Electronic Supplementary Information

A mitochondria-targeted ratiometric fluorescent probe for hypochlorite and its applications in bioimaging

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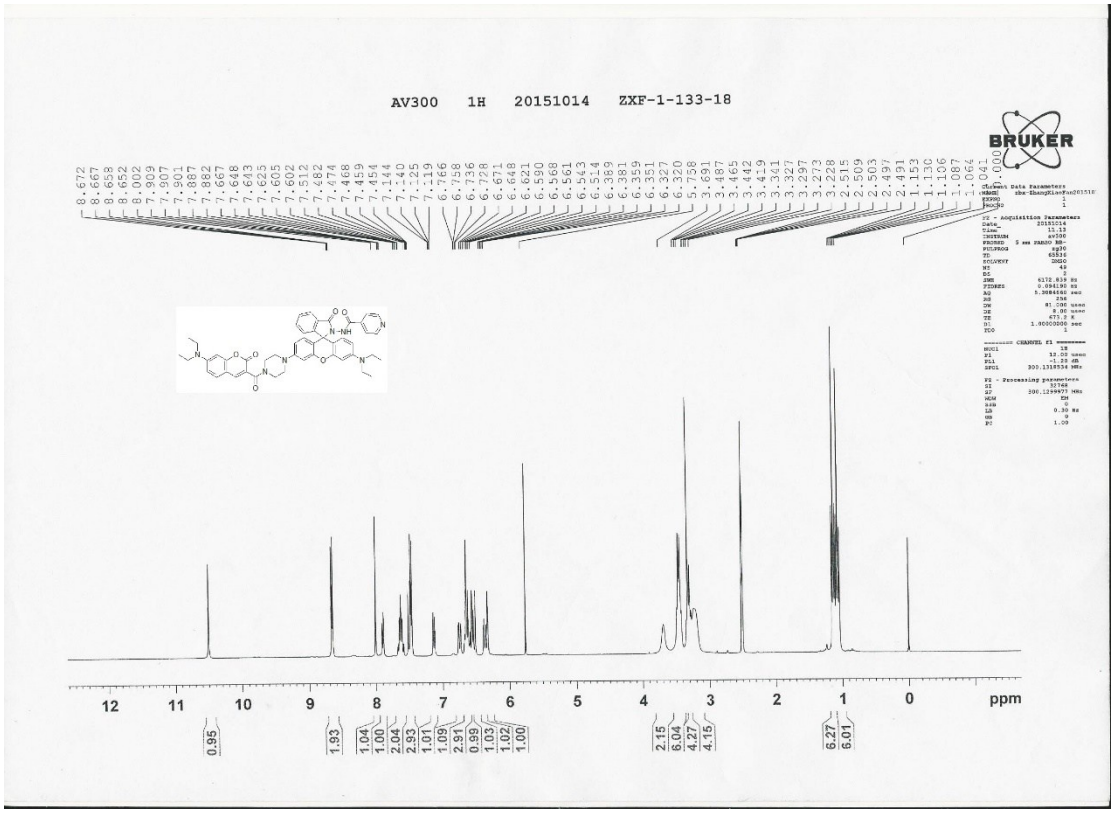


Fig. S1 ^{1}H NMR spectrum of compound **3**.

