Research Article

SYNTHESIS, PHARMACOLOGICAL EVALUATION AND LIGAND-PROTEIN INTERACTION STUDY OF HYBRID UREA AND THIOUREA DERIVATIVES AS ANTIHYPERGLYCEMIC AGENTS

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ABSTRACT

A series of hybrid urea/thiourea derivatives (5a-5f) with chalcone moiety were synthesized and pharmacological activity was evaluated using *invitro* α -glucosidase inhibition assay and *invivo* antidiabetic activity in streptozotocin (STZ) induced diabetic rat model. Among the synthesized molecules, compound 5d (1-[4'-N-(N'-p-chlorophenylurenyl) phenyl]-3-(4-methoxyphenyl)-2-propen-1-one) is more potent with IC₅₀ value 12.88 μ M when compared to the standard drug Acarbose (IC₅₀ 16.54 μ M) in *invitro* study. *Invivo* study demonstrated that compound 5d was more effective than other synthesized molecules by estimation of different biochemical parameters. For the understanding of ligand-protein interaction, molecular docking studies of the synthesized compounds were also performed. Overall, synthesized hybrid urea/thiourea derivatives might be potential a new class of compound for the treatment of type-II diabetes.

Keywords: Urea/thiourea derivatives; α -glucosidase inhibitor; antidiabetic; Molecular docking

INTRODUCTION

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate in both developed and developing countries¹. Uncontrolled hyperglycemia can lead to serious damage of vital organs including kidney damage, nerve damage and heart disease^{2,3}. Type II diabetes is the majorly affective diabetes which is associated with 'diabetes' and 'metabolic syndrome'. In context of genetic susceptibility in certain ethnic groups, type II diabetes is influenced by environmental and other factors such as a sedentary lifestyle, overly rich nutrition and obesity⁴. However, treatment of diabetes mellitus principally requires the reduction of blood glucose levels and controlling cell signalling cascade. Strikingly, α -glucosidase is a membrane-bound enzyme at the epithelium of the small intestine and play an important role in carbohydrate metabolism⁵. Growing evidences suggested that inhibition of α -glucosidase might significantly decrease the postprandial hyperglycemia⁶. Thus, α -glucosidase inhibition might be a potential therapeutic target for the treatment of type-II diabetes mellitus⁷. Amongst the α -glucosidase inhibitors, acarbose, miglitol, and voglibose are being clinically used for the cure of type II diabetes mellitus⁸⁻¹⁰. However, their adverse effects are also linked to them such as abdominal discomfort, diarrhoea, and flatulence^{11,12} in some cases and newly discovered additive effects (like stabling carotid plaques, and reducing inflammation)¹³ encouraging the development of new therapeutic agents is an utmost interest in medicinal chemistry research.

In this context, urea and thiourea derivatives are important functional groups in numerous natural products and drug intermediates¹⁴ and building blocks for various heterocycles. Urea and thiourea derivatives possess many promising biological activities, such as herbicidal¹⁵, antimicrobial^{16, 17}, antioxidant¹⁸,

antiviral¹⁹, anti-HIV²⁰, antitumor^{21, 22}, antimalarial²³ and antidiabetic activity²⁴⁻²⁶.

This present study involves the synthesis of a series of urea/thiourea derivatives using previously reported methods^{23, 27, 28}. Furthermore, hypoglycemic activity of urea/thiourea derivatives were investigated using well defined *in vitro* α -glucosidase assay and STZ induced diabetic rat model.

MATERIALS AND METHODS

Chemistry

¹H NMR (300 MHz) and ¹³C NMR (400MHz) spectra were recorded on Bruker spectrometer in DMSO- d₆ using TMS as internal standard. IR spectra were recorded on a Bruker Alpha FTIR spectrophotometer. Mass spectra were obtained on ESI mass instrument. Melting points were determined in Veego melting point apparatus. All reactions were monitored by thin layer chromatography (TLC) on pre-coated Silica Gel 60 F₂₅₄; spots were visualized under UV light. All the reactions were carried out using reagent-grade solvents, and the reagents were purchased from Sigma-Aldrich, Loba, Alpha aesar and Spectrochem.

General procedures for the synthesis of 4-(phenylurenyl/thiourenyl) acetophenone

A mixture of the p-aminoacetophenone (**1**) and phenylisocyanate/thiocyanate (**2a-b**) derivatives were dissolved in toluene. The mixture was refluxed; yellow solid was filtered out and dried. Recrystallization afforded the desired 4-(phenylurenyl/thiourenyl) acetophenone (**3a-b**) derivatives in pure form²⁸.

