# Synthesis and *In Vitro* Evaluationof C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4]resorcinaryl octabenzoate as the Sunscreen

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## ABSTRACT

*The present study was aimed to synthesize and evaluate sunscreen activity of C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4]resorcinaryl octabenzoate. The target compounds were synthesized in 2 steps. They were a synthesis of C-methylcalix[4]-resorcinarene via acid catalyzed the condensation of resorcinol and acetaldehyde by using HCl catalyst, followed by esterification using cinnamoyl chloride and benzoyl chloride. The characterization of the target compounds was performed by IR, $^{1}$H-NMR, $^{13}$C-NMR, and LC-MS spectrometers. The sunscreen activity test was conducted by spectroscopic method and MTT-assay. Commercial sunscreen p-amino benzoic acid (PABA) was used as a comparator to the MTT assay. The sunscreen activity test used spectroscopic showed that C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4]resorcinaryl octabenzoate can absorb the ultraviolet radiation between 280 and 320 nm (UV-B) with the maximum absorption at 290 nm (ε = 31.535 $M^{-1}$ $cm^{-1}$) and 282 nm (ε = 42.217 $M^{-1}$ $cm^{-1}$), respectively. The results of MTT-assay indicated that the $IC_{50}$ of C-methylcalix[4]resorcinaryl octacinnamate, C-methylcalix[4]resorcinaryl octabenzoate and PABA are 12.006, 20.568 and 12.564 ppm, respectively, it means that the order of sunscreen activity is C-methylcalix-[4]resorcinaryl octacinnamate, PABA and C-methylcalix[4]resorcinaryl octabenzoate.*

***Keywords***: *synthesis; esterification; sunscreen; activity; characterization*

## ABSTRAK

*Studi ini dimaksudkan untuk mensintesis dan menguji aktivitas tabir surya senyawa C-metilkaliks[4]resorsinaril oktasinamat dan C-metilkaliks[4]resorsinaril oktabenzoat. Senyawa target disintesis melalui dua tahap reaksi yaitu sintesis C-metilkaliks[4]resorsinarena melalui reaksi kondensasi resorsinol dan asetaldehida menggunakan katalis HCl, dilanjutkan dengan esterifikasi menggunakan sinamoilklorida dan benzoilklorida. Karakterisasi molekul target dilakukan menggunakan IR, $^{1}$H-NMR, $^{13}$C-NMR dan LC-MS spektrometer. Uji aktivitas tabir surya dilakukan menggunakan metode spektroskopi dan MTT-assay. Sebagai senyawa pembanding pada MTT-assay digunakan asam p-amino benzoat (PABA). Hasil ujiaktivitas tabir surya menggunakan metode spektrometri menunjukkan bahwa C-metilkaliks[4]-resorsinaril oktasinamat dan C-metilkaliks[4]resorsinaril oktabenzoat berturut-turut dapat menyerap radiasi ultraviolet diantara 280 sampai 320 nm (UV-B) dengan panjang gelombang maksimum pada 290 nm (ε = 31,535 $M^{-1}$ $cm^{-1}$) dan 282 nm (ε = 42,217 $M^{-1}$ $cm^{-1}$). Hasil MTT-assay menunjukkan bahwa nilai $IC_{50}$ C-metilkalix[4]resorcinaril oktasinamat, C-metilkalix[4]resorcinarilocta-benzoat dan PABA berturut-turut adalah 12,006, 20,568 dan 12,564 ppm, ini berarti urutan aktivitas tabir suryanya adalah C-metilkalix[4]resorcinaril oktasinamat, PABA dan C-metilkalix[4]resorcinaril octabenzoat.*

***Kata Kunci***: *sintesis; esterifikasi; tabir surya; aktivitas; karakterisasi*

## INTRODUCTION

Solar ultraviolet (UV) radiation reaching the earth is a combination of UV-B (280-320 nm) and UV-A (320-400 nm) wavelengths. It is well known that both acute and chronic sun exposure induce biologically and clinically detrimental damaging effects such as sunburn, immunosuppression, photoaging and skin cancer [1-2]. Mironava et al. [3] have reported that commercials compact fluorescent light (CFL) bulbs emit UV-A and UV-C light, which can induce to various types of skin cancer. The uses of sunscreen have increased

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worldwide attention to prevent sun damage of skin. Nowadays ultraviolet filter is regularly added to cosmetic product [4-5]. It means the people are conscious of the possible dangers of photoaging and skin cancer, occurring as a result of the sun overexposure. Every year, about one million people are diagnosed with skin cancer and about 10,000 die from malignant melanoma [4,6].

