Synthesis, docking molecule study and antibacterial activity of N’-(4-Fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(4-Fluorobenzylidene)-4-hydroxybenzohydrazide

S Suzana¹, M I Sulistyowaty¹, Isnaeni¹ and T Budiati¹*
¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60285
*Corresponding author: suzanarushadi@yahoo.com

Abstract. Many antibacterial are already resistant, so new antibacterial is needed. Objective: The compounds of N’- (4-Fluorobenzylidene) -4-hydroxybenzohydrazide, and N’ - (3-Bromobenzylidene) -4-hydroxybenzohydrazide as antibacterial generally contain azometin (HN-N=CH-) and halogen groups. Before the compounds to be synthesized were done docking molecule study, to predict its activity as antibacterial. The synthesis was carried out by microwave and identification of the results with FT-IR, MS, ¹H-NMR, and ¹³C-NMR, docking molecule study with the Molegro Virtual Docker program, and antibacterial activity by diffusion method. The obtained compounds of N’- (4-Fluorobenzylidene) -4-hydroxybenzohydrazide, and N’ - (3-Bromobenzylidene) -4-hydroxybenzohydrazide 87%, and 81% yield respectively. The results of docking molecule study obtained reranked score lower than the starting material (methyl 4-hydroxybenzoate). The compounds have antibacterial activity against Gram positive (Bacillus subtilis) and Gram negative (Escherichia coli). Conclusion: N’- (4-Fluorobenzylidene) -4-hydroxybenzohydrazide and N’ - (3-Bromobenzylidene) -4-hydroxybenzohydrazide have been synthesized, characterized and exhibited promised antibacterial.

1. Introduction
Infection is a disease that still affects many people in the world, both lower, middle and upper levels of society. According to the World Health Organization (WHO) infectious diseases are the second leading cause of death in the world [1]. Infection is a dominant disease in developing countries and many people suffer from microbial Enterobacteria from Escherichia, Salmonella, Shigella, Klebsiella. Pathogenic microbes that are resistant to certain antimicrobials are also growing, for example MRSA (Methicillin-resistant Staphylococcus aureus), VRSA (Vancomycin-resistant Staphylococcus aureus) and ESBL (Extended spectrum beta-lactamase producers).

Fat is one of the most important components in the formation of microbial cell walls, an important component of the composition of lipopolysaccharides and phospholipids in cell membranes. The pathway of fatty acid biosynthesis in microbes begins with acetylcholine. Fatty acid biosynthesis in bacteria through many stages and involves many enzymes. In the terminal stage there is a change in trans 2-enoyl ACP to acyl ACP which is affected by the enzyme enoyl-ACP reductase (ENR). Acyl ACP is needed as a phospholipid biosynthesis material, which is a constituent component of membranes in the microbial cell wall [2].

Antimicrobials that selectively inhibit the function of cell membranes through resistance to ENR enzymes, both against Gram negative (E. coli), Gram positive Staphylococcus aureus (S. aureus), B subtilis...
are nifuroxazida (N’ - (5-nitrofuran-2-ii) methylen) -4-hydroxybenzohydrazide). These compounds have hydroxyl groups on the benzene ring and azometin groups and are known as intestinal antiseptics [3].

Hydrazide derivative compounds are widely used because they have biological activity and clinical applications that vary among them as antimicrobials [4]. The presence of hydroxyl on the benzene and azometin (NN-N = CH-) as pharmacophore provide activity as antimicrobials [5]. In some antimicrobial structures, there is a hydroxyl group or an azometin group (-HN-N = CH-) which is a pharmacophore. In some compounds, these groups are in aromatic rings, aromatic heterocyclic rings or in nonaromatic heterocyclics, as -NH- or -N = C- [6].