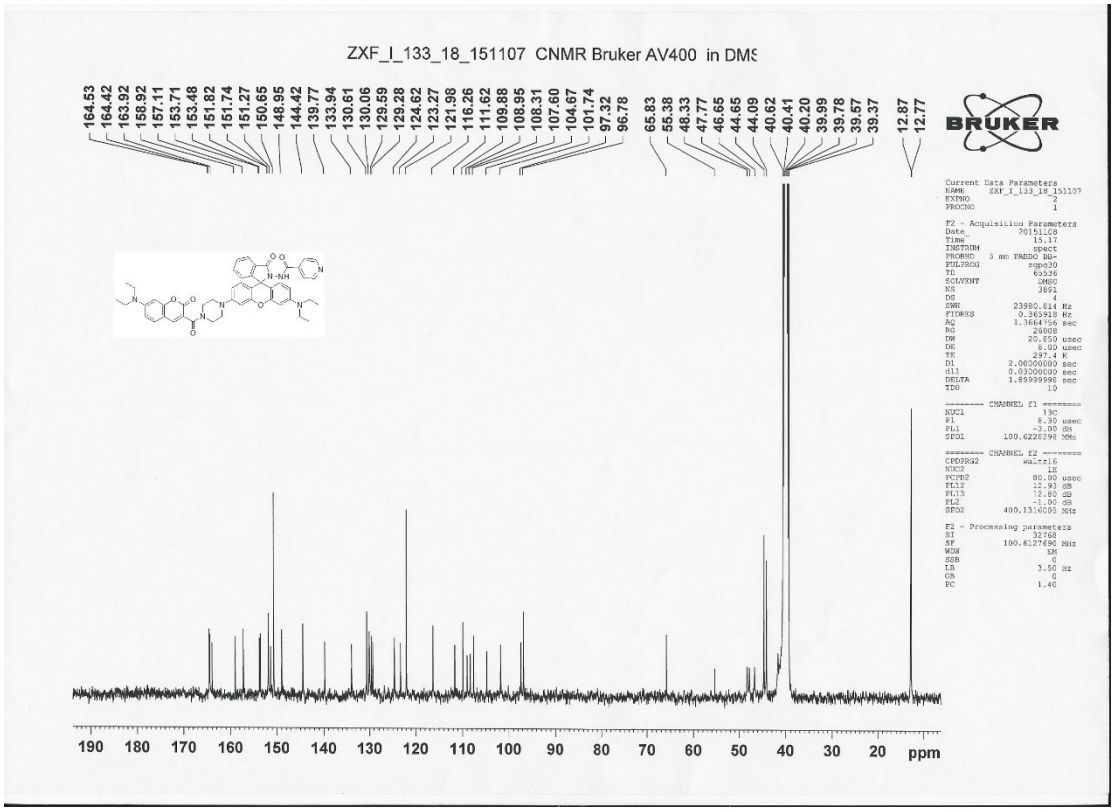


Fig. S2 ^{13}C NMR spectrum of compound **3**.

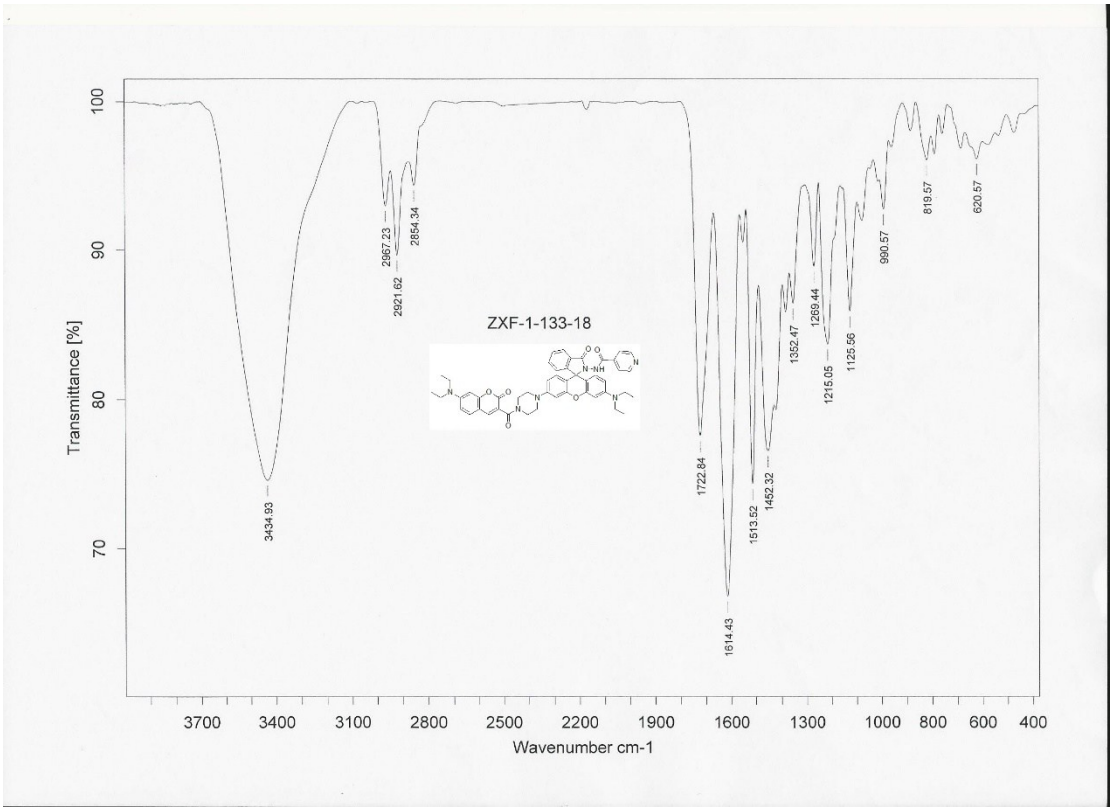


Fig. S3 Infrared spectrum of compound **3**.

