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REVIEW

The protein tyrosine phosphatase 1b as a drug target for the treatment of diabetes type II. Developing effective and selective PTP1B inhibitors

Phaedra Eleftheriou
Department of Medical Laboratory Studies, School of Health and Medical Care, Alexander Technological Educational Institute of Thessaloniki, Sindos, 57400, (GREECE)
E-mail: elfther@mls.teithe.gr
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Abstract : PTP1B is a protein tyrosine phosphatase involved in insulin receptor desensitization. PTP1B inhibition, resulting in prolonged maintenance of the activated state of the receptor, practically enhances insulin effect. Thus PTP1B has become a drug target for the treatment of Diabetes type II. Although, a great number of inhibitors have been developed, among which inhibitors binding to the catalytic site and allosteric inhibitors, development of novel highly effective and selective compounds based on profound knowledge of the structural characteristics of the binding sites and inhibitors still remains a challenge.

Keywords : Diabetes mellitus; PTP1b inhibitors; Competitive; Allosteric.

DIABETES MELLITUS, INCIDENCE, CATEGORIZATION AND TREATMENT

Diabetes mellitus is a metabolic disorder that affects 150 million people worldwide[1]. Diabetes type II, the non insulin dependent type (NIDDM), accounts for about 90-95% of all cases in America[2]. Although percentage varies from place to place, it remains high all over the world. It is a metabolic disorder of carbohydrate metabolism characterised by hyperglycaemia. However, disturbances of protein and fat metabolisms are also associated with the disease. The main types of Diabetes are Diabetes mellitus type I, Diabetes mellitus type II, gestational diabetes and Diabetes of other etiology. Diabetes type I, also known as “insulin-dependent diabetes mellitus” (IDDM) or “juvenile diabetes” is considered as an autoimmune disease and is characterized by the inability of the beta cells of the islets of Langerhans of pancreas to produce insulin. Diabetes type II, is characterized by insulin resistance and is associated with hyperglycemia and hyperinsulinemia at the first stages of the disease. As the disease progresses, diminished insulin production is observed. Diabetes type II is also

connected with atherosclerosis, hypertension and abnormal lipid profile[1].

THE PROTEIN TYROSINE PHOSPHATASE PTP1B AS A DRUG TARGET FOR THE TREATMENT OF NIDDM

The intracellular moiety of insulin receptor is a kinase which is activated by autophosphorylation following interaction of the extracellular part with insulin. This is the first step of a cascade leading to glucose uptake through glucose transporter GLUT4. The cascade is terminated by the dephosphorylation of the intracellular moiety of insulin receptor by the protein tyrosine phosphatase PTP1B. Thus, PTP1B inhibition, resulting in prolonged maintenance of the phosphorylated state, practically enhances insulin effect[1].

Protein tyrosine phosphatase 1B (PTP1B) also acts as a negative regulator of the leptin receptor pathway[3]. PTP1B-deficient mice as well as PTP1B knockout mice have revealed phenotypes of enhanced insulin sensitivity, improved glycemic control, and resistance to high fat diet induced obesity. Furthermore, treatment of diabetic mice with PTP1B antisense oligonucleotides reduced the expression level of the enzyme and subsequently normalized blood glucose and improved insulin sensitivity[1]. Thus, PTP1B has been considered as an attractive therapeutic target for type 2 diabetes and obesity.

Protein Tyrosine Phophatases (PTPs) represent a large family of enzymes. They play a very important role in cellular signaling. PTPs work antagonistically with Protein Tyrosine Kinases (PTKs) to regulate signal transduction in a cell. Perturbation of the balance between protein-tyrosine kinases (PTKs) and protein-tyrosine phosphatases (PTPs) disrupts cell function and has been implicated in a variety of diseases, including diabetes, obesity, and cancer[1]. Tyrosine phosphorylation offers a rich source of drug targets[4]. The existence of large number of PTPs makes necessary the development of selective inhibitors without serious side effects.

Various compounds for the treatment of diabetes have been developed: insulin and insulin mimetics[5], insulin release enhancers[6,7], inhibitors of hepatic glucose production[8,9], inhibitors of glucose intestinal uptake[8,9], etc. However, most of these can not be used for the treatment of diabetes type II at the first stage. Therefore, small molecular inhibitors of PTP1B have been developed by both industry and academia[10-12], some of them exhibiting IC_{50} values at the μM or nM range. Instability, low selectivity and inability of certain charged compounds to penetrate cell membrane were some of the most serious problems associated with the first inhibitors referred[1]. Although, a large number of PTP1B inhibitors have been found, the race for finding novel highly effective and selective inhibitors still continues.

CHEMICAL CATEGORIES OF KNOWN PTP1B INHIBITORS

The first synthetic PTP1B inhibitors were mimicking phospho-tyrosine containing oligopeptide of the natural substrate of the enzyme. Among them are phosphopeptides[13], peptides bearing phosphono(difluoromethyl)-phenylalanine[14], peptides containing dicarboxylic acid-based Tyr mimetics[15], *O*-malonyltyrosine and fluoro-*O*-malonyltyrosine derivatives[16], o-carboxymethyl salicylic acid containing peptides[17], aryl α-ketocarboxylic acids[18], and derivatives of thieno[2,3-c]pyridine-3-carboxylic acid[19], arylodifluoromethyleno-phosphoric acids[20], oxalylarylamino benzoic acid derivatives[21], bicyclic benzofuran and indole-based salicylic acids[22], dibenzo[b,d]furan carboxylic acids[23], isochroman carboxylic acid derivatives[25], aryl diketoacid derivatives[25], 1,2-naphthoquinones[26], quinoline derivatives[27], pyridazine analogues[28], piperazin derivatives[29], diarylsulfonamide derivatives[30], formylchromone derivatives[31], pyrrolo[2,3-c]azepine derivatives[32], bromo-retrochalcone derivatives[33], styrylbenzene derivatives[35], maslinic acid derivatives[35], (glycopyranosyl-triazolyl)-purines[36], thiazolidinedione[37,38] and thiazolidinone[39] derivatives, benzyl 6-triazolo(hydroxy)benzoic glucosides[40], triazole-linked glycosylated α-ketocarboxylic acid derivatives[41], Beta-C-glycosiduronic acids and beta-C-glycosyl compounds[25], triazole-linked beta-C-glycosyl dimers[42], α-bromo-acetophenon derivatives[43], oxovanadium (IV) complexes[44-50], copper complexes with multi-benzimidazole derivatives[49].

Certain natural products with PTP1B inhibitory action have also been found. Among them, oleic acid[50],

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Oleanolic acid derivatives[51], 1,2,3,4,6-Penta-O-galloyl-D-glucopyranose[52], phlorotannins[53], flavonoids[54-56], triterpenes[57,58] and triterpene derivatives such as dammaranes[59,60], alkaloids such as berberine[61], caffeoyl derivatives[62], benzofuran derivatives[63], chalcones[64] and chalcone derivatives[63], sesquiterpene quinones[48,65], pterocarpans[66,67], aquastatin A[68], depsidone and pseudodepsidone derivatives[69], brominated naphthalene[66], diphenyl ether and benzophenone derivatives[70].

