel pipette to a final DMSO concentration of 1% (v/v). The plates were sealed with clear plastic adhesive film and incubated at 30°C with intermittent shaking in a microplate spectrophotometer (TECAN) for 24 hours. Endpoint growth was quantified by measuring optical density (OD) at 600 nm. The relative growth in each chemical exposure condition was calculated by dividing the endpoint OD<sub>600</sub> reading in by the endpoint OD<sub>600</sub> reading in the DMSO solvent control. Three biological replicates were performed, and the relative growth was averaged across the replicates.

#### *Candida albicans* Dose-Response Assays

Compound potency against *C. albicans* (SN95) was assessed using two-fold dose-response assays following a standard protocol. Briefly, YPD medium was inoculated with  $\sim 1 \times 10^3$  cells/mL from saturated overnight cultures. Assays were performed in 384-well, flat-bottom microtiter plates (Corning) in a final volume of 40  $\mu$ L/well. Each compound was added to wells in a two-fold concentration gradient from 50  $\mu$ M to 0  $\mu$ M. Plates were then incubated in the dark at 30°C under static conditions for 48 hours. Endpoint growth was quantified by measuring OD<sub>600</sub> using a spectrophotometer (Molecular Devices) and the results were corrected for background media. Relative fungal growth for each compound treatment was defined by normalizing the OD<sub>600</sub> values in treated wells to the values observed in untreated controls. All dose-response assays were performed in technical triplicate and relative growth levels were averaged across replicates.

### Beneficial Rhizobacteria Dose-Response Assays

*P. simiae* WSC417 and *P. defensor* WSC374r strains were provided by Keiko Yoshioka (University of Toronto). Saturated overnight cultures were diluted in LB to an OD<sub>600</sub> of 0.01 and 100 µL of culture was dispensed into each well of 96-well flat-bottom culture plates. Compounds were added to the wells using a multichannel pipette to a final DMSO concentration of 0.6% (v/v). The plates were sealed with clear plastic adhesive film and incubated at 28°C with continuous shaking for 16 hours. Endpoint growth was quantified by measuring the OD<sub>600</sub> with a microplate spectrophotometer (BioTek Epoch 2). The relative bacterial growth in each chemical exposure condition was calculated by dividing the endpoint OD<sub>600</sub> reading by the endpoint OD<sub>600</sub> reading in the DMSO solvent control. Three biological replicates were performed with three technical replicates on each day and the relative growth was averaged across the replicates.

### HEK293 Cell Dose-Response Assays

HEK293 cells were seeded into 96-well plates, at 5000 cells per well, in 100 µL total volume of DMEM/10%FBS/1%PS media and grown overnight at 37°C in the presence of 5% CO<sub>2</sub>. Compounds were added to cells (final DMSO concentration of 0.5% v/v), and growth was continued for an additional 48 hours. Following growth, 10 µL of CellTiter-Blue Viability reagent (Promega) was added to each well, and plates were incubated for 4 hours at 37°C in the presence of 5% CO<sub>2</sub>. Fluorescence measurements (560 nm excitation/590 nm emission) were then performed using a CLARIOstar Plate Reader (BMG Labtech) to quantify reagent reduction and estimate cell viability. Fluorescence measurements were corrected for background from medium. Relative growth was calculated by dividing corrected fluorescence in the treatment wells by that measured in the corresponding DMSO control well. At least three replicates were performed and the relative growth was averaged across the replicates.

### HepG2 Cell Dose-Response Assays

HepG2 cells (ATCC, Cat# HB-8065) were counted using a haemocytometer and diluted to 5 x 10<sup>4</sup> cells/mL in 100 µL of RPMI-1640 (Sigma) medium supplemented with 10%

heat inactivated fetal bovine serum (Gibco) following a standard protocol. Cells were seeded in black, clear-bottom 384-well plates (Corning) to a final density of 2000 cells/well in 40  $\mu$ L. Cells were incubated at 37°C with 5% CO<sub>2</sub> for 24 hours. Subsequently, a 2-fold dilution series of test compound was added to seeded cells from 0  $\mu$ M to 50  $\mu$ M, and plates were incubated at 37°C with 5% CO<sub>2</sub> for 72 hours. After 72 hours, Alamar Blue (Invitrogen) was added to the HepG2 cells at a final concentration of 0.05X and plates were incubated at 37°C for 3 hours. Fluorescence was measured at Ex560nm/Em590nm using a TECAN Spark microplate fluorometer and values were corrected for background from the medium. Relative proliferation was calculated by dividing corrected fluorescence in the treatment wells by that measured in the corresponding DMSO control well. Three technical replicates were performed per experiment, and the relative proliferation was averaged across the replicates. The data presented is representative of two biological replicate experiments.

### **POR Knockdown Dose-Response Experiments**

*E. coli* HT115(DE3) containing an RNAi feeding vector expressing dsRNA targeting the *emb-8* gene or an empty RNAi feeding vector (L4440) were grown overnight in LB with 100  $\mu$ g/mL carbenicillin at 25°C with no shaking until the culture was in mid-log phase (OD<sub>600</sub> ~0.6). The culture was induced with 1 mM IPTG and grown at 37°C with shaking at 200 rpm for 1 hour. The bacteria were pelleted and concentrated ten-fold with liquid NGM containing 1 mM IPTG and 100  $\mu$ g/mL carbenicillin. This bacterial suspension was dispensed into the wells of a flat-bottomed 96-well culture plate (40  $\mu$ L per well). *C. elegans* temperature sensitive mutant strain MJ69 *emb-8(hc69)* and wild-type strain N2 synchronized L1s were obtained from an embryo preparation that was left to hatch at 15°C overnight. Approximately 20 N2 L1s were plated in 10  $\mu$ L of M9 per well containing L4440 empty vector control bacteria (“wild-type” condition). Approximately 20 *emb-8* L1s were plated in 10  $\mu$ L of M9 per well containing *emb-8* dsRNA expressing bacteria (“POR knockdown” condition). Worms were grown for 40 hours at the restrictive temperature of 25°C with shaking at 200 rpm at which point 120  $\mu$ M FUDR was added to each well and plates were returned to the 25°C incubator for another 30 hours. At this 72-hour timepoint the compounds were added to the wells using a multichannel pipette

to a final DMSO concentration of 1% v/v. After 48 hours of chemical exposure viability was quantified, and the data is presented as the proportion of viable worms in each condition relative to the DMSO controls. Three biological replicates were completed with at least two technical replicates on each day, and the proportion viable was averaged across the replicates.

### ***C. elegans* P450 RNAi Screen**

The P450 RNAi screen consisted of RNAi-mediated knockdown of 69 *C. elegans* microsomal P450 genes. Each P450 RNAi feeding vector and the L4440 empty vector control were grown in LB with 100 µg/mL carbenicillin at 25°C with no shaking until the culture was in mid-log phase (OD<sub>600</sub> ~0.6). The culture was induced with 1 mM IPTG and grown at 37°C with shaking at 200 rpm for 1 hour. The bacteria were pelleted and concentrated ten-fold with liquid NGM containing 1 mM IPTG and 100 µg/mL carbenicillin. This bacterial suspension was dispensed into the wells of a flat-bottomed 96-well culture plate (40 µL per well). Approximately 20 N2 L1s obtained from an embryo preparation were plated in 10 µL of M9 per well. Worms were grown for 48 hours at 20°C with shaking at 200 rpm at which point 120 µM FUDR was added to each well and plates were returned to the 20°C incubator for another 24 hours. At this 72-hour timepoint, 40 µM of cyprocode-B was added to each well (1% DMSO v/v). After 48 hours of chemical exposure, the proportion of mobile worms was quantified in each condition. Four replicate wells were analyzed for each P450 knockdown condition, and the mobility was averaged across the replicates.

### ***cyp-35D1* Mutant Dose-Response Experiments**

*E. coli* HB101 was grown overnight in LB at 37°C with shaking at 200 rpm. The saturated bacterial culture was pelleted and concentrated two-fold with liquid NGM. This bacterial suspension was dispensed into the wells of flat-bottomed 96-well culture plates (40 µL per well). *C. elegans* wild-type N2, *cyp-35D1(ean221)*, and *cyp-35D1(ean222)* synchronized L1s were obtained from an embryo preparation the previous day. Approximately 20 L1s were plated in 10 µL of M9 per well. Worms were grown for 48 hours at 20°C with shaking at 200 rpm at which point 120 µM FUDR was added to each

well and plates were returned to the 20°C incubator for another 24 hours. At this 72-hour timepoint, the compounds were added to the wells using a multichannel pipette to a final DMSO concentration of 1% v/v. After 48 hours of chemical exposure, viability was quantified, and the data were presented as the proportion of viable worms in each condition relative to the DMSO controls. These dose-response experiments were completed in three biological replicates with two technical replicates on each day, and the relative viability was averaged across the replicates.

## **NACET Experiments**

### *C. elegans* NACET Assays

*C. elegans* L1 +/- NACET viability assays were set up in liquid NGM in 96-well plate format as described above. NACET was dissolved directly in the + NACET media before dispensing at a final concentration of 5 mM and plates were incubated at 20°C with shaking at 200 rpm for 4 hours. After 4 hours, 25 µM of nematicide (cyproside-B, wact-11 or wact-55) or DMSO solvent were added to the wells (1% final concentration of DMSO) and returned to the 20°C incubator. After 24 hours of drug exposure, the proportion of mobile (living) and immobile worms in each condition was quantified manually under a dissection microscope. Three biological replicates with at least three technical replicates of each condition were performed, and the proportion mobile worms relative to DMSO solvent controls are reported.

### *D. dipsaci* NACET Assays

*D. dipsaci* +/- NACET viability assays were set up in distilled water in 96-well plate format as described above. NACET was dissolved directly in the + NACET media before dispensing at a final concentration of 90 mM and plates were incubated at 20°C with shaking at 200 rpm for 4 hours. After 4 hours, 60 µM of cyproside-B, 30 µM of fluopyram or DMSO solvent were added to the wells (1% final concentration of DMSO) and returned to the 20°C incubator. After three days of drug exposure, the proportion of mobile (living) and immobile worms in each condition was quantified manually under a dissection microscope after the addition of 2 µL of 1 M NaOH to the wells. Three biological replicates with at least three technical replicates of each condition were

performed, and the proportion mobile worms relative to DMSO solvent controls are reported.

### **1-ABT Experiments**

1-ABT viability assays were set up in 100  $\mu$ L liquid NGM + HB101 media (*C. elegans*) or 100  $\mu$ L distilled water (PPN species) in 96-well plate format as described above. 1-ABT or DMSO solvent was added to a final concentration of 1mM (0.5% DMSO v/v) in the media. Sealed plates were incubated at 20°C with shaking at 200 rpm for 24 hours. After 24 hours 20  $\mu$ M (*C. elegans*) or 60  $\mu$ M (PPNs) final concentration of cyproicide-B or DMSO was added to the well (0.5% DMSO v/v). After two (*M. incognita*) or three days (all other species) of compound exposure, the proportion of mobile (viable) worms in each condition was quantified manually under a dissection microscope. Four biological replicates were performed with at least three technical replicates of each condition per day, and the proportion mobile relative to DMSO controls was averaged across the replicates. For *M. incognita* six replicates were completed, and the proportion relative to DMSO controls was averaged across the replicates.

### ***Meloidogyne incognita* Soil-Based Root Infection Assays**

Ninety grams of soil (1:1 sand:loam mix) was added to each compartment of 6-compartment plastic garden packs. The soil was drenched with 18 mL of deionized water containing dissolved chemical or DMSO solvent alone. 500 infective *M. incognita* second-stage juveniles (J2s) (culture maintained and J2s collected as described in (28)) were then added to the soil in 2 mL of water, for a final chemical concentration of 60  $\mu$ M in 20 mL of water (0.1% DMSO v/v). The J2s were incubated in the soil and chemical for 24 hours, after which a three-week old tomato seedling was transplanted into each compartment. Plants were grown for six weeks in a greenhouse as described (28) under long-day conditions (16-h photoperiod) with 26/18°C day/night temperatures. After six weeks, the plants were harvested and the roots gently washed with water. Eggs were extracted from the roots using a 10% bleach solution with agitation at 300 rpm for 3 minutes. The roots were rinsed over nested 250- and 25.4- $\mu$ m sieves and eggs were collected in water from the fine sieve. The number of eggs found in the root system of

each plant was counted using a haemocytometer under a dissection microscope. After egg extraction, the roots were dried in a 65°C oven and the dry roots were weighed. The number of eggs per milligram of root was calculated for each plant by dividing the number of eggs quantified by the mass of the dried root material. The infection assays were repeated on two different days, and three plants were analyzed from each chemical exposure condition on each day.

### **Construction of P450-Expressing Yeast Strains**

The *C. elegans cyp-35D1* cDNA sequence was obtained from WormBase (WormBase web site, <http://www.wormbase.org>, release WS280, April 7, 2021) and codon-optimized for *S. cerevisiae* (see Supplementary Table 1 for sequence). The codon-optimized cDNA was synthesized by Integrated DNA Technologies. Using standard restriction cloning techniques, the *cyp-35D1* cDNA was cloned into the ATCC p416 GAL1 expression vector (URA3 selection marker; CEN6/ARSH4 origin of replication) and transformed into *S. cerevisiae* BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0). Transformants were selected for on URA- SD media.

Nineteen P450s that were representative of the diversity of sequences within the *M. incognita* P450 superfamily were selected for analysis ((8) for details). These 19 *M. incognita* P450 sequences were codon optimized for *S. cerevisiae* expression (see Supplementary Table 1 for sequences) and constructed by Twist Bioscience using the same ATCC p416 GAL1 expression vector. The 19 P450 expression vectors were individually transformed into *S. cerevisiae* BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0), and transformants were selected for on URA- SD media.

### **Dose-Response Experiments with P450-Expressing Yeast Strains**

Overnight cultures of *S. cerevisiae* strains containing either the *C. elegans* CYP-35D1 p416 GAL1 expression vector, the *M. incognita* CYP4731A3 p416 GAL1 expression vector or an empty vector control (strain construction described above) were set up in SD media with URA- selection containing 2% raffinose at 30°C with shaking. The cultures were diluted to an OD<sub>600</sub> of 0.03 in SD URA- with 2% raffinose and grown for 4

hours at 30°C with shaking. To induce P450 expression, galactose was spiked into the culture at a 2% final concentration and grown for a further 4 hours at 30°C with shaking. 200 µL of culture was dispensed into each well of a 96-well flat-bottom culture plate. Compounds or DMSO solvent control were added to the wells using a multichannel pipette to a final DMSO concentration of 1% (v/v). The plates were sealed with clear plastic adhesive film and incubated at 30°C with no shaking in a microplate spectrophotometer (BioTek Epoch 2) with OD<sub>600</sub> readings taken of each well every 30 minutes for 48 hours. The area under the growth curve was calculated for each chemical exposure condition and divided by that of the DMSO solvent controls for the strain to produce a value for the relative growth in each condition. Four replicates of the dose-response assays were performed, and the relative growth values presented are the average across the replicates.

