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Synthesis and Characterization of Novel Calix[4]arene Schiff Base Derivatives and Cytotoxicity Effect Evaluation on Cancer Cell Lines

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ABSTRACT

In this study, four stages were used to create brand-new *p*-tert-butyl-calix [4] arene Schiff base derivatives. First, *p*-tert-butyl-phenol and formaldehyde are reacted to create *p*-tert-butyl-calix [4] arene (1). In the following step, methyl bromoacetate and p-ter-butyl-calix [4] arene (1) were combined with acetone and reflux to create the *p*-tert-butyl-calix [4] arene diester complex (2). The third step involves reacting the diester compound (2) and hydrazine hydrate to create the *p*-tert-butyl-calix [4] arene hydrazinamide molecule (3). In the final stage, calix [4] arene Schiff base derivatives (4a-d) were produced in good yields by combining compound (3), *p*-tert-butyl-calix [4] arene hydrazinamide, and various aldehyde derivatives with reflux in EtOH. Through the use of ^{1}H-NMR, ^{13}C-NMR, infrared spectroscopy, and elemental analysis, the structures of produced compounds were verified. Four distinct cancer lines are linked to the antitumor activity of synthetic chemicals. (HT-29, a human colon cancer cell line, PC-3, a human prostate cancer cell line, C6, a rat glioma cell line and MCF-7, a human breast cancer cell line). Weak antitumor activity was seen in synthetic substances. However, only compound 4b was found to have potential efficacy against C6 and HT-29. It is clear that compound 4b, which has a nitro substitute on the phenyl ring, draws attention due to its increased activity.

Keywords: Calix[4]arene, Anticancer activity, Schiff Base.

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Introduction

A malignant condition characterized by unchecked and aberrant cell proliferation is referred to as cancer in general. Although different types of cancer have specific causes that lead to the abnormality, all types of cancer involve many mysterious phenomena [1]. Even though they were first developed as traditional anticancer medications, the most of them have negative side effects and primarily are unable to distinguish specifically between cancer cells and healthy cells. Selectivity is one of the primary issues with all current and forthcoming anticancer medications. As a result, throughout the past three decades, new developments in targeted chemotherapy have emerged. A scientific answer is clearly required in the area. From a comprehensive standpoint, it is possible to develop more potent medications and treatment plans to combat the tumor's resistance to therapy [2]. The use of supramolecular systems, such as macrocycles, has attracted attention in the realm of cancer therapy as a possible strategy to solve this issue.

Supramolecules typically form when two or more molecules come together under the influence of non-covalent bonding forces. Crown ethers, cyclodextrins, cucurbiturils, calixarenes, and pillar[n]arenes are examples of macrocyclic compounds [3-7]. The third generation of supramolecular chemistry is represented by a significant class of cyclic oligomers known as calixarenes. These are made of phenolic units linked by methylene in the ortho positions. There are numerous more structural features of calixarenes, such as the simplicity modification of their basic core and their low toxicity, and their lack of immunological reactions [8]. Among all its biological characteristics, calix[n]arenes' capacity to inhibit the growth of cancer cells is one of the most extensively researched therapeutic areas. The cause is most likely a result of their distinct physicochemical characteristics, biocompatibility, and a variety of biological functions [9]. Numerous research teams have looked at the anticancer properties of molecules based on calixarene. Clinical trial data for anticancer drugs based on calixarene are among the most crucial. Only one Phase I investigation of OTX008, a calixarene-based molecule and galectin-1 inhibitor with potential antiangiogenic and antineoplastic activity, has been documented to date in the US clinical trials database [10]. According to this viewpoint, there is still a critical need for further research into the molecular mechanisms underlying the effects of novel functionalized calixarenes as anticancer agents.

On the other hand, due to their adaptability, Schiff bases are significant chemical substances in a variety of domains, including inorganic, analytical, and

pharmaceutical chemistry. The carbonyl group of an aldehyde or beta diketone interacts with an amine moiety to generate Schiff bases. Because its active group (-N=CH-) contains active electrons, Schiff bases are excellent candidates for the development of novel medications [11-12]. Because of their extensive variety of pharmacokinetic properties and their popularity in drug development programs, the derivatives of Schiff bases represent a large category of substances that have found several applications in medicinal chemistry [13-14].

