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SYNTHESIS, CHARACTERIZATION AND NEUROTOXIC EFFECT OF SCHIFF BASE LIGANDS AND THEIR COMPLEXES

(Sintesis, Pencirian dan Pengaruh Nurotoksik Bes Schiff dan Kompleksnya)

Ahmad Fauzi Abu Bakar[1]*, Hadariah Bahron[1], Karimah Kassim[1], Mazatulikhma Mat Zain[2]

[1]Department of Chemistry, Faculty of Applied Sciences,
[2]Institute of Science (IOS),
Universiti Teknologi MARA
40450 Shah Alam, Selangor, Malaysia

**Corresponding author: fauzi_charming@yahoo.com*

Abstract

Two ligands and eight complexes are successfully synthesized by reacting 1,8-diaminonapthelene with aldehyde/ketone derivatives in 1:2 ratio for ligand formation and 1:1 ratio for complex formations. The compounds are characterized through CHN elemental anaysis, IR spectroscopy, ^{1}H NMR spectroscopy, melting point and magnetic susceptibility determination. The neurotoxicity of compounds is evaluated using neuroblastoma SH-SY5Y cell lines and it was indicate the cells were non-toxic either for ligands and complexes after 24 hours exposure.

Keywords: Schiff base, 1,8-diaminonaphthalene, neurotoxicity, SH-SY5Y cell lines

Abstrak

Dua ligan dan lapan kompleks berjaya disintesis dengan tindakbalas 1,8-diaminonapthelene dengan keton/aldehid terbitan dengan nisbah 1:2 untuk pembentukan ligan dan nisbah 1:1 untuk pembentukan kompleks. Sebatian dicirikan melalui analisis unsur, spektroskopi IR, spektroskopi ^{1}H NMR, takat lebur dan penetapan suseptibiliti magnet. Neurotoksisiti sebatian dinilai menggunakan baris sel neuroblastoma SH-SY5Y yang mendedahkan bahawa ligan dan kompleks tidak toksik ke atas sel-sel selepas 24 jam pendedahan.

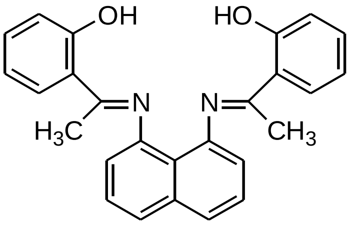
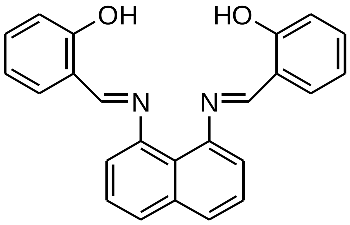
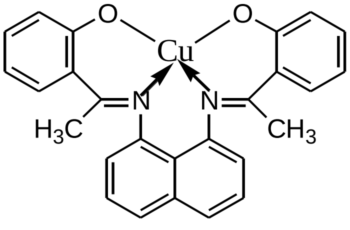
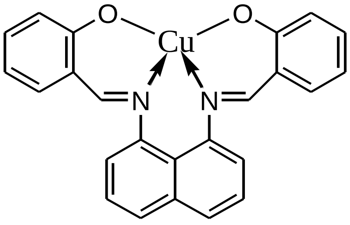
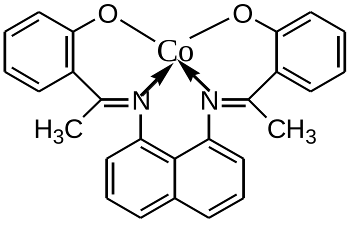
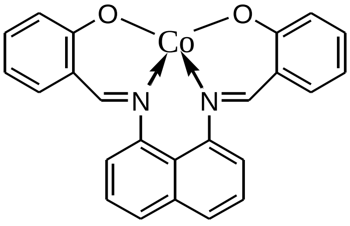
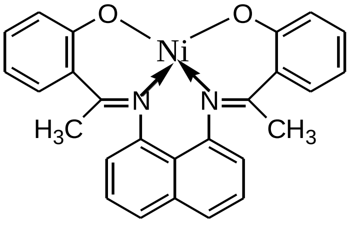
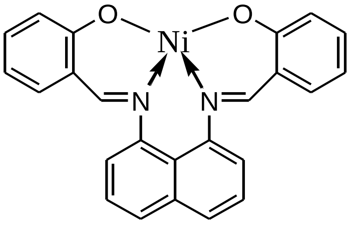
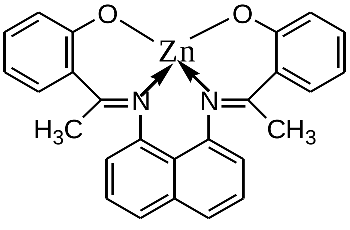
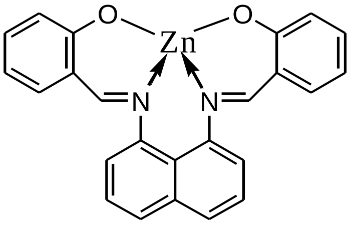
Kata kunci: Bes Schiff, 1,8-diaminonaftalena, neurotoksisiti, baris sel SH-SY5Y

