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A Caffeic Acid Derivative Potential for Anticancer Drug: Synthesis of *N*-(piperidinyl)caffeamide and Its Activity against P388 Leukemia Murine Cells

Firdaus[1]*, Nunuk Hariani Soekamto, Syadza Firdausiah, Musrifah Tahar

Abstract. Some esters and amide derivatives of *p*-hydroxycinnamic acid have anticancer activity. However, the amide compound is more stable to metabolic reactions compared to its ester derivative. In this research, the synthesis of a new compound, namely *N*-(piperidinyl)caffeamide (**M5**) and its anticancer activity assay, has been conducted. The compound **M5** was synthesized using *p*-hydroxycinnamic acid and piperidine as starting materials, and the activity assay was carried out against P388 Leukemia Murine Cells by the MTT method. By these methods, the compound **M5** was obtained a yellowish crystalline with a melting point of 212-214°C, and it was very active as an anticancer with an IC_{50} value of 0.861 μg/mL. This compound was more active than the analog compounds previously synthesized.

Introduction

Hydroxycinnamic acid is a phenolic compound that is found in fruits, vegetables, nuts, and cereals. The leading role of hydroxycinnamic acid in plants is in the process of pigmentation, growth, reproduction, and resistance to pathogens (Farah & Donangelo, 2006; Tošović, 2017). Hydroxycinnamic acid compounds chemically have three functional groups that have the potential as active sides in anticancer drugs, namely phenyl groups, carbonyl α,β-unsaturated, and hydroxyl group (OH) substituents in aromatic rings (De, Baltas, & Bedos-Belval, 2011; Magnani, Isaac, Correa, & Salgado, 2014; Nakamura, Nakajima, Aoyama, Okitsu, & Koyama, 2014; Sharma, 2011). Some of the compounds which include hydroxycinnamic acid derivatives are p-coumaric acid, caffeic acid, and ferulic acid (Figure 1), which are the primary sources in the treatment of various cancers and other diseases (Angelino et al., 2017; De et al., 2011; Rosa, et al., 2016; Russell & Duthie, 2011).

Caffeic acid is a hydroxycinnamic acid derivative which has two hydroxyls (OH) groups. Caffeic acid is found in fruits and vegetables such as plums, apples, apricots, blueberries, sunflower seeds, coffee beans, wheat, potatoes, carrots, olives, and tomatoes with caffeic acid content of more than 75% (Silva, Oliveira, & Borges, 2014; Tošović, 2017). The compound usually found as esters, amides, and glycosides. Because of their ability to inhibit the formation of a nitro compound, these compounds have activity as anticancer, antioxidant, antiproliferative, anti-inflammatory, and anti-carcinogenic(Damasceno, Dantas, & Ribeiro-filho, 2017; Huang, Lin, & Yan, 2013; Rosa et al., 2016; Sidoryk, Jaromin, Filipczak, Cmoch, & Cybulski, 2018).

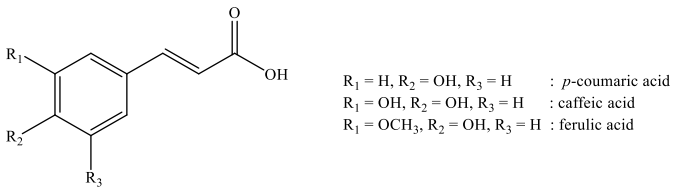

Figure 1. Structure of hydroxycinnamic acid derivatives

One of the most commonly found caffeic acid derivatives and has interesting bioactivity is caffeic acid phenethyl ester (CAPE). This compound has antiproliferation, antioxidant, anticancer, and anti-inflammatory activities, as well as inhibitor of tumor cell growth, which can be used as a substitution agent for radiation therapy (Catchpole, Mitchell, Bloor, Davis, & Suddes, 2015; Chiang et al., 2014;

[1] *Department of Chemistry, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Indonesia;Email:*

Gajek, Marciniak, & Kontek, 2020; Guzman, 2014). However, esters usually do not show excellent performance in their application because they are less stable in metabolism and hydrolyzed before reaching target cells in the body (Firdaus, Seniwati, Alamsyah, & Paramita, 2019). Therefore, to obtain a compound with intense activity as an anticancer, it is crucial to find their analog amide compounds.

