



## Short communication

# Design, synthesis and molecular modelling studies of novel 3-acetamido-4-methyl benzoic acid derivatives as inhibitors of protein tyrosine phosphatase 1B



Monika Rakse<sup>a,2</sup>, Chandrabose Karthikeyan<sup>a,2</sup>, Girdhar Singh Deora<sup>a,1</sup>,  
N.S.H.N. Moorthy<sup>a,b</sup>, Vandana Rathore<sup>c</sup>, Arun K. Rawat<sup>d</sup>, A.K. Srivastava<sup>d</sup>,  
Priyush Trivedi<sup>a,\*</sup>

<sup>a</sup> CADD Laboratory, School of Pharmaceutical Sciences, Rajiv Gandhi Proudyogiki Vishwavidyalaya, Airport Bypass Road, Gandhi Nagar, Bhopal, MP 462036, India

<sup>b</sup> Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, 687, Rua de Campo Alegre, Porto 4169-007, Portugal

<sup>c</sup> Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Gachibowli, Hyderabad 500046, India

<sup>d</sup> Division of Biochemistry, Central Drug Research Institute, Lucknow, UP 226001, India

## ARTICLE INFO

## Article history:

Received 4 March 2013

Received in revised form

9 October 2013

Accepted 11 October 2013

Available online 23 October 2013

## Keywords:

Diabetes

PTP1B

Acetamido benzoic acid derivatives

Molecular modelling

Lead optimization

## ABSTRACT

A novel series of 3-acetamido-4-methyl benzoic acid derivatives designed on the basis of vHTS hit ZINC02765569 were synthesized and screened for PTP1B inhibitory activity. The most potent compounds 3-(1-(5-methoxy-1H-benzo[d]imidazol-2-ylthio)acetamido)-4-methyl benzoic acid (**10c**,  $IC_{50}$  8.2  $\mu$ M) and 3-(2-(benzo[d]thiazol-2-ylthio)acetamido)-4-methyl benzoic acid (**10e**,  $IC_{50}$  8.3  $\mu$ M) showed maximum PTP1B inhibitory activity. Docking studies were also performed to understand the nature of interactions governing the binding mode of the designed molecules within the active site of the PTP1B enzyme.

© 2013 Elsevier Masson SAS. All rights reserved.

## 1. Introduction

Diabetes mellitus is a complex metabolic syndrome, has a major human health concern over the world and is estimated to affect 300 million people by the year 2025. Indians alone may have about 1/5 of the global diabetic population by 2025 [1–4] due to hereditary, ageing, poor diet, obesity and sedentary life style [5]. Severe diabetes may leads to poor wound healing, blurring vision, kidney damage, cardiovascular diseases and premature death [6]. Protein tyrosine phosphatase 1B (PTP1B) have emerged as a promising therapeutic target of diabetes mellitus as it down regulates insulin transduction mediated by receptor tyrosine kinases such as insulin receptor and epidermal growth factor [7,8]. Studies independently

generated by two laboratories, with PTP1B knockout mice, have demonstrated that the targeted disruption of the PTP1B gene in mice resulted in enhanced insulin sensitivity. Thus, PTP1B is considered as an attractive therapeutic target for type-II diabetes [9,10].

The crystal structure of PTP1B enzyme is well explored [11,12] and the main structural feature of PTP1B enzyme is the catalytic site which contains two aryl phosphate-binding sites: a high-affinity catalytic site (containing the nucleophile cysteine residue, Cys215) and a low-affinity non-catalytic site (demarcated by Arg24 and Arg254 residues). The inhibitor binding with high-affinity catalytic site is deemed to be significant for its potency whereas the low-affinity non-catalytic site has been frequently exploited by medicinal chemist for enhancing the ligand binding affinity and selectivity for inhibition of PTP1B over other PTPs.

The catalytic site of PTP1B enzyme accommodates pTyr, which contains two negative charges at physiological pH [13]. Because of the electrostatic properties of the enzyme active site, the most commonly adopted strategy in the design of PTP1B inhibitors is to

\* Corresponding author. Tel.: +91 755 2678883; fax: +91 755 2742001.

E-mail addresses: [piyush.trivedi@rgtu.net](mailto:piyush.trivedi@rgtu.net), [drtrivedislab@gmail.com](mailto:drtrivedislab@gmail.com) (P. Trivedi).

<sup>1</sup> Current address: The University of Queensland, School of Pharmacy, Brisbane, Qld 4072, Australia.

<sup>2</sup> These authors contributed equally to this work.

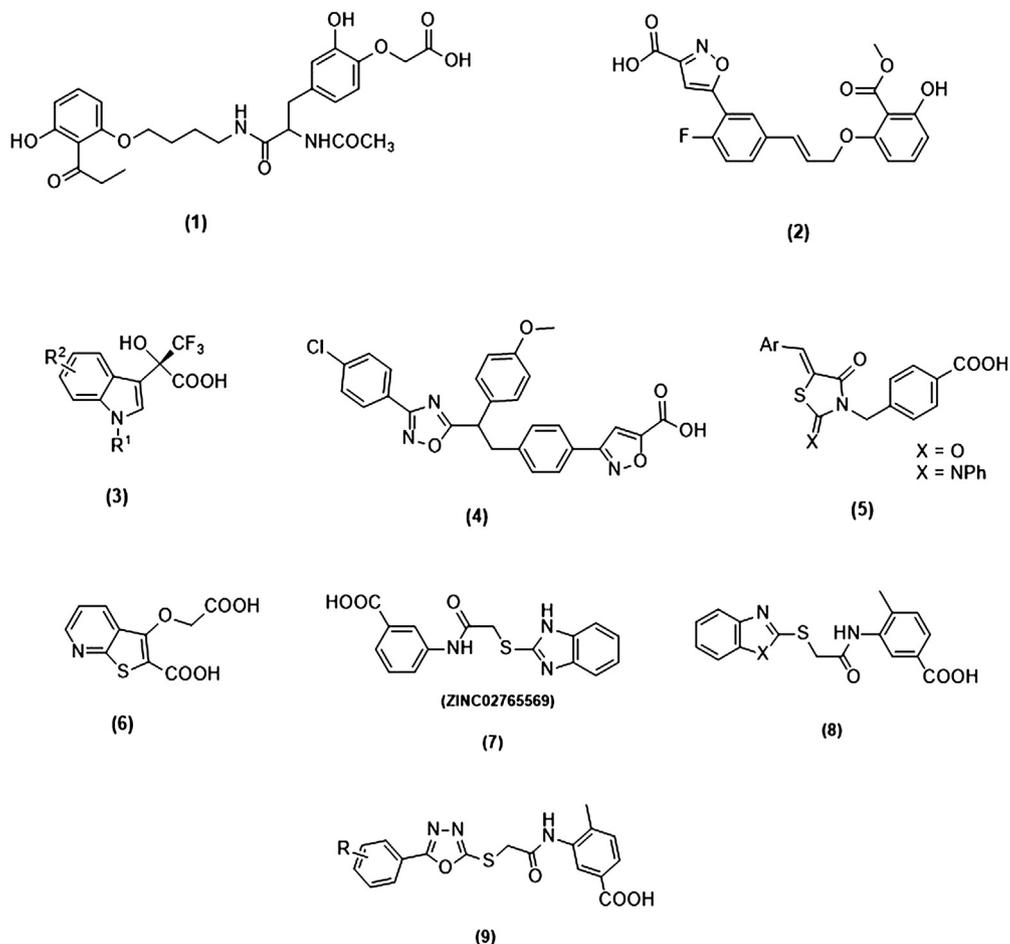


Fig. 1. Chemical Structures of some reported PTP1B inhibitors (1–7) and compound synthesized in the study (8).

insert non-hydrolysable pTyr-mimetic groups that are able to replicate the interactions of pTyr residue with the active site of the enzyme onto different optimal templates. Most of previously used pTyr mimetic groups such as phosphonates [14], carboxylic acids [15], sulphonamides [16], sulphanamic acids [17] have proven lack of cell permeability and oral bioavailability because of the strong negative charge carried by the pTyr mimetics as well as their high molecular weight [18]. Hence, there is an urgent need to discover new and orally bioavailable PTP1B inhibitors that can combat type-II diabetes. Several efforts aimed at developing potent and bioavailable PTP1B inhibitors integrating a carboxylic group as pTyr mimetic have been recorded in literature. Some examples of carboxylic acids endowed with cellular activity and/or oral availability are (2-hydroxyphenoxy) acetic acid derivatives (1) [19], isoxazole carboxylic acid derivatives (2) [20], 2-aryl-3,3,3-trifluoro-2-hydroxypropionic acids (3) [21], heterocyclic carboxylic acid derivatives (4) [22], 4-[(5-arylidene-2-arylimino-4-oxo-3-thiazolidinyl)methyl]benzoic acids (5) [23] and Thiophene carboxylic acid derivatives (6) [24] (Fig. 1).

