Muneer et al. Journal of Analytical Science and Technology (2017) 8:3
DOI 10.1186/s40543-017-0111-3

Journal of Analytical Science and Technology

RESEARCH ARTICLE Open Access

Synthesis and investigation of antimicrobial properties of pyrrolidine appended calix[4]arene

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Abstract

Background: Calixarenes are widely used macromolecules in supramolecular chemistry synthesized by simple phenol formaldehyde condensation reaction in the presence of base. This article describes the synthesis, antibacterial, and antifungal properties of calix[4]arene appended with pyrrolidine at the upper rim (**CAP3**).

Methods: Antimicrobial activity of **CAP3** was determined by Kirby-Bauer well agar diffusion method in Mueller-Hinton agar (MHA) medium of growth against *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 10231) and *Streptococcus viridans* (ATCC 12392), *Candida albicans* (ATCC 32333), *Aspergillus niger* (ATCC 16404), and *Aspergillus flavus* (ATCC 90906). Structure–activity relationship was also used to evaluate the active site of **CAP3**.

Results: Bacterial strains have shown minimum inhibitory concentration (MIC) values in 1.17–2.34 mg/mL range; whereas, the fungal strains have shown MIC values at the range of 0.58-2.34 mg/mL. It is observed that **CAP3** has excellent antifungal action for *A. niger* as well as antibacterial action for *S. viridans* having MIC values of 0.58 and 1.17 mg/mL, respectively. The SAR (structure–activity relationship) study of compound reveals that there is a significant antimicrobial activity shown by **CAP3** probably due to the pyrrolidine substituents at phenyl rings of calix[4]arene.

Conclusions: The results indicate that **CAP3** is an effective material against the microbial strains.

Keywords: Pyrrolidine, Calix[4]arene, Structure–activity relationship, MIC, Antimicrobial activity

Background

Because of diverse bioactive and potential pharmaceutical characteristics, pyrrolidine and related heterocyclic compounds have possessed specific position among diverse organic classes (Pinto and Abdala 2000). From the last few decades, these molecules have got considerable attention in pharmaceutical industry due to the extensive clinical requests (Mohammat et al. 2011; Feng et al. 2012). It is because of the outstanding antiviral and antibiotic properties of these molecules caused by heterocyclic core, such as pyrrolidine-2,4-dione (natural tetramic acids) (Gallardo et al. 2004; Gitterman et al. 1964; Chen et al. 2008; Gitterman 1965; la Croix et al. 1975; Janardhanan and Husain 1984; Suzuki et al. 1967; Mourer et al. 2009). Arun et al. has prepared and estimated the antimicrobial activity of dispirooxindole-pyrrolidine derivatives via 1,3-dipolar cycloaddition of an azomethine ylide obtained from sarcosine and isatin. Dandia et al. prepared spiro[acenaphthylene-1,2′-pyrrolidine] in the course of 1,3-dipolar cycloaddition reaction of azomethine ylide generated from sarcosin and di/tri ketone. The cycloadducts ketocarbazalo spiro N-methyl pyrrolidines have demonstrated highly remarkable antimicrobial activity even in very low concentrations (Dandia et al. 2013). However, importance in drug delivery and potentially enhanced neuroprotection and anticancer properties often found in novel compounds. In the last few decades, calixarene molecules are found to have multiple uses in basic as well as applied sciences. They are also a class of important macrocyclic molecules like cyclodextrins, crown ethers, and porphyrins, which act as host molecules. Structure of

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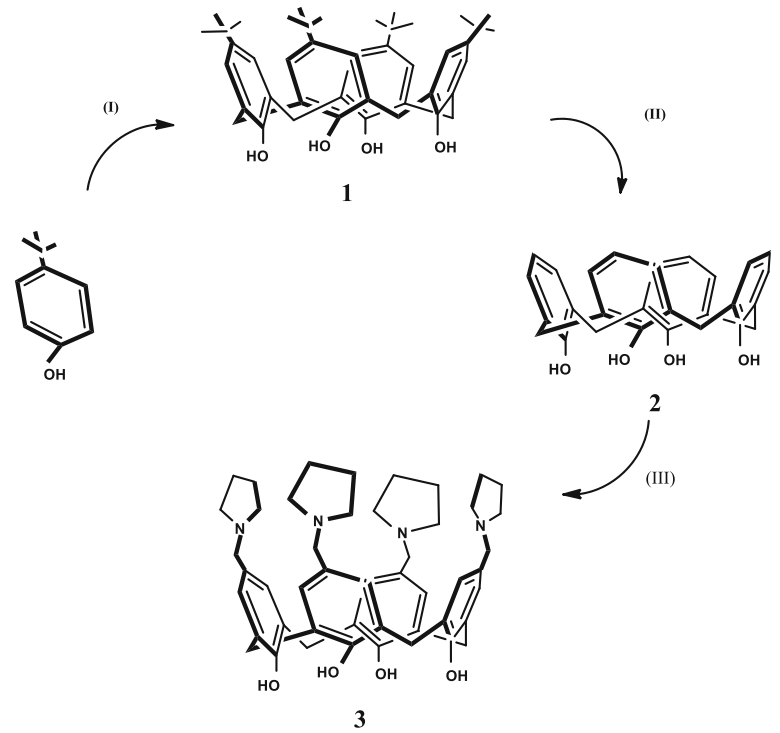

