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Research Article

DESIGN, SYNTHESIS AND EVALUATION OF PROTEIN TYROSINE PHOSPHATASE 1B (PTP1B) INHIBITORY ACTIVITY OF HETEROCYCLIC DERIVATIVES OF CHALCONE SCAFFOLD

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is considered one of the most common metabolic disorders which mainly associate with consequences of hyperglycemia. Recently many macromolecular targets for diabetes have been investigated and Protein tyrosine phosphatase 1B (PTP1B) is one such target for anti-diabetic medicinal agents. It is believed that inhibition of PTP1B offers anti-diabetic response by altering insulin resistance and signaling pathway. However agents possessing PTP1B inhibitory activity yet to come in market as anti-diabetic compounds therefore finding of such type of anti-diabetic agents is still in progress. Computational tools based on ligand and receptor interactions showed some prerequisite structural features for PTPIB inhibitory activity. *In silico* virtual screening was performed to design ligand with required structural features and on the basis of findings of virtual screening we designed some heterocyclic derivatives of chalcone scaffold. Designed ligands possess structural similarities with co-crystallized ligand bound with 3D crystal structure of PTP1B receptor. Therefore PTPIB inhibitory activity was expected with designed ligands. Moreover these ligands showed interactions with PTP1B receptor in molecular docking study, thus further synthesized and evaluated for their *in-vitro* PTPIB inhibitory activity using PTP1B enzyme colourimetric assay kit. Study observed 60.57 % inhibition of enzyme PTP1B when tested against compound **AS-4** ((*E*)-3-(3-nitrophenyl)-1-(thiophen-2-yl) prop-2-en-1-one) which was considered as most potent inhibitory agent.

Keywords: Chalcone, Heterocycles, Anti-diabetic, PTP1B, Enzyme

1. INTRODUCTION

The increased glucose level in blood resembles condition of Diabetes mellitus (DM) which is very common now-a-days and turned to chronic condition if preventive measure not adopted at early stage. Diabetes mellitus is mainly associated with impaired insulin secretion or peripheral insulin deficiency in adipose, liver and muscle tissues [1]. There are two types of diabetes mellitus Type 1 and Type 2, the severe form Type 2 diabetes mellitus (T2DM) can initiate or worsen pathogenesis of other health problems like cardiac diseases, atherosclerosis, diabetic retinopathy and kidney dysfunction, etc [2]. Insulin resistance and disruption in leptin can be attributed to the pathogenesis of T2DM [3].

As discussed above, insulin signal transduction pathways play important role in the pathogenesis of T2DM. Many macromolecular protein receptor possess ability to alter this signaling pathway and "Protein Tyrosine Phosphatase 1B" (PTP1B) is one such enzyme which modulates signal transduction pathways in tissues responsive to the insulin [4]. PTP1B negatively modulate insulin pathway responsible for the lipid and glucose metabolism in adipose and skeletal tissues [5, 6], therefore it is stated that hyper-activation of PTP1B can results metabolic disorder like type 2 diabetes mellitus [7, 8]. Researcher presented study showing enhanced insulin sensitivity and increased glucose tolerance level in mice models after the removal of PTP1B gene [9]. Hence, PTP1B can be taken as therapeutic target for the development of novel anti-diabetic agents [10].

It is clear that medicinal agents that inhibit negative regulation of PTP1B can help to treat type 2 diabetes mellitus [11,12]. The possible mode of action of PTP1B inhibitors as anti-diabetic agents is prolongation of half life of phosphorylated insulin receptor which ultimately enhances effects of insulin.

