Synthesis, Molecular Docking and PTP1B Inhibitory Activity of Functionalized 4,5-Dihydronaphthofurans and Dibenzofurans[#]

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Abstract: Protein tyrosine phosphatase 1B (PTP1B) is an enzyme that plays a critical role in down-regulating insulin signaling through dephosphorylation of the insulin receptor. Inhibitors of PTP1B showed increased insulin sensitivity and normalize plasma glucose level and thus are useful therapeutic agents for the treatment of diabetes. A series of functionalized 4,5-dihydronaphthofurans and dibenzofurans were synthesized, studied through molecular docking and evaluated for their PTP1B inhibitory activity.

Key Words: Diabetes, PTP1B inhibitor, 4,5-dihydronaphthofuran, dibenzofuran, molecular docking

INTRODUCTION

Type 2 diabetes is characterized by a defect in the insulin-signaling cascade, which occurred mainly by defective or inappropriate regulation of protein tyrosine phosphatases (PTPs) activity [1]. Several PTPs such as PTPα, PTPε, PTP1B, T-cell PTP, leukocyte antigen-related (LAR), and CD-45 have been implicated as negative regulators of the insulin-signaling pathway through dephosphorylation of the insulin receptor (IR) [1,2]. Among them, PTP1B plays a crucial role in the down regulation of insulin signaling [3]. Studies on PTP1B knockout mice displayed increased sensitivity to insulin in skeletal muscle and liver and increased IR autophosphorylation [4]. Thus, PTP1B inhibitors could potentially ameliorate insulin resistance and normalize plasma glucose and insulin without inducing hypoglycemia and could be potential pharmacological agents for the treatment of T2DM.

Over the last two decades, tremendous amount of efforts have been devoted on developing orally active PTP1B inhibitors for the treatment of diabetes. Majority of known inhibitors possess tyrosine mimetic structures functionalized with negatively charged moieties such as phosphonates [5], malonates [6], carboxylates [7], or cinnamates [8]. Although highly potent PTP1B inhibitors have been identified, most of these are either too large, too negatively charged or too lipophilic leading to lack of the necessary physicochemical properties that are required for bioavailability and high efficacy [1].

The development of small molecule PTP1B inhibitors has emerged only recently as a rapidly growing area of investigation in medicinal chemistry [9]. Recently Malamas and co-workers [10] reported two novel series of benzofuran/benzothiophene biphenyl oxo-acetic acids (**I**) and sulfonyl-salicylic acid as potent inhibitor of PTP1B with good antihyperglycemic activity. Wrobel and co-workers [11] reported series of 11-arylbenzo[*b*]naphtho[2,3-*d*]furans/thiophenes **(II)** derivatives as illustrated in Fig. (**1**), which showed 25 times more selectivity for PTP1B over other PTPs. Hansen and co-workers [12] developed a series of benzofuran-cored non-competitive PTP1B inhibitors in which one of the compounds of prototype **III** showed PTP1B enzyme inhibition with IC_{50} of 22 μM and displayed enhanced insulin signaling in cells. The inhibitor binds to a novel allosteric site roughly 20Å away from the catalytic pocket.

Based on the ligand-receptor binding interactions of known PTP1B inhibitors, it was envisaged that simulation of both hydrophilic and hydrophobic moieties such as carboxylates, hydroxy and methylsulfanyl onto a 4,5-dihydronaphthofuran and dibenzofuran scaffold might lead to potential PTP1B inhibitors. Molecular docking analysis of a series of 4,5-dihydronaphthofurans (**3**) and dibenzofurans (**5**) revealed that compounds of the prototype **5** fit nicely in the pocket when docked into a human PTP1B enzyme active site. In this paper, we report synthesis, molecular modeling studies, and PTP1B inhibitory activity of functionalized 4,5-dihydronaphthofurans and dibenzofurans, which are involved in several non-covalent interactions with the crucial residues of PTP1B catalytic site and have been found to demonstrate good inhibitory activity.

