The Biology Activities of Isonicotinohydrazide Derivatives as an Anti-tuberculosis Candidate

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Abstract. The compound N’-(3-chlorobenzoyl)isonicotinohydrazide, N’-(3-bromobenzoyl)isonicotinohydrazide, N’-(4-fluorobenzoyl)isonicotinohydrazide are isonicotinohydrazide derivative compounds which have antibacterial activity against bacteria Staphylococcus aureus, bacteria Bacillus subtilis as gram-positive Escherichia coli as gram-negative, and against strain Mycobacterium tuberculosis H37Rv. The purpose of this study was to obtain antituberculous candidates from isonicotinohydrazide compounds. The method used to test the antibacterial activity was the disc diffusion while for antimycobacterium using method Resazurin Microtiter Assay (REMA). The results of the antibacterial activity test on the bacteria Staphylococcus aureus compound N’-(3-chlorobenzoyl)isonicotinohydrazide had the lowest MIC value (0.26 ppm), while the bacteria Bacillus subtilis and Escherichia coli compound with the lowest MIC value of N’-(3-bromobenzoyl)isonicotinohydrazide with a MIC value of 0.30 and 0.24 ppm. In the bacterium M. tuberculosis, H37Rv compound isoniazid has the smallest MIC value (3.125 ppm).

1. Introduction
Tuberculosis is the most common contagious disease (about 80%) attacks the lungs, with the cause being gram-positive bacilli resistant to very slow growth, namely Mycobacterium tuberculosis. Based on the literature study that isoniazid is the strongest tuberculostatic against Mycobacterium tuberculosis which is bactericidal against rapidly growing bacilli, the mechanism of action is based on inhibition of the synthesis of mycolic acid which is needed by bacteria to build bacterial walls [1]. The emergence of resistant multidrug cases (MDR) is a major challenge to the success of tuberculosis treatment [2].

To reduce the resistance of antituberculosis drugs such as isoniazid many researchers are developing drugs to find new compounds with better activity. Previously, we have synthesized three isonicotinohydrazide derivatives, namely the compound N’-(4-fluorobenzoyl)isonicotinohydrazide, N’-(3-bromobenzoyl) isonicotinohydrazide, N’-(3-chlorobenzoyl)isonicotinohydrazide [3].
Based on the results of the interaction study that the three compounds have better interactions than isoniazid compounds. In the development of new drugs, it is necessary to know biological activity in vitro. From the another research, that was the synthesis and test of in vitro biological activity of cycondensation isoniazid derivative 1,2,3,4-tetrahydroxyrimine on gram-positive and antimycobacterial bacteria in the strain Mycobacterial tuberculosis H37Rv with MIC values ranging from 1.04-2.97 µg/mL [4].

Purnamasari et al (2015) added that using the Resazurin Microtiter Assay (REMA) method the results of testing were fast, relatively cheaper with simpler work and gave more sensitive test results. Based on the background described, the antibacterial activity of three isonicotinohydrazide derivatives against gram-positive bacteria, Staphylococcus aureus, Bacillus subtilis, in gram-negative Escherichia coli bacteria and M. tuberculosis bacteria H37Rv [5].

2. Experimental and Method
2.1. Instruments
Petri dish, test tube, stirring rod, needle ose, Erlenmeyer, beaker, micropipette, Bunsen, analytic scale, autoclave, incubator (Mammert), LAF (Laminar Air Flow), refrigerator.

2.2. Material
Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus), Escherichia coli gram-negative bacteria, H37Rv M. tuberculosis strains, Nutrient Agar (NA) media, Mueller Hilton Agar (MHA), DMSO, umbrella paper, cotton, gauze, wool threads, aquadest, NaCl, Middlebrook 7H9 broth (Difco), indicator of Resazurin, isoniazid, isonicotinohydrazide derivatives.

2.3. Experiments
2.3.1. Sterilization of tools and materials
Petri dishes, test tubes, and media were sterilized in an autoclave at 1210C for 15 minutes. An oil needle and tweezers are fixed by burning on a fire directly.

2.3.2. Oblique Media
Nutrient media must be made by dissolving 10 grams of NA media powder in 500 mL of aquadest in Erlenmeyer. The media solution is heated until it is completely dissolved, then cover the top of the Erlenmeyer mouth with a cotton plug and the surface layer with paper that is tied to the ketal. Sterilized using autoclave at 1210C, 1-2 atm pressure for 30 minutes [6].

2.3.3. Preparation of MHA Media
Weigh 38 gram media, then add 1 liter of aquadest. Heat and stir until dissolved. Autoclave sterilization at 1210C for 15 minutes. Wait until cool, pour into the petri dish then wait until it solidifies [7].

