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Discovery of pH-Selective Marine and Plant Natural Product Inhibitors of Cathepsin B Revealed by Screening at Acidic and Neutral pH Conditions

Von V. Phan, Charles Mosier, Michael C. Yoon, Evgenia Glukhov, Conor R. Caffrey, Anthony J. O'Donoghue, William H. Gerwick, and Vivian Hook*

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ABSTRACT: Dysregulation of cathepsin B, which involves the translocation of the enzyme from acidic pH lysosomes to the neutral pH cytosol, followed by the initiation of cell death and inflammation, occurs in numerous brain disorders. The wide difference in the acidic pH (4.6) of lysosomes compared to the neutral pH (7.2) of the cytosol suggests that screening at different pH conditions may identify pH-selective modulators of cathepsin B. Therefore, a collection of pure marine and plant natural product (NP) compounds, with synthetic compounds, was screened at pH 4.6 and pH 7.2 in cathepsin B assays, which led to the identification of GER-12 (Crossbyanol B) and GER-24 ((7Z,9Z,12Z)-octadeca-7,9,12-trien-5-ynoic acid) marine NP inhibitors at acidic pH but not at neutral pH. GER-12 was effective for the reversible inhibition of cathepsin B, with an IC_{50} of 3 μM. GER-24 had an IC_{50} of 16 μM and was found to be an irreversible inhibitor. These results show that NP screening at distinct biological pH conditions can lead to the identification of pH-selective cathepsin B modulators. These findings suggest that screening efforts for molecular probes and drug discovery may consider the biological pH environment of the target in the disease process.
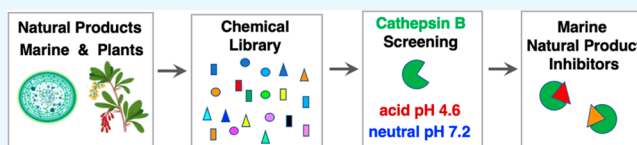
INTRODUCTION

Cathepsin B is a lysosomal cysteine protease that is normally involved in molecular pathways that maintain cellular homeostasis through proteolysis and protein catabolism.[1,2] Dysregulation of cathepsin B has been implicated in brain disorders, including Alzheimer's disease (AD), traumatic brain injury (TBI), and others,[2] as well as cancer[3] and inflammatory diseases.[4,5] The inhibition of cathepsin B activity has been an area of interest to assess the mechanistic role of cathepsin B in disease pathogenicity and drug discovery efforts.

Evidence for the participation of cathepsin B in brain disorder pathogenicity has spurred much investigation into agents that inhibit cathepsin B to ameliorate dysfunctions associated with neurodegenerative diseases. Cathepsin B is involved in the inflammatory pathways and cell death pathways of AD and TBI, which are hypothesized to involve the leakage of lysosomal cathepsin B into the cytosol.[2] *In vivo* inhibition or gene silencing of cathepsin B in AD mouse models results in improved memory deficits and alleviates neuropathology.[6−8] *In vivo* studies of TBI mouse models show that gene knockout of cathepsin B improves motor dysfunction and reduces brain tissue loss.[9] Furthermore, clinical patient studies indicate elevated levels of cathepsin B in AD and TBI.[10,11] Thus, there is interest in the field to discover new cathepsin B inhibitors.

Marine and plant natural products (NP) provide rich sources of chemically diverse compounds that are biologically active and useful as active therapeutic agents.[12,13] Studies have shown that potent and effective protease inhibitors can be

identified from NP sources.[14,15] For example, gallinamide A is a potent and selective inhibitor of the lysosomal cysteine protease cathepsin L discovered from marine cyanobacteria,[16] which has the potential to reduce SARS-CoV-2 infection.[17] Natural products, both marine and terrestrial, account for ~50% of small-molecule drugs approved for the treatment of cancer and related conditions; this includes their novel structures as well as their derivatives.[18] Thus, NP small-molecule libraries provide rich and novel resources to search for selective inhibitors of cathepsin B activity.

