ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

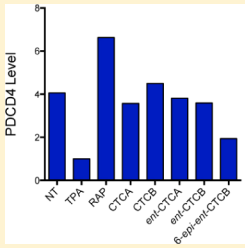
Cryptocaryol Structure–Activity Relationship Study of Cancer Cell Cytotoxicity and Ability to Stabilize PDCD4

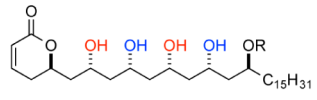
Michael F. Cuccarese,[†] Yanping Wang,[†] Penny J. Beuning,* and George A. O'Doherty*

Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115, United States

Supporting Information

ABSTRACT: The synthetic cryptocaryols A and B and a series of their analogues have been evaluated for their cytotoxicity and their ability to stabilize the tumor suppressor PDCD4. Cytotoxicities in the 3 to 30 μM range were found. Both the cytotoxicity and PDCD4 stabilizing ability were tolerant of large stereochemical changes to the molecule. Co-dosing studies with cryptocaryols A and B and several known cancer drugs showed no measuable enhancement in cancer drug cytotoxicity.



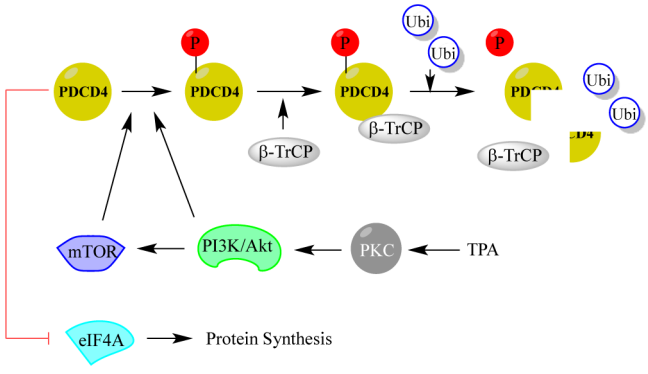


Compound	Cell Line, IC_{50} (μM) MCF-7	HT-29	H460
CTCA (R = H)	8.1	4.2	5.4
CTCB (R = Ac)	5.8	2.9	3.8

KEYWORDS: *PDCD4, PDCD4 stabilizer, cryptocaryols, cryptocaryol SAR*

Since the discovery of protein kinase C (PKC) as a potential cancer target, there has been a search for related downstream kinase targets (e.g., mTOR, Akt).[1] It is believed that modulation of these kinases will lead to selective tumor suppression.[2,3] Mouse epidermal cells that were resistant to tumor promotion were discovered to have elevated levels of programmed cell death 4 (PDCD4).[4] PDCD4 is regulated by mTOR[5] and Akt[6] and regulates protein synthesis by binding to translation initiation factor eIF4A.[7−9] Down-regulation of PDCD4 has been shown to increase translation and in turn tumor cell transformation and invasion (Scheme 1).[10,11]

Scheme 1. Tumor-Suppressive Effects of PDCD4/Inhibition of eIF4A[a]



[a]PDCD4 is an inhibitor of protein synthesis via inhibition of initiation factor eIF4A (red line). The E3 ubiquitin ligase β-TrCP binds to phosphorylated PDCD4, targeting it for ubiquitination and degradation via the 26S proteasome. TPA activates PKC, which initiates phosphorylation and degradation of PDCD4 via PI3K, Akt, or mTOR. Stabilizers of PDCD4 interfere with the degradation of PDCD4 (i.e., rapamycin inhibits mTOR, inhibiting PDCD4 phosphorylation).

Loss of PDCD4 is observed in lung, breast, colon, and prostate cancers.[12] Similarly, in a panel of 124 lung cancer patients, expression of PDCD4 in tumor cells was inversely related to poor prognosis.[13] Also, expression of PDCD4 has been shown to confer increased sensitivity to some anticancer drugs[14] and reduce the malignancy of ovarian cancer cells. Thus, PDCD4 is a target for the development of novel antineoplastic agents.[15]

