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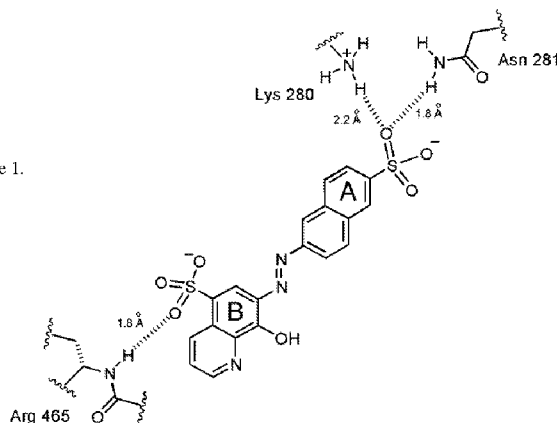
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(54) Title: INHIBITION OF SHP2/PTPN11 PROTEIN TYROSINE PHOSPHATASE BY NSC-117199 AND ANALOGS

Figure 1.



(57) **Abstract:** Protein tyrosine phosphatase (PTP) Shp2 is a non-receptor PTP that involved in cell signaling and regulation of cell proliferation, differentiation, and migration. Shp2 mediates activation of kinases that are involved in the pathogenesis of human carcinoma. NSC-117199 was identified as a potent inhibitor of the protein tyrosine phosphatase (PTP) Shp2. A focused library of analogs incorporating an isatin scaffold was designed and evaluated for inhibition of Shp2 and Shp1 PTP activities. Several compounds were identified that selectively inhibit Shp2 over Shp1 and PTP1B with low to sub-micromolar activity. Also disclosed are methods of inhibiting a protein tyrosine phosphatase in a cell and treating cancer through selective inhibition of Shp2.

5 **INHIBITION OF SHP2/PTPN11 PROTEIN TYROSINE
PHOSPHATASE BY NSC-117199 AND ANALOGS**

STATEMENT OF GOVERNMENT INTEREST

10 This invention was made with Government support under Grant No. CA077467 awarded by the National Institutes of Health. The Government has certain rights in the invention.

CROSS REFERENCE TO RELATED APPLICATIONS

15 This application is a continuation-in-part of pending U.S. Nonprovisional Patent Application 11/733,023, entitled "Inhibition of SHP2/PTPN11 Protein Tyrosine Phosphatase by NSC-87877, NSC-117199, and Their Analogs", filed April 9, 2007, which claims priority to U.S. Provisional Patent Application 60/744,431, entitled, "Shp2 Protein Tyrosine Phosphatase Inhibitor", filed April 07, 2006, the contents of which are herein incorporated by reference. This application also claims priority to U.S. Provisional Application 61/049,183, entitled "Oxindole Scaffold SHP-2 Inhibitors and Cancer Treatment Method", filed April 30, 2008, the contents of which are herein incorporated by reference.

20 **FIELD OF INVENTION**

 This invention relates to cancer therapy. More specifically, this invention relates to compounds useful in inhibiting Shp2/PTPN11 protein tyrosine phosphatase activity.

BACKGROUND OF THE INVENTION

25 The function of many proteins, particularly those involved in signal transduction pathways, is dependent upon their tyrosine phosphorylation status, which is finely regulated by the action of protein tyrosine kinases and protein tyrosine phosphatases (PTP). Many protein tyrosine kinases such as Bcr-Abl, c-kit, ErbB, and VEGFR are validated drug targets for cancer therapy (Bridges AJ. *Chem. Rev.* **2001**, *101*, 2541-2571), however little work has been performed on development of protein tyrosine phosphatase inhibitors as an alternative
30 strategy to modulate key target protein phosphorylation states (Bialy L and Waldmann H. *Angew. Chem.-Int. Ed.* **2005**, *44*, 3814-3839; Jiang Z-X and Zhang Z-Y. *Cancer Metast. Rev.* **2008**, *27*, in print (doi:10.1007/s10555-10008-19113-10553); Tautz L, et al. *Expert Opin. Ther. Targets* **2006**, *10*, 157-177; Dewang P et al. *Curr. Med. Chem.* **2005**, *12*, 1-22; Alonso A et al. *Cell* **2004**, *117*, 699-711). Shp2, encoded by the PTPN11 gene, is a
35 non-receptor PTP with two Src homology-2 (SH2) domains (N-SH2, C-SH2), (Alonso A, et al. *Cell* **2004**, *117*(6):699-711; Neel BG, et al. *Trends Biochem Sci.* **2003**, *28*(6):284-293) which mediates activation of Erk1 and Erk2 (Erk1/2, Erk) MAP kinases by receptor tyrosine kinases

5 such as ErbB1, ErbB2, and c-Met. (Nishida, K.; Hirano, T., *Cancer Sci.* **2003**, *94*, 1029-33; Gu, H.; Neel, B. G., *Trends Cell Biol.* **2003**, *13*, 122-30; Deb, T. B.; et al. *J Biol Chem* **1998**, *273*, 16643-6; Cunnick, J. M.; et al. *J. Biol. Chem.* **2000**, *275*, 13842-8; Maroun, C. R.; et al. *Mol. Cell Biol.* **2000**, *20*, 8513-25; Furge, K. A.; et al. *Oncogene* **2000**, *19*, 5582-9).

Shp2 is basally inactive due to auto-inhibition by its N-SH2 domain (Hof P, et al. *Cell* **1998**,
 10 92(4):441-450). In growth factor- and cytokine-stimulated cells, Shp2 binds to tyrosine-phosphorylated docking proteins through its SH2 domains, resulting in its activation (Cunnick JM, et al. *J Biol. Chem.* **2001**, *276*(26):24380-24387). Shp1 is mostly expressed in hematopoietic and epithelial cells and functions as a negative regulator of signaling pathways in lymphocytes. (Neel, B., Tonks, N., *Curr. Opin. Cell Biol.* **1997**, *9*, 193-204; Poole, A., Jones,
 15 M., *Cell. Signalling* **2005**, *17*, 1323-1332). The crystal structure of ligand-free Shp1 shows a similar arrangement of tandem SH2 domains that adopt a conformation blocking the PTP catalytic site. (Yang, J., et al., *J. Biol. Chem.* **2003**, *278*, 6516-20). Shp2 has been shown to bind Gab1 (or Gab2) in cells stimulated with EGF, HGF, or interleukin-6 (Cunnick JM, et al. *J Biol. Chem.* **2001**, *276*(26):24380-24387; Gu H and Neel BG. *Trends Cell. Biol.* **2003**,
 20 13(3):122-130; Maroun CR, et al. *Mol. Cell. Biol.* **2000**, *20*(22):8513-8525; Nishida K and Hirano T. *Cancer Sci.* **2003**, *94*(12):1029-1033). Gab1-Shp2 interaction as well as Shp2 PTP activity are necessary for Erk1/2 activation by these growth factors (Cunnick et al., 2002; Neel et al., 2003). (Cunnick JM, et al. *J Biol. Chem.* **2002**, *277*(11):9498-9504). Though growth factor-activation of Shp2 has been elucidated, the mechanisms by which Shp2 produces
 25 downstream signals, like activation of Ras-Erk1/2 MAP kinase pathway, are less clear (Mohi MG, et al. *Cancer Cell* **2005**, *7*(2):179-191).

Gain-of-function Shp2 mutants are found in childhood hematological malignancies such as juvenile myelomonocytic leukemia (JMML), some cases of solid tumors, and are associated with ~50% cases of Noonan syndrome. (Bentires-Alj, M.; et al. *Nat. Med.* **2006**, *12*, 283-285)
 30 (Bentires-Alj M, et al. *Cancer Res.* **2004**, *64*(24):8816-8820; Tartaglia M and Gelb BD. *Eur. J. Med. Genet.* **2005**, *48*(2):81-96). JMML is a progressive myelodysplastic/myeloproliferative disorder characterized by overproduction of tissue-infiltrating myeloid cells. Somatic mutations in PTPN11 account for about 35% of JMML patients, (Kratz CP, et al. *Blood* **2005**, *106*(6):2183-2185) and recent reports indicated JMML-associated Shp2 mutants can
 35 transform murine bone marrow and fetal liver cells (Chan RJ, et al. *Blood* **2005**, *105*(9):3737-3742; Mohi MG, et al. *Cancer Cell* **2005**, *7*(2):179-191; Schubbert S, et al. *Blood* **2005**, *106*(1):311-317). Noonan syndrome is a developmental disorder characterized by facial anomalies, short stature, heart disease, skeletal defects, and hematological disorders (Tartaglia M and Gelb BD. *Eur. J. Med. Genet.* **2005**, *48*(2):81-96), with about 50% of cases
 40 caused by germline PTPN11 mutations. All Shp2 mutants found in Noonan syndrome and JMML are gain-of-function mutations, mostly resulting from weaker autoinhibition of the N-

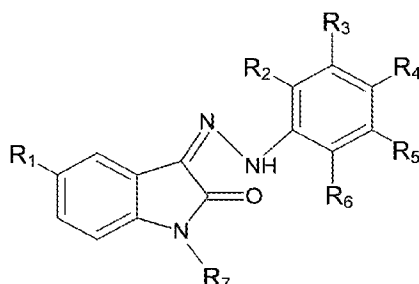
5 SH2 domain (Fragale A, et al. *Hum. Mutat.* **2004**, 23(3):267-277). It has also been reported that Shp2 is a key mediator of the oncogenic CagA protein of *Helicobacter pylori*, which causes gastric cancer. (Higashi, H.; et al. *Science* **2002**, 295, 683-6; Meyer-ter-Vehn, T.; et al. *J. Biol. Chem.* **2000**, 275, 16064-72).

PTP inhibitor development is an emerging area in the field of drug development (Bialy L and Waldmann H. *Angew Chem. Int. Ed. Engl.* **2005**, 44(25):3814-3839). Several compounds have been reported to non-selectively inhibit Shp2, with most efforts of PTP inhibitor discovery and design focused on PTP1B and Cdc25 inhibitors (Lazo JS, et al. *Mol. Pharmacol.* **2002**, 61(4):720-728; Zhang ZY. *Ann. Rev. Pharmacol. Toxicol.* **2002**, 42:209-234). No systematic effort to identify Shp2-selective PTP inhibitors has been reported. While PTP1B inhibitors that cross-inhibit Shp2 have been found (Huang P, et al. *Bioorg. Med. Chem.* **2003**, 11(8):1835-1849; Shen K, et al. *J. Biol. Chem.* **2001**, 276(50):47311-47319), none of them has demonstrated *in vivo* activity in cell cultures.

SUMMARY OF INVENTION

The development of a Shp2-specific inhibitor that does not cross-inhibit Shp1 is important for development of effective treatment modalities. Developing a Shp2-specific inhibitor is complicated by the similarity between Shp1 and Shp2, which share 60% overall sequence identity and approximately 75% similarity in their PTP domains. However, Shp1 and Shp2 catalytic domains have different substrate specificity (Tenev, T., et al., *J. Biol. Chem.* **1997**, 272, 5966-73; O'Reilly, A., Neel, B., *Mol. Cell Biol.* **1998**, 18, 161-77) suggesting that the catalytic cleft is not identical. Furthermore, the surface electrostatic potential of the catalytic cleft is much more positive in human Shp2 than in human Shp1. (Yang, J., et al., *J. Biol. Chem.* **1998**, 273, 28199-207). The PTP catalytic cleft consists of a base and four sides in the 3D structures (Hof P, et al. *Cell* **1998**, 92(4):441-450; Yang J, et al. *J. Biol. Chem.* **2003**, 278(8):6516-6520). Although amino acid residues present at the base of Shp1 and Shp2 PTP catalytic clefts are identical, all four sides of the catalytic cleft contain one or more residues that are different between Shp1 and Shp2.

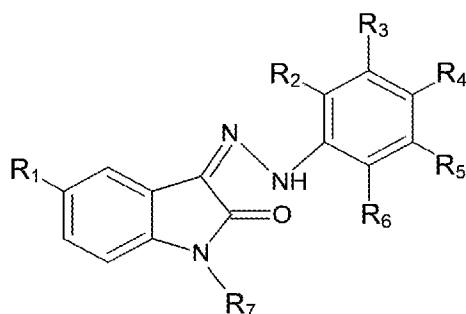
The present invention provides compounds and associated methods for inhibiting a protein tyrosine phosphatase. Experimental and virtual screenings of the NCI Diversity Set chemical library identified NSC-87877 and NSC-117199 as Shp2 PTP inhibitors. Significantly, NSC-117199 exhibited some selectivity between Shp1 and Shp2. NSC-117199 analogs were designed to enhance protein tyrosine phosphatase inhibition and provide specificity between Shp1 and Shp2. These compounds comprise the general formula



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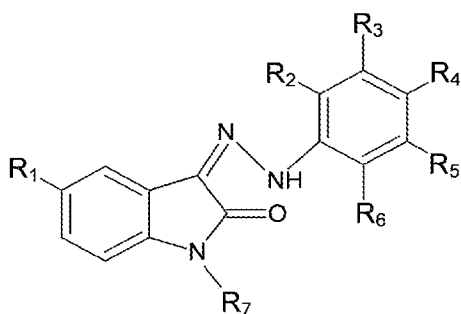
The indole R_1 is a functional group, includes SO_3H , CO_2H , CONHCH_2 (4- $\text{Cl-C}_6\text{H}_4$), $\text{SO}_2\text{NH}^i\text{Pr}$, SO_2NH_2 , SO_2NHCH_2 (2- $\text{Cl-C}_6\text{H}_4$), SO_2NHCH_2 (3- $\text{Cl-C}_6\text{H}_4$), SO_2NHCH_2 (4- $\text{Cl-C}_6\text{H}_4$), SO_2NHCH_2 (4- $\text{Me-C}_6\text{H}_4$), SO_2NHCH_2 (3- CF_3 -4- $\text{Cl-C}_6\text{H}_4$), SO_2NHCH_2 (4- FC_6H_4), SO_2NHCH_2 (3- Cl -4- $\text{F-C}_6\text{H}_4$), $(\text{CH}_2)_n\text{SO}_3\text{H}$, $(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $\text{SO}_2\text{NH}(\text{CH}_2)_2$ (2- Cl -4- $\text{Cl-C}_6\text{H}_4$), $\text{SO}_2\text{N-R}_8$, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO_3H , CO_2NH_2 , SO_2NH_2 , PO_3H , $\text{CF}_2\text{PO}_3\text{H}$, $(\text{CH}_2)_n\text{CO}_2\text{H}$, $(\text{CH}_2)_n\text{SO}_3\text{H}$, $(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, $(\text{CH}_2)_n\text{PO}_3\text{H}$, $\text{O}(\text{CH}_2)_n\text{CO}_2\text{H}$, $\text{O}(\text{CH}_2)_n\text{SO}_3\text{H}$, $\text{O}(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{PO}_3\text{H}$, $\text{NH}(\text{CH}_2)_n\text{CO}_2\text{H}$, $\text{NH}(\text{CH}_2)_n\text{SO}_3\text{H}$, $\text{NH}(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $\text{NH}(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, and $\text{NH}(\text{CH}_2)_n\text{PO}_3\text{H}$. The phenylhydrazone moieties R_2 through R_6 are independently hydrogen, NO_2 , COO^- , COO_2H , phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO_3H , CO_2NH_2 , SO_2NH_2 , PO_3H , $\text{CF}_2\text{PO}_3\text{H}$, $(\text{CH}_2)_n\text{CO}_2\text{H}$, $(\text{CH}_2)_n\text{SO}_3\text{H}$, $(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, $(\text{CH}_2)_n\text{PO}_3\text{H}$, $\text{O}(\text{CH}_2)_n\text{CO}_2\text{H}$, $\text{O}(\text{CH}_2)_n\text{SO}_3\text{H}$, $\text{O}(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, $\text{O}(\text{CH}_2)_n\text{PO}_3\text{H}$, $\text{NH}(\text{CH}_2)_n\text{CO}_2\text{H}$, $\text{NH}(\text{CH}_2)_n\text{SO}_3\text{H}$, $\text{NH}(\text{CH}_2)_n\text{CO}_2\text{NH}_2$, $\text{NH}(\text{CH}_2)_n\text{SO}_2\text{NH}_2$, and $\text{NH}(\text{CH}_2)_n\text{PO}_3\text{H}$. The R_7 group attached to the indole nitrogen may be either is hydrogen or methyl. In certain embodiments of the compound, the R_1 group at the 5-position of the oxindole moiety is either a carboxylic acid, a sulfonamide, and a carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, or *p*-halosulfonamide. Specifically, the *p*-halosulfonamide may be chloridesulfonamide. Additionally, specific embodiments of the compound possess a polar group on the oxindole moiety and a carboxylate or carboxylic acid on the phenylhydrazone moiety. The compound may comprise a *z*-configuration iastin hydrazone.

