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Mitochondria-specific imaging in living cells with two-photon absorption small molecule containing amino groups

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ABSTRACT

Two-photon fluorescent probes have emerged as promising molecular tools for imaging subcellular organelles. Here, the facile synthesis of three novel nitro molecules (**Z1-Z3**) with lager two-photon absorption cross-section and three novel amino molecules (**L1-L3**) based on mitochondrial probes with two-photon absorption are presented. Their photophysical properties have been investigated both experimentally and theoretically. The study of nonlinear optical property revealed that **Z1-Z3** possess more excellent two-photon absorption (2PA) cross-section values than **L1-L3** and the 2PA cross-section values were enhanced with increasing electron-donating strength of the end group when fixed the opposite group (nitro or amino). Furthermore, in consideration of the water solubility and fluorescent intensity, **L1-L3** possessing high specificity for mitochondrial localization were applied to biology, which is advantageous in comparison with commercially available mitochondrial trackers. Due to their low cytotoxicity, these small molecules **L1-L3** offer a promising platform to directly monitor mitochondria in living cells and zebra fish.

1. Introduction

Small molecular probes are dedicated to illustrate biological processes as well as providing practical tools for modulating them at the interface of advanced chemistry and biology [1]. The recent technologies that can exploit small molecular materials with two-photon absorption (2PA) property had significantly applied in the areas of chemistry, biology and photonics, such as three-dimensional micro-fabrication, high capacity data storage [2], optical limiting, two-photon laser scanning fluorescence imaging [3], and photodynamic therapy [4]. Therefore, considerable research efforts have been devoted to develop novel small molecule materials with strong 2PA activity for the last decade. One of these important applications is two-photon fluorescence imaging of living cells, which including the imaging of various organelles, such as lysosome [5], endoplasmic reticulum [6], Golgi [7], Mitochondria [8].etc. In addition, a lot of literature of two-photon fluorescence imaging [9–13] have been reported.

Over the past few decades, the design of a fluorescent chromophore targeting either specific intracellular structures or chemical species has progressed rapidly [14]. In recent years, derivatives of amidogen were explored for cancer imaging [15], modifications in DNA [16], and the detection of TNP (2,4,6-trinitrophenol) [17]. Furthermore, studies of the derivatives of amino as pH-Responsive Fluorescent False Neurotransmitters and TPF probe for live Pancreatic Islet Imaging were reported recently [18,19]. The application of fluorescent imaging of the derivatives of amidogen has a great prospect.

Spurred by this, we designed and synthesized six novel ligands (**Z1-Z3** and **L1-L3**) (Scheme 1 and Scheme S1). Firstly, hydroxyl is attached to the phenylamine group to enhances its electron-donation ability and improves the solubility of these compounds. Secondly, cyano-substituted compounds show good optical and electrical properties due to their high electron affinities [20]. Molecule including an electron-withdrawing cyano group on the central π-bridge exhibit high 2PA cross-section value. The photophysical properties of all compounds were systematically investigated. Relying on the comprehensive studies, compounds **L1-L3** were selected for bioimaging applications. Bioimaging study using two-photon microscopy showed that **L1-L3** would target mitochondria and they are capable of specifically monitoring the

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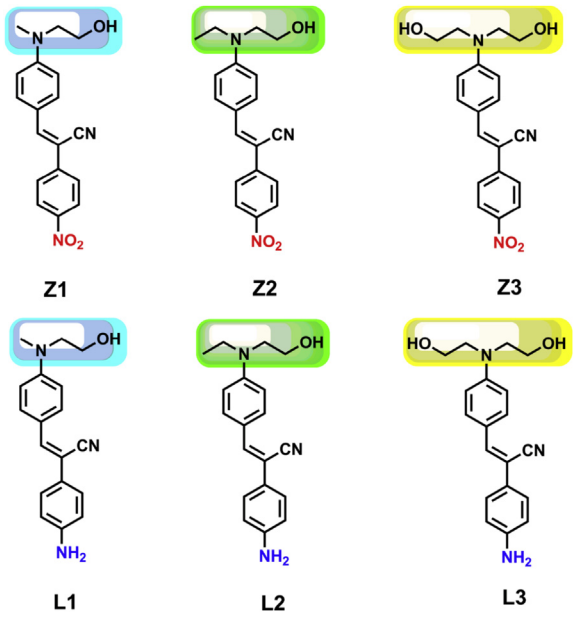

Scheme 1. The structural formulas of compounds **Z1-Z3** and **L1-L3**.

fluorescent signals in the intestinal system of living zebrafish. All these results suggest that **L1-L3** are potential two-photon probes for in *vitro* and *vivo* bioimaging.

