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Research Article

Design, synthesis and biological evaluation of some schiff base ligand as antimalarial agents

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Abstract

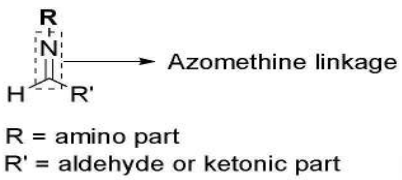
Schiff base ligand AA-**1**, was derived from ethylenediamine and salicylaldehyde for the development of pharmaceuticals and it was followed by complexation of ligand with Ni (II) metal ion **2**. The data of elemental analyses, molar conductance, ^{1}H-NMR, IR, EI-MS was used for the corroboration of structural features of Schiff base AA**1** and its complex AA**2** along with the data obtained from single X-ray crystallography. The magnetic moment and UV spectral data was employed to proposed square planar geometry to the complex which was strongly supported by the Single-Crystal X-Ray diffraction studies. On the basis of conductivity data, it was suggested that the complex AA**2** has non-electrolytic nature. The results of biological activities showed that the The most potent compound AA-5 showed inhibitory potency (IC50) of 2.1 mg/ml and 3mg/ml against chloroquine sensitive and chloroquine resistant strains respectively.The results of antimalerial activity showed that the ligand AA-**1** exhibits moderately active while complex AA-**2** exhibits good activity for chloroquine sanstive and resistant.

Keywords: Schiff base, Single-Crystal X-Ray diffraction, chloroquine sensitive strain and resistance strain, Antimalarial activity

1. Introduction

The class of ligands which has attained prime significance include Schiff bases because they are synthetically flexibile, selective and sensitive to the metal atom or ion, similar in structural aspects to the biological systems and have an imine (-N=CH-) group (Figure 1), which is considered as the most biological group for understanding and elucidating transformation and racemization mechanisms in a variety of biological systems with ease[1].

Figure. 1 General representation of the structure of a Schiff base.



The pharmaceutical and medicinal importance of these compounds enhance their remarkably significant utilization in medicine field[2] and most of the chemists are fascinated and attracted by the increasingly reported therapeutic potential in the Schiff base derivatives with novelty in structural framework[3]. The broad range of biological activities were attributed to

the heterocyclic ring of these compounds which include antiviral[4], anticancer[5], anticonvulsant, acetylcholinesterase inhibition[6], anti-HIV[7] and anti-PAF[8]. In general radiopharmaceuticals employed for cancer treatment involve the charming way of coordination of Schiff bases to the metal ion, and same is the case for models in biological systems and agrochemicals[9]. The most prominent intermediates for a number of enzymatic reactions which are involved in the interaction of amino or carbonyl of substrate with the enzyme, are Schiff bases. One of the most important types of catalytic mechanism is the biochemical process which involves the condensation reaction of a primary amine usually that of lysine residue with carbonyl of the substrate in an enzyme leads to the formation of a Schiff base or so called imine.

The enzyme which catalyzes the formation of ammonia and carbon dioxide by hydrolysis of urea is a nickel based metalloenzyme, is commonly known as urease enzyme[10]. A large variety of prokaryote, most of the plants and a fewer fungi possess this enzyme[11]. There are various negative implications caused by elevation of pH due to high concentration of ammonia produced by such type of reactions, in agriculture and medicine[12]. Such type of negative implications can be minimized by decreasing the activity of urease enzyme by employing suitable inhibitors. Inhibitors of urease can be broadly classified into two categories: (i) active site directed (substrate-like), (ii) mechanism-based directed. The urease due to its high substrate (urea) specificity can only bind to a few inhibitors with a similar binding mode as urea.

The oxidative stress associated with various important diseases and pathogens are controlled by ROS reactive oxygen species which include superoxide anions, hydrogen peroxide, hydroxyl and nitric oxide radicals[13]. The damages caused by free radicals are protected by antimalarias, the radical mediated toxicity and as a result these antimalarias serve as major defense. The prevention and treatment of complicated diseases such as atherosclerosis, stroke, cancer and diabetes is carried out by employing antimalaria agents[14].

