Reaction-Based Fluorescent Probes for rapid detection of hydrogen sulfide in vivo

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1. Spectroscopic properties of probe 1-2

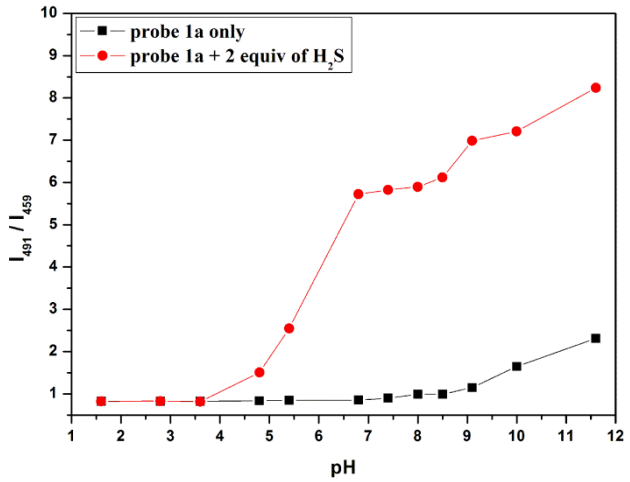

Figure S1. Fluorescence intensity of **1a** (5.0 μM) in the absence and presence of 2.0 equiv. of H_2S in water solution with different pH conditions, λex= 400 nm.

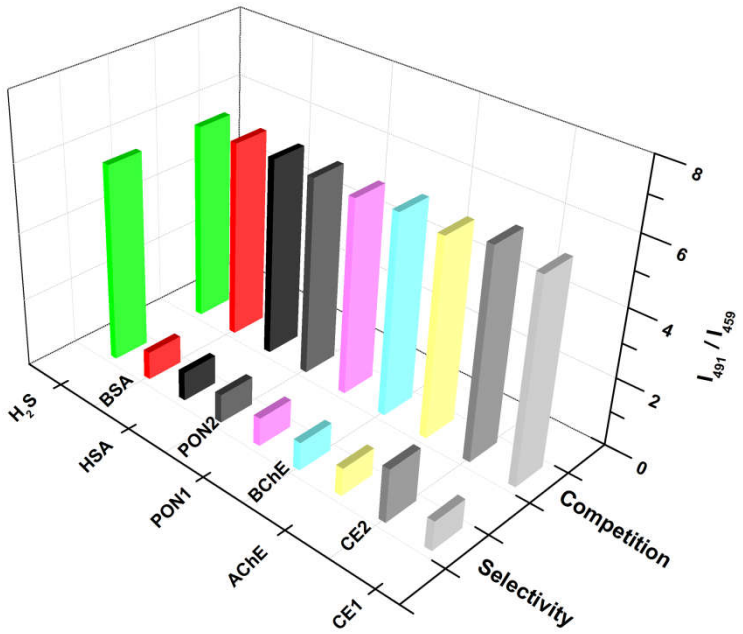

Figure S2. Fluorescence responses of probe **1a** (5.0 μM) upon addition of different esterases in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. Carboxylesterase 1 (CE1, 10.0 μg/mL), carboxylesterase 2(CE2, 10.0 μg/mL), acetylcholinesterase (AChE, 0.1 μg/L), butyrylcholinesterase (BChE, 20.0 U/L), paraoxonase 1 (PON1, 10.0 μg/mL), paraoxonase 2 (PON2, 10.0 μg/mL) and human serum albumin (HAS, 0.5 mg/L), bovine serum albumin (BSA, 0.5 mg/L).The pillars in the front row: probe **1a** (5.0 μM) + various biologically relevant species. The pillars in the back row: probe **1a** (5.0 μM) + NaHS (10.0 μM) + other biologically relevant species. λex = 400 nm.

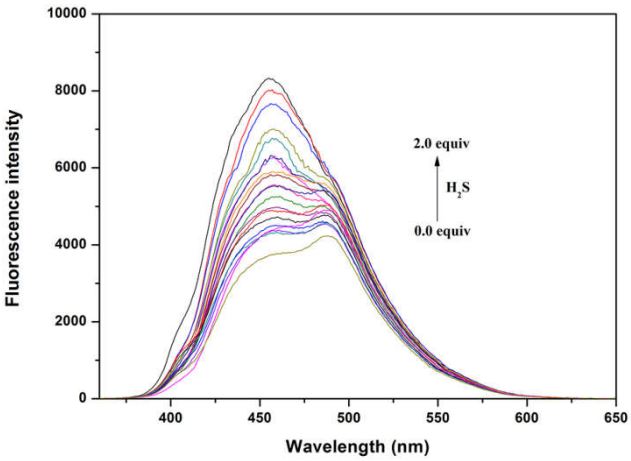

Figure S3. Fluorescence spectra of probe **1b** (10.0 μM) towards different concentration of NaHS (0.0-40.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

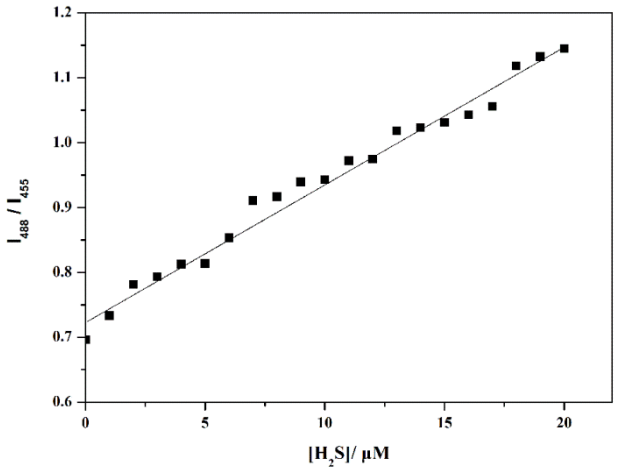

Figure S4. Plots of fluorescence intensity ratio $I_{488/455}$ towards different concentration of NaHS (0.0-20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

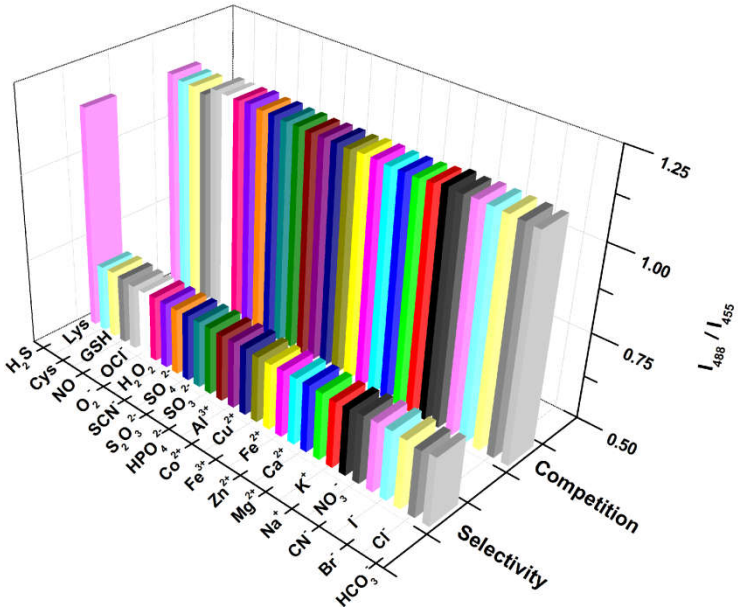

Figure S5. Selectivity of probe **1b** for NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. The pillars in the front row represent the value of fluorescence intensity ratio $I_{488/455}$ in the presence of various analytes. The pillars in the back row indicate the change in the fluorescence intensity ratio $I_{488/455}$ upon subsequent addition of NaHS (20.0 μM) to the solution containing probe **1b** and the respective analytes. λ_{ex} = 350 nm.

