Bioorganic & Medicinal Chemistry 20 (2012) 4371–4376

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Arylstibonic acids are potent and isoform-selective inhibitors of Cdc25a and Cdc25b phosphatases

Lok Hang Mak [a,b], Jessica Knott [a,b], Katherine A. Scott [c,d], Claire Scott [e], Gillian F. Whyte [a,b], Yu Ye [f], David J. Mann [b,c], Oscar Ces [a,b], James Stivers [f], Rudiger Woscholski [a,b,*]

[a] Department of Chemistry, Imperial College London, Exhibition Road, London SW7 2AZ, UK
[b] Institute of Chemical Biology, Imperial College London, Exhibition Road, London SW7 2AZ, UK
[c] Division of Molecular Biosciences, Imperial College London, Exhibition Road, London SW7 2AZ, UK
[d] Drug Discovery Centre, Imperial College London, St. Dunstan's Road, London, W6 8RP, UK
[e] Division of Cell and Molecular Biology, Imperial College London, Exhibition Road, London SW7 2AZ, UK
[f] Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205, US

ARTICLE INFO

Article history:
Received 13 February 2012
Revised 14 May 2012
Accepted 16 May 2012
Available online 24 May 2012

Keywords:
Arylstibonic acids
Protein tyrosine phosphatase
Cdc25 inhibitor

ABSTRACT

Arylstibonates structurally resemble phosphotyrosine side chains in proteins and here we addressed the ability of such compounds to act as inhibitors of a panel of mammalian tyrosine and dual-specificity phosphatases. Two arylstibonates both possessing a carboxylate side chain were identified as potent inhibitors of the protein tyrosine phosphatase PTP-ß. In addition, they inhibited the dual-specificity, cell cycle regulatory phosphatases Cdc25a and Cdc25b with sub-micromolar potency. However, the Cdc25c phosphatase was not affected demonstrating that arylstibonates may be viable leads from which to develop isoform specific Cdc25 inhibitors.

Introduction

Phosphatases are important regulators of many cellular functions, which in turn impact disease and human health. In particular, protein tyrosine phosphatases (PTPs), counteract the many protein tyrosine kinases involved in cellular signaling, and have a significant role in the development of cancer, diabetes and obesity.[1,2] The PTP family is defined by its conserved phosphatase domain, which is characterized by a cysteine separated by five amino acids from an arginine (CX_5R motif). Within this family of CX_5R phosphatases are the cell division cycle 25 (Cdc25) phosphatases, which play a key role in cell cycle regulation by controlling cyclin-dependent kinase dephosphorylation and activation.[3] There are three isoforms of Cdc25 phosphatases encoded by the human genome, namely Cdc25a, Cdc25b and Cdc25c. Cdc25a appears to regulate the G1/S transition, whereas Cdc25b and Cdc25c both are regulators of the S/G2 transition.[4] Cdc25a and Cdc25b are over-expressed in various primary human cancers,[5,6] making Cdc25 phosphatases potential targets for anticancer therapeutics.

Recently, arylstibonic acids have been characterized as potent inhibitors of the topoisomerases DNA repair endonuclease APE1 and type 1B topoisomerases.[7,8] Interestingly, APE1 shares some homology in its catalytic site with the type II phosphoinositide 5-phosphatase (5′-P) active site of the synaptojanins, which is distinct from the active sites of the CX_5R family of phosphatases.[9] Interestingly, in addition to its 5′-P activity, synaptojanin also contains an independent phosphatase active site, referred to as SAC1, which is related to the CX_5R family of the PTPs. From synaptojanin's homology with APE1, we surmised that the arylstibonates might selectively target its 5′-P active site. Instead, we surprisingly found that a subset of these arylstibonates were potent and isoform selective Cdc25 phosphatase inhibitors.

2. Results and discussion

We began by performing a preliminary screen of the arylstibonic acid library for inhibitors against the activities of representative phosphatases from the 5′-P and CX_5R families. With respect to synaptojanin, this screen had the potential to simultaneously assess the selectivity of these arylstibonates for either active site of this bifunctional enzyme. In addition, we also screened PTP-ß, another CX_5R family member. We used screening conditions where the substrate concentration was fixed near its K_M, employing just 5 μM of each arylstibonate. This screen revealed that some compounds provided 40–50% inhibition of the 5′-P activity of synaptojanin under these conditions, consistent with the expectation based on APE1 homology (6). However, the structurally unrelated CX_5R phosphatases SAC1 and PTP-β were found to be much better targets for several compounds (Fig. 1).

