Cy5-Glu-Pro-Asp-acyloxymethyl ketone

Cy5-AB50

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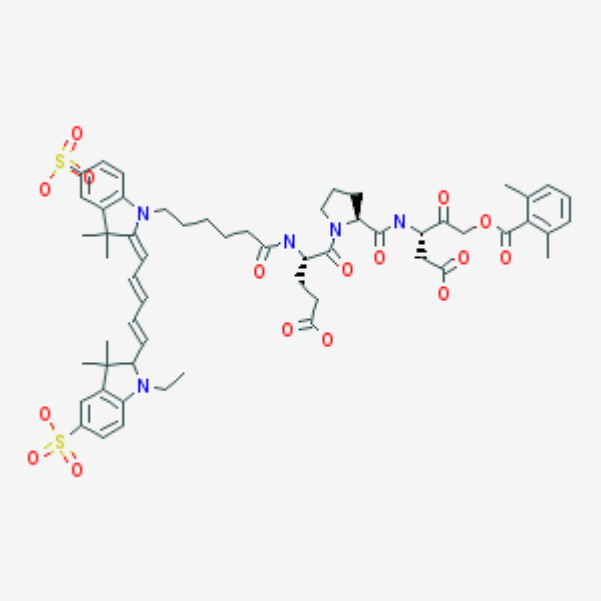
Chemical name:	Cy5-Glu-Pro-Asp-acyloxymethyl ketone	
Abbreviated name:	Cy5-AB50	
Synonym:		
Agent category:	Compound	
Target:	Caspase-3, caspase-7	
Target category:	Enzyme	
Method of detection:	Optical, near-infrared (NIR) fluorescence imaging	
Source of signal:	Cy5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on the above structure for additional information in PubChem.

Background

[PubMed]

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Optical fluorescence imaging is increasingly used to obtain biological functions of specific targets in small animals (1, 2). However, the intrinsic fluorescence of biomolecules poses a problem when fluorophores that absorb visible light (350–700 nm) are used. Near-infrared (NIR) fluorescence (700–1,000 nm) detection avoids the background fluorescence interference of natural biomolecules, providing a high contrast between target and background tissues. NIR fluorophores have wider dynamic range and minimal background as a result of reduced scattering compared with visible fluorescence detection. They also have high sensitivity, resulting from low infrared background, and high extinction coefficients, which provide high quantum yields. The NIR region is also compatible with solid-state optical components, such as diode lasers and silicon detectors. NIR fluorescence imaging is becoming a noninvasive alternative to radionuclide imaging in small animals.

Apoptosis (programmed cell death) plays an important role in the pathophysiology of many diseases, such as cancer, neurodegenerative disorders, vascular disorders, and chronic hepatitis, as well as in the biology of normal cells, such as epithelial cells and immune cells (3). Apoptosis is gene-regulated (4) and is the result of proteolysis of intracellular components by activation of a series of proteolytic enzymes called caspases preceding changes in the plasma membrane structure by translocase, floppase, and scramblase (5-7). As a result, there is rapid redistribution of phosphatidylserine (PS) from the inner membrane leaflet to the outer membrane leaflet, exposing the anionic head group of PS. On the other hand, PS is also accessible for annexin V binding in necrosis because of disruption of the plasma membrane.

Annexin V is a 36-kDa endogenous human protein produced in particular by epithelial cells in many tissues, such as the placenta, umbilical vessels, liver, spleen, kidney, heart, uterus, and skeletal muscle, as well as by erythrocytes, leukocytes, endothelial cells, and platelets (8). Annexin V binds to PS with high affinity (K_D = 7 nM) (5, 9, 10). Apoptosis can be induced by chemicals, radiation, cytokines, hormones, and various pathological conditions (7); therefore, the ability to monitor apoptosis in association with disease progression or regression should provide important information for clinical applications. Annexin V has been radiolabeled with ^{18}F, ^{123}I, ^{125}I, and ^{99m}Tc for imaging (11-14). However, annexin V is not able to distinguish between apoptosis and necrosis. The caspase ligand may be an attractive alternative for imaging cells undergoing apoptosis because caspases are key enzymes that mediate apoptosis (15). Berger et al. (16) reported that acyloxymethyl ketone peptides exhibited selective inhibition of caspase-3 and caspase-7 (executioner caspases) over caspase-8 and caspase-9 (initiator caspases). Glu-Pro-Asp-acyloxymethyl ketone (EPD-AOMK or AB50) has been conjugated with Cy5 for NIR imaging of caspase-3/7 activation in apoptosis (17).

Related Resource Links:

- Chapters in MICAD
- Gene information in NCBI (Caspase-3, Caspase-7).
- Articles in OMIM

- Clinical trials (Caspase inhibitors)
- Drug information in FDA
- Information about Cy5-AB50 in PubChem
- Information about caspase inhibitors in PubChem

Synthesis

[PubMed]

AB50 was synthesized with the solid-phase synthesis method (17). Cy5 was coupled to AB50 to form Cy5-AB50 using the methods as reported by Blum et al. (18). Cy5-AB50 was purified with column chromatography. The identity of Cy5-AB50 was confirmed with liquid chromatography mass spectroscopy with one Cy5 per molecule.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In vitro caspase enzyme assays using AB50 showed inhibition concentration value of ~8 nM for caspase-3 in competition with Cy5-AB46 (17). Induction of caspase-3/7 apoptosis with anti-Fas antibodies in Jurkat tumor cells increased accumulation of Cy5-AB50 (100 nM) over the untreated cells at 30 min and 120 min. The increased caspase activities were completely blocked with AB50 pretreatment. Cy5-AB50 did not bind to cathepsin B as expected.

Animal Studies

Rodents

[PubMed]

Edgington et al. (17) performed *ex vivo* accumulation studies in the thymus in mice treated with dexamethasone for 6, 12, or 24 h (n = 3 mice per group). The thymi were excised for analysis at 2 h after injection of Cy5-AB50 (50 nmol). Caspase-3/7 activity was visualized at 6 h (~12 arbitrary units (au)) after injection, peaked at 12 h (~40 au), and returned to background level at 24 h (~8 au) after injection. The amount of caspase-3/7 in the thymi was confirmed with immunoprecipitation. NIR imaging was performed in tumor-bearing mice treated with Apomab (a DR5-targeted pro-apoptotic receptor agonist) for 2, 5, 8, 11, and 17 h to induce apoptosis in the tumors (19). Tumor fluorescence signal was monitored at 1 h after injection of Cy5-AB50. Quantification of total tumor fluorescence signal correlated with caspase-3/7 levels as measured with gel analysis. The tumor fluorescence peaked at 12 h with more than three-fold increase over the background at 0 h. Whole-body NIR imaging at 12 h showed that there was a four-fold increase of tumor fluorescence intensity in mice treated with Apomab as compared with mice treated with vehicle. No blocking experiment was performed. Quantitation of the % ID/g was not reported.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

NIH Support

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