Supporting Information for

Potent inhibition of protein tyrosine phosphatase 1B by copper complexes: Implication to copper toxicity in biological systems

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S1 Reactants and physical measurements

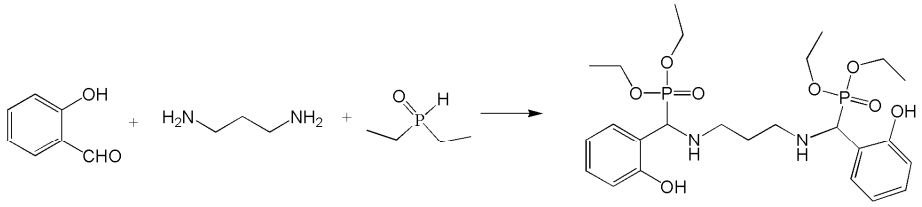
All reagents and solvents were purchased commercially and used as supplied. Double distilled water was used to prepare buffer solutions.

UV/Vis spectra were recorded on a Hewlett-Packard HP-8453 Chemstation spectrophotometer. Fluorescence emission spectra were recorded on a Cary Eclipse Spectrophotometer (Varian, USA). For titration experiments, aliquots of ***1*** (5 μl) were added to PTP1B solution (2 ml, in 20 mM MOPS, 500 mM NaCl, pH 7.2) and the mixture was left to equilibrate at 310 K for 35 min. Electrospray ionization mass spectra (ESI-MS) were recorded on a Quattro Micro API (Water, USA) in methanol aqueous solution (1:1, v:v). Elemental analyses were carried out by a VARI-EL elemental analyzer. Infrared spectra (4000-400 cm^{-1}) were recorded on a Shimadzu FT IR-8300 spectrometer in KBr disks. The X-ray data were collected on a Bruker SMART APEX 1K CCD diffractometer. Bioactivity assays of the complexes were carried out on a Bio-RAD model 550 microplate reader. EPR spectra were obtained on a Bruker-ER 200-D-SRC spectrometer at RT.

S2 Synthesis of ligand (Diethyl (propane-1,3-diylbis(azanediyl))bis((2-hydroxyphenyl) methylene)bis(hydrogen phosphonate)) (Scheme S1).

A 20 ml ethanol solution containing 4.18 ml (0.04 mol) of salicylaldehyde was added drop-wise into a stirred solution of 1.67 ml (0.02 mol) of propane-1,3-diamine in 20 ml of ethanol. The resulting solution was refluxed for 4 h with constant stirring. Then a 20 ml ethanol solution of diethyl phosphonate (0.04 mol, 5.13 ml) was added drop-wise into the solution. The mixture was refluxed for about 32 h. Light yellow solid was precipitated

and washed with ethanol and ether, respectively. The isolated yellow solid was 4.459 g (yield, 43.5%; m.p.: 483-485 K). Element analysis for $C_{21}H_{32}N_2O_8P_2 \cdot H_2O$, Calc: C 49.32, H 6.50, N 5.48, Found C 49.1, H 6.62, N 5.50. IR(cm^{-1}): 3421m, ν(O-H, N-H); 2979m, ν(C-H); 1231s, ν(P=O); 1074, 1048s, ν(P-O-C); 947 s, ν(P-C). UV: ε(220nm) = $7.44\times10^3 M^{-1}cm^{-1}$, ε(280nm) = $4.15\times10^3 M^{-1}cm^{-1}$.



Ligand

Scheme S1

S3 Crystal structure refinements

The X-ray data of crystal ***1*** were collected by ω-scan method on a SMART 1K CCD area detector single-crystal diffractometer that uses graphite monochromated Mo Kα radiation (λ=0.71073 Å). The structure was solved by direct methods and refined by least squares procedures on F^2 using SHELX-97 package. [R1] The space group choice was confirmed by successful convergence of the full-matrix least-squares refinement on F^2. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were generated geometrically or determined from the difference Fourier map and refined isotropically. The water O9 atom and the ethyl groups bonding to the phosphonates are in disorder.

