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THE SULFONIMIDAMIDE AS A NOVEL TRANSITION STATE ANALOG FOR ASPARTIC ACID AND METALLO PROTEASES

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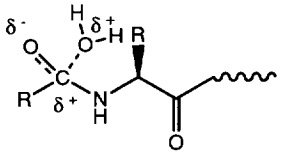
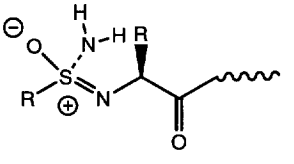
Abstract: We have developed a novel strategy for the preparation of tetrahedral transition state analogs for aspartic acid and metallo-proteases based upon the sulfonimidamide functional group. Our best α-des-amino dipeptide analog binds at least 100-fold tighter than the corresponding ground state structure (i.e., amide). A previously unpublished five-membered cyclic sulfonimidamide was also synthesized.

The mechanism of aspartic acid and metallo-proteases suggests a tetrahedral transition state in which an activated water molecule attacks the carbonyl carbon at the scissile amide bond. The overall tetrahedral geometry and electronics of the sulfonimidamide functional group (Figure 1) should mimic this transition state. The amide NH_2 of the sulfonimidamide should closely mimic the geometry and hydrogen bonding capability of the attacking water molecule, and the dative sulfur-oxygen bond should closely mimic the stretch and expected charge of the carbonyl group in the transition state. Stable mimics of the transition state in aspartic acid and metallo-protease catalyzed reactions have proven to be potent inhibitors for these types of enzymes. Most of the tetrahedral transition state analog inhibitors are either secondary alcohols or phosphorus-containing structures.[1–7] The use of sulfur-containing analogs has met with little success owing to the instability of sulfinamides and α-amino sulfonamides.[8–12] However, the sulfonimidamide has not been evaluated as a transition state analog for any protease to the best of our knowledge.

Figure 1. Comparison of the putative transition state structure for aspartic acid or metallo-protease catalyzed peptide bond hydrolysis with a sulfonimidamide-containing transition state analog.



Transition State



Transition State Analog

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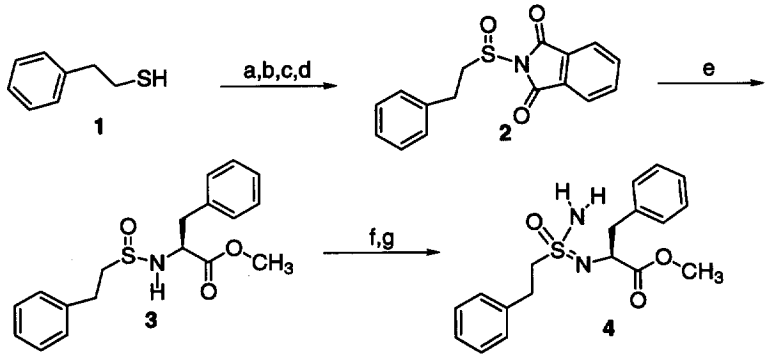
Since the first reported synthesis of a sulfonimidamide nearly 40 years ago,[13] this functional group has received very little attention. The bulk of the work published about the sulfonimidamide has revolved around the properties and reactivity of fully substituted, aryl-sulfonimidamides.[14–18] Our goal was to synthesize an alkyl-sulfonimidamide with an unsubstituted amido nitrogen. After incorporation of this functional group into peptide-like structures their potential as mimics of tetrahedral protease and amidase reaction intermediates could be evaluated.

Chemistry: The synthesis[19] (Scheme 1) began with oxidation of commercially available phenethyl mercaptan (**1**) to the disulfide under basic conditions with H_2O_2.[20] Following a literature procedure, the disulfide was converted to the sulfenyl phthalimide.[21] The sulfenyl phthalimide was recrystallized from the yellow oily residue in 90% yield in ethyl acetate/hexane (1/9). Typical yields for sulfenyl phthalimides range from 81% (*S*-benzyl) to 100% (*S*-*n*-hexyl).[22] Oxidation of the sulfenyl phthalimide with one equivalent of pure *m*-CPBA afforded the sulfinyl transfer reagent **2**[23] in 94% yield. Displacement of phthalimide from **2** by L-phenylalanine methyl ester provided the known compound **3** as a mixture of diastereomers.[24] Isolated yields were usually between 70 and 80%, and the diastereomers were partially separable by flash chromatography. Treatment of **3** with Cl_2 at -78 °C followed by displacement of chloride with ammonia (-78 °C) afforded a clean route to the dipeptide analog **4**.[16] Substitution of glycine benzyl ester or L-leucine methyl ester for phenylalanine methyl ester afforded a racemic mixture of compound **5** or an unresolved diastereomeric mixture of compound **6**, respectively.

