

SUPPLEMENTARY INFORMATION

1,5-Benzodiazepin-2(3H)-ones: *in vitro* evaluation as antiparkinsonian agents

Ana Ortiz de Zárate¹, Marta Pérez-Torralba², Iñigo Bonet Isidro¹, Concepción López², Rosa M. Claramunt^{2,*}, Diana Martínez-Casanova¹, Isabel Sánchez-Vera¹, Jesús Jiménez González¹ and José Luis Lavandera^{1,3,*}

¹Instituto de Medicina Molecular Aplicada (IMMA), Facultad de Medicina, Universidad San Pablo-CEU. CEU Universities. Campus de Montepríncipe, Boadilla, E-28668 Madrid, Spain.

²Departamento de Química Orgánica y Bio-Orgánica, Facultad de Ciencias, Universidad Nacional de Educación a Distancia (UNED), Paseo Senda del Rey, 9, E-28040 Madrid, Spain.

³ Department of Pharmacology, Physiology and Neuroscience. Medical School. Rutgers. The State University of New Jersey. 185 South Orange Avenue. Newark. NJ 07103. USA

*Correspondence: rclaramunt@ccia.uned.es; joseluis.lavandera@ceu.es

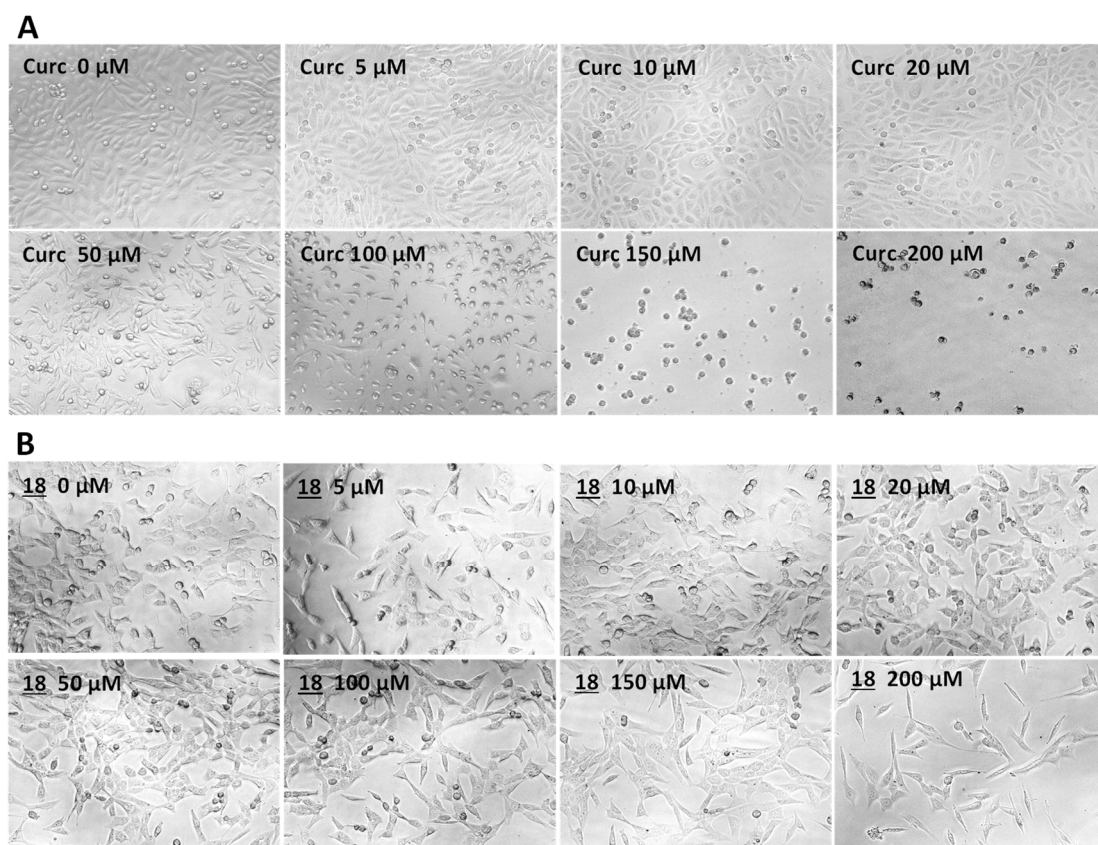


Figure S1. Cytotoxicity results were obtained for curcumin (A) and compound 18 (B) against SH-SY5Y cells. Images were taken with a Leica DM IL inverted microscope with a Leica EC3 camera at 10X.

