

Development trans-N-benzyl  
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# Development *trans*-*N*-benzyl hydroxyl cinnamamide based compounds from cinnamic acids and characteristics anticancer potency

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## Abstract

The derivatization of three hydroxycinnamamides becomes *trans*-*N*-benzylhydroxycinnamamides, and their potential assay as anticancer agents has been carried out. *N*-benzyl-*p*-coumaramide (**5a**), *N*-benzylcaffeamide (**5b**), and *N*-benzylferulamide (**5c**) were obtained from *p*-coumaric, caffeic, and ferulic acid, respectively, with benzylamine via four reaction steps, i.e., acetylation, chlorination, amidation, and deacetylation. All products characterize using FTIR, <sup>1</sup>H-NMR, and <sup>13</sup>C-NMR spectroscopy, and their cytotoxicity were tested against P388 leukemia murine cells by MTT method. Although compound **5b** and **5c** have high and low anticancer activity with IC<sub>50</sub> sequentially of 674.38 and 179.56 μg/mL, compound **5a** showed potentially use as an anticancer agent with IC<sub>50</sub> of 16.15 μg/mL. Molecular modelling studies were performed to understand the interactions with the activity against murine leukemia P388 cells.

**Keywords** Cytotoxic · Hydroxycinnamamides · Hydroxycinnamic · Leukemia · *N*-Benzyl · P388 cells

## Introduction

The hydroxycinnamic acid compounds (e.g., *p*-coumaric, ferulic, and caffeic acids) as a new drugs or agent is interesting research topic of scientist in the world due to the antioxidant [1–3, 12, 19, 30, 36, 38, 41], antiviral [4], anticancer [1, 23, 31], antimicrobial [2, 12, 14, 21, 30], and antifungal activities [22, 44, 46] of their ester and amide derivatives. The activities are thought to arise from *p*-hydroxycinnamoyl and amine moiety in amides or alcohol moiety in ester [3], coupled with the presence of phenolic nuclei conjugated with the olefin group of the carbonyl. Such a molecular framework will facilitate the resonance of phenoxy radicals, thus it becomes relatively stable [12]. In addition, the

α,β-unsaturated carbonyl group which can be considered as Michael's acceptor, with an active moiety commonly used in designing anticancer drugs [18, 26].

Esters and amides of hydroxycinnamic acids are more active than their free acid [12], so most of the researchers did the conversion of cinnamic hydroxyl compounds into their ester and amides before testing their bioactivity [3, 4, 6, 14, 33, 42]. The esterification and amidation of hydroxycinnamic acids are practical techniques to improve their solubility and emulsification properties as well as evaluated as potential antioxidant and antidiabetic activities [40, 45].

Although many hydroxycinnamic acids derived ester analogs display the potential activities, the ester groups in hydroxycinnamic acid are metabolically more labile than amides and have limited use [29]. In this study, we synthesized three hydroxycinnamide compounds from *p*-coumaric, caffeic and ferulic acid, and benzylamine to produce *N*-benzyl-*p*-coumaramide (**5a**), *N*-benzylcaffeamide (**5b**), and *N*-benzylferulamide (**5c**), respectively, as well as evaluated their cytotoxic activity against P388 murine leukemia cells. Based on the IC<sub>50</sub> value, compound **5a** was the most active between the synthesized compounds. Moreover, it was more active than *N*-(*o*-tolyl)-*p*-coumaramide, which

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was previously synthesized [26], and potentially used as an anticancer drug.

## Materials and method

### Materials

*p*-Coumaric acid, caffeic acid, ferulic acid, benzylamine, acetic anhydride (Ac<sub>2</sub>O), thionyl chloride, pyridine, triethylamine (TEA), 4-(dimethylamino)pyridine (DMAP), pyrrolidine, dichloromethane (DCM), ethyl acetate (EtOAc), benzene, methanol, *n*-hexane, NH<sub>4</sub>Cl, HCl, and anhydrous Na<sub>2</sub>SO<sub>4</sub> were pro synthesis and pro analysis grades and were used without purification. The FTIR spectra were recorded on a Spectrophotometer Shimadzu Prestige 21. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra were collected using Spectrometer Agilent operating at 500 MHz for <sup>1</sup>H-NMR and 125 MHz for <sup>13</sup>C nuclei, and the melting points were taken using electrothermal apparatus.

