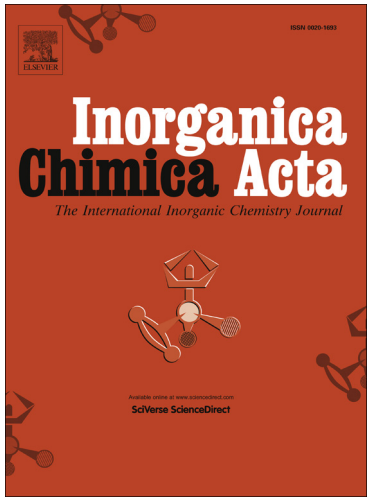

Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases

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PII: S0020-1693(13)00291-0
DOI: http://dx.doi.org/10.1016/j.ica.2013.05.018
Reference: ICA 15473

To appear in: *Inorganica Chimica Acta*

Received Date: 22 February 2013
Revised Date: 9 May 2013
Accepted Date: 16 May 2013

Please cite this article as: R. Zhu, L. Lu, M. Zhu, H. Han, C. Yuan, S. Xing, X. Fu, Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases, *Inorganica Chimica Acta* (2013), doi: http://dx.doi.org/10.1016/j.ica.2013.05.018

Cover Page

Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases

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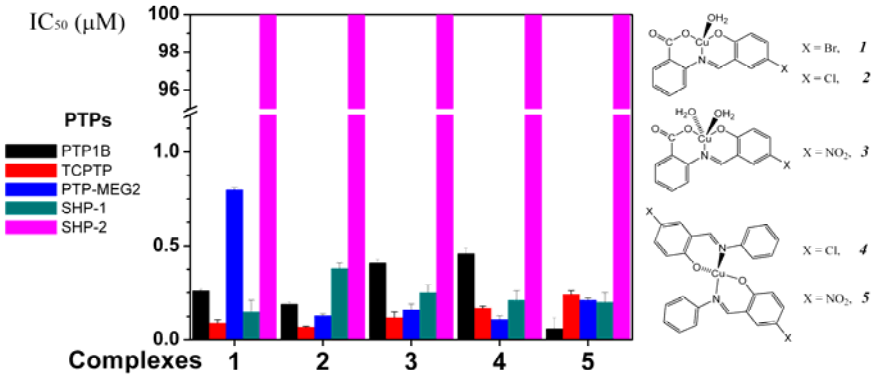
Index Abstract

Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases

Ruiting Zhu, Liping Lu, Miaoli Zhu*, Hong Han, Caixia Yuan, Shu Xing*, Xueqi Fu*



Five copper complexes of Schiff-base condensates from 5-substituted-2-hydroxy-benzaldehyde and 2-substituted- benzenamine were synthesized and characterized. The copper complexes noncompetitively inhibit PTP1B, TCPTP, PTP-MEG2 and SHP-1, but do not inhibit SHP-2. Complex ***5*** exhibits very strong inhibition and better selectivity against PTP1B (IC_{50}=0.059 μM).

Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases

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Keywords: copper complexes, protein tyrosine phosphatases, selective inhibitor, Schiff base.

Abstract

Five copper complexes, [Cu(bhbb,chbb,nhbb)(H_2O)$_n$] (tridentate-ligands: H_2bhbb=2-(5-bromo-2-hydroxylbenzylideneamino)benzoic acid, ***1***; H_2chbb=2-(5-chloro-2-hydroxylbenzylideneamino)benzoic acid, ***2***; H_2nhbb=2-(5-nitro-2-hydroxyl-benzylideneamino)benzoic acid, ***3***) and [Cu(cpmp,npmp)$_2$] (bidentate-ligands: Hcpmp=4-chloro-2-((phenylimino)methyl)phenol, ***4***; Hnpmp=4-bromo-2-((phenyl-imino)methyl)phenol, ***5***) have been prepared and characterized by EA, IR, EPR UV-vis, and ESI-MS. Structure-activity relationship of copper complexes in inhibiting protein tyrosine phosphatases (protein tyrosine phosphatase 1B, PTP1B; T-cell protein tyrosine phosphatase, TCPTP; megakaryocyte protein-tyrosine phosphatase, PTP-MEG2; Src homology phosphatase 1, SHP-1 and Src homology phosphatase 2, SHP-2) is investigated. Inhibitory activities of complexes against the five PTPs indicate that they potently inhibit PTP1B, TCPTP, PTP-MEG2 and SHP-1, but do not inhibit SHP-2. In the complexes, ***5*** exhibits very strong inhibition (IC_{50}, 0.059 μM) and better selectivity against PTP1B while ***1*** and ***2*** show very strong inhibition (IC_{50}=0.089 and 0.067 μM) and a little selectivity against TCPTP. Compared with the oxovanadium(IV) complexes of same ligands, the copper complexes increase the inhibitory ability against TCPTP, PTP-MEG2 and SHP-1 but decrease the inhibition against SHP-2. For complex ***5***, the inhibition over PTP1B, TCPTP, PTP-MEG2 and SHP-1 are all improved about 5~15-fold compared with the oxovanadium(IV) complex. The results demonstrate that both the ligand structures and the center metals influence the inhibition and selectivity against different PTPs.

1. Introduction

Protein tyrosine phosphatases (PTPs) are a large family of signaling enzymes playing a critical role in signal transduction and regulation of cellular processes. Many human diseases such as diabetes, obesity, cancer, and immune disorders are involved in the dysregulation of PTP activities [1-5]. PTPs have therefore emerged as potential therapeutic targets and attracted great interests in both academia and industrial in past decades [6-8]. PTP inhibitors are thus expected as promising targeted therapeutic drugs and dozens of new PTP inhibitors are reported each year [9, 10]. In recent years, one important progress is the investigation of metal-based PTPs inhibitors [11]. Except vanadium complexes that their insulin-sensitizing effects have been demonstrated to be at least partly related to the inhibition of PTPs [12-20], other metal complexes such as Au, Sb, Fe, Ru complexes are reported to potently inhibit PTPs and result in the increase of cellular phosphorylation [21-25].

