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Dual function inhibitors of relevance to chronic obstructive pulmonary disease

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ABSTRACT

The general strategy and rationale underlying the design of COPD therapeutics that possess protease inhibitory activity and are also capable of releasing a species that attenuates inflammation by inhibiting caspase-1, are described. The synthesis and in vitro biochemical evaluation of a dual function molecule that sequentially inhibits HNE and caspase-1 in a time-dependent manner is reported.

Chronic obstructive pulmonary disease (COPD) is a multi-factorial inflammatory disorder characterized by enlargement of the airspaces and airflow obstruction that is not fully reversible.[1] COPD is a leading cause of morbidity and mortality, and is currently the fourth most common cause of death in the US.[2] Cigarette smoking and genetic predisposition are risk factors associated with COPD development. Currently, there are no drugs on the market that halt or reverse the progression of COPD.[3]

The molecular mechanisms underlying the pathogenesis of COPD are poorly understood.[4] The disorder is characterized by an influx of neutrophils, macrophages and cytotoxic T lymphocytes which release an array of proteolytic enzymes, including serine (elastase, proteinase 3), cysteine (cathepsin S) and metallo- (MMP-12) proteases. These mediate a multitude of signaling pathways,[5] as well as participate in the degradation of lung connective tissue and other components of the extracellular matrix.[6] The protease/antiprotease imbalance[7] plays a prominent role in COPD, however, other processes are of paramount importance. These include an oxidant/antioxidant imbalance[8] that arises from the inhalation of cigarette smoke and the release of reactive oxygen species by phagocytic cells, alveolar septal cell apoptosis,[9] and chronic inflammation[10] (Fig. 1). The confluence and interplay of these processes and mediators ultimately leads to the initiation and progression of the disease. Effective therapeutic interventions for COPD may require the disruption of more than one of the aforementioned processes.

In light of the forgoing, we envisaged that an entity such as (I) capable of disrupting the cycle of events shown in Figure 1 by abrogating multiple processes (for instance, inhibition of elastase and prevention of apoptosis or amelioration of inflammation), may be more efficacious in treating the disorder. The biochemical rationale underlying the design of (I) rested on the following considerations: (a) previous studies have demonstrated that the 1,2,5-thiadiazolidin-3-one-1,1-dioxide scaffold docks to the active site of chymotrypsin-like serine proteases such as, for example, human

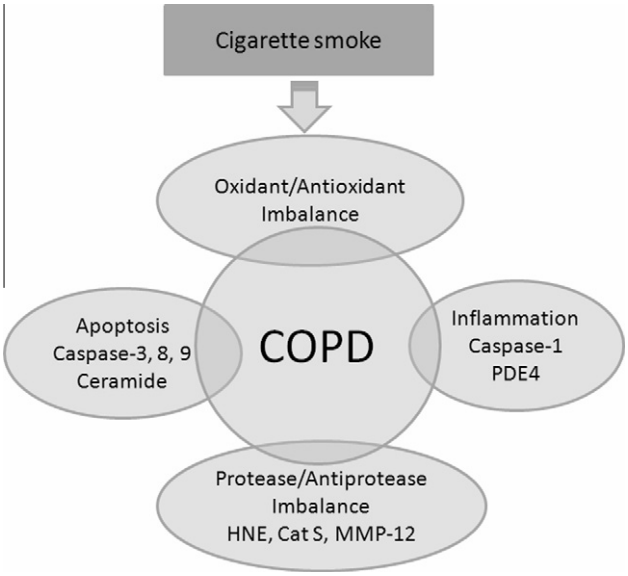

Figure 1. Interlinked processes involved in COPD pathogenesis.

