Supporting Information

A new GFP fluorophore-based probe for lysosomes labelling and tracing lysosomal viscosity in live cells

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Content

1. Comparison between the absorption and emission of HBDI and Lys-V

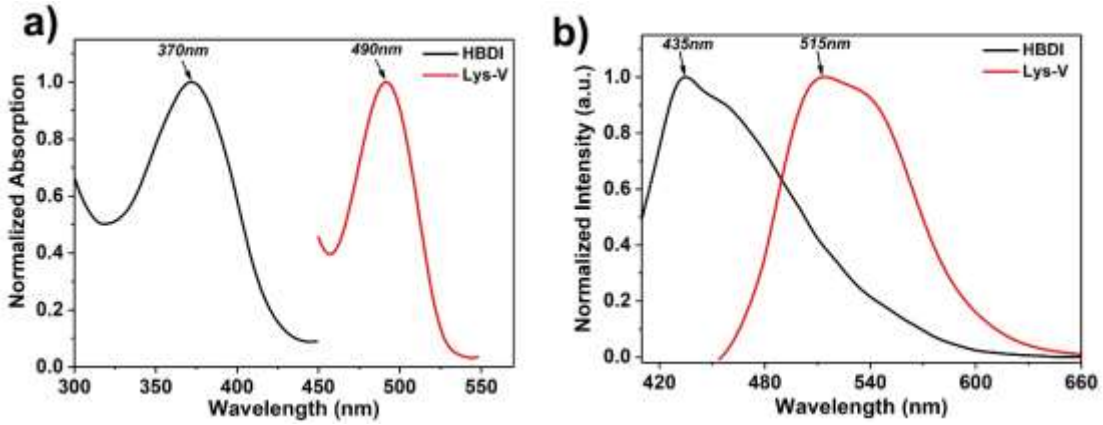


Figure S1. The normalized absorption and emission of HBDI and **Lys-V** in water (pH 7.0, containing 1% DMSO).

2. Solvent Effect

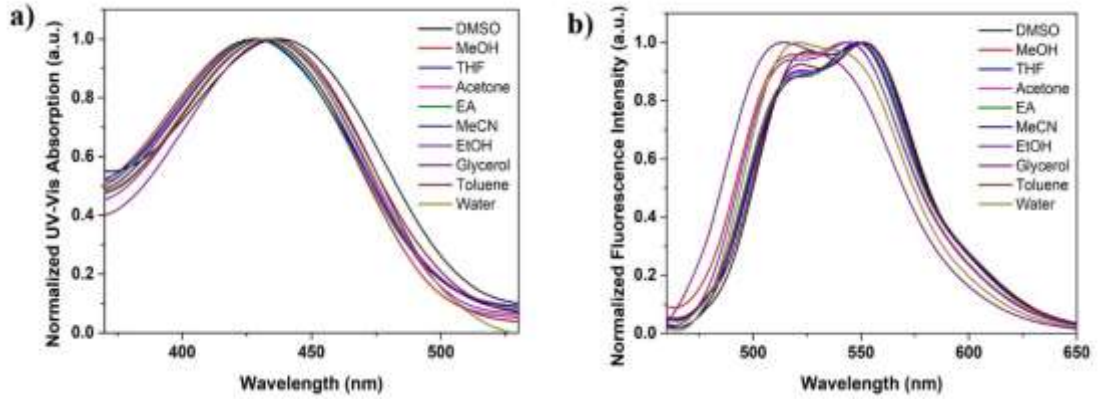


Figure S2. (a) Normalized UV-Vis absorption and (b) normalized fluorescence spectra of the probe **Lys-V** (10 μM) in different solvents.

3. pH Titration.

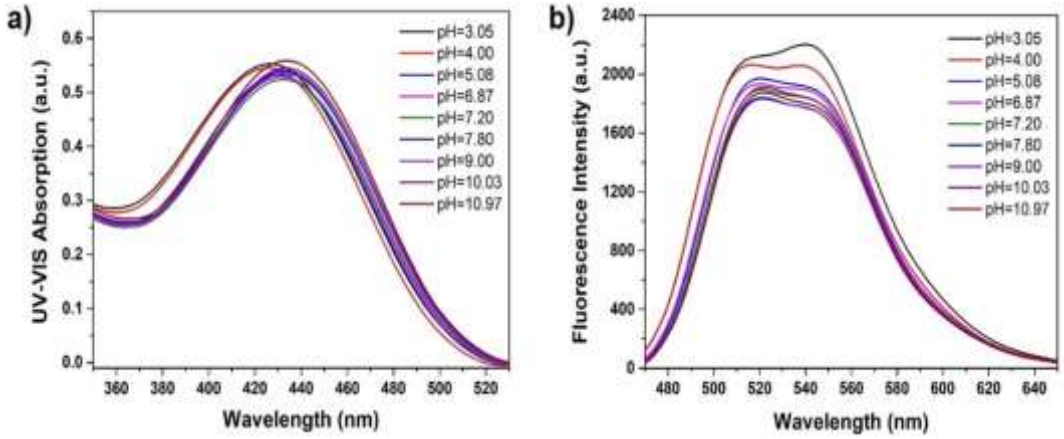


Figure S3. (a) UV-Vis absorption and (b) fluorescence spectra of the probe **Lys-V** (10 μM) in different pH aqueous solutions. The pH was adjusted by addition of HCl (0.1 M) and NaOH (0.1 M). λ_{ex} = 430 nm.