Also disclosed are methods of inhibiting a protein tyrosine phosphatase in a cell. A compound is administered to the cell, such as by contacting the cell with an effective amount of a the compound having the formula



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- The compound may comprise indole R₁ is a functional group, including includes SO₃H, CO₂H, CONHCH₂ (4-ClC₆H₄), SO₂NHⁱPr, SO₂NH₂, SO₂NHCH₂(2-ClC₆H₄), SO₂NHCH₂(3-ClC₆H₄), SO₂NHCH₂(4-ClC₆H₄), SO₂NHCH₂(4-MeC₆H₄), SO₂NHCH₂(3-CF₃-4-Cl-C₆H₄), SO₂NHCH₂(4-FC₆H₄), SO₂NHCH₂(3-Cl-4-F-C₆H₄), (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, SO₂NH(CH₂)₂(2-Cl-4-ClC₆H₄), SO₂N-R₈, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H. The phenylhydrazone moieties R₂ through R₆ are independently hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H. The R₇ group attached to the indole nitrogen may be either is hydrogen or methyl. In certain embodiments of the compound, the R₁ group at the 5-position of the oxindole moiety is either a carboxylic acid, a sulfonamide, and a carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, or *p*-halosulfonamide. Specifically, the *p*-halosulfonamide may be chloridesulfonamide.
- Additionally, specific embodiments of the compound possess a polar group on the oxindole moiety and a carboxylate or carboxylic acid on the phenylhydrazone moiety. The compound may comprise a *z*-configuration iastin hydrazone. In specific embodiments, the protein tyrosine phosphatase inhibited by the method is a Shp2 protein tyrosine phosphatase. More specifically, the Shp protein tyrosine phosphatase is selectively inhibited.
- Finally, a method of treating a disease in an animal is disclosed. A compound is administered to the cell, such as by contacting the cell with an effective amount of a the compound having the formula



5

- The compound may comprise indole R₁ is a functional group, including includes SO₃H, CO₂H, CONHCH₂ (4-ClC₆H₄), SO₂NHⁱPr, SO₂NH₂, SO₂NHCH₂(2-ClC₆H₄), SO₂NHCH₂(3-ClC₆H₄), SO₂NHCH₂(4-ClC₆H₄), SO₂NHCH₂(4-MeC₆H₄), SO₂NHCH₂(3-CF₃-4-Cl-C₆H₄), SO₂NHCH₂(4-FC₆H₄), SO₂NHCH₂(3-Cl-4-F-C₆H₄), (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, SO₂NH(CH₂)₂(2-Cl-4-ClC₆H₄), SO₂N-R₈, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H. The phenylhydrazone moieties R₂ through R₆ are independently hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H. The R₇ group attached to the indole nitrogen may be either is hydrogen or methyl. In certain embodiments of the compound, the R₁ group at the 5-position of the oxindole moiety is either a carboxylic acid, a sulfonamide, and a carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, or *p*-halosulfonamide. Specifically, the *p*-halosulfonamide may be chloridesulfonamide. Additionally, specific embodiments of the compound possess a polar group on the oxindole moiety and a carboxylate or carboxylic acid on the phenylhydrazone moiety. The compound may comprise a *z*-configuration iastin hydrazone.

The method may be used to treat a protein tyrosine phosphatase disease, including Noonan syndrome, juvenile myelomonocytic leukemia, Noonan-like disorder with multiple giant cell lesion syndrome, LEOPARD syndrome, acute lymphoblastic leukemia, acute myelogenous leukemia, *H. pylori*-associated gastritis, or gastric cancer. The method may target a protein tyrosine phosphatase for inhibition. Specifically, the protein tyrosine phosphatase is a Shp2 protein tyrosine phosphatase.

5 BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

Figure 1 illustrates the molecular model of NSC-87877 binding to the Shp2 PTP domain. The hydrogen bonds formed between the NSC-87877 and the protein, via Arg-465, Lys-280 and Asn-281 are shown schematically but not to scale. The hydrogen bonds are defined with a minimum donor angle of 90° and minimum acceptor angle of 60° and maximum length of 2.5 Å

Figure 2 depicts the inhibition of EGF-stimulated Shp2 activation by NSC-87877. Serum-starved HEK293 cells were pretreated with or without NSC-87877 (50 μM), stimulated with EGF or mock-treated, and Shp2 PTP activity was determined by the immune complex Shp2 PTP assay. The relative Shp2 PTP activity is shown in the histogram. Data were from two independent experiments performed in duplicate (n=4). After determination of Shp2 PTP activity, Shp2 immunoprecipitates were analyzed by immunoblotting with an antibody to Shp2 (bottom panel beneath the histogram). *, $p < 0.05$.

Figure 3 is a model depicting the overlay of **NSC-117199** (dark-shaded chemical structure) and **HL2-052-2** (light-shaded chemical structure) in the SHP2 active site.

Figure 4(a) and (b) are diagrammatic model of Shp2 ligand docking. 4(a) models compound **5** and annotates some identified, important structural features for activity. 4(b) models structure **6**, representing the oxindole pharmacophore for new inhibitor design.

Figure 5 is an illustration of reaction scheme 1. The following reagents and conditions were used: a) NBS, isopropanol:H₂O (95:5), 0 °C, 45 min; b) i: HCl (aq. 4M, 1-3 mL), μw, 150 °C, 5 min, ii: HCl (aq. 4M, 1-3 mL), ArNHNH₂, μw, 150 °C, 5 min; c) NaOH, EtOH:H₂O (1:1), 96%; d) pentafluorophenyl trifluoroacetate, pyridine, DMF, inert conditions, 70%; e) RNH₂, pyridine, acetonitrile; f) NBS, isopropanol:H₂O (95:5), 0 °C, 30 min; g) dry MeOH, ArNHNH₂, μw, 150 °C, 5 min (yield from 20-60%); h) HCl (aq. 1M, 2 drops) EtOH, μw, ArNHNH₂, 120 °C, 15 min (for **18c** irradiation time was only 2 min).

Figure 6 is an illustration of reaction scheme 2. The following reagents and conditions were used: a) NBS, isopropanol:H₂O (95:5), 0 °C, 45 min; b) i: HCl (aq. 4M, 1-3 mL), μw, 150 °C, 5 min, ii: HCl (aq. 4M, 1-3 mL), ArNHNH₂, μw, 150 °C, 5 min; c) NaOH, EtOH:H₂O (1:1), 96%; d) pentafluorophenyl trifluoroacetate, pyridine, DMF, inert conditions, 70%; e) RNH₂, pyridine, acetonitrile; f) NBS, isopropanol:H₂O (95:5), 0 °C, 30 min; g) dry MeOH, ArNHNH₂, μw, 150 °C, 5 min (yield from 20-60%); h) HCl (aq. 1M, 2 drops) EtOH, μw, ArNHNH₂, 120 °C, 15 min (for **18c** irradiation time was only 2 min)

- 5 Figure 7 is a table of Shp2 active compounds from the isatin library. ^a Values are means and standard deviations of at least 4 experiments, each performed in duplicate.

Figures 8(a) and (b) are model depicting the overlay of compound 5 and compound 14a. 8(a) models the overlay of **5** (NSC-117199) (black) and **14a** (gray) docked in the Shp2 PTP active site. 8(b) models compound **14a** docked to Shp1 PTP active site.

10 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Shp2 is a non-receptor protein tyrosine phosphatase (PTP) encoded by the PTPN11 gene. It is involved in growth factor-induced activation of mitogen-activated protein (MAP) kinases Erk1 and Erk2 (Erk1/2) and has been implicated in the pathogenicity of the oncogenic bacterium *Helicobacter pylori* (*H. pylori*). Moreover, gain-of-function Shp2 mutations have
15 been found in childhood leukemias and Noonan syndrome. Thus, small molecule Shp2 PTP inhibitors are much needed reagents for evaluation of Shp2 as a therapeutic target and for chemical biology studies of Shp2 function. By screening the National Cancer Institute (NCI) Diversity Set chemical library, NSC-87877 was identified as a potent Shp2 PTP inhibitor. Site-directed mutagenesis and molecular modeling studies suggested that NSC-87877 binds
20 to the catalytic cleft of Shp2 PTP. NSC-87877 cross-inhibited Shp1 *in vitro*, but it was selective for Shp2 over other PTPs (PTP1B, HePTP, DEP1, CD45, and LAR). Importantly, NSC-87877 inhibited EGF-induced activation of Shp2 PTP, Ras, and Erk1/2 in cell cultures but did not block EGF-induced Gab1 tyrosine phosphorylation or Gab1-Shp2 association. Furthermore, NSC-87877 inhibited Erk1/2 activation by a Gab1-Shp2 chimera but did not
25 affect the Shp2-independent Erk1/2 activation by phorbol 12-myristate 13-acetate (PMA). These results identified NSC-87877 as the first PTP inhibitor capable of inhibiting Shp2 PTP in cell cultures without a detectable off-target effect. This provides the first pharmacological evidence that Shp2 mediates EGF-induced Erk1/2 MAP kinase activation.

The term "administration" and variants thereof (e.g., "administering" a compound) in reference
30 to a compound of the invention means introducing the compound or a prodrug of the compound into the system of the animal. When a compound of the invention or prodrug thereof is provided in combination with one or more other active agents (e.g., a cytotoxic agent, etc.), "administration" and its variants are each understood to include concurrent and sequential introduction of the compound or prodrug thereof and other agents.

35 A "safe and effective amount" refers to the quantity of a component that is sufficient to yield a desired therapeutic response without undue adverse side effects (such as toxicity, irritation, or allergic response) commensurate with a reasonable benefit/risk ratio when used in the manner of this invention.

5 A "pharmaceutically acceptable carrier" is a carrier, such as a solvent, suspending agent or vehicle, for delivering the compound or compounds in question to the animal or human. The carrier may be liquid or solid and is selected with the planned manner of administration in mind. Liposomes are also a pharmaceutical carrier. As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal
10 agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated.

15 A "pharmaceutically acceptable" component is one that is suitable for use with humans and/or animals without undue adverse side effects (such as toxicity, irritation, and allergic response) commensurate with a reasonable benefit/risk ratio.

The term "therapeutically effective amount" as used herein means that amount of active compound or pharmaceutical agent that elicits the biological or medicinal response in a
20 tissue, system, animal or human that is being sought by a researcher, veterinarian, medical doctor or other clinician. In reference to cancers or other unwanted cell proliferation, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an
25 amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay occurrence and/or recurrence. An effective amount can be administered in one or more doses. In the case of cancer, the effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs;
30 (iv) inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

As used herein, "treatment" refers to obtaining beneficial or desired clinical results. Beneficial or desired clinical results include, but are not limited to, any one or more of: alleviation of one
35 or more symptoms (such as tumor growth or metastasis), diminishment of extent of cancer, stabilized (i.e., not worsening) state of cancer, preventing or delaying spread (e.g., metastasis) of the cancer, preventing or delaying occurrence or recurrence of cancer, delay or slowing of cancer progression, amelioration of the cancer state, and remission (whether partial or total). The methods of the invention contemplate any one or more of these aspects
40 of treatment.

5 An "animal" includes any animal, such as, but not limited to; mammals including humans, gorillas and monkeys, mice rats, pigs horses, cats, dogs, rabbits, sheep deer, cows, and goats.

An "alkyl", as used herein, means a hydrocarbon radical having from one to ten carbon atoms, which can be a straight or branched chain, and including from zero to four carbon-
10 carbon double or triple bonds. Representative of such radicals are methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, isobutyl, tert-butyl, 2-ethyl-hexyl and the like.

The term "dialkylammo" as used herein refers to $-NR_aR_b$, wherein R_a and R_b are independently selected alkyl groups, defined above.

The term "alkoxy" as used herein means an alkyl, as defined above, having an oxygen atom,
15 attached thereto. Representative alkoxy groups include — methoxy, ethoxy, propoxy, tert-butoxy and the like.

The term "alkoxycarbonyl" as used herein means an alkoxy radical, as defined above, including a carbonyl wherein Y and Z are each independently alkyl, as de- group, as defined below. fined above.

20 The term "amino" as used herein means a $-NH_2$ substituent.

NSC-87877 **4** was identified as a hit from the NCI Diversity set, is a potent Shp2 and Shp1 inhibitor (Chen, L. et al., *Mol. Pharmacol.* **2006**, *70*, 562-570). NSC-87877 potently inhibited Shp2 with an IC_{50} of 0.318 ± 0.049 (μM), but lacks selectivity between human Shp2 and Shp1 *in vitro*. NSC-87877 showed approximately 5-, 24-, 206-, 266-, and 475-fold selectivity for
25 Shp2 over PTP1B, HePTP, DEP1, CD45, and LAR. The oxindole **5** (NSC-117199) (obtained from the Developmental Therapeutics Program of the NCI/NIH; Milne, G., et al., *J. Chem. Inf. Comput. Sci.* **1986**, *26*, 159-68) was found to be a hit with only moderate potency (IC_{50} 47 μM). Compounds with an oxindole core have been studied by other groups as potential therapeutic agents. (Fong, T. et al., *Cancer Res.* **1999**, *59*, 99-106; Sun, L., et al., *J. Med. Chem.* **1999**, *42*, 5120-5130). Modeling of Shp2 PTP ligand docking (from pdb 2SHP¹⁵ using
30 GLIDE, Friesner, R., et al., *J. Med. Chem.* **2004**, *47*, 1739-1749) indicated the hydrazone aromatic ring system is pointing into the active site PTP signature motif (VHCSAGIGRTG) with the polar nitro group mimicking the substrate phosphate group, seen in Figures 1 and 2. The sulfonic acid group of oxindole **5** is hydrogen bonded with the basic residues Arg362 and
35 Lys366, as seen in Figure 1. The *ortho*-nitro group exhibited hydrogen bonding interactions with backbone atoms of the PTP loop residues Ser460, Cys459 and Arg465.

Computer docking GLIDE modeling of NSC-87877 to Shp2 suggested that the B-ring sulfonic acid group forms hydrogen bond with the backbone NH group of Arg-465. To evaluate this molecular model, two Shp2 PTP mutants were generated containing changes in the Lys-280

5 and Asn-281 residues, predicted to interact with NSC-87877. One (Shp2V280) mutant contained a Lys-280 to Val-280 mutation and the other (Shp2RD) mutant contained dual Lys-280/Asn-281 to Arg-280/Asp-281 mutations. *In silico* prediction gave both Shp2V280 and Shp2RD mutants a 0.6 kcal/mole increase (destabilization) in the GLIDE docking score. Sensitivity of mutated and wild-type Shp2 to NSC-87877 inhibition was then compared
10 experimentally by the PTP assay. Shp2V280 and Shp2RD were approximately 3-fold less sensitive to NSC-87877 inhibition ($p = 0.0015$ for comparison of IC_{50} s between Shp2 and Shp2V280; $p = 0.0062$ for comparison of IC_{50} s between Shp2 and Shp2RD). These data suggest that Lys-280 and/or Asn-281 are involved in NSC-87877 binding to Shp2.

