Cbz-Phe-Lys(Cy5)-methyl ketone-2,6,dimethylterephthalic amide-hexyl-QSY 21

GB137

Huiming Zhang, PhD[1]

Created: November 24, 2008; Updated: January 5, 2009.

Chemical name:	Cbz-Phe-Lys(Cy5)-methyl ketone-2,6,dimethylterephthalic amide-hexyl-QSY 21	
Abbreviated name:	GB137	
Synonym:		
Agent category:	Small molecule	
Target:	Cysteine cathepsin	
Target category:	Enzyme	
Method of detection:	Optical imaging	
Source of signal/contrast:	Cy5	
Activation:	Yes	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Cysteine cathepsins are proteolytic enzymes that belong to the papain subfamily of cysteine proteases (1). The human family of cysteine cathepsins consists of 11 members: cysteine cathpsin B, C, F, H, K, L, O, S, V, W and X (2). The active site in these enzymes contains a cysteine residue that acts as a nucleophile for attacking the peptide bond of

[1] National Center for Biotechnology Information, NLM, NIH, Bethesda, MD; Email: micad@ncbi.nlm.nih.gov.

NLM Citation: Zhang H. Cbz-Phe-Lys(Cy5)-methyl ketone-2,6,dimethylterephthalic amide-hexyl-QSY 21. 2008 Nov 24 [Updated 2009 Jan 5]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

substrates, and a His residue that acts as a general base for shuttling protons (3). As a key component in cell division, cell death and antigen presentation, cysteine cathepsins are found to be associated with various diseases, including cancer, arthritis, osteoporosis, cardiovascular diseases and neurodegenerative disorders (4). Increased expression, activity and mislocation of several cysteine cathepsins (B and L) are significantly correlated with malignant progressions in tumors (2). Like the other four catalytic types of mammalian protease (aspartic, metallo, serine and threonine), the cysteine cathepsins are synthesized as inactive zymogens and their activity is post-translationally regulated (5). Thus, the abundance of these enzymes may not directly correlate to their proteolytic activity. Activity-based proteomics is a novel technology that has been developed recently to characterize the activity of proteolytic enzymes (6, 7). In this method, small molecules known as activity-based probes (ABPs) covalently bind to the catalytic site of specific target enzymes in complex proteomes. ABPs comprise three basic elements: a reactive functional group known as a warhead for labeling enzymes via covalent binding, a linker for preventing steric congestions, and a reporter tag for identification, purification and visualization of the probe-labeled enzymes (8). For instance, acyloxymethyl ketone (AOMK) is a warhead specific for cysteine cathepsins (B, L and S). The ketone in AOMKs reacts with the cysteine in the enzyme active *via* a sulfhydryl nucleophile to produce a stable thiomethyl ketone adduct, leading to blockade of cysteine cathepsins (8). With an optical labeling as the tag, the ABPs can be used to image the activity of proteolytic enzymes *in vivo* (1).

CBz-Phe-Lys(Cy5)-methyl ketone-2,6,dimethylterephthalic amide-hexyl-QSY 21(GB137) is an optical agent used for imaging cysteine cathepsins *in vivo* (1). GB137 consists of four components: an AOMK type of warhead to bind specifically to cysteine cathepsin B and cysteine cathepsin L, a hexyl and a Lys as spacers to reduce steric congestion, the infrared fluorescent probe Cy5 (646/664 nm excitation/emission), and the commercial fluorescence quencher QSY 21 (absorption ~661 nm). As a quenched ABP (qABP), Cy5 and QSY 21 are separated by a Lys-methyl ketone in the molecule. In the absence of cysteine cathepsin, the fluorescence emitted by the Cy5 is quenched by the QSY 21, and no fluorescence is observed. Upon binding to the enzyme, GB137 interacts with the enzyme active site to release the quenching group QSY 21 as a side product, and produces a Cy5-labeled cysteine cathepsin that is detectable with infrared fluorescent imaging.

Synthesis

[PubMed]

Blum et al. reported the details of the synthesis of GB137 in multiple steps (1). Initially, 2-chloritrityl chloride resin was loaded with the commercial Fmoc 1, 6-diaminohexane hydrochloride in the presence of diisopropylethylamine (DIEA) in anhydrous dichloromethane (DCM). Then, the Fmoc was removed with 20% piperidine/dimethylformamide (DMF) (v/v). The produced resin-attached 6-aminohexane was reacted with 2, 6-dimethylterephthalic acid in the presence of DIEA, hydroxybenzotriazole (HOBT) and benzotriazol-1-yl-oxytripyrrolidinophosphonium

