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Synthesis and High Antioxidant Activity of C-Alkyl Calix[4]resorcinarene and C-Alkyl Calix[4]pyrogallolarene Derivatives

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ABSTRACT

In the present work, a successful synthesis and high antioxidant activity of C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives were reported. The C-alkylcalix[4]resorcinarenes were prepared from a cyclization reaction of resorcinol and either pentanaldehyde or octanaldehyde under acidic conditions. Meanwhile, the C-alkylcalix[4]pyrogallolarenes were prepared from a cyclization reaction of pyrogallol with pentanaldehyde or octanaldehyde. Four synthesized products, i.e., nBu-CR, nHep-CR, nBu-CP, and nHep-CP, were successfully prepared in 92.4-96.4% yield. Their chemical structure was elucidated by Fourier transform infrared (FTIR), liquid chromatography-mass spectrometry (LC-MS), and proton nuclear magnetic resonance (^{1}H-NMR) analysis. The antioxidant activity assay of these compounds was evaluated through an *in vitro* assay employing 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The DPPH assay showed that the half-maximal inhibitory concentration (IC_{50}) values of nBu-CR, nHep-CR, nBu-CP, and nHep-CP compounds were 25.1, 22.9, 11.5, and 21.9 μg mL^{-1}, respectively. The IC_{50} value of the synthesized compounds was 2.0-4.3 times lower than the positive standard of butylated hydroxytoluene (BHT, IC_{50} = 49.9 μg mL^{-1}), which was remarkable. This finding demonstrates that both C-alkylcalix[4]resorcinarenes and C-alkylcalix[4]pyrogallolarenes are better antioxidant agents than BHT. The nHep-CR compound was found as the best antioxidant agent among the other compounds due to weaker intramolecular and intermolecular hydrogen bondings and a longer alkyl chain.

Keywords: Antioxidant, Alkyl Chain, Calix[4]resorcinarene, Calix[4]pyrogallolarene, *In Vitro*

INTRODUCTION

Cancer and cardiovascular are the deadliest diseases in the world. In Indonesia, the prevalence percentage of cancer disease increased from 1.40 to 1.79% from 2013 to 2018. On the other hand, the prevalence percentage of cardiovascular disease was 1.50% in 2018 (Kementerian Kesehatan RI, 2018). It means that up to 18 and 15 people within the 1,000 population suffer from cancer and cardiovascular diseases, respectively. These numbers keep increasing every year due to a serious environmental pollution level and a lack of healthy lifestyle and sports activity (Martiningsih and Haris, 2019; Dewi *et al.*, 2020). It is reported that the leading cause of cancer and cardiovascular diseases is the presence of free radicals (Neha *et al.*, 2019). These free radicals are very reactive chemical species due to the presence of an

unpaired electron in their chemical structures. The free radicals damage the biomolecules, such as DNA and proteins to increase their stability in a chain reaction process. Consequently, these biomolecules become damaged and lose their biological activity. Moreover, the disruption of these biomolecules may convert them to toxic chemicals which enter the bloodstream system, thereby interfering with the cell metabolism, activating the oncogenes, and generating atherosclerosis (Ahangarpour *et al.*, 2019). These harmful mechanisms accumulate over time and lead to cancer and cardiovascular diseases in the human body. Because of that, the presence of free radicals must be minimized to suppress the potential for both cancer and cardiovascular diseases.

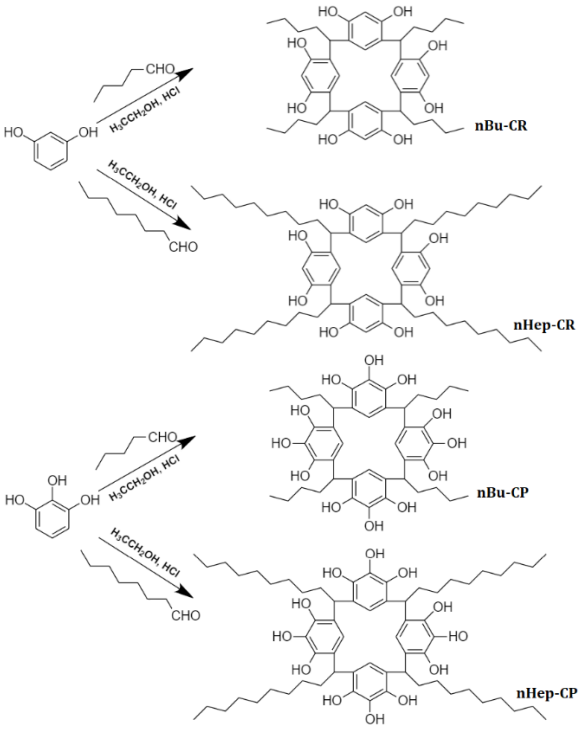
An antioxidant agent is a food supplement for deactivating the reactivity of free radicals in the human body (Shahidi and Ambigaipalan, 2015). From the chemistry point of view, active antioxidant agents usually contain a phenolic group that can donate its hydrogen atom due to its high resonance stability. A highly stable antioxidant agent is pivotal in silencing the chain reaction of free radicals, thus preventing the possibility of cancer and cardiovascular diseases (Purnomo and Yuliati, 2020). Because of that, research on the development of antioxidant agents either from natural sources or organic synthesis processes has been extensively developed (Pringgenies and Idris, 2019; Masduqi *et al.*, 2020). The level of antioxidant activity is reflected in the half-maximum inhibitory activity (IC_{50}) value. When the IC_{50} value is lower than 50 μg mL^{-1}, the antioxidant activity is categorized to be very strong. Strong, moderate, and weak antioxidant activity are determined when the IC_{50} value is 50-100 μg mL^{-1}, 101-150 μg mL^{-1}, and more than 150 μg mL^{-1}, respectively (Jumina *et al.*, 2019). The antioxidant agents derived from natural sources usually exhibit weak antioxidant activity (Lukitaningsih *et al.*, 2014; Fidrianny *et al.*, 2012; Asirvatham and Akhil, 2017; Chandra *et al.*, 2020). For example, the extract of *Datura stramonium* L. in methanol has the IC_{50} value of 435 μg mL^{-1}, which was categorized as a weak antioxidant agent (Christhudas *et al.*, 2013). Another example is the *Fusarium tricinctum* extract with IC_{50} value of 482 μg mL^{-1}, which was also categorized as a weak antioxidant agent (Vasundhara *et al.*, 2016). Because of that, the researchers' attention is gradually shifted to synthetic antioxidant agents. Synthetic antioxidant agents are mainly designed and prepared based on aromatic compounds (Malik *et al.*, 2017). Chalcone derivative with 3,4-methylenedioxy functional group gave the IC_{50} value of 159 μg mL^{-1} (Venkatachalam *et al.*, 2012). Even though the IC_{50} value of 3,4-methylenedioxychalcone was lower than both *Datura stramonium* L. (IC_{50} = 435 μg mL^{-1}) and *Fusarium tricinctum* (IC_{50} = 482 μg mL^{-1}) extracts, the antioxidant activity of 3,4-methylenedioxychalcone was still categorized as a weak antioxidant agent. Metal-organic complexes such as cadmium-macrocyclic Schiff base compound also exhibited low antioxidant activity (IC_{50} = 140 μg mL^{-1}) (Keypour *et al.*, 2021).

