Phenethyl ester and amide of Ferulic Acids: Synthesis and bioactivity against P388 Leukemia Murine Cells

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Abstract. Bioactivity of a compound is closely related to the molecular structure of the compound concerned, its strength being the quantitative relation of the strength of the activity of the group it possesses. The combining of moieties of the active compounds will produce more active compounds. Most phenolic compounds as well as compounds containing moiety phenethyl groups have potential activity as anticancer. Combining phenolic groups and phenethyl groups in a compound will result in compounds having strong anticancer bioactivity. This study aims to combine the feruloyl and phenethyl groups to form esters and amides by synthesis of phenethyl trans-3-(4-hydroxy-3-methoxyphenyl)acrylate (5) and trans-3-(4-hydroxy-3-methoxyphenyl)-N-phenethylacrylamide (6) from ferlic acid with phenethyl alcohol and phenethylamine, and to study their bioactivity as anticancer. The synthesis of both compounds was conducted via indirect reaction, including acetylation, chlorination, esterification/amidation, and deacetylation. Structures of products were characterized by FTIR and NMR data, and their bioactivity assay of the compounds against P388 Leukemia Murine Cells was conducted by an MTT method. Results showed that the compound 5 was obtained as a yellow gel with the IC50 of 10.79 µg/mL (36.21 µM), and the compound 6 was a yellowish solid with a melting point of 118-120°C and the IC50 of 29.14 µg/mL (97.79 µM). These compounds were more active than the analog compounds.

1. Introduction

Ferulic acid or trans-3-(4-hydroxy-3-methoxyphenyl) acrylic acid is a derivative compound of hydroxycinnamic acid having various interesting bioactivity [1]. The structure of these compounds consists of a phenolic group which has been associated with anticancer [2]. Ferulic acid and its derivatives have a therapeutic effect that is potentially useful in the treatment of cancer, diabetes, lung and cardiovascular diseases, as well as hepatic, neuro and photoprotective effects, and has antimicrobial and anti-inflammatory activities [3]. Hexacosyl-(E)-ferulate and leucosceptoside displayed significant antioxidant activity in the DPPH assay with the RC50 of 0.0976 mg/mL and 0.0148 mg/mL, respectively [4].

The same phenomenon, 2-phenyl ethanol exhibits a wide spectrum of activity as it is highly active against S. aureus, E. faecalis, E. coli and P. aeruginosa [5]. Several ester and amide compounds containing phenyl group exhibit biological activity such as antitumor [6], antioxidant [7], anti-inflammatory [8,9], immune regulation [10], antimicrobial [11], and antidiabetic [12]. These facts indicated that phenethyl group played an important role on bioactivity of compounds. Therefore, the merge of phenethyl and ferulic groups in an ester or amide compounds like 5 or 6 (figure 1) can be expected to be more active as anticancer.

Attempts to synthesize ester and amide derivatives of p-hydroxycinnamic acid directly by using a catalyst have been conducted. However, the method was failed. This is thought to be due to the reaction of condensation between hydroxycinnamic compounds involving the phenolic group with the
active carboxyl group. Based on these assumptions, the \( p \)-hydroxyic acid amide has been successfully synthesized after the phenolic group is protected with an acetyl group \([13]\). Therefore, the conversion of \textit{trans}-3-(4-hydroxy-3-methoxyphenyl)acrylic acid to ester \( 5 \) and amide \( 6 \) have been conducted via protection, chlorination, esterification/amidation, and deprotection reactions (figure 1). Their activities were assayed against P388 leukemia murine cells using the MTT method.

![Figure 1. The pathway of synthesis reaction of compounds 5 and 6.](image)

2. Methodology

2.1 Materials

\textit{Trans}-Ferulic acid, acetic anhydride (\( \text{Ac}_2\text{O} \)), phenethylalcohol, phenethylamine, ethyl acetate (\( \text{EtOAc} \)), pyridine, thionyl chloride, dichloromethane (\( \text{DCM} \)), benzene, methanol, pyrrolidine, triethylamine (\( \text{TEA} \)), \( n \)-hexane, 4-(dimethylamino)pyridine (\( \text{DMAP} \)), \( \text{NH}_4\text{Cl} \), \( \text{HCl} \), and anhydrous \( \text{Na}_2\text{SO}_4 \), were pro synthesis and pro analysis grades, used without purification.

