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Synthesis, Molecular Docking And Antimicrobial Of N'-Benzylidene-4-Hydroxybenzohydrazide And N'-(4-Methoxybenzylidene)-4-Hydroxybenzohydrazide.

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ABSTRACT

Synthesized N'-benzylidene-4-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide in the two-step reaction by using methyl 4-hydroxybenzoate as starting material has been performed. Methyl 4-hydroxybenzoat was treated with hydrazine hydrate to obtain 4-hydroxybenzohydrazide. The reaction was carried out by microwave irradiation resulting 91 % yield. The obtained compound was then reacted with benzaldehyde and 4-methoxybenzaldehyde to accomplish the target molecule, N'-benzylidene-4-hydroxybenzo hydrazide and N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide in 92% and 78% yield respectively. The purity of the products was determined by melting point, and Thin Layer Chromatography (TLC). Identification of N'-benzylidene-4-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-4-hydroxybenzohidroksida was performed by UV-VIS, FT-IR, ^{1}H-NMR, ^{13}C-NMR and MS spectroscopy. The molecular docking study was done with receptor PDB.1C14, based on the rerank score it was found that the target compounds exhibited a greater antimicrobial activity than methyl 4-hydroxybenzoate. The N'-benzylidene-4-hydroxybenzohydrazide exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* at 1000 ppm, *Bacillus subtilis* and *Candida albicans* at 500 ppm. Antimicrobial activity of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* were 1000 ppm, and 500 ppm against *Candida albicans*.

Keywords: Synthesis, 4-hydroxybenzohydrazide derivatives, molecular docking, antimicrobial

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INTRODUCTION

Synthesis of drugs interest in the development of the pharmaceutical industry. Modifying of the structures can change to its biological activity. Hydrazide derivatives are often used because they have biological activities and diverse clinical applications such as anticancer, antimicrobial and anti-tuberculosis [1,2,3]. Awathi *et al* (2007) has conducted research synthesis hydrazide derivative 2-hydroxybenzylidene and ((1H-indole-3-ilmetlen)hydrazide) using microwave irradiation, a mixture of ester and hydrazine hydrate (mole ratio 1:1), the results obtained about 85 -90% [4]. In another study conducted Jain et al, 2007, the synthesis of 2-hydroxybenzohydrazide using microwave irradiation within 120 seconds, the synthesis results obtained 90% [5]. In this research synthesis 4-hydroxybenzohydrazide and N'-benzylidene-4-hydroxybenzohydrazide and derivatives. Synthesis is done by using microwave irradiation method, without the toxic solvents and more environmentally friendly methods [6,7]. Reduced use of toxic solvents and energy savings in accordance with campaign *green chemistry.* Based on the background above, the problems are formulated in this study: whether can be synthesized 4-hydroxybenzohydrazide, N'-benzylidene-4-hydroxybenzohydrazide and derivative? What is their antimicrobial activity on N'-benzylidene-4-hydroxybenzohydrazide and derivatives? The benefits of research can yield new knowledge about the reaction method to synthesize of 4-hydroxybenzohydrazide and N'-benzylidene-4-hydroxybenzohydrazide and derivatives. The reaction is described as Figure 1.

