

Mitochondria-targeting EGCG derivatives protect H9c2 cardiomyocytes from H₂O₂-induced apoptosis: Design, synthesis and biological evaluation

Revathy Sahadevan^{a†}, Anupama Binoy^{a†}, Irene Shajan^a, and Sushabhan Sadhukhan^{ab*}

^aDepartment of Chemistry, ^bPhysical & Chemical Biology Laboratory and Department of Biological Sciences & Engineering, Indian Institute of Technology Palakkad, Kerala, India

[#]Equal contribution

*Corresponding author. *E-mail address:* sushabhan@iitpkd.ac.in

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1. HPLC analysis of MitoEGCG_n compounds

Solvents used for the HPLC analysis were LC-MS grade water with 0.1% acetic acid (solvent A) and LC-MS grade acetonitrile with 0.1% acetic acid (solvent B). The MitoEGCG_n compounds were eluted at a flow rate of 0.7 mL/min. The solvent gradient used for the HPLC analysis was of 5% solvent B for 1 min, followed by a linear gradient from 5% to 60% solvent B over 4 min, continued for 1 min, followed by changing the solvent B from 60% to 95% in 3 min, continued for another 3 min, and finally, it was brought down to 5% solvent B in 1.5 min and then continued for another 1.5 min before the method stopped. Column was always washed with 50% Solvent B followed by 95% Solvent B before sample injection.

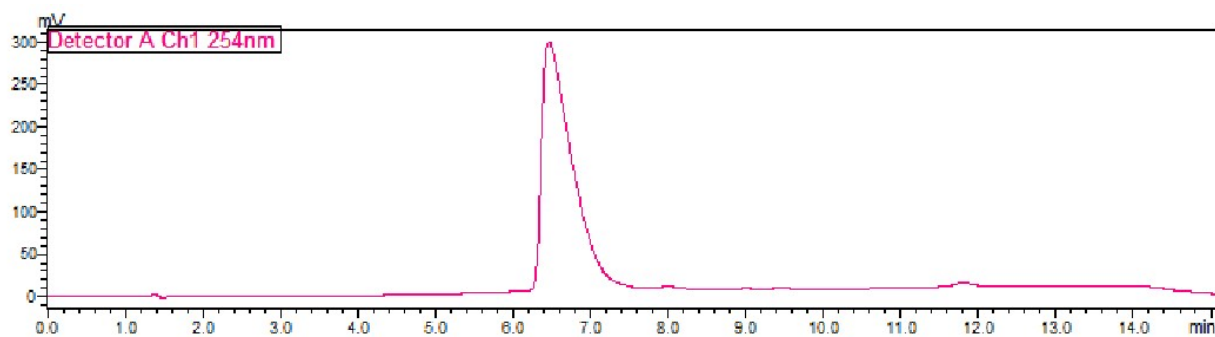


Figure S1. HPLC chromatogram of MitoEGCG₄ showing > 99.2% purity.

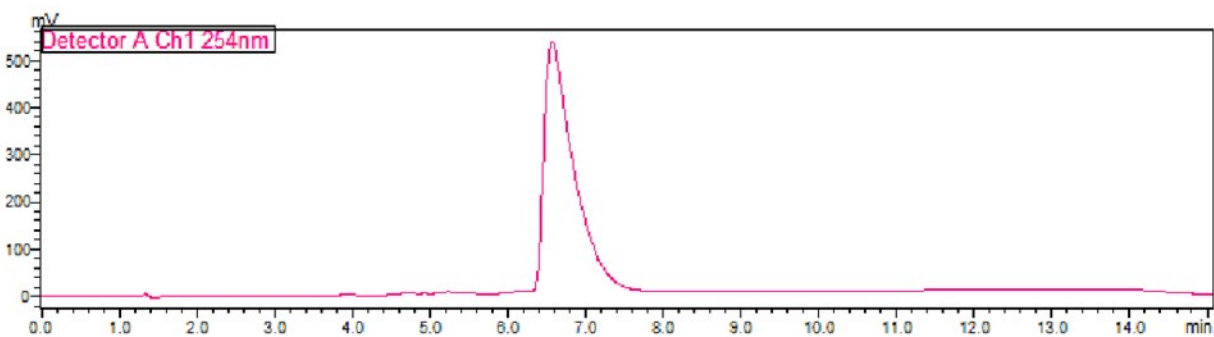


Figure S2. HPLC chromatogram of MitoEGCG₆ showing >99.4% purity.