METHODS AND MATERIALS

Molecular Structures and Optimization

PTP1B consists of 435 amino acids, which possesses a catalytic motif phosphate-binding loop (P-loop) defined by residues 214-221 (His-Cys-Ser-Ala-Gly-Ile-Gly-Arg) of which Cys215 and Arg221 amino acids are conserved in PTPs [13]. Apart from these residues, a number of other amino acids such as Lys120, Gln262, Val49, Arg47, Phe182 and Tyr46 contribute significantly to peptide substrate recognition by a combination of electrostatic, hydrophobic and hydrogen-bonding interactions [14].

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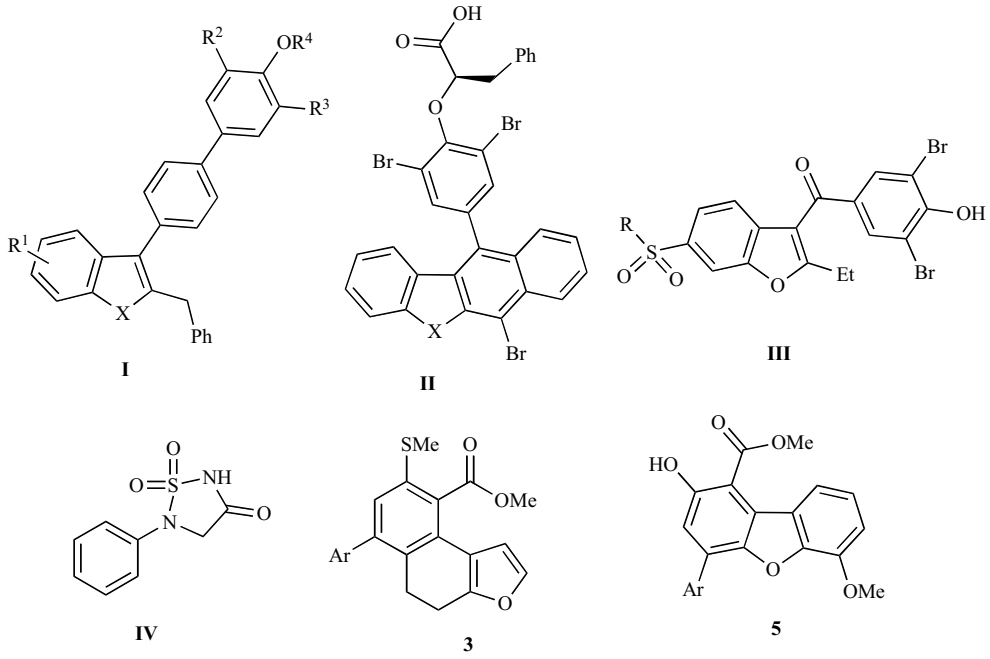


Fig. (1). Structures of known PTP1B inhibitors (**I**-**IV**) [10-12].

The compounds used for docking simulation were constructed using the Builder module of Insight II [15]. The geometries of these compounds were subsequently optimized by Sybyl7.0 [16] using Tripos force field and Gasteiger-Huckel charges [17]. Energy minimization was done using the Powell method with an energy convergence gradient of 0.001 kcal/mol. The high resolution X-ray structure of Human PTP1B protein (PDB Code 2BGE) [18] was used for the docking studies without further modification, i.e the protein neither underwent additional minimization nor were any side chain conformations changed. Docking simulations were carried out using FlexX program [19,20] interfaced with Sybyl7.0, which is a fast algorithm for the flexible docking of small ligands into fixed protein binding sites using an incremental construction algorithm. Based on the previously reported structural information, the active site regions for the comparative FlexX docking simulations of the compounds with Human PTP1B were constructed. The proposed interaction modes of the ligand with the PTP1B binding site were determined as the highest scored conformation among the thirty conformations. FlexX uses a pure empirical scoring function similar to that developed by Rarey [20] and BÖhm [21]. The binding free energy of a protein/ligand complex was estimated as the sum of free energy contributions from hydrogen bonding, ion-pair interactions, hydrophobic and π-stacking interaction of aromatic groups and lipophilic interactions.