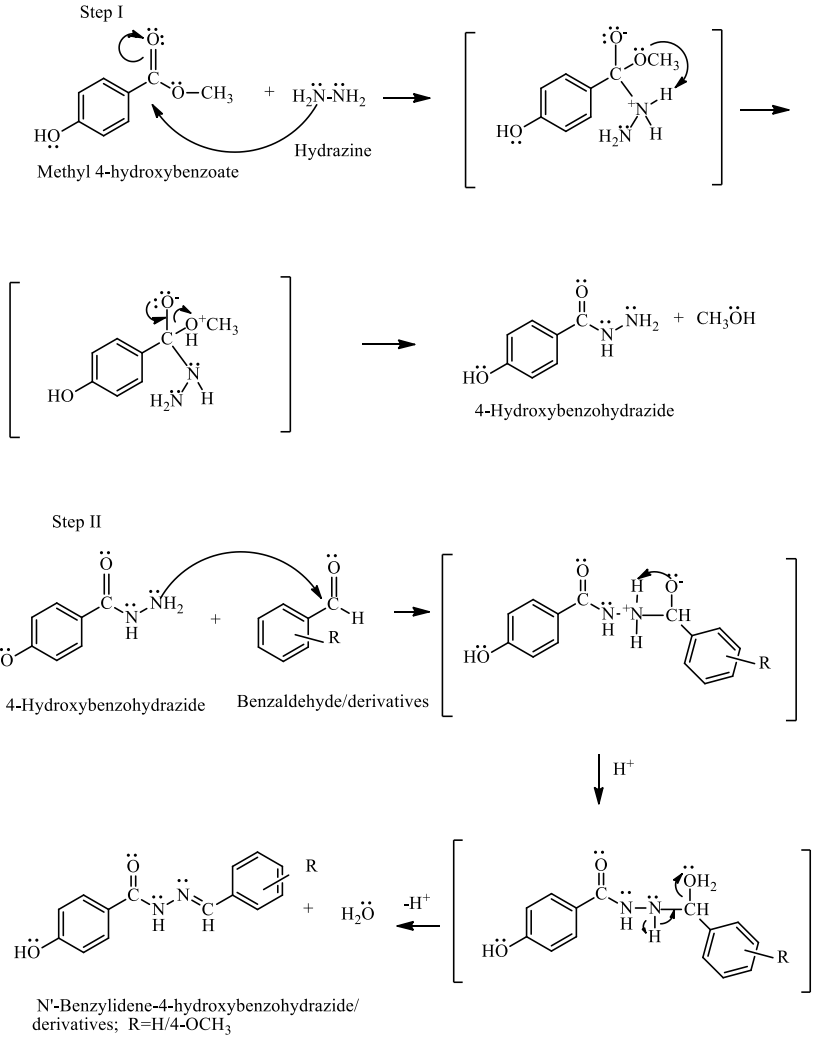

Figure 1. Reaction of N'-benzylidene-4-hydroxybenzohydrazide/derivatives formation from methyl 4-hydroxybenzoate

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MATERIALS AND METHODS

Materials

All chemicals were used in this study with a degree of purity p.a unless otherwise stated. The chemicals were methyl 4-hydroxybenzoate, hydrazine hydrate, benzaldehyde, 4-methoxybenzaldehyde, ethanol 95%, chloroform, ethyl acetate, acetone, hexane, KBr, silica gel 60 GF_{254}.

Instruments

Glasswares commonly used in the chemical synthesis laboratories; Sanyo microwave EM-S 400 Watt, UV-Vis Spectrophotometer HEWLETT PACKARD 8452A; IR Spectrophotometer M 500 Buck Scientific; GS-MS Agilent 6890N, FT- NMR spectrometer JEOL ECS -400.

Methods

Synthesis of 4- hydroxybenzohydrazide [8]

Methyl 4-hydroxybenzoate (10 mmol) and hydrazine hydrate (50 mmol) were mixtures enter in the reaction flask (25 ml). A mixture was stirred until homogeneous. The mixed solution was irradiated with microwave (perfection reaction was monitored by thin layer chromatography). The mixture was cooled to room temperature and then added to 20-30 ml of aquadest, washed with ethanol, filtered. Crystals were recrystallized with ethanol 95%. The purity tests use melting point and thin layer chromatography using three different eluent. Identification of compounds was performed using UV-VIS, FT-IR, ^{1}H-NMR spectroscopy.

Synthesis of N'-benzylidene-4-hydroxybenzohydrazide and derivatives [9,10]

4-Hydoxybenzohydrazide (10 mmol), benzaldehyde/4-methoxybenzaldehyde (20 mmol) were mixtured enter in the reaction flask (25 ml). A mixture was stirred until homogeneous. The mixed solution was irradiated with microwave (perfection reaction was monitored by thin layer chromatography). The mixture was cooled to room temperature and then added to 20-30 ml of aquadest, washed with ethanol, filtered. Crystals were recrystallized with ethanol 95%. The purity tests use melting point and thin layer chromatography using three different eluent. Identification of compounds was performed using UV-VIS, FT-IR, ^{1}H-NMR, ^{13}C-NMR, MS spectroscopy.

RESULTS AND DISCUSSION

Synthesis 4-hydroxybenzohydrazide derivatives were done by the nucleophilic substitution reaction [11]. 4-Hydroxybenzohydrazide is obtained as a white needle crystalline. Synthesized percentage is 91%. Purity test results with thin-layer chromatography shows one spot, with melting point 255-256^{o}C. Identification of 4-hydroxybenzohydrazide was done with UV-Vis, FT-IR, ^{1}H-NMR spectroscopy. N'-benzylidene-4-hydroxybenzohydrazide is white needle crystalline. Synthesized percentage is 92%. Purity test results with thin-layer chromatography shows one spot, m.p 243-244 ^{o}C . Identification of N'-benzylidene-4-hydroxybenzohydrazide was used UV - Vis, Infrared, ^{1}H-NMR , ^{13}C-NMR, and MS spectroscopy. N'-(4-methoxybenzylidene)-4-hydroxy benzohydrazide is white needle crystalline. Synthesized percentage is 78%. Purity test results with thin layer chromatography shows one spot, m.p 220-221^{o}C. Spectra data UV-Vis, IR, ^{1}H-NMR, ^{13}C-NMR, and MS of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide can be seen in below section [12,13].