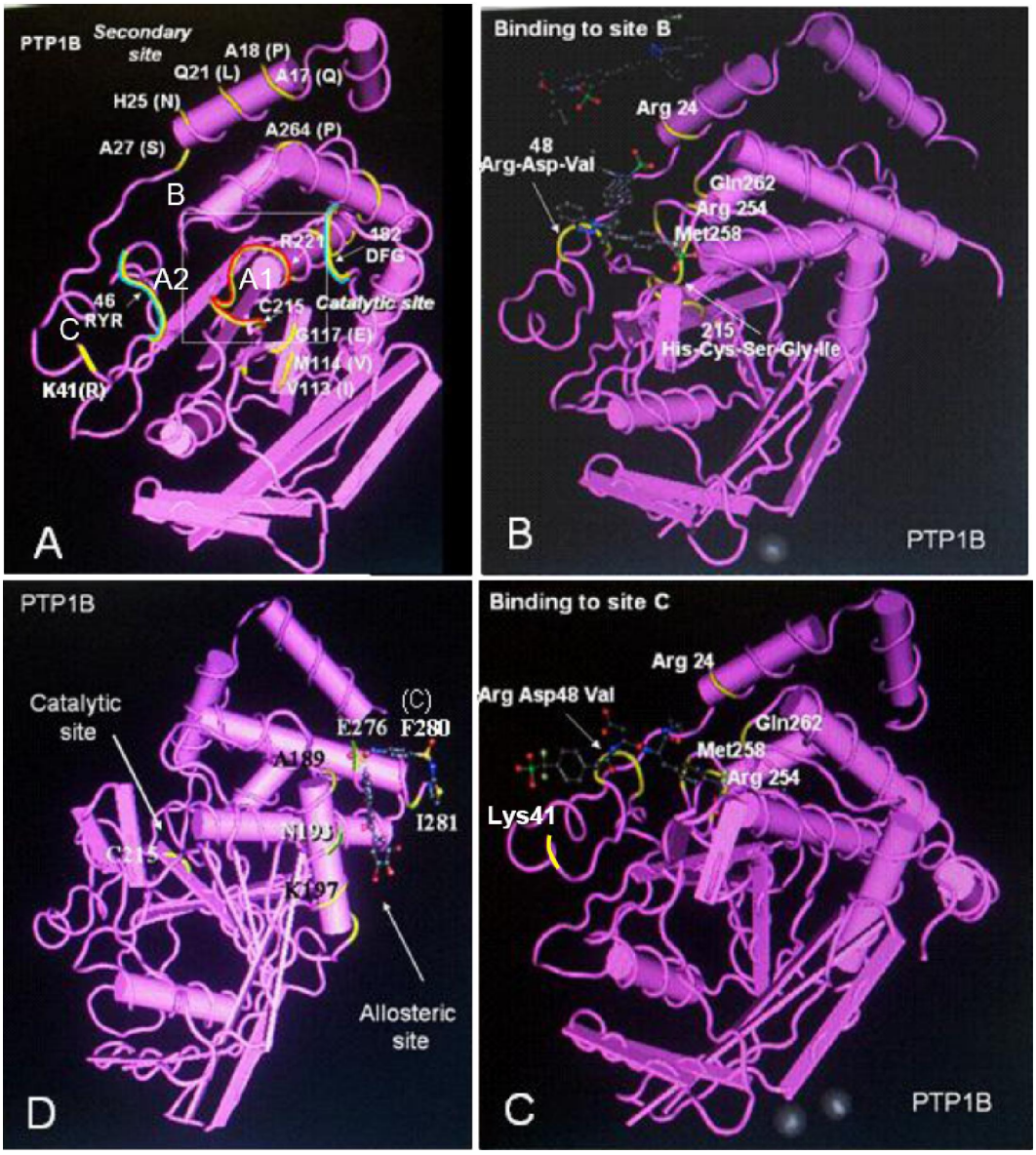

Figure 1 : A: Catalytic site of PTP1B and secondary binding sites. B: Binding of inhibitor to the active site also occupying the secondary binding site B. C: Binding of inhibitor to the active site also occupying the neighboring binding site C. D: Binding of inhibitor to the allosteric site. Aminoacids involved in interactions with the inhibitors are shown in yellow (In brackets: aminoacids at the same position of TC-PTP differing from that of PTP1B)[73,76-78].

CATEGORIZATION OF PTP1B INHIBITORS ACCORDING TO THE BINDING SITE

As far as interaction with PTP1B is concerned, the inhibitors developed till now are divided into two main categories. Inhibitors that bind to the active site of the enzyme[71,72] and allosteric inhibitors that bind to a site 20 Å from the catalytic center preventing mobility of certain enzyme loops[71,73].

The catalytic site of PTP1B consists of the aminoacids His214, Cys215, Ser216, Ala217, Gly218,

Ile219, Gly220 and Arg 221 with Cys215 being responsible for catalytic activity[74]. All these aminoacids may take part in hydrogen bond interactions with inhibitors, while Phe182 and Tyr46 may be involved in hydrophobic and π→π interactions[75] (Figure 1A).

The volume and structural characteristics of inhibitors interacting with the catalytic site vary. Small molecules which bind strictly to the core of the catalytic centre, interacting with aminoacids surrounding Cys 215 (area A1) have been developed. Compound 1ONZ in figure 7 represents an inhibitor of this kind. Existence of at least one acidic or hydrogen donor/acceptor moiety was the mandatory characteristic of such inhibitors. A second type of molecules, bearing two clusters of acidic or hydrogen donor/acceptor groups, could interact with the strict catalytic core aminoacids and aminoacids of the more distant loop consisted by Arg45, Tyr46, Arg47 and Asp48, designated in figure 1 as area A2. Compounds 1BZJ, 1BZC and 2VEW of figure 7 represent inhibitors of this kind. Orientation of the inhibitor 2VEW into the active site is shown in figure 2.

Although, there are some exceptions, the molecules capable to interact with both A1 and A2 sites form more stable complexes, thus exhibiting better inhibitory action. Since, hydrogen bond interactions have a major role in complex stabilization, the first mandatory characteristic for interaction with both A1 and A2 sites is the presence of two clusters of hydrogen donor/acceptor groups at a distance from about 9.3 to 11.5 Å as shown in the example of figure 2.

A secondary binding site B (Figure 1B)[76] adjacent to the catalytic centre in vicinity to the aminoacids Arg24, Ser 28, Gln262 and Arg254 or a nearby binding region C[71] oriented towards Lys41 (Figure 1C)[72] are also occupied by certain PTP1B inhibitors. Simultaneous occupation by the inhibitor of the main catalytic site A (A1, A2) and each of the secondary sites have been proposed by scientists as the choice of preference in order to achieve high and selective inhibition[71,75]. However, large molecules fulfilling the criterion of simultaneous interaction with main and secondary sites usually face the problem of cell membrane penetration inability.

