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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF GLYCOGEN SYNTHASE KINASE-3β INHIBITORS AS ANTIDIABETIC AGENTS

Keerti Jain [*1], Sapna Malviya [2], Arun Kumar Gupta [3] and Anil Kharia [4]

Department of Pharmaceutical Chemistry [*1], Smriti College of Pharmaceutical Education, Indore-452010, (M.P.), India.
Department of Pharmacy [2], Modern Institute of Pharmaceutical Sciences, Indore, (M.P.), India.
Department of Pharmacy [3], Central India Institute of Technology, Indore, (M.P.), India.

Keywords:

Glycogen Synthase Kinase-3β, Type 2 Diabetes mellitus, Phenylmethylene hydantoin, Drug design, Docking, Streptozotocin induced model, liver glycogen content.

Correspondence to Author:
Keerti Jain
Assistant Professor,
Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Chemistry, Modern Institute of Pharmaceutical Sciences, Indore, (M.P.), India.

E-mail: jainkeerti05@gmail.com

ABSTRACT: Purpose. To synthesize structurally modified novel Glycogen Synthase Kinase-3β inhibitors to overcome insulin resistance in type 2 diabetes mellitus and to overcome associated health problems with reducing morbidity, mortality and economic costs of diabetes. **Methods.** Nineteen Phenylmethylene hydantoin analogs were designed and docked in the ATP binding site of GSK-3β by Molegro Virtual Docker 2006.1.5. The synthesis of proposed six hydantoin analogs were performed based on docking results by two approaches, Knoevenagel condensation and Steglich Esterification reaction, keeping the hydantoin ring and different ester substitution at benzylidene ring system can afford potent and selective GSK-3β inhibitors involved in the control of glycogen metabolism. The antidiabetic activity and liver glycogen content were also determined against Streptozotocin induced diabetic rat model. **Results.** The synthesized compounds **H-a, H-c and H-e** increased hepatic glycogen content in range 410-420 mg/gm liver weight while compounds **H-b, H-d and H-f** cause increased in hepatic glycogen content in range of 357-370 mg/gm liver weight as compared to control having 242.01 mg/gm liver weight. **Conclusion.** The synthesized compounds decreased blood glucose level in glucose loaded hyperglycemia rats and also increased liver glycogen content. Structure activity relationship revealed that -ortho & -para chloro (electron withdrawing) substituted analogs favours for the activity while electron releasing (-methyl) substituted analogs having low potency.

INTRODUCTION: Diabetes mellitus, or simply diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. [1]

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It is one of the most common endocrine disorders affecting almost 6% of the world's population. The total number of people with diabetes is projected to raise from 171 million in 2000 to 366 million in 2030. More than 97% of these patients will have type 2 diabetes. [2] Type 2 diabetes is a leading cause of death in the developed world. This disease characteristically begins with insulin resistance in the peripheral tissues, and it is believed that potentiating insulin action may provide a valuable mode of treatment. [3] After meals, insulin controls blood glucose levels by promoting glucose transport into peripheral tissues and enhancing formation of glycogen. [4] At other times, glycogen

formation in resting cells is suppressed via phosphorylation and inactivation of the rate-limiting enzyme glycogen synthase (GS). [5]

Glycogen synthase kinase-3 (GSK-3) is the rate-limiting enzyme of glycogen biosynthesis has its ability to phosphorylate and inhibit glycogen synthase. [6] Mammalian GSK-3 exists as two isoforms, GSK-3α and GSK-3 β, sharing 98% homology in their catalytic domain. Both isoforms are ubiquitously expressed in cells and tissues, and have similar biochemical properties. [7] It is known that GSK-3 is involved in diverse cellular processes and might have multiple substrates. For example, GSK-3 phosphorylates and inhibits the functioning of insulin receptor substrate-1 (IRS-1) and GS, the two key targets in the insulin signaling pathway. [8, 9] Suppression of these targets may limit most insulin-mediated biological responses. In addition, elevated GSK-3 activity was found in diabetic tissues, reinforcing GSK-3 as a promising therapeutic target for insulin resistance and Type 2 diabetes. GSK 3 human consists of 420 amino acids sequence. [10]

METHODS:

The discovery was focussed on exploration of SAR around the Phenylmethylene hydantoin nucleus (**Figure 1)** with the aim of improving potency and kinase selectivity against GSK-3β and improving *in vivo* bioavailability.

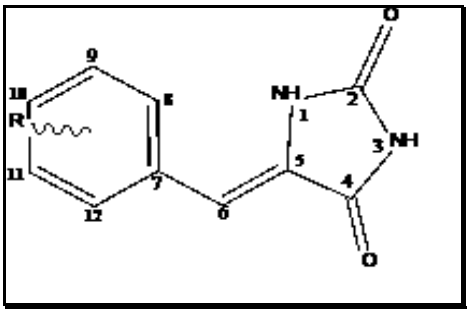


FIGURE 1: PHENYLMETHYLENE HYDANTOIN NUCLEUS

Docking of Designed Compounds:

The molecular docking was performed using Molegro Virtual Docker (MVD) 2006.1.5 and CS Chemoffice version 11.0. The docking scoring function of MolDock used is based on a piecewise linear potential (PLP) including new hydrogen bonding and electrostatic terms introduced by Gehlhaar et al., 2006.

Structures of all the compounds were sketched using builder module of the program. The sketched structures were subjected to energy minimization using molecular mechanics (MM2) until the RMS gradient value became smaller than 0.1 kcal/molA°. The energy minimized molecules were subjected to re-optimization via Austin model-1 (AM1) method until the RMS gradient attained a value smaller than 0.01 kcal/mol °A using MOPAC. The descriptor values for all the molecules were calculated using "compute properties" module of program. The minimized molecule was saved as MOL file format.

Following Steps were used for docking through MVD:

- **Importing and Preparing Molecules**

For importing the file the Molegro Virtual Docker Supports PDB, Mol2, SDF, and its own XML-based format, MVDML. Then the Molecular Surface is added in that structure and the binding site is predicted.

- **Running the Docking Simulation**

By selecting Docking, Docking Wizard option, choosing the structure to be docked and then definig the Region of interest. It starts docking and then after few minutes the results can be viewed. **Figure 2**.