### Synthesis method

The synthesis of **5a**, **5b**, and **5c** from *p*-coumaric, caffeic, and ferulic acid, and benzylamine here were conducted according to the procedure in reference [9]. This procedure consists of four stages of reactions, i.e., (1) acetylation reaction to protect the phenolic groups of each hydroxycinnamic acids gave acetic acetoxycinnamic anhydrides (**2a–c**), (2) chlorination reaction to activate the carboxyl group of the acetylation products gave acetoxycinnamoylchlorides, used for amidation without any purification (**3a–c**), (3) amidation reaction of the chlorination products using benzylamine to obtain *N*-benzylacetoxycinnamamides (**4a–c**), and (4) deacetylation reaction using pyrrolidine to deprotect the hydroxyl group of the amidation products gave *N*-benzylhydroxycinnamamides (**5a–c**) as the target compounds.

#### Acetic *p*-acetoxycinnamic anhydride **2a**

A yellowish crystalline solid with m.p. of 205–207 °C (71.0% of yield), FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3047.53 (C–H unsaturation), 2981.95 and 2827.64 (C–H saturation), 1745.58 (C=O acetyl), 1687.71 (C=O conj.), 1625.99 (C=C olefin), 1598.99 and 1506.41 (C=C Ar), 1427.32 and 1371.39 (CH<sub>3</sub>), 991.41 (*trans*-olefin), and 839.03 (*p*-substance Ar).

#### Acetic 3,4-diacetoxycinnamic anhydride **2b**

A white crystalline solid with m.p. of 184–185 °C (83.2% of yield), FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3024.38 (C–H unsaturation), 2985.81 (C–H saturation), 1764.87 (C=O acetyl), 1687.71

(C=O conj.), 1631.78 (C=C olefin), 1610.56 and 1504.48 (C=C Ar), 1431.18 and 1373.32 (CH<sub>3</sub>), 983.70 (*trans*-olefin), and 908.47 and 827.46 (tri-substance Ar).

#### Acetic 3-methoxy-4-acetoxycinnamic anhydride **2c**

A white crystalline solid with m.p. of 182–184 °C (82.3% of yield), FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3010.88 (C–H unsaturation), 2945.30 (C–H saturation), 1761.01 (C=O acetyl), 1687.71 (C=O conj.), 1631.78 (C=C olefin), 1600.92 and 1506.41 (C=C Ar), 1419.61 and 1371.39 (CH<sub>3</sub>), 985.62 (*trans*-olefin), and 933.55 and 856.39 (tri-substance Ar).

#### *N*-benzyl-*p*-acetoxycinnamamide **4a**

A yellowish solid, FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3290.56 (*sec*. N–H), 3062.96 and 3002.32 (C–H unsaturation), 2852.72 and 2922.16 (C–H saturation), 1761.01 (C=O ester), 1654.92 (C=O amide), 1622.13 (C=C olefin), 1537.27 (C–N amide), 1504.48 (C=C Ar), 1367.53 (N–C), 972.12 (*trans*-olefin), 844.82 (*p*-substance Ar), 700.16 and 746.45 (mono-substance Ar).

#### *N*-benzyl-3,4-diacetoxycinnamamide **4b**

A white crystalline solid with m.p. of 170–174 °C, FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3265.49 (*sec*. N–H), 3068.75 (C–H unsaturation), 2933.73 (C–H saturation), 1774.51 (C=O ester), 1656.85 (C=O amide), 1616.35 (C=C olefin), 1548.84 (C–N amide), 1504.48 (C=C Ar), 968.27 (*trans*-olefin), 966.34 and 829.39 (tri-subst. Ar), 700.16 and 742.59 (mono-subst. Ar).

#### *N*-benzyl-3-methoxy-4-acetoxycinnamamide **4c**

A white crystalline solid with m.p. of 118–122 °C, FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3184.48 (*sec*. N–H), 3080.75 (C–H unsaturation), 2941.44 (C–H saturation), 1762.94 (C=O ester), 1656.85 (C=O amide), 1656.85 (C=C olefin), 1552.70 (C–N amide), 1512.19 (C=C Ar), 987.55 (*trans*-olefin), 902.69 and 854.47 (tri-substance Ar), 700.16 and 756.10 (mono-substance Ar).