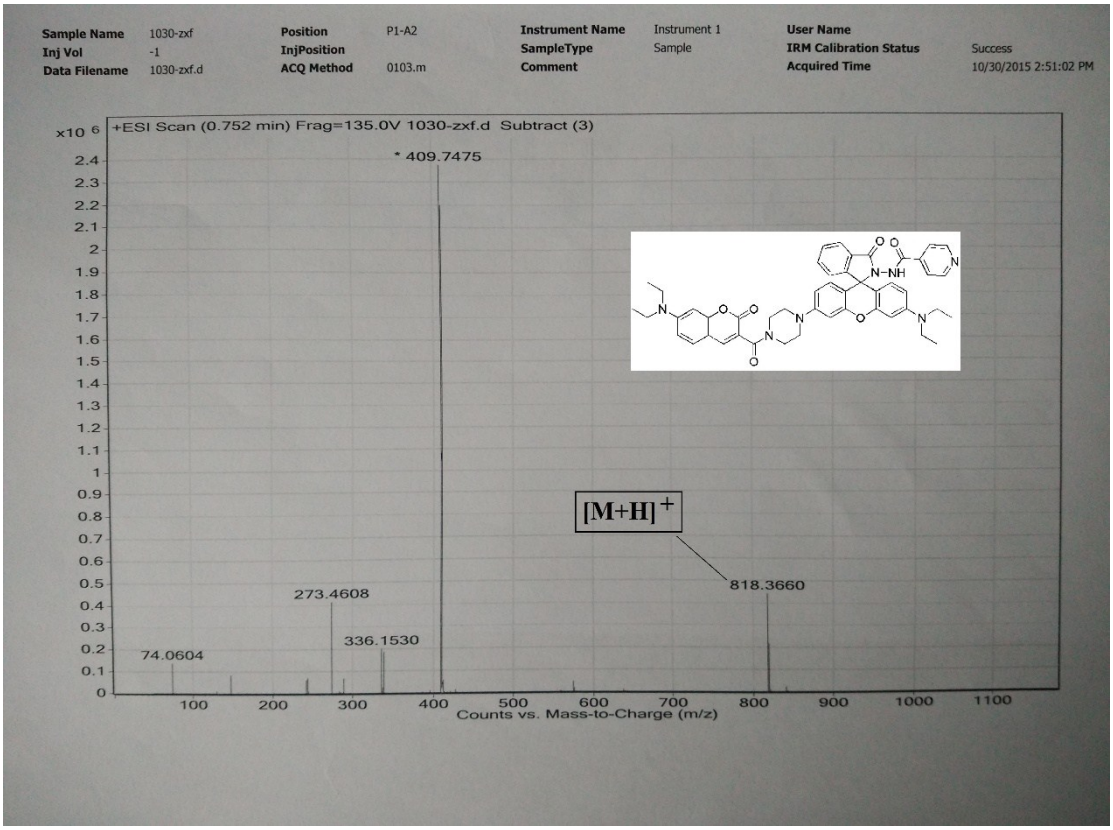


Fig. S4 High resolution mass spectrum of compound **3**.

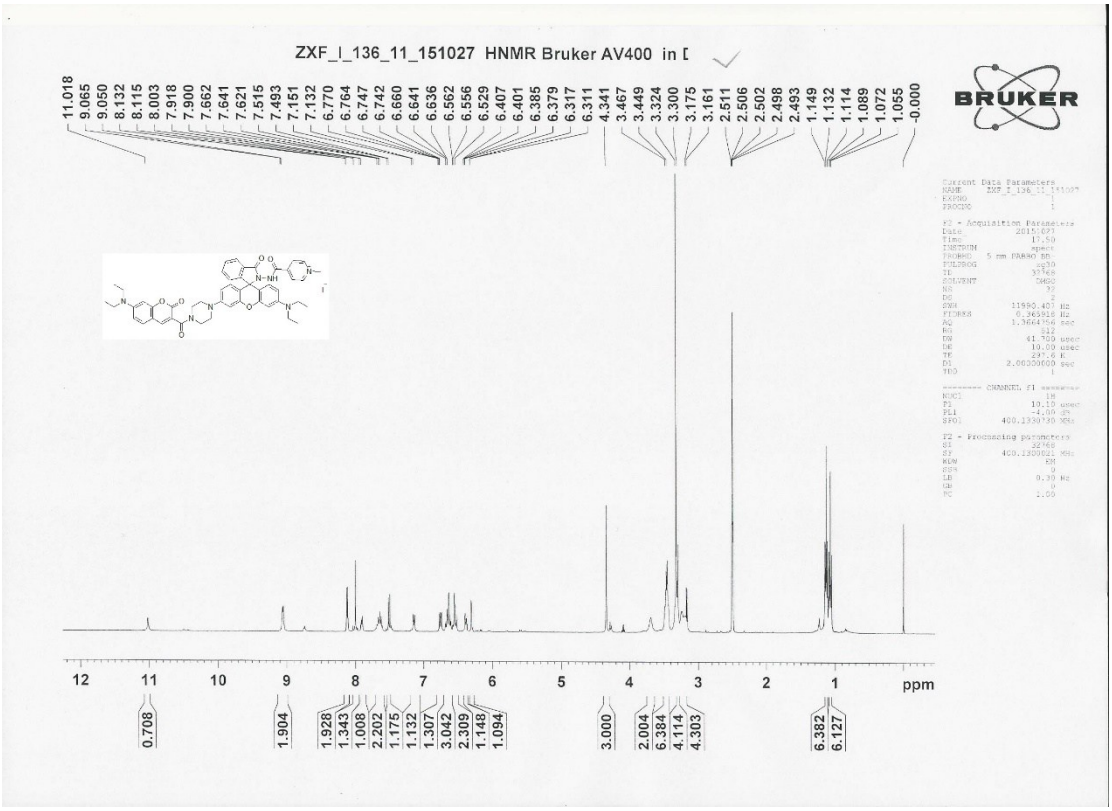


Fig. S5 ^{1}H NMR spectrum of **RCP**.

