International Journal of Drug Research and Technology

Available online at http://www.ijdrt.com

Research Article

SYNTHESIS OF CYCLOPYRROLIDINE CLUBBED WITH OXADIAZOLE BASES AND EVALUATION OF THEIR ANTI-DIABETIC ACTIVITY THROUGH *IN VIVO* MODEL

Megha Tukaram Salve*

Department of Pharmaceutical Chemistry, Modern College of Pharmacy, Nigdi, Pune, Maharastra -411044, India

ABSTRACT

Cyclopyrrolidine clubbed with oxadiazole bases (B-1 to B-16) were synthesized and characterized through IR, NMR, mass spectrometry, and elemental analysis. Docking studies were performed to assess interactions and binding modes of synthesized hits at the binding site of receptor DPP-4 (PDB 3W2T). Using vildagliptin as a standard drug, six of the synthesized compounds were tested for their antidiabetic activity in diabetic rats induced with HFD-STZ-Nicotinamide. The results showed that compound B-XIV (220*4.56B) resulted in the greatest reduction in blood glucose level from all synthesized compounds compared to that of vildagliptin (215*7.52B) in HFD-STZ-Nicotinamide. Other compounds showed moderate to good ant hyperglycemic activity.

Keywords: Fur Diazole**;** Hyperglycemic**;** HFD-STZ-Nicotinamide

Abbreviations: T2D: type 2 diabetes; DM: Diabetes mellitus; HFD: High-fat diet; STZ-Streptozotocin; DPP-IV- Dipeptidyl peptidase- IV;GPR40 -G-protein receptor 40;SAR structure-activity relationship;PPAR -peroxisome proliferator-activated receptor; STZ –Streptozotocin;IR-Infrared spectroscopy; 1HNMR- Hydrogen Nuclear Magnetic Resonance; 13CNMR- Carbon-13 nuclear magnetic resonance;GLP19- Glucagon-like peptide 1; T2DM Type 2 diabetes mellitus; DPP IV- dipeptidyl peptidase IV; 2D- two-dimensional; TLC -Thin layer chromatography; Fourier-transform infrared spectroscopy; TMS- Transcranial magnetic stimulation;CDCl3-Deuterated chloroform.

INTRODUCTION

Since the early 1970s, 1,2,4-oxadiazole heterocycles have been extensively investigated, yielding a variety of bioactive compounds, including anticancer [1], anti-inflammatory[2], anticonvulsant[3], antiviral[4], antibacterial[5], antifungal[6], antidepressant[7], antiangiogenic[8], analgesic[9], anti-insomnia, anti-oedema[10], antiphrastic[11], anti-Alzheimer[12], and antidiabetic[13]. In addition, they showed inhibition of dipeptidyl peptidase IV[14], α-Glucosidase[15], α-Amylase[16], Protein

tyrosine phosphatase 1B[17]. A number of 1, 2, 4-oxadiazoles were screened as GLP19 (Glucagon-like peptide 1) [18], PPAR (peroxisome proliferator-activated receptor) alpha/gamma agonists[19], GPR40 (G-protein receptor 40)[20] agonists as a antidiabetic activity.

Recently, GLP-1 has been identified as a potential target for treatment of T2DM. Gut hormone GLP-1 increases insulin secretion after eating. GLP-1 has been shown to improve glycemic control in patients with Type 2 diabetes [21]. As GLP-1 cleaves its N-terminal, DPP-IV controls its activity [7-36]-amide to form GLP-1[9-36]-amide, an inert compound. This method can be used to increase GLP-1 in the blood by inhibiting DPP-IV [22]. As a result, the investigation of DPP-IV inhibitors as possible treatments has been devoted a significant amount of time and effort (Figures 1 and 2).

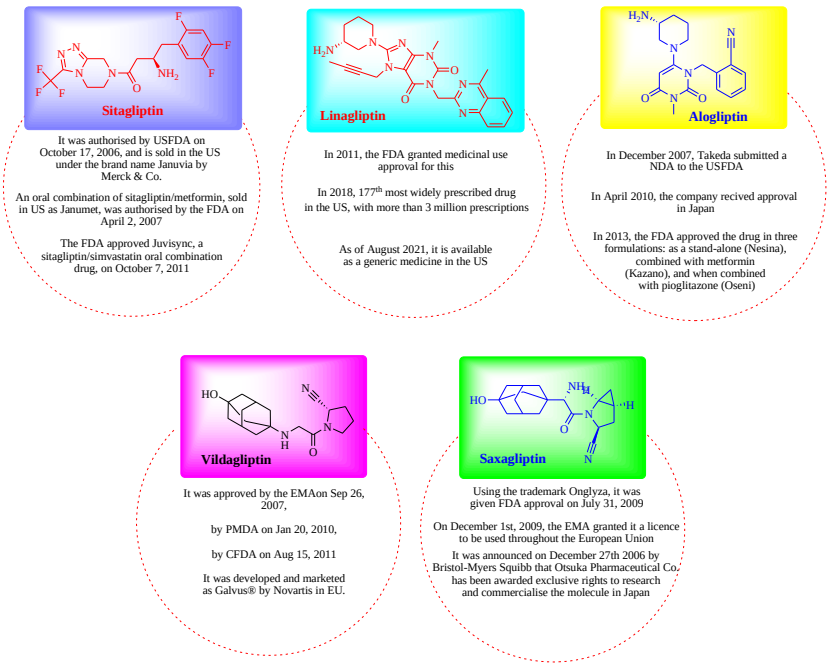


Figure 2. The structures of clinically approved DPP-IV inhibitors.

