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# Design, Synthesis and Biological Evaluation of Glycogen Synthase Kinase-3 $\beta$ Inhibitors as Antidiabetic Agent

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#### Abstract

Diabetes mellitus (DM) is a progressive disease characterized by hyperglycemia due to insulin deficiency and insulin resistance or both. Owing to the progressive nature of the disease, an evolving treatment strategy is necessary to maintain glycemic control. Inhibitors. Glycogen synthase kinase- $3\beta$  (GSK- $3\beta$ ) is a unique multifunctional serine/threonine kinase that is inactivated by phosphorylation in response to insulin binding; PKB/AKT phosphorylates GSK- $3\beta$  on serine9, which prevents the enzyme from phosphorylating glycogen synthase. Encouraged by the above literature we tried to prepare the analogs in which keeping the hydantoin ring, and substitution at C-9 or C-10 positions apart from this hydantoin ring is replaced with bioisosteric substitute ring (like rhodadine, oxindole) can afford potent and selective GSK- $3\beta$  inhibitor. In the present research work, an attempt has been made to synthesize phenyl methylene hydantoin derivatives, which are expected to have antidiabetic activity. Phenyl methyl hydantoin analogs were synthesized and their activity shows their potency. Some novel GSK- $3\beta$  were designed and synthesized.

Key-Words: Synthesis, Diabetes, GSK-3β, Antidiabetic activity

#### Introduction

The insulin-insensitive form of diabetes, type 2 diabetes mellitus (T2DM). characterized bv hyperglycemia (elevated blood glucose concentrations), most frequently arises as а consequence of obesity, represents approximately 95% of the overall incidence of diabetes. Additionally, diabetes-related complications exert a heavy toll on patients with poor metabolic control.<sup>1-4</sup>

Diabetes mellitus 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 rise from 171 million in 2000 to 366 million in 2030. More than 97% of these patients will have type II diabetes. India has the dubious distinction of being the 'diabetes capital' of the world. The International Diabetes Federation estimates that the number of diabetic patients in India has more than doubled from 1995 till now. Upto 11 per cent of India's urban population above the age of 15 has diabetes.<sup>5</sup>

Anti-diabetic agents seek to reduce hyperglycemia and, thus, diminish the elevated risk of micro- and macrovascular disease in T2D patients.Glycogen synthase kinase- $3\beta$  (GSK- $3\beta$ ) has recently emerged, in the field of medicinal chemistry, as one of the most attractive therapeutic targets for Type II diabetes.

\* Corresponding Author E.mail: sumeet6882@gmail.com The full potential of GSK-3 $\beta$  inhibitors is yet to be realized and the number of drug candidates being developed by both academic centers and pharmaceutical companies has increased exponentially in the last few years.<sup>1-4</sup>

Glycogen synthase kinase- $3\beta$  (gsk- $3\beta$ ) is a unique multifunctional serine/threonine kinase that is inactivated by phosphorylation in response to insulin binding; PKB/AKT phosphorylates GSK- $3\beta$  on serine9, which prevents the enzyme from phosphorylating glycogen synthase. Unphosphorylated glycogen synthase is active & able to synthesize glycogen.<sup>3,4</sup>

# Material and Methods

General Synthetic Scheme

The designed compounds were synthesized using following schemewhich isdivided in three steps as:-Step-I: Synthesis of Acid chloride



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Step-II: Synthesis of aldehyde ester:



II a - f

CHO

#### Alternative Step: - For Step I & II



Step-III: Synthesis of Phenylmethylene hydantoin analogs:



#### General method of synthesis: General synthesis of comp. (I a-f):

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.

#### General synthesis of comp. (II a-f):

Benzoyl chloride analogs 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. 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 comp. (II a-f): Alternative Step

To stirred solution of 10mmol of carboxylic acid in 10ml of anhydrous Dichloromethane is added 30-110 mg DMAP and 20-40 mmol alcohol. DCC is added to the reaction mixture at  $0^{0}$ C, which is then stirred for % min. at  $0^{0}$ C and 3 hrs at 20<sup>0</sup>C, precipitated urea is then filtered off and filterate evaporated down in vaccuo.

The residue is taken up in DCM and if necessary filtered free of any further precipitate urea. The DCM solution is washed twice with 0.5N HCl and with saturated NaHCO<sub>3</sub> solution and then dried over  $MgSO_{4.The}$  solvent is removed by evaporation and crystalline products can be obtained in pure form.

#### Synthesis of hydantoin analog ( H a-f )

Hydantoin (1.0g) was dissolved in 10 ml H<sub>2</sub>O while heating at 70<sup>0</sup> C on oil bath with continuous stirring.The pH was adjusted to7.0 using saturated NaHCO<sub>3</sub> solution after complete dissolution. The temperature was then raised to 90<sup>0</sup>C after the addition of 0.9 ml ethanolamine.Equimolar quantity of the ester substituted benzaldehyde solution in 5 ml C<sub>2</sub>H<sub>5</sub>OH 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<sub>2</sub>H<sub>5</sub>OH /H<sub>2</sub>O (1:5) before recrystallization from C<sub>2</sub>H<sub>5</sub>OH.

The analytical data of synthesized compounds are as follows:

**H-a**(C<sub>17</sub>H<sub>12</sub>N<sub>2</sub>O<sub>4</sub>): Practical yield and M.P. of the compound was found to be 58% and 237-240°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 317 nm.H-a is soluble in DMSO, DMF, Ethanol, Acetone, Methanol and shows R<sub>f</sub> value 0.53 in Petro ether: Ethyl Acetate(2:3) solvent system.

