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Biological evaluation and docking studies of natural isocoumarins as inhibitors for human kallikrein 5 and 7

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ABSTRACT

Human kallikrein 5 and 7 (KLK5 and KLK7) are trypsin-like and chymotrypsin-like serine proteases, respectively, and promising targets for the treatment of skin desquamation, inflammation and cancer. In an effort to develop new inhibitors for these enzymes, we carried out enzymatic inhibition assays and docking studies with three isocoumarin compounds. Some promising inhibitors were uncovered, with vioxanthin and 8,8′-paepalantine being the most potent competitive inhibitors of KLK5 (K_i = 22.9 μM) and KLK7 (K_i = 12.2 μM), respectively. Our docking studies showed a good correlation with the experimental results, and revealed a distinct binding mode for the inhibitors at the binding sites of KLK5 and KLK7. In addition, the docking results suggested that the formation of hydrogen bonds at the oxyanion hole is essential for a good inhibitor.

Human kallikrein 5 (KLK5) and 7 (KLK7) are members of the human tissue kallikrein family, which is comprised of 15 kallikrein-like serine peptidases (KLKs).[1–3] KLK5 is found in many tissues, including the breast, central nervous system, prostate, testis, and trachea; while KLK7 is expressed in the esophagus, kidney, liver, and salivary gland.[4,5] Moreover, both enzymes are most abundantly expressed in human skin,[5] where an important role in skin desquamation has been reported.[6,7] KLK5 and KLK7 are believed to contribute to desquamation by hydrolyzing extracellular proteins that are part of intercellular adhesion structures, called corneodesmosomes.[8,9] The function of these KLKs in tissues other than the skin is less clear; however, there is growing evidence that overexpression of KLK5 and KLK7 occurs in endocrine-related malignancies, including ovarian, breast and testicular cancer.[10–12] Thus, the development of inhibitors for KLK5 and KLK7 can not only help to elucidate their physiological and pathological functions, but also serve as an archetype for new drugs to treat diseases that result from their uncontrolled activity.

The isocoumarins are a class of natural polyphenolic compounds that are present in a variety of plant species[13] and display several pharmacological activities, including anti-tumor properties.[14–18] Several isocoumarins, including paepalantine (9,10-dihydroxy-5,7-dimethoxy-1*H*naptho(2, 3c)pyran-1-one), its dimer (8,8′-paepalantine) and vioxanthin (Fig. 1), have previously been isolated from *Paepalanthus bromelioides*, a Brazilian plant commonly found in the region of Cipó Mountain, Minas Gerais State, Brazil.[19–21] An antioxidant analysis of these compounds reveals that paepalantine, and to a lesser extent its dimer, are powerful agents that are able to protect against oxidative stress conditions imposed on mitochondria.[21] Additionally, it has been reported that compounds based on isocoumarin scaffolds are potent inhibitors of cysteine proteases,[22] aspartyl proteases,[23] and serine proteases, which include chymotrypsin-, trypsin-, and elastase-like enzymes.[24] Therefore, we assayed these three natural isocoumarins for activity against both KLK5 and KLK7 as a part of our ongoing research program aimed at discovering new protease inhibitors. Herein, we report the results of these inhibitory assays and associated docking studies.

Both proteases were obtained from an insect cell/baculovirus expression system as described previously,[25] and the fluorescence resonance energy transfer (FRET) peptides Abz-KLRSSKQ-EDDnp and Abz-KLYSSKQ-EDDnp (Abz, *o*-aminobenzoic acid; EDDnp, *N*-(2,4-dinitrophenyl) ethylenediamine) were synthesized by solid phase synthesis as earlier described.[26] In our experiments, the vioxanthin was the most potent of the three compounds against KLK5 (IC_{50} = 53.7 μM), followed by the 8,8′-paepalantine dimer

