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Identification of lipid-like salicylic acid-based derivatives as potent and membrane-permeable PTP1B inhibitors

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ABSTRACT

Developing protein tyrosine phosphatase-1B (PTP1B) inhibitors is an important strategy to treat type 2 diabetes mellitus (T2DM). Most existing ionic PTP1B inhibitors aren't of clinical useful due to their low cell-permeability, however. Herein, we introduced a series of lipid-like acid-based (salicylic acid) modules to prepare PTP1B inhibitors, and demonstrated a marked improvement of cell-permeability while maintaining excellent PTP1B inhibitory activity (e.g. compound **B12D**, IC_{50} = 0.37 μM against PTP1B and P_{app} = 1.5 × 10^{-6} cm/s). We believe that this strategy can be widely utilized to modify potent lead compounds with low cell-permeability.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) plays a crucial role in attenuating insulin sensitivity by dephosphorylating the phosphor-tyrosine residues on the proteins of insulin signal pathway [1]. The PTP1B knockout mice demonstrated increased insulin sensitivity and obesity resistance compared to the wild-type [2]. Additionally, PTP1B inhibition was reported as a potential target for preventing lung metastasis [3] and treating Rett syndrome [4]. Therefore, there is a considerable significance in developing PTP1B inhibitors for associated diseases such as T2DM, obesity, breast cancer, and Rett syndrome [2,5–8].

Over the past few decades, numerous structure-based PTP1B inhibitors have been developed [9]. Nevertheless, phosphorylated tyrosine (pTyr) is a charged substrate; thus, typical PTP1B inhibitors (Fig. 1) that fit well into the protein's active pocket shall carry a charged pharmacophore to achieve adequate PTP1B inhibitory activity. However, the ionic pharmacophore makes these compounds challenging to cross the cell membrane [14], and unsuitable for oral administration.

To solve the membrane permeability issue, lately, many efforts have been made for the identification of new PTP1B inhibitors by applying an uncharged pharmacophore (Fig. 2). Nevertheless, these efforts often led to reduced PTP1B inhibitory activity.

In this study, we demonstrated a novel strategy to produce a group of mono- or bis-anionic amphipathic PTP1B inhibitors by designing and synthesizing a series of lipid-like salicylic acid-based derivatives. The corresponding similarity to the amphiphilic lipids makes these compounds capable of crossing the cell membrane adequately despite the anionic pharmacophore. The acid groups were used to bind to the catalytic site of the protein, and the alkane tails with various lengths were applied to generate further hydrophobic interactions. Thus, we could reveal a rational strategy to provide potent and permeable PTP1B inhibitors. These novel inhibitors could simulate the structure of amphiphilic lipids and demonstrate excellent PTP1B inhibitory activity with sufficient membrane permeability. This study suggests a new scenario for the optimization of potent lead PTP1B inhibitors with low cell permeability.

2. Results

2.1. The lead: The acid-based derivatives with an amphipathic core

Focusing on the cell membranes nature, the compound **A16S** [19] (Fig. 3), which is originally used as a liposome substance, attracted our attention for its cell membrane crossing abilities. We found out that it also possessed an inhibitory, however weak, activity against PTP1B (IC_{50} = 174 μM, P_{app} = 1.7 × 10^{-6} cm/s) which collectively could make **A16S** a good option as a lead core in our designing strategy. Docking simulation studies depicted that the salicylic acid group in **A16S** could bind to the catalytic site (A site [20], Fig. 3) of PTP1B, while the hexadecyl group formed multiple hydrophobic interactions with PTP1B. Despite the poor inhibitory activity, the structural

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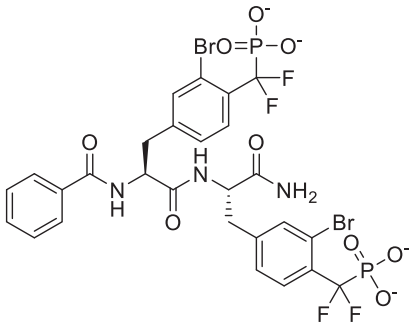
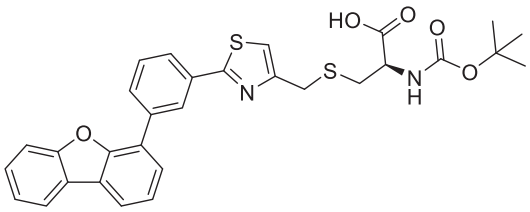


