Bioorganic & Medicinal Chemistry 18 (2010) 36–45

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and anti-HIV evaluation of water-soluble calixarene-based bithiazolyl podands

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ARTICLE INFO

Article history:
Received 24 July 2009
Revised 2 November 2009
Accepted 7 November 2009
Available online 12 November 2009

Keywords:
Calixarene
Bithiazole
Antiviral
HIV

ABSTRACT

Nine anionic water-soluble calix[4]arene species, incorporating sulfonate, carboxylate or phosphonate groups, six of them incorporating two 2,2′-bithiazole subunits in alternate position at the lower rim, have been synthesised and evaluated as anti-HIV agents on various HIV strains and cells of the lymphocytic lineage (HIV-1 III B/MT4, HIV-1 LAI/CEM-SS, HIV-1 Bal/PBMC), using AZT as reference compound. A toxicity was detected for a minority of compounds on PBMC whereas for the others no cellular toxicity was measured at concentrations up to 100 μM. Most of the compounds have an antiviral activity in a 10–50 μM range, and one of them, sulfonylated, displays its activity, whatever the tropism of the virus, at a micromolar concentration.

1. Introduction

As assessed by recent reviews,[1] very few studies relate to the therapeutical activities of calixarene compounds; for example, hydrophilic derivatives have shown interesting levels of activity against bacteria,[2] fungi, cancerous cells and enveloped viruses,[3] but also against thrombosis[4] or fibrosic diseases.[5] In the middle 50s, the calixarene derivative 'Macrocyclon',[6] and more recently some parent structures[7] have been studied in the treatment of tuberculosis and other mycobacterioses. The building of designed calixarenic mimics of vancomycin has also been studied, with the aim of targeting an antimicrobial activity,[8] and some biological studies related to plasmid DNA binding and cell transfection have notably been reported by Ungaro and co-workers.[9] In this field, our group is focusing on the development of calixarene platforms designed as molecular drug dispensers and offering at the lower rim penicillin or quinolone moieties attached via a labile bound,[10,11] as well as of new ionic calixarene derivatives displaying an intrinsic antimicrobial activity.[12]

Antiviral activity of calixarene derivatives have been very modestly investigated since the seminal patent of Hwang et al. in 1994,[3a] in which the acidic form of *para*-sulfonatocalix[4]arene **1** and parent compounds (phenyl or naphthyl; sulfonamides or/and carboxylic acid derivatives) were described as anti-HSV and -HIV agents; Harris,[3b,c] then Coveney and Costello[3d] developed, as inhibitors of fusion and integrase enzyme in HIV infection, phenol-, resorcinol- and pyrogallol calixarene derivatives incorporating notably carboxylate substituents; three of these compounds with high activity level were tested on humans. Kral et al.[3e] reported the anti-HIV properties, as protease inhibitors, of a series of anionic borane, carborane and Co/Fe/Ni/Ru-metallacarborane clusters, some of them being attached to a calix[4]arene platform. All the derivatives mentioned above are polyanionic compounds. More recently, Motornaya et al. reported the anti-HSV properties of two polycationic aminoadamantyl derivatives of calix[4]arene.[3g]

Taking into account the above mentioned results, and more specifically those relative to calix[4]arene **1**, we attempted, as done with polycationic derivatives,[12] to develop families of compounds structurally close to **1**, and incorporating at the upper rim anionic groups, among which sulfonates, carboxylates or phosphonates, and, at the lower rim, different kinds of substituents, some of them being heterocyclic (Fig. 1).

Among various water-soluble calixarene derivatives that we have prepared with this objective, we have selected some species including two 2,2′-bithiazole subunits disposed in alternate position at the lower rim. This choice was done in order to co-evaluate derivatives of a sulfonyl species obtained as a mixture of three non-separable conformers, and incorporating two 2,2′-bithiazole subunits in alternate position of the lower rim, that was shown to display, associated to a low cytotoxicity, very interesting

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doi:10.1016/j.bmc.2009.11.016

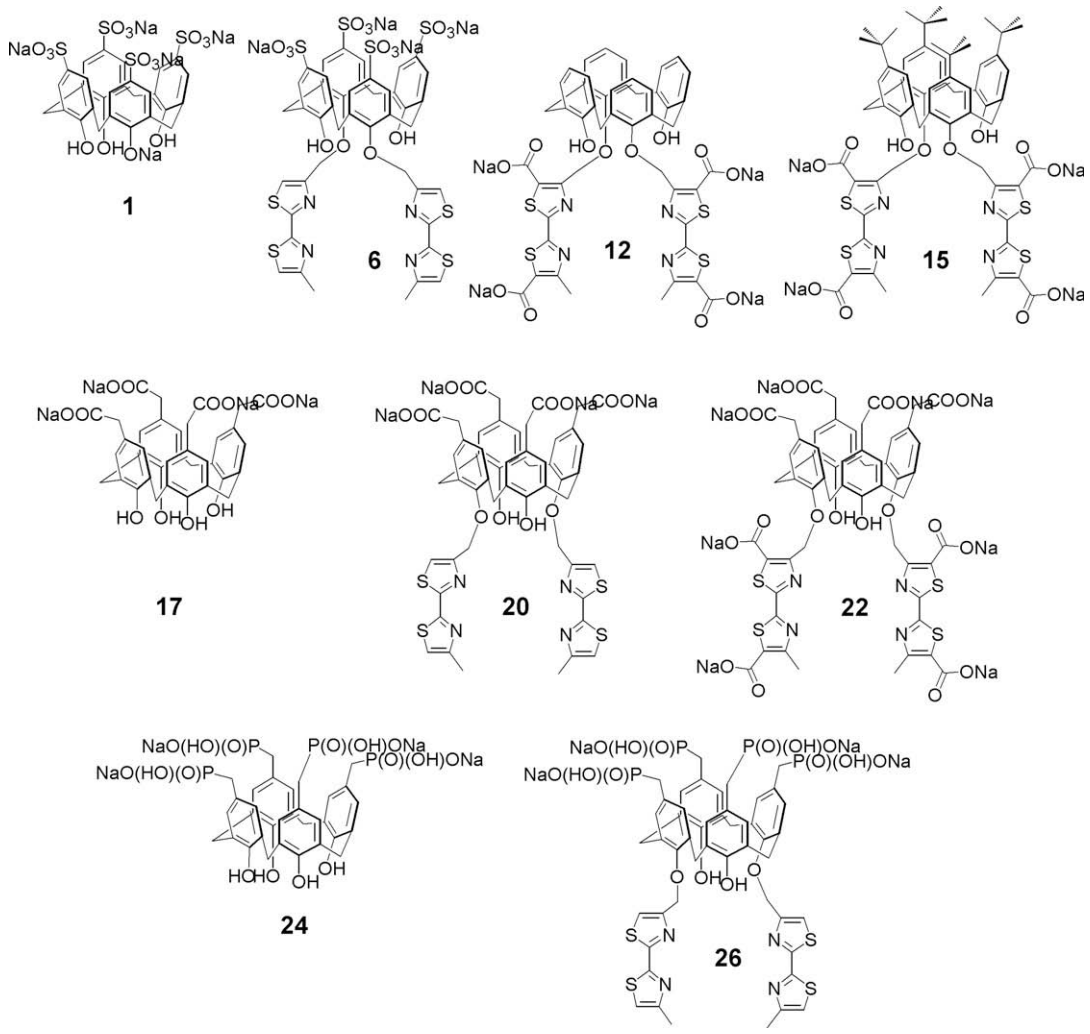


Figure 1. Water-soluble calixarene species of this study.

activities against HSV-1, HSV-2[13] and, at the origin of the present report, against HIV-1.

We relate here the synthesis and characterisation of a series of new calix[4]arene derivatives incorporating two bithiazole subunits at the lower rim, and evaluation of their activities against HIV-1 in MT4, CEM-SS and PBMC cells, in comparison with AZT as reference compound.

2. Chemistry

2.1. Sulfonate derivatives

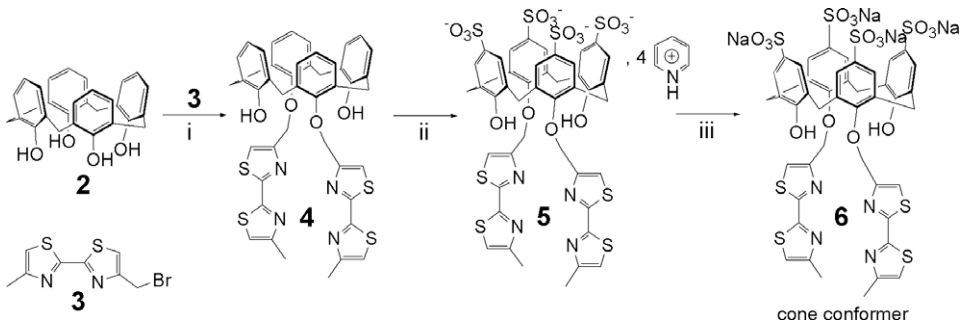
The calix[4]arene-tetra-*p*-sulfonic acid was prepared according to a patented procedure,[14] using a direct *ipso* sulfonation of tetra-*p*-(*tert*-butyl)calix[4]arene with concentrated sulfuric acid. Careful titration of the acid with aqueous NaOH, followed by lyophilisation, afforded the penta-sodium salt **1**,[15] associated to nine molecules of H_2O.

The synthesis of its bis-1,3-bis-(2,2′-bithiazolyl) analogue **6** was performed by a multistep process involving first the formation of the 1,3-bis-(2,2′-bithiazolyl)-calix[4]arene **4** by reaction of 4-bromomethyl-4′-methyl-2,2′-bithiazole **3**[16] with calix[4]arene **2** in the presence of K_2CO_3 and MeCN, then the direct formation of the tetra sulfonate by reaction of the podand **4** with chlorosulfonic acid, followed by basic hydrolysis of the resulting chlorosulfonate into the tetra-pyridinium salt **5** (mixture of two main conformers), then pyridine/sodium exchange. At this stage, **6** was obtained as a mixture of three main conformers, in variable ratio, we were until now unable to separate. Fortunately, obtention of the conic analogue **6** was achieved by treatment of the previous mixture by concentrated sulfuric acid that gave the conic tetraacid as a precipitate which was filtered, dissolved in MeOH and precipitated again by addition of an excess of AcOEt. The resulting solid was carefully titrated with NaOH to give, after dialysis and lyophilisation, the conic sodium salt **6**, associated, according to elemental analysis, to five molecules of H_2O and $6Na_2SO_4$ (Scheme 1).

