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Letter

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Onchocerca volvulus Molting Inhibitors Identified through Scaffold Hopping

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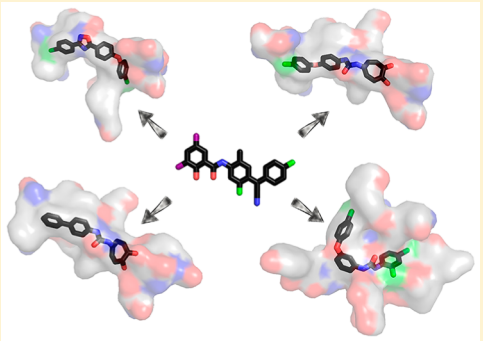
Supporting Information

ABSTRACT: The anthelmintic closantel has shown promise in abrogating the L3 molting of *Onchocerca volvulus*, the causative agent of the infectious disease onchocerciasis. In our search for alternative scaffolds, we utilized a fragment replacement/modification approach to generate novel chemotypes with improved chitinase inhibitory properties. Further evaluation of the compounds unveiled the potential of urea-tropolones as potent inhibitors of *O. volvulus* L3 molting.



KEYWORDS: *onchocerciasis, tropolones, molting*

The helminth *Onchocerca volvulus* is the causative pathogen of onchocerciasis, a neglected tropical disease that threatens approximately 100 million people, mostly in Africa. Currently, the only treatment option is the broad-spectrum antiparasitic agent ivermectin. The exact pharmacological mechanism of ivermectin is unclear, but research posits the glutamate-gated chloride channels in neurons and pharyngeal muscle cells to be activated, resulting in membrane hyperpolarization and eventual paralysis/death of the parasite.[1] In another study, Moreno et al. had proposed that ivermectin functions by disrupting the secretion of proteins imperative for eluding the human host immune system.[2] Although ivermectin has substantially reduced the microfilarial load in infected patients as well as transmission, it does not kill the adult filariae and, thus, does not totally eradicate the disease. Several papers have also documented the failure of infected patients in Ghana to respond after repeated treatment with ivermectin.[3,4] Given the paucity of preventive efforts and the possible emergence of ivermectin-resistant strains of *O. volvulus*,[5] the identification of novel targets and therapeutic strategies is a pressing necessity for effective intervention of this infectious malady that will meet the elimination target of 2025.[6]

O. volvulus undergoes a series of molts before reaching adult maturity. The L3-to-L4 molt is of particular interest as it represents the major developmental event necessary for active infection of the host and, hence, interruption of this transition is an attractive chemotherapeutic strategy. The stage-specific chitinase OvCHT1 has been hypothesized to mediate ecdysis and contribute to L3 infectivity and transmissibility.[7] Our group has previously disclosed the veterinary anthelmintic drug closantel (**1**) to exhibit potent inhibition of OvCHT1 with an IC_{50} value of 1.60 ± 0.08 μM.[8] Additionally, treatment with closantel inhibited the separation between the old L3 cuticle and the newly synthesized L4 cuticle, preventing the molting of the infective L3 larvae.[8]

A computational study of the closantel−OvCHT1 complex revealed that the diiodosalicylate moiety anchors closantel in the binding pocket of the chitinase, whereas the 4-(chlorophenyl)acetonitrile and the 5-chloro-2-methylaniline substituents form strong van der Waals interactions with important residues lining the active site.[9] We have conducted retro-fragment-based[8] and preliminary SAR[10] studies on closantel to map the structural requirements for inhibition of OvCHT1. Removal of the terminal *p*-Cl phenyl ring in **1** resulted in a >3-fold loss in potency,[8] indicating its relevance for chitinase inhibition. We have also delineated that the phenolic group is not crucial for activity,[10] suggesting that the diidosalicylate moiety is most likely interacting with nonspecific hydrophobic contacts within the active site and is therefore amenable for fragment replacement and manipulation.

