ZW800-1, a zwitterionic near-infrared fluorophore, and its cyclic RGD peptide derivative cyclo-(RGDyK)-ZW800-1

ZW800-1 and cRGD-ZW800-1

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Chemical name:	ZW800-1, a zwitterionic near-infrared fluorophore, and its cyclic RGD peptide derivative cyclo-(RGDyK)-ZW800-1	
Abbreviated name:	ZW800-1 and cRGD-ZW800-1	
Synonym:		
Agent Category:	Compound	
Target:	Non-targeted (ZW800-1); integrin $\alpha_v\beta_3$ (cRGD-ZW800-1)	
Target Category:	Non-targeted (ZW800-1); receptor (cRGD-ZW800-1)	
Method of detection:	Optical imaging (near-infrared fluorescence (NIRF) imaging)	
Source of signal / contrast:	ZW800-1	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Indocyanine green (ICG) is a noninvasive near-infrared (NIR) fluorescence (NIRF) imaging dye that is approved by the United States Food and Drug Administration (FDA) for ophthalmic angiography to determine cardiac output and liver blood flow and function. This dye is also used in cancer patients to map sentinel lymph nodes (SLN), for

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the detection of solid tumors, and for angiography during reconstructive surgery (2). Methylene blue (MB) is another NIRF dye that has been approved by the FDA for the treatment of drug-induced methemoglobinemia, but MB is also used to visualize SLNs with ultrasound imaging in the clinic (3). Two other commercially available NIRF dyes, Cy5.5 and IRDye800-CW (CW800), have been coupled with peptides or antibodies and successfully used for the targeted visualization of neoplastic tumors in rodents (4). Although the NIRF dyes exhibit low autofluorescence, tissue absorbance, and scatter at NIR wavelengths (700–900 nm), a major limitation of using these dyes in the clinic is that they often show high nonspecific binding and have high tissue uptake and retention (5). In addition, these dyes are cleared from circulation primarily through the hepatobiliary pathway and generate high background signals in the gastrointestinal tract, which results in the masking of signals from cancerous lesions that may be present in tissues located around the digestive system (5). The high background signals interfere with distinguishing the tumors from normal tissues during NIRF-guided resection of the lesions (6). Physiochemical properties such as positive/negative charge density, charge distribution, and hydrophilicity/hydrophobicity (or lipophilicity) are known to affect the protein binding, serum stability, and *in vivo* biodistribution characteristics of compounds and imaging agents, including probes of the NIRF variety (7). In this regard, MB carries a small net positive charge (+1), whereas ICG, Cy5.5, and CW800 carry a net negative charge (−1, −4, and −4, respectively) and are hydrophobic (Table 1) (6). The physiochemical properties of these NIRF dyes are discussed in detail by Gioux et al. (6).

Results obtained from a biodistribution study of NIRF nanoparticles (NPs) with different chemical compositions, hydrodynamic diameters, shapes, and surface charges administered to rats through the pulmonary route showed that zwitterionic NPs (i.e., NPs with a net zero charge or charge-neutral), compared with cationic or anionic charged NPs, were rapidly absorbed from the lungs and were moved to the SLN and into blood circulation for clearance through the kidneys (8). Similar results were reported with zwitterionic quantum dots (QDs) conjugated to CW800 and decorated with a limited number of small molecule (2-(3-amino-3-carboxypropyl)) or peptide (cyclo-RGD-yK) ligands that targeted the prostate-specific membrane antigen and the $\alpha_v\beta_3$ integrin receptor, respectively (9). On the basis of these studies, it was hypothesized that NIRF agents with a net zwitterionic charge would probably behave in a manner similar to the zwitterionic NPs and QDs under *in vivo* conditions (7). In addition, it was expected that the charge-neutral NIRF agents would generate superior images compared with NIRF probes that carried a net anionic or cationic charge. To test this hypothesis, the biodistribution of a series of heptamethine indocyanine NIRF probes with varying net charges, e.g., ICG (net charge −1), CW800 (net charge −4), RS800 (a derivative of CW800, net charge −2), ZW800-1 (a derivative of CW800, net charge 0), and ZW800-3a (a derivative of CW800, net charge +2) was investigated in mice (7). In another study, cyclo-(RGDyK) conjugated to ZW800-1 (cRGD-ZW800-1) was compared with cyclo-(RGDyK) conjugated to CW800 (cRGD-CW800) and cyclo-(RGDyK) conjugated to Cy5.5 (cRGD-Cy5.5) for the visualization of xenograft tumors that overexpressed integrin $\alpha_v\beta_3$ receptors in mice (5). In addition, fibrinogen (FBG) labeled with the NIRF dyes (FBG-

ZW800, FBG-CW800, and FBG-Cy5.5, respectively) was evaluated for the detection of fibrinogen-positive thrombi (blood clots) in mice (5).

