

## A POTENT UREASE INHIBITOR: 2-[[[2-[[[*E*]-[2-HYDROXYPHENYL] METHYLIDENE]AMINO]ETHYL]IMINO]METHYL] PHENOL-COPPER (II) COMPLEX

N. AFZA<sup>1\*</sup>, I. ANIS<sup>2</sup>, M. ASLAM<sup>1,2</sup>, A. HUSSAIN<sup>3</sup>, M. SAFDER<sup>3</sup>, A. H. CHAUDHRY<sup>4</sup>, L. IQBAL<sup>1</sup>, M. LATEEF<sup>1</sup>, A. QAYYUM<sup>5</sup>, M. NIAZ<sup>1</sup>

<sup>1</sup>Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi-75280, Pakistan, <sup>2</sup>Department of Chemistry, University of Karachi, Karachi-75270, Pakistan, <sup>3</sup>Department of Chemistry, Government College University, Faisalabad-38040, Pakistan, <sup>4</sup>GARL, GSP, Islamabad, Pakistan, <sup>5</sup>Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore 54000, Pakistan. Email: hocprc@yahoo.com. Dedicated to Dr. Zahra Noreen on the occasion of her birthday

Received: 27 December 2012, Revised and Accepted: 09 February 2013

### ABSTRACT

In order to develop new pharmaceutical agents, the Schiff base ligand, 2-[[[2-[[[*E*]-[2-hydroxyphenyl]methylidene]amino]ethyl]imino]methyl]phenol (**MA**), was derived from ethylenediamine and salicylaldehyde followed by complexation with copper (II) (**MA-Cu**). The chemical structures of Schiff base ligand (**MA**) and its complex (**MA-Cu**) were corroborated by means of elemental analyses, molar conductance and spectro-analytical data (<sup>1</sup>H-NMR, FT-IR, FAB-MS). The conductivity data of the complex (**MA-Cu**) suggested non-electrolytic nature. The electronic spectra and magnetic moment value confirmed octahedral geometry of the complex. The complex (**MA-Cu**) showed potent urease inhibitory activity, using thiourea as standard reference.

**Keywords:** Schiff base ligand; Copper (II) complex; Octahedral geometry; Potent urease inhibition activity; Antioxidant activity; Antibacterial activity.

### INTRODUCTION

The biologically significant transamination reactions involve Schiff base as an intermediate which reflects that Schiff bases have certain essential role in such reactions [1]. They are exploited by organic chemists for the protection of amino group during synthetic work and are involved in natural rubber as a protective agent. The field of coordination chemistry has become very popular among the scientific community due to wide scale utilization of Schiff bases as chelating ligands in coordination complexes with certain important biological activities. The coordination of various metals with metallo-biomolecules at their active site is considered to take place with the involvement of N and S atoms. The antifungal, industrial, anticancer, antibacterial and herbicidal applications of Schiff bases have drawn the attention of researchers to study the metal complexes of Schiff base [2]. These compounds are important models in the species of biological significance and have applications in asymmetric epoxidation, reduction of aromatic ketones, asymmetric aziridination of olefins and biomimetic catalytic reactions. Moreover, they are involved in the preparation of ion selective electrodes and extraction of metal ions via solid phase etc. A large variety of biological activities such as antibacterial [3], herbicidal [4], anticancer [5], anti-inflammatory [6], analgesic [7], antifungal [8] and anti-tumor [9] are shown by the complexes containing N, S and O atoms in the chelating ligands which are bonded to the metal ion in many ways. The ground reality for the presence of metal is that its presence enhances the activity of biologically active compounds. The number of ways in which ligands can bind to metal ions in different coordination environments and applications of complexes in various fields prompted us for the synthesis of such compounds [10].

Most of the enzymatic reactions involve Schiff bases as significant intermediates because such type of reactions take place via interaction of amino or carbonyl group of substrate with enzyme. The biochemical process involving catalytic mechanism is the condensation of carbonyl group of substrate with primary amine such as lysine residue to form a Schiff base or an imine.