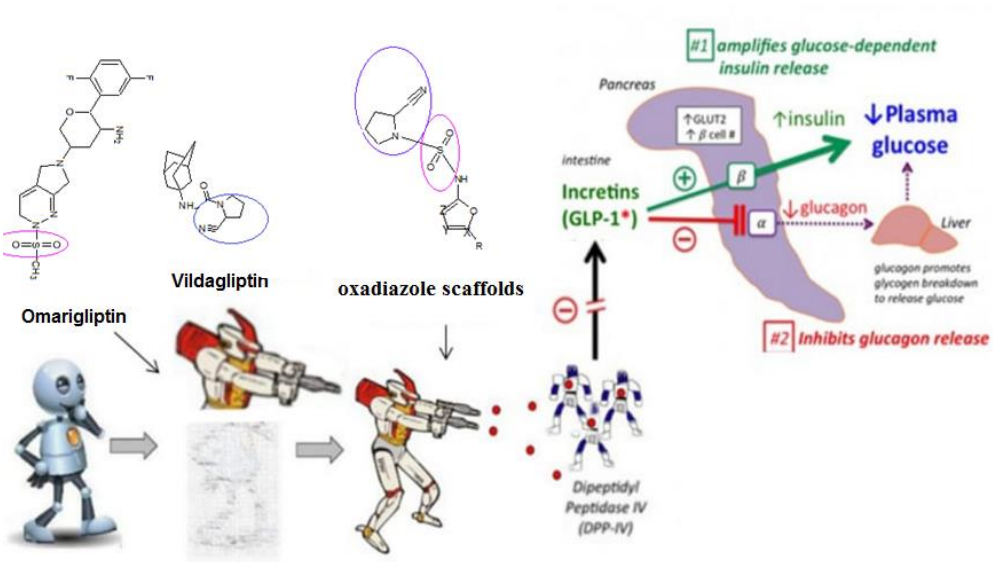


Figure 2. Synthesis of cyclopyrrolidine clubbed with oxadiazole bases: Graphical abstract.

MATERIALS AND METHODS

ChemSketch software was used to simulate the 2D structures of the compounds, and AutoDock version 1.5.6 was used to dock the hits. A protein (PDB NO: 3W2T) was used as DPP-IV. Chemicals of analytical grade came from Nice Chemicals Pvt. Ltd (India), Fizmerck (India), M Lychem chemical (India), and Poona chemical (India). We used the chemicals and solvents in their original forms, without purification.

Using thin-layer chromatography (TLC), a reaction was monitored to determine if the reactants had been consumed and if a new product had been formed. Using the Labronics LT-115 digital melting point apparatus, we measured the melting points of open capillary tubes. A silica gel cartridge (230-400 mesh, 40 m silica) was used to purify compounds using column chromatography. A Bruker FTIR was used to capture these FTIR spectra. Analyses of the synthesized compounds were conducted using $CDCl_3$ (unless specified), TMS as an internal reference (chemical deviation, ppm) and SND 400 MHz instruments (chemical deviation, ppm) 100183-SND and 100186-SND, respectively). It was conducted by Thermo Finnigan. Only 4% of theoretical values were obtained in the elemental analysis.

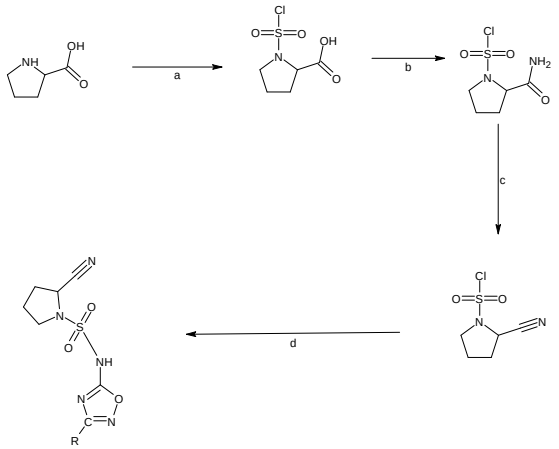
Evaluation of pharmacological activity

To study the anti-diabetic activity of the synthesized compounds, 72 female Albino Wistar rats were used. The animals were obtained from Animal House of National Institute of Biosciences Address: Plot no 5, Ambika C.H.S., Opp. Indraprasta Hall, Near Chaturshinghi mandir, Pune-411016. The Modern College of Pharmacy's Institutional Animal Ethics Committee approved the study (MCP/IAEP/010/2020). In polypropylene cages at 37^0C, rats were housed. Streptozotocin and nicotinamide-induced diabetic rats were used to induce diabetes in Albino Wistar rats.

For evaluation of anti-diabetic activity

High Fat Diet Streptozotocin and Nicotinamide-induced diabetes rats were given blood samples by retro-orbital puncture under light ether anesthesia [23,24]. Separation of serum was accomplished by collecting blood samples without anticoagulants. Blood samples were then centrifuged at 3500 rpm for 15 min, serum was extracted from blood and glucose is measured in the serum [25]. Moreover cholesterol [26], triglycerides [27] creatinine and urea levels were evaluated, respectively.

Synthesis of procedure for B-I to BXVI derivatives



a-chloro sulphonic chloride, sodium carbonate (3.0 mmol) in dichloromethane:water, stirred for 16 hours at room temperature

b-1-ethyl-3-(3dimethylaminopropyl)carbodiimide hydrochloride (EDCI) (1.5 mmol) and 1-hydroxy benzotriazole (HOBt) (1.0 mmol) in dichloromethane added to the sample at 10 to 15°C (duration 5.0

c-A mix of trifluoroacetic anhydride (2 mmol) and tetrahydrofuran (10 mL) was stirred at room temperature for five hours.

d- oxadiazole derivative compound (1.5mmol) was refluxed with anhydrous potassium carbonate for 2 hr

R=4-Hydroxy Phenyl, 3-Chloro Phenyl, 4-Nitro Phenyl, 2-Chloro Phenyl, 2-Acetoxy Phenyl, 4-Bromo Phenyl, 4-Chloro Phenyl, 2-Bromo Phenyl, 4-Methyl Phenyl, 3-Methyl Phenyl, 2-Nitro Phenyl, 4-Amino Phenyl, Trifluoromethyl Phenyl, 3,4-Dimethoxy Phenyl, 2-Hydroxy Phenyl, 4-Methoxy Phenyl.

Synthesis 1-(chlorosulfonyl) pyrrolidine-2-carboxylic acid

As monitored by TLC, water was added to L-proline (1.0 mmol) and sodium carbonate (3.0 mmol) in a dichloromethane (1:1) solution and the mixture was stirred at room temperature for 16 hr until the reaction was completed. After the reaction was complete, the reaction mixture was washed with petroleum ether (20 ml), then acidified with concentrated hydrochloride to a pH of acid. After filtering, the white solid was washed several times with water, dried, and observed on a single point of silica gel [25].

