ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 13, No. 1 (2012) 36-39

Bioactivity Assessment of Water Soluble Calix[4]arene Derivative

Ali Muhammad Soomro[1], Raj Kumar Oad[2], Shahabuddin Memon*[3] and Imdadullah Qureshi[4]

[1]*Department of Physiology, Faculty of Natural Sciences, University of Sindh, Jamshoro 76080, Pakistan*
[2]*Department of Pharmacology, Faculty of Pharmacy, University of Sindh, Jamshoro 76080, Pakistan*
[3]*National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan*
[4]*Department of Chemistry, University of Science & Technology, Bannu KPK, Pakistan*

Received 19 November 2011, Revised 15 December 2011, Accepted 19 December 2011

Abstract

The present study deals with the bioactivity assessment of 5,11,17,28-tetrakismorpholinomethyl-25,26,27,28-tetrahydroxycalix[4]arene (**3**) against a variety of microorganisms including Gram Positive; *Staphylococcus albus* ATCC 10231, *Streptococcus viridans* ATCC 12392, Gram Negative: *Bacillus procynous* ATCC 51189, *Enterobacter aerogenes* ATCC 13048, *Klebsiella aerogenous* ATCC 10031, *Escherichia coli* ATCC 8739, *Sallmonella* ATCC 6017 and Fungi: *Aspergillus Niger* ATCC 16404, *Aspergillus fumagatus* ATCC 90906, *Penicillium* ATCC 32333. The antimicrobial activity was found by using a modified disc diffusion method. All microorganisms were obtained from the American Type Culture Collection (ATCC) and selective agar media were employed for the growth of microbial strains. Results show that all the tested microorganisms are highly susceptible to compound **3**. The MIC of 4 μg/μL and 8 μg/μL was determined against most of the bacterial and fungal strains. The bioactivity of **3** could be a valuable addition in therapeutic index.

Keywords: Calixarene; Microorganism; Bioactivity; Bacteria; Fungi.

Introduction

Since decades consistent increasing infectious diseases and emergence of antibiotic drug resistance have enhanced the efforts to synthesize and assess new compounds for bioactivity. The parent compounds selected for synthesis usually are those which contain properties of known safety and probable efficacy against microorganisms. The calixarenes, a versatile class of synthetic macrocycles has attracted most of the researchers working in a wide range of fields. On the basis of their nontoxic nature, calixarenes have extensive applications in the biological and pharmaceutical area [1-3], and are considered in the assessment of antimicrobial activities; though, most of the calixarene components have been reported for their efficacy against few microorganism species including bacteria, fungi and viruses [4-8]. On the other hand, safety profile reported by Perret F. *et al.* indicated calixarenes as inert substances like glucose, but incidence of slight toxicity was found with sulfonate derivatives of calix[4]arene compounds [9].

Previous researches have reported that morpholine derivatives possessed antimicrobial activities [10-13]. The mode of action recorded for some of the compounds was targeting through enzyme pathway in fungi, therefore used as fungicide in agriculture fields [14]. As per Material Safety Data Sheet (MSDS), no complete data on morpholine toxicity is available however; it has been preferred in synthesis and development of

*Corresponding Author Email: shahabuddinmemon@yahoo.com

new drugs. Thus, in view of these reports and our previous expertise [15-18] we have synthesized 5,11,17,28-tetrakismorpholinomethyl-25,26,27,28-tetrahydroxycalix[4]arene (**3**), a macromolecule based on calixarene and morpholine units in order to explore their collective nature toward the microorganisms.

Material and Methods

Apparatus

Melting points were determined on a Gallenkamp apparatus (UK) in a sealed glass capillary tube and are uncorrected. FT-IR spectra were recorded on a Thermo Nicollet AVATAR 5700 FT-IR spectrometer using KBr pellets in the spectral range 4,000-400. Elemental analyses were performed using a CHNS instrument model Flash EA 1112 elemental analyzer. Analytical TLC was performed on precoated silica gel plates (SiO_2, Merck PF254).