It was envisaged that suitable calixarene derivatives would either function as efficient sun protectant screen harmful ultraviolet radiations themselves or as carrier molecules to encapsulate and established sun protections [7]. Calix[4]resorcinarenes are analogs of calixarene having two hydroxyl groups on benzene rings at an extra annular position forming a macrocycle. The compounds are synthesized by the acid-catalyzed cyclocondensation of resorcinol with various aliphatic or aromatic aldehyde [8-10]. The compounds are easily synthesized by well-established one-pot procedures [9]. They have found much application of Calix[4]resorcinarenes such as macrocyclic receptor [11], host molecules [12-14], dying fiber [10], building blocks for even larger supramolecular architectures assemblies such as cages, capsule and as starting materials for the preparation of more sophisticated molecules [15]. Unfortunately, the study of calix[4]resorcinarene as the ultraviolet light absorbers has attracted very little attention. Calix[4]resorcinarene has some advantages such as; stable at high temperature and low toxicity. When the molecules which able to absorb dangerous UV radiation were coupled to calix[4]resorcinarene, for examples cinnamoyl and benzoyl chloride, it will be obtained a compound with a good characteristic as a UV absorber with high stability.

Rojanathannes et al. [16] have reported photo switchable calix[4]arene incorporating different regioisomers of stilbene and azobenzene. It has been observed that the stilbene bridged derivative isomerized only under UV irradiation, while azobenzene derivatives undergo thermal or photochemically induced isomerization.

Budiana et al. [17] have synthesized benzoyl and cinnamoylcalix[4]resorcinarenes. It has been observed that both the compounds could absorb UV-B radiation, but the route of synthesis still long enough, so it has economically disadvantage. Chalwa [7] also reported that tetra propoxycalix[4]arene enones and cinnamate have the ability to absorb UV-B, but the yield of the reaction was only 25%. As it is known that conjugated double bonds in benzoyl and also cinnamoyl group can serve as an absorber of harmful UV rays (UV-B).

Recently, *in vitro* methods have shown a significant potential for assessing the toxicity of environmental and occupational health risk [18]. One such method is the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, which is based on the ability of viable cells to convert a soluble tetrazolium salt to a formazan product. This method has been proven to be user-friendly, rapid and highly sensitive [19].

The focus of this paper is to synthesize the new two derivates of calixresorcinarene (C-methylcalix[4] resorcinaryl octacinnamate and C-methylcalix[4] resorcinaryl octabenzoate through the shorter reaction pathway than that reported by Chalwa [7] and Budiana [17].

## EXPERIMENTAL SECTION

### Materials

All reagents used were resorcinol, acetaldehyde, hydrochloric acid, ethanol 96%, cinnamoyl chloride and pyridine. The reagents were commercially obtained from E. Merck with P.A. grade and directly used without any further purification.

### Instrumentation

Structure elucidation of the synthesized compounds was performed using $^{1}$H-NMR and $^{13}$C-NMR (Agilent 400), as well as FT-IR (Shimadzu, Prestige 21) and Lambda 35 UV/Vis spectrometer (Perkin Elmer Inc. USA) with 10 mm quarts and LC-MS (Mariner).

### Procedure

#### *Synthesis of C-metylcalix[4]resorcinarene (1)*

Resorcinol (5.50 g, 0.05 mole), ethanol 95% (25 mL) and water (25 mL) were mixed in the 100 mL round bottomed flask. The mixture was stirred for 15 min. Acetaldehyde 2.8 mL (0.05 mole) and 1.5 mL hydrochloric acid were then added to the homogeneous solution. The mixture was heated for 24 h at 80 °C. The product was collected as yellow solid, washed with water and characterized by IR, $^{1}$H-NMR, and mass spectroscopy.