Screening of small molecules in drug discovery efforts has traditionally utilized optimal enzyme assay conditions for the assessment of effective modulators. However, drug targets in biological systems may be located at different pH conditions within subcellular and extracellular locations in diseases compared to normal healthy conditions. For example, cathepsin B in numerous brain disorders[2] and cancer[3] mediates disease deficits at abnormal neutral pH locations, including the cytosol and extracellular locations; however, cathepsin B is normally located in lysosomes containing an acidic internal
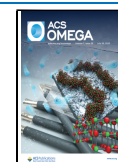
pH.[1,2] The large pH differences between pathogenic and normal enzyme locations in biological systems suggest that drug discovery should target the pathogenic pH form of the enzyme in screening programs.

Significantly, our previous studies showed that cathepsin B is selectively inhibited by Z-Arg-Lys-AOMK at neutral pH 7.2 with a nanomolar (nM) potency, with no inhibition at acidic pH 4.6 at nM inhibitor concentrations.[19] The Z-Arg-Lys-AOMK inhibitor was designed using the pH 7.2 preferences of residues adjacent to the cleavage sites of cathepsin B proteolysis. Cleavage profiling analysis of cathepsin B using a defined library of diverse peptide substrates led to the design of the neutral-pH-selective substrate Z-Arg-Lys-AMC and an acid-pH-selective substrate Z-Glu-Lys-AMC. We propose that the use of these pH-selective substrates in compound screening efforts may uncover pH-selective modulators of cathepsin B.

For these reasons, the goal of this study was to evaluate the hypothesis for differential screening outcomes of natural product (NP) modulators, from marine and plant organisms, of cathepsin B by comparing screening assays conducted at the acidic lysosomal pH of 4.6 to those conducted at the neutral cytosolic pH of pH 7.2. Indeed, results of such a screening approach identified NP inhibitors effective at acidic pH, but not at neutral pH, for inhibition of cathepsin B. These findings indicate that NP compound screening at distinct biological pH conditions can lead to the differential identification of cathepsin B enzyme modulators. Furthermore, the finding of pH-dependent NP modulators may reflect natural pH-dependent activities of NP molecules in their native ecological environments.

RESULTS AND DISCUSSION

Results. *Strategy to Assess Marine and Plant Natural Product (NP) Modulators of Cathepsin B at Acidic and Neutral pH Conditions Using pH-Selective Peptidic Substrates.* A collection of purified natural product compounds consisting of 151 chemical molecules from marine organisms of cyanobacteria, Rhodophyta, sponge, mollusk, and Phaeophyceae combined with NP molecules from plants were utilized to discover pH-dependent inhibitory modulators of human cathepsin B proteolytic activity (Figure 1). Our unique cathepsin B screening strategy was conducted at the lysosomal pH of 4.6 and the cytosolic pH of 7.2. These cathepsin B assays used pH-selective substrates Z-Glu-Lys-AMC and Z-Arg-Lys-AMC that monitor cathepsin B activity at acidic pH 4.6 and neutral pH 7.2, respectively.[19] NP molecules displaying at least 50% inhibition of cathepsin B activity were further assessed for potency by calculating IC$_{50}$ values, the reversible or irreversible mechanism of inhibition, and the specificity of the NP inhibition of cathepsin B compared to other cysteine cathepsins.

