Design and evaluation of chalconeimine derivatives as α-amylase inhibitors

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Abstract:
Alpha-amylase is a known target for type II diabetes. Therefore, it is of interest to design α-amylase inhibitors based on hydrazone scaffold. The structure of these hybrids was confirmed by spectroscopic analysis (IR, ¹H- and ¹³C NMR). All the compounds have potential inhibitory properties as shown by in vitro α-amylase inhibition activity. The compound 5-((1Z,3Z)-3-(benzo[d][1,3]dioxol-5-yl)-3-((2-chloropyridin-3-yl)limino)prop-1-en-1-yl)-2-(difluoromethoxy)phenol(4a) in 100 µg/mL concentration showed a high inhibition of 85.23%. In vitro α-amylase inhibition was further supported by docking studies of compound against the active site of pig pancreatic α-amylase (PDB ID: 3L2M). Docking studies revealed that the bonding interactions found between the compound and human pancreatic α-amylase are similar to those responsible for α-amylase inhibition by acarbose.

Keywords: Molecular docking, diabetes, alpha-amylase, hydrogen bond, hydrazone, chalcone.

Background:
Diabetes is a multi-factorial disorder of the pancreas, in which the pancreas fails to perform its function to produce insulin hormone properly in the body. It involves multiple disorders like hyperglycemia, glycosuria and abnormal metabolism of lipids, carbohydrates and proteins [1, 2]. This affects the human body at physiological, physical and social level. It has been known as the 3rd leading cause of death in humans along with other diseases such as cancer, cerebro-vascular and heart. Hypoglycemic medication is helps to lower the blood sugar level in body or treat the other severe symptoms and complications of diabetes mellitus [2]. The side effects of these medications include extreme hypoglycemia, liver cell injury, lactic acidosis, digestive discomfort, permanent neurological deficit, headache, dizziness and even death [3, 4]. The basic challenge in curing diabetes is to maintain blood glucose level close to normal levels [5-10]. These therapies are used as mono therapy or in combination for optimal control of glycaemia [11-14]. As mentioned before that these drugs are normally expensive and come with side effects. These drugs have their limitations due to low pharmacokinetic properties, secondary failure rates and relative bad effects [15-21]. Thus, there is a need for efficient class of compounds to reduce the side effects. Molecular docking is a competent tool for novel micro molecule drugs discovery for targeting protein. This study has been carried out in order to identify effective, selective and efficient antidiabetic Lead compound and its analogues.

Chalcone is a class of open-chain flavonoids that is not only biosynthesized by plants but also can be prepared synthetically. The simplest chalcone can be prepared by an aldol condensation between a benzaldehyde and an acetophenone in the presence of base [22-24]. Hydrazones of chalcones have shown a wide variety of pharmacological effects, including anti-inflammatory and anticancer activities [25-29]. Despite the comprehensive biological studies on chalcones, reports on their anti-diabetic activity are limited [30]. Significant advances have been made in the past few years in the isolation and preparation of several hydrazones of chalcones derivatives.

Material and methods:

Chemistry:
Thin layer chromatography (TLC) was used to examine the progress of the reaction. Open glass vessels were used to make a decision for the dissolving on outstanding softening mechanical assembly and were uncorrected. H1 and 13C atomic enticing reverberation (H1 proton magnetic resonance and 13CNMR)
Preparation of AC-CdO-TiO$_2$ nanocomposite material by precipitation method:
AC-CdO-TiO$_2$ nanocomposite material was synthesized by precipitation method. Initially cadmium acetate dihydrate (0.4 M) were dissolved in anhydrous ethanol solution beaker A. 0.4m citric acid and tetra isopropyl orthotitanate were in ethanol is taken as another solution beaker B and Activated carbon (AC) were dissolved in anhydrous ethanol (10 ml) and deionized water (90 ml). To this mixture 0.01 mol citric acid, 0.01 mol substituted aniline (4a-e) were dissolved in anhydrous ethanol solution beaker C, the solution A and solution C was added to Solution beaker B and stirred well. Then to this 2 drops NH$_4$OH is added at room temperature under vigorous stirring until the precipitate was formed. The obtained precipitate was washed with water and ethanol. Then the precipitate was collected and dried in oven at 100°C for 12 hrs. The resulting powder was finally calcinated at 500°C at 4 hrs.

