Molecular Pharmacology

Supplementary Materials

A resveratrol analogue promotes ERK^{MAPK}-dependent Stat3 serine and tyrosine phosphorylation alterations and antitumor effects *in vitro* against human tumor cells

Zachary L. Chelsky, Peibin Yue, Tamara P. Kondratyuk, David Paladino, John M. Pezzuto, Mark Cushman, and James Turkson

Figure Legends

Fig. S1. **Cmpd1 preferentially suppress colony formation of malignant cells.** Cultured human glioma (U251MG and SF295), breast (MDA-MB-231 and MCF7), and pancreatic (Panc-1) cancer cells, and normal NIH3T3 mouse fibroblast were seeded as single-cells and treated once with 0-20 μM Cmpd1 and allowed to culture until large colonies were visible, which were stained with crystal violet and imaged. Data are representative of three independent determinations.

Fig. S2. **Effects of Cmpd1 and resveratrol on constitutive or ligand-induced Stat3 and Stat1 activation in normal or mouse transformed fibroblasts.** Immunoprobes of pYStat3, pSer727Stat3, Stat3, pY701Stat1, Stat1 and β-actin from whole-cell lysate preparation from (A and B) v-Src-transformed mouse fibroblasts (NIH3T3/v-Src) harboring aberrantly-active Stat3 untreated or treated for 0-24 h with (A) 15 μM Cmpd1 or (B) 20 μM resveratrol (Res); or (C) mouse fibroblasts over-expressing the human EGF receptor (NIH3T3/hEGFR) pre-treated with or without 15 μM Cmpd1 for 0-24 h and stimulated with EGF for 15 min. The positions of proteins in the gel are labeled; bands corresponding to the phospho-protein levels in the gel were quantified by ImageQuant and calculated as a percent of control (DMSO) relative to the total proteins and the β-actin levels; control lane (-, 0) represents whole-cell lysates prepared from 0.025% DMSO-treated or un-stimulated cells. Data are representative of three independent determinations.

Fig. S3. **Differential effects of Cmpd1 and resveratrol on Src induction in v-Src-transformed mouse fibroblasts.** Immunoblots of pY416Src, Src and β-actin from whole-cell lysate preparation from

v-Src-transformed mouse fibroblasts (NIH3T3/v-Src) harboring aberrantly-active Stat3 untreated or treated for 0-24 h with (A) 15 μM Cmpd1 or (B) 20 μM resveratrol (Res). The positions of proteins in the gel are labeled; bands corresponding to the phospho-protein levels in the gel were quantified by ImageQuant and calculated as a percent of control (DMSO) relative to the total proteins and the β-actin; control lane (0) represents whole-cell lysates prepared from 0.025% DMSO-treated cells. Data are representative of three independent determinations.

Fig. S4. **Phospho-Erk1/2^{MAPK} induction mediated by Cmpd1 and pterostilbene is reversed by MEK inhibitor, PD98059, but not the general protein kinase inhibitor, staurosporine or the inhibitors of p38, mTOR, PI 3-kinase/Akt, or Src**. Immunoblotting analysis of whole-cell lysate preparation from U251MG cells pre-treated with (+) or without (-) the designated concentrations of PD98059, SB202190, LY294002 (LY), dasatinib (Das), rapamycin (Rap) or staurosporine (Staur) for 1 h prior to treatment with (A) 15 μM Cmpd1 or (B) 20 μM pterostilbene (PTE) and probing for pErk1/2^{MAPK} and Erk1/2^{MAPK}. The positions of proteins in the gel are labeled; control lane (-) represents whole-cell lysates prepared from 0.025% DMSO-treated cells. Data are representative of two independent determinations.

Fig. S5. **Lack of modulation by Cmpd1 of Stat3 and Erk1/2 ^{MAPK} phosphorylation in normal and v-Ras-transformed mouse fibroblasts**. Immunoblotting analysis of whole-cell lysate preparation from normal (NIH3T3) and v-Ras-transformed (NIH3T3/v-Ras) mouse fibroblasts pre-treated with (+) or without (-) 20 μM PD98059 for 1 h prior to treatment with 15 μM Cmpd1 and

probing for pY705Stat3, pS727Stat3, Stat3, pErk1/2^{MAPK}, and Erk1/2^{MAPK}. The positions of proteins in the gel are labeled; control lane (-) represents whole-cell lysates prepared from 0.025% DMSO-treated cells. Data are representative of two independent determinations.