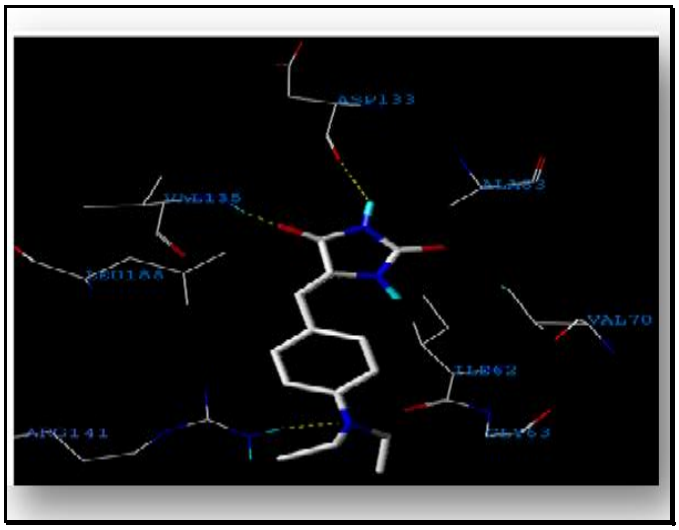


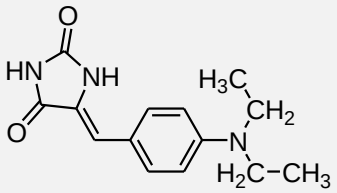
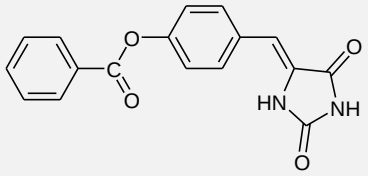
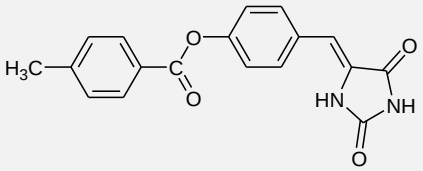
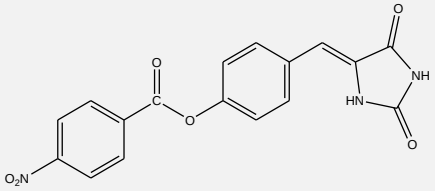
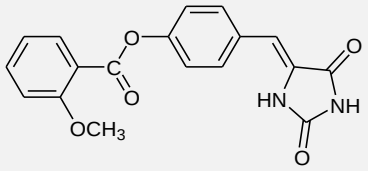
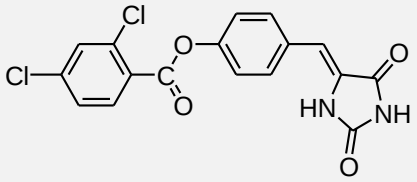
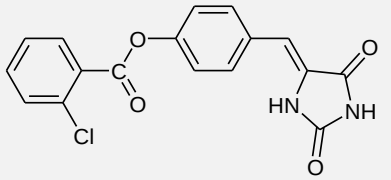
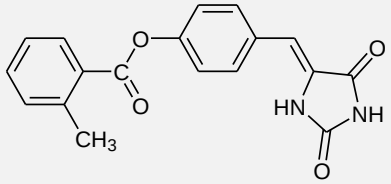
FIGURE 2: DOCKING POSE OF A PHENYLMETHYLENE HYDANTOIN MOEITY IN DIFFERENT VIEW ANGLE IN THE ATP BINDING SITE OF GSK-3β

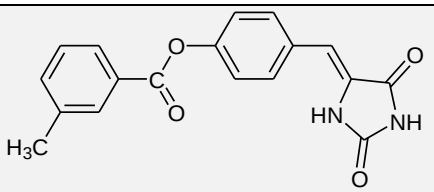
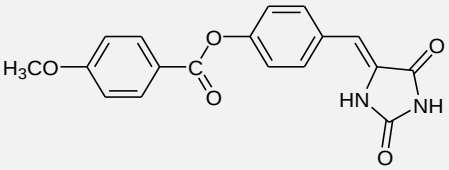
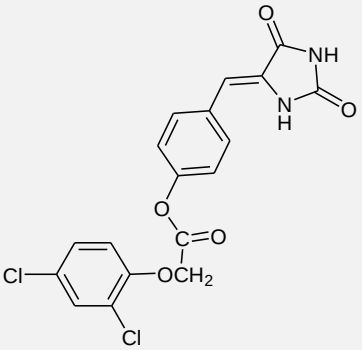
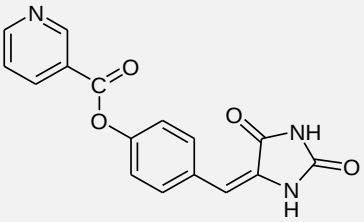
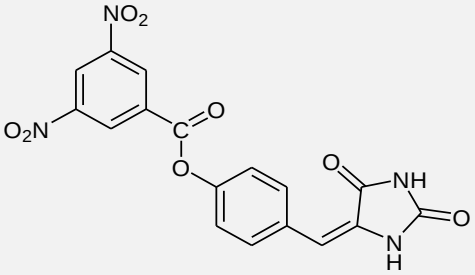
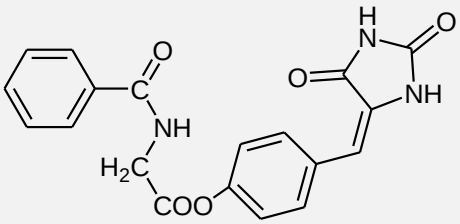
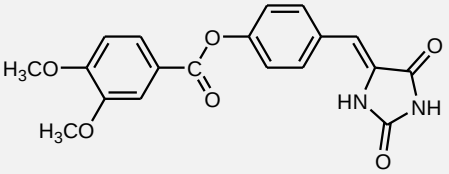
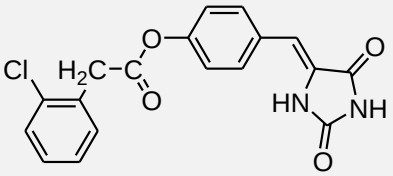
Structure of Designed Compounds

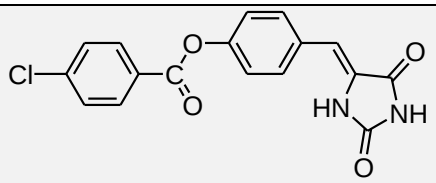
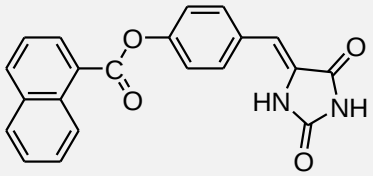
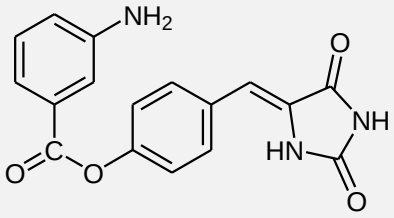
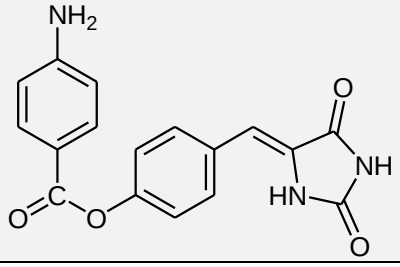
On the basis of literature study & substitution of different esters on Phenylmethylene hydantoin

following compounds were designed as GSK-3β inhibitors then do docking in enzyme cavity [11] and proposed for synthesis based on docking results. **(Table 1)**

TABLE 1: STRUCTURE OF DESIGNED COMPOUNDS WITH THEIR DOCKING SCORES

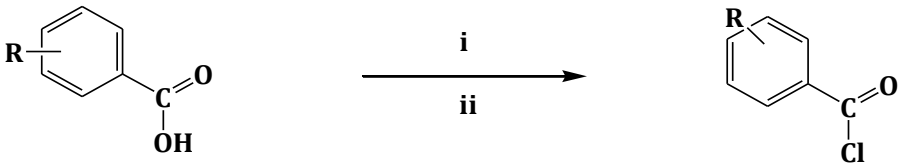
S.NO.	Designed Compound	MolDock Score (E-Total)	H-Bond	Torsions
Ref. Comp.		**-106.02**	**-4.01**	**4**
1.		**-113.32**	**-2.68**	**4**
2.		**-126.06**	**-3.36**	**4**
3.		**-124.92**	**-4.21**	**4**
4.		**-122.31**	**-6.03**	**5**
5.		**-118.83**	**-6.04**	**4**
6.		**-129.62**	**-3.02**	**4**
7.		**-117.23**	**-2.68**	**4**

8.		**-125.05**	**-4.71**	**4**
9.		**-123.83**	**-2.89**	**5**
10.		**-128.44**	**-4.06**	**6**
11.		**-125.27**	**-4.26**	**4**
12.		**-147.35**	**-5.82**	**6**
13.		**-147.53**	**-3.32**	**6**
14.		**-129.02**	**-6.74**	**6**
15.		**-112.83**	**-6.15**	**5**

16.		**-127.91**	**-3.14**	**4**
17.		**-139.89**	**-3.24**	**4**
18.		**-120.14**	**-6.44**	**4**
19.		**-115.91**	**-3.72**	**4**

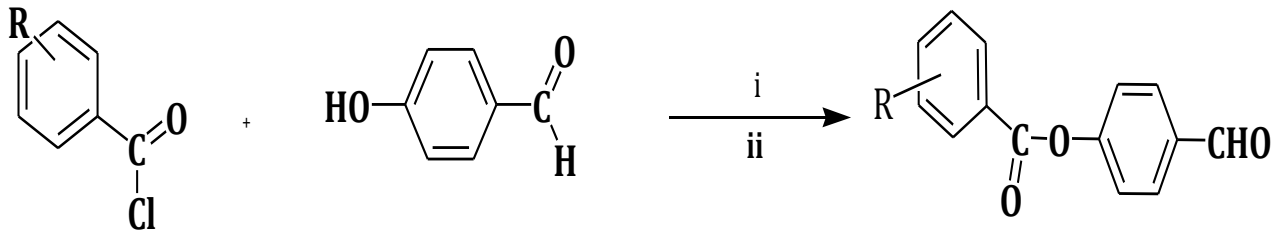
General Synthetic Scheme

Scheme1. The designed compounds were synthesized using following scheme which is divided in three steps as:-



