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Synthesis of Fluorescent Calix[4]arene Derivatives for the Detection of Hg(II) / Cr(VI) and Investigation of their Antimicrobial Properties

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Abstract: Calixarenes are noteworthy compounds in organic chemistry since they can be synthesized and modified more conveniently compared to many other supramolecules. They can undergo functionalization either through the phenolic–O groups or at the para-position of the phenolic rings. Moreover, substitutions at the upper and lower rims allow their conversion into various derivatives, including ethers, esters, carboxylates, and amides. Owing to these features, calixarenes have become valuable supramolecular platforms in areas such as molecular recognition, sensing, self-assembly, catalysis, biocatalysis, nanotechnology, and drug discovery. Their unique structural characteristics also provide opportunities for biomedical applications, particularly in the development of antitumor, antiviral, antimicrobial, antithrombotic, and antifungal agents. In the present study, 4-sulfo-1,8-naphthalimide derivatives of calix[4]arene were synthesized, followed by the replacement of potassium sulfonate salts with silver ions to obtain silver sulfonate salts. The resulting antimicrobial materials were used to form transparent biofilms in the presence of hyaluronic acid (HA), and their antimicrobial activity was subsequently evaluated.

Keywords: Calixarene, Fluorescent, Antimicrobial

Introduction

Increased industrial activities lead to the contamination of water and soil with heavy metal ions and harmful compounds, leading to environmental and water pollution. Most of the approximately 300 million tons of heavy metals extracted to date still reside in soil and groundwater (Liu et al., 2021). In recent years, concerns about the use of heavy metals and their environmental impacts have been growing. Studies show that human activities cause heavy metals to disrupt ecosystems and threaten life through bioaccumulation. Due to its mutagenic and carcinogenic properties, chromium is considered a hazardous metal (Cilamkoti & Dutta, 2023; Kayhan et al., 2023). Chromium, a frequently encountered pollutant in the environment, originates from both anthropogenic activities and natural sources and persists in groundwater and soil (Miretzky & Cirelli, 2010). Leather processing, stainless steel production, textiles, and photography are just a few of the industrial areas where chromium compounds are commonly used (Guo et al., 2011). Due to their high solubility in water, Cr(VI) salts are a serious and widespread source of pollution. Their high toxicity and carcinogenic nature make this metal considered a pollutant that needs to be urgently identified (Liu et al., 2024). Fluorescent probes used to detect metal ions are widely used not only in environmental studies but also in biological studies, attracting the interest of many researchers. The development of highly sensitive probes, particularly for the determination of biologically important metals, is a current research topic (Fateh et al., 2021; Xu et al., 2019). Recent research has revealed that water resource pollution, particularly due to harmful heavy metals, poses an increasing

ecological and public health risk. Heavy metals are reported to disrupt ecosystem balance and negatively impact wildlife through bioaccumulation, resulting from human activities (Phichi et al., 2020; Tripathy et al., 2020). Toxic Hg(II) and Cr(VI) analyses in biological and environmental samples havegenerally been performed by classical methods such as ion-selective electrodes, atomic absorption spectrometry, inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled argon plasma optical emission spectrometry (ICP-OES) (Minhas, Memon, & Bhanger, 2010). However, these methods are not always practical due to both the cost of sampling and the difficulty of procuring the required equipment. In contrast, fluorescence-based approaches offer a relatively easy and advantageous method for detecting these ions in terms of selectivity and response time (Tosun et al.,2024 & Guler, 2024).

In this study, the interactions of previously synthesized fluorescent compounds *4* and *7* with various anions and cations were investigated and their selectivity towards Hg(II) and Cr(VI) ions was determined. These compounds, which contain potassium sulfonate groups, were also converted into silver salts to investigate their antimicrobial properties. Fluorescent, antimicrobial, and transparent biofilms were prepared using hyaluronic acid (HA); biofilm tests revealed that these materials exhibited selective antimicrobial activity, particularly against the bacterium *S. lutea*.

