Identification and characterization of small-molecule inhibitors of hepsin

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Abstract

Hepsin is a type II transmembrane serine protease overexpressed in the majority of human prostate cancers. We recently demonstrated that hepsin promotes prostate cancer progression and metastasis and thus represents a potential therapeutic target. Here we report the identification of novel small-molecule inhibitors of hepsin catalytic activity. We utilized purified human hepsin for high-throughput screening of established drug and chemical diversity libraries and identified sixteen inhibitory compounds with IC50 values against hepsin ranging from 0.23-2.31 μM and relative selectivity of up to 86-fold or greater. Two compounds are orally administered drugs established for human use. Four compounds attenuated hepsin-dependent pericellular serine protease activity in a dose-dependent manner with limited or no cytotoxicity to a range of cell types. These compounds may be used as leads to develop even more potent and specific inhibitors of hepsin to prevent prostate cancer progression and metastasis. [Mol Cancer Ther 2008;7(10):3343–51]

Introduction

Prostate cancer is the most common cancer in United States males, with an estimated 186,320 new cases in 2008, accounting for 25% of cancer incidence and 10% of cancer deaths (1). Although significant progress has been made in recent years in understanding the molecular mechanisms responsible for prostate cancer initiation and progression, therapeutic approaches for the treatment of prostate cancer remain limited. Although localized prostate tumors are usually curable, diagnosis of prostate cancer remains a difficult, inexact process and treatment can result in side effects that significantly affect quality of life (2, 3). Metastatic prostate cancer is highly resistant to therapeutic intervention and almost uniformly fatal. Therefore, the development of effective novel targeted therapies to inhibit prostate cancer progression and metastasis will have a significant effect on prostate cancer mortality.

Multiple genetic and epigenetic changes take place during human prostate cancer initiation and progression (4, 5). Hepsin (HPN) is one of the most up-regulated genes in human prostate cancer and encodes a type II serine protease overexpressed in up to 90% of prostate tumors with levels often increased >10-fold (6–8). Hepsin is up-regulated early in prostate cancer initiation and maintained at this high-level throughout progression and metastasis. In addition, hepsin is also overexpressed in ovarian and renal carcinomas (9, 10).

Significant evidence indicates that hepsin overexpression plays an important role in the promotion of prostate cancer progression and metastasis. Hepsin up-regulation in a transgenic mouse model of localized prostate cancer promoted progression, causing the transition of nonmetastatic cancer into an aggressive carcinoma with metastasis to bone, liver, and lung (11). The cellular context and level of hepsin expression appear to be important to the phenotype, as high levels of hepsin overexpression in a prostate cancer cell line reduces cell proliferation and invasion (12). Whereas the molecular mechanisms responsible for hepsin function in prostate cancer in vivo are unknown, in vitro evidence indicates that hepsin can activate pro-urokinase plasminogen activator and pro-hepatocyte growth factor (13, 14). Activation of the urokinase plasminogen activator cell-surface serine protease system and hepatocyte growth factor-Met scattering pathway may be responsible for hepsin promoting metastasis and is consistent with the observed basement membrane disruption in mouse prostates overexpressing hepsin (11).

We sought to identify small molecules that specifically inhibit hepsin catalytic activity that may be used as lead compounds to develop targeted drugs to attenuate prostate cancer progression. Protease-targeted drugs have proven to be clinically useful for treatment of HIV and hypertension and show potential in the treatment of cancer, obesity, cardiovascular, inflammatory, and neurodegenerative diseases (15). WX-UK1 is a potent small-molecule inhibitor of urokinase plasminogen activator developed by Wilex and has shown antitumor and antimetastasis activity in a...
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Materials and Methods

Reagents

The DIVERSet and NINDS II compound libraries and reordered hit compounds from those libraries were purchased from Chembridge and MicroSource Discovery Systems, respectively. The chromogenic peptide pyroGlu-Pro-Arg-pNA (S-2366) was purchased through Diapharma Group. Rabbit anti-human hepsin polyclonal antibody was purchased from Cayman Chemical and goat anti-rabbit secondary antibody purchased from Jackson Immuno-Research. Polyclonal rabbit anti-mouse hepsin antisera was produced against a synthetic peptide corresponding to the last 18 amino acids of mouse hepsin. Molecular biology-grade DMSO was purchased from Fluka. Trypsin was purchased from ICN and thrombin was purchased from Sigma.