Table 1: Physiochemical properties of MB, ICG, Cy5.5, IRDye800-CW, and ZW800-1 NIRF dyes.

Dye	Molecular weight (Da)	Log D (at pH 7.4)	Net charge	Absorption (nm)	Emission (nm)	Quantum yield (%)	Primary clearance route	
							Renal	Hepatic
MB	320	−0.2	+1	670	690	3.8	+	++
ICG	775	7.88	−1	807	822	9.3	−	+++
Cy5.5*	1,069	1.44	−4	680	697	21.0	−	+++
CW800*	1,091	2.51	−4	786	800	14.2	+	++
ZW800-1	1,149	−3.56	0	772	788	15.1	+++	−

*Carboxylic acid derivatives. Abbreviations: MB, methylene blue; ICG, indocyanine green; 800-CW, IRDye800-CW, NIRF, near-infrared fluorescent. For complete data, see Gioux et al. (6).

Related Resource Links

Other NIRF imaging agent chapters in MICAD

Integrin-related chapters in MICAD

Fibrinogen-related chapters in MICAD

Clinical trials involving NIRF dyes

Synthesis

[PubMed]

The synthesis of ZW800-1 and ZW800-3a is described elsewhere (7). The chemical yields and chemical purities of ZW800-1 and ZW800-3a were reported to be >85% and 92%, and 97.1% and 98.7%, respectively (7).

ZW800-1 was also synthesized on a preparative scale using current good manufacturing practice (cGMP) procedures (10). With this method, the final yield of the NIRF dye was >90%, with a purity of >99%, as determined with fluorescence and evaporative light scatter detection.

In another publication, ZW800-1 was synthesized on a preparative scale synthesis, and the chemical yield of the dye was reported to be >85% (5). The final preparation of ZW800-1 had a purity of >98% as determined with analytical reverse-phased high-performance liquid chromatography and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) (5). *N*-Hydroxysuccinimide (NHS) esters of ZW800-1 (ZW800-1 NHS ester), CW800 (CW800 NHS ester), and Cy5.5 (Cy5.5 NHS ester) were prepared for conjugation with cRGD or FBG as described by Choi et al. (5). The purity and chemical structures of all the NIRF dye esters and the NIRF dye

conjugates of cRGD or FBG were confirmed with liquid chromatography-MS and MALDI-TOF MS, respectively (5).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The fluorescence signal with 1 mg ZW800-1 in 50 mM HEPES buffer (pH 7.4) was reported to be ~3.3-fold higher than the signal with the same amount of ICG under the same conditions (10). This indicated that smaller doses of ZW800-1, compared with those of ICG, can probably be used for the different preclinical and clinical studies.

MS analysis of urine obtained from mice or pigs injected intravenously with ZW800-1 showed that the dye was excreted unchanged from both animal species (10).

The binding specificity of cRGD-ZW800-1, cRGD-CW800, and cRGD-Cy5.5 was investigated in *in vitro* assays with M21 cells (human melanoma cell line that expresses integrin $\alpha_v\beta_3$) and M21-L cells (negative controls; do not express integrin $\alpha_v\beta_3$) (5). High background fluorescence signals due to nonspecific binding of the dye were obtained in both cell lines with cRGD-CW800 and cRGD-Cy5.5, but no background signals were observed in cells exposed to cRGD-ZW800-1. To confirm these observations, the NIRF dyes were conjugated to an anti-epidermal growth factor receptor 2 antibody (anti-HER2 Ab), and MDA-MB-231 cells that overexpress the HER2 receptor were exposed to the Ab-NIRF conjugates (5). Although all three conjugates showed specific binding to the HER2-positive MDA-MB-231 cells, a high background signal was observed with the CW800 and Cy5.5 conjugates, even in the control MDA-MB-231 cells that did not express HER2. In addition, the anti-HER2 Ab-Cy5.5 conjugate showed strong nonspecific binding to both cell lines (5). No blocking studies were reported.