Introduction

Schiff base formed by condensation of a primary amine and aldehyde/ketone resulting $R^1HC=N-R^2/R^1H_3C=N-R_2$. it is particularlyfor binding metal ion through N atom that has lone pair on it. It usually used for combining one or more donor atom to form polydentate or macrocyclic ligand [1]. Schiff bases also are characterized by the presence of –N=C– (imine) functional group [3] that can be derived from a large number of carbonyl compounds and amines which is important in biological systems [8]. It was found that Schiff bases produced from salicyldehyde and its derivatives yield polydentate ligands mostly which can form stable complexes with transition metals [8]. Most of the matel make the ration 1:1 to the metal formed complexes with Schiff base. [7]. Schiff base and their first row transition metal complexes [(Cu(II), Co(II), Ni(II) and etc.)] were reported to exhibit fungicidal, bactericidal, antiviral and antitubucular activity [1]. Metal complexes with Schiff base as ligand played an important role in development of coordination chemistry [1]. The antifungal and antibacterial properties of a range of Cu(II) complexes have been evaluated against several phatogenic and the bacterial [5]. Copper complex wih drugs are much more active in the presence of nitrogen donor heterocyclic ligand [5]. Co(II) complexes with N-donor ligands containing binding units suitable either for coordination of single metal ion or for assembling dimetallic center [6].

The research investigation concerns on Schiff base compounds synthesized from the condensation reaction of 1,8-diaminonaphthalene, an aromatic amine containing two fused benzene rings, with aldehydes and ketones containing electron donating and electron withdrawing substituents in the benzene ring. The compounds investigated are illustrated in Table 1. In this study, we prepared a variety of Schiff base ligands (L) and copper(II), cobalt(II), nickel(II) and zink(II).These compounds are then evaluated for their toxicity on SH-SY5Y neuroblastoma cell lines.

Table 1: Expected Schiff base ligands (L) and its complexes.

	 L1	 L2
$CuCl_2.2H_2O$	 CuL1	 CuL2
$CoCl_2.6H_2O$	 CoL1	 CoL2
$NiCl_2.6H_2O$	 NiL1	 NiL2
$ZnCl_2$	 ZnL1	 ZnL2

Experimental

Physical measurements

The elemental analysis was carried out on Thermo Finnigan Flash EA 110 Elemental Analyzer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Varian 300 Hz. Infrared spectra were obtained on Perkin Elmer 1750X FTIR spectrophotometer on KBr discs in the range 4000–350 cm^{-1} and melting points were measured using BÜCHI Melting Point B-545. Magnetic susceptibility balances of compounds were measured on Sherwood Auto Magnetic susceptibility balance.