Serum-starved HEK293 cells were pre-incubated with or without NSC-87877 and stimulated
15 with EGF or mock-treated to determine its ability to inhibit Shp2 in the cells. DiFMUP substrate assay of cell lysate Shp2 immunoprecipitates indicated Shp2 PTP activity increased 2.6-fold in response to EGF stimulation in the absence of NSC-87877 pretreatment whereas NSC-87877 incubation reduced basal Shp2 PTP activity by 45%. The EGF-stimulated Shp2 activation was inhibited by 97% when cells were pretreated with 50 μ M NSC-87877, seen in
20 Figure 2. To evaluate NSC-87877 inhibition of Shp2-dependent cell signaling, serum-starved HEK293 cells were pretreated with 0-50 μ M NSC-87877 for 3 h and stimulated with EGF for 5 min. Erk1/2 activation was determined by anti-phospho-Erk1/2 immunoblotting showed NSC-87877 inhibition is concentration dependent, with an IC_{50} for EGF-stimulated Erk1/2 activation of 6 μ M in two independent experiments. Chimeric Gab1PH-Shp2 Δ N protein, consisting of
25 the Gab1 PH domain and a constitutively active Shp2 N-SH2 domain deletion, shows Shp2 PTP is necessary for Erk1/2 activation by Gab1PH-Shp2 Δ N (Cunnick JM, et al. *J Biol. Chem.* **2002**, 277(11):9498-9504). Induction of Flp-In-T-Rex-293 cells with dox induced Gab1PH-Shp2 Δ N expression and Erk1/2 activation, which was abrogated in NSC-87877 treated cells. This result suggests that NSC-87877 inhibition of EGF-induced Erk1/2 activation is not
30 mediated by events upstream of Shp2 and the target of NSC-87877 is intracellular since there was no extracellular stimulation involved in the Erk1/2 activation by Gab1PH-Shp2 Δ N.

Previous studies have shown that PMA-induced Erk1/2 activation is not affected by overexpression of a PTP-inactive Shp2 mutant (Yamauchi K, et al. *Proc. Nat'l Acad. Sci. U S A* **1995**, 92(3):664-668), suggesting that the protein kinase C (PKC) activator-mediated Erk1/2
35 activation is Shp2-independent. We, therefore, compared the effect of NSC-87877 on EGF- and PMA-stimulated Erk1/2 activation. NSC-87877 inhibited EGF-stimulated Erk1/2 activation but, NSC-87877 did not affect PMA-induced Erk1/2 activation. Because PKC is known to activate the Erk1/2 MAP kinase cascade by directly phosphorylating Raf-1 (Carroll MP and May WS. *J. Biol. Chem.* **1994**, 269(2):1249-1256; Kolch W, et al. *Nature* **1993**, 364(6434):249-252), the data suggest that the target of NSC-87877 is upstream of Raf-1, consistent with the notion that NSC-87877 is acting on Shp2 to inhibit Erk1/2 activation.

- 5 NSC-87877 treatment did not inhibit EGF-stimulated Gab1 tyrosine phosphorylation or subsequent Shp2-Gab1 binding in HEK293 cells, indicating NSC-87877 does not affect EGF-activated signaling steps prior to Shp2 activation.

- Computer docking GLIDE of NSC-117199 to Shp2 indicated NSC-117199 also forms hydrogen bond with the B-ring sulfonic acid group at the backbone NH group of Arg-465, seen in Figure 3. Arg-465 is a conserved residue in the PTP signature motif (motif 9) VHCSXGXGR[T/S]G located at the base of the PTP catalytic cleft (Andersen et al., 2001). The A-ring sulfonic acid forms hydrogen bonds with the side-chain NH₃ group of Lys-280 and the side-chain NH₂ group of Asn-281. Lys-280/Asn-281 are non-conserved PTP residues located adjacent to the phosphotyrosine recognition loop (motif 1) (Andersen et al., 2001).
- 15 The interaction between aromatic rings of the compound and the protein contributes to the binding through hydrophobic stabilization. This formed the basis of the design of the library of NSC-117199 analogs (with the isatin core). The anionic groups on the isatin bind to the phosphate binding site of the PTP loop, shown as the grey tube in Figure 3, and to Arg262, Lys364 and Lys 366.
- 20 Using oxindole **5** and the models, PTP inhibitors were developed that display selectivity for Shp2 over Shp1 inhibition. The oxindole **5** appeared to fit well in the catalytic site suggesting the hydrazone unit should not be replaced by longer spacer groups, seen in Figures 1 and 3. The model also suggested the sulfonic acid could be replaced with small polar groups e.g. sulfonamide and carboxylamide. Thus, the library was biased to include small polar
- 25 replacements of the nitro and sulfonic acid groups to interact with the two polar binding sites. These were mostly at the 2 and 3 positions of the A-ring and the C5 of the indole ring, respectively. Inclusion of sulfonamide or carboxylamide groups at the 5-position, accessible from readily available precursors, adds a useful point of diversity into the oxindole pharmacophore. The hydrogen bonding interactions of the oxindole N-H group with Asp425
- 30 was thought to be optimal, and variation of N-H was limited to N-Me, as seen in Figure 4.

Example 1

- Several libraries of hydrazones (**10**, **14** and **16**) were prepared by combining a 5-substituted isatin with commercially available hydrazines; the sulfonyl and carboxyl groups were elaborated with a further set of amines to provide sulfonamides and amides. The novel
- 35 oxindole hydrazone sulfonamide library **10** was developed using commercially available building blocks as shown in Figure 5. The oxindolesulfonyl chloride **8** was obtained from commercially available isatin-5-sulfonic acid according to a literature reported procedure. (Lee, D., et al., *J. Med. Chem.* **2001**, *44*, 2015-2026). Isatin-5-sulfonyl chloride was coupled to a series of requisite amines to obtain the sulfonamide library **9**. Some members of the
- 40 isatin library **9** were isolated and analyzed by NMR and mass spectrometry. Attempts to

5 purify and isolate other members of the library **9** were not successful, however crude isatinsulfonamides **9** were successfully used. The hydrazone library **10** was obtained by microwave assisted coupling of the crude library **9** with an appropriate set of hydrazines in moderate yields.

The carboxylic acid library **14** was prepared from methyl indole-5-carboxylate **11a**, seen in
10 Figure 6. Treatment of **11a** with NBS produced a dibromooxindole intermediate (Parrick, J., et al., *J. Chem. Soc., Perkin Trans. 1* **1989**, 2009-2015) **12** in moderate yield, which was then converted into the desired library **14** in good yields by microwave assisted coupling with the requisite hydrazines. Hydrolysis of **12** is used to generate intermediate isatin **13**. A series of
15 carboxylamides **15** were prepared from the pentafluorophenyl ester of indole-5-carboxylic acid **11b**, seen in Figure 6. These carboxylamides were then reacted with NBS to form the dibromooxindole intermediates that were subsequently reacted with the requisite hydrazines to obtain the final library **16**, shown in Figure 6. The syntheses of the most potent compounds are highlighted in Figures 5 and 6. The 5-unsubstituted oxindoles **18** were synthesized in high yield by microwave assisted coupling of isatin and the required hydrazines as shown in
20 Figure 6.

Example 2

The libraries **10**, **14**, and **16** were analyzed by NMR (^1H and ^{13}C), low and high resolution mass spectroscopy. The ^1H NMR spectra of these compounds indicated formation a single
25 stereoisomer with > 95% purity. Isatin hydrazones have been reported to exist in the *Z* configuration in solution, presumably due to the intramolecular hydrogen bonding between NH of the hydrazone linkage and the carbonyl group of the indolinone. The analysis of the ^{13}C NMR spectra of the final compounds of the library **10** (**10a-e**, **10h**, **10i**, **10m**, **10o**, **10q**) revealed oxindole carbonyl chemical shifts around 163 ppm, indicative of the *Z*-hydrazone stereochemical configuration. Similarly, ^{13}C NMR analysis of the library **14** also revealed
30 carbonyl shifts around 163 ppm indicating that the members are also configured *Z*. However, the reaction of isatin **17** with 2-hydrazinobenzoic acid afforded a mixture of isomers. The ^1H NMR of the compound **18b** indicated formation of a mixture of stereoisomers approximately 1:3 ratio. Upon modifying the reaction conditions (microwave heating, 120 °C, 2 min.), exclusive formation (by ^1H and ^{13}C NMR) of what was previously the minor isomer (**18c**) was
35 produced in 48% yield. So far the major isomer of the **18b** has not been produced as a single compound.

The libraries were evaluated for Shp2 PTP inhibition by *in vitro* PTP assay using DiFMUP as the substrate.³³ The compounds that displayed low micromolar inhibitory activity against Shp2 are shown in Figure 7. These compounds were further screened against Shp1 and
40 PTP1B to determine their selectivity. It was found that the carboxylic acid, sulfonamides, and

5 carboxylamides at the 5-position of the oxindole moiety and nitro or carboxylic acid functional groups at the *ortho*-, *meta*- or *para*-positions of the phenylhydrazone moiety gave rise to the best Shp2 PTP inhibitory activity. Compounds **10a-r** from the sulfonamide library with either carboxylic acid or nitro (*ortho*, *meta* or *para*) groups on the aromatic hydrazine moiety showed good Shp2 inhibitory activities; IC_{50} = 1-10 μ M and selectivity, with over 5-fold Shp2
10 preference over Shp1. The sulfonamide library **10** compounds also displayed better solubility under the assay conditions.

The *bis*-carboxylic acid derivatives **14a** and **14b** displayed IC_{50} 0.8 and 15 μ M inhibitory activity, respectively, with 20 and 5 fold Shp2 selectivity. The compounds that lack the 5-position carboxylic acid, carboxyamides or sulfonamide groups (**18a-e**, Figure 6) showed poor
15 activity (IC_{50} > 60 μ M, data not shown) indicating that the 5-substitution with polar groups (carboxylic acid, sulfonamide or carboxyamide) is important for activity and suggesting that the interactions in this region with Lys366 and Arg362, seen in Figure 8, are pivotal. Members of both libraries **10** and **14** that possessed hydrogen, halogens (such as chloro or fluoro), primary amide or alkyl groups on the aromatic hydrazine moiety showed poor Shp2
20 inhibitory activity (not reported here) further demonstrating the importance of a carboxylate moiety (phosphotyrosine mimic) at this position, and its critical role in interacting with Cys459 and Arg465.

Varying degrees of selectivity for Shp2 versus PTP1B phosphatase inhibition were observed. For example the potent compounds **14a** and **10f** show a 2-fold Shp2:PTP1B selectivity,
25 whereas halosulfonamides **10g**, **10h** and **10m** show a better 4 to 15-fold difference.

The comparison of the docking modes of **5** (NSC-117199) and **14a** as shown in Figure 8(a). The 5-substituents of the oxindole ring are superimposed and, in the model, display favorable interactions with both Lys366 and Arg362 residues and most likely contribute towards the Shp2 affinity of the ligands. The lower activity of **10b** and the lack of activity found for all five
30 derivatives of **18** (IC_{50} > 60 μ M, Figure 6) which lack any 5-substituents is consistent with this observation.

The orientation of the hydrazine-aromatic ring positions the carboxylic acid moiety of **14a**, seen in Figures 8(a) and (b), so that it is capable of undergoing additional hydrogen bond interactions with Cys459, Gly464 and Ile463 in the catalytic site. This site binds substrate
35 phosphotyrosine residues, therefore it is not surprising that the carboxylate ion binds better than the nitro group. These interactions may explain the 40 fold increased potency of **14a** compared to **5** (IC_{50} values of 47 μ M versus 0.8 μ M). The indole nitrogen atoms do not overlay well, but both show a hydrogen bonding interaction with Shp2 amino acid residues (Asp425 in the case of NSC-117199 and Glu361 with **14a**). These observations are in
40 agreement with the design strategy to include both a polar group and phosphate mimic as a

5 model for Shp2 inhibition. The lead compound **14a** was docked into the Shp1 PTP-catalytic site (using the X-ray crystal structure from pdb 1FPR³⁴), as shown in Figure 8(b). In its lowest energy docking pose **14a** displayed a weaker binding affinity (by 1.9 kcal), which may explain its selectivity towards Shp2. In the Shp1 binding site it is now the carboxyl group of the phenylhydrazine that binds to the PTP loop. The residues Arg360 and Lys358 display H-bond interactions with carboxyl group of **14a**, in addition to the Lys362 and Ser456 H-bond interactions with carboxyl group of the indole ring. These different docking orientations of **14a** for Shp2 and Shp1 may also contribute to its large differences in inhibitory activities. Overall, the docking poses of other members of the isatin library **10** are similar to that of **14a** (data not shown).

15 **Example 3**

All reagents were purchased from commercial suppliers and used without further purification. Melting points were determined using a Barnstead international melting point apparatus and remain uncorrected.

¹H NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer with CDCl₃ or DMSO-*d*₆ as the solvent. ¹³C NMR spectra are recorded at 100 MHz. All coupling constants are measured in Hertz (Hz) and the chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm) relative to TMS (δ 0), which was used as the internal standard.

High resolution mass spectroscopy was carried out on an Agilent 6210 LC/MS (ESI-TOF).

Microwave reactions were performed in CEM 908005 model and Biotage initiator 8 machines.

25 HPLC analysis was performed using a JASCO HPLC system equipped with a PU-2089 Plus quaternary gradient pump and a UV-2075 Plus UV-VIS detector, using an Alltech Kromasil C-18 column (150 × 4.6 mm, 5 μm).

Thin layer chromatography was performed using silica gel 60 F254 plates (Fisher), with observation under UV when necessary. Anhydrous solvents (acetonitrile, dimethyl formamide, ethanol, isopropanol, methanol and tetrahydrofuran) were used as purchased from Aldrich. HPLC grade solvents (methanol, acetonitrile and water) were purchased from Burdick and Jackson for HPLC and mass analysis.

PTP phosphatase activity was measured using the fluorogenic 6, 8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, from Molecular Probes) as the substrate. Each reaction contained 25 mM MOPS (pH 7.0), 50 mM NaCl, 0.05% Tween-20, 1 mM DTT, 20 μM DiFMUP, 10 nM Microcystin LR, 20 nM PTP (Shp2, Shp1 or PTP1B), (See, Chen, L. et al. *Mol. Pharmacol.* **2006**, 70, 562-570) and 5 μl test compound or dimethyl sulfoxide (DMSO, solvent) in a total reaction volume of 100 μl in black 96-well plate. Reaction was initiated by

5 addition of DiFMUP and the incubation time was 30 min at room temperature. DiFMUP fluorescence signal was measured at an excitation of 355 nm and an emission of 460 nm with a Wallac Victor² 1420 plate reader. IC₅₀ was defined as the concentration of an inhibitor that caused a 50% decrease in the PTP activity. For IC₅₀ determination, 8 concentrations of compounds at 1/3 dilution (~ 0.5 log) were tested. Each experiment was performed in triplicate and IC₅₀ data were derived from at least three independent experiments. The curve-fitting program Prism 4 (GraphPad Software) was used to calculate the IC₅₀ value.

Example 4

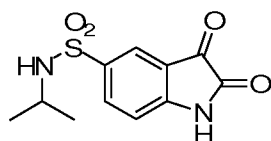
2,3-Dioxoindoline-5-sulfonyl chloride 8: This compound was prepared according to a procedure reported by Lee *et al.*³⁰ Mp = 200-202 °C (lit. Mp = 188-190 °C); ¹H NMR (400 MHz, CD₃CN:CDCl₃ 1:1) δ 9.49 (br s, NH, 1H), 8.24 (dd, *J* = 8.4, 2.0 Hz, 1H), 8.16 (d, *J* = 2.0 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H).

Example 5; Synthesis of isatin library 9:

The DIPEA (2.0 mmol) and appropriate amine (1.3 mmol) were added to a solution of **8** (1.0 mmol) in anhydrous THF (8 ml) at 0 °C under inert atmosphere. The reaction mixture was warmed to r.t. and stirred overnight (some reactions required 3 days for completion). The crude reaction mixture was poured into water (10 ml) and extracted with EtOAc (3 × 15 ml). The organic phase was dried (MgSO₄) and evaporated to obtain a beige/yellow solid. This crude product was taken to next stage without purification in the majority of the cases (see general procedure for library 7), except compounds **6a**, **6b**, **6c**, which were purified by trituration with cold ethyl acetate.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonamide (9a). Yellow solid,³⁶ 47%. Mp = 198-200 °C, decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.02 (d, *J* = 8.2 Hz, 1H), 7.38 (s, 2H), 7.82 (d, *J* = 1.8 Hz, 1H), 7.95 (dd, *J* = 8.2, 1.8 Hz, 1H), 11.35 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 112.90, 118.50, 122.44, 135.74, 139.13, 153.34, 160.26, 184.00; HRMS (ESI+ve) *m/z* calculated for C₈H₇N₂O₄S (M+H)⁺ 227.0127, found 227.0133.

