Synthesis and Characterization of Fe(III) Complex with N’-(3-Nitrobenzoyl)Isonicotinohydrazide as an Anti-tuberculosis Candidate

R Ruswanto1, Y Sarwatiningsih1, ATK Pratita1, Indra1, R Dewi1

1Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada, Tasikmalaya 46115, Indonesia

ruswanto@stikes-bth.ac.id

Abstract. Recently, we have been conducted synthesis of complex compounds of Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide using refluxing N’-(3-nitrobenzoyl)isonicotinohydrazide as ligands and FeCl3.6H2O as the metal at 75 °C for 6 hours in ethanol. The synthesis results obtained 82.45% recovery, and from the purity test are shown with a narrow melting range is 220-222°C. Based on identification with UV-Visible spectrophotometer, infrared spectrometer and Atomic Absorption Spectrophotometer (AAS) indicate that the complex compound have λmax of 272.00 nm, giving vibration of Fe-O at 495.1 cm⁻¹ and 599.6 cm⁻¹ with formula complex compounds, namely [Fe(C13H10N4O4(H2O)4)Cl3].10 H2O.

1. Introduction
Tuberculosis is an infectious disease that can attack various organs or tissues of the body and is caused by a Gram-positive result in Mycobacterium tuberculosis [1-2]. Isoniazid is a derivative of isonicotinic acid which is the most effective tuberculostatic against Mycobacterium tuberculosis (in the resting phase) and is bactericidal to rapidly growing bacilli. The mechanism of action is based on disruption of the synthesis of mycolic acid, one of the constituents of the bacterial wall[3]. Mycobacterium tuberculosis which has been induced by isoniazid and ethambutol can still grow gradually during four weeks of incubation [4].

Previously, we have successfully synthesized with acylation reaction [5] and characterized twelve N-benzoylisonicotinohydrazide derivatives, and they have good interactions with InhA receptors. But there was one compound that has the best activity, namely 3-nitro-N’-[(pyridin-4-yl) carbonyl] benzohydrazide[6].

Another research have performed to react the Fe(III) compounds with 3-aminoquixaline-2-carbonitrile N1 derivative, N4-dioxide can provide better antimycobacterial activity than their free ligands [7]. The Fe(III) complexes can act as bioactive ligands, increasing the concentration of molecules in the mycobacterial cell. The mechanism between complex compounds with bacteria is through the Chelation theory, where when a metal ion in a chelate with a ligand the polarity of a metal ion decreases due to overlapping of various ligand orbitals and contributes a portion of the positive charge
of a metal ion to a donor group so that its lipophilicity increases and the complex can penetrate or enter the bacterial membrane [8]. This also happened in the study of Zheng et al. (2008) that the antitumor activity of thiosemicarbazone increases with the presence of coordination bonds between ligands and metal cations which improve lipophilicity so that the compound will be more easily entered into cells [9].

The ability of a compound to be used as an antituberculosis depends on the ability to change the structure of the constituent mycolic acid in the bacterial cell wall. In the structure of mycolic acid, metals can form rings with O atoms of hydroxyl groups and O at carbonyl groups. By changing the structure of mycolic acid, the bacterial resistance decreases [10].

Based on the above background, the development of antituberculosis drugs has been carried out by synthesizing complex compounds between N’-(3-nitrobenzoyl) isonicotinohydrazide with Fe (III) metal. Identification and characterization of the compound were carried out using melting point determination, UV-Vis Spectrophotometry, Infrared Spectrophotometry, and Atomic Absorption Spectrophotometry (AAS).

2. Experimental and Method
2.1. Instruments
The tools used include analytic scales, measuring cups, watch glass, reflux set, magnetic stirrer, thermometer, hot plate, filter paper, 250 mL beaker, steam cup, oven, Melting Point Apparatus, UV-Vis Genesys 10S Spectrophotometer, Perkin Elmer Spectrum 100 FT-IR Spectrometer, and Atomic Absorption Spectrophotometry (AAS) Agilent Technology series 8453.

2.2. Material
The ingredients used are N, -(3-nitrobenzoyl) isonicotinohydrazide, FeCl$_3$.6H$_2$O p.a, ethanol p.a, HCl p.a, and aquadest.

2.3. Experiments
2.3.1. Synthesis of Fe (III) N’-(3-nitrobenzoyl) Isonicotinohydrazide compounds
The N’-(3-nitrobenzoyl) isonicotinohydrazide as much as 0.25 mmol was dissolved in 30 mL ethanol (solution A). The 0.13 mmol of FeCl$_3$.6H$_2$O metal was dissolved in ethanol (solution B), then dripped slowly into solution A. The dripping process was carried out consistently in low heating. Then, the were refluxed for 6 hours at 75 °C while continuing to be stirred using a magnetic stirrer. Then the evaporation of the solvent is carried out using a rotary evaporator, then the precipitate is dried [8].

