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SYNTHESIS, CHARACTERIZATION AND ANTI-DIABETIC ACTIVITY OF VANILLIN BASED ACETOHYDRAZIDE-HYDRAZONE DERIVATIVES

M. Upendar Reddy*[1,3] and M. C. Somasekhara Reddy[2]

[1]Department of Chemistry, Rayalaseema University, Kurnool-518007, Andhra Pradesh, India.

[2]Department of Basic Sciences, G.P.R Engineering College (Autonomous), Kurnool-518007, Andhra Pradesh, India.

[3]Dr. J.C.R Bio, Plot No. 79/80, Survey No-12, Chengicherla Village, Cherlapalli Phase III Industrial Park, Hyderabad-500039, Telangana State, India.

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***Corresponding Author**
M. Upendar Reddy
Department of Chemistry, Rayalaseema University, Kurnool-518007, Andhra Pradesh, India.

ABSTRACT

Hydrazide–hydrazone derivatives are present in many bioactive molecules and display a wide variety of biological activities, such as antibacterial, antitubercular, antifungal, anticancer, anti-inflammatory, anticonvulsant, antiviral and antiprotozoal action. The present paper describes the synthesis, characterization and anti-diabetic activity studies of vanillin based acetohydrazide-hydrazone derivatives. The synthesis involves the utilization of 2-phenylacetic acids and vanillin as starting materials. The direct conversion of 2-phenylacetic acids to 2-phenylacetohydrazides was accomplished in presence of HATU as peptide reagent, resulting in 88-92% yield**.** Coupling of vanillin **1** with 4-(bromomethyl)benzonitrile in 2-methyl-tetrahydrofuran in presence of potassium carbonate gave 4-((4-formyl-2-methoxyphenoxy)methyl)benzonitrile in 94% yield. Coupling of 4-((4-formyl-2-methoxyphenoxy)methyl)benzonitrile with 2-phenylacetohydrazides in ethanol at 75^{o}C resulted in the formation of corresponding substituted phenyl-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazides in 88-90% yield. The structures of these derivatives were determined by ^{1}H NMR, IR, mass spectroscopic techniques. These compounds were evaluated for their *in vivo* for their oral hypoglycemic activity by alloxan induced diabetic model in rat (anti-diabetic studies). Among all the derivatives, compound with substitution R = 2,3-dihydrobenzofuran was found to exhibit significant hypoglycemic

activity (65.35%) when compared to insulin as a reference drug which showed 69.77% blood glucose lowering activity.

KEYWORDS: Acetohydrazide, Anti-diabetic, Hydrazone, Synthesis, Vanillin.

1. INTRODUCTION

Natural products are imperative basis for the advancement of new drugs that attribute to exclusive modes of action, enviable biological activities, effortless decomposition, environmental friendliness, low mammalian toxicity and specificity to target species.[1,2] Vanillin (4-hydroxy-3-methoxybenzaldehyde), a natural product a resultant product from orchids (*Vanilla planifolia*, *V. pompona*, or *V. tahitiensis*)[3], has engrossed the consideration of biologists. Vanillin a flavoring compound, has extensive uses in the pharmaceutical industries, nutraceutical food and beverage.[4,5] It has a simple chemical structure and has desirable biological activities[4] such as antimicrobial[6,7], antimutagenic[8], antiproliferative[9], anti-inflammatory[10], antitumor[11-13], antioxidant[14,15] antifungal[16-18], activities. In addition, vanillin derivatives possess desirable antifungal[19] and antibacterial[20] activities.

Hydrazide–hydrazone derivatives are present in many bioactive molecules and display a wide variety of biological activities, such as antibacterial, antitubercular, antifungal, anticancer, anti-inflammatory, anticonvulsant, antiviral and antiprotozoal action.[21] Therefore, many medicinal chemists synthesize various hydrazide–hydrazones and evaluate them for biological activities. Among biological properties of this class of compounds, antimicrobial activity is the most frequently encountered in scientific literature.[22,27] Perusal of literature survey on the hydrazone derivatives exhibiting anti-diabetic have seldom appeared in the literature[28,29], the present paper describes the synthesis, characterization and anti-diabetic activity studies of vanillin based acetohydrazide-hydrazone derivatives.

