Rhodamine-based fluorescent sensors for the rapid and selective off-on detection of salicylic acid and their use in plant cell imaging

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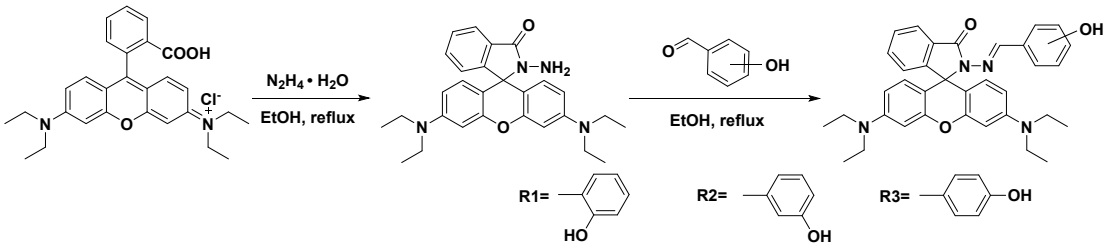
Synthesis of R1-R3



Scheme S1. Synthesis of sensors **R1-R3**

Synthesis of R1. Rhodamine B (3g, 6.26 mmol) was dissolved in 40 mL of anhydrous ethanol with 3 mL of hydrazine hydrate. The resulting mixture solution was refluxed for 5 h, and then cooled to room temperature. After that, the mixture solution was neutralized at pH = 7-8 by adjusting diluted hydrochloric acid, the solid was then precipitated, filtered, washed with double distilled water, and dried to obtain 2.604 g rhodamine hydrazine solid with a yield of 91.05%. Rhodamine B hydrazide (200 mg, 0.438 mmol) and 2- hydroxybenzaldehyde (46.7 μL, 0.438 mmol) were put into a pressure tube containing 5 mL of ethanol absolute. After the mixed system was heated under reflux for 10 h, the reaction mixture was then cooled to room temperature. The white precipitation was washed by ethanol for three times to yield the white pink (228.5 mg) with a yield of 92.7 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.83 (s, 1H), 9.23 (s, 1H), 7.98 (dd, J = 6.3, 1.8 Hz, 1H), 7.52 (pd, J = 7.4, 1.4 Hz, 2H), 7.20 – 7.14 (m, 2H), 7.10 (dd, J = 7.7, 1.5 Hz, 1H), 6.86 (d, J = 8.1 Hz, 1H), 6.78 (t, J = 7.4 Hz, 1H), 6.48 (t, J = 6.1 Hz, 4H), 6.26 (dd, J = 8.9, 2.6 Hz, 2H), 3.32 (q, J = 7.1 Hz, 8H), 1.15 (t, J = 7.1 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.33, 158.71, 153.66, 152.94, 150.82, 149.15, 133.61, 131.56, 131.34, 130.12, 128.70, 128.23, 124.28, 123.42, 119.00, 118.71, 117.08, 108.14, 105.38, 97.97, 66.56, 44.45, 12.71. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{35}H_{37}N_4O_3$ 561.2860; Found 561.2849.

Synthesis of R2. Rhodamine B hydrazine (200 mg, 0.438 mmol) and 3-hydroxybenzaldehyde (53.5 mg, 0.438 mmol) were dissolved in 15 mL pressure tube by adding 5 mL anhydrous ethanol. The solution was refluxed for 10 hours. After the reaction completion, the solution was cooled to room temperature, and white precipitation was occurred. After that, the precipitation was filtered, washed with

ethanol for 3 times and dried, and 208 mg of white solid was obtained, with a yield of 84.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.03 (dd, *J* = 6.1, 2.2 Hz, 1H), 7.54 – 7.42 (m, 2H), 7.37 (s, 1H), 7.12 (dd, *J* = 9.3, 6.5 Hz, 2H), 6.93 (d, *J* = 7.7 Hz, 1H), 6.84 (dd, *J* = 7.8, 2.0 Hz, 1H), 6.52 (d, *J* = 8.8 Hz, 2H), 6.44 (d, *J* = 2.4 Hz, 2H), 6.24 (dd, *J* = 8.9, 2.5 Hz, 2H), 3.32 (q, *J* = 7.0 Hz, 8H), 1.15 (t, *J* = 7.0 Hz, 12H).^{13}C NMR (101 MHz, $CDCl_3$) δ 164.54, 158.96, 153.90, 153.25, 151.06 , 149.40, 133.78, 131.76, 131.53, 130.36, 128.89, 128.42 , 124.50, 123.62, 119.19, 118.96, 117.30, 108.42, 105.70, 98.28, 66.80, 44.67, 12.93. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{35}H_{37}N_4O_3$ 561.2866; Found 561.2853.

Synthesis of R3. Rhodamine B hydrazine (200 mg, 0.438 mmol) and 4-hydroxybenzaldehyde (53.5 mg, 0.438 mmol) were dissolved in 15 mL pressure tube by adding 5 mL anhydrous ethanol. The solution was refluxed for 10 hours. After the end of the reaction, it was cooled to room temperature, and white precipitation occurred. After extraction, it was washed with ethanol for 3 times and dried, 229 mg white solid was obtained, with a yield of 92.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H), 7.97 (dd, *J* = 6.4, 1.6 Hz, 1H), 7.53 – 7.39 (m, 4H), 7.16 (s, 1H), 7.08 (dd, *J* = 6.4, 1.4 Hz, 1H), 6.89 (d, *J* = 8.6 Hz, 2H), 6.52 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 2.4 Hz, 2H), 6.23 (dd, *J* = 8.9, 2.5 Hz, 2H), 3.31 (q, *J* = 7.0 Hz, 8H), 1.14 (t, *J* = 7.0 Hz, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.17, 158.25, 152.86, 152.28, 148.97, 147.47, 133.31, 129.36, 128.82, 128.22, 127.97, 127.41, 123.63, 123.31, 115.58, 108.18, 105.71, 97.94, 65.99, 44.33, 12.60. HRMS (ESI) m/z: $[M + H]^+$ Calcd for $C_{35}H_{37}N_4O_3$ 561.2866; Found 561.2850.

