Synthesis and Toxicity Test of Schiff Base Compound from 4-Formylpyridine and *p*-Anisidine Using Stirrer Method with Water Solvent

Mohammad Alfin[1], Ahmad Hanapi[2], Rachmawati Ningsih[3]
[1,2,3] Chemistry Department, Universitas Islam Negeri (UIN) Maulana Malik Ibrahim Malang, Indonesia
Email: [1] 16630104@student.uin-malang.ac.id

***Abstract*- Schiff base is a compound that is produced when primary amine and aldehydes or ketones reacted under certain situations. Schiff base compounds have the characteristic of a C=N (imine) functional group. The Schiff base compound was synthesized from 4-formylpyridine and *p*-anisidine using a stirrer method with water as the solvent and a 15-minute synthesis time. FTIR spectrophotometer and GC-MS are being used to identify the product of Schiff base compound. The BSLT (Brine Shrimp Lethality Test) method has been used to test the toxicity and LC_{50} values are used as parameters for the toxic levels of Schiff base compound. The results showed the synthesis product have a yield 99.86%. The product of the synthesis of Schiff base compounds has a physical characteristics such as: greenish-gray solid with a melting point of 91-93.5 ºC. The results of characterization using FTIR showed a typical absorption of the C=N group with the target compound at a wavenumber of 1620 cm^{-1}. The results of the GC-MS characterization showed one signal with a retention time of 36.5 minutes and a 100% area. The molecular ion of the m/z was 212, which also corresponded to the molecular weight of the compound 4-methoxy-N-(pyridine-4-ylmethylene)aniline. The results of the toxicity test of Schiff's base compound using the BSLT (Bhrine Shrimp Lethality Test) method showed the presence of toxic properties that have potential as anti-cancer with an LC_{50} value of 18.66 ppm.**

Keywords—4-formylpyridine; p-anisidine; Schiff base; stirrer method;

I. INTRODUCTION

A Schiff base is a compound with a characteristic imine or azomethine functional group. [1]. The condensation process between the primary amine and the carbonyl functional group (aldehyde or ketone) can produce in the Schiff base compounds [2]. Schiff base compounds, both natural and synthetic, have biological applications such as antibacterial [3-5], antifungal [6], antimicrobial [7-8], antitubercular [9], antioxidant [10], and anticancer [11].

The synthesis of Schiff base compounds can be carried out in two ways: using conventional methods and green chemistry methods. The conventional method is a method of synthesizing Schiff base compounds using reflux which can be complete using an acid catalyst [12] and organic solvents [13].

Conventional synthesis of Schiff base compounds can increase the usage of environmental contaminants to humans and the environment, so a more effective synthesis technique, called the green chemistry method, is required for Schiff base compounds [14]. The synthesis of Schiff base compounds using the green chemistry method can be done using the stirrer method with water solvent [15], natural acid catalysts [16], grinding without using catalysts and organic solvents [17], grinding method using a natural acid catalyst [18], and using a microwave method with water solvent [19]. In this research, the Schiff base compound was synthesized using stirrer method with water solvent.

Toxicity test method is a preliminary test used to determine the various bioactivities of the sample [20]. In this study, the toxicity test of Schiff base compound using Brine Shrimp Lethality Test (BSLT). BSLT method is an analytical method that has been widely used for toxicity tests. The advantages of using a Brine Shrimp Lethality Test (BSLT) method are; fast, simple, low requirements, sturdy, inexpensive, and high repetition rate [21]. The Median Lethal Concentration (LC_{50}) can be used to express the result of the toxicity using the Brine Shrimp Lethality Test (BSLT) method. Lethal concentration is the optimum concentration that can kill 50% of organisms in treatment, in this case, shrimp larvae from *Artemia salina*. The level of toxicity of Schiff base compounds based on the LC_{50} value can be seen in table I [22].

TABLE I
TOXICITY LEVEL BASED ON LC_{50} VALUE

Toxicity level	LC_{50} (µg/mL)
Non Toxic	>1000
Low	500-1000
Medium	200-500
High	100-200
Very High	1-100

II. METHODOLOGY

This research was implemented on a laboratory scale at the Organic Laboratory of the Department of Chemistry, Faculty of Science and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang.