* Corresponding author. Tel.: +44 (0) 2075945305.
E-mail address: r.woscholski@imperial.ac.uk (R. Woscholski).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.040

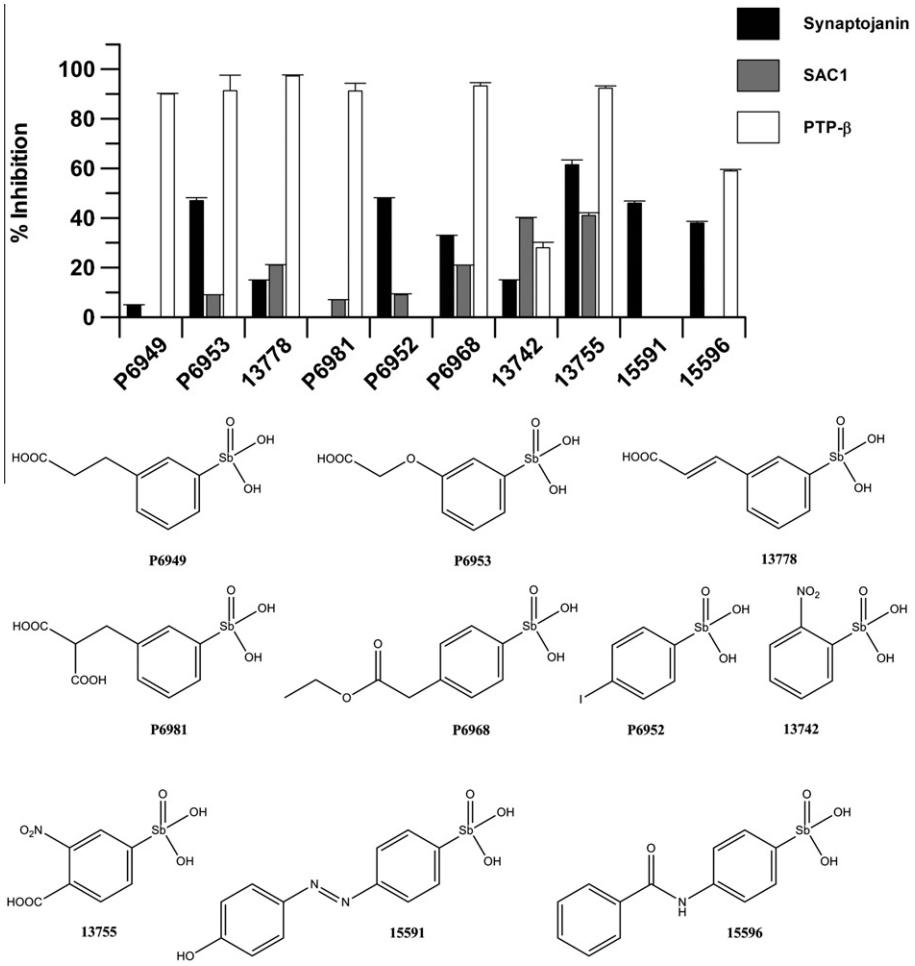


Figure 1. *Inhibition of synaptojanin, SAC1 and PTP-β by arylstibonic acids.* (Top panel) A library of arylstibonic acids was screened in activity assays against synaptojanin, SAC1 and PTP-β. Shown are the percentages of inhibition in the presence of 5 μM arylstibonic acid. The most potent compounds for synaptojanin (⩾40% inhibition), SAC1 (⩾40% inhibition) and PTP-β (⩾90% inhibition) are shown. The activity of synaptojanin was measured in the presence of 50 μM PI(4)P presented in octylglucoside mixed micelles; SAC1 and PTP-β activities were assessed using 50 μM I(1,4,5)P_3 and 1 mM p-NPP as the substrates, respectively. Error bars represent the standard error of triplicate measurments. (Bottom panel) Chemical structures of the arylstibonic acids that were found to inhibit synaptojanin, SAC1 domain and PTP-β. For the full list of tested arylstibonates refer to.[7] All other compounds showed less than 40% inhibition against the tested enzymes (data not shown).