In the absence of crystallographic data, we undertook in this study a "scaffold hopping" strategy in an attempt to explore the biological space of OvCHT1 and generate novel molecular frameworks with improved potency. We show that the urea and oxadiazole congeners efficiently occupy the active site of the

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chitinase. On the basis of docking studies, these analogues are able to extend to an adjacent binding pocket that is not readily accessible by closantel. Further analysis of the compounds inadvertently led to the discovery of urea-tropolones as promising inhibitors of *O. volvulus* L3 molting.

RESULTS AND DISCUSSION

To test the feasibility of our approach, we simplified the structure of closantel by replacing the diiodosalicylate moiety with a phenyl group bridged to a phenoxyphenyl system via an amide bond (**2a**). Although **2a** showed only marginal inhibition of OvCHT1, we were delighted to observe a significant improvement in activity using three different atom linkers as in compounds **2b–d** (Figure 1A), with the urea analogue **2d** displaying >50% inhibition at 5 μM (Figure 1B). We then focused on structural modification and expansion using the urea linkage to generate a tractable series of derivatives **2e–2k** (which were easily accessed by reaction of 4-(4-chloro)-phenoxyaniline with isocyanates, Scheme 1). Compounds were initially screened at 5 μM (Figure 1B) and further evaluated for determination of IC_{50} (values listed in Table 1). Surprisingly, inclusion of a chloro substituent in **2a** (as in **2e** and **2g**) dramatically increased the activity against OvCHT1, with IC_{50} values in the nanomolar range. Replacement of the phenyl ring with benzenesulfonamide (**2i**) or isoxazole (**2j**) did not yield a significant effect on potency, whereas adamantane (**2k**) and the more flexible ethylbenzene (**2h**) resulted in almost complete inhibition at 5 μM (Figure 1B).

An interesting scaffold is the nonbenzenoid aromatic tropolone **3a**, which was prepared by nucleophilic addition of 5-aminotropolone[11] to the activated PNP-(4-(4-chlorophenoxy)phenyl)carbamate under microwave irradiation conditions (Scheme 1). Compound **3a** fully inhibited OvCHT1 activity at 5 μM, to give an IC_{50} value of 0.84 ± 0.01 μM and a competitive inhibitory constant K_i of 0.22 ± 0.04 μM. Intrigued by this unusual class of natural product, we synthesized a few analogues based on the tropolone framework. Biphenyl (**3b**) and naphthyl (**3c**) urea derivatives displayed comparable potency as **3a** (Table 1), whereas monobenzene urea **3e** or coupling to a shorter amide spacer (**3f**) led to a significant decrease in activity (Figure 1B). These results echo the significance of the terminal phenyl ring and the urea functionality for chitinase inhibition. Of note, installation of the tropolone moiety greatly reduced the cLogP, compared to the highly lipophilic closantel (Table 1), although there was no clear correlation between the lipophilicity of the compounds and their potency against OvCHT1.

We also explored the use of the oxadiazole linkage as a secondary pharmacophore against OvCHT1. Refluxing the amidoxime **7** with the corresponding carboxylic acids in the presence of the coupling reagent HBTU furnished compounds **4a** and **4b** (Scheme 1). Oxadiazoles **4a** and **4b** exhibited potent inhibition of the chitinase, with IC_{50} values of 0.56 ± 0.18 and 0.49 ± 0.04 μM, respectively.

Sequence alignment of amino acids indicates that OvCHT1 shares 67% identity with the related filarial chitinase from *Brugia malayi* (BmCHT1) and about 13% identity with those of the parasitic protozoans *Entamoeba histolytica* (EhCHT1) and *Plasmodium falciparum* (PfCHT1). Representative compounds (**2g**, **3a**, **3b**, and **4a**) were examined for chitinase selectivity and were shown with a predilection toward OvCHT1 over the chitinases from other species (Figure S1 in the Supporting Information).