General procedure for the Synthesis of (E)-1-(4-((difluoromethoxy)-3-hydroxyphenyl)-3-phenylprop-2-en-1-one(3): 4-(difluoromethoxy)-3-hydroxybenzaldehyde 1 (0.02 mol) and 1-(benzo[d][1,3]dioxol-5-yl)ethanone 2 (0.02 mol) were dissolved in 30 ml of alcohol. To this reaction mixture 40% NaOH (10 ml) and AC-CdO-TiO$_2$ nanoparticles catalyst (0.003 g), in anhydrous ethanol (5 mL) and deionized water (90 mL), the mixture was heated on water bath at 85°C for 15 min. Further, the enzyme solution (1 unit/mL) was prepared by mixing 100 mg in 100 mL of 20 mM sodium phosphate buffer (pH 6.9). The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (DNSA) (20 mL),5.31 M sodium potassium tartrate in 2 M NaOH (8 mL) and deionized water (12 mL). Acarbose was used as a standard at the concentration of 1mg/mL. 100 µl of test solution and 100 µL of enzyme solution were mixed in vials and incubated at 25°C for 30 min. To this mixture 100 µL of color reagent was added and the mixture was heated on water bath at 85°C for 15 min. Further, the reaction mixture was removed from water bath, cooled and absorbance value determined at 595 nm. Individual blanks were prepared for correcting the background absorbance. Control experiment was conducted in the same manner by replacing the drug sample with 1 mL DMSO. Inhibition percentage of α-amylase was calculated by the formula [34]. Enzyme activity was calculated and percentage of inhibition was ((Control – Test)/100) x 100.

Table 1: Physical data of various synthesized compounds

| Compound | Color  | Mol. Formula | Mol. weight | Solubility | Melting point (°C) |
|----------|--------|-------------|-------------|------------|-------------------|
| 4a       | Yellow | C$_2$H$_2$ClF$_2$NO$_2$ | 444         | Ethanol    | 157               |
| 4b       | Yellow | C$_2$H$_2$ClF$_2$NO$_2$ | 444         | Ethanol    | 133               |
| 4c       | Yellow | C$_2$H$_2$ClF$_2$NO$_2$ | 444         | Ethanol    | 148               |
| 4d       | Pale   | C$_2$H$_2$F$_2$NO$_2$  | 424         | Ethanol    | 128               |
| 4e       | Yellow | C$_2$H$_2$F$_2$NO$_2$  | 424         | Ethanol    | 118               |

Table 2: Data from IR spectra of chalconeimine derivatives (4a-e)

| Compounds | FREQUENCY cm$^{-1}$ | C=O | C=N | CH=CH | ARO C-H |
|-----------|---------------------|-----|-----|-------|---------|
| 4a        | 1666                | 1597| 2966| 1452  | 3089    |
| 4b        | 1645                | 1586| 2924| 1425  | 3084    |
| 4c        | 1645                | 1589| 2924| 1448  | 3084    |
| 4d        | 1625                | 1586| 2926| 1452  | 3093    |
| 4e        | 1667                | 1597| 2924| 1450  | 3088    |

Table 3: Data from 1H NMR spectra of hyrazone derivatives (4a-e)

| Compounds | -CH$_3$ | CH$_2$ | Aromatic protons |
|-----------|---------|--------|------------------|
| 4a        | 6.30 (2H,singlet) | 7.48 (1H,singlet) | 7.43-8.86 (11H, multiplet) |
| 4b        | 6.60 (2H,singlet) | 7.47 (1H,singlet) | 7.47-7.95 (11H, multiplet) |
| 4c        | 6.55 (2H,singlet) | 7.49 (1H,singlet) | 7.43-8.38 (11H, multiplet) |
| 4d        | 6.58 (2H,singlet) | 7.46 (1H,singlet) | 7.28-7.96 (11H, multiplet) |
| 4e        | 6.55 (2H,singlet) | 7.41 (1H,singlet) | 7.14-8.38 (11H, multiplet) |
Results and Discussion:

Table 1 show all the physical data like color, molecular formula, molecular weight, solubility, melting point, of synthesized compounds. The IR frequencies of compounds 4a-e is shown in Table 2 in which the C=N stretching frequency appear at 1586-1667 cm\(^{-1}\). Aromatic (CH) stretching frequencies appear at 3084-3093 cm\(^{-1}\) and stretching frequency observed at 1625-1666 cm\(^{-1}\) C=O group present in the derivatives. The 1H NMR chemical shift values of compound (4a-e) given in Table 3. The singlet observed in the range 6.30-6.60 ppm is due to CH\(_2\) methylene proton of 3',4'-methylenedioxy acetophenone moiety proton. The singlet observed at 7.41-7.49 ppm is due to CH proton of –CH\(_2\) moiety. The signals appearing 7.14-8.38 ppm are obviously due to aromatic protons. The five chalconeimine derivatives (4a-e) shown in Figure 1a were taken for docking studies. These compounds are synthesized and their structures have been determined by IR,\(^{1}\)H and \(^{13}\)CNMR spectroscopy.

In vitro α-amylase inhibition:

All the synthesized compounds (4a-e) and standard drug were explored for their in vitro α-amylase inhibition studies at different concentrations (50, 100, 200 µg/mL) as shown in the Table 4. All the compounds showed good % inhibition of α-amylase when compared with standard drug acarbose. Compound 4b and 4d were found to be more potent among all the synthesized compounds when explored at the concentration of 50 µg/mL. Compound 4d shows 76.58% inhibition followed by 4b with 77.18% inhibition. There was a significant rise in % inhibition when concentration has been changed to 100 µg/mL from 50 µg/mL. Among all, 4b shows 81.35% inhibition followed by 4a which showed 85.23% inhibition at 100 µg/mL. Inspired by the results obtained at 100 µg/mL concentration, all the synthesized compounds were further screened for there in vitro α-amylase inhibition at 200 µg/mL. All compounds exhibited a linear rise in % inhibition.

Docking studies:

Interactions between inhibitors and active site of the target protein can be explored using molecular docking studies. The above results showed that all the synthesized molecules were stronger inhibitors of α-amylase as compared to acarbose. Therefore, for ascertaining the binding conformation and interactions responsible for the activity, docking simulation of compound 4a and 4d was performed against active site of pig pancreatic alpha-amylase (PDB ID: 3L2M). Ligands taken for the docking studies are shown in Figure 1a. Pig pancreatic alpha-amylase protein is considered as target protein for this study. Its structure was taken from RCSB Protein Data Bank (PDB) with PDB ID: 3L2M as shown in Figure 2.

Table 4: α-amylase inhibition activity of compounds 4a-e

| Compound | Concentration (µg/mL) | % Inhibition |
|----------|-----------------------|-------------|
| 4a       | 50                    | 70.84       |
|          | 100                   | 85.23       |
|          | 200                   | 86.84       |
|          | 50                    | 77.18       |
| 4b       | 100                   | 81.35       |
|          | 200                   | 83.64       |
|          | 50                    | 54.82       |
| 4c       | 100                   | 68.58       |
|          | 200                   | 73.34       |
|          | 50                    | 76.58       |
| 4d       | 100                   | 79.03       |
|          | 200                   | 77.84       |
|          | 50                    | 50.12       |
| 4e       | 100                   | 69.87       |
|          | 200                   | 71.93       |
|          | 50                    | 56.69       |
| Acarbose | 100                   | 63.85       |
|          | 200                   | 69.78       |

Table 5: Binding energy of docked compounds (4a-e)

| Compound | 4a | 4b | 4c | 4d | 4e | Co-ligand |
|----------|----|----|----|----|----|-----------|
| Binding energy | -8.9 | -8.9 | -8.3 | -8.7 | -8.5 | -7.8 |

Table 1: Binding energy of docked compounds (4a-e)

Docking energy obtained at 100 µg/mL concentration, all the synthesized compounds were further screened for there in vitro α-amylase inhibition at 200 µg/mL. All compounds exhibited a linear rise in % inhibition.
Figure 2: 3D Structure of X-ray crystallographic analysis of pig pancreatic alpha-amylase with alpha-cyclodextrin (PDB ID: 3L2M)

Figure 3: Binding of pig pancreatic alpha-amylase, with compound 4a

Figure 4: Binding of pig pancreatic alpha-amylase, with compound 4b

Figure 5: Binding of pig pancreatic alpha-amylase, with co-crystallized ligand
Target protein has its active sites where the compound shows maximum number of interaction with protein. The complete dataset was docked and found to bind at the same active site position. Amino acids are intimately involved in the binding ligand to protein and form a complex. The residue that is significant for binding interaction and thus comprising the binding pocket of target protein are shown in Table 4. Docking studies revealed that these amino acids present in the target proteins pocket involves in the binding interaction with the selected compounds.