4. Temperature Measurement

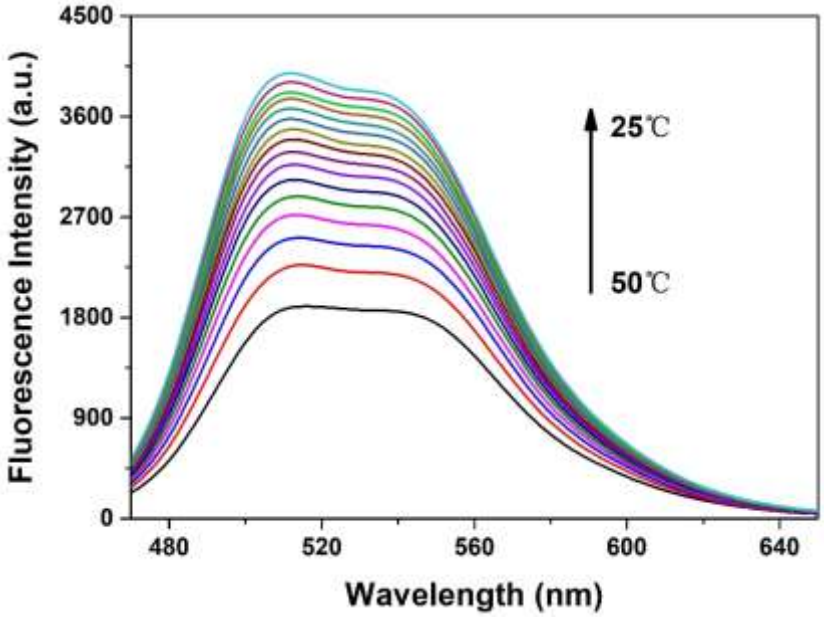


Figure S4. Temperature-varied fluorescence spectra of the probe **Lys-V** (10 μM) in water/glycerol (5/95) solution. λ_{ex} = 430 nm, pH = 7.0.

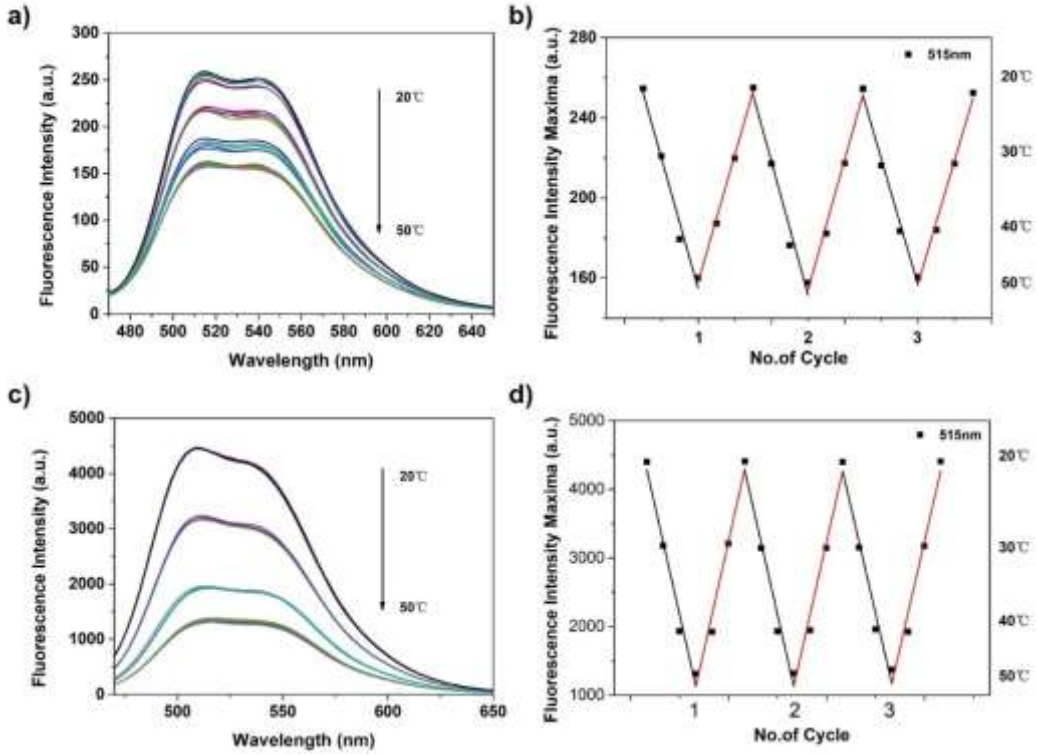


Figure S5. Fluorescence spectra of the probe **Lys-V** (10 μM) in (a) water and (c) glycerol at different temperature (20°C, 30°C, 40°C, 50°C). Fluorescence intensity maxima (515 nm) in (b) water and (d) glycerol. λ_{ex} = 430 nm, pH = 7.0.

5. Viscosity Measurement at different pH values

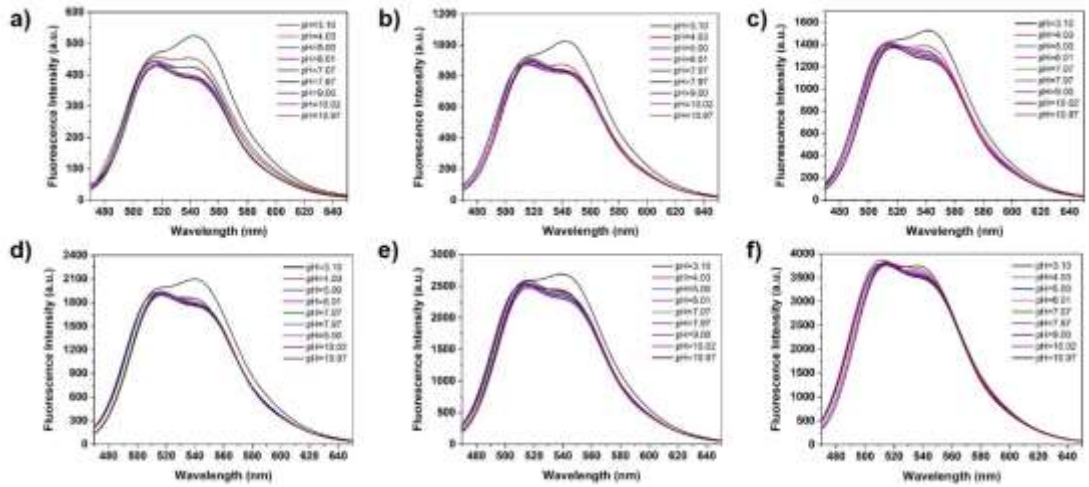


Figure S6. (a-f) Fluorescence spectra of the probe **Lys-V** (10 μM) in different pH aqueous buffer solutions at various viscosities, (a) 7.90 cP, (b) 32.50 cP, (c) 78.90 cP, (d) 140.60 cP, (e) 246.70 cP, (f) 438.40 cP. λ_{ex} = 430 nm.

