

Structural, Antioxidant and Antiviral Studies of C-3-nitrophenyl CALIX[4]resorcinarene

Bohari M Yamin[#], Hamza M Abosadiya^{*}, Siti Aisah Hasbullah and Jumina[#]

[#] School of Chemical Sciences, Universiti Kebangsaan Malaysia, Bangi, 43600, Selangor Malaysia
E-mail: bohari@ukm.edu.my

[#] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Gadjah Mada University, Indonesia
E-mail: pak_jumina@yahoo.com

Abstract— The calix[4]arene derivative C-3-nitrophenylcalix[4]resorcinarene was synthesized by using one-pot reaction of resorcinol with 3-nitrobenzaldehyde in the presence of concentrated HCl. The compound was characterized by IR, ¹H and ¹³C NMR spectroscopy. X-ray crystallographic study showed that this compound crystallized in a triclinic system with space group of P $\bar{1}$ and the unit cell dimensions, $a = 10.6143(3) \text{ \AA}$, $b = 13.6262(4) \text{ \AA}$, $c = 14.7971(5) \text{ \AA}$, $\alpha = 102.813(3)^\circ$, $\beta = 110.917(3)^\circ$, $\gamma = 90.885(2)^\circ$. $V = 1938.78(11) \text{ \AA}^3$ and $Z = 2$. The biological studies were also investigated. It has a strong antioxidant property and high antiviral activity against HSV-1. Cytotoxicity testing on Vero cells showed that it is non-toxic, with a CC_{50} of more than 0.4 mg/mL. Moderate antibacterial activity.

Keywords— C-3-nitrophenylcalix[4]resorcinarene, X-ray structural study and Biological Studies.

I. INTRODUCTION

Calixarene which was discovered by Adolf Von Baeyer in 1872 can now be considered as technology materials. The supramolecular future with cavitation that can display host-guest activity made the calixarene a good candidate as absorbant and material for sensor devices [1-5]. Therefore, it is not surprising that the development in these two areas of application has been quite rapid. In order to improve the performance of a device a new design of the active agent based on rational design and modelling is carried out before attempting the synthesis work. The synthesis work has always been the key step to a successful and continuing development in the product development. Calixarene is basically a phenolic macrocycle. Therefore it should display antioxidant property. The biological application of calixarene derivatives has currently become an interest to many researchers world-wide [6]. One major problem for the application in certain field is its low solubility and flexible existence of their conformers [7-10]. The presence of more than one conformer in the solution can be determined by NMR studies. However, in the solid state the CALIX[4]resorcinarene faced stability problem after crystallization. Therefore there are relatively less structures of the calix have been reported compare to their calix with

aliphatic linkers. In this paper the structural study of C-3-nitrophenylcalix [4]resorcinarene by X-ray crystallography and biological study including antiviral activity are presented.

II. EXPERIMENTAL

All the compounds utilized in this work were commercially available with high purity purchased from Acros Organics (Geel, Belgium) and Sigma-Aldrich (St Louis, MO, USA) and were used without further purification. All solvents were distilled before use. The microelemental analysis for CHNS-O was carried out using a Carlo Erba 1108 Elemental Analyzer (Milan, Italy). The infrared spectrum (IR) of the product (KBr pellets) was recorded using a Perkin Elmer Spectrum GX spectrophotometer (Perkin Elmer, Waltham, MA, USA) in the range of 400–4,000 cm^{-1} . Nuclear Magnetic Resonance (¹H and ¹³C) experiments were performed on a Bruker 600 MHz instrument using DMSO-d₆ as the solvent. Single-crystal X-ray experiment was performed on Bruker D-QUEST diffractometer (Bruker, AXS Inc., Madison, WI, USA) using graphite-monochromated Mo-K α radiation ($\lambda = 0.71073 \text{ \AA}$).

