Supporting information for

Development of a Fluorescent Probe for Measurement of Singlet Oxygen Scavenging Activity of Flavonoids

Darina Pronin,[†] Saarangan Krishnakumar,[§] Michael Rychlik,[†] Haixia Wu[*,‡,§] and Dejian Huang[*,§]

[‡]Department of Chemistry, College of Chemistry and Chemical Engineering, Inner Mongolia University, Hohhot 010021, People's Republic of China

[§] Department of Food Science and Technology, National University of Singapore, 3 Science Drive 3, Singapore 117543, Republic of Singapore

[†]Analytical Food Chemistry, Technical University of Munich, Maximus-von-Imhof-Forum 2, D-85354 Freising, Germany

***Corresponding author**

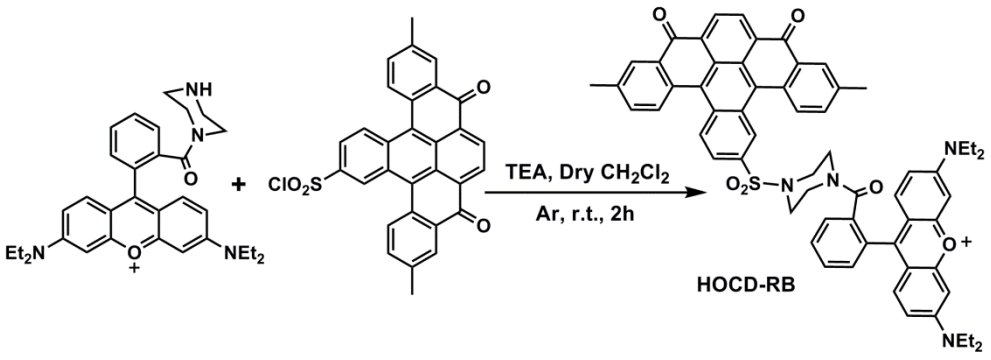
Haixia Wu. E-mail: chmwuhx@imu.edu.cn

Dejian Huang. E-mail: chmhdj@nus.edu.sg

Table S1. The final concentration solutions of antioxidants in PBS buffer.

Antioxidant[a]	Stock solution mg/mL	Final concentration μg/mL
Vitamin C	3.63	5.3 / 24.5 / 62.9 / 126 / 205.5 / 274
Trolox in MeOH	2.40	17.5 / 35 / 52.5 / 70 / 87.5 / 95
β-Carotene in MeOH	1.07	5.6 / 56 / 84 / 112 / 140 /168
Caffeic acid in MeOH	0.99	0.5 / 6.1 / 12.3 / 18.3 / 24.5 / 36.7
Epicatechin in DMSO	0.96	0.5 / 6.8 / 2.7 / 13.7 / 27.4 / 41.1
Catechin gallate	0.19	0.54 / 1.1 / 1.4 / 2.7 / 4.1 / 5.4
Epigallocatechin	0.67	0.4 / 0.9 / 1.9 / 4.8 / 13.9 / 19.2
Epigallocatechin galatte	0.98	0.2 / 0.3 / 0.5 / 1.1/ 1.4 / 2.2
Myricetin in DMSO	0.64	0.4 / 1.3 / 1.8 / 2.7 / 3.6 / 5.5
Quercetin in DMSO	0.60	0.9 / 1.7 / 2.1 / 2.3 / 2.6 / 3.5
Kaempferol in DMSO	0.57	2.2 / 2.5 / 2.9 / 3.8 / 5.0 / 5.7

[a] The following stock solutions were prepared in deionized water, unless otherwise described.



Scheme S1. Synthesis of probe HOCD-RB.

