Synthesis of Phenylurea Derivatives & Their Evaluation as Antihyperglycaemic Agents

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ABSTRACT: Literature survey reveals that in past few decades, some urea derivatives have been synthesized for the management of DM among which sulphonylurea have been gaining considerable recognition in the management of DM worldwide. In the present study N-phenyl-.N-(substituted) phenoxy acetyl ureas were synthesized using three- step reaction pathway. A new series of phenylurea's were prepared by treating phenylurea with chloroacetyl chloride to form the product which on further treatment with various substituted phenols gave the final product. All synthesized compounds were characterized on the basis of M.P range, R_F value, elemental analysis, IR, NMR spectral analysis. Out of the compounds tested few showed antihyperglycaemic activity. © 2011 IGJPS. All rights reserved.

**KEYWORDS:** Sulphonylureas; Diabetes Mellitus (DM); N-phenyl-.N-(substituted) phenoxy acetyl ureas.

INTRODUCTION

Urea and its derivatives constitute an important class of heterocyclic compounds which possess wide range therapeutic and pharmacological properties. Sulphonylureas become widely available in 1955 for the treatment of non-ketosis mild diabetes and are still being the drugs of choice. In the 1970 many so called "second generations" sulphonylureas have been developed which are 80-100 times more potent. A diguanide 'synthalin' was found to be hypoglycemic in 1920s, but was toxic. Clinically useful diguanide "a second class compound" in the form of single drug Phenformin has been used since 1957.Recently newer classes of drug viz. glucosidase inhibitors, Meglitinide analogues, and Thiazoledinediones have been included[1][2]. Because of synthetic utility and broad range of spectrum and pharmacological properties, it is an important active moiety in different therapeutic areas such as antihyperglycaemic [3]

sedative and hypnotics, anticonvulsants [4] antimicrobial and VLA-4 antagonist [5].

Diabetes mellitus is the most common chronic endocrine disorder and now became an epidemic that impairs glucose homeostasis resulting in retinopathy, angiopathy, nephropathy, neuropathy causing neurological disorder due to perturbation in utilization of glucose. It also results in faulty carbohydrate metabolism in which metabolism of protein and fat is disturbed. This results in hyperglycemia (excessive sugar in the blood) and glycosuria (presence of sugar in the urine). Type I diabetes is treated with exogenous insulin and Type II with Antihyperglycaemic agents (Sulphonylureas, Biguanides etc.). Hypoglycaemic agents are the drugs which lower blood glucose level and are effective orally. Sulphonylureas are most widely used hypoglycaemic agents. These drugs stimulate the

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release of insulin from the pancreas [6][7]. They are urea derivatives with an arylsulphonyl group to one nitrogen and another group attached to terminal nitrogen of urea viz. alkyl, alicyclic, heterocyclic etc. The agents of this category are:
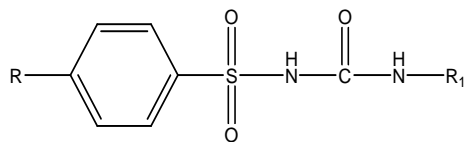

Where
R=CH$_3$ R$_1$=CH$_2$CH$_2$CH$_2$CH$_3$ - **Tolbutamide**
R=Cl   R$_1$=CH$_2$CH$_2$CH$_2$CH$_3$ - **Chlorpropamide**

So, because of synthetic utility of urea and broad range of pharmacological properties we have concentrated on further enlargement of urea moiety through derivatisation and modification and screened the synthesized compounds for antihyperglycaemic activity and found positive results.

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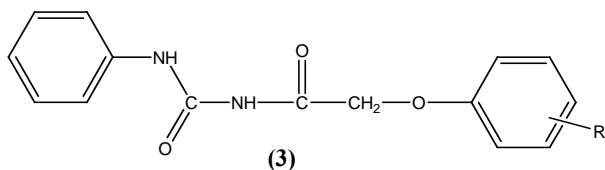
MATERIALS & METHODS

Most of the solvents used were of L.R. grade and purified before their use in different reactions. Chemicals used were obtained from Central Drug House Pvt. Ltd. (CDH), Qualigens and HIMEDIA, chemical suppliers. The melting points of the synthesized compounds as well as intermediates were determined by open capillary methods and are uncorrected. The IR spectra of the synthesized compounds were recorded in potassium bromide discs and on FT-IR spectrophotometer MODEL-8300 of SHIMADZU at College of Pharmacy, Institute of Foreign Trade and Management, Moradabad. The PNMR spectra of the synthesized compounds were recorded in DMSO (Dimethylsulphoxide) using MODEL AV-300 BROKE JEOL at 300MHz spectrophotometer, Central Drug Research Institute (CDRI), Lucknow. Chemical shift are reported in parts per million (ppm; δ) and the signals are described as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The thin layer chromatography of the compounds was performed on the precoated silica gel G plates using iodine vapors' for detection of the spots.

