ISSN: 1410-8917
e-ISSN: 2597-9914

Jurnal Kimia Sains dan Aplikasi 25 (3) (2022): 123–129

Jurnal Kimia Sains dan Aplikasi
Journal of Scientific and Applied Chemistry

Journal homepage: http://ejournal.undip.ac.id/index.php/ksa

Potential of *C*-Phenylcalix[4]Resorcinarene Epoxide Compound as Drug Delivery Agent in Breast Cancer Cells MCF-7

Dwi Rahmasari Fatmawati [a,*], Danny Nur Wahyu Hidayat [a], Diah Kartika Sari [a], Rizky Riyami Putri [a], Jumina [a], Yehezkiel Steven Kurniawan [a]

[a] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Yogyakarta, Indonesia

*Corresponding author: dwirahmasari@mail.ugm.ac.id

https://doi.org/10.14710/jksa.25.3.123-129

Article Info

Article history:

Received: 22nd October 2021
Revised: 20th February 2022
Accepted: 24th February 2022
Online: 31st March 2022

Keywords:
C-phenylcalyx[4]resorcinarene epoxide; drug delivery; breast cancer

Abstract

Cancer therapy through conventional chemotherapy has been widely applied; however, one of the main disadvantages of chemotherapy is the non-selective targeting of cancer cells which causes various adverse side effects. The development of drug delivery agents that are more selective and effective in cancer therapy needs to be performed so that the drugs have a therapeutic effect and minimize side effects. In this study, the compound *C*-phenylcalyx[4]resorcinarene epoxide (CFKRE) has acted as a drug delivery agent because it can form host complex interactions with ligands. The CFKRE compound was synthesized through two reaction steps: the condensation and alkylation reactions of the epoxide. The structure was analyzed using FTIR, ^{1}H-, and ^{13}C-NMR spectrophotometers and then tested for in vitro cytotoxicity using the MTT assay. The results showed that 70% yield of CFKRE was obtained. Molecular docking analysis of CFKRE compounds against PDGFR and EGFR proteins showed high binding energy compared to conventional chemotherapeutic agents. Molecular dynamic studies showed that CFKRE compounds could form a host-ligand complex with a -350.4 kcal/mol binding energy. Cytotoxic assay of CFKRE compound against MCF-7 breast cancer cells and Vero cells gave IC_{50} values of 4.04 and 29.59 µg/mL, respectively. These results indicated that CFKRE compounds are not toxic and have the potential to be utilized as new candidates for drug delivery agents.

1. Introduction

Cancer is a disease with a high incidence rate globally and is still a serious health problem today. Data from the Global Cancer Observatory in 2020 showed that the most common cancer cases in Indonesia were breast cancer, 16.59% of 396.914 cancer cases. Approximately 90% of cancer deaths are caused by metastatic mechanisms [1]. One of the properties possessed by cancer cells is the low level of apoptosis, which causes cells to continue to divide and not be controlled actively. Control of apoptosis plays an essential role in cancer treatment strategies [2]. Cancer treatment is tailored to the target cancer cells. Cancer treatments using radioactivity and surgery are applied to cancer cells that do not easily spread to surrounding normal cells, while chemotherapy is used for cancer that spreads quickly [3]. Conventional chemotherapy works by inhibiting mitosis and interfering with the DNA synthesis of cancer cells. Since chemotherapy agents are usually non-selective, they can damage healthy tissue and cause various side effects [4]. Chemotherapy has short-term side effects such as fatigue, alopecia, musculoskeletal pain, neurocognitive dysfunction, and chemical-induced peripheral neuropathy.

On the other hand, long-term side effects may include cardiomyopathy, neurocognitive dysfunction, psychosocial effects, secondary cancer, premature menopause, and cardiovascular disease [5]. Several chemotherapy drugs are alkylating agents, topoisomeric inhibitors, antimetabolites, antibiotics, mitotic spindle inhibitors, and tyrosine kinase inhibitors [6]. Anticancer drugs that have been widely applied in the medical world

include cisplatin, fluorouracil, doxorubicin, mercaptopurine, and methotrexate. Doxorubicin is an example of a chemotherapeutic agent belonging to the category of topoisomer II inhibitors [7]. The use of doxorubicin can cause resistance because it can induce over-expression of P-glycoprotein (P-gp), which causes efflux of chemotherapy drugs from the cell.

The development of chemotherapy drugs is an effort to overcome anticancer drug resistance. Protein tyrosine kinase (PTKS) is an intermediate in cell signaling pathways that control cell growth and apoptosis. Several pathological disorders, including unregulated cell proliferation, are caused by altered protein tyrosine kinase function [8]. Tyrosine kinase inhibitors can be considered anti-angiogenesis targets and applied as models in developing new compounds of chemotherapeutic drugs. The PDGFR receptor protein is directly involved in the survival of cancer cells, especially at the proliferative stage. Protein tyrosine kinase inhibitors such as imatinib can block Platelet-Derived Growth Factor (PDGFR) activity [9]. In addition, other protein tyrosine kinases, such as Epidermal Growth Factor Receptor (EGFR), can influence tumor growth, including metastasis, angiogenesis, proliferation, and inhibition in the process of apoptosis [10]. The tyrosine kinase inhibitor erlotinib can inhibit the activity of the EGFR protein. The search for new compounds as protein tyrosine kinase inhibitors can be studied by standard inhibitors binding (imatinib and erlotinib) to protein tyrosine kinases.

