TO DESIGN AND SYNTHESIZED OXADIAZOLE DERIVATIVES FOR ANTI-DIABETIC ACTIVITY

(Pharmaceutical chemistry)

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Abstract

Background: Oxadiazoles are synthetic heterocyclic compounds with biological activity against variety of diseases such as diabetes, malignancy and inflammatory conditions. There is constant research going on these moieties that may lead to development and discovery of newer agents. Aim: The aim of present study was to design as well as synthesize oxadiazole derivatives with anti-diabetic activity. Materials and methods: IR spectra were recorded using JASCO FT/IR-4100 spectrophotometer while H NMR spectra were recorded on Bruker (400 MHz and 100 MHz) by using TMS (internal standard). Mass spectra were observed using a JEOL JMS-D300 spectrometer operated at 70 eV. Elemental analyses were conducted on Flash EA1112 series CHNS-O analyzer. UV-Visible spectrophotometer was used for measuring absorbance for calculating DPPH scavenging assay. The reaction completion was assessed by thin layer chromatography on silica gel-coated aluminium sheets (silica gel 60 F254, Merck). Commercial grade reagents were used for performing reaction process without purification. Reaction scheme used for synthesis of main compound involved formation of intermediate compound- “6-methylpyridine-3-carbohydrazide” that was obtained by 6-methyl 3-methyl nicotinate hydazinolysis which was obtained from 2-methyl-5-ethyl pyridine. Derivatives of 2-[3-(6-Methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole were synthesized from 6-methylpyridine-3-carbohydrazide by reaction with aromatic acids which were dissolved in POCl3. Results: Compounds 3d, 5a and 6a demonstrated good α-amylase inhibitory activity whereas compounds 3d and 5a showed good α–glucosidase inhibitory activity. Conclusion: Thus, it was proven from given study that among all synthesized compounds, 3d and 5a have good potential against diabetes and must be further studied.

Key-words: oxadiazoles, synthesis, derivation, anti-diabetic.
Introduction

Diabetes is a metabolic disease that is characterized by elevated blood glucose levels due to defects in production of insulin or activity of insulin or both of these. The type II diabetes or diabetes mellitus is the most rapidly increasing health threat all over the world. In 2000, total numbers of cases of diabetes were estimated to be around 171 million and this number is expected to increase further to 366 million cases by the year, 2030.\(^1\),\(^2\)

Oxadiazoles were first of all synthesized by Ainsworth in 1965 via hydrazine thermolysis. It has a molecular formula- \(\text{C}_2\text{H}_2\text{ON}_2\) with molecular mass measuring 70.05 g/ml and is water-soluble. These are thermally stable molecules with 167.4 kJ/ml of resonance energy. There are 4 different isomers of oxadiazole- a) 1,2,3-oxadiazole; b) 1,3,4-oxadiazole; c) 1,2,5-oxadiazole and d) 1,2,4-oxadiazole based upon different position of nitrogen atom.

Oxadiazoles are 5-membered heterocyclic compounds containing 2 nitrogen and 1 oxygen atoms, 2 carbon atoms and 2 double bonds.\(^4\) These are synthesized by means of rearrangement and ring condensation.\(^5\) Oxazol-5-one contain various reactive sites that allows for various modifications. The oxazolone derivatives have agonistic activity which is exhibited by binding with the peroxisome proliferator-activated receptor-\(\gamma\) (PPAR-\(\gamma\)) that demonstrates preferential binding with DNA by activation of transcription of various regulators of metabolism which increase expressivity of variety of insulin-responsive genes that are involved in regulating metabolism of both glucose and lipid. A good concentration has been demonstrated between anti-hyperglycaemia and affinity of PPAR-\(\gamma\). This causes an increase in transport of glucose within muscles and adipocytic tissues by enhancement of synthesis as well as translocation of specific types of glucose transporters.\(^1\)