Copper complexes are widely explored as anticancer agents and expected relatively lower side effects than traditional platinum-based drugs. One of the main mechanisms proposed to explain the anticancer activity of copper complexes is the DNA damage by generating reactive oxygen species[26, 27]. Recent researches indicate copper complexes inhibit many enzymes such as topoisomerase II [28], chymotrypsin-like activity of proteasome[29], urease[30] and so on. Our study demonstrates that copper complexes are potent PTPs inhibitors [31]. The structures of the ligands coordinated to copper influence the inhibitory effects and the selectivity against different PTPs [32-36]. The in vitro screened copper complex exhibits consistent selectivity as in

cells [32], suggesting that in vitro screened metal-based selective PTP inhibitors may work in vivo.

Our previous study shows that copper complexes of Schiff base ligands containing 3,5-substituted-4-salicylideneamino-3,5-dimethyl-1,2,4-triazole, [Cu(RSal-dmta)$_2$], potently inhibit PTPs and exhibit some selectivity against PTP1B. In order to further check the influence of the ligands on PTPs inhibition, a series of copper complexes of Schiff bases condensated from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine have been synthesized and well characterized. Their inhibitory effects against five PTPs are evaluated. The results show these complexes are also potent PTPs inhibitors and one exhibits some selectivity against PTP1B.

2. Experimental

2.1. Materials and physical measurements

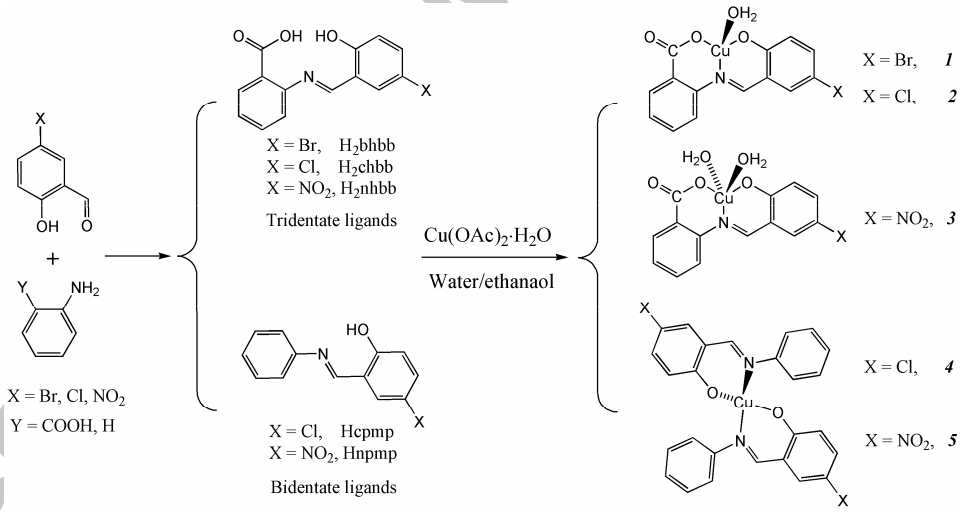
All reagents and solvents were obtained from commercial sources and used without further purification unless specially noted. Double distilled water was used to prepare buffer solutions.

The C, H and N analyses were performed on a VARI-EL elemental analyzer. IR spectra on KBr pellets were recorded on a Shimadzu FT IR-8300 spectrometer in the range of 4000-400 cm^{-1}(KBr disks). The electronic spectra were recorded on a Hewlett-Packard HP-8453 Chemstation spectrophotometer. Electrospray ionization mass spectra (ESI-MS) were recorded with a Quattro Micro API instrument (Waters, USA) in methanol solution. EPR spectrum was obtained in solid and DMSO solution

at 110 K on a Bruker-ER 200-D-SRC spectrometer. Bioactivity assays (IC_{50} values) of the complexes were carried out on a SpectraMax M5 Multi-Mode Microplate Readers (Molecular Devices, USA) as previously described [15-17, 20].

2.2. Synthesis of the complexes

The synthesis of Schiff bases and copper complexes ***1*** - ***5*** was described in Scheme 1. Tridentate and bidentate Schiff base ligands were synthesized from an equimolar mixture of 5-X-salicylaldehyde (X = Cl, Br and NO_2) and anthranilic acid or aniline using previously reported procedures [19, 37], respectively. Next, the object compouds were synthesized by the Schiff bases reacting with $Cu(OAc)_2 H_2O$ in water/ethanol solution.



Scheme 1. Schematic routes of the synthesis of the ligands and complexes ***1-5***

2.2.1. 2-(5-bromo-2-hydroxylbenzylidene-amino)benzoic acid (H_2bhbb) and [Cu(bhbb)(H_2O)] (***1***)

H_2bhbb: a 20.0 mmol of 5-bromosalicylaldehyde was thoroughly dissolved in 20 mL of absolute ethanol with a constant stirring. To it 20.0 mmol of anthranilic acid in 20 mL of absolute ethanol was added dropwise. The reaction mixture was heated under refluxing for 3 h. After cooling slowly, the yellow precipitates were separated out. The separated compound was filtered, washed thoroughly with absolute ethanol, and then dried in a vacuum desiccator with P_2O_5. Yield 74%, element analysis for H_2bhbb ($C_{14}H_{10}BrNO_3$): Calcd. (%) C 52.52, H 3.15, N 4.38; found (%) C 52.59, H 3.11, N 4.04; IR (υ/cm^{-1}, s = strong, m = medium, w = weak): $\nu_{C=O}$ 1620s, $\nu_{C=N}$ 1604s, ν_{C-O} 1226w.