More recently, our laboratory reported the discovery of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid (7) as inhibitor of PTP1B enzyme through a high throughput virtual screening of Zinc database [25]. The compound (ZINC02765569) presented modest PTP1B inhibition at 10  $\mu$ M concentration and good cellular permeability. Docking studies of the identified molecule revealed that the molecule shows all the relevant interactions essential for PTP1B inhibition. The acetamido benzoic acid moiety (ring A) of the molecule binds at the catalytic site of

PTP1B enzyme through a network of hydrogen bonds with the residues Cys215, Ser216 and Arg221 of catalytic site and the benzimidazole moiety (ring B) is accommodated inside the additional aryl phosphate binding site showing strong hydrogen bonding interaction with the residues Asp48 and Gln262 (Fig. 2). It has been previously described that inhibitor interaction with amino acid residues specifically Asp48 confers selectivity for PTP1B over

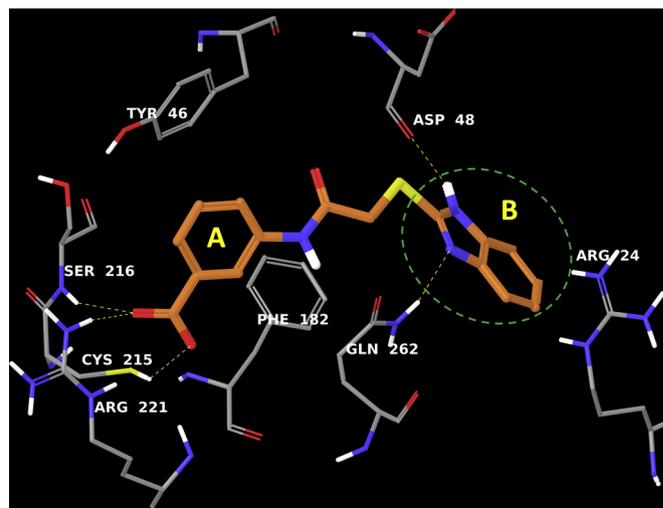


Fig. 2. Docked molecular model of ZINC02765569 in the active site of PTP1B enzyme.

other PTPs as this site and the channel communicating to the catalytic appear to be conserved in PTP1B [26]. These promising results alongwith the feasibility for structural derivatization of ZINC02765569 led us to expand our efforts in the synthesis of new diversely substituted 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid derivatives as potential PTP1B inhibitors. More particularly, we were interested in structural manipulations at ring A and ring B in order to derive 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid analogues with improved PTP1B inhibitory potency.

Our immediate goal was to identify the sites for structural manipulation through molecular docking studies. To this end, the results derived from docking of the lead compound ZINC02765569 with the active site of PTP1B enzyme were utilized. From the preliminary analysis of the inhibitor binding mode predicted by docking studies (Fig. 2), it was ascertained that a methyl substitution at position 5 in the ring A would be beneficial to facilitate additional interactions with the residue Tyr45 in the catalytic site. It was also hypothesized from the orientation of the benzimidazole ring in the non-catalytic site that substitution in the benzo ring of benzimidazole and replacement of benzimidazole ring with phenyl oxadiazole would be beneficial in terms of harnessing additional polar and hydrophobic interactions with amino acid residues in second aryl binding site.

Prompted by these findings, two series of compounds (**10a–e**, **15a–g**) (Tables 1 and 2) were prepared and their inhibitory activity against the PTP1B enzyme was assessed. The results from the binding assays were then used to re-evaluate the docking results in order to gain insight into their possible binding modes, allowing the development of more active compounds in the future.

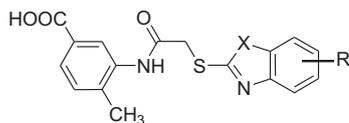
## 2. Result and discussion

### 2.1. Chemistry

Synthesis of the designed compounds (**10a–e**, **15a–g**) was accomplished by using known synthetic approaches [27–31]. The 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid derivatives (**10a–c**) alongwith 3-(2-(benzo[d]oxazol-2-ylthio)acetamido)-4-methylbenzoic acid (**10d**) and 3-(2-(benzo[d]thiazol-2-ylthio)acetamido)-4-methylbenzoic acid (**10e**) were prepared as outlined in Scheme 1. The synthesis began with chloroacetylation of 3-amino-4-methylbenzoic acid (**7**) in DMF under basic condition to afford 3-(2-chloroacetamido)-4-methylbenzoic acid (**7**). The obtained 3-(2-chloroacetamido)-4-methylbenzoic acid was then reacted with 1H-benzo[d]imidazole-2-thiols, benzo[d]oxazole-2-thiol and benzo[d]thiazole-2-thiol in acetone in the presence of anhydrous potassium carbonate to give the requisite compounds (**10a–e**). The preparation of 3-(2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid derivatives (**15a–g**)

**Table 1**

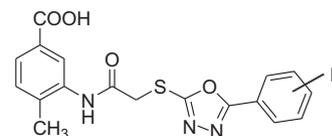
*In vitro* PTP1B enzyme inhibitory activity of the compounds (**10a–e**).



Compound	R	X	% PTP1B inhibition (at 10 $\mu$ M)	IC <sub>50</sub> ( $\mu$ M)
<b>10a</b>	H	NH	47.76	15.2
<b>10b</b>	5-NO <sub>2</sub>	NH	26.86	–
<b>10c</b>	5-OCH <sub>3</sub>	NH	57.46	8.2
<b>10d</b>	H	O	32.83	–
<b>10e</b>	H	S	57.46	8.5

**Table 2**

*In vitro* PTP1B enzyme inhibitory activity of the compounds (**15a–g**).