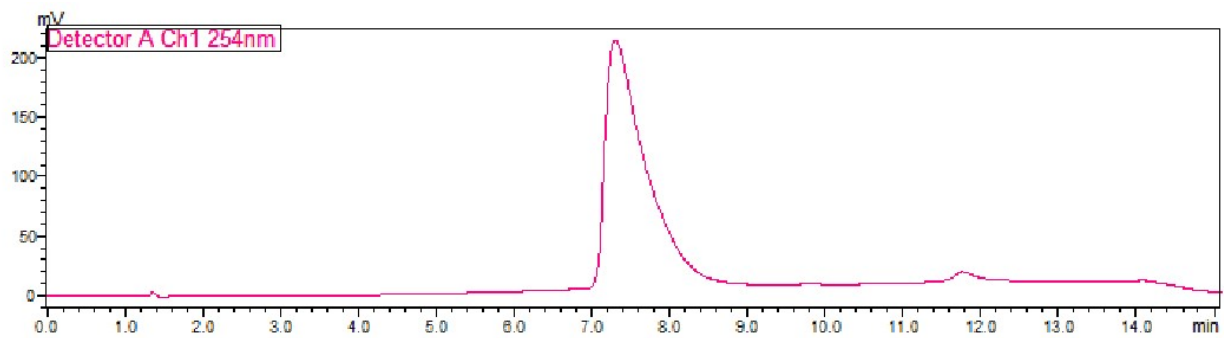


Figure S3. HPLC chromatogram of MitoEGCG₈ showing > 98.6% purity.

2. Mass spectra of MitoEGCG_n compounds

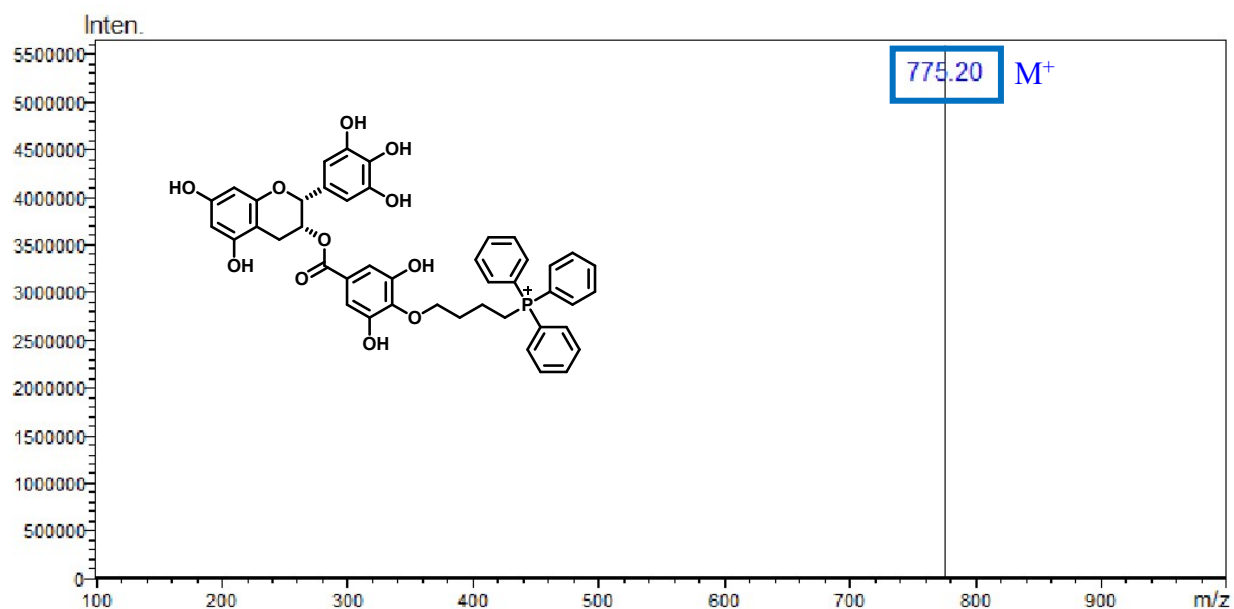


Figure S4. Mass spectrum of MitoEGCG₄ (Exact mass 775.23 Da).

