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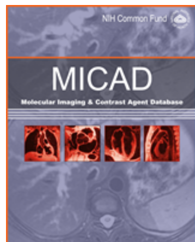

3,3-Diphenylpropylamido-indocyanine sulfonamide

VM315

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Chemical name:	2-{5-[1,1-Dimethyl-6,8-disulfo-3-(3-sulfo-propyl)-1,3-dihydro-benzo[e]indol-2-ylidene]-penta-1,3-dienyl}-6-{[3-(3,3-diphenyl-propylcarbamoyl)-propyl]-methyl-sulfamoyl}-1,1-dimethyl-3-(3-sulfo-propyl)-1H-benzo[e]indolium
Abbreviated name:	3,3-Diphenylpropylamido-indocyanine sulfonamide
Synonym:	VM315
Agent Category:	Small molecule
Target:	Albumin
Target Category:	Binding
Method of detection:	Near infrared fluorescence (NIRF)
Source of signal:	NIR Fluorophore
Activation:	Yes
Studies:	• In vitro • Rodents

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Background

[PubMed]

Near infrared fluorophores (NIRF), described for *in vivo* fluorescence imaging of disease (1, 2) have been generally classified into nonspecific enhancers (e.g. ICG (3)), molecularly targeted fluorochromes (e.g. RGD-Cy (4, 5) or enzyme activatable agents (e.g. cathepsin sensitive NIRF probes) (6). These agents have shown great potential for *invivo* imaging of specific molecular targets, biological processes and cells (7). Specific fluorophores

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have been used to image angiogenesis, apoptosis, protease activity, receptor status, macrophage activity and to track cells (2, 8).

Most of the affinity agents developed to date have molecular weights in excess of several thousand to millions (6, 9-11) to carry affinity ligands and/or to allow efficient quenching/de-quenching in large molecular weight constructs.

Small molecule fluorophores with affinity to albumin could potentially exhibit an increase in fluorescence upon binding; thus a family of “activatable” fluorophores could be developed that consequently would provide enhanced or superior in vivo target-to-background ratios in live animals. As proof-of-principal, VM315, an NIRF containing a 3,3-diphenylpropyl moiety that provides a structural feature capable of non-covalent albumin binding was developed and tested. Those studies showed that VM315 exhibited a remarkable increase in fluorescence upon albumin binding (12).

Such a molecule might be used to improve the detection of small cancers *in vivo*. Given the established role of fluorescent albumins in studying microvascular permeability in disease processes, VM315 may potentially represent a viable option for obtaining similar measurements clinically.

Synthesis

[PubMed]

VM315 was prepared by Montet et al. (12) as follows: VivoTag-S680® (1.1 mg, 1 µmol) and 3,3-diphenylpropylamine (1.1 mg, 5 µmol, Aldrich) were combined in approximately 100 µl of anhydrous DMF and kept at room temperature for one hour. The product of the reaction was then purified by RP-HPLC (25 mM triethylammonium acetate, pH 7, acetonitrile gradient) and isolated as a lyophilized solid (yield 90%). It was possible to complete the procedure of synthesis and purification in a day, with the time required for lyophilization dependent on the lyophilizer used. (ESI-MS m/e 1231.32065 $[M]^+$: calculated for $C_{59}H_{67}N_4O_{15}S_5^+$, was 1231.3207).

In Vitro Studies

[PubMed]

In vitro studies (12) showed that VM315 was fully soluble in water with an octanol/water partition coefficient of 11%. The absorption maximum was found to be at 685 nm and the emission maximum at 705 nm.

The fluorescence emission of VM315 in saline and serum exhibited an increase of over 210% in fluorescence upon albumin binding. Additional experiments at variable concentrations confirmed that the fluorescence increase was not due to quenching effects at the baseline state that can occur with less water soluble indocyanines such as indocyanine green (13).

VM315 showed selective albumin binding with >90% (determined by HPLC) of fluorochrome attached at diagnostic concentrations. The compound did not bind to any other plasma proteins (e.g. globulins) as determined by gel chromatography. K_i and EC50 were not available, but the the combined vascular half-life was approximately 108 min, as determined by intravital confocal microscopy (12).

Animal Studies

Rodents

[PubMed]

Montet et al. (12) investigated the use of VM315 in detecting small cancers in rodents by performing a series of imaging studies using intravital confocal microscopy, reflectance fluorescence and tomography imaging on 25 mice (5 groups of 5) bearing Lewis lung carcinoma (LLC), rodent gliosarcoma (9 L) and human colon adenocarcinoma (CT26). The animals were injected intravenously with VM315 (130 nmol/kg bodyweight corresponding to 4 nmol/mouse) prior to imaging.

Intravital confocal microscopy images of normal microvasculature of the ear were first obtained and signal intensity (SI) was recorded inside a vessel; mono-exponential decay function was used to calculate the blood half-lives of the compounds. Results showed a rapid (1 minute) increase in vascular fluorescence followed by a further steady but slower increase over the subsequent 30 minutes, presumably due to "albumin activation". Beyond 30 min, microvascular fluorescence slowly decreased due to elimination ($T_{1/2}$ 76 min). The apparent combined vascular half-life was found to be 108 min, and considerably longer than for other small molecule indocyanines (e.g. < 10 min for ICG). Over time, Montet et al. (12) observed a slow interstitial vascular leak, with kinetics similar to covalently FITC labeled albumins (14, 15).

Montet et al. (12) hypothesized that tumoral accumulation of brightly labeled serum albumin could be used as a marker for abnormal by enhanced permeability and retention (EPR effect) (19), and therefore studied the kinetics of tumoral accumulation of VM315 in a heterotopic CT26 colon cancer model, using reflectance fluorescence imaging. Serial images were obtained at 0.25, 5, 10, 20, 30, 60, 120, 240 and 1440 min after intravenous injection and SI was recorded for tumors and skin using a Cy5.5 channel. Regions of interest (ROI) were defined by the outlines of the tumor from the white light image and non-tumor ROIs were taken from adjacent skin. A third ROI was placed outside the animal to obtain the noise of the system. The tumor-to-background ratio (TBR) was calculated as: TBR = SI_{tumor}-noise / SI_{skin}-noise. Results showed that VM315 exhibited a pronounced tumoral accumulation over time with maximum contrast values obtained 2 hours after intravenous injection. Maximum tumor-background ratio was 2, corresponding to a 290% increase in signal intensity over baseline.

In order to evaluate the extent of accumulation enabling the detection of small orthotopic tumors (< 3 mm in diameter) and a greater depth penetration and quantification, Montet et al. (12) performed fluorescence molecular tomography (FMT) imaging (16) - instead of surface weighted reflectance imaging - on nude mice bearing 9 L, LLC and CT26 tumors injected intravenously with VM315, for a 2 h time period. All tumors were detectable by FMT, and fluorochrome mapping results in similar amounts per tumor were found to be consistent with those performed on excised specimen. *In vivo* measurements (in pmol of fluorochrome per tumor of ~ 4 mm, ± 5-10%) were as follows: 68 for lung cancer (LLC), 143 for glioma (9 L), and 46 for colon cancer (CT26). Histology confirmed the presence of tumors and fluorescence microscopy, the presence of fluorochrome within individual lesions.

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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