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REVIEW

Biologically active calixarene phosphonic acids

Oleksandr L. Kobzar[1], Sergii O. Cherenok[2], Sergiy O. Kosterin[3], Vitaly I. Kalchenko[2], Andriy I. Vovk[1]*

[1] *V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine, Kyiv, Ukraine*
[2] *Institute of Organic Chemistry of the NAS of Ukraine, Kyiv, Ukraine*
[3] *Palladin Institute of Biochemistry of the NAS of Ukraine, Kyiv, Ukraine*

Abstract: Phosphorylated derivatives of organic compounds are known to be capable of inhibiting the activities of enzymes and other proteins responsible for key metabolic pathways. In this connection, the calixarene phosphonic acids are of interest as macrocyclic agents interacting with targets that may be involved in pathological cellular processes. This review presents a literature survey on the synthesis and properties of calix[4]arene phosphonic acids as inhibitors of alkaline phosphatases, protein tyrosine phosphatases, Na,K-ATPase, nucleotide pyrophosphatase/phosphodiesterase 1, and some other enzymes and proteins. Brief information is also given about the inhibitory activity of calix[4]arene derivatives bearing alkyl phosphonate or phosphinic acid groups.

Keywords: calix[4]arene; phosphonic acids; enzymes; proteins; inhibition.

Introduction

Calixarenes are synthetically available nano-sized macrocyclic compounds with a cup-like structure [1]. Due to the unique receptor properties of calixarenes to biologically important cations and anions, neutral biomolecules, as well as biopolymers, they have broad prospects for biomedical applications [2, 3]. These macrocyclic molecules are considered as potential therapeutic agents [4] with antimicrobial [5] antibacterial [6], antiviral, anticoagulant, and antifungal activities [3]. The anticancer activity of functionalized calixarenes has been reported by several research groups [7].

One of the leading places in the field of calixarene chemistry is occupied by their phosphorylated derivatives [8, 9]. Phosphonic acid fragments of the synthetic compounds can be considered as non-hydrolyzable mimetics of phosphate groups of low-molecular bioorganic substrates and phosphate groups of proteins [10, 11]. The phosphonic acid derivatives of organic compounds are known to inhibit the activity of enzymes and some other biomolecules responsible for key metabolic pathways [12, 13].

Although several phosphonate inhibitors are known in the literature, these studies are still ongoing, including synthesis and investigation of bioorganic properties of various phosphorylated macrocycles [14, 15]. In this connection, the calixarene phosphonic acids are of interest as possible chemical agents interacting with targets involved in pathological cellular processes.

In this review, we summarize information on calix[4]arene phosphonic acids as inhibitors of biologically relevant enzymes such as alkaline phosphatases, protein tyrosine phosphatases, nucleotide pyrophosphatase/phosphodiesterase 1, ATPases, as well as glutathione *S*-transferase. In addition to the phosphonic acids, the activities of their mono- and diester forms as well as properties of calix[4]arene-based phosphinic acids are discussed. The analysis of possible mechanisms of inhibitory activity and details of the synthesis of the phosphorylated macrocycles can be found in the original papers which are referred to in the review.

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* Corresponding author. Tel.: +380-44-558-5388;
e-mail: vovk@bpci.kiev.ua (A.I. Vovk)
ORCID: 0000-0001-6167-076X

Inhibition of alkaline phosphatases

Alkaline phosphatases belong to the substrate non-specific enzymes catalyzing hydrolysis of phosphate monoesters and reactions of transphosphorylation [16]. Overexpression of these enzymes was observed in patients with bone diseases and some cancer types [17].

The inhibitory activity of calix[4]arene methylene-bisphosphonic acids **1** and **2** (Figure 1) towards alkaline phosphatases was investigated using *in vitro* enzymatic hydrolysis of *p*-nitrophenyl phosphate. Compounds **1** and **2** were synthesized in two steps. In the first stage, ethyl esters of calix[4]arene methylenebisphosphonic acids were obtained by the Arbuzov reaction of formyl calix[4]arenes with triethyl phosphite. The treatment of the phosphate esters with bromotrimethylsilane and methanol afforded the calix[4]arene methylenebisphosphonic acids [18].

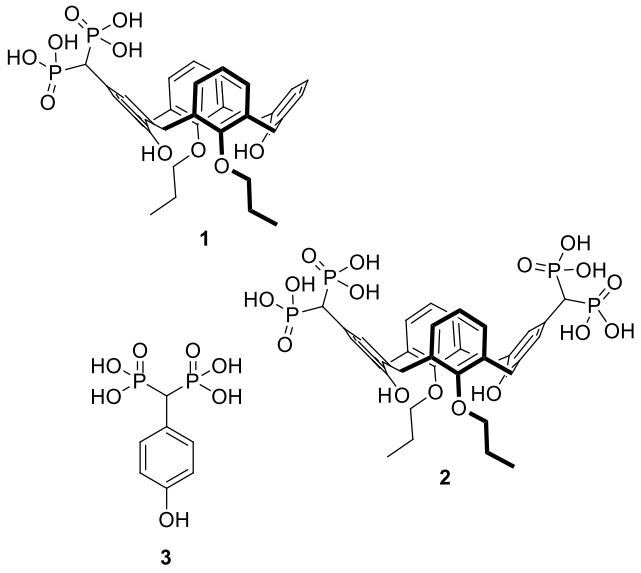


Figure 1. Calix[4]arene methylenebisphosphonic acids **1**, **2** and model compound **3**.

It was established that calix[4]arene methylene-bisphosphonic acids were stronger inhibitors of the calf intestine alkaline phosphatase in comparison with porcine kidney alkaline phosphatase and *E. coli* alkaline phosphatase. 4-Hydroxyphenyl methylenebisphosphonic acid **3** and methylenebisphosphonic acid exhibited significantly lower activity. The inhibition effects of *bis*-substituted calix[4]arene derivative **2** observed for alkaline phosphatase from calf intestine were more pronounced than in the case of compound **1**. The influence of compounds **1** and **2** on the enzyme activities is in agreement with mixed-type or partial mixed-type inhibition. According to this, the inhibitor competes with the substrate for binding to the enzyme's active site and binds with the enzyme-substrate complex. The competitive component in the inhibition mechanism prevailed over the non-competition one. The inhibition constants K_i of calix[4]arene methylenebisphosphonic acids **1** and **2** are 2.5 μM and 0.38 μM for calf intestine alkaline phosphatase, 6.3 μM and 0.40 μM for bovine intestine alkaline phosphatase, 8.8 μM, and 12 μM for bovine kidney alkaline phosphatase, and 230 μM and 190 μM for *E. coli* alkaline phosphatase. The strong inhibitory activity of calixarene **2** can be the consequence of the preorganization of two methylene-bisphosphonic acid moieties. The efficient coordination with the metal cations in the enzyme active site and the additional van der Waals interactions brought about by the macrocycle scaffold itself are probably responsible for the high activity of the compound **2** [18, 19].

The investigation of chiral calix[4]arene α-amino-phosphonic acids **4** and **5** (Figure 2) towards porcine kidney alkaline phosphatase was carried out. The macrocyclic derivatives were synthesized by the Pudovik reaction of chiral iminocalix[4]arenes with sodium diethyl phosphite and further dialkylation of obtained calix[4]arene aminophosphonates with bromotrimethylsilane and methanol [20].

Calix[4]arene derivatives **4** and **5** were found to be reversible inhibitors of porcine kidney alkaline phosphatase with a mixed-type inhibition mechanism. The alkaline phosphatase was shown to be sensitive to the absolute configuration of the chiral carbon atom of the calix[4]arene α-aminophosphonic acids. The K_i value for *S*-isomer **4b** was about two times smaller than for its *R*-counterpart **4a**. The inhibition of the enzyme increased drastically in the case of diastereomeric *bis*-aminophosphonic acids **5a** and **5b**. The binding affinity of the *RR*-diastereomer **5a** to the porcine kidney alkaline phosphatase with an inhibition constant of 1.7 μM was about 50 times higher than that of *SS*-diastereomer **5b**, which corresponds to a difference in free energy of 2.3 kcal/mol. However, a less pronounced enantioselectivity of inhibition by calix[4]arenes **5a** and **5b** was observed in the case of bovine intestinal alkaline phosphatase. The *S*-isomer of model compound **6** (Figure 2) showed significantly less inhibitory effect.

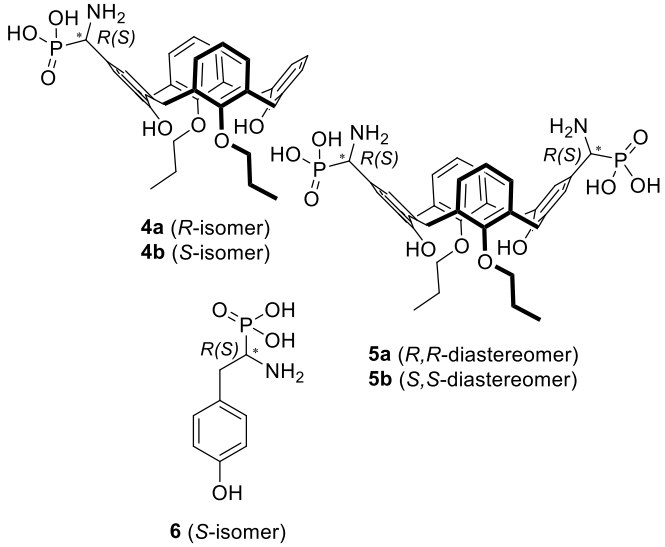


Figure 2. Calix[4]arene *α*-aminophosphonic acids **4**, **5** and model compound **6**.

