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Structure–activity relationships of hypervalent organochalcogenanes as inhibitors of cysteine cathepsins V and S

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ABSTRACT

A new series of organotelluranes were synthesized and investigated, and the structure–activity relationships in cysteine proteases inhibition were determinated. It was possible to identify the relevance of structural components linked to the reactivity of these compounds as inhibitors. For example, dibromo-organotelluranes showed to be more reactive than dichloro-organotelluranes towards cysteine cathepsins V and S. Besides, no remarkable enantio-selectivity was verified. In general the achiral organotelluranes were more reactive than the chiral congeners against cysteine cathepsins V and S. A reactivity order for organochalcogenanes and cysteine cathepsins was proposed after the comparison of the inhibitory potencies of organotelluranes with the related organoselenanes.

1. Introduction

Recent studies about selenium and tellurium-containing bioactive compounds have demonstrated the high potential of these non-conventional compounds to the medicinal chemistry. These compounds' ability to act as enzyme mimetic has guided the synthesis and evaluation of a large number of selenium and tellurium compounds with different oxidation state. The glutathione peroxidase (GPx)-like catalytic activity of selenium(II) compounds has been largely evaluated. Besides the antioxidant and anti-inflammatory properties[1] of selenium compounds, in vitro and in vivo assays have identified several biological activities of selenium compounds including neuroprotective and convulsant effects,[2] cancer prevention,[3] and apoptotic events.[4] The most explored organoselenium(II) compound is Ebselen which showed antioxidant and anti-inflammatory activity (Fig. 1).[5] On the other hand, some tellurium(II) compounds have also showed antioxidant properties including mimetics of GPx.[6] The most representative tellurium compound is AS-101, an inorganic tellurane (hypervalent tellurium(IV) compound) which has been investigated since 1990s by Sredni group (Fig. 1). The pleiotropic activities such as anti-inflammatory,[7] anti-microbial,[8] anti-myeloma[9,10] and melanoma treatment[11], Parkinson's disease,[12] immunomodulator,[13] besides treatment of anogenital warts,[14] hair growth induction,[15] stroke treatment where reducing brain damage was associated to caspases inhibition,[16] and tyrosine kinase[17] and cysteine (papain, cathepsin B) proteases inhibitors[18] have been described for AS-101.

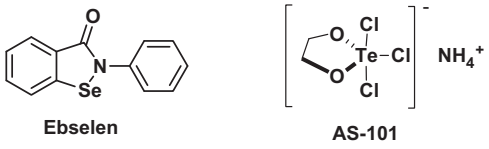

Figure 1. Ebselen, a selenium(II) compound, and AS-101, a inorganic tellurane. Representative chalcogenated compounds that present pleiotropic biological activities.

Organotelluranes have also been explored as inhibitors of cysteine proteases. The studies about organotelluranes as inhibitors of cysteine proteases have demonstrated that these compounds are more powerful cathepsin B inhibitors and for other cysteine catepsins than AS-101[19,20] and recently the potential of organotelluranes as anticonvulsant and inhibitors of caspases were reported.[21] It is noteworthy that, the published organotelluranes were chosen randomically and few conclusions about the possible structure–activity relationships could be identified. Thus, in this work we describe our investigation in an attempt to determine possible structure–activity relationships for a series of organotel-

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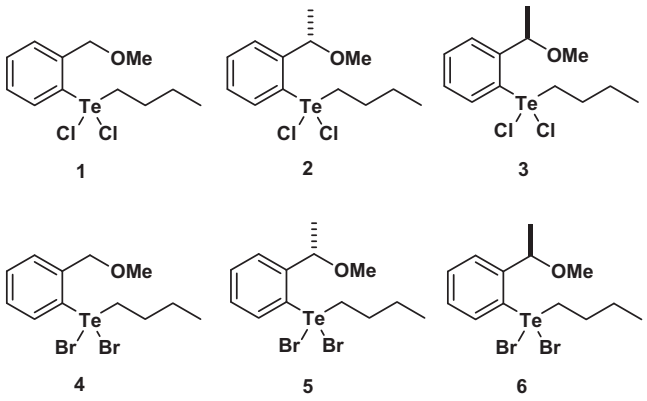


Figure 2. Organotelluranes with structural variations to be explored in the cysteine cathepsins inhibition.

luranes that was evaluated against two cysteine proteases, cathepsin V and S (Fig. 2).

