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Rhodanine-based PRL-3 inhibitors blocked the migration and invasion of metastatic cancer cells

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ABSTRACT

PRL-3, phosphatase of regenerating liver-3, plays a role in cancer progression through its involvement in invasion, migration, metastasis, and angiogenesis. We synthesized rhodanine derivatives, CG-707 and BR-1, which inhibited PRL-3 enzymatic activity with IC_{50} values of 0.8 μM and 1.1 μM, respectively. CG-707 and BR-1 strongly inhibited the migration and invasion of PRL-3 overexpressing colon cancer cells without exhibiting cytotoxicity. The specificity of the inhibitors on PRL-3 phosphatase activity was confirmed by the phosphorylation recovery of known PRL-3 substrates such as ezrin and cytokeratin 8. The compounds selectively inhibited PRL-3 in comparison with other phosphatases, and CG-707 regulated epithelial-to-mesenchymal transition (EMT) marker proteins. The results of the present study reveal that rhodanine is a specific PRL-3 inhibitor and a good lead molecule for obtaining a selective PRL-3 inhibitor.

Tumor progression is a complex process that includes malignant transformation, proliferation, invasion, and metastasis of cancer cells. Cancer cell invasion and metastasis are critical processes, and more than 90% of cancer deaths have been attributed to the metastatic spread of the disease rather than to the primary tumor.[1–3] Decades of intensive research have been focused on the search for therapeutic solutions targeting cancer cell migration, invasion, and angiogenesis.[4–6] From a clinical standpoint, the only major advance in the past 20 years has been the development of bone metastasis-targeting bisphosphonates and RANK-ligand inhibitors.

PRL-3, phosphatase of regenerating liver-3, belongs to the protein tyrosine phosphatase (PTP) family which regulates phosphorylation and dephosphorylation of many biologically significant molecules. The malfunction of PTP family members leads to various diseases including cancer, neurological disorder, and diabetes. Members of the PRL family, consisting of PRL-1, PRL-2, and PRL-3, are closely related and share the specific sequence CX_5R which is the PTP active site. PRL-3 is known to affect the cell cycle, cell proliferation, and differentiation, and it plays a role in cancer progression through its involvement in cancer cell invasion, migration, metastasis, and angiogenesis.[7,8] A relationship between PRL-3 and metastatic behaviors has been investigated since PRL-3 was shown to be expressed in 100% of metastatic colorectal tumor samples by Saha et al., in 2001.[9] PRL-3 expression was high in other metastatic tumors, but was low in non-metastatic carcinomas and normal epithelial cells. Increasing evidences from recent studies suggests that PRL-3 is an important regulator of cancer metastasis. Researches on PRL-3 have also tended to focus on metastasis-associated characteristics such as its stimulating effect on cell migration and invasion.[10] Recent studies showed that PRL-3 knockdown inhibited metastasis developments by decreasing the primary tumor size and also inhibited the invasion and growth of cancer cells.[11,12] Therefore, PRL-3 appears to be a diagnostic marker and a promising therapy target in cancer, especially colorectal cancer.

Rhodanine- and 1,3-thiazolidine-2,4-dione-based compounds have become a very important group of heterocyclic compounds in drug development, ever since the introduction of various glitazones into clinical use for the treatment of type II diabetes mellitus. Rhodanines have been reported to possess antibacterial, antifungal, antiviral, antimalarial, insecticidal, herbicidal, antitumor, anti-inflammatory and cardiotonic activities.[13,14] These rhodanine derivatives have been reported to inhibit cancer cell migration and the enzymatic activity of PRL-3.[15] In an effort to design and

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synthesize new rhodanine compounds as potential PRL-3 inhibitors, we have found that cinnamaldehyde-based rhodanines dramatically inhibit PRL-3 activity. A subsequent cell-based assay revealed that this structural modification allowed the compounds to inhibit human cancer cell migration and invasion. One such rhodanine derivative (CG-707) showed a strong ability to inhibit PRL-3 activity and cancer cell migration but did not exhibit apparent cellular toxicity in cell proliferation assays. This finding prompted us to design a series of rhodanine derivatives by varying the substitution groups on the cinnamaldehyde. We report here the preparation and biological activity evaluation of these new derivatives and a known PRL-3 inhibitor BR-1.

As shown in Figure 1, the designed derivatives were synthesized following the general procedures as detailed below. The condensation of rhodanine or thiazolidinedione with 2-substituted cinnamaldehydes by ammonium acetate and acetic acid afforded quantitative yield of rhodanines in refluxing toluene. After filtration, the product was recrystallized from acetone and hexane.

