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Research Article

SYNTHESIS AND PTP1B INHIBITORY ACTIVITY OF NOVEL BENZOTHIAZOLE AND 1, 2, 4-TRIAZOLE LINKED ACETAMIDO BENZOIC ACID DERIVATIVES AS ANTIDIABETIC ACTIVITY

Monika Rakse*, Ram Kishore Agrawal

Department of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh, India

**Corresponding author: mansi.rakse06@gmail.com*

ABSTRACT

The present research reports the synthesis and PTP1B inhibitory activity evaluation of a series of substituted benzthiazole/ 1, 2, 4-triazole linked acetamido benzoic acid derivatives designed based on a lead molecule, 4-methyl-3-(2-(5-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-2-ylthio) acetamido) benzoic acid identified previously in our laboratory. All the synthesized derivatives showed improved PTP1B inhibitory activity, out of which, compound **6c** (4-methyl-3-(2-(5-(pyridin-4-yl)-4H-1, 2, 4-triazol-3ylthio) acetamido) benzoic acid) elicited potent PTP1B inhibitory activity with IC_{50} value 6.45 µM. Furthermore, compound **6c** also showed good *in vivo* antidiabetic activity in streptozotocin-induced diabetes model. Molecular docking studies performed with **6c** revealed the binding mechanism of the molecule in the catalytic site of PTP1B. These results demonstrate that compound **6c** might serve as a valuable lead molecule for the development of promising antidiabetic agents targeting PTP1B.

Keywords: Protein tyrosine phosphatase 1B, Benzoic acid derivatives, Antidiabetic activity, Molecular docking.

1. INTRODUCTION

Type-2 diabetes is a major subtype of Diabetes mellitus *i.e.* approximately 90% of diabetes patients has Type-2 diabetes mellitus (T2DM). T2DM is a metabolic syndrome, which is characterized by insulin resistance resulting from relatively reduced insulin secretion. In India, more than 65.1 million people are afflicted with T2DM and this number is expected to exponentially increase to 89 million by 2030 [1]. The antidiabetics that are used presently for the clinical management of T2DM only focuses more towards lowering blood glucose ultimately leading to treatment failure. Hence, discovery of novel antidiabetic agents that not only lowers blood glucose but also delays disease progression is of utmost priority for medicinal chemists worldwide.

Protein tyrosine phosphatase 1B has received much attention as a tractable target for antidiabetic drug discovery owing to its critical regulatory role in insulin receptor and leptin signaling pathway [2-4]. The therapeutic potential of PTP1B in T2DM had been demonstrated in two independent studies. Gene knockout studies in mice model revealed that mice lacking PTP1B gene while remaining healthy, exhibited increased insulin sensitivity and obesity resistance [5-6]. Furthermore, oligonucleotides and small molecules targeting PTP1B demonstrated anti-diabetic and anti-obesity effects *in vivo* [7]. PTP1B targeted inhibitor discovery has been extensively undertaken in both pharmaceutical industries and academia resulting in the identification of several PTP1B inhibitors that targets the catalytic site of the enzyme [8-10]. Almost all the first-generation PTP1B inhibitors have a highly charged anionic group that mimics the natural pTyr substrate eg. DFMP (Difluoro methylphosphonate) hence exhibits poor cell permeability and oral bioavailability [11-13]. This resulted in the replacement of the phosphate group with bioisosteric monoanionic groups like carboxylates to yield PTP1B inhibitors of low polarity and good cell permeability [14-15]. Few examples of carboxylic acid containing PTP1B inhibitors are Cinnamic acid derivatives, (**1**) [16] Pyrrole phenoxy propionic acid derivatives, (**2**) [17] 3- carboxy-4-(O-carboxymethy)-tyrosine derivatives, (**3**) [18] 2-oxylamino benzoic acid, (**4**) [19] N-aryl oxamic acid, (**5**) [20] O- Hydroxy-(O-carboxymethyl)-tyrosine, (**6**) [21] Isoxazole acid (**7**) [22] and Hydroxy propionic acid derivatives (**8**) [23] (Fig. 1).

In continuation of our ongoing efforts on development of novel PTP1B inhibitors with 'drug like' properties, we had previously reported a series of 4-methyl-3-(2-((5-phenyl-1, 3, 4-oxadiazole-2-yl)thio)acetamido) benzoic acids as a new class of PTP1B inhibitors [24-25]. In this research, we sought to improve PTP1B inhibitory

potency of the aforementioned analogues by replacing the substituted 'oxadiazole' with bioisosteric 'thiazole/ triazole' moieties. This bioisosteric replacement strategy was corroborated through molecular docking wherein the substituted thiazole/triazole moiety was found to bind at the second aryl binding site in the enzyme similar to the phenyl oxadiazole moiety in the lead molecule. The synthesis and PTP1B inhibitory activity of the newly designed analogues are reported herein.