The organotelluranes **1–6** were designed considering the possibility of different activity between enantiomers or between chiral and achiral congeners. It is known that the tellurium atom is the electrophilic center of dihalogenated organotelluranes where one Te-X (X = Cl, Br, I) bond is replaced by a Enz–S–Te bond after the nucleophilic attack of the catalytic thiol residue from cysteine protease,[18–20] hence we decided to explore the relevance of the halogens (chlorine or bromine) as leaving groups bonded to the tellurium atom.

The choice of the cysteine cathepsins was based on their biological functions. Cathepsin V shares high protein sequence identity with Cathepsin L (80%), but in contrast to the ubiquitously expressed Cathepsin L and broadly reported as an important tumor treatment target, its expression is restricted to thymus, corneal epithelium and testis.[22] Cathepsin V has been involved in antigen presentation in humans[23] and also considered as a potential biomarker for colon tumors.[24] Moreover, together with cathepsins L, K and S, cathepsin V has been described to participate in atherosclerosis.[25] On the other hand, cathepsin S has been associated in several cancer stages, especially in angiogenesis and cell proliferation.[26] It was also described the role of cathepsin S in cerebral aneurysm indicating that this cysteine protease may promote the progression of atherosclerosis by altering vascular remodeling in atherosclerotic plaques.[27]

2. Results and discussion

2.1. Determination of the inhibitory activity of organotelluranes against cathepsins S and V

The inhibition of cathepsins V and S were investigated by a fluorimetric enzyme assay using optimal reactional conditions for each enzyme and the fluorogenic dipeptide Cbz-Phe-Arg-AMC (1 μmol L^{-1}) as previously described.[28] The percentage of residual enzymatic activity (%) was calculated by the ratio of the enzymatic activity of cathepsin in the presence of 1 μmol L^{-1} of the inhibitors **1–6** (A_2) and the pattern enzymatic activity of cathepsin in the absence of inhibitor (A_1) according to (Eq. 1). The residual activities of cathepsin V and S after reaction with the compounds **1–6** are shown in the Figure 3.

$$\%\,Residual\,enzymatic\,\text{activity} = (A_2/A_1) \times 100 \quad (1)$$

It was possible to observe that cathepsins V and S were strongly inhibited by all organotelluranes at 1 μmol L^{-1}. For cathepsin V, the most remarkable observation is that organotelluranes showed