Inhibitors of Acidic Cathepsin B Activity Revealed through Screening a Collection of Pure NP Compounds at Neutral and Acidic pH Assay Conditions. The library of pure NPs from marine and plant organisms was assessed for the modulation of human cathepsin B activity. Assays were conducted by preincubating (30 min) each NP with cathepsin B at pH 7.2 and pH 4.6, followed by adding the substrate and monitoring the proteolytic activity. Screening of the NP compounds utilized pH-selective substrates Z-Arg-Lys-AMC at pH 7.2 and Z-Glu-Lys-AMC at pH 4.6, which led to the identification of several active compounds that showed varying degrees of cathepsin B modulation (Figure 2). Seven
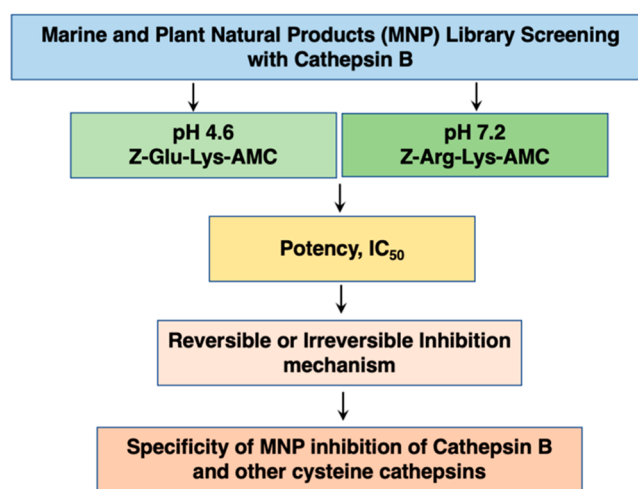

Figure 1. Workflow for screening marine and plant natural products (NP), and synthetic compounds, in cathepsin B assays at acidic and neutral pH conditions. A collection of pure NP compounds was assessed for modulators of human cathepsin B using acidic pH 4.6 and neutral pH 7.2 screening assay conditions. NP molecules that inhibited cathepsin B by >50% were characterized for potency, a reversible or irreversible inhibition mechanism, and specificity for cathepsin B compared to other cysteine cathepsins.
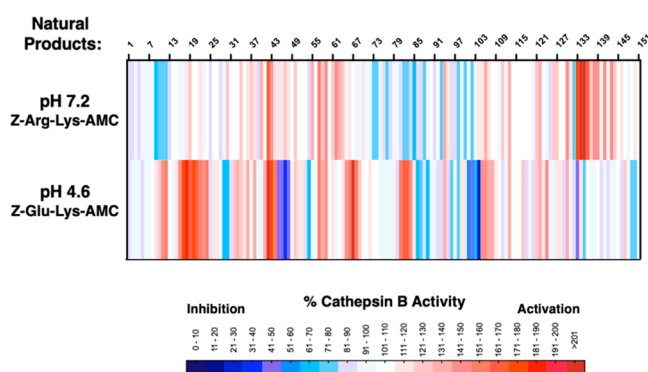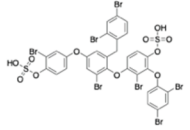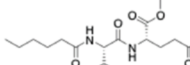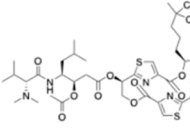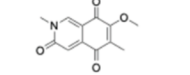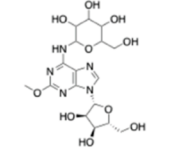

Figure 2. Heat map of the screening data illustrates pH-selective modulators of cathepsin B. NP molecules were preincubated with human cathepsin B (30 min) and then assessed for the modulation of proteolytic activity at pH 4.6 with the Z-E-K-AMC substrate and at pH 7.2 with the Z-R-K-AMC substrate. Modulators resulted from screening marine and plant natural products and synthetic compounds. Modulators of cathepsin B activity are illustrated in a heat map, which shows the inhibition (blue) and activation (red) by NPs (1−10 μM) compared to the controls (100%, no inhibitor).