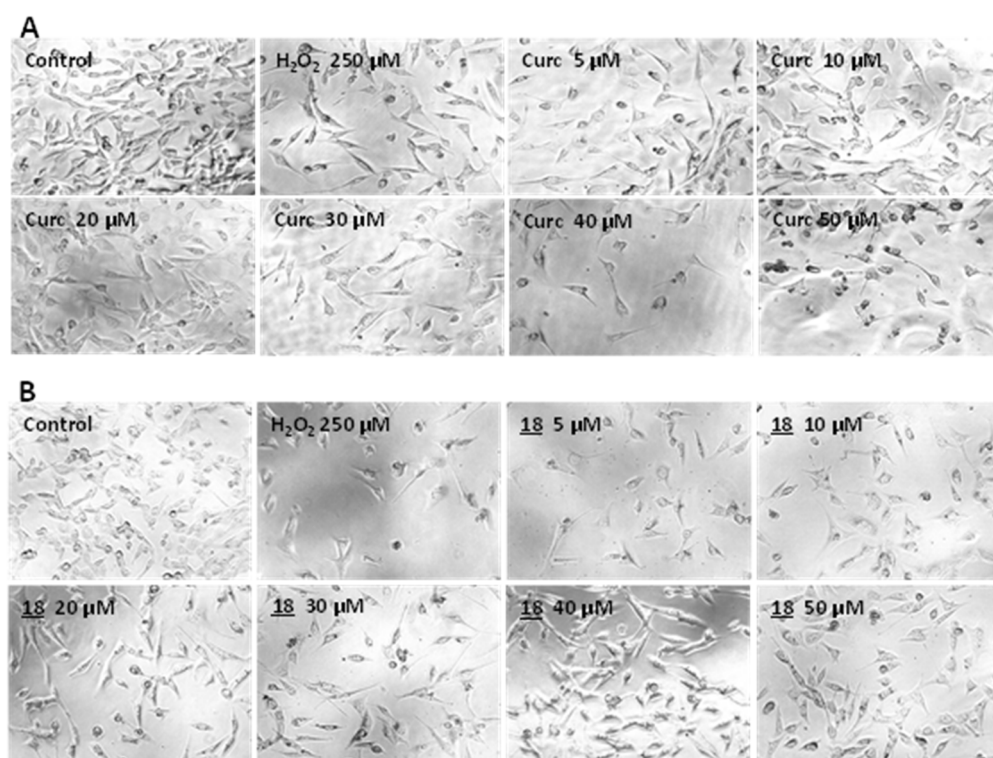


Figure S2. Results from protection experiment in SH-SY5Y cells with curcumin (A) and compound **18** (B) under oxidative stress induced by H_2O_2 250 μM . Images were taken with a Leica DM IL inverted microscope with a Leica EC3 camera at 10X.