4-N-(N'-p-chlorophenylurenyl) acetophenone (3a)

Yield: 96%; M.P.: 220-223°C (from ethanol); IR $\nu_{\max}$ 3374 (NH), 1714 (COMe), 1648 (CO) cm^{-1} ; ^1H NMR (300MHz, DMSO- d_6 , ppm) δ 2.49 (s, 3H, COMe), 7.33 (d, 2H, $J = 8.7\text{ Hz}$), 7.48 (d, 2H, $J = 8.7\text{ Hz}$), 7.56 (d, 2H, $J = 8.7\text{ Hz}$), 7.89 (d, 2H, $J = 8.7\text{ Hz}$), 8.93 (br s, 1H, NH), 9.12 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 26.30, 117.24, 119.97, 125.76, 128.65, 129.61, 130.56, 138.29, 144.15, 152.07, 196.25; MS-ESI: $\text{C}_{15}\text{H}_{13}\text{N}_2\text{O}_2\text{Cl}$ calcd $[\text{M}+\text{H}]^+$: 289.738, found 289.08.

4-N-(N'-phenylthiourenyl) acetophenone (3b)

Yield: 74%; M.P.: 157-160°C (from ethanol); IR $\nu_{\max}$ 3287 (NH), 1710 (COMe), 1642 (CO) cm^{-1} ; ^1H NMR (400MHz, DMSO- d_6 , ppm) δ 2.51 (s, 3H, COMe), 7.16 (t, 1H, $J = 8\text{ Hz}$), 7.36 (t, 2H, $J = 8\text{ Hz}$), 7.49 (d, 2H, $J = 8\text{ Hz}$), 7.70 (d, 2H, $J = 8\text{ Hz}$), 7.93 (d, 2H, $J = 8\text{ Hz}$), 10.12 (br s, 1H, NH), 10.06 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 26.98, 122.17, 124.14, 125.21, 129.01, 129.38, 132.60, 139.67, 144.64, 179.84, 197.07; MS-ESI: $\text{C}_{15}\text{H}_{14}\text{N}_2\text{OS}$ calcd $[\text{M}]^+$: 270.35, found 270.9.

General Procedure for synthesis of 4'-(phenylurenyl/thiourenyl) chalcone Derivatives

4'-(phenylurenyl/thiourenyl)chalcone (**5a-5f**) derivatives were synthesized by reacting equimolecular quantities of 4-(phenylurenyl/thiourenyl)acetophenone (**3a-b**) derivatives and the corresponding benzaldehyde (**4a-e**) in the presence of an excess sodium hydroxide in methanol. The mixture was stirred at room temperature; the resulting precipitate was filtered and dried in air. The precipitate was recrystallized from ethanol^{23, 27, 28}.

1-[4'-N-(N'-p-chlorophenylurenyl)phenyl]-3-(4-chlorophenyl)-2-propen-1-one (5a) Yield: 98%; M.P.: 258-261°C (from ethanol); IR $\nu_{\max}$ 3283 (NH), 1657 (CO-Urea), 1639 (CO, α , β unsaturated) cm^{-1} ; ^1H NMR (300MHz, DMSO- d_6 , ppm) δ 7.33 (d, 2H, $J = 8.7\text{ Hz}$), 7.49-7.52 (m, 4H), 7.63 (d, 2H, $J = 8.7\text{ Hz}$), 7.70 (d, 1H, Ha, $J = 15.6\text{ Hz}$), 7.92 (d, 2H, $J = 8.7\text{ Hz}$), 7.97 (d, 1H, $J = 16.5\text{ Hz}$), 8.13 (d, 2H, $J = 8.7\text{ Hz}$), 8.99 (br s, 1H, NH), 9.22 (br s, 1H, NH); ^{13}C NMR (400 MHz DMSO- d_6 , ppm): δ 117.87, 120.49, 123.25, 126.27, 129.15, 129.40, 130.63, 130.95, 131.48, 134.30, 135.35, 138.76, 142.09, 144.88, 152.56, 187.66; MS-ESI: $\text{C}_{22}\text{H}_{16}\text{N}_2\text{O}_2\text{Cl}_2$ calcd $[\text{M}+\text{H}]^+$: 412.28, found 412.10.