hexafluorophosphate (PyBOP) to yield resin-attached 2,6-dimethyl-4-(6-aminohexocarbonyl) benzoic acid. The carboxyl terminus was further conjugated with *N*-a-Fmoc-Lys (*N*-ε-Boc)-chloromethyl ketone in DMF to produce a resin-attached AOMK. After removal of the *N*-α-Fmoc with 5% diethanolamine(DEA)/DMF(v/v), CBz-protected Phe (ZF) was coupled to the *N*-α-amine in the Lys using solid-phase peptide synthesis in the presence of HOBT and disopropylcarbodiimide (DIC). The resulting ZF-Lys(*N*-ε-Boc)-2,6 dimethylterephthalic amide 6-aminohexane was released from the resin with 1% trifluoroacetic acid /DCM. In the presence of *N, N*-diisopropylethylamine (DIPEA), the released product (1.1% yield) was sequentially coupled with QSY 21 succinimidyl ester at the 6-aminohexane (75% yield) and with Cy5 succinimidyl ester at the *N*-ε-free amine of Lys (65% yield) to produce GB137 as a blue powder. The molecular weight of GB137 was determined to be 2019.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Blum et al. examined the specificity of GB137 for *in vitro* labeling of cysteine cathepsins (1). A series of cancer cells was used, including oncogene *Hras*1 transfected mouse skeletal myoblast cells (C2C12/Hras1), human breast cancer cells (MDA-MD 231, MDA-MB 435 and MDA-MB 231 MFP), murine fibroblast cells (NIH-3T3), and human glioblastoma cells (U87MG). These cells were pretreated with 5 μM GB111-NH_2, a potent cysteine cathepsin inhibitor, for 1.5 h, followed by incubation with 1.5 μM GB137 for 3 h. Then, the cells were lysed with a dounce homogenizer in detergent buffer. The obtained crude lysates were separated with SDS-PAGE, and visualized with fluorescent scanning to examine the GB137-labeled cysteine cathepsins. In all these cell lines, specifically labeled cysteine cathepsins were observed to be 20-30 kDa in size, which corresponded to GB137-labeled cysteine cathepsins B and/or L. However, the amount of labeled cysteine cathepsins varied over more than 10-fold, with the highest appeared in the C2C12/Hras1 cells. MDA-MD 231 cells, MDA-MB 435 cells, and MDA-MB 231 MFP cells exhibited a similar pattern for cysteine cathepsins with moderate amplitudes. As a control, GB137 was incubated with serum for 4 h to test its sensitivity to serum. No change in the labeling activity of GB137 was observed.

Animal Studies

Rodents

[PubMed]

Blum et al. examined the pharmacokinetics of GB137 *in vivo* with fluorescent imaging (1). BALB/c nude mice (n=3) were subcutaneously implanted with MDA-MB 231 MFP cells at ventral and dorsal locations. Two weeks later, the mice were injected intravenously with 2 μg/g GB137. After injection, fluorescent images were collected with a charge-coupled device (CCD) camera at various time points over a 24 h period. GB137 generated a

specific signal in the tumor location that increased with time and reached a maximum by 6-8 h after the injection. After collecting the images from the last imaging time point, the mice were euthanized and tissues were harvested for protein extraction. The extracts were separated with SDS-PAGE and visualized with fluorescent scanning. GB137-labeled cysteine cathepsins B and L were found in the tissues of tumors, liver, spleen and kidney.

Blum et al. used GB137 to evaluate the *in vivo* efficacy of small-molecule inhibitors for cysteine cathepsins with fluorescent imaging (1). BALB/c nude mice (n=3)with MDA-MB 231 MFP were treated with the general inhibitor of protease N-methyl-piperazine-phenylalanyl-homophenylalanyl-vinylsulfone phenyl (K11777) at a dose of 50 mg/kg twice daily starting at 5 d before the imaging in order to reduce cathepsin activity in tumor tissues. As a control, two mice bearing tumors were treated with DMSO vehicle. Mice were intravenously injected with 25 nmol GB137, and images were obtained at various times up to 10 h. At 10 h, the signal was reduced by 60-80% in the mice treated with K11777 compared to the control mice. To further confirm this observation, the mice were euthanized and the tissues were harvested for analysis. The tumor tissues were imaged with the same CCD camera. The signal intensity in the tumors treated with K11777 exhibited a reduced signal. All tissues were then lysed for protein extraction. The extracts were separated with SDS-PAGE. The GB137 labeled cysteine cathepsins were quantified, which demonstrated that the labeling of specific cysteine cathepsins was inhibited by 50-80% in tissues from the mice treated with K11777 compared to the control mice.

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

RR 20843, EB 05011, CA 72006

References

1. Blum G., von Degenfeld G., Merchant M.J., Blau H.M., Bogyo M. Noninvasive optical imaging of cysteine protease activity using fluorescently quenched activity-based probes. Nat Chem Biol. 2007;**3**(10):668–77. PubMed PMID: 17828252.
2. Palermo C., Joyce J.A. Cysteine cathepsin proteases as pharmacological targets in cancer. Trends Pharmacol Sci. 2008;**29**(1):22–8. PubMed PMID: 18037508.
3. Stoka V., Turk B., Turk V. Lysosomal cysteine proteases: structural features and their role in apoptosis. IUBMB Life. 2005;**57**(4-5):347–53. PubMed PMID: 16036619.
4. Blum G., Mullins S.R., Keren K., Fonovic M., Jedeszko C., Rice M.J., Sloane B.F., Bogyo M. Dynamic imaging of protease activity with fluorescently quenched activity-based probes. Nat Chem Biol. 2005;**1**(4):203–9. PubMed PMID: 16408036.
5. Blum G. Use of fluorescent imaging to investigate pathological protease activity. Curr Opin Drug Discov Devel. 2008;**11**(5):708–16. PubMed PMID: 18729022.
6. Saghatelian A., Cravatt B.F. Assignment of protein function in the postgenomic era. Nat Chem Biol. 2005;**1**(3):130–42. PubMed PMID: 16408016.
7. Evans M.J., Cravatt B.F. Mechanism-based profiling of enzyme families. Chem Rev. 2006;**106**(8):3279–301. PubMed PMID: 16895328.
8. Paulick M.G., Bogyo M. Application of activity-based probes to the study of enzymes involved in cancer progression. Curr Opin Genet Dev. 2008;**18**(1):97–106. PubMed PMID: 18294838.