#### *Synthesis of C-methylcalix[4]resorcinaryl octa cinnamates (2)*

The solution of 2.5 g (4.60 mmol) C-metylcalix[4]resorcinarene **(1)** in 22.7 mL pyridine was poured into 100 mL round bottomed flask. The mixture was stirred and heated at 40 °C for 15 min and 9.65 g (73.66 mmol) of cinnamoyl chloride was then added in five portions. The mixture was heated for 4 h at 65 °C. The product was collected as orange solid and washed with water until neutral. The C-methyl calix[4]resorcinaryl octacinnamate **(2)** was then characterized by IR, $^{1}$H-NMR, and mass spectroscopy.

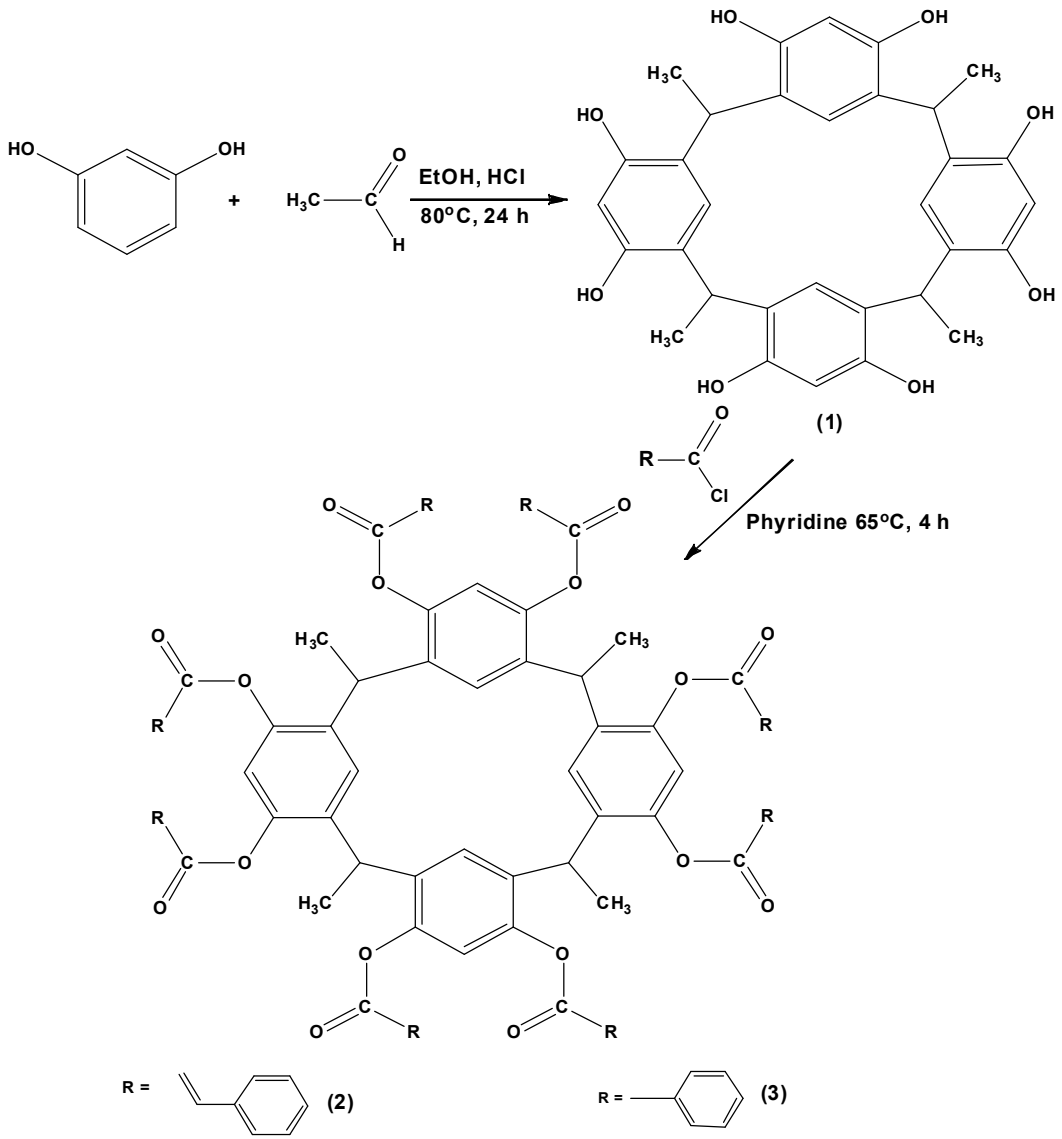


**Fig 1.** Scheme of C-metylcalix[4]resorcinaryl octacinnamate **(2)** and C-metylcalix[4]resorcinaryl octabenzoate synthesis **(3)**