In the light of this information, we report the preparation, characterization four new calix [4] arene Schiff base derivatives (4a-d) and evaluate their anticancer activity against C6 (a rat brain glioma adenocarcinoma cell line), HT-29 (human colon carcinoma cell line), PC-3 (human prostate cancer cell line), and MCF-7 (human breast cancer cell line).

Materials and Methods

Chemistry

The different derivatives of calixarenes presented in Figure 1 (1, 2, and 3) were synthesized according to the literature [15-17].

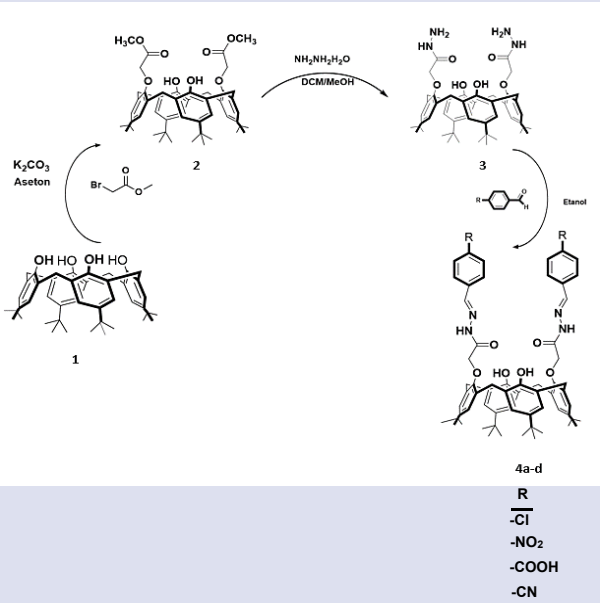

Figure 1. General synthesis pathways of the target compounds

Synthesis of 4a

Compound (3) (0.30 g, 0.38 mmol) and 4-chlorobenzaldehyde (0.13 g, 0.91 mmol) are in ethanol solution under reflux with stirring for 12 hours. The reaction was checked with thin layer chromatography, and after this time, the solvent is removed and the target product is crystallized from ethanol. Yield 0.35 g (% 90), M.p.: 299.8-301.5 oC. IR (ATR) vmax(cm-1): 1598 (HC=N), 1691 (C=O), 3373 (N-H). 1H-NMR (300 MHz, CDCl3): δ (ppm) 1.07 (18H, s, Bu^t), 1.31 (18H, s, Bu^t), 3.56 (4H, d, *J* = 13.44 Hz, $ArCH_2Ar$), 4.19 (4H, d, *J* = 13.38 Hz, $ArCH_2Ar$), 4.74 (4H, s, - OCH_2CO), 6.99 (4H, s, Ar-H), 7.17 (4H, s, Ar-H), 7.37 (4H, d, *J*=8.49 Hz, 1,4-disubstituebenzene), 7.60 (4H, d, *J*=8.52 Hz, 1,4-disubstituebenzene), 7.90 (2H, s, OH), 8.26 (2H, s, -CH=N), 11.43 (2H, s, -NH). ^{13}C-NMR (75 MHz, $CDCl_3$):δ (ppm) 30.95, 31.57, 34.23, 74.38, 125.90, 126.49, 127.34, 128.97, 129.13, 132.05, 144.12, 147.97, 149.17, 163.59. Anal. Calcd for $C_{62}H_{70}Cl_2N_4O_6$: C, 71.73; H, 6.80; N, 5.40 %. Found: C, 71.48; H, 6.81; N, 5.39 %.

