Pyro-Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys(BHQ3)

$PP_{MMP7}B$

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Chemical name:	Pyro-Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys(BHQ3)	
Abbreviated name:	$PP_{MMP7}B$	
Synonym:		
Agent category:	Peptide	
Target:	Matrix metalloproteinase subtype-7 (MMP-7)	
Target category:	Enzyme	
Method of detection:	Optical imaging, near-infrared (NIR) fluorescence	
Source of signal/contrast:	Pyropheophorbide α (Pyro)	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Photodynamic therapy (PDT), also known as photochemotherapy, uses light-activated photosensitizers (PS) in the presence of oxygen to kill cells (1). PDT has become a promising modality to treat skin, esophagus, and lung cancers, as well as other diseases such as atherosclerosis, macular degeneration, and rheumatoid arthritis (2). In PDT, light excites the singlet state of the PS, followed by intersystem transition from the singlet state to the triplet state; then, the energy is transferred from the triplet state of the PS to the triplet ground state of oxygen, $^3O_2(X^3\Sigma_g^-)$ ($^3O_2$ triplet state quenching) to generate singlet oxygen, $^1O_2(a^1\Delta_g)$ (3). The produced $^1O_2$ is a major cytotoxic agent that has a short life time (<200 ns) and an average diffusion range (~20 nm, which is smaller than the diameter of a cell) (2). Such a short diffusion range requires the delivery of target-

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specific PS agents into subcellular compartments such as cytoskeletal tubulin, lysosomes, mitochondria, plasma membrane, and the nucleus, where they can generate $^1O_2$ efficiently (2). A novel type of PS agent, called a photodynamic molecular beacon (PMB) or killer beacon, has been developed to meet this requirement (2, 4). A typical PMB consists of four modular components: a fluorescent PS, a quencher, a linker, and a delivery vehicle. The target-specific linkers keep the fluorescent PS and the quencher within effective distance of the Föster radius (3–6 nm) (2), which allows efficient fluorescence resonance energy transfer between the fluorescent PS and the quencher. As a result, the fluorescent PS is silent until the PMB meets the target, where the enzyme cleaves the linker and activates the fluorescence of the PS (4). Thus, the PS performs two functions by producing $^1O_2$ to kill cells and by illuminating detectable fluorescence to image its own therapeutic outcome (5).

Matrix metalloproteinases (MMPs) have been pharmaceutical targets for many years because they play important roles in many diseases such as atherosclerosis, lung pulmonary fibrosis, and cancer (4). MMPs in tumors aid the degradation of extracellular matrix, facilitate neoplastic cell motility, and direct cell invasion (4). Also known as matrilysin, MMP subtype-7 (MMP7) is one of only a few MMPs that are actually secreted by tumor cells (6). Pyro-Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys(BHQ3) ($PP_{MMP7}B$) is a PMB specific for MMP7, and it is detectable with near-infrared (NIR) fluorescence imaging (4). $PP_{MMP7}B$ consists of the infrared fluorescence PS pyropheophorbide α (Pyro), a black hole quencher 3 (BHQ3), and a peptide linker (Gly-Pro-Leu-Gly-Leu-Ala-Arg-Lys (GPLGLARK)) (4). The peptide contains the tripeptide motif Pro-Leu-Gly for MMP7 recognition, and the cleavage site is located between the Gly and Leu residues. Pyro acts as the intracellular delivery vehicle and as the PS (absorption, 665 nm; emission, 675 nm and 720 nm) with good $^1O_2$ production (50%). Pyro lacks dark toxicity (toxicity in absence of light) because of its low absorption between 450–600 nm. BHQ3 (absorption, 672 nm) can efficiently quench Pyro fluorescence *via* fluorescence resonance energy transfer (FRET). The cleavage of Pyro-Gly-Asp-Glu-Val-Asp-Gly-Ser-Gly-Lys(BHQ3) (PPB) by MMP7 separates the PS (Pyro) from the quencher (BHQ3) and restores the Pyro fluorescence for detection.

Synthesis

[PubMed]

Zheng et al. reported the detailed synthesis of $PP_{MMP7}B$ (4). A peptide of Fmoc-GPLGLAR(Pbf)K(Mtt)-Sieber resin (Pbf is 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl; Mtt is methyltrityl) was synthesized by the manual, Fmoc, solid-phase, peptide synthesis protocol with the use of commercially available N-α-protected amino acids as building blocks, Sieber amide resin as a solid support, and N-hydroxybenzotriazole (HOBt)/2-(benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) as amino acid activators. After the last Fmoc group cleavage, Pyro-acid was coupled to the N-terminal glycine of the peptide-resin at a molar ratio of 3:1. The produced peptide resin was treated with 3% trifluoroacetic acid (TFA) and 5% triisopropylsilane (Tis) in