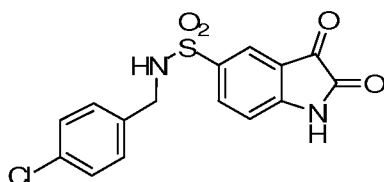
2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid isopropylamide (9b).



This was obtained from 2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonyl chloride and isopropylamine in a similar manner as described for preparation of 2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide. The pure compound 2,3-dioxo-2,3-dihydro-1H-indole-5-

- 5 sulfonic acid isopropylamide was obtained after trituration with ethyl acetate as a yellow solid (70%), Mp = 184-186 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 0.94 (d, *J* = 6.8 Hz, 6H), 3.11 (sept, *J* = 6.8 Hz, 1H), 7.04 (d, *J* = 8.2 Hz, 1H), 7.58 (d, *J* = 6.8 Hz, 1H), 7.77 (d, *J* = 1.8 Hz, 1H), 7.94 (dd, *J* = 8.2, 1.8 Hz, 1H), 11.38 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 23.90, 45.99, 113.15, 118.73, 123.00, 136.53, 136.65, 153.65, 160.20, 183.90; HRMS (ESI+ve) *m/z* calculated for C₁₁H₁₃N₂O₄S (M+H)⁺ 269.0596, found 269.0594.

2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide (9c).



- A yellow solid product was obtained from 2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonyl chloride and 4-chlorobenzylamine in a similar manner as described for preparation of 2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide. The pure compound 2,3-dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide was obtained after trituration with ethyl acetate as a yellow solid; 57%. Mp = 250 °C, decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 3.97 (d, *J* = 6.2 Hz, 2H), 7.00 (d, *J* = 8.1 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 1.9 Hz, 1H), 7.89 (dd, *J* = 8.1, 1.9 Hz, 1H), 8.22 (t, *J* = 6.2 Hz, 1H), 11.41 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 46.08, 113.10, 118.57, 123.24, 128.85, 130.20, 132.40, 135.52, 136.76, 137.12, 153.75, 160.15, 183.72; HRMS (ESI+ve) *m/z* calculated for C₁₅H₁₂ClN₂O₄S (M+H)⁺ 351.0206, found 351.0203.

Example 6; The general procedure for synthesis of isatin library 10.

- Method A.** A mixture of the crude intermediate from library **9** (100 mg) and hydrazinobenzoic acid (1 eq.) in ethanol (1 ml) with hydrochloric acid (2 drops 1M aq.) was heated in the Biotage microwave reactor at 120 °C for 15 min. A yellow solid precipitated on cooling the reaction vial in an ice bath. The solid obtained was filtered and washed with methanol to give the pure product **10**. The yields for these 2 steps were in the range of 15-80%.

- Method B.** A mixture of the pure isatin **9a-c** (0.419 mmol) and the appropriate hydrazine (0.461 mmol) in ethanol (3 ml) with hydrochloric acid (2 drops 1M aq.) was heated in the Biotage microwave reactor at 120 °C for 15 min. A yellow solid was precipitated on cooling in an ice bath. The solid obtained was filtered and washed with methanol to provide the pure final product.

(Z)-3-(2-(2-Nitrophenyl)hydrazono)-2-oxoindoline-5-sulfonic acid 5 (NSC-117199).

- 5 Orange solid, 50%. Mp = 272 °C decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.21 (s, 1H, disappeared on D₂O shake), 11.23 (s, 1H, disappeared on D₂O shake), 8.24 (dd, *J* = 8.4, 0.8 Hz, 2H), 8.21 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.84 (appd, *J* = 1.6 Hz, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 7.57 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.19-7.14 (m, 1H), 6.87 (d, *J* = 8.0 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 110.61, 116.59, 118.11, 120.25, 122.31, 126.46, 128.58, 133.59, 133.86, 137.33, 140.02, 142.14, 143.31, 163.38; HPLC 100% (*R*_t = 1.38, 80% methanol in water); HRMS (ESI-ve) *m/z* calculated for C₁₄H₉N₄O₆S (M-H)⁻ 361.0248, found 361.0250.
- 10

3-[*N*-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino]-benzoic acid (10a).

- Yellow solid, 57%. Mp = 275 °C decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.76 (s, 1H, disappeared on D₂O shake), 11.43 (s, 1H, disappeared on D₂O shake), 8.05-8.06 (m, 1H), 7.92 (d, *J* = 2.0 Hz, 1H), 7.67-7.70 (m, 3H), 7.47-7.51 (d, *J* = 7.6 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 3.21 (sept, *J* = 6.8 Hz, 1H), 0.93 (d, *J* = 6.8 Hz, 6H); ¹³C NMR (DMSO-*d*₆) δ 23.88, 45.88, 111.18, 115.36, 117.44, 119.75, 122.20, 124.74, 127.90, 127.97, 130.43, 132.79, 135.89, 143.24, 143.30, 163.76, 167.72; HPLC 99% (*R*_t = 5.20, 75% methanol in water); HRMS (ESI-ve) *m/z* calculated for C₁₈H₁₇N₄O₅S (M-H)⁻ 401.0925, found 401.0929.
- 15
- 20

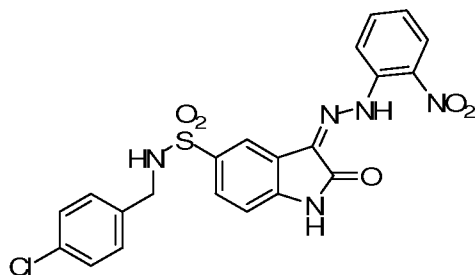
4-[*N*-(5-Isopropylsulfamoyl-2-oxo-1,2-dihydro-indol-3-ylidene)hydrazino] benzoic acid (10b).

- The product was obtained as a yellow solid, 75%. Mp = 290 °C decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H, disappeared on D₂O shake), 12.74 (bs, 1H, disappeared on D₂O shake), 11.47 (s, 1H, disappeared on D₂O shake), 7.95 (m, 1H), 7.94 (d, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.56 (1H, d, *J* = 8.0 Hz, 2H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.07 (d, *J* = 8.0 Hz, 1H), 0.93 (d, *J* = 6.0 Hz, 6H); ¹³C NMR (DMSO-*d*₆) δ 23.91, 45.90, 111.35, 114.77, 117.83, 121.95, 125.72, 128.35, 129.08, 131.78, 136.02, 143.59, 146.57, 163.70, 167.56; HPLC 99.9 % (*R*_t = 1.20, 90% methanol in acetonitrile); HRMS (ESI-ve) *m/z* calculated for C₁₈H₁₇N₄O₅S 401.0925 (M-H)⁻, found 401.0932.
- 25
- 30

3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1*H*-indole-5-sulfonamide (10c).

- Yellow solid, 58%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.24 (s, 1H, disappeared on D₂O shake), 11.53 (s, 1H, disappeared on D₂O shake), 8.21-8.24 (m, 1H), 8.07 (d, *J* = 1.6 Hz, 1H), 7.81-7.85 (m, 1H), 7.77 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.30 (s, 2H), 7.19-7.24 (m, 1H), 7.03 (d, *J* = 8.4 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 111.27, 116.44, 118.04, 121.18, 122.78, 126.56, 128.56, 132.74, 133.87, 137.31, 138.72, 139.63, 144.25, 163.26; HPLC 99% (*R*_t = 2.28, 90% acetonitrile in water); HRMS (ESI-ve) *m/z* calculated for C₁₄H₁₀N₅O₅S (M-H)⁻ 360.0408, found 360.0412.
- 35

- 5 **3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide (10d).**



- Yellow solid product was obtained(43%) from 2,3-Dioxo-2,3-dihydro-1H-indole-5-sulfonic acid 4-chlorobenzylamide and 2-nitrophenylhydrazine in a similar manner as described for preparation of 3-[(2-Nitrophenyl)hydrazono]-2-oxo-2,3-dihydro-1H-indole-5-sulfonic acid dimethylamide. 43%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.23 (s, 1H), 11.56 (s, 1H), 8.23-8.28 (m, 2H), 8.13 (t, *J* = 6.4 Hz, 1H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.82 (t, *J* = 8.4 Hz, 1H), 7.70 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.23-7.27 (m, 5H), 7.06 (d, *J* = 8.0 Hz, 1H), 3.98 (d, *J* = 6.4 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 46.14, 111.62, 116.69, 118.94, 121.39, 122.89, 126.58, 128.78, 129.59, 130.18, 132.38, 132.58, 133.95, 135.11, 137.32, 137.34, 139.69, 144.73, 163.26; HPLC 99% (*R*_t = 2.55, 90% acetonitrile in water); HRMS (ESI+ve) *m/z* calculated for C₂₁H₂₀ClN₆O₅S (M+NH₄)⁺ 503.0904, found 503.0909; calculated for C₂₁H₁₇ClN₅O₅S (M+H)⁺ 486.0639, found 486.0643.

- 20 **(Z)-3-(2-(5-(*N*-(4-Methylbenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10e).**

- Yellow solid, 27%. Mp = 297 °C decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H disappeared on D₂O shake), 11.43 (s, 1H disappeared on D₂O shake), 8.07 (s, 1H), 8.02 (t, *J* = 6.4 Hz, NH, 1H disappeared on D₂O shake), 7.84 (s, 1H), 7.72-7.62 (m, 3H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 8.4 Hz, 2H), 7.05-7.02 (m, 3H), 5.75 (s, 1H disappeared on D₂O shake), 3.90 (d, *J* = 6.0 Hz, 2H, CH₂, singlet on D₂O shake), 2.19 (s, CH₃, 3H); HPLC 92% (*R*_t = 1.57, 90% methanol in acetonitrile); HRMS (ESI-ve) *m/z* calculated for C₂₃H₁₉N₄O₅S (M-H)⁻ 463.1082, found 463.1087.

- (Z)-4-(2-(5-(*N*-Benzylsulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10f).**

- Yellow solid, 79%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.78 (s, 1H disappeared on D₂O shake), 11.48 (s, 1H disappeared on D₂O shake), 8.06 (t, *J* = 8.0 Hz, NH, 1H disappeared on D₂O shake), 7.95 (d, *J* = 8.0 Hz, 2H), 7.93 (s, 1H), 7.70 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 2H), 7.29-7.19 (m, 5 H), 7.06 (d, *J* = 8.0 Hz, 1H), 5.70 (s, 1H disappeared on D₂O shake), 3.96 (d, *J* = 8.0 Hz, 2H, CH₂, singlet on D₂O shake); ¹³C NMR (DMSO-*d*₆) δ 46.84, 111.33, 114.78, 118.01, 121.99, 125.73, 127.77, 128.28, 128.33, 128.54,

- 5 128.88, 131.77, 134.81, 138.28, 143.71, 146.57, 163.70, 167.56; HPLC 99% ($R_t = 1.15$, 90% methanol in acetonitrile); HRMS (ESI-ve) m/z calculated for $C_{22}H_{17}N_4O_5S$ (M-H)⁻ 449.0925, found 449.0940.

(Z)-3-(2-(5-(N-(3-Chlorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10g).

- 10 Yellow solid, 40%. Mp = 295 °C, decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H disappeared on D₂O shake), 11.44 (s, 1 H disappeared on D₂O shake), 8.17 (t, $J = 6.4$ Hz, NH, 1H disappeared on D₂O shake), 8.06 (s, 1H), 7.85 (d, $J = 1.6$ Hz, 1H), 7.70 (d, $J = 6.4$ Hz, 1H), 7.63 (d, $J = 8.0$ Hz, 2H), 7.49 (t, $J = 8.0$ Hz, 1H), 7.28-7.17 (m, 4 H), 7.01 (d, $J = 8.4$ Hz, 1H), 5.75 (s, 1H), 4.00 (d, $J = 6.4$ Hz, 2H, CH₂, singlet on D₂O shake); HPLC 99% ($R_t =$
15 1.63, 90% methanol in acetonitrile); HRMS (ESI-ve) m/z calculated for $C_{22}H_{16}ClN_4O_5S$ (M-H)⁻ 483.0535, found 483.0550.

(Z)-3-(2-(5-(N-(4-Chlorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10h).

- Yellow solid, 57%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H disappeared
20 on D₂O shake), 11.43 (s, 1 H disappeared on D₂O shake), 8.14 (t, $J = 6.4$ Hz, NH, 1H disappeared on D₂O shake), 8.06 (s, 1H), 7.82 (s, 1H), 7.71 (d, $J = 8.4$ Hz, 1H), 7.64 (t, $J = 7.2$ Hz, 2H), 7.49 (t, $J = 8.0$ Hz, 1H), 7.25 (dd, $J = 19.2, 8.4$ Hz, 4 H), 7.03 (d, $J = 8.4$ Hz, 1H), 5.75 (s, 1H disappeared on D₂O shake), 3.96 (d, $J = 6.0$ Hz, 2H CH₂, singlet on D₂O shake);
¹³C NMR (DMSO-*d*₆) δ 46.12, 111.12, 115.46, 117.59, 119.77, 122.19, 124.76, 127.77,
25 128.14, 128.75, 130.45, 130.46, 132.65, 132.82, 134.68, 137.36, 143.36, 143.77, 163.74, 167.72; HPLC 98.9 % ($R_t = 1.18$, 90% methanol in acetonitrile); HRMS (ESI-ve) m/z calculated for $C_{22}H_{16}N_4O_5SCl$ (M-H)⁻ 483.0535, found 483.0536.

(Z)-4-(2-(5-(N-(4-Chlorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10i).

- 30 Yellow solid, 55%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.78 (s, 1H disappeared on D₂O shake), 11.47 (s, 1H disappeared on D₂O shake), 8.12 (t, $J = 6.4$ Hz, NH, 1H disappeared on D₂O shake), 7.94 (d, $J = 8.0$ Hz, 2H), 7.84 (s, 1H), 7.66 (d, $J = 8.0$ Hz, 1H), 7.57 (d, $J = 8.0$ Hz, 2H), 7.25 (dd, $J = 17.6, 8.4$ Hz, 4H), 7.04 (d, $J = 7.6$ Hz, 1H), 5.75 (s, 1H disappeared on D₂O shake), 3.97 (d, $J = 6.4$ Hz, 2H, CH₂, singlet on D₂O shake); ¹³C NMR
35 (DMSO-*d*₆) δ 46.12, 11.29, 114.79, 117.96, 121.94, 125.73, 128.76, 128.92, 130.15, 131.79, 131.82, 132.34, 134.82, 137.34, 143.71, 146.61, 163.68, 167.57; HRMS (ESI-ve) m/z calculated for $C_{22}H_{16}ClN_4O_5S$ (M-H)⁻ 483.0535, found 483.0540.

(Z)-4-(2-(5-(N-(2-Chlorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10j).

- 5 Yellow solid, 28%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H, disappeared on D₂O shake), 11.47 (s, 1H, disappeared on D₂O shake), 8.14 (t, *J* = 5.6 Hz, NH, 1H disappeared on D₂O shake), 7.94 (d, *J* = 8.8 Hz, 2H), 7.92 (s, 1H), 7.69 (dd, *J* = 8.4, 1.6 Hz, 1H), 7.56 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 7.6 Hz, 1H), 7.34 (dd, *J* = 7.6, 1.6 Hz, 1H), 7.29-7.21 (m, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 4.04 (d, *J* = 5.6 Hz, 2H, CH₂, singlet on D₂O shake);
- 10 HRMS (ESI-ve) *m/z* calculated for C₂₂H₁₆ClN₄O₅S (M-H)⁻ 483.0535, found 483.0538.

(Z)-3-(2-(5-(N-(2-Chlorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10k).