2. MATERIALS AND METHODS

The uncorrected melting points of compounds were taken in an open capillary in a paraffin bath. All reagents used were commercial and laboratory grade, melting points were determined in open capillaries and are uncorrected. IR spectra were recorded on potassium bromide disks on a Perkin-Elmer 383 spectrophotometer. ^{1}H NMR spectra were obtained on Varian 400 MHz instrument and Varian 400 MHz, with TMS as internal Standard and chemical shifts are expressed in δ ppm solvent used in $CDCl_3$ (in case of intermediate compounds) and DMSO-d_6 (in case of final compounds) and mass spectrum on a Hewelett

Packard mass spectrometer operating at 70 ev, purity of the compounds were checked by TLC, which is performed with E. Merck pre coated silica gel plates (60 F-254) with iodine as a developing agent. Acme, India silica gel, 60-120 mesh for column chromatography is used

2.1 EXPERIMENTAL SECTION

CHEMISTRY

2.1.1 General method for the preparation of 2-phenylacetohydrazides (14-18)

Procedure 1: A mixture of 2-phenylaceticacids **4-8** (3.37 mmol) and Amberlyst -15 (500 mg) in ethanol (30 mL) was refluxed for 72 h and cooled to room temperature. The insoluble Amberlyst-15 was filtered and washed with ethanol (20 mL). The combined ethanol filtrates (containing corresponding ethyl-2-phenylacetates, **9-13)** was reacted with hydrazine hydrate (1.09 mL, 21.8 mmol) and heated to reflux for 10 h. After recovering back the ethanol solvent, the obtained residue was titurated with diethyl ether to obtain the corresponding 2-phenylacetoydrazides **(14-18).** Yields of the products ranged from 78-82%.

Procedure 2: To a stirred solution of 2-phenylaceticacids **4-8** (3.37 mmol) in 2-Methyl tetrahydrofuran (20 mL) was added triethyl amine (7.40 mmol) (cooled between 15-20°C) followed by HATU (4.0 mmol) and stirred at room temperature for 10 h. After completion of the reaction (monitored by TLC), the reaction was diluted with water and extracted with isopropylacetate (2 X 20 mL), the organic layer was separated, washed with water (2 X 20 mL) followed by brine solution. The isopropylacetate layer was dried over Na_2SO_4, filtered and evaporated under reduced pressure to isolate the corresponding 2-phenylacetohydrazides **(14-18).** Yields of the products ranged from 88-92%.

2-phenylacetohydrazide (14)

Off-white solid; Yield; 85%; M.p: 116-117^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 7.10 (d, J = 6.8 Hz, 1H), 6.82 (d, J = 6.8 Hz, 2H), 6.70 (d, J = 6.6 Hz, 1H0, 5.80 (d, J = 6.6 Hz, 1H), 5.30 (br.s, 1H), 3.56 (s, 2H).

2-(4-nitrophenyl)acetohydrazide (15)

Yellow solid; Yield: 88%; M.p.; 167^oC; ^1H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, J = 3.0 Hz, 2H), 7.42 (d, J = 2.6 Hz, 2H), 6.70 (br.s, 1H), 3.90 (br.s, 2H), 3.60 (s, 2H).

2-(3,5-dimethylphenyl)acetohydrazide (16)

Pale brown solid; Yield: 88%; M.p.: 94-95^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.90 (s, 1H), 6.82 (s, 2H), 6.70 (br.s, 1H), 3.90 (br.s, 2H), 3.50 (s, 2H), 2.40 (s, 6H).

2-(2-chloro-4-fluorophenyl)acetohydrazide (17)

White solid; Yield: 82%; M.p.: 118-119^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38-7.36 (m, 1H), 7.18-7.14 (m, 1H), 7.02-6.97 9m, 1H), 6.70 (br.s, 1H), 3.90 (br.s, 2H), 3.60 (s, 2H).

