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# 3-Substituted indolizine-1-carbonitrile derivatives as phosphatase inhibitors

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Dedicated to Rolf Huisgen on the occasion of his 85th birthday.

**Abstract**—In the course of studies directed toward the discovery of novel scaffolds for medicinal application, we synthesized a series of 3-substituted indolizine-1-carbonitrile derivatives. Some of them displayed activity against MPtpA/MPtpB phosphatases which are involved in infectious diseases. We report here the solid-phase synthesis and antiphosphatase activity of a series of indolizines.
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Protein phosphorylation and dephosphorylation reactions are employed by living organisms for the regulation of innumerable cellular processes. Aberrancies in protein phosphorylation contribute to the development of many human diseases such as cancer and diabetes.[1] Phosphorylation states are governed by protein kinases (PKs) catalyzing protein phosphorylation, and protein phosphatases (PPs), which are responsible for dephosphorylation. PKs are established targets for drug discovery.[2] Protein phosphatases have been classified by structure and substrate specificity into protein serine/threonine (PSTPs) and tyrosine phosphatases (PTPs). However, the development of small-molecule inhibitors of PTPs is emerging only very recently[3] as a rapidly growing area of investigation in clinical biology and medicinal chemistry.[4]

This development was undoubtedly triggered by the discovery that the disruption of the *ptp1b* gene in mice confers resistance to obesity and increases insulin sensitivity without negative side effects.[5] The treatment of type 2 diabetes and obesity could benefit from PTP inhibitors and therefore, PTP1b is currently a major target of medicinal chemistry research in the pharmaceutical industry.[6] Tyrosine phosphatases are essential for the virulence of several pathogenic bacteria. Bacteria like *Salmonella typhimurium* (the typhus pathogen) and *Yersinia pestis* (the plague pathogen) use their own phosphatases—such as YopH of *Yersinia*,[7] which blocks phagocytosis by macrophages—to infect their hosts or escape from an immune response. The restricted distribution of two putative tyrosine phosphatases (MPTPA and MPTPB), which are cloned from genomic DNA of *Mycobacterium tuberculosis*, makes it a good candidate for a virulence gene of *M. tuberculosis*.[8] The dual specific phosphatases of the Cdc25 family have attracted particular attention as regulators of the cell cycle, as they dephosphorylate the cyclin-dependent kinase which triggers key transitions in the process of eukaryotic cellular division.[9] Cdc25A has attracted considerable interest as its overexpression is correlated to the malignancy of tumors.[10] The vaccina VH1-related dual specific phosphatase VHR is a physiological regulator of extracellular regulated kinases of the MAP (mitogen-activated protein) kinase family and influences signaling through the MAP kinase pathway.[11]

PTP inhibition would thus constitute a valuable strategy against infectious diseases[12] and bioterrorism. The recent advances in the field of PTP inhibitor discovery with a focus on phosphatases could be the targets of the next drug generation as they play a major role in the development of diseases.[3a]

*Keywords*: Phosphatase; Solid-phase; 3-Substituted indolizine-1-carbonitrile.
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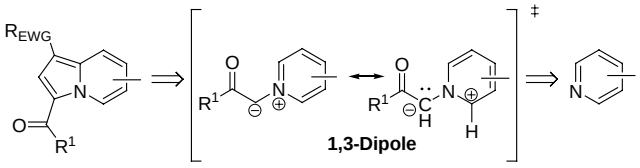
We here present 3-substituted indolizine-1-carbonitrile derivatives as phosphatase inhibitors. While searching for interesting molecular frameworks to use for the synthesis of combinatorial libraries on solid support, our attention was drawn to the formal [3+2]-cycloaddition of pyridinium ylides with electron deficient alkenes.[13] This chemistry has ample precedent in solution and is particulary attractive due to high yields and mild conditions typically involved.[14] The synthesis allows for the introduction of hydrophilic, electron-withdrawing substituents starting from several pyridine derivatives (Scheme 1).

