Discovery of Harmaline as a Potent Inhibitor of Sphingosine Kinase-1: A Chemopreventive Role in Lung Cancer

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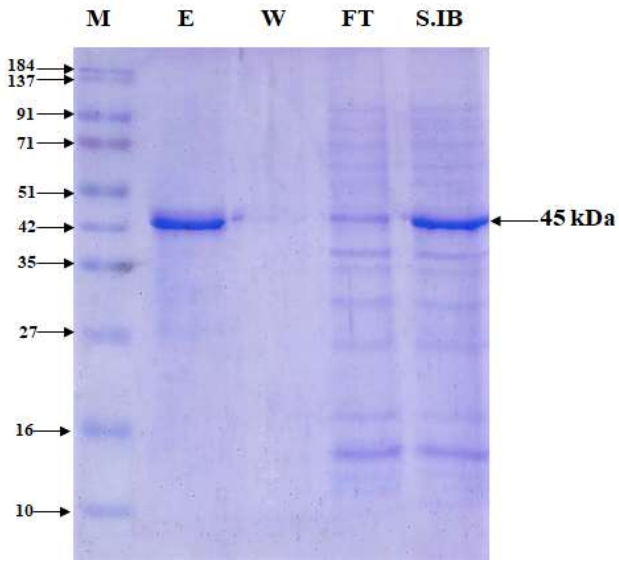

Figure S1. SDS-PAGE profile showing single band of SphK1 at approximately 45 kDa. The lanes S.IB, FT, W and E represent the solubilised inclusion bodies, flow through (unbound proteins) ,wash and final eluted SphK1.

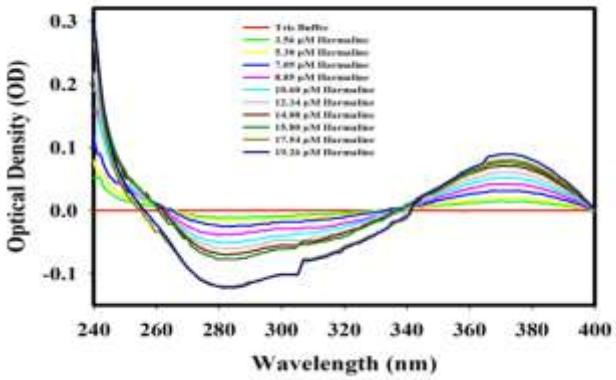

Figure S2. Absorption spectrum of increasing concentration of harmaline (3.56-19.26 µM) in 20 mM Tris, 100 mM NaCl buffer (pH 8.0). Harmaline absorbs in the range of 342-400 nm while no absorption is observed at 280 nm. After 260 nm, sharp increase in the absorbance of harmaline can be seen.