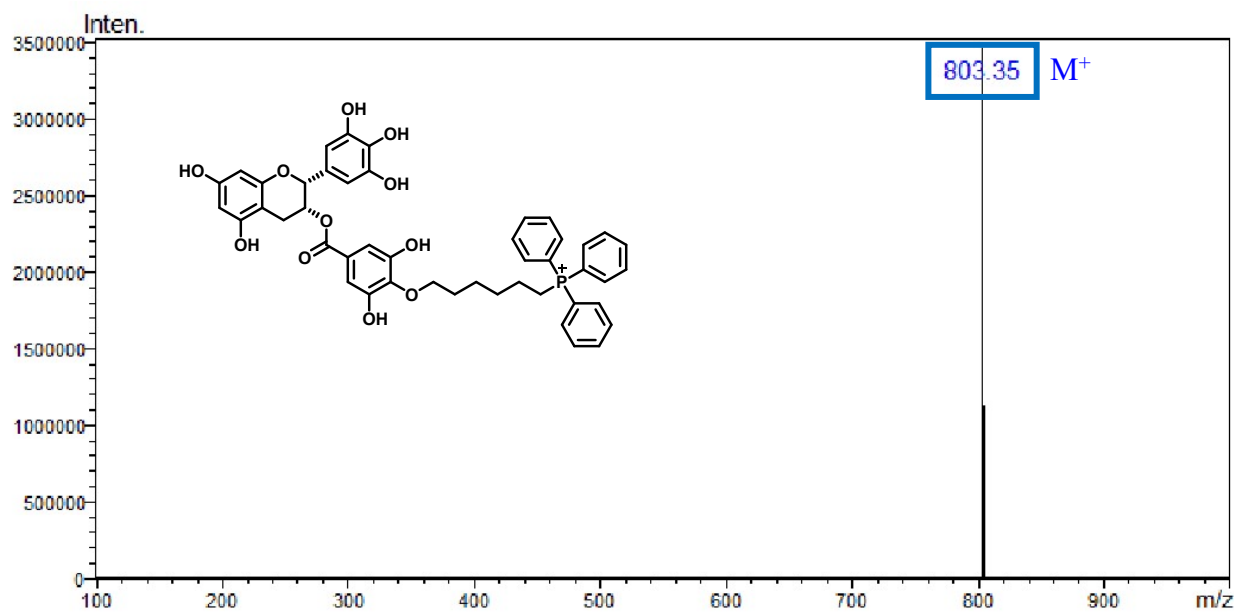


Figure S5. Mass spectrum of MitoEGCG₆ (Exact mass 803.26 Da).

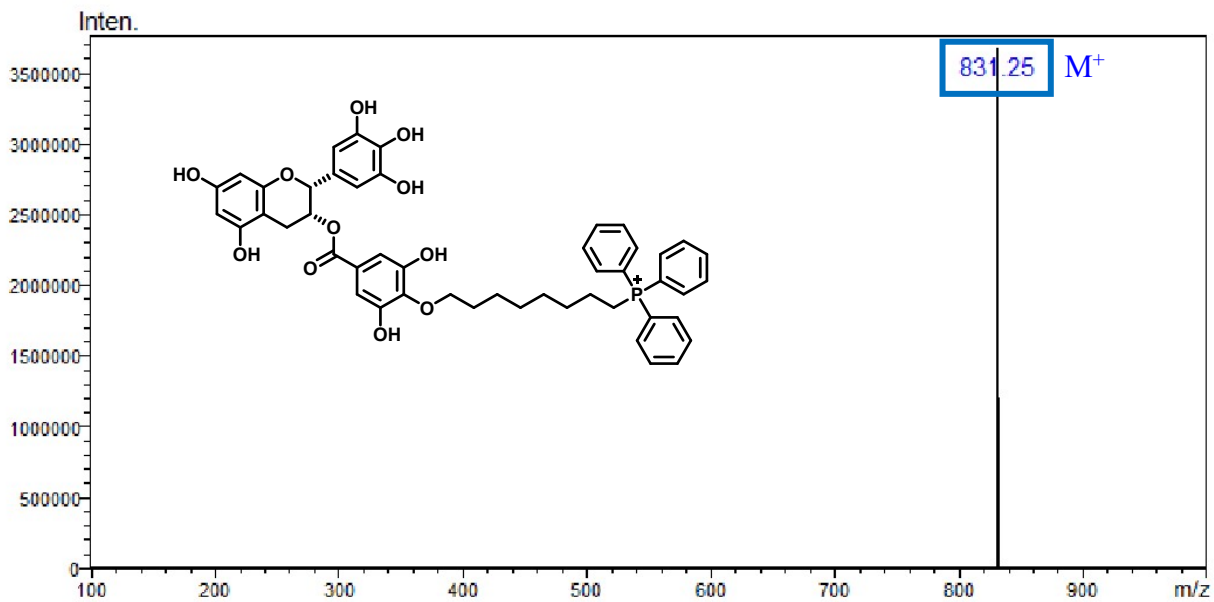


Figure S6. Mass spectrum of MitoEGCG₈ (Exact mass 831.29 Da).