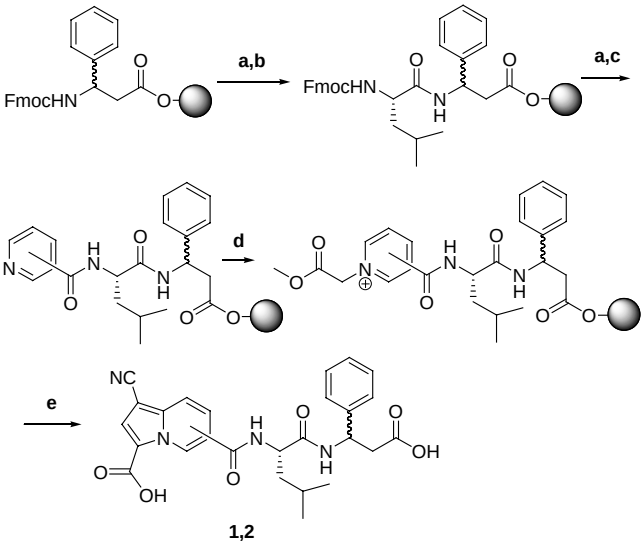
Initially, both nicotinic and isonicotinic acids were coupled under standard Fmoc solid-phase conditions to trityl chloride polystyrene resin (TCP) previously loaded with *N*-Fmoc-Leu β-phenylalanine followed by quaternization by treatment with excess of methyl-2-bromoacetate in DMF (Scheme 2). The resin bound pyridinium salt was simply washed with DMF and dichloromethane, and then treated with acrylonitrile in the presence of triethylamine in DMF for 2 h at 60 °C to furnish the resin bound tetrahydroindolizine. In order to preserve the bicyclic ring structure, an oxidative strategy was adopted. Hu et al. have developed the bimetallic complex TPCD $[Co(pyridine)_4(HCrO_4)_2]$ as a reagent for the one-pot synthesis of aromatic indolizines.[15] When the resin bound dipolar cycloaddition product was oxidized in the presence of TPCD at 80 °C in DMF, a clean reaction occurred to give the aromatic indolizines in good yields. The methyl esters were cleaved from the resin under acidic conditions and subsequently hydrolyzed to furnish the desired free acids **1** and **2**.[16]

The cycloaddition was regioselective in accordance with the electronic effects. Attempts to use both fumaric and maleic acid esters as electron deficient alkenes failed because of the putative incomplete oxidation of the tetrahydroindolizine intermediates (Schemes 3–8).

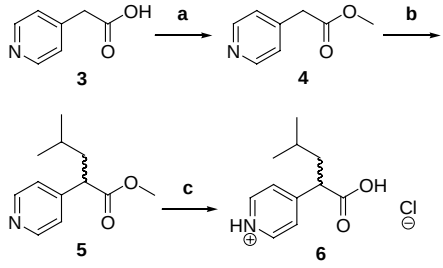
An amide bond can be substituted by aromatic ring surrogates mimicking the planar character.[17] Following this approach, (pyridin-4-yl)acetic acid (**3**) is a useful template due to the possibility of the easy introduction of substituents in α-position to the carbonyl group. The 2-substituted acid **6** can serve as a 1,3-dipole for the [3+2]-cycloaddition after quaternization. In the first step, the methyl ester **4** was alkylated with 1-bromo-2-methylpropane after deprotonation with sodium hydride followed by hydrolysis under acidic conditions to yield free acid **6**.



