Synthesis and anti-tuberculosis of Zn(II)Phenylalanine dithiocarbamate

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Abstract. Tuberculosis (TB) is caused by the infection with the bacterium Mycobacterium tuberculosis (M.tb), and according to the World Health Organization, was responsible for 1.6 million deaths and the emergence of 9.6 million new cases in 2017. A serious problem worldwide in the fight against TB is the rapid spread of the multidrug-resistant (MDR) TB due to inconsistent for protracted periods of treatment and the lack of new drugs in the market. New and effective drugs are needed for the treatment of tuberculosis by studying the synthesis of complex compounds that can be developed as anti-tuberculosis agents. A new complexes of dithiocarbamate, Zn(II) phenylalanine dithiocarbamate ligand were synthesized using an ‘in situ’ method by reaction complexes in a 1:2 molar ratio in refluxing ethanol. The complexes were characterized by using Ultra Violet Visible (UV-Vis), Fourier Transform Infra Red (FT-IR), HNMR, XRD, conductivity, and melting point. Complex Zn(II) each of them is 260 nm and 431 nm electronic transition is $\pi \rightarrow \pi^*$ of CS₂ and N-C-S. Infra-Red absorption spectra at wave number Zn(II) phenylalanine dithiocarbamate is 372 nm⁻¹ coordination occurred dithiocarbamate ligands and atoms M=S. Complex characterization using UV-Vis, IR, and HNMR showed that complexes are successfully synthesis. The bio-assay results show these complexes are potential as anti-tuberculosis agents.

1. Introduction

Phenylalanine is an amino acid found in many foods and used by our body to produce proteins and other important molecules. In addition to its role in protein production, phenylalanine is used to make other important molecules in our body, several of which send signals between different parts of our body [1]. Since phenylalanine is used to make these molecules in our body, it has been studied as a potential treatment for several medical conditions, including skin disorders, depression, and pain [2][3][4][5].

Phenylalanine has diverse chemical compositions and structures in two forms or arrangements: L-phenylalanine and D-phenylalanine. They’re nearly identical but have a slightly different molecular structure. The L-form is found in foods and used to produce proteins in our body, while the D-form can be synthesized for use in certain medical applications [6]. Although there are many studies that discuss phenylalanine as a potential molecule for the treatment, no study has investigated phenylalanine as an anti tuberculosis [7].

Tuberculosis (TB) is caused by bacteria Mycobacterium tuberculosis that most often affect the lungs. Tuberculosis mostly affects adults in their most productive years. However, all age groups are at risk. Over 95% of cases and deaths are in developing countries. One million children (0–14 years of age) fell ill with TB, and 230 000 children (including children with HIV associated TB) died from the disease in 2017. Along with the increase in this disease a new problem arises, namely multidrug Resistant Tuberculosis (MDR TB) reported by the World Health Organization (WHO) in 2017 the number of TB patients worldwide with cases of MDR TB continues to increase. MDR TB or Multidrug-Resistant TB (MDR TB) is TB (TB) that is resistant to the benefits of the two most powerful antituberculosis drugs, namely isoniazid, and rifampicin. Drug-resistant TB continues to be a
public health crisis. The best estimate is that, throughout the world in 2017, 558,000 people (range, 483,000-639,000) developed rifampicin-resistant TB (RR-TB), the most effective first-line drugs, and among them, 82% had multidrug-TB resistance (MDR-TB) [8].

MDR phenomenon is one of the stumbling blocks of TB control programs. Treatment of MDR Tb patients is more difficult, expensive, many side effects and the cure rate is relatively low. Therefore a new drug is needed to treat TB that has high inhibition and toxicity to M. tuberculosi bacteria, lower side effects with more effective treatment therapy and shorter treatment times and can treat MDR-TB. One attempt to find a new drug is through the synthesis of dithiocarbamate complex compounds, from several research results it is known to have a very wide application in various fields, one of which is as an anti bacterial [9]. In this study, phenylalanine was synthesis with dithiocarbamate in ethanol solvents. The synthesized compounds were characterized by UV-Vis spectroscopy, FT-IR, HNMR, conductometry, and melting point. The bioactivity of the compounds was tested against M. tuberculosis.

2. Material and Method

2.1 Materials

Chemicals used in this study were : L-Phenylalanine, CS₂, ZnCl₂, Test Bacteria (M.Tuberculosis), Medium Lowenstein Jensen, ethanol PA, methanol PA, aceton PA, methylene chloride PA, chloroform PA, n-hexane PA, acetonitrile PA, KBr.