The 1,3,4-oxadiazoles and their derivatives are said to possess anti-inflammatory, anti-bacterial, anti-fungal, analgesic and anticancerous properties.\(^6\) 1,3,4-oxadiazoles may be synthesized by aldehyde and aroylhydrazone condensation with the presence of acetic anhydride or by condensation of alkyl hydrazides with a substituted aromatic acid with \(\text{POCl}_3\) as an intermediary compound. The synthesized O-3 to O-13 compounds were assessed
for alloxan-induced type II diabetes. O-13 molecules demonstrated maximal decrease in serum glucose levels on 14\textsuperscript{th} day with good efficacy against diabetes. \textsuperscript{6} 1,3,4-oxadiazole may be derived from furan by substituting 2 methylene (-CH) groups with 2 pyridine type nitrogen (-N=). Agents that contain 1,3,4-oxadiazole and are currently being used in clinical medicine include- Zibotentan, an anti-cancer drug and Raltegravir, an anti-cancer agent.\textsuperscript{7} Various Oxadiazoles moieties have been used as anti-cancer, anti-microbial, anti-inflammatory, anti-convulsant, anti-oxidant and anti-HIV agents.\textsuperscript{8} 1,3,4-oxadiazoles demonstrate variety of biological functions like-P-glycoprotein inhibitors, anti-epileptic agent and as analgesic medicaments.\textsuperscript{9}

These heterocyclic compounds, specially which comprise of nitrogen are used as active biological agents. The azole group contains 1,3,4-thiazole nucleus which is a pharcophore with great versatility. Apart from this, there are three isomers- 1,2,3-thiadiazole, 1,2,4-thiadiazole and 1,2,5-thiadiazole. The thiaiazole cores are used as bioisostere for synthesizing other heterocyclic molecules by substituting oxygen by sulphur atom that can increase its biological function as well as increase their lipophilicity.\textsuperscript{10}

Oxadiazoles have demonstrated physiological activity as ligands of dopamine receptor, secretogogues for growth hormones, anti-spasmodic agent, anti-inflammatory and anti-thrombosis agents and partial agonists for benzodiazepine receptor. Oxadiazole synthesis involves reaction between acrylnitrile and hydroxyalanine as well as dehydrative cyclization of an O-acyl amidoxine. 1,2,4-oxadiazoles may be synthesized from derivatives of amidoxine and carboxylic acids by means of a one-pot reaction.\textsuperscript{11} The aim of the present study was to design and synthesize Oxadiazole derivatives for anti-diabetic activity.

**Materials and methods**

**Armamentarium used:**

Melting points of all compounds were estimated by open-capillary method. The IR spectra (in KBr pellets) were recorded using JASCO FT/IR-4100 spectrophotometer. H NMR spectra were recorded on a Bruker (400 MHz and 100 MHz) using TMS as internal standard. Mass spectra were observed using JEOL JMS-D 300
spectrometer which was operated at 70 eV. Elemental analyses were done on Flash EA 1112 series CHNS-O analyzer. UV-Visible spectrophotometer (Shimadzu UV-1800, Japan) was used for measuring absorbance for calculating the DPPH scavenging assay. The reaction completion was assessed by thin layer chromatography (TLC) on silica gel-coated aluminium sheets (silica gel 60 F254, Merck. Commercial grade solvents and reagents were used for performing reaction process without purification.

**Experiment**

The reaction scheme used for synthesizing main compound involved formation of intermediate compound- “6-methylpyridine-3-carbohydrazide” which was obtained by hydazinolysis of 6-methyl 3-methyl nicotinate which was obtained from 2-methyl-5-ethyl pyridine. 2-[3-(6-Methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole derivatives were synthesized from 6-methylpyridine-3-carbohydrazide by reacting with various aromatic acids dissolved in POCl3. 6-Methylpyridine-3-carbohydrazide on being refluxed with CS2 in alkaline solution of ethanol produced 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione. This compound on performing Mannich reaction with selective secondary amines produced corresponding Mannich bases. 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione on performing condensation reaction with different benzyl halides resulted in formation of 2-[3-(6-methylpyridinyl)]-5-substituted benzylthio-[1,3,4]-oxadiazole. The structures of all synthesized compounds were then established by IR, NMR, mass spectral along with elemental analysis.

**Synthesis of 6-methylpyridine-3-carbohydrazide:**

6-Methyl-3-methyl nicotinate was synthesized from 2-methyl 5-ethyl pyridine as follows:

6-Methyl-3-methyl nicotinate (0.05 m mol) and 99% hydrazine hydrate (0.05 m mol) were dissolved within ethanol (10 mL). The resultant solution was then refluxed for up to 2 hours. The resultant reaction product was then reduced to half of total volume and was then allowed cooling. The obtained solid mass was filtered followed by washing with minimal amount of chilled ethanol and was then, dried. Total yield of reaction product was 90%.