Bacillus cereus causes severe food poisoning, which produces nausea, diarrhea, mild illness, abdominal cramping, vomiting, and skin and wound infections. It is able to form spores and very resistant to low and high temperatures[15]. *Staphylococcus aureus* is a Gram-positive, facultative anaerobic coagulase-positive catalase-positive potential pathogen and found in skin, soft tissue, bone joint, endovascular and wound infections. It can cause a range of illnesses from minor skin infections like cellulitis folliculitis and scalded skin syndrome to life-threatening diseases as pneumonia, toxic shock syndrome and sepsis. Methicillin-resistant *S. aureus* is one of greatly feared strains of *S. aureus*, which have become resistant to most antibiotics. *Proteus mirabilis* causes community-acquired infection, high level of urease which leads to urinary tract infections and the formation of stones[16] but less commonly prostatitis, pneumonia include fever, chills, chest pain, rales and cough as well as food spoilage. *Escherichia coli* is a Gram-negative, facultative anaerobic and non-sporulating bacterium and is commonly found in lower intestine of warm-blooded organisms. Most strains of it are harmless but some serotypes can cause food poisoning in human and bacterial infections including cholecystitis, bacteremia, cholangitis, pneumonia, urinary tract infection (UTI), vomiting and bloody diarrhea. *Salmonella typhi* causes the typhoid (enteric fever) which affects the 17 million people and 60 thousands death annually in the world.

The above grounds prompted us to work and report the synthesis and characterization of nickel (II) complex **2** of the Schiff base ligand **1** conduct biological evaluation.

2. Experimental

2.1 Materials and methods: The chemicals and reagents purchased from Merck were used without any alteration. The hydrated metal salt was utilized like hydrated metal (II) acetate [$Ni(CH_3COO)_2.4H_2O$]. The use of thoroughly washed and oven dried glassware was made sure during the whole work.

2.2 Physical measurements: The process of weighing was accomplished by using electric Mettler Toledo balance, of AL 204 model. The melting points reported are uncorrected and were taken by using melting point apparatus of Gallenkamp. Perkin-Elmer 2400 Series II elemental analyzer was employed for elemental analysis. Thermo Nicolet Avatar 320 FT-IR spectrometer with a range of 400-4000 cm^{-1} was employed for taking IR spectra of the compounds. The conductivity meter with model Jenway 4010 was used for the measurement molar conductance of compounds. The UV spectra of compounds for studying electronic transitions were taken by using Specord 200 UV-Vis spectrophotometer. The recording of El-MS spectra was conducted by electron impact mode on Finnigan MAT-112 spectrometer (Finnigan, Waltham, MA, USA) and m/z (%) of $[M]^+$ ions reported. The compounds were subjected to TLC on precoated silica gel G-25-UV254 plates (E-Merck). The recording of ^{1}H-NMR spectra of compounds in DMSO-d_6 was made on a Bruker AMX-400 spectrometer. For

this purpose, chemical shift vlues (δ) are reported in ppm, based on the internal standard *i.e.,* tetramethylsilane and *J* values (*i.e*., scalar coupling constant) are shown in Hertz. Single-crystal X-ray diffraction data was collected on Bruker Smart APEX II, CCD 4-K area detector diffractometer[17]. Data reductions were performed by using SAINT program. The structure was solved by direct method[18] and refined by full-matrix least squares on F^2 by using the SHELXTL-PC package[19]. The figures were plotted with the aid of ORTEP program[20].

2.3 Synthesis of Schiff base ligand: The addition of 3-4 drops of conc. H_2SO_4 to a mixture of ethylenediamine (0.01 mol in 60 mL MeOH) and salicylaldehyde (0.02 mole in 60 mL MeOH) was carried out and it was refluxed in water bath for 4 hours at 70 ^oC and then it was kept for cooling in refrigerator. *n*-hexane was used for washing these yellow flakes and recrystallization in absolute methanol was accomplished. Desicator containing P_2O_5 was used for drying purpose.

2.4 2-{[(2-{[(E)-(2-hydroxyphenyl)methylidene]amino}ethyl)imino]methyl}phenol 1: The compound **1** was obtained with 91.03% yield as yellow flakes. m.p: 126 ºC; IR (KBr, ν_{max} cm^{-1}): 3471 (O-H), 3224 (C=N---H-O), 2932 (C-H), 1618 (C=N), 1491 (C=C); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 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); EI-MS: *m/z* (%) 268.1 $[M]^+$ (calcd. 268.1 for $C_{16}H_{16}N_2O_2$); Elemental analysis: found (calcd. %); C: 66.84 (71.62), H: 6.87 (6.01), N: 11.63 (10.44).

