Novel hydroxycinnamamide from morpholine and pyrrolidine: Synthesis, characterization, docking study, and anticancer activity against P388 leukemia murine cells

Firdaus1*, Nunuk Hariani Soekamto1, Syadza Firdausiah1, Herlina Rasyid1,2, Nur Asmi1, Muzdalifah Waelulu1
1Hasanuddin University, Makassar, Indonesia.
2Austrian-Indonesian Center for Computational Chemistry, Chemistry Department, Gadjah Mada University, Yogyakarta, Indonesia.

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ABSTRACT
This study aimed to synthesize N-(p-coumaroyl)morpholine (6a), N-caffeoylmorpholine (6b), N-(p-coumaroyl)pyrrolidine (7a), and N-caffeoylpypyrrolidine (7b) from p-coumaric and caffeic acid through acetylation, chlorination, amidation, and deacetylation reactions. The characterization of these compounds was conducted by Fourier transform infra-red and NMR spectroscopy, while the anticancer activity was studied against murine leukemia P388 cells. Compounds 6a, 6b, and 7b were found to have remarkable anticancer activity with IC50 values ≤ 50 μg/ml. Furthermore, 6b performed very active anticancer activity with IC50 of 1.48 μg/ml. The molecular docking study of compound 6b against the Top1 protein receptor showed the presence of hydrogen bond interactions on Asn722 and Thr718 amino acid residue. Thus, these compounds are promising candidates as anticancer agents.

INTRODUCTION
Cancer is still a significant health problem worldwide (Magalhaes et al., 2018; Siegel et al., 2019), which is the second leading cause of death after heart disease (Sudhakar, 2009). During 2018, there were 9.6 million deaths caused by cancer (Bray et al., 2018). The cancer cells are characterized by uncontrolled cellular growth, damage of tissue, and ability to spread to other organs that are not directly connected with them (Dutta et al., 2013; Sarkar et al., 2013). The majority of deaths by cancer were caused by metastases (Dillekäs et al., 2019).

Several approaches could be applied as cancer treatment; however, one of the most important treatments is chemotherapy (Chorawala et al., 2012). Chemotherapy is a treatment that uses chemicals to suppress the growth of cancer cells (Kakde et al., 2011). However, these chemicals can cause serious adverse side effects on the patient’s body because they do not only attack the cancer cells but also attack the healthy cells (Chorawala et al., 2012; Yao et al., 2014). Therefore, many researchers are still paying attention to the discovery of new drugs (Said et al., 2013).

Hydroxycinnamic compounds exhibited antioxidant activity, so it is supposed to be able to prevent diseases that accompany oxidative stress such as cancer, cardiovascular disease, and other chronic diseases (Rocha et al., 2012; Spencer et al., 2008). The compounds included in this group are p-coumaric acid, ferulic acid, caffeic acid, and cinnamic acid (Georgiev et al., 2012; Teixeira et al., 2013). There are many kinds of reported evidence about the truth of the allegation that have been reported. Caffeic acid phenethyl ester (CAPE) is an antioxidant and is believed to have potential use in the treatment of patients with advanced oral cancer (Kuo et al., 2015; Ozturk et al., 2012). CAPE also has the activity as 5-lipoxygenase (5-LO) inhibitors. 5-LO is a catalyst for the transformation of arachidonic acid into leukotrienes, which have been directly implicated in inflammatory diseases like asthma, atherosclerosis, and rheumatoid arthritis (Boudreau et al., 2012). Some amides derived from cinnamic acid showed antioxidant activity and also could act as inhibitors of acetylcholinesterase and butyrylcholinesterase; hence, they are potentially used for the prevention or treatment of Alzheimer’s disease.

*Corresponding Author
Firdaus, Hasanuddin University, Makassar, Indonesia.
E-mail: firdaus@unhas.ac.id

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Some amide derivatives from cinnamic acid have been synthesized, and their antioxidant, anti-inflammatory, and anticancer activities have also been evaluated, such as 4-O-(2″-O-acetyl-6″-O-p-coumaroyl-β-D-glucopyranosyl)-p-coumaric acid, which possess a potential use in the treatment of lung cancer (Peng et al., 2015), amides of caffeic acid which possessed moderate to good anti-inflammatory activities (Liu et al., 2014), and thiourea of p-methoxyxycinnamamide which showed activity as a chemopreventive agent on fibrosarcoma in mice (Ekwatwi et al., 2012).