3. ^1H NMR spectra of MitoEGCG_n compounds

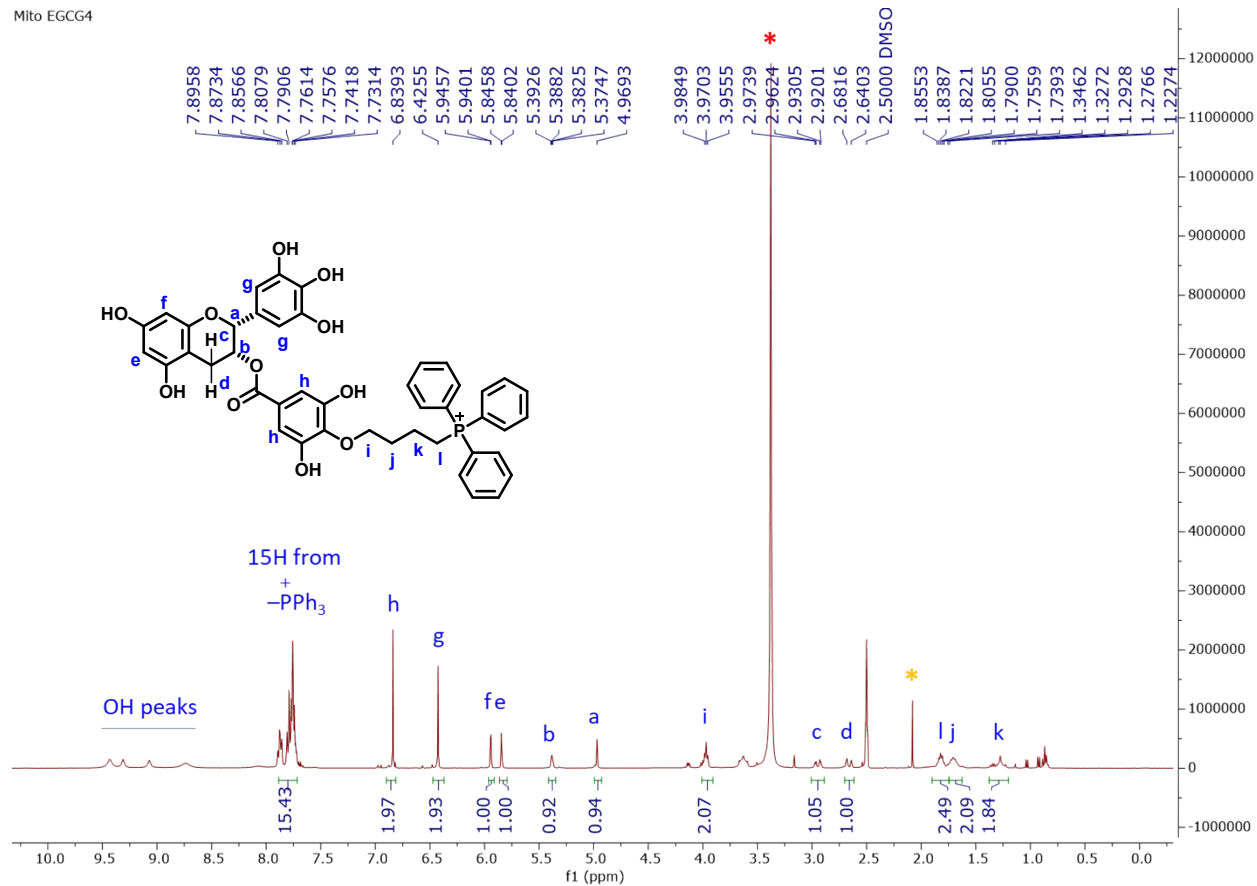


Figure S7. ^1H NMR spectrum of MitoEGCG₄. * and * denotes the residual solvent peaks for H₂O and acetone respectively.

Mito EGCG6

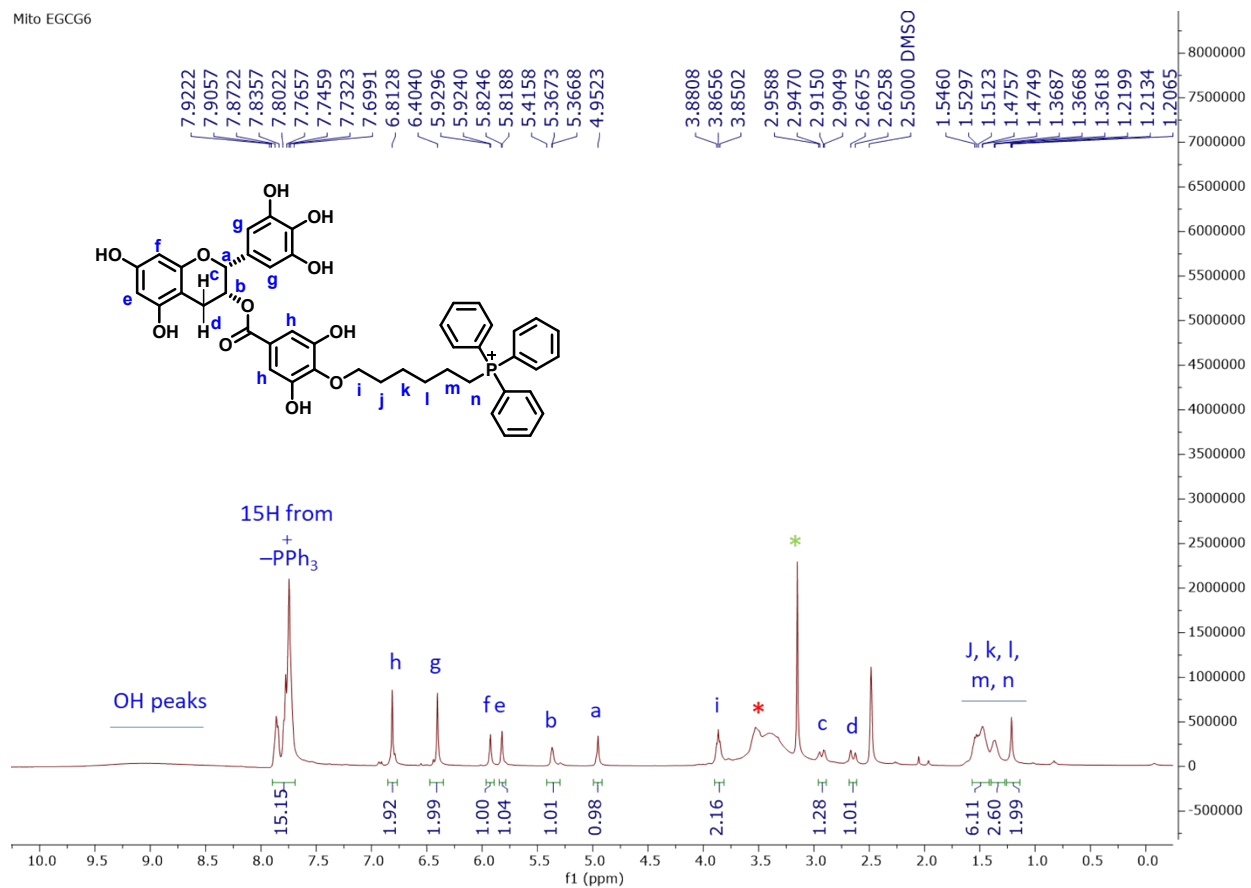


Figure S8. ^1H NMR spectrum of MitoEGCG₆. * and * denotes the residual solvent peaks for H₂O (and HDO) and methanol respectively.