Recombinant Hepsin Expression and Purification

Recombinant expression and chromatographic purification of human hepsin was done as described previously (17).

Compound Library Screening

The DIVERSet 10,000 compound and NINDS II 1,040 compound libraries were diluted from 20 and 10 mmol/L stock plates in DMSO, respectively, to 20 μmol/L 10× solutions in 10% DMSO. Purified hepsin was incubated with 2 μmol/L compounds in 30 mmol/L Tris-HCl, pH 8.4, 30 mmol/L imidazole, 200 mmol/L NaCl, and 1% DMSO for 30 min at room temperature. Chromogenic peptide was added and the reactions were allowed to proceed for 3 h. Endpoint absorbance was measured using a VersaMax microplate reader (Molecular Devices), corrected for background and residual activity observed relative to buffer/solvent controls on each plate.

Hepsin, Trypsin, and Thrombin Activity Assays

Titration of the chromogenic substrate pyroGlu-Pro-Arg-pNA was done for each enzyme and the resulting substrate-velocity data fit with nonlinear regression using GraphPad Prism 4 to calculate Vₘₐₓ and Kₘₑₜₐ. Enzyme assay concentration and observed Kₘₑₜₐ: 0.4 nmol/L hepsin, Kₘₑₜₐ = 170 μmol/L; 0.4 nmol/L trypsin, Kₘₑₜₐ = 78.6 μmol/L; thrombin 188 μmol/L, Kₘₑₜₐ = 106.2 μmol/L. Inhibitor activity was determined by incubating the individual enzymes with increasing concentrations of compounds in the library screen buffer for 30 min at room temperature followed by addition of the substrate at the observed Kₘₑₜₐ. The reactions were then followed using a kinetic microplate reader and the linear rates of increase in absorbance at 405 nm were expressed as residual percent activity (100% − vₒᵣᵣ/vₑᵢₙᵢᵣ). At least three independent experiments were done for each enzyme. IC₅₀ was calculated by fitting the data to a four-variable nonlinear regression using GraphPad Prism 4. The equilibration time dependence of inhibitor potency was determined by incubating hepsin with the respective inhibitor at its IC₅₀ value or buffer/solvent alone under the above conditions in triplicate. Samples were withdrawn at 30, 60, 120, and 180 min and activity was analyzed by the addition of substrate as above. Data are shown as percent inhibition relative to the respective buffer/solvent controls incubated for the same amount of time. The reversibility of inhibition was determined using a dilution technique. Hepsin was incubated with the inhibitors at their respective IC₅₀ values or buffer control as above for 1 h at room temperature in triplicate. Samples were then diluted with buffer to the additional percentage indicated and activity measured as above. Data are shown as percent inhibition relative to the respectively diluted buffer controls.

