Supplementary Information

Constructing *de novo* H_2O_2 Signaling Through Induced Protein Proximity

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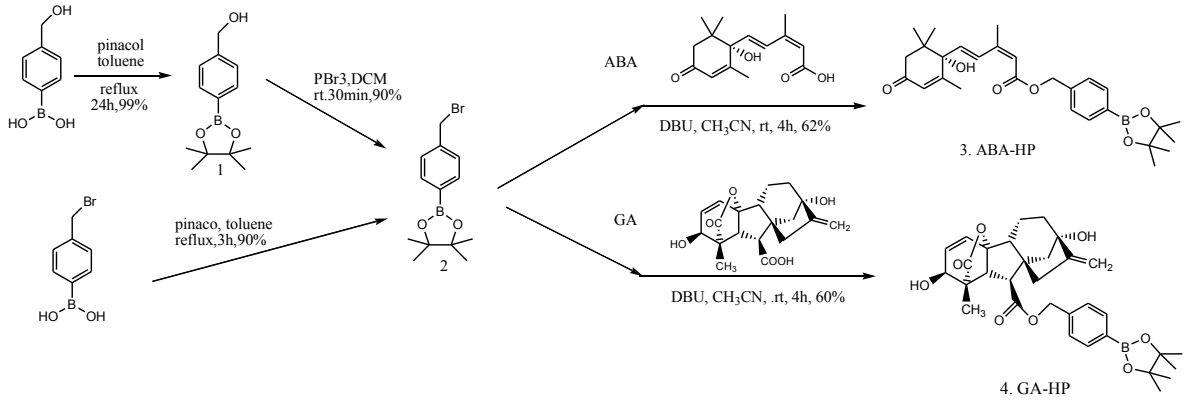
Materials and Methods

Chemical synthesis.

General. ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR were recorded on a Bruker Avance 500. All commercially available reagents were used without further purification. The progress on the reactions was monitored by analytical thin-layer chromatography (TLC) on Whatman silica gel plates with UV indicator. And Merk 60 silica gel was used for chromatography.



Scheme S1. Synthesis of ABA-HP and GA-HP

Synthesis of 2-[4-(Bromomethyl)phenyl]-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2). To 4-(bromomethyl)phenylboronic acid (200 mg, 0.93 mmol, Sigma Aldrich) in 1 mL toluene was added pinacol (165 mg, 1.40 mmol, Sigma Aldrich), then heated to reflux with Dean-Stark trap and stirred for 3 h. The solvent was removed by rotavapor, and the product was purified through column chromatography (EA : Hex = 1:3) and obtained as white solid (250 mg, 91%). Also compound 2 was synthesized through compound 1. ^{1}H-NMR (300 MHz, CDCl3), δ (ppm): 1.340 (12H, s), 4.488 (2H, s), 7.378-7.404 (2H, d, J = 7.8Hz), 7.772-7.798 (2H, d, J = 7.8Hz).

Synthesis of ABA-HP (3). To ABA (44 mg, 0.17 mmol, Gold Biotechnology, USA) in 10 mL CH_3CN was added compound 2 (55 mg, 0.19 mmol) and DBU (55 μL, 0.37 mmol, Sigma Aldrich), then stirred at RT for 4 h. The solvent was removed by rotavapor, then the product was purified by column chromatography (EA : Hex = 1:3) to give white solid (50 mg, 62%). The compound was characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry with >

95% purity based ^{1}H NMR. HPLC was used for further purification before biological activity test. ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 0.997 (3H, s), 1.097 (3H, s), 1.334 (12H, s), 1.896 (3H, s), 2.004 (3H, s), 2.165 (1H, s), 2.254-2.311 (1H, d, J = 17.1Hz), 2.443-2.500 (1H, d, J = 17.1Hz), 5.155 (2H, s), 5.802 (1H, s), 5.917 (1H, s), 6.130-6.183 (1H, d, J = 15.9Hz), 7.345-7.372 (2H, d, J = 8.1Hz), 7.785-7.812 (2H, d, J = 8.1Hz), 7.855-7.910 (1H, d, J = 16.5Hz). ^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 18.9, 21.3, 23.1, 24.3, 24.8, 41.5, 49.8, 65.7, 79.6, 83.8, 118.3, 127.0, 127.2, 128.1, 135.0, 136.5, 139.1, 149.7, 162.4, 165.6, 197.7. TOF-HRMS (m/z) found (calcd.) for $C_{28}H_{37}O_6B$ (M): $[M+Na]^+$, 503.2583 (503.2581) and [2M+Na]+, 983.5185 (983.5264).

Synthesis of GA-HP (4). To GA (100 mg, 0.29 mmol, Alfa Aesar) in 20 mL CH_3CN was added compound 2 (102.9 mg, 0.35 mmol) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU, 52 μL, 0.35 mmol, Sigma Aldrich), then stirred at RT for 4 h. The solvent was removed by rotavapor, and the product was purified by column chromatography (EA : Hex = 1:1) and obtained as white solid (97 mg, 60%). The compound was characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectrometry with > 95% purity based ^{1}H NMR. HPLC was used for further purification before biological activity test. ^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm): 1.198 (3H, s), 1.338 (12H, s), 1.632-2.172 (9H, m), 2.795-2.831 (1H, d, J = 10.8Hz), 3.200-3.236 (1H, d, J = 10.8Hz), 4.127 (1H, s), 4.876 (1H, s), 5.117-5.231 (3H, m), 5.867-5.910 (1H, dd, J = 3.6, 9.3Hz), 6.280-6.313 (1H, d, J = 9.9Hz), 7.318-7.345 (2H, d, J = 8.1Hz), 7.778-7.805 (2H, d, J = 8.1Hz). ^{13}C-NMR (75 MHz, $CDCl_3$), δ (ppm): 14.8, 17.3, 25.1, 38.4, 43.1, 45.1, 50.8, 51.0, 51.4, 53.1, 53.7, 67.1, 69.9, 75.4, 78.3, 84.2, 90.8, 107.8, 127.7, 132.7,133.0, 135.3, 138.5, 157.0, 172.3, 179.0. TOF-HRMS (m/z) found (calcd.) for $C_{32}H_{39}O_8B$ (M): $[M+Na]^+$, 585.2653 (585.2636).

Reverse-phase HPLC analysis

Chemical stability and reactivity of ABA-HP towards H_2O_2. ABA-HP chemical stability: 1 mM ABA-HP (in DMSO) was incubated in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) for 24 h at 37 ℃. HPLC results were detected at 0 min, 20 min, 40 min, 60 min, 80 min, 100 min, 120 min, 240 min, and 24 hrs. ABA-HP reactivity towards H_2O_2: 1 mM ABA-HP was incubated with 5 mM (5 eq) H_2O_2 in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) at 37 ℃. The cleavage products were detected at 0, 20, 40, 80, 100, 120 and 240 min by HPLC. 100 mM H_2O_2 was diluted with 11 μL of 30% (10 M) stock H_2O_2 (VWR) and 989 μL of ddH_2O. All concentrations shown were the final concentration. HPLC chromatograms were acquired using Dionex-UltiMate 3000 LC System

with Acclaim 120 Å, C18, 3 μm analytical (4.6 x 100 mm) column. Chromatographic conditions: eluent A: 0.1% v/v TFA in water; eluent B: 0.1% v/v TFA in acetonitrile. ABA in ethanol or ABA-HP in DMSO was eluted at a flow rate of 0.750 ml/min monitored at a wavelength of 260 nm. 0-12 min (linear): 95% A, 5% B to 5% A, 95% B; 12-15 min: 5% A, 95% B; 15-17 min (linear): 5% A, 95% B to 95% A, 5% B. Generation of ABA was quantified by the peak area of ABA out of the total peak area.

Reaction selectivity versus different molecules. 100 μM ABA-HP was incubated with 100 μM (1 eq) of different molecules as indicated in 50% HEPES/DMSO (pH 7.4) at 37℃ for 4 h and the cleavage products were detected by HPLC. H_2S was generated by 100 μM Na_2S solution in HEPES buffer, which can slowly release H_2S. •OH and •O^{t}Bu were generated by reaction of Fe^{2+} with H_2O_2 or tert-butyl hydroperoxide (TBHP), respectively. HPLC condition was the same as the stability and reactivity test of ABA-HP.

Chemical stability and reactivity of GA-HP towards H_2O_2. GA-HP chemical stability: 5 mM GA-HP (in DMSO) was incubated in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) for 24 h at 37 ℃ and the products were analyzed by HPLC. GA-HP reactivity towards H_2O_2: 5 mM GA-HP was incubated with 50 mM (10 eq) H_2O_2 in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) at 37 ℃. The products were analyzed at 5, 30, 60, 90 and 120 min using HPLC. HPLC chromatograms were acquired using Dionex-UltiMate 3000 LC System with Acclaim 120 Å, C18, 3 μm analytical (4.6 x 100 mm) column. Chromatographic conditions: eluent A: 0.1% v/v TFA in water; eluent B: 0.1% v/v TFA in acetonitrile. Solutions in DMSO were eluted at a flow rate of 1.000 ml/min monitored at a wavelength of 206 nm. 0-12 min (linear): 95% A, 5% B to 5% A, 95% B; 12-16 min: 5% A, 95% B; 16-18 min (linear): 5% A, 95% B to 95% A, 5% B.

Cloning and plasmid construction.

All DNA fragments were amplified by PCR (Polymerase chain reaction) from other intermediate constructs with the enzyme of Phusion DNA Polymerase (New England Biolabs), or PfuUltra II Fusion HotStart DNA Polymerase (Agilent Technologies) under S1000 thermal cycler with Dual 48/48 Fast Reaction Module (Bio-Rad). All the restriction enzymes used below are purchased from New England Biolabs. PYL-EGFP-Tiam1 construct was derived from pSV40-VP16-PYL-IRES-Gal4DBD-ABI[12] by inserting codon optimized PYL fragment (PCR amplified by primers CCGACAGAATTCGCCACCAT-GACCCAGGACGAGTTTACCCAG and CCGACA

GGCGCGCCGCTGCCGCCGTTCA-TAGCCTCAGTAATGCT) using EcoRI and AscI sites, Tiam1-SG linker fragment (amplified by primers GCTATGAACGGCGCGCCAAGTGCT GGTGGTAGTGCTGGT and CTAGAGTCGCGGCCGCTCAGATCTCAGTGTTCAGTTTC) using AscI and NotI sites, and eGFP-SG-linker fragment (amplified by primers CCGACAGGCGCGCCAGGTGGATCTGGAGGTTCAGGTGGATCTGGAGGTGTGAGCAAG GGCGAGGAGCTG and CCGACAGGCGCGCCCTTGTACAGCTCGTCCATGCC) using AscI site. NES-GID1 construct was generated from NES-ABI[25] by inserting GID1 fragment (amplified by primers CCGACAACGCGTGGATCTGGTGGAGCTGCGAGCGATGA-GTTAAT and CCGACAGCGGCCGCTCAACATTCCGCGTTTACAAACGC) using MluI and NotI sites. EGFP-GAI construct was generated from eGFP-PYL.[12] GAI fragment (amplified by primers CCGACAGGCGCGCCAGGATCTGGTGGAAAGAGAGATCATC-ATCATCAT and CCGACAGGATCCTCAAGGATTAAGGTCGGTGAGCAT) was inserted by AscI and blunt end ligation into AscI and blunted NotI sites on the vector.

Mammalian cell culture and transfection.

All cells were cultured in DMEM medium (Gibco) supplemented with 10% FBS, 2 mM GlutaMAX (life technologies), 100 U/ml penicillin (life technologies) and 100 μg/ml streptomycin (life technologies) at 37℃ in a humidified atmosphere containing 5% CO_2.

EGFP expression experiments. HEK 293T EGFP reporter cells were seeded 24 h prior to treatment in 24-well plates at 100,000/well. 10 μM ABA-HP was added to the cells 10 min prior to the addition of 10 μM ABA and 10, 50, or 100 μM of H_2O_2. Images were taken for living cells at 5, 7, 10 and 24 h.

