Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Two-photon excited red-emissive probe for imaging mitochondria with high fidelity and its application in monitoring mitochondrial depolarization via FRET

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Materials

All chemicals used are of analytical grade, 1-formylpyrene was purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 2-methyl quinoline etc. was purchased from J&K Chemical (Beijing, China). The solvents used in the spectral measurement are of chromatographic grade.

Calculation details

DFT calculation was performed with Gaussian 09 software package. The chemical structure of MVQ was optimized sequentially on the basic set of pm3, b3lyp/3-21g, b3lyp/6-31g, and cam-b3lyp/tzvp. The finally optimal chemical structure was used to calculate the frontier molecular orbitals and transition oscillator strength on the basic set of cam-b3lyp/tzvp.

Spectroscopic measurements

The UV-visible-near-IR absorption spectra of dilute solutions were recorded on a U2910 spectrophotometer using a quartz cuvette having 1 cm path length. One-photon fluorescence spectra of dilute solutions were obtained on a HITACH F-2700 spectrofluorimeter equipped with a 450-W Xe lamp. The in-situ emission spectra in nucleus and mitochondria were obtained with the spectra image function of Nikon A1R microscope, and the emission spectra of the solid probe were also acquired with this function.

Cell culture and staining methods

HeLa cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO_2 incubator at 37 $^{\circ}$C. For living cells imaging experiment of the probes, the culture medium surrounding the cells were firstly removed, and the cells were washed with PBS twice. Then the cells were incubated in 1 mL of culture medium. On the other hand, 1 mM stock solutions of the probe in DMSO were prepared. After that, 2 μL of stock solutions were mixed evenly with 1 mL culture medium (pH 7.4) in a tube. The cells were incubated with the above mixed solutions at 37 $^{\circ}$C. Cells were imaged immediately without washing procedures.

Fluorescent imaging methods

Confocal fluorescence images were obtained with a Nikon A1R confocal laser scanning microscope. The differential interference contrast (DIC) images were taken with 405 nm or 647 nm solid laser. Two-photon fluorescence images were obtained with the same microscope, with the excitation source of Ti:Sapphire femtosecond laser (800 nm). In the experiments to detect mitochondrial membrane potential changes, all the parameters should be fixed. The power of 405 nm laser was set as 0.5 mW, the pinhole was set as 1.5 au, and the background offset was set as 0.

Cytotoxicity tests

The cytotoxicity of MVQ and HVQ was testified with MTT assays. A 96-well plate was used to perform the cell viability experiment. Suspension of HeLa cells with cell concentration of 10000 cells/mL was firstly prepared, and the suspension was added to the plate (200 μL per well). At the same time, culture medium without cells was also introduced into the wells (200 μL per well) as blank. The cells were incubated with 2 μM of **HVQ** or **MVQ** for different time. Change the culture medium of all the wells, and after that 10 μL of MTT (5mg/mL) was added to each well. The well plate was incubated for 4 h, and then the culture medium in each well was removed. 200 μL of DMSO was added to dissolve the formazan, and finally the absorbance was measured with a microplate reader at 620 nm. The cell survival rates were finally calculated following the equation below:

$$\text{Survival rate (\%)} = \frac{A_{sample} - A_b}{A_c - A_b} \times 100 \quad (1)$$

Where A_{sample} is the absorbance of the wells with cells treated with the probes, A_b is the absorbance of wells pretreated with only culture medium, and A_c is the absorbance of wells with cells treated with no reagents.

