Published in final edited form as:

Org Lett. 2010 February 19; 12(4): 696–699. doi:10.1021/ol9027013.

Convergent synthesis of α -ketoamide inhibitors of Pin1

Guoyan G. Xu and Felicia A. Etzkorn*

Department of Chemistry, Virginia Tech, Blacksburg, Virginia 24061

Abstract

A convergent synthesis of α -ketoamide inhibitors of Pin1 is described. An α -hydroxyorthothioester derivative of Ser was reacted directly with an amine synthon. The reaction was catalyzed by HgO and HgCl₂ to form α -hydroxyamide. Thus, hydrolysis and coupling were combined in one step with 80% yield. Two diastereomers of a phospho-Ser-Pro α -ketoamide analogue were synthesized. The IC₅₀ values of 100 μ M and 200 μ M were surprisingly weak for Pin1 peptidyl-prolyl isomerase.

α -Ketoamides have been widely used in developing inhibitors of peptidases,¹ HDACs^{2,3} and peptidyl prolyl isomerases (PPIases).⁴ One of the best studied immunosuppressant drugs, FK506 has an α -ketoamide, and it is a transition state analogue inhibitor of FK506 binding proteins (FKBPs).⁵ α -Ketoamides have been used to inhibit several classes of proteases, such as serine proteases,^{6–10} cysteine proteases^{11–14} and HIV and FIV proteases.¹⁵

α -Ketoamides have an electron-deficient carbonyl due to the effect of the neighboring electron-withdrawing amide. α -Ketoamide containing inhibitors have been used to elucidate the mechanisms of enzymes, because they serve as transition state analogues to inhibit enzyme catalytic activity. This occurs for serine or cysteine proteases through the formation of a tetrahedral intermediate analogue (gem-diol or hemiketal) with the enzyme upon binding.¹⁶ FK506, a macrolide with an α -ketoamide functional group, has a dihedral angle of 95° to 100° between the two carbonyl groups.⁵ NMR studies of a ¹³C-labeled structure and a crystal structure of FK506 showed that the two carbonyls were orthogonal both in bound and unbound structures, indicating that FK506 acts as a “twisted amide” transition state analogue to inhibit FKBPs.⁵

Pin1, a PPIase enzyme belonging to the parvulin family, specifically recognizes pSer/pThr-Pro containing substrates, and regulates many cellular events such as cell cycle progression, transformation, and cell proliferation.^{17,18} Pin1 is overexpressed in many cancer cell lines, and plays an important role in oncogenesis.^{19,20} Pin1 is a potential diagnostic and therapeutic anti-cancer target.^{21–23} Thus, we are interested in developing potent and specific inhibitors of Pin1.^{24,25}

fetzorn@vt.edu.

Supporting Information Available: Experimental procedures, HPLC and spectroscopic data for compounds 1–10, and inhibition data for compound 1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Since α -ketoamides are an important class of enzyme inhibitors, numerous synthetic methods have been reported, including: cyano ylide coupling,²⁶ transition-metal-catalysis,²⁷ amidation of α -keto acids with amines,^{11,28} oxidation of α -hydroxyamides with Dess-Martin periodinane⁶ or TEMPO,^{29,30} and nucleophilic addition of a Grignard reagent to a Weinreb amide.³¹ A solid-phase method for synthesis of α -ketoamides has been developed.³² In this study, we have developed a convergent route to synthesize α -ketoamides by coupling a complex amine directly to an α -hydroxyorthothioester to form an α -hydroxyamide, which was then oxidized to an α -ketoamide with the Dess-Martin periodinane.³³

We have reviewed Pin1 inhibitors, including synthetic inhibitors, and natural products.^{4,23} α -Ketoamides are an important class of inhibitor for PPIases (e.g. FK506); the co-crystal structure of FK506 with FKBP was the main evidence cited to support the “twisted amide” transition state for PPIases.^{5,34} But α -ketoamides have not yet been reported as Pin1 inhibitors. Our impetus for synthesizing these challenging phosphorylated α -ketoamides was to investigate the ability of these compounds to inhibit Pin1, the only PPIase that regulates the cell cycle.