Chalcones or 1, 3-diaryl-2-propen-1-ones are three-carbon α, β-unsaturated carbonyl system joined by two aromatic rings, it is considered precursors of flavonoids and isoflavonoids in plants [13]. Chalcones and chalcone derivatives seek attention of researchers due to their

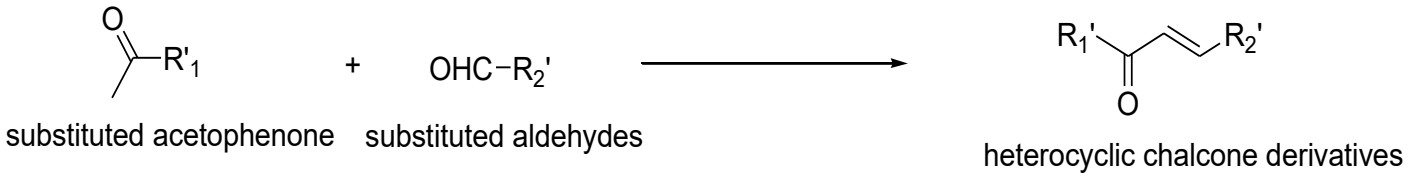
wide spectrum of biological activities [14, 15]. Similarly heterocyclic derivatives of chalcone also investigated recently for their various pharmacological activities in a view to develop novel synthetic agents of medicinal importance [16-18].

Considering all the facts discussed above, we planned to design and synthesize some heterocyclic derivatives of chalcones scaffolds as PTP 1B inhibitor. The strategy of work involves utilization of structural features of co-crystallized ligand; "5-{2-fluoro-5-[3-(3-hydroxy-2-methoxycarbonyl-phenoxy)-propenyl]-phenyl}-isoxazole-3-carboxylic acid" bound with 3D crystal structure (PDB ID: 1Q1M) of PTP 1B receptor retrieved from RCSB (Research Collaboratory for Structural Bio-informatics) data source. The co-crystallized ligand possesses some specific structural features through which it bound with active sites of enzyme (3D crystal structure of PTP 1B) as depicted in Fig. 1. Considering these features as prerequisite requirement for PTP 1B inhibitory activity some heterocyclic derivatives of chalcone scaffolds were designed and evaluated for their possible interactions with active sites of enzyme using molecular docking (Maestro) study. Ligands those exhibited prominent interactions with enzyme further synthesized and evaluated for their anticipated PTP 1B inhibitory activity using *in vitro* assay kit.

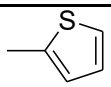
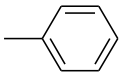
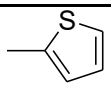
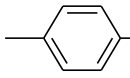
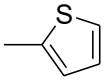
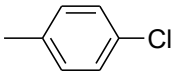
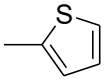
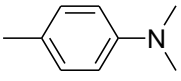
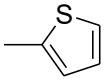
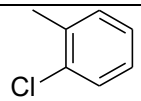
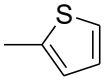
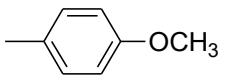
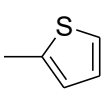
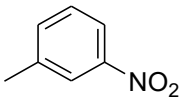
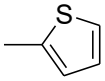
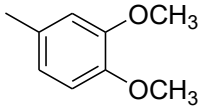
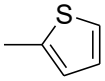
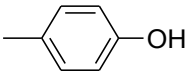
2. MATERIAL AND METHODS

2.1. Chemistry

The target derivatives were synthesized as mentioned in scheme 1, using Claisen-Schmidt reaction between substituted benzaldehyde and acetophenone.



Scheme 1: Synthetic route used for proposed derivatives

Compound Code	R_1'	R_2'	Compound Code	R_1'	R_2'
AS-1			**AS-6**		
AS-2			**AS-7**		
AS-3			**AS-8**		
AS-4			**AS-9**		
AS-5					

2.2. Synthesis of heterocyclic derivatives of chalcones (AS1-AS9)

Mixture of 2-acetyl thiophene and substituted benzaldehydes in ethanol wasstirred using magnetic stirrer and 5% solution of potassium hydroxide was added slowly to the mixture, temperature was maintained around 20 to 25°C. The reaction mixture was further stirred for 6 h; cooled and refrigerated overnight. The precipitate of crude heterocyclic chalcones **(*AS1-AS9*)** was filtered, dried and recrystallized using rectified spirit.

2.3. Molecular docking studies

Crystal structures of PTP1B (PDB codes: 1Q1M) was retrieved from RCSB Protein Data Bank and protein preparation was done to optimize docking features of receptor. Water molecules were removed and hydrogen atoms were added to ensure protein integrity followed

by correction in bonds orders sequences. The amino acids residues were considered important for interactions such as; Asp, His and Lys were assigned as protonated. OPLS 2005 force field model was employed to minimize heavy atoms with in required RMSD (0.3). Glide docking was performed using Schrödinger software. Rational geometry of 3D structures of protein and ligand were used for Glide docking with standard protocol [19].