A. Preparation of C-3-nitrophenylcalix[4]resorcinarene

Concentrated hydrochloric acid (7 mL) was added into a round-bottom flask containing a solution of 3-nitrobenzaldehyde (0.01 mol, 1.51 g) in absolute ethanol (60 mL). The mixture was stirred for 30 minutes and a solution of resorcinol (0.01 mol, 1.10 g) in absolute ethanol (20 mL) was added. The mixture was refluxed for 24 hours at 80 °C. The yellow precipitate formed was collected by filtration, washed with distilled water and acetone several times and dried under vacuum. Yield (82%); FTIR (KBr, cm⁻¹): 3399 (OH), 1516(C=C), 1313(C-N); 1204(C-O), ¹H-NMR(600 Hz; precipitate, DMSO-d₆) δ_H: 4.91 (2H, s, Ar-CH), 5.23 (2H, s, Ar-CH), 6.21 (4H, s, Ar-CH), 6.02 (4H, s, Ar-CH), 7.12 (4H, t, Ar-CH), 7.36 (4H, S, Ar-CH), 7.69 (4H, d, Ar-CH), 7.76 (4H, d, Ar-CH), 8.92 (4H, s, OH), 9.0 (4H, s, OH); ¹³C-NMR (150 MHz; DMSO-d₆) δ_C: 41.9 (4 × CH), 102.4 (4 × Ar-H), 119.6 (4 × Ar-CH), 120.5 (4 × Ar-CH), 120.5 (2 × Ar-CH), 128.6 (2 × Ar-CH), 129.8 (2 × Ar-H), 133.8 (4 × Ar-H), 135.2 (4 × Ar-H), 147.3(4×Ar-CH), 153.4(4 × Ar-OH), 153.7(4 × Ar-OH), 153.9(4 × Ar-NO₃). Analysis Calcd (%): C = 60.24 and H = 3.50 and N = 5.40. Found (%): C= 60.12 and H= 3.30 and N = 5.20.

B. Cytotoxicity Evaluation

The cytotoxicity of the compound was first determined on uninfected Vero cells (African monkey *Cercophiteus aetiops* kidney cells) with dilutions ranging from 5 mg/mL to 0.039 mg/mL in Dulbecco's Modified Eagle's Medium (DMEM, Flowlab, North Ride, Australia). Cytotoxicity was determined using the MTT assay [11]. The CC₅₀ value that is the compound concentration that kills 50% of the cell population was determined by the optical density of solubilized formazan. The percentage of growth inhibition was calculated using the following formula:

$$\% \text{ Cell viability} = 100 \times (\text{Abs}/\text{Ac}),$$

where Abs = Absorbance value of test compound and Ac = Absorbance value of control (cells only).

C. Antiviral Activity

The plaque reduction assay was performed to study the presence of antiviral activity of compound. HSV-1 stock was prepared and the viral titre of the stock was determined as 5.7 × 10⁸ pfu/mL. The concentration of compound used in this assay was set based on the concentration that allows growth of 70% or more Vero cells. This is to ensure that cell death is not due to the toxicity of the test compound which will affect the accuracy of the test results. In this study, the first test concentration of (I) is 0.35 mg/mL, which is below the CC₅₀ value and continues with two fold serial dilutions until 0.011 mg/mL. Virus was infected to 80% confluent cells and incubated for 48 hours for plaque formation. Plaques were stained with crystal violet and the numbers of plaques were counted. The EC₅₀ value was determined as the concentration that inhibited plaque formation by 50% of the untreated cells [12].

D. Antioxidant Test

A stock solution of DPPH was prepared by dissolving DPPH (0.4 g) in methanol (1 L) and the solution was kept in the dark at 4°C. A stock solution of the 3-nitrophenylcalix

[4]resorcinarene was prepared at 10 mg/5 mL in DMSO. A volume of 100 μL from the stock solution of the compound was added to 1 mL of DPPH. The mixture was shaken well and kept in the dark at room temperature for 2 hours. The absorbance of the mixture was measured at 517 nm using a spectrophotometer. The percentage of inhibition of radical scavenging ability was calculated as:

$$\text{Inhibition \%} = [(A_{\text{DPPH}} - A_{\text{Sample}})/(A_{\text{DPPH}})] \times 100 = [(1.012 - 0.176)/1.012] \times 100 = 82.60\%.$$

