Design, synthesis of guanidine derivatives and their anti-hyperglycemic evaluation

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ABSTRACT

A series of N-[2(dimethylamino)-2-iminoethanimidoyl-2-phenoxyacetamide derivatives have been synthesized by reaction of Metformin, Chloroacetyl chloride and an appropriate Phenol. Structures of these compounds were established by IR, ^{1}H NMR. All the compounds were evaluated for their Anti-diabetic activity. Fasting blood sugar level was estimated on hours 0,2,4,6 and 24 from rat tail vein using glucometer. Data was statistically analysed by Dunnett's test. Metformin derivatives of Phenol PHE-9, PHE-5 and PHE-6 produced a time dependent decrease in blood glucose level significantly compared to Metformin. However, this study shows hypoglycaemic action of Metformin.

Keywords: Anti-diabetic, Hypoglycaemic, Metformin, Dunnett's test, PHE.
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INTRODUCTION

Diabetes that is a most dreadful disease affecting 4.9 million people in India and more than 250 million people around the world have diabetes. This total is expected to rise to 380 million within 20 years. Each year a further 7 million people develop diabetes [1]. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia, hypertriglyceridaemia, resulting from defects insulin secretion or action or both. [2] Two group of oral hypoglycaemic drugs sulphonyl ureas and biguanides have been used in the treatment of diabetes mellitus. They act by lowering blood glucose level thereby delaying or preventing the onset of diabetic complications. [3] Biguanide was used for diabetes treatment in traditional medicine for centuries. In the 1920's guanidine compounds were discovered in Galega extracts. Animal study showed that these compounds lowered blood glucose levels. Some less toxic derivatives synthalin A and syntalin B, were used for diabetes treatment, but after the discovery of insulin they were forgotten for the next several decades. Biguanide were re-introduced in to type-2 diabetes treatment in the late 1950's. [4]

Biguanides lower blood glucose levels primarily by decreasing the amount of glucose produced by the liver. It can also decrease the amount of sugar absorbed into the body (from the diet) and can make insulin receptors in muscle tissue more sensitive, helping the body respond better to its own insulin. These diabetes pills improve insulin's ability to move sugar into cells especially into the muscle cells. All of these effects cause a decrease in blood sugar levels. It is usually taken two times a day. A side effect of metformin may be diarrhea, but this is improved when the drug is taken with food. Biguanides should not be used in people who have kidney damage or heart failure because of the risk of precipitating a severe build up of acid (called lactic acidosis) in these patients. Biguanides can decrease the HbA1c 1%-2%. Because the medication does not increase the amount of insulin produced by the body, it is less likely to cause dangerously low blood sugar (hypoglycemia), as many other diabetes medications can do. [5]

Metformin (1, 1-dimetyl biguanide) derivatives that has been widely used for a long time for the treatment of diabetes mellitus. Currently used hypoglycemic drugs metformin in the treatment of diabetes are not completely effective and ad associated with adverse effects such as lactic acidosis, sometimes fatal has occurred, marked

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anorexia, unexplained weight loss may indicate. Several works have been tried in various studies with the aim to prevent or delay type-2 diabetes.

The present study aims at investing the effect of metformin derivatives of phenol on the blood sugar level of Alloxan induced diabetic rat model is compare with standard drug.

MATERIALS AND METHODS

Melting points were determined in open capillaries and were uncorrected. R_f values were obtained using silica gel thin layer chromatography plates and a solvent system of Ethyl acetate/Hexane (3:1) were prepared. The infra-red spectra of all compounds were determined by a diffuse reflectance technique using potassium bromide powder on a FT-IR spectrophotometer MODEL-8300 of SHIMADZU. ^{1}H NMR spectra of all compounds were generated in DMSO (Dimethysulphoxide) using MODEL BRUKER AVANCE II 400 NMR Spectrometer.

