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

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ABSTRACT

Our current attempt was made to synthesize a new 2,4-dinitrophenyl hydrazone derivatives (**1–13**) compounds and explored their alpha amylase inhibitory potential. The thirteen new derivatives of 2,4-dinitrophenyl hydrazone (**1–13**) were achieved from the reaction of aliphatic aldehydes and aromatic aldehydes with dinitrophenyl hydrazine in methanol under reflux in the presence of catalyst used acetic acid. The molecular docking study was examined through standard software MOE (*Molecular Operating Environment)*. The result of docking shown that compounds in the catalytic site of enzyme is more potentially active for binding and arrangement. Our results predict compound **12** IC_{50} =16.42 μg/mL, **5** IC_{50} =12.16μg/mL, and **6** IC_{50} =15.03μg/mL more potent and excellent inhibitor than a standard **acarbose** IC_{50} = 42.47μg/mL for alpha amylase. It's concluded that compounds (**1–13**) can provide us a pathway for new antidiabetic drugs in the market the further analysis and exploration of these compound is important and valuable.

Keywords: *2,4-dinitrophenyl hydrazone; Schiff bases; MOE; alpha amylase activity; molecular docking.*

1. INTRODUCTION

Schiff bases are one of the novel organic compounds obtained by condensation reaction of carbonyl compounds (such as ketones and aldehydes) with primary amine. It's first time reported by Hugo Schiff in 1864 (German Chemist). General formula written as $R_1N{=}CR_2R_3$ it contain Imine or, azomethine functionality due to which it possess a lot of importance [1-3]. Schiff bases are organic compound and play a vital role in making biologically active compounds in various fields of chemistry. It shows antioxidant, antidepressant activities, antimalarial, anti-inflammatory, antiglycation and antimicrobial activity [4-9]. The basic nitrogen atom of imine make hydrogen bond formation with active sites of cell and is responsible for normal cell function [10, 11]. The structure of schiff bases is given in [Figure-1].

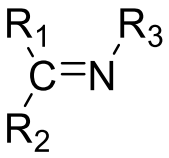


Figure 1. Structure of Schiff base.

Schiff bases also act as catalyst in polymer, organic synthesis inhibitor for corrosion and stabilizers [12]. Schiff bases in metal complexes show potent biological activities [13]. Schiff bases play a key role in the field of biochemistry, medicinal chemistry, coordination and inorganic chemistry and [14]. Several Schiff bases mainly used for synthesis of industrial compounds and cycloadditions such as of formazans, benzooxazines thiazolidinines ring closure [15]. Any synthesized or derived natural compound possessing imine or azomethine group always showed a valuable biological activities [5]. A number of important biological activities were found with Schiff bases such as antimalarial activity, antiviral activity, and antimicrobial activity with more potential [6-8]. The different Schiff bases derivatives of amines and aromatic aldehydes mainly employed for purposes in biological, analytical and inorganic chemistry [9-11]. Some reported novel significant biological activities with excellent results on synthesized Schiff bases such as the lipid oxidation potency[18], antitumor [12-15], anti-inflammatory[19, 20] and antioxidant activity[16-18]. In Pharmaceutical Medicinal field Schiff bases are most widely used and play key role for different types of activity [19, 21-23]. The inhibition of Urease was also reported on Schiff bass with excellent effects [24-26]. Due to lower side effects of Schiff bases show its novel nature in all other synthesized compounds[27, 28]. Throughout the world in the twenty first century in major health problem is the diabetic disease and approximately fifteen million people affected from it which is concerned with nephropathy, hyperglycemia and hypertension [29]. There are two types of diabetes mainly in type – I (from the pancreas insulin production rate retard either stop or decreased) while in type – II (the process of sugar level in blood changed), and [30, 31]. Mostly for diabetes oxidative stresses is accountable because it can change the type-IV enzyme and effect protein glycation structure and function also deactivate and reduced the antioxidant level of antiathero-sclerotic enzyme[32]. Recently diabetes is clinically treated through synthetic drugs like by Schiff bases due to novel imine or azomethine heteroatom groups [33, 34]. The Schiff bases compounds biological activities rates either enhance or decreased by donating or electron withdrawing groups [35, 36]. The highest noticeable biological activity of certain compounds is due to various aromatic or hetero atom linkages [37, 38]. The glucose level in the blood is lowered by alpha amylase enzyme which digests the carbohydrate and diabetes can be controlled through inhibition of this enzyme [39]. Throughout the world people are searching for full medication of diabetes mellitus in natural derived or synthetic drug sources