The development of drug delivery agents to target cancer cells increases the binding affinity of chemotherapy drugs with drug delivery agents, resulting in the effectiveness of chemotherapy. The drug delivery system controls drug release to target cells [11]. Drug delivery provides a pH response to normal and unhealthy tissues, such as cancer cells which have a slightly acidic pH than normal tissue and blood [12]. The pH-response of drug delivery is indicated by the conjugate drug delivery, which is hydrolyzed at an acidic pH resulting in the release of drug bound to drug delivery [11, 13]. Drug release from conjugated drug delivery by a pH gradient is caused by changes in the hydrophobic and hydrophilic balance; hence, the drug structure's hydrophobicity and drug delivery affect drug release. Not all drugs have an excellent binding affinity for drug delivery. Therefore, to improve the binding affinity of drug-drug delivery, macrocyclic compounds calix[4]arene and cyclodextrin [14, 15] can be used.

Many studies have been carried out on calixresorcinarene as a drug delivery agent. Calix-platin compound, namely the platinum-calixarene complex, has been tested for its anticancer activity and has shown effective anticancer activity, especially against human non-small cell lung cancer cells [16]. Mo *et al.* [17] have synthesized a compound p-phosphonate calix[4]arene vesicle (PCV) supplemented with paclitaxel (PTX) and conjugated with a ligand bound polyethyleneglycol (PEG)-folic acid.

Calix[4]arene and its derivatives are molecules useful in the pharmaceutical field for chiral molecular differentiation, drug delivery, diagnostics, and disease therapy [18]. Research on calix[4]arena as an anticancer has been studied. The anticancer activity of calix[4]arene derivative compounds is due to the geometric shape that can act as a drug delivery agent by forming complexes [19, 20]. The macromolecular compound calix[4]arene and its derivatives have activity as drug delivery agents through the cancer net conformation. Drug delivery agents in the form of a cancer net conformation will more selectively attack cancer cells to be safe for normal cells. The more selective the drug delivery agent, the more the inhibitory potential on cancer cells will increase.

In this research molecular docking approach has been conducted on calix[4]resorcinarene epoxide compounds to study the interaction of calix[4]resorcinarene epoxides with protein tyrosine kinases. Molecular dynamic studies were also performed to determine the interaction of calix[4]resorcinarene epoxide with ligands or drugs. The synthesis of *C*-phenylcalix[4]resorcinarene epoxide was carried out in two reaction steps: the condensation reaction and the alkylation of the epoxide. The synthesized compound *C*-phenylcalix[4]resorcinarene epoxide was analyzed for its structure using FTIR, ^{1}H- and ^{13}C-NMR spectrophotometer, and cytotoxicity was tested using the MTT assay with doxorubicin as a positive control against MCF-7 breast cancer cells and normal cells.

2. Methods

Several methods used in this research were molecular docking, molecular dynamic studies, synthesis of *C*-phenylcalix[4]resorcinarene, and synthesis of *C*- phenylcalix[4]resorcinarene epoxide, and cytotoxic assay.

2.1. Materials and Instrumentations

The tools used were a reflux device, hotplate, magnetic stirrer, digital balance, measuring cup, dropper, spatula, filter paper, and a glass funnel. Some of the molecular docking software were Chimera 1.13.1, AutoDockTools-1.5.6, Gaussian 5.0, and Discovery Studio 2017 R2. The materials used in this study consisted of synthetic materials, such as resorcinol, benzaldehyde, 37% (w/v) hydrochloric acid, 98% ethanol (Sigma-Aldrich, USA), n-hexane, ethyl acetate, epichlorohydrin, sodium hydroxide, methanol, and distilled water. On the other hand, the molecular docking study used PDGFR and EGFR receptor protein data with PDB ID: 1T46 and 1M17, respectively.

2.2. Experiment

2.2.1. Molecular Docking

Molecular docking was conducted by preparing protein tyrosine kinases bound to standard ligands. Optimization of the structure of the test compound was performed using the Gaussian program. The standard ligand is re-docked to the protein that has removed the standard ligand in the molecular docking validation stage. The analysis results will show the value of the docking

compound's Root Mean Square Distances (RMSD), which can be compared with the reference. The docking of the compound *C*-phenylcalix[4]resorcinarene epoxide to the protein, which had its standard ligand removed, was employed using the AutodockTools-1.5.6 program.

2.2.2. Molecular Dynamic Studies

The molecular dynamics study aimed to determine the interaction between the drug (imatinib) and the host (CFKRE) in creating the host-guest complex. The host-guest complex was designed by manually docking the guest through the ring and optimized using Gaussian 09 at the molecular mechanic level. The complex structure was further optimized at the B97D/6-31G (dp) level. The optimization results were followed by molecular dynamic studies with controlled electrostatic potential charge adjustments using the R.E.D server. The force field parameters were determined using ANTECHAMBER and PARMCHK and then solvated using the TIP3P water model periodically with a minimum 30.0 water shell around the complex to ensure that the system would not interact with its periodic image.