- Yellow solid, 35%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H, disappeared on D₂O shake), 11.44 (s, 1H, disappeared on D₂O shake), 8.16 (t, *J* = 5.6 Hz, NH, 1H disappeared on D₂O shake), 8.07 (s, 1H), 7.90 (d, *J* = 1.2 Hz, 1H), 7.69 (dt, *J* = 8.4, 2.0 Hz, 2H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 1H), 7.38 (dd, *J* = 7.6, 2.0 Hz, 1H), 7.33 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.29-7.21 (m, 2H), 7.05 (d, *J* = 8.0 Hz, 1H), 5.75 (s, 1H, disappeared on D₂O shake), 3.96 (d, *J* = 5.6 Hz, 2H, CH₂, singlet on D₂O shake); HRMS (ESI-ve) *m/z* calculated for C₂₂H₁₆ClN₄O₅S (M-H)⁻ 483.0535, found 483.0541.
- 15

- 20 **(Z)-2-(2-(5-(N-(4-Chloro-3-(trifluoromethyl)benzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10l).**

- Yellow solid, 35%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.25 (s, 1H disappeared on D₂O shake), 11.30 (s, 1H, disappeared on D₂O shake), 8.24 (t, *J* = 6.4 Hz, NH, 1H disappeared on D₂O shake), 8.04 (d, *J* = 8.4 Hz, 1H), 7.95 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.74 (d, *J* = 1.6 Hz, 1H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.60-7.52 (m, 4H), 7.12 (t, *J* = 7.6 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 4.14 (d, *J* = 6.4 Hz, 2H, CH₂, singlet on D₂O shake); HRMS (ESI-ve) *m/z* calculated for C₂₃H₁₅ClF₃N₄O₅S (M-H)⁻ 551.0409, found 551.0414.
- 25

(Z)-3-(2-(5-(N-(4-Fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10m).

- Yellow solid, 40%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.76 (s, 1H disappeared on D₂O shake), 11.43 (s, 1H disappeared on D₂O shake), 8.10 (t, *J* = 6.4 Hz, NH, 1H disappeared on D₂O shake), 8.06 (s, 1H), 7.86 (s, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.64 (t, *J* = 8.8 Hz, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.25 (dd, *J* = 8.0, 5.6 Hz, 2H), 7.08-7.02 (m, 3H), 3.94 (d, *J* = 6.4 Hz, 2H, CH₂, singlet on D₂O shake); ¹³C NMR (DMSO-*d*₆) δ 46.07, 111.12, 115.42, 115.55 (d, *J* = 21 Hz), 117.56, 119.78, 122.20, 124.76, 127.75, 128.16, 130.29 (d, *J* = 8 Hz), 130.45, 132.79, 134.49 (d, *J* = 3 Hz), 134.64, 143.31, 143.35, 161.97 (d, *J* = 241 Hz), 163.72, 167.72; HPLC 99% (*R*_t = 1.45, 90% methanol in acetonitrile); HRMS (ESI-ve) *m/z* calculated for C₂₂H₁₆FN₄O₅S (M-H)⁻ 467.0831, found 467.0844.
- 30
- 35

5 **(Z)-4-(2-(5-(N-(4-Fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (10n).**

Yellow solid, 41%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H disappeared on D₂O shake), 11.46 (s, 1 H disappeared on D₂O shake), 8.07 (t, *J* = 6.4 Hz, NH, 1H disappeared on D₂O shake), 7.93 (d, *J* = 8.8 Hz, 2H), 7.88 (s, 1H), 7.67 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.56 (d, *J* = 8.4 Hz, 2H), 7.27-7.23 (m, 2H), 7.08-7.03 (m, 3H), 3.95 (d, *J* = 6.4 Hz, 2H, CH₂, singlet on D₂O shake); HPLC 96% (*R*_t = 1.43, 90% methanol in acetonitrile); HRMS (ESI-ve) *m/z* calculated for C₂₂H₁₆FN₄O₅S (M-H)⁻ 467.0831, found 467.0846.

(Z)-3-(2-(5-(N-(3-Chloro-4-fluorobenzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10o).

15 Yellow solid, 87%. Mp = 293-295 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.76 (s, 1H disappeared on D₂O shake), 11.43 (s, 1 H, disappeared on D₂O shake), 8.17 (t, *J* = 5.6 Hz, NH, 1H disappeared on D₂O shake), 8.01 (s, 1H), 7.79 (s, 1H), 7.70 (d, *J* = 6.8 Hz, 1H), 7.64 - 7.58 (m, 2H), 7.49 (t, *J* = 7.6 Hz, 1H), 7.30 (d, *J* = 8.0 Hz, 1H), 7.24-7.22 (m, 2H), 6.99 (d, *J* = 8.8 Hz, 1H), 3.99 (d, *J* = 5.6 Hz, 2H, CH₂, singlet on D₂O shake); ¹³C NMR (DMSO-*d*₆) δ 45.59, 111.06, 115.44, 117.03, 117.24, 117.51, 119.61, 119.79, 122.17, 124.77, 127.68, 128.11, 128.98 (d, *J* = 7 Hz), 130.35 (d, *J* = 15 Hz), 132.79, 134.72, 136.15 (d, *J* = 3 Hz), 143.34 (d, *J* = 6 Hz), 156.88 (d, *J* = 244 Hz), 163.70, 167.72; HRMS (ESI-ve) *m/z* calculated for C₂₂H₁₅ClFN₄O₅S (M-H)⁻ 501.0441, found 501.0437.

(Z)-3-(2-(5-(N-(2,4-Dichlorophenethyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10p).

25 Yellow solid, 14%. Mp = 290-292 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.77 (s, 1H disappeared on D₂O shake), 11.43 (s, 1H disappeared on D₂O shake), 8.07 (s, 1H), 7.82 (s, 1H), 7.71 (d, *J* = 6.4 Hz, 2H), 7.64-7.61 (m, 2H, integrated to 1H on D₂O shake), 7.50 (t, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 1.6 Hz, 1H), 7.31-7.25 (m, 2H), 7.03 (d, *J* = 8.4 Hz, 1H), 5.75 (s, 1H disappeared on D₂O shake), 2.99 (q, *J* = 6.4 Hz, 2H, CH₂, triplet on D₂O shake), 2.76 (t, *J* = 6.4 Hz, 2H, CH₂); HRMS (ESI-ve) *m/z* calculated for C₂₃H₁₇Cl₂N₄O₅S (M-H)⁻ 531.0302, found 531.0304.

(Z)-4-(2-(5-(N-(4-Chloro-3-(trifluoromethyl)benzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10q).

35 Yellow solid, 34%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.76 (s, 1H disappeared on D₂O shake), 11.44 (s, 1 H disappeared on D₂O shake), 8.25 (t, *J* = 6.4 Hz, NH, 1H disappeared on D₂O shake), 7.94 (d, *J* = 8.8 Hz, 2H), 7.70 (s, 1H), 7.58-7.52 (m, 6H), 6.95 (d, *J* = 8.4 Hz, 1H), 5.75 (s, 1H disappeared on D₂O shake), 4.13 (d, *J* = 6.4 Hz, 2H, CH₂, singlet on D₂O shake); ¹³C NMR (DMSO-*d*₆) δ 45.72, 111.15, 114.76, 117.90, 121.79, 125.73,

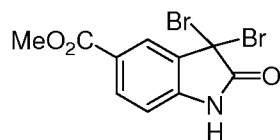
- 5 126.04 (q, $J = 272$ Hz), 126.64, 126.94, 127.46 (d, $J = 5$ Hz), 128.37, 128.72, 131.76, 131.93, 134.10, 134.93, 138.27, 143.68, 146.59, 163.64, 167.58; HRMS (ESI-ve) m/z calculated for $C_{23}H_{15}ClF_3N_4O_5S$ (M-H)⁻ 551.0409, found 551.0404.

(Z)-3-(2-(5-(N-(4-Chloro-3-(trifluoromethyl)benzyl)sulfamoyl)-2-oxoindolin-3-ylidene)hydrazinyl)benzoic acid (10r).

- 10 Yellow solid, 40%. Mp = 292-294 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.75 (s, 1H disappeared on D₂O shake), 11.41 (s, 1 H disappeared on D₂O shake), 8.28 (t, $J = 6.4$ Hz, NH, 1H disappeared on D₂O shake), 8.05 (s, 1H), 7.70-7.68 (m, 2H), 7.63 (d, $J = 7.6$ Hz, 1H), 7.57-7.47 (m, 5H), 6.95 (d, $J = 8.0$ Hz, 1H), 4.12 (d, $J = 6.4$ Hz, 2H, CH₂, singlet on D₂O shake); HRMS (ESI-ve) m/z calculated for $C_{23}H_{15}ClF_3N_4O_5S$ (M-H)⁻ 551.0409, found
15 551.0420.

Example 7

3,3-Dibromo-2-oxo-2,3-dihydro-1H-indole-5-carboxylic acid methyl ester (12).



- N*-Bromosuccinimide (13.41 g, 74.91 mmol) was added portion wise to a solution of 5-methyl
20 indole-2-carboxylate (4.50 g, 25.71 mmol) in isopropanol:H₂O (95:5, 350 ml) over 45 minutes under argon at room temperature. After the addition, the solvent was removed under reduced pressure and the solid residue was triturated with cold acetone (150 ml) to give the pure product as a yellow solid (4.90 g, 14.08 mmol, 55%). Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.71 (s, 1H), 8.05 (d, $J = 1.5$ Hz, 1H), 7.98 (dd, $J = 8.2, 1.5$ Hz, 1H), 7.06 (d, $J =$
25 8.2 Hz, 1H), 3.86 (s, 3H); ¹³C NMR (DMSO-*d*₆) □ 52.92, 111.96, 125.37, 126.83, 132.08, 134.15, 143.28 (2C), 165.88, 171.27. 7.96 (1H, dd, $J = 8.2, 1.6$ Hz, CH, Ar), 11.71

Example 8

(Z)-3-(2-(2-Carboxyphenyl)hydrazono)-2-oxoindoline-5-carboxylic acid (14a).

- Methyl dibromo-oxindole carboxylate **9** (40 mg, 0.114 mmol) was suspended in HCl (aq. 4 M,
30 2.00 ml) in a microwave vial and heated at 150 °C for 5 min. The intermediate **13** was not isolated, as seen in Figure 6. 2-Hydrazinylbenzoic acid (23 mg, 0.126 mmol) was added to the reaction mixture and heated using the Biotage microwave reactor at 150 °C for 15 min. The solid obtained upon cooling the reaction vial was filtered and washed with DCM to obtain the pure **14a** (15 mg, 40%) as an off white solid. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆)
35 δ 14.22 (s, 1H, disappeared on D₂O shake), 11.27 (s, 1H, disappeared on D₂O shake), 8.11 (appd, $J = 1.2$ Hz, 1H), 8.07 (d, $J = 8.4$ Hz, 1H), 7.93 (dd, $J = 8.0, 1.2$ Hz), 7.87 (dd, $J = 8.0,$

5 1.2 Hz, 1H); 7.63 (t, $J = 8.4$ Hz, 1H), 7.09 (t, $J = 7.2$ Hz, 1H), 6.99 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (DMSO- d_6) δ 110.94, 114.55, 114.88, 120.71, 121.93, 122.48, 124.98, 129.82, 131.53, 131.97, 135.21, 144.74, 145.26, 163.04, 167.82, 168.91; HPLC 100% ($R_t = 1.55$, 80% methanol in water); HRMS (ESI-ve) m/z calculated for $\text{C}_{16}\text{H}_{10}\text{N}_3\text{O}_5$ (M-H) $^-$ 324.0625, found 324.0622.

10 **(Z)-3-(2-(3-Carboxyphenyl)hydrazono)-2-oxoindoline-5-carboxylic acid (14b).**

The procedure was same as for the compound **14a**. 3-Hydrazinylbenzoic acid (23 mg, 0.126 mmol) was used to obtain the final compound **14b** (20 mg, 54%) as an off white solid. Mp > 300 °C; ^1H NMR (400 MHz, DMSO- d_6) δ 12.97 (broad s, 1H, disappeared on D₂O shake), 12.70 (s, 1H, disappeared on D₂O shake), 11.37 (s, 1H, disappeared on D₂O shake), 8.06 (s, 1H), 8.00 (s, 1H) 7.86 (dd, $J = 8.0, 1.2$ Hz, 1H), 7.72 (d, $J = 8.0$ Hz, 1H), 7.60 (d, $J = 7.6$ Hz, 1H), 7.49-7.45 (m, 1H), 7.00 (d, $J = 8.4$ Hz, 1H); HPLC 100% ($R_t = 1.60$, 80% methanol in water); HRMS (ESI-ve) m/z calculated for $\text{C}_{16}\text{H}_{10}\text{N}_3\text{O}_5$ (M-H) $^-$ 325.0625, found 324.0635.

Example 9

Pentafluorophenyl 1H-indole-5-carboxylate; intermediate for 15.

20 To a solution of 5-indolecarboxylic acid (0.5 g, 3.10 mmol) in DMF (3.00 ml) was added pentafluorophenyl trifluoroacetate (1.068 ml, 6.20 mmol) followed by pyridine (0.281 ml). The reaction mixture (a suspension was obtained at this stage) was stirred at room temperature under inert atmosphere for approximately 30 minutes. The reaction mixture was poured into ether (40 ml) and diluted with ethyl acetate (2 x 50 ml). The organic phase was washed with
25 water, dried (Na_2SO_4) and concentrated to obtain an off white solid (720 mg, 70%), t.l.c. $R_f = 0.71$ (EtOAc: hexane, 1:1). No purification was necessary: ^1H NMR (400 MHz, DMSO- d_6) δ 11.71 (s, 1H, NH), 8.49 (s, 1H), 7.85 (dd, $J = 8.4, 2.0$ Hz, 1H), 7.59-7.55 (m, 2H), 6.67 (broad t, 1H).

N-(4-Chlorobenzyl)-1H-indole-5-carboxamide (15).

30 The pentafluorophenyl ester (200 mg, 0.61 mmol) from the above experiment was suspended in dry acetonitrile under argon, pyridine (0.075 ml, 0.85 mmol) was added followed by 4-chlorobenzylamine (121 mg, 0.85 mmol) and stirred overnight (approximately 12 h). The resulting cloudy solution was diluted with EtOAc and washed with HCl (4M aq., 6 ml). The organic phase was separated, washed with water, dried (Na_2SO_4), and concentrated to give
35 the intermediate **15** (252 mg) as an orange solid. This compound was used in the next step without further purification. ^1H NMR (400 MHz, DMSO- d_6) δ 11.40 (s, 1H, disappeared on D₂O shake), 11.32 (s, 1H), 8.91 (t, $J = 6.4$ Hz, 1H, changed on D₂O shake), 8.15 (s, 1H), 7.64 (dd, $J = 8.4, 1.2$ Hz, 1H) 7.40-7.31 (m, 5H) 6.51 (m, 1H), 4.45 (d, $J = 6.0$ Hz, 2H, singlet on D₂O shake).

5 Example 10**3,3-Dibromo-*N*-(4-chlorobenzyl)-2-oxoindoline-5-carboxamide, Intermediate for 16.**

The indole **15** (252 mg, 0.89 mmol) was dissolved in aqueous isopropanol:H₂O (95:5, 5 ml), and NBS (0.471 g, 2.65 mmol) was added portion wise over 30 min. with stirring under argon atmosphere. The reaction was monitored by t.l.c (EtOAc: hexane, 1:1). T.L.C. indicated the
10 disappearance of the starting material. The reaction mixture was concentrated at room temperature and diluted with ether (approximately 10 ml). The succinimide precipitate was filtered and washed with ether. The ether phase was concentrated to obtain a pale yellow solid (225 mg, 55%). Attempts to purify this intermediate were not successful. The crude compound was used in the next step.

15 (Z)-3-(2-(5-(4-Chlorobenzylcarbamoyl)-2-oxoindolin-3-ylidene)hydrazinyl) benzoic acid (16).

