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The assessment of sulfonylcalix[4]arene derivatives as inhibitors of protein tyrosine phosphatases

Aim. To compare sulfonylcalix[4]arene derivatives containing ionizable or non-ionizable substituents at the upper rim of the macrocycle as inhibitors of protein tyrosine phosphatase 1B (PTP1B) and other PTPs.

Results and discussion. The properties of sulfonylcalix[4]arene with four phosphonic acid groups introduced at the upper rim were compared with those of the macrocycles containing four non-ionizable *tert*-butyl or trifluoroacetamide functions. The sulfonylcalix[4]arene tetrakis-methylphosphonic acid was found to inhibit PTP1B with IC₅₀ value in the low-micromolar range without selectivity over other PTPs, such as TC-PTP, MEG1, MEG2, SHP2, and PTPβ. At the same time, modification of sulfonylcalix[4]arene with trifluoroacetamide substituents led to inhibition of PTP1B with IC₅₀ of 1.4 μM and 4- to 28 fold selectivity over the other PTPs. In order to understand the ability of inhibiting PTP1B by sulfonylcalix[4]arene with introduced trifluoroacetamide groups the molecular docking and molecular dynamic simulations were performed. The inhibition mechanism was discussed.

Experimental part. The activities of the test compounds *in vitro* were examined spectrophotometrically measuring the rate of hydrolysis of *p*-nitrophenyl phosphate as a substrate of PTPs. The molecular docking was performed by AutoDock Vina.

Conclusions. This study can start an approach to develop new inhibitors of PTPs by variations in the non-ionogenic substituents on the upper rim of sulfonylcalix[4]arene scaffold.

Key words: sulfonylcalix[4]arene; protein tyrosine phosphatase; inhibition; molecular docking; molecular dynamics

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Оцінка похідних сульфонікалікс[4]арену як інгібіторів протеїнотирозинфосфатаз

Мета роботи. Порівняння похідних сульфонікалікс[4]арену, які містять здатні та нездатні до іонізації залишки на верхньому вінці макроциклу, як інгібіторів РТР1В та інших протеїнотирозинфосфатаз.

Результати та їх обговорення. Властивості сульфонікалікс[4]арену з чотирма фосфонатними групами, приєднаними до верхнього вінця макроциклу, порівнювалися з властивостями сполук, які містять чотири *tert*-бутильні або трифторацетамідні замісники. Було встановлено, що сульфонікалікс[4]арен-тетракіс-метилфосфоновая кислота інгібує РТР1В зі значенням IC₅₀ в низькомікромольному діапазоні без селективності стосовно інших РТРаз, таких як TC-PTP, MEG1, MEG2, SHP2 та РТРβ. Модифікація сульфонікалікс[4]арену залишками трифторацетаміду забезпечила інгібування РТР1В зі значенням IC₅₀ 1,4 мкМ з 4-28-кратною селективністю відносно інших РТРаз. Для з'ясування інгібувальної здатності похідної сульфонікалікс[4]арену з трифторацетамідними замісниками відносно РТР1В було застосовано молекулярний докінг та моделювання методом молекулярної динаміки. Обговорюється можливий механізм інгібування.

Експериментальна частина. Активність сполук досліджували спектрофотометрично, вимірюючи швидкість ферментативного гідролізу *p*-нітрофенілфосфату як субстрату протеїнотирозинфосфатаз. Молекулярний докінг було виконано за допомогою AutoDock Vina.

Висновки. Це дослідження може бути основою нового підходу до розробки інгібіторів РТРаз шляхом модифікації верхнього вінця сульфонікалікс[4]аренового каркасу неіоногенними замісниками.

Ключові слова: сульфонікалікс[4]арен; протеїнотирозинфосфатаза; інгібування; молекулярний докінг; молекулярна динаміка

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Оценка производных сульфониликаликс[4]арена как ингибиторов протеинтирозинфосфатаз

Цель работы. Сравнение производных сульфониликаликс[4]арена, содержащих способные и не способные к ионизации остатки на верхнем ободе макроцикла, как ингибиторов РТР1В и других протеинтирозинфосфатаз.

