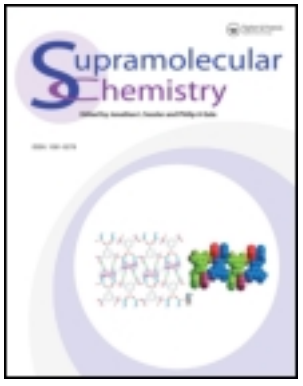

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Şeyda Çiğdem Özkan[a], Aydan Yilmaz[a] & İsmail Özmen[b]
[a] Department of Chemistry, Faculty of Science, Selcuk University, 42031, Konya, Turkey
[b] Department of Chemistry, Faculty of Science and Arts, Süleyman Demirel University, Isparta, Turkey
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Şeyda Çiğdem Özkan[a], Aydan Yilmaz[a]* and İsmail Özmen[b]

[a]*Department of Chemistry, Faculty of Science, Selcuk University, 42031 Konya, Turkey;* [b]*Department of Chemistry, Faculty of Science and Arts, Süleyman Demirel University, Isparta, Turkey*

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This study comprises the synthesis of new *p-tert*-butylcalix[4]arene with different amide functional groups and summarises an investigation of their DNA cleavage activities. The structural investigations of the synthesised compounds were examined by FTIR, ^{1}H NMR, ^{13}C NMR, elemental analysis and FAB-MS techniques. The interaction between these compounds and pBR322 plasmid DNA has been investigated via agarose gel electrophoresis and, according to the results, compounds **5**, **7**, **8** and **13** exhibit efficient DNA cleavage activity. In the electrophoresis images of **5**, **7** and **8**, Form IV which is small DNA fragment was observed in addition to supercoiled Form I, open circular Form II and linear Form III.

Keywords: calix[4]arene; DNA cleavage; amide; plasmid DNA

1. Introduction

Calix[*n*]arenes can be viewed as examples of [1$_n$] metacyclophanes that possess basket-shaped cavities which are composed of phenolic units ortho-linked by methylene bridges (*1*). Calixarenes can be used in many fields for a variety of purposes, including acting as cation and anion extractants (*2*), catalysts (*3*), sensor materials (*4*) and enzyme-mimic catalysts (*5*). Furthermore, the biological properties of calixarenes, i.e. antimicrobical agents (*6*), antiseptic and anticancer agents (*7*), cytotoxicity (*8*), DNA binding (*8*), DNA interaction recognition (*9*) and protection from ultraviolet radiation (*10*), have recently been investigated. Amide derivatives of calix[4]arenes have commonly been used in the creation of cation (*11*), anion (*12*) and neutral (*13*) extractants. Recently, amido-calixarenes have been used as nucleic acid recognisers (*14*), enzyme inhibitors (*15*) and potent-DNA binding agents (*16*). In this study, we prepared six new amide derivatives (mono- and di-) of calix[4]arenes that have some aliphatic and aromatic groups, and then investigated the activities of these compounds on the DNA.

