Bioorganic & Medicinal Chemistry Letters 30 (2020) 126894

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of a novel BODIPY-labeled PSMA inhibitor

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ARTICLE INFO

Keywords:
Prostate cancer
PSMA
BODIPY
Optical imaging

ABSTRACT

Prostate-specific membrane antigen (PSMA) is a zinc-bound metalloprotease which is highly expressed in metastatic prostate cancer. It has been considered an excellent target protein for prostate cancer imaging and targeted therapy because it is a membrane protein and its active site is located in the extracellular region. We successfully synthesized and evaluated a novel PSMA ligand conjugated with $BODIPY_{650/665}$. Compound **1** showed strong PSMA-inhibitory activity and selective uptake into PSMA-expressing tumors. Compound **1** has the potential to be utilized as a near infrared (NIR) optical imaging probe targeting PSMA-expressing cancers.

Introduction

Prostate-specific membrane antigen (PSMA) is a zinc metalloprotease belonging to the type II transmembrane protein family. PSMA, also referred to as glutamate carboxypeptidase II (GCP II), hydrolyzes endogenous *N*-acetylaspartylglutamate (NAAG) to *N*-acetylaspartate (NAA) and glutamate.[1,2] PSMA has been considered as an attractive target for imaging and therapy of prostate cancer because its expression is upregulated in nearly all stages of prostate cancer.[3,4] Moreover, the degree of PSMA expression positively correlates with the stage and grade of prostate cancer.[5] As PSMA has its enzymatic active site within the extracellular region, it can be targeted by small molecules as well as antibodies. A variety of small molecules and antibodies labeled with imaging probes (e.g., radionuclides, optical dyes) targeting PSMA have been extensively studied.[6–8] In particular, PSMA-targeted small molecules have been successfully developed due to the fact that they possess strong binding affinity for PSMA at nM concentrations *in vitro* and exhibit a high target-background ratio *in vivo*. The widely-applied functional groups of small molecules that interact with zinc ions in the active site are urea, thiol, and phosphonate.[9] Among these functional groups, the urea moiety has been extensively studied and used to make the class of glutamate-urea-lysine (Glu–urea–Lys). The glutamate of Glu–urea–Lys is the key pharmacophore group to interact with Arg210 and Lys699 in the S1′ site and the carbonyl group of the urea generates hydrogen bonds with zinc ions. The lysine of Glu–urea–Lys is more tolerant to structural modification and is utilized to conjugate with imaging prosthetic groups such as bulky optical dyes or radionuclide metal complexes.[10–13] Glu–urea–Lys is the most common scaffold of PSMA-targeted small molecules.

BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) is a valuable class of fluorophores discovered by Treibs and Kreuzer in 1968.[14,15] BODIPY is a biocompatible fluorophore which has several merits for *in vitro* and *in vivo* optical imaging including facile modulation of lipophilicity of the parent compound, large molar absorption coefficients, high fluorescence quantum yield, narrow emission bandwidths with high peak intensities, insensitivity to solvent polarity and pH, as well as thermal and photochemical stability.[15–17] Furthermore, its spectroscopic and photophysical properties can be finely tuned by attachment of appropriate substituents to the BODIPY framework. These attractive properties make them desirable for a wide range of applications such as fluorescent probes,[18–27] bioimaging,[28,29] and photodynamic therapy. In addition, as the non-radioactive ^{19}F atom of the core structure is replaced with radioactive ^{18}F, BODIPY is utilized as a dual-imaging prosthetic group for both optical and PET imaging simultaneously.[30–33]

The goal of this study was to identify PSMA-targeted NIR optical imaging agents based on the structure of urea-based PSMA ligand (Glu–urea–Lys). Here, we designed and synthesized a urea-based PSMA ligand (Glu–urea–Lys) and conjugated it to BODIPY which has 650 nm excitation and 665 nm emission wavelength. We also demonstrated selective cell uptake of the new NIR fluorophore-labeled ligands into

