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Research article

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SOME NOVEL POTENT TYPE II ANTIDIABETIC AGENTS.

VELINGKAR VS*, DANDEKAR VD, MURUGANANTHAN K.

*E mail- vsvelingkar@gmail.com/ vikrantdandekar@gmail.com, Tel: +91-02222164387/22164368
Department of Pharmaceutical Chemistry, K.M.Kundnani College of Pharmacy- Cuffe Parade, Mumbai-4000005
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ABSTRACT

Diabetes mellitus constitutes a major health problem in modern societies. A major role of antidiabetic therapy is to restrict blood glucose control, which approximates normal fasting levels and normal elevations of postprandial blood glucose. Sulfonylureas are the mainstay of antidiabetic therapy for many years.

The present work involves the synthesis of the lead molecule N-[4-{2-(5-chloro-2-methoxy benzamido) ethyl}phenyl sulfonyl]-N'-cyclohexyl urea known as Glibenclamide, second generation sulfonylurea and its novel analogs. The synthesized compounds were screened for antidiabetic activity .The structures of title compounds were established by elemental and spectral analysis.

Keywords : Sulfonylureas, Glibenclamide analogs, Hypoglycemia.

INTRODUCTION

Type II diabetes is a debilitating disease that arise from improper energy storage and utilization. The global prevalence of type II diabetes, which is presently estimated to affect more than 100 million people, is set to double by the year 2010[1]. Statistical data and epidemiological data clearly show increasing prevalence of diabetes with time. Hence, there is a crying need to synthesize more effective and oral antidiabetic agents.

Sulfonylureas are the mainstay of antidiabetic therapy for many years. Several structurally modified agents, which have been added in Sulfonylurea class, still there is need of efficacious agents, which are sufficiently nontoxic for chronic use[2,3].

The present work describes the synthesis (scheme 1) and antidiabetic evaluation of novel analogs of Glibenclamide, second-generation Sulfonylurea.

The various substituted carboxylic acids namely, 5-chloro-2-methoxy benzoic acid, 2-methoxy benzoic acid, benzoic

acid, 5-chloro-2-hydroxy benzoic acid, chloro acetic acid were refluxed with thionyl chloride to give corresponding acid chlorides. The individual acid chlorides were further treated with 2-phenethylamine in presence of base to produce corresponding amides. The corresponding sulfonamide derivatives were prepared by chlorosulfonation and amidation of theses prepared amides. Finally, the title compounds were obtained by reacting sulfonamide derivatives with isocyanates.

EXPERIMENTAL

Melting points of the synthesized molecules were determined by open capillary tube and were uncorrected. The purity of the compounds was ascertained by thin layer chromatography (TLC) on pre-coated silica gel G plate. I.R. spectra were recorded on Jasco FT/IR-5300 spectrometer using KBr pellet method. Nuclear magnetic resonance spectra were recorded using Bruker 200.13 MHz spectrometer. Elemental analysis was performed using Dumas method. The pharmacological evaluation was conducted at K.M.Kundnani college of pharmacy,Mumbai, after obtaining clearance from institutional animal ethics committee. Glibenclamide was taken as reference standard. It was obtained from U.S.Vitamins (India), Mumbai as gift sample. Blood glucose strips (Haemoglucotest 20-800R) and blood glucometer Obtained from Boehringer Mannhein,India were used to estimate the blood glucose level.

Synthesis of 5-chloro-2-methoxy-benzoyl chloride[4].

5-chloro-2-methoxy benzoic acid (0.13 mol), 75 ml of freshly distilled thionyl chloride and a drop of dimethyl formamide (DMF) were refluxed in a boiling water bath for 2hrs. The final product was purified by distillation under reduced pressure.

The various acid chlorides were synthesized from benzoic acid, 2-methoxy benzoic acid, 5-chloro-2-hydroxy benzoic acid, chloro acetic acid using the same thionyl chloride method as described above.

Synthesis of 5-chloro-2-methoxy-N-(2-phenethyl) benzamide[5].

