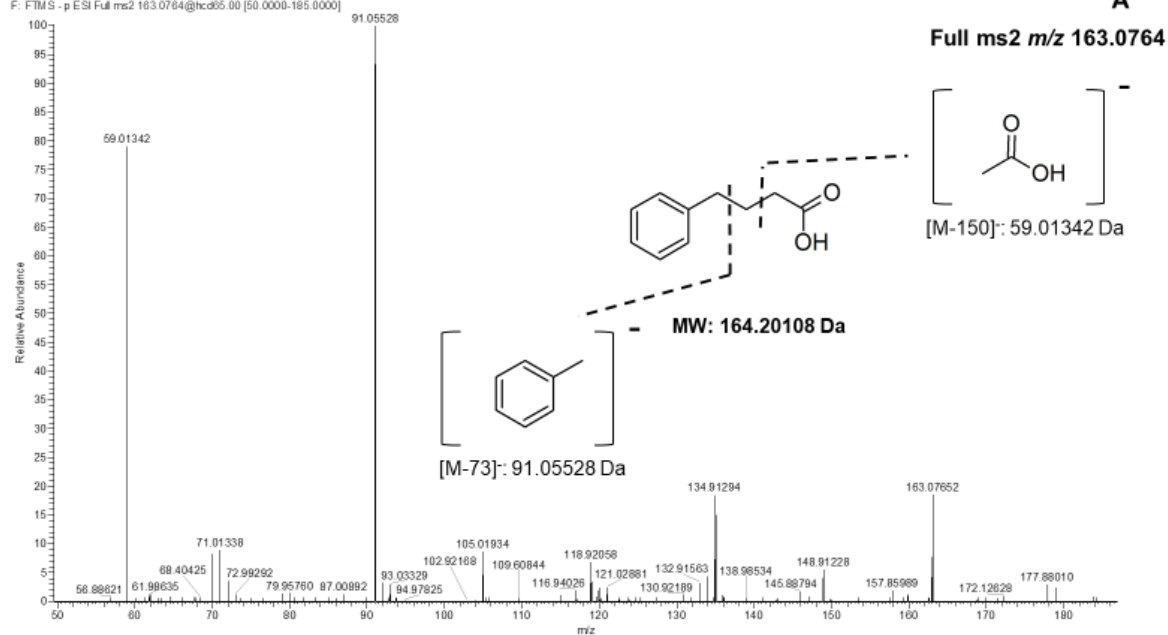


## Supplementary Material

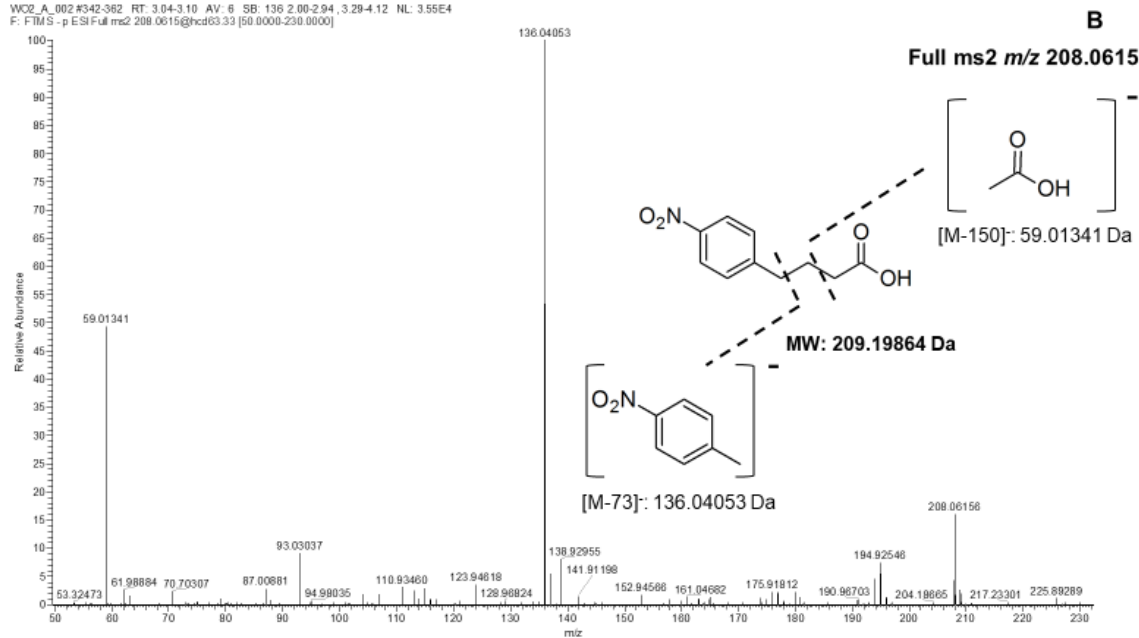
**Table S1. Instrumental LC-HRMS settings.** Representative scheme of the LC-HRMS method used to quantify 4-PBA, branched in ESI and LC conditions. The mass recording settings of SIM (Selected Ion Monitoring) and PRM (Parallel Reaction Monitoring) include the following m/z ions, in which MS1 and MS2 stand for precursor and product ions, respectively.

