Available online at www.derpharmachemica.com

Scholars Research Library

Der Pharma Chemica, 2015, 7(12):137-145
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Synthesis, characterization, antidiabetic and antioxidant activity of 1,3,4-oxadiazole derivatives bearing 6-methyl pyridine moiety

Shyma P. C.[a,b], Balakrishna Kalluraya[a,*], Peethambar S. K.[c] and Vijesh A. M.[d]

[a]*Department of Studies in Chemistry, Mangalore University, Mangalagangothri, Karnataka, India*
[b]*SeQuent Scientific Limited, No:120 A&B, Industrial Area, Baikampady, New Mangalore, Karnataka, India*
[c]*Department of Bio-Chemistry, Jnanasahyadri, Kuvempu University, Shankaraghatta, Karnataka, India*
[d]*Department of Chemistry, Payyanur College, Payyanur, Edat (PO), Kannur, Kerala, India*

ABSTRACT

*Three new series of 1,3,4-oxadiazole derivatives such as 2-[3-(6-methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole **(3a-e)**, 2-[3-(6-methylpyridinyl)]-4-substitutedaminomethyl-[1,3,4]-oxadiazole-5-thione **(5a-e)** and 2-[3-(6-methyl pyridinyl)]-5-substituted benzylthio-[1,3,4]-oxadiazole **(6a-e)** were synthesized. Newly synthesized compounds were characterized by spectral and analytical studies. All the synthesized compounds were screened for their anti-diabetic and antioxidant activity. The Mannich bases **(5a-e)** showed significant antioxidant activity comparable with that of the standard employed. Compounds **3d** and **5a** are showing good α-amylase inhibition activity and α-glucosidase inhibition activity against the standard.*

Key words: 1,3,4-Oxadiazole, Mannich bases, Antioxidant studies, Alpha amylase inhibition, Alpha glucosidase inhibition.

INTRODUCTION

Amylases (α-1,4-glucan-4-glucanohydrolase, EC3.2.1.1) are a group of enzymes which catalyze the hydrolysis of the (α-1,4) glycosidic linkages in starch and various other oligosaccharides[1]. Alpha-glucosidase inhibitors (AGIs; acarbose, miglitol, voglibose) are widely used in the treatment of patients with type 2 diabetes. AGIs delay the absorption of carbohydrates from the small intestine and thus have a lowering effect on postprandial blood glucose and insulin levels.

Enzyme inhibitors can be a potential target in many areas of disease control and treatment, as enzymes catalyse the most important biochemical pathways. Controlled carbohydrate digestion and monosaccharide absorption could be of great value in the avoidance of conditions such as diabetes, obesity, hyperlipoproteinaemia and hyperlipidaemia. In this aspect, amylase and glucosidase inhibitors are of particular importance [2]. Most of the commercially available amylase and glucosidase inhibitors are of microbial origin. Though these drugs help in maintaining constant level of glucose in blood by delaying the breakdown of starches, but however their usage has been limited due to their side-effects. Thus the search for novel anti-diabetic agents is on-going.

1,3,4-Oxadiazoles have occupied a unique place in the field of medicinal chemistry due to its wide range of biological activities. 1,3,4-Oxadizole moiety and its various derivatives are studied frequently and found to possess potent pharmacological activity. The 1,3,4-oxadiazole derivatives have been reported to have various biological activities such as antimicrobial [3,4,5], anti-inflammatory [6], , anti-tubercular [7], antioxidant[8], anticancer [9], anticonvulsant [10], antidiabetic [11] and analgesic[12]. It is observed that the structural modification of oxadiazoles by preparing Mannich bases or condensing with alkyl or aryl halides have yielded better results in terms of

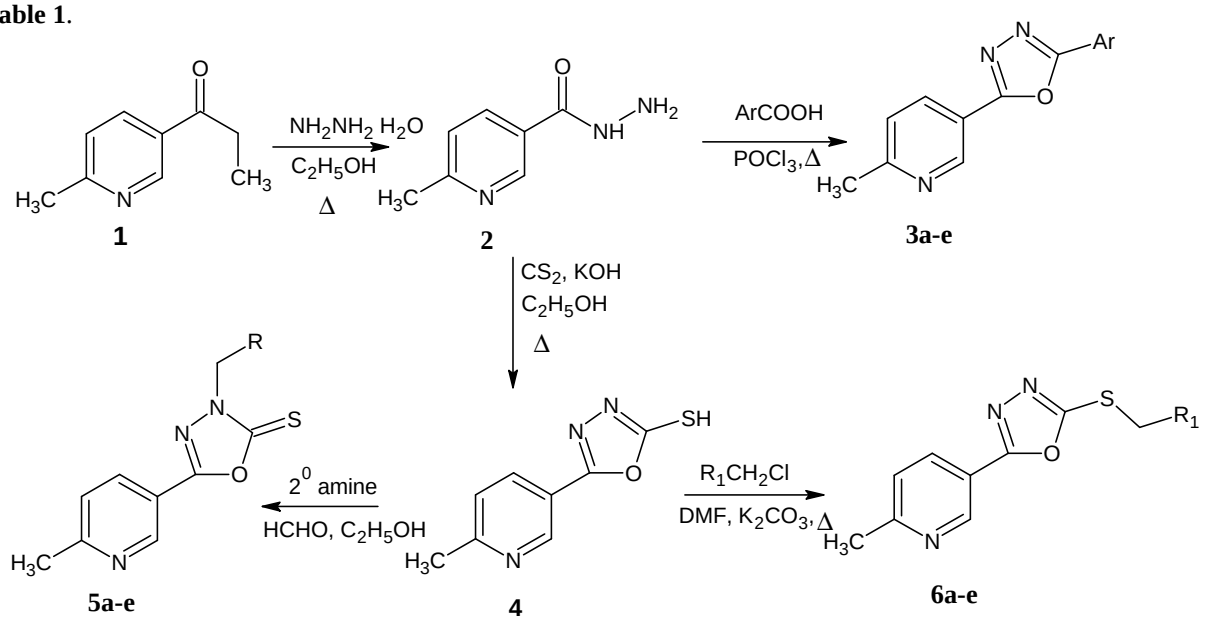
biological activities than the unsubstituted 1,3,4-oxadiazole. Similarly the current literature reveals that pyridine derivatives exhibit significant biological activities [13-15]. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyridine substituents.