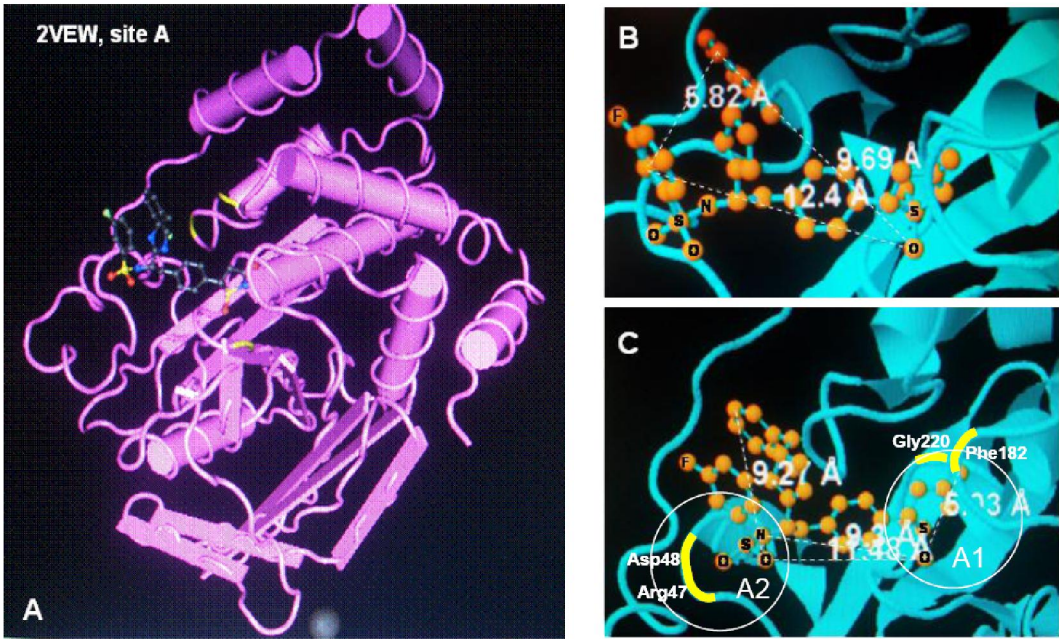

Figure 2 : Orientation of a competitive inhibitor binding to catalytic site A. Figure 2A. Docking of the PTP1B inhibitor 3-fluoro-N-[(1S)-1-[(4R)-4-[(2-fluorophenyl)methyl]imidazolidin-2-yl]-2-[4-[(5S)-1,1,3-trioxo-1,2-thiazolidin-5-yl] phenyl]ethyl]benzenesulfonamide (PDB: 2VEW) at catalytic site[79]. Figure 2B. Distances between the A1 hydrogen donor/ acceptor group and the hydrophobic moieties of the molecule. C. Distances between the main hydrogen acceptor/donor groups of the molecule involved in hydrogen bond interactions with the areas A1 and A2 of the enzyme[77,80].

The compounds 1Q1M and 1Q6T of figure 7 are examples of molecules interacting with sites A and B simultaneusly. Compound 1Q6T is a characteristic representative of molecules interacting with sites A1, A2 and B. According to crystallographic results, 1Q6T is placed in a position enabling hydrogen bond interactions

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between three donor/acceptor clusters of the compound and aminoacids of the regions A1, A2 and B of the enzyme being at appropriate distances to form hydrogen bonds (distances between 2.7 and 3.7 Å) (Figure 3).

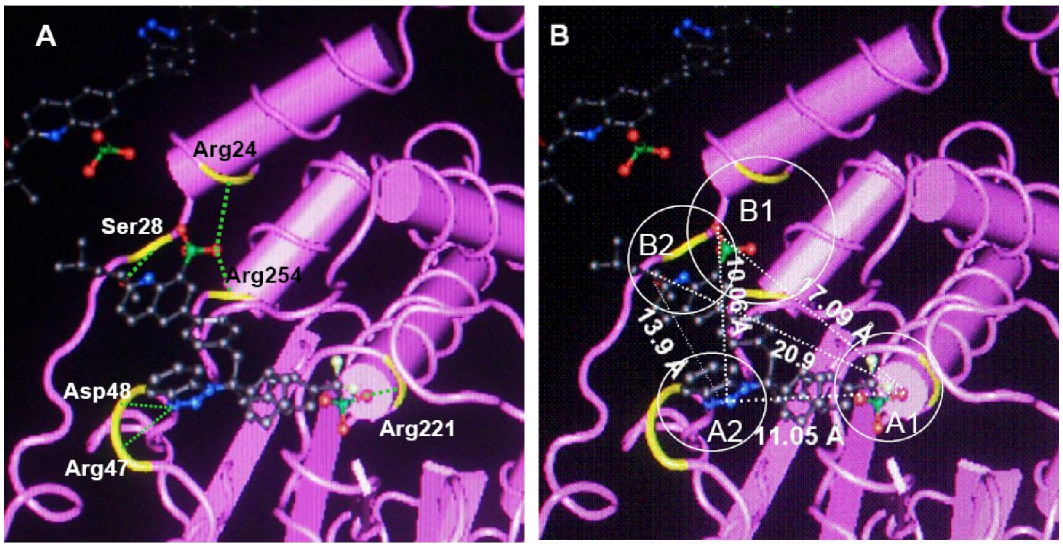

Figure 3 : Orientation of the inhibitor [6-[4-[(2R)-2-(benzotriazol-1-yl)-3-[4-[difluoro(phosphono)methyl]phenyl]-2-phenylpropyl]phenyl]-2-[(1S)-1-methoxy-3-methylbutyl]quinolin-8-yl]phosphonic acid (PDB: 1Q6T), which occupies both catalytic site A and the secondary site B into the active site of PTP1B. Figure 3A. Indicative hydrogen donor/acceptor groups with orientation enabling Hydrogen bond interactions (green lines). Figure 3B. Distances between the main hydrogen acceptor/donor groups of the molecule capable to be involved in hydrogen bond interactions with the areas A1, A2, B1 and B2 of the enzyme.

Attainment of this triple interaction shown in figure 3, demands a structure bearing the three hydrogen donor/acceptor clusters oriented at distances determined by the triangles shown in figure 3B.

Examples of molecules interacting with site B in a double interaction mode involving interaction with sites A1 and B and not with A1, A2 and B have been mentioned. Compound 1Q1M is indicative example of such an inhibitor[81].