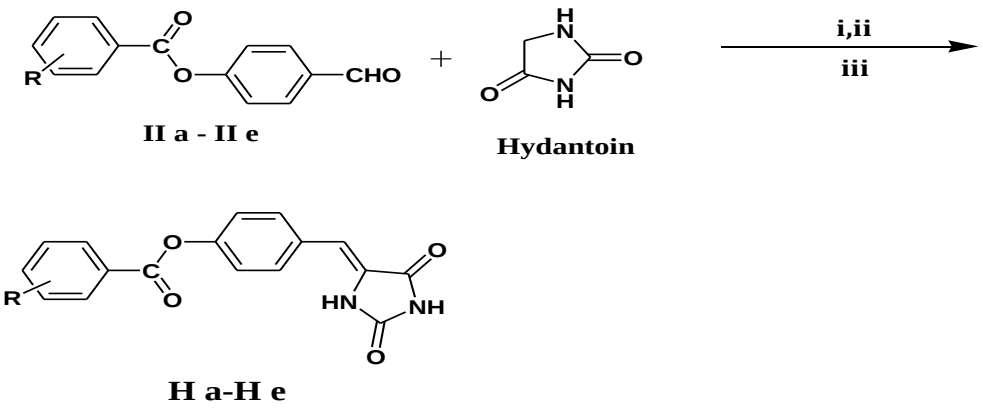
I **I a - I e**

Step-I: Reagents and conditions: (i) $SOCl_2$; (ii) Reflux for 3hrs; R = H, p-CH_3, o-Cl, m-CH_3, p-Cl for final test compounds H-a, H-b, H-c, H-d and H-e respectively.

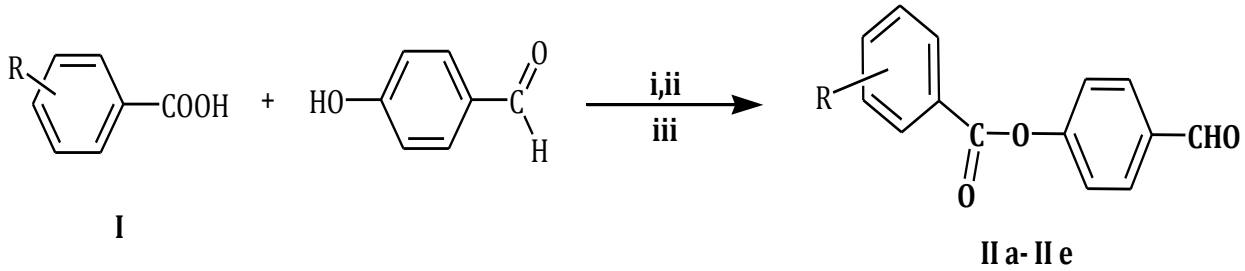


I a- I e **II** **II a- II e**

Step-II: Reagents and conditions: (i) Triethylamine; (ii) Dichloromethane (DCM)

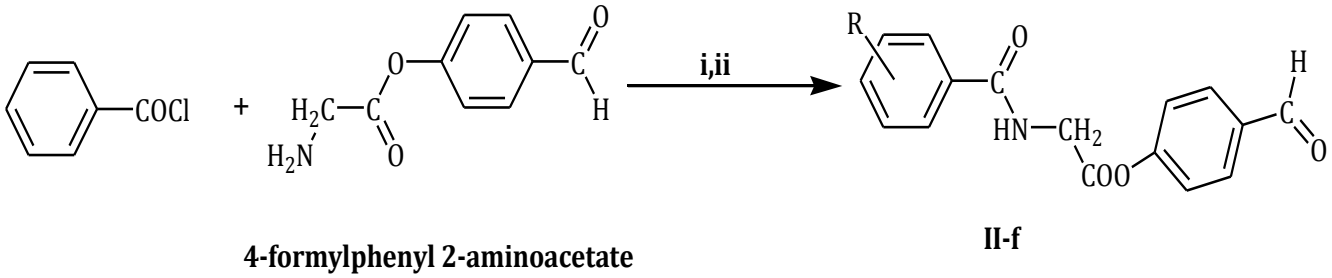


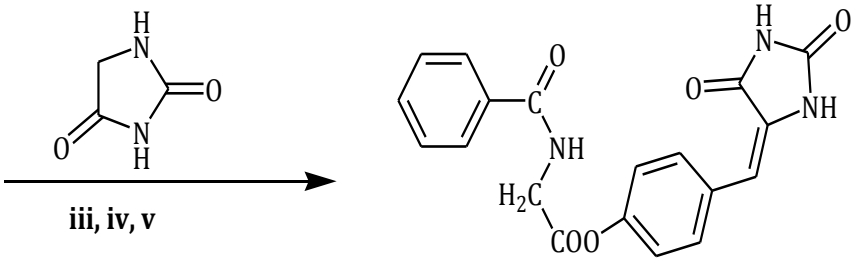
Step-III: Reagents and conditions: (i) $NaHCO_3$, (ii) Ethanolamine, (iii) Aq. EtOH



Scheme 2.

(Alternative to synthesize II a-II e): Reagents and Conditions: (i) Dicyclohexyl -Carbodiimide (DCC), (ii) DMAP, (iii) Dichloromethane (DCM); R = H, p-CH_3, o-Cl, m-CH_3, p-Cl for final test compounds H-a, H-b, H-c, H-d and H-e respectively.





Scheme 3. Reagents and Conditions: (i) Triethylamine; (ii) Dichloromethane (DCM); (iii) $NaHCO_3$, (iv) Ethanolamine, (v) Aq. EtOH

General Method of Synthesis

General synthesis of compounds (I a-f):

Benzoic acid derivatives (0.01 moles) were refluxed with thionyl chloride (10 ml) for 3- 4 hrs and progress of the reaction was monitored through TLC. On completion of the reaction excess thionyl chloride was removed under vacuum.

General synthesis of compound (II a-IIe):

Benzoyl chloride analogs were taken into 20 ml of dichloromethane in RBF and cooled to 0°C. To this reaction mixture triethylamine (0.03 moles) was added slowly with constant stirring. Followed by p-hydroxy benzaldehyde (0.01 moles), was added with stirring. The reaction mixture was stirred at 0°C for another 2 hrs and stirring continued at RT for overnight. Progress of the reaction mixture was checked through TLC. Then reaction mixture washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and pass through anhydrous Na_2SO_4. Solvent was removed under vacuum and recrystallize by ethanol.

General synthesis of compound (IIa-IIe): Alternative Step

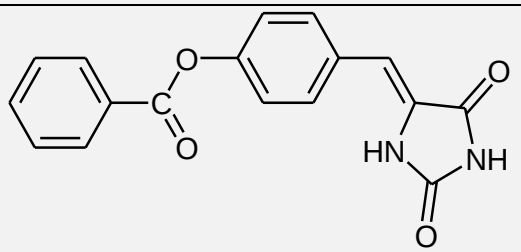
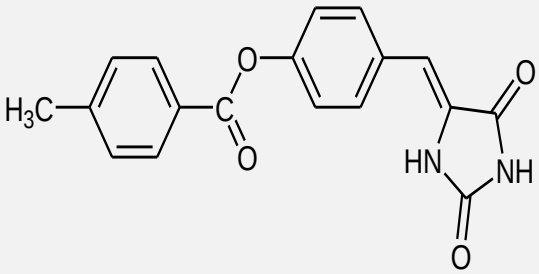
To the stirred solution of 10mmol of carboxylic acid and substituted benzaldedyde in 10ml of anhydrous Dichloromethane added 30-110 mg DMAP and 20-40 mmol alcohol. DCC was added to the reaction mixture at 0^0C, which was then stirred for 5 min. at 0^0C and 3 hrs at 20^0C, precipitated urea was then filtered off and filterate evaporated down in vaccuo.

