Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(16):46-56
(http://www.derpharmachemica.com/archive.html)

Synthesis of Some Novel Substituted 4-Morphionylphenyl-6-arylpyrimidine-2-imine Derivatives and Screening their Antihyperglycemic Activity

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ABSTRACT

Hyperglycemia is one of the major causes for various types of diseases and clinical complications. 4-Morphionylphenyl-6-arylpyrimidine-2-imines (8a-k) are good sources of medicinal compounds, and are novelty used to control diabetes mellitus. In this study the compounds (8a-k) are investigated by the molecular docking analysis, in vitro enzyme inhibitory activity, and in vivo antihyperglycemic activity. The molecular docking results of compounds (8a-k) suggest one potential agent as 1v4s protein activators for future hyperglycemic therapeutics. The compounds (8a-k) show the significant inhibiting activity of the alpha-amylase enzyme at the concentration of (100 µg/ml) as compared to the alpha-glucosidase enzyme (100 µg/ml). The in vivo antihyperglycemic activity of compounds (8a-k) 10 mg/kg (po) against nicotinamide 120 mg/kg (po)-streptozotocin 60 mg/kg/b.w (ip)-induced diabetes in healthy adult male albino Wistar rats are estimated and the entire designed compounds (8a-k) exhibits significant antihyperglycemic activity. These results suggest that the compounds (8a-k) may be a potential source for the development of new oral antihyperglycemic agent.

Keywords: Morpholine, 1v4s protein, Antihyperglycemia, IC_{50} values, Wistar rats

INTRODUCTION

Diabetes Mellitus (DM) is a complex metabolic disorder worldwide, caused by inherited and acquired deficiency in the production of insulin in beta cells of the pancreas, or by the desensitization of insulin receptors for insulin [1,2]. Such a deficiency results in the increased concentration of glucose in the serum which results in secondary complications affecting the kidneys, eyes, arteries and nerves [3]. Various types of diabetes mellitus have been identified and categorized as, type 1-Insulin Dependent Diabetes Mellitus (IDDM), type 2-Non-Insulin Dependent Diabetes Mellitus (NIDDM), gestational [4] and other specific types of diabetes mellitus. Antihyperglycemic medication seeks to maintain a normal glucose level. The classification of antihyperglycemic drug by origin, the chemical structure of action and mechanism includes insulin and insulin analogs and oral antihyperglycemic agents [5] used in T2DM. Current oral treatment options can be subdivided into the antihyperglycemic drugs (Figure 1) (α-glucosidase inhibitors, thiazolidinediones such as pioglitazone, and biguanides) is used as an insulin sensitizer to improve peripheral glucose uptake and antihypoglycemic [6,7] drugs (Benzoic acid derivatives and sulfonylureas) are insulinotropic and work as secretagogue for pancreatic cells. In the biguanide class metformin hydrochloride [8,9] (N, N-dimethylimidodicarbonimidic diamide hydrochloride) is primarily used for T2DM. Buformin [10] and phenformin [11-14] are closely related biguanides that have also been used to treat hyperglycemia, but their use has been discontinued due to the high risk of lactic acidosis. For this reasons metformin and not phenformin or buformin have been selected as this study. Some of the pharmaceutically important drugs contain morpholine [15,16] moiety in addition to N-heterocycles which are separated by one or more number of carbon atoms. In the present study, novel 4-Morphionylphenyl-6-arylpyrimidine-2-imines (8a-k) are synthesize adopting different schemes and results than compounds are characterize and evaluate for antihyperglycemic activity.

MATERIALS AND METHODS

General: The melting points are taken in electrothermal capillary melting points apparatus and are uncorrected. All the reactions are performed as per standard procedures and monitored on Merck aluminium thin layer chromatography plates using eluting solvents such as hexane and ethyl acetate (2:1). IR spectra are recorded on the KBr disc on an Agilent Cary 650 FTIR spectrometer. ^{1}H-NMR (400MHz) and ^{13}C-NMR (100 MHz) spectra are measured on an NMR Bruker Avance III in Deuterated Dimethyl Sulfoxide (DMSO-d_6) as a solvent, using Tetramethylsilane (TMS) as internal standard; chemical shifts are expressed as δ/ppm. Mass spectrometric data are determined using an API 3000 series mass spectrometer. Satisfactory microanalysis data are obtained on vario MICRO V2.2.0 CHN analyzer.

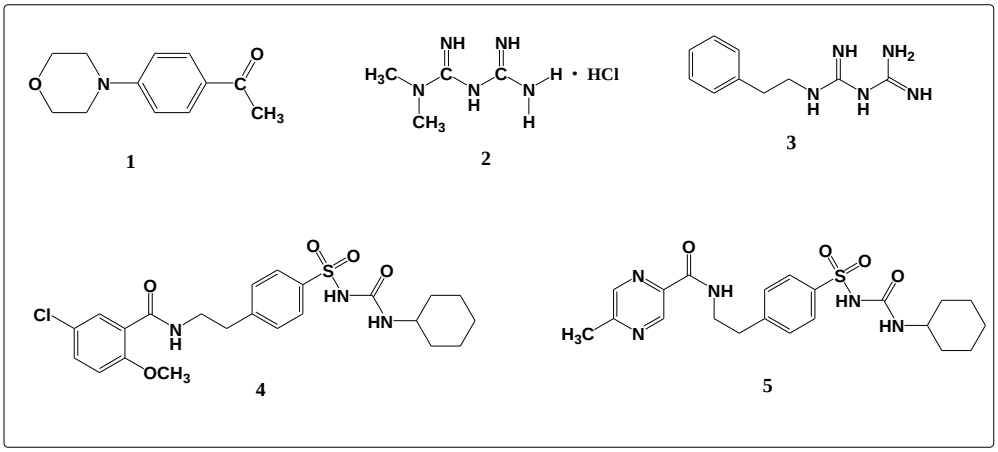


Figure 1: Chemical structures of some oral anti-diabetic drug: (1) 4-Morpholino acetophenone, (2) Metformin hydrochloride, (3) Phenformin, (4) Glyburide, (5) Glipizide

General procedure for synthesis of 4-morphionylphenyl-6-arylpyrimidine-2-imines (8a-k): A mixture of substituted 1- (4-morpholinophenyl)-3-phenylprop-2-en-1-one (8a-k) (Scheme 1) (0.01 mol) and metformin hydrochloride 2 (0.01 mol) are refluxed with methanol (25 ml) in the presence of anhydrous sodium methoxide (1 ml of 50%) at specified time. The flow of the reaction is monitored by Thin Layer Chromatography (TLC). After completion of the reaction, the reaction mixture is poured into crushed ice, filtered and recrystallized for its purification using ethanol as a solvent (70-80% yield) (8a-k) (Scheme 1).

