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Deleterious Consequences of UDP-Galactopyranose Mutase Inhibition for Nematodes

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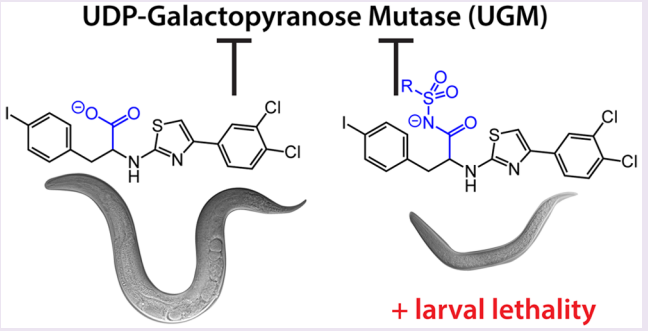
Supporting Information

ABSTRACT: Parasitic nematodes pose a serious threat to agriculture, livestock, and human health. Increasing resistance to antiparasitic agents underscores the need to replenish our anthelmintic arsenal. The nonpathogenic *Caenorhabditis elegans*, which serves as an effective model of parasitic helminths, has been used to search for new anthelmintic leads. We previously reported small-molecule inhibitors of the essential *C. elegans* protein UDP-galactopyranose mutase (UGM or Glf). This enzyme is required for the generation of galactofuranose (Gal*f*)-containing glycans and is needed in nematodes for proper cuticle formation. Though our first-generation inhibitors were effective *in vitro*, they elicited no phenotypic effects. These findings are consistent with the known difficulty of targeting nematodes. *C. elegans* is recalcitrant to pharmacological modulation; typically, less than 0.02% of small molecules elicit a phenotypic effect, even at 40 μM. We postulated that the lack of activity of the UGM inhibitors was due to their carboxylic acid group, which can be exploited by nematodes for detoxification. We therefore tested whether replacement of the carboxylate with an *N*-acylsulfonamide surrogate would result in active compounds. UGM inhibitors with the carboxylate mimetic can phenocopy the deleterious consequences of UGM depletion in *C. elegans*. These findings support the use of UGM inhibitors as anthelmintic agents. They also outline a strategy to render small-molecule carboxylates more effective against nematodes.



Nematodes include both parasitic organisms (helminths) and free-living species such as the model organism *Caenorhabditis elegans*.[1−6] Human infections by parasitic nematodes cause worldwide suffering, especially within the developing world. Over 1.5 billion people are infected with helminths, corresponding to 24% of the world's population.[7−9] Helminth damage extends beyond direct human infection. In the United States alone, helminths cause annual losses estimated at $2 billion in the cattle industry and $80 billion in crop damage.[10,11] The emergence of anthelmintic resistance has spurred the search for new strategies with which to combat the parasites.[12] The helminth glycocalyx can modulate host immune responses,[13−18] while serving as a critical protective barrier.[19] Thus, understanding the roles of glycans in parasitic nematodes could uncover new strategies to combat these pathogens.

Extracellular glycoconjugates are critical components of the protective nematode exoskeleton termed the cuticle. Cuticle glycoconjugates and the collective outer glycocalyx are needed for nematode motility and structural integrity. One proposed component of this glycocalyx is the thermodynamically disfavored isomer of galactose: galactofuranose (Gal*f*). The production of Gal*f*-containing glycoconjugates requires the enzyme uridine 5′-diphosphate (UDP) galactopyranose mutase (UGM or Glf). UGM, which is encoded by the *glf-1* ortholog present in the free-living nematode *C. elegans*,[20] catalyzes the interconversion of UDP-galactopyranose (UDP-Gal*p*) and UDP-galactofuranose (UDP-Gal*f*)[20,21] (Figure 1). Biochemical characterization of *C. elegans* UGM (CeUGM) authenticated its ability to interconvert UDP-Gal*p* and UDP-Gal*f*,[22] and deletion of this gene revealed a crucial role for UGM in nematode development.[23,24] Although Gal*f* residues have yet to be identified within any specific nematode glycoconjugate, the deleterious effects of UGM deletion suggest that Gal*f*-containing glycans have essential roles.[25−27] UGM orthologs are encoded by parasitic helminths including *Brugia malayi*, *Ascaris*, *Brugia*, and *Dirofilaria* spp.[22,28,29] In contrast, humans lack Gal*f* residues and therefore UGM. Together, these analyses provide impetus to elucidate UGM and Gal*f* function in nematodes.