#### *N*-benzyl-*p*-coumaramide **5a**

A white crystalline solid with m.p. of 108–110 °C (76.9% of yields), FTIR (KBr):  $\nu$  (cm<sup>-1</sup>) 3423.65 (O–H), 3300.20 (N–H), 3022.45 (C–H unsaturation), 1653.00 (C=O amide), 1610.56 (C=C olefin), 1514.12 (C=C Ar), 1546.91 (N–C), 977.91 (*trans*-olefin), 821.18 (*p*-subst. Ar), 698.23 and 732.96 (mono-substance). <sup>1</sup>H-NMR (500 MHz, acetone-*d*<sub>6</sub>)  $\delta$ , ppm: 4.51 (2H, *d*, *J*=6 Hz, H-1'), 6.58 (1H, *d*, *J*=15.7 Hz, H-2), 6.86 (2H, *d*, *J*=8.55 Hz, H-6), 7.23 (1H, *t*, *J*=14 Hz,

H-5'), 7.32 (3H, *m*, H-3' dan H-4'), 7.44 (2H, *d*,  $J=8.6$  Hz, H-5), 7.43 (1H, *d*,  $J=15.65$  Hz, H-3), 7.71 (1H, *b.s.*, NH), 8.88 (OH).  $^{13}\text{C-NMR}$  (100 MHz, acetone- $d_6$ )  $\delta$ , ppm: 43.64 (C-1'), 116.56 (C-6), 119.35 (C-2), 127.65 (C-5'), 127.71 (C-3'), 128.40 (C-4), 129.18 (C-4), 130.17 (C-4'), 140.57 (C-3), 140.63 (C-2'), 159.79 (C-7), 166.58 (C-1).

### *N*-benzylcaffeamide 5b

A white crystalline with m.p. of 140–143 °C (78.8% of yields), FTIR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) 3479.58 and 3234.62 (O–H), 3305.99 (N–H), 3032.10 (C–H unsaturation), 1656.85 (C=O amide), 1593.20 (C=C olefin), 1500.00 (C=C Ar), 1543.05 (N–C), 970.19 (*trans*-olefin), 850.61 and 813.96 (*trans*-substance Ar), 742.59 and 696.30 (mono substance).  $^1\text{H-NMR}$  (500 MHz, methanol- $d_4$ )  $\delta$ , ppm: 4.47 (2H, *s*, H-10), 4.85 (2H, *s*, O–H phenolic), 6.41 (1H, *d*,  $J=15.5$  Hz, H-2), 6.76 (1H, *d*, H-8), 6.91 (1H, *d*, H-9), 7.41 (1H, *s*, H-5), 7.1–7.2 (1H, *b.s.*, N–H amide), 7.24–7.31 (5H, *m*, H-12, H-14, H-15, and H-16), 7.43 (1H, *d*,  $J=15.4$  Hz, H-3).  $^{13}\text{C-NMR}$  (70 MHz, methanol- $d_4$ )  $\delta$ , ppm: 44.26 (C-10), 115.10 (C-5), 116.43 (C-8), 118.23 (C-2), 122.13 (C-9), 128.20 (C-4), 128.28 (C-12 and C-16), 128.59 (C-13 and C-15), 129.54 (C-14), 140.01 (C-11), 142.56 (C-3), 146.68 (C-6), 148.77 (C-7), and 169.16 (C-1).

### *N*-benzylferulamide 5c

A white crystalline with m.p. of 120–122 °C (83.31% of yields), FTIR (KBr):  $\nu$  ( $\text{cm}^{-1}$ ) 3493.09 (O–H), 3250.05 (N–H), 3084.18 (C–H unsaturation), 1653.00 (C=O amide), 1606.70 (C=C olefin), 1508.33 (C=C Ar), 1537.27 (N–C), 974 (*trans*-olefin), 923.83 and 815 (tri-subst. Ar), 758.02 and 700.16 (mono substance).  $^1\text{H-NMR}$  (500 MHz, chloroform- $d$ )  $\delta$ , ppm: 3.87 (3H, *s*, H-17), 4.58 (5H, *d*,  $J=5.75$  Hz, H-10), 6.01 (1H, *s*, OH phenolic), 6.29 (1H, *d*,  $J=15.6$  Hz, H-2), 6.89 (1H, *d*,  $J=8.2$  Hz, H-8), 6.96 (1H, *s*, H-5), 7.03 (4H, *dd*,  $J=8.2$  Hz, H-9), 7.26–7.4 (1H, *s*, N–H amide), 7.28 (2H, *m*, H-12 and H-16), 7.30 (1H, *m*, H-14), 7.31 (2H, *m*, H-13 and H-15), 7.56 (1H, *d*,  $J=15.6$  Hz, H-3).  $^{13}\text{C-NMR}$  (100 MHz, chloroform- $d$ )  $\delta$ , ppm: 43.97 (C-10), 56.02 (C-17), 109.82 (C-5), 114.90 (C-8), 118.09 (C-2), 122.23 (C-9), 127.41 (C-4), 127.67 (C-12 and C-16), 128.02 (C-13 and C-15), 128.85 (C-14), 138.41 (C-11), 141.55 (C-3), 146.87 (C-6), 147.60 (C-7), 166.26 (C-1).