α -Ketoamides **1a** and **1b** were designed based on the specificity of Pin1 for the pSer/pThr–Pro motif.¹⁸ An acetyl was chosen for the *N*-terminus, since co-crystal structures of Pin1-inhibitor complexes showed no electron density for *N*-terminal residues, and to improve water solubility over Fmoc derivatives.^{25,35,36} An aromatic naphthyl was introduced at the *C*-terminus based on substrate and inhibitor sequence specificities.^{18,36,37}

Boc-Pro-OH and 2-(2-naphthyl)-ethylamine (NEA) were coupled with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide-HCl (EDC) to give Boc-Pro-NEA **2** (Scheme 1). Free amine **3** was prepared by TFA deprotection of **2**, followed by neutralization with aqueous NaHCO₃.

Aldehyde **4** was obtained by LiAlH₄ reduction of the Weinreb amide of protected Ser (Scheme 2).³⁸ Lithio tris-methylthiomethane was added to aldehyde **4** to give orthothioester **5**.³⁹ The two diastereomers of orthothioester **5** were not separated at this stage, since the newly formed stereocenter would be eliminated in the subsequent oxidation of alcohol **7** to ketone **8**. Orthothioester **5** was converted directly to α -hydroxyamide **6** by aminolysis.

The Boc group of **6** was removed with TFA, and the resulting amine was selectively acetylated with one equivalent of acetic anhydride in the presence of the free hydroxyl to give **7** (Scheme 2). Alcohol **7** was oxidized with the Dess-Martin reagent to form α -ketoamide **8**.^{6,33} Although the Dess-Martin oxidation is known to produce the least epimerization α to electrophilic ketones,^{6,40,41} epimerization occurred either during oxidation or chromatography, but the diastereomers (1:1) were not separated at this stage. This epimerization occurs easily due to the strongly electron-withdrawing ketoamide group, and has been observed in several syntheses of α -ketoamides.^{15,29} Epimerization of α -ketoesters has been observed to occur in solution upon standing for one week.⁴¹ Both phospho-L-Thr and phospho-D-Thr containing inhibitors of Pin1 have been reported, and the D-Thr inhibitors were more potent.^{36,37} We

wanted to test both diastereomers of **1** for inhibition, so we did not attempt to improve the stereochemical purity of α -ketoamide **8**.

The benzyl on the side chain of serine was removed with BCl_3 to give alcohol **9**.^{42,43} Reverse-phase, semi-preparative HPLC purification gave a mixture of diastereomers of **9** in a 1:1 ratio as determined by ^1H NMR (Supporting Information). The 86 ppm ^{13}C NMR peak was assigned as the hydrated ketone carbon based on the literature, and the assignment below for **10a**.¹ The ketone carbonyl stretch did not appear in the IR at ca. 1720 cm^{-1} , which also indicates hydration of the ketone.

Alcohol **9** was phosphorylated with dibenzyl *N,N*-diethylphosphoramidite, followed by oxidation with *t*-BuOOH to produce dibenzylphosphate **10**.^{24,44} The diastereomers of **10** were isolated by reverse-phase semi-preparative HPLC. The ketone carbonyl for both diastereomers **10a** and **10b** appeared in the IR, but not in the ^{13}C NMR. The 84 ppm peak in the ^{13}C , and the 4.74 ppm peak in the ^1H NMR were assigned by HMBC as the hydrated ketone (Supporting Information). The ketone carbonyl stretch appeared in the IR at 1723 cm^{-1} , but much weaker than expected, indicating partial hydration.

Hydrogenolysis of each diastereomer of **10** under neutral conditions furnished two diastereomers of the final compound, **1a** and **1b**. Compounds **1a** and **1b** showed no ketone carbonyl in both the ^{13}C and IR spectra. Since we found that both diastereomers were weak Pin1 inhibitors, *vide infra*, we chose not to determine the absolute stereochemistry of the inhibitors.