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https://doi.org/10.1016/j.bmcl.2019.126894
Received 1 October 2019; Received in revised form 5 December 2019; Accepted 5 December 2019
Available online 18 December 2019
0960-894X/

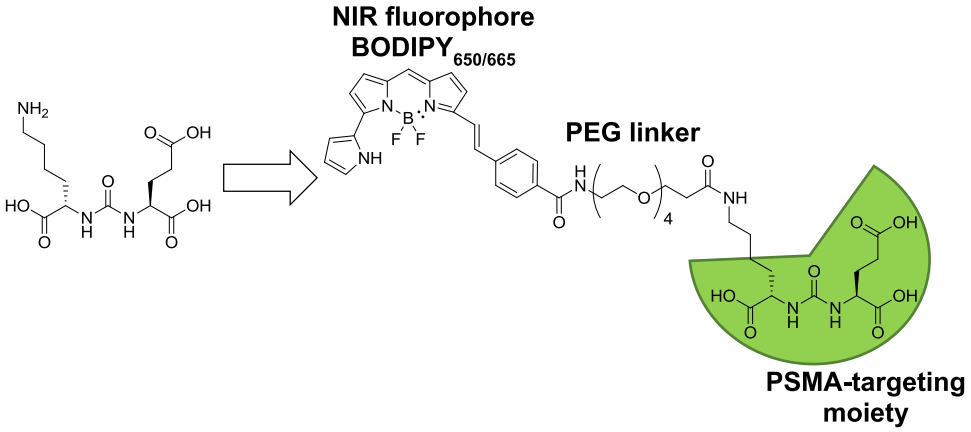
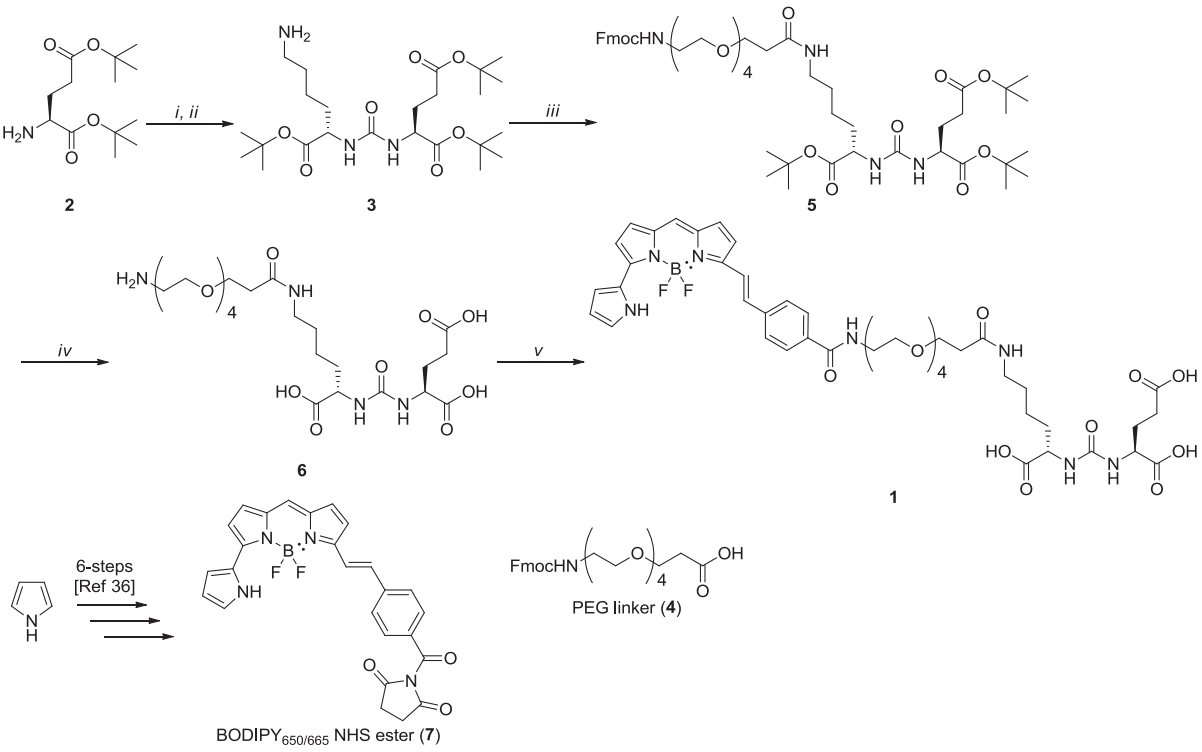