To determine the effects of the synthesized rhodanine derivatives on PRL-3 activity, we performed an in vitro DiFMUP assay with purified PRL-3 protein.[16,17] DiFMUP is a substrate for alkaline and acid phosphatase and its dephosphorylation yields fluorescence. Purified PRL-3 protein solution was added to the substrate and absorbance was read to measure the enzyme activity. As shown in Table 1, most of the synthetic derivatives displayed moderate or potent inhibitory activity against PRL-3, and 8 derivatives showed greater than 50% inhibition at a concentration of 5 μg/ml, except the thiazolidinedione derivative (CG-718). Both CG-707 and BR-1 dose-dependently inhibited the dephosphorylation of DiFMUP by PRL-3 as shown in Figure 2. CG-707 and BR-1 strongly inhibited PRL-3 phosphatase activity in a dose-dependent manner with IC_{50} values of 0.8 and 1.1 μM, respectively (Fig. 2). These data demonstrate that rhodanines are good lead molecules for PRL-3 inhibitors. Although we did not test the inhibitory activity of the compounds against PRL-1 and 2, rhodanines may not selectively inhibit PRL-3, because PRL-1, PRL-2, and PRL-3 share 76–87% amino acid sequence identity.[18]

Table 1
Biological activities of rhodanine derivatives

Compound	PRl-3 inhibition[a]	Migration inhibition[b]	Cell viability[c]
BR-1	65.2	95	98.1
CG-701	51.9	65	96.3
CG-702	30.6	40	99.2
CG-703	49.5	65	87.9
CG-704	52.8	73	75.5
CG-705	63.1	78	78.8
CG-706	40.8	80	41.5
CG-707	78.8	98	99.5
CG-708	52.9	82	87.1
CG-709	40.3	71	72.3
CG-710	64.2	80	83.1
CG-711	35.2	68	97.1
CG-712	53.9	80	81.9
CG-713	37.4	81	96.6
CG-714	41.7	69	98.8
CG-715	39.4	62	96.5
CG-716	47.1	45	105.4
CG-717	22.5	50	37.7
CG-718	3.8	45	37.2

[a] Inhibitory activity against PRL-3 at 5 μg/ml (%).
[b] Inhibition of DLD-1(PRL-3) cell migration at 10 μg/ml (%).
[c] Cell viability of DLD-1(PRL-3) cells at 10 μg/ml (%).

To determine the effects of the synthesized rhodanine derivatives on cancer cell migration, we prepared PRL-3 overexpressing DLD-1 colon tumor cells [DLD-1 (PRL-3)] because PRL-3 induces the invasion of human tumor cells.[19] We performed a transwell migration assay on each compound using the DLD-1 (PRL-3) cells. The cells were seeded at a density of 5×10^4 in serum-free medium in the upper chamber, with the lower chamber containing 5% FBS medium, and their ability to migrate in the presence and absence of the compounds was measured by counting the total