2.2.3. Synthesis of *C*-phenylcalix[4]resorcinarene

Resorcinol (1.10 g; 10 mmol) and benzaldehyde (1.06 g; 10 mmol) were transferred to a three-neck flask equipped with reverse cooling and magnetic stirrer and dissolved in 98% ethanol (25 mL). After the two reagents were dissolved, 37% HCl (1 mL) was added dropwise. Then the mixture was stirred and refluxed at 78°C for 8 hours. The mixture was then cooled to room temperature. The solid formed was filtered and washed with ethanol:distilled water (1:1) until it reached a neutral pH. The product was dried in a vacuum desiccator and then tested by TLC and characterization by FTIR, ^{1}H-, and ^{13}C-NMR.

2.2.4. Synthesis of *C*-phenylcalix[4]resorcinarene epoxide

The compound *C*-phenylcalix[4]resorcinarene (1.00 g; 1.26 mmol) was dissolved in ethanol (10 mL) and added with 3 M NaOH (6 mL). The mixture was heated at 60°C. Next, epichlorohydrin (1 mL) was added dropwise for 1 hour and heated at 80°C for 4 hours. The mixture was then cooled to room temperature, filtered, and washed with distilled water. The solids were then dried in a vacuum desiccator and characterized by FTIR, ^{1}H-, and ^{13}C-NMR.

2.2.5. Cytotoxicity assay

The cytotoxicity assay aimed to determine the cancer-net cytotoxic effect of *C*-phenylcalix[4]resorcinarene epoxide on cell viability using the MTT assay. MCF-7 cells (100 µL) with a density of 10,000 cells/mL were distributed into 96 well-plate wells containing DMEM with 10% FBS and incubated for 24 hours. Next, the concentration series of the test sample was added to the 96 well-plate wells. The mixture was incubated for 24 hours in a CO_2 incubator. After incubation, cells were washed with PBS solution and added 0.5 mg/mL (100 µL) MTT solution. After completion of incubation, 10% SDS stopper solution in 0.01 M HCl (100 µL) was added to dissolve the formazan MTT. The amount of MTT formazan formed was determined by measuring the absorbance with an ELISA reader at 595 nm.

2.2.6. Analysis Method

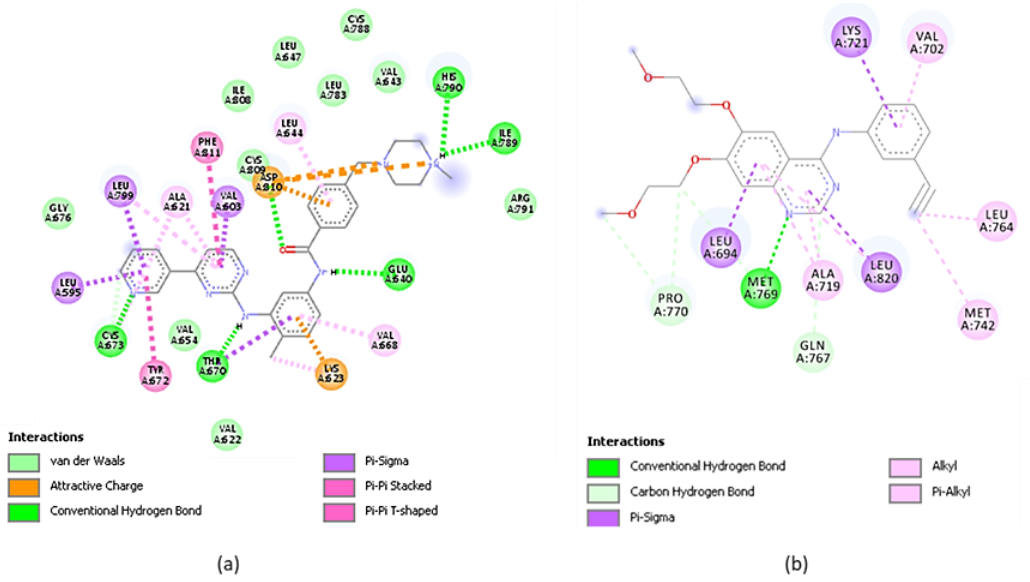
Validation of molecular docking of CFKRE compounds as protein tyrosine kinase inhibitors was evaluated from the results of the docking scores, which included binding energy and RMSD values of CFKRE compounds to PDGFR and EGFR proteins. The percentage of viable cells (% viability of cells) was calculated using the equation % viability of cells = ((absorbance treatment-absorbance control media)/(absorbance control cells-absorbance medium)) x 100%. The IC_{50} value was determined from a linear line equation formed from the data on the percentage of cell viability for each of the test compound concentration logs.