Validation of the Docking Method

To ensure that the ligand orientation and the position obtained from the docking studies were likely to represent valid and reasonable binding modes of the inhibitors, the FlexX program docking parameters had to be first validated for the crystal structure used (2BGE). The ligand 1,2,5-thiadiazolidin-3-one-1,1-dioxide (**IV**, Fig. **1**), in the conformation found in the crystal structure, was extracted and docked back to the corresponding binding pocket, to determine the ability of FlexX to reproduce the orientation and position of the inhibitor observed in the crystal structure. Results of the control docking showed that the FlexX determined the optimal orientation of the docked inhibitor **IV** (docking energy: -18.09 KJ/mol), close to that of the original orientation found in the crystal. The low r.m.s. of 0.62 Å deviation between the docked and crystal ligand coordinates indicate very good alignment of the experimental and calculated positions (Fig. **2**).

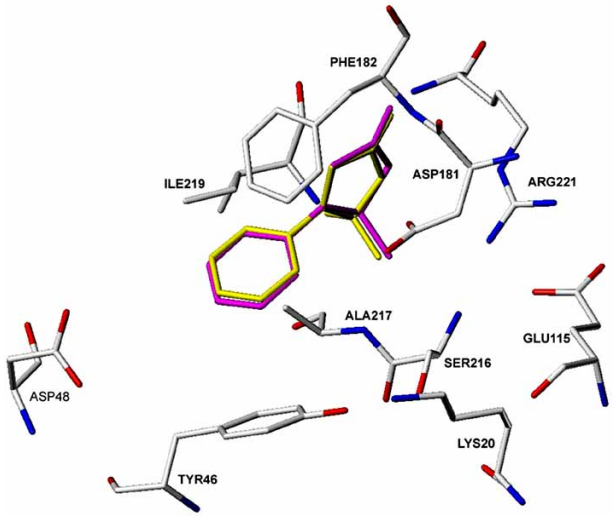


Fig. (2). Conformation of crystallographic 1,2,5-thiadiazolidin-3-one-1,1-dioxide (magenta) as compared to the docked conformation (Yellow).

We performed the docking experiments on the compounds 1,2,5-thiadiazolidin-3-one-1,1-dioxide (**IV**), 6-(4-fluorophe-

nyl)-8-methylsulfanyl-4,5-dihydronaphtho[2,1-*b*]furan-9-carboxylic acid methyl ester (**3a)** and 4-(4-fluorophenyl)-2-hydroxy-6-methoxy-dibenzofuran-1-carboxylic acid methyl ester (**5a)** with the aim to predict their proposed binding mode in the PTP1B catalytic site and to obtain information for the design of new potential PTP1B inhibitors. The 3-D structures of the docked compounds **3a** and **5a** were compared with the X-ray crystallographic structure of bound inhibitor 1,2,5-thiadiazolidin-3-one-1,1-dioxide (**IV**). A structural analysis of the binding mode found in these docked compound and 1,2,5-thiadiazolidin-3-one-1,1-dioxide showed that 4,5-dihydronaphtho[2,1-*b*]furan **3a** with -9.18 KJ/mol as FlexX docking energy, does not snugly fit into the catalytic site of PTP1B and was slightly off-set from the pocket.

Crucial interactions required for the inhibitor binding, involving residues Arg221, Ser216 and Asp181 were found to be missing in **3a**-PTP1B complex. However, only the carbomethoxy group of **3a** was involved in H-bond interaction with Tyr46 and Lys120 and the complex was mostly stabilized by non-covalent contacts including aromatic-aromatic interactions between **3a** and PTP1B catalytic site residues.

On the other hand, molecular modeling studies on dibenzofuran **5a** (docking energy: -15.77 KJ/mol) with adjacent hydroxy and carbomethoxy functionality showed that it bind the catalytic site in an extended conformation and engage in important contacts with the catalytic site residues such as electrostatic interactions with Arg221, H-bonds with the main chain nitrogen of Ser216 and carboxyl group of Asp181, van der Waals contact with several aliphatic side chains, and aromatic-aromatic interaction with Tyr46 and Phe182 (Fig. **3**).