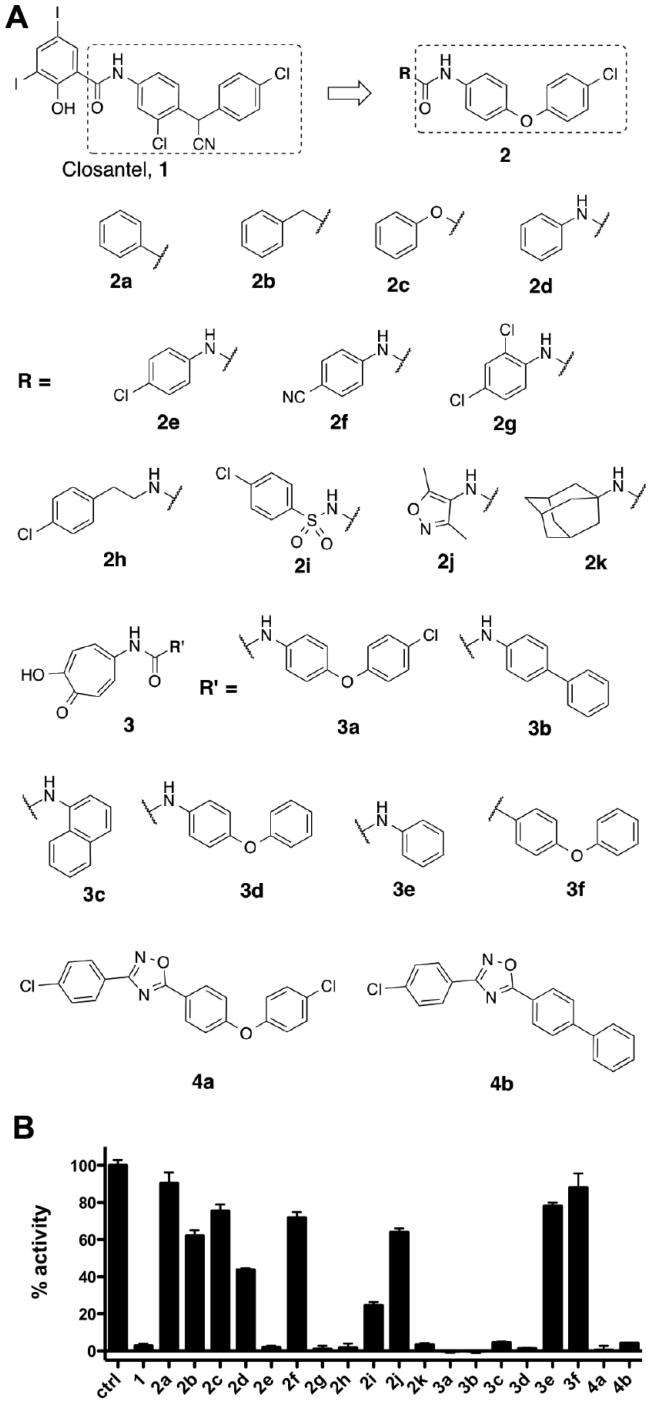

Figure 1. (A) Structures of novel OvCHT1 inhibitors; (B) evaluation of compounds using a fluorescence-based OvCHT1 inhibition assay. Data are shown as percent OvCHT1 activity in the presence of inhibitors at 5 μM, relative to control (0.5% DMSO).

Next, docking studies were undertaken using AutoDock Vina to provide structural insights into the binding mode of the inhibitors. We docked compounds **2g**, **3a**, **3b**, and **4a** into the active site of OvCHT1 using the homology model described by Segura-Cabrera et al.[9] All four compounds engage in van der Waals interactions with both hydrophobic and polar residues in the binding pocket (Figure 2; Figure S2 in the Supporting Information). Analysis of the docking poses revealed that binding of **3a** is augmented by π-stacking interactions with Tyr268 and Phe365, predisposing the seven-membered carbon

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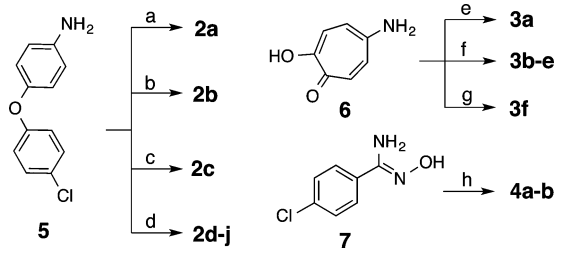
ACS Infect. Dis. 2015, 1, 198–202

Scheme 1. Synthesis of Compounds 2−4[a]