Synthesis and characterization

5,11,17,28-tetrakismorpholinomethyl-25,26,27,28-tetrahydroxycalix[4]arene (**3**) was synthesized according to the reported methods [19–21] as depicted in Fig. 1. Characterization of the compounds was made by various techniques such as, melting point, TLC, FT-IR, and elemental analysis, which confirm the structure and purity of the compounds.

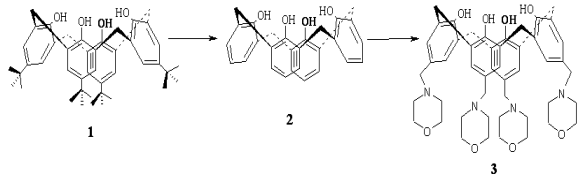

Figure 1. **5,11,17,28-tetrakis(morpholinomethyl)-25,26,27,28-tetrahydroxycalix[4]arene (3).**

Microbiological study

Antimicrobial activity of the test compound **3** (Figure 1) was carried out in vitro by using modified Kirby-Bauer disc diffusion method [22]. The antibacterial and antifungal activity was determined against variety of microorganisms from the American Type Culture Collection (ATCC). The selective agar media were employed for the growth of microorganism species including gram positive, gram negative and fungus. Table 1 shows distribution of culture strains (ATCC) and types of media obtained from Baltimore Biology Labs (BBL) USA, Oxoid AG Switzerland and Merck Frankfurter Germany. All the culture media were prepared and used according to the manufacturer guidelines.

Table 1. **Distribution of culture strains and media.**

Culture Strains	Media
Gram Positive	
Staphylococcus albus ATCC 10231	Tryptic soya agar (BBL)
Streptococcus viridans ATCC 12392	
Gram Negative	
Bacillus procynous ATCC 51189	Tryptic soya agar (OXOID)
Enterobacter aerogenes ATCC 13048	Cetrimide agar base (MERCK)
Klebsiella aerogenous ATCC 10031	Lactose broth (MERCK)
Escherichia coli ATCC 8739	
Sallmonella ATCC 6017	Violet red bile dextrose agar (OXOID)
Fungus	
Aspergillus Niger ATCC 16404	Sabourand dextrose agar (MERCK)
Aspergillus fumagatus ATCC 90906	
Penicillium ATCC 32333	

Bioactivity assay

Ten serial dilutions of the compound (3) yielded the concentrations of 2, 4, 8, 12, 16, 20, 24, 28, 32 and 36 µg/µL in double distilled water. Filter paper discs (Whatmans', no. 3) of 6 mm diameter were impregnated with 5 µL of each dilution prepared. The paper discs were allowed to dry completely at room temperature under sterile conditions and stored appropriately until used. The discs were placed on to bacterial and fungal agar plates seeded by streaking plate technique and were incubated at 37 ºC for 24 hours and 48 hours respectively. Simultaneously, negative control discs incubated were prepared using the same solvent employed to dissolve the test compound. After incubation period the antimicrobial activity was examined by measuring the diameter of inhibition zone in mm against each strain of microorganisms. Tests were performed in triplicate as suggested by Vanden Berghe 1991 [23]. The in vitro bioactivity of compound (**3**) was

quantitatively assessed by determining the MIC level; the lowest concentration of the substance that results in inhibition of macroscopic microbial growth after incubation time.

Results and Discussion

Primarily, the bioactivity assessment of the compound **3** was carried out by inhibition of growth against bacterial and fungal strains and activity was presented according to the four step criterion. In this criterion, inhibition zone diameters such as 9-11 mm, 7-9 mm, 5-7 mm and below 5 mm were considered as very high activity (+++), high activity (++), relatively high activity (+) and no antimicrobial activity (-), respectively. (Table 2) shows that compound **3** has very high antibacterial activity against two gram positive and four gram negative bacteria and high activity against rest of the selected bacteria and fungus whereas; no zone of inhibition was originated around control. The compound **3** has been found significantly active against bacterial and fungal strains.