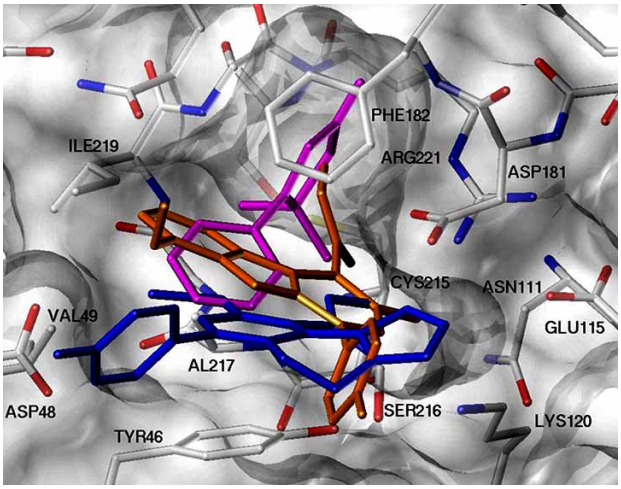


Fig. (3). Overlay of docked conformations of compounds **3a** (blue) and **5a** (orange) on the X-ray crystallographic structure of bound 1,2,5-thiadiazolidin-3-one-1,1-dioxide (magenta) in the PTP1B catalytic site. Residues colored by atom type.

Fig. (**4**) shows the binding interactions of the dibenzofuran **5a** with PTP1B enzyme. It is evident that **5a** bind deep into the pocket of PTP1B and is involved in a network of several hydrogen bonds with key residues in the catalytic site, especially residue Asp181, which is responsible for the substrate phosphate binding. The carbomethoxy group of **5a** is involved in a number of H-bond interactions with Ser216, Ala217, Asp181 and electrostatic interaction with Arg221. The hydrophobic methyl group of the ester formed favorable interactions with hydrophobic residues Phe182 and Ile219 in the active site. The hydroxyl group was involved in the H-bond network with –OH group of Tyr46 and backbone nitrogen; hydroxyl groups of Ser216; guanidinium nitrogen of Arg221; carboxyl groups of Asp181 and Glu115. The docking results showed that the aromatic ring of **5a** was sandwiched between Tyr46 and Phe182 and was involved in the formation of hydrophobic stacking interactions similar to the interaction of the phenyl ring of the pTyr residue of the substrate [22].

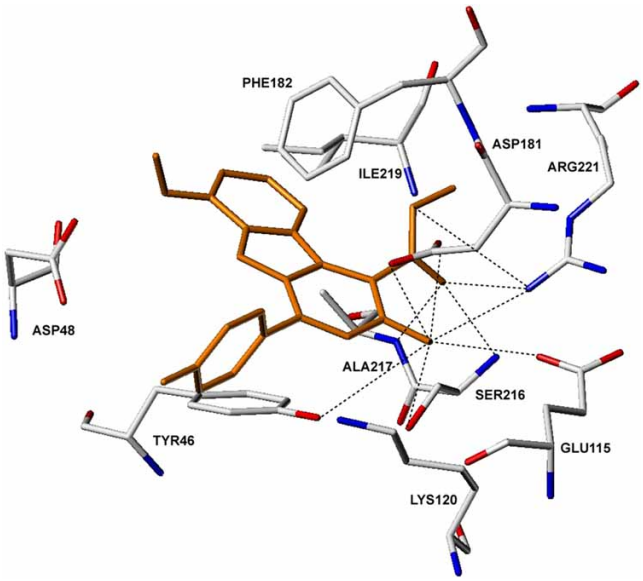


Fig. (4). Docked conformation of compounds **5a** (orange), showing interactions with neighbouring residues through H-bonding in the PTP1B catalytic site.

Our modeling studies also highlighted that, although many of the residues in contact with compound **5a** are not unique to PTP1B, the combination of all contact residues differ between PTP1B and a closely related enzyme leukocyte antigen-related (LAR), which share approximately 68% identity in the inhibitor binding site corresponding to PTP1B. This suggests that the binding surface defined by these residues may determine the selectivity of the inhibitor. To better understand the selectivity issue, the crystal structures of PTP1B and LAR [23] were compared, to identify residue that are unique to PTP1B in the catalytic site. Some of the residues are Asp48, Phe182 and Ile219, whereas the corresponding residues in LAR are Asn1357, His1491 and Val1526 respectively. Our docking studies demonstrates that the side chain of Ile219 clearly interact with the bound inhibitor **5a**, providing some degree of binding energy (Fig. **4**). Substitution of Ile with Val (as in LAR sequence), probably will have effect of reducing the extent of interaction between LAR and the inhibitor, with consequent loss of binding affinity. Comparison of the two structures (PTP1B and LAR) clearly indicate that a smaller side chain in position of 219 in PTP1B may not be able to exploit the same function as the

Ile, thus suggesting that Ile219 may act as selectivity determinant for the compound **5a** between PTP1B and LAR.