### Bioactivity assay against P388 leukemia murine cells

The activity of compound **5a–c** against P388 leukemia murine cells were analyzed using an MTT method according to the procedure in references [24, 35].

## Docking studies

### Preparation of protein receptor and ligand standard

Protein receptor human DNA topoisomerase (Top1) was downloaded from Protein Data Bank webpage <https://www.rcsb.org/structure/1T8I>. In order to obtain the active site of this protein, the coordinate of Camptothecin (Cpt) was retrieved from the protein complexes. All of residues were removed then prepared to dock in Chimera software [32]. Likewise, Camptothecin (Cpt) as a standard ligand was extracted from the protein complexes and saved as.pdb format file.

### Preparation of new ligands

There were two ligands **5a** and **5b** that docked into the active site of Top1. This two ligands then built using Avogadro software [15] and optimized in Chimera software using AM1-BCC semi empirical method then saved as.pdb format file.

### Molecular docking analysis

Docking analysis was exhibit using AutoDock 4.2 software with the help of AutoDock Tools program [28]. Each ligand was docked into the active site of Top1 protein receptor. Grid box size was set  $40 \times 40 \times 40$  Å with spacing 0.375 Å and saved as parameter file in.gpf format file. Docking procedure was set to produce 20 conformations and run for maximum energy evaluation of 25,000,000. 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 redocking standard ligand (Cpt) into the active site of protein receptor. Successful redocking process was known from low RMSD value about less than 2 Å [17]. Visualization of docking result was done by using Discovery Studio Visualizer software for windows [5]. LigPlot+ program was used to analyze the hydrophobic interactions of ligands against Top1 protein receptor [25].

## Results and discussion

### Chemistry

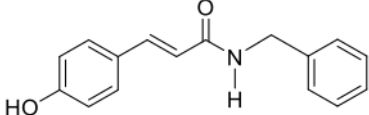
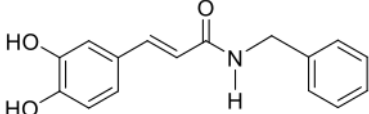
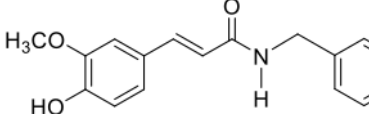
Boric acid has been successfully catalyzed the conversion of 4-phenylbutyric acid to *N*-benzyl-4-phenylbutyramide using benzylamine [39]. However, this catalyst failed to catalyze the conversion of hydroxycinnamic acid to its amide [8]. This failure is probably caused by the presence of a conjugate system in the hydroxycinnamic

acid structure and hydroxyl group on the phenolic moiety. Therefore, the conversion reaction must conduct by other methods. There are two general methods to convert hydroxycinnamic acid to an ester and amide, (1) directly conversion using a catalyst [27, 34, 39, 43], (2) indirect method involves four synthetic steps: (a) protection of the phenolic hydroxyl group, (b) activation of the carboxylic acid group, (c) condensation with alcohols or amines, and (d) deprotection of the phenolic hydroxyl group [16, 20, 27].

In this research, the synthesis of the target compounds was conducted by the indirect method (Fig. 1). The protection of the phenolic group in hydroxycinnamic acids (**1a–c**) were performed using acetic anhydride and produced acetoxy-cinnamic acids (**2a–c**). In addition, the conversion reaction of compound **2a–c** into *N*-benzylacetoxy-cinnamamides (**4a–c**) were performed through chlorination, and amidation reactions in situ generating a yellowish paste. The resulted compounds were then purified by column chromatography and continued by deprotection to give *N*-benzylhydroxycinnamamides (**5a–c**) as white crystalline. The structure of synthesized compounds with the yields from the last reaction step are given in Table 1.