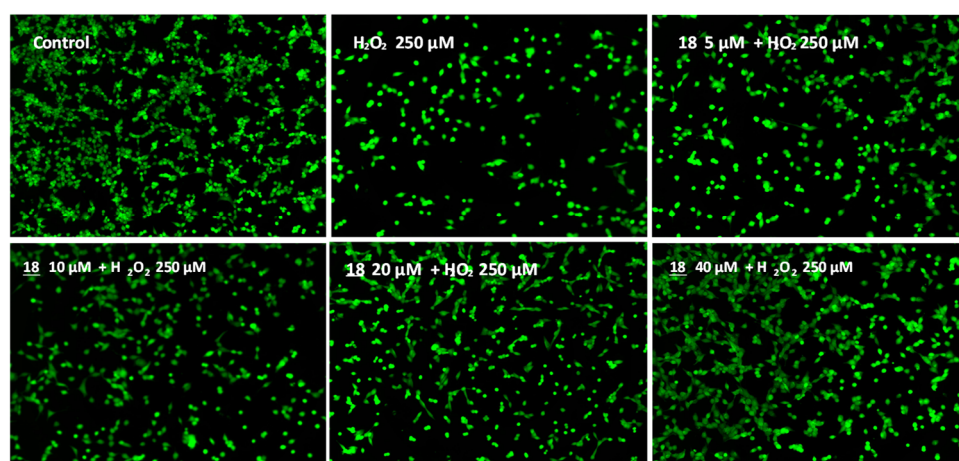


Figure S3. Representative fluorescent microscopy images from Calcein-AM staining experiments with compound **18** against H_2O_2 induced cytotoxicity in SH-SY5Y cells. Images were taken at 4X.

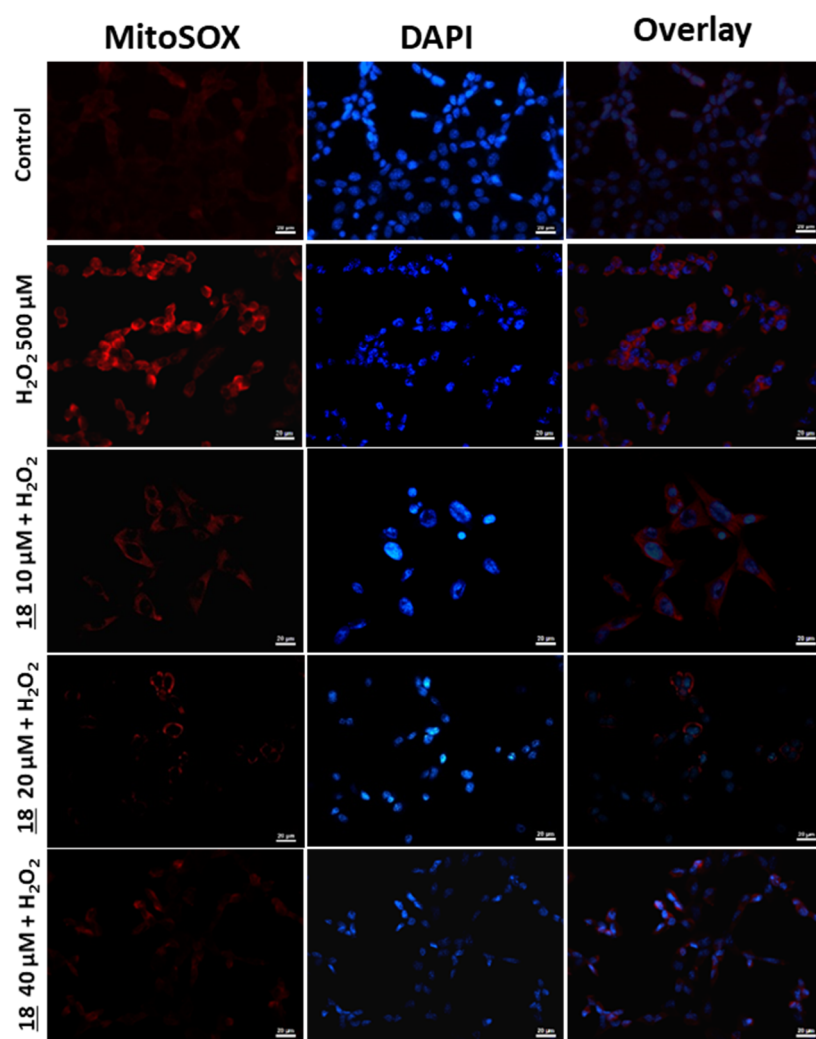


Figure S4. Images from MitoSOX experiments performed with compound 18 at 10, 20 and 40 μ M in SH-SY5Y cells under oxidative stress induced by H₂O₂ 250 μ M for 24h. Images were taken with a Leica DM 5500B microscope with a Leica DFC425FX camera.