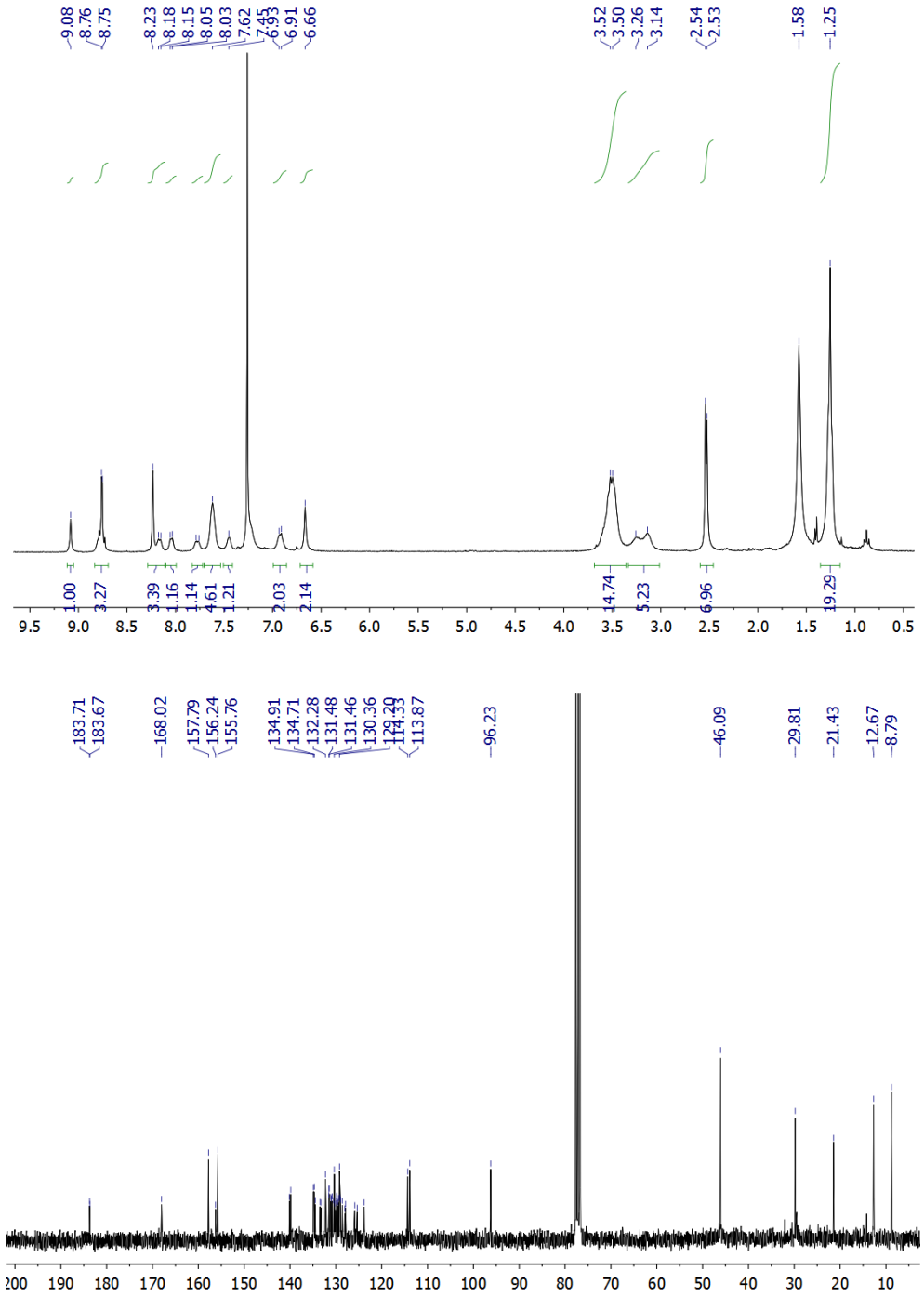


Figure S1. ^{1}H-NMR and ^{13}C-NMR spectra of HOCD-RB.