**H-b**(C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>): Practical yield and M.P. of the compound was found to be 64% and 159-161°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 282 nm.M<sub>1</sub> is soluble in DMSO, DMF, Ethyl acetate, Acetone, Methanol and shows R<sub>f</sub> value 0.61 in Petro Ether: Ethyl Acetate (2:3) solvent system.

**H-c**( $C_{17}H_{11}ClN_2O_4$ ): Practical yield and M.P. of the compound was found to be 56% and 156-159°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 272.5 nm.M<sub>3</sub> is soluble in DMSO, DMF, Acetone, Methanol



and shows  $R_{\rm f}$  value 0.65 in N-Hexane: Ethyl Acetate (2:3) solvent system.

**H-d**(C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>): Practical yield and M.P. of the compound was found to be 72% and 150-153°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 306 nm.M<sub>4</sub> is soluble in DMSO, DMF, Ethanol, Acetone, Methanol and shows R<sub>f</sub> value 0.56 in N-Hexane: Ethyl Acetate (2:3) solvent system.

**H-e**(C<sub>17</sub>H<sub>11</sub>ClN<sub>2</sub>O<sub>4</sub>): Practical yield and M.P. of the compound was found to be 61% and 154-157°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 290.5 nm.M<sub>5</sub> is soluble in DMSO, DMF, Ethanol, Ethyl acetate, Acetone, Methanol and shows R<sub>f</sub> value 0.58 in N-Hexane: Ethyl Acetate (2:3) solvent system.

**H-f**(C<sub>19</sub>H<sub>15</sub>N<sub>3</sub>O<sub>5</sub>): Practical yield and M.P. of the compound was found to be 38% and 147-150°C respectively. The  $\lambda_{max}$  of the compound was determined in methanol and it wasfound to be 293 nm.M<sub>6</sub> is soluble in DMSO, DMF, Ethanol, Acetone,

Methanol and shows  $R_f$  value 0.37 in N-Hexane: Ethyl Acetate(2:3) solvent system.

The IR spectrum of the compounds (**H a-f**) shows peaks C=C(aromatic) in the range 1532-1565 cm<sup>-1</sup>,C=Oester got merging broad peaks with C=C (alkene) in the range 1625-1670 cm<sup>-1</sup>,while C-H stretching fall between 2900-3010 cm<sup>-1</sup>. The IR spectrum of the compounds (**H-c, H-e**) shows peaks in the range720-760 cm<sup>-1</sup> for C-Cl stretching.

### **Biological Evaluation**

#### In- vivo Biological Evaluation

Hydantoin ester analogs were screened for their antidiabetic activity by Streptozotocin induced tail tipping method and GSK-3 $\beta$  inhibitory activity was determined by in-vivo comparision of increase in liver glycogen content in albino rat.

A) The anti-diabetic activities of the synthesized compounds are shown in Table & Fig

#### The Anti-diabetic Activities of the Synthesized Compounds

Compound –	Decrease in blood glucose level (mg/dl)		
	c1h	c3h	<sup>c</sup> 6h
<sup>a</sup> Control	4.27±0.61	8.18±1.90	17.29±2.17
Standard (Roziglitazone)	16.54±9.97*	50.24±27.88**	58.42±16.72***
<sup>b</sup> <b>H-a</b>	34.93±8.81**	51.67±8.97**	85.41±4.18***
<sup>b</sup> <b>H-b</b>	26.83±5.86*	44.78±3.40**	59.18±4.24***
<sup>b</sup> <i>H</i> -c	31.80±6.13**	52.39±12.62***	77.21±5.65***
<sup>b</sup> <i>H</i> -d	29.27±5.47**	42.34±7.53**	61.37±5.25***
<sup>b</sup> <i>H-e</i>	38.09±5.25**	57.48±5.25***	78.51±5.24***
<sup>b</sup> H-f	18.50±3.15*	45.52±15.27**	60.94±3.66***

<sup>a</sup>4 mg/kg body weight dose; <sup>b</sup>15 mg/kg body weight dose; <sup>c</sup> mean ± S.E.M.(n=6);\*\*\*P<0.001; \*\*P<0.01; \*P<0.05





Graphical Representation of Antidiabetic Activity of Hydantoin Analogs

**B**) Theincrease in liver glycogen contents by the synthesized compounds are shown in Table and fig..

### In-vivo Data of Liver Glycogen Content of Test Compounds

S.No.	<sup>b</sup> Compound	<sup>c</sup> Liver Glycogen Content (mg)	
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**	

<sup>b</sup>15 mg/kg body weight dose; <sup>c</sup> mean ± S.E.M.(n=3);\*\*\*P<0.001; \*\*P<0.01; \*P<0.05







Figure: A bar graph of increase in liver glycogen level by test compounds

Synthesized hydantoin ester analogswere screened for their antidiabetic activity by Streptozocin induced tail tipping method. The study was carried out in ten different groups of rats of either sex. Rosiglitazone (4 mg/kg body weight) was used as a standard drug. Compounds (Ha-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% after 3h while 60.38-85.53% 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 & -pera chloro (electron withdrawing) substituted analogs favours for

the activity while electron releasing (-methyl, methoxy) substituted analogs having low potency. **References** 

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