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Design, Synthesis and Antimycobacterial Evaluation of a Sulfonylacetamido-Functionalized Oxacalix[4]arene

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ABSTRACT

Tuberculosis remains a major global health challenge due to the emergence of multidrug-resistant strains of Mycobacterium tuberculosis, necessitating the development of new chemotherapeutic scaffolds. In the present study, a novel sulfonylacetamido-functionalized oxacalix[4]arene derivative, Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene, was designed and synthesized through a two-step synthetic strategy involving the formation of diacetamido-oxacalix[4]arene as a key intermediate followed by sulfonylation. The synthesized compound was purified and structurally characterized using ^{1}H NMR, FT-IR, and ESI-MS, which confirmed the successful incorporation of p-toluenesulfonylacetamido moieties onto the oxacalix[4]arene framework. The antimycobacterial potential of the synthesized compound was evaluated against Mycobacterium tuberculosis using the agar well diffusion (zone of inhibition) method. The compound exhibited concentration-dependent antimycobacterial activity, with increasing zones of inhibition observed at higher concentrations when compared to the solvent control, although the activity remained lower than that of the standard drug rifampicin. The observed activity may be attributed to the presence of aromatic sulfonylacetamido groups combined with the rigid macrocyclic oxacalix[4]arene scaffold. The results suggest that Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene represents a promising supramolecular scaffold for further structural optimization toward the development of new antitubercular agents.

INTRODUCTION:

Tuberculosis (TB) remains one of the most serious infectious diseases worldwide and continues to pose a significant public health challenge, particularly in developing countries. The disease is caused by Mycobacterium tuberculosis and primarily affects the lungs, although extrapulmonary manifestations are also common [1]. The emergence of multidrug-resistant and extensively drug-resistant strains of M. tuberculosis has severely limited the effectiveness of existing first-line and second-line antitubercular therapies, thereby highlighting the urgent need for new chemotherapeutic agents with improved efficacy and novel mechanisms of action [2].

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Supramolecular chemistry has emerged as a promising field in drug discovery due to its ability to design and construct complex molecular architectures through non-covalent interactions. Among supramolecular scaffolds, calixarenes and their heteroatom-bridged analogues have attracted considerable attention because of their rigid macrocyclic framework, tunable cavity size, and ease of functionalization at both the upper and lower rims. In particular, oxacalix[4]arenes, in which oxygen atoms replace methylene bridges of classical calixarenes, exhibit enhanced conformational flexibility, improved recognition properties, and favorable physicochemical characteristics for biological applications [3].

Functionalization of oxacalix[4]arene frameworks with biologically active moieties has been shown to significantly enhance their pharmacological potential. Sulfonamide and sulfonylacetamide groups are well-known pharmacophores in medicinal chemistry and are frequently associated with antibacterial and antitubercular activities. Incorporation of aromatic sulfonylacetamido units into a macrocyclic scaffold such as oxacalix[4]arene may promote stronger interactions with bacterial cell components and improve antimycobacterial efficacy through synergistic structural effects [4].

In this context, the present study focuses on the synthesis of a novel oxacalix[4]arene-based derivative, Bis(biphenylsulfonylacetamido) Oxacalix[4]arene, followed by its evaluation for anti-tuberculosis activity [5]. The synthesized compound was characterized using spectroscopic techniques, and its antimycobacterial potential was assessed using the zone of inhibition method against Mycobacterium tuberculosis [6]. The study aims to explore the suitability of sulfonylacetamido-functionalized oxacalix[4]arene as a promising supramolecular scaffold for the development of new antitubercular agents.

MATERIALS AND METHODS:

Materials:

All chemicals and reagents used in the present study were of analytical grade. p-Toluenesulfonyl chloride (≥99% purity), 2-chloroacetamide, triethylamine (TEA), phloroglucinol, and 1,5-difluoro-2,4-dinitrobenzene were procured from Sigma-Aldrich. Organic solvents such as acetone, ethyl acetate, hexane, and dimethyl sulfoxide (DMSO) were obtained from Finar Chemicals and used without further purification. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (E-Merck).

Instrumentation

Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy: ^{1}H NMR spectra were recorded using a Bruker spectrometer operating at 400 MHz. DMSO-d_6 was used as the solvent, and tetramethylsilane (TMS) served as the internal standard. Chemical shifts were reported in parts per million (ppm) [7].

Fourier Transform Infrared (FT-IR) Spectroscopy: Infrared spectra were obtained using an FT-IR spectrophotometer in the range of 4000–400 cm^{-1} to identify characteristic functional groups present in the synthesized compounds [8].

Mass Spectrometry (ESI-MS): Mass spectral analysis was carried out using an electrospray ionization mass spectrometer (ESI-MS) to confirm the molecular weight and structural integrity of the synthesized compounds [9].