2.5 Synthesis of Ni^{2+} Complex: 0.01 mole of nickel (II) acetate was added slowly with continuous stirring to the Schiff base ligand **1** (0.01 mole, 100 mL) in equimolar ratio and mixture was then refluxed for 45 minutes. A solution of 1M NaOH was added in dropwise manner and pH was gradually raised to a suitable pH value for complex formation. Maroon coloured crystals were obtained by reluxing the reaction mixture for 90 minutes under vacuum. It was concentrated in a way that the volume of reaction mixture reduced to half of its original volume. The product thus obtained was washed with cooled methanol after filteration. Absolute methanol was used for recrystallization.

2.6 2-{[(2-{[(E)-(2-hydroxyphenyl)methylidene]amino}ethyl)imino]methyl}phenol-nickel (II) 2: The percentage yield of complex **2** was 89.31% yield. in the form of maroon coloured crystals m.p: 203 ºC; IR (KBr, ν_{max} cm^{-1}): 2933 (C-H), 1524 (C=N), 1435 (C=C), 590 (Ni-N), 465 (Ni-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.88 (2H, s, H-5, -7), 7.24 (2H, d, J = 7.2 Hz, H-9, -18), 7.16 (2H, t, J = 7.2 Hz, H-11, -16), 6.69 (2H, d, J = 8.4 Hz, H-12, -15), 6.50 (2H, t, J = 7.2 Hz, H-10, -17), 3.41 (4H, s, H-1, -2); EI-MS: *m/z* (%) 324.0 $[M]^+$ (calcd. 324.0 for $C_{16}H_{14}N_2O_2Ni$); Elemental analysis: Found (calcd. %); 57.00 (59.13), H: 3.80 (4.34), N: 10.95 (8.62), Ni: 18.13 (18.06).

2.7 Biological studies: Ferheen *et al.* methodology was adopted for carrying out antimalaria (DPPH scavenging) and chloroquine sensitive strain and resistance strain of these compounds, however a modified method reported by Bibi *et al.* was employed for the measurement of diameter of zone of inhibition for antibacterial assay and then calculation of % inhibition was carried out[21].

3. Results and Discussion

3.1 Synthesis and characterization: Salicylaldehyde upon reaction with ethylenediamine resulted in the formation of Schiff base ligand **1**, however it was resulted in the formation of a complex **2** upon treatment with Ni (II) acetate. A very much clear evidence about the stability of complex **2** was determined by the fact that this compound possesses a high melting point *i.e.* 203 ^oC.

3.2 ^{1}H-NMR spectra: The presence of a hydrogen atom at δ 8.58 (2H, s) in ^{1}H-NMR spectrum indicated the presence of azomethine proton in ligand **1** and strengthened the idea that product contains azomethine linkage. The chemical shift of these hydrogens underwent a shift towards downfield region *i.e.* δ 7.88 (2H, s) and signals for two hydrogens at δ 13.34 (s) in ligand **1** disappeared from the spectrum of complex **2** which confirmed that oxygen atoms are involved in coordination.

3.3 Infrared spectra: The involvement of azomethine group in complex formation was determined by observing IR spectra of the complex **2** which showed shift in the position of a band at 1618 cm^{-1} due to the presence of ν(-C=N-) (azomethine) in ligand towards lower frequency region *i.e.* 1530 cm^{-1} in the spectrum of complex **2**[22]. The disappearance of distinguishing bands of carbonyl group ν(C=O) group and the amino group indicated the completion of condensation reaction. The

presence of a band at 3224 cm^{-1} for the ligand **1** in IR spectrum confirmed the presence of intramolecular hydrogen bonding. The bands at 590 and 465 cm^{-1} were assigned to the vibrations of (Ni-N) and (Ni-O)[23] respectively, in IR spectrum which is the direct evidence for involvement of heteroatoms (*i.e.* oxygen and nitrogen) of ligand in the coordination.

3.4 EI-MS spectra and microanalysis: The examination of stoichiometric composition of ligand AA-**1** and its complexAA- **2** was made by recording and comparing EI-MS spectra of ligand AA-**1** and complex AA-**2**. The ligand AA-**1** showed molecular ion peak $[M]^+$ at m/z 268.1 and the complex AA-**2** on the other hand was observed at m/z 324.0 which confirmed 1:1 ratio of metal and ligand in the complex. The data of EI-MS data was found in concurrence with the data of microanalysis.