Shintesized of N'-benzylidene-4-hydroxybenzohydrazide and N'-(4-methoxybenzyldene)-4-hydroxybenzo hydrazide are obtained respectively 92% and 78%. The presence of methoxy groups in positions *para* at aromatic ring cause to decline the reactivity of the carbonyl C. 4-Methoxy group act as positive inductive (+I) that can decline the electron density on the aromatic rings (from 4-methoxybenzaldehyde), so it becomes less electronegative. As a result, the carbonyl C atom becomes less electropositive. Thus C carbonyl of 4-methoxybenzaldehyde are more difficult attacked by nucleophiles (4-hydroxy benzohydrazide) [Figure 1]. So that the methoxy group at *para* positions in 4-methoxybenzaldehyde can decline the yield of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide[11].

Characterization of 4-hydroxybenzohydrazide (I) [12,13]

A white needle-shaped crystals were obtained, yield 91%; 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=C- aromatic), 1354 (C-N), 3005 (Csp^2-H), 850 (*para* di-substitution on benzena), 3197 ($-NH_2$). 1H-NMR (DMSO-d_6, δ, ppm): 6.74-6.72 (d, *J*=8.4 Hz, 2H, C_6H_4-), 7.65-7.62 (d, *J*=9.2 Hz, 2.H, C_6H_4-), 9.89 (s, 1H, OH),9.44 (s, 1H, NH), 4.32 (s, 2H, NH_2) .

Characterization of N'-benzylidene-4-hydroxybenzohydrazide (IIa) [12,13]

A white needle-shaped crystals were obtained, yield 92%; m.p. 245-246 °C. UV-Vis (EtOH), nm: 218 and 304. IR (KBr in cm^{-1}): 1618 (-C=O amide), 3225 (-OH phenolic), 1589 (-C=C- aromatic), 1551 (-NH), 1359 (C-N), 3038 (Csp^2-H), 849 (*para* substitution of benzene), 1574 (C=N). $[M^+]$ =240. ^{1}H-NMR (DMSO-d_6, δ, ppm): 11.61 (s, 1H, OH), 10.10 (s, 1H, NH), 8.38 (s, 1H, HC=N), 6.82 (dd, J=2 Hz, J=2Hz, 2H from aromatic ring), 763-743 (m, 3H from aromatic ring), 7.67 (d, J=6.0 Hz, 2H from aromatic ring), 7.76-7.79 (m,, 2H from aromatic ring). ^{13}C-NMR (DMSO-d_6, δ, ppm): 163.2 (1C, C=O), 161.2 (1C, C-OH), 147.3 (1C, C-N), 135.0, 130.4, 130.2 (2C), 129.4 (2C), 127.5 (2C), 124.4, 115.5 (2C).

Characterization of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide (IIb) [12,13]

A white needle-shaped crystals were obtained, yield 78%; m.p. 216-217 °C. UV-Vis (EtOH), nm: 218 and 316. IR (KBr in cm^{-1}): 1622 (-C=O amide), 3268 (-OH phenolic), 1603 (-C=C- aromatic), 1574 (-NH), 1361 (C-N), 3019 (Csp^2-H), 765 (*p*-disubstitution of benzene), 1628 (C=N), 1251 (phenylalkil ether), 2962 (Csp^3-H). $[M^+]$ =270. ^{1}H-NMR (DMSO-d_6, δ, ppm): 11.47 (s, 1H, OH), 10.04 (s, 1H, NH), 8.32 (s, 1H, HC=N), 6.81 (d, J=8.4 Hz, 2H from aromatic ring), 6.97 (d, J=8.8 Hz, 2H from aromatic ring), 7.60 (d, J=8.4 Hz, 2H from aromatic ring), 7.75 (d, J=8.8 Hz, 2H from aromatic ring), 3.77 (s, 3H, OCH_3). ^{13}C-NMR (DMSO-d_6, δ, ppm): 163.1(1C, C=O), 161.2 (1C, eter), 161.1(1C, C-OH), 147.2 (1C, C=N), 130.1 (2C), 129.1 (2C), 127.6, 124.5, 115.5 (2C), 114.8 (2C).

The active crystal structure of 1C14 interacted with pharmacophores of compounds (I, IIa, IIb, nifuroxazide). The rerank score of the compounds were showed on Table 1, which represents ligand-protein binding energy.