Fourier maps show that there were severe partial occupancies for two ethyl groups and one water molecule. The occupancies of these sites were freely refined with PART instruction. The final occupancies of all disorder atoms from the refinement were C11A

0.643(19), C11B 0.357(19), C12A 0.643(19), C12B 0.357(19); C20A 0.53(2), C21A 0.53(2), C20B 0.47(2), C21B 0.47(2); O9A 0.716(13), O9B 0.284(13), respectively. The ethyl group had still large U_{eq} values. This should be results of severe disordered side-chains (ethyl group). H-atoms except water were included in calculated positions and treated as riding atoms using the SHELXL default parameters. H atoms attached to O(water) were located in difference Fourier maps and their global U_{iso} values were refined.

Structure contains solvent accessible voids of 67.3 $Å^3$/per unit cell, i.e. some 4.7% of the total volume. Data were corrected for disordered electron density through the use of the SQUEEZE procedure. [R2] Attempts to refine peaks of residual electron density as solvent water molecules were unsuccessful. Derived values (formula weight, density, absorption coefficient) do not contain the contribution of the disordered solvent molecule.

ISOR instruction was employed to have ellipsoids of sites (C11A C11B C12A C12B C20A C20B C21A C21B O10 C16) be restraint to more appropriate values. So, 72 restraints were used for refinement anisotropically. 8 restraints were used for refinement to fix disordered ethyl chains binding to O7 and O3.

S4 UV/Vis titration of $CuCl_2$ with amino acids ssays

In order to mimic the situation when copper salts or complexes enter the body where amino acids are at a far higher concentration than that of copper and thus copper-amino acid complexes may be formed in vivo, we studied the PTP1B inhibition abilities of

copper complexes with four amino acids (glysine, histidine, glutamate and arginine). The complexes were prepared by mixing $CuCl_2$ with 20-fold excess of the indivadual amino acid in MOPS buffer(20 mM MOPS, 50 mM NaCl, pH 7.2). The solutions were directly used for PTP1B inhibition assays. In order to confirm the formation of the Cu-amino acid complexes under the conditions applied, UV/Vis titration were performed in the assay buffer. As shown in Fig. S5, all the 4 amino acids readily form copper-amino acid complexes in the form of $Cu(AA)_2$ (Fig. S5).

S5 The binding constants and the stoichiometry calculations

Maximum fluorescence intensities of PTP1B without and with the quencher (F_0 and F, respectively) were determined and F_0/F values were plotted against the quencher complex ***1***. The experimental data were fitted with the Stern–Volmer equation (1): [R3]

$$\frac{F_0}{F} = 1 + k_q \times \tau_0 \times [Q] = 1 + k_{sv} \times [Q] \qquad (1)$$

where F_0 and F are the fluorescence intensities before and after the addition of the quencher, respectively. K_{SV} is the Stern–Volmer quenching constant and [Q] is the concentration of quencher. k_q is the quenching rate constant of the PTP1B and $k_q = K_{SV}/\tau_0$. τ_0 is the average lifetime of the protein without any quencher, and 10^{-8} s is used as the fluorescence lifetime for proteins is ~ 10^{-8} s.[R4] The binding constants and the stoichiometry between PTP1B and quencher (complex ***1***) were calculated from the static quenching equation (2) by plotting lg ($(F_0-F)/F$) vs lg [Q]:[R5]

$$\lg\frac{F_0 - F}{F} = \lg K + n\lg[Q] \qquad (2)$$

Where K is the binding constant and n is the number of binding sites (stoichiometry).