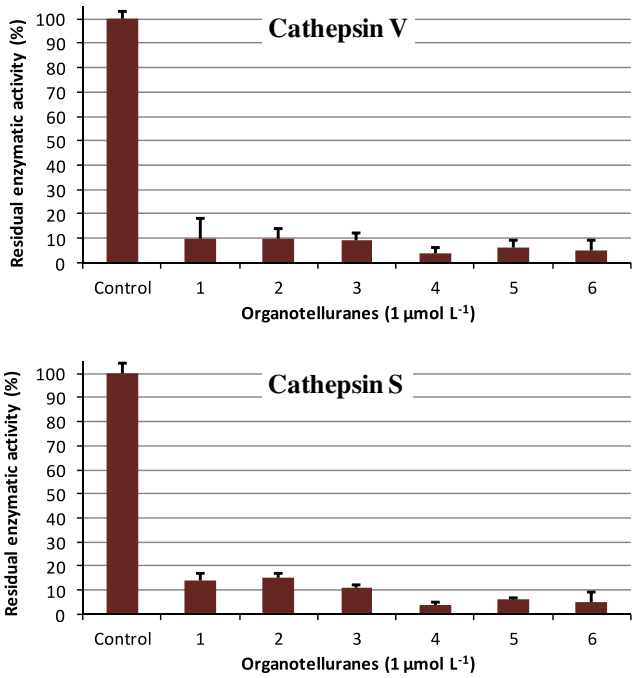


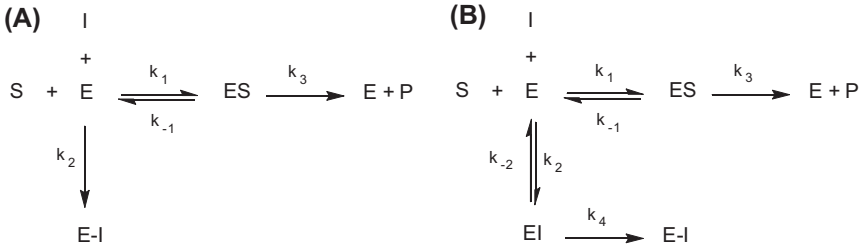
Figure 3. Inhibition profile for cathepsins V and S using organotelluranes **1–6**. Each compound (1 μmol L^{-1}) was pre-incubated with active cathepsin for 2 min prior to the addition of the substrate. The fluorogenic substrate Cbz-Phe-Arg-AMC (1 μmol L^{-1}) was used and the remaining enzyme activity was determined as a percentage of the enzymatic activity without any inhibitor. The assays were conducted in triplicate.

the highest inhibitory activity (around 90%). However, by these qualitative assays it was not possible to identify significant differences between the enantiomers (**2**–**3** or **5**–**6**) or between the congeners containing chlorine or bromine in their structure. In the assays with cathepsin S, all organotelluranes reduced the enzymatic activity around 90%. It was possible to observe that the dichloride telluranes inhibited around 85% of the activity of cathepsin S while dibromide telluranes **4**, **5** and **6** presented the highest inhibitory activity (>90%), especially the compound **4** that inhibited cathepsin S in 96%.

These assays were useful to identify the relevance of the chalcogen atom for the profile of the compounds **1–6** as inhibitor of cathepsins V and S. When these results are compared with that obtained to organoselenanes cogeners it is evident that the chalcogen atom is fundamental for the performance of the inhibitors since it was observed that the percentage of inhibition of cathepsins V and S by organotelluranes (**1–6**) are around 90 % while for organoselenanes the inhibitory activities were about 70–80 % at the same conditions.[29] Conclusions regarding the contributions of halogen and details involving the stereochemistry of these compounds could not be made by these tests. Thus, the second-order inactivation rate constants (k_2) of cathepsins V and S for compounds **1–6** were determined, then all structural factors that influence each compound activities against these enzymes were identified.

2.2. Second-order inactivation rate constants (k_2) determination for cathepsins V and S

Organotelluranes are very reactive irreversible inhibitors of cysteine proteases. Due to this high reactivity Cunha et al.[20] reported that by applying the Baici et al.[30] model the more appropriated kinetic parameter to described the organotelluranes activities is the second-order inactivation rate constants, k_2, in which the inhibition reaction occurs by a one-step mechanism according to

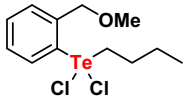
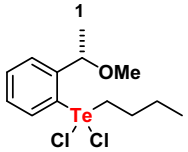
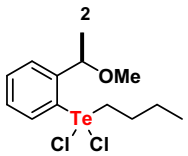
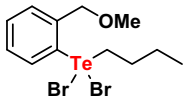
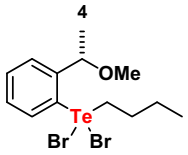
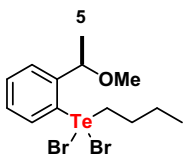


Scheme 1. Mechanism of irreversible inactivation (A) one step and (B) two steps.[30]

Scheme 1A. Thus, it is inconvenient to determine K_i for these class of inhibitor because the concentrations of these inhibitors are lower than K_i (k_{-2}/k_2) and the concentration of the reversible intermediate (EI) will be neglected.[20,30] However, K_i is an adequate parameter to less reactive inhibitors such as inorganic telluranes where the inhibition is a two-step reaction (Scheme 1B).

The second-order inactivation rate constants (k_2) of cathepsins S and V by organotelluranes **1**–**6** were fundamental to identify the structure–activity relationships for these compounds, they were determined as previously described (Table 1).[28] By the determination of k_2 of cathepsins V and S by the organochalcogenanes **1**–**6** it was possible to confirm that the telluranes are powerful inhibitors of these enzymes. The importance of halogen could be identified, and the dibromide compounds presented potency superior to the dichloride ones.