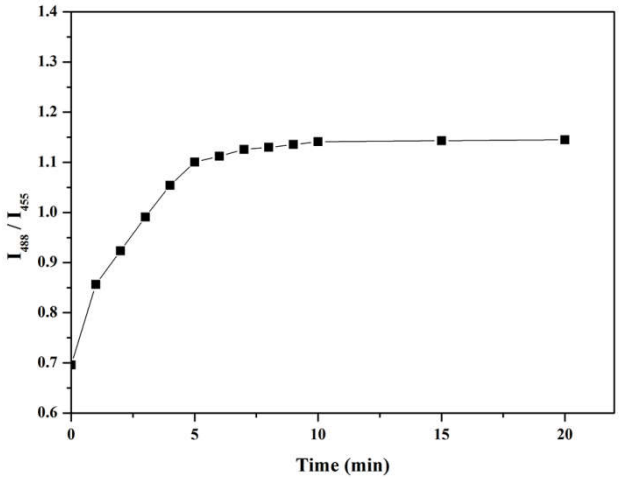

Figure S6. Time-dependent emission ratio changes observed for probe **1b** (10.0μM) upon interaction with NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

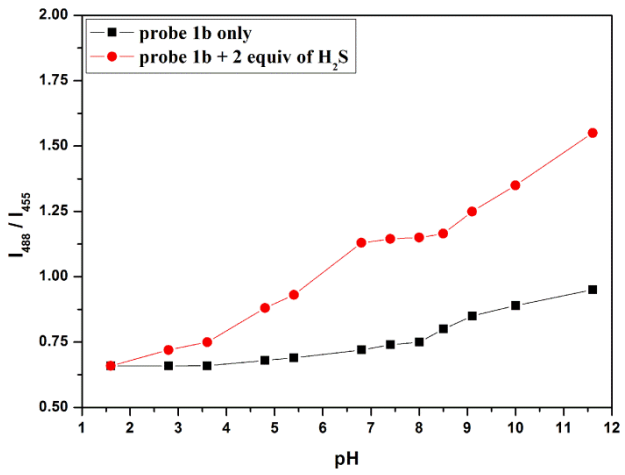

Figure S7. Fluorescence intensity of **1b** (10.0 μM) in the absence and presence of 2.0 equiv. of H_2S in water solution with different pH conditions, λex= 350 nm.

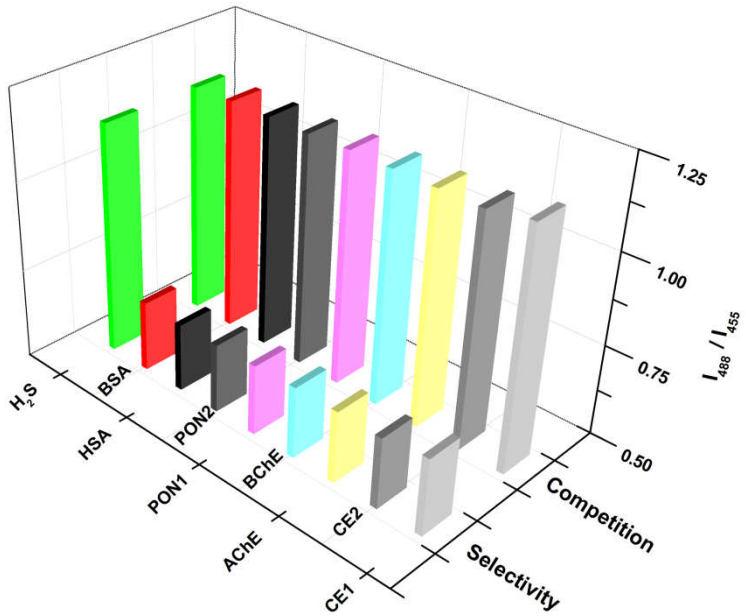

Figure S8. Fluorescence responses of probe **1b** (10.0 μM) upon addition of different esterases in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. Carboxylesterase 1 (CE1, 10.0 μg/mL), carboxylesterase 2(CE2, 10.0 μg/mL), acetylcholinesterase (AChE, 0.1 μg/L), butyrylcholinesterase (BChE, 20.0 U/L), paraoxonase 1 (PON1, 10.0 μg/mL), paraoxonase 2 (PON2, 10.0 μg/mL) and human serum albumin (HAS, 0.5 mg/L), bovine serum albumin (BSA, 0.5 mg/L).The pillars in the front row: probe **1b** (10.0 μM) + various biologically relevant species. The pillars in the back row: probe **1b** (10.0 μM) + NaHS (20.0 μM) + other biologically relevant species. λex = 350 nm.

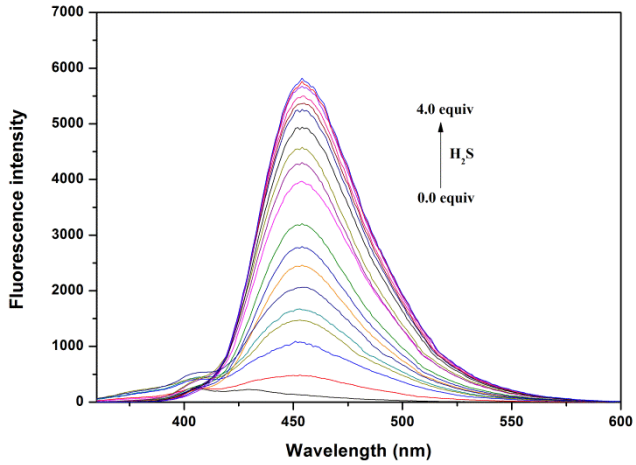

Figure S9. Fluorescence spectra of probe **2a** (10.0 μM) towards different concentration of NaHS (0.0-40.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

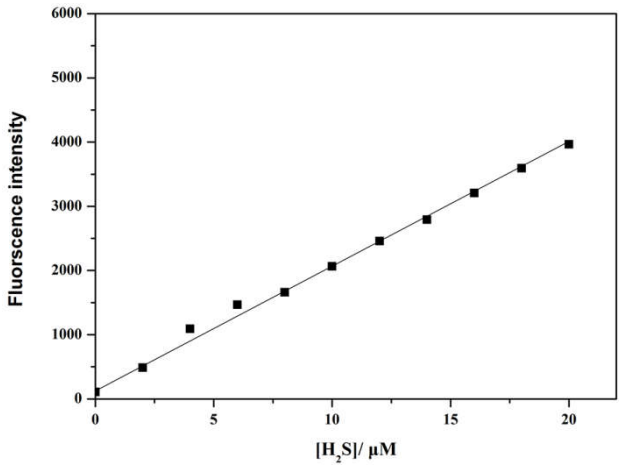

Figure S10. Plots of fluorescence intensity towards different concentration of NaHS (0.0-20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

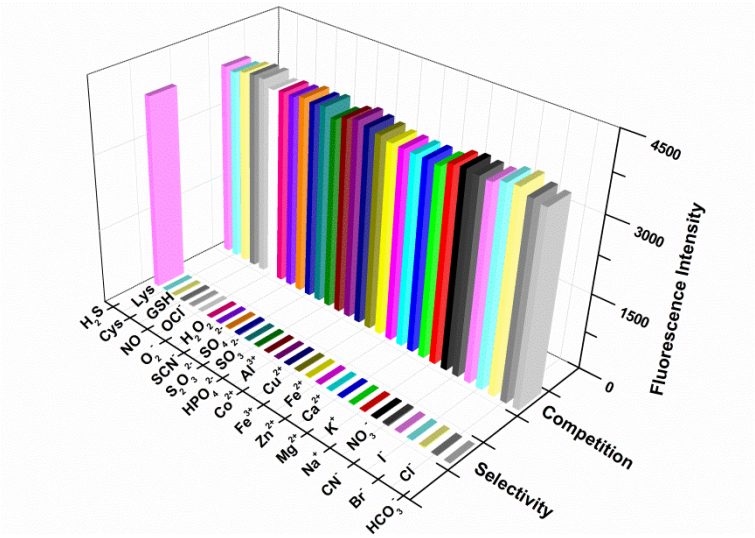

Figure S11. Selectivity of probe **2a** for NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. The pillars in the front row represent the value of fluorescence intensity in the presence of various analytes. The pillars in the back row indicate the change in the fluorescence intensity upon subsequent addition of NaHS (20.0 μM) to the solution containing probe **2a** and the respective analytes. λ_{ex} = 350 nm.