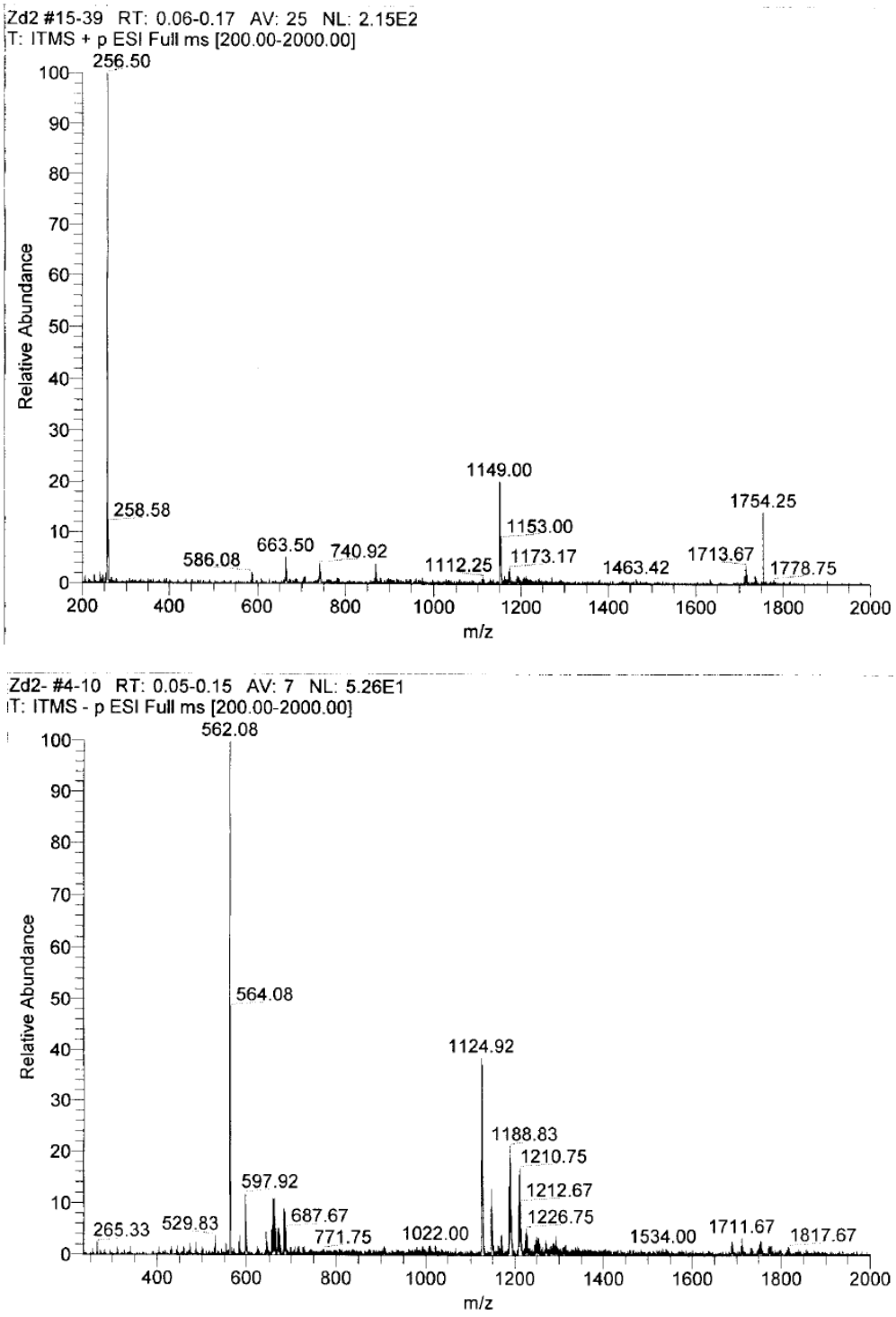


Fig. S1. ESI-MS spectra of the ***1*** in methanol aqueous solution (1:1, v:v). (Top) positive ion mode; (Bottom) negative ion mode. The species with m/z 256.50 observed in the positive ion mode may be assigned to $[\boldsymbol{1}+2CH_3OH+H^+ +4Na^+]^{5+}$ (m/z = 1283.17/5 = 256.63); the species with m/z 1754.25 may be assigned to $\{[\boldsymbol{1}]_3+4CH_3OH+2H^+\}^{2+}$ (m/z= 3508.58/2= 1754.29)..