The most potent inhibition was observed against PTP-ß, with several compounds (13755, P6953, 13778, P6968, P6981 and P6949) almost completely inhibiting phosphatase activity at 5 μM compound concentration. We then performed a full dose response study over the range 50 nM to 5 μM using the two most selective and effective PTP-ß phosphatase inhibitors and employing p-NPP as substrate (Fig. 2). We found that compounds 13778 and P6949 were the most potent inhibitors (IC_{50} ~100 nanomolar range).

The 40 compounds in the arystibonic acid library have discrete structural differences that allow assessment of useful structure-function relationships. Because the unsubstituted arylstibonate 13744 does not show any inhibitory effect (Table 1), one immediate conclusion is that substitutions in the aromatic ring are essential for inhibition of PTP-β (Table 1). Placement of anionic carboxylate groups or a zwitterionic nitro group at either the meta or para positions relative to the antimony substituent turns the inactive parent arylstibonate 13744 into a moderately active inhibitor. Interestingly, a neutral methyl ester or hydroxyl group placed at the same ortho or meta positions does not provide the same effect, suggesting that inhibition requires an electrostatic or hydrogen bonding interaction with the carboxylate or nitro groups. The importance of a carboxylic acid functionality in the inhibition of PTP- β is further revealed by the observation that twelve out of the thirteen carboxylate compounds in the library inhibited PTP-β by more than 60%. Indeed, many established PTP inhibitors also contain carboxylic acid functionality.[10,11] Finally, extending the length of the carboxylate side chain at the meta position by one

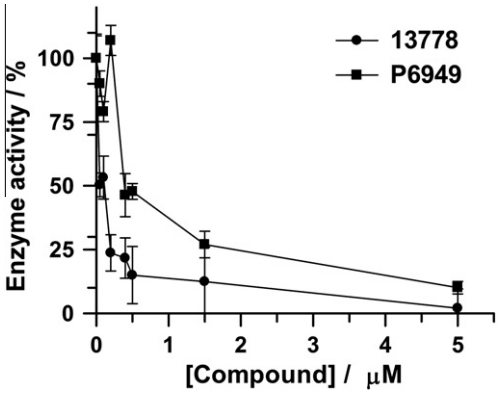
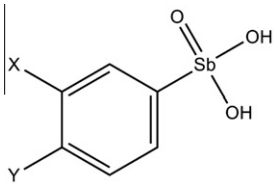


Figure 2. *Potency of selected arylstibonates on phosphatase activity of PTP-β.* The most potent PTP-β inhibitors identified in the primary screen were tested for their potency in the presence increasing concentrations of the arylstibonates 13778 and P6949. Error bars represent the standard errors from triplicate measurements.

Table 1
Structure activity relationships of arylstibonic acids with respect to PTP-β inhibition



Compound (5 μM)	X	Y	Inhibition of PTP-β
13744	H	H	No inhibition
13759	CO_2H	H	78%
13760	H	CO_2H	72%
P6966	CO_2H	CO_2H	51%
13771	CO_2CH_3	H	No inhibition
P6983	$CH_2CH(CO_2Et)_2$	H	No inhibition
13778	$C_2H_2CO_2H$	H	⩾90%
P6949	$C_2H_4CO_2H$	H	⩾90%
13743	NO_2	H	50%
P6981	$CH_2CH(CO_2H)_2$	H	⩾90%
P6982	H	$CH_2CH(CO_2H)_2$	67%
13755	NO_2	CO_2H	⩾90%
13745	H	OH	No inhibition

carbon greatly improves the potency to yield the highly potent compounds 13778 and P6949. The fact that a variety of compounds in the library gave excellent inhibition of PTP-β shows that these compounds could provide an alternative to previously reported vanadium and peptidomimetic-based PTP inhibitors.[12–14]