1-[4'-N-(N'-p-chlorophenylurenyl)phenyl]-3-(phenyl)-2-propen-1-one (5b) Yield: 96%; M.p.: 207-209°C (from ethanol); IR $\nu_{\max}$ 3293 (NH), 1713 (CO-Urea), 1641 (CO, α , β Unsaturated) cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6 , ppm) δ 7.34 (d, 2H, $J = 8.7\text{ Hz}$), 7.44-7.51 (m, 5H), 7.64 (d, 2H, $J = 8.7\text{ Hz}$), 7.72 (d, 1H, $J = 15.6\text{ Hz}$), 7.86-7.90 (m, 2H), 7.96 (d, 1H, $J = 15.6\text{ Hz}$), 8.13 (d, 2H, $J = 8.7\text{ Hz}$), 8.98 (brs, 1H, NH), 9.20 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 117.36, 119.97, 122.01, 125.75, 128.62, 128.69, 128.83, 130.03, 130.35, 131.09, 134.79, 138.25, 143.02, 144.26, 152.04, 187.28; MS-ESI: $\text{C}_{22}\text{H}_{17}\text{N}_2\text{O}_2\text{Cl}$ calcd $[\text{M}+\text{H}]^+$: 377.84, found 377.11.

1-[4'-N-(N'-p-chlorophenylurenyl)phenyl]-3-(2,4-dichlorophenyl)-2-propen-1-one (5c) Yield: 96%; M.P.: 244-247°C (from ethanol); IR $\nu_{\max}$ 3325 (NH), 1710 (CO-Urea), 1654 (CO, α , β Unsaturated) cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6 , ppm) δ 7.33 (d, 2H, $J = 8.7\text{ Hz}$), 7.48-7.56 (m, 3H), 7.62 (d, 2H, $J = 8.7\text{ Hz}$), 7.74 (s, 1H), 7.97 (d, 2H, $J = 13.8\text{ Hz}$), 8.14 (d, 2H, $J = 8.7\text{ Hz}$), 8.24 (d, 1H, $J = 8.7\text{ Hz}$), 8.96 (br s, 1H, NH), 9.19 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 117.36, 119.98, 125.33, 125.77, 127.86, 128.61, 129.41, 129.72, 130.23, 130.67,

131.50, 134.96, 135.34, 136.34, 138.21, 144.58, 152.01, 186.86; MS-ESI: $\text{C}_{22}\text{H}_{15}\text{N}_2\text{O}_2\text{Cl}_3$ calcd $[\text{M}]^+$: 445.72, found 445.19.

1-[4'-N-(N'-p-chlorophenylurenyl)phenyl]-3-(4-methoxyphenyl)-2-propen-1-one (5d) Yield: 93%; M.P.: 219-222°C (from ethanol); IR $\nu_{\max}$ 3339 (NH), 1708 (CO-Urea), 1638 (CO, α , β Unsaturated) cm^{-1} ; ^1H NMR (300 MHz, DMSO- d_6 , ppm) δ 3.83 (s, 3H, OCH₃), 7.03 (d, 2H, $J = 8.7\text{ Hz}$), 7.36 (d, 2H, $J = 9\text{ Hz}$), 7.52 (d, 2H, $J = 8.7\text{ Hz}$), 7.63 (d, 2H, $J = 8.7\text{ Hz}$), 7.69 (d, 1H, $J = 15.90\text{ Hz}$), 7.81 (d, 1H, $J = 13.80\text{ Hz}$), 7.85 (d, 2H, $J = 8.7\text{ Hz}$), 8.13 (d, 2H, $J = 8.7\text{ Hz}$), 8.98 (br s, 1H, NH), 9.18 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 55.31, 114.34, 117.33, 119.48, 119.95, 125.72, 127.43, 129.87, 130.56, 131.36, 138.27, 142.99, 144.04, 152.05, 161.16, 187.17; MS-ESI: $\text{C}_{23}\text{H}_{19}\text{N}_2\text{O}_3\text{Cl}$ calcd $[\text{M}+\text{H}]^+$: 407.86, found 407.10.

1-[4'-N-(N'-phenylthiourenyl)phenyl]-3-phenyl-2-propen-1-one (5e) Yield: 86%; M.P.: 162-165°C (from ethanol); IR $\nu_{\max}$ 3316 (NH), 1643 (CO, α , β Unsaturated) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , ppm) δ 7.16 (t, 1H), 7.36 (t, 2H, $J = 8\text{ Hz}$), 7.46-7.51 (m, 5H), 7.72-7.77 (m, 3H), 7.88-7.98 (m, 3H), 8.16 (d, 2H, $J = 9\text{ Hz}$), 10.10 (br s, 1H, NH), 10.18 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): δ 121.67, 121.99, 123.59, 124.69, 128.75, 128.85, 129.27, 130.44, 132.66, 134.73, 139.13, 143.35, 144.22, 179.26, 187.597; MS-ESI: $\text{C}_{23}\text{H}_{18}\text{N}_2\text{OS}$ calcd $[\text{M}+\text{H}]^+$: 359.46, found 359.0.