NES localization experiments in CHO cells. CHO cells were seeded over glass coverslips in 24-well plates at 50,000/well for 24 h. 0.4 μg NES-ABI and 0.2 μg EGFP-PYL DNA plasmids were mixed with 30 μL of Opti-MEM (life technologies) and 1.8 μL of PEI (Polysciences). After incubation at RT for 15 min, the mixture was added to the cells and cultured for 24 h. Then, 10 μM ABA or 10 μM ABA-HP plus 100 μM of H_2O_2 (ABA-HP added 10 min prior to H_2O_2), or 100 μM GA or 100 μM GA-HP plus 100 μM of H_2O_2 (GA-HP added 10 min prior to H_2O_2) was added to the cells. 30 min after adding compounds, the coverslips were washed with phosphate-buffered saline (PBS) and fixed with 300 μL of 4% paraformaldehyde (PFA, prepared in PBS) at room temperature for 20 min. The cells were then washed twice with PBS and incubated with 1x DAPI

in the dark at the room temperature for 5 min. After a final wash with PBS, the coverslips were mounted on a glass slide with Vectashield (VWR) mounting media and images of cells were then taken using a fluorescence microscope.

Live cells imaging experiment for CHO cells with nuclear export experiments and its analysis. CHO cells were seeded in 8 Chamber coverglass plate at 25,000/well for 24 h. 0.2 μg NES-ABI and 0.1 μg EGFP-PYL DNA plasmids were mixed with 15 μL of Opti-MEM (life technologies) and 0.9 μL of PEI (Polysciences). After incubation at RT for 15 min, the mixture was added to the cells and cultured for 24 h. Medium was changed 1 h before the addition of 10 μM ABA, 10 μM ABA-HP with and without 100 μM H_2O_2. Images were taken every 30 sec for duration of 20 min for the same area. Images generated were analyzed for fluorescent intensity using Slide Book v.6 software. Equal sized regions of interest were analyzed to get the EGFP fluorescent intensity ratio of the nucleus over cytoplasm. Three cells for each condition were analyzed to get the average ratio and normalized.

NES localization experiments in A431 cells. A431 cells were seeded over glass coverslips in 24-well plates at 100,000/well for 24 h. 0.4 μg NES-ABI and 0.2 μg EGFP-PYL DNA plasmids were mixed with 30 μL of Opti-MEM and 1.8 μL of PEI. After incubation at RT for 15 min, the mixture was added to the cells and cultured for 24 h. Then, 10 μM ABA or 10 μM ABA-HP plus 100 μM of H_2O_2 or plus 500 ng/mL hEGF (Sigma-Aldrich) (ABA-HP added 10 min prior to H_2O_2 or hEGF) was added to the cells. Slides were prepared 30 min after adding compounds and images were then taken using a fluorescence microscope.

NES localization experiments in A431 and CHO cells treated with catalase. Cells were seeded over glass coverslips in 24-well plates at 100,000/well for A431 cells and 50,000/well for CHO cells 24 h before the transfections. 0.4 μg NES-ABI and 0.2 μg EGFP-PYL DNA plasmids were mixed with 30 μL of Opti-MEM and 1.8 μL of PEI. After incubation at RT for 15 min, the mixture was added to the cells and cultured for 24 h. Then, cells were treated with 1mg/mL of catalase (Sigma-Aldrich, C1345) by changing the medium with catalase for 1 h incubation, followed by the addition of other drugs.

Ruffle generation experiment. CHO were seeded over glass coverslips in 24-well plates at 50,000/well for 24 h. 0.1 μg PYL-EGFP-Tiam1 and 0.1 μg myr-ABI DNA plasmids were mixed with 30 μL of Opti-MEM and 1.8 μL PEI. After incubation at room temperature for 15 min, the

mixture was added to the cells and cultured for 24 h. Then, 10 μM ABA or 10 μM ABA-HP plus 100 μM of H_2O_2 (ABA-HP added 10 min prior to H_2O_2) (or 5 μM ABA or 5 μM ABA-HP plus 50 μM of H_2O_2) was added to the cells. For the cells treated with rac1 inhibitor NSC 23766, 50μM of NSC 23766 was added to the medium 1h prior to drug addition. Slides were prepared 30 min after adding compounds and images were then taken using a Zeiss LSM 510 META Confocal Microscopy the next day.

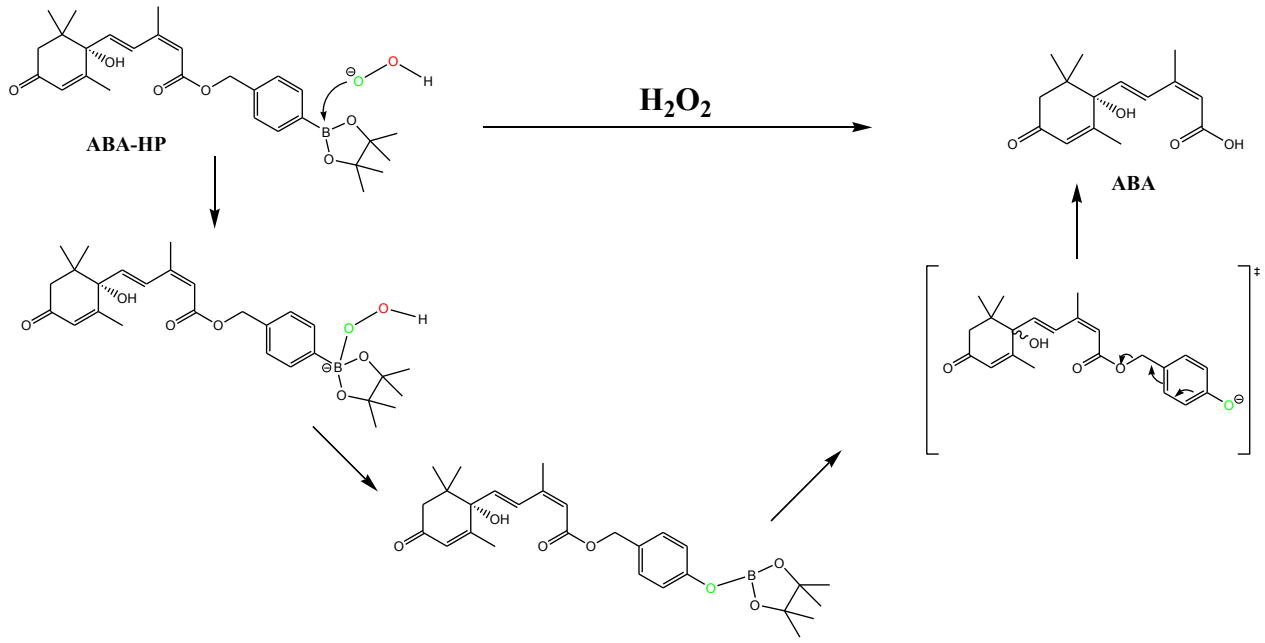
Luciferase reporter assay with CHO cells. CHO were seeded over glass coverslips in 24-well plates at 50,000/well for 24 h. 0.1 μg 5 × FL and 0.5 μg SV-VP-PYL-ires-Gal-ABI DNA plasmids were mixed with 30 μL of Opti-MEM and 1.8 μL PEI. After incubation at RT for 15 min, the mixture was added to the cells and cultured for 24 h. 10 μM ABA-HP or ABA was added to the cells. After 6 h, cells were washed with PBS and lysed with 100 μL of Reporter Lysis Buffer (Promega) by incubating and gently shaken at room temperature for 10 min. Cell lysates were centrifuged at 15,000 rpm in an Eppendorf Centrifuge 5424 and 25 μL of lysate was used for luciferase assay. 100 μL of luciferase assay reagent (5 mg luciferin (GoldBio) and 7 mg coenzyme A (Sigma) in 33 mL of Luciferase Assay Buffer [20 mM tricine, 1.07 mM $(MgCO_3)_4Mg(OH)2•5H_2O$, 2.67 mM $MgSO_4$, 0.1 mM EDTA, 33.3 mM dithiothreitol, and 0.53 mM ATP in water) was added to lysates. Luciferase assay reagent was added through the auto-injector of GLOMAX-Multi Detection System (Promega), and the signal was detected by the instrument with a 1.5 s delay and 0.5 s integration time. All experiments were conducted in triplicate.

Fluorescence microscopy. Zeiss Axio Observer. D1 outfitted with HBO 100 microscopy illumination system (GFP: excitation 470/40 and emission 525/50) and Zeiss LSM 510 META Confocal Microscopy outfitted with GFP/Alexa Fluor 488 (491 laser excitation, 528/38 emission) were used. *EGFP expression experiment:* Zeiss Axio Observer. D1 was used with the 20× objective and image areas were chosen randomly. *NES localization:* Zeiss Axio Observer. D1 was used with the 63× oil-immersion objective. *Ruffle formation*: Zeiss LSM 510 META Confocal Microscopy with 40× and 63× oil-immersion objectives. Fluorescent channels in all experiments were adjusted to the same intensity ranges. Acquisition times ranged from 100 to 1000 ms.

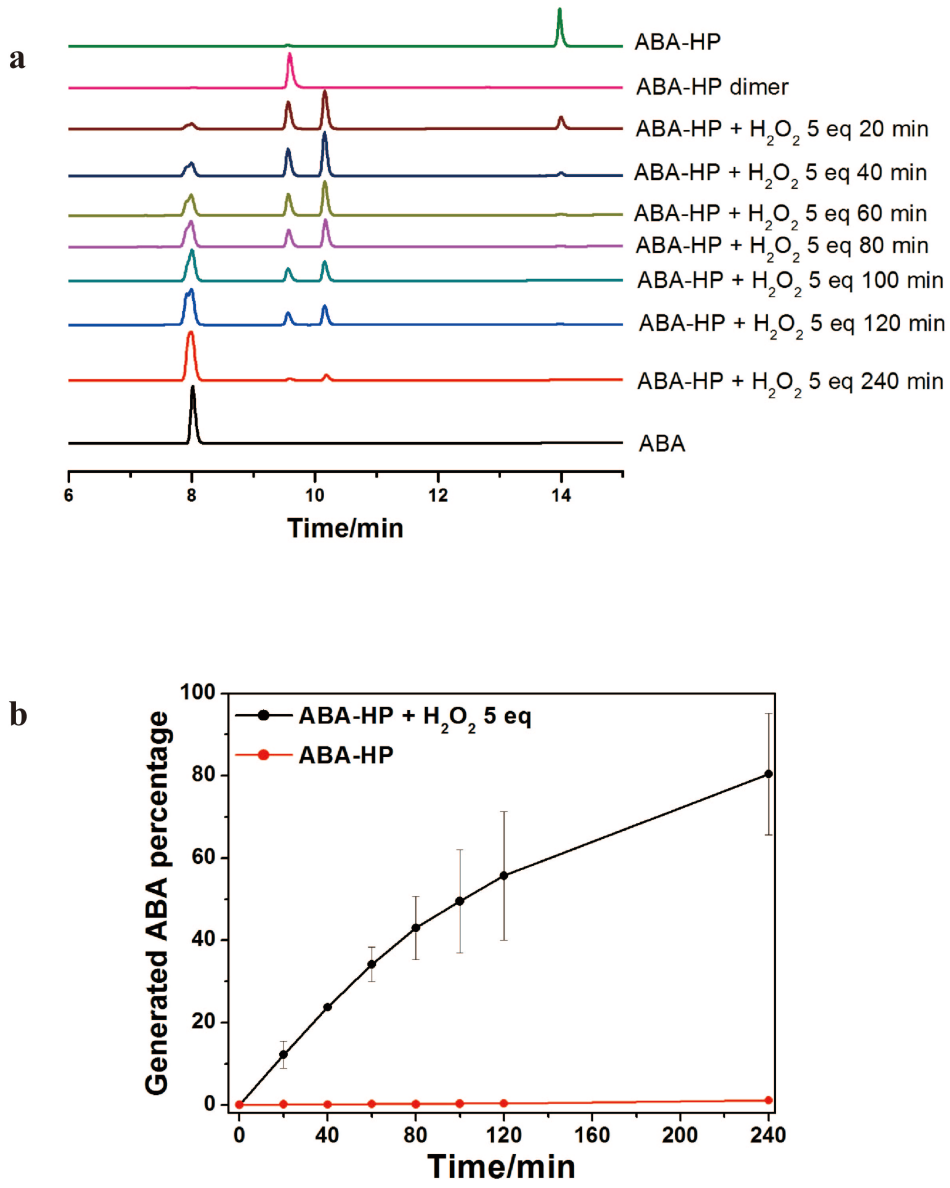
Statistical Analysis of Cell Population. Cell were categorized as displaying nuclear export of EGFP when the fluorescent intensity of the nucleus was less than 60% of the intensity over the

cytoplasm as shown in the representative pictures in Figure 2C, 2E and 3D. Cells were categorized as ruffled when they displayed broad extensions identifiable as lemellopodia or fillopodia from the GFP fluorescence from membrane localized EGFP-PYL-Tiam1 as shown in the representative pictures in Figure 2D. Cells were counted from 3 separate experiments with 5 different areas chosen randomly and over 700 cells were counted for each sample. P values were obtained using MS EXCEL with the function "CHISQ.TEST".

