



# Synthesis and Biological Evaluation of Some Novel Coumarin and Guanidine Derivatives by Oral Glucose Tolerance Test

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## ABSTRACT

**Objective:** The major drawback of current treatment of diabetes is challenging due to resistance to antidiabetic agents their side effects. Chromenes occurs naturally in plants and plays role of anticoagulant activity. Guanidine plays role of antidiabetic activity.

2H-chromenes are the heterocyclic compounds with considerable therapeutic and pharmacological properties. Guanidine is compounds with considerable therapeutic and pharmacological properties. In this view, it has been planned to combine both guanidine and coumarin derivatives. Biological activity for antidiabetic activity has been performed for guanidine derivatives.

**Experimental and Computational work done:** 2H-chromene was synthesized by Pechmann condensation of resorcinol, ethyl acetoacetate and sulphuric acid. Then coumarin was combined with guanidine. This combined compound was substituted by different substituted benzaldehyde and in other series by oxidation, chlorination and Schotten Baumann reaction.

The purity of the compounds was checked by TLC monitoring and all the synthesized compounds were characterized by UV, IR, Mass and some of by <sup>1</sup>H-NMR spectroscopy. The antidiabetic activities of synthesized compounds were tested by oral glucose tolerance test (OGTT).

**Results and discussion:** In antidiabetic activity, compounds 9-b have shown highest antidiabetic activity while compounds 9-a and 11-a have shown also antidiabetic activity against metformin as standard.

**Conclusion:** Evaluation of antidiabetic revealed that compounds with substituted benzaldehyde at the 4-position of the phenyl ring of 2H-chromenes and at oxidation, chlorination and Schotten Baumann reaction of synthesized intermediate i.e., 9-a is most active antidiabetic agent.

**Keywords:** 2*H*-chromene, antidiabetic activity, oral glucose tolerance test (OGTT).

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Diabetes is generally state of increased blood glucose level, Diabetes Mellitus mainly categorized in two categories: Type 1 (IDDM) & Type 2 (NIDDM).

On the basis of etiology Type 1 is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical Type 1 diabetes are Type 1a (about 90% of Type 1 cases in Europe) which is thought to be due to immunological destruction of pancreatic  $\beta$  cells resulting in insulin deficiency; and Type 1b (idiopathic, about 10% of Type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylases (anti-GAD), IA-2 or insulin antibodies that identify the autoimmune process with  $\beta$ -cell destruction.

Autoimmune diseases such as Grave's disease, Hashimoto's thyroiditis and Addison's disease may be associated with Type 1 diabetes mellitus.

Insulin deficiency in turn leads to chronic hyper glycaemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus diabetes covers a wide range of heterogeneous diseases.

According to the ADA recommendation changes in 1997, the fasting glucose concentration should be used in routine screening for diabetes as well as

epidemiological studies; the threshold for fasting glucose was changed from 7.8mmol/l (140mg/dl) to 7.0mmol/L (126mg/dl); however the 2-h glucose criterion remains as 11.1 mmol/l (200 mg/dl).<sup>1-3</sup>

## MATERIALS AND METHODS

The entire chemicals were supplied by S.D. Fine Chem. (Mumbai), Finar Chem. Ltd (Ahmedabad) and Loba Chemie. Pvt. Ltd. (Mumbai).

Melting points were determined by open tube capillary method and were uncorrected.

Purity of compounds was checked by thin layer chromatography (TLC) on silica gel-G in solvent system hexane-ethyl acetate (1:1) and the spots were located under iodine vapours and UV light.

IR spectra of all compounds were recorded on FT-IR 8400S Shimadzu spectrophotometer using KBr.

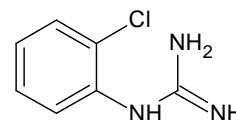
Mass spectra were obtained using 2010EV LCMS Shimadzu instrument.