[a]Reagents and conditions: (a) benzoyl chloride, DIPEA, DMF, rt, 0.5 h; (b) 2-phenylacetyl chloride, DIPEA, DMF, rt, 0.5 h; (c) phenyl chloroformate, DIPEA, DMF, rt, 0.5 h; (d) RNCO, DIPEA, DMF, rt or reflux, 3 h; (e) PNP-(4-(4-chlorophenoxy)phenyl)carbamate, THF, 95 °C, microwave; (f) RNCO, THF, 95 °C, microwave; (g) 4-phenoxybenzoic acid, EDC, Cl-HOBt, DMF, rt, 18 h; (h) RCOOH, HBTU, DIPEA, DMF, reflux, 3 h.

Table 1. CLogP and IC_{50} of OvCHT1 Inhibition

compd	cLogP	IC_{50} (μM)	compd	cLogP	IC_{50} (μM)
1	7.8	1.60 ± 0.08	**3a**	4.3	0.84 ± 0.01
2e	6.8	0.65 ± 0.10	**3b**	3.4	0.60 ± 0.01
2g	7.1	0.50 ± 0.14	**3c**	2.7	1.20 ± 0.08
2h	6.7	1.45 ± 0.17	**4a**	7.2	0.56 ± 0.18
2k	6.6	0.81 ± 0.06	**4b**	6.3	0.49 ± 0.04

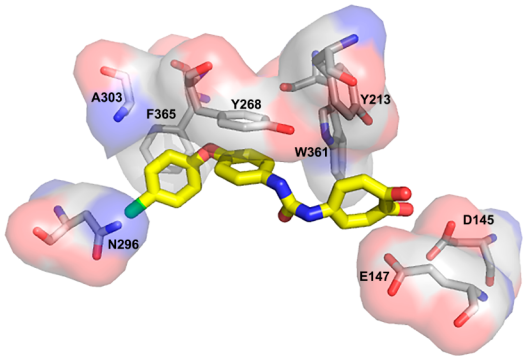


Figure 2. Lowest energy pose of compound **3a** docked into OvCHT1 using the program AutoDock Vina. Color scheme: oxygens in red, nitrogens in blue, carbons in yellow (compound **3a**) or gray (OvCHT1).

ring into a peripheral binding site (Figure 2). Exploitation of this additional binding pocket seems to be the prominent feature for all four compounds, which was not observed with the closantel molecule.[9]

Compounds **2g**, **3a**, **3b**, and **4a** were then evaluated for their effect on *O. volvulus* L3 molting. L3 larvae were cultured in the presence of the inhibitors at 10 μM until day 6, when molting was assessed for the empty cast of L3 and emergence of the L4 larvae.[8] Treatment with compound **2g** or **4c** resulted in up to 29% inhibition of molting, whereas tropolones **3a** and **3b** displayed better activity, with **3a** completely abolishing the L3-to-L4 molt (Figure 3A). At a dose of 1 μM, **3a** inhibited the shedding of the L3 cuticle by 23%.

The impervious cuticle of nematodes presents an impediment in the development of anthelmintics. As bioaccumulation is a predeterminant of in vivo efficacy, we sought to investigate whether the new OvCHT1 inhibitors could accumulate within the worm. Burns et al. had previously noted that of >1000 drug-like small molecules, fewer than 10% accumulated at