The finding that arylstibonates were potent PTP-β inhibitors prompted the testing of 13778 and P6949 against a panel of nine CX_5R family members using the artificial substrate *O*-methylfluorescein phosphate (OMFP) (Table 2). Included in this panel were several enzymes (Cdc25s, PTEN, MKP-3, VHR, and PTPMT1) that are members of the dual-specificity phosphatase (DSP) subfamily, which are characterized by a broader substrate specificity. Although they share the same catalytic mechanism as the classical PTPs, the DSP active site allows these enzymes to accommodate phosphoserine, phosphothreonine, phosphotyrosine, and even non-protein substrates. After determining the K_M and V_{max} values for each enzyme (not shown), dose response curves for arylstibonates 13778 and P6949 were then constructed using OMFP concentrations near the K_M value for each enzyme (Fig. 3). Arylstibonates 13778 and P6949 were found to inhibit some of the tested PTPs with even higher potency than PTP-β. The best potency was observed for arylstibonate 13778 against Cdc25a phosphatase with an IC_{50} = 0.11 ± 0.01 μM, followed by Cdc25b (IC_{50} = 0.60 ± 0.07 μM). Interestingly, the activity of Cdc25c was not substantially affected by even 10 μM of compound 13778, indicating that 13778 displays some isoform-selectivity for different Cdc25 phosphatases. Another phosphatase that was inhibited by 13778 was PTPMT1 (IC_{50} = 1.62 μM ± 0.19 μM), which was recently identified as a DSP anchored at the inner membrane of the mitochondria.[15] PTP1B is inhibited by 13778 with an IC_{50} of ~8 μM, which is over 10-fold higher than PTP-β, once again showing the significant selectivity of these simple compounds, even when the active site sequences of the enzymes are closely matched (see below).

Table 2
IC_{50} values of PTPs assayed in the presence of 13778 and P6949 using OMFP as substrate

Enzyme	IC_{50} – 13778/μM	IC_{50} – P6949/μM
Cdc25a	0.11 ± 0.01	3.15 ± 0.13
Cdc25b	0.60 ± 0.07	5.67 ± 0.48
Cdc25c	>>10	>>100
VHR	>>10	>>100
PTP-β	0.77 ± 0.29	1.62 ± 0.92
MKP-3	>>10	>>100
PTP 1B	7.93 ± 3.95	>>100
PTEN	~10	8.87 ± 1.02
PTP MT1	1.62 ± 0.19	13.44 ± 1.21

Similar selectivity by compounds 13778 and P6949 is expected based on their similar structures, which only differ by the presence of the double bond in the carboxylate side chain of 13778. Accordingly, arylstibonate P6949 also targeted Cdc25a, and Cdc25b (as well as PTP-β), while Cdc25c was not affected at the concentrations tested (Fig. 3b). Nevertheless, despite their similar selectivities, the potency of compound P6949 is lower than that of 13778 (~30-fold lower for Cdc25a and ~10-fold lower for Cdc25b), indicating a marked beneficial contribution from the double bond-containing side chain of 13778. We determined that the inhibition was reversible for both compounds using a standard dilution approach, in which the enzyme was pre-incubated with 4 times the IC_{50} concentration of the arylstibonate inhibitor, followed by a 10-fold dilution with reaction buffer (Fig. 4).[16]

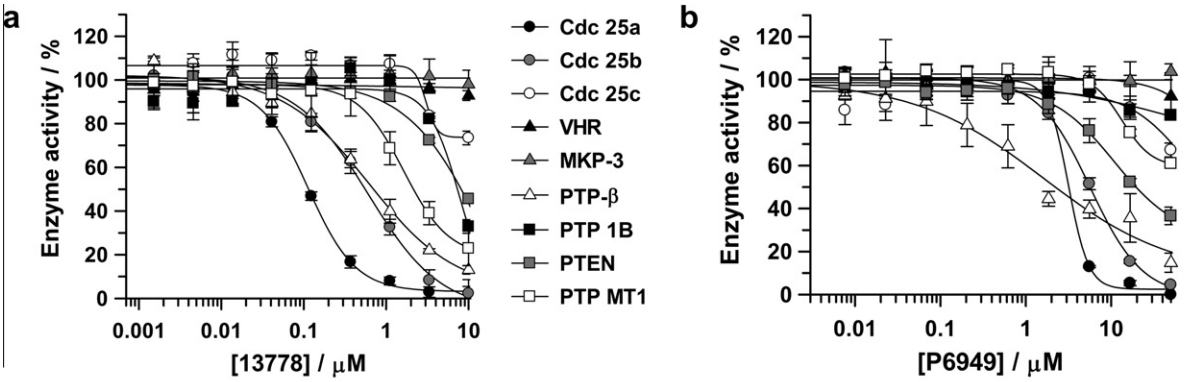
In order to explore the mode of inhibition, we measured the steady-state kinetic parameters of Cdc25a and Cdc25b in the presence of varying concentrations of 13778 and P6949. As shown in Figure 4, the apparent K_m values for both enzymes increased as the concentrations of 13778 and P6949 increased, and that V_{max} was simultaneously reduced, but not to zero. These trends indicate a partial mixed-type mode of inhibition, and that the inhibitors bind to a site different from that of the substrate. This behavior might explain why the arylstibonate inhibitors have different potency towards the three Cdc25 isoforms (period). Such selectivity would be difficult to explain if the active sites were targeted as the amino sequences of all three isoforms within the catalytic CX_5R motif are identical (Fig. 3c). Contrary to our initial expectations, these findings indicate that the arylstibonic acid moiety is not a mimic of the aryl phosphate substrate. Secondary plots of the enzymological data (not shown) revealed that 13778 has K_i values of 72 nM and 369 nM for Cdc25a and Cdc25b, respectively, while P6949 has K_i values of 1.7 μM for Cdc25a and 4.7 μM for Cdc25b.