The ^{1}H NMR, ^{13}C NMR and HMRS spectra of R1-R3

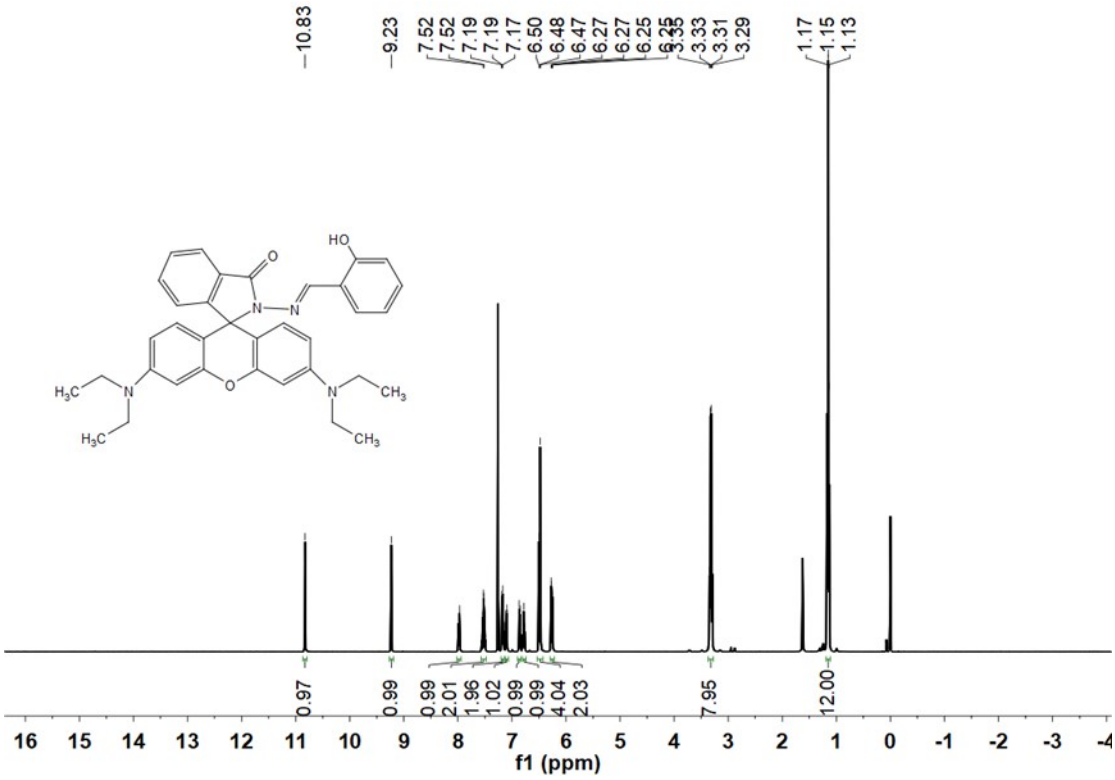


Figure S1. ^{1}H NMR spectrum of **R1** ($CDCl_3$, 400 MHz)

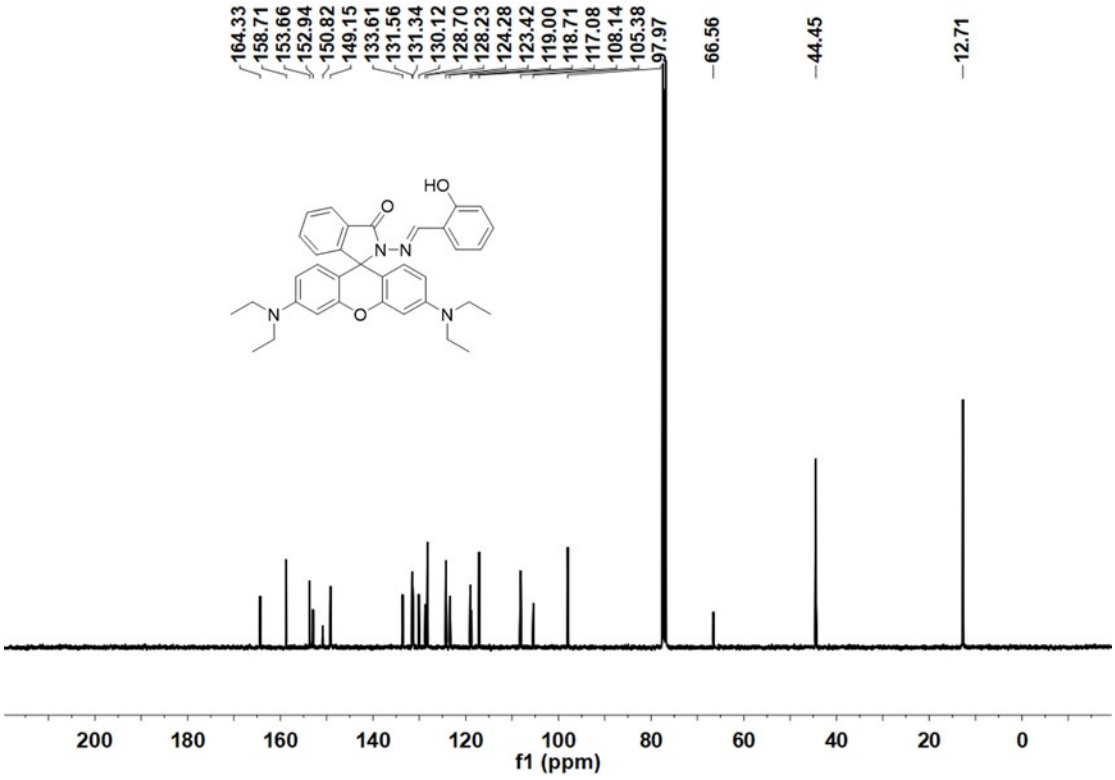


Figure S2. ^{13}C NMR spectrum of **R1** ($CDCl_3$, 101 MHz)

