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Synthesis and Evaluation of Constrained Phosphoramidate Inhibitors of Prostate-Specific Membrane Antigen

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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.

Keywords

phosphoramidate; inhibitor; prostate-specific membrane antigen; 4-hydroxyproline; prostate cancer

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.

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Supplemental Material

For detailed experimental procedures, refer to the Supplemental Material.

[24-27] Most recently, our lab developed, and radiolabeled with ^{18}F, the phosphoramidate inhibitors **5** and **6** (Figure 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** (Figure 1).[31]

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 and functionalized to provide globally protected precursors **20a-b**, **21a-b**, and **22a-b**. Global deprotection of these precursors under hydrogenolysis conditions yielded **8a-b**, **9a-b**, and **10a-b**.

To assess the stability of the *trans*-4-hydroxyproline scaffold compared to an acyclic conventional type of phosphoramidate PSMA inhibitor scaffold under mildly acidic conditions, the decomposition of **10a** and **6**[31], were monitored by ^{31}P NMR at pH 4.5. The compounds were analyzed at both 50 and 70 °C (see Supporting Information for

procedures). The respective rates of decomposition (Table 1) of **10a** and **6** confirmed that the *trans*-4-hydroxyproline scaffold exhibited enhanced the stability of the phosphoramidate P-N bond over the acyclic analog **6**. These results further support the contribution of the α-carboxylate of the P1 amino acid on P-N hydrolysis of this class of phosphoramidate inhibitors of PSMA.

While greater acid stability of the *trans*-4-hydroxyproline scaffold was observed, it was important to determine if this structural change resulted in a loss of inhibitory potency toward PSMA and/or a change in the mode of inhibition when compared to acyclic analogs. It was noted that the *trans*-4-hydroxyproline-based inhibitors of PSMA with unmodified *N*-terminal amines generally exhibited lower inhibitory potency than their respective 4-fluorobenzamide derivatives; a trend that is consistent with small molecule PSMA inhibitors based on phosphoramidate and urea scaffolds.[31] With respect to the impact of stereochemistry of the P1 4-*trans*-hydroxyproline residue on PSMA inhibition, it was observed that the natural L-isomer of this amino acid was preferred (e.g., **9a** and **10a**), which is consistent with our previous findings.[32] Of the 4-fluorobenzamide derivatives of the inhibitors based on the *trans*-4-hydroxyproline scaffold (**9** and **10**), the one that was most comparable to the analogs based on the hydroxypropylglycine scaffold (**5** and **6**)[31] was **10a** (Table 2) based on IC_{50} values. Interestingly, the mode of inhibition studies demonstrated that all the analogs based on the *trans*-4-hydroxyproline scaffold (**7**-**10**) exhibited slowly reversible binding, in contrast to the irreversible mode of inhibition for the compounds based on the acyclic scaffold **1**-**6**[31] suggesting that rigidity or increased molecular volume introduced by the *trans*-4-hydroxyproline residue does not allow the same interactions that the acyclic analogs experience with the binding site of PSMA that lead to irreversible binding.

With considerable inhibitory potency against PSMA and a slowly-reversible mode of inhibition, it was of interest to confirm that the 4-*trans*-hydroxyproline-based phosphoramidate inhibitor scaffold could also perform sufficiently *in vivo* as the targeting motif of a PET imaging agent for PSMA-positive tumors. For a preliminary assessment of this new scaffold's *in vivo* performance, the ^{18}F-radiolabled analog **[^{18}F]10a** was prepared as described previously using N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB).[15] As shown in the PET/CT image (Figure 2), there was significant and selective uptake of **[^{18}F]10a** tracer in CWR22Rv1 (PSMA+) tumor xenografts at 2 h post-injection. In addition, there was the expected uptake in the kidneys due to the expression of PSMA in mouse kidneys[23, 33, 34] but minimal uptake in all other organs.

