PTEN inhibitors

PTEN inhibitors: an evaluation of current compounds

Laura Spinelli[1,2, 3], Yvonne E. Lindsay[2, 3] and Nicholas R. Leslie[1,2, 4]

1. Institute of Biological Chemistry, Biophysics and Bioengineering, Nasmyth Building, Heriot Watt University, Edinburgh, EH14 4AS, UK

2. Division of Cell Signalling and Immunology, College of Life Sciences, University of Dundee, Dow Street, Dundee, DD1 5EH, UK

3. These authors contributed equally

4. Author for correspondence. Tel: 44-131-4518157 email: n.r.leslie@hw.ac.uk

ABSTRACT

Small molecule inhibitors of many classes of enzymes, including phosphatases, have widespread use as experimental tools and as therapeutics. Efforts to develop inhibitors against the lipid phosphatase and tumour suppressor, PTEN, was for some time limited by concerns that their use as therapy could result in increased risk of cancer. However, the accumulation of evidence that short term PTEN inhibition may be valuable in conditions such as nerve injury has raised interest. Here we investigate the inhibition of PTEN by four available PTEN inhibitors, bpV(phen), bpV(pic), VO-OHpic and SF1670 and compared this inhibition with that of only 3 other related enzymes, the tyrosine phosphatase SHP1 and the phosphoinositide phosphatases INPP4A and INPP4B. Even with this very small number of comparators, for all compounds, inhibition of multiple enzymes was observed and with all three vanadate compounds, this was similar or more potent than the inhibition of PTEN. In particular, the bisperoxovanadate compounds were found to inhibit PTEN poorly in the presence of reducing agents including the cellular redox buffer glutathione.

Keywords: Phosphatase, enzyme, inhibitor, drug, PI 3-Kinase, PTEN, phosphoinositide

Introduction

PTEN is a heavily studied lipid phosphatase and tumour suppressor. It metabolizes PtdIns(3,4,5)P_3, the principal product of the class I PI 3-Kinases and thus inhibits the activation of the growth-promoting PI3K-AKT signalling pathway (Song et al., 2012, Vanhaesebroeck et al., 2012). Structurally, PTEN is a 50kD cytosolic enzyme that interacts transiently with the plasma membrane to metabolize its lipid substrate (Das et al., 2003, Lee et al., 1999, Leslie et al., 2008, Vazquez et al., 2006) and its loss of function through several distinct mechanisms is observed at high frequency in many tumour types (Fragoso and Barata, 2014, Leslie and Foti, 2011, Song et al., 2012). PTEN activity has potent effects in many cell lineages on cell proliferation, growth, survival and associated changes in metabolism and in a more lineage-specific manner, can control cell polarization and movement (Song et al., 2012). Many of these effects are mediated through PTEN-mediated metabolism of the lipid PtdInsP_3, but evidence for the importance of PIP_3/PI3K independent functions of PTEN has been presented (Bassi et al., 2013, Leslie et al., 2007, Raftopoulou et al., 2004) and alternate mechanisms of action have been proposed (Shi et al., 2014, Song et al., 2011, Tamura et al., 1999), although their significance is currently hard to judge. In transgenic mouse models, tissue specific Pten deletion promotes tumorigenesis in many tissues (eg mammary gland, prostate, keratinocytes, B-cells, T-cells) but can also affect other processes such as oocyte maturation through mechanisms that are unclear (Davies et al., 2012, Suzuki et al., 2008). PTEN activity appears to be regulated in physiology at several levels, through regulation of PTEN expression and post-translational control of PTEN activity (Leslie et al., 2008, Song et al., 2012). It is relevant to the consideration of PTEN inhibition that PTEN, as with many related phosphatases, is highly sensitive to inhibition by reactive oxygen species (ROS) and reactive nitrogen species (RNS) and there is good evidence that this inhibition can occur in response to ROS/RNS generated endogenously within cells (Kwak et al., 2010, Lee et al., 2002, Leslie et al., 2003, Yu et al., 2005).

PTEN is a member of the Protein Tyrosine Phosphatase (PTP) super-family with a conserved catalytic mechanism reliant upon a reduced Cysteine nucleophile (Lee et al., 1999). There are just over 100 PTPs encoded in the human genome, sharing a short active site ‘signature motif’ and characteristic 3D phosphatase domain architecture (Alonso et al., 2004, Andersen et al., 2004). However, they are divergent enzymes, dephosphorylating lipids and nucleotide substrates in addition to phospho-serine, phospho-threonine and phospho-tyrosine residues within proteins (Alonso et al., 2004, Deshpande et al., 1999, Worby et al., 2006, Xiao et al., 2011). There is only one closely PTEN-related phosphatase in humans that is known to have catalytic activity, TPIP (amino acid identity approximately 50% through the phosphatase domain) (Walker et al., 2001), which has expression restricted largely to the testis and in contrast to PTEN probably dephosphorylates PI(4,5)P2 (Iwasaki et al., 2008, Kurokawa et al., 2012). Other PTP lipid and protein phosphatase domains range in similarity from 20-25% for closer relatives such as PTP4A1/PRL and CDC14 to the large number of distantly related PTPs, with amino acid identities in the 5-10% amino acid identity range. Although PtdInsP3 is also metabolised by phosphatases other than, PTEN, it appears that these enzymes are all of the structurally unrelated phosphoinositide 5-phosphatase family (Dyson et al., 2012, Elong Edimo et al., 2013).

There is a wealth of data generated in vitro and in vivo to show that the manipulation of PTEN activity could be of therapeutic benefit in several conditions. Initial consideration of developing a PTEN inhibitor were discouraged by concerns that long term systemic PTEN inhibition would lead to increased cancer risk and evidence that even modest reductions in PTEN expression level lead to increased frequencies of certain tumours, particularly breast (Alimonti et al., 2010a, Trotman et al., 2003). On the other hand, there is strong evidence that PTEN activity inhibits both cell survival during cerebral (and cardiac) ischemia (Ning et al., 2004, Ruan et al., 2009) and nerve regeneration after neuronal injury (Park et al., 2008). For example, in transgenic mice, Pten deletion from the heart protects against cardiac ischemia and deletion from either the spinal cord or optic nerve promotes nerve regeneration after injury (Liu et al., 2010, Park et al., 2008, Ruan et al., 2009). This work showed that viral Cre-driven Pten deletion promotes nerve regeneration after injury both in the spinal cord and optic nerve. This improved outcome was despite inefficient deletion of Pten from only a fraction of neurons and in some cases was evident when Pten was deleted only after injury (Liu et al., 2010, Sun et al., 2011). Therefore, interest has developed in the potential suppression of PTEN activity for the treatment of nerve injury and potentially cardiac ischemia with the expectation that treatment periods measured in days and weeks would have benefit without greatly elevating risks of cancer.