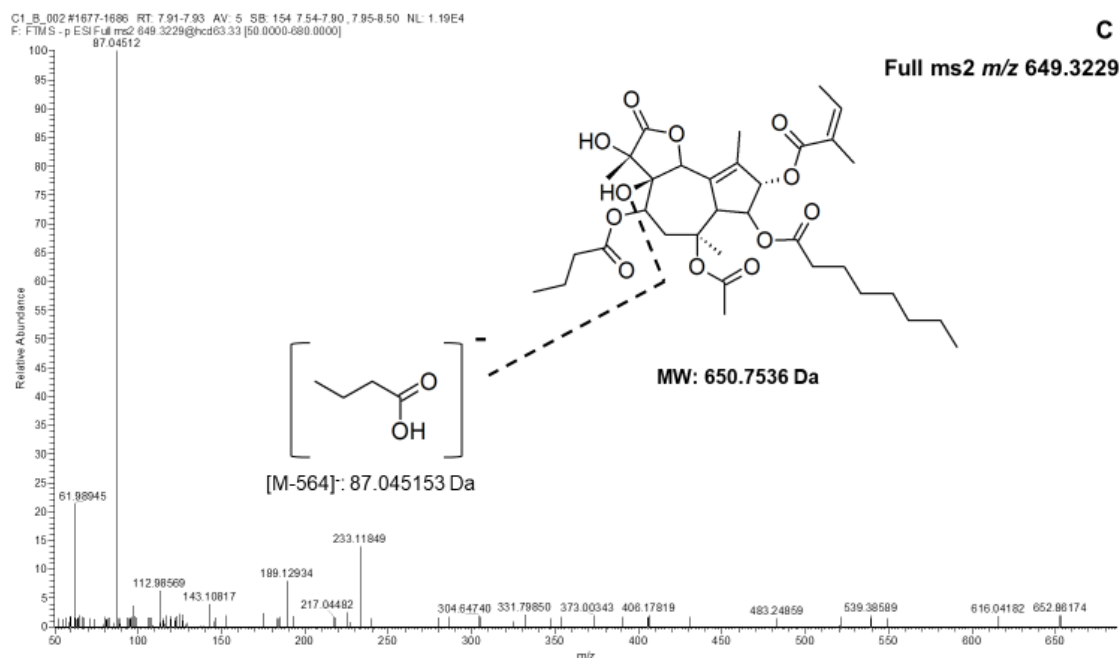
<b>Tune data – ESI source</b> <ul style="list-style-type: none"><li>• ESI- ionization</li><li>• Spray voltage: 2.80 kV</li><li>• Capillary temp.: 300 °C</li><li>• Sheath gas flow (N<sub>2</sub>): 45.00 L/min</li><li>• Sweep gas flow (N<sub>2</sub>): 0.00 L/min</li><li>• Aux. gas flow (N<sub>2</sub>): 10.00 L/min</li><li>• Aux gas temp.: 300 °C</li><li>• Source Current: 1.80 µA</li></ul>			<b>LC conditions</b> <ul style="list-style-type: none"><li>• Column: Synergi 4 µm Polar-RP 80 Å, LC 150 x 2 mm</li><li>• Column Temp.: 40 °C</li><li>• Mobile phases (UHPLC grade): A – H<sub>2</sub>O 0.1% FA, B – MeOH 0.1% FA</li><li>• Flow rate: 0.300 mL/min</li><li>• Gradient elution: A-B (40:60, v/v) for 5.0 min, A-B (0:100, v/v) in 0.1 min, A-B (0:100, v/v) from 5.1 to 8.0 min, A-B (40:60, v/v) from 8.1 to 10.5 min</li><li>• Total run time: 10.5 min</li><li>• Inj. volume: 5 µL</li></ul>		
<ul style="list-style-type: none"><li>• <b>Instrument:</b> Q-Exactive Plus UHMR Hybrid Quadrupole Orbitrap™ Mass Spectrometer equipped with a Vanquish™ Duo UHPLC system (Waltham, MA, USA)</li></ul>					
<b>Analyte</b>	<b>MW [g/mol]</b>	<b>Ion mode</b>	<b>MS1 [m/z]</b>	<b>MS2 [m/z]</b>	<b>Collision Energy [eV]</b>
4-PBA	164.20108	ESI-	163.07645	91.05528	65
NPBA	209.19864	ESI-	208.06153	136.04053	65
TG	650.75360	ESI-	649.32295	87.04515	65