The low antioxidant activity of the aromatic compounds is mostly caused by the absence of -OH (hydroxyl) groups. It was known that the hydroxyl group is reactive to donate its proton to the free radicals (Ahangarpour *et al.*, 2019). In our previous study, calix[4]resorcinarenes and calix[4]pyrogallolarenes were prepared from a cyclo-condensation reaction from resorcinol (1,3-dihydroxybenzene) and pyrogallol (1,2,3-trihydroxybenzene), respectively (Jumina *et al.*, 2019, 2020a, 2020b, 2021). These synthetic compounds exhibit strong antioxidant activity. For example, *C*-4-hydroxyphenylcalix[4]resorcinarene and *C*-4-hydroxyphenylcalix[4]pyrogallolarene exhibit high antioxidant activity with IC_{50} values of 22.7 and 88.2 μg mL^{-1}, respectively (Jumina *et al.*, 2019). The antioxidant activity of calix-[4]resorcinarenes and calix[4]pyrogallolarenes depend on the type and location of the attached functional group. The antioxidant activity of *C*-4-hydroxyphenylcalix[4]resorcinarene (IC_{50} = 22.7 μg mL^{-1}) was stronger than *C*-2-hydroxyphenylcalix[4]resorcinarene (IC_{50} = 77.4 μg mL^{-1}) and also stronger than *C*-4-methoxyphenylcalix[4]resorcinarene (IC_{50} = 79.0 μg mL^{-1}) (Handayani *et al.*, 2016; Ngurah, 2018; Jumina *et al.*, 2019).

It was reported that the IC_{50} value of C-methylcalix[4]-4-sulfonicresorcinarene acid reached 3.69 μg mL^{-1} which is categorized as a very strong antioxidant. In contrast, the IC_{50} value of C-phenylcalix[4]-4-sulfonicresorcinarene acid reached 248 μg mL^{-1} which is categorized as a weak antioxidant. From these data, C-alkylcalix[4]resorcinarene had a much higher antioxidant activity than the C-arylcalix[4]resorcinarene. However, other studies reporting on the antioxidant activity of C-alkylcalix[4]resorcinarene are rarely found to the best of the authors' knowledge.

Therefore, in the present work, we synthesized two C-alkylcalix[4]resorcinarenes and

two C-alkylcalix[4]pyrogallolarenes with n-butyl and n-heptyl substituents. The C-n-butylcalix[4]resorcinarenes (nBu-CR) and C-n-heptylcalix[4]resorcinarenes (nHep-CR) were prepared from a cyclo-condensation reaction between resorcinol and n-pentanaldehyde and n-octanaldehyde, respectively. Meanwhile, the C-n-butylcalix[4]pyrogallolarenes (nBu-CP) and C-n-heptylcalix[4]pyrogallolarenes (nHep-CP) were prepared from a cyclo-condensation reaction between pyrogallol and n-pentanaldehyde and n-octanaldehyde, respectively. The reaction scheme (Figure 1) and these synthetic compounds were characterized using Fourier transform infrared (FTIR), liquid chromatography-mass spectrometry (LC-MS), and proton nuclear magnetic resonance (^{1}H-NMR) analysis. Afterward, the antioxidant activity of these synthesized compounds was evaluated through *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.



Scheme 1. Reaction scheme on synthesis -\]].

MATERIAL AND METHODS

Resorcinol ($C_6H_6O_2$, 99%), pyrogallol ($C_6H_6O_3$, 99.5%), n-pentanaldehyde ($C_5H_{10}O$, 98%), n-octanaldehyde ($C_8H_{16}O$, 98%), ethanol (C_2H_5OH, >99.9%), concentrated hydrochloric acid (HCl, 37%), methanol (CH_3OH, 99.9%), dimethyl sulfoxide (C_2H_6OS, 99.9%), 2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$, 90%) were purchased from Merck (Darmstadt, Germany) and used without any further purification. The melting point of the product was measured using an Electrothermal 9100 apparatus. The LC-MS spectrum of the product was obtained from Waters Acquity HPLC-SQD MassLynx v4-1 SCN 805 (Milford, USA) with ESI+ ionization mode and C18 column as the stationary phase operated at 301 K. The mixture of acetonitrile and ethyl acetate in 1:1 volume ratio was used as a mobile phase at a flow rate of 0.2 mL min^{-1}. Meanwhile, the FTIR and ^{1}H-NMR spectra of the product were recorded using a Shimadzu Prestige 21 spectrophotometer (Tokyo, Japan) and a JEOL JNM-ECZ500 R/S1 500 MHz (Tokyo, Japan), respectively. The Jenway 6505 UV-Vis spectrophotometer was used for the evaluation of the antioxidant activity assay through a DPPH method at 517 nm.

