

## Synthesis, Characterization and Antibacterial Activity of Some New Neocuproine Schiff Bases

**Mohammad Habibi Juybari (PhD)**

Department of Chemistry, Gorgan Branch Islamic Azad University, Gorgan, Iran

**Hamidreza Pordeli (PhD)**

Department of Microbiology, Gorgan Branch Islamic Azad University, Gorgan, Iran

**Saeid Mikaeili (MSc)**

Department of Microbiology, Gorgan Branch Islamic Azad University, Gorgan, Iran

**Corresponding author:** Mohammad Habibi Juybari

**Email:** m.habibi@gorganiau.ac.ir

**Tel:** +98-1732160840

**Address:** Islamic Azad University, Gorgan Branch, Daneshjoo Avenue, Shahid Kalantari Boulevard, Gorgan, Iran

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### ABSTRACT

**Background and Objectives:** Schiff base ligands are prepared via the condensation reaction of 1, 10- dimethyl-phenantroline aldehyde derivative with some nitrogen donor ligands, such as benzene ring that have different functional groups (-OH, -SH, -OCH<sub>3</sub>, -CH<sub>2</sub>OH, -Br) in acetonitrile. Recent studies suggest that Schiff bases might have antibacterial activity. Therefore, we aimed to synthesize new Schiff base complexes and evaluate their antibacterial activity against a number of Gram-positive and Gram-negative bacteria.

**Methods:** Schiff base ligands and their complexes were characterized by mass spectrometry, infrared spectroscopy and nuclear magnetic resonance spectroscopy. The in vitro antibacterial activity of the Schiff base ligands and metal ions against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was evaluated by determining minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the broth dilution method.

**Results:** All synthesized Schiff bases exhibited favorable antibacterial activity against the tested microorganism, but the antibacterial effect of compounds 3OH and 3SH was more significant than that of other compounds.

**Conclusion:** Compound 3EOH has favorable antibacterial activity against the tested bacteria.

**Keywords:** Schiff bases, antibacterial effect, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*.

## INTRODUCTION

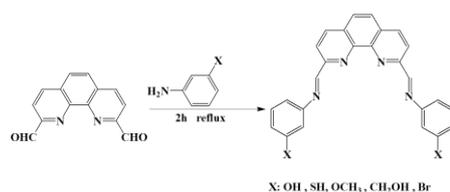
The design of new Schiff bases with antibacterial properties has received considerable attention over the past several years due to the presence of anions in biological systems and their crucial role in medicinal, catalytic and environmental chemistry (1, 2). Schiff bases are amongst the most widely used organic compounds. They are used as pigments and dyes, catalysts, intermediates in organic synthesis, and as polymer stabilizers. They also exhibit a broad range of biological activities, including antifungal, antibacterial, antimalarial, anti-proliferative, anti-inflammatory, antiviral, and antipyretic activities (3, 4). The first preparation of imines was reported in the 19<sup>th</sup> century by Schiff. The process involves condensation of a

Carbonyl compound with an amine under azeotropic distillation (Scheme 1).

Scheme 1. Preparation of phenylimino (methyl)-1,10-phenanthroline derivatives (Schiff based derivatives)

Moreover, molecular sieves are used to completely remove the water formed in the system (4).

The increase in the mortality rate associated with infectious diseases is directly linked to the emergence and spread of drug resistant bacteria (6). Therefore, developing a novel and effective antibacterial agent against such pathogens is urgently needed (7). Schiff bases have been proposed as promising antibacterial agents (8). Therefore, we aimed to synthesize, characterize and assess antibacterial activity of some new neocuproine Schiff bases.



## MATERIALS AND METHODS

We used 2,9-Dimethyl-1,10-phenanthroline (neocuproine) as a starting material for the synthesis of multidentate ligands (Scheme 2). Selenium dioxide gives a high yield in this reaction. Compound (II) was prepared according to the literature (9, 10).

Given the similar position of the methyl group in the composition of neocuproine, we thought that selenium would be suitable for the first phase of the reaction (Scheme 2).

All chemicals and solvents used in this study were purchased from Merck (Germany). Melting points (uncorrected) were determined on a Kofler hot-stage. Infrared spectra were obtained using a Shimadzu 470 spectrophotometer (potassium bromide disks). Nuclear magnetic resonance (H-NMR) spectra were recorded using a Varian 500 spectrometer and chemical shifts were reported in parts per million (ppm) relative to that of tetramethylsilane (TMS) as the internal standard. Chemical shift values were given in  $\delta$  scales. The mass spectra were recorded on LC-MS-Agilent 1100 series and API 2000 LC/MS system. Completion of the reaction was checked by thin layer chromatography

(TLC) on silica gel-coated aluminum sheets (silica gel 60 F254). Commercial grade solvents and reagents were used without further purification.

