Chemoenzymatic Synthesis of Organoselenium(IV) Compounds and their Evaluation as Cysteine Protease Inhibitors

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Uma série de organosselenanas foi sintetizada utilizando-se uma metodologia quimio-enzimática. Estas organosselenanas apresentam variações na estereoquímica e no halogênio ligado ao átomo de selênio. Devido à reação característica envolvendo compostos de selênio e tióis, estas organosselenanas foram avaliadas como inibidores de cisteíno proteases (catepsinas V e S). As constantes de inibição de segunda-ordem mostraram que a catepsina S é inibida mais rapidamente do que a catepsina V. Pode-se observar que as organosselenanas dibromadas são inibidores mais potentes do que as dicloradas. Desta forma, os resultados obtidos mostram que compostos hipervalentes de selênio podem ser aplicados como inibidores de cisteíno proteases.

A series of organoselenium dihalides (organoselenanes) was synthesized from organoselenenides using a chemoenzymatic approach. The organoselenanes have variations in their stereochemistry and in the halogen atom bonded to the selenium atom. Because of the unique selenium-thiol chemistry displayed by several organoselenium compounds, the organoselenanes were evaluated as new potential inhibitors of cysteine proteases (cathepsins S and V). By the analysis of the second-order rate constants of the inhibition of cathepsin S and V, it was possible to conclude that organoselenanes inhibited the cathepsin S faster than cathepsin V. It was observed higher inhibitory potencies for the dibromo organoselenanes derivatives than the dichloro analogues. In addition, the present data suggest the use of hypervalent selenium compounds as novel motifs for cysteine proteases inhibitors.

Keywords: selenium, cathepsin, inhibitors, biocatalysis

Introduction

The selenium compounds biological properties have been widely investigated,1 especially because selenium is an essential trace element for mammals and organisms.2 Selenium was found to compose glutathione peroxidase enzymes (GPx), which are pivotal protective enzymes of cells against oxidizing agents.3 The antioxidant activity has been the main studied biological property for organoselenium(II) compounds (organoselenenides)4 due to the possibility of these selenium-containing compounds act as mimetics of GPx. Because of their antioxidant properties, organoselenenides have been studied as antitumor, anti-inflammatory and anti-infective agents,5 furthermore, it has been widely reported that selenium acts as chemopreventive agents6 and is used as a micronutrient-containing foods.7 It was observed that selenium compounds increase the antitumor activity of anticancer drugs and protect against drug-induced toxicity,8 induce apoptosis mainly because of their interaction with thiol-dependent enzymes.9 This remarkable ability of
selenium derivatives to inhibit some enzymes relies on their unique selenium-thiol chemistry. In the case of hypervalent selenium(IV) compounds, the affinity by thiol groups is well known as the reduction of diorganoselenium dichloro to selenides mediated by thiourea, the reaction of selenoxides with thiols leading to selenides and the organoselenone moiety displacement by thiols. Biologically, it was demonstrated that organoseleninic acids also inhibit thiol-dependent protein tyrosine phosphatases, due to the particular reactivity of selenium(IV) atom in seleninic acids with thiols, but there is still a lack on the application of these particular reactivities of hypervalent selenium compounds in biological systems.

Inspired by the sulfur reactivity with selenium compounds, we looked at the cysteine cathepsins, which are lysosomal cysteine proteases, and are often upregulated in various human cancers. They have been implicated in distinct tumorigenic processes, such as angiogenesis, proliferation, and apoptosis and invasion. The human family of cysteine cathepsins has 11 members (cysteine cathepsin B, C, F, H, K, L, O, S, V, W and X), which share a conserved active site that is formed by cysteine, histidine and asparagine residues. Based on the structural catalytic properties of cysteine proteases and selenium(IV) chemistry, we report our investigation on the creation of a new class of selenium-containing compounds, organoselenanes (selenium(IV) compounds), to be used as novel inhibitors of human cysteine cathepsins S and V.

Results and Discussion

In this work, we prepared a small series of selenium-containing compounds to be investigated as possible inhibitors of cysteine cathepsins, through which was possible to evaluate the influence of structural elements, such as the chirality and the effect of halogen derivatives (Figure 1).