2.4. *In vitro* PTP1B enzyme inhibitory activity

Synthesized compounds were tested for their *in vitro* PTP1B enzyme inhibitory activity using colourimetric, assay kit obtained from Merck Millipore. Enzyme inhibitory activity was performed using human recombinant PTP1B and suramin was used as controlled drug provided with assay kit. Assay was performed using 96 well plates microtiter as per the manufacturer's protocol in which compounds were dissolved in DMSO. Method involves detection of free phosphate as per the principle of classic Malachite green assay [20]. The percentage inhibition of PTP1B enzyme by the test compounds was calculated using following formula; considering activity of the control tube (without inhibitor) as 100 %

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

3. RESULTS AND DISCUSSION

3.1. Chemistry

Mixture of 2-acetyl thiophene and substituted benzaldehydes in ethanol along with 5% solution of potassium hydroxide was utilized to synthesized heterocyclic derivative of chalcones scaffolds (*AS1-AS9*). Spectral analysis was performed to confirm purity and structural integrity of synthesized compounds. Melting points of synthesized compounds were determined using capillary melting point apparatus (Lab Hosp). Thin Layer Chromatography (TLC) was performed on silica Gel G coated plate to monitor progress of reaction and purity of compounds. Physicochemical characteristics of synthesized derivatives were mentioned in Table 1.

IR spectra were recorded in KBr on MB3000 (Make-ABB Bomen) spectrometer. The ^{1}H-NMR spectra were recorded in DMSO on Avance II 400 (Make-Bruker) NMR spectrometer. The Mass spectra were recorded on Jeol SX-102 (Make-Waters) spectrometer.

Table 1: Physicochemical characteristics of synthesized derivatives

Compound Code	Molecular Formula	TLC	M.P.
AS-1	$C_{13}H_{10}OS$	Rf= 0.51	120-122°C
AS-2	$C_{13}H_9ClOS$	Rf= 0.43	162-164°C
AS-3	$C_{13}H_9ClOS$	Rf= 0.46	135-137°C
AS-4	$C_{13}H_9NO_3S$	Rf= 0.48	132-134°C
AS-5	$C_{13}H_{10}O_2S$	Rf= 0.54	185-187°C
AS-6	$C_{13}H_9FOS$	Rf= 0.49	128-130°C
AS-7	$C_{15}H_{15}NOS$	Rf= 0.52	180-182°C
AS-8	$C_{14}H_{12}O_2S$	Rf= 0.56	160-162°C
AS-9	$C_{15}H_{14}O_3S$	Rf= 0.51	190-192°C

Rf value in solvent system: chloroform:ethylacetate (2:3)*

3.2. Spectral Analysis of synthesized compounds

3.2.1. (E)-3-phenyl-1-(thiophen-2-yl)prop-2-en-1-one (AS1)

Yield 71 %, ^{1}H NMR: δ 6.74 (1H, d, *J* = 15.7 Hz), 7.20 (1H, dd, *J* = 7.2, 5.0 Hz), 7.37-7.52 (5H, 7.48 (tt, *J* = 7.5, 1.5 Hz), 7.45 (dddd, *J* = 8.1, 2.3, 1.5, 0.5 Hz), 7.42 (dddd, *J* = 8.1, 7.5, 2.0, 0.5 Hz)), 7.56 (1H, d, *J* = 15.7 Hz), 7.73-7.78 (2H, 7.76 (dd, *J* = 7.2, 1.2 Hz), 7.76 (dd, *J* = 5.0, 1.2 Hz). Mass spectra: m/e: 215.2.