MATERIALS AND METHODS

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a JASCO FT/IR-4100 spectrophotometer. ^{1}H NMR spectra were recorded ($CDCl_3$) on a Bruker (400 MHz and 100 MHz) using TMS as internal standard. Chemical shift values are given in δ (ppm) scales. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Elemental analyses were performed on a Flash EA 1112 series CHNS-O analyser. UV-Visible spectrophotometer, Shimadzu UV-1800, Japan was used to measure the absorbance to calculate the DPPH scavenging assay. The completion of the reaction was checked by thin layer chromatography (TLC) on silica gel coated aluminium sheets (silica gel 60 F254) obtained from Merck. Commercial grade solvents and reagents were used without further purification.

Experimental

The reaction scheme employed for the synthesis of title compound is given in Scheme-1. The key intermediate, 6-methylpyridine-3-carbohydrazide **(2)** was obtained by the hydazinolysis of 6-methyl 3-methyl nicotinate **(1)** which in turn was obtained from 2-methyl-5-ethyl pyridine [16]. 2-[3-(6-Methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole **(3a-e)** derivatives were synthesized from 6-methylpyridine-3-carbohydrazide by reacting it with different aromatic acids in $POCl_3$. 6-Methylpyridine-3-carbohydrazide on refluxing with CS_2 in strong alkaline ethanol yielded 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione **(4)** which on Mannich reaction with selected secondary amines yielded the corresponding Mannich bases **(5a-e)**. Further 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione **(4)** on condensation with different benzyl halides gave the 2-[3-(6-methylpyridinyl)]-5-substituted benzylthio-[1,3,4]-oxadiazole **(6a-e)**. The structures of the newly synthesized compounds (**3a-e**, **5a-e** and **6a-e**) were established by IR, NMR, mass spectral and elemental analysis. The characterisation data of the synthesised compounds is given in **Table 1**.



Where Ar= 3,4-Diflourophenyl, 4-Thiomethylbenzyl, 3,4-Dinitrophenyl, 2,3-Dichlorophenyl, 3-Chloro-2-flourophenyl
R= Piperidinyl, Morpholinyl, N-Methyl piperazinyl, Imidazolyl, 2-methyl , Imidazolyl
R_1= 2,4-Dichloro benzyl, 4-Nitro benzyl,3-Methyl benzyl, 6-Chloro-2-triflouromethyl benzyl

Scheme 1: Synthetic route for the compounds 3a-e, 5a-e and 6a-e

Synthesis of 6-methylpyridine-3-carbohydrazide (3)

6-Methyl-3-metyl nicotinate (**1**) was prepared from 2-methyl 5-ethyl pyridine as per the literature procedure [16]. 6-Methyl-3-methyl nicotinate, **1** (0.05 mmol) and hydrazine hydrate (99%) (0.05 mmol) were dissolved in ethanol (10 mL) and the clear solution was refluxed for 2 hrs. The reaction mass was then reduced to half of its volume and allowed to cool. The solid mass thus separated out was filtered, washed with small amount of chilled ethanol and dried. (Yield = 90%).

General procedure for the synthesis of 2-[3-(6-methylpyridinyl)]-5-aryl-[1,3,4]-oxadiazole (3a-e)
6-Methylpyridine-3-carbohydrazide (0.006 mol) (**3**) is refluxed with substituted aromatic acids (0.006 mol) in phosphorous oxy chloride (5mL) for 8-10hours. The reaction mixture was slowly quenched to ice water and neutralized it with sodium bicarbonate solution. The solid separated was filtered, washed with water and dried. Compound is recrystallized from ethyl acetate.

