

## INHIBITION OF THERMOLYSIN WITH NITRONE-BEARING SUBSTRATE ANALOGS: A NEW TYPE OF THERMOLYSIN INHIBITORS

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**Abstract:** Nitrones are utilized as the active site zinc coordinating functionality in the design of inhibitors for thermolysin. This new type of thermolysin inhibitors are as potent as the existing inhibitors bearing a carboxylate or hydroxamate zinc ligating moiety. © 1998 Elsevier Science Ltd. All rights reserved.

Thermolysin (EC 3.4.24.4) is an extracellular thermostable zinc-containing endopeptidase isolated from *Bacillus thermoproteolyticus*.<sup>1</sup> Along with the mammalian digestive enzyme carboxypeptidase A, thermolysin is one of prototypical zinc-containing proteases, and has played an important role as a model enzyme in the development of inhibitor design strategies that can be translated to zinc proteases of physiological importance.<sup>2</sup>

Most of thermolysin inhibitors are characterized as being analogs of substrate having a moiety which is capable of coordinating to the active site zinc ion. Carboxylate, aldehyde and ketone, sulfhydryl, hydroxamate, and phosphorus oxy acid of various oxidation states have served as the zinc ligand in the design of inhibitors for thermolysin.<sup>3</sup> In this communication we wish to report a new type of thermolysin inhibitors which are designed by incorporating a nitron moiety into the substrate structural frame as the zinc coordinating functionality.

Nitrones (azomethine *N*-oxides) are highly valuable synthetic building blocks for the synthesis of various

types of nitrogen heterocycles,<sup>4</sup> and used as excellent 1,3-dipoles for cycloaddition reactions.<sup>5</sup> Reportedly, Lewis acid such as  $\text{Mg}^{2+}$  and  $\text{Zn}^{2+}$  brings about significant improvements of the rate as well as the regio- and stereo-specificity in the 1,3-dipolar cycloadditions, suggesting the negatively polarized oxygen atom of the nitron to form a coordinative bond to the Lewis acid.<sup>6</sup> We have exploited this unique property of the nitron to design a novel type of competitive reversible inhibitors for thermolysin. As illustrated in Figure 1, the nitron moiety in the inhibitors is expected to ligate to the active site zinc ion.

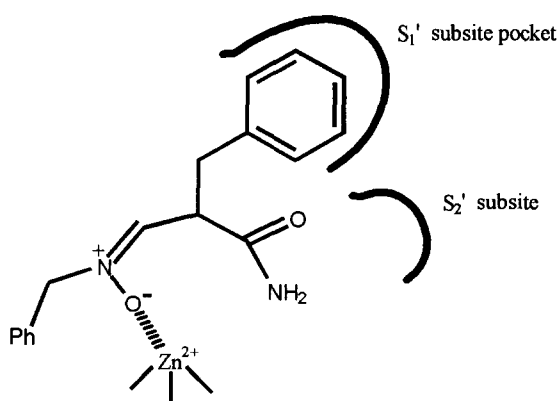


Figure 1. Postulated binding mode of a nitron bearing inhibitor to the active site of thermolysin.

We have synthesized six representative potential inhibitors<sup>7</sup> by oxidation of the corresponding secondary amines with hydrogen peroxide in the presence of a catalytic amount of sodium tungstate.<sup>8</sup> In the case of *N*-benzyl- $\beta$ -phenylalanine methyl ester, there was obtained a mixture of **3** and **4** (Table 1) in a nearly equal ratio, which was separated by column chromatography. Compound **6** was prepared by condensing *N*- $\beta$ -phenylalanidenebenzylamine *N*-oxide with methyl glycinate by a standard method and subsequent treatment of the product with dilute NaOH solution. Aldonitrones such as those synthesized in this study are known to exist in the thermodynamically more stable *Z*-form.<sup>9</sup>

