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

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In our current study thirteen new 2,4-dinitrophenyl hydrazone derivatives **1–13** have been 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 **1-13** have been obtained under reflux conditions by reacting dinitrophenyl hydrazine in methanol with different aromatic as well as aliphatic aldehydes in the presence of acetic acid act as a catalyst. The current results have shown that compounds **5** (IC_{50} =12.16μg/mL), **6** (IC_{50} =15.03μg/mL), and **12** (IC_{50} =16.42 μg/mL) have been found to be the more potent alpha amylase inhibitors as compared to the standard acarbose (IC_{50} = 42.47μg/mL). These compounds may provide better leads for alpha amylase inhibitor and further assessment of these compounds can be of great help in the discovery of new antidiabetic drugs.

Keywords: Schiff base, 2,4-dinitrophenyl hydrazone, alpha amylase activity, molecular docking

A German chemist, Joseph Schiff Ugo Hugo, modern Chemistry's father[1] reported Schiff bases having (C = N) azomethine functionality and are unique organic compound generally called imine[2]. The ketones or, aldehydes carbonyl compounds react with amines *via* a condensation reaction to produce an imine, Schiff base[3]. It contains nitrogen carbon double bond where the nitrogen does not have any hydrogen, but only possesses aryl/alkyl group while the carbon possesses R^2=H called azomethine secondary aldamine. R^3 is substituted phenyl, phenyl derived aniline[4]. General structure of Schiff bases is shown in Figure 1.

Compounds containing azomethine or imine functionality showed important biological activities due to the presence of imine reactive group found in natural, derived compounds or synthesized compounds[5]. A wide range of biological activities have been attributed to Schiff bases such as antiviral activity, antibacterial activity, antifungal activity, antimalarial activity[6-8], *etc.* Derived aromatic aldehyde and amine Schiff bases are widely used in analytical, inorganic, and biological chemistry[9-11]. Many novel biological activities have been reported with a significant result on synthesized Schiff base such as antitumor activity[12-15], antioxidant activity[16-18], anti-inflammatory activity[19,20] and lipid lowering ability[18]. Schiff bases play a key role in the medicinal and pharmaceutical field for different activities[19,21-23]. Urease inhibitory activity was also reported with significant results[24-26]. Schiff bases have a lower side effect and present novel behavior[27,28]. Our current study is focused on the evaluation of synthesized Schiff bases for alpha amylase inhibition activity.

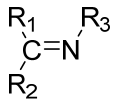


Figure 1 — General structure of Schiff base

Alpha amylase anti-diabetic activity

The major health problem of the twenty first century around the world is diabetic disease associated with hyperglycemia, hypertension, gastroparesis, ketoacidosis, and nephropathy, and affected around 15 million people globally[29]. Diabetes are mainly type – II (In which blood sugar level process effected), and in type – I (No or, little insulin production occur from pancreas)[30,31]. Oxidative stress is mainly held responsible in diabetes. It can change collagen type – IV enzyme function, structure and alter protein – glycation, reduced antioxidant level

deactivate antiathero – Sclerotic enzyme[32]. Diabetes now days can be controlled *via* synthetic drugs. One of the drugs, *i.e*. Schiff base derived drugs have heteroatom azomethine or imine functionality and are known to possess novel activity in clinical use[33,34]. The presence of electron withdrawing or donating groups change the biological activity rate of Schiff base compounds[35,36]. The hetero atom or aromatic linkage presence in certain compounds provides broader biological activity[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[39]. Around the world, diabetes patients have been multiplying. In the coming 25 years 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[40] therapies. Diabetes mellitus is disorder of carbohydrate metabolism characterized by hyperglycemia in which pancreas insulin level is altered and increase in blood sugar occurs[41]. It can be treated *via* an enzyme called alpha amylase[42]. Alpha amylase inhibition comprises the key step for the treatment of diabetes, intestinal absorption, digestion and breakdown of long chain carbohydrates[43].