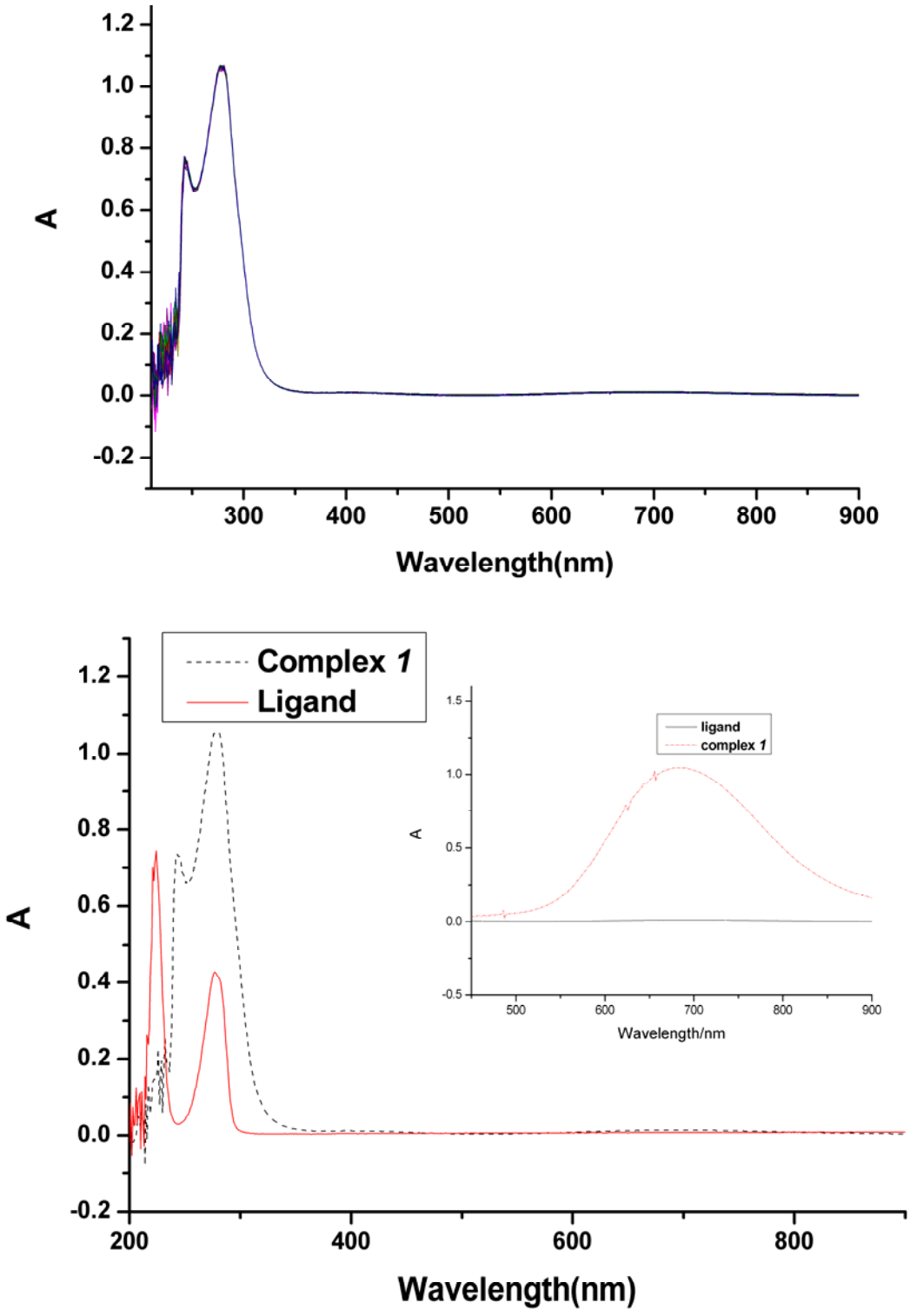


Fig.S2. UV–vis spectra of ***1*** (concentration, 10^{-4} M) recorded at 310 K in 20 mM MOPS buffer (pH=7.2) over 2 h with 5 min intervals (top); absorption spectra of the ligand and complex ***1*** in concentration of 10^{-4} M (inset 10^{-2} M) in DMSO (bottom).