Supplementary Figures

Fig. S1

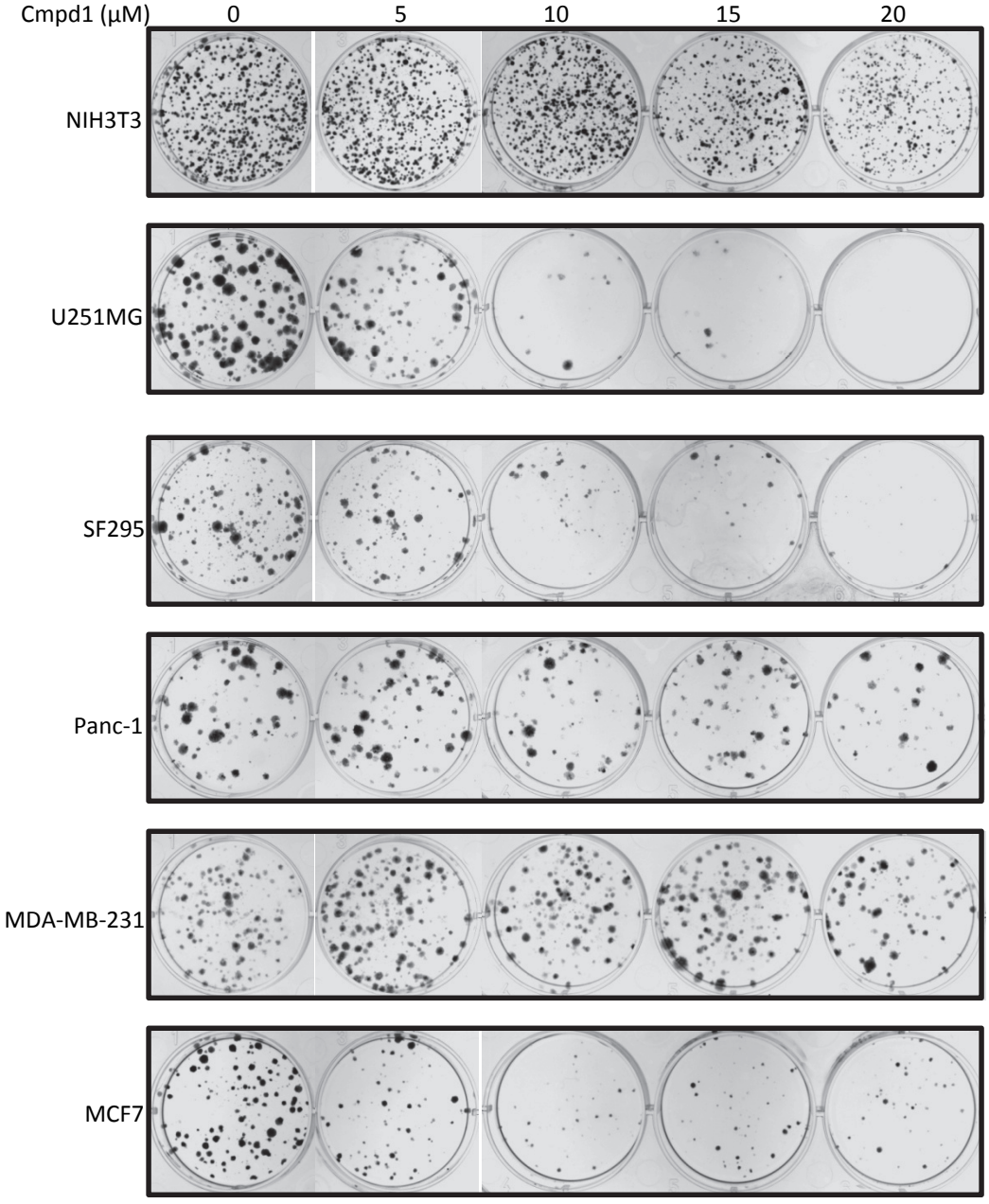


Fig. S2

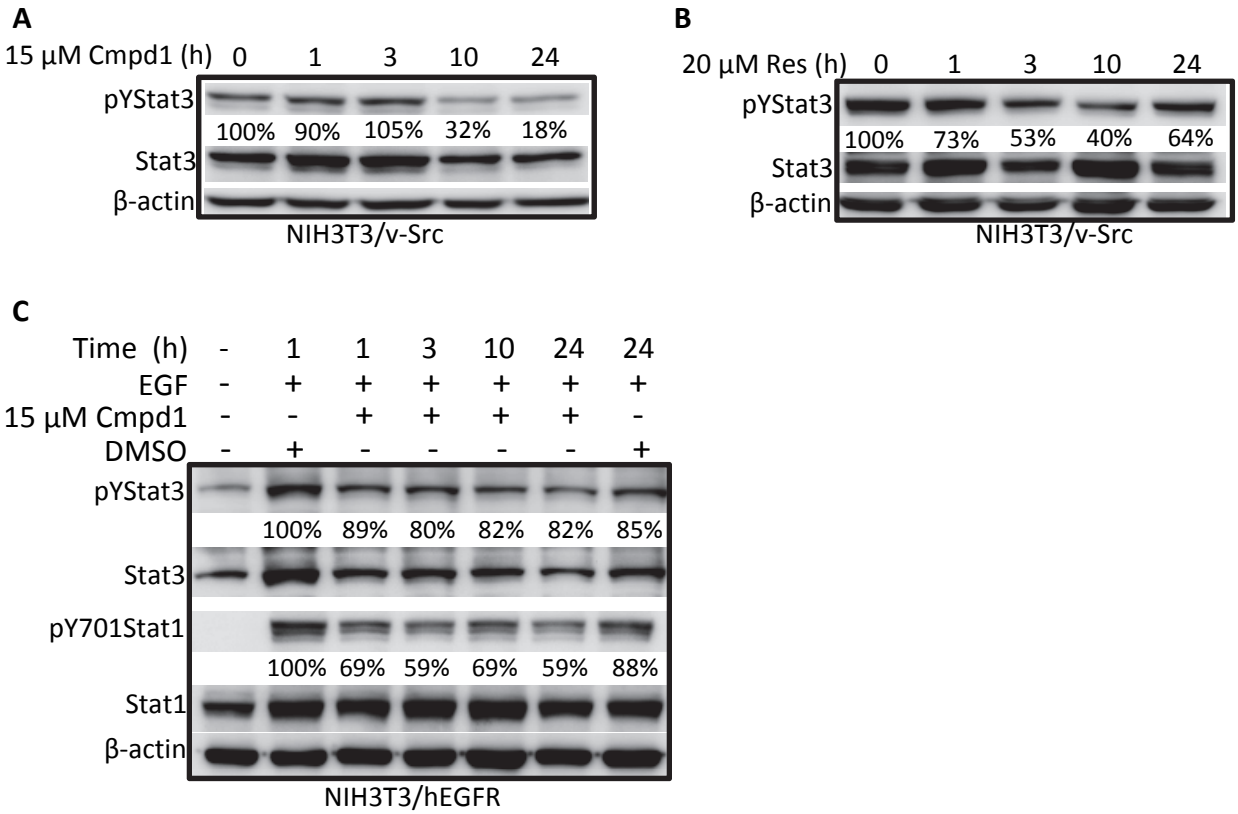

A
15 μM Cmpd1 (h) 0 1 3 10 24
pYStat3
100% 90% 105% 32% 18%
Stat3
β-actin
NIH3T3/v-Src
B
20 μM Res (h) 0 1 3 10 24
pYStat3
100% 73% 53% 40% 64%
Stat3
β-actin
NIH3T3/v-Src
C
Time (h) - 1 1 3 10 24 24
EGF - + + + + + +
15 μM Cmpd1 - - + + + + -
DMSO - + - - - - +
pYStat3
100% 89% 80% 82% 82% 85%
Stat3
pY701Stat1
100% 69% 59% 69% 59% 88%
Stat1
β-actin
NIH3T3/hEGFR

