Supporting information for

A highly sensitive and rapidly responding fluorescent probe based on a rhodol fluorophore for imaging endogenous hypochlorite in living mice

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1. Tautomerism equilibrium of ROA in diffrent solution

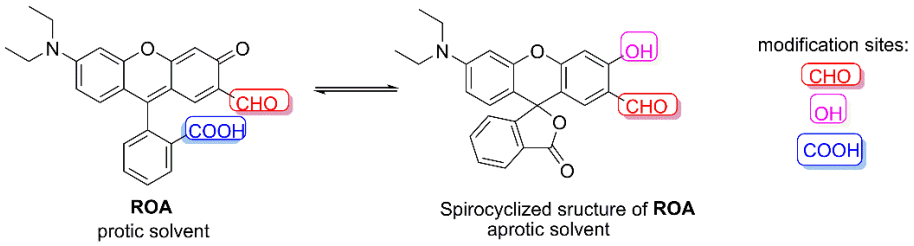
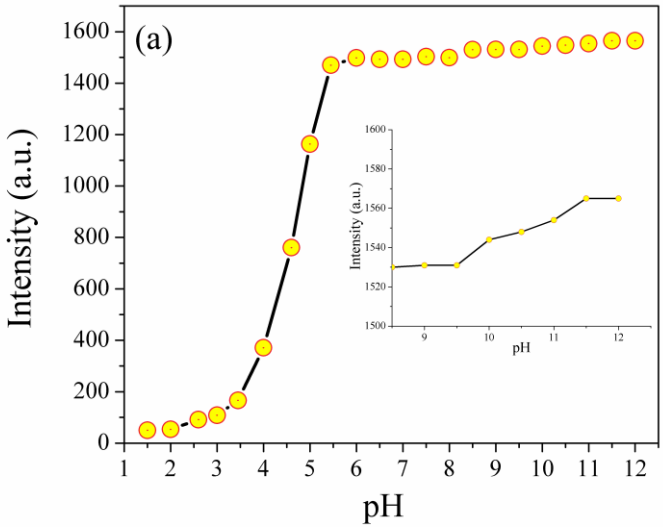


Fig. S1 Tautomerism equilibrium of **ROA** in different.

2. Acid-base equilibrium of ROA in different pH



(b) pH:

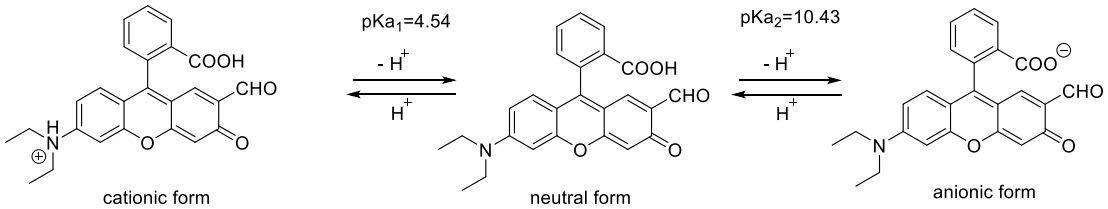


Fig. S2 (a) Fluorescent intensity of **ROA** (10 μM) at 577 nm under different pH conditions. (λ_{ex} = 515 nm, λ_{em} = 577 nm, slit: 5 nm/5 nm). (b) Acid-base equilibrium of ring-opened **ROA** (10 μM) in water containing 0.1% DMSO as a co-solvent.

3. Single crystal X-ray structure of spirocyclized ROA

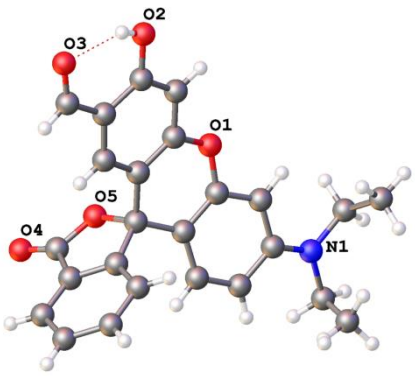


Fig. S3 Single crystal X-ray structure of spirocyclized **ROA.**

4. Crystal data and structure refinement for spirocyclized ROA

Empirical formula	$C_{25}H_{21}NO_5$
Formula weight	415.43
Temperature	296.15 K
Wavelength	0.71073 Å
Crystal system, space group	Monoclinic, C 1 2/c 1
Unit cell dimensions	a = 20.420(5) Å alpha = 90 deg. b = 7.929(2) Å beta = 91.616(6) deg. c = 29.104(8) Å gamma = 90 deg.
Volume	4710(2) $Å^3$
Z, Calculated density	8, 1.172 Mg/m^3
Absorption coefficient	0.082mm^{-1}
F(000)	1744
h, k, l max	24,9,34
Theta range for data collection	1.995 to 25.027 deg.
Limiting indices	-23<=h<=24, -8<=k<=9, -34<=l<=22