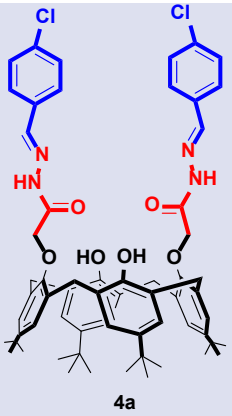

Figure 2. Compound 4a

Synthesis of 4b

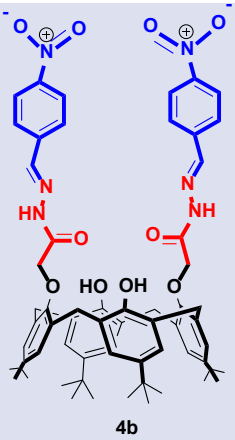

Figure 3. Compound 4b

Compound (3) (0.30 g, 0.38 mmol) and 4-nitrobenzaldehyde (0.14 g, 0.91 mmol) are in ethanol solution under reflux with stirring for 12 hours. The reaction was checked with thin layer chromatography, and after this time, the solvent is removed and the target product is crystallized from ethanol. Yield 0.37 g (% 92), M.p.: 327.2-329.4 °C. IR (ATR) v_{max}(cm-1): 1620 (HC=N), 1699 (C=O), 3234 (N-H). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.10 (18H, s, Bu^t), 1.31 (18H, s, Bu^t), 3.59 (4H, d, *J* = 13.47 Hz, $ArCH_2Ar$), 4.19 (4H, d, *J* = 13.35 Hz, $ArCH_2Ar$), 4.76 (4H, s, - OCH_2CO), 7.03 (4H, s, Ar-H), 7.18 (4H, s, Ar-H), 7.82 (4H, d, *J*=8.88 Hz, 1,4-disubstituebenzene), 8.09

(2H, s, OH), 8.28 (4H, d, *J*=8.82 Hz, 1,4-disubstituebenzene), 8.42 (2H, s, -CH=N), 11.73 (2H, s, -NH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 30.98, 31.56, 32.36, 74.37, 124.02, 126.04, 126.63, 127.28, 128.46, 132.08, 146.87, 148.95, 149.45, 163.91. Anal. Calcd. for $C_{62}H_{70}N_6O_{10}$: C, 70.3; H, 6.66; N, 7.93 %. Found: C, 70.12; H, 6.64; N, 7.94 %.

Synthesis of 4c

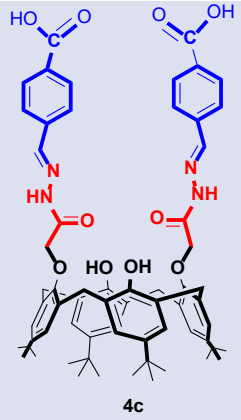

Figure 4. Compound 4c

Compound (3) (0.30 g, 0.38 mmol) and 4-carboxybenzaldehyde (0.14 g, 0.91 mmol) are in ethanol solution under reflux with stirring for 12 hours. The reaction was checked with thin layer chromatography, and after this time, the solvent is removed and the target product is crystallized from ethanol. Yield 0.29 g (% 75), M.p.: 321.8-323.1 °C. IR (ATR) ν_{max}(cm^{-1}): 1604 (HC=N), 1681 (C=O), 3498 (N-H). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.07 (18H, s, Bu^t), 1.31 (18H, s, Bu^t), 3.56 (4H, d, *J* = 13.17 Hz, $ArCH_2Ar$), 4.19 (4H, d, *J* = 13.83 Hz, $ArCH_2Ar$), 4.74 (4H, s, - OCH_2CO), 6.98 (4H, s, Ar-H), 7.17 (4H, s, Ar-H), 7.36 (4H, d, *J*=7.77 Hz, 1,4-disubstituebenzene), 7.47-7.51 (4H, m, 1,4-disubstituebenzene), 7.86 (2H, s, OH), 8.26 (2H, s, -CH=N), 11.43 (2H, s, -NH). ^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm) 30.95, 31.57, 34.01, 74.40, 125.90, 126.48, 127.36, 129.89, 130.79, 132.06, 135.06, 144.10, 148.30, 149.14, 175.31. Anal. Calcd. for $C_{62}H_{72}N_4O_{10}$: C, 72.70; H, 6.86; N, 5.30 %. Found: C, 72.51; H, 6.84; N, 5.28 %.

Synthesis of 4d

Compound (3) (0.30 g, 0.38 mmol) and 4-cyanobenzaldehyde (0.14 g, 0.91 mmol) are in ethanol solution under reflux with stirring for 12 hours. The reaction was checked with thin layer chromatography, and after this time, the solvent is removed and the target product is crystallized from ethanol.