1-[4'-N-(N'-phenylthiourenyl)phenyl]-3-(3-methoxyphenyl)-2-propen-1-one (5f) Yield: 97%; M.P.: 144-147°C (from ethanol); IR $\nu_{\max}$ 3316 (NH), 1646 (CO, α , β Unsaturated) cm^{-1} ; ^1H NMR (400 MHz, DMSO- d_6 , ppm) δ 3.83 (s, 3H, OCH₃), 7.03 (d, 1H, $J = 8\text{ Hz}$), 7.16 (t, 1H), 7.34-7.39 (m, 4H), 7.43-7.51 (m, 3H), 7.68-7.77 (m, 3H), 7.96 (d, 1H, $J = 12\text{ Hz}$), 8.16 (d, 2H, $J = 8\text{ Hz}$), 10.10 (br s, 1H, NH), 10.19 (br s, 1H, NH); ^{13}C NMR (400 MHz, DMSO- d_6 , ppm): 55.26, 113.20, 116.58, 121.60, 121.64, 122.23, 123.59, 124.69, 128.48, 129.31, 129.86, 132.63, 136.14, 139.13, 143.35, 144.24, 159.62, 179.26, 187.59; HRMS-ESI⁺: $\text{C}_{23}\text{H}_{20}\text{N}_2\text{O}_2\text{S}$ calcd $[\text{M}+\text{H}]^+$: 389.48, found 389.0.

α -glucosidase inhibition assay

The α -glucosidase inhibition assay was performed according to the modified method described by Patil *et al.*²⁹. 50 μL of yeast α -glucosidase (0.1 unit/ml) was mixed with 20 μL of each of the samples and incubated for 1 h at 37°C in 96-well microplate. Enzyme action for α -glucosidase was initiated by addition of 50 μL substrate (5 mM, *p*-nitrophenyl- α -D-glucopyranoside) and stopped by adding 2 mL of 0.1 M Na_2CO_3 after an incubation of 10 min at 37°C. Release of *p*-nitrophenol was measured at 420 nm. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 μL of phosphate buffer, pH 6.8. Control sample contained 10 μL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as $(1 - \text{B/A}) \times 100$, where [A] represents absorbance of control without test samples, and [B] represents absorbance in presence of test samples.

Acute toxicity study

Healthy Wistar albino rats (180 $\pm$ 20 g) of either sex were starved overnight and divided into different groups (n=4). The synthesized compounds were orally fed in increasing dose levels of 0.5, 1.0, 1.5 and 2.0 g/kg b.w.. The animals were observed continuously for the first 2 h for any gross change in behavioural, neurological and autonomic profiles and intermittently for the next 6 h and then again at 24 h, 48 h and 72 h for any lethality or death³⁰.

In vivo antidiabetic activity of synthesized compounds

A solution of streptozotocin (100 mM citrate buffer, pH 4.5) was dosed to overnight fasted rats (65 mg/kg b.w/rat) intraperitoneally³¹. 10% glucose solution was provided after 6 h for the next 24 h to prevent STZ induced hypoglycaemia³². The blood sugar level was measured by glucometer (Accucheck) after 24h of glucose feeding. Animals showing 200-400 mg/dL of blood sugar level were selected as a diabetic animal. The rats were divided into nine groups: group I (Normal), group II (diabetic control), group III-VIII (compounds **5a-5f**; 100 mg/kg body weight/day) and a group IX with standard drug Metformin (100 mg/kg body weight/day). Six rats in each group were taken.

Fasting blood glucose (FBG) level and body weight of each animal were measured at 0, 5th, 10th and 15th day. After 24 hrs of the last dose, blood was collected from overnight fasted rats for estimation of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT)³³ and alkaline phosphatase (ALP)³⁴. Plasma protein, blood urea, serum creatinine, lipid profile (Total cholesterol, Triglycerides, HDL, LDL and VLDL) were also estimated. Lipid peroxidation (LPO)³⁵, superoxide dismutase (SOD)³⁶, catalase (CAT)³⁷ and reduced glutathione (GSH)³⁸ were estimated in liver tissue.