III. RESULTS AND DISCUSSION

A. Synthesis and Characterization

The synthesis of C-3-nitrophenylcalix[4]resorcinarene was accomplished by refluxing mixture of equal molar amounts of resorcinol with 3-nitrobenzaldehyde in the presence of concentrated HCl in EtOH at 80 °C. The FTIR spectrum of the compound showed characteristic absorption at 3399 cm⁻¹ corresponds to O-H stretching vibration. The (C=C) and ν(C-N) stretching frequencies appeared at 1516 and 1313 cm⁻¹, respectively. ¹H-NMR spectrum of compound showed the resorcinol protons appeared at 8.92 and 9.0ppm, respectively. The different conformations of calix[4]resorcinarene can also be observed from the different chemical shift of methine linkage protons (4.91 and 5.23 ppm) indicated that the calix[4]arenes molecule adopts a chair conformation. The ¹³C-NMR spectrum showed the carbon chemical shifts of the two sets of the phenolic carbon at 153.4 and 153.7ppm. The four tertiary methine carbons appeared at 41.9ppm. The aromatic carbon chemical shifts appeared in the range of 102.4-153.9 ppm.

B. X-Ray Structure

X-ray investigation showed that the compound crystallized in triclinic system with space group of Pī. The unit cell dimension are a = 10.6143(3) Å, b = 13.6262(4) Å, c = 14.7971(5) Å, α = 102.813(3)°, β = 110.917(3)°, γ = 90.885(2)°. V= 1938.78(11) Å³ and Z =2. The crystal system and refinement parameters are given in Table 1.

TABLE 1
CRYSTALLOGRAPHIC DATA AND STRUCTURAL REFINEMENT OF C-3-NITROPHENYLCALIX[4]RESORCINARENE

Crystal parameters	Data/values
Empirical formula	C ₆₀ H ₆₂ N ₄ O ₂₅ S ₄
Temperature	293(2) K
Wavelength	1.54178 Å
Crystal system	Triclinic
Space group	Pī
Unit cell dimensions	a = 10.6143(3) Å b = 13.6262(4) Å c = 14.7971(5) Å α = 102.813(3)°. β = 110.917(3)°. γ = 90.885(2)°.
Volume	1938.78(11) Å ³
Z	2
Density (calculated)	1.399 Mg/m ³
Absorption coefficient	2.812 mm ⁻¹

F(000)	860
Crystal size	0.40 × 0.32 × 0.26
Theta range for data collection	3.296 to 71.486°.
Index ranges	-13 ≤ h ≤ 13, -16 ≤ k ≤ 16, -12 ≤ l ≤ 11
Reflections collected	21497
Independent reflections	3773 [R(int) = 0.0208]
Completeness to theta = 67.679°	51.5 %
Refinement method	matrix least-squares on F ²
Data / restraints / parameters	3773 / 0 / 498
Goodness-of-fit on F ²	1.045
Final R indices [I > 2σ(I)]	R1 = 0.0644, wR2 = 0.1570
R indices (all data)	R1 = 0.0668, wR2 = 0.1592
Extinction coefficient	n/a
Largest diff. peak and hole	1.179 and -1.200 e.Å ⁻³

The calix molecule adopts a chair conformation (C_{2h}) with two opposite resorcinol groups almost coplanar to each other and an other pair that are anti-parallel (Figure 1).