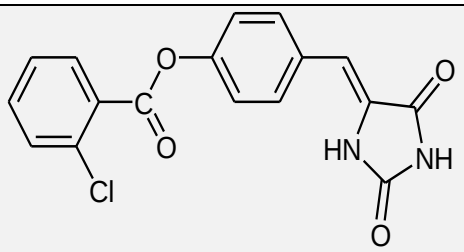
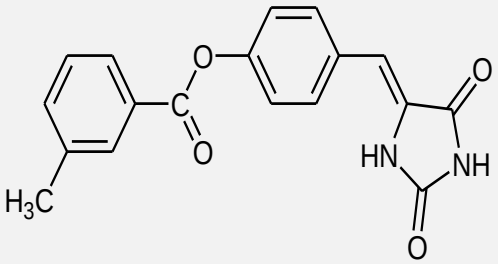
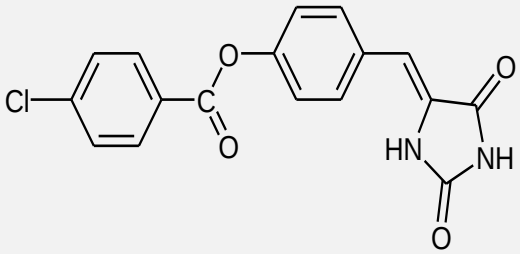
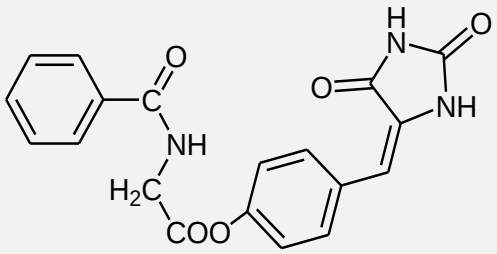
The residue was taken up in DCM and if necessary filtered free of any further precipitate urea. The DCM solution was washed twice with 0.5N HCl and with saturated $NaHCO_3$ solution and then dried over $MgSO_4$. The solvent was removed by evaporation and crystalline products obtained in pure form **(Scheme 2).**

Synthesis of Hydantoin analog (H a-f)

Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to 7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the ester substituted benzaldehyde solution in 5 ml C_2H_5OH was then added dropwise with continuous stirring. The reaction was kept under reflux for approximately 7 hr. The reaction was monitored by TLC every hour till completion of reaction. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. [12]

TABLE 2: STRUCTURE OF SYNTHESIZED COMPOUND

S.NO	SYNTHESIZED COMPOUND	IUPAC NAME
H-a		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl benzoate
H-b		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl 4-methylbenzoate

H-c		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl 2-chlorobenzoate
H-d		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl 3-methylbenzoate
H-e		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl 4-chlorobenzoate
H-f		4-((2,5-dioxoimidazolidin-4-ylidene)methyl)phenyl 2-benzamidoacetate

BIOLOGICAL EVALUATION:

In-Vitro Biological Evaluation

Selection of Animals

Healthy male adult Wistar Albino rats (120-200 g) were used for the study. The animals were stabilized for one week, housed in polypropylene cages, maintained under standard conditions (12 h light; 12 h dark cycle; 25± 30^0C). They were feed with standard rat pellet diet and water *ad libitum* **(Barik *et al.*, 2008)**. All experimental procedures were carried out as per Animal ethical norms **(OECD, 420).** The normoglycemic animals were selected for this experiment having the fasting blood glucose level around 80 mg/dl. The hyperglycemic animals were selected having fasting blood glucose concentration around 200-300 mg/dL.

Oral Glucose Tolerance Test

Animals were divided in eight groups, each group consisting of six rats (n=6). Overnight fasted rats were used for study. Group1 served as control group and received vehicle (Deionized water). Group 2 served as Standard, received Rosiglitazone (4 mg/kg) orally in acacia 2% suspension, Group 3-8 received test compounds **H-a, H-b, H-c, H-d H-e** and **H-f** in 15mg/kg dose suspended in vehicle (acacia 2% suspension) were administrated orally to the animals respectively. After 30 min. of drug and test compounds administration, the glucose (1.5 g kg^{-1}, p.o.) was administered.

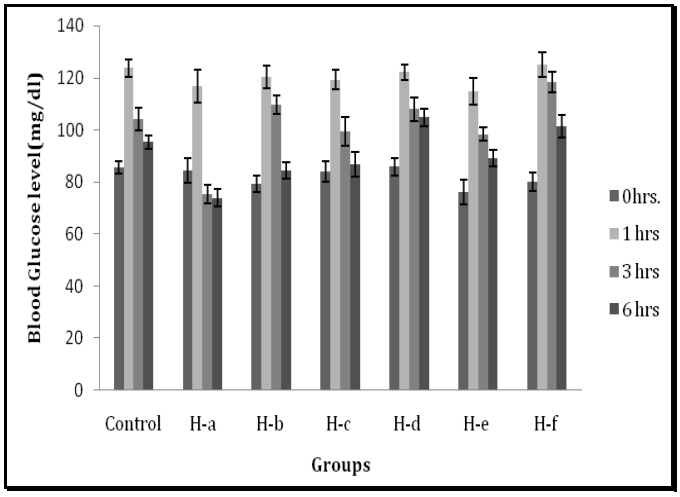
The blood samples were collected by snipping tail with surgically sterilized needle. Blood glucose was determined just prior to glucose administration at 0hr and 1hr, 3hr and 6hr after glucose loading. The fasting blood glucose level was estimated by glucose oxidase - peroxidase enzymatic method using Accu-chek Active TM Test strips in Accu-chek Active TM Test meter. [13]

TABLE 3: EFFECT OF TEST COMPOUNDS (HYDANTOIN ANALOGS) ON BGL OF GLUCOSE LOADED HYPERGLYCEMIC RATS

Groups	0hrs.	1 hrs	3 hrs	6 hrs
Control (Glucose 1.5g/kg)	85.56 ± 5.33	123.64 ± 3.36	104.26 ± 4.35	95.32 ± 2.54
H-a (15 mg/kg)	84.56 ± 5.71	116.67 ± 6.36	75.25 ± 3.66*** (35.50%)	73.88 ± 3.42*** (38.39%)
H-b (15 mg/kg)	79.32 ± 3.12	120.32 ± 4.31	109.74 ± 3.67* (8.79%)	94.31 ± 3.11*** (21.61%)
H-c (15 mg/kg)	83.89 ± 3.91	119.29 ± 3.65	99.41 ± 5.51** (16.66%)	86.73 ± 4.66*** (27.29%)
H-d (15 mg/kg)	85.81 ± 3.21	122.06 ± 2.89	107.92 ± 4.67** (11.58%)	104.78 ± 3.32** (14.16%)
H-e (15 mg/kg)	76.13 ± 4.57	114.72 ± 5.02	98.35 ± 2.63** (14.27%)	89.27 ± 3.22*** (22.18%)
H-f (15 mg/kg)	80.01 ± 3.68	125.11 ± 4.87	118.32 ± 4.02* (5.43%)	101.32 ± 4.34** (19.01%)

Tabular values are mean ± SEM, n = 6; significant difference from control,

***P<0.001; **P<0.01; *P<0.05 (OGTT was observed in the O^{th}, 1^{st}, 3^{rd} & 6^{th} hr), data were analyzed by ANOVA followed by Dunnett test.



Tabular values are mean ± SEM, n = 6; significant difference from control,
***P<0.001; **P<0.01; *P<0.05 (OGTT was observed in the O^{th}, 1^{st}, 3^{rd} & 6^{th} hr), data were analyzed by ANOVA followed by Dunnett test.

FIGURE 3: EFFECT OF TEST COMPOUNDS (HA-HF) ON BGL OF GLUCOSE LOADED HYPERGLYCEMIC RATS

Preparation of Streptozotocin solution

Streptozotocin solution was prepared by dissolving Streptozotocin in citrate buffer (P^H 4.5) with proper mixing. The preparation was used immediately within 15-20 minutes.

Induction of non-insulin-dependent diabetes mellitus

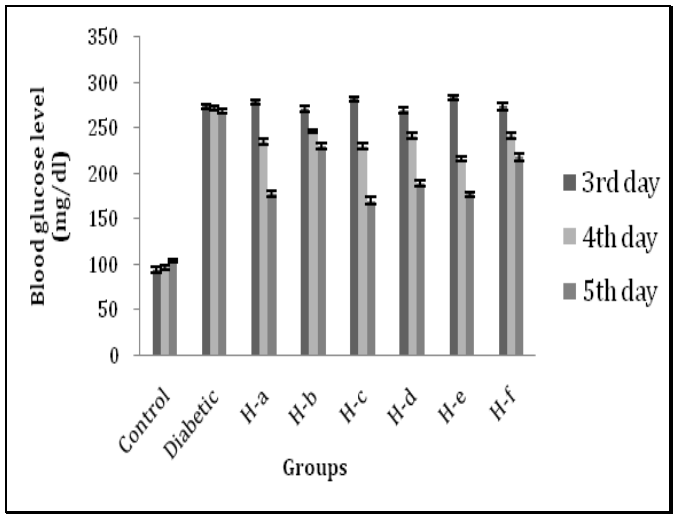
3.1.1.

