SYNTHESIS AND CHARACTERIZATION OF Co (II) PYRIDINE-2,6-DICARBOXYLATE COMPLEXES AS ANTICANCER COMPOUND

Fahimah Martak^{1,✉}, Endah Mutiara¹, Mahbub Alwathoni¹, Triyanda
Gunawan¹ and Soegeng Soegijanto²

¹Departement of Chemistry, Faculty of Science and Data Analitics, Institut Teknologi Sepuluh
Nopember, 60111 Sukolilo, Surabaya, Indonesia

²Dengue Study Group, Institut of Tropical Disease, Universitas Airlangga, Kampus C UNAIR,
Jalan Mulyorejo, Surabaya 60115, Indonesia

✉Corresponding Author: fahimahm@chem.its.ac.id

ABSTRACT

In this study, two cobalt [Co(II)] Complexes were synthesized using picolinic acid (2-pyridine carbocyclic) ligand under various pH conditions. Both Complexes were characterized using FTIR, UV-Vis, XRD, CHNS microanalysis, and thermogravimetric analysis (TGA). The Complex synthesized using metal ion: ligand mol ratio of 1:1 under pH ~2 has a molecular formula of $[Co^{II}(dipic)(\mu-dipic)-Co^{II}(H_2O)_5] \cdot 2H_2O$. On the other hand, the Complex synthesized using the higher ligand to metal ratio of 2 at pH ~1 has a molecular formula of $[Co^{II}(H_2dipic)-(dipic)] \cdot 3H_2O$. The $[Co^{II}(dipic)(\mu-dipic)-Co^{II}(H_2O)_5] \cdot 2H_2O$ Complex demonstrating toxicity toward *Artemia salina* larvae (BSL) with the LC_{50} value of 5.38 ppm. Meanwhile, the $[Co^{II}(H_2dipic)-(dipic)] \cdot 3H_2O$ possess an LC_{50} value of 8.61 ppm against the same larvae. Results showed that the Co (II)-2 Pyridine carbocyclic based Complex synthesized under acid environment demonstrating potential characteristic as anticancer cell

Keywords: Co (II) Complexes, Dipicolinate Ligand, Polymer Complexes, Toxicity Test, Anticancer Compound, BSL Test.

RASAYAN J. Chem., Vol. 14, No.3, 2021

INTRODUCTION

The study relating to picolinate Complexes showed the biological activity of inducing a murine leukemia HL-60 cell that can delay mycobacterium's *ovium* Complex growth.¹⁻³ Moreover, some reported metal-picolinate Complexes showing an effect in delaying the cancer cells. Van Rijt *et al.* synthesized Os(II) picolinate to slow the cancer cell growth.⁴ Their result showed that the synthesized Complex has similar properties as cisplatin, widely known as chemotherapy medicine. The LC_{50} of Os(II) picolinate value was 4.8 μM , slightly smaller than the carboplatin LC_{50} of 6 μM . The addition of one carbocyclic group in the picolinate ligand ring to produce a diplocinate structure (pyridine-2,6-dicarboxylate) was aimed to improve the toxicity against cancer cells and other bioactivities. To the best of our knowledge, the diplocinate Complexes properties against the cancer cell were not available in the literature. The research was limited to diabetes treatment and antibacterial. The Co (II) pyridine-2,6-dicarboxylates able to reduce the hyperlipidemia in diabetes.⁵ Moreover, reported that the metal dipicoclininate Complex act as the bacteria inhibitor growth.⁶ Thus, this research will carry out diplocinate based Complexes as the anticancer compound. Cobalt is one of the metals in the transition metal group in the periodic table widely used in medicinal chemistry due to the compatibility with the human body and acts as a trace element.^{5,7,8} In this study, cobalt used as an Osmium replacer in the 2-pyridine carboxylate Complexes aims to produce Complexes with similar LC_{50} (Lethal Concentration) value and better interaction in slowing the cancer cell growth.⁴ The Brine Shrimp Lethality Test (BSLT) is one of the most popular methods for the issue due to easy operation, fast and accurate by utilizing the *artemia salina* larvae as the test subject. The toxicity test result from the BSLT method usually relates to the cytotoxic of the anticancer compound and expressed in