In summary, the stability and inhibition studies demonstrated that limiting the interaction of the α-carboxylate of the P1 amino acid to the phosphoramidate core improved the acid and thermal stability of the P-N bond without completely sacrificing inhibitory potency against PSMA. As a result, it is expect that the *trans*-4-hydroxyproline scaffold could be radiolabeled with imaging and therapeutic radionuclides such as ^{68}Ga and ^{177}Lu that might require harsher conditions for the installation of the isotopes. Such findings would extend the versatility of the phosphoramidate scaffold in the targeting molecule motif for targeted imaging and therapeutic agents for prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Perner S, Hofer MD, Kim R, Shah RB, Li H, Moller P, Hautmann RE, Gschwend JE, Kuefer R, Rubin MA. Hum Pathol. 2007; 38:696. [PubMed: 17320151]
2. Bander NH, Milowsky MI, Nanus DM, Kostakoglu L, Vallabhajosula S, Goldsmith SJ. J Clin Oncol. 2005; 23:4591. [PubMed: 15837970]
3. Rosenthal SA, Haseman MK, Polascik TJ, Division of Radiation Oncology, R. A. o. S. M. G. I. C. U. S. A. Techniques in urology. 2001; 7(1):27. [PubMed: 11272670]
4. Chu TC, Shieh F, Lavery LA, Levy M, Richards-Kortum R, Korgel BA, Ellington AD. Biosens Bioelectron. 2006; 21:1859. [PubMed: 16495043]
5. Farokhzad OC, Khademhosseini A, Jon S, Hermmann A, Cheng J, Chin C, Kiselyuk A, Teply B, Eng G, Langer R. Analytical chemistry. 2005; 77:5453. [PubMed: 16131052]
6. Foss CA, Mease RC, Fan H, Wang Y, Ravert HT, Dannals RF, Olszewski RT, Heston WD, Kozikowski AP, Pomper MG. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005; 11:4022. [PubMed: 15930336]
7. Gao X, Cui Y, Levenson RM, Chung LW, Nie S. Nature biotechnology. 2004; 22:969.
8. Guilarte TR, McGlothan JL, Foss CA, Zhou J, Heston WD, Kozikowski AP, Pomper MG. Neurosci Lett. 2005; 387:141. [PubMed: 16006038]
9. Humblet V, Lapidus R, Williams LR, Tsukamoto T, Rojas C, Majer P, Hin B, Ohnishi S, De Grand AM, Zaheer A, Renze JT, Nakayama A, Slusher BS, Frangioni JV. Mol Imaging. 2005; 4:448. [PubMed: 16285907]
10. Milowsky MI, Nanus DM, Kostakoglu L, Sheehan CE, Vallabhajosula S, Goldsmith SJ, Ross JS, Bander NH. J Clin Oncol. 2007; 25:540. [PubMed: 17290063]
11. Pomper MG, Musachio JL, Zhang J, Scheffel U, Zhou Y, Hilton J, Maini A, Dannals RF, Wong DF, Kozikowski AP. Mol Imaging. 2002; 1:96. [PubMed: 12920850]
12. Smith KA, Sheppard SD, Johnson DW, Johnson RT. Journal of Engineering Education. 2005; 94:87.
13. Smith-Jones PM, Vallabhajosula S, Navarro V, Bastidas D, Goldsmith SJ, Bander NH. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2003; 44:610.
14. Tsukamoto T, Wozniak KM, Slusher BS. Drug Discov Today. 2007; 12:767. [PubMed: 17826690]
15. Lapi SE, Wahnishe H, Pham D, Wu LY, Nedrow-Byers JR, Liu T, Vejdani K, VanBrocklin HF, Berkman CE, Jones EF. Journal of nuclear medicine : official publication, Society of Nuclear Medicine. 2009; 50:2042.
16. Nedrow-Byers JR, Jabbes M, Jewett C, Ganguly T, He H, Liu T, Benny P, Bryan JN, Berkman CE. The Prostate. 2012; 72:904. [PubMed: 22670265]
17. Nedrow-Byers JR, Moore AL, Ganguly T, Hopkins MR, Fulton MD, Benny PD, Berkman CE. The Prostate. 2013; 73:355. [PubMed: 22911263]
18. Tasch J, Gong M, Sadelain M, Heston WD. Crit Rev Immunol. 2001; 21:249. [PubMed: 11642607]
19. Lu J, Celis E. Cancer research. 2002; 62(20):5807. [PubMed: 12384542]
20. Liu T, Wu LY, Berkman CE. Cancer letters. 2010; 296:106. [PubMed: 20452720]
21. Liu T, Wu LY, Choi JK, Berkman CE. International journal of oncology. 2010; 36:777. [PubMed: 20198319]

