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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 1-ARYL-2-PHENYL-4-GUANIDINO-4-THIOALKYL-1,3-DIAZABUTA-1,3-DIENES DERIVATIVES.

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ABSTRACT
Several 1-aryl-2-phenyl-4-guanidino-4-thioalkyl-1,3-diazabuta-1,3-dienes were prepared by the treatment of N-arylimino isothiocyanate and guanidine followed by S-alkylation with alkyl iodide in the presence of dry acetone .The constitution of the products was supported by IR , NMR and Mass spectral study.The synthesized compounds were evaluated for their effect on blood sugar and protective effect against alloxan induced hyperglycemia in mice .The structure-activity relationships(SAR) of these compounds are also presented.within this series of compounds, 12f is the most acive azadiene derivative.

Key words *Guanidine, S-methylisothiourea, Amidine ,Azadiene and Alkylation(S-methylation & S-ethylation)*

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INTRODUCTION

Diabetes mellitus is a common metabolic and endocrine disorder,characterized by chronic hyperglycemia and disturbance of carbohydrate ,fat and protein metabolism associated with absolute or relative deficiency of insulin secretion and/or insulin action[1].According to recent estimates approximately 215 million people all over the world suffer from diabetes mellitus and 80%-90% of them are from type II diabetes[2]. Type I diabetes is also a common and widespread disease occurring in every part of the world, is an autoimmune disorder that results from the immune-mediated inflammatory destruction of insulin producing β-cells in pancreatic islets.There are an estimated 500,000 to 1 million people with Type I diabetes in the US today. Although the specific pathogenic mechanisms in TypeI diabetes are not defined, it is clear that activated T cells and macrophages are required for initiation. Once activated macrophages secrete several inflammatory cytokines,such as interleukin1β (IL-1β),interleukin 12(IL-12) and tumour necrosis factor α (TNF-α) and trigger interferon-γ production from activated T cells[3] .These cytokines are cytotoxic to β-cells by inducing the formation

of oxygen free radicals,nitric oxide and lipid peroxides within β-cells and enhance the cell-mediated inflammatory responses which are responsible for β-cell destruction[4].Lisofylline(LSF) Fig.1 is a novel anti-inflammatory compound has been shown to protect β-cells from multiple inflammatory agents[5-6].

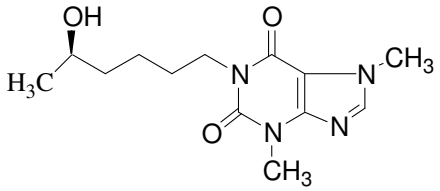


Fig. 1 Lisofylline(LSF) 1-(5-R-hydroxyhexyl)-3,7-dimethylxanthine

The discovery that certain organic compounds will lower the blood sugar level is not recent. In 1918, guanidine was shown to lower the blood sugar level .The discovery that certain trypanosomes need much glucose and will die in its absence was followed by the discovery that Galegine Fig.2 lowered the blood sugar level and was weakly trypanocidal; this led to the development of bisamidines, diisothioureas,bisguanidines and others.Synthalin Fig.3 and Pentamidine Fig.4 are outstanding examples. Synthalin lowers the blood sugar in normal, depancreatized,and completely alloxanized animals.This may be due to reduced oxidative activitiy of mitochondria, resulting from inhibition of the mechanisms that simultaneously promote phosphorylation of ADP and stimulate oxidation by nicotinamide adenine dinucleotide(NAD) in the citric acid cycle[7].The synthetic biguanides such as Metformin Fig.5 and its analogues[8-9],were synthesized on the basis of natural product leads,that is galegine which was isolated from the seeds of Galega officianalis[10].

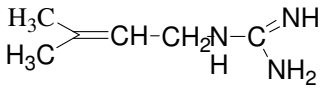


Fig.2 Galegine

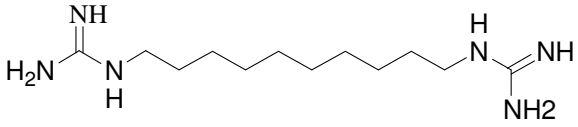


Fig.3 Synthalin

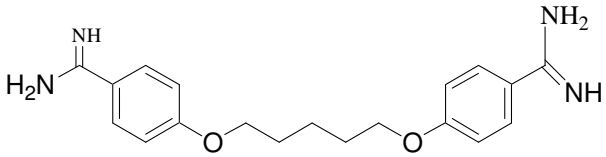


Fig.4 Pentamidine

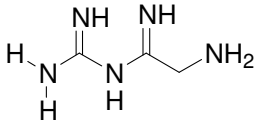


Fig.5 Metformin

CHEMISTRY

Formation of highly functionalised novel series of 1-aryl-2-phenyl-4-guanidino-4-thio-alkyl-1,3-diazabuta-1,3-dienes[11,12], has been shown to occur by the treatment of N-aryl-imino isothiocyanate[13], with guanidine in very good yields, commonly reffered to as thioureas. The alkylation of these thioureas with iodides viz methyl iodide and ethyl iodide and basification of resultant hydroiodides salts with 3N Sodium hydroxide resultant in excellent yields of 1-aryl-2-phenyl-4-guanidino-4-thioalkyl-1,3-diazabuta-1,3-dienes.