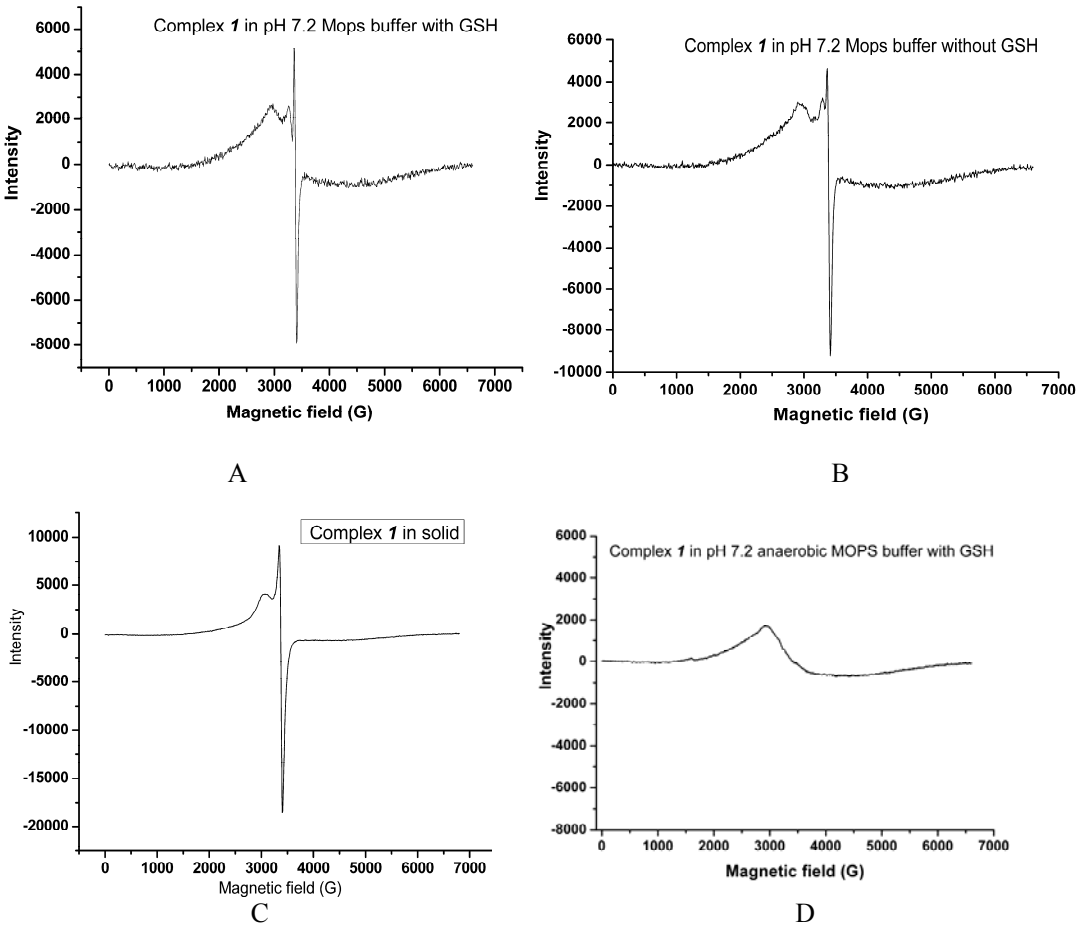


Fig. S3 EPR spectra at room temperature of complex ***1***. **(A)** in MOPS assay buffer (pH 7.2) in the presence of 2 mM GSH; (B) in MOPS buffer in the absence of GSH; (C) in solid state and (D) in anaerobic MOPS buffer with GSH.

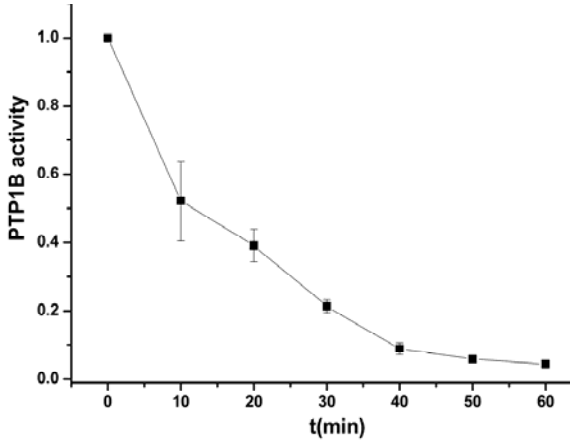


Fig. S4. Relative PTP1B activity after incubation with ***1*** (10^{-6} M) for different time (■, [PTP1B]=120 nM.