2.2 Instruments

Infrared spectra of the compounds were recorded as KBr discs using Infrared SHIMADZU Spectrophotometer, in frequency 4000-300 cm⁻¹. ¹H and ¹³C NMR spectral obtained using NMR JEOL spectroscopy. Electronic spectral obtained using UV-Vis Jenwey spectrophotometer 200-1100 nm for 10⁻³ M solutions in ethanolic at 25°C. Melting points were obtained on melting point type WRS-200, and Conductivity measurements were made with ethanolic solutions using a Eutech Con 510.2 at 25°C for complex concentration of 10⁻³ M.

2.3 Method

2.3.1 Synthesis of Phenylalanine dithiocarbamates ligand

L-Phenylalanine 0.61 gr (5 mmol) dissolved in 10 mL ethanol, followed by adding dropwise CS₂ 0.3 mL (5 mmol) into 10 mL ethanol solution under conditions under 10°C. The solution was allowed to reflux with stirrings for 25 minute.

![Diagram](image-url)
2.3.2 Synthesis of Zn(II)Phenylalanine dithiocarbamates
The phenylalanine dithiocarbamate ligand solution was added ZnCl₂·7H₂O 0.41 gr (3 mmol), which was dissolved in 10 mL ethanol and the solution was allowed to reflux with stirred for 30 minutes. Then the precipitate formed is then filtered and washed with ethanol and dried in a desiccator after recrystallization with the appropriate solvent, the mixture of acetonitrile and ethanol (1: 2 v/v), and the characterization of the product.

![Figure 2. Synthesis reaction of Zn(II)Phenylalanine dithiocarbamates](image)

3. Result and Discussion
3.1 Chemical Study
The results of the synthesis of complex compounds, melting point tests, conductivity measurements, and yield can be seen in Table 1.

| Compound          | Colour   | Yield (%) | Melting point (°C) | Molar conductance [S/m] |
|-------------------|----------|-----------|--------------------|-------------------------|
| Zn(II)L-PhenylalanineDtc | white    | 44.38     | 290-292            | 0.123                   |

The yield of synthesized complexes Zn(II)L-PhenylalanineDtc is 44.38 % and the melting point is 290-292 °C. The conductivity of the synthesized complexes is less than 65 [s/m] which indicates that the complexes are non-electrolytes.

3.2 Analysis UV-Vis spectra
The spectrum of UV-Vis spectroscopy of complex compounds using ethanol solvents can be seen in Figure 3.
Figure 3. UV-Vis Zn(II)L-PhenylalanineDtc

UV-Vis spectrum of compounds ditiokarbat usually appears 2 main absorption peaks show that in the complex there is interaction of repulsion between electrons [10]. Maximum absorption of Zn (II) complex compound phenylalanine dithiocarbamate occurs at wavelength 260 nm. The presence of absorption peaks appears indicating the occurrence transition of electrons to molecules. The electron transition that occurs is that there is an intraligan transition $\pi^*-\pi^*$ which is affected by the effect hyperconjugation of R groups to atoms nitrogen in the area 250-300 nm absorption (Table 2, Figure 3) [11].

Table 2. Electronic Spectra For Complexes Zn(II)L-PhenylalanineDtc

| Compound                  | $\lambda$ (nm) | Electronic transition |
|---------------------------|----------------|----------------------|
| Zn(II) L-PhenylalanineDtc | 260            | $\pi \rightarrow \pi^*$ |

3.3 Infrared Spectra

Infrared spectroscopy is done to determine the types of coordination bonds and bonding properties that occur in metal complex compounds [12]. Based on data from the IR spectrum of Zn (II) PhenylalanineDtc complex compounds in table 3, figure 4 it is known that for the compound dithiocarbamate, absorption $v$ (CN) lies in the wave number between single bonds (1350-1250) cm$^{-1}$ and double bonds (1450-1550) cm$^{-1}$, so that the bond is written as $v$ (CN), where this absorption is identified at wave number 1494 cm$^{-1}$. Another important absorption pathway is CS uptake written as $v$ (CS), because the wave number is between double bond wave numbers $C = S$ (1050-1200) cm$^{-1}$ and single bond CS (550-800) cm$^{-1}$ [13]. For Zn(II)phenylalanineDtc complexes the absorption path is indicated in the area of 997 cm$^{-1}$. Likewise, the absorption path of the metal-sulfur bond $v$ (M-S) is shown by the compound Zn (II) PhenylalanineDtc at wave number 372 cm$^{-1}$, is the result of stretching vibrations of M-S bonds in complex compounds [14]. From the data it can be concluded that Zn(II)phenylalanineDtc has been successfully synthesized.

