

Sorafenib induces cathepsin B-mediated apoptosis of bladder cancer cells by regulating the Akt/PTEN pathway. The Akt inhibitor, perifosine, enhances the sorafenib-induced cytotoxicity against bladder cancer cells

A

5637 cell line	Vehicle	Sorafenib 12h	Sorafenib 24h
PI ⁺	0.07 ± 0.04	1.07 ± 0.0	1.21 ± 0.9
AnnV ⁺ PI ⁺	0.52 ± 0.07	0.09 ± 1.3	8.86 ± 2.2 ^{**###}
AnnV ⁻ PI ⁻	98.46 ± 2.1	64.63 ± 1.6 ^{**}	44.14 ± 1.6 ^{**###}
AnnV ⁺	0.95 ± 0.5	34.21 ± 2.4 ^{**}	45.79 ± 1.7 ^{**###}

T24 cell line	Vehicle	Sorafenib 12h	Sorafenib 24h
PI ⁺	0.09 ± 0.0	0.73 ± 0.0	2.06 ± 0.7
AnnV ⁺ PI ⁺	1.02 ± 0.5	0.76 ± 0.9	11.17 ± 2.7 ^{**###}
AnnV ⁻ PI ⁻	97.55 ± 2.4	59.62 ± 1.2 ^{**}	40.54 ± 1.3 ^{**###}
AnnV ⁺	1.34 ± 0.3	38.89 ± 1.8 ^{**}	46.23 ± 2.4 ^{**###}

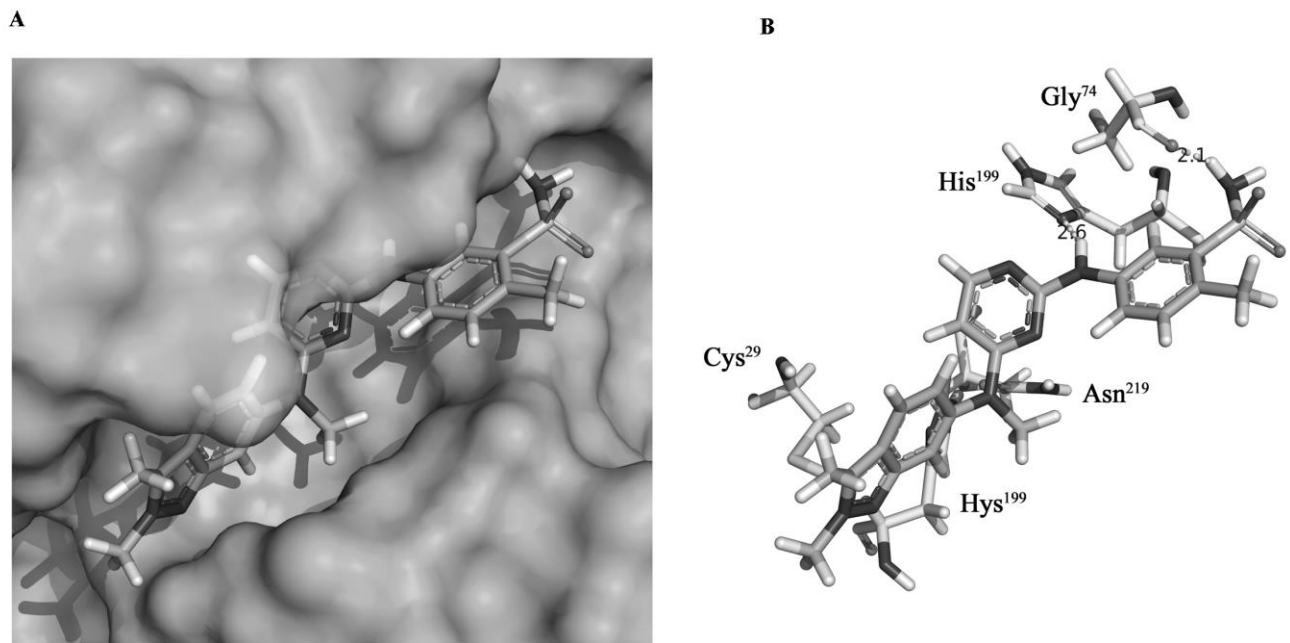
B

5637 cell line	Vehicle	Sorafenib 12h	Sorafenib 24h
PI ⁺	0.07 ± 0.0	0.00 ± 0.0	2.31 ± 0.3
AnnV ⁺ PI ⁺	0.63 ± 0.0	0.39 ± 0.0	15.63 ± 1.6 ^{**###}
AnnV ⁻ PI ⁻	93.84 ± 1.9	47.73 ± 1.8 ^{**}	36.74 ± 1.9 ^{**###}
AnnV ⁺	5.46 ± 0.3	51.88 ± 2.1 ^{**}	45.32 ± 1.4 ^{**###}

T24 cell line	Vehicle	Sorafenib 12h	Sorafenib 24h
PI ⁺	0.01 ± 0.0	0.00 ± 0.0	3.01 ± 0.4
AnnV ⁺ PI ⁺	0.02 ± 0.0	0.23 ± 0.0	25.14 ± 1.1 ^{**###}
AnnV ⁻ PI ⁻	96.79 ± 2.2	37.34 ± 0.9 ^{**}	18.34 ± 0.7 ^{**###}
AnnV ⁺	3.18 ± 0.2	62.43 ± 1.7 ^{**}	53.51 ± 1.5 ^{**###}

Supplementary Figure 1: A,B) 5637 and T24 cells treated with sorafenib (10 μM panel A and 20 μM panel B) or with vehicle for 12, 24 h, were stained with Ann V-FITC and PI and

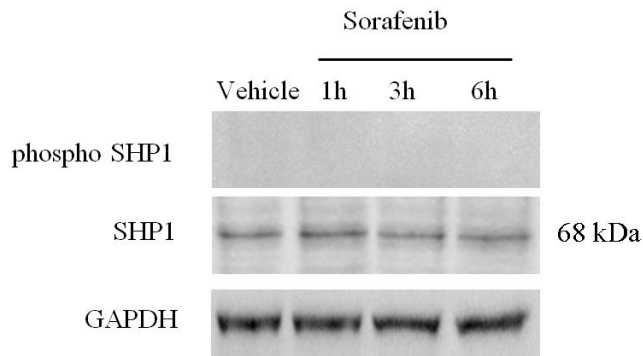
analyzed by FACS. Data are expressed as percentage of positive cells \pm SD of three separate experiments. ** $p < 0.01$ vs vehicle-treated cells; ## $p < 0.01$ vs 12h of sorafenib treatment. No statistical significant difference was found between untreated and vehicle-treated cells or comparing different times of vehicle-treatment each other (data not shown).



Supplementary Figure 2: Pazopanib docked into the catalytic groove of CB.

A) Pazopanib/CB complex is showed indicating the active site (light pink of the protein surface) inside the catalytic groove.

B) Cys²⁹ and the amino acids involved in the H-bond formation.



Supplementary Figure 3: Sorafenib does not modulate phosphorylation levels of SHP-1 in T24 BC cells. Lysates from T24 BC cells treated with sorafenib (20 μ M) for 1, 3 or 6 h or with vehicle for 1 h were separated via 9% SDS-PAGE and probed using anti-phospho-SHP-1, anti-SHP-1 and anti-GAPDH Abs. One representative experiment out of three independent experiments is shown. No statistical significant difference was found between untreated and vehicle-treated cells or comparing different vehicles-treatment each other (data not shown). Data shown are relative to T24 cell line and are representative of BC lines analyzed.