Bioorganic & Medicinal Chemistry Letters 25 (2015) 2536–2539

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of constrained phosphoramidate inhibitors of prostate-specific membrane antigen

Corinne R. Ley [a], Nathan R. Beattie [a], Shorouk Dannoon [b], Melanie Regan [b], Henry VanBrocklin [b], Clifford E. Berkman [a,*]

[a] Department of Chemistry, Washington State University, Pullman, WA 99164-4630, USA
[b] Department of Radiology and Biomedical Imaging, University of California, San Francisco, CA 94158, USA

ARTICLE INFO

Article history:
Received 6 February 2015
Revised 14 April 2015
Accepted 17 April 2015
Available online 29 April 2015

Keywords:
Phosphoramidate
Inhibitor
Prostate-specific membrane antigen
4-Hydroxyproline
Prostate cancer

ABSTRACT

Prostate-specific membrane antigen (PSMA) is a cell-surface enzyme-biomarker that is actively pursued for targeted delivery of imaging and therapeutic agents for prostate cancer. Our lab has developed PSMA inhibitors based on a phosphoramidate scaffold, which has shown both high selectivity for PSMA-positive tumors and rapid clearance in vivo when radiolabeled with ^{18}F. However, this scaffold exhibits hydrolytic instability under low pH and high temperature conditions, barring the use of other imaging or therapeutic radionuclides such as ^{68}Ga or ^{177}Lu. Previous studies in our lab have shown a trend in increasing acid stability as the distance between the phosphoramidate core and the α-carboxylate of the P1 residue is increased. Therefore, a new generation of phosphoramidate inhibitors was developed based on *trans*-4-hydroxyproline as the P1 residue to restrict the interaction of the α-carboxylate to the phosphoramidate core. These hydroxyproline inhibitors demonstrated comparable IC_{50} values to earlier generations as well as enhanced thermal and acid stability.

Prostate-specific membrane antigen (PSMA) is a cell-surface enzyme-biomarker[1,2] that continues to be actively pursued for targeted delivery of imaging[3–17] and therapeutic agents[18–22] for prostate cancer. PSMA has been found to be up-regulated and strongly expressed on cancer cells, including those that are metastatic.[23] As a consequence, enzyme inhibitors have been developed to selectively, and in some cases, irreversibly bind to PSMA.[24–27] Most recently, our lab developed, and radiolabeled with ^{18}F, the phosphoramidate inhibitors **5** and **6** (Fig. 1), which exhibits both high selectivity for PSMA-positive tumors and rapid clearance in vivo. However, this scaffold, like previous generations of this class of inhibitors, exhibits hydrolytic instability under low pH and high temperature conditions. These conditions are likely to be encountered when such scaffolds are labeled with other imaging or therapeutic radionuclides such as ^{68}Ga or ^{177}Lu.[28–30] Hence, it is desirable to optimize the phosphoramidate scaffold for this class of PSMA inhibitors for greater acid and thermal stability.

In our studies with earlier generations of phosphoramidate-based PSMA inhibitors, we observed a trend in increasing acid stability as the distance between the phosphoramidate centers and the α-carboxylate of the P1 residue was increased. This is evidenced by the observed rates of decomposition at pH 6.0 and 4.5 for scaffolds containing a P1 serine (**1**), homoserine (**2**), and hydroxypropylglycine residue (**3**).[31] The mechanism for the cleavage of the P–N bond of the phosphoramidate core under these conditions remains conjectural, however, the proximity of the P1 α-carboxylate to the phosphoramidate center does appear to contribute to its lability. While this mechanism is a topic for a future study, this observed trend inspired the design of a new scaffold based on *trans*-4-hydroxyproline as the P1 residue. Due to the conformational restrictions provided by the proline ring, the *trans*-orientation between the α-carboxylate and hydroxyl group was expected to afford enhanced acid stability. Therefore, the focus of this study was aimed at preparing and evaluating a limited series of phosphoramidate-based PSMA inhibitors comprised of a *trans*-4-hydroxyproline in the P1 position for both acid and thermal stability as well as inhibitory potency against PSMA. Because of the restricted conformational freedom imposed by the pyrrolidine ring, it was also expected that a degree of stereoselective inhibition of PSMA would be observed. In terms of assessing the impact of introducing a relatively inflexible pyrrolidine ring in the P1 position (e.g., **7a–b**, **8a–b, 9a–b**, and **10a–b**), we chose to compare the inhibitory potencies and mode of inhibition to the more conventional acyclic analog **3** (Fig. 1).[31]