Molecular modelling study

The x-ray crystal structure of a few bacterial α -glucosidase has been reported. However, the 3D structure of α -glucosidase used in biological assays from yeast has not yet been reported³⁹. In addition to using NCBI's PDB to search for 3D structures of homologous proteins, various online-based structure prediction tools were applied, including (i) Phyre2⁴⁰ (ii) Swiss Model⁴¹. Homology model for *Saccharomyces cerevisiae* α -glucosidase was built using the crystallographic structure of *Saccharomyces cerevisiae* isomaltase (PDB Code 3A47; Resolution 1.30Å) with 72% of sequence identity with α -glucosidase was selected as a template. Protein sequence for Baker's yeast α -glucosidase (MAL12) was obtained from uniprot (<http://www.uniprot.org/>). Sequence alignment and homology modeling were performed

using phyre2 model, which is fully automated homology modelling.

Docking Study

Protein-ligand docking study on urea/thiourea derivatives was carried out using patchDock software^{42,43}. From the protocol of patchDock, the recommended values are 4 Å for protein-protein docking and 1.5 Å for protein-small molecule docking (<http://bioinfo3d.cs.tau.ac.il/PatchDock/help.html>). Since the α -glucosidase (protein), we decided to use the value of 4.0 for the clustering of the RMSD. The PatchDock algorithm divides the Connolly dot surface representation of the molecules into concave, convex and flat patches. Then, complementary patches are matched in order to generate the candidate transformations of docked complex (the candidate transformations are the docked complexes of specified receptor and ligand molecule based on the patchDock theory). All molecular representations in this study were generated using Pymol (www.pymol.org/) and UCSF chimera⁴⁴.

RESULTS AND DISCUSSION

Chemistry

The 4'-(phenylurenyl/thiourenyl) chalcone (**5a-5f**) derivatives were synthesized as shown in the scheme. The structures of all the new synthesized compounds **5a-5f** were characterized by ¹H NMR, ¹³C NMR, FT-IR and Mass spectra. For instance, IR $\nu_{\max}$ of compound **5d** (R_2 - 4-Methoxy) is 3339 (NH), 1708 (CO-Urea), 1638 (CO, α , β -Unsaturated) cm^{-1} . The ¹H NMR spectrum of **5d** showed a singlet at δ 3.83 ppm due to methoxy proton of the phenyl ring. Two broad singlet signals at δ 8.98 and δ 9.18 ppm were corresponded to the protons of -NH-CO- and -CO-NH- respectively. The twelve aromatic protons were appeared in the region of δ 7.03-8.13 ppm. The signals at δ 7.69 and 7.81 corresponded to α and β protons of the enone system, respectively. The ¹³C NMR spectra of **5d** showed δ 187.17 (CO), 152.05 (CO(NH)₂), 144.04 (C $_{\beta}$) and 119.95 (C $_{\alpha}$). All these data are in agreement with the structure of compound **5d**.

Scheme - General synthetic pathway for the preparation of 4'-(phenylurenyl/thiourenyl) chalcone (**5a-5f**)

Compounds	X	R ₁	R ₂
5a	O	Cl	4-Cl
5b	O	Cl	H
5c	O	Cl	2,4-di-Cl
5d	O	Cl	4-OCH ₃
5e	S	H	H
5f	S	H	3-OCH ₃

α -glucosidase inhibition assay

All synthesized compounds were evaluated through inhibitory assay for their yeast α -glucosidase (*Saccharomyces cerevisiae*) inhibitory activity. It was observed that most of the urea/thiourea derivatives exhibited significant inhibitory activity against α -glucosidase (**Table 1**). Importantly, compound **5d** was found to be the most active, showing concentration-dependent inhibition of α -glucosidase activity with 97.73% inhibition at 100 μ M concentration. From the dose-response curve, IC₅₀ value of **5d** was calculated as 12.88 μ M (**Figure 1**) whereas Acarbose showed 77.69% inhibitory activity at 100 μ M concentration under similar assay conditions. IC₅₀ value of Acarbose was calculated as 16.54 μ M.

In vivo antidiabetic activity

In the acute toxicity study, synthesized compounds did not show any mortality or toxic effect up to the dose of 2 g/kg b.w.; accordingly, 100 mg/kg b.w./day was taken as the dose for the *in vivo* experiment. All the synthesized compounds were further evaluated for its antihyperglycemic activity in STZ-induced diabetic rats for 14-day experiment. The increased fasting blood glucose (FBG) level in STZ induced diabetic rats was significantly ($p < 0.001$) reduced after 14 days of experiment whereas compound **5b** showed reduced blood glucose level ($p < 0.01$) when compared to the diabetic control group (**Figure 2A**). Compound **5d** showed maximum reduction of FBG in contrast with the other synthesized molecules.

Diabetic group of rats displayed notable reduction in body weight when compared with normal group of rats. Compound **5a**, **5d** and **5f** showed significant ($P < 0.001$) increase in body weight after treatment of 14 days. However, there was no improvement in body weight of diabetic rats treated with **5b** and **5e** in contrast with diabetic control group (**Figure 2B**).