Based on the description above, it was determined that the lead compound in this study was N-benzilidene-4-hydroxybenzohydrazide. Substituted N-benzilidene-4-hydroxybenzohydrazide compounds synthesized based on ligand-based design molecular modeling using the Mollegro Virtual Docker (MVD) version 5.0 program. In molecular modeling, several derivatives of N-benzilidene-4-hydroxybenzohydrazide are used as ligands, whereas as receptors, ENR is used (pdb.1C14) [7]. The structure of IC14 is chosen because it is a target of compounds that have a working mechanism to inhibit ENR.

The methyl 4-hydroxybenzoate is a generally limited antimicrobial compound that used as a food preservative and cosmetics. Modified the structure of methyl 4-hydroxybenzoate to 4-hydroxybenzohydrazide, then modified to 4-hydroxybenzohydrazide-derived novel compounds. The compounds of 4-hydroxybenzohydrazide derivatives with some additional specific groups acting as chromophore such as the azometin group (-N-N = C-) and halogen (F, Br) groups, are expected to act as more antimicrobials and smaller toxicities [8]. The addition of groups also causes 4-hydroxybenzohydrazide derivatives to become more lipophyllable, so that the ability to penetrate cell membranes also increases. Based on these descriptions, the synthesis of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzo hydrazide, indicated in vitro indispensable antibacterial effect.

The antibacterial activity was performed on Gram negative (E. coli) and Gram positive (B. subtilis) to obtain minimum inhibitory concentration (MIC) of the compounds. Based on the background briefly above, the problems formulated in this study were: (1) Whether N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzo hydrazide may be synthesized? (2) How is their antimicrobial activity in these derivatives? This research will be informed new technology of the reaction method with microwave irradiation to synthesise of N’-benzilidene-4-hydroxybenzohydrazide derivatives (Figure 1).

Figure 1. Synthesis reaction of N’-benzilidene-4-hydroxybenzohydrazide derivatives

2. Materials and Methods

2.1 Synthesis of 4-hydroxybenzohydrazide

The methyl 4-hydroxybenzoate (5 mmol) was added hydrazine hydrate (50 mmol) in the flask. The mixture of this compound was stirred until a homogeneous solution obtained and then it was irradiated with microwave. The mixture was cooled, added aquadest, washed with ethanol, filtered and recrystallized with ethanol. The purity of compounds was tested by melting point and thin layer chromatography. Identification of compounds was performed using UV-VIS, FT-IR, 1H-NMR spectroscopy [9,10,11].

2.2 Synthesis of N’-benzilidene-4-hydroxybenzohydrazide derivatives

4-Hydroxybenzohydrazide (5 mmol) and 4-fluorobenzaldehyde/3-bromobenzaldehyde (7 mmol) were mixture in the flask. The mixture was stirred until a homogeneous. The mixed solution was irradiated with microwave. The mixture was cooled to room temperature and then added aquadest, washed with
ethanol, and filtered. Crystals were recrystallized with ethanol. The purity of compounds was tested by melting point and thin layer chromatography. Identification of compounds was performed using UV-VIS, FT-IR, MS, 1H-NMR, 13C-NMR spectroscopy [9,10,11].

3. Result and Discussion

Synthesis of compounds was done by two step reactions, (1) Synthesis 4-hydroxybenzohydrazide and (2) Synthesis N'-benzylidene-4-hydroxybenzohydrazide derivatives. Synthesis of 4-hydroxybenzohydrazide was done by the nucleophilic substitution reaction [12,13]. The 4-Hydroxybenzohydrazide is obtained as a white needle crystalline, with 91% yield. The thin-layer chromatogram showed one spot with UV detector. The melting point of the compound was 255-256°C. Characterization of 4-hydroxybenzohydrazide was done with UV-Vis, FT-IR, 1H-NMR spectroscopy. Synthesis of N'-benzylidene-4-hydroxybenzohydrazide derivatives on second step reactions were done by the nucleophilic addition reaction [12,13] The N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide is white needle crystalline with synthesized percentage 87%. The thin-layer chromatogram showed one spot, m.p 245-256°C. Identification of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide was done by UV-VIS, Infrared, MS, 1H-NMR spectroscopy. N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide is white needle crystalline with synthesized percentage 81%. The thin layer chromatogram showed one spot, m.p. 250-251°C.