2.3.4. Preparation of Middlebrook Media
Made by 4.7 g Middlebrook 7H9 broth base, 20 mL glycerol 10%, 880 mL aquadest [8].

2.3.5. The Breeding of bacteria
Test bacteria derived from pure culture, taken by one ole, then inoculated by slipping on slanted nutrient agar (NA) media then incubated for 24 hours at 370C (Nurhayati, 2011), while the Mycobacterium H37rv strain was subcultured in Lowenstein Jensen media (LJ ) [7].

2.3.6. Making bacterial suspension
The inoculated test bacteria were taken with sterile wire and then suspended into a tube containing 2 ml of 0.9% NaCl solution to obtain the same turbidity as the turbidity standard of Mc solution. Farland
0.5. The log phase suspension of M. tuberculosis H37Rv was made by NaCl so that the turbidity was in accordance with Mc. Farland's turbidity standard [7].

2.3.7. **Antibacterial Activity Test**

Antibacterial activity test was carried out by filling a petri dish with 1 mL of test bacterial suspension and aseptically 20 mL of MHA (Mueller Hilton Agar) media. The media is homogenized by rotating to form the number eight and allowed to solidify. The media that has been solidified is then marked into 4 sections. Paper discs that have been filled with test compounds, positive controls, and negative controls are placed on each part above the surface of the media that has condensed [9]. The media was then incubated at 37°C for 24 hours [10].

2.3.8. **Anti-mycobacterial Activity Test**

Middlebrook 7H9 as much as 100 µL was inserted into each well on the microtiter plate. Pipette each test compound that has been prepared. In this test also used bacterial growth control which consists of only medium and bacteria. Then the plate was closed and incubated at 37°C after 7 days of incubation, 30 µL of the resazurin indicator was added to each well and pre-incubated 24 hours. The results of incubation were observed whether there was a color change from blue to pink which indicates bacterial growth [5].

2.3.9. **Preparation of the test solution**

The test solution was prepared by dissolving the compound in dimethyl sulfoxide with a concentration range of 100 µg/mL - 0.195 ppm.

### 3. Result and Discussion

3.1. **The results of antibacterials of the compounds**

The results of testing the antibacterial activity of the three test compounds against gram positive and gram negative bacteria using well diffusion method can be seen in the table 1-3 below:

| Table 1. The Antibacterial activity test results of the N’-(3-chlorobenzoil)isonicotinohydrazide |
|---------------------------------|--------------------------------|-----------------|-----------------|
| Concentration (ppm) | Staphylococcus aureus | Bacillus subtilis | Escherichia coli |
|----------------------|-----------------------|------------------|------------------|
| 0.195                | 0                     | 0                | 0                |
| 0.3                  | 0.0 ± 0.041           | 0                | 0                |
| 0.78                 | 3.53 ± 0.021          | 3.35 ± 0.212     | 2.90 ± 0.283     |
| 1.56                 | 4.30 ± 0.070          | 4.50 ± 0.566     | 3.35 ± 0.495     |
| 3.125                | 6.50 ± 0.707          | 5.65 ± 0.354     | 4.50 ± 0.141     |
| 6.25                 | 7.00 ± 0.070          | 5.50 ± 0.414     | 6.00 ± 0.141     |
| 12.5                 | 7.00 ± 0.846          | 6.50 ± 0.283     | 6.45 ± 0.495     |
| 25                   | 8.00 ± 0.566          | 5.90 ± 0.414     | 6.00 ± 0.141     |
| 50                   | 7.80 ± 0.707          | 5.90 ± 0.414     | 6.00 ± 0.141     |
| 100                  | 8.45 ± 0.212          | 6.50 ± 0.283     | 6.45 ± 0.495     |

| Table 2. The Antibacterial activity test results of the N’-(3-bromobenzoil) isonicotinohydrazide |
|---------------------------------|--------------------------------|-----------------|-----------------|
| Concentration (ppm) | Staphylococcus aureus | Bacillus subtilis | Escherichia coli |
|----------------------|-----------------------|------------------|------------------|
| 0.195                | 0                     | 0                | 0                |
| 0.3                  | 3.40 ± 0.283          | 4.10 ± 0.141     | 4.90 ± 0.071     |
| 0.78                 | 5.45 ± 0.071          | 5.00 ± 0.566     | 4.70 ± 0.778     |
| 1.56                 | 5.35 ± 0.354          | 5.00 ± 0.566     | 5.00 ± 0.566     |
| 3.125                | 4.95 ± 0.919          | 5.10 ± 0.424     | 5.50 ± 0.778     |
| 6.25                 | 5.25 ± 0.636          | 5.45 ± 0.495     | 5.50 ± 0.778     |
| 12.5                 | 5.80 ± 0.566          | 5.80 ± 0.707     | 5.90 ± 0.495     |
| 25                   | 6.55 ± 0.636          | 6.35 ± 0.636     | 6.30 ± 0.061     |
| 50                   | 7.00 ± 0.849          | 6.55 ± 0.636     | 6.00 ± 0.273     |
| 100                  | 5.80 ± 0.283          | 7.75 ± 0.485     | 7.10 ± 0.061     |
Table 3. The Antibacterial activity test results of the N’-(4-fluorobenzoil)isonicotinohydrazid