Table 1
Second-order inactivation rate constants of the inhibition of cathepsins V and S by organotelluranes **1**–**6**

#	k_2 (mol L^{-1} s^{-1})	
	Cat. V	Cat. S
 **1**	11,600 ± 110	1300 ± 60
 **2**	2400 ± 80	1800 ± 100
 **3**	2500 ± 30	1800 ± 100
 **4**	26,400 ± 800	4400 + 300
 **5**	7400 ± 200	4900 ± 300
 **6**	3900 + 300	2500 ± 300

It is possible to verify from Table 1 that for each inhibitor the k_2 for cathepsin V are higher than that of cathepsin S, demonstrating that the organotelluranes **1**–**6** are more potent inhibitors for cathepsin V than cathepsin S. It is important to highlight that **1** is almost five-fold more potent against cathepsin V than the chiral congeners **2** and **3**. In the case of **4**, against cathepsin V this inhibitor is almost four-fold more potent than **5** and almost seven-fold more potent than **6**. In this way, it is clear that the achiral organotelluranes **1** and **4** are more potent inhibitors than their chiral congeners. In the case of the inhibition of cathepsin S few differentiations were observed between chiral and achiral congeners. Only in the comparison of dibromo-organotelluranes was possible to see that **5** is two-fold more potent than **6**.

An inhibitory potency detailed analysis of these organotelluranes was more evident when we compare the second-order inactivation rate constants (k_2) with those obtained for the organoselenanes congeners (Fig. 4).[29]

The first observation, by this comparison, is the opposite selectivity. While organotelluranes **1**–**6** inhibited cathepsin V faster than cathepsin S, in the case of organoselenanes cathepsins S was preferentially inhibited than cathepsin V. By consider the k_2 of organotelluranes **1**–**6** and organoselenane **7**–**12**, it is possible to distribute the compounds **1**–**12** in three groups according to the combinations of the chalcogen (Y = Se and Te) and halogens (X = Cl and Br) and correlate them with the inhibition constants. Thus, the first group would be formed by the compounds containing tellurium and bromine in their structures (**4**–**6**, Table 1). It was observed that these compounds are the most potent inhibitors with all k_2 greater than 3000 mol L^{-1} s^{-1}. This group includes the organotellurane **4**, which undoubtedly was the strongest inhibitor with a k_2 = 26,400 L mol^{-1} s^{-1} against cathepsin V. At the other extreme, in terms of potency of inhibitors, the compounds containing selenium and chlorine in their structures (**7**–**9**, Fig. 4) showed the lowest k_2. In this case, it was observed that the inhibition constants ranged between 500 and 1500 mol L^{-1} s^{-1}, including the organoselenane **7** that showed the lowest k_2 (500 L mol^{-1} s^{-1}) for inhibition of cathepsin V. Intermediates k_2 were observed to the compounds that present in their structures tellurium bonded to chlorine (**1**–**3**, Table 1), or selenium bonded to bromine (**10**–**12**, Fig. 4). In the case of these compounds, the k_2 ranged between 1300 and 4000 L mol^{-1} s^{-1}, indicating a strong similarity in the potency of compounds with different atomic composition. In this group, the exception was the dichloro-organotellurane **1** with k_2 = 11,600 L mol^{-1} s^{-1} in the inhibition of cathepsin V.

Based on these observations, it is possible to correlate the reactivity of compounds **1**–**12** toward the cysteine cathepsins, with the structural characteristics of these inhibitors. Compounds **1**–**12** are hypervalent tetracoordinated selenium(IV) and tellurium(IV) compounds with trigonal bipyramid geometry. The hypervalent selenium and tellurium compounds that exhibit this