Step1: Method of preparation of 2- Chloro-N- [2-(dimethylamino)-2-iminoethanimidoyl]acetamide
In a 500ml two necked round funnels bottomed flask provided with a Mechanical stirrer, and fitted with a 100ml dropping funnels was placed Metformin (5.5gm) dissolved in Methanol(40ml), Chloroacetyl chloride (0.06moles) was added dropwise in Reaction mixture. The temperature of the reaction mixture was kept between 90-100°C. Reaction was continued till single point. At the end of the reaction Methanol was evaporated and the crude product so formed was collected and recrystallised by Methanol.

Metformin   +   Chloroacteylchloride   →   2-Chloro-N-[2(dimethylamino)2-iminoetanimidoyl]acetamide

(stirring, 80-90C)
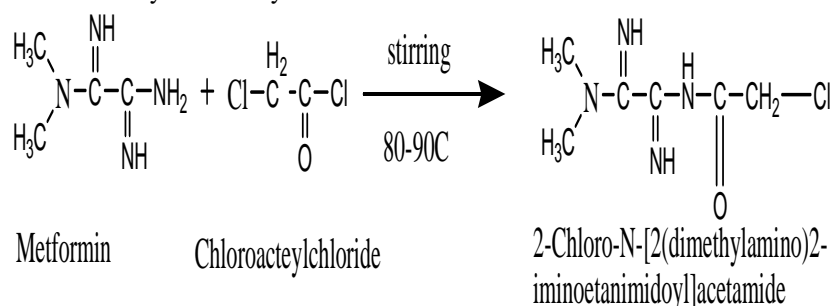
Step2: Method of preparation of N-[2- (dimethylamino)-2-iminoethanimidoyl]-2-phenoxyacetamide
A reaction assembly was arranged on Magnetic Stirrer. The Phenol (0.01mole) dissolved in sufficient quantity of acetone and anhydrous K_2CO_3 (0.01 mole) were placed in Iodine flask and were Refluxed for 1hr. After 1hr the chloro compound (2-chloro-N-[2-(dimethylamino)-2-iminoethanimodoyl] acetamide) (0.01 mole) dissolved in dry acetone was added to the above reaction mixture along with Pinch of KI(200mg).The reaction mixture was stirred by magnetic stirrer and was refluxed for 14-18hrs.

2-Chloro-N-[2(dimethylamino)2-imino etanimidoyl]acetamide   +   Phenol

(15-20C, stirring)

N-[2(dimethylamino)-2-iminoethanimidoyl-2-phenoxyacetamide

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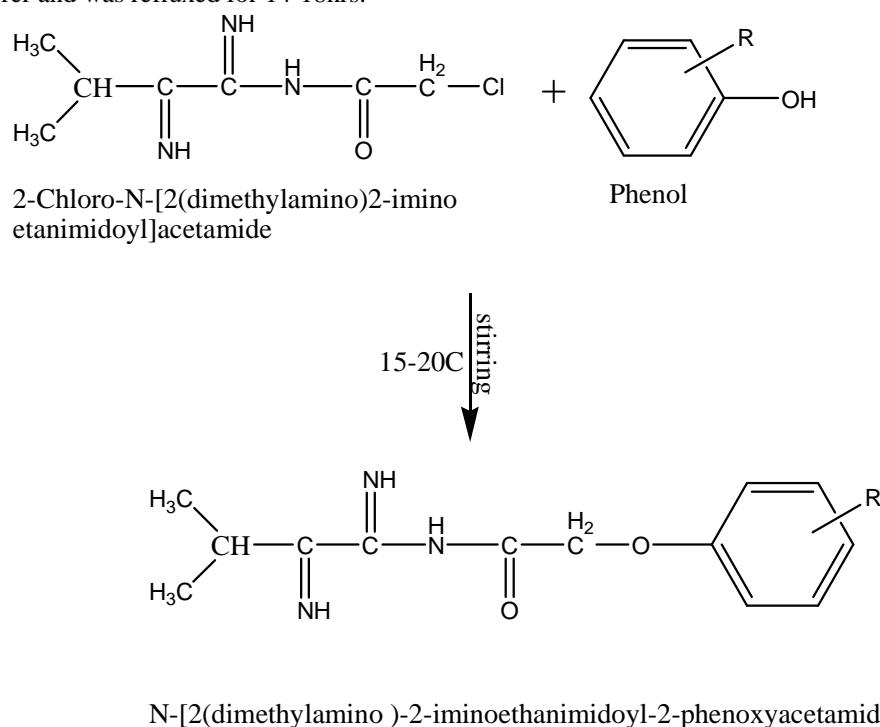
The progress of the reaction mixture was monitored by Thin Layer Chromatography. At the end of the reaction time, the flask was allowed to get cooled to room temperature. The compound was filtered; residue was then washed with 10% solution of Sodium Carbonate in order to remove the excess of phenol. This was again filtered and washed with water. The compound was transferred to a petridish and allowed to dry. The compound obtained was recrystallised using of Methanol.