Synthesis of C-n-butylcalix[4]resorcinarene

At first, as much as 0.55 g (5 mmol) resorcinol and 0.15 mL concentrated HCl were dissolved in 10 mL of ethanol at 288 K. Then 0.43 g pentanaldehyde (5 mmol, 1 equivalent) was added dropwise into the mixture and the mixture was refluxed at 351 K for 24 h. After that, the mixture was cooled to reach room temperature (303 K) and poured with 10 mL of distilled water. The solid residue was filtered and washed with ethanol:distilled water (1:1 volume ratio) until the pH reached neutral. The desired product was dried to obtain a yellow solid (0.86 g) in 96.4% yield. The melting point of the titled compound was measured, and the product was characterized using FTIR, LC-MS, and ^{1}H-NMR analysis.

Synthesis of C-n-heptylcalix[4]resorcinarene

A mixture of 5.50 g (50 mmol) resorcinol and 1.5 mL concentrated HCl were dissolved in 25 mL of ethanol at 288 K. Afterward, 7.90 g (50 mmol) octanaldehyde was added dropwise into the mixture and the mixture was refluxed at 351 K. After 24 h, the mixture was cooled to reach room temperature (303 K). As much as 10 mL of distilled water was added into the mixture and the formed precipitation was filtered. The solid residue was washed with ethanol:distilled water (1:1 volume ratio) until the pH reached neutral. The desired product was dried to obtain a pale-yellow solid (11.29 g) in 96.4% yield. The melting point of the product was measured, and the product was further characterized using FTIR, LC-MS, and ^{1}H-NMR analysis.

Synthesis of C-n-butylcalix[4]pyrogallolarene

As much as 0.63 g (5 mmol) pyrogallol and 0.15 mL concentrated HCl were dissolved in 10 mL of ethanol at 288 K. Then 0.43 g pentanaldehyde (5 mmol, 1 equivalent) was added dropwise into the mixture and the mixture was refluxed at 351 K for 24 h. After the reaction, the mixture was cooled to reach room temperature (303 K) and poured with 10 mL of distilled water. The solid residue was filtered and washed with ethanol:distilled water (1:1 volume ratio) until the pH reached neutral. The desired product was dried to obtain a pale red solid (0.90 g) in 92.4% yield. The melting point of the titled compound was measured, and the product was characterized using FTIR, LC-MS, and ^{1}H-NMR analysis.

Synthesis of C-n-heptylcalix[4]pyrogallolarene

As much as 3.20 g (25 mmol) pyrogallol and 0.75 mL concentrated HCl were added into 10 mL of ethanol at 288 K. Afterward, 3.21 (25 mmol) octanaldehyde was added dropwise into the mixture and the mixture was refluxed at 351 K for 24 h. Then, the mixture was cooled at room temperature with the addition of 10 mL of distilled water. The mixture was then cooled again at 283 K to completely precipitate the desired compound. The solid residue was filtered and rinsed with ethanol:distilled water (1:1 volume ratio) until the pH reached neutral. The desired product was dried to obtain a pale-red solid (6.03 g) in 96.3% yield. The melting point of the product was measured, and the product was further characterized using FTIR, LC-MS, and ^{1}H-NMR analysis.

Antioxidant activity assay

At first, the 100 µg mL^{-1} DPPH solution in methanol was prepared by dissolving 5 mg DPPH in 50 mL methanol. The stock solutions of C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene were separately prepared by dissolving 10 mg of each compound in 100 mL dimethyl sulfoxide at room temperature to obtain 100 µg mL^{-1} concentration. The working solution in other concentrations (5.0, 10, 15, 20, 25 and 75 µg mL^{-1}) of the synthesized compound was prepared by diluting the stock solution. The antioxidant activity assay was conducted in a dark condition at room temperature (303 K). As much as 1.0 mL of 100 µg mL^{-1} DPPH solution and 0.2 mL of 5.0 µg mL^{-1} working solution of the synthesized compound were mixed and diluted with methanol to reach the final volume of 4.0 mL. The mixture was shaken at 150 rpm for 30 min. Afterward, the absorbance of the mixture was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer. The same procedure was performed for the other concentrations (10, 15, 20, 25 and 75 µg mL^{-1}) of each synthesized compound. The butylated hydroxytoluene (BHT) was used as the positive control in this study. The percentage of radical scavenging activity was calculated using the following equation (1):

$$\%\text{Radical scavenging activity} = \frac{100\%(A_0\text{-}A)}{A_0} \quad \text{.....(1)}$$

where A_0 and A were the absorbances of negative control and sample at 517 nm, respectively. The IC_{50} value was obtained from the standard curve of %Radical scavenging activity vs sample concentration. Meanwhile, the antioxidant activity index (AAI) was calculated using the following equation (2):

$$AAI = \frac{[DPPH]}{IC_{50}} \quad \text{..... (2)}$$

where [DPPH] was the concentration of the DPPH solution (100 µg mL^{-1}) (Purnomo and Yuliati, 2020).

RESULTS AND DISCUSSION

Synthesis of C-alkylcalix[4]resorcinarenes

The C-alkylcalix[4]resorcinarenes were prepared from a cyclo-condensation reaction under an acidic condition. The reaction mechanism to produce the C-alkylcalix[4]resorcinarenes could be predicted from the previous report (Ngurah, 2018). At first, the carbonyl group of the aldehyde compound was protonated to form a carbonyl-oxonium ion with a positive charge at the carbon atom as the intermediate. This intermediate is an electrophile; hence, the electrophilic substitution reaction of resorcinol generates water as the by-product. A similar mechanism happened to reach the cyclization reaction as the final stage. Because of that, this reaction is called a cyclo**-**condensation reaction (Priyangga *et al*., 2020).

The nBu-CR and nHep-CR compounds were obtained as yellowish solid in high yield (96.4%). The melting points of nBu-CR and nHep-CR compounds were 523 and 503 K, demonstrating their high thermal stability (Table I). The high thermal stability was caused by strong intramolecular and intermolecular hydrogen bondings of C-alkylcalix[4]resorcinarenes (Handayani *et al.*, 2016). The chemical structure of nBu-CR and nHep-CR compounds is shown in Scheme 1.

Table I. Physicochemical properties of C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives.