(E)-dimethyl-2-(4-(4-morpholinophenyl)-6-phenylpyrimidin-2-yl)guanidine (8a): Chemical formula: $C_{23}H_{26}N_6O$; Mol. Wt.: 402, m.p.: 80-82°C, (0.79 mg, 77% yield). Color: Pale brown, elemental analysis (%): calculated: C, 68.66; H, 6.47; N, 20.89; found: C, 71.95; H, 6.04; N, 23.12; FTIR (KBr/cm^{-1}); 3423 (-NH_2), 2959 (Aromatic, –CH str.), 2919 (Aliphatic, -CH str.), 1596 (-C=N str.), 1518 (Aromatic, -C=C), 1383 (C-N morphionyl), 1118 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.03 (2H, s), 7.91 (1H, d), 6.86-7.48 (9H, m), 3.88 (4H, t), 3.29 (4H, t), 3.17 (6H, s); ^{13}C NMR (100 MHz, DMSO-d6); (δ, ppm), 164.9, 163.4, 162.7, 157.1, 130.7, 128.9, 128.8, 128.5, 128.3, 127.2, 121.9, 114.6, 113.4, 103.5, 65.1, 47.8, 36.3; MS-m/z: 403 (M+H$^+$).

(E)-dimethyl-2-(4-(4-morpholinophenyl)-6-p-tolylpyrimidin-2-yl)guanidine (8b): Chemical formula: $C_{24}H_{28}N_6O$; Mol. Wt.: 416, m.p.: 76-78°C, (0.92 mg, 80% yield). Color: Pale brown, elemental analysis (%): calculated: C, 69.23; H, 6.73; N, 20.19; found: C, 69.26; H, 6.75; N, 20.24; FTIR (KBr/cm^{-1}); 3390 (-NH_2), 2962 (Aromatic, –CH str.), 2856 (Aliphatic, -CH str.), 1592 (-C=N str.), 1501 (Aromatic, -C=C), 1383 (C-N morphionyl), 1115 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.02 (2H, s), 7.93 (1H, d), 6.87-7.93 (8H, m), 3.87 (4H, t), 3.26 (4H, t), 3.12 (6H, s), 1.94 (3H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.2, 164.7, 163.1, 157.3, 129.5, 128.5, 128.4, 128.3, 127.1, 127.0, 114.8, 114.5,104.3, 66.7, 48.2, 29.7, 29.4; MS-m/z: 417 (M+H$^+$).

(E)-2-(4-(4-methoxyphenyl)-6- (4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8c): Chemical formula: $C_{24}H_{28}N_6O_2$; Mol. Wt.: 432, m.p.: 75-77°C, (0.79 mg, 76% yield). Color: Pale brown, elemental analysis (%): calculated: C, 66.67; H, 6.48; N, 19.44; found: C, 66.69; H, 6.53; N, 19.49; FTIR (KBr/cm^{-1}); 3453 (-NH_2), 2962 (Aromatic, –CH str.), 2851 (Aliphatic, -CH str.), 1592 (-C=N str.), 1509 (Aromatic, -C=C), 1357 (C-N morphionyl), 1118 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.00 (2H, s), 7.98 (1H, d), 6.87-7.43 (8H, m), 3.86 (3H, s), 3.78 (4H, t), 3.19 (4H, t), 3.00 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ ppm), 164.3, 163.7, 161.6, 157.1, 128.7, 128.4, 127.9, 127.6, 115.7, 114.5, 114.3, 114.1, 103.7, 66.7, 55.4, 48.2, 37.8; MS-m/z: 433 (M+H$^+$).

(E)-dimethyl-2-(4-(4-morpholinophenyl)-6- (4-nitrophenyl)pyrimidin-2-yl)guanidine (8d): Chemical formula: $C_{23}H_{25}N_7O_3$; Mol. Wt.: 447, m.p.: 148-150°C, (0.66 mg, 70% yield). Color: Pale brown, elemental analysis (%): calculated: C, 61.74; H, 5.59; N, 21.92; found: C, 61.79; H, 5.64; N, 21.97; FTIR (KBr/cm^{-1}); 3441 (-NH_2), 2957 (Aromatic, –CH str.), 2851 (Aliphatic, -CH str.), 1601 (-C=N str.), 1518 (Aromatic, -C=C), 1351 (C-N morphionyl), 1115 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.47 (2H, s), 8.45 (1H, d), 7.06-8.37 (8H, m), 3.76 (4H, t), 3.26 (4H, t), 3.06 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.5, 164.3, 163.7, 157.0, 130.7, 130.1, 129.9, 128.3, 121.7, 116.1, 115.3, 114.7, 102.7, 65.9, 47.3, 36.9; MS-m/z: 448 (M+H$^+$).

(E)-2-(4-(4-fluorophenyl)-6- (4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8e): Chemical formula: $C_{23}H_{25}N_6OF$; Mol. Wt.: 420, m.p.: 74-78°C, (0.72 mg, 74% yield). Color: Pale brown, elemental analysis (%): calculated: C, 65.71; H, 5.95; N, 20.00; found: C, 65.73; H, 5.99; N, 20.05; FTIR (KBr/cm^{-1}); 3449 (-NH_2), 3069 (Aromatic, –CH str.), 2961 (Aliphatic, -CH str.), 1598 (-C=N str.), 1508 (Aromatic, -C=C), 1358 (C-N morphionyl), 1121 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.05 (2H, s), 7.89 (1H, d), 6.95-7.87 (8H, m), 3.86 (4H, t), 3.28 (4H, t), 3.17 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.3, 164.6, 163.1, 154.3, 130.7, 130.1, 129.9, 128.3, 121.7, 116.1, 115.3, 114.7, 103.3, 66.7, 47.51, 36.9; MS-m/z: 421 (M+H$^+$).

(E)-2-(4-(4-chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8f): Chemical formula: $C_{23}H_{25}N_6OCl$; Mol. Wt.: 436, m.p.: 76-78°C, (0.83 mg, 78% yield). Color: Pale yellow, elemental analysis (%): calculated: C, 63.30; H, 5.73; N, 19.27; found: C, 63.35; H, 5.77; N, 19.32; FTIR (KBr/cm^{-1}); 3428 (-NH_2), 2920 (Aromatic, –CH str.), 2852 (Aliphatic, -CH str.), 1605 (-C=N str.), 1518 (Aromatic, -C=C), 1359 (C-N morphionyl), 1121 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.01 (2H, s), 7.76 (1H, d), 6.90-7.71 (8H, m), 3.86 (4H, t), 3.34 (4H, t), 3.15 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.2, 164.8, 163.3, 154.3, 136.0, 133.8, 130.7, 129.2, 128.6, 122.4, 114.6, 113.4, 104.7, 66.6, 47.4, 29.7; MS-m/z: 437 (M+H$^+$).

(E)-2-(4-(4-cyanophenyl)-6-(4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8g): Chemical formula: $C_{24}H_{25}N_7O$; Mol. Wt.: 427, m.p.: 80-82°C, (0.59 mg, 70% yield). Color: Pale brown, elemental analysis (%): calculated: C, 67.45; H, 5.85; N, 22.95; found: C, 67.48; H, 5.90; N, 23.01; FTIR (KBr/cm^{-1}); 3337 (-NH_2), 3197 (Aromatic, –CH str.), 2956 (Aliphatic, -CH str.), 2225 (-C≡N str.), 1593 (-C=N str.), 1557 (Aromatic, -C=C), 1352 (C-N morphionyl), 1111 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.38 (2H, s), 7.09 (1H, d), 6.95-

8.09 (8H, m), 3.76 (4H, t), 3.30 (4H, t), 3.25 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 164.1, 163.9, 161.9, 158.1, 132.2, 129.5, 128.1, 127.7, 127.6, 127.2, 126.4, 119.0, 118.7, 114.1, 102.5, 65.9, 46.7, 36.8; MS-m/z: 428 ($M+H^+$).