Synthesis of the achiral organoselenanes 1-2

The achiral organoselenanes 1-2 were synthesized from the bromo-benzylic alcohol 7. Initially, this alcohol was transformed into methyl ether 8, which was converted into selenide 9 by a bromine/lithium exchange reaction followed by the capture of the organolithium intermediate with dibutyl diselenide leading to the selenide 9 in 70% yield. When the selenide 9 was reacted with SO2Cl2, the dichloro organoselenane 1 was obtained (> 99% yield). In the same way, the dibromo organoselenane 2 was prepared from 9 by Se-oxidative reaction with Br2 in high yield (> 99%; Scheme 1).

Synthesis of the chiral organoselenanes 3-4 and 5-6

In order to synthesize the chiral organoselenanes 3-4 and 5-6 a chemoenzymatic methodology was developed. Initially, the ketone 11 was synthesized in 45% yield from 2'-amino-acetophenone 10 by using arenediazonium salt and lithium butylselenolate (Scheme 2). Then, reduction of the ketone 11 afforded the desired chiral seleno-alcohol 12.

Enzymatic kinetic resolution of the alcohol (RS)-12 through lipase-catalyzed acetylation was employed to obtain each enantiomer separately. The enzymatic resolution of racemic alcohol 12 was carried out with vinyl acetate as an acyl donor and CAL-B as biocatalyst (Scheme 3). This reaction was performed in hexane during 24 h, and the alcohol (S)-12 and ester (R)-13 were obtained in high enantiomeric excess (up to 99%).

After enzymatic kinetic resolution, the compounds (S)-12 and (R)-13 were purified by flash chromatography and both compounds were obtained in high isolated yield.

Scheme 1. Reagents and conditions. (i) a: NaH, THF (0 °C, 30 min); b: MeI, (rt, 2 h); (ii) a: t-BuLi, THF (−78 °C, 30 min); b: BuSeSeBu, THF (rt, 3 h); (iii) SO2Cl2, or Br2, THF (0 °C, 15 min).
Chemoenzymatic Synthesis of Organoselenium(IV) Compounds and their Evaluation


10

Se^2 + nBuLi
BuSeLi

ii

SeBu

OH

-SeBu

OH

SeBu

rac-12 (90%)

(45%). The alcohol (R)-12 was obtained by hydrolysis of the ester (R)-13. Thus, by using enzymatic kinetic resolution it was possible to obtain separately both alcohol enantiomers 12 in excellent enantiomeric excess (> 99%).

The next step, the enantiomerically pure seleno-alcohols (S)-12 and (R)-12 were transformed into their respective ethers (S)-14 and (R)-14 in high yields (86% and 89%, respectively). The organoselenides (S)-14 and (R)-14 were reacted with SO_2Cl_2 and the dichloro organoselenanes 3 and 4 were obtained in high yields (> 99%). In the same way, the dibromo organoselenanes 5 and 6 were prepared from (S)-14 and (R)-14 by Se-oxidative reaction with Br_2 in high yield (> 99%; Scheme 3).

Inhibition of cysteine cathepsins S and V by organoselenanes

In order to evaluate the relative potency of the organoselenanes 1-6 as possible inhibitors of cysteine proteases, we selected the cysteine cathepsin S and V and a fluorimetric assay to follow the enzymatic activity (Figure 2). The reaction progress was monitored continuously by the fluorescence of the released product (7-amino-4-methylcoumarin, AMC).^{17,18}

![Scheme 2](image)

**Scheme 2.** Reagents and conditions. (i) NaN_3, H_2SO_4, H_2O (0-5 °C); (ii) NaHCO_3, pH 7; (iii) THF (0 °C, 1 h); (iv) NaBH_4, MeOH (rt, 1 h).