Reflections collected / unique	11330 / 4149 [R(int) = 0.0635]
Completeness to theta = 25.10	99.6 %
Max. and min. transmission	0.7455 and 0.6520
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4149 / 104 / 331
Goodness-of-fit on F^2	1.011
Final R indices [I>2sigma(I)]	R1 = 0.0748, wR2 = 0.2080
R indices (all data)	R1 = 0.1534, wR2 = 0.2577
Largest diff. peak and hole	0.266 and -0.263 e.Å^{-3}

Table S1 Crystal data and structure refinement for spirocyclized **ROA.**

5. UV-vis spectra of RO610 with increasing concentrations of ClO^-

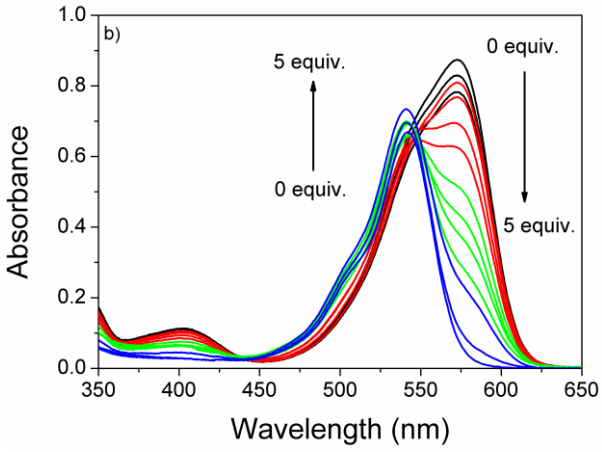


Fig. S4 UV-vis spectra of **RO610** (10 μM) upon the addition of increasing concentrations of sodium hypochlorite (0–5 equiv.) in aqueous solution (20 mM phosphate buffer, pH 7.4, containing 30% EtOH as a co-solvent).

6. Fluorescence intensity changes in different pH of RO610

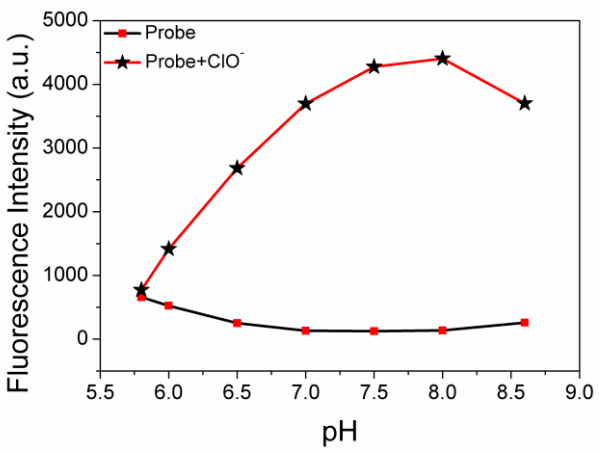


Fig. S5 Fluorescence intensity changes in different pH of **RO610** (10 μM) in the absence (black) and presence (red) of 50 μM ClO^- in aqueous solution (20 mM phosphate buffer, pH 7.4, containing 30% EtOH as a co-solvent) (λ_{ex} = 535 nm, λ_{em} = 577 nm, slit: 5.0 nm/5 nm).