When the NIRF dyes were conjugated with secondary Abs and used for immunohistochemical and Western blot analysis of human prostate or breast cancer tissue samples (for a detailed description of these studies, see Choi et al. (5)), it was observed that the zwitterionic ZW800-1 Ab conjugates generated no or very little background signal compared with the anionic CW800 or Cy5.5 Ab conjugates. This again showed that the anionic dyes generated higher background signals compared with the zwitterionic NIRF dyes.

Animal Studies

Rodents

[PubMed]

The biodistribution of CW800, RS800, ICG, ZW800-1, and ZW800-3a was investigated in CD-1 mice and Sprague-Dawley rats (7). The animals (n = 6 animals/NIRF dye) were intravenously injected with 40 pmol dye/g body weight (BW), and the abdominal organs and tissues were surgically exposed at 4 h postinjection (p.i.) for fluorescence imaging.

The amount of dye accumulated in the various organs of the mice and rats is presented as contrast/background ratios (CBR; defined as difference of the fluorescence signal minus the system background, with the difference divided by the system background) in Table 2. Only ZW800-1 was excreted primarily through the kidneys of both rodent species. All the cationic or anionic NIRF dyes showed a high uptake in different organs; all these agents were secreted into the bile and exhibited high fluorescence in the gastrointestinal tract of the animals. The blood circulation half-lives of the NIRF agents in the rodents were reported to be ICG>RS800>ZW800-1>CW800>ZW800-3a (7).

The biodistribution of ZW800-1 manufactured under cGMP conditions was investigated in normal CD-1 mice (10). The mice (n = 3 animals/dose) were intravenously injected with three different doses (30 nmol, 60 nmol, and 120 nmol/mouse) of the dye, and the abdominal organs and tissues were surgically exposed at 4 h p.i. for NIRF imaging. The images showed there was very low uptake of the dye in all the tissues. As discussed above (7), ZW800-1 was excreted primarily through the renal route (61.3 ± 2.15% ID/g) in all the animals, even at the maximum dose (10).

The use of cRGD-ZW800-1, cRGD-CW800, and cRGD-Cy5.5 was evaluated for the visualization of xenograft tumors generated with M21 cells ($\alpha_v\beta_3$-positive) and M21-L cells ($\alpha_v\beta_3$-negative) in mice (5). The animals (n = 5 mice/NIRF conjugate) were injected intravenously with 3 nmol (100 pmol/g BW) cRGD-ZW800-1 or cRGD-CW800 and 10 pmol (500 pmol/g BW) cRGD-Cy5.5, and NIRF images of the animals were acquired at 4 h p.i. Subsequently the CBR, the tumor/background ratios (TBR; defined as CBR of tumor/CBR of surrounding normal tissues), and the positive/negative tumor ratios (PNR; calculated as CBR of receptor positive tumor/CBR of receptor negative tumor) were calculated from the images (Table 3). Data in Table 3 shows that cRGD-ZW800-1 had higher TBR, CBR, and PNR compared with either cRGD-CW800 or cRGD-Cy5.5 up to 24 h p.i. This was primarily because the background signals in the normal tissues and the receptor-negative tumors were higher with cRGD-CW800 and cRGD-Cy5.5 than with cRGD-ZW800-1. The cRGD-NIRF conjugates were also evaluated for the visualization of metastatic tumors in the liver and lungs of mice (5). Although each NIRF dye was observed to accumulate in the hepatic tumors of the animals, only ZW800-1 generated a very low background, not only in this tissue, but in all the other organs also. In comparison, cRGD-CW800 and cRGD-Cy5.5 showed a high background with almost all tissues, including the liver.