**Procedure used for synthesis of 2-[3-(6-methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole:**

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6-Methylpyidine-3-carbohydrazide (0.006 mol) was refluxed with 0.006 mol substituted aromatic acids in phosphorous oxy chloride (5millilitres) for a duration of 8 to 10 hours. The obtained reaction mixture was then slowly quenched with ice water and was then neutralized with sodium bicarbonate solution. The separated solid separated was then filtered, washed using water and was then dried. The final compound was re-crystallized from ethyl acetate.

**Procedure for synthesis of 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione:**

0.02 mol 6-Methylpyidine-3-carbohydrazide (3.0 g) was treated with potassium hydroxide (0.04 mol) solution which was dissolved in 50 millilitres of ethanol with constant stirring. 3.0 g of Carbon disulfide (0.04 mol) was then slowly added to reaction mixture which was then slowly heated and was refluxed for duration of 8 hours. The resultant solvent was then distilled under conditions of vacuum and resultant residue was then dissolved in water. The resulting solution was then acidified using acetic acid and was resultant solid was then collected using filtration with 80% yield. Final product was a cream colored powder with melting point ranging between 224 to 226 °C.

**General procedure for synthesizing Mannich bases:**

1.0 g of 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione (0.006 mol) in 5mL ethanol was mixed with 0.5 ml of 37% formaldehyde and 0.006 mol of amine. The reaction mixture was continuously stirred over night and following cooling, the obtained precipitate was then filtered and subsequently, crystallized using ethanol.

**General procedure for the synthesis of 2-[3-(6-methylpyridinyl)]-5-substitutedbenzylthio-[1,3,4]-oxadiazole:**

2.0 g of 0.013 mol 2-[3-(6-Methylpyridinyl)]-1,3,4-oxadiazole-5-thione was dissolved in 10 mL of DMF and 0.019 mol potassium carbonate was then added. Substituted 0.013 mol benzyl halide was then added and was then heated to 80°C for duration of 2 hours. The resultant mixture was then cooled and was quenched with water. The obtained solid product was then filtered and crystallized from ethanol.

**Anti-diabetic studies**

**Assay method for α-Amylase inhibition:**

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The α-amylase inhibitory activity of newly synthesised compounds was performed as under-

0.5% w/v of starch solution was obtained by boiling and stirring 0.25 grams potato starch in 50 ml of de-ionized water for duration of 15 minutes. 0.5 unit/ml of enzyme solution was prepared by dissolving 0.001 g α-amylase in 100 ml of 20 mM sodium phosphate buffer (with pH= 6.9) which contained 6.7 mM sodium chloride. The synthesised products were then dissolved in DMSO to obtain following concentrations- 25, 50, 75 and 100 μg/ml. 1 ml of each concentration of synthesized compounds and 1 ml of enzymatic solution were then mixed in a test-tube followed by incubation at 25 ºC for duration of 30 minutes. 1 ml of prepared solution was then mixed with 1 ml starch solution and tube incubated at 25 ºC for 3 minutes. 1 ml of color reagent which was solution containing 20 ml of 96 mM 3,5-dinitrosalicylic acid, 8 ml of 5.31 M sodium potassium tartrate in 2 M sodium hydroxide and 12 ml of deionized water. The tube was then placed into a water-bath maintained at temperature of 85ºC. After 15 minutes, the reaction mixture was then removed from water bath and was then cooled, followed by dilution with 9 ml of distilled water.

The absorbance value was then measured at a wavelength of 540 nm using a spectrophotometer. Blanks were prepared for correction of any absorbance in background. The color reagent solution was then added before adding starch solution in a test tube which was then placed into a water-bath.