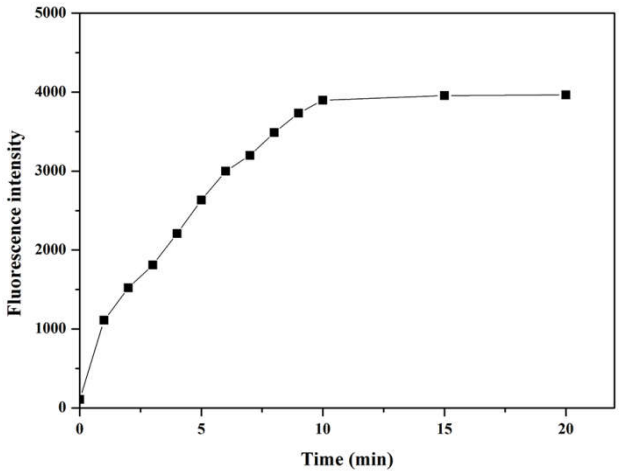

Figure S12. Time-dependent emission ratio changes observed for probe **2a** (10.0 μM) upon interaction with NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

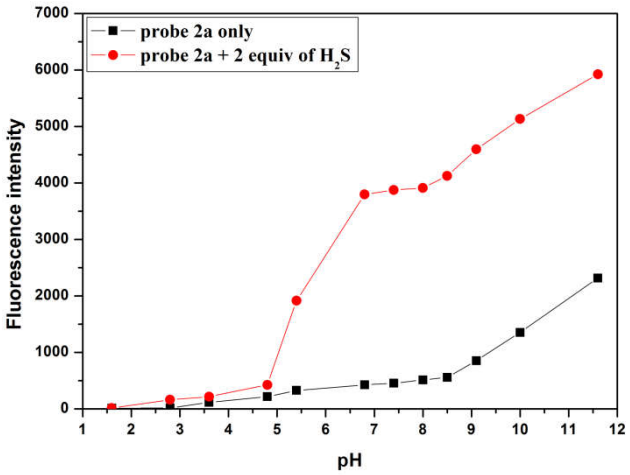

Figure S13. Fluorescence intensity of **2a** (10.0 μM) in the absence and presence of 2.0 equiv. of H_2S in water solution with different pH conditions, λex= 350 nm.

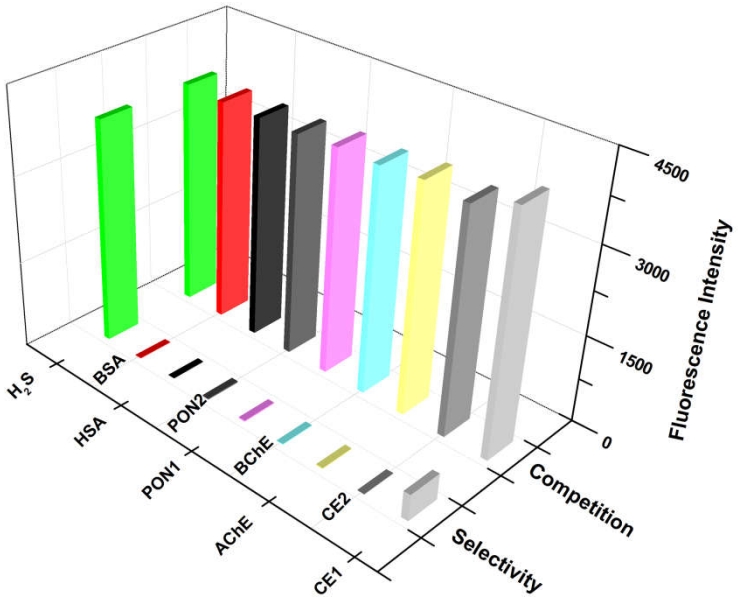

Figure S14. Fluorescence responses of probe **2a** (10.0 μM) upon addition of different esterases in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. Carboxylesterase 1 (CE1, 10.0 μg/mL), carboxylesterase 2(CE2, 10.0 μg/mL), acetylcholinesterase (AChE, 0.1 μg/L), butyrylcholinesterase (BChE, 20.0 U/L), paraoxonase 1 (PON1, 10.0 μg/mL), paraoxonase 2 (PON2, 10.0 μg/mL) and human serum albumin (HAS, 0.5 mg/L), bovine serum albumin (BSA, 0.5 mg/L).The pillars in the front row: probe **2a** (10.0 μM) + various biologically relevant species. The pillars in the back row: probe **2a** (10.0 μM) + NaHS (20.0 μM) + other biologically relevant species. λex = 350 nm.

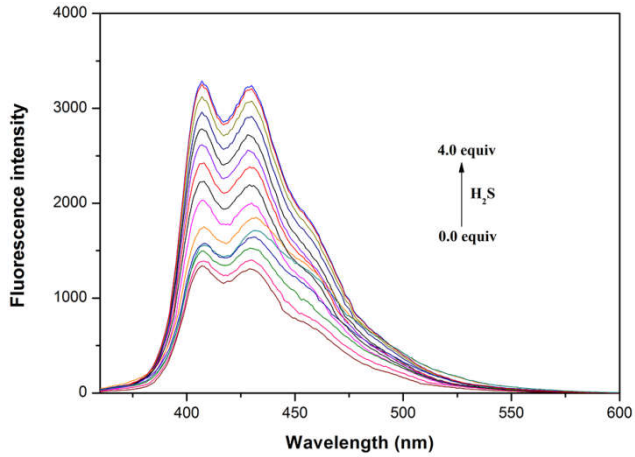

Figure S15. Fluorescence spectra of probe **2b** (10.0 μM) towards different concentration of NaHS (0.0-40.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

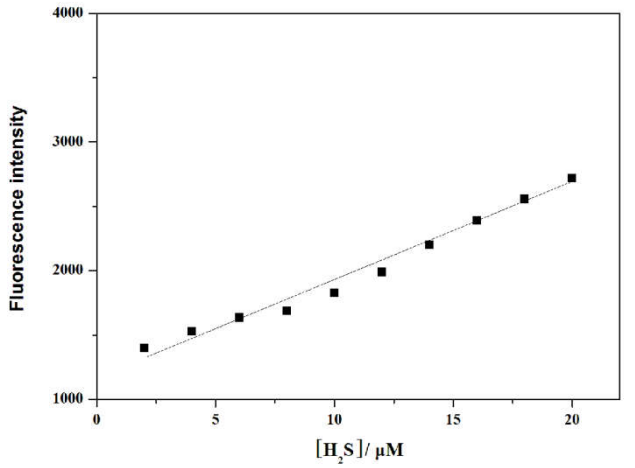

Figure S16. Plots of fluorescence intensity towards different concentration of NaHS (0.0-20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

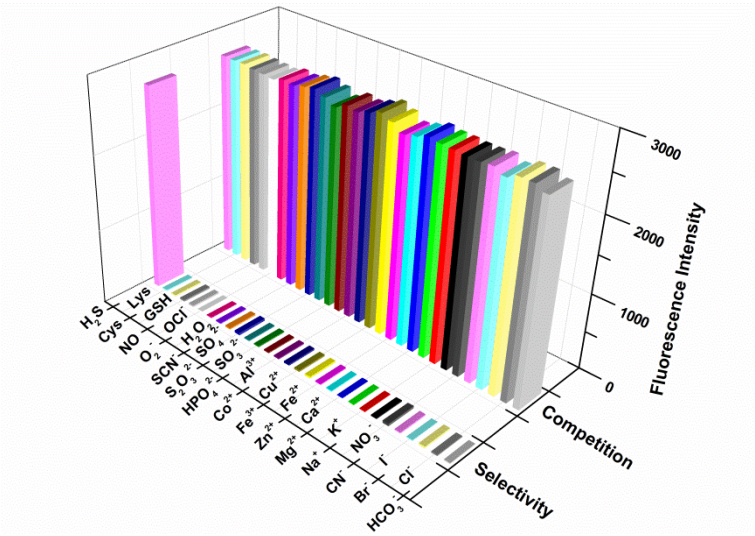

Figure S17. Selectivity of probe **2b** for NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. The pillars in the front row represent the value of fluorescence intensity in the presence of various analytes. The pillars in the back row indicate the change in the fluorescence intensity upon subsequent addition of NaHS (20.0 μM) to the solution containing probe **2b** and the respective analytes. λ_{ex} = 350 nm.

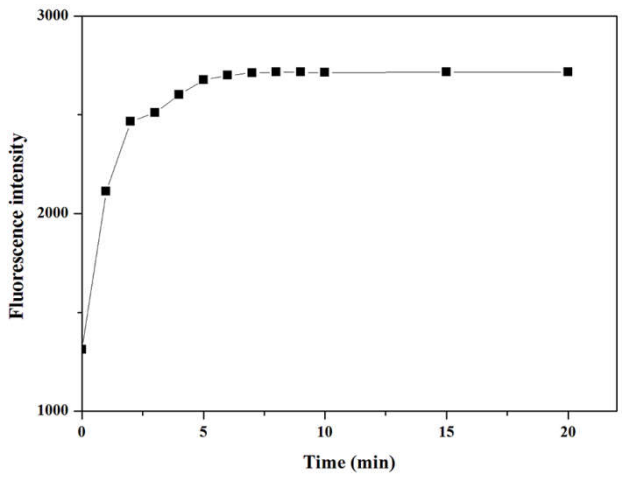

Figure S18. Time-dependent emission ratio changes observed for probe **2b** (10.0 μM) upon interaction with NaHS (20.0 μM) in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3). λ_{ex} = 350 nm.