24\_2\_001 #310-351 RT: 2.92-3.05 AV: 11 SB: 174 2.00-2.86, 3.52-5.06 NL: 9.90E3  
F: FTM S - p ESI Full ms2 163.0764@hcd05.00 [50.0000-185.0000]



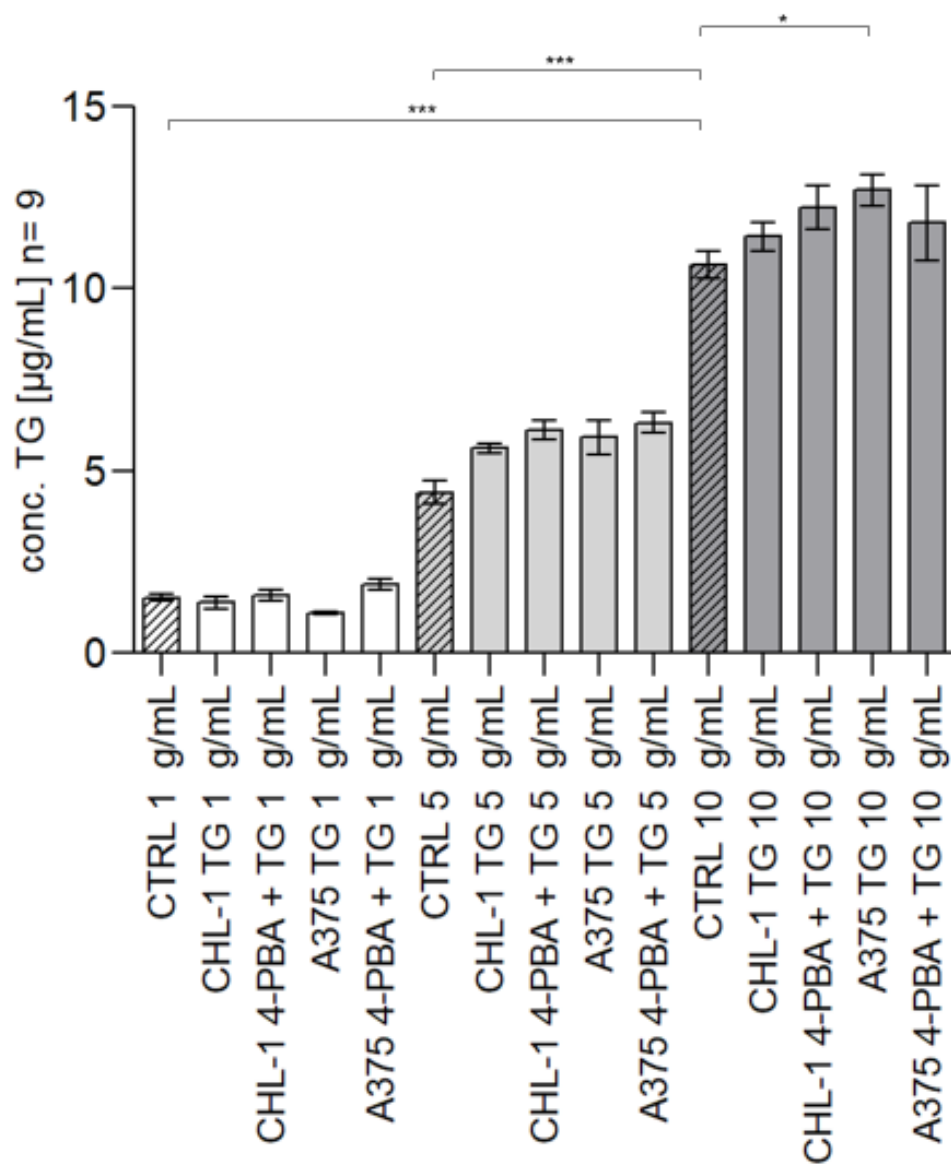
WC2\_A\_002 #342-362 RT: 3.04-3.10 AV: 8 SB: 136 2.00-2.94, 3.29-4.12 NL: 3.55E4  
F: FTM S - p ESI Full ms2 208.0615@hcd03.33 [50.0000-230.0000]