Small molecule probes of UGM would be valuable tools to examine the importance of the enzyme in nematodes. Indeed,

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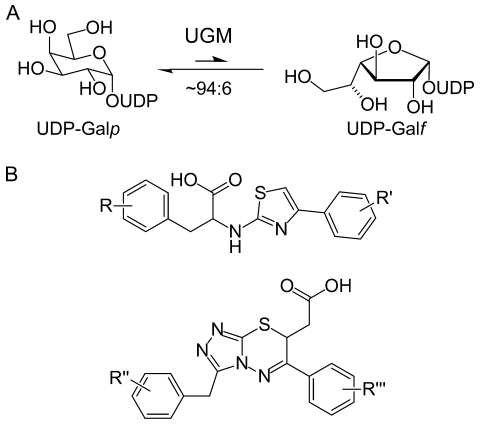

Figure 1. (A) UDP-galactopyranose mutase (UGM) catalyzes the interconversion of UDP-Gal*p* and UDP-Gal*f*. (B) Core structures of UGM small-molecule inhibitor scaffolds.

compounds that have been reported to block UGM activity also act against *Brugia malayi*.[30] There are two concerns with these observations, however. First, UGM produced from all other organisms is dimeric, while the *B. malayi* ortholog was reported to be decameric. The unexpected oligomerization state raises questions about whether the active form of the enzyme was isolated, and the experiments purported to quantify enzyme activity did not directly monitor UDP-Gal*f* formation. Second, the putative inhibitors are members of the PAINS (pan assay interference compounds) family. Conclusions about their activity and mechanism of action cannot be drawn without additional experiments.[31] As a result, evidence was lacking that UGM inhibitors are deleterious to nematodes.

We sought inhibitors of UGM that could assess its suitability as a target for anthelmintics. Our plan was to adapt the prokaryotic UGM inhibitors previously described by our group[32−34] for use in nematodes. We had identified two distinct classes of competitively binding small-molecule UGM inhibitors with antimycobacterial potency (Figure 1). The first class includes compounds containing a 2-aminothiazole core[35,36] that can impede both mycobacterium tuberculosis UGM activity and mycobacterial growth.[37] Virtual screening afforded a second class of inhibitors, triazolothiadiazines, which also were effective against mycobacteria.[38] Despite little sequence homology between CeUGM and prokaryotic UGM enzymes,[20] mechanistic and active site similarities between UGM orthologs allow compounds from each inhibitor class to function as potent inhibitors of CeUGM.[22,38]

Parasitic nematodes have complex life-cycles that complicate anthelmintic screening. As a nonpathogenic organism *C. elegans* is a free-living nematode that effectively models helminth biology and therefore serves as a simple model organism.[1−3,6] These properties, combined with our previous experience characterizing CeUGM, led us to focus on testing inhibitors against *C. elegans*. Still, like other nematodes, *C. elegans* is challenging to target with small molecules.[39] Pharmacological probes are typically used in worms at concentrations that are orders of magnitude higher than those employed in tissue culture.[40,41] The need for high probe concentrations to elicit phenotypic effects on nematodes has been attributed to two factors. First, the *C. elegans* genome encodes an array of enzymes to disarm xenobiotics; these include 86 cytochrome P450 enzymes, 72 glutathione S-transferases (GSTs), and 48 glucuronosyltransferases (UGTs).[42] Second, the nematode cuticle, composed of a network of collagen and glycoconjugates, creates a formidable and highly impermeable barrier to small molecules.[43] Herein, we describe inhibitors of CeUGM that can circumvent these barriers and thereby disrupt the nematode life cycle.

RESULTS AND DISCUSSION

Targeting UGM within *C. elegans*. Deletion of the gene encoding CeUGM (*glf-1*) significantly impairs *C. elegans* development.[23] The most notable phenotype is 95% lethality, which occurs in the late stages of embryogenesis and early stages of larval development. Similarly, RNA interference of *glf-1* in the parasitic species *Brugia malayi* results in larval lethality.[24] Experiments by Novelli *et al.* indicate that CeUGM production can first be detected after completion of cuticle synthesis, 1 h prior to hatching. In L4 larvae and adults, the enzyme is found predominantly in the seam cells, which are responsible for producing the carbohydrate-rich surface coat.[44] Nematodes deficient in *glf-1* exhibit distinctive phenotypes that are indicative of a defective surface coat, including impaired motility and compromised cuticle integrity.[45−47] These data suggest Gal*f* glycoconjugates are important constituents of the cuticle.

