2, 4-Dinitrophenyl hydrazone derivatives as potent of alpha amylase inhibitors

Abstract
In our current study thirteen new 2,4-dinitrophenyl hydrazone derivatives\textsuperscript{1–13} were evaluated for alpha amylase activity. The molecular docking results indicate that compounds potentially bind in the catalytic site of the enzyme with excellent result. Molecular Operating Environment (MOE) software was used for docking study. 2,4-dinitrophenyl hydrazone\textsuperscript{11,13} were obtained under reflux conditions by reacting dinitrophenyl hydrazine in methanol with different aromatic as well as aliphatic aldehydes using acetic acid act as a catalyst. Our results has shown that compounds 5 (IC\textsubscript{50}=12.16\mu g/mL), 6 (IC\textsubscript{50}=15.03\mu g/mL), and 12(IC\textsubscript{50}=16.42\mu g/mL), were found to be the more potential alpha amylase inhibitors as compared to the standard acarbose (IC\textsubscript{50}=24.7\mu g/mL). These compounds may lead better for alpha amylase inhibitor and further assessment of these compounds provide great help in the discovery of new anti diabetic drugs.

Keywords: schiff’s bases, 2,4-dinitrophenyl hydrazone, alpha amylase activity, molecular docking

Introduction

A German chemist Joseph C. Schiff Ugo Hugo Modern Chemistry father\textsuperscript{1} reported Schiff bases having (C = N) azomethine functionality are present and are unique organic compound generally called Imine;\textsuperscript{2} The ketones or, aldehydes carbonyl compounds react with amines via a condensation reaction to produce Imine, Schiff base.\textsuperscript{3} It contain nitrogen carbon double bond where nitrogen don’t have any hydrogen, only possess aryl/alkyl group while carbon possess R=H called azomethine secondary aldamine R=H is substituted phenyl, phenyl derived imine.\textsuperscript{3} Compound containing azomethine or Imine functionality showed an important biological activities due to the imine reactive group found within natural, derived compounds or synthesized compounds.\textsuperscript{4} A widespread range of biological function was reported on Schiff bases such as antiviral activity, antibacterial activity, antifungal activity, anti malarial activity.\textsuperscript{4–4} Derived aromatic aldehyde and amines Schiff bases widely used in analytical, inorganic, and biological chemistry.\textsuperscript{4–4} Many novel biological activities reported with a significant result on synthesized Schiff base such as antitumor activity,\textsuperscript{10,11} antioxidant activity,\textsuperscript{10,11} anti-inflammatory activity,\textsuperscript{12,13} and lipid lowering ability.\textsuperscript{14}

Schiff bases play a vital role in Medicinal and Pharmaceutical field for different activity.\textsuperscript{15–21} Urease inhibitory activity was also reported with a significant result.\textsuperscript{22–24} Schiff have a lower side effect present a novel behavior.\textsuperscript{25,26} Our current study is focused only to evaluate synthesized Schiff bases for alpha amylase inhibition activity. The major health problem of twenty first century around the world is diabetic disease associated with hyperglycemia, hypertension, gastro paresis, keto acidosis, and nephropathy, affected 15million people approximately.\textsuperscript{27} Diabetes are mainly type – II (In which blood sugar level process effected), and in type – I (No or, little insulin production occur from pancreas).\textsuperscript{30,31} Oxidative stress mainly responsible in diabetes it can change collagen type – IV enzyme function, structure and alter protein–glycation, reduced antioxidant level deactivate anti athero – Sclerotic enzyme.\textsuperscript{32} Diabetes now days control via synthetic drugs. One of the drug i.e. Schiff derived drug have heteroatom azo methine or Imine functionality possess a novel activities in clinical use.\textsuperscript{33,34}

Also the electron withdrawing presence or donating group can change the biological activity rate of Schiff bases compounds.\textsuperscript{35,36} The hetero atom or aromatic linkage presence in certain compound provides a broader biological activity.\textsuperscript{37,38} Diabetes can be treated via the inhibition of alpha amylase enzyme involved in digestion of carbohydrate by lowering the glucose level in blood.\textsuperscript{39} Around the world wide diabetes patients multiply, in upcoming 25year diabetes will be the most dangerous health killer. Around the world people are investigating full treatment of diabetes mellitus via a synthetic drug or, natural derived.\textsuperscript{40} Diabetes mellitus is disorder of carbohydrate metabolism characterized by hyperglycemia in which pancreas insulin level altered and increase in blood sugar occur.\textsuperscript{41} It can be treated via an enzyme called alpha amyrase.\textsuperscript{42} Alpha amyrase inhibition possess the key role for the treatment of diabetes, intestinal absorption, digestion and breakdown of long chain carbohydrate.\textsuperscript{43}