Characterization of 4-hydroxybenzohydrazide. The yield 91%, a white needle-shaped crystal; m.p. 255-256°C; UV-Vis (EtOH), nm: 208 and 254. IR (KBr in cm-1): 1674 (-C=O amide), 3318 (-OH phenolic), 1590 and 1467 (-C= aromatic), 1354 (C-N), 3005 (Csp2-H), 850 (para di-substitution on benzene), 3197 (-NH2). 1H-NMR (DMSO-d6, ppm): 6.73 (dd, J=8.4 Hz, 2H, C6H4-), 7.64 (dd, J=9.2 Hz, 2H, C6H4-), 9.89 (s, 1H, OH), 4.32 (s, 2H, NH2) [14,15].

Characterization of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide. The yield 87%, a white needle-shaped crystal; m.p. 245-246°C; UV-Vis (EtOH), nm: 224 and 306. IR (KBr in cm-1): 1629 (-C=O amide), 3436 (-OH phenolic), 1599 and 1472 (-C=C- aromatic), 1367 (C-N), 3038 (Csp2-H), 848 (para substitution of benzene), 1606 (-NH- bending). [M+Na] =281,0698. 1H-NMR (400MHz, DMSO-d6, ppm): 11.62 (s, 1H, -HC=N), 10.08 (s, 1H, -NH-), 8.39 (s, 1H, OH), 6.83 (d, J=8.8 Hz, 2H from benzene), 7.26 (t, J =8 Hz, 2H from benzene), 7.79-7.71 (m, 4H from benzene). 13C-NMR (400MHz, DMSO-d6, ppm): 164.7 (=C-F), 163.3(C, C=O), 161.2 (C, Csp2-OH), 146.2 (C, -CH=N), 131.7, 131.2, 130.2, 129.6, 124.4, 116.5 (2C), 116.3 (2C), 115.6 (10C from two aromatic rings) [14,15].

![Figure 2. Hydrogen bonding (a) and steric interaction (b) of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide with pdb.1C14 (2D)](image-url)

Characterization of N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide. The yield 81%, a white needle-shaped crystal; m.p. 250-251°C; UV-Vis (EtOH), nm: 208, 226 and 306. IR (KBr in cm-1): 1624 (-C=O amide), 3458 (-OH phenolic), 1591 and 1474 (-C=C- aromatic), 1429 (C-N), 847 (para...
substitution of benzene), 3236 (\(-\text{NH}\)- stretching) and 1606 (\(-\text{NH}\)- bending). \([\text{M+Na}] = 340,9896 (99\%),\)
\([\text{M+Na} + 2] = 342,9896 (100\%).\) 1H-NMR (400MHz,DMSO-d6, ppm): 11.76 (s, 1H, HC=N), 10.10 (s, 1H, -NH), 8.36 (s, 1H, OH), 6.85 (d, J=6.8 Hz, 2H from benzene), 7.34 (t, J=7.6 Hz, 1H from benzene), 7.54 (d, J=7.6 Hz, 1H from benzene), 7.80 (d, J=8.8 Hz, 1H from benzene), 7.86 (s, 1H from benzene). 13C-NMR (400 MHz, DMSO-d6, ppm): 163.3 (1C, C=O), 161.4 (1C, Csp2-OH), 145.6 (1C, -CH=N), 137.5, 132.9, 131.4, 130.3, 129.5 (2C), 126.6, 124.2, 122.7, 115.6 (2C) (11C from two aromatic rings) [14,15].

\[\text{Figure 3.} \text{ Hydrogen bonding (a) and steric interaction (b) of N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide with pdb.1C14 (2D)}\]

\[\text{Figure 4.} \text{ Hydrogen bonding (a) and steric interaction (b) of Methyl 4-hydroxybenzoate with pdb.1C14 (2D)}\]