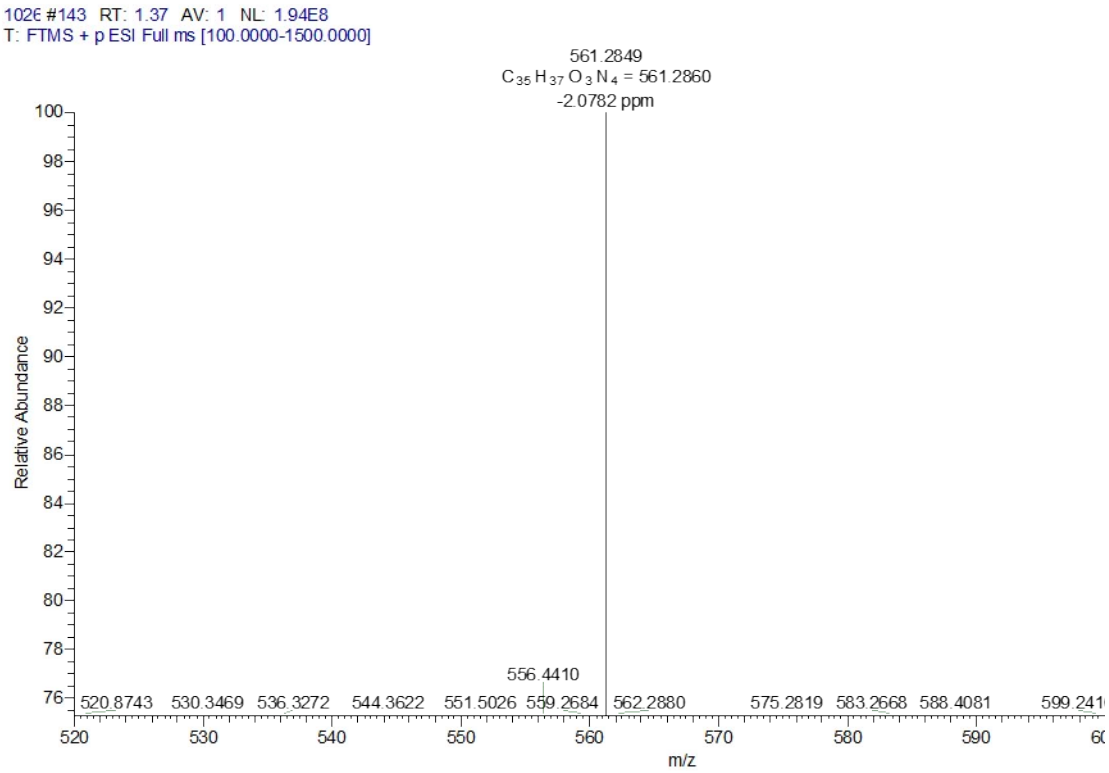


Figure S3. HRMS spectrum of R1

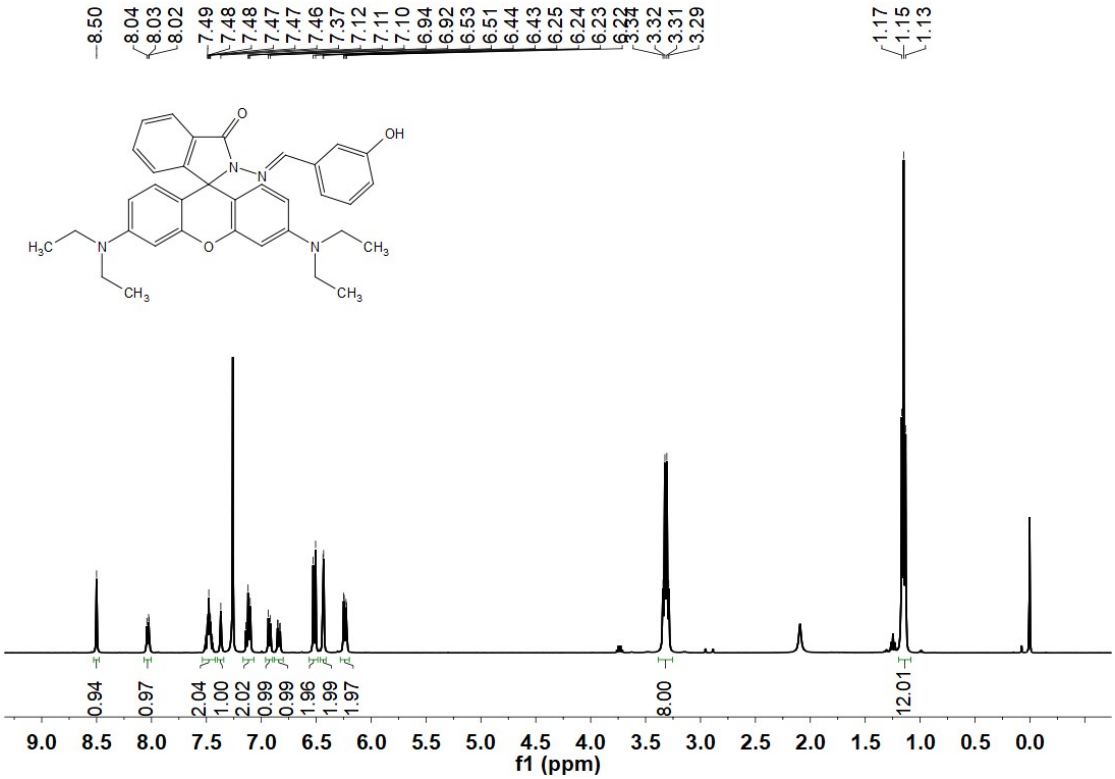


Figure S4. ^{1}H NMR spectrum of **R2** ($CDCl_3$, 400 MHz)