5-chloro-2-methoxy-benzoyl chloride (0.055 mol) was added in small portions to a solution of 2-phenethylamine (0.11 mol) and 120 ml of 10% potassium hydroxide with constant shaking and intermittent cooling. The separated white solid was then filtered at the pump

and washed thoroughly with water. It was then triturated with saturated solution of sodium bicarbonate to remove the traces of acid. The crude product was filtered, washed with water and then recrystallized using chloroform.

The various substituted amides were prepared by similar procedure.

Synthesis of 4-[(5-chloro-2-methoxy benzamido) ethyl] benzene sulfonyl chloride[6].

Chlorosulphonic acid (0.16 mol) was added dropwise to a cooled and stirred solution of 5-chloro-2-methoxy-N-(2-phenethyl) benzamide (0.02 mol) in 100 ml chloroform. The reaction mixture was taken in a stoppered flask and stirring was continued for 4 hr at room temperature and then the mixture was poured into one liter of ice-cold water. The organic layer was separated and the aqueous layer extracted with chloroform. The combined organic layer was dried over magnesium sulphate and the organic solvent was evaporated. On standing 4-[(5-chloro-2-methoxy benzamido) ethyl] benzene sulfonyl chloride solidified. The other substituted sulfonyl chlorides were synthesized in similar manner.

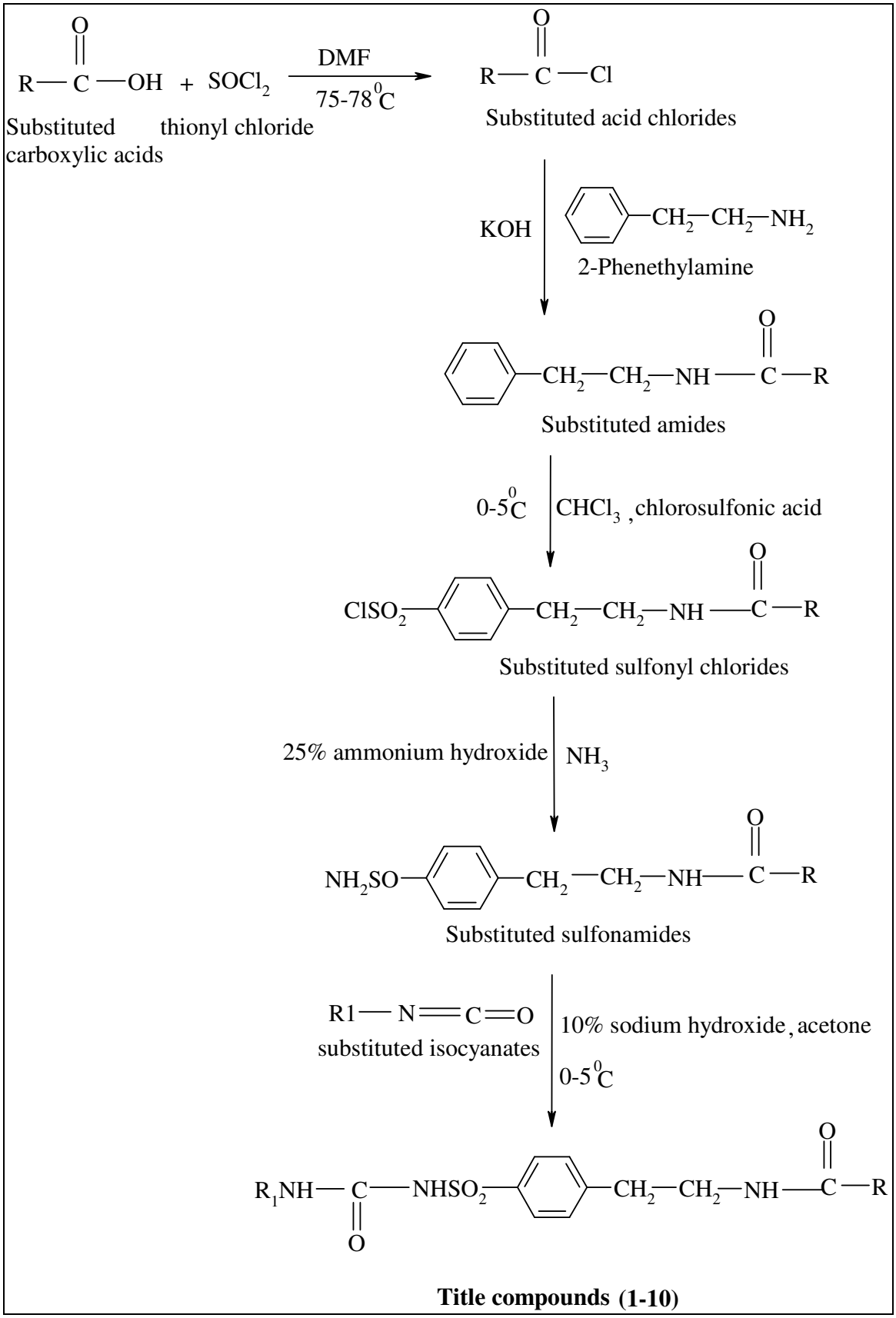
Synthesis of 4-[(5-chloro-2-methoxy benzamido) ethyl] benzene sulfonamide.

