ORGANIC LETTERS

XXXX
Vol. XX, No. XX
000–000

Serine Protease Inhibition by a Silanediol Peptidomimetic

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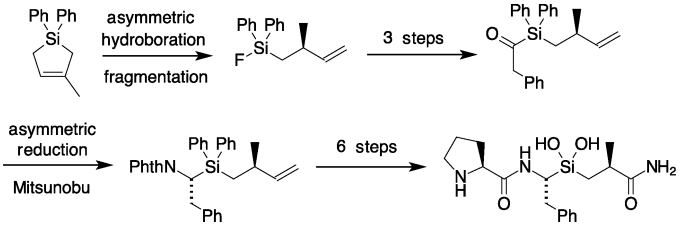
Received July 12, 2012

ABSTRACT



Silanediol peptidomimetics are demonstrated to inhibit a serine protease. Asymmetric synthesis of the inhibitor was accomplished using Brown hydroboration and CBS reduction of an acylsilane intermediate. The silanediol product was found to inhibit the serine protease chymotrypsin with a K_i of 107 nM. Inhibition of the enzyme may involve exchange of a silane hydroxyl with the active site serine nucleophile, contrasting with previous silanediol protease inhibitors.

Design of protease inhibitors is a focus of many pharmaceutical research programs because of the myriad of biological processes that these enzymes mediate.[1] More than 30 protease inhibitors have been approved for clinical use, and many of these have reached the marketplace.[2] Protease inhibitors are used to control hypertension,[3] AIDS, thrombosis,[4] and cancer[5] but have the potential for many additional applications. Each inhibitor is tailored to the protease class, based on the active site mechanism, and employ the recognition elements that flank the active site to give the inhibitors specificity and potency.

Within each protease class, certain functionally relevant replacements for the tetrahedral intermediate have found broad utility.[6] Typical inhibitors of metalloproteases utilize zinc ion coordinating groups.[7] For aspartic proteases, a carbinol is used to hydrogen bond with the active site carboxylic acids.[8] Cysteine protease inhibitors often employ electrophilic functional groups to capture the cysteine nucleophile.[9] Serine proteases are inhibited by electrophilic groups that interact with the active site serine alcohol nucleophile. Useful electrophiles include α-fluoro ketones and 1,2-dicarbonyls.[10] A similar effect is found with the recently introduced anticancer agent bortezomib, which inhibits the closely related threonine protease found in the proteasome. This inhibition is effected by coordination of the active site hydroxyl by a Lewis acidic boronic acid.[11]

(1) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359–1472. Leung, D.; Abbenante, G.; Fairlie, D. P. *J. Med. Chem.* **2000**, *43*, 305–341.

(2) Turk, B. *Nat. Rev. Drug Discovery* **2006**, *5*, 785–799.

(3) Matchar, D. B.; McCrory, D. C.; Orlando, L. A.; Patel, M. R.; Patel, U. D.; Patwardhan, M. B.; Powers, B.; Samsa, G. P.; Gray, R. N. *Ann. Intern. Med.* **2008**, *148*, 16–29.

(4) Mackman, N. *Nature* **2008**, *451*, 914–918.

(5) Kessenbrock, K.; Plaks, V.; Werb, Z. *Cell* **2010**, *141*, 52–67. Page-McCaw, A.; Ewald, A. J.; Werb, Z. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 221–233.

(6) Meanwell, N. A. *J. Med. Chem.* **2011**, *54*, 2529–2591.

(7) Lead references: **Thiols**: Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484–5491. **Carboxylates**: DePriest, S. A.; Mayer, D.; Naylor, C. B.; Marshall, G. R. *J. Am. Chem. Soc.* **1993**, *115*, 5372–5384. **Phosphinates**: Akif, M.; Schwager, S. L.; Anthony, C. S.; Czarny, B.; Beau, F.; Dive, V.; Sturrock, E. D.; Acharya, K. R. *Biochem. J.* **2011**, *436*, 53–59. Collinsova, M.; Jiracek, J. *Curr. Med. Chem.* **2000**, *7*, 629–647. **Hydroxamic acids**: Jacobsen, J. A.; Major Jourden, J. L.; Miller, M. T.; Cohen, S. M. *Biochim. Biophys. Acta, Mol. Cell Res.* **2010**, *1803*, 72–94.