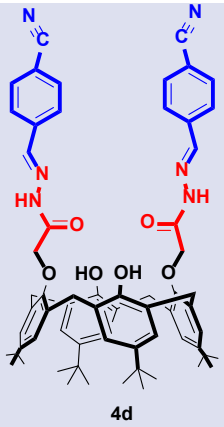

Figure 5. Compound 4d

Yield 0.35 g (% 88), M.p.: 328.9-330.2 °C. IR (ATR) ν_{max}(cm^{-1}): 1600 (HC=N), 1707 (C=O), 3278 (N-H). ^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.08 (18H, s, Bu^t), 1.31 (18H, s, Bu^t), 3.55 (4H, d, *J* = 13.44 Hz, $ArCH_2Ar$), 4.20 (4H, d, *J* = 13.35 Hz, $ArCH_2Ar$), 4.75 (4H, s, - OCH_2CO), 7.00 (4H, s, Ar-H), 7.16 (4H, s, Ar-H), 7.30-7.43 (4H, m, 1,4-disubstituebenzene), 8.02-8.10 (4H, m, 1,4-disubstituebenzene), 8.74 (2H, s, -CH=N), 11.79 (2H, s, -NH). ^{13}C-NMR (75 MHz, DMSO-d_6): δ (ppm) 31.32, 31.82, 34.11, 74.05, 112.39, 126.08, 127.33, 128.28, 133.42, 133.56, 138.56, 142.20, 148.23, 150.05, 165.18. Anal. Calcd. for $C_{64}H_{70}N_6O_6$: C, 75.41; H, 6.92; N, 8.25 %. Found: C, 75.21; H, 6.93; N, 8.22 %.

Anticancer Activity Studies

L929, a healthy human fibroblast cell line; PC-3, the human prostate cancer cell line; MCF-7, the human breast cancer cell line; and the C6, rat brain glioma adenocarcinoma cell line are all purchased from the Americam Type Culture Collection and grown in Dulbecco's modified Eagle' s medium (DMEM; Gibco, Thermo Fisher Scientific), which is supplemented with 10% fetal bovine serum (FBS; Sigma Aldrich), the cultivated cells were kept at 37 °C in a humid environment with 5% CO2. All recently created substances were dissolved in DMSO, and stock solutions were diluted with DMEM because the final DMSO concentration was less than 0.5%.

The MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) cell proliferation test was used to determine the viability of the cells. In a common colorimetric experiment, the mitochondrial activity of living cells is determined by splitting tetrazolium salts with mitochondrial dehydrogenases. [18,19]. The MTT assay was used to examine how the compounds in range 4a-4d affected the survival of the C6, HT-29, PC-3, MCF-7, and L929 cell lines. The cells were treated with 100 µM doses of each after being seeded at a density of 1×10^4 cells per well for 48 hours.

The control group consisted of untreated cells. To allow the metabolically active cells to convert MTT dye into

formazan crystals, the cells were treated with 20 µL of MTT solution (5 mg/mL in PBS, Sigma) and incubated at 37 °C for 3 h. DMSO was used to dissolve the formazan crystals for medicinal chemistry research (Sigma). Utilizing a microplate reader to measure the absorbance at 540 nm, the decrease of MTT was measured. The IC_{50} values were represented as mean ± standard deviation (± SD).