Scheme 1 Synthesis of **CAP3** (I) HCHO/NaOH, (II) $AlCl_3$/Phenol and (III) CH_3COOH/THF, Pyrrolidine/HCHO

calixarenes possesses the lower and upper rims with central annulus part joined via methylene groups, while hydroxyl functionality at meta-position (Memon and Bhatti 2015; Fátima and Fernandes 2009). Because of easy functionalization at the upper and lower rims, calixarenes are widely used in various fields like molecular recognition, nanotechnology, catalysis, sensing, drug delivery, and to mimic the biological processes (Nimsea and Kim 2013). The molecular recognition property makes them a versatile tool for complexation of organic and biomolecules. In this regard, the pioneer work is done by Cornforth et al. against tuberculosis (TB), which will prove to explore the calixarenes' for examining their biological properties. Furthermore, several pharmacological properties including antibacterial, antifungal, antiviral, and anticancer activities (Colston et al. 2004) of calixarenes have also been reported. Thus, herein, we report the synthesis and investigation of calix[4]arene (**CAP3**) containing pyrrolidine moiety and divulge the remarkable screening results against its antibacterial and antifungal activities.

Methods

For synthesis, all reagents were of analytical grade and used without further purification. Mueller-Hinton agar was purchase from Sigma-Aldrich; antimicrobial growth was performed locally prepared at laminar flow cabinet. Preparation of medium was done on vertical autoclave (i.e., Robus Technologies). Antimicrobial growth was carried out on Thermo Scientific incubator. Gallenkamp melting point apparatus was used for the melting point determination and was uncorrected. Elemental analysis was carried out using a CHNS instrument model Flash EA 1112 elemental analyzer. FT-IR spectra were obtained by applying KBr pellet method in the spectral range 400–4000 cm^{-1} on a Thermo Nicollet 5700 FT-IR spectrometer. TLC was carried out on pre-coated silica gel plates (SiO2, Merck PF254).

Synthesis of compound

5,11,17,23-tetra-tert-butyl-25,26,27,28-tetrahydroxycalix[4]arene (**1**), 25,26,27,28-tetrahydroxycalix[4]arene (**2**) as well as 5,11,17,23-tetrakis(N-pyrrolidinomethyl)-25,26,27,28-tetrahydroxycalix[4]arene) (**3**) have been prepared (Scheme 1) following the literature methods (Gutsche and Nam 1988). Various types of instrumental techniques used to validate the synthesis of **CAP3** such as melting

Table 1 Distribution of culture strains and medium

	G +ve bacteria	G -ve bacteria	Fungi
Microorganisms	*S. aureus*	*E. coli*	*A. niger*
	S. viridans		*A. flavus*
			C. albicans
Medium	Mueller-Hinton agar		

Table 2 Zones of inhibition for antimicrobial activities of CAP3

Microorganism strain	Concentration (mg/mL)								MIC value (mg/mL)
		0.58	1.17	2.3	4.7	9.4	18.7	37.5	75	
Bacterial strain	Zones of inhibition (mm)									
G +ve bacteria	*S. aureus*	–	–	17	19	21	25	27	28	2.3
	S. viridans	–	16	17	19	25	25	28	31	1.17
G -ve bacteria	*E. coli*	–	–	13	15	19	20	23	24	2.3
Fungi	*A. niger*	16	19	20	22	27	29	31	32	0.58
	A. flavus	–	15	16	18	21	21	23	26	1.17
	C.albicans	–	–	13	14	17	19	19	20	2.3
Control (DMSO)[†]		–ve	–ve	–ve	–ve	ve	–ve	–ve	–ve	–

[†]DMSO solvent was used as –ve control

point, TLC, and elemental analysis as well as FT-IR techniques.

