

Supplemental Data 1 : 4-Cl-PVP assay by LC-MS/MS

4-Cl-PVP concentrations were measured by liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using a Xevo TQ-XS quadrupole mass spectrometer with an ESI ionization source. The instrument was coupled to a Waters Acquity Class-H Plus equipped with a quaternary pump system (Waters, MA, USA).

The chromatographic separation was performed using an ACQUITY HSS C18 column (150 × 2 mm, 1.8 μm, Waters) during 15 minutes. the injection volume was 2 μL, the column was held at 40°C and the autosampler was set at 15°C. Gradient of elution was at a flow rate of 0.4 mL/min, consisting of ammonium formiate 5 mM/0.1% formic acid (pH=3) (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient started with 13% solvent B for 0.5 min and then ramped up to 50% solvent B over a period of 9.5 min, and up to 95% solvent B over another period of 2.25 min. The gradient was recycling back to 13% solvent B in 0.25 min and then held at 13% solvent B for 2.5 min.

The MS/MS data were acquired in multiple reaction ion monitoring (MRM) mode using positive electrospray ionization. One MRM transitions were set up for quantitation and one for confirmation; only one MRM transition for the internal standard (ketamine-D4). The following conditions were used: capillary 3 kV; source temperature 150°C, desolvation temperature 550°C, cone gas flow 150 L/h, desolvation gas flow 1000 L/h, nebulizer 7 bars, declustering potential 30 V. Optimized parameters and multiple reaction monitoring transitions for quantitation of 4-Cl-PVP and internal standard are reported in the table below :

Compound	RT (min)	Parent ion (<i>m/z</i>)	Product ion (<i>m/z</i>)	Collision energy (V)
4-Cl-PVP	6.1	266.1	125.0*	24
			195.1	16
Ketamine-D4 (IS)	3.1	242.0	129.0	26

* Product ion (*m/z*) used for quantitation

Supplemental Data 2 : Analysis by LC-HRMS/MS.

Liquid chromatography-mass spectrometry (LC-MS) analyses were carried out using Orbitrap Q Exactive™ mass spectrometer coupled to an UltiMate 3000 pump (Thermo Scientific, San Jose, CA). LC separation was performed on a Accucore Phenyl Hexyl (100 x 2.1 mm, 2.6 μm) (Thermo Scientific, San Jose, CA). The mobile phases were composed of ammonium formate at 2 mM and formic acid 0.1% in water (phase A) and ammonium formate at 2 mM and formic acid 0.1% in methanol and acetonitrile (50/50) (phase B). LC gradient (15 min) begin in an isocratic mode at 1% phase B for 1 min and was increased from 1 to 99% of phase B during 9 min followed by a 1.5 min plateau with 99%, then a 3.5 min equilibration step with 1% of phase B. The flow rate was 500 μL/min, the column temperature was maintained at 40 °C, the injection volume was 10 μL and the samples were maintained at 15 °C in the autosampler. A heated electrospray ionization source (HESI-II) was used for the ionization of the target compounds. Data acquisition, calibration and instrument control were performed using Xcalibur® 2.1 (Thermo Scientific) software. For mass spectrometry, the instrument operated alternately in ESI positive mode and the range for acquisition was 100-700 m/z. Ion precursor selection was performed in the data dependent mode of operation where the most intense ion from the previous scan was selected for fragmentation. Full scan (MS1) data were acquired for each ionization mode at a resolution of 35,000 FWHM, with an AGC target of 1e6 and a maximum injection time of 120 ms. Source parameters were as follows: source voltage + 3.0 and – 4.0 kV, sheath gas flow 60 units, auxiliary gas flow 10 units, capillary temperature 320 °C, S-Lens RF level 60 units. MS/MS (MS2) data were acquired at a resolution of 17,500 FWHM with an AGC target of 1e5, maximum injection time was 50 ms, a TopN of 5, an isolation window of 2.0 m/z. The normalized collision energy (NCE) was stepped at 17.5, 35 and 52.5, and the dynamic exclusion time set at 3 s.