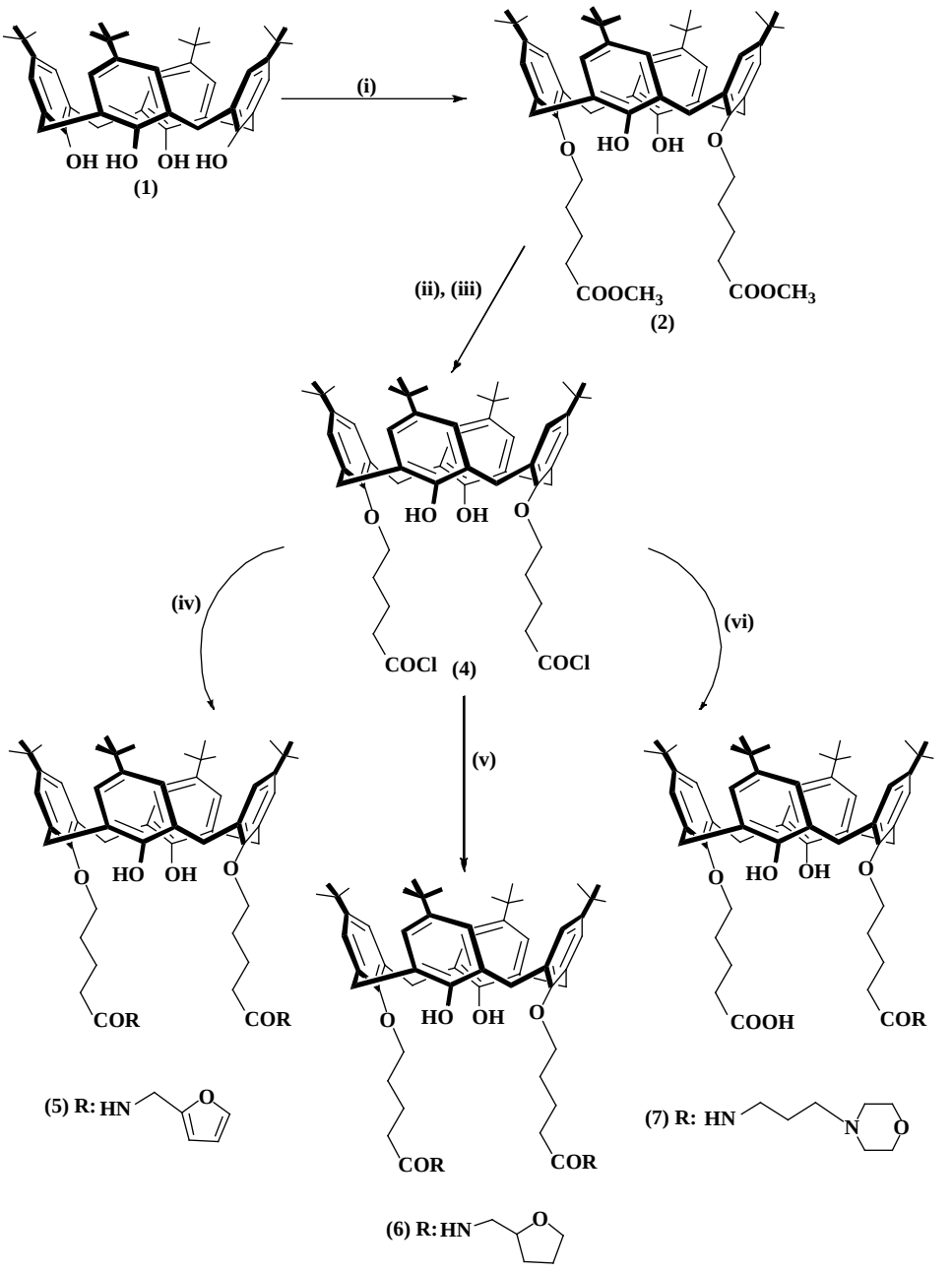
2. Results and discussion

2.1 *Synthesis of calix[4]arene derivatives*

More specifically, in this study, we synthesised the amide derivatives of *p-tert*-butylcalix[4]arene with different functional groups. First, different calixarenes with aliphatic and aromatic ester groups were synthesised. For this purpose, carboxylic acid derivatives (i.e. 5-bromovaleric acid and 4-(bromomethyl)benzoic acid) were converted to methyl esters with methanol in the presence of sulphuric acid through an esterification reaction. Then they were connected to calixarene in the form of an ester (*17*, *18*). The obtained calixarene diester derivatives were hydrolysed for a period of 5–10 min via a microwave device, and through the continuation of the reaction with oxalyl chloride, the acid chloride derivatives were obtained. Calixarenes with diamide and monoamide derivatives were synthesised by treating calixarene compounds in the acid chloride form with various primary amines at room temperature. The synthetic process for the preparation of *p-tert*-butylcalix[4]arene amide derivatives is described in Schemes 1 and 2.