[Cu(bhbb)(H_2O)]: a 2.0 mmol of H_2bhbb was thoroughly dissolved in 15 mL of absolute ethanol with a constant stirring. To it 0.399 g (2.0 mmol) of $Cu(OAc)_2$ H_2O in 10 mL of water was added dropwise and the pH of the reaction solution was adjusted to neutral by use of 0.1 M NaOH solution. The reaction mixture was heated under refluxing for 4 h. After cooling slowly, the green precipitates were separated out. The separated compound was filtered, washed thoroughly with absolute ethanol and water, and then dried in a vacuum desiccator with P_2O_5. Yield 83%, element analysis for ***1***($C_{14}H_{10}BrCuNO_4$): Calcd. (%) C 42.07, H 2.52, N 3.50; found (%) C 42.11, H 2.24, N 3.39; IR: $\nu_{C=O}$ 1597s, $_{C=N}$ 1564s, ν_{C-O} 1329m; EPR: g = 2.126; ESI-MS(m/z, a positive mode): observed molecular ion peak in methanol 414.42 for [***1***-H_2O+CH_3OH+H]$^+$ (Calcd. 414.72); UV-Vis(DMSO): $_{max}$/nm (ε 10^4/M^{-1}cm^{-1}), 264 (1.926), 420 (0.788).

2.2.2. 2-(5-chloro-2-hydroxylbenzyl-idene-amino)benzoic acid (H_2chbb) and [Cu(chbb)(H_2O)] (***2***)

H_2chbb: following the same procedures as described in synthesis of H_2bhbb, received orange H_2chbb with 5-chlorosalicylaldehyde instead of 5-bromo-salicylaldehyde. Yield 80%, element analysis for H_2chbb ($C_{14}H_{10}ClNO_3$): Calcd. (%) C 60.99, H 3.66, N 5.08; found (%) C 61.039, H 3.60, N 4.69; IR: $\nu_{C=O}$ 1620s, $\nu_{C=N}$ 1604s, ν_{C-O} 1228w.

[Cu(chbb)(H_2O)]: following the same procedures as described in synthesis of ***1***, received ***2*** with H_2chbb instead of H_2bhbb. Yield 82%, element analysis for ***2***($C_{14}H_{10}ClCuNO_4$), Calcd. (%) C 47.34, H 2.84, N 3.94; found (%) C 47.28, H 3.12, N 3.90; IR(cm^{-1}): $\nu_{C=O}$ 1520s, $_{C=N}$ 1597s, ν_{C-O} 1314w; EPR: g = 2.126; ESI-MS(m/z, a positive mode): observed molecular ion peak in methanol 436.75 for [***2***+H_2O+2CH_3OH+H]$^+$ (Calcd. 437.03); UV-Vis(DMSO): $_{max}$/nm (ε $10^4/M^{-1}cm^{-1}$), 263 (1.857), 421 (0.778).

2.2.3. 2-(5-nitro-2-hydroxylbenzylidene-amino)benzoic acid (H_2nhbb) and [Cu(nhbb)(H_2O)$_2$] (***3***)

H_2nhbb: following the same procedures as described in synthesis of H_2bhbb, received orange-yellow H_2chbb with 5-nitrosalicylaldehyde instead of 5-bromosalicylaldehyde. Yield 89%, element analysis for H_2nhbb ($C_{14}H_{10}N_2O_5$): Calcd. (%) C 58.74, H 3.52, N 9.79; found (%) C 58.17, H 3.49, N 9.32; IR: $\nu_{C=O}$ 1641s, $\nu_{C=N}$ 1611s, ν_{C-O} 1244m.

[$Cu(nhbb)(H_2O)_2$]: following the same procedures as described in synthesis of ***1***, received ***3*** with H_2nhbb instead of H_2bhbb. Yield 80%, Element analysis for ***3***($C_{14}H_{12}CuN_2O_7$): Calcd. (%) C 43.81, H 3.15, N 7.30; found (%) C 44.02, H 2.92, N 7.39; IR (υ/cm^{-1}): $\nu_{C=O}$ 1597s, $\nu_{C=N}$ 1562s, ν_{C-O} 1336s; EPR: g = 2.125; ESI-MS(m/z, a positive mode): observed molecular ion peak in methanol 384.67 for $[\boldsymbol{3}+H]^+$ (Calcd. 384.81); UV-Vis(DMSO): $_{max}$/nm (ε $10^4/M^{-1}cm^{-1}$), 270 (1.798), 387 (2.094).

2.2.4. 4-chloro-2-((phenylimino)methyl)phenol (Hcpmp) and [$Cu(cpmp)_2$] (***4***)

Hcpmp: a 20.0 mmol of 5-chlorosalicylaldehyde was thoroughly dissolved in 30 mL of absolute ethanol with a constant stirring. To it 20.0 mmol of aniline in 20 mL of absolute ethanol was added dropwise. The reaction mixture was heated under refluxing for 3 h. After cooling slowly, the orange-yellow precipitates were separated out. The separated compound was filtered, washed thoroughly with absolute ethanol, and then dried in a vacuum desiccator with P_2O_5. Yield 82%, element analysis for Hcpmp ($C_{13}H_{10}ClNO$): Calcd. (%) C 67.39, H 4.35, N 6.05; found (%) C 67.54, H 4.35, N 5.82; IR: $\nu_{C=N}$ 1614s, ν_{C-O} 1276s.

