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Abstract. Cannabinoid CB2 receptors (CB2R) hold promise as therapeutic targets for treating diverse diseases, such as cancers, neurodegenerative diseases, pain, inflammation, osteoporosis, psychiatric disorders, addiction, and immune disorders. However, the fundamental role of CB2R in the regulation of diseases remains unclear, largely due to a lack of reliable imaging tools for the receptors. The goal of this study was to develop a CB2R-targeted molecular imaging probe and evaluate the specificity of the probe using human tumor cells that naturally overexpress CB2R. To synthesize the CB2R-targeted probe (NIR760-Q), a conjugatable CB2R ligand based on the quinolone structure was first prepared, followed by bioconjugation with a near-infrared (NIR) fluorescent dye, NIR760. In vitro fluorescence imaging and competitive binding studies showed higher uptake of NIR760-Q than free NIR760 dye in Jurkat human acute T-lymphoblastic leukemia cells. In addition, the high uptake of NIR760-Q was significantly inhibited by the blocking agent, 4-quinolone-3-carboxamide, indicating specific binding of NIR760-Q to the target receptors. These results indicate that the NIR760-Q has potential in diagnostic imaging of CB2R positive cancers and elucidating the role of CB2R in the regulation of disease progression.

Keywords: CB2 receptor; near-infrared; optical imaging; leukemia; fluorescence imaging.

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1 Introduction

Since its discovery in 1993, an increasing amount of effort has been invested in studying the pharmacology and physiopathological role of type 2 cannabinoid receptors (CB2R). Presently, both academia and the pharmaceutical industries consider this receptor as a major therapeutic target. Specifically, CB2R represents a promising target to treat inflammation, pain, osteoporosis, autoimmune diseases, addiction, psychiatric disorders, diabetes, cardiovascular diseases, cancers, and neurodegenerative diseases. For example, recent studies have shown that CB2R plays a fundamental role in tumorigenesis and that the CB2R agonists may represent promising venues to develop cancer treatments. Specifically, some of the most exciting recent research carried out by several laboratories demonstrated that CB2R agonists potently inhibited viability, proliferation, adhesion, and migration of various cancer cells, such as breast, prostate, glioma, colon, lung, thyroid, lymphoma, skin, pancreas, and liver cancers. These data were collected from both cellular systems and preclinical animal models. Additional evidence indicates that the CB2R agonists exhibit promising therapeutic value for treating neurodegenerative diseases. CB2R agonists attenuate Alzheimer’s disease pathogenesis by blocking β-amyloid peptide-induced activation of microglial cells and play a neuroprotective role in Huntington’s disease and amyotrophic lateral sclerosis. The richness of CB2R’s regulatory roles has rendered this receptor as an attractive target to study a variety of diseases and biological processes. However, the precise role of CB2R in the regulation of diseases remains unclear. The ability to specifically image CB2R would contribute to develop reliable CB2R-based therapeutic approaches with a better understanding of the mechanism of CB2R action in these diseases.

Little has been done to target CB2R for imaging studies and therapeutic evaluations. The current imaging techniques to identify CB2R rely heavily on immunostaining; however, all currently available CB2R antibodies have significant nonspecific binding issues, leading to unreliable imaging results. As such, the development of reliable contrast agents for CB2R imaging is critically needed in the field. Few laboratories have developed CB2R imaging agents for positron emission tomography (PET) imaging, and CB2R imaging using other modalities such as optical imaging has been virtually unexplored. Although PET is a great imaging technique for clinical imaging and translational research due to high sensitivity and no limitation in tissue penetration, the spatial resolution is comparatively low. Optical imaging is widely used for biomedical imaging due to its high sensitivity and resolution, as well as low instrument cost. In clinical settings, optical imaging is a promising technology for intraoperative guidance and optical biopsies. Near-infrared (NIR) fluorescent dyes are typically used as the fluorophores for in vivo imaging applications.
because of the relatively deep tissue penetration and negligible autofluorescence in the NIR region (650 to 900 nm).