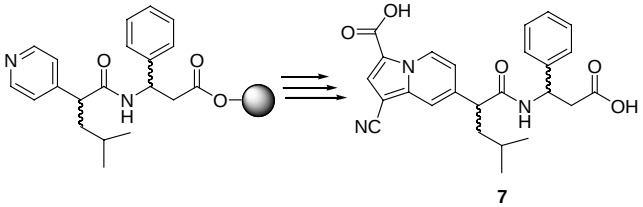

**Scheme 1.** Pyridine derivatives initiate the synthesis of indolizine scaffolds.



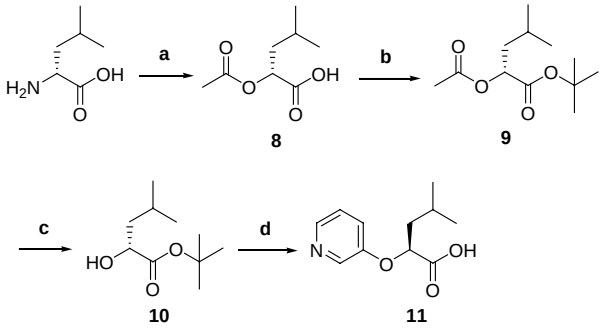

**Scheme 2.** Solid-phase synthesis of indolizines. Reagents and conditions: (a) i—20% piperidine/NMP; ii—wash with NMP; (b) i—*N*-Fmoc-Leu-OH, TBTU, HOBT, DIPEA, NMP; ii—wash with NMP; (c) i—nicotinic/isonicotinic acid, TBTU, HOBT, DIPEA, NMP; ii—wash with NMP and DCM; (d) i—20 equiv methyl-2-bromoacetate, DMF, 40 °C; ii—wash with DMF and DCM; (e) i—2 equiv $NEt_3$, 20 equiv acrylonitrile, DMF, 60 °C, 2 h; ii—wash with DMF and DCM; iii—TCPD, DMF, 80 °C, 10 h; iv—wash with DMF and DCM; v—TFA/DCM = 1:10; vi—NaOH, MeOH.



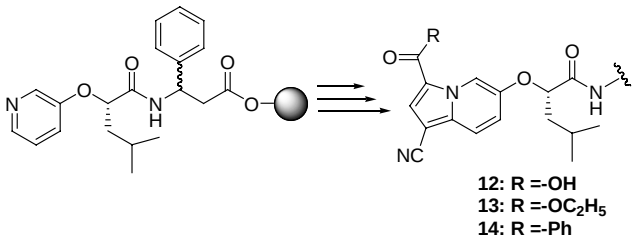

**Scheme 3.** Synthesis of 4-methyl-2-(pyridin-4-yl)pentanoic acid (**6**). Reagents and conditions: (a) i—MeOH, cat. $H_2SO_4$, reflux; ii—NaOH, $H_2O$ (quant.); (b) i—NaH, DMF, 0 °C; ii—1-bromo-2-methylpropane, 50 °C (68%); (c) 6 M HCl (quant.).



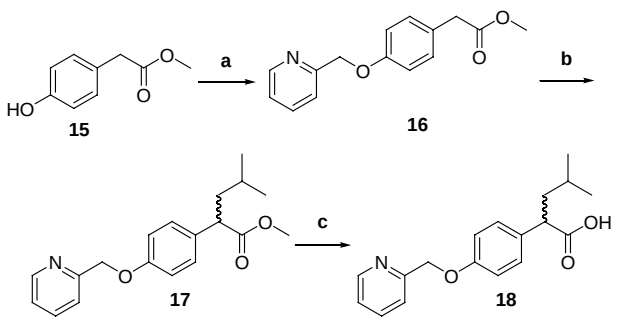

**Scheme 4.** Solid-phase synthesis of indolizine **7**. Reagents and conditions: (a) i—20% piperidine/NMP; ii—wash with NMP; (b) i—4-methyl-2-(pyridin-yl)pentanoic acid (6), TBTU, HOBT, DIPEA, NMP; ii—wash with NMP; (c) i—20 equiv methyl-2-bromoacetate, DMF, 40 °C; ii—wash with DMF and DCM; (d) i—2 equiv $NEt_3$, 20 equiv acrylonitrile, DMF, 60 °C, 2 h; ii—wash with DMF and DCM; iii—TCPD, DMF, 80 °C, 10 h; iv—wash with DMF and DCM; v—TFA/DCM = 1:10; vi—NaOH, MeOH.



**Scheme 5.** Synthesis of 4-methyl-2-(pyridin-3-yl-oxy)pentanoic acid (**11**). Reagents and conditions: (a) $NaNO_2$, acetic acid (85%); (b) DCC, 4.0 equiv *tert*-butanol, cat. DMAP (70%); (c) $K_2CO_3$, MeOH/$H_2O$ (63%); (d) i—pyridine-3-ol, DIAD, $PPh_3$, THF, 0 °C; ii—TFA/DCM = 1:1 (75%).