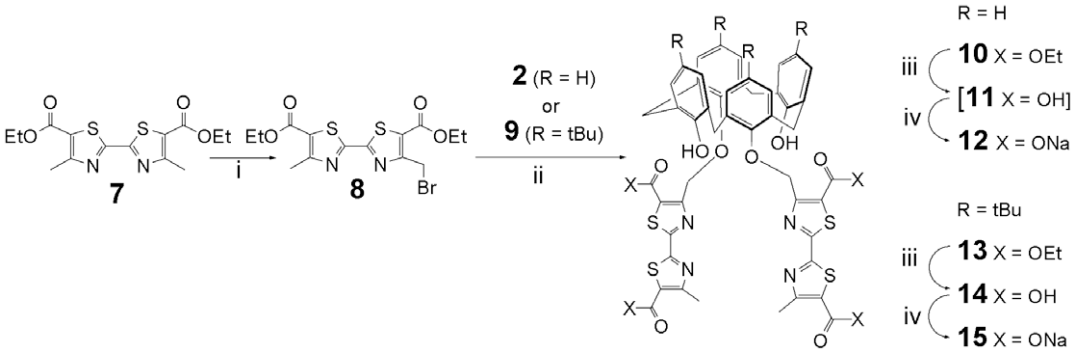
2.2. Carboxylate derivatives

The 4-bromomethyl-4′-methyl-5,5′-bis(carbethoxy)-2,2′-bithiazole **8**,[17] prepared by radical bromination of the bithiazole **7**[18] with NBS, was reacted with calix[4]arene **2** or with tetra-*p*-*tert*-butylcalix[4]arene **9** in refluxing MeCN and in the presence of K_2CO_3 as base, affording the corresponding tetraester podands **10** and **13**, with yields of 65% and 82%, respectively.

The ester functions were hydrolysed in an hydroalcoholic medium to give the corresponding tetraacids **11** (ca. 80%; partially characterised) and **14** (89%). A careful pH-meter monitored titration of these acids by aqueous NaOH afforded the corresponding



Scheme 1. Reagents and conditions: (i) K_2CO_3, MeCN, reflux, 86%; (ii) (a) $ClSO_3H$, CH_2Cl_2, rt; (b) pyridine, Me_2CO, H_2O, reflux, 80%; (iii) (a) NaOH, H_2O, argon, 100%; (b) H_2SO_4; (c) NaOH, H_2O, pH 7.5, dialysis (cut-off 100 D); 80%.



Scheme 2. Reagents and conditions: (i) NBS, AIBN, CCl_4, hν, reflux, 39%; (ii) K_2CO_3, MeCN, reflux, 65% (**10**), 82% (**13**); (iii) NaOH, H_2O, MeOH, 80% (**11**), 89% (**14**); (iv) NaOH, H_2O, pH 7.0 (**12**), pH 8.0 (**15**), dialysis (cut-off 100 D); 95% (**12**), 96% (**15**).

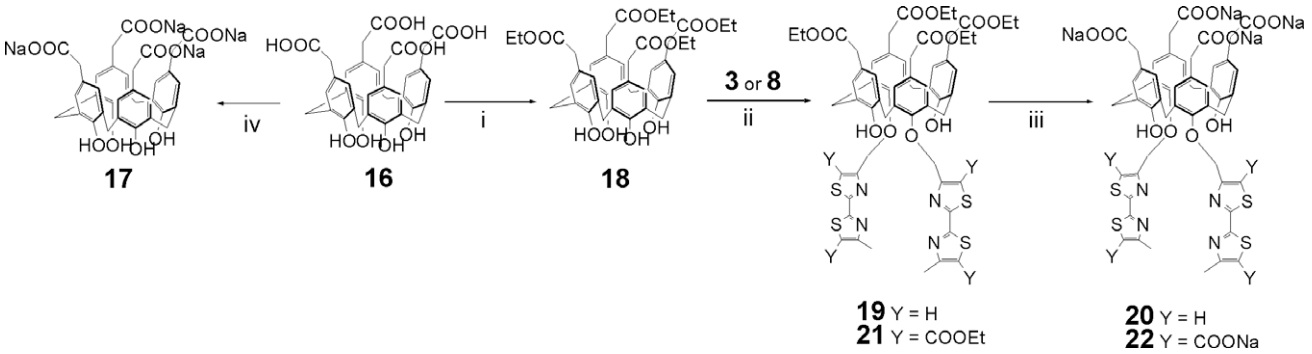
tetra sodio-derivatives **12** and **15**, with yields of 95% and 96%, respectively. Elemental analyses were consistent with the presence of 7 molecules of water in **12**, and 6.5 in **15** (Scheme 2).

The tetra-*p*-acetyl calix[4]arene **16** was prepared according to the procedure of Gutsche and colleagues,[19] involving a Mannich dimethylaminomethylation of calix[4]arene, quaternisation of amines followed by eliminative nitrilation, acidic hydrolysis of nitrile groups; a careful titration of **16** by aqueous NaOH gave the water-soluble tetra-sodium salt **17**. The tetra ethyl ester **18** was obtained in classical conditions with a yield of 53%. The reaction of **3** or **8** with **18**, in MeCN and in the presence of K_2CO_3 afforded the tetraester **19** and the octaester **21**, with yields of 54% and 48%, respectively. Compounds **19** and **21** were saponified to give after acidification the raw tetraacids which were carefully titrated with aqueous NaOH, dialysed and lyophilised. The resulting tetra sodio-derivative **20** and octa sodio-derivative **22** were obtained with overall yields of 71% and 67%, respectively. The calixarene unit is associated to 1NaCl and $6H_2O$ in **20**, and to 5NaCl and $7H_2O$ in **22** (Scheme 3).

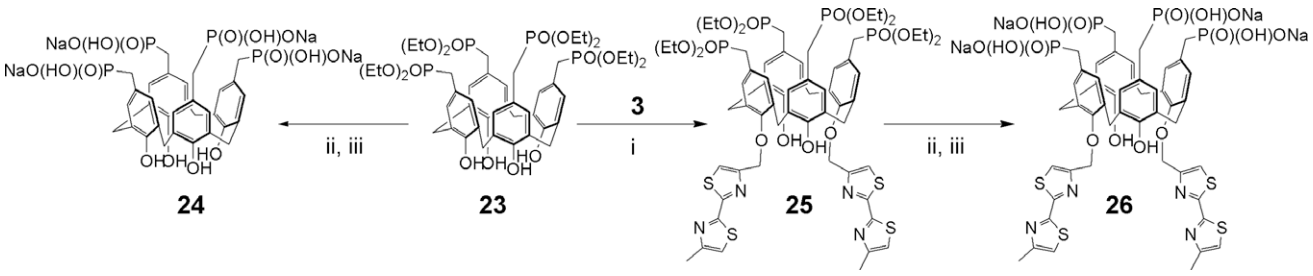
2.3. Phosphonate derivatives

The unsubstituted tetra-phosphonate **24** was prepared according to a slightly modified Ungaro's process,[20,21] involving the formation and the hydrolysis of its octa-ethylester analogue **23**.

As for carboxylate species, the reaction of **3** with **23**, in similar conditions, afforded the podand **25** with a yield of 86%. The ester functions of **25** were hydrolysed with BTMS, to give the resulting acid with a yield of 89%. The latter was carefully neutralised by NaOH, affording after dialysis and lyophilisation, the water-soluble sodium salt **26**, in which the calixarene unit is associated to 5.5 H_2O (Scheme 4).



Scheme 3. Reagents and conditions: (i) EtOH, H_2SO_4, 53%; (ii) K_2CO_3, MeCN, reflux, 54% (**19**), 48% (**21**); (iii) (a) NaOH, EtOH:H_2O, reflux; (b) HCl 1 M; (c) NaOH 0.1 M, pH 7.1 (**20**), pH 8.0 (**22**), dialysis (cut-off 100 D), 71% (**20**), 67% (**22**); (iv) NaOH 0.1 M, pH 7.4 dialysis (cut-off 100 D), 74%.



Scheme 4. Reagents and conditions: (i) MeCN, K_2CO_3, reflux, 86%; (ii) CH_2Cl_2, BTMS, rt, Ar, 89%; (iii) NaOH, H_2O, pH 7.4 (**26**), dialysis (cut-off 100 D), 95**%** (**26**).

As the final water-soluble species remains sometimes uneasy to purify in this state, all possible efforts have been brought to the purification of their organosoluble intermediates. Each of them has been fully analysed, notably by mass spectrometry, elemental analysis and by NMR spectroscopy, that confirmed the presence of upper and lower rim substituents in expected numbers and positions, as well as the cone conformation of the calixarene core, at the exception of the pyridinium salt **5** that consists in a mixture of two main isomers (78% and 22%).

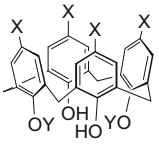
The final water-soluble derivatives gave also satisfactory analytical data, consistent with the proposed formulas integrating residual salts and/or water molecules. Molecular formulas issued from elemental analyses are given in Table 1. All compounds display a cone conformation, as assessed by Ar–CH_2–Ar ^{13}C resonance signals located at 31–32 ppm, and their AB-like shape in ^1H NMR.

3. Biological evaluations

3.1. Toxicity

Under the experimental conditions employed in this study, all of the nine water-soluble calixarene derivatives evaluated on the two cell lines MT4 and CEM-SS display a very weak or not toxicity as the CC_{50} was not reached at 100 μM. A similar very low toxicity level was also detected on PBMC (peripheral blood mononuclear cells) for the sulfonated species **1** and **6** and for the carboxylated bithiazole species **12** and **15**, while the cytotoxic concentration CC_{50} measured for the acetate derivatives **17**, **20**, **22** and the phosphonates **22** and **24** was in the range of 100 μM. For **6**, the introduction of the bithiazole subunits on the sulfonated calixarene core of **1**, as well as the increase of amphiphilic character by introduction of *tert*-Butyl groups at the upper rim of the calixarene for the two carboxylated species **12** and **15**, does not result in evolution of cytotoxicity, in the limit of the measure.

Table 1
Substituent types, formula and molecular weights of compounds of the study



	X	Y	Formula (mol. weight)
1	SO_3Na	H	$C_{28}H_{19}O_{16}Na_5S_4$, $9H_2O$ (1016.79)
6	SO_3Na	Btz	$C_{44}H_{32}O_{16}N_4S_8Na_4$, $6Na_2SO_4$, $5H_2O$ (2163.39)
12	H	Btz(COONa)$_2$	$C_{48}H_{32}N_4O_{12}Na_4S_4$, 4NaCl, $6H_2O$ (1418.87)
15	$C(CH_3)_3$	Btz(COONa)$_2$	$C_{64}H_{64}N_4O_{12}Na_4S_4$, $6H_2O$ (1409.53)
17	CH_2COONa	H	$C_{36}H_{28}O_{12}Na_4$, Na_2SO_4, $3H_2O$ (940.68)
20	CH_2COONa	Btz	$C_{52}H_{40}O_{12}N_4S_4Na_4$, $6H_2O$, NaCl (1299.59)
22	CH_2COONa	Btz(COONa)$_2$	$C_{56}H_{36}O_{20}N_4S_4Na_8$, $7H_2O$, 5NaCl (1815.2)
24	$CH_2P(O)(OH)ONa$	H	$C_{32}H_{32}O_{16}P_4Na_4$, $6H_2O$, Na_2HPO_4 (1299.59)
26	$CH_2P(O)(OH)ONa$	Btz	$C_{48}H_{44}O_{16}P_4S_4N_4Na_4$, $4H_2O$ (1349.00)

3.2. Antiviral activity

Three domains of activity can be deduced from Table 2 for the compounds tested on MT4 and CEM-SS cell lines infected with X4 isolates: 86–38 μM for the unsubstituted sulfonated derivative 1, and for the unsubstituted acetate **17**; 28–14 μM for the bithiazolyl acetate derivative **20**, the two carboxylates **12** and **15**, and the bithiazolyl phosphonate derivative **26**; 2.2–1.5 μM for bithiazolyl sulfonated species **6**, that appears as the leader of this series. One particular case is observed: the unsubstituted phosphonate **24** has no activity at 100 μM. The carboxylated bithiazolyl acetate derivative **22** is slightly more active on infections of CEM-SS cells than on MT4.