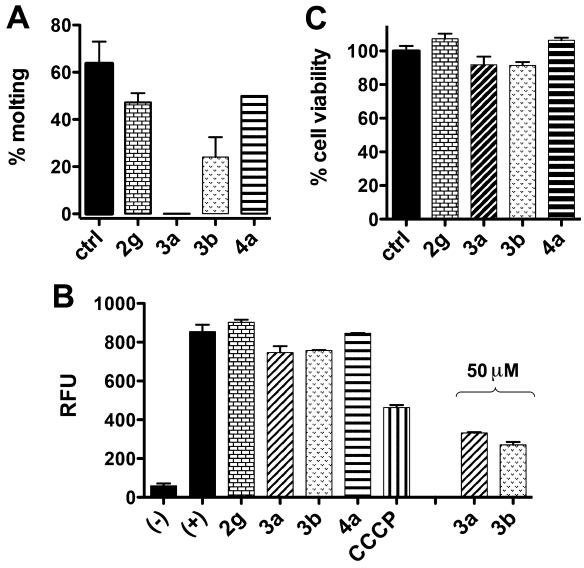


Figure 3. (A) Molting of *O. volvulus* L3 larvae in the presence of inhibitors at 10 μM. Data are presented as percent molting in a total of two to three wells containing on average 5−10 larvae per well. (B) Evaluation of mitochondrial uncoupling activity. HEK 293T/17 cells were incubated with compound (10 μM) and subsequently stained with TMRE. Data shown are the mean fluorescence intensity ± SD (n = 3). Unstained cells (no TMRE) and 0.5% DMSO were used as negative (−) and positive (+) controls, respectively. RFU, relative fluorescence units (λ_{ex} = 488 nm, λ_{em} = 575 nm). (C) Percent viability of HEK 293T/17 cells in the presence of inhibitors (10 μM), as assessed by MTS assay (n = 3).

concentrations >50% of the initial dose in a bioaccumulation assay using *C. elegans* as a model nematode.[13] Employing a similar procedure, we incubated late-stage L4 worms with urea **2g**, tropolone **3a**, and oxadiazole **4a** (at a final concentration of 10 μM) for 6 h and then analyzed the worm homogenates by LC-MS. Identification of the compounds in the lysates was confirmed by comparison of LC retention time and mass spectrum with those of the authentic synthetic standards (Figure S3 in the Supporting Information). Our results demonstrate that the urea-tropolone and oxadiazole scaffolds are capable of penetrating the worm cuticle and thus increasing the feasibility of engagement with the biological target(s) in *O. volvulus*.

Taking into account that the tested compounds have comparable potencies against OvCHT1, the superior efficacy of tropolones **3a** and **3b** on L3 molting suggested additional functions that might be vital to their bioactivity. We surmise that because **3a** and **3b** are lipophilic weak acids and metal chelators, they could act as ionophores capable of shuttling protons/cations across biological membranes to uncouple mitochondrial respiration. To shed light on this matter, we evaluated the ionophoric activity of tropolones **3a** and **3b** using the fluorescent mitochondrion-selective probe TMRE as a membrane polarization sensor. Human HEK 293T/17 cells were cultured in the presence of 10 μM **3a** and **3b**, subsequently stained with TMRE, and analyzed by spectrofluorometry. As shown in Figure 3B, the fluorescence intensity is decreased in the presence of **3a**/**3b** or CCCP (a proton ionophore), indicating mitochondrial depolarization, whereas no uncoupling effect was observed upon incubation with compounds **2g** and **4a**. The ionophoric activity of tropolones **3a**/**3b** was even more pronounced at a higher concentration of

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ACS Infect. Dis. 2015, 1, 198−202

50 μM (Figure 3B). Investigations on the morphology of *O. volvulus* L3 larvae disclosed the glandular esophagus as the predominant structure and the major site of protein synthesis.[12] Inhibition of mitochondrial respiration in the esophageal granules of L3 larvae may thus affect the molting cycle of the parasite. We add that no significant effect on the viability of human HEK 293T/17 cells (>90%) was observed upon incubation with **3a**/**3b** at the effective concentration of 10 μM (Figure 3C).

Indeed, tropolones have been demonstrated to possess a broad range of biological activities including antibacterial,[14] anticancer,[15] antifungal,[16] and antiviral.[17] The pharmacology of tropolones is largely attributed to their ability to chelate metalloenzymes such as histone deacetylases,[15] carboxypeptidase A,[18] and elastase LasB.[11] In filarial nematodes, metalloproteinases have been suggested to be crucial for host tissue invasion and parasite survival as was shown for the infective L3 larvae of *Ancylostoma caninum*,[19] *Strongyloides stercoralis*,[20] and *Trichinella spiralis*.[21] In the case of *O. volvulus*, juvenile and adult male worms secrete an astacin-like zinc endopeptidase Ov-AST-1 or onchoastacin[22] to facilitate larval migration and host skin tissue damage, resulting in atrophy and hanging groins observed in infected individuals. Onchoastacin is also differentially expressed in L3 larvae,[23] presumably to aid in the infection process and possibly in L3 molting as the involvement of astacins in cuticle ecdysis has been documented in other nematodes.[24] As onchoastacin is expressed in both the infective and migratory phases of *O. volvulus*, pharmacological intervention of the protease could provide a novel approach in the search for an effective drug with both micro- and macrofilaricidal activities. Whether tropolones **3a**/**3b** can potentially inhibit the metalloprotease will be the subject of a future investigation.