Compound 1PXH of figure 7 is an example of a molecule simultaneously occupying the sites A1, A2 and C, as shown in figure 4. Three clusters of hydrogen donor/acceptor molecules can also be recognized being able to interact with aminoacids at A1 and A2 sites and with Lys41 of site C, respectively. Approximate distances and positions of these three clusters are indi-

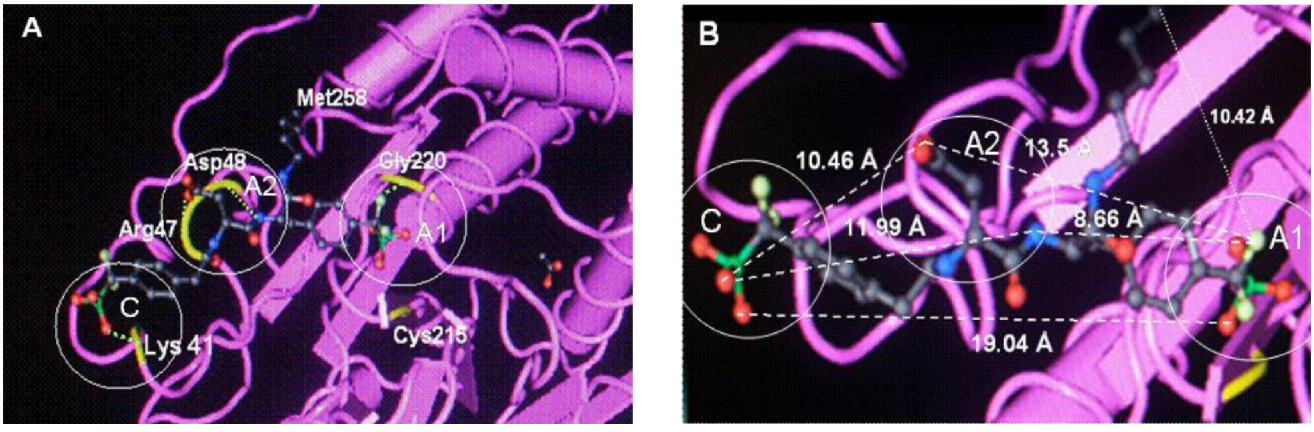

Figure 4 : Orientation of the inhibitor 3-[[2-[4-[difluoro(phosphono) methyl]phenyl]acetyl] amino]-4-[[(2S)-3-[4-[difluoro(phosphono)methyl]phenyl]-1-oxo-1-(pentylamino) propan-2-yl] amino]-4-oxobutanoic acid (PDB: 1PXH), which occupies both catalytic site A and the nearby site C. Figure 4A. Indicative hydrogen donor/acceptor groups with orientation enabling Hydrogen bond interactions (green lines). Figure 4B. Distances between the main hydrogen acceptor/donor groups of the molecule capable to be involved in hydrogen bond interactions with the areas A1, A2 and C of the enzyme.

cated in the triangles of Figure 4B.

Allosteric inhibition of PTP1B has also been observed and an allosteric site was identified about 20 Å away from the catalytic center[73] (Figure 1D). The allosteric center is placed in a cavity formed between helix 5 and helix 9 and consists of the aminoacids Ala189, Asn193, Lys197, Glu200, Glu276, Lys279, Phe280, Ile281 and Met282 which participate mainly in hydrogen bond interactions with the inhibitors (Figure 5). Chlorogenic acid, cichoric acid and the synthetic analogues such as 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-[4-(1,3-thiazol-2-ylsulfamoyl)phenyl]-1-benzofuran-6-sulfonamide[73], and 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N,N-dimethyl-1-benzofuran-6-sulfonamide are examples of molecules mentioned in the literature, while certain phlorotanins have been found to act as non competitive inhibitors[53] (Figure 8). Since the number of allosteric inhibitors known is small, there is little information concerning the mandatory structural characteristics of such molecules and the aminoacids involved in interactions. Studies on the best conformations adopted by chlorogenic acid and cichoric acid in the inhibitor-enzyme complex indicate involvement of hydrogen bond interactions between hydroxyl- or carbonyl- groups of the inhibitor and aminoacids Ile281, Phe280, Glu276 and Asn193 for cichoric acid and Ala189, Lys197 and Glu200 for chlorogenic acid. Cichoric acid adopts a bended conformation, forming a triangle with groups involved in hydrogen bond interactions arranged at each corner. Similar bended structures could be adopted by other non competitive inhibitors (figure 8). Chlorogenic

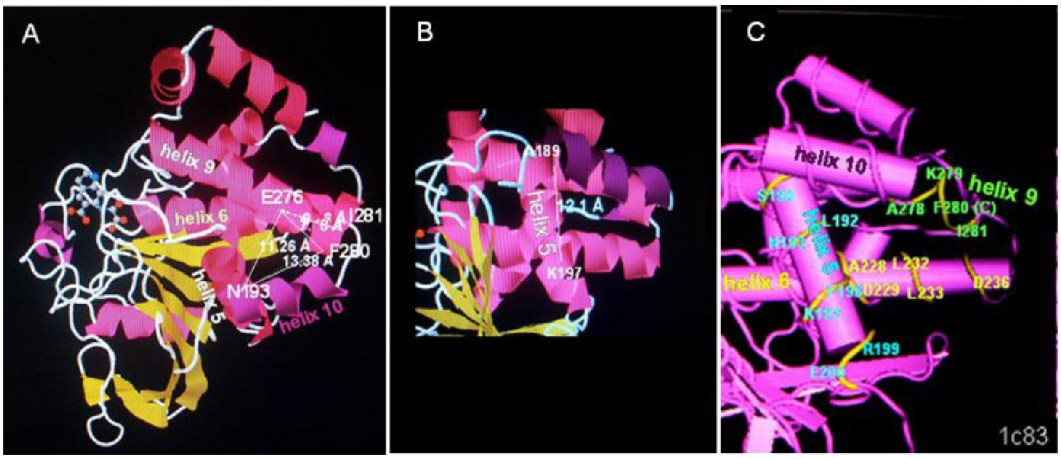

Figure 5 : PTP1B allaosteric site. A. Distances between aminoacids involved in hydrogen bond formation with cichoric acid [73] (Swish pro database). B. Distances between aminoacids involved in hydrogen bond formation with chlorogenic acid [73] (Swish pro database). C. aminoacids surrounding the allosteric site of PTP1B[77].

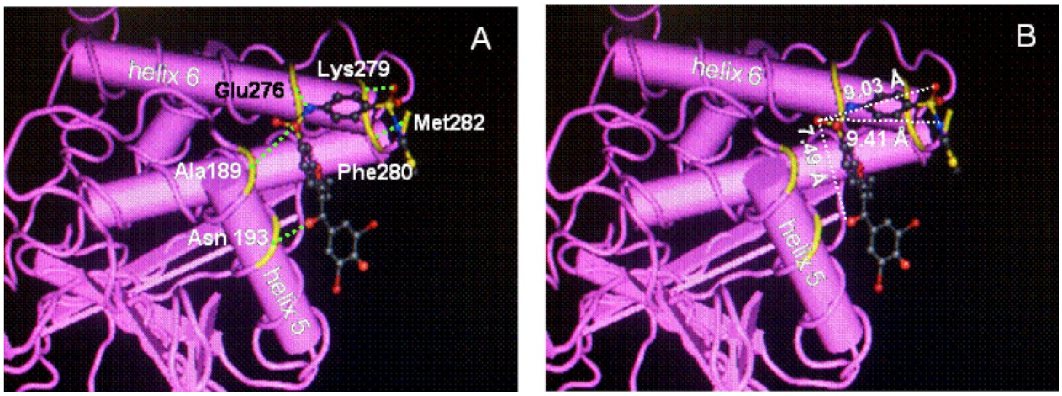

Figure 6 : Binding of the allosteric inhibitor 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-[4-(1,3-thiazol-2-ylsulfamoyl)phenyl]-1-benzofuran-6-sulfonamide (PDB: 1T4J) [73] to the allosteric site of PTP1B. A. Indicative aminoacids at appropriate distances from hydrogen donor/acceptor groups of the molecule enabling hydrogen bonds formation (green). B. Distances between the main hydrogen acceptor/donor groups of the molecule capable to be involved in hydrogen bond interactions.