2-[3-(6-Methylpyridinyl)]-5-(3,4-diflouro phenyl)-1,3,4-oxadiazole (3a)
White micro crystals; IR (KBr) $\gamma_{/}cm^{-1}$: 2935 (ArC-H), 1595 (C=N), 1334 (C-O-C); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.69 (s, 3H, CH_3), 7.28 (d, 1H, pyridine ring 5H), 7.37 (m, 1H, Ar-H), 7.92-8.01 (m, 2H, Ar-H), 8.32 (dd, 1H, pyridine 4H), 9.23 (s, 1H, pyridine 2H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 24.78, 116.33, 116.52, 117.35, 118.41, 118.59, 123.54, 123.76, 134.43, 147.34, 162.43, 163.12; LC-MS (m/z): 274 (M^+ + 1, 100%), (M.F: $C_{14}H_9F_2N_3O$).

2-[3-(6-Methylpyridinyl)]-5-(4-thiomethylbenzyl)-1,3,4-oxadiazole (3b)
Brown micro crystals; IR (KBr) $\gamma_{/}cm^{-1}$: 2938 (ArC-H), 1605 (C=N), 1330 (C-O-C); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.53 (s, 3H, SCH_3), 2.62 (s, 3H, CH_3), 4.54 (s, 2H, CH_2), 7.29 (d, 1H, pyridine ring 5H), 7.67 (d, 2H, Ar-H), 7.84-8.06 (m, 2H, Ar-H), 8.31 (dd, 1H, pyridine 4H), 9.25 (s, 1H, pyridine 2H); LC-MS (m/z): 298 (M^++ 1, 100%), (M.F: $C_{16}H_{15}N_3OS$).

2-[3-(6-Methylpyridinyl)]-5-(3,4-dinitrophenyl)-1,3,4-oxadiazole (3c)
Brown microcrystals; IR (KBr) $\gamma_{/}cm^{-1}$: 2932 (ArC-H), 1599 (C=N), 1328 (C-O-C); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.69 (s, 3H, CH_3), 7.44 (d, 1H, pyridine ring 5H), 8.40 (d, 1H, pyridine 4H), 9.18-9.29 (m 4H, pyridine 2H and Ar-H); LC-MS (m/z): 328 (M^+ + 1, 100%) , (M.F: $C_{14}H_9N_5O_5$).

2-[3-(6-Methylpyridinyl)]-5-(2,3-dichlorophenyl)-1,3,4-oxadiazole (3d)
Brown micro crystals; IR (KBr) $\gamma_{/}cm^{-1}$: 2945 (ArC-H), 1612 (C=N), 1328 (C-O-C); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.67 (s, 3H, CH_3), 7.47 (d, 1H, pyridine ring 5H), 7.56-8.07 (m, 3H, Ar-H), 8.34 (d, 1H, pyridine 4H), 9.19 (s, 1H, pyridine 2H); LC-MS (m/z): 307 (M^++ 1, 100%), (M.F:$C_{14}H_9Cl_2N_3O$).

2-[3-(6-Methylpyridinyl)]-5-(3-chloro-2-fluorophenyl)-1,3,4-oxadiazole (3e)
White micro crystals; IR (KBr) $\gamma_{/}cm^{-1}$: 2925 ArC-H, , 1594 (C=N), 1342 (C-O-C); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.34 (s, 3H, CH_3), 7.28-7.66 (m, 4H, Ar-H & pyridine 5H), 8.27 (d, 1H, pyridine 4H), 9.17 (s, 1H, pyridine 2H); LC-MS (m/z): 290 (M^+ + 1, 100%), (M.F: $C_{14}H_9ClFN_3O$).

General procedure for the synthesis of 2-[3-(6-methylpyridinyl)])-1,3,4-oxadiazole-5-thione (4)
6-Methylpyridine-3-carbohydrazide (3.0 g, 0.02 mol) was treated with a solution of potassium hydroxide (0.04 mol) dissolved in ethanol (50 mL) under stirring. Carbon disulfide (3.0 g, 0.04 mol) was added slowly to the reaction mixture. The reaction mixture was slowly heated to reflux and refluxed for 8 h. The solvent was distilled under vacuum and the residue was dissolved in water. Acidified the solution using acetic acid and collected the resulting solid by filtration. Yield 80%.

Creamish powder, m.p.: 224-226 ^{0}C, Lit: <250 ^{0}C [17]; LC-MS (m/z): 194 (M^+ + 1, 100%), (M.F: $C_8H_7N_3OS$).

General procedure for the synthesis of Mannich bases (5a-e)
To a mixture of 2-[3-(6-methylpyridinyl)])-1,3,4-oxadiazole-5-thione (**4**) (1.0 g, 0.006 mol) in ethanol (5mL) was added formaldehyde (0.5 ml, 37%) and appropriate primary or secondary amine (0.006mol). The reaction mixture was stirred overnight. After cooling, the precipitate was filtered and crystallized from ethanol.

2-[3-(6-Methylpyridinyl)]-4-(piperidin-1-ylmethyl)-1,3,4-oxadiazole-5-thione (5a)
Yield 80%, m.p. 156-158 ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2935 (C-H), 1617 (C=N), 1294 (C=S), 1012 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 1.42(m, 2H, CH_2), 1.61(m, 4H, CH_2), 2.66 (s, 3H, CH_3), 2.81(m, 4H, CH_2), 5.06(s, 2H, N-CH_2-N) 7.30 (d, 1H, pyridine 5H), 8.10 (d, 1H, pyridine 4H), 9.06 (s, 1H, pyridine 2H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 23.60, 24.79, 25.85, 51.61, 71.68, 116.37, 123.43, 133.83, 147.07, 157.12, 162.67, 178.09; DEPT: CH and CH_3 δ: 23.63, 24.82, 25.88, 51.63, 71.71, 123.46, 133.87, 147.10; LC-MS (m/z): 291 (M^+ + 1, 100%) , (M.F: $C_{14}H_{18}N_4OS$).