Inhibition of PTEN by vanadium compounds

Vanadium compounds, such as sodium orthovanadate have been recognized as inhibitors of several classes of phosphatase enzymes since the 1970s, in some cases with reasonable potency (eg. human liver alkaline phosphatase Ki<1μM) (Seargeant and Stinson, 1979, VanEtten et al., 1974). Although this broad spectrum phosphatase inhibition by vanadate appears to be mediated by simple reversible competitive inhibition, a more selective irreversible inhibition of several members of the protein tyrosine phosphatase family appears to be achieved by aqueous peroxovanadium compounds due to oxidation of the active site cysteine thiol (Bevan et al., 1995, Huyer et al., 1997). Subsequently, peroxovanadium compounds such as bisperoxovanadium 1,10 phenanthroline (bpV(phen)) and bisperoxovanadium 5-hydroxypiridine-2-carboxyl (bpV(HOpic)) were studied due to their increased biological potency and evidence that these vanadium complexes have greater target selectivity than the simple vanadate compounds. For example, bpV(phen) and bisperoxovanadium 2-carboxypyridine (bpV(pic)) were shown to inhibit Cdc25A with some selectivity, displaying IC50s determined in vitro in the presence of 1mM DTT in the 10-50nM range (Scrivens et al., 2003).

Materials and Methods

All methods, including protein purification, and phosphatase assays have been previously described (Leslie et al., 2003, Leslie et al., 2007, Ross et al., 2007). IC_{50} values were calculated from the presented data by sigmoid curve fitting with SIGMAplot software.

Results

Bisperoxovanadium compounds

In 2004 data were presented showing substantially greater potency of bpV(phen), bpV(pic) and bpV(HOpic) against PTEN than against the classical PTPs, PTP1B and PTPβ. This work also showed a bpV-mediated enhancement of insulin stimulated AKT activation that was observed in cells expressing PTEN (NIH3T3 fibroblasts), but not in a PTEN null cell line (UM-UC-3 bladder cancer) (Schmid et al., 2004). This stimulated significant interest in these compounds as PTEN inhibitors and lead the authors to then show that a related vanadium complex with alternate ligand structure, hydroxy(oxo)vanadium 3-hydroxypyridine-2-carboxylic acid (VO-OHpic), showed the greatest apparent selectivity for PTEN (Mak et al., 2010, Rosivatz et al., 2006). VO-OHpic (Alimonti et al., 2010b, Bolduc et al., 2013, Silva et al., 2011) and the bpV compounds bpV(phen) and bpV(pic) (Faratian et al., 2009, Mao et al., 2013, Obeidat et al., 2014, Zhou et al., 2007) have all now been used as tools in functional studies from many groups to study the function of PTEN at concentrations ranging from 50nM to 5μM (Alimonti et al and Silva et al both 500nM, Faratian et al 50nM, Mao et al, 100nM, Zhou et al 5uM). For structures of bpV(phen) and VO-OHpic, see Fig. 1. This led us to test the selectivity of some of these vanadate compounds in vitro. In addition, since the original selectivity analysis of Schmid et al assayed PTEN without reducing agents in the assay buffer and comparator phosphatases in the presence of 5mM DTT, we elected to investigate the effect of thiol reducing agents on the potency of these inhibitors. bpV(phen) and bpV(pic) inhibited both PTEN and the classical PTP, SHP1, with IC_{50} values around 100nM in the addition of reducing agents to their assay buffers. However, in the presence of 2mM DTT, the IC_{50} was raised in each case by at least 100 fold, to 10μM or higher (Fig. 2). In contrast, the phosphoinositide 4-phosphatses, INPP4A and INPP4B were somewhat more sensitive to these inhibitors, with IC_{50}s around 20nM, but importantly, there appeared to be little or no effect on this inhibition by 2mM DTT. Given that inhibitor and enzyme were briefly pre-incubated in the absence of substrate, this selective result also argues that the loss of inhibition is not simply caused by reductive modification of the bpV inhibitor before the addition of substrate. This suppression by thiols of bpV-mediated inhibition is not limited to the relatively strong reducing agent DTT, as even physiological concentrations of the cellular redox buffer glutathione were sufficient to suppress the inhibition of PTEN by bpV compounds (Fig. 3). These data together not only identify other phosphatases that are inhibited by bpV(phen) and bpV(pic) with greater potency than PTEN but imply that in cellular glutathione conditions, the nanomolar range concentrations of these inhibitors used in several publications may have little or no effect to inhibit PTEN. We think this adds to existing data indicating potent inhibition of other PTPs in the presence of mM concentrations of thiol reducing agents to argue strongly against the use of bpV(Phen) and bpV(pic) as selective cellular PTEN inhibitors.

VO-OHpic

We also studied the inhibition by VO-OHpic of PTEN and SHP1 using soluble PtdIns(3,4,5)P_3 and pNPP as respective substrates. Pre-incubation of the enzymes with

inhibitor had little or no effect on inhibition by VO-OHpic, consistent with data showing that it acts reversibly (Mak et al., 2010). However, we found much weaker inhibition of PTEN by VO-OHpic than previously reported, requiring concentrations in the micromolar range (IC_{50} 6.74μM) and also observed inhibition of SHP1 that was at least as potent (IC_{50} 975nM). The dramatic effect of DTT to reduce the inhibition of PTEN or SHP1 seen with the bisperoxovanadium compounds was not observed with VO-OHpic (Fig. 4). A number of explanations exist for the weak inhibition we have observed using VO-OHpic, including our commercial sources of inhibitor. However, the inhibition by VO-OHpic of SHP1 with similar or greater potency to PTEN using the same reagents and the potent inhibition of PTEN observed with other inhibitors fail to provide support for VO-OHpic as a selective PTEN inhibitor.