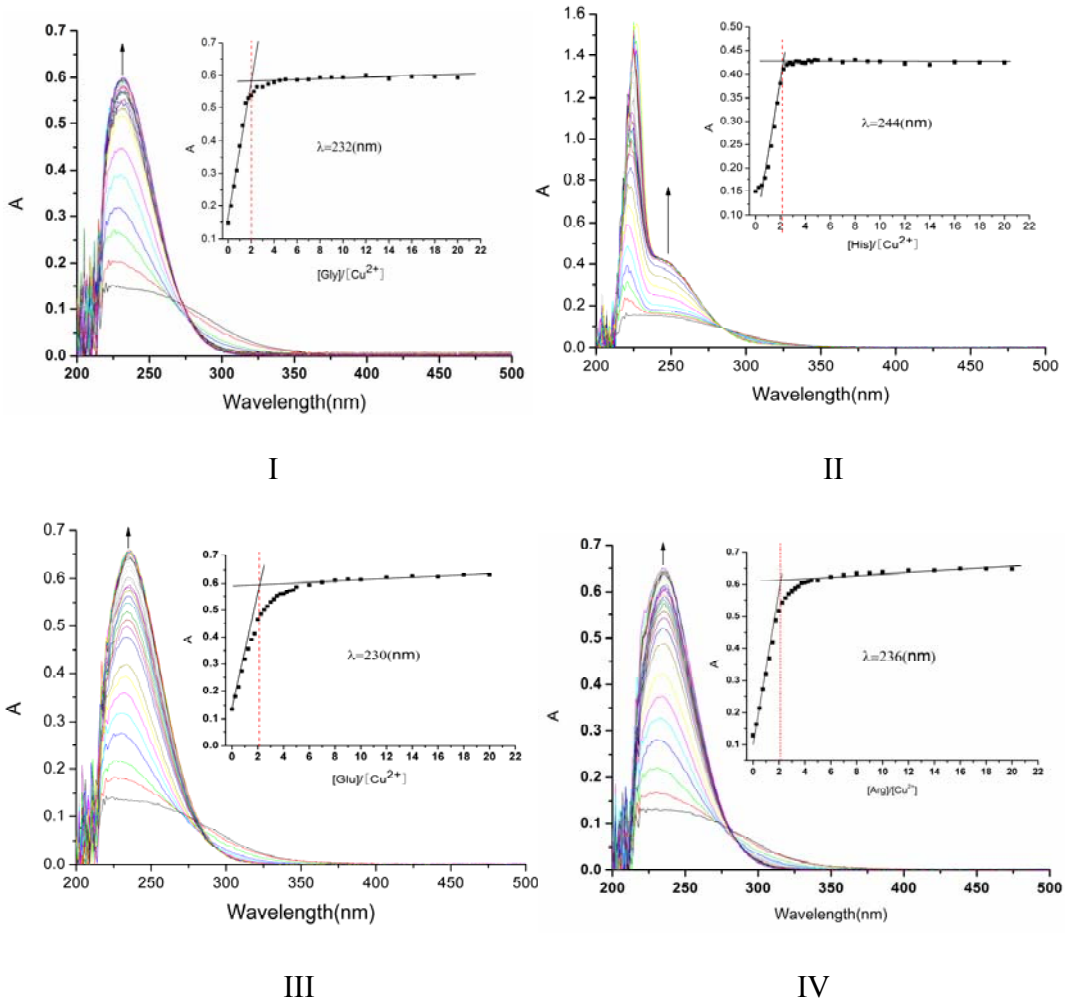


Fig. S5 UV/Vis spectra of titrations of $CuCl_2$ (2 ml, 10^{-4} M) with the 4 amino acids (0.1 M) used in the study in MOPS buffer at room temperature. (I). $CuCl_2$-glycine system; (II). $CuCl_2$-histidine system; (III). $CuCl_2$-glutamate system; (IV). $CuCl_2$-arginine system.