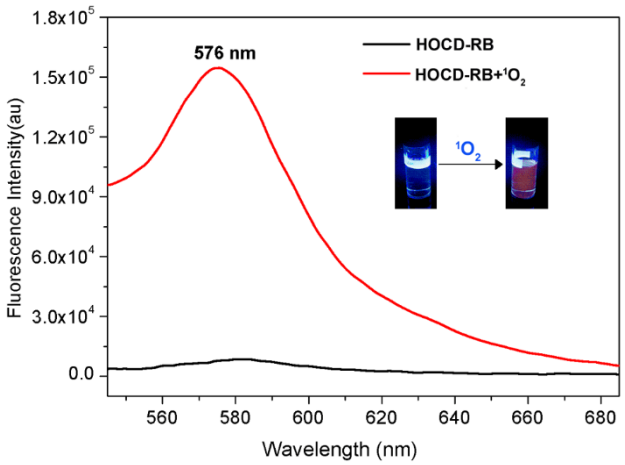


Figure S2. The fluorescence emission spectra of HOCD-RB (0.1 μM) delivered using 20 equiv DOTAP in 10 mM PBS buffer (pH 7.4), in the presence or absence of $^1O_2$ for 10 min at r.t. (λ_{ex} = 480 nm). $^1O_2$ is generated by photo-irradiation of rose Bengal.

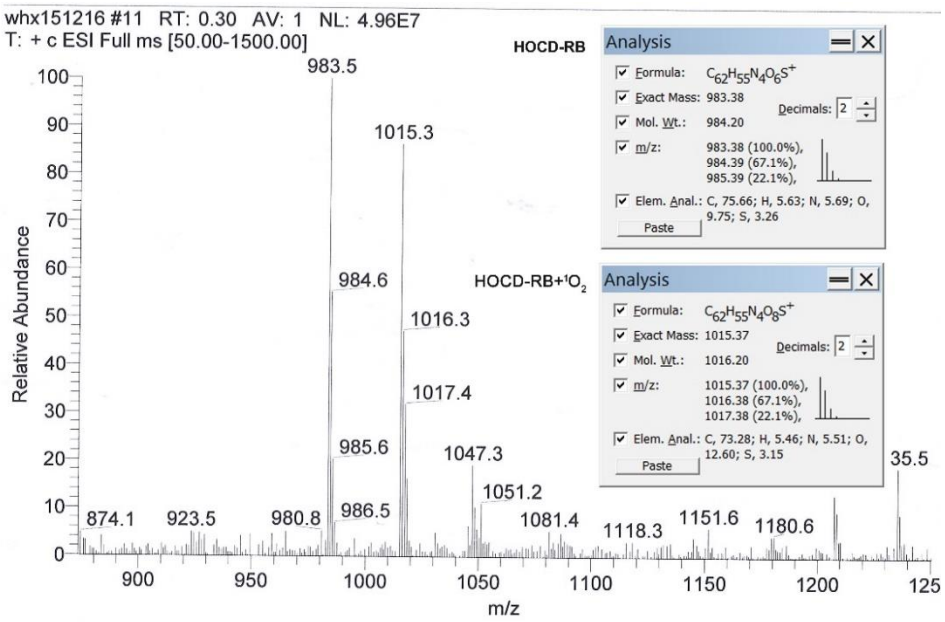
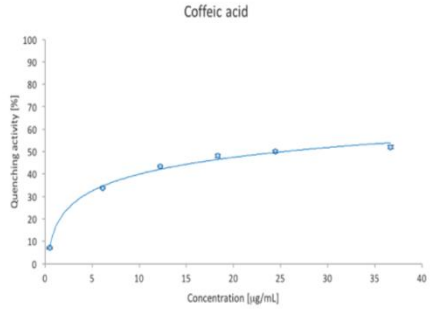
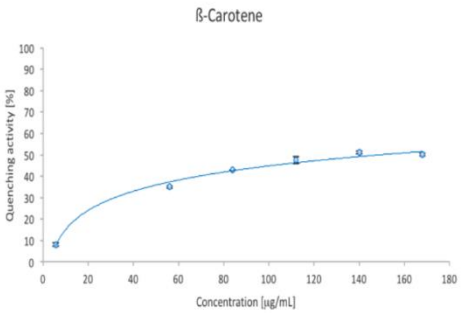
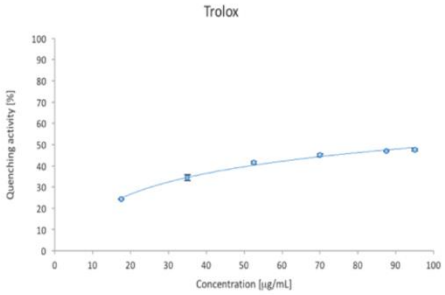
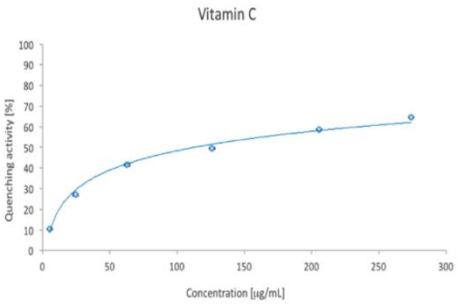


