

## Supplementary material

### Experimental

#### Synthesis and Physico-chemical data of compounds

All solvents and reagents were purchased from Merck and Sigma-Aldrich. The structures were identified using melting points (m.p.), infrared spectroscopy (IR),  $^1\text{H}$  and  $^{13}\text{C}$  nuclear magnetic resonance spectroscopy (NMR), and the unpublished ones were also submitted to elementary analyses. Melting points were determined with a Microquímica MGAPF-301 apparatus and are uncorrected. Infrared spectra were determined with a Perkin Elmer 16PC spectrophotometer (Perkin Elmer, Wellesley, MA, USA).  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AC-200F spectrometer (Rheinstetten, Germany) (at 400 MHz and 100 MHz, respectively).  $\text{CDCl}_3$  and  $\text{DMSO}-d_6$  were used as solvents with tetramethylsilane (TMS) as the internal standard. For the CHNS analysis, a CHNS elemental analyser PERKIN ELMER 2400 (Boston, MA, USA) was used. In the thin layer chromatography, aluminium sheets with silica gel 60 F-254 and 0.2 mm thickness were utilized. The synthesis of compounds **7-20** were already described by us [24].

**21 - 1-methyl-4-[(2R,6S)-3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0<sup>2,6</sup>]dec-8-en-4-yl]-N'-[(1E)-(4-bromophenyl)methylene]benzene sulfonyl-hydrazone.** The 4-(3,5-dioxo-10-oxa-4-azatricyclo[5.2.1.0<sup>2,6</sup>]dec-8-en-4-yl)benzenesulfonyl hydrazine (**13**) (0.50 g, 1.49 mmol) was added in a solution of *p*-bromobenzaldehyde (0.28 g, 1.49 mmol) in ethanol. The mixture was stirred at room temperature during two hours. The product was filtered off with suction and recrystallized with hexane-ethyl acetate (1:3). Yield: 71%. m. p.: 127.4-129.1 °C. IR (KBr): 3458 (v NH), 1709 large [v N(C=O)<sub>2</sub>], 1382 and 1172 (v SO<sub>2</sub>), 593 (v ArH)  $\text{cm}^{-1}$ . Anal. Calcd. for  $\text{C}_{21}\text{H}_{16}\text{N}_3\text{O}_5\text{Br}$  (%): C (51.17), H (3.51), N (8.14), S (6.21). Found (%): C (50.76); H (3.49); N (8.06); S (6.18).  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 11.74 (s, 1H, -NH-N), 8.00-7.98 (d, 2H, ArH,  $J = 8.79$  Hz), 7.92 (s, 1H, -N=CH-), 7.59-7.57 (d, 2H, ArH,  $J = 8.59$  Hz), 7.53-7.51 (d, 2H, ArH,  $J = 8.59$  Hz), 7.50-7.48 (d, 2H, ArH,  $J = 8.79$  Hz), 6.58-6.56 (d, 1H, -CH=CH-;  $J = 5.67$  Hz), 6.42-6.41 (d, 1H, -CH=CH-;  $J = 5.67$  Hz), 5.11 (d, 1H, -CH-O-,  $J = 1.95$  Hz), 3.21-3.20 (d, 1H, -(CH-C=O)<sub>2</sub>;  $J = 6.45$  Hz), 2.95-2.93 (d, 1H, -(CH-C=O)<sub>2</sub>;  $J = 6.45$  Hz), 1.60 (s, 3H, -CH<sub>3</sub>).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 16.22 (CH<sub>3</sub>), 51.31 (O-C-CH), 51.50 (O-CH-CH), 81.29 (C-CH<sub>3</sub>), 88.55 (O-CH), 124.16, 128.13, 128.76, 129.42, 132.51, 133.47, 136.54, 139.10 (C Ar), 147.10 (CH=N), 174.94 (CH-CH-C=O), 175.94 (C-CH-C=O).