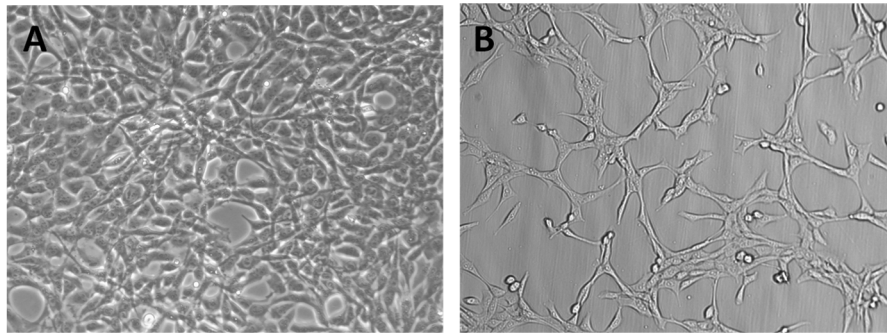


Figure S5. Undifferentiated (A) versus differentiated (B) SH-SY5Y cells. SHSY5Y cells were differentiated for 8 days by RA 10 μ M treatment in DMEM:F12 with FBS 1%. Medium with RA was replaced on Days 2, 4, 6, and 8. Pictures taken on day 8 allows the identification of a more neuronal-like morphology with axon-like projections. Images were taken at 10X magnification using a Leica DM IL inverted microscope with a Leica EC3 camera.