3.5 Molar conductance: The non electrolytic nature of complex was suggested by the molar conductance (λm) of complex which was measured in dimethyl formamide (10^{-3} M) and was found 6.21 ohm^{-1} cm^2 mol^{-1}.

3.6 Electronic spectra and magnetic moment: The π – π* transition of the azomethine (>C=N–) chromophore in ligand was observed at 329 nm in UV/Vis spectrum. The involvement of azomethine nitrogen in coordination/ complexation was also supported by the shift of azomethine band towards longer wavelength in the spectrum of complex **2**. The appearance of bands at 381, 461 and 492 nm in UV/Vis spectrum of complex was observed and these bands were assigned to $^1A_1g \rightarrow {}^1A_2g$, $^1A_1g \rightarrow {}^1B_1g$ and $^1A_1g \rightarrow {}^1E_1g$ transitions which confirmed that geometry of the complex **2** is square planar[24]. 2.01 B.M. was the observed value for magnetic moment of complex and is in full concurrence with the literature reported data[25].

3.7 Single-crystal X-Ray diffraction analysis: It was carried out to establish the structure of complex **2**. The ORTEP diagrams of complex **2** (Figure 2) showed that mononuclear nickel (II) complex, $[Ni(C_{16}H_{14}N_2O_2)]$, the Ni atom is coordinated with four donor atoms of the Schiff base ligand to adopt square-planar geometry. The two benzene rings are each planner with the dihedral angle of 6.61(13). The five member ring adopts an envelope conformation with maximum deviation of 0.156(2) for N1 atom from the atom from the least square plane. The bond lengths and angles are similar to those in other structurally related compounds[26]. In the crystal structure, the H atoms of C_8 and C_9 are involved in hydrogen bonding with atoms O_1 and O_2 of a neighboring molecule *via* C_8---H_{8B}---O_2 and C_9---H_{9A}---O_2 intermolecular interactions and lead to the formation of chain running parallel along the *c* axis (Figure 3).

Figure. 2 ORTEP diagram of Ni (II) complex 2.

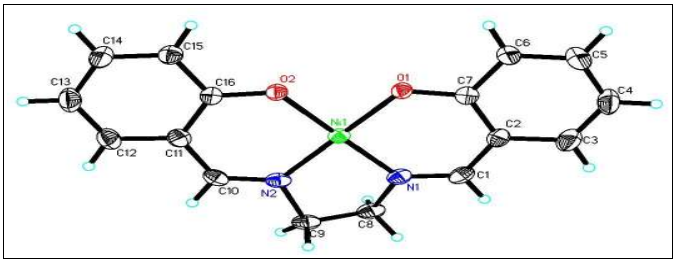


Figure. 3 Packing of the Ni (II) complex 2. Intermolecular C—H···O hydrogen bonds are shown as dashed lines.

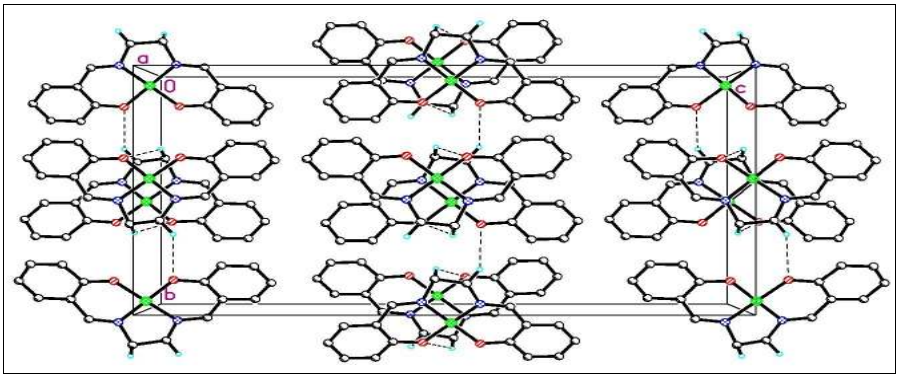


Figure. 4 (a) Removal of a hydrogen atom from the compound. (b) Hydrogen atom donated to DPPH radical by compound.