Method

Synthesis

Synthesis of calix[4]arene derivatives (1–7) was performed by following the literature procedure for alkylation and amide synthesis reported elsewhere (Chrisstoffels et al., 1999; Collins et al., 1991; Yildirim et al., 2021).

General Procedure for Fluorescence Study

The sensing abilities and fluorescence responses of compounds *4* and *7* towards various anions and cations were investigated using a fluorescence spectrophotometer. For this purpose, stock solutions of compounds *4* and *7* at 1×10^{-3} M were prepared in ethanol and then diluted with distilled water to a concentration of 1.5×10^{-5} M. Fluorescence emissions were then measured using different anions and cations.

Preparation of Antimicrobial Biofilms of HA-4Ag and HA-7Ag

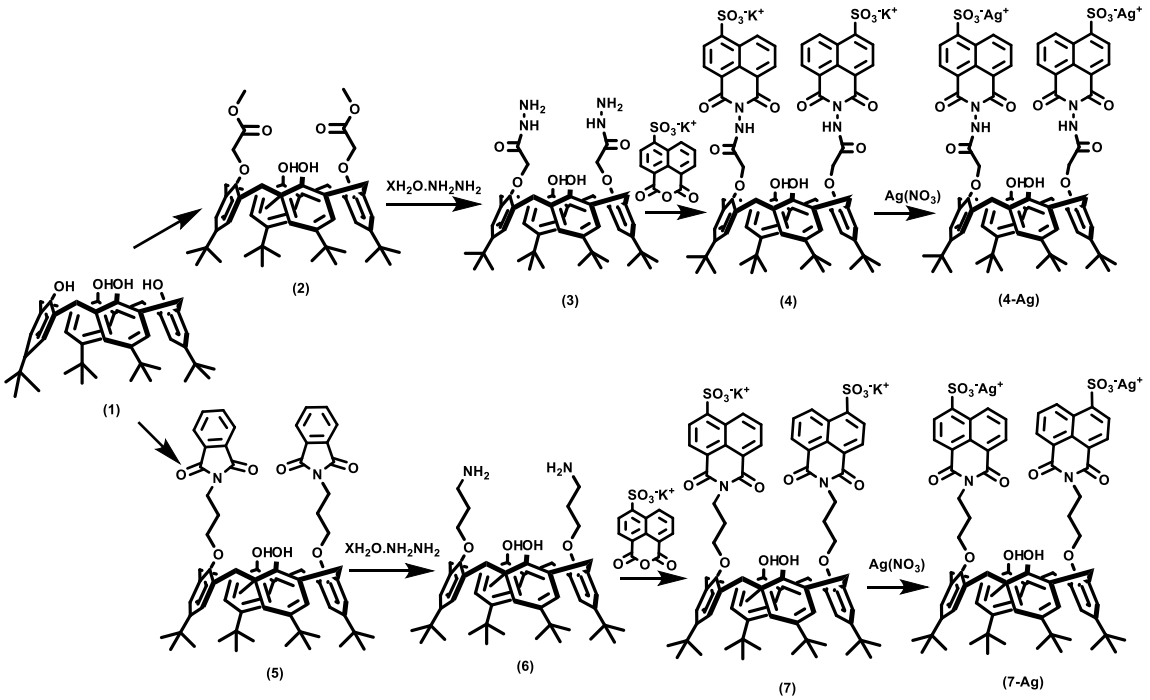

Figure 1. Synthesized antimicrobial compounds

The K^+ ions of the potassium sulfonate groups in the synthesized compounds *4* and *7* were replaced with Ag(I) ions by ion exchange for use in antimicrobial studies. For this purpose, 100 mg of compounds (*4* and *7*) were treated with 0.1 M $AgNO_3$ solution (4.0 mL) in the dark for 4 hours. Following the reaction, the solution was separated by precipitation in an ethanol-water mixture and dried in a vacuum oven at 90 °C (Yildirim et al., 2021).