4-[(5-chloro-2-methoxy benzamido) ethyl] benzene sulfonyl chloride (0.011mol) was suspended in 80ml of 25% ammonium hydroxide and the mixture was stirred at room temperature for 5 hr. The mixture was diluted with 100 ml of water and 20ml of chloroform to yield crude product. The crude product was filtered, washed with water and then recrystallized from ethanol.

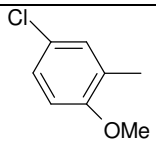
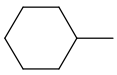
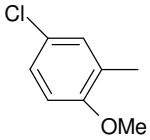
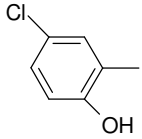
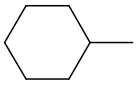
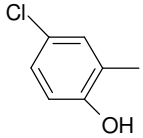
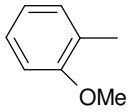
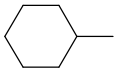
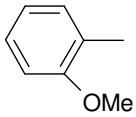
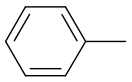
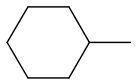
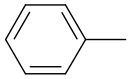
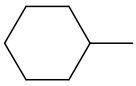
The substituted sulfonamides were synthesized by similar procedure as described above.

Synthesis of N-[4-{2-(5-chloro-2-methoxy benzamido) ethyl}phenyl sulfonyl]-N'-cyclohexyl urea[7,8].

To a solution of 4-[(5-chloro-2-methoxy benzamido) ethyl] benzene sulfonamide in 8ml of 10% sodium hydroxide and 20ml acetone at 0-5ºC, cyclohexylisocyanate was added dropwise and the mixture was kept aside for 3 hr. It was then diluted with water: methanol mixture (1:1), filtered and acidified with HCl. The separated solid was further treated with sodium methoxide and the salt obtained was recrystallized from acetic acid to yield pure title compound.


DMF
75-78 0C
Substituted carboxylic acids
thionyl chloride
Substituted acid chlorides
KOH
2-Phenethylamine
Substituted amides
0-5 0C
CHCl3 , chlorosulfonic acid
Substituted sulfonyl chlorides
25% ammonium hydroxide
NH3
Substituted sulfonamides
substituted isocyanates
10% sodium hydroxide, acetone
0-5 0C
Title compounds (1-10)

Scheme 1

Compound no.	R	R^1
1		
2		—CH_3
3		
4		—CH_3
5		
6		—CH_3
7		
8		—CH_3
9	—CH_2Cl	
10	—CH_2Cl	—CH_3