[$Cu(cpmp)_2$]: a 2.0 mmol of Hcpmp in 15 mL of absolute ethanol was heated under refluxing until thoroughly dissolved and 0.399 g (2.0 mmol) of $Cu(OAc)_2$ H_2O in 10 mL of water was added dropwise with a constant stirring. The reaction mixture was adjusted to neutral pH with NaOH solution, and then it heated under refluxing for 4 h. After cooling slowly, the brown-yellow precipitates were separated out. The separated compound was filtered, washed thoroughly with absolute ethanol and water, and then

dried in a vacuum desiccator with P_2O_5. Yield 38%, Element analysis for ***4***($C_{26}H_{18}Cl_2CuN_2O_2$), Calcd. (%) C 58.60, H 3.44, N 5.26; found (%): C 59.49, H 3.46, N 5.34; IR: $\nu_{C=N}$ 1557s, ν_{C-O} 1333s; EPR: g = 2.085; ESI-MS (m/z, a positive mode): observed molecular ion peak in methanol 546.17 for $[\boldsymbol{4}+Na]^+$ (Calcd. 545.99); UV-Vis (DMSO): $_{max}$/nm (ε $10^4/M^{-1}cm^{-1}$), 265 (2.556), 390 (1.181).

2.2.5. 4-nitro-2-((phenylimino)methyl)phenol (Hnpmp) and $[Cu(npmp)_2]$ (***5***)

Hnpmp: following the same procedures as described in synthesis of Hcpmp, received orange-yellow Hnpmp with 5-nitrosalicylaldehyde instead of 5-chlorosalicylaldehyde. Yield 91%, element analysis for Hnpmp ($C_{13}H_{10}N_2O_3$): Calcd. (%) C 64.46, H 4.16, N 11.56; found (%) C 64.45, H 4.10, N 11.14; IR: $\nu_{C=N}$ 1620s, ν_{C-O} 1288w.

$[Cu(npmp)_2]$: Following the same procedures as described in synthesis of ***4***, received ***5*** with Hnpmp instead of Hcpmp, yield 54%, element analysis for ***5***($C_{26}H_{18}CuN_4O_6$ $4.5H_2O$), Calcd. (%) C 49.80, H 4.34, N 8.93; found (%): C 49.86, H 3.76, N 8.9; IR (υ/cm^{-1}): $\nu_{C=N}$ 1555s, ν_{C-O} 1336s; EPR: g = 2.091; ESI-MS (m/z, a positive mode): observed molecular ion peak in methanol 578.83 for $[\boldsymbol{5}+CH_3OH+H]^+$ (Calcd. 579.04); UV-Vis(DMSO): $_{max}$/nm (ε $10^4/M^{-1}cm^{-1}$), 264 (1.758), 375 (2.986).

2.3. Protein tyrosine phosphatase inhibition assays

Human PTPs were expressed and purified as described previously [15, 33, 38-40]. PTP activities were measured by use of *p*NPP as the substrate. The assays were

performed in 20 mM MOPS buffer (pH 7.2, 50 mM NaCl). The complexes ***1*** **-** ***5*** were dissolved in DMSO ($1.0\ 10^{-2}$ M), and diluted to various concentration gradients, and further diluted 10 times into enzyme-MOPS buffer solutions for activity evaluations. Inhibition assays were performed in the same buffer on a 96-well plate in 100 μl volumes. Namely, 10 μl of complex with various concentrations were mixed to 83 μl enzyme solution for 30 min. Then 2 μl of *p*NPP (0.1 M) substrate was added to initiate enzyme reactions. After incubation for 30 min at room temperature, the reactions were terminated by the addition of 5 μl of 2 M NaOH. The optical density at 405 nm was measured on a microplate reader. IC_{50} values were obtained by fitting the concentration-dependent inhibition curves using the Origin program. All data points were carried out in triplicates. Solutions of the copper complexes were all freshly prepared before each experiment.

The inhibiting kinetic analysis was performed according to Eq. (1) for noncompetitive inhibition mode, where $V_{\max}$ is the maximum initial velocity, K_m for the corresponding Michaelis-Menten constant, S for the substrate, I for the inhibitor, K_i for the inhibition constant at varied substrate concentrations, derived from the slope of the Lineweaver-Burk plots.

$$\frac{1}{v} = \frac{K_m}{V_{\max}}\left(1+\frac{[I]}{K_i}\right)\frac{1}{S} + \frac{1}{V_{\max}}\left(1+\frac{[I]}{K_i}\right) \qquad (1)$$

Inhibition constants were determined by measuring initial hydrolysis rates at different concentrations of substrate and inhibitor. The apparent K_{app} values measured

at the various inhibitor concentrations were plotted against concentration of the inhibitor to calculate the K_i values.

Phosphatase inhibition of cell extracts was carried out as described in reference [36]. C6 rat glioma cells were harvested after growing in DMEM medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin and 100 lg/ml of streptomycin for 2 days. After washed with 0.85% NaCl (pH 7.4), cells were lysed by sonication in 20 mM MOPS, 50 mM NaCl, pH 7.2, 0.002% PMSF. Cell lysates were cleared by centrifugation at 15,000g for 30 min. The protein concentration of the cell lysates was estimated using the Bradford Method. Next, 82 µl of cell lysates with protein concentration of 1.55 mg/ml were treated with 30 µM complexes ***1-5*** for 1 h at 310 K. Then, 2µl of pNPP (0.1 M) substrate was added. After incubation for 30 min at 310 K, the reactions were terminated by the addition of 6 µl of 2 M NaOH. The A_{405} was measured on a microplate reader.