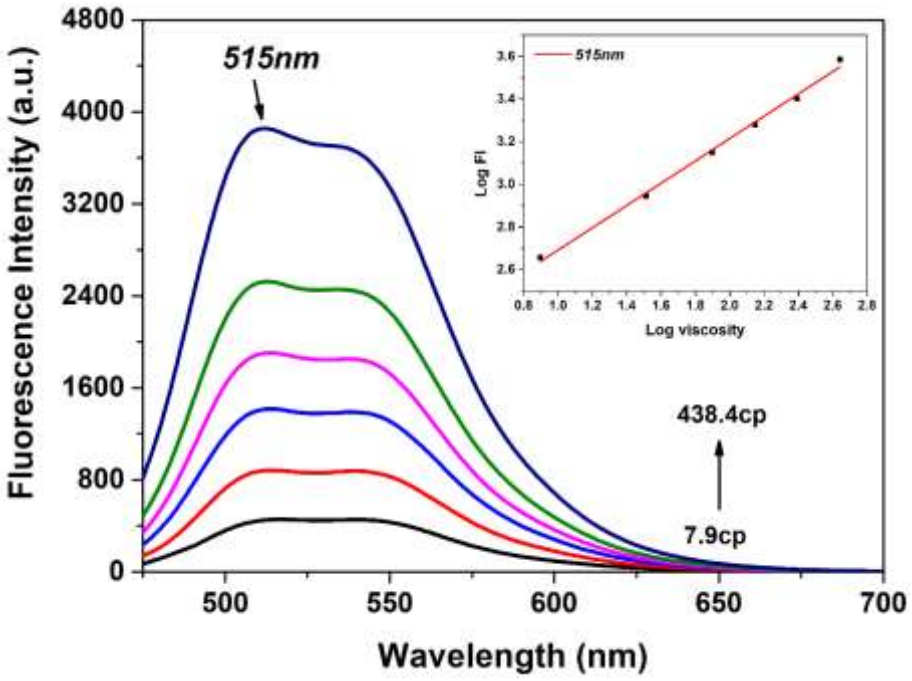


Figure S7. Fluorescence spectrum of **Lys-V** (10 μM) with increasing of solvents viscosity (from 7.9 cP to 438.4 cP, pH 4.0, containing 1% DMSO). (Inset: linear relation between the Log FI and Log Viscosity).

λ_{ex} = 430 nm. Temperature: 25℃.

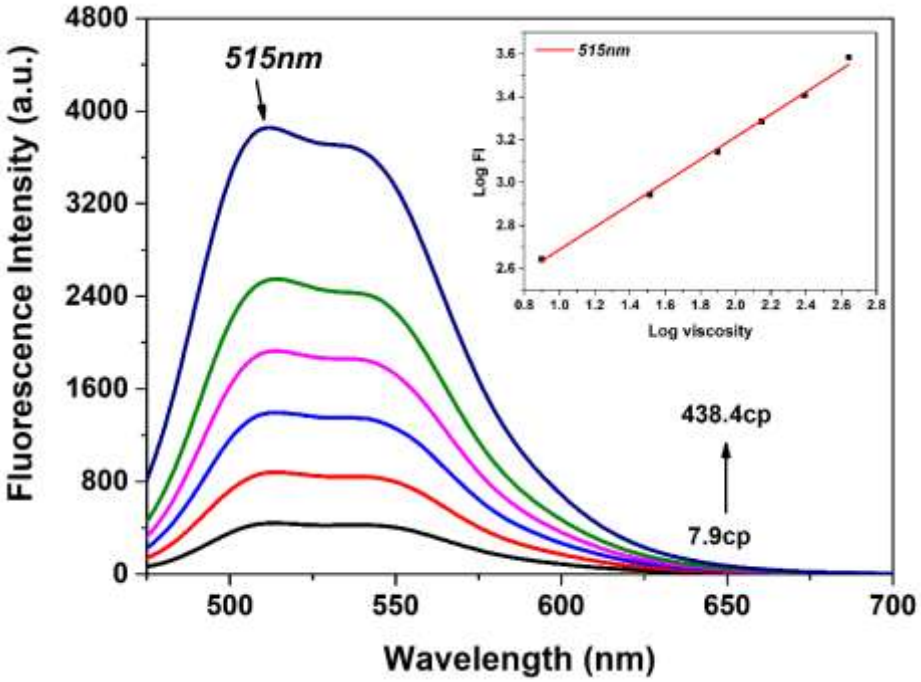


Figure S8. Fluorescence spectrum of **Lys-V** (10 μM) with increasing of solvents viscosity (from 7.9 cP to 438.4 cP, pH 5.0, containing 1% DMSO). (Inset: linear relation between the Log FI and Log Viscosity).

λ_{ex} = 430 nm. Temperature: 25℃.