HA-based transparent biofilms were then prepared using the resulting *4-Ag* and *7-Ag* derivatives. For this process, 25 mg of HA was dissolved in 0.5 mL of DMSO at room temperature overnight. Separately, 25 mg of the compounds (*4-Ag* and *7-Ag*) were dissolved in 0.5 mL of DMSO and added dropwise to the HA solution. The pH of the mixture was adjusted to 6 and stirred at room temperature overnight. Once the mixture reached a gel consistency, it was poured into molds and dried under vacuum at 50 °C to obtain transparent biofilms.

Results and Discussion

In our previous study, the anticancer properties of synthesized calix[4]arene-1,8-naphthalimide derivatives were investigated, and it was determined that these compounds were effective against colon cancer (Yildirim, Karakurt, et al., 2021). The summary of the research is as follows: First, calix[4]arene derivatives were synthesized, then, under appropriate reaction conditions, compounds *4* and *7* were successfully obtained by reacting dihydrazide (3) and diaminopropyl (6) compounds with 4-sulfo-1,8-naphthalic anhydride. The synthesis steps are shown step by step in Scheme 1.

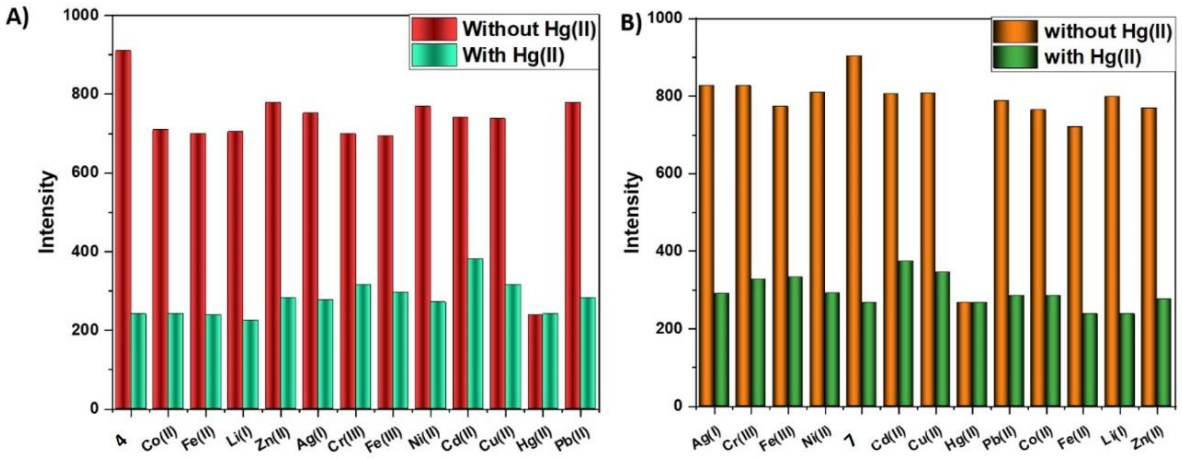

Figure 2. Fluorescence intensity change of 4-Hg(II) & 7-Hg(II) complexes (1.5×10^{-5} M) at 390 nm upon addition of different metal ions (10.0 eq.) in EtOH:H_2O (1:1, v/v).