3. Results and Discussion

3.1. Synthesis and general aspects

The copper complexes are prepared from a typical synthetic procedure, in which $Cu(OAc)_2\,H_2O$ is reacted with dianionic or monoanionic Schiff bases in ethanol aqueous as shown in Scheme 1. The complexes are soluble in DMSO and DMF, slightly soluble in methanol, and almost insoluble in water as well. The compositions of the complexes deduced from the elemental analysis were confirmed by ESI-MS study (see section experimental). In the infrared spectra, the strong bands at ca. 1555 ~ 1597 cm^{-1}, assignable to the C=N stretching frequency, shifted 7 ~ 57 cm^{-1} and

clearly indicates that C=N was coordinated to copper for these complexes. Crystal structure of complex ***4*** had been determined by the single crystal X-ray diffraction method with monoclinic, space group $P_{2_1/n}$ and cell parameters: a = 13.142(7), b = 9.7950(9), c = 9.7028(15) Å; = 110.923(2)°, which agreed with a reported result [41], CCDC refcode YIDFEW in CSD [42]. It revealed the structure of ***4*** was mononuclear and had perfectly planar *trans*-tetra-coordinated $[CuO_2N_2]$. Unfortunately, we failed to get better data of X-ray single crystal diffracting for the other four complexes. From CSD [42], similar crystal structures were disclosed, such as [Cu(hmbb)(H_2O)] (CCDC refcode NUVBOV, H_2hmbb = 2-(2-hydroxy-3-methoxy-benzylideneamino)benzoic acid) [43], [Cu(hbb)(H_2O)$_2$] (CCDC refcode WEHWEM, H_2hbb = 2-(2- hydroxylbenzylideneamino)benzoic acid) [44], which revealed that the molecular structures in ***1*** & ***2*** or ***3*** with tridentate ligands had distorting planar tetra-coordinated $[CuO_3N]$ or square-pyramidal coordinated geometries $[CuO_4N]$. Complexes ***1*** - ***5*** have also been characterized by electron paramagnetic resonance (EPR). The g_{ev} values of five complexes were in range of 2.085 ~ 2.126 (see section experimental), showing that all copper cations had d^9 electron configuration.

In order to explore the stoichiometries of the ligand bonding to metal cation in aqueous solution, the analysis of UV-Vis titration is employed. Here we take H_2bhbb and Hnpmp as representatives of tridentate and bidentate ligands. UV-Vis titration of complexes ***1*** and ***5*** are performed in 10% DMSO aqueous solution. As shown in Figure 1, ligands H_2bhbb or Hnpmp and Cu^{2+} ion easily form the stable complexes [Cu(bhbb)] and [Cu(npmp)$_2$], respectively, in which the ratio of tridentate and

bidentate Schiff bases binding to Cu^{2+} is 1 1 and 2:1. The results agree with those deduced from the elemental analysis and ESI-MS studies of complexes ***1*** and ***5***.

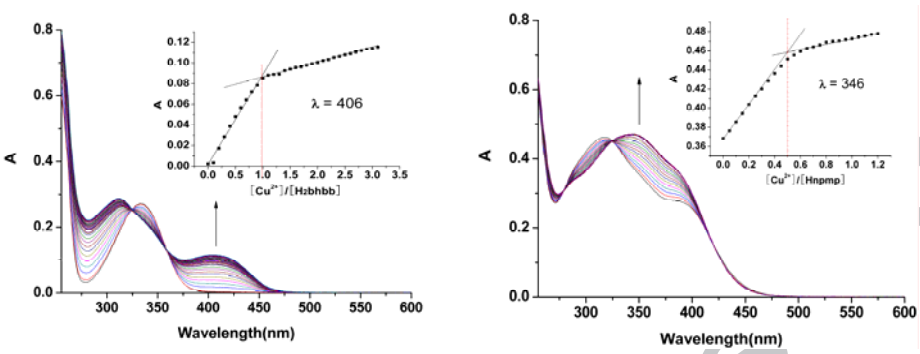


Figure 1 UV-Vis spectra of titrations of H_2bhbb (left) and Hnpmp (right) (2ml, 5×10^{-5}M) with Cu^{2+} ion (5×10^{-3}M) used in the study in 10% DMSO aqueous solution at room temperature.

The stabilities of the five complexes in PTP activity assay buffer are further investigated. The results are shown in Figure 2 with complexes ***1*** and ***5*** as representatives. The UV-Vis spectra are almost not changed in 2 h when the complexes are added to the buffer, suggesting the complexes are stable in the solution.

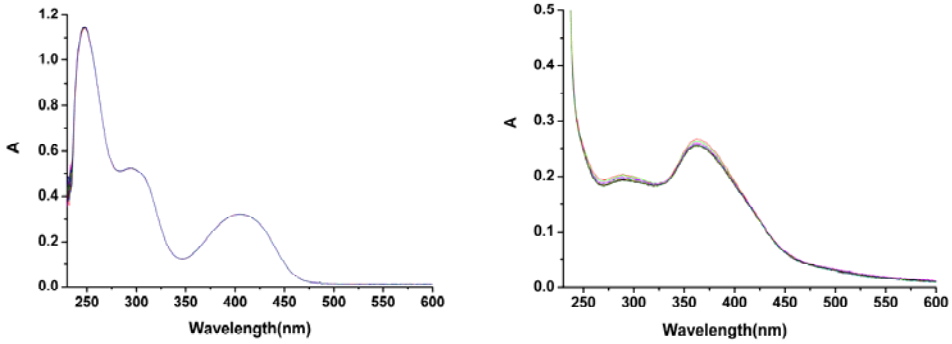


Figure 2 UV-Vis spectra of complexes ***1*** (left) (5×10^{-5}M) and ***5*** (right) (1×10^{-5}M) in PTP activity assay buffer in 2 h at room temperature. UV-Vis spectra are recorded every five minutes.