In conclusion, a focused 40 member arylstibonic acid library was tested against the synaptojanin I-5-P and various members of the PTP family. Compounds 13778 and P6949 were identified as inhibitors of Cdc25a and Cdc25b phosphatases, with 13778 showing nanomolar potency, and favorable selectivity. Within the Cdc25 family, 13778 and P6949 show 100-fold isoform selectivity for Cdc25a and Cdc25b over Cdc25c, which makes both compounds valuable additions to the existing portfolio of isoform-selective Cdc25 inhibitors.[17] As over-expression of Cdc25a and/or Cdc25b, but not Cdc25c, has been detected in numerous cancers, these compounds comprise potentially valuable leads for selective inhibition of Cdc25a and Cdc25b without affecting the activity of Cdc25c, a feature that could be exploited in future drug development.[5]

3. Material and methods

3.1. Compounds

All arylstibonic acids were obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute, Bethesda, MD. Compounds were originally screened from DMSO solutions and the inhibitory activities of P6949 and 13778 containing the antimony element were confirmed from dry powders. Because antimony is paramagnetic, the fine features of the 400 MHz proton NMR spectra were broadened



c

PTP	Amino acid sequences												
Cdc 25a	V	F	H	C	E	F	S	S	E	R	G	P	R
Cdc 25b	I	F	H	C	E	F	S	S	E	R	G	P	R
Cdc 25c	V	F	H	C	E	F	S	S	E	R	G	P	R
VHR	L	V	H	C	R	E	G	Y	S	R	S	P	T
MKP-3	L	V	H	C	L	A	G	I	S	R	S	V	T
PTP-β	V	V	H	C	S	A	G	V	G	R	T	G	T
PTP 1B	V	V	H	C	S	A	G	I	G	R	S	G	T
PTEN	A	I	H	C	K	A	G	K	G	R	T	G	V
PTP MT1	Y	V	H	C	K	A	G	R	S	R	S	A	T
Consensus	X	X	H	C	X	X	S/G	X	X	R	X	X	X

Figure 3. *Potency of arylstibonate 13778 and P6949 on members of the PTP family.* (a) IC_{50} curves of PTPs in the presence of arylstibonate 13778. (b) IC_{50} curves of PTPs in the presence of arylstibonate P6949. Data shown are the average of three independent experiments performed in triplicate. (c) Alignment of amino acid sequences of the catalytic motif of Cdc25 phosphatases, VHR, MKP-3, PTP-β, PTP 1B, PTEN and PTPMT1.

extensively, and carbon spectra were not obtainable due to due unfavorable relaxation effects. The purity of each compound was determined as greater than 97% based on proton NMR spectra. Microanalysis was performed by A P Dickerson, University of Cambridge.

P6949 3-(3-stibonophenyl)propanoic acid, ^{1}H NMR (D_2O): δ 2.0–3.0 (m, 4H), 7.0–8.0 (m, 4H); ESI-MS: m/z 320.9, 322.9 [MH^+].

13778 (*E*)-3-(3-stibonophenyl)acrylic acid, ^{1}H NMR (DMSO-d_6): δ 6.0–6.5 (m, 1H), 7.0–8.2 (m, 5H), 12.0–12.8 (br, 1H). ESI-MS: no signal obtained.

Microanalysis calculated for $C_9H_9O_5Sb$: C, 33.89; H, 2.84; N, 0.00. Found: C, 33.90; H, 2.70; N, 0.00.