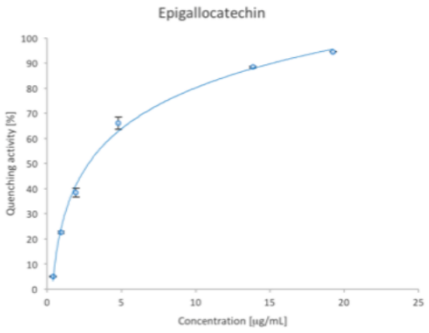
Figure S3. ESI-MS of endoperoxide obtained from the reaction of HOCD-RB with $^1O_2$ in 10 mM PBS buffer (pH 7.4).

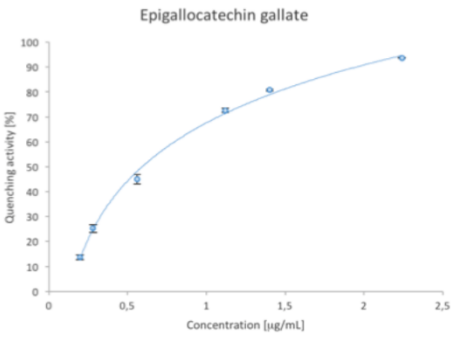

Coffeic acid
Quenching activity [%]
Concentration [µg/mL]

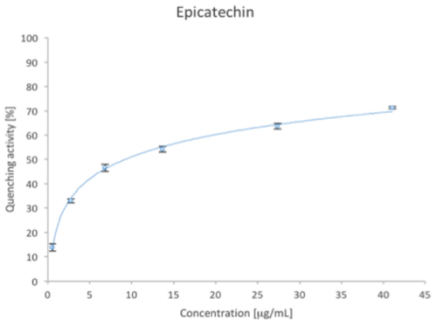

ß-Carotene
Quenching activity [%]
Concentration [µg/mL]

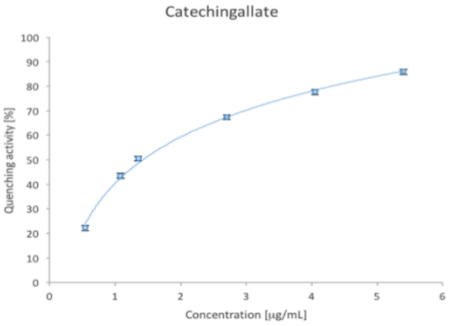

Trolox
Quenching activity [%]
Concentration [µg/mL]


Vitamin C
Quenching activity [%]
Concentration [µg/mL]


Epigallocatechin
Quenching activity [%]
Concentration [µg/mL]


Epigallocatechin gallate
Quenching activity [%]
Concentration [µg/mL]


Epicatechin
Quenching activity [%]
Concentration [µg/mL]


Catechingallate
Quenching activity [%]
Concentration [µg/mL]