The crude dibromoisatinamide intermediate from the above experiment (70 mg, 0.152 mmol) was suspended in dry MeOH (2.0 ml) in a microwave vial (CEM, 10.0 ml), and 3-hydrazinylbenzoic acid (31 mg, 0.166 mmol) was added and irradiated for 5 minutes at 150
20 °C in CEM microwave reactor. The reaction vial was left in an ice bath until a precipitate formed. The compound **16** (15 mg, 22%) was isolated as a pale yellow solid. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.10 (broad s, 1H, disappeared on D₂O shake), 12.79 (s, 1H, disappeared on D₂O shake), 11.31 (s, 1H, disappeared on D₂O shake), 9.13 (broad t, 1H), 8.12 (s, 1H), 8.04 (s, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 6.8 Hz, 1H), 7.61 (d, *J* = 7.6
25 Hz, 1H), 7.48 (t, *J* = 6.8 Hz, 1H), 7.37- 7.33 (m, 4H), 6.99 (d, *J* = 8.0 Hz, 1H), 4.45 (d, *J* = 5.2 Hz, 2H); HPLC 95% (*R*_t = 1.70, 80% methanol in water); LRMS (ESI+ve) *m/z* 449 [100, (M+H⁺)]; HRMS (ESI-ve) *m/z* calculated for C₂₃H₁₇ClN₄O₄ (M-H)⁻ 447.0860, found 447.0861.

Example 11**(Z)-3-(2-(2-Oxoindolin-3-ylidene)hydrazinyl)benzoic acid (18a).**

30 Yellow solid, 84%. Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 13.05 (broad s, 1H), 12.77 (s, 1H), 11.04 (s, 1H), 9.91 (d, *J* = 8.0 Hz, 1H), 7.96 (s, 1H), 7.54-7.64 (m, 3H), 7.44 (t, *J* = 8.0 Hz, 1H), 7.24 (t, *J* = 7.2 Hz, 1H), 7.04 (t, *J* = 8.0 Hz, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 111.23, 115.06, 119.16, 119.54, 121.66, 122.62, 124.12, 129.24, 129.52, 130.39, 132.74, 140.77, 143.57, 163.75, 167.75; HRMS (ESI-ve) *m/z* calculated for C₁₅H₁₀N₃O₃ (M-H)⁻
35 280.0727, found 280.0736.

(E) and (Z)-2-(2-(2-Oxoindolin-3-ylidene)hydrazinyl)benzoic acid (18b).

Yellow solid, 72% Yellow solid, As a mixture of isomers (*Z*:*E* 3:1), Major isomer (*Z*) (75%): ¹H NMR (400 MHz, DMSO-*d*₆) δ 14.19 (s, 1H), 10.92 (s, 1H), 8.01 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J*

5 = 7.6 Hz, 1H), 7.58-7.69 (m, 2H, overlap with the minor isomer), 7.25 (t, J = 7.6 Hz, 1H), 7.21-7.11 (m, 2H, overlap with the minor isomer), 6.90 (d, J = 6.8 Hz, 1H); Minor isomer (*E*) (25%): 12.39 (s, 1H), 10.72 (s, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.97 (dd, J = 8.0 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.58-7.69 (m, 1H, overlap with the major isomer), 7.37 (t, J = 7.2 Hz, 1H), 7.21-7.11 (m, 2H, overlap with the major isomer), 6.94 (d, J = 7.2 Hz, 1H); HRMS (ESI-ve) m/z
10 calculated for $C_{15}H_{10}N_3O_3$ (M-H)⁻ 280.0727, found 280.0732.

(*E*)-2-(2-(2-Oxoindolin-3-ylidene)hydrazinyl)benzoic acid (18c).

A mixture of isatin (0.060 g, 0.419 mmol) and 2-carboxylphenylhydrazine (0.085 g 0.461 mmol) and HCl (aq. 1 M, 2 drops) in ethanol (3 mL) was heated in the Biotage microwave at 120 °C for 2 min. After cooling to room temperature, pure product **18c** was collected as a
15 yellow precipitate by filtration and dried *in vacuo* (0.057 g, 0.20 mmol, 48%). Mp > 300 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.41 (s, 1H), 10.71 (s, 1H), 8.04 (d, J = 6.8 Hz, 1H), 7.97 (dd, J = 1.2, 8.0 Hz, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.64-7.68 (m, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.06 (m, 2H), 6.94 (d, J = 7.6 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 111.33, 133.33, 114.38, 116.43, 121.97, 122.21, 122.82, 131.72, 131.97, 132.72, 135.56, 142.87, 146.20, 165.60, 170.63;
20 HRMS (ESI-ve) m/z calculated for $C_{15}H_{10}N_3O_3$ (M-H)⁻ 280.0727, found 280.0736.

(*Z*)-3-(2-(2-Nitrophenyl)hydrazine)indolin-2-one (18d).

Orange solid, 38%. Mp = 279 °C, decomposed; ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.56 (s, 1H, disappeared on D₂O shake), 10.84 (s, 1H, disappeared on D₂O shake), 8.22 (d, J = 7.2 Hz, 1H), 8.01 (d, J = 8.4 Hz, 1H), 7.87-7.80 (m, 2H), 7.44-7.40 (m, 1H), 7.18 (q, J = 14.4, 7.2 Hz, 2H), 6.96 (d, J = 7.6 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 111.76, 116.27, 116.66, 122.44, 122.68,
25 123.69, 126.60, 132.92, 134.18, 135.96, 137.43, 140.57, 143.85, 165.17; HRMS (ESI+ve) m/z calculated for $C_{14}H_{11}N_4O_3$ (M+H)⁺ 283.0826, found 283.0834.

(*Z*)-3-(2-(3-Nitrophenyl)hydrazine)indolin-2-one (18e).

Yellow solid, 73%. Mp = 267-269 °C; ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.75 (s, 1H), 11.07 (s, 1H), 8.25 (t, J = 4.0 Hz, 1H), 7.85 (ddd, J = 8.0, 2.8, 0.8 Hz, 1H), 7.81 (ddd, J = 8.0, 2.0, 0.8 Hz, 1H), 7.62-7.57 (m, 2H), 7.26 (td, J = 7.6, 1.2 Hz, 1H), 7.04 (td, J = 7.2, 1.2 Hz, 1H), 6.90 (d, J = 7.6 Hz, 1H); ¹³C NMR (DMSO-*d*₆) δ 108.91, 111.30, 117.34, 119.95, 121.14, 122.71, 130.07, 130.53, 131.53, 141.24, 144.24, 144.80, 149.46, 163.49; HRMS (ESI+ve) m/z
30 calculated for $C_{14}H_{11}N_4O_3$ (M+H)⁺ 283.0826, found 283.0835.

35 **Example 12; General Materials and Methods.**

Chemical Library: The NCI Diversity Set chemical library of 1981 compounds was provided by the NCI Developmental Therapeutics Program. After the initial identification of NSC-87877 from the NCI Diversity Set, the authentic, 98% pure NSC-87877 [8-hydroxy-7-(6-

5 sulfonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid] was obtained from Acros for subsequent experiments.

Recombinant PTP Proteins: Plasmids for expression of glutathione S-transferase (GST)-PTP fusion proteins of human Shp2 (residues 205-593), Shp1 (residues 205-597), and PTP1B (residues 1-435) were constructed in pGEX-2T by PCR subcloning techniques. A plasmid for
10 GST fusion protein of human HePTP (residues 1-399) was constructed in pGEX-2T-KG. GST-Shp2 PTP containing Lys-280 to Val (V280) and Lys-280/Asn-281 to Arg/Asp (R280D281) mutants were generated by PCR-based mutagenesis. All constructs were verified by DNA sequencing.

GST-PTP fusion proteins were expressed in *E. coli* DH5 α and affinity purified with glutathione
15 Sepharose. After elution from glutathione affinity column, GST-fusion proteins were dialyzed with dialysis buffer (12.5 mM Tris-Cl, pH 7.5, 25 mM NaCl, 1 mM dithiothreitol (DTT), and 0.1% β -mercaptoethanol) at 4 °C for 40 h and then stored in dialysis buffer plus 20% glycerol at -80 °C. Recombinant CD45 (residues 584-1281) and LAR D1 domain were obtained from Calbiochem. Recombinant DEP1 was from Abcam. GST fusion proteins of murine Shp2
20 (GST-Shp2 Δ N) and Shp1 have been reported (Cunnick JM, et al. *Biochem Mol Biol Int* **1998**, 45(5):887-894; Cunnick JM, et al. *J Biol. Chem.* **2001**, 276(26):24380-24387) and were used in the chemical library screening and initial *in vitro* characterization.

PTP Activity Assay: PTP activity was measured using the fluorogenic 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP, from Invitrogen) as the substrate. Unless otherwise
25 specified, each reaction contained 25 mM MOPS (pH 7.0), 50 mM NaCl, 0.05% Tween-20, 1 mM DTT, 20 μ M DiFMUP, 10 nM Microcystin LR, 20 nM GST-PTP, and 5 μ l test compound or dimethyl sulfoxide (DMSO, solvent) in a total reaction volume of 100 μ l in black 96-well plates. Reaction was initiated by addition of DiFMUP and the incubation time was 30 min at room temperature. DiFMU fluorescence signal was measured at an excitation of 355 nm and
30 an emission of 460 nm with a Wallac Victor² 1420 plate reader. IC₅₀ was defined as the concentration of an inhibitor that caused a 50% decrease in the PTP activity. For IC₅₀ determination, 8 concentrations of NSC-87877 at 1/3 dilution (~ 0.5 log) were tested. The ranges of NSC-87877 concentrations used in each PTP assay were determined from preliminary trials. Each experiment was performed in triplicate and IC₅₀ data were derived
35 from at least three independent experiments. The curve-fitting program Prism 4 (GraphPad Software) was used to calculate the IC₅₀ value.

Computer Docking: Computer docking was performed using the X-ray crystal structure of human Shp2 (PDB identification code: 2SHP) (Hof P, et al. *Cell* **1998**, 92(4):441-450) using the GLIDE (Grid-Based Ligand Docking from Energetics, as part of the FirstDiscovery Suite
40 from Schrödinger, L.L.C.) program (Friesner RA, et al. *J Med Chem* **2004**, 47(7):1739-1749;

- 5 Halgren TA, et al. *J Med Chem* **2004**, 47(7):1750-1759). The N-SH2 domain of Shp2, which blocks the catalytic site, was removed from the 3D structure prior to the computer docking analysis. The GLIDE program relies on the Jorgensen OPLS-2001 force field. The optimal binding geometry for each model was obtained by utilization of Monte Carlo sampling techniques coupled with energy minimization.
- 10 Preparation of HEK293 Cell Line for Doxycycline-inducible Expression of a Gab1-Shp2 Chimera: Plasmid pcDNA5/FRT/TO-Gab1PH-Shp2 Δ N was constructed by subcloning the coding sequence for Flag-Gab1PH-Shp2 Δ N (Cunnick JM, et al. *J Biol. Chem.* **2002**, 277(11):9498-9504) from pcDNA3.1 into pcDNA5/FRT/TO (Invitrogen) through HindIII and
- 15 Apal sites. pcDNA5/FRT/TO-Gab1PH-Shp2 Δ N and pOG44 was then co-transfected into the Flp-In-T-Rex-293 cells (Invitrogen). Transfected cells were selected in Dulbecco's modified Eagle medium (DMEM)/10% tetracycline-free fetal bovine serum (FBS) medium containing 100 μ g/ml hygromycin. Individual Hygromycin-resistant cell lines were screened for dox-inducible expression of Flag-tagged Gab1PH-Shp2 Δ N by immunoblotting analysis of cell
- 20 lysates with an anti-Flag antibody (M2, from Sigma). Among 24 hygromycin-resistant cell lines that we have screened, 21 cell lines showed dox-inducible expression of Gab1PH-Shp2 Δ N. One of these 21 cell lines was randomly selected for use in the subsequent experiments.
- Cell Culture, Immunoprecipitation and Immunoblotting: Cells were cultured in DMEM/10% FBS. Sub-confluent cells were serum-starved in DMEM/0.1% BSA for 18 h prior to treatment
- 25 with NSC-87877 and stimulation with EGF or PMA. Cells were lysed on ice with Lysis Buffer A (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 25 mM NaF, 5 mM sodium pyrophosphate, 1 mM DTT, 20 mM *p*-nitrophenyl phosphate, 1% Triton X-100). Immunoprecipitation and immunoblotting analyses of Gab1, Shp2, paxillin, and Erk1/2 were performed essentially as described (Cunnick JM, et al. *J Biol. Chem.* **2001**, 276(26):24380-
- 30 24387; Ren Y, et al. *J. Biol. Chem.* **2004**, 279(9):8497-8505). Erk1/2 kinase assay was performed as reported except that endogenous Erk1/2 kinase activity was measured (Cunnick JM, et al. *J Biol. Chem.* **2001**, 276(26):24380-24387).
- Immune Complex PTP Assay: Serum-starved cells were pretreated with NSC-87877 (50 μ M, 3 h) or DMSO (solvent) and then stimulated with EGF (100 ng/ml, 5 min) or left untreated.
- 35 Cells were lysed in ice-cold PTP Lysis Buffer [25 mM Hepes pH7.4, 150 mM NaCl, 2 mM EDTA, 0.5% Triton X-100, 1:50 diluted protease inhibitor cocktail (Roche)]. Shp2 or Shp1 in cell lysate supernatants (0.5 mg/each) was immunoprecipitated with an antibody to Shp2 or an antibody to Shp1 (Santa Cruz) plus Protein A-Sepharose for 2 h at 4 C. Immunoprecipitates were washed twice with the PTP lysis buffer and twice with Reaction
- 40 Buffer (20 mM Hepes pH 7.4, 1 mM EDTA, 5% Glycerol, 1 mM DTT) (Tartaglia M, et al. *Nat.*

5 *Genet.* **2003**, 34(2):148-150). Each Shp2 or Shp1 immune complex was resuspended in 100 μ l Reaction Buffer containing 50 μ M DiFMUP and then incubated at room temperature for 20 min. After a brief centrifugation, supernatants were transferred into 96-well plates and the DiFMU fluorescence signal was measured. The remaining immune complexes were used for immunoblotting analysis of Shp2 or Shp1.

10 Ras Activation Assay: Active Ras in MDA-MB-468 cells was detected by means of Ras-GTP bound to a GST fusion protein of the Ras-GTP binding domain of Raf fragment (GST-RBD) (Cunnick JM, et al. *J Biol. Chem.* **2002**, 277(11):9498-9504) followed by immunoblotting with an anti-Ras antibody (Santa Cruz).

Statistical Analysis: Statistical analyses were performed using unpaired t test with Welch's
15 correction using the GraphPad Prism 4 program (GraphPad Software).

In the preceding specification, all documents, acts, or information disclosed do not constitute an admission that the document, act, or information of any combination thereof was publicly available, known to the public, part of the general knowledge in the art, or was known to be relevant to solve any problem at the time of priority.

20 The disclosures of all publications cited above are expressly incorporated herein by reference, each in its entirety, to the same extent as if each were incorporated by reference individually.

It will be seen that the advantages set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the
25 above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

While there has been described and illustrated specific embodiments of Shp2 inhibitors, it will be apparent to those skilled in the art that variations and modifications are possible without
30 deviating from the broad spirit and principle of the present invention. It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween.