The antiviral activity was also evaluated for an R5 virus on PBMC. The sulfonated species **1** and **6** and the carboxylated bithiazole species **12** and **15** have an inhibitory activity similar to that measured for the X4 HIV-1 strains on lymphocytic cell lines and it can be noted that the unsubstituted acetate species **17** is more active on the system HIV-1 Bal/PBMC than on MT4 and CEM-SS cell lines, infected with an X4 HIV-1, nevertheless it is also more toxic for PBMC. Of interest, compound **6** displays on the three cell types a micromolar activity.

The calculated selectivity indexes (CC_{50}/IC_{50}) are not precise as, in the majority of cases, no toxicity was recorded. Considering the three assays used to evaluate the properties of these new compounds, **1**, **12, 15** and **17** have a modest antiviral activity ($SI \geqslant [1.0–2.5]$). The carboxylated bithiazolyl acetate derivative **22** and the phosphonate **26** have also a modest activity, only in transformed lymphocytic cells infected with X4 HIV-1 ($SI > [6–26]$). The phosphonate derivative **24** has no specific activity on HIV replication in PBMC ($SI = 1$) and no effects in the other systems. Excepting PBMC for **17** (higher), **12**, **15**, **22** and **26** ($SI > [3–9]$), excepting PBMC for **26** (lower) or CEM-SS for **22** (higher), the IC_{50} are close to the highest concentration tested; thus, **6** and **20** appear to be the most interesting antivirals in this series ($SI > [8.3–70]$), with a significant advantage for **6**, given that **20** is less active on PBMC ($SI = 8.3$) and that higher concentrations are needed to achieve virus inhibition.

To check if an early stage of the virus replication cycle is inhibited, the most active compound **6** has been evaluated using GHOST cells (Table 3) expressing the co-receptors CxCR4 or CCR5, and infected with HIV-1 LAI and HIV-1 BaL, respectively. The infection by both viruses was inhibited with an IC_{50} of 3.7 and 2.2 μM for the sulfonated species **6**. This indicates that an active viral Tat protein was not produced when viruses were added to the cells, in presence of high concentrations of the compound. No effect on infec-

Table 2
Antiviral activity and cytotoxicity data of compounds **1**, **6**, **12**, **15**, **17**, **20**, **22**, **24** and **26** determined in various cells and with different HIV-1 strains (X4 strains: HIV-1 LAI and HIV-1 IIIB; R5 strain: HIV-1 Bal)

Compd	MT4 (HIV-1 III B)			CEM-SS (HIV-1 LAI)			PBMC (HIV-1 Bal)		
	IC_{50}[a] μM	CC_{50}[b] μM	SI[c]	IC_{50}[a] μM	CC_{50}[b] μM	SI[c]	IC_{50}[a] μM	CC_{50}[b] μM	SI[c]
1	38	>100	>2.6	86	>100	>1.2	45	>100	>2
6	1.6	>100	>70	2.2	>100	>45	1.5	>100	>60
12	17	>100	>6	27	>100	>3.5	12	>100	>8.5
15	15	>100	>6.5	19.5	>100	>5	14.5	>100	>7
17	44	>100	>2.3	81	>100	>1.2	9.5	100	10.5
20	16.5	>1000	>60	28	>1000	>36	27.7	230	8.3
22	14	>100	>7	5.3	>100	>26	>37	37	<1
24	>100	>100		>100	>100		70	78	1>
26	14	>100	>7	15	>100	>6	57	92	1.7>
AZT[e]	0.010	>100		0.0051	>100		0.0077	75	

[a] IC_{50}: concentration required to protect 50% of the virus-infected MT4 cells against virus cytopathogenicity or concentration required to inhibit 50% of virus replication by reverse transcriptase activity measurement on CEM-SS cells and PMBC. All data represent average values for at least two or three separate experiments. The variation of these results under the standard procedures is below ±10%.
[b] CC_{50}: concentration required to cause 50% death of uninfected cells.
[c] SI: selectivity index as CC_{50}/IC_{50}.

tion was noticed when **6** was added after virus adsorption. Given that, under these experimental conditions, a greater percentage of cells was infected with X4 than with R5 virus, a strict comparison of the efficiency of inhibition of the two viruses is difficult.

Overall, these preliminary results tend to confirm that **6** is active as anti-HIV agent and impairs the first steps of cell infection.

The association of its good IC_{50} value and selectivity index confers to **6** a label of lead compound that is the starting point for the development of this family as anti-HIV agents.

4. Conclusion

In this study, we have prepared a set of nine calixarene derivatives, incorporating sulfonate, carboxylate and phosphonate water-solubilising groups and, for six of them two 2,2′-bithiazole pendant arms at the lower rims. The nine compounds have been evaluated as anti-HIV agents on PBMC, MT4 and CEM-SS cells infected with Bal, LAI and III-b HIV strains, respectively. All of these compounds do not display a cellular toxicity at 100 μM (for most of them, the highest concentration tested), except on PBMC in case of the three acetate and the two phosphonate derivatives. For the unsubstituted derivatives **1** and **17** with a modest antiviral activity, the introduction of bithiazole units resulted in a gain of activity. The major gains were obtained in the sulfonated family for compound **6**, with micromolar activities and apparent selectivity indexes superior to 45.

5. Experimental

5.1. Chemistry

5.1.1. General remarks

Melting points (°C, uncorrected) were determined on an Electrothermal 9200 in Capillary apparatus. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 (chemical shifts in ppm). Mass spectra (electronic ionisation—EI, and electrospray—ES) were recorded on a Nermag R-1010C apparatus or a Micromass Platform II apparatus, respectively, at the Service Commun de Spectrométrie de Masse Organique, Nancy. Infrared was performed on a Bruker Vector 22 apparatus (KBr, ν in cm^{-1}) and UV spectra were recorded on a SAFAS *UV mc²* apparatus, λ_{max} in nm, ε in $mol^{-1}\,dm^3\,cm^{-1}$. Elemental analyses were performed at the Service de Microanalyse, Nancy. Merck TLC plates were used for chromatography analysis (SiO_2, ref. 1.05554; Al_2O_3, ref. 1.05581). All commercially available products were used without further purification unless otherwise specified.

Table 3
Antiviral activity of **6** on HIV-1 infected GHOST cells

	HIV-1 LAI/GHOST CxCR4 IC_{50} μM	HIV-1 BaL/GHOST CCR5 IC_{50} μM
6	3.7	2.2

5.1.2. 25,27-Bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 4

A suspension of calixarene **2** (1 g, 2.35×10^{-3} mol) and K_2CO_3 (0.30 g, 2.35×10^{-3} mol) in anhydrous MeCN (50 mL) was refluxed under Ar during 30 min. The 4-bromomethyl-4′-methyl-2,2′-bithiazole **3** (1.88 g, 4.95×10^{-3} mol) was then added and reflux was continued during 3 h (TLC monitoring; Al_2O_3, CH_2Cl_2/hexane 1:1). The solvent was evaporated to dryness, and the resulting solid was extracted with CH_2Cl_2 (3×25 mL). The organic phase was concentrated and addition of MeOH resulted in the precipitation of raw **4**, that was finally chromatographed (Al_2O_3, CH_2Cl_2/hexane 3:2); (1.66 g, 86%). Yellow-green powder. Mp: 221–225 °C. IR (KBr): 1509 (C=N). UV–vis (CH_2Cl_2): 234 (18,700), 286 (10,800); 332 (25,300). ^1H NMR (400 MHz, $CDCl_3$): 2.57 (s, 6H, *Me*btz); 3.44–4.40 (AB, J_{AB} = 13.3 Hz, 4H, ArCH_2Ar); 5.29 (s, 4H, OCH_2btz); 6.71 (t, J = 7.4 Hz, 2H, H(4) of ArOH); 6.81 (t, J = 7.8 Hz, 2H, H(4) of ArOR); 6.95 (d, J = 7.7 Hz, 4H, H(3) and H(5) of ArOR); 7.01 (s + s, 2H, OH); 7.11 (d, J = 7.4 Hz, 4H, H(3) and H(5) of ArOH); 7.82 (s, 2H, H(5′) of btz); 8.02 (s, 2H, H(5) of btz). ^{13}C NMR (100 MHz, $CDCl_3$): 17.59 (*Me*btz); 31.71 (ArCH_2Ar); 74.75 (OCH_2btz); 116.36, 118.10, 119.69, 126.23 (C(5), C(5′) of btz, C_p of Ar); 129.05, 129.61 (C_m of Ar); 128.26, 133.50 (C_o of Ar); 152.02, 153.57, 154.12, 154.81, 160.81, 162.31 (C(2), C(2′), C(4), C(4′) of btz; C_{ipso} of Ar). Anal. Calcd for $C_{56}H_{52}N_4O_{12}S_4 \cdot 0.25H_2O$ (817.55): C, 64.64; H, 4.50; N, 6.85. Found: C, 64.65; H, 4.61; N, 6.72. EI-MS (pos. mode): 812 $[M]^+$; 617 $[M-CH_3btzCH_2]^+$; 195 $[CH_3btzCH_2]^+$; 71$[C_3H_3S]^+$.