6. MTT Assay of Lys-V

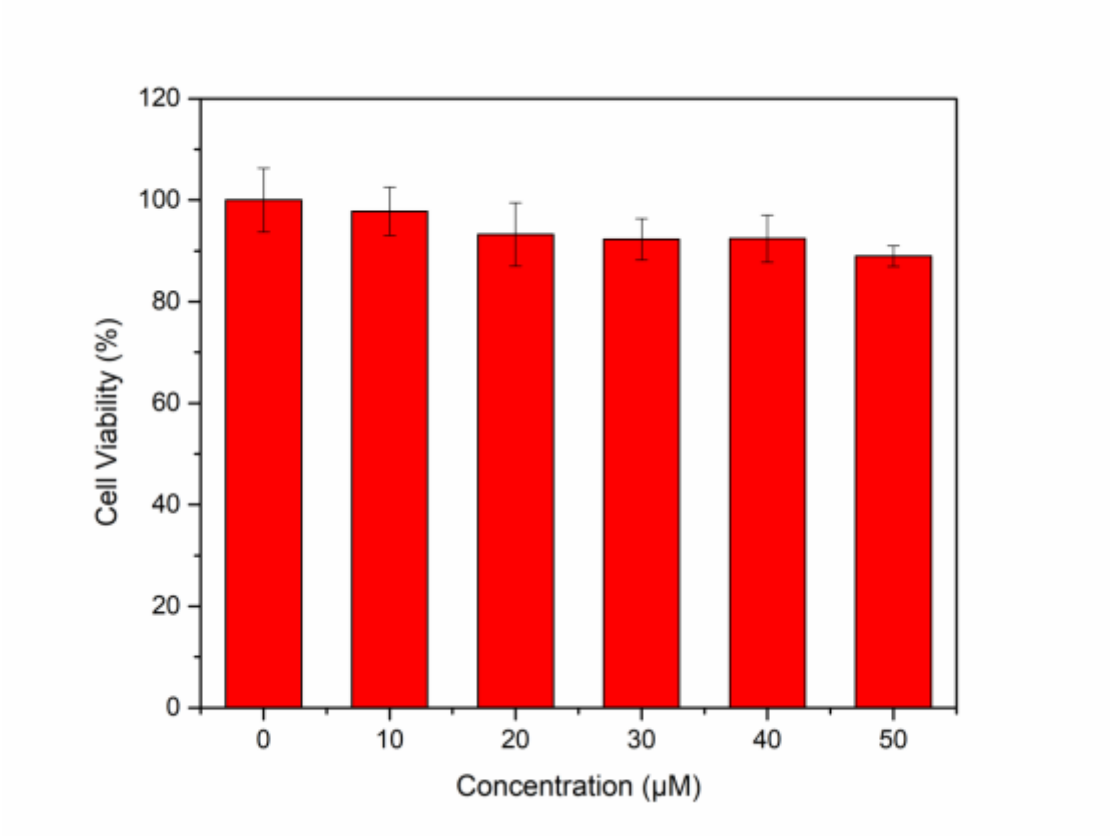


Figure S9. Cell viability of MCF-7 cells after incubation with different concentrations of **Lys-V** for 12

hours. (Concentration: 0 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM).

7. Fluorescence Images

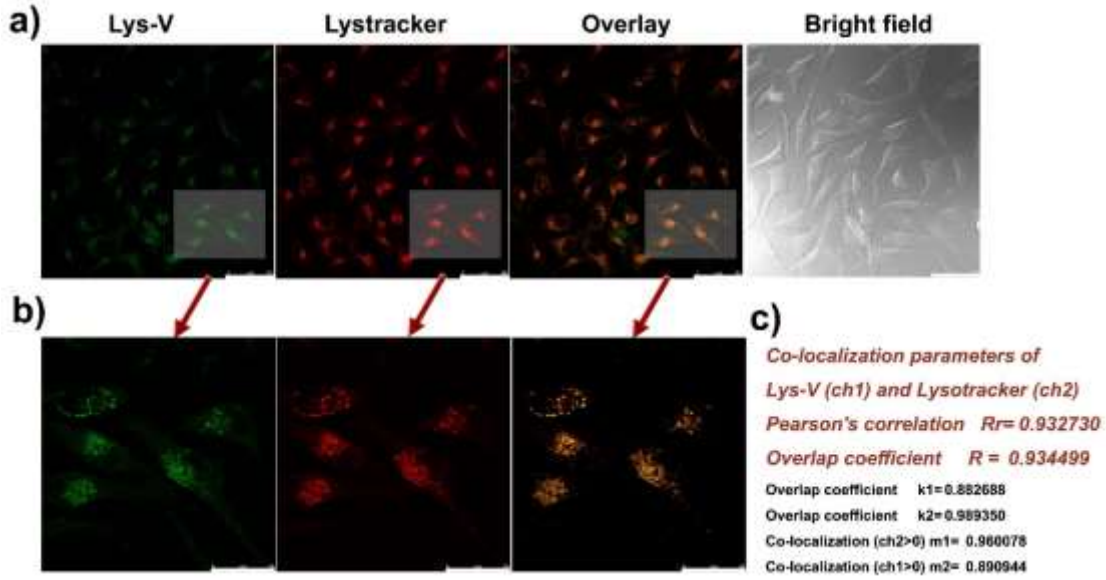
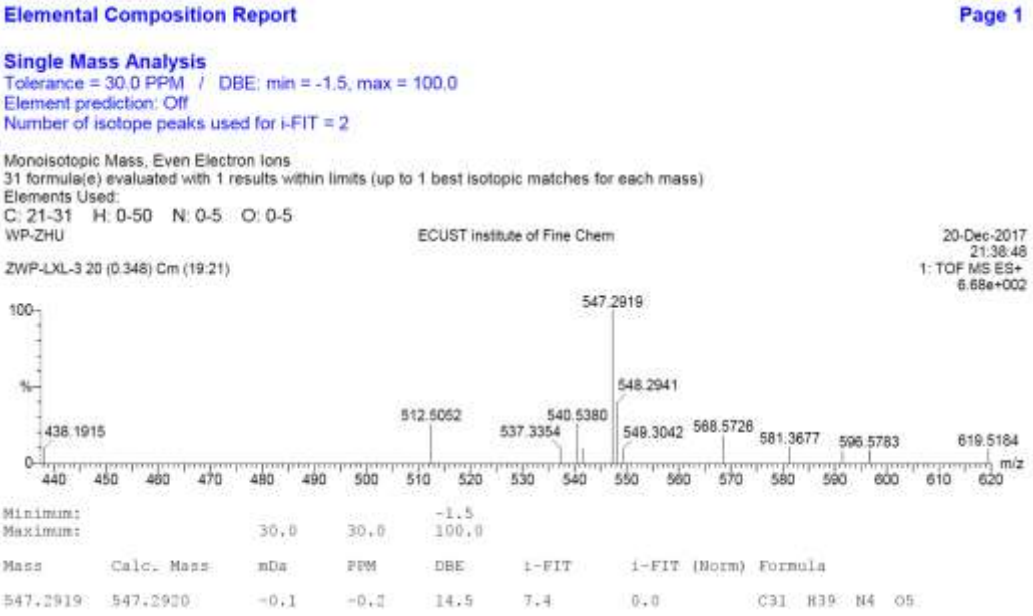


Figure S10. The Lysosome-targeting properties of probe **Lys-V** in live MCF-7 cells. The colocalization imaging of (a) **Lys-V** and (b) Lyso-Tracker Red costained living MCF-7 cells. Green image: the probe **Lys-V** stained signal collected from channel 1 (490 nm-610 nm). Red image: Lyso-Tracker labeled signal collected at 650 nm−800 nm (Pearson's correlation Rr = 0.933 and overlap coefficient R = 0.934). Scale bar in a) is 50 μm, in b) is 25 μm. Excited at 405 nm.