2.2 Equipment

The melting points were taken using electro-thermal apparatus, the NMR spectra were recorded on a Spectrometer Agilent operating at 500 MHz for \( ^1\text{H} \) and 125 MHz for \( ^1\text{C} \) nuclei, and the FT-IR were recorded on a Spectrophotometer Shimadzu Prestige 21, and the activity against P388 Leukemia murine cells were analyzed using a MTT method in the Natural Product Laboratory, Institute Technology of Bandung (ITB).

2.3 procedures

2.3.1 Synthesis of compound 1. The mixture of \textit{trans}-3-(4-hydroxy-3-methoxyphenyl)acrylic acid (10.0 mmol) and acetate anhydride (28.0 mmol) was dissolved into pyridine and stirred at room temperature for 30 minutes. Cold water was added into the reaction product while stirred until the formation of white precipitate has been completed. The precipitate was filtered, washed with water, dried, and recrystallized from hot methanol to obtain the compound 1 as white solids with a melting point of 194-196°C (56.42% yields). The pure compound 1 was analyzed with FT-IR spectrometer. IR (\( \text{KBr} \)): \( \nu \) (cm\(^{-1}\)) = 2500-3200 (O-H, carboxyl), 1761.01 (C=O, acetyl ester), 1687.71 (C=O, conj. carboxyl), 1631.78 (C=C, olefin), 3010.88 (unsat. C-H), 1600.99 & 1506.41 (C=C, Ar), 2976.16, 2943.37 & 2841.15 (sat. C-H), 1485.90 & 1371.39 (CH\(_3\)), 985.64 (\textit{trans}-olefin), 856.39 & 837.11 (1,2,4-trisubst. Ar).
2.3.2 Synthesis of compound 2. The compound 1 (2.56 mmol) was dissolved in 20 mL benzene and added with thionyl chloride (14.4 mmol). After atmosphere of reaction system has been changed with nitrogen, the reaction mixture was refluxed for 4 hours. The reaction product was cooled until room temperature, and the solvent was removed by evaporation to give compound 2 as an orange solids. This solid was used in further procedure without purification.

2.3.3 Synthesis of compound 3. The compound 2 was dissolved in 20 mL of dichloromethane and added with 2-phenylethanol (4.17 mmol), pyridine (4.0 mmol), and triethylamine (4.0 mmol). The solution was stirred at room temperature for 4 hours. The reaction product was diluted with 20 mL of dichloromethane and washed with saturated ammonium chloride solution and water respectively, dried with anhydrous sodium sulfate, filtered, and evaporated to give a solid. Purification the product was conducted by gravity column chromatography and recrystallized to give compound 3 as a white crystal with a melting point of 79-80°C (41.58%). The pure compound 3 was analyzed with FT-IR spectrometer. IR (KBr): \( \nu (\text{cm}^{-1}) = 1761.01 \) (C=O, acetyl ester), 1707.00 (C=O, conj. ester), 1641.42 (C=C, olefin), 3030.17 & 3070.88 (unsat. C-H), 1595.13 & 1510.26 (C=C, Ar), 2968.45, 2943.37 & 2843.07 (sat. C-H), 1452.40 & 1371.39 (CH₃), 989.48 (trans-olefin), 852.54 & 829.39 (1,2,4-trisubst. Ar), 754.17 & 702.09 (monosubst. Ar).