LC₅₀(Lethal Concentration)percent.⁹⁻¹¹ The compounds are classified as less toxic when the LC₅₀ value in the range of 300 - 1000 mg/ml while the LC₅₀ < 100 mg/ml were for active compounds.¹¹⁻¹³

EXPERIMENTAL

Material

Cobalt chloride (CoCl₂·6H₂O) as the metal source, diplocinate acid (H₂dipic) as ligand, KBr, NaOH, HCl, HNO₃ as pH controller demineralized water, and ethanol absolute, which were purchased from Merck. CoCl₂ (anhydrous) was obtained from the calcined of CoCl₂·6H₂O at a temperature of 140°C for 4 hours. The toxicity test was conducted on shrimp larvae *Artemia salina*, and the seawater was taken from Kenjeran beach in Surabaya, Indonesia.

Methods

The Co^{II}(dipic)(μ-dipic)Co^{II}(H₂O)₅·2H₂O(1) Complex was synthesized from the reaction of CoCl₂ and H₂dipic with the ratio of 1:1 under acid environment (pH=1-3). The [Co^{II}(H₂dipic)dipic]·3H₂O Complex was synthesized by reacting CoCl₂·6H₂O and H₂dipic with a molar ratio of 1:2. The solution was conditioned at pH=1-3 by adding HCl 5M. The solution was stirred at a temperature of 90°C for 2h, cooled naturally afterward, and followed by aging for 5 days. Finally, the solution was filtered and recrystallized using demineralized water followed by drying at ambient conditions.

The Complexes characterization was involving ¹H NMR (Bruker AMX 400 NMR at 400 MHz), UV-Vis (Thermo scientific-Genesys 10S), X-ray diffractogram (CuKα radiation, λ=0.154 Å at 40 kV and 30 mA) on an Expert PAN Analytical, Atomic Adsorption Spectroscopy (Shimadzu AA-680), the thermal stability of the Complexes were evaluated using Thermal gravimetric analysis (Mettler Toledo TGA/SDTA 851). The lethal concentration (LD₅₀) of the synthesized Complexes was determined using Brine Shrimp Lethality (BSL) method.¹³ The LD₅₀ was measured by varying the concentration (0, 1, 10, 100, and 1000 ppm) of Complex (1) and (2) as gram-positive and repeated five times with the contact time of 24h.

RESULTS AND DISCUSSION

Effect of pH in the Complex Formation

The binuclear Complex of [Co^{II}(dipic)(μ-dipic)Co^{II}(H₂O)₅].xH₂O formation was initiated at the pH=1 and increasing as the acidity level decreased. The maximum absorbance was observed at pH=3 with maximum wavelength and absorbance of (λ_{maks}) 496 nm and 0.115. Thus, the Complex (1) was formed at the optimum pH=3. The increment of basic (NaOH) lead to the deprotonation of H₂dipic from ligand into dipic²⁻ ion and is followed by the coordination formation of Co in the Oxygen donor atom to form the binuclear Complex of [Co^{II}(dipic)(μ-dipic)-Co^{II}(H₂O)₅].xH₂O. NaOH was chosen as a basic reagent due to the Na⁺ has high solubility. The addition of NH₃ was also conducted to increase the pH. The Complex with NH₃ addition posses a *luteo* (yellowish) color, and it is suggested that CoCl₂·6NH₃ was formed.