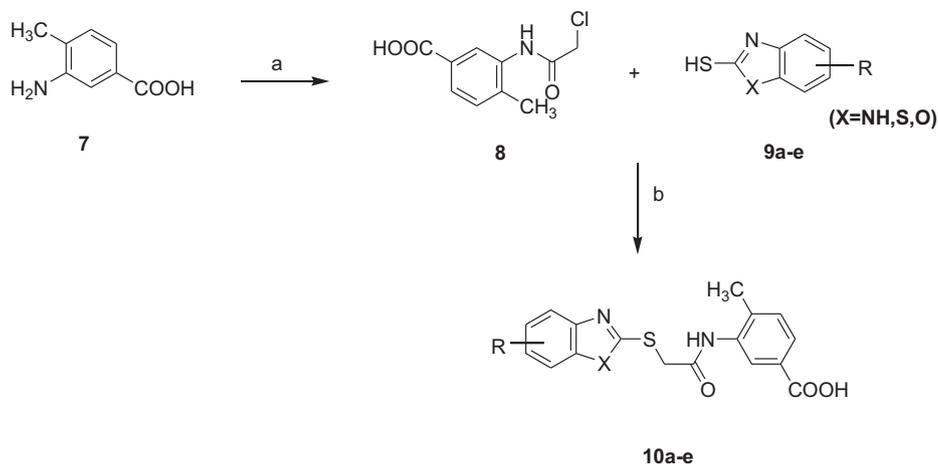


Compound	R	% PTP1B inhibition (at 10 $\mu$ M)	IC <sub>50</sub> ( $\mu$ M)
<b>15a</b>	2,3-di-Cl	29.85	–
<b>15b</b>	2-Cl	27.46	–
<b>15c</b>	2,4-di-Cl	43.28	–
<b>15d</b>	2-OH	38.80	–
<b>15e</b>	4-OH	47.01	10.3
<b>15f</b>	4-CH <sub>3</sub>	51.49	8.9
<b>15g</b>	3,4,5-tri-OCH <sub>3</sub>	50.74	9.7
ZINC02765569	–	24.7	–

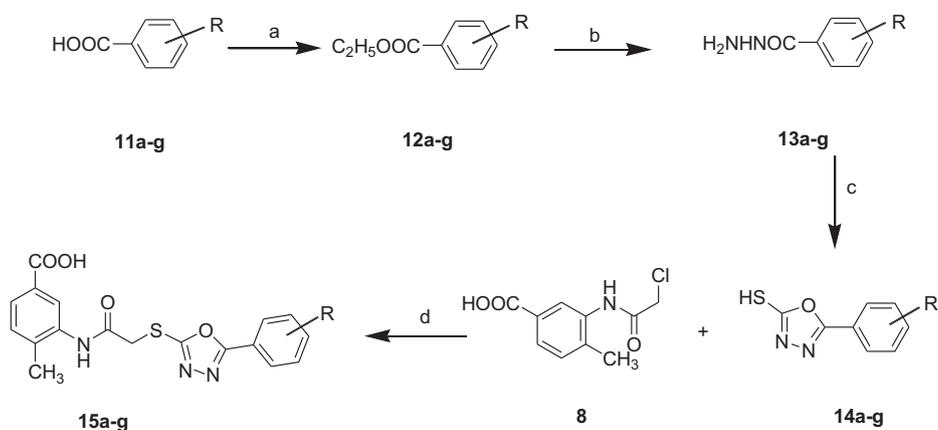
required a longer synthetic sequence included esterification, cyclization and elimination reactions, as shown in Scheme 2. Initially, the substituted 5-phenyl-1,3,4-oxadiazole-2-thiol derivatives were synthesized in three steps according to the reported procedure [30,31] using appropriate benzoic acids. The synthesized 5-phenyl-1,3,4-oxadiazole-2-thiol derivatives were subsequently linked to 3-(2-chloroacetamido)-4-methylbenzoic acid to give the desired 3-(2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid derivatives (**15a–g**).

### 2.2. *In vitro* PTP1B inhibition

The effect of synthesized compounds on protein tyrosine phosphatase inhibition was studied using colorimetric, non-radioactive PTP1B tyrosine phosphatase drug discovery kit-BML-AK 822 from Enzo Life Sciences, USA. The assay was done according to the Kit manufacturer's protocol. The results of the enzymatic assay are shown in Tables 1 and 2. In general, the compounds are moderate to potent inhibitors of PTP1B with the exception of compound **10b** and **15b**, which are significantly less active toward PTP1B. The unsubstituted benzimidazole derivative showed almost twice the potency exhibited by the lead compound ZINC02765569 which confirms the positive influence of the methyl substitution in benzoic acid moiety to PTP1B inhibition. Incorporation of electron withdrawing group in the fifth position of phenyl ring (**10b**) resulted in decreased PTP1B inhibitory potency whereas an electron releasing methoxy substitution (**10c**) in the same position improved PTP1B inhibitory activity. The best active compounds in the series are **10c**, and **10e** which showed PTP1B inhibitory activity greater than 50%. Interestingly, benzothiazole derivative (**10e**) has found to be equipotent with the benzimidazole derivative with methoxy substitution at fifth position of the phenyl ring (**10c**). However, replacement of the benzimidazole ring (**10a**) with a benzoxazole ring (**10d**) leads to drop in PTP1B inhibitory potency. Further, the compounds showing around 50% inhibition of PTP1B enzyme are further tested using same PTP1B drug discovery kit-BML-AK 822 at six different concentrations for calculation of their IC<sub>50</sub> value. The derived data has been analysed on Microsoft excel software and the IC<sub>50</sub> value is calculated (Tables 1 and 2) from the



**Scheme 1.** Reagent and conditions: (a) DMF, chloroacetyl chloride, RT, 24 h; (b)  $\text{K}_2\text{CO}_3$ , acetone, reflux, 6 h.



**Scheme 2.** Reagent and conditions: (a) ethanol, reflux, 8 h; (b)  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$ , reflux, 6 h; (c)  $\text{CS}_2$ , ethanol, reflux, 5 h; (d)  $\text{K}_2\text{CO}_3$ , acetone, reflux, 6 h.

values of percent inhibition obtained at different concentrations of the compounds.

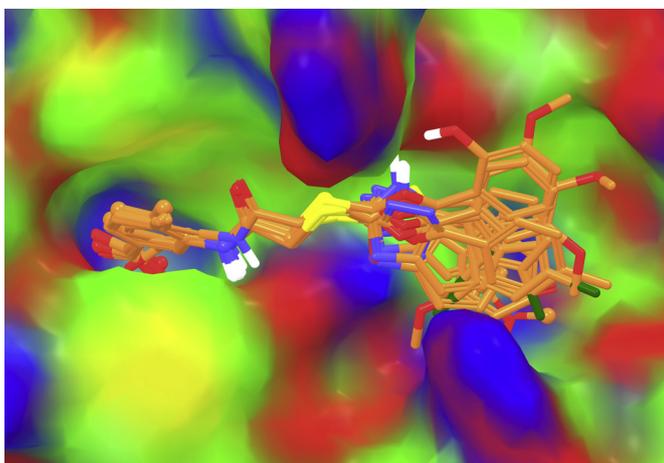
However, majority of the compounds in the phenyl oxadiazole series showed good PTP1B inhibitory potency except for compound **15b** which suggest that phenyl oxadiazole moiety is well tolerated at second aryl binding site of PTP1B enzyme. The most potent compound of the series **15f** bears a methyl group in the para position of the phenyl ring attached to the oxadiazole moiety.

Compound **15g** with 3,4,5 trimethoxy substitution in the phenyl ring also showed comparable potency to that of compound **15f**. Both the compounds manifest around 50% enzyme inhibition at 10  $\mu\text{M}$  concentration. A chlorine at ortho position (**15b**) of the phenyl ring resulted in drastic decrease in PTP1B inhibitory potency. Multiple chlorine substitutions in the phenyl ring favours PTP1B inhibitory activity if it is in ortho and para positions of the phenyl ring (**15c**) whereas it is detrimental when it is present in ortho and meta position of the phenyl ring (**15a**). Hydroxyl substituent at para position (**15e**) moderately enhanced PTP1B inhibitory activity relative to hydroxyl group in ortho position (**15d**).

Furthermore, the most active compound **10c** was screened for general toxicity studies using zebrafish embryos. The embryos were grown in the presence of the compound for 24, 48 and 72 h. Control embryos were incubated in 0.1% DMSO. Finally, embryos were observed using visible light microscopy at regular intervals after compound treatment to document general toxicity related effects such as developmental delays, deformations, oedema and death. The results indicate that tested compound **10c** having no any significant toxic effect on zebrafish embryos.