***Synthesis of C-methylcalix[4]resorcinarylocta benzoates (3)***

The compound **(3)** was prepared from C-methylcalix[4]resorcinarene **(1)** (2.5 g, 4.60 mmol), pyridine (22.7 mL) and benzoyl chloride (10.35 g, 73.66 mmol) under stirring and heating at 65 °C for 4 h. The product was collected as yellow solid and washed with water until neutral. The route of target compounds synthesis was displayed in Fig. 1.

***Ultraviolet analysis***

The UV analysis was conducted based on the method described in the literature [17]. Maximum wavelength ($\lambda_{max}$) and molar absorption (ε) were determined as follows: two solutions of (**2**) and (**3**) in the concentration $10^{-3}$, $0.25\times10^{-3}$, $0.5\times10^{-3}$, $0.75\times10^{-3}$ and $10^{-4}$ M were prepared in dichloromethane. Their absorbance (A) was recorded at respective peak wavelengths ($\lambda_{max}$) using quartz cuvets of 1 cm path length. A plot of A versus molar concentration at $\lambda_{max}$ was prepared and ε was obtained from the slope of the straight line. The absorptions spectra of samples in solution were obtained in the range of 200 to 400 nm using 1 cm quartz cell, and dichloromethane as a blank. The maximum wave length and molar absorption of **(2)** are 290 nm ($\lambda_{max}$) and ε = 11.535 $M^{-1}$ $cm^{-1}$. Mean while, the maximum wave length and molar absorption of **(3)** are 282 nm ($\lambda_{max}$) and ε = 42.217 $M^{-1}$ $cm^{-1}$.

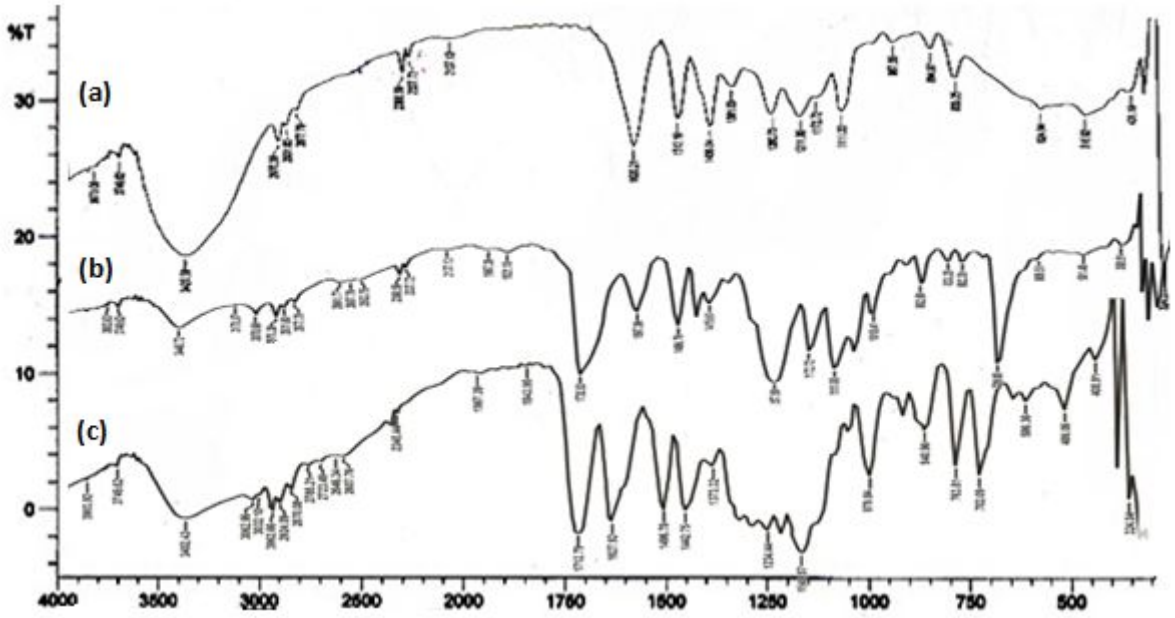


**Fig 2.** The IR Spectrum of C-Methylcalix[4]resorcinarene (a), C-Methylcalix[4]resorcinaryl octacinnamate (b) and C-Methylcalix[4]resorcinaryl octabenzoate (c)