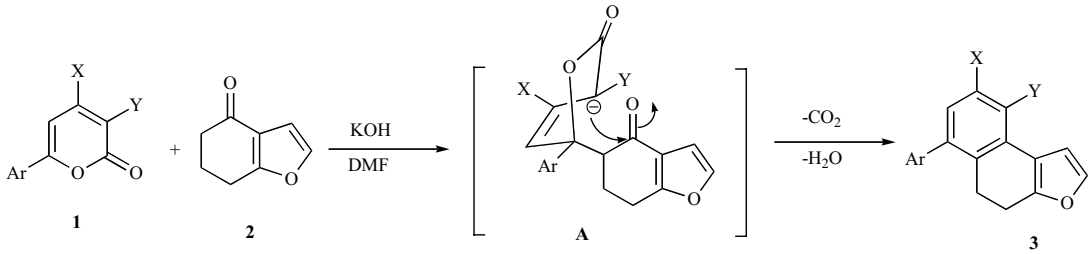
CHEMISTRY

Synthesis of Substituted 4,5-dihydronaphthofurans (3a-g) and Functionalized Dibenzofurans (5a-d)

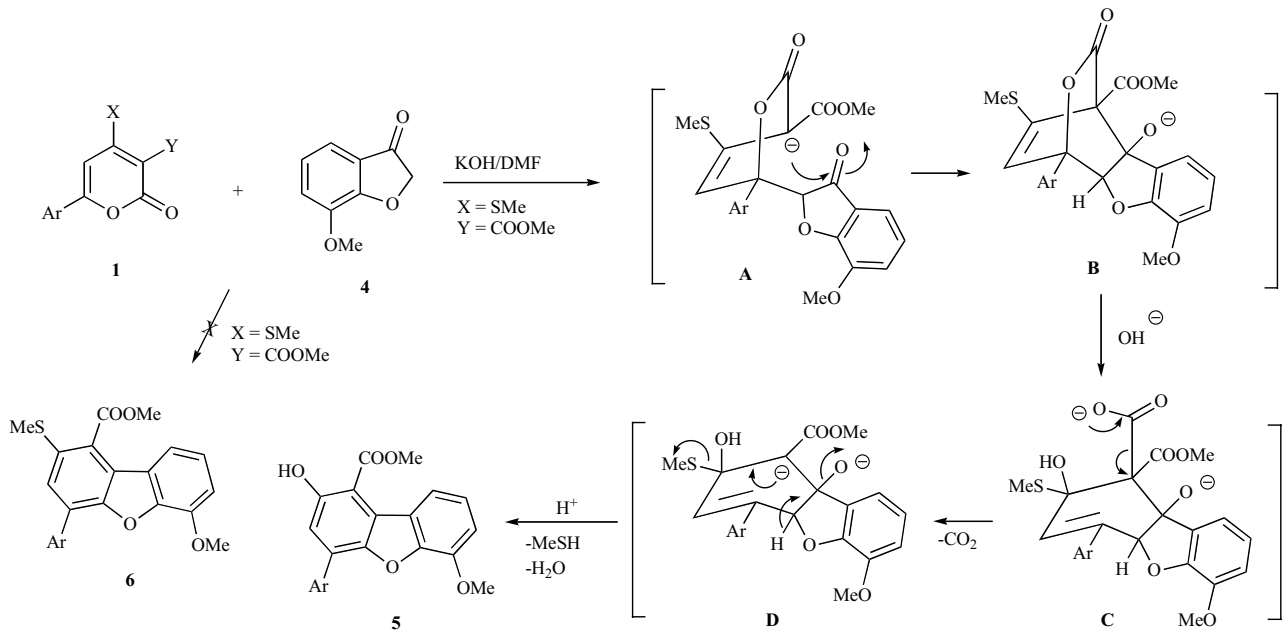
The 2*H*-pyran-2-ones (**1**) used as a parent precursor have been conveniently prepared by the reaction of methyl 2-carbomethoxy/cyano-3,3-dimethylsulfanylacrylate with acetophenone in high yield as described earlier [24]. The unique feature of 2*H*-pyran-2-ones **1** is the presence of three electrophilic centres; C2, C4 and C6, which can be exploited regioselectively by reacting with various C-, N- and S-nucleophiles to generate molecular diversity [25]. The presence of three electrophilic centres in lactone **1**, position C6 is highly susceptible to nucleophiles due to the extended conjugation and the presence of the electron withdrawing substitutent at position 3 of the pyranone ring. We have recently developed [26] an efficient route for the synthesis of 4,5-dihydronaphtho[2,1-*b*]furans (**3a-g**) by stirring an equimolar mixture of substituted 2*H*-pyran-2-one **1**, 6,7-dihydro-5*H*-benzofuran-4-one **2** and powdered KOH in dry DMF at room temperature for 24-30 hours as shown in Scheme **1** (Table **1**). The reaction is initiated by attack of the carbanion generated *in situ* from 6,7-dihydro-5*H*-benzofuran-4-one **2** at position C6 of the pyran-2-one, followed by cyclization involving carbonyl group and C3 of the pyran ring to form a bicyclic intermediate. This intermediate on decarboxylation, protonation and dehydration furnished 4,5-dihydronaphtho[2,1-*b*]furans in good yields.