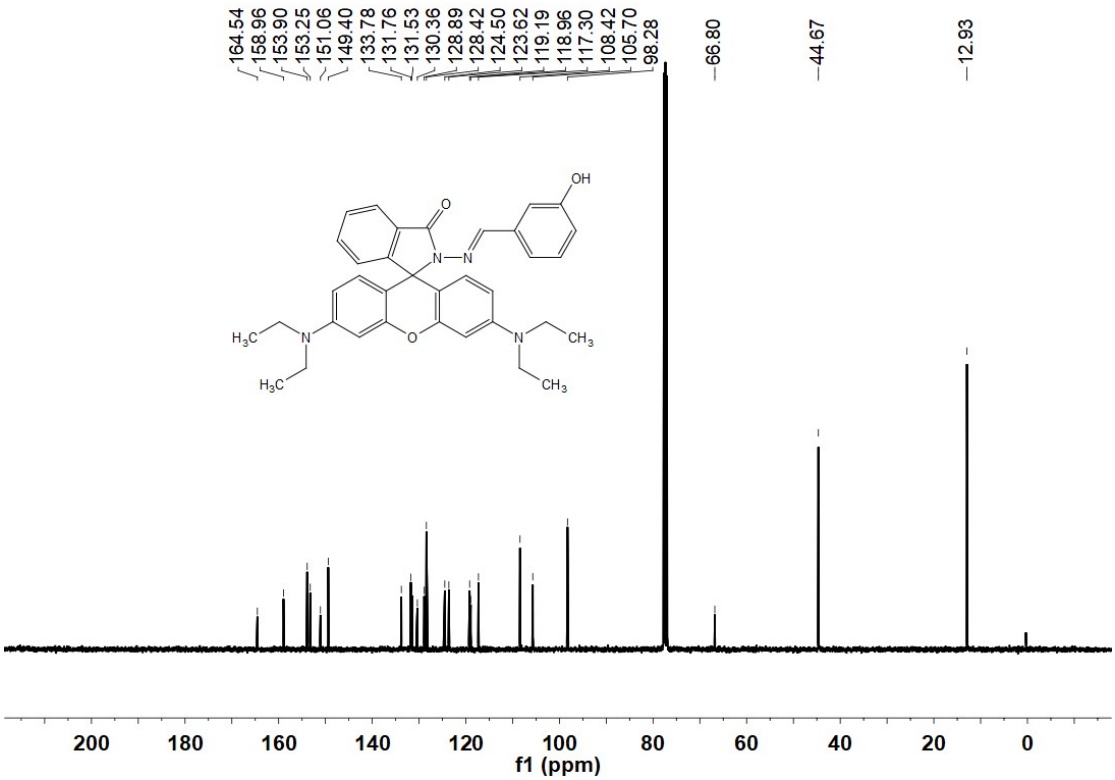


Figure S5. ^{13}C NMR spectrum of **R2** ($CDCl_3$, 101 MHz)