***In vitro Evaluation of C-methylcalix[4]resorcinaryl octacinnamates***

Fibroblast was obtained from human skin biopsy and cultured by using DMEM with 10% FCS supplemented with Penicillin-Streptomycin (100 UmL/100 µL) and 2 Mm L-Glutamine and cultures were incubated at 37 °C in a humidified atmosphere of 5% $CO_2$ and 95% air. The media was replaced every 2 or 3 days until confluence was reached. Fibroblast used for this study were between their 3$^{rd}$ and 5$^{th}$ passage in culture. Cell survival was analyzed by using a non radioactive cell proliferation assay system (MTT-assay) consisting of 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide. The cells were plated at $2\times10^5$ cell/mL per well into 96 well plates in various concentrations of C-methylcalix[4]resorcinaryl octa cinnamates and C-methylcalix[4]resorcinaryl octa benzoate were added with serum free DMEM in a total volume of 100 µL/well. The fibroblast cell without a sample is used as the control. After further culture for 24 h at 37 °C in a humidified atmosphere of 5% $CO_2$ in the air, both of test groups well and controls groups well were radiated by using UV-B radiation (100 mJ/cm$^2$) for 2 h. Fibroblast growth-induction activity was determined by the MTT solution (5 mg/mL) which was added to each well of the plate and plates were incubated for 4 h at 37 °C in a humidified atmosphere of 5% $CO_2$ in the air. The medium in each well was changed by 100 µL dimethylsulfoxide (DMSO) and mixed thoroughly to dissolve the dark blue crystal for 10 min at room temperature in order to ensure all crystals are solvable. The plates were measured with ELISA reader, using test wavelength of 570 nm. The absorbance (optical density) is proportional to the amount of fibroblast cell. The higher sunscreen activity of the compound indicates the higher ability of the compound to inhibit the fibroblast cell died.

$$\%\text{Inhibition} = \frac{(a-b)}{b} x100\%$$

a: optical density value of the test groups well
b: optical density value of the controls groups well

## RESULT AND DISCUSSION

The C-methylcalix[4]resorcinarene **(1)** was synthesized via acid catalyzed condensation and cyclization of resorcinol and acetaldehyde. Esterification of **(1)** by using cinnamoyl chloride and benzoyl chloride produced the target compounds C-methylcalix[4]resorcinaryl octacinnamate **(2)** and C-methylcalix[4]resorcinaryl octabenzoate **(3)**. The yield of every compound is 80.25% **(1)**, 78.65% **(2)** and 79.95% **(3)**, respectively.

The C-methyl calix[4]resorcinarene was obtained as a yellow solid, melting point >390 °C, IR spectroscopy (KBr pellet, cm$^{-1}$): 3425 (-OH), 1512 and 1620 (Ar-C=C) and 1435 (C-H bridge), $^1$H-NMR (DMSO) δ in ppm from TMS: 4.4 (C-H bridge), 6.2 and 6.8 (Ar-H), 8.6 (H of Ar-OH) and 1.3 (H-$CH_3$). The IR spectrum showed strong absorptions at 3425 and 1435 cm$^{-1}$ indicated the presence of the hydroxyl and C-H bridge of calix[4]resorcinarene, respectively (Fig. 2a). As shown in the $^1$H-NMR spectrum (Fig. 3a),