3.2. Inhibition of PTPs

The five ligands and complexes as well as $CuCl_2$ were tested for their abilities in inhibiting PTP1B, TCPTP, PTP-MEG2, SHP-1 and SHP-2 by use of *p*NPP as the

substrate. The IC_{50} values for the complexes and $CuCl_2$ inhibiting the five PTPs and the comparison with other complexes are listed in Table 1. The results tell us that almost all the five complexes and $CuCl_2$ display strong inhibition against PTP1B, TCPTP, PTP-MEG2 and SHP-1, but do not inhibit SHP-2, similar to Schiff bases copper complexes of [Cu(RSal-dmta)$_2$][33]. However, the ligands do not inhibit any PTP (the data not shown here). Obviously, $CuCl_2$ inhibits PTP1B, TCPTP, PTP-MEG2 and SHP-1 with similar potency. However, the five copper complexes exhibit some changes in the inhibition. In the three complexes possessing ligand/Cu^{2+} ratio of 1:1, ***1*** and ***2*** exhibit strongest inhibition against TCPTP with IC_{50} of 0.089 and 0.067 M, about 2 ~ 10-fold stronger than against SHP-1, PTP1B and PTP-MEG2 while ***3*** have not obvious selectivity over the four PTPs, suggesting substitute nitro group for chloride or bromine slightly affect inhibition against different PTPs. The selectivity of ***1*** and ***2*** against TCPTP is obviously different from [Cu(RSal-dmta)$_2$] complexes which either inhibit PTPs in ligand/Cu^{2+} ratio of 1:1 but have selectivity against PTP1B over other four PTPs, demonstrating substitute anthranilic acid for 3,5-dimethyl-1,2,4-triazole changes the selectivity. For the complexes with ligand/Cu^{2+} ratio of 2 1, ***5*** exhibits about 4-fold stronger inhibition against PTP1B (IC_{50}, 0.059 M) than against TCPTP, PTP-MEG2 and SHP-1(IC_{50}, 0.21 ~ 0.24 M), displaying very strong inhibition and better selectivity against PTP1B, similar to [Cu(RSal-dmta)$_2$] complexes. This similarity seems show that substitute anthranilic acid for 3,5-dimethyl-1,2,4-triazole do not obviously influence the inhibitory potency and selectivity in these bidentate Schiff base ligands. However, complex ***4*** with only a

substitute chloride for nitro group displays weaker inhibition against 1B than other three PTPs. All the results illustrate that the structures of ligands influence the inhibitory effects and selectivity of copper complexes over different PTPs but the influence is complicated. To screen potent and selective copper-based PTPs inhibitors against specific PTP by properly modification of the organic ligand moieties is a challenge.

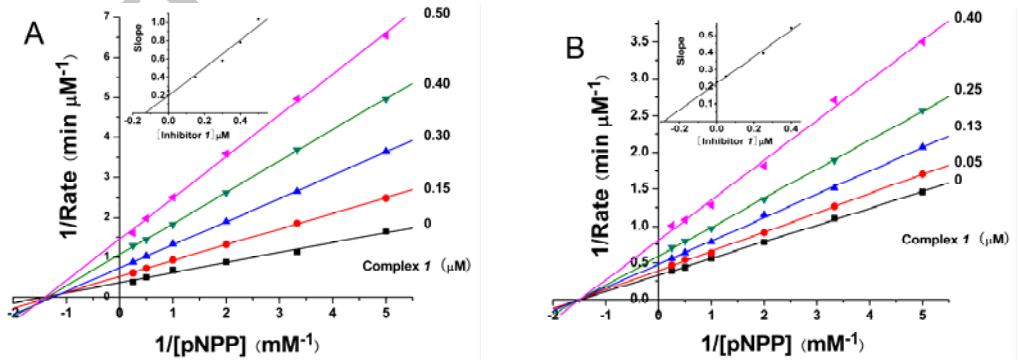
Our previous researches indicate that oxovanadium(IV) complexes which have same ligands with these copper complexes (Table 1) show better selectivity to PTP1B [19]. Comparing the copper complexes with the oxovanadium(IV) complexes with same ligands, it is not difficult to find that the copper complexes increase the inhibitory ability against TCPTP, PTP-MEG2 and SHP-1 but decrease the inhibition against SHP-2. For complex ***5***, the inhibition over PTP1B, TCPTP, PTP-MEG2 and SHP-1 are all improved about 5 ~ 15-fold compared with the oxovanadium(IV) complexes with same ligands. The results suggest the metal ions of coordination sphere influence the inhibition of PTPs. Thus, both the structures of ligands and the metal ions of metal complexes influence the inhibition against different PTPs and the selectivity.

Table 1 IC_{50}(S.D.)(μM) of metal complexes on five PTPs.

Compoud	PTP1B	TCPTP	PTP-MEG2	SHP-1	SHP-2	Ref
[Cu(bhbb)(H_2O)], ***1***	0.26(1)	0.089(19)	0.80(1)	0.15(6)	>100	This work
[Cu(chbb)(H_2O)], ***2***	0.19(1)	0.067(5)	0.13(1)	0.38(3)	>100	This work
[Cu(nhbb)(H_2O)$_2$], ***3***	0.41(2)	0.12(3)	0.16(3)	0.25(4)	>100	This work
[Cu(cpmp)$_2$], ***4***	0.46(3)	0.17(1)	0.11(2)	0.21(5)	>100	This work
[Cu(npmp)$_2$], ***5***	0.059(5)	0.24(2)	0.21(1)	0.20(3)	>100	This work
$CuCl_2$	0.14	0.15	0.20	0.18	>1000	This work
[VO(bhbb)(H_2O)$_2$]	0.21(2)	0.19(3)	0.97(6)	3.0(3)	49(9)	[19]
[VO(nhbb)(H_2O)$_2$]	0.23(1)	1.3(1)	0.60(9)	1.9(2)	4.2(4)	[19]
[VO(cpmp)$_2$]	0.80(9)	8.3(7)	3.6(4)	1.0(2)	2.3(6)	[19]