An equimolar mixture of 1, 10-phenanthroline-2, 9-dicarbaldehyde (1 mmole) and 3-Aminophenol (2 mmole) was refluxed in a little acetonitrile for 2 hours. The precipitate was filtered and recrystallized from acetone giving (3OH) a yield of 90%, m.p:220-222 °C. IR (cm<sup>-1</sup>): 3367(O-H), 3236(Ar-H), 3055(C-H), 1604 (C=N), 1278(C-O), <sup>1</sup>HNMR (500Mhz, DMSO): [ $\delta$  = 9.64 (2H<sub>m</sub>)], [ $\delta$  = 8.92( 2He,)], [ $\delta$  = 8.79 ( 2Ha)], [ $\delta$  =8.66 (2Hb)], [ $\delta$  = 8.56 (2Hc)], [ $\delta$  = 8.18( 2H<sub>h</sub>,)], [ $\delta$  = 7.28 ( 2H<sub>f</sub>)], [ $\delta$  =6.92 (2Hg)], [ $\delta$  =6.92 (2H<sub>k</sub>)].

An equimolar mixture of 1,10-phenanthroline-2,9-dicarbaldehyde (1 mmole) and 3-aminobenzenethiol (2 mmole) was refluxed in a little acetonitrile for 2 hours. The precipitate was filtered and recrystallized from acetone giving (3SH) a yield of 94%, m.p:210-212 °C. IR (KBr, v<sub>max</sub>, cm-1): 3068(Ar-H), 2807(C-H), 2684(S-H), 1616(C=N), 1508(C=N), 1278(C-O), <sup>1</sup>H NMR (500 MHz, DMSO): [ $\delta$  =

9.72 (2H<sub>m</sub>), [δ = 8.99 (2H<sub>e</sub>)], [δ = 8.63 (2H<sub>a</sub>)], [δ = 8.53 (2H<sub>b</sub>)], [δ = 8.12 (2H<sub>c</sub>)], [δ = 7.46 (2H<sub>h</sub>)], [δ = 7.44 (2H<sub>f</sub>)], [δ = 6.89 (2H<sub>g</sub>)], [δ = 6.87 (2H<sub>k</sub>)].

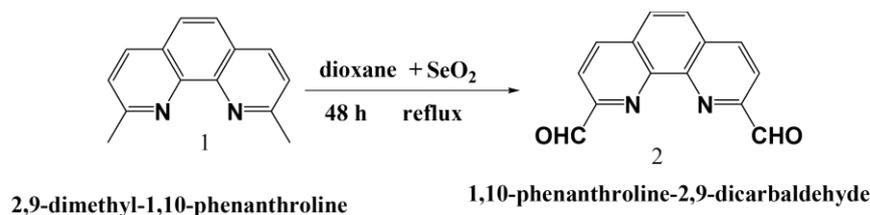
An equimolar mixture of 1,10-phenanthroline-2,9-dicarbaldehyde (1 mmole) and 3-methoxybenzenamine (2 mmole) was refluxed in a little acetonitrile for 2 hours. The precipitate was filtered and recrystallized from acetone giving (3M) a yield of 85%, m.p.: 185-188 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 2998(Ar-H), 2902(Ar-H), 1579(-C=N), 1504(-C=N), 1288(Ar-O), 1247(Ar-O-CH<sub>3</sub>), 1033(-O-CH<sub>3</sub>), 869(Ar-H), <sup>1</sup>H NMR (500 MHz, DMSO): [δ = 9.02 (2H<sub>e</sub>)], [δ = 8.66 (2H<sub>a</sub>)], [δ = 8.56 (2H<sub>b</sub>)], [δ = 8.15 (2H<sub>c</sub>)], [δ = 7.56 (2H<sub>h</sub>)], [δ = 7.55 (2H<sub>f</sub>)], [δ = 7.54 (2H<sub>g</sub>)], [δ = 7.54 (2H<sub>k</sub>)], [δ = 7.06 (3H<sub>m</sub>)].

An equimolar mixture of 1,10-phenanthroline-2,9-dicarbaldehyde (1mmole) and 3-bromo-benzenamine (2 mmole) was refluxed in a little acetonitrile for 2 hours. The precipitate was filtered and recrystallized from acetone giving (3Br) a yield of 80%, m.p.: 225-228 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3411(Ar-H), 1621(-C=N), 1581(-C=N), 1498(C-C)Ar, 1085(C-Br), 856(Ar-H). <sup>1</sup>H NMR (500 MHz, DMSO): [δ = 8.98 (2H<sub>e</sub>)], [δ = 8.70 (2H<sub>a</sub>)], [δ = 8.57 (2H<sub>b</sub>)], [δ = 8.12 (2H<sub>c</sub>)], [δ = 7.67(2H<sub>h</sub>)], [δ = 7.54 (2H<sub>f</sub>)], [δ = 7.49 (2H<sub>g</sub>)], [δ = 7.45 (2H<sub>k</sub>)].