### **Yeast-Based Screen for *M. incognita* P450s that Bioactivate Cyprocide-B**

Overnight cultures of the 19 *M. incognita* P450-expressing yeast strains (construction described above) and the empty vector (EV) control strain were set up in SD media with URA- selection containing 2% raffinose at 30°C with shaking. The cultures were diluted to an OD<sub>600</sub> of 0.05 in SD URA- with 2% raffinose and grown for 2 hours at 30°C with shaking. To induce P450 expression, galactose was spiked into the culture at a 2% final concentration and grown for a further 2 hours at 30°C with shaking. 100 µL of culture was dispensed into each well of a 96-well flat-bottom culture plate and each strain was treated with 50 µM cyprocide-B or DMSO solvent control (1% v/v). The plates were sealed with clear plastic adhesive film and incubated at 30°C with intermittent shaking in a microplate spectrophotometer (TECAN) with OD<sub>600</sub> readings taken of each well every 15 minutes for 48 hours. Six replicates were completed for each condition. The growth data was background corrected by subtracting the OD<sub>600</sub> reading from the first time point from all points in the growth curve. The timepoint at which the OD<sub>600</sub> was equal to 95% of the maximum of the curve was determined in the DMSO solvent control for each strain and the area under the curve (AUC) was calculated up to that time point for both the DMSO control and paired cyprocide-B-treated condition for the strain. The AUC for

the cyprocide-B treatment condition divided by the AUC of the DMSO control is reported as the 'normalized AUC' in response to cyprocide-B for each strain.

## **Incubations for HPLC and LC-MS Experiments**

### *C. elegans* Wild-Type and POR Knockdown Incubations for HPLC Analysis

Wild-type N2 and *emb-8(hc69)* L1s, and empty vector (L4440) and *emb-8* targeting RNAi bacteria were grown and prepared as described for the POR knockdown dose-response experiments. For the wild-type condition, 2 mL of L4440 bacterial suspension and 500  $\mu$ L of M9 buffer containing 1250 N2 L1s were added to a 15 mL conical tube. For the POR knockdown condition, *emb-8* targeting RNAi bacteria and *emb-8(hc69)* L1s were used. Tubes were incubated on a nutator for 40 hours at 25°C at which point 120  $\mu$ M FUDR was added to the tubes, and they were returned to 25°C for another 30 hours. At the 72-hour timepoint, 100  $\mu$ M cyprocide-B or DMSO were added to the tubes (0.5% DMSO v/v) and incubated for 6 hours on a nutator at 25°C. After the incubation, the worms were washed three times with M9 buffer and transferred to the wells of a Pall AcroPrep 96-well filter plate (0.45- $\mu$ m wwPTFE membrane, 1 mL well volume). The wells were cleared by vacuum, worms were resuspended in 50  $\mu$ L of M9 buffer, transferred to a 1.5mL microcentrifuge tube, and immediately stored at -80°C. Three replicates were performed for each condition.

### *C. elegans* L1 and PPN Incubations for LC-MS Analysis of Lysate

Synchronized *C. elegans* wild-type N2 L1s were obtained using an embryo preparation. In 500  $\mu$ L of M9 buffer, 150,000 L1s were incubated with 100  $\mu$ M cyprocide-B or DMSO (0.5% v/v). Microcentrifuge tubes were incubated on a nutator for 6 hours at 20°C. After the incubation, the worms were transferred to the wells of a Pall AcroPrep 96-well filter plate (0.45- $\mu$ m wwPTFE membrane, 1 mL well volume). The wells were cleared by vacuum and washed once with 600  $\mu$ L of M9 buffer. The worms were resuspended in 50  $\mu$ L of M9 buffer, transferred to a 1.5 mL microcentrifuge tube, and immediately stored at -80°C. The *D. dipsaci* were collected as described above, and incubations were carried out similarly but with the following modifications: the incubations were performed in 2.5 mL of distilled water in 15 mL falcon tubes, 2500 *D. dipsaci* were used

per incubation, and the incubation length was 24 hours. *P. penetrans* were collected as described above, and incubations were carried out similarly to *D. dipsaci* but 20,000 mixed stage *P. penetrans* were used per incubation and the incubation length was 16 hours. *M. hapla* J2s were collected as described above, and incubations were performed identically to *P. penetrans* but 15,000 *M. hapla* were used per incubation.

#### *D. dipsaci* Incubations for +/- 1-ABT LC-MS Analysis

*D. dipsaci* incubations for the +/- 1-ABT LC-MS analysis of incubation buffer were performed similarly to the incubations for lysate analysis with some modifications: the incubations were performed in a 1.5 mL microcentrifuge tube with 2000 worms in a 1 mL volume of distilled water. The worms were incubated with a 1 mM final concentration of 1-ABT or DMSO solvent (0.25% DMSO v/v) for 24 hours before adding 100  $\mu$ M cyproside-B or paired DMSO solvent (0.5% DMSO v/v) and incubated for an additional 24 hours. After the incubation, the worms were transferred to the wells of a Pall AcroPrep 96-well filter plate (0.45- $\mu$ m wwPTFE membrane, 1 mL well volume), and the incubation buffer was cleared by vacuum into a collection plate. These incubation buffers were transferred to 1.5 mL microcentrifuge tubes and immediately frozen at -80°C.

#### Yeast Incubations for Lysate LC-MS Analysis

Overnight cultures of *S. cerevisiae* strains containing either the *C. elegans* CYP-35D1 p416 GAL1 expression vector, the *M. incognita* CYP4731A3 p416 GAL1 expression vector or an empty vector control (strain construction described above) were set up in SD media with URA- selection containing 2% raffinose at 30°C with shaking. The cultures were diluted to an OD<sub>600</sub> of 0.3 in SD URA- with 2% raffinose in a 4.95 mL final volume in glass test tubes. The yeast cultures were grown for 4 hours at 30°C on a rotator. To induce P450 expression, 550  $\mu$ L of 20% galactose was spiked into the culture for a 2% final concentration and grown for a further 4 hours at 30°C on a rotator. 100  $\mu$ M cyproside-B or cyproside-E were added to the cultures (1% DMSO v/v). The cultures were vortexed and incubated for 6 hours at 30°C on a rotator. At the end point, the cells were transferred to 1.5 mL microcentrifuge tubes and pelleted, and all media

was aspirated. The cells were washed in 0.5 mL of water, pelleted, and the water was removed. The cells were resuspended in 0.5 mL of M9 buffer and pelleted once again. All the media was removed, and the cell pellet was stored at -80°.

### **Sample Lysis for HPLC and LC-MS Experiments**

*C. elegans* worm pellets were thawed and 50 µL of 2X worm lysis buffer (20 mM Tris-HCl pH 8.3, 0.2% SDS, 240 µg/mL proteinase K) was added to each tube. Tubes were vortexed and incubated in a water bath at 56°C for 80 minutes, vortexing every 20 minutes. Tubes were bath sonicated for 20 minutes in a Branson 1510 bath sonicator at room temperature and lysates were frozen at -80°C. PPN worm pellets were lysed using a similar methodology to *C. elegans* with the following modifications: the 2x worm lysis buffer was composed of 20 mM Tris-HCl pH 8.3, 0.2% SDS and 720 µg/mL proteinase K, and after lysis buffer was added the tubes were incubated in a water bath at 65°C for 2 hours (*D. dipsaci*) or 3 hours (*M. hapla* and *P. penetrans*) with vortexing every 15 minutes.

The volume of the frozen *S. cerevisiae* pellets was adjusted to 50 µL with a solution of M9 buffer containing 1 M sorbitol and 300 U/mL zymolase. The cells were incubated at 37°C for 60 minutes with vortexing every 10 minutes. 50 µL of 2X yeast lysis buffer (20 mM Tris-HCl pH 8.3, 0.2% SDS, 720 µg/mL proteinase K) was added to the tubes and the cells were incubated for 2 hours at 56°C with vortexing every 10 minutes. Tubes were bath sonicated for 20 minutes at room temperature and lysates were frozen at -80°C.

### **Incubation Buffer Processing for LC-MS Analysis**

*D. dipsaci* incubation buffers were processed for LC-MS analysis using Sep-Pak Light C8 Cartridges (Waters) fitted to a 10 mL BD Luer-Lok Syringe using a flow rate of ~1 mL per minute. The column was activated with 3 mL of 100% acetonitrile (ACN) and then washed with 3 mL of Milli-Q water. The incubation buffer was then passed through the column and the column was washed with 1 mL of water. Three sequential elutions

were performed using 250  $\mu$ L each of 20%, 50% and 100% ACN. The eluates were combined, dried using an Eppendorf Vacufuge Concentrator set at 60°C and stored at -80°C.

### **HPLC-DAD Analysis and Quantification**

The frozen *C. elegans* wild-type and POR knockdown worm lysates were thawed and 50  $\mu$ L of acidified acetonitrile (ACN) solution (50% ACN, 0.2% acetic acid) was added. The samples were vortexed and centrifuged at 17,949g for 1 minute. Chromatographic separation was performed by reversed-phase chromatography (ZORBAX SB-C8 column, 4.6 x 150 mm, 5-micron particle size, with ZORBAX RX-C8 4.6 x 12.5 mm guard column) using an HP1050 system equipped with an autosampler, vacuum degasser, and variable wavelength diode-array detector (DAD). The column was maintained at ~22°C. 50  $\mu$ L of lysate sample was injected and eluted over 8.65 minutes with the solvent and flow rate gradients shown in Supplementary Table 2. Absorbance was measured every 2 nm between 190 and 602 nm. Prior to processing the worm lysates, 20 nmol of cyprocode-B was processed to determine elution time and absorbance spectrum. HPLC analyses of unmodified cyprocode-B parent and the five metabolites visible by HPLC-DAD (M1-M5) in worm lysates were performed three times, and area under the curve (AUC) for each analyte was quantified using the HP ChemStation peak integration tool, using default settings. To control for differences in biomass between samples all AUC values from a particular lysate were divided by the AUC of the endogenous worm contents in the lysate (21) and this value is reported as the normalized abundance. AUCs for the unmodified cyprocode-B parent and metabolite M3 were calculated at the absorbance intensity maximum of 280 nm, AUC for M1 and M2 were calculated at 300 nm, M4 at 250 nm, and M5 at 245 nm.

### **LC-MS Analysis and Quantification**

#### Lysate LC-MS Analysis

Frozen *C. elegans* L1, *D. dipsaci*, *P. penetrans*, *M. hapla* and yeast lysates were thawed and 50  $\mu$ L of acidified acetonitrile (ACN) solution (50% ACN, 0.2% acetic acid) was added. The samples were vortexed and centrifuged at 17,949g for 2 minutes.

Chromatographic separation was performed by reversed-phase chromatography (Kinetex C18 analytical column, 100 x 2.1 mm, 2.5-Micron particle size, and ZORBAX C18 3.5-Micron particle size guard column) using an HPLC system (Agilent 1260 Infinity series binary pump with G1367D 1200 series HP autosampler) and mobile phase A (H<sub>2</sub>O, 0.1% formic acid) and B (ACN, 0.1% formic acid). Five  $\mu$ L of lysate sample was injected and eluted with the solvent and flow rate gradients described in Supplementary Table 3. Electrospray ionization mass spectrometry (ESI-MS) analyses were carried out using an Agilent 6538 UHD quadrupole time-of-flight mass (Q-TOF) analyzer. The Q-TOF instrument was operated in positive scanning mode (90–2000 *m/z*) with the following parameters: VCap, 3500 V; fragmentor, 175 V; gas temperature, 325 °C; drying gas, 8 L/min; nebulizer, 30 psig. The Agilent MassHunter Qualitative Analysis software (version 10.0) was used for EIC extraction, peak integration and accurate mass measurement (Supplementary Table 4).

Tandem MS/MS was performed with the chromatographic separation and source parameters described above, using the targeted MS/MS acquisition mode of the instrument with isolation width set to 'Medium' (~4 amu) and collision energy set to 20 or 30 eV. LC-MS/MS of nematode and yeast lysates was run by the AIMS Mass Spectrometry Laboratory in the Department of Chemistry at the University of Toronto, Canada.

#### Buffer LC-MS Analysis

Dried buffer samples were solubilized in 120  $\mu$ L of acidified acetonitrile (ACN) solution (20% ACN, 0.1% formic acid). The samples were vortexed and centrifuged at 17,949g for 2 minutes. Chromatographic separation was performed by reversed-phase chromatography (ZORBAX Eclipse Plus C18 column, 2.1 x 50 mm, 1.8-Micron particle size, and 2.1 x 5 mm guard column) using an Agilent 1260 Infinity II HPLC system and mobile phase A (H<sub>2</sub>O, 0.1% formic acid) and B (ACN, 0.1% formic acid). The column compartment was maintained at 40°C. 2.5  $\mu$ L of sample was injected at 100% A and 0.250 mL/min flow, followed by a linear gradient to 75% B over 22 minutes (0.250 mL/min flow), and finally a linear gradient to 100% B over 1 minute (0.250 mL/min flow). ESI-MS analyses were carried out using an Agilent 6545 quadrupole time-of-flight mass

analyzer operated in positive scanning mode (100-1000  $m/z$ ) with the following parameters: VCap, 3500 V; fragmentor, 110 V; gas temperature, 275 °C; drying gas, 10 L/min; nebulizer, 35 psig; sheath gas temperature, 350 °C; sheath gas flow, 12 L/min. The Agilent MassHunter Qualitative Analysis software (version 10.0) was used for EIC extraction, integration and quantification of EIC peak area.

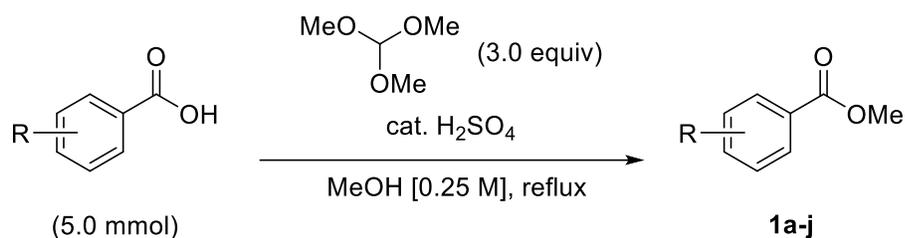
## Synthesis of Cyprocide Analogs

### Chemistry-General Considerations

Unless otherwise stated, all reactions were carried out under an atmosphere of dry argon, using glassware that was either oven (120 °C) or flame-dried. Work-up and isolation of compounds was performed using standard benchtop techniques. All commercial reagents were purchased from chemical suppliers (Sigma-Aldrich, Combi-Blocks, or Alfa Aesar) and used without further purification. Dry solvents were obtained using standard procedures (dichloromethane and acetonitrile were distilled over calcium hydride). Togni's reagent I was prepared according to literature procedures (39). Reactions were monitored using thin-layer chromatography (TLC) on EMD Silica Gel 60 F254 plates. Visualization was performed under UV light (254 nm) or using potassium permanganate (KMnO<sub>4</sub>) stain. Flash column chromatography was performed on Siliaflash P60 40-63  $\mu\text{m}$  silica gel purchased from Silicycle. NMR characterization data were obtained at 293 K on a Varian Mercury 300 MHz, Varian Mercury 400 MHz, Bruker Advance III 400 MHz, Agilent DD2 500 MHz equipped with a 5 mm Xses cold probe or Agilent DD2 600 MHz. <sup>1</sup>H spectra were referenced to the residual solvent signal (CDCl<sub>3</sub> = 7.26 ppm, DMSO-*d*<sub>6</sub> = 2.50 ppm). <sup>13</sup>C{<sup>1</sup>H} spectra were referenced to the residual solvent signal (CDCl<sub>3</sub> = 77.16 ppm, DMSO-*d*<sub>6</sub> = 39.52 ppm). Data for <sup>1</sup>H NMR are reported as follows: chemical shift ( $\delta$  ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, h = sextet, m = multiplet, br = broad), coupling constant (Hz), integration. NMR spectra were recorded at the University of Toronto Department of Chemistry NMR facility (<http://www.chem.utoronto.ca/facilities/nmr/nmr.html>). Infrared spectra were recorded on a PerkinElmer Spectrum 100 instrument equipped with a single-bounce diamond/ZnSe ATR accessory in the solid state and are reported in wavenumber (cm<sup>-1</sup>) units. High resolution mass spectra (HRMS) were obtained on a JEOL JMS-T200GC AccuTOF

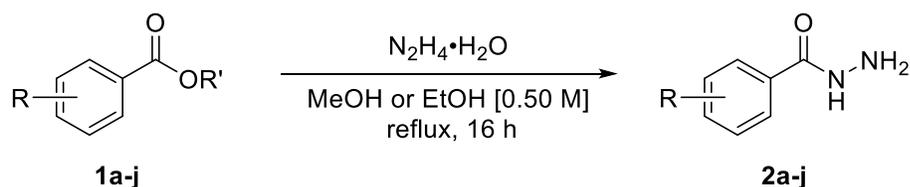
GCx plus(EI) or an Agilent 6538 UHD Q-TOF (ESI) or a JEOL JMS-100LP AccuTOFLC- plus 4G mass spectrometer equipped with an IONICS® Direct Analysis in Real Time (DART) ion source at the Advanced Instrumentation for Molecular Structure (AIMS) facility of the Department of Chemistry at the University of Toronto (<https://sites.chem.utoronto.ca/chemistry/facilities/massspec/about.htm>). Melting point ranges were determined on a Fisher-Johns melting point apparatus and are reported uncorrected.