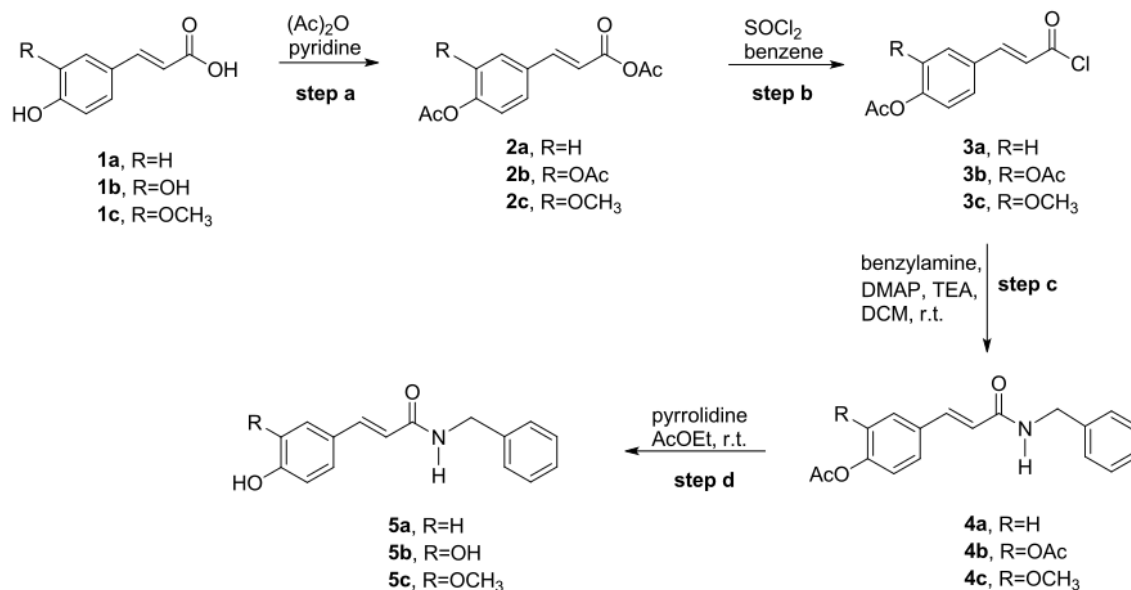
The success of pyrrolidine in 95% ethanol in removing the acetyl protecting groups without affecting the hydroxycinnamate bond is quite convenient [16]. Therefore, the method still applied in this research. All the main products in this synthesis reaction stages were analyzed by FTIR spectrometer, except for the chlorination products.

**Table 1** The structure and yield of each synthesized compounds

Symbol	Compound structure	Yield (%)
5a		76.9
5b		78.8
5c		83.31

Furthermore, deacetylation products were analyzed using  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectrometers.

The FTIR spectra of acetylation products no longer showed any O–H absorption band, either O–H of phenolic or O–H of carboxylate. This data means, acetylation did not only occur in phenolic OH groups but also



**Fig. 1** The pathway of synthesis reaction of *trans*-*N*-benzylhydroxycinnamamides

occurred in carboxylic groups. Besides, the spectra also show the absorption of carbonyl esters that appear around 1745–1761  $\text{cm}^{-1}$ , which indicate the presence of the acetyl group in the compounds. All of this data indicate the success of the acetylation reaction.

The success of the in situ amidation reaction was indicated by the presence of a sharp band with strong intensity in each spectrum around 1650  $\text{cm}^{-1}$  as an amide I band and a sharp band with medium intensity from N–H bond at around 3184–3290  $\text{cm}^{-1}$  as an amide band II in the FTIR spectrum of the reaction products. When the amidation products were deacetylated, the crystalline solid products were obtained.