The inhibitory properties of functionalized calixarenes can be dependent not only on the modification of lower rim substituents but also on a change in scaffold structure. The effect of the thiacalix[4]arene platform on the inhibition of

alkaline phosphatases was demonstrated on the example of *tetrakis*-dihydroxyphosphorylmethyl derivatives of thiacalix[4]arene and calix[4]arene. The derivatives **7** and **8** (Figure 3) were obtained by the Arbuzov reaction of the chloromethylated macrocycles with triethyl phosphite followed by transformation of the obtained esters into corresponding acids by treatment with hydrochloric acid solution [21].

As expected, the *tetrakis*-dihydroxyphosphorylmethyl thiacalix[4]arene **7** was an effective inhibitor of bovine intestinal alkaline phosphatase, while showing lower inhibitory effects against human placental alkaline phosphatase and shrimp alkaline phosphatase. The activity of calix[4]arene **8** against these enzymes was less pronounced as compared to that of compound **7**. The value of inhibition constant for thiacalix[4]arene **8** as an inhibitor of bovine intestinal alkaline phosphatase was approximately one order of magnitude lower than for calix[4]arene methylenebisphosphonic acid **2**. Both compounds were found to be mixed-type inhibitors of the enzymes from human placenta and bovine intestinal mucosa with a larger contribution of the competitive component. At the same time, these compounds were competitive inhibitors of the alkaline phosphatase from shrimp. Based on molecular docking results, it was suggested that the properties inherent to thiacalix[4]arene scaffold can provide a more extensive network of hydrogen bonds between phosphonate groups and amino acid residues of the active site [21].

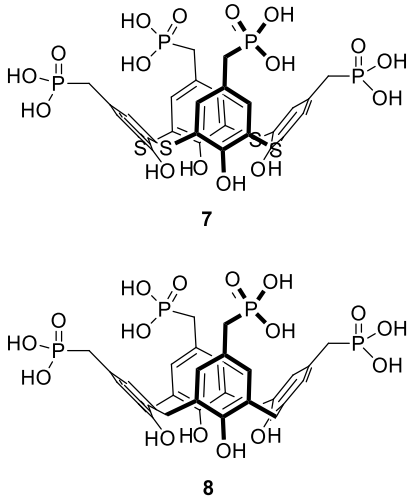


Figure 3. Thiacalix[4]arene and calix[4]arene *tetrakis*-methylphosphonic acids **7** and **8**.

Inhibition of ATPases

Na,K-ATPase is a membrane protein that catalyzes the hydrolysis of ATP and pumps Na^+ and K^+ ions across the cellular membranes. This enzyme is responsible for generating the membrane potential and maintaining the osmotic equilibrium [22]. Numerous attempts have been made to elucidate the inhibitory properties of calix[4]arene phosphonic acids as possible regulators of ATPase activities. In this field, the effects of calix[4]arene derivatives **1**, **9**, and **10** (Figures 1 and 4) with methylenebisphosphonic, aminophosphonic, and *α*-hydroxyphosphonic acid groups on the enzymatic activity of ouabain-sensitive Na,K-ATPase and ouabain resistant basal Mg-ATPase were studied. In suspension of myometrium cell plasma membranes, the calix[4]arene phosphonic acids in the concentration of 100 μM inhibited the enzymatic activity of Na,K-ATPase by 86-98% and did not practically affect the activity of Mg-ATPase. These calix[4]arenes were more effective than ouabain in suppressing the enzymatic activity of the sodium pump. Among the compounds studied, calix[4]arene methylenebisphosphonic acid **1** exhibited the best inhibitory effect on Na,K-ATPase with I_{50} of 33 nM [23].The synthesis of calix[4]arene **9** and **10** were described in papers [23, 24].

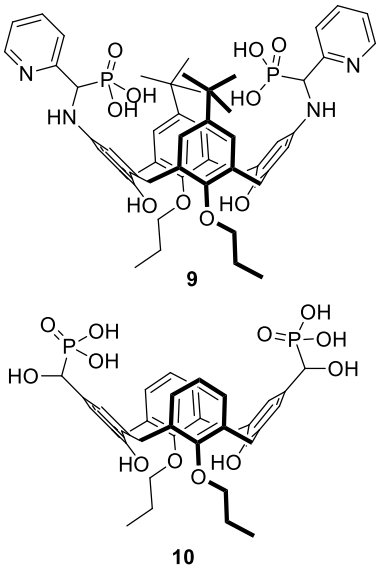


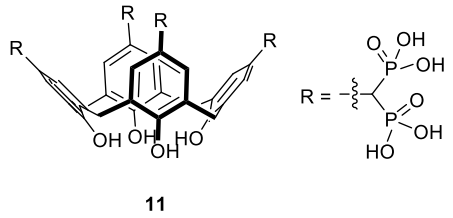
Figure 4. Calix[4]arene **9** and **10** studied as inhibitors of ATPases.

Other experimental results showed that compound **9** at a concentration of 100 μM can activate the actomyosin ATPase. At the same time, 100 μM calixarenes **1** and **10** inhibited the activity of this ATPase by 70% and 50%, respectively. In the case of myosin subfragment-1 ATPase, calix[4]arene **9** (100 μM) increased ATP hydrolysis more than twice, while 100 μM calix[4]arene **1** inhibited activity by 77% ($I_{0.5}$ = 43 μM) [25]. Calix[4]arene **9** is also able to inhibit myometrium Na,K-ATPase (I_{50} = 54 nM) with selectively over other ATPases of the plasma membrane and simultaneously activated the enzymatic activity of the myosin ATPase of smooth muscles (A_{50} = 9.6 μM). It was shown that inhibition of Na,K-ATPase and activation of myosin ATPase by calix[4]arene **9** cause stimulation of isolated smooth muscle contractile activity [26]. It was also shown that the inhibitors of plasma membrane Ca,Mg-ATPase have been identified among sulfonylamidine derivatives of calix[4]arenes [27, 28].

Inhibition of protein tyrosine phosphatases

The first investigation of calix[4]arene phosphonic acids as inhibitors of PTPases was undertaken on the *Yersinia* protein tyrosine phosphatase [29]. This enzyme is an essential virulence factor of *Yersinia pestis* in infected cells

causing dephosphorylation of multiple focal adhesion proteins to disrupt the signaling pathways and to escape the immune responses [30]. The i*n vitro* study revealed that calix[4]arene methylenebisphosphonic acids **1**, **2**, and **11** (Figures 1 and 5) as well as thiacalix[4]arene *tetrakis*-methylphosphonic acid **7** and calix[4]arene *tetrakis*-methylphosphonic acids **8** (Figure 3) are competitive inhibitors of *Yersinia* PTPase. The inhibition constants of the calix[4]arene methylene-bisphosphonic acid **1** and calix[4]arene *bis*-methylene-bisphosphonic acid **2** were 7.1 μM and 1.4 μM, respecti-vely, which are approximately 80-fold and 400-fold better than of 4-hydroxyphenyl methylenebisphosphonic acid **4** (Figure 2). At the same time, compound **11** bearing four methylenbisphosphonic acid fragments at the wide rim of the calix[4]arene scaffold showed decreased inhibition of the enzyme compared with the effects of compounds **1** and **2**. The most effective inhibitors of *Yersinia* phosphatase were thiacalix[4]arene- and calix[4]arene-based *tetrakis*-methylphosphonic acids **7** and **8** with K_i values of 0.92 μM and 0.22 μM, respectively. According to molecular docking results, the calix[4]arene inhibitors may occupy the active site region of the enzyme, thereby preventing substrate binding [29].



11

Figure 5. Calix[4]arene *tetrakis*-methylenebisphosphonic acid **11**.

Further systematic studies of calix[4]arene phosphonic acids have been conducted using protein tyrosine phosphatase 1B (PTP1B), T-cell protein tyrosine phosphatase (TC-PTP), megakaryocyte protein tyrosine phosphatases 1 and 2 (MEG1 and MEG2), Src homology region 2 containing protein tyrosine phosphatase (SHP2), leukocyte common antigen protein tyrosine phosphatase (CD45), and receptor-type protein tyrosine phosphatase beta (PTP*β*), also known as vascular endothelial-protein tyrosine phosphatase (VE-PTP). PTP1B is involved in the insulin and leptin signal transduction by dephosphorylation of activated insulin receptor or leptin receptor-associated kinase JAK2. As a negative regulator of insulin and leptin signaling, this enzyme is one of the most promising therapeutic targets for the treatment of type 2 diabetes and obesity [31, 32]. TC-PTP having a sequence identity of the catalytic domain to PTP1B of 74% [33] plays a role in inflammation control [34]. MEG2 is a regulator of hematopoietic signaling and blood glucose homeostasis and is considered a potential therapeutic target in the treatment of myeloproliferative disorders and type 2 diabetes [35-37]. Activation of SHP-2 was observed in many pathological conditions including different cancer types [38]. CD45 is implicated in immune system function and therefore can be involved in the development of autoimmune diseases [39]. Overactivity of PTP*β* upregulated by hypoxia can lead to a variety of endothelial dysfunctions in patients with diabetes [40]. These data suggest that the inhibitors of PTPs may be developed as possible therapeutic agents.