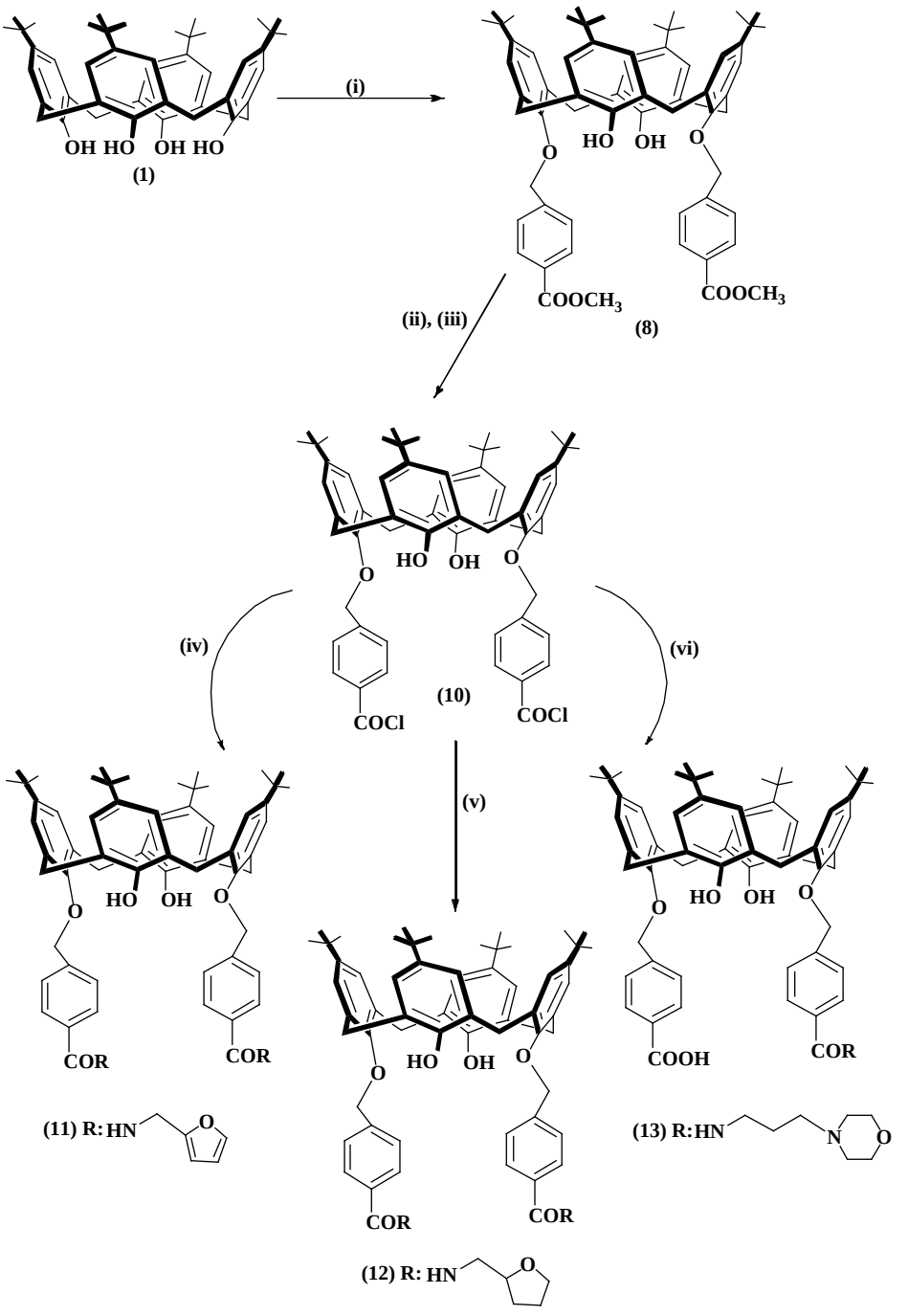
The diamide derivatives **5**, **6**, **11** and **12** were characterised via FTIR, ^{1}H NMR, ^{13}C NMR, elemental analysis and FAB-MS techniques. The formation of the diamide derivatives of *p-tert*-butylcalix[4]arene (**5**, **6**, **11** and **12**) was confirmed by the appearance of characteristic amide bands at 1648, 1650, 1639 and 1706 cm^{-1} respectively, and by the disappearance of acid carbonyl band at about 1700 cm^{-1} in the IR spectra. The conformational characteristics of calix[4]arenes were conveniently estimated by the splitting pattern of the ArCH$_2$Ar methylene protons in the ^{1}H NMR spectrum (*19*, *20*). The ^{1}H NMR spectroscopic data showed that compounds **5**, **6**, **11** and **12** were in the cone conformation. A typical AX pattern was observed for the methylene bridge of the ArCH$_2$Ar protons as doublets 3.30 and 4.14

*Corresponding author. Email: aydan@selcuk.edu.tr



Scheme 1. A schematic representation showing synthesis pathway of calix[4]arene amide derivatives **5**–**7**. (i) Methyl 5-bromovalerate, K_2CO_3, acetone, 42%; (ii) ethanol, NaOH, MW, 600 W (% 100), 10 min, 82%; (iii) oxalyl chloride, DCM/DMF; (iv) furfuryl amine, THF, 50%; (v) tetrahydrofurfuryl amine, THF, 65%; (v) 3-morpholinopropyl amine, THF, 75%.

($J = 13.1$ Hz) for **5**, 3.33 and 4.25 ($J = 13.1$ Hz) for **6**, 3.34 and 4.28 ($J = 13.1$ Hz) for **11** and 3.31 and 4.27 ($J = 13.0$ Hz) for **12** in the ^{1}H NMR data. Likewise, the monoamide derivatives **7** and **13** were characterised using the same techniques. The formation of these new compounds was confirmed by the appearance of new amide carbonyl bands at about 1650 cm^{-1} and acid carbonyl bands at about 1720 cm^{-1} in the IR spectra. In the ^{1}H NMR spectra, the chemical shift for the amide NH was recorded as a triplet (1H) at 7.89 ($J = 5.4$ Hz) for **7** and 8.67 ($J = 5.2$ Hz) for **13**. The $ArCH_2Ar$ methylene protons of compound **7** showed two doublet signals at 3.31 and 4.22 ($J = 13.1$ Hz). These signals indicated that compound **7** is in the cone conformation. The $ArCH_2Ar$ methylene protons for compound **13** showed one doublet at 4.27 ($J = 13.3$ Hz) and an overlapped peak in the range of 2.94–3.47. The ^{13}C NMR spectral data confirmed the obtained results.