### General Procedure A



This reaction was performed in air using glassware directly from the drawer. A round bottom flask equipped with a PTFE-coated magnetic stir bar was charged with carboxylic acid (5.0 mmol, 1.0 equiv). Methanol (20 mL), followed by concentrated sulfuric acid (1 drop per mmol), and trimethyl orthoformate (1.64 mL, 15.0 mmol, 3.0 equiv) were added sequentially at room temperature. The solution was stirred at reflux for 16 h. The solution was cooled to room temperature and concentrated *in vacuo*. The residue was then diluted with ethyl acetate and the organic layer was washed twice with a saturated solution of NaHCO<sub>3</sub>, water, and brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo* to afford the methyl benzoates (**1a-j**). The crude material was used in the following step without further purification.

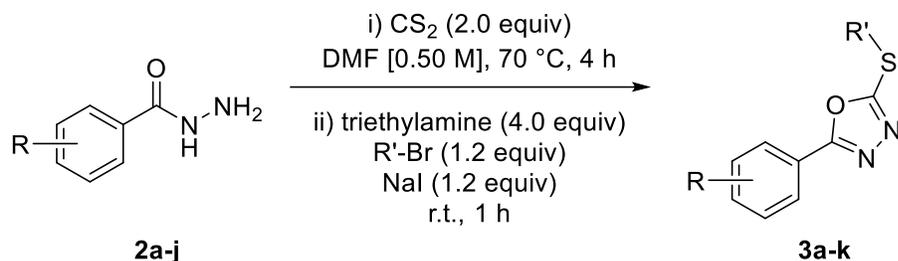
### General Procedure B



This reaction was performed in air using glassware directly from the drawer. A round bottom flask was equipped with a PTFE-coated magnetic stir bar was charged with methyl or ethyl benzoates (5.0 mmol). Methanol (10 mL) or ethanol (10 mL) was added, followed by N<sub>2</sub>H<sub>4</sub>·H<sub>2</sub>O (0.20 mL per mmol). The solution was stirred at reflux for 16 h.

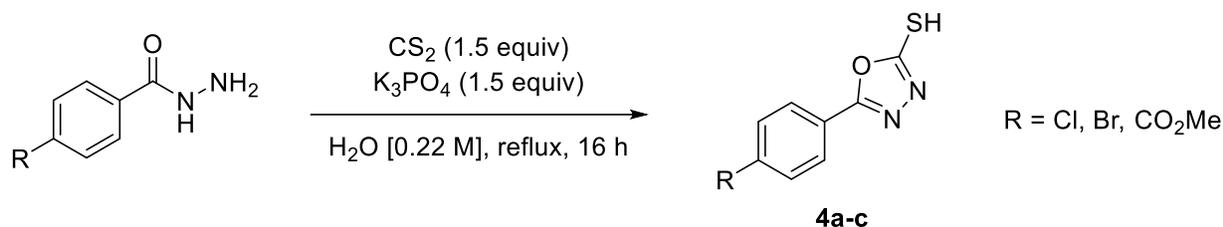
The solution was cooled to room temperature and concentrated *in vacuo*. The crude solid was recrystallized from methanol or ethanol to afford the aryl hydrazides (**2a-j**).

### General Procedure C



A modified literature procedure for the one-pot synthesis of oxadiazoles was followed (40). A flame-dried 9-dram vial was charged with a PTFE-coated magnetic stir bar and cooled under a positive pressure of argon. To the vial was added hydrazide (1.0 mmol, 1.0 equiv), anhydrous DMF (2.0 mL), followed by  $\text{CS}_2$  (0.18 mL, 3.0 mmol, 3.0 equiv). The solution was stirred at 70 °C for 4 h. The mixture was then cooled to ambient temperature. To the vial were added triethylamine (0.50 mL, 4.0 mmol, 4.0 equiv), alkyl bromide (1.2 mmol, 1.2 equiv), and NaI (0.18 g, 1.2 mmol, 1.2 equiv) sequentially, and the resulting mixture was stirred over night at ambient temperature. The solution was diluted with water and extracted twice with ethyl acetate. The combined organic layers were washed four times with brine, dried over  $\text{MgSO}_4$ , filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography with the indicated eluent to afford the alkylated oxadiazoles (**3a-k**).

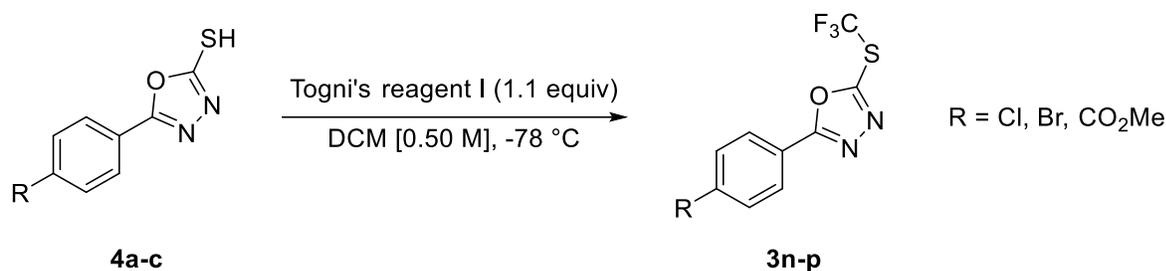
### General Procedure D



A modified literature procedure was followed (41). This reaction was not performed under inert atmosphere and the glassware was not flame- or oven-dried. A round bottom flask equipped with a PTFE-coated magnetic stir bar was charged with hydrazide (380 mg, 2.2 mmol, 1.0 equiv),  $\text{K}_3\text{PO}_4$  (709 mg, 3.3 mmol, 1.5 equiv), distilled

water (10 mL), and CS<sub>2</sub> (0.20 mL, 3.3 mmol, 1.5 equiv). The solution was stirred at reflux for 16 h. The mixture was allowed to cool to ambient temperature, and 1 M HCl was added dropwise until pH 7, leading to the precipitation of a white solid. The solid was collected by vacuum filtration and washed with ice cold distilled water. The thiols **4a-c** were used as is for future steps.

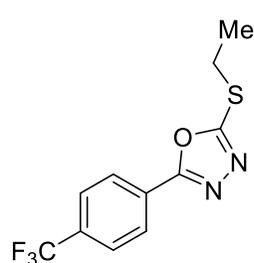
### General Procedure E



A flame-dried 9-dram vial was charged with a PTFE-coated magnetic stir bar and cooled under a positive pressure of argon. To the vial was added thiol (106 mg, 0.5 mmol, 1.0 equiv.) and DCM (1 mL). The solution was cooled to -78 °C, and Togni's reagent I (263 mg, 0.8 mmol, 1.6 equiv) dissolved in DCM (1 mL) was added dropwise. The mixture was stirred at -78 °C for three hours and allowed to warm to room temperature. The mixture was concentrated *in vacuo*, and purified by flash column chromatography with the indicated eluent to afford products **3n-p**.

### Characterization data for products

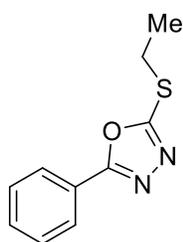
#### **2-(ethylthio)-5-(4-(trifluoromethyl)phenyl)-1,3,4-oxadiazole (3a)(cyprocide-V)**



Synthesized according to general procedures A, B, and C from 4-(trifluoromethyl)benzoic acid. The crude material was purified by flash column chromatography (2.5% → 5% EtOAc in pentanes) to afford **3a** as an off-white solid (183 mg, 67% yield from last step), (mp = 76–78 °C). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 8.13 (dd, *J* = 8.9, 0.8 Hz, 2H), 7.76 (dd, *J* = 8.9, 0.7 Hz, 2H), 3.34 (q, *J* = 7.4 Hz, 3H), 1.53

(t, *J* = 7.4 Hz, 3H). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ 165.5, 164.6, 133.3 (q, *J* = 32.9 Hz), 127.1, 126.2 (q, *J* = 3.8 Hz), 124.8, 122.6, 27.2, 14.8. <sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>) δ -63.1. IR (neat): 2942, 1622, 1559, 1505, 1469, 1414, 1310, 1201, 1113. HRMS (DART): calc for C<sub>11</sub>H<sub>10</sub>N<sub>2</sub>OF<sub>3</sub>S 275.0460 [M+H]<sup>+</sup>, found 275.0469.

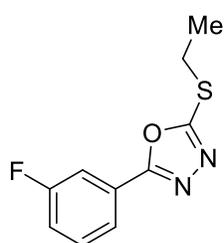
#### **2-(ethylthio)-5-phenyl-1,3,4-oxadiazole (3b)(cyprocide-X)**



Synthesized according to general procedures B and C from methyl benzoate. The crude material was purified by flash column chromatography (gradient elution, 2.5% → 10% EtOAc in pentanes) to afford **3b** as a white solid (48.2 mg, 23% yield from last step).

Characterization data matched that previously reported (42). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.03 – 7.96 (m, 2H), 7.58 – 7.43 (m, 2H), 3.31 (q, *J* = 7.4 Hz, 1H), 1.51 (t, *J* = 7.4 Hz, 1H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.8, 164.5, 131.7, 129.1, 126.8, 123.8, 27.2, 14.9.

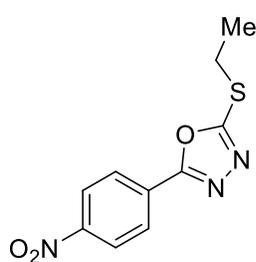
### 2-(ethylthio)-5-(3-fluorophenyl)-1,3,4-oxadiazole (**3c**)(cyprocide-P)



Synthesized according to general procedure C from 3-fluorobenzohydrazide. The crude material was purified by flash column chromatography (gradient elution, 5% → 10% EtOAc in pentanes) to afford **3c** as an off-white solid (98.2 mg, 44% yield from last step). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.80 (ddd, *J* = 7.8, 1.5, 1.0 Hz, 1H), 7.72 – 7.68 (m, 1H), 7.50 – 7.43 (m, 1H), 7.21 (tdd, *J* = 8.4, 2.6, 1.0 Hz, 1H), 3.32 (q, *J* = 7.3 Hz, 2H), 1.52 (t, *J* = 7.4 Hz, 3H).

**<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.0, 164.8 (d, *J* = 3.4 Hz), 162.9 (d, *J* = 247.7 Hz), 131.0 (d, *J* = 8.1 Hz), 125.7 (d, *J* = 8.6 Hz), 122.5 (d, *J* = 3.3 Hz), 118.8 (d, *J* = 21.3 Hz), 113.8 (d, *J* = 24.4 Hz), 27.2, 14.8. **<sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>)** δ -111.2. **IR (neat):** 2925, 1557, 1486, 1458, 1341, 1308, 1267, 1230, 1172. **HRMS (DART):** calc for C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>OFS 225.0492 [M+H]<sup>+</sup>, found 225.0494.

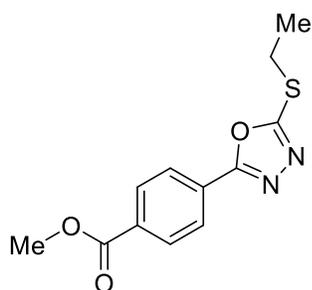
### 2-(ethylthio)-5-(4-nitrophenyl)-1,3,4-oxadiazole (**3d**)(cyprocide-T)



Synthesized according to general procedures B and C from methyl 4-nitrobenzoate. The crude material was purified by flash column chromatography (gradient elution, 5% → 15% EtOAc in pentanes) to afford **3d** as a yellow solid (157 mg, 62% yield from last step), (mp = 98–100 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.41 – 8.32 (m, 2H), 8.23 – 8.16 (m, 2H), 3.36 (q, *J* = 7.4 Hz, 2H), 1.54 (t, *J* = 7.4 Hz, 3H).

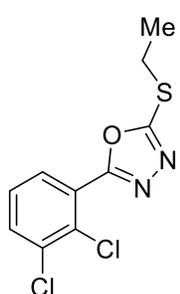
**<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.3, 164.0, 149.5, 129.3, 127.6, 124.5, 27.3, 14.8. **IR (neat):** 3108, 1603, 1558, 1513, 1436, 1416, 1361, 1343, 1191. **HRMS (DART):** calc for C<sub>10</sub>H<sub>10</sub>N<sub>3</sub>O<sub>3</sub>S 252.0437 [M+H]<sup>+</sup>, found 252.0438.

### methyl 4-(5-(ethylthio)-1,3,4-oxadiazol-2-yl)benzoate (**3e**)(cyprocide-U)



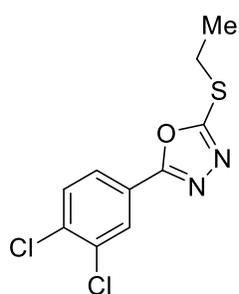
Synthesized according to general procedures B and C from dimethyl terephthalate. General procedure C was modified using iodoethane *in lieu* of bromoethane and NaI. The crude material was purified by flash column chromatography (gradient elution, 5% → 15% EtOAc in pentanes). The material was recrystallized from pentanes to afford **3e** as a white solid (40.2 mg, 15% yield from last step), (mp = 93–95 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.18 – 8.12 (m, 2H), 8.11 – 8.05 (m, 2H), 3.95 (s, 3H), 3.34 (q, *J* = 7.4 Hz, 2H), 1.52 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.2, 165.4, 165.0, 132.8, 130.4, 127.6, 126.7, 52.6, 27.2, 14.8. **IR (neat):** 2954, 1724, 1616, 1584, 1498, 1469, 1438, 1410, 1311. **HRMS (DART):** calc for 265.0641 C<sub>12</sub>H<sub>13</sub>N<sub>2</sub>O<sub>3</sub>S [M+H]<sup>+</sup>, found 265.0646.

### 2-(2,3-dichlorophenyl)-5-(ethylthio)-1,3,4-oxadiazole (**3f**)(cyprocide-R)



Synthesized according to general procedures A, B, and C from 2,3-dichlorobenzoic acid. The crude material was purified by flash column chromatography (gradient elution, 5% → 10% EtOAc in pentanes) to afford **3f** as an off-white solid (135 mg, 49% yield from last step), (mp = 28–30 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.84 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.63 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.34 (t, *J* = 8.0 Hz, 1H), 3.33 (q, *J* = 7.4 Hz, 2H), 1.53 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.6, 163.6, 135.2, 133.2, 131.7, 129.5, 127.7, 125.3, 27.2, 14.9. **IR (neat):** 2961, 1526, 1466, 1458, 1443, 1396, 1263, 1199, 1184. **HRMS (DART):** calc for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>OSCl<sub>2</sub> 274.9807 [M+H]<sup>+</sup>, found 274.9812.