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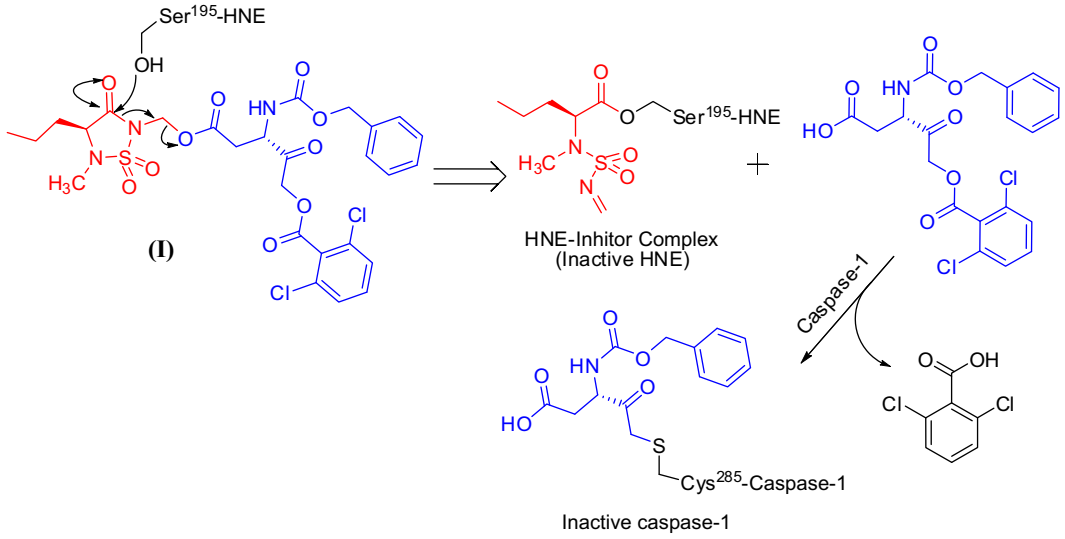
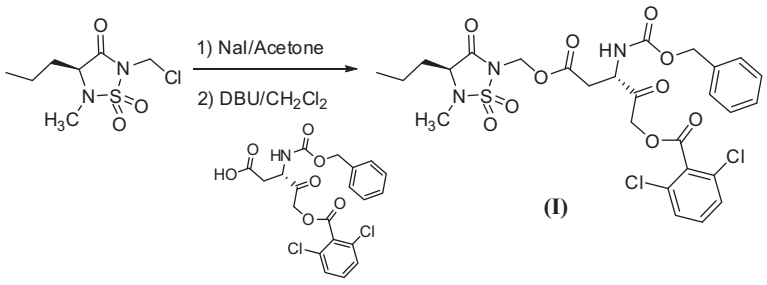


Figure 2. Mechanism of inhibition of human neutrophil elastase and caspase-1 by dual function inhibitor (I).



Scheme 1. Synthesis of inhibitor (I).