Control studies were carried out in a similar fashion by replacing compound solutions with 1 ml of DMSO. Acarbose solution (at equal concentrations as that of the synthesised compounds) was used as a ‘positive’ control. The inhibitory effects of synthesized organic compounds was then compared with standard salivary α-amylase inhibitor ‘acarbose’. The α-amylase inhibitory activity was expressed as ‘percentage(% age) inhibition.

\[ \% \text{ inhibition} = \frac{(\Delta A_{\text{control}} - \Delta A_{\text{sample}})}{\Delta A_{\text{control}}} \times 100 \]

Where, \( A_{\text{sample}} \) = Absorbance of the test sample and \( A_{\text{control}} \) = Absorbance of the control

b) α-Glucosidase inhibition assay

α-Glucosidase activity was determined as described below-
Appropriate dilutions of synthesized compounds (0 to 200 μL) and 100 μL of α-glucosidase solution (1.0 U/mL) prepared in 0.1 mol/L of phosphate buffer with pH= 6.9 were incubated at 25 °C for 10 minutes. Following this, 50 μL 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/L of phosphate buffer (pH 6.9) was subsequently added. All mixtures were then incubated at a temperature of 25 °C for duration of 5 minutes, before recording the absorbance at 405 nm in spectrophotometer. α-glucosidase inhibitory activity was expressed as “percentage inhibition (table 1).

\[
\text{% Inhibition} = \left(\frac{\text{AbsControl} - \text{AbsSamples}}{\text{AbsControl}}\right) \times 100
\]

**Results**

2-[3-(6-Methylpyridinyl)]-5-(3,4-diflouro phenyl)-1,3,4-oxadiazole (3a):

White micro-crystals were obtained and assessed with IR (KBr) γ/cm⁻¹: 2927 (Ar C-H), 1584 (C=N), 1352 (C-O-C); 1H NMR (CDCl₃, 400MHz): δ 2.70 (s, 3H, CH₃), 7.24 (d, 1H, pyridine ring 5H), 7.49 (m, 1H, Ar-H), 7.89-8.02 (m, 2H, Ar-H), 8.28 (dd, 1H, pyridine 4H), 9.45 (s, 1H, pyridine 2H); 13C NMR (CDCl₃, 100MHz): δ 24.56, 114.21, 113.21, 113.42, 114.32, 114.32, 117.09, 128.67, 125.45, 135.36, 149.57, 169.82, 165.12; LC-MS (m/z): 274 (M++ + 1, 100%), (M.F: C₁₄H₉F₂N₃O).

2-[3-(6-Methylpyridinyl)]-5-(4-thiomethylbenzyl)-1,3,4-oxadiazole (3b):

Brown micro-crystals were obtained; on assessing using IR (KBr) γ/cm⁻¹: 2940 (Ar C-H), 1623 (C=N), 1329 (C-O-C); 1H NMR (CDCl₃, 400MHz): δ 2.67 (s, 3H, SCH₃), 2.68 (s, 3H, CH₃), 4.59 (s, 2H, CH₂), 7.34 (d, 1H, pyridine ring 5H), 7.45 (d, 2H, Ar-H), 7.87-8.02 (m, 2H, Ar-H), 8.28 (dd, 1H, pyridine 4H), 9.34 (s, 1H, pyridine 2H); LC-MS (m/z): 299 (M++ 1, 100%), (M.F:C₁₆H₁₅N₃OS).

2-[3-(6-Methylpyridinyl)]-5-(3,4-dinitrophenyl)-1,3,4-oxadiazole (3c):

The final reaction product was seen as brown micro-crystals which were assessed as under- IR (KBr) γ/cm⁻¹: 2967 (Ar C-H), 1647 (C=N), 1334 (C-O-C); 1H NMR (CDCl₃, 400MHz): δ 2.67 (s, 3H, CH₃), 7.75 (d, 1H, pyridine ring 5H), 8.35 (d, 1H, pyridine 4H), 9.19-9.30 (m 4H, pyridine 2H and Ar-H); LC-MS (m/z): 330 (M+ + 1, 100%), (M.F: C₁₄H₉N₅O₅).

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2-[3-(6-Methylpyridinyl)-5-(2,3-dichlorophenyl)-1,3,4-oxadiazole (3d):

The reaction product was obtained as brown micro-crystals which was analyzed using IR (KBr) $\gamma$/cm-1: 2943 (Ar C-H), 1610 (C=N), 1323 (C-O-C); 1H NMR (CDCl3, 400MHz): $\delta$2.78 (s, 3H, CH3), 7.49 (d, 1H, pyridine ring 5H), 7.54-8.02 (m, 3H, Ar-H ), 8.32 (d, 1H, pyridine 4H), 9.20 (s, 1H, pyridine 2H); LC-MS (m/z): 304 (M++ 1, 100%), (M.F:C14H9Cl2N3O).