SF1670

In 2007, a patent presented a series of phenanthrene-9, 10-diones as PTEN inhibitors (Garlich et al, 2007, Pten inhibitors, Patent EP1755574 A2). These compounds had been developed from chemical series originally protected as inhibitors of the tyrosine phosphatase CD45 (Chapdelaine et al, 2001, CD45 inhibitors, Patent WO2001046125 A2). However, several of the compounds described showed 20x or greater selectivity for PTEN over comparator phosphatases and had cellular effects consistent with PTEN inhibition. These inhibitors included SF1670 (structure in Fig. 1) which has now been shown to have extensive effects on neutrophil function which are consistent with action as a PTEN inhibitor (Li et al., 2011). However, further evidence for the target selectivity of SF1670 has not been presented. We investigated the ability of SF1670 to inhibit PTEN, SHP1 and INPP4A and INPP4B in the presence and absence of additional DTT. We found little or no inhibition by SF1670 of the phosphatase activity of SHP1 against pNPP up to 100μM inhibitor concentration and similarly only weak inhibition of INPP4A or INPP4B dephosphorylation of soluble PtdIns(3,4)P_2 at this highest concentration used. The inhibition of PTEN by SF1670 appeared only marginally more potent and unexpectedly was consistently stronger in the presence of 1mM DTT than in the absence of additional reducing agents. However, a 15 minute pre-incubation of the enzyme and inhibitor led to much stronger inhibition, suggesting that inhibition may be irreversible (Fig. 5).

Discussion

Current drug discovery aims to develop agents with recognized mechanisms of action and for which side-effects caused in most cases by interaction with molecules other than the drug target are tolerable. This has led to the concepts of clean drugs which act selectively on their targets and dirty drugs which have many “off-target” effects. The advent in some drug discovery areas of large selectivity panels, for example testing protein kinase inhibitors against hundreds of other kinases (Bain et al., 2007, Davies et al., 2000, Goldstein et al., 2008), has supported this aim towards high target selectivity. In the development of PTEN inhibitors, obtaining target selectivity over other members of the large and diverse Protein Tyrosine Phosphatase superfamily is a significant hurdle. However, academic laboratories have demonstrated the ability to compare the inhibition of small panels of (10-20)

phosphatase enzymes (Ross et al., 2007, Sergienko et al., 2012) and at least one phosphatase selectivity panel is available as a commercial inhibitor screening service (Millipore phosphatase profiler). It may be possible to develop active site inhibitors for PTEN given that its substrate binding pocket is much larger than those of the classical PTPs. Also, evidence for the conformational activation of the PTEN catalytic domain when contacting membrane surfaces supports the possibility of developing allosteric inhibitors not necessarily targeting the active site. Our understanding of the biology of PTEN and the rich provision of the assays and counter-screens required in an inhibitor programme provide strong motivation to target the phosphatase. However, the data shown here do not provide support for the high target selectivity of the available PTEN inhibitors and indicate that more effort and target selectivity analysis is required in this area.

Conflict of interest

The authors declare they have no conflict of interest

References

Alimonti A, Carracedo A, Clohessy JG, Trotman LC, Nardella C, Egia A, et al. Subtle variations in Pten dose determine cancer susceptibility. Nature genetics. 2010a;42:454-8.
Alimonti A, Nardella C, Chen Z, Clohessy JG, Carracedo A, Trotman LC, et al. A novel type of cellular senescence that can be enhanced in mouse models and human tumor xenografts to suppress prostate tumorigenesis. J Clin Invest. 2010b;120:681-93.
Alonso A, Sasin J, Bottini N, Friedberg I, Osterman A, Godzik A, et al. Protein tyrosine phosphatases in the human genome. Cell. 2004;117:699-711.
Andersen JN, Jansen PG, Echwald SM, Mortensen OH, Fukada T, Del Vecchio R, et al. A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage. Faseb J. 2004;18:8-30.
Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007;408:297-315.
Bassi C, Ho J, Srikumar T, Dowling RJ, Gorrini C, Miller SJ, et al. Nuclear PTEN controls DNA repair and sensitivity to genotoxic stress. Science (New York, NY. 2013;341:395-9.
Bevan AP, Drake PG, Yale JF, Shaver A, Posner BI. Peroxovanadium compounds: biological actions and mechanism of insulin-mimesis. Mol Cell Biochem. 1995;153:49-58.
Bolduc D, Rahdar M, Tu-Sekine B, Sivakumaren SC, Raben D, Amzel LM, et al. Phosphorylation-mediated PTEN conformational closure and deactivation revealed with protein semisynthesis. eLife. 2013;2:e00691.
Das S, Dixon JE, Cho W. Membrane-binding and activation mechanism of PTEN. Proc Natl Acad Sci U S A. 2003;100:7491-6.
Davies EM, Sheffield DA, Tibarewal P, Fedele CG, Mitchell CA, Leslie NR. The PTEN and Myotubularin Phosphoinositide 3-Phosphatases: Linking Lipid Signalling to Human Disease. Sub-cellular biochemistry. 2012;58:281-336.
Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. Biochem J. 2000;351:95-105.
Deshpande T, Takagi T, Hao L, Buratowski S, Charbonneau H. Human PIR1 of the protein-tyrosine phosphatase superfamily has RNA 5'-triphosphatase and diphosphatase activities. J Biol Chem. 1999;274:16590-4.
Dyson JM, Fedele CG, Davies EM, Becanovic J, Mitchell CA. Phosphoinositide phosphatases: just as important as the kinases. Sub-cellular biochemistry. 2012;58:215-79.
Elong Edimo W, Vanderwinden JM, Erneux C. SHIP2 signalling at the plasma membrane, in the nucleus and at focal contacts. Advances in biological regulation. 2013;53:28-37.
Faratian D, Goltsov A, Lebedeva G, Sorokin A, Moodie S, Mullen P, et al. Systems biology reveals new strategies for personalizing cancer medicine and confirms the role of PTEN in resistance to trastuzumab. Cancer Res. 2009;69:6713-20.
Fragoso R, Barata JT. PTEN and leukemia stem cells. Advances in biological regulation. 2014;56:22-9.
Goldstein DM, Gray NS, Zarrinkar PP. High-throughput kinase profiling as a platform for drug discovery. Nat Rev Drug Discov. 2008;7:391-7.
Huyer G, Liu S, Kelly J, Moffat J, Payette P, Kennedy B, et al. Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. J Biol Chem. 1997;272:843-51.
Iwasaki H, Murata Y, Kim Y, Hossain MI, Worby CA, Dixon JE, et al. A voltage-sensing phosphatase, Ci-VSP, which shares sequence identity with PTEN, dephosphorylates phosphatidylinositol 4,5-bisphosphate. Proc Natl Acad Sci U S A. 2008;105:7970-5.
Kurokawa T, Takasuga S, Sakata S, Yamaguchi S, Horie S, Homma KJ, et al. 3' Phosphatase activity toward phosphatidylinositol 3,4-bisphosphate [PI(3,4)P2] by voltage-sensing phosphatase (VSP). Proc Natl Acad Sci U S A. 2012;109:10089-94.
Kwak YD, Ma T, Diao S, Zhang X, Chen Y, Hsu J, et al. NO signaling and S-nitrosylation regulate PTEN inhibition in neurodegeneration. Molecular neurodegeneration. 2010;5:49.

Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, et al. Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell. 1999;99:323-34.
Lee SR, Yang KS, Kwon J, Lee C, Jeong W, Rhee SG. Reversible inactivation of the tumor suppressor PTEN by H2O2. J Biol Chem. 2002;277:20336-42.
Leslie NR, Batty IH, Maccario H, Davidson L, Downes CP. Understanding PTEN regulation: PIP2, polarity and protein stability. Oncogene. 2008;27:5464-76.
Leslie NR, Bennett D, Lindsay YE, Stewart H, Gray A, Downes CP. Redox regulation of PI 3-kinase signalling via inactivation of PTEN. The EMBO journal. 2003;22:5501-10.
Leslie NR, Foti M. Non-genomic loss of PTEN function in cancer: not in my genes. Trends Pharmacol Sci. 2011;32:131-40.
Leslie NR, Yang X, Downes CP, Weijer CJ. PtdIns(3,4,5)P3-dependent and -independent roles for PTEN in the control of cell migration. Curr Biol. 2007;17:115-25.
Li Y, Prasad A, Jia Y, Roy SG, Loison F, Mondal S, et al. Pretreatment with phosphatase and tensin homolog deleted on chromosome 10 (PTEN) inhibitor SF1670 augments the efficacy of granulocyte transfusion in a clinically relevant mouse model. Blood. 2011;117:6702-13.
Liu K, Lu Y, Lee JK, Samara R, Willenberg R, Sears-Kraxberger I, et al. PTEN deletion enhances the regenerative ability of adult corticospinal neurons. Nat Neurosci. 2010;13:1075-81.
Mak LH, Vilar R, Woscholski R. Characterisation of the PTEN inhibitor VO-OHpic. Journal of chemical biology. 2010;3:157-63.
Mao L, Jia J, Zhou X, Xiao Y, Wang Y, Mao X, et al. Delayed administration of a PTEN inhibitor BPV improves functional recovery after experimental stroke. Neuroscience. 2013;231:272-81.
Ning K, Pei L, Liao M, Liu B, Zhang Y, Jiang W, et al. Dual neuroprotective signaling mediated by downregulating two distinct phosphatase activities of PTEN. J Neurosci. 2004;24:4052-60.
Obeidat M, Li L, Ballermann BJ. TIMAP promotes Angiogenesis by suppressing PTEN-mediated Akt inhibition in Human Glomerular Endothelial Cells. Am J Physiol Renal Physiol. 2014.
Park KK, Liu K, Hu Y, Smith PD, Wang C, Cai B, et al. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. Science (New York, NY. 2008;322:963-6.
Raftopoulou M, Etienne-Manneville S, Self A, Nicholls S, Hall A. Regulation of cell migration by the C2 domain of the tumor suppressor PTEN. Science (New York, NY. 2004;303:1179-81.
Rosivatz E, Matthews JG, McDonald NQ, Mulet X, Ho KK, Lossi N, et al. A small molecule inhibitor for phosphatase and tensin homologue deleted on chromosome 10 (PTEN). ACS Chem Biol. 2006;1:780-90.
Ross SH, Lindsay Y, Safrany ST, Lorenzo O, Villa F, Toth R, et al. Differential redox regulation within the PTP superfamily. Cell Signal. 2007;19:1521-30.
Ruan H, Li J, Ren S, Gao J, Li G, Kim R, et al. Inducible and cardiac specific PTEN inactivation protects ischemia/reperfusion injury. Journal of molecular and cellular cardiology. 2009;46:193-200.
Schmid AC, Byrne RD, Vilar R, Woscholski R. Bisperoxovanadium compounds are potent PTEN inhibitors. FEBS letters. 2004;566:35-8.
Scrivens PJ, Alaoui-Jamali MA, Giannini G, Wang T, Loignon M, Batist G, et al. Cdc25A-inhibitory properties and antineoplastic activity of bisperoxovanadium analogues. Molecular cancer therapeutics. 2003;2:1053-9.
Seargeant LE, Stinson RA. Inhibition of human alkaline phosphatases by vanadate. Biochem J. 1979;181:247-50.
Sergienko E, Xu J, Liu WH, Dahl R, Critton DA, Su Y, et al. Inhibition of hematopoietic protein tyrosine phosphatase augments and prolongs ERK1/2 and p38 activation. ACS Chem Biol. 2012;7:367-77.