(E)-2-(4-(4-dimethylamino)phenyl)-6-(4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8h): Chemical formula: $C_{25}H_{31}N_7O$; Mol. Wt.: 445, m.p.: 77-78°C, (0.77 mg, 78% yield). Color: Pale brown, elemental analysis (%): calculated: C, 67.41; H, 6.97; N, 22.02; found: C, 67.46; H, 6.99; N, 22.07; FTIR (KBr/cm^{-1}); 3439 (-NH_2), 2918 (Aromatic, –CH str.), 2850 (Aliphatic, -CH str.), 1599 (-C=N str.), 1516 (Aromatic, -C=C), 1358 (C-N morphionyl), 1119 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.01 (2H, s), 8.0 (1H, d), 6.71-7.99 (8H, m), 3.88 (4H, t), 3.30 (4H, t), 3.15 (6H, s), 3.08 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.3, 164.5, 163.9, 154.6, 142.2, 138.3, 127.2,123.4, 120.4, 118.3, 114.3, 111.6, 104.7, 66.8, 48.5, 40.3, 29.7; MS-m/z: 446 ($M+H^+$).

(E)-dimethyl-2-(4-(4-morpholinophenyl)-6-o-tolylpyrimidin-2-yl)guanidine (8i): Chemical formula: $C_{24}H_{28}N_6O$; Mol. Wt.: 416, m.p.: 60-62°C, (0.65 mg, 72% yield). Color: Pale brown, elemental analysis (%): calculated: C, 69.23; H, 6.73; N, 20.19; found: C, 68.94; H, 7.08; N, 19.65; FTIR (KBr/cm^{-1}); 3397 (-NH_2), 2960 (Aromatic, –CH str.), 2855 (Aliphatic, -CH str.), 1605 (-C=N str.), 1511 (Aromatic, -C=C), 1380 (C-N morphionyl), 1115 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.03 (2H, s), 8.01 (1H, d), 6.88-7.57 (8H, m), 3.78 (4H, t), 3.26 (4H, t), 3.16 (6H, s), 2.98 (3H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm),165.9, 164.4, 163.8, 157.2, 144.2, 136.9, 127.2, 127.7, 126.3, 125.8, 115.2, 114.6, 107.3, 66.9, 48.5, 37.2, 19.0; MS-m/z: 417 ($M+H^+$).

(E)-2-(4-(2-chlorophenyl)-6-(4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8j): Chemical formula: $C_{23}H_{25}N_6OCl$; Mol. Wt.: 436, m.p.: 94-96°C, (0.73 mg, 79% yield). Color: Pale yellow, elemental analysis (%): calculated: C, 63.30; H, 5.73; N, 19.27; found: C, 63.35; H, 5.77; N, 19.32; FTIR (KBr/cm^{-1}); 3425 (-NH_2), 2961 (Aromatic, –CH str.), 2855 (Aliphatic, -CH str.), 1598 (-C=N str.), 1520 (Aromatic, -C=C), 1233 (C-N morphionyl), 1119 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 8.02 (2H, s), 7.99 (1H, d), 6.53-7.03 (8H, m), 3.82 (4H, t), 3.27 (4H, t), 3.15 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm),165.2, 164.7, 163.5, 152.7, 138.5, 132.7, 128.7, 128.1, 127.6, 126.8, 126.5, 124.8, 104.2, 66.7, 48.3, 37.3; MS-m/z: 437 ($M+H^+$).

(E)-2-(4-(2-bromophenyl)-6-(4-morpholinophenyl)pyrimidin-2-yl)dimethylguanidine (8k): Chemical formula: $C_{23}H_{25}N_6OBr$; Mol. Wt.: 480, m.p.: 94-96°C, (0.88 mg, 72% yield). Color: Pale brown, elemental analysis (%): calculated: C, 57.50; H, 5.21; N, 17.50; found: C, 71.95; H, 6.04; N, 23.12; FTIR (KBr/cm^{-1}); 3441 (-NH_2), 2960 (Aromatic, –CH str.), 2855 (Aliphatic, -CH str.), 1604 (-C=N str.), 1517 (Aromatic, -C=C), 1398 (C-N morphionyl), 1119 (C-O morphionyl); ^{1}H-NMR (400 MHz, DMSO-d6): (δ, ppm) 7.59 (2H, s), 7.50 (1H, d), 6.52-7.22 (8H, m), 3.85 (4H, t), 3.22 (4H, t), 3.06 (6H, s); ^{13}C-NMR (100 MHz, DMSO-d6); (δ, ppm), 165.8, 164.1, 163.5, 156.8, 132.6, 131.1, 129.9, 127.7, 123.7, 122.7, 115.2, 114.7, 105.8, 66.8, 48.3, 37.0; MS-m/z: 481 ($M+H^+$).

Molecular docking studies

Identify the receptor drug molecules, it is very important to act the protein-ligand docking, the modeling experiment used to identify the orientation and the position of the ligand upon docking. For the present investigation, the CDOCKER, available on the Discovery Studio is adopted. CDOCKER specifically employ the CHARMm- based molecular dynamics method and further generates confirmation adoption the high temperature and are later escorted onto the binding site for binding pose studies.

Protein preparation

The selection of protein for the current investigation is one of the most crucial aspects. The selected crystal structure of glucokinase (1v4s) protein is prepared prior to the docking analysis by correcting the chemistry of the losing hydrogen. Therefore the 1v4s protein is subjected to energy minimization by applying the CHARMm force field until a satisfactory gradient tolerance is obtained.

Ligand preparation

The ligand preparation process consists of several steps that perform conversions; corrections are applied to the structures, optimize the structure and eliminate unwanted structures. These compounds are drawn in ChemDraw and their respective three-dimensional structures are produced on the Discovery Studio (v 16.1.0.15350). The CHARMm force field is applied as a measure to minimize the ligand molecules. the process like convert the format of the structure, select the structures, hydrogen atom added, unwanted molecules are removed, charged groups neutralized, ionization states are generated, generate low-energy ring conformations to get the output file.

***In vitro* enzyme inhibition assay**

***In vitro* α-amylase inhibition assay**: The α-amylase (0.5 mg/ml) is premixed with the tested compounds (8a-k) at various concentrations (20-100 µg/ml) and starch as a substrate is added as a 0.5% starch solution to start the reaction. The reaction is carried out for 5 min at 37°C and terminated by the addition of 2 ml of 3,5-Dinitrosalicylic acid (DNS) reagent. The reaction mixture is heated for 15 min at 100°C and diluted with 10 ml of distilled water in an ice bath. α- amylase activity is determined by measuring spectrum at 540 nm. The % α-amylase inhibitory activity is calculated by the following formula:

$$\% \text{ Inhibition} = (\text{Control OD-Sample OD/Control OD}) \times 100$$

The IC_{50} value is defined as the concentration of the tested compounds (8a-k) to inhibit 50% of α-amylase activity under assay condition.