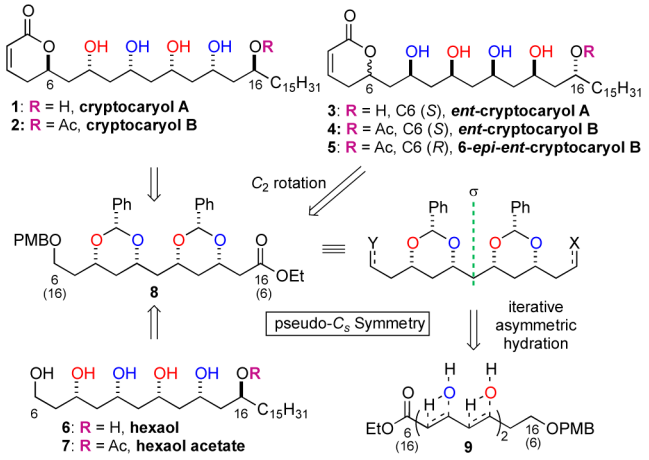
PDCD4 is degraded within the cell via a discrete pathway. Phosphorylation of PDCD4 by Akt leads to ubiquitination and proteasomal degradation.[16] This degradation (aka, destabilization) appears to be increased in some tumors.[17] Given the benefits of PDCD4 expression, stabilization of PDCD4 is an attractive way to elevate PDCD4 levels and holds the potential to increase cancer cell sensitivity to chemotherapy. Rapamycin is known to both stabilize PDCD4 and synergize with anticancer drugs;[18] unfortunately, the use of rapamycin (RAP) in cancer treatment is hindered by its immunosuppressive effects.[19] In an effort to find compounds that sensitize cells to cancer drugs, we have developed a synthesis of cryptocaryols A and B (CTCA and CTCB) as well as a series of analogues (Scheme 2).[20,21]

Cryptocaryols A and B are a class of natural products that share a 5,6-dihydro-α-pyranone and a 1,3-polyol segment. They were isolated from *Cryptocarya* spp. and identified by Gustafson in a high-throughput assay to stabilize PDCD4.[22] They are structurally interesting compounds in that the C-2 symmetry of the polyol can be leveraged to simplify the synthesis of cryptocaryol analogues, their enantiomers, and other analogues. Our interest in the cryptocaryols was three-fold. First, we were interested in elucidating their 3D structure by means of

Received: December 9, 2013
Accepted: February 17, 2014
Published: February 17, 2014

 © 2014 American Chemical Society dx.doi.org/10.1021/ml4005039 | ACS Med. Chem. Lett. 2014, 5, 522−526

Scheme 2. Retrosynthetic Analysis for Cryptocaryols A/B and Analogues[a]



[a]The seven cryptocaryol analogues were elucidated from the key intermediate **8**. In turn protected pentaol **8** with pseudo-C_s symmetry was synthesized from dienoate **9** with iterative asymmetric hydration (see Supporting Information).

asymmetric synthesis. Second, we were interested in determining the structure–activity relationship (SAR) as it relates to cancer cell cytotoxicity. Third, we were interested in studying their cytotoxicity and relating it to their ability to stabilize PDCD4. Recently, we disclosed our successful synthetic efforts at elucidating the structure of the cryptocaryols and providing material for its initial SAR studies. This effort led to the synthesis of cryptocaryols A (**1**) and B (**2**), their enantiomers **3** and **4**, and a diastereomer of cryptocaryol B (**5**), where all but one of the stereocenters were inverted. In addition, two analogue structures of cryptocaryols A and B, **6** and **7**, were prepared that lacked the pyranone ring. Herein, we detail our efforts to determine the cytotoxicity in three cell lines (MCF-7, HT-29, and H460), demonstrate/quantify their ability to stabilize PDCD4, and explore their potential to sensitize cancer cells to anticancer agents (camptothecin (CPT), digitoxin (DIG),[23−26] etoposide (ETO), 5-fluorouracil (5FU), and oxaliplatin (OXA)).

