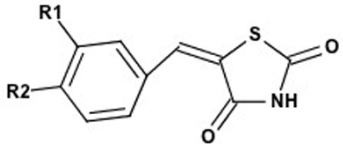
SUPPLEMENTAL DATA

Table S1. Structures of benzylidene-thiazolidine-2,4-diones compounds.



Compound	R_1	R_2
1a	CH_3	H
2a	H	CH_3
3a	CF_3	H
4a	H	CF_3
8a	C_2H_5	H
9a	$(CH_3)_2CH$	H
10a	CH_3O	H
11a	CF_3O	H
12a	H	CH_3O
13a	H	CF_3O
14a	C_2H_5O	H
15a	H	CF_2HCF_2O
16a	C_3H_7O	H
17a	$(CH_3)_2N$	H
18a	F	H
20a	H	F
21a	Cl	H
22a	H	Cl
23a	Br	H
24a	H	Br

SUPPLEMENTAL DATA FIGURE LEGENDS:

Figure S1. *A*, Comparison of Pim-1 and DYRK1a in vitro kinase activity with peptide substrates. Pim-1 and DYRK1a activity were measured using the coupled kinase assay as described in Materials and Methods with recombinant DYRK1a (Millipore, Billerica, MA) and Woodtide (Millipore, Billerica, MA) as the substrate. Compounds (**4a**, **16a**, harmine) were added at 5 μM and values shown with the addition of inhibitors represent the percent activity remaining compared to DMSO control. Some combinations were not tested (NT). *B*, growth inhibition of MV4;11 cells treated for 72 h with Pim inhibitors (5 μM), harmine (5 μM) with or without rapamycin (5 nM). Leukemic cells were treated, an MTS assay perfomed as described in Methods and Materials and data analyzed as described in Figure 1C.

Figure S2. Amino acid sequence alignment of DYRK1a, Pim-1, and Pim-2. Amino acids identified to contribute to potential inhibitor interactions in the ATP-binding domain of Pim-1 and DYRK1a are shown in bold and numbered with respect to the Pim-1 amino acid sequence. The hinge region is denoted as amino acids 121-128.

Figure S3. The Pim inhibitors **4a** and **16a** reduce the level of c-Myc protein in the human leukemic cell line K562. Cells were treated for 4 h with **4a** or **16a** (5 μM) under serum-free conditions and the level of c-Myc protein was determined by SDS-PAGE followed by Western blotting.

Figure S1

A

	Bad peptide (RSRHSSYPAGT)			Woodtide (KKISGRLSPIMTEQ)			
	none	4a	16a	none	4a	16a	harmine
Pim-1	100	2	8	0	NT	NT	NT
DYRK1a	0	NT	NT	100	0	13	4

B

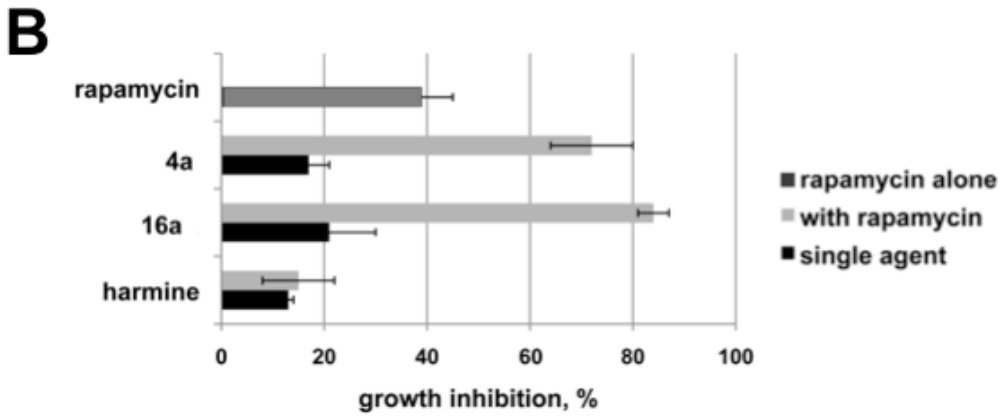


Figure S2

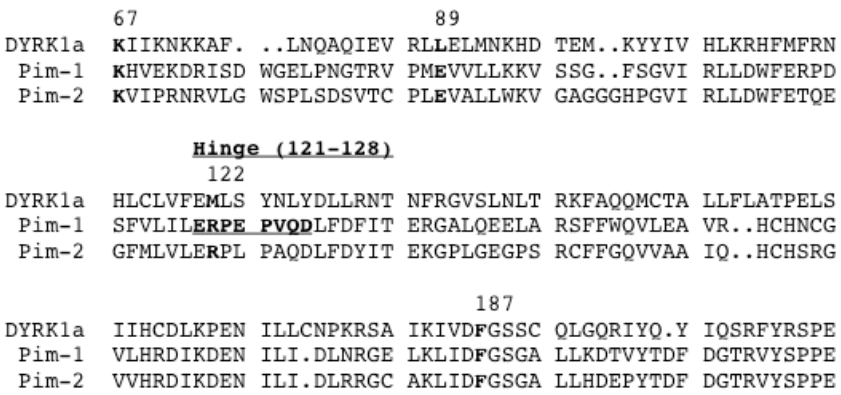

67
89
DYRK1a KIIKNKKAF. ..LNQAQIEV RLLELMNKHD TEM..KYYIV HLKRHFMFRN
Pim-1 KHVEKDRISD WGELPNGTRV PMEVVLLKKV SSG..FSGVI RLLDWFERPD
Pim-2 KVIPRNRVLG WSPLSDSVTC PLEVALLWKV GAGGGHPGVI RLLDWFETQE
Hinge (121-128)
122
DYRK1a HLCLVFEMLS YNLYDLLRNT NFRGVSLNLT RKFAQQMCTA LLFLATPELS
Pim-1 SFVLILERPE PVQDLFDFIT ERGALQEELA RSFFWQVLEA VR..HCHNCG
Pim-2 GFMLVLERPL PAQDLFDYIT EKGPLGEGPS RCFFGQVVAA IQ..HCHSRG
187
DYRK1a IIHCDLKPEN ILLCNPKRSA IKIVDFGSSC QLGQRIYQ.Y IQSRFYRSPE
Pim-1 VLHRDIKDEN ILI.DLNRGE LKLIDFGSGA LLKDTVYTDF DGTRVYSPPE
Pim-2 VVHRDIKDEN ILI.DLRRGC AKLIDFGSGA LLHDEPYTDF DGTRVYSPPE

Figure S3