Результаты и их обсуждение. Свойства сульфониликаликс[4]арена с четырьмя фосфонатными группами, закрепленными на верхнем ободе макроцикла, сравнивались со свойствами соединений, содержащих *tert*-бутильный или трифторацетамидный остатки. Было установлено, что сульфониликаликс[4]арен-тетракіс-метилфосфоновая кислота ингибирует РТР1В со значением IC₅₀ в низькомікромольном диапазоне без селективности по отношению к другим РТРазам, таким как TC-PTP, MEG1, MEG2, SHP2 и РТРβ. Модификация сульфониликаликс[4]арена остатками трифторацетаміда привела к ингибированию РТР1В со значением IC₅₀ 1,4 мкМ и 4-28-кратной селективности по отношению к другим РТРазам. Для выяснения ингибирующей способности сульфониликаликс[4]арена с трифторацетамідными заместителями относительно РТР1В были выполнены молекулярный докінг и моделирование методом молекулярной динаміки. Обсуждается возможный механизм ингибирования.

Экспериментальная часть. Активность соединений исследовали спектрофотометрически, измеряя скорость ферментативного гидролиза *p*-нітрофенілфосфата, используемого в качестве субстрата протеинтирозинфосфатаз. Молекулярный докінг был выполнен с помощью AutoDock Vina.

Выводы. Это исследование может быть основой нового подхода к разработке ингибиторов РТРаз путем модификации верхнего обода сульфониликаликс[4]аренового каркаса неіоногенными заместителями.

Ключевые слова: сульфониликаликс[4]арен; протеинтирозинфосфатаза; ингибирование; молекулярный докінг; молекулярная динамика

The addition or removal of a phosphoryl group in proteins is known to control their stability, and modulate the enzyme activity. Approximately 30 % of cellular proteins can be in a phosphorylated form [1]. Tyrosine phosphorylation is essential for many cellular processes, such as growth, differentiation, survival, cell communications, cell migration, and the immune response [2]. Aberrant tyrosine phosphorylation proved to be associated with human diseases, including cancers, diabetes, rheumatoid arthritis, and hypertension [3, 4]. During the last decade, the extensive data concern the activity of PTP1B leading to inhibition of insulin signaling, and the activity of SHP-2 as an oncogene and promoter of the growth factor signaling [2, 5]. In those instances where the PTPs activity is inappropriately high the inhibitors of these enzymes can be regarded as possible therapeutic agents. Although such agents are permanently studied, PTP-targeted drugs have not yet been available. It is all because the highly charged and highly conserved nature of the PTP active site. For this reason, potent inhibitors of PTPs tend to have a negatively charged group, which may limit their cell permeability and bioavailability.

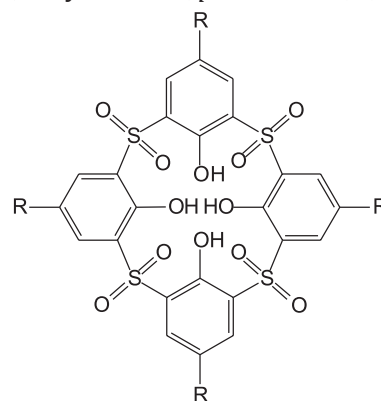
Such synthetic macrocycles as calixarenes have diverse applications in chemistry and biology [6-8] due to their unique conformational features and the possibility for modifications both at lower and upper rims [9, 10]. It has been shown that calix[4]arenes can be useful as a platform for designing bioactive compounds with the possibility of targeting enzymes and other proteins [11]. For instance, calix[4]arene derivatives with covalently bound phosphonic acid groups exhibit inhibitory effects towards PTP1B and some other PTPs [12]. We also observed that phosphonate monoesters on the thiacalix[4]arene framework were more effective and selective PTP1B inhibitor [13]. An unsubstituted sulfonylcalixarene containing four bridging SO_2 -groups turned out to be promising bioactive compound with the inhibitory activity towards protein tyrosine phosphatase MEG2 and selectivity over some other PTPs [14]. These data provided a basis for further investigation of sulfonylcalix[4]arene derivatives in searching for substances with desired biological properties. Our next efforts for designing the macrocyclic inhibitors of PTPs directed towards sulfonylcalixarenes contained: i) non-cleavable phosphonic acid groups, which mimicked the phosphorylated tyrosine fragment and ii) non-ionizable functions, such as *tert*-butyl or trifluoroacetamide groups, which could improve the cell permeability. The aim of this work was to compare the structural diverse sulfonylcalix[4]arene derivatives containing ionizable or non-ionizable substituents at the upper rim of the macrocycle as inhibitors of PTP1B and other PTPs.