### Scheme of Synthesis

See scheme-1

### Procedure of guanidine derivatives

#### (1) Preparation of 1-(2-chlorophenyl) guanidine

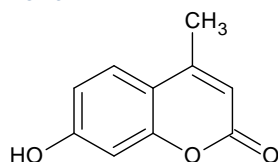


(3)

The 0.5mol of 2-chloro aniline and 2mol of guanidine were taken in a round bottomed flask. 4ml of concentrated HCl and 4ml of glacial acetic acid were added into it

and refluxed for 30 min with porcelain piece. Fine white crystals appeared which was recrystallized with methanol with the addition of a little decolorizing carbon to obtain gives pure product.<sup>4</sup>

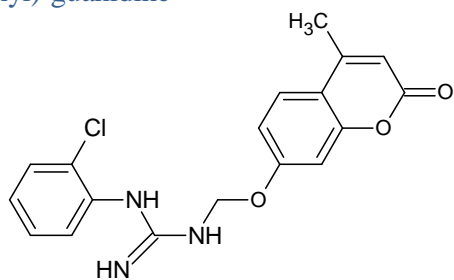
(2) Preparation of 7-hydroxy-4-methyl-2*H*-chromene-2-one



(6)

0.91mol of resorcinol and 1.03mol of ethyl acetoacetate mixture was added in concentrated H<sub>2</sub>SO<sub>4</sub> in drop wise by maintaining the temperature below 10°C on ice bath. It was heated for 30minutes on water bath and then added in a mixture of crushed ice and cool water. Product obtained was collected and recrystallized with methanol.<sup>5</sup>

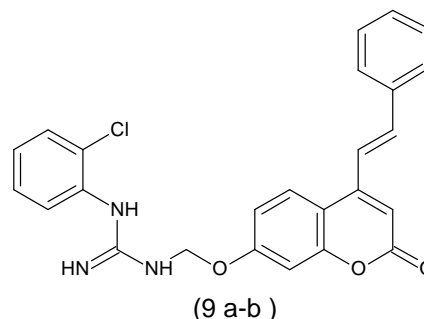
(3) Preparation of 1-(2-chlorophenyl)-3-((4-methyl-2-oxo-2*H*-chromen-7-yl-oxy)-methyl)-guanidine



(7)

4-methyl-7-hydroxy coumarin (0.045mol), 2-chloro phenyl guanidine (0.045mol) and dichloromethane (0.045) were taken in a round bottom flask and refluxed with methanol as a solvent and refluxed for 1hour. The obtained product was recrystallized with methanol by activated carbon.<sup>6</sup>

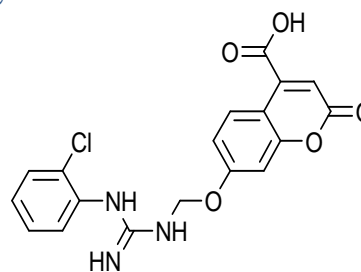
(4) Preparation of (*E*)-1-(2-chlorophenyl)-3-((2-oxo-4-styryl-2*H*-chromene-7-yloxy)-methyl)-guanidine



(9 a-b)

0.016mol of 1-(2-chloro-phenyl)-3-((4-methyl-2-oxo-2*H*-chromen-7-yloxy)-methyl)-guanidine and 0.016mol of benzaldehyde were mixed in alcoholic NaOH and refluxed for 4-5hours. The obtained product was recrystallized with *N,N*-Dimethyl formamide.<sup>7</sup>

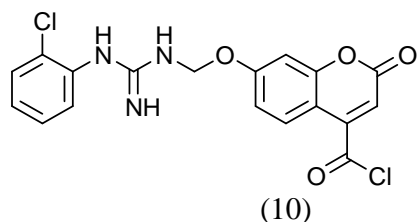
(6) Preparation of 7-((3-(2-chlorophenyl)-guanidino)-methoxy)-2-oxo-2*H*-chromene-4-carboxylic acid



(8)

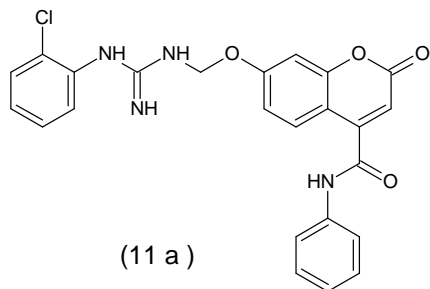
In the oxidation step we have to place compound in the solution of potassium permanganate .Then basified with the potassium hydroxide solution. Methanol was added to the solution to solubilize the oxidize product. It was filtered and after adding of the conc. HCl to acidify the product was obtained. I was recrystallized with methanol.