(8) Rich, D. H.; Bernatowicz, M. S.; Agarwal, N. S.; Kawai, M.; Salituro, F. G.; Schmidt, P. G. *Biochemistry* **1985**, *24*, 3165–3173. Dunn, B. M. *Chem. Rev.* **2002**, *102*, 4431–4458. Eder, J.; Hommel, U.; Cumin, F.; Martoglio, B.; Gerhartz, B. *Curr. Pharm. Des.* **2007**, *13*, 271–285.

(9) Powers, J. C.; Asgian, J. L.; Ekici, D.; James, K. E. *Chem. Rev.* **2002**, *102*, 4639–4750.

(10) Zhong, J.; Groutas, W. C. *Curr. Top. Med. Chem.* **2004**, *4*, 1203–1216. Bachovchin, D. A.; Cravatt, B. F. *Nat. Rev. Drug Discovery* **2012**, *11*, 52–68.

(11) Groll, M.; Berkers, C. R.; Ploegh, H. L.; Ovaa, H. *Structure* **2006**, *14*, 451–456.

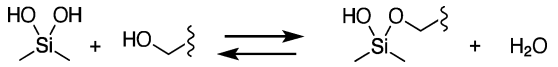
10.1021/ol301933n

Each of these functional groups have steric, electronic, and metabolic consequences that present challenges and opportunities in pharmaceutical design.

Serine proteases are contemporary targets for treatment of Alzheimer's disease, cancer, obesity, diabetes, thrombosis, and more.[12] Novel functionality for serine protease inhibitor design may therefore have broad utility.

Silanols have potential as serine protease inhibitors because a silanol hydroxyl will rapidly exchange with water and with alcohols (Scheme 1).[13] A covalent interaction between the silicon and the enzyme would contrast with previously described silanediol inhibitors that have been inhibitors of aspartic and metallo proteases where water is the nucleophilic group.[14,15]

Scheme 1. Silanols Readily Exchange with Alcohols



Chymotrypsin is an archetype serine protease, characterized by a requirement for an aromatic side chain at P_1 (Figure 1).[16] The use of the phenylalanine isostere **1** by Imperiali and Abeles underscored the importance of this aromatic substituent in chymotrypsin inhibitor design.[17] To evaluate a silanediol as an inhibitor of this enzyme, we elected to utilize **2**, an Ala−[Si]−Phe dipeptide analog. The choice of alanine substitution for P_1' in **2** was based on its effective use as a steric shield for the silanediol to minimize oligomerization.[18] Chymotrypsin is relatively insensitive toward substitution at P_1' in substrates,[19] and methyl substitution was effective in phosphoramide inhibitors developed by Bartlett et al.[20] Termination of **2** with a primary amide ensures that the polarity and hydrogen bonding capabilities of the substrate are retained. Evaluation of **2** as an inhibitor used the commercially available chymotrypsin substrate **3** (vide infra).

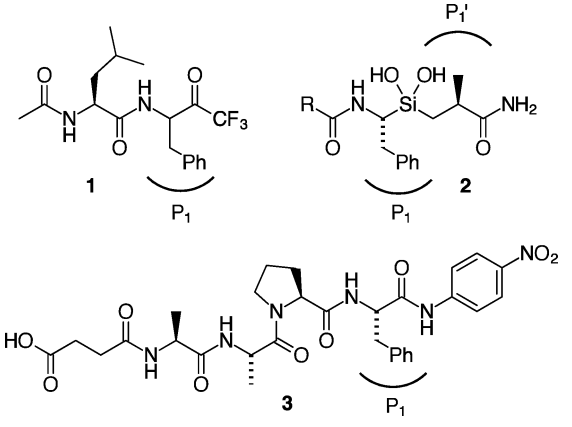
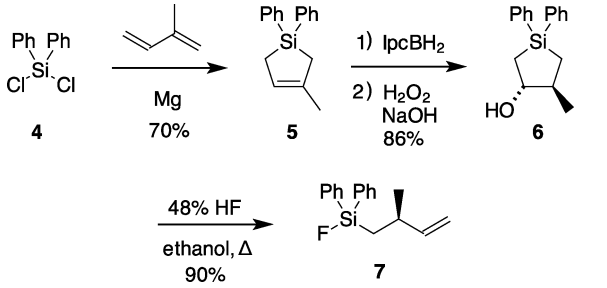