2-(2,3-dihydrobenzofuran-5-yl)acetohydrazide (18)

White solid; Yield: 85%; M.p.: 112-113^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.10 (s, 1H), 6.94 (d, J = 7.2 Hz, 1H), 6.78 (d, J = 7.2 Hz, 1H), 6.62 (br.s, 1H), 4.58 (t, J = 5.8 Hz, 2H), 3.84 (br.s, 2H), 3.42 (s, 2H), 3.18 (t, J = 5.8 Hz, 2H).

2.1.2 Preparation of 4-((4-formyl-2-methoxyphenoxy)methyl)benzonitrile (3)

To a solution of vanillin **1** (1g, 6.58 mmol) in 2-methyl-tetrahydrofuran (20 mL) was added potassium carbonate (1.10g, 7.88 mmol) followed by 4-(bromomethyl)benzonitrile **2** (1.80g, 6.71 mmol) and stirred at room temperature for 7h. The solvent was evaporated and the pale yellow residue was diluted with water (15 mL) to afford white solid which was filtered applying vacuum and dried to obtain compound **3**.

White solid; Yield: 1.64g, 94%; M.p: 112-113^{o}C; IR (KBr): υ_{max} 2229 (-CN str), 1680 (-CHO str) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.84 (s, 1H), 7.92 (d, J = 8.2 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 7.58 (d, J = 8.0 Hz, 1H), 7.42 (s, 1H), 7.22 (t, J = 8.4 Hz, 1H), 5.38 (s, 2H), 3.88 (s, 3H); ESI-MS: m/z, 267.9 $(M-1)^{+}$.

2.1.3 General experimental procedure for the synthesis of acetohydrazide-hydrazone derivatives (19-23)

To a stirred solution of compound **3** (100mg, 0.375mmol) in ethanol was added arylacetohydrazides **14-18** (0.375 mmol) and heated to 75^{o}C for 1h. The reaction mixture was cooled to room temperature and the obtained solids were washed with diethyl ether to obtain the pure compounds **19-23**. Yields of the products obtained were about 88-90%.

Phenyl-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazide (19)

White solid; Yield: 88%; M.p.: 124-125^{o}C; IR (KBr): υ_{max} 3443 (-NH str), 2229 (-CN str),1659 (-C=O str) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d6): 11.52 (* 11.22, s, 1H), 8.18 (* 7.98, s, 1H), 7.86 (d, J = 7.6 Hz, 2H), 7.62 (d, J = 7.2 Hz, 2H), 7.38-7.34 (m, 5H), 7.26-7.24

(m, 1H), 7.18 (brs, 1H), 7.0 9 (t, J = 6.8 Hz, 1H), 5.22 (s, 2H), 4.0 (* 3.56, s, 2H), 3.84 (* 3.82, s, 3H); ESI-MS: m/z, 400.0 $(M+1)^+$.

(4-Nitro-phenyl)-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazide (20)

Yellow solid; Yield: 90%; M.p.: 99-101^oC; IR (KBr): υ_{max} 3433 (-NH str), 2229 (-CN str), 1650 (-C=O str), 1373 (-NO_2 str) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 11.60 (* 11.40, s, 1H), 8.18 (* 7.94, s, 1H), 8.20 (d, J = 7.8 Hz, 2H), 7.86 (d, J = 7.8 Hz, 2H), 7.64 (d, J = 7.2 Hz, 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.38 (d, J = 7.2 Hz, 1H), 7.20 (d, J = 7.6 Hz, 1H), 7.00 (dd, J = 3.2, 6.6 Hz, 1H), 5.20 (s, 2H), 4.20 (* 3.78, s, 2H), 3.82 (* 3.80, s, 3H); ESI-MS: m/z, 445.0 $(M+1)^+$.

(3,5-Dimethyl-phenyl)-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazide (21)

Off-white solid; Yield: 84%; M.p.: 89-90^oC; IR (KBr): υ_{max} 3321 (-NH str), 2230 (-CN str), 1658 (-C=O str) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 11.42 (* 11.22, s, 1H), 8.18 (* 7.88, s, 1H), 7.90 (d, J = 7.0 Hz, 2H), 7.62 (d, J = 7.2 Hz, 2H), 7.38 (dd, J = 3.4, 7.4 Hz, 1H), 7.18 (d, J = 6.8 Hz, 1H), 7.12 (d, J = 6.8 Hz, 1H), 6.92 (d, J = 7.2 Hz, 2H), 6.84 (d, J = 7.2 Hz, 1H), 5.22 (s, 2H), 3.88 (* 3.86, s, 3H), 3.82 (* 3.42, s, 2H), 2.92 (s, 3H), 2.0 (s, 3H); ESI-MS: m/z, 428.0 $(M+1)^+$.