Mito EGCG8

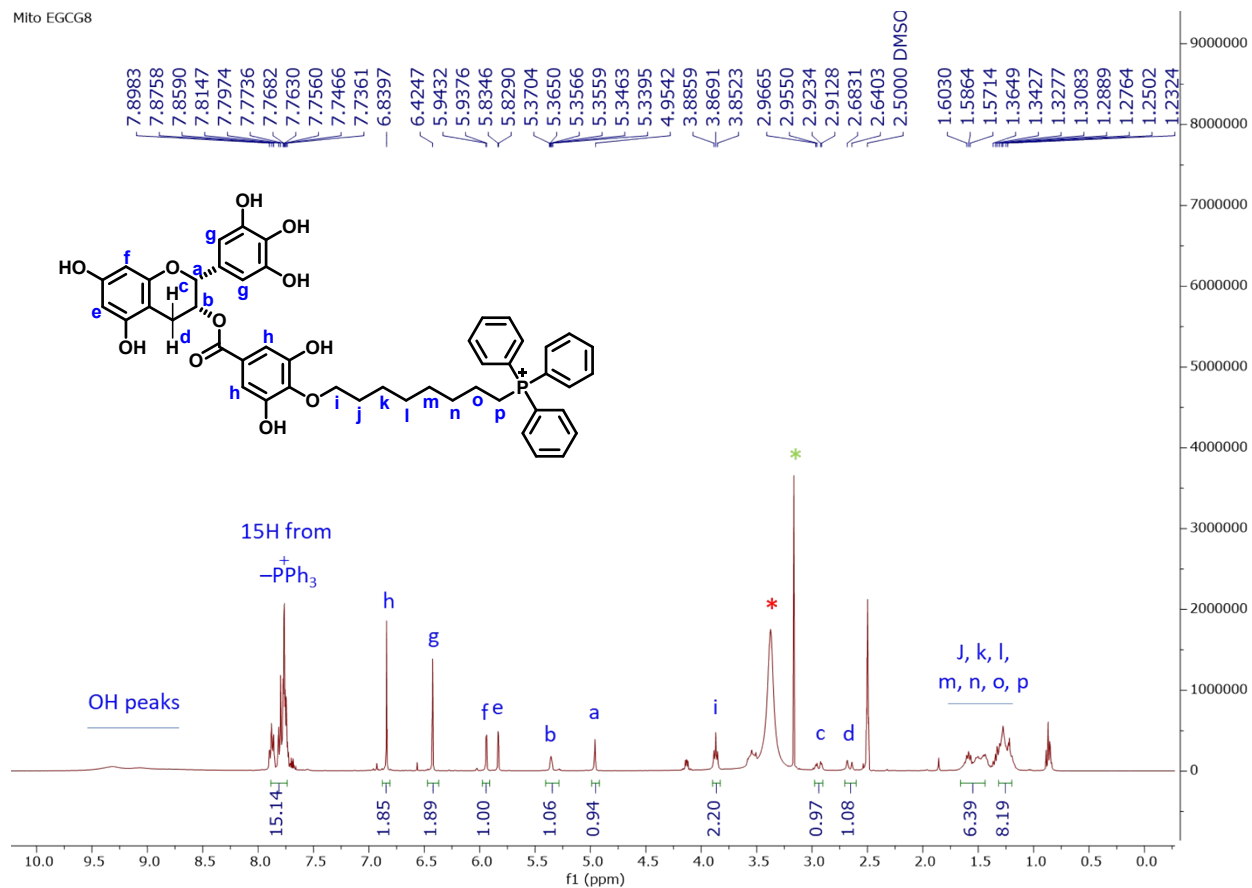


Figure S9. ¹H NMR spectrum of MitoEGCG₈. * and * denotes the residual solvent peaks for H₂O (and HDO) and methanol respectively.