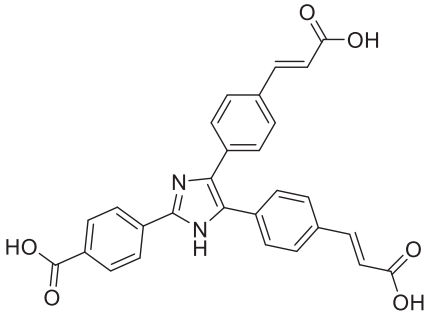
I

PTP1B K_i = 0.5 nM



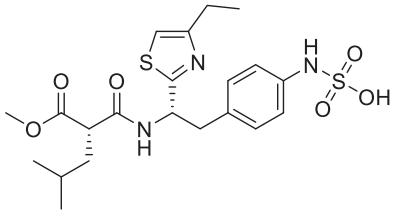
II

PTP1B IC_{50} = 1.08 nM



III

PTP1B IC_{50} = 72 nM



IV

PTP1B IC_{50} = 0.07 μM

Fig. 1. Representative anionic PTP1B inhibitors [10–13].

similarity to the cell membrane lipids inspired a path through identification and designing of a new group of lipid-like PTP1B inhibitors.

Utilizing the structure of **A16S**, we designed and synthesized a series of **A16S** homologs (Table 1) to investigate the structure-activity-relationships (SAR). These homologs which contained a single salicylic acid group and a hydrophobic tail, all exhibited weak inhibitory activity and even varying the hydrophobic tail could not exert much difference on the activity. Subsequently, the compound **A16D**, designed with two groups of salicylic acids on the core, demonstrated a graver inhibitory activity (Table 2). This finding led us to further delve into the SARs of bis-salicylic acid-based derivatives for the identification of more potent and membranophilic PTP1B inhibitors.

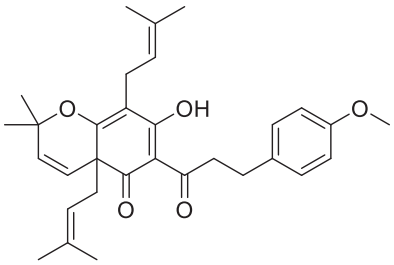
Briefly, synthetic strategies for both mono- or bis-salicylic acid-based PTP1B inhibitors were illustrated (Scheme 1). We initiated the synthesis by utilizing 2-hydroxy-4-methyl benzoic acid and proceeded through a series of synthetic sequences, including protection, brominating, alkylation, and deprotection. First, 2-hydroxy-4-methyl benzoic acid was converted to compound **2** by using acetone as a protecting group; then compound **2** was bromized by NBS to yield compound **3**. Compound **3** reacted with the corresponding amines and then deprotected by 1 N NaOH/ACN to yield mono and bis-salicylic acid-based inhibitors of PTP1B (**5** and **7** series). The ratio of compound **3** to amine is 1:1 and 2.5:1 for mono and bis-salicylic acid-based products, respectively.

2.2. Length of the tail: Essential benefit

Following the discovery of **A16D**, various bis-salicylic acid-based derivatives were designed and synthesized (Table 2). A short hydrophobic tail such as hexyl could not manifest enough PTP1B inhibitory activity (**A6D**). Then, mounting a longer hydrophobic tail such as dodecyl or hexadecyl demonstrated a vast improvement in the inhibitory activity by 2- and 7-time respectively **(A12D, A16D)**. However, the tail elongation was limit-dependent; for example, **A18D** with an octadecyl tail (the most extended hydrophobic tail) demonstrated lower inhibitory activity than **A12D** or **A16D**. We found that the replacement of four linear carbon atoms with a benzene ring could rectify the PTP1B inhibitory activity (**A12D/B8D**). Following, increasing the alkane chain length on the benzene ring from C2 to C6 doubled the inhibitory activity (**B2D/B6D**), and C6 to C8 caused a 14-time leap in activity (**B6D/B8D**). Although, the further increase of length slightly could enhance the inhibitory activity (**B8D/B12D**). Interestingly, the elongation of the hydrophobic tail, which was initially intended to give the molecule a lipid-like structure, could benefit the PTP1B inhibitory activity gravely.