Yield: 52%; Melting point: 118 - 120oC; IR (KBr):1200(S=O sym), 1400(S=O asym), 600(C-Cl), 1200(S=O sym), 1400(S=O asym), 1450 (CH2), 1600 (C-H), 3550 (COOH): 1H NMR (400 MHz, CDCl3): 1.2-2.4(m,6H), 3.6 (s,1H), 11.4 (s,1H),; 13C NMR (400 MHz, CDCl3): δ 22.3, 28.3, 62.3, 70.3, 174; ESI MS (m/z): 214.09[M+H]; Anal. Calcd. For found C5H8ClNO4S: C (28.11%), H (3.77%), N (6.56%).

Synthesis 2-carbamoyl pyrrolidine-1-sulfonyl chloride

As a result, the mixture of dicyclohexylcarbodiimide in methylene chloride was connected to the mixture of phase-i a) in dichloromethane (10 mL solution) in a (1mmole) gradually at 10-15 °C (duration 5.0 minutes), and the solution was stirred at room temperature for 5 hours. Stir gently for 1 hour with 5.0 mm of bicarbonate. Thin-layer chromatography analysis of 5% methanol, 3 percent chloroform, 3% anisaldehyde, and 2% iodine. After the process was completed, the sample was stirred in, and the residues were cleaned with dichloromethane. After collecting and pooling the filtrates, they were washed twice with water, dried over anhydrous sodium sulphate, and evaporated under decreased pressure to provide a crude product that was then purified using silica gel by column chromatography. With ethyl acetate used as eluent, silica gel has been spotted with a single spot when tested with the pure required product [24].

Yield: 58%; Melting point: 140-1470C; IR (KBr):600 (C-Cl), 1200(S=O sym), 1400(S=O asym), 1450(CH2),1600(C-H), 1650 (NH2-C=O); 1H NMR (400 MHz, CDCl3): 1.64-2.8 (m,6H), 3.7 (s,1H), 7.21(s,1H); 13C NMR (400 MHz, CDCl3): δ 21.9, 28.3, 62.3,71, 176.7; ESI MS (m/z):214 [M+2H]; Anal. Calcd. For found C5H9ClN2O3S: C (28.22%), H (4.22%), N (13.12%).

Synthesis 2-cyanopyrrolidine-1-sulfonyl chloride

Trifluoroacetic anhydride (2.0 mmol) suspension in tetrahydrofuran (10 ml) was added to amide (1.0 mmol) at 0-5°C and was mixed for 4 hours at room temperature. The response was tracked

using thin-layer chromatography. Ammonium bicarbonate (7 mmol) was added (after completion of reaction) to the solution (later 15 minutes). The mixture was condensed in vacuum at 40° C and stirred for one hour at room temperature, then purified using dichloromethane and methanol (95:5) as elution to give 2-cyano-pyrrolidine-1-sulfonyl chloride as a white solid pure desired product and identified at a single point on silica gel using dichloromethane: methanol (95:5) as elution to give 2-cyano-pyrrolidine-1-sulfonyl chloride as a white solid pure desired product [24].

Yield: 55%; Melting point: 65-670C; IR (KBr): 650 (C-Cl), 1200 (S=O sym), 1400 (S=O asym), 1450 (CH2), 1600 (C-H), 2200 (−C≡N); 1H NMR (400 MHz, CDCl3): 1.64-2.8 (m,6H), 3.8 (S,1H); 13C NMR (400 MHz, CDCl3): δ 30.2, 52.8, 62.2, 116.2; ESI MS (m/z): 195 [M+H]; Anal. Calcd. For found C5H7ClN2O2S: C (30.80%), H (3.60%), N (14.32%).

Catalyst-free Synthesis of 2-cyano-N-(5-substituted-oxadiazol-3-yl) pyrrolidine-1-sulphonamide

3-amino-5-substituted-1,2,4-oxadiazole (2 mmol) (Step-I amine compound) and 2-cyanopyrrolidine-1-sulfonyl chloride (1 mmol) were added to ethanol (2 ml) at room temperature and stirring was continued until the reaction was complete (monitored by TLC). It was filtered and the residue was washed with ethyl acetate. We collected the filtrate and evaporated the solvent under reduced pressure to give the crude product. Using ethyl acetate and petroleum ether as stationary phases, our crude product was purified by column chromatography (7:3) as a mobile phase to yield the product [25,26].

R=4-Hydroxy Phenyl, 3-Chloro Phenyl, 4-Nitro Phenyl, 2-Chloro Phenyl, 2-Acetoxy Phenyl, 4-Bromo Phenyl, 4-Chloro Phenyl, 2-Bromo Phenyl, 4-Methyl Phenyl, 3-Methyl Phenyl, 2-Nitro Phenyl, 4-Amino Phenyl, 4-Trifluoromethyl Phenyl, 3,4-Dimethoxy Phenyl, 2-Hydroxy Phenyl, 4-Methoxy Phenyl.

RESULTS

2-cyano-N-[3-(4-hydroxyphenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 82%; Melting point: 156-1580C; IR (KBr): 1100(C=O), 1250(C-O), 1350 (O-H), 1450(CH2), 1550(S=O), 1650(C-H.), 2250(−C≡N), 3050(C-H), 3400(N-H); 1H NMR (400 MHz, CDCl3): 1.40-2.80 (m,6H), 3.8 (s,1H), 6.88 (d,2H), 7.90 (d,2H), 9.67 (s,1H),10.5 (s,1H); 13C NMR (400 MHz, CDCl3): δ 20-30, 50, 55,56.1, 125-135, 144.6, 164.5-169.3; ESI MS (m/z): 388.06[M+H]; Anal. Calcd. For found C13H13N5O4S: C (46.56%), H (3.91%), N (20.88%).