![Figure 2](image)

**Figure 2.** Fluorimetric enzyme assay for new inhibitors of cysteine cathepsin. (7-amino-4-methylcoumarin).

![Scheme 3](image)

**Scheme 3.** Reagents and conditions. (i) vinyl acetate, CAL-B, hexane (32 °C, 24 h); (ii) K_2CO_3, MeOH, H_2O (rt, overnight); (iii) a: NaH (0 °C, 30 min); b: MeI, THF (rt, 2 h); (iv) SOCl_2 or Br_2, THF (0 °C, 15 min).
Fluorimetric enzyme assays were used to evaluate the organoselenanes 1-6 (at 1 μmol L\(^{-1}\)) as cysteine cathepsins inhibitors (S and V). Initially, the enzyme activities were determined by the hydrolysis of the fluorogenic substrate Cbz-FR-AMC (1 μmol L\(^{-1}\)) in the absence of selenanes (Control assay). Then, each organoselenane 1-6 (1 μmol L\(^{-1}\)) was pre-incubated with active cathepsin S (Figure 3) and cathepsin V (Figure 4) for 2 min prior to the addition of the substrate Cbz-FR-AMC. The residual enzyme activities in the presence of each organoselenane were determined and the percentage of inhibition was determined by direct comparison with the control assay.

In the assays with the cathepsin S, no significant differences on the percentage of inhibition for dibromo and dichloro organoselenanes was observed. All compounds 1-6 inhibited about 70-80% of the cathepsin S enzymatic activity, demonstrating that for this enzyme steric factors or different leaving group attached to the selenium atom did not lead to selective interactions. Therefore, the low selectivity showed by this enzyme indicates that all selenanes 1-6 can be equally used as inhibitor without remarkable differentiation. In addition, the possibility of the organoselenides intermediates 9, 11, 12 and 14 being applied as inhibitor of cathepsins S and V was also evaluated. As expected, none of these organoselenides caused any inhibition at the concentration of 1 μmol L\(^{-1}\) (For all data, see the Supplementary Information, SI).

On the other hand, assays with the cathepsin V showed that the percentages of inhibition observed for dibromo derivatives 2, 5 and 6 were higher than the inhibition observed for dichloro analogues 1, 3 and 4 (Figure 4). The achiral dibromo organoselenane 2 inhibited 95% of the activity of this enzyme and the chiral dibromo organoselenanes 5 and 6 showed 80% of inhibition. From these results, it was possible to see that the methyl group attached to the benzyl carbon provides a decrease of almost 20% in the percentage of inhibition of cathepsin V by the chiral organoselenanes 5 and 6 when compared with the organoselenane 2, which has no methyl group at that position. However, a discrete difference in the inhibition for the organoselenanes 5 and 6 showed that the stereochemistry of the benzyl carbon was not relevant and, consequently no enantiomeric preference was observed. Meanwhile, the dichloro organoselenanes 1, 3 and 4 inhibited about 60% of cathepsin V activity. Surprisingly, the structural differences of 1, 3 and 4 did not show any significant influence on the inhibition of cathepsin V, as observed for the dibromo compounds. In this case it was possible to notice that cathepsin V was preferably inhibited by dibromo organoselenanes including the influence of chirality. It is also important to highlight that this qualitative assay showed almost the same inhibitory profile of selenanes 1-6 against cathepsins V and S. However, kinetic constants of the inhibition reaction for each selenane were determined to get a detailed study about inhibitors potency and selectivity.

**Inhibition kinetics of cathepsins S and V**

The second-order rate constants for inactivation of cathepsins S and V by organoselenanes 1-6 were determined\(^{17,18}\) as illustrated in Figure 5 to cathepsin V and organoselenane 2. Initially, it was determined the pseudo-first order rate constants (\(k_{\text{app}}\)) for inactivation of cathepsin V by different concentrations of selenane 2 (Figure 5A). Finally, the second-order rate constants (\(k_2\)) were determined by the linear correlation between each
$k_{obs}$ and its respective inhibitor concentration, (Figure 5B). The second-order inhibitory constant is obtained from the slope of the curve $k_{obs}$ versus inhibitor concentration. This method was applied to all compounds against cathepsins V and S (see SI and Table 1).

Organoselenanes showed both time-dependent and concentration-dependent behavior on the inhibition of cathepsins S and V, as observed in Figure 5A. Dibromo organoselenanes (2, 5 and 6) were found to inhibit both enzymes more rapidly (Table 1, entries 2, 5 and 6) than dichloro analogues (1, 3 and 4; Table 1, entries 1, 3 and 4). These experimental results can be explained by the nucleophilic substitution of a halogen atom of organoselenanes by the thiol group of cysteine cathepsin, so the best leaving group attached at the selenium atom the highest inhibitory potency was observed. Then, dibromo organoselenanes 2, 5 and 6 are much more potent than chlorine organoselenanes 1, 3 and 4.