Cell Culture

LNCaP, HepG2, and HEK 293FT cells were purchased from the American Type Culture Collection. The spontaneously transformed mouse prostate epithelial cell line MP-1 was established by passaging C57/B6 primary mouse prostate epithelial cells. Cell culture components and suppliers were as follows: DMEM, F-12, and RPMI base media (Invitrogen), hydrocortisone (Calbiochem), and insulin, T3, and cholera toxin (Sigma). Cells were incubated in a humidified (37°C, 5% CO₂) incubator and passaged at 80% confluency with trypsin/EDTA. Mouse prostate epithelial cells MP-1 were maintained in E-medium containing 3:1 DMEM/F-12, 37 mmol/L sodium bicarbonate, 0.42 μg/mL hydrocortisone, 0.89 mg/mL chola toxan, 5.3 μg/mL insulin, 5.3 μg/mL transferrin, 2.1 × 10⁻¹¹ mol/L T3, penicillin/streptomycin, and t-glutamine with 15% FCS. LNCaP cells were maintained in RPMI supplemented with 10% FCS and penicillin/streptomycin. HepG2 cells were maintained in DMEM supplemented with 10% FCS and penicillin/streptomycin. 293FT cells were maintained in DMEM supplemented with 10% FCS, t-glutamine, nonessential amino acids and penicillin/streptomycin.

Cell Cytotoxicity Assay

The general cytotoxicity of the compounds was determined using the CellTiter-Glo Assay from Promega. Mouse prostate epithelial, LNCaP and HepG2 cells were seeded in 96-well black culture plates at 2 × 10⁴ per well and allowed to attach. Medium was aspirated and compounds were administered at 20 μmol/L in the appropriate medium and medium with compounds was changed at 24 and 48 h. CellTiter-Glo reagent was added to the cells and ATP-luciferase coupled activity recorded on a microplate luminometer.
Pericellular Serine Proteolytic Activity Assay

Plasmids encoding full-length mouse wild-type, or active-site S352A mutant hepsin (Genbank: NM008281) cDNA, or the empty pLNCX2 vector were transfected into HEK 293FT cells using a calcium phosphate protocol. After 3.5 h, transfect medium was replaced with fresh medium containing 20 or 50 μmol/L of test compounds or solvent control (0.5% DMSO in medium) and cells incubated to 24 h post-transfection. Attached cell monolayers were then washed twice with PBS and once with assay buffer (5% CO2 equilibrated phenol red-free DMEM with 1% bovine serum albumin) to remove residual serum proteases/inhibitors. Cells were then incubated in assay buffer containing 20 or 50 μmol/L compounds for 30 min at 37°C. Peptide substrate was then added to a final concentration of 369 μmol/L (observed K_m in this system) and the reactions were incubated at 37°C for 30 min. The chromogenic serine protease substrate pyroGlu-Pro-Arg-pNA was then added and enzyme activity was observed as a linear increase in absorbance at 405 nm over time.

Figure 1. Characterization of recombinant active hepsin. A, chromatographically purified recombinant human hepsin was produced in P. pastoris and analyzed by silver staining of SDS-PAGE gel and immunoblotting with anti-hepsin catalytic domain antibodies. B, purified hepsin is proteolytically active and inhibited by a broad-spectrum serine protease inhibitor. Purified hepsin (0.4 nmol/L) was incubated at room temperature for 30 min in buffer alone (diamonds) or in the presence of 4 mmol/L PEFABloc (triangles). The chromogenic serine protease substrate pyroGlu-Pro-Arg-pNA was then added and enzyme activity was observed as a linear increase in absorbance at 405 nm over time.

Figure 2. Chemical structures of identified hepsin inhibitors. Compounds 1 to 12 were identified from the Chembridge DIVERSet library. Compounds 13 to 16 (meclizine, probucol, anthralin, and 2,3-dihydroxy-6,7-dichloroquinoxaline) were identified from the NINDS II library of known drugs and bioactives.
allowed to proceed at 37°C. Samples were withdrawn at 20, 40, and 60 min and quenched into an equal volume of 7% acetic acid and absorbance at 405 nm was measured with a microplate reader. Percent inhibition was calculated as residual activity relative to solvent control. Matched samples were used in parallel to determine toxicity of the test compounds to HEK 293FT cells in this system using the CellTiter-Glo assay.

