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ORIGINAL ARTICLE

# Synthesis of new water-soluble phosphonate calixazacrowns and their use as drug solubilizing agents

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**Abstract** This study presents the selective chloromethylation of calix[4](aza)crown ethers **2a–c**, using chloromethyl *n*-octyl ether and $SnCl_4$ in chloroform at room temperature in good yield for the first time. Chloromethylated products **2a–c** are used as key intermediates to synthesize new water-soluble *p*-phosphonato calix[4](aza)crown ethers **5a–c**. Liquid–liquid phase extraction and phase solubility studies with poor water soluble drug molecules such as nifedipine, niclosamide and furosemide are performed to evaluate their binding properties. Among the studied drugs, furosemide was the most effectively dissolved drug by *p*-phosphonato calix[4](aza)crown ethers **5a–c** in water.

**Keywords** Calixarene · Chloromethylation · Niclosamide · Furosemide · Nifedipine

## Introduction

The importance of calixarenes has been entirely recognized since the pioneering studies of Gutsche [1, 2]. Calixarenes are cyclic oligomers made of several phenolic units bound with methylene bridges, which can adopt various conformations and form hydrophobic cavities. Calixarenes can be decorated with a wide variety of functional groups on the aromatic rings and/or the O-centres of the phenolic groups, the so-called upper (or wide) and lower (or narrow) rims of the calixarenes, respectively [3, 4]. Calixarenes are sparingly soluble in aqueous media and this property is the major problem for calixarene use in biopharmaceutical applications. To overcome these limitations, water-soluble groups containing positive or negative charges, such as amine [5], phosphonate [6] and sulphonate [7] groups, or with neutral groups, such as sulfonamides [8], sugars [9] and polyoxyethylene [10], can be located on the lower or upper rim of the calixarene skeleton. The first example of a water-soluble calixarene introduced by four carboxy methyl groups on the phenolic oxygen of *p-tert*-butylcalix[4]arene was reported by Ungaro and co-workers in 1984 [11]. In the same year, Shinkai reported the preparation of *p*-sulfonato calix[6]arene [12]. Although the most widely studied water soluble calixarenes are the *p*-sulfonato derivatives that show solubility greater than 0.1 M in aqueous solutions, the least studied are *p*-phosphonatocalixarene derivatives, especially due to the concerned synthetic procedures required to bind phosphonate groups directly to *para* position of calixarene via bromination [13]. To overcome this, a synthetic method was reported by Ungaro and co-workers in 1989 by introducing chloromethyl groups onto the para position of calixarene (tetramer, hexamer or octamer) [6]. These chloromethylated compounds are useful intermediates for introducing useful functional groups at this position. A number of researchers have carried out the chloromethylation of calixarenes but no selective product has been observed until now [14–17]. Calix(aza)crown ether derivatives have been extensively used in chemical applications such as cations, anions and neutral recognitions [18–23]. Therefore, selective chloromethylated calix(aza)crown ethers may be useful precursors to obtain several new *p*-substituted calixcrown ethers.

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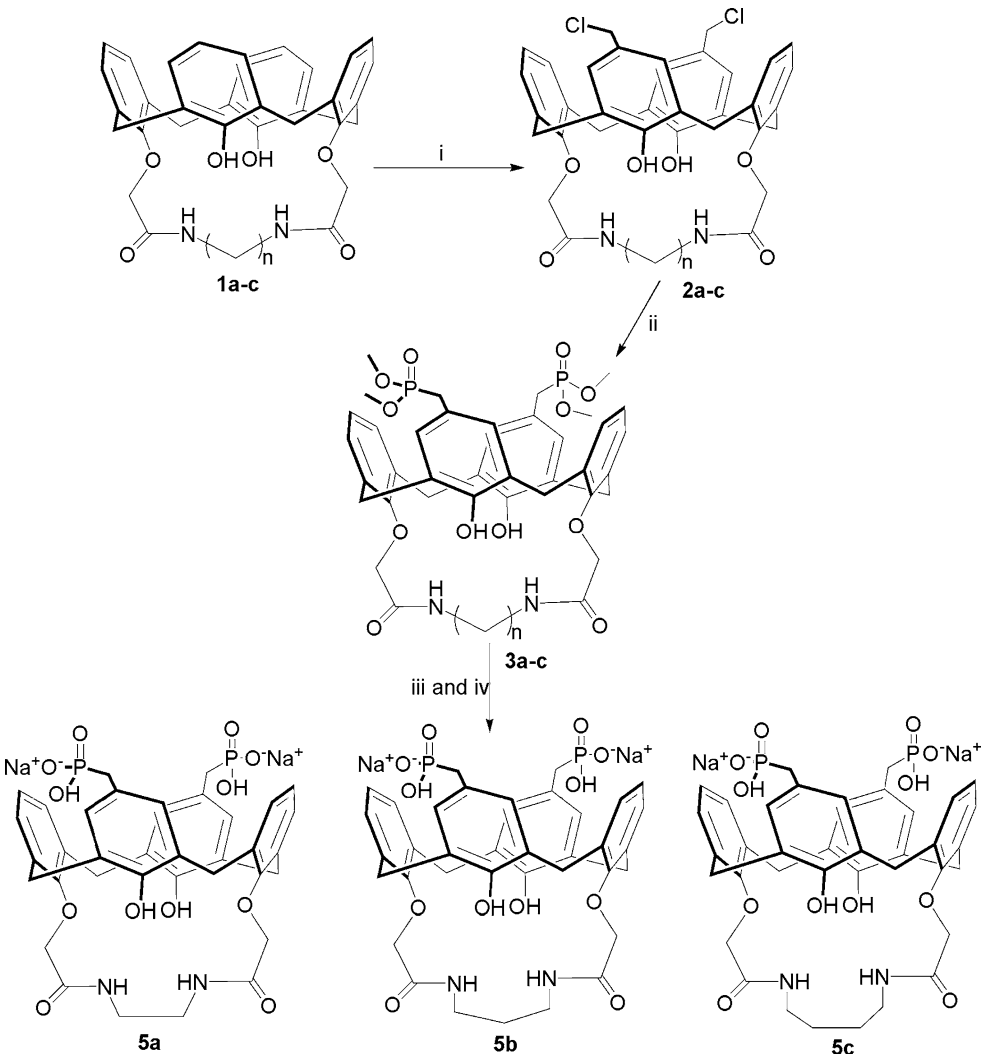
Niclosamide 5-chloro-*N*-2-chloro-4-nitrophenyl-2-hydroxybenzamide, nifedipine 3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate and furosemide 5-(aminosulfonyl)-4-chloro-2-[(2-furanyl methyl) amino] benzoic acid are poorly water soluble drug molecules and are used as an anthelmintic, a calcium channel blocker and a loop diuretic, respectively [24, 25]. The main problem of these molecules is poor aqueous solubility. A commonly used technique to increase the solubility of drugs is through supramolecular complexation [26–28]. Cyclodextrins are the most widely used macromolecules to solubilize drugs [29]. Cyclodextrins are a family of three major well-known cyclic oligosaccharides. The negligible cytotoxic effects of cyclodextrins are an important contribution in application of drug carriers [30]. Although the FDA has currently not approved the use of calixarenes in medicines to date the calixarenes have showed neither toxicity nor immune responses [31]. This situation increases interest in their use in biopharmaceutical applications beyond their current use for the chiral separation of molecules [32] and as complex forming agents to remove molecules from the environment [33–35].

