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

Abstract

In our current study thirteen new 2,4-dinitrophenyl hydrazone derivatives (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 an excellent result. Molecular Operating Environment (MOE) software was used for docking study. 2,4-dinitrophenyl hydrazone (1-13) 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$_{50}$=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$_{50}$=2.47µg/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 Schiff Ugo Hugo Modern Chemistry father I reported Schiff bases having (C = N) azomethine functionality are present and are unique compound generally called Imine. The ketones or, aldehydes carbonyl compounds react with amines via a condensation reaction to produce Imine, Schiff base. 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 aldime R is substituted phenyl, phenyl derived aniline. 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. A widespread range of biological function was reported on Schiff bases such as antiviral activity, antibacterial activity, antifungal activity, anti malarial activity. Derived aromatic aldehyde and amines Schiff bases widely used in analytical, inorganic, and biological chemistry. Many novel biological activities reported with a significant result on synthesized Schiff base such as antitumor activity, antioxidant activity, anti-inflammatory activity, and lipid lowering ability.

Schiff bases play a vital role in Medicinal and Pharmaceutical field for different activity. Urease inhibitory activity was also reported with a significant result. Schiff have a lower side effect present a novel behavior. 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 150million people approximately. Diabetes are mainly type – II (In which blood sugar level process effected), and in type – I (No or, little insulin production occur from pancreas). 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. 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.

Also the electron withdrawing presence or donating group can change the biological activity rate of Schiff bases compounds. The hetero atom or aromatic linkage presence in certain compound provides a broader biological activity. Diabetes can be treated via the inhibition of alpha amylase enzyme involved in digestion of carbohydrate by lowering the glucose level in blood. Around the world wide diabetes patients multiply, in upcoming 25 year 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. Diabetes mellitus is disorder of carbohydrate metabolism characterized by hyperglycemia in which pancreas insulin level altered and increase in blood sugar occur. It can be treated via an enzyme called alpha amylase. Alpha amylase inhibition possess the key role for the treatment of diabetes, intestinal absorption, digestion and breakdown of long chain carbohydrate.

Material and methods

Methodology of compounds 1–13

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. Anhydrous 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 $\alpha$-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, (1-13) were evaluated for α-amylase
activity. Inhibition Potential Activity was determined by Worthington–
Enzymatic Manual Method.8 The various diluted concentration of
synthesized compound ranging from 10–100µl prepared in Di methyl
sulfoxide (DMSO). A sodium phosphate of 0.02M, Concentration
500µl buffer pH [Exact 6.9, including Sodium Chloride (NaCl)
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 1.0M Dinitro salicylic Acid (DNS).

The mixture obtained then transformed for incubation into water
bath containing–boiled distilled water for 15minutes cool at 20–25°C
room temperature. Again 10M. 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.

\[
\text{Alpha- amylase percentage} = \left(\frac{A - B}{X - Y}\right) \times 100
\]

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 (1-13)

The general root for the synthesis of the given hydrazone
derivatives1–13 followed the general procedure which involves the
use of round bottom flask, condenser and hot plate. A weighed amount
of 2,4-dinitrophenyhydrazone 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-dinitrophenyhydrazone 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,(1-13) 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-br omo-2-methoxy benzylidene)-2-(2,4 dinitrophenyl
-hydrazone) has IC\(_50\) 12.16(µg/mL) value while compound
(2,6-dimethoxybenzylidene)-2-(2,4 dinitrophenyl)hydrazone) show
IC\(_50\) 15.03(µg/mL) and 12 has IC\(_50\) 16.42(µg/mL) while compound
(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 above compound
showed a less inhibition potential have IC\(_50\) 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
powerly deactivates the ring and further compel the compounds to
show less activity, whereas these groups making the aromatic ring
electron-poor (δ+) as compared to benzene, therefore, they too much
displace the catalytic residues including; the electrically charged
amino acid residues 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 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 (1–13)

| Compound | R¹       | Compound | R¹       | Compound | R¹       |
|----------|----------|----------|----------|----------|----------|
| 1        | Cl       | 6        | H₃CO⁻    | OCH₃     | 11       |
|          | Cl       | 2        | OMe      |          |          |
| 3        | NO₂      | 8        | N        | Me       | 13       |
| 4        | OH       | 9        | H₃CO⁻    | F        |          |
| 5        | H₃CO⁻    | 10       | Br       |          |          |

Table 2: IC₅₀ values of synthesized compounds (1–13)

| Compound | IC₅₀ (µg/mL) | Compound | IC₅₀ (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 |

Citation: Yousaaf M, Hassan A, Ahmad S, et al. 2,4-Dinitrophenyl hydrazone derivatives as potent of alpha amylase inhibitors. J And Pharm Res. 2019;8(6):222–226. DOI: 10.15406/japr.2019.08.00342
2, 4-Dinitrophenyl hydrazone derivatives as potent alpha amylase inhibitors displayed average alpha-amylase potential also showed some favorable key interactions with catalytic residues includes; R\textsuperscript{343}, Q\textsuperscript{389} and W\textsuperscript{388} 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,5,6,12 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 potential inhibition activity.\textsuperscript{35,36} According to the different aromatic or, heteroatom linking in a certain compound reported with a broader biological activity. Also according to\textsuperscript{35,36} 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\textsubscript{50}=12.16µg/mL), 6 (IC\textsubscript{50}=15.03µg/mL), and 12 (IC\textsubscript{50}=16.42µg/mL), were found to be the more potential alpha amylase inhibitors as compared to the standard acarbose (IC\textsubscript{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.