2.3.2. Atomic Absorption Spectrophotometry
Iron content was measured by atomic absorption spectrophotometer (AAS) at maximum wavelength $\lambda_{max}$ 248.28 nm. The parent standard solution was made by dissolving FeCl$_3$.6H$_2$O in 6 N HCl so that Fe$^{3+}$ 1000 ppm standard was obtained, then Fe$^{3+}$ 50 ppm standard solution was prepared. The concentration of standard solutions for measurements was made at concentrations of 2, 4, 6, 8, and 10 ppm taken from the standard 50 ppm solution. Then the absorbance is measured, and a standard curve was made. The sample solution in 6 N HCl was made with a concentration of not more than 5 ppm, and the absorbance was measured and then plotted on a standard curve [11].

3. Result and Discussion
3.1. Synthesis of Fe(III)N’-(3-nitrobenzoyl)Isonicotinohydrazide compounds
Complex compounds can be formed by reacting ligands with metal; the ligands act as donors of electron pairs because one of the atoms has a free electron pair that can covalently coordinate with metal elements. The synthesis of this complex compound is carried out with a mole ratio of 1: 1 metal ligand and one of them must be excess so that the equilibrium of the reaction shifts to the right because FeCl$_3$.6H$_2$O will react entirely with the compound N’-(3-nitrobenzoyl) isonicotinohydrazide.
This reflux method was chosen because it used a temperature of 75 °C which can accelerate the reaction process and reflux time for 6 hours. The longer the time used, the better the process of forming these complex compounds. Besides, a stirring process was carried out which will affect the kinetic energy of the molecule. Where with this process, particles will collide with each other so that the kinetic energy will increase. Refflux compounds were carried out by evaporation of solvents using a rotary evaporator. The aim is that the remaining synthesized residue can be evaporated and collisions occur between deposits, thus forming a larger aggregate. After that, washing using aquadest was done, then the residue is dried. So that obtained synthesis results with% recovery as much as 82.45%. Synthesized compounds in the form of powder, yellow in color, smell distinctive, slightly soluble in water, and dissolved in ethanol.

3.2. The Purity Test Results
Melting point test is used to ensure that the compounds produced are new compounds, not ligands, or salts. A compound is said to be pure if it has a melting point of the compound from starting to melt until it melts completely is 2 °C. The results of melting point distance test are listed in Table 1.

| Compound                                         | Melting Point distance (°C) |
|--------------------------------------------------|-----------------------------|
| FeCl$_3$.6H$_2$O                                  | 37.00                       |
| N’-(3-nitrobenzoyl)isonicotinohydrazide            | 194.50-195.70               |
| Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide    | 220.00-222.00               |

The difference of melting point distance between the complex compound and the comparison can describe that the complex compound has been successfully synthesized.

3.3. Identification and Characterization of Synthesized Compounds
Identification and characterization of the structure of the synthesized compounds were carried out with various instruments including UV-Vis spectrophotometry, Infrared Spectrophotometry, Mass Spectrophotometry, and SSA. Identification and characterization of the first structure using UV-Vis spectrophotometry aim to determine the maximum wavelength shift to obtain a spectrum profile of the synthesized compound which is then compared with the spectrum profile of the compound N’- (3-nitrobenzoyl) isonicotinohydrazide. The result is that the maximum wavelength value (λ$_{max}$) of the absorbance value (A) of the synthesized compound in DMSO solvent was shown in Table 2 below.

| Senyawa                               | A    | λ$_{max}$ (nm) |
|---------------------------------------|------|---------------|
| FeCl$_3$.6H$_2$O                      | 3.88 | 298.00        |
|                                       | 3.82 | 298.00        |
|                                       | 3.83 | 297.00        |
| Mean                                  | 3.84 | 297.67        |
| Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide | 2.96 | 272.00        |
|                                       | 3.02 | 272.00        |
|                                       | 2.98 | 272.00        |
| Mean                                  | 2.98 | 272.00        |

The more polar solvents will give a shift in the absorption band toward shorter wavelengths (hypochromic shifts). This occurs in the transition n → π* where the organic molecule has a functional group which is unsaturated so that the double bond in the group gives the required π orbitals. In most particles that show the transition, the ground state is more polar than the excited state. In particular, hydrogen-binding solvents interact more strongly with unpaired electron pairs on molecules in the ground than in particles in the excited state [12]. The formation of this complex compound is indicated by a shift in the maximum wavelength towards the smaller (25.67 nm) spectrum of Fe$^{3+}$ from
the solution of FeCl₃.6H₂O to the spectrum of Fe³⁺ from the solution of Fe (III) N'-(3-nitrobenzoyl) isonicotinohydrazide complex. The magnitude of the shift of λmax, absorbance (A) and a molar absorptivity (ε) and the cleavage energy (10 Dq) of Fe(III)N'-(3-nitrobenzoyl) isonicotinohydrazide complexes are shown in Table 3 below.