5,11,17,23-tetrakis (N-pyrrolidinomethyl)-25,26,27,28-tetrahydroxycalix[4]arene)

2.8 mmol of compound (**2**) was dissolved in 45 mL of THF with of 37% formaldehyde (12 mmol) added drop wise to reaction mixture at room temperature. By the addition of pyrrolidine (12 mmol), the reaction mixture start to become yellowish wax type. The solution was stirred for 24 h and filtered, washed with water and crystallized in methanol to furnished (**3**).

Antimicrobial studies

The biological activities of pyrrolidine appended calix[4]-arene **CAP3** have been explored, which exhibited excellent antibacterial and antifungal activities. These activities were evaluated using well diffusion method. The dimethylsulphoxide (DMSO) has been used as –ve control with standard solution of (150 mg/mL) has been prepared (Volonterio and Zanda 2008). The antifungal property has been carried out against *Aspergillus niger* and *Candida albicans* as well as *Aspergillus flavus.* Likewise, antibacterial property has been studied against Gram +ve (*Staphylococcus aureus* and *Streptococcus viridans*) as well as Gram -ve (*Escherichia coli*). Study has revealed that **CAP3** is significantly effective against both the strains. Inhibition zones have been measured in millimeter (mm) for all microorganisms. MIC (minimum inhibitory concentration) data has also been evaluated for the same species (Table 1) provide classification of bacterial, fungal species, and medium.

Microbial assay

Various methods were employed for the determination of the antimicrobial activity. Antimicrobial activity of **CAP3** was tested by using Kirby-Bauer well diffusion method (Perret and Coleman 2011). Different concentrations were used for compound **CAP3** ranging from 75.0, 37.5, 18.7, 9.37, 4.68, 2.34, 1.17, and 0.58 mg/mL in dimethylsulphoxide (DMSO) (Zgoda and Porte 2001). A well was developed in the sterile Petri dish with medium

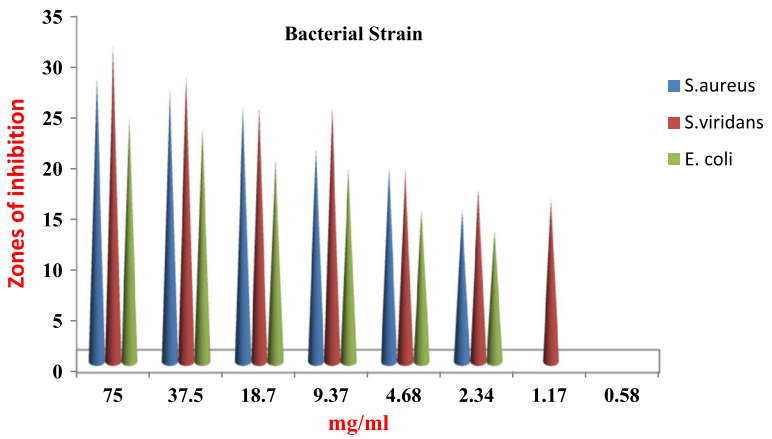

Fig. 1 Antibacterial activity of various concentrations of **CAP3** against different bacterial strains obtained by well diffusion method. The concentration was used 0.58–75.0 mg/mL at room temperature

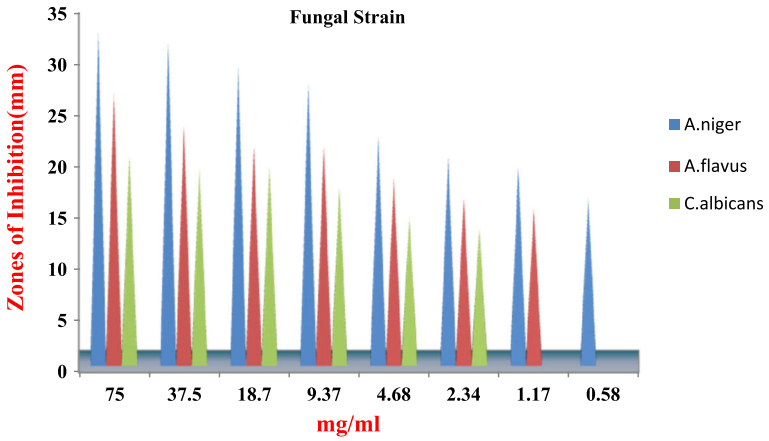

Fig. 2 Antifungal activity of various concentrations of **CAP3** obtained by way of well diffusion method. At room temperature and concentration was used 0.58–75.0 mg/mL

plates were prepared with the help of cork borer (6 mm in diameter) and (50 μL) solution of each concentration was poured into the wells. Under sterilized condition, wells were filled with diluted solution; micropipette was used for filling purpose. This work was done at room temperature and stored them for further use. Sterilized Petri dishes were incubated for 48 h at 37 °C. At the same time, DMSO as –ve control was employed, and the solution of **CAP3** was also prepared in the same solvent. While the relative antifungal and antibacterial activities in millimeter were measured for evaluation. The results were studied in triplicate. MIC data have also been appraised (Dumazet et al. 1997; Mistry and Desai 2014).