dichloromethane to give Pyro-GPLGLAR(Pbf)K, followed by reaction with BHQ3-N-hydroxysuccinimide (NHS) to produce Pyro-GPLGLAR(Pbf)K(BHQ3). The resulting peptide was treated with 95% trifluoroacetic acid and 5% triisopropylsilane to produce $PP_{MMP7}B$. The labeling was confirmed with ultraviolet-visible spectroscopy by identifying Pyro-specific absorbance at 414 nm and BHQ3-specific absorbance at 676 nm.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The specificity of $PP_{MMP7}B$ to MMP-7 was tested in aqueous solution by measuring the fluorescence emitted from the Pyro in $PP_{MMP7}B$ and the production of singlet oxygen $^1O_2$ induced by the activated Pyro (4). $PP_{MMP7}B$ emitted 15-fold less fluorescence than PP_{MMP7} (the positive control without attached BHQ3), which demonstrated an efficient quenching of Pyro fluorescence by BHQ3. In the presence of the enzyme MMP7, an immediate increase in the Pyro fluorescence of $PP_{MMP7}B$ was observed, which reached a plateau at 3 h with a 12-fold increase in fluorescence. However, in the presence of MMP2 or the co-presence of MMP7 and its inhibitor, no noticeable increase in Pyro fluorescence was observed. No cleavage by MMP7 occurred when $PP_{MMP7}B$ was replaced with its scrambled sequence (Pyro-GDEVDGSGK-BHQ3). The results were further confirmed with matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF). The production of $^1O_2$ was evaluated through the NIR luminescence of $^1O_2$ at 1,270 nm. $^1O_2$ production in $PP_{MMP7}B$ was 18-fold lower than in PP_{MMP7} or in a mixture of PP_{MMP7} and BHQ3. This demonstrated that the close proximity of Pyro and BHQ3 to the self-folding of the MMP7-cleavable peptide effectively inhibited the $^1O_2$ production of Pyro. Adding MMP7 to $PP_{MMP7}B$ restored the quenched $^1O_2$ production by 19-fold, whereas the presence of MMP7 and its inhibitor prevented this restoration. This confirmed that MMP7 is suitable specifically for cleavage of $PP_{MMP7}B$, which leads to the separation of Pyro and BHQ3 and the photo-activation of Pyro.

Zheng et al. used $PP_{MMP7}B$ to study MMP-7–triggered PDT in cancer cells *in vitro* (4). KB cells (human nasopharyngeal epidermoid carcinoma cells) with high MMP7 expression were used as $MMP7^+$ cells, and BT20 cells (human breast cancer cell line) with deficient MMP7 expression were used as $MMP7^-$ cells. After incubation with $PP_{MMP7}B$ or its scrambled sequence (Pyro-GDEVDGSGK-BHQ3 (C-PPB)), confocal microscopy was performed with excitation at 633 nm and detection at >650 nm. A strong fluorescence signal was observed in KB cells incubated with $PP_{MMP7}B$, whereas minimal fluorescence was found in BT20 cells incubated with $PP_{MMP7}B$ and in KB cells or BT20 cells incubated with C-PPB. High-performance liquid chromatography was used to analyze the cell medium collected at the end of drug incubation, which demonstrated that the cleavage of MMP7 occurred in the KB cell medium and produced two fragments as found in the solution studies. No cleavages were found in the BT20 cell medium. These results suggest that the Pyro on $PP_{MMP7}B$ also serves as a delivery vehicle to cross the cell membrane in addition to being a fluorescence dye and a PS. The subcellular location of $PP_{MMP7}B$ and its cleaved fragments was examined with confocal microscopy *via* the

measurement of Pyro fluorescence and MitoTracker (stain for mitochondria) fluorescence (4). The Pyro fragment of $PP_{MMP7}B$ was located in nearby mitochondria but was absent from the nucleus. The $^1O_2$ production was evaluated through the measurement cell viability with the use of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) in KB cells and BT20 cells before and after PDT treatment. Compared with cells not treated with drugs or light, no noticeable dark toxicity was found for treatment with up to 4 μM $PP_{MMP7}B$ or C-PPB. Upon PDT treatment, only $PP_{MMP7}B$ reduced the viability of the KB cells. To further confirm whether this PDT-induced cytotoxicity occurred *via* the apoptotic mechanism, Apoptag Plus *in situ* fluorescein was used to stain the KB cells. KB cells with $PP_{MMP7}B$ and PDT treatment demonstrated a strong signal in the Apoptag fluorescein channel (excitation, 488 nm; emission, 497–580 nm), whereas KB cells with $PP_{MMP7}B$ but without PDT treatment exhibited no significant apoptosis, which suggests that apoptosis may be the primary cytotoxic mechanism.

Animal Studies

Rodents

[PubMed]

Zheng et al. studied the cleavage of $PP_{MMP7}B$ *in vivo* with fluorescence imaging (4). A mouse bearing two KB tumors (one on each flank) was injected intravenously with 80 nmol $PP_{MMP7}B$. Compared with the prescan images or with the drug-free mouse, no increase in Pyro fluorescence was observed immediately after injection. However, the fluorescence in the tumor increased 20 min later and peaked at 3 h, indicating MMP7-triggered $PP_{MMP7}B$ activation. At 3 h, PDT treatment was administered to the tumor on the left flank, whereas the tumor on the right flank served as a dark control. One hour after PDT treatment (4 h after injection), the treated tumor became edematous, whereas the untreated tumor showed no changes in size or fluorescence. For comparison, a drug-free mouse bearing two tumors was treated in the same way; no changes in size or fluorescence were observed in either tumor. Three days after PDT, the treated tumor in the drugged mouse decreased in size, whereas the untreated tumor and both tumors in the drug-free mouse continued to grow. These data demonstrate that $PP_{MMP7}B$ that accumulated in $MMP7^+$ tumors is photodynamically activatable.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

NIH Support

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