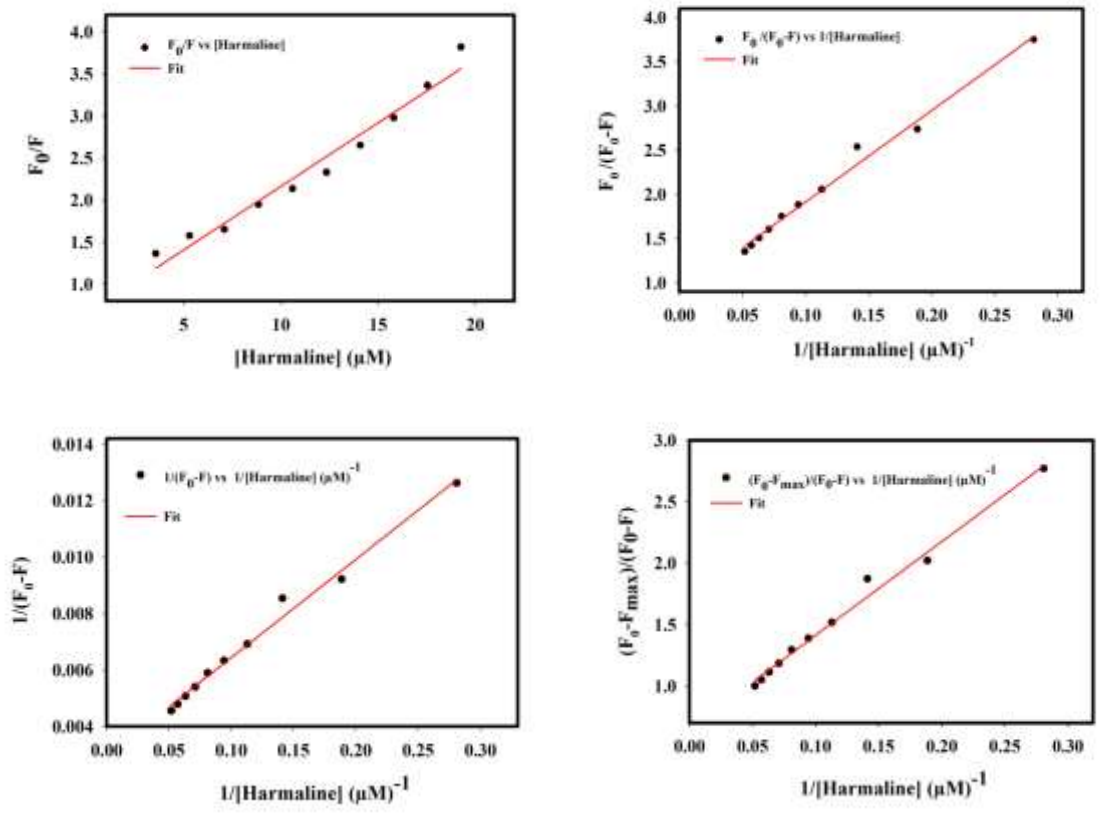

Figure S3. (A) Stern -Volmer plot for quenching of SphK1 (4µM, pH 8.0) by harmaline at 25 °C. (B) Modified Stern-Volmer plot for the estimation of the fraction of accessible fluorophore (SphK1). (C) The modified Stern–Volmer (Lineweaver-Burk) curve of harmaline quenching SphK1 fluorescence at 25 ˚C. (D) Plot of Johansson equation.

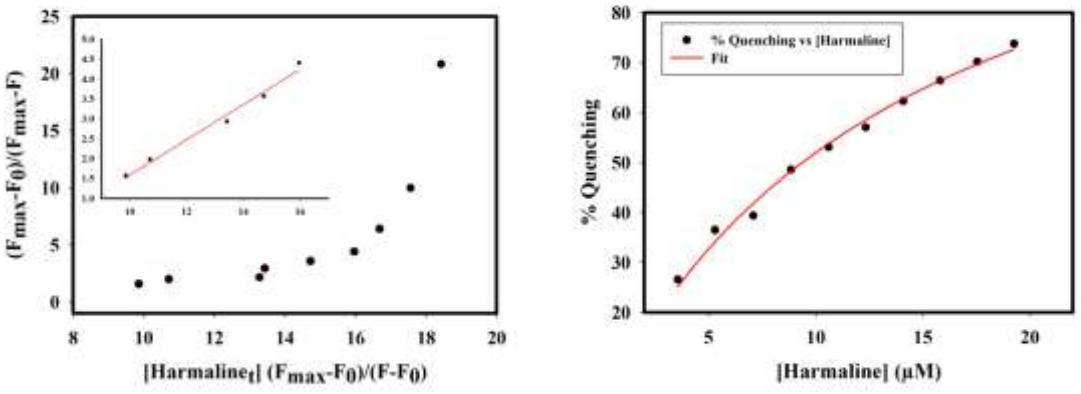

Figure S4. (A) The Scatchard plot of Harmaline quenching SphK1 fluorescence at 25 ˚C. (B) Langmuir binding Fitting curve (one site saturation) of the quenching of the fluorescence of SphK1 by Harmaline.

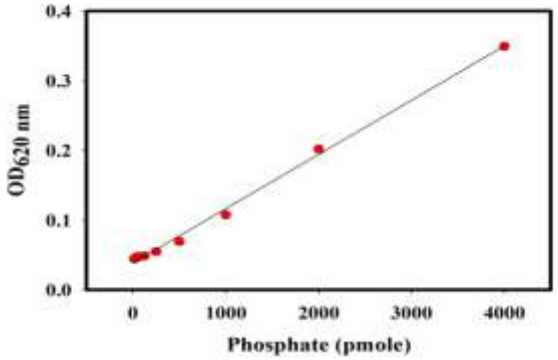

Figure S5. Standard phosphate hydrolysis curve showing the quantity of phosphate measured.