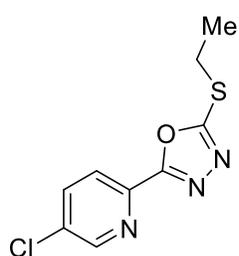
### 2-(3,4-dichlorophenyl)-5-(ethylthio)-1,3,4-oxadiazole (**3g**)(cyprocide-S)



Synthesized according to general procedures A, B, and C from 3,4-dichlorobenzoic acid. The crude material was purified by flash column chromatography (gradient elution, 5% → 10% EtOAc in pentanes) to afford **3g** as a white solid (138 mg, 50% yield from last step), (mp = 63–65 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.09 (d, *J* = 2.0 Hz, 1H), 7.84 (dd, *J* = 8.4, 2.0 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 3.33 (q, *J* = 7.4 Hz, 2H), 1.52 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.3, 164.0, 136.2, 133.8, 131.4, 128.4, 125.7, 123.6, 27.2, 14.8. **IR (neat):** 3087, 1602,

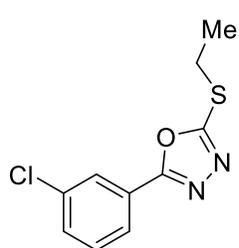
1542, 1454, 1393, 1374, 1270, 1256, 1188. **HRMS (DART):** calc for C<sub>10</sub>H<sub>9</sub>N<sub>2</sub>OSCl<sub>2</sub> 274.9807 [M+H]<sup>+</sup>, found 274.9812.

### 2-(5-chloropyridin-2-yl)-5-(ethylthio)-1,3,4-oxadiazole (3h)(cyprocide-Z)



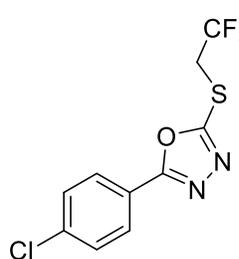
Synthesized according to general procedures A, B, and C from 5-chloropicolinic acid. The crude material was purified by flash column chromatography (gradient elution, 5% → 10% EtOAc in pentanes) to afford **3h** as an off-white solid (116 mg, 48% yield from last step), (mp = 63–65 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.68 (dd, *J* = 2.4, 0.8 Hz, 1H), 8.14 (dd, *J* = 8.5, 0.7 Hz, 1H), 7.84 (dd, *J* = 8.5, 2.4 Hz, 1H), 3.34 (q, *J* = 7.4 Hz, 2H), 1.51 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.4, 164.2, 149.4, 141.5, 137.2, 134.5, 123.6, 27.2, 14.7. **IR (neat):** 3047, 2968, 2927, 1563, 1538, 1472, 1455, 1418, 1112. **HRMS (DART):** calc for C<sub>9</sub>H<sub>9</sub>N<sub>3</sub>OSCl 242.0149 [M+H]<sup>+</sup>, found 242.0156.

### 2-(3-chlorophenyl)-5-(ethylthio)-1,3,4-oxadiazole (3i)(cyprocide-O)



Synthesized according to general procedures B, and C from ethyl 3-chlorobenzoate. The crude material was purified by flash column chromatography (gradient elution, 2.5% → 10% EtOAc in pentanes) to afford **3i** as an off-white solid (147 mg, 61% yield from last step), (mp = 40–42 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.98 (ddd, *J* = 2.1, 1.7, 0.5 Hz, 1H), 7.89 (ddd, *J* = 7.7, 1.7, 1.2 Hz, 1H), 7.48 (ddd, *J* = 8.1, 2.1, 1.2 Hz, 1H), 7.43 (ddd, *J* = 8.1, 7.7, 0.5 Hz, 1H), 3.32 (q, *J* = 7.4 Hz, 2H), 1.52 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.1, 164.6, 135.3, 131.7, 130.5, 126.7, 125.4, 124.8, 27.2, 14.8. **IR (neat):** 2964, 2927, 2867, 1553, 1352, 1412, 1175, 1088, 1065. **HRMS (DART):** calc for C<sub>10</sub>H<sub>10</sub>N<sub>2</sub>OSCl 241.0197 [M+H]<sup>+</sup>, found 241.0208.

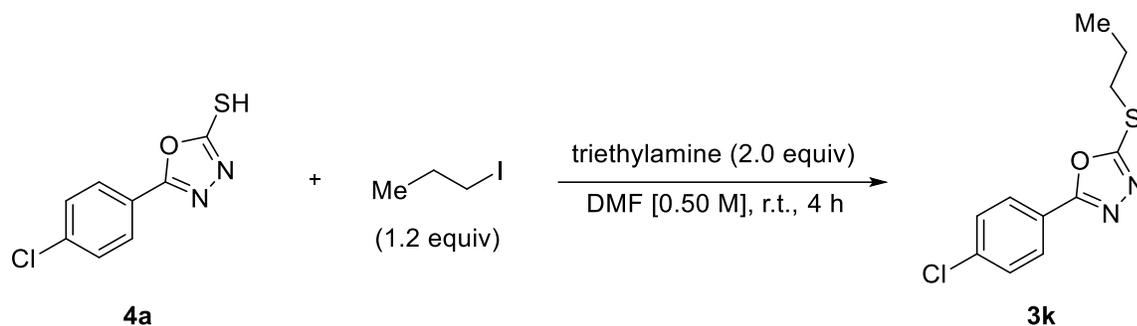
### 2-(4-chlorophenyl)-5-((2,2,2-trifluoroethyl)thio)-1,3,4-oxadiazole (3j)(cyprocide-G)



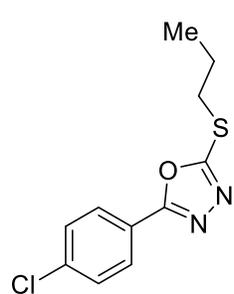
Synthesized according to general procedures B and C from methyl 4-chlorobenzoate. General procedure C was modified using alkyl iodide 1,1,1-trifluoro-2-iodoethane *in lieu* of alkyl bromide and NaI. The crude material was purified by flash column chromatography (gradient elution, 5% → 10% EtOAc in pentanes). The material was recrystallized from pentanes to afford **3j** as a beige solid (30.3 mg, 10% yield from last

step), (mp = 77–79 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.97 – 7.93 (m, 2H), 7.52 – 7.47 (m, 2H), 3.99 (q, *J* = 9.3 Hz, 2H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.0, 161.8, 138.5, 129.7, 128.2, 124.3 (q, *J* = 276.7 Hz), 121.8, 34.4 (q, *J* = 35.3 Hz). **<sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>)** δ -66.9. **IR (neat):** 3009, 2957, 1608, 1553, 1476, 1316, 1279, 1180, 1125. **HRMS (DART):** calc for C<sub>10</sub>H<sub>7</sub>N<sub>2</sub>OF<sub>3</sub>SCI 294.9914 [M+H]<sup>+</sup>, found 294.9918.

#### Synthesis of 2-(4-chlorophenyl)-5-(propylthio)-1,3,4-oxadiazole (3k)

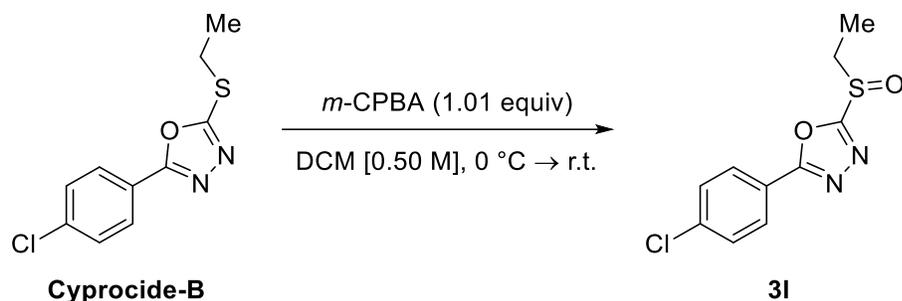


#### **2-(4-chlorophenyl)-5-(propylthio)-1,3,4-oxadiazole (3k)(cyproicide-J)**

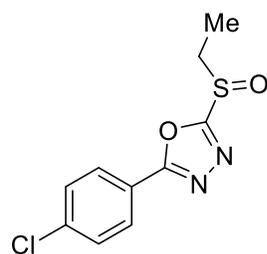


A flame-dried 9-dram vial was charged with a PTFE-coated magnetic stir bar and cooled under a positive pressure of argon. To the vial were added thiol **4** (149 mg, 0.7 mmol, 1.0 equiv.), DMF (1.4 mL), 1-iodopropane (82 μL, 0.84 mmol, 1.2 equiv.), and triethylamine (0.20 mL, 1.4 mmol, 2.0 equiv), and the resulting mixture was stirred at ambient temperature for 4 h. The solution was diluted with water and extracted twice with ethyl acetate. The combined organic layers were washed four times with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (gradient elution, 2.5% → 10% EtOAc in pentanes) to afford **3k** as an off-white solid (150 mg, 84% yield), (mp = 55–57 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.95 – 7.92 (m, 2H), 7.49 – 7.44 (m, 2H), 3.28 (dd, *J* = 7.5, 6.9 Hz, 2H), 1.88 (h, *J* = 7.4 Hz, 2H), 1.08 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 165.0, 165.0, 137.9, 129.5, 128.0, 122.3, 34.7, 22.8, 13.3. **IR (neat):** 2952, 2927, 2867, 1607, 1487, 1460, 1412, 1368, 1125. **HRMS (DART):** calc for C<sub>11</sub>H<sub>12</sub>N<sub>2</sub>O SCl 255.0353 [M+H]<sup>+</sup>, found 255.0369.

#### Synthesis of 2-(4-chlorophenyl)-5-(ethylsulfinyl)-1,3,4-oxadiazole (3l)

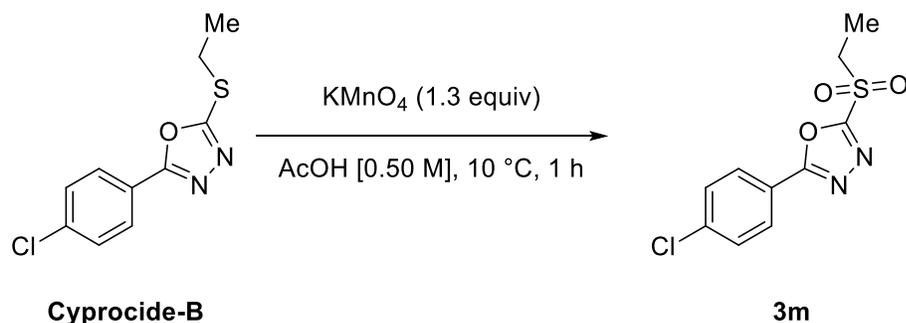


### 2-(4-chlorophenyl)-5-(ethylsulfinyl)-1,3,4-oxadiazole (**3I**)(cyprocide-K)

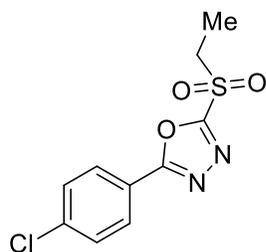


**Cyprocide-B** was prepared according to general procedures B and C from methyl 4-chlorobenzoate. A flame-dried 9-dram vial was charged with a PTFE-coated magnetic stir bar and cooled under a positive pressure of argon. To the vial was added **Cyprocide-B** (122 mg, 0.51 mmol, 1.0 equiv) and DCM (5mL). The solution was cooled to 0 °C and *m*-CPBA (89.7 mg, 0.52 mmol, 1.01 equiv) was added portionwise. The mixture was then stirred at 0 °C for 2 h and then warmed to ambient temperature. The mixture was diluted with DCM, washed twice with a saturated aqueous solution of NaHCO<sub>3</sub> and once with brine. The organic layer was dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (gradient elution, 30% → 35% EtOAc in pentanes) to afford **3I** as a white solid (84.8 mg, 65% yield), (mp = 56–58 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.11 – 8.01 (m, 2H), 7.57 – 7.50 (m, 2H), 3.47 (qd, *J* = 7.4, 2.1 Hz, 2H), 1.48 (t, *J* = 7.5 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.7, 165.9, 139.4, 129.9, 128.9, 121.2, 47.7, 6.4. **IR (neat):** 2934, 1600, 1544, 1478, 1470, 1403, 1185, 1116, 1089. **HRMS (DART):** calc for C<sub>9</sub>H<sub>5</sub>N<sub>2</sub>OF<sub>3</sub>SCI 280.9758 [M+H]<sup>+</sup>, found 280.9768.

### Synthesis of 2-(4-chlorophenyl)-5-(ethylsulfonyl)-1,3,4-oxadiazole (**3m**)

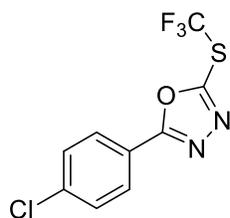


### 2-(4-chlorophenyl)-5-(ethylsulfonyl)-1,3,4-oxadiazole (**3m**)(cyprocide-L)



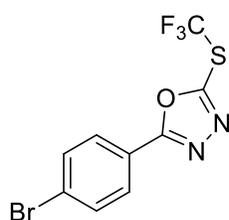
**Cyprocide-B** was prepared according to general procedures B and C from methyl 4-chlorobenzoate. A modified literature procedure was followed for the oxidation of sulfide to sulfone (42). A flame-dried 9-dram vial was charged with a PTFE-coated magnetic stir bar and cooled under a positive pressure of argon. To the vial was added **Cyprocide-B** (96.3 g, 0.51 mmol, 1.0 equiv) and glacial acetic acid (1 mL). The solution was cooled to 10 °C and KMnO<sub>4</sub> (82.2 mg, 0.52 mmol, 1.3 equiv) was added as a 5% aqueous solution over 10 min. The mixture was then stirred at 10 °C for 1 h and then warmed to ambient temperature after which a 40% NaHSO<sub>3</sub> solution was added until the colour dissipated. The mixture was extracted with twice with ethyl acetate and the combined organic layers were washed with brine, dried over MgSO<sub>4</sub>, filtered, and concentrated *in vacuo*. The crude material was purified by flash column chromatography (gradient elution, 10% → 20% EtOAc in pentanes) to afford **3m** as a white solid (72.1 mg, 66% yield). Characterization data matched that previously reported (42). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.17 – 7.99 (m, 2H), 7.59 – 7.48 (m, 2H), 3.62 (q, *J* = 7.4 Hz, 2H), 1.55 (t, *J* = 7.4 Hz, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 166.0, 161.5, 140.0, 130.0, 129.1, 120.7, 50.2, 7.0.

### **2-(4-chlorophenyl)-5-((trifluoromethyl)thio)-1,3,4-oxadiazole (3n)(cyprocide-I)**



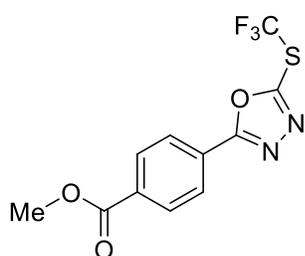
Synthesized according to general procedures A, B, E, and D from methyl 4-chlorobenzoate. The crude material was purified by flash column chromatography (gradient elution, 0% → 10% EtOAc in pentanes). The material was recrystallized from pentanes to afford the product **3n** as a white solid (33.1 mg, 24% yield), (mp = 27–29 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.07 – 7.99 (m, 2H), 7.58 – 7.50 (m, 2H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 167.9, 154.3 (q, *J* = 3.3 Hz), 139.3, 129.7, 128.5, 127.1 (q, *J* = 313.3 Hz), 121.2. **<sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>)** δ -38.7. **IR (neat):** 3090, 2918, 1603, 1544, 1479, 1407, 1299, 1280, 1148. **HRMS (DART):** calc for C<sub>9</sub>H<sub>5</sub>N<sub>2</sub>OF<sub>3</sub>SCl 280.9758 [M+H]<sup>+</sup>, found 280.9768.