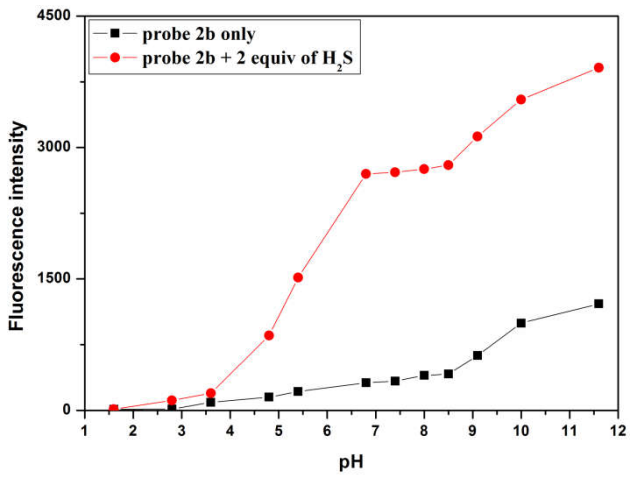

Figure S19. Fluorescence intensity of **2b** (10.0 μM) in the absence and presence of 2.0 equiv. of H_2S in water solution with different pH conditions, λex= 350 nm.

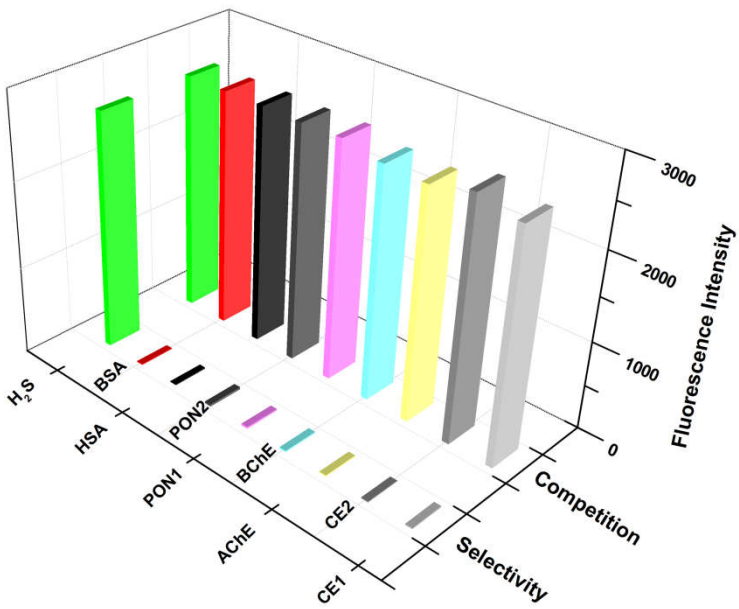

Figure S20. Fluorescence responses of probe **2b** (10.0 μM) upon addition of different esterases in PBS buffer (20.0 mM, pH 7.4, 1 % CH_3COCH_3) solution. Carboxylesterase 1 (CE1, 10.0 μg/mL), carboxylesterase 2(CE2, 10.0 μg/mL), acetylcholinesterase (AChE, 0.1 μg/L), butyrylcholinesterase (BChE, 20.0 U/L), paraoxonase 1 (PON1, 10.0 μg/mL), paraoxonase 2 (PON2, 10.0 μg/mL) and human serum albumin (HAS, 0.5 mg/L), bovine serum albumin (BSA, 0.5 mg/L).The pillars in the front row: probe **2b** (10.0 μM) + various biologically relevant species. The pillars in the back row: probe **2b** (10.0 μM) + NaHS (20.0 μM) + other biologically relevant species. λex = 400 nm.