Inhibitory activities of sulfonylcalix[4]arene derivatives **1-3** (Fig. 1) were assessed *in vitro* against PTP1B, T-cell protein tyrosine phosphatase (TC-PTP), Src homology-2 domain containing protein tyrosine phosphatase 2 (SHP2), megakaryocyte protein-tyrosine phosphatases MEG1 and MEG2, and PTP β . In the experiments, *p*-nitrophenyl phosphate was used as a substrate. The values of IC_{50} were calculated from dose-dependent curves as concentrations of the test compound, which decreased the enzyme activity to 50 %.

As seen from Tab. 1, sulfonylcalix[4]arene tetra-kismethylphosphonic acid **1** demonstrated approximately the same inhibition effects on the activities of all PTPs in the low micromolar range of IC_{50} values, and there was no selectivity for one over the other. Replacement of the phosphonic acid groups with *tert*-butyl substituents resulted in a dramatic decrease in the inhibitory effect of the less charged compound. The sulfonylcalix[4]arene **2** functionalized by the non-ionizable *tert*-butyl group was a weak inhibitor of PTP1B, MEG2, SHP2, and PTP β and did not affect the activity of TC-PTP, MEG1. At the same time, the modification of sulfonylcalix[4]arene with trifluoroacetamide substituents (compound **3**) provided the inhibition of PTP1B with IC_{50} value of 1.4 μM and 4- to 28 fold selectivity over the other PTPs. In this case, the selective inhibition of PTP1B can be attributed to the non-phosphonate upper rim substituents of sulfonylcalix[4]arene **3**.

According to the Lineweaver–Burk plots, the effect of inhibitors **3** on the activity of PTP1B was in agreement with a competitive type of inhibition (Fig. 2). The apparent K_i value was of 1.5 μM . The inhibition mechanism suggests that sulfonylcalix[4]arene derivative **3** bound by the enzyme at the substrate binding site.

The molecular modeling by AutoDock Vina was performed to get insights into the binding mechanism of compound **3** with PTP1B. Two groups of X-ray structures of PTP1B having the open or closed WPD-loop were used for the calculations. It is known that the calix[4]arene backbone exists in four different conformations, they are *cone*, *partial cone*, *1,2-alternate*,



R = $\text{CH}_2\text{PO}(\text{OH})_2$ (**1**); R = *tert*-But (**2**); R = $\text{NHC}(\text{O})\text{CF}_3$ (**3**)

Fig. 1. Chemical structures of sulfonylcalix[4]arene derivatives **1-3**

Table 1

Sulfonylcalix[4]arene derivatives **1-3** as inhibitors of protein tyrosine phosphatases*

| Inhibitor | PTP-1B | TC-PTP | MEG2 | MEG1 | SHP2 | PTP-β |
|-----------|-------------|------------|-------------|------------|-------------|------------|
| 1 | 0.17 ± 0.04 | 0.4 ± 0.01 | 0.23 ± 0.04 | 0.2 ± 0.04 | 0.35 ± 0.01 | 0.3 ± 0.04 |
| 2 | 8.13 ± 1.1 | n/i** | 18 ± 4.1 | n/i | 14 ± 5.9 | 11 ± 1.4 |
| 3 | 1.4 ± 0.26 | 9.6 ± 0.26 | 5.73 ± 0.5 | 40 ± 2 | 11 ± 4 | 38 ± 4 |