2. Experimental section

2.1. Materials and apparatus

All solvents were dried and purified by usual methods. IR spectra (4000-400 cm^{-1}), as KBr pellets, were recorded with a Nicolet FT-IR 170 SX sepctrophotometer. Mass spectrum was performed with a Micromass GCT-MS (ESI source). Elemental analysis was carried out on a Perkin-Elmer 240 analyzer. The ^1H NMR and ^{13}C NMR spectra recorded on at 25 °C using Bruker Avance 400 spectrometer were reported as parts per million (ppm) from TMS. UV spectra were recorded on a SHIMADZU UV-3600 spectrophotometer. The fluorescence spectra measurement was performed with the use of a HITACHI F-2500 Spectro fluorophotometer.

2.2. Synthesis

2.2.1. Synthesis of Z1

M1 (1.79 g, 10 mmol) was dissolved in ethanol solution (30 mL) in the flask (150 mL), then 2-(4-nitrophenyl)-acetonitrile (1.62 g, 10 mmol) and several drops piperidine were added to it. The reaction mixture was refluxed for 24 h. Resulting mixtures was cooled and filtered. The brown solid was obtained (1.69 g, yield 87%). IR (KBr, cm^{-1}) selected band: 3418 (m), 2216 (s), 1615 (w), 1580 (s), 1511 (s), 1396 (s), 1326 (s), 1038 (m), 807 (s). ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.07 (s, 3 H), 3.52–3.55 (t, 2 H, J = 6.0 Hz), 3.56–3.60 (q, 2 H, J = 4.0 Hz), 4.75–4.77 (t, 1 H, J = 4.6 Hz), 6.85 (s, 2 H), 7.92 (d, 2 H), 7.945 (d, 2 H, J = 8.0 Hz), 8.06 (s, 1 H), 8.28 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 53.80, 58.20, 99.19, 111.50, 118.85, 120.00, 124.23, 125.54, 132.13, 141.71, 146.03, 146.08, 151.78. ESI-MS: 324.14 ($[M+1]^+$), calcd: 323.15.

2.2.2. Synthesis of Z2, Z3

The synthesis routes of compounds **Z2** and **Z3** are similar with **Z1**.

Z2: 1.65 g, yield 85%. IR (KBr, cm^{-1}) selected band: 3420 (m), 2401 (s), 1592 (m), 1535 (s), 1350 (s), 1200 (s), 1060 (m), 841 (w), 714 (s). ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 1.12–1.15 (t, 3 H, J = 6.0 Hz), 3.47–3.50 (t, 2 H, J = 6.0 Hz), 3.53 (d, 2 H), 3.57–3.61 (q, 2 H, J = 3.0 Hz), 4.79–4.81 (t, 1 H, J = 4.0 Hz), 6.84 (d, 2 H, J = 8.0 Hz), 7.91 (d, 2 H, J = 8.0 Hz), 7.93 (d, 2 H, J = 8.0 Hz), 8.05 (s, 1 H), 8.18 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 11.94, 44.92, 51.92, 58.25, 98.92, 111.35, 118.90, 119.79, 124.25, 125.51, 132.33, 141.77, 146.01, 150.69. ESI-MS: 338.15 ($[M+1]^+$), calcd: 337.37.

Z3: 1.68 g, yield 87%. IR (KBr, cm^{-1}) selected band: 3429 (m), 2390 (s), 1569 (m), 1535 (s), 1326 (s), 1188 (m), 1072 (m), 829 (s), 629 (w). ^1H NMR (400 MHz, d_6-CH_3COCH_3, ppm): δ 3.56–3.58 (t, 4 H, J = 4.0 Hz), 3.59–3.62 (t, 4 H, J = 4.0 Hz), 4.81–4.83 (t, 2 H, J = 4.0 Hz), 6.87 (d, 2 H, J = 8.0 Hz), 7.92 (d, 2 H, J = 4.0 Hz), 7.94 (d, 2 H, J = 8.0 Hz), 8.07 (d, 1 H), 8.29 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 53.08, 58.13, 99.10, 111.53, 118.88, 119.94, 124.25, 125.54, 132.19, 141.73, 146.00, 146.04, 151.11. ESI-MS: 354.15 ($[M+1]^+$), calcd: 353.37.