To date, although several works about the effect of water-soluble *p*-sulphonic calix[n]arenas on the solubility of drugs have been reported [36–38], there are no other published extraction and/or phase solubility studies between drug molecules and phosphorylated calixarene derivatives in literature. Furthermore, there are no studies about the synthesis of selectively chloromethylated calixarenes by using chloromethyl *n*-octyl ether. Therefore, the aim of the present study was to synthesize new water-soluble *p*-phosphonato calix(aza)crown ethers by selective introducing chloromethyl groups onto the *para* position of calix[4](aza)crown ethers and exploring the effect of phosphorylated calix[4](aza)crown receptors on the extraction and solubility of nifedipine, niclosamide and furosemide drug molecules.

## Results and discussion

The required starting material, calix(aza)crown ethers **1a–c** was obtained by using a literature procedure [18]. Water soluble derivatives **5a–c** were obtained in four steps as shown in Scheme 1. In the first step, calix(aza)crown ethers **1a–c** were chloromethylated using chloromethyl *n*-octyl ether and $SnCl_4$ in chloroform at room temperature in good yields following the modified literature procedure of Ungaro [6]. In the second step, obtained chloromethylated compounds **2a–c** were refluxed with an excess amount of trimethyl phosphite instead of triethyl phosphite to obtain the corresponding phosphonato methyl ester of calix(aza)crown ethers **3a–c** for 6 h. In this step, trimethyl phosphite was chosen as a new phosphorylation reagent instead of triethyl phosphite owing to the easy elimination of the unreacted phosphite compound. In the third step, compounds **3a–c** were subsequently converted into the phosphonic acid analogues **4a–c** by the reaction of phosphonato methyl ester of calix(aza)crown ethers **3a–c** with bromotrimethylsilane (BTMS) and methanol at room temperature and under a dry atmosphere. Finally, the phosphonic acids **4a–c** were converted in their corresponding sodium salts **5a–c** by the controlled neutralization of crude products **4a–c** with 0.05 M of NaOH.

All compounds were fully characterized. The $^1$H NMR spectra of newly synthesized compounds **2a–c**, **3a–c** and **5a–c** showed two sets of doublets for the bridging methylene protons. A typical AX pattern was observed for the methylene bridge $ArCH_2Ar$ protons around 3.47–3.50 ppm ($J_{AB}$ = 13.1 Hz) and 4.12–4.16 ppm for compounds **2a–c**, **3a–c** and **5a–c** in the $^1$H NMR. The high field doublets around 3.47–3.50 ppm for compounds **2a–c**, **3a–c** and **5a–c** are assigned to the equatorial protons of methylene groups, whereas the low field signals around 4.12–4.16 ppm for compounds **2a–c**, **3a–c** and **5a–c** are assigned to the axial protons in the $^1$H NMR. This NMR data demonstrated that these compounds were in the cone conformation. Also, this situation was supported by $^{13}$C NMR data with $ArCH_2Ar$ resonance signals comprised between 31.00 and 31.50 ppm. While the $^1$H NMR spectra of the chloromethylated calix(aza)crown ethers **2a–c** showed one singlet peak assigned to chloromethyl protons around 4.48 ppm, the same peak was observed at 3.00 ppm attributed to the phosphonomethylene protons for compounds **3a–c** as doublet ($J_{PH}$ = 21.1 Hz). The new peak for compounds **3a–c** was seen as doublet ($J_{POCH_3}$ = 10.0 Hz) around 3.60–3.63 ppm attributed to the phosphonomethylester protons (Fig. 1). The $^1$H NMR analysis of water-soluble phosphonatocalix(aza)crown ethers **5a–c** were performed in $D_2O$. $^1$H NMR data for compounds **5a–c** showed the presence of the expected alkyl resonance signals, especially one doublet ($J_{PH}$ = 20.1 Hz) around 2.75 ppm attributed to the phosphonomethylene protons, and an AB system ($J_{AB}$ = 13.2 Hz) around 3.50 and 4.00 ppm corresponding to the $ArCH_2Ar$ groups, respectively. In the $^1$H NMR spectra of the compounds **5a–c**, a peak attributed to the phosphonomethylester protons around 3.60–3.65 ppm was not observed. This data showed that desterification of phosphonomethylester protons of compounds **3a–c** with BTMS was completed. The presence of the phosphonate groups of compounds **3a–c** and **5a–c** was confirmed by $^{31}$P resonance signals at 29.00 ppm for compounds **3a–c** ($CDCl_3$) and 21.00 ppm for compounds **5a–c** ($D_2O$). All other data




**Scheme 1** Synthesis of compounds **2a–c**, **3a–c** and **5a–c**. Reagents and conditions: (i) $C_8H_{17}OCH_2Cl$, $SnCl_4$, $CHCl_3$, rt, 90%; (ii) $(CH_3O)_3P$, $CHCl_3$, reflux, 80%; (iii) BTMS, $CH_3OH$, rt (v) 0.05 M NaOH, $H_2O$

were in agreement with the proposed structures of compounds **2a–c**, **3a–c** and **5a–c**.

## Liquid–liquid extraction and phase-solubility studies

### Furosemide

Furosemide is a derivative from anthranilic acid, whose structure is presented in Fig. 2. Furosemide represents a powerful loop diuretic that is widely used in the treatment of hypertension and edema. It is usually commercialized as tablets or parenteral solutions. The orally bioavailability of furosemide is very poor due to aqueous solubility in gastrointestinal pH, making solubility the rate-determining step in the gastric absorption of furosemide [37]. Several techniques have been used to increase its aqueous solubility, including cyclodextrin complexation [39, 40].