N-[4-{2-(5-chloro-2-methoxy benzamido) ethyl}phenyl sulfonyl]-N'-cyclohexyl urea (1)

IR(KBr;cm^{-1}):3032 (C-H stretching - Ar), 756(C-H bending-Ar), 2932 (C-H stretching-Aliphatic), 1718 (carbonyl stretching), 3368 (NH stretching amide), 1618 (NH bending amide), 1157 (S=O stretching), 883 (C-Cl stretching); nitrogen analysis 8.52 (calc.), 8.56(obs.); ^{1}NMR ($CDCl_3$,δ(ppm):7.10-7.90 (m, 7H,aromatic), 3.40 (s,3H,OCH_3), 2.64(t,2H,R-CH_2-Ar), 5.84(s, 1H, -CONH), 1.44(m, 6H,cyclohexane).

N-[4-{2-(5-chloro-2-methoxy benzamido) ethyl} phenyl sulfonyl]-N'-methyl urea (2)

IR(KBr;cm^{-1}): 2988 (C-H stretching - Ar), 771(C-H bending-Ar), 2854 (C-H stretching-Aliphatic), 1715 (carbonyl stretching), 3398 (NH stretching amide),

1523(NH bending amide), 1154 (S=O stretching), 864 (C-Cl stretching)**;** nitrogen analysis 9.88 (calc.), 9.80 (obs.); ^{1}NMR ($CDCl_3$, δ (ppm): 7.24-7.88(m, 7H,aromatic), 3.34 (s,3H,OCH_3), 2.58 (t,2H,R-CH_2-Ar), 6.18 (s, 1H, -CONH).

N-[4-{2-(5-chloro-2-hydroxy benzamido) ethyl}phenyl sulfonyl]-N'-cyclohexyl urea (3)

IR(KBr;cm^{-1}): 3092 (C-H stretching - Ar), 788(C-H bending-Ar), 2852 (C-H stretching-Aliphatic), 1682 (carbonyl stretching), 3364 (N-H stretching amide), 1610 (N-H bending amide), 1153,1325 (S=O stretching), 827 (C-Cl stretching); nitrogen analysis 8.77 (calc.), 8.67 (obs.); ^{1}NMR ($CDCl_3$, δ(ppm): 6.90-7.40 (m,7H,aromatic), 4.54 (s,1H,Ar-OH),2.60(t,2H,R-CH_2-Ar), 5.90(s, 1H, -CONH), 1.30(m, 6H, cyclohexane).

N-[4-{2-(5-chloro-2-hydroxy benzamido) ethyl}phenyl sulfonyl]-N'-methyl urea (4)

IR(KBr;cm^{-1}): 3090 (C-H stretching - Ar), 790 (C-H bending-Ar), 2866 (C-H stretching-Aliphatic), 1685 (carbonyl stretching), 3394 (NH stretching amide), 1525 (NH bending amide), 1153 (S=O stretching), 827(C-Cl stretching; nitrogen analysis 10.22 (calc.), 10.21 (obs.); ^{1}NMR ($CDCl_3$,δ (ppm):6.84-7.40 (m,7H,aromatic), 4.54(s,1H,ArOH),2.54(t,2H,R-CH_2-Ar), 5.12(s, 1H, -CONH).

N-[4-{2-(2-methoxy benzamido) ethyl} phenyl sulfonyl]-N'-cyclohexyl urea (5)

IR (KBr; cm^{-1}): 3053 (C-H stretching - Ar), 758(C-H bending Ar), 2953(C-H stretching aliphatic), 1622 (carbonyl stretching) 3369 (N-H stretching amide), 1595 (N-H bending amide), 1155,1319 (S=O stretching); nitrogen analysis 9.15 (calc.), 9.18 (obs.); ^{1}NMR ($CDCl_3$, δ(ppm): 7.10-7.68 (m,8H,aromatic), 3.28(s,3H,OCH_3), 2.62 (t,2H,R-CH_2-Ar), 6.28 (s,1H,-CONH),1.42 (m, 6H, cyclohexane).