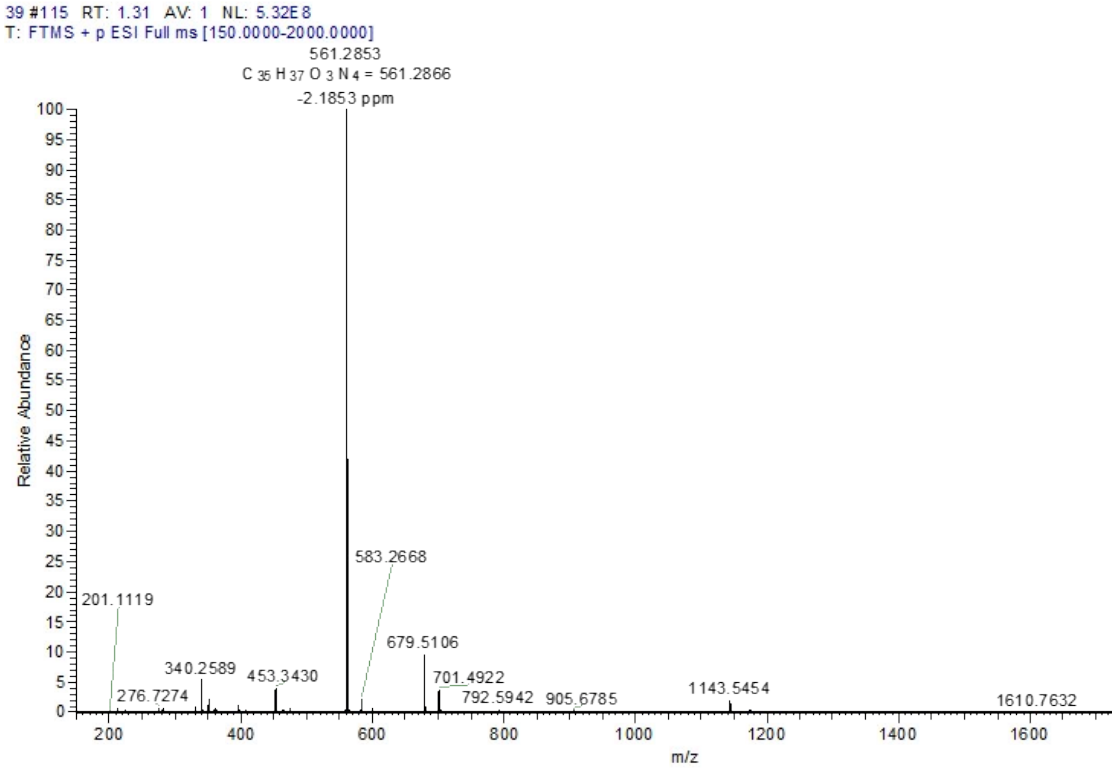


Figure S6. HRMS spectrum of **R2**

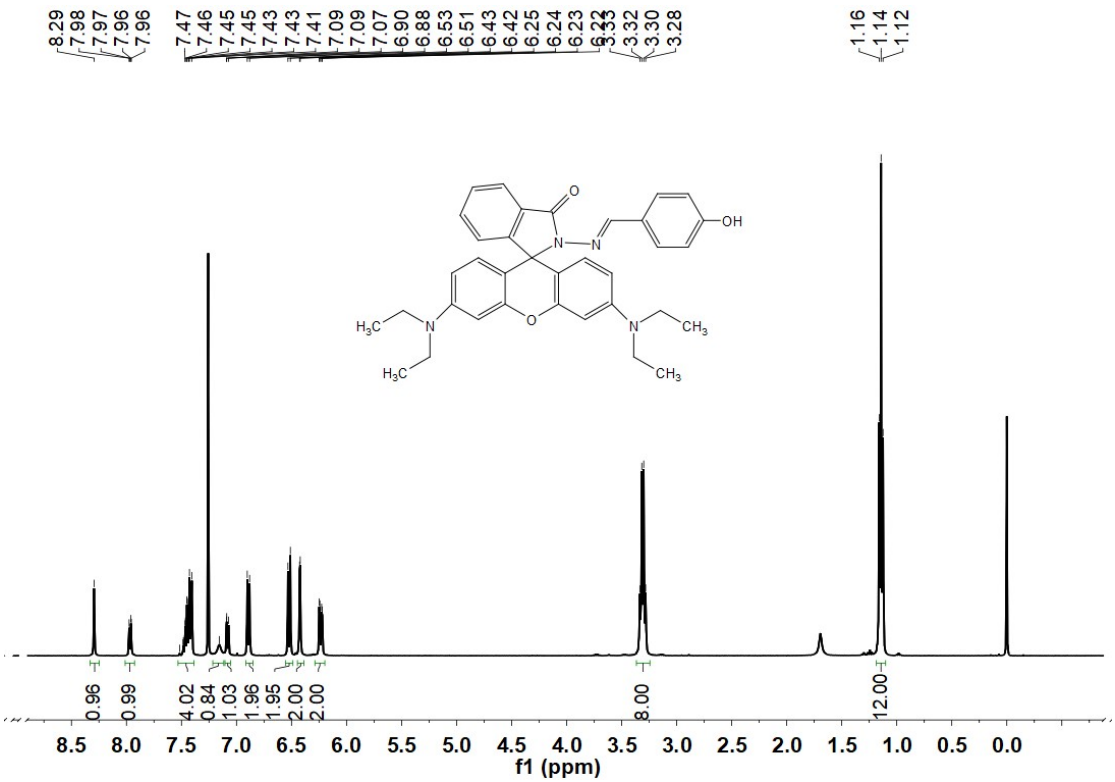


Figure S7. ^{1}H NMR spectrum of **R3** ($CDCl_3$, 400 MHz)

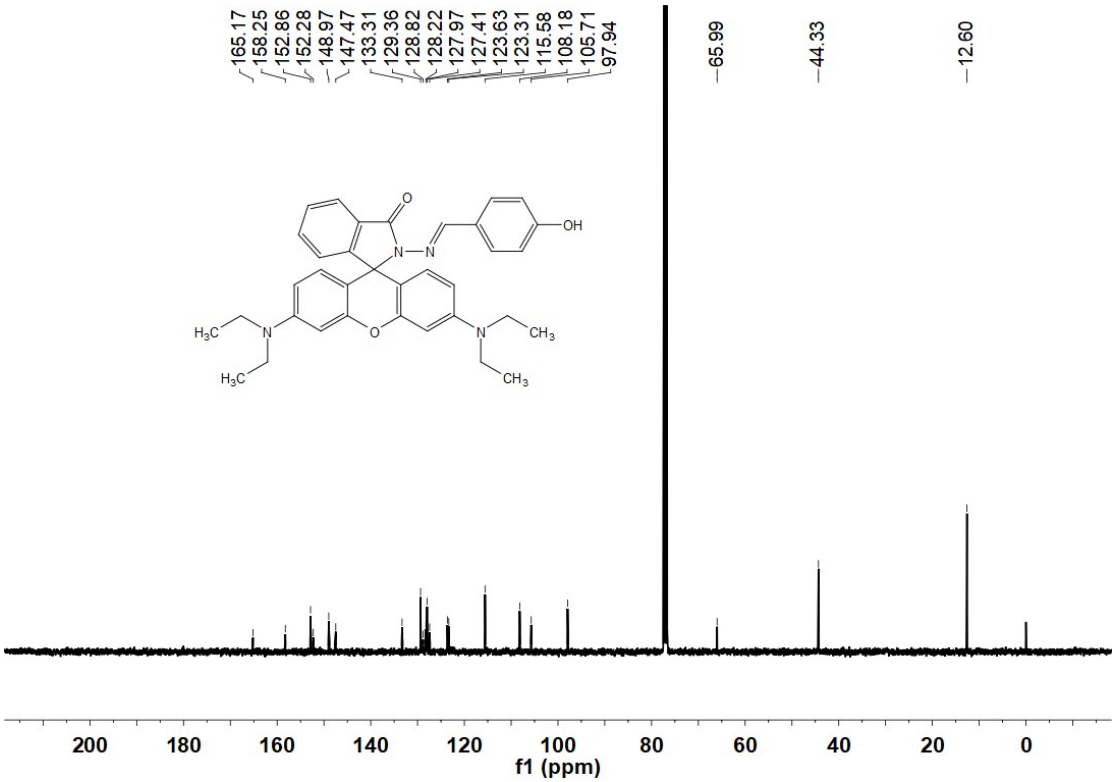


Figure S8. ^{13}C NMR spectrum of **R3** ($CDCl_3$, 101 MHz)