In the Complex (2) formation, the optimum pH condition was observed at pH 1.0 as indicated with the highest absorbance value at the maximum wavelength of the Complex (2) formation (λ_{maks}) 465 nm. At pH 1.0, most of the pyridine-2,6 dicarboxylate acid was protonated into dipic²⁻ ion. The rest was not protonated and still in the H₂(dipic) form after forming coordination bonding with the central cobalt atom. The absorbance value at fewer acid conditions (pH 1.5 and 2.0) was decreased. The significant absorbance value drop was further observed at a later stage (pH 2.5 dan 3.0) and suggested the other cobalt-dipicolinate Complexes' formation. The possibility of the other cobalt Complex such as [Co(dipic)].3H₂O was observed at higher pH conditions of the metal: ligand reaction.⁵

The AAS Analysis

The light element analysis of each Complex was listed in Table. The Complex (1) element composition synthesized in this study was close to the calculated structure of [Co(dipic)(μ-dipic)Co(H₂O)₅].2H₂O, which indicated a similar amount in cobalt element. However, a slight difference in the C, H, and N percentage of the Complex (1) compared to the calculated structure was due to the impurities present, resulting in a lower amount of those elements than its calculated structure. Despite having a lower ligand to metal ratio of 1, the cobalt amount in the Complex (1) has a higher amount (20.34%) as compared to the Complex (2), 12.77%, which successfully synthesized at a higher ligand to metal ratio of 2. This result indicated that

Complex **(1)** possesses two nuclei (binuclear) of the central cobalt atom, while Complex **(2)** has one nucleus (mononuclear). The Complex **(2)** light element composition was close to the theoretically calculated structure of $[\text{Co}(\text{H}_2\text{dipic})(\text{dipic})].3\text{H}_2\text{O}$ with a percentage difference of less than 0.5%. This result confirms the assumption that Complex **(2)** is a mononuclear Complex while Complex **(1)** is a binuclear Complex. The pH and ligand to metal ratio were essential in determining the final product of the cobalt-dipicolinate based Complexes.

Table-3: The Element (C, H, N) Analysis of the Synthesized Complexes

Molecular Formula	%Co	%C	%H	%N
Complex (1)				
Sample (experiment)	20.34	28.82	3.42	4.64
$[\text{Co}(\text{dipic})(\mu\text{-dipic})\text{Co}(\text{H}_2\text{O})_5].2\text{H}_2\text{O}$	20.52	29.28	3.51	4.87
Complex (2)				
Sample (experiment)	12.77	37.98	3.31	6.96
$[\text{Co}(\text{H}_2\text{dipic})(\text{dipic})].3\text{H}_2\text{O}$	13.23	37.76	3.16	6.29

The FTIR Analysis of Synthesized Complexes

Fig.-1 shows the FTIR spectra of both Complexes. The broad peak at wavenumber around $3448 - 3101.54 \text{ cm}^{-1}$ corresponds to the O-H from the Complexes crystal water and water ligand. The C=N vibration of the pyridine ring in the Complex **(1)** was observed at the peak at $1620 - 1630 \text{ cm}^{-1}$.¹⁴ Medium peak intensity observed at 1712.79 cm^{-1} refers to the C=O vibration in the carboxylic group. The formation of the binuclear Complex **(1)** was confirmed in the peak that appeared at 1080.14 cm^{-1} , which corresponds to the Co-O vibration with O-dipicolinate as a bridging ligand. In comparison, the coordination bonding of oxygen with Co was observed at 424.37 and 331.76 cm^{-1} . The strong absorption peak at 686.66 cm^{-1} corresponded to the Co-N vibration.⁵ Similar peaks were also observed in the FTIR spectra of Complex **(2)**. The noticeable differences were in the fingerprint region of $1400 - 300 \text{ cm}^{-1}$. The peak observed at $671.23 - 432.05$ and 316.33 cm^{-1} were referred to as the Co-O and Co-N vibrations, respectively. Moreover, a significant difference was observed in the C-C peaks at 1373.32 cm^{-1} . The Complex (2) low rigidity structure leads to the higher absorption peak of C-C and C-O compared to the Complex **(1)**.

