The host-guest system of resorcinolcalix[4]arene and pyridine-containing compounds: their syntheses, structures, antioxidant and antibacterial activity analyses

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Abstract

Self-assembly of C-iso-butyl-resorcinolcalix[4]arene (CBCR) with 4-aminopyridine (4-AP) or 3-dimethylaminopyridine (3-DMAP) in ethanol afforded two host-guest complexes CBCR4-AP (1) and CBCR2(3-DMAP) (2), respectively. Complexes 1 and 2 were characterized by FT-IR, 1H NMR spectroscopies and single crystal X-ray diffraction analysis. The antioxidant and antibacterial experiments were conducted on complexes 1 and 2. The DPPH and ABTS free radical scavenging rate could reach 91.73% and 98.55% when the concentration of complex 2 was 500 and 31.25 mg/mL, respectively. The clearance rate of complex 1 for both free radicals is weaker than 2. When the concentration of 1 was 31.25 mg/mL, the ABTS free radical scavenging rate was 74.60%. The antibacterial test of complexes 1 and 2 showed that the antibacterial circle diameter of complex 1 against Escherichia coli was 10.25 mm, with a MIC value of 12.5 mg/mL, and the antibacterial circle diameter against Staphylococcus aureus was 14.33 mm with a MIC value of 3.12 mg/mL; complex 2 has a diameter of 11.27 mm and a MIC value of 12.5 mg/mL for Escherichia coli, and a diameter of 16.40 mm and a MIC value of 1.56 mg/mL for Staphylococcus aureus. Co-crystallization of pyridine-containing compounds and CBCR might enhance antioxidant and antibacterial activity.

Introduction

Resorcinolcalix[4]arene is a kind of the third-generation supramolecular macrocyclic compound with excellent properties after crown ether and cyclodextrin [1], which has broad application prospects in molecular self-assembly, host-guest chemistry and other fields. Self-assembly of C-alkyl-calix[4]resorcinarenes with organic molecules have been extensively investigated to study host-guest interactions, molecular recognition, container catalysis, and encapsulation of a guest within discrete capsules [2−5]. Therefore, the hydrocarbons of the resorcinolcalix[4]arene have a unique ability to contain guest species, especially in constructing molecular containers with large cavities [6]. For example, Aoki et al. demonstrated the formation of host-guest 1:1 multi-component complexes between resorcin[4]arene as a host and DL-pipecolinic acid as a guest, and found that C−H π interaction was a major factor responsible for this complexation [7]. Maija Nissinen, Kari Rissanen et. al. [8] studied the hydrogen bonding self-assembly of chair type C-methyl-resorcinol calix[4]arene, using the calix[4]arene host and melamine to form a multi-component complex. In addition, a major advantage of calixarenes in biochemistry is their lack of obvious cytotoxicity and immunogenicity [9,10], and their flexible conformation, making them a platform for synthesizing bioactive compounds [11]. This makes calixarenes one of the valuable candidate drugs for anticancer, antioxidant, and antibacterial activities, and even as drug carriers [12−14]. In addition, the introduction of pyridine-containing small molecules has biological activity, and the introduction of amino and methyl groups can further enhance the biological activity of compounds, such as antibacterial properties [15], and the amino group at the C-4 position of the pyridine ring should be an important factor affecting antioxidant capacity [16]. Intramolecular hydrogen bonding (IHB) also plays an important role in the mechanism of antioxidant activity as revealed by noncovalent interaction analysis and rotational barrier calculations [17]. Due to its
properties, including alkalinity, water solubility, stability, ability to establish hydrogen bonds, and its tiny molecular size, pyridine fractions are often used in pharmaceuticals [18,19]. The incorporation of pyridine motifs into pharmaceutical products can improve the biochemical potency and metabolic stability of the product [20], as well as its permeability and difficulty in forming protein-binding interactions [21]. In 2013, Kim reported that 3-aminoconjugates containing pyridine groups have activity against most Gram-positive bacteria in vitro [22]. Hu proposed that the amino group at the C-4 position of the pyridine ring should be an important factor affecting the antioxidant capacity of synthetic inulin derivatives [16].