Figure 1. Inhibition of the serine protease chymotrypsin requires an aromatic substitutent at P_1.

The central Ala−[Si]−Phe dipeptide of **2** had been previously prepared as part of a study of inhibitors of an angiotensin-converting enzyme, but this synthesis employed a lengthy sequence.[21] In this original preparation, the P_1' stereogenic center of **2** was derived from the commercially available, enantiomerically pure Roche ester.[22] Subsequent separation of diastereomers led to the correct stereochemistry at P_1. We have recently reported an asymmetric method for constructing the P_1' methyl stereogenic center, and we employed this chemistry to prepare **2** (Scheme 2).[23] Magnesium-mediated cycloaddition of isoprene with dichlorodiphenylsilane can be performed on a >100 g scale.[24] Hydroboration of **5** with monoisopinocampheylborane at -20 °C leads to silacyclopentane **6** in 93% ee. Warming this β-hydroxysilane with a 3:1 mixture of 48% HF in ethanol leads to Peterson fragmentation and formation of fluorosilane **7** in 90% distilled yield.[19]

Scheme 2. Setting the P_1' Methyl Stereochemistry[23]



Construction of the α-aminosilane component utilized a Brook−Corey acylsilane approach (Scheme 3).[25] Addition

(12) Bachovchin, D. A.; Cravatt, B. F. *Nat. Rev. Drug Discovery* **2012**, *11*, 52–68.

(13) Tacke, R.; Linoh, H.; Ernst, L.; Moser, U.; Mutschler, E.; Sarge, S.; Cammenga, H. K.; Lambrecht, G. *Chem. Ber.* **1987**, *120*, 1229–1237.

(14) A silanediol inhibitor of the serine protease elastase was reported: Showell, G. A.; Montana, J. G.; Chadwick, J. A.; Higgs, C.; Hunt, H. J.; MacKenzie, R. E.; Price, S.; Wilkinson, T. J. Silicon diols, effective inhibitors of human leukocyte elastase. In *Organosilicon Chem VI, [Eur Silicon Days]*, 2nd ed.; Auner, N., Weis, J., Eds.; Wiley-VCH Verlag GmbH & Co. KGaA: 2005; pp 569−574. This silanediol has also been prepared by Skrydstrup et al.: Hernández, D.; Lindsay, K. B.; Nielsen, L.; Mittag, T.; Bjerglund, K.; Friis, S.; Mose, R.; Skrydstrup, T. *J. Org. Chem.* **2010**, *75*, 3283–3293.

(15) Sieburth, S. McN.; Chen, C.-A. *Eur. J. Org. Chem.* **2006**, 311–322.

(16) Schecter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

(17) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760–3767.

(18) Mutahi, M. wa; Nittoli, T.; Guo, L.; Sieburth, S. McN. *J. Am. Chem. Soc.* **2002**, *124*, 7363–7375.

(19) Schellenberger, V.; Braune, K.; Hofmann, H.; Jaubke, H. *Eur. J. Biochem.* **1991**, *199*, 623–636.

(20) Bartlett, P. A.; Lamden, L. A. *Bioorg. Chem.* **1986**, *14*, 356–377.

(21) Kim, J.; Hewitt, G.; Carroll, P.; Sieburth, S. McN. *J. Org. Chem.* **2005**, *70*, 5781–5789.