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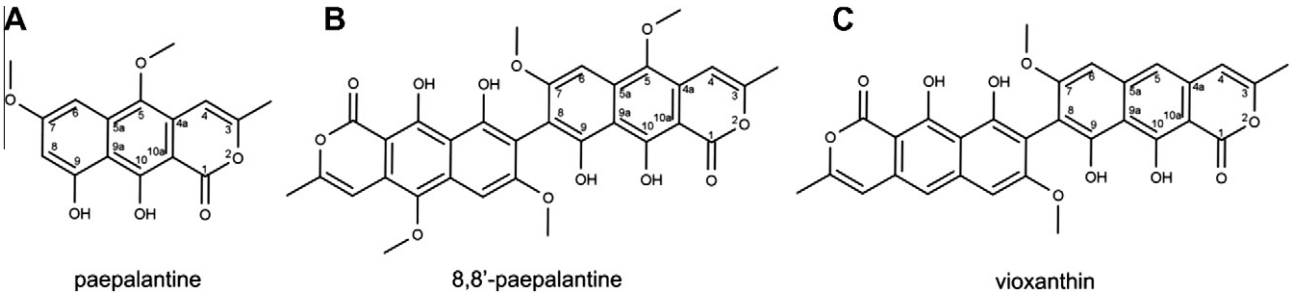

Figure 1. Structure of the three isolated isocoumarins used as possible inhibitors: paepalantine (A), 8,8′-paepalantine dimer (B), vioxanthin (C). The compounds were isolated by chromatographic procedures and compared to spectroscopic data previously published.[19–21]

(IC_{50} = 72.9 μM) and paepalantine itself (IC_{50} = 367.4 μM). With respect to KLK7, the 8,8′-paepalantine dimer has the lowest IC_{50} (112.4 μM), followed by vioxanthin (IC_{50} = 135.3 μM) and then paepalantine (IC_{50} = 165.8 μM). These preliminary results suggest that both dimers are able to inhibit the enzymatic activity of KLK5 and KLK7. To better understand the interaction between these kallikreins and the three natural isocoumarins assayed, we have performed a detailed kinetic study to determine the mechanism of inhibition. Lineweaver–Burk double-reciprocal plots show the intercepts of all lines, both in the absence of and at three different inhibitor concentrations, converging at the *y*-axis ($1/V_{max}$), whereas the slope (K_m/V_{max}) and *x*-axis intercepts ($1/K_m$) vary with inhibitor concentration. Therefore, the V_{max} values remain constant, whereas the apparent K_m values increase with increasing inhibitor concentrations. This behavior is consistent with a mutually exclusive binding mode between the inhibitor and substrate (Fig. 2). Since IC_{50} values can vary with substrate concentration for competitive inhibitors, the enzyme dissociation constant (K_i) represents a better way to compare different molecules as potential inhibitor scaffolds. Using the data depicted in Figure 2, it is possible to determine the K_i values of each competitive inhibitor by plotting the reciprocal of the initial velocity ($1/V_0$) versus a series of inhibitor concentrations at constant substrate concentrations. On this plot, the *x*-intercept indicates the K_i (Table 1). In this analysis, vioxanthin remains the most potent inhibitor of KLK5 (K_i = 22.9 μM), followed by 8,8′-paepalantine (K_i = 24.4 μM) and paepalantine (K_i = 47.5 μM). With respect to KLK7, however, 8,8′-paepalantine (K_i = 12.2 μM) is the best inhibitor, followed by vioxanthin (K_i = 37.2 μM) and paepalantine (K_i = 70.7 μM).