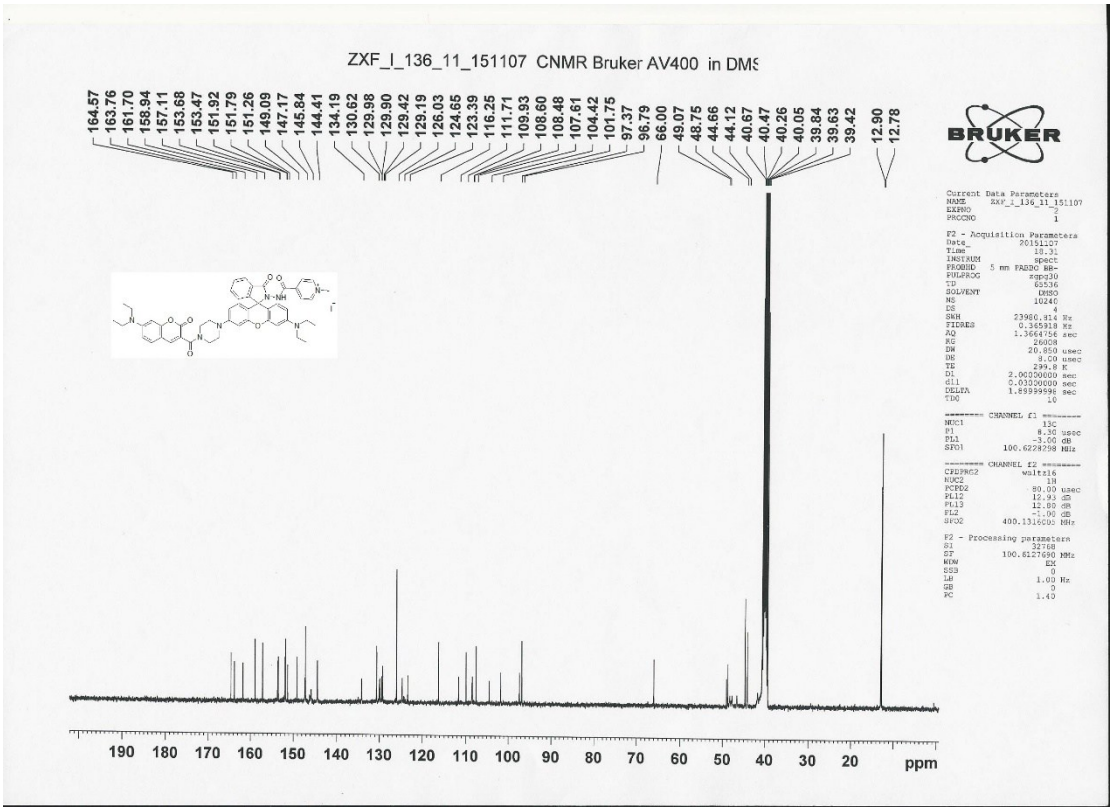


Fig. S6 ^{13}C NMR spectrum of **RCP**.