PHARMACOLOGICAL ACTIVITY
Antidiabetic activity/Anti-Hyperglycemic Agent
The most widely used primary test to screen new antihyperglycaemic agents' measures the ability of a compound to reduce blood glucose level in the rats which rose by induction of the drug Alloxan- monohydrate.

Experimental Animals and Research Protocol Approval
Male wistar albino rats weighing 150-200gm obtained from Animal Facility Centre were used for the study. The animals were housed and maintained in an air conditioned room at $22\pm2℃$ and relative humidity of 45-55% and light (12:12hr- light: dark cycle. The animals had free access to standard food pellets and water was available ad. libitum. The experimental protocol was approved by Institutional Ethical Committee (IAEC) and constituted in accordance with the rules and guidelines of the committee for the purpose of control and supervision on experiments on animals (CPCSEA), India.

Drugs and dose
The standard drugs and the test compounds were administered in the form of suspension:
a) Control vehicle: Appropriate volumes of water.
b) Standard Drug: Metformin at a dose level of 100mg/kg body wt. was given as standard drug for comparison.
c) Test drugs: the test drugs (PHE-1, PHE-2, PHE-3, PHE-4, PHE-5, PHE-6, PHE-7, PHE-8 and PHE-9) were given at dose levels of 100mg/kg body wt to test groups.

Induction of Experimental Diabetes and Determination of Blood Glucose Level
Rats were deprived from food for 16-18 hrs (fasted state) before the induction of diabetes. The baseline plasma glucose levels were determined prior to administration. Diabetes was induced in male wistar rats by an intravenous injection through tail vein using alloxan monohydrate (80mg/kg) solution. They were left for 7 days at the end of which the plasma glucose levels were determined by using glucometer. The rats showing BGL above 250mg/dl (diabetic state) were selected for this study.

RESULTS AND DISCUSSION

All the various synthesized compounds were characterized with Physicochemical data (shown in Table-1), and spectral analysis with respect to 1H NMR spectra and IR spectra.

Synthesized compounds were also screened for analgesic activity. Compounds were screened for peripheral analgesic activity by acetic acid induced writhing test (shown in Table -2).

PHYSICOCHEMICAL CHARACTERISATION:

Table1: The result of the reaction of various N-[2-dimethylamino)-2-iminoethanimidoyl]-2-Phenoxyacetamide"

S.No.	Compound Code	R	M.Pt.($℃$)	%Yield	R_f Value
1.	PHE-1	H	125-128	75.47	0.52
2.	PHE-2	$3\text{-}NH_2$	130-132	66.03	0.56
3.	PHE-3	$4\text{-}NO_2$	115-118	66	0.80
4.	PHE-4	$2\text{-}NO_2$	112-115	70	0.72
5.	PHE-5	2-Cl	110-112	70.70	0.66
6.	PHE-6	$4\text{-}NH_2$	100-105	61.32	0.58
7.	PHE-7	$2\text{-}NH_2$	123-125	60.84	0.54
8.	PHE-8	$4\text{-}C_6H_5$	122-125	67.45	0.88
9.	PHE-9	$4\text{-}CH_3CO$	118-125	68.86	0.62