Research on antibacterial mechanisms has shown that this compound can directly damage the integrity of bacterial cell membranes, leading to bacterial cell contents leakage and death. This membrane active mode of action provides a new design approach for the development of new membrane active antibacterial agents.

However, researches in this field are still worth more exploring. Our group has a long term interest in the functionalization of resorcinolcalix[4]arene [23,24] and syntheses of supramolecules involving resorcinolcalix[4]arene and 4,4'-bipyridine [25,26]. In this paper, we will introduce the syntheses and structures of two host-guest multi-component complexes formed by C-iso-butylcalix[4]arene and two small molecules containing pyridine groups (4-aminopyridine and N,N'-dimethylpyridine-3-amine) (Scheme 1), and the biological activity of the synthesized complexes was tested through antioxidant and antibacterial experiments.

**Experimental section**

**General**

All solvents were commercial products of high purity and used as received. C-iso-butylcalix[4]resorcinarene (CBCR) was prepared according to procedures described in the literature [27]. 4-Aminopyridine (4-AP) and 3-dimethylaminopyridine (3-DMAP) was purchased from Alfa Aesar Ltd. Tryptone, peptone, yeast extract, and agar are all biological grade reagents from Shanghai Yuanye Biotechnology Co., Ltd; Kanamycin solution is a 100 mg/mL aqueous solution (sterile) from Shanghai McLean Biochemical Technology Co., Ltd; DPPH, ascorbic acid, and ABTS all have a purity of 99%, sourced from Shanghai McLean Biochemical Technology Co., Ltd; Bacterial Escherichia coli ATCC 25922 from Xinjiang University; Bacterial staphylococcus aureus CMCC (B) 26003 comes from Shanghai Luwei Technology Co., Ltd; 1H NMR spectra were recorded on a Bruker DPX-400 using tetramethylsilane as internal standard. Infrared spectra were recorded on a PerkinElmer 16 PC FT-IR spectrophotometer with use of pressed KBr pellets in the region of 400–4000 cm⁻¹.

**Preparation of multi-component complex CBCR·(4-AP) (1)**

To a warm solution of CBCR (70 mg, 0.10 mmol) in ethanol (10 mL) was added 4-AP (10 mg, 0.10 mmol) with stirring. The mixture was stirred for 2 h until a clear solution was obtained. The solution was cooled to room temperature and allowed to stand for crystallization. Brown bulk-shaped crystals were found
after 7 days and identified as CBCR-(4-AP). The crystals were isolated and allowed to dry for 1 day in air. Yield: 50 mg, 63%. IR spectrum (KBr, cm⁻¹): ν (-NH₂: 3330, 3220); ν (-alkyl: 2960, 2867); ν (C = C_AR/C = N_AR) 1650, 1500; ν (C−O/C−N) 1260, 1090. ¹H NMR (400 MHz, DMSO-d₆) δ 8.91 (s, 8H, ArOH), 8.00 (d, J = 8.0 Hz, 2H, CH= N), 7.15 (s, 4H, ArHₜₐₚₜ), 6.54 (d, J = 8.0 Hz, 2H, CH = CNH₂), 6.50 (s, 2H, NH₂), 6.16 (s, 4H, ArHₙₙₙₙ), 4.38 (t, J = 8.0 Hz, 4H, ArCH₂), 1.90 (m, 8H, CH₂CH(CH₃)₂), 1.34 (m, 4H, CH₂CH(CH₃)₂), 0.89 (d, J = 4.0 Hz, 24H, CH₂CH(CH₃)₂). In these data, we arbitrarily normalized the peaks corresponding to the resorcinarene and 4-AP groups. The ratio of resorcinarene to 4-AP is 1:1.