Fig. 1. Design of PSMA-targeted optical imaging agents based on Glu–urea-Lys.



Scheme 1. Synthesis of $BODIPY_{650/665}$-labeled PSMA ligand **1**. Reagents and reaction condition: (*i*) H-Glu(O*t*Bu)-O*t*Bu·HCl, triphosgene, Et_3N, CH_2Cl_2, –78 °C to rt, 12 h, 56% yield; (*ii*) H_2, 10% Pd/C, MeOH, 4 h; (*iii*) PEG linker (**4**), HATU, DIPEA, DMF, 12 h, 61% yield; (*iv*) 20% piperidine in DMF, rt, 2 h and then 50% TFA in CH_2Cl_2, rt, 2 h, 40% yield in two steps; (*v*) $BODIPY_{650/665}$ NHS ester (**7**), Tri-HCl buffer, DMSO, rt, 16 h, 47% yield.

Table 1
Summary of analytic results of compound **1**.

Compound	Fluorescence properties		Mass spectrometric data		HPLC	
	λ_{exc} (nm)	λ_{em} (nm)	*m/z* calculated	*m/z* found	R_t (min)	Purity
1	650	665	950.3924	950.4039	17.53	> 98%

HPLC was performed with Phenomenex C18 column (150 × 4.6 mm, 3 μm, 110 Å), gradient elution of 10% to 90% of solvent B (A = 0.1% formic acid in water and B = 0.1% formic acid in acetonitrile) in 20 min, flow of 1 mL/min, detection of 650 nm.

PSMA-positive cells.

The designed PSMA ligand (**1**) labeled with $BODIPY_{650/665}$ consists of a Glu–urea–Lys structure, polyethylene glycol (PEG) linker, and

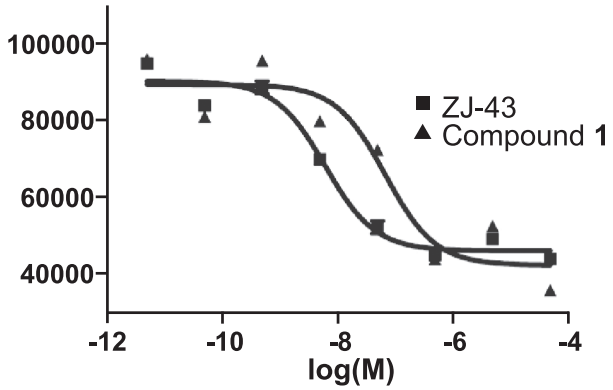


Fig. 2. PSMA inhibitory curves for compound **1** and ZJ-43. Experiments were performed with a triplicate of samples.

Table 2
PSMA inhibitory activities of compound **1** and ZJ-43.

Compound	IC_{50}	95% CI (IC_{50})[a]	K_i	95% CI (K_i)[a]
ZJ-43	6.21 nM	3.93–9.82 nM	1.42 nM	0.79–1.96 nM
1	68.8 nM	30.4–155 nM	13.8 nM	6.07–31.2 nM

[a] 95% Confidence interval.

$BODIPY_{650/665}$, as shown in Fig. 1. The PEG linker was assumed to be located in the tunnel region of the PSMA protein. As it is fairly hydrophilic and biocompatible, it was successfully conjugated with bulky optical dyes such as Cy7 and IRDye800CW.[34] This strategy was expected to decrease the adverse effect of the bulky and lipophilic BODIPY moiety on PSMA binding affinity.