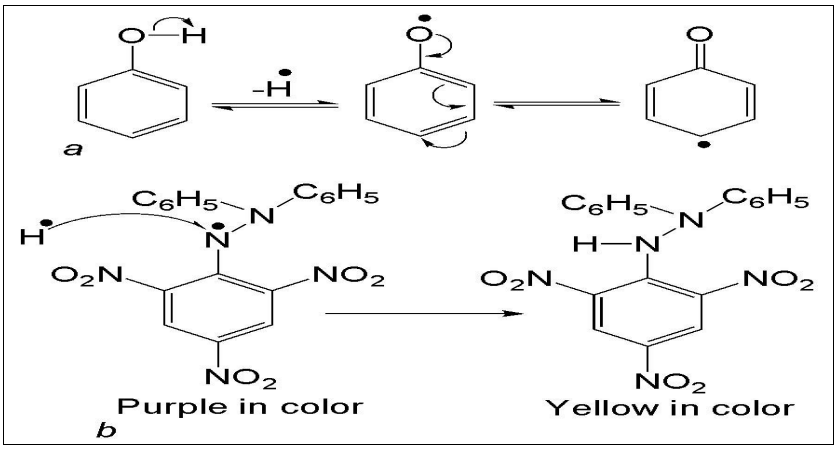
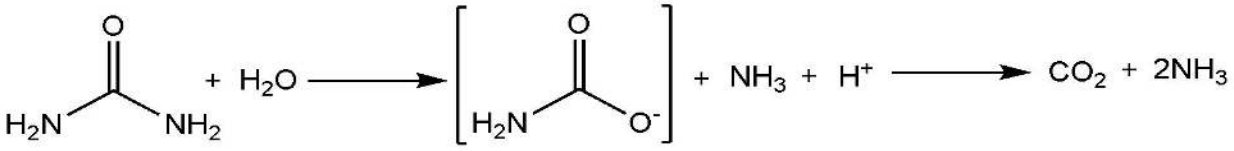


Figure. 5 The breakdown of urea to ammonia and carbamate.



Crystal data of complex **2:** Ni($C_{16}H_{14}N_2O_2$), Mr = 325.00, Orthorhombic, space group *Pbca*, *a* = 7.4785(4) Å , *b* = 13.8271(7) Å , *c* = 26.1344(14) Å , *V* = 2702.5(2)Å ^{3} , *Z* = 8, r_{calc} = 1.598 mg/m^3, *F*(000) = 1344, μ(Mo Kα = 0.71073 Å, max/min transmission 0.8935/0.6105 crystal dimensions 0.38 x 0.28 x 0.08, 0.70◦ < q< 25.5◦, 14710 reflections were collected, of which 2506 reflections were observed (R_{int} = 0.0387). The *R* values were: R_1 = 0.0318, wR_2 = 0.0763 for I > 2s(I), and R_1 = 0.0464, wR_2 = 0.0853 for all data; max/min residual electron density: 0.527/-0.226 e A$^{\circ -3}$.

3.8 Suggested structural formula of the complex 2: It has been established by the foregoing discussion that nitrogen atoms of imino group and oxygen atoms of Schiff base ligand are involved in complexation. Scheme 1 illustrates the tentative proposed structure for complex **2**.

3.9 Biological studies: The antimalaria, urease inhibition and antibacterial activities of complex **2** and Schiff base ligand AA-**1** were conducted by screening these compounds.

The reason for the non-significant antimalaria ability of the compounds **1-2** can be explained by looking into the structures of these compounds. It is well known that the compounds with structures containing one or more functional groups such as –OH, –SH, –COOH, –N, –S–, –O– can show antimalaria activity (Figure 4). But in Schiff base ligand **1**, hydroxyls are involved in hydrogen bonding and in case of complex AA-**2**, these are utilized in complex formation. That is why; the compounds have non-significant antimalaria activity.

3.9.2 Urease inhibition study: The urease (EC 3.5.1.5) is a protein and found in bacteria, yeast, higher plants and exceptional in *Helicobacter pylori*. Many gastrointestinal or urinary tract pathogens also produce urease. It is a nickel-enzyme, which catalyzes the hydrolysis of urea to ammonia and carbamate, which decomposes to ammonia and carbonic acid (Figure 5), consequently pH is increased. It causes the gastric ulceration, urinary stone formation, pyelonephritis and other dysfunctions[27]. The Schiff base ligand **1** has significant result (40.7 ± 0.11) as compared with the complex **2** for urease inhibition as is evident from Table-1, using thiourea (21.6 ± 0.12) as standard.