***In vitro* α-glucosidase inhibition assay**: The enzyme α-glucosidase inhibitory activity is determined by premixing α-glucosidase (0.07 Units) with 20-100 µg/ml of tested compounds (8a-k). Then 3 mM p-nitro phenyl glucopyranoside is added as a substrate. This reaction mixture is incubated for 30 min at 37°C and the reaction is terminated by the addition of 2 ml of sodium carbonate. The α-glucosidase activity is determined by measuring the p-nitro phenyl release from p-nitrophenyl glucopyranoside at 400 nm. The % α-glucosidase inhibitory activity is calculated by the following formula:

$$\% \text{ Inhibition} = (\text{Control OD-Sample OD/Control OD}) \times 100$$

The IC_{50} value is defined as the concentration of the tested compounds (8a-k) to inhibit 50% of α-glucosidase activity under assay condition.

***In vivo* antihyperglycemic assay**

Animals and maintenance: Healthy adult male albino Wistar rats aged 6 weeks and weighing between 150-200 g are (Sri Venkateshwara Enterprises, Pvt. Ltd, Bangalore) housed in clean polypropylene cages and maintained in a well-ventilated temperature controlled animal house with a constant 24 h dark and light schedule. The animals are fed with standard rat pelleted diet and clean drinking water is made available ad libitum. All animal procedures are performed after approval from the KMCH College of Pharmacy, Department of Pharmacology, Coimbatore (Registration number: KMCRET/PhD/01/2016-17) ethics committee and in accordance with the recommendations for the proper use of laboratory animals.

Induction of diabetes: The rats are kept overnight fasting and test the fasting blood glucose at an initial time from the tip of rat tail vein. Nicotinamide is dissolved in normal saline and streptozotocin is dissolved in a 0.1 mol/l citrate buffer (pH 4.5) at a dosage of 35 mg/kg body weight. NIDDM is induced in overnight fasted rats by a single intraperitoneal injection of 60 mg/kg streptozotocin, 30 min after the i.p administration of 120 mg/kg of nicotinamide. Thirty minutes following the synthesized compounds or metformin treatment, each rat is given an oral glucose load of 3 g/kg body weight. Diabetes is confirmed by the elevated level of blood glucose are measured at 72 h. The animals with blood glucose concentration more than 250 mg/dl will be used for the study.

Treatment design

The albino Wistar rats are divided into 14 groups of six animals each. Group 1: Normal control. Group 2: Nicotinamide 120mg/kg (po)+Streptozotocin 60 mg/kg/b.w (ip). Group 3: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with Metformin 10 mg/kg. (po). Group 4: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8a 10 mg/kg. (po). Group 5: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8b 10 mg/kg. (po). Group 6: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8c 10 mg/kg. (po). Group 7: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8d 10 mg/kg. (po). Group 8: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8e 10 mg/kg. (po). Group 9: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8f 10 mg/kg. (po). Group 10: Nicotinamide 120mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8g 10 mg/kg. (po). Group 11: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8h 10 mg/kg. (po). Group 12: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8i 10 mg/kg. (po). Group 13: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8j 10 mg/kg. (po) Group 14: Nicotinamide 120 mg/kg (po)+Streptozotocin (60 mg/kg) animals treated with compound 8k 10 mg/kg (po).

The normal saline, test compounds, and metformin are administered to the respective group animals given once daily by oral gavage for 28 days. During the period of study, test compounds and metformin are freshly dispersed in normal saline and distilled water before to the administration. The fasting animal blood glucose level is estimated on 1st, 10th, 15th and the 28th day from the tip of rat tail vein. The body weight of the entire animal in each group is also noted during the experiment period.

Statistical analysis

All the tests of fasting blood glucose and body weight estimations are carried out in triplicates. The values are expressed as Mean ± SD (n=3) analyzed by One-way Analysis of Variance (ANOVA) and post hoc Dunnett's-test. Differences between groups are considered to be significant if $p<0.05$. The IC_{50} values are determined from plots of percentage inhibition versus log inhibitor concentration and are calculated by non-linear regression analysis from the mean inhibitory values.

RESULTS AND DISCUSSION

Chemistry

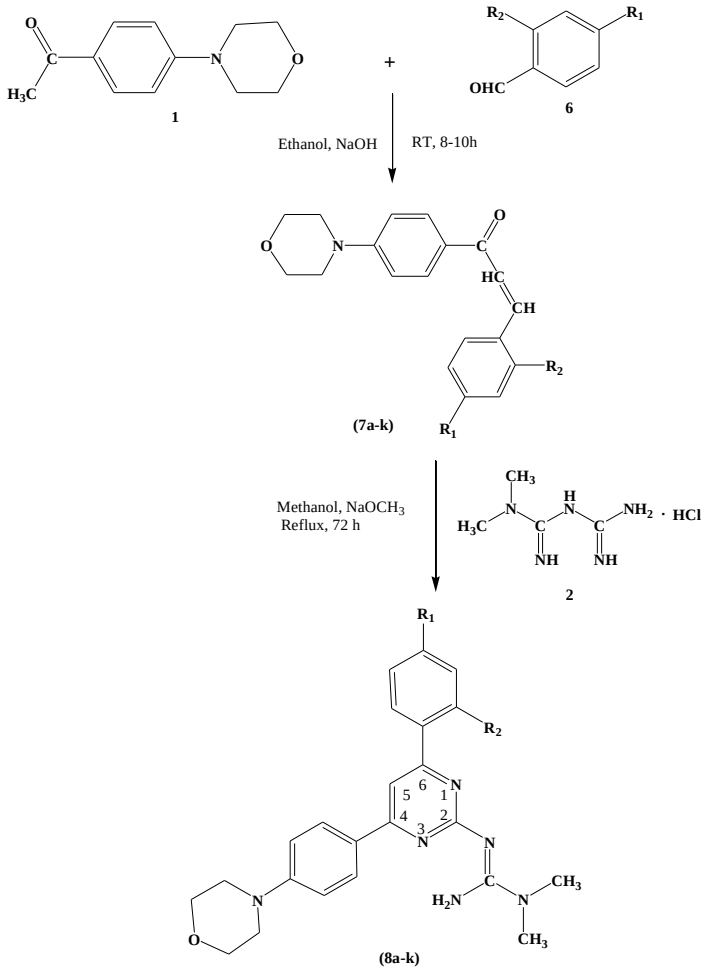
A series of various substituted 1-(4-morpholino phenyl)-3-phenylprop-2-en-1-one (7a-k) (Figure 2) (Scheme 1) are prepared by reacting varies substituted benzaldehyde 6 with 4-Morpholino acetophenones 1 in the presence of aqueous basic medium and synthesized chalcones are reacted with metformin hydrochloride 2 in alkali medium methanol as a solvent form varies substituted (*E*)-dimethyl-2-(4-(-morpholinophenyl)-6-phenylpyrimidin-2-yl)guanidine (8a-k) (Figure 2) (Scheme 1) compounds.

