Cy5.5-Polyethylene glycol-CGS25966 inhibitor of matrix metalloproteinases

Cy5.5-AF489

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Chemical name:	Cy5.5-Polyethylene glycol-CGS25966 inhibitor of matrix metalloproteinases	
Abbreviated name:	Cy5.5-AF489	
Synonym:	Cy5.5-PEG-CGS25988, CGS-Cy5.5	
Agent category:	Compound	
Target:	Matrix metalloproteinases (MMPs)	
Target category:	Enzyme	
Method of detection:	Optical, near-infrared (NIR) fluorescence	
Source of signal/contrast:	Cy5.5	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure is currently not available in PubChem.

Background

[PubMed]

Extracellular matrix (ECM) adhesion molecules consist of a complex network of fibronectins, collagens, chondroitins, laminins, glycoproteins, heparin sulfate, tenascins, and proteoglycans that surround connective tissue cells, and they are mainly secreted by fibroblasts, chondroblasts, and osteoblasts (1). Cell substrate adhesion molecules are considered essential regulators of cell migration, differentiation, and tissue integrity and

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remodeling. These molecules play a role in inflammation and atherogenesis, but they also participate in the process of invasion and metastasis of malignant cells in the host tissue (2). Tumor cells adhere to the ECM, which provides a matrix environment for permeation of tumor cells through the basal lamina and underlying interstitial stroma of the connective tissue. Overexpression of matrix metalloproteinases (MMPs) and other proteases by tumor cells allows intravasation of tumor cells into the circulatory system after degrading the basement membrane and ECM (3).

Several families of MMPs are involved in atherogenesis, myocardial infarction, angiogenesis, and tumor invasion and metastasis (4-7). MMP expression is highly regulated in normal cells, such as trophoblasts, osteoclasts, neutrophils, and macrophages. Elevated levels of MMPs have been found in tumors associated with a poor prognosis for cancer patients (8). There are four members of endogenous tissue inhibitors of metalloproteinases (TIMP1-4), which regulate the activity of MMPs and lead to the inhibition of tumor growth and metastasis (9, 10). CGS25966 is a broad-spectrum, small-molecule inhibitor of MMPs (11). Faust et al. (11) inserted a polyethylene glycol (PEG) linker with an amino functional group for conjugation with the near-infrared (NIR) fluorescence dye Cy5.5 to form Cy5.5-PEG-CGS25966 (CY5.5-AF489). Waschkau et al. (12) evaluated Cy5.5-AF489 for use with *in vivo* NIR fluorescence imaging of tumors with high or low MMP-2/MMP-9 expression in nude mice.

Related Resource Links:

- Chapters in MICAD (MMP, TIMP)
- Gene information in NCBI (TIMP-2, MMP-2, MMP-8, MMP-9, MMP-13)
- Articles in Online Mendelian Inheritance in Man (OMIM) (TIMP-2, MMP-2, MMP-8, MMP-9, MMP-13)
- Clinical trials (TIMP, MMP)
- Drug information in FDA (TIMP, MMP)

Synthesis

[PubMed]

A solution of Cy5.5-*N*-hydroxysuccinimide ester (0.9 μmol) and the amino derivative ((*R*)-2-(*N*-(4-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)benzyl)-4-methoxyphenylsulfon-amido)-*N*-hydroxy-3-methylbutanamide) (1.7 μmol) was incubated for 2 h at 25°C (12). Cy5.5-AF489 was purified with high-performance liquid chromatography, with 43% yield and >97% purity. Mass spectroscopy analysis confirmed conjugation of Cy5.5 to the amino derivative. Cy5.5-AF489 exhibited a molecular weight of 1.45 kDa. Fluorescence absorption maximum of Cy5.5-AF489 was 678 nm, with an excitation coefficient of 250,000 $M^{-1}cm^{-1}$.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Using *in vitro* enzyme activity inhibition assays, Cy5.5-AF489 exhibited IC_{50} values of 1.5 ± 0.6 nM, 1.8 ± 0.2 nM, 2.1 ± 0.1 nM, and 1.7 ± 0.3 nM for MMP-2, MMP-8, MMP-9, and MMP-13 (12), respectively. *In vitro* cellular fluorescence MMP-binding assays of Cy5.5-AF489 were performed with tumor sections of human rhabdomyosarcoma A-673 (high MMP-2/MMP-9 expression), fibrosarcoma HT-1080 (weak MMP-2/MMP-9 expression), and mammary carcinoma BT-20 (low MMP-2/MMP-9 expression). High fluorescence intensity rank order (Cy5.5-AF489 binding) was A-673 > HT-1080 > BT-20. Furthermore, Cy5.5-AF489 fluorescence was colocalized with MMP-2 and MMP-9 in the A-673 tumor sections.

Animal Studies

Rodents

[PubMed]

Waschkau et al. (12) performed *ex vivo* optical imaging studies of Cy5.5-AF489 (2 nmol/mouse) in nude mice bearing A-673 tumors at 6 h (n = 12), 24 h (n = 12), and 72 h (n = 20). High NIR fluorescence levels were observed for the tumor (282.4 ± 56.9 arbitrary units, AU) and kidneys (197.1 ± 14.5 AU), followed by the lungs (150.5 ± 39.5 AU) and liver (113.7 ± 25.5) at 6 h after injection. Low fluorescence signal was observed in the spleen, muscle, heart, and brain. NIR fluorescence intensity in the tumor and normal tissues was reduced by 50% at 24 h and was mostly eliminated by 72 h. The level of Cy5.5-AF489 remained at 20% of injected dose in the blood at 6 h and <10% at 24 h.

Whole-body fluorescence reflectance imaging was performed for up to 72 h in mice bearing A-673, HT-1080, MDA-MB 231, or BT-20 xenografts after injection of Cy5.5-AF489 (2 nmol/mouse) (12). A rapid and clear visualization of the tumors was observed at 30–45 min, and visualization remained consistent for ~5 h before declining to the fluorescence levels of the abdomen and muscle by 72 h. A-673 tumors (727.7 ± 44.2 AU) exhibited higher signal than HT-1080 (569.9 ± 14.1 AU), MDA-MB-231 (519.9 ± 15.2 AU), and BT-20 (468.3 ± 37.3 AU) tumors. Fluorescence intensities were 320–350 AU for the abdomen and muscle at 6 h. The maximal tumor/muscle ratios were 2.3, 1.8, 1.6, and 1.6 for A-673, HT-1080, MDA-MB 231, and BT-20 tumors, respectively. NIR fluorescence signal in A-673 tumors was inhibited by 34% after pretreatment with the MMP inhibitor CGS27023A (200 nmol, 15 min) ($P < 0.05$). There was a significant correlation between NIR fluorescence intensities and MMP-2/MMP-9 levels in the four tumors ($P < 0.001$).

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.

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