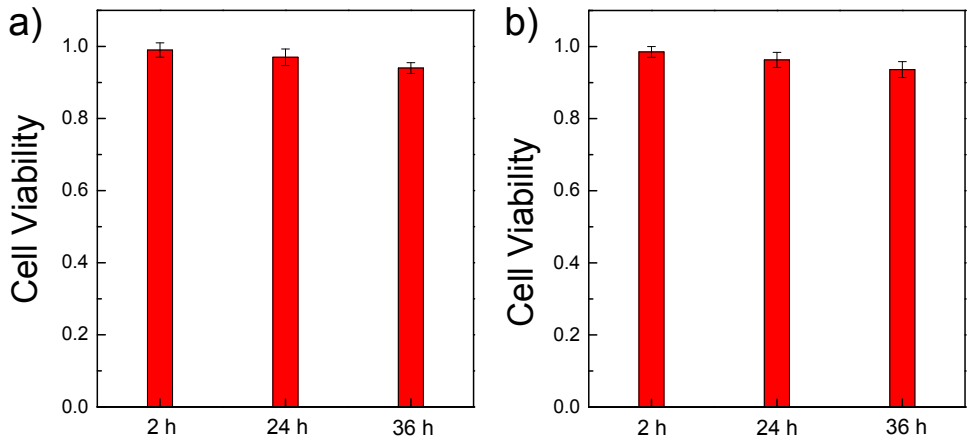


Figure S1. The cell viability of Hepg2 cells incubated with 2 μM MVQ (a) and HVQ (b) for different time (2 h, 24 h, and 36 h).

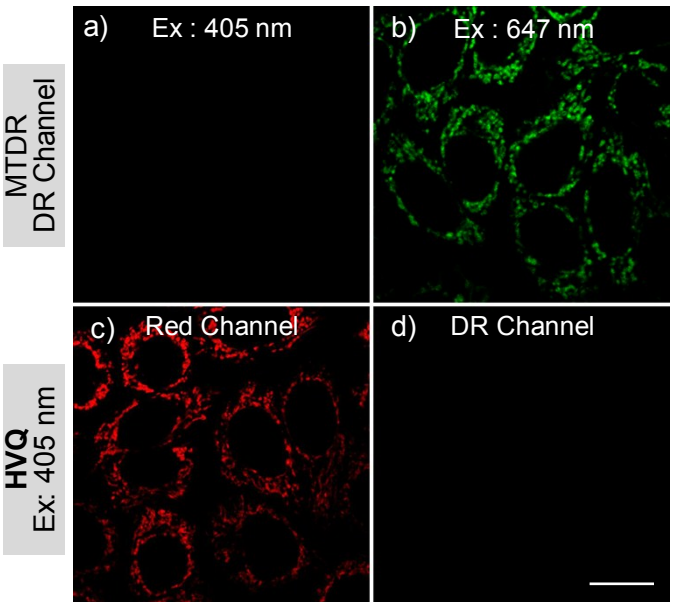


Figure S2. The fluorescence images of Hepg2 cells solely stained with MTDR (a,b) and HVQ (c,d). (a,b) was acquired in DR channel with the excitation of 405 nm and 647 nm, respectively. (c,d) was excited with 405 nm and the emission was acquired in Red and DR channel, respectively.

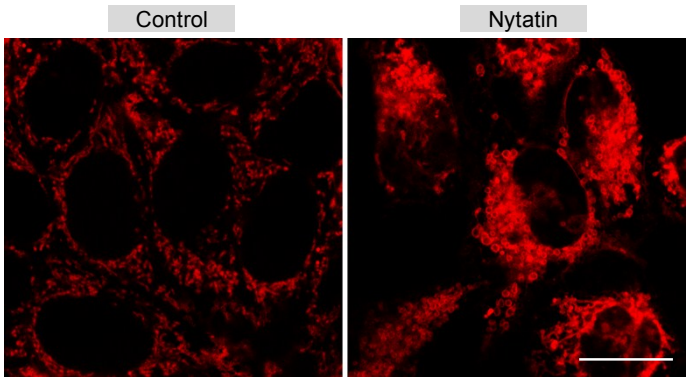
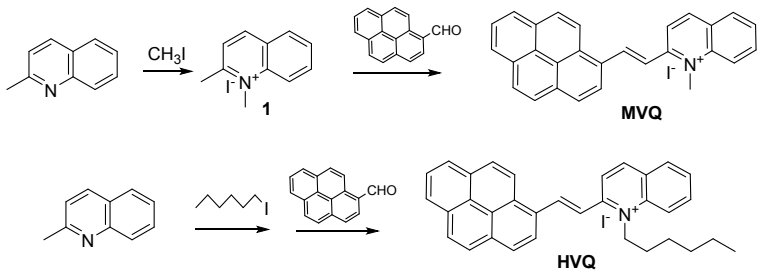