These complex structures reveal essential interactions between the inhibitor and the protein and these interactions are taken as the reference for the hydrazine derivative (4a-e). The co-crystallized ligand are forms hydrogen bond interaction with the residues GLY 309, GLN 302, ARG 346, ASP316, ARG 267 (Figure 5) which are present within the ATP binding pocket. The ligand is also further stabilized by a number of hydrophobic contacts with the residues. The five hydrazine derivatives (4a-e) shown in Figure 1a were taken for docking studies. These compounds are synthesized and their structures have been determined by IR, 1H and 13C-NMR spectroscopy. The docking studies clearly reveal that some of these compounds bind efficiently to the enzymes of pig pancreatic alpha-amylase. Binding score of autodock 4.2 varies between -7.8 to -8.9 for compounds 3a-g tested for pig pancreatic alpha-amylase (Table 5) Out of the five hydrazine derivatives analyzed, compound 4b and 4d forms the best interaction with pig pancreatic alpha-amylase.

The compound 4a and 4d has the highest binding score of -8.9 and -8.7. The fluoride, oxygen atom on hydrazine compound forms hydrogen bond with the hydrogen atom of ALA 198, ARG 195, and HIS 299 of pig pancreatic alpha-amylase (Figure 3 and Table 6). Compound 4d having a binding score of -8.9 makes hydrogen bonds with the active site residue ASP 300, GLU 233, LYS200 and ILE 235 of enzyme (Figure 4). Re-docking of the inhibitor from the co-crystallized complex structure (Figure 5) of pig pancreatic alpha-amylase resulted in a binding score of -7.8, which is comparable to the scores found for compound 4b and 4d (Table 5). The re-docked conformation of co-crystallized ligand (Figure 2) resembles the conformation of the hydrazine derivative (compound 4b and 4d respectively).

Conclusion:
We describe the synthesis and evaluation of five hydrazine derivatives as α-amylase inhibitors. The structures of all synthesized compounds were confirmed by elemental and spectroscopic analysis (IR, 1H and 13C-NMR). The biological potential of synthesized compounds was investigated through in vitro α-amylase inhibition activity. The results showed that some of the synthesized compounds exhibited significant inhibitory activities. The compound 5-((1Z,3Z)-3-(benzo[d][1,3]dioxol-2-ylimino)prop-1-en-1-yl)-2-(difluoromethoxy) phenol (4b) in 100 μg/mL concentration showed remarkable inhibition of 81.35%. Docking studies of compound 4a-e were performed against active site of pig pancreatic alpha amylase (PDB ID: 3LM2). It has been revealed from docking studies that the binding interactions found between 4b and 4d with pig pancreatic α-amylase are similar to those responsible for α-amylase inhibition by acarbose.

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Table 6: Binding interactions of docked compounds

| Compound | Type of interaction | Between | Distance | Type of interaction | Between | Distance |
|----------|--------------------|---------|----------|--------------------|---------|----------|
| 4a       | Hydrogen bond      | NH-O (GLC 701) | 3.04      | Halogen bond       | F-O (GLU 233) | 2.99     |
| 4b       | Hydrogen bond      | NH-O (ALA 198) | 2.72      | Halogen bond       | F-O (GLU 233) | 2.99     |
| 4c       | Charge-transfer    | Halogen bond | 2.83      | Halogen bond       | F-O (GLU 233) | 2.99     |
| 4d       | Hydrogen bond      | NH-O (GLU 233) | 5.53      | Halogen bond       | F-O (GLU 233) | 2.99     |
| 4e       | Hydrogen bond      | NH-O (HIS 299) | 2.55      | Halogen bond       | F-O (GLU 233) | 2.99     |

Co-ligand

| Hydrogen bond | Distance | Donor -Donor | H-H (ARG 346) | 2.35 |

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