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Benzimidazolone as potent chymase inhibitor: Modulation of reactive metabolite formation in the hydrophobic (P_1) region

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ABSTRACT

A new class of chymase inhibitor featuring a benzimidazolone core with an acid side chain and a P_1 hydrophobic moiety is described. Incubation of the lead compound with GSH resulted in the formation of a GSH conjugate on the benzothiophene P_1 moiety. Replacement of the benzothiophene with different heterocyclic systems such as indoles and benzoisothiazole is feasible. Among the P_1 replacements, benzoisothiazole prevents the formation of GSH conjugate and an in silico analysis of oxidative potentials agreed with the experimental outcome.

Chymase is a chymotrypsin-like serine protease that is stored in a latent form in secretory granules of mast cells.[1] Upon stimulation, mast cells degranulate and release an active form of chymase into the local tissue.[2] In addition to angiotensin converting enzyme, chymase has been demonstrated to catalyze the formation of angiotensin II (Ang II) from angiotensin I,[3] although the physiological significance of this remains unclear since the role of chymase on the regulation of blood pressure is minimal.[4] Besides generating Ang II, chymase is also believed to be involved in the activation of transforming growth factor-β (TGF-β)[5] and the production of collagen type I.[6] During pathological states, the release of chymase in local tissues such as the heart may result in vascular injury, fibrosis, and cardiac remodeling upon chronic exposure.

Heart failure is one of the leading causes of death in the United States and continues to represent an unmet medical need. A link between heart failure and chymase has been documented,[7] and there is an interest to develop a specific chymase inhibitor as a new therapeutic regimen for the disease. A number of small molecules inhibitors for chymase have been reported.[8] **TPC-806** (Fig. 1) is the first reported non-covalent chymase inhibitor[9] and has been in phase II clinical trials for heart failure since 2007. In silico analysis of the binding mode of **TPC-806** with human chymase suggested a possible replacement of the benzimidazole core of **TPC-806** with a benzimidazolone. As shown in Figure 1, lead compounds **1** and **2** were prepared and tested against **TPC-806** in a human chymase (h-Chy) enzyme assay,[10] compound **1** is only threefold weaker than **TPC-806** (IC_{50} of 70 nM vs 22 nM). We believe that benzimidazolones represent an interesting new class of inhibitor with perhaps better physical chemical properties than **TPC-806** (*c* log *P* for **TPC-806**: 5.5; *c* log *P* for **1**: 3.7).

An X-ray co-crystal structure of lead compound **2** bound to human chymase was obtained which allowed determination of the binding mode of the inhibitor (Fig. 2). The benzothiophene, namely the P_1 moiety of the inhibitor, occupies a well defined hydrophobic pocket, namely the S_1 pocket with a size of approximately 10 Å deep, 7 Å wide and 7 Å high. The benzimidazolone core orientates perpendicular to the P_1 moiety and anchors the carboxylic acid side chain to interact with two basic amino acid residues, LYS^{40} and LYS^{192}. This acid–base interaction is critical to the affinity of the inhibitor.[11]

Assessment of the target independent properties of this new class of inhibitor revealed reactive metabolite formation as a liability of compound **1**. Upon incubation of compound **1** with glutathione (GSH) in the presence of human liver microsomes, a GSH

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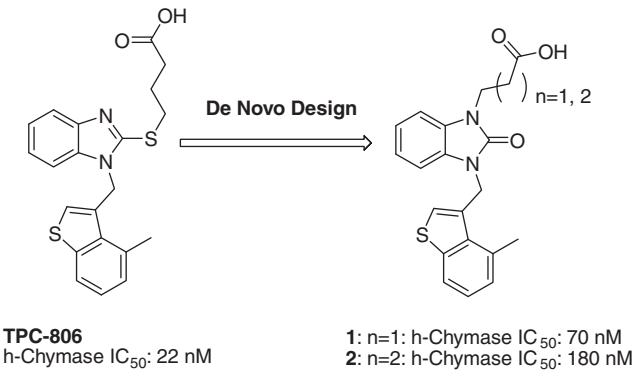


Figure 1. De novo design for benzimidazolone core.

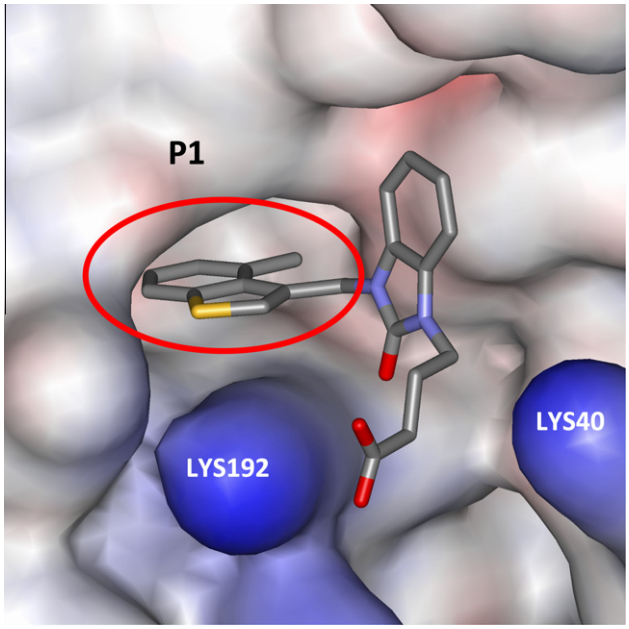


Figure 2. X-ray co-crystal structure of **2** in human chymase.