N-[3-(3-chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-cyanopyrrolidine-1-sulfonamide

Yield: 84%; Melting point: 120-1220C; IR (KBr): 780(C-Cl), 1100(-C=O), 1450(CH2), 1550(-S=O), 1650(-C-H), 2250(−C≡N), 3050(C-H), 3400(N-H);1H NMR (400 MHz, CDCl3): 1.40- 2.8 (m,6H), 3.8 (s,1H), 8.16(m,1H), 7.97(d,2H), 7.48(s,1H), 10.5 (s,1H); 13C NMR (400 MHz, CDCl3): δ 20.9, 29.3, 45.6, 45-55, 55, 116.2, 134, 125-135, 164.5-169.3; ESI MS (m/z): 354.78[M+H]; Anal. Calcd. For found C13H12ClN5O3S: C (44.13%), H (3.42%), N (19.8%).

2-cyano-N-[3-(4-nitrophenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 85%; Melting point: 168-1700C; IR (KBr): 1100(C=O), 1400(-NO2), 1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N), 3050(C-H), 3400(N-H);1H NMR (400 MHz, CDCl3):1.40-2.8(m,6H), 3.8(S,1H), 8.3(d,2H), 8.87(d,2H), 10.58(S,1H); 13C NMR (400 MHz, CDCl3): δ20.9,

29.3, 45.6, 45-55, 55, 116.2, 134, 125-135, 164.5-169.3; ESI MS (m/z): 366.05[M+2H]; Anal. Calcd. For found C13H12N6O5S: C (42.86%), H (3.32%), N (23.07%).

N-[3-(2-chlorophenyl)-1,2,4-oxadiazol-5-yl]-2-cyanopyrrolidine-1-sulfonamide

Yield: 80%; Melting point: 125-1270C; IR (KBr): 680(C-Cl), 1050(C=O), 1455(CH2), 1560(C-H), 1600(S=O), 2200(−C≡N), 3050(CH), 3300(N-H);1H NMR (400 MHz, CDCl3): 1.40-2.80 (m,6H), 3.8 (s,1H), 7.61 (d,2H),7.71 (m,2H), 10.5 (S,1H); 13C NMR (400 MHz, CDCl3): 20-29, 55,45.6, 116.2, 130-145, 166.7,169.3; ESI MS (m/z): 354.78[M+H]; Anal. Calcd. For found C13H12ClN5O3S: C (44.13%), H (3.42%), N (19.8%).

2-{5-[(2-cyanopyrrolidine-1-sulfonyl)amino]-1,2,4-oxadiazol-3-yl}phenyl acetate

Yield:84%; Melting point: 165-1670C; IR (KBr): 1455(CH2), 1560(C-H), 1600(S=O), 1690(-C=O), 2200(−C≡N), 3050(CH), 3300(NH);1H NMR (400 MHz, CDCl3): 1.40-2.80(m,6H), 2.45(m,3H), 3.8(S,1H), 7.45(d,2H), 7.77(m,2H), 10.5(S,1H); 13C NMR (400 MHz, CDCl3): 20.3, 20.9-29.3, 45.6, 55,116.2, 123.2-151.1, 169, 166.7,169.3; ESI MS (m/z): 378.08 [M+H];Anal. Calcd. For found C15H15N5O5S: C(47.74%) H(4.01%) N(18.56%).

N-[3-(4-bromophenyl)-1,2,4-oxadiazol-5-yl]-2-cyanopyrrolidine-1-sulfonamide

Yield: 79%; Melting point: 128-130 0C; IR (KBr): 780(C-Br), 1100(-C=O), 1450(CH2), 1550(-S=O), 1650(-C-H), 2250(−C≡N), 3050(C-H), 3400(N-H); 1H NMR (400 MHz, CDCl3): 1.40-2.80(m,6H),3.8(d,1H), 7.38(d,2H), 7.71(d,2H), 10.5(S,1H); 13C NMR (400 MHz, CDCl3): δ20.9, 29.3, 45.6, 45-55, 55, 116.2, 134, 125-135, 164.5-169.3; ESI MS (m/z): 354.78[M+H]; Anal. Calcd. For found C13H12BrN5O3S: C (39.13%), H (3.42%), N (19.8%).

N-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2-cyanopyrrolidine-1-sulfonamide

Yield: 72%; Melting point: 158-1600C;IR (KBr): 780(C-Cl), 1100(-C=O), 1450(CH2), 1550(-S=O), 1650(-C-H), 2250(−C≡N), 3050(C-H), 3400(N-H); 1H NMR (400 MHz, CDCl3):1.40-2.8(m,6H), 3.8 (s,1H), 7.97(d,2H), 7.48(d,2H), 10.5 (s,1H); 13C NMR (400 MHz, CDCl3): δ 20.9, 29.3, 45.6, 45-55, 55, 116.2, 134, 125-135, 164.5-169.3; ESI MS (m/z): 354.78[M+H]; Anal. Calcd. For found C13H12ClN5O3S: C (44.13%), H (3.42%), N (19.8%).

N-[3-(2-bromophenyl)-1,2,4-oxadiazol-5-yl]-2-cyanopyrrolidine-1-sulfonamide

Yield: 69%; Melting point: 122-1250C; IR (KBr): 710(C-Br), 1100(-C=O), 1450(CH2), 1550(-S=O), 1650(-C-H), 2250(−C≡N), 3050(C-H), 3400(N-H); 1H NMR (400 MHz, CDCl3): 1H NMR (400 MHz, CDCl3): 1.40- 2.8 (m,6H), 3.8 (s,1H), 7.97(d,2H), 8.16(m,2H), 10.5 (s,1H); 13C NMR (400 MHz, CDCl3): δ20.9, 29.3, 45.6, 45-55, 55, 116.2, 134, 125-135, 164.5-169.3; ESI MS (m/z): 354.78[M+H]; Anal. Calcd. For found C13H12BrN5O3S: C (39.13%), H (3.42%), N (19.8%).

2-cyano-N-[5-(4-methyl phenyl)-1,2,4-oxadiazol-2-yl]pyrrolidine-1-sulfonamide

Yield: 74%; Melting point: 137-1390C; IR (KBr): 1050(C=O),1450(CH2), 1550(S=O),1650(C-H), 2250(−C≡N),3050(C-H), 2810(O-CH3),2900(-CH3), 3050(C-H), 3450(NH), ;1HNMR(400MHz,CDCl3):1.40-2.4(m,6H),3.6(S,1H),3.8(m,3H),7.03(d,1H),8.02(d,1H),10.5(S,1H);13C NMR (400 MHz, CDCl3): 14.16-18.76, 50,55.9, 56.5,62.5, 123.52,130.68,135.85, 144.6, 166.5-169.3; ESI MS (m/z):350.09

[M+H]; Anal. Calcd. For found C14H15N5O3S: C (48.13%), H (4.33%), N (20.05%).