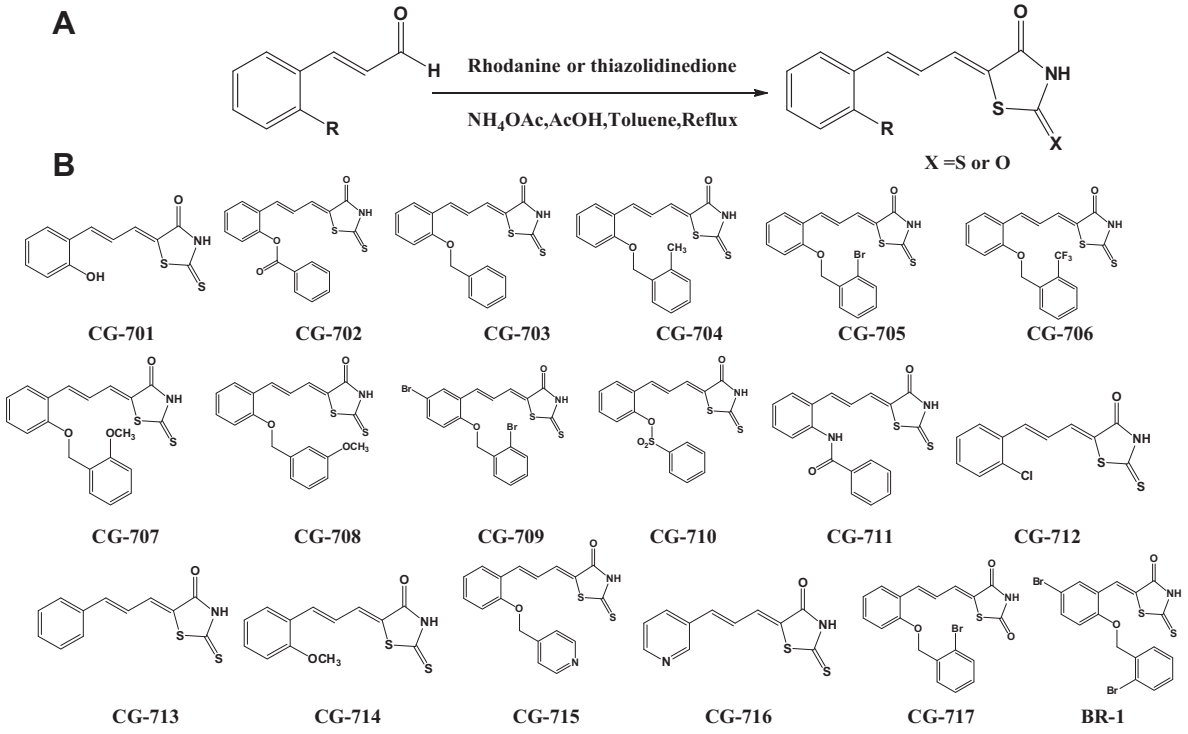

Figure 1. (A) Synthetic scheme. (B) Structures of the rhodanine derivatives.

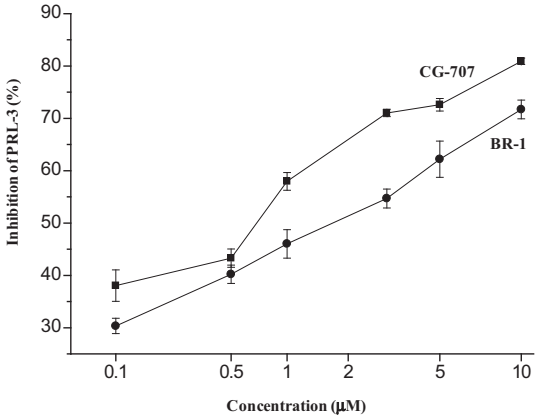

Figure 2. Inhibition of PRL-3 phosphatase activity by CG-707 and BR-1 was observed using a DiFMUP assay.

number of cells in the lower chamber after 20 h. As shown in Table 1, most of the synthetic derivatives strongly inhibited the migration of DLD-1 (PRL-3) cells at a 10 μg/ml concentration. The thiazolidinedione derivative (CG-18) displayed moderate anti-migratory activity but this compound causes cell cytotoxicity.

Given the potent anti-migration efficacy demonstrated by most of the rhodanine derivatives, we decided to study the dose-response of the most potent derivatives to obtain their IC_{50} values for suppressing the transwell migration of the DLD-1 (PRL-3) cells. As shown in Figure 3A, CG-707 and BR-1 inhibited the migration of DLD-1 (PRL-3) cells in a dose-dependent manner with IC_{50} values of 5 and 7 μM, respectively. After the cells were treated with 20 μM of the compounds, more than 90% of cell migration was blocked and cell invasion was blocked by over 60% at the same concentration (Fig. 3B).

We next performed proliferation assays on DLD-1 (PRL-3) cancer cells treated with the compounds to rule out any indirect effects on cell migration and invasion due to cytotoxicity. DLD-1 (PRL-3) cells were allowed to grow for 24 h in 96-well plates in the presence or absence of the synthetic compounds at a concentration of 10 μg/ml. The cell toxicity data for all derivatives are listed in Table 1, along with the anti-migration data for comparison. The 14 compounds all inhibited cell migration by over 60% (62–98%) without significant cell toxicity when the cells were treated with the derivatives at a dose of 10 μg/ml, the exception was CG-6, which significantly inhibited cell growth by approximately 60%. A few other anti-migratory derivatives greatly inhibited DLD-1 (PRL-3) cell proliferation. For example, CG-17 and CG-18 blocked cell migration (approximately 50%) and also inhibited the cell proliferation (approximately 63%). For these derivatives, cytotoxicity may have partially contributed to their overall anti-migratory effect. However, the potent PRL-3 inhibitors, CG-707 and BR-1, did not inhibit the proliferation of DLD-1 (PRL-3) cells, even at concentration as high as 50 μM (Fig. 3C). These results indicate that these compounds significantly inhibit cell migration and invasion without cytotoxicity and may be good lead molecules for designing a new PRL-3 inhibitor.