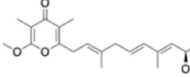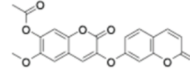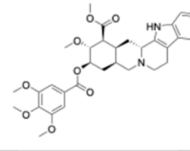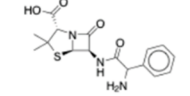compounds were observed to significantly ($p < 0.05$) inhibit cathepsin B activity at pH 4.6 by at least 50% at NP concentrations of 1−10 μM, with no inhibition of cathepsin B at neutral pH 7.2 (Table 1). Furthermore, at pH 4.6, one NP molecule activated cathepsin B at least 200% or greater compared to controls (100%), with no effects at pH 7.2. Additionally, two other NPs activated cathepsin B at pH 7.2 without effects at pH 4.6 (Table 1). These results illustrate the differential discovery of NP inhibitors or activators of cathepsin B activity at acidic pH 4.6 compared to neutral pH 7.2. Inhibition of cathepsin B has been predicted in the field to ameliorate several conditions of brain disorders, cancer, infectious disease, and others.[2−9] Therefore, we continued with the characterization of NP inhibitors of cathepsin B.

Table 1. Identification of pH-Selective Marine Natural Product Modulators of Cathepsin B[a]

Natural Product Compound	Concentration	Cathepsin B Activity		Compound Structure
		pH 4.6 Z-E-K-AMC	pH 7.2 Z-R-K-AMC	
None	0	100%	100%	
GER-12	1.6 μM (2 μg/ml)	43 ± 5 *	78 ± 15	
GER-24	8.0 μM (2 μg/ml)	23 ± 1 *	117 ± 5	
GER-151	5.2 μM (2 μg/ml)	205 ± 21 *	109 ± 11	
GER-163	2.2 μM (2 μg/ml)	48 ± 7 *	118 ± 11	
GER-165	8.6 μM (2 μg/ml)	47 ± 4 *	120 ± 13	
GER-169	4.4 μM (2 μg/ml)	38 ± 7 *	128 ± 7	
GER-170	6.0 μM (2 μg/ml)	50 ± 4 *	118 ± 3	
ST024730	2.0 μM	50 ± 7 *	173 ± 33	
ST024752	2.0 μM	111 ± 33	241 + 21 *	
ST024772	2.0 μM	73 ± 22	205 ± 21 *	

[a]A collection of pure marine natural products (purified) were assessed for the modulation of human cathepsin B activity (with preincubation) by screening them at pH 4.6 and pH 7.2 using pH-selective substrates of Z-E-K-AMC and Z-R-K-AMC, respectively. the asterisk indicates compounds that resulted in a significant inhibition of <50% (blue) or activation of >200% (green) with *$p < 0.05$.

GER-12 (Crossbyanol B) and GER-24 ((7Z,9Z,12Z)-Octadeca-7,9,12-trien-5-ynoic Acid) NP Inhibition of Cathepsin B at Acidic pH. To evaluate the seven inhibitors at the same concentration, inhibitors were tested at 5 μM since the initial inhibitor testing was performed in the micromolar range (shown in Table 1). Reassessment of the seven inhibitors (without preincubation) resulted in two compounds, GER-12

and GER-24, that demonstrated the inhibition of cathepsin B (Table 2). Therefore, continued studies examined GER-12 and GER-24 in concentration-dependent studies (Figures 3 and 4, respectively).

The potencies of the NPs were assessed by calculating the concentration of each compound that inhibited cathepsin B by 50% (IC$_{50}$ value). The GER-12 inhibition of cathepsin B at pH

Table 2. Inhibitors with No Preincubation in Cathepsin B Assays at pH 4.6 with the Z-EK-AMC Substrate[a]

inhibitor (5 μM)	% control cathepsin B activity
none	100
GER-12	28
GER-24	80
GER-163	103
GER-165	112
GER-169	99
GER-170	101
ST024730	110

[a]The indicated NP molecules were assessed at 5 μM, with no preincubation of inhibitor and cathepsin B enzyme, in assays using the substrate Z-EK-AMC at pH 4.6.

4.6 had an IC$_{50}$ value of 3 μM (Figure 3b). The GER-24 inhibition of cathepsin at pH 4.6 had an IC$_{50}$ value of 16 μM (Figure 4b). These results show the effective inhibition of cathepsin B at acidic pH by GER-12 and GER-24 at micromolar levels of inhibitors.