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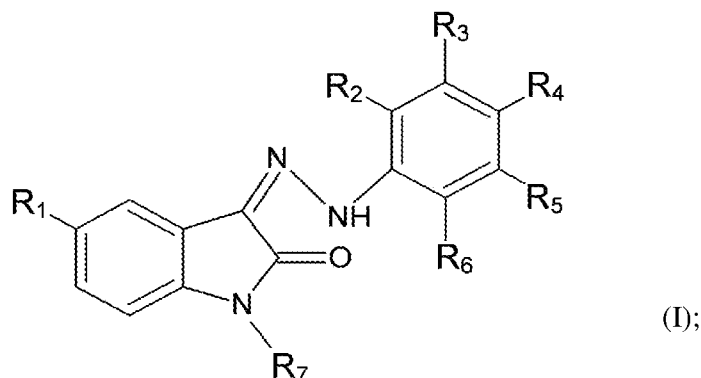
5 [0029]

[0030] Val His Cys Ser Ala Gly Ile Gly Arg Thr Gly

[0031] 1 5 10

5 What is claimed is:

1. A method of inhibiting a protein tyrosine phosphatase in a cell comprising the step of contacting the cell with an effective amount of a compound having the formula (I):

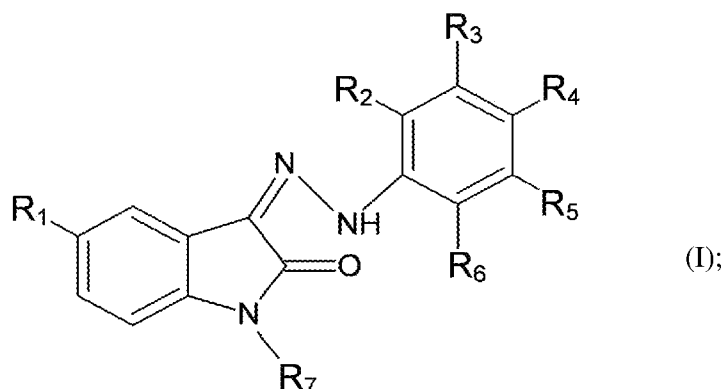


- 10 wherein R₁ is selected from the group consisting of SO₃H, CO₂H, CONHCH₂ (4-ClC₆H₄), SO₂NHⁱPr, SO₂NH₂, SO₂NHCH₂(2-ClC₆H₄), SO₂NHCH₂(3-ClC₆H₄), SO₂NHCH₂(4-ClC₆H₄), SO₂NHCH₂(4-MeC₆H₄), SO₂NHCH₂(3-CF₃-4-Cl-C₆H₄), SO₂NHCH₂(4-FC₆H₄), SO₂NHCH₂(3-Cl-4-F-C₆H₄), (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, SO₂NH(CH₂)₂(2-Cl-4-ClC₆H₄), SO₂N-R₈, phenyl, nitro, carboalkoxy,
- 15 carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, and NH(CH₂)_nPO₃H.
- 20 wherein R₂ is selected from the group consisting of hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;
- 25

- wherein R₃ is selected from the group consisting of hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
- 30

- 5 O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;
wherein R₄ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
10 CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;
wherein R₅ is selected from the group consisting of hydrogen, NO₂, COO⁻,
15 COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
20 NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;
wherein R₆ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
25 (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H; and
wherein R₇ is selected from the group consisting of hydrogen and methyl.
2. The method of claim 1, wherein the R₁ group at the 5-position of the oxindole moiety
30 is selected from the group consisting of a carboxylic acid, a sulfonamide, a
carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, and *p*-
halosulfonamide.
3. The method of claim 2, wherein the *p*-halosulfonamide is chloridesulfonamide.
4. The method of claim 1, wherein the compound having the formula (I) possesses a
35 polar group on the oxindole moiety and a carboxylate or carboxylic acid on the
phenylhydrazone moiety.
5. The method of claim 1, wherein R₈ is selected from the group consisting of straight-
chained alkyl, branched alkyl, cyclic alkyl, phenyl, and benzyl.

- 5 6. The method of claim 5, wherein at least one carbon on the alkyl, phenyl, or benzyl is substituted with at least one compound selected from the group consisting of a halogen, F, Cl, alkyl, haloalkyl, and CF₃.
7. The method according to claim 1, wherein the protein tyrosine phosphatase is a Shp2 protein tyrosine phosphatase.
- 10 8. The method according to claim 7, wherein the Shp protein tyrosine phosphatase is a selective inhibitor of the Shp protein tyrosine phosphatase.
9. The method according to claim 1, wherein the compound comprises a z-configuration iastin hydrazone.
- 15 10. A method of treating a disease in an animal comprising the step of administering to the subject in need thereof an effective amount of a compound having the formula (I):



wherein R₁ is selected from the group consisting of SO₃H, CO₂H, CONHCH₂ (4-CIC₆H₄), SO₂NHⁱPr, SO₂NH₂, SO₂NHCH₂(2-CIC₆H₄), SO₂NHCH₂(3-CIC₆H₄), SO₂NHCH₂(4-CIC₆H₄), SO₂NHCH₂(4-MeC₆H₄), SO₂NHCH₂(3-CF₃-4-Cl-C₆H₄), SO₂NHCH₂(4-FC₆H₄), SO₂NHCH₂(3-Cl-4-F-C₆H₄), (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, SO₂NH(CH₂)₂(2-Cl-4-CIC₆H₄), SO₂N-R₈, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H.

wherein R₂ is selected from the group consisting of hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,

5 (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

wherein R₃ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
10 chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

15 wherein R₄ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
20 O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

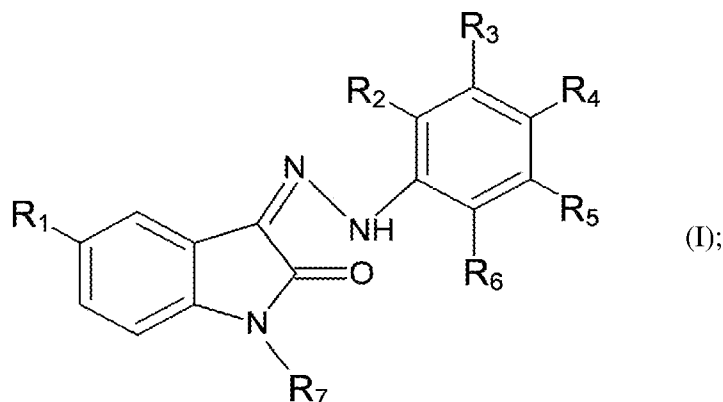
wherein R₅ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
25 CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

wherein R₆ is selected from the group consisting of hydrogen, NO₂, COO⁻,
30 COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
35 NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H; and

wherein R₇ is selected from the group consisting of hydrogen and methyl.

11. The method of claim 10, wherein the R₁ group at the 5-position of the oxindole moiety
is selected from the group consisting of a carboxylic acid, a sulfonamide, a

- 5 carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, and *p*-halosulfonamide.
12. The method of claim 11, wherein the *p*-halosulfonamide is chloridesulfonamide.
13. The method of claim 10, wherein R₈ is selected from the group consisting of straight-chained alkyl, branched alkyl, cyclic alkyl, phenyl, and benzyl.
- 10 14. The method of claim 13, wherein at least one carbon on the alkyl, phenyl, or benzyl is substituted with at least one compound selected from the group consisting of a halogen, F, Cl, alkyl, haloalkyl, and CF₃.
- 15 15. The method of claim 10, wherein the compound having the formula (I) possesses a polar group on the oxindole moiety and a carboxylate or carboxylic acid on the phenylhydrazone moiety.
16. The method according to claim 10, wherein the protein tyrosine phosphatase is a Shp2 protein tyrosine phosphatase.
17. A compound having the formula (I):



- 20 wherein R₁ is selected from the group consisting of SO₃H, CO₂H, CONHCH₂ (4-CIC₆H₄), SO₂NHⁱPr, SO₂NH₂, SO₂NHCH₂(2-CIC₆H₄), SO₂NHCH₂(3-CIC₆H₄), SO₂NHCH₂(4-CIC₆H₄), SO₂NHCH₂(4-MeC₆H₄), SO₂NHCH₂(3-CF₃-4-Cl-C₆H₄), SO₂NHCH₂(4-FC₆H₄), SO₂NHCH₂(3-Cl-4-F-C₆H₄), (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, SO₂NH(CH₂)₂(2-Cl-4-CIC₆H₄), SO₂N-R₈, phenyl, nitro, carboalkoxy,
- 25 carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H, CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂, (CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂, O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂, NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H.

5 wherein R₂ is selected from the group consisting of hydrogen, NO₂, COO⁻, COO₂H, phenyl, nitro, carboalkoxy, carboxamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
10 O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

wherein R_3 is selected from the group consisting of hydrogen, NO_2 , COO^- , COO_2H , phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO_3H , CO_2NH_2 , SO_2NH_2 , PO_3H , CF_2PO_3H , $(CH_2)_nCO_2H$, $(CH_2)_nSO_3H$, $(CH_2)_nCO_2NH_2$, $(CH_2)_nSO_2NH_2$, $(CH_2)_nPO_3H$, $O(CH_2)_nCO_2H$, $O(CH_2)_nSO_3H$, $O(CH_2)_nCO_2NH_2$, $O(CH_2)_nSO_2NH_2$, $O(CH_2)_nPO_3H$, $NH(CH_2)_nCO_2H$, $NH(CH_2)_nSO_3H$, $NH(CH_2)_nCO_2NH_2$, $NH(CH_2)_nSO_2NH_2$, and $NH(CH_2)_nPO_3H$;

wherein R₄ is selected from the group consisting of hydrogen, NO₂, COO⁻,
COO₂H, phenyl, nitro, carboalkoxy, carboxyamide, benzylcarboxamide, straight-
chained alkyl, branched alkyl, cyclic alkyl, SO₃H, CO₂NH₂, SO₂NH₂, PO₃H,
CF₂PO₃H, (CH₂)_nCO₂H, (CH₂)_nSO₃H, (CH₂)_nCO₂NH₂, (CH₂)_nSO₂NH₂,
(CH₂)_nPO₃H, O(CH₂)_nCO₂H, O(CH₂)_nSO₃H, O(CH₂)_nCO₂NH₂, O(CH₂)_nSO₂NH₂,
O(CH₂)_nPO₃H, NH(CH₂)_nCO₂H, NH(CH₂)_nSO₃H, NH(CH₂)_nCO₂NH₂,
NH(CH₂)_nSO₂NH₂, and NH(CH₂)_nPO₃H;

wherein R_5 is selected from the group consisting of hydrogen, NO_2 , COO^- , COO_2H , phenyl, nitro, carboalkoxy, carboxamide, benzylcarboxamide, straight-chained alkyl, branched alkyl, cyclic alkyl, SO_3H , CO_2NH_2 , SO_2NH_2 , PO_3H , CF_2PO_3H , $(CH_2)_nCO_2H$, $(CH_2)_nSO_3H$, $(CH_2)_nCO_2NH_2$, $(CH_2)_nSO_2NH_2$, $(CH_2)_nPO_3H$, $O(CH_2)_nCO_2H$, $O(CH_2)_nSO_3H$, $O(CH_2)_nCO_2NH_2$, $O(CH_2)_nSO_2NH_2$, $O(CH_2)_nPO_3H$, $NH(CH_2)_nCO_2H$, $NH(CH_2)_nSO_3H$, $NH(CH_2)_nCO_2NH_2$, $NH(CH_2)_nSO_2NH_2$, and $NH(CH_2)_nPO_3H$;

wherein R_6 is selected from the group consisting of hydrogen, NO_2 , COO^- , COO_2H , phenyl, nitro, carboalkoxy, carboxamide, benzylcarboxamide, straight-
35 chained alkyl, branched alkyl, cyclic alkyl, SO_3H , CO_2NH_2 , SO_2NH_2 , PO_3H , CF_2PO_3H , $(CH_2)_nCO_2H$, $(CH_2)_nSO_3H$, $(CH_2)_nCO_2NH_2$, $(CH_2)_nSO_2NH_2$, $(CH_2)_nPO_3H$, $O(CH_2)_nCO_2H$, $O(CH_2)_nSO_3H$, $O(CH_2)_nCO_2NH_2$, $O(CH_2)_nSO_2NH_2$, $O(CH_2)_nPO_3H$, $NH(CH_2)_nCO_2H$, $NH(CH_2)_nSO_3H$, $NH(CH_2)_nCO_2NH_2$, $NH(CH_2)_nSO_2NH_2$, and $NH(CH_2)_nPO_3H$; and

40 wherein R₇ is selected from the group consisting of hydrogen and methyl.

- 5 18. The compound of claim 17, wherein R₈ is selected from the group consisting of straight-chained alkyl, branched alkyl, cyclic alkyl, phenyl, and benzyl.
19. The compound of claim 18, wherein at least one carbon on the alkyl, phenyl, or benzyl is substituted with at least one compound selected from the group consisting of a halogen, F, Cl, alkyl, haloalkyl, and CF₃.
- 10 20. The compound of claim 17, wherein the R₁ group at the 5-position of the oxindole moiety is selected from the group consisting of a carboxylic acid, a sulfonamide, and a carboxylamide, bis-carboxylic acid, bis-carboxylic acid derivative, and *p*-halosulfonamide.
21. The compound of claim 20, wherein the *p*-halosulfonamide is chloridesulfonamide.
- 15 22. The compound of claim 17, wherein the compound having the formula (I) possesses a polar group on the oxindole moiety and a carboxylate or carboxylic acid on the phenylhydrazone moiety.
23. The compound according to claim 17, wherein the compound comprises a *z*-configuration in the hydrazone.

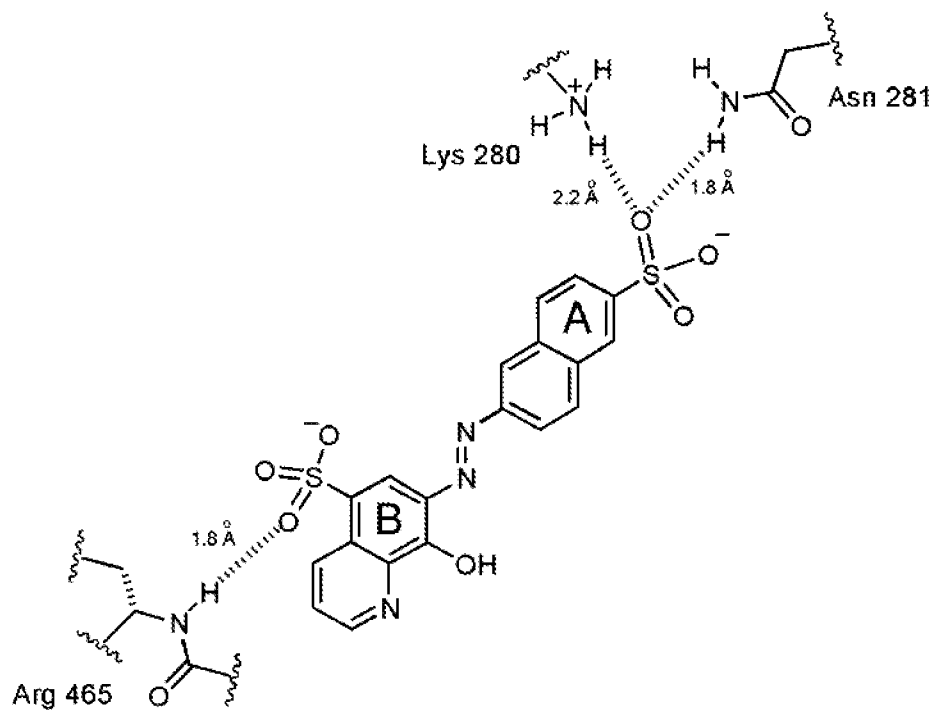


Figure 1.

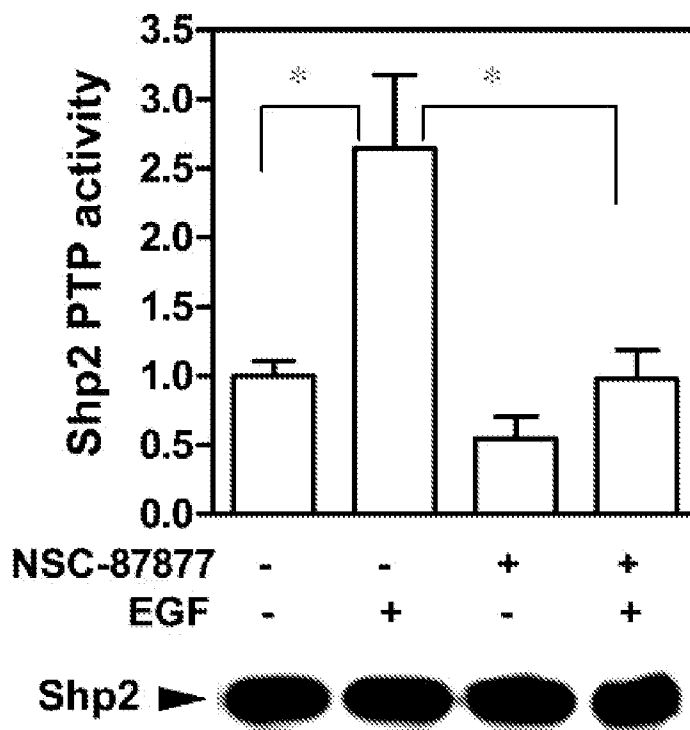


Figure 2.