N-[4-{2-(2-methoxy benzamido) ethyl} phenyl sulfonyl]-N'-methyl urea (6)

IR (KBr;cm^{-1}): 2932 (C-H stretching - Ar), 758(C-H bending-Ar), 2854 (C-H stretching-Aliphatic), 1620 (carbonyl stretching), 3369 (NH stretching amide), 1521 (NH bending amide), 1152 (S=O stretching)**;** nitrogen analysis 10.74 (calc.), 10.83 (obs.); ^{1}NMR ($CDCl_3$,δ (ppm): 6.98-7.62 (m,8H,aromatic), 3.42 (s,3H,OCH_3), 2.64 (t,2H,R-CH_2-Ar), 5.38 (s, 1H, -CONH).

N-[4-{2-(benzamido) ethyl} phenyl sulfonyl]-N'-cyclohexyl urea (7)

IR (KBr; cm^{-1}): 3082 (C-H stretching - Ar), 771 (C-H bending-Ar), 1630 (carbonyl stretching), 3393 (NH stretching amide), 1574 (NH bending amide), 1157, 1309 (S=O stretching)**;** nitrogen analysis 9.79 (calc.), 9.73(obs.); ^{1}NMR ($CDCl_3$, δ(ppm): 7.10-7.94

(m,9H,aromatic), 2.68 (t,2H,RCH$_2$Ar), 5.20 (s,1H,CONH), 1.28 (m,6H, cyclohexane).

N-[4-{2-(benzamido) ethyl} phenyl sulfonyl]-N'-methyl urea (8)

IR (KBr; cm^{-1}): 3086 (C-H stretching - Ar), 787(C-H bending-Ar), 2854 (C-H stretching-Aliphatic), 1676 (carbonyl stretching), 3242 (NH stretching amide), 1575 (NH bending amide), 1151, 1361 (S=O stretching)**;** nitrogen analysis 11.63 (calc.), 11.59 (obs.); ^{1}NMR (CDCl$_3$, δ(ppm): 6.84-7.32 (m,9H, aromatic), 2.48 (t,2H,R-CH$_2$-Ar),5.24 (s,1H,CONH).

N-[4-{2-(1-chloro acetamido) ethyl} phenyl sulfonyl]-N'-cyclohexyl urea(9)

IR (KBr; cm^{-1}): 2992 (C-H stretching - Ar), 760(C-H bending-Ar), 2945 (C-H stretching-Aliphatic), 1620 (carbonyl stretching), 3364 (NH stretching amide), 1537 (NH bending amide), 1155, 1309 (S=O stretching), 825 (C-Cl stretching)**;** nitrogen analysis 10.47 (calc.), 10.51 (obs.); ^{1}NMR (CDCl$_3$,δ(ppm): 3.42 (s,2H, Cl-CH$_2$-), 2.42 (t,2H,R-CH$_2$-Ar), 6.20(s,1H,CONH).

N-[4-{2-(1-chloro acetamido) ethyl} phenyl sulfonyl]-N'-methyl urea(10)

IR (KBr; cm^{-1}): 2937 (C-H stretching - Ar), 767 (C-H bending-Ar), 2856 (C-H stretching-Aliphatic), 1645 (carbonyl stretching), 3366 (NH stretching amide), 1595 (NH bending amide), 1157 (S=O stretching), 843 (C-Cl stretching)**;** nitrogen analysis 12.61 (calc.), 12.61(obs.); ^{1}NMR (CDCl$_3$, δ(ppm): 3.48 (s,2H,Cl-CH$_2$-), 2.42 (t,2H,R-CH$_2$-Ar), 5.94 (s,1H,CONH), 1.30(m, 6H, cyclohexane).

Table 1: Physical data of the title compounds.