2-[3-(6-Methylpyridinyl)-5-(3-chloro-2-fluorophenyl)-1,3,4-oxadiazole (3e):

The reaction product was obtained as white micro-crystals which were assessed using IR (KBr) $\gamma$/cm-1: 2923 Ar C-H, 1592 (C=N), 1340 (C-O-C); 1H NMR (CDCl3, 400MHz): $\delta$ 2.32 (s, 3H, CH3), 7.29 - 7.69 (m, 4H, Ar-H & pyridine 5H), 8.19 (d, 1H, pyridine 4H), 9.25 (s, 1H, pyridine 2H); LC-MS (m/z): 292 (M+ + 1, 100%), (M.F: C14H9ClFN3O).

2-[3-(6-Methylpyridinyl)]-4-(piperidin-1-ylmethyl)-1,3,4-oxadiazole-5-thione (5a):

80% yield of reaction product with melting point ranging between 156 to 158 ºC was obtained. Product analysis using IR (KBr) $\gamma$/cm-1 : 2933 (C-H), 1614 (C=N), 1294 (C=S), 1010 (C-O); 1H NMR (CDCl3, 400MHz): $\delta$ 1.39 (m, 2H, CH2), 1.56 (m, 4H, CH2), 2.67 (s, 3H, CH3), 2.82(m, 4H, CH2), 5.02(s, 2H, NCH2-N) 7.28 (d, 1H, pyridine 5H), 8.07 (d, 1H, pyridine 4H), 9.02 (s, 1H, pyridine 2H); 13C NMR (CDCl3, 100MHz): $\delta$ 23.34, 24.56, 25.43, 51.59, 71.56, 113.82, 121.39, 132.45, 143.02, 153.12, 160.52, 172.07; DEPT: CH and CH3 $\delta$: 22.32, 23.46, 24.67, 56.53, 70.56, 121.45, 132.87, 147.09; LC-MS (m/z): 291 (M+ + 1, 100% ), (M.F:C14H18N4OS).

2-[3-(6-Methylpyridinyl)]-4-(morpholin-4-ylmethyl)-1,3,4-oxadiazole-5-thione (5b):

72 % reaction product yield with melting point ranging between 143-149 ºC which were assessed with IR (KBr) $\gamma$/cm-1 : 2849 (C-H), 1618 (C=N), 1297 (C=S), 1004 (C-O); 1H NMR (CDCl3, 400MHz): $\delta$ 2.56 (s, 3H, CH3), 2.56 (m, 4H, CH2), 3.68 (m, 4H, CH2) 5.02 (s, 2H, N-CH2-N) ,7.21 (d, 1H, pyridine 5H), 8.02 (d, 1H, pyridine 4H), 9.01 (s, 1H, pyridine 2H); LC-MS (m/z): 290 (M+ + 1, 100%), (M.F: C13H16N4O2S).

2-[3-(6-Methylpyridinyl)]-4-[(4-methylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-5-thione (5c):