7. Selecitivity of RO610 towards various species

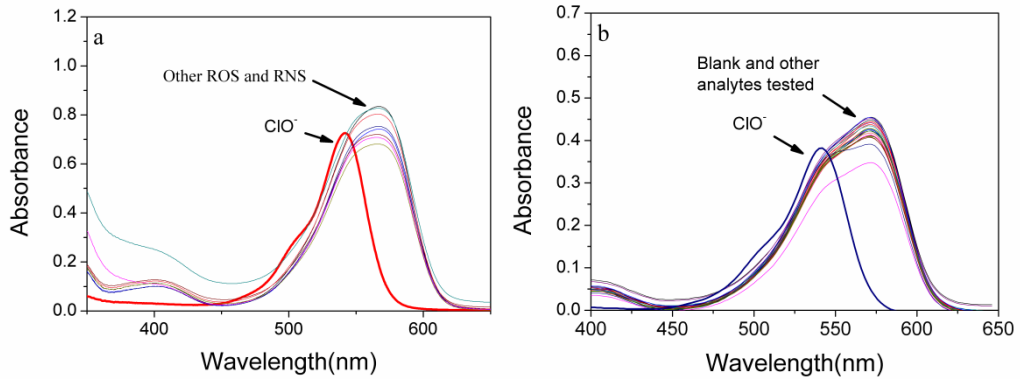


Fig. S6 (a) Absorbance spectra of probe **RO610** (10 μM) in phosphate buffer:EtOH = 7: 3 (V/V, 20 mM, pH = 7.4) in the presence of 200 μM analytes (H_2O_2, $^{\bullet}OH$, O_2^-, $^1O_2$, $NO^{\bullet}$, TBHP, $TBO^{\bullet}$) and 50 μM ClO^-; (b) in the presence of various anions (200 μM) and biothiols (200 μM).

8. Mechanism verification

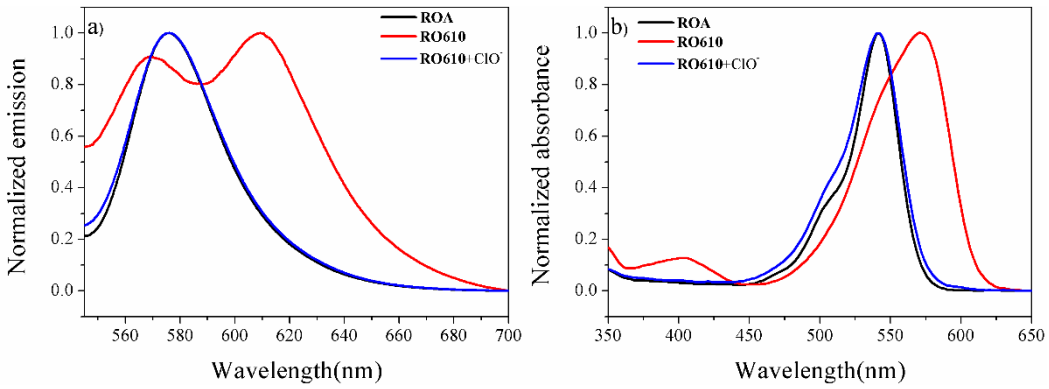


Fig. S7 (a) Normalized emission and (b) Normalized absorbance of **ROA**, the probe **RO610** and the present system of probe **RO610** (10 μM) with ClO^- (50 μM) in phosphate buffer: EtOH = 7: 3 (V/V, 20 mM, pH = 7.4) (λ_{ex} = 535 nm, λ_{em} = 577 nm, slit: 5 nm/5 nm).

9. ^{1}H NMR Spectrum of the Reaction Mixture of ClO^- with RO610

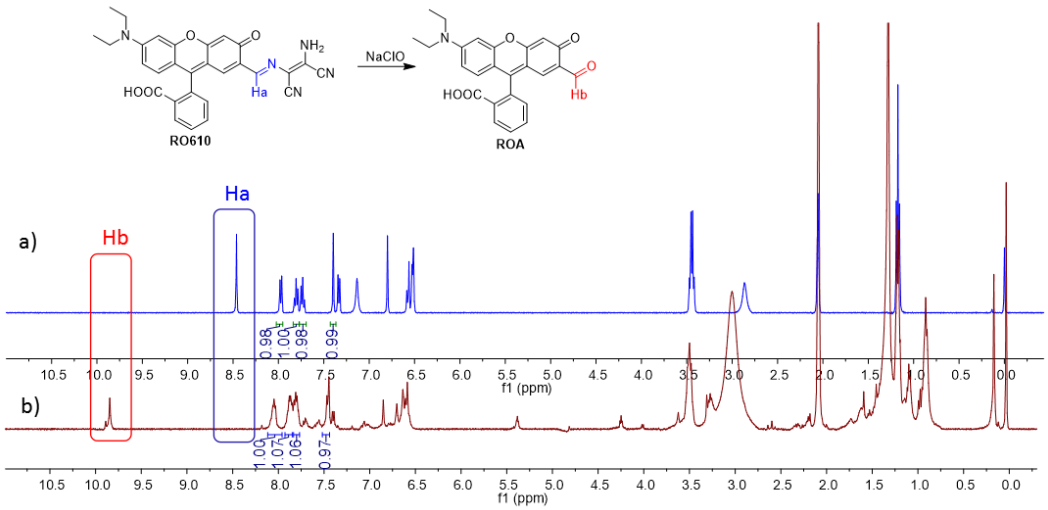


Fig. S8 ^{1}H NMR spectrum of (a) probe **RO610** in Actone-d_6; (b) the product of the complete reaction mixture of the probes **RO610** with 5 equiv. ClO^- in Actone-d_6.