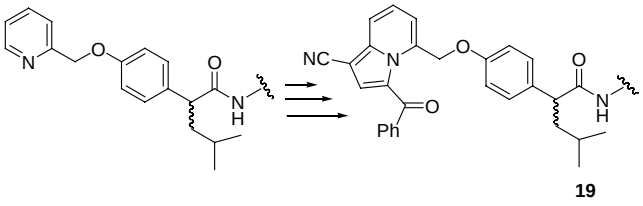



**Scheme 6.** Solid-phase synthesis of indolizines **12**–**14**. Reagents and conditions: (a) i—20% piperidine/NMP; ii—wash with NMP; (b) i—4-methyl-2-(pyridin-3-yl-oxy)pentanoic acid (11), TBTU, HOBT, DIPEA, NMP; ii—wash with NMP; (c) i—20 equiv methyl-2-bromoacetate, DMF, 40 °C; ii—wash with DMF and DCM; (d) i—2 equiv $NEt_3$, 20 equiv acrylonitrile, DMF, 60 °C, 2 h; ii—wash with DMF and DCM; iii—TCPD, DMF, 80 °C, 10 h; iv—wash with DMF and DCM; v—TFA/DCM = 1:10; vi—NaOH, MeOH.



**Scheme 7.** Synthesis of 2-substituted 2-(4-((pyridin-2-yl)methoxy)phenyl)acetic acid (**17**). Reagents und conditions: (a) (pyridine-2-yl)methanol, DIAD, $PPh_3$, THF, 0 °C (75%); (b) i—NaH, DMF, 0 °C; ii—1-bromo-2-methylpropane, 50 °C (67%); (c) NaOH, MeOH.

The [3+2]-cycloaddition on solid support offers a convenient approach to construct a small library. In the following variation, the planar amide bond is replaced by an aromatic surrogate but the usage of (pyridin-3-yl-oxy)-derivatives extended the backbone with an ether unit, isosteric to a methylene group, which imparts an additional hydrogen bond acceptor. With reference to the naturally occurring L-configuration of amino acids an enantioselective synthesis of the leucine surrogate was desirable. 4-Methyl-2-(pyridin-3-yl-oxy) pentanoic



**Scheme 8.** Synthesis of indolizine scaffold **19**. Reagens and conditions: (a) i—20% piperidine/NMP; ii—wash with NMP; (b) i—2-(4-((pyridin-2-yl)methoxy)phenyl)acetic acid (17), TBTU, HOBT, DIPEA, NMP; ii—wash with NMP; (c) i—20 equiv methyl-2-bromoacetate, DMF, 40 °C; ii—wash with DMF and DCM; (d) i—2 equiv $NEt_3$, 20 equiv acrylonitrile, DMF, 60 °C, 2 h; ii—wash with DMF and DCM; iii—TCPD, DMF, 80 °C, 10 h; iv—wash with DMF and DCM; v—TFA/DCM = 1:10; vi—NaOH, MeOH.

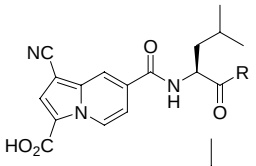
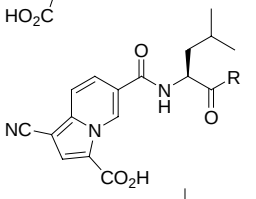
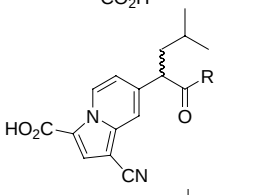
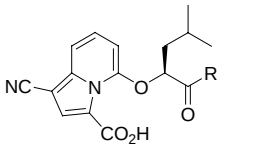
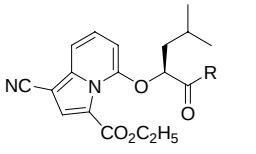
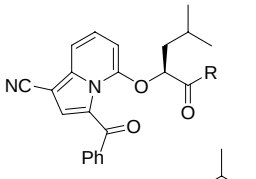
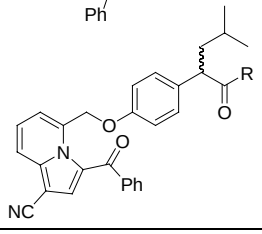
acid (**11**) is prepared as follows: D-leucine was converted to the diazonium compound and reacted with acetic acid to ester **8**, which was subsequently transformed into the *tert*-butyl ester **9**. After hydrolysis of the acetic acid ester moiety, the hydroxyl functionality was converted to an ether via Mitsunobu reaction with 3-hydroxy pyridine. Cleavage of the *tert*-butyl ester afforded the free acid **11** required for the indolizine synthesis on solid support.