The hydrolysis of urea to carbon dioxide and ammonia is catalyzed by urease (urea amidohydrolase) enzyme which is a metalloenzyme of nickel. Most of the plants, some fungi and a wide range of prokaryotes possess this enzyme. These reactions result in the elevation of pH due to an increase in the concentration of ammonia which has negative implications in agriculture and medicine to a significant extent. Such type of negative effects can be counteracted by the use of inhibitors of urease. The oxidative stress is the result of

reactive oxygen species (ROS) like hydrogen peroxide, nitric oxide radicals, superoxide anions and hydroxyl radicals which has a relation with the pathogenesis of various diseases. The toxicity due to radical formation is controlled by employing antioxidants which are the major defense against such radicals. The prevention and treatment of complex diseases, like atherosclerosis, diabetes, stroke and cancer is carried out with the help of antioxidants. The significance of Schiff bases in various areas has developed our interest to synthesize such compounds.

Herein we report the synthesis and characterization of Schiff base ligand (**MA**), derived from the condensation of salicylaldehyde with ethylenediamine, followed by complexation with Cu (II) and their biological activities, such as antioxidant, lipoxigenase and urease inhibition activity.

### MATERIALS AND METHODS

#### Reagent and solvents

The solvents and reagents were purchased from Merck and they were used as such. The metal (II) chloride [CuCl<sub>2</sub>.2H<sub>2</sub>O] was used as a metal salt. All the glass-wares were dried in an oven after washing with distilled water.

#### Physical measurements

The weighing was carried out on an electric Mettler Toledo balance, model AL 204. The uncorrected melting points are reported which were recorded by using Gallenkamp melting point apparatus. The percentage of elements was determined on a Perkin-Elmer 2400 elemental analyzer of Series II. FT-IR spectra of the compounds were recorded by using Thermo Nicolet Avatar 320 FT-IR spectrometer. The measurements for electrical conductivity were conducted on conductivity meter model Jenway 4010. The masses of compounds were confirmed by taking FAB mass spectra on JEOL SX102/DA-6000 mass spectrometer by employing glycerol as matrix and data was analyzed as *m/z* (%). The pre-coated silica gel G-25-UV<sub>254</sub> plates (E-Merck) were used for taking TLC. DMSO-*d*<sub>6</sub> was used for recording <sup>1</sup>H-NMR spectra on a Bruker AMX-400 spectrometer. The internal standard used for <sup>1</sup>H-NMR spectra was tetramethylsilane. The scalar coupling constants (*J*) are reported in Hz while chemical shift ( $\delta$ ) values are presented in ppm.

#### Synthesis of Schiff base ligand

The Schiff base (**MA**) was obtained by refluxing a mixture of salicylaldehyde (0.02 mole in 60 mL MeOH) and ethylenediamine

(0.01 mol in 60 mL MeOH) in the presence of a few drops of conc.  $\text{H}_2\text{SO}_4$  at 70 °C on water bath with stirring for 4 h. The reaction mixture was placed in the refrigerator for cooling. The synthesized compound was filtered, washed with *n*-hexane and methanol was used for recrystallization. The recrystallized product was dried in desiccators over anhydrous  $\text{CaCl}_2$ .

### Synthesis of $\text{Cu}^{2+}$ Complex (MA-Cu)

The methanolic solution of copper (II) [ $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ] was added to a hot methanolic solution of schiff base (MA) in 1:1 (M:L) molar ratio and the mixture was refluxed with stirring for 45 min. 1M NaOH in MeOH was added in drop wise fashion to maintain the pH of reaction mixture for complex formation and the precipitation of product. The complex, thus obtained in the form of precipitates were filtered off and dried under vacuum in the presence of anhydrous  $\text{CaCl}_2$ .