The active crystal structure of 1C14 interacted with pharmacophores of compounds [Figure 3-5]. The structure of the ENR inhibitor (pdb. 1C14) was obtained from www.rcsb.org. The rerank score of compounds based on molecular docking with receptor pdb. 1C14 program MVD (Molegro Virtual Docker 5.0) is smaller than the rerank score of methyl 4-hydroxybenzoate. This indicated that the modified compound has smaller bond energy, so that the binding is more stable [16,17]. The modified compounds be expected to have greater biological activity by docking molecule study. The rerank score of the compounds and the amino acids involved in the interaction [Table 1] which represents as ligand-protein binding energy.
Figure 5. Hydrogen bonding (a) and steric interaction (b) of Nifuroxazide with pdb.1C14 (2D)

Table 1. Molecular docking results of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide, N’-(3-bromo benzylidene)-4-hydroxybenzohydrazide, nifuroxazide and methyl 4-hydroxybenzoate with pdb. 1C14 program MVD 5.0

| Compounds                                      | Rerank Score (kcal/mol) | Amino Acid                      | Hydrogen Bonding | Interaction steric (Van der waals) |
|------------------------------------------------|-------------------------|---------------------------------|------------------|-----------------------------------|
| Methyl 4-hydroxybenzoate                       | -62.5223                | Asp 1064, Val 1065, Cys 1063, Gln 1040, Ile 1092 | 2                | 5                                 |
| Nifuroxazide                                   | -98.5737                | Asp 1064, Val 1065, Ser 1070, Ala 1066, Cys 1063, Ile 1119 | 4                | 6                                 |
| N’-4-Fluorobenzylidene-4-hydroxybenzohydrazide | -87.5696                | Asp 1064, Val 1065, Thr 1038, Gln 1040, Cys 1063, Ile 1119, Ala 1066, Glu 1067 | 2                | 8                                 |
| N’-(3-Bromobenzylidene)-4-hydroxybenzohydrazide | -89.5923                | Asp 1064, Val 1065, Thr 1038, Gln 1040, Cys 1063, Ile 1119, Ala 1066, Glu 1067 | 2                | 8                                 |

The lowest energy (RS) should represent the best-found binding mode. N’-benzylidene-4-hydroxybenzohydrazide derivatives have a better rerank score (RS=-87.5696 and -89.5923 kcal/mol) than methyl 4-hydroxybenzoate (RS=-62.5223 kcal/mol) which indicate high affinity of the compounds against 1C14. Besides hydrogen bonds, there are steric interaction performed by compounds (methyl 4-hydroxybenzoate, N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide) with the sum of amino acid residues: Asp 1064, Val 1065, and Cys 1063. Nifuroxazide as an intestinal antiseptic, in molecular docking has hydrogen bonds involving 4 kinds of amino acids (ie Asp 1064, Val 1065, Ser 1070, Ala 1066). Whereas in N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide hydrogen bonds only with two kinds of amino acids. N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzo
hydrazide have an interaction with the amino acid residue of the 1C14 receptor, which is almost the same.

The antibacterial activity of testing compounds against Bacillus subtilis FNCC 0059 and Escherichia coli ATCC 25922. Used nifuroxazide as reference standards. The antibacterial activity of target compounds was carried out by well diffusion method [17]. The N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide has MIC 15.7 ppm against B. subtilis and E. coli respectively. The MIC of N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide against E. coli was 15.7 ppm, and 15.7 ppm against B. subtilis. The antibacterial activity of the compounds was detected because of the halogen and hydroxyl on benzene (–C₆H₄-X) [18] and azometin groups (-HN-N=CH-) [19, 20, 21]. Based on a docking molecule study, N’-(4-fluoro benzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide have the rerank score value lower than methyl 4-hydroxybenzoate. This is in accordance with the results of antibacterial tests on E. coli and B. subtilis. These compounds have a higher activity than methyl 4-hydroxybenzoate

4. Conclusion

The yields of N’-(4-fluorobenzylidene)-4-hydroxybenzohydrazide and N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide were obtained 87%, 81% respectively. (N’-(3-bromobenzylidene)-4-hydroxybenzohydrazide), N’- (4-Fluorobenzylidene) -4-hydroxybenzohydrazide and N’ - (3-Bromobenzylidene) -4-hydroxybenzohydrazide have been exhibited promised antibacterial.