Preparation of multi-component complex CBCR·2(3-DMAP) (2)

To a warm solution of CBCR (70 mg, 0.10 mmol) in ethanol (10 mL) was added 3-DMAP (24 mg, 0.20 mmol) with stirring. The mixture was heated for 30 min until a clear solution was obtained. The solution was cooled to room temperature and allowed to stand for crystallization. Brown bulk-shaped crystals were found after 10 days and identified as CBCR·2(3-DMAP). The crystals were isolated and allowed to dry for 1 day in air. Yield: 65 mg, 80%. IR spectrum (KBr, cm⁻¹): ν (-alkyl: 2961, 2866); ν (C = C_AR/C = N_AR) 1650, 1500; ν (C−O/C−N) 1260, 1100. ¹H NMR (400 MHz, DMSO-d₆) δ 8.91 (s, 8H, ArOH), 8.13 (d, J = 8.0 Hz, 4H, CH= N), 7.15 (s, 4H, ArHₜₐₚₜ), 6.68 (d, J = 8.0 Hz, 4H, CH = CNH₂), 6.16 (s, 4H, ArHₙₙₙₙ), 4.38 (t, J = 8.0 Hz, 4H, ArCH₂), 3.01 (s, 12H, N(CH₃)₂), 1.91 (m, 8H, CH₂CH(CH₃)₂), 1.34 (m, 4H, CH₂CH(CH₃)₂), 0.88 (d, J = 4.0 Hz, 24H, CH₂CH(CH₃)₂). In these data, we arbitrarily normalized the peaks corresponding to the resorcinarene and 3-DMAP groups. The ratio of resorcinarene to 3-DMAP is 1:2.

X-Ray crystallography

Crystallographic data and experimental detail for compounds 4, 5 and 6 are summarized in Table S1 (see Supporting Information). Suitable single crystals were selected and mounted on a Bruker SMART Apex CCD area-detecting diffractometer for study using graphite-monochromated Mo-Kα (λ = 0.71073 Å) radiation at room temperature. Data were measured using ω scans of 0.5° per frame, such that a hemisphere was collected. Cell parameters were retrieved using SMART software and refined using SAINT on all observed reflections [28]. Data reduction was performed with SAINT software which corrects for Lorentz polarization and decay. Absorption corrections were applied using SADABS [29]. Structure was solved by direct methods using SHELXS-97 and refined by least squares on F² (SHELXL-97) [30,31]. Hydrogen atoms were placed in the idealized positions and refined employing a riding model with thermal parameters 1.5× or 1.2× those of the bond carbon atoms.

Crystallographic data for complexes 1 and 2 have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 2297121 and 2297122, respectively. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: (+44)1233-336-033; e-mail: deposit@ccdc.cam.ac.uk].

DPPH (2,2-diphenyl-1-picrylhydrazine) radical scavenging experiment [32,33]
A total of 10 mg of DPPH was inserted into a 100 mL flask and dissolved with DMSO up to the boundary marker, then stored in dark. Dissolved complex 1/2 (10.0 mg) in DMSO (10 mL), which was then diluted with additional DMSO to obtain testing solutions. Add the corresponding reagents to a 10 mL colorimetric tube according to Table 1, and reacted in dark for 30 minutes at room temperature (37°C). The incubated mixture poured into the cuvette then measured the absorbance by UV-Vis spectrophotometer at wave length of 517 nm. DMSO as the blank, DMSO and DPPH mixed solutions as the control, and ascorbic acid as the positive control. Measured the absorbance values of each tube, and repeated the experiment three times to take the average value. The formula to measure the capacity of antioxidant from absorbance of each test solution is as follows:

\[
\text{Clearance Rate } \% = \frac{A_0 - (A_s - A_c)}{A_0} \times 100 \%
\]

\(A_0: 2\) mL DPPH + 2 mL DMSO; \(A_s: 2\) mL of DPPH + 2 mL of sample solution; \(A_c: 2\) mL sample solution + 2 mL DMSO.