Table 1. Result of docking molecule N'-benzylidene-4-hydroxybenzohydrazide and N'-(4-methoxybenzylidene-4-hydroxybenzohydrazide with receptor pdb.1C14 program MVD 5.0

Compounds	Rerank score (kcal/mol)	Amino acid	Hydrogen bonding	Steric (Van der Waals)
Methyl 4-hydroxybenzoate (a)	-66.4976	Asp 1064, Val 1065, Cys 1063, Gln 1040, Ile1092	2	13
Nifuroxazide (b)	-106.9710	Asp 1064, Val 1065, Ser 1070, Ala 1066, Cys 1063, Ile 1119	4	16
N'-Benzylidene-4-hydroxybenzohydrazide (IIa)	-100.3780	Asp 1064, Val 1065, Val 1011, Thr 1038, Thr 1012, Gly 1013, Ile 1092, Ala 1066	2	18
N'-(4-Methoxybenzylidene)-4-hydroxybenzohydrazide (IIb)	-107.3360	Asp 1064, Val 1065, Thr 1038, Gln 1040, Gln 1062, Cys 1063, Ile 1119, Ala 1066, Gly 1067	3	17

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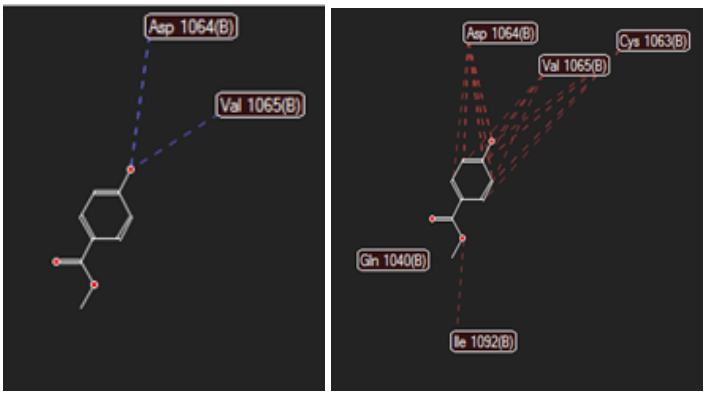
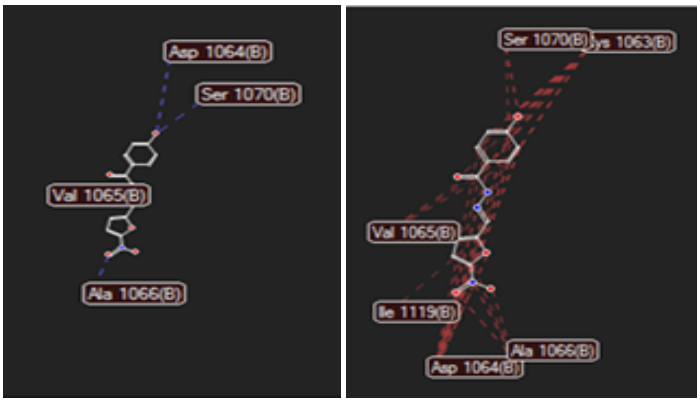


(1a) (2a)

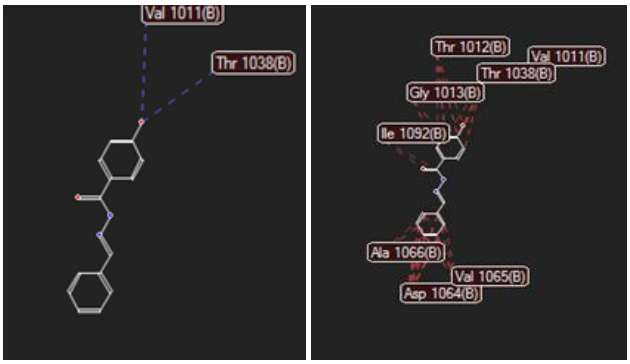
Hydrogen (1a) and steric bond (2a) in interaction of methyl 4-hydroxybenzoate-1C14 (2D)



(1b) (2b)

Hydrogen (1b) and steric bond (2b) in interaction of nifuroxazide-1C14 (2D)

Figure 2. Molecular docking of methyl 4-hydroxybenzoate and nifuroxazide with 1C14 (2D)



(1c) (2c)

Hydrogen (1c) and steric bond (2c) in interaction of N'-benzylidene-4-hydroxybenzohydrazide-1C14 (2D)