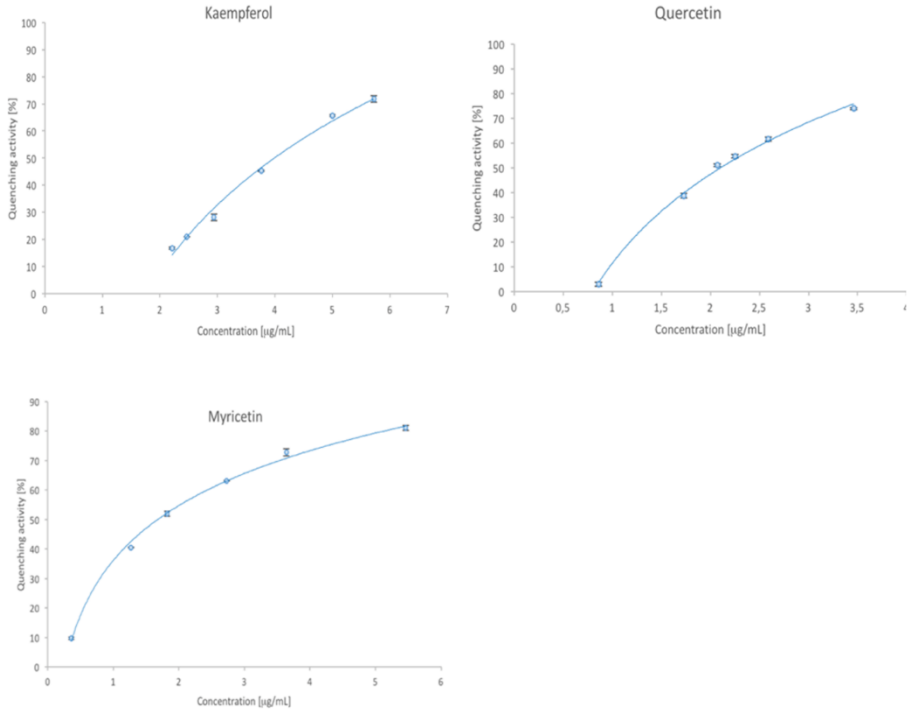


Figure S4. Dose response curves of antioxidants. Error bars represent means (n=3) ± standard deviation.

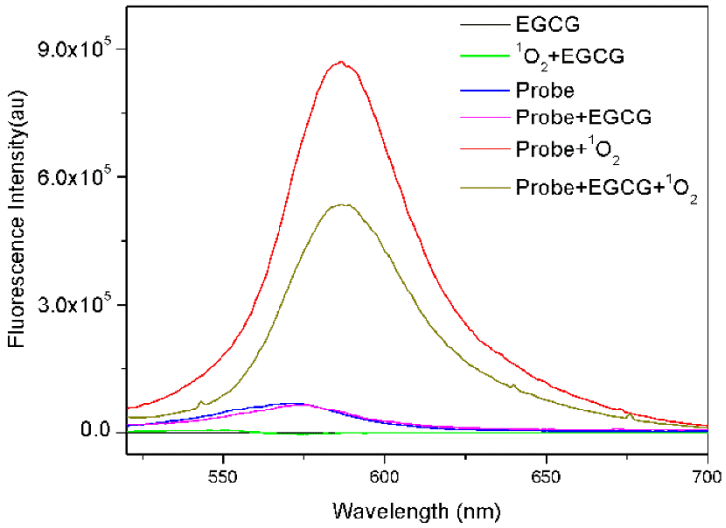


Figure S5. The fluorescence emission spectra of samples consist of ECGC with or without $^1O_2$ or probe in 100 mM PBS buffer (pH 6.8, λ_{ex} = 500 nm).

Table S2. Synergistic effects of flavonoids mixture (1:1 molar ratio), n=3.