2-[3-(6-Methylpyridinyl)]-4-(morpholin-4-ylmethyl)-1,3,4-oxadiazole-5-thione (5b)
Yield 72%, m.p. 146-148 ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2852 (C-H), 1621 (C=N), 1297 (C=S), 1010 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.66 (s, 3H, CH_3), 2.86 (m, 4H, CH_2), 3.71(m, 4H, CH_2) 5.06 (s, 2H, N-CH_2-N) ,7.33 (d, 1H, pyridine 5H), 8.09 (d, 1H, pyridine 4H), 9.06 (s, 1H, pyridine 2H); LC-MS (m/z): 293 (M^+ + 1, 100%), (M.F: $C_{13}H_{16}N_4O_2S$).

2-[3-(6-Methylpyridinyl)]-4-[(4-methylpiperazin-1-yl)methyl]-1,3,4-oxadiazole-5-thione (5c)
Yield 73%, m.p. 160-164ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2895 (C-H), 1625 (C=N), 1289 (C=S), 1015 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.43 (s, 3H, CH_3) 2.60 (s, 3H, CH_3), 2.78 (m, 4H, CH_2), 2.85(m, 4H, CH_2) 5.13 (s, 2H, N-CH_2-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-ylmethyl)-1,3,4-oxadiazole-5-thione (5d)
Yield 70%, m.p. 234-236ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2895 (C-H), 1622 (C=N), 1290 (C=S), 1017 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.63 (s, 3H, CH_3), 5.15 (s, 2H, N-CH_2-N), 7.12 (s, 1H, Imidazole proton), 7.34 (d, 1H, pyridine 5H), 7.75(m, 2H, Imidazole proton), 8.10 (d, 1H, pyridine 4H), 9.09 (s, 1H, pyridine 2H);LC-MS (m/z): 274 (M^+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-4-[(1H-2-methylimidazol-1-yl)methyl]-1,3,4-oxadiazole-5-thione (5e)
Yield 75%, m.p. 240-242ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2889 (C-H), 1618 (C=N), 1295 (C=S), 1019 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.45 (s, 3H, CH_3), 2.67 (s, 3H, CH_3), 5.10 (s, 2H, N-CH_2-N) , 7.01 (m, 2H, Imidazole proton), 7.34 (d, 1H, pyridine 5H), 8.10 (d, 1H, pyridine 4H), 9.09 (s, 1H, pyridine 2H);LC-MS (m/z): 288 (M^+ + 1, 100%)

General procedure for the synthesis of 2-[3-(6-methylpyridinyl)]-5-substitutedbenzylthio-[1,3,4]-oxadiazole (6a-e)
2-[3-(6-Methylpyridinyl)]-1,3,4-oxadiazole-5-thione ***(4)*** (2.0 g, 0.013 mol) was dissolved in DMF (10 mL) and potassium carbonate (0.019 mol) was added. Substituted benzyl halide (0.013 mol) was added and heated to80ºC for 2hrs. The mixture was cooled and quenched to water. The solid obtained was filtered and then crystallized from ethanol.

2-[3-(6-Methylpyridinyl)]-5-[(2,4-dichlorobenzyl)thio]-[1,3,4]-oxadiazole (6a)
Yield 78%, m.p. 82-84 ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2923 (C-H), 1606 (C=N), 1186 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.59 (s, 3H, CH_3), 4.62 (s, 2H, S-CH_2), 7.35-7.66 (m, 4H, pyridine 5H and ArH), 8.16 (d, 1H, pyridine 4H), 8.99 (s, 1H, pyridine 2H); LC-MS (m/z): 353 (M^+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-[(4-nitrobenzyl)thio]-[1,3,4]-oxadiazole (6b)
Yield 82%, m.p. 118-120ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2923 (C-H), 1606 (C=N), 1186 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.59 (s, 3H, CH_3), 4.67 (s, 2H, S-CH_2), 7.23 (d, 1H, pyridine 5H),7.55-7.69 (m, 4H, ArH), 8.19 (d, 1H, pyridine 4H), 9.09 (s, 1H, pyridine 2H); LC-MS (m/z): 329 (M^+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-[(3-methylbenzyl)thio]-[1,3,4]-oxadiazole (6c)
Yield 72%, m.p. 90-92 ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2915 (C-H), 1607 (C=N), 1075 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.35(s, 3H, CH_3), 2.65 (s, 3H, CH_3), 4.50 (s, 2H, S-CH_2), 7.12(d, 1H, pyridine 5H), 7.22 -7.31 (d, 4H, ArH), 8.17 (d, 1H, pyridine 4H), 9.08 (s, 1H, pyridine 2H); ^{13}C NMR ($CDCl_3$, 100MHz): δ 21.32, 24.71, 36.86, 117.39, 123.41, 126.16, 128.70, 128.93, 129.79, 134.09, 135.25, 138.58, 147.07, 161.98, 163.93, 164.45; DEPT: CH and CH_3 δ: 21.34, 24.74, 36.89, 123.44, 126.19, 128.73, 128.96, 129.83, 134.12; LC-MS (m/z): 298 (M^+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-(benzylthio)-[1,3,4]-oxadiazole (6d)
Yield 84%, m.p. 84-86ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 2925 (C-H), 1610 (C=N), 1180 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.64 (s, 3H, CH_3), 4.53 (s, 2H, S-CH_2), 7.26-7.37 (m, 4H, pyridine 5H and ArH), 7.45-7.47 (d, 2H, J=7.2, ArH), 8.16 (d, 1H, pyridine 4H), 9.07 (s, 1H, pyridine 2H); LC-MS (m/z): 329 (M^+ + 1, 100%).