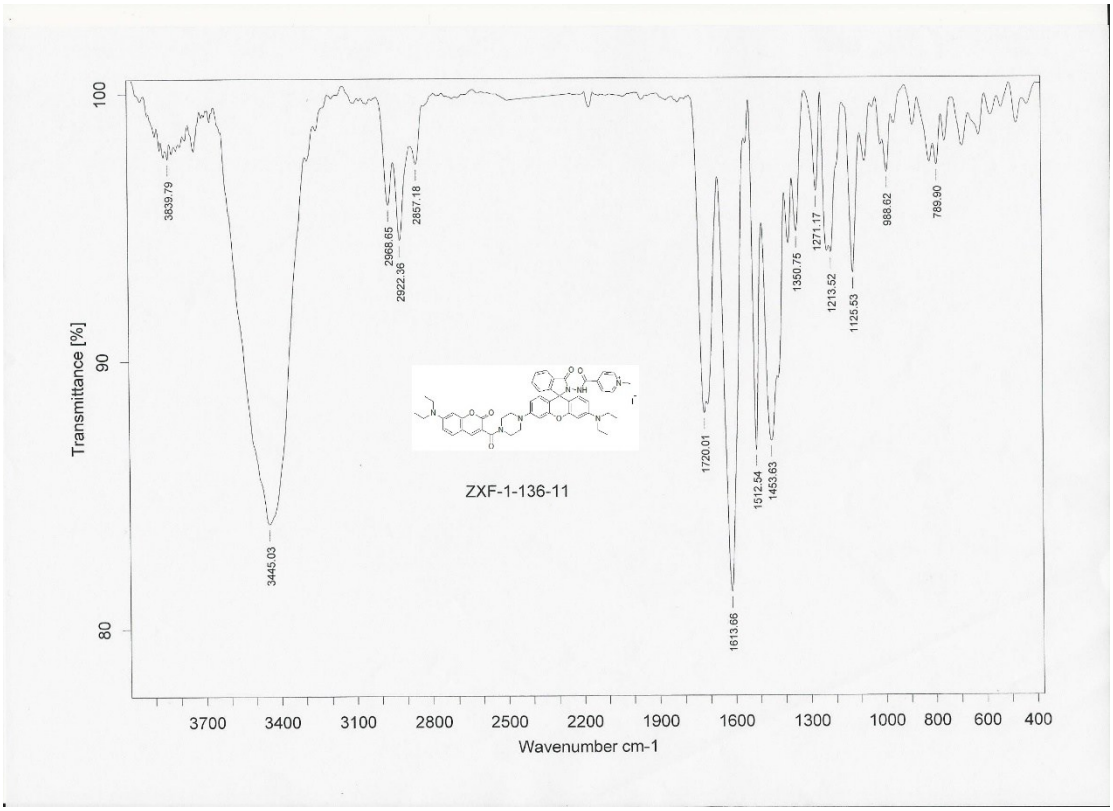


Fig. S7 Infrared spectrum of **RCP**.

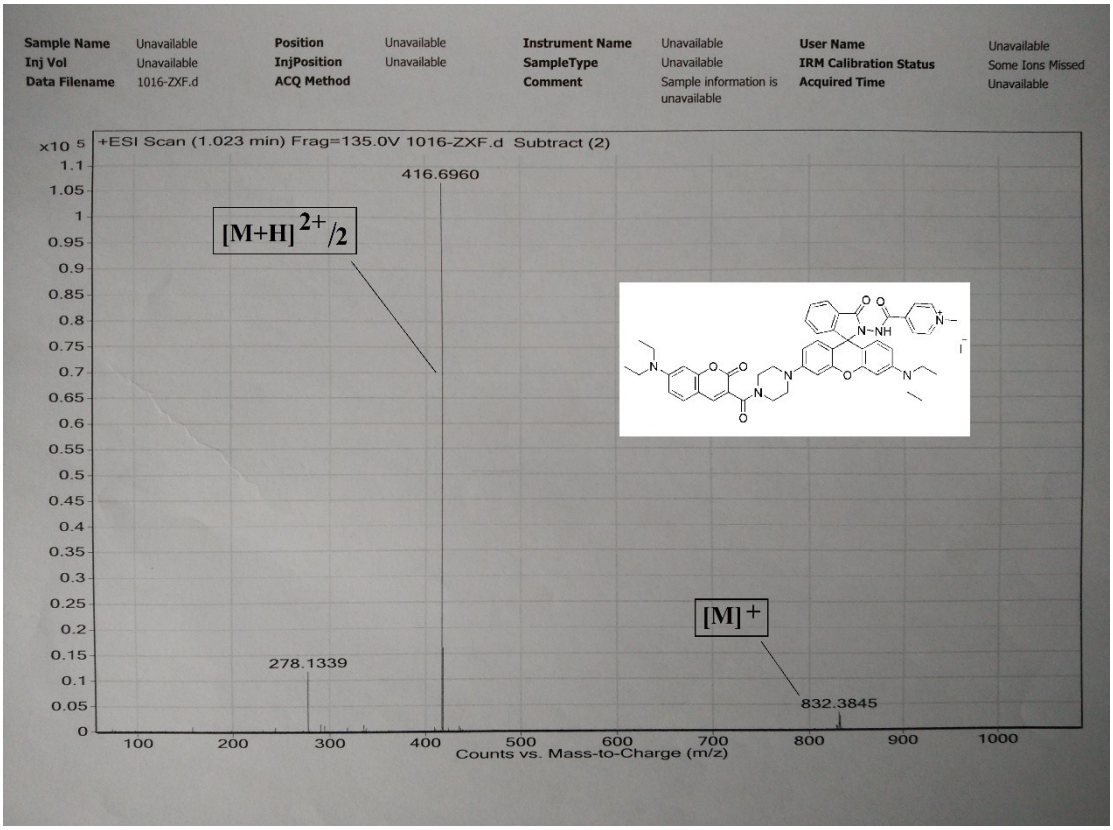


Fig. S8 High resolution mass spectrum of **RCP**.