Mixture (1:1)	SE	Effect
EGCG + K	0.66 ± 0.04	Antagonistic
EGCG + Q	0.82 ± 0.02	Antagonistic
EGCG + M	0.45 ± 0.05	Antagonistic
CG + K	1.18 ± 0.05	Synergistic
CG + Q	0.87 ± 0.06[a]	Additive
CG + M	0.94 ± 0.03[a]	Additive
EGC + K	0.67 ± 0.01	Antagonistic
EGC + Q	0.71 ± 0.07	Antagonistic
EGC + M	0.77 ± 0.03	Antagonistic
EC + K	0.71 ± 0.02	Antagonistic
EC + Q	0.80 ± 0.04	Antagonistic
EC + M	0.71 ± 0.02	Antagonistic

[a] No significant difference between experimental and theoretical quenching value $p < 0.01$.

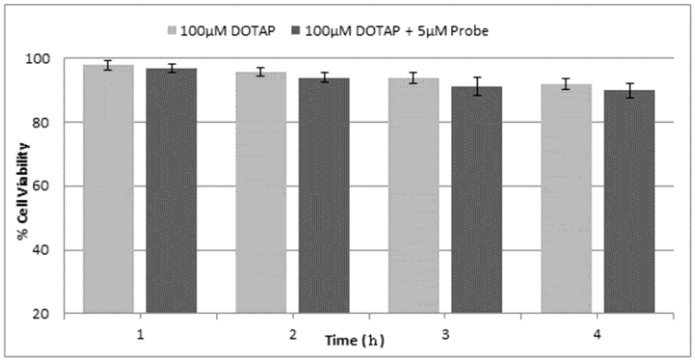


Figure S6. Cytotoxicity effects of HOCD-RB-DOTAP treatment on RAW 264.7 cells. RAW 264.7 cells were treated with 5 μM probe + 100 μM DOTAP mixture or 100 μM DOTAP alone for 4 h, the cell viability was measured using MTT assay. Data were presented as mean ± SDE of 3 independent experiments.

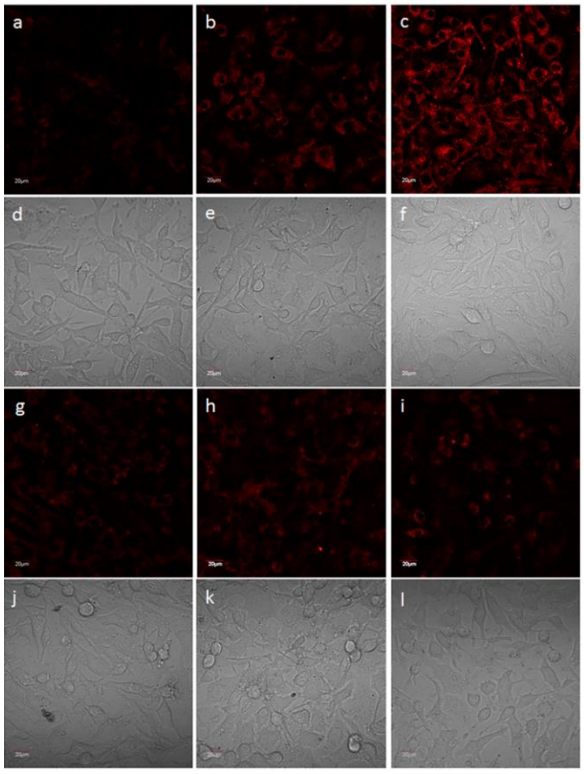


Figure S7. The 2D fluorescent images of cells treated with HOCD-RB (5 μM) for 30 min. (a) HOCD-RB alone as control, that is non-stimulated with PMA; (b) and (c) stimulated with PMA (5 and 10 μM); (g) histidine (400 μM) pre-treatment followed by PMA stimulation (10 μM); (h) EGC (100 μM) pre-treatment followed by PMA stimulation (10 μM); (i) EGCG (100 μM) pre-treatment followed by PMA stimulation. d-f and j-l are corresponding DIC images.

