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Supplemental Data

Table S1. Anti-migratory activity of locostatin in different cell types

Cell Line	**IC_{50}**[a]	**Activity**[b]
A549	> 50 μM (virtually no subtoxic activity), 24 hr[c]	29%, 75 μM
MCF-7	24.1 μM, 12 hr[c]	39%, 50 μM
B16-BL6	16.7 μM, 6 hr[c]	79%, 50 μM
MDCK	12.0 μM, 12 hr[c]	72%, 50 μM
MDCK	17.9 μM, 24 hr[c]	96%, 50 μM
MDCK (serum-free conditions)	12.5 μM, 24 hr[c]	69%, 20 μM
MDCK scatter[d]	≥ 20 μM	

[a]All IC_{50} values were calculated for inhibition of wound closure at 24 hr post-wounding with normalization to parallel controls for each compound, as described in Table 1.

[b]Values correspond to the percent inhibition of wound closure post-wounding normalized to parallel controls for the highest subtoxic concentrations, as indicated. The higher the percentage, the more inhibitory the compound relative to parallel controls for each cell type.

[c]Time after wounding for which IC_{50} and activity values were determined from digital images.

[d]All assays were scratch-wound closure assays using confluent cultures of the cells indicated with the exception of the MDCK cell scatter assay, which involved treating subconfluent MDCK cell cultures with HGF/SF.

Table S2. Calculated second-order association or inactivation rate constants for different locostatin-binding proteins.

Protein	$\boldsymbol{k}_{\mathbf{assoc}}$ ($M^{-1}s^{-1}$, mean ± standard deviation; $n = 4$)
Raf kinase inhibitor protein (RKIP)	13.09 ± 1.36
Aldehyde dehydrgenase 1A1 (ALDH1A1)	107.4 ± 17.0
Prolyl oligopeptidase (POP)	2.08 ± 0.04
Glutathione *S*-transferase omega 1-1 (GSTO1-1)	0.108 ± 0.008 (DHA reductase assay) 0.063 ± 0.008 (thiol transferase assay)

Supplemental Experimental Procedures

In experiments involving serum-free conditions, MDCK cells were serum starved for 36 hr prior to wounding and then maintained in serum-free conditions. A549 cells were cultured in F12K medium with 10% fetal bovine serum (FBS). B16-BL6 cells were grown in MEM with 10% FBS and switched to 5% FBS for wound closure assays. MCF7 cells were cultured in MEM with 10% FBS.

For wound closure assays involving A549, B16-BL6 and MCF7 cells, a grid of 100 pixels was imposed over time-lapse images for tracking specific points along the wound edge over time, and the distance of migration was measured. In the case of the HGF/SF-induced scatter experiments, subconfluent MDCK cell cultures were treated with 5 ng/ml HGF/SF and degree of scatter was determined 24 hr later from digital images.