10. Cytotoxicity of probe RO610 in A549 cells

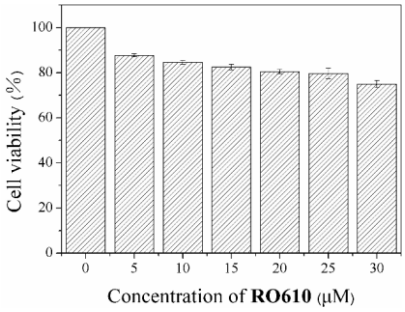


Fig. S9 Percentage of viable A549 after treatment with **RO610** (5, 10, 15, 20, 25, 30 μM) after 12 hours.

11. Theoretical calculations

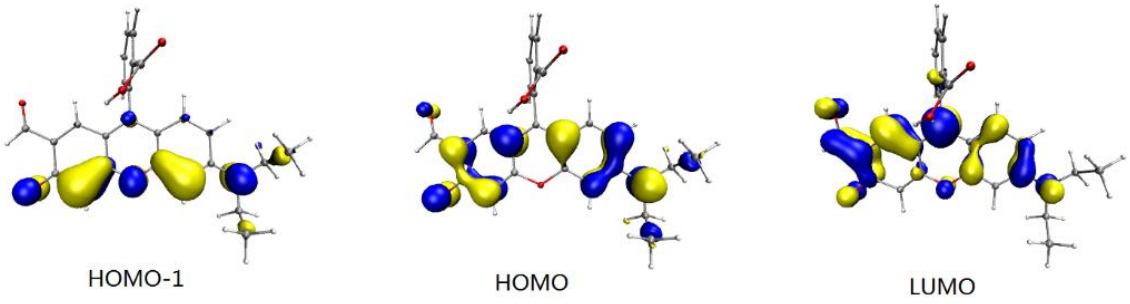


Fig. S10 Molecular orbital plots (LUMO and HOMO) of **ROA** (ring-opened form) in MeOH based on the

optimized ground-state geometry (S_0).

12. HPLC spectra of the reaction mixture of ClO^- with RO610

Data File C:\HPCHEM\1\DATA\GUOYUA15.D

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Sample Name     :                                   Vial :   1
Acq. Operator   : guo
Acq. Method     : C:\HPCHEM\1\METHODS\G.M
Last changed    : 12/6/17 10:14:09 AM by guo
                  (modified after loading)
Analysis Method : C:\HPCHEM\1\METHODS\G.M
Last changed    : 12/6/17 11:28:17 AM by guo
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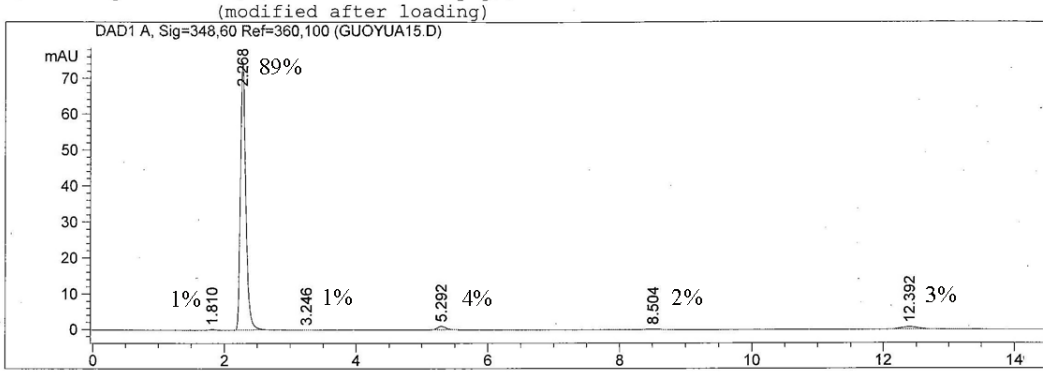