73 % product yield with melting point ranged between 160 to 164ºC; IR (KBr) $\gamma$/cm-1 : 2895 (C-H), 1625 (C-N), 1289 (C=S), 1015 (C-O); 1H NMR (CDCl3, 400MHz): $\delta$ 2.43 (s, 3H, CH3) 2.60 (s, 3H, CH3), 2.78 (m,
4H, CH2), 2.85(m, 4H, CH2) 5.13 (s, 2H, N-CH2-N), 7.36 (d, 1H, pyridine 5H), 8.12 (d, 1H, pyridine 4H), 9.10 (s, 1H, pyridine 2H); LC-MS (m/z): 306 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)-4-(1H-imidazol-1-yl methyl)-1,3,4-oxadiazole-5-thione (5d):]
70% final product yield with melting point ranged between 234 to 236 ºC analyzed with IR (KBr) γ/cm-1 : 2893 (C-H), 1619 (C=N), 1223 (C=S), 1001 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.59 (s, 3H, CH3), 5.13 (s, 2H, N-CH2-N), 7.09 (s, 1H, Imidazole proton), 7.29 (d, 1H, pyridine 5H), 7.69 (m, 2H, Imidazole proton), 8.06 (d, 1H, pyridine 4H), 9.03 (s, 1H, pyridine 2H); LC-MS (m/z): 272 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)-4-[(1H-2-methylimidazol-1-yl)methyl]-1,3,4-oxadiazole-5-thione (5e):]
75% final product yield with melting point ranged between 240 to 242 ºC which were analyzed at IR (KBr) γ/cm-1 : 2824 (C-H), 1612 (C=N), 1292 (C=S), 1002 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.37 (s, 3H, CH3), 2.52 (s, 3H, CH3), 5.08 (s, 2H, N-CH2-N), 7.0 (m, 2H, Imidazole proton), 7.21 (d, 1H, pyridine 5H), 8.08 (d, 1H, pyridine 4H), 9.01 (s, 1H, pyridine 2H); LC-MS (m/z): 282 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)-5-[(2,4-dichlorobenzyl)thio]-1,3,4-oxadiazole (6a):]
78 % product yield was obtained. The final product had melting point ranging between 82 to 84 ºC which was analyzed at IR (KBr) γ/cm-1 : 2319 (C-H), 1601 (C=N), 1183 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.34 (s, 3H, CH3), 4.59 (s, 2H, S-CH2), 7.32-7.42 (m, 4H, pyridine 5H and ArH), 8.12 (d, 1H, pyridine 4H), 8.68(s, 1H, pyridine 2H); LC-MS (m/z): 350 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)-5-[(4-nitrobenzyl)thio]-1,3,4-oxadiazole (6b):]
82% product yield with melting point ranged between 118 to 120 ºC which was assessed at IR (KBr) γ/cm-1 : 2912 (C-H), 1603 (C=N), 1145 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.53 (s, 3H, CH3), 4.59 (s, 2H, S-CH2), 7.12 (d, 1H, pyridine 5H), 7.43-7.53 (m, 4H, ArH), 8.15 (d, 1H, pyridine 4H), 9.01 (s, 1H, pyridine 2H); LC-MS (m/z): 323 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)-5-[(3-methylbenzyl)thio]-1,3,4-oxadiazol (6c):]
72 % product yield with melting point ranging between 90 to 92 ºC with IR (KBr) γ/cm-1 : 2912 (C-H), 1603 (C=N), 1071 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.29 (s, 3H, CH3), 2.34 (s, 3H, CH3), 4.48 (s, 2H, S-CH2),

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7.09 (d, 1H, pyridine 5H), 7.21 -7.29 (d, 4H, ArH), 8.14 (d, 1H, pyridine 4H), 9.02 (s, 1H, pyridine 2H); 13C NMR (CDCl3, 100MHz): δ 20.29, 23.52, 32.46, 116.28, 121.23, 122.13, 124.59, 124.76, 123.72, 131.02, 132.12, 131.23, 144.02, 162.98, 161.33, 164.44; DEPT: CH and CH3 δ: 21.32, 22.45, 32.34, 122.52, 122.29, 125.69, 126.03, 125.34, 132.84; LC-MS (m/z): 245 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-(benzthio)-[1,3,4]-oxadiazole (6d):
84% product yield with melting point ranged between 84 to 86 ºC analyzed at IR (KBr) γ/cm-1 : 2922 (C-H), 1608 (C=N), 1179 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.45 (s, 3H, CH3), 4.45 (s, 2H, S-CH2), 7.24-7.37 (m, 4H, pyridine 5H and ArH), 7.43-7.45 (d, 2H, J=7.1, ArH), 8.12 (d, 1H, pyridine 4H), 9.03 (s, 1H, pyridine 2H); LC-MS (m/z): 323 (M+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-[(6-chloro-2-trifluoromethylbenzyl)thio]-[1,3,4]-oxadiazol (6e):
68% product yield with melting point ranged between 96 to 98 ºC which were analyzed at IR (KBr) γ/cm-1 : 29129 (C-H), 1613 (C=N), 1168 (C-O); 1H NMR (CDCl3, 400MHz): δ 2.45 (s, 3H, CH3), 4.45 (s, 2H, S-CH2), 7.23-7.34 (m, 4H, pyridine 5H and ArH), 8.07 (d, 1H, pyridine 4H), 9.05 (s, 1H, pyridine 2H); LC-MS (m/z): 383 (M+ + 1, 100%).