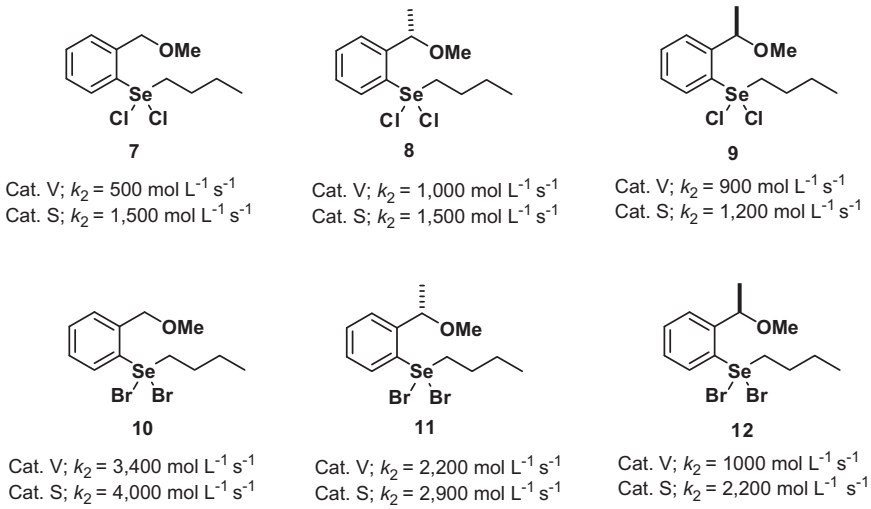
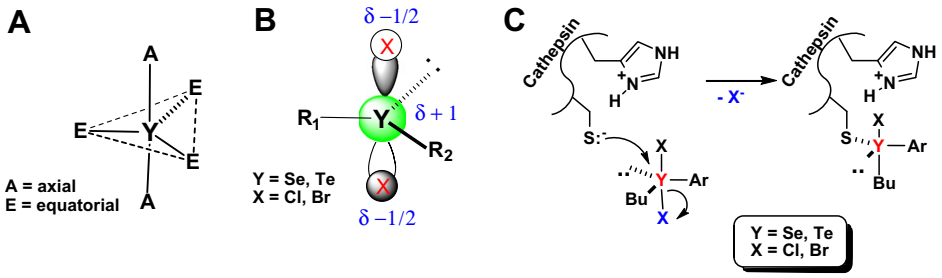


Figure 4. Second-order inactivation rate constants of the inhibition of cathepsins V and S by organoselenanes **7**–**12**.[29]

type of geometry, exhibit some structural restriction: (i) the more electronegative atoms occupy the axial position, while the less electronegative atoms occupy the equatorial position in the molecule. The non-bonding electrons pair is located in the equatorial position. (ii) Another detail is the charge distribution in molecules, since each atom in the axial positions accommodates a charge −½ while the central element accommodates a charge of +1. The electronegativity of the atoms that compose the structure can be taken as a factor to predict stability and reactivity of hypervalent compounds of selenium (IV) and tellurium (IV). Due to geometry and charge distribution it is known for these compounds that the lower the electronegativity of central atom and the higher electronegative of the element at the axial position, more stable will be the compound. Other important structural aspect is that compounds **1**–**12** are *ortho*-substituted and a non-bonded Y···O (Y = Se, Te) intramolecular interaction occurs. Due to this interaction, the strength of the bond Y–X (X = Cl, Br),[31] could facilitate the leaving of X^- after a nucleophilic attack on the chalcogen atom.

Given these considerations about the structure of hypervalent compounds of selenium(IV) and tellurium(IV), one can use these features to explain the reactivity of these compounds with the cysteine cathepsins V and S. In Scheme 2 it is shown the possible mechanism of inhibition of cysteine proteases by organoselenanes and organotelluranes. Under this proposal, the chalcogen (Y = Se, Te) is the electrophilic center in the inhibitor **1**–**12** that undergoes nucleophilic attack of thiol at the active site of the enzyme. In this reaction a Y–X bound (X = Cl, Br) is broken and a new bound Y–S is formed (Scheme 1). Therefore, knowing that the nucleophile is always the same (a thiol group from cathepsins S and V), it can simplify the mechanism in terms of the electrophilicity of chalcogen and the ability to stabilize the negative charge generated on the leaving group X^-.

Considering the electrophilicity of the chalcogen, it is known that tellurium is less electronegative than selenium and, due to its greater capacity to stabilize the negative charge, bromide is a better leaving group than the chloride, we can explain the highest reactivity of the dibromo-organotelluranes toward cysteine cathepsins. Moreover, the Y–Br bond is longer and weaker than the Y–Cl bond, favoring the bromide as a better leaving group than chlorine. From these considerations, the results in Table 1 can be interpreted as follows: (i) the inhibitors that contain in their structures Te-Br bounds are the most potent. In this case, the most electrophilic center is combinated to better leaving group (k_2 >2400 mol L^{-1} s^{-1}); (ii) the inhibitors composed by Se–Cl are those with the worst combination exhibiting smaller second-order inactivation rate constants (k_2 = 500–1500 mol L^{-1} s^{-1}). In this case, the less electrophilic chalcogen and the worst leaving group were combined; (iii) with intermediate potency are the inhibitor that contain Se–Br bound, in this case the inhibitors have a good leaving group but the lower electrophilicity of the chalcogen is unfavorable to the reaction with the thiol moiety from cathepsins (k_2 = 1300–4000 mol L^{-1} s^{-1}). In the case of Te–Cl containing inhibitors, the electrophilic chalcogen favors the reaction with the cathepsins but the leaving group is not so good. Because of these features, the combination of chalcogen and halogen, one can then explain