3.2. Protein expression and purification

Human PTEN and rat synaptojanin (amino acids 1–1042), the latter containing the SAC1 and 5-phosphatase activities were expressed as glutathione S-transferase (GST)-fusion proteins and purified as described previously.[18] Protein expression was induced in the *Escherichia coli* strain XL-1 blue for 24 h using 1 mM isopropyl β-D-1-thiogalactopyranoside at 18 °C for synaptojanin and at 23 °C for PTEN. Cells were harvested and stored at −20 °C. The harvested cells were re-suspended in lysis buffer containing 50 mM Tris (pH 7.4), 10 mM benzamidine hydrochloride, 100 μg/mL soybean trypsin inhibitor, 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride, 2 mM dithiothreitol (DTT) and 1% Triton X-100. Lysis was performed by adding lysozyme to the cell suspension at a concentration of 2 mg/mL and sonication. Cell debris was removed by centrifugation at 18,000*g* for 1 h at 4 °C. The supernatant was loaded onto a glutathione sepharose column, pre-equilibrated with 50 mM Tris (pH 7.4), 140 mM NaCl and 2.7 mM KCl. After loading, the column was washed twice with 50 mM Tris (pH 7.4), 140 mM NaCl, 2.7 mM KCl and 2 mM DTT. Another two washes were performed using the same buffer with 500 mM NaCl. The GST-tagged PTEN was eluted using 20 mM glutathione in 50 mM Tris (pH 7.4), 250 mM NaCl, 20% glycerol and 2 mM DTT. Protein concentration was determined using Bradford assay. Protein integrity was confirmed by Western blot using GST antibody (Novagen). The catalytic domains of Cdc25a, Cdc25b and Cdc25c with N-terminal His tags were expressed from pET21a in *E.coli* strain BL21 (DE3) and purified by ion exchange (Sepharose SP Fast Flow) and gel filtration (Sephadex G-50) chromatography. Recombinant human His-VHR was obtained from Enzo Life Sciences (Exeter, UK) and PTP-β was purchased from Sigma-Aldrich (Dorset, UK).

4. Phosphatase assays

All enzyme preparations were tested for linearity to ensure that suitable amounts of enzyme were employed in all kinetic studies.

4.1. Colorimetric quantification of phosphate release using malachite green assay

The phosphatase activities of synpatojanin and the SAC1 phosphatase were determined by measuring the amount of phosphate released using PI(4)P and IP_3 as substrates.[18] In the case of lipid substrates the phosphatase reaction was started by adding the corresponding substrates presented in octylglucoside mixed micelles as described before.[19] Reactions were carried out in a 96-well plate with buffer containing 4 mM $MgCl_2$, 100 mM Tris (pH 7.4) in a total volume of 80 μL at 37 °C. For a negative activity control, appropriate amounts of denatured enzyme (prepared from active enzyme by heat denaturation at 95 °C for 10 min) were employed in place of active enzyme. In order to stop the reaction, same volume of color reagent (5 mM malachite green, 17 mM ammonium heptamolybdate, 77 mM bismuth citrate and 1.7 M HCl) was added to the assay. The mixture was allowed to develop for 10 min and the absorbance was read at 625 nm.