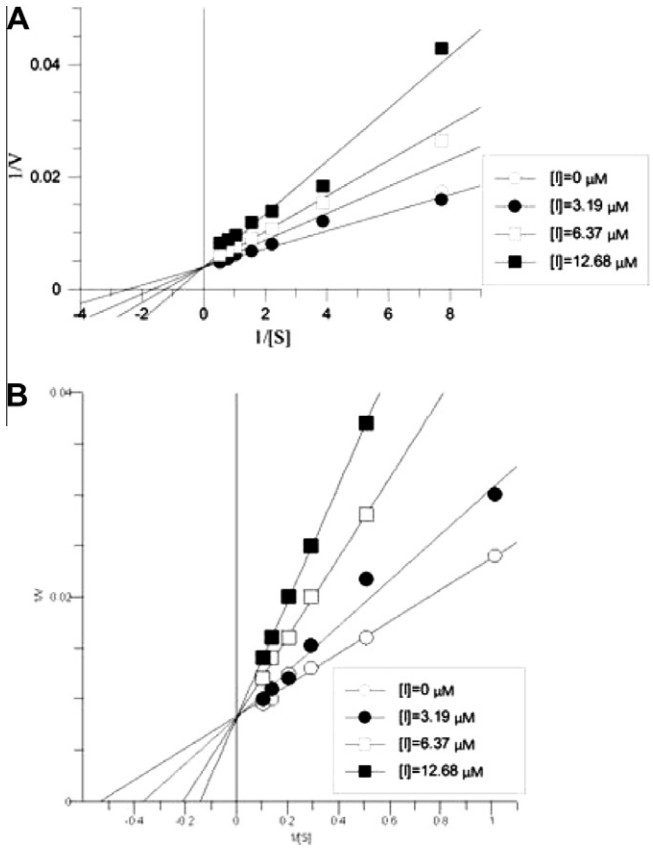

Figure 2. Lineweaver–Burk representation of the 8,8′-paepalantine dimer inhibitor for KLK5 (A) and for KLK7 (B). Both inhibitors compete with the substrate for the free catalytic site. Kinetic assays were conducted in the presence of increasing concentrations of inhibitors.

We performed docking studies to further investigate the mode of interaction between these isocoumarins and kallikreins. These docking studies focused on the analysis of two parameters: the docking score, which represents the energy of interaction between the enzyme–inhibitor complex, and hydrogen bond interactions. Docking scores calculated using the Glide program[27,28] indicate that the 8,8′-paepalantine dimer had the highest docking score, and therefore energy of interaction, for KLK5. This score (−7.68 kcal/mol) was slightly better than those calculated for vioxanthin and paepalantine, −7.49 kcal/mol and −6.73 kcal/mol, respectively. The inversion in 8,8′-paepalantine's and vioxanthin's ranking relative to their inhibition constants for KLK5 can be attributed to the inability of the Glide scoring function to distinguish between complexes with nearly equivalent binding affinities. Actually, affinity prediction is the biggest weakness associated with all scoring functions. According to the Gibbs free energy equation and the logarithmic relationship between binding free energy and affinity, a 10-fold difference in activity represents a difference of merely 1.4 kcal/mol.

Consequently, it is rarely possible to accurately predict binding affinity differences of ⩽10-fold because the error in the scoring function and in other more rigorous computations, such as the free energy perturbation, is in the range of 1.4–2.0 kcal/mol. Our docking studies revealed a good correlation with the K_i results with respect to KLK7; ranking the 8,8′-paepalantine dimer first (−9.47 kcal/mol), vioxanthin second (−7.59 kcal/mol), and paepalantine last (−7.49 kcal/mol).

Docking results suggested that vioxanthin and 8,8′-paepalantine share a common binding mode with KLK5. Half of the dimer is positioned at the entrance of the S1 pocket, which is formed by Asp189, Ser190, Cys191, and Tyr228, whereas the other half of the dimer fits into the S3 (Gln192 and Trp218) and S4 (Gln174 and Trp215) pockets (Fig. 3a). Alternatively, paepalantine only occupies the entrance of the S1 pocket. In contrast, 8,8′-paepalantine and vioxanthin bind inside KLK7's S1 pocket, in addition to the S1′ pocket; while paepalentine binds almost completely inside of the S1 pocket only (Fig. 3c).