Notes: * – IC₅₀ values are the means of 2-3 assays ± standard deviations; ** – No inhibition was observed at 10 μM

and *1,3-alternate*, depending on the number and nature of substituents at the lower or upper rim [15]. The molecular docking of these four conformers of compound **3** to the active site of PTP1B (PDB codes were 1NL9, 1PH0, 1Q6M, 2CM8) showed that there were no sufficient differences in the docking energy calculated for the conformations with the open and closed WPD-loop (Tab. 2).

The docking results obtained for sulfonylcalix[4]arene-tetrakis-trifluoroacetamide **3** were further optimized by 1 ns molecular dynamics simulations to assess the complex stability. After the molecular docking operations, the most favorable PTP1B structure was chosen to be with the open WPD loop (PDB code 1NL9). The 1 ns MD simulation was carried out for PTP1B alone, as well as in complexes with compound **3** represented by four conformations. The root mean square deviation (RMSD) was calculated for backbone atoms of the crystal structure in order to monitor its structural changes. As shown in Fig. 3, the final RMSD values for all simulation trajectories were less than 1.5 Å, indicating that the enzyme structures had reached the equilibrium states with little alterations during the entire simulations. It should be noted that the cone conformation of compound **3** had a little higher alterations during the complexation with PTP1B sug-

gesting its lower preferability in comparison with three others. The analysis of the ligand positioning after 1 ns molecular dynamics simulation was followed by rescoring with Autodock Vina to find out the final free energies of binding, which were -8.25, -7.7, -9.34, and -6.91 kcal/mol for *cone*, *partial cone*, *1,2-alternate*, *1,3-alternate* conformers, respectively. This results suggested that the *1,2-alternate* conformation was the most preferable.

The root mean square fluctuations (RMSF) being a significant parameter to study the motion of the key residues interacted with the ligand were calculated for all amino acid residues of the PTP1B. The key amino acids residues that are important for the substrate and inhibitors binding to PTP1B belong to P-loop (His214-Arg221), WPD loop (Thr177-Pro185), the substrate recognition loop (Lys36, Val49, and Lys120), and the secondary binding site (Tyr20-Phe52, and Arg254) [16-18]. As compared to free protein, most of the residues of the enzyme-inhibitor complex had lower RMSF values less than 1.4 Å (Fig. 4). These data point that binding of compound **3** reduced the flexibility of the active site residues, thus leading to the stable complex.

The analysis of the complex of compound **3** with PTP1B (Fig. 5) showed that one of phenyl rings of the inhibitor was involved in the π-π interaction with Phe182 of the WPD-loop. There were hydrogen bonds between SO₂ groups of the sulfonylcalixarene skeleton and amino acid residues Lys116, Gly183, Arg221, Gln266, and Gln262. One OH group on the bottom rim of the macrocyclic scaffold formed hydrogen bonds with Gln266 and Thr263. An additional set of hydrogen bonds was provided by trifluoroacetamide functions and residues of Arg24 and Arg221. Two of the

Table 2

Free energy of binding of compound **3** into the active site of PTP1B

| Conformation of the ligand | ΔG (kcal/mol) | | | |
|----------------------------|---------------|------|------|------|
| | 1NL9 | 1PH0 | 1Q6M | 2CM8 |
| Cone | -8.6 | -8.5 | -8.1 | -6.3 |
| Partial cone | -7.2 | -7.5 | -7.7 | -7.4 |
| 1,2-Alternate | -7.4 | -7.4 | -7.4 | -5.7 |
| 1,3-Alternate | -7.2 | -6.7 | -6.9 | -6.1 |