### **2-(4-bromophenyl)-5-((trifluoromethyl)thio)-1,3,4-oxadiazole (3o)(cyprocide-N-2)**



Synthesized according to general procedures A, B, E, and D from methyl 4-bromobenzoate. The crude material was purified by flash column chromatography (gradient elution, 2.5% → 10% EtOAc in pentanes). The material was recrystallized from pentanes to afford the product **3o** as a white solid (20.6 mg, 13% yield), (mp = 48–50 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 7.98 – 7.93 (m, 2H), 7.73 – 7.68 (m, 2H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 168.1, 154.5 (q, *J* = 3.2 Hz), 132.9, 128.8, 127.9, 127.2 (q, *J* = 312.6 Hz), 121.8. **<sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>)** δ -38.7. **IR (neat):** 3087, 1599, 1543, 1478, 1403, 1278, 1148, 1092, 1068. **HRMS (DART):** calc for C<sub>9</sub>H<sub>5</sub>N<sub>2</sub>OF<sub>3</sub>SBr 324.9253 [M+H]<sup>+</sup>, found 324.9259.

**methyl 4-(5-((trifluoromethyl)thio)-1,3,4-oxadiazol-2-yl)benzoate (3p)(cyprocide-U-2)**



Synthesized according to general procedures A, B, E, and D from dimethyl terephthalate. The crude material was purified by flash column chromatography (gradient elution, 2.5% → 10% EtOAc in pentanes). The material was recrystallized from pentanes to afford the product **3p** as a white solid (33.1 mg, 24% yield), (mp = 61–63 °C). **<sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):** δ 8.24 – 8.19 (m, 2H), 8.19 – 8.15 (m, 2H), 3.97 (s, 3H). **<sup>13</sup>C NMR (CDCl<sub>3</sub>, 125 MHz):** δ 168.0, 166.0, 154.9 (q, *J* = 3.3 Hz), 134.0, 130.6, 127.4, 127.2 (q, *J* = 312.9 Hz), 126.6, 52.8. **<sup>19</sup>F NMR (375 MHz, CDCl<sub>3</sub>)** δ -38.6. **IR (neat):** 2968, 1718, 1545, 1478, 1446, 1417, 1279, 1158, 1105. **HRMS (DART):** calc for C<sub>11</sub>H<sub>8</sub>N<sub>2</sub>O<sub>3</sub>F<sub>3</sub>S 305.0200 [M+H]<sup>+</sup>, found 305.0198.

## ACKNOWLEDGEMENTS

The authors thank Benjamin Mimee (Agriculture and Agri-Food Canada) and Nathalie Dauphinais (Agriculture and Agri-Food Canada) for providing the *Meloidogyne hapla* and *Pratylenchus penetrans* cultures and for advice on culture methods; Qing Yu (Agriculture and Agri-Food Canada) for providing the *Ditylenchus dipsaci* culture and for advice on culture methods; Keiko Yoshioka (University of Toronto) for providing the *Pseudomonas simiae* and *Pseudomonas defensor* strains; and Nicole Robbins for advice on the *S. cerevisiae* P450 expression assays. Some *C. elegans* strains were provided by the CGC, which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440).

## FUNDING

J.K. is supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) Alexander Graham Bell Canada Graduate Scholarship. E.P. is supported by an EvoFunPath fellowship, through the NSERC Research and Training Experience (CREATE) program (555337-2021). L.E.C. is a Canada Research Chair (Tier 1) in Microbial Genomics & Infectious Disease and co-Director of the CIFAR Fungal Kingdom: Threats & Opportunities program and is supported by CIHR Foundation grant (FDN-154288). P.J.R. is a Canada Research Chair (Tier 1) in Chemical Genetics. P.J.R. is supported by Canadian Institutes of Health Research Project Grants 173448 and 186156.

## COMPETING INTEREST STATEMENT

L.E.C. is co-founder and shareholder in Bright Angel Therapeutics, a platform company for development of novel antifungal therapeutics, and is a Science Advisor for Kapoose Creek, a company that harnesses the therapeutic potential of fungi. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer. J.K., A.R.B., B.C. and P.J.R. have a patent application related to the Cyprocides.

## **AUTHOR CONTRIBUTIONS**

Conceptualization: JK, ARB, PJR

Methodology: JK, ARB, PJR

Investigation: JK, ARB, BC, SRC, MK, JC, JMPC, EP, JS, EK, JBC

Visualization: JK, ARB, PJR

Funding acquisition: PJR, IZ, ML, LEC, IS, ECA

Project administration: PJR

Supervision: PJR, IZ, ML, LEC, IS, ECA

Writing – original draft: JK

Writing – review & editing: JK, PJR

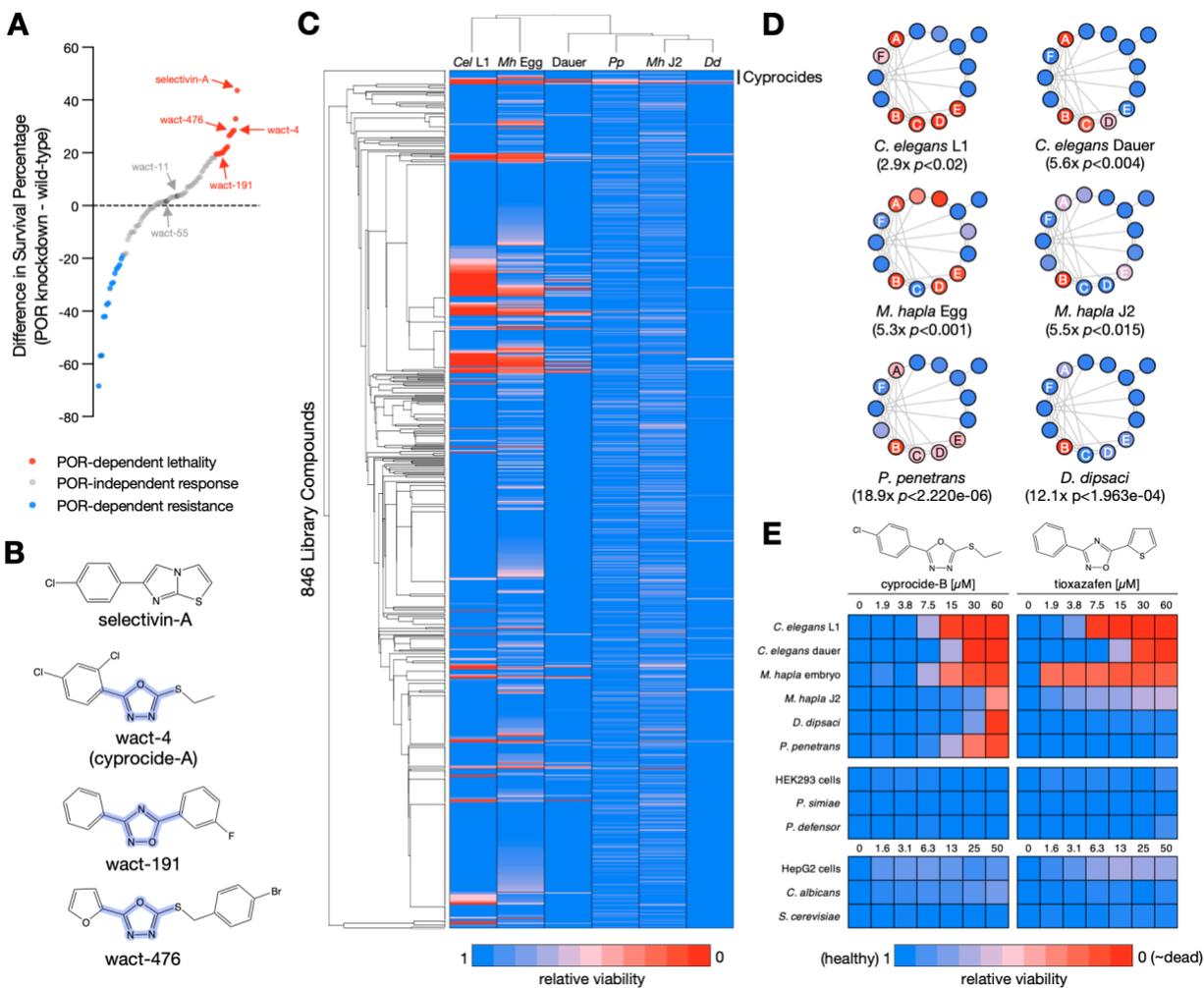
## REFERENCES

1. M. B. Cole, M. A. Augustin, M. J. Robertson, J. M. Manners, The science of food security. *npj Sci Food*. **2**, 14 (2018).
2. N. Alexandratos, J. Bruinsma, World Agriculture towards 2030/2050: the 2012 revision. *ESA Working Paper No. 12-03, Food and Agriculture Organization (FAO), Rome* (2012).
3. M. M. M. Abd-Elgawad, T. H. Askary, Impact of phytonematodes on agriculture economy. *Biocontrol agents of phytonematodes*, 3–49 (2015).
4. M. A. Hassan, T. H. Pham, H. Shi, J. Zheng, Nematodes threats to global food security. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*. **63**, 420–425 (2013).
5. I. A. Zasada, J. M. Halbrendt, N. Kokalis-Burelle, J. LaMondia, M. V. McKenry, J. W. Noling, Managing nematodes without methyl bromide. *Annu Rev Phytopathol*. **48**, 311–328 (2010).
6. L. G. Costa, G. Giordano, M. Guizzetti, A. Vitalone, Neurotoxicity of pesticides: a brief review. *FBL*. **13**, 1240–1249 (2008).
7. N. Donley, The USA lags behind other agricultural nations in banning harmful pesticides. *Environmental Health*. **18**, 44 (2019).
8. A. R. Burns, R. J. Baker, M. Kitner, J. Knox, B. Cooke, J. R. Volpatti, A. S. Vaidya, E. Puumala, B. M. Palmeira, E. M. Redman, J. Snider, S. Marwah, S. W. Chung, M. H. MacDonald, J. Tiefenbach, C. Hu, Q. Xiao, C. A. M. Finney, H. M. Krause, S. A. MacParland, I. Stagljar, J. S. Gilleard, L. E. Cowen, S. L. F. Meyer, S. R. Cutler, J. J. Dowling, M. Lautens, I. Zasada, P. J. Roy, Selective control of parasitic nematodes using bioactivated nematicides. *Nature*. **618**, 102–109 (2023).
9. M. Zhao, J. Ma, M. Li, Y. Zhang, B. Jiang, X. Zhao, C. Huai, L. Shen, N. Zhang, L. He, S. Qin, Cytochrome P450 Enzymes and Drug Metabolism in Humans. *Int J Mol Sci*. **22**, 12808 (2021).
10. F. P. Guengerich, Cytochrome P450 and Chemical Toxicology. *Chem. Res. Toxicol*. **21**, 70–83 (2008).
11. S. M. Attia, Deleterious Effects of Reactive Metabolites. *Oxidative Medicine and Cellular Longevity*. **3**, 238–253 (2010).
12. P. R. Ortiz de Montellano, Cytochrome P450-activated prodrugs. *Future Med Chem*. **5**, 213–228 (2013).
13. D. R. Nelson, Cytochrome P450 diversity in the tree of life. *Biochim Biophys Acta*. **1866**, 141–154 (2018).

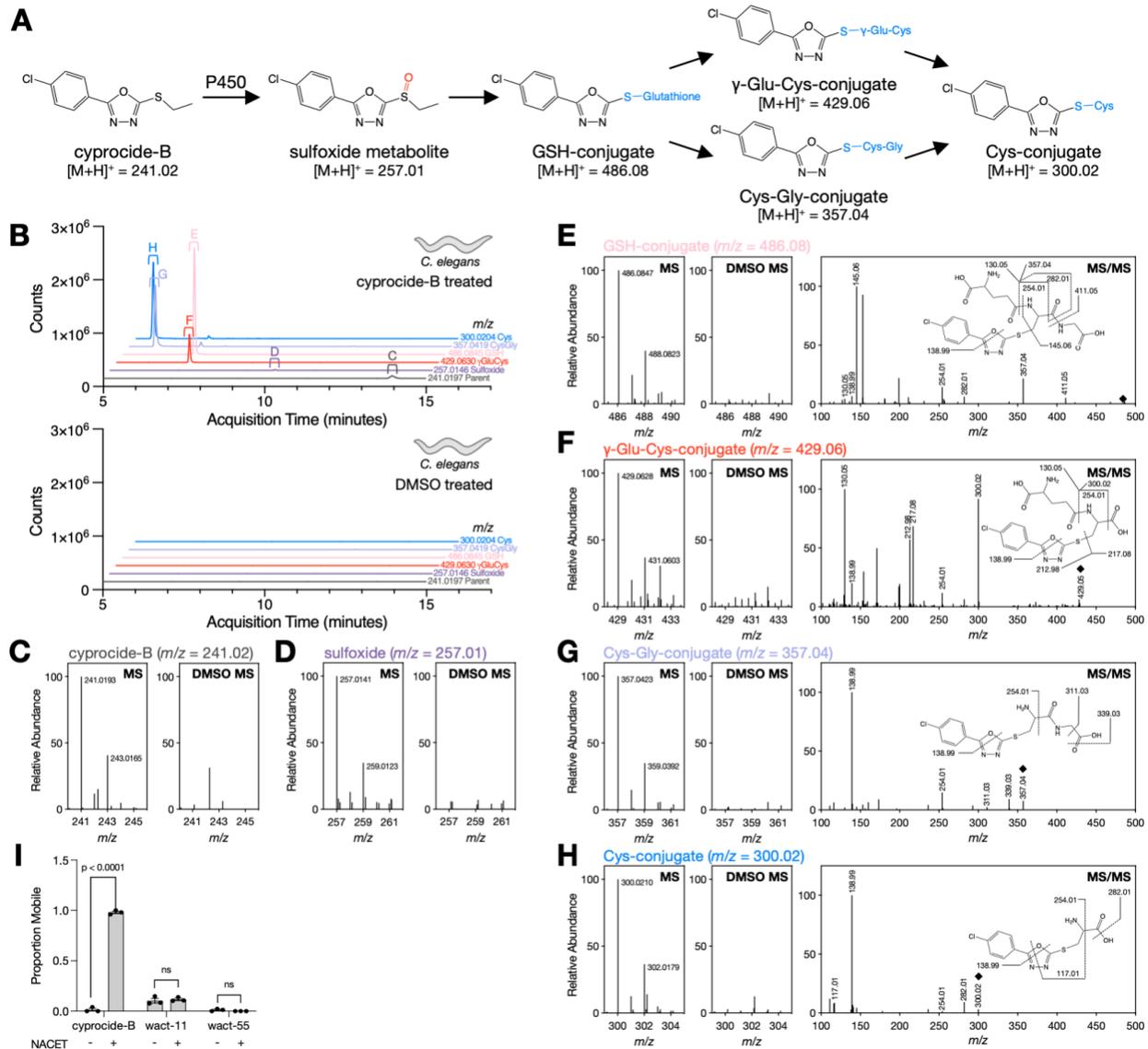
14. A. R. Burns, G. M. Luciani, G. Musso, R. Bagg, M. Yeo, Y. Zhang, L. Rajendran, J. Glavin, R. Hunter, E. Redman, S. Stasiuk, M. Schertzberg, G. Angus McQuibban, C. R. Caffrey, S. R. Cutler, M. Tyers, G. Giaever, C. Nislow, A. G. Fraser, C. A. MacRae, J. Gilleard, P. J. Roy, *Caenorhabditis elegans* is a useful model for anthelmintic discovery. *Nat Commun.* **6**, 7485 (2015).
15. J. Kulas, C. Schmidt, M. Rothe, W.-H. Schunck, R. Menzel, Cytochrome P450-dependent metabolism of eicosapentaenoic acid in the nematode *Caenorhabditis elegans*. *Archives of Biochemistry and Biophysics.* **472**, 65–75 (2008).
16. P. H. Harlow, S. J. Perry, A. J. Stevens, A. J. Flemming, Comparative metabolism of xenobiotic chemicals by cytochrome P450s in the nematode *Caenorhabditis elegans*. *Sci Rep.* **8**, 13333 (2018).
17. M. C. K. Leung, J. V. Goldstone, W. A. Boyd, J. H. Freedman, J. N. Meyer, *Caenorhabditis elegans* Generates Biologically Relevant Levels of Genotoxic Metabolites from Aflatoxin B1 but Not Benzo[a]pyrene In Vivo. *Toxicological Sciences.* **118**, 444–453 (2010).
18. J. Miwa, E. Schierenberg, S. Miwa, G. von Ehrenstein, Genetics and mode of expression of temperature-sensitive mutations arresting embryonic development in *Caenorhabditis elegans*. *Developmental Biology.* **76**, 160–174 (1980).
19. G. Benenati, S. Penkov, T. Müller-Reichert, E. V. Entchev, T. V. Kurzchalia, Two cytochrome P450s in *Caenorhabditis elegans* are essential for the organization of eggshell, correct execution of meiosis and the polarization of embryo. *Mech Dev.* **126**, 382–393 (2009).
20. J. T. Jones, A. Haegeman, E. G. J. Danchin, H. S. Gaur, J. Helder, M. G. K. Jones, T. Kikuchi, R. Manzanilla-López, J. E. Palomares-Rius, W. M. L. Wesemael, R. N. Perry, Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology.* **14**, 946–961 (2013).
21. A. R. Burns, I. M. Wallace, J. Wildenhain, M. Tyers, G. Giaever, G. D. Bader, C. Nislow, S. R. Cutler, P. J. Roy, A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nat Chem Biol.* **6**, 549–557 (2010).
22. H. F. Motiwala, Y.-H. Kuo, B. L. Stinger, B. A. Palfey, B. R. Martin, Tunable Heteroaromatic Sulfones Enhance in-Cell Cysteine Profiling. *J. Am. Chem. Soc.* **142**, 1801–1810 (2020).
23. R. M. LoPachin, B. C. Geohagen, L. U. Nordstroem, Mechanisms of Soft and Hard Electrophile Toxicities. *Toxicology.* **418**, 62–69 (2019).
24. D. Giustarini, A. Milzani, I. Dalle-Donne, D. Tsikas, R. Rossi, N-Acetylcysteine ethyl ester (NACET): A novel lipophilic cell-permeable cysteine derivative with an unusual pharmacokinetic feature and remarkable antioxidant potential. *Biochemical Pharmacology.* **84**, 1522–1533 (2012).