The consequences of *glf-1* deletion suggest that UGM could serve as an anthelmintic target. When known UGM inhibitors were assessed with wild-type *C. elegans* (N2), however, no effect on proliferation, development, or morphology was detected. The disparity between *in vitro* and *in vivo* efficacy of CeUGM inhibitors was disappointing, yet consistent with the difficulties of targeting *C. elegans* using small molecules. Nematodes employ the previously mentioned defensive features, their cuticle and detoxification mechanisms, to shield themselves from small molecule probes.[42,48−50] We hypothesized that at least one of these protective features mitigated the effects of the UGM inhibitors.

To circumvent nematode defense, we modified the inhibitor structure. A shared feature of the inhibitors tested is a carboxylate group, which we identified as a potential liability. Though the negatively charged carboxylate is not conducive to membrane permeability, our major concern was that carboxylate groups act as reactive sites for detoxification enzymes. Enzymes such as UDP-glucuronosyltransferase can convert a small-molecule carboxylate into an acylglucuronide, thereby rendering it inactive (Figure 2).[51] SAR studies of UGM inhibitors, however, indicate the carboxylate negative charge is critical for potency.[52] We therefore sought a carboxylate mimetic likely to evade detoxification enzymes. We previously

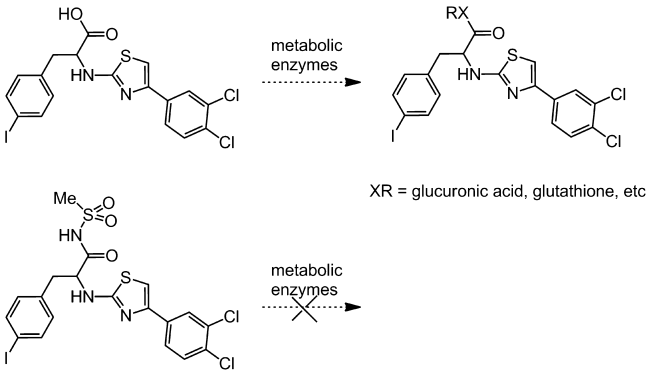

Figure 2. Modification of carboxylic acids by metabolic enzymes.

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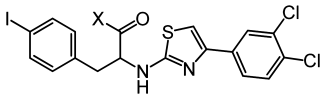
found *N*-acylsulfonamide groups were effective carboxylate surrogates, as they are anionic at physiological pH values.[52] Inhibitors with an *N*-acylsulfonamide group not only blocked MtbUGM enzymatic activity but were superior to their carboxylate counterparts at permeating the mycobacterial cell wall and therefore were more potent antimycobacterial agents. Accordingly, we evaluated their activity against nematodes.

_N_-Acylsulfonamide Analogs Inhibit CeUGM Catalysis. To compare inhibitor potency, we synthesized compounds **1**–**14**.[52] These analogs were tested for their ability to impede the catalytic activity of CeUGM.[22] Carboxylate-containing compounds **1** and **11** were active, but the corresponding methyl esters (compounds **10** and **14**) were significantly less potent. In contrast, each derivative bearing an anionic *N*-acylsulfonamide group was at least equivalent, if not superior, at inhibiting CeUGM compared to the corresponding carboxylate (Table 1, Table 2). These results support our previous findings that the *N*-acylsulfonamide group functions as an effective carboxylate surrogate[52] (Table 1). The most potent inhibitor identified was the chlorophenylsulfonamide **8** (IC_{50} = 0.7 ± 0.3 μM).