The syntheses of calix[4]arene *α*-hydroxymethylene-bisphosphonic acids **12** and **13** (Figure 6) were performed starting from calix[4]arene carboxylic acids and their acyl chlorides. Corresponding *α*-ketophosphonic acid silyl esters obtained in the Arbusov reaction were transformed into silyl esters of *α*-hydroxymethylenebisphosphonic acid, which gave free acids **12** and **13** after treatment by methanol [41].

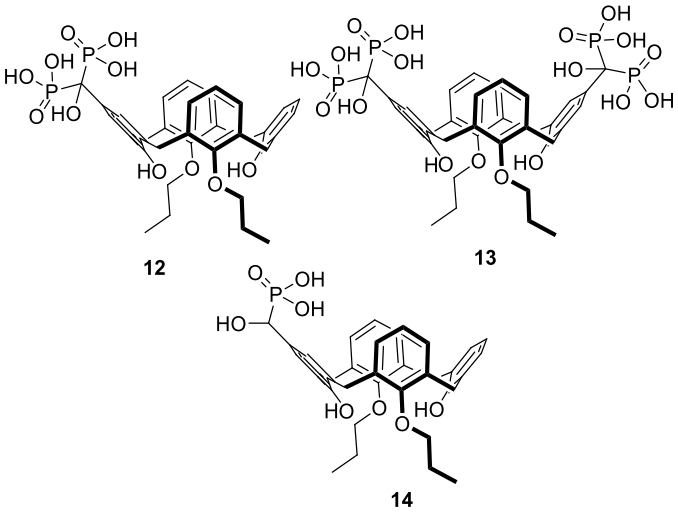


Figure 6. Calix[4]arene α-hydroxymethylenebisphosphonic acids **12**-**14**.

Calix[4]arene methylenebisphosphonic acids **1** and **2** and calix[4]arene *α*-hydroxymethylenebisphosphonic acids **12** and **13** were found to be micromolar inhibitors of PTP1B. Comparison of IC_{50} values of the compounds **1**, **2**, **12**, and **13** (Figures 1 and 6) showed that the replacement of one or two *α*-hydroxy-methylenebisphosphonate fragments by methylene-bisphosphonate ones leads to a decrease in enzyme inhibition. The macrocyclic inhibitors of PTP1B showed good selectivity over PTP*β* (with exception of *bis*-*α*-hydroxymethylenebisphosphonic acid **13**) and 4-9-fold selectivity over CD45. Mono-substituted calix[4]arenes **1** and **12** exhibited 8- and 14-fold selectivity over TCPTP. Kinetic studies revealed that the calix[4]arene derivative **1** is a slow-binding inhibitor of PTP1B with a competitive-type inhibition mechanism. Docking results suggested that the macrocyclic inhibitors are capable of occupying the active site region of PTP1B with the predicted free energies that were in agreement with experimental data [41].

The inhibitory effects of the calix[4]arene *α*-hydroxy-methylphosphonic acids **14** (in racemic form; Figure 6) and *bis*-substituted derivative **10** (as (*RS*)-stereoisomer; Figure 4) against PTPs were found to be similar to the inhibitory effects of calix[4]arene methylenebisphosphonic acids **1** and **2**. However, compound **14** exhibited high inhibitory activity against CD45 (IC_{50} values of 0.64 μM) with selectivity over TC-PTP and modest selectivity over PTP1B, PTP*β*, and SHP2 [42]. As compared to this the described activities of *α*-hydroxyphosphonates screened against CD45 were much lower [43].

Calix[4]arene *α*-ketophosphonic acids **15** and **16** (Figure 7) turned out to be slightly less effective inhibitors of the PTPases than the calix[4]arene methylenebisphosphonic acids **1** and **2** (Figure 1). At the same time, they showed preferred inhibition of PTP1B over TC-PTP, MEG1, MEG2, SHP2, and LAR. The position of the *α*-ketophosphonate fragment of the inhibitor **15** mimics the substrate binding at the active site of PTP1B [44].

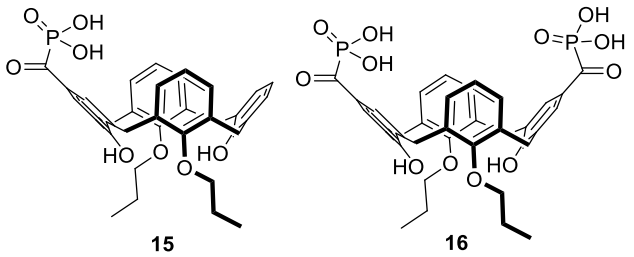


Figure 7. Calix[4]arene *α*-ketophosphonic acids **15** and **16**.

The IC_{50} values of thiacalix[4]arene *tetrakis*-methylphosphonic acid **7** and calix[4]arene *tetrakis*-methylphosphonic acid **8** for PTP1B with IC_{50} values of 0.17 μM and 1.3 μM were comparable with the values obtained for PTP*β* (0.13 μM and 3.8 μM, respectively). The activity of sulfonylcalix[4]arene **17** (Figure 8) with four phosphonic acid groups introduced at the upper rim was the same as compared to its thiacalix[4]arene analog **7** (Figure 3), without selectivity over other PTPases such as TC-PTP, MEG1, MEG2, SHP2, and PTP*β* [45]. Synthesis of sulfonylcalix[4]arene phosphonic acid **17** was performed by oxidation of thiacalix[4]arene scaffold followed by hydrolysis of the alkyl phosphonate groups of the obtained sulfonylcalix[4]arene derivative [46].

Quite unexpectedly, the tetraethyl ester of the *tetrakis*-methylphonic acid was found to exhibit inhibitory activity against PTP1B and MEG2 with IC_{50} values of 0.7 μM and 0.8 μM, respectively [45]. Unsubstituted sulfonylcalix[4]arene was also able to inhibit protein tyrosine phosphatase MEG2 with an IC_{50} value of 9.1 μM demonstrating lower activity against PTP1B, MEG1, TC-PTP, SHP2, and PTP*β* [47]. Sulfonylcalix[4]arene **18** functionalized by the *tert*-butyl group was a weak inhibitor of PTP1B, MEG2, SHP2, and PTP*β* and did not influence the activity of TC-PTP, MEG1. Modification of sulfonylcalix[4]arene scaffold with trifluoroacetamide substituents (compound **20**) led to inhibition of PTP1B with IC_{50} of 1.4 μM and 4- to 28-fold selectivity over the other PTPs [45].

Given that thiacalix[4]arene-based full ester **22** (Figure 8) was non-active against PTPases at a concentration of 10 μM, the thiacalix[4]arene *tetrakis*-methylphosphonate monoesters **20** and **21** were designed as compounds which have the improved bioavailability and do not lose the capacity to interact with polar regions of PTPs active sites. These monoesters were synthesized by consecutive treatment of octaesters **22** with lithium bromide and hydrochloric acid according to previously developed synthetic protocols [46]. The derivatives **20** and **21** were

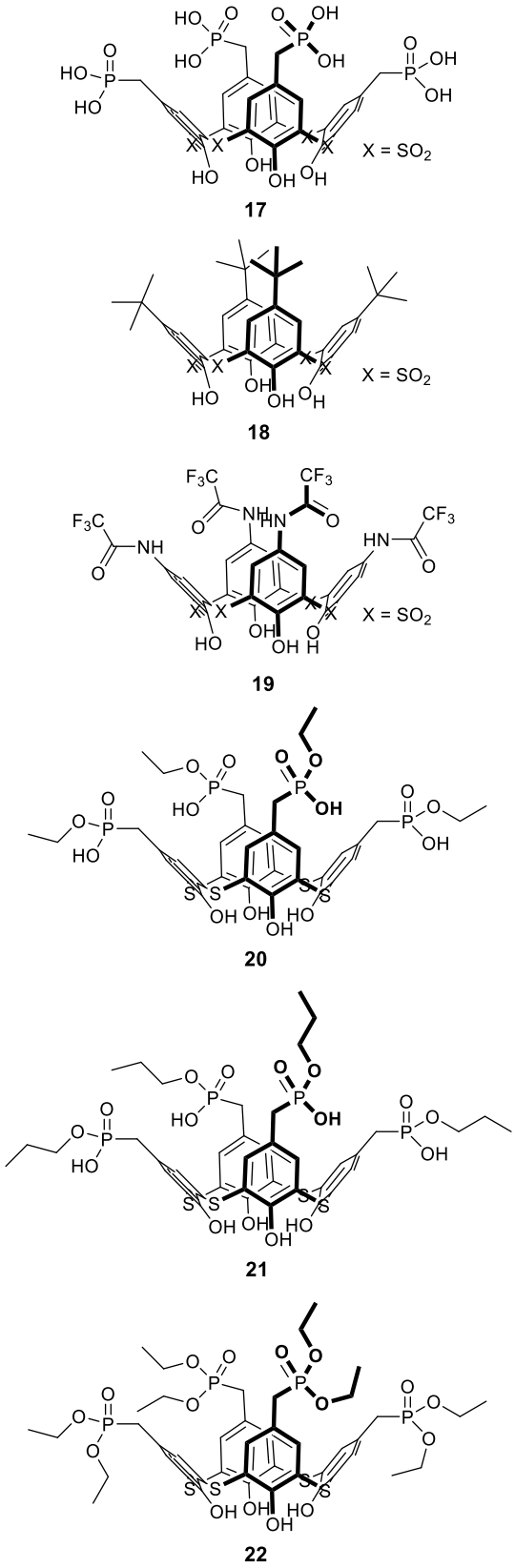


Figure 8. Derivatives of sulfonylcalix[4]arene (**17**-**20**) and thiacalix[4]arene (**20**-**22**).