Reduction in the levels of activities of SGOT ($P < 0.001$), SGPT ($P < 0.001$) and ALP ($P < 0.001$) was noted in the diabetic rats treated with **5d** and **5f** (**Figure 3**). But there was no alteration in the levels of SGOT in rats treated with **5c** when compared to diabetic control rats. The significant reversal in SGOT, SGPT and ALP activities in diabetic rats indicate the tissue protective nature of the compounds.

The increased levels of urea and creatinine and reduced levels of plasma protein in diabetic rats were significantly ($P < 0.001$) improved in **5a** and **5d** treated diabetic rats (**Figure 4**). Whereas, the diabetic rats treated with **5e** showed significant improvement in urea ($P < 0.05$) and creatinine ($P < 0.01$) levels but not in protein levels.

It was observed that there was an increase in the triglyceride (TG), total cholesterol (TC), LDL and VLDL level and decrease in the HDL levels in the diabetic rats when compared with normal groups (**Table 2**). There was significant ($P < 0.001$) reduction in triglyceride levels in all groups when compared to the diabetic control group while **5b** ($p < 0.01$) and **5c** ($p < 0.05$) exhibited the said significance. The total cholesterol (**5d** ($p < 0.001$)) and LDL (**5d**, **5e** ($p < 0.001$)) level were also significantly reduced to the normal level. The VLDL level was significantly reduced to the normal level except **5c**. There was significant ($p < 0.001$) increase in HDL level with compounds **5d** and **5f** in treated animal when compared with the diabetic control group.

The LPO level was significantly ($P < 0.001$) increased whereas reduced GSH, SOD and Catalase level were significantly ($P < 0.001$) depleted in diabetic control animals as compared to normal group (**Table 3**). Treatment with **5d** significantly ($P < 0.001$) reduced LPO level when compared with diabetic control animals. Reduced GSH level was found to be significant ($P < 0.001$) elevated towards normal level in all groups whereas **5b** and **5c** ($p < 0.05$) showed less significance when compared with diabetic control group. The level of SOD was significantly ($P < 0.01$) improved with the synthesized compounds **5d** and **5f**. The administration of **5a** and **5d** recovered CAT activity significantly ($P < 0.001$) towards normal level.

Histologic examination revealed degeneration and necrosis of pancreatic islets in the diabetic control group. The cytoplasm of peri-acinar hepatocytes showed either a single large or multiple small round empty vacuoles. Degenerated cortex and medulla as well as necrosis of tubules were observed in nephrons of diabetic groups. These histopathological changes (**Figure 5**) were restored to normal condition with compound **5d** treated animal.

3D Protein structure modelling of α -Glucosidase and molecular docking

We made a preliminary biocomputational analysis and we observed that experimental 3D protein α -Glucosidase is not determined. Therefore, in the present study, we used *insilico* methods including, Phyre2 and Swiss model to resolve α -Glucosidase's 3D protein structure. Phyre2 suggested the 3A47_A (589 AAs) template as one of the best homologous templates for a possible 3D α -Glucosidase MAL12 protein structure. Phyre2 predicted the 3D α -Glucosidase MAL12 (584 AAs) protein structure with 581 AAs based on the 3A47_A template (589 AAs). Submission of α -Glucosidase MAL12 (584 AAs) to the SWISS-MODEL server generated two 3D α -Glucosidase MAL12 protein structure model using two different templates (3AJ7_A (589AAs) and 3AXH_A (589AAs)). The proposed 3D α -Glucosidase MAL12 protein structures were pre-checked by Ramachandran plot and other methods. Based on

stereochemical properties, we observed that model obtained from Phyre2 method is most suitable for further ligand-protein interaction analysis.

For the binding ligand-protein interaction analysis, we performed a geometry-based molecular docking algorithm PatchDock for all synthesized molecules **5a-5f** (Table 4). The applied PatchDock docking program accurately defines specific binding sites of **5a-5f** molecules. According to docking score the highest docking

score was selected for further studies. The docked structure showed atomic contact energy in the range of -65.50 to -260.64 Kcal/mol. Among the synthesized compounds, **5d** showed highest docking score and lowest atomic contact energy. Based on overall results, most effective molecule, **5d** interacts with Tyr 71, Asp 68, Val 108, His 111, Lys 155, Phe 157, Phe 177, Asp 214, His 239, Asn 241, His 279, Tyr 313, His 348, Asp 349, Asp 408, Asn 412, Arg 439 of α -Glucosidase (Figure 6).