8. Movie S1

The dynamic changes of intracellular lysosomal viscosity through direct observing of the fluorescence signal changes was captured by merging the fluorescence images taken from every five minutes after dexamethasone stimulation.

9. HRMS and NMR Spectra



Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 30.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
31 formula(e) evaluated with 1 results within limits (up to 1 best isotopic matches for each mass)
Elements Used:
C: 21-31 H: 0-50 N: 0-5 O: 0-5

WP-ZHU ECUST institute of Fine Chem 20-Dec-2017 21:38:48
ZWP-LXL-3 20 (0.348) Cm (19:21) 1: TOF MS ES+ 6.68e+002

Minimum:				-1.5			
Maximum:		30.0	30.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
547.2919	547.2920	-0.1	-0.2	14.5	7.4	0.0	C31 H39 N4 O5

Figure S11. HRMS of **Lys-V**.

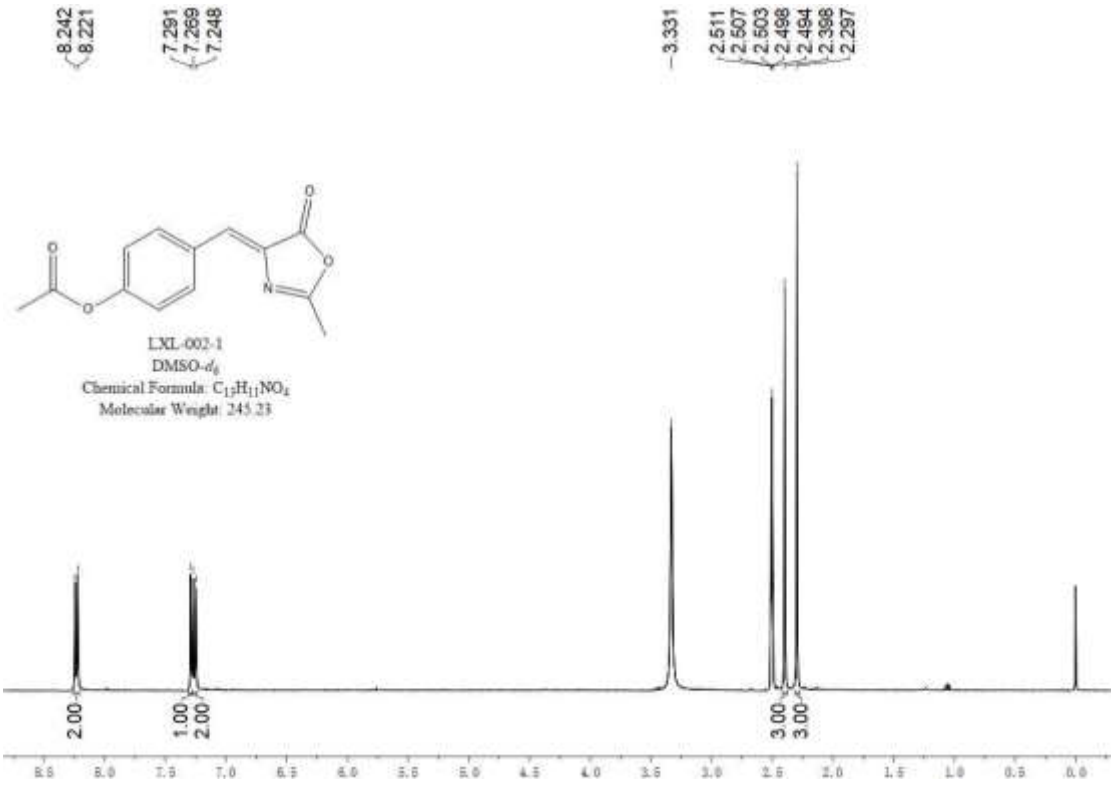


Figure S12. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum of Compound 1.