2.3.4 Synthesis of compound 5. The compound 3 (0.75 mmol) was dissolved in 1 mL pyrrolidine while stirred, and added with 50 mL of ethyl acetate. Stirring was continued for 1 hour. The reaction product was washed with unsaturated solution of ammonium chloride and water sequently, dried with anhydrous sodium sulfate, and the solvent was removed by evaporation to give compound 4 as a yellow gel (51.96% yields). The pure compound 4 was analyzed with FT-IR, and NMR spectrometer. IR (KBr): \( \nu (\text{cm}^{-1}) = 3412.08 \) (O-H fenolic), 1705.07 (C=O furate ester), 1631.78 (C=C olefin), 3062.96 & 3028.31 (unsat. C-H), 1597.08 & 1514.12 (C=C Ar), 2956.87, 2927.94 & 2852.72 (sat. C-H), 1458.18 & 1384.89 (CH₃), 981.77 (trans-olefin), 848.68 & 817.62 (1,2,4-trisubst. Ar), 748.38 & 702.09 (monosubst. Ar); \( ^{13} \text{C}-\text{NMR (CDCl}_{3}) \): \( \delta \) (ppm) = 36.37, 56.08, 65.02, 109.40, 114.83, 115.51, 123.27, 126.69, 127.09, 128.65, 129.06, 138.07, 145.09, 146.87, 148.09, and 167.34; \( ^{1} \text{H}-\text{NMR (CDCl}_{3}) \): \( \delta \) (ppm) = 3.02 (t, 2H, \( J = 7.05 \) Hz, CH₂), 3.93 (s, 3H, OCH₃), 4.42 (t, 2H, \( J = 7.05 \) Hz, CH₂), 5.90 (s, 1H, O-H), 6.29 (d, 1H, \( J = 15.95 \) Hz and 7.62 (d, 1H, \( J = 15.90 \) Hz, trans olefin), 6.93 (d, 1H, \( J = 8.15 \) Hz, H-Ar), 7.07 (dd, 1H, \( J = 1.75 \) & 8.15 Hz, H-Ar), 7.24 (m, 3H, H-Ar), 7.27 (m, 2H, H-Ar), 7.32 (dd, 2H, \( J = 7.2 \) & 0.95 Hz, H-Ar).

2.3.5 Synthesis of compound 4. The compound 2 was added with phenethylamine (1.1 eq. mol) and dissolved into 30 mL of dichloromethane, added with pyridine and triethylamine (1:1) and stirred at room temperature for 4 hours. The product of this reaction was washed with 20 mL of HCl 1 N, the organic layer was washed again with 20 mL of saturated NH₄Cl, dried with anhydrous Na₂SO₄, and evaporated to give a white solid. The solid was purified with gravity chromatography column to obtain a white solid with a melting point of 118-120°C (63.47% yields). The pure compound 5 was analyzed with FT-IR, and NMR spectrometer. IR (KBr): \( \nu (\text{cm}^{-1}) = 3435.22 \) (sec. N-H), 1753.29 (C=O, ferulate ester), 1651.57 (C=O, amide), 3070.68 (C=H unsat.), 1612.99 (C=C olefin), 1583.56 & 1496.26 (C=C Ar), 2962.66 & 2926.01 (C-H sat.), 1456.26 & 1365.60 (CH₃), 977.91 (trans-olefin), 910.40 & 833.25 (1,2,4-trisubst. Ar), 756.02 & 709.80 (monosubst. Ar).