3. Results and Discussion

3.1. Analysis of Molecular Docking

The imatinib ligand attached to the PDGFR protein gave binding energy of -8.06 kcal/mol and an RMSD value of 0.48 Å. On the other hand, the erlotinib ligand attached to the EGFR protein has a binding energy of -3.04 kcal/mol and an RMSD value of 1.81 Å. The AutoDock experimental parameters were entirely accurate, with an RMSD value of less than 2 Å. The binding pose of the imatinib ligand to the PDGFR protein occurred at the amino acid residues of Asp810 and Cys673 through hydrogen bonds. In contrast, the erlotinib ligand was bound to the EGFR protein through the amino acid residue Met769 (Figure 1).

Molecular docking of doxorubicin and CFKRE compounds was performed using the analysis results of the imatinib ligand binding site to the PDGFR protein. All compounds were arranged to have the same position as the imatinib ligand and docked to the PDGFR protein. Doxorubicin and CFKRE docking scores on PDGFR protein, respectively, showed the lowest binding energies of -3.45 Å and +3680.16 kcal/mol with RMSD values of 2.97 and 3.36 Å, respectively (Table 1). Based on the docking score, CFKRE compounds have higher binding energy than imatinib and doxorubicin ligands, so it is possible that CFKRE compounds do not act as an inhibitor of PDGFR protein.



(a) (b)

Figure 1. Visualization of ligand docking hydrogen bonds with protein amino acid residues (a) imatinib and (b) erlotinib

Table 1. Results of docking with PDGFR

Compounds	Binding Energy (kcal/mol)	Types of Bonding	Binding Interaction	RMSD (Å)
Imatinib	-8.06	Hydrogen Bonding	Cys673 (1.897) Asp810 (1.912)	0.48
Doxorubicin	-3.45	Hydrogen Bonding	Arg791 (2.011) Asp810 (2.464)	2.97
CFKRE	+3680.16	Unfavorable Bump	0	3.36

Table 2. Results of docking with EGFR

Compounds	Binding Energy (kcal/mol)	Types of Bonding	Binding Interaction	RMSD (Å)
Erlotinib	-3.04	Hydrogen Bonding	Met769 (1.804)	1.81
Doxorubicin	-5.49	Hydrogen Bonding	Met769 (1.812) Lys721 (1.778)	1.57
CFKRE	+300.54	Hydrogen Bonding	Lys692 (2.202) Lys704 (2.549)	4.34

The CFKRE compound bound to the EGFR protein had a higher binding energy value than the erlotinib and doxorubicin ligands. The docking scores of doxorubicin and CFKRE compounds against EGFR protein showed the lowest binding energies were -5.49 and +300.54 kcal/mol with RMSD values of 1.57 and 4.34 Å, respectively, as presented in Table 2. The CFKRE compound did not interact with EGFR protein when docked (Unfavorable Bump) due to its high binding energy value. The bond was weak and indicated that this compound had no activity as an inhibitor of EGFR protein. Visualization and pose of CFKRE compound binding to EGFR protein are shown in Figure 2.

The interactions of CFKRE compounds with proteins are not only hydrogen bonds but also Van der Waals, -sigma, and -alkyl interactions. The CFKRE compounds attached to protein tyrosine kinases had weak hydrogen bond interactions. Furthermore, they did not show an interaction (Unfavorable Bump) with protein amino acid residues, indicating that these compounds had no activity as protein tyrosine kinase inhibitors.

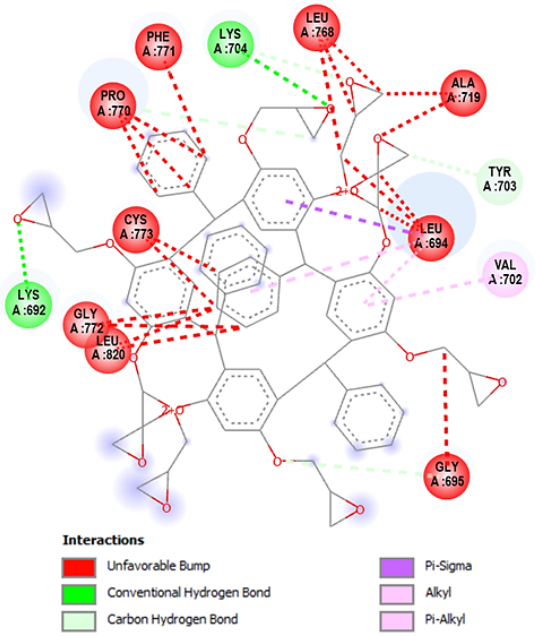


Figure 2. Visualization of the interaction of CFKRE compounds with EGFR protein

3.2. Study of Molecular Dynamic (MD) Conformation of Calix[4]resorcinarene Derivatives

Molecular dynamics studies conducted in previous studies resulted in 72 conformational complexes of calix with drugs with varying bond energy values. A total of 20 conformations were known to have low binding energies and selected as primary candidates in finding suitable host conformations for ligands. In general, the conformation of the ligands in calixarene with MD 72 100 ns simulation observations is as follows: (A) the ligand (imatinib) is in the calixarene ring; (B) the ligand interacts with the inside of the calixarene without crossing it, and (C) the ligand interacts with the outside of the calixarene ring (Figure 3). Based on the resulting complex interactions, the ligand interactions in the calixarene ring showed the most stable binding energy of −350.4 kcal/mol [21].

