

Supplementary materials for:

A novel, glutathione-activated prodrug of Pimasertib loaded in liposomes for targeted cancer therapy

Arianna Amenta ^{a†}, Susanna Comi ^{b†}, Marcelo Kravicz ^b, Silvia Sesana ^b, Antonia Antoniou ^c, Daniele Passarella ^a, Pierfausto Seneci ^a, Sara Pellegrino ^c, Francesca Re ^{b*}

¹ School of Medicine and Surgery, university of Milano-Bicocca, Monza, Italy

² Department of Chemistry, University of Milan, Milan, Italy

³ Department of Pharmaceutical Sciences, University of Milan, Milan, Italy

[†] These Authors have contributed equally

* Correspondence to Francesca Re, Email: francesca.re1@unimib.it

Table of contents:

1. HPLC chromatogram (Figure S1)	1
2. H ¹ and C ¹³ NMR Spectra of PROPIMA (Figure S2)	2
3. LC-MS of PROPIMA (Figure S3)	3

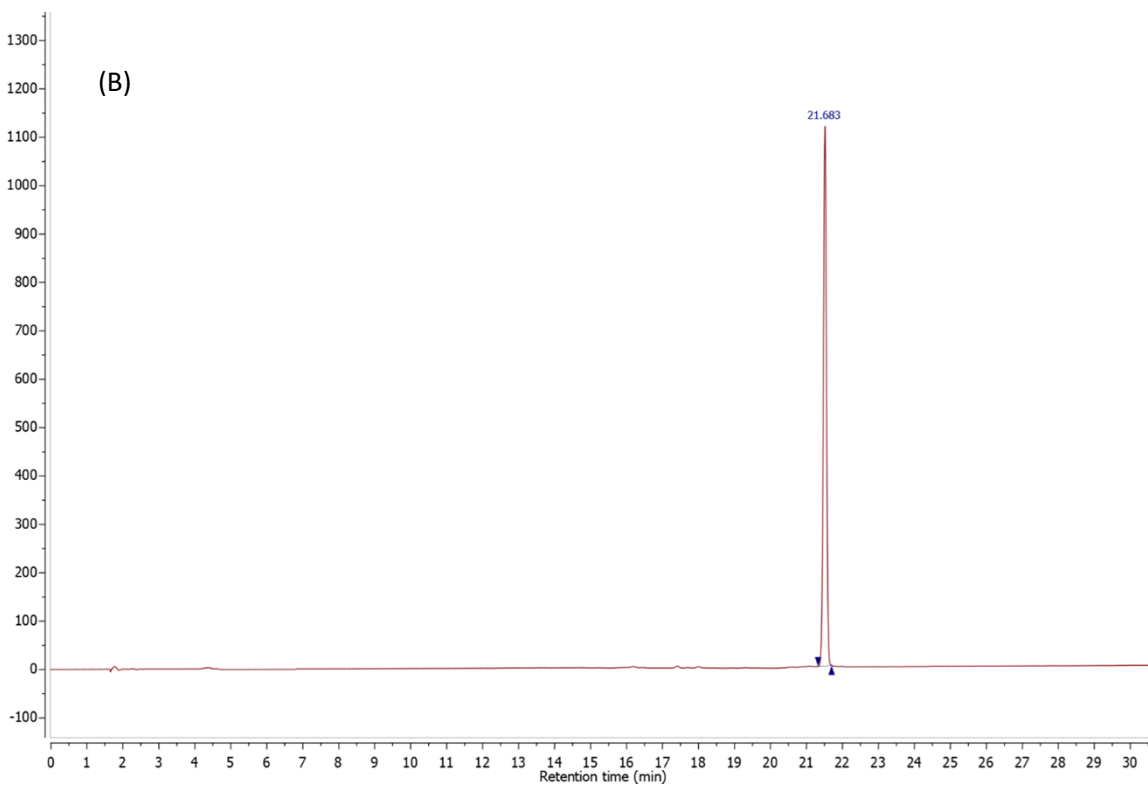
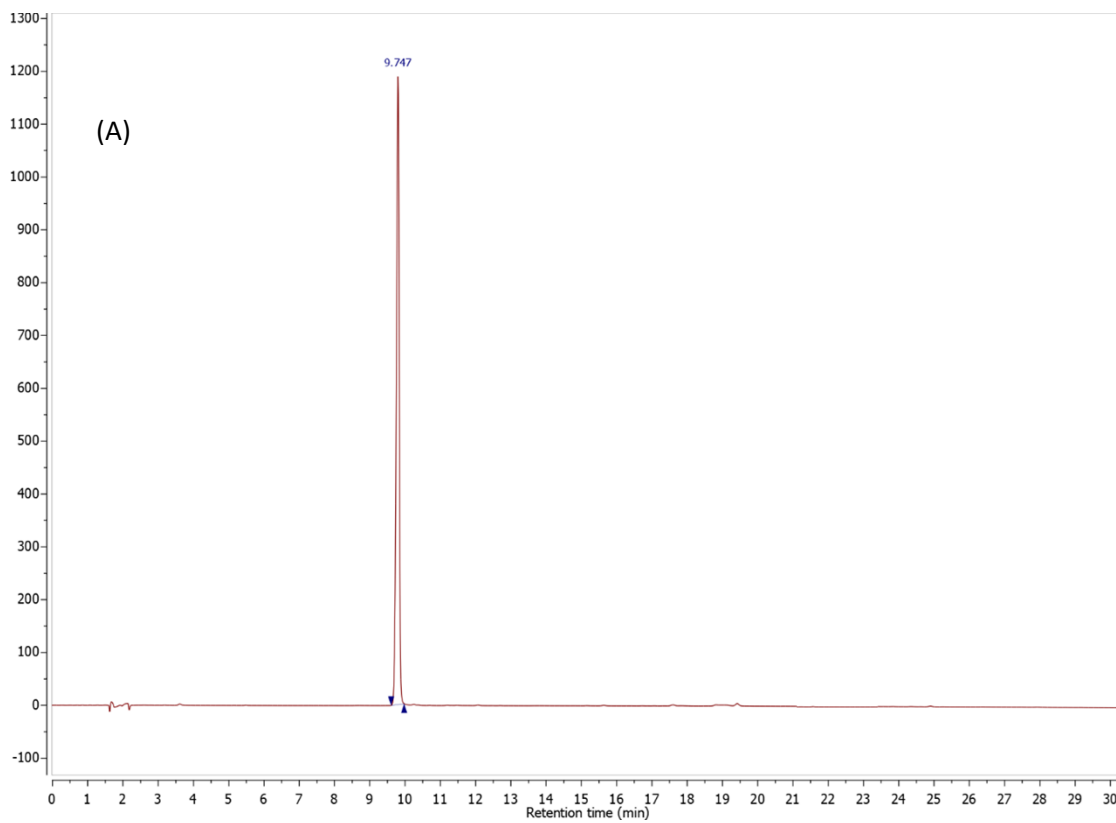
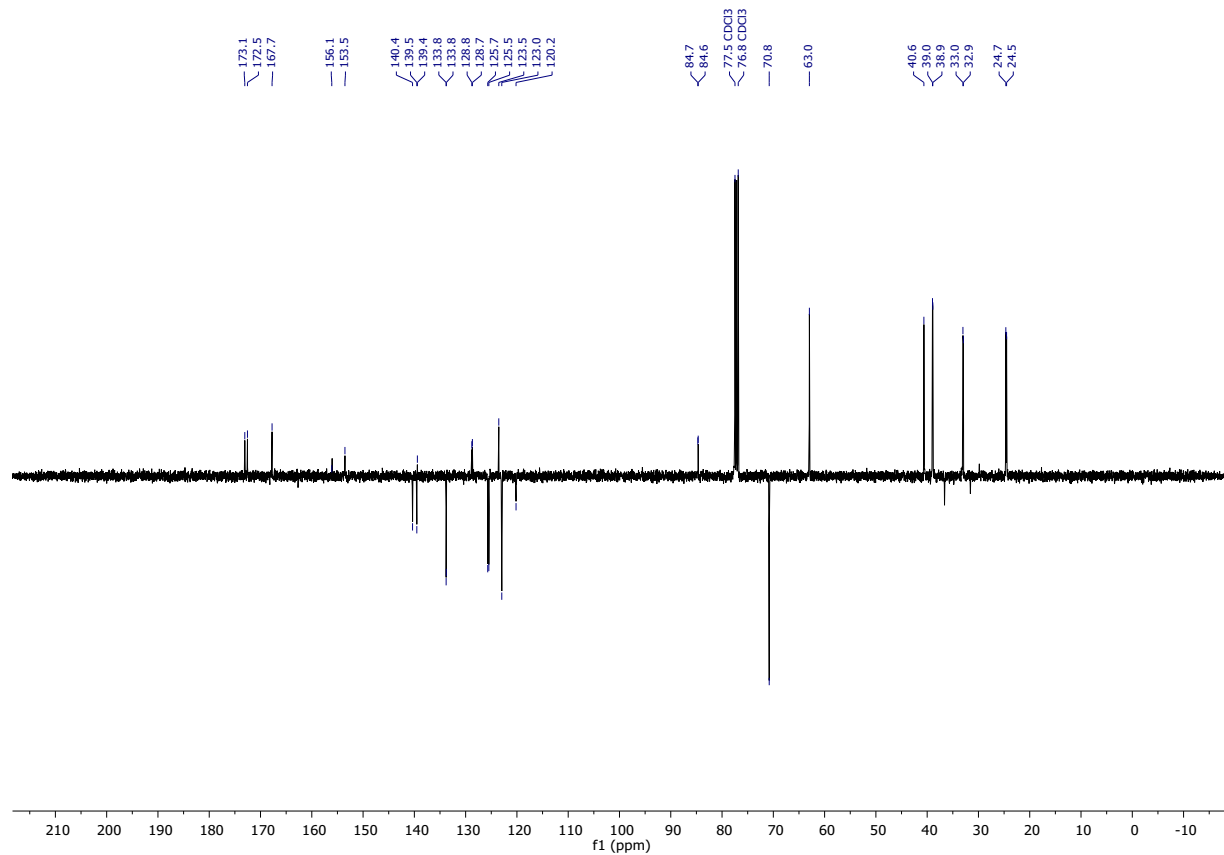
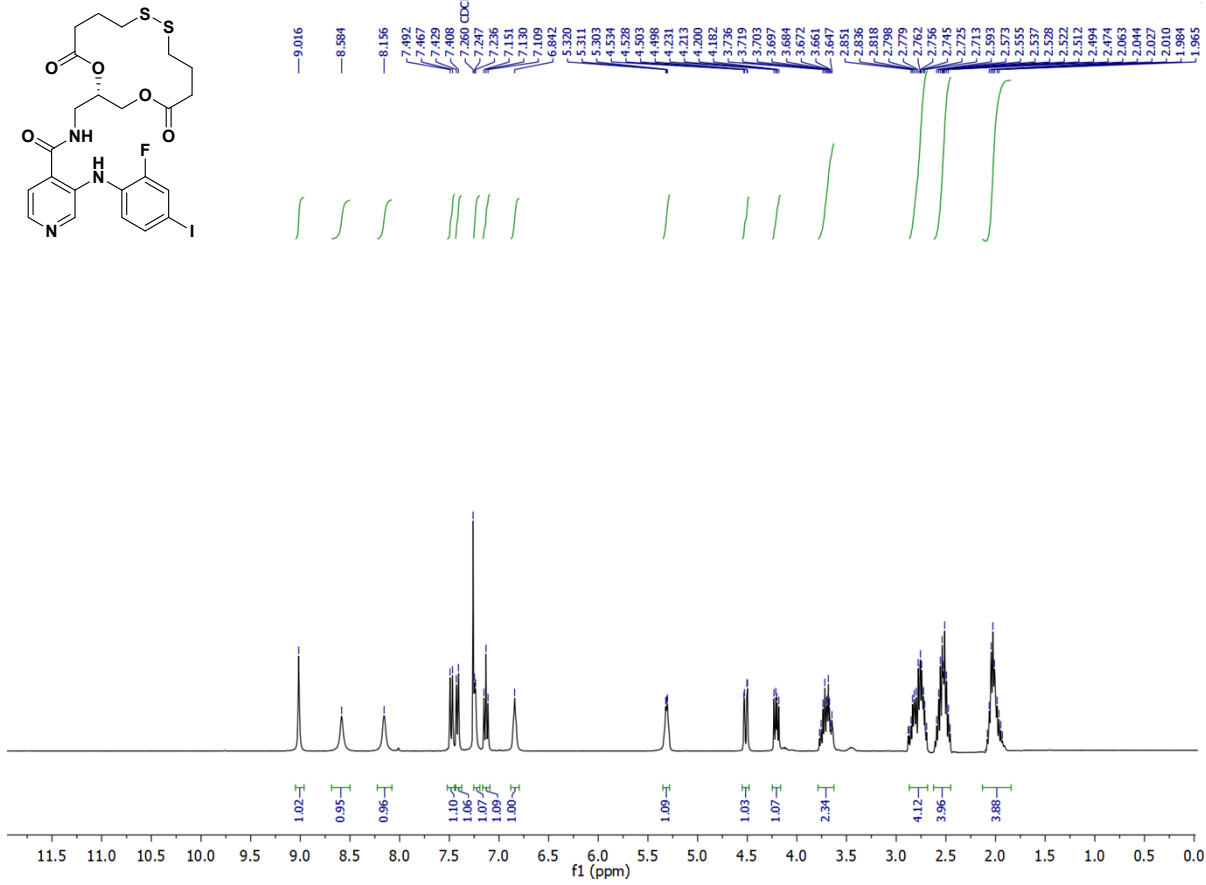


