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Short communication

A new method for filtering of reactive "warheads" of transition-state analog protease inhibitors

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ABSTRACT

In light of the major contribution of the reactive warhead to the binding energy trend in reversible covalent transition-state analog inhibitors of serine and cysteine hydrolases, would it be possible to rationally design and quickly filter such warheads, especially for large-scale screening? The previously defined W1 and W2 covalent descriptors quantitatively account for the energetic effect of the covalent bonds reorganization, accompanying enzyme-inhibitor covalent binding. The quantum mechanically calculated W1 and W2 reflect the warhead binding energy by modeling of the enzyme-inhibitor reaction core. Here, we demonstrate the use of these descriptors for warhead filtering, and examine its scope and limitations. The W1 and W2 descriptors provide a tool for rational design of various warheads as universal building blocks of real inhibitors without the requirement of 3D structural information about the target enzyme or QSAR studies. These warheads could then be used as hit structural templates in the subsequent optimization of inhibitors recognition sites.

1. Introduction

A dominating paradigm in drug development is the design of non-covalent inhibitors. However, this approach has been insufficient for the selective manipulation of a variety of disease-relevant targets [1,2]. Indeed, approximately one-third of all FDA-approved enzyme targeted drugs are covalent inhibitors [3]. Covalent inhibitors may provide rapid onset of inhibition, greater potency, longer duration of drug action, and potent and persistent activity against mutations that would otherwise lead to drug resistance [1,4–6]. About 5–10% of all biological targets of drug development are proteases, with current global sales of protease inhibitors based drugs of several billion US$ [7].

The acylation step in serine, threonine and cysteine proteases (or hydrolases) involves formation of a tetrahedral intermediate [7–10]. Depending on the protease family, the enzymatic nucleophile is either an oxygen or a sulfur atom. A neutral tetrahedral complex stabilized by protonation of the former carbonyl oxygen atom, TC(OH), is formed in cysteine proteases [11], in contrast to serine proteases (hydrolases) that form anionic tetrahedral complexes TC(O^-) [7–10]. The active sites of proteases and hydrolases with serine, threonine, and cysteine catalytic nucleophiles have high propensity to accommodate inhibitors with reactive electrophilic warheads [7–10]. A protease substrate can be converted to either irreversible or reversible covalent inhibitor by replacing the scissile amide group with a chemically reactive warhead [12,13]. Lipscomb introduced the term "reaction-coordinate analogs" (RCA) for the subtype of transition-state analog inhibitors which interact covalently and reversibly with the enzyme nucleophile, similar to the native substrate [14]. We will use the term 'chemical site' (CS) in addition to warhead in the following discussion. In this work we consider CS fragments of RCA inhibitors of serine and cysteine proteases and hydrolases that have a carbonyl reactivity center, in analogy to a native substrate.

RCA inhibitors can be considered as formally comprised of two parts: the chemical site (CS) responsible for the covalent binding, and the recognition site (RS) dominating the selectivity of the inhibitor towards the target enzyme [15,16]. Based on our previous studies of the stability trend of TC's in series of isoselective inhibitors (constant RS in series), we have recently introduced the Enzyme Mechanism Based Method (EMBM) aimed at rational design of chemical sites (CS) of RCA inhibitors [17–19]. We have defined two covalent descriptors, W1 and W2 [17]. W1 and W2 quantitatively account for the energetic contribution (heats of formation, H_f) from the enzyme nucleophile (Nuc)-inhibitor (Inh)

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covalent binding and reorganization of the covalent bonds of the CS fragment of the inhibitor during formation of TC(O^-) or TC(OH), respectively:

$$W1 = H_f[TC(O^-)] - H_f(Inh) - H_f(Nuc) \quad (1)$$

$$W2 = H_f[TC(OH)] - H_f(Inh) - H_f(Nuc) \quad (2)$$

W1 accounts for formation of the enzyme-inhibitor new covalent bond, reflecting the modulation of the electrophilicity of the carbonyl by the variation of CS, whereas W2 accounts for two energetic effects – formation of the enzyme-inhibitor covalent bond and the proton affinity (PA) of the carbonyl oxygen or pK_a of TC(OH). W1 and W2 are calculated quantum mechanically on small molecular clusters that simulate the reaction core of the formed covalent tetrahedral complex, either anionic TC(O^-) or neutral TC(OH) (Fig. 1) [17–19].

W1, accounting for the modulation of the inhibitor warhead electrophilicity, dominates the binding trend in serine proteases (hydrolases). The contribution of H-bonds in the oxyanion hole to the TC(O^-) stabilization is much smaller than the energy released in the formation of the enzyme-inhibitor covalent bond [13]. Thus, in serine proteases, W2 plays a minor role in comparison with W1. Conversely, PA/pKa of TC(OH) dominates the CS covalent binding trend in cysteine proteases, so W2 plays an exclusive role there, while W1's contribution is insignificant [17–19]. Detailed physical reasons for the different role of W1 and W2 in CS reactivity trend can be found in ref 17. Modeling on a reaction core (Fig. 1) allows generation of various CS and corresponding TC(O^-) and TC(OH) as universal building blocks of real inhibitors and their covalent complexes without requirement of any 3D structural information about the target enzyme. We have pre-calculated W1 and W2 indices for about 300 different CS groups forming covalent TC's with two types of nucleophiles CH_3O^- and CH_3S^-, corresponding to serine and cysteine hydrolases. The results are accumulated in our Chemical Site of Inhibitors (CSI) databank (see Supporting Information) [17]. To the best of our knowledge, EMBM, applied for both ligand-based [17] and structure-based [19] RCA inhibitors design, is the first systematic attempt of rational design of covalent inhibitor warheads. It provides a comprehensive analysis of all possible interactions, both covalent and non-covalent, of inhibitor CS fragments with their target enzyme active site.