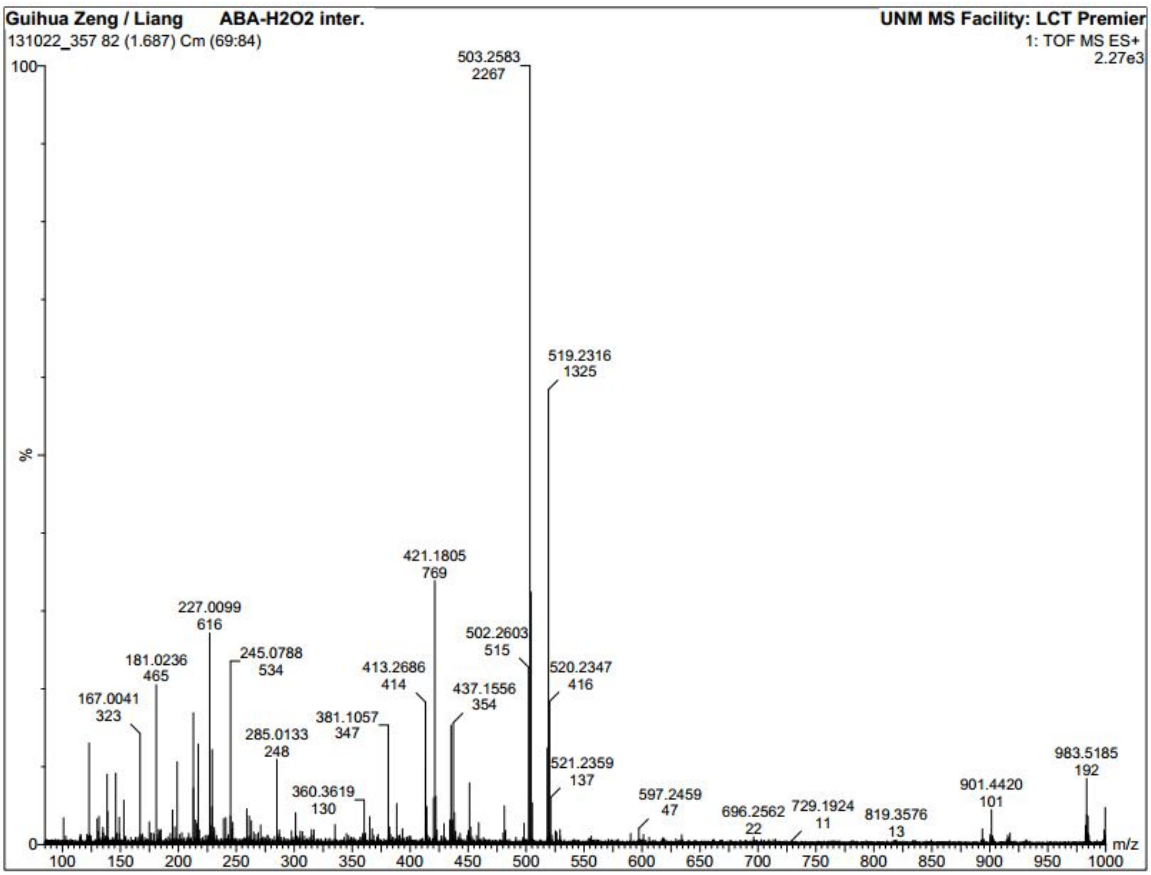
Supplementary Figures



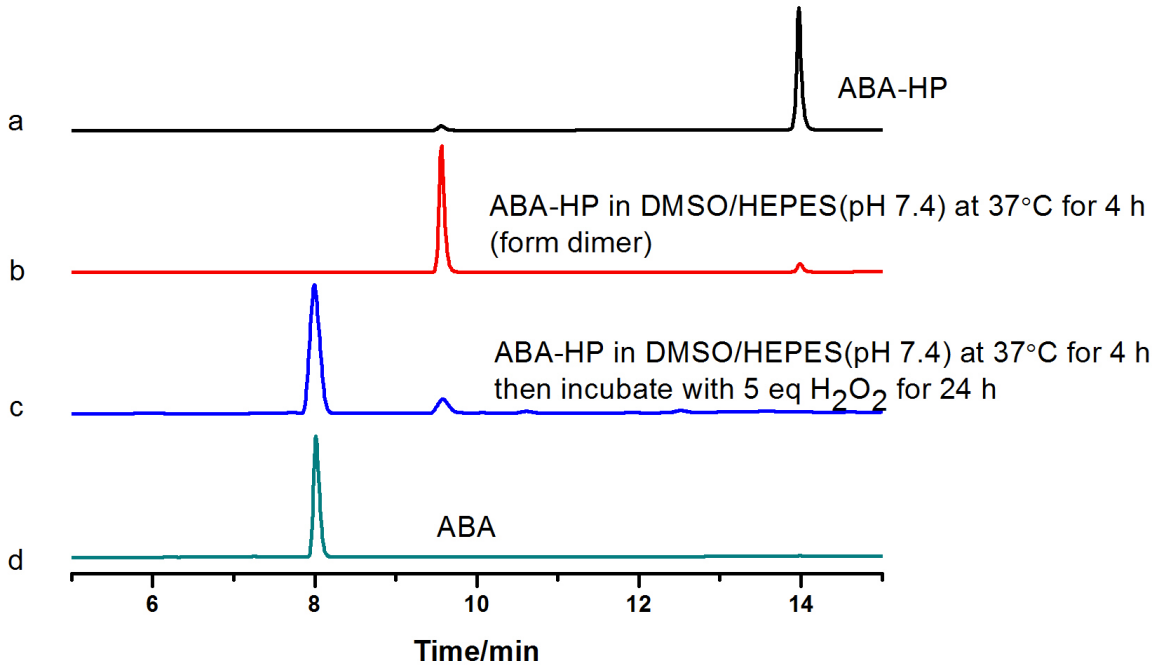
Supplementary Figure S1. A proposed mechanism of ABA-HP cleavage to form ABA in the present of H_2O_2 based on the reported oxidative cleavage mechanism of boronate ester.[22]



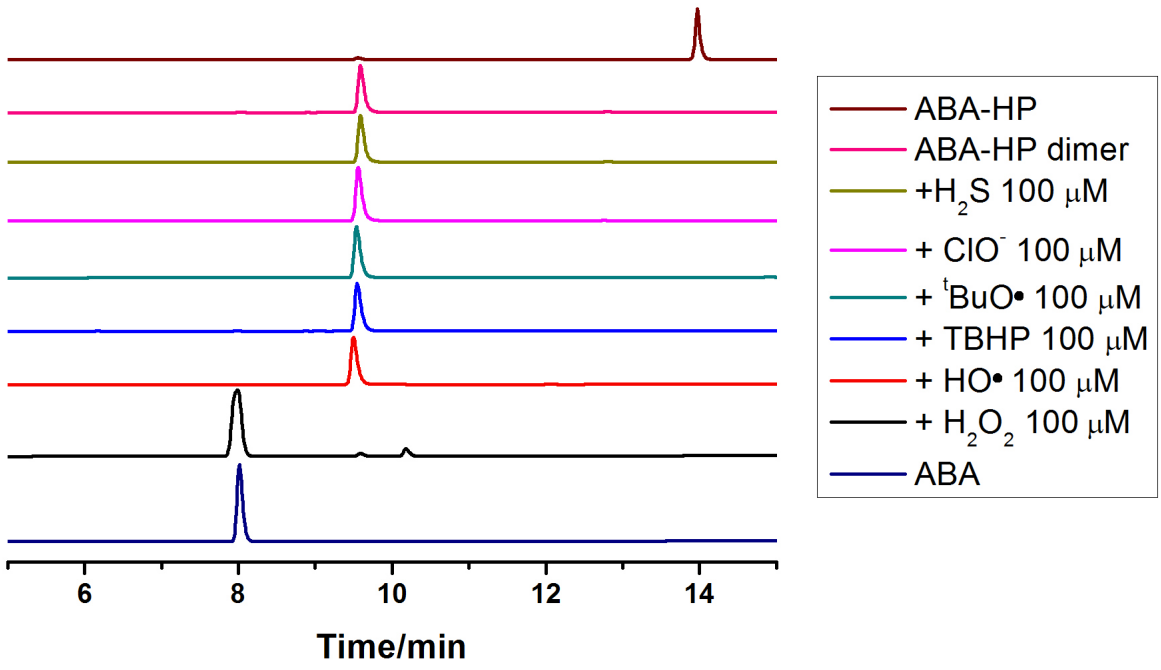
Supplementary Figure S2. Time dependent cleavage of ABA-HP by H_2O_2 detected by HPLC. 1 mM ABA-HP was treated with 5 mM H_2O_2 in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) from 0 to 240 min at 37℃. (**a**) The representative HPLC chromatograms from 3 independent experiments. (**b**) The quantitative analysis of generated ABA from ABA-HP in the presence of H_2O_2. The results were quantified by integrating the peak area corresponding to the ABA peak over the total areas of all peaks to give the generated ABA %. The shown result was the average from 3 independent experiments. Error bars are SD (N=3).



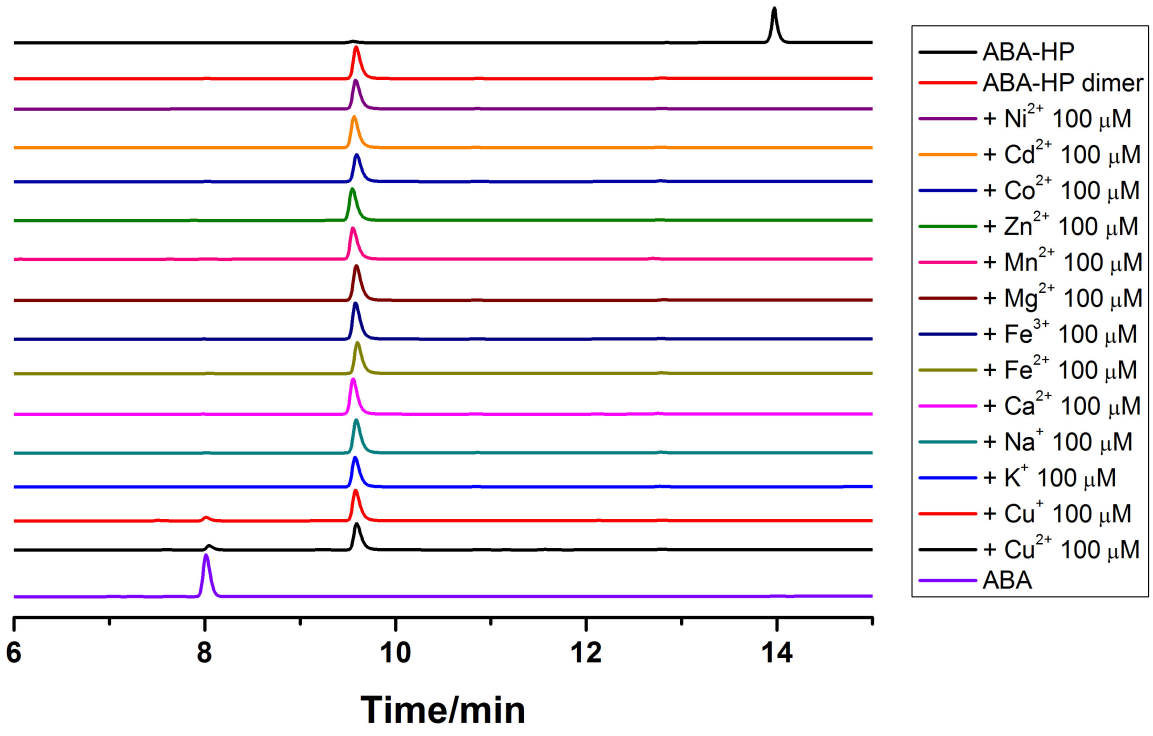
Supplementary Figure S3. MS of ABA-HP and ABA-HP dimer. TOF-HRMS (m/z) found (calcd.) for $C_{28}H_{37}O_6B$ (M): $[M+Na]^+$, 503.2583 (503.2581) and [2M+Na]+, 983.5185 (983.5264).