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<th>DMSO/mL</th>
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**ABTS (2,2'-hydrazine bis(3-ethylbenzothiazoline 6-sulfonic acid) radical scavenging experiment**
The ABTS assay was tested using the method described before [34,35]. The ABTS (4.00 mg) was dissolved in ultra-pure water (0.98 mL) into a 7.40 mM concentration. ABTS●+ was produced by reacting the ABTS stock solution with 2.60 mM potassium persulfate, which was allowed to stand in the dark at room temperature (37°C) for 12–16 h before use. Prior to the assay, the stock solution was diluted in ethanol (about 1:89 v/v) and equilibrated at room temperature to give an absorbance of 0.700 ± 0.02 in a 1 cm cuvette detected at 734 nm. The reaction mixture was prepared by mixing 3.2 mL of ABTS solution, and 0.8 mL of samples at different concentrations in a 50% (v/v) methanol solution. After a mild vortex, the samples were kept at room temperature for 6 min and measured at 734 nm to obtain their absorbance values. The scavenging percentage was calculated according to the following equation:

\[ \text{Clearance Rate} \% = \frac{A_0 - A_1}{A_0} \times 100\% \]

\((A_0: 3.2 \text{ mL ABTS dilution + 0.8 mL DMSO}; A_1: 3.2 \text{ mL ABTS dilution + 0.8 mL sample for test solution}).\)

The test was repeated three times to take the average, with absolute ethanol as blank and DMSO as the control.

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**Antimicrobial experiments** [36]

Configuration of LB solid medium: tryptone (1 g), yeast extract (0.5 g) and NaCl (1 g) were added to deionized water (50 mL), agar (1.5 g) was added (pH = 7.0). Then set the volume to 100 mL, and sterilized it at 121°C for 20 min. The medium was incubated at 37°C for 24 hours to observe whether it was infected. Bacterial culture: pipette 10 µL of bacteria and incubate for 24 h in an air incubator, inoculate single colonies of S. aureus and E. coli in LB liquid culture, and incubate at 37°C for 24 h.

Preparation of drug discs: the sample (200 mg) was dissolved in dimethyl sulfoxide (DMSO, 1000 µL) to obtain a sample solution (200 mg/mL), sterilized and diluted with DMSO to obtain sample dilutions (100,
50, 25, 12.5, 6.25, 3.12, 1.56, 0.781 mg/mL), three discs were soaked in diluent, UV sterilized and dried under sterile conditions. Agar disc diffusion method for measuring drug inhibitory activity: Bacterial suspension (100 µL) was coated on an agar plate and placed medication disc, samples were screened. Place different concentrations of antimicrobial active drug disc on an agar plate and incubate at 37°C for 12 hours (bacteria). Measure the diameter of inhibition zone in millimeters. Repeat the experiment three times and take the average value.

Results and discussion

Co-crystallization of CBCR with 4-AP or 3-DMAP in ethanol solution afforded a 1:1 multi-component complex CBCR⋅4-AP (1) and 1:2 complex CBCR⋅2(3-DMAP) (2), respectively (Scheme 1). IR spectrum of complex 1 showed two stretching vibrations at 3330 and 3220 cm⁻¹, assigned as the -NH₂ stretching frequency of 4-AP. The iso-butyl groups in complexes 1 and 2 were observed at about 2960 cm⁻¹ as strong peaks. The C – N/C – O stretching vibrations of complexes 1 and 2 were observed at about 1650 1250 and 1100 cm⁻¹. In the ¹H NMR spectra of complexes 1 and 2, the chemical shift of Ar-H_upper and Ar-H_lower protons exhibited as two singlets at 7.15 and 6.16 ppm, respectively, indicative of the C₄ᵥ isomer character. The bridging Ar₂CHCH₂ protons exhibited as a triplet at around 4.38 ppm with a coupling constant of 8.0 Hz, an indication of the integrity of CBCR. Additionally, two double peaks at 8.00 and 6.54 ppm were observed in ¹H NMR spectrum of complex 1, assigned as NCH=CHC protons on the 4-AP ring (Fig. 1). Similarly, the 3-DMAP component was clearly observed in ¹H NMR spectrum of complex 2 (SI). For example, the typical N(CH₃)₂ protons presented as a singlet at 3.01 ppm. The integral value of CBCR and 4-AP/3-DMAP presented their molar ratio was 1:1 and 1:2, respectively, in their ¹H NMR spectra.