**Table 3.** The electronic spectrum and 10 Dq value [13]

| Compound                                      | Mr     | λmaxs   | ν (cm⁻¹) | A     | ε (L.mol⁻¹.cm⁻¹) | 10 Dq (KJ.mol⁻¹) |
|-----------------------------------------------|--------|---------|----------|-------|-----------------|-----------------|
| FeCl₃.6H₂O                                    | 270.30 | 297.67  | 33594.25 | 3.84  | 1081.69         | 401.94          |
| Fe(III)N'-(3-nitrobenzoyl)isonicotinohydrazide| 700.66 | 272.00  | 36764.71 | 2.98  | 2811.32         | 439.87          |

The next test is Infrared Spectrophotometry to find out the description of the functional groups contained in the molecule resulting from the synthesis compound. The presence of sharp absorption around 3700-2900 cm⁻¹ indicates that the uptake is XH absorption (X = O, N) [14]. In this infrared spectrum, NH absorption is seen in the wave number 3497.3 cm⁻¹. Vibration absorption of C=C aromatic theoretically can occur at wave numbers 1475-1600 cm⁻¹. This occurs in comparators that appear at 1489.0 cm⁻¹ and 1532.7 cm⁻¹. Whereas in complex compounds appear at 1533.9 cm⁻¹ and 1574.4 cm⁻¹.

There is a vibration shift of C-O from the Fe complex to its free ligand of 1683.6 cm⁻¹ to 1669.6 cm⁻¹. This C-O vibration shifts towards lower wavenumbers because the vibration of C-O in this compound is bound to Fe metal so that the vibration is reduced [15].

Bond vibration absorption between Fe metal and ligand is seen at wave numbers 300-600 cm⁻¹. There is a vibration area of 600-450 cm⁻¹ for M-O vibrations and 400-310 cm⁻¹ for M-N vibrations [16]. The vibration of Fe-O at 495.1 cm⁻¹ and 599.6 cm⁻¹. In the Fe(III)N'-(3-nitrobenzoyl) isonicotinohydrazide complex, coordination between the N'-(3-nitrobenzoyl)isonicotinohydrazide ligand on the central ion of Fe (III) is monodentate through the O atom of the carbonyl group. The infrared data contained in Table 4 and the infrared spectrum can be seen in Figure 1 below.

![Figure 1](image-url)
Table 4. The comparison of infrared data of Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide complex and N’-(3-nitrobenzoyl)isonicotinohydrazide compound.

| Functional group | N’-(3-nitrobenzoyl)isonicotinohydrazide | Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide |
|------------------|----------------------------------------|-----------------------------------------------|
| N-H              | 3222.5                                 | 3497.3                                        |
| C-O              | 1669.6                                 | 1683.6                                        |
| C=C aromatik     | 1489.0                                 | 1533.9                                        |
|                  | 1532.7                                 | 1574.4                                        |
| Fe-O             | -                                     | 495.1                                         |
|                  |                                        | 599.6                                         |

Determination of the formula of complex compounds based on measurements of Fe(III) levels was carried out using SSA. The concentration series for the calibration curve of Fe$^{3+}$ was made from FeCl$_3$.6H$_2$O dissolved in HCl 6N solution[17]. From the Fe(III) levels measurements in Fe(III)-N’-(3-nitrobenzoyl)isonicotinohydrazide complex using SSA were 0.39 ppm while the theoretical results are shown in Table 5 below.

Table 5. The measurement of Fe(III) level by theoretical

| Formula number | Composition of complex | Mr       | % Fe(III) Theoretis |
|----------------|------------------------|----------|---------------------|
| 1              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)Cl$_3$. 12 H$_2$O | 664.63   | 8.40 %              |
| 2              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)Cl$_3$. 13 H$_2$O | 682.65   | 8.18 %              |
| 3              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)Cl$_3$. 14 H$_2$O | 700.66   | 7.97 %              |
| 4              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)$_2$Cl$_3$. 5 H$_2$O | 824.59   | 6.77 %              |
| 5              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)$_2$Cl$_3$. 6 H$_2$O | 843.05   | 6.62 %              |
| 6              | Fe(C$_{13}$H$_{10}$N$_4$O$_4$)$_2$Cl$_3$. 7 H$_2$O | 860.62   | 6.49 %              |

Based on the results of the calculation, obtained % Fe(III) levels in complex compounds formed by 7.80% approaching formula 3 which has% Fe(III) theoretically at 7.97%. So it can be concluded that the synthesized complex compound has the formula Fe (C$_{13}$H$_{10}$N$_4$O$_4$)$_2$Cl$_3$.14H$_2$O. Estimates of the structure of Fe(III)N’-(3-nitrobenzoyl) isonicotinohydrazide complexes are shown in Figure 2 below.