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acid is a smaller molecule wich adopts a not bended conformation. The synthetic inhibitor, 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-[4-(1,3-thiazol-2-ylsulfamoyl)phenyl]-1-benzofuran-6-sulfonamide, adopts a double bended conformation enabling hydrogen bond interaction with the aminoacids Ala 189, Asn193, Glu276, Lys279, Met280 and Phe 280 (Figure 6). Moreover, orientation of thiazolyl moiety enables interaction with Phe280, further stabilizing the complex. Orientation of the synthetic compound 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N,N-dimethyl-1-benzofuran-6-sulfonamide into the allosteric centre is shown in figure 11. Interactions with aminoacids Ala 189, Asn193, Glu276 are favored in this case.

The existence of a great number of polar aminoacids in the two helixes forming the allosteric center strongly favors inhibitor structures with many hydrogen donor/acceptor groups. Hydroxyl and carbonyl groups as well as SO_2 groups are mainly observed in most known allosteric inhibitors. The multiple possibilities of hydrogen bond interactions with the aminoacids of helixes 5 and 9 allow many favorable orientations of these groups within the molecule.

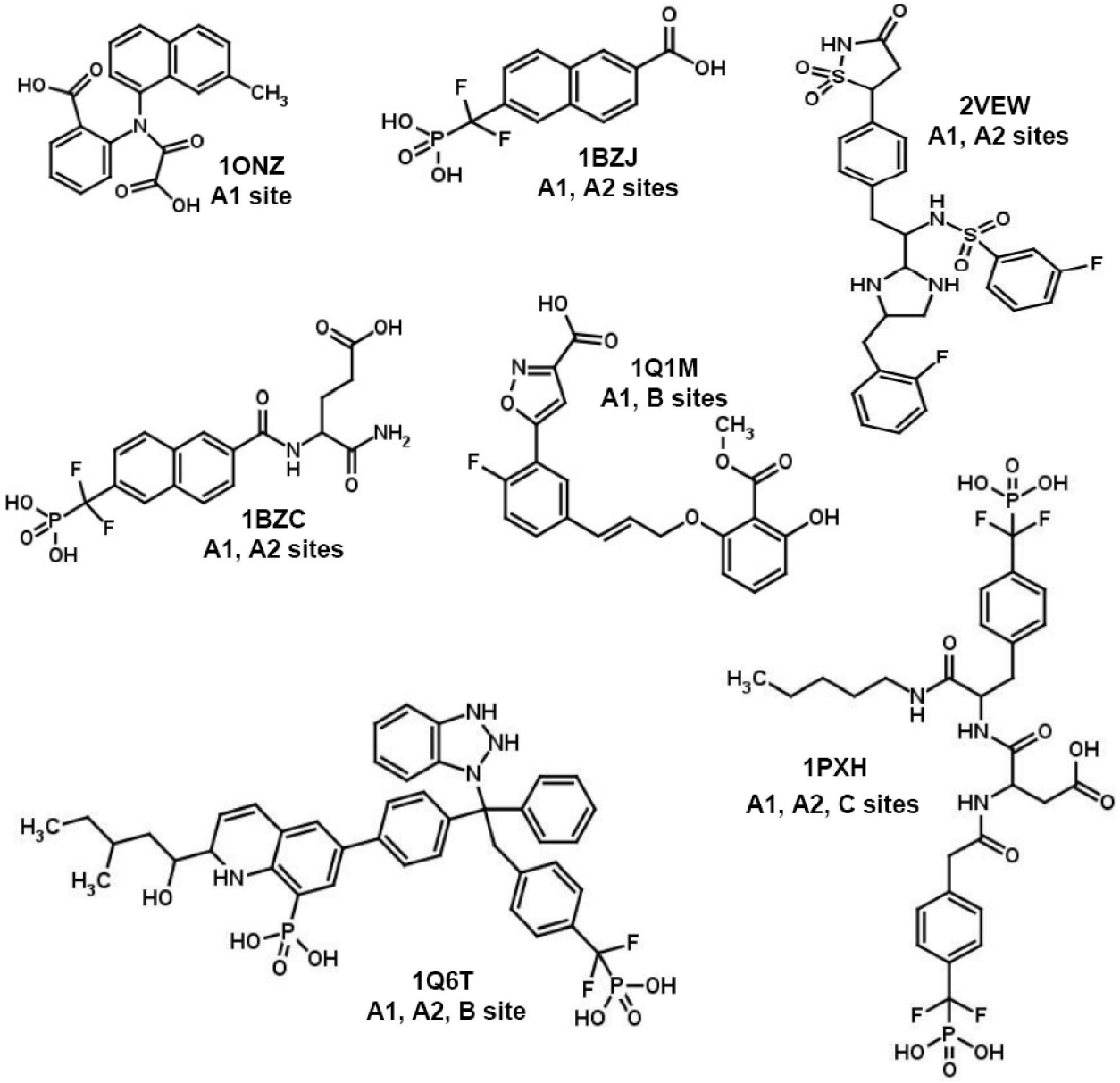

Figure 7 : Structures of PTP1B inhibitors interacting with the sites A, B and C of the enzyme. For each compound the Protein Data Bank code is given[77].