Immunoblotting
Total protein lysates from transfected, compound-treated HEK 293FT cells were separated on SDS-PAGE and transferred to Immobilon-P membrane. The membrane was blocked overnight in TBS-Tween 20 buffer containing 5% nonfat milk, 2% normal goat serum in 50 mmol/L Tris-HCl (pH 8.0), 100 mmol/L NaCl, and 0.1% Tween 20. The membrane was then incubated in 3% bovine serum albumin in TBS-Tween 20 with polyclonal anti-mouse hepsin antibody (1:1,000) for 2 h at room temperature, washed 3 × 5 min in TBS-Tween 20, and incubated for 1 h in 0.1% bovine serum albumin in TBS-Tween 20 containing goat anti-rabbit horseradish peroxidase secondary antibody (1:2,000), washed 3 × 5 min in TBS-Tween 20, and developed with enhanced chemiluminescence (Pierce). Protein loading was confirmed by stripping the membrane and reprobing with anti-β-actin antibodies. Expression levels were quantified by densitometry using ImageQuantTL.

Figure 3. Inhibition of hepsin activity by identified compounds. A, compounds 1 to 4. B, compounds 5 to 8. C, compounds 9 to 12. D, compounds 13 to 16. Purified recombinant hepsin was preincubated with indicated compounds for 30 min at room temperature. The residual percent activity of the enzyme toward the chromogenic substrate was then determined with a kinetic microplate reader at 405 nm. Data are the mean of three independent experiments. IC50 was calculated by four-variable nonlinear regression curve fitting.
Table 1. Protease inhibitory activities of the compounds identified from library screening

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<th>Compound</th>
<th>Library</th>
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<th>IC₅₀ hepsin (µmol/L)</th>
<th>IC₅₀ trypsin (µmol/L)</th>
<th>IC₅₀ thrombin (µmol/L)</th>
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<td>1 Chembridge DIVERSet</td>
<td>5133201</td>
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<td>16 NINDS II</td>
<td>N-methyl-D-aspartate receptor antagonist</td>
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*Compound 1 activated thrombin activity, approximately 3-fold at 1 µmol/L.

Results

Expression and Purification of the Recombinant Extracellular Region of Hepsin

The extracellular region of human hepsin consists of residues Ser⁴⁶ to Leu⁴¹⁷ (Genbank: BC025716) and contains the catalytic and scavenger receptor cysteine-rich domain. The yeast Pichia pastoris was stably transfected with the hepsin expression construct and the secreted 41-kDa hepsin zymogen was purified from the medium using several steps of affinity and ion exchange chromatography. During purification to homogeneity, the enzyme spontaneously activated as reported previously (17). Protein identity was confirmed by silver stain of SDS-PAGE separated samples activated as reported previously (17). Protein identity was confirmed by silver stain of SDS-PAGE separated samples and immunoblotting with anti-hepsin catalytic domain polyclonal antibodies (Fig. 1A). The purified hepsin was enzymatically active (Fig. 1B), cleaving the chromogenic proteolytic activity was completely inhibited by the serine protease substrate pyroGlu-Pro-Arg-pNA. This enzymatically active (Fig. 1B), cleaving the chromogenic peptide. In addition, the NINDS II library of 1,040 compounds was screened to identify hepsin inhibitors among established drugs and known bioactive molecules. Screens were done in a 96-well format at a final compound concentration of 2 mol/L. To minimize false positives, positions 1 and 12 of each row contained DMSO/saline controls. As a measure of reproducibility, the Z’ score for this assay was 0.78 (18). Compounds that showed ≥90% inhibition were individually reproduced. Reproduced hits were reordered from the supplier and their inhibitory activity was confirmed (Fig. 2). IC₅₀ values for these compounds were determined by titration of the compounds against kinetic hepsin activity (Fig. 3). Relative specificity was determined by titration against the serine proteases trypsin and thrombin (Table 1).