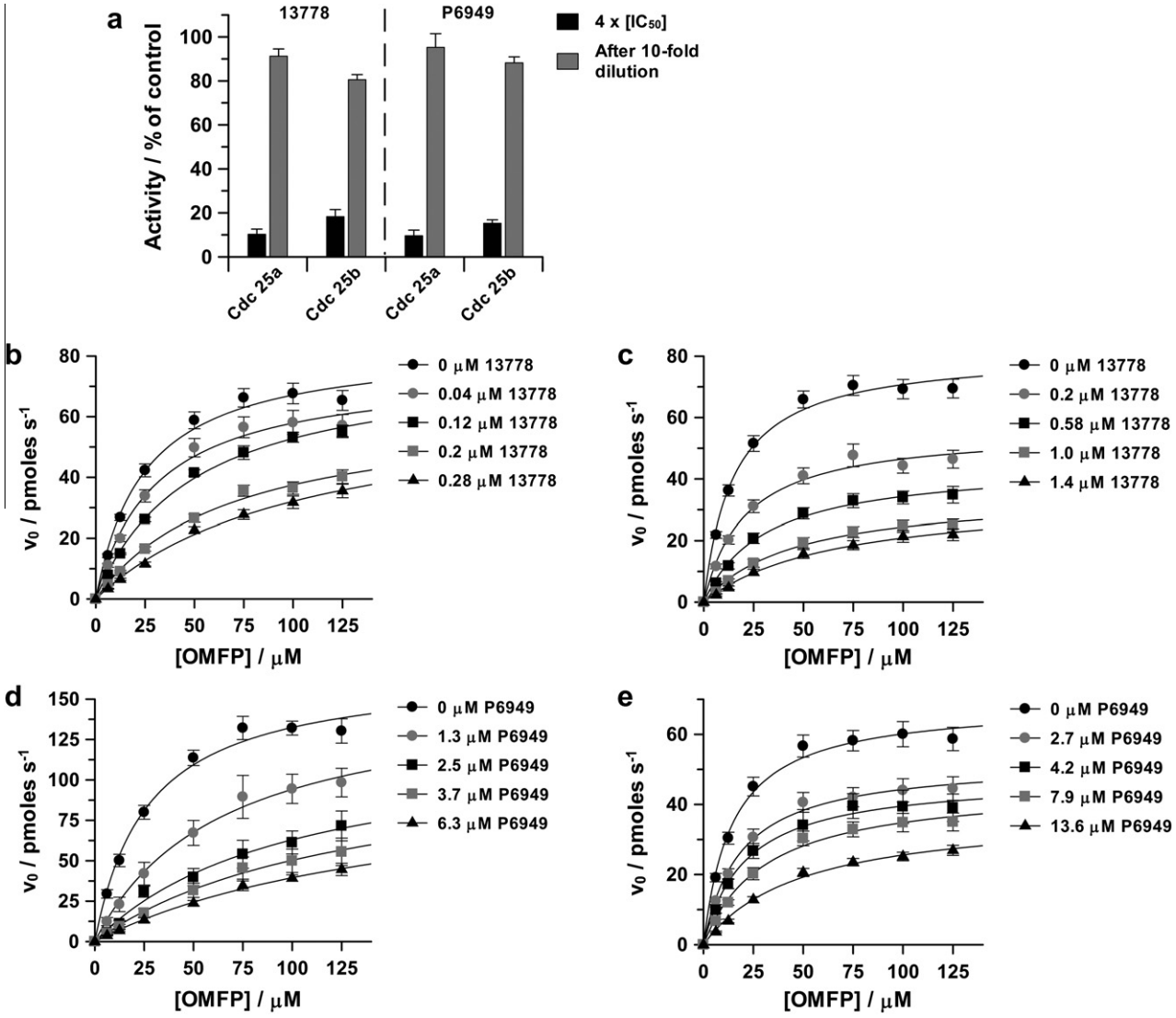


Figure 4. *Mode of inhibition of Cdc25a and Cdc25b by 13778 and P6949 is reversible and mixed-type.* (a) Reversibility of inhibition as determined by the dilution method. Cdc25 phosphatases were incubated with 4 times the IC_{50} concentrations of either 13778 or P6949, respectively. The enzyme-inhibitor mixture was diluted 10-fold using reaction buffer and enzyme activity was assayed as above. Activity is shown as the percentage of the uninhibited reaction. (b and c) Michaelis–Menten plots of Cdc25a activity using OMFP as the substrate in the presence of either 13778 or P6949. (d and e) Michaelis–Menten plots of Cdc25b activity using OMFP as substrate in the presence of either 13778 or P6949 at the indicated concentrations.

4.2. Colorimetric PTP-β assay using para-nitrophenol phosphate as substrate (p-NPP)

The phosphatase activity of PTP-β was measured using p-NPP as substrate.[20] As above, reactions were carried out in a 96-well plate in the presence of 25 mM Hepes (pH 7.4), 50 mM NaCl and 5 mM DTT. The reaction was initiated by the addition of p-NPP dissolved in H_2O. The enzymatic release of *p*-nitrophenol was monitored at 405 nm.

4.3. 3-O-Methylfluorescein phosphate (OMFP) assay

Enzyme kinetic studies using OMFP were performed as previously described.[16] OMFP was dissolved in DMSO to a concentration of 20 mM and then further diluted with 1% DMSO to the tested concentrations. Assays were performed in 96-well plates at room temperature (20 °C) using 100 mM Tris (pH 7.4) containing 2 mM DTT. For the inhibition studies, enzymes were pre-incubated with the arylstibonates at rt for 10 min. Reactions were then initialized by adding OMFP. The hydrolysis of OMFP to OMF was monitored by measuring the change in fluorescence (excitation at 485 nm and emission at 525 nm) using a Varian fluorescence spectrophotometer.