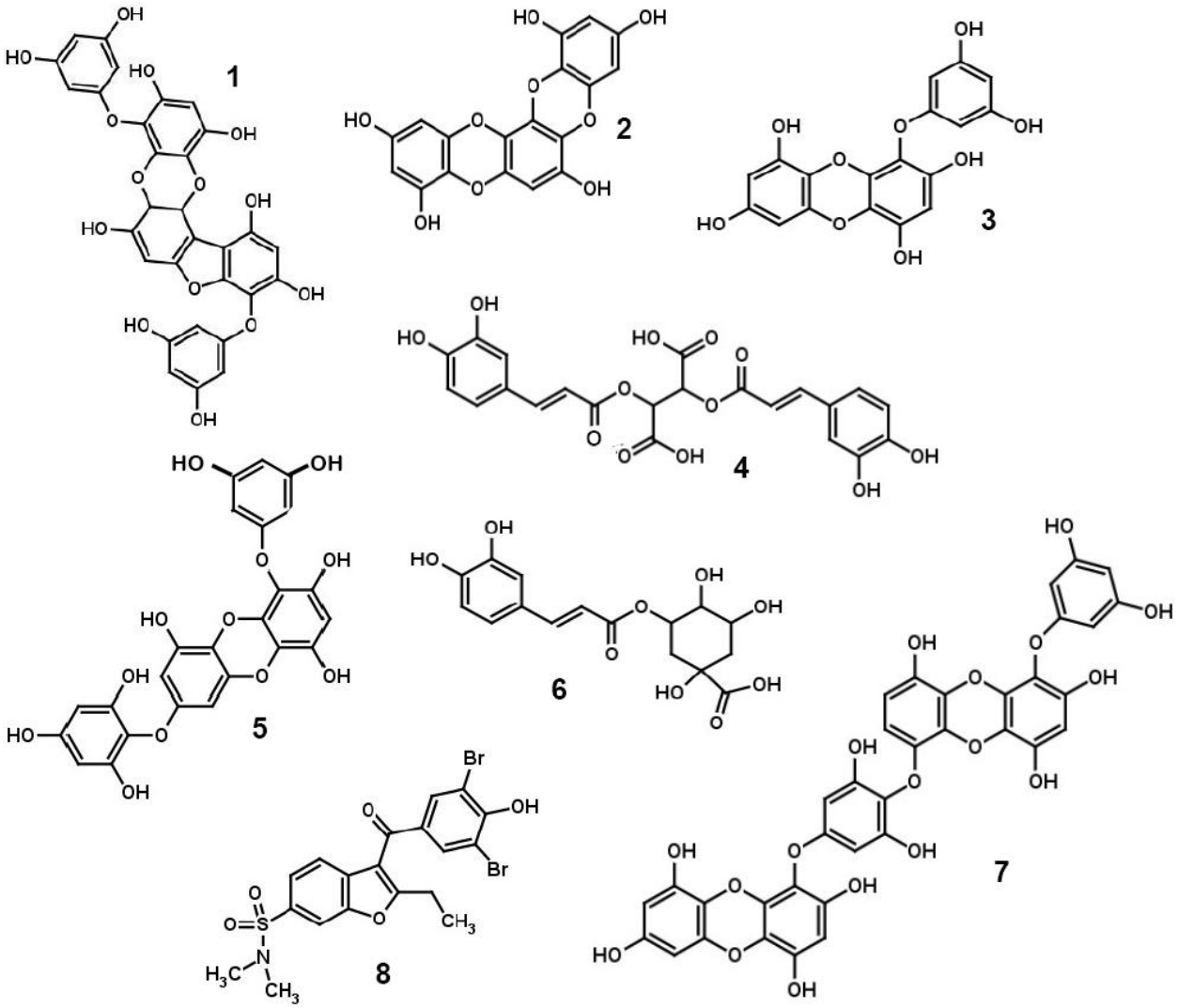

Figure 8 : PTP1B inhibitors binding to the allosteric center. 1. phlorofurofucoeckol, 2. dopxinodehydroeckol, 3. eckol, 4. cichoric acid, 5. 7-phloroectol, 6. chlorogenic acid, 7. dieckol, 8. allosteric inhibitor PDB:1T48

SPECIFICITY OF PTP1B INHIBITORS

Since, a great number of tyrosine phosphatases are involved in crucial cell signaling processes, selective inhibition of PTP1B is an important target and a mandatory property of an effective inhibitor with low side effect profile. Although, all tyrosine phosphatases exhibit increased protein sequence similarity, protein tyrosine phosphatase LAR (Leucocyte antigene related) and T-cell PTP share the most common characteristics with PTP1B.

Tyrosine phosphatase LAR is a transmembrane protein consisted of an extracellular portion containing one Ig-like and one fibronectin type III-like domains and an intracellular portion. The intracellular portion consists of two domains: The D1 domain of LAR has enzymatic phosphatase activity. LAR is involved in neuronal developement, regulation of cell-cell adhesion and modulation of insulin signaling[82]. It also acts on insulin receptor dephosphorylation and could also be a drug target for diabetes mellitus type II treatment according to some scientists[83]. Although, PTP1B and LAR are present in almost all kind of cells, they seem to have mainly different functions: on one hand PTP1B is mostly responsible for dephosphorylation in liver and muscle cells while on the other hand, the PTP LAR is responible for 40 % of the dephosphorylation in adipose tissue.

In spite of homology, LAR has substantial differences from PTP1B. Most importantly, the active site of LAR is clearly less basic than that of PTP1B. So it does not interact so easily with acidic inhibitors[84].

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T-cell PTP shares the greatest similarity with PTP1B. The catalytic sites of the two enzymes exhibit absolute sequence match (Figure 9). However, differences exist mainly in aminoacids in the neighbourhood of the secondary binding site B such as S28→H, A27→S, K26→E, H25→N, and the more distant aminoacids Q21→L, A18→P, A17→Q, in Lys 41 interacting with C-site binding inhibitors (41K→R) and in one of the aminoacids involved in interactions with the allosteric inhibitors, F280→C. So, inhibitors, placed in vicinity of Arg24 of secondary site B, inhibitors interacting with Lys41 of site C or allosteric inhibitors interacting with Phe280 have increased probability to exhibit low or now interaction with T-cell PTP. Examples of selective PTP1B inhibitors are shown in figure 12.

Comparison of the 3D structures of PTP1B to TCPTP in the absence of inhibitor reveals a nearly perfect alignment in almost all parts of the molecules (Figure 10). The most significant difference is observed in the portion of the protein chain between Glu115 and Leu119 which does not seam to affect inhibitor interaction, while minor disturbances in 3D structure are observed between Leu61 and Asp63 with no effect on enzyme inhibition and between aminoacids Ala27 and Asp31 in the region of site B.

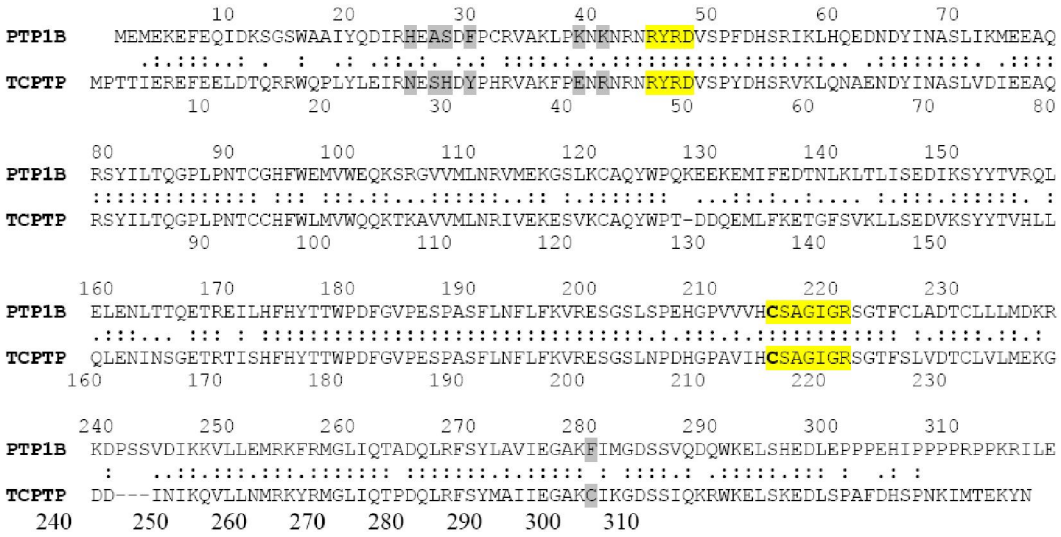

Figure 9 : Comparison of protein sequences between PTP1B and TCPTP. Aminoacids of sites A1 and A2 are highlighted in yellow. Differences in aminoacids between the two enzymes in the regions of site B, site C and allosteric site are marked in grey.