slow-binding selective inhibitors of PTP1B with IC_{50} values of 0.34 μM and 0.32 μM, respectively, which were more than 5 times better than IC_{50} values obtained for TC-PTP, MEG1, MEG2, and SHP2. Kinetic studies revealed that thiacalix[4]arene *tetrakis*-methylphosphonate monoester **20** was a competitive inhibitor of PTP1B. As in the

case of acids **7** and **8**, compound **20** is more favorable to bind to the closed conformation of PTP1B [48].

Thiacalix[4]arene phosphinic acid **24** (Figure 9) can be considered a structural analog of the monoester form of *tetrakis*-methylphosphonate **20**. It is known that phosphinic acid derivatives are widely used in designing biologically active compounds, in particular, enzyme inhibitors [49, 50]. Calix[4]arene phosphinic acid **23** and its thiacalix[4]arene and sulfonylcalix[4]arene analogs **24** and **25**, respectively (Figure 9), were synthesized from chloromethyl calix[4]arene or chloromethyl thiacalix[4]arene by the Arbuzov reaction of the corresponding chloromethyl derivatives with phenyl diisopropyl phosphonite in dry chloroform. The oxidation of thiacalix[4]arene intermediate with sodium perborate in absolute trifluoroacetic acid gave sulfonylcalix[4]arene derivative in form of isopropyl esters**.** The dealkylation of corresponding isopropyl esters with trimethylbromosilane and methanolysis of the silyl intermediates provided the methyl-phenylphosphinic acid derivatives of calix[4]arene, thiacalix[4]arene, and sulfonylcalix[4]arene (compounds **23-25**, respectively) [51].

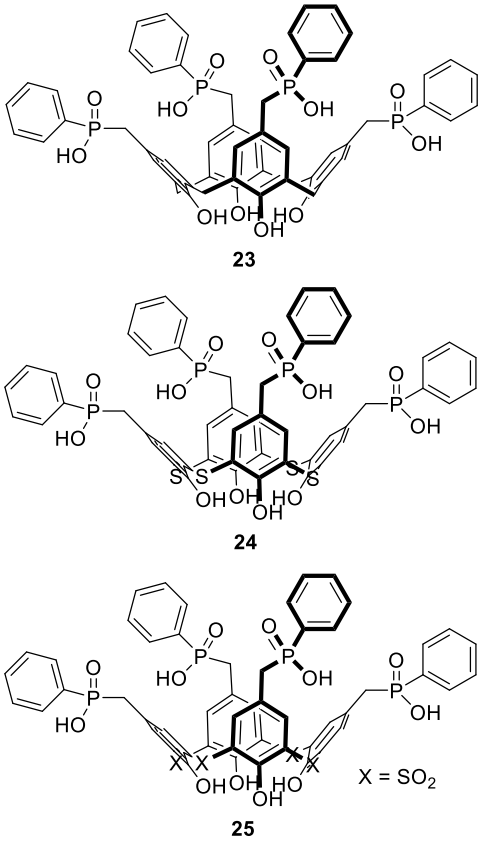


Figure 9. Calix[4]arene, thiacalix[4]arene, and sylfonylcalix[4]arene phosphinic acids **23-25**.

The compound **24** and corresponding calix[4]arene **23** and sulfonylcalix[4]arene **26** turned out to be effective inhibitors of PTP1B with IC_{50} values in the micromolar to nanomolar concentration range depending on the scaffold type. The inhibition effects towards PTPases increased from calix[4]arene **23** to thiacalix[4]arene **24** and sulfonylcalix[4]arene **25**, however, the selectivity of inhibition of PTP1B decreased. Thiacalix[4]arene and sulfonylcalix[4]arene phosphinic acids **24** and **25** were competitive inhibitors of PTP1B with K_i values of 0.14 μM and 0.032 μM. Modeling data revealed that the compounds can occupy the active site region of the open conformation of PTP1B forming many hydrogen bonds [51].

Considering the results obtained by the studies of calix[4]arene phosphonic acids as inhibitors of PTPases, it is worth emphasizing the role of macrocyclic scaffold for obtaining selectivity for individual enzymes such as PTP1B. The structural factors of scaffolds responsible for selectivity to PTP1B can be illustrated by the comparison of calix[4]arene, thiacalix[4]arene, and sulfonylcalix[4]arene derivatives [45, 51]. At the same time, some anionic fullerene derivatives considered a new class of protein tyrosine phosphatase inhibitors are potent inhibitors of CD45 showing IC_{50} values in the low micromolar to the high nanomolar range and also exhibiting less inhibitory activities against PTP1B [52]. α,α-Difluoro-β-ketophosphonate derivatives of tetraazamacrocycles were found to be potential inhibitors of T-cell protein tyrosine phosphatase with IC_{50} values in micromolar to nanomolar range and selectivity over PTP1B, CD45, SHP2, and PTPβ [15].

Inhibition of nucleotide pyrophosphatase/phosphodiesterase 1

Thiacalix[4]arene and calix[4]arene *tetrakis*-methylphosphonic acids **7** and **8** (Figure 3) were found to inhibit nucleotide pyrophosphatase/phosphodiesterase 1 (NPP/PDE1). This enzyme hydrolyzes a wide range of phosphodiester bonds being involved in various biological processes such as bone mineralization or cancer cell proliferation [53]. The inhibition constants of compounds **7** and **8** as inhibitors of NPP/PDE1 were in the low micromolar range [54]. However, the influence of these inhibitors on the enzyme activity was sufficiently lower than their effects on alkaline phosphatase from bovine intestinal mucosa. At the same time, methylphosphonic acid derivative **17** bearing sulfonylcalix[4]arene skeleton (Figure 8) demonstrated inhibitory activity only against NPP/PDE1 and did not influence the activity of alkaline phosphatases. These results indicated that the selectivity of sulfonylcalix[4]arene derivative **17** as an inhibitor of NPP1 can be attributed to the modified macrocyclic scaffold. The inhibition of NPP/PDE1 by sulfonylcalix[4]arene *tetrakis*-methylphosphonic acids **17** was found to be of the competitive type with a K_i value of 0.39 μM. *Tetrakis-tert*-butyl sulfonylcalix[4]arene **18** showed a low inhibition effect on NPP/PDE1, while *tetrakis*-3-fluoromethylacetamide sulfonylcalix[4]arene **19** had an IC_{50} value of 0.2 μM with selectivity over alkaline phosphatases.

Glutathione *S*-transferase as a target for calix[4]arene-based phosphonic acids

Glutathione *S*-transferases (GSTases) belong to the phase II detoxification system, catalyzing the conjugation of glutathione with a variety of endogenous and exogenous electrophilic compounds, including chemotherapeutic

drugs. Overexpression of GSTases can be observed in cancer cells [55].

Calix[4]arene *α*-hydroxymethylphosphonic acids **26**-**28** (Figure 10) were found to be inhibitors of GSTases showing different effects on the enzymes from equine liver and human placenta. These compounds as a mixture of stereoisomers or *meso*-form for *bis*-substituted derivative **27** can be obtained by the reaction of formylcalix[4]arenes with triisopropyl phosphite or diisopropylphosphite sodium salt, and further treatment of alkylphosphonate esters by trimethylbromosilane and methanol [24]. The inhibition activities of calix[4]arene *α*-hydroxymethylphosphonic acids against GSTases, especially in the case of GST from equine liver, were higher than those of corresponding methylenebisphosphonic or *α*-aminophosphonic acids [24, 56].