Figure S3. The cell images of Hepg2 cells pretreated with non-reagent and nystatin (20 μM, 30 min) then incubated with 2 μM **HVQ** for 20 min. Bar = 20 μm.

Synthesis and characterization of MVQ and HVQ



Scheme S1. The synthetic routine of **MVQ** and **HVQ**.

Synthesis of **1,2-dimethylquinolin-1-ium iodide** (**1**)

Toward a round-bottom flask with 5 mL ethanol, 1.0 mL (7.4 mmol) of 2-methylquinoline and 0.46 mL (7.4 mmol) iodomethane were added. The mixture was stirred evenly and heated to reflux at 80 °C for 24 h to accomplish the reaction. The system was then cooled down to room temperature, and dark yellow powder could be precipitated. The product was filtered and washed with ethanol three times without further purification, with yield of 76 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J = 8.5 Hz, 1H), 8.60 (d, J = 9.0 Hz, 1H), 8.41 (dd, J = 8.2, 1.6 Hz, 1H), 8.24 (ddd, J = 8.8, 7.0, 1.6 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.00 (t, J = 7.6 Hz, 1H), 4.45 (s, 3H), 3.09 (s, 3H).

Synthesis of **(E)-1-methyl-2-(2-(pyren-1-yl)vinyl)quinolin-1-ium iodide** (**MVQ**)

1-Formylpyrene (150 mg, 0.65 mmol) and compound **1** (186 mg, 0.65 mmol) were added into a

round-bottom flask, followed by the addition of 5 mL ethanol. The mixture was stirred evenly and 400 μL of pyrrolidine was added in. The system was stirred for 24 h at room temperature to accomplish the reaction. Dark red powder was precipitated, and the crude product can be obtained by filtration. **MVQ** could be final purified by recrystallization in ethanol, and the yield was 57%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.30 (d, J = 15.6 Hz, 1H), 9.17 (d, J = 9.0 Hz, 1H), 9.03 (d, J = 9.1 Hz, 2H), 8.97 (d, J = 8.4 Hz, 1H), 8.63 (d, J = 9.0 Hz, 1H), 8.52 – 8.41 (m, 5H), 8.40 – 8.14 (m, 5H), 8.00 (t, J = 7.6 Hz, 1H), 4.69 (s, 3H). HRMS (ESI): m/z calculated for $C_{28}H_{20}N$ 370.1590 [M^+], found: 370.1597.

Synthesis of **(E)-1-hexyl-2-(2-(pyren-1-yl)vinyl)quinolin-1-ium iodide** (**HVQ**)

Compound **HVQ** was synthesized in one-pot method. Iodohexane (0.15 mL, 1 mmol) and 2-methylquinoline (0.14 mL, 1 mmol) were firstly added into a round-bottom flask with 5 mL ethanol. The mixture was then stirred evenly and heated at 80 ^{o}C for 24 h. Afterwards, the system was cooled down to room temperature, and 1-formylpyrene (0.231 mg, 1 mmol) was added into the flask and stirred evenly. 400μL of pyrrolidine was then added in and the system was stirred at room temperature for 24 h to accomplish the reaction. Dark red powder was precipitated and isolated by filtration. Pure product was obtain by recrystallization in ethanol, with a yield of 48 %. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (d, J = 15.2 Hz, 1H), 9.19 (d, J = 8.9 Hz, 1H), 9.03 (dd, J = 12.8, 9.1 Hz, 2H), 8.92 (d, J = 8.3 Hz, 1H), 8.63 (d, J = 9.1 Hz, 1H), 8.52 – 8.42 (m, 5H), 8.39 – 8.30 (m, 2H), 8.28 – 8.17 (m, 3H), 8.01 (t, J = 7.5 Hz, 1H), 5.24 (d, J = 8.4 Hz, 2H), 2.00 (d, J = 7.5 Hz, 2H), 1.60 (q, J = 7.6 Hz, 2H), 1.45 – 1.21 (m, 4H), 0.84 (t, J = 7.2 Hz, 3H). HRMS (ESI): m/z calculated for $C_{33}H_{30}N$ 443.2373 [M^+], found: 443.2380.