25. L. Larigot, D. Mansuy, I. Borowski, X. Coumoul, J. Dairou, Cytochromes P450 of *Caenorhabditis elegans*: Implication in Biological Functions and Metabolism of Xenobiotics. *Biomolecules*. **12**, 342 (2022).
26. P. R. O. de Montellano, 1-Aminobenzotriazole: A Mechanism-Based Cytochrome P450 Inhibitor and Probe of Cytochrome P450 Biology. *Med chem*. **08** (2018), doi:10.4172/2161-0444.1000495.
27. Y. Oka, From Old-Generation to Next-Generation Nematicides. *Agronomy*. **10**, 1387 (2020).
28. C. L. Wram, I. A. Zasada, Short-Term Effects of Sublethal Doses of Nematicides on *Meloidogyne incognita*. *Phytopathology*®. **109**, 1605–1613 (2019).
29. C. L. Wram, C. N. Hesse, I. A. Zasada, Transcriptional response of *Meloidogyne incognita* to non-fumigant nematicides. *Sci Rep*. **12**, 9814 (2022).
30. T. C. Sparks, A. J. Crossthwaite, R. Nauen, S. Banba, D. Cordova, F. Earley, U. Ebbinghaus-Kintscher, S. Fujioka, A. Hirao, D. Karmon, R. Kennedy, T. Nakao, H. J. R. Popham, V. Salgado, G. B. Watson, B. J. Wedel, F. J. Wessels, Insecticides, biologics and nematicides: Updates to IRAC's mode of action classification - a tool for resistance management. *Pesticide Biochemistry and Physiology*. **167**, 104587 (2020).
31. J. Desaegeer, C. Wram, I. Zasada, New reduced-risk agricultural nematicides - rationale and review. *J Nematol*. **52**, e2020-91 (2020).
32. J. A. Lewis, J. T. Fleming, "Basic Culture Methods" in *Methods in Cell Biology*, H. F. Epstein, D. C. Shakes, Eds. (Academic Press, 1995), vol. 48 of *Caenorhabditis elegans: Modern Biological Analysis of an Organism*, pp. 3–29.
33. T. Stiernagle, Maintenance of *C. elegans*. *WormBook*, 1–11 (2006).
34. A. R. Burns, T. C. Y. Kwok, A. Howard, E. Houston, K. Johanson, A. Chan, S. R. Cutler, P. McCourt, P. J. Roy, High-throughput screening of small molecules for bioactivity and target identification in *Caenorhabditis elegans*. *Nat Protoc*. **1**, 1906–1914 (2006).
35. S. R. Cammalleri, J. Knox, P. J. Roy, Culturing and Screening the Plant Parasitic Nematode *Ditylenchus dipsaci*. *JoVE (Journal of Visualized Experiments)*, e63438 (2022).
36. H. S. Atamian, P. A. Roberts, I. Kaloshian, High and Low Throughput Screens with Root-knot Nematodes *Meloidogyne* spp. *JoVE (Journal of Visualized Experiments)*, e3629 (2012).
37. Y. Cao, A. Charisi, L.-C. Cheng, T. Jiang, T. Girke, ChemmineR: a compound mining framework for R. *Bioinformatics*. **24**, 1733–1734 (2008).

38. P. Shannon, A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski, T. Ideker, Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res.* **13**, 2498–2504 (2003).
39. D. C. Powers, E. Lee, A. Ariafard, M. S. Sanford, B. F. Yates, A. J. Canty, T. Ritter, Connecting binuclear Pd(III) and mononuclear Pd(IV) chemistry by Pd-Pd bond cleavage. *J Am Chem Soc.* **134**, 12002–12009 (2012).
40. M. Soleiman-Beigi, M. Alikarami, T. Hosseinzadeh, One-pot Synthesis of 2-Alkylthio-1,3,4-oxadiazole and Bis-(1,3,4-oxadiazole-2-yl)thio alkyl Derivatives from Acid Hydrazides and CS<sub>2</sub>. *Asian J. Chem.* **25**, 4939–4942 (2013).
41. X. Yan, S. Zhou, Y. Wang, Z. Ge, T. Cheng, R. Li, Propylene oxide assisted one-pot, tandem synthesis of substituted-1,3,4-oxadiazole-2(3H)-ones in water. *Tetrahedron.* **68**, 7978–7983 (2012).
42. W.-M. Xu, F.-F. Han, M. He, D.-Y. Hu, J. He, S. Yang, B.-A. Song, Inhibition of Tobacco Bacterial Wilt with Sulfone Derivatives Containing an 1,3,4-Oxadiazole Moiety. *J. Agric. Food Chem.* **60**, 1036–1041 (2012).



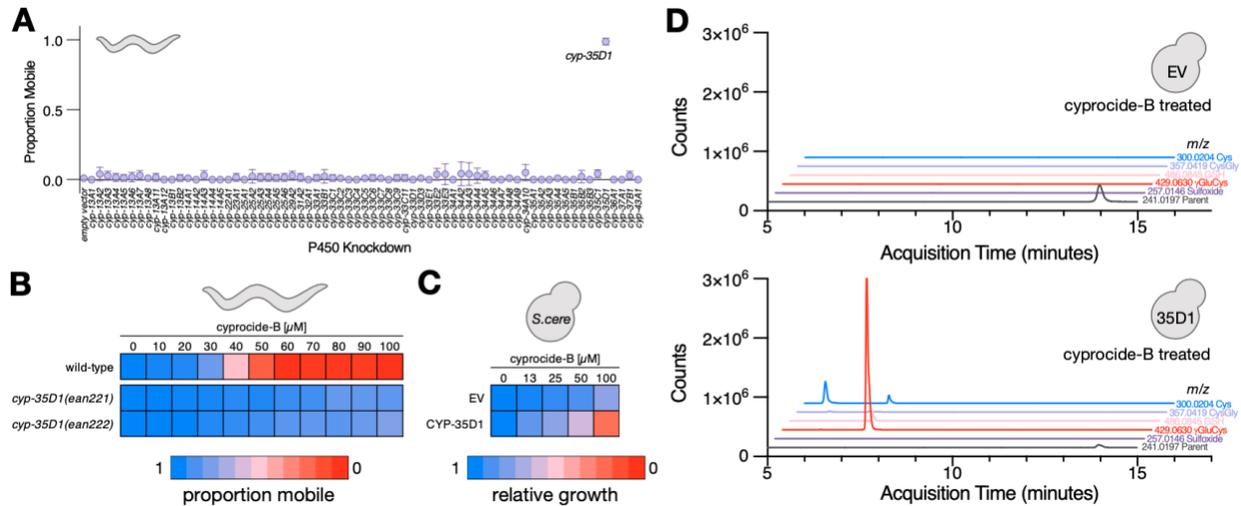
**Figure 1. Cyprocide-B is a selective nematicide with broad-spectrum activity in PPNs.** (A) POR knockdown screen results. Difference in survival percentage between POR knockdown and wild-type conditions is plotted for the 87 unique nematicides tested. (B) Structure of selectivin-A and the three POR-dependent selective nematicides. The shared disubstituted oxadiazole (DODA) core scaffold is highlighted in purple. (C) Summarized screening results for the DODA nematicide library in *C. elegans* larval (Cel L1) and dauer stages, *M. hapla* embryo (Mh Egg) and infective juvenile (Mh J2) stages, *D. dipsaci* (Dd), and *P. penetrans* (Pp). Relative viability is represented by the colour-coded scale. Library compounds are organized by structural similarity. (D) The Cyrocides are shown clustered by structural similarity with screening results from (C) overlaid. Family members (nodes) are connected by an edge if they share a Tanimoto structural similarity score > 0.725. Named analogs are labeled on the nodes. The enrichment of Cyrocides within screen hits and associated hypergeometric p-values are indicated. (E) Dose-response analysis of cyprocide-B and tioxazafen on nematode and non-target organisms, including human cells (HEK293 and HepG2), fungi (*Saccharomyces cerevisiae* and *Candida albicans*), and plant beneficial rhizobacteria (*Pseudomonas simiae* and *P. defensor*). Compound activity is represented by the colour-coded scale.



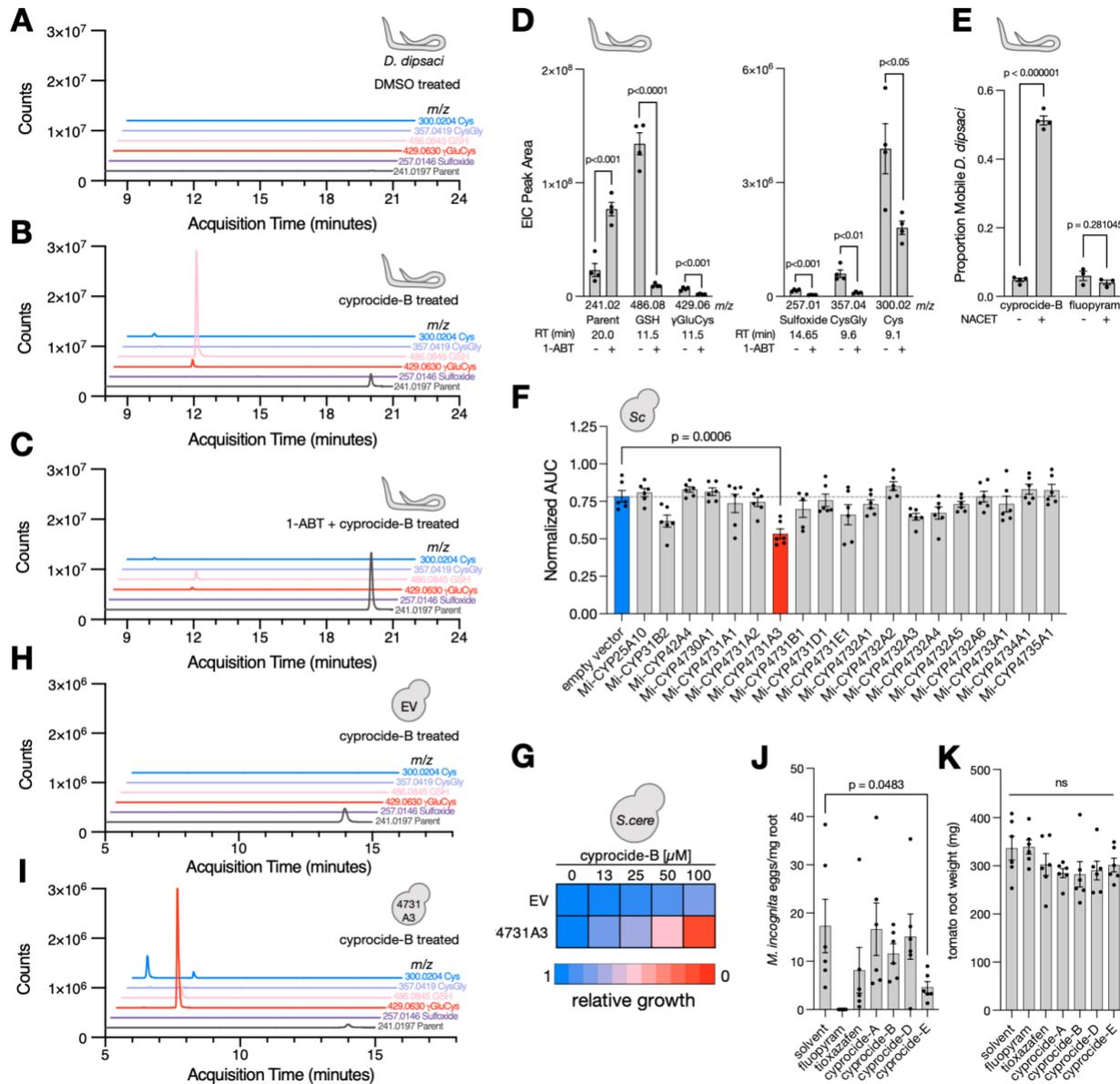
**Figure 2. Cyprocode-B is bioactivated to produce a reactive electrophile. (A)**

Schematic of the proposed cyprocode-B sulfoxidation and low-molecular-weight thiol conjugation pathway. P450s likely catalyze the S-oxidation of cyprocode-B, which produces an electrophilic sulfoxide metabolite. This sulfoxide metabolite reacts with glutathione (GSH) and is likely further processed to  $\gamma$ -glutamylcysteine ( $\gamma$ -Glu-Cys), cysteinylglycine (Cys-Gly) and cysteine (Cys) conjugates. The cyprocode-B sulfoxide metabolite can also react directly with  $\gamma$ -glutamylcysteine, cysteinylglycine and/or cysteine (B) Extracted ion chromatograms (EICs) for indicated m/z values found in cyprocode-B treated *C. elegans* lysate (above) and paired DMSO solvent control (below). (C-H) Mass spectrometry (MS) data for the unmodified cyprocode-B parent (C), the sulfoxide metabolite (D), and the GSH-,  $\gamma$ -Glu-Cys-, Cys-Gly- and Cys-conjugates (E-H) from lysates of *C. elegans* treated with cyprocode-B and paired solvent-treated

controls. The predicted structures of the thiol conjugates are supported by tandem mass spectrometry (MS/MS) (E-H). (I) *C. elegans* L1 larvae were preincubated with NACET or solvent control for four hours before a 24-hour exposure to 25  $\mu$ M cyproside-B, wact-11, or wact-55. The proportion of mobile worms in each condition is reported (n=3 biological replicates (BRs)). Error bars indicate SEM, p-values obtained from unpaired two-tailed Student's t-tests, comparing means of the NACET and solvent preincubation conditions, ns is not significant ( $p>0.05$ ).