In our method, orthothioester **5** was directly reacted with the fully formed right half of the molecule, the amine H-Pro-NEA **3**, as the nucleophile to produce amide **6** (Scheme 2). Orthothioester **5** was used as a carboxylic acid synthon instead of a nitrile to avoid the toxicity of NaCN or KCN. In addition, conditions for the hydrolysis of a nitrile are incompatible with amides.²⁹

This synthesis of the target compound was convergent compared with previous linear syntheses of α -ketoamides via α -hydroxy acids. Previously, orthothioesters were either hydrolyzed to a carboxylic acid and coupled with an amine, or methanolized to a methyl ester.^{39,45,46} Methyl esters require the additional step of hydrolysis before coupling, while α -hydroxy acids are difficult to isolate and purify. Instead, the H-Pro-NEA amine was coupled in one step to the α -hydroxyorthothioester. We attempted ozonolysis of a cyano ylide to form the α -ketoamide functionality using Wasserman's method.⁹ However, due to the instability of the α -ketoamide in subsequent Pro ester hydrolysis and coupling to NEA, we chose to use aminolysis of thioester **5** with amine **3** to form the complete skeleton prior to oxidation to the ketoamide.

The aminolysis was catalyzed by HgO and HgCl_2 , similar to hydrolysis of orthothioesters.^{39,45} Thus, the α -hydroxyamide **6** was formed without additional coupling reagents or steps. We propose that the key step in the reaction involves Hg(II)-catalyzed hydrolysis of the orthothioester to a methyl thioester intermediate **11** (Scheme 3).^{45,47} The THF used as the solvent was wet, which provided the water for the hydrolysis. Aminolysis with the amine nucleophile present in situ then gave amide **6** directly. Thus, hydrolysis and coupling were combined in a single step with 80% yield. Although the overall yield in this 12-step synthesis is not high (6% for the 9 linear steps), the aminolysis to form the key intermediate for α -ketoamides should be very useful in a variety of syntheses.

The α -chymotrypsin protease-coupled assay, with succinyl-Ala-Glu-*cis*-Pro-Phe-*p*-nitroanilide (*p*NA) as the substrate, was used to measure inhibition of Pin1 by the two diastereomers of **1**.²⁴ The absorbance of *p*NA released from the PPIase trans product by

chymotrypsin was recorded continuously at 390 nm by UV/Vis. The IC₅₀ values of the two diastereomers were determined to be 100 ± 20 μM and 200 ± 20 μM.

Surprisingly, neither diastereomer was a potent inhibitor of Pin1. These inhibitors were less potent than a similarly substituted, ground-state analogue, Fmoc-pSer-Ψ[(Z)CH=C]-Pro-(2)-N-(3)-ethylaminoindole (IC₅₀ = 28 μM).²⁵ Since the α-ketoamide of FK506 acts as a transition state analogue to inhibit FKBP, we expected more potent inhibition of Pin1 by at least one of the α-ketoamide stereoisomers. There are two proposed mechanisms for Pin1 catalysis: the twisted-amide mechanism,^{5,48,49} and the Cys-113 tetrahedral intermediate mechanism.⁵⁰ The hemiketal of an α-ketoamide has been shown to act as a tetrahedral transition-state analogue for a serine protease.¹⁶ We anticipated that the ketoamide could act as a potent electrophile if the Cys-113 thiol of Pin1 were positioned for nucleophilic addition. The twisted amide mechanism is not supported by this poor inhibition either. Although a hydrated ketone would preclude either Cys-113 nucleophilic addition, or the orthogonal carbonyl conformation necessary to mimic the twisted amide transition state, as in FK506, the hydration is reversible. The ketone state should be accessible for binding to the enzyme if it were a favorable analogue of the transition state.

Although the two diastereomers of ketoamides were weak inhibitors of Pin1, we have provided a convergent method for synthesizing α-ketoamide peptidomimetics, which is a very useful class of enzyme inhibitors, especially for proteases. Many α-ketoamides have been developed as drugs, and our convergent method contributes to the available methods for their synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the NIH for Grant No. R01 CA110940 for financial support.