The presence of the methyl group at the benzylic carbon in the dichloro organoselenanes 3 and 4 (Table 1, entries 3 and 4) showed a 2-fold improvement in the second-order inactivation rate constant of the inhibition of cathepsin V when compared to the achiral analogue 1 (Table 1, entry 1), however, for cathepsin S no difference was observed among these compounds. Notably, a distinct pattern was observed for dibromo organoselenanes (Table 1, entries 2, 5 and 6) in which the presence of methyl group decreased the inhibition rate of cathepsin V. Then, the inhibition of cathepsin S by the organoselenane 5 (Table 1, entry 5) was 5-fold faster than its enantiomer 6 (Table 1, entry 6).

Gel permeation chromatography assays with cathepsin V were used to discriminate between reversible and irreversible mechanism for inhibition of cathepsins by organoselenanes. Firstly, cathepsin V was passed

![Figure 5](image.png)

**Figure 5.** Time course of the inactivation of cathepsin V with organoselenane 2. (A) The kinetics of inactivation of cathepsin V was performed in sodium acetate buffer (50 mmol L\(^{-1}\); pH 5.5) containing EDTA (2.5 mmol L\(^{-1}\)), and different concentration of 2: control, 0.5, 1.0, 1.5, 3.0 and 5.0 µmol L\(^{-1}\) of 2. The reaction progress was monitored continuously by the fluorescence of the released product (methylcoumarin). (B) Cathepsin V first-order rate inactivation in function of organoselenane concentration.

<table>
<thead>
<tr>
<th>entry</th>
<th>Selenane</th>
<th>$k_2/(mol \cdot L^{-1} \cdot s^{-1})$</th>
<th>Cathepsin V</th>
<th>Cathepsin S</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image.png" alt="Structure 1" /> OMe</td>
<td>500 ± 100</td>
<td>1500 ± 400</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><img src="image.png" alt="Structure 2" /> Br Se</td>
<td>3400 ± 200</td>
<td>4000 ± 200</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image.png" alt="Structure 3" /> Br</td>
<td>1000 ± 200</td>
<td>1500 ± 380</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><img src="image.png" alt="Structure 4" /> OMe Se</td>
<td>900 ± 200</td>
<td>1200 ± 300</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><img src="image.png" alt="Structure 5" /> Br Se</td>
<td>2900 ± 560</td>
<td>4900 ± 200</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><img src="image.png" alt="Structure 6" /> OMe Br</td>
<td>2200 ± 240</td>
<td>1000 ± 430</td>
<td></td>
</tr>
</tbody>
</table>
through the gel permeation chromatography column and around 18 fractions were collected. The enzyme activity was monitored by adding the substrate, Cbz-FR-AMC, to each fraction, which indicated the presence of cathepsin V in the fractions 6-13 (Figure 6, (○)). Secondly, cathepsin V was incubated with the most active organoselenane 2 followed by submitting the mixture to a gel permeation chromatography, which resulted around 18 fractions. Each fraction was evaluated for cathepsin V activity, however, as observed in the Figure 6 none of the fraction collected from the column showed enzyme activity (Figure 6, (●)). The protein content for each fraction was verified by measuring the absorption (Figure 6, (---)). In order to show the presence of inactive cathepsin V in the fractions with protein content (fractions 8-11), the enzyme activity was recovered by the addition of 5 mmol L\(^{-1}\) of the reducing agent dithiothreitol (DTT), which reduces the sulfur-selenium bond in the inactive enzyme, restoring its functionality (data not shown).

The inhibition of cathepsins by organoselenanes can be based on the reactivity of the selenium(IV) moiety toward the thiol group of these enzymes. A nucleophilic substitution reaction of the sulfur from cysteine cathepsin to the selenium species led to the formation of an S–Se covalent bond, which affords the selenium-cathepsin adduct, resulting in the irreversible inhibition of these enzymes (Figure 7).