Obtained results show that furosemide drug molecules are encapsulated into the hydrophobic cavity of cyclodextrins; this significantly increases the solubility and dissolution rate of furosemide. Also, calixarene compounds might form host–guest complexes with furosemide. Therefore, we performed some preliminary evaluations to investigate the binding efficiencies of the extractants **5a–c** for furosemide by using solvent extraction. The results showed that furosemide could be extracted from organic phase into aqueous phase at neutral pH values. The results are summarized in Table 1. From Table 1, it is clear that maximum extraction percentage toward furosemide occurs at 59.9% for **5a**, 58.1% for **5b** and 54.5% for **5c**. From the extraction data, water-soluble *p*-phosphonate derivative **5a–c** was found to be an effective extractant for the phase transfer of furosemide. At the same time, a phase-solubility study of furosemide by phosphonate calixarenes **5a–c** was performed to show the possible interaction between calixarenes and drug molecules. From the phase solubility study of furosemide (Fig. 3), the solubility of furosemide in water increased significantly when the concentrations of the *p*-phosphonate derivatives **5a–c** were increased, and so

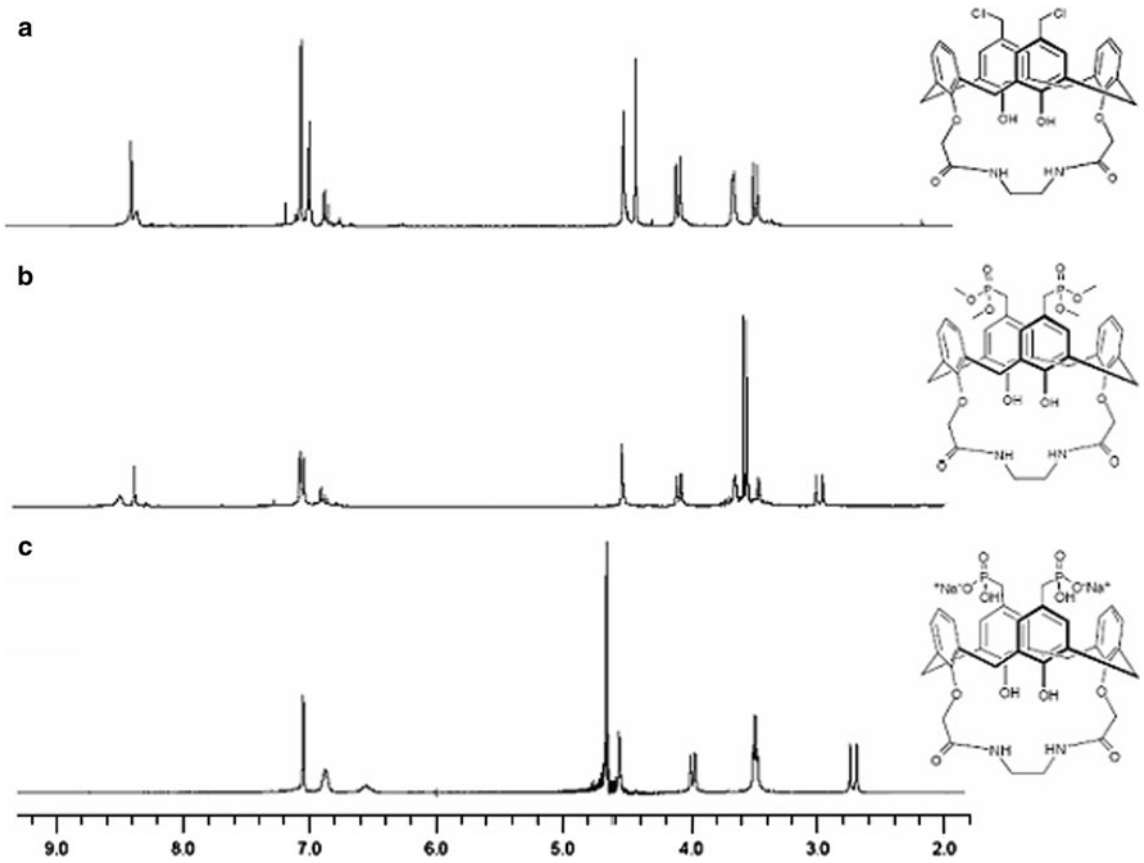
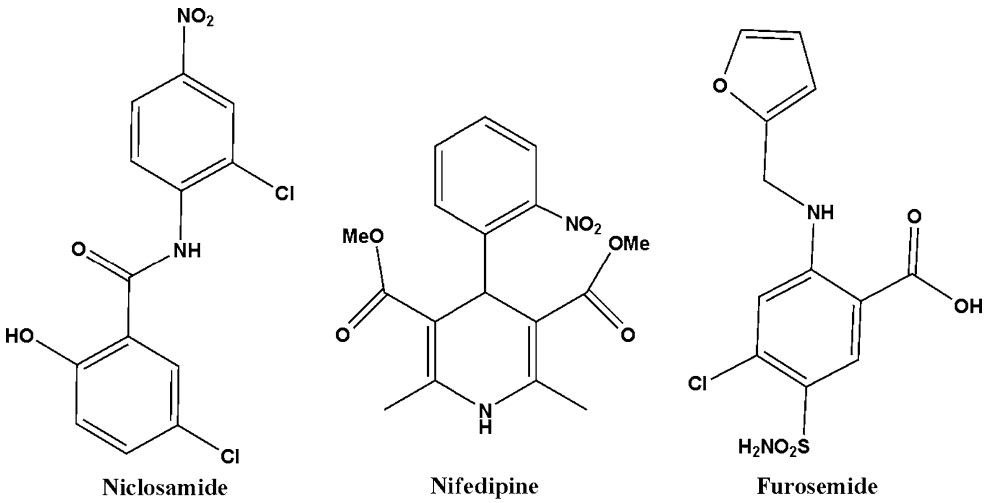



**Fig. 1** $^{1}$H NMR of **a** compound **2a** ($CDCl_3$, 400 MHz), **b** compound **3a** ($CDCl_3$, 400 MHz) and **c** compound **5a** ($D_2O$, 400 MHz)

**Fig. 2** Molecular structures of drugs




it was clear that the possible more-soluble host–guest complex between furosemide and calixarene backbone was formed. The largest increase in solubility from $(4.80 \pm 0.03) \times 10^{-3}$ M to $(18.20 \pm 0.03) \times 10^{-3}$ M for compound **5b** and from $(5.00 \pm 0.03) \times 10^{-3}$ M to $(18.80 \pm 0.03) \times 10^{-3}$ M for compound **5c** was observed at 0.007 M (Fig. 3; Table 2). From the phase solubility profiles of the furosemide, a linear increase in solubility of furosemide as shown in Fig. 3 represents Type $A_L$ phase solubility profiles attributable the formation of 1:1 furosemide:calixarene complexes [41]. Higuchi and Connors [41] have classified complexes based on their effect on solubility of substrate. A-type phase-solubility profiles are obtained when the solubility of the drug increases with increasing ligand concentration. The $A_L$ model shows that the association constant of $K_{1:1}$ indicates one molecule of