Compound no.	Empirical formula	Melting point (^{0}C)	Yield (%)
1	$C_{28}H_{28}N_3O_5S$	172	78.45
2	$C_{18}H_{20}N_3ClO_5S$	166	74.79
3	$C_{22}H_{26}N_3O_5S$	171	54.0
4	$C_{17}H_{18}N_3O_5S$	168	44.50
5	$C_{23}H_{29}N_3O_5S$	182	53.01
6	$C_{18}H_{21}N_3O_5S$	170	45.90
7	$C_{22}H_{27}N_3O_4S$	192	72.89
8	$C_{17}H_{19}N_3O_4S$	163	42.31
9	$C_{17}H_{24}N_3O_4S$	135	25.86
10	$C_{12}H_{16}N_3O_4S$	143	29.42

Biological activity

Hypoglycemic assay[7, 8,9,10]

Female sprague dawley rats weighing between 170-250 gm were fasted for 18 hrs with excess water and libitum. The compounds were grounded and 1% suspension in carboxymethyl cellulose were prepared and dosed orally by gavage at 50mg/kg to the rats. The animals were divided into three different groups of five animals each. First group received vehicle and served as reference control .The second group of animals was administered with Glibenclamide (5mg/kg). Remaining group of animals received the test compounds at a dose of 50mg/kg. Blood glucose concentration was estimated at 0,1,2,3,4,6,12,24 hours by using blood glucose strips and glucometer. The Mean blood glucose concentration and the mean percent reduction of glucose level were calculated and results are tabulated in table1 and 2.

Table 1 : Mean blood glucose concentration (mg/dl) ± standard error after drug administration.

Treatment group (n=5)	Time (hours)							
	0	**1**	**2**	**3**	**4**	**6**	**12**	**24**
Control	48.24 (±3.61)	47.42 (±3.36)	46.12 (±2.92)	45.34 (±3.44)	43.40 (±3.21)	42.64 (±4.64)	41.52 (±3.34)	40.91 (±5.54)
Compound 1	55.62 (±2.97)	40.34 (±7.06)	38.84 (±7.18)	32.68 (±5.55)	23.12 (±6.35)	22.34 (±4.48)	19.42 (±8.35)	32.34 (±5.24)
Compound 2	36.64 (±4.05)	32.52 (±3.17)	30.16 (±4.46)	28.44 (±4.06)	27.82 (±3.20)	26.28 (±3.62)	15.72 (±4.28)	28.74 (±4.35)
Compound 3	34.88 (±4.92)	28.66 (±3.96)	22.42 (±3.52)	20.85 (±3.70)	18.54 (±2.85)	12.60 (±2.51)	10.21 (±1.98)	18.20 (±2.38)
Compound 4	36.54 (±6.52)	34.38 (±6.16)	30.44 (±7.51)	29.62 (±7.21)	28.92 (±6.74)	27.96 (±7.16)	24.74 (±7.24)	32.76 (±6.54)
Compound 5	37.82 (±4.09)	34.64 (±3.52)	32.81 (±3.45)	31.64 (±3.64)	30.28 (±3.72)	28.55 (±3.24)	20.26 (±3.05)	26.42 (±4.16)
Compound 6	38.81 (±6.72)	37.84 (±6.18)	35.53 (±7.28)	30.57 (±7.28)	26.52 (±7.28)	27.62 (±7.48)	22.28 (±6.66)	32.65 (±4.83)
Compound 7	31.41 (±4.32)	29.54 (±3.58)	24.82 (±3.56)	21.84 (±2.58)	18.20 (±3.82)	16.25 (±1.48)	10.44 (±0.89)	27.22 (±6.66)
Treatment group (n=5)	**Time (hours)**							
	0	**1**	**2**	**3**	**4**	**6**	**12**	**24**
Compound 8	32.24 (±4.14)	28.52 (±4.04)	24.88 (±3.45)	22.44 (±2.15)	18.50 (±1.52)	16.32 (±1.68)	10.13 (±0.38)	25.20 (±2.89)
Compound 9	37.40 (±6.66)	35.61 (±5.22)	27.82 (±7.12)	25.64 (±7.46)	22.83 (±7.58)	20.33 (±6.82)	12.10 (±6.15)	27.82 (±6.15)
Compound 10	39.64 (±4.12)	32.55 (±5.20)	29.74 (±6.55)	27.66 (±7.54)	26.74 (±5.28)	24.36 (±7.42)	14.54 (±6.60)	34.36 (±7.16)
Standard	47.42 (±5.55)	45.22 (±5.70)	38.64 (±6.28)	32.41 (±6.48)	28.90 (±5.72)	27.62 (±7.47)	15.80 (±4.32)	22.64 (±5.43)