### 2-[[2-[[E)-(2-Hydroxyphenyl)methylidene]amino]ethyl]imino]methyl]pheno l (MA)

Yellow flakes; yield 91.03%; m.p.: 126 °C; IR (KBr,  $\nu_{\text{max}}$   $\text{cm}^{-1}$ ): 3471 (O-H), 3224 (C=N--H-O), 2932 (C-H), 1618 (C=N), 1491 (C=C);  $^1\text{H-NMR}$  (400 MHz,  $\text{DMSO}-d_6$ )  $\delta$ : 13.34 (s, -OH), 8.58 (2H, s, H-5, -7), 7.40 (2H, dd, J = 7.6, 1.6 Hz, H-9, -18), 7.30 (2H, ddd, J = 8.8, 7.6, 1.6 Hz, H-11, -16), 6.85 (4H, m, H-10, -12, -15, -17), 3.91 (4H, s, H-1, -2); FAB-MS  $m/z$ : 269.3  $[\text{M}+\text{H}]^+$  (calcd. 268.1 for  $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2$ ); Elemental analysis: found (calcd. %): C: 66.84 (71.62), H: 6.87 (6.01), N: 11.63 (10.44).

### 2-[[2-[[E)-(2-Hydroxyphenyl)methylidene]amino]ethyl]imino]methyl]pheno l-copper (II) [ $\text{Cu}(\text{MA})(\text{H}_2\text{O})_2$ ] (MA-Cu)

Black solid; yield: 72 %; m.p.: 225 °C; IR (KBr)  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3327 (OH), 3037 (C-H), 1590 (C=N), 1440 (C=C), 592 (Cu-N), 458 (Cu-O); Molar conductance ( $\text{DMSO}$ )  $\lambda_m$  ( $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ ): 11.97; FAB-MS  $m/z$ : 366.1  $[\text{M}+\text{H}]^+$  (calcd. 365.0 for  $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_4\text{Cu}$ ); Elemental analysis (%): C 52.58, H 5.02, N 7.71, Cu 17.43 (calcd. C 52.52, H 4.96, N 7.66, Cu 17.37).

### Biological studies

The method reported by Aslam *et al.* was employed for the measurements of antioxidant (DPPH scavenging) activity and urease inhibition activity and also for the measurement of inhibition of LOX activity [11].

## RESULTS AND DISCUSSION

### Chemistry and characterization

The condensation of salicylaldehyde and ethylenediamine was carried out to synthesize schiff base ligand (MA) and then its

complex (MA-Cu) (Scheme 1) was synthesized by treating Schiff base with transition metal (II). synthesize Schiff base ligand (MA) and then its complex (MA-Cu) have sharp m.p. and are fairly stable compounds which is a clear evidence for the purity of compound. TLC comparison method (*i.e.* comparison of spots of reactants and products) was employed to recognize the formation of Schiff base ligand (MA) and complex (MA-Cu).

### $^1\text{H-NMR}$ spectroscopy

$^1\text{H-NMR}$  and spectra were used for further confirmation of Schiff base ligand (MA) and complexes (MA-Cu). A singlet was observed for the ligand (MA) at  $\delta$  8.58 ppm due to C-H of azomethine group, confirming the fact that Schiff base ligand possesses an azomethine linkage. The signals for aromatic region of schiff base ligand were observed at 7.40 and 6.85 ppm whereas  $-\text{CH}_2$  was observed at 3.91 ppm.

