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# Nonpeptide Inhibitors of Cathepsin G: Optimization of a Novel β-Ketophosphonic Acid Lead by Structure-Based Drug Design

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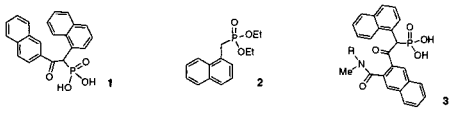
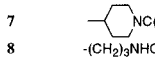
The serine protease cathepsin G (EC 3.4.21.20; Cat G) is stored in the azurophilic granules of neutrophils (polymorphonuclear leukocytes) and released on degranulation.[1] This chymotrypsin-like enzyme has been implicated in a variety of pathological conditions associated mainly with inflammation.[2] For example, Cat G is involved in tissue remodeling at sites of injury via the cleavage of matrix components, including proteoglycans, collagen, fibronectin, and elastin.[3] Thus, specific inhibitors of Cat G could be useful for treating emphysema, asthma, reperfusion injury, psoriasis, and rheumatoid arthritis. We now report the discovery of nonpeptide inhibitors of Cat G possessing a novel β-ketophosphonic acid subunit, by use of a structure-based drug design approach.

There are several reports on Cat G inhibitors,[2c−e,4] some of which are reasonably potent; however, the inhibitors have generally been peptide-like structures or irreversible inactivators.[2c−e,4a−j] We were able to identify bis-naphthyl β-ketophosphonic acid **1**[5] as a moderately potent, reversible Cat G inhibitor (Table 1) by high-throughput screening of a diverse chemical library with a chromogenic assay.[6] This compound is very intriguing because of its novel nonpeptide structure and competitive, reversible kinetics. To enhance the potency, and to develop an initial structure−activity profile, we synthesized numerous analogues with alterations of the aromatic rings and ketone unit. Unfortunately, this exercise did not improve the $IC_{50}$ value into the nM range. For instance, replacement of the 2-naphthyl ring of **1** with phenyl or 1-naphthyl, or replacement of the 1-naphthyl ring with phenyl or 2-naphthyl, abolished Cat G activity ($IC_{50}$ > 100 μM). Unfavorable results were also realized on converting the C=O group to a CHOH, $CH_2$, or $SO_2$ group. Since **1** appeared to represent a minimum-acceptable pharmacophore, we resorted to structure-based drug design to enhance potency.



With the work of Hof et al.[4a] as a backdrop, we prepared an X-ray diffractable crystal of **1**·Cat G by the hanging-drop method and determined the molecular structure.[7] The tetragonal crystals, space group *P*41 ($a = b = 59.44$ Å, $c = 130.62$ Å), diffracted X-rays to 3.0 Å resolution. Refinement of 7065 reflections (of 9299 independent reflections; *R*-merge = 9.8%) gave an *R*-factor of 0.24 using 15 to 3.5 Å data. The positions of the two ligand/protein complexes in the asymmetric unit were established. The two complexes are essentially the same when superimposed except for the Ile-35/Arg-41 loop in the S1′ region, which has two distinctly different conformations. The two ligands had a displacement of RMSD = 1.79 Å, while the α-carbons of the proteins (minus the 35−41 loop) had RMSD = 0.08 Å. One of the complexes is shown in Figure 1.

***Table 1.*** Effect of Carboxamide Substituents on Bioactivity

| cmpd | R in **3** | $IC_{50}$ ± SEM, μM (*N*) |
|---|---|---|
| **1** | | 4.1 ± 0.3 (6) |
| **4** | -$CH_2$Ph | 1.0 ± 0.2 (11) |
| **5** | -$CH_2C(O)NHCH_2CH_2Ph$ | 1.3 ± 0.2 (9) |
| **6** | -$CH_2C(O)NHCH_2CHPh_2$ | 0.50 ± 0.10 (12) |
| **7** |  | 0.053 ± 0.012 (10) |
| **8** | -$(CH_2)_3NHC(O)Ph$ | 0.80 ± 0.20 (5) |

In the complex, the active site of Cat G is occupied by the enantiomer of **1** possessing the *R* absolute configuration. The 2-naphthyl group resides in the hydrophobic S1 specificity pocket and the 1-naphthyl group resides in the S2 region (Figure 2). Significantly, the distal benzene ring of the 1-naphthyl appears to be involved in a π-stacking interaction with the imidazole ring of the catalytic His-57, with the nearly parallel planes having a closest approach of 3.6 Å. The phosphonic acid is strategically deployed, with one oxygen atom H-bonded to Nε of His-57,[8] another partially inserted into the "oxyanion hole", H-bonded to Nα of Gly-193, and a third H-bonded to the side chain Nε of Lys-192 (Figure 3). The unenolized ketone of **1** is also H-bonded to Nε of Lys-192. Notably, this structure reveals a vacant S3 region in the active-site cleft, which offers an opportunity for occupation by a suitable appendage to enhance the potency (Figures 2 and 3). The hydrophobic surfaces provided by the side chains of Ile-99, Phe-172, and Tyr-215 are attractive for added interactions. By employing computer-assisted molecular modeling, we tested different ideas for substituting **1** with probes of the S3 subsite of Cat G. Substituents on the 1-naphthyl ring were perceived to have difficulty in reaching the S3 pocket because of impeded access by Ile-99. Our analysis indicated that attachment would be preferred on the 2-naphthyl ring at the 3-position. Thus, we designed versions of **1** with a carboxamide group located on the 2-naphthyl ring to anchor a substituent that could nestle comfortably in the S3 region with **2** and 2,3-naphthalic anhydride serving as building blocks.[9] Since our modeling gave priority to structures containing an aromatic group tethered by 6−8 Å to the 3-carboxyl, we incorporated arene-bearing amines of varying chain lengths to obtain ligands of general type **3**,[9] namely **4**−**8**, which were assayed for inhibition of Cat G (Table 1; *N* = number of experiments).[6a]