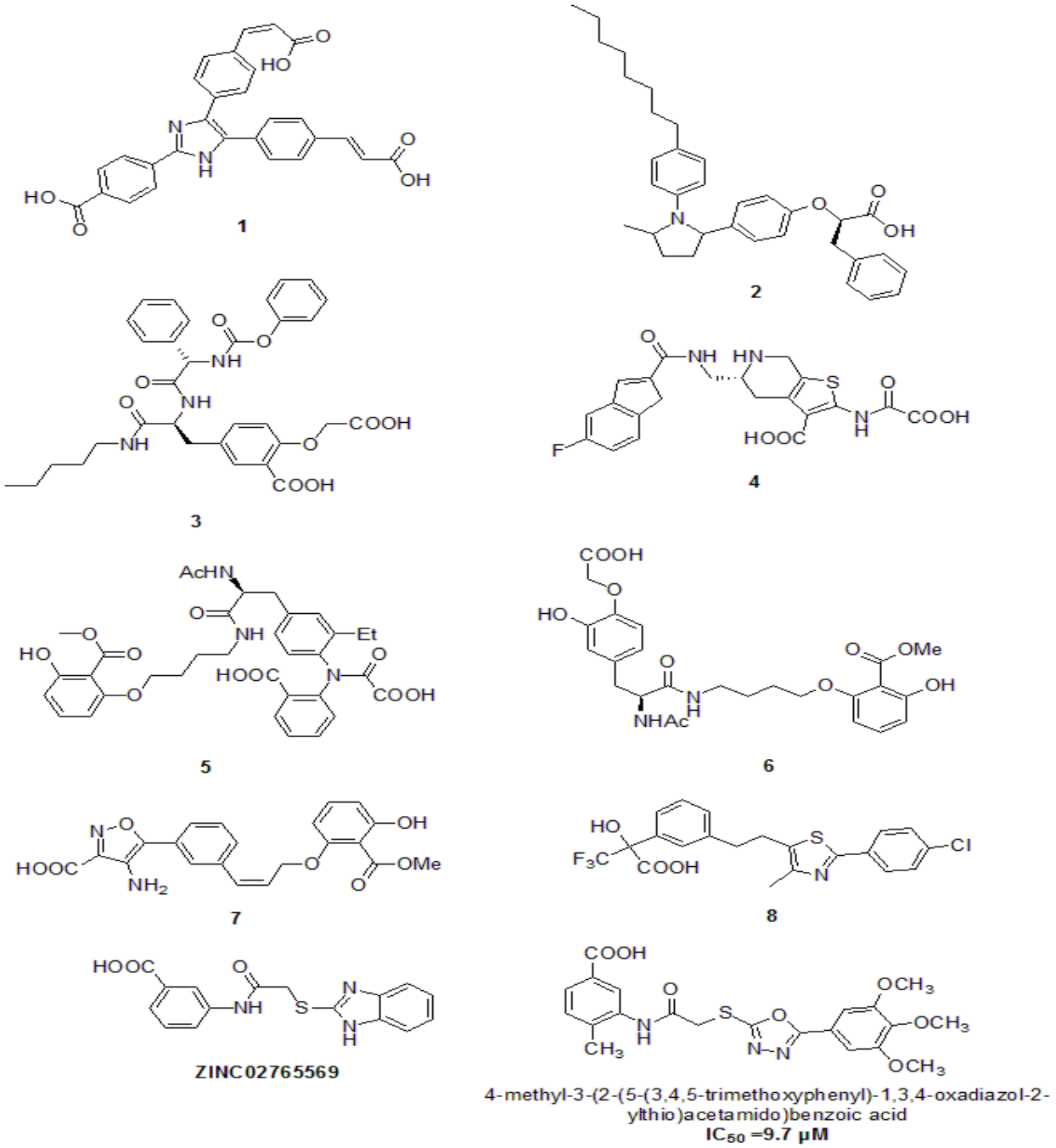


Fig. 1: Molecular structures of PTP1B inhibitors

2. MATERIAL AND METHODS

2.1. General information

The melting points of synthesized compounds were found out by digital melting point apparatus Electronic India (EI) and are uncorrected. For the synthesis of compounds, all materials are purchased from commercial supplier were used without further purification. TLC checks synthesis completion and it

was performed on precoated silica gel plate (F^{254} Merck) using the solvent system as chloroform-Methanol in the ratio 9:1. IR spectra were recorded on FT-IR Shimadzu Prestige-21 (Japan) spectrophotometer (V_{max} in cm^{-1}). ^1H and ^{13}C CNMR spectra were recorded on a Bruker 500 MHz spectrometer in DMSO-*d6* solvent using TMS as the internal reference and chemical shift value was measured in δppm. HR-LCMS an LCMS were recorded on Micro TOF-Q-II mass spectrometer manufactured by Bruker Daltonics. Elemental analysis was performed on Elemental (CHNSO) analyzer (Elementar Vario Micro Cube). The gradual improvement of the reaction in each step of synthesis was done on ascending TLC precoated plates of Silica gel G (manufactured by Merck) and it is visualized under UV lamp.

2.2. Procedure for the preparation of substituted 3-(2-chloro acetamido) benzoic acid

Substituted 3-Amino benzoic acid (**1**) (0.137g, 1mmol) was dissolved in DMF (10ml) and chloroacetyl chloride (0.225g, 2mmol) was added drop wise to the reaction mixture for 20 min. 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 [24].^1H NMR (DMSO-*d6*) δ (ppm) 10.3921 (s, 1H, NHCO), 8.2130 (s, 1H, Ar-CH), 7.83-7.88 (m, 1H, Ar-CH), 7.6844 (d, 1H, Ar-CH, J= 7.70), 7.4028 (t, 1H, Ar-CH, J= 7.89Hz), 4.2023 (s, 2H, CH_2).

2.2.1. 3-(2-chloro acetamido) 4-methyl benzoic acid

1H NMR (400 MHz, DMSO-*d6*) δ ppm 2.30 (s, 3 H) 4.27 (s, 2 H) 7.31 (d, *J*=8.07 Hz, 1 H) 7.70 (dd, *J*= 7.89, 1.65 Hz, 1H) 8.03 - 8.07 (m, 1H) 9.70(s, 1 H).

2.2.2. General procedure for the preparation of substituted 3-(2-(5-phenylthiazol-2-ylthio) acetamido)benzoic acid (4 a-d)

A mixture of substituted 3-(2-chloroacetamido) benzoic acid (**2**) (0.227g, 1mmol), substituted 5-phenylthiazole-2-thiol (**3**) (1mmol) and K_2CO_3 (0.207g, 1.5mmol) was refluxed in acetone (20ml) for 5-6 h. Excess of solvent was evaporated at room temperature, concentrated the reaction mixture and diluted with water (about 200ml). The product precipitated out on acidification with dilute hydrochloric acid was filtered, thoroughly washed with cold water [24].

2.2.3. General procedure for the preparation of substituted 3-(2-(4H-1,2,4-triazol-3 ylthio) acetamido)benzoic acid (6 a-d)

A mixture of substituted 3-(2-chloroacetamido) benzoic acid (**2**) (0.227g, 1mmol), substituted 4H-1,2,4-triazole-3-thio (**3'**) (1mmol) and K_2CO_3 (0.207g, 1.5mmol) was refluxed in acetone (20ml) for 5-6 h. Excess of solvent was evaporated at room temperature, concentrated the reaction mixture and diluted with water (about 200ml). The product precipitated out on acidification with dilute hydrochloric acid was filtered, thoroughly washed with cold water [24, 26-29].