The determined IC₅₀ values for these compounds against hepsin displayed a range of 0.28 to 2.31 mol/L. Compounds 1, 3, 7, 9, and 10 are subject to nucleophilic addition and may react with the active site serine γ-O. Compounds 2 and 8 share a chlorophenyl-substituted thiadiazolurea core, with compound 2 displaying higher selectivity. Compounds 5 and 6 share a tetrahydro-3H-cyclopenta[c]-quinoline core, with compound 5 displaying both higher potency and selectivity. Compounds 2 and 6 display a hydrogen bond to the γ-O of the catalytic serine of thrombin.

We observed four total hits from the NINDS II library of drugs and bioactive molecules (compounds 13-16). Interestingly, compounds 13 and 14 (meclizine and probucol) are established human-use drugs with oral dosing. Compound 15 (anthratin) is a topically administered antipsoriatic agent. Compound 16 (2,3-dihydroxy-6,7-dichloroquinoxaline, a N-methyl-D-aspartate receptor antagonist) shares a tetrasubstituted pyrazine with amirolide, a selective, moderately potent urokinase plasminogen activator inhibitor (20).

High-Throughput Screening and Characterization of Hit Compounds

To identify novel inhibitors of hepsin, we screened the Chembridge DIVERSet 10,000 compound high-diversity library using an assay based on the cleavage of the chromogenic peptide. In addition, the NINDS II library of 1,040 compounds was screened to identify hepsin inhibitors among established drugs and known bioactive molecules. Screens were done in a 96-well format at a final compound concentration of 2 mol/L. To minimize false positives, positions 1 and 12 of each row contained DMSO/buffer controls. As a measure of reproducibility, the Z’ score for this assay was 0.78 (18). Compounds that showed ≥90% inhibition were individually reproduced. Reproduced hits were reordered from the supplier and their inhibitory activity was confirmed (Fig. 2). IC₅₀ values for...
an ATP-luciferase coupled assay. Compounds 1 and 2 displayed substantial toxicity to LNCaP cells without affecting MP-1 or HepG2 cells. Compound 12 was substantially toxic to LNCaP and MP-1 without affecting HepG2. Compound 15 (a known inhibitor of cellular respiration, metabolism, and DNA synthesis; ref. 21) was toxic to all cell types, particularly to LNCaP and HepG2 cells. Compounds 3 to 11, 13, 14, and 16 displayed limited or no cytotoxicity to these cells at this concentration.

Inhibition of Hepsin-Dependent Pericellular Proteolytic Activity

To determine whether the identified compounds were able to inhibit cell-based hepsin activity, we developed an assay to measure hepsin-dependent pericellular serine proteolytic activity (Fig. 4B; refs. 22–26). For this purpose, we used HEK 293FT cells expressing full-length wild-type and catalytically inactive (S352A) mutant mouse hepsin proteins or vector alone. Attached cell monolayers were

Figure 4. Cellular toxicity of identified inhibitors and hepsin-dependent pericellular serine protease assay. A, mouse prostate epithelial cell line MP-1 (white columns), human prostate cancer cell line LNCaP (gray columns), and human hepatoma cell line HepG2 (black columns) were incubated for 72 h with 20 μmol/L indicated compounds in medium with 0.5% DMSO. Media and drugs were replaced every 24 h. Cell viability was then determined by an ATP-luciferase coupled assay. Puromycin at 5 μg/mL and DMSO at 0.5% were used as positive and negative controls, respectively. B, 293FT cells expressing full-length wild-type, catalytically inactive mouse hepsin mutant, or empty vector were incubated for 30 min in serum-free medium containing vehicle alone or the broad-spectrum serine protease inhibitor PEFAbloc. The chromogenic serine protease substrate pyroGlu-Pro-Arg-pNA was then added to the medium. The medium with cleaved substrate was collected at indicated times, quenched and pericellular proteolytic activity was observed as absorbance at 405 nm. Note that only the cells expressing wild-type hepsin, but not the cells expressing inactive mutant hepsin, vector alone, or wild-type hepsin in the presence of PEFAbloc, displayed pericellular proteolytic activity. C, toxicity of the compounds over the course of the pericellular protease assay was evaluated by 24-h treatment of cells with 20 μmol/L (white columns) or 50 μmol/L (black columns) compounds relative to vehicle control. Cell viability was then determined by an ATP-luciferase coupled assay. Puromycin at 5 μg/mL was used as a positive control.
incubated in assay buffer with peptide substrate and the reactions were allowed to proceed at 37°C. Samples were withdrawn at 20, 40, and 60 min and quenched into an equal volume of 7% acetic acid and absorbance at 405 nm was measured with a microplate reader. Activity levels were adjusted by altering hepsin expression levels to within the linear range of detection. A positive linear rate of activity was observed only for wild-type hepsin-expressing cells and was abolished in the presence of the broad-spectrum serine protease inhibitor PEFAbloc.