Shi Y, Wang J, Chandarlapaty S, Cross J, Thompson C, Rosen N, et al. PTEN is a protein tyrosine phosphatase for IRS1. Nat Struct Mol Biol. 2014;21:522-7.
Silva SR, Zaytseva YY, Jackson LN, Lee EY, Weiss HL, Bowen KA, et al. The effect of PTEN on serotonin synthesis and secretion from the carcinoid cell line BON. Anticancer Res. 2011;31:1153-60.
Song MS, Carracedo A, Salmena L, Song SJ, Egia A, Malumbres M, et al. Nuclear PTEN regulates the APC-CDH1 tumor-suppressive complex in a phosphatase-independent manner. Cell. 2011;144:187-99.
Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012;13:283-96.
Sun F, Park KK, Belin S, Wang D, Lu T, Chen G, et al. Sustained axon regeneration induced by co-deletion of PTEN and SOCS3. Nature. 2011;480:372-5.
Suzuki A, Nakano T, Mak TW, Sasaki T. Portrait of PTEN: messages from mutant mice. Cancer Sci. 2008;99:209-13.
Tamura M, Gu J, Takino T, Yamada KM. Tumor suppressor PTEN inhibition of cell invasion, migration, and growth: differential involvement of focal adhesion kinase and p130Cas. Cancer Res. 1999;59:442-9.
Trotman LC, Niki M, Dotan ZA, Koutcher JA, Di Cristofano A, Xiao A, et al. Pten dose dictates cancer progression in the prostate. PLoS biology. 2003;1:E59.
VanEtten RL, Waymack PP, Rehkop DM. Letter: Transition metal ion inhibition of enzyme-catalyzed phosphate ester displacement reactions. Journal of the American Chemical Society. 1974;96:6782-5.
Vanhaesebroeck B, Stephens L, Hawkins P. PI3K signalling: the path to discovery and understanding. Nature reviews Molecular cell biology. 2012;13:195-203.
Vazquez F, Matsuoka S, Sellers WR, Yanagida T, Ueda M, Devreotes PN. Tumor suppressor PTEN acts through dynamic interaction with the plasma membrane. Proc Natl Acad Sci U S A. 2006;103:3633-8.
Walker SM, Downes CP, Leslie NR. TPIP: a novel phosphoinositide 3-phosphatase. Biochem J. 2001;360:277-83.
Worby CA, Gentry MS, Dixon JE. Laforin, a dual specificity phosphatase that dephosphorylates complex carbohydrates. J Biol Chem. 2006;281:30412-8.
Xiao J, Engel JL, Zhang J, Chen MJ, Manning G, Dixon JE. Structural and functional analysis of PTPMT1, a phosphatase required for cardiolipin synthesis. Proc Natl Acad Sci U S A. 2011;108:11860-5.
Yu CX, Li S, Whorton AR. Redox Regulation of PTEN by S-Nitrosothiols. Mol Pharmacol. 2005;68:847-54.
Zhou J, Wulfkuhle J, Zhang H, Gu P, Yang Y, Deng J, et al. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proc Natl Acad Sci U S A. 2007;104:16158-63.

Figure Legends

Fig. 1. Structures for the inhibitors used are shown: (A) bpV(phen), (B) VO-OHpic and (C) SF1670.

Fig. 2. Concentration inhibition curves are shown for the effects of bpV(Phen) (A, C, E and G) and bpV(Pic) against PTEN (A,B), SHP1 (C,D), INPP4A (E, F) and INPP4B (G,H). Each set of assays was performed in parallel in the presence and absence of 2mM DTT. Enzymes were pre-incubated for 5 minutes in the presence of inhibitor before the addition of substrate. Activity data in arbitrary units are shown as the mean activity from duplicate assays +/- the range/2. The experiments were performed on at least three occasions with similar results.

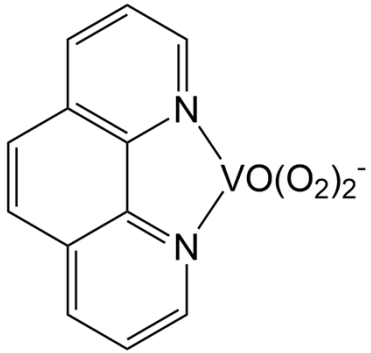
Fig. 3. The inhibition of (A) PTEN and (B) SHP1 by bpVPic was tested in the presence and absence of the physiological redox buffer, reduced glutathione (GSH). Activity data in arbitrary units are shown as the mean activity from duplicate assays + the range/2. The experiments were performed twice with similar results.

Fig. 4. Concentration inhibition curves are shown for the effects of VO-OHpic on (A) PTEN and (B) SHP1. Activity data in arbitrary units are shown as the mean activity from duplicate assays +/- the range/2. The experiments were performed on at least three occasions with similar results.

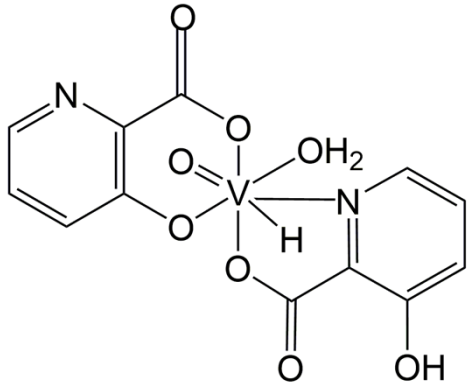
Fig. 5. The inhibition of (A, B) PTEN and (C) INPP4B by SF1670 in the presence (A) and absence (B) of 1mM DTT. Inhibition by bpV(Phen) and bpV(pic) are used as positive controls. Activity data in arbitrary units are shown as the mean activity from duplicate assays + the range/2. These experiments were performed twice with similar results.