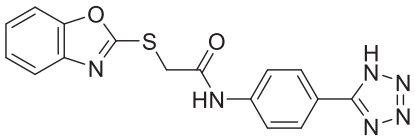
2.3. Membrane permeability and selectivity over TCPTP

Next, the permeability of bis-salicylic acid-based derivatives was assessed. We evaluated the membrane permeability by parallel artificial



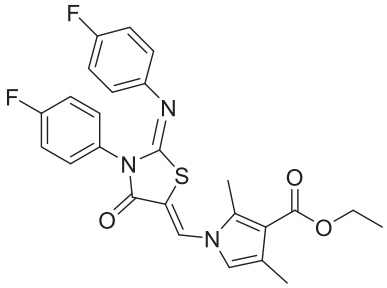
V

PTP1B IC_{50} = 6.5 μM



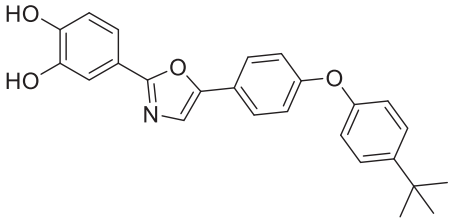
VI

PTP1B IC_{50} = 4.48 μM



VII

PTP1B IC_{50} = 6.09 μM



VIII

PTP1B IC_{50} = 0.487±1.3 μM

Fig. 2. Representative nonionic PTP1B inhibitors [15–18].

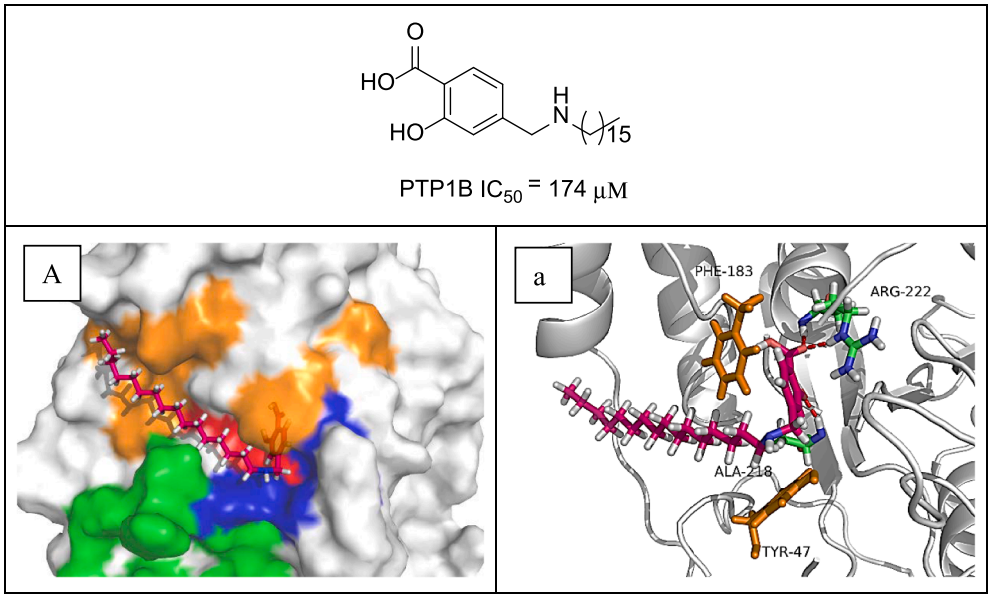
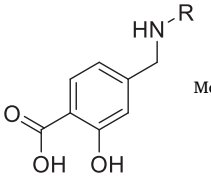