PHARMACOLOGICAL ACTIVITY:
Effects of Metformin Derivatives on Blood Glucose Level in Alloxan induced Diabetic Rats
The alloxan induced diabetic rats were divided into 11 groups of 2 male rats each. The test group received 100mg/kg metformin analogues while the control group received appropriate volume of water orally respectively. All the compounds were also given orally. The study involves the determination of BGL at 0, 2, 4, 6, 24 hrs respectively by

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taking 0.5 ml blood from the tail vein of the rats. The blood was dropped on the strip of glucometer and was inserted into glucometer and the reading noted.

Statistical Analysis

Data were analyzed using **"Dunnett's test"** to determine the statistical significance of the change in BGL $p<0.01$ was considered significant.

Table-2- Anti-diabetic activity of synthesized compounds on Blood Glucose Level (Mean±SEM) In Diabetic Rats:

Treatment	Dose	Mean reduction in Blood Glucose Level				
		0 hrs Blood Glucose level (mg/dl)	2 hrs Blood Glucose level (mg/dl)	4hrs Blood Glucose level (mg/dl)	6 hrs Blood Glucose level (mg/dl)	24 hrs Blood Glucose level (mg/dl)
Control (water)	100 mg/kg	99.67±18.04	99±24.06	100± 26.38	99.68±26.3	100±32.82
Standard Metformin	100 mg/kg	225.5±2.5	150.5±3.8	119.2±20.2	69.8±10.1	56.4±7.7
PHE-1	100 mg/kg	283.67±32	211±15.36	199±18.06	170.56±14	166.12±17.8
PHE-2	100 mg/kg	320.5±14.7	255±31.17	226.5±49.14	154±16.74*	142±9.244*
PHE-3	100 mg/kg	239.67±8.3	291±10.38	371.8±20.78	366.8±1.16	372.5±11.26
PHE-4	100 mg/kg	221.76±2.2*	210±10.23*	180.9±12.3*	160±5.8*	145.4±6.5*
PHE-5	100 mg/kg	226.3±0.8	205.8±16.8	150.9±20.3	131.3±11.3	125±10.1
PHE-6	100 mg/kg	223±2.1	201.6±15.6	130.4±13.45	118±9.3*	131±23.2
PHE-7	100 mg/kg	225.4±2.5	196.3±18.8	119.5±15*	107±7.1*	137.8±22.1
PHE-8	100 mg/kg	222±1.3*	231.2±22*	191.3±16.4*	181.3±11.1*	165.3±3.8*
PHE-9	100 mg/kg	222.3±2.1*	189.3±18.3*	129.1±10.1*	87.3±13.1*	69.2±6.7*

No. of animals in each group= 6
Each value represents the Mean± SEM
**represents (p<0.05) compared to control vs. treated group*

Fig1: Shows the effects of Standard and synthesized compounds on Blood Glucose Level (Mean + SEM) in Diabetic Rats on different hours Treatment.