NMR spectra of all the compounds shows different substituent –C (C-1 to C-16), and –H (H-2 to H-14) have been analyzed from their NMR spectra. We have performed the analysis with a 1D experiment like  $^1\text{H}$ ,  $^{13}\text{C}$ , and compare chemical shifts with different substituents at C (3),  $^1\text{H}$ ,  $^{13}\text{C}$  NMR of all compounds. The  $^1\text{H}$  chemical shifts of all three series (51, 5b, and 5c) of compounds obtained from the  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectra have been compared.  $^1\text{H}$  chemical shift of –NH– was observed for H-2 to H-14, the X-ray crystal structures of these compounds reveal intra and intermolecular N–H $\cdots$ O interactions. Besides, the C (3) substitution may affect the  $^1\text{H}$  chemical shift of –NH–. The downfield  $^2\text{H}$  chemical shift was noticed for the H-2 and C-2. The aromatic proton H(5) takes part intermolecular C–H $\cdots$ O interaction in most of the crystal structures of derivatives. The  $^1\text{H}$  chemical shift implies that the intermolecular C–H $\cdots$ O interaction. The differences for the  $^{13}\text{C}$  chemical shift of C(2), C(10), C(12), and C(13) of the series are transparent. The chemical shift for C-2 to C-14, showed the carbon chemical shift. For proton (H-2 to H-14) which ascertains the intramolecular hydrogen bonding between C–O–N–H is stronger in hydrogen. The differences observed in the carbon's chemical shift may be due to the electronegativity differences. The  $^{13}\text{C}$  chemical shift of *n*-alkylamino carbons of analogs compounds was found in a similar range except for the terminal methyl carbons. The variation in chemical shift can be correlated to hydrogen bond strength (intra and intermolecular) and the presence of electronegative substituent.

### Cytotoxicity study

Many of ester and amide derivatives of hydroxycinnamic acid have been synthesized and tested for their cytotoxic activity against P388 [7, 9–11]. According to the  $\text{IC}_{50}$  values of *trans*-3-(4-hydroxy-3-methoxyphenyl)acrylate (10.79  $\mu\text{g}/\text{mL}$ ) and *trans*-3-(4-hydroxy-3-methoxyphenyl)-*N*-phenethylacrylamide (29.14  $\mu\text{g}/\text{mL}$ ), the ester compound was more active than amide compound [7]. However, the amide compound should be more effective than the ester analogue due to the stability of the amide compounds. Furthermore, the  $\text{IC}_{50}$  of both *N*-(*o*-tolyl)caffeamide (0.91  $\mu\text{g}/\text{mL}$ ) and

*N*-(*o*-tolyl)-*p*-coumaramide (16.97  $\mu\text{g}/\text{mL}$ ) (Fig. 2) indicated that the hydroxycinnamoyl group has a significant effect on the activity against P388 murine leukemia cells.

Table 2 shows the cytotoxic activity of 5a–c. The results show that the substituent in the phenyl ring of hydroxycinnamic moieties affect the activity. In our previous reports, caffeamide compounds showed higher cytotoxic activity than coumaramide analogues [9, 10]. This is due to the presence of more phenolic groups in caffeamide than in *p*-coumaramide and ferulamide. However, in this study we found that 5a and 5c showed higher cytotoxic activity than 5b. One possible explanation of this fact is that 5a and 5c which have lower deprotonation state can penetrate into the cell deeper than 5b [13]. The effect of amine moiety of hydroxycinnamide compounds against P388 murine leukemia cells can be predicted by comparing the  $\text{IC}_{50}$  values of both 5a and 5b to *N*-(*o*-tolyl) analogues. The  $\text{IC}_{50}$  of 5a is lower than the  $\text{IC}_{50}$  value of the former compound (16.97  $\mu\text{g}/\text{mL}$ ), but the difference is not significant. However, *N*-(*o*-tolyl)caffeamide showed strong cytotoxic activity with  $\text{IC}_{50}$  of 0.91  $\mu\text{g}/\text{mL}$ , which is contrast to 5b. The facts indicate that both the hydroxycinnamic and amine moieties play crucial role in the cytotoxicity of hydroxycinnamide compounds.