2.3. *In vitro* PTP1B enzyme inhibition

The *In vitro* antihyperglycaemic activity of all the synthesized compounds was performed against PTP1B enzyme using colourimetric, non-radioactive drug discovery Assay kit. Expressed in *E. coli*, human recombinant PTP1B along with suramin as a controlled drug were provided with the kit to determine the inhibitory potential of test compounds. 96 well flat-bottomed micro titer plates were used to perform the assay given in the manufacturer's protocol. DMSO was used to dissolve the synthesized test compounds at 10μM concentration. Further, IC_{50} values were calculated for those compounds which show more than 50% inhibition at a 10μM concentration by testing them at different concentrations. The principal of classic Malachite green assay was followed for detection of free phosphate released in the assay solution. The % inhibition of the PTP1B enzyme by test compounds is calculated on the basis of activity in the control well (without inhibitor) taking as 100 % by using the following formula:

% Activity = {[Test Sample ($nmolPO_4^{-2}$)-time zero ($nmolPO_4^{-2}$)] / [Control ($nmolPO_4^{-2}$)-time zero ($nmolPO_4^{-2}$)]}× 100

2.4. *In vivo* anti-hyperglycemic activity in a streptozotocin-induced diabetic Wistar rat model

Compound **6c** was evaluated for *in vivo* anti-hyperglycaemic activity in streptozotocin induced type II diabetes Wistar rat model [30]. Healthy Wistar male rats (200-250 gm body weight and age of 3 months) were obtained from in-house laboratory (Reg. No. 2004/ GO/ReBi/S/18/CPCSEA dated: 28.02.2018), chosen for the studies which were maintained under moderate temperature (22 ± 2°C), humidity (55±5 %) and maintained the light-dark cycle of 12h/12h. All the

rats were fed with "rat feed pallets" and purified water during complete study period.

2.5. Treatment Protocol

Animals were then divided randomly into four groups (N=5)

Group 1 contains normal control animals; Group 2 contains diabetic control animals, Group 3 contains diabetic animals treated with Pioglitazone with the dose of 30 mg/kg and Group 4 contains diabetic animals treated with synthesized compound 6c with the dose of 30 mg/kg.

2.6. Induction of Type 2 diabetes using a low dose of streptozotocin

Diabetes was induced in 12h overnight fasted rats by a single dose with freshly prepared streptozotocin at 40 mg/kg, intra peritoneal in 0.1M citrate buffer (pH 4.5). After that animals were left aside and diabetes was confirmed after 72 h of STZ administration by checking the blood glucose level. Initially, the serum glucose level of diabetes-induced rats was measured on day 0, 7^{th}, 14^{th}, 21^{st} day. After induction of diabetes through streptozotocin, the treatment was given for 21 days [31]. The test compound **(6c)** and a standard drug (pioglitazone) were given orally once a day at a dose of 30 mg/kg [32]. An equal amount of vehicle, 0.5 % of carboxy methyl cellulose were administered to the normal control and diabetic control groups. All animals were supervised regularly to notice the changes in body weight, food & water intake and mortality of animals during the course of study period.

2.7. Statistical Analysis

All the results were carried out as the mean ± SEM. Two-way ANOVA, accompanied by Bonferroni multiple comparison tests was used to determine the significant difference. Statistical analysis was performed using Graph Pad prism-5 software (San Diego, CA, USA). Differences were studied statistically significant when $P < 0.05$.

2.8. Molecular Docking Studies

Molecular docking studies were performed with reported X-ray crystal structure of PTP1B co-complexed with Isoxazole Carboxylic Acid (PDB entry: 1XBO; resolution, 2.5 Å) using Flex X docking algorithm available on Lead IT 2.3.2 molecular modeling platform. Prior to docking, the protein was prepared using Receptor Preparation Wizard implemented in Lead IT software [30] wherein polar hydrogens are added and correct protonation and tautomeric states are assigned. The ligand binding site was defined using the co-complexed ligand and the residues of amino acids were selected within 8.0 Å of the ligand. The structures of the lead molecule and the most active compound 6c were built using ISIS 2.4 package [31] and subsequently energy minimized in Avogadro software using MMF94 force field [32, 33]. The energy minimized 3D structures of the ligand molecules were then docked into the ligand binding site of PTP1B using the Flex X. The docking parameters were set to their default values [34]. The docking poses generated by Flex X for each ligand is scored and ranked accordingly. The best scoring pose of each ligand were then analyzed for favorable binding interactions and visualized using Discovery Studio Visualizer (version 20.1.0.19295) [35, 36].