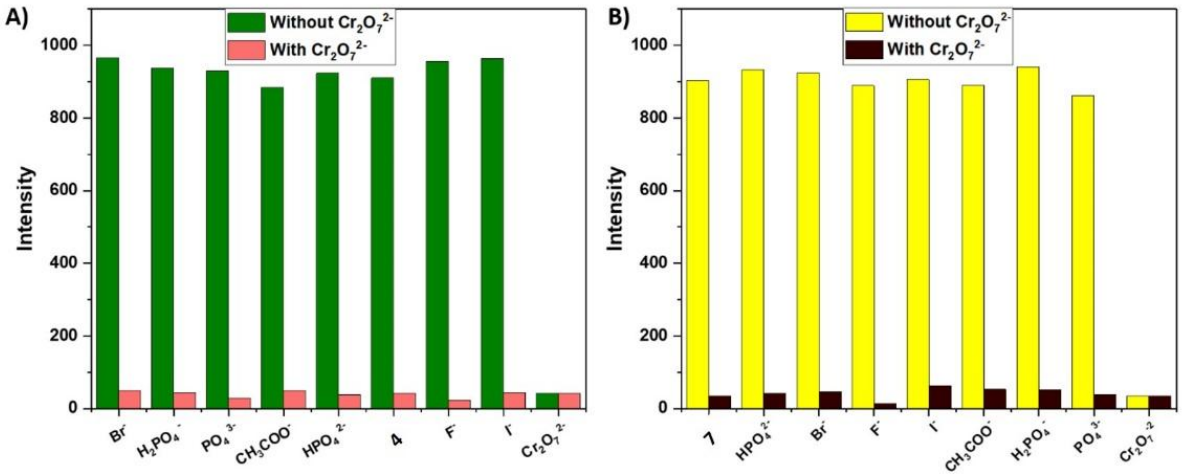

Figure 3. Fluorescence intensity change of 4-Cr(VI) & 7-Cr(VI) complexes (1.5×10^{-5} M) at 390 nm upon addition of different anions (10.0 eq.) in EtOH:H_2O (1:1, v/v).

After characterization, spectrophotometric analyses were performed on compounds 4 and 7 to determine different anions and cations. In this study, the antimicrobial properties of derivatives obtained by replacing the K(I) ions in compounds *4* and *7* with Ag(I) ions were investigated. Additionally, antimicrobial gel films (*HA-4Ag* and *HA-7Ag*) were prepared with *4-Ag* and *7-Ag* compounds using hyaluronic acid as the biopolymer.

In order to verify the selectivity and potential applications of compounds *4* and *7*, a competitive study was carried out by adding 10 equivalents of metal ions to their aqueous solutions. The density of *4*-Hg(II) and *7*-Hg(II) complexes was measured in the presence of various metal ions such as Pb(II), Cu(II), Zn(II), Cr(III), Ni(II), Co(II), Ag(I), Cd(II), Fe(II), Fe(III) and Li^+ and the results are shown in *Figure 2*. The selectivity of compounds *4* and *7* towards Hg(II) ion remained at a satisfactory level despite the addition of another 10 equivalents of cations. Only minor changes in the density were observed, confirming that both compounds exhibit effective selectivity towards Hg(II) ion. If we look at the *4*-Cr(VI) & *7*-Cr(VI) complexes in Figure 3.