Inhibitory activities of the synthesized nitron derivatives for thermolysin were estimated at pH 7.2 (Tris buffer) by the literature method<sup>10</sup> using 2-furylacryloyl-Gly-Leu-NH<sub>2</sub> as substrate, and are summarized in Table 1. No significant thermolysin inhibitory activity was observed with the amine precursor of **5** at concentration up to 3.3 mM, suggesting strongly that the enzyme inhibitory activity of these nitron derivatives arises from the zinc coordinating propensity of the nitron moiety. The most potent inhibitor in this study is shown to be **5** having the  $K_i$  value of 40  $\mu\text{M}$ . In general, amides are more potent than esters,



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7. **1**: mp 174 - 175 °C; IR (thin film) 1735, 1570 (C=N)  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.55 (dd,  $J$  = 14.2, 4.4 Hz, 1H), 3.66 (dd,  $J$  = 14.2, 10.3 Hz, 1H), 3.78 (s, 3H), 4.64 (dd,  $J$  = 10.3, 4.4 Hz), 7.06 (s, 1H,  $-\text{CH}=\text{N}$ ), 7.17 - 7.38 (m, 8H), 8.11 (m, 2H, ArH ortho to  $\text{CH}=\text{N}^+-\text{O}^-$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  35.5, 53.6, 79.8, 127 - 137, 167; Anal. Calcd for  $\text{C}_{17}\text{H}_{17}\text{NO}_3$ : C, 72.07; H, 6.05; N, 4.94. Found: C, 71.72; H, 6.25; N, 5.14. **2**: mp 190 - 192 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.48 - 3.12 (m, 2H), 4.61 (m, 1H,  $\alpha$ -H), 7.08 - 7.53 (m, 10H), 7.76 (d,  $J$  = 7.1 Hz, 2H), 8.45 (d,  $J$  = 8.5 Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  38.1, 55.6, 127.0 - 138.4, 174.2. **3**: IR (neat) 1720, 1570, 1555  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  2.94 (m, 1H), 3.10 (m, 1H), 3.67 (s, 3H), 3.72 (m, 1H), 4.0 (dd,  $J$  = 12.0, 4.8 Hz, 1H), 4.25 (dd,  $J$  = 12.0, 8.7 Hz, 1H), 7.19 - 7.49 (m, 9H), 8.20 - 8.23 (m, 2H, ArH ortho to  $\text{CH}=\text{N}^+-\text{O}^-$ );  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  36.2, 44.8, 52.5, 66.9, 127 - 137.0, 174.0. **4**: IR (neat) 1740, 1590, 1570  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.11 (m, 1H), 3.21 (m, 1H), 3.68 (s, 3H), 4.21 (q,  $J$  = 6.9 Hz, 1H), 4.88 (s, 2H), 6.79 (d,  $J$  = 6.6 Hz, 1H,  $-\text{CH}=\text{N}$ ), 7.04 - 7.07 (m, 2H), 7.19 - 7.41 (m, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  35.1, 44.9, 52.6, 69.9, 126.3 - 137.8, 171.7. **5**: mp 150 - 152 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.08 - 2.94 (m, 2H), 3.4 (m, 1H), 4.31 (m, 2H), 7.16 - 7.35 (m, 10H), 9.54 (t,  $J$  = 6.3 Hz, 1H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  36.7, 47.7, 53.3, 123.0 - 141.5, 177.5. **6**: mp 186 - 188 °C;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  2.96 - 3.15 (m, 2H), 3.49 (dd,  $J$  = 8.3, 6.2 Hz, 1H), 3.76 (d,  $J$  = 5.7 Hz, 2H), 4.13 (dd,  $J$  = 15.3, 5.4 Hz, 1H), 4.30 (dd,  $J$  = 15.3, 5.4 Hz, 1H), 7.0 - 7.26 (m, 10H), 8.12 (t,  $J$  = 5.7 Hz, 1H), 8.32 (t,  $J$  = 6.1 Hz, 1H), 12.8 (br, 1H,  $\text{CO}_2\text{H}$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  36.1, 41.8, 43.0, 55.4, 124.5 - 128.7, 139.8, 170.0, 172.0; Anal. Calcd for  $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_4$ : C, 67.05; H, 5.92; N, 8.23. Found: C, 67.62; H, 5.89; N, 8.49.
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