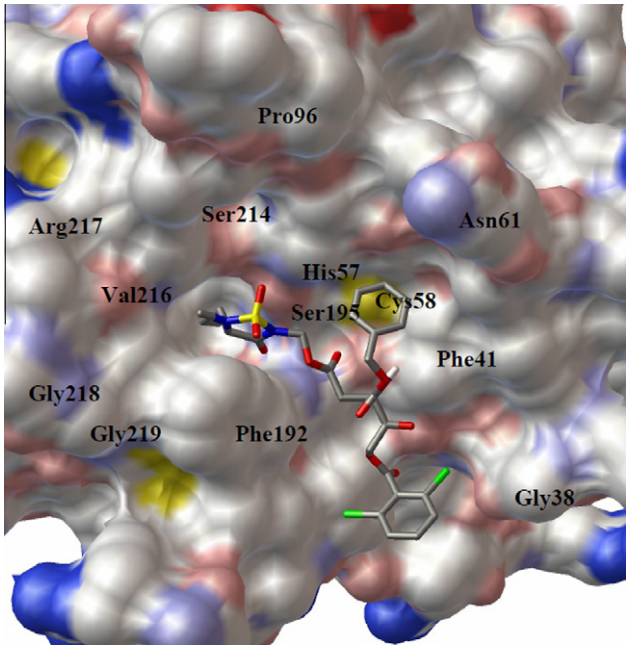


Figure 3. Inhibitor (I) bound to the active site of human neutrophil elastase. Molecular docking simulations were performed using AUTODOCK4.0[18] (The Scripps Research Institute). The receptor model was prepared using the human neutrophil elastase-turkey ovomucoid inhibitor complex (HNE-OMTKY3 complex/PDB code:1PPF[20]), stripped of all water molecules and OMTKY3 ligand. Inhibitor (I) was constructed in SYBYL8.0[21] and was structurally optimized to default convergence thresholds using the Tripos Force Filed[22] and Gasteiger–Marsili partial atomic charges.[23] The ligand is colored by their atom type and the enzyme surface is colored using David Goodsell colors.[18]

neutrophil elastase (HNE) and proteinase 3 (Pr 3), in a substrate-like fashion, making possible the exploitation of multiple binding interactions with both the S and S′ subsites of a protease;[11,12] (b) carboxylate derivatives linked to the aforementioned heterocyclic scaffold function as potent mechanism-based inhibitors of HNE and Pr 3;[13] (c) exploratory studies have shown that exploitation of binding interactions with the S′ subsites yields highly selective and potent inhibitors of HNE; (d) the S′ subsites of HNE are hydrophobic and tolerate structurally-diverse recognition elements of variable size;[14]

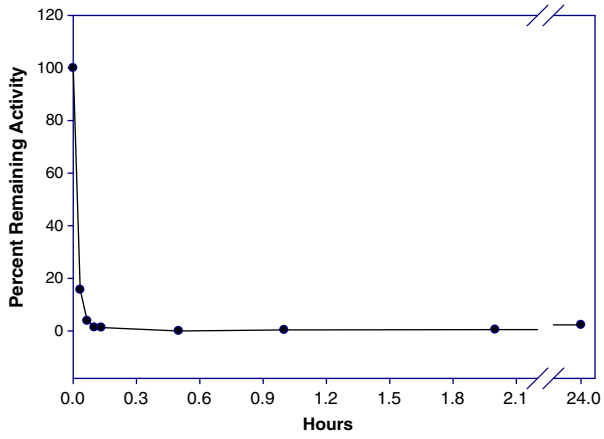


Figure 4. Time-dependent loss of enzymatic activity with compound (**I**). Percent remaining activity versus time plot was obtained by incubating inhibitor (I) (10.5 μM) with human neutrophil elastase (700 nM) in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25, and 1% DMSO. Aliquots were withdrawn at different time intervals and assayed for enzymatic activity using MeOSuc-AAPV *p*-NA by monitoring the absorbance at 410 nm.

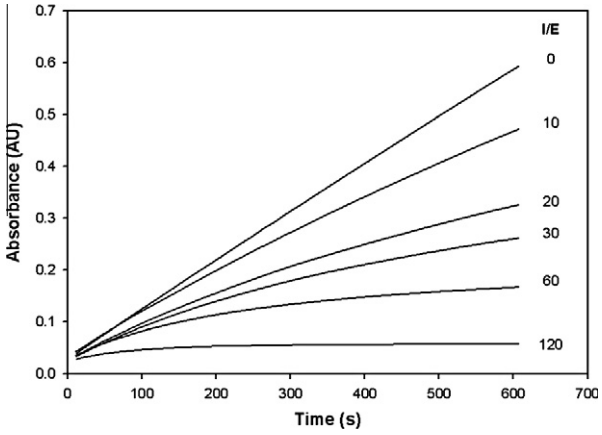


Figure 5. Progress curves for the inhibition of human neutrophil elastase (HNE) by compound (**I**). Absorbance was monitored at 410 nm for reaction solutions containing 10 nM HNE, 105 μM MeOSuc-AAPV *p*-nitroanilide, and inhibitor (I) at the indicated inhibitor to enzyme ratios in 0.1 M HEPES buffer containing 0.5 M NaCl, pH 7.25, and 2.5% DMSO. The temperature was maintained at 25 °C, and reactions were initiated by the addition of enzyme.