because around the world in next 25 year diabetes patients are multiplying and is major health killer problem [40]. The diabetes mellitus arises from carbohydrate metabolism disorder hyperglycemia where the level of sugar in blood from pancreas insulin is totally altered [41]. Alpha amylase enzyme is responsible for control [42]. The inhibition of this enzyme is key role for the medication of diabetes, carbohydrate digestion and intestinal absorption [43]. Our current attempt is to examine potential of synthesized Schiff bases against alpha amylase inhibition activity.

2. MATERIALS AND METHODS

2.1. Synthesis of Compounds (1–13)

The various aliphatic and also aromatic aldehydes react with equimolar 2,4-dinitrophenyl hydrazine in presences of solvent absolute methanol for at least 4-6 hours under reflux at a fixed temperature of100 ^{0}C. The catalyst used was anhydrous acetic acid for increasing rate of reaction. The progress and reaction completion with interval was monitored via chromatography thin layer under UV-visible lamp. All the derivatives obtained with excellent yields, pure solid products in each case and were recrystallized in methanol after washing with sterilized water.

2.2. Molecular docking of Compounds (1–13)

Molecular Docking was explored to show the binding potential capacity possibility of all the synthesized compounds tested against alpha-amylase enzyme via a standard MOE (Molecular Operating Environment) a well-developed modeling tool [44].

The three dimensional (3D) structure and coordinates for all the synthesized compounds were made in Molecular Operating Environment software builder wizard and the common parameter for minimal energy were subjected to protonation in MOE during molecular docking study. The known crystal structure of alpha-amylase was saved from (www.rcsb.org) web server using PDB and 3BAJ common codes which contain protein data bank. In the MOE the structure then evaluated and prepared to confirm the possible lower energy level for molecular docking. At last under the common requirement of molecular operating environment for each ligands only five conformations was allowed for generation and minimum energy was utilized to explore the docking.

All the ligands were marked based on using their docking score the lowered score showed excellent results and more reasoning of possibilities. Finally the obtained PLI (Protein Ligands Interactions) were explored to show the molecular interactions using (software **PyMol v 1.7.)**

2.3. Alpha-amylase Assay

All the synthesized compounds **(1–13)** were explored for inhibition potential activity against alpha-amylase using the enzymatic method of Worthington [45].

All the synthesized compounds were dissolve and diluted at the concentration raging from 10-100μl in DMSO (dimethylsulfoxide). The sodium phosphate buffer of 0.02 M at500μl concentration having pH (6.9) with Sodium Chloride (NaCl) of 0.006 M was incubated at 25^{0}C for 15 minute containing amylase solution (0.5 mg/mL). The 1 % 500μl starch solution added to each test tube containing a buffer of 0.02 M sodium phosphate having pH (6.9) with Sodium Chloride (NaCl) of 0.006 M again subjected the reaction mixture for incubation period at 25^{0}C for 15 minute and for control placed DNS (dinitrosalicyclic acid) 1.0 mL. The obtained mixture again placed in the water bath for incubation at room temperature 20-25^{0}C to cool for about minute. The sterilized water 10 mL for dilution added to reaction mixture.

The absorbance recorded at 540 nm on UV – spectrophotometer. The standard control **acarbose** were also prepared in DMSO and treated same as above the sample compounds. The **Equation-1** was used to determine the percent inhibition of alpha amylase given below.