2. ^{1}H NMR, ^{13}C NMR and MS spectra

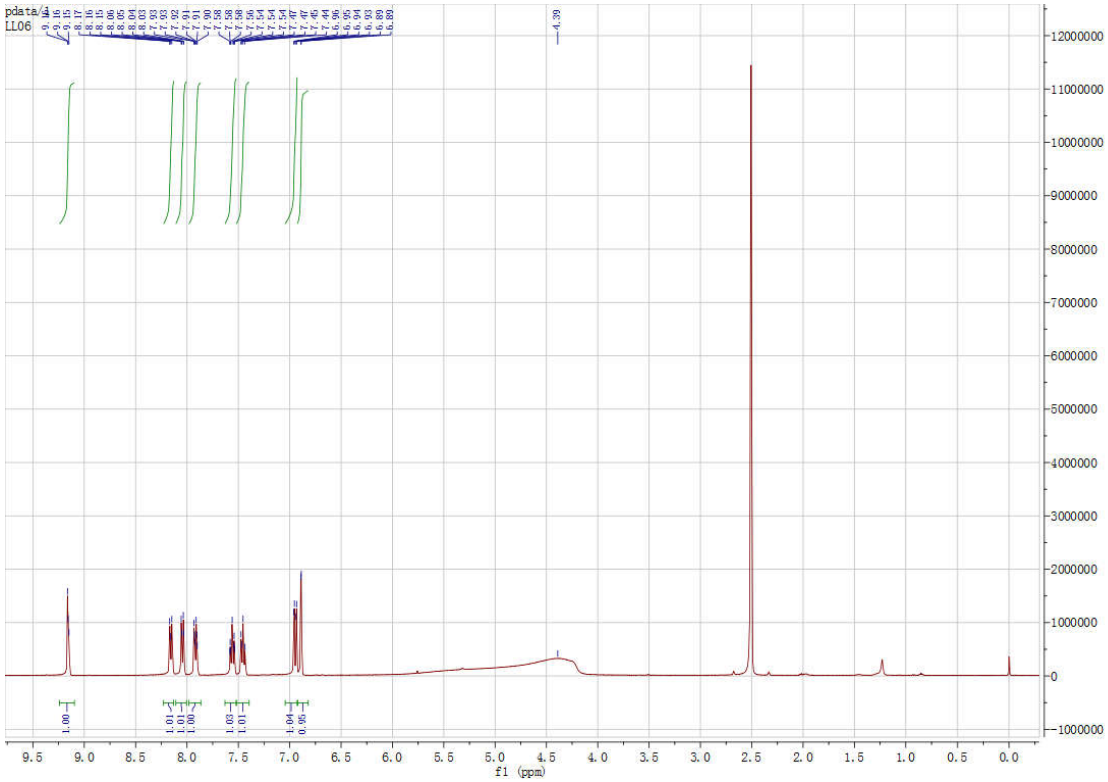

Figure S21. ^{1}H NMR spectrum of **3** in DMSO

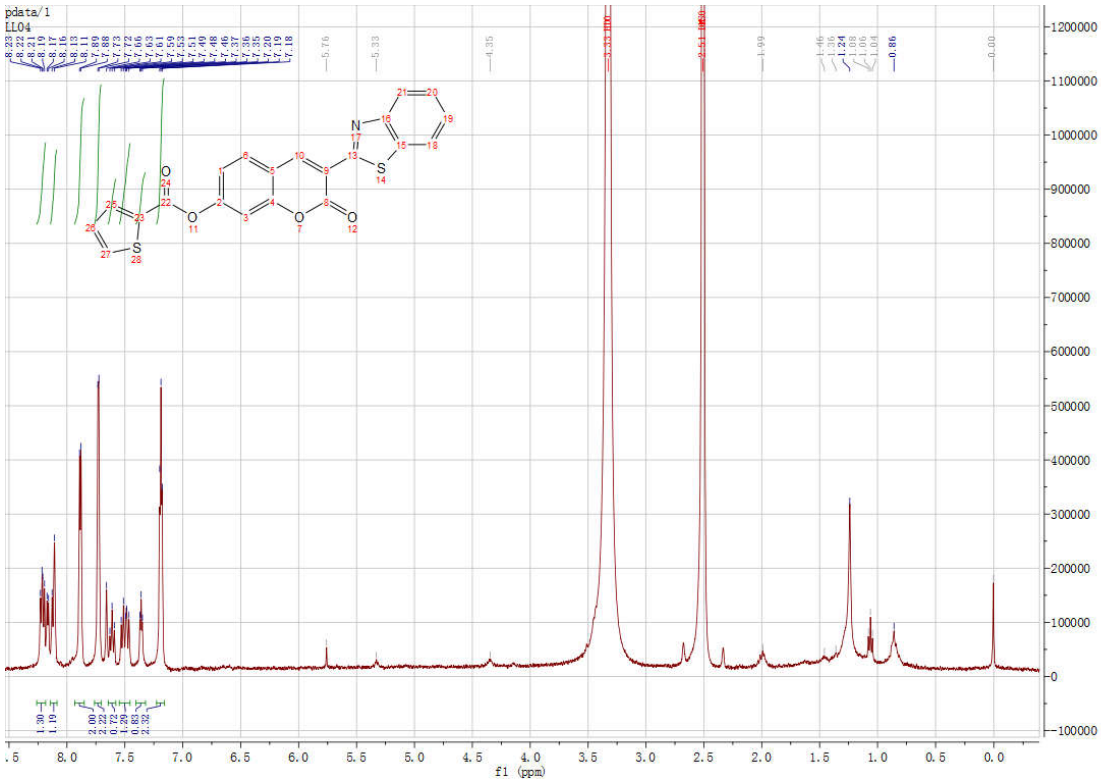

Figure S22. ^{1}H NMR spectrum of **1a** in DMSO

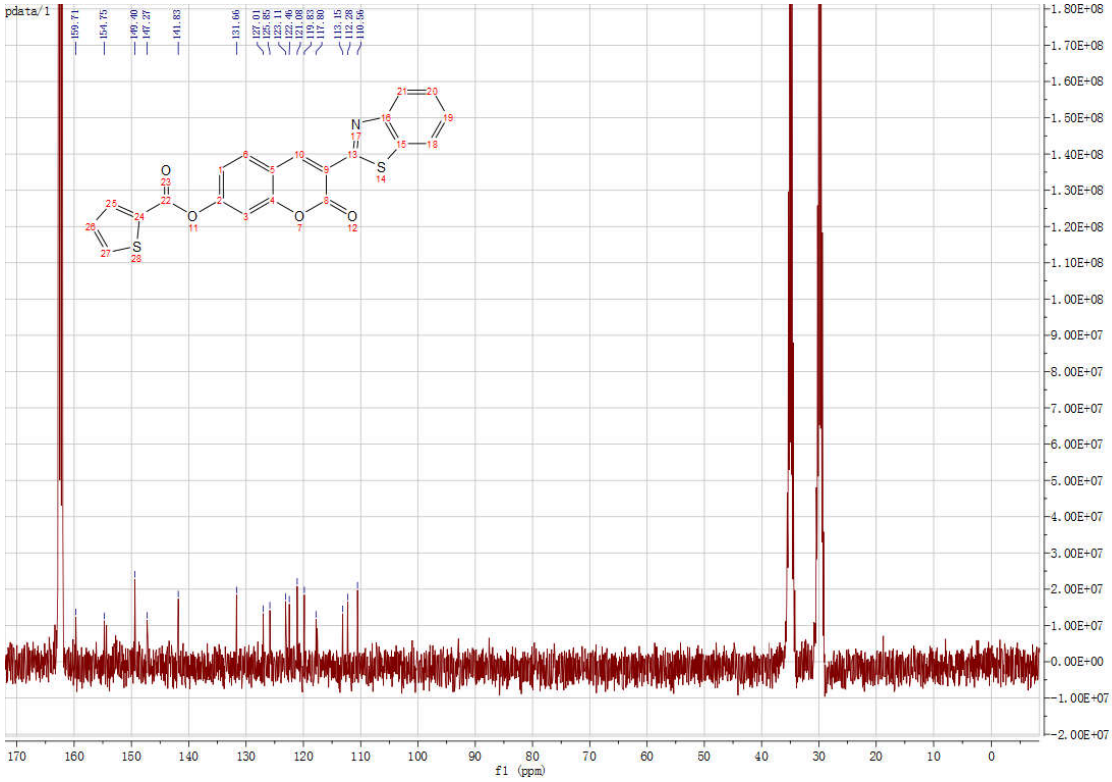

Figure S23. ^{13}C NMR spectrum of **1a** in DMF.

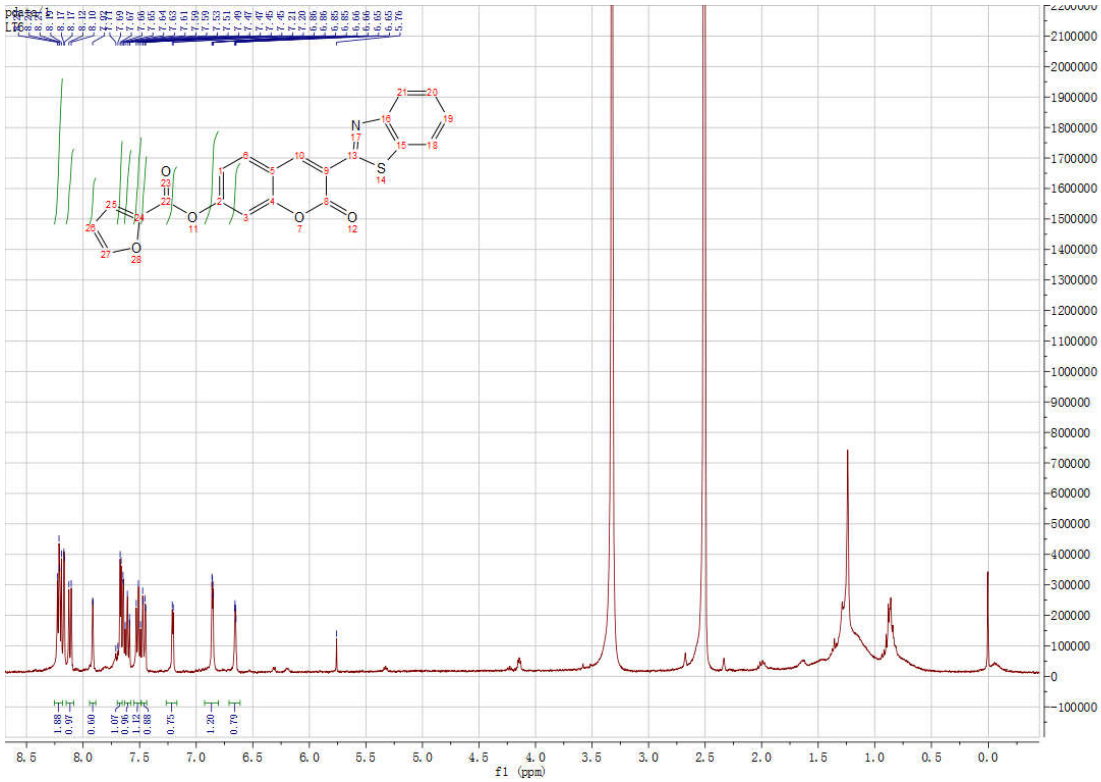

Figure S24. ^{1}H NMR spectrum of **1b** in DMSO

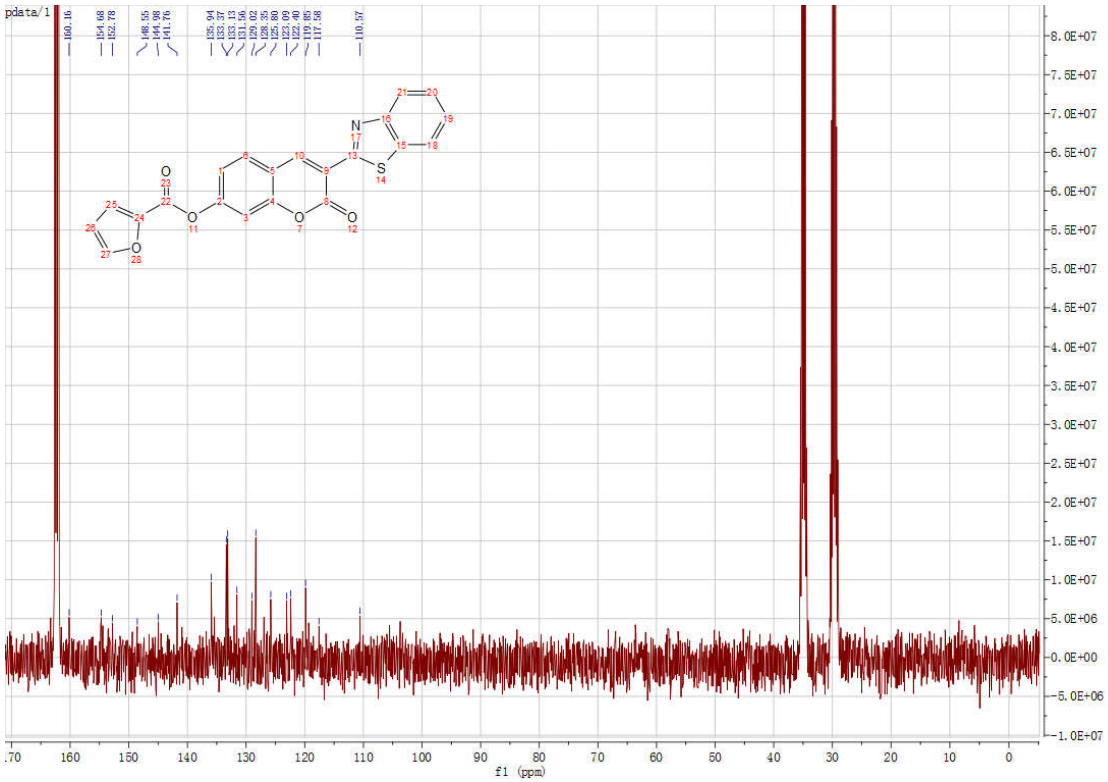

Figure S25. ^{13}C NMR spectrum of **1b** in DMF.