When an inhibitor is attached to the binding site, a significant movement of the loop containing the aminoacids D181, F182, G183 is observed resulting in a significant difference with the non complexed TCPTP (Figure 11A). No significant additional difference in 3D structure of PTP1B complex with an allosteric inhibitor and non complexed TCPTP is observed (Figure 11B).

STRUCTURAL CHARACTERISTICS OF PTP1B INHIBITORS

Mimicking phospho-tyrosine residues of the natural substrate, most synthetic PTP1B inhibitors contain one or two aromatic rings bearing acidic groups. Carboxylic and phosphate substituents were the most commonly used. Ionic interactions and hydrogen bonds between these groups and the crucial aminoacids of the active site are involved in complex stabilization. Hydroxy- and oxo-, alkoxy-, amino- and $-SO_2$ groups also take part in Hb formation[51]. A favorable structure was proposed on 2007 by Bharatham et. al. based on structural similarities of known inhibitors. The model proposes the existence of an aromatic moiety a second aromatic or hydrophobic moiety at a distance of about 5.8 – 7.8 Å and a third H-bond acceptor moiety at a distance of 9.3-12.3 Å and 10.6-13.0 Å from the other two moieties respectively[85].

Based on crystal structures of known inhibitors, one can not overlook the significance of hydrogen bonds in

complex stabilization and the commonly observed presence of two or three clusters of hydrogen donor/acceptor groups capable to interact with aminoacids placed on sites A1, A2, B and C respectively.

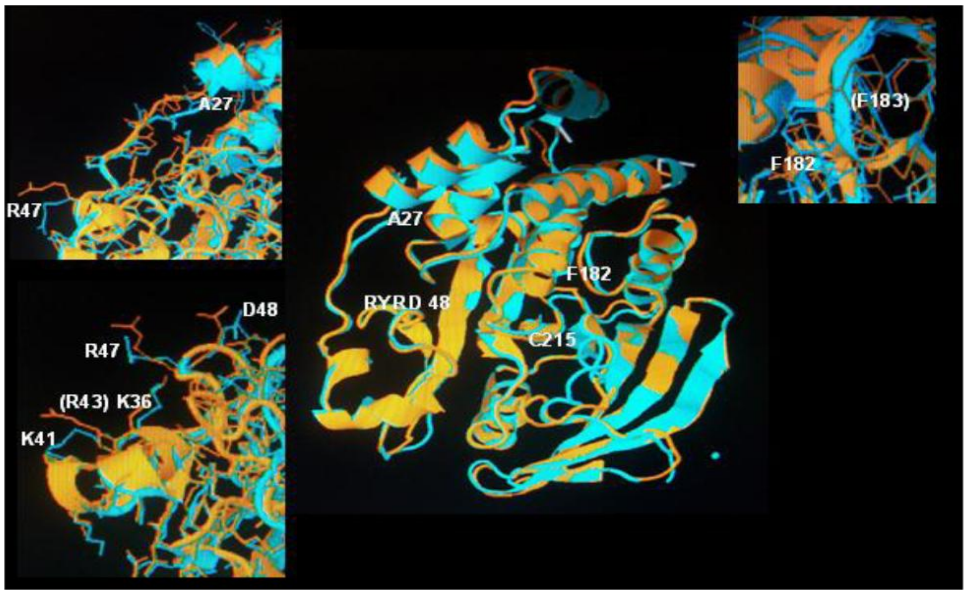

Figure 10 : 3D structure alignment between PTP1B and TCPTP in the absence of inhibitor. PTP1B is indicated with blue. TCPTP corresponds to the yellow structure. TCPTP aminoacids when referred is shown in brackets. Alignment was performed between non complexed PTP1B structure, PDB: 3A5K, and non complexed TCPTP structure, PDB: 1L8K, by the method jFATCAT_rigid, using the Structure comparison tool of RCSB Protein Data Bank.