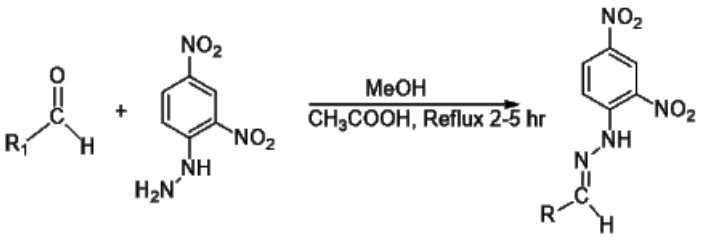
$$Alph\ amylase\ percentage = \frac{A-B}{X-Y} \times 100$$

Whereas **A** = After Incubation absorbance of amylase, sample and starch. **B** = After Incubation absorbance of starch sample. **X** = After Incubation absorbance of and starch amylase. **Y** = After Incubation absorbance of starch only.

3. RESULTS

3.1. Chemistry of Compounds (1-13)

The pathway for the hydrazone derivatives **(1-13)** synthesis followed the most common procedure which includes the usage of hot plates, round bottom flask and condenser. In round bottom flask a solvent absolute methanol was added with weighed amount of 2,4-dinitrophenylhydrazine and was stir continuously under refluxed. After some time to 2,4-dinitrophenyl hydrazine the aldehydes was added to initiate the chemical reaction exact 2-3 drop of acetic acid was added which act as catalyst in the reaction mixture. The reaction at fixed temperature of about 100^{0}C was refluxed for 3 hours. The entire desired product obtained under **scheme 1** various derivatives present in **Table 1**. The reaction was monitored with interval of time through TLC (Thin layer chromatography) the obtained crystal products was precipitated in cold ice water then clean-dried and were recrystallized to achieve final pure crystal products after subjection to solvent methanol.



Scheme 1. The 2, 4-Diniyrophenyl hydrazone derivatives (**1-13**).

All the hydrazone derivatives (**1-13**) showed a potent antidiabetic activity when compared to a standard control acarbose of alpha amylase inhibitor given in [**Table-2**] were used the more potent antidiabetic character found in synthesized compounds such as compound **4** (2,4-Dinitrophenyl)-N'-(2',3'dihydroxybenzylidene) hydrazone showed IC_{50} =31.54(µg/mL), compound **13** (2,4-Dinitrophenyl)-N'-(4'-methoxybenzylidene)-hydrazone) showed

IC_{50} =23.78(µg/mL) while compound **6** (2,6 dimethoxy benzylidene)-2-(2,4 dinitrophenyl)hydrazine) show IC_{50}= 15.03(µg/mL) and Compound **5** (5-bromo-2-methoxy benzylidene)-2-(2,4dinitrophenyl)-hydrazine) showed IC_{50}= 12.16(µg/mL) value of inhibition and compound **11** (N-(2,4-Dinitrophenyl N(2',3',4'trihydroxybenzyli-dene)hydrazone has IC_{50} =27.27(μg/mL) and **12** showed IC_{50} =16.42(μg/mL) values respectively.

While the standard antidiabetic control **acarbose** used for comparison with the synthesized hydrazones derivatives (**1-13**) showed less potential against amylase with IC_{50}=42.47(μg/mL) given in [**Table 2**]. The other hydrazones derivatives in the series were also active with not a greater potential as compared to a standard use given in [**Figure 2**] and [**Error! Reference source not found.**].

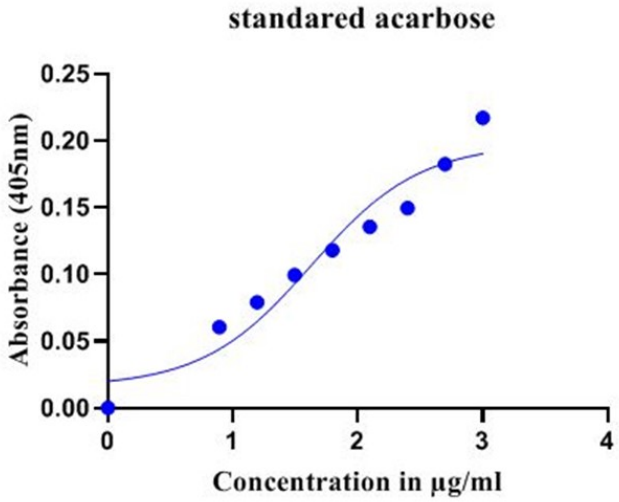


Figure 2. Standard Acarbose Absorbance at various concentrations.