In the present study, it would seem that the robust inhibition of L3 molting by tropolones **3a**/**3b** occurs as a result of the additive effects of chitinase inhibitory and ionophoric activities, as compounds **2g** and **4a** (OvCHT1 inhibitors only) exhibited less potency compared to the dual mechanistic compounds **3a**/**3b**. Moreover, compounds **3a**/**3b** could likely target metalloprotease(s) that may be necessary for active infection and transmission of the parasite. As such, the tropolone analogues could present a multitarget scheme for effective treatment of onchocerciasis.

The infective L3 larva is the critical stage for human host invasion, and thus halting its development would avoid subsequent insidious damage within the host. Moreover, the persistence of microfilarial infection despite multiple treatments of ivermectin[5,25] (suggesting possible appearance of *O. volvulus* strains resistant to ivermectin monotherapy) is alarming in its breadth and urges immediate action. Starting with our initial screening hit closantel, we initiated a "scaffold hopping" approach by fragment replacement to identify novel structures with OvCHT1 inhibitory activity. Our efforts resulted in the identification of urea and oxadiazole analogues with improved activity against the chitinase that are also able to penetrate the worm cuticle. Although compounds **2g** and **4a** are potent OvCHT1 inhibitors, they gave only modest inhibition of L3 molting when dosed at 10 μM. The present work culminated in the serendipitous discovery of tropolones **3a**/**3b** as auspicious inhibitors of *O. volvulus* L3 molting. These compounds could plausibly operate by collective modulation of multiple targets to impart superior efficacy and prevent drug resistance, thereby illustrating polypharmacology as a viable strategy to eliminate onchocerciasis.

■ ASSOCIATED CONTENT

Supporting Information

The following file is available free of charge on the ACS Publications website at DOI: 10.1021/acsinfecdis.5b00017.

Experimental procedures (syntheses and characterization of compounds, molecular docking, mitochondrial uncoupling, *O. volvulus* L3 molting, bioaccumulation in *C. elegans*, cell viability; supplementary figures on chitinase selectivity, molecular docking, and bioaccumulation (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

C. elegans, *Caenorhabditis elegans*; *O. volvulus*, *Onchocerca volvulus*; OvCHT1, *Onchocerca volvulus* chitinase; L3, third larval stage; L4, fourth larval stage; PNP, *p*-nitrophenyl; CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; TMRE, tetramethylrhodamine ethyl ester

■ REFERENCES

(1) Wolstenholme, A. J., and Rogers, A. T. (2005) Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology 131* (Suppl.), S85−95 DOI: 10.1017/S0031182005008218.

(2) Moreno, Y., Nabhan, J. F., Solomon, J., Mackenzie, C. D., and Geary, T. G. (2010) Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. *Proc. Natl. Acad. Sci. U.S.A. 107*, 20120−20125 DOI: 10.1073/pnas.1011983107.

(3) Awadzi, K., Attah, S. K., Addy, E. T., Opoku, N. O., Quartey, B. T., Lazdins-Helds, J. K., Ahmed, K., Boatin, B. A., Boakye, D. A., and Edwards, G. (2004) Thirty-month follow-up of sub-optimal responders to multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. *Ann. Trop. Med. Parasitol. 98*, 359−370 DOI: 10.1179/000349804225003442.