Despite of having very limited existing structure–activity relationships (SAR), the compounds with benzyl group showed more promising activity against PRL-3 compared with the other compounds, and a dramatic loss of inhibitory potency was seen when the rhodanine group was substituted by a thiazolidinedione (CG-18). The replacement of the oxybenzyl group with a sulfonylbenzyl (CG-10) provided more promising starting points for the design of a new PRL-3 inhibition, because CG-10 strongly inhibited both PRL-3 activity and the migration of DLD-1 (PRL-3) cells. However, compounds with an amide (CG-11) or a pyridine ring (CG-15 and 16) only moderately inhibited PRL-3 activity and DLD-1 (PRL-3) cell migration.

The recovery of phosphorylation was examined in an attempt to confirm the inhibition of PRL-3 phosphatase activity by CG-707 and BR-1. Two PRL-3 substrates were used: ezrin and cytokeratin 8. Ezrin, a direct target of PRL-3, interacts with radixin and moesin, constituting the ERM complex, which connects the actin cytoskeleton with cell surface receptor. Phosphorylation at amino acid Thr567 activates ezrin and controls the structural transitions of this protein, which plays a pivotal role in tumor progression and metastasis, therefore, we detected the residue phosphorylation of the Thr567 residue.[20,21] Cytokeratin 8 (KRT8) is reported to colocalize and interact with PRL-3 at lamellipodias, participating in cellular movement.[22] Treatment with 20 μM CG-707 or BR-1 in DLD-1 (PRL-3) cells increased the level of phospho-ezrin more than three-fold when compared with DMSO-treated cells. CG-707 and BR-1 also demonstrated the ability to rescue phospho-cytokeratin 8 which is a PRL-3 mediated effector protein (Fig. 4). These data suggests that CG-707 and BR-1 specifically inhibited the phosphatase activity of PRL-3.

The expression of PRL-3 has been shown to increase cell migratory and invasive ability. We reported that DLD-1 colon cancer cells overexpressing PRL-3 exhibited increased migration and invasion rates when compared with DLD-1 cells.[22] To observe the relationship between PRL-3 expression level and the anti-migratory activity of CG-707 and BR-1, the two compounds were treated to cell lines other than DLD-1 (PRL-3). The numbers of cells seeded were varied depending on the size and mobility of each cell line. The migration assay was stopped when the cells in a control well had moved and penetrated the membrane to approximately 70% confluence. As shown in Figure 5A, CG-707 and BR-1 inhibited the migration of DLD-1, A375P, MIA PaCa-2, and AsPC-1 cells which represent a colon cancer cell line, a melanoma cell line, and 2 pancreatic cancer cell lines, respectively.[17] However, the compounds did not affect the migration of DU 145 or MDA-MB-231, which have relatively low level of PRL-3 expression and are derived from prostate cancer and breast cancer, respectively. CG-707 and BR-1 strongly blocked the migration of A375P and MIA PaCa-2 cells which have higher endogenous PRL-3 expression levels than the cell lines such as DU 145 and MDA-MB-231, that were not affected by the compounds. These results showed that the anti-migratory effects of the compounds correlated with the PRL-3 expression level of each cell lines (Fig. 5B).

Our primary screen revealed a class of rhodanine molecules as potent PRL inhibitors. The structure and activities of selected compounds, CG-707 and BR-1, are shown in Figures 1 and 2. These compounds exhibited similar IC_{50} values, 0.8 μM for CG-707 and 1.1 μM for BR-1 in PRL-3 phosphatase assays, respectively. To examine the selectivity of these compounds, we further examined the inhibitory activities of the rhodanines by performing a phosphatase assay with 9 other phosphatases that represent different classes of phosphatases, including tyrosine and dual-specific phosphatases. As shown in Table 2, using DiFMUP as the substrate for all of the protein phosphatase (PTPase) assays, the compounds displayed excellent selectivity for the PRL-3 phosphatase, but showed minimal effects on other phosphatases.