GER-12 Reversible Inhibition and GER-24 Irreversible Inhibition of Cathepsin B. The mechanism of GER-12 and GER-24 inhibition was assessed by preincubating cathepsin B (at 100× the enzyme concentration for the assay) for 30 min with the inhibitor (at 10× times its IC$_{50}$). A 100-fold dilution was then performed on the mixture, and the Z-E-K-AMC substrate was added to the mixture to the monitor the proteolytic activity over a time-course up to 120 min.

After GER-12 preincubation, the progress of cathepsin B activity in the time course was similar to that for the enzyme incubated alone (no inhibitor), demonstrating reversible inhibition by GER-12 (Figure 3c). Further assessment of the reversible inhibition showed that GER-12 inhibition of cathepsin B was not observed in the presence of the nonionic detergent triton X-100 (Supplemental Figure S1).

After GER-24 preincubation, cathepsin B inhibition occurred over the entire time course, which demonstrated the irreversible inhibition of cathepsin B by GER-24 (Figure 4c). These data demonstrate the different reversible and irreversible inhibition mechanisms of GER-12 and GER-24, respectively.

Kinetics of GER-12 and GER-24 Inhibition. Kinetics of GER-12's reversible inhibition of cathepsin B was assessed by Michael−Menten and inverse Lineweaver−Burk plots (Supplemental Figure S2). A K_i value of 1.4 μM was calculated for

the noncompetitive inhibition of cathepsin B by GER-12. GER-24's irreversible inhibition of cathepsin B was observed to possess the kinetic constant k_{inact}/K_I of 4.2 × 10^5 μM^{-1} s^{-1} (Supplemental Figure S3).

GER-12 and GER-24 Inhibition of Cathepsin B Compared to Other Cysteine Cathepsins. GER-12 was evaluated for the inhibition of cathepsin B compared to members of the cysteine cathepsin family consisting of cathepsins C, H, K, L, S, V, and X. At 10 μM (pH 4.6), GER-12 inhibited cathepsin B up to 14% of the control with no inhibitor (100%) and inhibited cathepsins C, H, K, L, S, V, and X up to 26%, 8%, 21%, 89%, 13%, 3%, and 54% compared to control (Table 3). These data indicate that GER-12 inhibits several cysteine cathepsins in addition to cathepsin B.

At 50 μM (pH 4.6), GER-24 inhibited cathepsin B up to 0% of control (100%) and inhibited cathepsins C, H, K, L, S, V, and X up to 65% 21%, 7%, 84%, 0%, 17%, and 67% compared to the no-inhibitor control (100%) (Table 3). Thus, GER-24 at a high concentration of 50 μM inhibits cathepsin B and several cysteine cathepsin proteases.

These data show that the inhibition of cathepsin B by GER-12 and GER-24 occurs without selectivity, as shown by inhibition of other cysteine cathepsin proteases.

■ DISCUSSION

Results of this study highlight the incorporation of biological pH conditions for screening NP modulators of cathepsin B at acidic pH compared to neutral pH conditions. The use of different pH screening conditions for cathepsin B resulted in the identification of different NP compounds that inhibited cathepsin B at lysosomal acidic pH 4.6 compared to cytosolic neutral pH 7.2. These findings indicate the importance of considering the distinct pH environments of the biological locations of enzyme targets in the design of screening conditions to identify NP or other types of chemical modulators. Most chemical library screens for enzyme modulators utilize the optimum *in vitro* enzymatic assay conditions, including pH. However, differential pH properties of enzymes, such as those for cathepsin B, suggest that the target enzyme may be treated as distinct types of enzymes at different pH levels. Our previous finding of pH-selective modulators of cathepsin B may reflect the enzyme's pH-selective cleavage properties at acid and neutral pH conditions.[19] The discovery and development of modulators through chemical screening will benefit from specifying the pH

Figure 3. GER-12 (crossbyanol B) potency and reversible inhibition of cathepsin B. (a) The GER-12 NP structure, (b) the potency for inhibition illustrated by its IC$_{50}$ value, and (c) the reversible mechanism of inhibition are illustrated. Potency was assessed at different concentrations of GER-12 to assess IC$_{50}$ values for the inhibition of cathepsin B, and the reversible mechanism of GER-12 inhibition was determined by dilution experiments, as explained in the section Experimental Procedures.