In our previous study, we reported the first CB2R-targeted NIR fluorescent probe, NIRmbc94, which was synthesized by coupling a conjugable pyrazole-based CB2R ligand, mbc94, with an NIR fluorescent dye, IRDye800CW. We selected the pyrazole structure to develop mbc94 because SR144528, a pyrazole molecule, is a well-known selective CB2R inverse agonist with subnanomolar binding affinity and well-characterized biology. NIRmbc94 was successfully used to image CB2R in a transfected mouse malignant astrocytoma delayed brain tumor (DBT) cell line, CB2-mid DBT, that expresses CB2R at endogenous levels. Later on, NIRmbc94 was also used to select specific CB2R ligands by high-throughput screening in the receptors' native environment. Recently, we reported the first in vivo optical imaging study using another NIR fluorescent probe based on mbc94, NIR760-mbc94, which has an NIR dye (NIR760) with easy synthesis, and high fluorescence quantum yield, stability, and molar extinction coefficient.

In this study, we developed a novel CB2R-targeted NIR fluorescent probe based on a quinoline structure. Quinoline-based molecules were recently reported as highly selective CB2R ligands with binding affinities as high as 0.2 nM. To develop the quinoline-based CB2R probe (NIR760-Q), we synthesized a novel conjugable CB2R ligand based on the quinoline structure, followed by bioconjugation with the NIR760 dye. To demonstrate the translational potential of this new CB2R probe, NIR760-Q was used to image CB2R in Jurkat human acute T-lymphoblastic leukemia cells that naturally overexpress CB2R. To our best knowledge, this is the first CB2R-targeted cellular imaging of human cancer cells that naturally overexpress the target receptor.

2 Methods and Materials

2.1 Synthesis of the CB2R-Targeted NIR Probe, NIR760-Q

The solvents used are of American Chemical Society (ACS) or high-performance liquid chromatography (HPLC) grade. The dye NIR760 and compound 3 were synthesized according to the method reported previously. A Biotage (Charlotte, North Carolina) microwave reactor (model: Initiator+ US/ JPN 356007) was employed for the synthesis of compound 3. Flash column chromatography was run through a Teledyne Isco (Lincoln, Nebraska, combiflash RF) purification system with silica gel (standard grade, 60A, Sorbtech, Norcross, Georgia) or C18-reversed phase silica gel (20 to 40 μm, RediSepRf, Lincoln, Nebraska). 1H and 13C NMR spectra were recorded on a Bruker Avance (Billerica, Massachusetts) III 400-MHz NMR instrument. Mass spectra were recorded on a Waters (Milford, Massachusetts) LCT Premier mass spectrometer. UV/Vis spectra were recorded on a Cary (Santa Clara, California) 100 Bio UV-Vis spectrophotometer, and fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer.

Compound 4. Under argon protection, sodium ascorbate (79 mg, 0.4 mmol) in water (1 mL) and 11-azido-3,6,9-trioxanodecan-1-amine (48 μL, 0.24 mmol) followed by copper (II) sulfate (29 mg, 0.2 mmol) in water (0.5 mL) were added to a solution of compound 3 (83 mg, 0.2 mmol) in ethanol (10 mL). The resulting reaction mixture was stirred at room temperature for 2 h and then poured into water (10 mL), followed by extraction with ethyl acetate (30 mL × 5). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed by rotary evaporation. The crude product was purified by silica gel column chromatography using dichloromethane/Methanol/NH3 H2O (100/10/1) as the eluent. Compound 4 (0.103 g, 81%) was obtained as a colorless oil. 1H NMR (CDCl3): δ = 9.85 (s, 1 H), 8.67 (s, 1 H), 8.61 (s, 1 H), 8.38 (d, 1 H, J = 8.8 Hz), 8.26 (s, 1 H), 7.56 (d, 1 H, J = 8.8 Hz), 4.64 (brs, 2 H), 4.22 (t, 2 H, J = 7.2 Hz), 3.93 (brs, 2 H), 3.56-3.69 (m, 12 H), 2.13 (brs, 6 H), 2.08 (brs, 3 H), 1.84–1.87 (m, 2 H), 1.65-1.73 (m, 6 H), 1.33–1.35 (m, 4 H), 0.87 (t, 3 H, J = 7.2 Hz). 13C NMR (CDCl3): δ = 176.38, 163.53, 147.31, 146.12, 138.48, 130.28, 128.06, 127.85, 123.40, 122.14, 116.89, 112.87, 70.45, 70.53, 69.57, 54.47, 51.71, 50.60, 50.51, 41.88, 36.56, 29.55, 28.84, 28.74, 22.26, 13.88. MS (ESI): calculated for C33H32N6O2S [M + H] m/z 655.39, found m/z 635.02.