While other PDCD4 stabilizers are known to be cytotoxic, there is very little data to correlate their cytotoxicity to PDCD4 stabilization.[27] We chose three cancer cell lines to evaluate cytotoxicity for the seven polyols. Three cell lines selected for study were chosen based on their basal PDCD4 content as measured by RNA microarray and protein content analysis by immunoblot. The first cell line studied was MCF-7, which has been shown to have high expression levels of PDCD4. The second cell line chosen was HT-29, which has a medium expression level of PDCD4. The third cell line studied was H460, which has very low expression levels of PDCD4.[14]

Both cryptocaryols A and B possessed growth inhibitory activity against the three cell lines in the micromolar range. The relative cytotoxicity of the cryptocaryols was consistent with their PDCD4 stabilizing activity (i.e., **2** slightly more active than **1**) for each cell line; however, the cell line sensitivity to a given compound did not correlate with the cell line's PDCD4 expression levels. That is to say, HT-29 cell lines, with the medium level of PDCD4 expression, were the most sensitive (e.g., 4.2 μM for **1**); whereas MCF-7 cells, with the highest level of PDCD4 expression, were the least sensitive (e.g., 8.1 μM for **1**). This trend held true for cryptocaryols A and B (**1**/**2**) as well as the diastereomers **3**–**5**. The pyranone functionality was identified to be an important pharmacophore, as the two analogues, **6** and **7**, without a pyranone ring lost cytotoxicity (>30-fold). The stereochemistry of the pyranone ring has some importance for cytotoxicity, as the diastereomer **5** (with only the C-6 pyrano-stereocenter retained) had a small loss in cytotoxicity (~2-fold). The effect of C-16 acylation could be seen in the comparison between cryptocaryols A and B (**1**/**2**) and **6**/**7**, which lacked the pyranone ring. Surprisingly, the stereochemistry of natural products did not have a significant effect on cytotoxicity as *ent*-cryptocaryol B (**3**) and

Table 1. PDCD4 Stabilization and Cytotoxicity Data

	[rel.]	cell line, IC_{50} (μM)[b]		
compd (1 μM)	PDCD4[a]	MCF-7	HT-29	H460
CTCA (**1**)	3.6	8.1	4.2	5.4
CTCB (**2**)	4.5	5.8	2.9	3.8
ent-CTCA (**3**)	3.8	25.8	4.1	7.9
ent-CTCB (**4**)	3.6	9.0	2.4	4.0
6-*epi*-*ent*-CTCB (**5**)	1.9	13.3	4.9	7.8
hexaol (**6**)	1.8	>500	>1500	>1500
hexaol Ac (**7**)	2.8	162	1278	>1000

[a]PDCD4 stabilization is presented as a relative value over cells treated only with TPA (see Figure 1). [b]IC_{50} was determined via MTT colorimetric analysis and curve fitting in Graphpad Prism. The most cytotoxic cryptocaryol analogue CTCB is also the best PDCD4 stabilizer.

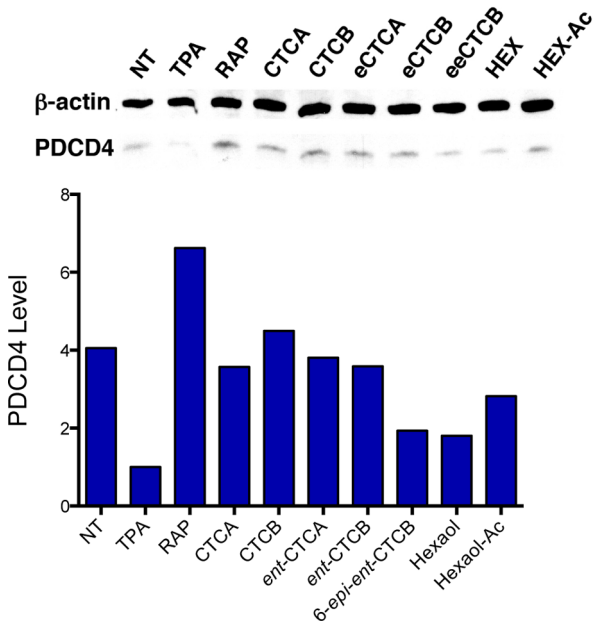


Figure 1. MCF-7 cells were seeded at 100,000 cells per well in 12-well plates. After 24 h, cells were treated with RAP (10 nM) or cryptocaryol analogues (1 μM). After 30 min, cells were treated with TPA (20 nM). After an additional 6 h, cells were harvested by scraping and lysed with RIPA buffer. Gel electrophoresis was carried out with 16 μL of cell lysate on a 12% polyacrylamide gel and transferred to a PVDF membrane. Western analysis was performed with antibodies for PDCD4 (Abcam ab87678) and β-actin (Abcam ab8226), and visualized with chemiluminescence. Contrast was adjusted uniformly over the image for clarity. Band density measurements (Imagestudio) were made prior to contrast-adjustment. Bars are band density relative to TPA-only, which was set to 1.