Scheme 1



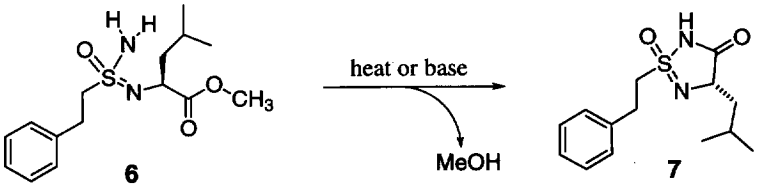
(a) 1 equiv NaOH, 1 equiv H_2O_2, 70% EtOH, 0 °C, 30 min; (b) 1 equiv SO_2Cl_2, $CHCl_3$, 0 °C, 30 min; (c) 1 equiv phthalimide, 1.3 equiv triethylamine, DMF, 0 °C, 30 min; (d) 1 equiv *m*-CPBA, $CHCl_3$, 0 °C, 1 h; (e) 1.5 equiv L-phenylalanine methyl ester, $CHCl_3$, 0 °C, 1 h; (f) Cl_2, minimal ether, -78 °C, 10 min; (g) NH_3, -78 °C, 10 min

In an attempt to resolve the diastereomers of **6** by recrystallization, a novel transformation of **6** into the cyclic sulfonimidamide **7** was observed (Scheme 2). After heating a solution of **6** in ethyl acetate/hexane (1/20) to 60 °C and allowing the solution to cool slowly to room temperature, needle-like crystals formed. ^{1}H NMR of the new crystalline material indicated that the methyl ester singlet of **6** was absent, and a broad singlet appeared at δ 6.09 that integrated to one proton. Mass spectral analysis indicated a mass change from 312 to 280 consistent

with a loss of methanol. The spectral and chemical data of the crystalline solid were consistent with the structure of the cyclic sulfonimidamide **7**.[25] Acyclic sulfonimidamides like **6** test positively with ninhydrin, staining dark purple on TLC plates. Neither the cyclic sulfonimidamide **7** nor any of the corresponding sulfinamides (e.g., **3**) tested positively with ninhydrin.

To confirm the structure and test the stability of **7**, a ring opening back to the acyclic form **6** was attempted under two different sets of conditions. First, **7** was refluxed in 6% HCl in methanol for 6 h. Second, a methanolic solution of **7** was saturated with anhydrous ammonia at 0 °C, and the reaction was allowed to warm to room temperature in a sealed container overnight. In either case **7** was quantitatively recovered indicating that the cyclic sulfonimidamide is quite stable. Similarly, when anhydrous ammonia was introduced into a solution of **6** in methanol at room temperature, the cyclic sulfonimidamide **7** was recovered, not the methyl ester ammonolysis product (C-terminal amide).

Scheme 2



Saponification of **4** was attempted in order to obtain a compound with a free carboxylic acid to test as an inhibitor of carboxypeptidase A. Under these basic conditions, a cyclic sulfonimidamide analogous to **7** was recovered. Hydrolysis of the methyl ester of **4** was achieved under acidic conditions to yield compound **8**.

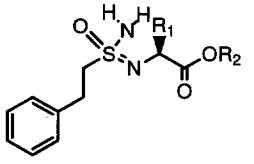
Biological Evaluation: Sulfonimidamide dipeptide analogs were tested together with their ground state (amide) dipeptide counterparts against various proteases by use of an enzyme-coupled spectrophotometric assay.[26] Pepsin and HIV Protease were employed as examples of aspartic acid proteases, while carboxypeptidase A was employed as a representative of the metallo-proteases. Pre-incubation of enzyme with inhibitor under assay conditions for at least 20 min prior to initiating assays by addition of substrate was compared with appropriate controls (no inhibitor). Carboxypeptidase A and the coupling enzymes employed in the assay were not inhibited by the sulfonimidamide dipeptide methyl ester analogs (**4** and **5**). With each enzyme tested, neither the ground state analogs nor the cyclic sulfonimidamides showed any sign of inhibitory activity at concentrations up to 0.3 mM. Table 1 summarizes the results of the inhibition studies.