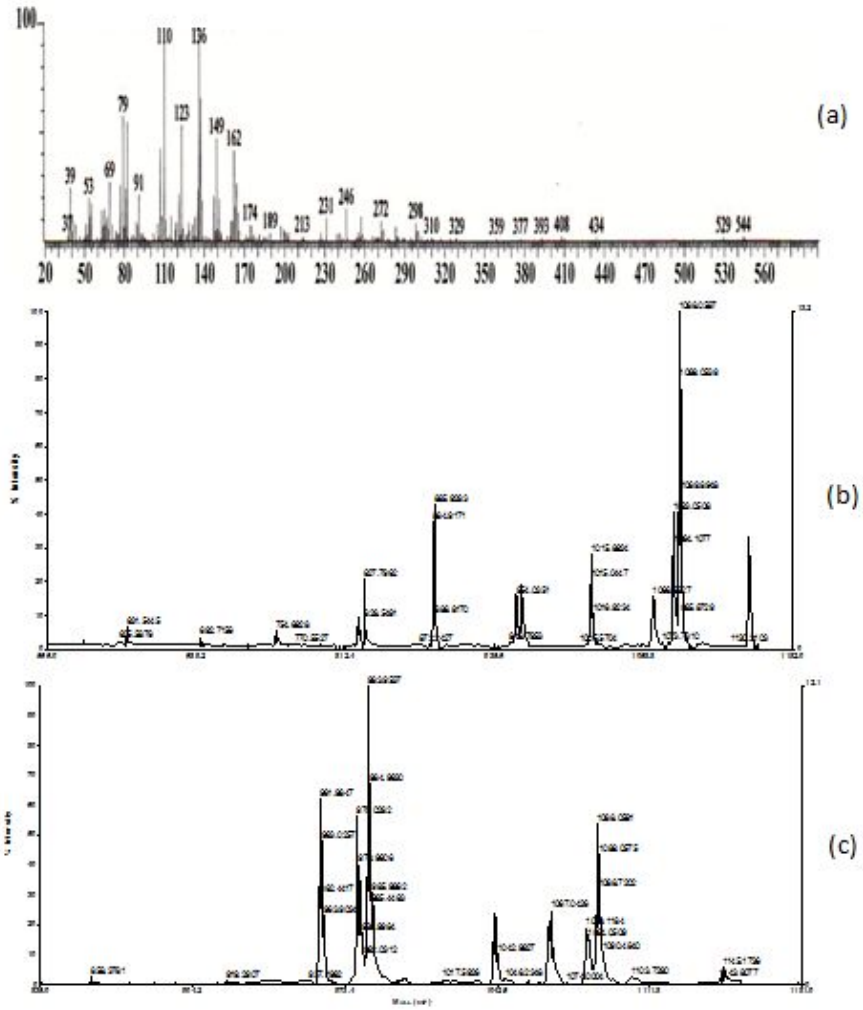


**Fig 4.** The MS Spectrum of C-Methylcalix[4]resorcinarene (a), C-Methylcalix[4]resorcinaryl octacinnamate (b) and C-Methylcalix[4]resorcinaryl octabenzoate(c)

the peak at 4.42 ppm could be considered as the H-bridge. The aromatic proton gave two singlets at 6.15 and 6.76 ppm. The appearance of a peak at 8.60 ppm could be ascribed to the hydroxyl proton.

The MS spectrum of **(1)** showed the appearance of a molecular peak at m/z 544 [$M^+$] which indicated the molecular mass of C-methylcalyx[4]resorcinarene (Fig. 4a). The high melting point of **(1)** (> 390 °C) is caused by hydrogen bonding in the compound.

The target compounds of C-methylcalix[4] resorcinaryl octacinnamate **(2)** were obtained as an orange solid, melting point at 121-124 °C, IR spectroscopy (KBr pellet, $cm^{-1}$): 1712 (-C=O ester), 1512 (Ar-C=C), 1627 (-C=C) and 1435 (C-H bridge). As shown in $^1$H-NMR ($CDCl_3$) δ in ppm from TMS (Fig. 3b), the peak at 4,25 ppm could be considered (C-H bridge), 6.85 and 7.94 (Ar-H),6.53, 7.14, 7.35 and 7.56 (H of cinnamoyl groups) and 1.3 (H-$CH_3$). The IR spectrum showed strong absorptions at 1712 and 1753 $cm^{-1}$ indicated the presence of ester carbonyl group. The presence of carbonyl group and disappeared of strong absorptions of the hydroxyl group at 3425 $cm^{-1}$ indicated that the esterification process conducted well (Fig. 2b).

The MS spectrum of **(2)** showed the appearance of a molecular peak at m/z 1130 [$M^+$] which indicated the molecular mass of C-methylcalyx[4]resorcinaryl octacinnamate (Fig. 4b). The melting point of **(2)** which was 121-124 °C. The decreasing of melting point is caused by the disappeared of hydrogen bonding in the compound, and the high yield of the reaction showed that the C-methylcalix[4]resorcinaryl octacinnamate conducted very easy.