* Corresponding author. Tel.: +1 (509) 335 7613; fax: +1 (509) 335 8867.
E-mail address: cberkman@wsu.edu (C.E. Berkman).

http://dx.doi.org/10.1016/j.bmcl.2015.04.047
0960-894X/

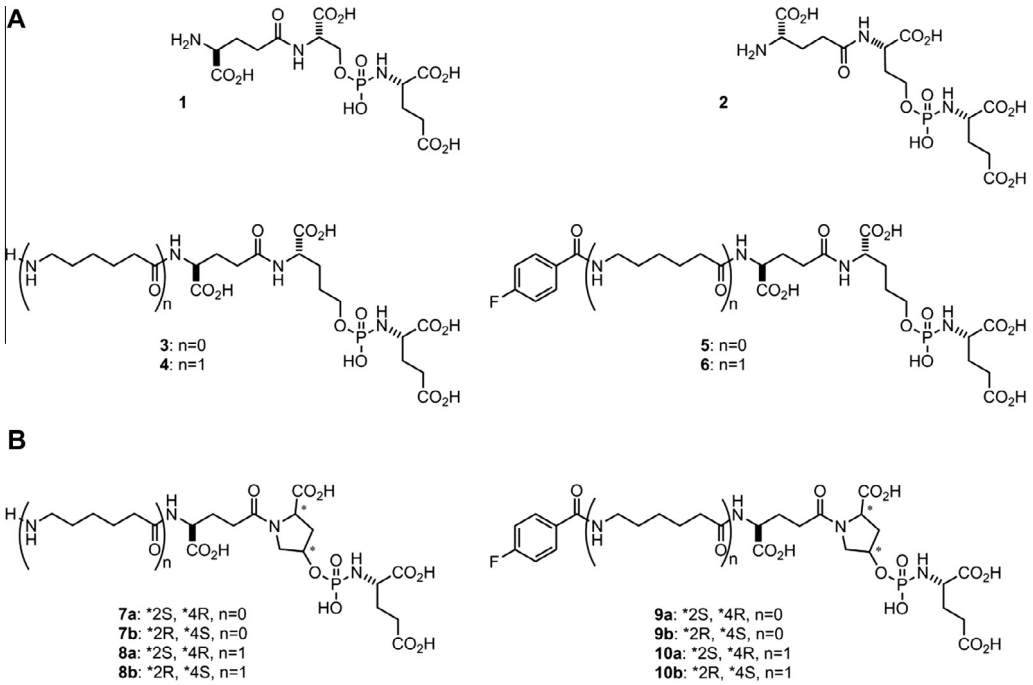
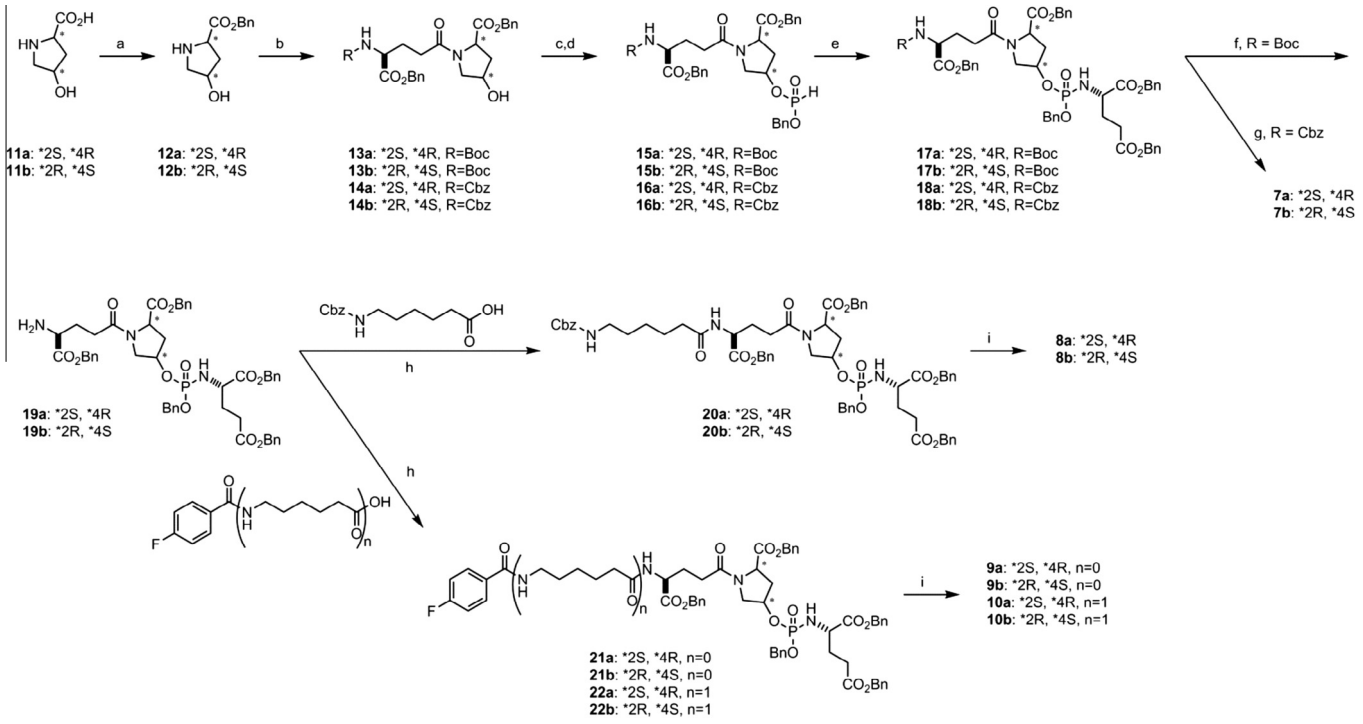