Synthesis of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene

Step I: Synthesis of Diacetamido-Oxacalix[4]arene

Oxacalix[4]arene was initially synthesized via a nucleophilic aromatic substitution reaction using phloroglucinol and 1,5-difluoro-2,4-dinitrobenzene under reported conditions. The obtained oxacalix[4]arene was then reacted with 2-chloroacetamide in a 1:2 molar ratio in acetone in the presence of triethylamine (TEA) as a base. The reaction mixture was stirred for 30 minutes and subsequently refluxed at 80 °C for 12 hours. Progress of the reaction was monitored by thin-layer chromatography (TLC). After completion, the reaction mixture was quenched with water and extracted with ethyl acetate to afford diacetamido-oxacalix[4]arene as the intermediate.

Step II: Synthesis of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene

The obtained diacetamido-oxacalix[4]arene was dissolved in acetone and treated with p-toluenesulfonyl chloride in a 1:2 molar ratio in the presence of triethylamine. The reaction mixture was refluxed for 24 hours. Upon completion, the mixture was poured into ice-cold water, resulting in the precipitation of the final product. The solid was filtered, washed thoroughly with water, and purified by solvent evaporation under reduced pressure to yield Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene.

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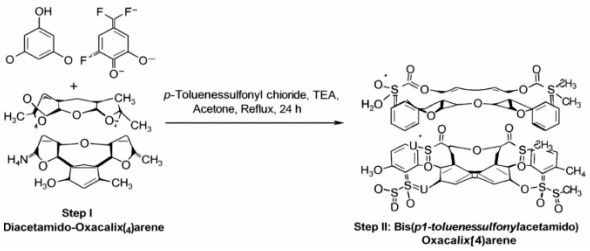

Figure 1. Synthesis of Bis(biphenylsulfonylacetamido) Oxacalix[4]arene

Anti-Tuberculosis Activity

The anti-tuberculosis activity was evaluated using the zone of inhibition (ZI) method against Mycobacterium tuberculosis. Mueller Hinton agar (MHA) plates were uniformly inoculated with the bacterial culture, and wells (cavities) were aseptically punched into the agar surface. Different concentrations of the test compound were introduced into the respective cavities. A cavity containing only the solvent served as the negative control, while a Rifampicin disc (10 µg) was used as the standard anti-tuberculosis drug for comparison. The plates were incubated at 37 °C for 24 hours, after which the zones of inhibition around each cavity were measured.

RESULTS AND DISCUSSION

The present investigation reports the successful synthesis and biological evaluation of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene, an oxacalix[4]arene-based sulfonamide derivative. The compound was characterized using spectroscopic techniques and evaluated for anti-tuberculosis.

Physicochemical Properties

The physicochemical data presented in Table 1 indicate that Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene was obtained in moderate yield as a yellowish-brown solid with a well-defined melting point range, confirming the formation of a stable sulfonyl-functionalized oxacalix[4]arene framework. The solubility and TLC behaviour (Table 1) are consistent with previously reported oxacalix[4]arene sulfonamide derivatives.

Table 1. Physicochemical parameters of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene

Name of compound	Solubility	Colour	Melting point (°C)	Yield (%)	TLC system
Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene	Ethyl acetate	Yellowish brown	170–185	55	Ethyl acetate : Hexane (7:4)

Spectral Characterization

^{1}H NMR Analysis

Spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene shows characteristic methylene, aromatic, and sulfonamide NH proton signals, confirming successful functionalization of the oxacalix[4]arene framework (Figure 2).

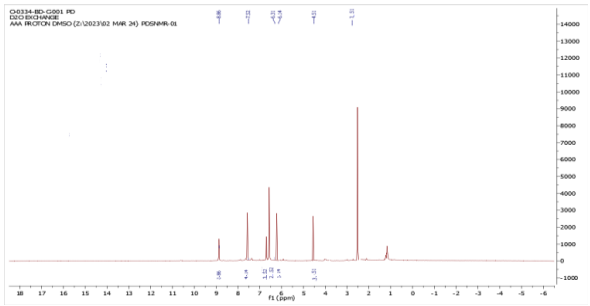

Figure 2. ^{1}H NMR spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene (DMSO-d_6)

FT-IR Spectrum

The spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene confirms the successful incorporation of p-toluenesulfonylacetamido moieties onto the oxacalix[4]arene framework. A characteristic broad absorption band observed at 3399 cm^{-1} corresponds to N–H stretching vibrations of the sulfonamide and amide functionalities. The strong absorption band at 1659 cm^{-1} is attributed to C=O stretching of the amide group, confirming acetamido linkage formation. The intense band appearing at ~1419 cm^{-1} is associated with aromatic C–C stretching vibrations of the oxacalix[4]arene and p-tolyl rings. The presence of sulfonyl functionality is evidenced by characteristic S=O stretching vibrations, observed in the region ~1350–1150 cm^{-1}. The absorption band at 757 cm^{-1} corresponds to aromatic C–H out-of-plane bending, confirming the para-substituted toluene ring. Additional bands below 700 cm^{-1} are attributed to skeletal vibrations of the macrocyclic oxacalix[4]arene framework (Figure 3).