Scheme 2. A schematic representation showing synthesis pathway of calix[4]arene amide derivatives **11**–**13**. (i) Methyl 4-(bromomethyl)benzoate, K_2CO_3, acetone, 75%; (ii) ethanol, NaOH, MW, 600 W (% 100), 5 min, 78%; (iii) oxalyl chloride, DCM/DMF; (iv) furfuryl amine, THF, 65%; (v) tetrahydrofurfuryl amine, THF, 60%; (v) 3-morpholinopropyl amine, THF, 70%.

2.2 pBR322 DNA–compound interactions

The compound–DNA interactions were investigated via electrophoresis. In experimental studies, synthesised amide compounds in concentrations of 10,000 μM and pBR322 plasmid DNA were used. Figure 1 shows that modification of gel electrophoretic mobility of pBR322 DNA after treated with the compounds.

We determined whether the compounds were effective based on the calculations obtained from the electrophoresis images. Table 1 presents the percentages that were obtained from the intensity calculations of the bands revealed in the electrophoresis images of the compounds.

When the numerical data obtained from the electrophoresis images of compound **2** were compared with that

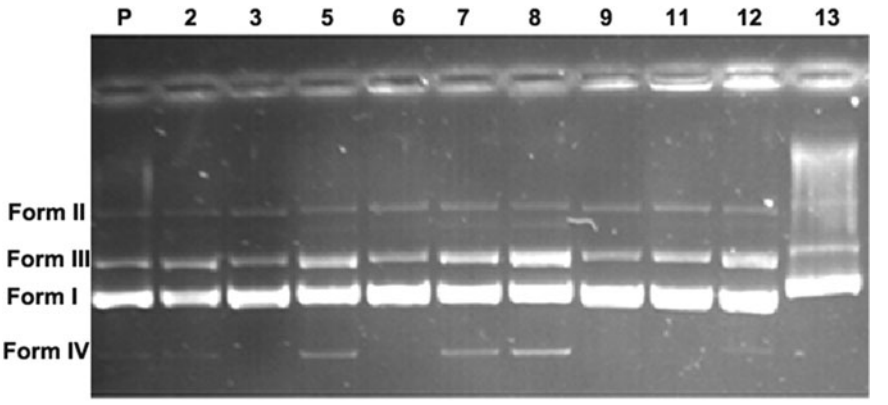


Figure 1. The electrophoresis image of pBR322 DNA after treated with the compounds (P: untreated plasmid DNA with compounds).

Table 1. The percentages of Form I, Form II, Form III and Form IV of pBR322 DNA after treated with the compounds.

Compounds	% SC Form I	%NC Form II	% LC Form III	% SF Form IV
P	86.9	0.9	12.2	–
2	74.8	2.6	22.6	–
3	83.1	3.9	13.0	–
5	62.1	7.3	26.2	4.4
6	73.3	11.2	15.5	–
7	57.0	12.3	23.9	6.8
8	51.7	9.7	31.2	7.4
9	71.3	13.1	15.6	–
11	67.1	13.1	19.8	–
12	60.4	11.8	27.8	–
13	55.1	27.9	17.0	–

Note: Form I, SC; Form II, NC; Form III, LC and Form IV, small DNA fragments.

of the plasmid DNA, the intensity of Form I decreased and the intensities of Form II and Form III increased. This result also indicated that the supercoiled structure of the plasmid DNA was reduced, and the linear and open circular structure of the plasmid DNA was increased by compound **2**. In a comparison of compound **3** with plasmid DNA, Form I, Form II and Form III did not significantly change. In contrast, compound **5** reduced the intensity of Form I of the plasmid DNA and increased the densities of Form II and Form III. In addition, the electrophoresis gel image of compound **5** reveals that the DNA is fragmenting into smaller units in Form IV. When compound **6** is compared with plasmid DNA, the density of Form I partially decreased and the density of Form III partially increased; however, the density of Form II significantly increased. The numerical data obtained from the electrophoresis images of compound **7** showed that the density of Form I significantly decreased and the densities of Form II and Form III increased. At the same time, the electrophoresis image of this compound revealed the formation of Form IV.

The electrophoresis image of compound **8** showed that the density of Form I significantly decreased and the density of Form II and Form III increased. Furthermore, as compounds **5** and **7** were formed, Form IV was revealed. When compounds **9** and **11** were compared with plasmid DNA, the intensity of Form I significantly declined; however, the intensities of Form II and Form III partially increased. Compound **12** showed a significant reduction in the intensity of Form I, while the intensities of Form II and Form III partially increased. The electrophoresis image of compound **13** showed that Form I, Form II and Form III progressed more slowly than the other compounds, because the mass of the plasmid DNA increased as the calixarene held onto the DNA molecule, thus causing it to migrated more slowly.