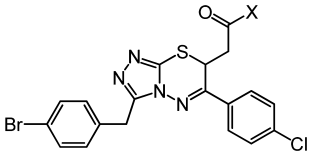
Table 1. Inhibition Potency of Compounds 1–10



compound	X	%inhibition at 2.5 μM[a]	IC_{50} (μM)[b]	EC_{100} (μM)[c]
1	−OH	70 ± 1	1.6 ± 0.6	>250
2	$-NHSO_2CH_3$	81 ± 1	1.1 ± 0.4	100
3	$-NHSO_2CF_3$	85 ± 2	0.9 ± 0.2	100
4	$-NHSO_2Ph$	92 ± 0.2	1.1 ± 0.4	250
5	$-NHSO_2$(4-NO_2−Ph)	93 ± 0.1		150
6	$-NHSO_2$(4-OCH_3−Ph)	92 ± 1		>250
7	$-NHSO_2$(4-CH_3−Ph)	90 ± 0.2		>250
8	$-NHSO_2$(4-Cl-Ph)	100	0.7 ± 0.3	250
9	$-NHSO_2$(2-NO_2−Ph)	92 ± 1		150
10	$-OCH_3$	14 ± 3		>250

[a]The relative activity of recombinant CeUGM was evaluated in the presence of inhibitor. Error values represent the standard deviation from the mean ($n \geq 2$). [b]The relative activity of recombinant CeUGM was evaluated for a range of inhibitor concentrations. Data were analyzed using nonlinear regression analysis with a one-site binding model. The calculated half-maximal inhibition concentration (IC_{50}) is provided with the standard deviation ($n \geq 2$). [c]All EC_{100} values ≤250 μM represent data from synchronized embryos cultured on compound-embedded agar (see methods).

Table 2. Inhibition Potency of Compounds 11–14



compound	X	%inhibition at 10 μM[a]	IC_{50} (μM)[b]	EC_{100} (μM)[c]
11	−OH	53 ± 3		>250
12	$-NHSO_2CH_3$	68 ± 2		>250
13	$-NHSO_2Ph$	92 ± 0.5	3.6 ± 1	250
14	$-OCH_3$	14 ± 3		>250

[a]The relative activity of recombinant CeUGM was evaluated in the presence of inhibitor. Error values represent the standard deviation from the mean ($n \geq 2$). [b]The relative activity of recombinant CeUGM was evaluated for a range of inhibitor concentrations. Data were analyzed using nonlinear regression analysis with a one-site binding model. The calculated half-maximal inhibition concentration (IC_{50}) is provided with the standard deviation ($n \geq 2$). [c]All EC_{100} values ≤250 μM represent data from synchronized embryos cultured on compound-embedded agar (see methods).

Activity of UGM Inhibitors in Nematodes. We assessed the effects of the *N*-acylsulfonamide CeUGM inhibitors on *C. elegans*. Initial testing was performed by synchronizing *C. elegans* as embryos and exposing them to compounds **1**–**14** (250 μM) dissolved in agar. As anticipated from our previous findings, neither carboxylate **1** nor **11** affected growth, and animals treated with either carboxylate appeared phenotypically normal and healthy. We also tested the corresponding methyl esters **10** and **14**. Though ineffective as CeUGM inhibitors as esters, they could serve as pro-inhibitors if unmasked by cellular esterases. However, neither methyl ester variant restricted reproduction or development. In contrast, several *N*-acylsulfonamide derivatives impeded nematode development and proliferation (Tables 1 and 2). When nematodes were maintained on nematode growth medium (NGM) supplemented with compound **4** or **8**, their development was severely restricted. Four days following synchronization, only larva were present. These larva either failed to produce progeny upon reaching adulthood or failed to reach adulthood altogether. More effective inhibitors, such as **2**, **3**, **5**, and **9**, resulted in near complete embryonic lethality; no viable animals were present 4 days following synchronization. Thus, strategic substitution of the carboxylic acid moiety affords inhibitors that act on nematodes.

To compare inhibitor potency quantitatively, we evaluated the concentration dependence of *C. elegans* inhibition (Tables 1 and 2). We assessed the effective concentration at which growth and proliferation is eliminated (EC_{100} value). Compounds **4** and **8** had little effect on nematode proliferation at concentrations lower than 250 μM. In contrast, compounds **5** and **9** had EC_{100} values of 150 μM. Compounds **2** and **3**, which blocked nematode proliferation at 100 μM, were the most efficacious.