Acknowledgments

The author is acknowledge to all those who supported directly or, indirectly this experimentally workup.

Conflicts of interest

There author declares that there are no conflicts of interest.

Funding

None.

References

1. Schiff H. Mittheilungen aus dem Universitätslaboratorium in Pisa: eine neue Reihe organischer Basen, Justus Lie, Anna der Chem. 1864;1311:118–119.
2. Patai S. The Chemistry of the carbon-nitrogen double bond. John Wiley & Sons. 1970.
3. Silva CM, Silva DL, Modolo LV, Alves RB, et al. Schiff bases, A short review of their antimicrobial activities. J of Adv Research. 2011;2:1–8.
4. Nic M, Jirat J, Kosata B. IUPAC Compendium of Chemical Terminology. 2006.
5. Prakash A, Adhikari D. Application of Schiff bases and their metal complexes-A Review. Int J Chem Tech Res. 2011;34:1891–1896.
6. Hameed A, M al-Rashida, M Uroos, et al. Schiff bases in medicinal chemistry: a patent review. Exp opin on therapeut patents. 2017;27(1):63–79.
7. Bringmann G, Dreyer M, Faber JH, et al. Ancistrotanazaine C and Related 5, 1'-and 7, 3'-Coupled Naphthyliso quinoline Alkaloids from Ancistrocladus t anzaniensis. J nat prod. 2004;67(5):743–748.
2, 4-Dinitrophenyl hydrazone derivatives as potent of alpha amylase inhibitors. Asian J of Chem. 2010;22(7):5289–5296.

Singh P, Goel R, Singh B. Synthesis, Characterization and Biological Activity of Schiff Bases. J Indian Chem Soc. 1975;52:958–959.

Berry P, A Beezer, Miles RJ, et al. Evaluation of microcalorimetry as a drug bioactivity screening procedure: application to a series of novel Schiff base compounds. Microbiots. 1988;45(184):181–91.

Elmali A, Kabak M, Kavakoglu E, Elerman Y, et al. Tautomeric properties, conformations and structure of N-(2-hydroxy-5-chlorophenyl) salicyldiamine. J mol structure. 1999;510(1-3):207–214.

Kraicheva I, Bogomilova A, Tsacheva I, et al. Synthesis, NMR characterization and in vitro antitumor evaluation of new aminophosphonic acid diesters, Euro J med chem. 2009;448:3363–3367.

Li Z, Gu Z, Yin K, Zhang R, et al. Synthesis of substituted-phenyl-1, 2, 4-triazol-3-thione analogues with modified d-glucopyranosyl residues and their antiproliferative activities. Euro J medi chem. 2009;44(11):4716–4720.

Ren S, Wang R, Komatsu K, et al. Synthesis, biological evaluation, and quantitative structure–activity relationship analysis of new Schiff bases of hydroxysemiacbazide as potential antitumor agents. J medi chem. 2002;45(2):410–419.

Hranjec M, Starčević K, Pavičik Š, et al. Synthesis, spectroscopic characterization and antiproliferative evaluation in vitro of novel Schiff bases related to benzimidazoles. Euro J of medi chem. 2011;46(6):2274–2279.

Li YF, Liu ZQ, Ferrocenyl Schiff base as novel antioxidant to protect DNA against the oxidative damage, Euro J of Pharma Scies. 2011;442(1):158–163.

Nechoritis CG, Zargas-Tzitzikas T, Tsoleridis CA, et al. One-pot microwave assisted synthesis under green chemistry conditions, antioxidant screening, and cytotoxicity assessments of benzimidazole Schiff bases and pyrimido [1, 2-a] benzimidazol-3 (4H)-ones. Euro J of medi chem. 2011;46(1):297–306.

Sashidhara KV, Rosaih JA, Bhatia G, et al. Novel keto-enamine Schiff bases from 7-hydroxy-4-methyl-2-oxo-2H-benzo [h] chromene-8, 10-dicarbaldehyde as potential antiinflammation and antioxidant agents, Euro J of medi chem. 2008;43(11):2592–2596.

El-Sayed NA, Awadallah FM, Ibrahim NA, et al. Synthesis, anti-inflammatory and ulcerogenicity studies of some substituted pyrimido [1, 6-a] azepine derivatives. Indian J of Pharm Res. 2012;5:2044-2047.