During surgery, bleeding can occur in the gastrointestinal tract, and it is important to repair the damaged blood vessels to stop the bleeding. However, it may not be easy to detect the exact location of bleeding in the injured tissue. Therefore, the use of FBG-ZW800, FBG-CW800, and FBG-Cy5.5 was evaluated to detect intraoperative blood clots (thrombi, which are fibrinogen-positive) in the stomach and mesenteric vessels of rats (5). For this study, 1 h after mucosal resection in the stomach or mesenteric vessels, groups of mice (number of animals/group not reported) were injected intravenously with FBG-NIRF (40 pmol/g BW). Fluorescence intensities generated by the targeted FBG-NIRF conjugates were similar at the site of thrombosis in all the animal groups, but a marked

difference was observed in the background signal intensity observed with each conjugate. At 60 min p.i., the CBRs of FBG-ZW800-1 at the mucosal and mesenteric thrombus sites were ~3.5 and ~4, respectively. At this time point, the CBRs for the mucosal and mesenteric thrombi with FBG-CW800 and FBG-Cy5.5 were ~1.9 and ~1, and ~1 and ~0.5, respectively. The higher CBRs obtained with FBG-ZW800-1 were attributed to the lower background generated by this NIRF conjugate compared with the CW800 and Cy5.5 derivatives.

Table 2: Biodistribution of NIRF dyes CW800, RS800, ICG, ZW800-1, and ZW800-3a in mice and rats. Data are presented as CBR.

NIRF dye	Animal species	Organs					
		Kidneys	Muscle	Liver	Intestine	Rectum	Bladder
CW800	**Mouse**	+	+	–	+++	++	+++
	Rat	++	+	+	+++	+	+++
RS800	**Mouse**	+	–	–	++	+++	++
	Rat	+	–	–	+++	++	–
ICG	**Mouse**	–	–	++	+++	+	–
	Rat	–	–	++	+++	+	–
ZW800-1	**Mouse**	–	–	–	–	–	+++
	Rat	+	–	–	–	–	+++
ZW800-3a	**Mouse**	+	+	++	+++	+	+++
	Rat	++	++	+++	++	–	+++

The contrast/background ratio (CBR) was calculated as (fluorescence signal – system background (BG))/BG. The CBR of each tissue was relative to the abdominal wall and was quantified as follows: –: 0 to 1; +: 1 to 2; ++: 2 to 5; and +++: >5. For complete data set, see Choi et al. (7)

Table 3: CBR, TBR, and PNR of tumors at 4 h p.i. in mice injected with cRGD-ZW800-1, cRGD-CW800, or cRGD-Cy5.5 (5).

cRGD-NIRF dye conjugate	TBR		CBR	PNR
	4 h p.i.	24 h p.i.*		
ZW800-1	17.2 ± 1.2	4.8	5.1 ± 0.7	4.2 ± 0.9
CW800	5.1 ± 1.2	2.0	2.6 ± 0.2	2.1 ± 1.0
Cy5.5	2.7 ± 1.4	1.3	1.4 ± 0.2	1.4 ± 1.1

*Standard deviation not available. Abbreviations: TBR, CBR of tumor/CBR of surrounding normal tissues; PNR, CBR of receptor positive tumor/CBR of receptor negative tumor.

Other Non-Primate Mammals

[PubMed]

The biodistribution of ZW800-1 manufactured under cGMP conditions was compared with that of ICG in normal Yorkshire pigs (10). The animals (n = 3 pigs/group) were

injected intravenously with different doses of either ZW800-1 (1.2–12 μmol) or ICG (1.5–15 μmol). Subsequently, NIRF images of the surgically exposed organs/tissues of the pigs were acquired at 1 h p.i. Blood half-lives of ZW800-1 and ICG in the animals were 3.7 min and 17.9 min, respectively. The majority of the ZW800-1 was excreted through the renal system of the pigs at all doses (81.9 ± 10.7% ID for the1.2-μmol dose and 56.7 ± 10.3% ID for the 12-μmol dose). In contrast, ICG was detected in the urinary bladder only after 4 h p.i. Compared with ICG, no uptake of ZW800-1 was observed in other organs/tissues of the animals.

With results obtained from the above studies, the investigators concluded that ZW800-1 is probably superior for use with NIRF imaging compared with either CW800 or Cy5.5 (5). The investigators also mentioned that ZW800-1 has been identified as a first-in-class molecule by the United States National Cancer Institute's Experimental Therapeutics Program for rapid translation into human clinical studies however, they do not mention weather ZW800-1 will be evaluated for the visualization of SNL or for the visualization of tumors during surgical resection (7).

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

No information is currently available.

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