Fig. S3

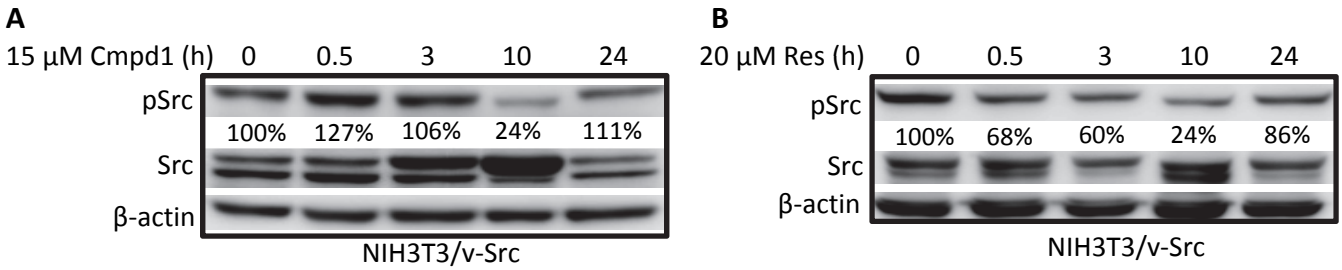
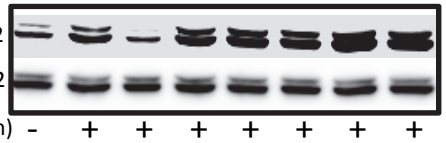

A
15 μM Cmpd1 (h) 0 0.5 3 10 24
pSrc
100% 127% 106% 24% 111%
Src
β-actin
NIH3T3/v-Src
B
20 μM Res (h) 0 0.5 3 10 24
pSrc
100% 68% 60% 24% 86%
Src
β-actin
NIH3T3/v-Src

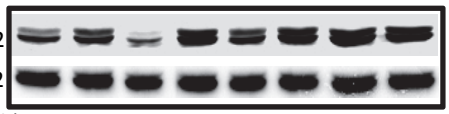
Fig. S4

A



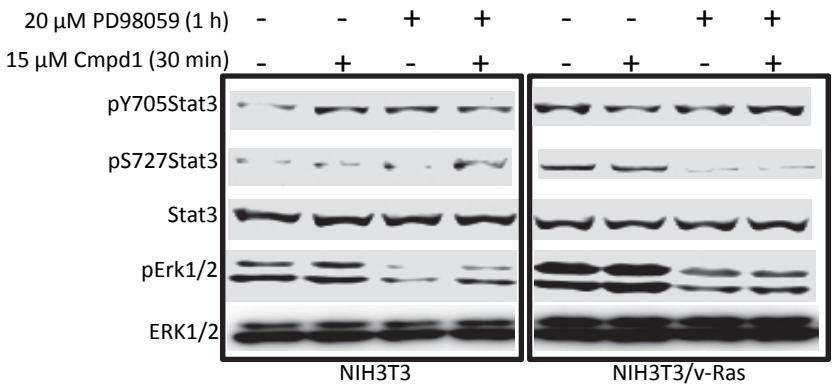
15 μM Cmpd1 (0.5 h)	-	+	+	+	+	+	+	+
20 μM PD98059 (1 h)	-	-	+	-	-	-	-	-
10 μM SB202190 (1 h)	-	-	-	+	-	-	-	-
0.1 μM LY (1 h)	-	-	-	-	+	-	-	-
0.1 μM Das (1 h)	-	-	-	-	-	+	-	-
0.1 μM Rap (1 h)	-	-	-	-	-	-	+	-
0.1 μM Staur (1 h)	-	-	-	-	-	-	-	+

B



20 μM PTE (0.5 h)	-	+	+	+	+	+	+	+
20 μM PD98059 (1 h)	-	-	+	-	-	-	-	-
10 μM SB202190 (1 h)	-	-	-	+	-	-	-	-
0.1 μM LY (1 h)	-	-	-	-	+	-	-	-
0.1 μM Das (1 h)	-	-	-	-	-	+	-	-
0.1 μM Rap (1 h)	-	-	-	-	-	-	+	-
0.1 μM Staur (1 h)	-	-	-	-	-	-	-	+

Fig. S5


20 μM PD98059 (1 h) - - + + - - + +
15 μM Cmpd1 (30 min) - + - + - + - +
pY705Stat3
pS727Stat3
Stat3
pErk1/2
ERK1/2
NIH3T3
NIH3T3/v-Ras