3. Conclusions

This study focused on the synthesis of calix[4]arene derivatives (i.e. diester, diacide, diamide and monoamide) with different functional groups. The characterisation of the compounds was accomplished via FTIR, ^{1}H NMR, ^{13}C NMR, elemental analysis and FAB-MS techniques. In addition, the DNA interaction activities of the synthesised compounds were investigated. The DNA interaction studies were carried out via agarose gel electrophoresis and by using pBR322 plasmid DNA (*21*, *22*). The investigation of the DNA cleavage activities of the valerate derivatives of calix[4]arene (**2**, **3**, **5** and **6**) showed that compound **5** is more effective in this area. The electrophoresis image of this compound showed a decrease in Form I and an increase in Form III. The investigation of the DNA cleavage activities of the benzoate derivatives of calix[4]arene (**8**, **9**, **11** and **12**) showed that compound **8** is the most effective in this area. The electrophoresis image of this compound revealed the formation of Form IV. The investigation of the DNA interaction properties of mono-morpholine amide derivatives of calix[4]arene (**7** and **13**) revealed that compound

13 is more effective in this area due to the benzoate group in the structure. The electrophoresis image of this compound, revealed that the migration of Form I, Form II and Form III occurred more slowly. Consequently, calix[4]arene compounds that have aromatic groups generally showed more DNA interaction activity. These results confirm the significant biological activity due to the topological and hydrophobic nature of aromatic arms of calixarenes (*14*).

As, scientists have expressed the need to develop new materials due to the drug-resistant nature of pathogenic micro-organisms, these results reveal the potential for new biomedical applications of calixarenes.

4. Experimental

4.1 Materials and methods

Chemicals and solvents were obtained from commercial sources (Merck; Darmstadt, Germany and Sigma; Steinheim, Germany) and used without further purification. DNA [supercoiled (SC) pBR322] was purchased from Fermantas (Vilnius, Lithuania). Measurements of ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian MR 400 MHz spectrometer (UK) using tetramethylsilane as an internal standard. A Perkin-Elmer 1605 FT-IR spectrophotometer was used to record the infrared spectra of all compounds (4000–400 cm^{-1}). Elemental analyses (C, H and N) were carried out using a Leco CHNS-932 analyzer (USA). FAB-MS spectra were taken on a Varian MAT 312 spectrometer (Netherland). Microwave irradiated reactions were carried out by using a CEM MDS-2000. Determination of the melting points was carried out using a Büchi B-540. Analytical TLC was carried out on precoated silica gel plates (SiO_2, Merck PF254).

4.2 Synthesis

Compounds **1**, **3**, **8** and **9** were synthesised according to previously described methods (*17–20*, *23*). The synthesis of the compounds **2**, **4–7** and **10–13** was firstly reported in this study.

*4.2.1 Synthesis of compound **2***

p-tert-Butylcalix[4]arene (5 g, 7.7 mmol), potassium carbonate (2.04 g, 14.8 mmol) and 5-bromomethyl valerate (3.1 g, 15.1 mmol) dissolved in an anhydrous acetone (250 ml) and refluxed by stirring under a nitrogen atmosphere for 72 h. The cooled solution was filtered, the solvent was removed in vacuo to dryness. The remaining crude product was recrystallised from a mixture of dichloromethane–ethanol to obtain compound **2**. Yield: 2.84 g (42%). Mp: 160–162°C IR: 3331 cm^{-1} (OH), 1736 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ ppm 0.96 (s, 18H, But), 1.28 (s, 18H, But), 1.98–2.10 (m, 8H, $OCH_2\underline{CH_2CH_2}$), 2.50 (t, 4H, $J = 6.8$ Hz $COCH_2$), 3.30 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 3.69 (s, 6H, OCH_3), 3.98 (t, 4H, $J = 5.9$ Hz, OCH_2), 4.25 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 6.80 (s, 4H, ArH), 7.04 (s, 4H, ArH), 7.48 (s, 2H, OH). ^{13}C NMR ($CDCl_3$): δ 21.5 ($ArOCH_2CH_2\underline{CH_2}$), 28.6 ($ArOCH_2\underline{CH_2}$), 31.3, 31.5, 32.2, 33.7, 34.1, 34.6, 51.3 (OCH_3), 69.4 ($ArOCH_2$), 125.4, 126.3, 128.2, 130.1, 132.7, 148.7, 150.8, 152.3, 174.2 (C=O). FAB-MS *m/z*: 900.1 $(M + Na)^+$. Anal. calcd for $C_{56}H_{76}O_8$ (877.27): C, 76.6; H, 8.7. Found: C, 77.0; H, 8.5.

