1,1′-bis-(4-sulfobutyl)indotricarbocyanine-5,5′-dicarboxylic acid diglucamide monosodium salt

SIDAG

The MICAD Research Team

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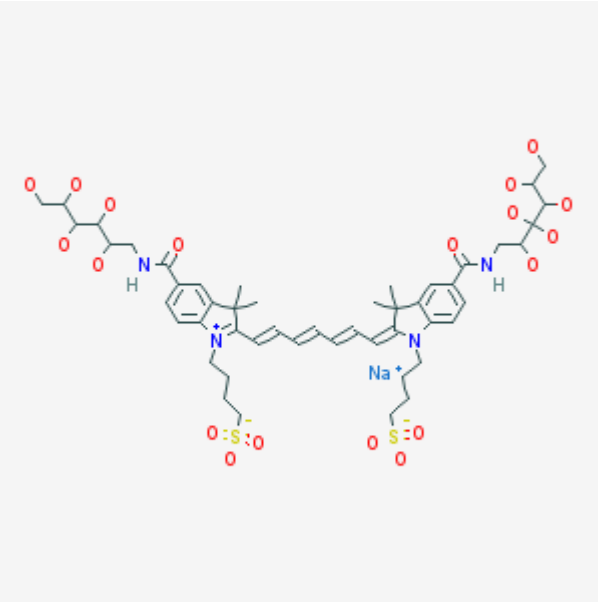
Chemical name:	1,1'-bis-(4-sulfobutyl)indotricarbocyanine-5,5'-dicarboxylic acid diglucamide monosodium salt
Abbreviated name:	SIDAG
Synonym:	
Agent Category:	Peptide
Target:	Non-targeted
Target Category:	Binding
Method of detection:	Optical imaging (NIRF)
Source of signal:	SIDAG
Activation:	No
Studies:	• *In vitro* • Rodents



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Background

[PubMed]

In recent years, an increasing number of imaging studies have been carried out using all-optical, imaging technology that absorb and emit in the near-infrared (NIR) spectrum (1-4). NIR imaging has the advantage of reducing the background and scattering through

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biological tissue usually encountered with fluorescent imaging in live animals with short wavelengths of light.

The indocyanine green (ICG) is a well-known, clinically approved, carbocyanine non-specific NIR-fluorescent dye (5, 6) used for example for testing hepatic functions (7). It has been investigated as a potential contrast agent in animals (8) and in humans for the detection of breast tumors (9). ICG presents major disadvantages that make its use very limited: a high level of plasma protein binding (up to 98% a few seconds after injection), a rapid clearance by the liver, a low stability in aqueous media and a poor fluorescence efficiency (5, 10).

Major effort is being made in designing new NIR-absorbing and fluorescing probes with more favorable characteristics, such as a high hydrophilicity and low levels of plasma protein binding. One of them, 1,1'-bis-(4-sulfobutyl)indotricarbocyanine-5,5'-dicarboxylic acid diglucamide monosodium salt (SIDAG), is a highly hydrophilic derivative based on a carbocyanine chromophore identical to ICG, and showing a low plasma binding of ~10%.

SIDAG It has an estimated blood half-life of ~200 min, is 60 times more biocompatible than ICG (5), less toxic (11), and has a significantly greater fluorescent yield compared with ICG (5, 12). Its absorption and emission maximum wavelengths are 800 and 830 nm respectively.

Synthesis

[PubMed]

Synthesis details for preparing SIDAG were reported by Licha et al. (5) in 2000. The procedure described by the authors involved a derivatization of the symmetric dicarboxylated indotricarbocyanine bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-dicarboxylic with the amino-sugar D-glucamine, leading to the final symmetric diglucamide SIDAG (0.75g, 53% yield). After purification by chromatography, SIDAG was obtained as a salt. The indotricarbocyanine was obtained in 77% yield by reaction of the carboxylated indolenine, 5-carboxy-1-(4-sulfobutyl)-2,3,3,-trimethyl-*3H*-indolenine with 1-(4-sulfobutyl)-2,3,3-trimethyl-3H-indolenine, both synthesized following the procedures previously described in the literature (13, 14). Details on the various intermediary steps involved in the synthesis of SIDAG can be found in (5).