### Infrared spectroscopy

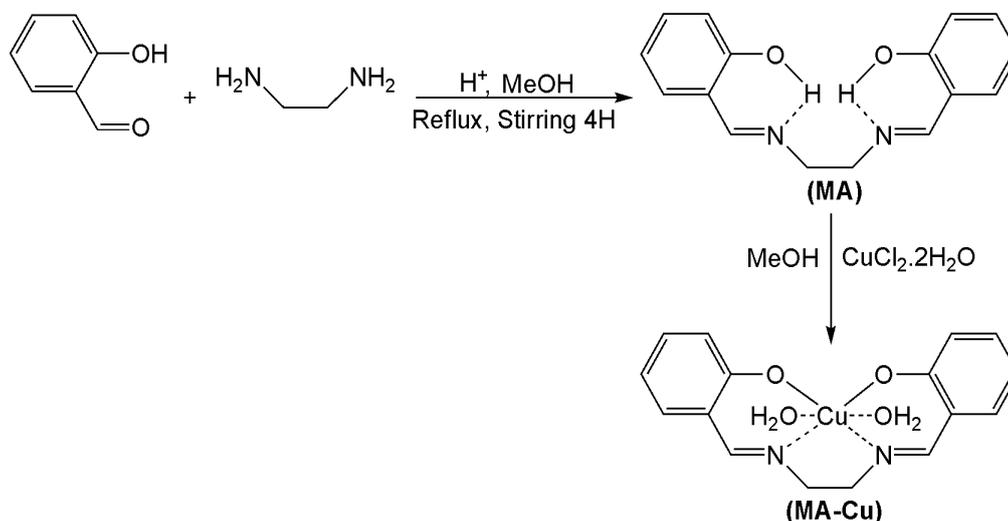
The Schiff base ligand (MA) has a strong band at 3224  $\text{cm}^{-1}$  was observed for schiff base ligand in IR spectrum confirmed that intramolecular hydrogen bonding is found in this compound (Scheme 1). The position for (C=N-) stretching at 1618  $\text{cm}^{-1}$  for (MA) shifted towards lower frequency region *i.e.* 1590  $\text{cm}^{-1}$  in case of complex (MA-Cu), which indicated the involvement of azomethine linkage in complex formation [12]. The vibrations associated with (Cu-N) and (Cu-O) were observed in the IR spectrum at 592 and 458  $\text{cm}^{-1}$  respectively which is a clear indication that nitrogen atoms of ligand take part in coordination.

### Mass spectroscopy and microanalysis

The stoichiometric composition of metal complex (MA-Cu) and free ligand was compared with the help of FAB (positive) mass spectra. The molecular ion peak  $[\text{M}+\text{H}]^+$  for free ligand was observed at  $m/z$  269.3, which was in agreement to that of theoretical molecular weight *i.e.* 268.1 of the compound. The molecular ion peak representing the complex (MA-Cu) was observed for determining stoichiometry and it was found that ligand to metal ratio was 2:1. It was found that the data for elemental analyses for the Schiff base ligand (MA) and complex (MA-Cu) was in concurrence with peak representing molecular ion.

### Molar conductance

The molar conductivities were measured by dissolving the complex in DMSO and preparing  $1 \times 10^{-3}$  M solutions at room temperature. The molar conductance of metal complex was observed in the range 11.97  $\text{ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ , which in turn depicts that all the complexes have non-electrolytic nature.



Scheme 1: Synthesis of Schiff base ligand (MA) and complex (MA-Cu).

Table 1: IC<sub>50</sub> (μM) values of Schiff base ligand (MA) and complex (MA-Cu) in the antioxidant, lipoxygenase and urease assay.

Compound	DPPH Scavenging activity IC <sub>50</sub> (μM)	Lipoxygenase inhibition activity IC <sub>50</sub> (μM)	Urease inhibition activity IC <sub>50</sub> (μM)
MA	212.1	+	+
MA-Cu	+	+	19.0
BHA	44.2	-	-
Baicalein	-	22.6	-
Thiourea	-	-	21.6

(+ = Non-significant)

### Electronic spectra and magnetic moment

The electronic spectra were used for assigning the stereochemistry of the metal ion in the complex based on the number of d-d and charge transfer transitions. The bands at 19630 and 16500 cm<sup>-1</sup> in the electronic spectrum of Cu (II) complex, assigned to <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>E<sub>1g</sub> and <sup>2</sup>B<sub>1g</sub> → <sup>2</sup>B<sub>2g</sub> transitions, showing octahedral geometry, further confirmed by its magnetic moment value 1.87 B.M [13].

### Suggested Structural formula of the complex

The analytical and spectral data illustrated the bonding of schiff base ligand (MA) to the metal ion through nitrogen and oxygen atoms of imino group. In this connection, tentative proposed structure for the complex is shown in scheme 1.