Materials

The starting materials used in this work were 1,8-diaminonaphthalene, 2-hydroxyacetophenone, salicyldehyde, copper(II) chloride dihydrate, cobalt(II) chloride hexahydrate, nickel(II) chloride hexahydrate and zinc(II) chloride purchased from Sigma Aldrich. All chemicals and solvents were of analytical grade and used as received without prior purification.

Synthesis of L1

Ethanolic solution of 2-hydroxyacetophenone (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for 8 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of CuL1

Complex CuL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of copper(II) chloride dehydrate (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of CoL1

Complex CoL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of cobalt(II) chloride hexahydrate (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of NiL1

Complex CuL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. The nickel(II) chloride hexahydrate (1 mmol) solution was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of ZnL1

Complex CuL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of zink(II) chloride (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of L2

Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for 8 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of CuL2

Complex CuL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of copper(II) chloride dehydrate (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of CoL2

Complex CoL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of cobalt(II) chloride hexahydrate (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of NiL2

Complex NiL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of nickel(II) chloride hexahydrate (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Synthesis of ZnL2

Complex ZnL1 was prepared by the *in-situ* method. Ethanolic solution of salicyldehyde (2 mmol) was added dropwise to a stirred ethanolic solution of 1,8-diaminonaphthalene (1 mmol). The reaction mixture was refluxed for about 3 hours. Then ethanolic of zink(II) chloride (1 mmol) was added into the mixture. After all mixture was completed the mixture was heated under reflux for 5 hours. Then the solution was allowed to slowly evaporate at room temperature, upon which a precipitate was obtained. The product was filtered off, washed with cold ethanol and vacuum dried.

Cytotoxicity effect

Human neuroblastoma SH-SY5Y cell lines were given as a gift from cell culture laboratory of Universiti Teknologi MARA (UiTM). The cells differentiated to neutral phenotype with addition of retinoic acid (RA). Incubation of cells in humidified atmosphere at 37°C in 5% of CO_2 conducted in 6 days before testing. Media changed after 3 days.

Small volumes of ligands were added to each well to make the dilution 10nM, 100nm, 1µM, 10µM, 100µM and 1mM. Cultures incubated in humidified atmosphere at 37°C in 5% of CO_2 for 24 hours.

After incubated for 24 hours, 20µl of MTS solution added to each well and incubated in humidified atmosphere 37°C in 5% of CO_2 for 2-4 hours in the dark. The quantity of formazan product present was determined by measuring its absorbance at 490nm using GloMax Multi Detection System. Cell viability was determined. Compound will be considered as non toxic if the viability of cells is above 50% [4].

Results and Discussion

The Schiff bases were synthesized by condensation reaction between ketone/aldehyde derivatives and 1,8-diaminonaphthalene in 2:1 molar ratio in ethanol and in 1:1 molar ratio between ligand and metal salt. All the Schiff bases are stable at room temperature and soluble in DMSO.

Elemental Analysis (C, H, N)

From the Table 2, comparing the experimental and theoretical values of the C, H and N percentages, it could be seen that the two sets of Schiff base compounds (L1 and L2 with metal complexes) that reported are acceptable. Therefore it could be suggested that the intended Schiff bases have been successfully isolated. The melting point of each ligands were found below 200°C while for complexes were all found above 200°C. This suggested that the compound were stable in air.