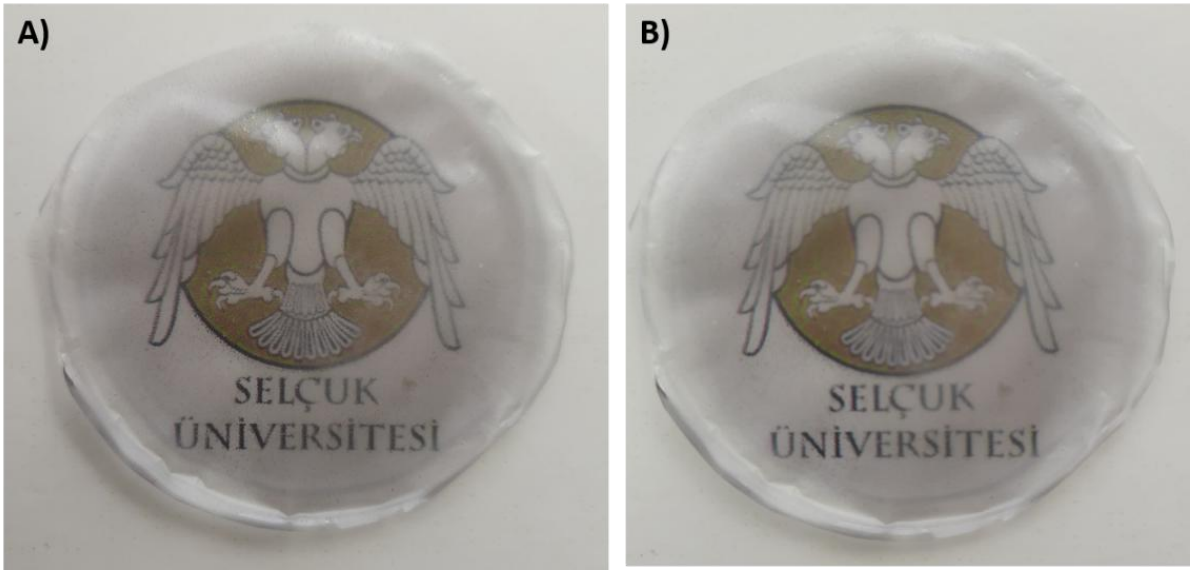

Figure 4. Transparent biofilms of HA-4Ag & HA-7Ag in A and B

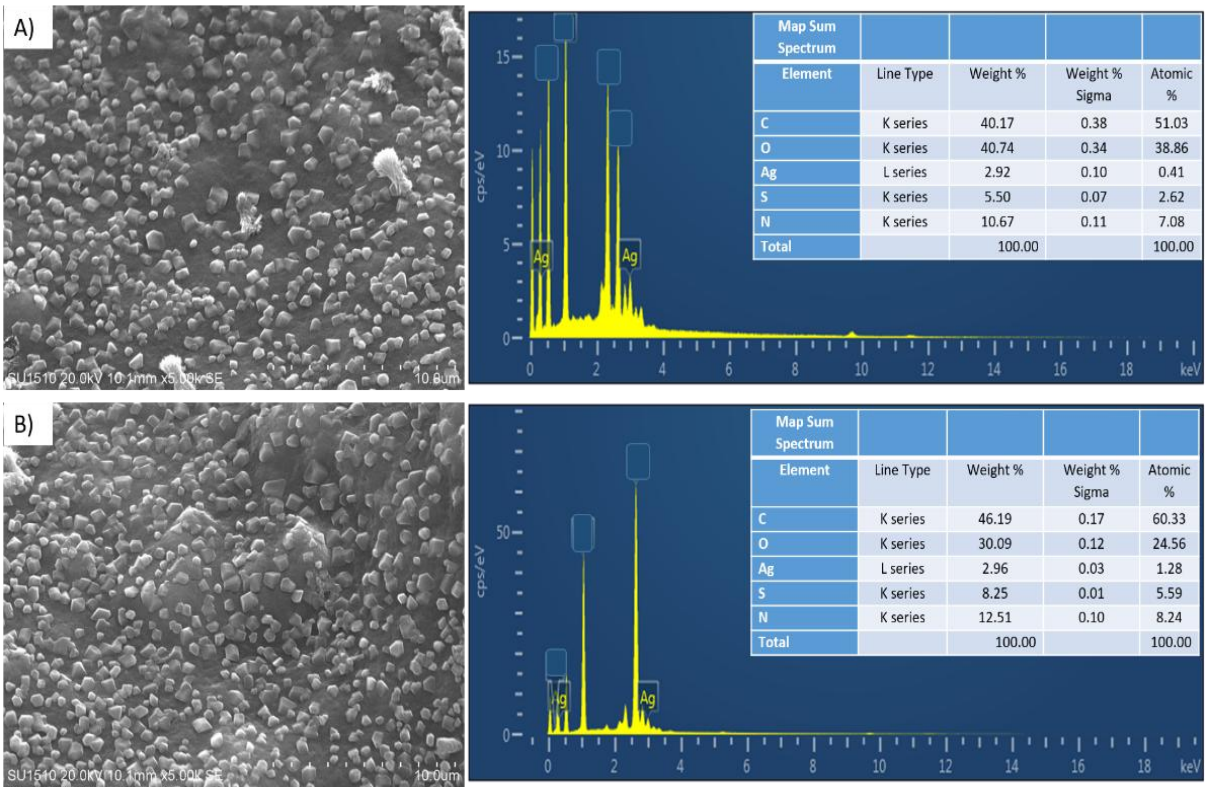

Figure 5. EDX spectral data and SEM images of A) HA-4Ag and B) HA-7Ag.