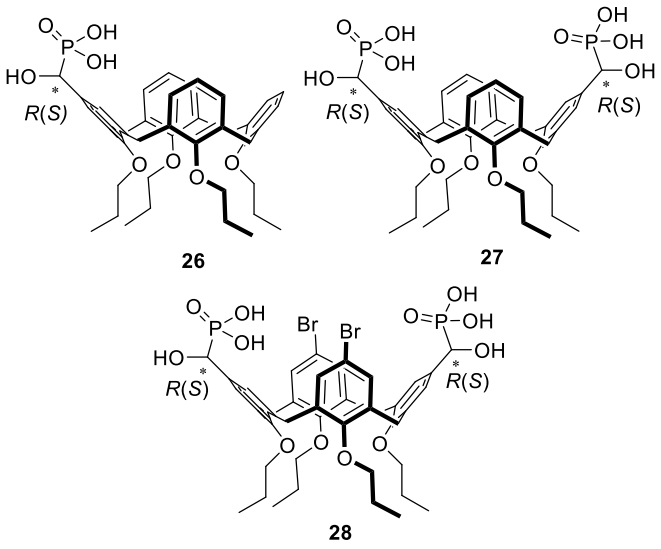


Figure 10. Calix[4]arene α-hydroxymethylphosphonic acids **26**-**28**.

Calix[4]arene-, thiacalix[4]arene-, and sulfonylcalix[4]arene-based phosphinic acids **23**-**25** turned out to be more effective inhibitors of the GST from equine liver and human recombinant GSTA1-1 than calix[4]arene *α*-hydroxymethylphosphonic acids **26**-**28**. At the same time, the inhibitory effects of these compounds were negligible for GST from the human placenta and GSTP1-1. The thiacalix[4]arene phosphinic acid inhibitor **24** that showed IC_{50} values of 0.085μM and 0.05 μM for GST from equine liver and GSTA1-1, respectively, was one order magnitude better than calix[4]arene or sulfonylcalix[4]arene derivatives. Docking results suggest that the thiacalix[4]arene being noncompetitive inhibitor of the GSTs can bind at the bottom of a V-shaped cleft formed between subunits of the homodimer structure of the enzyme [57].

It is known that peptides containing the benzoylphosphonate group were found to exhibit photoactive inhibitory effects towards PTP1B, MptpA, and STAT5b [58, 59]. The photoactive effects of low-molecular alkyl and aryl *α*-ketophosphonates were also observed in the case of GSTases [60]. Assuming that the UV-induced inhibition by the *α*-ketophosphonic acid group can be improved by macrocyclic scaffold, the calix[4]arene *α*-ketophosphonic acids **15**, **16**, **29**-**32**, and diethyl ester **33** (Figures 7 and 11) were studied as light-activated irreversible inhibitors of GSTases [61].

The *α*-ketophosphonic acid fragments were inserted at the calix[4]arene upper rim by the interaction of corresponding dialkoxycalix[4]arene acyl chlorides with triethylphosphite in the Arbusov reaction condition. The mono- or *bis*-substituted calix[4]arene ethyl *α*-ketophosphonates gave corresponding acids **15**, **16**, **29**-**31** by treatment with trimethylbromosilane and methanol. In the case of compound **32** one of the *α*-ketophosphonate substituents of calix[4]arene was transformed into *α*-hydroxymethylene bisphosphonate group using triethylphosphite in the presence of pyridinium perchlorate [44, 61].

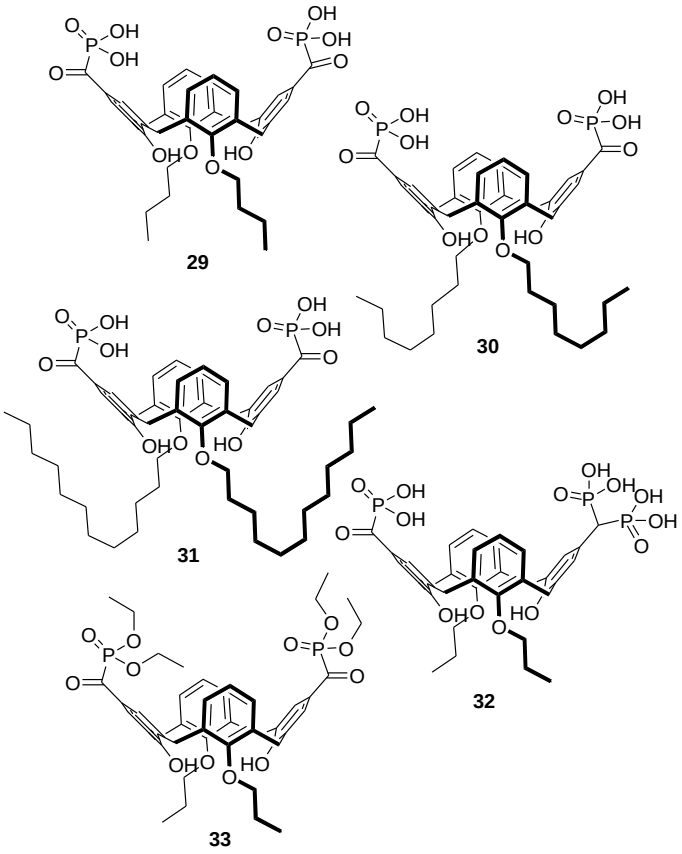


Figure 11. Calix[4]arene α-ketophosphonic acids **29**-**32** and tetraethyl calix[4]arene α-ketophosphonate **33**.

Without irradiation, the *α*-ketophosphonic acids **15**, **16**, and **29**-**32** demonstrated low to high inhibitory potency against GST from equine liver and GSTA1-1. Lengthening of alkyl substituents of calix[4]arene scaffold to *n*-octyl and *n*-dodecyl (compounds **30** and **31**) increased their inhibitory activity against GST from equine liver and GSTA1-1, providing micromolar IC_{50} values. At the same time, these calix[4]arene derivatives were devoid of such activity against GST from human placenta and GSTP1-1. Under UV irradiation at 365 nm, the inhibitory properties of some of the calix[4]arenes were significantly improved, showing IC_{50} values in the low micromolar range. Inactivation of the enzymes was described by pseudo-first-order rate constants. The calculated second-order rate constants indicated that the photoactivated inhibitors were more effective towards GST

from human placenta than GST from equine liver. The most potent UV-activated inhibitor was compound **16** with IC_{50} of 0.21 μM. The second-order rate constant for this calix[4]arene is up to 10 times higher than that for other derivatives. It was suggested that the mechanism of the inactivation by photoactive calix[4]arenes includes formation of reversible complex with the enzyme followed by irreversible radical transformation of some of catalytically important amino acid residues [61].

Interaction with proteins

Molecular interactions between the calix[4]arene phosphonic acids and human serum albumin (HSA) influence the possible mechanisms of their bioavailability. Using the fluorescence-quenching method, the albumin-binding properties of thiacalix[4]arene *tetrakis*-methyl-phosphonic acid **7** and its phosphonate monoester **20** (Figures 3 and 8) were studied [48]. HSA is the most abundant protein, responsible for drug delivery and transport of metabolites and other molecules [62]. The results obtained indicate that fluorescence quenching of HSA by the thiacalix[4]arene derivatives **7** and **20** can be initiated by complex formation rather than by dynamic collision. Double logarithmic Stern-Volmer plot allowed determining stoichiometry and apparent binding constants of the ligands to HSA. According to this, the binding capacity (at least one binding site) of thiacalix[4]arene *tetrakis*-methylphosphonic acid **7** to HSA was enhanced with the increasing temperature. In the case of monoester derivative **20**, the values of K_b and n decreased with increasing temperature, indicating the reduction of the stability of the ligand-protein complex. Such differences between the binding modes may be explained by the nature of binding forces playing a major role in the binding process. According to the ΔG values calculated from van't Hoff equation, the binding of compounds **7** and **20** to HSA is a spontaneous process. The hydrophobic interaction forces might play a major role in the interaction of free acid **7** with the protein as confirmed by positive values of ΔS and ΔH. In the case of monoester derivative **20**, negative values of ΔH and ΔS suggest that hydrogen bonds and van der Waals forces are dominant.

It was previously established that water-soluble anionic calix[n]arenes with sulfo groups exhibit anti-thrombotic activity [63]. According to the results of other researches, the phosphorylated calix[4]arene derivatives turned out to be inhibitors of fibrin polymerization [64]. The influence of calix[4]arene *tetrakis*-methylenebisphosphonic acid **11** (Figure 5) [65, 66] led to a decrease in the maximum rate in the fibrinogen–thrombin reaction with IC_{50} value of 0.52 μM at a molar ratio of compound to starting fibrinogen of 1.7 : 1. The IC_{50} value of 1.26 μM was obtained in case of desAABB fibrin polymerization at a molar ratio of compound **11** to fibrin monomer of 4 : 1. In human blood plasma, the calixarene *tetrakis*-methylenebisphosphonic acid **11** increased both the prothrombin time and the activated partial thromboplastin time. Electron microscopy confirmed that compound **11** inhibits the formation of protofibrils in the first stage of fibrin polymerization. At the same time, calix[4]arene *bis*-methylenebisphosphonic acid **2** (Figure 1) inhibited fibrin desAABB polymerization with an IC_{50} of 131 μM. This data showed that methylenebisphosphonic acid derivative **11** can be a specific inhibitor of fibrin polymerization and blood coagulation and may be useful for designing new antithrombotic agents.