We further evaluated the impact of UGM inhibitors on *C. elegans*. We synchronized nematodes and assessed the results of UGM inhibitor exposure after 48 h (Figure 3A). As expected, compounds **2** and **4**, which both have measurable EC_{100} values, afforded substantial lethality. Compared to untreated controls, many dead embryos were visible, and the number of animals decreased. Additionally, animals treated with **2** or **4** exhibited developmental delays. At 48 h following synchronization, the surviving animals were at the L2 or L3 stages, and very few animals treated with **4** had reached adulthood. Consistent with our EC_{100} determinations, proliferation of animals treated with **2** was completely blocked, and no animals that hatched reached adulthood, even 12 days following synchronization. At 48 h after treatment, animals treated with **1** were healthy and progressed through development at the same rate as untreated controls (Figure 3B). However, as observed with exposure to compounds **2** and **4**, carboxylate **1** led to many dead embryos. Indeed, this inhibitor caused a statistically significant reduction in viable animals. The activity of **1** came as a surprise, because

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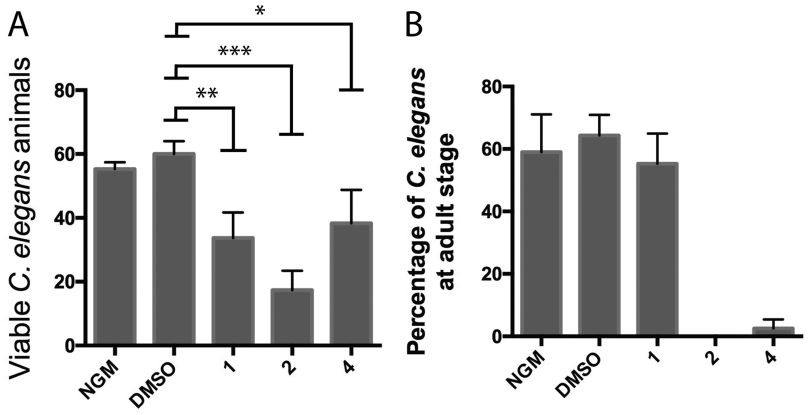

Figure 3. (A) Live *C. elegans* animals were counted after synchronization. All UGM inhibitors (100 μM) induced a statistically significant decrease in survival 48 h following synchronization (*p value < 0.05, **p value < 0.01, ***p value < 0.005). (B) Number of adults at 48 h. Animals that survived late embryogenesis in the presence of compound **1** were healthy and developed normally. In contrast, animals treated with **2** or **4** were developmentally delayed. NGM: nematode growth medium.

our previous experiments indicated compound **1** had no detectable EC_{100} concentration. These data indicate that the impact of carboxylate-containing compound **1** is short-lived relative to that of *N*-acylsulfonamide-containing compounds **2** and **4**.

The basis for the enhanced biological potency of acylsulfonamides relative to their carboxylate counterparts was assessed. Installing a carboxylate mimetic could either improve inhibitor permeability or circumvent detoxication enzymes such as glucuronosyltransferases. To assess permeability, we exposed *C. elegans* to compound **1** or **2** and quantified inhibitor levels (see Supporting Information). The concentration of each compound accumulated within *C. elegans* was similar, suggesting the enhanced potency of carboxylate surrogates is not due to differences in permeability. The comparable accumulation levels of **1** and **2** and the transient action of **1** relative to **2** suggest that the *N*-acylsulfonamide derivatives resist detoxification and therefore persist as UGM inhibitors.

Phenotype Induced by UGM Inhibitors. We examined the phenotypic effects elicited by the *N*-acylsulfonamide UGM inhibitors. Sublethal concentrations (50 μM and 25 μM) of compounds **2** or **3** elicited phenotypic responses. Specifically, inhibitor-treated animals displayed lengthy development delays. Typically, untreated animals took approximately 48 h to reach adulthood, while animals exposed to **3** (25 μM) took 4−6 days (Figure 4A). Additionally, UGM inhibitor exposure led to a wide range of developmental delays, and some animals failed to progress to adulthood altogether. For example, some animals cultivated with 50 μM **3** persisted as larvae for up to 19 days. The ability of UGM inhibitors to induce these deleterious effects is notable; fewer than 0.02% of compounds tested (40 μM) in a high throughput nematode screen exhibited phenotypic effects.[40]