4. ^{13}C NMR spectra of MitoEGCG_n compounds

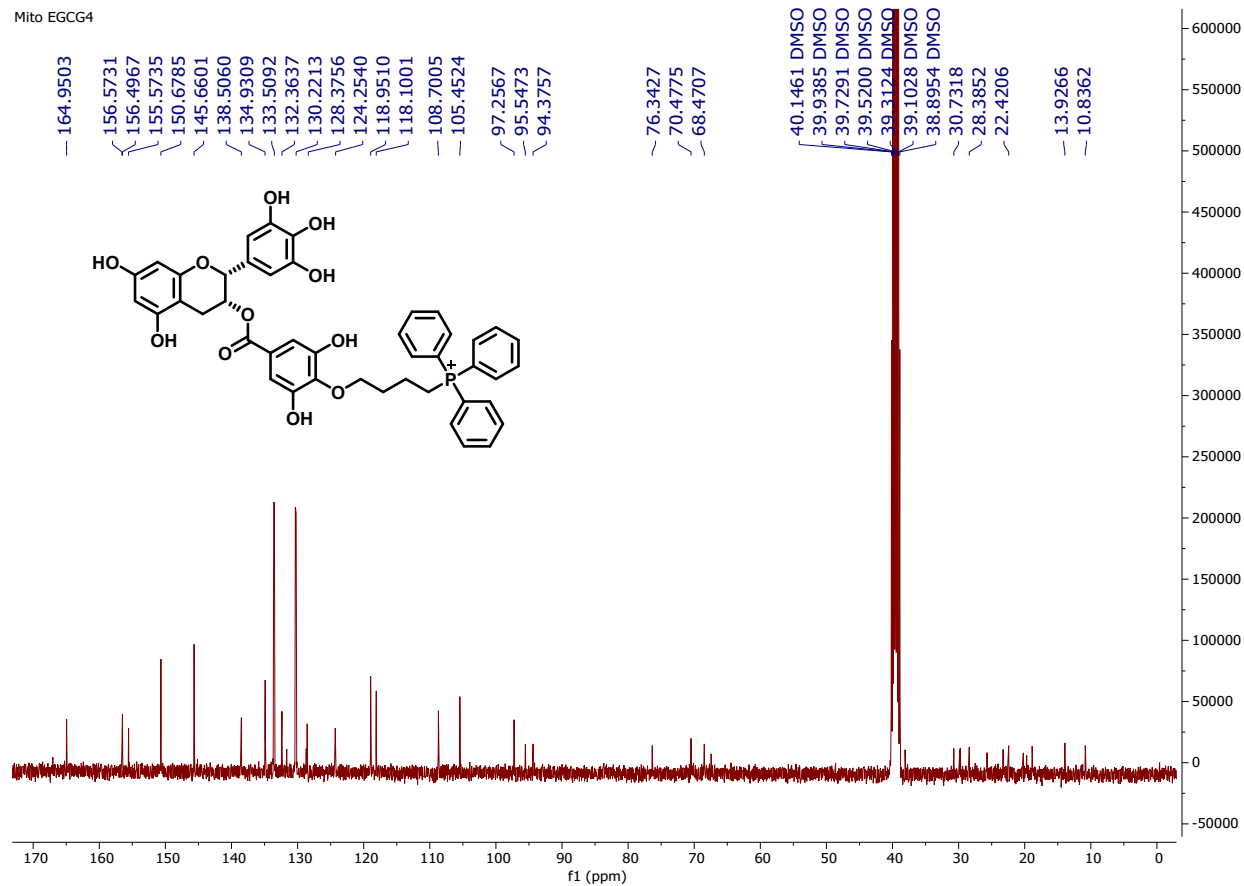


Figure S10. ^{13}C NMR spectrum of MitoEGCG₄.