3. RESULTS AND DISCUSSION

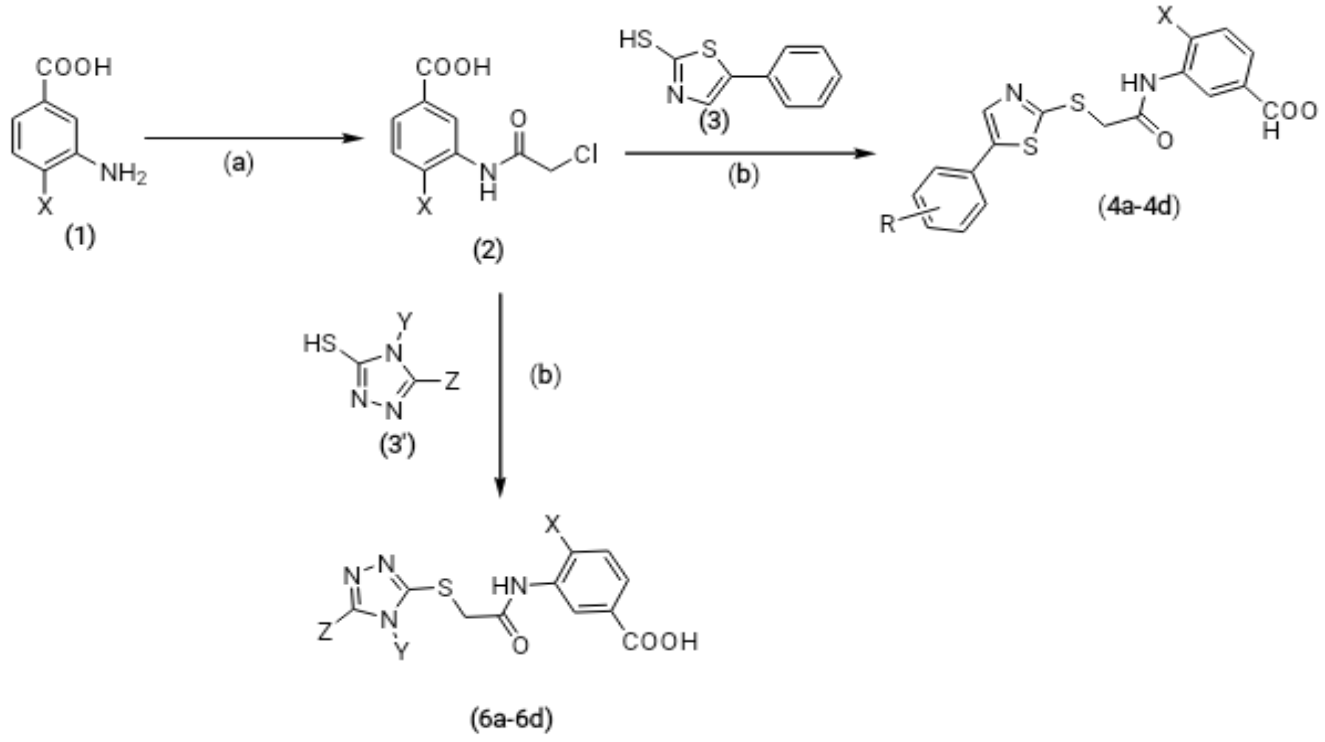
3.1. Chemistry

The title compounds (4a-4d, 6a-6d) were synthesized in two steps employing synthetic protocols previously established in our laboratory (Scheme 1) [26, 27]. In short, chloroacetylation of 3-amino benzoic acid and 3-amino 4-methyl benzoic acid **(1)** under basic conditions in DMF yielded 3-(2-chloroacetamido)4-methyl benzoic acid intermediates **(2).** The intermediates **(2)** were then reacted with substituted 5-phenylthiazole-2-thiols **(3) or** substituted 4*H*-1,2,4-triazole-3-thiols **(3')** in acetone under basic conditions to provide the title compounds in good yields (4a-4d, 6a-6d). The molecular structures of synthesized compounds were established using NMR, Mass, IR spectral data and elemental analysis data [27].

3.1.1. Characterization of 3-(2-(5-phenylthiazol-2-ylthio)acetamido)benzoic acid (4a).

Yield 52%, Mp 224°C. IR (KBr, cm^{-1}) 1710.86 (CO st. of COOH), 1651.07 (amide CO st.), 3500 (NH st.), 3251.98 (OH st. of COOH), 3059.10 (Ar C-H st.), 1550.77 (Ar C-C st.). ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 10.64 (s, 1H, CONH), 8.28 (s, 1H, Ar-CH), 8.01 (s, 1H, Ar-CH), 7.88-7.94 (m, 2H, Ar-CH), 7.80-7.88 (m, 1H, Ar-CH), 7.66 (d, 1H, Ar-CH, J=7.63), 7.46 (t, 1H, Ar-CH, J=7.93), 7.33-7.40 (m, 2H, Ar-CH), 7.28-7.33 (m, 1H, Ar-CH), 4.27 (s, 2H, CH_2). ^{13}C NMR (500MHz, DMSO-d6) δ: 38.66, 114.82, 120.39, 123.68, 124.80, 126.46, 128.64, 129.14, 129.58, 132.01, 133.95, 135.79, 139.56, 154.42, 163.76, 166.95, 167.64. MS (HR-LCMS)

m/z: 371.0519 (M^++H) Found 370.0446, Anal. Calcd. For $C_{18}H_{14}N_2O_3S_2$: C, 58.1230; H, 3.9725; N, 7.9624; S, 17.3248% Found: C, 58.36; H, 3.81; N, 7.56; S, 17.31%.



(a) DMF, Chloroacetyl Chloride, RT, 24 Hrs. (b) K_2CO_3, acetone, reflux, 6 Hrs

Scheme 1: Synthesis of 3-(2-(5-phenylthiazol-2-ylthio)acetamido)benzoic acid analogues (4a- 4d) and 3-(2-(4*H*-1,2,4-triazol-3-ylthio)acetamido)benzoic acid analogues (6a-6d).

3.1.2. Characterization of 3-(2-(5-(4-bromo-phenyl) thiazol-2-ylthio) acetamido)benzoic acid (4b)

Yield 56% Mp 248°C. IR (KBr, cm^{-1}) 1697.36 (CO st. of COOH), 1658.78 (amide CO st.), 3261.63 (OH st. of COOH), 3074.53 (Ar C-H st.), 2970.38 (Ali-CH st.) 1546.91 (Ar C-C st.), 750.31 (C-S st.). ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 10.64 (s, 1H, CONH), 8.26 (t, 1H, Ar-CH, J= 1.68), 8.07 (s, 1H, Ar-CH), 7.81-7.91 (m, 3H, Ar-CH), 7.66 (d, 1H, Ar-CH, J=7.63), 7.51-7.58 (m, 2H, Ar-CH), 7.46 (t, 1H, Ar-CH, J=7.93), 4.27 (s, 2H, CH_2). ^{13}C NMR (500MHz, DMSO-d6) δ: 39.44, 115.67, 120.37, 121.76, 123.70, 124.83, 128.44, 129.61, 132.02, 132.06, 133.15, 139.52, 153.12, 164.23, 166.21, 168.55. MS (HR-LCMS) m/z: 448.9624 (M^++H) Found 447.9551, Anal. Calcd. For $C_{18}H_{13}BrN_2O_3S_2$: C, 48.3264; H, 2.7925; N, 6.0234; S, 14.0014% Found: C, 48.11; H, 2.92; N, 6.23; S, 14.27%.