The synthesized compound (8a-k) structures are characterized by its FTIR spectroscopy, ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectrometry analysis. The IR spectrum of the synthesized compounds reveals a broad, strong band around 3453-3337 cm^{-1} representing the presence of $–NH_2$ group of guanidine group. The appearance of the characteristic band at 1605-1592 cm^{-1} is due to –C=N- group. The band at 1501-1557 cm^{-1} confirms the aromatic C=C group. The morpholine group C-N and C-O stretching vibrations are observed at 1233-1398 cm^{-1} and 1111-1121 cm^{-1} respectively. The structure of the synthesized products (8a-k) further confirmed by the corresponding ^{1}H-NMR (DMSO-d_6) spectra. A broad singlet from δ=7.59-8.47 ppm which indicates the signal of the NH_2 resonance of compounds (8a-k). The down field doublet of one proton in the region of δ=7.09-8.45 ppm represents the pyrimidine ring proton which reveals that the further confirmation of cyclization compounds (8a-k). The multiplets of aromatic protons appeared in the desired range. The CH_2-O-CH_2 and CH_2-N-CH_2 protons of a morpholine ring appeared as a two triplet at the range of δ=3.76-3.88 ppm and δ=3.19-3.34 ppm respectively. The guanidine group equivalent protons of six hydrogens showed a sharp, strong singlet at the range of δ=3.00-3.25 ppm. The singlet of phenyl ring attached -CH_3, -OCH_3, -N $(CH_3)_2$ appeared in the corresponding range of δ=1.94 ppm, δ=3.86 ppm and δ=3.08 ppm respectively.

On the basis of ^{13}C-NMR (DMSO-d_6) the following signal is assigned to the compounds (8a-k). The guanidine group ipso carbon shows a peak at the most down field region of δ=150.8-165.2 ppm. The ^{13}C-NMR spectrum of compounds (8a-k) shows the signals in the range of δ=164.1-128.8 ppm which represents the pyrimidine ring ipso carbons and the one proton attached to the carbon of the pyrimidine ring is recorded in the range of δ=102.5-107.3 ppm confirm the formation of cyclized products. Two aliphatic intense signals of compound (8a-k) at δ=65.9-66.9 ppm (CH_2-O-CH_2) and δ=47.4-55.4 ppm (CH_2-N-CH_2) indicate the presence of morpholine ring. The signal at the range of δ=29.7-48.2 ppm represents the guanidine group N $(CH_3)_2$ carbon atoms. Finally, the signal of -CH_3, -OCH_3, -N $(CH_3)_2$ and -CN attached to the phenyl ring appeared in the range of δ=29.4, 37.8, 29.7 and 114.1 ppm respectively. The aromatic carbons are seen at the expected chemical shifts. The compounds (8a-k) are further confirmed by mass spectral data and C, H, N elemental analysis.

Scheme 1: Substituted 1-(4-morpholinophenyl)-3-phenylprop-2-en-1-one (8a-k)

Scheme-1



Compound	8a	8b	8c	8d	8e	8f	8g	8h	8i	8j	8k
R1	H	CH_3	OCH_3	NO_2	F	Cl	CN	N $(CH_3)_2$	H	H	H
R2	H	H	H	H	H	H	H	H	CH_3	Cl	Br

Figure 2: Synthetic scheme of 4-morphionylphenyl-6-arylpyrimidine-2-imine derivatives (8a-k)

Molecular docking study with 1v4s protein

Glucokinase (1v4s) is a monomeric cytoplasmic enzyme found in the liver and pancreas. The main function is the regulation of glucose levels in these organs. Standard metformin docked with 1v4s protein with the results of very low cdocker energy of (-21.6 kcal/mol), hence the synthesized compounds contain metformin moiety are docked against 1v4s protein and compare the values of cdocker energy [17,18]. The compounds are developed which contains metformin moiety, which increase the maximal activity and glucose affinity of the enzyme and lower blood glucose in hyperglycemic animals by curbing glucose production by the liver [19]. The results of the docking study, the selection of best 10 poses which are saved for analysis and compared to the standard metformin (-21.6 kcal/mol) (Table 1 and Figure 3).

At last, the pose with least CDOCKER energy [20,21] is used for further study. The compound 8b emerged has a good ligand, demonstrating a lowest CDOCKER energy of -16.81 kcal/mol and its corresponding lowest CDOCKER interaction value of -49.33 kcal/mol respectively. Figure 4 shows the 3D structure of the interaction between compound 8b and the 1v4s binding site. Discovery Studio is used to find out if the active site contains amino acids such as ILE A: 159, TYR A: 61, VAL A: 452, VAL A: 62, VAL A: 455, PRO A: 66, GLN A: 98, GLU A: 67, MET A: 235, TYR A: 214, ILE A: 211, ALA A: 456 shows in 2D structure (Figure 3). Figure 5 represents the interaction of compound 8b with active site residues of Glucokinase receptor. The interaction energies of the compounds with 1v4s protein (glucokinase) affect their antihyperglycemic activities *in vivo*. The cdocker energy value lower which indicate the strong binding of the ligand and the receptor, so the synthesized compounds are enhancing the glucokinase activity and control the blood glucose level of diabetic rats in this study [22]. Further medicinal candidates are required to validate these compounds.

Table 1: Docking results of the designed compounds (8a-k) towards 1v4s

S. No	Entry	-CDOCKER Energy kcal/mol	-CDOCKER Interaction Energy kcal/mol
1	8a	10.01	43.8
2	8b	**16.81**	49.33
3	8c	10.72	52.4
4	8d	5.56	53.69
5	8e	8.44	44.92
6	8f	6.62	52.4
7	8g	10.22	47.83
8	8h	12.4	50.57
9	8i	12.28	47.06
10	8j	2.16	52.49
11	8k	5.09	44.9
12	Metformin	21.6	28.75

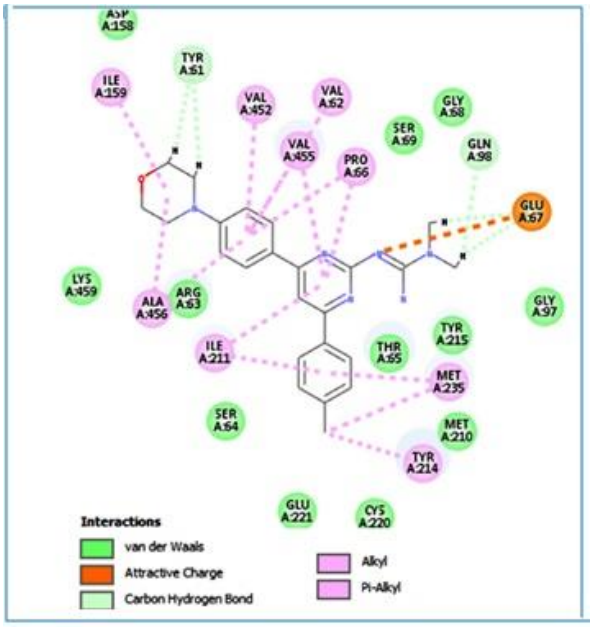


Figure 3: Two-dimensional model of the interaction between compound 8b and the 1v4s binding site

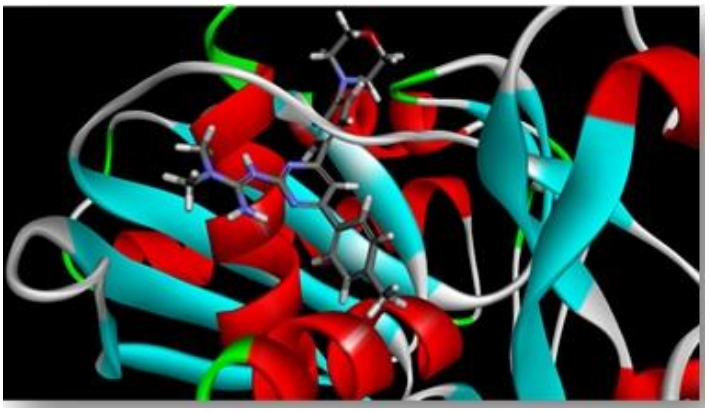


Figure 4: Three-dimensional model of the interaction between compound 8b and the 1v4s binding site