Results and discussion

Calixarenes have been proved to be a good platform for number of valuable bioactive compounds/molecules (Fátima and Fernandes 2009). The antimicrobial activity of **CAP3** compound was studied using well diffusion method against Gram +ve and Gram -ve bacteria as well as fungi. Thus, it is observed that the antibacterial as well as antifungal action of **CAP3** derivative is significant as presented in (Table 2).

It shows that **CAP3** exhibits good antibacterial action against Gram +ve bacteria comparative to Gram -ve. It also shows better antifungal activity against *A. niger* than all other selected fungal strains due to morphological change in their structures.

Antibacterial activity

Number of polymers like calix[4]arene have shown antibacterial action, the first study emerged in 1955; in that study, macrocyclon has efficiently worked against tuberculosis, vancomycin, and some other types of mycobacteriosis models (Casnati et. al. 1996; Cornforth et al. 1955). The antibacterial action of newly prepared **CAP3** derivative has been comprehensively explored and calculated against various species of Gram +ve bacteria such as *S. viridans* and *S. aureus* and bacteria like *E. coli* (Gram -ve) (Muneer et al. 2016; Rao and Chakraborty 2014). **CAP3** shows appreciably good antibacterial action for *S. viridans* (Gram +ve bacteria), while it shows variations against other antibacterial strains (Fig. 1) (Saba et al. 2015).

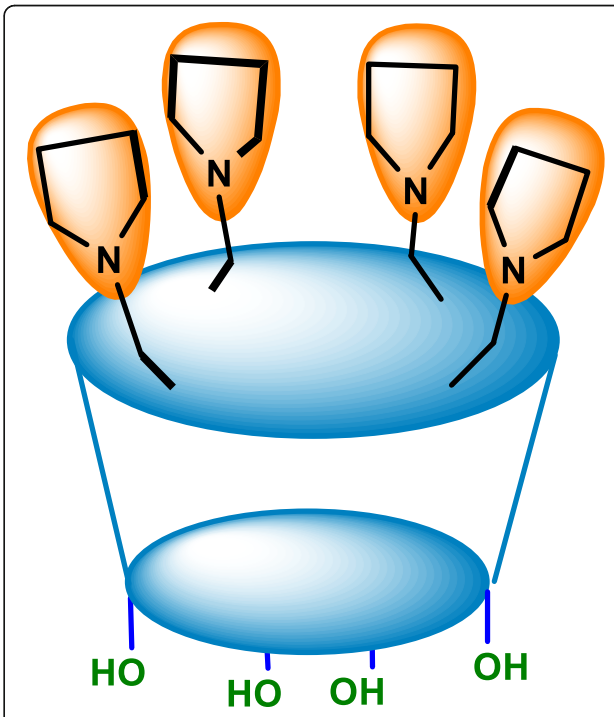

Fig. 3 Proposed mechanism showing active functionalities for structure–activity relationship

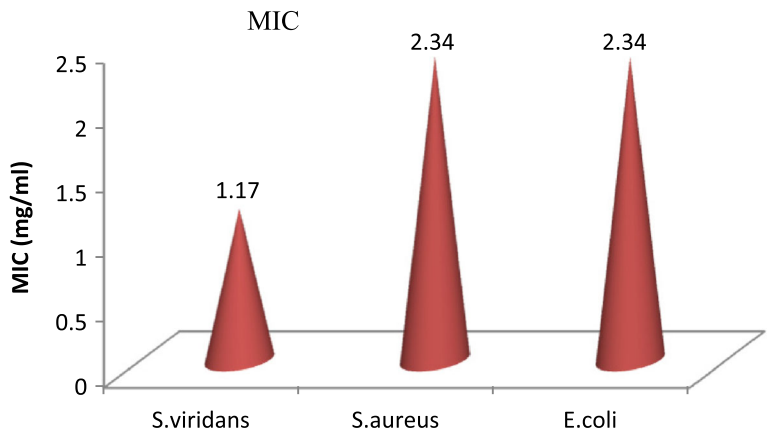

Fig. 4 CAP3 showing MIC data against bacterial species at room temperature and 1.17 mg/mL concentration

Antifungal activity

CAP3 has also been examined for antifungal action against *C. albicans*, *A. flavus*, and *A. niger*. The results show that it effectively inhibit fungal species. Among them, *A.niger* has displayed the biggest zone of inhibition than remaining fungal species due to morphologically changed structures; thus, **CAP3** is more active against *A. niger* for antifungal strain (Fig.2).