$[VO(bpmp)_2]$	0.69(3)	1.9(3)	2.9(4)	6.5(3)	35(3)	[19]
$[VO(npmp)_2]$	0.93(9)	1.4(1)	3.2(6)	2.5(5)	2.6(2)	[19]
$[Cu(RSal\text{-}dmta)_2]$	0.038(5) - 0.091(6)	0.069(9) - 0.23(5)	0.12(8) - 0.26(4)	0.27(8) - 0.50(5)	>100	[33]

Complex ***1*** as a representative of five complexes was chosen to further investigate the inhibition mode of the four PTPs. As shown in Figure 3, for PTP1B, TCPTP, PTP-MEG2 and SHP-1, the Lineweaver–Burk double-reciprocal plot of the kinetics data of complex ***1*** all show the lines converged at an intersection on the x-axis left the y-axis, implying a noncompetitive inhibition mode versus *p*NPP. The apparent inhibition constants (K_i) for PTP1B, TCPTP, PTP-MEG2 and SHP-1 are calculated to be 0.13, 0.28, 0.22 and 0.46 M, respectively (Figure 2, inset). The noncompetitive inhibition modes of complex ***1*** agree with the copper complexes of $[Cu_2(phen)_2(ida)(NO_3)]NO_3$ which exhibits obvious selectivity against TCPTP[32], but differ from copper complexes of $[Cu(RSal\text{-}dmta)_2]$ which exhibit competitive inhibition modes and selectivity against PTP1B[33], suggesting the binding sites of copper complexes bonding to PTPs will be changed with the structural changes of copper complexes, and may be related to their selectivity.



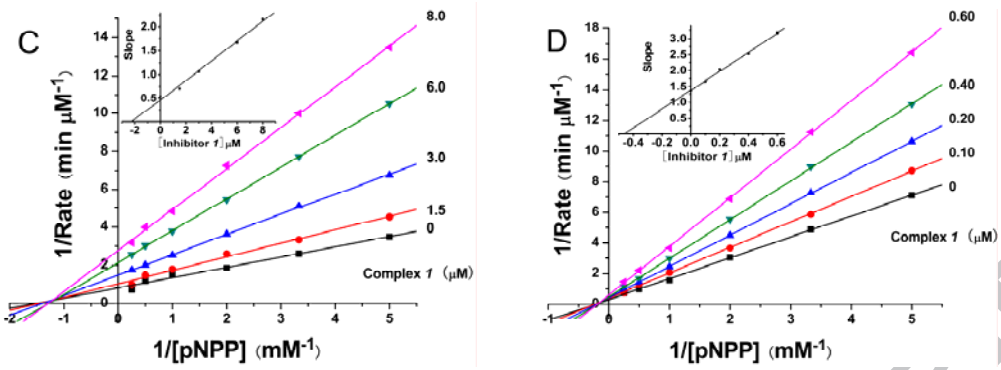


Figure 3. Lineweaver–Burk plots of $1/v$ (min mM^{-1}) *vs*. the reciprocal of the *p*NPP concentrations (mM^{-1}) at five fixed concentrations of complex ***1*** for PTP1B (A), TCPTP (B), PTP-MEG2 (C), and SHP-1 (D). Inset: determination of K_i for complex ***1*** inhibiting PTP1B, TCPTP, PTP-MEG2, and SHP-1, respectively.

Additionally, the phosphatase inhibitory activity on cell extracts from C6 rat glioma cells was evaluated. As shown in Figure 4, complexes ***1*** - ***5*** inhibit about 35% - 55% phosphatase activity at 30 μM. Complexes ***3*** and ***5*** show stronger inhibition. The inhibitory effects are slightly weaker than the copper complexes with multi-benzimidazole derivatives [36], but comparable to quinquedentate binuclear copper complexes [35]. This seems not consistent with the inhibition over the recombinant PTPs since these Schiff base copper complexes exhibit evidently stronger inhibition against SHP-1 or SHP-1 and PTP-MEG2 compared with the two type copper complexes respectively, and they should then have stronger inhibitory effects in inhibit cellular total phosphatases from cell extracts. The fact is that there are various phosphatases such as PTPs which are far more than the five, protein serine/threonine phosphatases, acidic and alkaline phosphatases existing in cell extracts. Different copper complexes may produce different phosphatase inhibitory effects by inhibiting different phosphatases activity. Although the Schiff base copper complexes exhibit stronger inhibition against PTP1B, TCPTP, SHP-1 and PTP-MEG2,

they may weakly inhibit other phosphatases, and then display weaker inhibition against cell extracts compared with the copper complexes with multi-benzimidazole derivatives. On the other hand, the protein components in cell extracts are complicated. Many metal complexes including some copper complexes are illustrated to bind to various proteins such as topoisomerase II, proteasome, carbonic anhydrases[28, 29, 45]. The interaction of copper complexes with these protein will decrease the inhibitory ability over PTPs. Other non-protein components in cell extracts may also complex with copper complexes and block the inhibition on PTPs. Thus, the PTP inhibition against cell extracts is intricate.