Experimental Section

General procedure for synthesis of compounds 1–13

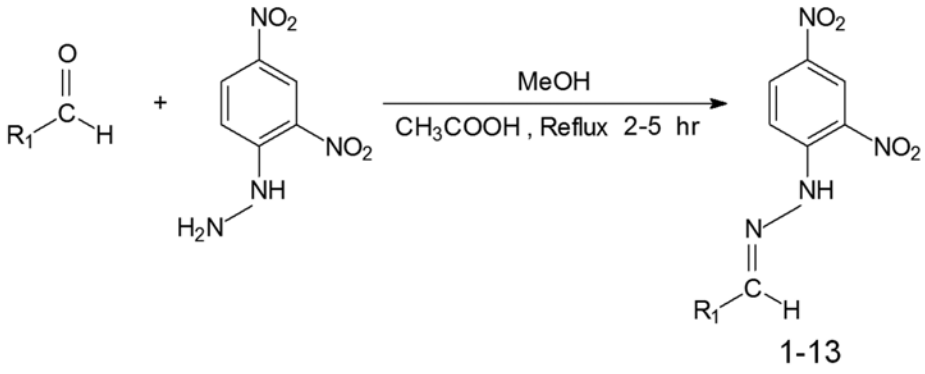
Equimolar amounts of 2,4-dinitrophenylhydrazine and different aromatic as well as aliphatic aldehydes were refluxed in absolute methanol for about 4-6 hr at a temperature of 100°C. Anhydrous acetic acid was used as a catalyst to speed up the chemical reaction. Progress of reaction was monitored through TLC. In all the cases solid product was obtained, which was washed with water and further recrystallized with methanol to obtain compounds **1-13** (Scheme I, Table I).

Molecular docking studies

Molecular Docking study was performed to explore the possible binding mode of the synthesized tested compounds against α-amylase enzyme using a well-developed modelling tool Molecular Operating Environment (MOE)[44]. The 3D coordinates for all compounds were generated using MOE-builder wizard and were protonated and energy minimized using the default parameters for molecular docking study in MOE. The crystal structure of α-amylase was retrieved from Protein Data Bank (www.rcsb.org) using PDB code 3BAJ. The structure was subjected to MOE for preparation to get the minimal energy conformation for molecular docking. Finally, the minimal energy conformation was used to perform docking using the default parameters of MOE and total five conformations for each ligand was allowed to generate. The ligands were ranked based on docking score; lower scores indicate more favorable poses. Finally, the predicted protein-ligand interactions (PLI) were analyzed for molecular interactions using PyMol v 1.7.

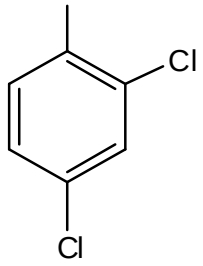
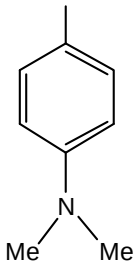
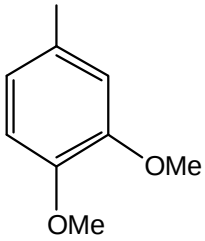
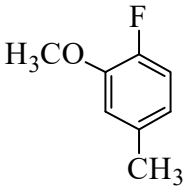
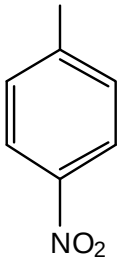
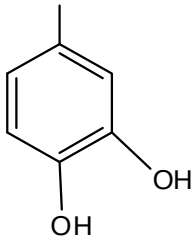
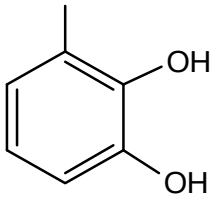
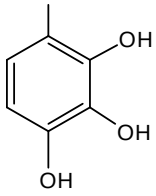
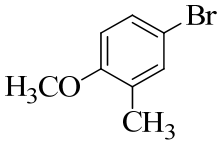
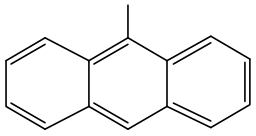
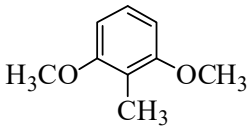
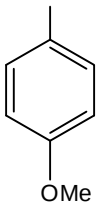
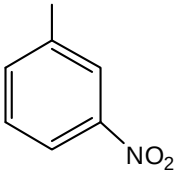
Alpha amylase inhibitory assay

