

Cytotoxic Activity of Pentacyclic Triterpene-3-Heptadecanoate Ester against Hela Cell Line and Its Docking Study

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ABSTRACT: Pentacyclic triterpene-3-heptadecanoate (12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate) ester refers to the compound as a result of reaction between pentacyclic triterpene-3-ol (12,13-dihydro- α -amyrin-20,30-en-3-ol) and heptadecanoic acid. This research was aimed to conduct cytotoxicity test of pentacyclic triterpene-3-heptadecanoate; 12,13-dihydro- α -amyrin-20,30-en-3-ol and 12,13-dihydro- α -amyrin-20,30-en-3-acetate esters against Hela cell line. The activity assay was carried out using MTT method. The results indicated that IC_{50} value of 12,13-dihydro- α -amyrin-3-heptadecanoate; 12,13-dihydro- α -amyrin-20,30-en-3-acetate and 12,13-dihydro- α -amyrin-20,30-en-3-ol were 111.0, 151.1 and 944.4 μ g/ml, respectively. The compound of 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate had the lowest IC_{50} value, suggesting it has a potency to be synthesized as an anticancer drug.

Keywords: Hela cells line; pentacyclic triterpene-3-heptadecanoate ester; 12,13-dihydro- α -amyrin-20,30-en- α -amyrin-3-ol; 12,13-dihydro- α -amyrin-20,30-en-3-acetate ester; cytotoxic activity

1. Introduction

Cancer is a disease that cause the loss of cell control towards both the regulation of cell cycle and *homeostatic* function leading to fast and uncontrolled proliferation [1]. A number of researchs have been conducted to create new anticancer compound. Isolation of natural substances known to have anticancer activity have also been conducted. By finding the lead compound having some activities as anticancer, therefore, the chemical groups playing a role of having corresponding activities can be determined. This, as a result, offers an opportunity to modify a lead compound in order to find a new more potential compound. The natural compounds useful as the anticancer are 12,13-dihydro- α -amyrin-20,30-en-3-ol and 12,13-dihydro- α -amyrin-20,30-en-3-acetate that are categorized as the pentacyclic triterpenes.

Pentacyclic triterpene-3-heptadecanoate ester (Figure 1) is the compound as the result of the synthesis of pentacyclic triterpene-3-ol and heptadecanoic acid [2]. 12,13-dihydro- α -amyrin-20,30-en-3-ol (pentacyclic triterpene) is also present in the *Eupatorium inulifolium* H.B.K leaves. Compound that can be isolated from *Eupatorium inulifolium* is 12,13-dihydro- α -amyrin-20,30-en-3-acetate [3]. Mulyadi [4], reported that 12,13-dihydro- α -amyrin-20,30-en-3-ol and 12,13-dihydro- α -amyrin-20,30-en-3-acetate are useful as inhibitors of protein kinase enzyme target in Hela cells (cervix cancer cell) in vitro, meaning to have the cytotoxic activity. 12,13-dihydro- α -amyrin-20,30-en-3-acetate (es-

ter with C1) has IC_{50} value of 268.8 $\mu\text{g/ml}$ while 12,13-dihydro- α -amyrin-20,30-en-3-ol has IC_{50} value of 628.9 $\mu\text{g/ml}$. [Figure 1]

Based on the calculation of the molecular docking score with the PLANTS program [5] towards the protein kinase enzyme target (code 3HMP), the result of the docking of pentacyclic triterpene ester with C9 was as much as (-80, 7), C14 (-84, 3), C16 (-88, 5) and C15 (-87, 9). From those results, the pentacyclic triterpene ester with C16 was chosen to be tested its cytotoxic activity in which the pentacyclic triterpene ester with C16 was as the pentacyclic triterpene-3-heptadecanoate ester that was possible to be more active than the pentacyclic triterpene-3-acetate ester. The selection of Hela cell line for the research was because it was in line with the purpose to be applied to human.

Some problems emerging from this research included how the cytotoxic activity of the triterpene pentacyclic-3-heptadecanoate ester (C16) and whether it is more effective compared to the pentacyclic triterpene-3-acetate ester (C1). Thus, the objective of this research is to find out the cytotoxic activity of triterpene pentacyclic-3-heptadecanoate ester as the anticancer towards Hela cell line. Hela cell line is cervix cancer cell that can be used for cancer research [6].