5.1.3. Sulfonate derivatives

5.1.3.1. 5,11,17,23-Tetra-(sulfonic acid)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra pyridinium salt 5. A mixture of **4** (2 g, 2.46×10^{-3} mol) and chlorosulfonic acid (3.27 mL, 4.91×10^{-2} mol) in dry CH_2Cl_2 (120 mL) was stirred under Ar at rt during 4 h (TLC monitoring, Al_2O_3, CH_2Cl_2). The solvent was evaporated, then the solid residue was dissolved in a mixture of H_2O (2 mL), Me_2CO (60 mL) and pyridine (60 mL) and refluxed during 20 h. The resulting mixture was

evaporated to dryness, and the solid residue was dissolved in MeOH. Addition of EtOH gave a precipitate that was filtered and rinsed with EtOH (3×10 mL) to give **5** as a white powder (2.35 g, 60%). Two main isomers (78 and 22%). Partial analyses. Mp: 175–176 °C. IR (KBr): 1192.5 (SO_3^-); 1623.9 (C=N$^+$). UV–vis (DMSO): 330 (8151). ^{1}H NMR (400 MHz, DMSO-d_6, integrations relative to each isomer): 2.47 (br s, *Me*btz); 3.29–4.06 (AB, J_{AB} = 12.8 Hz, 4H of Ar–C*H*$_2$–Ar$_{major}$); 3.85–4.65 (AB, J_{AB} = 14.7 Hz, 4H of Ar–C*H*$_2$–Ar$_{major}$); 3.14–4.78 (AB, J_{AB} = 12.5 Hz, 4H of Ar–C*H*$_2$–Ar$_{minor}$); 3.62–4.54 (AB, J_{AB} = 15.5 Hz, 4H of Ar–C*H*$_2$–Ar$_{minor}$); 4.57–5.28 (AB, J_{AB} = 12.0 Hz, 4H of OC*H*$_2$btz$_{major}$); 4.78–5.06 (AB, J_{AB} = 12.97 Hz, 4H of OC*H*$_2$btz$_{minor}$); 6.92 (br s, 2H, OH); 7.21–7.50 (m, Ar*H*$_{major}$ and Ar*H*$_{minor}$); 7.65 (s, 2H of btz$_{minor}$); 7.72 (br t, H$_m$ pyridinium$_{major}$, H$_m$ pyridinium$_{minor}$, and 2H of btz$_{major}$); 7.85 (s, 2H of btz$_{major}$); 7.94 (s, 2H of btz$_{minor}$); 8.32 (br t, H$_p$ pyridinium$_{major}$, and H$_p$ pyridinium$_{minor}$); 8.64 (br d, H$_o$ pyridinium$_{major}$ and H$_o$ pyridinium$_{minor}$). Anal. Calcd for $C_{64}H_{56}N_8O_{16}S_8{\cdot}3C_6H_5N{\cdot}3.5H_2SO_4{\cdot}0.5C_2H_5OH$ (2034.86): C, 47.17; H, 3.99; N, 7.56. Found: C, 47.39; H, 3.61; N, 7.10.

5.1.3.2. 5,11,17,23-Tetra-(sulfonic acid)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra sodium salt 6. A solution of **5** (1.02 g, 0.63×10^{-3} mol) in water (20 mL) was titrated to pH 7.6 with 0.1 M aqueous NaOH, under a stream of Ar. The resulting aqueous phase was evaporated to dryness and the residue was dissolved again in water prior dialysis (Float-A-Lyser, cellulose acetate, MWCO 100 D) and lyophilisation to give the mixture of conformers. The latter (0.6 g, 0.45 mmol) was dissolved in water (3 mL) and about 3 mL of 96% H_2SO_4 was carefully added (exothermic). After cooling to rt, the voluminous precipitate of tetra sulfonic acid that was formed was collected by suction-filtration, and was dried under vacuum over P_2O_5 (0.48 g). The tetraacid (0.3 g, 0.26×10^{-3} mol) was solubilised in H_2O (90 mL), and was carefully titrated by 0.1 M NaOH (pH-meter, initial pH 1.6, final pH 7.5). The resulting solution was evaporated to dryness and the solid residue was triturated in a mixture of EtOH and Et_2O. The resulting precipitate was collected by filtration, dried and dissolved in H_2O prior dialysis (Float-A-Lyser, cellulose acetate, MWCO 100D) to give **6** (0.26 g, overall yield 31%). Mp: >230 °C (dec). IR (KBr): 3448.7 (OH), 1142.6 (SO_3^-). UV–vis (H_2O): 330 (13,711). ^{1}H NMR (400 MHz; DMSO-d_6): 2.45 (s, 3H, *Me*btz); 3.50–4.30 (AB, J_{AB} = 12.9 Hz, 8H, ArC*H*$_2$Ar); 5.16 (s, 4H, OC*H*$_2$btz); 7.25 (s, 4H, Ar*H* **B**); 7.43 (s, 4H, Ar*H* **A**), 7.45 (s, 2H, H(5′) btz); 7.93 (s, 2H, *H*(5) btz); 7.94 (s, 2H, O*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.04 (*Me*btz); 31.22 (ArCH$_2$-Ar); 73.56 (OCH$_2$btz); 117.97 (C(5′) btz); 119.72 (*C*(5) btz); 126.29 (*C*$_o$ of **A**); 126.79 (*C*$_m$ of **A**); 127.02 (*C*$_m$ of **B**); 132.55 (*C*$_o$ of **B**); 139.50 (*C*$_p$ of **A**); 144.36 (*C*$_p$ of **B**); 152.55 (*C*$_{ipso}$ of **B**); 153.45 (*C*$_2$ btz); 153.82 (*C*$_{ipso}$ of **A**); 154.13 (C(2′) btz); 159.80 (C(4′) btz); 161.56 (C(4) btz). Anal. Calcd for $C_{44}H_{32}O_{16}N_4S_8Na_4{\cdot}6Na_2SO_4{\cdot}5H_2O$ (2163.39): C, 24.42; H, 1.95; N, 2.58. Found: C, 24.55; H, 1.79; N, 2.21. ES-MS (neg. mode): 1196.33 [M−Na$^+$]$^-$; 587.09 [M−2Na$^+$]$^{2-/2}$; 383.85 [M−3Na$^+$]$^{3-/3}$.

5.1.4. Carboxylate derivatives

5.1.4.1. 25,27-Bis(4-methyleneoxy-4′-methyl-5,5′-bis(ethoxycarbonyl)-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 10. A suspension of calix[4]arene **2** (1 g, 2.35×10^{-3} mol) and K_2CO_3 (0.31 g, 2.35×10^{-3} mol) in anhydrous MeCN (50 mL) was refluxed under Ar during 30 min. The bromide **8** (1.88 g, 4.95×10^{-3} mol) was then added and reflux was continued over ca. 4 h (TLC monitoring, Al_2O_3, CH_2Cl_2/hexane (1:1)). The solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2 (75 mL), filtered then mixed with an excess of MeOH. The resulting solution was concentrated under vacuum while cooling, affording a precipitate that was collected and finally chromatographed (Al_2O_3, CH_2Cl_2/hexane (3:2)) to give **10** (1.7 g, 65%). Mp: 275 °C (dec). IR (KBr): 1518.3 (C=N–), 1713.0 (COOEt). UV–vis (CH_2Cl_2): 232 (25,500); 345 (37,100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.39 (t, J = 7.0 Hz, 6H, C*H*$_3$CH$_2$O); 1.47 (t, J = 7.2 Hz, 6H, C*H*$_3$CH$_2$O); 2.80 (s, 6H, C*H*$_3$btz); 3.45–4.57 (AB, J_{AB} = 12.8 Hz, 4H,, ArC*H*$_2$Ar); 4.31 (q, J = 7.1 Hz, 4H, CH$_3$C*H*$_2$O); 4.38 (q, J = 7.2 Hz, 4H, CH$_3$C*H*$_2$O); 5.43 (s, 4H, OC*H*$_2$btz); 6.65 (t, J = 6.4 Hz, 2H, *H*$_p$ of Ar); 6.86 (t, J = 7.5 Hz, 2H, *H*$_p$ of Ar); 7.03 (d, J = 6.4 Hz, 4H, *H*$_m$ of Ar); 7.11 (d, J = 7.6 Hz, 4H, *H*$_m$ of Ar); 8.60 (s, 2H, ArO*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 14.46, 14.91 (*C*H$_3$CH$_2$); 17.66 (*Me*btz); 32.17 (ArCH$_2$Ar); 62.05, 62.46 (CH$_3$CH$_2$); 72.77 (OCH$_2$btz); 118.59, 125.98 (*C*$_p$ of Ar); 128.14, 133.97 (*C*$_o$ of Ar); 128.84, 129.41 (*C*$_m$ of Ar); 123.92, 125.15, 152.51, 154.43, 160.38, 161.17, 161.90, 162.15, 163.15 (*C*(2), *C*(2′), *C*(4), *C*(4′), *C*(5), *C*(5′), *C*$_{ipso}$ of Ar, –COO). EI-MS (pos. mode): 1100 [M]$^+$, 340 [CH$_3$btz(COOEt)$_2$CH$_2$]$^+$, 311 [CH$_3$btz(COOEt)$_2$CH$_2$−C$_2$H$_5$]$^+$, 71 [C$_3$H$_3$S]$^+$. Anal. Calcd for $C_{56}H_{52}N_4O_{12}S_4$ (1101.30): C, 61.07; H, 4.76; N, 5.09. Found: C, 61.01; H, 4.77; N, 4.93.

5.1.4.2. 25,27-Bis(4-methyleneoxy-4′-methyl-5,5′-bis(carboxylic acid)-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra sodium salt 12 (via tetraacid 11). A mixture of **10** (0.2 g, 1.82×10^{-4} mol), NaOH (0.08 g, 2×10^{-3} mol), H_2O (20 mL) and MeOH (40 mL) was refluxed during 24 h (TLC monitoring, SiO_2, CH_2Cl_2/CH_3OH (95:5)). After cooling to rt, the solution was acidified to pH 3–4 with 1 M aqueous HCl. The resulting yellow precipitate was collected, rinsed with H_2O (4×10 mL) then with CH_2Cl_2 (3×10 mL). A final dissolution in THF followed by evaporation of the solvent to dryness afforded the tetraacid **11** (0.177 g). The latter was dispersed in H_2O (50 mL), and carefully solubilised at pH 7 by controlled addition of 0.1 M aqueous NaOH. The resulting solution was evaporated to dryness to give the tetra sodio-derivative **12** (0.210 g, 95%). Compound **11**: partial characterisation. Yellow powder. Mp: 245 °C (dec). IR (KBr): 1518.3 (C=N); 1709.1 (COOH). UV–vis (THF): 244 (12,500); 344 (37,100). ^{1}H NMR (400 MHz, DMSO-d_6): 2.67 (s, 6H, C*H*$_3$btz); 3.43–4.46 (AB, J_{AB} = 13.2 Hz, 8H, ArC*H*$_2$Ar); 5.31 (s, 4H, OC*H*$_2$btz); 6.55 (t, J = 7.6 Hz, 2H, *H*$_p$ of Ar); 6.84 (t, J = 7.6 Hz, 2H, *H*$_p$ of Ar); 7.10 (d, J = 7.6 Hz, 4H, *H*$_m$ of Ar); 7.14 (d, J = 7.6 Hz, 4H, *H*$_m$ of Ar); 8.73 (s, 2H, ArO*H*); 13.37 (s, 2H, COO*H*); 13.82 (s, 2H, COO*H*). Anal. Calcd for $C_{48}H_{36}N_4O_{12}S_4{\cdot}2H_2O$ (1025.11): C, 56.24; H, 3.93; N, 5.47. Found: C, 56.34; H, 3.69; N, 5.22.