dx.doi.org/10.1021/ml4005039 | ACS Med. Chem. Lett. 2014, 5, 522−526

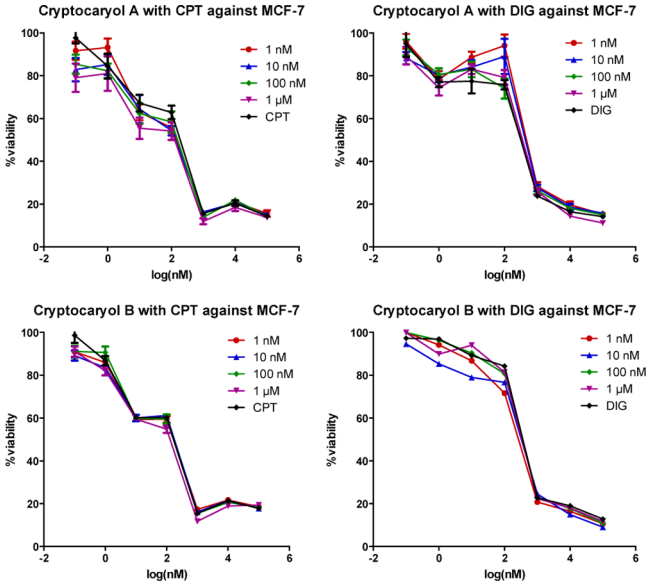


Figure 2. Effect of CTCA and CTCB on the cytotoxicity of CPT and DIG against MCF-7 cells. Cells were pretreated with 1 nM to 1 μM CTCA or CTCB followed by CPT or DIG, and cytotoxicity was measured by MTT after 72 h. No enhancement of anticancer activity was observed following treatment with cryptocaryols.

Table 2. Co-dosing Effects of the Cryptocaryols in MCF-7 Cells

co-drug	IC_{50} (10^2 nM)[a]			
	CTCA		CTCB	
drug	CPT	DIG	CPT	DIG
0 nM	1.39	3.27	0.99	2.04
1 nM	1.24	4.35	0.86	3.46
10 nM	1.33	3.06	1.05	2.75
100 nM	1.08	4.74	1.38	2.86
1 μM	0.81	4.86	1.13	2.99
CI @ ED_{75}[b]	0.78	1.34	1.12	1.04
Rel. PDCD4[c]	3.6		4.5	

[a]IC_{50} for CPT and DIG in combination with cryptocaryols. [b]CI @ ED_{75} was calculated via Chou–Talalay (Calcusyn). No synergistic relationship was observed. [c]Cryptocaryols with the highest PDCD4 stabilization activity were chosen. PDCD4 stabilization activity does not enhance the cytotoxicity of CPT or DIG in MCF-7.

ent-cryptocaryol A (**4**) had only a 2- to 3-fold loss in cytotoxicity.

In addition, the seven compounds were also evaluated for their ability to stabilize PDCD4. This analysis by immunoblot followed the protocol described by Tobias Schmid,[27] which uses TPA to initiate PDCD4 degradation, with the known PDCD4 stabilizer, rapamycin, as the positive control. The high expression level of MCF-7[13] made it the ideal cell line for PDCD4 stabilization studies. These results are outlined in Table 1 and Figure 1. Cryptocaryols A and B both showed significant ability to stabilize PDCD4 levels, with cryptocaryol B being a slightly better stabilizer than A, which was in line with the results found by Gustafson's high throughput screen. To our surprise, the cryptocaryol diastereomers also showed the ability to stabilize PDCD4 levels. It is also noteworthy that both hexaol **6** and hexaol acetate **7**, which lack the pyranone ring, retained significant PDCD4 stabilizing ability, while essentially losing all cytotoxicity (>10-fold). Once again, the polyol with the C16 acetate was a better stabilizer than the one without the acetate.