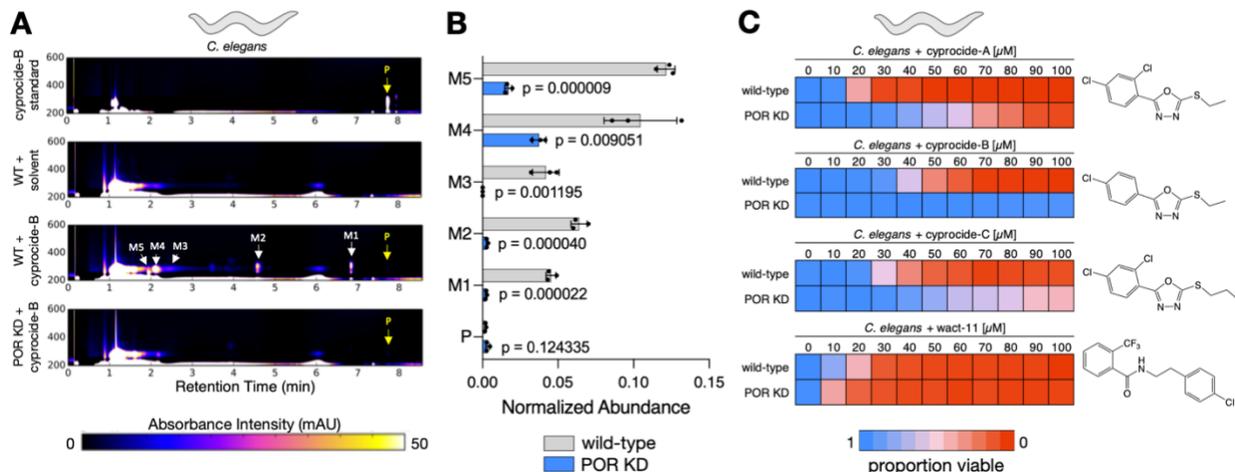


**Figure 3. Cyprodicide-B is bioactivated by *C. elegans* CYP-35D1.** (A) Cytochrome P450 (P450) RNAi knockdown screen results. The proportion of mobile adult *C. elegans* worms is reported in each knockdown condition after a 48-hour exposure to 40  $\mu$ M cyprodicide-B (n=4 technical replicates (TRs)). Error bars indicate standard deviation, p-value comparing the mean of the empty vector to *cyp-35D1* knockdown condition ( $p < 0.0001$ ) was obtained from an unpaired two-tailed Student's t-test. (B) Cyprodicide-B dose-response in adult *C. elegans*. The proportion of mobile worms in each condition is indicated by the colour-coded scale in the wild-type and *cyp-35D1* deletion mutants after a four-day exposure to the indicated concentrations of cyprodicide-B (n=3 BRs). (C) Cyprodicide-B dose-response for a *S. cerevisiae* strain expressing *C. elegans* CYP-35D1 and one carrying an empty vector (EV) that does not express a P450. The mean area under the growth curve in each condition over 48-hours of cyprodicide-B exposure relative to solvent controls is indicated by the colour-coded scale (n=4 BRs). (D) Yeast expressing CYP-35D1 and the EV control strain were exposed to 100  $\mu$ M cyprodicide-B for six hours. Yeast lysates were analyzed by LC-MS and extracted ion chromatograms for the indicated cyprodicide-B parent and metabolite m/z values are shown for both EV and CYP-35D1-expressing strains.



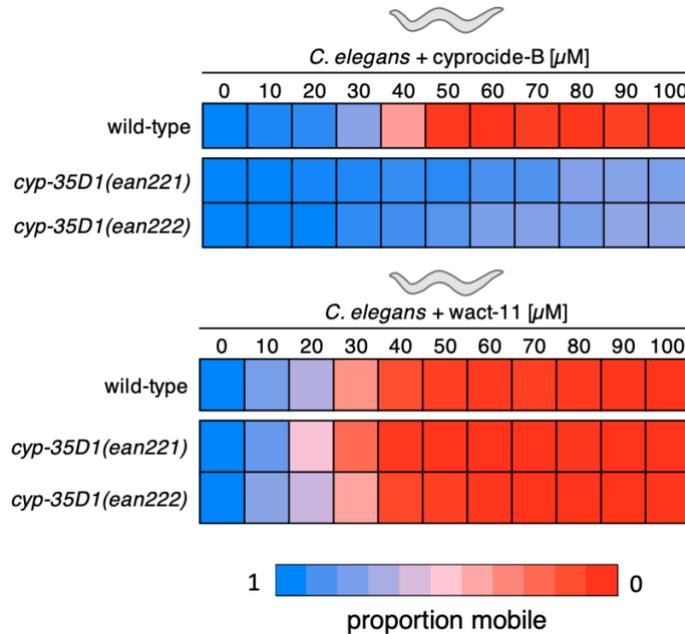
**Figure 4. Cyprodicide-B is bioactivated into a toxic electrophile by P450s in PPNs.** EICs for indicated cyprodicide-B parent and metabolite m/z values from *D. dipsaci* buffer after exposure to (A) DMSO, (B) cyprodicide-B, and (C) 1-ABT plus cyprodicide-B. (D) EIC peak area at indicated retention time (RT) for cyprodicide-B parent and metabolites in *D. dipsaci* buffer after incubation with cyprodicide-B +/- 1-ABT (n=4 BRs). (E) *D. dipsaci* were preincubated +/- NACET before a three-day incubation in cyprodicide-B or fluopyram. The proportion of mobile worms after chemical exposure is reported (n=4 BRs for cyprodicide-B exposures, n=3 BRs for fluopyram exposures). (F) Mean AUC of *M. incognita* P450-expressing yeast strains exposed to 50  $\mu$ M cyprodicide-B, normalized to the solvent control for each strain (n=6 BRs). (G) Cyprodicide-B dose-response for yeast strains expressing CYP4731A3 and EV controls. Mean AUC relative to solvent controls is indicated by the colour-coded scale (n=4 BRs). (H-I) EICs for indicated cyprodicide-B parent and metabolite m/z values from yeast lysates after cyprodicide-B

exposure in EV and CYP4731A3-expressing strains. **(J)** Number of *M. incognita* eggs per milligram of tomato root tissue after six weeks of infection in soil drenched with solvent or indicated chemical is reported (n=6 BRs). **(K)** Tomato root weight at infection assay endpoint in each condition (n=6 BRs). Error bars represent SEM, p-values were obtained from unpaired two-tailed Student's t-tests, ns is not significant (p>0.05).



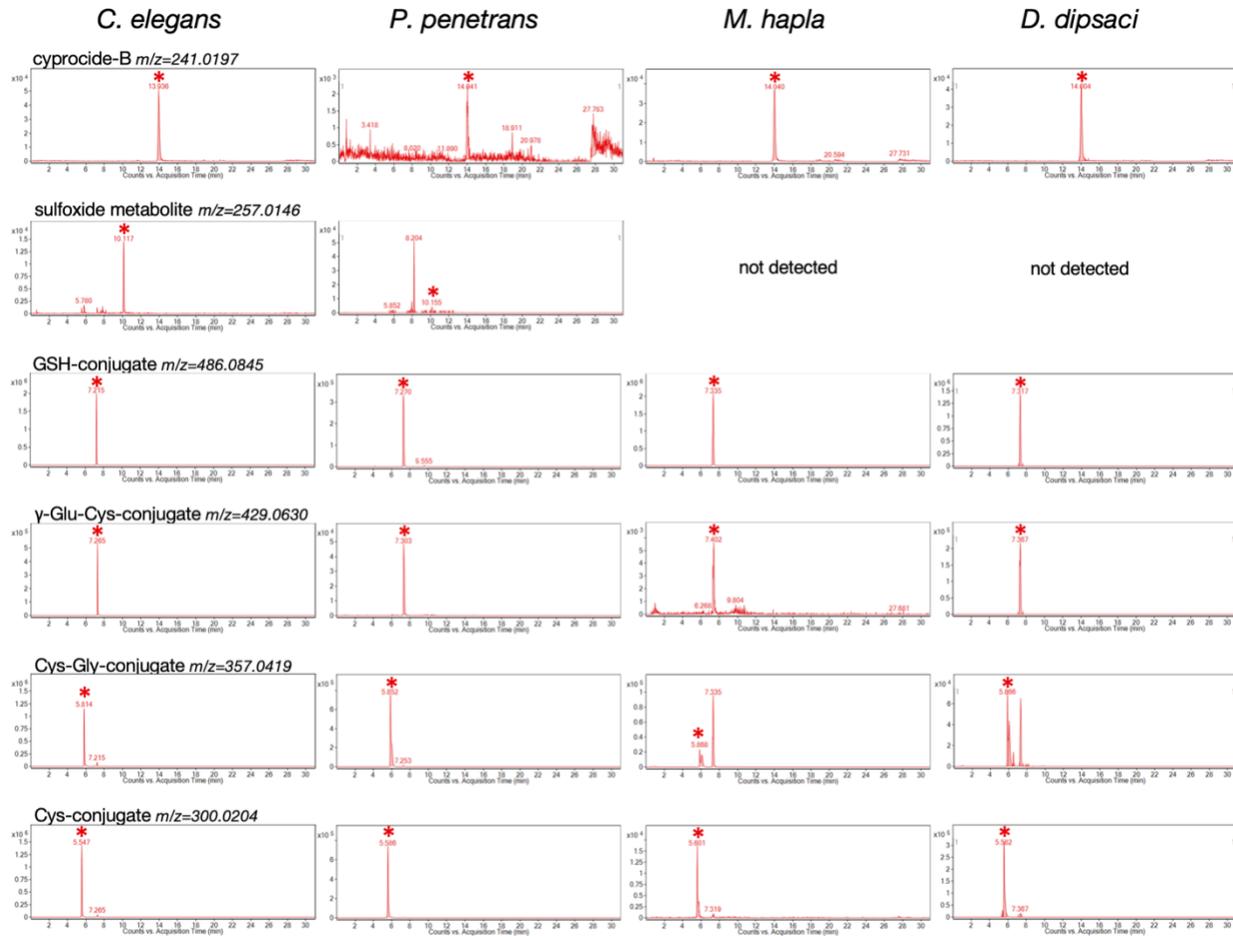
**Figure S1. The Cyprocides are metabolically bioactivated by P450s in *C. elegans*.**

(A) HPLC chromatograms of *C. elegans* lysates after six-hour incubation with 100  $\mu\text{M}$  cyprocide-B. Cyprocide-B is transformed into five metabolites visible by HPLC-DAD. Peaks corresponding to unmodified cyprocide-B parent (P) and metabolites (M1-M5) are indicated in wild-type (WT) and POR knockdown (POR KD) conditions. The Y-axis indicates absorbance wavelength in nm. (B) HPLC-DAD quantification of parent and metabolite abundance from (A) in WT and POR KD conditions ( $n=3$  BRs). Error bars represent SD, p-values were obtained from unpaired two-tailed Student's t-tests comparing the mean normalized abundance in WT and POR KD conditions (C) Viability of adult *C. elegans* wild-type or POR KD worms after a 48-hour incubation in each chemical exposure condition is indicated by the colour-coded scale ( $n=3$  BRs). Structures of assayed compounds are shown.

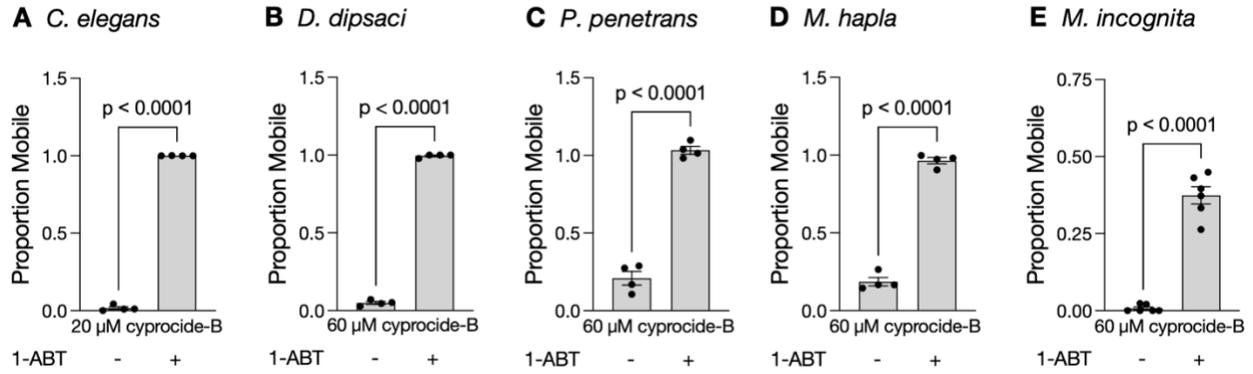


**Figure S2. *C. elegans* CYP-35D1 is required for cyproside-B-induced lethality.**

Adult *C. elegans* wild-type (N2), *cyp-35D1(ean221)*, and *cyp-35D1(ean222)* deletion mutants were exposed to the indicated concentrations of cyproside-B (A) or wact-11 (B) for four days. The average proportion of mobile worms in each condition at the endpoint of the assay is indicated by the colour coded scale (n=3 BRs).