Complex 1 crystallized in the monoclinic space group P2₁/n. Its molecular structure is shown in Fig. 2a. There are obvious intermolecular hydrogen bonds in the multi-component complex 1, which could be classified into two categories: hydrogen bonds between the hydroxyl groups of resorcinolcalix[4]arene, i.e. O – H···O hydrogen bonds [O1···O8 = 2.829(7) Å, O1 – H1···O8 = 171.7º; O2···O3 = 2.536 Å, O2 – H2···O3 = 160º; O4···O5 = 2.810(9) Å, O4 – H4···O5 = 157.0º; O6···O7 = 2.626(7) Å, O6 – H7···O7 = 147.3º]; the hydrogen bond between the hydroxyl group and nitrogen atoms from 4-AP, i.e. [O1···N2 = 3.149(8) Å, O1 – H1···N2 = 161.1º; O3···N1 = 2.875(13) Å, O3 – H3···N1 = 149.6º]. It could be concluded that the O – H···N hydrogen bond is the major factor responsible for the formation of complex 1. The small 4-AP molecules cannot be totally encapsulated in the cavity, as shown in Fig. 2b. Encapsulating the guest molecule in the calixarene bowl cavity is also a direction for further research. From the a-axis side view Fig. 2c, it can be seen that the two calixarene molecules were arranged in a staggered manner with the cup mouths facing each other. As shown in top view Fig. 2d, the units of resorcinolcalix[4]arene and 4-AP were alternately arranged, and the molecules of resorcinolcalix[4]arene were arranged in parallel.

Complex 2 crystallized in the triclinic space group P, whose asymmetric unit is displayed in Fig. 3a. As with complex 1, four intramolecular hydrogen bonds [O3···O7 = 2.629(8) Å, O3 – H3···O7 = 156.4º; O5···O8 = 2.691(7) Å, O5 – H5···O8 = 160.3º; O1···O6 = 2.625(7) Å, O1 – H1···O6 = 158.0º; O4···O2 = 2.765(7) Å, O4 –
H4···O2 = 173.1°] were formed between the hydroxyl groups in complex 2 (Fig. 3b). Unlike multi-component complex 1, the intermolecular hydrogen bond in multi-component complex 2 is formed by the hydrogen of the methyl group of 3-DMAP and the hydroxyl group of CBCR [C46···O1 = 2.651(5) Å, C(46) – H(46)···O(1) = 159.3°, C41···O4 = 2.654(7) Å, C41 – H41···O4 = 161.5°], displayed in Fig. 3b. The 3-DMAP molecules assemble with the CBCRs through C–H···O hydrogen bonds. The guest molecule might be encapsulated by two CBCRs. As shown in Fig. 3c, it could be seen that two calix[4]arene molecules were arranged with the cup mouths facing each other.

DPPH radicals are stable free radicals centered around nitrogen, its stability comes from the spatial barriers and resonance stabilization of the three benzene rings, which make unpaired electrons on the nitrogen atom sandwiched in the middle unable to exert their expected electron pairing effect [37]. The peaks of the benzene ring around 300 nm can be seen in Figs. 4a, 4c, 4e, while looking at Figs. 4b, 4d, and 4f, new broad peaks have been generated in the range of 400–600 nm in all three figures except for the peaks of the benzene ring. In Fig. 4d, there was a great peak difference between the test sample and the control group (7) at 517 nm, followed by Fig. 4b. Figure 4f shows that there was no significant peak difference between the test sample and the control. Therefore, it could be seen that complex 2 has the most obvious scavenging effect on DPPH free radicals, while complex 1 has a poor effect on DPPH free radicals. In addition, the DPPH radical scavenging formula was used to calculate the DPPH radical scavenging rate of each compound, and Fig. 4g was obtained. When the concentration of complex 2 reached 62.5 mg/mL, the upward trend of the curve tends to flatten out, and at this concentration, the DPPH clearance rate of complex 2 reached 73.16%, while the DPPH clearance rate of CBCR was only 45.10%. When the concentration of complex 2 was 500 mg/mL, the DPPH clearance effect was equivalent to that of the positive control VC, indicating that complex 2 interacts with DPPH free radicals, and the addition of 3-DMAP can enhance the DPPH free radical scavenging effect of the raw material CBCR. The possible reason is the formation of C–H···O hydrogen bonds between calixarenes and 3-DMAP, which have many hydrogen bonds and strong bond energy. The addition of 4-AP weakens the DPPH radical scavenging effect, indicating that the co-crystallization of compound 4-AP with CBCR was not conducive to the DPPH radical scavenging effect of CBCR.