**Table 1** Extraction percentage of drug molecules by receptors

| Compound | Nifedipine | Niclosamide | Furosemide |
|---|---|---|---|
| **5a** | 17.5 ± 0.1 | 40.3 ± 0.2 | 56.9 ± 0.1 |
| **5b** | 20.1 ± 0.2 | 42.4 ± 0.2 | 58.8 ± 0.1 |
| **5c** | 22.8 ± 0.2 | 38.7 ± 0.2 | 54.5 ± 0.1 |
| Blank | <4.1 ± 0.3 | <3.5 ± 0.3 | <1 ± 0.3 |

Averages and standard deviations calculated for data obtained from three independent extraction experiments

Aqueous phase [ligand]: $4 \times 10^{-3}$ M; organic phase: [drug molecule]: $2 \times 10^{-4}$ M; at 25 °C, for 5 h

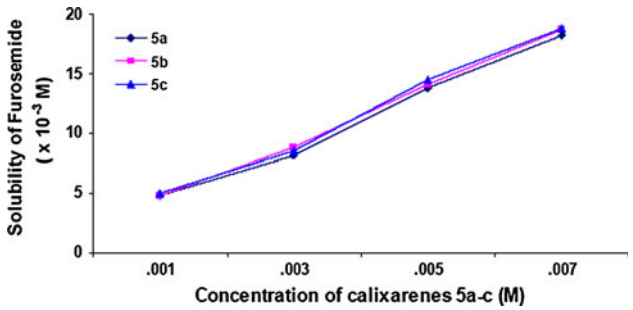



**Fig. 3** Phase solubility diagrams of furosemide in the presence of increasing concentrations of phosphonate calixarene hosts **5a–c** in water

drug forms a complex with one molecule of ligand and a linear relationship exhibit. A type $A_P$ system indicates that one molecule of drug forms a complex with two molecules of ligand and a positive deviation from linearity is obtained. Also, an $A_N$ type profile, which is the least encountered system, shows a negative deviation, indicating decreasing drug concentration with increasing ligand concentrations. Generally, the most common stoichiometry of drug/calixarene inclusion complexes is 1:1, and is often studied by phase solubility studies. B type phase-solubility profiles indicate formation of complexes with limited solubility in the aqueous complexation medium. $^1$H NMR and FTIR spectra of the solid inclusion complex of furosemide:calixarene **5b** was used to clarify the possible interaction between the furosemide drug molecule and the cavity of calixarene compound **5b**. In the IR spectrum of the calixarene:furosemide inclusion complex the characteristic absorption of the calixarene skeleton was superposed over the furosemide structure. FTIR spectra of the furosemide showed a sharp signal around 1,559 $cm^{-1}$ due to the sulphonamide group ($–SO_2NH_2$) of the furosemide structure. However, intensity and sharpness of this signal at 1,559 $cm^{-1}$ drastically reduced the inclusion complex formation. This situation indicated that an interaction between the $NH_2$ group of furosemide and the cavity of calixarene skeleton occurred. Also, the spectrum of solid inclusion complex did not show any new peaks, which indicates that no new chemical bonds are formed in the complex. This situation is in accordance with the literature results [42, 43] of inclusion complex of furosemide drug molecules with macrocyclic ligands by supramolecular complexation. Furthermore, in the $^1$H NMR spectra of the furosemide in $CDCl_3$ the characteristic absorptions of aromatic protons of furosemide were observed in the range of 7.1–7.8 ppm. After complexation the signals become more or less shifted in the $^1$H NMR ($D_2O$) spectra of the furosemide drug molecule. The slight difference of the furan protons of the furosemide drug molecule in the inclusion complex showed that the complexation did not occur close to the furan group. This interaction is attributed to the weak interaction forces, including hydrogen bonding, $\pi$–$\pi$ interactions, dipole–dipole bonding or electrostatic interaction between hydrophobic cavities, nitrogen donor groups in crown moiety or alky phosphonate groups of receptors and/or substituted groups of furosemide. Calixarenes, with both phosphoryl (P=O) groups and nitrogen groups in the azacrown ring, are capable of effectively binding different cations and organic molecules with hydrogen bond donors [44–46]. With the help of one or a combination of these forces, furosemide most probably formed by non-covalent inclusion complexes with the *p*-phosphonate derivative similar to the complexes it forms with 4-sulphonic calix[n]arenes [37].

## Nifedipine

Nifedipine as a L-type calcium-channel blocker is used extensively for the clinical management of a number of

**Table 2** Concentration values of furosemide with increasing calixarene concentrations in water

| Compounds | Concentration values (mol·$L^{-1}$ in water) | | | |
|---|---|---|---|---|
| | 0.001[a] | 0.003[a] | 0.005[a] | 0.007[a] |
| **5a** | $4.8 \times 10^{-3} \pm 0.03$[b] | $8.1 \times 10^{-3} \pm 0.03$ | $13.8 \times 10^{-3} \pm 0.03$ | $18.2 \times 10^{-3} \pm 0.03$ |
| **5b** | $4.8 \times 10^{-3} \pm 0.03$ | $8.8 \times 10^{-3} \pm 0.03$ | $14.1 \times 10^{-3} \pm 0.03$ | $18.7 \times 10^{-3} \pm 0.03$ |
| **5c** | $5.0 \times 10^{-3} \pm 0.03$ | $8.5 \times 10^{-3} \pm 0.03$ | $14.5 \times 10^{-3} \pm 0.03$ | $18.8 \times 10^{-3} \pm 0.03$ |

[a] Concentration values of calixarenes **5a–c**

[b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments

**Table 3** Concentration values of nifedipine with increasing calixarene concentrations in water

| Compounds | Concentration values (mol·L$^{-1}$ in water) | | | |
|---|---|---|---|---|
| | 0.001[a] | 0.003[a] | 0.005[a] | 0.007[a] |
| **5a** | $3.4 \times 10^{-5} \pm 0.03$[b] | $4.8 \times 10^{-5} \pm 0.03$ | $5.1 \times 10^{-5} \pm 0.03$ | $5.2 \times 10^{-5} \pm 0.03$ |
| **5b** | $3.8 \times 10^{-5} \pm 0.03$ | $4.9 \times 10^{-5} \pm 0.03$ | $5.3 \times 10^{-5} \pm 0.03$ | $5.3 \times 10^{-5} \pm 0.03$ |
| **5c** | $3.8 \times 10^{-5} \pm 0.03$ | $4.9 \times 10^{-5} \pm 0.03$ | $5.3 \times 10^{-5} \pm 0.03$ | $5.2 \times 10^{-5} \pm 0.03$ |