By use of the enzyme-coupled assay, compound **11** proved to be a substrate for carboxypeptidase A. The rate of hydrolysis was linear with respect to both enzyme and substrate but displayed marked substrate inhibition at concentrations above 4 mM. The apparent second order rate constant for carboxypeptidase-catalyzed hydrolysis of **11,** calculated from the velocities at low substrate concentration (1 mM) and using a molecular weight for carboxypeptidase A of 32,000, is 5 x 10^4 M^{-1} s^{-1}. The second order rate constant for carboxypeptidase-catalyzed hydrolysis of hippuryl-phenylalanine is 1.5 x 10^4 M^{-1} s^{-1}. Thus, the des-amino

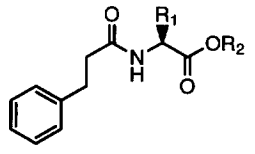
dipeptide amide **11** is actually a better substrate for carboxypeptidase A than the commonly employed substrate hippuryl-phenylalanine.

Summary: Inhibition of representative aspartic acid and metallo-proteases indicates that sulfonimidamide dipeptide analogs bind significantly more tightly than their amide (ground state) counterparts. However, for the most potent inhibitor presented here (**8**), there appears to be little potential for improvement. Since the second order rate constant for a substrate is expected to correlate with inhibitor potency of corresponding transition state mimics,[27] and based on the substrate activities of the ground state analog **11** and hippuryl-phenylalanine, increasing the size of a peptide analog for carboxypeptidase A by extension in the amino-terminal direction would probably not increase inhibitor potency. An IC_{50} of 20 μM is not particularly potent, but it is 100-fold lower than the K_m for the analogous substrate **11**. This indicates that although the sulfonimidamide appears to act as a transition state analog for carboxypeptidase A, it simply does not impart the potency of other types of transition state mimics (e.g., phosphoramidates and phosphonates).

Table 1. Comparison of the inhibition constants between the sulfonimidamide transition state analogs and their ground state (amide) counterparts.



4 R_1 = Bn, R_2 = CH_3
5 R_1 = H, R_2 = Bn
8 R_1 = Bn, R_2 = H



9 R_1 = Bn, R_2 = CH_3
10 R_1 = H, R_2 = Bn
11 R_1 = Bn, R_2 = H

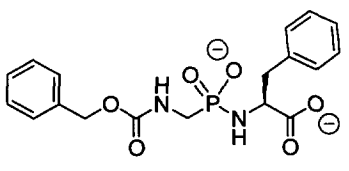
Enzyme	Sulfonimidamide	IC_{50} (mM)[a]	Amide	IC_{50} (mM)[a]
Pepsin	**4**	0.7[b], 0.7[c]	**9**	>3
HIV PR	**4**	1[b], >5[c]	**9**	>3
Pepsin	**5**	0.7	**10**	>3
HIV PR	**5**	>5	**10**	>3
CP-A	**8**	0.02[d]	**11**	2.1[e]

[a]Concentration of inhibitor that produces one-half maximal enzyme activity
[b]first pure eluting diastereomer of **4**
[c]second pure eluting diastereomer of **4**
[d]tested as a mixture of diastereomers
[e]K_m for compound **11**

An explanation for the unexpectedly low potency of this compound could be made by analogy to much more potent phosphonamidate- and phosphonate-containing inhibitors of carboxypeptidase A (Figure 2).[28,29] Although it is possible that the phenethyl group of compound **8** may not be in position for binding in the appropriate carboxypeptidase A subsite, it seems more likely that the uncharged sulfonimidamide may simply not be a sufficiently avid chelator of the active site zinc to impart the kind of potency seen with these structurally similar phosphorus-containing structures.

For proteases that rely more heavily on interactions with the P1 α-amino group and other P-side determinants, increased potency may be achieved by adding this functionality to the current sulfonimidamide-containing dipeptide analogs. We are currently developing methods to add an amino group in the alpha position relative to the sulfonimidamide to enable extension of these dipeptide analogs in the amino terminal direction while still maintaining the peptide backbone.

Figure 2. Potent phosphorus-containing transition state analog inhibitors of carboxypeptidase A. The phosphonamidate **12** has a K_i of 90 nM[28] while the phosphonate **13** has a K_i of 11 fM.[29]



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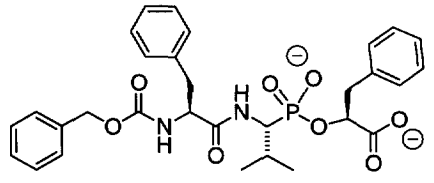

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