### 2.3. Molecular modelling

The docking studies reveal a common binding orientation of all the synthesized compounds in the catalytic binding pocket of PTP1B (Fig. 3). The benzoic acid moiety plays an important role in the binding, as both the oxygen atoms of carboxylic group are



**Fig. 3.** Binding orientation of synthesized molecules at the catalytic site of PTP1B.

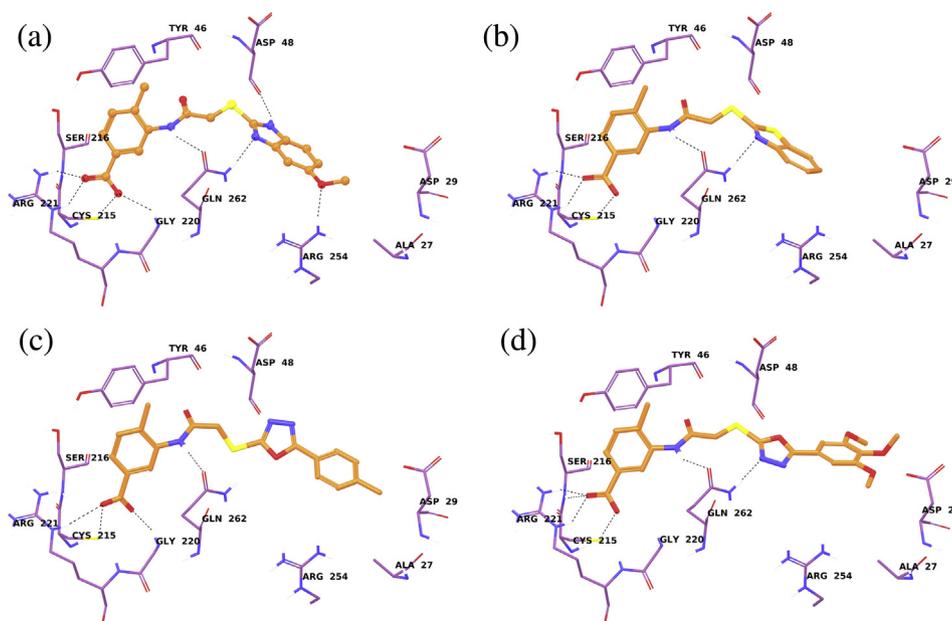


Fig. 4. Binding mode and interaction of synthesized compounds **10c** (a), **10e** (b), **15f** (c) and **15g** (d) with catalytic site residues of PTP1B.

involved in hydrogen bonding interactions with amino acid residues Cys215, Ser216, Gly220 and Arg221 at the catalytic site as shown in Fig. 4. A  $\pi$ - $\pi$  stacking interaction is also observed between phenyl ring of the benzoic acid moiety and the phenyl ring of the amino acid residue Phe182 in all the compounds. In addition, the methyl group in the benzoic acid moiety shows hydrophobic interaction with phenyl ring of the Tyr45 residue and the NH group of the acetamido benzoic acid moiety is involved in the hydrogen bonding interaction with the carbonyl oxygen atom of Gln262 amino acid. In addition to the above interactions, the nitrogen atom of benzimidazole/benzothiazole/benzoxazole rings of **10a–e** and oxadiazole ring of **15a–g** formed hydrogen bonding with Gln262 residue of the additional aryl phosphate binding site. Most notably, the benzimidazole derivatives **10a–c** showed extra hydrogen bonding interaction between the NH group of imidazole and Asp48. The most potent compound of the series **10c** shows an additional hydrogen bonding interaction with the Arg254 residue of the additional aryl phosphate binding site of PTP1B enzyme (Fig. 4a).

### 3. Conclusion

In conclusion, we describe herein structure guided optimization of a lead molecule (3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid) obtained through virtual high throughput screening. Incorporation of a methyl group vicinal to amino group on ring A and substitution on phenyl ring of benzimidazole ring (ring B) in the lead molecule provided two fold improvement in the PTP1B inhibitory potency of these compounds. Further, the results of the study also established that phenyl oxadiazole moiety is well tolerated in the second aryl phosphate binding site of PTP1B enzyme. Molecular interaction pattern of studied compounds with the receptor has successfully generated with the help of docking simulations and the results indicate that the compounds show all the relevant interactions requisite for PTP1B inhibition. Further medicinal chemistry efforts are in progress to develop more structurally diverse analogues from this compound to optimize the PTP1B inhibitory potency and establish structure activity relationship which will help in the development of the studied acetamido benzoic acid derivatives into probable antidiabetic drug candidates.

## 4. Experimental section

### 4.1. Chemistry

Melting points were determined by Open Capillary Method using VEEGO, Programmable Digital Melting Point Apparatus and are uncorrected. Unless stated otherwise, all materials obtained from commercial suppliers were used without further purification. TLC controls were carried out on precoated silica gel plates ( $F^{254}$  Merck) using Chloroform-Methanol (9:1) as solvents. IR spectra were recorded on FT-IR 470 plus spectrophotometer (JASCO) using KBr as the internal standard ( $\nu_{\max}$  in  $\text{cm}^{-1}$ ).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in DMSO on a Bruker 400 MHz spectrometer using tetramethylsilane (TMS) as the internal reference (chemical shift was measured in  $\delta$  ppm). Mass spectra (ESI-MS) were measured on Applied Biosystems 3200 Q-TRAP LC-MS/MS. The progress of the reaction and the purity of the synthesized compounds were verified on ascending thin layer chromatography (TLC) plates coated with silica gel G (Merck). An iodine chamber and UV lamp were used for the visualization of the TLC spots.

#### 4.1.1. Procedure for the preparation of 3-(2-(2-chloro acetamido) 4-methyl benzoic acid (**8**))

3-Amino, 4-methyl benzoic acid (**7**) (0.151 g, 1 mmol) was dissolved in DMF (10 mL) and chloroacetyl chloride (0.225 g, 2 mmol) was added drop wise to the reaction mixture for 20 min. The reaction mixture was stirred for 24 h, at room temperature and on completion of the reaction, the reaction mixture was poured in to crushed ice and stirred. Precipitated product was filtered and washed with water to remove the traces of acetic acid.  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  (ppm) 9.6193 (s, 1H, NHCO), 8.0857 (s, 1H, Ar-CH), 7.7321 (d, 1H, Ar-CH,  $J = 7.88$  Hz), 7.2883 (d, 1H, Ar-CH,  $J = 7.96$  Hz), 4.2393 (s, 2H,  $\text{CH}_2$ ), 2.3158 (s, 3H,  $\text{CH}_3$ ).

#### 4.1.2. General procedure for the preparation of 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-4-methylbenzoic acids (**10a–e**)

A mixture of 3-(2-(2-chloroacetamido)-4-methyl benzoic acid (**8**) (0.227 g, 1 mmol), substituted 2-mercapto benzimidazoles (**9a–c**) or benzo[d]oxazole-2-thiol (**9d**) or benzo[d]thiazole-2-thiol (**9e**) (1 mmol) and  $\text{K}_2\text{CO}_3$  (0.207 g, 1.5 mmol) was refluxed in acetone

(20 mL) for 5–6 h. Excess of solvent was evaporated at room temperature, concentrated the reaction mixture and diluted with water (about 200 mL). The product precipitated out on acidification with dilute hydrochloric acid was filtered, thoroughly washed with cold water.

**4.1.2.1. 3-(2-(1H-benzo[d]imidazol-2-ylthio)acetamido)-4-methylbenzoic acid, 10a.** Yield 44.05%, Mp 245 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1698.24 (CO st. of COOH), 3250.25 (OH st. of COOH), 2869.20 ( $\text{CH}_3$  st.), 1618.23 (amide CO st.), 758.34 (C–S st.), 3389.68 (NH st.), 3060.91 (Ar C–H st.), 1580.23 (Ar C–C st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 12.4158 (s broad peak, 1H, COOH), 10.1371 (s, 1H, NHCO), 7.6811 (m, 3H, Ar–CH), 7.4409 (m, 2H, Ar–CH), 7.1378 (m, 2H, Ar–CH), 7.0849 (m, 1H, Ar–CH), 4.5788 (s, 2H,  $\text{CH}_2$ ), 2.3156 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.5, 36.5, 110.5, 115.8, 123.8, 125.6, 125.9, 129.4, 135.2, 136.8, 139.1, 147.8, 165.3, 168.7. MS (ESI)  $m/z$ : 339.05 (M – H).