(e) caspase-1 is a cysteine protease that converts interleukin-1 to interleukin-1β (IL-1β), an inflammatory cytokine present in COPD;[15] and (f) the primary substrate specificity residue (P1) of caspase-1 is Asp. Consequently, we reasoned that attachment of a known inhibitor of caspase-1 to the 1,2,5-thiadiazolidin-3-one-1,1-dioxide scaffold via the carboxylate group would result in dual function inhibitor (I) capable of inhibiting HNE, thereby preventing the hydrolysis of elastin, the hydrophobic component of lung connective tissue, and also capable of releasing a caspase-1 inhibitor, moderating inflammation (Fig. 2).

Inhibitor (I) was readily constructed by reacting (L)-4-*n*-propyl-5-methyl-2-chloromethyl 1,2,4-thiadiazolidin-3-one-1,1-dioxide[14] with Z-Asp-CH_2DCB (DCB = (2,6-dichlorobenzoyl)oxy)[16] in the presence of DBU in methylene chloride[17] (Scheme 1).

Molecular modeling simulations using AUTODOCK4.0[18] provided a measure of assurance regarding the binding mode of (I) to the active site of HNE (Fig. 3). Specifically, it was anticipated that inhibitor (I) would dock to the active site of the enzyme with the *n*-propyl group nestled in the primary specificity pocket (S_1) and the hydrophobic leaving group/caspase-1 inhibitor oriented toward the S′ subsites. The nature of the interaction of (I) with HNE was investigated by incubating (I) with HNE. This led to rapid, time-dependent, and irreversible loss of enzymatic activity (Fig. 4).

The bimolecular rate constant (k_{inact}/K_I M^{-1} s^{-1}), an index of inhibitor potency, was determined using the progress curve method[19] (Fig. 5) and found to be 24,700 M^{-1} s^{-1}. Inhibitor (I) was devoid of any inhibitory activity toward proteinase 3 ($[I]/[E] = 250$, 30 min incubation time). In a separate experiment, a 10-fold excess of compound (**I**) was incubated with HNE for 2 min, an aliquot (50 μL) was withdrawn and added to a cuvette containing Ac-YVAD-AMC and 10 mM Tris buffer containing 1 mM DDT, 0.1% CHAPS, pH 7.5, and 4% DMSO. The reaction was initiated by adding 4 nM caspase-1 and monitoring the fluorescence emission at 460 nm and excitation at 360 nm (Fig. 6). The temperature of the solution was maintained at 37 °C. It is evident from Figure 6 that the interaction of (I) with HNE results in the rapid release of caspase-1 inhibitor which then inhibits caspase-1. The effectiveness of the proposed strategy rests on many factors, including the ability of (I) to possess high inhibitory activity toward HNE, ensuring rapid release of a secondary species (in this case a caspase-1 inhibitor), which is also a highly efficient inhibitor ($k_{obs}/[I]$ 7100 M^{-1} s^{-1}).[16] In principle, the nature of the secondary species can include agents that possess antioxidant, anti-inflammatory, or anti-MMP-12 activity. It should be noted that inhibitor (I) was devoid of any inhibitory activity toward caspase-1 without previous incubation with HNE (Fig. 6).