2-[3-(6-Methylpyridinyl)]-5-[(6-chloro-2-triflouromethylbenzyl)thio]-[1,3,4]-oxadiazole (6e)
Yield 68%, m.p. 96-98ºC; IR (KBr) $\gamma_{/}cm^{-1}$: 29230 (C-H), 1615 (C=N), 1179 (C-O); ^{1}H NMR ($CDCl_3$, 400MHz): δ 2.64 (s, 3H, CH_3), 4.50 (s, 2H, S-CH_2), 7.25-7.39 (m, 4H, pyridine 5H and ArH), 8.10 (d, 1H, pyridine 4H), 9.10 (s, 1H, pyridine 2H); LC-MS (m/z): 386 (M^+ + 1, 100%).

Antioxidant studies: DPPH radical scavenging assay
Free radical-scavenging capacities of the compounds **3a-e**, **5a-e** and **6a-e** were determined using the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH)[18] . The stock solution of test compounds (1 mg/mL) and DPPH (0.004%) were prepared using 95% methanol. Freshly prepared DPPH solution were taken in test tubes and organic compounds were then added (100 μg) to every test tube. The absorbance was measured after 10 minutes at 517 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). BHT was used as a reference standard. Control sample was prepared without any extract or BHT. 95% Methanol was used as blank.

Oxadiazole derivatives **5a-e** showed significant antioxidant property when compared with the standard BHT. Similarly compounds **3b** and **3d** showed moderate activity when compared with the standard BHT. Results were presented in **Table-2** and **Fig. 1** shows the graphical representation of antioxidant activity.

α-Amylase inhibition assay

The α-amylase inhibitory activity of newly synthesised compounds was carried out using method of Giancarlo *et. al.*[19] with slight modifications. The Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium choride. The synthesised compounds were dissolved in DMSO to give concentrations from 25 to 100 µg/ml (25, 50, 75, 100 µg/ml). 1 ml of each different concentration of synthesised compounds and 1 ml enzyme solution were mixed in a tube and incubated at 25ºC for 30 min. To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25ºC for 3 min. Then, 1 ml of the color reagent (The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml)) was added and the closed tube placed into an 85ºC water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value was measured at 540 nm by spectrophotometer (Shimadzu-1800). Individual blanks were prepared for correcting the background absorbance. In this case, the colour reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing compound solutions with 1 ml DMSO. Acarbose solution (at the concentrations same as that of synthesised compounds) was used as positive control. The inhibitory effect of organic compounds was compared with standard salivary α-amylase inhibitor acarbose. The α-amylase inhibitory activity was expressed as percentage inhibition. The result is given in **Table-3** and **Fig. 2** shows the graphical representation of α-amylase inhibitory activity.

$$\% \text{ inhibition } I_{\alpha\text{-amylase}} = (\Delta A_{control} - \Delta A_{sample})/ \Delta A_{control} X100$$

Where, A_{sample}= Absorbance of the test sample and

$A_{control}$= Absorbance of the control

Compounds **3d**, **5a** and **6a** are showing good α-amylase inhibitory activity against the standard.

α-Glucosidase inhibition assay

α -Glucosidase activity was determined according to the method described by Kim *et. al*. [20]. Appropriate dilutions of the compounds (0 - 200 µL) and 100 µL of α-glucosidase (EC 3.2.1.20) solution (1.0 U/mL) in 0.1 mol/L phosphate buffer (pH 6.9) were incubated at 25 °C for 10 min. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mol/L phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25 °C for 5 min, before reading the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition. The percentage inhibition of the synthesised compounds is given in **Table-4** and **Fig 3** shows the graphical representation of α- glucosidase inhibitory activity.

$$\% \text{ Inhibition} = [(Abs_{Control} - Abs_{Samples})/Abs_{Control}] \times 100$$

Compounds **3d** and **5a** are showing good α – glucosidase inhibition activity against the standard.

Acute Toxicity Studies

The experimental studies revealed that the compounds were quite safe up to 250, 500, 750, 1000 and 2000 mg/kg and no death of animals were recorded. Further, no significant gross behavioural changes were observed in experimental animals except in the 3000 mg/kg of 3a, 3c, 3e, 5c, 5e, 6a, 6d and 6e compounds, which showed depression on the first day and dead on second day.