Three steps are involved in the methodology used in the above research work for preparation of sulphonylurea analogues.

Step1- Synthesis of Phenylurea:

Aniline (65gm) and urea (120gm) was dissolved in water (200ml) followed with HCl (4ml) and glacial acetic acid (4ml) in a reflux condenser. The content was boiled for 30min. Fine white crystals appeared within 15min of refluxing. At the end of reaction time the flask was cooled in ice and the solid was collected. The mixture of phenylurea and diphenylurea thus formed and separated. Phenylurea dissolved in boiling water while diphenylurea remain undissolved. This was filtered and the filtrate cooled thoroughly. The Phenylurea crystallized out was collected by filtration and dried in steam oven.

- Yield: 10gm   Melting Point: 147 ^{o}C

- Molecular Formula: C$_7$H$_8$N$_2$O   R$_f$ Value: 0.54

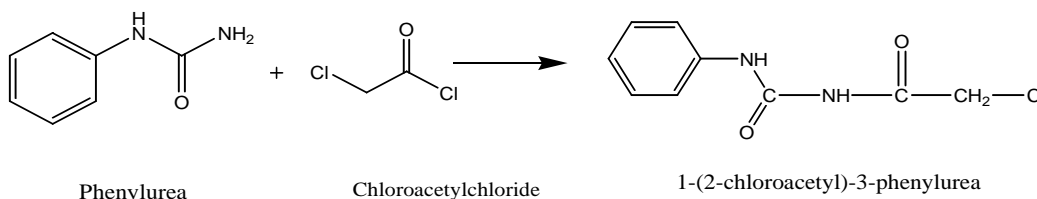- Mobile phase for TLC: Ethyl acetate: n-Hexane {3:1}
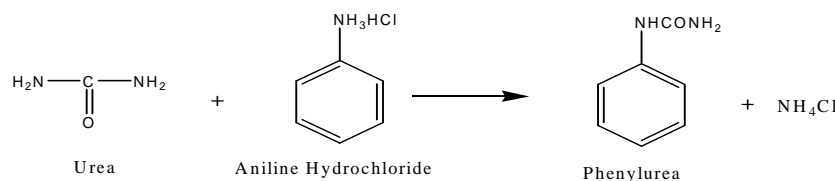

Step 1

Step2: Synthesis of 1-(2-chloroacetyl)-3-phenylurea:

Phenylurea (5.5gm) dissolved in ethyl methyl ketone was placed in a 500ml three necked round bottomed flask. Solution of chloroacetyl chloride (0.06 moles) in ethyl methyl ketone and sodium carbonate (saturated solution) in distilled water were taken into the dropping funnels. At first, some volume of the sodium carbonate was added to the reaction flask and this was followed by simultaneous drop wise addition of the solution of chloroacetyl chloride and solution of sodium carbonate from the dropping funnels. The assembly was left as such as for half an hour. Two layers of aqueous and non-aqueous solutions were formed. The aqueous layer was discarded and the organic layer was washed with water and further distilled by simple distillation method. The crude product so formed was collected and was recrystallised from ethanol.

- Yield: 5.3 (62.5%)   MeltingPoint:120-122°C
- Molecular Formula: $C_9H_9N_2OCl$ R_fValue:0.5
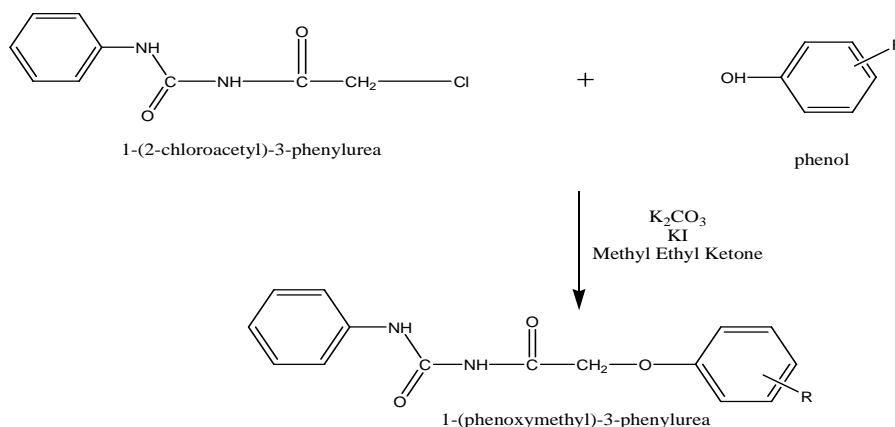
- Mobile phase for TLC: Ethylacetate : n-Hexane {3:1}