2. Methods

2.1. Instrument and materials

Laminar air flow cabinet (Labcono), 96-well microplate (Nucleon), eppendorf, Elisa reader (BioRad mode 680), incubator CO_2 (Labcono),

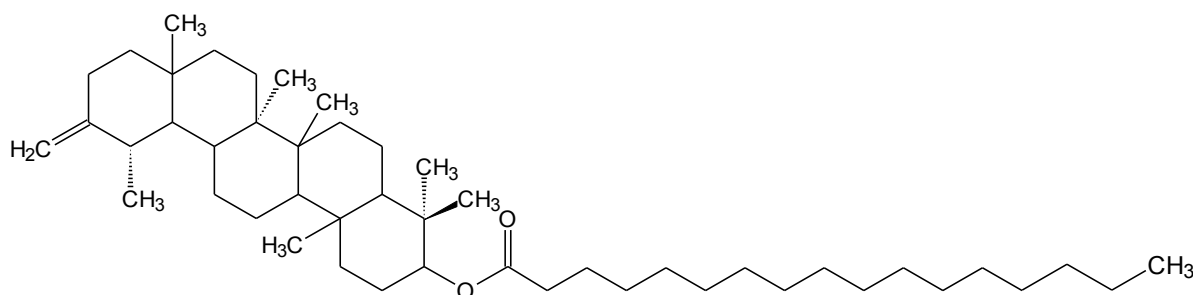


Figure 1. Structure of triterpene pentacyclic-3-heptadecanoate ester

centrifuge (PLC 01), pipette, tissue culture flask, vortex (Thermolyne Maximi), micropipette (Finnipipet Campus), refrigerator, Nebauer haemocytometer, analytical scale, camera and a set of computer for docking.

The materials used are pentacyclic triterpene-3-acetate, pentacyclic triterpene-3-ol, pentacyclic triterpene-3-heptadecanoate ester (result of esterification), MTT, Phosphate Buffered Saline (PBS), Natrium Dodecyl Sulphate (SDS) and Hydrochloride Acid (HCl). Hela cell line was obtained from LPPT UGM (Laboratory of Integrated Research and Testing of Universitas Gadjah Mada).

2.2. Cytotoxicity test

Stock solution of 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate was prepared by dissolving in dimethyl sulfoxide (DMSO) and media culture of RPMI 1640 was initially done in order to obtain a series of concentration from 15.6 to 500 $\mu\text{g/ml}$. The 12,13-dihydro- α -amyrin-20,30-en-3-acetate and 12,13-dihydro-amyrin-20,30-en-3-ol was also prepared in the series of concentration from 15.6 to 500 $\mu\text{g/ml}$. The Hela cell line with concentration of 20,000 cells/well were placed into 96-well plates containing RPMI 1640 with the volume of 100 μl /well were incubated within 1 day. A series of concentrations of pentacyclic triterpene-3-heptadecanoate ester were placed into the 100 μl wells. Here, triterpene pentacyclic triterpene -3-heptadecanoate ester had a series of concentrations of 15.6, 31.3, 62.5, 250 and 500 $\mu\text{g/ml}$. Then, it was incubated within 24 hours in an incubator with 5% CO_2 at the temperature of 37°C. MTT with the concentration of 5 mg/ml in PBS was added to this compound before the compound was placed into each of 10- μl well and incubated for 4 hours in an incubator with 5% CO_2 at 37°C. The stop solution of 10% SDS in hydrochloride acid 0.01 N at 100 μl , subsequently, was added and incubated at room temperature. In this case, the absorbance of it was read using Elisa reader with the wavelength of 550 nm.

The IC_{50} value was obtained from the analysis

of regression from the result of absorbance obtained. Based on the equation of $y = Bx + A$ with x = the level of pentacyclic triterpene-3-heptadecanoate ester, pentacyclic triterpene-3-acetate ester, pentacyclic triterpene-3-ol and y = mean of the cell viability (%). Thus, the IC_{50} could be found by correlating percentage of cell viability at 50% at the equation. In this case, the IC_{50} value can be calculated as $\mu\text{g/ml}$ and then divided with the molecular weight. The calculation of cell viability is as follows:

$$\text{viability} = \frac{\text{sample Ab} - \text{media control Ab}}{\text{cell control of Ab} - \text{media control Ab}} \times 100\%$$

Remark: Ab = absorbance

3. Results and discussion

The results of cytotoxicity test of pentacyclic triterpene-3-heptadecanoate ester, pentacyclic triterpene-3-acetate ester and triterpene pentacyclic triterpene-3-ol against the Hela cell line (Table 1, 2, and 3) show that all of the compounds could inhibit the growth of Hela cells line with the IC_{50} value in the level of $\mu\text{g/ml}$.