Table 3. Selected Infrared Spectral Of The Complexes Zn(II)L-PhenylalanineDtc

| Complexes            | $v$(C=)N cm$^{-1}$ | $v$(C=S) cm$^{-1}$ | V(M-S) cm$^{-1}$ |
|----------------------|--------------------|--------------------|-------------------|
| Zn(II) Zn(II)L-      | 1494.83            | 997.20             | 372.26            |
| PhenylalanineDtc     |                    |                    |                   |
3.4 NMR spectra
The selected NMR chemical shifts of the synthesized complexes are shown in Table 4.
H-NMR spectroscopic analysis showed a singlet proton signal in the area of $\delta$ 8.48 ppm which indicated that the synthesized compound had protons from a carboxylic acid group (1H, -COOH). The multiplet proton signal in the area of $\delta$ 7.28 ppm, 7.29 ppm, 7.32 ppm, 7.34 ppm, and 7.36 ppm comes from the aromatic proton group (5H, H Aromatic). The existence of a doublet proton signal in the area of $\delta$ 2.51 ppm and triplets in the area of $\delta$ 3.14 ppm each shows the presence of a methylene proton signal (2H, CH2) and a meth proton signal (1H, CH). The presence of triplet proton signals in the area of $\delta$ 4.15 ppm and the proton shift of N-H at $\delta$ 2.50 ppm indicates that the synthesized compound has been formed where protons are bounded to N-H from the N-C-S group. The C-NMR spectrum of compound products has 9 carbon atoms. The carbon atom signal in the area of $\delta$ 170.78 ppm indicates the presence of the carbonyl group C = O (C-9) of a carboxylic acid (1C, RCOOH). The existence of four signals of carbon atoms in the region of $\delta$ 135.44 ppm; $\delta$ 129.98 ppm; $\delta$ 129.01 ppm and $\delta$ 127.64 ppm, each of which comes from the carbon position (1C, C-3); carbon atom ortho position (2C, C-1 and C-5); meta position carbon atoms (2C, C-2 and C-4); and C-terminal phenyl carbon atoms (1C, C-6) aromatic compounds. The signals of carbon atoms in the area of $\delta$ 53.63 ppm and $\delta$ 36.15 ppm each come from carbon atoms metin (1C, C-8) and methylene carbon atoms (1C, CH2). Based on the NMR C spectrum, the synthesized compound showed a carbon signal from the N-C-S bond in the area of $\delta$ 170.78 ppm [15].

| Complexes          | $^{13}$C NMR (ppm)         | $^1$H NMR (ppm)              |
|--------------------|---------------------------|----------------------------|
| Zn(II)PhenylalanineDtc | 170.78, 135.44, 129.98, 129.01, 127.64, 53.63, 36.15 | 8.48, 7.28, 7.29, 7.32, 7.34, 7.36, 2.51(d), 3.14(d), 4.15(t), 2.50 |
3.5 Antimicrobial Activity

Compounds that have been synthesized tested its activity as anti tuberculosis by method Lewinstein Jensen. In this study used rifampicin drug as a positive control and dimethylsulfoxide (DMSO) as a negative control. The results of the antituberculosis test are presented in Table 5.

| Compound              | Concentration (ppm) | Population M.Tb complex |
|-----------------------|---------------------|-------------------------|
| Zn(II) PhenylalanineDtc | 0.002               | -                       |
|                       | 0.004               | -                       |
|                       | 0.006               | -                       |

The ability of a compound to be used as an anti tuberculosis depends on the ability to change structure micolate acid composer on the wall bacterial cell. On acidic structures mikolat, metal can form ring with hydroxyl group O atoms and O carbonyl group atoms. On this study, Phenylalanine ditiokarbamat ligands used as a metal mobilizer to form bonds with acid mikolat in
bacteria. With change the structure of mycolic acid, then the resistance of bacteria is getting more down hill [16].