Fig.-1: FTIR Spectra of Complex (1) and (b) (2)

XRD Analysis of the Synthesized Complexes

The diffraction pattern of both Complexes was shown in Fig. The Complex (1) diffractogram pattern showed high-intensity peaks that indicated high crystallinity of the synthesized Complex. The nine highest peaks were further analyzed with the FULPROF 2006 software. The resulting calculation was that the crystal has a monoclinic shape with the space group of P2/m, $a=13.36$, $b=3.23$, $c=8.62$. The Complex (2) diffractogram pattern shows different patterns compared to the Complex (1), which confirms that both Complexes have a different crystal structure. The analysis result showed that the crystal has a similar monoclinic shape with the space group P2/m, $a=11.06$, $b=11.61$, and $c=6.46$, which was consistent with the single crystal data reported previously.^{5,15} Overall, the XRD study confirms that both Complexes have been successfully synthesized with high crystallinity.

Fig.-2: Diffractogram Pattern of Complex (a) (1) and (b) (2)

Thermogravimetric Study of the Complexes

Fig shows the thermograph profile of the synthesized Complexes. The thermograph profile of both Complexes showing a similar trend. The first mass loss occurred at a temperature of 70–120°C, which refers to the Complex crystal water loss. The mass reduction in this stage was equal to the ~3 mol of crystal water. The second step was the decomposition of the H₂dipic (~0.5 mol), which occurred at a temperature of 220–280°C. The last step was the ligand of pyridine-2,6-dicarboxylate loss at a temperature of 360–480°C. Theoretically, 1 mol of dipic ligand equal to the 37.1% mass, thus it was confirmed that the third mass reduction referred to the 1 mol of dipic ligand. The residue around 31.5% corresponded to the cobalt and the undecomposed ligand, and theoretically, 1 mol of Co and 0.5 mol of dipic equal to the 31.76% total mass of the Complex. Overall, the Complex thermographic profile was consistent with the dipic-based Complex reported previously.^{5,16}

Fig.-3: The Thermograph Profile of the Complex (a) (1) and (b) (2)

Structure of the Complexes

Based on the characterization data discussed previously, the synthesized Complexes molecular structure was illustrated in Fig.-2. The computational approaches toward the diffractogram data showed the single crystal and spacing groups similar to the monoclinic P2/m.⁵ The thermograph profile matched with the AAS analysis in terms of total residue produced after the heating process. Similar to the Complex (1), all the characterization data of the Complex (2) strongly suggest that the structure of the Complex was [Co(H₂dipic)(dipic)].3H₂O. The AAS analysis showed a difference in element content with less than 0.5% compared to the simulated structure.

Moreover, the FTIR spectra revealed less O-H from the water molecule (3402.43 cm⁻¹) than the Complex (1). The monoclinic P2/m crystal system. The percentage of residue in the thermograph profile of the Complex (2) matched with the AAS data that suggests the structure of the Complex (2) was [Co(H₂dipic)(dipic)].3H₂O.

Fig.-2 Structure Prediction of Synthesized Complex (a) (1) and (b) (2)

Toxicity Test

The Complex concentration treatment led to the disorientation movement of *Artemia*, which was spinning at one point. The factors that caused the *Artemia* to fall was an external factor that was outside the researcher control. As can be seen in Fig.-3, the linear regression obtained were $y=0.0451x-1.519$ and $y=0.049x-1.516$ for Complex (1) and (2), respectively. Thus, the LD_{50} value of the Complex (1) and (2) was 5.38 and 8.61, respectively.