22. Liu T, Nedrow-Byers JR, Hopkins MR, Wu LY, Lee J, Reilly PT, Berkman CE. Bioorganic & medicinal chemistry letters. 2012; 22:3931. [PubMed: 22607680]
23. Bacich DJ, Pinto JT, Tong WP, Heston WD. Mammalian genome : official journal of the International Mammalian Genome Society. 2001; 12(2):117. [PubMed: 11210180]
24. Liu T, Toriyabe Y, Kazak M, Berkman CE. Biochemistry. 2008; 47:12658. [PubMed: 18983168]
25. Liu T, Wu LY, Hopkins MR, Choi JK, Berkman CE. Bioorganic & medicinal chemistry letters. 2010; 20:7124. [PubMed: 20947349]
26. Majer P, Jackson PF, Delahanty G, Grella BS, Ko YS, Li W, Liu Q, Maclin KM, Polakova J, Shaffer KA, Stoermer D, Vitharana D, Wang EY, Zakrzewski A, Rojas C, Slusher BS, Wozniak KM, Burak E, Limsakun T, Tsukamoto T. J Med Chem. 2003; 46:1989. [PubMed: 12723961]
27. Thomas AG, Wozniak KM, Tsukamoto T, Calvin D, Wu Y, Rojas C, Vornov J, Slusher BS. Adv Exp Med Biol. 2006; 576:327. [PubMed: 16802724]
28. Wunderlich G, Schiller E, Bergmann R, Pietzsch H-J. Nuclear Medicine and Biology. 2010; 37:861. [PubMed: 21055615]
29. Liu S, Cheung E, Ziegler MC, Rajopadhye M, Edwards DS. Bioconjugate Chemistry. 2001; 12:559. [PubMed: 11459461]
30. Sosabowski JK, Mather SJ. Nat. Protocols. 2006; 1:972. [PubMed: 17406332]
31. Ganguly, T. Ph.D. Thesis. Washington State University; 2013.
32. Wu LY, Anderson MO, Toriyabe Y, Maung J, Campbell TY, Tajon C, Kazak M, Moser J, Berkman CE. Bioorganic & medicinal chemistry. 2007; 15:7434. [PubMed: 17869524]
33. Troyer JK, Beckett ML, Wright GL Jr. Int J Cancer. 1995; 62:552. [PubMed: 7665226]
34. Israeli RS, Powell CT, Corr JG, Fair WR, Heston WD. Cancer research. 1994; 54:1807. [PubMed: 7511053]

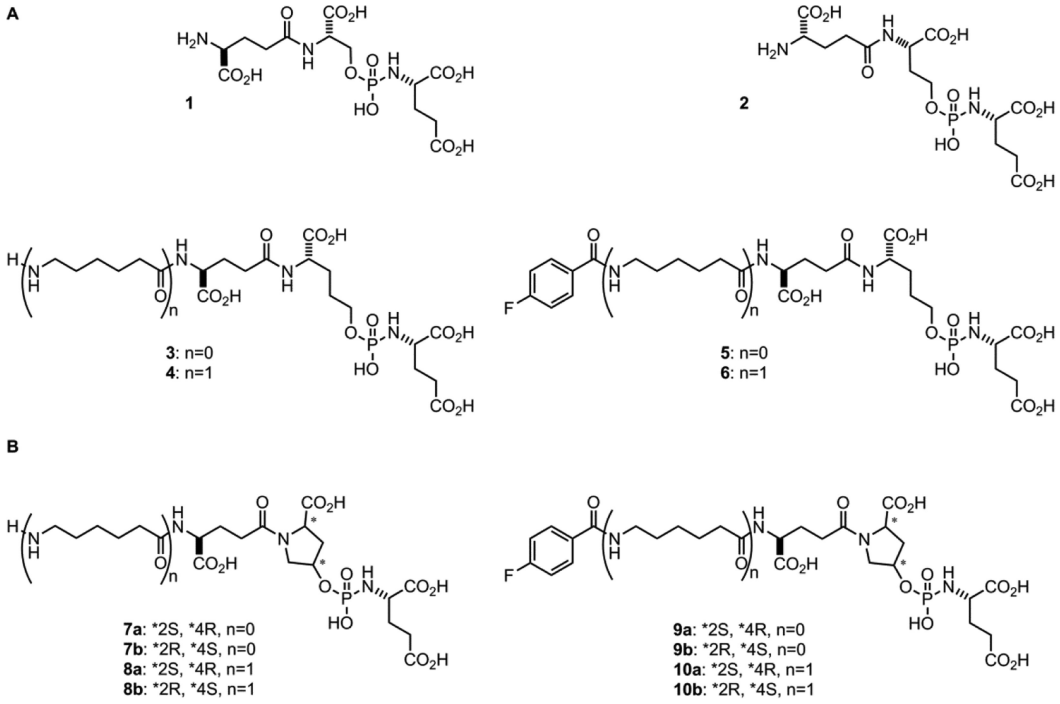
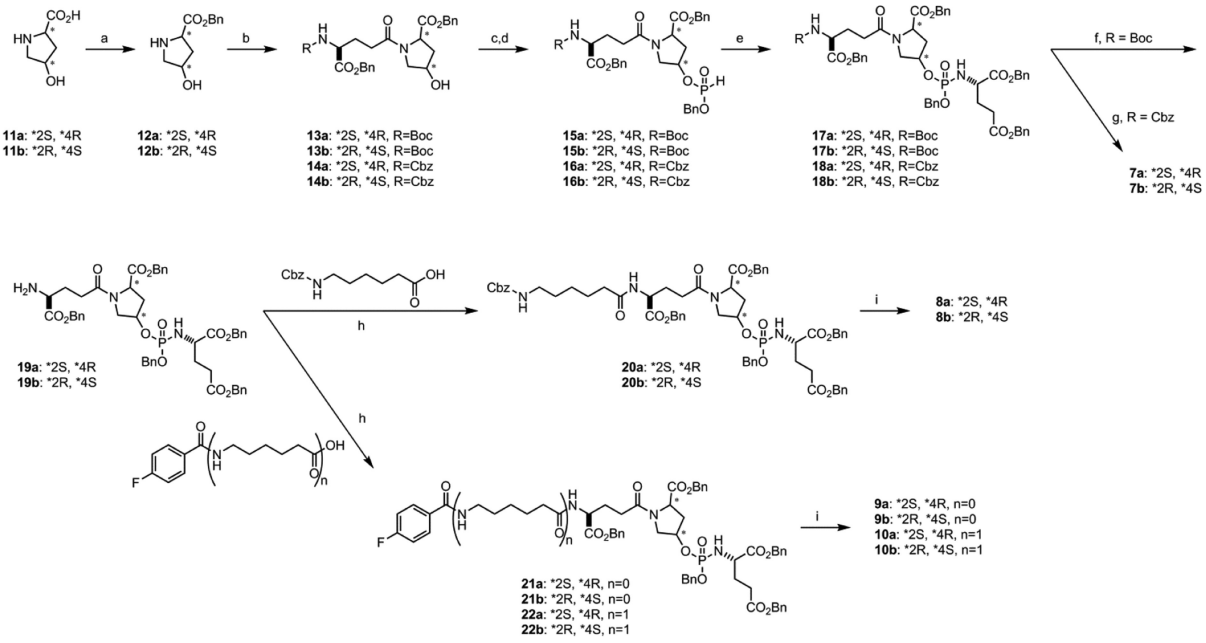