It was envisaged that the reaction of 2*H*-pyran-2-one with benzofuran-3-one would analogously furnish dibenzofurans **5a-d** following same reaction mechanism as described for the synthesis of 4,5-dihydronaphtho[2,1-*b*]furans. Thus, diversely substituted dibenzofurans were prepared by stirring an equimolar mixture of the 2*H*-pyran-2-one **1**, 7-methoxybenzofuran-3-one **4** and powdered KOH in dry DMF in an inert atmosphere at ambient temperature for 4-6 hours as shown in Scheme **2** (Table **1**). The spectroscopic analysis of a ring transformed product **5a** revealed that the reaction of 6-(4-fluorophenyl)-3-carbomethoxy-4-methylsulfanyl-2*H*-pyran-2-ones (**1**) with 7-methoxybenzofuran-3-one **4** afforded 2-hydroxy-6-methoxy-4-aryl-dibenzofuran-1-carboxylic acid methyl ester (**5a**) instead of corresponding 2-methylsulfanyl derivatives (**6**).

The mechanism, depicted in Scheme **2**, implies that the reaction is initiated by attack of the carbanion generated *in situ* from **4** at position C6 of the 2*H*-pyran-2-one **1**, followed



Scheme 1.



Scheme 2.

Table 1. *In Vitro* PTP1B Enzyme Inhibitory Activity for the Compounds 3a-g, 5a-d

Entry	Ar	X	Y	PTP1B enzyme inhibitory activity			
				-Triton[b] X-100	+Triton[c] X-100		
				Inhibition[a] (%)	Inhibition[a] (%)	IC_{50} (μM)	*Ki* (μM)
3a	4-FC_6H_4	SMe	COOMe	52.5	55.5	90	25
3b	4-ClC_6H_4	SMe	COOMe	69.2	64.6	72	86
3c	4-BrC_6H_4	SMe	COOMe	62.8	52.6	96	56
3d	3,4-$Cl_2C_6H_3$	SMe	COOMe	73.0	69.7	70	50
3e	thien-2-yl	SMe	COOMe	74.0	64.4	71	37
3f	3,4-$CH_2O_2C_6H_3$	piperidine	CN	11.4	-	-	-
3g	4-ClC_6H_4	4-methyl-piperidine	CN	6.3	-	-	-
5a	4-FC_6H_4	--	--	95.1	77.2	53	30
5b	4-BrC_6H_4	--	--	76.8	74.8	69	29
5c	4-$OMeC_6H_4$	--	--	89.0	74.6	64	38
5d	4-MeC_6H_4	--	--	50.0	58.1	80	40
Sodium vanadate		--	--	56			

[a]Values are mean from three independent sets of experiments tested at 100 μM concentration.

[b]Compounds were examined without Triton X-100.