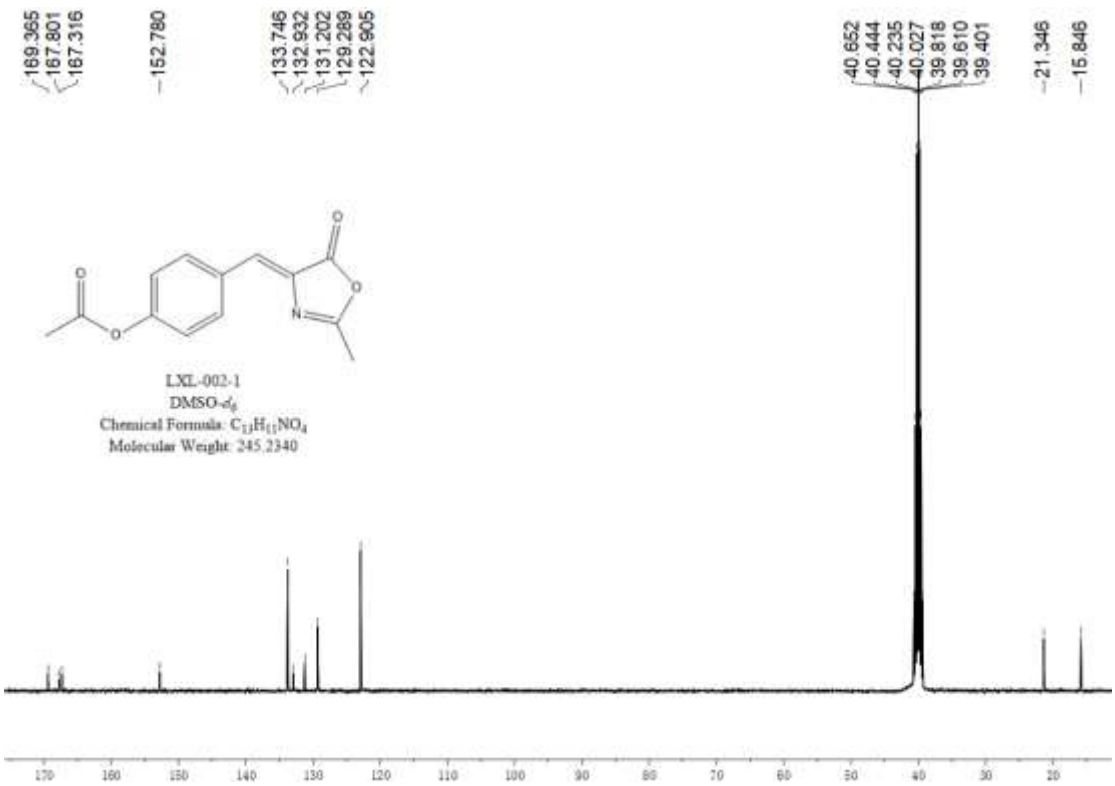


Figure S13. ^{13}C-NMR (101MHz, DMSO-d_6) spectrum of Compound **1**.

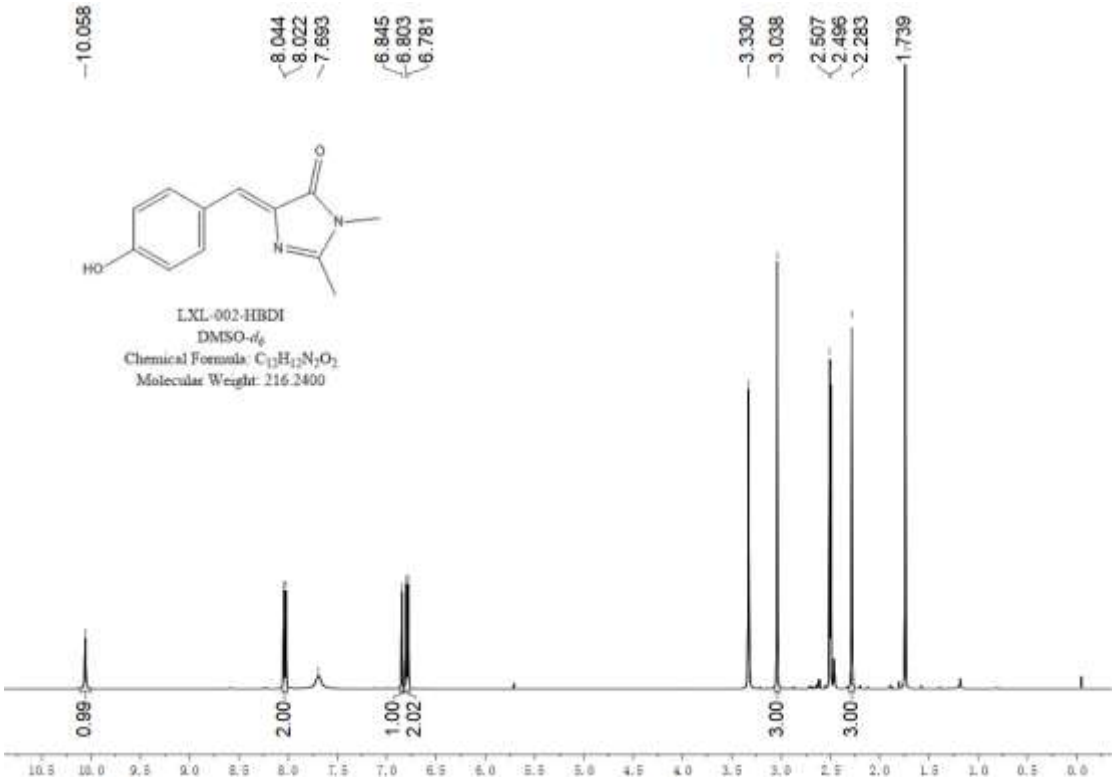


Figure S14. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum of Compound **HBDI**.

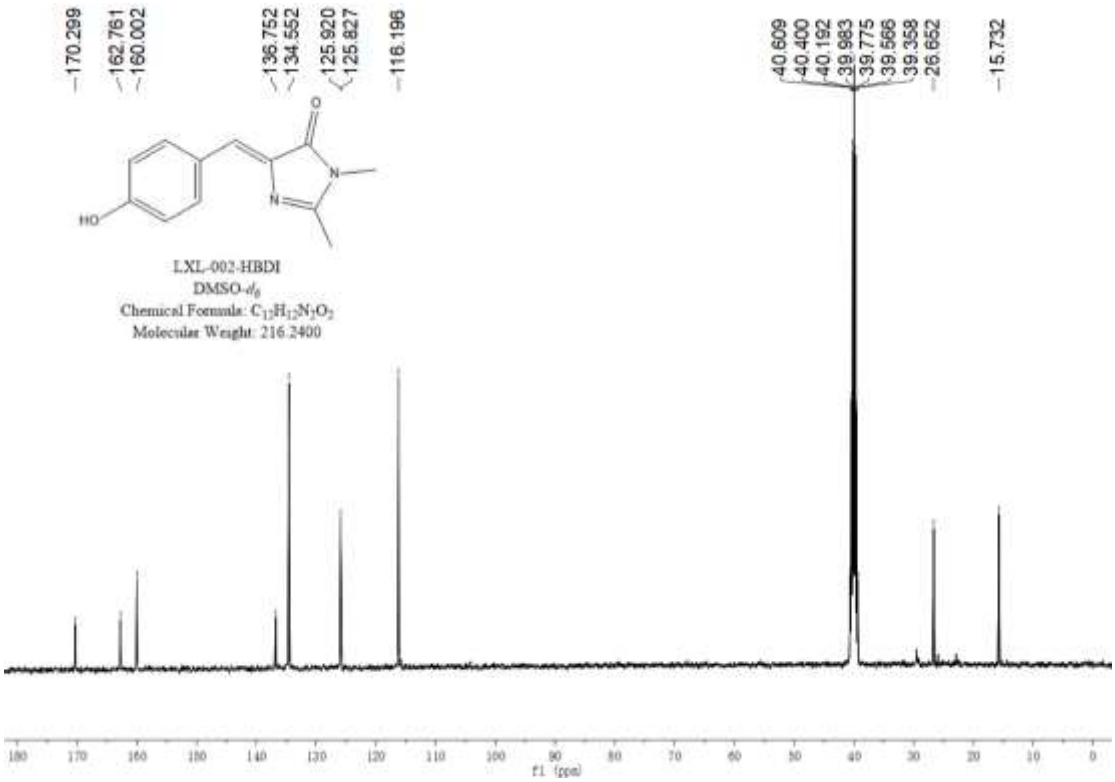


Figure S15. ^{13}C-NMR (101MHz, DMSO-d_6) spectrum of Compound **HBDI**.

