Published in final edited form as:

Tetrahedron Lett. 2012 August 8; 53(32): 4065–4069. doi:10.1016/j.tetlet.2012.05.105.

Progress towards the synthesis of Urukthapelstatin A and two analogues

Chung-Mao Pan^b, Chun-Chieh Lin^b, Seong Jong Kim^a, Robert P. Sellers^b, and Shelli R. McAlpine^a

^aSchool of Chemistry, University of New South Wales, Sydney, NSW 2052 Australia

^bDepartment of Chemistry and Biochemistry, 5500 Campanile Dr., San Diego State University, San Diego, CA 92182-1030 USA

Abstract

We report our progress towards the synthesis of Urukthapelstatin A (Ustat A) and two analogues. Our retrosynthetic strategy involved the synthesis of three fragments: a tri-heteroaromatic moiety, a phenyl oxazole fragment, and a dipeptide. Described are the syntheses of three unique tri-heteroaromatic moieties. In addition, the corresponding linear precursors of Ustat A and two analogues are presented.

Natural products provide original pharmacophores, offering new scaffolds with novel mechanisms of action. Urukthapelstatin A (Ustat A) is one such natural product (Figure 1), with cytotoxicity against cancer cells in the desirable low nanomolar range (average GI₅₀ = 5.6 nM).¹ It does not share structural homology with other classes of marketed cancer therapeutics, and it has a distinct activity profile compared to structurally similar compounds, indicating that it likely has a unique mechanism of action.¹ Given that there are considerable ongoing efforts to expand the arsenal of drugs that target cancer, the search for new compounds that inhibit novel cell-survival pathways is of paramount importance to the evolution of cancer medicine. Herein, we report our synthetic strategy aimed at completing the first synthesis of the natural product Ustat A and two analogues (Figure 1). Our efficient, modular approach is the first report that targets this unique pharmacophore, or that outlines a method for analogue development. Our approach is a flexible method intended for building Ustat A derivatives, thereby providing access to a series of unique molecular scaffolds.

Several natural product peptidomimetic structures that contain oxazoles and thiazoles have been isolated and synthesized.^{2–8} Among these natural products, telomestatin is the only natural product whose mechanism of action has been thoroughly studied. Although Ustat A is structurally similar to telomestatin, it does not appear to target telomerase (using the traditional TRAP assay),¹ and thus its highly cytotoxic potency is associated with an as yet unknown mechanism of action. The isolated natural product was also tested in numerous anticancer assays, which revealed that it is also unlikely to be an HDAC, farnesyl transferase, or proteasome inhibitor.¹ The activity profile of Ustat A using COMPARE analysis resembles that of the known anticancer agent cytarabine, which is an

© 2012 Elsevier Science

Supplementary data

Supplementary data associated with this article can be found in the online version at:

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

antimetabolite.¹ Indeed, the COMPARE profile suggests that this molecule acts via a unique mechanism, indicating the value of further investigation and analogue synthesis.

Herein we outline an efficient synthetic strategy for three fragments. Using a Hantzsch thiazole synthesis to couple **fragments 1** and **2**, followed by a peptide coupling of **fragment 3** completes the linear precursor. Although cyclization conditions are currently on-going, we anticipate completing the macrocycle by coupling between alanine and threonine. Subsequent elimination to the *Z*-enamine⁹ would give the final product. In this paper, we focus on developing linear precursors with modified heterocyclic components. Oxazoles form stronger hydrogen bonds than thiazoles, but are significantly more rigid when placed in a macrocyclic conformation.¹⁰ Thus, syntheses of the natural product and two analogues were designed to investigate how modification of the heterocycles would alter: a) the ability of these analogs to hydrogen bond, and b) the ability of the rings to close. The synthesis of **fragment 1** differed for each of the three macrocycles, with Schemes 1, 2, and 3 describing the synthetic approaches for compounds **1**, **2**, and **3**, respectively. However, the synthesis of **fragments 2** and **3** were the same for all three linear precursors.