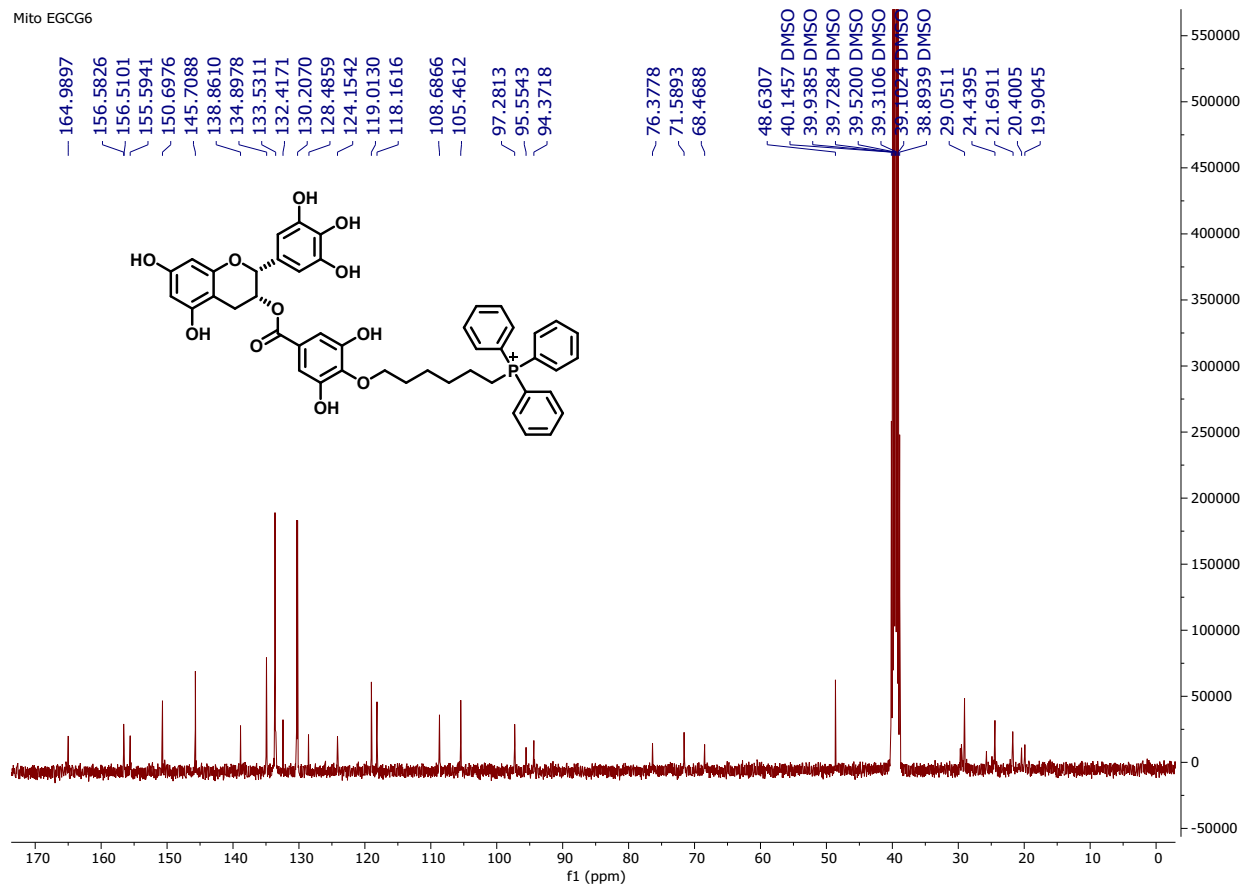


Figure S11. ^{13}C NMR spectrum of MitoEGCG₆.

Mito EGCG8

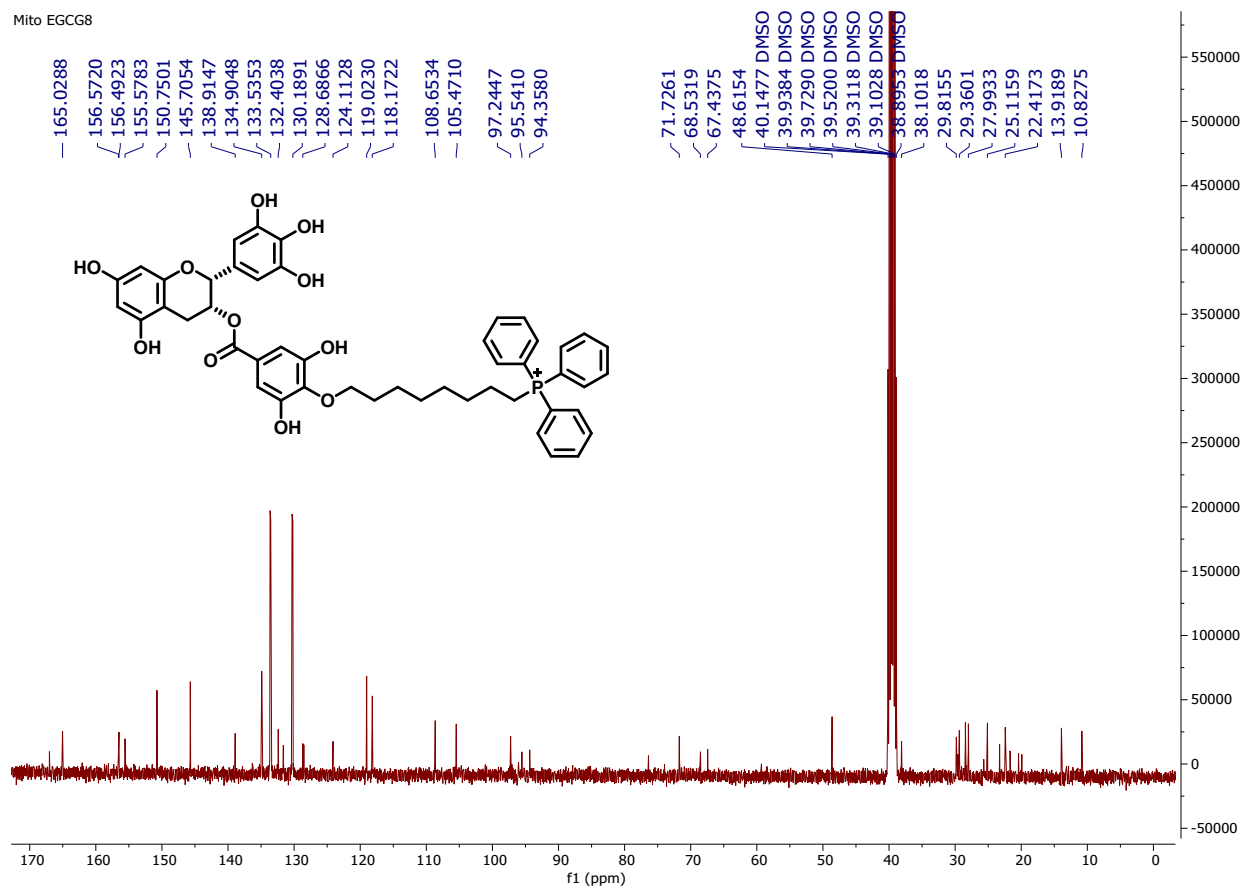


Figure S12. ¹³C NMR spectrum of MitoEGCG₈.