\(^{77}\)Se NMR analysis: evidence of the formation of organoselenane/thiol adduct

Finally, after the kinetic measurements of the cysteine cathepsins inhibition, another evidence of the interaction of organoselenanes with thiols was accessed by \(^{77}\)Se NMR analysis using cysteine (L-Cys) as a model thiol compound (Figure 8). In a NMR tube, the organoselenane 1 was incubated with increasing ratios of L-Cys (0, 1 and 2 equiv.) in DMSO-\(d_6\). The \(^{77}\)Se NMR spectra of the reaction were taken after 1 h of the addition of 1 equiv. of cysteine (Figure 8A) and 1 h after the addition of the second equivalent (Figure 8B).

By adding the first equivalent of the cysteine, the original chemical shift of 1 (549 ppm) disappeared and a single new signal at 406 ppm was observed. Two hours after the addition of the second equivalent of cysteine, only the same signal at 406 ppm was observed. These results indicated that the selenium specie that was observed by adding one or two equivalents of cysteine was the same. Therefore, it suggests that there is a substitution of only one atom of chlorine from organoselenane 1 by cysteine nucleophilic attack on the selenium atom, indicating that the reaction stoichiometry is 1:1. The exchange of one chlorine atom at selenium moiety by one sulfur atom lead to an increase in the electronic density around the selenium atom due to the difference in the electronegativity of the ligands, thus a decrease in the \(^{77}\)Se chemical shift is observed (549 ppm to 406 ppm).

**Experimental**

Unless otherwise noted, commercially available materials were used without further purification. Lipase from *Candida antarctica* (fraction B, CAL-B) immobilized,
spectra were obtained on a Bruker DRX 300 spectrometer, using the appropriate decoupling accessories. All $^{77}$Se chemical shifts were referenced to internal standard PhSeSePh (463 ppm).

Infrared spectra were recorded from KBr discs or from a thin film between NaCl plates on FTIR spectrometer (Bomem Michelson model 101) with internal reference. Absorption maxima ($\nu_{\text{max}}$) are reported in wavenumbers (cm$^{-1}$).

High-resolution mass spectra (HRMS) were acquired using a Bruker Daltonics MicroTOF instrument, operating electrospray ionization (ESI) mode.

Optical rotations were measured on a Perkin Elmer-343 digital polarimeter in a 1 mL cuvette with a 1 dm pathlength. All values are reported in the following format: $[\alpha]_{D}$(temperature of measurement) = specific rotation (concentration of the solution reported in units of 10 mg sample per 1 mL solvent used).

High performance liquid chromatograph (HPLC) analyses for measurement of enantiomeric excesses were performed on a Shimadzu, LC-10AD liquid chromatograph equipped with an autosampler and a variable wavelength UV detector (deuterium lamp 190-600 nm). Chiral columns: Chiralcel® OJ-H (0.46 cm$^2$× 25 cm) from Daicel Chemical Ind. i-PrOH and hexane (60% n-hexane) HPLC grade purchased from J. T. Baker were used as the eluting solvents.
**General procedure for O-methylation reactions (8 and 14)**

To a two-necked round-bottomed flask (25 mL) containing the appropriate alcohol [7, (S)-12 or (R)-12] (1 mmol) in dry THF (10 mL), sodium hydride (48 mg; 2 mmol) was added. This mixture was stirred at 0 °C (30 min), then iodomethane (280 mg; 2 mmol) was added. The mixture was stirred for 2 h at room temperature. After that, ammonium chloride saturated solution (10 mL) was added and the organic phase was diluted with ethyl acetate (20 mL). The organic phase was washed with brine (3 × 10 mL), separated and dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography using a mixture of n-hexane and ethyl acetate (9:1) as eluent to afford the ethers 8, (S)-14 and (R)-14.