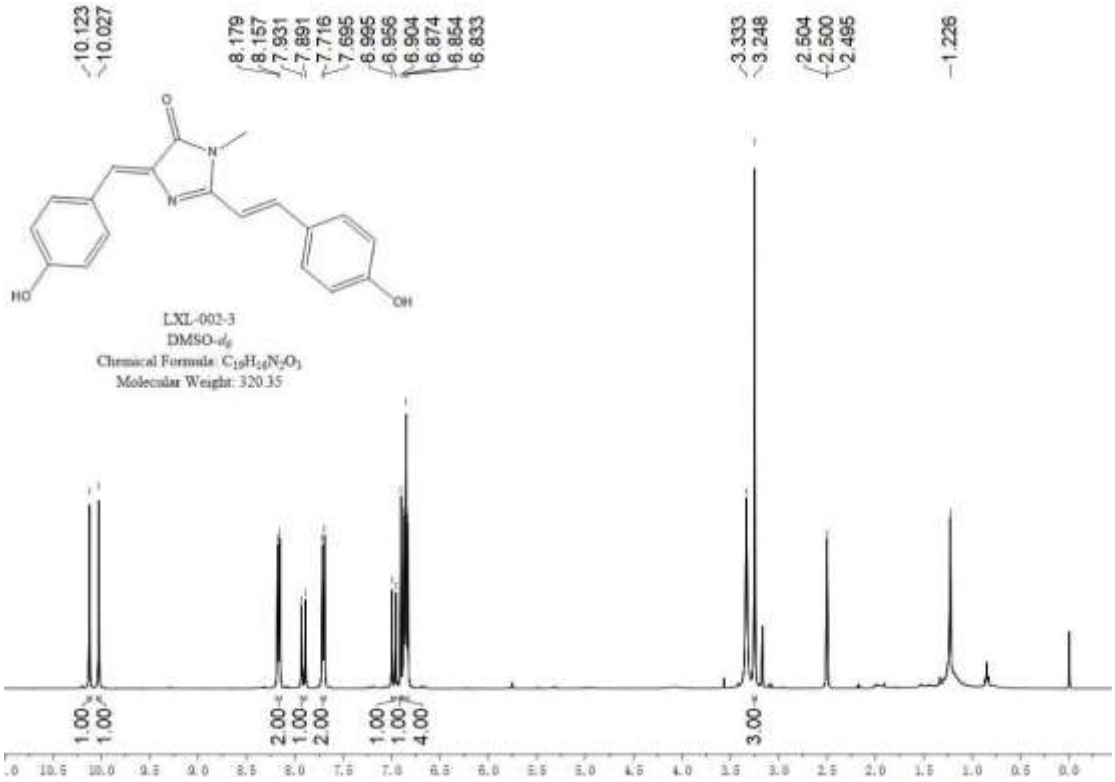


Figure S16. ^{1}H-NMR (400MHz, DMSO-d_6) spectrum of Compound 3.

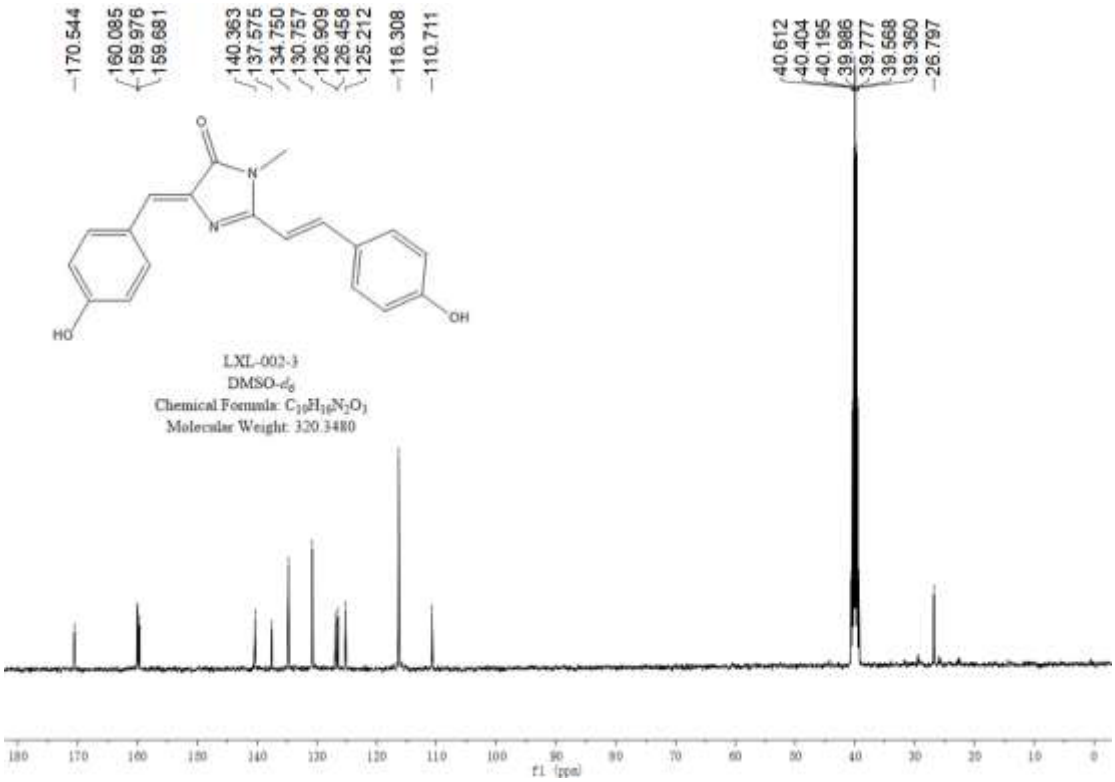


Figure S17. ^{13}C-NMR (101MHz, DMSO-d_6) spectrum of Compound **3**.