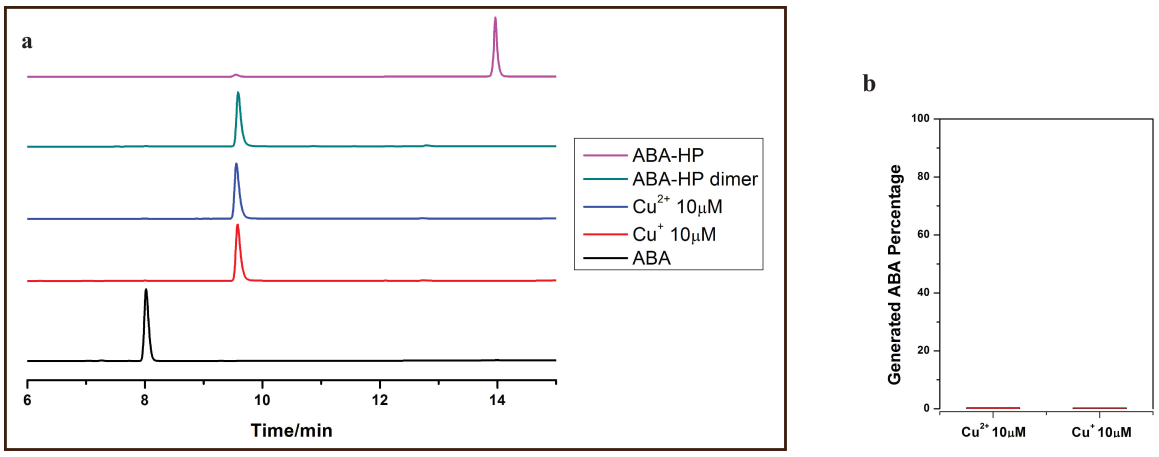
Supplementary Figure S4. Formation of ABA-HP dimer and its cleavage in the presence of H_2O_2. 1 mM ABA-HP was incubated in 50% DMSO/HEPES (10 mM HEPES, pH 7.4) for 4 h (**b**), and then treated with 5 mM H_2O_2 for 24 h (**c**). The chromatograms are representative results from 3 independent experiments.



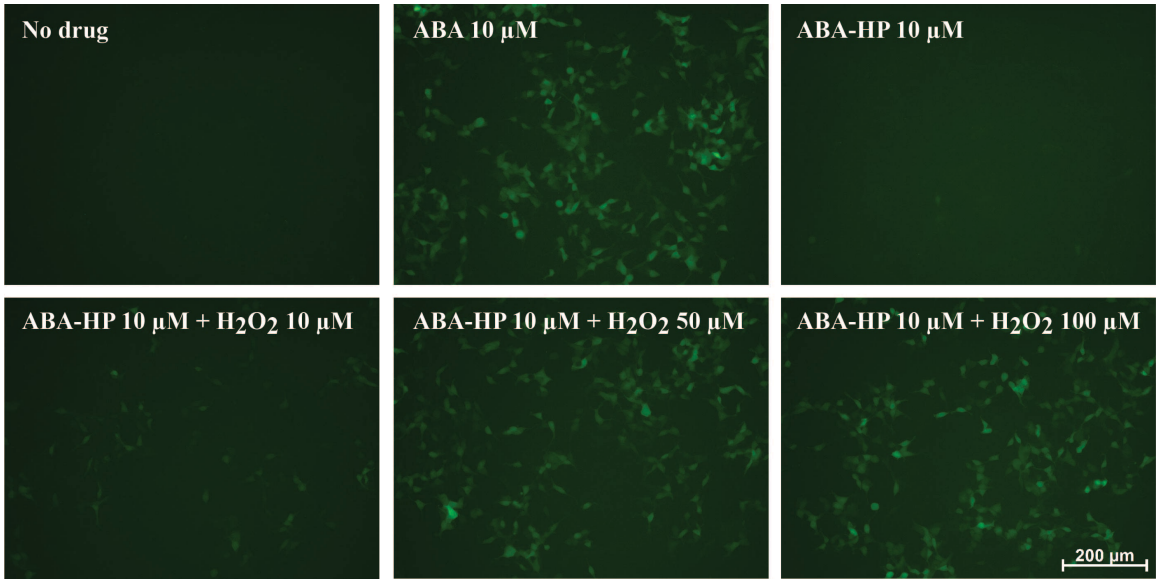
Supplementary Figure S5. Reaction selectivity of ABA-HP (100 μM) against H_2S and ROS (100 μM) in 50% HEPES/DMSO. The results were analyzed by HPLC after 4-h incubation at 37℃. H_2S was generated by Na_2S in HEPES buffer (pH 7.4). •OH and •O^{t}Bu were generated by reaction of Fe^{2+} with H_2O_2 or tert-butyl hydroperoxide (TBHP), respectively. The HPLC chromatograms shown are representative results from 3 independent experiments. The results were quantified by integrating the peak area corresponding to the ABA peak over the total areas of all peaks to give the generated ABA % as shown in **Figure 1D**.



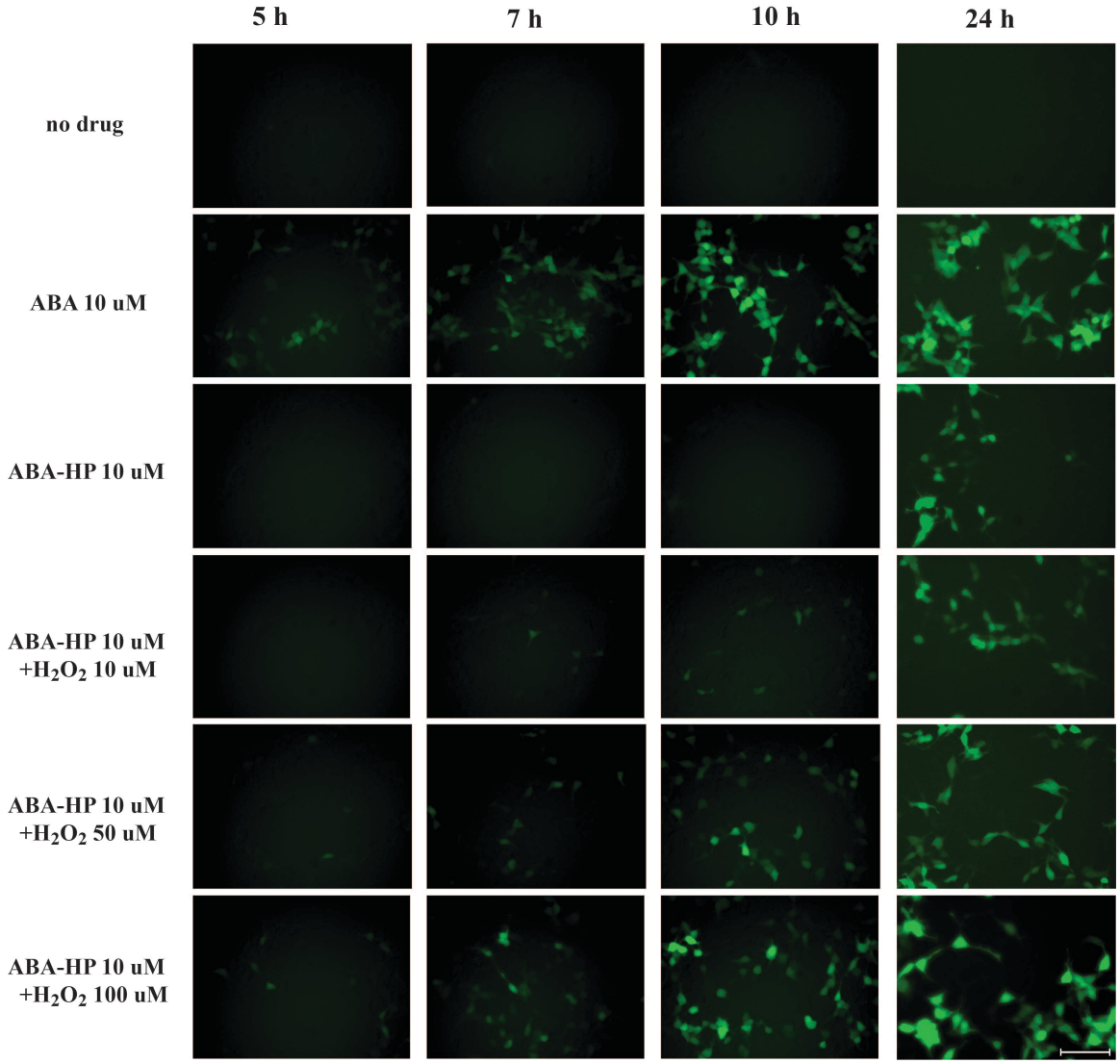
Supplementary Figure S6. Reaction selectivity of ABA-HP (100 μM) against common cellular metal ions (100 μM) in 50% HEPES/DMSO. The results were analyzed by HPLC after 4-hr incubation in 37℃. The HPLC chromatograms shown are representative results from 3 independent experiments. The results were quantified by integrating the peak area corresponding to the ABA peak over the total areas of all peaks to give the generated ABA % as shown in **Figure 1D**.



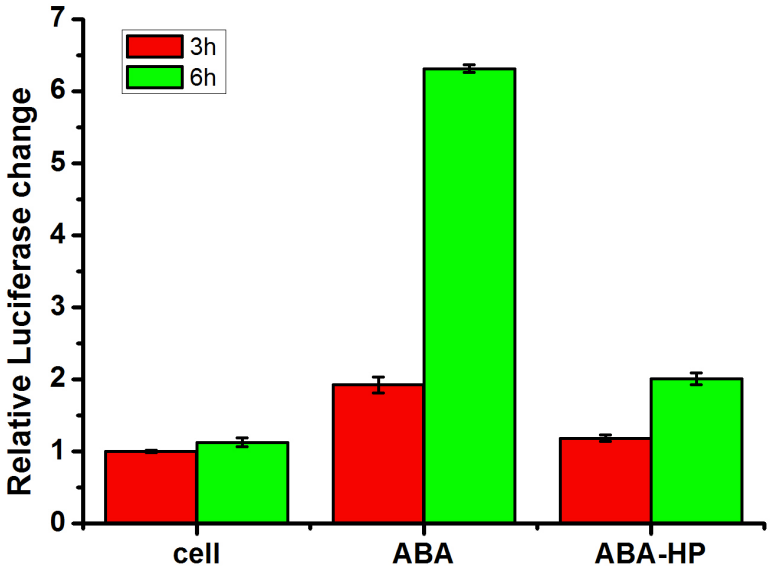
Supplementary Figure S7. Reaction selectivity of ABA-HP (100 μM) against Cu^{+} and Cu^{2+} (10 μM) in 50% HEPES/DMSO. The results were analyzed by HPLC after 4-hr incubation in 37℃. (**a**) The representative HPLC chromatograms of 3 independent experiments. (**b**) The results were quantified by integrating the peak area corresponding to the ABA peak over the total areas of all peaks to give the generated ABA % as shown.