**4.1.2.2. 4-Methyl-3-(2-(5-nitro-1H-benzo[d]imidazol-2-ylthio)acetamido)benzoic acid, 10b.** Yield 85.23%, Mp 191 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1695.31 (CO st. of COOH), 3197.76 (OH st. of COOH), 2889.17 ( $\text{CH}_3$  st.), 1620.09 (amide CO st.), 757.97 (C–S st.), 3444.63 (NH st.), 3105.18 (Ar C–H st.), 1583.45 (Ar C–C st.), 1340.43 ( $\text{NO}_2$  st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 9.973 (s, 1H, NHCO), 8.321 (s, 1H, Ar–CH), 8.119 (s, 1H, Ar–CH), 8.076 (d, 1H, Ar–CH,  $J = 8.8$  Hz), 7.633 (d, 2H, Ar–CH,  $J = 12.8$  Hz), 7.33 (d, 1H, Ar–CH,  $J = 8.0$  Hz), 2.296 (s, 3H,  $\text{CH}_3$ ), 4.364 (s, 2H,  $\text{CH}_2$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.8, 37.2, 109.5, 111.7, 115.9, 118.3, 125.1, 125.9, 129.4, 136.1, 136.8, 137.9, 139.8, 143.4, 147.8, 166.3, 169.7. MS (ESI)  $m/z$ : 384.6 (M – H).

**4.1.2.3. 3-(2-(5-Methoxy-1H-benzo[d]imidazol-2-ylthio)acetamido)-4-methylbenzoic acid, 10c.** Yield 67.54%, Mp 221.5 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1695.31 (CO st. of COOH), 3255.62 (OH st. of COOH), 2837.09 ( $\text{CH}_3$  st.), 1649.02 (amide CO st.), 761.83 (C–S st.), 3452.34 (NH st.), 3020.32 (Ar C–H st.), 1581.52 (Ar C–C st.), 1157.21 ( $\text{OCH}_3$  st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 12.354 (s, 1H, COOH), 10.255 (s, 1H, NHCO), 8.372 (s, 1H, Ar–CH), 6.5–8.0 (m, 5H, Ar–CH), 4.020 (s, 2H,  $\text{CH}_2$ ), 3.755 (s, 3H,  $\text{OCH}_3$ ), 2.251 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.8, 37.5, 56.3, 100.9, 109.8, 111.2, 117.9, 125.6, 125.9, 129.8, 133.0, 136.8, 137.7, 139.9, 147.8, 153.2, 165.3, 168.7. MS (ESI)  $m/z$ : 369.6 (M – H).

**4.1.2.4. 3-(2-Benzo[d]oxazol-2-ylthio)acetamido)-4-methylbenzoic acid, 10d.** Yield 43.85%, Mp 235.5 °C, IR (KBr,  $\text{cm}^{-1}$ ) 1706.88 (CO st. of COOH), 3286.48 (OH st. of COOH), 2854.45 ( $\text{CH}_3$  st.), 1650.95 (amide CO st.), 754.12 (C–S st.), 3359.77 (NH st.), 3055.03 (Ar C–H st.), 1581.52 (Ar C–C st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 12.6405 (s, 1H, COOH), 9.7768 (s, 1H, NHCO), 8.4080 (s, 1H, Ar–CH), 7.8420 (d, 1H, Ar–CH,  $J = 8.72$  Hz), 7.6330 (d, 2H, Ar–CH,  $J = 12.8$  Hz), 7.472 (t, 1H, Ar–CH,  $J = 7.6$  Hz), 7.1070 (d, 1H, Ar–CH,  $J = 7.6$  Hz), 7.270 (t, 1H, Ar–CH,  $J = 7.6$  Hz), 4.1108 (s, 2H,  $\text{CH}_2$ ), 2.251 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.5, 37.8, 109.8, 110.8, 119.5, 123.9, 124.9, 125.3, 125.9, 129.4, 136.9, 137.8, 139.9, 141.8, 152.2, 166.3, 168.1. (ESI)  $m/z$ : 340.8 (M – H).

**4.1.2.5. 2-Benzo[d]thiazol-2-ylthio)acetamido)-4-methylbenzoic acid, 10e.** Yield 87.75%, Mp 194 °C, IR (KBr,  $\text{cm}^{-1}$ ) 1693.38 (CO st. of COOH), 3272.98 (OH st. of COOH), 2852.52 ( $\text{CH}_3$  st.), 1656.74 (amide CO st.), 767.97 (C–S st.), 3411.84 (NH st.), 3056.96 (Ar C–H st.), 1579.59 (Ar C–C st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 9.508 (s, 1H, NHCO), 8.194 (s, 1H, Ar–CH), 7.781 (d, 2H, Ar–CH,  $J = 14.4$  Hz), 7.6435 (d, 1H, Ar–CH,  $J = 7.6$  Hz), 7.471 (t, 1H, Ar–CH,  $J = 7.6$  Hz), 7.279 (t, 1H, Ar–CH,  $J = 7.6$  Hz), 7.170 (d, 1H, Ar–CH,  $J = 7.6$  Hz), 4.235 (s, 2H,  $\text{CH}_2$ ), 2.230 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.8, 38.2, 109.6, 121.5, 121.8, 124.3, 125.5, 125.6, 125.8,

129.2, 134.5, 136.5, 137.2, 152.5, 166.1, 166.6, 168.1. MS (ESI)  $m/z$ : 356.6 (M – H).

#### 4.1.3. General procedure for the preparation of substituted aromatic ethyl esters (12a–g)

A mixture of substituted benzoic acids (**11a–g**) (0.001 M, 2.12 g) and ethanol (20 mL) were heated under reflux until the benzoic acid was dissolved in ethanol then few drops of concentrated  $\text{H}_2\text{SO}_4$  was added to the mixture and reflux for 8 h. The resulting mixture was cooled to room temperature and a saturated solution of sodium bicarbonate was added to the mixture to neutralise the benzoic acid. The precipitated product was filtered and washed with water and dried. The dried product was recrystallized with ethanol.

#### 4.1.4. General procedure for the preparation of substituted benzohydrazide (13a–g)

A mixture of substituted ethyl benzoate (**12a–g**) (0.1 M) and hydrazine hydrate (0.3 M) was heated under reflux for 30 min. Ethanol (20 mL) was added to the refluxing mixture as a solvent in order to homogenize the solution, the resulting mixture was further allowed for 6 h. Excess of ethanol was distilled out and the content was allowed to cool. The crystals formed were filtered and washed thoroughly with water and dried.

#### 4.1.5. General procedure for the preparation of substituted 5-phenyl-1,3,4-oxadiazole-2-thiol (14a–g)

To a solution of substituted benzohydrazide (**13a–g**) (0.01 M) in ethanol (15 mL) at 0 °C, carbon disulphide (2 mL) and potassium hydroxide (0.6 g) were added, and the reaction mixture was refluxed until the evolution of  $\text{H}_2\text{S}$  gas ceased (around 12 h). Excess solvents were evaporated under reduced pressure and the residue was dissolved in water and then acidified with dilute hydrochloric acid (10%) to pH 5. The precipitate was filtered off, dried and crystallized from ethanol.

#### 4.1.6. General procedure for the preparation of substituted 4-methyl-3-(2-(5-phenyl-1,3,4-oxadiazol-2-ylthio)acetamido)benzoic acid (15a–g)

A mixture of 3-(2-chloroacetamido)-4-methyl benzoic acid (**8**) (0.227 g, 1 mmol), substituted 5-phenyl-1,3,4-oxadiazole-2-thiol (**14a–g**) (1 mmol) and  $\text{K}_2\text{CO}_3$  (0.207 g, 1.5 mmol) was refluxed in acetone (20 mL) for 5–6 h. The excess of solvent was evaporated at room temperature, concentrated the reaction mixture and diluted with water (about 200 mL). The product precipitated out on acidification with dilute hydrochloric acid was filtered and thoroughly washed with cold water.