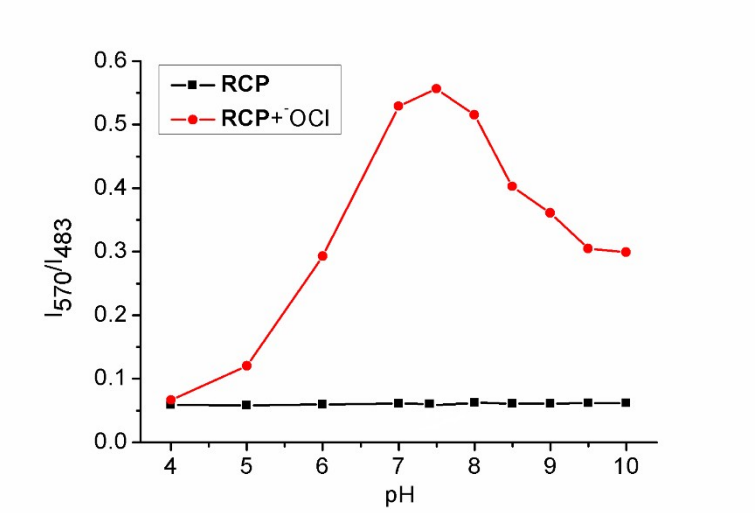


Fig. S9 Fluorescence intensity ratio (I_{570}/I_{483}) of **RCP** versus pH values in the absence (■) or presence (●) of $^{-}$OCl (8 equivalent). Condition: [**RCP**] = 5 μM, [$^{-}$OCl] = 40 μM, PBS buffer (pH 4.0-10.0, containing 0.5% EtOH), incubation time = 30 min. λex = 420 nm.

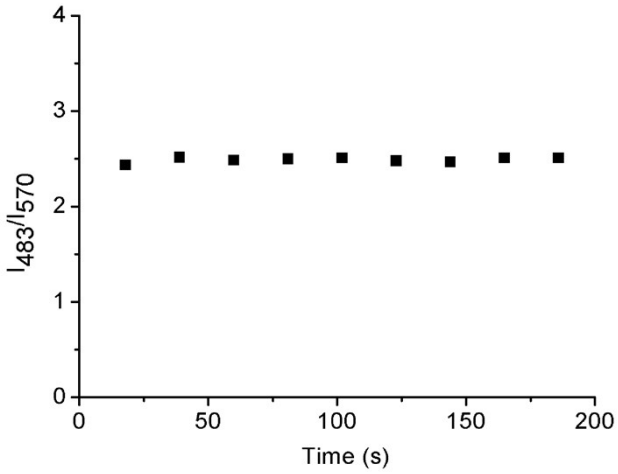
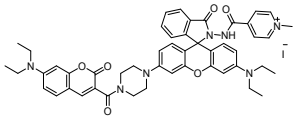
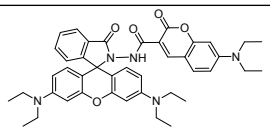
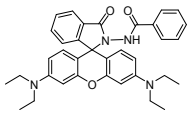
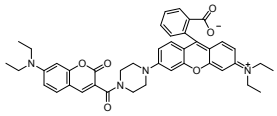
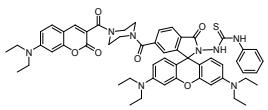
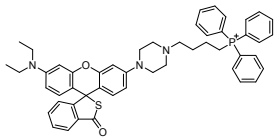
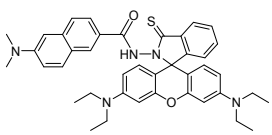
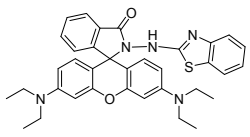


Fig. S10 Time-dependent fluorescence intensity ratio (I_{483}/I_{570}) changes of **RCP** upon addition of $^{-}$OCl (6 equivalent). Condition: [**RCP**] = 5 μM, [$^{-}$OCl] = 30 μM, PBS buffer (pH 7.4, containing 0.5% EtOH), λex = 420 nm.

Table S1. The performances of **RCP** and other $^{-}$OCl probes

Probes	Probe concentr-ation (μM)	Solvent	λex/λem (nm)	Detection limit (nM)	Reaction time	Ref.
	5	EtOH:PBS (pH 7.4) = 0.5:99.5 (v:v)	420/ 483,570	70	within 1 min	This work
	5	DMF:potassium phosphate buffer (pH 8.5) = 4:6 (v:v)	410,554/ 501,578	24	within 1 min	4
	10	THF:$Na_2B_4O_7$/NaOH (pH 12) = 3:7 (v:v)	520/578	27	20 min	26
	10	DMF:NaH_2PO_4 (pH 5) = 4:6 (v:v)	410/ 470,580	_	within 100 s	46
	5	DMF:PBS (pH 7.4) = 1:1 (v:v)	414/ 473,594	52	_	33
	10	DMSO:PBS (pH 7.4) = 1:99 (v:v)	553/ 558	9	2 min	30
	5	EtOH:Na_2HPO_4 (pH 6) = 3:7 (v:v)	350/ 440,585	100	2 min	38
	5	MeCN:PBS (pH 7.4) = 3:7 (v:v)	550/ 575	1.06	40 min	47