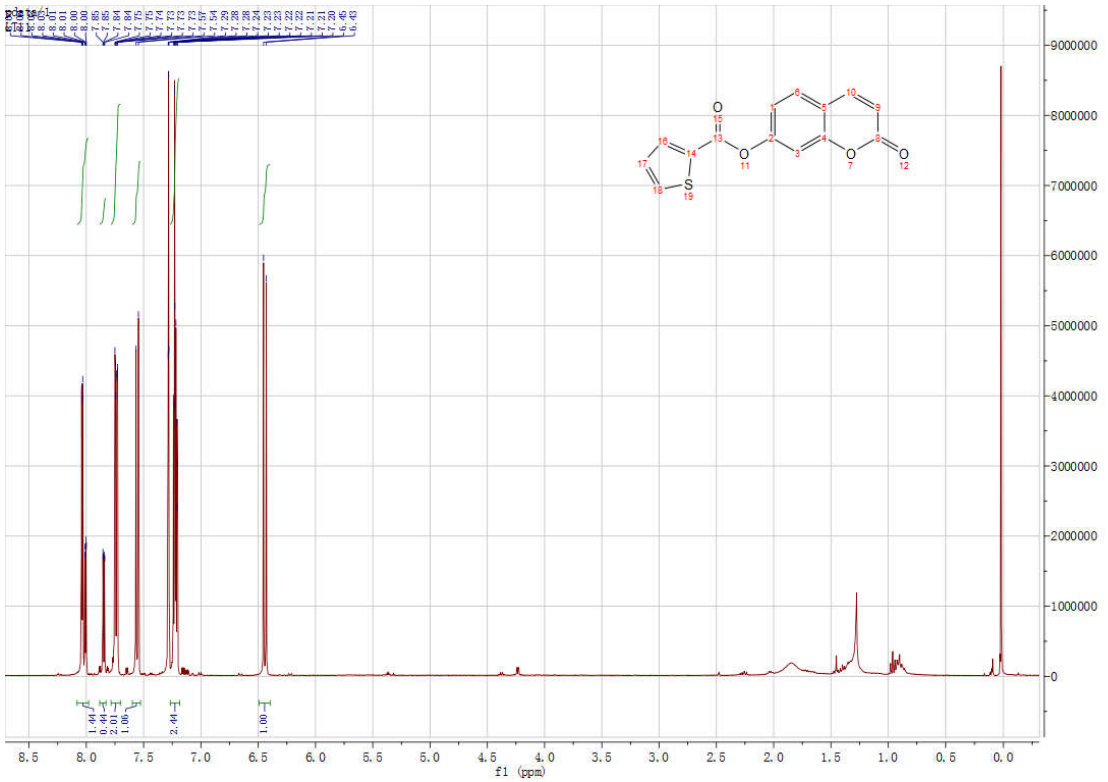

Figure S26. ^{1}H NMR spectrum of **2a** in $CDCl_3$

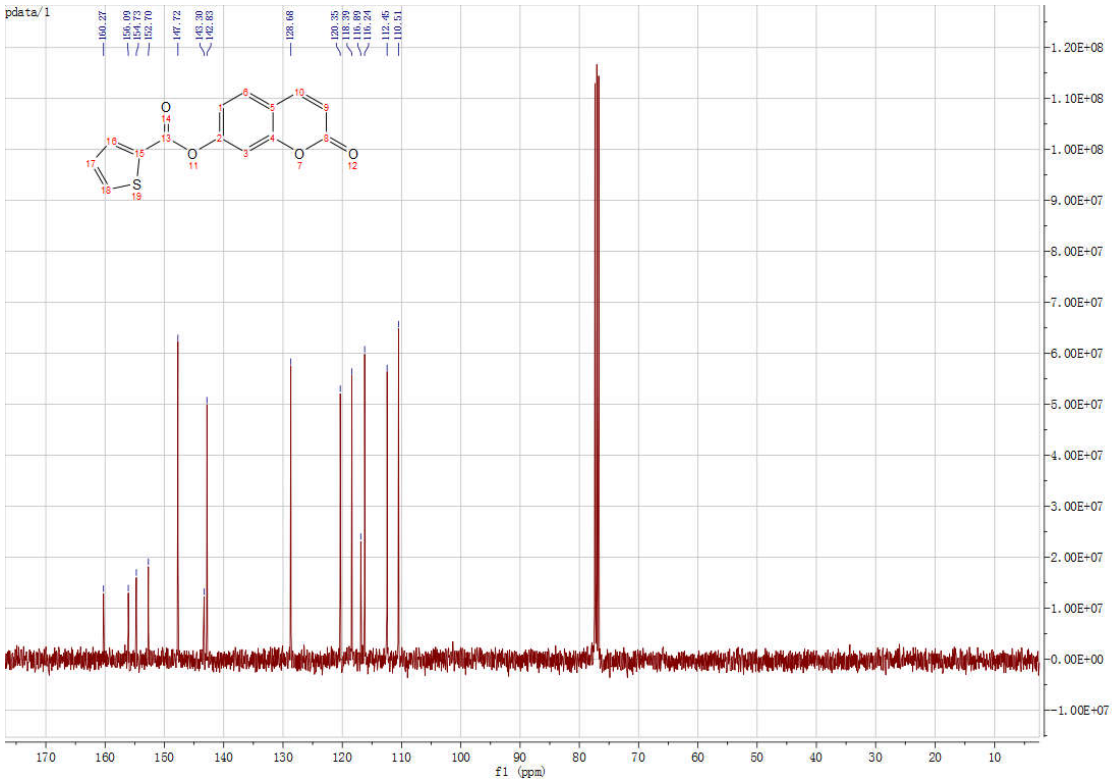

Figure S27.^{13}C NMR spectrum of **2a** in DMF.