3.1.3. Characterization of 4-methyl-3-(2-(5-phenylthiazol-2-ylthio)acetamido)benzoic acid (4c)

Yield 57% Mp 231.5°C. IR (KBr, cm^{-1}) 1707.00 (CO st. of COOH), 1676.14 (amide CO st.), 3267.41 (OH st. of COOH), 3012.81 (Ar C-H st.), 2914.44 (Ali-CH st.), 2848.86 (CH_3 st.), 1533.41 (Ar-C-C st.), 763.81 (C-S st.). ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 9.92 (s, 1H, CONH), 7.99-8.10 (m, 2H, Ar-CH), 7.96 (d, 2H, Ar-CH, J=7.93), 7.67 (d, 1H, Ar-CH, J=7.93), 7.38-7.47 (m, 2H, Ar-CH), 7.34 (s, 1H, Ar-CH), 7.26 (d, 1H, Ar-CH, J=7.93), 4.33 (s, 2H, CH_2), 2.21 (s, 3H, CH_3). ^{13}C NMR (500MHz, DMSO-d6) δ: 18.38, 38.24, 114.92, 126.26, 126.49, 126.72, 128.68, 129.22, 130.57, 132.66, 133.95, 134.02, 135.95, 136.23, 154.60, 163.98, 166.62, 168.55. MS (HR-LCMS) m/z: 385.0675 (M^++H) Found 384.0602, Anal. Calcd. For $C_{19}H_{16}N_2O_3S_2$: C, 59.2583; H, 4.3265; N, 7.2142; S, 16.1017% Found: C, 59.35; H, 4.19; N, 7.29; S, 16.68%.

3.1.4. Characterization of 3-(2-(5-(4-bromophenyl) thiazol-2-ylthio)acetamido)-4-methylbenzoic acid (4d)

Yield 58% Mp 245°C. IR (KBr, cm^{-1}) 1689.64 (CO st. of COOH), 1662.64 (amide CO st.), 3261.63 (OH st. of COOH), 3016.67 (Ar C-H st.), 2976.16 (Ali-CH st.), 2837.29 (CH_3 st.), 1543.05 (Ar C-C st.), 740.67 (C-S st.). ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 9.86 (s, 1H, CONH), 8.11 (s, 1H, Ar-CH), 8.04 (s, 1H, Ar-CH), 7.91 (d, 2H, Ar-CH, J=8.54 MHz), 7.68 (d, 1H, Ar-CH, J=7.63), 7.61 (d, 2H, Ar-CH, J=8.54), 7.29 (d, 1H, Ar-CH, J=7.93), 4.33 (s, 2H, CH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (500MHz, DMSO-d6) δ: 18.39, 38.22, 115.80, 121.82, 126.16, 126.72, 128.47, 130.78, 132.15, 133.21, 136.35, 153.35, 164.36, 166.35. MS (HR-LCMS) m/z: 462.9780 (M^++H) Found 461.9707, Anal. Calcd. For $C_{19}H_{15}BrN_2O_3S_2$: C, 49.6530; H, 3.6501; N, 5.9432; S, 13.5917% Found: C, 49.25; H, 3.26; N, 6.05; S, 13.84%.

3.1.5. Characterization of 3-(2-(5-(pyridin-4-yl)-4H-1,2,4-triazol-3-ylthio)acetamido) benzoic acid (6a)

Yield 48% Mp 254.5°C. IR (KBr, cm^{-1}) 1708.93 (CO st. of COOH), 1666.50 (amide CO st.), 3329.14 (OH st. of COOH), 3097.68 (Ar C-H st.), 2985.81 (Ali-CH st.), 1417.68 (pyridine st.), 765.74 (C-S st.). ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 10.54 (s, 1H, CONH), 8.65-8.74 (m, 2H, Ar-CH), 8.24 (t, 1H, Ar-CH, J=1.68 MHz), 7.85-7.91 (m, 2H, Ar-CH), 7.78-7.83 (m, 1H, Ar-CH), 7.62-7.68 (m, 1H, Ar-CH), 7.45 (t, 1H, Ar-CH, J=7.93 MHz), 4.19 (s, 2H, CH_2). ^{13}C NMR (500MHz, DMSO-d6) δ: 37.06, 120.38, 120.41, 123.73, 124.81, 129.57, 131.95, 139.50, 150.94, 166.77, 167.61. MS (HR-LCMS) m/z: 356.0812 (M^++H) Found 355.0739, Anal. Calcd. For $C_{16}H_{13}N_5O_3S$: C, 54.1253; H, 3.9932; N, 19.2812; S, 8.9230% Found: C, 54.08; H, 3.69; N, 19.71; S, 9.02%.

3.1.6. Characterization of 3-(2-(4-methyl-4H-1,2,4-triazol-3-ylthio)acetamido)benzoic acid (6b)

Yield 40% Mp 199°C. ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 10.50 (s, 1H, CONH), 8.56 (s, 1H, Ar-CH), 8.20 (s, 1H, Ar-CH), 7.72-7.79 (m, 1H, Ar-CH), 7.65 (d, 1H, Ar-CH, J=7.63), 7.45 (d, 1H, Ar-CH, J=7.63), 4.06-4.09 (m, 2H, CH_2), 3.61 (s, 3H, CH_3). ^{13}C NMR (500MHz, DMSO-d6) δ: 31.30, 38.11, 120.32, 123.67, 124.84, 129.58, 131.94, 139.41, 146.72, 149.16, 166.56, 167.57. MS (HR-LCMS) m/z: 293.1 (M^++H) Found 292.063.