To determine the potential cytotoxicity of the previously identified hepsin inhibitors in this model system, HEK 293FT cells were incubated under identical conditions with 20 and 50 μmol/L of the compounds and cytotoxicity was determined using the ATP-luciferase coupled assay as described above (Fig. 4C). Compounds 12 and 15 displayed substantial toxicity at 50 μmol/L and were not further characterized. The remaining compounds at 20 and 50 μmol/L final concentration of were incubated overnight with hepsin-expressing cells and pericellular proteolytic activity was determined in the presence of the compounds as described above (Fig. 5). As treatment with chemical compounds may alter hepsin expression level (and affect pericellular proteolytic activity), hepsin levels in drug-treated cells were determined via immunoblotting. Data are displayed as normalized pericellular proteolytic activity/expression level relative to vehicle-treated wild-type hepsin-expressing cells. We found that compounds 3 to 5 and 13 attenuated pericellular proteolytic activity in a dose-dependent manner without substantially affecting hepsin expression levels or displaying overt toxicity. Compounds 4 and 5 offered the most potent inhibition, attenuating activity approximately 60% at 50 μmol/L. Compounds 3 and 13 reduced activity approximately 50% and 30% (respectively) at 50 μmol/L. To further characterize these four compounds, we evaluated the time dependence and reversibility of their biochemical inhibition of hepsin. Inhibition of hepsin by compounds 3 to 5 and 13 significantly increased with extended equilibration time (Supplementary Fig. S1) and this inhibition was not reversible by dilution (Supplementary Fig. S2), indicating that these compounds are slow-binding, irreversible inhibitors.

**Discussion**

We report here the identification of several small molecules that display potent and selective inhibition of the type II cell-surface serine protease hepsin. Hepsin is overexpressed...
Hepsin Inhibitors

in human prostate, renal, and ovarian cancers and significant evidence implicates hepsin as a metastasis-promoting protease in human prostate cancer. Therefore, specific hepsin inhibitors may be useful to attenuate prostate cancer progression and prevent metastasis.

In this study, we identified 16 hepsin inhibitors using high-throughput screening of small-molecule libraries. To determine the relative selectivity of the newly identified compounds for hepsin, we evaluated their inhibitory activity toward the physiologically relevant serine proteases trypsin and thrombin. Trypsin is a broad-spectrum serine protease with roles in digestion, defense, development, and blood coagulation. Thrombin is a chymotrypsin-like serine protease that converts fibrinogen to fibrin and has other roles in blood coagulation. Several of the compounds identified in this study have substantial selectivity for hepsin, with some of the molecules displaying up to 78-fold selectivity toward hepsin versus trypsin and >87-fold selectivity toward hepsin versus thrombin. These IC_{50} values were determined with relatively short incubation times and it is possible that they were reflective of enzyme-inhibitor association rate differences. Indeed, compounds 3 to 5 and 13 displayed a time-dependent inhibition of enzyme-inhibitor association. Indeed, these IC_{50} values were determined with relatively short incubation times and it is possible that they were reflective of enzyme-inhibitor association rate differences. Indeed, compounds 3 to 5 and 13 displayed a time-dependent increase in hepsin inhibition. In addition, dilution experiments showed that compounds 3 to 5 and 13 irreversibly inhibit hepsin. Compound 3 is subject to nucleophilic addition through 1,4-addition and may form a covalent bond with the active site serine γO. The mechanisms that may be responsible for the irreversibility of the inhibition by compounds 4, 5, and 13 are less clear. In the future studies, it will be important to rigorously characterize the inhibitory potency and specificity of these compounds under fully equilibrated conditions and to determine their mode of inhibition.