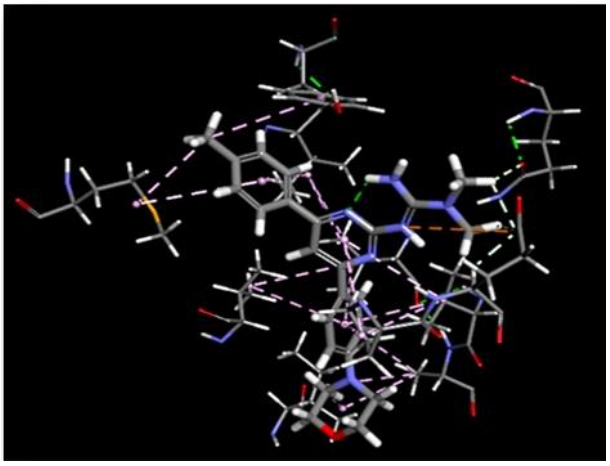


Figure 5: Interaction of compound 8b with active site residues of glucokinase receptor

Table 2: IC_{50} values for *in vitro* alpha-glucosidase and alpha-amylase inhibition by compound (8a-k) Results are expressed in terms of mean ± S.D

Entry	α- Glucosidase inhibition concentration (µg/ml)					IC_{50} (µg/ml)	α- Amylase inhibition concentration (µg/ml)					IC_{50} (µg/ml)
	20	40	60	80	100		20	40	60	80	100	
8a	17.33	30.54	54.65	62.32	87.34	59.25	28.56	44.72	61.33	71.3	94.21	53.07
8b	18.87	38.66	58.21	71.33	90.11	**55.3**	29.32	48.55	67.22	77.23	92.89	**49.78**
8c	15.12	36.22	59.32	64.31	82.37	**56.76**	28.33	45.66	68.71	71.97	90.32	**51.02**
8d	18.32	36.23	55.43	63.23	87.68	60.7	26.33	41.23	58.31	69.21	91.62	56.56
8e	18.28	35.28	52.78	61.73	85.22	57.77	27.45	43.33	62.34	76.22	93.54	51.22
8f	19.31	31.83	58.22	66.21	88.28	57.06	24.42	49.32	63.88	73.21	91.43	51.92
8g	19.86	35.22	50.26	62.63	87.88	59.07	26.44	48.53	62.83	72.55	90.33	51.92
8h	17.18	32.54	57.33	67.33	79.22	59.8	27.32	41.34	67.32	76.31	92.12	**51.03**
8i	19.23	34.78	53.86	68.33	89.22	**56.45**	33.56	48.45	68.43	71.66	92.56	**50.26**
8j	16.54	32.66	51.55	60.49	89.54	59.38	31.23	47.22	65.23	72.34	94.36	51.34
8k	17.11	34.23	48.55	57.26	86.44	61.62	34.43	47.45	64.56	70.73	94.87	51.46

***In vitro* enzyme inhibition activity**

The two enzymes (alpha-amylase and alpha-glucosidase) used in this study are responsible for the metabolism of carbohydrate and helps to control the postprandial hyperglycemia [23]. Alpha-amylase is officially known as 1,4-a-D-Glucan glucanohydrolase. It is one of the most important proteins in saliva, where it breaks down starch by hydrolysis to release maltose. Alpha-glucosidase breaks down starch and disaccharides to glucose. Alpha-amylase [24] and alpha-glucosidase inhibitors are oral hyperglycemia drugs utilized for NIDDM that work by preventing the carbohydrate metabolism. Table 2 summarized the results of percentage inhibition of alpha-amylase and alpha-glucosidase values [25] and IC_{50} values [26] of newly synthesized compounds (8a-k). The oral anti-diabetic drug metformin inhibit the α-glucosidase and α-amylase enzymes to lower the post prandial hyperglycemia. Hence, it is analyze if the synthesized compounds (8a-k) contain metformin residues also inhibit the α-glucosidase and α-amylase enzymes are tested. In this *in vitro* enzyme inhibition study; one should not compare the activities of alpha-amylase and alpha-glucosidase with the positive control.

Only the inhibitory activity of synthesized compounds for the above two enzymes (alpha-amylase and alpha-glucosidase) are analysed and reported. From the results, the compounds 8b, 8c and 8i shows high potent of yeast alpha-glucosidase inhibitory activity 55.30, 56.76 and 56.45 µg/ml respectively. The compounds (8a-k) possess the alpha-amylase inhibitory activity. Compounds 8b, 8c, 8h and 8i IC_{50} values are 49.78, 51.02, 51.03 and 50.26 µg/ml respectively. Figures 6 and 7 depicted the percentage inhibition of alpha-glucosidase and alpha-amylase enzymes respectively. It reveals that the higher concentration 100 µg/ml shows the maximum percentage of inhibition. The inhibition percentage values of compounds (8a-k) gradually increase with an increase in the concentration of both alpha-amylase and alpha-glucosidase. The IC_{50} values of compounds (8a-k) for alpha-amylase and alpha-glucosidase plotted are shown in Figures 8 and 9. From the above results in the compounds 8b possess a least IC_{50} value of alpha-glucosidase inhibition 55.30 µg/ml and alpha-amylase inhibition 49.78 µg/ml which indicate that the compound 8b having higher inhibitory activity than all other synthesized compounds. From the above results in the compound 8b possess a least IC_{50} value of alpha-glucosidase inhibition (55.30 µg/ml) and alpha-amylase inhibition (49.78 µg/ml) which indicate that the compound 8b having higher inhibitory activity than all other synthesized compounds. Presence of bioactive compounds such as morpholine moiety of the phenyl rings attached to C-4 carbon of the pyrimidine moiety, methyl (8b), methoxy (8c) and halogen (8e and 8f) substituent at para position, methyl (8i), chloro (8j) and bromo (8k) at ortho position of the phenyl rings attached to C-6 carbons of the pyrimidine moiety increases the inhibitory activity of the two enzymes such as alpha-amylase and alpha-glucosidase.

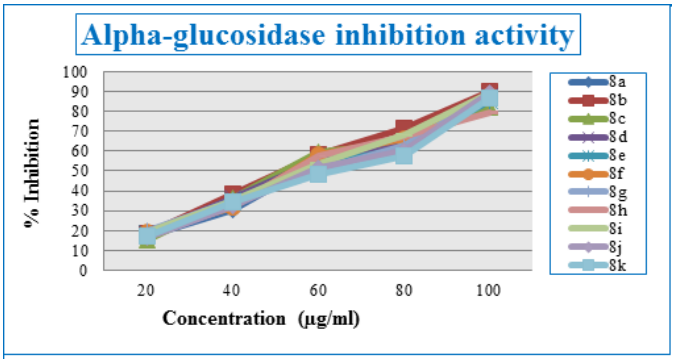


Figure 6: Inhibitory activity of compounds (8a-k) against the alpha-glucosidase enzyme.

Values were expressed as Mean ±SD (n=3) analyzed by one-way analysis of variance (ANOVA) and post hoc Dunnett's-test.