Pandey A, Rajavel R, Chandraker S, et al. Synthesis of Schiff bases related to benzimidazoles. Indian J of Pharm Res. 2012;4(4):2324–2331.

http://www.asianjournalofchemistry.co.in/User/SearchArticle. aspx?Volume=23&Issue=5&Article&Criteria=

Barbachyn MR, Ford CW. Oxazolidinone structure–activity relationships leading to linozolid. Angewandte Chemie Inter Ed. 2003;42(18):2010–2023.

Panchal AD, Patel PM. Synthesis of N-(5-Substitutedphényl)-4, 5-dihydro-1H-pyrazol-3-yl]-4H-1, 2, 4-triazol-4-amine from 4-Amino-4H-1, 2, 4-triazole. J Chem. 2011;53:1180–1185.

Perez H, Iqbal MS, Tahir MY, et al. In vitro cytotoxic, antibacterial, antifungal and urease inhibitory activities of some N 4-substituted isatin-3-thiosemicarbazones. J of enzyme inhibition and medi chem. 2008;23(6):848–854.

Arshia A, Khan A, Khan KM, et al. Synthesis and urease inhibitory activities of benzophenone semicarbazides/thiosemicarbazones. Medi Chem Research. 2016;25(11):2666–2679.

Hameed A, Khan KM, Zehr ST, et al. Synthesis, biological evaluation and molecular docking of N-phenyl thiosemicarbazones as urease inhibitors. Bioorg Chem. 2015;61:51–57.

Zaborska W, Kot M, K Superata. Inhibition of jack bean urease by 1, 4-benzoquinone and 2, 5-dimethyl-1, 4-benzoquinone. Evaluation of the inhibition mechanism. J of enzyme inhibition and medi chem. 2002;174:247–253.

Krajewska B. Ureases I: Functional, catalytic and kinetic properties: a review. J of Molec Catalysis B: Enzymatic. 2009;59(1):9–21.

Soltni A, Pourian M, Davani BM. Correction to Does this patient have Pherochromocytoma? a systematic review of clinical signs and symptoms. J of Diaie & Meta Disorders. 2016;15(6):42.

Krishan P, Singh G, O Bedi. Carbohydrate restriction ameliorates nephropathy by reducing oxidative stress and upregulating HIF-1α levels in type-1 diabetic rats. J of Diaie & Meta Disorders. 2017;161:47.

Pratley RE. The early treatment of type 2 diabetes. Am J Med. 2012;126(9):52–59.

LeBlu VS, MacDonald B, Kalluri R. Structure and function of basement membranes. Expert bio and medi. 2007;232(9):1121–1129.

Nalini P, Poornam Y. Synthesis and Biological activities of some new Phthalides. Orient J Chem. 2012;2:57–61.

Golcu A, Turner M, Demirell H, et al. Cd (II) and Cu (II) complexes of polyydentate Schiff base ligands: synthesis, characterization, properties and biological activity. Inorg Chim Acta. 2005;358(6):1785–1797.

Yalcin I, Kocyiigit Kaymacioglu B, Ören I, et al. Synthesis and microbiological activity of some novel N-(2-hydroxy-5-substitutedphenyl) benzacetamides, phenoxy acetamides and thiophenoxyacetamides as the possible metabolites of antimicrobial active benzoazolmes. Farmaco. 1997;52:685–689.

Thangadurai TD, Natarajan K. Tridentate Schiff base complexes of ruthenium (III) containing ONS/ONO donor agents. J of Medicinal Chem. 2001;44(6):717–722.

Zhang LX, Liu Y, Cia LH, et al. Inhibitory study of some novel Schiff base derivatives on Staphylococcus aureus by microcalorimetry. Thermochim acta. 2006;440(1):51–56.

Mezeiöva E, Spilovska K, Nepovimova E, et al. Profiling donepezil template into multipotent hybrids with antioxidant properties. J of enzyme inhibition and medi chem. 2018;33(1):583–606.

Rabasa-Lhoret R, J Chiasson. “Alpha-glucosidase inhibitors International textbook of diabetes mellitus. Wiley and Sons. 2004.

Singh R, Rajasree P, Sankar C. Screening for anti-diabetic activity of the ethnologic extract of Barleria cristata seeds. Int J Pharm Life Sci. 2012;3:2044-2047.

West IC. Radicals and oxidative stress in diabetes. Diabetic Medi. 2000;173:171–180.

Bhosal U, Hallale B. Gamma radiation induced mutations in black gram (Vigna mungo (L.) Hepper). Asian J of Plant Scie and Resea. 2016.

Subramanian AAR, A Sadikun, Asmawi MZ. In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. J Pol Biochem Soc. 2008;55:391–398.

MOE (MOE), 1010 Sherbrooke St. West, Canada: Chemi Computing Group Inc. 2016.

V. Worthington. Worthington Enzyme Manual. Freehold Biochemical Corp. 1993;36–41.

Citation: Yousaf M, Hassan A, Ahmad S, et al. 2, 4-Dinitrophenyl hydrazone derivatives as potent of alpha amylase inhibitors. J And Pharm Res. 2019;8(6):222–226. DOI: 10.15406/japlr.2019.08.00342