(22) Cohen, N.; Eichel, W. F.; Lopresti, R. J.; Neukom, C.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 3505–3511.

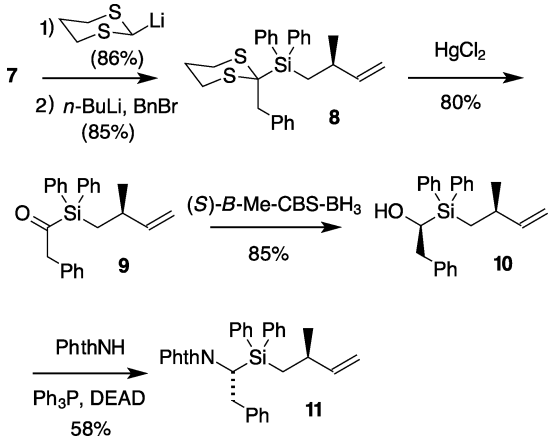
(23) Sen, S.; Purushotham, M.; Qi, Y.; Sieburth, S. McN. *Org. Lett.* **2007**, *9*, 4963–4965.

(24) Manuel, G.; Mazerolles, P.; Lesbre, M.; Pradel, J.-P. *J. Organomet. Chem.* **1973**, *61*, 147–165.

(25) Brook, A. G.; Duff, J. M.; Jones, P. F.; Davis, N. R. *J. Am. Chem. Soc.* **1967**, *89*, 431–434. Corey, E. J.; Seebach, D.; Freedman, R. *J. Am. Chem. Soc.* **1967**, *89*, 434–436.

of 2-lithio-1,3-dithiane to fluorosilane **7**, followed by deprotonation and alkylation with benzyl bromide, gave **8**. Hydrolysis of the dithiane then gave the silyl ketone **9**. Reduction of this ketone using the (*S*)-*B*-Me-CBS reagent gave a clean reduction with a diastereoselectivity of >90% dr (NMR).[26] The resulting alcohol **10** underwent Mitsunobu inversion with phthalimide to produce **11**.

Scheme 3. Asymmetric Construction of the α-Amino Silane

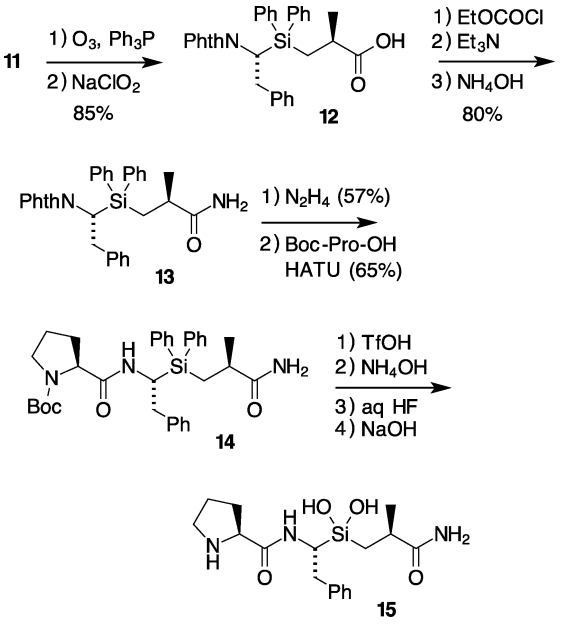


Completion of the inhibitor (Scheme 4) began with ozonolysis of the terminal alkene, followed by Pinnick oxidation of the resulting aldehyde. Acid **12** was then converted to the primary amide through the mixed anhydride using ethyl chloroformate. Hydrolysis of the phthalimide with hydrazine gave the corresponding amine, which was then coupled with Boc-protected proline using HATU to give tripeptide analog **14**.