The C-methylcalix[4]resorcinaryl octabenzoate **(3)** was obtained as a yellow solid, melting point at 116-120 °C, IR spectroscopy (KBr pellet, $cm^{-1}$): 1753 (-C=O ester), 1510 (Ar-C=C), and 1420 (C-H bridge). As shown in $^1$H-NMR ($CDCl_3$) δ in ppm from TMS (Fig. 3c),

the peak at 4,44 ppm could be considered (C-H bridge), 7.15 and 7.78 (Ar-H),7.46, 7.56 and 8.14 (H of benzoyl groups) and 1.64 (H-$CH_3$). The characterization result of **(3)** using mass spectrophotometer (Fig. 4c), showed that the reaction product releases the benzoyl group. This was indicated by the presence of fragments with a value of 1067 caused by the release of three benzoyl groups, 961 (release four benzoyl groups) and 858 (release five benzoyl groups). The released of benzoyl group by C-methylcalix[4]resorcinaryl octabenzoate is very likely to occur because of the bond between benzoyl group and the main calix[4]resorcinarenes is an ester bond.

The UV spectral behavior in relation to the absorption maximum and absorption range in the UV region from 280 to 400 nm were examined. The spectrophotometer analysis (Fig. 5) showed that the C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4]resorcinaryl octabenzoate had a both of wide range of UV-B absorption and high molar extinction coefficient ε = 31.535 $M^{-1}$ $cm^{-1}$ and ε = 42.217 $M^{-1}$ $cm^{-1}$. The bandwidth of C-methylcalix[4]resorcinaryl octacinnamate is larger than those C-methylcalix[4] resorcinaryl octabenzoate.

The C-methylcalix[4]resorcinaryloctacinnamate can absorb UV-B very well, so the compound potent to be applied as a sunscreen. UV-B is responsible for the more detrimental delayed sunburn and tanning. UV-B radiation also acts directly on biological molecules, causing mutations that are potentially cancerous.

The ability of both compounds (**2** and **3**) as a protection against UV-B rays, were demonstrated by its ability to inhibiting skin fibroblast cell death. The relation between test agent (C-methylcalix[4] resorcinaryl octacinnamate, C-methylcalix[4] resorcinaryl octabenzoate and PABA) and inhibition after 2 h exposure using MTT assay are presented in Fig. 6. Inhibition or cell viability was significantly increased in increasing of test agent concentration. This is due to the higher concentration of the test substance means more active substances that could inhibit cell death due to exposure to UV-B rays.

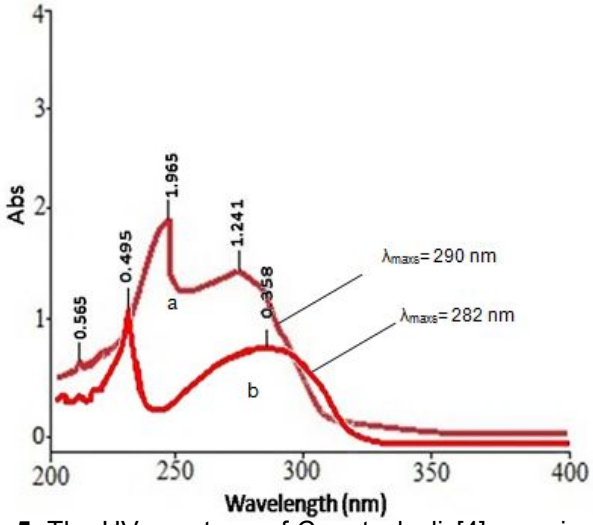


**Fig 5.** The UV spectrum of C-metnylcalix[4]resorcinaryl octacinnamate (a) and C-methylcalix[4]resorcinaryl octabenzoate (b)

