Quinolinium, 1,1'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[4-[3-(3-methyl-2(3*H*)-benzothiazolylidene)-1-propen-1-yl]-,iodide (1:4)

TOTO-3

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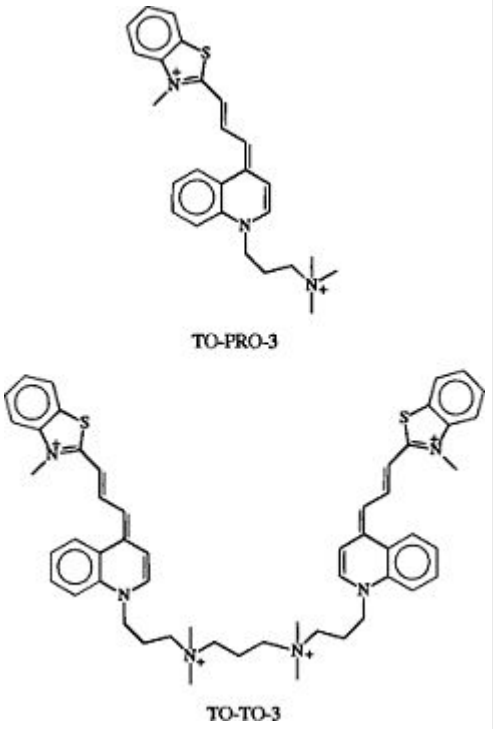
Chemical name:	Quinolinium, 1,1'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[4-[3-(3-methyl-2(3*H*)-benzothiazolylidene)-1-propen-1-yl]-,iodide (1:4)
Abbreviated name:	TOTO-3
Synonym:	
Agent Category:	Compounds
Target:	Nucleic acids (DNA and RNA)
Target Category:	Nucleic acids
Method of detection:	Optical imaging
Source of signal / contrast:	TOTO-3
Activation:	Yes
Studies:	• *In vitro* • Rodents



Structures of TO-PRO-3 and TOTO-3 (1).

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Background

[PubMed]

Quinolinium, 1,1'-[1,3-propanediylbis[(dimethyliminio)-3,1-propanediyl]]bis[4-[3-(3-methyl-2(3*H*)-benzothiazolylidene)-1-propen-1-yl]-,iodide (1:4), abbreviated as TOTO-3, is a cell-impermeable cyanine acid dye that has a high affinity for nucleic acids (1, 2). TOTO-3 shows weak fluorescence in solution but generates significantly increased quantum yield (100- to 1,000-fold increase) when binding to DNA or RNA (1, 3, 4). Furthermore, TOTO-3 has a relatively long wavelength (maximum excitation/emission, 640/660 nm). These optical properties make it suitable to assess plasma membrane integrity and to detect DNA with optical imaging (2, 5). TOTO-3 is the dimeric form of quinolinium, 4-[3-(3-methyl-2(3*H*)-benzothiazolylidene)-1-propenyl]-1-[3-(trimethylammonio)propyl]-,diiodide (TO-PRO-3).

To date, chemotherapy remains the treatment of choice for advanced cancer. However, anticancer agents are often much less efficient against tumor cells *in vivo* compared with their effect *in vitro*. Several characteristics of tumors cause this inefficiency, such as high interstitial pressure, increased collagen fibers in the extracellular matrix, acidic microenvironment, and cell membrane (2). Ultrasound has been shown to facilitate the penetration of drugs into tumors by several mechanisms, such as ultrasound-induced hyperthermia, which can enhance drug accumulation in tumor tissue and trigger the release of the drug payload from temperature-sensitive carriers, and ultrasound-induced pores in cell membrane, which can improve cell internalization by increasing the membrane permeability (6, 7).

Molecular imaging has been investigated to monitor and quantify the processes of ultrasound-mediated intracellular drug delivery (2). For this purpose, contrast agents have usually been coupled to or loaded into drug carriers. However, coupling contrast agents to small molecules, as with chemotherapeutic drugs, may change their pharmacokinetics and pharmacodynamics (2). An alternative strategy is co-delivery of drug-mimicking contrast agents and small molecule drugs. Deckers et al. tested the feasibility of using a fluorescent nuclear acid staining dye (TOTO-3) as a model drug to monitor ultrasound-mediated delivery in real time (2). *I*n vivo fluorescence imaging demonstrated that an optical contrast agent with characteristics similar to an anti-cancer drug may be used for in vivo monitoring of the drug delivery process in real time (2). This chapter summarizes the data obtained with TOTO-3.

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Synthesis

[PubMed]

TOTO-3 is commercially available.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Deckers et al. analyzed the cell internalization of TOTO-3 after incubation with mouse rectal carcinoma line CMT-93 cells without or with preliminary permeabilization with 0.2% Tween (2). When cells were permeabilized before incubation with TOTO-3, strong fluorescent staining of the cytoplasmic RNA and nucleoli was observed. On the contrary, the TOTO-3 fluorescence was not detectable in cells without permeabilization, confirming that TOTO-3 is a cell-impermeable dye.

Animal Studies

Rodents

[PubMed]

Deckers et al. tested the feasibility of TOTO-3 as a smart probe for in vivo monitoring of ultrasound-mediated intracellular drug delivery (2). RAG 2/g_C mice bearing a CMT-93 tumor on both flanks (n = 8 mice) were first co-injected with TOTO-3 and microbubbles directly into both tumors, and ultrasound (1.5 MHz for 1 min) was then applied to one tumor with the second tumor serving as a control. The time course of ultrasound-mediated intracellular delivery of TOTO-3 was measured with fluorescence imaging. A distinct difference in the fluorescence intensity was observed between the ultrasound-treated and control tumors at 2 h and 4 h after ultrasound application. At these time points, two-fold higher mean signal intensity and an even larger difference in the total and maximum intensities were observed in the ultrasound-treated tumor.

Epifluorescence microscopy was performed on the excised tumor sections to demonstrate the internalization of TOTO-3 (2). Relatively large areas with TOTO-3 signal were observed in the ultrasound-exposed tumor sections. In contrast, control tumor sections typically showed no fluorescence or only single cells with TOTO-3 uptake. The overall signal intensity was quite weak in both cases.

The investigators hypothesized that phagocytic cells, such as macrophages, caused ultrasound-independent uptake of TOTO-3, which may explain the observed fluorescence in the control tumors (2). Anti-CD68 staining revealed a significant presence of macrophages in both control and ultrasound-treated tumors. However, TOTO-3 signal was strongly associated with macrophages only in the control tumors. Ultrasound-independent uptake was further confirmed with isolated intraperitoneal macrophages

from female Rag-gamma mice after incubation of TOTO-3 with the macrophages (2). Flow cytometry and confocal microscopy confirmed that macrophages could take up TOTO-3 in an ultrasound-independent manner. The peak fluorescent intensity was in the range of 1×10^3 to 1×10^4, comparable to the intensity observed for the positive control macrophages (permeabilized with 0.2% Tween before incubation with TOTO-3). The negative control sample (incubation without TOTO-3 and Tween) showed negligible fluorescence signal.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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