External Standard Report

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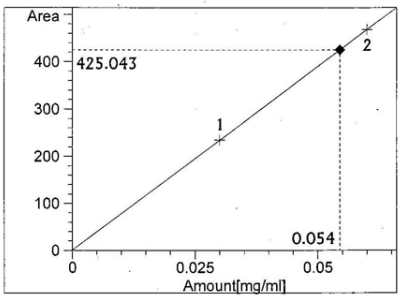
Sorted By             :      Signal
Calib. Data Modified  :      12/6/17 11:28:17 AM
Multiplier            :      1.0000
Dilution              :      1.0000

Signal 1: DAD1 A, Sig=348,60 Ref=360,100
```

RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mg/ml]	Grp	Name	Theoretical amount [mg/ml]	Yield [%]
2.268	BB	425.04291	1.28178e-4	5.44813e-2		roa	6.1e-2	89
Totals :				5.44813e-2				

Results obtained with enhanced integrator!

Calibration Curves

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roa at exp. RT: 2.279
DAD1 A, Sig=348,60 Ref=360,100
Correlation:              1.00000
Residual Std. Dev.:       0.44707
Formula: y = mx + b
      m:   7798.27932
      b:      1.82515e-1
      x: Amount[mg/ml]
      y: Area
```

Instrument 1 12/6/17 11:29:00 AM guo Page 1 of 1

Fig. S11 The yield of reaction of probe **RO610** with ClO^- was calculated by the normalization method [Agilent C_{18} chromatographic column (4.6 mm × 150 mm, 5 mm), Liquid phase : PBS (10 mM, pH = 2.15) :

Acetonitrile = 40 : 60 (V/V), Flow rate of 1 ml min^{-1}, Detection wavelength 248 nm, The sampling amount is 10 μL].

13. NMR, Mass and IR spectra

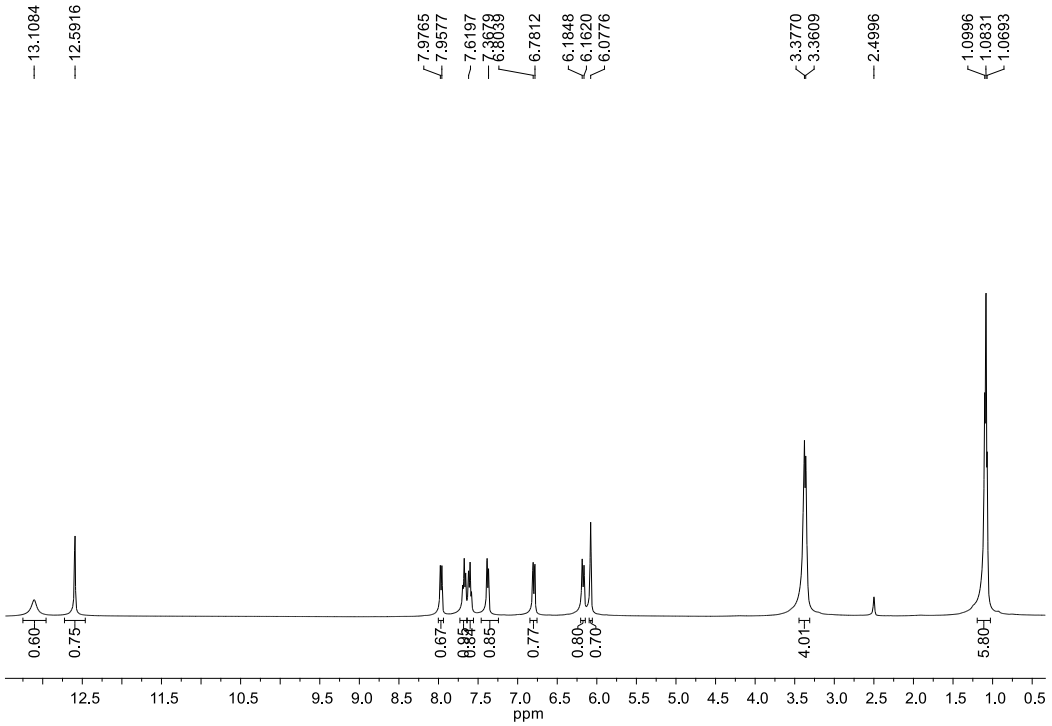

Fig. S12 ^{1}H NMR spectrum of compound **3** in DMSO-d_6.