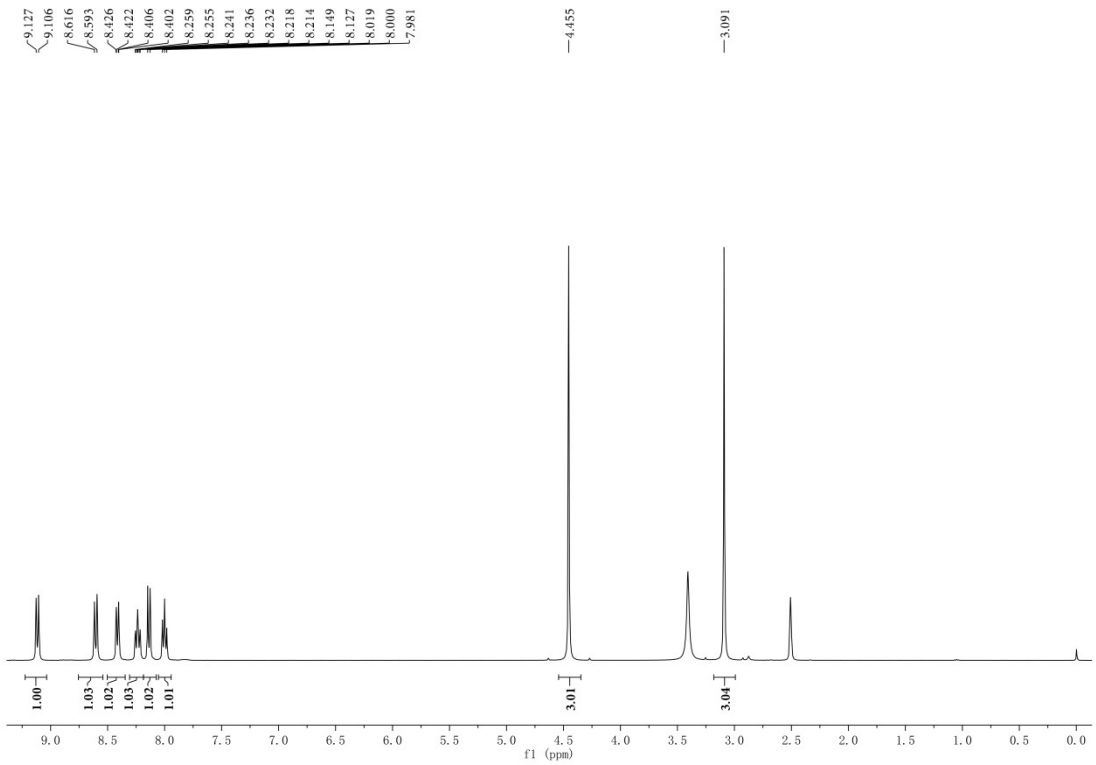


Figure S4. The ^{1}H NMR spectra of compound **1** in DMSO-d_6.