The non-insulin-dependent diabetes mellitus was induced in overnight fasted adult Wister Albino rats, by single intraperitonial injection of freshly prepared solution of Streptozotocin in normal saline at a dose of 60 mg/kg, body weight. After administration of Streptozotocin, blood glucose was monitored after 72 hours to detect hyperglycemia. Diabetes was developed and stabilized in the Streptozotocin treated animals for 3 days and then antidiabetic activity was performed for a period of 3-6 days. The rats found with permanent non-insulin dependent diabetes mellitus (NIDDM - blood glucose level > 250 mg/dL) were considered to be diabetic and were used in experiment. [14, 15]

Experimental designs for antidiabetic study

In this antidiabetic experiment, the rats were divided into nine groups of six rats each after induction of diabetes, Group 1 served as normal control (0.9 % w/v Saline 10 ml/kg b.w.), Group 2 served as diabetic control received Streptozotocin 60 mg/kg b.w.**,** Group 3 served as reference standard received Rosiglitazone, 4 mg/kg b.w. by oral route, suspended in vehicle. Group 4-9 served as test standard received dose of 15 mg/kg b.w. of test compounds (**H-a, H-b, H-c, H-d, H-e** and **H-f**) respectively. In antihyperglycaemic study treatment continued with a daily single oral administration of vehicle (saline), standard suspension and test

compounds in morning by oral feeding needle for 5 days. The blood samples was collected from rats by retro orbital plexus bleeding method and the fasting blood glucose level was monitored by using a glucose oxidase-peroxidase reactive strips and a glucometer.[14]



Values are mean ± SEM, n = 6;
(Antidiabetic activity was observed in the 3rd, 4th, 5th day) data were analyzed by ANOVA followed by Dunnett test.

FIGURE 4: EFFECT OF TEST COMPOUNDS ON BGL OF STREPTOZOTOCIN INDUCED DIABETIC RATS

Statistical Analysis

The values are expressed as Mean±SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A value of $p<0.001$ was used as a criterion of significance.

***In-vivo* Determination of liver glycogen content**

All animals were housed in the same conditions and separated randomly to seven groups. Seven groups (n=6) used to investigate test compounds received 15 mg/kg dose were administered orally in 2% acacia suspension and one group as control. On the day of experiment, food and water were removed 6h before the drug administration. Then animal's livers were immediately removed for glycogen determination.

The tissue samples were homogenized with appropriate volume of 5% trichloro acetic acid over 5 min. The homogenate was centrifuged at 3000 rpm for 5 min. The supernatant fluid was taken and filtered using acid-washed filter paper. The glycogen of 1.0 mL of this filtrate was precipitated using ethanol (95%, 5 mL), incubated in water bath at 37-40ºC for 3h, and centrifuged at 3000 rpm for 15 min. The clear liquid is gently decanted from the packed glycogen, and the tubes were allowed to drain in an inverted position for 10 min. The glycogen was dissolved in distilled water (2 mL) and mixed with 10 mL of the anthrone reagent (0.05% anthrone, 1.0% thiourea in 72% H_2SO_4).

The mixture incubated for 30 min, and subsequently, the absorbance was measured at 620 nm by a UV-Vis spectrophotometer. [13]

The liver glycogen content is estimated using the following formula:

Amount (mg) of glycogen liver tissue
= (DU/DS) × (Volume of Extract (mL)/Weight of Liver Tissue (g)) ×0.09

Where, DU is the absorbance of the unknown sample and DS is the absorbance of the standard.

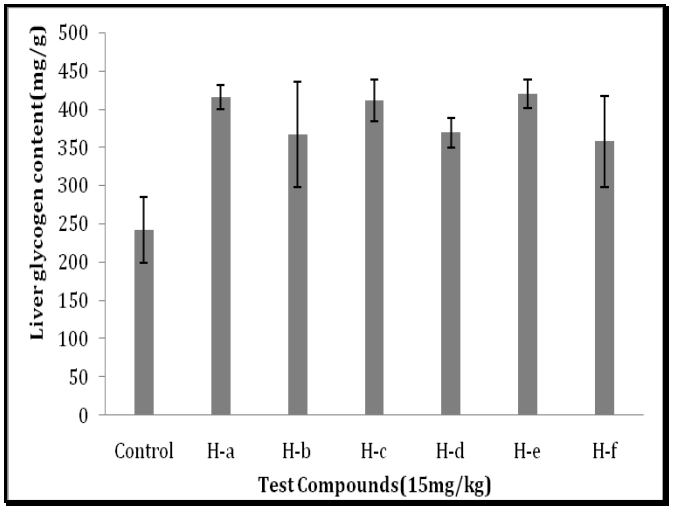
This *In-vivo* activity was approved by the Institutional Animal Ethics Committee (IACE) **[Registration No.- IAEC/SCOPE/11-12/78]**.

TABLE 4: EFFECT OF TEST COMPOUNDS (HYDANTOIN ANALOGS, H-A TO H-F) ON GLYCOGEN LEVEL OF STREPTOZOTOCIN INDUCED DIABETIC RATS

S.No.	[b]Compound	Liver Glycogen Content (mg/g)
1.	Control	242.01±42.80
2.	H-a	415.36±15.45**
3.	H-b	366.33±68.73**
4.	H-c	411.06±27.05**
5.	H-d	369.27±19.67**
6.	H-e	420.16±18.23**
7.	H-f	357.46±59.45**

[b]15 mg/kg body weight dose; Values are mean ± SEM, n = 6; significant difference from control,

***P<0.001; **P<0.01; *P<0.05 (Liver glycogen



Values are mean ± SEM, n = 6; significant difference from control,
***P<0.001; **P<0.01; *P<0.05 (Liver glycogenwas observed on the 6th day), data were analyzed by ANOVA followed by Dunnett test

FIGURE 5: EFFECT OF TEST COMPOUNDS (HYDANTOIN ANALOGS, HA-HF) ON GLYCOGEN LEVEL OF STREPTOZOTOCIN INDUCED DIABETIC RATS

RESULT:

Synthesized Phenylmethylene hydantoin ester analogs were screened for their antidiabetic activity by Streptozotocin induced tail tipping method. The study was carried out in nine different groups of rats of either sex. Rosiglitazone (4 mg/kg body weight) was used as a standard drug. Compounds **(Ha-H_f)** at 15 mg/kg body weight shown significant (P<0.001) decreasing in blood glucose levels. Synthesized compound shows decrease in blood glucose level in the range of 45.44-57.54 (±4.1) % after 3h while 60.38-85.53 (± 3.6) % after 6h **(Table 4.4)**.

The compounds were also tested for change in hepatic glycogen content. H-a, H-c and H-e increased hepatic glycogen content in range 410-420 mg/gm liver weight while compounds H-b, H-d and H-f cause increased in hepatic glycogen content in range of 357-370 mg/gm liver weight as compared to control having 242.01 mg/gm liver weight. Preliminary study revealed that amino substituted Phenylmethylene hydantoin analogs are more potent anti-diabetic agents. Primary structure activity relationship revealed that -ortho & -para chloro (electron withdrawing) substituted analogs favours for the activity while electron releasing (-methyl, -methoxy) substituted analogs having low potency.

DISCUSSION:

The worldwide epidemic of type 2 diabetes (NIDDM) has been stimulating the search for new concepts and targets for the treatment of this incurable disease. Over the past few years there has been much interest within the pharmaceutical industry in identifying compounds that inhibit GSK-3β as possible insulin mimetic sensitizing drugs. This interest has been heightened by the report that the level and activity of GSK-3β is moderately elevated in diabetic and obese strains of mice. The wide chemical diversity of possible inhibitors and the existence of multiple sites for potential inhibition constitute strong encouragement to pursue the development and evaluation of GSK-3β inhibitors as potential drugs.