[c]Compounds were examined in the presence of 0.01% Triton X-100.

by intramolecular cyclization involving the carbonyl group of benzofuran-3-one and C3 of the pyranone to form a intermediate B. The intermediate **B** is electrophilic in nature and hydroxide may attack at this position to form intermediate **C**, followed by decarboxylation, protonation and elimination of methyl mercaptan and water furnished **5a-d** in high yields. All the synthesized compounds were characterized by spectroscopic analysis [26].

RESULT AND DISCUSSION

Our objective was to design annulated benzofuran-based PTP1B inhibitors to identify lead structures through an *in silico* approach and evaluate their inhibitory activity. Vanadate is a non-selective inhibitor of PTPs, and studies have shown that treatment with vanadate can normalize blood glucose level in diabetics [1]. Taking sodium vanadate as a control, we evaluated PTP1B inhibitory activity [27] of functionalized 4,5-dihydronaphthofurans and dibenzofurans at 100μM concentration and their results are summarized in (Table **1**). It is evident from the activity profile of 4,5-dihydronaphthofurans functionalized with methylsulfanyl and carbomethoxy groups (**3a-e**) that the compounds demonstrated moderate to good PTP1B inhibitory activity (52-74%) at 100μM concentration. The compounds functionalized with amine and nitrile functionalities **3f,g** were found to be incapable of inhibiting PTP1B enzyme possibly due to the lack of ester functionality which forms several non-covalent interactions with the active residues of PTP1B (Fig. **4**). The predicted binding affinities obtained with the docking simulation of dibenzofurans **5a-d** were found to correlate well with the experimental values. The best-docked compound **5a** ranked on the basis of the total FlexX score was found most active in the inhibition assay (95%). Other structurally very similar compounds **5b,c** also displayed good inhibition against PTP1B enzyme.

Recent studies have demonstrated that the formation of aggregates of nonspecific inhibitors (promiscuous inhibitors) sometimes play a major role in displaying enzyme inhibitory activity rather than a single 1:1 ligand-protein interaction [28]. These aggregates have shown inhibition by interacting with protein through adsorption or absorption mechanism. It has also been demonstrated that promiscuous inhibition can be prevented and reversed using an appropriate concentration of nonionic detergents such as Triton X-100, saponin, or digitonin without compromising the enzyme assay performance [29]. In order to ruled out the possibility of promiscuous inhibition in our screening results, we examined the compounds **3a-g**, **5a-d** at 25, 50, 75, and 100 μM concentration in the presence of a detergent 0.01% Triton X-100. The IC_{50} and the dissociation constant *Ki* of the compounds are shown in Table **1**, which suggests that the inhibitory activity of these compounds did not significantly changed by the addition of an appropriate concentration of Triton X-100, as expected for promiscuous inhibitors. In contrast, compounds **3a** and **5d** showed increased catalytic enzyme activity in the presence of Triton X-100 probably due to the detergent causing a reduction in nonspecific protein binding

onto the experimental plates. A decrease in enzyme inhibitory activity was observed for the compounds **3b-g** and **5a-c** in the presence of Triton X-100, which suggests that the inhibitory activity may be due to the partial formation of aggregates of these compounds. Among all the screened compounds, three compounds **5a-c** showed PTP1B enzyme inhibition in the range of 74-77% in the presence of Triton X-100 with IC_{50} and *K*i in the range of 53-69 μM and 29-38 μM, respectively.

In conclusion, we have demonstrated synthesis, molecular modeling studies of a new series of 4,5-dihydronaphthofurans and dibenzofurans, which possessed good inhibitory activity against PTP1B. Molecular docking studies showed that hydroxy and carbomethoxy functionalities at adjacent positions on dibenzofuran scaffold are crucial for inhibitory activity as they are involved in a number of H-bond interactions with Ser216, Ala217, Asp181, Tyr46 and electrostatic interaction with Arg221. The compound **5a,** which showed docked energy –15.77 KJ/mol demonstrated 95 % inhibition against PTP1B. A series of dibenzofurans **5a-c** demonstrated good inhibition against PTP1B and are useful candidates as leads for the development of potential antihyperglycemic agents. Further studies in this area are currently in progress.

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