Table 2 : Mean percentage reduction of blood glucose ± standard error after administration of test compounds.

Treatment group (n=5)	Time (hours)						
	1	**2**	**3**	**4**	**6**	**12**	**24**
Control	1.52 (±1.92)	4.12 (±3.52)	6.32 (±4.17)	7.21 (±5.54)	9.22 (±4.63)	12.45 (±5.17)	18.65 (±4.20)
Compound 1	26.57 (±8.99)	48.28 (±11.38)	54.68 (±10.51)	60.51 (±9.16)	68.30 (±8.24)	86.28 (±5.45)	58.87 (±6.23)
Compound 2	9.28 (±3.95)	25.24 (±9.27)	32.62 (±6.26)	42.75 (±5.16)	58.58 (±7.27)	68.24 (±6.15)	48.28 (±5.28)
Compound 3	16.99 (±7.68)	18.28 (±6.92)	38.80 (±5.74)	48.35 (±6.48)	62.58 (±4.75)	88.71 (±7.43)	64.48 (±7.22)
Compound 4	8.58 (±2.42)	24.34 (±5.84)	26.78 (±5.34)	30.41 (±4.18)	32.68 (±4.54)	54.35 (±5.60)	25.30 (±3.23)
Compound 5	18.68 (±1.46)	27.68 (±4.58)	38.38 (±3.24)	42.52 (±4.45)	47.20 (±9.10)	68.84 (±6.72)	32.73 (±12.25)
Compound 6	9.65 (±2.82)	18.32 (±4.92)	24.62 (±4.22)	34.68 (±5.62)	38.24 (±3.86)	58.35 (±4.60)	38.18 (±5.26)
Compound 7	8.52 (±5.42)	14.75 (±10.17)	37.12 (±12.52)	44.72 (±12.10)	52.19 (±7.10)	78.25 (±10.52)	38.54 (±7.82)
Treatment group (n=5)	**Time (hours)**						
	1	**2**	**3**	**4**	**6**	**12**	**24**
Compound8	16.28 (±2.14)	27.85 (±5.72)	38.45 (±4.62)	44.82 (±5.18)	48.64 (±7.25)	64.72 (±8.91)	40.63 (±11.20)
Compound 9	4.74 (±1.54)	16.45 (±5.76)	28.32 (±6.18)	33.47 (±7.82)	42.83 (±6.26)	68.74 (±5.22)	47.98 (±5.30)
Compound10	18.25 (±3.28)	28.14 (±5.68)	28.58 (±6.26)	38.21 (±5.48)	54.68 (±4.22)	72.38 (±5.20)	38.30 (±4.18)
Standard	1.82 (±1.34)	16.56 (±6.54)	22.32 (±5.18)	34.84 (±3.75)	58.32 (±6.14)	72.55 (±6.62)	42.74 (±7.23)

RESULTS AND DISCUSSION

Various analogs of N-[4-{2-(5-chloro-2methoxy benzamido) ethyl}phenyl sulfonyl]-N'-cyclohexyl urea were synthesized and purified. These compounds were then characterized by physical, chemical and spectral analysis. The analogs were also evaluated for hypoglycemic activity using experimental animal model. All compounds showed significant reduction in blood glucose level. Compound 1 and 3 were found to exhibit good and significant hypoglycemic activity. Compound 2, 5, 8 and 10 showed comparable activity to standard Glibenclamide.

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