**4.1.6.1. 3-(2-(5-(2,3-Dichlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid, 15a.** Yield 57.20%, Mp 236.5 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1697 (CO st. of COOH), 3201.61 (OH st. of COOH), 2931.60 ( $\text{CH}_3$  st.), 1650.95 (amide CO st.), 760.89 (C–S st.), 3463.92 (NH st.), 3074.32 (Ar C–H st.), 1596.95 (Ar C–C st.), 1049.20 (Cl st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 10.8162 (s, 1H, NHCO), 7.9803 (m, 1H, Ar–CH), 7.8874 (m, 1H, Ar–CH), 7.4776 (m, 1H, Ar–CH), 7.2918 (m, 3H, Ar–CH), 7.5793 (d, 1H, Ar–CH,  $J = 7.84$  Hz), 4.1923 (s, 2H,  $\text{CH}_2$ ), 2.3411 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  17.2, 38.2, 109.6, 125.4, 125.8, 127.0, 127.8, 129.5, 130.3, 131.3, 134.1, 136.5, 137.2, 138.4, 166.1, 166.6, 167.3, 168.3. MS (ESI)  $m/z$ : 435.6 (M – H).

**4.1.6.2. 3-(2-(5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid, 15b.** Yield 52.10%, Mp 194 °C. IR (KBr,  $\text{cm}^{-1}$ ) 1718.45 (CO st. of COOH), 3207.40 (OH st. of COOH), 2979.82 ( $\text{CH}_3$  st.), 1654.81 (amide CO st.), 756.04 (C–S st.), 3388.70 (NH st.), 1596.95 (Ar C–C st.), 1037.63 (Cl st.).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )

$\delta$  (ppm): 12.447 (s, 1H, COOH), 9.742 (s, 1H, NHCO), 8.083 (s, 1H, Ar-CH), 7.1–8.0 (m, 6H, Ar-CH), 4.302 (s, 2H, CH<sub>2</sub>), 2.272 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  17.3, 38.2, 109.6, 125.5, 125.9, 127.2, 127.8, 129.6, 129.9, 131.5, 134.5, 136.5, 137.2, 138.4, 166.2, 166.5, 167.3, 168.3. MS (ESI)  $m/z$ : 401.6 (M – H).

4.1.6.3. 3-(2-(5-(2,4-Dichlorophenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid, **15c**. Yield 80.01%, Mp 257.5 °C. IR (KBr, cm<sup>-1</sup>) 1691.46 (CO st. of COOH), 3226.69 (OH st. of COOH), 2995.25 (CH<sub>3</sub> st.), 1643.46 (amide CO st.), 761.83 (C–S st.), 3461.99 (NH st.), 3082.04 (Ar C–H st.), 1596.95 (Ar C–C st.), 1033.17 (Cl st.). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 10.7706 (s, 1H, NHCO), 7.9819 (d, 2H, Ar–CH,  $J = 7.92$  Hz), 7.4715 (m, 4H, Ar–CH), 4.1909 (s, 2H, CH<sub>2</sub>), 2.33374 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  17.8, 38.3, 109.4, 125.4, 125.9, 127.4, 127.8, 129.6, 130.4, 131.5, 134.6, 136.8, 137.4, 138.6, 166.3, 166.6, 167.3, 168.3. MS (ESI)  $m/z$ : 437.8 (M – H).

4.1.6.4. 3-(2-(5-(2-Hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid, **15d**. Yield 60.36%, Mp 257.5 °C. IR (KBr, cm<sup>-1</sup>) 1689.53 (CO st. of COOH), 3228.62 (OH st. of COOH), 2923.88 (CH<sub>3</sub> st.), 1645.17 (amide CO st.), 752.19 (C–S st.), 3382.91 (NH st.), 3072.39 (Ar C–H st.), 1552.59 (Ar C–C st.), 1356.51 (OH st.). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 11.7969 (s broad peak, 1H, OH), 10.9380 (s, 1H, NHCO), 7.9758 (d, 1H, Ar–CH,  $J = 7.88$  Hz), 7.9082 (d, 1H, Ar–CH,  $J = 1.64$  Hz), 7.8378 (d, 1H, Ar–CH,  $J = 4.58$  Hz), 7.4771 (d, 1H, Ar–CH,  $J = 8.04$  Hz), 7.3481 (m, 1H, Ar–CH), 6.9293 (d, 1H, Ar–CH,  $J = 8.12$  Hz), 6.8748 (t, 1H, Ar–CH,  $J = 7.72$  Hz), 4.2406 (s, 2H, CH<sub>2</sub>), 2.3359 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  17.3, 38.4, 109.6, 118.2, 121.8, 125.5, 125.3, 126.5, 129.2, 129.9, 131.5, 136.4, 137.2, 155.3, 166.1, 166.3, 167.1, 168.5. MS (ESI)  $m/z$ : 383.8 (M – H).

4.1.6.5. 3-(2-(5-(4-Hydroxyphenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)-4-methylbenzoic acid, **15e**. Yield 59.05%, Mp 230.5 °C. IR (KBr, cm<sup>-1</sup>) 1695.31 (CO st. of COOH), 3272.98 (OH st. of COOH), 2829.38 (CH<sub>3</sub> st.), 1662.52 (amide CO st.), 763.76 (C–S st.), 3385.42 (NH st.), 3028.03 (Ar C–H st.), 1581.52 (Ar C–C st.), 1374.15 (OH st.). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 8.4369 (d, 1H, Ar–CH,  $J = 1.52$  Hz), 7.7174 (d, 1H, Ar–CH,  $J = 7.84$  Hz), 7.3674 (d, 2H, Ar–CH,  $J = 8.4$  Hz), 7.3211 (d, 1H, Ar–CH,  $J = 7.88$  Hz), 7.1962 (m, 1H, Ar–CH), 7.1050 (m, 1H, Ar–CH), 4.1379 (s, 1H, CH<sub>2</sub>), 2.4161 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  17.3, 38.4, 109.6, 116.6, 116.8, 118.8, 125.3, 125.9, 129.2, 136.7, 137.3, 158.3, 166.4, 166.6, 167.1, 168.5. MS (ESI)  $m/z$ : 383.6 (M – H).

4.1.6.6. 4-Methyl-3-(2-(5-*p*-tolyl)-1,3,4-oxadiazol-2-ylthio)acetamido)benzoic acid, **15f**. Yield 89.03%, Mp 246 °C. IR (KBr, cm<sup>-1</sup>) 1693.88 (CO st. of COOH), 3220.90 (OH st. of COOH), 2999.10 (CH<sub>3</sub> st.), 1645.17 (amide CO st.), 754.12 (C–S st.), 3542.99 (NH st.), 1539.09 (Ar C–C st.), 1741 (C<sub>6</sub>H<sub>5</sub>CH<sub>3</sub> st.). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 10.597 (s, 1H, NHCO), 7.0–8.0 (m, 7H, Ar–CH), 4.102 (s, 2H, CH<sub>2</sub>), 2.329 (s, 6H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>)  $\delta$  17.3, 21.7, 38.4, 109.6, 123.1, 125.3, 125.8, 126.7, 127.8, 129.2, 131.9, 136.7, 137.3, 164.4, 166.6, 167.1, 168.8. MS (ESI)  $m/z$ : 381.8 (M – H).