[a] Concentration values of calixarenes **5a–c**

[b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments

cardiovascular diseases, such as essential hypertension, congestive heart failure and cerebral ischemia [24]. A major pharmaceutical problem associated with nifedipine is its poor aqueous solubility, 5–6 μg/cm$^3$ over a pH range of 2–10, which may account for its highly variable bioavailability in humans [47]. Obtained extraction results showed that the nifedipine drug molecule could be transported from organic phase into aqueous phase by compounds **5a–c**, as shown in Table 1. Comparing the extraction data between furosemide and nifedipine, extractability of nifedipine was not significantly changed with compounds **5a–c**. From Table 1 it is clear that maximum extraction percentage towards nifedipine occurs at 17.5% for **5a**, 20.1% for **5b** and 22.8% for **5c**. Obtained results show that extraction of nifedipine by compounds **5a-c** is limited (Table 3). Also, this situation was supported by the phase solubility study of nifedipine (Fig. 4). Although there was no significant difference in the increasing solubility of nifedipine by compounds **5a–c**, the solubility of nifedipine in water was increased to some extent with increasing the concentrations of the *p*-phosphonate derivatives **5a–c**. In literature [38], 4-Sulphonic calix[8]arene has shown a good interaction with nifedipine owing to the suitably large cavity of calix[8]arene. Calix[8]arenes are more flexible than calix[4]arenes owing to stronger intra-molecular hydrogen bonding in the calix[4]arenes [1]. Weak interaction forces such as $\pi$–$\pi$ interactions, dipole–dipole bonding and/or electrostatic attraction, as mentioned above for furosemide, may be other important contributions to the interaction between receptors **5a–c** and nifedipine, as well as hydrogen binding between OH groups of *p*-phosphonate calix[4]arenes and substituted groups of nifedipine [48].

## Niclosamide

Niclosamide is active against most tapeworms, including the beef tapeworm, the dwarf tapeworm, and the dog tapeworm [25]. This drug is also used as a molluscicide for the treatment of water in schistosomiasis control programs [36]. Niclosamide is practically insoluble in water (230 ng/cm$^3$), which may severely limit its efficacy [49]. From the extraction results (Fig. 4.), it has been observed that extraction percentage towards niclosamide occurred at 40.3% for **5a**, 42.4% for **5b** and 38.7% for **5c**. These are not surprising results because in our previous extraction study [48] it was observed that the *p*-phosphonate calix[6]arene receptor extracted the niclosamide drug molecules at around 38% from the organic phase to the aqueous phase. In contrast to the obtained extraction percentage, solubility of niclosamide by compounds **5a–c** in water was increased by some extent with increasing the concentrations of the *p*-phosphonate derivatives **5a–c**, as mentioned for nifedipine (Fig. 5). The largest increase in solubility of niclosamide from $(1.40 \pm 0.03) \times 10^{-6}$ M to $(3.30 \pm 0.03) \times 10^{-6}$ M for compound **5b** and from

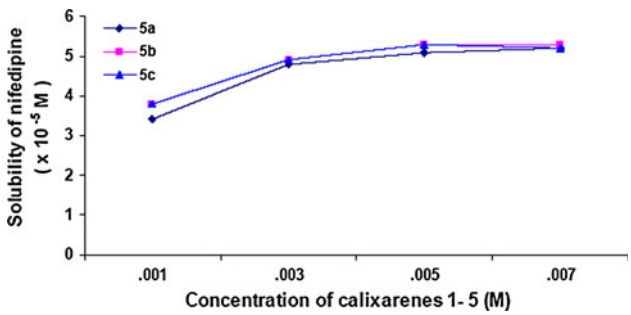



**Fig. 4** Phase solubility diagrams of nifedipine in the presence of increasing concentrations of phosphonate calixarene hosts **5a–c** in water

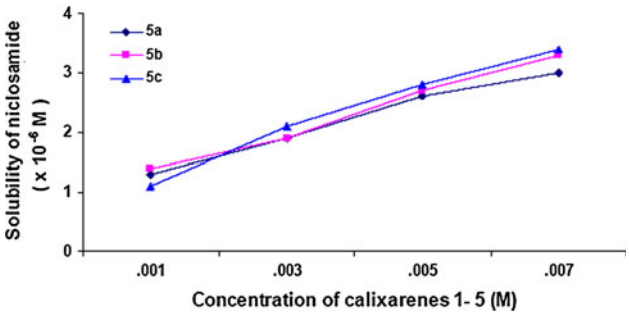



**Fig. 5** Phase solubility diagrams of niclosamide in the presence of increasing concentrations of phosphonate calixarene hosts **5a–c** in water

**Table 4** Concentration values of niclosamide with increasing calixarene concentrations in water

| Compounds | Concentration values (mol·L$^{-1}$ in water) | | | |
|---|---|---|---|---|
| | 0.001[a] | 0.003[a] | 0.005[a] | 0.007[a] |
| **5a** | $1.3 \times 10^{-6} \pm 0.03$[b] | $1.9 \times 10^{-6} \pm 0.03$ | $2.6 \times 10^{-6} \pm 0.03$ | $3.0 \times 10^{-6} \pm 0.03$ |
| **5b** | $1.4 \times 10^{-6} \pm 0.03$ | $1.9 \times 10^{-6} \pm 0.03$ | $2.7 \times 10^{-6} \pm 0.03$ | $3.3 \times 10^{-6} \pm 0.03$ |
| **5c** | $1.1 \times 10^{-6} \pm 0.03$ | $2.1 \times 10^{-6} \pm 0.03$ | $2.8 \times 10^{-6} \pm 0.03$ | $3.4 \times 10^{-6} \pm 0.03$ |

[a] Concentration values of calixarenes **5a–c**

[b] Averages and standard deviations calculated for data obtained from three or four independent solubility experiments