(7) Preparation of 7-((3-(2-chlorophenyl)-guanidine)-methoxy)-2-oxo-2*H*-chromene-4-carbonyl chloride



In the chlorination step oxidized product was refluxed with thionyl chloride for 1-2 hours. After that solution of reaction mixture was cooled to get the product in liquid form. Take the precaution while handling.

(8) Preparation of 7-((3-(2-chlorophenyl)-guanidino)-methoxy)-2-oxo-N-phenyl-2H-chromene-4-carboxamide



This is the condensation reaction. In this aniline was added in to the solution of chlorinated product. Then product obtained in solid form which was collected and recrystallized with methanol.

Spectral characteristics of synthesized compounds

- 9-a:** 1-(2-chlorophenyl)-3-((2-oxo-4-styryl-2H-chromen-7-yloxy)-methyl)-guanidine I.R. spectra showed absorption band at 3294 (NH), 1726 (C=O), 1643 (HC=CH), 1132(-O-), 898 (Ar-Cl). Mass spectra showed a characteristics M+ and M+2 447.1 and 447.4 respectively. NMR spectra showed a characteristics at 1.95 (s, 1H, NH), 3.73 (s, 1H, NH), 4.42 (s, 1H, NH), 5.32 (s, 2H, CH<sub>2</sub>), 6.55-6.57 (d, 2H, C=CH), 7.6-7.99(m, 13H, Ar-H)
- 9-b:** 1-((4-(4-hydroxystyryl)-2-oxo-2H-chromen-7-yloxy)-methyl)-3-(2-chlorophenyl)-guanidine I.R. spectra

showed absorption band at 3423 (NH), 1710 (C=O), 1602 (HC=CH), 1137 (-O), 748 (Ar-Cl). Mass spectra showed a characteristics M+ and M+2 463.2 and 463.8 respectively.

- 11-a:** 7-((3-(2-chlorophenyl)-guanidino)-methoxy)-2-oxo-N-phenyl-2H-chromene-4-carboxamide I.R. spectra showed absorption band at 3454 (-NH), 1711 (C=O), 1101 (-O-), 715 (Ar-Cl). Mass spectra shown a characteristics M+ and M+2 464.8 and 465.1 respectively.

*In-Vivo* Antidiabetic Activity

Oral glucose tolerance test

*In-vivo* study of synthesized compounds by OGTT

The oral glucose tolerance test (OGTT) measures the body's ability to use a type of sugar, called glucose that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favour of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus.

Anti-diabetic activity

The anti-diabetic activity of newly synthesized benzimidazole derivatives was carried out using Oral glucose tolerance test method.

**Method:** Oral glucose tolerance test

**Animals used:** Albino Wistar rat

**No. of animals used:** 3 (in each group)

**Dose of std. drug:** 60mg/kg (metformin)

**Route of administration:** Oral

**Group I:** normal control group.

**Group II:** metformin control group (60mg/kg)

**Group-III-X:** were treated with synthesized compounds. The synthesized compounds

were dissolved in suspension of 0.5% CMC.

**Requirements:**

**Instruments:** Glucometer.

**Chemicals:** 0.5% CMC

**Standard drug:** Metformin (60 mg/kg) aq. solution was prepared using 0.5% CMC.

**Test compounds:** Solution of compounds was prepared and administered orally similar to that of standard drug.

**Apparatus:** feeding needles (for oral dosing), syringes (1ml, 2ml)

### Experimental design and procedure

A total number of 26 Albino Wistar rats weighing about 200-250gm. Group I served as a normal control group while Group II for metformin control group. Group III-X was treated with synthesized compounds.

Special diets are fed for 30 to 90days prior to the OGTT. We carry out the OGTT by fasting animals for 18hours, taking a blood sample from the tail under local anesthesia and then gavaging with 12ml/kg of a 50% glucose solution, which delivers 6g of glucose per kg of body weight. Blood samples are taken 30, 60, 90 and 120 minutes after the glucose meal and analyzed for blood glucose with a clinical glucometer. The reference drug and the synthesized compounds were administered orally with oral feeding tube to the rats.

OGTT for non-diabetic rats were performed according to the standard method. In short, Group I to Group V was selected for OGTT test after starving at water for 16hours. The baseline glucose level was measured by glucometer.