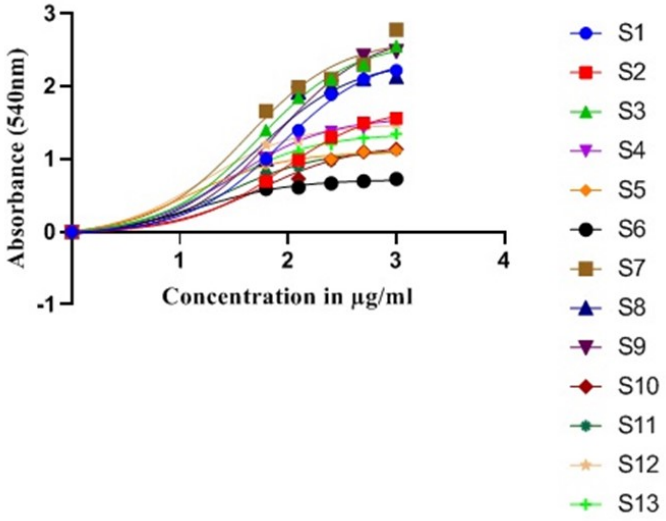


Figure 3. The IC_{50} of compounds **(1–13)** at various concentrations.

3.2. Molecular docking Study

In the current study we have performed the molecular docking studies to predict the potential of the all the synthesized hydrazone derivatives (**1-13**) against alpha-amylase. From the results of molecular docking concluded that the compounds with more potential bind in the active catalytic site of the enzyme. The catalytic site was depicted in **Fig 4A** from the given enzyme zoomed-in from the surface representation. We have concluded that that some compounds which contain EWG's (electron-withdrawing group) showed more potential in binding sites, while those compounds which possess EDG (electron-donating groups) showed less inhibition potential and binding capacity because these group make the aromatic ring electron-poor (δ+) as compared benzene. When they too much highly deactivate the ring compound their possibility increases for interaction and enhances the inhibitory potential activity.

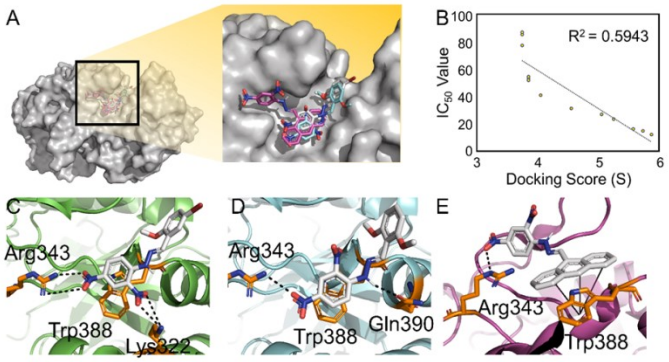


Figure 4. The PLI (Protein Ligand Interaction) profiles with alpha-amylase enzyme.
(A) The surface representation of the alpha-amylase enzyme and shows the PLI profile for compound **5**, **6** and **12**. Single-side arrow represents the arene-arene interaction.

The correlation among predicated docking score (S) and IC_{50} values were plotted and shown in **Fig 4B**. The PLI (Protein Ligand Interaction) profile for active compounds represents that **Compound 5** showed excellent and adopted interaction with catalytic site of enzyme and inhibition potential while in **Fig 4C** representing the hydrophobic **W388** and electrically negative-positive charged residue **K322**, **R343**.

The high potent activity is because of compound bearing the EWG's (electron-withdrawing group) such as bromine (Br) placed at *-meta* position in the skeleton which have higher potential and is more electronegative as compared to OCH_3 and phenyl groups respectively might be therefore enhancing the enzymatic potency.