EMT, epithelial-to-mesenchymal transition, is a critical step in the metastatic progression and invasion of cancer cells during which epithelial cells gain fibroblastic properties and lose cell adhesive ability. A decreased level of the epithelial cell marker protein, E-cadherin, resulted in a loss of cell-cell adhesion junctions and was followed by an increased invasiveness of tumor cells. It has been reported that PRL-3 is associated with EMT, where the overexpression of PRL-3 promotes the mesenchymal transition.[23] To observe the modulation of EMT marker proteins, such as E-cadherin

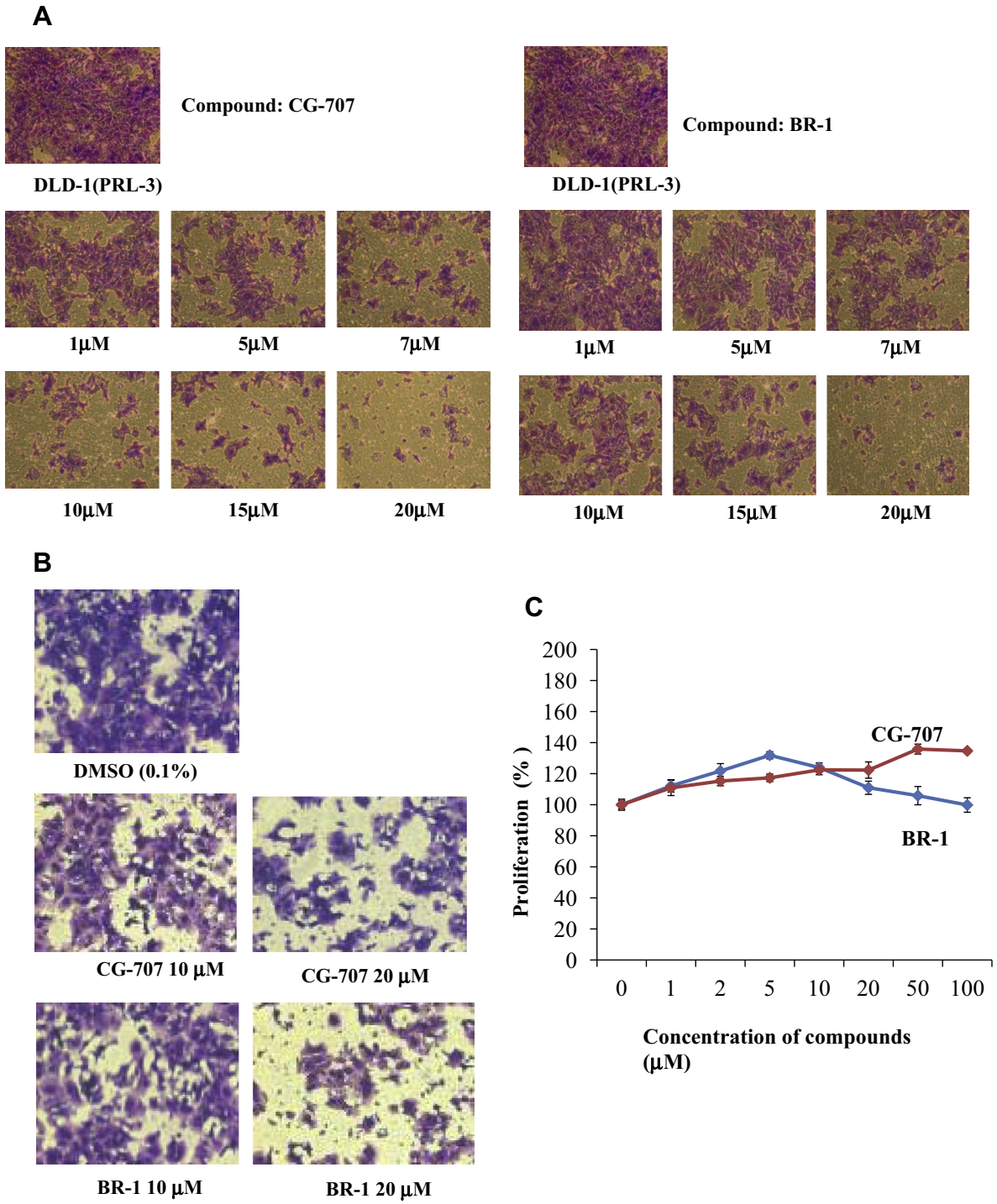

Figure 3. CG-707 and BR-1 inhibit the migration and invasion of DLD-1 cells overexpressing PRL-3. (A) The cell migration assay was performed with DLD-1 (PRL-3) cells that were treated with various concentrations of the compounds for 20 h. Migrating cells were stained with crystal violet and were counted under a light microscope. (B) The cell invasion assay was performed with DLD-1 (PRL-3) cells which were treated with various concentration of the compound for 20 h. Cell invasion was investigated using Matrigel-coated 8.0 μm filter invasion chambers. Invaded cells were stained with crystal violet and were counted under a light microscope. (C) The proliferation assay with DLD-1 (PRL-3) cells in the presence of the compound. The cells were seeded at a density of 5000 cells per well in 96-well plates in RPMI 1640 medium containing 10% FBS. The cells were incubated with fresh complete medium containing the compounds or 0.1% DMSO. After 24 h of incubation, the cell proliferation reagent WST-1 (Roche Applied Science) was added to each well. WST-1 formazan was quantitatively measured at 450 nm using an enzyme-linked immunosorbent assay reader (Bio-Rad). Each experiment was repeated three times, and each value is expressed as the mean ± SD. Statistical significance (p value) is $p < 0.01$.