Step3: Synthesis of N-phenyl-N-(substituted) phenoxy acetyl urea:

The phenol (0.01 mole) dissolved in sufficient quantity of dry acetone and anhydrous K_2CO_3 (0.01 mole) were placed in the round-bottomed flask and were refluxed for 1hr. After 1hr the chloro compound (N-chloroacetyl phenylurea) (0.01 mole) was added to the above reaction mixture with pinch of KI (200mg). The reaction mixture was stirred by magnetic stirrer and was refluxed for 8-10 hrs. The progress of the reaction mixture was monitored by thin layer chromatography. At the end of reaction time, the flask was allowed to get cooled to room temperature. The compound was filtered and 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 obtained was recrystallised using appropriate solvent.

Phenylurea   Chloroacetylchloride   1-(2-chloroacetyl)-3-phenylurea

Step 2

1-(2-chloroacetyl)-3-phenylurea   phenol

K_2CO_3
KI
Methyl Ethyl Ketone

1-(phenoxymethyl)-3-phenylurea

Step 3

Pharmacological Evaluation

The compounds synthesized during my project were evaluated for their pharmacological activity in experimental animals, in order to get information regarding their pharmacological profile. Amongst all derivatives only a few of them were tested due to restriction on availability of experimental animals. These animals were tested for *Antihyperglycaemic* activity.

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: Male wistar albino rats weighing 150-200gm obtained from Animal Facility Centre were used for the study.

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.

Effects of Phenylurea Derivatives on Blood Glucose Level in Alloxan induced Diabetic Rats:

The alloxan induced diabetic rats were divided into four groups of 2 male rats each. The test group received 100mg/kg phenylurea 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.

RESULTS & 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 CHARACTERIZATION**

S. No.	List of various phenylurea derivatives	R	M.Pt.(^{o}C)	%Yield	R_f Value
1.	1-(2-phenoxyacetyl)-3-phenylurea	H	125-128	56.29	0.52
2.	1-(2-(2-nitrophenoxy)acetyl)-3-phenylurea	2-NO$_2$	85-88	56.96	0.56
3.	1-(2-(m-tolyloxy)acetyl)-3-phenylurea	3-CH$_3$	115-118	56.33	0.80
4.	1-(2-(o-tolyloxy)acetyl)-3-phenylurea	2-CH$_3$	112-115	58.30	0.72
5.	1-(2-(4-chlorophenoxy)acetyl)-3-phenylurea	4-Cl	110-112	60.39	0.66
6.	1-(2-(2,6-dichlorophenoxy)acetyl)-3-phenylurea	2,6-(Cl)$_2$	100-105	63.44	0.58
7.	1-(2-(4-bromophenoxy)acetyl)-3-phenylurea	4-Br	123-125	61.63	0.54
8.	1-(2-(4-aminophenoxy)acetyl)-3-phenylurea	3-CH$_3$,4-Cl	132-135	63.24	0.69
9.	1-(2-(2-methylcarboxyphenoxy)acetyl)-3-phenylurea	4-NH$_2$	122-125	56.54	0.88

Table 1 The result of the reaction of various N-phenyl-N-(substituted) phenoxy acetyl urea.

- **PHARMACOLOGICAL ACTIVITY**

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	99.6718.04	9924.06	10026.38	99.6826.3	10032.82
Standard (Glibenclamide)	100 mg/kg	225.52.5	150.53.8	119.220.2	69.810.1	56.47.7
SAV-1	100 mg/kg	283.6732	21115.36	19918.06	170.5614	166.1217.8
SAV-2	100 mg/kg	320.514.7	25531.17	226.549.14	15416.74*	1429.244*
SAV-3	100 mg/kg	239.678.3	29110.38	371.820.78	366.81.16	372.511.26
SAV-4	100 mg/kg	221.762.2*	21010.23*	180.912.3*	1605.8*	13.56.5*
SAV-5	100 mg/kg	226.30.8	205.816.8	150.920.3	131.311.3	12510.1
SAV-6	100 mg/kg	2232.1	201.615.6	130.413.45	1189.3*	15123.2
SAV-7	100 mg/kg	225.42.5	196.318.8	119.515*	1077.1*	137.822.1
SAV-8	100 mg/kg	2221.3*	231.222*	191.316.4*	181.316.1*	105.33.8*
SAV-9	100 mg/kg	222.32.1*	189.318.3*	129.110.1*	87.313.1*	69.26.7*