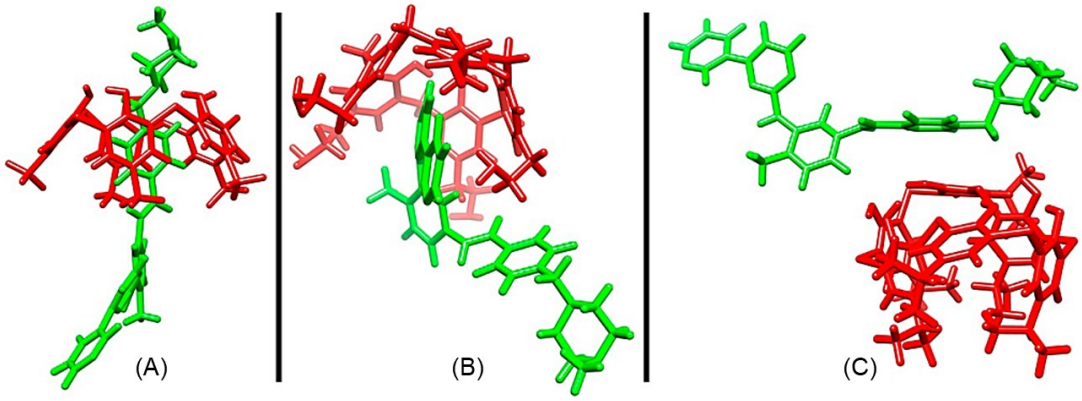


Figure 3. Conformation of imatinib in calixarene with the simulated observation of MD 72 100 ns

3.3. Synthesis of *C*-phenylcalix[4]resorcinarene

The compound *C*-phenylcalix[4]resorcinarene was synthesized from benzaldehyde and resorcinol in ethanol solvent under acidic conditions. The obtained product was a pale-yellow powder solid with a yield of 84% and had a melting point of 289°C. The results of the FTIR characterization of the compound *C*-phenylcalix[4] resorcinarene are presented in Figure 4.

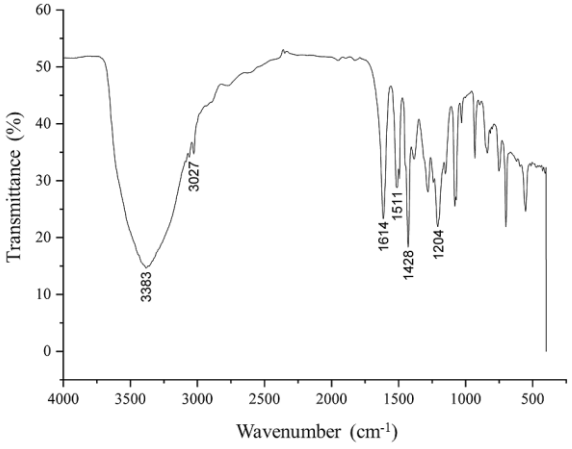


Figure 4. FTIR spectrum of *C*-phenylcalix[4]resorcinarene

The FTIR spectrum shows absorption at 1428 cm^{-1}, which is the absorption peak of the methine bridge connecting the aromatic ring of resorcinol with benzaldehyde. Another absorption peak is the presence of two vibrational absorptions -C=C- at 1614 and 1511 cm^{-1}, which are characteristic absorptions of aromatic compounds. Meanwhile, the broad absorption at 3383 cm^{-1} is a stretching vibration of the O-H group bound to the aromatic resorcinol ring, stretched due to hydrogen bonds between molecules.

Characterization of CFKR compounds using ^{1}H-NMR (in DMSO solvent) shows all protons in the corresponding chemical shift (Figure 5). The methine bridge proton is presented at a chemical shift of 5.64 ppm, while the aromatic proton appeared at a shift of 6.13 and 6.95 ppm. The chemical shift of the hydroxyl proton is found at 8.53 ppm. There are peaks at chemical shifts of 2.50 and 3.48 ppm, originating from DMSO and H_2O solvents (possibly coming from DMSO, which absorbs water).

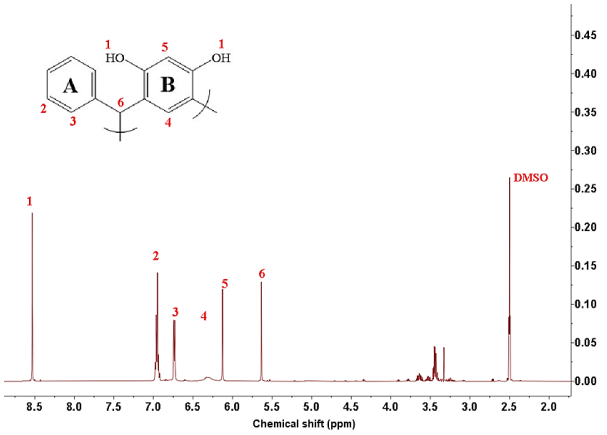


Figure 5. ^{1}H-NMR spectrum of *C*-phenylcalix[4]resorcinarene

The ^{13}C-NMR spectrum (Figure 6) shows 9 carbon peaks, including a chemical shift of 153.12 ppm (peak 1), which is the peak of the resorcinol aromatic ring carbon bound directly to the oxygen atom of the OH group. The peaks at the chemical shifts of 145.76–119.70 ppm are the peak of the aromatic ring carbon. The chemical shift of 55.20 and 41.39 ppm are assigned to methine CH carbon atom. The synthesized *C*-phenylcalix[4]resorcinarene compound will be used as a precursor for the synthesis of *C*-phenylcalix[4]resorcinarene epoxide (CFKRE) compound.