Some studies proved that hydroxycinnamoyl and cyclic amines moieties play a role in antioxidant and anticancer activities of the compounds (Firdaus et al., 2017; Rajan et al., 2001). In designing the target of active compounds, in this study, two groups have been considered. For the first target compound, we combined hydroxycinnamoino moiety with morpholine because the compounds containing the morpholine group, which showed a multidrug resistance (MDR) effect on MDR cancer cells (Yao et al., 2014). For the second target compound, hydroxycinnamoino moiety was combined with a heterocyclic amine, pyrrolidine. Other chemical constituents bearing morpholine and pyrrolidine moieties, such as tetrazole (Łukowska-Chojnacka et al., 2019), pyridazinoquin (Kamble et al., 2017), pyrazolidine (Surendra Kumar et al., 2017), and koeic acid derivatives (Karakaya et al., 2019), have been obtained and have showed moderate-to-high biological activities, such as antifungal, antioxidant, antimicrobial, cytotoxic, and tyrosinase inhibitory activities.

Several methods have been applied to synthesize the analogous of p-coumaramide and caffeamide. Here, we report four new p-coumaramide and caffeamide compounds from morpholine and pyrrolidine, and also evaluated their in vitro cytotoxic activity against P388 leukemia cells. Furthermore, a molecular docking study was conducted to investigate the interaction of the active compound against the protein receptor, which plays a role in the inhibition of cancer cell growth (Lu et al., 2013; Qin et al., 2016). Compound 6b performed vigorous anticancer activity with an IC_{50} value < 10 μg/ml. It also showed a lower docking score than the other compounds and had an intermolecular interaction against the amino acid residue of the protein receptor.

**MATERIALS AND METHODS**

Melting points were measured using the thermal melting point apparatus, the Fourier transform infra-red (FTIR) spectra were recorded from Shimadzu IR-Prestige-21 spectrophotometer, and the nuclear magnetic resonance (NMR) spectra were obtained from Agilent 500 MHz spectrometer.

**Materials**

Except p-coumaric and caffeic acid that were purchased from Sigma Aldrich, all materials used in this research were purchased from Merck.

**Synthesis and characterization**

These reactions were performed according to the previously reported method (Firdaus et al., 2017).

**Synthesis of Acetic Anhydride 2a and 2b**

In this reaction step, both compounds were obtained as yellow solids which were recrystallized by using methanol for 2a to obtain a pale yellow crystalline and ethyl acetate and n-hexane for 2b to obtain a white crystalline.

**Compound 2a.** m.p. of 205°C–207°C. IR (KBr) \( \nu \), cm\(^{-1}\): 1,747.51 (C=O acetyl), 1,681.93 (C=O conjugated), 2,819.93 & 2,981.95 (C-H sat.), 1,371.39 & 1,427.32 (CH\(_3\)), 3,047.53 (C-H unsat.), 1,627.92 (C=C, olefine), 1,506.41 & 1,595.13 (C=C, Ar), 993.34 (trans-olefin), 839.03 (p-subst. Ar).

**Compound 2b.** m.p. of 182°C–184°C. IR (KBr) \( \nu \), cm\(^{-1}\): 1,764.87 (C=O acetyl), 1,687.71 (C=O conjugated), 2,823.79 & 2,987.74 (C-H sat.), 1,373.32 & 1,431.18 (CH\(_3\)), 3,055.24 (C-H unsat.), 1,629.85 (C=C, olefine), 1,502.55 (C=C, Ar), 985.62 (trans-olefin), 910.40 & 829.39 (C-H tri-substituted aromatic).

**Synthesis of Coumaramide 4a and 5a and Caffeamide 4b and 5b**

This synthesis was initiated with the chlorination of the compounds 2a and 2b using thionyl chloride to produce compounds 3a and 3b, followed by an in situ amidation reaction using an appropriate amine to produce compounds 4a and 4b (from morpholine) as well as 5a and 5b (from pyrrolidine). After recrystallization, the pure crystals were obtained as white crystals.

**Compound 4a.** m.p. of 144°C–146°C. IR (KBr) \( \nu \), cm\(^{-1}\): 3,047.53 (C-H unsat.), 2,829.57 & 2,981.95 (C-H sat.), 1,747.51 (C=O acetyl), 1,683.86 (C=O amide), 1,627.92 (C=C olefin), 1,508.41 & 1,600.92 (C=C, Ar), 1,371.39 & 1,427.32 (CH\(_3\)), 1,323.17 (C-N), 993.34 (trans-olefin), 838.03 (p-subst. Ar).