Table 2 The Antihyperglycaemic effects of the synthesized compounds on Blood GlucoseLevel (Mean SEM) in Diabetic Rats.
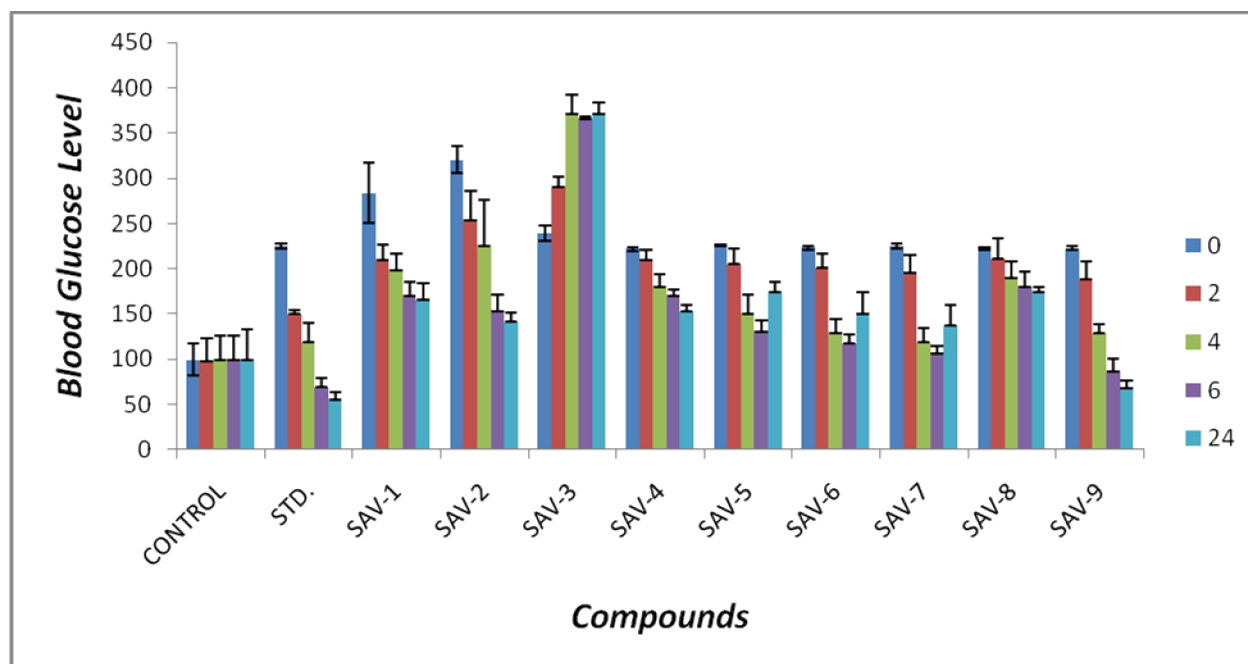

Figure 1 Shows the effects of Standard and synthesized compounds on Blood Glucose Level (Mean + SEM) in Diabetic Rats on different hours Treatment.

- **REPRESENTATIVE SPECTRAL ANALYSIS**

SAV1: 1-(2-phenoxyacteyl)-3-phenylurea: 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 10.187 (s, 1H, NH-urea), δ 6.087 (s, 1H, NH-imide), δ 6.77-7.64 (m, 10H ,Ar-H), δ 4.82 (s,1H,CH$_2$).

SAV2:1-(2-[2-nitrophenoxy] acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3324.35 (N-H str), 1065.27 (C-N str), 2894.58 (Ar-H), 1726.22 (NH-C=O), 1689.21 (C=O), 1153.2 (C-O-C), 1625 (NO$_2$ str). NMR (DMSO, d$_6$): δ 10.187 (s, 1H, NH-urea), δ 6.087 (s, 1H, NH-imide), δ 6.77-7.64 (m, 10H, Ar-H), δ 4.82 (s, 1H, CH$_2$).