Compound **12** Mp: 240 °C (dec). IR (KBr): 1514.0 (C=N–); 1601.9 (COO$^-$). UV–vis (H_2O): 276 (970); 346 (34,400). ^{1}H NMR (400 MHz, DMSO-d_6): 2.60 (s, 6H, *Me*btz); 3.23–4.26 (AB, J_{AB} = 13 Hz, 8H, ArC*H*$_2$Ar); 5.31 (s, 4H, OC*H*$_2$btz); 6.54 (t, J = 7.3 Hz, 2H, *H*$_p$ of Ar); 6.66 (t, J = 7.4 Hz, 2H, *H*$_p$ of Ar); 6.86 (d, J = 7.5 Hz, 4H, *H*$_m$ of Ar); 7.06 (d, J = 7.6 Hz, 4H, *H*$_m$ of Ar); 7.83 (s, 2H, ArO*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.15 (*Me*btz); 31.30, 31.50 (ArCH$_2$Ar); 72.77 (OCH$_2$btz); 119.67, 125.54 (*C*$_p$ of Ar); 128.75, 134.44 (*C*$_o$ of Ar); 129.31, 129.33 (*C*$_m$ of Ar); 141.80, 151.79, 153.15, 153.54, 158.83, 159.66, 164.24, 165.03, 175.46 (*C*(2), *C*(2′), *C*(4), *C*(4′), *C*(5), *C*(5′) of btz, *C*$_{ipso}$ of Ar, –COO$^-$). ES-MS (pos. mode): 989.1 [M−4Na$^+$+5H$^+$]$^+$, 1011.1 [M−3Na$^+$+4H$^+$]$^+$, 1033.1 [M−2Na$^+$+3H$^+$]$^+$, 1055.1 [M−Na$^+$+2H$^+$]$^+$. (neg. mode): 987.1 [M−4Na$^+$+3H$^+$]$^-$, 1009.1 [M−3Na$^+$+2H$^+$]$^-$, 1031.1 [M−2Na$^+$+H$^+$]$^-$. Anal. Calcd for $C_{48}H_{32}N_4O_{12}Na_4S_4{\cdot}7H_2O$ (1203.12): C, 47.92; H, 3.85; N, 4.66. Found: C, 48.08; H, 3.59; N, 4.67.

5.1.4.3. 5,11,17,23-Tetra-(*tert*-butyl)-25,27-bis(4-methyleneoxy-4′-methyl-5,5′-bis(ethoxycarbonyl)-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 13. A mixture of *p*-*tert*-butyl-calixarene **9** (1 g, 1.54×10^{-3} mol), K_2CO_3 (0.23 g, 1.54×10^{-3} mol) and anhydrous MeCN (50 mL) was refluxed under Ar during 30 min. The bromide **8** (1.375 g, 3.25×10^{-3} mol) was then added and reflux was continued during 3 h (TLC monitoring, SiO_2, CH_2Cl_2). The solvent was evaporated to dryness, and the residue was dissolved in CH_2Cl_2

(25 mL). The solid materials were filtered off and the filtrate was concentrated and added to MeOH. The resulting precipitate was collected and chromatographed (SiO_2, CH_2Cl_2/CH_3OH (95:5)) to give pure **13** (1.690 g, 82%). Mp: 265 °C (dec). IR (KBr): 1254.2 (OC_2H_5); 1518.2 (C=N–); 1714.5 (COOEt). UV–vis (CH_2Cl_2): 232 (33,900); 292 (13,400); 345 (38,700). ^{1}H NMR (400 MHz, $CDCl_3$): 1.06 (s, 18H, Me_3C); 1.31 (s, 18H, Me_3C); 1.38 (t, J = 7.1 Hz, 6H, CH_3CH_2); 1.46 (t, J = 7.1 Hz, 6H, CH_3CH_2); 2.79 (s, 6H, *Me*btz); 3.37–4.51 (AB, J_{AB} = 12.8 Hz, 8H, ArCH_2Ar); 4.30 (q, J = 7.0 Hz, 4H, CH_3CH_2); 4.37 (q, J = 7.1 Hz, 4H, CH_3CH_2); 5.44 (s, 4H, OCH_2btz); 6.95 (s, 4H, H_m of Ar); 7.10 (s, 2H, H_m of Ar); 8.15 (s, 2H, ArO*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 14.47, 14.90 (CH_3CH_2);17.67 (*Me*btz); 31.50, 32.17 (Me_3C); 32.42 (Ar–CH_2–Ar); 34.19, 34.44 (Me_3C); 61.98, 62.36 (CH_3CH_2); 72.44 (OCH_2btz); 125.36, 125.89 (C_m of Ar); 127.77, 133.45 (C_o of Ar); 124.24, 125.22 140.84, 147.33, 150.36, 151.83, 160.54, 161.23, 161.81, 162.22, 162.27, 163.08 (*C*(2), *C*(2′), *C*(4), *C*(4′), *C*(5), *C*(5′) of btz, C_{ipso} and C_p of Ar, –COO$^-$). ES-MS (pos. mode): 1325.55 [M+H$^+$]$^+$, 1347.41 [M+Na$^+$]$^+$, 987.50 [M−CH_3btzCH_2+H$^+$]$^+$. Anal. Calcd for $C_{72}H_{84}N_4O_{12}S_8$ (1325.72): C, 65.23; H, 6.39; N, 4.23. Found: C, 65.02; H, 6.26; N, 4.23.

5.1.4.4. 5,11,17,23-Tetra-(*tert*-butyl)-25,27-bis(4-methyleneoxy-4′-methyl-5,5′-bis(carboxylic acid)-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 14. A mixture of ester **13** (1.0 g; 0.754×10^{-3} mol), NaOH (0.3 g, 7.5×10^{-3} mol), H_2O (30 mL) and MeOH (100 mL) was refluxed during 48 h (TLC monitoring, SiO_2, CH_2Cl_2/CH_3OH (96:4)). The solvent were evaporated to dryness, and the residue was dissolved in H_2O (20 mL), then 1 M aqueous HCl was added up to pH 3. The resulting suspension was triturated, filtered, then rinsed with H_2O (5 × 15 mL) and CH_2Cl_2 (3 × 10 mL). The solid residue was dissolved in THF then evaporated to dryness to give the tetraacid **14** (0.818 g, 89%). Yellow powder. Mp: 230–235 °C (dec). IR (KBr): 1518.0 (C=N–); 1708.5 (COOH). ^{1}H NMR (400 MHz, DMSO-d_6): 1.17 (s, 18H, Me_3C); 1.20 (s, 18H, Me_3C); 2.67 (s, 6H, *Me*btz); 3.40–4.44 (AB, J_{AB} = 12.4 Hz, 8H, ArCH_2Ar); 5.24 (s, 4H, OCH_2btz); 7.13 (s, 4H, H_m of Ar); 7.19 (s, 2H, H_m of Ar); 8.75 (s, 2H, ArO*H*); 13.35 (s, 2H, COO*H*); 13.78 (s, 2H, COO*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.67 (*Me*btz); 31.79, 32.35 (Me_3C); 32.54 (Ar–CH_2–Ar); 34.40, 34.91 (Me_3C); 72.95 (OCH_2btz); 126.12, 126.38 (C_m of Ar); 127.57, 134.05 (C_o of Ar); 125.54, 127.04, 141.05, 147.90, 151.21, 152.00, 159.26, 160.49, 161.35, 161.99, 162.31, 163.15 (*C*(2), *C*(2′), *C*(4), *C*(4′), *C*(5), *C*(5′) of btz, C_{ipso} and C_p of Ar, –COO$^-$). ES-MS (pos. mode): 1235.15 [M+Na$^+$]$^+$, 1257.32 [M−H$^+$+2Na$^+$]$^+$. Anal. Calcd for $C_{64}H_{68}N_4O_{12}S_8$ (1213.51): C, 63.34; H, 5.65; N, 4.62. Found: C, 63.35; H, 5.71; N, 4.56.

5.1.4.5. 5,11,17,23-Tetra-(*tert*-butyl)-25,27-bis(4-methyleneoxy-4′-methyl-5,5′-bis(carboxylic acid)-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra sodium salt 15. A suspension of tetraacid **14** (0.4 g, 0.33×10^{-3} mol) in H_2O (20 mL) was titrated with aqueous NaOH until pH 8.1 was raised. The resulting solution was evaporated to dryness and the solid residue was triturated in MeOH to give, after evaporation, the tetra sodio-derivative 53 (0.45 g, 96%). Light yellow powder. Mp: 220–230 °C (dec). IR (KBr):1515.0 (C=N–); 1603.0 (COO$^-$). UV–vis (H_2O): 282 (5940); 348 (21,008). ^{1}H NMR (400 MHz, DMSO-d_6): 0.99 (s, 18H, Me_3C); 1.17 (s, 18H, Me_3C); 2.58 (s, 6H, *Me*btz); 3.18–4.19 (AB, J_{AB} = 12.7 Hz, 8H, ArCH_2Ar); 5.63 (s, 4H, OCH_2btz); 6.87 (s, 4H, *H*m of Ar); 7.04 (s, 2H, *H*m of Ar); 7.81 (s, 2H, ArO*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 17.01 (*Me*btz); 31.64, 32.27 (Me_3C); 32.11 (ArCH_2Ar); 34.36, 34.57 (Me_3C); 71.86 (OCH_2btz); 125.89 (C_m of Ar); 128.40, 133.52 (C_o of Ar); 138.74, 141.80, 146.83, 150.79, 151.26, 152.04, 152.94, 158.18, 159.81, 164.51, 164.69 (*C*(2), *C*(2′), *C*(4), *C*(4′), *C*(5), *C*(5′) of btz, C_{ipso} and C_p of Ar, –COO$^-$). ES-MS (neg. mode): 403.5 [M−4Na+H]$^{3-/3}$, 605.7 [M−4Na+2H]$^{2-/2}$, 616.7 [M−3Na+H]$^{2-/2}$. Anal. Calcd for $C_{64}H_{64}N_4O_{12}Na_4S_8{\cdot}6.5H_2O$ (1418.53): C, 54.19; H, 5.47; N, 3.95. Found: C, 54.29; H, 5.38; N, 3.73.

5.1.4.6. 5,11,17,23-Tetra-(ethoxycarbonylmethyl)-25,26,27,28-tetrahydroxycalix[4]arene 18. A suspension of the tetraacid calixarene **16** (2.0 g, 2.77×10^{-3} mol) in a mixture of EtOH (40 mL) and 96% H_2SO_4 (8 mL) was refluxed during 7 h. EtOH was evaporated and the oily residue was added to ice-cold water (200 mL) under vigorous stirring. After 1 h, the resulting precipitate was filtered off and washed with water. After drying under vacuum, the residue was chromatographed (SiO_2, CH_2Cl_2/MeOH 99:1) to give the tetraester **18** (1.13 g, 53%). White powder. Mp: 214–215 °C. IR (KBr): 1741 (COO). UV–vis (CH_2Cl_2): 279 (6778). ^{1}H NMR (400 MHz, $CDCl_3$): 1.13 (t, J = 7.3 Hz, 12H, OCH_2CH_3); 3.40 (s, 8H, CH_2COOEt); 3.51–4.23 (AB, J_{AB} = 12 Hz, 8H, ArCH_2Ar); 4.14 (q, J = 7 Hz, 12H, OCH_2CH_3); 6.99 (s, 4H, Ar*H*); 10.16 (s, 4H, O*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 14.63 (OCH_2CH_3); 32.12 (ArCH_2Ar); 40.94 (ArCH_2COOEt); 61.22 (OCH_2CH_3); 128.05 (C_p); 128.61 (C_o); 130.26 (C_m); 148.31); (C_{ipso}); 172.09 (COOEt). Anal. Calcd for $C_{44}H_{48}O_{12}$ (768.85): C, 68.73; H, 6.29. Found: C, 68.77; H, 6.17. ES-MS (pos. mode): 806.91 [M+K$^+$]$^+$, 790.94 [M+Na$^+$]$^+$, 768.96 [M+H$^+$]$^+$. ES-MS (neg. mode): 766.99 [M−H$^+$]$^-$.