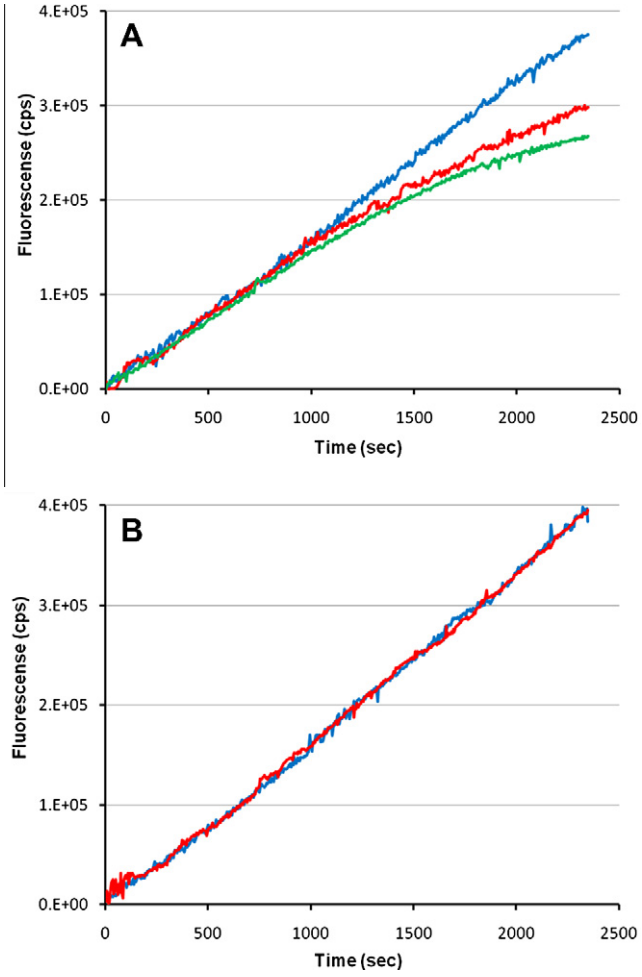


Figure 6. (Panel A) Progress curves for the inhibition of caspase-1 by Z-Asp-CH_2DCB released from dual inhibitor (I) after incubation with HNE for 2 min. The fluorescence emission at 460 nm (excited at 380 nm) was monitored for reaction solutions containing 4 nM caspase-1, 46 μM Ac-YVAD-AMC, and the inhibitor at the indicated inhibitor to enzyme ratios in 10 mM Tris buffer containing 1 mM DTT, 0.1% CHAPS, pH 7.5, and 4% DMSO. The temperature was maintained at 37 °C, and reactions were initiated by the addition of enzyme. Blue line: hydrolysis control (inhibitor (I)/HNE/caspase-1 = 0:0:1); red line: inhibitor (I)/HNE/caspase-1 = 200:20:1; green line: inhibitor (I)/HNE/caspase-1 = 400:40:1. (Panel B) Screening of dual functional inhibitor (I) under the same conditions as in panel A but in the absence of HNE. Blue line: hydrolysis control; red line: inhibitor (I).

In conclusion, the design of a dual function species capable of disrupting multiple pathogenic mechanisms associated with COPD is described.

Acknowledgment

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17. The *N*-chloromethyl compound (0.96 g; 4 mmol) was dissolved in dry acetone (20 mL), sodium iodide (0.66 g; 4.4 mmol) was added, and the reaction was stirred at room temperature for 10 h. The precipitate was removed using a silica gel column and eluting with methylene chloride. Evaporation of the solvent left a residue which was dissolved in methylene chloride (10 mL). The solution was treated with Z-Asp-CH_2DCB (1.70 g; 4.4 mmol), followed by DBU (0.66 g; 4.4 mmol), and the reaction mixture was stirred at room temperature overnight. The solvent was removed, ethyl acetate (60 mL) was added and the resulting solution was washed with 5% HCl (20 mL) and brine (20 mL). The solution was dried, filtered and the solvent evaporated in vacuo, leaving a crude product that was purified using flash chromatography (silica gel/ethyl acetate/methylene chloride/hexanes) to give the desired product (0.59 g; 25% yield), as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.88–0.98 (t, 3H), 1.25–1.56 (m, 2H), 1.68–2.00 (m, 2H), 2.82 (s, 3H), 2.90–3.20 (m, 2H), 3.80–3.88 (m, 1H), 4.71–4.79 (m, 1H), 5.09–5.20 (m, 4H), 5.57–5.75 (m, 2H), 5.98–6.05 (t, 1H), 7.28–7.40 (m, 8H). HRMS (ESI) calculated for $C_{27}H_{29}Cl_2N_3O_{16}SNa$ $[M+Na]^+$ 680.0848, found 680.0847.
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