SAV3: 1-(2-[m-tolyloxy]acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3482 (N-H str), 1047.8 (C-N str), 3011.21 (Ar-H), 1726.2 (NH-C=O), 1645 (C=O) 1, 152.3(C-O-C), 2875.2 (C-H, CH$_3$). NMR (DMSO, d$_6$): δ 10.117 (s, 1H, NH-urea), δ 6.458 (s, 1H, NH-imide), δ 6.57-7.64 (m, 9H, Ar-H), δ 4.82 (s,1H, CH$_2$), δ 2.597 (s,1H, CH$_3$).

SAV4: 1-(2-[o-tolyloxy]acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3457 (N-H str), 1059.17 (C-N str), 3075.82 (Ar-H), 1708.2 (NH-C=O), 1675.3 (C=O), 1149(C-O-C), 2940 (C-H, CH$_3$). NMR (DMSO,d$_6$): δ 10.012 (s, 1H,NH-urea), δ 6.048 (s, 1H,NH-imide), δ 6.64-7.760 (m,9H,Ar-H), δ 4.835 (s,1H,CH$_2$), δ 2.337 (s,1H, CH$_3$).

SAV5:1-(2-[4-chlorophenoxy] acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3458.1(N-H str), 1054.03(C-N str), 3337 (Ar-H), 1689 (NH-C=O), 1624.3 (C=O), 1217 (C-O-C). NMR (DMSO, d6): δ 10.417 (s, 1H, NH-urea), δ 6.090 (s, 1H, NH-imide), δ 6.7-7.64 (m, 9H, Ar-H), δ 4.845 (s, 1H, CH$_2$).

SAV6: 1-(2-[2, 6-dichlorophenoxy] acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3457.4(N-H str), 1052.61(C-N str), 3341.02 (Ar-H), 1685.4 (NH-C=O), 1625 (C=O), 1159.6 (C-O-C). NMR (DMSO, d$_6$): δ 9.817 (s, 1H, NH-urea), δ 6.051 (s, 1H, NH-imide), δ 6.146-7.92 (m, 8H, Ar-H), δ 4.82 (s, 1H, CH$_2$).

SAV7: 1-(2-[4-bromophenoxy] acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3418.15 (N-H str), 1056.17 (C-N str), 3078.2 (Ar-H), 1703.1 (NH-C=O), 1705 (C=O), 1145.5 (C-O-C). NMR (DMSO, d$_6$): δ 10.217 (s, 1H, NH-urea), δ 6.042 (s, 1H, NH-imide), δ 6.048-8.07 (m, 9H, Ar-H), δ 4.905 (s, 1H, CH2).

SAV8: 1-(2-[4-aminophenoxy] acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3455.8 (N-H str), 1051.88 (C-N str), 3340.90 (Ar-H), 1686.52 (C=O), 1626.02 (NH-C=O), 1158.86 (C-O-C). NMR (DMSO, d$_6$): δ 10.073 (s, 1H, NH-urea), δ 6.037 (s, 1H, NH-imide), δ 6.35-8.120 (m, 9H, CH), δ 4.815 (s, 1H, CH$_2$), δ 4.07 (s, 1H, c-NH).

SAV9: 1-(2-(2-methylcarboxy) phenoxy) acetyl)-3-phenylurea: IR (KBr) cm^{-1}: 3456.98 (N-H str), 1052.26 (C-N str), 3409.20 (Ar-H), 1684.71 (NH-C=O), 1627.02 (C=O), 1155.87 (C-O-C). NMR (DMSO,d$_6$): δ 11.22 (s,1H,OH), δ 10.135 (s, 1H,NH-urea), δ 6.285 (s, 1H,NH-imide), δ 6.96-8.04 (m,10H,CH), δ 4.816 (s,1H,CH$_2$).

CONCLUSION

The present study was conducted to synthesize a series of N-phenyl-N-(substituted)-phenoxy acetyl urea by using a three-step reaction pathway. The synthesized compounds were characterized by their melting points, R$_f$ value, IR and H^1 NMR spectral analysis. All the synthesize compounds have been evaluated for their antihyperglycaemic activity in the alloxan induced rat diabetic model. The antihyperglycaemic profile indicates that some of the synthesized compounds (SAV4, SAV8, and SAV9) have shown significant activity, which is comparable to the control. The present study reveals that some N-phenyl-N-(substituted)-phenoxy acetyl urea could be used as a template for the future development through modification or derivatisation to design more potent therapeutic agents.

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