Research Article

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

Tanmoy Guria, Puspita Roy, Tapan kumar Maity *

Division of Synthetic and Natural Product Research Laboratory, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

*Corresponding Author Email: jutkmait@gmail.com

Article Received on: 29/04/18 Approved for publication: 20/05/18

DOI: 10.7897/2230-8407.09571

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 µM when compared to the standard drug Acarbose (IC50 16.54 µM) 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 disease2,3. Type II diabetes is the majorly affective diabetes which is associated with ‘diabetesy’ 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, the 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 flatulence11,12 in some cases and newly discovered additive effects (like stabilizing 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, antimicrobial16,17, antioxidant18, antiviral19, anti-HIV20, antitumor21,22, antimalarial23 and antidiabetic activity24-26. This present study involves the synthesis of a series of urea/thiourea derivatives using previously reported methods23,27,28. Furthermore, hypoglycemic activity of urea/thiourea derivatives were investigated using well defined invitro 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 Veegeo 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’-p-chlorophenylurenyl) acetophene (3a)

Yield: 96%; M.P.: 220-223°C (from ethanol); IR vmax 3374 (NH), 1714 (CO-Me), 1648 (CO) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆, ppm) δ 2.49 (s, 3H, COMe), 7.33 (d, 2H, J = 8.7 Hz), 7.48 (d, 2H, J = 8.7 Hz), 7.56 (d, 2H, J = 8.7 Hz), 7.89 (d, 2H, J = 8.7 Hz), 8.93 (br s, 1H, NH), 9.12 (br s, 1H, NH); 13C NMR (400 MHz, DMSO-d₆, 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: C₁₉H₁₄N₂O₂Cl calculated [M+H⁺]: 378.738, found 378.08.

4-(N’-phenylthiourenyl) acetophene (3b)

Yield: 74%; M.P.: 157-160°C (from ethanol); IR vmax 3287 (NH), 1710 (CO-Me), 1642 (CO) cm⁻¹; 1H NMR (400 MHz, DMSO-d₆, ppm) δ 2.51 (s, 3H, COMe), 7.16 (t, 1H, J = 8 Hz), 7.36 (t, 2H), 7.49 (d, 2H, J = 8 Hz), 7.70 (d, 2H, J = 8 Hz), 7.93 (d, 2H, J = 8 Hz), 10.12 (br s, 1H, NH), 10.06 (br s, 1H, NH); 13C NMR (400 MHz, DMSO-d₆, 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: C₁₉H₁₄N₂O₂ calculated [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)acetophene (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.

1-[4’-(N’-p-chlorophenylurenyl)phenyl]-3-(4-chlorophenyl)-2-propen-1-one (5a)

Yield: 98%; M.P.: 258-261°C (from ethanol); IR vmax 3283 (NH), 1657 (CO-Urea), 1639 (CO, α, β-unsaturated) cm⁻¹; 1H NMR (300 MHz, DMSO-d₆, ppm) δ 7.33 (d, 2H, J = 8.7 Hz), 7.49-7.52 (m, 4H), 7.63 (d, 2H, J = 8.7 Hz), 7.70 (d, 1H, J = 15.6 Hz), 7.92 (d, 2H, J = 8.7 Hz), 7.97 (d, 1H, J = 16.5 Hz), 8.13 (d, 2H, J = 8.7 Hz), 8.99 (br s, 1H, NH), 9.22 (d, 1H, NH), 13C NMR (400 MHz, DMSO-d₆, 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: C₁₉H₁₄N₂O₂Cl calculated [M⁺]: 412.28, found 412.10.

Acute toxicity study

Healthy Wistar albino rats (180 ± 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 death.
**Invivo 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) intraperitoneally\(^{31}\). 10% glucose solution was provided after 6 h for the next 24 h to prevent STZ induced hypoglycaemia\(^{32}\). The blood sugar level was measured by glucometer (Accuchek) after 24 h 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/bdy 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 reported\(^{39}\). 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) Phyre2\(^{40}\); (ii) Swiss Model\(^{41}\). 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 Bake’s yeast α-glucosidase (MAL12) was obtained from uniprot (http://www.uniprot.org/). Sequence alignment and homology modelling 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\(^{42}\). 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 conically 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 chimera\(^{44}\).