Synthesis of the precursor for compound **1** was accomplished as described in Scheme 1. Briefly, the PSMA-binding motif (**3**) consisting of glutamine and lysine, linked via their α-amino groups by a carbonyl forming a urea group, was prepared in two steps from the commercial (L)-glutamic acid di-*tert*-butyl ester (**2**) by applying a previously reported synthetic procedure.[35] Amide coupling of compound **3** with PEG linker (Fmoc-15-amino-4,7,10,13-tetraoxapentadecanoic acid) **4** in the presence of 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*] pyridinium 3-oxide hexafluorophosphate (HATU) and *N*,*N*-diisopropylethylamine (DIPEA) was achieved to generate compound **5** in 61% yield. The Fmoc group of compound **5** was removed using 20% piperidine in DMF, followed by the hydrolysis of the *tert*-butyl ester (O*t*-Bu) groups using 25% trifluoroacetic acid (TFA) in dichloromethane (DCM) to afford compound **6** with an 2-step yield of 40%. *N*-Hydroxysuccinimide (NHS) ester of $BODIPY_{650/665}$ (**7**) was prepared according to our previously reported method.[36] The $BODIPY_{650/665}$ NHS ester **7** was obtained in 6-steps starting from pyrrole and pyrrole aldehyde. The reaction of compound **6** with **7** in the presence of Tris-HCl buffer in dimethyl sulfoxide (DMSO) at room temperature for 16 h afforded the final compound **1** in 47% yield. Compound **1** was purified by reversed-phase high-performance liquid chromatography (RP-HPLC) using a linear gradient of acetonitrile (ACN) and water containing 0.1% formic acid. The chemical structure of compound **1** was analyzed by ^1H NMR, HPLC, and high-resolution MS (see Table 1 and supplementary data).

Compound **1** exhibited strong binding affinity to PSMA with a K_i value of 13.8 nM (Fig. 2 and Table 2), which is in the range of successful *in vivo* molecular imaging. The known potent PSMA inhibitor ZJ-43 was used as control compound.

We evaluated cellular uptake and retention of compound **1** using PSMA-expressing PC3/ML/PSMA and PSMA-negative PC3/ML cell. As shown in Fig. 3, compound **1** was able to bind to PC3/ML/PSMA cells more selectively than PC3/ML cells at lower concentrations, indicating its selectivity for PSMA. However, compound **1** at a concentration of 1 μM showed substantial cellular uptake in the PSMA-negative cell line. It appears that the lipophilic property of $BODIPY_{650/665}$ causes non-specific binding at 1 μM concentration. However, treatment with compound **1** at low concentrations (10 and 100 nM) showed its selective binding for PC3/ML/PSMA cells over PC3/ML cells.

We then evaluated the utility of compound **1** for detecting PSMA-positive tumors in a murine xenograft model. We developed xenograft tumors of PSMA-positive PC3-PIP cells and PSMA-negative PC3-flu cells in the same mouse. Mice were injected with 20 nmol of compound **1** or