Table 2: Data of Elemental Analysis

Comp.	**Color**	**M.P (°C)**	**C**		**H**		**N**	
			Theo	Expt	**Theo**	Expt	**Theo**	Expt
L1	Reddish Brown	150	**79.16**	80.91	**5.62**	5.82	**7.10**	7.58
CuL1	Grey	265	**68.48**	67.31	**4.42**	4.06	**6.14**	5.32
CoL1	Dark Brown	268	**69.18**	68.76	**4.47**	3.41	**5.80**	5.45
NiL1	Dark Brown	260	**69.22**	68.75	**4.47**	4.67	**6.21**	6.02
ZnL1	Dark Brown	274	**68.21**	68.35	**4.40**	4.22	**6.12**	6.77
L2	Light Brown	179	**78.67**	77.66	**4.95**	5.34	**7.65**	7.45
CuL2	Grey	257	**67.36**	65.79	**3.77**	3.19	**6.55**	7.14
CoL2	Black	267	**68.09**	68.66	**3.81**	3.11	**6.62**	6.51
NiL2	Dark Brown	262	**68.13**	67.65	**3.81**	3.59	**6.62**	5.16
ZnL2	Black	267	**67.07**	66.14	**3.75**	3.83	**6.52**	6.71

Infrared Spectroscopy

Using Perkin Elmer 1750X FTIR spectrophotometer on KBr discs, Table 3 show the data of infrared spectroscopy investigation of the Schiff bases revealed strong bands in the region 1600 cm^{-1} due to ν(C=N) [8]. Due to ν(-OH), IR peaks appear in the spectra of each Schiff bases in the region 2800 cm^{-1} [2]. The peaks of ν(-H_2O) appear around 3500 cm^{-1}. Peak at ν(-OH) will be disappeared when it formed complexes.

^{1}H Nuclear Magnetic Resonance Spectroscopy

By using ^{1}H NMR spectroscopy, the number of hydrogen can be quantified in the Schiff base compounds. The results obtained compared positively with the predicted ^{1}H NMR from ChemDraw Ultra 7.0.

From ^{1}H NMR spectrum data for the free ligand L1 shows the multiplet in the range 7.38- 8.10 ppm due to the aromatic proton. Singlet O-H proton will appeared at 12.98 ppm. Singlet methyl proton will appear at around 1.82 ppm while free lidand L2 shows the multiplet in the range 7.00- 8.20 ppm due to the aromatic proton. Singlet O-H proton will appear around 10.66 ppm.

For the complexes L1, the multiplet spectrum that represent aromatic proton will appear at range 6.73- 7.84. Singlet methyl proton will appear around 1.65 ppm while complexes L2, the multiplet in the range 7.24- 8.22 ppm that indicates aromatic proton and the azomethine proton will appear around 8.54 ppm.

Table 3: Data of Infrared Spectroscopy

Peaks	L1	CuL1	CoL1	NiL1	ZnL1	L2	CuL2	CoL2	NiL2	ZnL2
Broad ν (-OH)	3376.81	3446.53	3367.97	3260.22	3134.32	3424.35	3467.11	3400.49	3400.03	3544.17
ν (-OH)	2846.79	-	-	-	-	2848.70	-	-	-	-
ν (C=N)	1600.69	1628.13	1628.11	1618.11	1631.33	1601.38	1621.21	1621.31	1638.52	1618.54
ν (M-O)	-	535.12	576.22	521.52	524.47	-	563.11	574.45	568.06	523.66
ν (M-N)	-	443.86	463.13	458.54	476.36	-	446.48	471.22	436.75	451.49

Magnetic susceptibility determination

Magnetic susceptibility was determined using Sherwood Auto Magnetic susceptibility balance and the data was shown at Table 4. This data indicated that Cu(II) [Ar]d^9 are found to have 1.62-1.69 B.M and its correspond to the one unpaired electron. The paramagnetic properties agree that the Cu(II) will be formed tetrahedral or square planar geometry. Co(II) [Ar]d^7 are found to have 3.23-3.64 B.M and shows as paramagnetic property because it has three lone pair in splitting diagram. From the splitting diagram it shows the Co(II) complexes has tetrahedral geometry. Since Ni(II) [Ar]d^8 has diamagnetic property and it will formed square planar geometry because the splitting diagram shows the electron are filling in all stage while Zn(II) [Ar]d^{10} has diamagnetic property to formed either tetrahedral or square planar geometry.