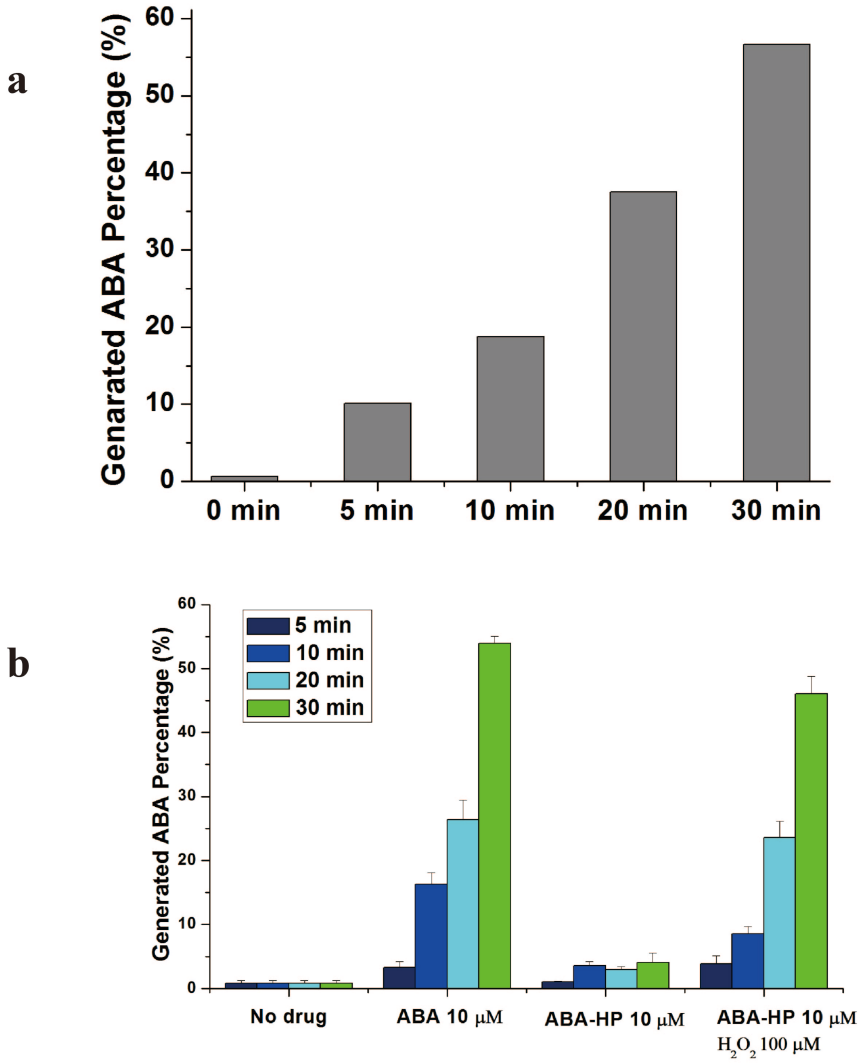
Supplementary Figure S8. EGFP expression in HEK293T EGFP reporter cells with ABA, ABA-HP or ABA-HP pre-cleaved by H_2O_2. Cells were treated with indicated molecules and the EGFP expression was observed under a fluorescence microscope after 7 h. Images shown are therepresentative results from 3 independent experiments.



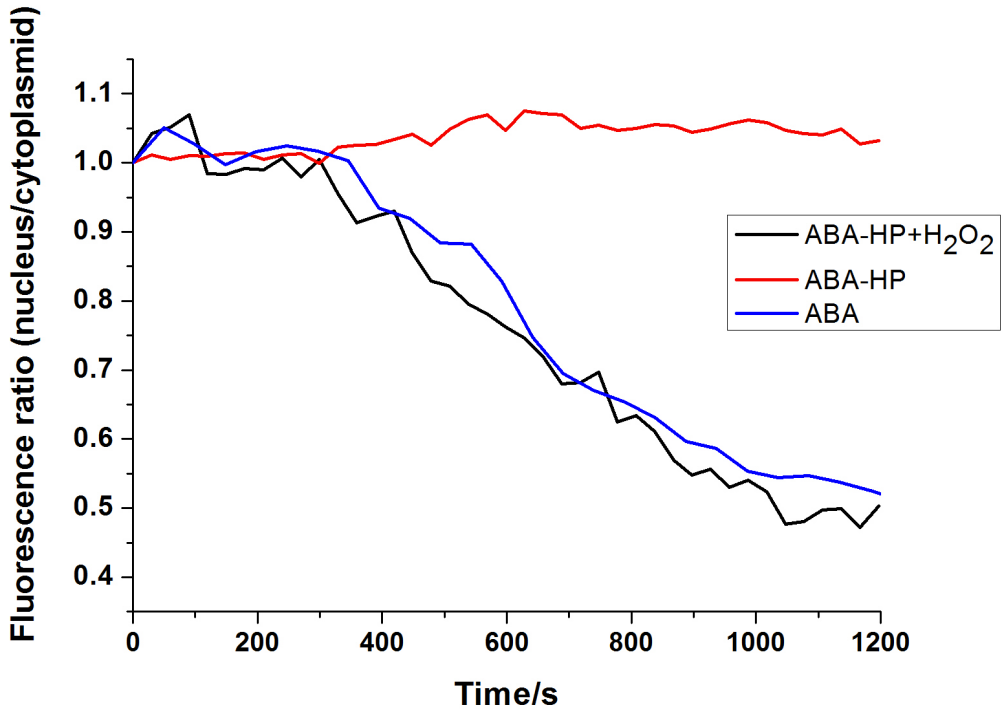
Supplementary Figure S9. Dose and time dependent EGFP expression controlled by H_2O_2 in HEK293T EGFP reporter cells. Cells were treated with indicated molecules and the EGFP expression was observed under a fluorescence microscope after indicated times. The scale bar is 100 μm. Images shown are the representative results from 3 independent experiments.



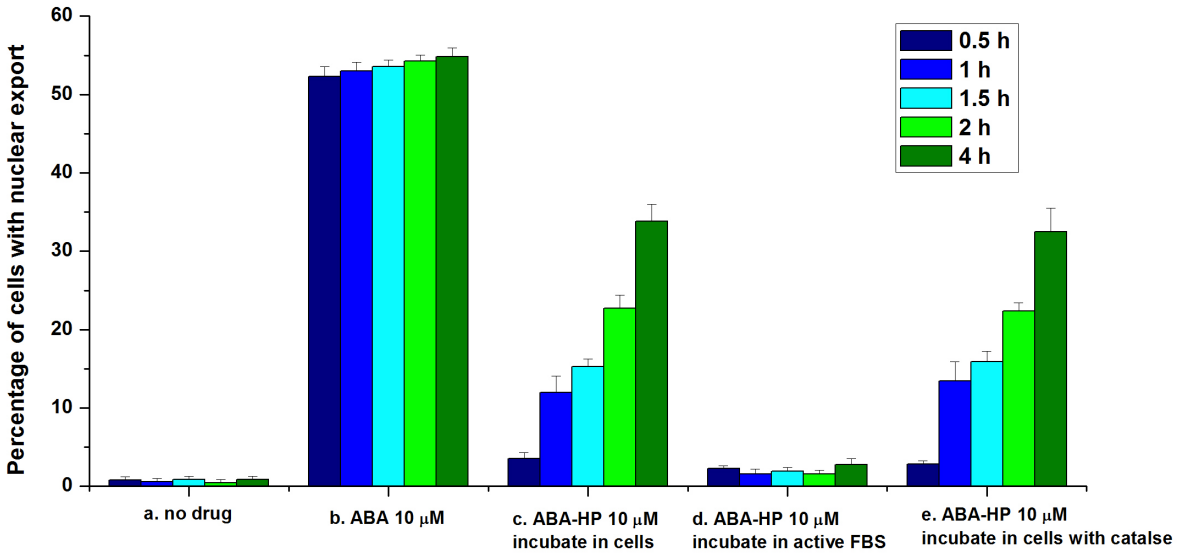
Supplementary Figure S10. Stability assay of ABA-HP in CHO cells analyzed by the luciferase assay. CHO cells were transfected with ABA-inducible luciferase reporter constructs. Drugs were added for 3 or 6 h and then the cells were lysed for luciferase assays. Error bars are SD (N = 4).



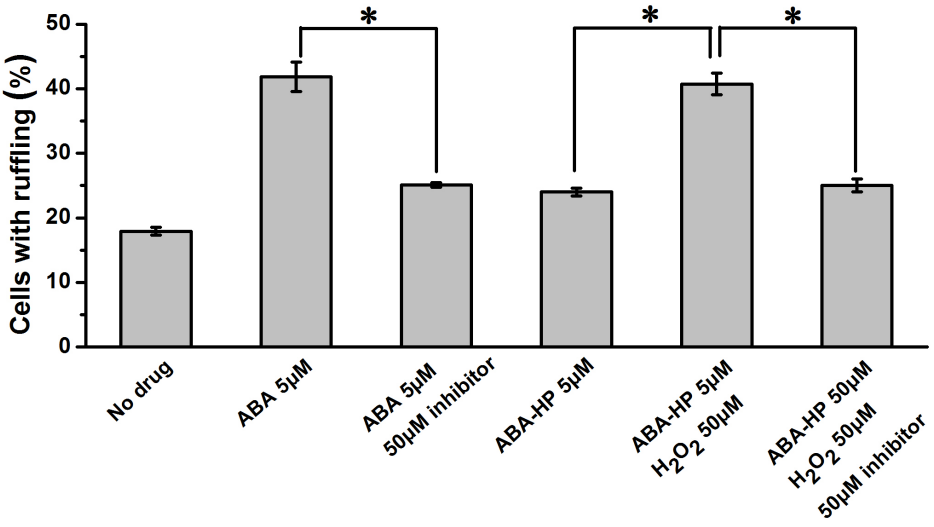
Supplementary Figure S11. Time dependent cleavage of ABA-HP *in vitro* and in cells. (**a**) 10 μM ABA-HP was incubated with 100 μM H_2O_2 in 50% DMSO/HEPES (10mM, pH 7.4) at 37℃ for 5, 10, 20 and 30 min, and then analyzed using HPLC to give the generation of ABA percentage. The results were quantified by integrating the peak area corresponding to the ABA peak over the total areas of all peaks to give the generated ABA %. (**b**) CHO cells were transfected with NES-ABI & PYL-EGFP plasmids for 24 h. Cells were then treated with no drug, 10 μM ABA, 10 μM ABA-HP or 10 μM ABA-HP plus 100 μM H_2O_2 for 30 min. Cells were counted to obtain the percentage of the cells showing unclear export over the total cell number over 700 cells were counted for each sample). The shown results were from 3 independent experiments. Error bars are SD (N = 5).