**22 - 2-benzyl-1H-benzo[de]isoquinoline-1,3(2H)-dione.** A mixture of naphthalic anhydride (3.00 g, 15 mmol) and benzylamine (1.65 mL, 15 mmol) in ethanol was refluxed for 3 h. The formed compound separated out on cooling and was filtered through a Büchner funnel and washed twice with 20 mL of ethanol. Yield: 91%. m.p. 197.9-198.6 °C (Lit. 192 °C) [31].  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  5.37 (s, 2H, CH<sub>2</sub>), 7.22-7.26 (t, 1H, ArH), 7.20-7.33 (t, 2H, ArH), 7.56-7.58

(dd, 2H, ArH, J=7.03 Hz), 7.65-7.68 (t, 2H, ArH), 8.08-8.10 (dd, 2H, ArH, J=8.01 Hz), 8.52-8.54 (dd, 2H, ArH, J=7.03 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 43.49; 122.46, 126.83, 127.47, 127.98, 128.40, 129.03, 131.28, 131.42, 133.93, 137.29 (C Ar); 164.10 (C=O).

**23 - 2-benzyl-6-chloro-1H-benzo[de]isoquinoline-1,3(2H)-dione.** Imide (**23**) was obtained as described in the compound (**22**). Yield: 85%. m.p. 176.0-176.2 °C (Lit. 168.5-170.5 °C) [32]. IR (KBr) 1689 and 1656 [ν N(C=O)<sub>2</sub>], 1340 (ν -CN), 741 (ν Ar.). Anal. Calcd. for C<sub>19</sub>H<sub>12</sub>ClNO<sub>2</sub>: C, 70.92; H, 3.76; N, 4.35. Found: C, 70.51; H, 3.74; N, 4.37. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.40 (s, 2H, CH<sub>2</sub>), 7.04-7.06 (d, 2H, ArH, J=8.20Hz), 7.14-7.16 (d, 2H, ArH, J=8.20Hz), 7.39-7.41 (d, 1H, ArH, J=7.42 Hz), 7.43 (t, 1H, ArH), 7.81-7.83 (d, 1H, ArH, J=7.42Hz), 8.40-8.38 (d, 1H, ArH, J=7.80 Hz), 9.43-9.41 (d, 1H, ArH, J=7.42 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 43.60 (CH<sub>2</sub>); 121.41, 122.90, 127.29, 127.56, 127.76, 128.44, 128.91, 129.00, 129.13, 130.61, 131.22, 132.12, 137.01, 139.05 (C Ar); 163.37 (C=O), 163.62 (C=O).

**24 - 4-[(1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl]benzenesulfonyl chloride.** The compound (**22**) (2.00 g, 7 mmol) was added on chlorosulphonic acid cold (2.76 mL; 42 mmol), in small portion. The resulting mixture was stirred and heated at 60 °C for around 10 min, until the evolution of HCl ceased. The reaction mixture was poured onto ice. The solid was filtered and washed with distilled water. Yield: 93%. m.p. 113.1-115.5 °C. IR (KBr) 1699 and 1655 [ν N(C=O)<sub>2</sub>], 1337 and 1172 (ν -SO<sub>2</sub>), 1235 (ν -CN), 776 (ν Ar.). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 5.24 (s, 2H, CH<sub>2</sub>), 7.30-7.32 (d, 2H, ArH, J=8.01 Hz), 7.51-7.54 (d, 2H, ArH, J=8.20 Hz), 7.85-7.80 (t, 2H, ArH), 8.48-8.46 (d, 2H, ArH, J=8.01 Hz), 8.51-8.49 (d, 2H, ArH, J=8.01 Hz). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>) δ 43.51 (CH<sub>2</sub>); 109.99, 122.60, 126.27, 127.53, 127.97, 131.71, 132.03, 135.27, 138.44 (C Ar); 164.18 (C=O).