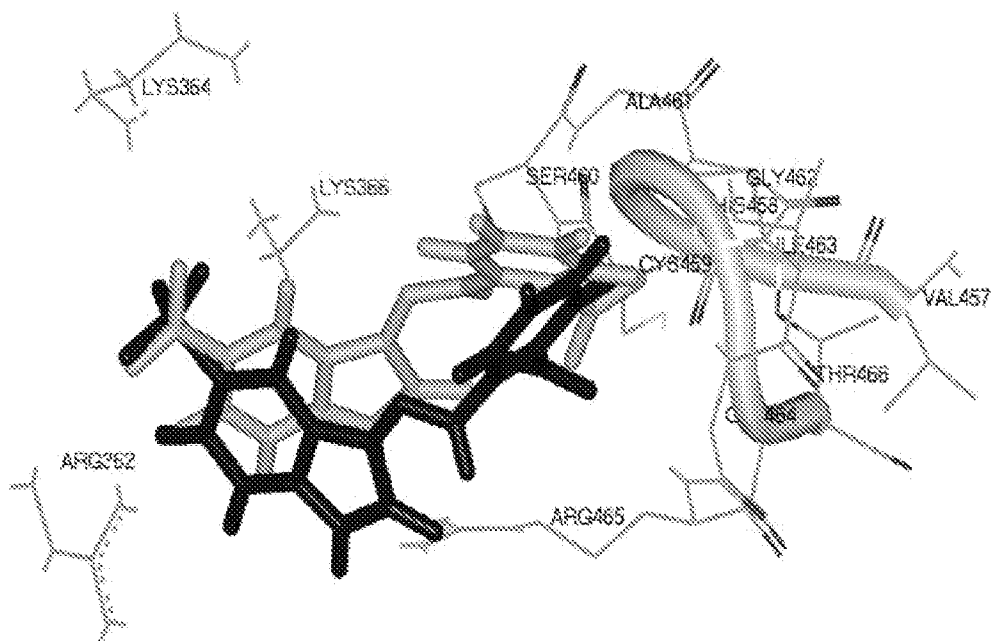


Figure 3.

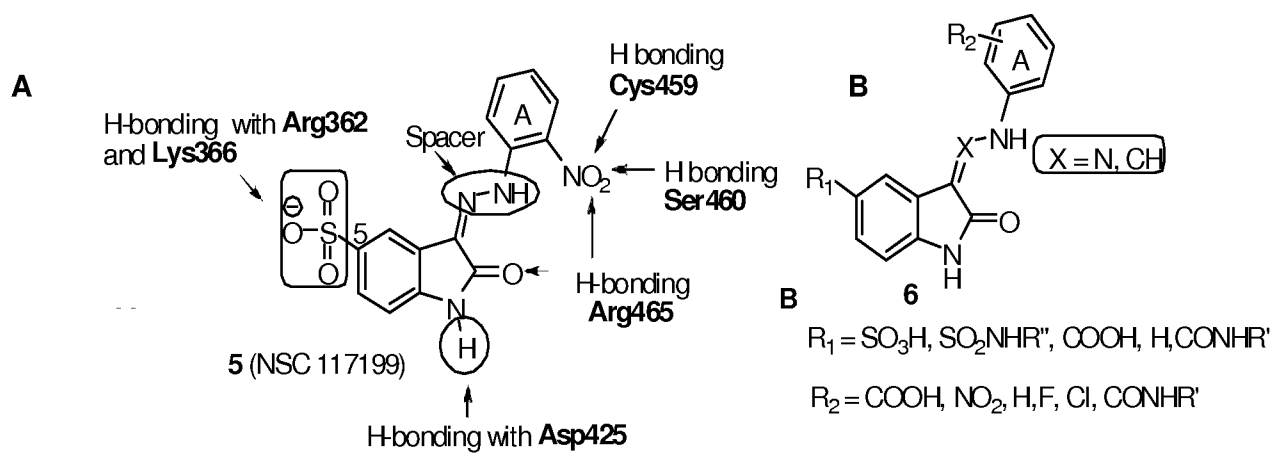


Figure 4.

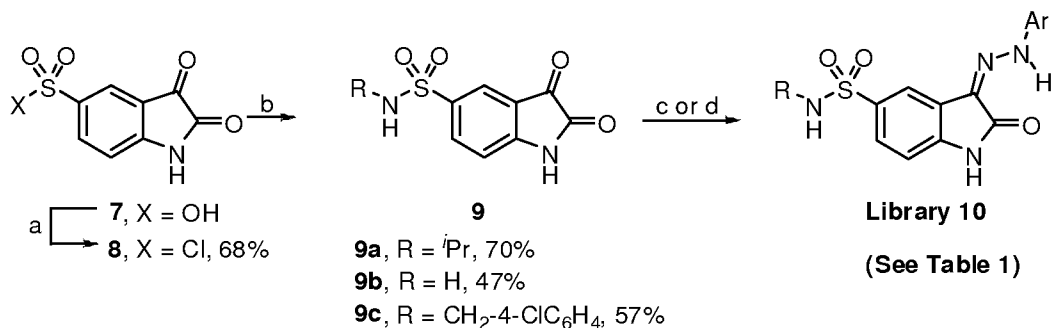


Figure 5.

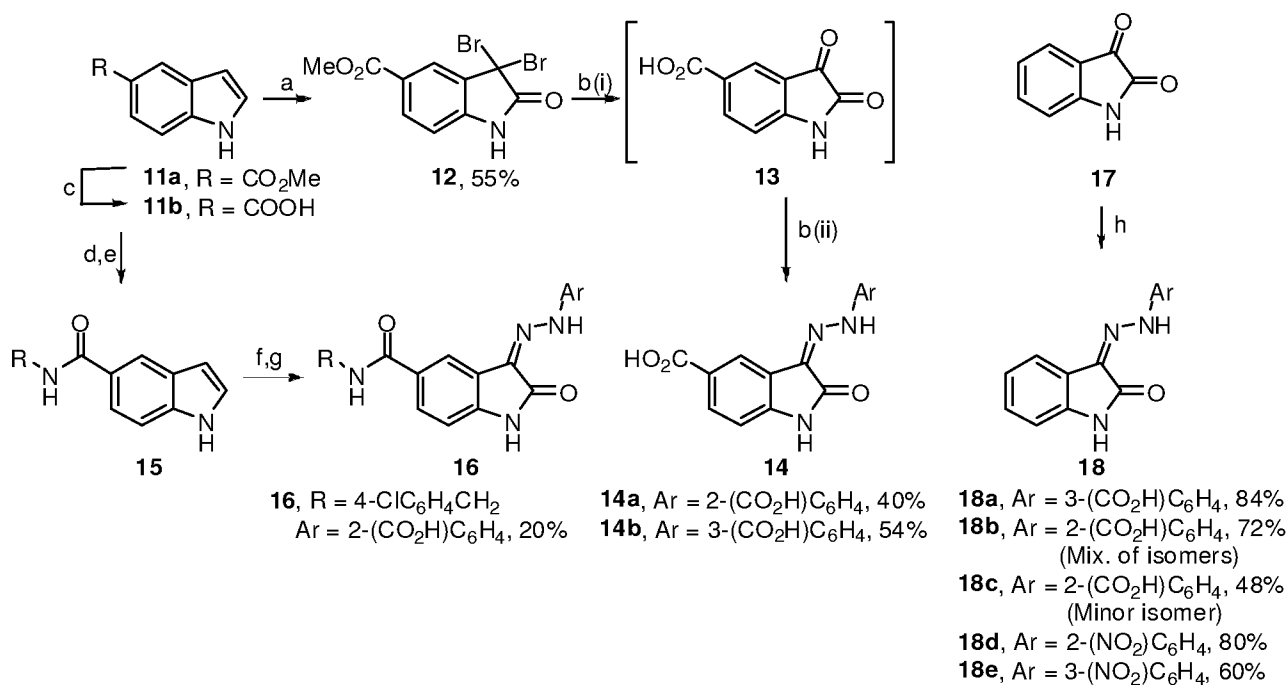
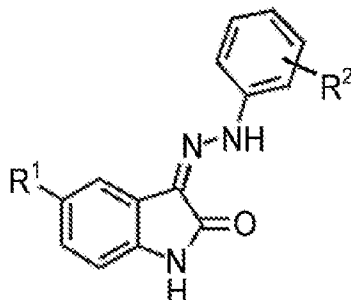


Figure 6.



Compound	R ¹	R ²	IC ₅₀ (μM) ^a		
			Shp2	Shp1	PTP1B
5	SO ₃ H	2-NO ₂	46.8±10.2	68±17.1	96.7±29.9
14a	CO ₂ H	2-CO ₂ H	0.8±0.2	15.4±2.1	1.5±0.6
14b	CO ₂ H	3-CO ₂ H	15.8±6.6	72.5±19.3	38.2±13.9
16	CONHCH ₂ (4-ClC ₆ H ₄)	3-CO ₂ H	22.3±5.2	19.4±1.8	>300
10a	SO ₂ NH ⁱ Pr	3-CO ₂ H	4.5±1.7	15.7±1.7	37.1±4.9
10b	SO ₂ NH ⁱ Pr	4-CO ₂ H	4.5±1.0	27.7±9.0	9.3±1.6
10c	SO ₂ NH ₂	2-NO ₂	11.9±1.5	103.7±17.4	156.6±14.1
10d	SO ₂ NHCH ₂ (4-ClC ₆ H ₄)	2-NO ₂	4.4±2.4	40.9±15.3	9.8±2.6
10e	SO ₂ NHCH ₂ (4-MeC ₆ H ₄)	3-CO ₂ H	5.0±1.9	32.6±8.5	11.3±4.1
10f	SO ₂ NHCH ₂ C ₆ H ₅	4-CO ₂ H	1.3±0.9	7.1±1.1	2.5±1.5
10g	SO ₂ NHCH ₂ (3-ClC ₆ H ₄)	3-CO ₂ H	3.8±0.2	42.5±7.2	15.4±6.2
10h	SO ₂ NHCH ₂ (4-ClC ₆ H ₄)	3-CO ₂ H	1.4±0.6	18.0±7.2	18.8±5.1
10i	SO ₂ NHCH ₂ (4-ClC ₆ H ₄)	4-CO ₂ H	5.9±1.4	18.1±3.7	7.5±1.1
10j	SO ₂ NHCH ₂ (2-ClC ₆ H ₄)	4-CO ₂ H	7.4±2.4	60.8±26.1	11.7±2.4
10k	SO ₂ NHCH ₂ (2-ClC ₆ H ₄)	3-CO ₂ H	5.5±0.4	15.9±1.7	19.4±10.2
10l	SO ₂ NHCH ₂ (3-CF ₃ -4-Cl-C ₆ H ₄)	2-CO ₂ H	22.9±7.0	32.4±17.9	25.7±6.6
10m	SO ₂ NHCH ₂ (4-FC ₆ H ₄)	3-CO ₂ H	1.0±0.2	18.3±5.2	14.5±1.5
10n	SO ₂ NHCH ₂ (4-FC ₆ H ₄)	4-CO ₂ H	6.3±2.3	10.8±4.1	11.4±0.4
10o	SO ₂ NHCH ₂ (3-Cl-4-F-C ₆ H ₄)	3-CO ₂ H	4.8±0.6	122.8±74.5	54.9±13.3
10p	SO ₂ NH(CH ₂) ₂ (2-Cl-4-ClC ₆ H ₃)	3-CO ₂ H	5.2±1.7	73.4±36.9	19.3±13.8
10q	SO ₂ NHCH ₂ (3-CF ₃ -4-Cl-C ₆ H ₄)	4-CO ₂ H	8.3±1.7	43.1±7.2	32.8±8.0
10r	SO ₂ NHCH ₂ (3-CF ₃ -4-Cl-C ₆ H ₄)	3-CO ₂ H	10.6±1.5	74.2±8.0	63.1±24.7

Figure 7.

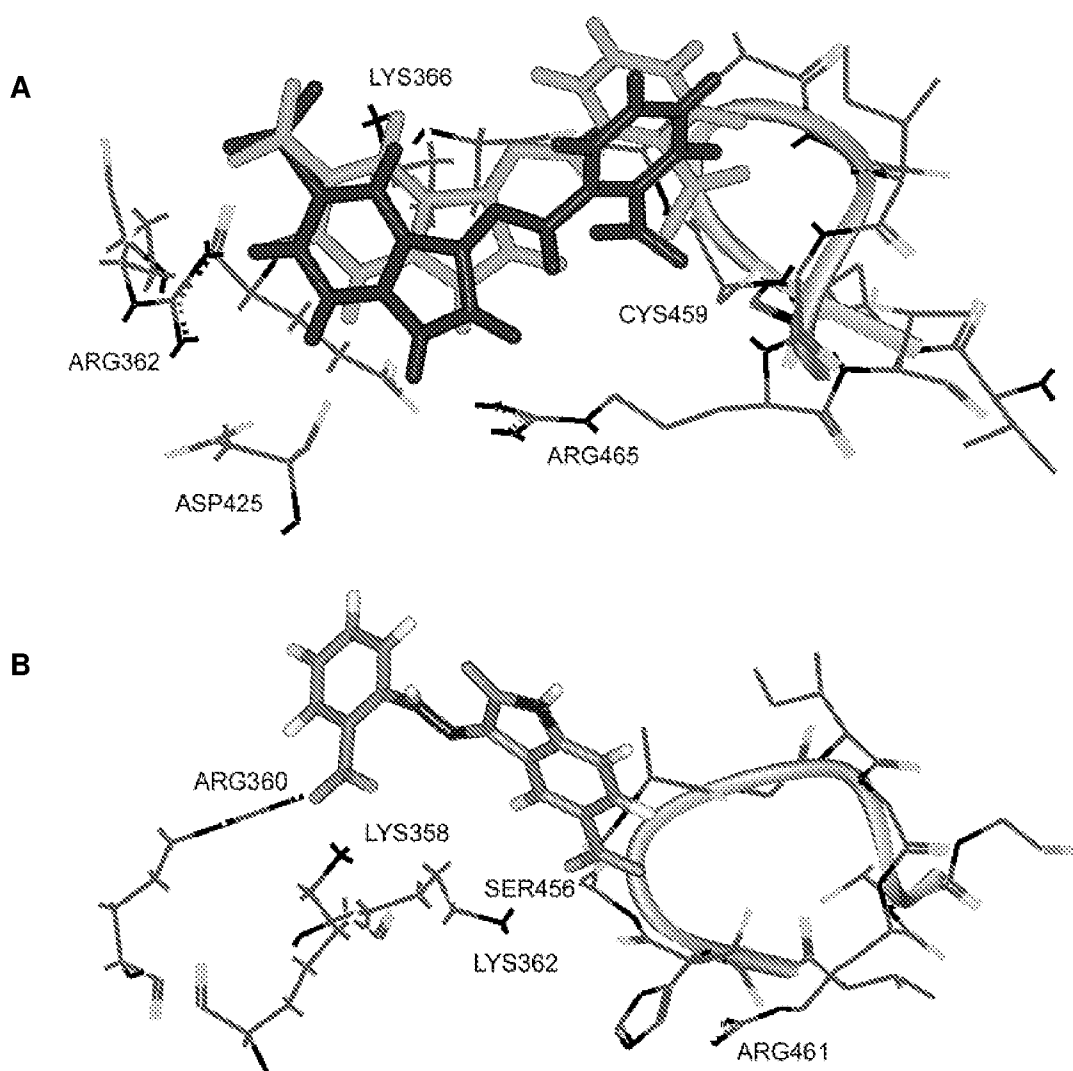


Figure 8.