NIR760-Q. A mixture of NIR760 (17 mg, 19 μmol), HBTU (11 mg, 29 μmol), and HOBt (3.8 mg, 28 μmol) in dry dimethylformamide (DMF) (1 mL) was stirred under argon at room temperature for 5 min. N,N-diisopropylethylamine (DIEA) (5 μL, 29 μmol) was then added and the resulting mixture was stirred for another 10 min. Next, compound 4 (12 mg, 19 μmol) in anhydrous DMF (2 mL) was added to the dye solution. The resulting mixture was stirred at room temperature in the absence of light for 20 h. The solvent was then removed by rotary evaporation and the resulting solid was purified by C18-reversed phase column chromatography using H2O/Methanol (20% MeOH to 75% MeOH) as the eluent resulting in a relatively pure probe. The green solid was further purified by preparative HPLC using a Phenomenex Jupiter C4 column (250×21.20 mm) at a flow rate of 10 mL/min. Flow A was 0.1% triethylamine (TEA) in water and flow B was 0.1% TEA in acetonitrile. The elution method started with a linear gradient from 30% to 70% B over 20 min, then from 70% to 100% B over 10 min, held at 100% B for 3 min, and finally returned to 30% B over 10 min. After being dried by lyophilization, NIR760-Q (7.1 mg, 24%) was obtained as a green solid. 1H NMR (d6-DMso): δ = 9.98 (s, 1 H), 8.77-8.78 (m, 2 H), 8.76 (s, 1 H), 8.70 (t, 1 H, J = 5.6 Hz), 8.28 (dd, 1 H, J = 2 & 8.8 Hz), 8.12 (d, 2 H, J = 8 Hz), 8.00 (d, 1 H, J = 9.2 Hz), 7.57-7.59 (m, 4H), 7.36 (d, 2 H, J = 8.4 Hz), 7.33 (3d, 2 H, J = 8.4 Hz), 7.03 (d, 2 H, J = 13.6 Hz), 6.41 (d, 2 H, J = 14.4 Hz), 4.60 (t, 2 H, J = 4.8 Hz), 4.46 (t, 2 H, J = 7.2 Hz), 4.26 (brs, 4 H), 3.90 (t, 2 H, J = 5.2 Hz), 3.55-3.59 (m, 12 H), 2.71 (brs, 4 H), 2.55 (t, 4 H, J = 6.8 Hz), 2.07 (brs, 9 H), 1.90-1.97 (m, 6 H), 1.79 (brs, 2 H), 1.67 (s, 6 H), 1.30–1.32 (m, 4 H), 1.08 (s, 12 H), 0.84 (t, 3 H, J = 6.8 Hz). 13C NMR (d6-DMso): δ = 175.44, 171.35, 165.55, 162.79, 160.43, 147.83, 146.96, 145.17, 144.92, 142.20, 141.77, 139.97, 138.31, 133.76, 131.51, 129.94, 129.36, 127.62, 127.59, 127.43, 126.19, 122.52, 122.15, 119.65, 111.71, 110.44, 101.03, 69.75, 69.71, 69.62, 68.87, 68.58, 50.61, 49.73, 48.13, 47.82, 42.69, 41.70, 38.28, 36.08, 28.91, 28.44, 28.03, 27.03, 24.34, 23.26, 21.78, 13.87. MS (ESI): calculated for C79H79N15O17S2 [M + H] m/z 1560.57, found m/z 1560.35.

2.2 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Jurkat cells were seeded into a T75 flask and cultured at 37°C for 72 h. Total ribonucleic acid (RNA) was extracted from Jurkat cells using RNAzol Reagent (Invitrogen, Grand Island, New York). Single strand cDNA was synthesized from total RNA using the SuperScript III first-strand synthesis system.

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2.3 **In Vitro Saturation Binding Assay of NIR760-Q**

We carried out intact cell saturation binding assay to determine binding affinity of NIR760-Q to CB2R. Briefly, Jurkat cells were seeded into 96 well optical bottom plates (1.5 × 10^5 cells per well). Cells were incubated for 30 min with an increasing concentration (0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 80.0, and 160.0 nM) of NIR760-Q at 37°C. For nonspecific binding measurements, 1 μM (final concentration) of the blocking agent 4-quinolone-3-carboxamide (4Q3C, Cayman Chemical, Ann Arbor, Michigan) was added with NIR760-Q to each well, while for total binding measurements, no blocking agent was added. Cells were then rinsed with serum free medium and the fluorescence intensity at 790 nm (relative fluorescence units) was recorded with a Synergy H4 Hybrid Multi-Mode Microplate Reader. DRAQ-5, a commonly used cell nuclear DNA labeling dye, was used to normalize cell numbers based on a fluorescence intensity at 690 nm (excited at 650 nm) using the protocol provided by the manufacturer. The specific binding of total binding was determined by the subtraction of nonspecific binding from total binding. The dissociation constant (K_d) and receptor density (B_max) were estimated from the nonlinear fitting of specific binding versus NIR760-Q concentration using Prism software (GraphPad Prism 6.01, San Diego, California).