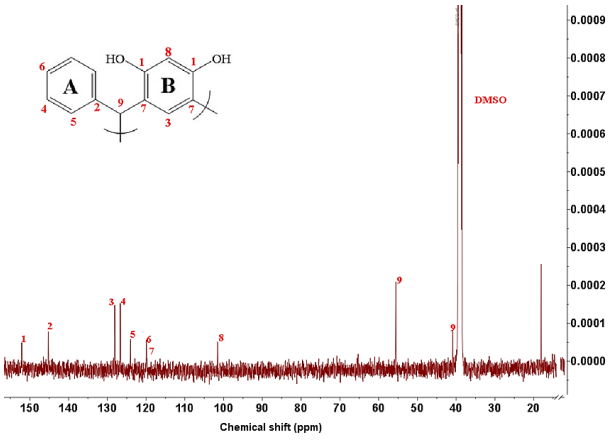


Figure 6. ^{13}C-NMR spectrum of *C*-phenylcalix[4]resorcinarene

3.4. Synthesis of *C*-phenylcalix[4]resorcinarene epoxide

The compound *C*-phenylcalix[4]resorcinarene epoxide (CFKRE) was synthesized from *C*-phenylcalix[4]resorcinarene by alkylation of epoxide with the addition of epichlorohydrin in alkaline conditions. The product obtained was a brownish-cream colored powder solid with 70% yield. The results of the FTIR spectrum of CFKRE compounds are presented in Figure 7. The FTIR spectrum obtained shows absorption at 1607, 1452, and 841 cm^{-1} for the C=C, CH methine, and CO epoxide groups, respectively, in CFKRE compounds.

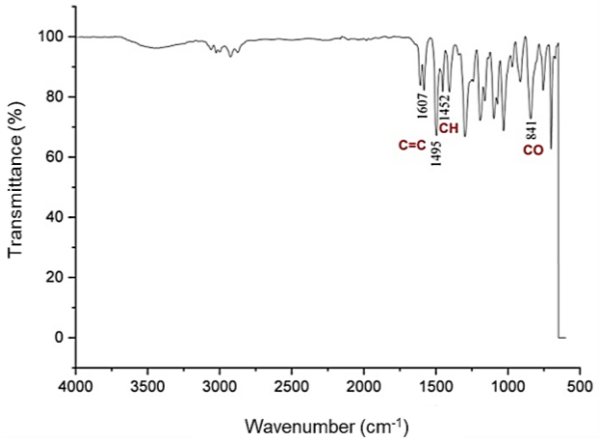


Figure 7. FTIR spectrum of CFKRE Compounds

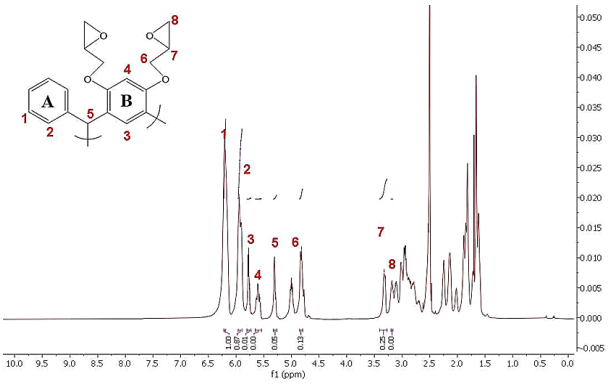


Figure 8. ^{1}H-NMR spectrum of CFKRE Compound

Based on the ^{1}H-NMR spectrum (Figure 8), the peak at 6.21 ppm chemical shift (peak 1) indicates the appearance of a multiplet which is the absorption of vibration -C=C-. The success of epoxidation was indicated by the appearance of protons in Csp^2-H and CH_2 epoxides at chemical shifts of 3.33 and 3.30 ppm (peaks 7 and 8) with multiplet and doublet appearances, and 8 protons integrated. The CFKRE compound was further analyzed with ^{13}C-NMR, as shown in Figure 9. The characteristics of CFKRE that emerged were the presence of a carbon epoxide group as shown in the chemical shift 70.26, 49.76, and 43.50 ppm (peaks 8, 9, and 10). In addition, the loss of the hydroxyl group peak indicates that the epoxide alkylation reaction has been carried out successfully.