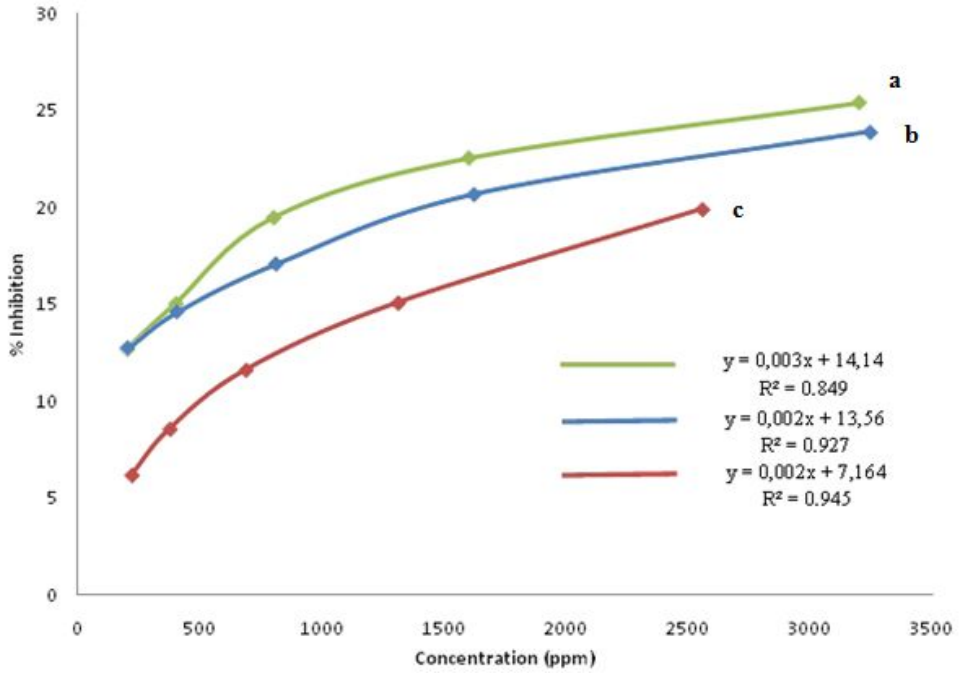


**Fig 6.** Concentration-% inhibition curve of **(2)** (a), concentration-% inhibition curve of *p*-amino benzoic acid (PABA) (b) and concentration-% inhibition curve of **(3)** (c)

The sunscreen activity of the test compounds is indicated by $IC_{50}$ values which were calculated from the linear regression equation curve. The lower $IC_{50}$ value of the compounds, is the higher their activities. *In vitro* evaluation result indicated that the C-methylcalix[4] resorcinaryl octacinnamate has sunscreen activity ($IC_{50}$ = 12.006 ppm). The sunscreen activity of the C-methylcalix[4]resorcinaryl octacinnamate is stronger than this commercial sunscreen *p*-amino benzoic acid (PABA) ($IC_{50}$ = 12.564 ppm) and C-methylcalix[4] resorcinaryl octabenzoate (20.568 ppm).This fact is caused by differences in the amount and type of active group contained in the test compound. In the C-methyl calix[4]resorcinaryl octacinamate there are eight cinnamic active group conjugated, while in the PABA conjugated system is a unity of the acid group to amino group. The more group that can absorb UV radiation in a molecule, are the better their sunscreen activity. The activity of C-methylcalix[4]resorcinaryl octabenzoateis the lowest compared to C-methylcalix[4]resorcinaryl octacinamate and PABA. This fact is caused by a benzoyl group attached to the C-[4]resorcinaryl octabenzoate has the shortest conjugation system, so the ability to absorb UV-B is also the lowest. An electron in conjugated double bond system can undergo excitation to the higher energy level or be converted to another harmless molecule as it irradiated by UV-B. Gasparro et al. [20] reported that the organic sunscreens absorb a specific portion of the UV-B spectrum that is generally re-emitted at a less energetic and longer wavelength, that harmless to the skin. The concentration allowing for sunscreen is 10% or 100.000 ppm.

## CONCLUSION

C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4]resorcinaryl octabenzoate can be synthesized trough the shorter reaction pathway compared to the benzoyl and cinnamoyl calix[4]arenes series that have been previously synthesized. The compounds displayed a good ability in absorb UV-B rays and inhibit the fibroblast cell death. The sunscreen activity of C-methylcalix[4]resorcinaryl octacinnamate ($IC_{50}$ = 12.006 ppm) stronger than that of the commercial sunscreen PABA ($IC_{50}$ = 12.564 ppm).

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