1-Bromo-2-(methoxymethyl)benzene (8): Isolated yield: 90%; [α]_D = −78.0 (c = 1.06; CHCl₃); (R)-14: Isolated yield: 89%; Enantiomeric excess = 99%; [α]_D = +70.5 (c = 1.00; CHCl₃); (S)-14: Isolated yield: 96%; Enantiomeric excess = 99%; [α]_D = +70.5 (c = 1.00; CHCl₃);

Butyl(2-(1-methoxyethyl)phenyl)selenole (14): (S)-14: Isolated yield: 96%; Enantiomeric excess = 99%; [α]_D = +70.5 (c = 1.00; CHCl₃);

**Procedure for the Br/Li exchange reaction**

This procedure was done in an oven-dried two-necked round-bottomed flask (25 mL) containing the 2′-bromobenzyl ether 8 (187 mg; 1 mmol) in dry THF (10 mL), cooled to −78 °C by using dry-ice/acetone bath, tert-butyl lithium (0.8 mL of a solution 1.4 mol L⁻¹; 1.1 mmol) was added dropwise. Afterwards, the mixture was stirred at 0 °C for 30 min and dibutyl diselenide (301 mg; 1.1 mmol) was added, and the mixture was stirred for 2 h at room temperature. The reaction was quenched by adding ammonium chloride saturated solution (10 mL), and the organic phase was diluted with ethyl acetate (20 mL). The organic phase was washed with brine (3 × 10 mL), separated and dried over anhydrous magnesium sulphate and filtered. The solvent was removed under reduced pressure and the residue was purified by flash chromatography, using a mixture of n-hexane and ethyl acetate (9:1) as eluent to afford 9 in 90% yield.

Butyl(2-(methoxymethyl)phenyl)selenole (9): ¹H NMR (200 MHz, CDCl₃) δ: 7.49 (1H, m); 7.22 (1H, m); 7.19 (2H, m); 4.55 (2H, s); 3.41 (3H, s); 2.89 (2H, t, J 7.0 Hz); 1.71 (2H, q, J 6 Hz); 1.58 (2H, t, J 7.45 Hz); 0.90 (3H, t, J 7.02 Hz); ¹³C NMR (75 MHz, CDCl₃) δ: 141.6; 136.6; 128.4; 127.9; 126.9; 116.6; 78.0; 57.5; 36.3; 13.3; 7.49; ⁷Se NMR (57.24 MHz, CDCl₃) δ: 240.0; IR (film) ν max/cm⁻¹: 3058; 2982; 2926; 2871; 1436; 1195; 1106; 748; HRMS (ESI), [M+Na]+: calculated for C₁₂H₁₆NaOSe = 281.0421; Found 281.0413.

**General procedure for the oxidation of selenides (1-6)²¹**

To an one-necked round-bottomed flask (25 mL) the appropriate selenide [8, (S)-14 or (R)-14] (1 mmol) was solubilized in dry THF (5 mL). The mixture was cooled at 0 °C and then, a cooled solution of sulfuryl chloride (1 mmol) or bromine (1 mmol) dissolved in THF (2 mL), was added dropwise. The resulting mixture was stirred at 0 °C for 20 min and then the solvent was removed under reduced pressure. The organoselenanes 1-6 were purified by recrystallization from a mixture of diethyl ether and hexane in quantitative yields.

1-[Butyl(dichloro)-λ⁴-selanyl]-2-(methoxymethyl)benzene (1): ¹H NMR (200 MHz, CDCl₃) δ: 7.82 (1H, dd, J₅, J₆ 3.6 Hz, J₆, J₇ 2.1 Hz); 7.50 (2H, dd, J₅, J₆ 3.9 Hz, J₆, J₇ 3.3 Hz, J₅, J₆ 2.4 Hz); 7.4 (1H, m); 4.92 (2H, s); 4.14 (2H, t, J 8.1 Hz); 3.48 (3H, s); 2.29 (2H, q, J 7.5 Hz); 1.66 (2H, t, J 7.5 Hz); 1.08 (3H, t, J 7.2 Hz). ¹³C NMR (50 MHz, CDCl₃) δ: 141.5; 137.4; 131.4; 128.8; 126.9; 126.5; 73.7; 60.9; 58.7; 25.8; 22.6. ⁷Se NMR (57.24 MHz, CDCl₃) δ: 549.4; IR (KBr) ν max/cm⁻¹: 3437; 2956; 2927; 2813; 1461; 1445; 1212; 1199; 1106; 1096. Elemental analysis: Calculated for C₁₂H₁₀Cl₂OSe: C 49.92; H 5.53; Cl 21.61%. Found: C 49.01; H 5.23; Cl 21.43%.