2-cyano-N-[3-(3-methyl phenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield:85%;Melting point:145-1470C;IR(KBr): 1050(C=O),1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N),2900(-CH3),3050(C-H),3450(N-H);1HNMR(400MHz,CDCl3): 1.40-2.08 (m,6H), 2.34(m,3H), 3.8(d,1H), 7.1(s,1H), 7.35 (d,2H), 7.97(m,1H), 10.58(S,1H);13C NMR (400 MHz, CDCl3): 20.9-29.3, 21.3, 55,46.5, 116.2, 123.1,127.4,126.3, 164.5-169.3; ESI MS (m/z): 334.09 [M+H]; Anal. Calcd. For found C14H15N5O3S:C(50.44%), H(4.54%),N(21.01%).

2-cyano-N-[3-(2-nitrophenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 81% Melting point: 192-1940C; IR (KBr): 1450(CH2); 1550(S=O), 1550(asym -NO2),1570 (asym -NO2), 1650(C-H), 2250(−C≡N), 3050(C-H),3400(N-H); 1HNMR(400MHz,CDCl3): 1.40-2.8(m,6H), 3.8(S,1H), 7.72(d,2H), 8.08(m,2H), 10.5(S,1H); 13C NMR (400 MHz, CDCl3): 20.9-29.3, 45.6-55, 116.2, 124.4-150.1,150.1, 166.5-169.3;ESI MS (m/z): 365.06 [M+H]; Anal. Calcd. For found C13H12N6O5S: C(42.86%),H(3.32%), N(23.07%).

2-cyano-N-{3-[4-(trifluoromethyl) phenyl]-1,2,4-oxadiazol-5-yl}pyrrolidine-1 sulfonamide

Yield: 87% Melting point: 137-1390C; IR (KBr): 1200CF3,1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N), 3050(C-H), 3400(N-H);1H NMR (400 MHz, CDCl3): 1.4-2.8(m,6H), 3.5(s,1H), 7.9(d,2H), 8.2(d,2H), 10.4(S,1H);13C NMR (400 MHz, CDCl3): 20.9-29.3,45.6-55,116.2,124.1,125.6-131,164.5-169.3; ESI MS (m/z): 388.06 [M+H]; Anal. Calcd. For found C14H12F3N5O3S: C Composition: C(43.41%),H(3.12%), N(18.08%).

2-cyano-N-[3-(4-aminophenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 85% Melting point: 145-1470C; IR (KBr): 1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N), 3050(C-H), 3350(PrimN-H2), 3400(N-H);1H NMR (400 MHz, CDCl3):1.40-2.8(m,6H), 3.8(S,1H), 5.24(S,2H), 6.7(d,2H), 7.90(d,2H), 10.58(S,1H); 13C NMR (400 MHz, CDCl3): 20.9-29.3, 46.6-55, 116.2,115.1-145.6, 164.5-169.3;ESI MS (m/z): 335.09; [M+H];Anal. Calcd. For found C13H14N6O3S: C(46.70%), H(4.22%), N(25.14%).

2-cyano-N-[3-(3,4-dimethoxyphenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 75%; Melting point: 126-1280C; IR (KBr): 1250(C-O), 1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N),3050(C-H), 3400(N-H); 1H NMR (400 MHz, CDCl3):1.40-2.8(m,6H), 3.8(S,1H), 3.85(m,3H), 3.92(m,3H), 7.0(d,1H), 7.29(d,1H), 7.76(d,1H), 10.58(S,1H); (400 MHz, CDCl3): δ20.9-29.3, 45.6-55, 56.1, 116.2, 111-150.3,164.5-169.3 ESI MS (m/z): 380.1[M+H]; Anal. Calcd. For found C15H17N5O5S: C(47.49%) H(4.52%) N(18.46%).

2-cyano-N-[3-(2-hydroxyphenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 82%; Melting point: 156-1580C; IR (KBr): 1250(C-O), 1350(O-H), 1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N), 3050(C-H), 3400(N-H);1H NMR (400 MHz, CDCl3): 1.40-2.8(m,6H), 3.8(s,1H), 7.0 d,1H), 7.06(d,1H), 7.32(d,1H), 7.6(d,1H), 9.61(S,1H), 10.58(S,1H); 13C NMR (400 MHz, CDCl3): 20.9-29.3, 50, 55,56.1, 116.2, 154.1-117.8, 164.5-169.3; ESI MS (m/z): 350.09[M+H]; Anal. Calcd. For found C13H13N5O4S: C (46.56%), H (3.91%), N (20.88%).

Table 1. Random glucose level (mg/dl) of synthesized analogs and standard drug in Streptozotocin-Nicotinamide+high-fat diet-induced diabetic rats.

Group	**Dose**	**1st**	**7th**	**14th**	**21st**	**28th**
Normal control	1ml Tap water	98 ± 3.0	95 ± 4.2	98 ± 1.5	105 ± 7.2	102 ± 3.2
Diabetic control	0.25% CMC	295 ± 8.2	289 ± 7.5	295 ± 8.5	275 ± 6.2	280 ± 4.6
Vildagliptin	10 mg/kg	280 ± 6.5#	230 ± 8.2 #	215 ± 7.2 B	205 ± 4.1B	198 ± 3.4B
Compound B-I	100 mg/kg	287 ± 2.36#	235 ± 3.1 #	222 ± 3.62 B	215 ± 2.20 B	212 ± 5.67 B
Compound B-II	100 mg/kg	309 ± 2.36#	257 ± 3.1 #	242 ± 3.62 B	236 ± 2.20 B	229 ± 5.67 B
Compound B- III	100 mg/kg	290 ± 2.94#	238 ± 3.09#	225 ± 3.77 B	218 ± 5.48 B	210 ± 4.87 B
Compound B-XI	100 mg/kg	282 ± 5.18#	233 ± 2.86#	220 ± 4.56 B	215 ± 3.18 B	209 ± 2.92 B
Compound B-XIV	100 mg/kg	307 ± 4.49#	255 ± 3.32#	242 ± 4.64 B	235 ± 6.73 B	232 ± 2.76 B
Compound B-XV	100 mg/kg	310 ± 2.96#	255 ± 6.98#	240 ± 5.30 B	234 ± 4.79 B	228 ± 5.70 B
Data is presented as mean ± SD of 8 animals per group. Values with different superscripts down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.						