(2-Chloro-4-fluoro-phenyl)-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazide (22)

Pale yellow solid; Yield: 82%; M.p.: 132-133^oC; IR (KBr): υ_{max} 3432 (-NH str), 2221 (-CN str), 1671 (-C=O str) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 11.58 (* 11.40, s, 1H), 8.18 (* 7.96, s, 1H), 7.82 (d, J = 7.2 Hz, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.48-7.42 (m, 2H), 7.38 (s, 1H), 7.28-7.18 (m, 2H), 7.0 (t, J = 6.8 Hz, 1H), 5.22 (s, 2H), 4.18 (* 3.72, s, 2H), 3.84 (s, 3H); ESI-MS: m/z, 452.1 $(M+1)^+$.

(2,3-Dihydro-benzofuran-5-yl)-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazide (23)

Off-white solid; Yield: 84%; M.p.: 140-141^oC; IR (KBr): υ_{max} 3263 (-NH str), 2221 (-CN str), 1654 (-C=O str) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): 11.40 (* 11.0, s, 1H), 8.18 (* 7.88, s, 1H), 7.76-7.66 (m, 4H), 7.58 (d, J = 7.6 Hz, 2H), 7.36-7.26 (m, 3H), 7.18-7.10 (m, 3H), 7.0

(t, J = 6.8 Hz, 1H), 6.24 (dd, J = 3.6, 7.2 Hz, 1H), 5.18 (s, 2H), 4.44 (t, J = 6.4 Hz, 2H), 3.84 (* 3.83, s, 3H), 3.80 (* 3.40, s, 2H), 3.10 (t, J = 7.2 Hz, 2H); ESI-MS: m/z, 511.1 $(M+1)^+$.

2.2 Biology Experimental

2.2.1 Pharmacological evaluation

The acute toxicity studies and anti-diabetic activity studies were conducted following the previous reported literature protocols.[30,34] All the compounds were screened *in vivo* for their oral hypoglycemic activity by alloxan induced diabetic model in rat. LD_{50} cut-off value of the test compounds was fixed as 50 mg kg^{-1}, so that 500 mg kg^{-1} i.e., 1/10 of cut-off value was taken as screening dose for evaluation of antidiabetic activity. All the animal experiments were conducted by the approval of Institutional Animal Ethics Committee, Anurag Group of Institutions (formerly Lalitha college of Pharmacy), Hyderabad, India. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals.

Animals were divided into 10 groups of 6 animals (n = 6): Group 1 diabetic animals (vehicle) received 0.5% CMC (1 mL); Group 2 diabetic animals received insulin 50 mg/Kg. Groups (3–10) diabetic animals received compounds **19–23** in a single dose of 50 mg/kg body weight per oral respectively for 7 days continuously. Blood was withdrawn from the tail vain each time. Blood glucose was measured at 0, 3rd and 7th days interval. At the end of 0, 3rd and 7th day, blood samples were withdrawn from a tail vein by snipping the tip of the tail and the blood glucose level was measured by Accu Sure Blood Glucose Monitoring System (Dr. Gene Health & Wellness).

Statistical analysis

Values are represented as mean ± SEM. Data were analyzed using analysis of variance and group means were compared with Tukey–Kramer Post ANOVA test. The values were considered when $P < 0.01$.