Material and methods

Methodology of compounds1–13

Equimolar amounts of 2,4 dinitrophenyl hydrazine and different aromatic as well as aliphatic aldehydes were refluxed in absolute methanol for about 4-6hr at a temperature of 100 °C. Anhydro acetic acid was used as a catalyst to enhance the rate of chemical reaction. The completion of reaction was controlled through thin layer chromatography. In all the cases solid purified product was achieved, which was clean with water and further re crystallized with methanol.

Molecular docking studies

Molecular Docking study was evaluated to predict the possible - binding mode of the synthesized tested compounds against α-amylase
enzyme using a well-developed modeling tool Molecular Operating Environment (MOE), the 3D coordinates for all compounds were made using builder–MOE wizard and the general parameters for minimized energy were protonated in molecular docking study in MOE. The alpha-amylase known crystal structure of was taken from server contains Protein Data Bank through common codes using 3BAJ and PDB. The structure was examined in MOE for preparation to achieve and confirmed the lowered energy level as possible for molecular docking. In last, the minimal energy conformation was used to perform docking under the common requirement of MOE and total five conformations for each ligand was allowed to generate. The ligands were ranked based on docking score; lowest scores highlighted more reasonable, poses. Finally, the predicted protein-ligand interactions (PLI) were examined to check molecular interactions using PyMol v 1.7.

**Alpha-amylase inhibitory assay**

The synthesized compounds, were evaluated for α-amylase activity. Inhibition Potential Activity was determined by Worthington–Enzymatic Manual Method. The various diluted concentration of synthesized compound ranging from 10–100µl prepared in Dmethyl sulfoxide (DMSO). A sodium phosphate of 0.02M, Concentration 500µl buffer pH [Exact 6.9, including Sodium Chloride (NaCl) of 0.006M] containing alpha – amylase solution [0.5mg/mL] for 15minute at 25°C was incubated. Then starch solution of 1% 500µl added to each test – tube containing a sodium phosphate of 0.02M buffer [Exact 6.9, including Sodium Chloride (NaCl) of 0.006 M] then again 15minutes at 25°C reaction mixture incubated, and control by addition 1.0ml Dinitro salicylic Acid (DNS).

The mixture obtained then transformed for incubation into water bath containing–boiled distilled water for 15minute cool at 20–25°C room temperature. Again 10ml sterilized–water subjected to a reaction mixture for dilution. On UV–spectrophotometer absorbance at 540nm are recorded. The control is acarbose in DMSO prepared same as above. The percent inhibition of the alpha–amylase activity is calculated on the following given Equation 1.

$$\text{Alpha- amylase percentage } = \frac{A - B}{X - Y} \times 100$$

Whereas A = after incubation absorbance of sample, amylase, starch. B=after incubation absorbance of sample, starch. X = after incubation absorbance of amylase and starch. Y=after incubation absorbance of starch only.

**Results & discussion**

**A. Chemistry of Compounds**

The general root for the synthesis of the given hydrazone derivatives followed the general procedure which involves the use of round bottom flask, condenser and hot plate. A weighed amount of 2,4-dinitrophenyldrazine was taken in R.B containing methanol as a solvent and was refluxed with continuous stirring. After some time aldehyde was added to 2,4-dinitrophenyldrazine to initiate the chemical reaction and about 2 to 3drops of acetic acid was subjected to the reaction mixture which acts as a catalyst. The reaction was refluxed for 3hrs at a fixed temperature of about 100°C.

The reaction was controlled with a passage of time with thin layer chromatography and crystals of the obtained product was precipitated in ice cold water, washed and dried and were re-crystallized after subjection to methanol to get pure crystals of final product.