Acknowledgments

We thank Bob Shoemaker and Rhone Akee and the Developmental Therapeutics Program at the National Cancer Institute, Frederick Maryland for providing the arylstibonic acid small molecule library. We would also like to thank Jack E. Dixon (University of California at San Diego) for the generous gift of the expression plasmid of PTPMT1. This work was supported by an EPSRC Centre for Doctoral Training Studentship from the Institute of Chemical Biology (Imperial College London) awarded to J.K., Cancer research UK (D.M.) and a NIH Grants GM068626 and GM056834 to J.T.S.

References and notes

1. Burke, T. R.; Zhang, Z.-Y. *Peptide Science* **1998**, *47*, 225.
2. Tonks, N. K. *Nat. Rev. Mol. Cell Biol.* **2006**, *7*, 833.

3. Rudolph, J. *Biochemistry* **2007**, *46*, 3595.
4. Boutros, R.; Lobjois, V.; Ducommun, B. *Nat. Rev. Cancer* **2007**, *7*, 495.
5. Gasparotto, D.; Maestro, R.; Piccinin, S.; Vukosavljevic, T.; Barzan, L.; Sulfaro, S.; Boiocchi, M. *Cancer Res.* **1997**, *57*, 2366.
6. Kristjansdottir, K.; Rudolph, J. *Chem. Biol.* **2004**, *11*, 1043.
7. Seiple, L. A.; Cardellina, J. H.; Akee, R.; Stivers, J. T. *Mol. Pharmacol.* **2008**, *73*, 669.
8. Kim, H.; Cardellina Ii, J. H.; Akee, R.; Champoux, J. J.; Stivers, J. T. *Bioorg. Chem.* **2008**, *36*, 190.
9. Whisstock, J. C.; Romero, S.; Gurung, R.; Nandurkar, H.; Ooms, L. M.; Bottomley, S. P.; Mitchell, C. A. *J. Biol. Chem.* **2000**, *275*, 37055.
10. Andersen, H. S.; Iversen, L. F.; Jeppesen, C. B.; Branner, S.; Norris, K.; Rasmussen, H. B.; Møller, K. B.; Møller, N. P. H. *J. Biol. Chem.* **2000**, *275*, 7101.
11. Doman, T. N.; McGovern, S. L.; Witherbee, B. J.; Kasten, T. P.; Kurumbail, R.; Stallings, W. C.; Connolly, D. T.; Shoichet, B. K. *J. Med. Chem.* **2002**, *45*, 2213.
12. Kotoris, C. C.; Chen, M. J.; Taylor, S. D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3275.
13. Schmid, A. C.; Byrne, R. D.; Vilar, R.; Woscholski, R. *FEBS Lett.* **2004**, *566*, 35.
14. Huyer, G.; Liu, S.; Kelly, J.; Moffat, J.; Payette, P.; Kennedy, B.; Tsaprailis, G.; Gresser, M. J.; Ramachandran, C. *J. Biol. Chem.* **1997**, *272*, 843.
15. Pagliarini, D. J.; Wiley, S. E.; Kimple, M. E.; Dixon, J. R.; Kelly, P.; Worby, C. A.; Casey, P. J.; Dixon, J. E. *Mol. Cell* **2005**, *19*, 197.
16. Mak, L., Vilar, R., Woscholski, R. *J. Chem. Biol.*
17. Vintonyak, V. V.; Antonchick, A. P.; Rauh, D.; Waldmann, H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 272.
18. Rosivatz, E.; Matthews, J. G.; McDonald, N. Q.; Mulet, X.; Ho, K. K.; Lossi, N.; Schmid, A. C.; Mirabelli, M.; Pomeranz, K. M.; Erneux, C.; Lam, E. W. F.; Vilar, R.; Woscholski, R. *ACS Chem. Biol.* **2006**, *1*, 780.
19. Schmid, A. C.; Wise, H. M.; Mitchell, C. A.; Nussbaum, R.; Woscholski, R. *FEBS Lett.* **2004**, *576*, 9.
20. Picha, K. M.; Patel, S. S.; Mandiyan, S.; Koehn, J.; Wennogle, L. P. *J. Biol. Chem.* **2007**, *282*, 2911.