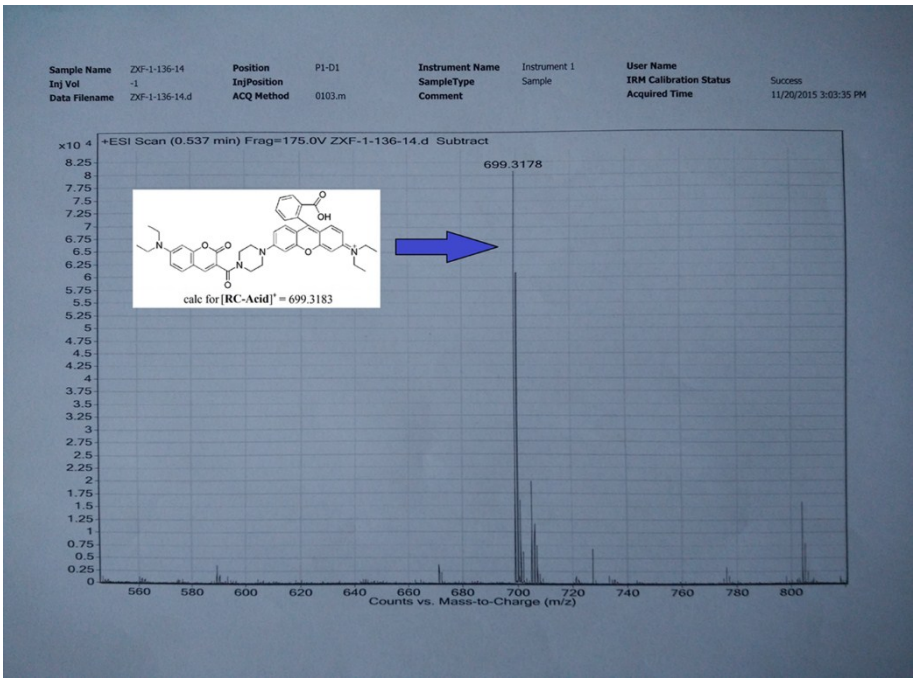


Fig. S11 HRMS spectra of the crude product after treatment of **RCP** with $^{-}$OCl.

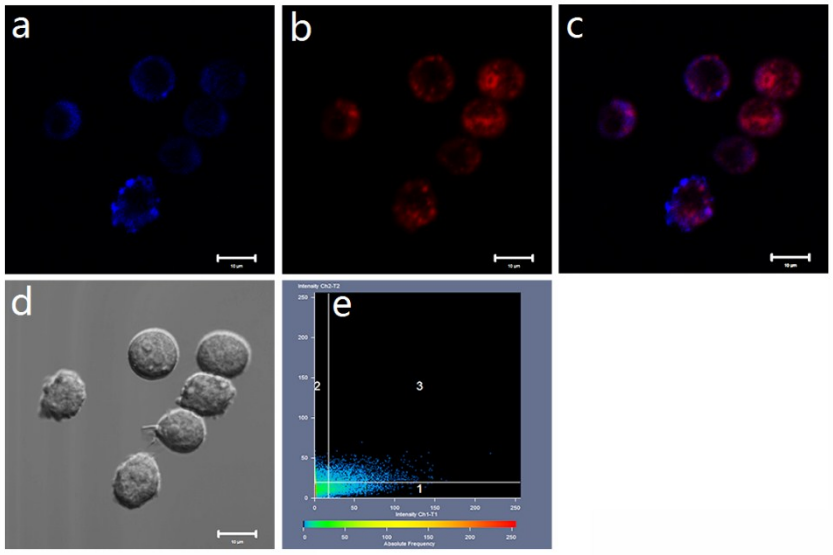


Fig. S12 Fluorescence images of RAW264.7 cells co-stained with **RCP** (5 μM) and Lyso Tracker Deep Red (0.1 μM). (a) Blue fluorescence of **RCP** (405-555 nm), λex = 405 nm. (b) Red fluorescence of Lyso Tracker Deep Red, λex = 640 nm. (c) Merge images of (a) and (b). (d) Bright field images. (e) Quantitation of co-localization coefficient (Pearson's coefficient): 0.59. Scale bar = 10 μm.