Table 1: Percentage inhibition of α -Glucosidase by synthesized compounds

Compounds	Concentration (μ M)			
	10	25	50	100
5a	51.64 $\pm$ 0.95	56.04 $\pm$ 2.04	80.40 $\pm$ 1.11	86.42 $\pm$ 1.92
5b	14.09 $\pm$ 1.72	31.15 $\pm$ 1.3	64.61 $\pm$ 1.42	72.69 $\pm$ 1.46
5c	9.76 $\pm$ 0.58	26.35 $\pm$ 2.10	33.05 $\pm$ 1.49	55.36 $\pm$ 1.59
5d	43.87 $\pm$ 1.38	64.62 $\pm$ 1.21	85.28 $\pm$ 1.24	97.73 $\pm$ 0.75
5e	14.95 $\pm$ 1.46	32.46 $\pm$ 1.19	37.75 $\pm$ 1.18	63.47 $\pm$ 1.03
5f	38.78 $\pm$ 1.78	62.10 $\pm$ 0.77	82.10 $\pm$ 0.91	91.07 $\pm$ 0.75
Acarbose	40.28 $\pm$ 1.12	60.61 $\pm$ 1.00	65.10 $\pm$ 0.94	77.69 $\pm$ 1.29

Values are mean $\pm$ SEM; experiment performed in triplicate

Table 2: Level of Total cholesterol, Triglycerides, HDL, LDL, and VLDL in diabetic control and experimental groups of rats after 14 days experimental period

Groups	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL(mg/dL)	LDL(mg/dL)	VLDL(mg/dL)
I(Normal)	127.00 $\pm$ 2.59	104 $\pm$ 2.65	51.00 $\pm$ 2.52	55.20 $\pm$ 2.62	20.80 $\pm$ 1.47
II (Diabetic Control)	157.00 $\pm$ 3.34	212 $\pm$ 5.68	22.00 $\pm$ 1.59	92.60 $\pm$ 1.32	42.40 $\pm$ 1.90
III (STZ+5a)	141.00 $\pm$ 4.14*	140 $\pm$ 3.15***	30.00 $\pm$ 2.95	83.00 $\pm$ 2.12*	28.00 $\pm$ 2.07**
IV(STZ+5b)	150.00 $\pm$ 3.89	172 $\pm$ 2.89**	30.00 $\pm$ 2.94	85.60 $\pm$ 3.23	34.40 $\pm$ 1.13*
V (STZ+5c)	147.00 $\pm$ 2.65	185 $\pm$ 4.34*	29.00 $\pm$ 2.52	81.00 $\pm$ 2.11**	37.00 $\pm$ 3.45
VI (STZ+5d)	133.00 $\pm$ 0.97***	127 $\pm$ 1.59***	47.00 $\pm$ 2.24***	60.60 $\pm$ 2.81***	25.40 $\pm$ 1.73**
VII (STZ+5e)	135.00 $\pm$ 4.12**	132 $\pm$ 3.90***	33.00 $\pm$ 2.27*	75.60 $\pm$ 4.19*	26.40 $\pm$ 3.28**
VIII (STZ+5f)	134.00 $\pm$ 2.58**	128 $\pm$ 3.77***	40.00 $\pm$ 1.79***	68.40 $\pm$ 2.77***	25.60 $\pm$ 1.63**
IX (STZ+Metformin)	128.00 $\pm$ 2.58***	112 $\pm$ 2.82***	49.00 $\pm$ 3.23***	56.60 $\pm$ 2.23***	22.40 $\pm$ 1.72***

Values represent the mean $\pm$ S.E.M. (n=6). *p < 0.05, **p<0.01 and ***p< 0.001 compared with diabetic control group.

Table 3: Tissue lipid peroxide (LPO), reduced glutathione (GSH) Superoxide dismutase (SOD) and catalase (CAT) levels in diabetic control and experimental groups of rats after 14 days experimental period