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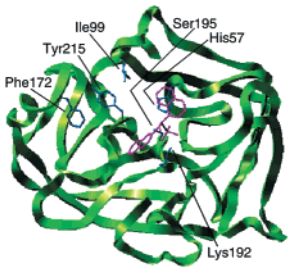


***Figure 1.*** Structure of **1** (magenta) and Cat G (green ribbon), showing key side chains (blue).

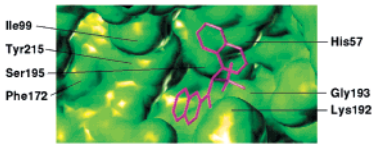


***Figure 2.*** View of **1** (magenta) within the active site of Cat G (green electron-density surface); the vacant S3 pocket is on the left side.

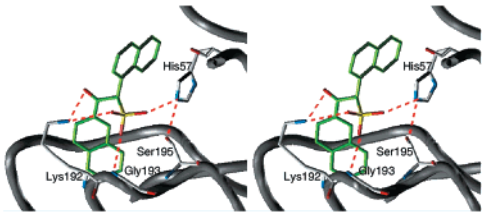


***Figure 3.*** Stereoview of the interactions of with Cat G. Hydrogen bonds are indicated by the broken red lines.

Compounds **4** and **5** exhibit a 3-fold potency increase over **1**, while **6** exhibits an 8-fold increase, indicating that added hydrophobicity can enhance affinity. There was a notable 80-fold improvement over **1** with the aromatic moiety attached via a cyclic tether, as in **7**. Presumably, the conformational constraint imparted by the piperidine ring favorably orients the phenyl portion of the ligand within Cat G's cleft. The 15-fold weaker potency of acyclic variant **8**, compared with **7**, supports this view. For additional insight, we carried out a simulated annealing experiment in which **7** was docked into the active site of Cat G (Figure 4).[10] The interactions in the S1, S2, and catalytic regions of the **7**·Cat G model are analogous to those observed for the X-ray crystal structure of **1**·Cat G (Figure 1). However, the piperidine ring (chair form) serves as a scaffold to position the phenyl ring of **7** within the S3 pocket, such that it makes hydrophobic contacts with the side chains of Phe-172, Tyr-215, and Ile-99. A more detailed picture of the interaction between **7** and Cat G awaits an X-ray structure determination of the complex.

Compound **7** shows reversible, competitive inhibition with $IC_{50}$ and $K_i$ values of 53 ± 12 ($N$ = 10) and 63 ± 14 nM ($N$ = 5), respectively. Another attribute of **7** relates to its selectivity vs other serine proteases. It weakly inhibits chymotrypsin ($K_i$ = 1.5 ± 0.2 $\mu$M), and poorly inhibits (<50% inhibition at 100 $\mu$M) thrombin, factor Xa, factor IXa, plasmin, trypsin, tryptase, proteinase 3, and human leukocyte elastase.

Serine protease inhibitors with phosphonate or phosphinate groups that can occupy the active site in the vicinity of the key catalytic machinery, that is, Ser-195 and His-57, are known.[5a,11] These have generally been P-ester forms, especially diphenyl phosphonates, which operate by slow-tight or irreversible binding.[5a,11] The formation of a covalent bond with O$\gamma$ of Ser-195 to yield a phosphonylated enzyme species has been confirmed by three different X-ray studies.[5a,11e,h] In the reports that describe serine protease inhibitors with a free phosphonic or phosphinic acid end-group, all of the compounds have an acylamino group on the carbon $\alpha$ to phosphorus.[11a−c,12] The known phosphonate/phosphinate ester inhibitors also usually possess this structural element. Consequently, we have identified a new inhibitor motif involving a ketone on the carbon $\beta$ to phosphorus. Such a $\beta$-ketophosphonic acid core structure has the potential for wider applicability. One can imagine designing potent inhibitors for other types of serine proteases by incorporating structural features that achieve favorable interactions within the S1−S3 subsites.[13]

**Acknowledgment.** We thank Stephen Yabut for technical contributions and Dr. Michael Costanzo for discussions and advice.

**Supporting Information Available:** Experimental details and compound characterization, Figure 4, schematic of the interactions of **1** with Cat G, stereoview of Figure 1, diagrams of the electron density of **1** in Cat G (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) (a) Details of the Cat G assay and $IC_{50}$ determinations are presented in the Supporting Information. (b) We screened our proprietary chemical library of ca. 250 000 compounds.

(7) Details of the X-ray work will be published separately.

(8) The O$\gamma$ of Ser-195 is proximal to this oxygen atom (3.3 Å) and may be involved in a hydrogen bond.

(9) The adduct from acylation of the lithio anion of **2** with anhydride was reacted with dicyclohexylcarbodiimide and RMeNH, followed by TMS−Br/pyridine, then 1 N HCl. Synthetic details are contained in the Supporting Information. Use of a primary amine in the DCC coupling gave an undesired phthalimide product and little of the desired NH amide.

(10) See Supporting Information for Figure 4 and for details of the simulated annealing studies, performed with AMBER (version 5.0).

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(13) Note Added in Proof: The X-ray coordinates for **1** Cat G were deposited in the PDB under the code number 1KYN.

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