*4.2.2 Synthesis of compounds **3** and **9***

Compound **2** or **8** (82.2 mmol), aqueous NaOH solution (6.5 ml) and ethanol (95 ml, 15%) were kept in microwave device for about 5–10 min (by TLC monitoring) and then the reaction was terminated. According to the literature procedures (*24*), reaction mixture was pured.

*4.2.3 Synthesis of **4** and **10***

To a solution of **3** or **9** (1.77 mmol) in a mixture of dichloromethane (20 ml) was added oxalyl chloride (1 ml, 7.85 mmol) and four drops of DMF. The reaction mixture was stirred at room temperature for 1 h and then refluxed for 3 h. The solvent removed under reduced pressure.

*4.2.4 General synthesis method of **5**, **6**, **11** and **12***

Acid chloride compound of calixarene (**4** or **10**) (1.77 mmol) was dissolved in THF, primary amine (8.85 mmol) was added, the reaction mixture was stirred at room temperature for 3 h. It was filtered, the filtrate was removed under vacuo. The residue was dissolved in DCM, later it was extracted with water. The organic phase was separated and dried over $MgSO_4$. The solvent was removed and recrystallised from hot ethanol.

*4.2.4.1 Compound **5***. Yield 50%. Mp > 88°C (decomp.). IR: 1648 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$): δ ppm 0.90 (s, 18H, Bu^t), 1.31 (s, 18H, Bu^t), 1.79–2.10 (m, 8H, $OCH_2\underline{CH_2CH_2}$), 2.51 (t, 4H, $J = 7.2$ Hz, $COCH_2$), 3.30 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 3.90 (t, 4H, $J = 5.6$ Hz, OCH_2),4.14 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 4.43 (d, 4H, $J = 5.2$ Hz, $NHCH_2$), 6.12–6.17 (m, 2H, furfuryl), 6.18–6.24 (m, 2H, furfuryl), 6.73 (s, 4H, ArH), 6.77–6.79 (m, 2H, furfuryl), 7.07 (s, 4H, ArH), 7.16 (s, 2H, OH), 7.38–7.44 (m, 2H, NH). ^{13}C NMR ($CDCl_3$): δ 22.2 ($ArOCH_2\underline{CH_2CH_2}$), 24.6 ($ArOCH_2\underline{CH_2}$), 29.6, 31.6, 32.1, 34.5, 34.6, 37.1 ($NHCO\underline{CH_2}$), 37.3 ($NHCH_2$), 61.4 ($ArOCH_2$), 108.1 (furfuryl =$\underline{CH}$), 110.9 (furfuryl =CH), 125.7, 126.2, 128.6, 132.9, 133.1, 142.7, 147.7, 150.2, 150.8, 152.2, 174.6 (C=O). FAB-MS *m/z*: 1030.17 $(M + Na)^+$.

Anal. calcd for $C_{64}H_{82}O_8N_2$ (1007.34): C, 76.3; H, 8.2; N, 2.8. Found: C, 76.5; H, 8.1; N, 3.1.

4.2.4.2 Compound ***6***. Yield 65%. Mp: 100–102°C. IR:1650 cm^{-1} (C═O). ^{1}H NMR ($CDCl_3$): δ ppm 0.93 (s, 18H, Bu^t), 1.29 (s, 18H, Bu^t), 1.73–2.14 (m, 16H, $OCH_2\underline{CH_2CH_2}$ CH_2CO, $OCH_2\underline{CH_2CH_2}$), 2.51 (t, 4H, $J = 7.2$ Hz, $\underline{COCH_2}$), 3.33 (d, 4H, $J = 13.1$ Hz, $ArCH_2$-Ar), 3.39–3.52 (m, 4H, $NHCH_2$), 3.66–3.76 (m, 4H, OCH_2), 3.88–4.06 (overlapped, 6H, OCH_2, OCH), 4.25 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 6.76 (s, 4H, ArH), 7.07–7.11 (overlapped, 6H, ArH, OH), 7.37 (t, 2H, $J = 5.4$ Hz, NH). ^{13}C NMR ($CDCl_3$): δ 23.8 ($ArOCH_2CH_2\underline{CH_2}$), 25.9 ($CHCH_2\underline{CH_2}$), 29.0 ($ArOCH_2\underline{CH_2}$), 31.2, 31.8, 31.9, 34.0, 34.3, 37.1 ($NHCO\underline{CH_2}$), 43.8 ($\underline{NHCH_2}$), 64.5 ($CHOCH_2$), 66.8 ($ArOCH_2$), 77.6 ($NHCH_2\underline{CH}$), 125.3, 125.7, 128.2, 132.6, 141.7, 146.5, 148.9, 150.2, 174.1 (C═O). FAB-MS *m/z*: 1038.24 $(M + Na)^+$. Anal. calcd for $C_{64}H_{90}O_8N_2$ (1015.41): C, 75.7; H, 8.9; N, 2.7%. Found: 75.5; H, 8.5; N, 2.9%.