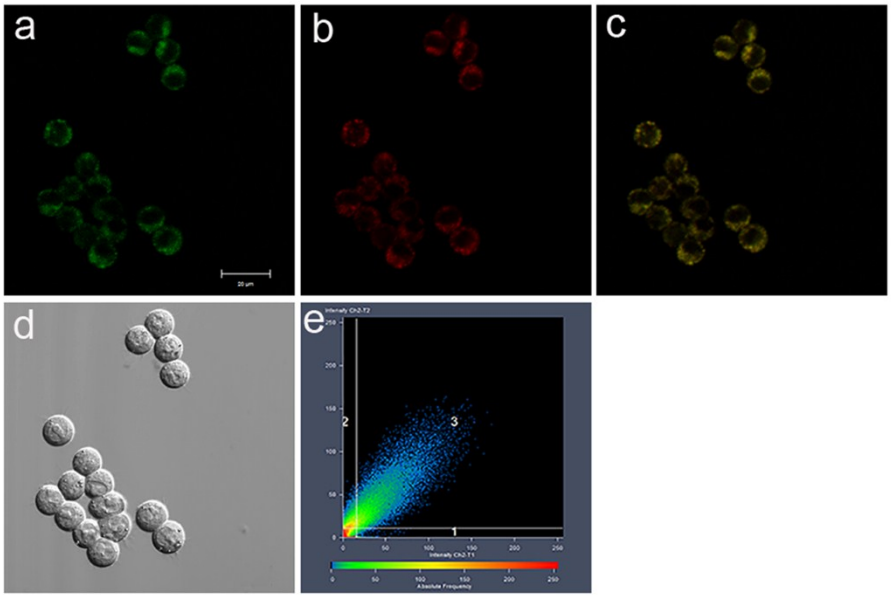


Fig. S13 Fluorescence images of RAW264.7 cells co-stained with **RCP** (5 μM) and Mito Tracker Deep Red (0.3 μM). (a) Red fluorescence of **RCP** (560-700 nm), λex = 405 nm. The red fluorescence was coloured as green for discrimination. The signal has been amplified to emphasize the probe's location. (b) Red fluorescence of Mito Tracker Deep Red, λex = 644 nm. (c) Merge images of (a) and (b). (d) Bright field images. (e) Quantitation of co-localization coefficient (Pearson's coefficient): 0.91. Scale bar = 20 μm.

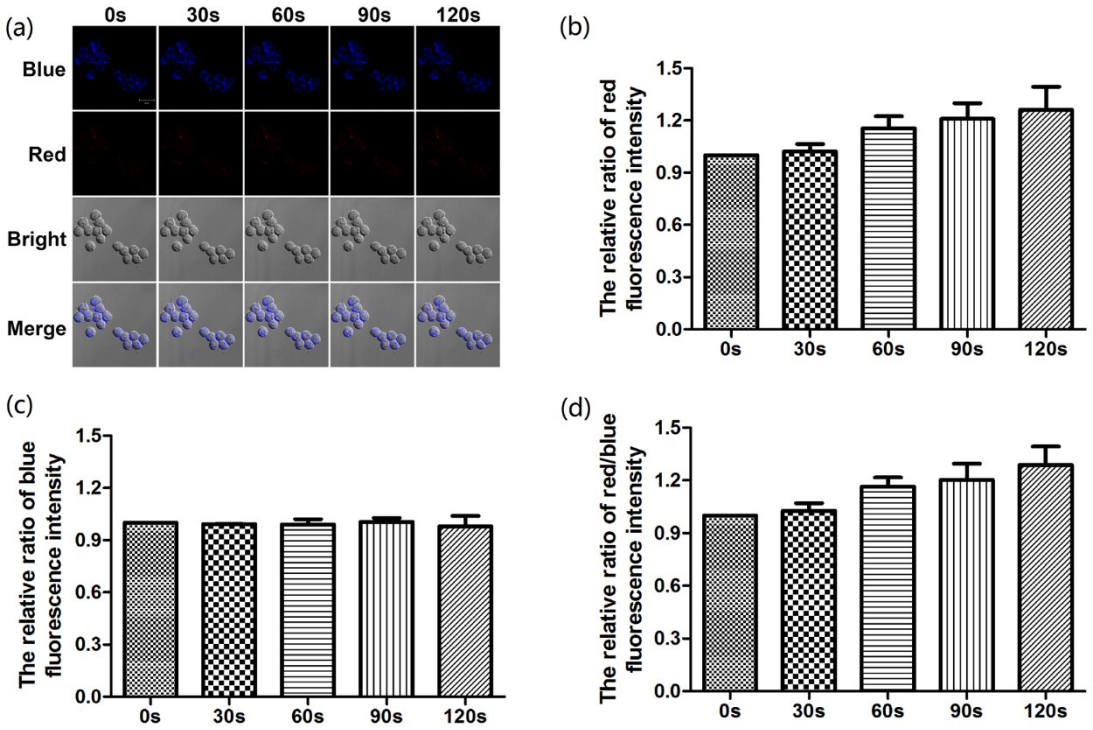


Fig. S14 Photostability of **RCP** (5 μM) in RAW264.7 cells. (a) Fluorescence images of RAW264.7 cells after 0, 30, 60, 90 and 120 s of continuous irradiation. λex = 405 nm. First line: fluorescence images at blue channel (405-555 nm), second line: fluorescence images at red channel (560-700nm), third line: bright field images, fourth line: merge images of first, second and third line. (b) The relative ratio of red fluorescence intensity (rhodamine moiety) in cells at different periods of time. (c) The relative ratio of blue fluorescence intensity (coumarin moiety) in cells at different periods of time. (d) The corresponding relative ratio of red/blue fluorescence intensity in cells at different periods of time [the initial red/blue fluorescence intensity ratio (i.e., at about 0 s) was defined as 1.0]. Fluorescence intensity quantitation was analyzed by the Image J. The results were presented as means ± SE with replicates n = 3. Scale bar = 20 μm.