Result and Discussion

Data on ^{1}H-NMR, ^{13}C-NMR, IR, and elemental analysis results were used to deduce the structures of four produced original compounds, 4a–4d. Reaction times are reduced, efficiency is raised, fewer resources are needed, and it is more environmentally and economically friendly when the final compounds are synthesized. With the aid of spectroscopic data, the IR investigations of four novel compounds derived from calix [4] arene Schiff bases that were synthesized as part of the study were clarified. All synthetic substances have an aromatic ring system when their chemical structures are analyzed. The voltage band bee of the HC=N group in the range of 1598 – 1620 cm^{-1}. In the literature, it has been reported that C=N stretching vibrations in Schiff bases are generally observed as a sharp peak at 1610-1640 cm^{-1}, and peaks are observed at 1600-1637 cm^{-1} in those that do not carry the methylene group attached to the azomethine group, that is, those that are directly attached to the aromatic ring [20]. Therefore, the obtained data are in accordance with the literature data. The 1,4-disubstituebenzene rings are a further structure that is present in all synthesis products. For this ring, the unique non-plane deformation bands were measured between 812 and 871 cm^{-1}. The voltage bands of the C=O group in the range of 1681 to 1707 cm^{-1} and the N-H group in the region of 3234 to 3498 cm^{-1} are additional structures that are present in all synthesis products. In the ^{1}H-NMR spectra, due to the presence of two *p*-tert butyl signals (-C(CH_3)$_3$) at 1.07-1.10 ppm and 1.31 ppm. Protons of ArCH_2Ar methylene bridges located on *p*-tert-butyl-calix [4] arene were observed as two doublets, in the range of 3.55-3.59 ppm and 4.19-4.20 ppm. Therefore, our observation of both doublets in this way provides evidence that the calix [4] arene compound was synthesized in the cone conformation. When the benzene ring is in the 1,4-disubstituted state, it conforms to the AA'BB' spin system. In this case, there are changes in the value and shape of the peak that the aromatic protons of the benzene ring give as singlet at 7.27 ppm, according to the electron donating or electron withdrawing properties of the substituents attached to the ring. The excess electron density around the proton causes resonance at higher field intensity by shielding the proton, and therefore the ppm value decreases accordingly. The low electron density around the proton, on the other hand, does not shield the proton, causing resonance at lower field strength, causing the current peak to be higher in ppm. When an electron withdrawing group is attached to the aromatic ring, since the electron density in the ring will decrease, the shielding of the ring protons also decreases, and accordingly, the chemical shift values of the aromatic ring protons shift downward. While the benzene ring is in the 1,4-disubstituted state, singlet, doublet or quartet-shaped peaks can be observed in the spectrum depending on the electronic properties of the substituents on the ring. The 1,4-disubstituted phenyl ring peaks, which are usually observed as two doublets, can be obtained in quartet or even singlet form if the electronic properties of the substituents are very close to each other [21, 22]. In this context, the presence of the 1,4-disubstituted benzene ring is demonstrated at 7.37 and 8.28 ppm and Ar-H signals at 6.98 ppm and 7.18 ppm and the doublet four proton peaks. The signals belonging to -CH=N- proton was found at 8.26-8.74 ppm. Around 11.43-11.79 ppm, a wide singlet caused by the -N-H proton of the phenyl ring was detected. For the compounds included by the study, percentage studies of the C, H, and N components were carried out. The results show a 0.4% difference between experimental outcomes and theoretically predicted element percentages. This result suggests that there aren't many contaminants in the compounds. The anticancer activity of compounds 4a–4d was assessed against the cancer cell lines C6, HT-29, PC-3, and MCF-7 as well as the healthy cell line L929. The IC_{50} value of four new compounds synthesized was determined by MTT. Anticancer activity results of compounds 4a-4d are presented in Table 1. When the activity results of compounds were evaluated, it was seen that compounds 4a, 4c and 4d were ineffective cancer cells. However, compound 4b shows activity against C6, HT-29 cancer cell lines. The IC_{50} value of 93.421±4.282 and 96.622±4.518 µM, respectively. It was also found that compound 4b shows activity against L929 cell line. The IC_{50} value of compound 4b against L929 was 48,35±2.328 µM. It is a disadvantage that show cytotoxic effects are observed on healthy fibroblast cells (L929).

As a result of the study, four novel compounds were synthesized and characterized. Although synthesized compounds 4a, 4c, and 4d are ineffective against cancer, compound 4b seems to provide hope in anticancer activity. Future research aims to reach more potent molecules with various changes via this primary structure.

Table 1. IC_{50} values (0-100 µM) of calix[4]arene Schiff base derivatives on different cells

Compounds	C6	HT-29	PC-3	MCF-7	L929
4a	>100	>100	>100	>100	>100
4b	93.421±4.282	96.622±4.518	>100	>100	48,35±2.328
4c	>100	>100	>100	>100	>100
4d	>100	>100	>100	>100	>100

Conclusions

Using FT-IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis, the structures of four novel calix [4] arene containing Schiff base derivatives that were developed and synthesized in this study were clarified. C6, HT-29, PC-3, MCF-7 cancer cell lines and L929 healthy cell line were tested for anticancer activity. The compounds **4a**, **4c**, **4d** were show no anticancer activity. However, compound **4b** shows activity against C6 (IC_{50}= 93.421±4.282 µM), HT-29 (IC_{50}=96.622±4.518 µM) cancer cell lines and L929 (IC_{50}= 48,35±2.328 µM) cell line. In future studies, by making use of the findings of this study, new compounds with similar chemical structures that are thought to be more effective can be synthesized and contribute to the pharmaceutical industry.

Acknowledgment

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Conflicts of interest

There are no conflicts of interest in this work.

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