![Antibacterial test result against M. tuberculosis](image)

**Figure 7.** Antibacterial test result against *M. tuberculosis* I<sub>1</sub> Complexes 0.002; I<sub>2</sub> Complexes 0.004; I<sub>3</sub> Complexes 0.006

4. Conclusion

Complexes dithiocarbamate has been successfully synthesized by in-situ method and has the potential as an anti-tuberculosis

Acknowledgments

The author would like to thank the Education Fund Management Institute (LPDP), for funding support in our research, we would like to thank Ms. Haslinda S.Si, Ms. Kartini SP, Mr. Sugeng and Mr. Markus who participated in the analysis of *Mycobacterium tuberculosis* at the Microbiology Research Center of Hasanuddin University, Makassar, Indonesia

References

[1] John D F and Madelyn H F 2007 Tyrosine, Phenylalanine, and Catecholamine Synthesis and Function in the Brain *The Journal of Nutrition* 137 1539S–1547S

[2] A L Russell and M F McCarty 2000 L-phenylalanine Markedly Potentiates Opiate Analgesia – An Example of Nutrient/Pharmaceutical Up-Regulation of The Endogenous Analgesia System. *Medical Hypotheses* 55(4) 283–288

[3] Dong Fang Li, Pan Pan Hu, Mu Song Liu, Xiao Le Kong, Jin Chao Zhang, Robert C Hider and Tao Zhou 2013 Design and Synthesis of Hydroxypyridinone-L-phenylalanine Conjugates as Potential Tyrosinase Inhibitors. *Journal of agricultural and food chemistry* 61 6597–6603

[4] Khaled Ezzedine, Viktoria Eleftheriadou, Maxine Whitton Nanja van Geel 2015 Vitiligo Seminar 15 1-11

[5] By Jhon Mann, M D Eric, D Peselow M D, Selma nyderman M D, And Samuel Gershon M D 1980 D-phenylalanine in Endogenous Depression. *Am J Psychiatry* 137 12

[6] Susheel Durani 2007 Protein Design with L- and D-r-Amino Acid Structures as the Alphabet Accounts of chemical research *Vol* 41 No. 10 1301-1308

[7] Krishan Kumar, et all 2009 Phenylalanine-Rich Peptides Potently Bind ESAT6 a Virulence Determinant Of Mycobacterium Tuberculosis And Concurrently Affect The Pathogen’s Growth *Plos One* 4(11) 1-11
[8] WHO 2017 World Tuberculosis Report (online) http://www.who.int/mmediacentre (accessed 30 March 2019: 10 am)

[9] Manav N, Mishra AK, Kaushik NK 2005 In vitro antitumor and antibacterial studies of some Pt(IV) dithiocarbamate complexes. Spectrochimica Acta Part A 65 32-35

[10] Raya I, Baba I, Yamin BM 2006 New mixed ligands complexes of samarium(III) with dithiocarbamates and 1,10-phenanthroline. MJAS 10(1) 93-98

[11] Faraglia G, Fregon D, Sitran S, Giovagnini L, Marzano C Baccichetti, F Casellato U and Graziani R 2001 Platinum (II) and palladium (II) complexes with dithiocarbamates and amines synthesis characterization and cell assay J. Inorg. Biochem 83 31–40

[12] Ahmed T Numan, Synthesis 2017 Characterization and Biological Evaluation of New Dithiocarbamate Ligand and Its Complexes with some Metal Ions Ibn Al-Haitham Jour. for Pure & Appl. Sci Vol. 30 (3) 211-215

[13] Bernal C Neves, E A and Cavalheiro T G Differences in thermal decomposition of Ag(I), Mn(II), Fe(II), and Fe(III) complexes of cyclic dithiocarbamate. ThermocemicaActa 370(1-2) 49-55

[14] Nakamoto N 2009 Infrared and Raman Spectra of Inorganic and Coordination Compounds. John Wiley & Sons Inc 6th Ed New Jersey

[15] Safa’a Fares Kayed Yang Farina And Ibrahim Baba 2009 Spectral Studies Of Zinc (II) Complex Of 2-Acetylbenzothiophene 3-Thiosemicarbazone Prosiding Seminar Kimia Bersama UKM-ITB VIII 9-11

[16] Porcheron G Garenaux A Proulx J Sabri M Dozois CM 2013 Iron, Copper, Zink, and Manganese Transport and Regulation in Pathogenic Enterobacteria : correlations between strains, site of infection and the relative importance of the different metal transport system for virulence. Front cell infect microbial of the different metal transport system for virulence Front cell infect microbial Vol 3 6-8