Encouraged by the significant PDCD4 stabilization, we further explored the potential use of cryptocaryols in combination with other anticancer drugs to determine if PDCD4 stabilization could result in an enhanced anticancer effect. These studies were carried out in both MCF-7 (Figures 2 and 3) and HT-29 (Figure 4) cell lines. The co-dosing studies were performed first with just the combination of cryptocaryol and cancer drug and later with the addition of TPA, which might better mimic the tumor environment where PDCD4 is more rapidly degraded.

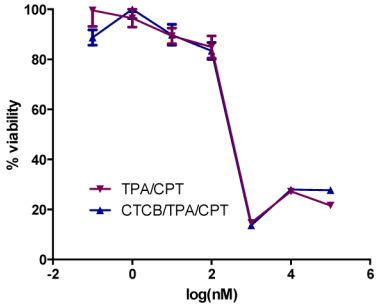


Figure 3. Changes in CTC/anticancer drug profiles depending on the presence of 20 nM of TPA. When MCF-7 cells are treated with 1 μM CTCA or CTCB and anticancer drugs, the presence of TPA has no effect, indicating that the degradation of PDCD4 with TPA and recovery with CTCB does not play a role in cytotoxicity.

Table 3. Co-Dosing Effect of CTCB and CPT in the Presence of TPA in MCF-7[a]

treatment	CPT IC_{50} (10^2 nM)
no combination	1.41
20 nM TPA	2.14
1 μM CTCB + TPA	2.02

[a]An increase in cell viability was observed in the presence of TPA, whereas pretreatment with CTCB had no further effect on cytotoxicity of CPT. Drugs with lower cytotoxicity (ETO, 5FU, and OXA; see Supporting Information) were also tested, but accurate IC_{50} measurements could not be calculated.

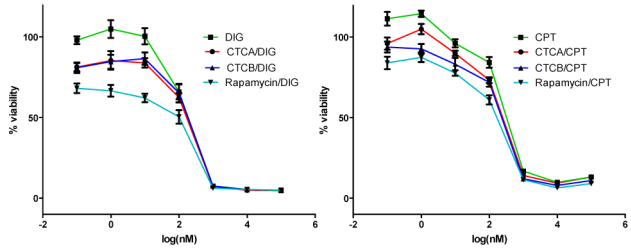


Figure 4. HT-29 cells were pretreated with PDCD4 stabilizers CTCA, CTCB (1 μM), and rapamycin (10 nM) followed by DIG or CPT.

We initially studied whether simple co-dosing of cryptocaryols A and B would reveal a synergistic effect in MCF-7 cells. Adopting a drug-combination strategy from our gentamicin-induced sensitization studies,[28] our results are outlined in Figure 2 and Table 2. MCF-7 cells were treated with several concentrations of CTCA and CTCB followed by CPT and DIG. Similar results were observed with HT-29 cells (Figure 4).

dx.doi.org/10.1021/ml4005039 | ACS Med. Chem. Lett. 2014, 5, 522–526

Table 4. Perturbation in IC_{50} for CPT and DIG in the Presence of CTCA, CTCB or Rapamycin; No Significant Change in IC_{50} was Observed

co-drug	PDCD4 stabil.	drug, IC_{50} (10^2 nM)	
		CPT	DIG
no co-drug	0	2.07	1.52
1 μM CTCA (**1**)	3.6	1.87	2.04
1 μM CTCB (**2**)	4.5	2.14	2.24
10 nM rapamycin	6.6	1.84	2.00

No enhancement of cytotoxicity for either anticancer drug was observed. Chou–Talalay analysis of this data gave CI @ ED_{75} values in the range of 1.3 to 0.8, which are consistent with no synergistic relationship between the two drugs.

To further probe for a sensitization for the PDCD4 stabilizers we decided to perform the co-dosing studies in the presence of TPA, which reduces PDCD4 levels by interaction with PKC. This experiment was performed for four cancer drugs (CPT, ETO, 5FU, and OXA), but only clean dose–response curves could be obtained for camptothecin (Figure 3). Thus, MCF-7 cells were exposed to a range of camptothecin doses in the presence of TPA (20 nM) and CTCB (1 μM). Using similar conditions to our Western blot studies (i.e., where PDCD4 stabilization was observed), the cells were dosed with CTCB 30 min prior to the addition of TPA. After an additional 8 h, cells were treated with a range of CPT concentrations. Under these conditions, no sensitization effect could be seen. In fact, TPA had a larger protective effect than cryptocaryol B (Table 3).