The results of the cytotoxicity test of three pentacyclic triterpene compounds showed effect on the inhibition of the Hela cells' growth. The IC_{50} value of pentacyclic triterpene-3-heptadecanoate ester, pentacyclic triterpene-3-acetate ester and pentacyclic triterpene-3-ol were 110.0, 151.1 and 944.40 $\mu\text{g/ml}$, respectively. This shows that pentacyclic triterpene-3-heptadecanoate ester as the result of modification had the lowest IC_{50} value. In other words, esterification of pentacyclic triterpene-3-ol with heptadecanoic acid increased the activity.

The highest concentration of compound selected was 500 $\mu\text{g/ml}$. The relationship between percentage of viability of Hela cell line and the concentration of each compound are shown in Figure 2.

It can be seen from Figure 2 that the higher the concentration of the compounds, the smaller

Table 1. The viability test of triterpene pentacyclic-3-heptadecanoate ester (ETPH) against Hela cell line

Concentration of triterpene pentacyclic-3-heptadecanoate ester ($\mu\text{g/ml}$)	Absorbance			Viability (%)				IC_{50} ($\mu\text{g/ml}$)
	1	2	3	1	2	3	Average	
500	0.060	0.050	0.040	2.49	1.63	0.77	1.63	111.0
250	0.165	0.168	0.168	11.50	11.75	11.76	11.67	
125	0.519	0.655	0.549	41.89	53.56	44.46	46.64	
62.5	0.665	0.812	0.735	54.42	67.04	60.43	60.63	
31.25	0.663	0.874	0.865	54.43	72.36	71.59	66.06	
15.625	0.771	0.850	0.790	63.52	70.30	65.15	66.32	

Table 2. The viability test of triterpene pentacyclic-3-acetate ester (ETPA) against Hela cell line

Concentration of triterpene pentacyclic-3-acetate ester ($\mu\text{g/ml}$)	Absorbance			Viability (%)				IC_{50} ($\mu\text{g/ml}$)
	1	2	3	1	2	3	Average	
500	0.145	0.144	0.110	9.78	9.70	6.78	8.75	151.1
250	0.452	0.421	0.427	36.14	33.48	33.99	34.53	
125	0.748	0.661	0.730	61.54	54.08	60.00	58.54	
62.5	0.714	0.680	0.962	58.63	55.71	79.91	64.75	
31.25	0.738	0.768	0.000	60.69	63.26	0.00	61.97	
15.625	0.789	0.673	0.808	65.06	55.11	62.29	62.29	

Table 3. The viability test of triterpene pentacyclic-3-ol (TPOL) against Hela cell line

Concentration of triterpene pentacyclic-3-ol ($\mu\text{g/ml}$)	Absorbance			Viability (%)				IC_{50} ($\mu\text{g/ml}$)
	1	2	3	1	2	3	Average	
500	0.785	0.730	0.706	64.72	60.00	57.94	60.89	994.4
250	0.864	0.760	0.810	71.50	62.57	66.87	66.98	
125	0.871	0.000	0.804	72.10	0.00	66.35	69.23	
62.5	0.873	0.882	0.848	72.27	73.05	70.13	71.82	
31.25	0.834	1.170	0.802	68.93	97.77	66.18	77.73	
15.625	0.886	0.815	0.828	73.39	67.30	68.41	69.70	

Table 4. The results of the docking score between the triterpene pentacyclic-3-heptadecanoate ester, triterpene pentacyclic-3-acetate ester and triterpene pentacyclic-3-ol ester with the target of protein kinase enzymes (code 3HMP)

Compounds	Docking score
Triterpene pentacyclic-3-heptadecanoate ester	-88.5
Triterpene pentacyclic-3-acetate ester	-64.2
Triterpene pentacyclic-3-ol	-61.2

the percentage of the viability is. It means that the pentacyclic triterpene-3-ol had small effect to the percentage of viability. Moreover, the effect of pentacyclic triterpene-3-heptadecanoate ester on the growth of the Hela cell line at the concentration of 500 $\mu\text{g/ml}$ was 1.63%, whereas at the concentration of 15.6 $\mu\text{g/ml}$ was 66.32%. It means that there was a significant effect of the compound on the viability of Hela cell line.