On the basis of literature study Phenylmethylene hydantoin analogs were designed **(Table 1).** These designed analogs were docked in the ATP binding site of GSK-3β by Molegro Virtual Docker 2006.1.5. Phenylmethylene hydantoin (PMH) forms strong interactions with the hinge region of GSK-3β; carbonyl oxygen at position 2 form a H-bonding with backbone nitrogen of Val135 and the NH at position 3 to the carbonyl oxygen of Asp200 **(Figure 2).**

Furthermore, the substituted benzylidene ring system builds an H-bonding interaction with the guanidine moiety of Arg141. Targeting Arg141 is important to improve the activity in the process of designing new derivatives because it is considered the selectivity residue for GSK-3β. By showing the above docking results, we tried to prepare the analogs in which keeping the hydantoin ring, and different ester substitution at benzylidene ring system can afford potent and selective GSK-3β inhibitors as (Ha-H_f) **(Table 2).**

The part of the design compounds were synthesized by Knoevenagel condensation **(Scheme 1)** and Steglich Esterification reaction **(Scheme 2).** The progress of the reaction was monitored through thin layer chromatography and by melting point determination of intermediates. The synthesized compounds were purified through column

chromatography. The synthesized compounds were characterized by melting point, λ_{max}, IR, NMR & Mass spectroscopy. Structure of synthesized compounds was elucidated by IR Spectral data and Mass spectral.

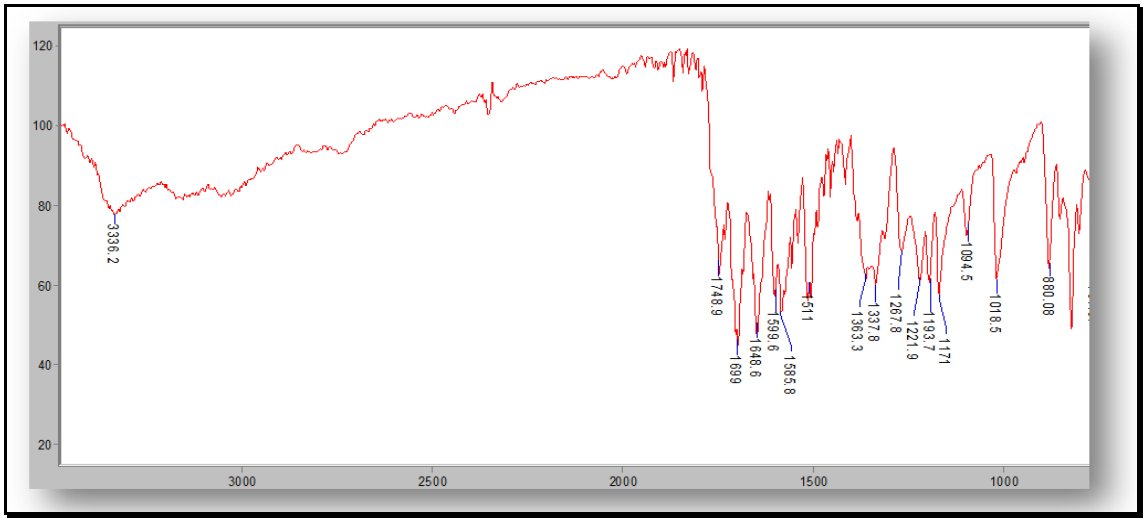


FIGURE 6: IR SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL BENZOATE (H-a)

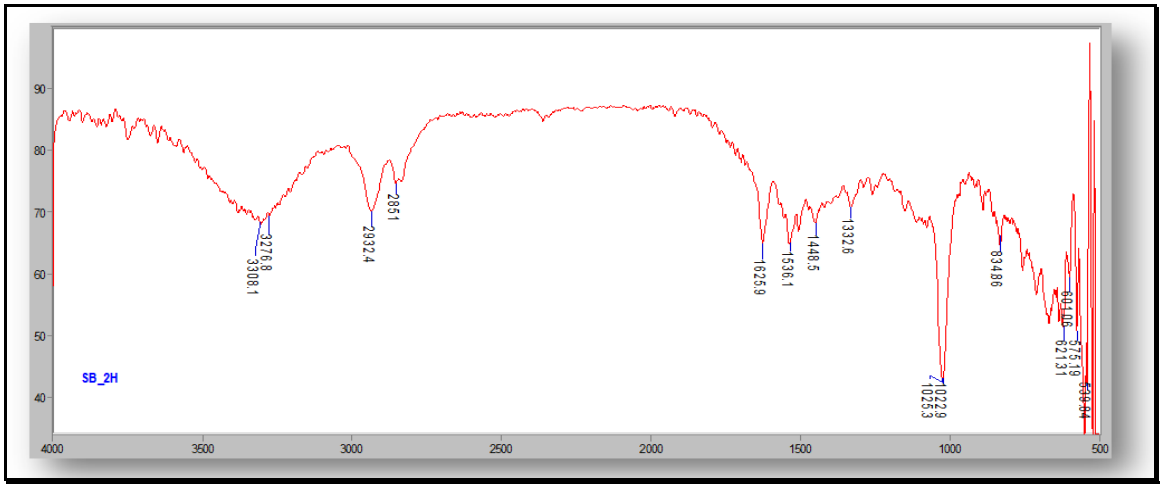


FIGURE 7: IR SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 4-METHYLBENZOATE (H-b)

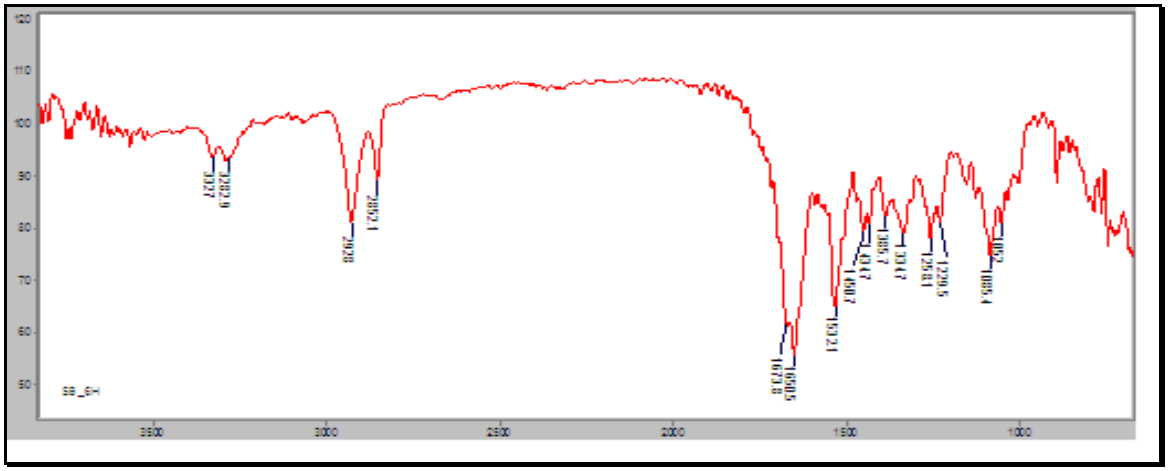


FIGURE 8: IR SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 2-CHLOROBENZOATE (H-c)

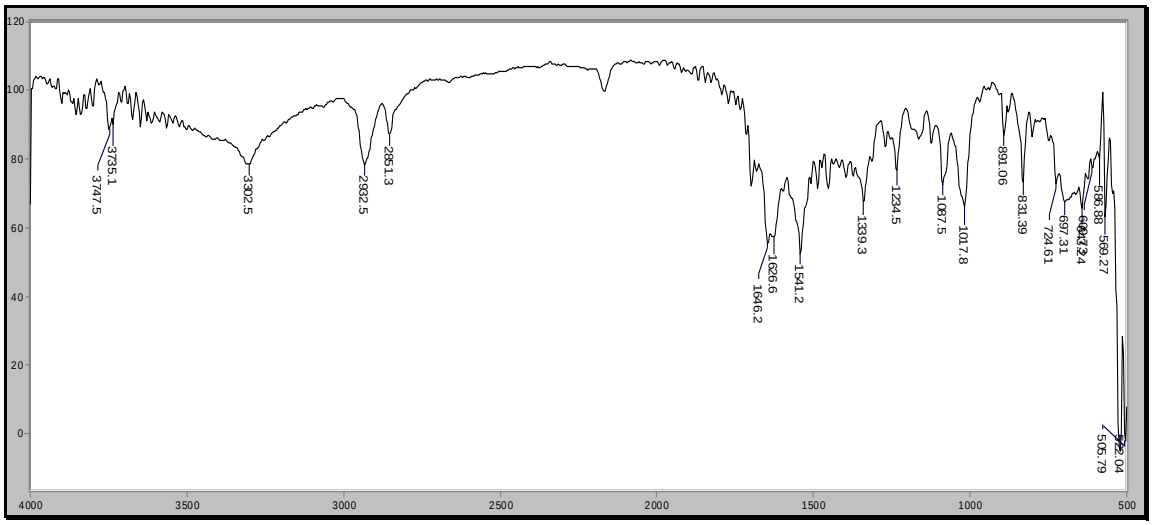


FIGURE 9: IR SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 3-METHYLBENZOATE (H-d)