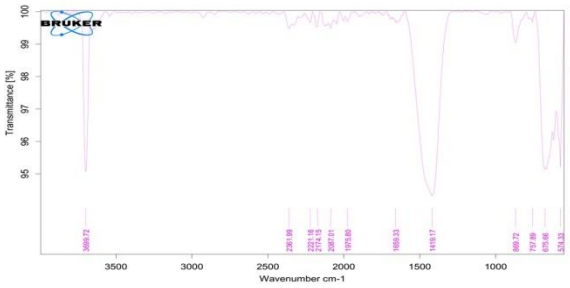

Figure 3. FT-IR spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene)

ESI–MS Analysis

The ESI–MS spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene exhibits a prominent molecular ion peak corresponding to the expected molecular weight, confirming the successful formation of the target compound (Figure 4).

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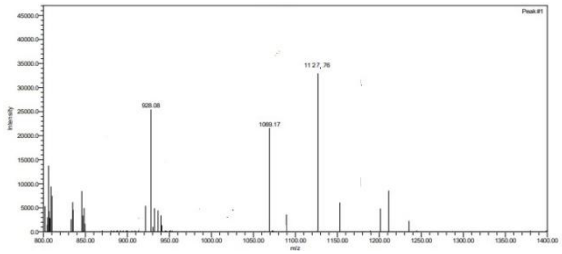

Figure 4. ESI–MS spectrum of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene

Anti-Tuberculosis Activity

The anti-tuberculosis activity of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene was evaluated against *Mycobacterium tuberculosis* using the agar well diffusion method. As shown in Figure 8, the agar plate contained wells loaded with different concentrations of the test compound along with appropriate controls. Cavity-1, containing the solvent control, did not exhibit any zone of inhibition, confirming that the solvent had no inhibitory effect on bacterial growth. At the lowest concentration, Cavity-2 (25 µg/mL) produced a small but clearly detectable zone of inhibition measuring 6.2 ± 0.3 mm, indicating initial antimycobacterial activity. An increase in the inhibition zone was observed with rising concentration, where Cavity-3 (50 µg/mL) showed a zone of 8.5 ± 0.4 mm and Cavity-4 (75 µg/mL) exhibited a further increase to 10.9 ± 0.5 mm, demonstrating a clear concentration-dependent response. The maximum zone of inhibition was observed for Cavity-5 (100 µg/mL), measuring 13.6 ± 0.6 mm, indicating stronger antimycobacterial activity at higher concentration. For comparison, the standard anti-tuberculosis drug rifampicin (10 µg) produced a significantly larger zone of inhibition (21.8 ± 0.7 mm). The quantitative zone of inhibition data is in Table 2, which corroborate the visual observations from the agar diffusion assay and confirm the moderate, concentration-dependent antimycobacterial activity of the synthesized compound.

Table 2. Zone of inhibition of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene against *Mycobacterium tuberculosis*

Cavity No.	Sample / Concentration	Zone of Inhibition (mm)
Cavity-1	Solvent control	No inhibition
Cavity-2	25 µg/mL	6.2 ± 0.3
Cavity-3	50 µg/mL	8.5 ± 0.4
Cavity-4	75 µg/mL	10.9 ± 0.5
Cavity-5	100 µg/mL	13.6 ± 0.6
Standard	Rifampicin (10 µg)	21.8 ± 0.7

Values are expressed as mean ± SD (n = 3).

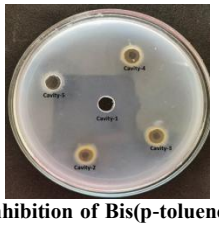

Figure 5. Zone of inhibition of Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene against *Mycobacterium tuberculosis*

CONCLUSION

In the present study, a novel sulfonylacetamido-functionalized oxacalix[4]arene derivative, Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene, was successfully designed and synthesized through a two-step synthetic route involving diacetamido-oxacalix[4]arene as a key intermediate. The structure of the synthesized compound was unambiguously confirmed by ^{1}H NMR, FT-IR, and ESI-MS analyses, which collectively verified the successful incorporation of p-toluenesulfonylacetamido moieties onto the oxacalix[4]arene framework. The physicochemical properties further supported the formation of a stable and well-defined macrocyclic system. The antimycobacterial evaluation performed using the agar well diffusion method against Mycobacterium tuberculosis demonstrated a concentration-dependent zone of inhibition, indicating moderate antimycobacterial activity of the synthesized compound when compared with the standard drug rifampicin. Although the observed activity was lower than that of the reference drug, the results highlight the potential of sulfonylacetamido-functionalized oxacalix[4]arene scaffolds as promising supramolecular platforms for antitubercular drug development. The activity may be attributed to the combined effect of the rigid oxacalix[4]arene macrocycle and the biologically relevant sulfonylacetamido groups, which together may facilitate interactions with the mycobacterial cell envelope. this study establishes Bis(p-toluenesulfonylacetamido) Oxacalix[4]arene as a valuable lead structure for further investigation. Future studies focusing on structural modification, detailed mechanistic evaluation, and advanced biological assays may help enhance antimycobacterial potency and provide deeper insight into structure–activity relationships, thereby supporting the development of new supramolecular antitubercular agents.

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