3.2.2. (E)-3-(4-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS2)

Yield 51 %, ^{1}H NMR: δ 6.70 (1H, d, *J* = 15.7 Hz), 7.20 (1H, dd, *J* = 7.2, 5.0 Hz), 7.50-7.59 (5H, 7.54 (d, *J* = 15.7 Hz), 7.56 (ddd, *J* = 8.1, 1.4, 0.5 Hz), 7.54 (ddd, *J* = 8.1, 1.5, 0.5 Hz)), 7.72-7.78 (2H, 7.75 (dd, *J* = 7.2, 1.2 Hz). Mass spectra: m/e: 249.2

3.2.3. (E)-3-(2-chlorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS3)

Yield: 48%%, ^{1}H NMR: δ 6.70 (1H, d, *J* = 15.7 Hz), 7.20 (1H, dd, *J* = 7.2, 5.0 Hz), 7.34 (1H, ddd, *J* = 7.9, 7.4, 1.3 Hz), 7.50-7.61 (3H, 7.55 (d, *J* = 15.7 Hz), 7.57 (ddd, *J* = 8.1, 7.4, 1.5 Hz), 7.53 (ddd, *J* = 8.1, 1.3, 0.5 Hz). Mass spectra: m/e: 249.2

3.2.4. (E)-3-(3-nitrophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS4)

Yield: 66%, ^{1}H NMR: δ 6.76 (1H, d, *J* = 15.7 Hz), 7.12-7.25 (3H, 7.15 (ddd, *J* = 8.2, 2.4, 1.6 Hz), 7.20 (dd, *J* = 7.2, 5.0 Hz), 7.21 (ddd, *J* = 8.2, 8.0, 0.5 Hz)), 7.34-7.41 (2H, 7.37 (ddd, *J* = 1.9, 1.6, 0.5 Hz), 7.37 (ddd, *J* = 8.0, 2.4, 1.9 Hz). Mass spectra: m/e: 260.2

3.2.5. *(E)-3-(4-hydroxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS5)*

Yield: 52%, ^{1}H NMR: δ 6.63 (1H, d, *J* = 15.7 Hz), 6.90 (2H, ddd, *J* = 8.3, 1.6, 0.4 Hz), 7.20 (1H, dd, *J* = 7.0, 5.0 Hz), 7.49 (1H, d, *J* = 15.7 Hz), 7.57 (2H, ddd, *J* = 8.3, 1.9, 0.4 Hz). Mass spectra: m/e: 231.2

3.2.6. *(E)-3-(4-fluorophenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS6)*

Yield: 59%, ^{1}H NMR: δ 6.67 (1H, d, *J* = 15.7 Hz), 7.13-7.23 (3H, 7.16 (ddd, *J* = 8.1, 1.3, 0.6 Hz), 7.20 (dd, *J* = 7.2, 5.0 Hz)), 7.53 (1H, d, *J* = 15.7 Hz), 7.75 (1H, dd, *J* = 7.2, 1.2 Hz). Mass spectra: m/e: 233.2

3.2.7. *(E)-3-(4-(dimethylamino)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (AS7)*

Yield: 52%, ^{1}H NMR: δ 6.21 (1H, d, *J* = 15.9 Hz), 6.97 (2H, ddd, *J* = 8.1, 1.6, 0.5 Hz), 7.19 (1H, dd, *J* = 7.0, 5.0 Hz), 7.33 (1H, d, *J* = 15.9 Hz), 7.56 (2H, ddd, *J* = 8.1, 1.5, 0.5 Hz), Mass spectra: m/e: 258.2

3.2.8. *(E)-3-(4-methoxyphenyl)-1-(thiophen-2-yl) prop-2-en-1-one (AS8)*

Yield: 64%, ^{1}H NMR: δ 3.83 (3H, s), 6.62 (1H, d, *J* = 15.7 Hz), 7.16-7.23 (3H, 7.20 (ddd, *J* = 8.8, 1.2, 0.5 Hz), 7.20 (dd, *J* = 7.0, 5.0 Hz)), 7.43-7.54 (3H, 7.51 (ddd, *J* = 8.8, 1.9, 0.5 Hz), 7.47 (d, *J* = 15.7 Hz)), 7.72-7.77 (2H, 7.74 (dd, *J* = 7.0, 1.2 Hz). Mass spectra: m/e: 245.2

3.2.9. *(E)-3-(3,4-dimethoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one (AS9)*