Recently, the inhibition of nucleocapsid protein chaperone activity by calixarene phosphonic acids have been described [67]. The nucleocapsid protein is a highly conserved protein that plays key roles in HIV-1 replication and is a promising target for antiviral therapy. The phosphonate derivatives of thiacalix[4]arene were found to be able to compete with nucleic acids for the binding to nucleocapsid protein. In addition, the thiacalix[4]arene *tetrakis*-methylphosphonate (tetrasodium salt of compound **7**; Figure 3) at low micromolar concentrations inhibited in cells the infectivity of wild-type and drug-resistant HIV-1 strains, primarily targeting the early steps of HIV-1 replication. It was shown also that the thiacalix[4]arene **7** can inhibit the flipping and polymerization activity of reverse transcriptase. Thus the calix[4]arene phosphonic acids can represent a new class of nucleocapsid protein inhibitors with multitarget antiviral activity.

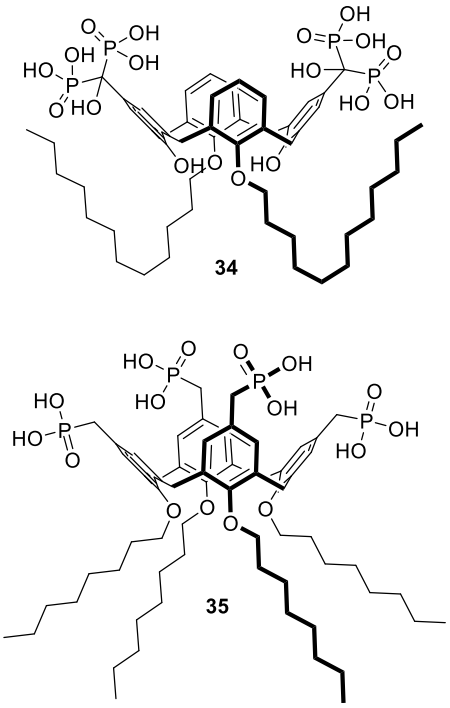


Figure 12. Amphiphilic calix[4]arene derivatives **34** and **35**.

Newly synthesized calixarenes **34** and **35** (Figure 12) bearing four phosphonate groups at the upper rim and two or four long alkyl chains at the lower rim of the macrocycle were found to complex with oligo-lysine and biologically relevant HIV-1 nucleocapsid peptide forming small nanoparticles (20-40 nm). At the same time, anionic calixarene with short alkyl chains (compound **13**; Figure 6) did not form small nanoparticles with peptides, highlighting the importance of micellar assembly of the amphiphilic macrocycles for peptide complexation. Thus anionic

amphiphilic macrocycles can be considered as promising building blocks for the preparation of peptide delivery vehicles [68].

Conclusions

The main works in the literature on the bioactivity of calix[4]arenes were carried out during the last 25 years. These studies became possible due to the development of new synthetic methods for obtaining the various functionalized macrocycles. A series of the new derivatives carrying a calix[4]arene scaffold were synthesized for assays of their biological properties. These investigations afforded new information about calix[4]arenes which demonstrated activity *in vitro*. However, many efforts are still required to identify the advantages of the calix[4]arenes in comparison with known agents with the same activity, including investigation of efficacy, toxicity, resistance, and other biomedical profiles. Further studies should also be directed to elucidate the mechanisms of action for compounds of this class.

The studies of calix[4]arene phosphonic acids are quite attractive since their phosphonate motif as a phosphate bioisoster in the structure of these compounds can regulate the enzymatic phosphorylation and the cellular signaling pathways. The examples of the inhibitory activity of the calix[4]arene phosphonic acids against the alkaline phosphatases, ATPases, protein tyrosine phosphatases, and nucleotide pyrophosphatase phosphodiesterase, which are represented in this review, can only outline some targets in a wide list of protein structures with potential affinity to phosphorylated macrocycles. Some other enzymes such as glutathione *S*-transferase and proteins were also considered here. It can be suggested that many new possible targets will be identified soon. All these data can be used as a tool for a more detailed study of the physiological role of the calix[4]arene phosphonic acids to search for compounds with selective action in cells. However, this also requires the development of new approaches to the synthesis of the diverse derivatives of phosphorus-containing calix[4]arenes. These synthetic approaches can include modification of macrocyclic scaffold, separation of stereoisomers in case of molecules functionalized with chiral substituents or inherently chiral calix[4]arenes, and introduction of other structurally motivated functional fragments in addition to phosphonate ones.

Notes

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The authors declare no conflict of interest.

References

1. *Calixarenes and beyond*, Neri, P., Sessler, J.L., Wang M.-X., Eds.; Springer: Switzerland, **2016**.
2. Pan, Y.-C.; Hu, X.-Y.; Guo, D.-S. Biomedical applications of calixarenes: state of the art and perspectives. *Angew. Chem. Int. Ed.* **2021**, *60*, 2768-2794.
3. Tauran, Y.; W. Coleman, A.; Perret, F.; Kim, B. Cellular and in vivo biological activities of the calix[n]arenes. *Curr. Org. Chem.* **2015**, *19*, 2250-2270.
4. Naseer, M.M.; Ahmed, M.; Hameed, S. Functionalized calix[4]-arenes as potential therapeutic agents. *Chem. Biol. Drug Des.* **2017**, *89*, 243-256.
5. Shurpik, D.N.; Padnya, P.L.; Stoikov, I.I.; Cragg, P.J. Antimicrobial activity of calixarenes and related macrocycles. *Molecules*. **2020**, *25*, 5145.
6. Fang, S.; Dang, Y.-Y.; Li, H.; Li, H.; Liu, J.; Zhong, R.; Chen, Y.; Liu, S.; Lin, S. Membrane-active antibacterial agents based on calix[4]arene derivatives: synthesis and biological evaluation. *Front. Chem.* **2022**, *10*, 816741.
7. Isik, A.; Oguz, M.; Kocak, A.; Yilmaz, M. Calixarenes: recent progress in supramolecular chemistry for application in cancer therapy. *J. Inc. Phenom. Macrocycl. Chem.* **2022**, *102*, 439-449.
8. Komisarenko, S.V.; Kosterin, S.O.; Lugovskoy, E.V.; Cherenok, S.O.; Tanchuk, V.Y.; Vovk, A.I.; Kalchenko, V.I. Calix[4]arene methylene bisphosphonic acids as effectors of biochemical processes. In *Ligands: synthesis, characterization and role in biotechnology*; Gawryszewska, P. and Smolenski, P., Eds.; Noza Science Publishers, Inc.: New York, **2014**, 67-116.
9. Rodik R.V.; Boyko V.I.; Kalchenko V.I. Calixarenes in biotech-nology and bio-medical researches. In *Frontiers in Medicinal Chemistry*; Rahman, A., Choudhary, M I., Reitz, A.B., Eds.; Vol. 8; Benthame Books, **2016**, 206-301.
10. Makukhin, N.; Ciulli, A. Recent advances in synthetic and medicinal chemistry of phosphotyrosine and phosphonate-based phosphotyrosine analogues. *RSC Med. Chem.* **2021**, *12*, 8-23.
11. Downey, A.M.; Cairo, C.W. Synthesis of α-brominated phosphonates and their application as phosphate bioisosteres. *Med. Chem. Comm.* **2014**, *5*, 1619-1633.
12. Kafarski, P. Phosphonopeptides containing free phosphonic groups: recent advances. *RSC Adv.* **2020**, *10*, 25898-25910.
13. Groaz, E.; De Jonghe, S. Overview of biologically active nucleoside phosphonates. *Front. Chemi.* **2021**, *8*, 616863.
14. Shevchuk, M.; Wang, Q.; Pajkert, R.; Xu, J.; Mei, H.; Röschenthaler, G.-V., Han, J. Recent advances in synthesis of difluoromethylene phosphonates for biological applications. *Adv. Synth. Catal.* **2021**, *363*, 2912-2968.
15. Kobzar, O.L.; Shevchuk, M.V.; Lyashenko, A.N.; Tanchuk, V.Yu.; Romanenko, V.D.; Kobelev, S.M.; Averin, A.D.; Beletskaya, I.P.; Vovk, A.I.; Kukhar, V.P. Phosphonate derivatives of tetraaza-macrocycles as new inhibitors of protein tyrosine phosphatases. *Org. Biomol. Chem*. **2015**, *13*, 7437-7444.
16. Al-Rashida, M.; Iqbal, J. Inhibition of alkaline phosphatase: an emerging new drug target. *Mini Rev. Med. Chem.* **2015**, *15*, 41-51.
17. Zaher, D.M.; El-Gamal, M.I.; Omar, H.A.; Aljareh, S.N.; Al-Shamma, S.A.; Ali, A J.; Zaib, S.; Iqbal, J. Recent advances with alkaline phosphatase isoenzymes and their inhibitors. *Arch. Pharm.* **2020**, *353*, e2000011
18. Vovk, A.I.; Kalchenko, V.I.; Cherenok, S.A.; Kukhar, V.P.; Muzychka, O.V.; Lozynsky, M.O. Calix[4]arene methylene-bisphosphonic acids as claf intestine alkaline phosphatase inhibitor. *Org. Biomol. Chem*. **2004**, *2*, 3162-3166.
19. Muzychka, O.V.; Tanchuk, V.Yu.; Vovk, A.I.; Muravyova, I.V.; Kukhar, V.P.; Cherenok, S.A.; Kalchenko, V.I. Calix[4]arene methylenebisphosphonic acids: the features of alkaline phosphatases inhibition. *Ukr. Biokhim. Zh.* **2007**, *79*, 26-33 (*In Ukrainian*).
20. Cherenok, S.; Vovk, A.; Muravyova, I.; Shivanyuk, A.; Kukhar, V.; Lipkowski, J.; Kalchenko, V. Calix[4]arene α-aminophosphonic acids: asymmetric synthesis and enantioselective inhibition of an alkaline phosphatase. *Org. Lett*. **2006**, *8*, 549-552.
21. Vovk, A.I.; Kononets, L.A.; Tanchuk, V.Yu.; Drapailo, A.B.; Kalchenko, V.I.; Kukhar, V.P. Thiacalix[4]arene as molecular platform for design of alkaline phosphatase inhibitors. *J. Incl. Phenom. Macrocycl. Chem.*, **2010**, *66*, 271-277.
22. Yoneda, J.S.; Sebinelli, H.G.; Itri, R.; Ciancaglini, P. Overview on solubilization and lipid reconstitution of Na,K-ATPase: enzyme kinetic and biophysical characterization. *Biophys. Rev.* **2020**, *12*, 49-64.
23. Veklich, T.O.; Kosterin, S.O.; Rodik, R.V.; Cherenok, S.O.; Boĭko, V.I.; Kal'chenko, V.I. Effect of calixarene-phosphonic acid on Na^+, K^+-ATPase activity in plasma membranes of the smooth-muscle cells. *Ukr. Biokhim. Zh.* **2006**, *78*, 70-86 (*In Ukrainian*).