An equimolar mixture of 1,10-phenanthroline-

2,9-dicarbaldehyde (1mmole) and 4-Aminobenzyl alcohol (2 mmole) was refluxed in a little acetonitrile for 2 hours. The precipitate was filtered and recrystallized from acetone giving (3Ben) a yield of 90%, m.p.: 175-178 °C. IR (KBr, v<sub>max</sub>, cm<sup>-1</sup>): 3378(O-H), 3056(Ar-H), 1498(-C=N), 1467(-C=N), 856(Ar-H), <sup>1</sup>H NMR (500 MHz, DMSO): [δ = 8.91 (2H<sub>m</sub>)], [δ = 8.67 (2H<sub>e</sub>)], [δ = 8.56 (2H<sub>a</sub>)], [δ = 8.10 (2H<sub>b</sub>)], [δ = 7.46 (2H<sub>c</sub>)], [δ = 7.40(2H<sub>h</sub>)], [δ = 7.38 (2H<sub>f</sub>)], [δ = 7.33 (2H<sub>g</sub>)], [δ = 7.31 (2H<sub>k</sub>)], [δ = 4.59 (4H<sub>i</sub>)]. All the newly synthesized compounds were screened for antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* by determining minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the 2-fold dilution method (3, 11). Several colonies of *S. aureus*, *E. coli* and *P. aeruginosa* were taken from fresh cultures and inoculated in separate tubes containing 5 mL of trypticase soy broth. The tubes were incubated at 37 °C for 6 hours until achieving visible growth. Turbidity was adjusted to 0.5 McFarland Standard by adding 0.05 mL of 1% w/v BaCl<sub>2</sub>.2H<sub>2</sub>O in phosphate buffered saline (PBS) to 9.95 mL of 1% v/v H<sub>2</sub>SO<sub>4</sub> in PBS. Finally, 10 mg of each compound were dissolved in 10 mL of dimethylformamide to obtain concentration of 1 mg/mL.

Scheme 2- Preparation of 1,10-phenanthroline-2,9-dicarbaldehyde



## RESULTS

Chemical structure of some of the most important derivatives are shown in scheme 3.

The results of the MBC and MIC assays are reported in Table 1.

Schem3- Chemical structure of the new Schiff base derivatives

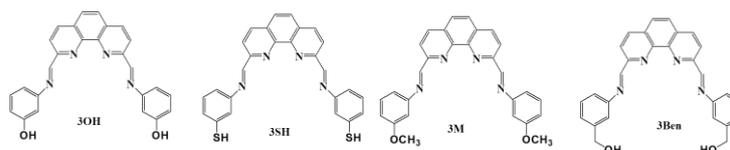


Table 1-The MIC and MBC of the synthesized Schiff bases against the tested bacteria

Bacterium	Concentration (mg/ml)	3EOH	3ESH	3M	3Br	3AB
<i>P. aeruginosa</i>	0.5	-	-	-	-	-
	0.25	-	-	-	-	-
	0.125	-	-	-	-	-
	0.0625	+	-	+	-	+
	0.031	+	+	+	+	+
	0.016	+	+	+	+	+
	MIC	0.125	0.0625	0.0625	0.125	0.125
	MBC	0.125	0.125	0.25	0.125	0.125
<i>E. coli</i>	0.5	-	-	-	-	-
	0.25	-	-	-	-	-
	0.125	-	-	-	-	-
	0.0625	+	-	+	-	-
	0.031	+	+	+	+	+
	0.016	+	+	+	+	+
	MIC	0.125	0.0625	0.0625	0.125	0.125
	MBC	0.125	0.0625	0.5	0.0625	0.125
<i>S. aureus</i>	0.5	-	-	-	-	-
	0.25	-	-	-	-	-
	0.125	-	-	-	-	-
	0.0625	+	-	+	-	+
	0.031	+	+	+	+	+
	0.016	+	+	+	+	+
	MIC	0.125	0.0625	0.0625	0.125	0.125
	MBC	0.25	0.25	0.25	0.125	0.25

MIC and MBC are reported in mM.

## DISCUSSION

All the synthesized compounds were tested against one gram-positive and two gram-negative strains of bacteria. Among the tested compounds, compound 2ESH showed good MIC, and compounds 3EOH and 3ESH showed moderate to good antibacterial activity. All synthesized compounds exhibited favorable antibacterial effect against *S. aureus*. However, given the smaller MIC and MBC values, compound 4EOH showed the highest antibacterial activity against *S. aureus*. Although all synthesized compounds had relatively favorable effects against *E. coli*, this effect was more significant in the case of compound 2ESH. Given the results of the MBC and MIC assay, all synthesized compounds had good antibacterial effects against *P. aeruginosa*, but the activity of compounds 3ESH and 3EOH against this bacterium were more profound.

## CONCLUSION

The results show that compound 3EOH has the highest inhibitory and bactericidal

Effects against the gram-negative bacteria, while compounds 3EOH, 3ESH and 3M have equal inhibitory and bactericidal effects against *P. aeruginosa*, *E. coli* and *S. aureus*.

In addition, compound 3EOH has a relatively better MIC and MBC values against the tested bacteria.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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