(4) Awadzi, K., Boakye, D. A., Edwards, G., Opoku, N. O., Attah, S. K., Osei-Atweneboana, M. Y., Lazdins-Helds, J. K., Ardrey, A. E., Addy, E. T., Quartey, B. T., Ahmed, K., Boatin, B. A., and Soumbey-Alley, E. W. (2004) An investigation of persistent microfilaridermias despite multiple treatments with ivermectin, in two onchocerciasis-endemic foci in Ghana. *Ann. Trop. Med. Parasitol. 98*, 231−249 DOI: 10.1179/000349804225003253.

(5) Lustigman, S., and McCarter, J. P. (2007) Ivermectin resistance in *Onchocerca volvulus*: toward a genetic basis. *PLoS Negl. Trop. Dis. 1*, No. e76, DOI: 10.1371/journal.pntd.0000076.

DOI: 10.1021/acsinfecdis.5b00017
ACS Infect. Dis. 2015, 1, 198−202

(6) Mackenzie, C. D., Homeida, M. M., Hopkins, A. D., and Lawrence, J. C. (2012) Elimination of onchocerciasis from Africa: possible? *Trends Parasitol. 28*, 16−22 DOI: 10.1016/j.pt.2011.10.003.
(7) Wu, Y., Egerton, G., Underwood, A. P., Sakuda, S., and Bianco, A. E. (2001) Expression and secretion of a larval-specific chitinase (family 18 glycosyl hydrolase) by the infective stages of the parasitic nematode, *Onchocerca volvulus*. *J. Biol. Chem. 276*, 42557−42564 DOI: 10.1074/jbc.M103479200.
(8) Gloeckner, C., Garner, A. L., Mersha, F., Oksov, Y., Tricoche, N., Eubanks, L. M., Lustigman, S., Kaufmann, G. F., and Janda, K. D. (2010) Repositioning of an existing drug for the neglected tropical disease onchocerciasis. *Proc. Natl. Acad. Sci. U.S.A. 107*, 3424−3429 DOI: 10.1073/pnas.0915125107.
(9) Segura-Cabrera, A., Bocanegra-Garcia, V., Lizarazo-Ortega, C., Guo, X., Correa-Basurto, J., and Rodriguez-Perez, M. A. (2011) A computational analysis of the binding mode of closantel as inhibitor of the *Onchocerca volvulus* chitinase: insights on macrofilaricidal drug design. *J. Comput. Aided Mol. Des. 25*, 1107−1119 DOI: 10.1007/s10822-011-9489-y.
(10) Gooyit, M., Tricoche, N., Lustigman, S., and Janda, K. D. (2014) Dual protonophore-chitinase inhibitors dramatically affect *O. volvulus* molting. *J. Med. Chem. 57*, 5792−5799 DOI: 10.1021/jm5006435.
(11) Fullagar, J. L., Garner, A. L., Struss, A. K., Day, J. A., Martin, D. P., Yu, J., Cai, X., Janda, K. D., and Cohen, S. M. (2013) Antagonism of a zinc metalloprotease using a unique metal-chelating scaffold: tropolones as inhibitors of *P. aeruginosa* elastase. *Chem. Commun. 49*, 3197−3199 DOI: 10.1039/c3cc41191e.
(12) Strote, G., and Bonow, I. (1991) Morphological demonstration of essential functional changes after in vitro and in vivo transition of infective *Onchocerca volvulus* to the post-infective stage. *Parasitol. Res. 77*, 526−535 DOI: 10.1007/BF00928422.
(13) Burns, A. R., Wallace, I. M., Wildenhain, J., Tyers, M., Giaever, G., Bader, G. D., Nislow, C., Cutler, S. R., and Roy, P. J. (2010) A predictive model for drug bioaccumulation and bioactivity in *Caenorhabditis elegans*. *Nat. Chem. Biol. 6*, 549−557 DOI: 10.1038/nchembio.380.
(14) Oblak, E. Z., Bolstad, E. S., Ononye, S. N., Priestley, N. D., Hadden, M. K., and Wright, D. L. (2012) The furan route to tropolones: probing the antiproliferative effects of β-thujaplicin analogs. *Org. Biomol. Chem. 10*, 8597−8604 DOI: 10.1039/c2ob26553b.