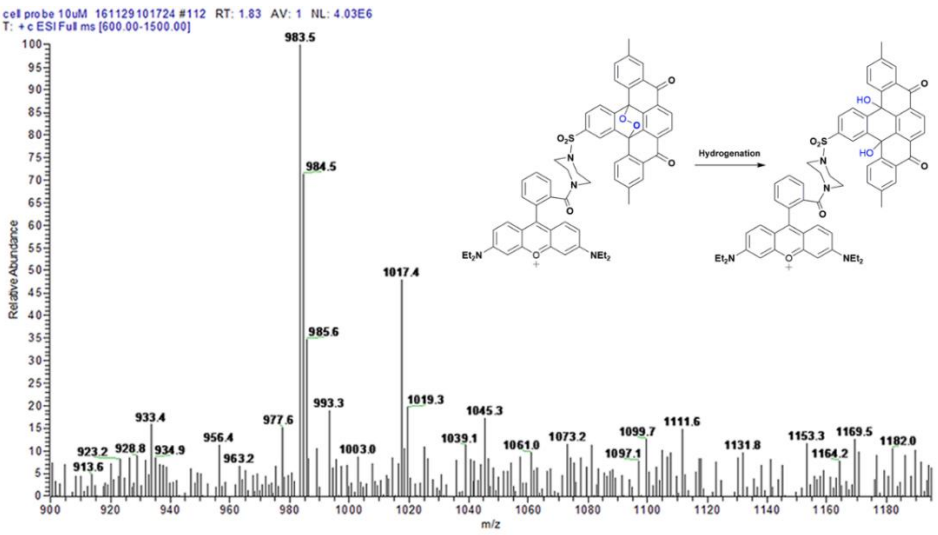


Figure S8. ESI-MS of the products obtained from HOCD-RB reacted with $^1O_2$ in cells.

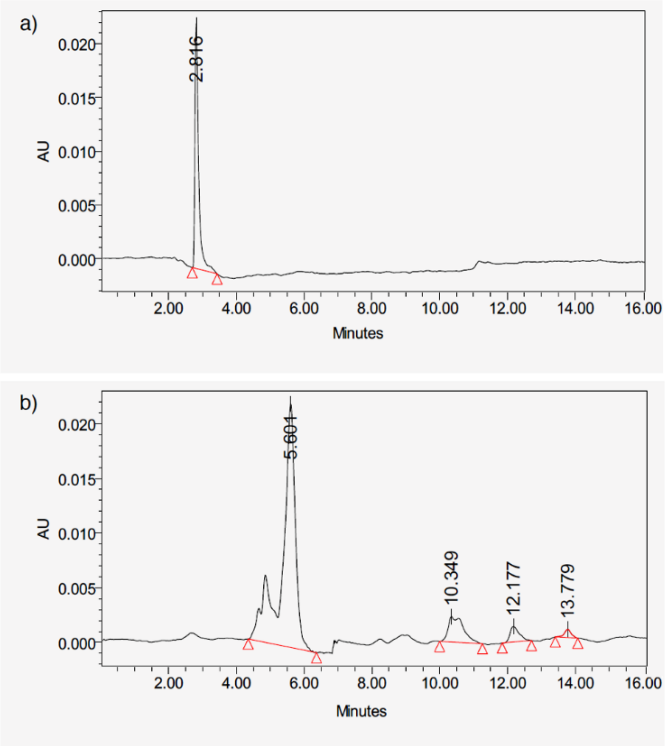


Figure S9. HPLC spectra of EGCG with rose Bengal before (a) and after (b) irradiation.