$(1.10 \pm 0.03) \times 10^{-6}$ M to $(3.40 \pm 0.03) \times 10^{-6}$ M for compound **5c** was observed at 0.007 M in water (Fig. 5; Table 4). Niclosamide is a weak acid with an acidic p$K_a$ of 7.3 [47]. In water, ionized states of both the basic calixarenes and drug molecules show a good interaction leading to an increase in solubility [50]. Conversely, niclosamide is a highly hydrophobic molecule, such as nifedipine and furosemide. Therefore, similar interactions between calixarenes and niclosamide were probably occurred as mentioned, in both nifedipine and furosemide. From the phase solubility study of niclosamide, in literature, the larger cavities would geometrically be more suited for a stronger interaction with niclosamide [36] owing to the "host-size selectivity" in host–guest-type complexation with calixarenes [1, 31]. Hydrogen bonding and weak interaction forces as $\pi$–$\pi$ interactions and dipole–dipole bonding are the most important forces responsible for the extraction of niclosamide (Fig. 5). Furthermore, the other interaction is not micellar aggregation of the calixarenes **5a–c** around the drug molecules such as furosemide, nifedipine and niclosamide. This is because the *p*-phosphonate calix[4]arenes form the micellar aggregate at concentrations of more than 0.2 M in water [51]. The concentrations of *p*-phosphonate calix[4]arenes used in this study were therefore below the critical micelle concentrations. Also, this situation supports the inclusion complexation phenomena between calixarene receptors **5a–c** and drug molecules. With the help of both this situation and weak forces drug molecules most probably form non-covalent inclusion complexes with the *p*-phosphonate calix[4]arenes **5a–c** similar to the complexes it forms with cyclodextrins. In the IR spectra of the solid inclusion complex, broad bands of calixarene overlapped the main niclosamide characteristic peaks. Nevertheless, niclosamide characteristic peaks could be detected in the IR spectra. The shape and location of the bands was shifted to some extent. While the characteristic absorptions of aromatic protons (nitro phenyl) of niclosamide were seen around 8.1–8.5 ppm in the $^1$H NMR ($CDCl_3$) spectra of the drug, these protons more or less shifted after inclusion complexation of the niclosamide:calixaren **2**. Especially, the slight difference of the phenolic ring protons of niclosamide around 8.1 and 7.1 ppm showed that the complexation did not occur close to this aromatic group.

## Conclusion

In conclusion, we have successfully synthesized the first examples of chloromethylated calix[4](aza)crown ether and water-soluble *p*-phosphonato calix[4](aza)crown ether derivatives. All procedures are simple and materials can be obtained in large quantities. Chloromethylated calixcrown ethers **2a–c** may be useful precursors to synthesize further relevant calixcrown derivatives. Furthermore, both drug extraction and phase solubility studies were performed to evaluate use of calixcrown derivatives as drug solubilizing agents. The complexation studies showed that compounds **5a–c** were effective receptors for niclosamide and furosemide drug molecules. It could be concluded that the complexation of drug molecules depends on the structural properties of the water-soluble *p*-phosphonate calix[4](-aza)crown, such as hydrophobic cavity diameters, hydrogen binding ability and stability or rigidity, and also depends on ion–dipole attraction or electrostatic interaction between *p*-phosphonate calixarene and drug molecules.

The inclusion complexation of the drugs with the water-soluble calixarenes provides an opportunity to modify different characteristics of the active molecule to improve stability, crystallize amorphous drugs, prevent polymorphism and increase solubility. The biopharmaceutical application of water-soluble calixarenes by their complexation can be expected to be of great importance in the future.

## Experimental

All of the reagents used in this study were obtained from Merck, Sigma Aldrich or Fluka and used without further purification. Thin layer chromatography (TLC) was performed using silica gel on glass TLC plates (silica gel H, type 60, Merck). Generally solvents were dried by storing

them over molecular sieves (Aldrich; 4 Å, 8–12 mesh). All aqueous solutions were prepared with deionized water that had been passed through a Millipore Milli-Q Plus water purification system. $^{1}H$, $^{13}C$ and $^{31}P$ NMR spectra were obtained using a Varian 400 MHz spectrometer operating at 400 MHz. IR spectra was recorded on a PerkinElmer spectrum 100 FT-IR spectrometer (ATR). UV–Visible spectra were obtained on Jenway 6105 and Shimadzu 160 A UV–Visible recording spectrophotometers. Elemental analyses were performed using a Leco CHNS-932 analyzer. A Crison MicropH 2002 digital pH meter was used for the pH measurements.

Synthesis of chloromethylated calix[4](aza)crown ether derivatives **2a–c** (general procedure)

To a cooled solution of corresponding 1.2 mmol of calix[4](aza)crown ethers (**1a–c**) and 40.5 mmol of chloromethyl-*n*-octyl ether in 50 mL of $CHCl_3$, 20.15 mmol of $SnC1_4$ were added dropwise at −10 °C in about 10 min and then the reaction mixture stored at room temperature for additional 50 min. After a drop of $SnCl_4$ reaction mixture turned white turbidity and also pink turbidity occured in the end of the reaction. Water was then added slowly and two phases separated. The organic layer was washed twice with distilled water and then aqueous layer was extracted with $CHCl_3$ again and combined organic phases were evaporated to give a residue. Residue was dissolved a minimum amount of $CHCl_3$ and treated with n-hexane and obtained precipitate was filtered off and washed with diethyl ether to give corresponding pure chloromethylated calix[4](aza)crown products **2a–c**.

*Compound **2a***

Yield = (90%, pale pink) m.p. >300 °C. $^{1}H$ NMR ($CDCl_3$): $\delta$ 8.48 (s, 2H, OH), 8.44 (bs, 2H, NH), 7.13 (s, 4H, ArH), 7.07 (d, 4H, $J = 7.6$ Hz, ArH), 6.95–6.93 (m, 2H, ArH), 4.57 (s, 4H, $OCH_2CO$), 4.48 (s, 4H, $CH_2Cl$), 4.15–4.12 (d, 4H, $J = 13.3$ Hz, $ArCH_2Ar$), 3.70 (bs, 4H, $NHCH_2$) 3.54–3.51 (d, 4H, $J = 13.4$ Hz, $ArCH_2Ar$). $^{13}C$ NMR ($CDCl_3$): 167.65, 152.32, 149.13, 132.84, 129.97, 129.89, 129.81, 127.84, 127.41, 74.96, 46.36, 39.50, 31.50. Anal. Calc.: $C_{36}H_{34}Cl_2N_2O_6$. C 65.36, H 5.18, Cl 10.72, N 4.23%. Found: C 65.39, H 5.22, Cl 10.68, N 4.28%.

*Compound **2b***

Yield = (90%, pale yellow) m.p. >300 °C. $^{1}H$ NMR ($CDCl_3$): $\delta$ 8.75 (bs, 2H, NH), 8.55 (s, 2H, OH), 7.14 (s, 4H, ArH), 7.05 (d, 4H, $J = 7.6$ Hz, ArH), 6.95–6.91 (m, 2H, ArH), 4.58 (s, 4H, $OCH_2CO$), 4.48 (s, 4H, $CH_2Cl$), 4.13–4.09 (d, 4H, $J = 13.3$ Hz, $ArCH_2Ar$), 3.55–3.51 (m, 8H, $NHCH_2$ and $ArCH_2Ar$) 2.34–2.31 (m, 2H, $NHCH_2CH_2$). $^{13}C$ NMR ($CDCl_3$): 168.11, 150.32, 149.43, 132.81, 130.07, 129.85, 127.81, 127.35, 127.01, 74.77, 46.30, 39.48, 31.22, 23.41. Anal. Calc.: $C_{37}H_{36}Cl_2N_2O_6$. C 65.78, H 5.37, Cl 10.50, N 4.15%. Found: C 65.74, H 5.32, Cl 10.48, N 4.18%.