and Snail, by CG-707, we observed changes in the EMT marker expression levels in DLD-1(PRL-3) cells. As shown in Figure 6, the E-cadherin expression level was decreased and Snail expression level was increased in DLD-1 (PRL-3) cells. In a phosphatase-activity-mutated cell line, DLD-1 (C104S), E-cadherin expression was increased and Snail expression was decreased in contrast to PRL-3 overexpressing cells. When the DLD-1 (PRL-3) cells were treated with the rhodanine compound, E-cadherin expression increased

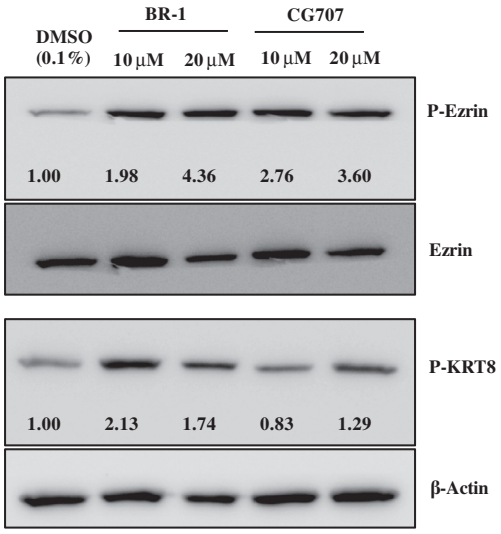

Figure 4. The phosphorylation rescue of ezrin and cytokeratin 8 (KRT8) after CG-707 or BR-1 treatment in DLD-1 (PRL-3) cells. The lysates of cells that were treated with the compound were prepared using RIPA lysis buffer (50 mM Tris, pH 7.0, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 1% deoxycholic acid, 0.1% SDS, 30 mM Na_2HPO_4, 50 mM NaF, and 1 mM $NaVO_4$) containing a protease inhibitor cocktail (Roche Applied Science). The protein (45 μg) was resolved using 7.5 or 10% SDS–PAGE and transferred to a PVDF membrane (Roche Applied Science). The membrane was blocked with 5% nonfat dried milk in TBS-T (50 mM Tris–HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween 20) and was probed with primary antibodies (Cell Signaling Technology) for 2–2.5 h. The blot was washed, exposed to HRP-conjugated anti-rabbit IgG for 1.5 h, and examined using chemiluminescence POD reagents (Roche Applied Science).

and snail expression decreased in a similar pattern to that seen in the phosphatase-activity-mutated cell line. The ability of CG-707 to block cell migration and invasion was confirmed by the changes in the expression level of EMT markers.

Table 2
Selectivity assays of CG-707 and BR-1 against PRL-3 and 9 other phosphatases using DiFMUP as a substrate

Phosphatase[a]	BR-1	CG-707
PRL-3	64	73
CD45	12	11
DUSP22	30	10
LMPTP-A	0	6
MKP5	5	1
PP2A	0	29
PTP-1B	11	8
SHP-2	27	18
TCPTP	9	1
VHR	7	3
YopH	14	9

[a] Inhibitory activity against phosphatases at 5 μM (%).