Yield: 66%, ^{1}H NMR: δ 3.81 (3H, s), 3.97 (3H, s), 6.63 (1H, d, *J* = 15.7 Hz), 6.74 (1H, dd, *J* = 8.4, 0.4 Hz), 7.16-7.25 (2H, 7.24 (dd, *J* = 1.9, 0.4 Hz), 7.20 (dd, *J* = 7.2, 5.0 Hz)), 7.57 (1H, d, *J* = 15.7 Hz), 7.69-7.77 (2H, 7.72 (dd, *J* = 8.4, 1.9 Hz). Mass spectra: m/e: 275.2

3.3. Molecular docking studies

Computational method of virtual screening was used to identify binding mode of designed ligands in the active sites of 3D crystal structures of PTP1B receptor, these binding interaction provides idea about the possible enzyme inhibitory activity of compounds. The co-crystallized ligand bound with 3D crystal structure (PDB ID: 1Q1M) of PTP 1B receptor retrieved from RCSB data source showed some key interactions as depicted in Fig. 1. The similar interactions were expected from designed ligands with active sites of PTP1B receptor. Protein preparation wizard of Schrödinger suite was used to optimize crystal structure of PTP1B receptor. Binding position of co-crystal ligand was used to generate receptor grids which ensure confined area of drug-receptor interaction [21, 22]. Glide docking was used to detect binding interactions of designed ligands with 3D crystal structure of enzyme PTP1B (PDB ID: 1Q1M).

Literature revealed closed proximity of important binding areas in active site of enzyme PTP1B as sub-pockets A, B and C, these sub-pockets of enzyme were considered important for inhibition of insulin signaling pathway [23, 24]. Fortunately some of the designed ligands (AS-4, AS-5, AS-8 & AS-9) exhibited interaction in sites A as well as n site B. Compounds binds with Arg 221 and Tyr 46 residues of receptor in site A and site C respectively. Interestingly polar interactions observed with compounds AS4 and AS5, moreover these compounds also showed prominent enzyme inhibitory activity when tested using *in vitro* enzyme assay kit. Compound AS5 exhibited polar interaction with Arg 221 in Site A & hydrophobic interaction with Tyr 46 in Site C. The oxygen of compound AS5 ((*E*)-3-(4-hydroxyphenyl) - 1 - (thiophen -2-yl) prop-2-en -1-one) bind with Arg 221 residue through polar interaction in site A and it bind with Tyr 46 in Site C through hydrophobic interaction. Compound AS4 ((*E*)-3-(3-nitrophenyl)-1-(thiophen-2- yl) prop - 2 - en - 1 - one) interact with Arg 221 through hydrophobic interaction, it exhibited polar interaction with Gln 262 residue of receptor, here oxygen of nitro group of compound AS4 bind with Gln 262 residue. The study observed that presence of hydrogen bond acceptor or donor in terminal rings at *para* position play crucial role in ligand-receptor interactions. The docking study revealed that all compounds interacts at least one or more catalytic sites of PTP1B. Potent compounds such as AS4 and AS5 exhibited prominent interactions (fig. 2) almost in similar manner as like co-crystallized ligand bound with crystal structure of PTP1B (fig.1). These interactions suggested PTP1B inhibitory activity of tested compounds.

3.4. *In vitro* PTP1B enzyme inhibitory activity

The synthesized derivatives were tested for their PTP1B enzyme inhibitory activity as per the standard protocol mentioned in assay kit. The synthesized compounds were evaluated at level of concentration 30 μM.

Compounds AS4 & AS5 which showed appreciable interactions in molecular docking study also showed ≥50 % inhibition of PTP1B enzyme during *in-vitro* assay (Table 2). Compound AS4 & AS5 observed as most potent agents with 60.57 & 54.09% inhibition of enzyme respectively. These findings suggested these compounds can be developed as anti-hyperglycemic agent based on their PTP1B enzyme inhibitory potential.