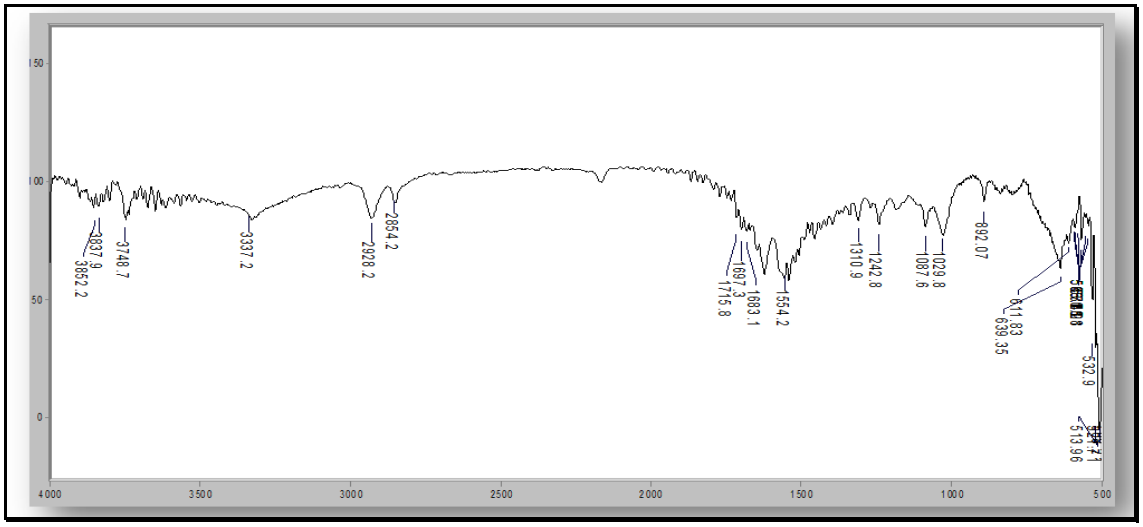


FIGURE 10: IR SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 4-CHLOROBENZOATE (H-e)

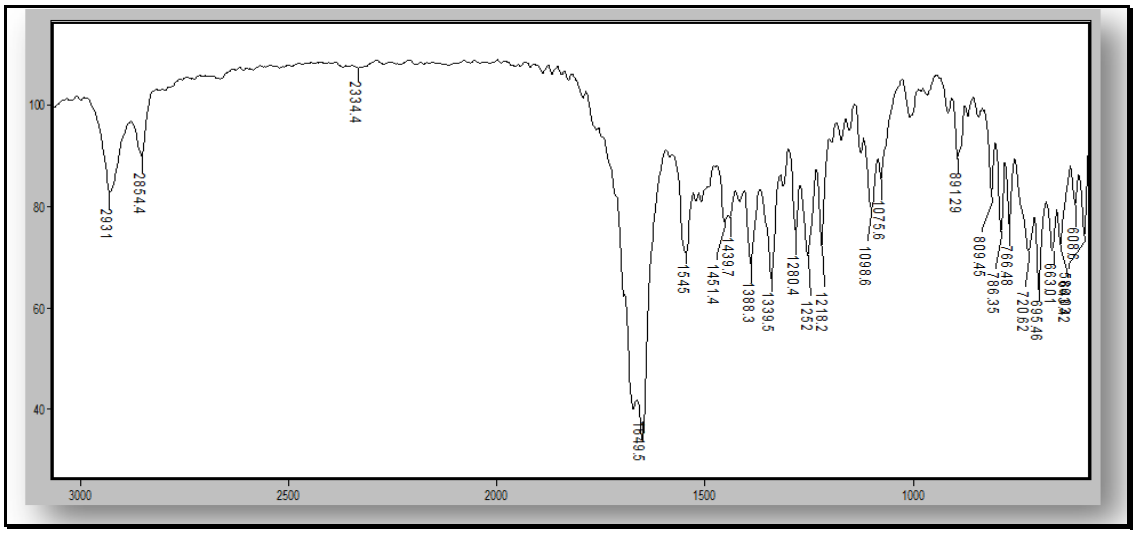


FIGURE 11: IR SPECTRA OF 4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 2-BENZAMIDOACETATE (H-f)

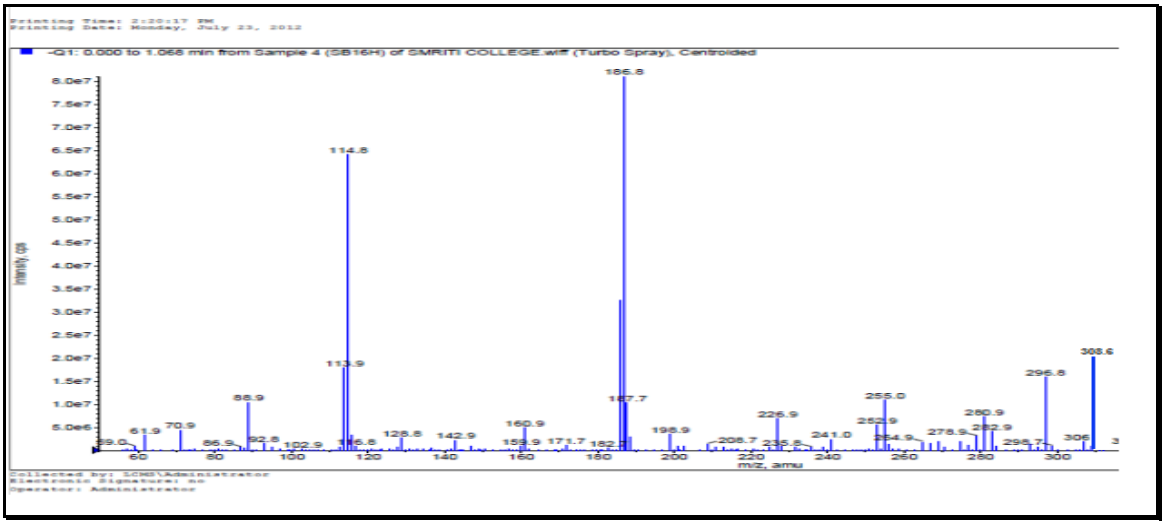


FIGURE 12: MASS SPECTRA OF 4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL BENZOATE (H-a)

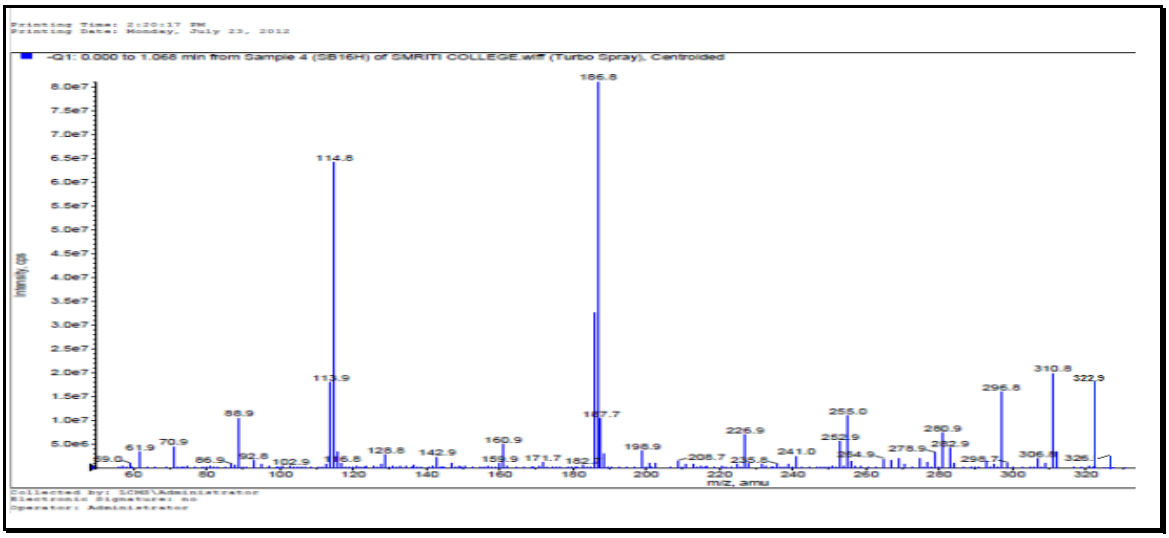


FIGURE 13: MASS SPECTRA OF (Z)-4-((2, 5-DIOXOIMIDAZOLIDIN-4-YLIDENE) METHYL) PHENYL 3-METHYLBENZOATE (H-d)