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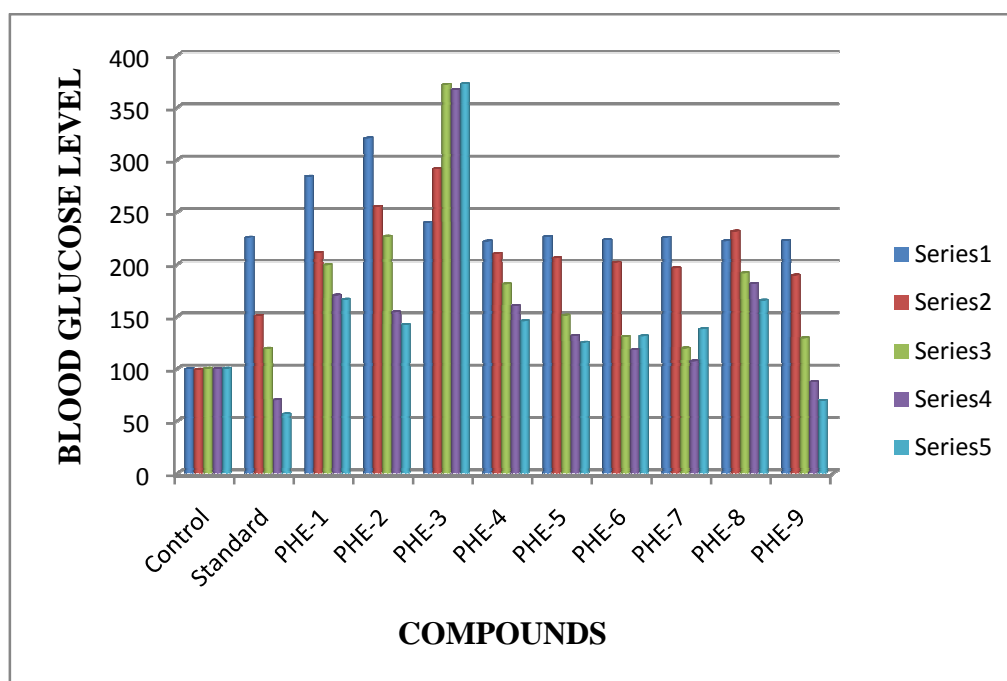
From the results of antihyperglycaemic effect, it can be concluded that synthesized compounds have shown significant activity (P<0.05), when compared to the control group. Some of the synthesized compounds have shown reduction in BGL comparable to the control. However, the hypoglycaemic effect is incomparable to that of the standard drug (METFORMIN; 100mg/kg).
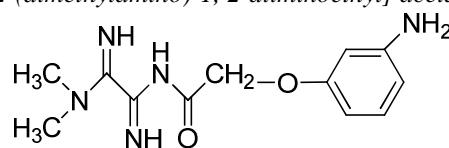
REPRESENTATIVE SPECTRAL ANALYSIS:
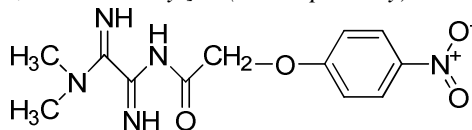**3.1(PHE-1)***N-(2-(dimethylamino-1,2-diiminoethyl)-2-phenoxyacetamide.*
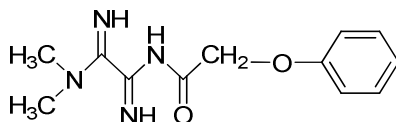
IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C). NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ3.30 (s, 1H, CH$_3$), δ 6.77-7.64 (m, 5H, Ar-H), δ 4.63 (s, 1H, CH$_2$).

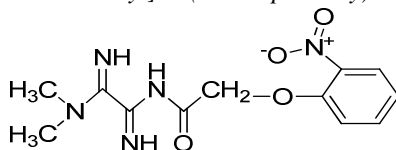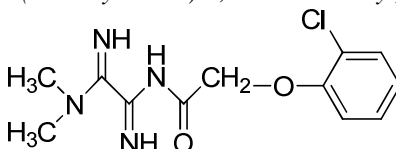**3.2(PHE-2)** *[3-(aminophenoxy)-N-(2-(dimethylamino)-1, 2-diiminoethyl] acetamide.*

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C),3310 (NH$_2$). NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ 3.30 (s, 1H, CH$_3$), δ 6.77-7.64 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$), δ6.27 (s, 1H, NH$_2$).