RESULTS AND DISCUSSION

Chemistry

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-diflourophenyl)-1,3,4-oxadiazole **(3a)** was confirmed by the presence of absorption peak at 1599 cm^{-1} in IR spectrum which is due to C=N stretching and 1081 cm^{-1} is due to C-O . The ^1H NMR spectrum of compound **3a** showed singlet at δ 2.69 which is due to pyridine $–CH_3$. The C-5 proton of oxadiazole appears 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

appears as a singlet at δ 9.23. ^{13}C 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 is in agreement with the molecular formula $C_{14}H_9F_2N_3O$.

In case of the Mannich base prepared from 2-[3-(6-methylpyridinyl)]-1,3,4-oxadiazole-5-thione **5a** the $-CH_2$ protons of piperidine ring resonated at δ 1.42, δ 1.60 and δ 2.81 as multiplets. The $-CH_2$ in the midst of oxadiazole ring and piperidine ring appeared as a singlet at 5.06. The methyl carbon of pyridine ring appeared at δ 24.79 in ^{13}C NMR. The $-CH_2$ 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 is in agreement with the molecular formula $C_{14}H_{18}N_4OS$.

Table-1: Characterization data of compounds 3a-e, 5a-e and 6a-e

Comp. No	Ar	M.p. (°c)	Yield (%)	Molecular formula	Elemental analysis found(calculated) (%)		
					C	H	N
3a	3,4-Diflouro phenyl	160-162	69	$C_{14}H_9F_2N_3O$ (273.24)	61.52 (61.54)	3.29 (3.32)	15.35 (15.38)
3b	4-Thiomethyl benzyl	206-208	68	$C_{16}H_{15}N_3OS$ (297.37)	64.60 (64.62)	5.05 (5.08)	14.10 (14.13)
3c	3,4-Dinitro phenyl	192-194	77	$C_{14}H_9N_5O_5$ (327.25)	51.35 (51.38)	2.75 (2.77)	21.37 (21.40)
3d	2,3-Dichloro phenyl	156-158	72	$C_{14}H_9Cl_2N_3O$ (306.15)	54.90 (54.92)	2.95 (2.96)	13.70 (13.73)
3e	3-Chloro-2-flouro phenyl	142-144	73	$C_{14}H_9ClFN_3O$ (289.69)	58.01 (58.04)	3.11 (3.13)	14.48 (14.51)
5a	Piperidinyl	156-158	80	$C_{14}H_{18}N_4OS$ (290.39)	57.89 (57.91)	6.24 (6.25)	19.27 (19.29)
5b	Morpholinyl	146-148	72	$C_{13}H_{16}N_4O_2S$ (292.36)	53.38 (53.41)	5.50 (5.52)	19.13 (19.16)
5c	N-Methyl piperazinyl	160-164	73	$C_{14}H_{19}N_5OS$ (305.39)	55.03 (55.06)	6.26 (6.27)	22.90 (22.93)
5d	Imidazolyl	234-236	70	$C_{12}H_{11}N_5OS$ (273.31)	52.70 (52.73)	4.04 (4.06)	25.60 (25.62)
5e	2-Methyl imidazolyl	240-242	75	$C_{13}H_{13}N_5OS$ (287.34)	54.31 (54.34)	4.55 (4.56)	24.35 (24.37)
6a	2,4-Dichloro benzyl	82-84	78	$C_{15}H_{11}Cl_2N_3OS$ (352.24)	51.12 (51.15)	3.14 (3.15)	11.90 (11.93)
6b	4-Nitro benzyl	118-120	82	$C_{15}H_{12}N_4O_3S$ (328.35)	54.88 (54.87)	3.65 (3.68)	14.60 (14.62)
6c	3-Methyl benzyl	90-92	72	$C_{16}H_{15}N_3OS$ (297.37)	64.60 (64.62)	5.07 (5.08)	14.11 (14.13)
6d	Benzyl	84-86	84	$C_{15}H_{13}N_3OS$ (283.35)	63.55 (63.58)	4.60 (4.62)	14.80 (14.83)
6e	6-Chloro-2-triflouromethyl benzyl	96-98	68	$C_{16}H_{11}ClF_3N_3OS$ (385.79)	49.79 (49.81)	2.85 (2.87)	10.86 (10.89)

Table-2: DPPH scavenging activity of the compounds 3a-e, 5a-e and 6a-e

Compounds	DPPH Assay in %
3a	---
3b	52.2
3c	49.1
3d	18.3
3e	14.5
5a	82.6
5b	83.2
5c	87.6
5d	82.3
5e	85.4
6a	10.5
6b	38.8
6c	3.9
6d	12.7
6e	35.4
BHT	90.42

The ^{1}H NMR spectrum of 2-[3-(6-methylpyridinyl)]-5-(3-methylphenylthio)-1,3,4-oxadiazole **6c,** the $-CH_3$ protons of phenyl ring and pyridine ring resonated at δ 2.35 and δ 2.65 as singlet. The $-SCH_2$ appeared as a singlet at 4.50.