2-cyano-N-[3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl]pyrrolidine-1-sulfonamide

Yield: 79%; Melting point: 112-1140C; IR (KBr): 1250(C-O); 1450(CH2), 1550(S=O), 1650(C-H), 2250(−C≡N), 3050(N-H), 3400(N-H);1H NMR (400 MHz, CDCl3): 1.40-2.8(m,6H), 3.8(S,1H), 3.81(m,3H), 7.29(d,2H), 7.76(d,2H), 10.58(S,1H); 13C NMR (400 MHz, CDCl3): 20.9-29.3,45.6-55, 55.8, 111-150.3, 164.5-169.3; ESI MS (m/z): 350.09[M+H]; Anal. Calcd. For found C14H15N5O4S: C (48.13%), H (4.33%), N (20.05%) (Tables 1-6).

Table 2. Effect of synthesized compounds on serum LDL level (mg/dl) in Streptozotocin-Nicotinamide+high-fat diet-induced diabetic rats.

Group	**Dose**	**Day 1st day**	**14th day**	**28th day**
Normal control	0.25% CMC	61 ± 2.65	63 ± 2.7	60 ± 3.86
Diabetic control	0.25% CMC	147 ± 3.63	145 ± 4.02	149 ± 2.78B
Vildagliptin	10 mg/kg	125 ± 2.25#	120 ± 4.63B	118 ± 3.38B
B-I	100 mg/kg	125 ± 1.05#	120 ± 3.39B	117 ± 2.36 B
B-XI	100 mg/kg	122 ± 2.73#	118 ± 2.78 B	115 ± 3.71 B
Data is presented as mean ± SD of 8 animals per group. Values with different superscripts down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.				

Table 3. Effect of synthesized compounds on serum HDL level (mg/dl) in Streptozotocin-Nicotinamide+high-fat diet-induced diabetic rats.

Group	**Dose**	**1st day**	**14th day**	**28th day**
Normal control	0.25% CMC	55 ± 5.1	57 ± 3.2	54 ± 3.2
Diabetic control	0.25%CMC	19 ± 2.3	18 ± 3.2	17 ± 4.2
Vildagliptin	10 mg/kg	$23 \pm 4.1^{\#}$	38 ± 3.2^{B}	40 ± 4.2^{B}
B-I	100 mg/kg	$20 \pm 2.5^{\#}$	29 ± 2.3^{B}	32 ± 4.5^{B}
B-XI	100 mg/kg	$22 \pm 2.6^{\#}$	31 ± 3.1^{B}	34 ± 1.6^{B}
Data is presented as mean ± SD of 8 animals per group. Values with different superscripts down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.				

Table 4. Effect of synthesized compounds on serum total cholesterol level (mg/dl) in Streptozotocin-Nicotinamide+high-fat diet-induced diabetic rats.

Group	**Dose**	**1st day**	**14th day**	**28th day**
Normal control	0.25% CMC	80 ± 3. 56	85 ± 4.23	80 ± 5.32
Diabetic control	0.25% CMC	158 ± 4.45	164 ± 6.64	169 ± 5.43
Vildagliptin	10 mg/kg	$145 \pm 5.43^{\#}$	140 ± 5.71^{B}	138 ± 3.72^{B}
B-I	100 mg/kg	$149 \pm 3.41^{\#}$	143 ± 2.31^{B}	138 ± 1.92^{B}
B-XI	100 mg/kg	$147 \pm 2.07^{\#}$	141 ± 5.62^{B}	135 ± 3.58^{B}
Data is presented as mean ± SD of 8 animals per group. Values with different superscripts down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.				

Table 5. Effect of synthesized compounds on serum creatinine level (mg/dl) in Streptozotocin-Nicotinamide+high-fat diet-induced diabetic rats.

Group	**Dose**	**1st day**	**14th day**	**28th day**
Normal control	0.25% CMC	0.63 ± 0.14	0.64 ± 0.17	0.65 ± 0.25
Diabetic control	0.25% CMC	0.96 ± 0.11	1.20 ± 0.26	1.44 ± 0.20
Vildagliptin	10 mg/kg	$0.97 \pm .016^{\#}$	0.81 ± 0.34^{B}	0.72 ± 0.19^{B}
B-I	100 mg/kg	$0.95 \pm 0.06^{\#}$	0.81 ± 0.02^{B}	0.71 ± 0.12^{B}
B-XI	100 mg/kg	$0.92 \pm 0.08^{\#}$	0.78 ± 0.05^{B}	0.70 ± 0.06^{B}
Data is presented as mean ± SD of 8 animals per group. Values with different superscripts down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.				

Table 6. Effect of synthesized compounds on serum urea level (mg/dl) in Streptozotocin-Nicotinamide+high-fat diet.

Group	Dose	1st day	14th day	28th day
Normal control	0.25% CMC	15 ± 1.23	16 ± 1.54	15 ± 1.52
Diabetic control	0.25%CMC	22 ± 2.32	25 ± 3.41	28 ± 3.58
Vildagliptin	10 mg/kg	22 ± 4.35#	18 ± 3.24*	16 ± 2.76*
B-I	100 mg/kg	21 ± 1.82#	21 ± 1.82	18 ± 2.21*
B-XI	100 mg/kg	22 ± 1.31#	20 ± 2.84*	17 ± 1.68*
Data is presented as mean ± SD of 8 animals per group. Values with different superscript down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value. Data is presented as mean ± SD of 8 animals per group. Values with different superscript down the column indicate a significant difference (P<0.05). # means non-significant value. B means significant value.				