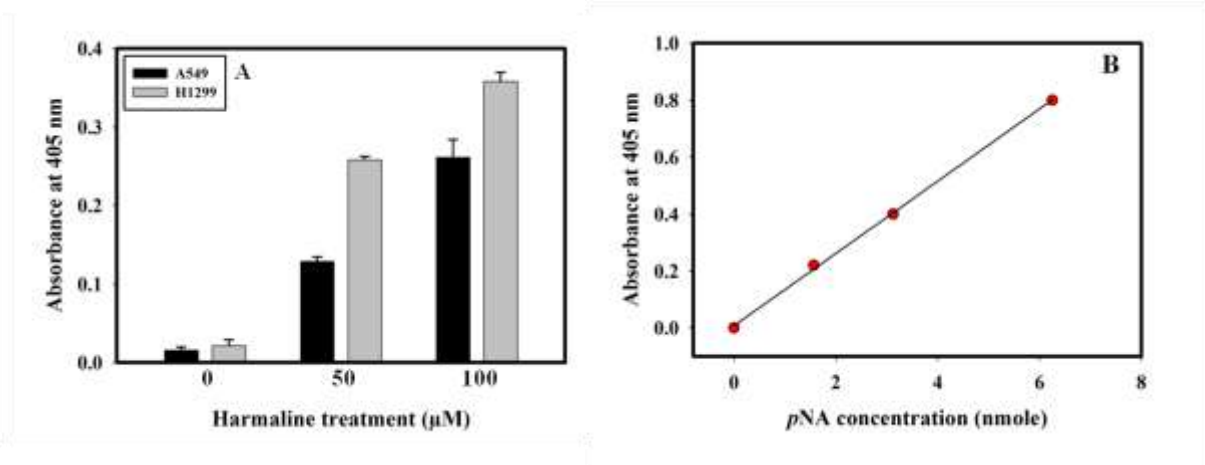

Figure S6. (A) An increase in the absorbance at 405 nm (corresponding to free pNA liberated) for both A549 and H1299 cells was recorded after treatment with 0, 50 and 100 μM of harmaline for 24 h. (B) Standard *p*NA curve that depicts the absorbance of free *p*NA at different concentrations.

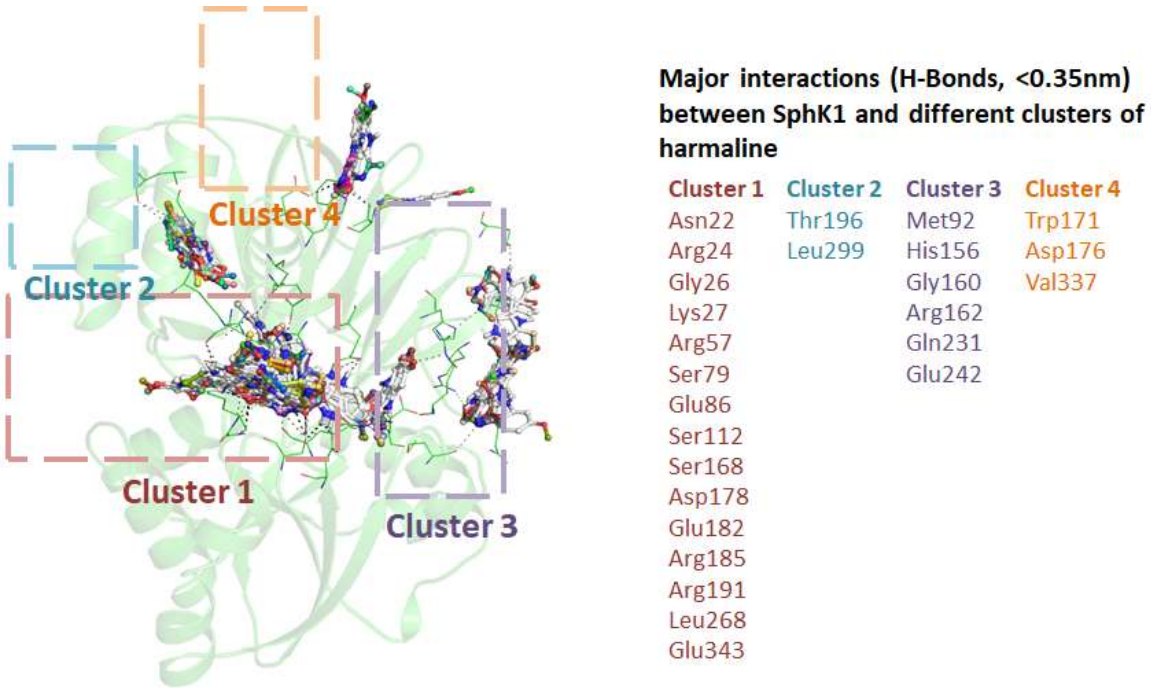

Figure S7. Plausible docking sites for harmaline on SphK1. Cartoon representation of SphK1 showing 4 plausible binding sites for harmaline docked conformers. Dotted boxes showing the clusters of harmaline conformers ranked based on their binding energy and number of conformers binding at the same site. +Cluster 1 has the lowest binding energy (thus highest affinity) and highest number of docked conformations and so on. Average binding affinity for Cluster 1, Cluster 2, Cluster 3, and Cluster 4 was estimated as −7.9, −7.4, −6.8, and −6.4 kcal/mol, respectively. Major hydrogen bonding interactions between SphK1 and different clusters of harmaline are also shown on the right side.

Table S1. The K_a and n of harmaline and SphK1 calculated using different models.

Equation	K_a	***n***	R/R^2
(1)	$1.5 \times 10^5\ M^{-1}$	-	0.97/0.98
(2)	$8.5 \times 10^4\ M^{-1}$	1.14	0.99/0.99
(3)	$7.1 \times 10^5\ M^{-1}$	1.2	0.99/0.98
(4)	$8.62 \times 10^4\ M^{-1}$	-	0.99/0.99
(5)	$1.3\times 10^5\ M^{-1}$	-	0.99/0.98
(6)	$4.4 \times 10^5\ M^{-1}$	1.6	0.99/0.98
(7)	$6.9\times 10^4\ M^{-1}$	1	0.99/0.99