Using the optimized conditions we found for MCF-7 (Table 2, entry 5), we also screened for a sensitizing effect in HT-29 cells. With 12 h pretreatment with PDCD4 stabilizers, sensitivity of HT-29 cells to 5FU and CPT was unchanged and appeared to have an antagonistic effect with DIG. The HT-29 cell line was the most sensitive to the cryptocaryols (Figure 4 and Table 4) and expressed a moderate level of PDCD4. These studies were conducted with 1 μM cryptocaryols A or B and at a range of cancer drug doses (camptothecin and digitoxin) without the use of TPA. As was observed for MCF-7, no enhancement in cytotoxicity between either cryptocaryols (CTCA and CTCB) and anticancer drugs (CPT and DIG) could be observed. This dose–response assay was also performed for CPT with CTCB in the presence of TPA, once again no significant enhancement in cytotoxicity was observed (see Supporting Information). Similar studies were also carried out with 5FU, but these gave inconclusive dose–response curves without observable IC_{50}s.[21] Interestingly, rapamycin, a well-known PDCD4 stabilizer, also showed no significant enhancement in this co-dosing cytotoxicity assay.

In summary, we have determined the cytotoxicity of both cryptocaryols A and B in three cell lines as well as for several analogues. In addition, the ability to stabilize the tumor suppressor PDCD4 was also determined for these compounds. Rudimentary structure–activity relationships could be drawn as structures with the best PDCD4 stabilizing ability tended to be the most cytotoxic. However, changes to the structures that removed cytotoxicity (e.g., **6** and **7**) did not completely remove the PDCD4 stabilizing activity. To our surprise, both the cytotoxicity and PDCD4 stabilizing ability were tolerant to changes in stereochemistry, as enantiomers (e.g., **3** and **4**) and diastereomers (e.g., **5**) retained significant activity in both assays. For the two most potent PDCD4 stabilizers, cryptocaryols A and B, no sensitization effects could be seen in co-dosing studies with several cancer drugs (CPT, DIG, and 5FU) in two different cancer cell lines (MCF-7 and HT-29). Further efforts to elucidate the mechanism of action for this class of natural products are ongoing.

■ ASSOCIATED CONTENT

Supporting Information

Assay protocols, statistical analysis data, synthetic procedures, characterization data, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*(G.A.O.) E-mail: g.odoherty@neu.edu.

*(P.J.B.) E-mail: p.beuning@neu.edu.

Author Contributions

†Co-first authors, the order is alphabetical. The manuscript was written with contributions from all authors. M.F.C. and Y.W. performed all experiments. P.J.B. and G.A.O. developed the hypotheses and aims.

Funding

We thank NIH (GM090259), NSF (CHE-1213596), and the American Cancer Society (RSG-12-161-01-DMC) for their support of our research.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Prof. Yon Rojanasakul (West Virginia University) and Dr. Todd A. Stueckle (West Virginia University) for the cancer cell lines (i.e., NCI-H460, A549, and MCF-7).

■ ABBREVIATIONS

FBS, fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate; PVDF, polyvinylidene fluoride

■ REFERENCES

(1) Mochly-Rosen, D.; Das, K.; Grimes, K. V. Protein kinase C, an elusive therapeutic target? *Nat. Rev. Drug. Discovery* **2012**, *11*, 937–957.

(2) Watanabe, R.; Wei, L.; Huang, J. mTOR signaling, function, novel inhibitors, and therapeutic targets. *J. Nucl. Med.* **2011**, *52*, 497–500.

(3) Cheng, J. Q.; Lindsley, C. W.; Cheng, G. Z.; Yang, H.; Nicosia, S. V. The Akt/PKB pathway: Molecular target for cancer drug discovery. *Oncogene* **2005**, *24*, 7482–7492.

(4) Yang, H. S.; Jansen, A. P.; Nair, R.; Shibahara, K.; Verma, A. K.; Cmarik, J. L.; Colburn, N. H. A novel transformation suppressor, Pdcd4, inhibits AP-1 transactivation but not NF-kappaB or ODC transactivation. *Oncogene* **2001**, *20*, 669–676.