Acknowledgements

This research was financially funded by RISTEK-DIKTI through Grant “Penelitian Disertasi Doktor (PDD)” scheme 2018. We are grateful to Prof. Dr. Siswandono, Apt., MS., (Faculty of Pharmacy, Universitas Airlangga) for facilitation of the MVD program license.

References

[1] Romero J C L, Rios H G, Borges A and SimoesM 2015 Escherichia coli and Staphylococcus aureus Evidence-Based Complementary and Alternative Medicine Volume 2015 Article ID 795435 44-55
[2] George S, Basheer R M, Ram S V, Selvaraj S K., Rajan S and Ravi T K. 2014 Bangladesh Journal Pharmacological 9 49-53
[3] Asif M 2012 International Journal of Advanced Chemistry 2 (2) pp. 85-103.
[4] Negi VJ, Sharma AK, Negi JS and Ram V 2012 International Journal of Pharmaceutical Chemistry 2 (4) 100-109.
[5] Wang Q, Pan Y, Wang J, Peng Q, Luo H and Zheng J 2011 African Journal of Biotechnology 10 (78) 18012-18021.
[6] Pangal A, Ahmed K and Shaikh S 2013 International Research Journal of Pharmacy 4 (8) 172-176.
[7] Kapoor M, Mukhi P L S, Surolia N, Kusuga K. and Surolia A 2004 Biochemistry Journal 381 725-733.
[8] Kostecka M 2012 Molecules 17 3560-73.
[9] Budiati T, Stephani D A and Widjajakusuma E C 2012 Indonesia Journal of Chemistry 12 (2) 163-165.
[10] Jain AK, Gupta PK, Ganesan K, Pande A and Malhotra RC 2007 Defense Science Journal 57 (2) 267-270.
[11] Habibi D and Marvi O 2006 General paper Arkivoc (xiii) 8-15.
[12] McMurry J 2008 Organic Chemistry 7th Edition Thomson Learning Inc. USA 877-884.
[13] Solomons G T W and Fryhile C B 2011 Organic Chemistry 10th Ed. United States: John Willey & Sons Inc., 833-834.
[14] Pavia DL, Lampman GM, Kriz GS and Vyvyan JR 2009 Introduction to Spectroscopy 4th Ed. Brooks/Cole, USA 381-403.
[15] Silverstein RM, Webster FX and Kiemle DJ 2005 Spectrofotometric Identification of Organic Compound, 7th Edition, New York: John Wiley and Sons, Inc. 14-37.
[16] Behera SM, Mohanta RK, Sahu SK, Banerjee M and Mohanta L 2013 Molecular docking: a review. World Journal of Pharmaceutical Research 3 (1) 236-257.

6
[17] Kitchen DB, Decornez H, Furr JR and Bajorath J 2004 Nature Reviews Drug Discovery 3 (11) 935-949.
[18] Balouri M, Sadiki M and Ibnsouda SK 2016 Journal of Pharmaceutical Analysis 11 (5) 1-10.
[19] Merkl R, Hradkova L, Filip V and Smidrkal J 2010 Czech J. Food Sci. 28 275-279.
[20] Narasimhan B, Pradeep K and Sharma D 2010 Biological activity of hydrazide derivatives in the new millennium. Acta Pharmceutica Sciencia; 52 169-180.
[21] Ali MR, Marella A, Alam MT, Naz R, Akhter M, Shaquiquzzaman M, Saha R, Tanwar O, Alam MM and Hooda J 2012 Indonesian Journal Pharmacy 23 (4) 193-202.