Transparent biofilms (*HA-4Ag* and *HA-7Ag*) obtained as a result of the interaction of *4-Ag* and *7-Ag* compounds with HA are shown in Figure 4. The surface profiles of the transparent biofilms prepared with *HA-4Ag* and *HA-7Ag* were examined using SEM and EDX analyses. The results indicate that the crystals of *4-Ag* and *7-Ag* compounds are distributed homogeneously within the structure of the HA biofilms. Furthermore, the EDX analyses of *HA-4Ag* and *HA-7Ag* (*Figure 5A* and *B*) reveal that, in addition to the main elements C, O, and N, the Ag(I) ions successfully replace the K(I) ions.

Antimicrobial tests revealed that compound *4* exhibited antibacterial activity against S. aureus (MRSA) at 3.125 mg/mL, while the other tested microorganisms were resistant at the same concentrations. Notably, Candida albicans was sensitive to compound *4* at a lower dose of 1.562 mg/mL. In contrast, compound *7* showed no significant activity against the tested bacteria, with the exception of C. albicans.

Table 1. Detected MIC values (mg/mL) of compounds against pathogen bacteria

Strains	**4**	**HA-5**	**HA-6**	**HA-7**	**Gentamicin (µg/ml)**
***Escherichia coli* ATCC 25922**	-	1.562	-	1.562	1.95
***Pseudomonas aeruginosa* ATCC27853**	-	1.562	1.562	0.781	<0.97
***Klebsiella pneumonia* ATCC700603**	-	1.562	-	-	7.81
***Staphylococcus aureus*(MRSA)ATCC 43300**	-	3.125	3.125	1.562	1.95
***Sarcina lutea* ATCC 9341**	-	1.562	-	0.097	1.95

The *HA-4Ag* material exhibited high efficacy against the tested pathogenic microorganisms, showing pronounced antibacterial and antifungal activity. Among the bacteria, S. lutea was the most susceptible, with an MIC value of 0.012 mg/mL. Overall, the silver-modified compounds *HA-4* and *HA-7* demonstrated moderate to strong antimicrobial effects against both bacteria and yeasts, outperforming the unmodified compounds *4* and *7,* with S. lutea being the most affected by the silver-containing derivatives.

Conclusion

As a result, the fluorescent naphthalimide-conjugated calix[4]arene chemosensors were studied for their selective ion transport capabilities toward cations and anions. The results demonstrated that these compounds are both selective and sensitive to Hg(II) and Cr(VI) ions, with low detection limits. Upon incorporation of *4-Ag* and *7-Ag* into hyaluronic acid, transparent biofilms were produced, and their antimicrobial performance was assessed. These biofilms exhibited moderate to strong activity against various bacteria and yeasts, with S. lutea showing the highest susceptibility

Recommendations

In future studies, paper sensors can be designed to more practically determine Hg(II) and Cr(VI) ions in aqueous solutions by appropriately designing the synthesized fluorescent naphthalimide-conjugated calix[4]arene compounds. Furthermore, the antimicrobial properties of 4-Ag and 7-Ag can be used in many areas for transparent biofilms prepared with hyaluronic acid.

Scientific Ethics Declaration

*The authors declares that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Conflict of Interest

*The authors declare that they have no conflicts of interest

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* In conclusion, the materials synthesized in this thesis demonstrated efficacy in antimicrobial studies.

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