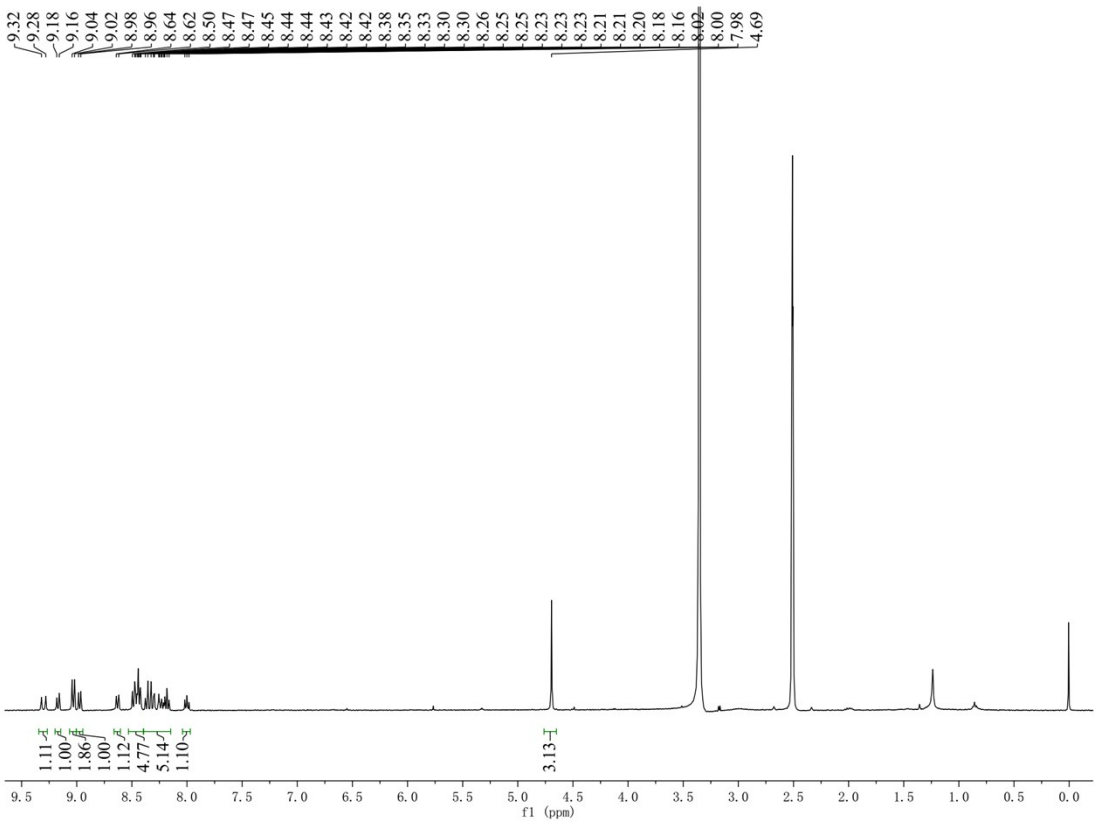


Figure S5. The ^{1}H NMR spectra of **MVQ** in DMSO-d_6.