Figure 2. The chemical structure prediction of Fe(III)N’-(3-nitrobenzoyl)isonicotinohydrazide complex
4. Conclusion
Based on the research that has been carried out it can be concluded that Fe(III)N'(3-nitrobenzoyl)isonicotinohydrazide complex compounds can be synthesized through a reaction between FeCl$_3$.6H$_2$O with N'(3-nitrobenzoyl)isonicotinohydrazide with 85.73% of percentage recovery. The results of the identification and characterization showed that the synthesized compound had $\lambda_{\text{max}}$ of 272.00 nm, giving the Fe-O vibration at 495.1 cm$^{-1}$ and 599.6 cm$^{-1}$ with the complex formula, namely [Fe(C$_{13}$H$_{10}$N$_4$O$_4$(H$_2$O)$_4$)Cl$_3$].10H$_2$O.

5. References
[1] Widoyono, 2011, 2nd edition Jakarta: Penerbit Erlangga.
[2] Siswandono, 2016, 2nd edition. Surabaya: Airlangga University Press.
[3] Tjay, T.H., dan Rahardja, K. 2002 Jakarta: PT. Elex Media Komputindo.
[4] Purwanti, R.D., Ws, A.H., dan Oekar, N.K. 2013 Indonesian Journal of Pharmaceutical Science and Technology Vol.II, No.2.
[5] Ruswanto R, Mardianingrum R, Lestari T, Nofianti T, Siswandono S 2018 Molbank M1005; https://doi.org/10.3390/M1005
[6] Ruswanto, R., Mardianingrum, R., Nofianti, T., dan Rahayuningsih, N. 2018 Malaysian Journal of Fundamental and Applied Sciences14(3) (accepted).
[7] Tarallo, M.B., Urquiola, C., Monge, A., Costa, B.P., Ribeiro, R.R., Costa-Filho, A.J., Mercader, R.C., Pavan, F.R., Leite, C.Q.F., Torre, M.H Gambino, D. 2010 Journal of Inorganic Biochemistry104, pp 1164–1170.
[8] Kirubavathy SJ, Velmurugan R, Parameswari K, Chitra S. 2014 Arab J Chem [Internet]. King Saud University pp. 0–5. Available from: http://dx.doi.org/10.1016/j.arabjc.2014.10.013.
[9] Zheng, L. P., Chen, C. L., Zhou, J., Li, M. X. 2008 Verlag der Zeitschrift für Naturforschung. 63b 1257–1261.
[10] Hasminisari, J., Raya, I., dan Usman, H. 2014 Makasar: FMIPA Universitas Hasanudin.
[11] Ruswanto R, Mardianingrum R, Apriliani AY, Ramdaniah FK, Sarwiningsih Y, Tri A, et al. 2018 Journal of Pharmacopolum1(2), pp.100–6.
[12] Gandjar, I.G., dan Rohman, A. 2007 Kimia Farmasi Analisis. Yogyakarta: Pustaka Pelajar.
[13] Kusyanto, A., dan Sugiyarto, K.H. 2017 Yogyakarta: Jurnal Kimia Dasar Volume 6 No 1.
[14] Ruswanto R, Mardianingrum R, Apriliani AY, Ramdaniah FK, Sarwiningsih Y, Tri A, et al. 2018 Journal of Pharmacopolum1(2), pp.100–6.
[15] Setyawati, H., dan Murwani, I.K. 2010 Prosiding Seminar Nasional Sains 2010.
[16] Revanasiddappa, M., Suresh, T., Syed K., Raghavendra, S.C., Basavaraja, C., S.D. Angadi. 2008 E-Journal of Chemistry. Vol. 5. No 2. 395-403.
[17] Cotton FA, Wilkinson G 1989 Kimia Anorganik Dasar. Jakarta: UI Press.

Acknowledgment
The authorst hank to the Ministry of Research, Technology and Higher Education of Indonesia for a Fundamental Research Grant in 2017 and 2018, and Bakti Tunas Husada of Health Science College Tasikmalaya for the provision of the facilities to complete our research.