**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 \(^1\)H NMR, \(^13\)C NMR, FT-IR and Mass spectra. For instance, IR \(\nu_{\text{max}}\) of compound 5d (R2: - 4-Methoxy) is 3339 (NH), 1708 (CO), 1638 (CO, α, β-Unsaturated) cm\(^{-1}\). The \(^1\)HNMR 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 6.76 and 7.81 corresponded to α and β protons of the enone system, respectively. The \(^13\)C NMR spectra of 5d showed δ187.17 (CO), 152.05 (CO(NH)\(_2\)), 144.04 (C\(_9\)) and 119.95 (C\(_8\)). 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)
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 µM concentration. From the dose-response curve, IC_{50} value of 5d was calculated as 12.88 µM (Figure 1) whereas Acrabose showed 77.69% inhibitory activity at 100 µM concentration under similar assay conditions. IC_{50} value of Acrabose was calculated as 16.54 µM.

Invivo 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 invivo 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 5e 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 (3A7J_A (589AAs) and 3AXH_A (589AAs)). The proposed 3D α-Glucosidase MAL12 protein structures were pre-checked by Ramachandran plot and other methods.
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, Asp 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>5a</td>
<td>51.6±0.95</td>
</tr>
<tr>
<td>5b</td>
<td>14.9±1.72</td>
</tr>
<tr>
<td>5c</td>
<td>9.76±0.58</td>
</tr>
<tr>
<td>5d</td>
<td>43.87±1.38</td>
</tr>
<tr>
<td>5e</td>
<td>14.95±1.46</td>
</tr>
<tr>
<td>5f</td>
<td>38.78±1.78</td>
</tr>
<tr>
<td>Acarbose</td>
<td>40.28±1.12</td>
</tr>
</tbody>
</table>

Values are mean ± 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>l(Normal)</td>
<td>127.00±2.59</td>
<td>104.00±2.65</td>
<td>51.00±2.52</td>
<td>55.20±2.62</td>
<td>20.80±1.47</td>
</tr>
<tr>
<td>II (Diabetic Control)</td>
<td>157.00±3.34</td>
<td>212.5±5.68</td>
<td>22.00±1.59</td>
<td>92.60±1.32</td>
<td>42.40±1.90</td>
</tr>
<tr>
<td>IH (STZ+5a)</td>
<td>141.00±4.4*</td>
<td>140.00±3.15***</td>
<td>30.00±2.95</td>
<td>83.00±2.12*</td>
<td>28.00±2.07**</td>
</tr>
<tr>
<td>IV(STZ+5b)</td>
<td>150.00±3.89</td>
<td>172.5±2.89***</td>
<td>30.00±2.94</td>
<td>85.60±3.23</td>
<td>34.40±1.13*</td>
</tr>
<tr>
<td>V(STZ+5c)</td>
<td>147.00±2.65</td>
<td>185.4±3.4**</td>
<td>29.00±2.52</td>
<td>81.00±2.11**</td>
<td>37.00±3.45</td>
</tr>
<tr>
<td>VI(STZ+5d)</td>
<td>133.00±3.97***</td>
<td>127.2±1.99***</td>
<td>47.00±2.24***</td>
<td>60.60±2.81***</td>
<td>25.40±1.73**</td>
</tr>
<tr>
<td>VII(STZ+5e)</td>
<td>135.00±4.12**</td>
<td>132.3±3.90***</td>
<td>33.00±2.27*</td>
<td>75.60±4.19*</td>
<td>26.40±3.28**</td>
</tr>
<tr>
<td>VIII(STZ+5f)</td>
<td>134.00±2.58**</td>
<td>128.3±3.77***</td>
<td>40.00±1.70**</td>
<td>68.40±2.77**</td>
<td>25.60±1.63**</td>
</tr>
<tr>
<td>IX(STZ+Metformin)</td>
<td>128.00±2.58***</td>
<td>112.2±2.82***</td>
<td>49.00±3.23**</td>
<td>56.60±2.23**</td>
<td>22.40±1.72**</td>
</tr>
</tbody>
</table>