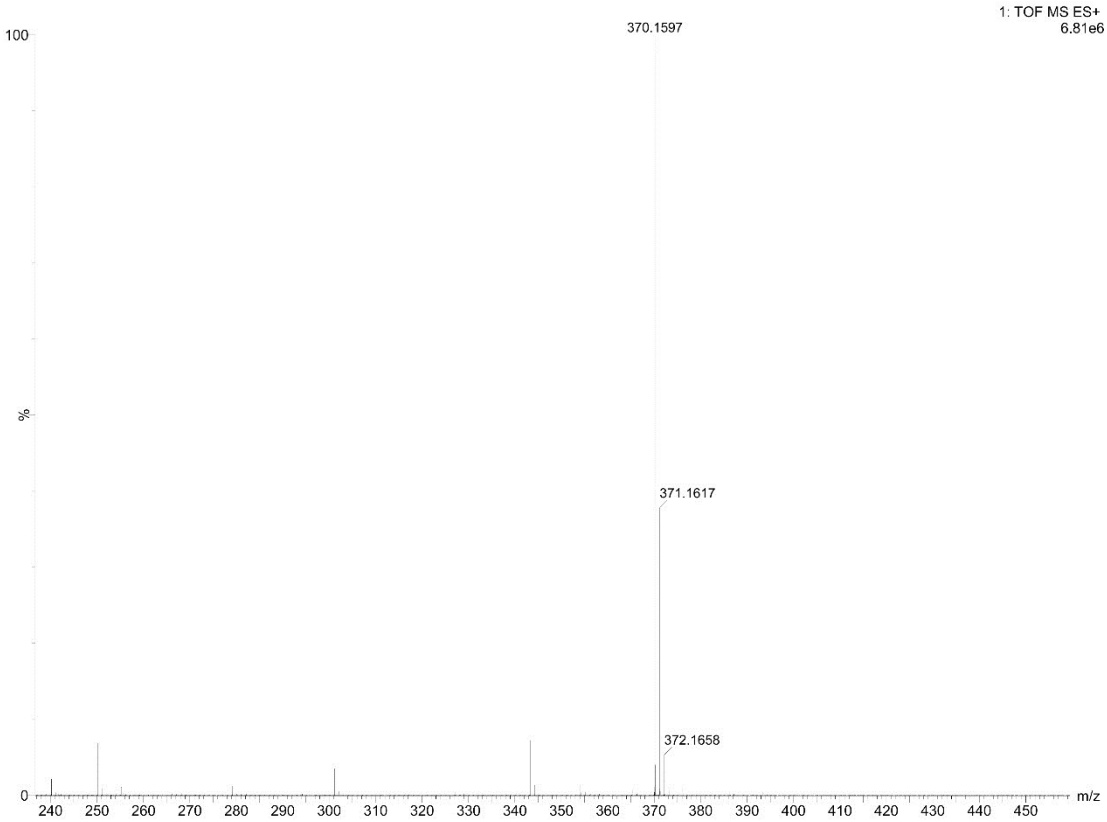


Figure S6. HRMS spectra of **MVQ**.