### Biological studies

The metal complex (MA-Cu) and its schiff base ligand were checked for the activities like antioxidant, lipoxygenase and urease inhibition activity (Table 1). The Schiff base ligand (212.1 μM) and complex were found to have non-significant IC<sub>50</sub> values on comparison of results with the standards for lipoxygenase and DPPH scavenging. The reference/standard used for urease inhibition was thiourea and results showed that Schiff base ligand (MA) has non-significant activity but complex (MA-Cu) as a potent urease inhibitor which showed even stronger activity than standard (19.0 μM).

### CONCLUSIONS

The electronic spectra and magnetic moment value confirmed the octahedral geometry of the complex. The Schiff base ligand (MA) has non-significant biological activity but upon complexation with copper (II) ions, the enhancement of activity was observed and as a result complex (MA-Cu) of the same ligand appears as potent urease inhibitor.

### ACKNOWLEDGMENTS

The authors express their truthful thanks to Pakistan Council of Scientific and Industrial Research Laboratories Complex, Karachi for financial support and Department of Chemistry, University of Karachi, Karachi for providing research facilities.

### REFERENCES

- Spinu C, Kriza A. Co(II), Ni(II) and Cu(II) complexes of bidentate Schiff bases. *Acta Chim. Slov.* **2000**; 47: 179-185.
- Cozzi PG. Metal-Salen Schiff base complexes in catalysis: practical aspects. *Chem. Soc. Rev.* **2004**; 33: 410-421.
- More PG, Bhalvankar RB, Pattar SC. Synthesis and biological activities of Schiff bases of aminothiazoles. *J. Indian Chem. Soc.* **2001**; 78: 474-475.
- Samadhiya S, Halve A. Synthetic utility of Schiff bases as potential herbicidal agents. *Orient. J. Chem.* **2001**; 17: 119-22.
- Shkawat DR, Sabnis SS, Deliwala CV. Potential anticancer agents, Schiff bases from p-(3-azaspiro[5,5]undec-3-yl)benzaldehydes. *Bull. Haffkine. Inst.* **1973**; 1: 35-39.
- Sathe BS, Jagtap VA, Sreenivasa GM. Synthesis, characterization and *in vitro* anti-inflammatory evaluation of new fluorobenzothiazole Schiff bases. *Int. J. Phar. Tech. Res.* **2011**; 3: 164-69.
- Chinnasamy RP, Sundararajan R, Govindraj S. Synthesis, characterization and analgesic activity of novel Schiff base of isatin derivatives. *J. Adv. Pharm. Tech. Res.* **2010**; 3: 342-47.
- Chaitanya MS, Nagendrappa G, Vaidya VP. Synthesis, biological and pharmacological activities of 2-methyl-4Hpyrimido[2,1-b][1,3]benzothiazoles. *J. Chem. Pharm. Res.* **2010**; 2(3): 206-213.
- Hodnett EM, Dunn WJ. Structure-antitumour activity correlation of some Schiff bases. *J. Med. Chem.* **1970**; 13: 768-70.
- Aslam M, Anis I, Afza N, Mehmood R, Hussain A, Bokhari TH, Hussain MT, Nawaz H, Khalid M. Synthesis, characterization and antibacterial investigation of divalent metal complexes of NO-donor ligand: {5-chloro-2-[(4-chlorobenzylidene)-amino]phenyl}(phenyl)methanone. *J. Saudi Chem. Soc.* **2012**; <http://dx.doi.org/10.1016/j.jscs.2012.09.016>.
- Aslam M, Anis I, Afza N, Iqbal L, Iqbal S, Hussain A, Mehmood R, Hussain MT, Khalid M, Nawaz H. Biological evaluation of potent antioxidant, lipoxygenase inhibitor and antibacterial: A comparative study. *J. Saudi Chem. Soc.* **2012**; <http://dx.doi.org/10.1016/j.jscs.2012.09.009> (b).
- Lever ABP. *Inorganic Electronic Spectroscopy*, 2<sup>nd</sup> Edn. (Elsevier, Amsterdam, **1984**), pp. 205-207.
- Rana VB, Singh DP, Singh P, Teotia MP. Trivalent chromium, manganese, iron and cobalt chelates of a tetradentate N6 macrocyclic ligand. *Transition Met. Chem.* **1982**; 7(3): 174-177.