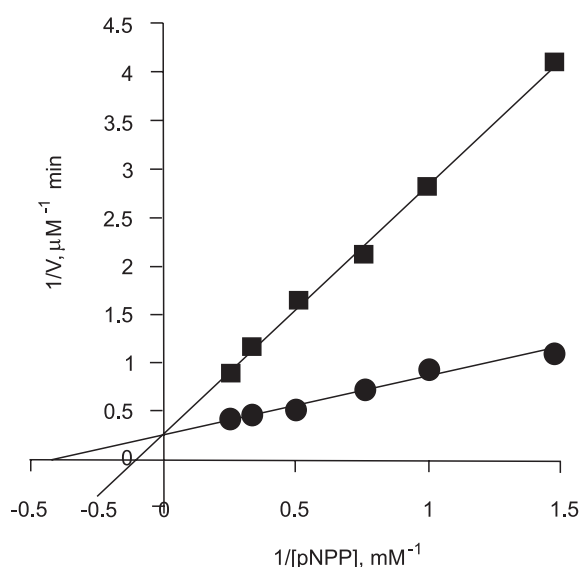


Fig. 2. The Lineweaver-Burk plot for inhibition of PTP1B by trifluoroacetamide derivative of sulfonylcalix[4]arene. The concentration of inhibitor **3** was 1.5 μM

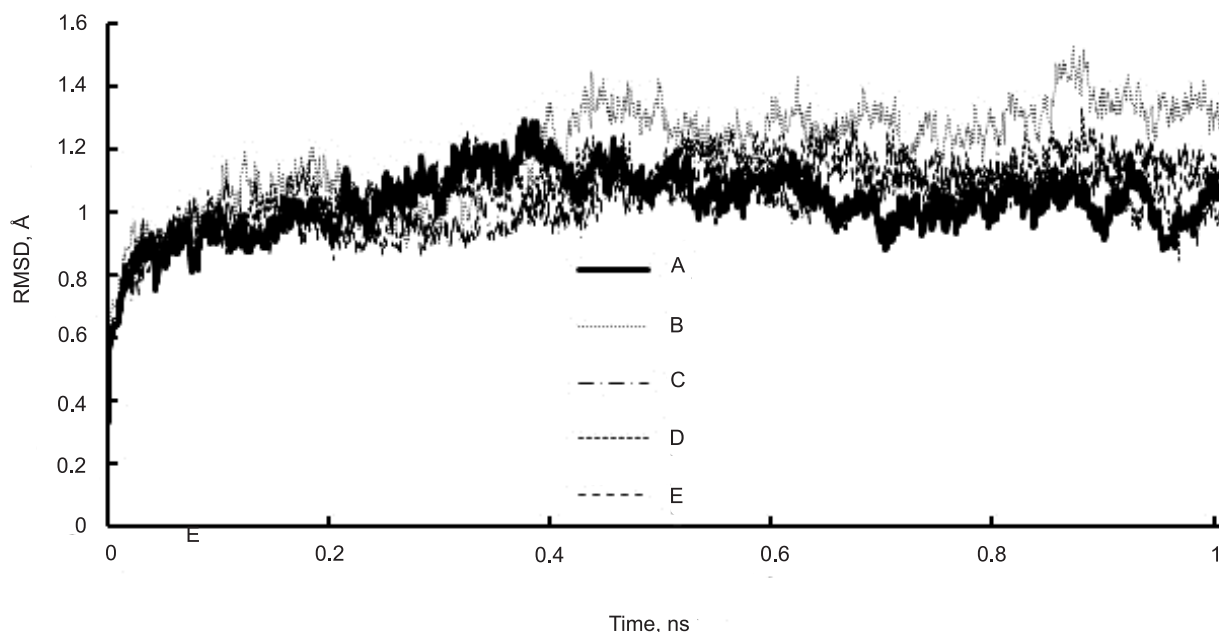


Fig. 3. The RMSD results of molecular dynamic simulations for PTP1B in complex with compound **3**. (A) Free enzyme; (B) PTP1B in complex with the inhibitor in the *cone* conformation; (C) PTP1B in complex with the inhibitor in the *partial cone* conformation; (D) PTP1B in complex with the inhibitor in the *1,2-alternate* conformation; (E) PTP1B in complex with the inhibitor in the *1,3-alternate* conformation

upper-rim substituents were directed to the region of the active site of PTP1B preventing the access of the substrate to Cys215.