According to Figs. 5a, b and c, it was found that all the three compounds produced relatively weak peaks in the range of 700 ~ 750 nm. By calculating the ABTS radical scavenging rate formula, Fig. 5d was obtained. It can be seen that the ABTS free radical scavenging effects of the three compounds are relatively similar, with complex 2 having the best ABTS free radical scavenging effect. Since the concentration of complex 2 decreased to 31.25mg/mL, the curve has flattened, and the clearance rate has reached as high as 98.55%. At this concentration, the clearance rate of CBCR is only 78.49%, and the clearance rate of complex 1 is only 74.60%. This also indicates that co-crystallization of 3-DMAP with the raw material can enhance the ABTS radical scavenging effect of the raw material CBCR, and complex 2 can serve as a good antioxidant. However, co-crystallization of 4-AP with the raw material did not enhance the ABTS radical scavenging effect of the raw material CBCR, indicating that compound 4-AP has an antagonistic effect on antioxidant activity against CBCR.
After bacteriostatic activity test, Table S2 (see Supporting Information) was obtained and these data were shown in Fig. 6. As can be seen from the Fig. 6a, compared 4-AP and complex 1, 3-DMAP and complex 2, it can be found that CBCR has no obvious effect on both Gram bacteria, and the effects of compounds 4-AP and 3-DMAP were not satisfactory. The inhibition zone diameters of complexes 1 and 2 on both Grams were close in diameter to kanamycin, indicating that 4-AP and 3-DMAP co-crystallized with CBCR can enhance the inhibitory effect on Gram bacteria. The pyridine skeleton generates a suite of flexibility, leading to the formation of libraries of compounds bearing a variety of functional groups. This is due to its characteristic solubility, basicity, and ability of hydrogen bond-formation chemistry [38]. Then, compared the inhibition zone diameters of complexes 1 and 2 against E. coli and S. aureus, multi-component complex 1 had an antibacterial zone diameter of 10.25 mm on E. coli, and a diameter of 14.33 mm on S. aureus; multi-component complex 2 has a diameter of 11.27 mm on E. coli, and a diameter of 16.40 mm on S. aureus, it can be found that the inhibition zone diameters of both complexes against S. aureus were larger, indicating that the antibacterial effect on S. aureus is better. It is known that Gram-positive bacteria are more sensitive than Gram-negative ones [39], because the cell structure of their wall, with a principal peptidoglycan, p enables the hydrophobic compounds to infiltrate the cells and proceed on the wall as well as on the cell membrane and inside the cytoplasm. The cell structure of the Gram-negative bacteria’s wall is more complex with a reduced amount of peptidoglycan and with an external membrane composed of a phospholipid’s double-layer connected with the internal membrane by lip polysaccharides [40]. In addition, the minimum inhibitory concentration of complexes 1 and 2 for both Grams was determined, and as the concentration decreases, the inhibitory zone disappeared at a certain concentration, and the minimum inhibitory concentration was obtained. Figure 6b showed the minimum inhibitory concentration (MIC) values of each compound against Gram bacteria, with a MIC value of complex 1 for S. aureus was 3.12 mg/mL, and a MIC value of complex 2 for S. aureus was 1.56 mg/mL, it further indicated complexes 1 and 2 have a relatively good inhibitory effect on Gram negative bacteria S. aureus. Compound 2 has better inhibitory effect on both gram bacteria, possibly due to the excellent antimicrobial biological properties of heterocyclic aminopyridine [41], and the possible reason is the formation of C−H···O hydrogen bonds between calixarenes and 3-DMAP, which have more hydrogen bonds and strong bond energy. Ionic interactions provide the release of ion responsive fungicides in the physiological environment [42].