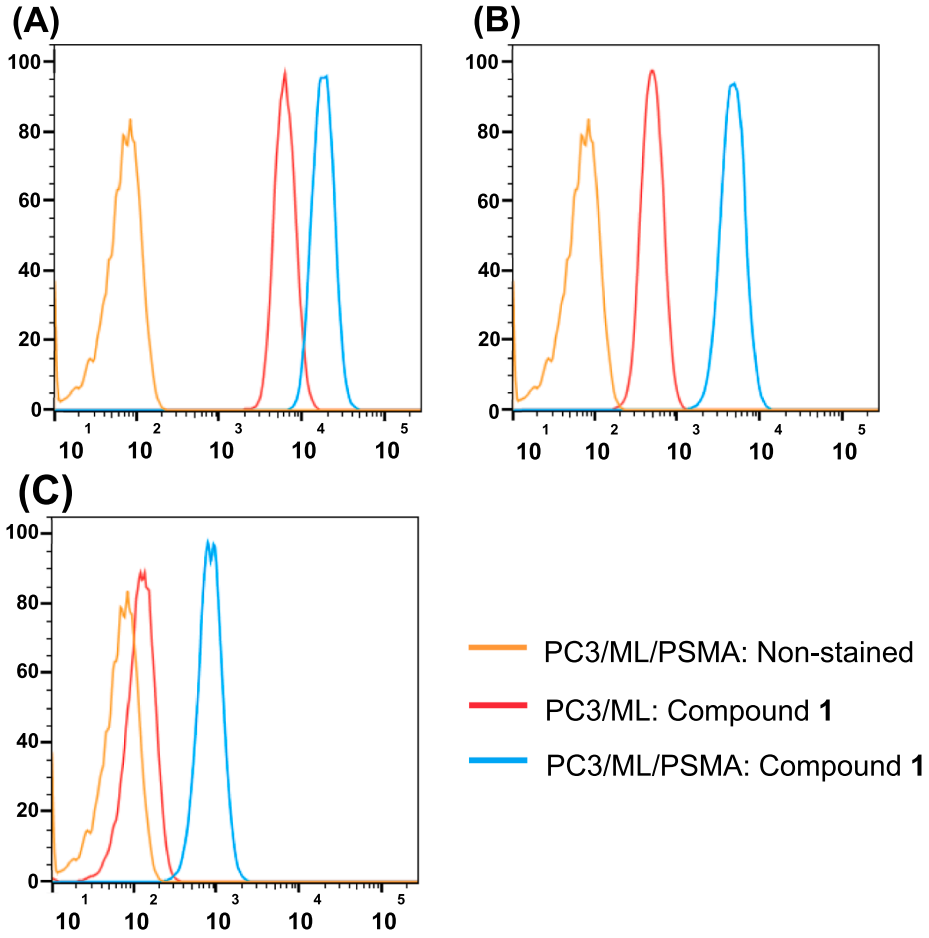


Fig. 3. *In vitro* cellular uptake study of compound **1** in PC3/ML and PC3/ML/PSMA cell lines. Flow cytometric analyses of cells incubated with (A) 1000 nM, (B) 100 nM, and (C) 10 nM of compound **1**.

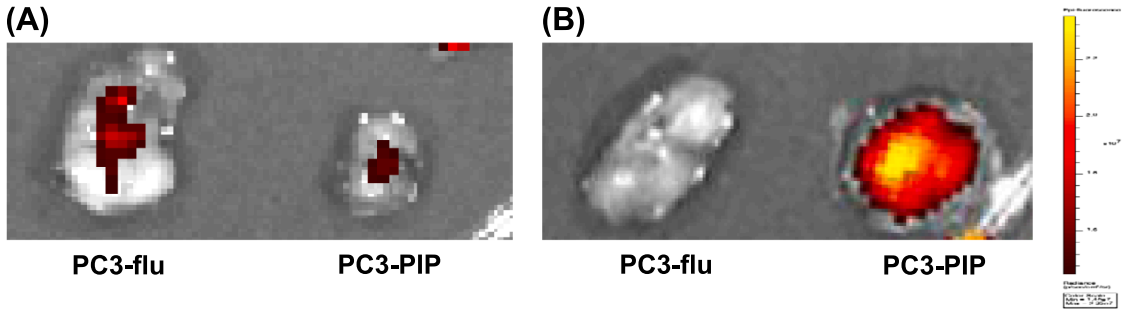


Fig. 4. *Ex vivo* fluorescent imaging of compound **1**. (A) Tumors from a mouse injected with saline. (B) Tumors from a mouse injected with 20 nmol of compound **1**. Fluorescent intensity was referenced to the same scale for both samples. PC3-flu: PSMA-negative human prostate cancer cell line, PC3-PIP: PSMA-positive human prostate cancer cell line.