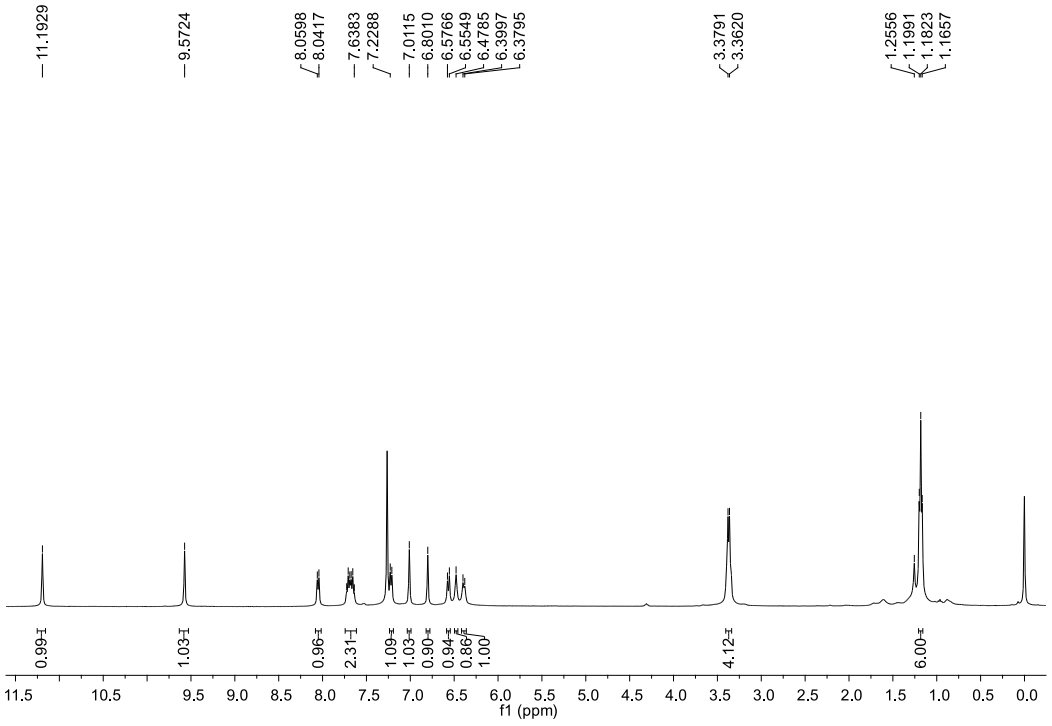

Fig. S13 ^{1}H NMR spectrum of compound **ROA** in $CDCl_3$.

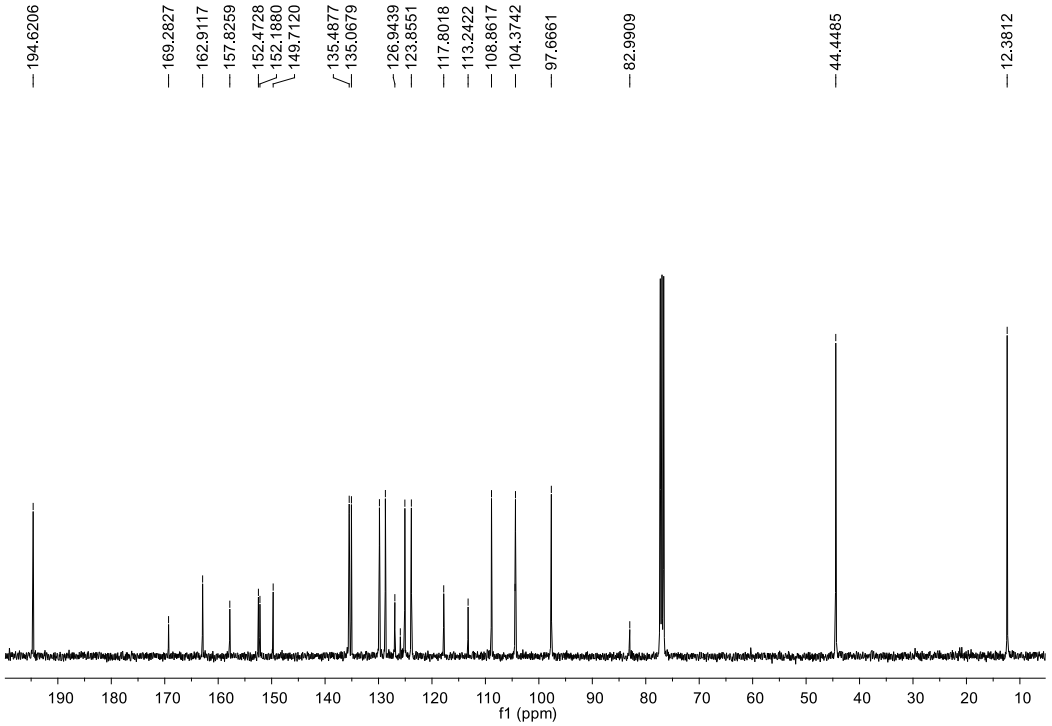

Fig. S14 ^{13}C NMR spectrum of compound **ROA** in $CDCl_3$.