References

1. Ocain TD, Rich DH. *J Med Chem* 1992;35:451–456. [PubMed: 1738140]
2. Curtin M, Glaser K. *Curr Med Chem* 2003;10:2373–2392. [PubMed: 14529480]
3. Wada CK, Frey RR, Ji Z, Curtin ML, Garland RB, Holms JH, Li J, Pease LJ, Guo J, Glaser KB, Marcotte PA, Richardson PL, Murphy SS, Bouska JJ, Tapang P, Magoc TJ, Albert DH, Davidsen SK, Michaelides MR. *Bioorg Med Chem Lett* 2003;13:3331–3335. [PubMed: 12951120]
4. Wang XJ, Etzkorn FA. *Biopolymers* 2006;84:125–146. [PubMed: 16302169]
5. Rosen MK, Standaert RF, Galat A, Nakatsuka M, Schreiber SL. *Science* 1990;248:863–866. [PubMed: 1693013]
6. Wipf P, Kim H. *J Org Chem* 1993;58:5592–5594.
7. Maryanoff BE, Zhang HC, Greco MN, Glover KA, Kauffman JA, Andrade-Gordon P. *Bioorg Med Chem* 1995;3:1025–1038. [PubMed: 7582977]
8. Ogilvie W, Bailey M, Poupart MA, Abraham A, Bhavsar A, Bonneau P, Bordeleau J, Bousquet Y, Chabot C, Duceppe JS, Fazal G, Goulet S, Grand-Maitre C, Guse I, Halmos T, Lavallée P, Leach M, Malenfant E, O'Meara J, Plante R, Plouffe C, Poirier M, Soucy F, Yoakim C, Déziel R. *J Med Chem* 1997;40:4113–4135. [PubMed: 9406601]
9. Wasserman HH, Petersen AK. *J Org Chem* 1997;62:8972–8973.
10. Cacciola J, Fevig JM, Stouten PF, Alexander RS, Knabb RM, Wexler RR. *Bioorg Med Chem Lett* 2000;10:1253–1256. [PubMed: 10866393]
11. Li Z, Ortega-Vilain AC, Patil GS, Chu DL, Foreman JE, Eveleth DD, Powers JC. *J Med Chem* 1996;39:4089–4098. [PubMed: 8831774]