No	Compound	Appearance	Melting point (K)	Yield (%)
1	nBu-CR	Yellow solid	523 K	96.4%
2	nBu-CP	Pale red solid	> 553 K	92.4%
3	nHep-CR	Pale yellow solid	503 K	96.4%
4	nHep-CP	Pale red solid	> 553 K	96.3%

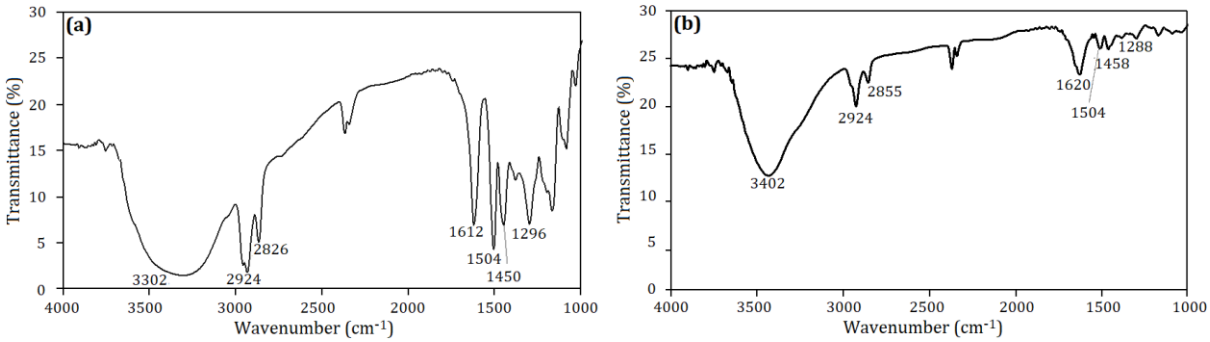


Figure 1. FTIR spectrum of (a) C-N-Butylcalix[4]Resorcinarene, and (b) C-N-Heptylcalix[4]Resorcinarene.

The O-H functional group is observed as a broad signal at 3302 and 3402 cm^{-1} for nBu-CR and nHep-CR compounds, respectively (Figure 1). The C-H methine and alkyl group stretching and bending signals are found at 2862-2924 and 1450 cm^{-1} for nBu-CR while the C-H methine stretching and bending signals of nHep-CR showed up at 2855-2924 and 1458 cm^{-1}. The C=C aromatic signals of nBu-CR and nHep-CR appeared at 1504-1612 and 1504-1620 cm^{-1}, respectively. On the other hand, the C-O phenolic signal showed up at 1296 and 1288 cm^{-1} for nBu-CR and nHep-CR compounds, respectively. These spectral patterns were similar to the other reported calix[4]resorcinarenes (Jumina *et al.*, 2021).

The LC chromatograms of nBu-CR and nHep-CR compounds (Figure 2 (a) and (c)). Generally, calix[4]resorcinarenes exist in several conformations, such as crown-C4, boat-C2v, chair-C2h, diamond-Cs, and saddle-S4 (Gubaidullin *et al.*, 2002); hence, several peaks were found on the chromatogram of C-alkylcalix[4]resorcinarenes. The MS spectrum of nBu-CR at 7.84 min as the retention time (Figure 2b). The molecular ion ($C_{44}H_{55}O_8^+$) was detected at m/z = 711.26 while its dimer ($C_{88}H_{110}O_{16}$+Na^+) was detected at m/z = 1445.39. When the dimer lost the butene (C_4H_8) from the alkyl chain, it forms a molecular fragment ($C_{84}H_{102}O_{16}^+$) at m/z = 1366. Meanwhile, the fragment $C_{84}H_{102}O_{16}^+$ lost one water molecule (H_2O) and one more butane (C_4H_{10}) to form a molecular fragment $C_{80}H_{90}O_{15}^+$ at m/z = 1290. On the other hand, Figure 2 (d) shows the MS spectrum of nHep-CR at 14.35 min as the retention time. The molecular ion ($C_{56}H_{79}O_8^+$) was detected at m/z = 878.98 while its dimer with three heptyl chains lost ($C_{21}H_{45}$) was detected at m/z = 1463 ($C_{91}H_{115}O_{16}^+$). When the molecular ion forms a complex with seven acetonitrile molecules, it forms a molecular fragment at m/z = 1166.65 ($C_{56}H_{79}O_8$+$7CH_3CN^+$).

The ^{1}H-NMR spectra of nBu-CR and nHep-CR compounds (Figure 3 (a) and (b). The successful formations of both C-alkylcalix[4]resorcinarenes are indicated by the absence of a C-H aldehyde signal at around 9.00 ppm from either pentanaldehyde or octanaldehyde. The O-H of nBu-CR appeared as a singlet signal at 8.53 ppm while the aromatic protons of resorcinol showed up as multiplet signal at 6.23-6.41 ppm. The C-H methine is observed as a singlet signal at 3.91 ppm, while the -CH_2- and -CH_3 protons of nBu-CR are found as multiplet signals at 1.20-1.32 ppm. The signal assignment on the chemical structure of nBu-CR is shown in Figure 3 (a). The O-H of nHep-CR showed up as a singlet signal at 8.85 ppm while the aromatic protons of resorcinol appeared as multiplet signals at 6.27-7.08 ppm. The C-H methine is found as a singlet signal at 4.05 ppm.