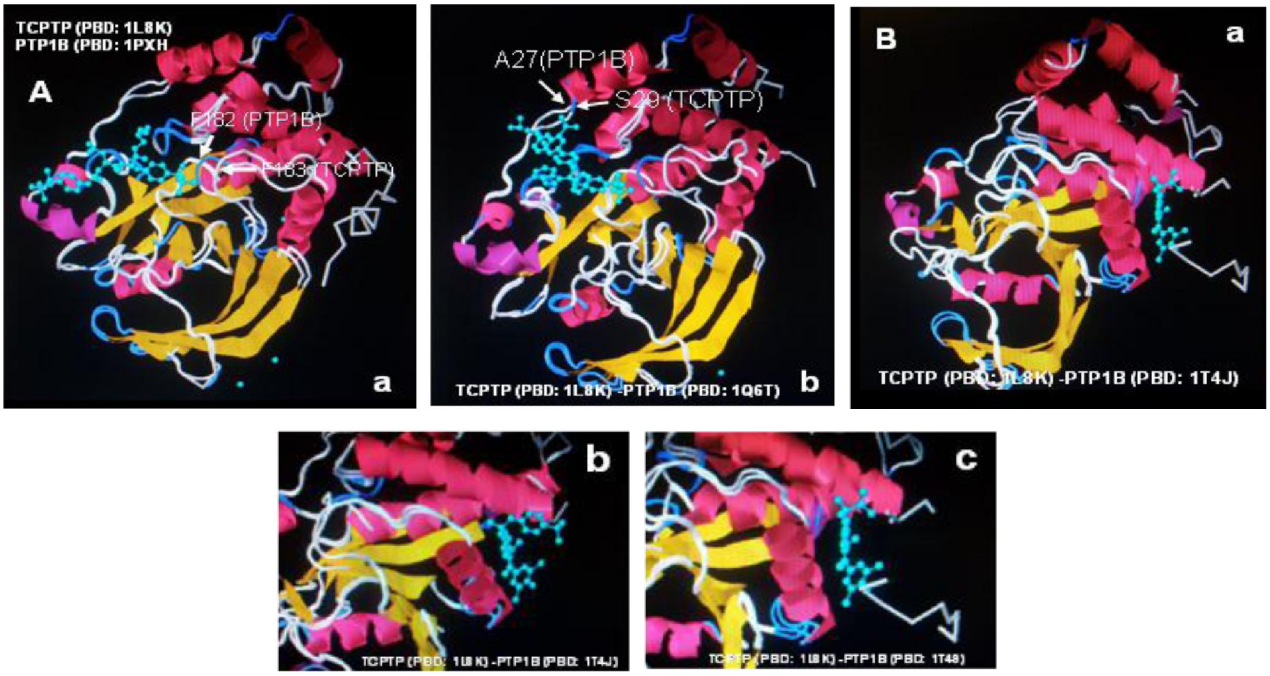

Figure 11 : Comparison between the non complexed structure of TCPTP and the structure of PTP1B complexed with the nhibitor 3-[[2-[4-[difluoro(phosphono) methyl]phenyl]acetyl] amino]-4-[[(2S)-3-[4-[difluoro(phosphono)methyl]phenyl]-1-oxo-1-(pentylamino) propan-2-yl] amino]-4-oxobutanoic acid (PDB: 1PXH) (Figure 11Aa), with the inhibitor [6-[4-[(2R)-2-(benzotriazol-1-yl)-3-[4-[difluoro(phosphono)methyl]phenyl]-2-phenylpropyl]phenyl]-2-[(1S)-1-methoxy-3-methylbutyl]quinolin-8-yl]phosphonic acid (PDB: 1Q6T) (Figure 11Ab),with the inhibitor 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N,N-dimethyl-1-benzofuran-6-sulfonamide (PBD:1T48, figure 11Ba and 11Bc) or the inhibitor 3-(3,5-dibromo-4-hydroxybenzoyl)-2-ethyl-N-(4-sulfamoylphenyl)-1-benzofuran-6-sulfonamide. (PBD:1T49, Figure Bb), (RCSB PDB Protein Comparison Tool).

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Combining the observations concerning the molecules occupying simultaneously the sites A1, A2 and B or A1, A2 and C, one can notice that existence of three hydrogen donor/acceptor moieties at distances of 8.6-13,5 Å and 10-14 Å with each other, with the appropriate angle or a bending capacity round the central donor/acceptor group may enable simultaneus interaction with active site A (A1,A2) and the secondary site B or C. Interactions with the secondary sites increase the possibility of developing more effective and highly selective inhibitors, since, more differences in aminoacids between PTP1B and the highly similar phosphatase TCPTP are located in these areas.

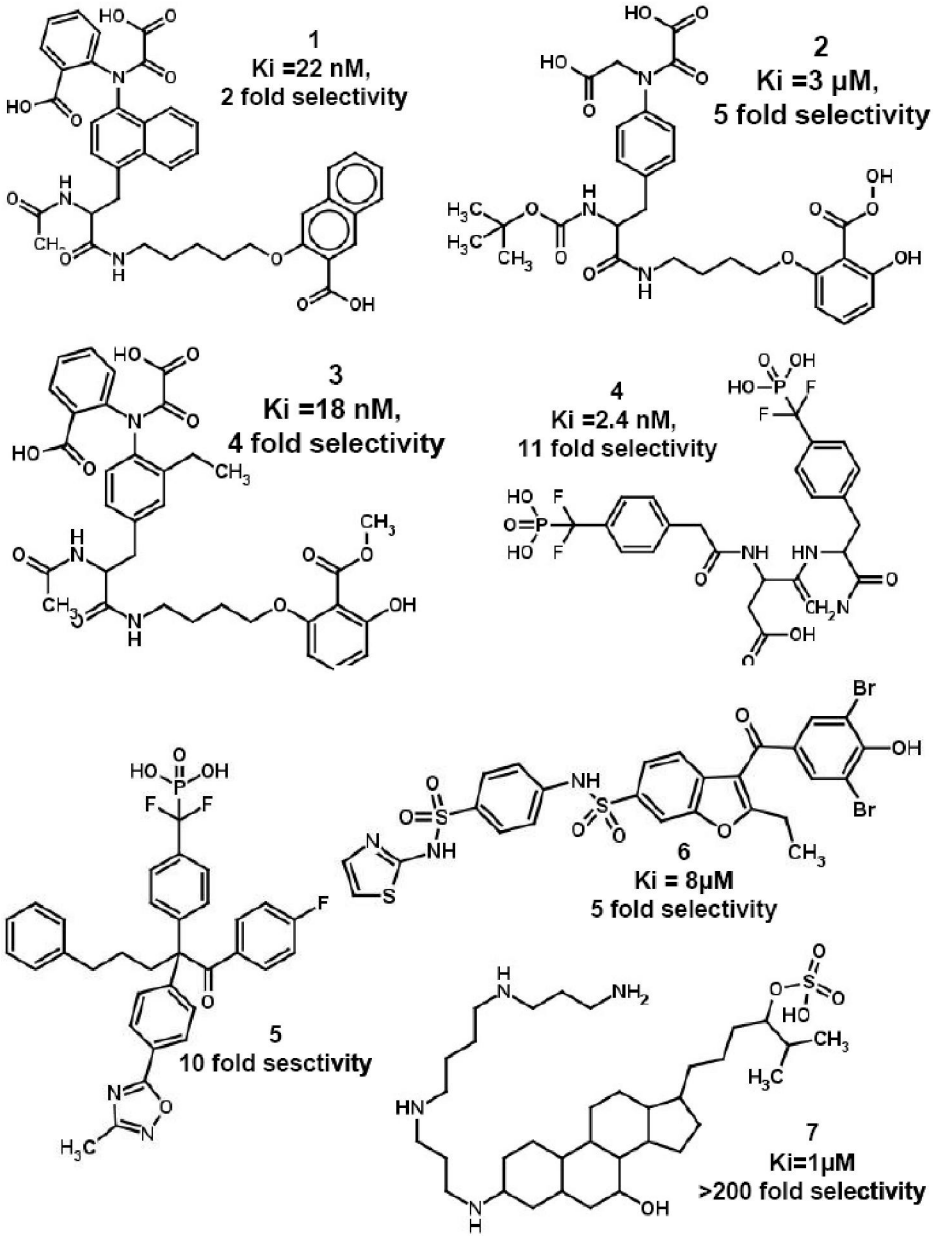

Figure 12 : Indicative structures of selective PTP1B inhibitors. Molecules 1-4 are believed to interact with the active site of the enzyme. Compounds 5, 6 and 7 are considered to interact with an allosteric site.

Structures with many hydrogen donor/acceptor groups seam to favor interaction with the allosteric centre. Hydroxyl and carbonyl groups as well as SO_2 groups may represent the polar parts of the molecule, while many favorable orientations of these groups within the structure may occur. Existence of an aromatic moiety at appropriate position may increase activity and selectivity of the inhibitors.

Cell penetration is one of the problems commonly faced in the effort of development of potent PTP1B inhibitors. The first molecules, mimicking phospho-tyrosine residues of the natural substrate contained negatively charged groups such as phosphate and carboxylic groups. These charged molecules had a great difficulty in penetrating cell membranes. However, recent results have indicated that other[73] hydrogen donor/acceptor groups may be used, such as hydroxy- and oxo-, alkoxy-, $-SO_2$ and $-NO_2$ group[86]. Incorporation of such non charged groups may lead to the development of molecules with improved cell permeability properties.

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