The IR spectrum of the compounds (**Figure 6 to Figure 11**) shows peaks C=C (aromatic) in the range 1532-1565 cm^{-1}, C=O ester got merging broad peaks with C=C (alkene) in the range 1625-1670 cm^{-1}, while C-H stretching fall between 2900-3010 cm^{-1}. The IR spectrum of the compounds (**Figure 8, Figure 10**) shows peaks in the range 720-760 cm^{-1} for C-Cl stretching. Further MASS data supported the synthesis of H-a and H-d. The MASS spectrum of compound H-a **(Figure 12)** and H-d **(Figure 13)** showed molecular ion peak at *m/z* 308.6 and 322.9, in conformity with the molecular formula $C_{17}H_{12}N_2O_4$ and $C_{18}H_{14}N_2O_4$ respectively. The spectral data was in conformation of the anticipated structure of synthesized compounds.

Phenylmethylene Hydantoin analogs were screened for their anti-diabetic activity by Streptozotocin induced tail tipping method **(Figure 4)** and GSK-3β inhibitory activity was determined by in-vivo comparision of increase in liver glycogen content in albino rat **(Table 4, Figure 5).**

EXPERIMENTAL CHEMISTRY

General information:

Electrospray mass spectra (MS-ES) were recorded on a Shimadzu LCMS 2010 eV **(RGPV University, Bhopal)**. High resolution mass spectra (HRMS) were obtained for two compounds **(H-a, H-d)** on a Micromass Autospec. E.spectrometer. Melting points were determined in open capillary tubes with a Thomas–Hoover apparatus and were

uncorrected. To determine the purity of the compounds thin layer chromatography for compounds was performed using silica gel-G on glass plate in different solvent systems. Iodine vapor and UV detector (long wavelength) were used as detecting agents. The R_f value is characteristic for each of the compound. Functional group modification was studies through IR spectra and results are obtained for each synthesized compound. The IR spectra of the intermediate and synthesized compounds were recorded on FTLA 2000 spectrophotometer at SCOPE, Indore.

Substituted Acid Chloride (Ia-Ie):
Benzoic acid derivatives (0.01 mole) were refluxed with thionyl chloride (10 ml) for 3- 4 hrs and progress of the reaction was monitored through TLC. On completion of the reaction excess thionyl chloride was removed under vacuum.

4-formylphenyl Benzoate derivatives (IIa-IIe):
Benzoyl chloride analogs **(Ia-Ie)** were taken into 20 ml of dichloromethane in RBF and cooled to 0°C. To this reaction mixture triethylamine (0.03 mole) was added slowly with constant stirring. Followed by p-hydroxy benzaldehyde (0.01 mole), was added with stirring. The reaction mixture was stirred at 0°C for another 2 hrs and stirring continued at RT for overnight. Then reaction mixture washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and pass through anhydrous Na_2SO_4. Solvent was removed under vacuum and recrystallize by ethanol.

4-((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl benzoate (H-a).
Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl Benzoate **(II-a)** solution in 5 ml C_2H_5OH was then added drop wise with continuous stirring. The reaction was kept under reflux for approximately 7 hr. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. Practical yield and M.P. of the compound was found to be 58% and 237-240°C respectively. The λ_{max} of the compound was determined in methanol and it was found to be 317 nm. Ha is soluble in DMSO, DMF, Ethanol, Acetone, and Methanol and shows R_f value 0.53 in Petro ether: Ethyl Acetate (2:3). MS (ES) m/z 308.6 ($M+H^+$).

4-((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl 4-methylbenzoate (H-b).
Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl 4-methylbenzoate **(II-b)** solution in 5 ml C_2H_5OH was then added drop wise with continuous stirring.

The reaction was kept under reflux for approximately 7 hr. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. Practical yield and M.P. of the compound was found to be 64% and 159-161°C respectively. The λ_{max} of the compound was determined in methanol and it was found to be 282 nm. H-b is soluble in DMSO, DMF, Ethyl acetate, Acetone, Methanol and shows R_f value 0.61 in Petro Ether: Ethyl Acetate (2:3).

4-((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl 2-chlorobenzoate (H-c).
Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl 2-chlorobenzoate **(II-c)** solution in 5 ml C_2H_5OH was then added dropwise with continuous stirring. The reaction was kept under reflux for approximately 7 hr. Practical yield and M.P. of the compound was found to be 56% and 156-159°C respectively. The λ_{max} of the compound was determined in methanol and it was found to be 272.5 nm. H-c is soluble in

DMSO, DMF, Acetone, and Methanol and shows R_f value 0.65 in Petro ether: Ethyl Acetate (2:3).

4-((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl 3-methylbenzoate (H-d).

Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to 7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl 3-methylbenzoate **(II-d)** solution in 5 ml C_2H_5OH was then added dropwise with continuous stirring.

The reaction was kept under reflux for approximately 7 hr. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. Practical yield and M.P. of the compound was found to be 72% and 150-153ºC respectively. The λ_{max} of the compound was determined in methanol and it was found to be 306 nm. H-d is soluble in DMSO, DMF, Ethanol, Acetone, and Methanol and shows R_f value 0.56 in Petro ether: Ethyl Acetate (2:3); MS (ES) m/z 322.9($M+H^+$).

4 - ((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl 4-chlorobenzoate (H-e).

Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to 7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl 4-chlorobenzoate **(II-e)** solution in 5 ml C_2H_5OH was then added dropwise with continuous stirring.

The reaction was kept under reflux for approximately 7 hr. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. Practical yield and M.P. of the compound was found to be 61% and 154-157ºC respectively. The λ_{max} of the compound was determined in methanol and it was found to be 290.5 nm. H-e is soluble in DMSO, DMF, Ethanol, Ethyl acetate, Acetone, Methanol and shows R_f value 0.58 in Petro ether: Ethyl Acetate (2:3).

4-formylphenyl 2-benzamidoacetate (II-f).

Benzoyl chloride were taken into 20 ml of dichloromethane in RBF and cooled to 0°C. To this reaction mixture triethylamine (0.03 mole) was added slowly with constant stirring. Followed by 4-formylphenyl 2-aminoacetate (0.01 mole), was added with stirring. The reaction mixture was stirred at 0°C for another 2 hrs and stirring continued at RT for overnight. Then reaction mixture washed with saturated solution of sodium bicarbonate, brine solution and water. Organic phase was separated and pass through anhydrous Na_2SO_4. Solvent was removed under vacuum and recrystallize by ethanol.

4 - ((2, 5-dioxoimidazolidin-4-ylidene) methyl) phenyl 2-benzamidoacetate (H-f).

Hydantoin (1.0g) was dissolved in 10 ml H_2O while heating at 70^0 C on oil bath with continuous stirring. The pH was adjusted to 7.0 using saturated $NaHCO_3$ solution after complete dissolution. The temperature was then raised to 90^0C after the addition of 0.9 ml ethanolamine. Equimolar quantity of the 4-formylphenyl 2-benzamidoacetate **(II-f)** solution in 5 ml C_2H_5OH was then added dropwise with continuous stirring.

The reaction was kept under reflux for approximately 7 hr. After complete depletion of the starting aldehyde, the mixture was cooled and the precipitate was filtered and washed with C_2H_5OH /H_2O (1:5) before recrystallization from C_2H_5OH. The λ_{max} of the compound was determined in methanol and it was found to be 293 nm. H-f is soluble in DMSO, DMF, Ethanol, Acetone and Methanol, and shows R_f value 0.37 in Petro ether: Ethyl Acetate (2:3).

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