Utilizing a peptide approach to generate the heterocycles provided the fragments in the fewest number of steps (versus a cross-coupling approach-theoretical calculation) and gave high overall yields. The synthesis of **fragment 1** for compound **1** began with the construction of the dipeptide **4** (Scheme 1). This dipeptide was generated by coupling free amine H₂N-Ser(Bn)-OMe and free acid Boc-Thr(O^tBu)-OH using 2-(1-*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (TBTU) and diisopropylethylamine (DIPEA) in anhydrous methylene chloride (CH₂Cl₂) yielding **4** in 90% yield. Removal of the benzyl ether protecting group was accomplished via hydrogenolysis using Pd/C (10%) as catalyst. A two-step procedure involving an intramolecular cyclization using the fluorinating agent diethylaminosulfur trifluoride (DAST) and K₂CO₃ to yield the intermediate oxazoline, which was oxidized using bromochloroform (BrCCl₃) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), generated **5** in 85% overall yield over three steps. Hydrolysis of the ester **5** using LiOH and subsequent coupling between the free acid and free amine H₂N-Ser(Bn)-OMe was performed using TBTU and DIPEA. This yielded compound **6** in 95% yield. Hydrogenolysis of **6** and subsequent conversion of the free serine into an oxazole using DAST/BrCCl₃ resulted in **7** (85% overall yield over three steps). The formation of thioamide **8** was carried out using ammonium hydroxide in methanol followed by conversion of the resulting amide into the thioamide using Lawesson's reagent (77% yield over two steps). A base-induced Hantzsch thiazole synthesis¹¹ was performed using an excess of ethyl bromopyruvate and KHCO₃ to afford the intermediate thiazoline, which was dehydrated using trifluoroacetic anhydride (TFAA) and pyridine to give compound **9** (77% yield over two steps). Compound **10** was generated using ammonium hydroxide in methanol followed by treatment with Lawesson's reagent (70% yield over two steps).

The synthesis of **13** en route to the Ustat A analogue utilized the dioxazole **7**, which was converted into the free acid using LiOH in methanol (Scheme 2). Subsequent peptide bond formation between the free acid Boc-Thr(O^tBu)-dioxazole-OH and the free amine H₂N-Ser(Bn)-OMe using TBTU and DIPEA in anhydrous CH₂Cl₂ gave **11** (92% yield, 2 steps). The benzyl ether of **11** was removed via hydrogenolysis followed by the formation of the third oxazole moiety, **12**, using DAST/BrCCl₃ in 70% yield over the three steps. Conversion of the ester into a thioamide to generate **13** was achieved using ammonium hydroxide followed by sulfur installation using Lawesson's reagent (55% yield, 2 steps).

The construction of Ustat A analogue **20** is summarized in Scheme 3. Starting with protection of the free acid Boc-Thr(O^tBu)-OH using trimethylsilyl diazomethane (TMSD) in methanol and then conversion into the thioamide via ammonium hydroxide and Lawesson's

reagent resulted in the formation of **14** (60% yield over three steps). The Hantzsch thiazole synthesis was then performed reacting **14**, ethyl bromopyruvate, and KHCO_3 to yield the intermediate thiazoline, whereupon subsequent dehydration was facilitated using TFAA and pyridine at 0 °C to give **15** (78% over two steps). Generation of **16** was accomplished using a two-step procedure involving ammonium hydroxide and Lawesson's reagent (53% yield, two steps). The next two thiazole moieties were installed by repeating the Hantzsch thiazole synthesis process, whereby the thioamide was reacted with ethyl bromopyruvate and KHCO_3 . Dehydration using TFAA and pyridine and then treatment with NaOEt in EtOH gave **17** (97% yield over three steps). This approach was repeated on **17** to generate thioamide **18** (73% yield over two steps), and then tri-thiazole **19** (87% yield over three steps). Conversion of **19** into a thioamide using ammonium hydroxide and Lawesson's reagent provided **20** (73% yield over two steps).

Fragments 2 (23) and **3 (25)** were synthesized next (Scheme 4). Phenylserine **21** was generated by coupling 3-bromo-2,2-dimethoxypropionic acid with (2*R*,3*S*)/(2*S*,3*R*)-racemic H_2N - β -hydroxy-Phe-OMe using TBTU and DIPEA in anhydrous CH_2Cl_2 (92% yield). Racemic **21** was then subjected to intramolecular cyclization induced by DAST and then pyridine in CH_2Cl_2 to afford the intermediate phenyloxazoline. This intermediate was then advanced to the phenyloxazole through oxidation using BrCCl_3 and DBU (45% overall yield 2 steps). Deprotection of the ketone was achieved using formic acid induced hydrolysis at 50 °C for 20 minutes yielding **23** (quantitative yield). **Fragment 3 (25)**, was generated via a peptide coupling reaction between free acid Boc-D-allo-Ile-OH and free amine H_2N -Ala-OMe using TBTU and DIPEA (99% yield). The dipeptide was then amine deprotected using TFA, providing **25** (quantitative yield).