A. synthesis of 4-methoxy-N-(pyridin-4-ylmethylene)aniline [15]

To a mixed solution of 1,1042-gram of *p*-anisidine compounds (0.01 mole) and 10 mL of water, add 1,2567-gram 4-formylpyridine compounds (0.01 mole). The resulting was then stirred at 450 rpm in a glass beaker for 15 minutes at room temperature. The solid formed is then filtered and dried in a desiccator until the weight is constant

B. Characterization of 4-methoxy-N-(pyridin-4-ylmethylene) aniline

Product of the Schiff base compound was characterized for its properties in the form of shape, color, mass, and melting point. Product characterization using FTIR was obtained using Shimadzu Infrared Spectrophotometer (Brawijaya University of Malang), using KBr plate. While GC-MS characterization using QP2010S/Shimadzu was obtained from the Gajah Mada University of Yogyakarta.

C. Toxicity Test [23-24]

Stock solution with a concentration of 500 ppm of Schiff's base compound can be done by weighing 12.5 mg of Schiff's base compound, then put into a 25 mL volumetric flask with the addition of chloroform solvent to the mark and homogenized. The sample solution concentrations of 10, 15, 20, 25, 30, and 35 ppm were pipetted from the stock solution. The solvent is evaporated to dryness after pouring the solution into a vial. 2 mL saltwater was added to the vial and mixed thoroughly. Then 100 µL of dimethyl sulfoxide (DMSO) solution was added. After that, add a drop of bread yeast solution and 10 *Artemia salina* (L.) shrimp larvae into the vial glass. Finally, add seawater until the volume becomes 10 mL. Each concentration was repeated 3 times. The LC50 value was defined using probit analysis with Minitab software where confidence level is 95%.

III. Result and Discussion

Physical characterization of the base compound Schiff 4-methoxy-N-(pyridine-4-ylmethylene)aniline consisted of phase, yield, color, and melting point characterization. This can be seen in table I. The difference in color and melting point between the reactants and the product compounds indicates the formation of a new Schiff base compound. Figure 1 depicts the reaction formation of a Schiff base compound.

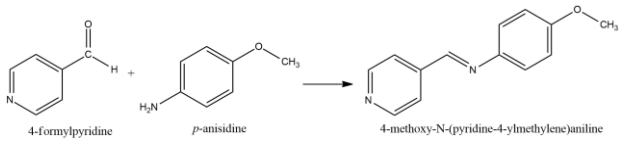

Fig. 1. Reaction formation of a Schiff base compound

FTIR spectrophotometer was carried out to determine the functional group product and to ensure that the resulting of the compound was 4-methoxy-N-(pyridine-4-ylmethylene)aniline,

TABLE II
PHYSICAL OBSERVATIONS REACTANTS AND PRODUCTS OF SCHIFF BASES

Observation	4-Formylpyridine	*p*-Anisidine	Schiff base
Phase	Liquid	Solid	Solid
Color	Clear brown	Black	Greenish-gray
Mass (gram)	1,1042	1,2567	2,1181
Yield (%)	–	–	99,86%
Melting point (ºC)	-4 [25]	57,2 [26]	91-93,5

Source; [25] Saglam et al., 2007 [26] S. Abed et al., 2015

which the results of the spectrum of was carried out by comparing the product compound with the two reactants. Figure 2 shows Schiff base product has sharp and strong absorption of the C=N functional group at wavenumber region of 1620 cm^{-1}, while the 4-formylpyridine and *p*-anisidine have a characteristically functional group C=O and N-H which indicated aldehyde and primary amine compounds at wavenumber 1712 cm^{-1} and 3323-3347 cm^{-1}. The disappearance of the C=O and N-H functional groups in the product spectra, which are characteristic of primary aldehyde and amine compounds, indicated the formation of a new Schiff base compound with an imine functional group (C=N).

The characterization of the synthetic product using GC-MS was carried out to determine the purity of the synthesized product and to estimate the presence of the target 4-methoxy-N-(pyridine-4-ylmethylene)aniline from the structure of the chemical formed based on the analysis of molecular ions, base peak spectra, and patterns fragmentation of the m/z molecular ion. On the chromatogram, Figure 4.4, the synthesized product consists of one chromatogram signal with a retention time of 36 minutes and a % area of 100%, which indicating the product has a compound purity of 100%. According to the mass spectra in Figure 4.5, the synthesis compound product has a molecular ion (M+) m/z 212, which also corresponded to the target compounds molecular weight of 212.09, strengthening the suspicion of the formation of the Schiff base compound 4-methoxy-N-(pyridine -4-ylmethylene)aniline.