Figure 1

A: bpV(phen)



B: VO-OHpic



C: SF1670

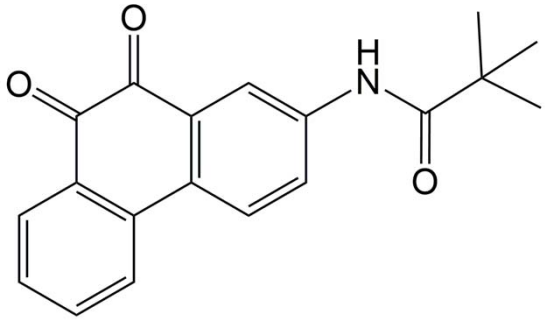


Figure 2

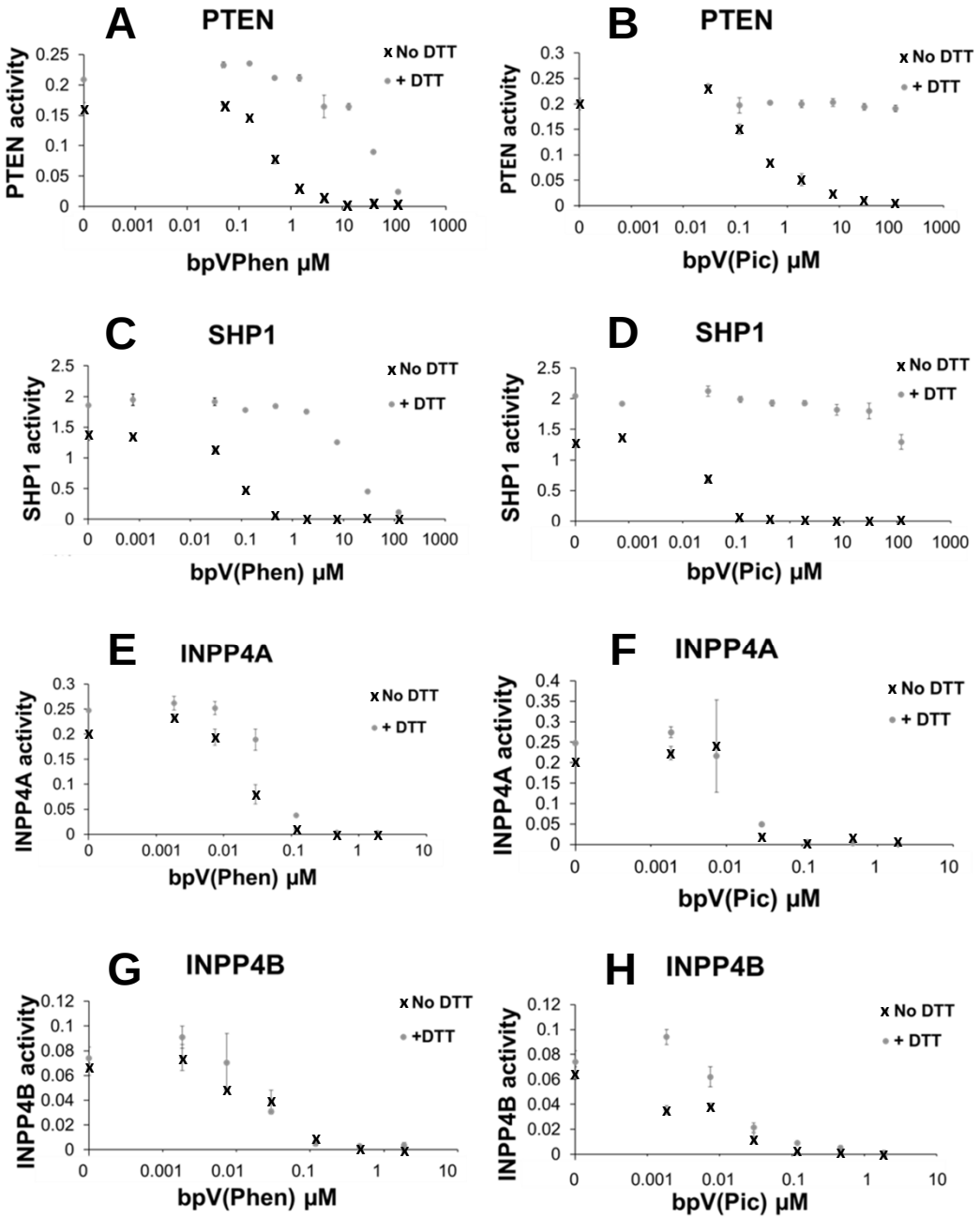

A
PTEN
PTEN activity
bpVPhen µM
B
PTEN
PTEN activity
bpV(Pic) µM
C
SHP1
SHP1 activity
bpV(Phen) µM
D
SHP1
SHP1 activity
bpV(Pic) µM
E
INPP4A
INPP4A activity
bpV(Phen) µM
F
INPP4A
INPP4A activity
bpV(Pic) µM
G
INPP4B
INPP4B activity
bpV(Phen) µM
H
INPP4B
INPP4B activity
bpV(Pic) µM
x No DTT
+ DTT
+DTT