4.1.6.7. 4-Methyl-3-(2-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio)acetamido)benzoic acid, **15g**. Yield 54.70%, Mp 163.5 °C. IR (KBr, cm<sup>-1</sup>) 1718.46 (CO st. of COOH), 3311.55 (OH st. of COOH), 2945.10 (CH<sub>3</sub> st.), 1654.81 (amide CO st.), 751.03 (C–S st.), 3496.70 (NH st.), 1589.23 (Ar C–C st.), 1128.28 (OCH<sub>3</sub> st.). <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>)  $\delta$  (ppm): 10.7274 (s broad peak, 1H, COOH), 9.7648 (s, 1H, NHCO), 8.0864 (s, 1H, Ar–CH), 7.8527 (s, 1H, Ar–CH), 7.6501 (d, 1H, Ar–CH,  $J = 7.48$  Hz), 7.4099 (d, 1H, Ar–CH,  $J = 8.04$  Hz), 7.2233 (d, 1H, Ar–CH,  $J = 7.92$  Hz), 4.2744 (s, 2H, CH<sub>2</sub>), 3.8046 (s, 6H, OCH<sub>3</sub>), 3.7720 (s, 3H, OCH<sub>3</sub>), 2.2754 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (100 MHz,

DMSO-d<sub>6</sub>)  $\delta$  17.3, 38.4, 56.4, 61.0, 104.3, 109.6, 120.6, 124.9, 125.4, 129.3, 135.6, 137.8, 139.3, 152.8, 164.3, 166.3, 167.2, 168.2. MS (ESI)  $m/z$ : 457.6 (M – H).

#### 4.2. In vitro PTP1B enzyme inhibition

PTP1B enzyme inhibitory activity was determined by using the colorimetric, non-radioactive assay (PTP1B assay kit, BML-AK 822, Enzo life sciences, USA). The test compounds on PTP1B enzyme activity were studied by incubating these with human recombinant PTP1B enzyme and determined the PTP1B activity using phosphate detection reagent, Biomol red. The reaction was carried out in 96 well, flat-bottomed microtiter plates at 10  $\mu$ M concentration using DMSO as control. Further, those compounds show around 50% inhibitions were tested at six different concentrations for the calculation of IC<sub>50</sub> values. The detection of free phosphate released is based on classic Malachite green assay [32]. The percentage inhibition by test compounds on PTP1B enzyme was calculated based on the activity in the control tube (without inhibitor) as 100% from three independent sets of experiments.

#### 4.3. Evaluation of in vivo toxicity using zebrafish embryos

All procedures using zebrafish were in accordance with ethical guidelines for animal use and followed those published by the NIH. An indigenous wild type adult zebrafish strain from India was used for this study (obtained from Vikrant Aquaculture, Mumbai, India). They were maintained in a recirculation system using purified ELIX System (Millipore, Billerica, US) grade water containing 200 mg/l sea salt at 28 °C under a 14:10 h light and dark cycle. Fish were fed three times daily with a combination of freshly hatched live brine shrimp and dry food. Males and females were kept in separate tanks for four days before they were allowed to spawn. On day five, 300–400 embryos were obtained by natural mating of both adult sexes (3:2 female and male ratios) in a breeding tank setup. Embryos were collected and raised in 60 mm Petri dishes containing E3 medium (5 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl<sub>2</sub>, and 0.33 mM MgSO<sub>4</sub>) for 24 h. Compound stock was diluted in E3 medium to obtain final concentrations of 10  $\mu$ M in 0.1% DMSO. Zebrafish embryos at 24 h post fertilization were dechorionated with 500  $\mu$ g/ml, Pronase K for 10 min. Six embryos per well were placed in 24-well plates containing E3 medium and compounds added to a final concentration of 10  $\mu$ M (in 0.1% DMSO). The embryos were grown in the presence of the compound for 24 h, 48 h and 72 h. Control embryos were incubated in 0.1% DMSO. Finally, embryos were observed using visible light microscopy at regular intervals after compound treatment to document general toxicity-related effects.

#### 4.4. Molecular modelling

For better understanding of binding mode of substituted 3-acetamido-4-methyl benzoic acid derivatives at the molecular level, we carried out molecular docking simulations of synthesized molecules (**10a–e** and **15a–g**) at the PTP1B catalytic ligand binding site. The docking simulations of synthesized compounds (**10a–e** and **15a–g**) were performed using Maestro, version 9.2 implemented from Schrodinger software suite [33]. The ligands were sketched in 3D format using build panel and were prepared for docking using ligprep application. The protein for docking study was taken from Protein data bank (PDB ID: 1XBO) and prepared by removing solvent, adding hydrogen and further minimization in the presence of bound ligand (IX1) using protein preparation wizard. Grids for molecular docking were generated with bound co-crystallized ligand. For the validation of docking parameters the co-crystal ligand (IX1) was re-docked at the catalytic site of protein

and the RMSD between co-crystal and re-docked pose was found to be 0.255 Å. All compounds (**10a–e** and **14a–g**) were docked using Glide extra-precision (XP) mode, with up to three poses saved per molecule.

## Acknowledgements

The authors gratefully acknowledge the Central Instrumentation Facility; Jamia Hamdard Deemed University, New Delhi, India for the NMR spectral analysis of the compounds used in this study. The authors AKR and C. Karthikeyan are recipients of a senior research fellowship of CSIR, New Delhi. GSD wishes to thank AICTE, New Delhi for a postgraduate fellowship.