Evidence indicates that the phenotypic effects result from UGM inhibition. Deletion of *glf*-1 in *C. elegans* results in lethality during embryonic and larval stages, which is consistent with observations that *glf-1* is most highly expressed in late embryonic development.[53,23] Similarly, worms exposed to UGM inhibitors afforded extensive lethality during late embryonic development. Moreover, UGM inhibitor-treatment in nematodes resulted in motion defects reminiscent of the Skd phenotype. This phenotype, also displayed by *glf-1* null animals, is characterized by poor traction on agar surfaces.[23] Animals treated with 25 μM compound **3** move normally within the bacterial lawns but display a traction defect on agar (Figure 4B, videos in Supporting Information). This rare phenotype has been predominantly observed in *C. elegans* mutants of cuticle glycosylation.[3] Another characteristic of impaired cuticle formation displayed by both *glf-1* null mutants and *glf-1* knock-down worms is hypersensitivity to hypochlorite-induced rupturing. We therefore subjected UGM inhibitor-treated worms to the hypochlorite-induced rupture assay (Figure 4C). Nematodes exposed to **2** or **4** (50 μM) ruptured more rapidly (in about half the time) than untreated nematodes. Notably, the rapid-rupture phenotype was not elicited by carboxylate **1**. Thus, the *N*-acylsulfonamide but not carboxylate UGM inhibitors were effective against adult nematodes. Together, the data reveal that UGM inhibitors phenocopy unique aspects of *glf-1* deletion, indicating that the characteristic phenotypic defects result from CeUGM inhibition.

A more stringent test of the target is to ascertain whether *glf-1* depletion sensitizes *C. elegans* to UGM inhibitors. Reduction of UGM levels should augment the effects of UGM inhibitor treatment. In accord with this prediction, the EC_{100} of **3** decreased to 50 μM in *glf-1* knock-down worms, and the developmental delays observed in *glf-1* knock-down worms were longer than those undisturbed by genetic interference (see Supporting Information). These results, in conjunction with our other data, are consistent with a model in which the acylsulfonamide UGM inhibitors act on target.

Conclusions. Nematodes possess powerful defense mechanisms. Their cuticle and rapid metabolism of xenobiotic compounds are attributes that function as a formidable barrier to small-molecule probes and drugs. Our observation that previous generations of UGM inhibitors lacked antinematode activity, despite efficacy against purified enzymes, was consistent with these challenges. We postulated that the Achilles heel of these inhibitors was their carboxylate group and therefore generated inhibitors that could circumvent nematode defense mechanisms. In replacing the carboxylic acid with an *N*-acylsulfonamide, we produced inhibitors that phenocopy many aspects of the *glf-1* deletion mutants. Our findings indicate that carboxylate group replacement improved the metabolic stability of the UGM inhibitors. We anticipate that the use of carboxylate surrogates will be a general strategy

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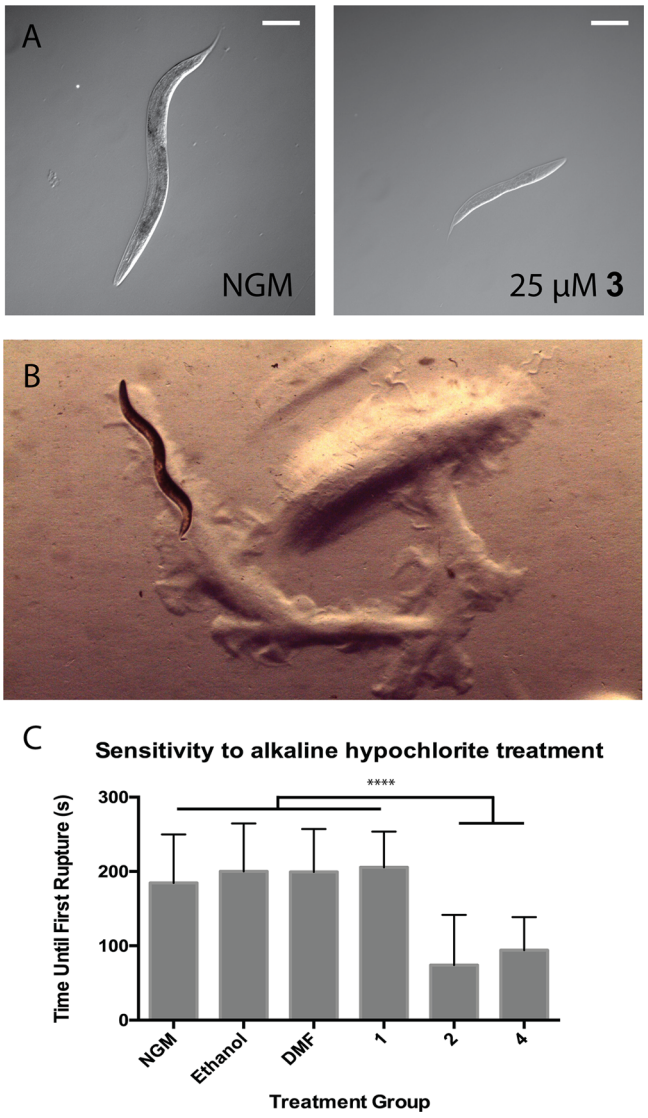