2.3.6 Synthesis of compound 6. The compound 4 was dissolved in 1 mL of pyrrolidine and added with 50 mL of ethyl acetate, and stirred at room temperature for 4 hours. The product of this reaction was washed with 20 mL of 1 M H₂SO₄, the organic layer was washed again with 20 mL of saturated NH₄Cl dried with anhydrous Na₂SO₄, and evaporated to give a white solid. The solid was dissolved into ethyl acetate, added with n-hexane to give turbid solution, and stored at room temperature until the crystal was completely formed. The crystal was filtered and dried to obtain white crystalline with a melting point of 94-96°C (63.47% yields). The pure compound 6 was analyzed with FT-IR, and...
NMR spectrometer. IR (KBr): \(\nu (\text{cm}^{-1}) = 3352.26\) (N-H, amide), 3288.63 (O-H, phenolic), 3062.96 & 3024.36 (unsat. C-H), 1595.13 & 1514.12 (C=C, Ar), 2926.01, & 2852.72 (C-H sat.), 1454.33 & 1368.46 (CH\(_2\)), 977.91 (trans-olefin), 904.61 & 817.82 (1,2,4-trisubst., Ar), 748.36 & 700.16 (monosubst. Ar); \(^1\)C-NMR (CDCl\(_3\)): \(\delta (\text{ppm}) = 35.76, 40.91, 55.97, 109.83, 114.90, 118.13, 122.17, 126.61, 127.31, 128.75, 129.90, 138.99, 141.24, 146.91, 147.60, \) \& 166.49; \(^1\)H-NMR (CDCl\(_3\)): \(\delta (\text{ppm}) = 2.88 (t, 2H, J = 6.95 \& 13.90 \text{ Hz}, \text{CH}_2), 3.64 (dd, 2H, J = 6.75 \& 13.20 \text{ Hz}, \text{CH}_2), 3.85 (s, 3H, OCH\(_3\)), 5.85 (t, 1H, \ J = 5.15 \text{ Hz}, \text{N-H}), 6.21 (d, 1H, J = 15.50 \text{ Hz} \& 7.53 (d, 1H, J = 15.90 \text{ Hz, trans olefin}), 6.87 (d, 1H, J = 8.20 \text{ Hz, H-Ar}), 6.94 (d, 1H, J = 1.60 \text{ Hz, H-Ar}) 7.00 (dd, 1H, J = 1.55 \& 8.15 \text{ Hz, H-Ar}), 7.20 (m, 1H, H-Ar), 7.22 (m, 2H, H-Ar), 7.30 (m, 2H, H-Ar).

3. Discussion

Approximately a quarter registered medicines have molecules containing amide groups and, consequently, amidation is the most commonly executed conversion to obtain a particular medicine. [14]. However, the common methods for conversion of carboxylic acids to amides suffer from inherent drawbacks. This case is much more pronounced in the conversion of \(p\)-hydroxycinnamic acids to their amides, due to the presence of phenolic groups capable of performing dimerization reactions with activated carbonyl groups [15]. Therefore, the conversion of \(p\)-coumaric acid to its amide was successful when the phenolic group was protected by an acetyl group [13]. This method has been adopted and successfully used in converting ferulic acid compounds into ester and amide, i.e compound 5 and compound 6.

The activity both compounds against P388 murine leukemia cells has been evaluated by a MTT method, and gave the IC\(_{50}\) of 10.79 \(\mu\)g/mL for compound 5 and 29.14 \(\mu\)g/mL for compound 6. Pursuant to these values, the compound 5 can be thought gave strong activity and the compound 6 was moderate [16]. This fact indicated that the activity of the ester compound were stronger than the amide compound. Structurally, this phenomenon is a proper case. When the compounds released hydrogen radicals leave phenolic radicals, the phenolic radical of ester was easily to delocalize its single electron to its carbonyl group; but in the phenolic radical of amide, the delocalization was interfered by the resonance of lone pair electron of nitrogen to its carbonyl group.

Although the activity of ester compounds was higher than amide compounds, the amide derivatives were the most widely used in the medicinal field. It is due to the amide compounds which are neutral, stable, and have hydrogen-bond receptor accepting and donating properties [7]. Moreover, some ester compounds are toxic to the cells [17], so the ester compounds should pass the toxicity assay against the cell before it becomes a drug.

Comparing the result of the previous research about bioactivity of analog amide compounds against P388 murine leukemia cells, i.e N-feruloylpiperidine with IC\(_{50}\) = 46.67 \(\mu\)g/mL and N-feruloylmorpholine IC\(_{50}\) = 57.10 \(\mu\)g/mL [13], the activity of compound 6 was higher than both compounds. This fact indicated that beside the \(p\)-hydroxycinnamyl group, the residue amine groups also influenced the bioactivity of \(p\)-hydroxycinnamamide compounds.

4. Conclusion

The compounds 5 and 6 can be synthesized from ferulic acid with 2-phenethylalcohol and phenethylamine, respectively via indirect conversion giving compound 5 as a yellow gel, and compound 6 as a yellowish solid with a melting point of 118-120\(^\circ\)C. Both compounds were active against P388 murine leukemia cells with the IC\(_{50}\) of 10.79 and 29.14 \(\mu\)g/mL, respectively. These compounds were more active than the analog compounds synthesized in the previous research [13].

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