DISCUSSION

Chemistry

As shown in Scheme 1, we synthesized a library of sixteen title compounds (B1-B16). We have synthesized several small molecule inhibitors of DPP-IV based on using amino acids: L-proline amide. L-proline on reaction with chlorosulphonyl chloride gave 1-(chlorosulfonyl) pyrrolidine-2-carboxylic acid, which on dehydration with trifluoroacetic anhydride gave 2-cyanopyrrolidine-1-sulphonyl chloride. The compounds were all characterized by IR, 1H NMR, 13C NMR, ESI-MS and CHN.

Anti-diabetic study on HFD-STZ-Nicotinamide induced diabetic rat model

Activation of inflammatory p-65 proteins and MAPKs was induced by STZ/nicotinamide in HFD rats. The anti-diabetic activity of six compounds was examined in diabetic Wistar rats induced with HFD-STZ-Nicotinamide and selected based on their molecular docking scores. Six analogs (B-I, Compound B-II, Compound B-III, Compound B-XI, Compound B-XIV, Compound B-XV) were selected based on their molecular docking scores, *in vitro* assay and were investigated for their anti-diabetic activity in induced HFD-STZ-Nicotinamide diabetic rats by using Vildagliptin as a standard drug. It is noteworthy that all the compounds displayed moderate to excellent anti-diabetic effects. Our B-I and B-XI derivatives have shown significant results in our *in vivo* models but it was lesser than standard Vildagliptin. A clear reduction in Random blood glucose level was observed from the day after treatment with B-I and B-XI ($p<0.05$) when compared to STZ-Nicotinamide and HFD induced diabetic control values. Random blood glucose levels lowered with Vildagliptin as standard when compared with diabetic control. On 14th day ($P < 0.05$) fall was noted of total cholesterol, LDL, creatinine and urea in serum on the treatment of B-XI, and B-I oxadiazole derivatives. A significant increase in HDL levels in serum was observed. A normal control rat was used in this experiment.

Structure-activity relationship (SAR)

An SAR study was conducted based on *in vivo* antihyperglycemic results of the newly developed

series. These results were used to evaluate the possibility of SAR using substituents attached to the phenyl rings of the oxadiazole scaffold. Chlorine substitution on phenyl ring at 3rd position (B-II) exhibited greater activity than hydroxy substitution at 2nd (B-XV) and 4th position (B-I). 4-hydroxy group on phenyl ring (B-I) showed more activity than 2-hydroxy group substitution on phenyl ring (B-XV). For trifluoromethyl 4th position (B-XI) showed lower activity than the 3nd position substitution of chlorine on phenyl ring (B-II). Nitro substitution on 4rd position on phenyl ring (B-III) gave better activity than that of 3,4-disubstituted methyl position (B-XIV). Thus we can say that compounds containing electron-withdrawing groups (trifluoromethyl, chlorine, nitro, hydroxy) displayed a good anti-diabetic effect [28-30].

CONCLUSION

The ligand-binding interactions of synthesized analogs were also studied using molecular docking. *In-vivo* anti-hyperglycemic activity was determined for the six selected compounds based on higher docking scores. The anti-diabetic effects of all compounds were moderate to excellent. Out of all synthesized compounds B-I and B-XI were shown significant results in our *in vivo* models but it was lesser than of standard drug. SAR developed indicated that compounds with electron-withdrawing groups as substitution on phenyl ring of oxadiazole derivatives display pronounced anti-diabetic effect as compared to compounds with electron-donating group. Compound B-XI contacting electron-withdrawing group i.e. trifluro methyl at para position shown significant result but it is lesser than standard Vildagliptin. The oxadiazole hybrids discovered in this study seem to have the potential to become a valuable lead molecule in designing new compounds with potential anti-diabetic activity.

ACKNOWLEDGMENT

The authors are thankful to the management and Principal of Modern College of Pharmacy for providing infrastructure and other research facilities.

REFERENCES

1. Kumar D, Patel G, Chavers AK, Chang KH, Shah K (2011). Synthesis of novel 1, 2, 4-oxadiazoles and analogues as potential anticancer agents. ***Eur J Med Chem*** 46:3085-3092.
2. Gobec M, Tomašič T, Markovič T, Mlinarič-Raščan I, Dolenc M S et al. (2015). Antioxidant and anti-inflammatory properties of 1, 2, 4-oxadiazole analogs of resveratrol. ***Chem Biol Interact*** 240: 200-207.
3. Lankau H J, Unverferth K, Grunwald C, Hartenhauer H, Heinecke, et al. (2007). New GABA-modulating 1, 2, 4-oxadiazole derivatives and their anticonvulsant activity. ***Eur J Med Chem*** 42:873-879.
4. Kim J, Shin J S, Ahn S, Han S B, Jung Y S (2018). 3-Aryl-1, 2, 4-oxadiazole derivatives active against human rhinovirus. ***ACS Med. Chem. Lett*** 9:667-672.
5. Zhu L, Zeng H, Liu D, Fu Y, Wu Q, et al. (2020). Design, synthesis, and biological activity of novel 1, 2, 4-oxadiazole derivatives. ***BMC Chem Biol*** 14:1-12.