Values represent the mean ± 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>LPO level (mM/mg wet tissue)</th>
<th>GSH level (µg/mg wet tissue)</th>
<th>SOD level (unit/min/mg tissue)</th>
<th>CAT level (µM of H2O2 decomposed/min/mg wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal)</td>
<td>2.67±0.30</td>
<td>59.08±0.47</td>
<td>6.06±0.08</td>
<td>69.95±0.78</td>
</tr>
<tr>
<td>II (Diabetic Control)</td>
<td>5.33±0.22</td>
<td>27.60±0.44</td>
<td>4.25±0.18</td>
<td>40.31±1.00</td>
</tr>
<tr>
<td>IH (STZ+5a)</td>
<td>3.77±0.27**</td>
<td>51.54±0.70***</td>
<td>4.47±0.33</td>
<td>50.64±0.83***</td>
</tr>
<tr>
<td>IV(STZ+5b)</td>
<td>4.03±0.23*</td>
<td>34.58±1.72*</td>
<td>4.93±0.12*</td>
<td>45.57±1.20*</td>
</tr>
<tr>
<td>V(STZ+5c)</td>
<td>3.83±0.25**</td>
<td>33.63±1.61*</td>
<td>4.83±0.14</td>
<td>44.27±1.32</td>
</tr>
<tr>
<td>VI(STZ+5d)</td>
<td>3.02±0.24***</td>
<td>49.11±0.60***</td>
<td>5.48±0.11**</td>
<td>57.44±1.32**</td>
</tr>
<tr>
<td>VII(STZ+5e)</td>
<td>3.91±0.21**</td>
<td>40.04±1.41***</td>
<td>4.88±0.08**</td>
<td>43.34±0.86</td>
</tr>
<tr>
<td>VIII(STZ+5f)</td>
<td>3.22±0.35**</td>
<td>46.21±1.60***</td>
<td>5.26±0.13**</td>
<td>47.97±1.29**</td>
</tr>
<tr>
<td>IX(STZ+Metformin)</td>
<td>3.66±0.20**</td>
<td>48.14±0.60***</td>
<td>5.75±0.06**</td>
<td>59.59±2.44**</td>
</tr>
</tbody>
</table>

Values represent the mean ± 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

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Score</th>
<th>Area</th>
<th>ACE*</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>5656</td>
<td>677.9</td>
<td>-124.35</td>
<td>-0.73 -0.56 2.30 32.55 -18.38 13.73</td>
</tr>
<tr>
<td>5b</td>
<td>5556</td>
<td>656.9</td>
<td>-118.30</td>
<td>-1.11 -0.68 -0.29 26.81 -9.49 9.54</td>
</tr>
<tr>
<td>5c</td>
<td>5822</td>
<td>716.0</td>
<td>-116.58</td>
<td>-0.52 -0.49 2.49 32.84 -18.46 15.33</td>
</tr>
<tr>
<td>5d</td>
<td>5980</td>
<td>700.10</td>
<td>-65.50</td>
<td>-1.15 0.27 0.06 25.76 -6.68 6.97</td>
</tr>
<tr>
<td>5e</td>
<td>5656</td>
<td>623.9</td>
<td>-117.64</td>
<td>-0.94 -0.52 -0.00 27.64 -8.28 10.12</td>
</tr>
<tr>
<td>5f</td>
<td>5640</td>
<td>696.50</td>
<td>-260.64</td>
<td>-0.61 -1.20 1.94 35.52 -13.79 13.44</td>
</tr>
</tbody>
</table>

*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± 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.
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 (IC₅₀ 12.88 μM) having 4- methoxy substitution at phenyl ring was found to be the most active compound when compared to the standard drug Acarbose (IC₅₀ 16.54 μM). In invitro 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 invitro 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.

ACKNOWLEDGMENTS

The authors are thankful to the Council of Scientific and Industrial Research (CSIR), New Delhi, India for providing the financial support (Scheme No.-02(0098)/12/EMR-II) and Jadavpur University for providing the necessary research facility. We also thank Dr. Subrata Pramanik for assistance with structure solution and refinement.

REFERENCES

15. Yonova PA, Stoilova GM. Synthesis and biological activity

Cite this article as: Tanmoy Guria et al. Synthesis, pharmacological evaluation and ligand-protein interaction study of hybrid urea and thiourea derivatives as antihyperglycemic agents. Int. Res. J. Pharm. 2018;9(5):36-44 http://dx.doi.org/10.7897/2230-8407.09571

Source of support: Council of Scientific and Industrial Research (CSIR), New Delhi, India, Conflict of interest: None Declared

Disclaimer: IRJP is solely owned by Molsha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the content published in our Journal. IRJP cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of IRJP editor or editorial board members.