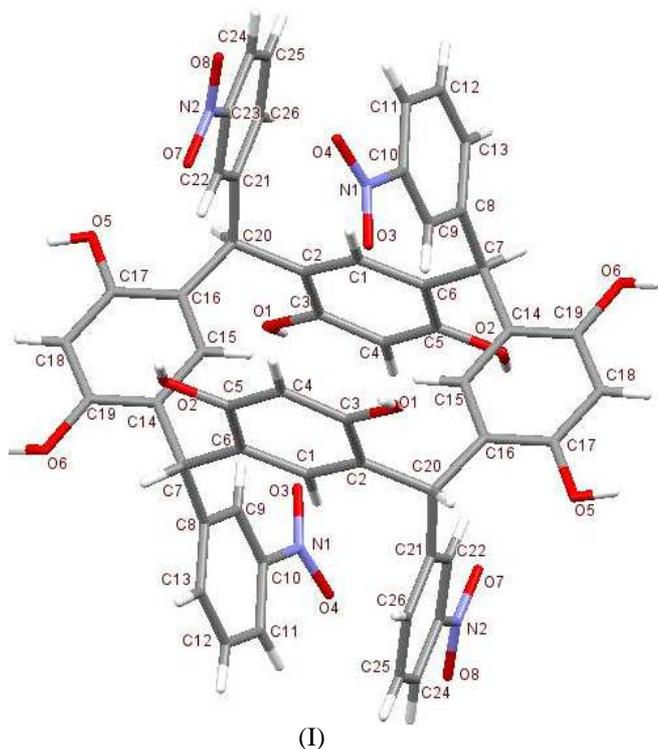


Figure 1. The molecular structure of C-3-nitro phenyl calix[4]resorcinarene(I). The solvent molecules are not shown for clarity.

The alternate resorcinol rings (C1–C6) and (C14–C19) are perpendicular with a dihedral angle of 84.1(3)°. The dihedral angle between the resorcinol ring (C1–C6) and 3-nitrophenyl linkage group (C8–C13) is 87.4(3)°, indicate a high degree of co-planarity between the resorcinol rings and lead to the adoption of a chair conformation. A similar conformation with approximately C_{2h} symmetry due to the

presence of a crystallographic inversion center has also been observed for tetraarylboronic acid resorcinarene [10].

C. Biological Studies

The cytotoxicity test indicated that C-3-nitrophenylcalix[4]resorcinarene is safe to be used as an antimicrobial therapeutic agent due to its non-toxicity against Vero cells with a CC₅₀ value of 5 mg/mL. The CC₅₀ was obtained from the graph of percentage of cell survival viability versus compound concentration (Figure 2).

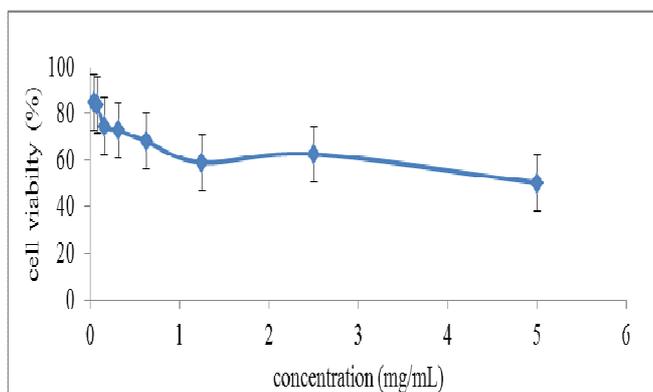


Figure 2. Percentage of cell survival against concentration of compound that C-3-nitrophenylcalix[4]resorcinarene

Antiviral tests showed that the compound C-3-nitrophenyl calix[4]resorcinarene is a good candidate as an antiviral agent because of its ability to inhibit 100% plaque formation, even at the lowest concentration of 0.011 mg/mL. Thus, the EC₅₀, which is the concentration when the presence of test compound caused 50% reduction of plaques or cytopathic effect, is much lower than the minimum inhibitory concentration of 0.011 mg/mL. The selectivity index (SI = CC₅₀/EC₅₀) of compound I is more than 36. This indicates that compound can be considered as a potentially safe antiviral agent with low cytotoxicity and high potency. SI values greater than 10 indicate potential antiviral therapeutic safety and efficacy.

Antioxidant properties measured as radical scavenging activity are due to the transfer of electrons or hydrogen atoms of the hydroxyl groups to an oxidizing agent. Compound being a polyphenolic compound, could inhibit the oxidation of other molecules such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH) by donating hydrogen atoms to form the stable non-radical form of DPPH as shown by the formation of a pale yellow color. The antioxidant activity exhibited by compound was 82.60%.

IV. CONCLUSIONS

C-3-nitrophenylcalix[4]resorcinarene was successfully synthesized by the cyclocondensation of 3-nitro benzaldehyde and resorcinol in the presence of concentrated HCl. The X-ray structure was in good agreement with the NMR data and the calix molecule adopted a chair C_{2h} conformation. The compound showed good anti-HSV-1 and antioxidant activity at non-cytotoxic concentrations.