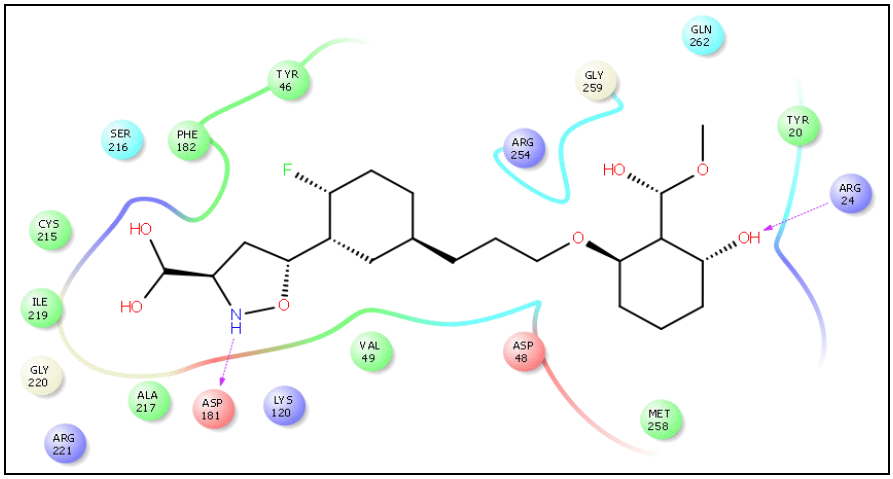


Fig. 1: Interaction between ligand and PTP1B enzyme in the co-crystallized enzyme inhibitor complex (PDB ID: 1Q1M, 2.3 Å).

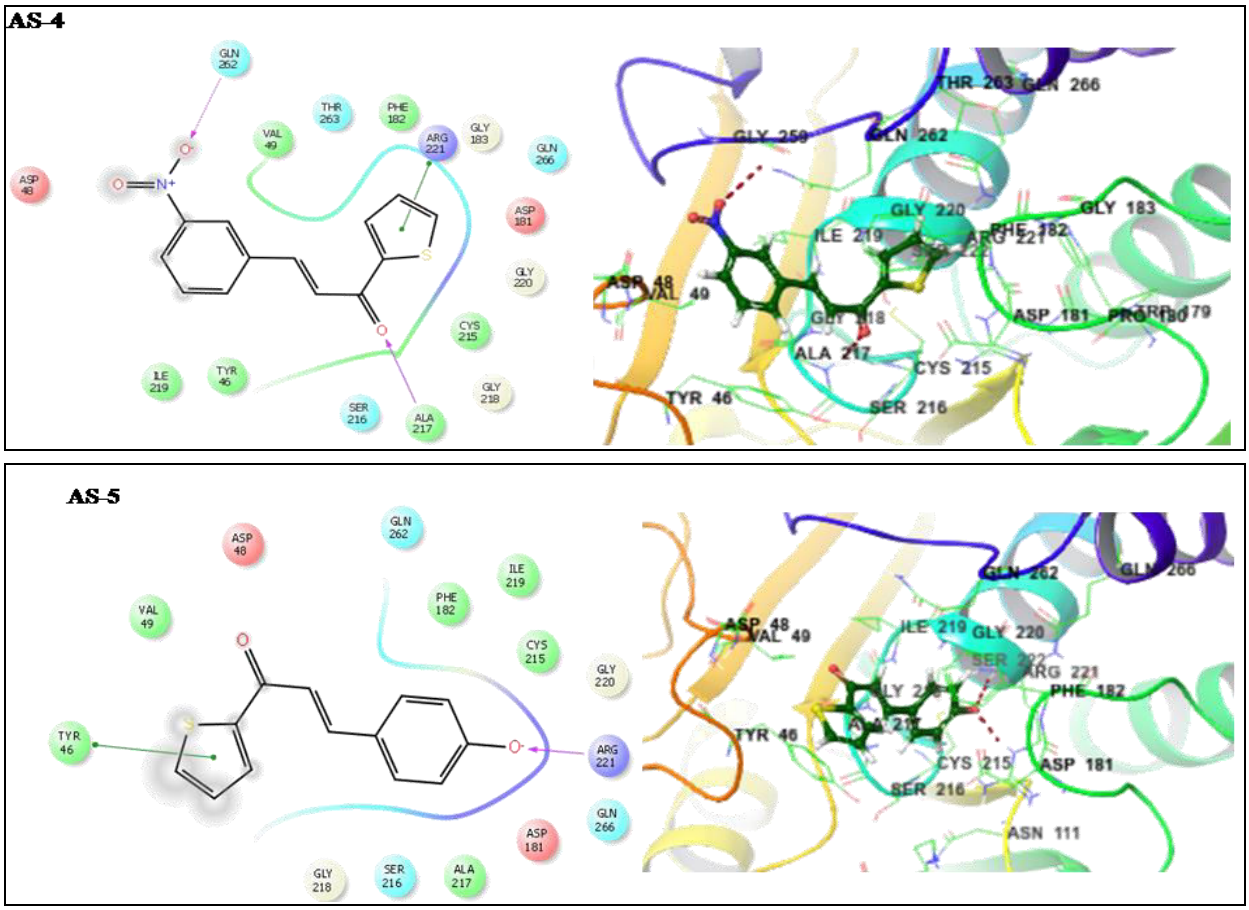
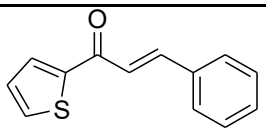
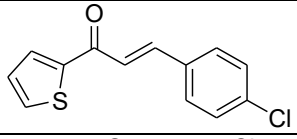
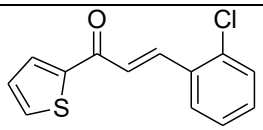
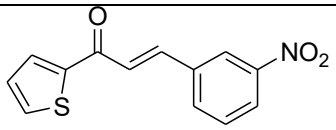
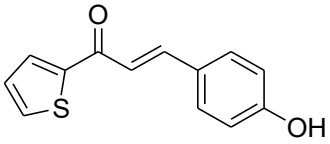
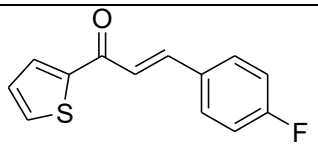
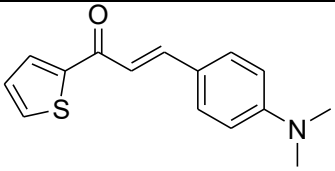
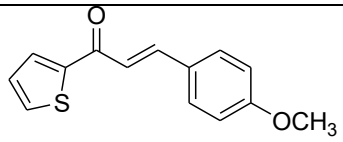
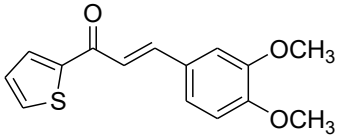