Figure 4. (A) Representative images of *C. elegans* treated with compound **3** (25 μM) 48 h after synchronization to the L1 stage. Scale bars are ~0.1 mm. (B) *C. elegans* exposed to compound **3** (25 μM) were monitored for the Skd phenotype seen in mutants of cuticle glycosylation. (C) Animals treated with compounds **1**, **2**, and **4** (50 μM) were assessed for their sensitivity to rupture in bleach (p value < 0.0001).

to boost the efficacy of carboxylic acid-bearing small molecules against nematodes.

We anticipate that the UGM inhibitors described herein can be used to probe the role of Gal*f* in nematode glycoconjugates. The available data suggest that nematodes generate galactofuranose-containing cell-surface glycans that function as vital constituents of the cuticle, but as yet are unidentified. Because surface glycoconjugates of *C. elegans* are similar to those of parasitic nematodes, we anticipate our UGM inhibitors can be used to probe helminth biology and further evaluate the utility of UGM as an anthelmintic target. An additional impetus to pursue this direction is found in the results of disruption of *glf-1* in the parasite *Brugia malayi*, which afforded impaired motility and embryogenesis and reduced microfilarial release.[24] As helminth glycoconjugates likely facilitate evasion of host immune defenses, a deeper understanding of the composition and roles of nematode glycoconjugates could lead to new strategies with which to combat infection. To this end, we note that *glf*-1 deficient *C. elegans* display hypersensitivity to ivermectin and other anthelmintics,[23] thereby raising the prospect that UGM inhibitors could be used to enhance drug sensitivity in anthelmintic-resistant nematodes.

METHODS

Compound Synthesis. The carboxylate 2-aminothiazole **1** was synthesized according to published protocols.[37] Synthetic procedures for carboxylate modification to *N*-acylsulfonamide or ester derivatives of either **1** or the commercially available **11** were performed as described.[52]

Evaluation of CeUGM Activity. Recombinant CeUGM was produced according to published protocols,[22] and enzyme activity was evaluated using a previously described HPLC assay.[32] Briefly, CeUGM in sodium phosphate buffer (50 mM sodium phosphate, pH 7.0) was mixed with dithionite (10 mM) and the substrate UDP-Gal*f* (12 μM) in either the absence or presence of an inhibitor (added as a DMSO stock for a final concentration of 1% DMSO). Inhibitor concentrations ranged from 50 nM to 25 μM. After 4 min, the enzymatic reaction was quenched by the addition of an equal volume of 1:1 methanol/chloroform. The aqueous portion was separated by centrifugation and analyzed by HPLC using a Dionex Carbopac PA-100 column (200 mM ammonium acetate, pH 8.0, 0.6 mL/min) to quantify conversion of UDP-Gal*f* to UDP-Gal*p*. Relative enzyme activity was derived by normalizing activity in the presence of inhibitors against the activity of the enzyme alone. Inhibition curves were plotted using GraphPad Prism 6, and IC_{50} values were derived using a one-site linear regression model.