Fig.-3 The Toxicity Properties of the Synthesized Complexes

A compound could show toxicity properties in the BSL test if it caused 50% death of subject test (LC_{50}) in less than 1000 $\mu\text{g/ml}$ sample concentration, while the pure compound was $LC_{50} < 200 \mu\text{g/ml}$.¹⁰ Based on the parameters mentioned above, the sample of Cobalt(II) chloride, $[\text{Co}(\text{dipic})(\mu\text{-dipic})\text{Co}(\text{H}_2\text{O})_5] \cdot 2\text{H}_2\text{O}$ (1) and $[\text{Co}(\text{H}_2\text{dipic})(\text{dipic})] \cdot 3\text{H}_2\text{O}$ (2) showing high toxicity against BSL sample. The binuclear Complex of $[\text{Co}(\text{dipic})(\mu\text{-dipic})\text{Co}(\text{H}_2\text{O})_5] \cdot 2\text{H}_2\text{O}$ (1) showed the highest LC_{50} of 5.38 ppm. The Complex (1) possessed two ligand types of dipicolinate and aqua. Aqua ligand has coordination interaction with cobalt and a weak ligand that is easily substituted by integral protein from membrane cell. This results in the blocking of the Na^+ and K^+ active transport. Stopped active transport led to the uncontrollable Na^+ ion entrance and caused membrane cell breaking.^{14,17,18} It is suggested that the hydrogen bonding in the Complex (1) contributed to reducing LC_{50} value. The hydrogen bonding deteriorated the membrane cell through the groove binding mechanism. This mechanism was highly influenced by the Complex molecule molecular geometry that interacted with the DNA and the electric field around the DNA framework, Van der Waals forces, hydrogen bonding, and hydrophobic effect.⁸ For Complex (2), the -OH groups from dipicolinate ligand bonded with the integral protein from the membrane cell and destroys the cell.

CONCLUSION

Two Complexes based on the cobalt (II) pyridine-2,6-dicarboxylate have been synthesized. The characterization results showed that the Complexes have a molecular formula of $[\text{Co}^{\text{II}}(\text{dipic})(\mu\text{-dipic})\text{Co}^{\text{II}}(\text{H}_2\text{O})_5] \cdot 2\text{H}_2\text{O}$ (1) and $[\text{Co}^{\text{II}}(\text{H}_2\text{dipic})(\text{dipic})] \cdot 3\text{H}_2\text{O}$ (2). Complex (1) was formed at optimum pH of 3 and yield of 47.9 %, while Complex (2) was formed at optimum pH of 1 and a yield of 50.45%. Complex (1)

have binuclear with the mixed ligand, while Complex (2) was mononuclear with one type of ligand. Based on the toxicity test on Brine Shrimp Lethality (BSL), the binuclear Complex of $[\text{Co}(\text{dipic})(\mu\text{-dipic})\text{Co}(\text{H}_2\text{O})_5]\cdot 2\text{H}_2\text{O}$ (1) exhibited an LC_{50} value of 5.38 ppm, while for Complex (2) showed LC_{50} value of 8.61 ppm. The binuclear interaction, groove binding, intercalation, and lower LC_{50} value of Complex (1) indicated more toxic properties than Complex (2). Thus, it can be concluded that the binuclear system of the Co-dipicolinate based Complex shows promising characteristics as the new anticancer compounds.

ACKNOWLEDGEMENT

The author acknowledges the Ministry of Research, Technology, and Higher Education of Indonesia for supporting the research under PDUPT (Penelitian Dasar Unggulan Perguruan Tinggi) grant, contract number: 881/PKS/ITS/2018, as well as the financial support from Hibah Riset Mandat Universitas Airlangga, 2019 grant, contract number: 380/UN3/14LT/2019.