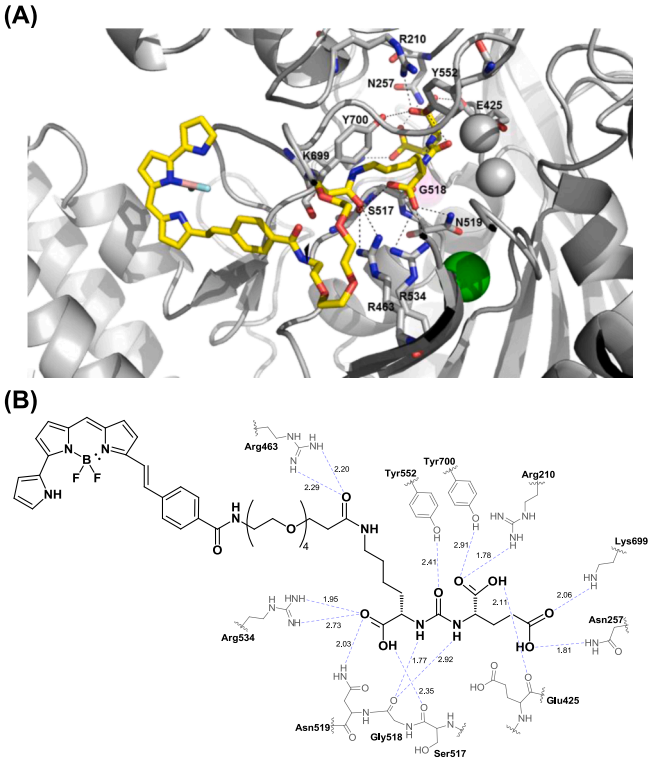


Fig. 5. The best-docked pose of compound **1** in the active site of PSMA. (A) The binding mode of compound **1** at the binding cavity. (B) Hydrogen-bonding interactions between compound **1** and key residues of PSMA protein.

saline, and euthanized at 24 h post injection. Each tumor was dissected and imaged. *Ex vivo* imaging data revealed that compound **1** accumulated only in the PC3/PIP tumor suggesting selective targeting ability of compound **1** *in vivo* (Fig. 4).

In order to investigate interactions and binding mode between compound **1** and PSMA, compound **1** was docked into the active site of the PSMA X-ray crystal structure (PDB ID: 5O5R) using the *Surflex-Dock GeomX* module of *SYBYL-X 2.1.1*. The protein minimization for molecular docking was performed by the '*POWELL*' method with '*AMBER7 FF99*' Force Field setting. '*Surflex-Dock protomol*' was used to guide docking site as defined by the '*Ligand*' method. Other docking parameters were kept to the default values. The boron atom was replaced with a C. 3 atom type for docking studies because the *SYBYL-X* program does not provide a boron atom type to coordinate with the lone pair electron of nitrogen of the $BODIPY_{650/665}$ core (for details see supplementary data). As shown in Fig. 5, the glutamate moiety of Glu–urea–Lys was oriented and bound in the S1′ pharmacophore pocket of PSMA through hydrogen bonds with the side chains of Arg210, Asn257, and Lys699. The carboxylic group of the lysine moiety provided hydrogen bonding with Arg534, Asn519, and Ser517. The PEG linker amide bond between Glu–urea–Lys and $BODIPY_{650/665}$ formed a hydrogen bond with Arg463 and was projected into the tunnel region. The bulky $BODIPY_{650/665}$ moiety was positioned on the surface of the protein without direct interaction with PSMA.

In summary, we designed and synthesized a novel PSMA-binding optical ligand which was labeled with NIR dye $BODIPY_{650/665}$. The *in vitro* PSMA inhibition assay and cell uptake study showed strong and specific binding for PSMA. As the BODIPY moiety can be applied for optical imaging as well as PET imaging by replacing ^{19}F with radioactive ^{18}F, compound **1** has the potential to be utilized as a NIR imaging probe as well as dual-modality to combine PET and optical imaging.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We acknowledge the supports from the National Research Foundation (2017R1A2B4005036 and 2019R1A6A1A03031807) and Ministry of Health & Welfare, Republic of Korea (HI16C1827).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.126894.

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