The simplest method for converting caffeic acid into an amide is consists of four reaction stages (Firdaus, Soekamto, Seniwati, Islam, & Sultan, 2018). This method was adopted to synthesize **M5** compounds from caffeic acid and piperidine (Figure 2).

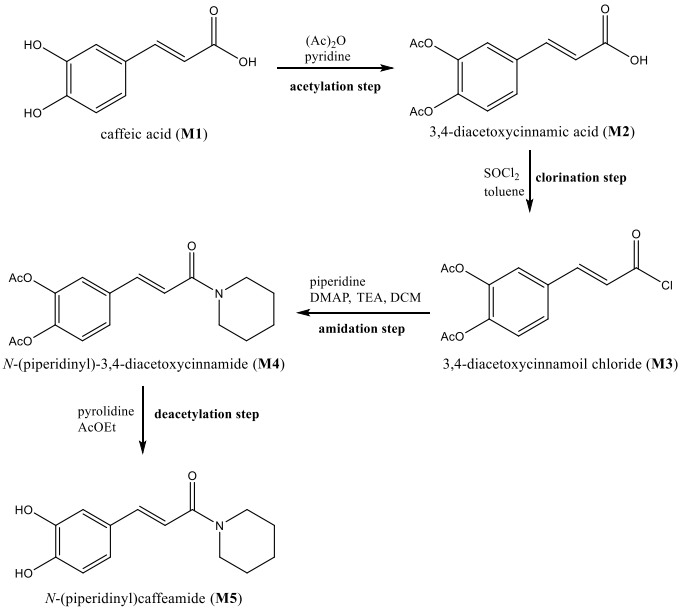

Figure 2. The synthesis pathway of compound **M5**

Experimental

Material and Methods

The materials used in this study were caffeic acid, acetic anhydride, thionyl chloride, pyridine, aquades, toluene, triethylamine, piperidine, dichloromethane, saturated NH4Cl, HCl 3%, acetone, Na_2SO_4 anhydrous, ethyl acetate, H_2SO_4 1 M, Whatman filter paper, and P388 Leukemia Murine Cells.

The equipment used in this study were UV lamps, Electrothermal Melting Point Apparatus, Buchi Rotavapor R-20, FTIR spectrophotometer Shimadzu Prestige-21, NMR Agilent 500 MHz spectrometer, tri-neck round flask, condenser, thermometer, analytical balance, hotplate stirrers, desiccators, and glassware commonly used in laboratories.

Procedures

The synthesis procedure in this research was adopted from reference (Firdaus et al., 2018) with slight modification, which use piperidine as the amide. The bioactivity test of compound **M5** was carried out using P388 Murine Leukemia Cells based on reference (Widiyarti, Hanafi, Kosela, & Budianto, 2016) by MTT method.

*Synthesis of **M2***. This reaction step gave a white solid (83.20% yields), melted at 180-183°C. IR (KBr): (cm^{-1}) 1764.87 (C=O, acetyl ester), 2987.74, & 2823.79 (C-H sat.), 1373.32 & 1431.18 (methyl), 1687.71 (C=O, conj. carboxyl), 1629.85 (C=C, olefin), 985.62 (trans-olefin), 3055.24 (C-H unsat.), 1581.63 & 1502.55 (C=C, Ar), 910.40 & 829.39 (1,2,4-trisubst. Ar).

*Synthesis of **M4**.* Compound **M4** was obtained through two steps of reaction, namely acetylation and amidation in situ gave a white crystalline solid (14.02% yields), melted at 139-141°C. IR (KBr): (cm^{-1}) 1647.21 (C=O amide), 1764.87 (C=O ester), 985.62 (trans olefin), 3032.10 (C-H unsat.), 1504.48 & 1602.85 (C=C Ar), 910.40 & 831.32 (1,2,4-trisubst. Ar), 2858.51 & 2943.37 (C-H sat.), 1369.46 & 1442.75 (methyl).