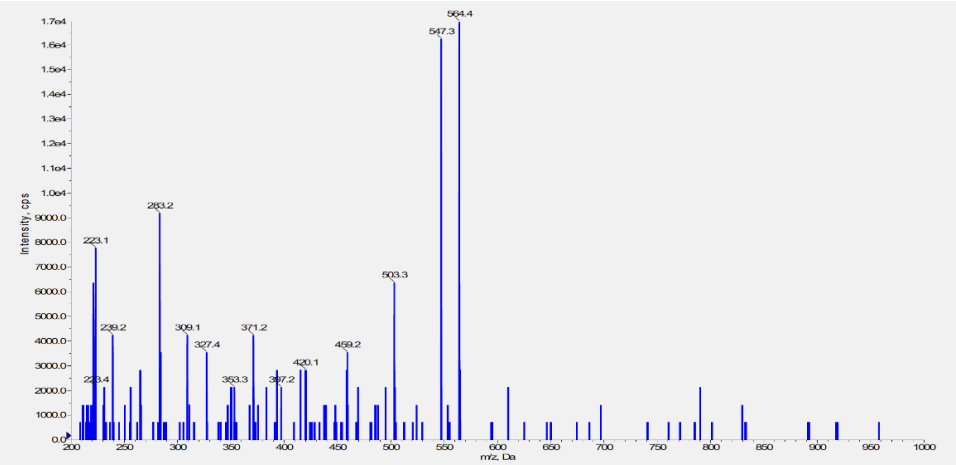


Figure S10. LC-MS/MS spectrum of oxidation product of EGCG with the retention time at 13.78 min and m/z 564.4.

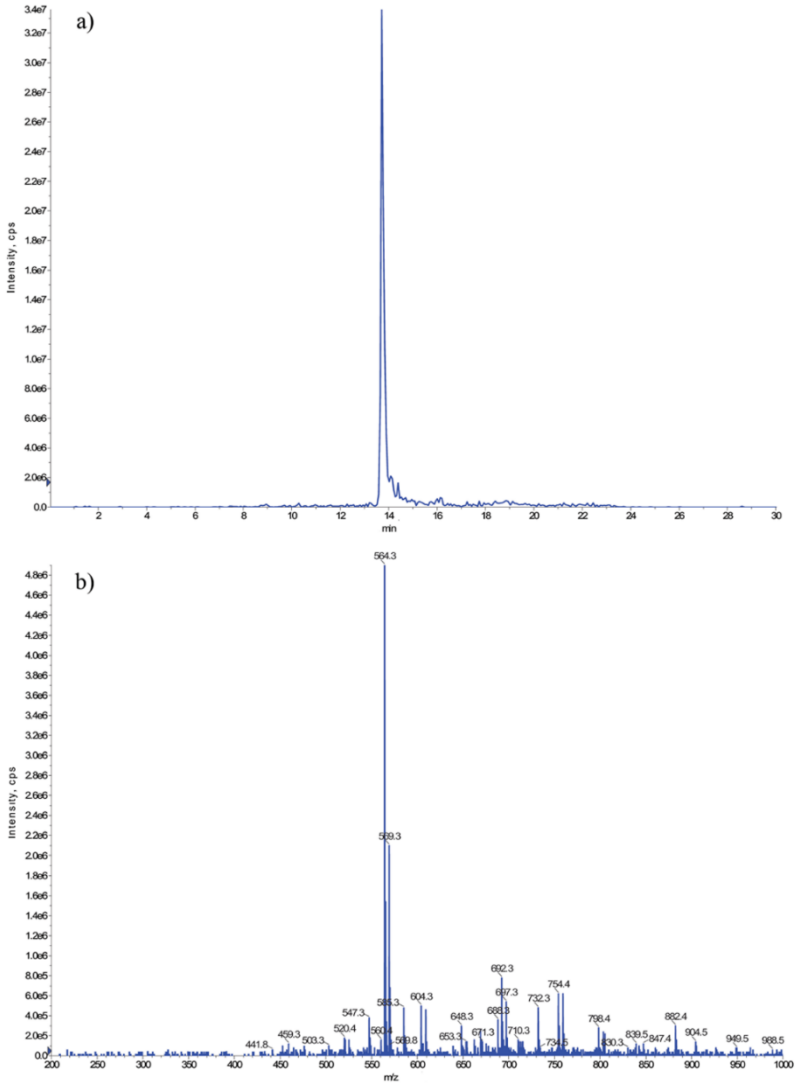


Figure S11. Theaflavine standard LC chromatogram (a) and mass spectrum (b).