**Compound 4b.** m.p. of 149°C–151°C. IR (KBr) \( \nu \), cm\(^{-1}\): 3,001.24 & 3,068.75 (C-H unsat.), 2,968.45, 2,924.09 & 2,852.72.07 (sat. C-H), 1,757.15 (C=O acetyl), 1,651.07 (C=O amide), 1,610.56 (C=C, olefin), 1,502.55 & 1,610.56 (C=C, Ar), 1,435.04 (C=N amide), 1,377.17 (CH\(_3\)), 1,111.0 (C-O ether), 972.12 (trans-olefin), 906.54 & 831.32 (trisubst. Ar).

**Compound 5a.** m.p. of 105°C–107°C. IR (KBr) \( \nu \), cm\(^{-1}\): 1,761.01 (C=O, acetyl), 1,683.86 (C=O, amide), 2,870.08 & 2,972.31 (C-H sat.), 1,369.76 & 1,444.68 (CH\(_3\)), 3,043.67 (C-H unsat.), 1,651.07 (C=C, olefin), 1,510.26 & 1,595.13 (C=C, Ar), 993.34 (trans-olefin), 840.96 (p-subst. Ar), 1,309.67 (C-N).

**Compound 5b.** m.p. of 156°C–158°C. IR (KBr) \( \nu \), cm\(^{-1}\): 1,261.45 (C-N, amide), 1,651.07 (C=O, amide), 1,768.72 (C=O, acetyl ester), 1,109.07 & 1,193.94 (C=O, acetyl ester), 2,966.52 & 2,875.85 (C-H, sat.), 1,369.46 (-CH\(_3\)), 1,435.04 (-CH\(_2\), pyrrolidine heterocyclic ring), 3,034.67 (C-H, unsat.), 1,506.41 cm\(^{-1}\) (C=C Ar).

**Synthesis of N-(p-Coumaroylamide)amide (6a and 7a) and N-(p-Caffeoyl)amide (6b and 7b)**

In this reaction step, recrystallization using ethyl acetate-hexane produced a crystalline form for compounds 6a, 6b, 7a, and 7b.

**Compound 6a.** m.p. of 217°C–219°C. IR (KBr) \( \nu \), cm\(^{-1}\): 3,219.19 (OH Ar), 3,088.96 (C-H unsat.), 2,860.43, 2,926.01, & 2,960.73 (C-H sat.), 1,643.35 (C=O amide), 1,606.70 (C=C olefin), 1,514.12 & 1,585.49 (C=C Ar), 1,440.83 (CH\(_3\)), 985.82 (trans-olefin), 833.25 (p-subst.), \(^1\)H-NMR (500 MHz, chloroform-d) \( \delta \), ppm: 3.50–3.80 (8H, overlapped signal (H-1′+H-4′)-(H-2′+H-3′), 5.95 (1H, s, OH), 6.70 (1H, d, \( J_{1,5} = 15.4 \) Hz, H-2), 6.85 (2H, d, \( J_{2,6} = 8.2 \) Hz, H-6+H-8), 7.41 (2H, d, \( J_{4,5} = 8.2 \) Hz, H-5+H-9), 7.6 (1H, d, \( J_{7,8} = 15.4 \) Hz, H-3). \(^1\)C-NMR (100 MHz, acetone-d\(_6\)) \( \delta \), ppm: 46.46 (C-1′+C-4′), 67.49 (C-2′+C-3′), 115.13
Compound 6b. m.p. of 222°C–224°C. IR (KBr) ν, cm\(^{-1}\): 3,421.72 (O-H Ar), 2,854.65 (sat. C-H), 1,641.42 (C=O amide), 1,604.77 & 1,571.99 (C=C Ar), 1,436.97 (C-N amide), 1,111.0 (C-O ether), 970.19 (trans-olefin), 973.75 & 808.17 (trisubst. Ar). \(^1\)H-NMR (500 MHz, chloroform-d) d, ppm: 3.71 (s, 8H, H-1+H-2), 6.78 (d, 1H, J = 8.05, H-4), 6.87 (d, 1H, J = 15.35, H-1), 6.99 (d, 1H, J = 8.75, H-3), 7.05 (s, 1H, H-5), 7.50 (d, 1H, J = 17.95, H-2), 7.90 (s, 1H, -OH). \(^1\)C-NMR (100 MHz, chloroform-d d/ppm): 66.45 (C-2′), 112.72 (C-9), 113.99 (C-6), 115.04 (C-2), 120.89 (C-5), 127.06 (C-4), 143.79 (C-3), 145.28 (C-7), 147.56 (C-8), and 166.99 (C-1).