24. Cherenok, S.O.; Yushchenko, O.A.; Tanchuk, V.Yu.; Mischenko, I.M.; Samus, N.V.; Ruban, O.V.; Matvieiev, Y.I.; Karpenko, J.I.; Kukhar, V.P.; Vovk, A.I.; Kalchenko, V.I. Calix[4]arene-α-hydroxyphosphonic acids. Synthesis, stereochemistry, and inhibition of glutathione *S*-transferase. *Arkivoc* **2012**, *4*, 278-298.
25. Bevza A.A.; Labyntseva R.D.; Rodik R.V.; Cherenok S.O.; Kosterin S.O.; Kal'chenko V.I. Effect of calix[4]arenes on the activity of actomyosin ATPase and actomyosin subfragment-1 ATPase from the myometrium. *Ukr. Biokhim. Zh.* **2009**, *81*, 49-58 (*In Ukrainian*).
26. Veklich, T.O.; Labyntseva, R.D.; Shkrabak, O.A.; Tsymbalyuk, O.V., Rodik, R.V., Kalchenko, V.I.; Kosterin, S.O. Inhibition of Na^+,K^+-ATPase and activation of myosin ATPase by calix[4]arene C-107 cause stimulation of isolated smooth muscle contractile activity. *Ukr. Biochem. J.* **2020**, *92*, 21-30.
27. Mazur, Iu.I.; Veklich, T.O.; Shkrabak, O.A.; Mohart, M.A.; Demchenko, A.M.; Gerashchenko, I.V.; Rodik, R.V.; Boyko, V.I.; Kalchenko, V.I.; Kosterin, S.O. Selective inhibition of smooth muscle plasma membrane transport Ca^{2+}, Mg^{2+}-ATPase by calixarene C-90 and its activation by IPT-35 compound. *Gen. Physiol. Biophys*. **2018**, 37, 223-231.
28. Shkrabak, O.A.; Veklich, T.O.; Rodik, R.V.; Kalchenko, V.I.; Kosterin, S.O. Inhibition of plasma membrane Ca^{2+},Mg^{2+}-ATPase by calixarene sulfonylamidines. Structure-activity relationship. *Ukr. Biochem. J.* **2022**, *94*, 18-35.
29. Vovk, A.I.; Kononets, L.A.; Tanchuk, V.Yu.; Cherenok, S.O.; Drapailo, A.B.; Kalchenko, V.I.; Kukhar, V.P. Inhibition of Yersinia protein tyrosine phosphatase by phosphonate derivatives of calixarenes. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 483-487.
30. Fällman, M.; Deleuil, F.; McGee, K. Resistance to phagocytosis by *Yersinia*. *Int. J. Med. Microbiol.* **2001**, *291*, 501-509.
31. Koren, S.; Fantus, I. G. Inhibition of the protein tyrosine phosphatase PTP1B: potential therapy for obesity, insulin resistance and type-2 diabetes mellitus. *Best Pract. Res. Clin. Endocrinol. Metab.* **2007**, *21*, 621-640.
32. Singh, S.; Grewal, A.S.; Grover, R.; Sharma, N.; Chopra, B.; Dhingra, A.K.; Arora, S.; Redhu, S.; Lather, V. Recent updates on development of protein-tyrosine phosphatase 1B inhibitors for treatment of diabetes, obesity and related disorders. *Bioorg. Chem.* **2022**, *121*, 105626.
33. Iversen, L.F.; Møller, K.B.; Pedersen, A.K.; Peters, G.H.; Petersen, A.S.; Andersen, H.S.; Branner, S.; Mortensen, S.B.; Møller, N.P.H. Structure determination of T cell protein-tyrosine phosphatase. *J. Biol. Chem.* **2002**, *277*, 19982-19990.
34. Heinonen, K.M.; Nestel, F.P.; Newell, E.W.; Charette, G.; Seemayer, T.A.; Tremblay, M.L.; Lapp, W.S. T-cell protein tyrosine phosphatase deletion results in progressive systemic inflammatory disease. *Blood* **2004**, *103*, 3457-3464.
35. Cho, C.Y.; Koo, S.-H.; Wang, Y.; Callaway, S.; Hedrick, S.; Mak, P.A.; Orth, A.P.; Peters, E.C.; Saez, E.; Montminy, M.; Schultz P.G. Identification of the tyrosine phosphatase PTP-MEG2 as an antagonist of hepatic insulin signaling. *Cell Metab.* **2006**, *3*, 367-378.
36. Zhang, S.; Liu, S.; Tao, R.; Wei, D.; Chen, L.; Shen, W.; Yu, Z.-H.; Wang, L.; Jones, D.R.; Dong, X.C.; Zhang, Z.-Y. A highly selective and potent PTP-MEG2 inhibitor with therapeutic potential for type 2 diabetes. *J. Am. Chem. Soc.* **2012**, *134*, 18116-18124.
37. Xu, M.-j.; Sui, X.; Zhao, R.; Dai, C.; Krantz, S.B.; Zhao, Z.J. PTP-MEG2 is activated in polycythemia vera erythroid progenitor cells and is required for growth and expansion of erythroid cells. *Blood* **2003**, *102*, 4354-4360.
38. Zheng, H.; Alter, S.; Qu, C.-K. SHP-2 tyrosine phosphatase in human diseases. *Int. J. Clin. Exp. Med.* **2009**, *2*, 17-25.
39. Tchilian, E.Z.; Beverley P.C.L. Altered CD45 expression and disease. *Trends Immunol.* **2006**, *27*, 146-153.
40. Campochiaro, P.A.; Peters K.G. Targeting Tie2 for treatment of diabetic retinopathy and diabetic macular edema. *Curr. Diab. Rep.* **2016**, *16*, 126.
41. Trush V.V.; Cherenok S.O.; Tanchuk V.Yu.; Kukhar V.P.; Kalchenko V.I.; Vovk A.I. Calix[4]arene methylenebisphosphonic acids as inhibitors of protein tyrosine phosphatase 1B. *Bioorg. Med. Chem. Lett*. **2013**, *23*, 5619-5623.
42. Trush V.V.; Tanchuk V.Y.; Cherenok S.O.; Kalchenko V.I.; Vovk A.I. Calix[4]arene α-hydroxymethylphosphonic acids as potential inhibitors of protein tyrosine phosphatase. *J. Org. Pharm. Chem.* **2014**, *12*, 39-42.
43. Frechette, R.F.; Ackerman, C.; Beers, S.; Look, R.; Moore, J. Novel hydroxyphosphonate inhibitors of CD-45 tyrosine phosphatase. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2169-2172.
44. Trush, V.; Cherenok, S.; Tanchuk, V.; Kalchenko, V.; Vovk, A. Evaluation of inhibition of protein tyrosine phosphatase 1B by calixarene-based α-ketophosphonic acids. *Chem. Biol. Lett*. **2015**, *2*, 1-5.
45. Buldenko, V.M.; Trush, V.V.; Kobzar, O.L.; Drapailo, A.B.; Vyshnevsky, S.G.; Kalchenko, V.I.; Vovk, A I. The assessment of sulfonilcalix[4]arene derivatives as inhibitors of protein tyrosine phosphatases. *J. Org. Pharm. Chem.* **2018**, *16*, 24-29.
46. Kharchenko, S.G.; Drapailo, A.B.; Kalchenko, O.I.; Yampolska, G.D.; Shishkina, S.V.; Shishkin, O.V.; Kalchenko, V I. Thia- and sulfonyl-calix[4]arene methylphosphonous acids: synthesis, structure, and amino acids binding. *Phosphorus Sulfur Silicon Relat. Elem.* **2013**, *188*, 243-248.
47. Buldenko, V.M.; Kobzar, O.L.; Trush, V.V.; Drapailo, A.B.; Kalchenko, V.I.; Vovk, A.I. Sulfonyl-bridged calix[4]arene as an inhibitor of protein tyrosine phosphatases. *Fr. Ukr. J. Chem.* **2017**, *5*, 144-151.
48. Trush, V.V.; Kharchenko, S.G.; Tanchuk, V.Yu.; Kalchenko, V.I.; Vovk, A.I. Phosphonate monoesters on thiacalix[4]arene framework as potential inhibitors of protein tyrosine phosphatase 1B. *Org. Biomol. Chem.* **2015**, *13*, 8803-8806.
49. Abdou, M.M.; O'Neill, P.M.; Amigues, E.; Matziari, M. Phosphinic acids: current status and potential for drug discovery. *Drug Discov.* **2019**, *24*, 916-929.
50. Vassiliou, S.; Pagoni, A.; Węglarz-Tomczak, E.; Talma, M.; Tabor, W.; Grabowiecka, A.; Berlicki, Ł.; Much, A. Phosphinic acid-based enzyme inhibitors. *Phosphorus Sulfur Silicon Relat. Elem.* **2022**, *197*, 451-456.
51. Buldenko, V.M.; Trush V.V.; Kobzar, O.L.; Drapailo, A.B.; Kalchenko, V.I.; Vovk, A.I. Calixarene-based phosphinic acids as inhibitors of protein tyrosine phosphatases. *Bioorg. Med. Chem. Lett*. **2019**, *29*, 797-801.
52. Kobzar, O.L.; Trush, V.V.; Tanchuk, V.Yu.; Zhilenkov, A.V.; Troshin, P.A.; Vovk, A.I. Fullerene derivatives as a new class of inhibitors of protein tyrosine phosphatases. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 3175-3179.
53. Lee, S.-Y., Müller, C.E. Nucleotide pyrophosphatase/phosphor-diesterase 1 (NPP1) and its inhibitors. *Med. Chem. Comm.* **2017**, *8*, 823-840.
54. Buldenko, V.M.; Kononets, L.A.; Kobzar, O.L.; Drapailo, A.B.; Vyshnevsky, S.G.; Kalchenko, V.I.; Vovk, A.I. The inhibitory potential of calixarenes against nucleotide pyrophosphatase/phosphordiesterase 1. *J. Org. Pharm. Chem.* **2017**, *15*, 41-47.
55. Hanna, P.E.; Anders, M.W. The mercapturic acid pathway. *Crit. Rev. Toxicol.* **2019**, *49*, 819-929.
56. Vovk, A.I.; Mischenko, I.M.; Cherenok, S.O.; Tanchuk, V.Yu.; Kalchenko, V.I.; Kukhar, V.P. Phosphorylated calix[4]arenes as inhibitors of glutathione S-transferase. *Phosphorus Sulfur Silicon Relat. Elem.* **2011**, *186*, 961-963.
57. Kobzar, O.L.; Shulha, Yu.V.; Buldenko, V.M.; Drapailo, A.B.; Kalchenko, V.I.; Vovk, A.I. Inhibition of glutathione *S*-transferases by calix[4]arene-based phosphinic acids. *Ukr. Bioorg. Acta* **2022**, *17*, 86-91.
58. Horatscheck, A.; Wagner, S.; Ortwein, J.; Kim, B.G.; Lisurek, M.; Beligny, S.; Shütz, A.; Rademann, J. Benzoylphosphonate-based photoactive phosphopeptide mimetics for modulation of protein tyrosine phosphatases and highly specific labeling of SH2 domains. *Angew. Chem. Int. Ed. Engl*. **2012**, *51*, 9441-9447.
59. Wagner, S.; Schütz, A.; Rademann, J. Light-switched inhibitors of protein tyrosine phosphatase PTP1B based on phosphonocarbonyl phenylalanine as phosphoactive phosphotyrosine mimetic. *Bioorg Med Chem*. **2015**, *23*, 2839-2847.
60. Kobzar, O.L.; Shulha, Yu.V.; Buldenko, V.M.; Mrug, G.P.; Kolotylo, M.V.; Stanko, O.V.; Onysko, P.P.; Vovk, A.I. Alkyl and aryl α-ketophosphonate derivatives as photoactive compounds targeting glutathione *S*-transferases. *Phosphorus Sulfur Silicon Relat. Elem.* **2021**, *196*, 672-678.
61. Kobzar, O.; Shulha, Yu.; Buldenko, V.; Cherenok, S.; Silenko, O.; Kalchenko, V.; Vovk, A. Inhibition of glutathione *S*-transferases by photoactive calix[4]arene α-ketophosphonic acids. *Bioorg. Med. Chem. Lett*. **2022**, *77*, 129019.
62. Fanali, G.; di Masi, A.; Trezza, V.; Marino, M.; Fasano, M.; Ascenzi, P. Human serum albumin: from bench to bedside. *Mol. Asp. Med.* 2**012**, *33*, 209-290.
63. Silva, E.D.; Ficheux, D.; Coleman, A.W. Anti-thrombotic activity of water-soluble calix[n]arenes. *J. Incl. Phenom. Macrocycl. Chem.* **2005**, *52*, 201-206.
64. Komisarenko, S.V.; Kosterin, S.O.; Lugovskoy, E.V.; Kalchenko, V.I. Calixarene methylene bisphosphonic acids as promising effectors of biochemical processes. *Ukr. Biochem. J.* **2013**, *85*, 106-128.