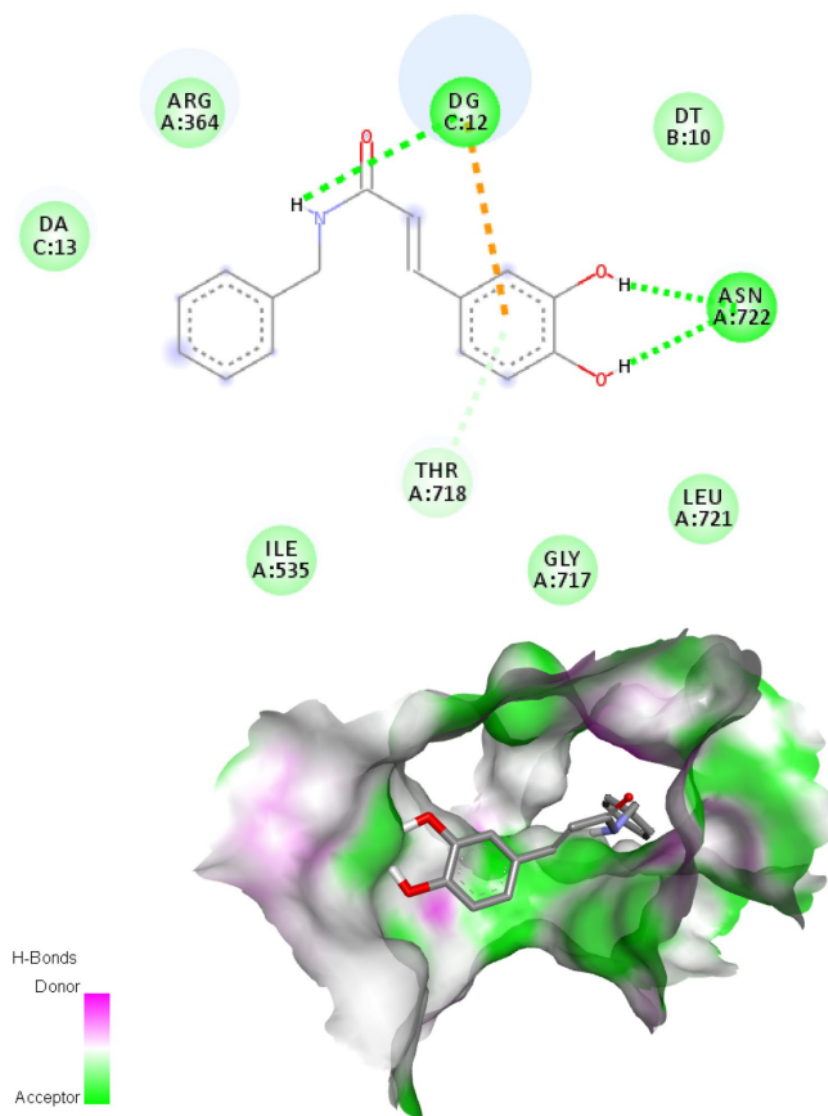
In addition, when comparing compound 5a and 5c, there are a huge difference in their cytotoxic activity. Both compounds have only a difference in the substituent on C-8, which 5c has a methoxy group while no substituent in 5a. This substituent caused the presence of intramolecular hydrogen bond between H atom of hydroxyl group and O atom of methoxy group in 5c. Due to this hydrogen bond, the ability of 5c to release a radical hydrogen is decrease, thus the cytotoxic activity of this compound is lower than 5a.

### Docking study

Docking analysis of compounds 5a, 5b, and 5c was done through Autodock4 program. Result of analysis as shown in Table 3 with compound 5b have a smaller binding energy than the others compound. This phenomenon is due to the more hydrogen bond interactions of 5b against Top1 receptor. Visualization of interaction between 5b and Top1 is shown in Fig. 2. The presence of two hydroxyl groups in compound 5b causes this compound to have more hydrogen bonds then resulted a greater binding energy value than 5a and 5c. In 3D visualization, it was shown that the position of two hydroxyl groups as a donor hydrogen bond meet very close to the site of acceptor hydrogen bond at the Top1 surface.