1-[Dibromo(butyl)-λ⁴-selanyl]-2-(methoxymethyl)benzene (2): ¹H NMR (300 MHz, CDCl₃) δ: 7.78 (1H, m); 7.50 (2H m); 7.38 (1H, m); 4.87 (2H, s); 4.08 (2H, t, J 8.1 Hz); 3.49 (3H, s); 2.26 (2H, q, J 6 Hz); 1.66 (2H, t, J 7.5 Hz); 1.03 (3H, t, J 7.2 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 138.3; 137.7; 131.5; 130.0; 129.9; 129.3; 127.7; 73.8; 58.8; 25.8; 22.7. ¹⁷Se NMR (57.24 MHz, CDCl₃) δ:
solution was continuously stirred at room temperature. The solution was added dropwise in a 25 mL round-bottomed flask. The solution was stirred in an orbital shaker at 32 °C for 24 h (160 rpm). Following that, the enzyme was filtered off and washed with dichloromethane (3 × 20 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography using a mixture of n-hexane and ethyl acetate (9:1) as eluent to afford 11 in 45% yield.

\[\text{J} \text{-} (2\text{-}(\text{butylselanyl})\text{phenyl})\text{ethanone (11): Isole}t\text{d yield: 45%}; 1\text{H NMR (200 MHz, CDCl}_3\text{)}: 7.90 (d, 1H, J 7.9 Hz); 7.44 (m, 2H); 7.23 (m, 1H); 2.84 (t, 2H, J 7.5 Hz); 2.63 (s, 3H); 1.72 (qt, 2H, J 6.9 Hz); 1.50 (st, 3 H, J 7.3 Hz); 0.95 (t, 3H, J 7.2 Hz); 1\text{C NMR (50 MHz, CDCl}_3\text{)}: 98.9; 138.5; 132.4; 131.8; 128.4; 124.3; 30.8; 27.7; 24.8; 23.5; 13.8; IR (film) \nu_{\text{max}}/\text{cm}^{-1}: 2957, 2928, 1666, 1457, 1428, 1248; HRMS (ESI), [M+Na]$: calculated for C_{18}H_{25}OSeNa = 279.0264. Found 279.0264.

Enzymatic kinetic resolution of 1\-\text{-}(2\-\text{-}(\text{butylselanyl})\text{phenyl})\text{ethanol (12)}

To an one-necked round-bottomed flask (100 mL) containing 1\-\text{-}(2\-\text{-}(\text{butylselanyl})\text{phenyl})\text{ethanol rac-12 (1.285 g; 5 mmol) in hexane (20 mL), CAL-B (Novozyme 435; 400 mg) and vinyl acetate (0.86 g; 15 mmol) were added. The mixture was stirred in an orbital shaker at 32 °C for 24 h (160 rpm). Following that, the enzyme was filtered off and washed with dichloromethane (3 × 20 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography using a mixture of n-hexane and ethyl acetate (9:1) as eluent to afford (S)-12 (ee 99%) and (R)-13 (ee 99%) in 45% yield each one.

\[\text{(S)}\-\text{-}\text{-}(2\text{-}(\text{butylselanyl})\text{phenyl})\text{ethanol (12): Isole}t\text{d yield: 45%; Enantiometric excess > 99%}; 1\text{H NMR (200 MHz, CDCl}_3\text{)}: 7.50 (m, 2H); 7.23 (m, 2H); 5.30 (qd, 1H, J 6.3 Hz); 2.91 (t, 2H, J 7.5 Hz); 2.3 (s, 1H); 1.70 (qt, 2H, J 7.4 Hz); 1.44 (m, 5H); 0.96 (t, 3H, J 7.24); 1\text{C NMR (50 MHz, CDCl}_3\text{)}: 146.9; 132.7; 129.4; 128.0; 127.4; 125.6; 69.3; 32.2; 28.2; 24.3; 23.2; 13.7; IR (film) \nu_{\text{max}}/\text{cm}^{-1}: 3361, 3057, 2961, 2928, 2870, 1463, 1008, 754; HRMS (ESI), [M+Na]$: calculated for C_{18}H_{25}OSeNa = 281.0421. Found 281.0416.