The mass spectrum of **5a** showed molecular ion peak at m/z = 298 (M^+ + 1) which is in agreement with the molecular formula $C_{16}H_{15}N_3OS$. Similarly the spectral values for all the synthesized compounds and C, H, N analyses are given in the experimental part and the characterization data is provided in **Table-1.**

Table 3: In vitro *α*-amylase inhibitory activity of synthesized compounds 3a-e, 5a-e and 6a-e

Compounds	% inhibition
3a	20.34
3b	45.87
3c	56.12
3d	70.38
3e	41.89
5a	69.73
5b	20.45
5c	14.30
5d	18.38
5e	22.31
6a	63.27
6b	34.17
6c	---
6d	---
6e	54.82
Acarbose	82.76

Table 4: *α* – Glucosidase inhibition assay of compounds 3a-e, 5a-e and 6a-e

Compounds	% inhibition
3a	11.43
3b	23.15
3c	40.58
3d	59.12
3e	30.64
5a	62.48
5b	13.74
5c	31.67
5d	28.71
5e	11.47
6a	28.93
6b	41.38
6c	---
6d	---
6e	43.91
Acarbose	75.53

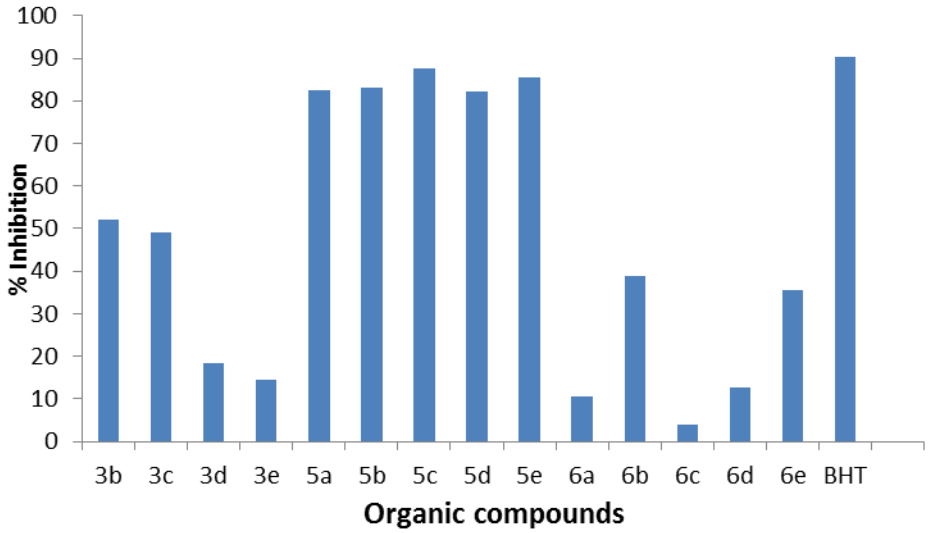


Fig 1: DPPH scavenging activity of the compounds 3a-e, 5a-e and 6a-e. The experiment was performed in triplicate and the values expressed are as Mean ±SD

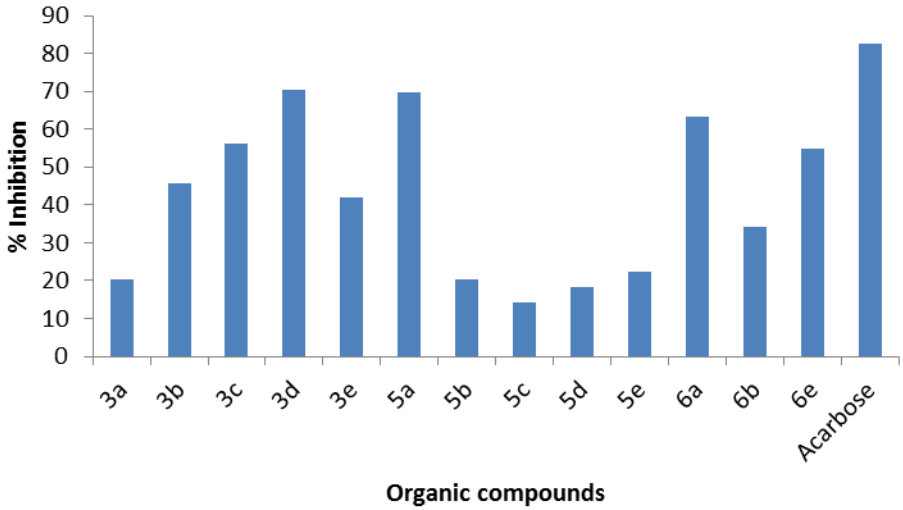


Fig 2: α-Amylase inhibition assay of the compounds 3a-e, 5a-e and 6a-e. The experiment was performed in triplicate and the values expressed are as Mean ±SD