**25 - 4-[(6-chloro-1,3-dioxo-1H-benzo[de]isoquinolin-2(3H)-yl)methyl]benzenesulfonyl chloride (**25**).** Chloride (**25**) was obtained as described in the compound (**22**). Yield: 67%. m.p. 136.0-138.2 °C. IR (KBr) 1702 and 1659 [ν N(C=O)<sub>2</sub>], 1343 and 1173 (ν -SO<sub>2</sub>), 1233 (ν -CN), 780 (ν Ar.). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sup>6</sup>) δ 5.16 (s, 2H, CH<sub>2</sub>), 7.32-7.34 (d, 2H, ArH, J=8.20Hz), 7.57-7.55 (d, 2H, ArH, J=8.20 Hz), 7.77-7.81 (m, 2H, ArH), 8.20-8.22 (d, 1H, ArH, J=7.81 Hz), 8.34-8.32 (d, 1H, ArH, J=7.42 Hz), 8.37-8.39 (d, 1H, ArH, J=7.42Hz). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sup>6</sup>) δ 43.47 (CH<sub>2</sub>); 121.58, 122.87, 126.26, 127.86, 128.21, 128.83, 129.09, 130.72, 131.63, 132.35, 137.44, 138.30, 138.43, 147.14 (C Ar); 163.22 (C=O), 163.51 (C=O).

**General procedures for the preparation of sulfonamides (**26-28**):** Two equivalents of amines (pyrrolidine or morpholine) were added dropwise to in a solution of the imides (**23** or **24**) in methanol at around 0°C. After that, the reaction mixture was stirred for about 30 min, at temperature 0°C approximately. The product was filtered through a Büchner funnel and

washed twice with 20 mL of methanol cold. The product was recrystallized in methanol-ethyl acetate (1:2).

**26 - 2-[4-(pyrrolidin-1-ylsulphonyl)benzyl]-1H-benzo[de]isoquinoline-1,3(2H)-dione.**

Sulfonamide (**26**) was obtained as described previously. Yield: 61%. m.p. 207.2-207.8 °C. IR (KBr) 1702 and 1658 [v -N(C=O)<sub>2</sub>], 1331 and 1161 (v SO<sub>2</sub>), 770 (v arom.). Anal. Calcd. for C<sub>23</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>S: C, 65.70; H, 4.79; N, 6.66; S, 7.63. Found: C, 65.44; H, 4.80; N, 6.63; S, 7.61. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.48-8.50 (d, 2H, ArH., J= 7.22 Hz), 8.45-8.46 (d, 2H, ArH, J=7.22 Hz), 7.84-7.88 (t, 2H, ArH), 7.71-7.73 (d, 2H, ArH, J= 8.20 Hz), 7.57-7.59 (d, 2H, ArH, J=8.20 Hz), 5.32 (s, 2H, -CH<sub>2</sub>-), 3.06-3.10 (t, 4H, -CH<sub>2</sub>-N-CH<sub>2</sub>-), 1.54-1.62 (m, 4H, -(CH<sub>2</sub>)<sub>2</sub>-). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 164.23 (C=O), 143.17, 135.61, 135.37, 132.83, 132.03, 131.76, 128.82, 128.20, 127.99, 122.55 (C Ar.); 48.41 (CH<sub>2</sub>-N-CH<sub>2</sub>-); 43.40 (CH<sub>2</sub>-N-ArH), 25.33 (-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-).

**27 - 2-[4-(morpholin-1-ylsulphonyl)benzyl]-1H-benzo[de]isoquinoline-1,3(2H)-dione.**

Sulfonamide (**27**) was obtained as described previously. Yield: 62%. m.p. 221.7-223.3 °C. IR (KBr) 1703 and 1660 [v N(C=O)<sub>2</sub>], 1336 and 1163 (v SO<sub>2</sub>), 1227 and 1104(v -COC-). Anal. Calcd. for C<sub>23</sub>H<sub>20</sub>N<sub>2</sub>O<sub>5</sub>S: C, 63.29; H, 4.62; N, 6.42; S, 7.35. Found: C, 63.04; H, 4.60; N, 6.39; S, 7.37. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.50-8.52 (d, 2H, ArH., J=7.22 Hz), 8.47-8.49 (d, 2H, ArH., J=8.20 Hz), 7.86-7.90 (t, 2H, ArH), 7.65-7.67 (d, 2H, ArH., J= 8.20 Hz), 7.61-7.63 (d, 2H, ArH., J=8.20 Hz), 5.54 (s, 2H, -CH<sub>2</sub>-), 3.57-3.59 (t, 2H, -CH<sub>2</sub>-O-CH<sub>2</sub>-), 2.79-2.82 (t, 4H, -CH<sub>2</sub>-N-CH<sub>2</sub>-). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 164.89, 143.39, 135.10, 132.39, 130.44, 128.77, 127.76, 123.05, 66.75, 46.58, 43.72.