Structure–activity relationship hypothesis

Structure–activity relationship (SAR) evaluation clarifies that electron-donating/electron-withdrawing groups in compounds are found to have more antimicrobial properties relative to their precursors. The pyrrolidine ring type compounds due to structural variation show more bioactivity and attract attention of many research groups. Tsou and coworkers have also reported some natural molecules, which include 2-aryl pyrrolidine type skeleton (Han et al. 2012). However, the derivative has increased its antimicrobial action because of electron releasing moieties causing net increase than precursor as shown in graphical structure (Fig.3).

Minimum inhibitory concentration

The necessary concentration required for inhibiting completely the growth of bacterial and fungal strain is called minimum inhibitory concentration (MIC) (Wang et al. 2015; Mojab et al. 2008). However, quantitatively valuable MIC data have been assessed for **CAP3** using different concentration extending from 75.0, 37.5, 18.7, 9.37, 4.68, 2.34, 1.17 up to 0.58 mg/mL for testing the fungal and bacterial strains. The data have been provided in (Table 2) as well as illustrated in (Fig. 4). MIC data have described a significant antibacterial activity of compound **CAP3** for *S.viridans* at 1.17 mg/mL as given in (Fig. 4). *S. aureus* is found to have lower antibacterial action at 2.34 mg/mL

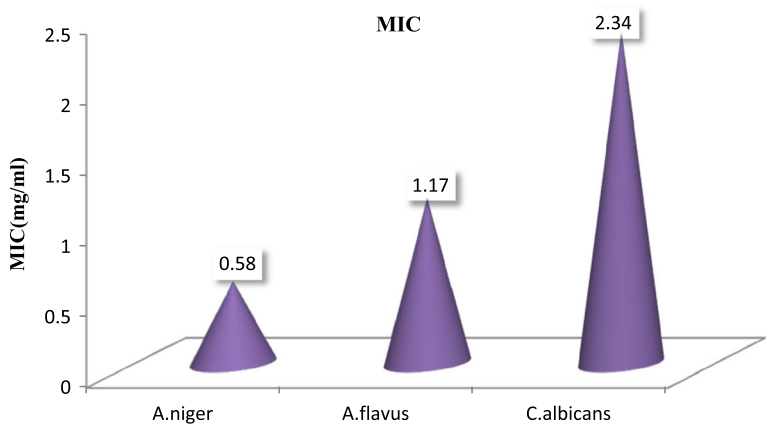

Fig. 5 Minimum inhibitory concentration (MIC) of **CAP3** against fungal strains. The concentration was used 0.58 mg/mL at room temperature

MIC value. In addition, *E. coli* has also lower activity showing MIC value of 2.34 mg/mL.

MIC value of compound **CAP3** for antifungal activity has been observed in 0.58–2.3 mg/mL range. It illustrates good antifungal activity against *A. niger* at the concentration of 0.58 mg/mL, while the MIC data of **CAP3** against *A. flavus* is 1.17 mg/mL which seem to be the moderate antifungal activity; whereas it has shown very low activity at 2.34 mg/mL concentration against *C. albicans* (Fig. 5).

Conclusions

In the present study, pyrrolidine appended calix[4]arene (**CAP3**) was synthesized and explored for its antimicrobial activity using Kirby-Bauer well agar diffusion method. The study revealed that **CAP3** show good antimicrobial activity against selected bacterial and fungal strains. The MIC values calculated are in the range 1.17–2.34 and 0.58–2.34 mg/mL for bacterial and fungal strains, respectively. The convenient synthetic scheme and low toxicity as well as cost effectiveness of the starting materials make **CAP3** a promising candidate for the pharmaceutical formulations against the tested microorganisms. Consequently, the study shows a good impact for controlling the infections created by different microorganism in future.

Authors' contribution

All authors read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interests.

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Received: 16 August 2016 Accepted: 24 January 2017
Published online: 09 February 2017

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