The results in the docking score (Table 4) between the compounds from the result of modification include pentacyclic triterpene com-

ound of 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate (ETPH) and the target of protein kinase enzymes (code 3HMP) at -88.5 as a quite good result in comparison to the compound of pentacyclic triterpene 12,13-dihydro- α -amyrin-20,30-en-3-acetate (ETPA) at -64.2 and the compound of pentacyclic triterpene 12,13-dihydro- α -amyrin-20,30-en-3-ol (TP-ol) at -61.2. The docking result was in line with the result of its cytotoxicity test showing that the IC_{50} value for the compound of 12,13-dihydro- α -amyrin-20,30-en-heptadecanoate was the lowest one (111.0 $\mu\text{g/}$

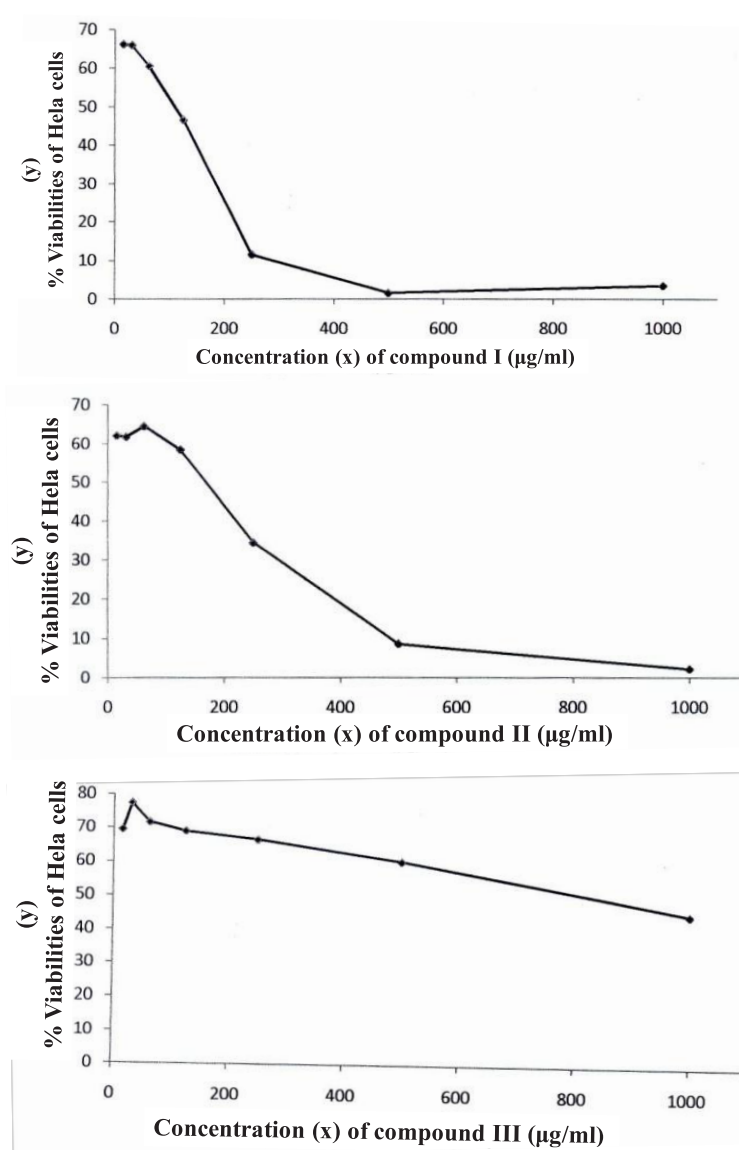


Figure 2. The relationship between percentage of viability (y) of Hela cell line and the concentration (x) of three different compounds ($\mu\text{g/ml}$)
 I. Triterpene pentacyclic-3-heptadecanoate ester
 II. Triterpene pentacyclic-3-acetate ester
 III. Triterpene pentacyclic-3-ol