Formation of the final compounds: 3a-e, 5a-e and 6a-e were confirmed by recording their IR, NMR and mass spectra. All the compounds were characterized after recrystallization from appropriate solvents. Formation of 2-[3-(6-methylpyridinyl)]-5-(3,4-difluorophenyl)-1,3,4-oxadiazole (3a) was confirmed by presence of peak of absorption at 1599 cm-1 in IR spectrum due to C=N stretching and 1081 cm-1 due to C-O . The 1H NMR spectrum of compound 3a showed singlet at δ 2.69 which was due to pyridine –CH3. The C-5 proton of oxadiazole appeared at δ 7.28 as singlet. The aromatic protons of phenyl ring resonated at 7.28 as a singlet and at 7.92-8.01 as a multiplet. The two pyridine ring –CH appears as a doublet at δ 7.37 and δ 8.32. The proton at the pyridine C-2 appeared as a singlet at δ 9.23. 13C NMR spectrum of 3a showed signals at δ 24.78 due to pyridine ring attached methyl carbon. Signals at δ 118.59, δ 123.76, δ 134.47, δ 147.37 and δ 162.43 corresponds to C-3, C-5, C-4 C-2 and C-6 of pyridine ring respectively. Signals observed at δ 116.33, δ 116.52, δ 117.35, 118.41,
123.54 and δ 123.76 are due to phenyl ring carbons. The mass spectrum of 3a showed molecular ion peak at m/z = 274 (M+1) which was in agreement with the molecular formula C14H9F2N3O.

In case of the Mannich base prepared from 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione 5a, the –CH2 protons of piperidine ring resonated at δ 1.42, δ 1.60 and δ 2.81 as multiplets. The –CH2 in the middle of oxadiazole ring and piperidine ring appeared as singlet at 5.06. The methyl carbon of pyridine ring appeared at δ 24.79 in 13C NMR.

The –CH2 carbons resonated at δ 23.60, δ 25.85, δ 51.61 and δ 71.68. The values are further confirmed through DEPT spectrum of the compound. The mass spectrum of 5a showed molecular ion peak at m/z = 291 (M+1) which was in agreement with the molecular formula C14H18N4OS.

The mass spectrum of 5a showed molecular ion peak at m/z = 298 (M+ + 1) which was in agreement with the molecular formula, C16H15N3OS.

Most significant anti-diabetic properties were studied by means of analyzing the inhibitory activities against α-amylase and α–Glucosidase enzymes. It was found that synthesized compounds: 3d, 5a and 6a demonstrated excellent α-amylase inhibitory activity while compounds: 3d and 5a demonstrated good α–glucosidase inhibitory activity when compared against standard (blank).

**Discussion**

Compounds 3d, 5a and 6a demonstrated good α-amylase inhibitory activity when compared against standard (table 1). Compounds 3d and 5a demonstrated good α–glucosidase inhibitory activity when compared against standard (table 2). Thus, synthesized compounds- 3d and 5a showed potential usage against anti-diabetic activity.

In support to our study, Bhutani et al (2019) reported moderate to excellent levels of anti-hyperglycaemic function 6 hours after administration of synthesized benzothiaole-1,3,4-oxadiazole-4-thiazolidinone analogues-Tz21, Tz17 and Tz10. Similarly, Selvaraj et al in 2019 in their observational study reported anti-diabetic functioning against α-amylase assays.12
Selvaraj et al (2017) synthesized series of 3-(5-cyclohexyl-1,3,4-oxadiazole-2-yl)-N-substituted aniline using multi-step reaction while using the benzohydride as starting compound. All the synthetic compounds were analyzed for their activities against diabetes, inflammation and cancers. Most of the synthesized compounds showed good to moderate anti-diabetic activity against diabetes by demonstrating inhibitory effects on α-amylase enzymatic activity.  