Figure 3

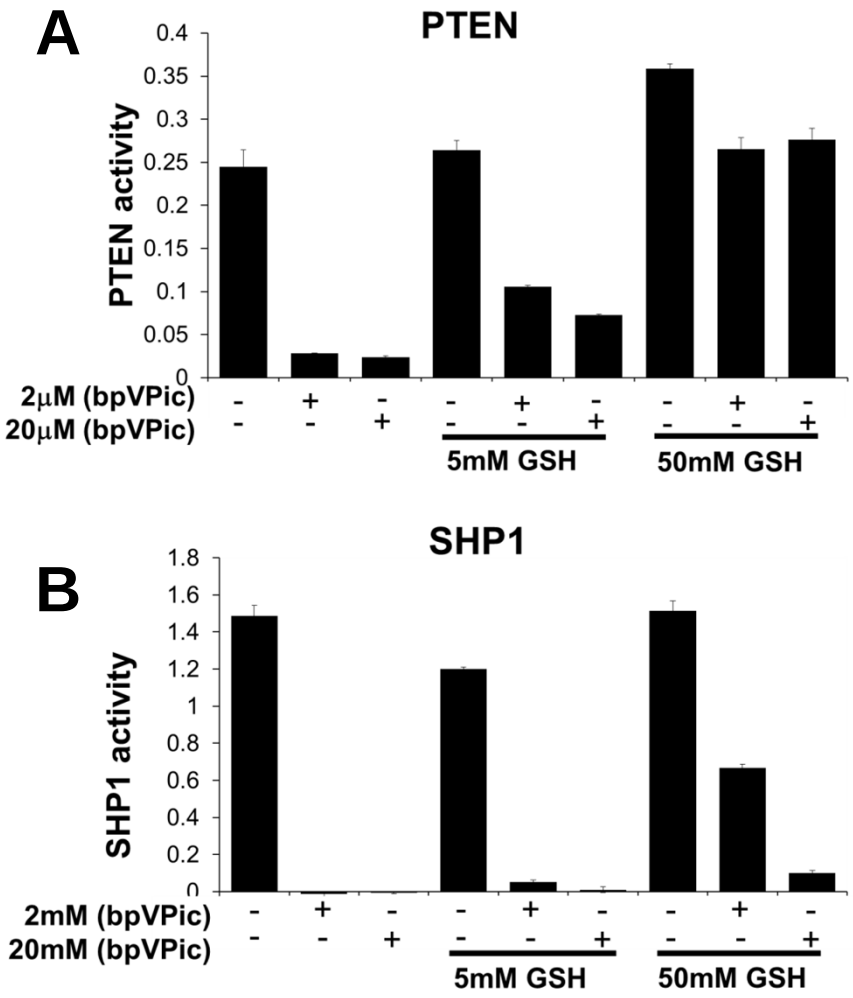

A
PTEN
PTEN activity
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
2μM (bpVPic)
20μM (bpVPic)
5mM GSH
50mM GSH
B
SHP1
SHP1 activity
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
2mM (bpVPic)
20mM (bpVPic)
5mM GSH
50mM GSH

Figure 4

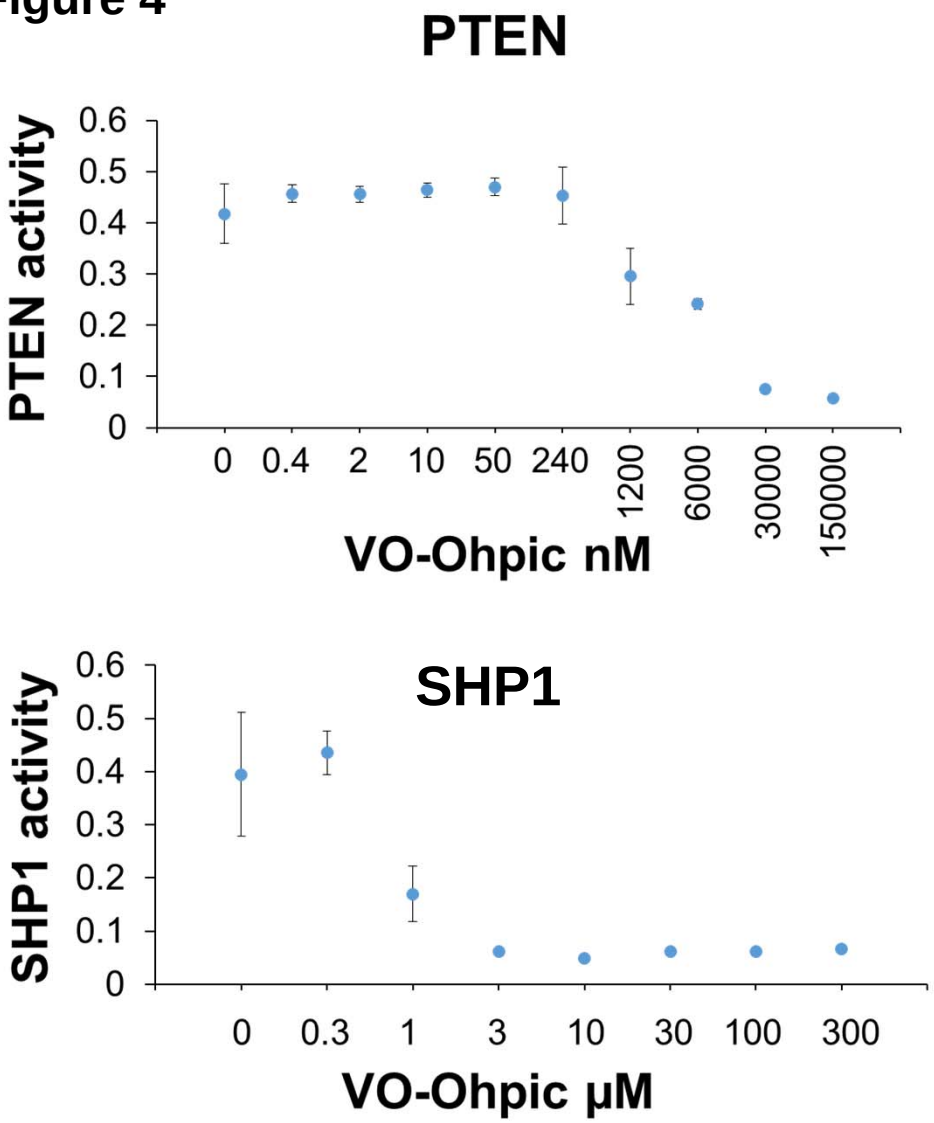

PTEN
PTEN activity
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.4
2
10
50
240
1200
6000
30000
150000
VO-Ohpic nM
SHP1
SHP1 activity
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.3
1
3
10
30
100
300
VO-Ohpic µM