Fig. 2: Binding mode of compounds AS4 and AS5 at PTP1B binding sites (PDB ID: 1Q1M). Important amino acids are depicted as sticks, whereas the lead ligand is shown in green colour with nitrogen and oxygen atom in blue and pink, respectively. Brown dotted lines represent hydrogen bonding in the active site of PTP1B.

Table 2: Results of *in-vitro* PTP1B enzyme inhibitory activity

S. No.	Compound code	Structure of target derivatives	% Inhibitory activity (30 µM)	IC_{50} value (µM)
1	AS-1		36.76	—
2	AS-2		28.01	—
3	AS-3		32.32	—
4	AS-4		60.57	18.23
5	AS-5		54.09	—
06	AS-6		21.00	—
07	AS-7		24.79	—
08	AS-8		42.59	—
09	AS-9		18.97	—
10	Suramin	—	23.04	—

4. CONCLUSION

In summary, heterocyclic derivatives of chalcone scaffolds were designed and synthesized based on prerequisite structural features required for PTP1B inhibition. The synthesized derivatives were evaluated for their Protein Tyrosine Phosphatase 1B inhibitory potential using *in-vitro* assay kit. Compounds AS4 was observed as potent inhibitory agent of enzyme PTP1B (IC50 < 20 µM). The compound AS4 also exhibited remarkable binding interactions with catalytic sites of PTP1B enzyme during molecular docking study. The finding of this study suggested that proposed heterocyclic chalcone derivatives possess excellent scope for further development and evaluation of their anti-hyperglycemic potential in animal model.

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Conflict of interest

No conflict of interest associated with this work.

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