The synthesized compounds **1–13** were evaluated for alpha - amylase activity. Inhibition Potential Activity was determined by Worthington – Enzymatic Manual Method[45]. The various diluted concentration of synthesized compound ranging from 10 – 100 µL prepared in Dimethylsulfoxide (DMSO). A sodium phosphate of 0.02 M, Concentration 500 µL buffer *p*H [Exact 6.9, including Sodium Chloride (NaCl) of 0.006 M] containing alpha – amylase solution [0.5 mg/mL] for 15 minute at 25°C was incubated. Then starch solution of 1 % 500 µL added to each test – tube containing a sodium phosphate of 0.02 M buffer [Exact 6.9, including Sodium Chloride (NaCl) of 0.006 M] then again 15 minute at 25°C reaction



Scheme I — Synthesis of 2,4-diniyrophenyl hydrazone derivatives

Table I — 2,4-Diniyrophenyl hydrazone derivatives **1-13**

Compd	R^1	Compd	R^1
1		**8**	
2		**9**	
3		**10**	
4		**11**	
5		**12**	
6		**13**	
7			

mixture incubated, and control by addition 1.0 mL Dinitrosalicyclic Acid (DNS). The mixture obtained then transformed for incubation into water bath containing – boiled distilled water for 15 – minute cool to 20-25°C (room temperature). Again 10 mL distilled – water added to a reaction mixture for dilution. On UV – spectrophotometer absorbance at 540 nm are recorded (Sindhu *et al*., 2013; K. Balan *et al*., 2014). 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.

$$\textit{Alpha} - \textit{amylase percentage} = \left(\frac{A-B}{X-Y}\right) \times 100 \qquad Eq-1$$

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 and Discussion

Chemistry

The general route for the synthesis of the hydrazone derivatives**(1-13)** followed the general procedure which involves the use of round bottom flask, condenser and hot plate. A weighed amount of 2,4-dinitrophenylhydrazine 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-dinitrophenylhydrazine to initiate the chemical reaction and few drops of acetic acid was added to the reaction mixture which act as a catalyst. The reaction was refluxed for 3 hrs at a temperature of about 100°C. The reaction was monitored with TLC and crystals of the obtained product was precipitated in ice cold water, washed and dried which were recrystallized from methanol to get pure crystals of final product.

Synthetic Procedure

All the synthesized compounds (**1–13**) were evaluated for alpha amylase activity. Inhibition Potential was determined by Worthington enzymatic manual method **[45]**. The various diluted concentration of synthesized compound prepared ranging from 10 – 100 µL was used in the assay. All the compounds (**1–13**) showed a potential

antidiabetic activity in comparison with a standard acarbose alpha amylase inhibitor as shown in Table II were used the more potential activity in synthesized compound is shown by Compound **5**(*5-bromo-2-methoxybenzylidene)-2-(2,4-dinitrophenyl)hydrazine*) has IC_{50} 12.16(μg/mL) value while compound **6** (*2,6-dimethoxybenzylidene)-2-(2,4 dinitrophenyl) hydrazine*) show IC_{50} 15.03(μg/mL) and 12 has IC_{50} 16.42(μg/mL) while compound **13** (*N-(2,4-Dinitrophenyl)-N'-(4'-methoxybenzylidene)hydrazone*) has IC_{50} 23.78(μg/mL) while compound **11** (*N-(2,4-Dinitrophenyl)-N' (2',3',4'trihydroxybenzylidene) hydrazone*) has IC_{50} 27.27(μg/mL) and **4** *N-(2,4-Dinitrophenyl)-N'-(2',3'-dihydroxybenzylidene)hydrazone* has IC_{50} 31.54(μg/mL) respectively. While the standard antidiabetic acarbose used in comparison to the above compound showed a less inhibition potential have IC_{50} 42.47(μg/mL) shown in (Table II). All other remaining compound is also active in activity but show less potential in comparison to the standard used shown (Figure 2 and Figure 3).