2.4 **Cell Fluorescent Imaging of NIR760-Q**

Jurkat cells were treated with 5 μM of NIR760-Q or NIR760 at 37°C for 30 min, with or without coincubation with 10 μM of 4Q3C as the blocking agent. After being washed three times with serum free medium, cells were fixed with 4% paraformaldehyde/PBS for 20 min at room temperature. The cell nucleus was stained with 1 μg/mL 4',6-diamidino-2-phenylindole (DAPI) for 15 min at room temperature. Cells were mounted and then imaged using a Zeiss (Thornwood, New York) Axio Observer fluorescence microscope equipped with the ApoTome 2 imaging system. NIR760-Q or NIR760 fluorescence images were captured using an NIR camera with ICG filter set (excitation/emission: 750 to 800 nm/820 to 875 nm). Nuclear images were obtained with a DAPI filter set (excitation/emission: 335 to 383 nm/420 to 470 nm). Differential interference contrast (DIC) images were obtained through Trans light DIC.

A multiplate reader system (Synergy H4 Hybrid) was used for the quantitative CB2R binding assay measurements. Cells were divided into four groups: (1) Jurkat cells treated with 5 μM of NIR760-Q at 37°C for 30 min, without blocking agent; (2) Jurkat cells treated with 5 μM of NIR760-Q, together with 10 μM of 4Q3C as the blocking agent at 37°C for 30 min; (3) Jurkat cells treated with 5 μM of free NIR760 dye without targeting moiety at 37°C for 30 min; and (4) wild type DBT-cells (CB2R+) treated with 5 μM of NIR760-Q at 37°C for 30 min. To prepare for the measurements, DBT-cells were seeded into 96-well optical plates 24 h before the treatment. Jurkat cells were grown to 90% confluence in T75 flasks, harvested, and seeded into 96-well optical plates (1.5 × 10^5 cells per well).

Since most Jurkat cells were in suspension, the 96-well plates were centrifuged before 5 μM of NIR760-Q or NIR760 with or without 4Q3C were added in culture medium. After being incubated for 30 min, cells were washed three times with serum free medium. A Synergy H4 Hybrid MultiMode Microplate Reader was used to record fluorescence intensity 790 nm (excitation at 740 nm). Assays for each group were triplicated.

2.5 **Data Processing and Statistics**

All of the data are given as the mean ± standard error of the mean of n independent measurements. Statistical analysis was performed using a two-tailed unpaired student’s t test (IBM SPSS Statistics version 21), with p values < 0.05 considered statistically significant.

3 **Results and Discussion**

The design of NIR760-Q has the following considerations: (1) The quinolone structure was chosen as the targeting moiety because certain quinolone molecules, such as 4-quinolone-3-carboxamide, were recently reported to have high CB2R selectivity and binding affinity (K_d as high as 0.2 nM). (2) A terminal amino group was introduced to the targeting moiety to allow for universal conjugation with various labeling moieties, such as fluorescent dyes (for optical imaging) and metal chelators (for PET, single photon emission-computed tomography, and magnetic resonance imaging); (3) NIR760 was selected as the fluorophore for NIR fluorescence imaging, which has high fluorescence quantum yield, stability, and molar extinction coefficient and can be synthesized with only two steps. In addition, the four sulfonate groups on NIR760 introduce high hydrophilicity to the imaging probe; (4) A di(ethylene glycol) linker between the targeting moiety and NIR760 dye provides ether oxygens as hydrogen bond acceptors that can potentially facilitate the binding. To synthesize NIR760-Q (Fig. 1), we first prepared quinolone compound 3 through 7-step reactions using previously reported methods. The following click reaction between compound 3 and 11-azido-3,6,9-trioxaundecan-1-amine provided the CB2R ligand 4 with a terminal amine group, which was conjugated with fluorescent dye NIR760 to yield the desired probe, NIR760-Q.