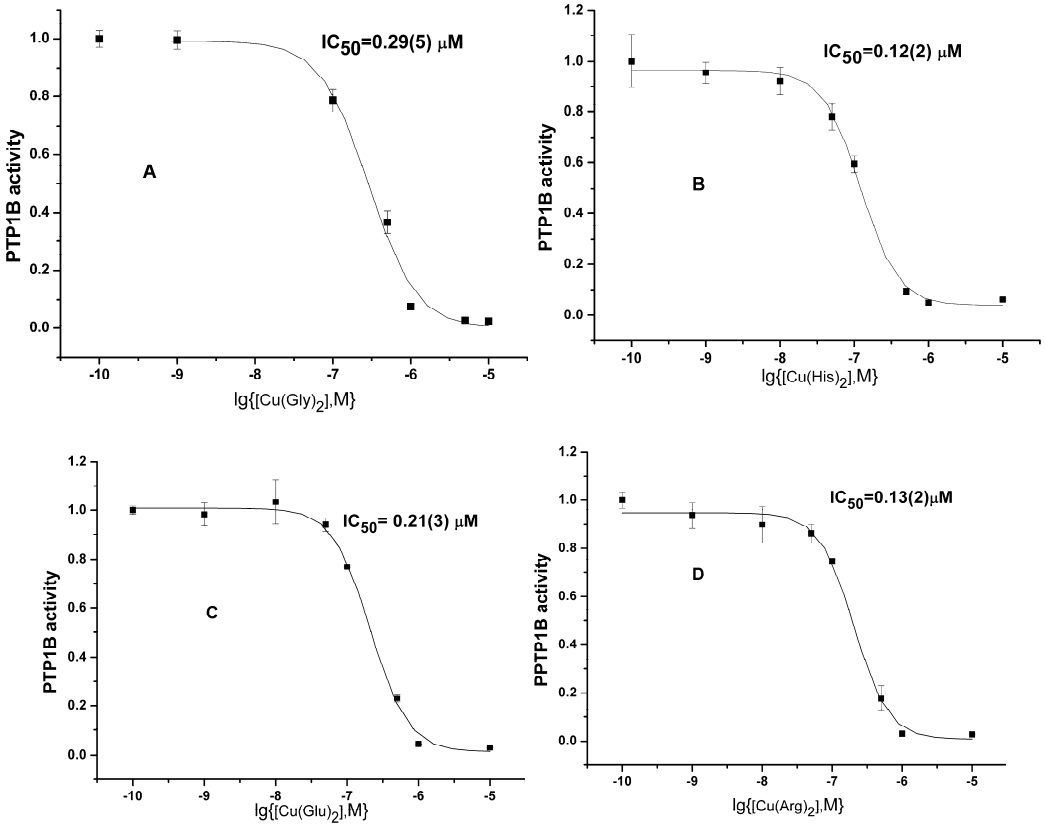
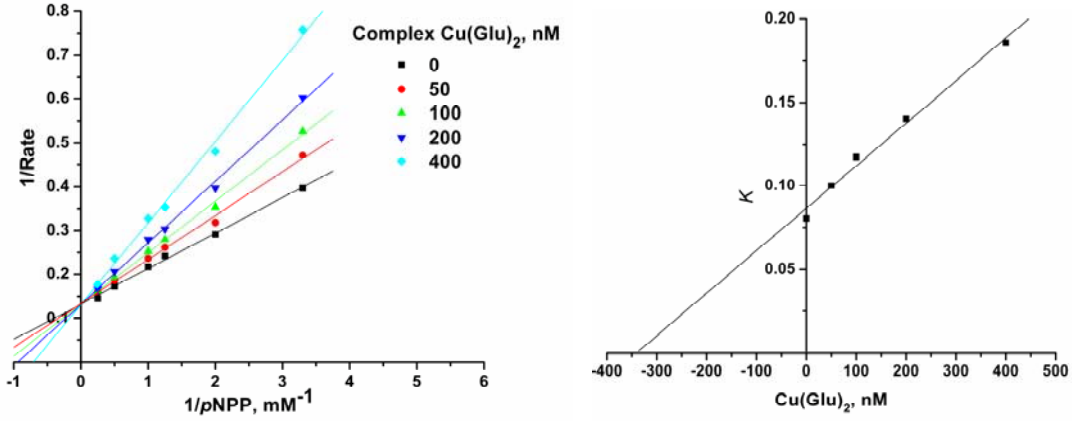


Fig. S6. Concentration-dependent inhibitions of PTP1B by 4 Cu-amino acid complexes with IC_{50} values shown in insets.A, $Cu(Gly)_2$; B, $Cu(His)_2$; C, $Cu(Glu)_2$; D, $Cu(Arg)_2$.



A B

Fig. S7. A) Lineweaver–Burk plot of 1/Rate (min μM^{-1}) versus the reciprocal of the pNPP concentration (mM^{-1}) at five fixed concentrations (nM) of the Cu-Glu_2 complex. (B) The plot of apparent Michaelis constant (*K*) versus the concentrations (nM) of Cu-Glu_2 to determine the inhibition constant K_i.