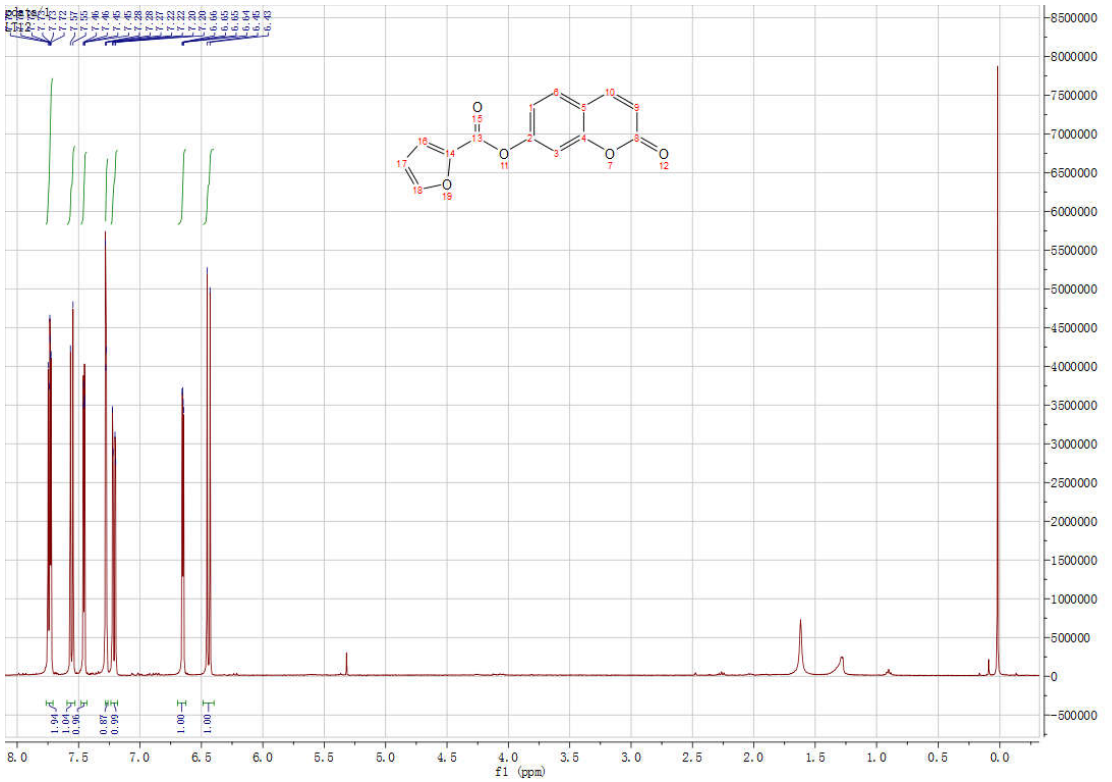

Figure S28. ^{1}H NMR spectrum of **2b** in $CDCl_3$

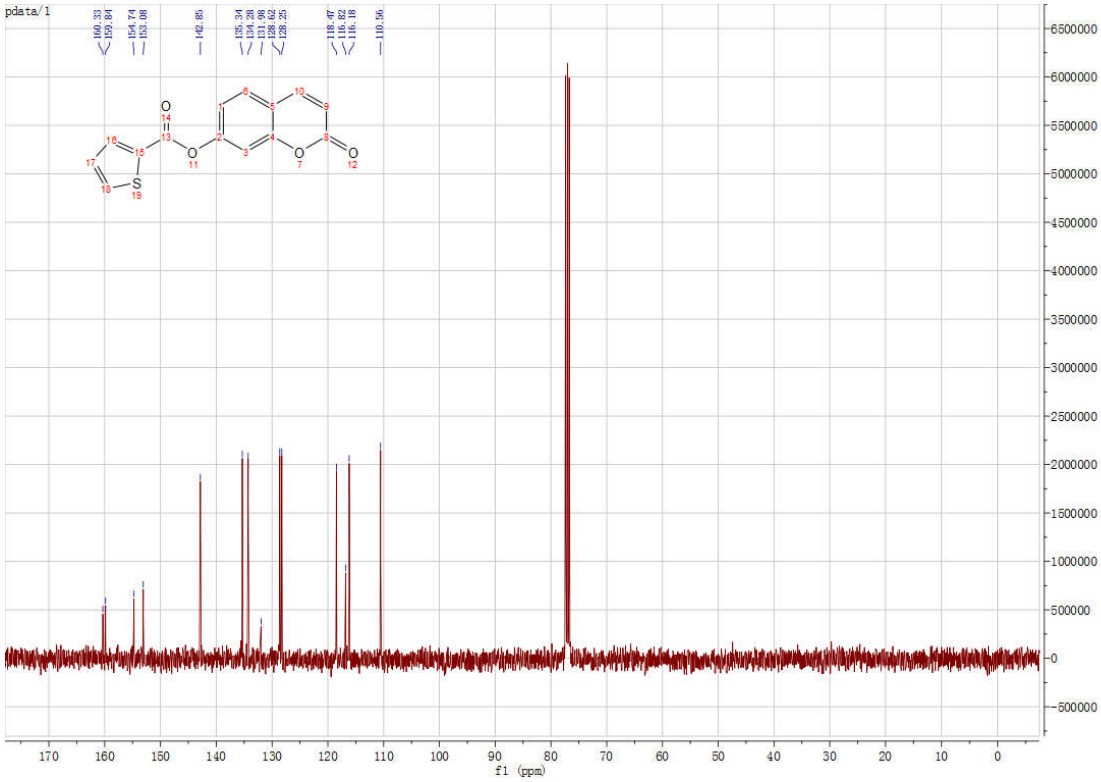

Figure S29. ^{13}C NMR spectrum of **2b** in DMF.

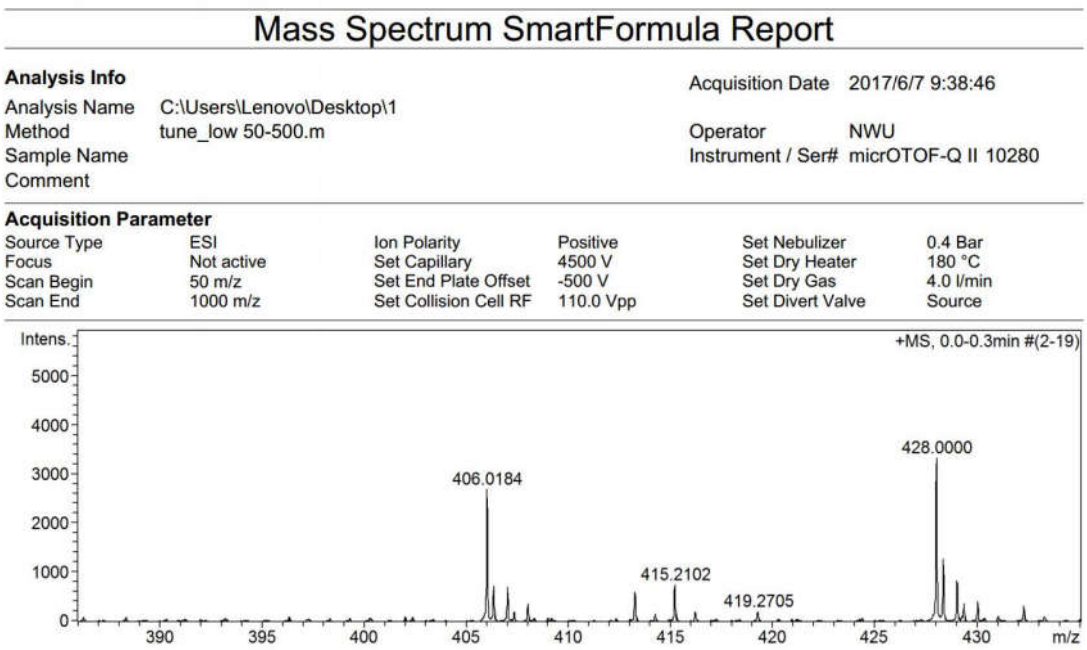

Figure S30. Mass spectrum of **1a**

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	hanyf-LY047	Instrument / Ser#	micrOTOF-Q II 10280
Comment			

Acquisition Parameter

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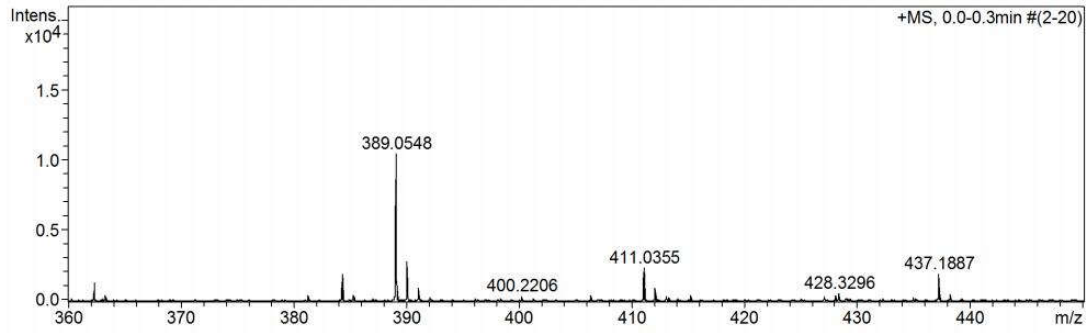

Figure S31. Mass spectrum of **1b**

Mass Spectrum SmartFormula Report

Analysis Info

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Method	tune_low 50-500.m	Operator	service
Sample Name		Instrument / Ser#	micrOTOF-Q II 10280
Comment			

Acquisition Parameter

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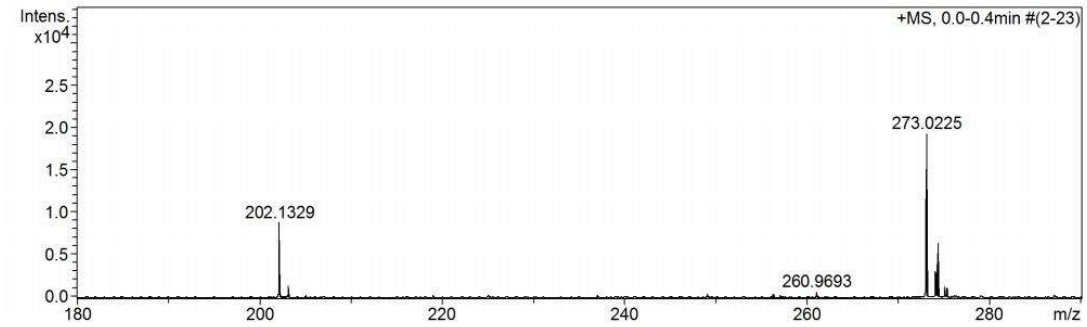