4.2.4.3 Compound ***11***. Yield 63%. Mp: 226–229°C. IR: 1639 cm^{-1} (C═O). ^{1}H NMR ($CDCl_3$): δ ppm 0.92 (s, 18H, Bu^t), 1.30 (s, 18H, Bu^t), 3.34 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 4.28 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 4.69 (d, 4H, $J = 11.5$ Hz, $NHCH_2$), 5.08 (s, 4H, OCH_2), 6.25–6.34 (m, 4H, calix-ArH), 6.78 (s, 4H, calix-ArH), 7.07–7.09 (m, 6H, furfuryl-H), 7.34 (s, 2H, OH), 7.56 (t, 2H, $J = 5.6$ Hz, NH), 7.62 (d, 4H, $J = 8.4$ Hz, ArH), 7.96 (d, 4H, $J = 8.4$ Hz, ArH). ^{13}C NMR ($CDCl_3$): δ 31.0 ($C\underline{C}H_3$), 31.5 ($C\underline{C}H_3$), 31.8 ($ArCH_2Ar$), 33.9 ($\underline{C}(CH_3)_3$), 34.1 ($\underline{C}(CH_3)_3$), 37.1 ($NHCH_2$), 63.5 (OCH_2), 107.4 (furfuryl ═CH), 110.6 (furfuryl ═CH), 125.2, 125.7, 126.8, 127.6, 127.8, 132.3, 133.6, 140.9, 141.6, 142.0, 147.4, 149.6, 150.7, 152.0, 167.5 (C═O). FAB-MS *m/z*: 1098.21 $(M + Na)^+$. Anal. calcd for $C_{70}H_{78}O_8N_2$ (1075.38): C, 78.1; H, 7.3, N, 2.6. Found: C, 77.6; H, 7.5, N, 2.2.

4.2.4.4 Compound ***12***. Yield 60%. Mp: 150–153°C. IR: 1657 cm^{-1} (C═O). ^{1}H NMR ($CDCl_3$): δ ppm 0.93 (s, 18H, Bu^t), 1.29 (s, 18H, Bu^t), 1.59–1.77 (m, 4H $OCH_2\underline{CH_2}$), 1.86–1.98 (m, 4H, $OCH\underline{CH_2}$), 3.31 (d, 4H, $J = 13.0$ Hz, $ArCH_2Ar$), 3.44–3.54 (m, 2H, $NHCH_2$), 3.73–3.84 (m, 4H, OCH_2), 3.86–3.95 (m, 2H, $NHCH_2$), 4.14 (p, 2H, $J = 5.4$ Hz, OCH), 4.27 (d, 4H, $J = 13.0$ Hz, $ArCH_2Ar$), 5.09 (s, 4H, OCH_2Ar), 6.78 (s, 4H, calix-ArH), 7.05 (s, 4H, calix-ArH), 7.13 (s, 2H, OH), 7.28 (t, 2H, $J = 5.4$ Hz, NH), 7.75 (d, 4H, $J = 8.3$ Hz, ArH), 7.91 (d, 4H, $J = 8.4$ Hz, ArH). ^{13}C NMR ($CDCl_3$): δ 25.9 ($OCH_2\underline{CH_2}$), 28.9, 30.9, 31.6, 31.7, 33.8, 33.9, 43.8 ($NH\underline{CH_2}$), 68.2, 68.4, 77.9 (OCH), 125.0, 125.6, 126.8, 127.5, 132.4, 134.0, 140.8, 141.5, 147.2, 149.5, 150.6, 152.0, 167.5 (C═O). FAB-MS *m/z*: 1106.27 $(M + Na)^+$. Anal. calcd for $C_{70}H_{86}O_8N_2$ (1083.44): C, 77.6; H, 8.0; N, 2.5. Found: C, 78.1; H, 7.8; N, 2.3.

*4.2.5 Synthesis of monoamide derivatives (****7, 13****)*

Acid chloride compound of calixarene (**4** or **10**) (3 mmol) was dissolved in THF, 3-morpholinopropyl amine (3 mmol) was added, the reaction mixture was stirred at room temperature for 3 h. It was pured with same procedure of **5**, **6**, **11** and **12**.