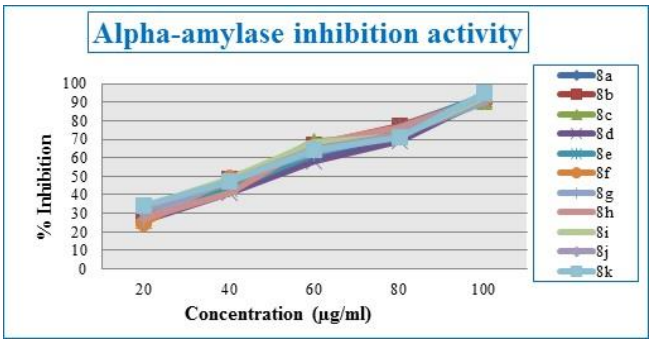


Figure 7: Inhibitory activity of compounds (8a-k) against the alpha-amylase enzyme

Values were expressed as Mean ± SD (n=3) analyzed by one-way analysis of variance (ANOVA) and post hoc Dunnett's-test.

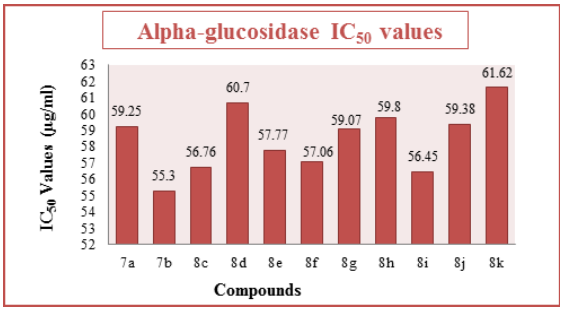


Figure 8: Evaluation of IC_{50} values of compounds (8a-k) on alpha-glucosidase enzyme

Results are expressed in terms of mean ± SD. The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values.

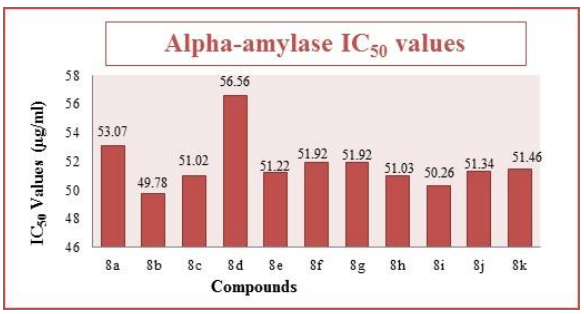


Figure 9: Evaluation of IC_{50} values of compounds (8a-k) on alpha-amylase enzyme

Results are expressed in terms of mean ± SD. The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values.

***In vivo* antihyperglycemic activity**

Effect of compounds on blood glucose level

In normal control rats, the blood sugar level also normal at the initial to the 28th day. Our study is based on T2DM. Nicotinamide-streptozotocin (STZ-NA) induces the T2D in the normal control mice. Hence that rat utilize for further *in vivo* antihyperglycemic studies [27]. There is suddenly increasing the blood glucose level of nicotinamide 120 mg/kg (po)-streptozotocin 60 mg/kg/b.w (ip) (STZ-NA)-induced **[**28-31**]** hyperglycemic rats from initial to final days of the experiment. The standard drug metformin 10 mg/kg (p.o)-treated on hyperglycemic rats, there is a significant reduction in the blood glucose level [32**]** of the hyperglycemic rats. The hyperglycemic animals are treated with compounds (8a-k) 10 mg/kg (p.o.) show a significant reduction in 10th, 15th and 28th day. Compound 8b and 8i treated diabetic animals blood sugar levels (124.7 ± 12.7 mg/dl) are (127.3 ± 31.2 mg/dl) lower than the standard drug metformin (130.3 ± 14.2 mg/dl) at the 28th day and entire compounds results showing close to the normal blood glucose level. It reveals that the overall newly synthesized compounds of (8a-k) show a significant decrease in the blood glucose levels when a comparison with the diabetic-induced [33-35] group (Group 2) at 10th to 28th day. The results of the blood glucose level of animals treated with standard metformin 10 mg/kg and compounds (8a-k) 10 mg/kg are shown in Table 3 and Figure 10.

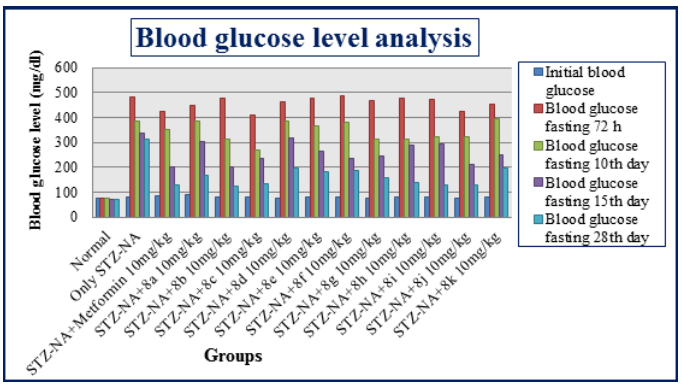


Figure 10: Fasting blood glucose level analysis of compounds (8a-k)

All the tests of fasting blood glucose and body weight estimations were carried out in triplicates. Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's ***P<0.001, **P<0.01,*P<0.05 calculated by comparing treated group with control group.

Table 3: Effect of compounds (8a-k) on blood glucose level analysis

Groups	Blood glucose level (mg/dl)				
	Initial blood glucose	**Blood glucose fasting 72 h**	**Blood glucose fasting 10th day**	**Blood glucose fasting 15th day**	**Blood glucosefasting 28thday**
Normal	78.1211 ± 4.221	73.432 ± 6.3122***	77.543 ± 6.312***	69.321 ± 4.9928**	73.228 ± 2.0123*
Only STZ-NA	81.65 ± 3.453	482.35 ± 30.54	384.76 ± 22.564***	337.74 ± 21.55***	311.59 ± 21.88***
STZ-NA+ Metformin 10 mg/kg	84.55 ± 5.6459	422.77 ± 48.28***	352.21 ± 47.77**	200.86 ± 20.31***	130.33 ± 14.22***
STZ-NA+8a 10 mg/kg	91.23 ± 3.4521	450.43 ± 59.97***	384.45 ± 24.564***	300.76 ± 17.864***	170 ± 14.5643**
STZ-NA+8b 10 mg/kg	81.5623 ± 3.5421	477.65 ± 42.765***	310.43 ± 25.76***	203.78 ± 8.967***	124.730 ± 12.672***
STZ-NA+8c 10 mg/kg	78.213 ± 5.908	410 ± 48.549***	267.543 ± 12.75	234.84 ± 11.945**	132.429 ± 14.970***
STZ-NA+8d 10 mg/kg	73.67 ± 9.765	464.75 ± 43.21**	384.97 ± 23.88***	317.97 ± 8.786**	198.76 ± 40.77
STZ-NA+8e 10 mg/kg	80 ± 3.87298	478.33 ± 54.1888**	365 ± 38.449***	266.66 ± 26.7914***	182.5 ± 11.529***
STZ-NA+8f 10 mg/kg	79.66 ± 21.673	486 ± 32.5512**	382.241 ± 31.95**	234.21 ± 34.21***	186.645 ± 21.77***
STZ-NA+8g 10 mg/kg	75 ± 3.65148	466.66 ± 67.26***	315 ± 13.102*	245 ± 17.4643**	158.333 ± 12.7584*
STZ-NA+8h 10 mg/kg	81.65 ± 7.99	475 ± 42.34***	311.55 ± 21.77***	289.53 ± 25.88**	137.32 ± 21.896***
STZ-NA+8i 10 mg/kg	79.412 ± 42.11	470.412 ± 36.99***	321.341 ± 42.33**	292.532 ± 12.22***	127.312 ± 31.223***
STZ-NA+8j 10 mg/kg	77.21345 ± 5.6754	423.22 ± 39.876***	321.76 ± 27.986***	210.75 ± 8.943***	130.121 ± 12.987***
STZ-NA+8k 10 mg/kg	78.43 ± 7.4312	455.32 ± 31.432***	393.22 ± 28.4428**	247.7721 ± 45.32***	194.32 ± 17.64**