Scheme 2. (A) Spatial arrangement of tetracoordinated hypervalent selenium(IV) or tellurium(IV) compounds showing the equatorial (E) and axial (A) position in the molecule. (B) The charge distribution in the hypervalent selenium(IV) or tellurium(IV) compounds. (C) Probable mechanism for inhibition of cysteine proteases by selenanes and telluranes.

the similarities between the Se–Br and Te–Cl containing inhibitors, and consequently the intermediate potency of these inhibitors.

Another important observation is the influence of chirality in the potency of inhibitors **1**–**12**. It was noted that the second-order inactivation rate constants for cathepsins by these inhibitors, most pairs of enantiomers showed similar potency to each other. Only in the case of dibromides–organotelluranes one enantiomer (**5**) is two-fold more potent than the other (**6**). Thus, one can conclude that for the inhibitors **1**–**12** against cysteine cathepsins V and S no significant differentiation between pairs of enantiomers was observed. However, comparing a pair of enantiomers with the achiral congeners, the achiral compounds showed higher second-order inactivation rate constant than the chiral congeners.

3. Conclusions

By a rational design of a series of organotelluranes and by comparison with the organoselenanes congeners, it was possible to conclude that the structural aspects are directly linked to the reactivity of these hypervalent compounds. The combination Te and Br lead to very powerful cysteine proteases inhibitors while the worst combination was Se and Cl, that lead to less powerful inhibitors. Intermediates potencies were observed to the inhibitors that combined Se and Br or Te and Cl in their structures. These results indicate that the potency of this family of hypervalent compounds can be modulated using the correct combination among Se, Te, Cl and Br.

4. Experimental section

4.1. Screening of inhibitory activity of cathepsins by organotelluranes

The cathepsins kinetics of Cbz-Fr-MCA hydrolysis were performed in the optimal conditions for each enzyme: Cathepsin B: 50 mmol L^{-1} sodium phosphate buffer (pH 6.0) containing 200 mmol L^{-1} NaCl and 1 mmol L^{-1} EDTA; Cathepsin S: 50 mmol L^{-1} sodium phosphate buffer (pH 6.5) containing 2.5 mmol L^{-1} EDTA; Cathepsin V and K: 50 mmol L^{-1} sodium acetate buffer (pH 5.5) containing 2.5 mmol L^{-1} EDTA. The screening of the inhibition of cathepsins was performed by the incubation of each cathepsin with the organotelluranes **1–6** (1 μmol L^{-1}) for two min in the appropriate buffer solution at 37 °C, then the fluorogenic substrate Cbz-FR-MCA was added and the remaining enzyme activity was determined. The remaining cathepsin activities were expressed as a percentage of the activity of the control experiment.

4.2. Inactivation kinetics

Cathepsins activities were monitored spectrofluorometrically using the fluorogenic substrate Cbz-FR-MCA 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 was employed and the inhibition of activated cysteine peptidases were carried out in the presence of substrate and different concentrations of each organotellurane, as described previously. The substrate concentrations were kept 10-fold below the K_M values. The kinetics of cathepsin inactivation by organotellurane was obtained in pseudo-first-order conditions. The inhibition reaction was monitored continuously by the fluorescence of the enzymatic-released AMC. The hydrolysis progress curves were obtained in pseudo-first-order conditions and treated by non-linear regression according to Eq. (1) as reported previously:

$$P = (v_z/k_{obs})[1 - \exp(-k_{obs}t)] + d$$

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 organotellurane-induced enzyme inactivation and d is the basal fluorescence before addition of the enzyme.

Acknowledgments

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Supplementary data

Supplementary data (enzyme kinetics for Cathepsins V and S) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.01.054.

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