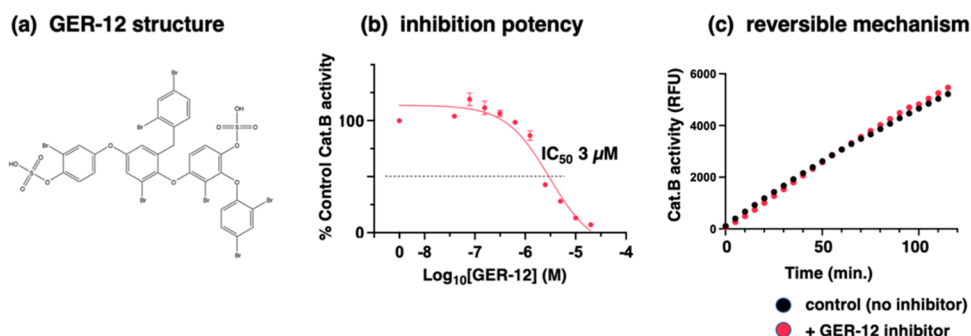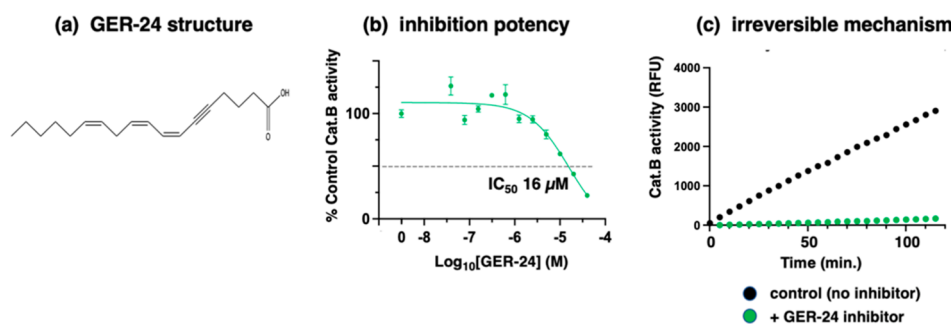**Figure 4.** GER-24 ((7Z,9Z,12Z)-octadeca-7,9,12-trien-5-ynoic acid) potency and irreversible inhibition of cathepsin B. (a) The GER-24 NP structure, (b) the potency for inhibition illustrated by its IC$_{50}$ value , and (c) the irreversible mechanism of inhibition are illustrated. Potency was assessed at different concentrations of GER-12 for the inhibition of cathepsin B, as assessed by the IC$_{50}$ value, and the irreversible mechanism of GER-12 inhibition was determined by dilution experiments, as explained in the section Experimental Procedures.

Table 3. Evaluation of the GER-12 and GER-24 Inhibition of Cathepsin B Compared to Other Cysteine Cathepsin Proteases[a]

protease	GER-12 (10 μM) % control activity (100%, no inhibitor)	GER-24 (50 μM) % control activity (100%, no inhibitor)
cathepsin B	14	0
cathepsin C	26	65
cathepsin H	8	21
cathepsin K	21	7
cathepsin L	89	84
cathepsin S	13	0
cathepsin V	3	17
cathepsin X	53	67

[a]GER-12 and GER-24 inhibition (no preincubation) of cathepsin B and the cysteine cathepsins C, H, K, L, S, V, and X were compared. Assay conditions (no preincubation) for these human cysteine cathepsins are provided in the procedures.

condition of the enzyme target to address pH-specific cellular or physiological functions.