Molecular docking

In the current study, we have performed molecular docking study to evaluate the inhibition potential of all the synthesized compounds against α-amylase enzyme. The molecular docking results indicate the compounds potentially bind in the catalytic site of the enzyme. The surface representation of the enzyme with zoomed-in the catalytic site was depicted in Figure 4A. 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 make the aromatic ring electron-poor (δ+) relative to benzene, therefore, they strongly deactivate the ring and further compel the compounds to adopt favorable interactions, and hence raised the inhibitory activity. The correlation among IC_{50} and predicted docking score (S) were plotted and depicted in Figure 4B. The protein-ligand interaction (PLI) profile for potent compounds revealed that **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 4C.

The high potency might be due to the EWG (Br) at *-meta* position whose have higher magnitude of deactivation than the phenyl and OCH_3 group respectively and, hence raising the enzymatic potential. The 2nd and 3rd ranked compound in the series (**6** and **12)** which also displayed average α-amylase potential also showed some favorable key interactions with catalytic residues includes; R343, Q389 and W388 as shown in Figure 4D and Figure 4E respectively. The less potential might be due to the strong and weak magnitude of deactivation

Table II — IC_{50}valuesof synthesized compounds **1–13**

Compd	IC_{50} (μg/mL)	Compd	IC_{50} (μg/mL)
1	86.31	**8**	52.36
2	87.96	**9**	78.24
3	53.61	**10**	55.19
4	31.54	**11**	27.27
5	12.16	**12**	16.42
6	15.03	**13**	23.78
7	41.43	Standard Acarbose	42.47

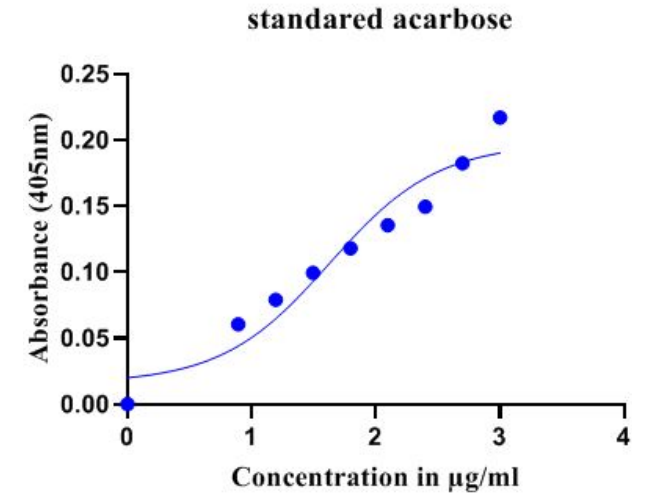


Figure 2 — Standard acarbose value at various concentrations

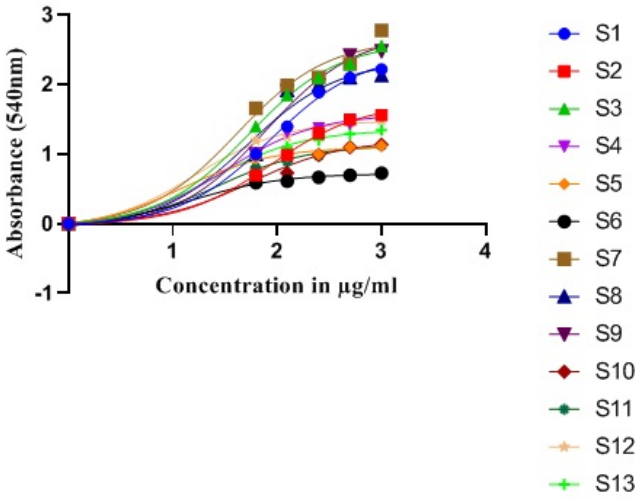


Figure 3 — Comparison of median inhibitory concentration values of compounds **1–13**