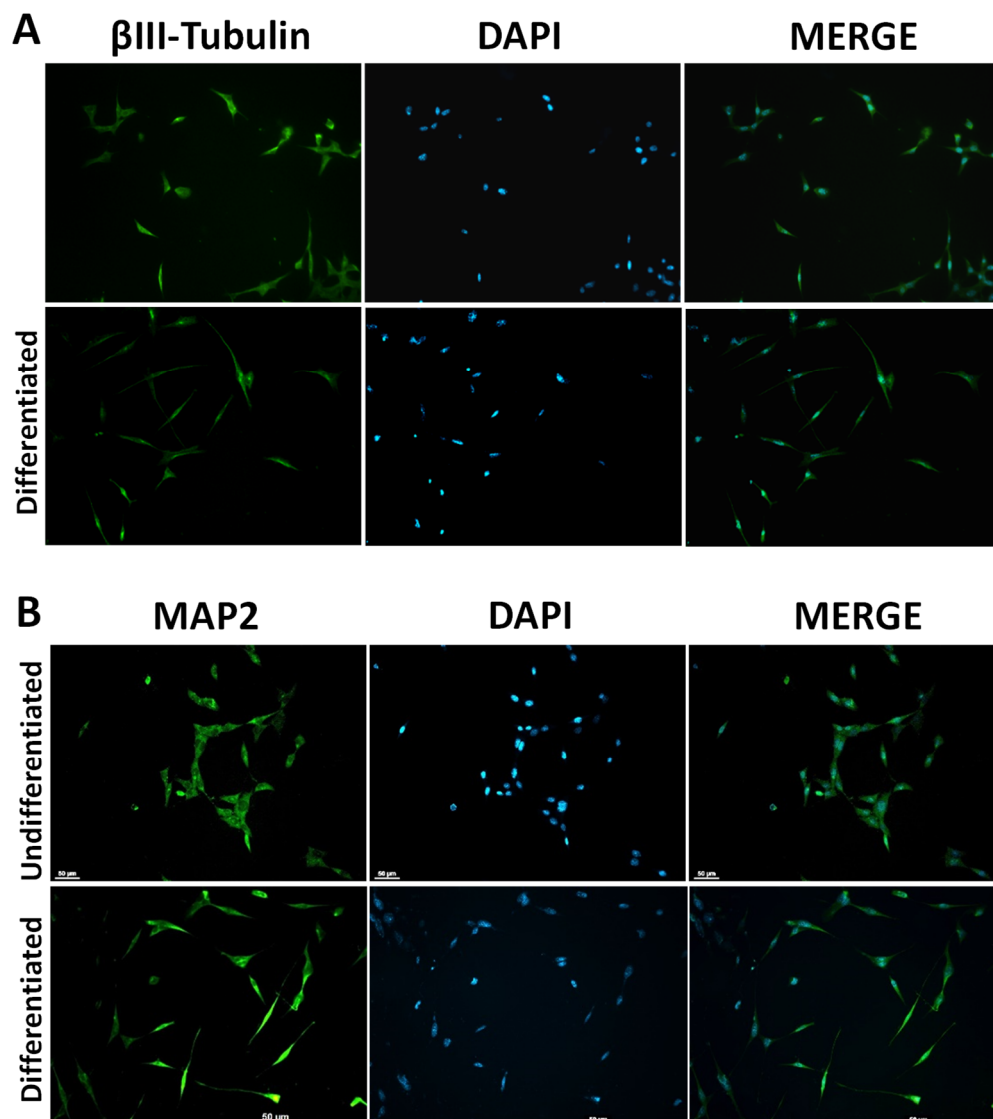


Figure S6. Fluorescence microscopy images of undifferentiated and differentiated SH-SY5Y cells using β III-Tubulin and MAP2 to highlight their different morphology features. Images were obtained at 50X magnification.

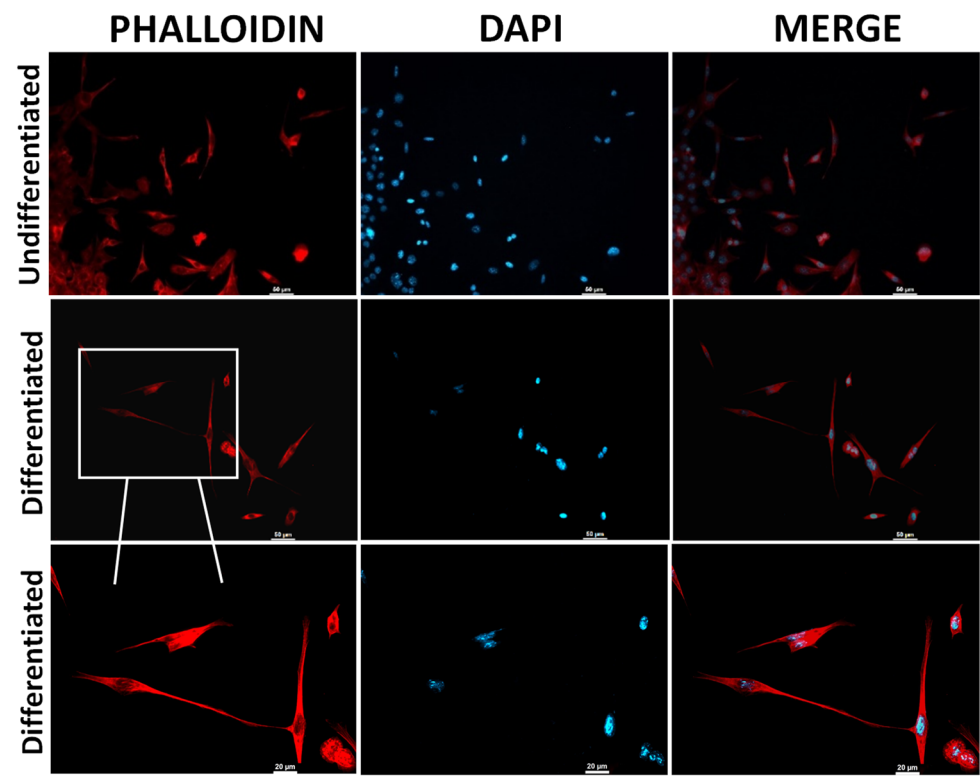


Figure S7. Staining of undifferentiated and differentiated SH-SY5Y cells with phalloidin and DAPI. Images were taken at 50 µm with a Leica DM5500 microscope.