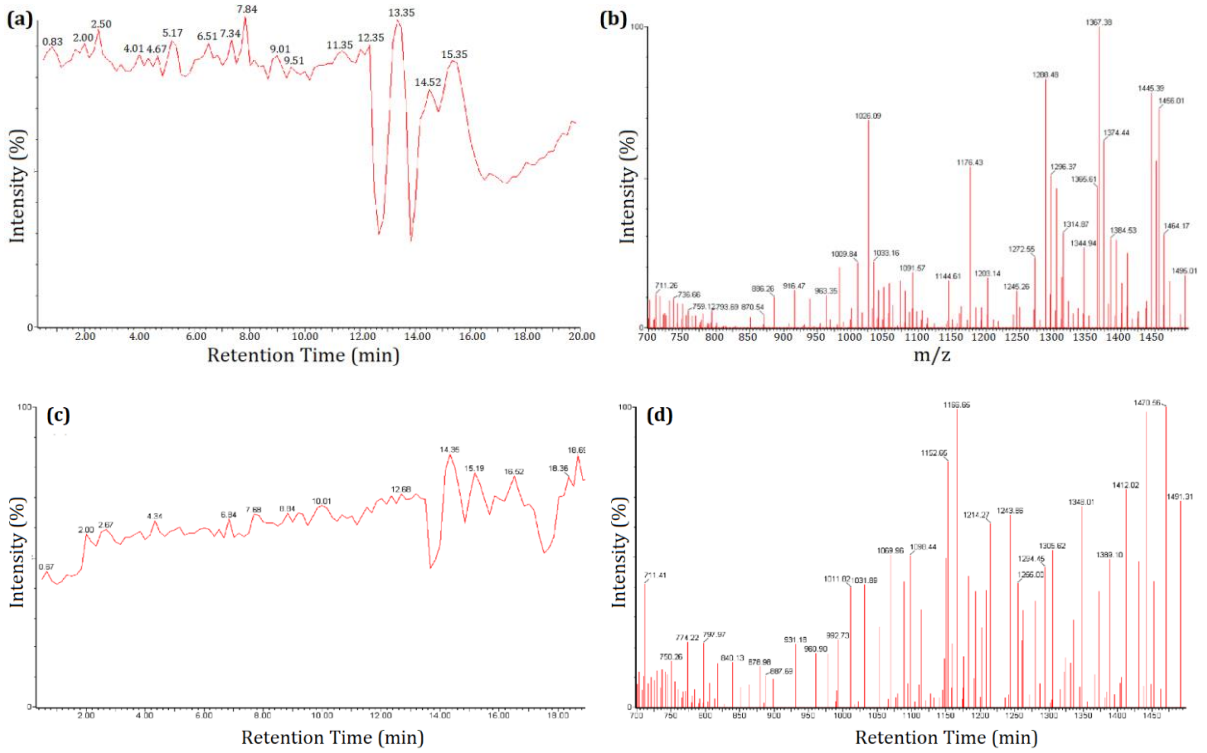


Figure 2. LC chromatogram of (a) C-n-butylcalix[4]resorcinarene, and (c) C-n-heptylcalix[4]resorcinarene, and MS spectrum of (b) C-n-butylcalix[4]resorcinarene, and (d) C-n-heptylcalix[4]resorcinarene.

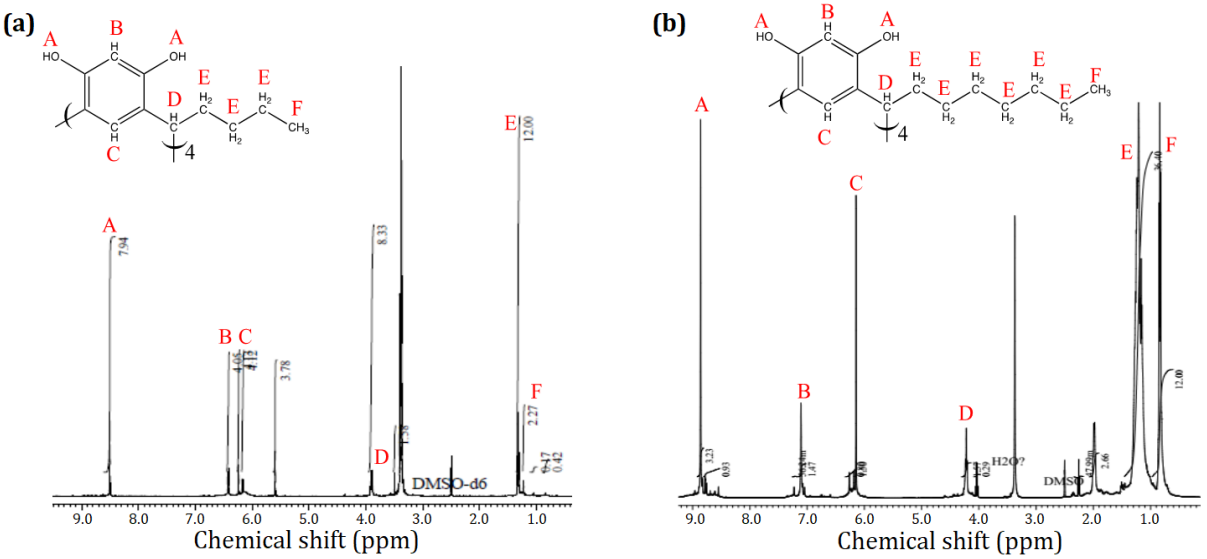


Figure 3. ^{1}H-NMR spectrum of (a) C-n-butylcalix[4]resorcinarene and (b) C-n-heptylcalix[4]resorcinarene.

Meanwhile, the -CH_2- and -CH_3 protons of nHep-CR are observed as multiplet signal at 0.85-1.23 ppm. The signal assignment on the chemical structure of nHep-CR (Figure 3 (b)). From the FTIR, LC-MS, and ^{1}H-NMR analyses, it can be concluded that both nBu-CR and nHep-CR derivatives have been successfully synthesized.

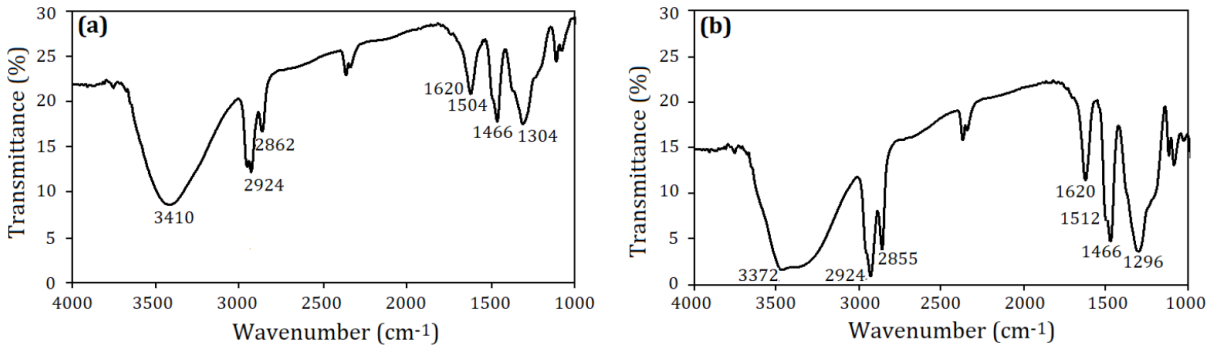


Figure 4. FTIR spectrum of (a) C-n-butylcalix[4]pyrogallolarene and (b) C-n-heptylcalix[4]pyrogallolarene.