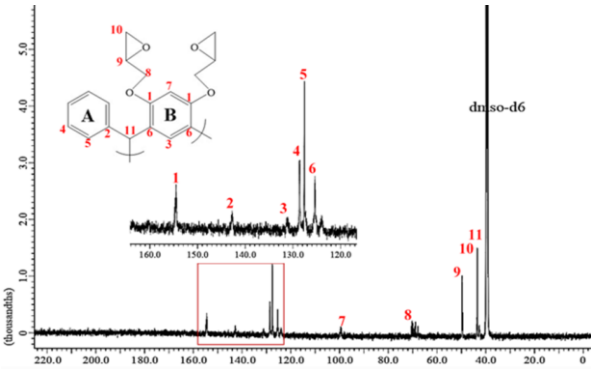


Figure 9. ^{13}C-NMR spectrum of CFKRE Compound

3.5. Cytotoxicity assay

An anticancer activity assay using the MTT assay was conducted on MCF-7 cancer cells and Vero cells. The principle of the MTT assay is based on the ability of the oxidoreductase enzyme found in live cancer cells to reduce tetrazolium to formazan. The cytotoxicity assay in this study aimed to determine the effect of the concentration of CFKRE compounds on the viability of MCF-7 cancer cells and Vero cells, as indicated by the IC_{50} value. Cytotoxicity assay showed differences between the colony morphology of control cells MCF-7 (0 g/mL CFKRE) and MCF-7 cells treated with CFKRE compounds.

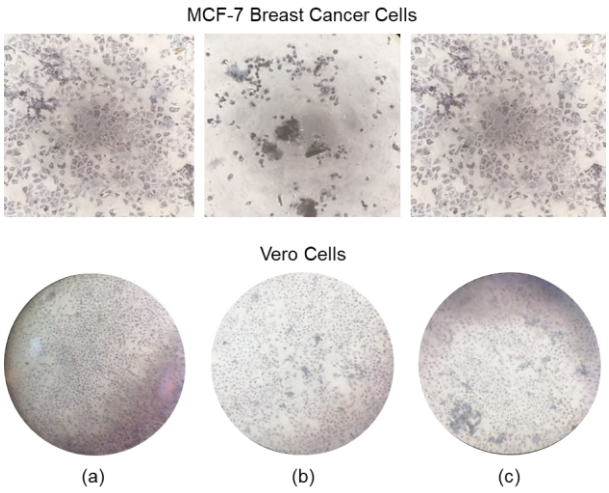


Figure 10. Effect of CFKRE compound treatment on the viability of MCF-7 cancer and Vero cells (100x magnification) (a) Concentration 0 µg/mL; (b) concentration 1.5625 µg/mL; (c) concentration 100 µg/mL

Figure 10 shows that concentrations of 1.5625 g/mL and 100 g/mL still resulted in the high growth of MCF-7 cancer cells because they almost filled the well surface. The same phenomenon was also found for the colony morphology of Vero control cells (0 g/mL CFKRE) treated with CFKRE compounds. Figure 10 shows that given concentrations of 1.5625 g/mL and 100 g/mL resulted in high normal cell growth of Vero because it filled all well surfaces. Quantitative analysis was done by measuring the absorbance, and the absorbance data obtained were used to determine the IC_{50} value.

Based on absorbance data and probit log analysis, the CFKRE compound had an IC_{50} value of 4.04 g/mL against

MCF-7 cells, while the CFKRE compound had an IC_{50} value of 29.59 g/mL against Vero cells. The IC_{50} value was higher than the positive doxorubicin control (3.286 g/mL). These results indicated that CFKRE compounds are toxic to MCF-7 cancer cells but not to Vero cells.

4. Conclusion

The CFKRE compounds have activity as a weak inhibitor of the PDGFR and EGFR protein receptors. CFKRE compounds could interact to form complexes with guests (drugs) with low and stable binding energies to have the potential for drug delivery. The CFKRE compounds could be synthesized, which produced a yield of 70%. CFKRE compounds are not toxic to Vero/normal cells but toxic to the MCF-7 cancer cells.

References

[1] Christine L. Chaffer, Robert A. Weinberg, A perspective on cancer cell metastasis, *Science*, 331, 6024, (2011), 1559-1564 https://doi.org/10.1126/science.1203543

[2] Rebecca S. Y. Wong, Apoptosis in cancer: from pathogenesis to treatment, *Journal of Experimental & Clinical Cancer Research*, 30, 1, (2011), 1-14 https://doi.org/10.1186/1756-9966-30-87

[3] Bruce A. Chabner, Thomas G. Roberts, Chemotherapy and the war on cancer, *Nature Reviews Cancer*, 5, 1, (2005), 65-72 https://doi.org/10.1038/nrc1529

[4] Sudipta Senapati, Arun Kumar Mahanta, Sunil Kumar, Pralay Maiti, Controlled drug delivery vehicles for cancer treatment and their performance, *Signal Transduction and Targeted Therapy*, 3, 1, (2018), 1-19 https://doi.org/10.1038/s41392-017-0004-3

[5] Jessica J. Tao, Kala Visvanathan, Antonio C. Wolff, Long term side effects of adjuvant chemotherapy in patients with early breast cancer, *The Breast*, 24, (2015), S149-S153 https://doi.org/10.1016/j.breast.2015.07.035