All blood glucose levels are recorded in mg/dl. Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's ***P<0.001, **P<0.01,*P<0.05 calculated by comparing treated group with control group

Effect of compounds on body weight

After the treatment of the 28 day period, it is observed that, slight increase in the body weight of normal control rats. It may be due to their normal growth. From the Table 4 the results are showing that a significant decrease in the body weight of animals after nicotinamide 120 mg/kg (po)-streptozotocin 60 mg/kg/b.w (ip) (STZ-NA)-treated hyperglycemic rats [36,37]. The decrease body weights [38] of the rats are significantly increase after treatment with the compounds (8a-k) 10 mg/kg (p.o.) and standard drug metformin 10 mg/kg (p.o.) when compared with the hyperglycemic control group. The changes in body weight [39] of the animals during initial, 72 h, 10th, 15thand 28th days are shown in Figure 11. Presence of morpholine moiety, methyl, methoxy and halogen substituent at para position of the phenyl rings attached to C-4 and C-6 carbons of the pyrimidine moiety are responsible for the decrease of fasting blood glucose and increases fasting body weight of the diabetic animals near to the normal control animals.

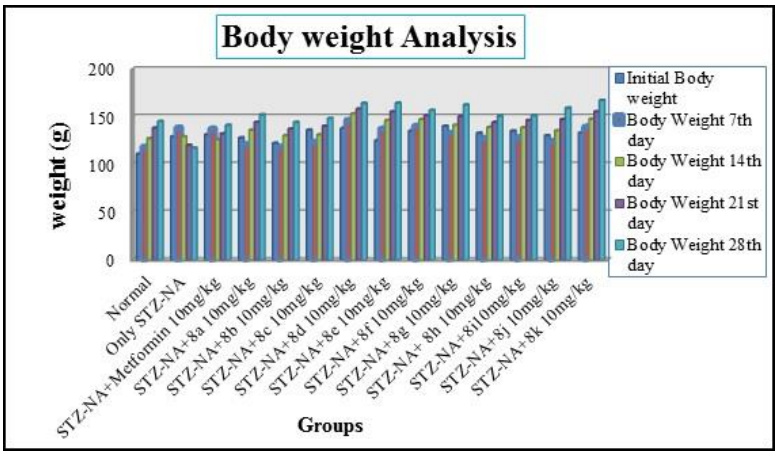


Figure 11: Fasting body weight analysis of compounds (8a-k)

All the tests of fasting body weight estimations were carried out in triplicates. Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$ calculated by comparing treated group with control group.

Table 4: Effect of compounds (8a-k) on fasting body weight analysis

Body weight (g)					
Groups	**Initial body weight**	**Body weight 7th day**	**Body weight 14th day**	**Body weight 21st day**	**Body weight 28th day**
Normal	111.833 ± 0.997	118.5 ± 1.423	127.33 ± 1.278***	138 ± 0.991	145 ± 1.231***
Only STZ-NA	129 ± 0.856349	138.26 ± 1.324**	129.21 ± 0.979***	120.12 ± 1.323	117.25 ± 1.855***
STZ-NA+Metformin 10 mg/kg	131.212 ± 1.99231	137.75 ± 1.32***	126.98 ± 1.98061***	132 ± 1.112***	141 ± 2.129**
STZ-NA+8a 10 mg/kg	127.83 ± 0.542	120.66 ± 0.843***	136.5 ± 0.885**	144.5 ± 1.147	152 ± 1.033***
STZ-NA+8b 10 mg/kg	122.16 ± 1.327	118.33 ± 1.308***	130.33 ± 1.282	137.5 ± 1.310***	144 ± 1.154**
STZ-NA+8c 10 mg/kg	136 ± 1.095	123.33 ± 1.520**	131.5 ± 1.147***	140.16 ± 0.980**	148.83 ± 1.077**
STZ-NA+8d 10 mg/kg	138.667 ± 0.802773	146.167 ± 0.872***	153.333 ± 0.557***	158.833 ± 1.327**	164.8 ± 1.249
STZ-NA+8e 10 mg/kg	125.24 ± 0.927	137.14 ± 1.121	146 ± 1.421***	155.07 ± 1.223	164.21 ± 0.932**
STZ-NA+8f 10 mg/kg	135.17 ± 1.470	140.83 ± 1.400***	147.17 ± 1.137*	151.17 ± 1.351***	156.66 ± 1.475
STZ-NA+8g 10 mg/kg	140.11 ± 1.091	132.21 ± 1.301*	141.41 ± 1.237***	150 ± 1.551	162.28 ± 1.088
STZ-NA+8h 10 mg/kg	132.67 ± 1.308	127.33 ± 1.115***	138.67 ± 1.891**	144.5 ± 1.056***	150.5 ± 1.231*
STZ-NA+8i 10 mg/kg	135.31 ± 1.870	128.03 ± 1.378**	138.43 ± 1.329***	146.32 ± 1.221**	151.05 ± 0.910
STZ-NA+8j 10 mg/kg	130.31 ± 1.007	124.25 ± 1.324	135.21 ± 1.328***	146.93 ± 1.324	159.21 ± 1.23***
STZ-NA+8k 10 mg/kg	133.45 ± 0.992	139.01 ± 1.620***	147.23 ± 1.281	155.24 ± 1.559***	167.01 ± 1.423

Values are expressed as the mean ± S.D; Statistical significance (p) calculated by one-way ANOVA followed by Dunnett's $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$ calculated by comparing treated group with control group. All body weights are recorded in grams

CONCLUSION

Almost all the ligand molecules (8a-k) are showing the best interaction energies with the protein molecule 1v4s. These studies reveal that the compound would be significant use in the drug discovery process for the development of novel antihyperglycemic drug molecules. Our *in vitro* enzyme inhibition assay results indicate that among these eleven compounds, the potential top four (8b, 8c, 8h,= and 8i) compounds have shown effective alpha-amylase and alpha-glucosidase inhibition. The results of the in vivo antihyperglycemic study indicate that compounds (8a-k) at the doses 10 mg/kg (p.o.) possess significant antihyperglycemic activity against nicotinamide–streptozotocin-induced hyperglycemic rats. The result reveals that the compounds (8a-k) are reduce the blood glucose level and increase the body weight of the hyperglycaemic-induced animals. The entire studies shows that the presence of the methyl group of these compounds (8a-k) might be the reason for these inhibitions and that the compounds may essentially contain pharmacological compounds which require further structural characterization.

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