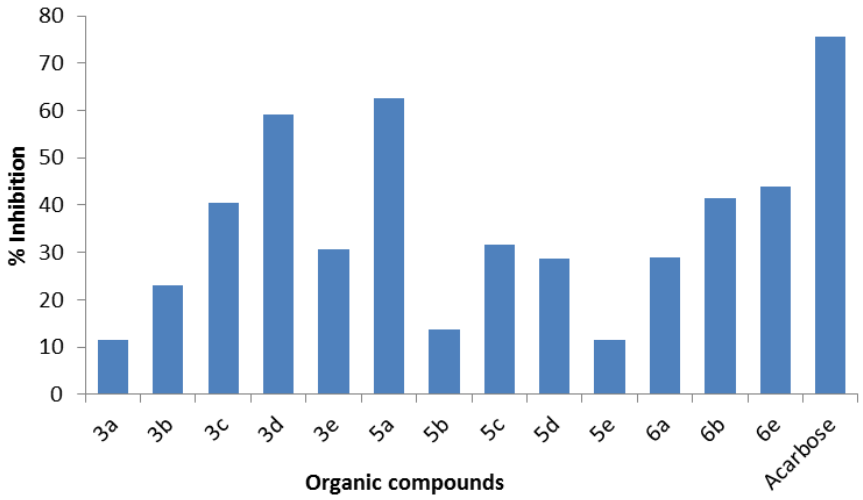


Fig 3: α- Glucosidase inhibition assay of the compounds 3a-e, 5a-e and 6a-e. The experiment was performed in triplicate and the values expressed are as Mean ±SD

CONCLUSION

Antioxidant activity results of synthesised compounds shows that the Mannich base series **5a-e** as efficient radical scavengers. Compound **5e** showed significant activity against all the microbial strains. Compounds **3d** and **5a** are showing good antidiabetic activity. Thus considering all these we can conclude that 1,3,4-oxadiazole derivatives incorporating pyridine moiety can be studied as potent biomolecules of pharmacological importance.

Acknowledgement
The authors are grateful to the management, SEQUENT SCIENTIFIC LTD, New Mangalore, India for their invaluable support and allocation of resources for this work. Authors are also thankful to Chairman, Dept. of Chemistry, Mangalore University. SPC thanks to Prof. Shrikrishna and MIT, Manipal for his technical help.

REFERENCES

[1] K. Karthic, K. S. Kirthiram, S. Sadasivam, B. Thayumanavan, *Indian Journal of Experimental Biology*., **2010**, 46, 677.
[2] N. D. Kim, H. J. Nho, et al. *J. Microbiol*., **2004**, 42, 223.

[3] R. Chawla, A. Arora, M. K. Parameswaran, P. Chan, D. Sharma, S. Michael, T. K. Ravi, *Acta. Pol. Pharm*., **2010**, 67(3), 247.
[4] K. K. Jha, A. Samad, Y. Kumar, Shaharyar, R. L. Khosa, J. Jain, V. Kumar, P. Singh, *Eur. J. Med. Chem.*, **2010**, 45(11), 4963.
[6] S. Wagle, V. Adhikari, N. S. Kumari, *Indian. J. Chem*., **2008**, 47B, 439.
[7] C. S. de Oliveira, B. F. Lira, J. M. Barbosa-Filho, J. G. F. Lorenzo, P. F. de Athayde-Filho, *Molecules*., **2012**, 17, 10192.
[8] E. A. Musad, R. Mohamed, B. A. Saeed, B. S. Vishwanath, K. M. Lokanatha Rai. *Bioorganic. Med. Chem. Lett*., 2011; 21: 3536-3540.
[9] Juan Sun, Hui Zhu, Zhong-Ming Yang, Hai-Liang Zhu, *Eur. J. Med. Chem.*, **2013**, 60, 23.
[10] A. Zarghi, Z. Hajimahdi, A. Shafiee, *Chem. Pharm. Bull(Tokyo).*, **2008**, 56, 509.
[11] S. Nazreen, S. A. Mohammad, H. Hamid, S. Y. Mohammad, S. Shafi, D. Abhijeet, Perwez Alam, M. A. Q. Pasha, B. Sameena, M. A. Mohammad, H. Saqlain, Yakub Ali, K. Chetna, K. K. Pillai, *Eur. J. Med Chem*., **2014**, 87, 175.
[12] G. C. Ramaprasad, B. Kalluraya, B. Sunil Kumar, R. K. Hunnur, *Eur. J. Med Chem*., **2010**, 45, 4587.
[13] R. M. Mohareb, A. E. M. Abdallah, M. A Abdelaziz, *Med. Chem. Res.*, **2014**, 23, 564.
[14] A. M. Vijesh, A. M. Isloor, S. K. Peethambar, K. N. Shivananda, T. Arulmoli, N. A. Isloor, *Eur. J. Med. Chem.*, **2011**, 46, 5591.
[15] S. H. Paul, B. Simon, V. A. Jonathon, J. B. Sandra, J. C. Thomas, C. C. Lance *Bioorg. Med. Chem. Lett.*, **2006**, 16(23), 6120.
[16] Grayson JI, US 4,579,953, **1986**.
[17] W. B. Williams, M. E. Cuvelier, C. Berset, *LWT-Food Sci Technol.*, **1995**, 28, 25.
[18] S. Giancarlo, L. M. Rosa, F. Nadjafi, M. Francesco, *Nat Prod Res.*, **2006**, 20, 882.
[19] Y. M. Kim, Y. K. Jeong, M. H. Wang, W. Y. Lee, H. I. Rhee, *Nutrition*, **2005**, 21(6), 756.
[20]R. Nyfeler, W. Eckhardt, E. Beriger, O. Kristiansen, US 4,894,380, Jan 16, **1990**.