**28 - 2-[4-(morpholin-1-ylsulphonyl)phenylethyl]-1H-benzo[de]isoquinoline-1,3(2H)-dione.**

Sulfonamide (**28**) was obtained as described previously. Yield: 74%. m.p. 171.1-172.7 °C. IR (KBr) 1701 and 1663 [v -N(C=O)<sub>2</sub>], 1341 and 1168 (v SO<sub>2</sub>), 1227 and 1105 (v -COC-). Anal. Calcd. for C<sub>23</sub>H<sub>19</sub>ClN<sub>2</sub>O<sub>5</sub>S: C, 58.66; H, 4.07; N, 5.95; S, 6.81. Found: C, 58.45; H, 4.06; N, 5.94; S, 6.84. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.51-8.48 (m, 2H, ArH.), 8.85-8.33 (d, 1H, ArH, J=7.81Hz), 7.94-7.92 (m, 2H, ArH.), 7.54-7.52 (d, 2H, ArH, J=8.00Hz), 7.32-7.30 (d, 2H, ArH, J=8.00Hz), 5.30 (s, 2H, -CH<sub>2</sub>-), 3.58-3.55 (t, 4H, -CH<sub>2</sub>-O-CH<sub>2</sub>), 2.81-2.78 (t, 4H -CH<sub>2</sub>-N-CH<sub>2</sub>-). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 163.76 (C=O), 163.48 (C=O), 143.47 (C Ar.), 138.44, 133.79, 132.51, 131.78, 130.93, 129.12, 129.07, 128.54, 128.36, 127.65, 126.25, 123.18, 121.88 (C Ar.), 65.91 (CH<sub>2</sub>-O-CH<sub>2</sub>-), 46.50 (CH<sub>2</sub>-N-CH<sub>2</sub>-), 43.57 (CH<sub>2</sub>-N-ArH).

**PtpB: expression and purification [25, 27]**

In order to express recombinant PtpB protein from *M. tuberculosis*, plasmid pET28a-Mt\_PtpB (a kind gift from Dr. Pedro Alzari and Dr. Andrea Villarino - Institut Pasteur Paris) was transformed into *Escherichia coli* BL21(DE3). *E. coli* cells containing the pET28a-Mt\_PtpB

recombinant plasmid were inoculated into 10 ml of Luria-Bertani (LB) broth containing 50 µg/ml kanamycin. Overnight cultures were transferred into 250 ml of the same medium and were grown at 37 °C until an optical density value of 0.8 at 600 nm was reached. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 mM, cultures were further grown overnight at 15 °C. Cells were harvested by centrifugation (6,000g for 30 min at 4 °C) and re-suspended in cold lysis buffer (20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 10 mM imidazole, 10% glycerol) containing protease inhibitor cocktail (Complete, Mini, Boehringer-Mannheim). The cells were disrupted by gentle sonication (6 cycles, 20s) on ice and centrifuged (10,000g for 30 min at 4 °C).

*Mt\_PtpB* carrying the hexahistidine tag (*His-tag*) at the N-terminus was purified under native conditions by chelating sepharose fast flow affinity chromatography, following the supplier's instructions (Amersham Biosciences, Uppsala, Sweden). The hexahistidine tag *Mt\_PtpB* was eluted with 250 mM imidazole. The purity of the protein preparations was assessed by SDS-PAGE in 16% acrylamide slab gels, under reducing conditions. Fractions containing purified recombinant protein were pooled and dialyzed at 4°C in four steps for 2 h against the elution buffer with decreasing concentrations of imidazole (150 mM, 75 mM, 35 mM and without imidazole). The last dialysis buffer contained 20 mM Tris-HCl pH 8.0, 50 mM NaCl, 5 mM EDTA, 20% glycerol, 5 mM DTT. Then, proteins were concentrated by ultrafiltration with a 10 kDa pore membrane (Amicon Ultra-15 Millipore) and stored at -20°C. The protein content was determined using the method of Bradford [33] with bovine serum albumin as standard.