Upon the synthesis of NIR760-Q, absorption and emission spectra were collected and compared with NIR760 as shown in Fig. 5. NIR760 has intense NIR absorption and emission in water with peaks at 760 and 781 nm, respectively. After conjugation with CB2R ligand 4, the dye showed redshifted absorption and emission spectra. The maximum absorption of NIR760-Q is located at 768 nm with a high molar extinction coefficient of 2.5 × 10^4 M^-1 cm^-1, which is comparable with that of NIR760 (ε = 2.4 × 10^4 M^-1 cm^-1). NIR760-Q also exhibits the intense fluorescence centered at 787 nm with a high quantum yield (16.5% in water), which is roughly 60 times higher than that of indocyanine green (ICG) (Φ = 0.28% in water). ICG is the only Food and Drug Administration-approved NIR fluorescent dye and typically serves as the gold standard for NIR fluorescence imaging.

We recently reported a CB2R-targeted NIR fluorescent probe, NIR760-mbc94, which was successfully used to image CB2R+ cells and tumors. NIR760-Q and NIR760-mbc94 share the same fluorescent dye, NIR760, while the targeting molecules are based on quinolone and pyrazole structures, respectively. Compared to NIR760-mbc94, NIR760-Q has similar maximum absorption (768 versus 766 nm) and emission...
(787 versus 785 nm) wavelengths, but higher molar extinction coefficient \(\varepsilon = 2.5 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}\) versus \(1.44 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}\) in water) and quantum yield \(\Phi = 16.5\%\) versus 15.2\% in water). The enhanced absorption and emission near 800 nm, and excellent water solubility render NIR760-Q as an outstanding fluorescent probe for biomedical imaging.

We selected Jurkat human acute T-lymphoblastic leukemia cells to test the binding affinity and \textit{in vitro} imaging potential of NIR760-Q. In our previous CB2-R-targeted imaging studies,\cite{42,52} we used a transfected mouse malignant astrocytoma DBT cell line, CB2-mid DBT, that expresses CB2-R at endogenous levels.\cite{44} Along with CB2-mid DBT cells, we used WT DBT cells as the CB2-R-control cells. Although CB2-DBT and WT-DBT cells are an excellent pair for studying CB2-R targeting specificity, these cells are mouse cell lines and CB2-R is not naturally expressed. To demonstrate the translational potential of the developed CB2-R-targeted probe, we used Jurkat cells that have been reported to naturally express CB2-R.\cite{26} Before Jurkat cells were used for imaging, we verified their CB2-R expression using RT-PCR. RT-PCR was performed using primers for the human CB2 receptor. As shown in Fig. 3, a CB2-R positive band at 1415 base pairs was detected, indicating positive mRNA expression of CB2-R in Jurkat cells.

To determine NIR760-Q’s binding affinity to CB2-R in living Jurkat cells, we used an \textit{in vitro} saturation binding assay to measure the equilibrium dissociation constant and the maximum specific binding. A large excess of 4Q3C was added to a parallel set of cells to saturate receptor binding sites and account for nonspecific binding. Figure 4 shows a representative saturation binding curve. NIR760-Q binds to CB2-R with a \(K_d\) of \(75.51 \pm 27.97\) nM and \(B_{\text{max}}\) of \(440.9 \pm 71.75\) pmol/mg.

The CB2-R targeting specificity and imaging potential of NIR760-Q was evaluated using Jurkat cells that were incubated with 5 \(\mu\)M of NIR760-Q or NIR760. For blocking studies, Jurkat cells were treated with 5 \(\mu\)M of NIR760-Q and 10 \(\mu\)M of 4Q3C. We observed strong fluorescence signal from cells...
incubated with NIR760-Q, which primarily localized in the cytoplasm [Fig. 5(a)]. In contrast, no significant fluorescence signal was seen from cells incubated with the same concentration of free dye (NIR760) control. Moreover, challenged cells treated with both NIR760-Q and 4Q3C showed lower fluorescence signal than unchallenged cells. These results indicate specific binding of NIR760-Q to the target receptor.