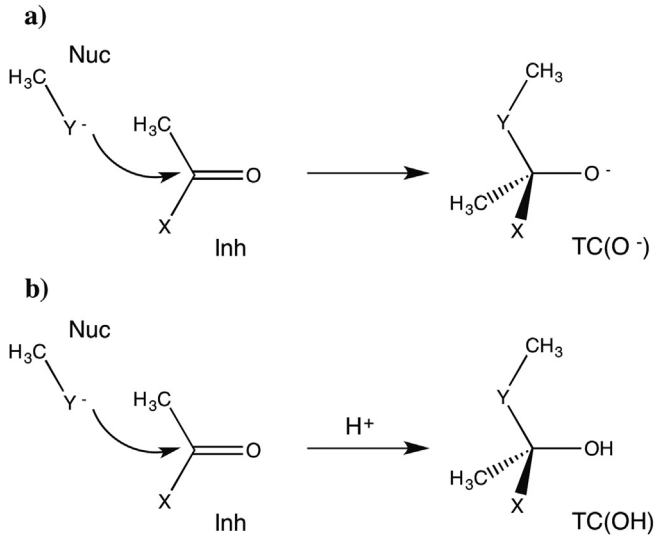

Fig. 1. Molecular clusters of the enzyme (Nuc)-inhibitor (Inh) reaction core, simulating the first covalent catalytic step in proteases. a) Formation of an anionic TC(O^-), and b) formation of a neutral TC(OH). Y = O or S, in serine or cysteine proteases, respectively. X is the varied substituent modifying the reactivity of the carbonyl group of the ligand.

2. Results and discussion

In light of the major contribution of the enzyme-inhibitor covalent bond to the binding energy trend in RCA inhibitors [13], would it be possible to filter CS fragments by the covalent interaction only, especially for large-scale screening? Or, in other words, can the W1 and W2 descriptors, pre-calculated on reaction cores and collected in the CSI databank, be used for filtering and prediction of the reactivity of CS fragments of RCA inhibitors without any need of QSAR or 3D enzyme structure? The present work addresses this question in order to define the scope of such a simple screening protocol. Table 1 presents the applicability of filtering by W1 of warheads for series of RCA inhibitors with experimental pK_i data of the serine proteases thrombin [20,21], trypsin [20,21], and human neutrophil elastase (HNE) [22], and fatty acid amide hydrolase (FAAH) [23]. Filtering of CS by W2, applied to series of RCA inhibitors of the cysteine proteases HRV 3C [24], cathepsin K [25], and caspase 3 [26], with experimental pK_i or pIC_{50} values, is presented in Table 2. In the considered examples the warheads of the inhibitors are either α-keto heterocycles, α-keto amides, or N-benzylisatins. The CS reaction center in all cases is a carbonyl group. The electrophilicity of the carbon atom and the proton affinity of the oxygen atom of the carbonyl group have an opposite influence on the binding trend of a series of warheads [15–17]. It defines an opposite role of the W1 and W2 descriptors in the warheads activity trend: the activity of warheads towards a serine nucleophile increases with decreasing of the W1 values, and oppositely a larger W2 descriptor defines a more active warhead towards a cysteine nucleophile [17–19]. The values of W1 and W2 are sorted in descending order in Tables 1 and 2. We used a conditional color format of the tables to facilitate data analysis: the color of cells in a column changes from green to red with the descending of the numerical values.

Two factors influence the binding affinity of CS fragments [17–19]. The first is the energy of reorganization of covalent bonds of the CS during the chemical transformation in the enzyme active site, accounted for by the W1 and W2 covalent descriptors. This factor strongly depends on the level of influence of the varied fragment of CS on the electron density variation on the reaction center (electrophilicity of C and pKa of O atoms of the carbonyl group). The second factor is the proper alignment of the warhead in the enzyme active site for efficient chemical interaction with the attacking enzyme nucleophile. With poor alignment, the chemical reaction cannot be efficiently realized for geometrical reasons, leading to a high activation barrier. Consequently, only very active warheads will react in such a case, whereas weaker CS's will not, despite being relevant if optimally aligned. The quality of CS alignment depends on its own non-covalent interactions and on the selectivity of the RS fragment of the inhibitor. Therefore, we can formulate the following criterion: W1 and W2 can be used for filtering and identification of the most active warheads when the varied substituents in CS significantly influence the electrophilicity (W1) or pKa (W2) of the carbonyl of CS. In most literature publications the activity of warheads were discovered in screening assays by hindsight [27,28], and the experimental data used in this work are no exception. It is rather difficult to find literature experimental data regarding sets of RCA isoselective (constant RS fragment) inhibitors spanning a wide interval of K_i or IC_{50} values. Nevertheless, there are a few such examples, which are analyzed here. One subset of data includes thrombin, trypsin (columns a, b, and e in Table 1), and HNE serine protease inhibitors (column c,