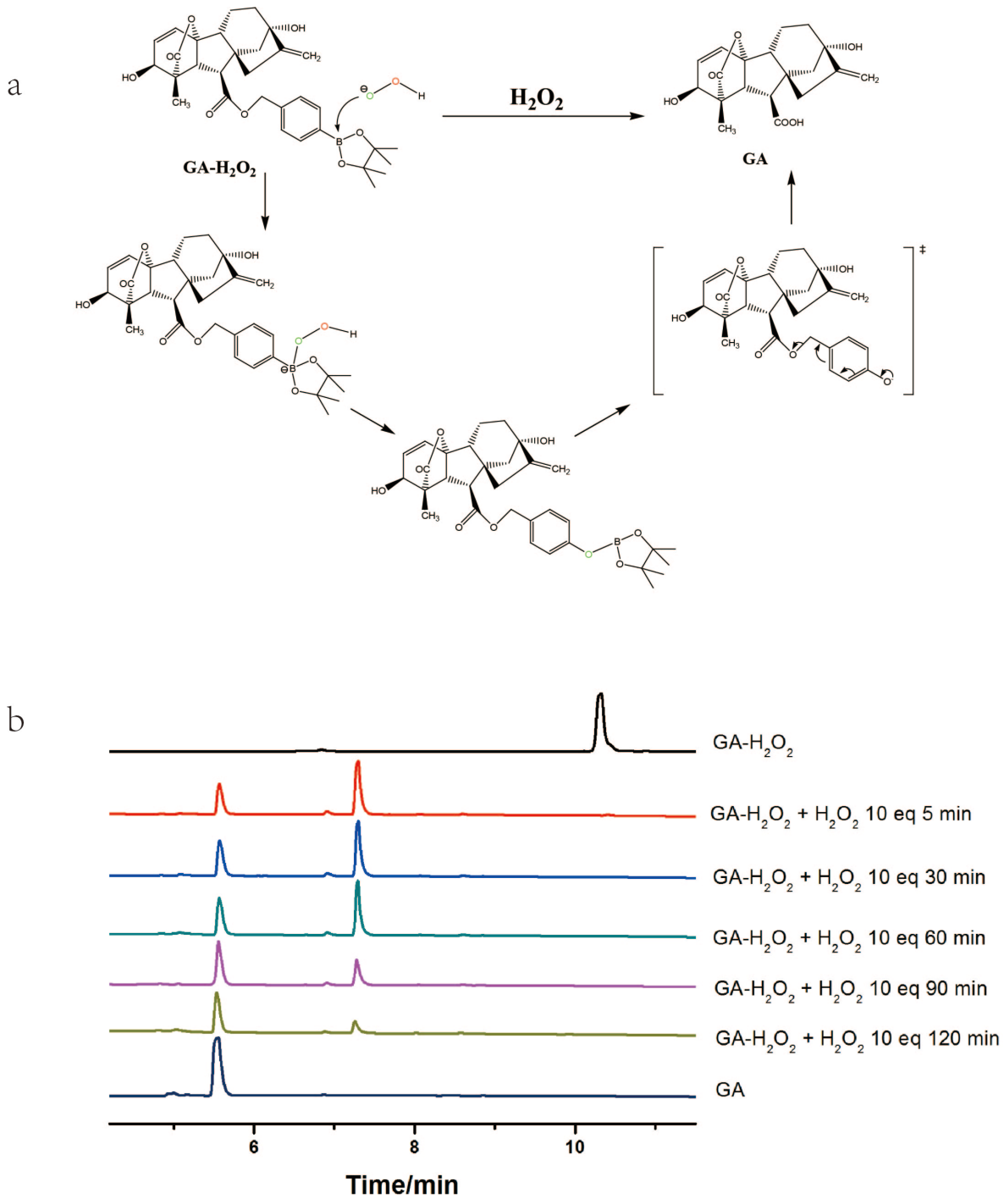
Supplementary Figure S12. Live cell analysis of EGFP nuclear export in CHO cells. Cells were transfected with NES-ABI & PYL-EGFP plasmids for 24 h and then were treated with 10 μM ABA, 10 μM ABA-HP, or 10 μM ABA-HP plus 100 μM H_2O_2 and the chosen cells were followed under a confocal microscope in real time. The fluorescence intensity was quantified using Slide Book v.6 software as described in methods. The results were the average of fluorescence intensity ratio of nuclear/cytoplasm from 3 cells in 3 independent experiments.



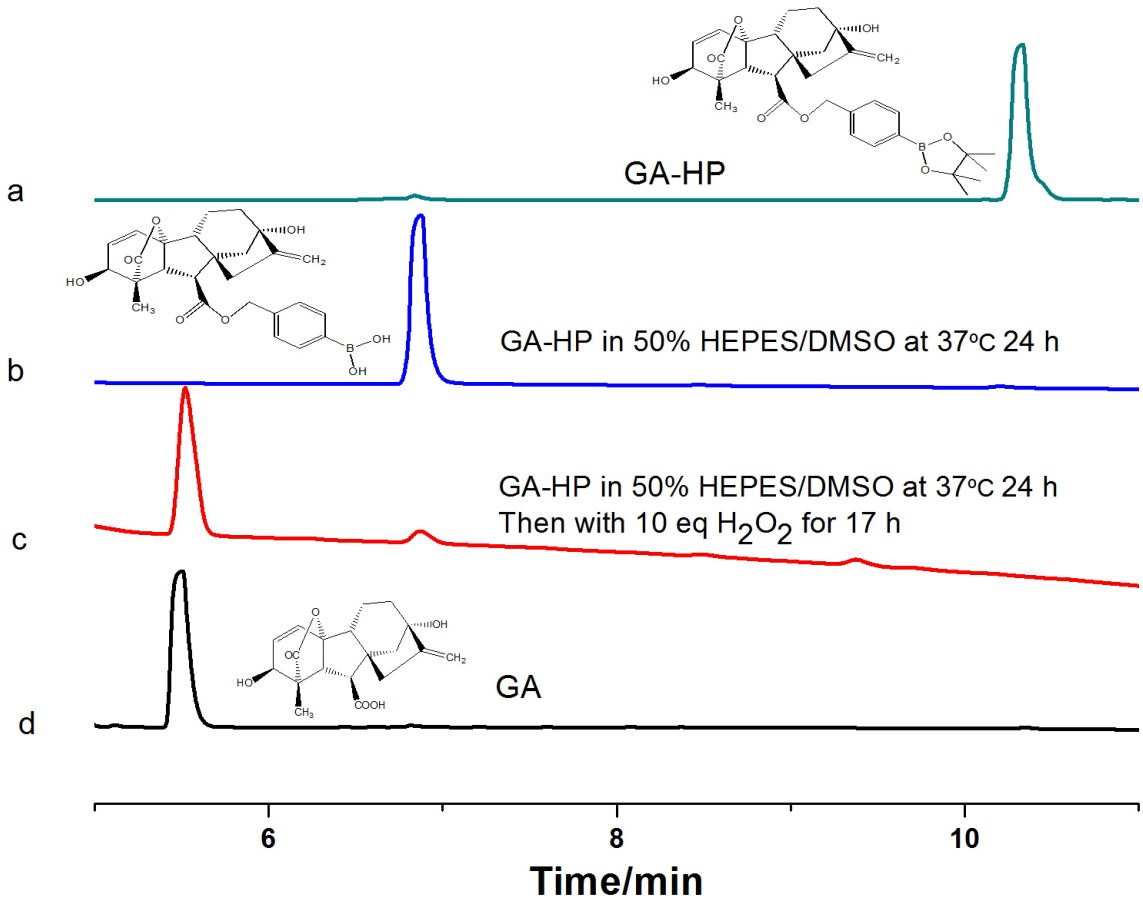
Supplementary Figure S13. Stability of ABA-HP in cells and in FBS. CHO cells were transfected with NES-ABI & PYL-EGFP plasmids for 24 h before the drugs. Cells were treated with **(a)** no drug; (**b**) ABA for 0.5, 1, 1.5, 2 and 4 h; (**c**) ABA-HP for 0.5, 1, 1.5, 2 and 4 h; (**d**) FBS pre-incubated ABA-HP (at 37 ℃ for 0.5, 1, 1.5, 2 and 4 h) for 30 min; (**e**) 1 mg/mL of catalase for 1 h and then ABA-HP for 0.5, 1, 1.5, 2 and 4 h. The results were analyzed by counting the cells showing EGFP unclear export over the total cell number (over 700 cells were counted for each sample). The shown results were from 3 independent experiments. Error bars are SD (N = 5).



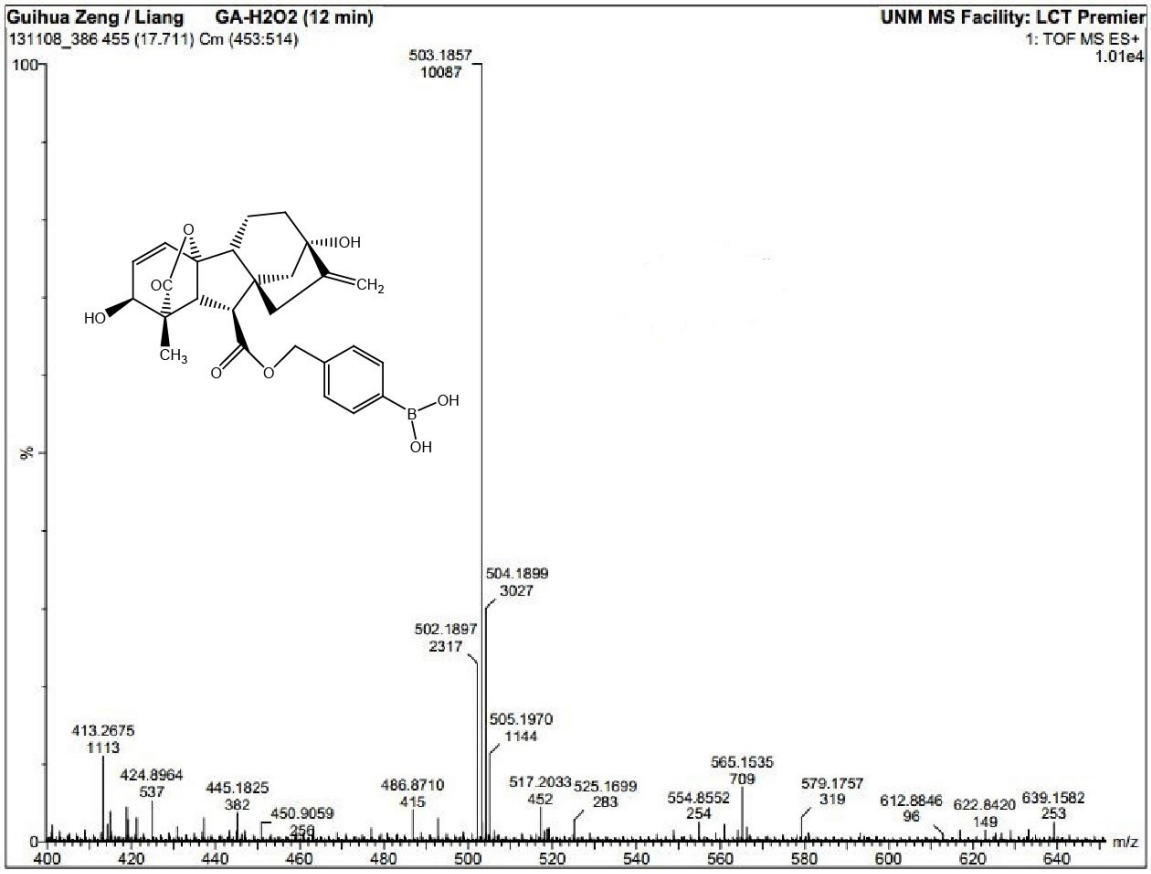
Supplementary Figure S14. Ruffle formation of CHO cells in 30 min. The statistic data were obtained by counting the number of cells to give the percentage of cells showing ruffling over the total number of EGFP positive cells. Cells were counted from 3 separate experiments with 5 different areas chosen randomly and over 700 cells were counted for each sample. Error bars are SD (N=3). *P-value < 0.001.