It is of interest that cathepsin B displays pH-dependent properties for inhibitors through compound screening at acidic pH 4.6 and neutral pH 7.2 conditions. Protease inhibitors often mimic peptide substrate-binding properties with the protease enzyme. The substrate profiling analysis of cathepsin B showed its pH-dependent preferences for amino acid residues adjacent to the P1–P1′ substrate cleavage sites.[19] Notably, glutamate (Glu) is the preferred P2 residue of cathepsin B at acidic pH 4.6, but the Glu residue is not preferred at pH 7.2. It is of interest that GER-24 possesses a functional group of −CH$_2$−CH$_2$−COOH that resembles the Glu side chain of substrates that interact with the S2 subsite (Glu-245) of the enzyme.[19] Thus, it is possible that the −CH$_2$−CH$_2$−COOH group of GER-24 may interact with cathepsin B at its S2 subsite. With respect to GER-12, its inhibitory activity was not observed in the presence of the detergent triton X-100, suggesting nonionic detergent-dependent interactions; however, a future structural analysis will be needed. Interactions of inhibitors with target proteases are best assessed by structural analysis via X-ray crystallography, which has, for example, been achieved for the selective CA-074 inhibitor of cathepsin B.[20−22] CA-074 interacts with the enzyme's active site region at the S2−S2′ subsites. It will be of interest to assess pH-selective inhibitor interactions with cathepsin B through future structural analyses.

Proteases such as cathepsin B may have dual functions within acidic lysosomes and extra-lysosomal neutral cellular compartments, including the cytosol, nuclei, and extracellular locations.[1,2,23] Cathepsin B, for example, displays distinct substrate and inhibitor specificity at acidic pH compared to neutral pH functions.[19,24] Therefore, it will be valuable to develop inhibitors of neutral pH cathepsin B to allow the assessment of its functions in extra-lysosomal locations, including cell death, inflammation, and cancer. Selective inhibitors of acidic cathepsin B functions will enhance the understanding the enzyme's role in protein health, namely proteostasis, which is important for cellular functions in health and disease. Furthermore, pH-selective activators may be utilized to gain an additional understanding of the function of cathepsin B.

Furthermore, the results of our NP screens for modulators of cathepsin B indicate that marine natural product molecules such as GER-12 (crossbyanol B from cyanobacteria) and GER-24 ((7Z,9Z,12Z)-octadeca-7,9,12-trien-5-ynoic acid from Rhodophyta) may possess pH-dependent modulating functions for target enzymes. This study opens avenues to assess natural products for the pH-selective inhibition or activation of proteases and enzymes. Therefore, natural product screening under different biological pH conditions, as demonstrated by this study, can be an important avenue to advance our understanding of the multiple roles of cathepsin B and enzymes in cellular compartments of differing pH conditions.

In summary, the results of this study indicate that using different pH conditions to screen NP chemical libraries can lead to the identification of pH-selective modulators of targeted protease enzymes in designated biological pH environments.

■ EXPERIMENTAL PROCEDURES

Experimental procedures are summarized here. Detailed descriptions of protocols are provided in the Supporting Information.

Materials and Reagents. The sources of enzymes, peptide substrates, and reagents are provided in the Supporting Information.

Collection of Purified Marine and Plant Natural Products (NP). Natural products have been collected and purified from multiple marine organisms of cyanobacteria, Rhodophyta, sponges, mollusk, Phaeophyceae, and plant sources. The list of NP molecules investigated is provided in Supporting Information Table S1. Marine NP compound stocks were 1 mg/mL and were screened at a final

concentration of 2 μg/mL. The plant NP compounds stocks were at 1 mM and were screened at a final concentration of 2 μM. The structures of the NP molecules are provided in Supporting Information Table S2, which indicates diverse NP chemical structures. These molecules were utilized for screening in the cathepsin B assays.