Figure 1. (A) Current phosphoramidate inhibitors of PSMA **1**–**6**. (B) Phosphoramidate inhibitors of PSMA with enhanced stability **7a**–**10b**.



Scheme 1. Reagents and conditions: (a) BnOH, *p*-toluene-SO_3H, benzene, 125 °C, 20 h reflux; (b) R-Glu-OBzl (R = Cbz or Boc), HBTU, Et_3N, DMF; (c) $(PhO)_2P(O)H$, pyridine, −5 °C to rt, 2 h; (d) BnOH, rt, 3 h; (e) H-Glu(OBzl)-OBzl HCl, CCl_4, Et_3N, CH_3CN; (f) 30% TFA/CH_2Cl_2, rt, 1.5 h; (g) H_2, 10% Pd/C, K_2CO_3, ddH_2O, 1,4-dioxanes; (h) HBTU, Et_3N, DMF; (i) H_2, 10% Pd/C, K_2CO_3, ddH_2O, 1,4-dioxanes.

A common method was employed to prepare the *trans*-4-hydroxyproline-based phosphoramidate inhibitors (Scheme 1). The starting amino acids **11a–b** were protected as the benzyl esters **12a**–**b**, which were subsequently coupled to *N*-protected glutamic acid to provide the corresponding alcohols **13a–b** and **14a–b**. The reaction of these alcohols with diphenyl phosphite followed by the addition of benzyl alcohol provided H-phosphonates **15a–b** and **16a–b**, which were then subjected to standard Atherton–Todd conditions to generate the protected phosphoramidate intermediates **17a–b** and **18a–b**. Deprotection of **18a–b** under hydrogenolysis conditions yielded the stereoisomeric inhibitors **7a**–**b**. The Boc-protected intermediates **17a–b** were *N*-deprotected