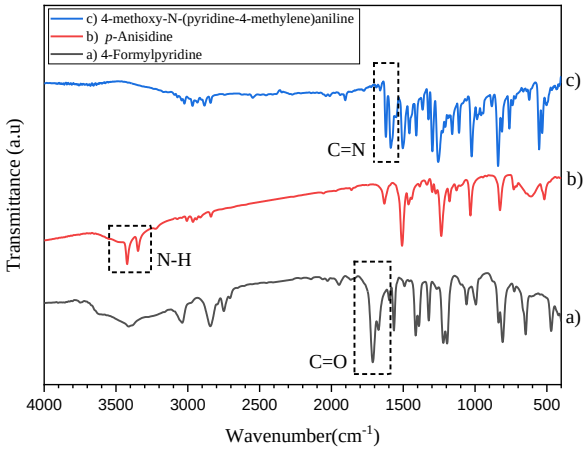

Fig.2. Spectrum IR of Schiff base and reactant

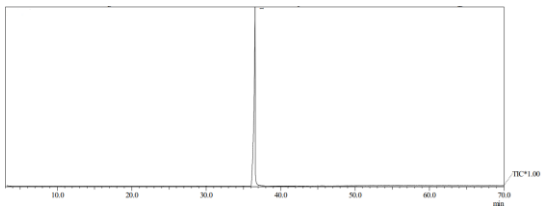

Fig. 3. Synthetic product chromatogram results

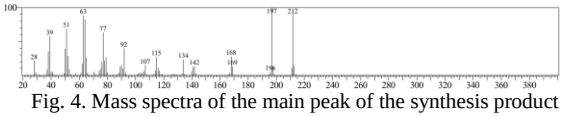

Fig. 4. Mass spectra of the main peak of the synthesis product

The results of toxicity test using probit analysis with Minitab 17 software where the confidence level is 95% in table III, the value of LC_{50} (Median Lethal Concentration) of Schiff base compound 4-methoxy-N-(pyridine-4-ylmethylene)aniline is 18.66 ppm. This means that the Schiff base compound with a concentration of 18.66 ppm can kill 50% of the test organisms, in this case, *Artemia salina* L. shrimp larvae. Schiff base compound 4-methoxy-N-(pyridine-4-ylmethylene)aniline has an LC_{50} value of 18.66 ppm ($LC_{50} \leq$ 100-30 ppm) [21], so it can be assumed that the Schiff base compound product has high toxicity and has potential as an anti-cancer [13]. As compared to the reactants, Schiff base 4-methoxy-N-(pyridine-4-ylmethylene)aniline has a greater toxicity value. The LC_{50} value for the compound 4-formylpyridine and *p*-anisidine was 43,64 and 22,42 ppm [27-28].

TABLE III
THE RESULTS OF TOXICITY TEST USING PROBIT ANALYSIS

Concentration (ppm)	% Mortality	Total shrimp larvae	LC_{50} (ppm)
10	24,14	10	18,66
15	38,34	10	
20	54,34	10	
25	69,65	10	
30	82,12	10	
35	90,75	10	

IV. CONCLUSION

The synthesis of Schiff base compounds produced a greenish-gray solid product with a melting point of 91-93.5 ºC and a yield of 99.86%. The C=N functional group in the IR spectra is shown at a wavenumber of 1620 cm^{-1}. The purity of the product compound in GC is 100% with the molecular ion peak (m/z) in the MS spectra is 212, which corresponds to the molecular weight of the target compound 4-methoxy-N-(pyridine-4-ylmethylene)aniline. Schiff base compound has potential as an anti-cancer with an LC_{50} value of 18.66 ppm

ACKNOWLEDGMENT

Gratefully acknowledge of financial support from the Indonesian Ministry of Religious and Sains and Technology Faculty of State Islamic University (UIN) Maulana Malik Ibrahim Malang.

REFERENCES

[1] A. Hameed, M. al-Rashida, M. Uroos, S. Abid Ali, and K.M. Khan, "Schiff Bases in Medicinal Chemistry: A Patent Review (2010-2015)," *Expert opinion on therapeutic patents*, vol. 27, No. 1, pp.63-79, May 2017.