With **fragments 1** and **2** in hand, we performed the Hantzsch thiazole synthesis (Scheme 5) to generate the heterocyclic hemisphere of Ustat A and analogues (overall yield for two steps **26a**, **b** and **c** = 69%, 72%, and 88%, respectively). Upon gaining access to penta-heterocycle **fragments 26a–c**, we performed an acid deprotection and coupled the resulting free acids to **fragment 3** using TBTU and DIPEA in CH_2Cl_2 , thereby generating **27a–c** (61%, 65%, and 47% yields, respectively). The ester group in compounds **27a–c** was deprotected using LiOH, and the Boc and *tert*-butyl groups were removed using TFA, generating compounds **28a–c**. NMR spectroscopic studies and high resolution mass spectroscopy confirmed the structures of the three linear precursors **28a–c**.

In summary, we have outlined an efficient and viable synthetic approach to three different rigid penta-heterocycles. We then converted these into the linear precursors in preparation for cyclization into Urukthapelstatin A (**1**) and two analogues **2** and **3**. We are currently investigating cyclization strategies,¹² which will be reported in due course. These linear analogs will be tested for their ability to inhibit colon and pancreatic cancer cell growth.^{13,14}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the University of New South Wales for the support of S.R.M. and S.J.K., the Frasch foundation (658-HF07) for support for C.M.P. and C.C.L. We thank Dr. Lafferty for his assistance in NMR experiments.

References and notes

1. Matuso Y, Kanoh K, Yamori T, Kasai H, Katsuta A, Adachi K, Shin-ya K, Shizuri Y. J Antibiot. 2007; 60:251–255. [PubMed: 17456975]

2. Doi T, Yoshida M, Shin-ya K, Takahashi T. *Org Lett.* 2006; 8:4165–4167. [PubMed: 16928100]
3. Shin-ya K, Wierzba K, Matuo K, Ohtani T, Yamada Y, Furihata K, Hayakawa Y, Seto H. *J Am Chem Soc.* 2001; 123:1262–1263. [PubMed: 11456694]
4. Hernández D, Vilar G, Riego E, Cañedo LM, Cuevas C, Albericio F, Álvarez M. *Org Lett.* 2007; 9:809–811. [PubMed: 17266322]
5. Dalisay DS, Rogers EW, Edison AS, Molinski TF. *J Nat Prod.* 2009; 72:732–738. [PubMed: 19254038]
6. Hamada Y, Kato S, Shioiri T. *Tetrahedron Lett.* 1985; 26:3223–3226.
7. Wipf P, Miller CP. *J Am Chem Soc.* 1992; 114:10975–10977.
8. Nakamura M, Shibata T, Nakane K, Nemoto T, Ojika M, Yamada K. *Tetrahedron Lett.* 1995; 36:5059–5062.
9. Nicolaou KC, Zak M, Safina BS, Estrada AA, Lee SH, Nevalainen M. *J Am Chem Soc.* 2005; 127:11176–11183. [PubMed: 16076225]
10. Dehner A, Furrer J, Richter K, Schuster I, Buchner J, Kessler H. *ChemBioChem.* 2003; 4:870–877. [PubMed: 12964162]
11. Aguilar E, Meyers AI. *Tetrahedron Lett.* 1994; 35:2473–2476.
12. Styers TJ, Rodriguez RA, Pan PS, McAlpine SR. *Tetrahedron Lett.* 2006; 47:515–517.
13. Otrubova K, McGuire KL, McAlpine SR. *J Med Chem.* 2007; 50:1999–2002. [PubMed: 17411027]
14. Pan PS, Vasko RC, Lapera SA, Johnson VA, Sellers RP, Lin CC, Pan CM, Davis MR, Ardi VC, McAlpine SR. *Bioorg Med Chem.* 2009; 17:5806–5825. [PubMed: 19643615]

Figure 1.
Retrosynthetic approach for Ustat A and analogues

Scheme 1.
Synthetic approach to Ustat A, **Fragment 1**

Scheme 2.
Synthetic approach to compound **2**, **Fragment 1**

Scheme 3.
Synthetic approach to compound 3, **Fragment 1**

Scheme 4.
Synthetic approach to **Fragments 2** and **3**

Scheme 5.
Synthetic approach to the linear precursors