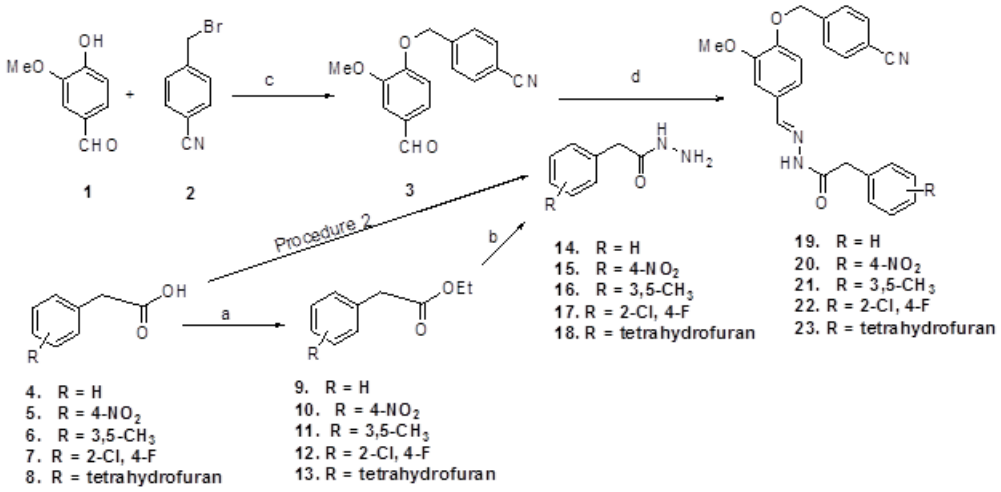
3.0 RESULTS AND DISCUSSION

3.1CHEMISTRY

The synthesis of acetohydrazide-hydrazone derivatives (**19-23**) is illustrated in Scheme 1. The synthesis begins with the utilization of 2-phenylacetic acids as starting materials. Initially, 2-phenylacetohydrazides **14-18** was prepared in two procedures involving single

step synthesis i.e procedure 2 and a two step synthesis involving procedure 1. Procedure 1 involves the conversion of 2-phenylacetic acids **4-8** to the corresponding 2-phenylethyl benzoates **9-13** followed by immediate conversion into corresponding 2-phenylacetohydrazides **14-18** resulting in 78-82% yield, while the procedure 2 involves the direct conversion of 2-phenylacetic acids **4-8** to 2-phenylacetohydrazides **14-18** in presence of HATU, resulting in 88-92% yield**.** On the other hand, reaction of vanillin **1** with 4-(bromomethyl)benzonitrile **2** in 2-methyl-tetrahydrofuran in presence of potassium carbonate at room temperature for 7h gave 4-((4-formyl-2-methoxyphenoxy)methyl)benzonitrile **3** in 94% yield. Coupling of the aldehyde **3** with 2-phenylacetohydrazides **14-18** in ethanol at 75^{o}C for 1h resulted in the formation of corresponding substituted phenyl-acetic acid [4-(4-cyano-benzyloxy)-3-methoxy-benzylidene]-hydrazides **19-23** in 88-90% yield.

The structural elucidation of the synthesized hydrazide-hydrazone derivatives **19-23** were determined by ^{1}H NMR, mass and IR spectral data. ^{1}HNMR elucidation of compound 20 is described here, the broad singlet at 11.60 (* 11.40 ppm) and 8.18 ppm (* 7.94 ppm) corresponds to the protons representing to –CO-**NH**- and -CO-NH-N=**CH-** groups respectively. In the aromatic region, the protons of 4-nitro-phenyl ring, 4-cyano-phenyl ring and vanillin ring protons appeared as expected. The protons resonating at 8.20 ppm, 7.64 ppm as doublet with two proton integration corresponds to the 4-nitro-phenyl ring and the protons resonating at 7.86 ppm and 7.58 ppm as doublet with two proton integration corresponds to the 4-cyano-phenyl ring couple to the vanillin moiety. The vanillin aromatic ring protons appeared at 7.38 ppm, 7.20 ppm and 7.00 ppm respectively. The protons in the aliphatic region at 5.20 ppm as singlet, 4.20 (* 3.78) ppm and 3.82 (*3.80) ppm is assigned to the following groups –OCH_2-, -C=O-CH_2 and OCH_3 groups respectively. '*' indicates that these compounds exist as a mixture of two rotameric forms in solution[35] e.g. antiperiplanar (*ap*) and synperiplanar (*sp*) as indicated by their ^{1} H NMR spectra. The mass spectrum of the compound **20** showed m/z, 445.0 as (M+1) peak corresponding to the desired molecular ion, and is in agreement with the structure. In the IR spectra, the functional groups appeared in the expected region (presented in experimental section). The peaks in the IR spectra region at 3263-3443, 2221-2230, 1650-1671cm^{-1} corresponds to –NH (str), -CN (str) and -C=O (str) respectively. The above description of ^{1}H NMR, mass and IR thus confirms the formation of desired compound **23**. Similarly, the ^{1}H NMR, mass and IR data of the remaining hydrazide-hydraone derivatives is in agreement with the desired structure.