Synthesis of C-alkylcalix[4]pyrogallolarenes

The C-alkylcalix[4]pyrogallolarenes were synthesized from a cyclo-condensation reaction between pyrogallol and aldehyde. Initially, the carbonyl group of the aldehyde compound was protonated to form carbonyl-oxonium ion in the acidic condition. The carbonyl-oxonium intermediate is connected to the pyrogallol through an electrophilic substitution reaction. Then further stepwise mechanism happened to produce the C-alkylcalix[4]pyrogallolarenes.

The nBu-CP and nHep-CP compounds were obtained as pale red solid in 92.4 and 96.3% yield, respectively. The melting points of both compounds were higher than 553 K (Table II), demonstrating their high thermal stability due to strong intramolecular and intermolecular hydrogen bondings of C-alkylcalix[4]pyrogallolarenes (Priyangga *et al.*, 2020). The chemical structure of nBu-CP and nHep-CP compounds is shown in Scheme 1.

The O-H functional group is found as a broad signal at 3410 and 3372 cm^{-1} for nBu-CP and nHep-CP, respectively (Figure 4). The C-H methine and alkyl group stretching and bending signals appeared at 2862-2924 and 1466 cm^{-1} for nBu-CP while the C-H methine stretching and bending signals of nHep-CP were found at 2855-2974 and 1466 cm^{-1}. The C=C aromatic signals of nBu-CP and nHep-CP are observed at 1504-1620 and 1512-1620 cm^{-1}, respectively. On the other side, the C-O phenolic signal showed up at 1304 and 1296 cm^{-1} for nBu-CP and nHep-CP compounds, respectively (Figure 4). These spectral patterns were similar to the other calix[4]pyrogallolarenes (Jumina *et al.*, 2020a).

The LC chromatograms of nBu-CP and nHep-CP compounds (Figure 5a,c) it was reported that calix[4]pyrogallolarenes may exist in several conformations (Gubaidullin *et al.*, 2002). Therefore, several peaks are reasonable to be found on the chromatogram of C-alkylcalix[4]pyrogallolarenes. MS spectrum of nBu-CP at 17.52 min as the retention time (Figure 5b). The molecular ion ($C_{44}H_{56}O_{12}^{+}$) is detected at m/z = 776.29 while its complexes, i.e. ($C_{44}H_{56}O_{12}$+$8H_2O^{+}$) and ($C_{44}H_{56}O_{12}$+$10CH_3CN^{+}$) are detected at m/z = 920.20 and 1186.87, respectively. On the other hand (Figure 5d) the MS spectrum of nHep-CR at 4.01 min as the retention time. The molecular ion ($C_{56}H_{80}O_{12}^{+}$) is detected at m/z = 944.39 while its complexes, i.e. ($C_{56}H_{80}O_{12}$+CH_3CN+Na+$2H^{+}$) and ($C_{56}H_{80}O_{12}$+CH_3CN+$5H_2O$+Na+H^{+}) are detected at m/z = 1010.69 and 1099.76, respectively.

The successful formation of both C-alkylcalix[4]pyrogallolarenes is indicated by the absence of the C-H aldehyde signal of either pentanaldehyde or octanaldehyde. The O-H of nBu-CP appeared as two separated singlet signals at 8.10 and 8.64 ppm. Meanwhile, the aromatic proton of pyrogallol showed up as a singlet signal at 6.87 ppm. The C-H methine is found as a singlet signal at 4.14 ppm. The -CH_2- and -CH_3 protons of nBu-CP are observed as multiplet signal at 0.85-1.33 ppm (Figure 6 a). On the other hand, the O-H of nHep-CP showed up as two separated singlet signals at 8.52 and 9.02 ppm. Meanwhile, the aromatic proton of pyrogallol appeared as a singlet signal at 7.21 ppm. The C-H methine is observed as a singlet signal at 4.56 ppm. The -CH_2- and -CH_3 protons of nHep-CP are detected as multiplet signal at 1.26-1.66 ppm (Figure 6b). From the FTIR, LC-MS, and ^{1}H-NMR analyses, it can be concluded that both nBu-CP and nHep-CP derivatives have been successfully synthesized.