To elucidate the influence of extension of the aromatic moiety on activity, the use 2-(4-hydroxyphenyl)acetic acid (**15**) is useful as the phenyl ring might mimic the planar structure of the amide bond and the hydroxyl functionality can easily be derivatized with (pyridin-2-yl)methanol via Mitsunobu reaction. 2-(4-((Pyridin-2-yl)methoxy)phenyl)acetic acid (**16**) imparts an additional hydrogen bond acceptor. The methyl ester **16** was alkylated with 1-bromo-2-methylpropane after deprotonation with sodium hydride and subsequently hydrolyzed under basic conditions to give the substituted phenyl-benzyl ether core **18**. The aromatic moiety can be extended by quaternization by treatment with 2-bromo-1-phenylethanone.

The inhibitory effects of a representative number of indolizine derivatives against different phosphatases were examined in an in vitro assay system. The results are shown in Table 1. The compound displaying the 2-(4-((pyridin-2-yl)methoxy)phenyl)acetic acid moiety **19** is proposed to interact with the active site, whereas the peptidic backbone bearing the alkyl side chains was proposed to exert hydrophobic interactions with the enzyme.[18] The 5-(phenoxymethyl)indolizine scaffold is not essential but is preferred over the other scaffolds as indolizine **14** bearing an indolizine-6-ol moiety also shows activity. The benzoyl substituent on the indolizine scaffold seems to be essential for the activity.

In conclusion, compound **19** incorporates a structural framework not present in any of the phosphatase inhibitors described so far. These data also make clear that the 5-(phenoxymethyl)indolizine scaffold provides a promising lead structure and certainly deserves further studies to establish pertinent structure–activity relationships. The scaffold **14** was found to inhibit MPTPB but not MPTPA, while compound **19** was found to be

**Table 1.** $IC_{50}$ values (μM) of biased indolizine scaffolds against different phosphatases[a]

| Compound | Structure[b] | Cdc25A | PTP1b | MPTPA | MPTPB | VHR |
|---|---|---|---|---|---|---|
| **1** |  | >100 | >100 | >100 | >100 | >100 |
| **2** |  | >100 | >100 | >100 | >100 | >100 |
| **7** |  | >100 | >100 | >100 | >100 | >100 |
| **12** |  | >100 | >100 | >100 | >100 | >100 |
| **13** |  | >100 | >100 | >100 | >100 | >100 |
| **14** |  | 70.7 ± 5.3 | 85.1 ± 7.2 | >100 | 22.0 ± 2.7 | 82.9 ± 3.9 |
| **19** |  | 69.2 ± 8.9 | 80.6 ± 5.2 | 74.9 ± 8.8 | 7.5 ± 1.9 | 77.8 ± 1.8 |

[a] For assay conditions, see Ref. 19.

[b] The compounds accrued as diastereomers. R = 3-amino-3-phenylpropionic acid. The assigned structures were confirmed by $^1H$ NMR and mass spectroscopy.

10-fold more selective for MPTPB. Generally, the [3+2]-cycloaddition of electron deficient alkenes to pyridinium ylides provides a convenient method to substitute indolizines.