In summary, the present work has demonstrated the formation of a host–guest 1:1 complex with CBCR as the host and 4-AP as the guest. And the formation of a host–guest 1:2 complex with CBCR as the host and 3-DMAP as the guest. It was obvious that the interaction of intramolecular and intermolecular hydrogen bonds was the main factor in forming the host guest complex network structure. The intramolecular hydrogen bonds of two complexes were mainly O−H···O hydrogen bonds, while intermolecular hydrogen bonds for complex 1 were mainly O−H···N hydrogen bonds, and for complex 2 were mainly C−H···O hydrogen bonds. The antioxidant tests of multi-component complexes 1 and 2 showed that complex 2 had good free radical scavenging effects on both DPPH and ABTS. When its concentration was 62.5 mg/mL, the DPPH clearance rate had reached 73.16%, while at a concentration of 500 mg/mL, the DPPH free radical scavenging rate reached 91.73%; and complex 2 had a more
significant effect on the free radical scavenging of ABTS. When its concentration was 31.25 mg/mL, the ABTS free radical scavenging rate reached 98.55%. The clearance rate of complex 1 for both free radicals was weaker than complex 2. The preliminary screening results showed that complex 1 had an antibacterial circle diameter of 10.25 mm and a MIC value of 12.5 mg/mL for E. coli, and a diameter of 14.33 mm and a MIC value of 3.12 mg/mL for S. aureus; complex 2 has a diameter of 11.27 mm and a MIC value of 12.5 mg/mL for E. coli, and a diameter of 16.40 mm and a MIC value of 1.56 mg/mL for S. aureus, this was also the best inhibitory effect achieved, indicating that the co-crystallization of 3-DMAP and CBCR can enhance antioxidant and antibacterial activity. With these results, we are interested in continuing to study the molecular structure of calixarenes and drug molecules containing pyridine groups.

**Declarations**

**Acknowledgements**

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**References**


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**Scheme 1**

Scheme 1 is available in the Supplementary Files section.

**Figures**
Figure 1

$^1$H NMR spectrum of complex 1
Figure 2

(a) The asymmetric unit of multi-component complex 1. (b) Filling diagram of complex 1. (c) The alternating cup to cup arrangement structure of complex 1 viewed along $a$-axis. (d) The two alternating layers of complex 1 viewed along $c$-axis.
Figure 3

(a) The asymmetric unit of multi-component complex 2. (b) Filling diagram of complex 2. (c) The two alternating layers of complex 2 viewed from a-axis (hydrogen bonds are shown as dashed lines).
Figure 4

UV–vis spectra of CBCR (a), complex 2 (c), and complex 1 (e) dissolved in DMSO solution; full wavelength spectra of CBCR (b), complex 2 (d), and complex 1 (f) for DPPH radical scavenging; (g) DPPH free radical scavenging rates of CBCR, complexes 1 and 2 (Details of the specific concentrations of 1-13 containing the samples were shown in Table 1. The test wavelength for DPPH radical scavenging is 517 nm).
Figure 5

(a) full wavelength spectra of CBCR (a), complex 2(b), and complex 1 (c) for ABTS radical scavenging; (d) ABTS free radical scavenging rates of CBCR, complexes 1 and 2 (Details of the specific concentrations 1-7 containing the samples were shown in Table 2. The test wavelength for ABTS radical scavenging is 734 nm).
Figure 6

(a) Zone of inhibition of compounds against two bacterial strains; (b) MIC value of compounds against two bacterial strains.

Supplementary Files

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- clssupportinginformation20231012.docx
- Scheme1.png