In summary, we have designed and synthesized 18 rhodanine derivatives, based on BR-1, to be novel anti-migration and anti-invasion agents. Structural modification of the substitution groups on the cinnamaldehyde group led to the identification of several potent PRL-3 inhibitors that strongly suppressed cell migration in metastatic cancer cells. The compounds rescued the phosphorylation of ezrin and KRT8, which are known PRL-3 substrates. More importantly, CG-707 and BR-1 exhibited no apparent cytotoxicity and displayed a selectivity that inhibited cancer cell migration in proportion to the level of PRL-3 expression. These data support the idea that the anti-migration activity of these compounds may be a result of the inhibition of PRL-3 activity in cells. Potent PRL3 inhibitors also selectively inhibited PRL-3, when compared other phosphatases, and modulated the expression of EMT marker proteins. Thus, our study provides a novel type of small molecule

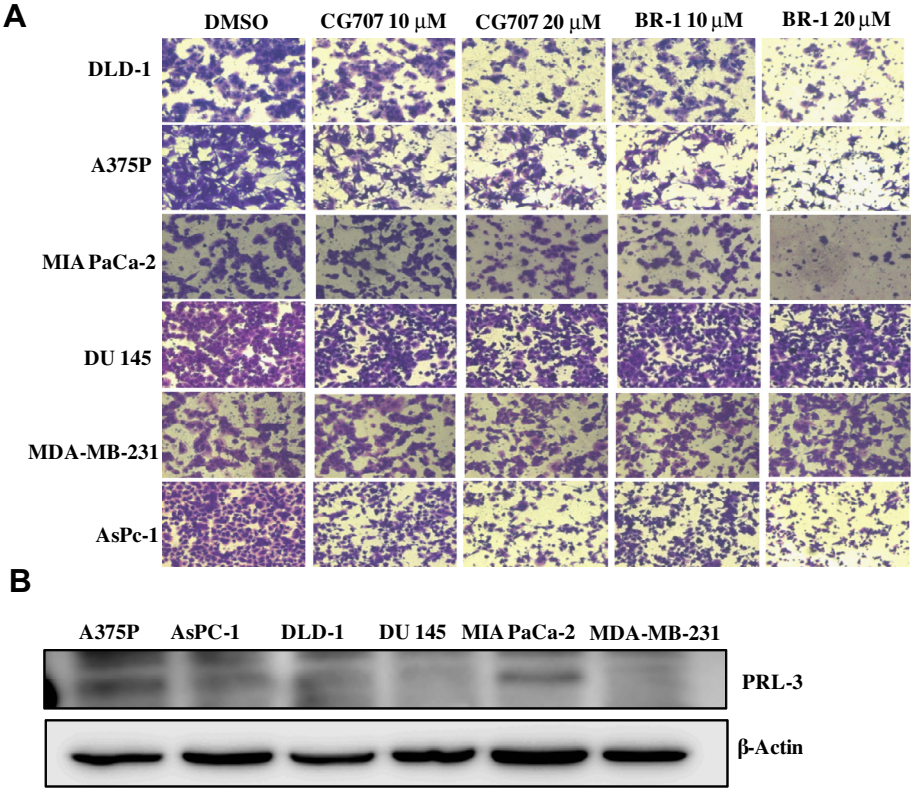

Figure 5. (A) Migration assay of CG 707 and BR-1 in various cancer cell lines. (B) Expression level of PRL-3 in various tumor cells.

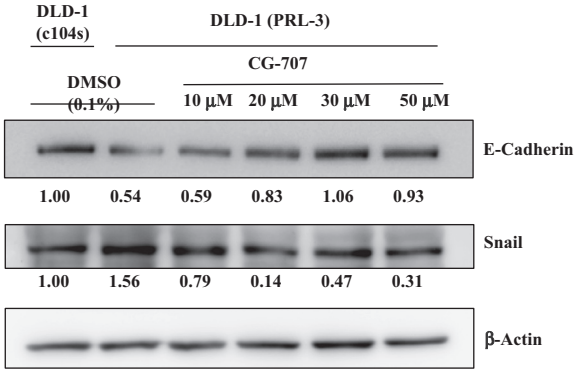

Figure 6. Protein expression levels of EMT markers, E-cadherin and Snail. Cells were seeded at a density of 60×10^4 in 60 mm plates. After a 24 h incubation, cells were treated with 0.1% DMSO or different concentrations of the compounds for another 24 h. Cells were lysed as described above. The protein (25 μg) was resolved using 7.5% SDS–PAGE and transferred to a PVDF membrane (Roche Applied Science). The membrane was blocked with 5% nonfat dried milk in TBS-T and incubated with primary antibodies (Abcam) overnight at 4 °C. The blot was washed, incubated with secondary anti-rabbit, anti-goat, and anti-mouse antibodies for 1.5 h, and examined using chemiluminescence POD reagents (Roche Applied Science).

therapeutic agents that aim to block cancer cell migration and invasion without exerting cell toxicity.