3.1.7. Characterization of 4-methyl-3-(2-(5-(pyridin-4-yl)-4H-1, 2, 4-triazol-3ylthio) acetamido)benzoic acid (6c).

Yield 45% Mp 268.5°C. ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 9.87 (br. s, 1H, CONH), 8.70 (d, 2H, Ar-CH, J=5.80), 8.06 (s, 1H, Ar-NH), 7.91 (d, 2H, Ar-CH, J=5.49), 7.66 (d, 1H, Ar-CH, J=7.93), 7.30 (d, 1H, Ar-CH, J=7.93), 4.20 (s, 2H, CH_2), 2.22 (s, 3H, CH_3). ^{13}C NMR (500MHz, DMSO-d6) δ: 18.35, 39.43, 120.39, 126.00, 126.57, 130.90, 131.29, 136.06, 136.56, 136.79, 150.91, 156.05, 157.63, 166.96, 168.11, 174.49. MS (LCMS) m/z: 370.0968 (M^++H) Found 369.0896.

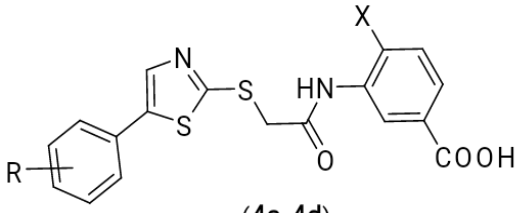
3.1.8. Characterization of 4-methyl-3-(2-(4-methyl-4H-1,2,4-triazol-3-ylthio)acetamido)benzoic acid (6d)

Yield 47% Mp 203.5°C. ^{1}H NMR (500MHz, DMSO-d6) δ (ppm): 9.86 (s, 1H, CONH), 8.57 (s, 2H, Ar-CH), 8.17 (s, 1H, Ar-CH), 8.02 (s, 2H, Ar-CH), 7.42 (s, 1H, Ar-CH), 4.12 (s, 2H, CH_2), 2.28 (s, 6H, CH_3). ^{13}C NMR (500MHz, DMSO-d6) δ: 18.43, 31.32, 37.50, 125.62, 127.67, 131.02, 131.31, 136.13, 136.17, 146.75, 149.32, 165.65, 166.87. MS (LCMS) m/z: 307.1 (M^++H) Found 306.0787.

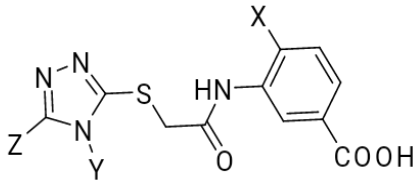
3.2. *In Vitro* PTP1B Enzymatic Inhibition

The PTP1B inhibitory potency of the synthesized compounds was ascertained using a colorimetric assay method (Protein Tyrosine Phosphatase 1B Assay Kit, 539736 from Merck) [27]. The results obtained are presented in Table 1. The synthesized compounds at the beginning were tested for PTP1B inhibitory activity at 10 μM concentrations and only those compounds that elicit inhibition of more than 50 % were regarded as active against PTP1B enzyme. Furthermore, the active compounds were evaluated for PTP1B inhibition at different concentrations and the IC_{50} values were determined. It is evident from the results summarized in table 1 that almost all the synthesized compounds except for compound **4a**, showed two-fold greater PTP1B inhibition than the reference standard Suramin at 10 μM concentration. Overall, the compounds with 4-methyl substitution in phenyl ring of 3-acetamido-4-

methyl benzoic acid exhibited enhanced PTP1B inhibition than the unsubstituted analogues. In case of 3-(2-((5-phenylthiazol-2-yl) thio) acetamido) benzoic acid derivatives (**4a**-**4d**), presence of 4-Bromo substituent in phenyl ring caused a modest improvement in PTP1B inhibition shown by these analogues. Presence of a 4-pyridinyl substitution in 1,2,4-triazole-3-thiol moiety caused a slight improvement the PTP1B inhibitory potency in 3-(2-((4H-1,2,4-triazol-3-yl) thio) acetamido) -4-methylbenzoic acid derivatives (**6a**-**6d**). Between the two series, the analogues containing 1,2,4-triazole-3-thiol moiety (**6a**-**6d**) elicited slightly better PTP1B inhibitory potency than the analogues with thiazole-2-thiol moiety (**4a**-**4d**). Infact, compound **6c** with a 4-pyridinyl substitution in the C5 of 1,2,4-triazole-3-thiol exhibited more than 50% PTP1B inhibition at 10 μM concentration and the IC_{50} value of **6c** was determined to be 6.45 μM.



(4a-4d)



(6a-6d)

Table 1: Molecular Structures and PTP1B inhibition data of the compounds reported in this study (4a-4d) and (6a-6d).

Compound Code	R	X	Y	Z	% PTP1B Inhibition (at 10 μM)	IC_{50} μM
4a	4-H	4-H	--	--	32.96%	-
4b	4-Br	4-H	--	--	42.03%	-
4c	4-H	4-CH_3	--	--	41.41%	-
4d	4-Br	4-CH_3	--	--	43.90%	-
6a	--	4-H	H	4-pyridine	47.37%	-
6b	--	4-H	CH_3	H	46.73%	-
6c	--	4-CH_3	H	4-pyridine	**53.12%**	**6.45 μM**
6d	--	4-CH_3	CH_3	H	48.34%	-
Suramin	-	-	-	-	21.02%	-