4.2.5.1 Compound ***7***. Yield 75%. Mp > 110°C (decomp.) IR: 1650 cm^{-1} (C═O) 1729 cm^{-1} (COOH). ^{1}H NMR ($CDCl_3$): δ ppm 0.95 (s, 18H, Bu^t), 1.27 (s, 18H, Bu^t), 1.93–2.12 (m, 10H, $NHCH_2\underline{CH_2}$, $COCH_2\underline{CH_2CH_2}$), 2.40–2.60 (m, 4H, $COCH_2$), 2.95–3.11 (m, 6H, $N(CH_2)_3$), 3.31 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 3.41 (q, 2H, $J = 6.6$ Hz, $NH\underline{CH_2}$), 3.92–4.02 (m, 8H, $ArOCH_2$, CH_2OCH_2), 4.22 (d, 4H, $J = 13.1$ Hz, $ArCH_2Ar$), 6.72–6.84 (m, 4H, ArH), 7.03–7.11 (m, 4H, ArH), 7.47 (s, 2H, OH), 7.89 (t, 1H, $J = 5.4$ Hz, NH). ^{13}C NMR ($CDCl_3$): δ 22.8 ($ArOCH_2CH_2\underline{CH_2}$), 23.9 ($ArOCH_2CH_2\underline{CH_2}$), 29.3 ($ArOCH_2\underline{CH_2}$), 29.5 ($ArOCH_2\underline{CH_2}$), 30.3, 31.0, 31.5, 31.7, 33.8, 33.9, 34.0, 34.2, 36.0, 36.6, 52.1 ($NHCH_2$), 55.4 (NCH_2), 64.0 (CH_2NCH_2), 64.2 (CH_2OCH_2), 72.3 ($ArOCH_2$), 125.5, 127.9, 128.2, 132.4, 133.4, 135.8, 142.1, 147.1, 149.7, 150.4, 150.8, 151.6, 174.5 (C═O), 177.1 (C═O). FAB-MS *m/z*: 998.17 $(M + Na)^+$. Anal. calcd for $C_{61}H_{86}O_8N_2$ (975.34): C, 75.1; H, 8.9; N, 2.9. Found: C, 75.7; H, 8.5; N, 2.6.

4.2.5.2 Compound ***13***. Yield 70%. Mp > 155°C (decomp.). IR: 1640 cm^{-1} (C═O) 1713 cm^{-1} (COOH). ^{1}H NMR ($CDCl_3$): δ ppm 0.91 (s, 18H, Bu^t), 1.29 (s, 18H, Bu^t), 2.26–2.28 (m, 2H, $CH_2\underline{CH_2}CH_2$), 2.32 (t, 2H, $J = 5.4$ Hz, NCH_2) 2.94–3.47 (overlapped, 10H, $NHCH_2$, CH_2NCH_2O, $ArCH_2Ar$), 4.05 (bs, 4H, CH_2OCH_2), 4.27 (d, 4H, $J = 13.3$ Hz, $ArCH_2Ar$), 5.08 (s, 4H, OCH_2), 6.75–6.81 (m, 4H, ArH), 7.02–7.14 (overlapped, 6H, ArH, OH), 7.81 (d, 4H, $J = 8.0$ Hz, ArH), 8.09 (d, 4H, $J = 8.0$ Hz, ArH), 8.67 (t, 1H, $J = 5.2$ Hz, NH) ^{13}C NMR ($CDCl_3$): δ 23.8 ($NHCH_2\underline{CH_2}$), 29.5, 30.3, 30.9, 31.7, 33.8, 33.9, 34.2, 37.2 ($NH\underline{CH_2}$), 52.1, 55.5 (NCH_2), 60.1 (CH_2NCH_2), 60.9 (CH_2OCH_2), 63.7 (OCH_2), 125.0, 125.5, 125.6, 126.6, 127.0, 127.6, 127.9, 128.2, 129.9, 132.3, 132.5, 133.0, 135.8, 141.3, 141.6, 142.1, 147.3, 149.5, 150.5, 151.4, 166.4 (C═O), 167.9 (C═O). FAB-MS *m/z*: 1066.21 $(M + Na)^+$. Anal. calcd for $C_{67}H_{82}O_8N_2$ (1043.38): C, 77.1; H, 7.9; N, 2.7. Found: C, 76.7; H, 8.1; N, 2.5.

4.3 DNA interaction studies

For the agarose gel electrophoresis experiments, 0.5 μg/μl SC pBR322 DNA (0.5 μg/μl) was treated with 1 μl of 1 mM tested ligand and its complexes in DMF and 2 μl of 0.1 M Tris–HCl (pH 8.0) buffer. After incubation at 37°C for 2 h, 1 μl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol in H_2O) was added to each tube and the mixed solution was loaded on 1% agarose gel. The electrophoresis was carried out for 1.5 h at 100 V in TBE buffer (89 mM Tris–borate, pH 8.3, 2.5 mmol l–1 EDTA). Gels were stained with ethidium bromide (1 mg ml^{-1}) for 10 min prior to being photographed under UV light. The efficiency of the DNA cleavage was measured by determining the ability of the compound to form linked circular (LC) or nicked circular (NC) DNA from its SC form by quantitatively estimating the intensities of the bands using the Biolab UVItec gel documentation system. The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane.

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