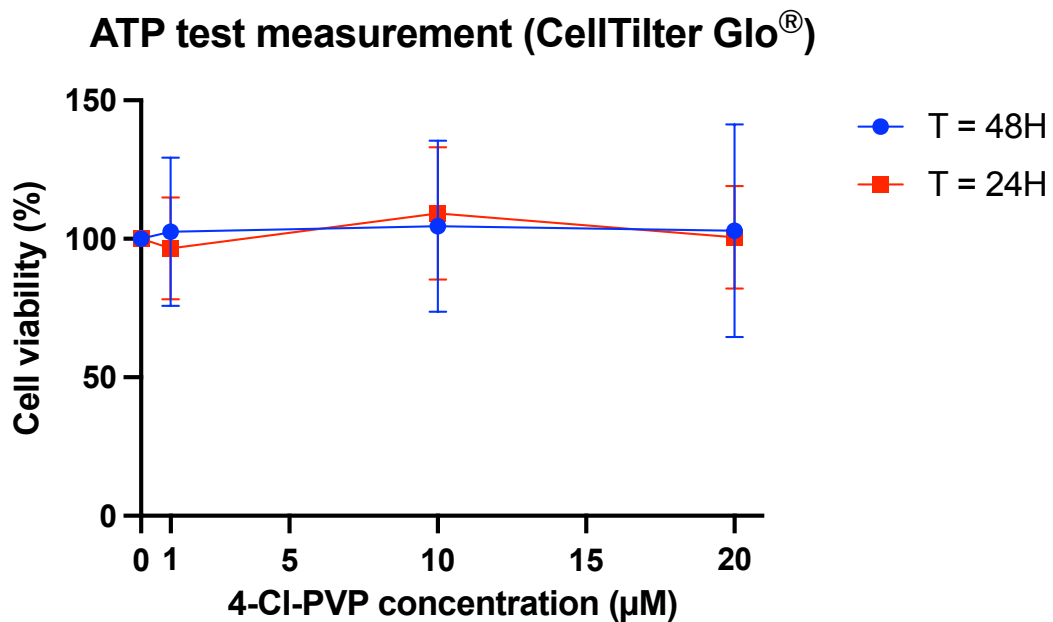
Supplemental Data 3 : Molecular networking generation.

Spectral data allowed us to generate molecular network using semi-quantitative bioinformatics approach. After acquisition of data by LC-HRMS/MS in a data dependant manner, raw spectral data files were converted from Thermo Fisher's proprietary .raw format into the .mzXML open mass format. This step was performed with the command line version of MSConvert (<http://proteowizard.sourceforge.net>). Spectra were converted from profile to centroid mode. Preprocessing of the data was required for the generation of multisample molecular networks, and the use of precursor ion abundance for relative quantification. This also allows the retention time to be taken into account, and thus to separate isomers into individual nodes. The different preprocessing steps for molecular network generation using MZmine 2.4.1 software (<http://mzmine.github.io>) have been described by Olivon et al. (2017)(Pluskal et al. 2010; Olivon et al. 2017). The main steps were deconvolution, alignment, and annotation of MS1 data using a custom database (a .csv file) containing the following features: the compound's name, the exact mass of the protonated molecule, the retention time, and the molecular formula. The single .mgf output file was uploaded on the Global Natural Products Social networking (GNPS) web-based platform (<http://gnps.ucsd.edu>) in order to generate the multimatrix molecular network (Wang et al. 2016). To the use of high resolution data, the basic parameters were modified to m/z 0.02 for the mass tolerance of precursor and fragment ions used for MS/MS spectral library searching, and m/z 0.02 for the mass tolerance of fragment ions used for molecular networking. The minimum cluster size was set to 1. In addition, links between nodes were created when the cosine score was greater than 0.70, and the minimum number of common fragment ions shared by two MS/MS spectra was 6. Links between two nodes were only kept in the network if each node was in the top 10 most similar nodes. The molecular network was visualized using Cytoscape 3.8.0 software (<http://www.cytoscape.org>) (Shannon et al. 2003). The nodes were annotated by comparison with reference standards, by spectral matching with the curated GNPS, mzCloud online mass spectral libraries and information propagation (node identified are used as a starting point to identify other nodes in the same cluster).

Supplemental Data 4: Cytotoxicity study

A cell viability study was performed to estimate a possible cell toxicity at the doses of 4-Cl-PVP used. For this purpose, we used a kit to measure cell ATP by the CellTiter-Glo® 3D Cell Viability Assay kit (Promega, Charbonnières, France). Culture medium was collected after 24 and 48 hours of treatment and cells were rinsed with 100 μ L of PBS at room temperature before incubating for 15 minutes with ATP reagent (100 μ L/well) reconstituted according to the manufacturer's instructions. One hundred microliters of each well were transferred to an opaque 96-well plate for reading with a microplate reader (POLARstar Omega®, BMG labtech®, Ortenberg, Germany).

Measurement of cellular ATP showed no decrease at the three doses tested (1, 10, and 20 μ M) either after 24 hours or after 48 hours. Morphological study also showed no major cellular damage.



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