[2] H.H. Abass, R.A. Salih, and A.A. Salih, "Synthesis, Characterization and Biological Activity of Two Phenol-Schiff Bases and Formaldehyde Resin Cobalt (II)-Complexes," *Global Journal of Pure and Applied Chemistry Research*, vol. 3, No. 2, pp.14-23, July 2015.

[3] E.B. Poormohammadi, M. Behzad, Z. Abbasi, and S.D.A. Astaneh, "Copper Complexes of Pyrazolone-Based Schiff Base Ligands: Synthesis, Crystal Structures and Antibacterial Properties," *Journal of Molecular Structure*, 1205, 127603, December 2019.

[4] Y. Xu, Y. Shi, F. Lei, and L. Dai, "A Novel and Green Cellulose-Based Schiff Base-Cu(II) Complex and its Excellent Antibacterial Activity," *Carbohydrate Polymers*, 230, 115671, November 2019.

[5] L. Zhang, P. Yan, Y. Li, X. He, Y. Dai, and Z. Tan, "Preparation and Antibacterial Activity of a Cellulose-Based Schiff Base Derived from Dialdehyde Cellulose and L-Lysine," *Industrial Crops and Products*, 145, 112126, January 2020.

[6] R.K. Mohapatra, A.K. Sarangi, M. Azam, M.M. El-ajaily, M. Kudrat-E-Zahan, S.B. Patjoshi, and D.C. Dash, "Synthesis, Structural Investigations, DFT, Molecular Docking and Antifungal Studies of Transition Metal Complexes with Benzothiazole Based Schiff Base Ligands," *Journal of Molecular Structure*, 1179, pp.65–75, October 2018.

[7] W.H. Mahmoud, R.G. Deghadi, and G.G. Mohamed, "Metal Complexes of Ferrocenyl-Substituted Schiff Base: Preparation, Characterization, Molecular Structure, Molecular Docking Studies, and Biological Investigation," *Journal of Organometallic Chemistry*, 121113, January 2020.

[8] C.M. Sharaby, M.F. Amine, and A.A. Hamed, "Synthesis, structure characterization and biological activity of selected metal complexes of sulfonamide Schiff base as a primary ligand and some mixed ligand complexes with glycine as a secondary ligand," *Journal of Molecular Structure*, 1134, pp.208–216, December 2016.

[9] R.S. Hunoor, B.R. Patil, D.S. Badiger, R.S. Vadavi, K.B. Gudasi, V.M. Chandrashekhar, and I.S. Muchchandi, "Spectroscopic, Magnetic and Thermal Studies of Co(II), Ni(II), Cu(II) and Zn(II) Complexes of 3-Acetylcoumarin–Isonicotinoylhydrazone and Their Antimicrobial and Anti-Tubercular Activity Evaluation," *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, vol. 77, pp.838–844, August 2010.

[10] Y. Chen, Y. Mi, Q. Li, F. Dong, and Z. Guo, "Synthesis of Schiff Bases Modified Inulin Derivatives For Potential Antifungal and Antioxidant Applications," *International Journal of Biological Macromolecules*, vol. 143, pp.714–723, September 2019.

[11] S. Shokrollahi, A. Amiri, F. Fadaei-Tirani, and K. Schenk-Joß, "Promising Anti-Cancer Potency of 4,5,6,7-Tetrahydrobenzo[D]Thiazole-Based Schiff-Bases," *Journal of Molecular Liquids*, 300, 112262, December 2019.

[12] J. M. Dinore, A. A. Yelwande, M. P. Palve and A.V. Sapkal, 2016. Citric Acid Catalyzed Synthesis of Hydrazones Schiff Bases of 2,4-Dinitrophenyl Hydrazine. *International Journal of Pharmacy & Pharmaceutical Research*, vol. 6, No. 1, pp.349-354, April 2016.

[13] S. Shamim, S. Murtaza, and M.F. Nazar, "Synthesis of Schiff Bases of Pyridine-4-Carbaldehyde and their Antioxidant and DNA Binding Studies," *Journal of the Chemical Society of Pakistan*, vol. 38, No. 3, January 2016.

[14] A.B. Thomas, P.N. Tupe, R.V. Badhe, R.K. Nanda, L.P. Kothapalli, O.D. Paradkar, P.A. Sharma and A.D. Deshpande, "Green Route Synthesis of Schiff's Bases of Isonicotinic Acid Hydrazide," *Green Chemistry Letters and Reviews*, vol. 2, No. 1, pp.23-27, March 2009.