Group I stands for normal control group. Group II is treated with metformin (60mg/kg body weight). The synthesized compounds were dissolved 0.5% CMC in according to 50mg/kg of body weight. Then the solution was administered orally to the glucose fed rats and blood was collected from the rat by cutting the tail. Blood

sample was taken in a strip and then measured the glucose concentration level by glucometer and plasma glucose level in mg/dl was being monitored at 0, 30, 60 90, 120 minutes for three rats/group. Data were expressed as Mean±Standard Error of Mean (SEM). Statistical comparisons were performed by one-way ANOVA followed by Dunnett's Multiple Comparison Test and the values were considered statistically significant when  $P < 0.05$ .<sup>8</sup>

### CONCLUSION

All the synthesized compounds were tested antidiabetic activity and compared with metformin as a standard.

From the above activity Table-2 it was evident that all synthesized compound give a good antidiabetic activity.

Compound 9-b was found to be having significant decrease glucose level as compared to standard.

Other compounds 9-a and 11-a were found to be less active as compared to other compounds.

Synthesized compounds were screened for antidiabetic activity by oral glucose tolerance test method.

Upon data shown in Table-2, it can be said that the synthesized compound 9-b has lowering the glucose level significantly with time and result are approximately near with standard metformin, so it having similar antidiabetic activity like standard.

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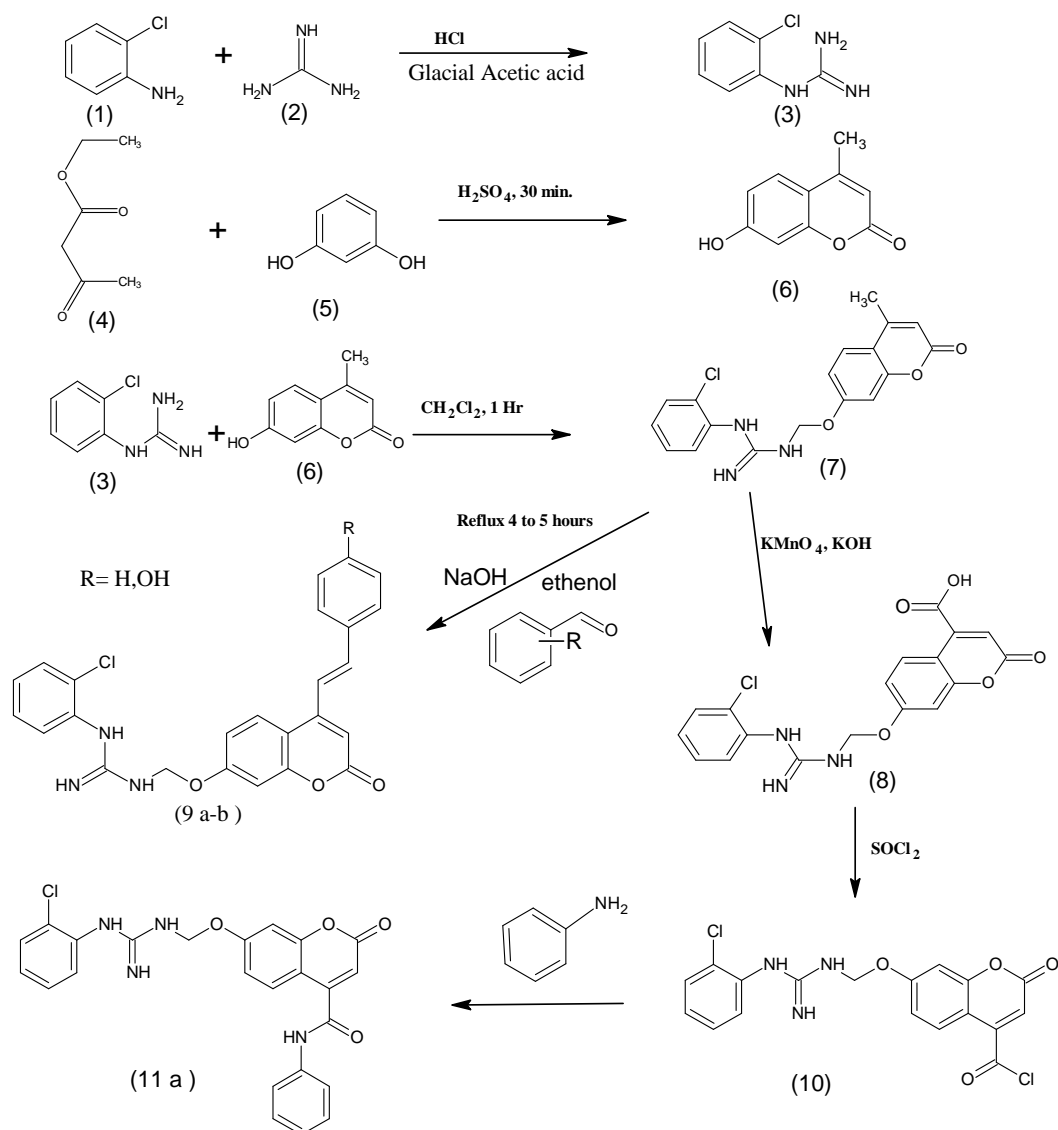
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**Table 1. Physical characteristic of synthesized compounds**