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References and notes

1. Chambers, A. F.; Groom, A. C.; MacDonald, I. C. *Nat. Rev. Cancer* **2002**, *2*, 563.
2. Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144*, 646–674.
3. Weiss, L. *Cancer Metastasis Rev.* **2000**, *19*, 193.
4. Guzinska-Ustymowicz, K.; Pryczynicz, A. *Anticancer Agents Med. Chem.* **2011**, *11*, 99.
5. Sethi, N.; Kang, Y. *Nat. Rev. Cancer* **2011**, *11*, 735.
6. Gaorav, P.; Gupta, G. P.; Massagué, J. *Cell* **2006**, *127*, 679.
7. Zeng, Q.; Dong, J. M.; Guo, K.; Li, J.; Tan, H. X.; Koh, V.; Pallen, C. J.; Manser, E.; Hong, W. *Cancer Res.* **2003**, *63*, 2716.
8. Waldmann, H.; Bialy, L. *Angew. Chem. Int., Ed.* **2005**, *24*, 814.
9. Saha, S.; Bardelli, A.; Buckhaults, P.; Velculescu, V. E.; Rago, C.; Croix, B. S.; Romans, K. E.; Choti, M. A.; Lengauer, C.; Kinzler, K. W.; Vogelstein, B. *Science* **2001**, *294*, 1343.
10. Bardelli, A.; Saha, S.; Sager, J. A.; Romans, K. E.; Xin, B.; Markowitz, S. D.; Lengauer, C.; Velculescu, V. E.; Kinzler, K. W.; Vogelstein, B. *Clin. Cancer Res.* **2003**, *9*, 5607.
11. Wang, L.; Shen, Y.; Song, R.; Sun, Y.; Xu, J.; Xu, Q. *Mol. Pharmcol.* **2009**, *76*, 1238.
12. Stephens, B. J.; Han, H.; Gokhale, V.; Von Hoff, D. D. *Mol. Cancer Ther.* **2005**, *4*, 1653.
13. Tomašić, T. Peterlin Mašič *L. Curr. Med. Chem.* **2009**, *16*, 1596.
14. Tomašić, T. Peterlin Mašič *L. Expert Opin. Drug Discov.* **2012**, *7*, 549.
15. Ahn, J. H.; Kim, S. J.; Park, W. S.; Cho, S. Y.; Ha, J. D.; Kim, S. S.; Kang, S. K.; Jeong, D. G.; Jung, S. K.; Lee, S. H.; Kim, H. M.; Park, S. K.; Lee, K. H.; Lee, C. W.; Ryu, S. E.; Choi, J. K. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2996.
16. Han, Y. M.; Lee, S. K.; Jeong, D. K.; Ryu, S. E.; Kim, D. K.; Kwon, B. M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 323.
17. Daouti, S.; Li, W. H.; Qian, H.; Huang, K. S.; Holmgren, J.; Levin, W.; Reik, L.; McGady, D. L.; Gillespie, P.; Perrotta, A.; Bian, H.; Reidhaar-Olson, J. F.; Bliss, S. A.; Olivier, A. R.; Sergi, J. A.; Fry, D.; Danho, W.; Ritland, S.; Fotouhi, N.; Heimbrook, D.; Niu, H. *Cancer Res.* **2008**, *68*, 1162.
18. Zeng, Q.; Hong, W.; Tan, Y. H. *Biochem. Biophys. Res. Commun.* **1998**, *244*, 421.
19. Lee, S. K.; Han, Y. M.; Yun, J.; Lee, C. W.; Shin, D. S.; Ha, Y. R.; Kim, J.; Koh, J. S.; Hong, S. H.; Han, D. C.; Kwon, B. M. *Int. J. Cancer* **2012**, *131*, E190.
20. Forte, E.; Orsatti, L.; Talamo, F.; Barbato, G.; De Francesco, R.; Tomei, L. *Biochim. Biophys. Acta* **2008**, *1783*, 334.
21. Ren, L.; Hong, S. H.; Chen, Q. R.; Briggs, J.; Cassavaugh, J.; Srinivasan, S.; Lizardo, M. M.; Mendoza, A.; Xia, A. Y.; Avadhani, N.; Khan, J.; Khanna, C. *Cancer Res.* **2012**, *1001*, 72.
22. Mizuuchi, E.; Semba, S.; Kodama, Y.; Yokozaki, H. *Int. J. Cancer* **1802**, *2009*, 124.
23. Wang, H.; Quah, S. Y.; Dong, J. M.; Manser, E.; Tang, J. P.; Zeng, Q. *Cancer Res.* **2007**, *67*, 2922.