*Compound **2c***

Yield = (90%, pale pink) m.p. >300 °C. $^{1}H$ NMR ($CDCl_3$): $\delta$ 7.64 (bs, 2H, NH), 7.33 (s, 2H, OH), 7.16 (s, 4H, ArH), 6.91–6.85 (m, 6H, ArH), 4.53 (s, 4H, $OCH_2CO$), 4.51 (s, 4H, $CH_2Cl$), 4.16–4.12 (d, 4H, $J = 13.6$ Hz, $ArCH_2Ar$), 3.61 (bs, 4H, $NHCH_2$) 3.51 3.47 (d, 4H, $J = 13.6$ Hz, $ArCH_2Ar$) 1.76 (bs, 4H, $NHCH_2CH_2CH_2$). $^{13}C$ NMR ($CDCl_3$): 168.33, 152.46, 150.59, 132.38, 129.78, 127.83, 126.68, 126.63, 120.78, 75.26, 46.63, 37.88, 31.41, 25.33. Anal. Calc.: $C_{38}H_{38}Cl_2N_2O_6$. C 66.18, H 5.55, Cl 10.28, N 4.06%. Found: C 66.14, H 5.51, Cl 10.33, N 4.02%.

Synthesis of *p*-phosphonato calix[4](aza)crown ether derivatives **3a–c** (general procedure)

0.48 mmol of corresponding chloromethylated calix[4](-aza)crown derivative **2a–c** in 5 mL of chloroform was refluxed for 5 h with 5 mL of trimethyl phosphite. Excess amount of unreacted trimethyl phosphite $P(OCH_3)_3$ was then distilled under reduced pressure and the obtained yellow oily residue was dissolved in minimum amount of chloroform and then precipitated with excess amount of diethyl ether. Obtained white precipitates were filtered off and washed with diethyl ether to give corresponding pure of *p*-phosphonato calix[4](aza)crown ether derivatives **3a–c**.

*Compound **3a***

Yield = (80%, white) m.p. 296–297 °C. $^{1}H$ NMR ($CDCl_3$): $\delta$ 8.46 (bs, 2H, NH), 8.35 (s, 2H, OH), 7.06–7.03 (m, 8H, ArH), 6.91–6.87 (m, 2H, ArH), 4.56 (s, 4H, $OCH_2CO$), 4.13–4.10 (d, 4H, $J = 13.4$ Hz, $ArCH_2Ar$), 3.67 (bs, 4H, $NHCH_2$), 3.62–3.59 (d, 12H, $J = 10.7$ Hz, $POCH_3$), 3.53–3.49 (d, 4H, $J = 13.4$ Hz, $ArCH_2Ar$), 3.05–2.99 (d, 4H, $J = 21.2$ Hz, $CH_2PO$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 167.62, 151.34, 149.06, 133.04, 130.55, 129.79, 127.84, 127.27, 123.11, 74.85, 53.21, 39.47, 32.71, 31.49. $^{31}P$ NMR ($CDCl_3$): $\delta$ 29.21. Anal. Calc.: $C_{40}H_{46}N_2O_{12}P_2$. C 59.40, H 5.73, N 3.46, P 7.66%. Found: C 59.37, H 5.72, N 3.51, P 7.61%.

*Compound **3b***

Yield = (80%, white) m.p. >300 °C. $^{1}H$ NMR ($CDCl_3$): $\delta$ 8.77 (bs, 2H, NH), 8.40 (s, 2H, OH), 7.06–6.88 (m, 10H,

ArH), 4.57 (s, 4H, $OCH_2CO$), 4.10–4.07 (d, 4H, $J = 13.4$ Hz, $ArCH_2Ar$), 3.63–3.50 (m, 20H, $NHCH_2$, $POCH_3$, $ArCH_2Ar$), 3.05–3.00 (d, 4H, $J = 21.2$ Hz, $CH_2PO$), 2.31 (bs, 2H, $NHCH_2CH_2$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 168.15, 151.24, 149.59, 132.94, 130.63, 129.83, 127.82, 127.22, 123.26, 74.73, 53.22, 36.28, 32.69, 31.55, 23.67. $^{31}P$ NMR ($CDCl_3$): $\delta$ 29.19. Anal. Calc.: $C_{41}H_{48}N_2O_{12}P_2$. C 59.85, H 5.88, N 3.40, P 7.53%. Found: C 59.87, H 5.94, N 3.39, P 7.51%.

*Compound **3c***

Yield = (76%, white) m.p. 226–227 °C. $^1H$ NMR ($CDCl_3$): $\delta$ 7.68 (bs, 2H, NH), 7.13–6.77 (m, 12H, OH, ArH), 4.49–4.47 (bs, 4H, $OCH_2CO$), 4.13–4.09 (m, 4H, $ArCH_2Ar$), 3.77–3.44 (m, 20H, $NHCH_2$, $POCH_3$, $ArCH_2Ar$), 3.09–3.03 (m, 4H, $CH_2PO$), 1.74 (bs, 4H, $NHCH_2CH_2CH_2$). $^{13}C$ NMR ($CDCl_3$): $\delta$ 167.95, 152.48, 149.33, 131.88, 130.03, 128.68, 127.18, 126.32, 122.26, 74.63, 54.03, 36.12, 32.33, 31.47, 23.07. $^{31}P$ NMR ($CDCl_3$): $\delta$ 29.29. Anal. Calc.: $C_{42}H_{50}N_2O_{12}P_2$. C 60.28, H 6.02, N 3.35, P 7.40%. Found: C 60.21, H 5.97, N 3.30, P 7.38%.

Synthesis of water-soluble *p*-phosphonato calix[4](aza)crown ether derivatives **5a–c** (general procedure)

A solution of 4 mmol of **1a** and 5 mL of trimethylsilyl bromide (BTMS) in dry chloroform (2 mL) was stirred at room temperature for 24 h. After most of bromotrimethylsilane was removed under vacuum 2 mL of methanol were added and stirred overnight. After methanol removal, obtained white solid product was washed with water, methanol and chloroform and then dried under vacuum to give corresponding *p*-phosphonic acid calix[4](aza)crown ethers **4a–c**. Obtained solid product **4a–c** (yield: 75–80%) was suspended in water and potentiometrically titrated with 0.05 N NaOH solution until the first equivalence point was observed around 7.5 value of pH which resulted in the salification of the first OH group of the phosphonic acid **4a–c**. The solvent was removed under vacuum and obtained pure solid water-soluble *p*-phosphonato calix[4](aza)crown ether derivatives **5a–c** were dried under vacuum for 24 h.