3.3 (PHE-3) *N-[2-(dimethylamino)-1, 2-diiminoethyl]-2-(4-nitrophenoxy) acetamide.*
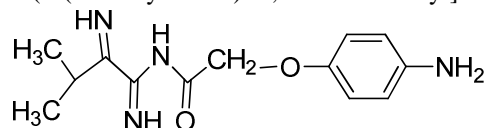

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C), 1625(NO$_2$).NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ3.30 (s, 1H, CH$_3$), δ 7.25-8.15 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$).

3.4 (PHE-4) *N-[2-(dimethylamino)-1, 2-diiminoethyl]-2-(2-nitrophenoxy) acetamide.*

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C), 1625(NO$_2$).NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ3.30 (s, 1H, CH$_3$), δ 6.94-8.15 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$).

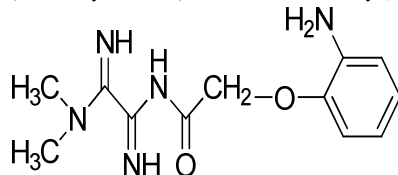**3.5-(PHE-5)** *2-[2-(chlorophenoxy)-N-(2-(dimethylamino)-1, 2-diimino ethyl] acetamide.*
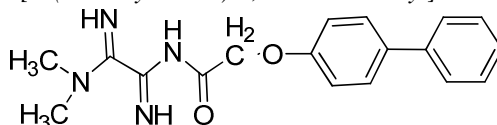

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C), 1150(C-Cl). NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Sec. amide), δ3.30 (s, 1H, CH$_3$), δ 6.95-7.38 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$).

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3.6 (PHE-6) 2-[4-(aminophenoxy)-N-(2-(dimethylamino) -1, 2-diimino ethyl] acetamide.

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C), 1600(NH$_2$). NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ3.30 (s, 1H, CH$_3$), δ 6.66-6.74 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$), δ 6.27 (s, 1H, NH$_2$).

3.7(PHE-7) *2-[2-(aminophenoxy)-N-(2-(dimethylamino)-1, 2-diimino ethyl] acetamide.*

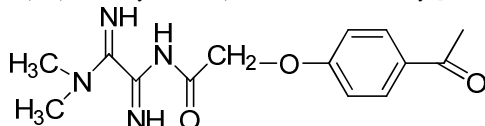IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C), 1600(NH$_2$).NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ3.30 (s, 1H, CH$_3$), δ 6.66-6.74 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$), δ 6.27 (s, 1H, NH$_2$).

3.8 (PHE-8) *2-(biphenyl-4-yloxy)-N-[2-(dimethylamino)-1, 2-diimino ethyl] acetamide*

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C).NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ 3.30 (s, 1H, CH$_3$), δ 7.05-7.68 (m, 9H, Ar-H), δ 4.63 (s, 1H, CH$_2$).

3.9 (PHE-9) *2-[4-(acetylphenoxy)-N-(2-(dimethylamino) -1, 2-diimino ethyl] acetamide.*

IR (KBr) cm^{-1}: 3340 (N-H str), 1065.27 (C-N str), 3307.58 (Ar-H), 1703.1 (NH-C=O), 1654 (C=O), 1215 (C-O-C). NMR (DMSO, d$_6$):δ 8.0 (s, 1H, NH-Secondary amide), δ 3.32 (s, 1H, CH$_3$), δ 7.10-7.83 (m, 4H, Ar-H), δ 4.63 (s, 1H, CH$_2$), δ 2.50 (s, 1H, CH$_3$).

CONCLUSION

We have prepared nine compounds by reacting substituted phenols in Dry Acetone. All synthesized compounds were characterized by TLC, MP and Spectral analysis. All compounds were screened for anti-diabetic and compounds PHE-9, PHE-5 and PHE-6 exhibited significant anti-diabetic activity comparable or superior to Metformin. Compounds PHE-7, PHE-4 and PHE-2 exhibited moderate hypoglycemic activity while compounds PHE-8, PHE-1 and PHE-3 were found to be less potent as compared to standard and other analogue.

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