**Figure S1. Analytes and IS mass spectra.**

**(A). MS/MS spectra of 4-PBA precursor ion.** (A) Precursor ion fragmentation of 4-PBA showed product ion peaks with masses of [M-73]<sup>-</sup> and [M-150]<sup>-</sup>. (B). MS/MS spectra of NPBA precursor ion. (B) Interestingly, precursor ion of NPBA displayed a fragmentation related to 4-PBA, since there are still product ion peaks with masses of [M-73]<sup>-</sup> and [M-150]<sup>-</sup>. (C). MS/MS spectra of TG precursor ion. (C) Precursor ion fragmentation of thapsigargin (TG) figured an intense product ion peak with a mass of [M-564]<sup>-</sup>.

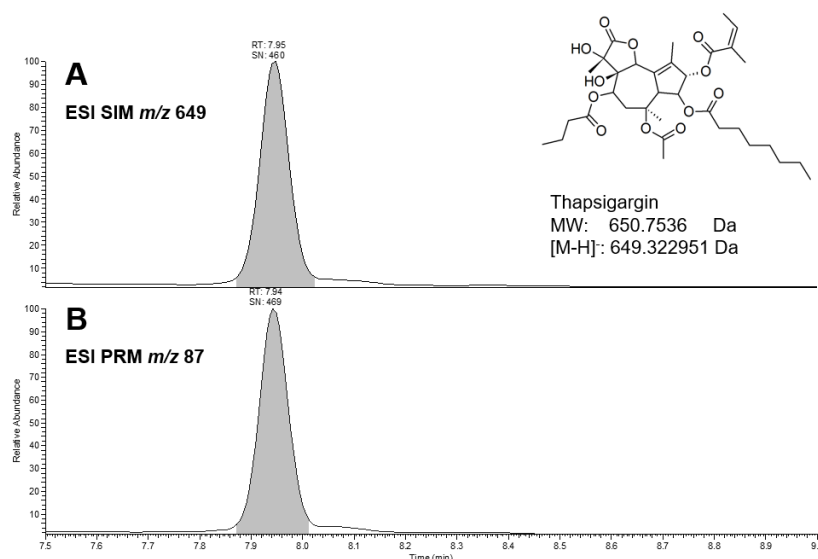


**Figure S2.** Monitoring of operative TG concentration by semi-quantitative LC-HRMS.

Concentrations of TG were evaluated in CHL-1 and A375 conditioned cell culture media at 24h, by LC-HRMS analysis. TG initial treatment concentrations at initial time (t0) were used as an internal control (CTRL). TG concentrations were expressed as  $\mu\text{M} \pm \text{SE}$ .

Experiments were performed in triplicate and repeated three times (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ;

\*\*\*  $p < 0.001$ ; by one- way Anova, Sidak's multiple comparison).



**Figure S3.** Chemical structure and chromatograms of thapsigargin (TG). (A) and (B) show the chromatograms recorded in SIM and PRM acquisition modes, respectively.

**Supplemental S1. Details for a general bioanalytical validation for ICH, FDA and EMA guidelines.**

It is possible to find all the recommendations for the bioanalytical validation on these links.

- [https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf)
- <https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf>
- [https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5\\_en.pdf](https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5_en.pdf)