NP Screening Conducted at pH 4.6 and pH 7.2 to Identify Modulators of Cathepsin B. Assays of cathepsin B (activated) with 151 pure marine natural products were conducted in triplicate with 5 mM DTT, 1 mM EDTA, 100 mM NaCl, and 1.5% DMSO using 0.2 ng/μL cathepsin B at pH 4.6 in 40 mM citrate phosphate buffer with 60 μM Z-Glu-Lys-AMC substrate and at pH 7.2 in 40 mM citrate phosphate buffer with 60 μM Z-Arg-Lys-AMC substrate. Each pure NP (151 compounds in total) was preincubated with the enzyme for 30 min at RT. To the mixture was then added the substrate, and the sample was incubated at RT for 30 min. Fluorescence was measured at 360 nm excitation and 460 nm emission. Cathepsin B activity in the absence and presence of each NP was assessed for significance using Student's t test with $p <$ 0.05. As a positive inhibitor control, each screening plate included CA-074 (1 μM final concentration), a known selective inhibitor of cathepsin B;[21,22] CA-074 is a natural product originally isolated from *Aspergillus japonicus*.[25]

It is noted that the Z-Glu-Lys-AMC substrate at pH 4.6 and the Z-Arg-Lys-AMC substrate at pH 7.2 had similar K_m values of 460 and 429 μM, respectively. These similar K_m values of both substrates at a concentration of 60 μM in the screening assays represent equivalent substrate conditions.

Potency of NP Inhibition of Cathepsin B as Assessed by IC$_{50}$ Values. GER-12 (crossbyanol B) and GER-24 ((7Z,9Z,12Z)-octadeca-7,9,12-trien-5-ynoic acid) at different concentrations were incubated with cathepsin B (activated) without preincubation in cathepsin B assay conditions (described above). IC$_{50}$ values were calculated as the inhibitor concentration that reduced cathepsin B activity by 50%. Curve-fitting of the inhibition curves and the calculation of IC$_{50}$ values were performed with Prism software (version 8). Confidence intervals of 95% were obtained for GER-24 and GER-12 at 7.9−36.5 and 2.1−4.4 μM, respectively, using a nonlinear regression curve fit model.

Assessment of Inhibitors for a Reversible or Irreversible Mechanism of Inhibition. Cathepsin B (100×) was preincubated with GER-12 or GER-24 at 10× the determined IC$_{50}$ concentration for 30 min at room temperature (25 °C). Then, a 1:100 dilution of the mixture was performed. The Z-Glu-Lys-AMC substrate was added to the diluted mixture, and the fluorescence was monitored for proteolytic activity every minute for 2 h. The lack of inhibition of cathepsin B activity following dilution indicates a reversible inhibitory mechanism. The reduction of cathepsin B activity following dilution indicates an irreversible inhibitory mechanism.

Kinetics of the Reversible and Irreversible Inhibition of Cathepsin B. GER-12 reversible inhibition kinetics was assessed by Michaelis−Menten and Lineweaver−Burke inverse plots to determine the K_i value for the noncompetitive inhibition of cathepsin B by GER-12 using the equation $^{app}V_{max} = V_{max}(K_i /[I]_o + K_i)$.[26] The irreversible inhibition by GER-24 was assessed by determining the K_I value as we previously described.[19]

Evaluation of GER-12 and GER-24 for the Inhibition of Cathepsin B Compared to Other Cysteine Cathe-

psins. GER-12 at 10 μM and GER-24 at 50 μM were evaluated for the inhibition of cathepsin B compared to cathepsins C, H, K, L, S, V, and X. Assay conditions for these cathepsin proteases are described in the Supporting Information.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

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Author Contributions

screening. V.V.P. and C.M. conducted the experiments and data calculations for analysis. A.J.O. and C.R.C. provided advice and evaluated the results. V.H. and V.V.P. wrote the manuscript text. All authors provided editorial input for the final manuscript submitted.

Notes

The authors declare the following competing financial interest(s): V.H. has an equity position at American Life Science Pharmaceuticals (ALSP) and is a founder of ALSP. V.H. is an advisor to ALSP. V.H.'s conflict has been disclosed and is managed by her employer, the University of California, San Diego. The other authors have no conflicts of interest.

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