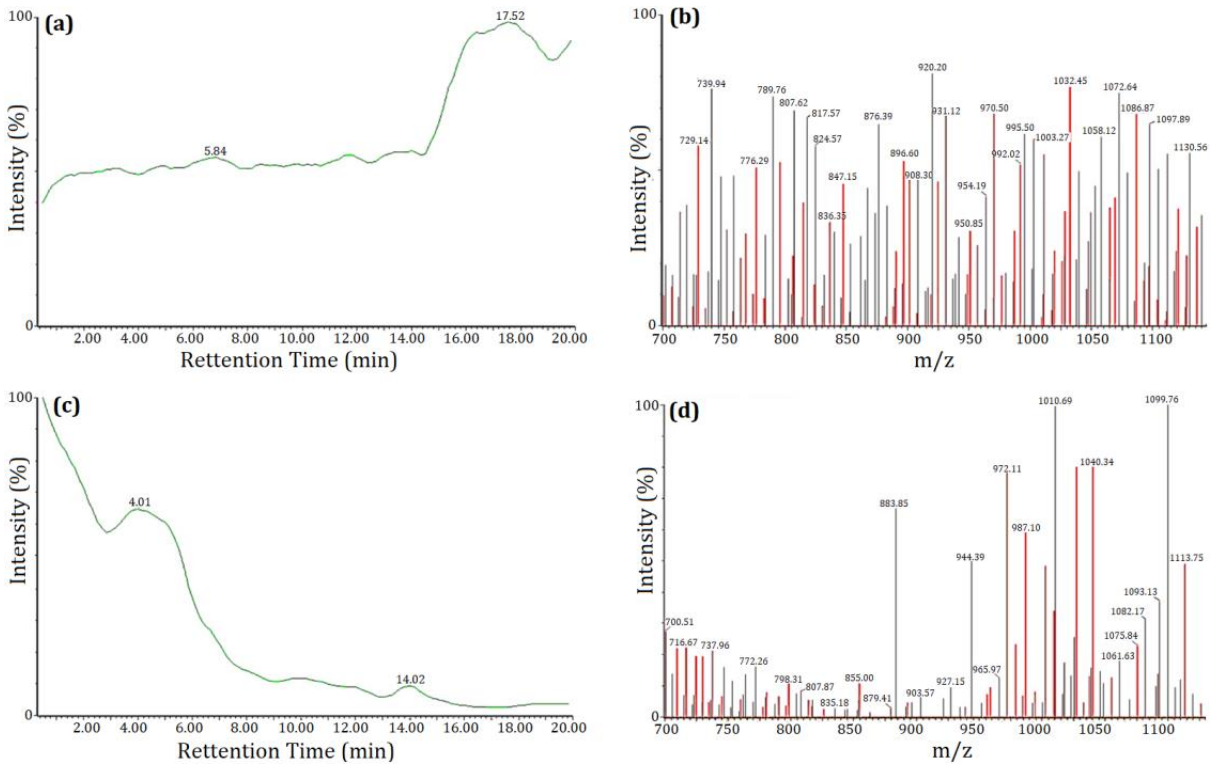


Figure 5. LC chromatogram of (a) C-n-butylcalix[4]pyrogallolarene and (c) C-n-heptylcalix[4] pyrogallolarene, and MS spectrum of (b) C-n-butylcalix[4]pyrogallolarene and (d) C-n-heptylcalix[4] pyrogallolarene.

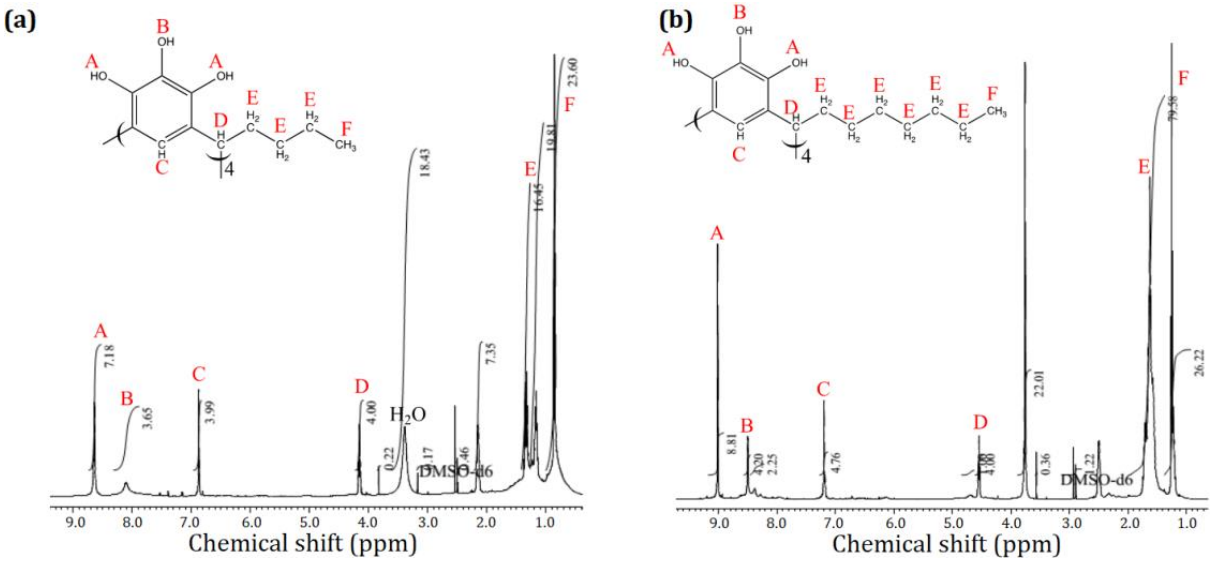


Figure 6. 1H-NMR spectrum of (a) C-n-butylcalix[4]pyrogallolarene and (b) C-n-heptylcalix[4] pyrogallolarene.

Evaluation of the antioxidant activity of C-alkylcalix[4]resorcinarenes and C-alkylcalix[4]pyrogallolarenes through *in vitro* DPPH assay

The antioxidant activities of C-alkylcalix[4]resorcinarene and C-alkylcalix[4] pyrogallolarene derivatives were evaluated using a DPPH *in vitro* assay as a standard method for assessing the antioxidant activity. The commercially available butylated hydroxytoluene (BHT) was selected as the positive control due to its similar aromatic structure with a hydroxyl group. The %Radical scavenging activity was calculated from the absorbance of the solution with and without the presence of the antioxidant agent (see equation (1)). The %Radical scavenging activities of C-alkylcalix[4]resorcinarenes, C-alkylcalix[4] pyrogallolarenes and BHT (Figure 7). All synthesized compounds give higher %radical scavenging activity than BHT. The IC_{50} value of C-alkylcalix[4]resorcinarenes, C-alkylcalix[4] pyrogallolarenes and BHT (Table II). Since the IC_{50} value of C-alkylcalix[4]resorcinarenes and C-alkylcalix[4]pyrogallolarenes (11.5-25.1 μg mL^{-1}) is lower than 50 μg mL^{-1}, they are categorized as very strong antioxidant agents. Furthermore, it should be noted that all synthesized compounds give a lower IC_{50} value than BHT (49.9 μg mL^{-1}), which is remarkable.