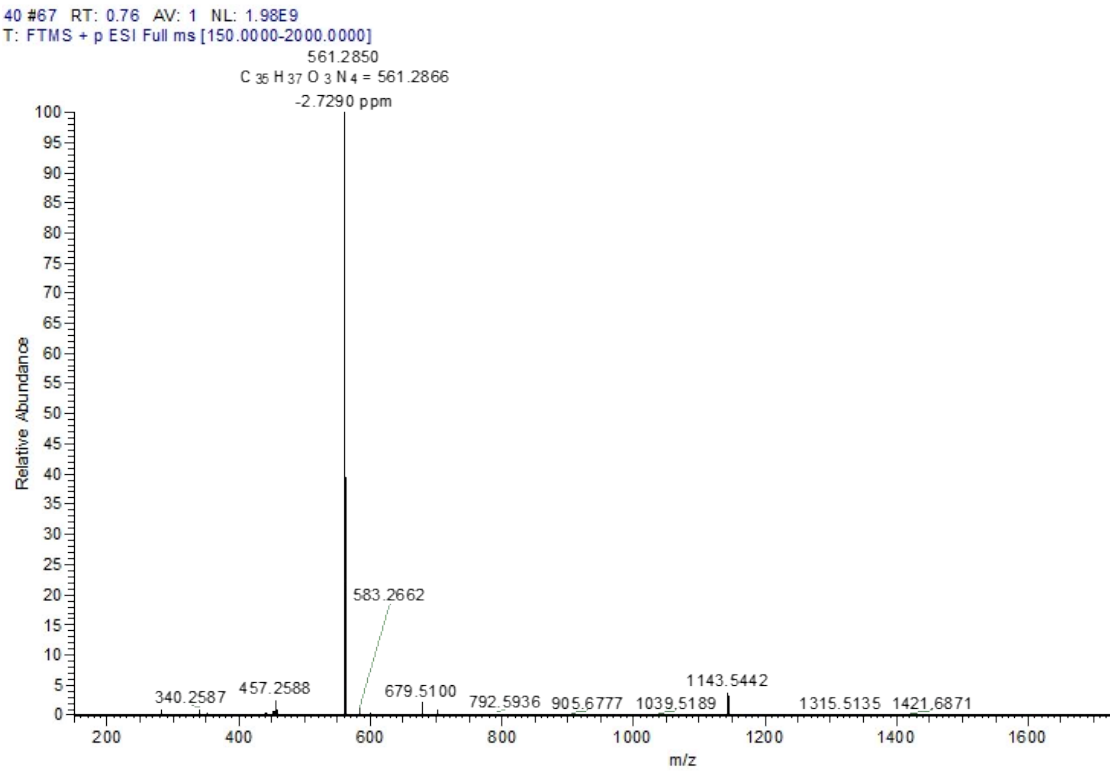


Figure S9. HRMS spectrum of **R3**

The sensitivity of R2 for SA in different solvents

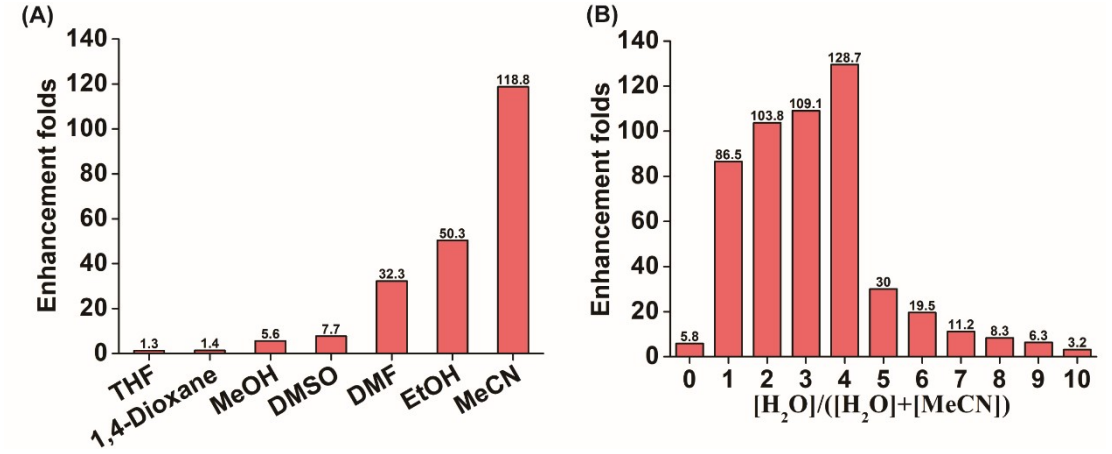


Figure S10. (A) The fluorescence intensity of **R2** upon addition of SA in different solvent; (B) The fluorescence intensity of **R2** upon addition of SA in MeCN solvents with different water fractions.

UV–vis absorption spectra and fluorescence spectra of R1-R3

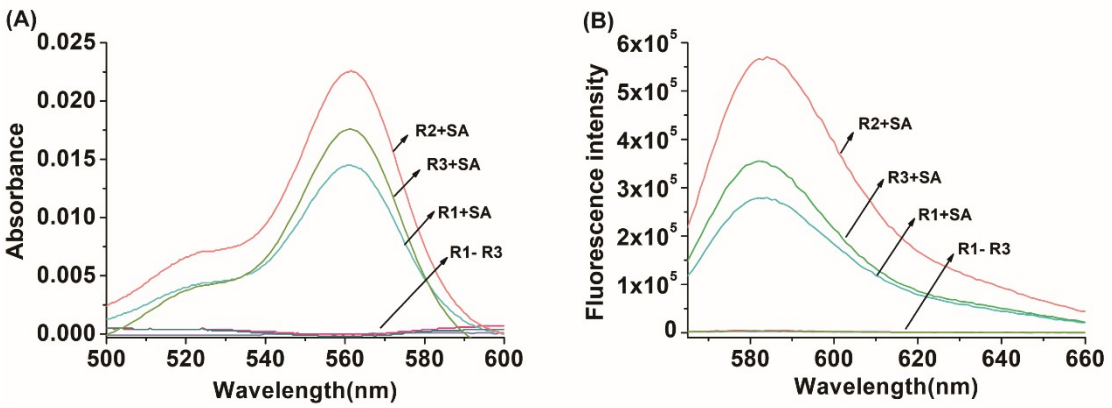


Figure S11. (A) UV–vis absorption spectra and (B) Fluorescence spectra of **R1-R3** (10 μM) and their addition SA (50 μM). (λ_{ex} = 560 nm, slits: 2 nm/2 nm, MeCN: H_2O = 3: 2, v/v).