Cell-based efficacy is a significant barrier to the development of inhibitors identified with biochemical screens due to target accessibility, matrix effects, and potential nonspecific cytotoxicity. To determine the ability of these compounds to inhibit cell-based hepsin activity, we developed a hepsin-dependent pericellular serine protease activity assay. Four of the identified compounds (3-5 and 13) were able to attenuate pericellular proteolytic activity with limited or no cytotoxicity at effective concentrations. Compounds 4 and 5 were among the most potent inhibitors of hepsin in the purified biochemical assay; however, compounds 3 and 13 were among the least potent. This observation, in addition to the increased dosage required to attenuate pericellular activity, may be attributable to the presence of albumin in the cell-based system. Albumin is known to reversibly bind drugs, reduce their concentration free in solution, and alter dose-response relationships (27, 28). For example, the nonnucleoside HIV reverse transcriptase inhibitor efavirenz (used as part of highly active antiretroviral therapy) is more than 99% protein bound in plasma, mainly to albumin (29). Alternatively, it is possible that a portion of the pericellular serine protease activity observed on hepsin overexpression is due to the hepsin-mediated activation of other serine proteases, which may take place in the Golgi or endoplasmic reticulum, before proteins are delivered to the cell surface. Therefore, lower potency of hepsin inhibitors in the cell-based assay may reflect lower plasma membrane and/or Golgi/endoplasmic reticulum permeability of these compounds.

Two of the compounds that were identified as nonselective hepsin inhibitors are orally administered human use drugs. Probucol is an antihyperlipidemic agent developed for use in coronary artery disease and was one of the most potent and specific in vitro inhibitors of hepsin proteolytic activity. Paradoxically, this drug did not reduce pericellular serine protease activity in the cell-based assay. It is possible that probucol showed no activity in the cell-based assay due to the previously mentioned effects of albumin or its high hydrophobicity. This compound has an approximate logP value of 10, is known to be transported almost exclusively by lipoprotein vesicles in serum and delivered from these directly into the cell membrane (30, 31). Water-soluble analogues of probucol (32, 33) have been synthesized and it will be interesting to determine whether these compounds show inhibition of hepsin proteolytic activity and function in a cell-based assay. Meclizine is an antinausea drug and available as an over-the-counter remedy for motion sickness. It displayed moderate potency, >10-fold selectivity, and was able to attenuate hepsin-mediated pericellular proteolytic activity by 30% at 50 μmol/L. Presently, meclizine is one of the most promising lead compounds and provides a template for hepsin inhibitor optimization via medicinal chemistry approaches.

Prostate cancer develops slowly in the majority of cases; however, progression to metastasis is highly lethal and can occur rapidly. Treatments to prevent metastasis include radical prostatectomy and radiation therapy, both of which carry significant risk to urinary and sexual function. Metastatic prostate cancer can be treated with androgen ablation therapy but almost uniformly results in hormone-refractory disease leading to mortality. Effective agents to prevent disease progression would reduce the need for surgical or radiation-based therapies and have a significant effect on prostate cancer-related mortality. Hepsin inhibitors derived from the lead compounds identified here may serve this purpose.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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Identification and characterization of small-molecule inhibitors of hepsin


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