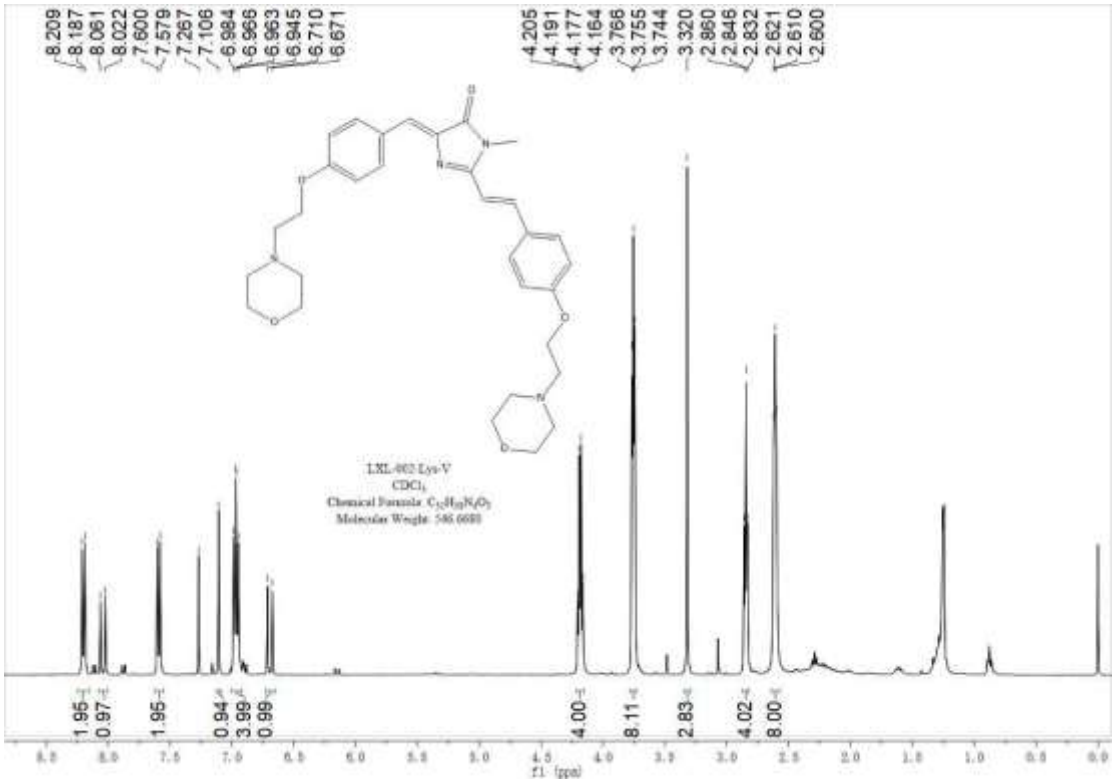


Figure S18. ^{1}H-NMR (400MHz, $CDCl_3$) spectrum of Compound **Lys-V**.

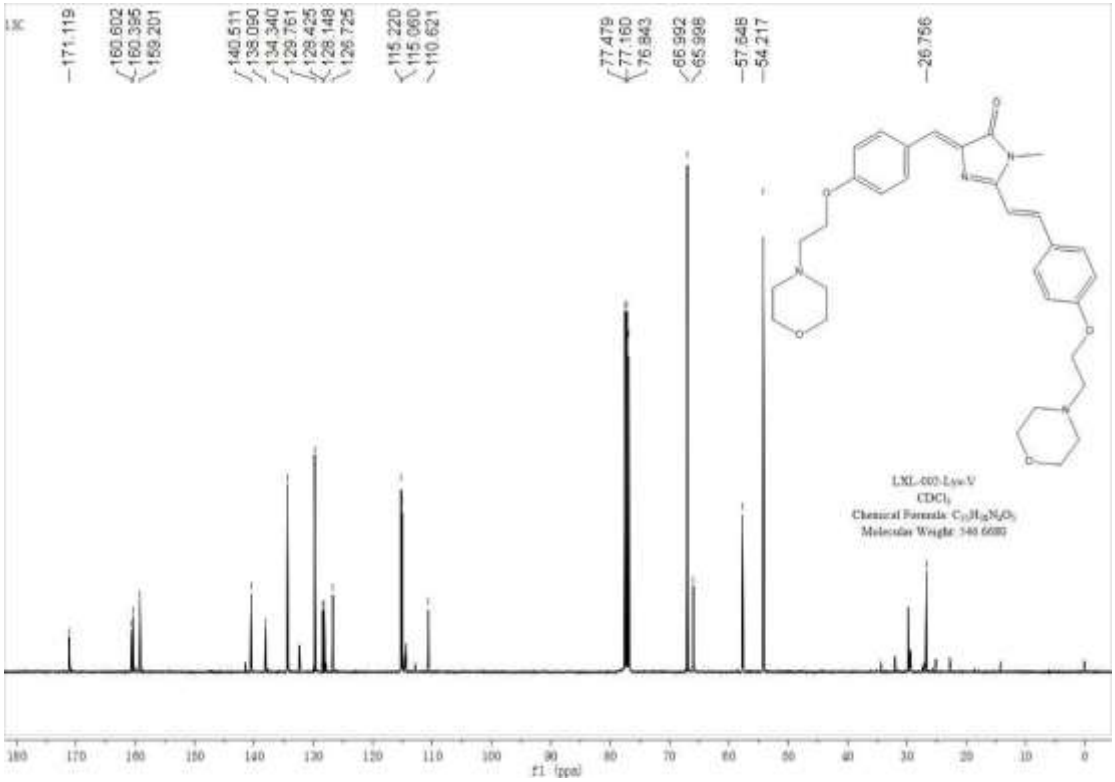


Figure S19. ^{13}C-NMR (101MHz, $CDCl_3$) spectrum of Compound **Lys-V**.