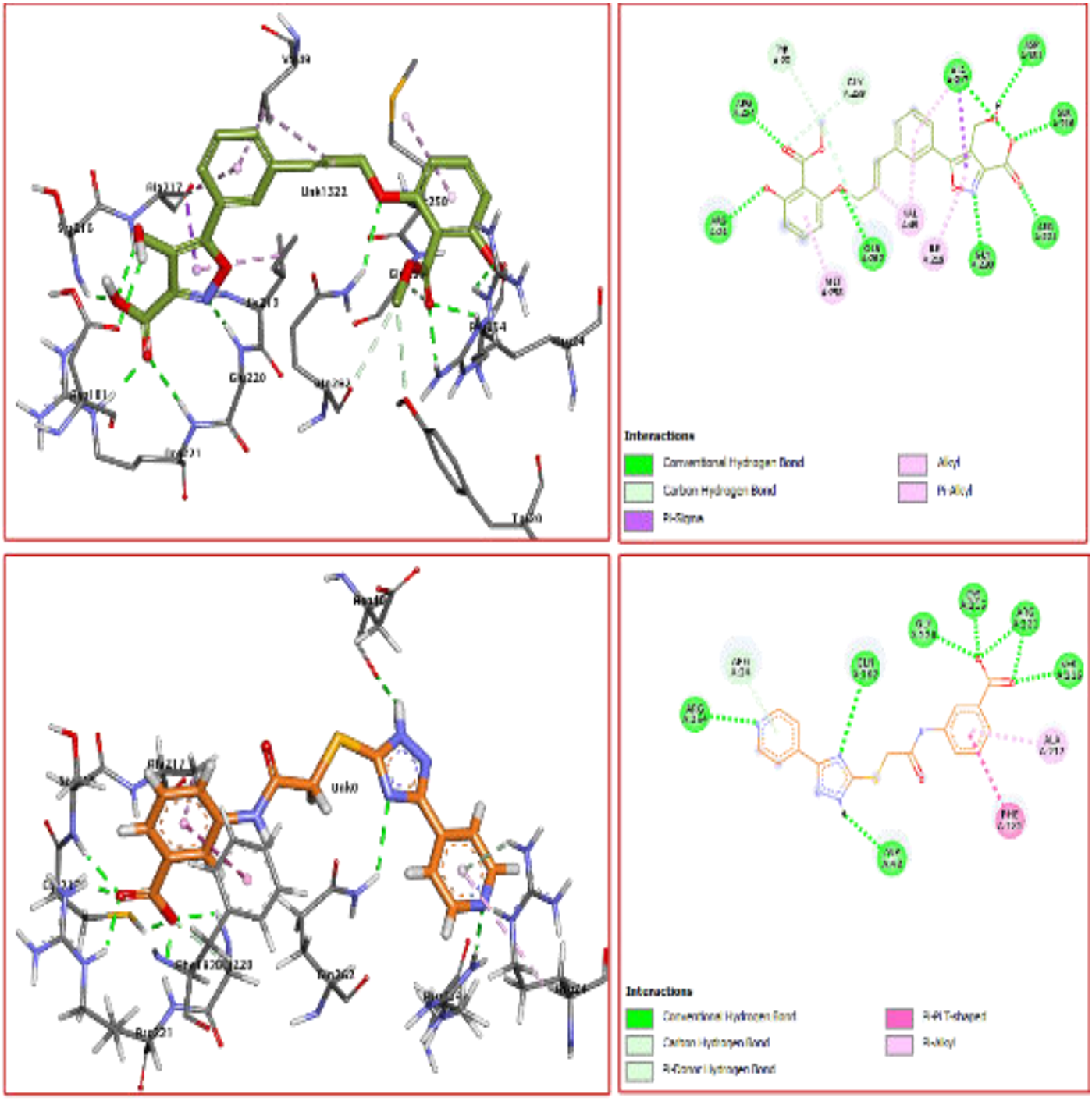
3.3. *In Vivo* Antihyperglycemic Activity

The most active compound **6c** was also assessed for in *vivo* antidiabetic activity in streptozotocin (STZ) induced type II diabetic rat model by using Wistar rats [27, 30]. The test compound **(6c)** and standard drug (pioglitazone) were administered orally, 30 mg/kg single dose once a day for 21 days after induction of diabetes [31]. The level of serum glucose was measured on day 0, 7th, 14th, 21st day. The *in vivo* efficacy of compound **6c**, and pioglitazone were compared with control (Fig. 3). As shown in Fig. 3, the reduction of blood glucose level affected by compound **6c** in diabetic animals is same as that of pioglitazone suggesting that the test compound produced almost comparable antidiabetic efficacy to the standard pioglitazone. The body weights of rats were also measured and recorded during twenty-one days study period (Fig. 4). The Wistar rats treated with compound **6c** exhibited a marginal decrease in body weight whereas pioglitazone treated rats showed a marginal increase in the body weight.

3.4. Molecular Docking Studies

Molecular docking studies were also carried out with the most active compound **6c** using Flex X docking algorithm [28] to elucidate the binding mode of **6c** in the catalytic site of the PTP1B (PDB ID: 1XBO) [27, 29]. Initially, the reliability of the docking protocol employed was established by redocking the co-crystallized ligand into the active site and the binding conformation of co-complexed ligand was successfully reproduced with highest accuracy (RMSD < 1). Fig. 2 presents the top scoring pose of **6c** in the ligand binding site of PTP1B. In this complex, the acetamido benzoic acid moiety of compound **6c** is favorably placed in the high affinity phosphate binding site enabling multiple H-bond interactions amongst carboxylate group and various amino acid residues like Cys215, Ser216,

Gly220 and Arg221. This placement of acetamido benzoic acid moiety also positions the 4-Pyridinyl substitution at C3 position of 1, 2, 4-triazole-3-thiomethyl moiety in the low-affinity non-catalytic site permitting hydrogen bonding between the pyridinyl nitrogen with Arg24 and Arg224. Lastly, the ring nitrogens N1 and N4 of 1,2,4-triazole ring formed hydrogen bonds with amino acid residues Asp48 and Gln262 in the second aryl binding site.



Interacting active site acid residues are represented as sticks and the co-complexed ligand is depicted as sticks with green carbons. ***(B)*** *Docked confomation of compound* ***6c*** *in the active site of PTP1B (PDB id: 1XBO). Interacting active site residues are depicted as sticks and the compound* ***6c*** *is shown as sticks with orange carbons.*

Fig. 2: Binding mode of co-complexed ligand in the active site of PTP1B (PDB id: 1XBO).