5.1.4.7. 5,11,17,23-Tetra-(ethoxycarbonylmethyl)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 19. A suspension of tetra ethylester **18** (0.55 g, 0.72×10^{-3} mol) and K_2CO_3 (0.1 g, 0.72×10^{-3} mol) in dry MeCN (50 mL) was refluxed under Ar during 30 min. The bromide **3** (0.40 g, 1.44×10^{-3} mol) was then added and reflux was continued during ca. 6 h (TLC monitoring: Al_2O_3, CH_2Cl_2). After cooling to rt, the solvent was evaporated to dryness, and the resulting solid material was dissolved in CH_2Cl_2. The insoluble inorganic materials were filtered off, and the organic phase was concentrated then chromatographed (Al_2O_3, CH_2Cl_2) to give **19** (0.45 g, 54%). White powder. Mp: 109–110 °C. IR (KBr): 1734.7 (COOEt). UV–vis (CH_2Cl_2): 293 (15,954); 330 (35,028). ^{1}H NMR (400 MHz, $CDCl_3$): 1.21 (t, J = 7.2 Hz, 6H, OCH_2CH_3 of **A**); 1.27 (t, J = 7.2 Hz, 6H, OCH_2CH_3 of **B**); 2.53 (s, 6H, CH_3btz); 3.32 (s, 4H, CH_2COOEt of **B**); 3.41–4.35 (AB, J_{AB} = 13.3 Hz, 8H, ArCH_2Ar); 3.50 (s, 4H, CH_2COOEt of **B**); 4.14 (m, 8H, OCH_2CH_3 of **A** and **B**); 5.24 (s, 4H, OCH_2btz); 6.87 (s, 4H, Ar*H* of **B**); 6.97 (s, 2H, H(5′)btz); 7.02 (s, 4H, Ar*H* of **A**); 7.94 (s, 2H, O*H*); 7.96 (s, 2H, H(5)btz). ^{13}C NMR (400 MHz, $CDCl_3$): 14.54 (OCH_2CH_3 of **A**); 14.66 (OCH_2CH_3 of **B**); 17.55 (*Me*btz); 31.87 (ArCH_2Ar); 41.00 (CH_2COOEt of **A**); 41.21 (CH_2COOEt of **B**); 61.12 (OCH_2CH_3 of **A**); 61.19 (OCH_2CH_3 of **B**); 74.67 (OCH_2btz); 116.43 (*C*(5′)btz); 118.22 (*C*(5)btz); 125.00 (C_p de **A**); 128.10 (C_o of **A**); 129.88 (C_m of **A**); 130.60 (C_m of **B**); 131.52 (C_p de **B**); 133.57 (C_o of **B**); 151.41 (C_{ipso} of **B**); 152.73 (C_{ipso} of **A**); 153.96 (*C*(2)btz); 154.73 (*C*(2′)btz); 160.77 (*C*(4′)btz); 162.25 (*C*(4)btz); 172.12 (COOEt of **B**); 172.52 (COOEt of **A**). Anal. Calcd for $C_{60}H_{60}O_{12}N_4S_4$ (1157.41): C, 62.26; H, 5.23; N, 4.84. Found: C, 61.98; H, 5.29; N, 4.71. ES-MS (pos. mode): 1156.59 [M]; 1157.54 [M+H$^+$]$^+$; 1179.71 [M+Na$^+$]$^+$, 1200.86 [M+2Na$^+$−H$^+$]$^+$.

5.1.4.8. 5,11,17,23-Tetra-(ethoxycarbonylmethyl)-25,27-bis(4-methyleneoxy-4′-methyl-5,5′-diethoxycarbonyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 21. Same procedure. From tetra ethylester **18** (0.1 g, 0.13×10^{-3} mol) and K_2CO_3 (0.018 g, 0.13×10^{-3} mol); MeCN (10 mL); bromide **8** (0.110 g, 0.26×10^{-3} mol); TLC monitoring: SiO_2, CH_2Cl_2/MeOH 98:2. After filtration of insoluble inorganic materials, addition to the CH_2Cl_2 solution of an excess MeOH and concentration under vacuum while freezing, resulting in the formation of a gel that was filtered and dried prior chromatography. Chromatography: Chromatotron, Al_2O_3, 1 mm thickness, CH_2Cl_2/Hex 80:20. Tetraester **21** (0.09 g, 48%). Yellow

powder. Mp: 218–219 °C. IR (KBr): 1712.6 (COO). UV–vis (CH_2Cl_2): 292 (11,934); 346 (37,464). ^{1}H NMR (400 MHz, $CDCl_3$): 1.27 (m, 12H, OCH_2CH_3 of Ar **A** and **B**); 1.39 (t, J = 7.05 Hz, 6H, OCH_2CH_3 btz); 1.46 (t, J = 7.05 Hz, 6H, OCH_2CH_3 btz); 2.79 (s, 6H, CH_3btz); 3.39 (s, 4H, CH_2COOEt of **B**); 3.42–4.52 (AB, J_{AB} = 12.84 Hz, 8H, ArCH_2Ar); 3.49 (s, 4H, CH_2COOEt of **A**); 4.16 (m, 8H, OCH_2CH_3 of Ar **A** and **B**); 4.31 (q, J = 7.05 Hz, 4H, OCH_2CH_3 btz); 4.39 (q, J = 7.05 Hz, 4H, OCH_2CH_3 btz); 5.40 (s, 4H, OCH_2 btz); 6.95 (s, 4H, Ar*H* of **B**); 7.03 (s, 4H, Ar*H* of **A**); 8.66 (s, 2H, O*H*). ^{13}C NMR (100 MHz, $CDCl_3$): 14.46 (CH_3CH_2 of btz); 14.59 (CH_3CH_2O of Ar **A** or **B**); 14.67 (CH_3CH_2O of btz); 14.94 (CH_3CH_2O of Ar **A** or **B**); 17.63 (*Me* bpy); 32.23 (ArCH_2Ar); 41.02 (CH_2COO of **A**); 41.46 (CH_2COO of **B**); 61.05, 61.20 (CH_3CH_2O of Ar **A** and **B**); 62.07, 62.46 (CH_3CH_2O of btz); 72.89 (OCH_2btz); 123.72 (C_p of **A**); 123.74 (*C*(5) btz); 125.11 (*C*(5′) btz); 127.95 (C_o of **A**); 129.65 (C_m of **A**); 130.41 (C_m of **B**); 131.07 (C_p of **B**)133.99 (C_o of **B**); 151.91 (C_{ipso} of **B**); 153.57 (C_{ipso} of **A**); 160.32 (*C*(4) btz); 161.11 (btz*C*OOEt); 161.87 (*C*(4′) btz); 162.02 (C(2) or C(2′) of btz); 162.12 (btz*C*OOEt); 163.11 (C(2) or C(2′) of btz); 172.38 (CH_2COOEt of **B**); 172.70 (CH_2COOEt of **A**). Anal. Calcd for $C_{72}H_{76}O_{20}N_4S_4$ (1445.65): C, 59.82; H, 5.30; N, 3.88. Found: C, 59.94; H, 5.30; N, 3.90. ES-MS (pos. mode): 1444.52 $[M+H^+]^+$, 1467.61 $[M+Na^+]^+$.

5.1.4.9. 5,11,17,23-Tetra-(acetic acid)-25,27,26,28-tetrahydroxycalix[4]arene, tetra sodium salt 17. The acid **16** (0.3 g, 0.415 × 10^{-3} mol) was suspended in distilled water (25 mL), and 0.1 M aqueous NaOH was carefully added until solubilisation was complete (pH-meter monitoring, final pH 7.4). The resulting solution was filtered, then lyophilised. The resulting solid was dissolved in distilled water, dialysed (Float-A-Lyser, cellulose acetate, cut-off 100 D), and finally lyophilised to give **17** (0.31 g, 74%). Purple solid. Mp: 250 °C (dec). IR (KBr): 3396.2 (OH); 1571.7 (COO). UV–vis (DMSO): 293 (13,099). ^{1}H NMR (400 MHz, DMSO-d_6): 2.98 (s, 8H, CH_2COONa); 2.99–4.17 (AB, J_{AB} = 12.08 Hz, 8H, ArCH_2Ar); 6.80 (s, 8H, Ar*H*); 13.02 (br s, 4H, O*H*). ^{13}C NMR (100 MHz, DMSO-d_6): 32.19 (ArCH_2Ar); 43.88 (CH_2COONa); 129.21 (C_m); 131.37 (C_o and C_p); 151.07 (C_{ipso}); 182.05 (*C*O). Anal. Calcd for $C_{36}H_{26}Na_6O_{12}{\cdot}8H_2O$ (932.65): C, 46.36; H, 4.54. Found: C, 46.73; H, 4.10.

5.1.4.10. 5,11,17,23-Tetra-(acetic acid)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra sodium salt 20. The tetraester **19** (0.39 g, 0.34 × 10^{-3} mol) was suspended in a 1:1 mixture of EtOH and H_2O (30 mL). Solid NaOH (0.54 g, 13.6 × 10^{-3} mol) was added to the mixture. The latter was maintained at reflux during 7 h (TLC monitoring Al_2O_3, CH_2Cl_2). The solution was cooled to rt, and EtOH was evaporated under vacuum; the resulting aqueous solution was acidified with 1 M HCl, affording a precipitate that was filtered (with or without centrifugation) and dried over P_2O_5 to give the corresponding acid (0.33 g, 95%). An analytical sample was conserved for analyses. The acid derivative (0.33 g, 0.32 × 10^{-3} mol) was suspended in distilled water (30 mL), and 0.1 M aqueous NaOH was carefully added until solubilisation was complete (pH-meter monitoring, final pH 7.1). The resulting solution was filtered, then lyophilised. The resulting solid was dissolved in distilled water, dialysed (Float-A-Lyser, cellulose acetate, cut-off 100 D), and finally lyophilised to give **20** (0.361 g, 82%).