To evaluate the targeting specificity of NIR760-Q in a quantitative manner, we used a multiwell plate reader system to perform Jurkat cell binding assays. Similar to the fluorescence imaging study described above, Jurkat cells were divided into three groups: (1) cells incubated with 5 \( \mu \)M of NIR760-Q; (2) cells incubated with 5 \( \mu \)M of free dye; (3) cells incubated with 5 \( \mu \)M of NIR760-Q and 10 \( \mu \)M of 4Q3C. We also used DBT-cells as the CB2R-cell line for comparison. As shown in Fig. 4(b), Jurkat cells treated with NIR760-Q showed a 2.8-fold higher fluorescence signal than those treated with NIR760 (10469.00 \pm 495.26 versus 3755.67 \pm 311.81, \( p < 0.0001 \)). Treatment with 4Q3C reduced the uptake of NIR760-Q in Jurkat cells by 40% (from 10469.00 \pm 495.26 to 6274.33 \pm 350.55, \( p = 0.0001 \)). In addition, CB2R-WT DBT cells treated with NIR760-Q showed 40% less fluorescence signal than Jurkat cells (6297.67 \pm 250.68 versus 10469.00 \pm 495.26, \( p = 0.0001 \)). These results indicate specific binding of NIR760-Q to the target receptor and are consistent with our previous cellular imaging studies using NIR760-mbc94, whose uptake was also blocked by 40% when challenged with a CB2R ligand. The partial inhibition indicates nonspecific binding of NIR760-Q, which may be due to its net negative surface charge. In a recent study, Choi et al. reported that replacing the negatively charged NIR fluorescent dyes with a zwitterionic dye that has no net surface charge significantly reduced the nonspecific binding of NIR fluorescent imaging probes. With a net surface charge of \(-4\), NIR760-Q is likely to have nonspecific binding. We noticed that the cell uptake of NIR760-Q in DBT-WT cells is higher than that of NIR760 in Jurkat cells. Although both uptakes are due to nonspecific binding, the higher lipophilicity of NIR760-Q than NIR760 could have caused the enhanced level of nonspecific binding. Future study will involve developing zwitterionic CB2R probes with reduced nonspecific binding.

![Fig. 4](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/076016-5/July-2014/Vol.-19(7)/Wu-et-al-Molecular-imaging-of-human-tumor-cells-that-naturally-overexpress-type-2-cannabinoid-receptors/terms-of-use)

**Fig. 4** In vitro CB2R saturation binding assay of NIR760-Q using intact cells. (a) Total binding data (solid) were represented as relative fluorescent units (RFU) on the y-axis as a function of NIR760-Q concentration (x-axis) in the absence of 4Q3C (blocking agent). Nonspecific binding data (dash) were represented as RFU as a function of NIR760-Q concentration in the presence of 4Q3C. (b) Specific binding data were represented as RFU (y-axis) as a function of NIR760-Q concentration (x-axis). Data in y-axis were obtained by subtracting the nonspecific binding data from the total binding data. The dissociation constant (\( K_d \)) and receptor density (\( B_{max} \)) were estimated from the nonlinear fitting of the specific binding versus the concentration of NIR760-Q using Prism software. Each data point represents the mean \( \pm \) SEM based on triplicate samples.

![Fig. 5](https://www.spiedigitallibrary.org/journals/Journal-of-Biomedical-Optics/076016-5/July-2014/Vol.-19(7)/Wu-et-al-Molecular-imaging-of-human-tumor-cells-that-naturally-overexpress-type-2-cannabinoid-receptors/terms-of-use)

**Fig. 5** NIR760-Q specifically binds to CB2R in Jurkat cells. Jurkat cells were incubated for 30 min with 5 \( \mu \)M of NIR760-Q or free NIR760, with or without 10 \( \mu \)M of blocking agent 4Q3C. Cells were then washed three times with serum free medium before imaging. (a) Fluorescence imaging using a Zeiss Axio Observer fluorescent microscopy with a Synergy H4 Hybrid Multimode Microplate Reader. Wild type DBT-cells were used as a CB2R negative control group for comparison. Each data point represents the mean \( \pm \) SEM based on triplicate samples. (**\( p < 0.001 \), ***\( p < 0.0001 \)).
binding to allow for enhanced imaging contrast at the target site. In addition, we plan to develop a xenograft mouse model using Jurkat cells and evaluate the potential of NIR760-Q and new zwitterionic CB₂R probes in vivo.

4 Summary

In summary, we have developed a novel CB₂-targeted NIR probe NIR760-Q, which demonstrated specific binding in Jurkat human cells. Compared to our previously reported CB₂R probe, NIR760-mbc94, NIR760-Q has enhanced absorption and emission and comparable binding affinity and specificity. We also report the first CB₂R-targeted optical imaging of human tumor cells that naturally express CB₂R. The combined data indicate that NIR760-Q is a promising imaging probe for CB₂R-targeted imaging with the potential of translational studies. Such an imaging tool may have great value in elucidating the regulatory role of CB₂R in various diseases.

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