*Synthesis of **M5**.* This reaction step gave a yellow crystalline (56.11% yields), melted at 212-214°C. IR (KBr): ((cm-1) 3354.21 (O-H), 1653.00 (C=O amide), 3026.31 (C-H unsat.), 981.77 (trans olefin), 1512.19 & 1600.92 (C=C Ar), 935.48 & 864.11(1,2,4-trisubst. Ar), 2868.15 & 2929.87 (C-H sat.). ^{13}C-NMR {$(CD_3)_2CO$}: δ (ppm) 164.80, 146.75, 145.25, 141.84, 128.03, 120.72, 115.28, 115.07, 114.19, 46.24, 26.71, and 24.53. ^{1}H-NMR {$(CD_3)_2CO$}: δ (ppm) 8.21 & 8,53 (s, 2H, OH phenol), 7.005 & 6.835 (d, 1H, J= 10 Hz, H9 & H6-Ar), 7.15 (s, 1H, H5-Ar), 7.45 & 6.97 (d, 2H, J= 15 Hz, H2 & H3-trans-olefin), 1.68 (m, 2H, J= 5 Hz, H3'-pip), 1.82 (m, 2H, J= 5 Hz , H4'-pip), 1.94 (m, 2H, J= 5 Hz , H2'-pip), 3.33 (m, 2H, J= 5 Hz, H5'-pip), and 3.50 (m, 2H, J= 5 Hz, H1'-pip).

Cytotoxic assay. The testing procedure of compound **M5** against P388 murine leukemia cells was performed by the MTT method [21]. By this method, the compound **M5** gave IC_{50} values of 0.861 μg/mL.

Result and Discussion

Synthesis and characterization of M5

Conversion of caffeic acid compounds into amide derivatives is done to increase the activity of compounds and is more stable than their esters (Firdaus et al., 2019). The amide compound was synthesized through an indirect conversion method (Firdaus et al., 2018). This reaction begins by protecting the phenolic hydroxyl group in caffeic acid using an acetyl group from the acetic anhydride reagent gave compound **M2**. This protection needs to be done to avoid the dimerization reaction at the chlorination stage.

After chlorination, *in situ* amidation gave the compound **M4**, releasing the acetyl group from compound **M4** to produce **M5**. In this method, the compound **M3** did not isolate because it considers the instability of the compound.

The IR spectrum of each product proves the success of each reaction stage in this synthesis reaction. The first stage product provides an absorption band of the carbonyl ester group at 1764.87 cm^{-1} accompanied by loss of OH phenolic absorption band. The third stage products provide carbonyl amide absorption bands at 1647.21 cm^{-1}. The fourth stage product gives the phenolic OH absorption band at 3354.21 cm^{-1} accompanied by loss of absorption of esters carbonyl at wave number of 1764.87 cm^{-1}.

Besides FTIR spectroscopy, the compound target synthesis (**M5**) has been characterized using ^{1}H-NMR and ^{13}C-NMR. Data from FTIR and NMR spectroscopy analysis displayed in Table 1.

Table 1. FTIR and NMR data of N-(piperidinyl)caffeamide (**M5**)

Compound	FTIR	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
N-(piperidinyl)caffeamide (**M5**)	3354.21 (O-H phenol), 1653.00 (C=O amide), 3026.31 (C-H unsat.), 2868.15 & 2929.87 (C-H sat.), 1585.49 (C=C olefin), 981.77 (*trans* olefin), 1512.19 & 1600.92 (C=C Ar), 935.48 & 864.11(1,2,4-trisubst. Ar)	8.21 & 8,53 (*s*, 1H, O-H), 7.005 & 6.835 (*d*, 1H, *J*= 10 Hz, H9 & H6-Ar), 7.15 (*s*, 1H, H5-Ar), 7.45 & 6.97 (*d*, 2H, *J*= 15 Hz, H2 & H3-*trans*-olefin), 1.68 (*m*, 2H, *J*= 5 Hz, H3'-pip), 1.82 (*m*, 2H, *J*= 5 Hz , H4'-pip), 1.94 (*m*, 2H, *J*= 5 Hz , H2'-pip), 3.33 (*m*, 2H, *J*= 5 Hz, H5'-pip), and 3.50 (*m*, 2H, *J*= 5 Hz, H1'-pip).	164.80, 146.75, 145.25, 141.84, 128.03, 120.72, 115.28, 115.07, 114.19, 46.24, 26.71, and 24.53