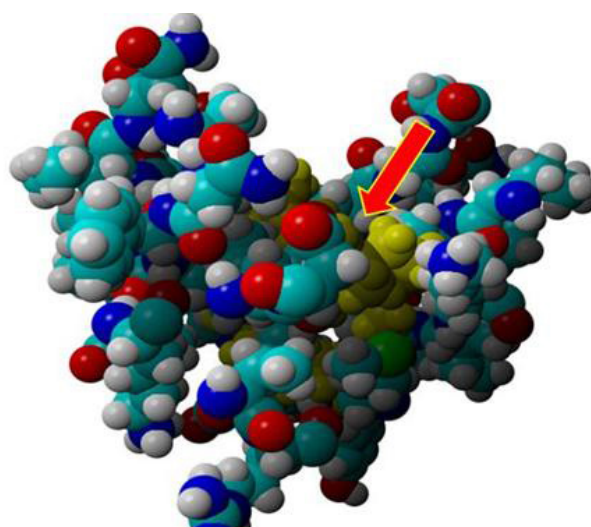


Figure 3. The bond between triterpene pentacyclic-12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate ester (ETPH) and the target of protein kinase enzyme (3HMP)

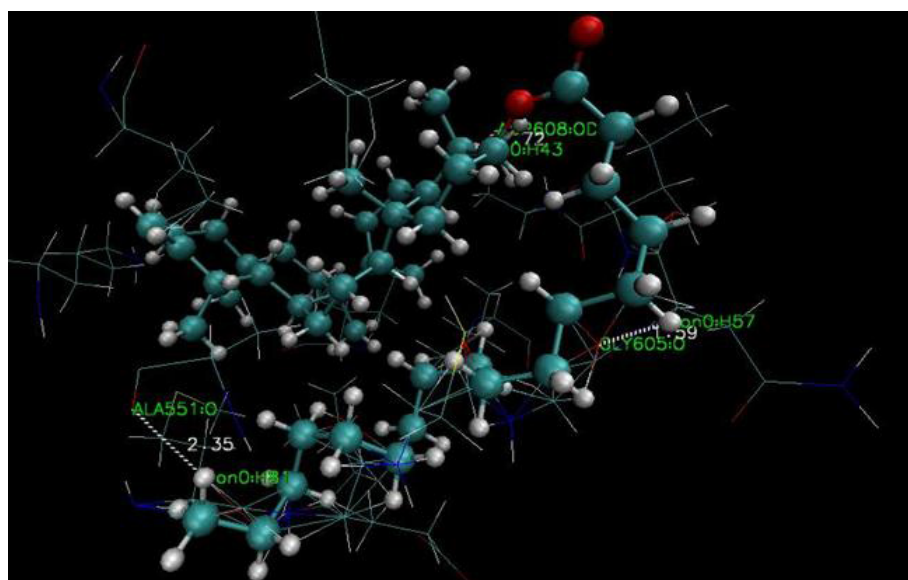


Figure 4. The bond between triterpene pentacyclic-12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate ester (ETPH) and amino acid from the target of protein kinase enzymes (code 3HMP)

ml) if it was compared to that of 12,13-dihydro- α -amyrin-20,30-en-3-acetate (151.1 $\mu\text{g/ml}$) and 12,13-dihydro- α -amyrin-20,30-en-3-ol (944.4 $\mu\text{g/ml}$). The supports for the result of the docking between the compound of pentacyclic triterpene 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate with the target of enzymes code of 3HMP can be seen in Figure 3 and 4.

The results showed a binding between the 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate ester and the target of protein

kinase enzyme (code 3HMP) through its binding to Alanin 551 (Ala 551), Glycin 605 (Gly 605) and Aspartate 608 (Asp 608).

4. Conclusion

It can be concluded that pentacyclic triterpene-3-heptadecanoate could influence the growth of Hela cell line or it was cytotoxic to the Hela cell line. The IC_{50} value of pentacyclic

triterpene-12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate ester (ETPH), pentacyclic triterpene-12,13-dihydro- α -amirin-20,30-en-3-acetate ester and pentacyclic triterpene-12,13-dihydro- α -amyrin-20,30-en-3-ol were 111.0, 151.1 and 944.4 $\mu\text{g/ml}$, respectively. Those compounds were bound to the target of protein kinase enzymes (code 3HMP) with the docking score -88.5, -64.2 and -61.2, respectively. It was also found that pentacyclic triterpene-12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate (ester with C16) was more active than pentacyclic triterpene-12,13-dihydro- α -amyrin-20,30-en-3-acetate (ester with C1) towards Hela cell line. It also had the most stable binding on target of protein kinase enzyme. Thus, the compound of 12,13-dihydro- α -amyrin-20,30-en-3-heptadecanoate can be suggested as the anticancer drug to the cervical cancer.

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