Table 4: Data of Magnetic susceptibility determination

Complexes	μ_{eff} (B.M.)	d^n	Suggested Geometry
CuL1	1.62	d^9	Tetrahedral/square planar
CoL1	3.64	d^7	Tetrahedral
NiL1	Diamagnetic	d^8	Square planar
ZnL1	Diamagnetic	d^{10}	Tetrahedral/square planar
CuL2	1.69	d^9	Tetrahedral/square planar
CoL2	3.23	d^7	Tetrahedral
NiL2	Diamagnetic	d^8	Square planar
ZnL2	Diamagnetic	d^{10}	Tetrahedral/square planar

Neurotoxic Effect

The percentages of viability of neuroblastoma SH-SY5Y cell lines after treatment with 2 Schiff bases and 8 complexes are illustrated in Figure 1. The results revealed that all the percentages of viable cells were well above 50% with the minimum cell viability (most toxic) just below 60% for the NiL1 and NiL2. This observation indicated that the Schiff bases ligands and its complexes under study were all considered as non-toxic to the neuroblastoma SH-SY5Y cell lines [4]. It is also interesting to note that L1 seemed to enhance the growth of these cells.

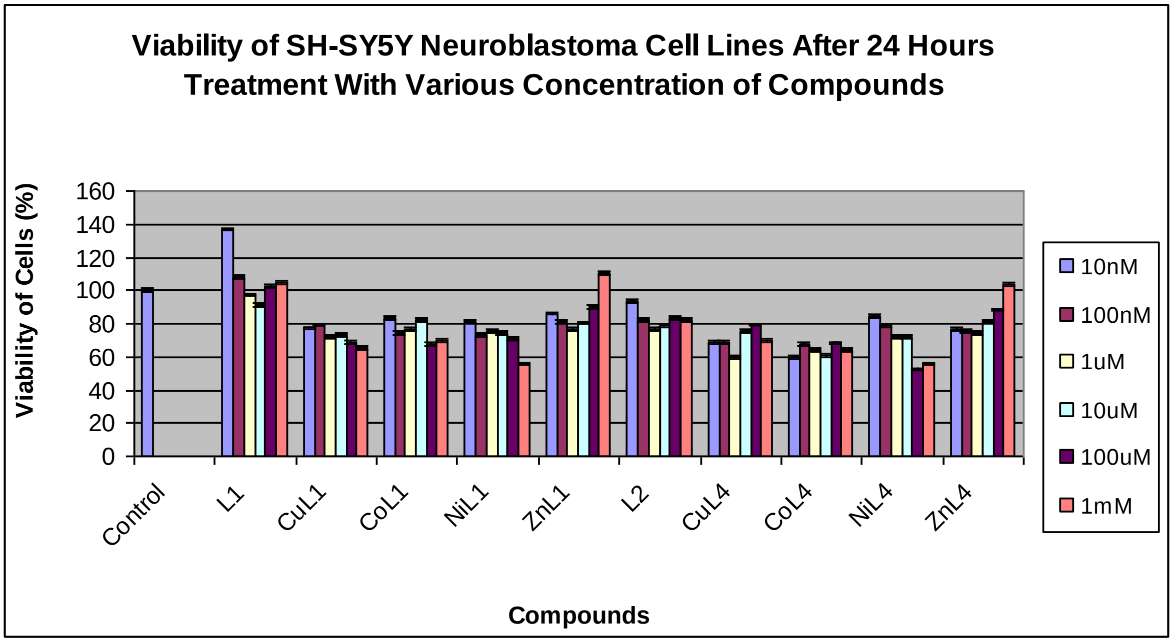


Figure 1: Viability of SH-SY5Y neuroblastoma cell lines after a 24-hour treatment with various concentrations of compounds.

Conclusion

The Schiff bases and their complexes were successfully synthesized and characterized. The compounds were found to be non-toxic to the SH-SY5Y neuroblastoma cell lines after a 24-hour treatment with L1 being the best compound that enhanced the cell growth.

Acknowledgement

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