## References

- [1] N.C. Turner, J.C. Clapham, Insulin resistance, impaired glucose tolerance and non-insulin-dependent diabetes, pathologic mechanisms and treatment: current status and therapeutic possibilities, *Prog. Drug Res.* 51 (1998) 33–94.
- [2] D.E. Kelley, K.V. Williams, Metabolic consequences of weight loss on glucose metabolism and insulin action in type 2 diabetes, *Diabetes Obes. Metab.* 2 (2001) 121–129.
- [3] A.P. Babenko, L. Aguilar-Bryan, J. Bryan, A view of sur/KIR6.X, KATP channels, *Annu. Rev. Physiol.* 60 (1998) 667–687.
- [4] L. Aguilar-Bryan, J.P. Clement, G. Gonzalez, K. Kunjilwar, A. Babenko, J. Bryan, Toward understanding the assembly and structure of KATP channels, *Physiol. Rev.* 78 (1998) 227–245.
- [5] U. Riserus, W.C. Willett, F.B. Hu, Dietary fats and prevention of type 2 diabetes, *Prog. Lipid Res.* 48 (2009) 44–51.
- [6] D.W. Cooke, L. Plotnic, Type 1 diabetes mellitus in pediatrics, *Pediatr. Rev.* 29 (2008) 374–385.
- [7] O.J. Theodore, E. Jacques, R.J. Michael, Protein tyrosine phosphatase 1B inhibitors for diabetes, *Nat. Rev. Drug Discov.* 1 (2002) 696–709.
- [8] H.G. Cheon, S.M. Kim, S.D. Yang, J.D. Ha, J.K. Choi, Discovery of a novel protein tyrosine phosphatase-1B inhibitor, KR61639: potential development as an antihyperglycemic agent, *Eur. J. Pharmacol.* 485 (2004) 333–339.
- [9] L.D. Klamon, O. Boss, O.D. Peroni, J.K. Kim, J.L. Martino, N. Moghal, M. Lubkin, Y.B. Kim, G.I. Sharpe, B.G. Neel, B.B. Khan, Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice, *Mol. Cell Biol.* 20 (2000) 5479–5489.
- [10] V.S. Murthy, V.M. Kulkarni, 3D-QSAR CoMFA and CoMSIA on protein tyrosine phosphatase 1B inhibitors, *Bioorg. Med. Chem.* 10 (2002) 2267–2282.
- [11] D. Barford, A.J. Flint, N.K. Tonks, Crystal structure of human protein tyrosine phosphatase 1B, *Science* 263 (1994) 1397–1404.
- [12] J.P. Sun, A.A. Fedorov, S.Y. Lee, X.L. Guo, K. Shen, D.S. Lawrence, S.C. Almo, Z.Y. Zhang, Crystal structure of PTP1B complexed with a potent and selective bidentate inhibitor, *J. Biol. Chem.* 278 (2003) 12406–12414.
- [13] M. Sarmiento, Y.A. Puius, S.W. Vetter, Y.F. Keng, L. Wu, Y. Zhao, D.S. Lawrence, S.C. Almo, Z.Y. Zhang, Structural basis of plasticity in protein tyrosine phosphatase 1B substrate recognition, *Biochemistry* 39 (2000) 8171–8179.
- [14] K. Shen, Y.F. Keng, L. Wu, X.L. Guo, D.S. Lawrence, Z.Y. Zhang, Acquisition of a specific and potent PTP1B inhibitor from a novel combinatorial library and screening procedure, *J. Biol. Chem.* 276 (2001) 47311–47319.
- [15] S. Shrestha, B.R. Bhattarai, K.H. Lee, H. Cho, Mono- and disalicylic acid derivatives: PTP1B inhibitors as potential anti-obesity drugs, *Bioorg. Med. Chem.* 15 (2007) 6535–6548.
- [16] D. Patel, M. Jain, S.R. Shah, R. Bahekar, P. Jadav, A. Joharapurkar, N. Dhanesha, M. Shaikh, K.V.V.M. Sairam, P. Kapadnis, Discovery of potent, selective and orally bioavailable triaryl-sulfonamide based PTP1B inhibitors, *Bioorg. Med. Chem. Lett.* 22 (2012) 1111–1117.
- [17] K.K.D. Amarasinghe, A.G. Evidokimov, K. Xu, C.M. Clark, M.B. Maier, A. Srivastava, A.-O. Colson, G.S. Gerwe, G.E. Stake, B.W. Howard, M.E. Pokross, J.L. Gray, K.G. Peters, Design and synthesis of potent, non-peptidic inhibitors of HPTPβ, *Bioorg. Med. Chem. Lett.* 16 (2006) 4252–4256.
- [18] S. Zhang, Z.Y. Zhang, PTP1B as a drug target: recent developments in PTP1B inhibitor discovery, *Drug Discov. Today* 12 (2007) 373–381.
- [19] Z. Xin, G. Liu, C. Abad-Zapatero, Z. Pei, B.G. Szczepankiewicz, X. Li, T. Zhang, C.W. Hutchins, P.J. Hajduk, S.J. Ballaron, M.A. Stashko, T.H. Lubben, J.M. Trevillyan, M.R. Jirousek, Identification of a monoacid-based, cell permeable, selective inhibitor of protein tyrosine phosphatase 1B, *Bioorg. Med. Chem. Lett.* 13 (2003) 3947–3950.
- [20] H. Zhao, G. Liu, Z. Xin, M.D. Serby, Z. Pei, B.G. Szczepankiewicz, P.J. Hajduk, C. Abad-Zapatero, C.W. Hutchins, T.H. Lubben, S.J. Ballaron, D.L. Haasch, W. Kaszubska, C.M. Rondinone, J.M. Trevillyan, M.R. Jirousek, Isoxazole carboxylic acids as protein tyrosine phosphatase 1B (PTP1B) inhibitors, *Bioorg. Med. Chem. Lett.* 14 (2004) 5543–5546.
- [21] D.R. Adams, A. Abraham, J. Asano, C. Breslin, C.A.J. Dick, U. Ixkes, B.F. Johnston, D. Johnston, J. Kewnay, S.P. Mackay, S.J. MacKenzie, M. McFarlane, L. Mitchell, D. Spinks, Y. Takano, 2-Aryl-3,3,3-trifluoro-2-hydroxypropionic acids: a new class of protein tyrosine phosphatase 1B inhibitors, *Bioorg. Med. Chem. Lett.* 17 (2007) 6579–6583.
- [22] S. Basu, U.V. Prasad, D.A. Barawkar, S. De, V.P. Palle, S. Menon, M. Patel, S. Thorat, U.P. Singh, K.D. Sarma, Y. Waman, S. Niranjana, V. Pathade, A. Gaur, S. Reddy, S. Ansari, Discovery of novel and potent heterocyclic carboxylic acid derivatives as protein tyrosine phosphatase 1B inhibitors, *Bioorg. Med. Chem. Lett.* 22 (2012) 2843–2849.
- [23] R. Ottanà, R. Maccari, S. Amuso, G. Wolber, D. Schuster, S. Herdinger, G. Manao, G. Camici, P. Paoli, New 4-[(5-arylidene-2-arylimino-4-oxo-3-thiazolidinyl)methyl]benzoic acids active as protein tyrosine phosphatase inhibitors endowed with insulinomimetic effect on mouse C2C12 skeletal muscle cells, *Eur. J. Med. Chem.* 50 (2012) 332–343.
- [24] A.F. Moretto, S.J. Kirincich, W.X. Xu, M.J. Smith, Z.K. Wan, D.P. Wilson, B.C. Follows, E. Binnun, D. Joseph-McCarthy, K. Foreman, D.V. Erbe, Y.L. Zhang, S.K. Tam, S.Y. Tam, J. Lee, Bicyclic and tricyclic thiophenes as protein tyrosine phosphatase 1B inhibitors, *Bioorg. Med. Chem.* 14 (2006) 2162–2177.
- [25] P. Joshi, G.S. Deora, V. Rathore, O.P. Tanwar, A.K. Rawat, A.K. Srivastava, D. Jain, Identification of ZINC02765569: a potent inhibitor of PTP1B by vHTS, *Med. Chem. Res.* 22 (2013) 28–34.
- [26] A.P. Combs, Recent advances in the discovery of competitive protein tyrosine phosphatase 1B inhibitors for the treatment of diabetes, obesity, and cancer, *J. Med. Chem.* 53 (2009) 2333–2344.
- [27] P. Joshi, G.S. Deora, V. Rathore, A.K. Rawat, A.K. Srivastava, D. Jain, Molecular modeling and synthesis of ZINC02765569 derivatives as protein tyrosine phosphatase 1B inhibitors: lead optimization study, *Med. Chem. Res.* 22 (2013) 1618–1623.
- [28] O.P. Bansal, J.S. Srinivas, C.V. Reddy Sastry, Synthesis and pharmacology of some new 3,4-diaryl-5-aryloxymethyl-1,2,4-triazoles, *Ind. J. Chem.* 23 (1992) 289–292.
- [29] R. Kumar, A. Mittal, U. Ramachandran, Design and synthesis of 6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrimidine-5-carboxylic acid derivatives as PPAR gamma activators, *Bioorg. Med. Chem. Lett.* 17 (2007) 4613–4618.
- [30] A. Zarghi, S. Hamed, F. Tootooni, B. Amini, B. Sharifi, M. Faizi, S.A. Tabatabai, A. Shafiee, Synthesis and pharmacological evaluation of new 2-substituted-5-[(2-(2-halobenzyl)thio)phenyl]-1,3,4-oxadiazoles as anticonvulsant Agents, *Sci. Pharm.* 76 (2008) 185–201.
- [31] G.S. Deora, C. Karthikeyan, N.S.H.N. Moorthy, V. Rathore, A.K. Rawat, A.K. Tamrakar, A.K. Srivastava, P. Trivedi, Design, synthesis and biological evaluation of novel arylidene-malononitrile derivatives as non-carboxylic inhibitors of protein tyrosine phosphatase 1B, *Med. Chem. Res.* 22 (2013) 5344–5348.
- [32] B. Martin, C.J. Pallen, J.H. Wang, D.J. Graves, Use of fluorinated tyrosine phosphates to probe the substrate specificity of the low molecular weight phosphatase activity of calcineurin, *J. Biol. Chem.* 260 (1985) 14932–14937.
- [33] Maestro, Version 9.2, Schrödinger, LLC, New York, NY, 2011.