| Concentration (ppm) | Staphylococcus aureus | Bacillus subtilis | Escherichia coli |
|---------------------|-----------------------|-------------------|-----------------|
| 0.195               | 0                     | 0                 | 0               |
| 0.3                 | 0                     | 0                 | 0               |
| 0.78                | 0                     | 0                 | 3.55±0.071      |
| 1.56                | 0                     | 0                 | 4.33±0.414      |
| 3.125               | 0                     | 0                 | 4.55±0.495      |
| 6.25                | 0                     | 0                 | 3.65±0.354      |
| 12.5                | 4.50±0.566            | 6.80±0.919        | 5.10±0.141      |
| 25                  | 6.40±0.424            | 8.30±0.212        | 5.05±0.071      |
| 50                  | 7.80±0.919            | 9.90±0.354        | 5.35±0.212      |
| 100                 | 9.40±0.566            | 10.5±0.071        | 6.00±0.707      |

Figure 1. The picture of antibacterial activity test

The results of the negative control test, namely DMSO, showed 0 mm inhibition, which means that DMSO as a solvent does not provide antibacterial activity so it will not affect the antibacterial activity of the test compound. From the table above, the compound N’-(3-chlorobenzoyl) isonicotinohydrazide has the best activity in Staphylococcus aureus bacteria with a concentration of 0.391 ppm. Meanwhile, the N’-(3-chlorobenzoyl) isonicotinohydrazide compound had better antibacterial activity against Bacillus subtilis and Escherichia coli bacteria with the same concentration of 0.39 ppm. The diameter of inhibition ranges from 5-10 mm and is categorized into moderate inhibitory power because based on the provisions of the antibacterial inhibitory power of 10-20 mm (strong indicated), 5-10 mm (medium) and less than 5 mm (weak) [11].

3.2. Minimum Inhibitory Concentration (MIC)

After we have known the compounds with the best antibacterial activity, the concentration dilution was then performed to find the Minimum Inhibitory Concentration (MIC) value of each test compound. The results are as follows:

Table 4. The data of Minimum Inhibitory Concentration (MIC) of the compounds

| Compounds                     | MIC (ppm) |
|-------------------------------|-----------|
|                               | B. subtilis | S. aureus | E. colli | H37Rv |
| N’-(3-chlorobenzoyl)isonicotinohydrazid (a) | 0.65±3.70  | 0.26±2.35  | 0.48±3.00 | 25    |
| N’-(3-bromobenzoyl)isonicotinohydrazid (b) | 0.30±3.40  | 0.48±4.60  | 0.24±2.20 | 6.25  |
| N’-(3-fluorobenzoyl)isonicotinohydrazid (c) | 9.00±3.10  | 9.70±2.90  | 0.32±1.80 | 25    |
| INH                           | 1.04±4.30  | 18.0±2.80  | 1.13±4.30 | 3.125 |
| DMSO                          | 0          | 0          | 0        | 0     |
Based on the table above it was obtained, with a low MIC value in the Staphylococcus aureus bacteria, namely compound (a). While for Escherichia coli and Bacillus bacteria subtilized compounds with the lowest MIC value, compound (b). The three compounds have better antibacterial activity than isoniazid. The inhibitory power for the three test compounds is moderate because it is in the range of 5-10 mm [11].

The H₃7Rv M. tuberculosis bacteria test was carried out using the method of Resazurin Microtiter Assay (REMA) where the results of the testing of antimycobacterial activity were characterized by the formation of colors in blue and pink. The blue color shows the absence of bacterial growth while the pink color indicates the growth of bacteria which in this study can be observed as a value of Minimum inhibitory concentration (MIC). While the isoniazid M. tuberculosis bacteria is more potential than the three test compounds because the lower isoniazid MIC value is 3.125 ppm.

4. Conclusion
Based on the results of the research that has been carried out it can be concluded that the three isonicotinohydrazide derivative test compounds have antibacterial and antimycobacterial activity. The best activity is the N’-(3-bromobenzoyl) isonicotinohydrazid against the E. colli (MIC 0.24 ± 2.20 ppm).

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