Figure S32. Mass spectrum of **2a**

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name		Instrument / Ser#	micrOTOF-Q II 10280
Comment			

Acquisition Parameter

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Scan End	1000 m/z	Set Collision Cell RF	110.0 Vpp	Set Divert Valve	Source

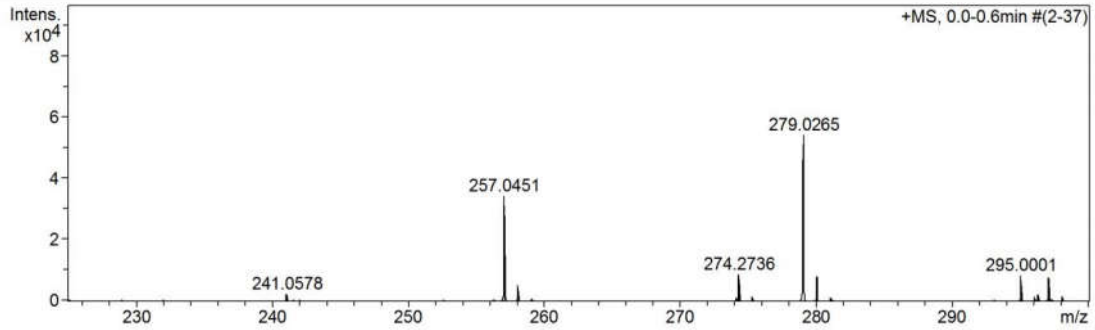

Figure S33. Mass spectrum of **2b**

Mass Spectrum SmartFormula Report

Analysis Info

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Comment			

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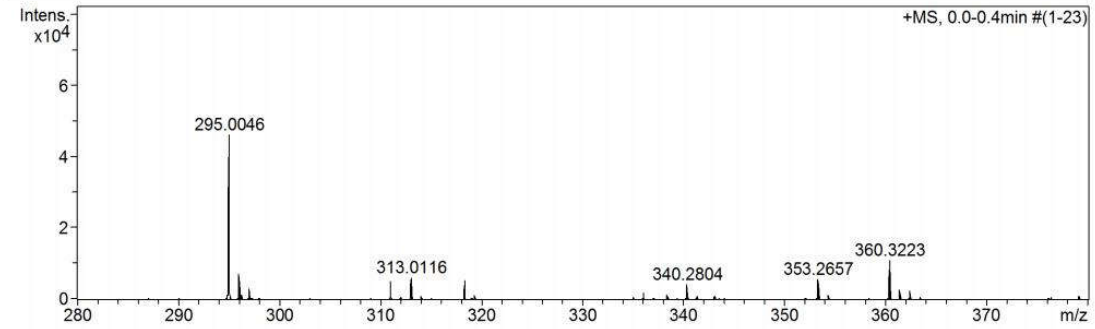

Figure S34. Mass spectrum of **4**

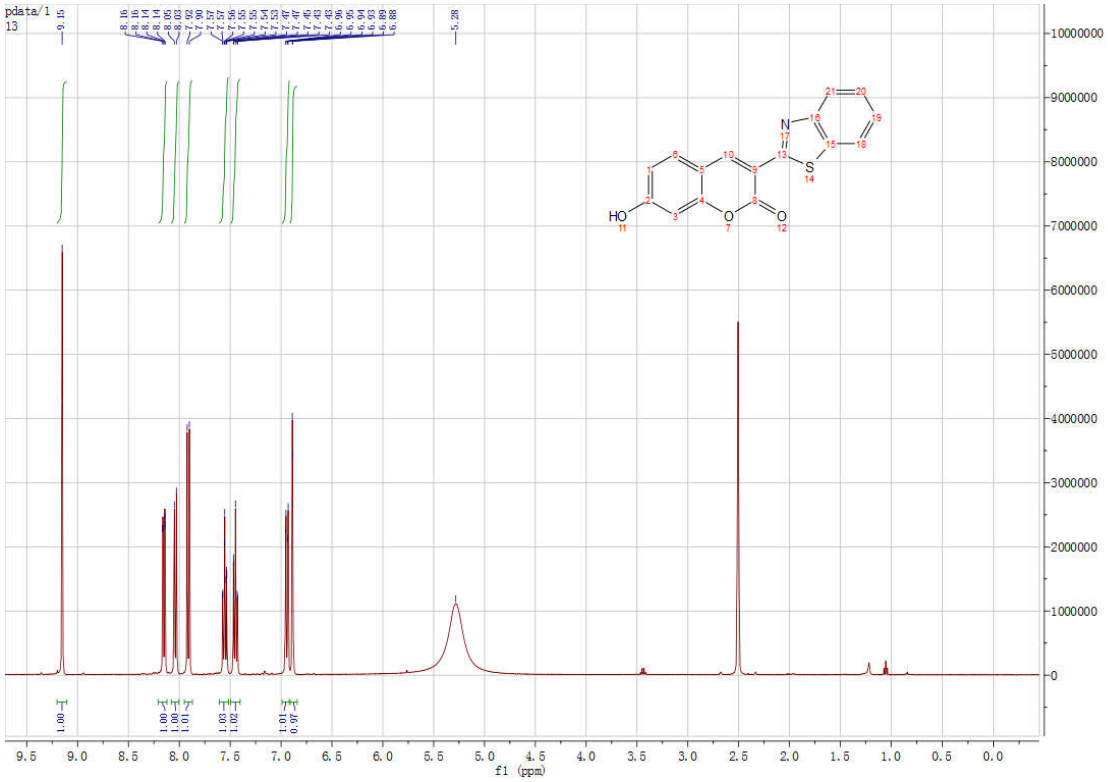

Figure S35. ^{1}H NMR spectrum of **4** in DMSO

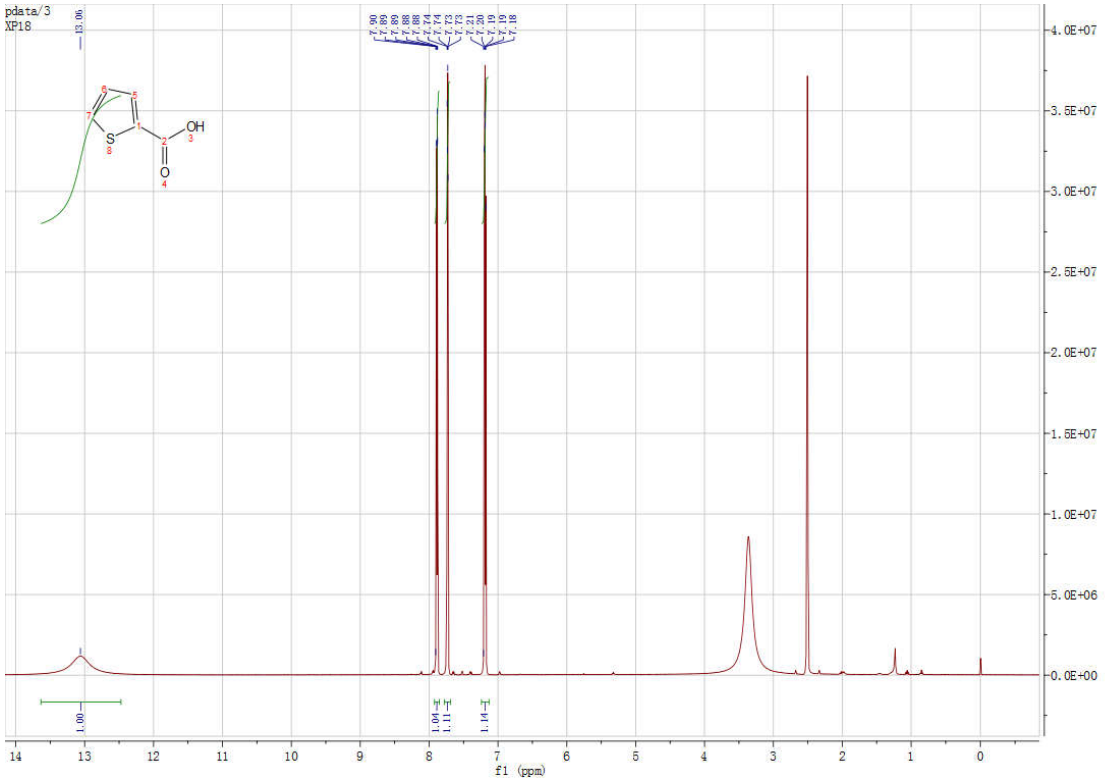

Figure S36. ^{1}H NMR spectrum of **5** in DMSO