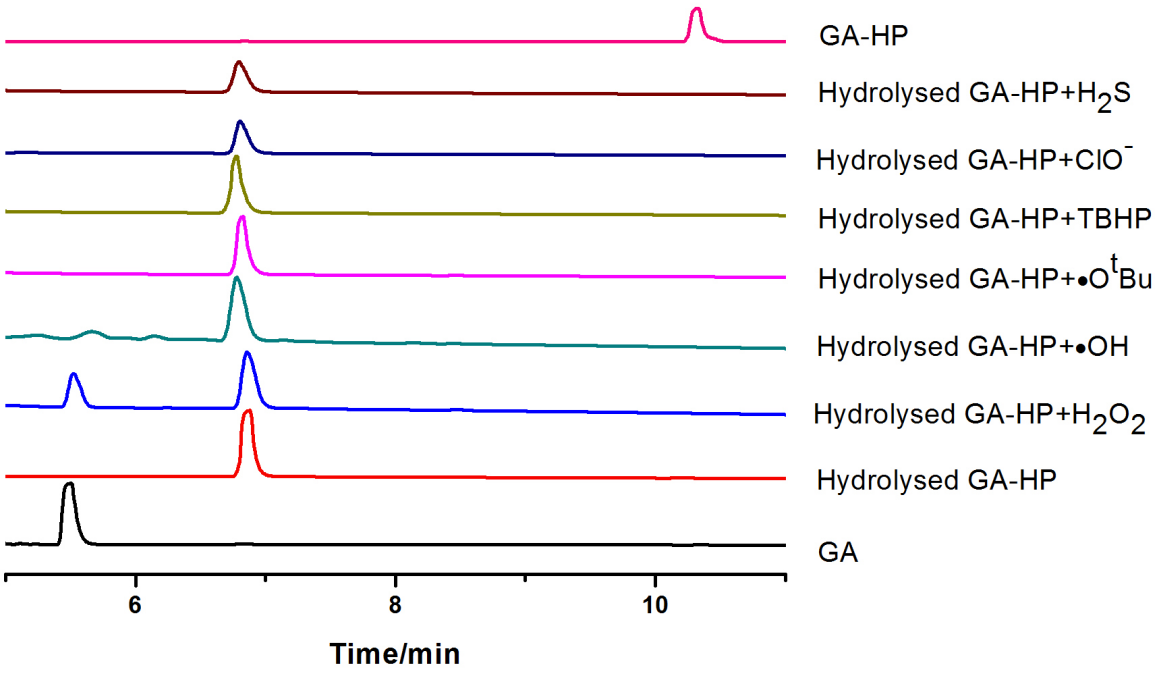
Supplementary Figure S15. Time dependent cleavage of GA-HP by H_2O_2 detected by HPLC. (**a**) A proposed mechanism of generating GA from GA-HP in the presence of H_2O_2. (**b**) 5 mM GA-HP was treated with 50 mM H_2O_2 in 50% HEPES/DMSO (10 mM HEPES, pH 7.4) from 0 to 120 min at 37℃. The HPLC chromatograms shown are representative from 3 independent experiments.



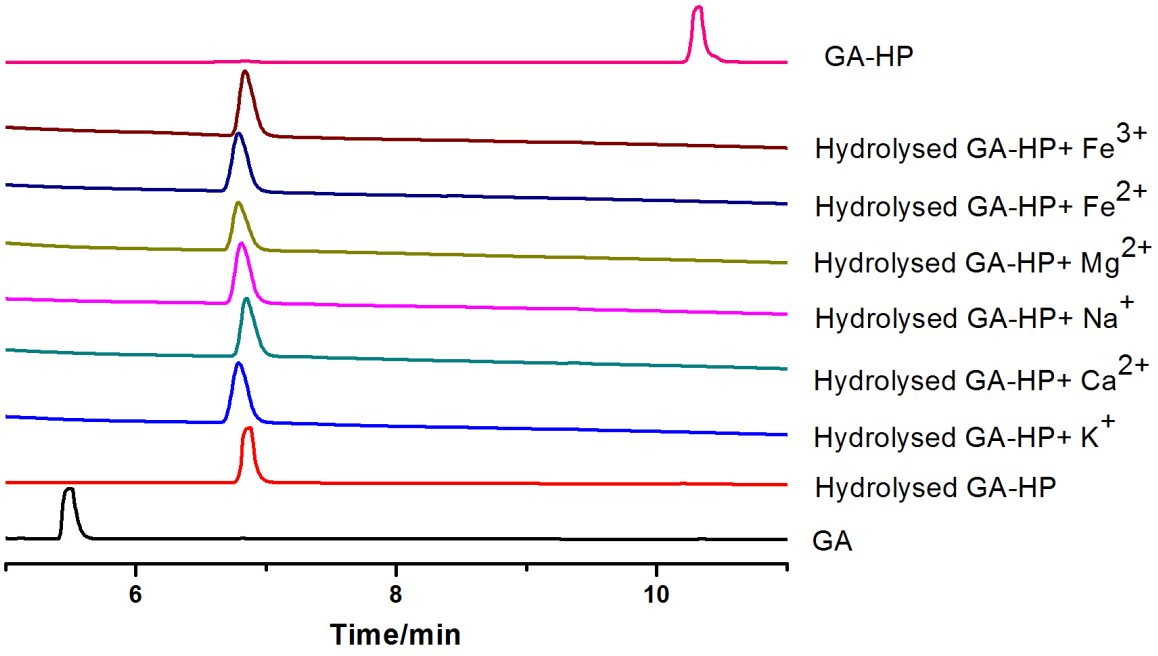
Supplementary Figure S16. Hydrolyzed GA-HP and its cleavage when treated with H_2O_2. GA-HP was incubated in 50% DMSO/HEPES at 37℃ for 24 h (**b**), and following the treatment with 10 eq H_2O_2 for 17 h (**c**). The shown HPLC chromatograms are representative results from 3 independent experiments.



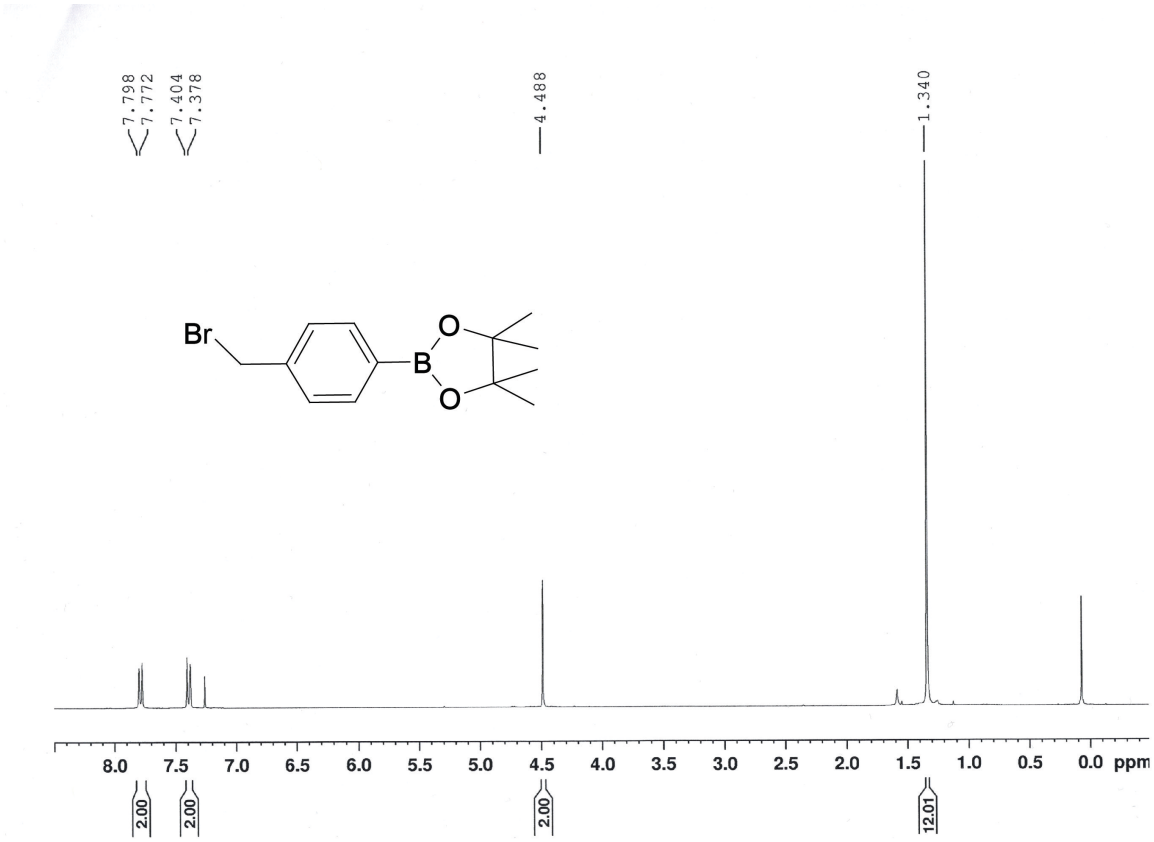
Supplementary Figure S17. MS of partial hydrolyzed GA-HP. TOF-HRMS (m/z) found (calcd.) for $C_{26}H_{29}O_8B$ (M): $[M+Na]^+$, 503.1857 (503.1853).



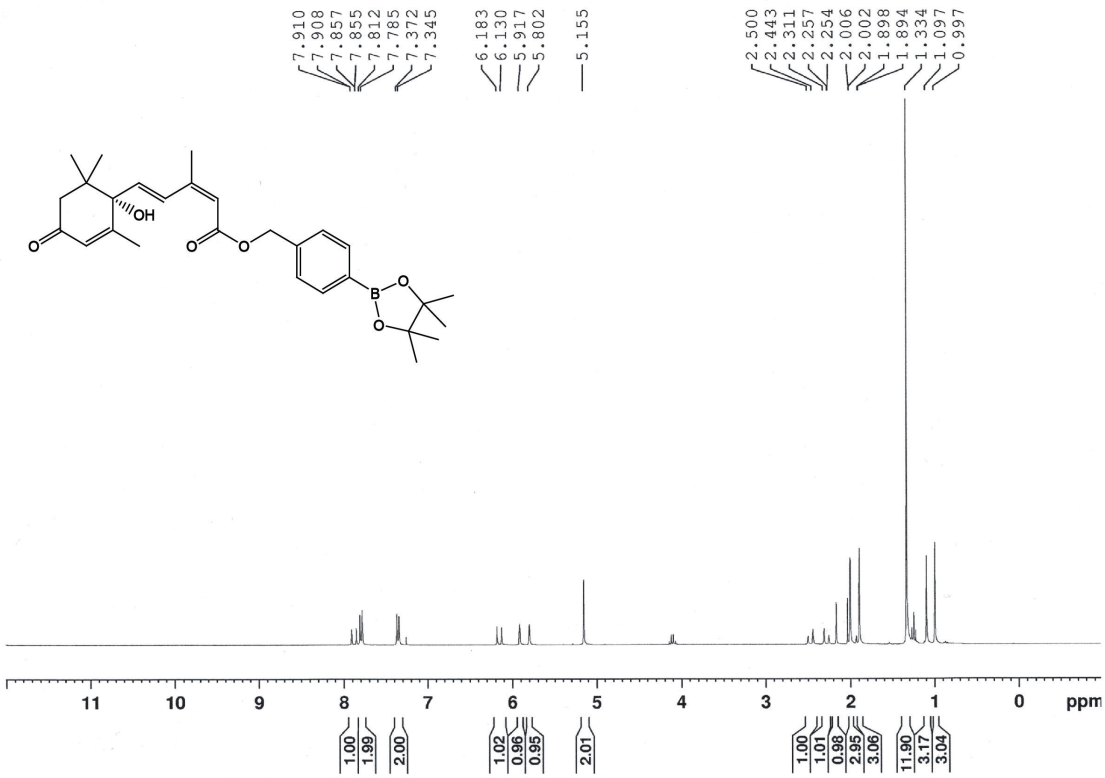
Supplementary Figure S18. Reaction selectivity of GA-HP (1 mM) against H_2S and different ROS (1 mM) in 50% DMSO/HEPES at 37℃ for 4 h. The results were analyzed by HPLC. H_2S was generated by Na_2S in HEPES buffer (pH 7.4). •OH and •O^{t}Bu were generated by reaction of Fe^{2+} with H_2O_2 or tert-butyl hydroperoxide (TBHP), respectively. The HPLC chromatograms shown are representative from 3 independent experiments.