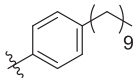
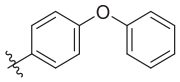
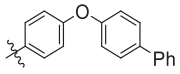
Fig. 3. Molecular docking analysis of **A16S** bound to PTP1B (**A**) binding conformation of **A16S** with PTP1B (different sites of PTP1B are marked with different colors respectively: red - A site - catalytic site, orange - B site- secondary binding site, green - C site - negatively charged flat region, blue - D site - small pocket next to A site [20]). (**a**) interactions with PTP1B. H-bonds are marked in red dashes (PDB: 2CNE).

membrane permeability assay (PAMPA [21]) using the compounds with IC_{50} values ≃5 μM against PTP1B (**A16D, A18D, C2D, B4D, B6D**, **B8D**, **B10D** and **B12D**). Permeability of **Atenolol** and **Propranolol** as the choice references in PAMPA are also given. Clog P values explained the hydrophobicity of these compounds. As shown in Table 3, tested compounds with two acid groups exhibited acceptable membrane

Table 1
PTP1B inhibitory activities of **A16S** homologs. [a]



Mono-salicylic acid-based scaffold

Cmpd	R'	IC_{50} (μM)	Cmpd	R'	IC_{50} (μM)
A6S		> 200	**B0S**		> 200
A12S		> 200	**B10S**		55
A16S		174	**C1S**		> 200
A18S		152	**C2S**		155
Sodium vanadate		2.9			

[a] Experiments are conducted in triplicates.

permeability (P_{app} = 0.6–1.5 × 10^{-6} cm/s). The lipid-like structure could highly enhance the hydrophobicity and ability of cell-membrane crossing. Permeability of **A18D** was not detected, and by the speculation, it might be stuck in the membrane due to excessive lipophilicity.

Simultaneously, the selectivity of these salicylic acid-based derivatives over TCPTP was assessed. It is suggested that the inhibition of TCPTP, the homologous of PTP1B, caused undesired side effects such as impaired glucose tolerance and attenuated glucose-stimulated insulin secretion [22]. As shown in Table 3, these compounds showed a mild selectivity for TCPTP. Overall, 3- to 10-fold PTP1B selectivity was achieved by **A16D, A18D, C2D, B4D, B6D**, **B8D**, **B10D**, and 16-fold by **B12D** over TCPTP.

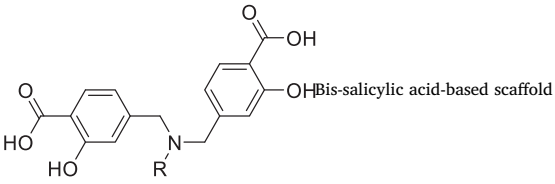
2.4. Docking studies analyses

The bis-salicylic acid-based compounds demonstrated highly potent PTP1B inhibitory activity in comparison with mono-salicylic acid-based ligands, which could implicate the dynamic interactions with external sites of PTP1B. Thus, initially, molecular docking results of the most potent bis-acid ligand called **B12D** was scrutinized (Fig. 4**A, a**). As depicted, one of the salicylic acid groups bound tightly to the catalytic site (A site) by forming three H-bonds with residues Ala218 and Arg222 while another salicylic acid group formed one H-bond with Lys117 and bound to D site [20]. Five benzene rings, including three on the molecule's core and two on the side chain of Tyr47 and Phe183, were spaced in the close range and could form multiple π-π stacking interactions. Furthermore, the dodecyl chain of **B12D** formed other hydrophobic interactions with Ala28, Val50, Ile220, and Met259.

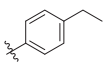
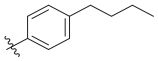
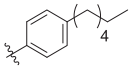
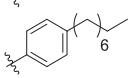
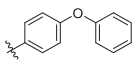
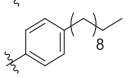
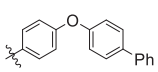
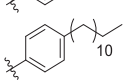
To make a comparison with mono-acid-based compounds which demonstrated weaker potency, simultaneously, we investigated the docking results of the most potent ligand among our monoacid compounds: **B10S** (Fig. 4**B, b)**. The salicylic acid group bound to the catalytic site (A site) by forming three H-bonds with residues Ala218 and Arg222, while Tyr47 and Phe183 formed π-π stacking interactions. Decyl of **B10S** formed hydrophobic reactions with mentioned above amino acids of PTP1B.