conjugate was formed.[12] Identification of the pathways leading to potential reactive metabolites is considered as one of the key studies in understanding the mechanism of toxicity associated with a compound of interest because there are numerous reports linking the formation of glutathione adducts with xenobiotics to either liver toxicity or idiosyncratic reaction.[13] Glutathione has been frequently used to trap potential reactive metabolites generated through bioactivation in systems such as liver microsomes. Glutathione adducts are able to be detected by a mass spectrometer, and the results are used not only to confirm the presence of reactive metabolites, but also to provide information on their potential structures. To further elucidate the structure of the glutathione adduct, an enhanced product ion scan was performed. Based on the enhanced product ion spectra, the site of conjugation was tentatively assigned to the P_1 benzothiophene group (Fig. 3).

With this information in hand, it is possible to alleviate this liability by replacing the benzothiophene P_1 with other systems that are resistant to glutathione conjugation. In this Letter, an effort to identify an alternative P_1 moiety is reported.

The general synthetic route of compound **1** is depicted in Scheme 1. Starting with 1-isopropenyl-2-benzimidazolidinone, Michael addition to ethyl acrylate followed by deprotection with

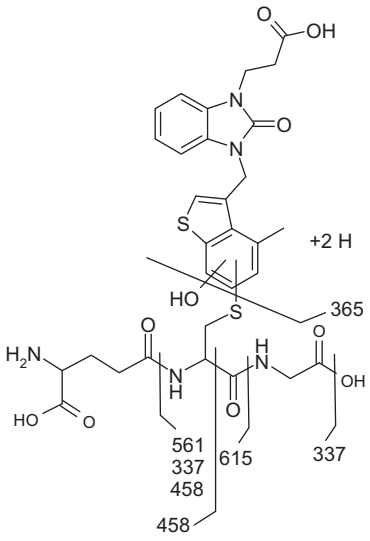
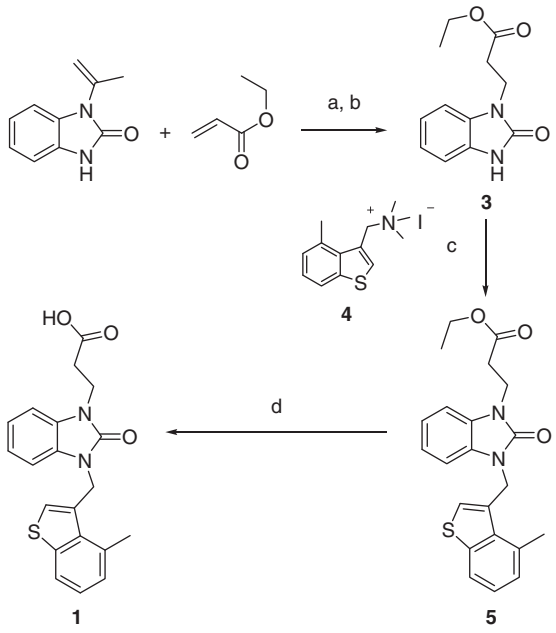


Figure 3. GSH conjugate of **1** (with LC/MS/MS fragments).



Scheme 1. Synthesis of benzimidazolone **1**; key: Reagents and conditions: (a) benzyltrimethylammonium hydroxide, MeOH, rt, 76%; (b) HCl, MeOH/H_2O, 60 °C, 92%; (c) **4**, K_2CO_3, DMF, 90 °C, 50%; (d) LiOH, 1,4-dioxane/H_2O, rt, 100%.

HCl gave benzimidazolone **3**. S_N2 reaction of **3** with benzothiophene iodide **4** provided benzimidazolone ester **5**. The acid moiety in lead compound **1** was finally unmasked with LiOH in quantitative yield.

Based on molecular modeling, the first set of alternative P_1s was designed to focus on ligand similarity with respect to benzothiophene. Different bicyclic systems, which have appropriate steric requirements, were prepared. The syntheses of those analogs are similar to **1** with replacement of intermediate **4** with other bicyclic systems (Scheme 1). The synthetic routes of some representative bicyclic P_1 intermediates and analogs are described in Schemes 2–6.

As shown in Scheme 2, the synthesis of indole P_1 analog **31** started with methylation of 3-methyl indole to give dimethyl indole **6**. Friedel–Crafts formylation of **6** with pyrophosphoryl