Cytotoxic activity of compound M5

Cytotoxic assay of compound **M5** against P388 Murine Leukemia cells was carried out by the MTT method (Widiyarti et al., 2016). The activity test results of compound **M5** gave an IC_{50} value of 0.861 µg/mL, classified as very active (Hadi Kuncoro, Rijai, Julaeha, & Supratman, 2003). These IC_{50} values indicate that the compound **M5** is more active compared to analog compounds, namely *N*-feruloylpiperidine (Firdaus, Naid, Soekamto, Sumarna, & Islam, 2017) and piperidinyl-*p*-coumaramide (Firdaus et al., 2012) with IC_{50} values of 46.67 µg/mL and 5.34 µg/mL, respectively (Figure 3).

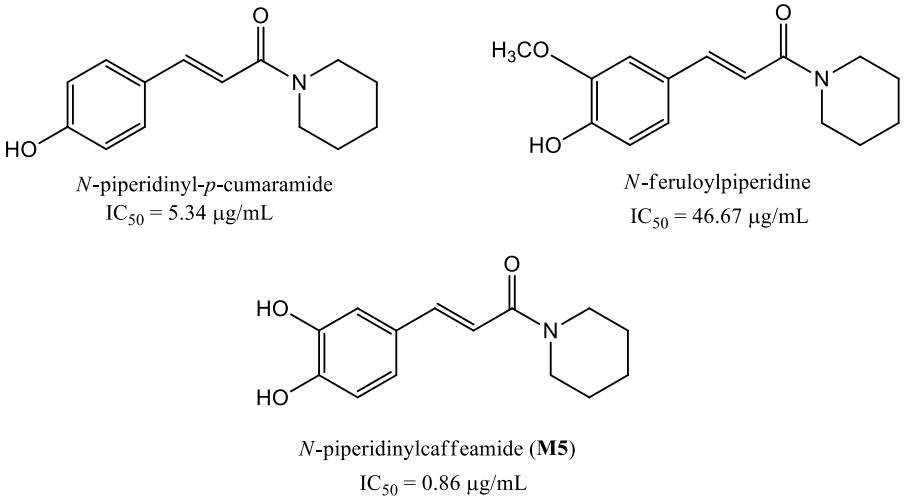

Figure 3. Bioactivity of compounds **M5** and its analog compounds

Structurally, the three compounds in Figure 3 are only distinguished by their cinnamate moiety. Therefore, the superior of compound **M5** to the two analog compounds causes the presence of two hydroxyls groups in the structure of caffeic moiety. The hydroxyl group in meta-position will facilitate radicals hydrogen release from hydroxyl in para-position (Georgiev et al., 2012). Thus, the more hydroxyl in the phenolic group of cinnamic compounds, the stronger the anticancer activity (Sidoryk et al., 2018; Touaibia & Doiron, 2011). In contrast, in the *N*-feruloylpiperidine compound, intramolecular hydrogen bonds between the methoxide and hydroxyl groups will inhibit the release of hydrogen radicals (Figure 4).

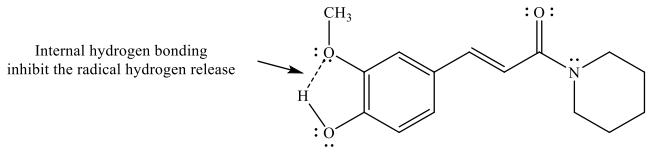

Figure 4. Internal hydrogen bonding in *N*-feruloyl-piperidine

Conclusion

Compound **M5** can be synthesized from caffeic acid and piperidine through an indirect conversion method in the form of yellow crystals with a melting point of 212-214°C and a yield of 56.11%. The activity of compound **M5** against P388 Murine Leukemia cells gave IC_{50} values of 0.861 μg/mL, which more active than other hydroxycinnamic acid derivatives.

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Conflict of Interest

The authors disclose no conflicts.

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