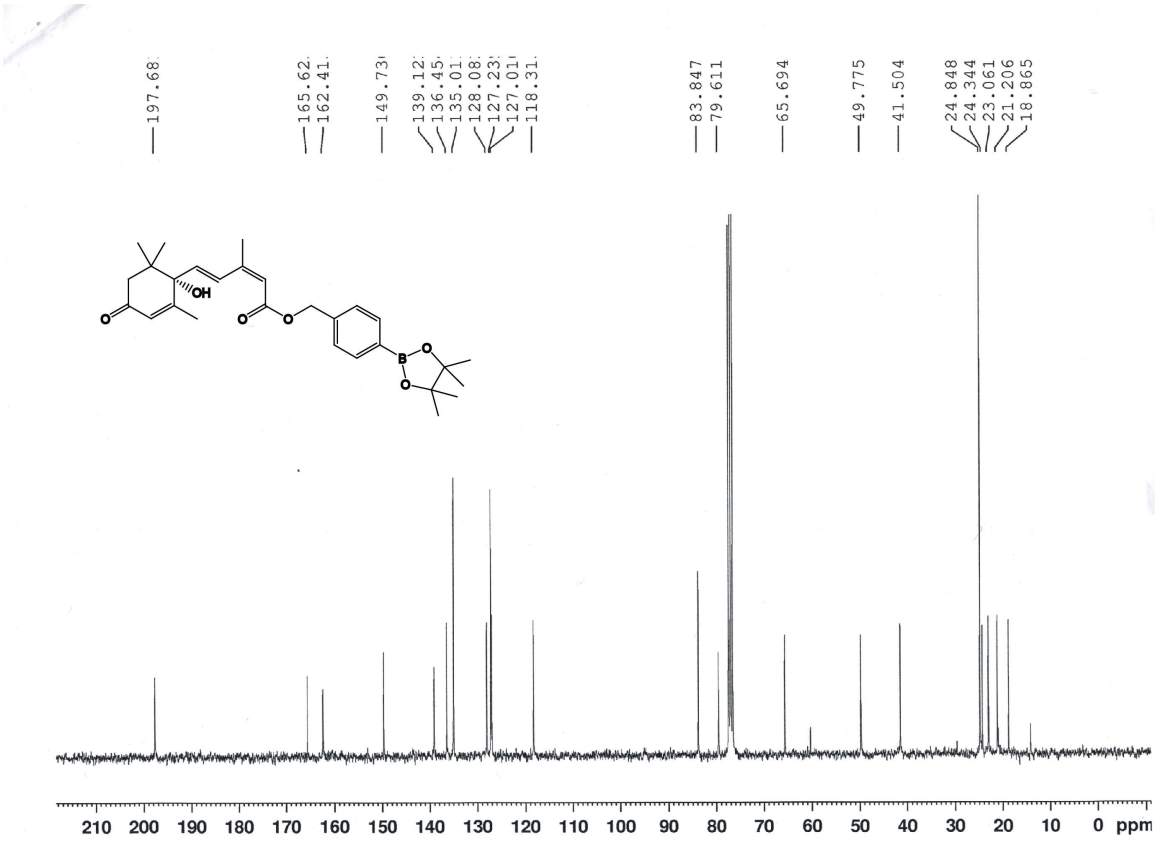
Supplementary Figure S19. Reaction selectivity of GA-HP (1 mM) against common cellular metal ions (1 mM) in 50% HEPES/DMSO. The results were analyzed by HPLC after 4-h incubation in 37℃. Shown HPLC chromatograms are representative from 3 independent experiments.



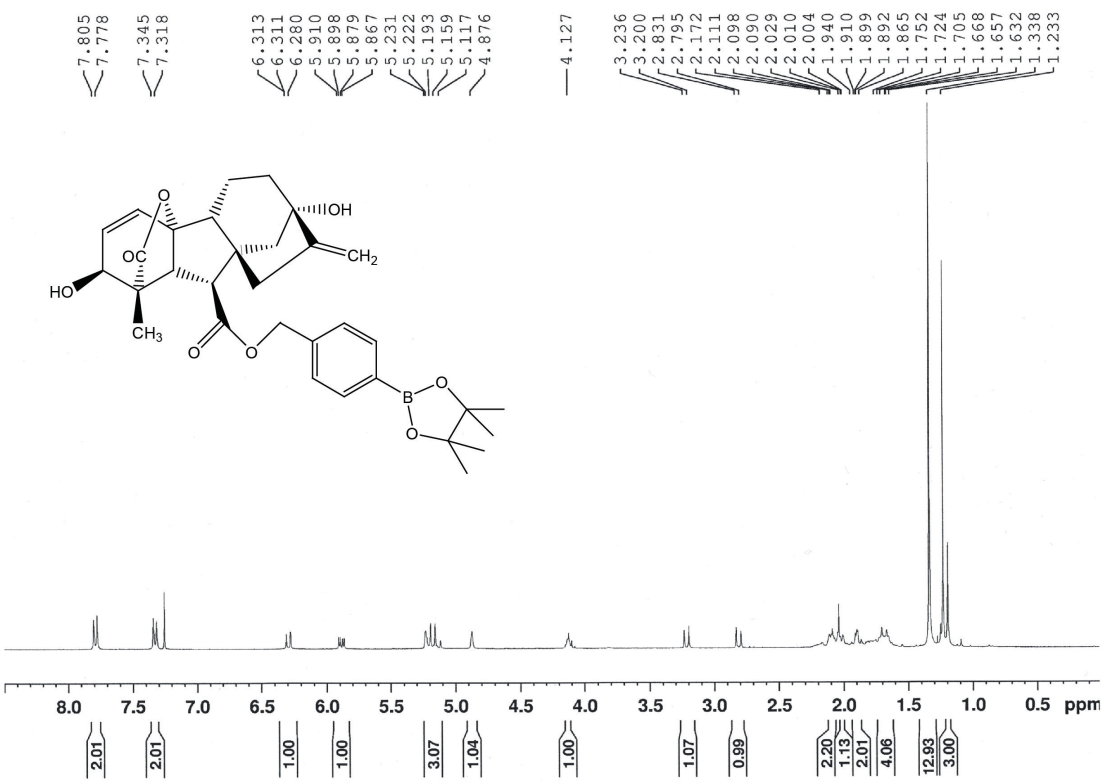
Supplementary Figure S20. ^{1}H-NMR of compound **2**.



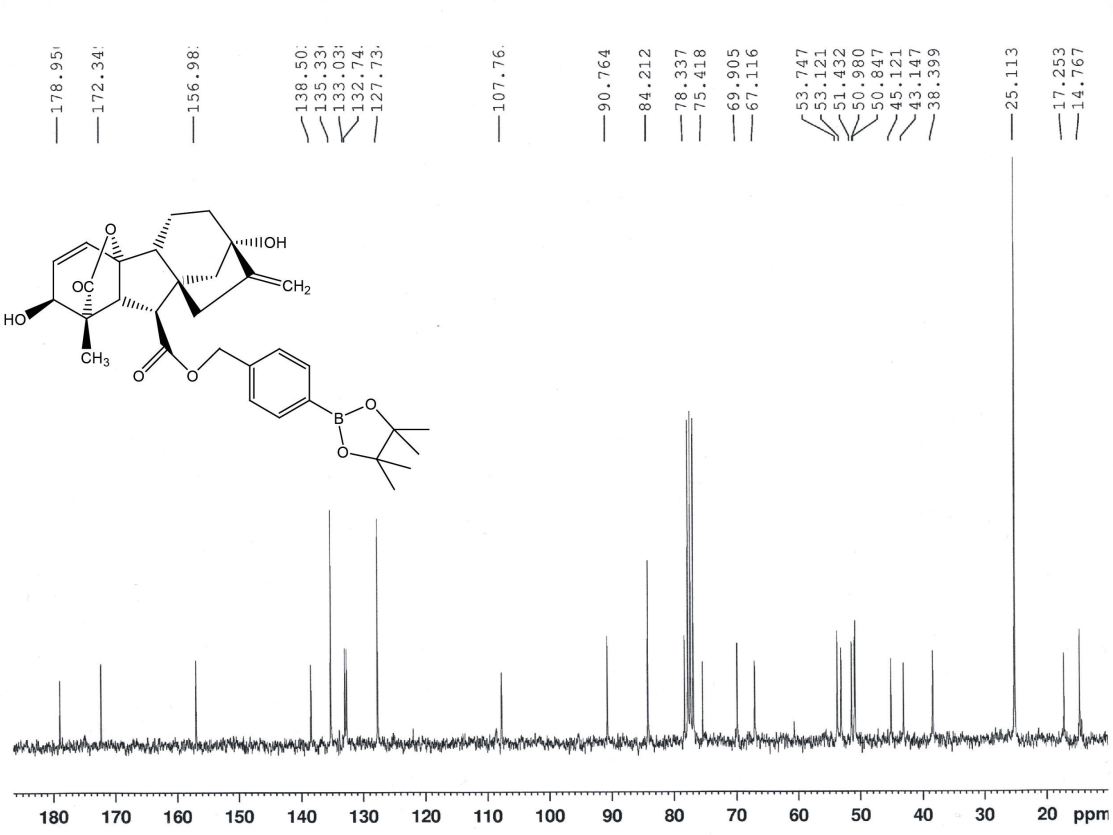
Supplementary Figure S21. ^{1}H-NMR of compound **3** (ABA-HP).



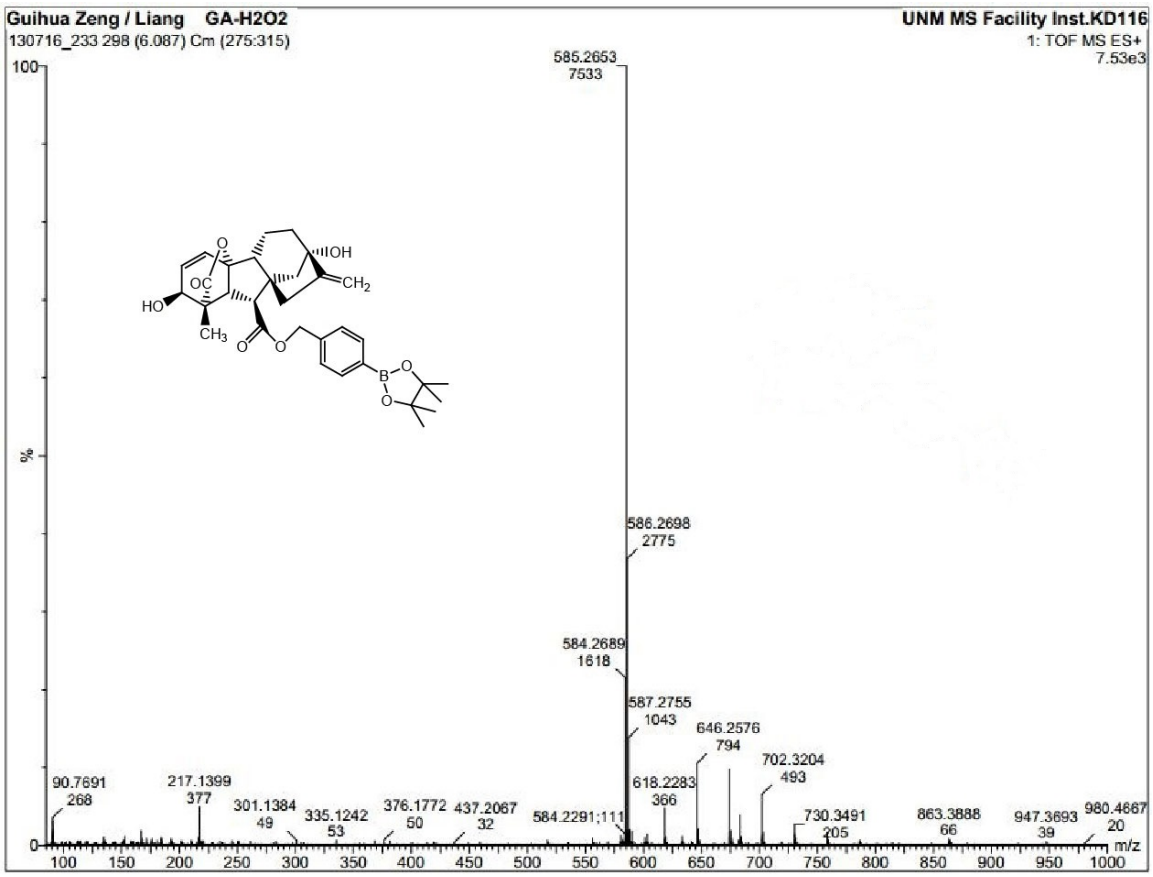
Supplementary Figure S22. ^{13}C-NMR of compound **3** (ABA-HP).



Supplementary Figure S23. ^{1}H-NMR of compound **4** (GA-HP).



Supplementary Figure S24. ^{13}C-NMR of compound **4** (GA-HP).



Supplementary Figure S25. MS of GA-HP.