### Measurement of phosphatase activity

Phosphatase activity assays were carried out in 96-well plates containing 25 µM of diluted compound or solvent (100% DMSO), 20 mM imidazol [pH 7.0], 40 mM *p*-nitrophenylphosphate [pNPP], and MilliQ water, in a final volume of 200 µl, in each well. After dilution in on equal volume of PtpB buffer (20 mM Tris-HCl [pH 8.0], 50 mM NaCl, 5 mM EDTA, 20% glycerol and 5 mM dithiothreitol), 2 µl of recombinant PtpB (0.4 µg/µl) was added in order to initiate the reaction. The absorbance was measured for 10 min, at 37°C (410 nm with readings every 1 minute) on an ELISA plate spectrophotometer (TECAN). Negative controls were performed in the absence of enzyme, and positive controls were carried out in the presence of enzyme with DMSO 100% instead of compound. The relative activity was calculated by the absorbance difference between positive control and each inhibitor tested (average of two experiments carried out in triplicate). The IC<sub>50</sub> values were determined using at least six inhibitor concentrations, in triplicate in two independent experiments.

### Enzyme kinetics

To determine the type of inhibition of phosphatase activity promoted by inhibitors, the **14**, **15**, **19** and **21** inhibitors were selected and screened for each concentration of pNPP (0.5, 1.5, 3, 6, 8, 10, 15, 20 and 25mM), at the same conditions described in "Measurement of phosphatase activity"

Section (above). All assays were done in triplicate. The *p*-nitrophenol released (*V*) was quantified as described and plotted in a Lineweaver–Burk plot ( $1/V \times 1/[S]$ ) using SigmaPlot Program 9.0. The values of  $K_i$ ,  $V_{max}$ ,  $K_M$  of the compounds were determined from the collected data by linear regression analysis. To estimate the inhibition constants, a range of pNPP concentrations was used for each assay and four or five concentrations of inhibitors, to determine the *p*-nitrophenol released (*V*).  $K_{Mapp}$  values obtained for each compound concentration were plotted versus  $[I]$ , with the intercept of the curve and X-axis corresponding to  $-K_i$ .

### Molecular modeling

The 3D structures of the inhibitors were constructed using standard geometric parameters of the molecular modeling software package SYBYL 8.0. Each single optimized conformation of each molecule in the data set was energetically minimized employing the Tripos force field and the Powell conjugate gradient algorithm [34] with a convergence criterion of 0.05 kcal/mol.Å and Gasteiger–Hückel charges [35].

Molecular docking and scoring protocols as implemented in FlexX [36] (BioSolveIT GmbH, Sankt Augustin - Germany) were used to investigate the possible binding conformations of the ligands within the PtpA binding pocket. The X-ray crystallographic data for PtpB determined at 2 Å (PDB ID 2OZ5) [21] used in the docking simulations were retrieved from the Protein Data Bank (PDB). All the water molecules were removed from the binding pocket for docking simulation. Hydrogen atoms were added in standard geometry using the Biopolymer module implemented in SYBYL 8.0. Histidines, glutamines, and asparagines residues within the binding site were manually checked for possible flipped orientation, protonation, and tautomeric states with Pymol 1.2 (DeLano Scientific, San Carlos, USA) side chain wizard script. The binding site was defined as all the amino acid residues encompassed within a 6 Å radius sphere centered on both the proximal and distal bound inhibitors [21]. The docking procedures were repeated 30 times for each inhibitor. The implemented FlexX scoring function and visual inspection were employed to select the representative conformation for each inhibitor.

### References

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