Experimental section

Commercially available protein tyrosine phosphatases PTP1B, TC-PTP, MEG1, MEG2, SHP2, and PTP β were purchased from Sigma-Aldrich. Previously de-

veloped protocols were used for the synthesis of sulfonylcalix[4]arene tetrakis-methylphosphonic acid **1** [19], tetrakis-*tert*-butylsulfonylcalix[4]arene **2** [20, 21], sulfonylcalix[4]arene-tetrakis-trifluoroacetamide **3** [22].

The *in vitro* study of PTPs inhibition

The assay solution contained 50 mM Bis-Tris (pH 7.2), 1 vol % of dimethyl sulfoxide, 100 mM NaCl, 2mM DTT, 3 mM EDTA. Concentrations of *p*-nitrophenyl phos-

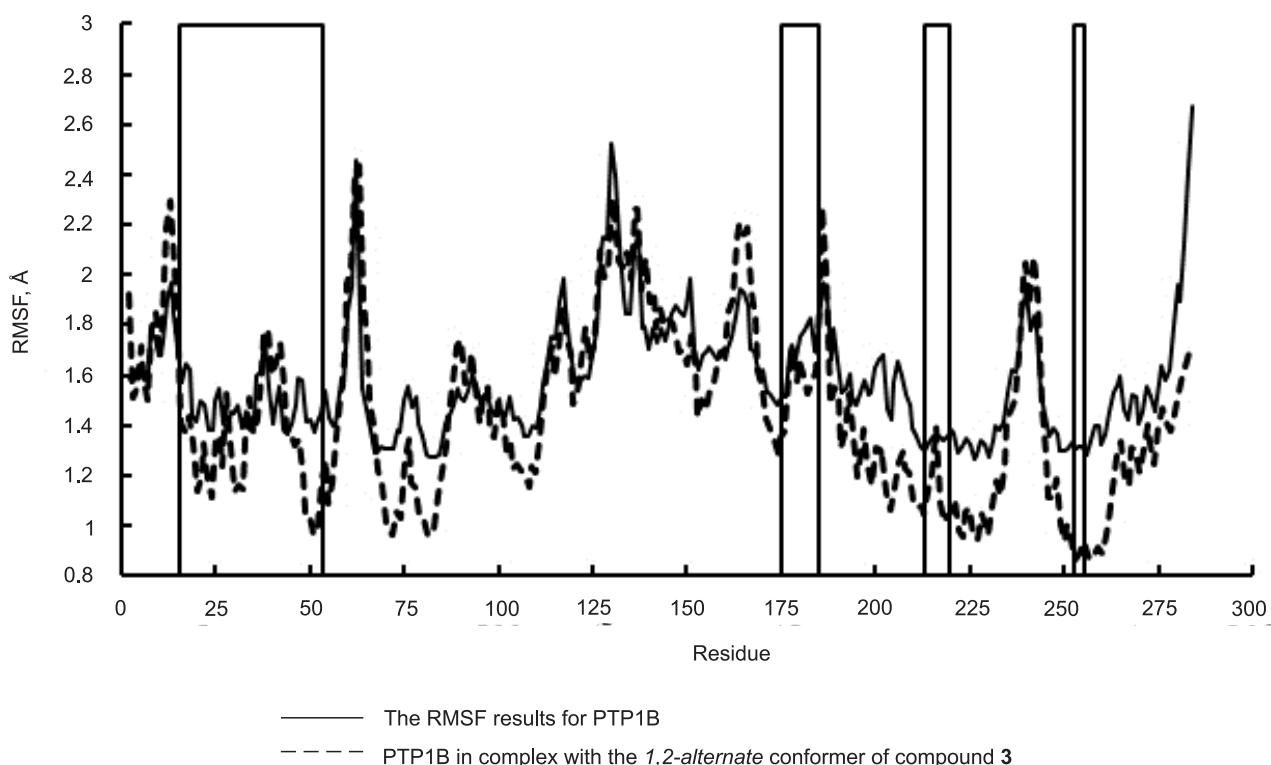


Fig. 4. The RMSF results for PTP1B and PTP1B in complex with the *1,2-alternate* conformer of compound **3**