Figure 1.

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



Scheme 1.

(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

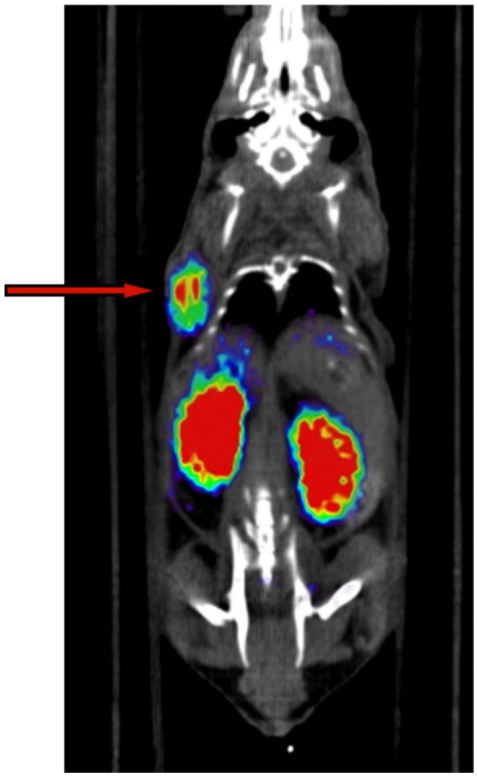


Figure 2.
PET/CT image (20 minute static scan) of a male nude mouse bearing a CWR22Rv1 tumor xenograft at 2 h post-injection of **[^{18}F]10a**. Arrow indicates tumor placement.

Table 1

Comparative stability of representative phosphoramidates at pH 4.5

Entry	Temp (°C)	$t_{½}$ (min)
6	50	75
6	70	12
10a	50	105
10a	70	32

Table 2

Inhibition potency of phosphoramidate inhibitors of PSMA

Entry	IC_{50} (nM)[a]
3	27 (3)
4	19 (1)
5	1.3 (0.08)
6	0.4 (0.05)
7a	60 (11)
7b	357 (29)
8a	79 (6)
8b	112 (8)
9a	2.6 (0.2)
9b	3.0 (0.3)
10a	1.3 (0.2)
10b	11 (0.8)

[a]Standard deviation in parentheses.