Determination of EC_{100}. Compounds were supplemented into NGM (nematode growth medium) at the indicated concentration from a DMSO stock solution.[54] *C. elegans* were then synchronized *via* treatment with alkaline hypochlorite solution.[54] Embryos were then transferred to plates supplemented with the compound and grown at 22 °C using OP-50 as a food source. Preliminary screening for efficacy was performed in 24-well tissue culture plates; all compounds (250 μM) were tested in triplicate. Those compounds that showed activity in whole worms were evaluated at lower concentrations in triplicate (150 μM, 100 μM, 50 μM) by using compound-supplemented NGM in 10 mm plates. The EC_{100} value, the concentration of compound that blocked all *C. elegans* proliferation, was determined in two stages. For EC_{100} testing at 250 μM, growth was monitored 4 days following synchronization. Those compounds that appeared most active were examined further. For all subsequent testing at 150 μM, 100 μM, and 50 μM, growth was monitored for 12 days following synchronization.

Quantification of Embryonic Inhibitor Lethality and Assessment of Developmental Delays. Compounds were added to NGM (nematode growth medium) at the indicated concentration.[54] *C. elegans* were synchronized *via* treatment with alkaline hypochlorite solution, and an egg suspension (50−75 eggs/plate) was pipetted onto NGM plates.[54] Unless otherwise noted, the number of animals/total number of adult animals was tabulated 48 h after synchronization. Results are representative of independent biological replicates, each conducted in triplicate. Development was typically monitored for 10 days or until plates were depleted of nutrients.

RNAi Knock-Down of *glf-1* and Treatment with UGM Inhibitors. RNAi knock-down of *glf-1* (Gene H04M03.4) was performed by feeding as previously described using the relevant strain from the RNAi feeding library.[55] The compound was supplemented into NGM (Nematode Growth Media) from a DMSO stock solution at the indicated concentration, along with carbenicillan to 25 μg/mL and IPTG to 1 mM.[54] Assays were conducted in 24-well tissue culture plates.

Prior to determination of UGM inhibitor sensitivity, *C. elegans* were maintained on either H04M03.4 knockdown or L4440 empty vector control. Gravid hermaphrodites were then synchronized *via* treatment with alkaline hypochlorite solution. An egg suspension of either *glf-1* knockdown or control *C. elegans* was then pipetted onto NGM plates supplemented with compound. The day prior to synchronization,

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either H04M03.4 knock-down *E. coli* or L4440 empty vector control *E. coli* were added to allow ample time for induction of knock-down RNAi.[54]

Compounds **1**, **3**, and **8** were tested along with a DMSO control. Each compound was tested in triplicate at 25 μM, 50 μM, 100 μM, or 200 μM, with final DMSO concentrations of 0.05%, 0.1%, 0.2%, and 0.4%, respectively. Observations regarding development of *C. elegans* grown in the presence of **8** were made 72 h postsynchronization. Determinations of EC_{100} were made 12 days following synchronization.

Hypochlorite Sensitivity. Sensitivity to alkaline hypochlorite was measured as a proxy for cuticle integrity.[3] Animals were grown on NGM supplemented with the compound of interest (50 μM). The compound was applied from an ethanol stock solution (50 mM). Adult worms were individually transferred to an NGM plate, and 20 μL of alkaline hypochlorite solution was dropped onto the worm. Reagent grade sodium hypochlorite solution (Sigma, available chlorine 10−15%) was used. Prior to use in the assay, sodium hypochlorite solution was diluted 4-fold, and sodium hydroxide was added to 0.25 M. The time until first rupture was measured. Statistical outliers were determined by the Thompson tau technique.[56] Each treatment group was composed of ≥20 nematodes. Data are representative of two separate experiments.

Evaluation of Skd Phenotype. *C. elegans* were synchronized by hypochlorite treatment. Embryos were transferred to a 24 well plate, with wells containing NGM supplemented with 25 μM **3**. Upon reaching adulthood, animals were transferred to NGM agar plates and traction was observed. Representative videos of animals displaying a Skd phenotype were collected (Videos S1−S5).

Nematode Permeability of Small Molecules. The permeability assay was conducted by adapting a known protocol (see the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acschembio.7b00487.

Representative inhibition curves for compounds **1**, **2**, **4**, and **8** with CeUGM; control experiments for the hypochlorite sensitivity assay; analysis of compound uptake in *C. elegans*; and analysis (PDF)

C. elegans untreated with UGM inhibitor display normal locomotion on agar surfaces. (MOV)

C. elegans traction defects upon UGM inhibitor treatment (MOV)

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Notes

The authors declare no competing financial interest.

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