(5) Schmid, T.; Bajer, M. M.; Blees, J. S.; Eifler, L. K.; Milke, L.; Rübsamen, D.; Schulz, K.; Weigert, A.; Baker, A. R.; Colburn, N. H.; Brüne, B. Inflammation-induced loss of Pdcd4 is mediated by phosphorylation-dependent degradation. *Carcinogenesis* **2011**, *32*, 1427–1433.

(6) Palamarchuk, A.; Efanov, A.; Maximov, V.; Aqeilan, R. I.; Croce, C. M.; Pekarsky, Y. Akt phosphorylates and regulates Pdcd4 tumor suppressor protein. *Cancer Res.* **2005**, *65*, 11282–11286.

(7) Suzuki, C.; Garces, R. G.; Edmonds, K. A.; Hiller, S.; Hyberts, S. G.; Marintchev, A.; Wagner, G. PDCD4 inhibits translation initiation by binding to eIF4A using both its MA3 domains. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3274–3279.

(8) Carayol, N.; Katsoulidis, E.; Sassano, A.; Altman, J. K.; Druker, B. J.; Platanias, L. C. Suppression of programmed cell death 4 (PDCD4) protein expression by BCR-ABL-regulated engagement of the mTOR/p70 S6 kinase pathway. *J. Biol. Chem.* **2008**, *283*, 8601−8610.
(9) Dorrello, N. V.; Peschiaroli, A.; Guardavaccaro, D.; Colburn, N. H.; Sherman, N. E.; Pagano, M. S6K1- and βTRCP-mediated degradation of PDCD4 promotes protein translation and cell growth. *Science* **2006**, *314*, 467−471.
(10) Yang, H. S.; Jansen, A. P.; Komar, A. A.; Zheng, X. J.; Merrick, W. C.; Costes, S.; Lockett, S. J.; Sonenberg, N.; Colburn, N. H. The transformation suppressor Pdcd4 is a novel eukaryotic translation initiation factor 4A binding protein that inhibits translation. *Mol. Cell. Biol.* **2003**, *23*, 26−37.
(11) Wang, Q.; Sun, Z.; Yang, H. S. Downregulation of tumor suppressor Pdcd4 promotes invasion and activates both [beta]-catenin/Tcf and AP-1-dependent transcription in colon carcinoma cells. *Oncogene* **2007**, *27*, 1527−1535.
(12) Göke, A.; Göke, R.; Knolle, A.; Trusheim, H.; Schmidt, H.; Wilmen, A.; Carmody, R.; Göke, B.; Chen, Y. H. DUG is a novel homologue of translation initiation factor 4G that binds eIF4A. *Biochem. Biophys. Res. Commun.* **2002**, *297*, 78−82.
(13) Chen, Y.; Knösel, T.; Kristiansen, G.; Pietas, A.; Garber, M. E.; Matsuhashi, S.; Ozaki, I.; Petersen, I. Loss of PDCD4 expression in human lung cancer correlates with tumour progression and prognosis. *J. Pathol.* **2003**, *200*, 640−646.
(14) Jansen, A. P.; Camalier, C. E.; Stark, C.; Colburn, N. H. Characterization of programmed cell death 4 in multiple human cancers reveals a novel enhancer of drug sensitivity. *Mol. Cancer Ther.* **2004**, *3*, 103−110.
(15) Lankat-Buttgereit, B.; Göke, R. Programmed cell death protein 4 (PDCD4): A novel target for antineoplastic therapy? *Biol. Cell* **2003**, *95*, 515−519.
(16) Sonenberg, N.; Pause, A. Signal trnasduction: Protein synthesis and oncogenesis meet again. *Science* **2006**, *314*, 428−429.
(17) Schmid, T.; Jansen, A. P.; Baker, A. R.; Hegamyer, G.; Hagan, J. P.; Colburn, N. H. Translation inhibitor PDCD4 is targeted for degradation during tumor promotion. *Cancer Res.* **2008**, *68*, 1254−1260.