65. Lugovskoy, E.V.; Gritsenko, P.G.; Koshel, T.A.; Koliesnik, I.O.; Cherenok, S.O.; Kalchenko, O.I.; Kalchenko, V.I.; Komisarenko, S.V. Calix[4]arene methylenebisphosphonic acids as inhibitors of fibrin polymerization. *FEBS J.* **2011**, *278*, 1244-1251.
66. Cherenok, S.O.; Yuschenko, O.A.; Gritsenko, P.G.; Lugovskoy, E.V.; Koshel, T.A.; Chernishov, V.I.; Kalchenko, O.I., Komisarenko, S.V.; Kalchenko V.I. Synthesis of calixarenemethylenebisphosphonic acids and their influence on fibrin polymerization. *Phosphorus Sulfur Silicon Relat. Elem.* **2011**, *186*, 964-965.
67. Humbert, N.; Kovalenko, L.; Saladini, F.; Giannini, A.; Pires, M.; Botzanowski, T.; Cherenok, S.; Boudier, C.; Sharma, K.K.; Real, E.; Zaporozhets, O.A.; Cianférani, S.; Seguin-Devaux, C.; Poggialini, F.; Botta, M.; Zazzi, M.; Kalchenko, V.I.; Mori, M.; Mély, Y. (Thia)calixarenephosphonic acids as potent inhibitors of the nucleic acid chaperone activity of the HIV-1 nucleocapsid protein with a new binding mode and multitarget antiviral activity. *ACS Infect. Dis*. **2020**, *6*, 687-702.
68. Rodik, R.V.; Cherenok, S.O.; Postupalenko, V.Y.; Oncul, S.; Brusianska, V.; Borysko, P.; Kalchenko. V.I.; Mely, Y.; Klymchenko, A.S. Anionic amphiphilic calixarenes for peptide assembly and delivery. *J. Colloid Interface Sci*. **2022**, *624*, 270-278.

Біологічно активні каліксаренфосфонові кислоти

О.Л. Кобзар[1], С.О. Черенок[2], С.О. Костерін[3], В.І. Кальченко[2], А.І. Вовк[1]

[1] *Інститут біоорганічної хімії та нафтохімії ім. В.П. Кухаря Національної академії наук України, Київ, Україна*
[2] *Інститут органічної хімії Національної академії наук України, Київ, Україна*
[3] *Інститут біохімії ім. О.В. Палладіна Національної академії наук України, Київ, Україна*

Резюме: Відомо, що фосфорильовані похідні органічних сполук здатні інгібувати активність ензимів та інших протеїнів, відповідальних за перебіг ключових метаболічних перетворень. У цьому зв'язку каліксаренфосфонові кислоти є цікавими як макроциклічні агенти, які взаємодіють з мішенями, що можуть брати участь в патологічних клітинних процесах. У цьому огляді наведено літературні дані щодо синтезу та властивостей калікс[4]аренфосфонових кислот як інгібіторів лужних фосфатаз, протеїнтирозинфосфатаз, Na,K-АТФази, нуклеотидпіро-фосфатази/фосфодіестерази 1 та деяких інших ензимів і протеїнів. Також подано короткі відомості про інгібувальні властивості похідних калікс[4]арену, що містять залишки фосфінової кислоти або алкілфосфонатні групи.

Ключові слова: калікс[4]арен; фосфонові кислоти; ензими; протеїни; інгібування.