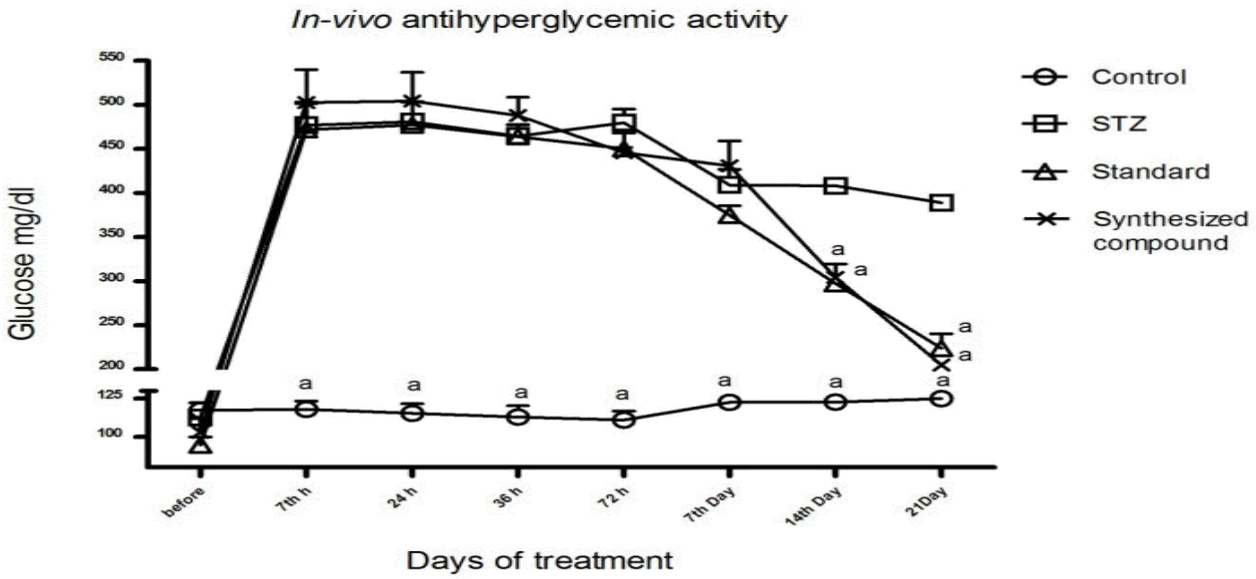


Fig. 3: Antihyperglycemic activity of compound 6c in STZ induced diabetic rats

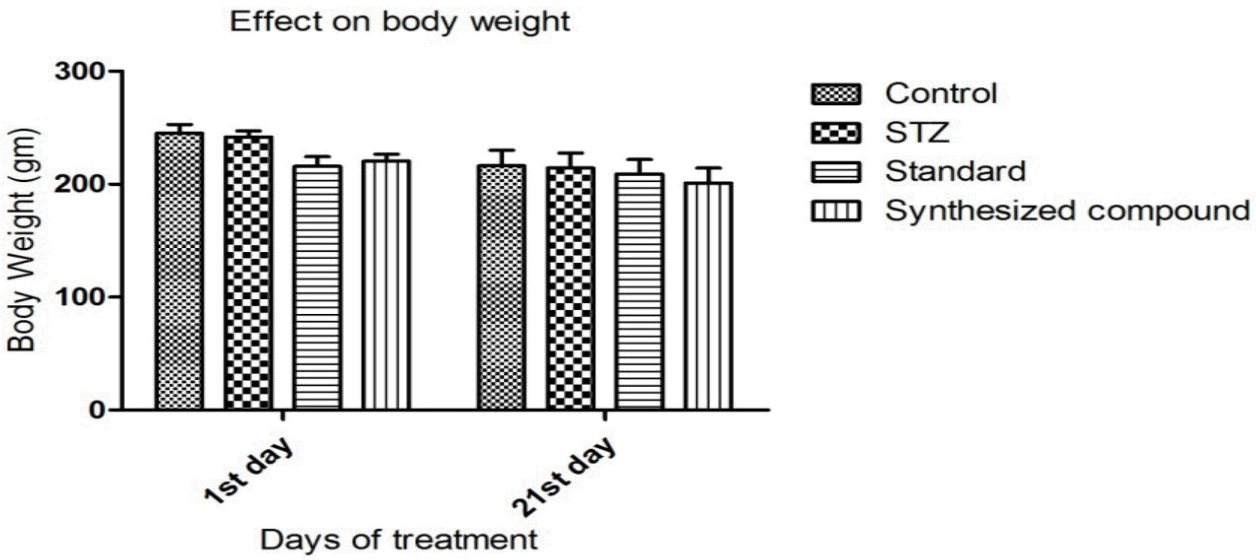


Fig. 4: Effect on body weight by 6c in STZ induced diabetic rats

4. CONCLUSION

In summary, this report describes the synthesis and evaluation of antidiabetic activity of a series of PTP1B inhibitors developed based on a lead molecule discovered in our laboratory. Bioisoteric replacement of phenyl substituted oxadiazole in the lead molecule with substituted 'thiazole' and 'triazole' moieties yielded compounds with greater PTP1B inhibitory potency. Between the two, the analogues containing 1,2,4-triazole moiety showed slightly better PTP1B inhibition than the analogues with thiazole moiety. Compound **6c** with a 5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thio group linked to 3-acetamido-4-methylbenzoic acid exhibited potent PTP1B inhibition (IC_{50} value = 6.45 μM). Molecular docking studies with **6c** revealed that the compound interacted with key residues deemed important for the catalytic activity of the PTP1B enzyme. In addition, the antidiabetic activity shown by compound **6c** in Streptozotocin-induced diabetic model was found comparable to that of standard antidiabetic drug pioglitazone. All these findings establish compound **6c** as a valuable prototype for the development of potent PTP1B inhibitors with antidiabetic potential.

Ethics approval and consent to participate

Ethical approval for this study was prevailed from Institutional Animal Ethics Committee (IAEC), Indira Gandhi National Tribal University Amarkantak, Madhya Pradesh (Reg. No. 2004/GO/ReBi/S/18/CPCSEA), the protocol code is IGNTU/IAEC/16.

Human and animal rights

The experimental protocol regarding animal studies were approved by the 'Institutional Animal Ethical Committee' (IAEC) and conducted according to the 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) guidelines on the use and care of experimental animals.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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