Supporting Information

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SI Text

Methods. ***Synthesis of closantel derivatives.*** Compounds 2–5 were purchased from commercial sources: Compounds 2, 4, and 5 were purchased from Sigma Aldrich and compound 3 is a product of Alfa Aesar.

General procedure for the preparation of compounds 6 and 7: PyBrOP (Bromotripyrrolidinophosphonium hexafluorophosphate) (2.0 mmol) and diisopropylethylamine (4.0 mmol) were added to a stirring solution of carboxylic acid (2.0 mmol) in dichloromethane (2 mL, 0.5 M) at 25 °C. After stirring the resulting solution for 20 min at 25 °C, amine (1.0 mmol) and DMAP (4-dimethylaminopyridine) (0.2 mmol) were added and the mixture was stirred overnight at 25 °C and then concentrated in vacuo. The crude extract was purified via flash column chromatography (0–40% EtOAc in hexanes) to yield the pure compound. The ^{1}H NMR and MS were consistent with the literature for compounds 6 and 7 (1).

General procedure for the preparation of compound 8: A scintillation vial was charged with aryl bromide (1.2 mmol), dimethylformamide (5.9 mL, 0.17 M), chlorophenylacetonitrile (1.0 mmol) and cesium carbonate (2.0 mmol) at 25 °C. Upon deprotonation of the phenylacetonitrile, a red color persisted and palladium acetate (0.1 mmol) and triphenylphosphine (0.1 mmol) were added. The mixture was capped and heated to 120 °C for 2 h. After cooling to room temperature, diethyl ether was added (15 mL) and the organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. The crude extract was purified via flash column chromatography (0–50% EtOAc in hexanes). The ^{1}H NMR and MS were consistent with the literature for compound 8 (1).

Library screening and chitinase activity. The library was purchased from D.Sullivan (JHU Bloomberg School of Public Health) as 10 mM stocks in DMSO or water in a 96-well plate format. Each compound-containing well was subsequently diluted to 500 μM using 1 × PBS (pH 7.4) and stored at −20 °C until use. All screening reactions were performed in 50-μL volumes at 37 °C containing 1.25 units OvCHT1, 20 μM 4-methylumbelliferyl-*N*-*N*′-*N*″-*β*-chitotrioside and 50 μM compound in 200 mM NaCl with 20 mM Na_3PO_4 (pH 6.0) and 1.0 mM EDTA. As a control, a sample was prepared as described above but without compound. In all reactions, the DMSO concentration was kept constant at 0.5%, and no influence on enzymatic turnover was observed. The reactions were monitored over 10 min for the release of fluorescent 4-methylumbelliferone using SpectraMax M2^e Microplate Reader, Molecular Devices; $\lambda_{ex} = 350$ nanometer, $\lambda_{em} = 440$ nanometer. The slopes of the enzymatic turnover were compared to the control with no inhibitor added. Analyses were performed using GraphPad Prism (version 5.02 for Windows, GraphPad Software, www.graphpad.com).

Determination of IC_{50}, K_i and mode of inhibition. Identified hit compounds were tested using the fluorescence assay described in the "*Library screening*" section, but with varying concentrations of compound to determine the IC_{50}. The reactions were performed in triplicates and slopes of the linear enzymatic turnover were plotted against the logarithm of compound concentration to allow for nonlinear fitting using a four-parameter model in GraphPad Prism. K_i and the mode of inhibition for closantel and allosamidin were determined using the same fluorescence assay with various concentrations of inhibitor and substrate 1. Velocities of enzymatic turnover were calculated by comparing slopes of enzymatic turnover to an established standard curve using 4-methyllumbelliferone. Velocities for different inhibitor concentrations were subsequently plotted against substrate concentrations and evaluation was performed using GraphPad Prism. For each, error was determined from the mean and standard deviation.

Determination of IC_{50} using BmCHT1, EhCHT1, PfCHT1 and human chitotriosidase. Reaction buffer for BmCHT1, EhCHT1, and PfCHT1 was the same as for OvCHT1 reaction buffer. Human chitotriosidase assays contained McIlvain buffer (100 mM citric acid, 250 mM sodium phosphate, 1 mg/mL BSA, pH 5.0) with a final enzyme concentration of 2 nanomolar. Reactions were run as described in the "*Library screening*" section and turnover was monitored over time. All reactions were performed in triplicate. Analyses were performed using GraphPad Prism as previously described. Error was determined from the mean and standard deviation.

Sequence alignments. Amino acid sequences were taken from PubMed (http://www.ncbi.nlm.nih.gov/pubmed) using the following accession numbers: Q25615 for OvCHT1, P29030 for BmCHT1, P90546 for EhCHT1, Q9U4I9 for PfCHT1, and Q13231 for HsCHT1. Alignments were performed online using standard settings in CLUSTALW from PBIL (2). The amino acid sequence of each enzyme was compared to the sequence of OvCHT1, and the percentage of identical amino acids was denoted in the manuscript.

1. Macielag MJ, et al. (1998) Substituted salicylanilides as inhibitors of two-component regulatory systems in bacteria. *J Med Chem* 41:2939–2945.

2. Thompson JD, Higgins DG, & Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680.