## References and notes


1. Hunter, T. *Cell* **2000**, *100*, 113.
2. (a) Bridges, A. J. *Chem. Rev* **2001**, *101*, 2541; (b) Shawver, L. K.; Slamon, D.; Ullrich, A. *Cancer Cell* **2002**, *1*, 117; (c) (c) Grosios, K.; Traxler, P. *Drug Future* **2003**, *28*, 679; (d) Noble, M. E. M.; Endicott, J. A.; Johnson, L. N. *Science* **2004**, *303*, 1800.
3. (a) Zhang, Z. Y. *Annu. Rev. Pharmacol.* **2002**, *42*, 209; (b) Szczepankiewicz, B. G. et al. *J. Am. Chem. Soc.* **2003**, *125*, 4087; (c) Bialy, L.; Waldmann, H. *Angew. Chem., Int. Ed.* **2005**, *44*, 3814.
4. (a) Lyon, M. A.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Nat. Rev. Drug Discov.* **2002**, *1*, 961; (b) van Huijsduijnen, R. H.; Bombrun, A.; Swinnen, D. *Drug Discov. Today* **2002**, *7*, 1013; (c) Liu, G. *Curr. Med. Chem.* **2003**, *10*, 1407.
5. (a) Elchebly, M.; Payette, P.; Michaliszyn, E.; Cromlish, W.; Collins, S.; Loy, A. L.; Normandin, D.; Cheng, A.; Himms-Hagen, J.; Chan, C. C.; Ramachandran, C.; Gresser, M. J.; Tremblay, M. L.; Kennedy, B. P. *Science* **1999**, *283*, 1544; (b) Klaman, L. D.; Boss, O.; Peroni, O. D.; Kim, J. K.; Martino, J. L.; Zabolotny, J. M.; Moghal, N.; Lubkin, M.; Kim, Y. B.; Sharpe, A. H.; Stricker-Krongrad, A.; Shulman, G. I.; Neel, B. G.; Kahn, B. B. *Mol. Cell Biol.* **2000**, *20*, 5479.
6. Johnson, T. O.; Ermolieff, J.; Jirousek, M. R. *Nat. Rev. Drug Discov.* **2002**, *1*, 696.