**Synthetic procedure**

The various diluted concentration of synthesized compound prepared ranging from 10–100µl was used in the assay. All the compounds, showed a potential antidiabetic activity in comparison with a standard acarbose alpha amylase inhibitor as shown in (Table 1) were used the more potential activity in synthesized compound is shown by Compound 5 (5-br omo-2-methoxy-benzylidene)2-(2,4 dinitrophenyl)-hydrazine) has IC₅₀ 12.16(µg/mL) value while compound 6 (2,6-dimethoxybenzylidine)-2-(2,4 dintrhino)hydrazine) show IC₅₀ 15.03(µg/mL) and 12 has IC₅₀ 16.42(µg/mL) while compound 13 (N-(2,4-Dinitrophenyl)-N'-(4'-methoxybenzylidene)-hydrazone) has IC₅₀ 23.78(µg/mL) while compound 11(N-(2,4-Dinitrophenyl N(2′,3′,4′trihydroxybenzylidene)hydrazone) has IC₅₀ 27.27(µg/mL) and 4 N-(2,4-Dinitrophenyl)-N′(2′,3′,4′trihydroxybenzylidene)hydrazone has IC₅₀ 31.54(µg/mL) respectively. While the standard anti diabetic acarbose used in comparison to the above compound showed a less inhibition potential have IC₅₀ 42.47(µg/mL) shown in (Table 2). All other remaining compound is also active in activity but show less potential in comparison to the standard used shown (Figure 1,2).

**B. Molecular docking study**

In the present study, we have explored molecular docking study to examine the inhibition potency of all the given synthesized compounds with alpha-amylase enzyme. The molecular docking results indicate the compounds potentially bind within the catalytic site of the enzyme. The surface representation of the given enzyme with zoomed-in the catalytic site was depicted in (Figure 3A). We have noticed that the compounds bearing electron-withdrawing group (EWG)’s showed best binding potential, while bearing electron-donating groups (EDG) showed less activity, whereas these groups making the aromatic ring electron-poor (δ+) as compared to benzene, therefore, they too much powerless deactivates the ring and further compels the compounds to adopt favorable interactions, and hence raised the inhibitory activity.

The correlation among IC₅₀ and predicted docking score (S) were plotted and depicted in (Figure 3B). The protein-ligand interaction (PLI) profile for potent compounds revealed that Compound 5 showed excellent amylase inhibitory potential and adopted favorable interaction with catalytic residues including; the electrically charged positive and negative residues R343, K322 and hydrophobic W388 as shown in (Figure 3C).
Table 1 2,4-Dinitrophenyl hydrazone derivatives\textsuperscript{1–13}

<table>
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<th>Compound</th>
<th>R\textsuperscript{1}</th>
<th>Compound</th>
<th>R\textsuperscript{1}</th>
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Table 2 IC\textsubscript{50} values of synthesized compounds\textsuperscript{1–13}

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<td>Standard Acarbose</td>
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displayed average α-amylase potential also showed some favorable key interactions with catalytic residues includes; R343, Q389 and W388 as shown in (Figure 3D & 3E) respectively. The less potential might be due to the strong and weak magnitude of deactivation of EWg and EDG attached respectively. Our experimental activity correlates well with molecular docking. Concluded that the more potential activity of these compounds, is due to electron donating groups such as methoxy and methyl group is present in their basic skeleton structure. While other remaining compound contain different electron-withdrawing group such as chlorine and nitro groups therefore possess less potent inhibition activity. According to the different aromatic or, heteroatom linking in a certain compound reported with a broader biological activity. Also according to the biological activity of tested compound is always different it is because of structural-relationship when it contain different electron donating or, withdrawing group. The electron-withdrawing group showed less potency while in comparison to electron-donating is reported with highest potential activity.

**Conclusion**

Our current study has shown that our compounds 5 (IC$_{50}$=12.16µg/mL), 6 (IC$_{50}$=15.03µg/mL), and 12 (IC$_{50}$=16.42µg/mL), were found to be the more potential alpha amylase inhibitors as compared to the standard acarbose (IC$_{50}$=42.47µg/mL). These compounds may lead better for alpha-amylase inhibitor the further assessment of these compounds is important and provide a great help in the discovery of new anti diabetic drugs.

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**Conflicts of interest**

There author declares that there are no conflicts of interest.

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None.

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