Compounds	Molecular Formula	Molecular weight (gm/mol)	Melting point (°C)
9-a	C <sub>25</sub> H <sub>20</sub> ClN <sub>3</sub> O <sub>3</sub>	445	190-192°C
9-b	C <sub>25</sub> H <sub>20</sub> ClN <sub>3</sub> O <sub>4</sub>	461	108-110°C
11-a	C <sub>24</sub> H <sub>19</sub> ClN <sub>4</sub> O <sub>4</sub>	462	110-113°C

**Table 2. Antidiabetic screening**

n=3	Control±SEM	Standard±SEM	Test-1±SEM	Test-2±SEM	Test-3±SEM
Base line	61.33±2.40	66±1.15	58±1.15	63±0.57	59.33±1.76
30 min	250±1.15	136±1.15	185.66±2.02	146.33±1.20	195.33±0.88
60 min	222.33±3.17	99±1.15	142±1.73	112±1.15	151.66±2.60
90 min	199±2.08	93±1.15	123.66±0.88	100±1.15	128.66±0.88
120 min	195±1.76	85±1.15	110.33±2.02	95.33±1.20	112.33±1.45



Scheme-1



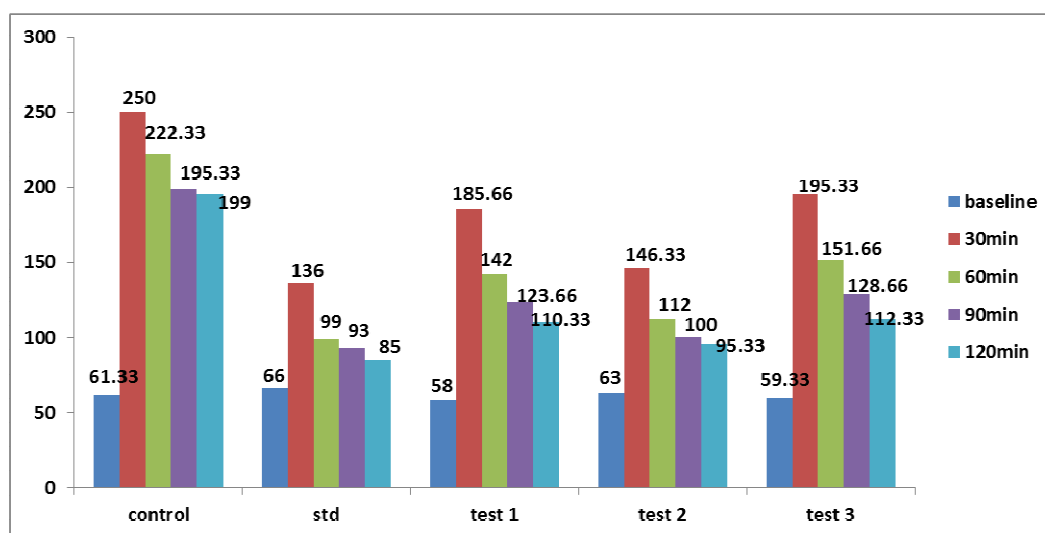


Figure-1- Histogram of Antidiabetic Screening