(18) Buck, E.; Eyzaguirre, A.; Brown, E.; Petti, F.; McCormack, S.; Haley, J. D.; Iwata, K. K.; Gibson, N. W.; Griffin, G. Rapamycin synergizes with the epidermal growth factor receptor inhibitor erlotinib in non-small-cell lung, pancreatic, colon, and breast tumors. *Mol. Cancer Ther.* **2006**, *5*, 2676−2684.
(19) Sehgal, S. N. Rapamune (RAPA, rapamycin, sirolimus): mechanism of action immunosuppressive effect results from blockade of signal transduction and inhibition of cell cycle progression. *Clin. Biochem.* **1998**, *31*, 335−340.
(20) Wang, Y.; O'Doherty, G. A.; Cryptocaryols, A.; Total, B. Cryptocaryols A and B: Total Syntheses, Stereochemical Revision, Structure Elucidation, and Structure−Activity Relationship. *J. Am. Chem. Soc.* **2013**, *135*, 9334−9337.
(21) For other PDCD4 stablizers, see: Zhao, L.-X.; Huang, S.-X.; Tang, S.-K.; Jiang, C.-L.; Duan, Y.; Beutler, J. A.; Henrich, C. J.; McMahon, J. B.; Schmid, T.; Blees, J. S.; Colburn, N. H.; Rajski, S. R.; Shen, B. Actinopolysporins A−C and tubercidin as a PDCD4 stabilizer from the halophilic actinomycete actinopolyspora erythraea YIM 90600. *J. Nat. Prod.* **2011**, *74*, 1990−1995.
(22) Grkovic, T.; Blees, J. S.; Colburn, N. H.; Schmid, T.; Thomas, C. L.; Henrich, C. J.; McMahon, J. B.; Gustafson, K. R. Cryptocaryols A−H, α-pyrone-containing 1,3-polyols from *Cryptocarya* sp. implicated in stabilizing the tumor suppressor Pdcd4. *J. Nat. Prod.* **2011**, *74*, 1015−1020.
(23) Pongrakhannon, V.; Stueckle, T. A.; Wang, H.-Y. L.; O'Doherty, G. A.; Dinu, C. Z.; Chanvorachote, P.; Rojanasakul, Y. Monosaccharide digitoxin derivatives sensitize human non-small cell lung cancer cells to anoikis through Mcl-1 proteasomal degradation. *Biochem. Pharmacol.* **2013**, DOI: 10.1016/j.bcp.2013.10.027.
(24) Hinds, J. W.; McKenna, S. B.; Sharif, E. U.; Wang, H.-Y. L.; Akhmedov, N. G.; O'Doherty, G. A. C3′/C4′-Stereochemical effects of digitoxigenin α-L-/α-D-glycoside in cancer cytotoxicity. *ChemMedChem* **2013**, *8*, 63−69.
(25) Wang, H.-Y. L.; Wu, B.; Zhang, Q.; Kang, S.-W.; Rojanasakul, Y.; O'Doherty, G. A. C5′-Alkyl substitution effects on digitoxigenin α-L-glycoside cancer cytotoxicity. *ACS Med. Chem. Lett.* **2011**, *2*, 259−263.
(26) Iyer, A.; Zhou, M.; Azad, N.; Elbaz, H.; Wang, L.; Rogalsky, D. K.; Rojanasakul, Y.; O'Doherty, G. A.; Langenhan, J. M. A direct comparison of the anticancer activities of digitoxin MeON-neoglycosides and O-glycosides: Oligosaccharide chain length-dependant induction of caspase-9-mediated apoptosis. *ACS Med. Chem. Lett.* **2010**, *1*, 326−330.
(27) Blees, J. S.; Bokesch, H. R.; Rubsamen, D.; Schulz, K.; Milke, L.; Bajer, M. M.; Gustafson, K. R.; Henrich, C. J.; McMahon, J. B.; Colburn, N. H.; Schmid, T.; Brune, B. Erioflorin stabilizes the tumor suppressor PDCD4 by inhibiting its interaction with the E3-ligase beta-TrCP1. *PLoS One* **2012**, *7*, e46567.
(28) Cuccarese, M. F.; Singh, A.; Amiji, M.; O'Doherty, G. A.; Novel, A. Use of gentamicin in the ROS-mediated sensitization of NCI-H460 lung cancer cells to various anticancer agents. *ACS Chem. Biol.* **2013**, *6*, 2771−2777.