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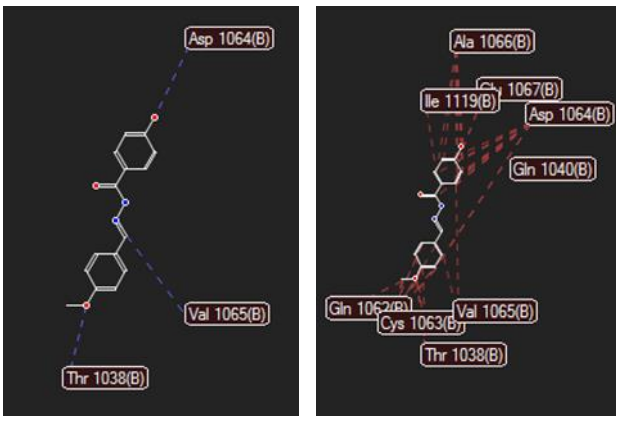

(1d) (2d)

Hydrogen (1c) and steric bond (2c) in interaction of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide-1C14 (2D)

Figure 3. Molecular docking of of N'-benzylidene-4-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide with 1C14 (2D)

Molecular docking study was done with receptor pdb.1C14 program MVD (Mollegro Virtual Docker 5.0), based on the rerank score it was found that the target compounds exhibited a greater antimicrobial activity than methyl 4-hydroxybenzoate. The rerank score of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide exhibited a greater than nifuroxazide [14,15].

Antimicrobial Activity Method

The antibacterial activity of testing compounds was carried out by the well diffusion method [16]. The antimicrobial activity of testing compounds was conducted against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Candida albicans.* Kanamycin 50 ppm and Gentamycin 50 ppm were used as reference standards to compare the results. The N'-benzylidene-4-hydroxybenzohydrazide exhibited antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* at 1000 ppm, *Bacillus subtilis* and *Candida albicans* at 500 ppm. Antimicrobial activity of N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide against *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis* was 1000 ppm, and 500 ppm against *Candida albicans*. Antimicrobial activity of target compounds because compounds have hydroxyl phenolic (-C_6H_4-OH) [17] and azometin groups (-HN-N=CH-) [18, 19, 20, 21].

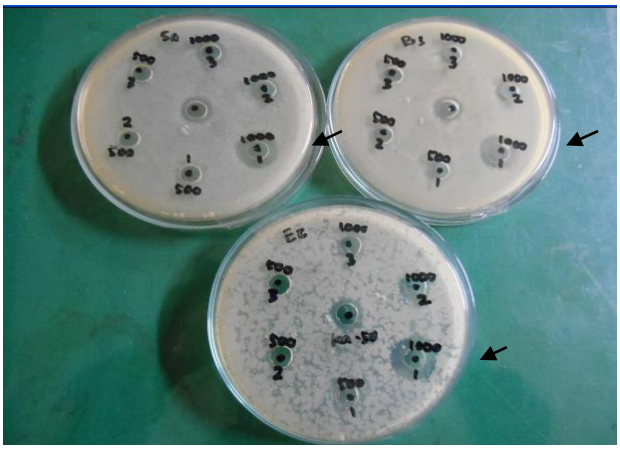

Figure 4. Bioassay of N'-benzylidene-4-hydroxybenzohydrazide (1) and N'-(4-methoxy benzylidene)-4-hydroxybenzohydrazide (3), 500 ppm and 1000 ppm against *S. aureus, E. coli* and *B. subtilis*

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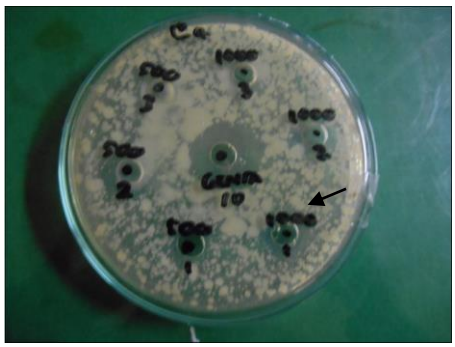

Figure 5. Bioassay of N'-benzylidene-4-hydroxybenzohydrazide (1) and N'-(4-methoxy benzylidene)-4-hydroxybenzohydrazide (3) against *C. albicans*

CONCLUSION

4-Hydroxybenzohydrazide and its derivatives can be synthesized by microwave irradiation. The derivatives were N'-benzylidene-4-hydroxybenzohydrazide, and N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide. The yields were obtained 78%-92%. From molecular docking study data the smallest of rerank score value was -107.3360 kcal/mol (N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide). The preliminary biological tests indicated that N'-benzylidene)-4-hydroxybenzohydrazide and N'-(4-methoxybenzylidene)-4-hydroxybenzohydrazide have antimicrobial activity against *S. aureus*, *E. coli, B. subtilis* and *C. albicans.*

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