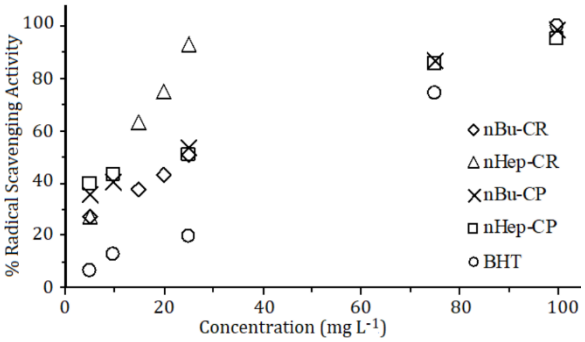


Figure 7. Percentages of radical scavenging activity of C-alkylcalix[4]resorcinarees and Calkylcalix[4] pyrogallolarenes from the DPPH assay.

The results reveal that the alkyl chain influenced the IC_{50} value of C-alkylcalix[4] resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives. The IC_{50} value of nHep-CR (11.5 μg mL^{-1}) is two times lower than the IC_{50} value of nBu-CR (25.1 μg mL^{-1}). Meanwhile, the IC_{50} value of nHep-CP (21.9 μg mL^{-1}) is just slightly lower than the IC_{50} value of nBu-CP (22.9 μg mL^{-1}). A longer alkyl chain gave a lower IC_{50} value for both C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives. This phenomenon may be caused by the longer alkyl chain (heptyl group) preventing the intermolecular hydrogen bondings. Consequently, each hydroxyl group of C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives openly serves as a proton donor to the DPPH radicals.

Table II. Antioxidant activity of C-alkylcalix[4] resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives

No	Compound	IC_{50} (μg mL^{-1})
1	nBu-CR	25.1
2	nBu-CP	22.9
3	nHep-CR	11.5
4	nHep-CP	21.9
5	BHT	49.9

The nBu-CR and nBu-CP gave similar IC_{50} values due to the presence of intermolecular hydrogen bondings, thus limiting the ability of hydroxyl groups to donate the protons for DPPH radicals. Meanwhile, the IC_{50} value of nHep-CR is two times lower than that of nHep-CP. The presence of three hydroxyl groups in the C-alkylcalix[4]pyrogallolarenes yields stronger intra-molecular hydrogen bondings, thus limiting their ability to donate the protons. Such strong intra-molecular hydrogen bondings are not available in the structure of C-alkylcalix[4] resorcinarenes. Therefore, nHep-CR exhibits the lowest IC_{50} value (or the highest antioxidant activity).

The AAI value of nHep-CR with the other reported antioxidant agents (Table III). The reason for using AAI value rather than IC_{50} value is because AAI values demonstrate the antioxidant activity with no dependence on the DPPH concentration (Purnomo and Yuliati, 2020). A higher AAI value reflects a higher antioxidant activity, it shows that nHep-CR compound exhibit a higher AAI value than BHT (Table III row 2), natural extracts (Table III row 3-6), inorganic-organic hybrid (Table III row 7), synthetic chalcones (Table III row 8-12), and other C-arylcalix[4]resorcinarenes and C-arylcalix[4]pyrogallolarenes (Table III row 13-19), which is quite remarkable. It is proved that the alkyl group could enhance the antioxidant activity of the calix structure. However, the AAI value of nHep-CR is still lower than C-methylcalix[4]-4-sulfonic resorcinarene acid (Table III row 20), thus further experimental and computational studies are still required.

Table III. Comparison of the IC_{50} and AAI values of nHep-CR with other antioxidant agents

No	Compound	AAI	References
1	nHep-CR	8.69	This work
2	BHT	2.00	This work
3	*Crotalaria pallida*	0.04	Govindappa *et al.*, 2011
4	*Curcuma longae*	0.14	Zhong *et al*., 2011
5	*Fusarium tricinctum*	0.08	Vasundhara *et al*., 2016
6	*Datura stramonium* L.	0.34	Christhudas *et al*., 2013
7	cadmium-macrocyclic Schiff base	0.84	Keypour *et al*., 2021
8	4-(2-pyridyl)chalcone	0.22	Venkatachalam *et al.*, 2012
9	3,4-(methylenedioxy)chalcone	0.62	Venkatachalam *et al.*, 2012
10	4-(methylthio)chalcone	0.70	Venkatachalam *et al.*, 2012
11	4-benzyloxychalcone	0.81	Venkatachalam *et al.*, 2012
12	4-[(2-cyanoethyl)methylaminochalcone	1.63	Venkatachalam *et al.*, 2012
13	*C*-4-hydroxyphenylcalix[4]pyrogallolarene	1.13	Jumina *et al*., 2019
14	*C*-4-hydroxy-3-methoxyphenylcalix[4]pyrogallolarene	1.26	Jumina *et al*., 2019
15	*C*-4-methoxyphenylcalix[4]resorcinarene	1.27	Ngurah, 2018
16	*C*-2-hydroxyphenylcalix[4]resorcinarene	0.56	Handayani *et al*., 2016
17	*C*-4-hydroxyphenylcalix[4]resorcinarene	4.41	Jumina *et al*., 2019
18	*C*-4-hydroxy-3-methoxyphenylcalix[4]resorcinarene	5.80	Jumina *et al*., 2019
19	C-phenylcalix[4]-4-sulfonicresorcinarene acid	0.23	Handayani *et al*., 2020
20	C-methylcalix[4]-4-sulfonicresorcinarene acid	70.3	Valand *et al*., 2015

CONCLUSIONS

The C-alkylcalix[4]resorcinarenes and C-alkylcalix[4]pyrogallolarenes were successfully prepared from a cyclo-condensation reaction in an acidic condition to give the desired product in 92.4-96.4% yield. From the FTIR, LC-MS, and ^{1}H-NMR analyses, it can be concluded that the chemical structures of all C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives have been correctly elucidated. Through DPPH *in vitro* assay, all synthesized compounds gave a lower IC_{50} value than BHT as the positive control. A longer alkyl chain yields a higher antioxidant activity of C-alkylcalix[4]resorcinarene and C-alkylcalix[4]pyrogallolarene derivatives. On the other hand, more hydroxyl groups on C-alkylcalix[4]pyrogallolarene structure lead to stronger intramolecular hydrogen bondings, thus C-alkylcalix[4]pyrogallolarenes show a weaker antioxidant activity than the C-alkylcalix[4]resorcinarenes.

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