*Compound **4a***

Anal. Calc.: $C_{36}H_{38}N_2O_{12}P_2$. C 57.45, H 5.09, N 3.72, P 8.23%. Found: C 57.48, H 5.07, N 3.70, P 8.28%.

*Compound **4b***

Anal. Calc.: $C_{37}H_{40}N_2O_{12}P_2$. C 57.96, H 5.26, N 3.65, P 8.08%. Found: C 57.89, H 5.21, N 3.72, P 8.02%.

*Compound **4c***

Anal. Calc.: $C_{38}H_{42}N_2O_{12}P_2$. C 58.46, H 5.42, N 3. 59, P 7.93%. Found: C 58.45, H 5.47, N 3.63, P, 7.99%.

*Compound **5a***

$^1H$ NMR ($D_2O$): $\delta$ 7.05–6.87 (m, 10H, ArH), 6.54 (bs, 2H, NH), 4.56 (s, 4H, $OCH_2CO$), 4.00–3.97 (d, 4H, $J = 13.7$ Hz, $ArCH_2Ar$), 3.49 (m, 8H, $NHCH_2$, $ArCH_2Ar$), 2.74–2.69 (d, 4H, $J = 20.1$ Hz, $CH_2PO$). $^{31}P$ NMR ($D_2O$): $\delta$ 20.25. Anal. Calc.: $C_{36}H_{36}N_2Na_2O_{12}P_2{\cdot}4H_2O$. C 49.78, H 5.11, N 3.22, P 7.13, Na 5.29%. Found: C 49.71, H 5.14, N 3.19, P 7.16, Na 5.23%.

*Compound **5b***

$^1H$ NMR ($D_2O$): $\delta$ 7.06–6.89 (m, 10H, ArH), 6.64 (bs, 2H, NH), 4.03–3.97 (bs, 4H, $ArCH_2Ar$), 3.50–3.41 (m, 8H, $NHCH_2$, $ArCH_2Ar$), 2.74–2.67 (bs, 4H, $CH_2PO$), 2.08–2.00 (bs, 2H, $NHCH_2CH_2$), 4 protons ($OCH_2CO$) not observed due to overlap with solvent signal. $^{31}P$ NMR ($D_2O$): $\delta$ 20.61. Anal. Calc.: $C_{37}H_{38}N_2Na_2O_{12}P_2{\cdot}4H_2O$. C 50.35, H 5.25, N 3.17, P 7.02, Na 5.21%. Found: C 50.37, H 5.29, N 3.21, P 7.06, Na 5.28%.

*Compound **5c***

$^1H$ NMR ($D_2O$): $\delta$ 7.09–6.76 (m, 10H, ArH), 6.40 (bs, 2H, NH), 4.45–4.40 (bs, 4H, $OCH_2CO$), 4.00–3.93 (bs, 4H, $ArCH_2Ar$), 3.42–3.27 (m, 8H, $NHCH_2$, $ArCH_2Ar$), 2.79–2.70 (bs, 4H, $CH_2PO$), 1.51–1.40 (bs, 4H, $NHCH_2CH_2CH_2$). $^{31}P$ NMR ($D_2O$): $\delta$ 19.96. Anal. Calc.: $C_{38}H_{40}N_2Na_2O_{12}P_2{\cdot}5H_2O$. C 49.90, H 5.51, N 3.06, P 6.77, Na 5.03%. Found: C 49.84, H 5.56, N 3.00, P 6.80; Na 4.97%.

Liquid–liquid extraction

Into a vial was pipetted an aqueous solution (10 mL) containing calixarene ligand (**5a–c**) at a concentration of $4\bullet10^{-3}$ M and 10 mL of $2 \times 10^{-4}$ M drug molecule in chloroform. The mixture was shaken vigorously in a stoppered glass tube with a mechanical shaker for 2 min and then magnetically stirred in a thermostated water bath at 25 °C for 5 h respectively, and finally left standing for an additional 30 min. The concentration of drug molecule remaining in the organic phase was then determined spectrophotometrically at 347 for furosemide, 339 for niclosamide and 319 nm for nifedipine drug molecule [43]. Blank experiments showed that drug extraction occurred in the absence of calixarene ligand (**5a–c**). But, the percentage of the drug molecule in the absence of calixarene

ligand (**5a–c**) was observed around 4.1, 3.5 and 1% for nifedipine, niclosamide and furosemide respectively.

The percent extraction ($E$ %) has been calculated as:

$$E\% = [(C_0 - C)/C_0] \times 100$$

where $C_0$ and $C$ are the initial and final concentrations of the drug molecule in organic phase before and after the extraction, respectively.

## Solubility measurements

The aqueous solubility of niclosamide, furosemide and nifedipine in water was determined at increasing concentrations of the *p*-phosphonate calixarenes. The solubility method of Higuchi and Conners was used [41]. An excess amount of drug powders was added into the screw capped amber vials containing 3 mL of water solution and the complexing agents at increasing concentrations (1.0–7.0 $\times$ $10^{-3}$ M). The vials were rotated at 60 rpm while being kept at 30 °C. After equilibrium was reached (24 h), the solutions was filtered through 0.45-μm cellulose acetate filters and analyzed for drug content by HPLC. All of the solubility experiments and HPLC analysis were carried out in the dark to prevent photodegradation of the drug molecules. Phase solubility diagrams were constructed by plotting the molar concentration of drugs dissolved versus the molar concentration of complexing agents.

## HPLC analysis of drugs

Drug content was analyzed by an HPLC Agilent 1200 Series were carried out using a 1200 model quaternary pump, a G1315B model diode array and multiple wavelength UV–Vis detector, a 1200 model standard and preparative autosampler, a G1316A model thermostated column compartment, a 1200 model vacuum degasser and an Agilent Chemstation B.02.01-SR2 Tatch data processor at 254, 342 and 338 nm for niclosamide, furosemide and nifedipine respectively. Niclosamide, furosemide and nifedipine eluted on a Supelco Discovery RP Amide C16 column (25 cm $\times$ 4.6 mm, 5 μm, Bellefonate, PA) after 14, 8 and 13 min respectively. The mobile phase was 75:25 (methanol:0.05 M $NH_4H_2PO_4$ v/v) for niclosamide, 60:40:1 (water:acetonitrile:acetic acide v/v) for furosemide and nifedipine, flow rate of 1 mL/min, and injection volume of 20 mL. Each determination was conducted in triplicate.

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