The absorbance of Rhodamine B (50 μM) and R2 (10 μM)/SA (50 μM)

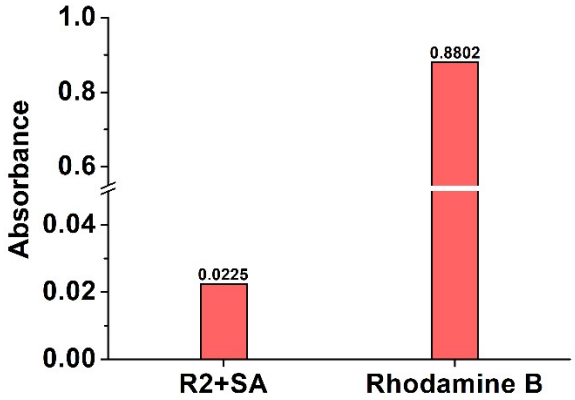
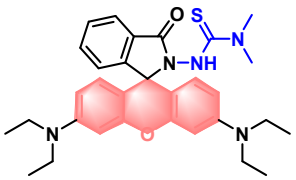
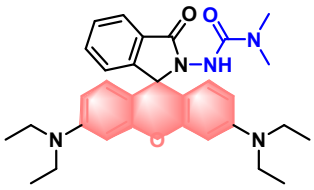
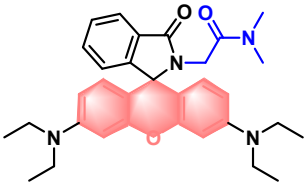
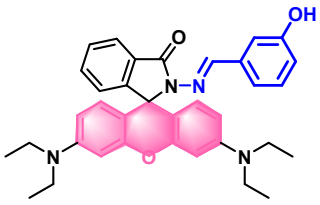


Figure S12. The absorbance of Rhodamine B (50 μM) and **R2** (10 μM)/SA (50 μM).

Comparison of previous work for SA detection

Table S1 Comparison of previous work for SA detection

Chemosensor structure	application	SA imaging concentration (μM)	SA incubation time (h)	References
	NRK-52E cell	50	2	[1]
	NRK-52E cell	400	2	[1]
	A549 cells,	100	1	[2]
	M. asiatica nakai callus		720	
	*Nicotiana glutinosa* L. callus	25	2.5	This work
	radish seedling		2.5	

Fluorescence enhancement folds of R1-R3

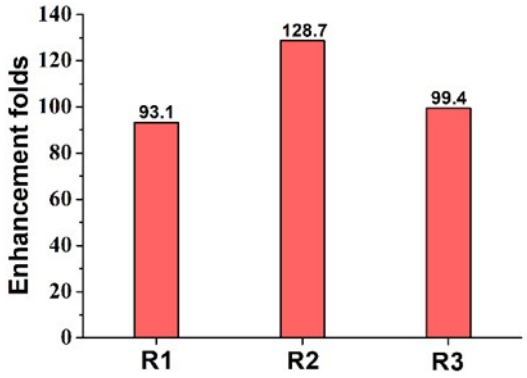


Figure S13. The fluorescence enhancement folds of **R1-R3** after addition SA.

The selectivity and anti-interference testing

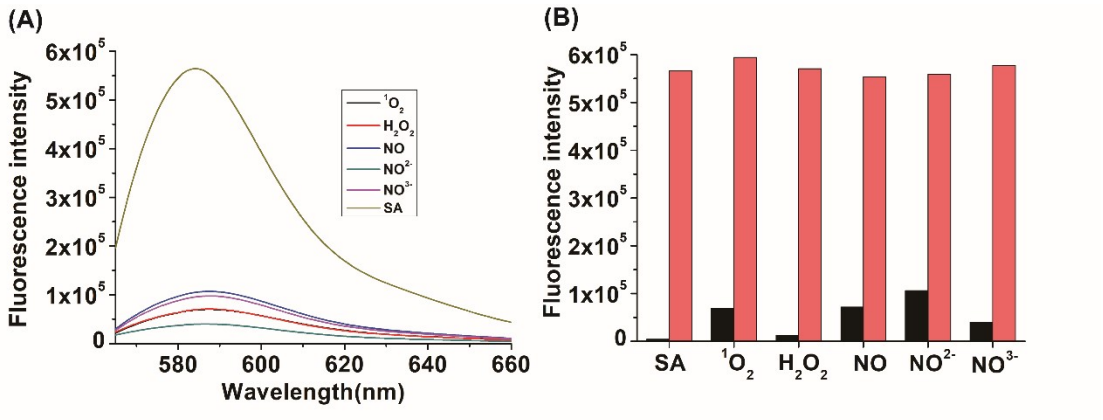


Figure S14. (A) Fluorescence response of **R2** (10 μM) after adding SA and various reactive species (50 μM). λ_{ex} = 560 nm, slits: 2 nm/2 nm, MeCN:H_2O (3:2, v/v). (B) Fluorescence response of **R2** towards SA and various reactive species. Black bars: fluorescence intensity at 585 nm for **R2** with reactive species, Red bars: after adding SA into the premixed **R2** with reactive species.

Calculation of the detection limit

The detection limit of probe for SA was calculated by the signal-to-noise ratio (S/N). The fluorescence intensity of the probe without target analyte was measured 20 times at the specified wavelength. On the basis of these data, the average fluorescence intensity ($average_{blank}$) and its associated standard deviation (SD_{blank}) were determined,

and SD_{blank} was taken as the noise (N) of our detection system. Subsequently, probe was added to a relatively low concentration of the corresponding analyte and the fluorescence intensity was measured five times and the average (mean sample) was recorded. Finally, S/N is calculated as follows:

$$S/N = (|average_{sample} — average_{blank}|)/SD_{blank}$$

When the S/N value is in the range of 3 ~ 5, the corresponding concentration can be determined as the detection limit.