Compound 7a. m.p. of 227°C–229°C. IR (KBr) ν, cm\(^{-1}\): 3,101.54 (OH Ar), 3,064.89 & 3,003.17 (C-H unsat.), 2,968.45 & 2,877.79 (C-H sat.), 1,647.21 (C=O amide), 1,570.06 (C=C olefin), 1,510.26 (C=C Ar), 1,446.61 (CH), 970.19 (trans-olefin), 827.46 (p-subst.). \(^1\)H-NMR (500 MHz, methanol-d) d, ppm: 1.92 (2H, m, J= 6.8 Hz, H-2′), 2.02 (2H, m, J= 6.7 Hz, H-3′), 3.52 (2H, t, J= 6.7 Hz, H-1′), 3.68 (2H, t, J= 6.8 Hz, H-4′), 4.88 (1H, s, OH), 6.73 (1H, d, J = 15.6 Hz, H-2), 6.80 (2H, d, J= 7.4 Hz, H-6-H-8), 7.47 (2H, d, J= 7.4 Hz, H-5-H-9), 7.52 (1H, d, J = 15.4 Hz, H-3). \(^1\)C-NMR (100 MHz, methanol-d) d, ppm: 25.30 (C-3′), 26.99 (C-2′), 47.26 (C-1′), 49.92 (C-4′), 116.13 (C-2′), 116.72 (C-6+C-8), 127.84 (C-4′), 130.90 (C-5+C-9), 143.52 (C-3), 160.74 (C-7), 167.60 (C-1′).

Compound 7b. m.p. of 204°C–206°C. IR (KBr) ν, cm\(^{-1}\): 3,134.67–3,442.94 (OH Ar), 3,043.17 & 3,003.17 (C-H unsat.), 2,994.09 (C-H sat.), 1,643.35 (C=O amide), 1,560.41 & 1,535.34 (C=C Ar), 1,446.61 (CH), 970.19 (trans-olefin), 827.46 (p-subst.). \(^1\)H-NMR (500 MHz, chloroform-d) d, ppm: 1.50 (2H, m, J= 6.8 Hz, H-2′), 2.03 ppm (2H, m, J= 6.7 Hz, H-3′), 3.53 ppm (2H, t, J= 6.7 Hz, H-1′), 3.69 ppm (2H, t, J= 13.55 Hz, H-4′), 6.67 (1H, d, J = 15.45 Hz, H-2), 6.78 (1H, d, J = 8.15 Hz, H-8), 6.97 (1H, d, J = 8.15 Hz, H-9), 7.05 (1H, s, H-5), 7.46 (1H, d, J = 15.4 Hz, H-3). \(^1\)C-NMR (100 MHz, methanol-d) d, ppm: 23.87 (C-3′), 25.57 (C-2′), 45.83 (C-4′), 46.48 (C-1′), 113.88 (C-5), 114.68 (C-8), 115.05 (C-2′), 120.94 (C-9), 127.02 (C-4′), 142.49 (C-3′), 145.30 (C-6′), 147.55 (C-7′), 166.20 (C-1′).

Docking study

**Preparation of protein receptor and ligand standard**

The crystal structure of the protein receptor human DNA topoisomerase (Top1) was downloaded from the Protein Data Bank webpage (https://www.rcsb.org/structure/1T8I). All of the residues were removed and selected under the docking preparation menu in Chimera software (Petterson et al., 2004). Camptothecin (Cpt) as a standard ligand was extracted from the protein complexes file and saved in a .pdb file format.

**Preparation of ligand**

A set of novel hydroxycinnamamides was constructed using Avogadro software (Hanwell et al., 2012). Then, all ligands were prepared to dock by optimizing the structure in Chimera software using the AM1-BCC semiempirical method and saved in a .pdb file format.

**Molecular docking**

The docking process was done using AutoDock 4.2 software with the help of AutoDockTools program (Morris and Huey, 2009). Each ligand was docked into the active site of Top1 protein receptor. Grid box size was set at 40 × 40 × 40 Å with spacing 0.375 Å and saved as parameter file in .gpf format file. The docking procedure was set to produce 10 conformations and run for a maximum energy evaluation of 2,500,000. The Lamarckian genetic algorithm was used to obtain data in the form of binding energy (kcal/mol) and predicted inhibition constant. Validation of docking was determined from the value of root mean square deviation (RMSD) of the redocking standard ligand into the active site of the protein receptor. The successful the redocking process was known from a low RMSD value of about less than 2 Å (Huey et al., 2007). Visualization of docking results was exhibited by using Discovery Studio Visualizer software for windows (Dassault Systemes, 2019).