Table 1. Antimalaria and chloroquine sensitive strain and resistance strain of Schiff base ligand AA-1 - AA-10

Compound Code	IC_{50} ^{3D7} (AACSS)	IC_{80} ^{3D7} (AACSS)	IC_{50} ^{W} (AACRS)
AA-1	22.1	50.6	32.8
AA-2	>50	ND	ND
AA-3	16.3	42.3	51.1
AA-4	3.6	12.3	18.3
AA-5	2.1	6	2
AA-6	4.3	8	10
AA-7	10.2	19.3	25.2
AA-8	19.6	ND	ND
AA-9	>50	ND	ND
AA-10	4.1	12	18

AACSS - activity against chloroquine sensitive strain

AACRS - activity against chloroquine resistance strain

3.9.3 Anti-bacterial study: Metal ions are adsorbed on the cell walls of the microorganisms, disturbing the respiration processes of the cells and thus blocking the protein synthesis that is required for further growth of the organisms. Hence, metal ions are essential for the growth-inhibitory effects[28]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials, so lipophilicity is an important factor controlling the antifungal activity. Upon chelation, the polarity of the metal ion will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. In addition, chelation allows for the delocalization of π-electrons over the entire chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity facilitates the penetration of the complexes into lipid membranes, further restricting proliferation of the microorganisms. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the microbial cells or on differences in the ribosomes of the cells[29]. All of the metal complexes possess higher antifungal activity than the ligand[30].

3.9.3.1 Mode of action: Although the exact biochemical mechanism is not completely understood, the mode of action of antimicrobials may involve various targets in the microorganisms. These targets include the following: (i) The higher activity of the metal complexes may be due to the different properties of the metal ions upon chelation. The polarity of the metal ions will be reduced due to the overlap of the ligand orbitals and partial sharing of the positive charge of the metal ion with donor groups. Thus, chelation enhances the penetration of the complexes into lipid membranes and the blockage of metal binding sites in the enzymes of the microorganisms[31]. (ii) Tweedy's chelation theory predicts that chelation reduces the polarity of the metal atommainly because of partial sharing of its positive charge with donor groups and possible electron delocalization over the entire ring. This consequently increases the lipophilic character of the chelates, favoring their permeation through the lipid layers of the bacterial membrane[32]. (iii) Interference with the synthesis of cellular walls, causing damage that can lead to altered cell permeability characteristics or disorganized lipoprotein arrangements, ultimately resulting in cell death. (iv) Deactivation of various cellular enzymes that play a vital role in the metabolic pathways of these microorganisms. (v) Denaturation of one or more cellular proteins, causing the normal cellular processes to be impaired. (vi) Formation of a hydrogen bond through the azomethine group with the active centers of various cellular constituents, resulting in interference with normal cellular processes[33].

In vitro antibacterial effects of the investigated compounds were tested against activity against chloroquine sensitive strain and resistance strain. The results showed that the AA-5 exhibits moderate activity, but complex AA-**10** exhibits good activity for chloroquine sensitive strain and resistance strainspecies and significant for Gram-negative species.

3.9.3.2 Effect of azomethine (-C=N-) group: The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (-C=N-) with the active centers of cell constituents, resulting in interferences with the

normal cell process[34].

4. Conclusions

On the basis of this work, it is concluded that Schiff base ligand **AA-5** and its complex **AA-10** are non-significant as compared to standard BHA and Schiff base AA-5 has significant result while the complex AA-**5** shows non-significant urease inhibition as compared with thiourea. *In vitro* antibacterial effects of the investigated compounds showed that the ligand AA-**10** exhibits moderate activity, but complex **AA-5 and** AA-10 exhibits good activity for chloroquine sensitive strain and resistance strainspecies and significant for Gram-negative species

Supplementary Material

CCDC-880349 contains the supplementary crystallographic data for compound **2**. The data can be obtained free of charge from http://www.ccdc.cam.ac.uk/conts/retrievinghtml or by e-mailing: deposit@ccdc.cam.ac.uk; or by contacting: The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(0)1223-336-033.

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