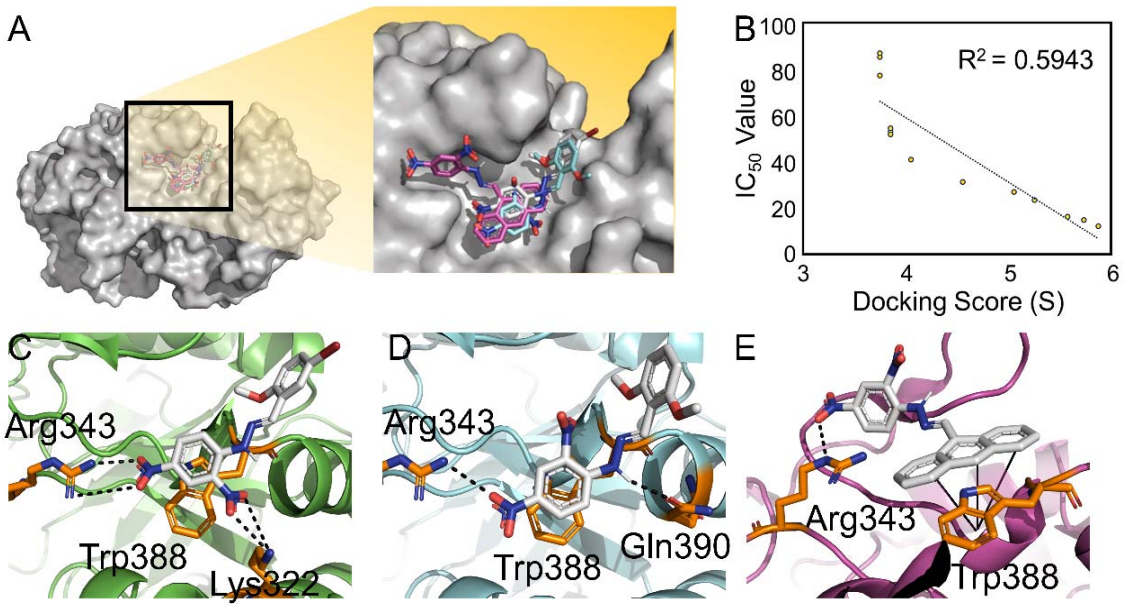


Figure 4 — The Protein-Ligand Interaction (PLI) profiles against α-amylase enzyme. (A) Indicates the surface representation of the α-amylase enzyme and represent the PLI profile for compounds **5**, **6** and **12**. Single-side arrow represents the arene-arene interaction.

of EWG and EDG attached respectively. Our experimental activity correlates well with molecular docking.

Concluded that the more potential activity of these compounds (**5**, **6** and **12**) 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[37,38]. The different aromatic or, heteroatom linking in a certain compound reported with a broader biological activity. Also, the biological activity of tested compound is always different[35,36] 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

The current studies have shown that compounds **5** (IC_{50} =12.16μg/mL), **6** (IC_{50}=15.03μg/mL), and **12** (IC_{50} =16.42 μg/mL), are found to be the more potent alpha amylase inhibitors as compared to the standard acarbose (IC_{50} =42.47μg/mL). These compounds may provide better leads for preparation of novel alpha-amylase inhibitors. Further assessment of these compounds is important and can be of great help in the discovery of new antidiabetic drugs.

Conflict of interest

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

Acknowledgement

Declared none.

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