12. Choe Y, Brinen LS, Price MS, Engel JC, Lange M, Grisostomi C, Weston SG, Pallai PV, Cheng H, Hardy LW, Hartsough DS, McMakin M, Tilton RF, Baldino CM, Craik CS. *Bioorg Med Chem* 2005;13:2141–2156. [PubMed: 15727867]
13. Shirasaki Y, Miyashita H, Yamaguchi M. *Bioorg Med Chem* 2006;14:5691–5698. [PubMed: 16651001]
14. Barrett DG, Catalano JG, Deaton DN, Hassell AM, Long ST, Miller AB, Miller LR, Ray JA, Samano V, Shewchuk LM, Wells-Knecht KJ, Willard DH Jr, Wright LL. *Bioorg Med Chem Lett* 2006;16:1735–1739. [PubMed: 16376075]
15. Slee DH, Laslo KL, Elder JH, Ollmann IR, Gustchina A, Kervinen J, Zdanov A, Wlodawer A, Wong CH. *J Am Chem Soc* 1995;117:11867–11878.
16. Maryanoff BE, Qiu X, Padmanabhan KP, Tulinsky A, Almond HR Jr, Andrade-Gordon P, Greco MN, Kauffman JA, Nicolaou KC, Liu A, et al. *Proc Natl Acad Sci U S A* 1993;90:8048–8052. [PubMed: 8367461]
17. Lu KP, Hanes SD, Hunter T. *Nature* 1996;380:544–547. [PubMed: 8606777]
18. Yaffe MB, Schutkowski M, Shen M, Zhou XZ, Stukenberg PT, Rahfeld JU, Xu J, Kuang J, Kirschner MW, Fischer G, Cantley LC, Lu KP. *Science* 1997;278:1957–1960. [PubMed: 9395400]
19. Bao L, Kimzey A, Sauter G, Sowadski JM, Lu KP, Wang DG. *Am J Pathol* 2004;164:1727–1737. [PubMed: 15111319]
20. Ryo A, Liou YC, Lu KP, Wulf G. *J Cell Sci* 2003;116:773–783. [PubMed: 12571275]
21. Finn G, Lu KP. *Curr Cancer Drug Targets* 2008;8:223–229. [PubMed: 18473735]
22. Lu KP. *Cancer Cell* 2003;4:175–180. [PubMed: 14522251]
23. Xu GG, Etzkorn FA. *Drug News Perspect* 2009;22:399–407. [PubMed: 19890497]
24. Wang XJ, Xu B, Mullins AB, Neiler FK, Etzkorn FA. *J Am Chem Soc* 2004;126:15533–15542. [PubMed: 15563182]
25. Zhao S, Etzkorn FA. *Bioorg Med Chem Lett* 2007;17:6615–6618. [PubMed: 17935986]
26. Wasserman HH, Ho WB. *J Org Chem* 1994;59:4364–4366.
27. Ozawa F, Soyama H, Yanagihara H, Aoyama I, Takino H, Izawa K, Yamamoto T, Yamamoto A. *J Am Chem Soc* 1985;107:3235–3245.
28. Chiou A, Verger R, Kokotos G. *Lipids* 2001;36:535–542. [PubMed: 11432468]
29. Harbeson SL, Abelleira SM, Akiyama A, Barrett R 3rd, Carroll RM, Straub JA, Tkacz JN, Wu C, Musso GF. *J Med Chem* 1994;37:2918–2929. [PubMed: 8071940]
30. Han W, Jiang X, Hu Z, Wasserman ZR, Decicco CP. *Bioorg Med Chem Lett* 2005;15:3487–3490. [PubMed: 15982872]
31. Sibi MP, Marvin M, Sharma R. *J Org Chem* 1995;60:5016–5023.
32. Papanikos A, Meldal M. *J Comb Chem* 2004;6:181–195. [PubMed: 15002966]
33. Dess DB, Martin JC. *J Org Chem* 1983;48:4155–4156.
34. Fischer S, Michnick S, Karplus M. *Biochemistry* 1993;32:13830–13837. [PubMed: 7505615]
35. Etzkorn, FA.; Noel, JP.; Zhang, Y.; Wang, XJ. *Understanding Biology Using Peptides*. In: Blondelle, SE., editor. *Proc. 19th Am. Pept. Symp.*; San Diego: Springer, Inc; 2006. p. 759-762.
36. Zhang Y, Daum S, Wildemann D, Zhou XZ, Verdecia MA, Bowman ME, Lucke C, Hunter T, Lu KP, Fischer G, Noel JP. *ACS Chem Biol* 2007;2:320–328. [PubMed: 17518432]
37. Wildemann D, Erdmann F, Alvarez BH, Stoller G, Zhou XZ, Fanghanel J, Schutkowski M, Lu KP, Fischer G. *J Med Chem* 2006;49:2147–2150. [PubMed: 16570909]
38. Wang XJ, Hart SA, Xu B, Mason MD, Goodell JR, Etzkorn FA. *J Org Chem* 2003;68:2343–2349. [PubMed: 12636401]
39. Hu Z, Han W. *Tetrahedron Lett* 2008;901–902.
40. Burkhart JP, Peet NP, Bey P. *Tetrahedron Lett* 1988;29:3433–3436.
41. Patel DV, Rielly-Gauvin K, Ryono DE, Free CA, Smith SA, Petrillo EW Jr. *J Med Chem* 1993;36:2431–2447. [PubMed: 8355245]
42. Williams DR, Brown DL, Benbow JW. *J Am Chem Soc* 1989;111:1923–1925.
43. Xie J, Menand M, Valery JM. *Carbohydrate Research* 2005;340:481–487. [PubMed: 15680604]

44. de Bont HB, Liskamp RM, O'Brian CA, Erkelens C, Veeneman GH, van Boom JH. *Int J Pept Protein Res* 1989;33:115–123. [PubMed: 2707968]
45. Mamane V, Aubert E, Fort Y. *J Org Chem* 2007;72:7294–7300. [PubMed: 17696549]
46. Braukmüller S, Brückner R. *Eur J Org Chem* 2006:2110–2118.
47. Orito K, Seki Y, Suginome H, Iwadare T. *Bull Chem Soc Jpn* 1989;62:2013–2017.
48. Schroeder OE, Carper E, Wind JJ, Poutsma JL, Etzkorn FA, Poutsma JC. *J Phys Chem A* 2006;110:6522–6530. [PubMed: 16706410]
49. Wiederrecht G, Etzkorn FA. *Perspect Drug Discov Des* 1994;2:57–84.
50. Ranganathan R, Lu KP, Hunter T, Noel JP. *Cell* 1997;89:875–886. [PubMed: 9200606]

Scheme 1.
Synthesis of H-Pro-NEA 3.

Scheme 2.
Synthesis of inhibitors **1a** and **1b**.

Scheme 3.
Proposed mechanism for hydroxyamide **6** formation.