Figure S1. HPLC chromatograms for Pimasertib (A) and PROPIMA (B).



re S2. ¹H and ¹³C NMR spectra of PROPIMA.

Fig

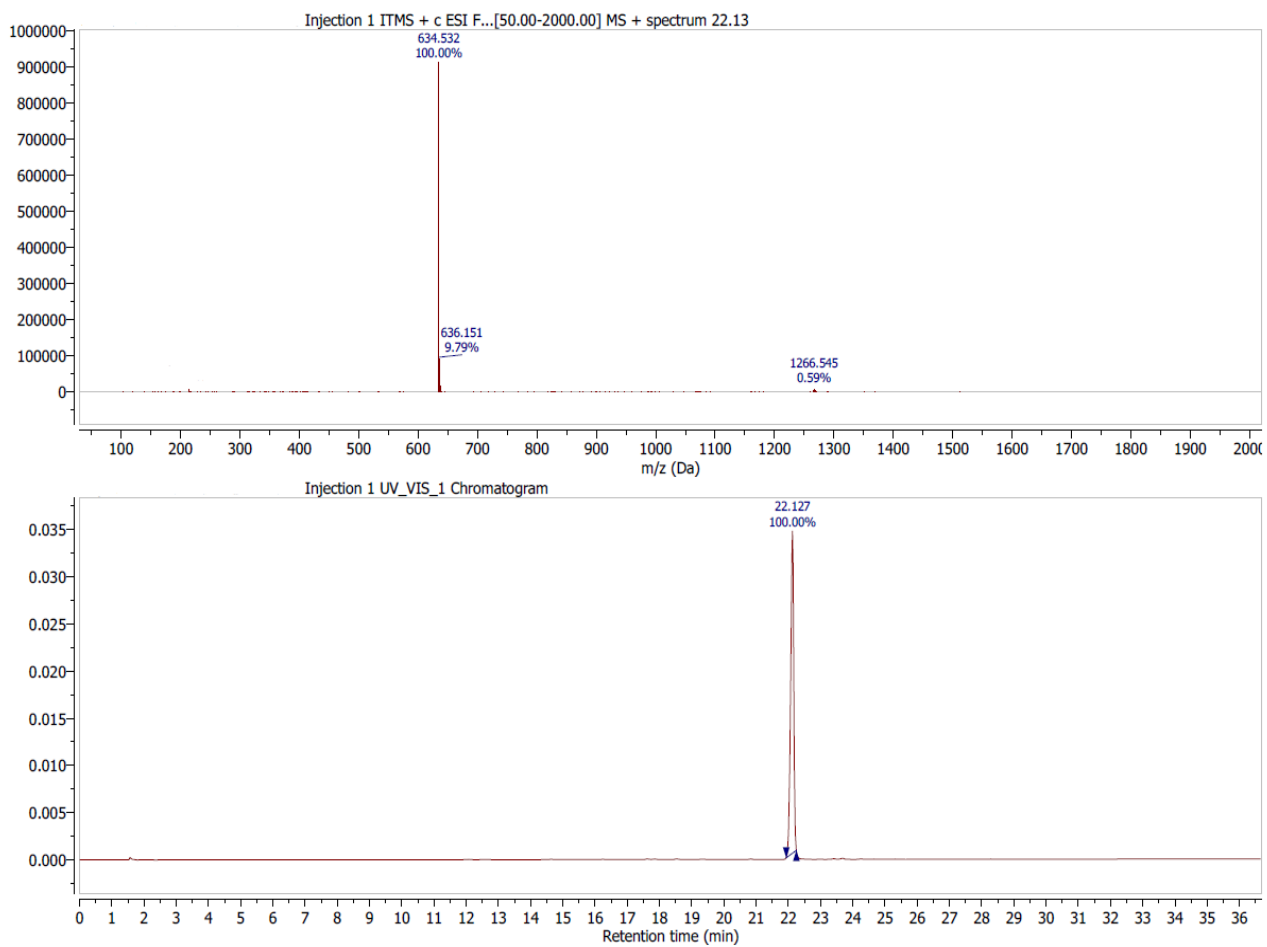


Figure S3. LC-MS of PROPIMA.

Predicted m/z $[M+H]^+$: 633.03, found 634.532.

Supplementary Methods

Determination of Glutathione peroxidase (GpX) activity. Either A375 or hCMEC/D3 cells were seeded in Petri dishes (300000 cells/cm² and 400000 cells/cm², respectively) and cultured in complete medium for 48 hours. Then, whole cell lysates were obtained, and the total protein content was measured as previously described [PMID: 35740641]. To measure GpX activities, we used an enzyme assay kit (Abcam ab219926) [PMID: 31562333], that entails as a first reaction the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). Later, GSSG and NADPH chemically interact to produce GSH and NADP⁺, with the help of glutathione reductase. In this GpX activity assay, we used a NADP sensor specific for NADP⁺ to produce a fluorescent product at 420/480 nm (Ex/Em). The fluorescence was recorder with the microplate reader TECAN (infinite F200 PRO) in kinetic modality every 3 minutes for 30 minutes. The GpX activity is directly proportional to the signal obtained.