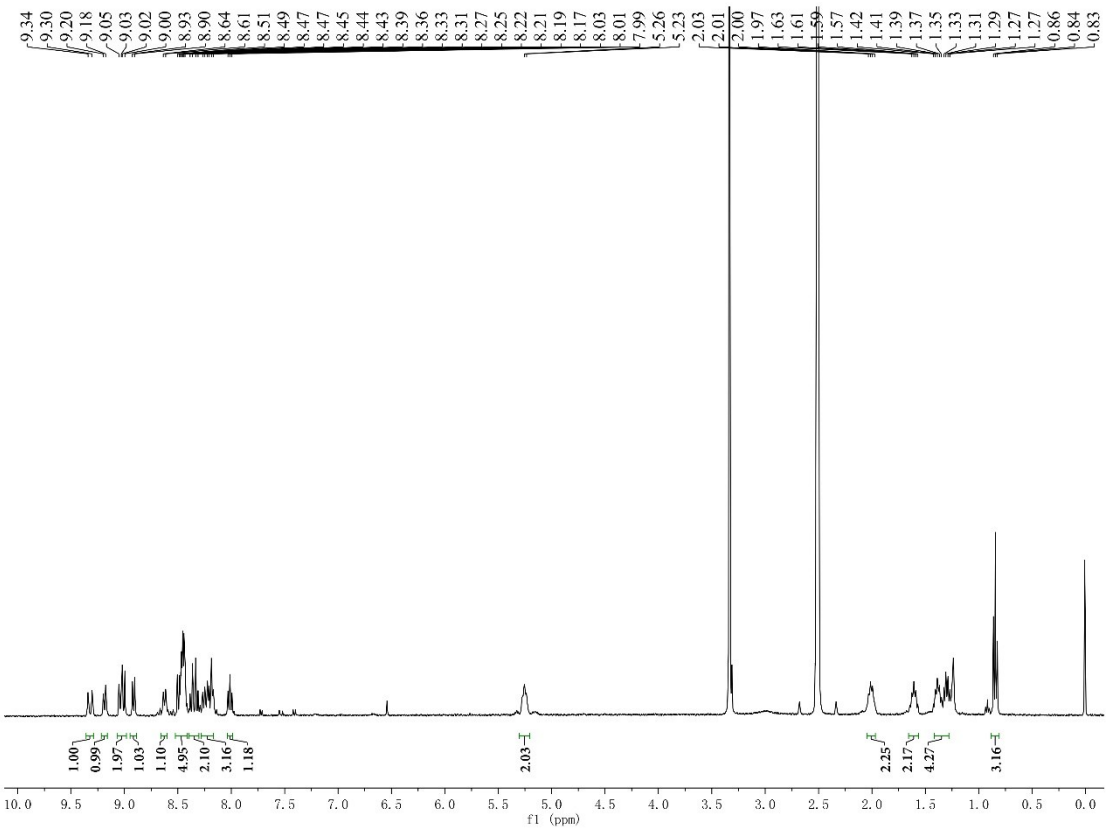


Figure S7. The ^{1}H NMR spectra of **HVQ** in DMSO-d_6.

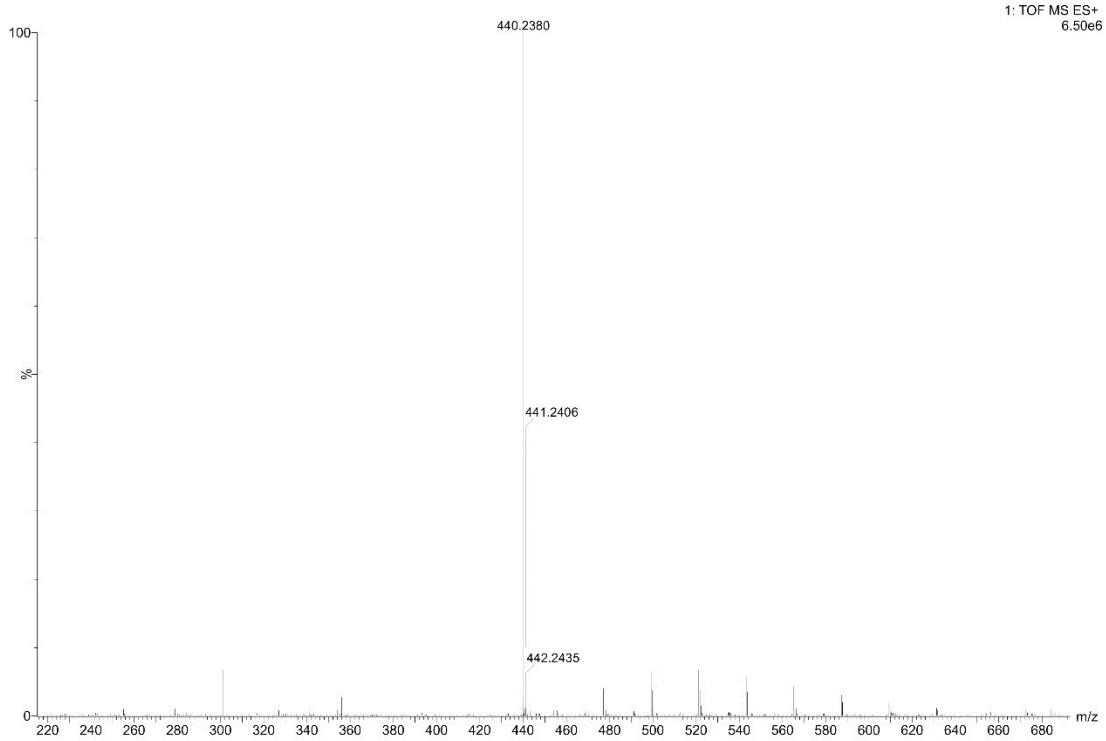


Figure S8. HRMS spectra of **HVQ**.