As seen, both **B10S** and **B12D** formed H-bonds with residues Ala218 and Arg222 while Tyr47 and Phe183 were engaged in π-π stacking interactions, and they also formed multiple hydrophobic interactions with PTP1B. The H-bond formed by the second salicylic acid with

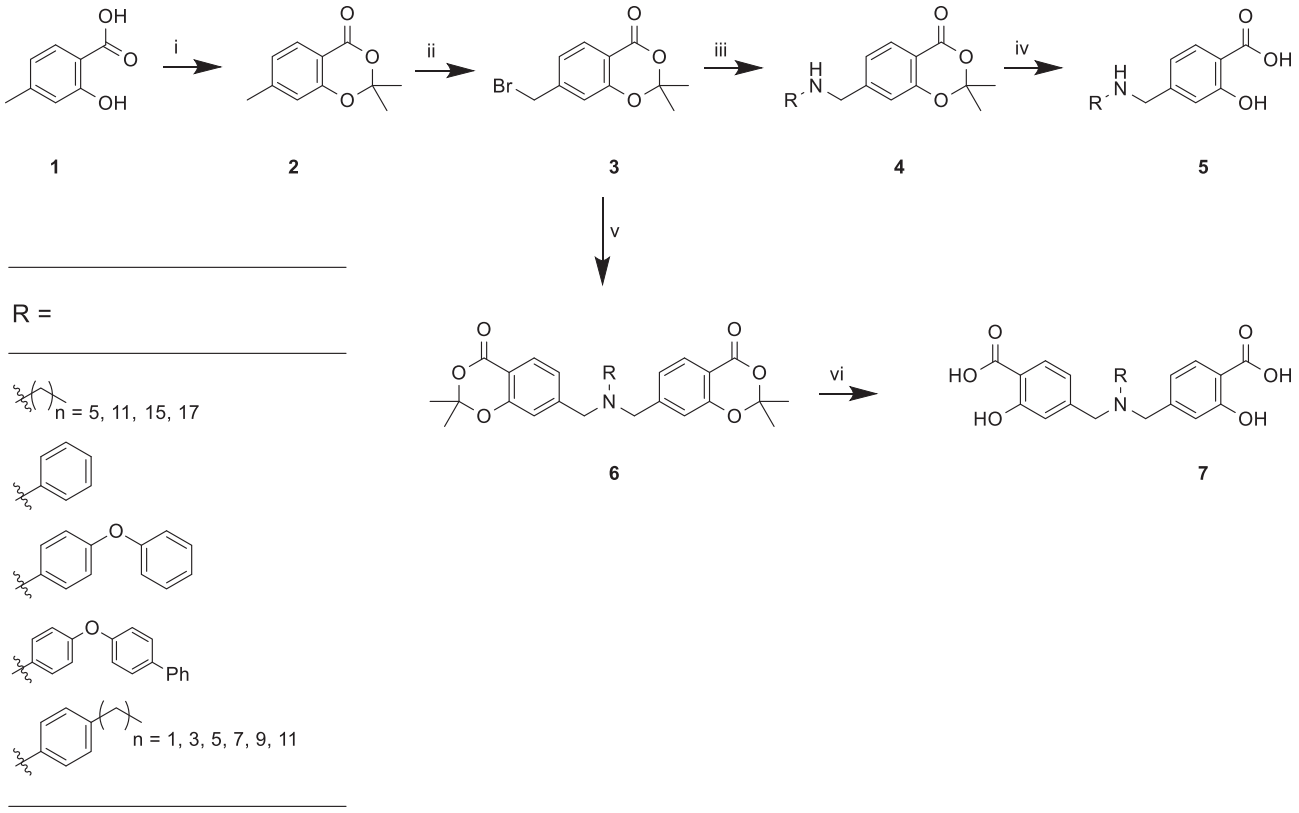
Table 2
PTP1B inhibitory activities of bis-salicylic acid-based inhibitors [a].