**Activity study against P388 leukemia murine cells**

The activity assay procedure against P388 leukemia murine cells of compounds 6a, 6b, 7a, and 7b was conducted according to a previous method (Kuncoro et al., 2003) in a single performed test.

**RESULTS AND DISCUSSION**

**Synthesis and characterization**

Theoretically, a proper synthetic method of amide can be undertaken from carboxylic acid with an amine, through the amidation reaction using a boric acid catalyst (Tang et al., 2012). However, that reaction did not succeed in the conversion of the hydroxycinnamic acid to its amide. This is presumably alleged due to the presence of the phenolic group in the benzene ring, which has an acidic property, so that it may turn into unexpected products. Therefore, it is necessary to protect the hydroxyl group, usually using an acetyl group (Lu and Ralph, 1998). Without a protective group in the phenolic hydroxyl group, a polymerization reaction will occur when the carboxylic group has been converted to its halide.

In this study, the hydroxyl groups of p-coumaric (1a) and p-caffeic acid (1b) were protected with acetyl using acetic anhydride (Figure 1). The products from each reaction step were analyzed with an FTIR spectrometer for identification purpose, except for the chlorination products. Specifically, for the deacetylation products, the FTIR analysis was continued with a low RMSD value of about less than 2 Å (Huey et al., 2007).
The selectivity of these chlorinating agents has also been confirmed by Greenberg and Sammakia (2017), which reported that benzyl ester is unreactive to thionyl chloride. Thus, it cannot be converted into acid chloride.

The successful conversions of compounds 3a–b to compounds 4a–b and 5a–b were indicated by the existence of an IR absorption band of C-N at about 3,100 cm\(^{-1}\) for secondary amide. In the IR spectra of all compounds, the absorption band of acetyl C=O was around 1,770–1,740 cm\(^{-1}\). The loss of the carboxylate ester band and the emergence of the hydroxyl absorption band of the phenolic group around 3,200–3,400 cm\(^{-1}\) were used as an indication of successful deacetylation, which produced compounds 6a–b and 7a–b.

The \(^{13}\)C-NMR spectra of all compounds also provided signals that corresponded to the structures of 6a–b and 7a–b. The \(^{13}\)C-NMR spectra of compound 6a–b conveyed nine signals representing 13 carbon atoms. In this case, there were four equivalent pairs of carbon atoms, namely, C-5 and C-9, C-6 and C-8, C-1' and C-4', and C-2' and C-3'. However, the \(^{13}\)C-NMR spectra of compounds 7a–b showed 11 signals, more than the
signal displayed by compounds 6a–b, due to the absence of equivalent carbon in the five-membered amino ring moieties. The different environments of four carbons in pyrrolidine moiety are presumably due to the nonplanar geometry, which is expected to have an envelope conformation. This conformation cannot be inverted at room temperature due to its high inversion energy (El-Gogary and Soliman, 2001).

In the 1H-NMR spectra, all compounds were also distinguished by the signals that emerged from their amino group protons. Each of the compounds 6a and 6b gave two doublet signals with the coupling constant from the group which were 15.4 and 15.35 Hz, respectively. On the other hand, each of the compounds 7a and 7b showed two triplet signals and two multiplet signals with coupling constants of 6.7–6.8 Hz and 13.55–27.65 Hz, respectively. The other signals from all compounds have the same profile and shift value, which are also almost similar to the signals coming from equivalent carbon atoms.

The structure of compound 7a has also been confirmed by the HSQC and HMBC methods. Both spectra are shown in Figures 2 and 3.

Based on the HSQC spectra, the correlation of one bond between a hydrogen atom and a carbon atom in the structure of compound 7a is shown in Figure 4. Also, the multibond correlation between a hydrogen atom and carbon atoms, according to the HMBC spectra, is shown in Figure 5. Figure 4 shows that all hydrogen atoms are positively correlated to the carbon atoms in which they are bonded. Furthermore, Figure 5 shows two types of correlations between hydrogen atoms and carbon atoms with two spaced bonds as a short distance correlation (blue curved arrows) and long-distance correlations, three and four bonds (red curved arrows). Figure 5 also shows a positive correlation between the hydrogen atoms and the carbon atoms of the pyrrolidinyl group. In addition to the correlation between the two bonds, the atoms also show the correlation between the three bonds.