Nevertheless, the interesting part is the inappropriate value of the inhibition activity with the resulting binding energy data. Compound 5b has the lowest inhibition activity compared to 5a and 5c but has the highest bonding energy to Top1 proteins. To know the reason of this phenomenon,

**Fig. 2** 2D and 3D Visualization of compound **5b** against Top1 protein receptor



**Table 2** The cytotoxicity of synthesized compounds against leukemia murine P388 cell

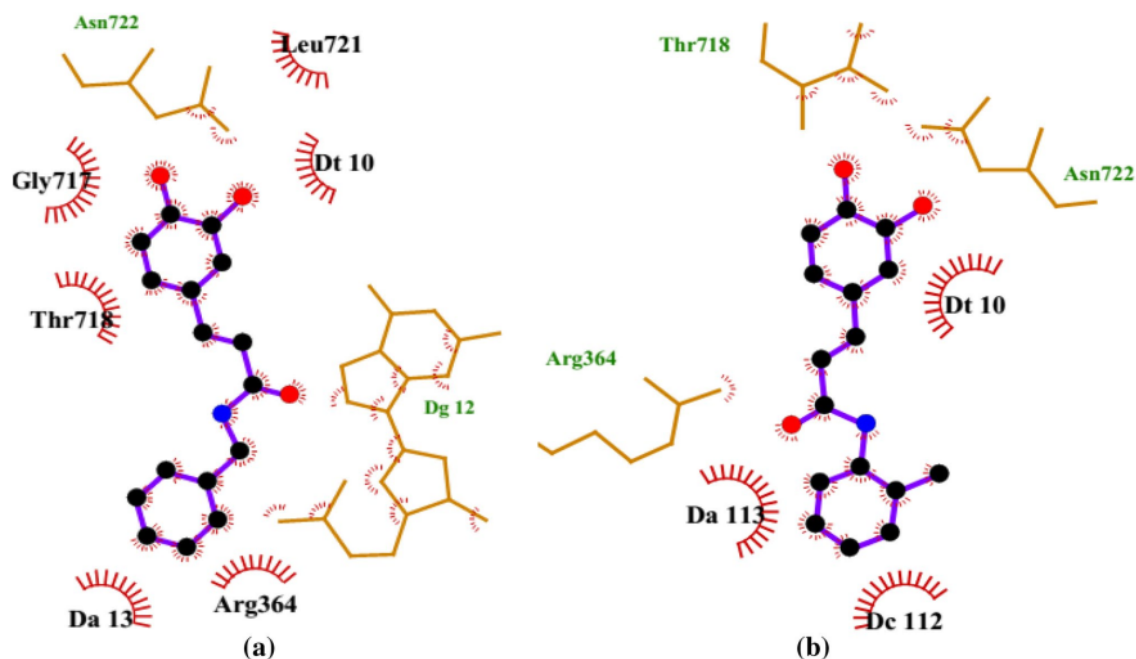
Compound	IC <sub>50</sub> value (μg/mL)
<b>5a</b>	16.15
<b>5b</b>	674.38
<b>5c</b>	179.56

we compared compound **5b** and *trans-N-(o-tolyl)caffeamide* (IC<sub>50</sub> = 0.91 μg/mL) by hydrophobic analysis of that has been synthesized [9] using LigPlot+ program. Figure 3 showed the result of hydrophobic analysis of these two compounds. These two compounds have a two hydroxyl groups in phenyl ring but has a very difference inhibition activity value.

**Table 3** Docking analysis result of compounds **5a**, **5b**, and **5c** against Top1

Compound	ΔG (kcal/mol)	Ki (μM)	Hydrogen bond interaction
<b>5a</b>	-6.40	20.43	Asn722; DG12
<b>5b</b>	-6.64	13.47	Asn722; Asn722; DG12
<b>5c</b>	-6.62	14.08	Asn722; DG12

Hydrophobic interactions of compound **5b** was resulted in Leu721, Gly717, Dt10, Da13, Thr718, and Arg364. Meanwhile, compound *trans-N-(o-tolyl)caffeamide* has at least three interaction at residue Dt10, Da113, and Dc112 for



**Fig. 3** Hydrophobic analysis result of compound **a 5b** and **b** *trans-N-(o-tolyl)caffeamide* against Top1

hydrophobic and hydrogen bond interactions also found at residue Arg364 and Thr718 while in **5b** these two residue only has hydrophobic interaction. It cause of *trans-N-(o-tolyl)caffeamide* having a better activity than **5b** because hydrogen bond interaction with Arg364 has an important role in inhibition mechanism of Top1 receptor [37].

## Conclusion

Compound **5a–5c** have been successfully synthesized from hydroxycinnamic acids by indirect method. Compound **5a** showed moderate cytotoxicity against murine leukemia P388 cells, thus it has a highly potential to be used as an anticancer drug.

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## Declarations

**Conflict of interest** All authors declare no conflict of interest.

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