ACKNOWLEDGMENT

The authors thank the Ministry of Higher Education of Malaysia and Universiti Kebangsaan Malaysia for the research grant DIP-2012-11 and FRGS/1/2013/ST01/UKM/03/4. One of us HAMZA would like to thank the Ministry of Higher Education of Libya for the scholarship

REFERENCES

- [1] Zhang, Z.; Luo, Y.; Chen, J.; Dong, S.; Yu, Y.; Ma, Z.; Huang, F. Formation of linear supramolecular polymers that is driven by C–H \cdots π interactions in solution and in the solid state. *Angew. Chem. Int.* 2011, *50*, 1397–1401.
- [2] Han, C.; Ma, F.; Zhang, Z.; Xia, B.; Huang, F. DIBPillar[n]arenes (n = 5, 6): Syntheses, X-ray crystal structures, and complexation with n-Octyltriethyl ammonium hexafluorophosphate. *Org. Lett.* **2010**, *12*, 4360–4363.
- [3] Zhang, M.; Xu, D.; Yan, X.; Chen, J.; Dong, S.; Zheng, B.; Huang, F. Self-healing supramolecular gels formed by crown ether based host–Guest interactions. *Angew. Chem. Int.* 2012, *51*, 7011–7015.
- [4] Zheng, B.; Wang, F.; Dong, S.; Huang, F. supramolecular polymers constructed by crown ether-based molecular recognition. *Chem. Soc. Rev.* 2012, *41*, 1621–1636.
- [5] Turshatov, A.A.; Melnikova, N.B.; Semchikov, Y.D.; Ryzhkina, I.S.; Pashirova, T.N.; Mobius, D.; Zaitsev, S.Y. Interaction of monolayers of calix[4]resorcinarene derivatives with copper ions in the aqueous subphase. *Colloids Surf. A Physicochem. Eng. Aspects.* 2004, *240*, 101–106.
- [6] Abosadiya, H. M., Hasbullah, S. A., Mackeen, M. M., Low, S. C., Ibrahim, N., Koketsu, M., & Yamin, B. M. .Synthesis, Characterization, X-ray Structure and Biological Activities of C-5-Bromo-2-hydroxy phenyl calix [4]-2-methyl resorcinarene. *Molecules*, 2013, *18*, 13369-13384.
- [7] Makinen, M.; Jalkanen, J.P.; Vainiotalo, P. Conformational properties and intramolecular hydrogen bonding of tetraethyl resorcinarene: An ab initio study. *Tetrahedron.* 2002, *58*, 8591–8596.
- [8] Thondorf, I.; Brenn, J.; Bohme, V. Conformational properties of methylene-bridged resorcarenes. *Tetrahedron.* 1998, *54*, 12823-12828
- [9] Iwanek, W.; Wzorek, A. Introduction to the chirality of resorcinarenes. *Mini Rev. Org. Chem.* 2009, *6*, 398–411.
- [10] Davis, C.J.; Lewis, P.T.; Billodeaux, D.R.; Fronczek, F.R.; Escobedo, J.O.; Strongin, R.M. Solid-state supramolecular structures of resorcinol-arylboronic acid compounds. *Org. Lett.* 2001, *3*, 2443–2445.
- [11] Andrighetti-Frohner, C.R.; Antonio, R.; Creczynski-Pasa, T.; Barardi, C.; Simoes, C. Citotoxicity and potential antiviral evaluation of violacein produced by *Chromobacterium violaceum*. *Mem. Inst. Oswaldo Cruz.* 2003, *98*, 843–848
- [12] Macedo, N.R.P.V.; Ribeiro, M.S.; Villaca, R.C.; Ferreira, W.; Pinto, A.M.; Teixeira, V.L.; Cirne-Santos, C.; Paixao, I.C.N.P.; Giongo, V. Caulerpin as a potential antiviral drug against herpes simplex virus type 1. *Braz. J. Pharmacogn.* 2012, *22*, 861–867.