7. DeVinney, I.; Steele-Mortmer, I.; Finlay, B. B. *Trends Microbiol.* **2000**, *8*, 29.
8. Koul, A.; Choidas, A.; Treder, M.; Tyagi, A. K.; Drlica, K.; Singh, Y.; Ullrich, A. *J. Bacteriol.* **2000**, *182*, 5425.
9. Obaya, A. J.; Sedivy, J. M. *Cell Mol. Life Sci.* **2002**, *59*, 126.
10. (a) Galaktionov, K.; Lee, A. K.; Eckstein, J.; Draetta, G.; Meckler, J.; Loda, M.; Beach, D. *Science* **1995**, *269*, 1575; (b) Dixon, D.; Moyana, T.; King, M. J. *Exp. Cell Res.* **1998**, *240*, 236.
11. (a) Alonso, A.; Saxena, M.; Williams, S.; Mustelin, T. *J. Biol. Chem.* **2001**, *276*, 4766; (b) Todd, J. L.; Rigas, J. D.; Rafty, L. A.; Denu, J. M. *Oncogene* **2002**, *21*, 2573.
12. Manger, M.; Scheck, M.; Prinz, H.; von Kries, J.-P.; Langer, T.; Saxena, K.; Schwalbe, H.; Fürstner, A.; Rademann, J.; Waldmann, H. *ChemBioChem*, accepted for publication.
13. (a) Huisgen, R. *Angew. Chem., Int. Ed. Engl.* **1968**, *7*, 321; (b) Huisgen, R.; Temme, R. *Eur. J. Org. Chem.* **1998**, *2*, 387; (c) Druta, I. I.; Andrei, M. A.; Ganj, C. I.; Aburel, P. S. *Tetrahedron* **1999**, *55*, 13063; (d) Goff, D. A. *Tetrahedron Lett.* **1999**, *40*, 8741.
14. (a) Uchida, T.; Matsumoto, K. *Synthesis* **1976**, *209*; (b) Swinbourne, F. T.; Hunt, J. H.; Klinkert, K. In *Advances in Heterocyclic Chemistry*; Katrizky, A. R., Ed.; Academic Press: New York, 1978; Vol. 32; (c) Molina, P.; Fresneda, P. M.; Lajara, M. C. *J. Heterocycl. Chem.* **1985**, *22*, 113; (d) Sliwa, W. *Heterocycles* **1986**, *24*, 181.
15. (a) Hu, Y.; Hu, H. *Synth. Commun.* **1992**, *22*, 1491; (b) Wei, X.; Hu, Y.; Li, T.; Hu, H. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2487; (c) Zhang, X.; Cao, W.; Wei, X.; Hu, H. *Synth. Commun.* **1997**, *27*, 1395.
16. Typical experimental procedure: the indolizine precursor was coupled to the TCP resin via standard Fmoc-solid-phase chemistry. After the coupling, the resin was washed with DMF and dichloromethane, dried, and subsequently treated with a solution of ethyl 2-bromoacetate (20 equiv) in DMF for 2 h at 40 °C. The resin was washed with DMF and DCM. Afterwards the resin was transferred into a glass vial and treated with a solution of acroylnitrile (20 equiv) and triethylamine (20 equiv) in DMF at 60 °C for 2 h. Washing with DMF and dichloromethane furnished the resin bound tetrahydroindolizine, which was treated with TCPD (3.0 equiv) in DMF. The oxidation was run for 6 h at 80 °C to afford the indolizine after washing with DMF and DCM. The desired product was cleaved with 1:10 TFA/DCM and the ethyl ester was hydrolyzed under basic conditions to furnish the crude product, which was purified by HPLC. The [3+2]-cycloaddition and subsequent oxidation afforded the indolizines in a clean reaction and good yields.
17. Rockwell, A.; Melden, M.; Copeland, R. A.; Hardman, K.; Decicco, C. P.; DeGrado, W. F. *J. Am. Chem. Soc.* **1996**, *118*, 10337.
18. (a) Barford, D.; Flint, A. J.; Tonks, N. K. *Science* **1994**, *263*, 1397; (b) Pannifer, A. D. B.; Flint, A. J.; Tonks, N. K.; Barford, D. *J. Bio. Chem.* **1998**, *273*, 10454.
19. All enzyme assays were performed by means of an automated system consisting of a Zymark SciClone ALH 500 in conjunction with a Twister II and a Bio-Tek Power Wave 340 reader. The reaction volume was 10 μL. The reaction was started by the addition of 5 μL *p*-nitrophenyl phosphate to 5 μL of a solution containing the respective enzymes which had been pre-incubated for 10–15 min with different concentrations from twofold dilution series of inhibitors. Reaction velocity was determined from the slope of the absorbance change at 405 nm and related to control values in absence of the inhibitor. $IC_{50}$ values were calculated from linear extrapolations of reaction velocity as a function of the logarithm of concentration. This non-biased approach did not allow for the determination of $IC_{50}$ values larger than 100 μM. The error indicated in Table 1 is the standard deviation. The overall experimental error, including the water content of DMSO stock solutions and weighing errors, is approximately 50% of the respective $IC_{50}$ values. All buffered solutions contained 2 mM DTE (1,4-dithio-D,L-threitol added on the day of the experiment from 100 mM stock) and 0.025% (v/v) of the detergent NP-40 (Calbiochem 492015). The buffers consisted of 50 mM Tris, 50 mM NaCl, 0.1 mM EDTA in the case of CDC25A, or 25 mM HEPES, 50 mM NaCl, 2.5 mM EDTA in the case of PTP1b, MPTPA, and MPTPB, or 25 mM MOPS, 5 mM EDTA in the case of VHR. The twofold dilution series were obtained from 10 μL of a buffered enzyme solution containing 200 μM of inhibitor. Of this, 5 μL was removed and mixed with 5 μL buffered enzyme solution resulting in a twofold dilution. This step was repeated 5 times. Five microlitres of the final dilution was removed, so that each well consisted of 5 μL buffered enzyme inhibitor mix. After addition of 5 μL *p*-nitrophenyl phosphate, the concentrations for the enzyme reaction were 50 mM in the case of CDC25A or 1 mM for all other phosphatases. The inhibitor concentrations were 100, 50, 25, 12.5, 6.25, or 3.125 μM, respectively. For all enzymes, their concentration was adjusted to an initial absorbance change of 1–2 $OD_{405}$/h. All reactions were performed as quadruplets from identical manual dilutions (1:10 in buffer from 10 mM stock solutions in DMSO).