Similarly, the attachment of the olefin group to the carbonyl group is shown by the correlation of olefin hydrogen atoms in two bonds and the correlation in three bonds. Furthermore, the attachment of the olefin group to the aromatic group is shown by the correlation of the H-2 olefin group to the C-4 aromatic group.

![Figure 2. HSQC spectra of compound 7a.](image-url)
A molecular docking study has been conducted between 6a, 6b, 7a, and 7b against the human topoisomerase I (Top1) receptor. This protein receptor is the molecular target of a diverse set of anticancer compounds (Laco et al., 2002; Ismail et al., 2010). Table 1 shows the result of molecular docking of all compounds against the Top1 receptor. As can be seen from Table 1, compound 6b performed the lowest binding energy than the other compounds due to the presence of hydrogen bonds and other intermolecular interactions. Figure 6 shows a 2D intermolecular interaction of compound 6b with the Top1 receptor. There were three hydrogen bonds, two pi-pi stacking, and pi-alkyl stacking interactions.

### Docking study

Activity against P388 leukemia murine cells

The activity assay of all compounds is shown in Table 2. Based on the IC\textsubscript{50} values, compounds 6a, 6b, and 7b exhibited intense anticancer activity against P388 leukemia murine cells, especially for compound 6b, which performed IC\textsubscript{50} below 10 μg/ml. Caffeamide of both morpholine and
pyrrolidine showed more vigorous anticancer activity than the analog coumaramides. Compared to 6a, which has a difference only in the presence of the hydroxyl group on C-6, the activity of 6b was superior. It showed that this –OH group has an essential role in its activity against P388 murine leukemia cells. As shown in the docking study, it is due to the presence of an intramolecular hydrogen bond between the adjacent hydroxyl groups, which can stabilize the structure after releasing hydrogen radicals. This result is consistent with the previous report related to the comparison of anticancer activity between caffeamide and p-coumaramide (Firdaus et al., 2019).

Meanwhile, compared to the activity of p-coumaramide that was isolated from Kleinhovia Hospita L. (IC₅₀ of 44.00 μg/ml) (Firdaus et al., 2014) and N-feruloyl morpholine which previously synthesized (IC₅₀ of 46.67) (Firdaus et al., 2017), compound 6a was considerably more active. The different activities of 6a compared to p-coumaramide may be due to the difference in the polarity of the two compounds. The polarity of 6a with amine morpholine moiety is lower than the p-coumaramide compound, which is a primary amide. Therefore, it is easier for 6a to penetrate the lipophilic cell shield. However, this concept cannot be used to explain the differences in the activity of compounds 6a and N-feruloyl morpholine. Structurally, 6a and N-feruloyl morpholine have a difference only in the phenolic groups. The N-feruloyl morpholine contains a methoxy group at the ortho position to a hydroxyl group, where both groups are ready to form an intramolecular hydrogen bonding. This bond decreases the polarity of the compound so that its ability to penetrate cell shields increases, and it is expected to increase its activity. However, this is contrary to the fact.

To explain the difference in activity between compound 6a and N-feruloyl morpholine, we must review other factors that have a perspective of influencing a compound’s ability to transfer hydrogen radicals (Hatfield et al., 2008; Zhang et al., 2015). The p-hydroxycinnamic compounds are ready to transfer hydrogen radicals because the release of hydrogen radicals will leave relatively stable radicals due to the delocalization of the unpaired electrons to carbonyl groups (Georgiev et al., 2012). Therefore, the hydrogen bond at N-feruloyl morpholine obstructs the release of hydrogen radicals, and this effect is reflected in the low activity of this compound rather than compound 6a.

CONCLUSION

Four amide compounds have been synthesized from p-coumaric and caffeic acid via acetylation, chlorination, amidation, and deacetylation, sequentially. Docking study showed that compound 6b had the lowest binding energy value and had some intermolecular interaction against Top1 receptor. All compounds were active against P388 murine leukemia cells with the IC₅₀ of 19.35, 1.48, 53.46, and 11.35 μg/ml for compounds 6a, 6b, 7a, and 7b, respectively.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest in this work.

ETHICAL APPROVAL

This study does not involve the use of animals or human subjects.

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