While other compound ranked as 2nd and 3rd in the whole series is compounds **(6 & 12)** which also showed excellent potential against alpha-amylase and possible interaction with the catalytic sites including charges and residue **W388**, **Q389** and **R343** given in **Figure 4D and 4E**. The less inhibition potency may be due containing weak and strong electron withdrawing and donating groups or more electronegative or electropositive atoms attached which altered the activity rate. Our predicated result from experiments in well modes correlates with molecular docking.

It's concluded that the highest more potent activity of the given compounds such as **5**, **6** and **12** is due to methyl and methoxy electron donating groups found in the main skeleton structure of these compounds. The other compounds in the series showed less potential activity because they possess various electron-withdrawing groups like nitro and chlorine.

The Schiff bases compounds biological activities rates either enhance or decreased by donating or electron withdrawing groups[35, 36]. The highest noticeable biological activity of

certain compounds is due to various aromatic or hetero atom linkages[37, 38] the structural-relationship is the key factor in the results of activity the high potential always found with electron donating groups while withdrawing group possess less activity.

Table-1. 2,4-Diniyrophenyl hydrazone derivatives **(1-13).**

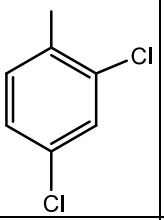
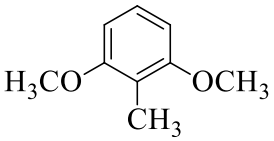
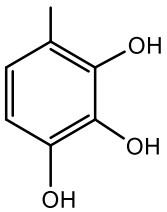
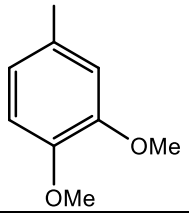
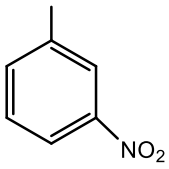
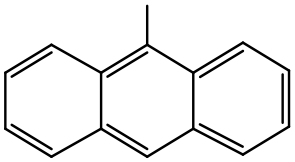
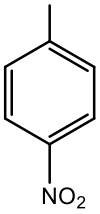
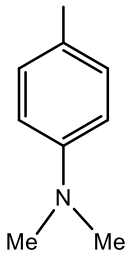
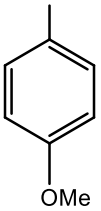
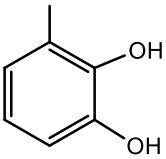
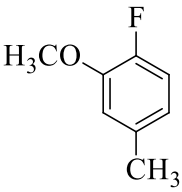
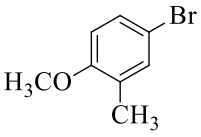
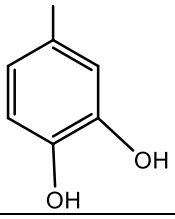
Compound	R^1	Compound	R^1	Compound	R^1
1		**6**		**11**	
2		**7**		**12**	
3		**8**		**13**	
4		**9**			---
5		**10**			---

Table-2.IC_{50}valuesof synthesized compounds (**1–13**).

Compound	IC_{50} (μg/mL)	Compound	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**

4. CONCLUSIONS

Our current attempt was made to synthesize a new 2,4-dinitrophenyl hydrazone derivatives (**1–13**) compounds and explored their alpha amylase inhibitory potential. The molecular docking study was examined through standard *Molecular Operating Environment* and shown that compounds in the catalytic site of enzyme is more potentially active for binding. Our results predicted that compounds **12** IC_{50} =16.42 μg/mL, **5** IC_{50} =12.16μg/mL, and **6** IC_{50} =15.03μg/mL is more potent and excellent inhibitor than a standard **acarbose** IC_{50} = 42.47μg/mL for alpha amylase inhibitor. It's concluded that these compounds can provide us a pathway for new antidiabetic drugs in the market the further analysis and exploration of these compounds is important and valuable.

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6. ACKNOWLEDGEMENTS

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