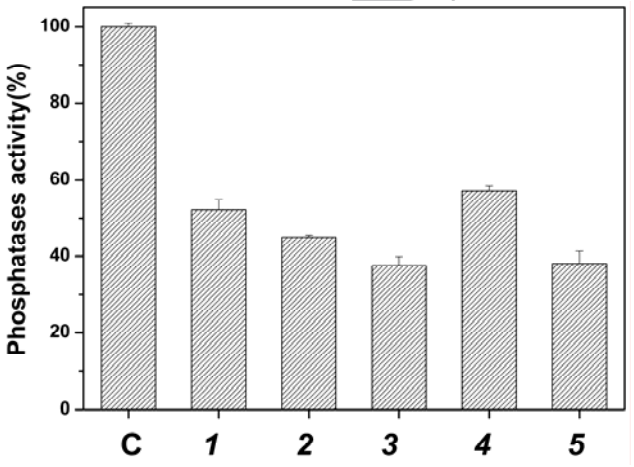


Figure 4. The inhibitory effects of complexes ***1-5*** on phosphatases from cell extracts, C for control.

4. Conclusion

In summary, we have synthesized and characterized five copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine. Complexes ***1-3*** have the 1 1 molar ratio of tridentate ligands Cu^{2+} while complexes ***4-5*** possess 2:1 molar ratio of bidentate ligands

Cu^{2+}. The inhibitory activities of the complexes against PTP1B, TCPTP, PTP-MEG2, SHP-1 and SHP-2 indicate that the complexes potently inhibit PTP1B, TCPTP, PTP-MEG2 and SHP-1, but do not inhibit SHP-2. In the complexes, ***5*** with a ratio of 2 1 ligand/copper exhibits very strong inhibition and better selectivity against PTP1B while ***1*** and ***2*** show very strong inhibition and a little selectivity against TCPTP. Compared with the oxovanadium(IV) complexes with same ligands, the copper complexes increase the inhibitory ability against TCPTP, PTP-MEG2 and SHP-1 but decrease the inhibition against SHP-2. For complex ***5***, the inhibition over PTP1B, TCPTP, PTP-MEG2 and SHP-1 are all improved about 5~15-fold. Usually, copper and vanadium complexes inhibit PTPs with competitive, non competitive or mixed inhibition models, implying they unactivate the enzymes by reversibly binding to them not oxidating SH to SO_3 at catalytic domain[13, 15, 17, 19, 31, 33-36, 46]. Thus, the differences in the PTP inhibition between the two complexes more possibly result from the dissimilarity of coordination sphere for the two metals.

The results demonstrate both the structures of ligands and the metal ions of coordination sphere influence the inhibition and the selectivity against different PTPs.

Acknowledgements

This work was supported financially by the National Natural Science Foundation of China (21001070, 21171109 and 21271121), SRFDP (20111401110002 and 20121401110005), the Natural Science Foundation of Shanxi Province of China (2010011011-2, 2011011009-1 and 2011021006-2) and the Shanxi Scholarship Council of China (2012-004).

Appendix A. Abbreviations

CSD	Cambridge Structural Database
ESI-MS	electrospray ionization mass spectra
Hcpmp	4-chloro-2-((phenylimino)methyl)phenol
Hnpmp	4-bromo-2-((phenylimino)methyl)phenol
H_2bhbb	2-(5-bromo-2-hydroxylbenzylideneamino)benzoic acid
H_2chbb	2-(5-chloro-2-hydroxylbenzylideneamino)benzoic acid
H_2nhbb	2-(5-nitro-2-hydroxylbenzylideneamino)benzoic acid
K_{app}	apparent Michaelis-Menten constant
K_i	inhibition constant
K_m	Michaelis-Menten constant,
MOPS	3-morpholinopropanesulfonic acid
*p*NPP	*p*-nitrophenol phosphate
PTP1B	protein tyrosine phosphatase 1B
PTP-MEG2	megakaryocyte protein-tyrosine phosphatase
PTPs	protein tyrosine phosphatases
SHP-1	Src homology phosphatase 1
SHP-2	Src homology phosphatase 2
TCPTP	T-cell protein tyrosine phosphatase

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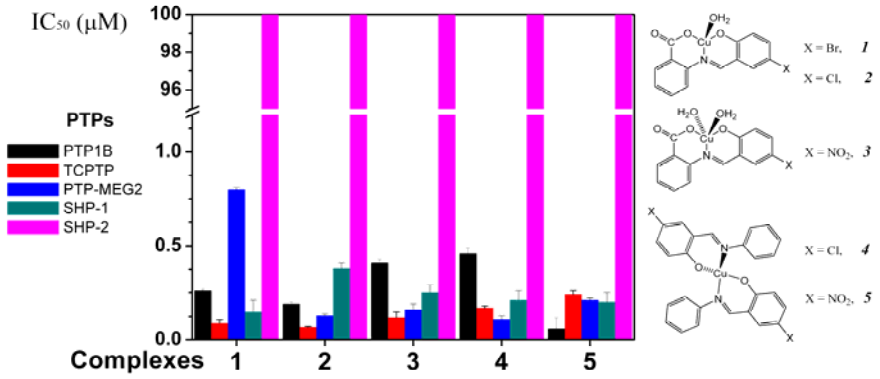
Index Abstract

Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted-benzenamine as selective inhibitors of protein tyrosine phosphatases

Ruiting Zhu, Liping Lu, Miaoli Zhu*, Hong Han, Caixia Yuan, Shu Xing*, Xueqi Fu*



Synthesis and evaluation of copper complexes of Schiff-base condensates from 5-substituted-2-hydroxybenzaldehyde and 2-substituted- benzenamine as selective inhibitors of protein tyrosine phosphatases

Liping Lu, Ruiting Zhu, Miaoli Zhu, Hong Han, Caixia Yuan, Shu Xing, Xueqi Fu**

Highlights

- Schiff-base copper complexes were synthesized and characterized.
- The complexes noncompetitively inhibit PTP1B, TCPTP, PTP-MEG2 & SHP-1 but SHP-2.
- Complex ***5*** exhibits very strong inhibition and better selectivity against PTP1B.