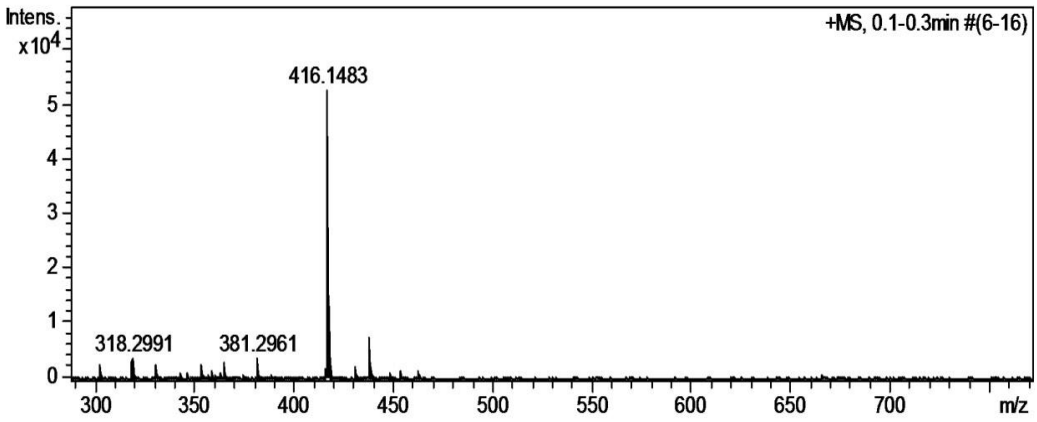

Fig. S15 ESI-MS spectrum of compound **ROA**.

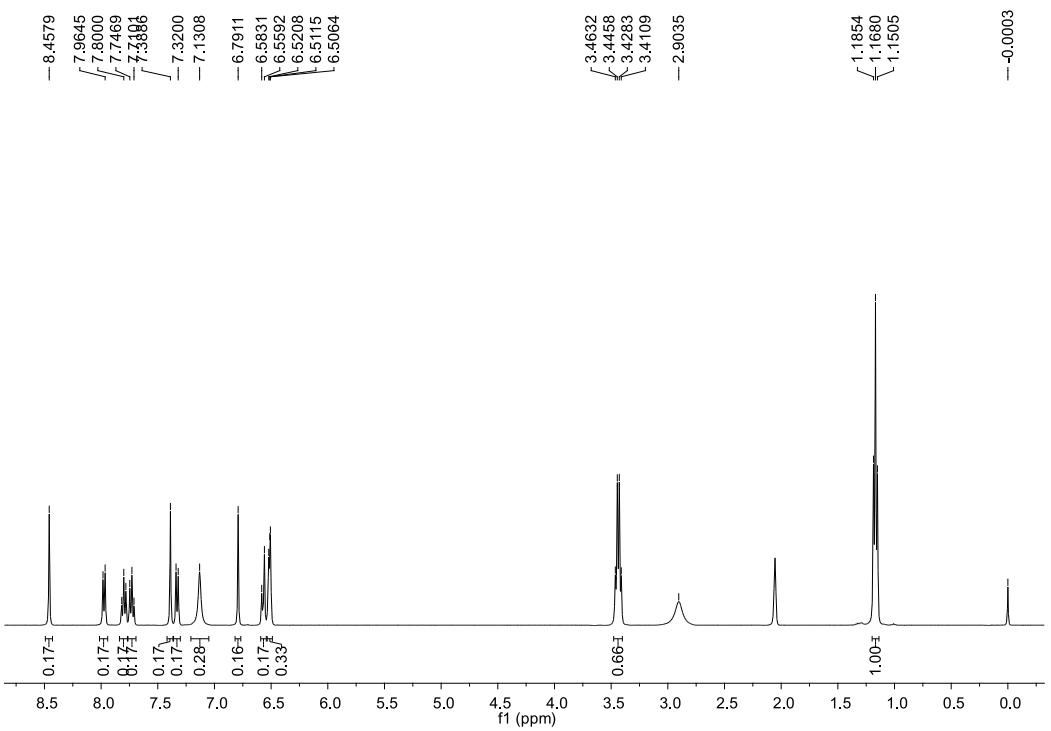

Fig. S16 ^{1}H NMR spectrum of compound **RO610** in Actone-d_6.