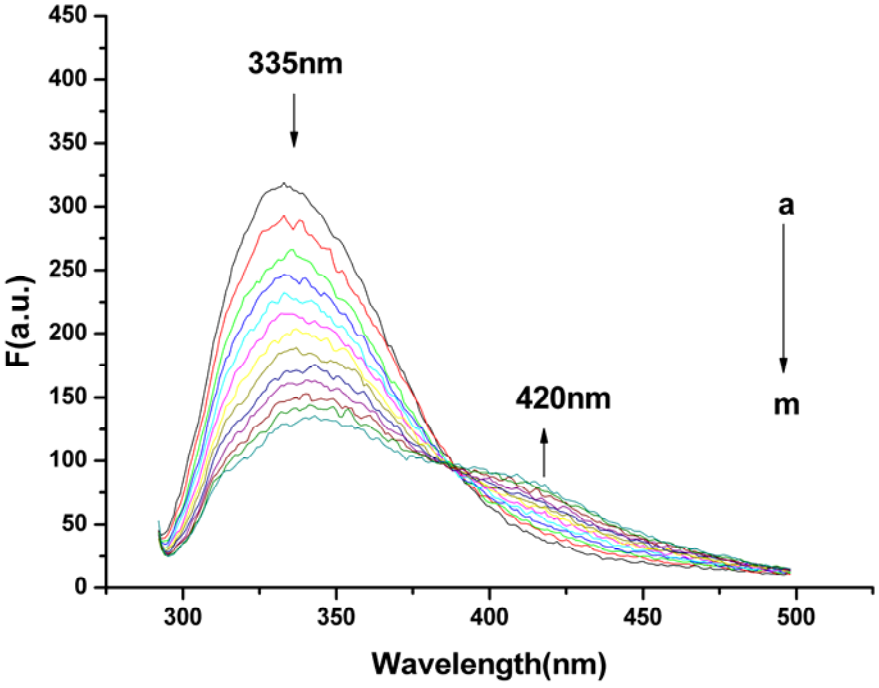


Fig. S8. Fluorescence emission spectra for the titration of PTP1B with ***1***. Experimental conditions:, λ_{ex} = 282 nm, 303K., [PTP1B] = 2.0×10^{-7} mol/L, [***1***]: (a) 0; (b) 0.25; (c) 0.5; (d) 0.75; (e) 1.0; (f) 1.25; (g)1.5; (h) 1.75; (i) 2.0; (j) 2.25; (k) 2.5; (l) 2.75; (m) 3.0 (10^{-7} mol/L).

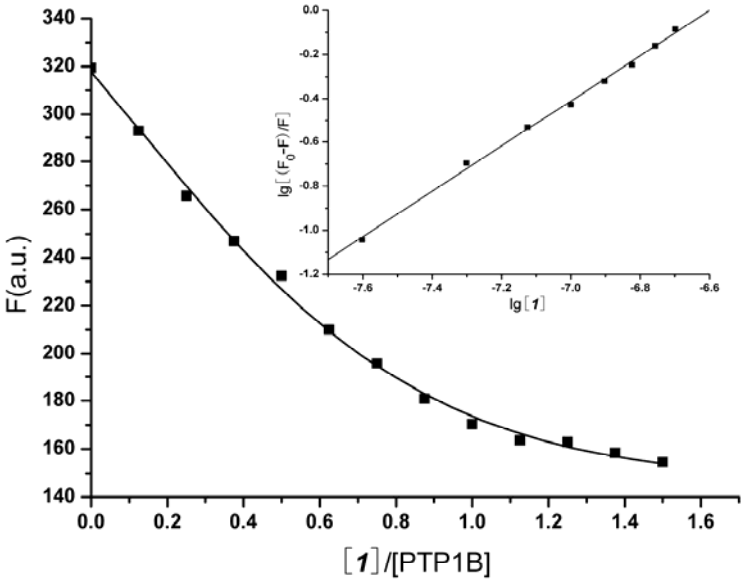


Fig.S9. The fluorescence intensity at 335 nm is plotted against the ratio of [***1***]/[PTP1B], insert presenting the plot for the binding constant (K) and stoichiometry (n) determination at 303 K. The calulations give n=1 and $K_{303}=2.67\times10^{6}$ M^{-1}.

References

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