2.2.3. Synthesis of L1

Z1 (2.25 g, 6.97 mmol) was dissolved in ethanol solution (25 mL) in the flask (100 mL), then $SnCl_2 2H_2O$ (7.86 g, 34.85 mmol) was added to it slowly. The reaction mixture was reflux for 4 h, cooled and Na_2CO_3 was added to adjust the pH under stirring. Resulting mixtures was filtered after the pH to the value of 7 and filtrate was extracted with ethyl acetate. The solution was dried by saline solution and the resulting solution was evaporated. The cured product was recrystallized by ethanol. The yellow solid was obtained (1.68 g, yield 75%). IR (KBr, cm^{-1}) selected band: 3414 (m), 3369 (m), 2915 (w), 2210 (s), 1608 (m), 1524 (s), 1433 (w), 1216 (w), 1186 (vs), 1066 (m), 813 (s). ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.01 (s, 3 H), 3.45–3.48 (t, 2 H, J = 7.0 Hz), 3.55–3.59 (q, 2 H,J = 4.0 Hz), 4.72–4.74 (t, 1 H, J = 4.6 Hz), 5.43 (s, 2 H), 6.62 (d, 2 H), 6.67 (d, 2 H, J = 8.0 Hz), 7.34 (d, 2 H, J = 8.0 Hz), 7.46 (s, 1 H), 7.75 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 40.01, 53.79, 57.83, 103.37, 110.96, 114.08, 119.79, 121.19, 121.46, 125.49, 129.80, 1337.39, 148.59, 149.77. ESI-MS: 294.15 ($[M+1]^+$), calcd: 293.15. Anal. Calc. for $C_{18}H_{19}N_3O$: C, 73.69; H, 6.53; N, 14.32. Found: C, 73.61; H, 6.52; N, 14.30.

2.2.4. Synthesis of L2, L3

The synthesis routes of compounds **L2** and **L3** are similar with **L1**.

L2: 1.64 g, yield 70%. IR (KBr, cm^{-1}) selected band: 3407 (m), 3329 (m), 3031 (w), 2965 (w), 2201 (s), 1607 (s), 1520 (s), 1401 (m), 1180 (s), 831 (m). ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 1.09–1.12 (t, 3 H, J = 4.0 Hz), 3.54–3.57 (t, 2 H, J = 4.0 Hz), 3.75–3.79 (q, 2 H, J = 4.0 Hz), 3.92–3,.94 (t, 2 H, J = 4.0 Hz), 4.76–4.78 (t, 1 H, J = 4.0 Hz), 5.42 (s, 2 H), 6.63 (d, 2 H, J = 8.0 Hz), 6.75 (d, 2 H, J = 8.0 Hz), 7.35 (d, 2 H, J = 12.0 Hz), 7.46 (s, 1 H), 7.74 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 11.41, 39.67, 44.61, 51.32, 58.03, 102.27, 110.63, 113.82, 119.49, 120.85, 121.97, 125.80, 130.03, 137.45, 148.39. ESI-MS: 308.17 ($[M+1]^+$), calcd: 307.17. Anal. Calc. for $C_{19}H_{21}N_3O$: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.16; H, 6.88; N, 13.68.

L3: 1.74 g, yield 71%. IR (KBr, cm^{-1}) selected band: 3459 (m), 3359 (m), 3218 (m), 2963 (w), 2206 (s), 1607 (m), 1521 (vs), 1355 (s), 924 (m). ^1H NMR (400 MHz, d_6-CH_3COCH_3, ppm): δ 3.04–3.06 (t, 4 H, J = 4.0 Hz), 3.10–3.13 (t, 4 H, J = 4.0 Hz), 4.35–4.37 (t, 2 H, J = 4.0 Hz), 4.99 (s, 2 H), 6.17 (d, 2 H, J = 8.0 Hz), 6.32 (d, 2 H, J = 12.0 Hz), 6.88 (d, 2 H, J = 8.0 Hz), 7.00 (s, 1 H), 7.29 (d, 2 H, J = 8.0 Hz); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ 52.82, 58.13, 103.43, 111.08, 113.95, 119.29, 120.92, 121.72, 126.10, 130.33, 137.58, 148.74, 149.02. ESI-MS: 324.17 ($[M+1]^+$), calcd: 323.16. Anal. Calc.