Job's and Benesi-Hildebrand plot of R2 with SA

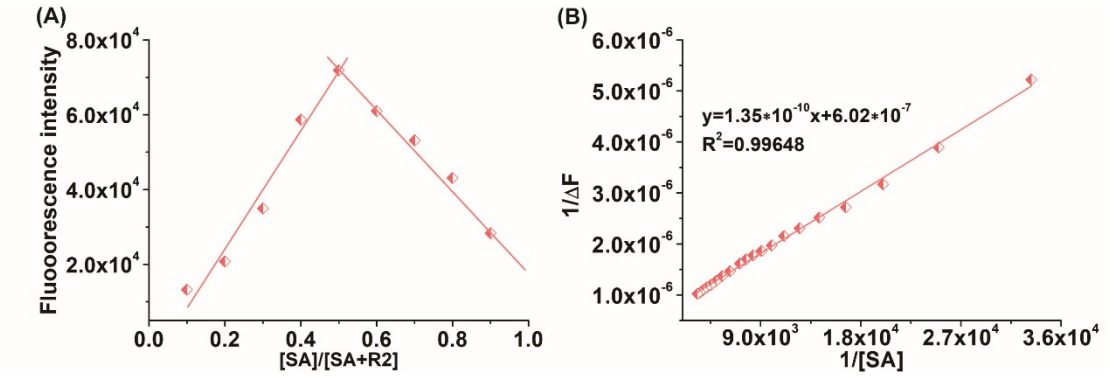


Figure S15. (A) Job's plot for determination of stoichiometry of complex **R2** + SA. Total concentration of **R2** and SA was kept constant at 20 μM. (B) Benesi-Hildebrand plot of **R2** with SA. Binding constant (K_a = 4.46 × 10^3 M^{-1}) was determined by fluorescence method.

Effect of pH on SA detection

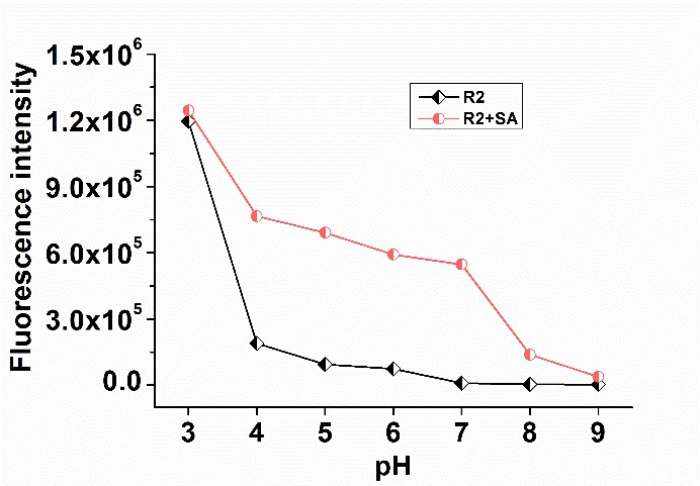


Figure S16. R2 (10 μM) was used in MeCN/H_2O(3/2) to measure the fluorescence intensity of SA (50 μM) at different pH conditions.

HRMS spectrum of R2 after addition SA

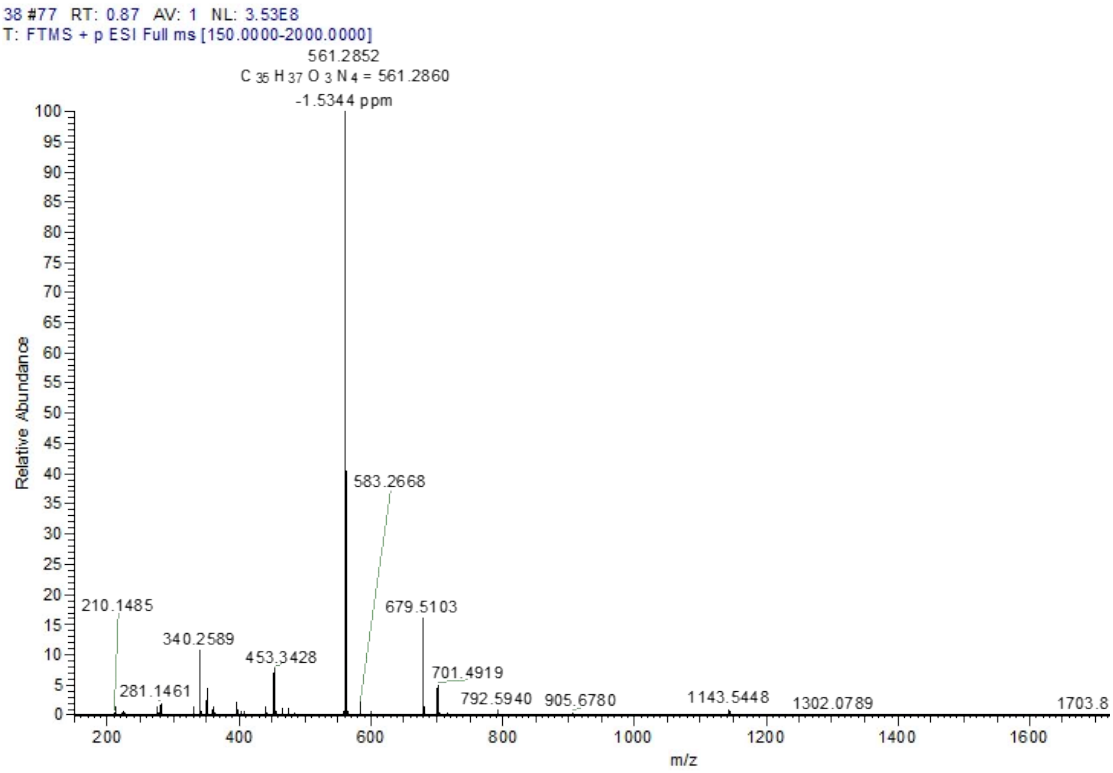


Figure S17. HRMS spectrum of **R2** after addition SA.

Reference：

[1] Wang, P. Y.; Luo, X.; Yang, L. L.; Zhao, Y. C.; Dong, R.; Li Z.; Yang, S. A rhodamine-based highly specific fluorescent probe for the in situ and in vivo imaging of the biological signalling molecule salicylic acid. *Chem. Commun.* **2019**, *55*, 7691-7694.

[2] Yang, L. L.; Zou, S. Y.; Fu, Y. H.; Li, W.; Wen, X. P.; Wang, P. Y.; Wang, Z. C.; Ouyang, G. P.; Li, Z.; Yang, S. Highly Selective and Sensitive Detection of Biogenic Defense Phytohormone Salicylic Acid in Living Cells and Plants Using a Novel and Viable Rhodamine-Functionalized Fluorescent Probe. *J. Agric. Food Chem.* **2020**, *68*, 4285−4291.