Srinivas et al (2013) prepared various derivatives of 3-[2-(methylamino)methyl]-5-{{2-phenylquinazolin-4-yl}oxy}methyl]-1,3,4-oxadiazole-3(3H)-thione and assessed their inhibitory activity on GSK-3β. Compounds 2, 3 and 4 of oxadiazole-quinazoline series were shown to exhibit excellent hypoglycaemic effects.  

Iqbal et al (2012) synthesized few novel derivatives of thiazolidinone by incorporation of oxadiazole moiety. These synthesized compounds were then evaluated under in vivo conditions for testing their hypoglycaemic activity. The compound ‘7’ exhibited good anti-hypoglycaemic activity.  

Kun et al (2011) synthesized derivatives of 2-(β-D-glucopyranyl)-5-(4,hydroxymethyl-1,2,3-trazole-1-ylmethyl)-1,3,4-oxadiazole and assays were performed against the rabbit muscle glycogen phosphorylase b. 2-phenyl-5-[1-(β-D-glucopyranosyl)-1,2,3-traizol-4-yl]-1,3,4-oxadiazole was observed to have best anti-hyperglycemic inhibitory activity.  

**Conclusion**

1,3,4-oxadiazole is an important scaffold that has shown good anti-diabetic activity. According to current study, synthesized oxadiazole derivatives- 3d and 5a have demonstrated favorable results for anti-diabetic activity. Further, modifications in structure of basic scaffold may result in discovery of a novel drug candidate.

Table 1: Table illustrating α-amylase inhibitory activity of synthesized compounds 3a-e, 5a-e and 6a-e under in vitro conditions

<table>
<thead>
<tr>
<th>Synthesized compounds</th>
<th>% inhibition</th>
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<table>
<thead>
<tr>
<th>Compound</th>
<th>Activity</th>
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<tbody>
<tr>
<td>3a</td>
<td>20.33</td>
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<tr>
<td>3b</td>
<td>46.95</td>
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<tr>
<td>3c</td>
<td>57.14</td>
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<tr>
<td>3d</td>
<td>75.39</td>
</tr>
<tr>
<td>3e</td>
<td>43.85</td>
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<tr>
<td>5a</td>
<td>70.76</td>
</tr>
<tr>
<td>5b</td>
<td>22.54</td>
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<tr>
<td>5c</td>
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<td>5d</td>
<td>19.39</td>
</tr>
<tr>
<td>5e</td>
<td>24.35</td>
</tr>
<tr>
<td>6a</td>
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<tr>
<td>6b</td>
<td>35.19</td>
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<tr>
<td>6c</td>
<td>-</td>
</tr>
<tr>
<td>6d</td>
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</tr>
<tr>
<td>6e</td>
<td>56.89</td>
</tr>
<tr>
<td>Acarbose</td>
<td>89.79</td>
</tr>
</tbody>
</table>

**Graph 1:** Graph showing α-amylase inhibitory activity of synthesized compounds 3a-e, 5a-e and 6a-e.
Table 2: α-Glucosidase inhibition assay of compounds 3a-e, 5a-e and 6a-e

<table>
<thead>
<tr>
<th>Synthesized compounds</th>
<th>% inhibition</th>
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<tbody>
<tr>
<td>3a</td>
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<tr>
<td>3b</td>
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<tr>
<td>3c</td>
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<tr>
<td>3d</td>
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<tr>
<td>3e</td>
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<td>5a</td>
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<td>5b</td>
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<tr>
<td>5c</td>
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<tr>
<td>Compound</td>
<td>Activity (%)</td>
</tr>
<tr>
<td>----------</td>
<td>--------------</td>
</tr>
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</table>

Graph 2: Graph illustrating α–Glucosidase inhibition assay of compounds: 3a-e, 5a-e and 6a-e
References


5. Zhang MZ, Mulholland M, Bzattie D. Synthesis and antifungal activity of 3-(1,3,4-oxadiazole-5-yl)-indoles and 3-(1,3,4-oxadiazole-5-yl) methyl indoles. Eur J Med Chem 2013;63:22-32.


15. Kun S, Nagy GZ, Toth M, czece L, Nbien ANV, Docsat T et al. Synthesis of variously coupled conjugates of D-glucose, 1,3,4-oxadiazole and 1,2,3-triazole for inhibition of glycogen