[6] Karol Bukowski, Mateusz Kciuk, Renata Kontek, Mechanisms of multidrug resistance in cancer chemotherapy, *International Journal of Molecular Sciences*, 21, 9, (2020), 3233 https://doi.org/10.3390/ijms21093233

[7] Oktay Tacar, Pornsak Sriamornsak, Crispin R. Dass, Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems, *Journal of Pharmacy and Pharmacology*, 65, 2, (2013), 157-170 https://doi.org/10.1111/j.2042-7158.2012.01567.x

[8] Dengguo Wei, Xiaolu Jiang, Lu Zhou, Jing Chen, Zheng Chen, Chong He, Kun Yang, Ying Liu, Jianfeng Pei, Luhua Lai, Discovery of multitarget inhibitors by combining molecular docking with common pharmacophore matching, *Journal of Medicinal Chemistry*, 51, 24, (2008), 7882-7888 https://doi.org/10.1021/jm8010096

[9] Emmy Yuanita, Pranowo Harno Dwi, Mustofa Mustofa, Swasono Respati Tri, Jufriza Syahri, Jumina Jumina, Synthesis, characterization and molecular docking of chloro-substituted hydroxyxanthone derivatives, *Chemistry Journal of Moldova*, 14, 1, (2019), 68-76 http://dx.doi.org/10.19261/cjm.2018.520

[10] Jose Baselga, Why the epidermal growth factor receptor? The rationale for cancer therapy, *The Oncologist*, 7, S4, (2002), 2-8 https://doi.org/10.1634/theoncologist.7-suppl_4-2

[11] Mengrui Liu, Hongliang Du, Wenjia Zhang, Guangxi Zhai, Internal stimuli-responsive nanocarriers for drug delivery: Design strategies and applications, *Materials Science and Engineering: C*, 71, (2017), 1267-1280 https://doi.org/10.1016/j.msec.2016.11.030

[12] Zhe Wang, Xiangping Deng, Jinsong Ding, Wenhu Zhou, Xing Zheng, Guotao Tang, Mechanisms of drug release in pH-sensitive micelles for tumour targeted drug delivery system: A review, *International Journal of Pharmaceutics*, 535, 1-2, (2018), 253-260 https://doi.org/10.1016/j.ijpharm.2017.11.003

[13] Sandeep J. Sonawane, Rahul S. Kalhapure, Thirumala Govender, Hydrazone linkages in pH responsive drug delivery systems, *European Journal of Pharmaceutical Sciences*, 99, (2017), 45-65 https://doi.org/10.1016/j.ejps.2016.12.011

[14] Yue Zhou, Hui Li, Ying-Wei Yang, Controlled drug delivery systems based on calixarenes, *Chinese Chemical Letters*, 26, 7, (2015), 825-828 https://doi.org/10.1016/j.cclet.2015.01.038

[15] Anis Abdul Karim, Qingqing Dou, Zibiao Li, Xian Jun Loh, Emerging supramolecular therapeutic carriers based on host–guest interactions, *Chemistry–An Asian Journal*, 11, 9, (2016), 1300-1321 https://doi.org/10.1002/asia.201501434

[16] Fazel Nasuhi Pur, Karim Akbari Dilmaghani, Calixplatin: novel potential anticancer agent based on the platinum complex with functionalized calixarene, *Journal of Coordination Chemistry*, 67, 3, (2014), 440-448 https://doi.org/10.1080/00958972.2014.890718

[17] Jingxin Mo, Paul K. Eggers, Zhi-Xiang Yuan, Colin L. Raston, Lee Yong Lim, Paclitaxel-loaded phosphonated calixarene nanovesicles as a modular drug delivery platform, *Scientific Reports*, 6, 1, (2016), 1-12 https://doi.org/10.1038/srep23489

[18] M. D. Shah, Y. K. Agrawal, Calixarene: A new architecture in the analytical and pharmaceutical technology, *Journal of Scientific and Industrial Research (JSIR)*, 71, (2012), 21-26

[19] Ângelo de Fátima, Sergio A. Fernandes, Adão Aparecido Sabino, Calixarenes as new platforms for drug design, *Current Drug Discovery Technologies*, 6, 2, (2009), 151-170 http://dx.doi.org/10.2174/157016309788488302

[20] Ali Yousaf, Shafida Abd Hamid, Noraslinda M. Bunnori, A. A. Ishola, Applications of calixarenes in cancer chemotherapy: facts and perspectives, *Drug Design, Development and Therapy*, 9, (2015), 2831 https://doi.org/10.2147/DDDT.S83213

[21] Rodrigo Galindo-Murillo, María Eugenia Sandoval-Salinas, Joaquín Barroso-Flores, In silico design of monomolecular drug carriers for the tyrosine kinase inhibitor drug imatinib based on calix-and thiacalix [n] arene host molecules: a DFT and molecular dynamics study, *Journal of Chemical Theory and Computation*, 10, 2, (2014), 825-834 https://doi.org/10.1021/ct4004178