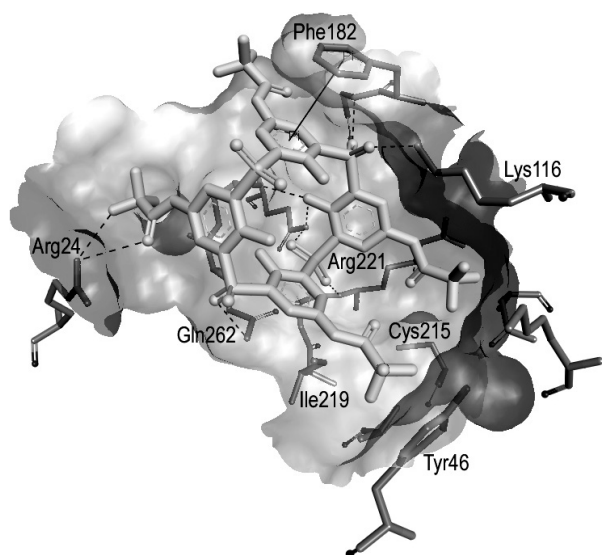


Fig. 5. The possible binding mode of compound **3** at the active site of PTP1B after MD

phate were of 2 mM for PTP1B, TC-PTP, 5 mM for MEG1, MEG2, 7 mM for SHP2, and 1 mM for PTP β corresponding to K_m values of the enzymes. The final volume of the assay solution was 0.5 ml. The mixture was thermostated at 25 °C (MEG1, MEG2), 37 °C (PTP1B) and 30 °C (TC-PTP, SHP2, PTP β) for 5 min, and the reaction was initiated by addition of the enzyme (4–8 nM in the reaction mixture). The *p*-nitrophenol released was determined by reading the absorbance at 410 nm ($\epsilon = 18.000 \text{ M}^{-1}\text{cm}^{-1}$).

Computer modeling

The molecular docking studies were performed by AutoDock Vina [23] program using X-ray crystals

of four centroids of PTP1B clusters (PDB codes: 1NL9, 1PHO, 1Q6M, 2CM8) [24]. The structures of four ligand conformers were prepared by program Avogadro and optimized in MMFF94s force field [25]. The conformers were docked to the active site region of PTP1B. The ligands presented and water molecules were removed from PDB-files before calculations.

Molecular dynamic simulations (MD) of the complexes was performed with NAMD 2.10 [26] using the CHARMM27 and the CHARMM36 force field. The topology parameters for all conformers of compound **3** were built using SwissParam server [27]. The preliminary preparation of the parameter files for PTP1B was performed by means of VND 1.9.2 [28]. MD was performed in an NPT ensemble using the Langevin thermostat and pressure control. Energy minimization was performed for 10 ps before the simulation, and the subsequent molecular dynamics for 100 ps was conducted at the temperature of 310 K and 1 atm pressure. One step of MD was 1 fs with preservation of trajectories for further analysis after every 50 fs.

Conclusions

Sulfonylcalix[4]arene can be used as a novel scaffold for designing PTPs inhibitors. The sulfonylcalix[4]arene-tetrakis-trifluoroacetamide was found to inhibit PTP1B with IC_{50} value in the micromolar range and selectivity over other PTPs, such as TC-PTP, MEG1, MEG2, SHP2, and PTP β . This approach provides a way to new PTP1B inhibitors functionalized by non-ionizable groups on the upper rim of the macrocycle.

Conflicts of Interests: authors have no conflict of interests to declare.

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