Tetraacid (partial characterisation): light yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): 2.46 (s, 6H, CH_3btz); 3.36 (m, 12H, ArCH_2Ar, CH_2COOH); 4.28 (d, J = 12.8 Hz, 4H, ArCH_2Ar); 5.17 (s, 4H, OCH_2btz); 6.92 (s, 4H, Ar*H* of **A** or **B**); 7.05 (s, 4H, Ar*H* of **A** or **B**); 7.47, 7.98, 8.05 (3s, 6H, *H* btz, O*H*); 12.22 (br s, 4H, COO*H*). Compound **20**: light yellow cotonous powder. Mp: >230 °C. IR (KBr): 1565.9 (COO^-). UV–vis (H_2O): 297 (s, 14,534); 330 (30,504). ^{1}H NMR (400 MHz, D_2O): 2.25 (s, 6H, CH_3btz); 3.09 (s, 4H, CH_2COONa of **B**); 3.44 (s, 4H, CH_2COONa of **A**); 3.48–4.35 (AB, J_{AB} = 13.3 Hz, 8H, ArCH_2Ar); 4.94 (s, 4H, OCH_2btz); 6.83 (s, 4H, Ar*H* of **B**); 7.07 (s, 6H, Ar*H* of **A** and *H*(5′) btz); 7.71 (s, 2H, *H*(5) btz). ^{13}C NMR (100 MHz, D_2O): 16.09 (*Me*btz); 30.75 ($ArCH_2$-Ar), 43.94 (CH_2COONa of **A** and **B**); 73.11 (OCH_2 btz); 117.81 (*C*(5) of btz); 121.97 (*C*(5′) of btz); 127.84 (C_o of **A** or **B**); 128.86 (C_p of **A**); 130.05 (C_m of **A**); 130.29 (C_m of **B**); 133.41 (C_o of **A** or **B**); 135.19 (C_p of **B**); 150.77, 150.83 (C_{ipso} of **A** and **B**); 152.21 (*C*(2) of btz); 153.92 (*C*(2′) of btz); 160.02, 161.52 (*C*(4) and *C*(4′) of btz); 180.96 (*C*OONa of **B**); 181.75 (*C*OONa of **A**). Anal. Calcd for $C_{52}H_{40}O_{12}N_4S_4Na_4{\cdot}6H_2O{\cdot}$NaCl (1299.60): C, 48.06; H, 4.03; N, 4.31. Found: C, 48.04; H, 3.89; N, 4.32. ES-MS (pos. mode): 1155.28 $[M+Na^+]^+$, 788.42 $[2\,M+2\,K^+{+}Na^+]^{3+/3}$, 777.70 $[2\,M+3Na^+]^{3+/3}$, 597.04 $[M+Na^+{+}K^+]^{2+/2}$, 589.12 $[M+2Na^+]^{2+/2}$, 577.98 $[M+Na^+{+}H^+]^{2+/2}$. ES-MS (neg. mode): 1108.45 $[M-Na^+]^-$; 1086.52 $[M-2Na^+{+}H^+]^-$; 1064.59 $[M-3Na^+{+}2H^+]^-$; 1042.61 $[M-4Na^+{+}3H^+]^-$; 543.24 $[M-2Na^+]^{2-/2}$; 532.16 $[M-3Na^+{+}H^+]^{2-/2}$; 521.19 $[M-4Na^+{+}2H^+]^{2-/2}$.

5.1.4.11. 5,11,17,23-Tetra-(acetic acid)-25,27-bis(4-methyleneoxy-4′-methyl-5,5′-dicarboxylic acid-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, octa sodium salt 22. Same procedure from octaester **21** (0.43 g, 0.29 × 10^{-3} mol), EtOH/H_2O (30 mL), NaOH (0.46 g, 11.6 × 10^{-3} mol), reflux 7 h; TLC monitoring, SiO_2, CH_2Cl_2. Octaacid (0.40 g, 97%). Salification: H_2O (30 mL), 0.1 M NaOH, final pH 8.0. Octa sodium salt **71** (0.36 g, 67%). Octaacid (partial characterisation): light yellow powder. ^{1}H NMR (400 MHz, DMSO-d_6): 2.66 (s, 6H, CH_3 btz); 3.34 (m, 12H, ArCH_2Ar, ArCH_2COOH); 4.43 (d, J = 12.8 Hz, 4H, ArCH_2Ar); 5.28 (s, 4H, OCH_2btz); 6.93 (s, 4H, Ar*H* of **A** or **B**); 7.02 (s, 4H, Ar*H* of **A** or **B**); 8.67 (s, 2H, O*H*); 12.23, 13.34, 13.79 (br s 8H, COO*H*). Compound **22**: yellow powder. Mp: >240 °C. IR (KBr): 1578.4 (COO). UV–vis (H_2O): 287 (s, 10,995); 347 (38,803). ^{1}H NMR (400 MHz, D_2O): 2.64 (s, 6H, *Me*btz); 3.12 (s, 4H, CH_2COONa of **B**); 3.42 (s, 4H, CH_2COONa of **A**); 3.47–4.40 (AB, J = 13.4 Hz, 8H, ArCH_2Ar); 5.53 (s, 4H, OCH_2btz); 6.8 (s, 4H, Ar*H* of **B**); 7.10 (s, 4H, Ar*H* of **A**). ^{13}C NMR (100 MHz, D_2O): 16.34 (*Me*btz); 31.19 (Ar*C*H_2Ar); 43.86 (CH_2COONa); 71.50 (OCH_2btz); 128.51 (C_o of **A**); 128.63 (C_p of **A**); 129.89 (C_m of **A**); 130.15 (C_m of **B**); 133.95 (C_o of **B**); 134.21 (C(5′) btz); 134.81 (C_p of **B**); 135.86 (C(5) btz); 151.31 (C_{ipso} of **A**); 151.65 (C_{ipso} of **B**); 154.03 (C(2) btz); 155.81 (*C*(2′) btz); 160.17, 160.52 (*C*(4) and *C*(4′) btz); 167.32 (C(5)*C*OONa); 168.69 (C(5′)*C*OONa); 181.18 (*C*OONa of **B**); 182.03 (*C*OONa of **A**). Anal. Calcd for $C_{56}H_{36}O_{20}N_4S_4Na_8{\cdot}5NaCl{\cdot}7H_2O$ (1815.24): C, 37.05; H, 2.77; N, 3.08. Found: C, 37.05; H, 2.80; N, 3.08. ES-MS (neg. mode): 685.88 $[M-Na^+{-}H^+]^{2-/2}$; 674.92 $[M-2Na^+]^{2-/2}$; 663.90 $[M-3Na^+{+}H^+]^{2-/2}$; 652.93 $[M-4Na^+{+}2H^+]^{2-/2}$; 641.97 $[M-5Na^+{+}3H^+]^{2-/2}$; 631.01 $[M-6Na^+{+}4H^+]^{2-/2}$; 619.92 $[M-7Na^+{+}5H^+]^{2-/2}$; 609.02 $[M-8Na^+{+}6H^+]^{2-/2}$; 598.00 $[M-5Na^+{+}3H^+{-}2COO]^{2-/2}$; 586.97 $[M-6Na^+{+}4H^+{-}2COO]^{2-/2}$; 575.95 $[M-7Na^+{+}5H^+{-}2COO]^{2-/2}$; 564.99 $[M-8Na^+{+}6H^+{-}2COO]^{2-/2}$; 554.02 $[M-5Na^+{+}3H^+{-}4COO]^{2-/2}$; 543.06 $[M-6Na^+{+}4H^+{-}4COO]^{2-/2}$; 532.04 $[M-7Na^+{+}5H^+{-}4COO]^{2-/2}$; 521.07 $[M-8Na^+{+}6H^+{-}4COO]^{2-/2}$.

5.1.5. Phosphonate derivatives

5.1.5.1. 5,11,17,23-Tetra-(methylphosphonic acid)-25,27,26,28-tetrahydroxycalix[4]arene, tetra sodium salt 24. A solution of phosphonoester **23** (0.5 g; 0.49 × 10^{-3} mol) and trimethylsilylbromide (3 mL) in CH_2Cl_2 (15 mL) was stirred under argon at rt during 12 h. The mixture was then evaporated to dryness and the solid residue was triturated in H_2O (10 mL), filtered then rinsed with H_2O (3 × 10 mL) and CH_2Cl_2 (3 × 10 mL) to give the corresponding tetraacid (0.33 g; 72%). The latter (0.2 g; 2.14 × 10^{-4} mol) was suspended in H_2O (40 mL) and 0.1 M aqueous NaOH was added up to reach pH 7, while solubilisation occurs at pH 4–5. The resulting solution was evaporated to dryness and the solid residue taken off with MeOH then evaporated again to give **24** (0.212 g; 87%).

Tetraacid: white powder. Mp: 290–300 °C (dec). IR (KBr): 1003.5 (P–OH); 3193.3(Ar–OH). UV–vis (THF): 281 (8800). ^{1}H NMR (400 MHz, DMSO-d_6): 2.72 (d, J = 20.8 Hz, 8H, CH_2P); 3.40–4.15 (br signal, 8H, ArCH_2Ar); 6.97 (s, 8H, ArH); 9.63 (br s, 4H, ArOH). ^{13}C NMR (100 MHz, DMSO-d_6): 31.52 (ArCH_2Ar); 127.31 (C_p Ar); 129.07 (C_o Ar); 130.81 (C_m Ar); 148.38 (C_{ipso} Ar); Ar–CH_2–P not found. Anal. Calcd for $C_{32}H_{36}O_{16}P_4{\cdot}2H_2O{\cdot}H_3PO_4$ (934.54): C 41.13; H 4.64. Found: C, 41.06; H, 4.39. ES-MS (pos. mode): 823.1 $[M+Na^+]^+$, 845.2 $[M-H^++2\ Na^+]^+$. ES-MS (neg. mode): 799.2 $[M-H^+]^-$, 418.2 $[M+K^+-3H^+]^{2-/2}$, 399.2 $[M-2H^+]^{2-/2}$. Compound **24**: slight purple powder. Mp: 263–266 °C (dec). IR (KBr): 3400 (Ar–OH). UV–vis (H_2O): 284 (8400). ^{1}H NMR (400 MHz, CD_3OD): 2.71 (d, J = 20.2 Hz, 8H, CH_2P); 3.24–4.39 (AB, J_{AB} = 12.6 Hz, 8H, ArCH_2Ar); 6.94 (d, J = 2.1 Hz, 8H, ArH). ^{13}C NMR (100 MHz, D_2O): 32.04 (ArCH_2Ar); 35.23 (d, J = 129.0 Hz, ArCH_2P); 127.44 (d, J_{PC} = 8.7 Hz), 129.63 (d, J_{PC} = 5.1 Hz), 131.10, 150.51 (C_p, C_o, C_m, C_{ipso} of Ar). Anal. Calcd for $C_{32}H_{32}O_{16}Na_4P_4{\cdot}Na_2HPO_4{\cdot}6H_2O$ (1138.49): C, 33.76; H, 3.98. Found: C, 33.41; H, 3.57. ES-MS (neg. mode): 432.2 $[M-Na^+-H^+]^{2-/2}$; 421.25 $[M-2Na^+]^{2-/2}$; 418.3 $[M-3Na^++H_2O+H^+]^{2-/2}$, 410.3 $[M-3Na^++H^+]^{2-/2}$, 399.3 $[M-4Na^++2H^+]^{2-/2}$, 280.6 $[M-2Na^+-H^+]^{3-/3}$, 278.56 $[M-3Na^++H_2O]^{3-/3}$, 273.2 $[M-3Na^+]^{3-/3}$, 265.6 $[M-4Na^++H^+]^{3-/3}$.