[15] V. K. Rao, S. S. Reddy, B. S. Krishna, K. R. M. Naidu, C. N. Raju and S. K. Ghosh, "Synthesis of Schiff's bases in aqueous medium: a green

alternative approach with effective mass yield and high reaction rates," *Green Chemistry Letters and Reviews*, vol. 3, No. 3, pp.217-223, September 2010.

[16] D. Bhai R., C.R. Girija, and R. Reddy K., "Green Synthesis of Novel Schiff Bases Derived from 2, 6 Diamino Pyridine-Characterization and Biological Activity," *Journal of Advances in chemistry*, vol. 10, No. 5, pp.2705-2710, September 2014.

[17] U. Hasanah, A. Hanapi, and R. Ningsih, "Synthesis of Schiff Base Compound from Vanillin and p-Toluidine by Solvent Free-Mechanochemical Method," *In Proceedings of the International Conference on Green Technology*, vol. 8, No. 1, pp.278-281, October 2017.

[18] S. Patil, S.D. Jadhav, M.B. Deshmukh and U.P. Patil, "Natural Acid Catalyzed Synthesis of Schiff under Solvent-Free Condition: As a Green Approach," *International Journal of Organic Chemistry*, vol. 2, No. 2, pp.166-171, 2012.

[19] S. Bhagat, N. Sharma and T.S. Chundawat, "Synthesis of Some Salicylaldehyde-Based Schiff Bases in Aqueous Media," *Journal of Chemistry*, vol. 2013, August 2012.

[20] Z. Zuraida, "Analisis Toksisitas Beberapa Tumbuhan Hutan dengan Metode Brine Shrimp Lethality Test (BSLT)," *Jurnal Penelitian Hasil Hutan*, vol. 36, No. 3, pp.239-246, November 2018.

[21] M.R. Hamidi, B. Jovanova, and T.K. Panovska, "Toxicological Evaluation of The Plant Products Using Brine Shrimp *(Artemia Salina L.)* Model," *Maced pharm bull*, vol. 60, No. 1, pp.9-18, April 2014.

[22] H.D. Salusu, E. Obeth, A.R. Zarta, E. Nurmarini, H. Nurkaya, I.W. Kusuma, and E.T. Arung, "The Toxicity and Antibacterial Properties of *Calamus ornatus* Bl," *Rattan Fruit. agriTECH*, vol. 39, No. 4, pp.350-354, September 2019.

[23] M.F. Jasmarullah, "The Activity Test of Antioxidant and Toxicity Test of Schiff Base Compound from Vanilin and Aniline," *Theses*, Department of Chemistry, Faculty of Sains and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang, 2018.

[24] N. Millati, "Toxicity Test using BSLT Method of Steroid Compound in Petroleum ether Fraction from Microalgae *Chlorella sp*.," *Theses*, Department of Chemistry, Faculty of Sains and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang, 2016.

[25] A. Saglam, F. Ucun, and V. Guclu, "Molecular Structures and Vibrational Frequencies 2,3 and 4-Pyridinecarboxaldehydes by Abinition Hartree Fock and Density Functional Theory Calculations," *Spectrochimica Acta Part A: Moleculer and Biomolecular Spectroscopy*, vol. 67, No. 2, pp.465-471, August 2007.

[26] A.S. Abed, K.M. Ziadan, and A.Q. Abdullah, "Synthesis and Study Some Optical Properties of Conducting Polymer Poly p-Anisidine (PPANS) Doped with Camphor Sulphonic Acid (CSA)" *Basrah Journal of Science*, vol. 33, No 1A, pp.137-155, 2015.

[27] Mustofa "Toxicity Test of Schiff Base Compound of Synthesis Result from Vanilin and *p*-Anisidine," *Theses*, Department of Chemistry, Faculty of Sains and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang, 2019.

[28] Z.B. Laili, "Synthesis of Schiff Base Compound from 4-Formylpyridine and p-Toluidine Using Stirring Method (Water Solvent) and its Toxicity Test agains *Artemia Salina* L," *Theses*, Department of Chemistry, Faculty of Sains and Technology, State Islamic University (UIN) Maulana Malik Ibrahim Malang, 2020.