Figure 5

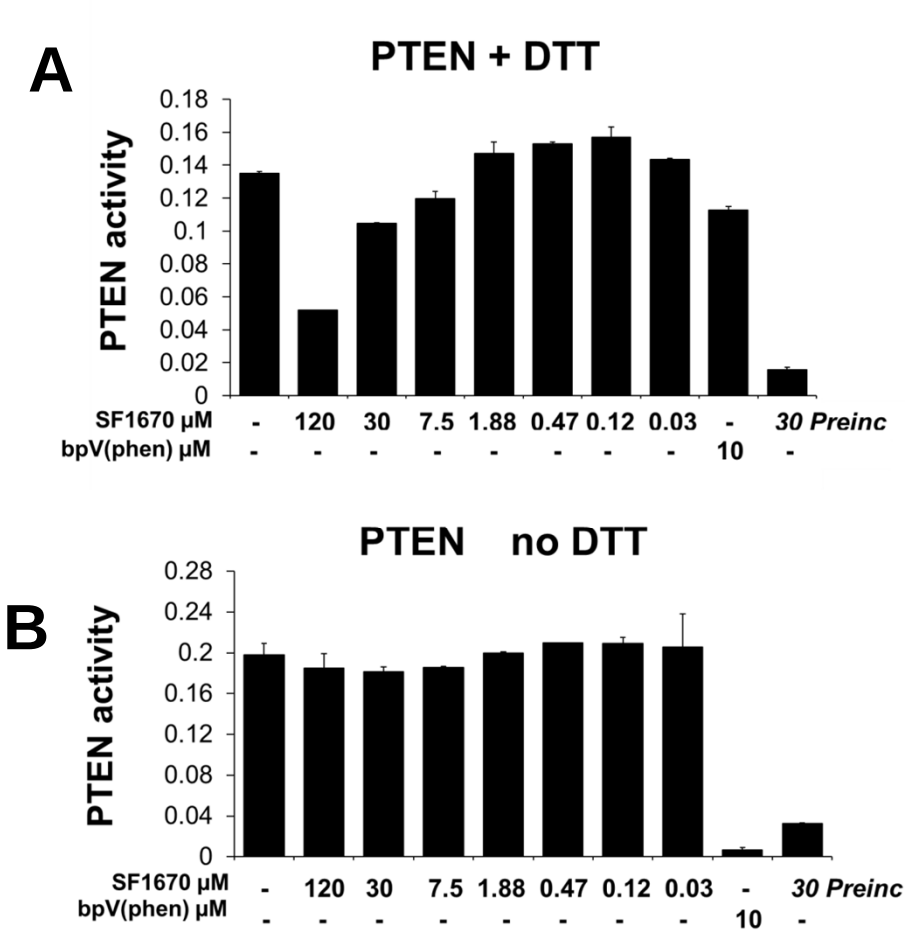
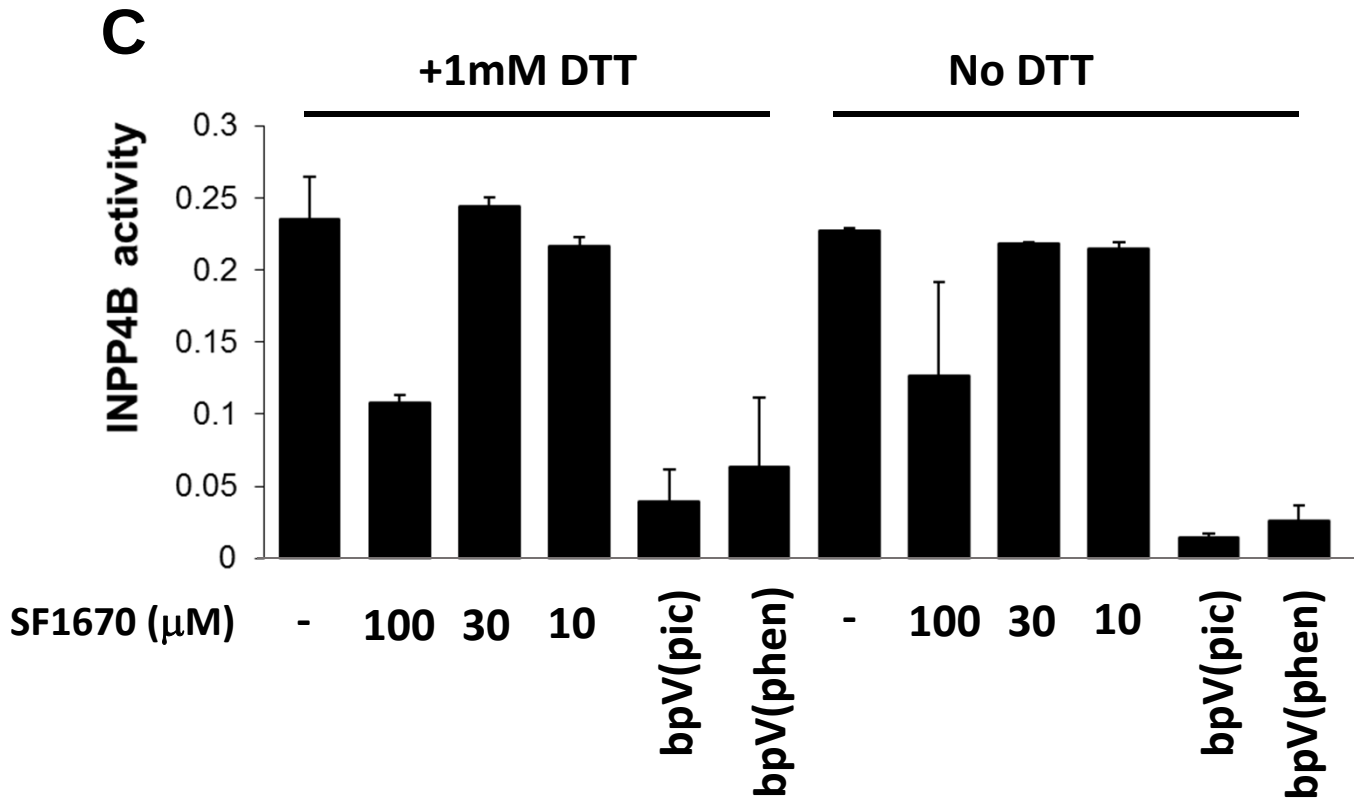

A
PTEN + DTT
PTEN activity
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
SF1670 µM - 120 30 7.5 1.88 0.47 0.12 0.03 - 30 Preinc
bpV(phen) µM - - - - - - - - 10 -
B
PTEN no DTT
PTEN activity
0.28
0.24
0.2
0.16
0.12
0.08
0.04
0
SF1670 µM - 120 30 7.5 1.88 0.47 0.12 0.03 - 30 Preinc
bpV(phen) µM - - - - - - - - 10 -

Figure 5


C
+1mM DTT
No DTT
INPP4B activity
0.3
0.25
0.2
0.15
0.1
0.05
0
SF1670 (μM)
-
100
30
10
bpV(pic)
bpV(phen)
-
100
30
10
bpV(pic)
bpV(phen)