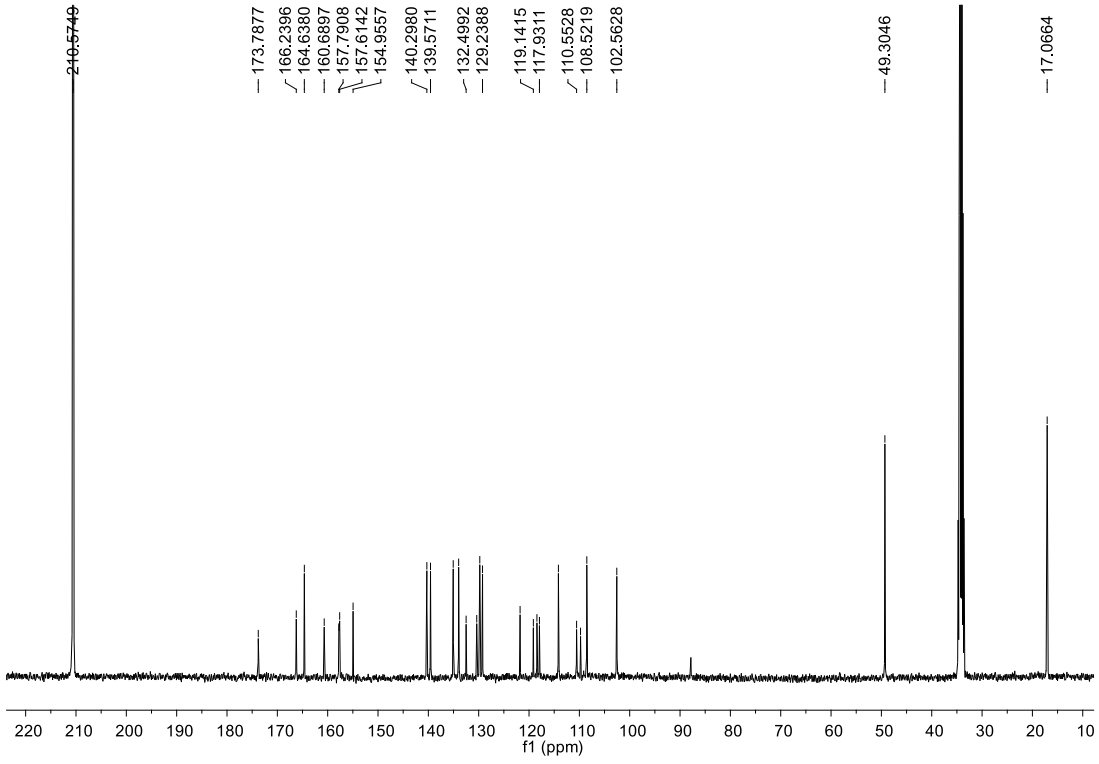

Fig. S17 ^{13}C NMR spectrum of compound **RO610** in Actone-d_6.

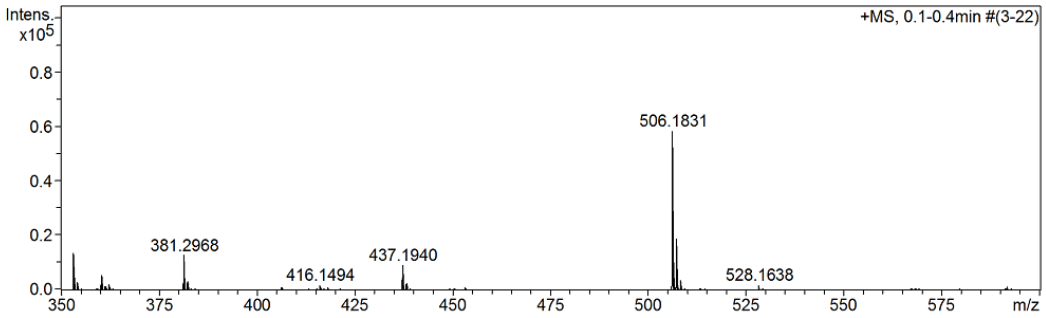

Fig. S18 ESI-MS spectrum of compound **RO610**.