(15) Ononye, S. N., VanHeyst, M. D., Oblak, E. Z., Zhou, W., Ammar, M., Anderson, A. C., and Wright, D. L. (2013) Tropolones as lead-like natural products: the development of potent and selective histone deacetylase inhibitors. *ACS Med. Chem. Lett. 4*, 757−761 DOI: 10.1021/ml400158k.
(16) Morita, Y., Matsumura, E., Okabe, T., Shibata, M., Sugiura, M., Ohe, T., Tsujibo, H., Ishida, N., and Inamori, Y. (2003) Biological activity of tropolone. *Biol. Pharm. Bull. 26*, 1487−1490 DOI: 10.1248/bpb.26.1487.
(17) Budihas, S. R., Gorshkova, I., Gaidamakov, S., Wamiru, A., Bona, M. K., Parniak, M. A., Crouch, R. J., McMahon, J. B., Beutler, J. A., and Le Grice, S. F. (2005) Selective inhibition of HIV-1 reverse transcriptase-associated ribonuclease H activity by hydroxylated tropolones. *Nucleic Acids Res. 33*, 1249−1256 DOI: 10.1093/nar/gki268.
(18) Inamori, Y., Shinohara, S., Tsujibo, H., Okabe, T., Morita, Y., Sakagami, Y., Kumeda, Y., and Ishida, N. (1999) Antimicrobial activity and metalloprotease inhibition of hinokitiol-related compounds, the constituents of *Thujopsis dolabrata* S. and Z. hondai MAK. *Biol. Pharm. Bull. 22*, 990−993 DOI: 10.1248/bpb.22.990.
(19) Williamson, A. L., Lustigman, S., Oksov, Y., Deumic, V., Plieskatt, J., Mendez, S., Zhan, B., Bottazzi, M. E., Hotez, P. J., and Loukas, A. (2006) *Ancylostoma caninum* MTP-1, an astacin-like metalloprotease secreted by infective hookworm larvae, is involved in tissue migration. *Infect. Immun. 74*, 961−967 DOI: 10.1128/IAI.74.2.961-967.2006.
(20) Gomez Gallego, S., Loukas, A., Slade, R. W., Neva, F. A., Varatharajalu, R., Nutman, T. B., and Brindley, P. J. (2005) Identification of an astacin-like metallo-proteinase transcript from the infective larvae of *Strongyloides stercoralis*. *Parasitol. Int. 54*, 123−133 DOI: 10.1016/j.parint.2005.02.002.
(21) Lun, H. M., Mak, C. H., and Ko, R. C. (2003) Characterization and cloning of metallo-proteinase in the excretory/secretory products of the infective-stage larva of *Trichinella spiralis*. *Parasitol. Res. 90*, 27−37 DOI: 10.1007/s00436-002-0815-0.
(22) Borchert, N., Becker-Pauly, C., Wagner, A., Fischer, P., Stocker, W., and Brattig, N. W. (2007) Identification and characterization of onchoastacin, an astacin-like metalloproteinase from the filaria *Onchocerca volvulus*. *Microbes Infect. 9*, 498−506 DOI: 10.1016/j.micinf.2007.01.007.
(23) Lizotte-Waniewski, M., Tawe, W., Guiliano, D. B., Lu, W., Liu, J., Williams, S. A., and Lustigman, S. (2000) Identification of potential vaccine and drug target candidates by expressed sequence tag analysis and immunoscreening of *Onchocerca volvulus* larval cDNA libraries. *Infect. Immun. 68*, 3491−3501 DOI: 10.1128/IAI.68.6.3491-3501.2000.
(24) Page, A. P., Stepek, G., Winter, A. D., and Pertab, D. (2014) Enzymology of the nematode cuticle: a potential drug target? *Int. J. Parasitol. Drugs Drug Resist. 4*, 133−141 DOI: 10.1016/j.ijpddr.2014.05.003.
(25) Osei-Atweneboana, M. Y., Awadzi, K., Attah, S. K., Boakye, D. A., Gyapong, J. O., and Prichard, R. K. (2011) Phenotypic evidence of emerging ivermectin resistance in *Onchocerca volvulus*. *PLoS Negl. Trop. Dis. 5*, No. e998, DOI: 10.1371/journal.pntd.0000998.

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