Bis-salicylic acid-based scaffold

Cmpd	R'	IC_{50} (μM)	Cmpd	R'	IC_{50} (μM)
A6D		> 10	**B2D**		> 10
A12D		4.0	**B4D**		4.6
A16D		1.5	**B6D**		6.1
A18D		5.4	**B8D**		0.45
C1D		> 10	**B10D**		0.47
C2D		0.87	**B12D**		0.37

[a] Experiments are conducted in triplicates.



Scheme 1. The synthesis of mono- and bis-salicylic acid-based derivatives. Reagents and conditions: (i) acetone, TFA, TFAA, r.t., overnight, 58%; (ii) NBS, AIBN, reflux, 2 h, 39%; (iii) K_2CO_3, ACN, reflux, 2 h; (iv) NaOH, H_2O, ACN, reflux, 2 h, 25%-38%; (v) K_2CO_3, ACN, reflux, 48 h; (vi) NaOH/H_2O, ACN, reflux, 2 h, 17%-39%.

Lys117 on the D site of PTP1B in bis-acid ligand might be quite critical for the dramatic difference between inhibitory activities of mono- and bis-acid ligands (IC_{50} = 55, 0.37 μM respectively against PTP1B). Besides, the second salicylic acid also could involve in few other π-π stacking interactions with PTP1B. The superimposition of these two ligands emphasized the fit conformation of bis-acid ligands lucidly (Fig. 5).

Table 3
The effects of various hydrophobic tail of salicylic acid-based inhibitors on permeability, Clog P, PTP1B potency, and selectivity over TCPTP.

Cmpd	Clog P [a]	Permeability (10^{-6} cm/s) [b]	PTP1B IC_{50} (μM)	TCPTP IC_{50} (μM)	Selectivity [c]
A16D	11.8	0.9	1.5	15.3	10.2
A18D	12.8	ND[d]	5.4	25.2	4.7
B4D	5.61	0.6	4.7	14.8	3.1
B6D	6.45	0.8	6.1	24.2	4.0
B8D	7.28	1.0	0.45	3.2	7.1
B10D	8.11	1.2	0.47	3.9	8.3
B12D	8.95	1.5	0.37	5.8	15.7
C2D	9.82	ND[d]	0.87	6.7	7.7
Atenolol	−0.11	1.2	–	–	–
Propranolol	2.76	18.1	–	–	–

[a] Predicted by ChemBioDraw Ultra 14.0.
[b] Values detected by Parallel Artificial Membrane Permeability Assay.
[c] TCPTP IC_{50}/PTP1B IC_{50}.
[d] Not detected.

3. Discussion

Despite various efforts, such as the molecule charge manipulations and hydrophobicity enhancements for the cell-membrane permeability improvement of PTP1B inhibitors [23], the identification of the potent and bioavailable inhibitors and specifically transforming those into therapeutic applications is still a great challenge. In this research, we introduced a new scenario by simulating the structure of the amphiphilic lipids and created a series of lipid-like anionic molecules. By this strategy, we were able to rectify the potency and bioavailability of PTP1B inhibitors extensively. These lipid-like salicylic acid-based derivatives were inspired by a liposome substance (**A16S**) and could exhibit a notable PTP1B inhibitory activity and improved bioavailability (e. g., **B12D**, IC_{50} = 0.37 μM against PTP1B and $P_{app} = 1.5 \times 10^{-6}$ cm/s).

While our study has solely focused on the discovery of salicylic acid-based inhibitors of PTP1B, the designing strategy created and brought to the surface by this study can also be applied to other phosphatase inhibitors containing anionic moieties. This strategy supports precise cell-membrane penetration without significant decrease of phosphatase inhibitory activity. These compounds and the strategy behind confidently offer a potential path for the future potent and membranophilic PTP1B inhibitors targeting T2DM.