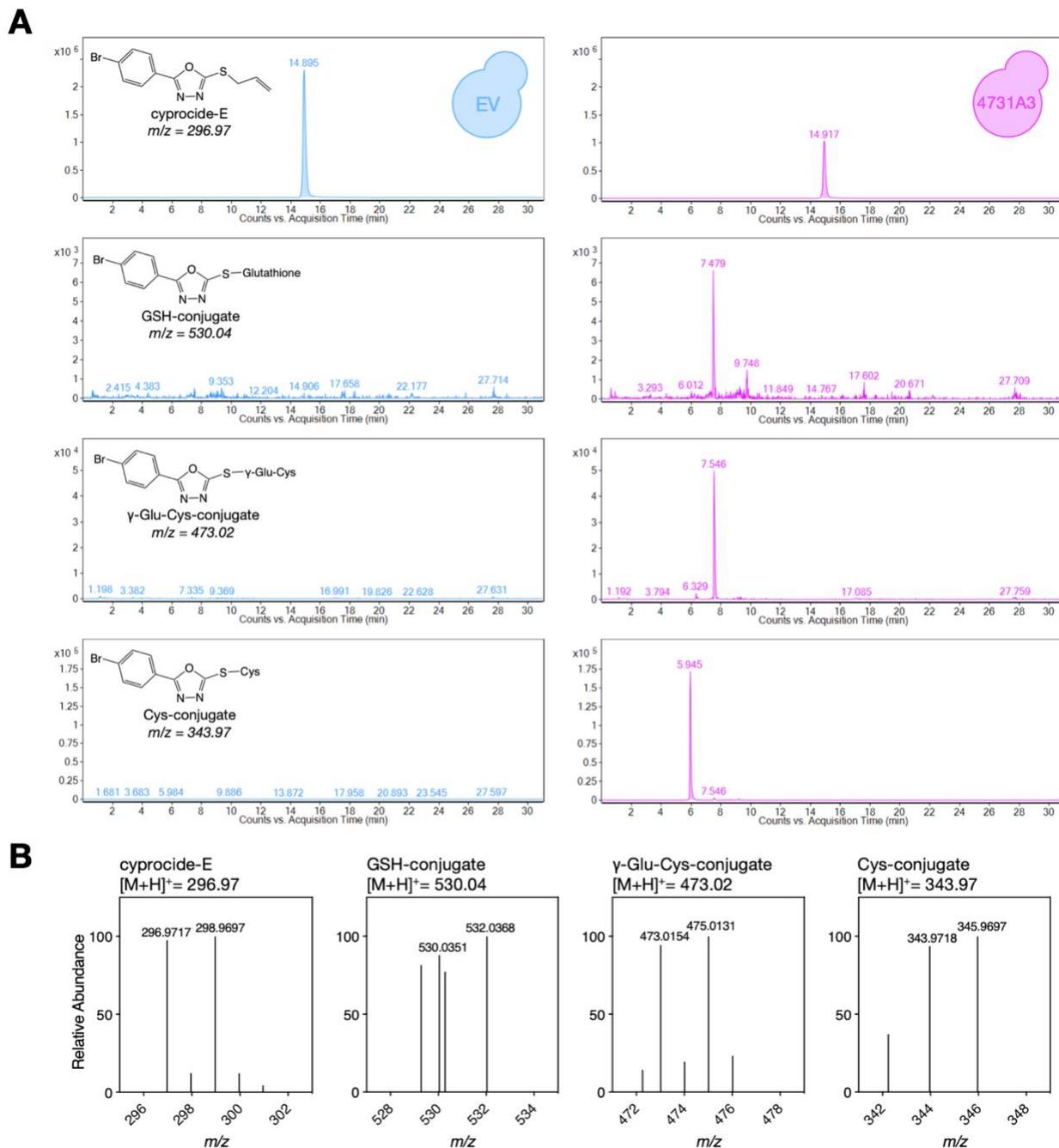


**Figure S3. Cyprodicide-B is metabolized in diverse nematode species.** Nematode lysates were analysed by LC-MS after a six-hour (*C. elegans*), 16-hour (*P. penetrans* and *M. hapla* J2), or 24-hour (*D. dipsaci*) incubation with 100  $\mu$ M cyprodicide-B. EICs for the indicated  $m/z$  values of the cyprodicide-B parent and metabolites are shown for each species. Asterisks indicate the analyte peak at the retention time of interest. Analyte peaks with an abundance below the threshold of  $1 \times 10^3$  counts were considered 'not detected'.



**Figure S4. 1-ABT suppresses cyproicide-B activity in *C. elegans* and PPNs.**

Preincubation with 1 mM of the non-specific, irreversible P450 inhibitor 1-ABT suppresses the activity of cyproicide-B in *C. elegans* (n=4 BRs) (A), *D. dipsaci* (n=4 BRs) (B), *P. penetrans* (n=4 BRs) (C), *M. hapla* (n=4 BRs) (D), and *M. incognita* (n=6 TRs) (E). The proportion of mobile worms after chemical exposure is reported (see methods for details). Error bars represent SEM, p-values were obtained from unpaired Student's t-tests comparing the mean proportion mobile in conditions with and without 1-ABT.





**Table S1. Cytochrome P450 cDNA sequences codon optimized for *S. cerevisiae* expression. Contact authors for full sequence.**

Species	P450 Name	<i>S. cerevisiae</i> Codon Optimized cDNA Sequence
<i>C. elegans</i>	CYP-35D1	ATGATACTTATACTTCTTTTTCTTACAGCGATTACCGTTATAACCGTCAGGCTGTATAGGAAGGTGTTACG
<i>M. incognita</i>	CYP4732A1	ATGTTGTTGCTCTTGTGTTGATTACTATCTCTATTATTTCTTGTCTGGGCTGCCATCAGAAACAATA
<i>M. incognita</i>	CYP4732A2	ATGTTGTTGTTATTGCTTCTCATTATCACTATTTCTACTTTGTTTTGTTCTGGGCTGCCATCAGAAACAATA
<i>M. incognita</i>	CYP4732A3	ATGTTCTTGTGATCTTGTGTTGATCACTATCTCTTCTTGTGTTGTTCTGGACTGCTATTAGAAAAAAC/
<i>M. incognita</i>	CYP4732A4	ATGTTGTTAATTTTGATTATTTCTGCTTCTTCTTGTGTTCTGGACTGCTATCAGAAAGAACAACACTACTGGAA/
<i>M. incognita</i>	CYP4732A5	ATGTTCTTGTAAATTTTGCTGTTGATCACCATTCTCTTGTGTTGTTCTGGTCTGCCAAGAGAAAGTCC/
<i>M. incognita</i>	CYP4732A6	ATGTTCTTGTAAATTTTGCTGTTGATCACCATTCTCTTGTGTTGTTCTGGTCTGCCAAGAGAAAGTCC/
<i>M. incognita</i>	CYP4731B1	ATGTTGCCATCTTGTATGGTTGTTCAATTTCTCTTTCATCATCTGTTTCTGGAACCTTCGTCTGGAAGAGAT
<i>M. incognita</i>	CYP4731A3	ATGTTCTTACTTCTTTCTTTTATTCTTCACCACCTTGCTGTTCTGGAACCTTGTCTGGAAAAGAAAGGGT
<i>M. incognita</i>	CYP4733A1	ATGATCATTGGTTGATTTTGCTACATTTGTTCACTTTGATTTTCTGGAACCTTGTCTGGAAAAGAAAGAA/
<i>M. incognita</i>	CYP4731A1	ATGTTCTTATTGTTGTTCTTGTCTTCACCACCTTGTGTTCTGGAACTTCGTCTGGAAGAGAAAGGGT
<i>M. incognita</i>	CYP4731D1	ATGGCTTGTGTTATCCTCCCTATTTCCCTATTTAATCAACTGTATCTTATCTTGATCATCTTTACAACCTT
<i>M. incognita</i>	CYP4731A2	ATGTTCTAATCTAATTTTGCTATTGATCATCTTGCCTTCTGGAACCTTATCTGGAAGAGAAGAAACTT
<i>M. incognita</i>	CYP4731E1	ATGTTCTGCTGCTATCATTTTATTGTTTATTCTTGTCTTCTACTGGAACCTTCATATACAAGAGACAAGGT
<i>M. incognita</i>	CYP4734A1	ATGTTGTTTTGTTCTTCGGTATCTTGCTAGCTTTGTTCCAATTGTTGTTATTCTACAACCTTTACTGGAAGA
<i>M. incognita</i>	CYP4730A1	ATGCCACCAGGTCCATCCATTTCCATTGATCGGTAACCTTGCCAAGTTGGACTTGAGATATCCGCACA/
<i>M. incognita</i>	CYP4735A1	ATGCCAGAACAATGTTTCTTGAGATGGAGAAGAAAGTACGGTAACATCTTCACTATCTGGTTCGGTGAA
<i>M. incognita</i>	CYP42A4	ATGCTTTCTGGGATTTTAATTGTCACCAATTTTCTCTTGGTTTTTCGTTTGGTTCCTAAGAAAGTACG
<i>M. incognita</i>	CYP31B2	ATGTTGATTGCTCCTCAATCTTGTGTTCTCTTTCATCATCTTCTGCGCTATTTTGTGTACAGACACATCA
<i>M. incognita</i>	CYP25A10	ATGTACTCAATTCGGTTGGGTGACGAATTTGCTATTAATTTAATGTTGATCTTCGTCTTCTACAAGTTCACCI

**Table S2. Solvent and flow rate gradients for HPLC analysis of *C. elegans* lysates**

<b>Time</b>	<b>Flow Rate (mL/min)</b>	<b>% Solvent A Acid)</b>	<b>% Solvent B 95:4.9:0.1 (ACN:H2O:Acetic Acid)</b>
0.00	1.3	85.0	15.0
4.49	1.3	43.5	56.5
4.50	1.0	43.5	56.5
5.54	1.0	42.0	58.0
6.54	2.0	30.0	70.0
8.04	3.0	0.0	100.0
8.34	3.0	0.0	100.0
8.35	2.0	85.0	15.0
8.65	2.0	85.0	15.0

**Table S3. Solvent and flow rate gradients for LC-MS analysis of nematode and yeast lysates**

Time	Flow Rate (mL/min)	% Solvent A		% Solvent B	
		H2O, 0.1% Formic Acid		ACN, 0.1% Formic Acid	
0.00	0.35	95.0		5.0	
1.00	0.35	95.0		5.0	
21.00	0.35	5.0		95.0	
21.01	0.35	0.0		100.0	
26.00	0.35	0.0		100.0	
26.01	0.35	95.0		5.0	
31.00	0.35	95.0		5.0	

**Table S4. Exact mass and accurate mass values for cyprocide-B, cyprocide-E and metabolites**

System	Analyte	Ionic Formula	Ion Species	Exact Mass	Accurate Mass	+/- (ppm)
<i>C. elegans</i>	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0193	-1.659615
<i>C. elegans</i>	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	257.0141	-1.945415
<i>C. elegans</i>	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0847	0.411451
<i>C. elegans</i>	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0628	-0.466132
<i>C. elegans</i>	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0423	1.120317
<i>C. elegans</i>	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0210	1.999864
<i>S. cerevisiae</i> + EV	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0212	6.223558
<i>S. cerevisiae</i> + EV	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	not detected	not detected
<i>S. cerevisiae</i> + EV	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	not detected	not detected
<i>S. cerevisiae</i> + EV	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	not detected	not detected
<i>S. cerevisiae</i> + EV	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	not detected	not detected
<i>S. cerevisiae</i> + EV	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	not detected	not detected
<i>S. cerevisiae</i> + CYP-35D1	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0207	4.149038
<i>S. cerevisiae</i> + CYP-35D1	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	not detected	not detected
<i>S. cerevisiae</i> + CYP-35D1	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0865	4.114511
<i>S. cerevisiae</i> + CYP-35D1	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0658	6.525848
<i>S. cerevisiae</i> + CYP-35D1	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0436	4.761346
<i>S. cerevisiae</i> + CYP-35D1	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0223	6.332903
<i>S. cerevisiae</i> + CYP4731A3	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0207	4.149038
<i>S. cerevisiae</i> + CYP4731A3	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	not detected	not detected
<i>S. cerevisiae</i> + CYP4731A3	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0871	5.348864
<i>S. cerevisiae</i> + CYP4731A3	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0664	7.924244
<i>S. cerevisiae</i> + CYP4731A3	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0439	5.601583
<i>S. cerevisiae</i> + CYP4731A3	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0226	7.332835
<i>D. dipsaci</i>	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0177	-8.298077
<i>D. dipsaci</i>	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	not detected	not detected
<i>D. dipsaci</i>	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0809	-7.40612
<i>D. dipsaci</i>	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0593	-8.670055
<i>D. dipsaci</i>	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0390	-8.122296
<i>D. dipsaci</i>	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0182	-7.332835
<i>P. penetrans</i>	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0176	-8.712981
<i>P. penetrans</i>	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	257.0142	-1.556332
<i>P. penetrans</i>	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0805	-8.229022
<i>P. penetrans</i>	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0594	-8.390376
<i>P. penetrans</i>	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0396	-6.413813
<i>P. penetrans</i>	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0187	-5.666281
<i>M. hapla</i>	cyprocide-B	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> OS	(M+H) <sup>+</sup>	241.0197	241.0174	-9.542788
<i>M. hapla</i>	sulfoxide	C <sub>10</sub> H <sub>10</sub> ClN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	257.0146	not detected	not detected
<i>M. hapla</i>	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> ClN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	486.0845	486.0806	-8.023296
<i>M. hapla</i>	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> ClN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	429.0630	429.0589	-9.555706
<i>M. hapla</i>	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> ClN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	357.0419	357.0383	-10.08285
<i>M. hapla</i>	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> ClN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	300.0204	300.0175	-9.666009
System	Analyte	Ionic Formula	Ion Species	Exact Mass	Accurate Mass	+/- (ppm)
<i>S. cerevisiae</i> + EV	cyprocide-E	C <sub>11</sub> H <sub>10</sub> BrN <sub>2</sub> OS	(M+H) <sup>+</sup>	296.9692	296.9707	5.051029
<i>S. cerevisiae</i> + EV	sulfoxide	C <sub>11</sub> H <sub>10</sub> BrN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	312.9641	not detected	not detected
<i>S. cerevisiae</i> + EV	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> BrN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	530.0340	not detected	not detected
<i>S. cerevisiae</i> + EV	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> BrN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	473.0125	not detected	not detected
<i>S. cerevisiae</i> + EV	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> BrN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	400.9914	not detected	not detected
<i>S. cerevisiae</i> + EV	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> BrN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	343.9699	not detected	not detected
<i>S. cerevisiae</i> + CYP4731A3	cyprocide-E	C <sub>11</sub> H <sub>10</sub> BrN <sub>2</sub> OS	(M+H) <sup>+</sup>	296.9692	296.9716	8.081646
<i>S. cerevisiae</i> + CYP4731A3	sulfoxide	C <sub>11</sub> H <sub>10</sub> BrN <sub>2</sub> O <sub>2</sub> S	(M+H) <sup>+</sup>	312.9641	not detected	not detected
<i>S. cerevisiae</i> + CYP4731A3	GSH-conjugate	C <sub>18</sub> H <sub>21</sub> BrN <sub>5</sub> O <sub>7</sub> S	(M+H) <sup>+</sup>	530.0340	530.0371	5.848681
<i>S. cerevisiae</i> + CYP4731A3	γ-Glu-Cys-conjugate	C <sub>16</sub> H <sub>18</sub> BrN <sub>4</sub> O <sub>6</sub> S	(M+H) <sup>+</sup>	473.0125	473.0151	5.496683
<i>S. cerevisiae</i> + CYP4731A3	Cys-Gly-conjugate	C <sub>13</sub> H <sub>14</sub> BrN <sub>4</sub> O <sub>4</sub> S	(M+H) <sup>+</sup>	400.9914	not detected	not detected
<i>S. cerevisiae</i> + CYP4731A3	Cys-conjugate	C <sub>11</sub> H <sub>11</sub> BrN <sub>3</sub> O <sub>3</sub> S	(M+H) <sup>+</sup>	343.9699	343.9720	6.105185

Accurate mass was not determined for those analytes with an abundance below the threshold of 1 x 10<sup>3</sup> counts which were considered 'not detected'

**Data S1. (separate file)**

Details on the EMB-8 disruption survey for bioactivated worm-active molecules (wactives). Available upon request.

**Data S2. (separate file)**

Details on the DODA screen for molecules that kill *C. elegans* and plant parasitic nematodes. Available upon request.