Groups	LPO level (nM/mg wet tissue)	GSH level (μ g/mg wet tissue)	SOD level (unit/min/gm tissue)	CAT level (μ M of H ₂ O ₂ decomposed/min/mg wet tissue)
I (Normal)	2.67 $\pm$ 0.30	59.08 $\pm$ 0.47	6.06 $\pm$ 0.08	69.95 $\pm$ 0.78
II (Diabetic control)	5.33 $\pm$ 0.22	27.60 $\pm$ 0.44	4.25 $\pm$ 0.185	40.31 $\pm$ 1.00
III (STZ+5a)	3.77 $\pm$ 0.27**	51.54 $\pm$ 0.79***	4.47 $\pm$ 0.33	50.64 $\pm$ 0.83***
IV(STZ+5b)	4.03 $\pm$ 0.23*	34.58 $\pm$ 1.72*	4.92 $\pm$ 0.12*	45.57 $\pm$ 1.20*
V (STZ+5c)	3.83 $\pm$ 0.25**	33.63 $\pm$ 1.61*	4.83 $\pm$ 0.14	44.27 $\pm$ 1.32
VI (STZ+5d)	3.02 $\pm$ 0.24***	49.11 $\pm$ 0.60***	5.48 $\pm$ 0.11**	57.44 $\pm$ 1.32***
VII (STZ+5e)	3.91 $\pm$ 0.21**	40.04 $\pm$ 1.41***	4.88 $\pm$ 0.08*	43.34 $\pm$ 0.86
VIII (STZ+5f)	3.22 $\pm$ 0.35**	46.21 $\pm$ 1.69***	5.26 $\pm$ 0.13**	47.97 $\pm$ 1.29**
IX (STZ+Metformin)	3.66 $\pm$ 0.20**	48.14 $\pm$ 0.69***	5.75 $\pm$ 0.06***	59.59 $\pm$ 2.44***

Values represent the mean $\pm$ S.E.M. (n=6). *p < 0.05, **p<0.01 and ***p< 0.001 compared with diabetic control group.

Table 4: Ligand-protein docking study of synthesized molecules (5a-5f) with α -Glucosidase model based on 3a47 template using Patch Dock method

Compounds	Score	Area	ACE*	Transformation
5a	5656	677.90	-124.35	-0.73 -0.56 2.30 32.55 -18.38 13.73
5b	5556	656.90	-118.30	-1.11 -0.68 -0.29 26.81 -9.49 9.54
5c	5822	716.00	-116.58	-0.52 -0.49 2.49 32.84 -18.46 15.33
5d	5980	700.10	-65.50	1.15 0.27 0.06 25.76 -6.68 6.97
5e	5658	623.90	-117.64	-0.94 -0.52 -0.00 27.64 -8.28 10.12
5f	5640	696.50	-260.64	-0.61 -1.20 1.94 35.52 -13.79 13.44

*ACE-Atomic contact energy

Figure 1: Dose response curve of compound 5d of α -glucosidase inhibition

Figure 2: Level of fasting blood glucose levels (A) and body weight (B) in diabetic control and experimental groups of rats after 14 days of treatment. Values represent the mean $\pm$ S.E.M. (n=6). *p < 0.05, **P<0.01 and ***P< 0.001 compared with diabetic control group

Figure 3: The activities of SGOT, SGPT and ALP in serum of diabetic control and experimental groups of rats after 14 days experimental period

Figure 4: The levels of plasma protein (A), blood urea (B) and serum creatinine (C) in diabetic control and experimental groups of rats after 14 days experimental period

Figure 5: Histological analysis of rat pancreatic tissues, hepatic tissues and nephritic tissues. DM-Diabetes Mellitus, IL-Islets of Langerhans, CV-Central Vein, GL-Glomerulus

Figure 6: Molecular docking analysis of the ligand binding pocket of α -glucosidase MAL12 model structure obtained from the phyre2 server, based on the 3A47 template. The docked complexes were analyzed using Chimera 3d viewer. Compound 5d and the interacting amino acids (a-His 111, Lys 155, Phe 157, Phe 177, His 239, Asn 241, His 279; b- Tyr 71, Asp 68, Val 108, Asp 214, His 348, Asp 349; c- Tyr 313, Asp 408, Asn 412, Arg 439) are depicted. Molecular Docking were done by using online Patch Dock Server.

CONCLUSION

We synthesized a series of urea/thiourea derivatives (**5a-5f**). All the synthesized compounds were tested for their *invitro* α -glucosidase inhibitory activity. Among them, compound **5d** (IC_{50} 12.88 μ M) having 4- methoxy substitution at phenyl ring was found to be the most active compound when compared to the standard drug Acarbose (IC_{50} 16.54 μ M). In *invivo* study, compound **5d** showed significant improvement of the body weight, blood glucose level, serum enzyme parameter, lipid profile as well as tissue antioxidant parameters. Further, molecular docking studies showed that these urea/thiourea derivatives were binding to the active site of α -glucosidase enzyme with the hydrophobic interactions, noncovalent and hydrogen bond interactions. The docking studies are in good agreement with the *invitro* and *invivo* studies. Hence, this study identified a new structural type of α -glucosidase inhibitors which could be used as the lead molecules for further research and development of potent α -glucosidase inhibitors for the treatment of diabetes.

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