6. Yang S, Ren C L, Ma T Y, Zou W Q , Dai L, et al. (2021). 1, 2, 4-Oxadiazole-based bio-isosteres of benzamides: Synthesis, biological activity and toxicity to zebrafish embryo. ***Int J Mol Sci*** 22:2367.
7. Biernacki K, Daśko M, Ciupak O, Kubiński K, Rachon J, et al(2020). Novel 1, 2, 4-oxadiazole derivatives in drug discovery. ***Pharmaceuticals*** 13:111.
8. Dewangan D, T Nakhate K, K Tripathi D, Kashyap P, Dhongde H (2015). Synthesis, characterization and screening for analgesic and anti-inflammatory activities of 2, 5-disubstituted 1, 3, 4-oxadiazole derivatives. Anti-Inflammatory & ***Antiallergy Agents Med Chem*** (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents) 14:138-145.
9. Ranjan C, Kumar J, Sharma K, Akhter M, Siddiqui A A, et al. (2018). 1, 2, 4-Oxadiazole Incorporated Ketoprofen Analogues in Search of Safer Non-steroidal Anti-inflammatory Agents: Design, Syntheses, Biological Evaluation and Molecular Docking Studies. ***Lett Drug Des Discov*** 15:590-601.
10. Haugwitz R D, Martinez A J, Venslavsky J, Angel R G, Maurer B V, et al. (1985). Antiparasitic agents. 6. Synthesis and anthelmintic activities of novel isothiocyanatophenyl-1, 2, 4-oxadiazoles. ***J Med Chem*** 28:1234-1241002E.
11. Wang M, Liu T, Chen S, Wu M, Han J, et al. (2021). Design and synthesis of 3-(4-pyridyl)-5-(4-sulfamido-phenyl)-1, 2, 4-oxadiazole derivatives as novel gsk-3β inhibitors and evaluation of their potential as multifunctional anti-alzheimer agents. ***Eur J Med Chem*** 209: 112874.
12. Kaur P, Bhat Z R, Bhat S, Kumar R, Kumar R, et al. (2020). Synthesis and evaluation of new 1, 2, 4-oxadiazole based trans-acrylic acid derivatives as potential PPAR-alpha/gamma dual agonist. ***Bioorg Chem*** 100:103867.
13. Kumar K A, Lokeshwari D M, Pavithra G, Kumar G V (2012). 1, 2, 4-Oxadiazoles: A potential pharmacological agents-An overview. ***Res J Pharm Technol*** 5:1490-1496.
14. Xu J, Wei L, Mathvink R J, Edmondson S D, Eiermann G J, et al. (2006). Discovery of potent, selective, and orally bioavailable oxadiazole-based dipeptidyl peptidase IV inhibitors. ***Bioorganic Med Chem.KLet*** 16:5373-5377.
15. Kayukova L A, Uzakova A B, Baitursynova G P, Dyusembaeva G T, Shul'gau Z T, et al.(2020). Study on the interaction of triaryl-dihydro-1, 2, 4-oxadiazoles with α-glucosidase. ***DARU J Pharm Sci*** 28:109-117.
16. Liu J, Chen Y, Li J Y, Luo C, Li J,et al. (2019). Inhibition of α-Amylase and α-Glucosidase by New β-Aminopropionamidoxime Derivatives. ***Pharm chem j*** 53:129-133.

17. Matsumoto K, Yoshitomi T, Ishimoto Y, Tanaka N, Takahashi K, et al. (2018). Function-oriented synthesis of marine phidianidine derivatives as potential PTP1B inhibitors with specific selectivity. ***Mar Drugs*** 16:97.
18. Kaur P, Bhat Z R, Bhat S, Kumar R, Kumar R, et al.(2018). DS-8500a, an orally available G protein-coupled receptor 119 agonist, upregulates glucagon-like peptide-1 and enhances glucose-dependent insulin secretion and improves glucose homeostasis in type 2 diabetic rats. ***J Pharmacol Exp Ther*** 367:509-517.
19. Kaur P, Bhat Z R, Bhat S, Kumar R, Kumar R, et al.(2015). Phenoxymethyl 1, 3-oxazoles and 1, 2, 4-oxadiazoles as potent and selective agonists of free fatty acid receptor 1 (GPR40). ***Bioorg Med Chem Lett*** 25:3105-3111.
20. Zander M, Madsbad S, Madsen J L, Holst J J (2002). Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: a parallel-group study. ***The Lancet*** 359:824-830.
21. Deacon C F, Ahrén B, Holst J J (2004). Inhibitors of dipeptidyl peptidase IV: A novel approach for the prevention and treatment of Type 2 diabetes? ***Expert Opin Investig Drugs*** 13:1091-1102.
22. Zare A, Hasaninejad A, Moosavi-Zare A R, Parhami A, Khalafi-Nezhad A (2009). A conversion of carboxylic acids to amides under microwave irradiation. ***Asian J Chem*** 21:1090.
23. Sharma R, SomanS S (2015). Design and synthesis of sulfonamide derivatives of pyrrolidine and piperidine as anti-diabetic agents. ***Eur J Med Chem*** 90:42-350.
24. Trinder P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. ***Ann Clin Biochem*** 6:24-27. Van Herck, H Baumans, V Brandt, C J W M, Boere H A G (1953). A new method for the direct determination of serum cholesterol. ***J Lab Clin Med*** 41:486-492.
25. Vardarli I Nauck M A, Köthe L D, Deacon C F, Holst J J, Schweizer A, et al.(2011). Inhibition of DPP-IV with vildagliptin improved insulin secretion in response to oral as well as “isoglycemic” intravenous glucose without numerically changing the incretin effect in patients with type-II diabetes. ***J Clin Endocrinol Metab*** 96:945-954.
26. Kroll M H, Roach N A, Poe B, Elin R J (1987). Mechanism of interference with the Jaffé reaction for creatinine. ***Clin Chem*** 33:1129-1132.
27. Wills M R, Savory J O H N. (1981). Biochemistry of renal failure. ***Ann Clin Lab Sci*** 11:292-299.

28. Zarei M (2018). A mild and efficient one-pot preparation of 1, 2, 4-oxadiazoles from nitriles and carboxylic acids using vilsmeier reagent. ***Chemistry Select*** 3:11273-11276.

29. Van Herck, H Baumans, V Brandt, C J W M, Boere H A G, et al. (2001). Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: Comparative effects on selected behavioural and blood variables. ***Lab Anim*** 35:131-139.

Correspondence Author:

Megha Tukaram Salve*

Department of Pharmaceutical Chemistry, Modern College of Pharmacy, Nigdi, Pune, Maharastra -411044, India

E-mail: meghasalve77@gmail.com
Tel: 7620156366

Date of Submission: 02-October-2022, Manuscript No. IJDRT-22-76407; **Editor assigned:** 05-October-2022, Pre QC No. P-76407; **Reviewed:** 18- October-2022, QC No. Q-76407; **Revised:** 25- October-2022, Manuscript No. R-76407; **Published:** 29- October-2022, DOI: 10.37421/2277-1506.2022.11.376

Cite This Article: Salve MT, (2022) "Synthesis of Cyclopyrrolidine Clubbed With Oxadiazole Bases and Evaluation of Their Anti-Diabetic Activity Through *In Vivo* Model." ***International Journal of Drug Research and Technology*** Vol. 11 (10) 1-16.