Scheme 1: Synthesis of hydrazide-hydrazone derivatives 19-23.

Reaction conditions: Procedure-1: a) Amberlyst-15, Ethanol, reflux, 72h; b) Hydrazine-hydrate, ethanol, reflux, 10h; b) 2-phenylacetic acids **4-8**, HATU, Triethlamine, 2-Methyl tetrahydrofuran, room temperature, 10h; c) 4-(bromomethyl)benzonitrile **2,** 2-Methyl tetrahydrofuran, K_2CO_3, room temperature, 10h; d) Arylacetohydrazides **14-18**, ethanol, 75^oC, 1h.

3.2 Antidiabetic activity

All the compounds were screened *in vivo* for their oral hypoglycemic activity by alloxan induced diabetic model in rat. The results of the hypoglycemic property of the synthesized hydrazone derivatives are presented in **Table 1**. A major increase in blood glucose was determined in diabetic rats. All the compounds **19**–**23** had shown a significant reduction in blood glucose as compared to control diabetic rats at 50 mg/kg body weight for 3rd and 7th days.

Insulin was taken as a reference drug which showed 69.77% blood glucose lowering activity at the dose of 50 mg/kg.p.o. Among all the synthesized derivatives, compound **23** (65.35%) with substitution R = 2,3-dihydrobenzofuran was found to exhibit significant hypoglycemic activity when compared to the compounds within the series, while the compound **19** (54.62%) and compound **21** (58.22%) with substitution R = H and 3,5-dimethyl showed moderate hypoglycemic activity. The intermediate aldehyde **3** (49.22%) and the compounds **20** (52.44%) and **22** (53.22%) with substitution R = 4-NO_2 and 2-Cl, 4-Fluoro displayed weak hypoglycemic activity.

Table 2: Hypoglycemic effects of synthesized hydrazide-hydrazone derivatives 19-2.

Treatment (mg/kg b.w p.o)	Blood glucose level (mg/dl)			
	0-day	3-day	7-day	% of hyperglycemic activity
Control (0.5% CMC	345.0 ± 2.48	376.3 ± 3.54**	395.1 ± 3.03**	
Insulin	351 ± 3.98	140.60 ± 3.54**	106.1 ± 3.56**	69.77
3	344.4 ± 1.75	169.51 ± 1.82**	174.89 ± 3.16**	49.22
19	336.5 ± 1.75	183.79 ± 3.26**	152.71 ± 2.29**	54.62
20	350.8 ± 2.26	183.95 ± 2.08**	166.85 ± 2.31**	52.44
21	348.6 ± 3.16	202.95 ± 2.06**	145.65 ± 1.76**	58.22
22	337.5 ± 3.20	179.61 ± 2.03**	157.89 ± 3.69**	53.22
23	338.2 ±2.25	221.01 ± 1.58**	117.19 ±1.45**	65.35

Values are expressed as mean ± SEM; (n=6), **P < 0.001.

% of hyperglycemic activity = 351-106.1/ 351X 100 = 69.77

4.0 CONCLUSION

All the compounds were screened *in vivo* for their oral hypoglycemic activity by alloxan induced diabetic model in rat. Insulin was taken as a reference drug which showed 69.77% blood glucose lowering activity at the dose of 50 mg/kg.p.o. Among all the synthesized derivatives, compound **23** (65.35%) with substitution R = 2,3-dihydrobenzofuran was found to exhibit significant hypoglycemic activity when compared to the compounds within the series, while the compound **19** (54.62%) and compound **20** (58.22%) with substitution R = H and 3,5-dimethyl showed moderate hypoglycemic activity.

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6.0 CONFLICT OF INTEREST

"The author(s) declare(s) that there is no conflict of interest regarding publication of this article".

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