Hydrolysis of diphenylsilane **14** to silanediol **15** used our protocol developed in earlier work.[27] Treatment of **14** with triflic acid in methylene chloride at 0 °C cleaved the Si–phenyl bonds (as well as the Boc group). This was followed by dilution with ammonium hydroxide. The resulting product was then treated with 48% aqueous HF, to form the difluorosilane. This monomeric product is readily hydrolyzed to the corresponding silanediol under mildly basic aqueous conditions.

Evaluation of **15** as an inhibitor of chymotrypsin employed the method of Bartlett et al. in which hydrolysis of substrate **3** was followed by the increase in UV absorbance at 410 nm.[28,29] Chymotrypsin is inhibited by **15** in a concentration-dependent manner. The results are shown in a Dixon plot (Figure 2). These experiments found the K_i for this inhibition to be 107 nM.

Scheme 4. Completion of the Inhibitor Synthesis



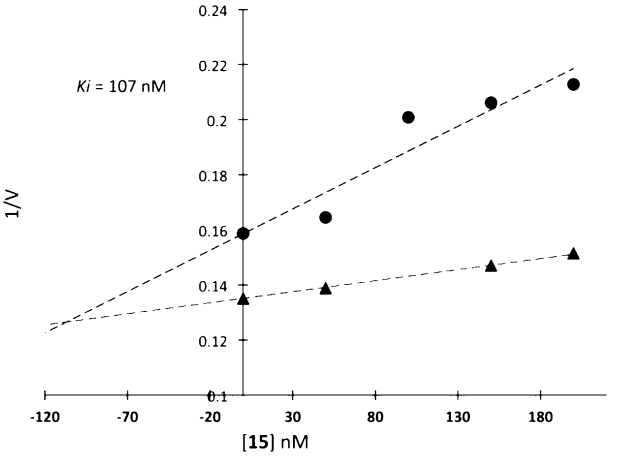


Figure 2. Dixon plot showing inhibition of chymotrypsin by **15**, with concentration of substrate **3** at 50 μM (●) and 45 μM (▲).

Inhibition of chymotrypsin by **15** is consistent with exchange of a silanediol hydroxyl group with the nucleophilic active site serine hydroxyl of the enzyme. It is likely that this hydroxyl exchange occurs through a pentavalent intermediate.[30]

(26) Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986–2012.
(27) Kim, J.; Sieburth, S. McN. *J. Org. Chem.* **2004**, *69*, 3008–3014.
(28) Delmar, E. G.; Largman, C.; Brodrick, J. W.; Geokas, M. C. *Anal. Biochem.* **1979**, *99*, 316–320.
(29) Hansen, K. B.; Grosch, B.; Greiveldinger-Poenaru, S.; Bartlett, P. A. *J. Org. Chem.* **2003**, *68*, 8465–8470.
(30) Lickiss, P. D.; Rappoport, Z.; Apeloig, Y. Polysilanols. *The Chemistry of Organic Silicon Compounds (3)*; John Wiley & Sons: New York, 2001; pp 695–744. Trivalent species have also been postulated: Oostendorp, D. J.; Bertrand, G. L.; Stoffer, J. O. *J. Adhes. Sci. Technol.* **1992**, *6*, 171–191. It is unlikely however that a pentavalent silanol is the inhibitor: Karsch, H. H.; Bienlein, F.; Sladek, A.; Heckel, M.; Burger, K. *J. Am. Chem. Soc.* **1995**, *117*, 5160–5161. Bassindale, A. R.; Parker, D. J.; Taylor, P. G.; Auner, N.; Herrschaft, B. *Chem. Commun.* **2000**, *7*, 565–566.

Synthesis of this inhibitor took advantage of an asymmetric hydroboration and an asymmetric borane reduction to control the two stereogenic centers flanking the silicon. The inhibition of chymotrypsin illustrates the use of silanediols as inhibitors of a third family of proteases, adding to metallo and aspartic examples.[15] Additional studies are in progress.

Acknowledgment. The authors thank Yingmei Qi for preliminary studies and the National Institutes of Health (R01 GM076471) for generous support for this program.

Supporting Information Available. Experimental details and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.