The partition coefficient (*n*-butanol/water) reported by Licha et al. (5) was <0.005 and the equilibrium plasma protein binding was ~ 10% (maximum standard deviation of 10% of value, 3 measurements performed). Measurements of the absorption maxima $\lambda_{max,abs}$ (nm), fluorescence emission maxima $\lambda_{max,em}$ (nm), molar absorption coefficients ϵ ($l.mol^{-1}.cm^{-1}$) and fluorescence quantum yields Φ (based on ICG as standard, Φ_{ICG} = 0.13) were also made by the authors and the following respective values were obtained: 753, 790, 201 000, 0.066 with PBS as solvent, and 755, 70, 252 000, 0.076 in plasma.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

A value of ~10% plasma protein binding for SIDAG (obtained by equilibrium dialysis) was reported in the literature by Paumgarten et al. (15) in 1970. Other measurements performed by ultrafiltration using 400µl of a 0.5mg/ml solution of SIDAG in spiked mouse serum were reported in details by Perlitz et al. (16) in 2005. The experimental protocol followed by Perlitz et al. involved incubating the dye solution at 37°C for 1h, centrifuging it for 8 min at 1500g, and subsequently performing absorbance and emission measurements with an excitation wavelength of 700nm. The resulting quantum yield obtained for SIDAG was 6.5% (with the quantum yield from ICG in DMSO ($\Phi_{ICG} = 0.13$) used as standard (9)), and the measured free fraction was 40%. Those values appeared to be in agreement with the previous results reported by Paumgarten et al. (15).

Animal Studies

Rodents

[PubMed]

Perlitz et al. (16) investigated the ability of SIDAG to be used as a contrast agent in *in vivo* imaging of subcutaneously grown tumors, using F9-teratocarcinoma bearing mice (n=8, tumor volumes = 100 to 950 mm^3, manganese-free diet to reduce auto-fluorescence). The experimental protocol involved injecting intravenously a physiological saline solution of dye (2 µmol/kg of body) into the tail vein, and recording fluorescence signals at 1, 10, 30 min and 1, 4, 7, 24 and 48 h after injection. Tumor tissue and non-diseased tissue (control region) were selected as regions of interest (ROIs). Quantification of the fluorescence intensities in the ROIs were made using a special NIR-XP software, as detailed in the article (16).

A maximum of fluorescence intensity of approximately a million counts was reached at 10 min after injection (versus 1,100,000 counts in the control regions at that same time). After 4 h, the fluorescence intensity was below 100,000 counts, with higher values in the tumor than in the control region. A discrimination of the tumor from its surrounding areas was observed 4 h after injection, and was still clearly visible for 48 h. A strong fluorescence from kidneys was recorded at 4 and 7 h post-injection, suggesting renal uptake. Because differences in fluorescence intensities between tumor and control regions remained constant for the all 48 h, Perlitz et al. (16) suggested that a tubular re-absorption of a certain amount of dye was re-entering the circulation and leading to prolonged contrasts between tumor and control tissue. Using a two-compartment model, the authors estimated the renal elimination half-life to be 220 min.

Fischer et al. (11) performed NIRF studies using ICG- and SIDAG-NIRF to determine whether normal and inflammatory joints can be differentiated through kinetic properties of those non-specific dyes. The experimental setup involved using 20 healthy mice and 20

mice with induced Lyme arthritis (n=10 for ICG, n=10 for SIDAG for both healthy and arthritic groups). Each group of mice injected with either dye was divided into two sub-groups (n=5): sub-group 1 one received the dye at 1.0µmol/kg of tissue, and sub-group 2 was injected with a solution at 2.0µmol/kg. Data were acquired from ankle joints of the mice, using short exposure times (0.2 s).

Results showed that such technique was indeed able to detect early stages of arthritis, and that SIDAG was retained more readily in inflamed tissues than ICG, providing a sufficient spatial resolution even for small structures within the joints. After injection of SIDAG, a slow increase in fluorescence signal was observed throughout the 150 min observation period, with AUCs of 220 ± 60 counts (sub-group 1) and 290 ± 90 counts (sub-group 2). Histological examination showed a difference of histological score between control and arthritis groups of ~3 (1.7 ± 1.7 versus 6.2 ± 3.2; $P < 0.05$). The detection of inflammation in the joints was found possible at 24h post-injection with SIDAG, but not with ICG. Nevertheless, several limitations were noted by the authors, including the fact that the interval of image acquisition after bolus injection had to be very short, explaining why the peak enhancement after SIDAG was missed.

Licha et al. (5) investigated the efflux behavior of SIDAG as a function of its physicochemical properties, both for healthy and tumor tissues in 9L-glioma-bearing Fischer rats (animal weight = 290 to 350g, tumor volume = ~5 ml). SIDAG was intravenously injected at a concentration of 0.5 µmol/kg of body weight, 2 min after signal recording. A slow clearance of the dye was observed, with calculated half-life times of 75 min for the tumor and 115 min for the control site (assuming a mono-exponential decay). The authors reported a maximal contrast of 1.78 reached at 7 min post-injection, with a subsequent decrease to the native level of 1.3 at ~100 min post-injection.

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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