5.1.5.2. 5,11,17,23-Tetra-(diethyl-methylphosphonate)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene 25. A solution of **23** (0.85 g; 8.10×10^{-4} mol) in MeCN (50 mL) was refluxed under argon in the presence of K_2CO_3 (0.112 g, 8.10×10^{-4} mol) during 5 min. Bromide **3** (0.491 g, 1.78×10^{-3} mol) was added and reflux was continued during 2.5 h. (TLC monitoring; SiO_2, CH_2Cl_2/CH_3OH 95:5). The solvent was evaporated to dryness and the solid residue dissolved in CH_2Cl_2. The insoluble material was filtered off and the filtrate concentrated for column chromatography (SiO_2, CH_2Cl_2/CH_3OH 95:5). The fractions containing the desired product were evaporated to dryness, dissolved in CH_2Cl_2 and added to excess cyclohexane. The resulting suspension was frozen then lyophilised to give **25**. White powder (1.0 g; 86%). Mp: 71–75 °C. IR (KBr): 1026.8 (P–O–R); 1241.3 (P=O); 1738.5(ArO-CH_2); 3406.8(Ar–OH). UV–vis (CH_2Cl_2): 231 (38,100); 294 (11,500); 330 (25,100). ^{1}H NMR (400 MHz, $CDCl_3$): 1.02 (t, J = 7.1 Hz, 12H, CH_3CH$_2$); 1.23 (t, J = 7.1 Hz, 12H, CH_3CH$_2$); 2.56 (s, 6H, *Me*btz); 2.82 (d, J = 21.6 Hz, 4H, CH_2P); 3.05 (d, J = 21.0 Hz, 4H, CH_2P); 3.41–4.35 (AB, J_{AB} = 13.2 Hz, 8H, ArCH_2Ar); 3.83 (m, 8H, CH$_3$CH_2); 3.98 (m, 8H, CH$_3$CH_2); 5.23 (s, 4H, OCH_2btz); 6.88 (d, J = 2.3 Hz, 4H, ArH); 7.05 (d, J = 2.3 Hz, 4H, ArH); 7.86 (s, 2H, H(5) or H(5′) btz); 7.94 (s, 2H, H(5′) or H(5) btz). ^{13}C NMR (100 MHz, $CDCl_3$) : 16.47, 16.53, 16.80, 16.86 (*C*H$_3$CH$_2$); 17.57 (*C*H$_3$ btz); 31.73 (ArCH_2Ar); 32.98 (d, J_{PC} = 138.8 Hz, ArCH_2P); 33.51 (d, J_{PC} = 138.4 Hz, ArCH_2P); 62.43, 62.48 (CH$_3$CH_2); 77.65 (OCH_2btz); 116.39, 118.03 (*C*(5), *C*(5′) btz), 153.85, 154.82, 160.71, 162.38 (*C*(2), *C*(2′), *C*(4), *C*(4′) btz); 122.21 (d, J = 9.1 Hz), 128.04 (d, J = 2.5 Hz), 128.97 (d, J = 8.3 Hz), 130.46 (d, J = 6.2 Hz), 130.98 (d, J = 6.2 Hz), 133.64 (d, J = 2.5 Hz), 151.32 (d, J = 4.0 Hz), 152.62 (d, J = 3.3 Hz), (C_{ipso}, C_o, C_m and C_p Ar). Anal. Calcd for $C_{64}H_{84}N_4O_{16}P_4S_4{\cdot}0.25(C_2H_5O)_3P$ (1454.03): C, 54.07; H, 5.80; N, 3.85. Found: C, 54.25; H, 5.78; N, 3.53. ES-MS (pos. mode): 1435.4 $[M+Na^+]^+$, 1430.4 $[M-C_2H_5+H+2Na^+]^+$, 1241.4 $[M-CH_3btzCH_2+H+Na^+]^+$, 1236.4 $[M-CH_3btzCH_2-C_2H_5+H+2Na^+]^+$, 737.4 $[M-C_2H_5-H^++4Na^+]^{2+/2}$, 729.4 $[M+2Na^+]^{2+/2}$, 726.4 $[M-C_2H_5+3Na^+]^{2+/2}$, 718.5 $[M+Na^++H^+]^{2+/2}$, 707.4 $[M+2H^+]^{2+/2}$.

5.1.5.3. 5,11,17,23-Tetra-(methylphosphonic acid)-25,27-bis(4-methyleneoxy-4′-methyl-2,2′-bithiazolyl)-26,28-dihydroxycalix[4]arene, tetra sodium salt 26. A solution of **25** (0.59 g; 4.17×10^{-4} mol) and bromotrimethylsilane (2.7 mL) in dry CH_2Cl_2 (15 mL) was stirred at rt under Ar during 20 h. The solvent were evaporated to dryness and the solid residue triturated with water, filtered, rinsed with water (3 × 10 mL), MeOH (3 × 10 mL) and CH_2Cl_2 (3 × 15 mL) to give the corresponding tetraacid (0.44 g; 87%). The latter (0.2 g; 1.66×10^{-4} mol) was suspended in water (40 mL) and was treated with 1 M NaOH until pH 7 was raised, while solubilisation occurred at pH 4.5. The resulting neutral solution was evaporated to dryness, the residue was treated with MeOH then dried again to give **26** after a final dialysis/lyophilisation process (0.22 g; 96%). Tetraacid (partial characterisation): white powder. Mp: 250–254 °C (dec). IR (KBr): 987.4 (P–OH); 1244.1 (P=O); 3366.2 (Ar–OH). UV–vis (THF): 330 (26,700). ^{1}H NMR (400 MHz, DMSO-d_6): 2.46 (s, 6H, *Me*btz); 2.71 (d, J = 21.2 Hz, 4H, CH_2P); 2.77 (d, J = 21.2 Hz, 4H, CH_2P); 3.35–4.31 (AB, J_{AB}: 12.8 Hz, 8H, ArCH_2Ar); 5.17 (s, 4H, OCH_2btz); 6.99 (s, 4H, ArH); 7.01 (s, 4H, ArH); 7.48 (s, 2H, H(4) or H(4′), btz); 8.03 (s, 2H, H(4′) or H(4) btz); 8.13 (s, 2H, ArOH). Anal. Calcd for $C_{48}H_{48}N_4O_{16}P_4S_4{\cdot}H_2O$ (1207.09): C, 47.76; H, 4.18; N, 4.64. Found: C, 47.71; H, 4.11; N, 4.45. ES-MS (neg. mode): 1187.2 $[M-H^+]^-$; 1093.3 $[M-(CH_2P(O)(OH)_2^+)]^-$; 593.3 $[M-2H^+]^{2-/2}$; 546.3 $[M-CH_2P(O)(OH)_2-H^+]^{2-/2}$; 496.3 $[M-CH_3btzCH_2-H]^{2-/2}$. Compound **26**: yellow powder. Mp: 250–260 °C (dec). IR (KBr): 1152.1 (P=O); 3490.0 (Ar–OH). UV–vis (H_2O): 330 (24,700). ^{1}H NMR (400 MHz, CD_3OD): 2.49 (s, 6H, *Me*btz); 2.74 (d, J = 20.6 Hz, 4H, CH_2P); 2.77 (d, J = 20.2 Hz, 4H, CH_2P); 3.40–4.39 (AB, J_{AB} = 12.6 Hz, 8H, ArCH_2Ar); 5.18 (s, 4H, 0CH_2btz); 7.05 (d, J = 2.1 Hz, 4H, ArH); 7.14 (d, J = 2.1 Hz, 4H, ArH); 7.25 (s, 2H, H(4) or H(4′), btz); 7.97 (s, 2H, H(4′) or H(4) btz). ^{13}C NMR (100 MHz, D_2O): 16.03 (*C*H$_3$ btz); 30.83 (ArCH$_2$Ar); 35.30 (d, J_{PC} = 129.8 Hz, ArCH_2P); 35.49 (d, J_{PC} = 127.6 Hz, ArCH_2P); 73.05 (OCH_2btz); 116.40, 117.83 (*C*(5), *C*(5′) btz); 127.13 (d, J = 8.0 Hz), 133.54 (d, J = 8.8 Hz) (C_p Ar); 127.99 (d, J = 1.5 Hz), 133.3 (d, J = 1.5 Hz) (C_o Ar); 130.42 (d, J = 5.8 Hz), 130.55 (d, J = 5.1 Hz) (C_m Ar); 150.60 (C_{ipso} Ar); 152.27, 153.43 (*C*(2), *C*(2′) btz); 160.08, 161.52 (*C*(4), *C*(4′) btz). Anal. Calcd for $C_{48}H_{44}N_4O_{16}Na_4P_4S_4{\cdot}5.5H_2O$ (1376.08): C, 41.90; H, 4.03; N, 4.07. Found: C, 41.79; H, 3.66; N, 3.92. ES-MS (pos. mode): 683.1 $[M-2H^++4Na^+]^{2+/2}$, 672.1 $[M-H^++3Na^+]^{2+/2}$, 661.0 $[M+2Na^+]^{2+/2}$. ES-MS (neg. mode): 615.1 $[M-2Na^+]^{2-/2}$, 604.0 $[M-3Na^++H^+]^{2-/2}$, 593.2 $[M-4Na^++2H^+]^{2-/2}$, 402.6 $[M-3Na^+]^{3-/3}$, 395.3 $[M-4Na^++H^+]^{3-/3}$.

5.2. Biology

The in vitro antiviral activity and cytotoxicity (respectively estimated by the IC_{50} and CC_{50}, defined in Table 2) of the compounds in CEM-SS, MT4 and PBMC cells were determined according to published procedures.[22] For the CEM-SS and PBMC cells, the replication of HIV-1 (LAI and Bal strains, respectively) was evaluated by measuring the reverse transcriptase activity (RT) in the culture supernatant, which reveals the presence of virus particles released from the cells. The replication of HIV-1 IIIB in MT4 cells was followed by the cytopathogenic effect induced by the virus replication.

GHOST cells (human osteogenic sarcoma) transformed to express human CD4 plus CXCR4 or CCR5, and the green fluorescent protein (GFP) under the control of an HIV-2 LTR, were cultured in Dulbecco medium supplemented with 10% FCS. Cells were distributed in 24-well plates at a density of 2.5×10^5 cells per well in 500 μL medium. Twenty four hours later, the medium was eliminated and cells were pre-treated with different concentrations of the compound **6** diluted in medium for 1 h at 37 °C, then infected in presence of the drug with HIV-1 LAI (GHOST-CXCR4) or HIV-1 Bal (GHOST-CCR5) as described previously.[23] Mock-treated cells were infected in parallel. After 2 h adsorption, cells were washed and cultured in absence of drugs for 48 h. At the end of the incubation period, cells were treated with trypsin, washed, suspended in 700 μL 1.5% *para*-formaldehyde and analysed by flow cytometry to detect the presence of GFP resulting from LTR transactivation med-

iated by Tat which is produced upon cell infection by HIV. The percentage of cells expressing GFP, infected in the absence or in the presence of different concentrations of antivirals was measured and the 50% inhibitory concentration of cell infection (IC_{50}) was derived from the computer-generated median effect plot of the dose–effect data.[24]

Acknowledgements

We are grateful to the MRES and CNRS for financial support, especially M. Mourer for a PhD grant. We thank ANRS (Agence nationale de recherches sur le SIDA et les hépatites virales) for funding antiviral assays. The authors thank E. Dubs for preparing some of the chemical intermediates, J.-M. Ziegler and F. Dupire of Service Commun de Spectrometrie de Masse, Nancy, as well as S. Sanglier (LSMBO, Strasbourg) for mass measurements and S. Adach for elemental analyses.

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