5. Bright field images of DCFH-DA stained H9c2 cells

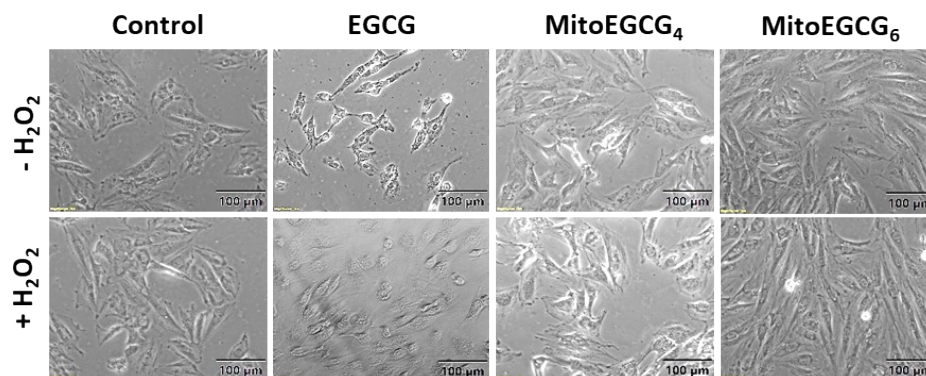


Figure S13: Bright field images of DCFH-DA stained H9c2 cells pretreated with 50 μM of EGCG, MitoEGCG₄, and MitoEGCG₆.

6. Bright field images of TMRE-stained H9c2 cells

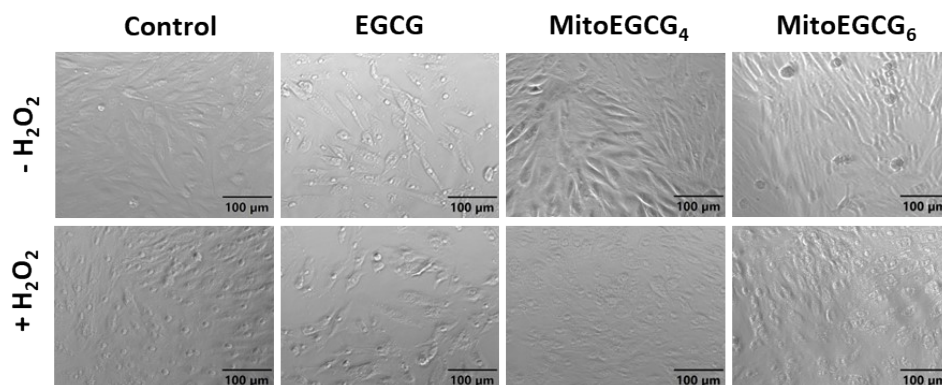


Figure S14: Bright field images of TMRE stained H9c2 cells pretreated with 50 μM of EGCG, MitoEGCG₄, and MitoEGCG₆.

7. Uncropped Western blot images

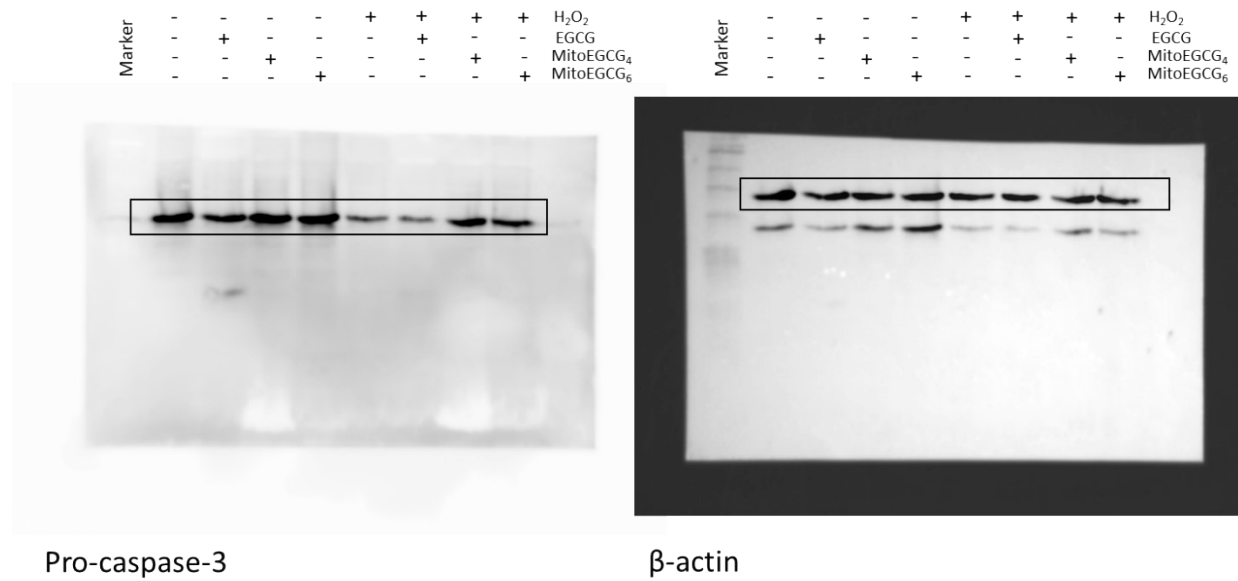


Figure S15: Uncropped Western blot images of Figure 6a.