Table 2. **Primary screening of bioactivity of the tested compound 3.**

Microbial Strains	Zone of Inhibition (mm)	Antibacterial Activity
Gram Positive		
Staphylococcus albus ATCC 10231	9.4	+++
Streptococcus viridans ATCC 12392	9.6	+++
Gram Negative		
Bacillus procynous ATCC 51189	10.5	+++
Enterobacter aerogenes ATCC 13048	11.2	+++
Klebsiella aerogenous ATCC 10031	10.6	+++
Escherichia coli ATCC 8739	7.2	++
Sallmonella ATCC 6017	11.9	+++
Fungus		
Aspergillus Niger ATCC 16404	7.8	++
Aspergillus fumagatus ATCC 90906	7.8	++
Penicillium ATCC 32333	9.6	+++
Control	0.0	-

The *in vitro* quantitative activity of compound **3** was assessed with determination of minimum inhibitory concentration (MIC) values. It showed antibacterial activity against Gram-positive, *Staphylococcus albus* with MIC level equal to 16 μg/μL, while against *Streptococcus viridians*, it appeared more sensitive and found inhibited at MIC 4 μg/μL. Similarly, zone inhibition diameter was larger at lowest concentration (MIC 4 μg/μL) among the Gram-negative bacteria, i.e. *Bacillus procynous*, *Klebsiella aerogenous*, *Escherichia coli*, *Sallmonella* than *Enterobacter aerogenes* with MIC level 8 μg/μL. The lower potency of compound **3** was found analogously effective to inhibit growth of the fungal strains with MIC 4 μg/μL for *Aspergillus fumagatus*, *Penicillium* and MIC determined for *Aspergillus Niger* was 8 μg/μL (Table 3). The very high activity by means of potency of calixarene derivative (**3**) against most of the bacterial and fungal strains revealed the high level of significance in this study, leading towards the future control over infections caused by problem pathogens. Previously, the noticeable differences in MIC values for bacterial and fungal strains were recorded but present study revealed the MIC level of **3** that was exceptionally simultaneous in action against bacterial and fungal strains at lower concentration excluding one *Staphylococcus albus* (Table 3).

Table 3. **Minimum inhibitory concentration of compound 3 against bacterial and fungal strains.**

Microbial Strains	Disc Content (μg)										MIC μg/μL
	10	20	40	60	80	100	120	140	160	180	
Gram Positive											
Staphylococcus albus	+	+	+	+	–	–	–	–	–	–	16
Streptococcus viridans	+	–	–	–	–	–	–	–	–	–	04
Gram Negative											
Bacillus procynous	+	–	–	–	–	–	–	–	–	–	04
Enterobacter aerogenes	+	+	–	–	–	–	–	–	–	–	08
Klebsiella aerogenous	+	–	–	–	–	–	–	–	–	–	04
Escherichia coli	+	–	–	–	–	–	–	–	–	–	04
Sallmonella	+	–	–	–	–	–	–	–	–	–	04
Fungus											
Aspergillus Niger	+	+	–	–	–	–	–	–	–	–	08
Aspergillus fumagatus	+	–	–	–	–	–	–	–	–	–	04
Penicillium	+	–	–	–	–	–	–	–	–	–	04

Conclusion

New calixarene derivative 5,11,17,28-tetrakismorpholinomethyl-25,26,27,28-tetrahydroxycalix[4]arene (**3)** was synthesized and screened for bioactivity. The results show that all the tested microorganisms including various strains from gram positive, gram negative and fungi were susceptible to compound **3**. Since, compound **3** showed better antimicrobial profile against most of the bacterial and fungal strains therefore; this compound would be extended to further analysis of bioavailability and toxicity.

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