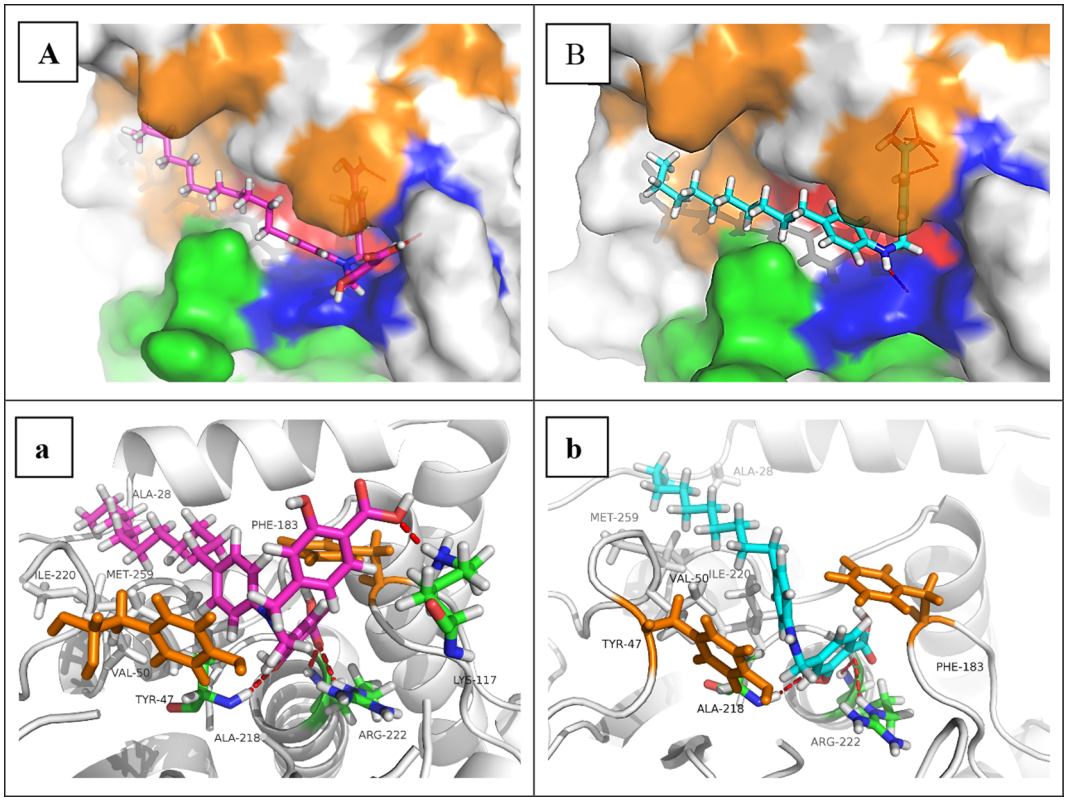


Fig. 4. Molecular Docking comparisons of compounds **B12D (bis-acid)** and **B10S** (**mono-acid**) bound to PTP1B. **(A)** Binding conformation of **B12D** with PTP1B. **(B)** Binding conformation of compound **B10S** with PTP1B (different sites of PTP1B are marked with different colors respectively: red - A site - catalytic site, orange - B site- secondary binding site, green - C site – negatively charged flat region, blue - D site – small pocket next to A site). (**a**) Demonstration of interactions between compound **B12D** and PTP1B [20]. (**b**) Demonstration of interactions between compound **B10S** and PTP1B. H-bonds are marked in red dashes.

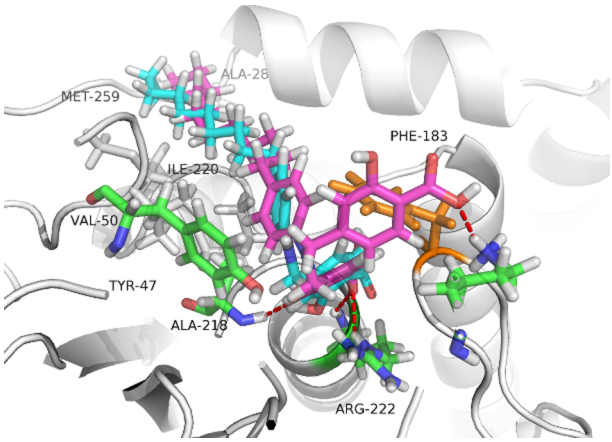


Fig. 5. Superimposition of compounds **B12D (bis-acid)** and **B10S** (**mono-acid**). H-bonds are marked in red dashes.

Acknowledgments

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