\[\text{(R)}\-\text{-}\text{-}(2\text{-}(\text{butylselanyl})\text{phenyl})\text{ethanol (13): Isole}t\text{d yield: 45%; Enantiometric excess > 99%}; 1\text{H NMR (200 MHz, CDCl}_3\text{)}: 7.51 (1H, dd, J 1.30 Hz); 7.41 (1H, dd, J 1.70 Hz); 7.22 (m, 2H); 6.29 (qd, 1H, J 6.58 Hz); 2.91 (t, 2H, J 7.5 Hz); 2.92 (2H, t, J 7.5 Hz); 2.08 (1H, s); 1.70 (2H, qt,
Inactivation kinetics

Cathepsins activities were monitored spectrofluorometrically using the fluorogenic substrate Cbz-FR-AMC on a Hitachi F-2000 spectrofluorometer equipped with a thermostated cell holder. The excitation and emission wavelengths were set at 380 and 460 nm, respectively. The continuous method\textsuperscript{16} was employed and the inhibition of activated cysteine proteases were carried out in the presence of substrate and different concentrations of each organoselenane, as described before.\textsuperscript{17} The substrate concentrations were kept 10-fold below the $K_{m}$ values. The kinetics of cathepsin inactivation by organoselenananes were obtained in pseudo-first-order conditions. The inhibition reaction was monitored continuously by the fluorescence of the enzymatic-released Cbz-FR-AMC. The hydrolysis progress curves were obtained in pseudo-first-order conditions and treated by non-linear regression according to equation 1 as reported previously:\textsuperscript{22}

$$P = \left(\frac{v_z}{k_{obs}}\right) \left[1 - \exp\left(-k_{obs} t\right)\right] + d \quad (1)$$

where $P$ is the product concentration (in our case it is proportional to the fluorescence of AMC) at a given time, $v_z$ is velocity of substrate hydrolysis for zero time and $k_{obs}$ is the observed first-order rate of organoselenane-induced enzyme inactivation and $d$ is the basal fluorescence before adding the enzyme.

Determination of inhibition mechanism by gel permeation chromatography

In order to show the irreversible inhibition of cathepsins by organoselenanes, the most potent inhibitor, organoselenane 2, was chosen and used as follows.

Gel permeation chromatography of cathepsin V: A 2.5 mL solution of activated cathepsin V was loaded on a 1×10-cm Sephadex G-25 column (Amersham PD-10 column, Amersham), pre-equilibrated with sodium acetate buffer (50 mmol L\textsuperscript{-1}, pH 5.5) containing EDTA (2.5 mmol L\textsuperscript{-1}) at 27 °C. Around 18 fractions (500 µL fractions) were collected. The enzyme activity was monitored by adding the substrate, Cbz-FR-AMC, to each fraction.

Gel permeation chromatography of cathepsin V and organoselenane 2: A solution of activated cathepsin V was incubated with the most active organoselenane 2 (10 mmol L\textsuperscript{-1}), in a total volume of 2.5 mL, for 10 min. Then, this mixture was loaded on a 1×10-cm Sephadex G-25 column (Amersham PD-10 column, Amersham), pre-equilibrated with sodium acetate buffer (50 mmol L\textsuperscript{-1}, pH 5.5) containing EDTA (2.5 mmol L\textsuperscript{-1}) at 27 °C. Around 18 fractions (500 µL fractions) were collected. The enzyme activity was monitored by adding the substrate, Cbz-FR-AMC, to each fraction. The protein content for each fraction was verified by measuring the absorption $A_{280\text{nm}}$.

Conclusions

We have synthesized a series of hypervalent organoselenium compounds (organoselenananes), and it has been demonstrated, for the first time, that these compounds can be efficiently used as cysteine protease inhibitors. The inhibition of these enzymes is irreversible and showed a time-dependent and concentration-dependent behavior. In addition, the enzyme activity was totally restored after the treatment of the selenium-cathepsin adduct (inactive enzyme) with the reducing agent dithiothreitol (DTT). We expect that the present report will provide a good basis for the development of novel cysteine protease inhibitors based on selenium(IV) compounds.
Supplementary Information

Supplementary information, with characterization data (1H, 13C and 77Se NMR, IR and mass spectrometry) for all compounds and enantiomeric separation for chiral selenium compounds by chiral HPLC, are available, free of charge, at http://jbcs.sbq.org.br, as PDF file.

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