REFERENCES

1. A. M. Hamil, K. M. Khalifa, A. Al-Houni, and M. M. El-Ajaily, *Rasayan Journal of Chemistry*, **2**(2), 261(2009).
2. P. Rajkumar, G. Saraswathy, Z. Abdul Vaheith, and A. Fiaz Ahamed, *Rasayan Journal of Chemistry*, **12**(3), 1127(2019), <https://doi.org/10.31788/RJC.2019.1235318>
3. G. R. Pandhare, V. M. Shinde, and Y. H. Deshpande, *Rasayan Journal of Chemistry*, **1**(2), 337(2008).
4. S. H. van Rijt, A. F. A. Peacock, R. D. L. Johnstone, S. Parsons, and P. J. Sadler, *Inorganic Chemistry*, **48**(4), 1753(2009), <https://doi.org/10.1021/ic8020222>
5. L. Yang, D. C. Crans, S. M. Miller, A. La Cour, O. P. Anderson, P. M. Kaszynski, M. E. Godzala, L. T. D. Austin, and G. R. Willsky, *Inorganic Chemistry*, **41**(19), 4859(2002), <https://doi.org/10.1021/ic020062l>
6. A. T. Çolak, F. Çolak, O. Z. Yeşilel, and O. Büyükgüngör, *Journal of Molecular Structure*, **936**(1–3), 67(2009), <https://doi.org/10.1016/j.molstruc.2009.07.026>
7. F. Blasco, L. Perelló, J. Latorre, J. Borrás, and S. Garcíá-Granda, *Journal of Inorganic Biochemistry*, **61**(2), 143(1996), [https://doi.org/10.1016/0162-0134\(95\)00053-4](https://doi.org/10.1016/0162-0134(95)00053-4)
8. B. J. Pages, D. L. Ang, E. P. Wright, and J. R. Aldrich-Wright, *Dalton Transactions*, **44**(8), 3505(2015), <https://doi.org/10.1039/C4DT02700K>
9. S. A. Gadir, *Journal of Chemical and Pharmaceutical Research*, **4**(12), 5145(2012).
10. J. J. Martínez Medina, L. G. Naso, A. L. Pérez, A. Rizzi, N. B. Okulik, M. Valcarcel, C. Salado, E. G. Ferrer, and P. A. M. Williams, *Biomedicine and Pharmacotherapy*, **111**, 414(2019), <https://doi.org/10.1016/j.biopha.2018.12.098>
11. S. Muthukrishnan, T. Senthil Kumar, and M. V. Rao, *Journal of Environmental Chemical Engineering*, **5**(2), 1685(2017), <https://doi.org/10.1016/j.jece.2017.03.004>
12. T. ur Rehman, A. U. Khan, A. Abbas, J. Hussain, F. U. Khan, K. Stieglitz, and S. Ali, *Saudi Pharmaceutical Journal*, **26**(3), 422(2018), <https://doi.org/10.1016/j.jsps.2017.12.019>
13. S. Pisutthanant, P. Plianbangchang, N. Pisutthanant, S. Ruanruay, and O. Muanrit, *Naresuan University Journal*, **12**(2), 13(2004).
14. A. Klanicová, Z. Trávníček, I. Popa, M. Čajan, and K. Doležal, *Polyhedron*, **25**(6), 1421(2006), <https://doi.org/10.1016/j.poly.2005.09.032>
15. A. Geete, B. D. Shrivastava, and A. Mishra, *Rasayan Journal of Chemistry*, **13**(3), 1878(2020), <https://doi.org/10.31788/RJC.2020.1335777>
16. Z. A. Siddiqi, M. Khalid, S. Kumar, M. Shahid, and S. Noor, *European Journal of Medicinal Chemistry*, **45**(1), 264(2010), <https://doi.org/10.1016/j.ejmech.2009.10.005>
17. P. C. Harrington and R. G. Wilkins, *Journal of Inorganic Biochemistry*, **12**(2), 107(1980), [https://doi.org/10.1016/S0162-0134\(00\)80122-1](https://doi.org/10.1016/S0162-0134(00)80122-1)
18. İ. Uçar, A. Bulut, A. Karadağ, and C. Kazak, *Journal of Molecular Structure*, **837**(1–3), 38(2007), <https://doi.org/10.1016/j.molstruc.2006.09.029>

[RJC-5823/2020]