EXPERIMENTAL

The compounds having 4-guanidine at C-4 position have been synthesized and were evaluated for antihyperglycemic activity in rats .We introduced alkyl groups onto the sulphur at the 4-position of compounds Viz methyl or ethyl group.The synthetic route that was used to synthesized 1,3-diazabuta-1,3-dienes having primary amine and S-alkyl substitutes..The treatment of N-aryl imino isothiocyanates with a primary amine guanidine have been shown to result in very good yields of 1-aryl-2-Phenyl-4-amino-4-thiocarbonyl-1,3-diazabuta-1,3-dienes.The alkylation of these thioureas with alkyl iodides(methyl or ethyl iodide) and basification of the resultant hydroiodide salts with 10% sodium hydroxide solution resulted in

excellent yields of 1-aryl-2-Phenyl-4-Primary.amino-4-thioalkyl-1,3-diazabuta-1,3-dienes. Substitution with an electron-donating chlorine group and electron-donating methyl group both result in good activity.Moreover methyl and chlorine group on N-Phenyl ring increases the stability of the azadiene and product in the solid form and in more yield.All these azadiene derivatives were characterized by NMR, Mass and I.R spectral evidences.TLC plates were used for checking purity of compounds.All compounds were homogenous on TLC and gave proper spectral characteristics. The detailed spectral features of these 1,3-diazabuta-1,3-dienes are given in the experimental section while the main features are being mentioned here..In these compounds for e.g mass spectrum of showed the molecular peak at m/z 339 and IR spectra showed absorption bands at the regions 3340-3050 cm^{-1} (broad, NH, NH_2) and sharp band at 1600cm^{-1} in its IR spectrum (KBr disc) was assigned to carbon nitrogen double bond stretching frequency. Its ^1H NMR spectrum ($CDCl_3$) showed four singlet at δ_H 2.91 and δ_H 1.31 and δ_H 2.0 due to NH , NH and NH_2 proton exchangeable with D_2O appeared as broad singlet respectively and δ_H 2.35 due to CH_3 on phenyl ring.The multiplets at δ_H 2.91 and δ_H 1.31 due to SCH_2 and CH_3 respectively. The aromatic protons were observed as multiplet at , δ_H 7.1- δ_H 7.29

SCHEME OF SYNTHESIS:

General Reaction:

Fig. 6 Fig. 7 Fig.8

R_1= H, CH_3 & Cl

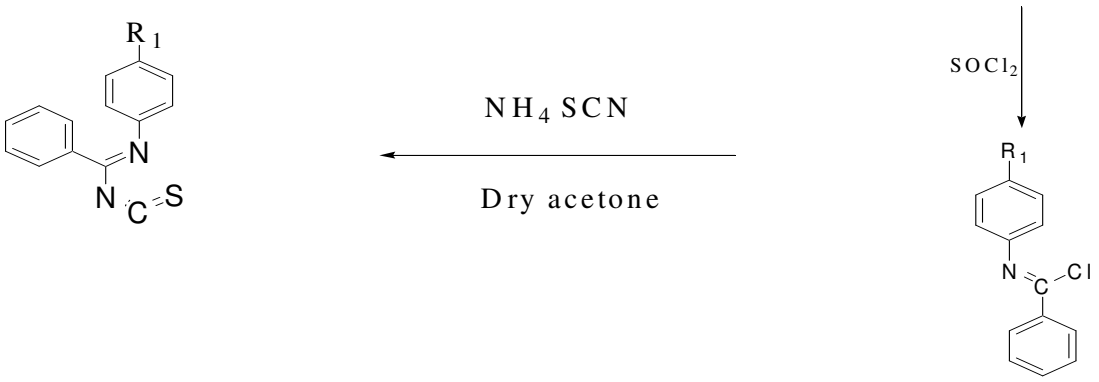


Fig.10 Fig. 9

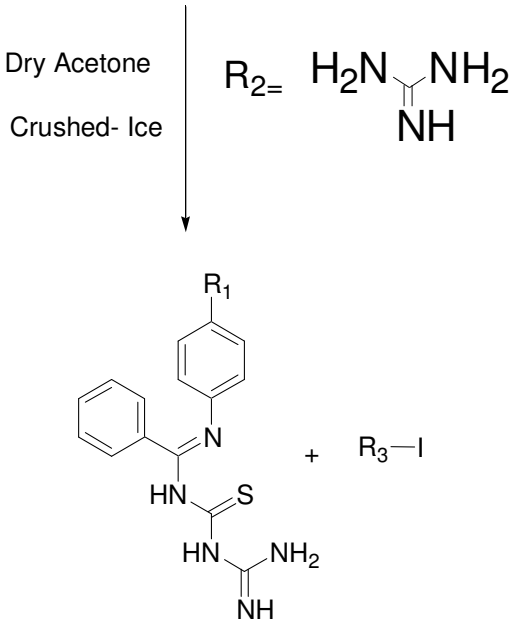


Fig. 11

Fig. 12a-f
R_3= CH_3 & CH_2CH_3

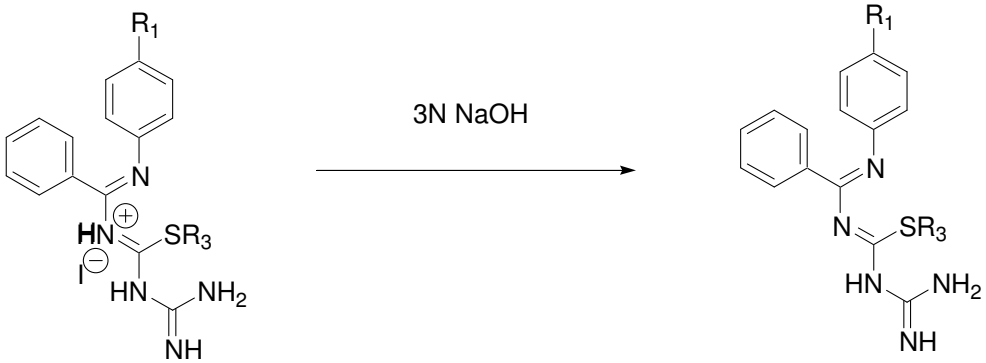
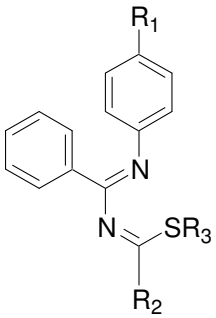


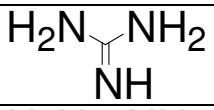
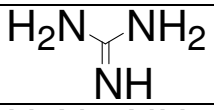
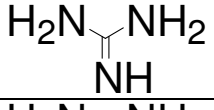
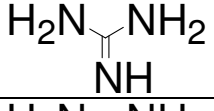
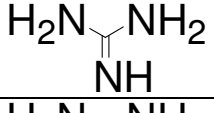
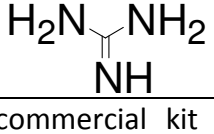
Fig.13

Fig.14



General Structure

TABLE 1: Series of Compounds Synthesized and Characterized

S.No	Code	R_1	R_2	R_3
12a	H6	H		CH_3
12b	H4	H		CH_2CH_3
12c	H3	CH_3		CH_3
12d	H2	CH_3		CH_2CH_3
12e	H5	Cl		CH_3
12f	H1	Cl		CH_2CH_3

BIOLOGICAL EVALUATION

EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY:

Diabetes was induced in the animals by single injection of alloxan monohydrate (150mg/Kg i.p). It was confirmed after 48 hours (on third day). Animals found hyperglycaemic were divided into different groups. Control group received no drug treatment, standard group received metformin(75mg/Kg p.o) and test group received test compounds H1-H6 (Table1))(50mg/Kg i.p) once a day up to 14th day of alloxan.Blood was estimated in all groups on 15th day. Rats were anesthetized by diethylether. Blood was withdrawn from retrooribital plexus using glass capillary containing heparin to prevent clotting of blood. Blood was centrifuged and plasma was separated for estimation at 2000rpm for 15min .Fasting blood samples was collected at 15th day(TABLE3) and estimated by GOD-POD Kit Method.

MATERIALS AND METHODS:

Fasting blood glucose:

(A) Estimation of Fasting Blood Sugar (FBS):

Fasting blood sugar was determined by GOD/POD Kit Method was estimated by using commercial kit (Span Diagnostics Ltd, Surat, India).

Principle: Glucose Oxidase (GOD) oxidizes Glucose to Gluconic Acid and Hydrogen Peroxide. In presence of enzyme Peroxidase, released Hydrogen Peroxide is coupled with Phenol and 4-Aminoantipyrine (4-AAP) to form coloured Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose concentration in the sample.

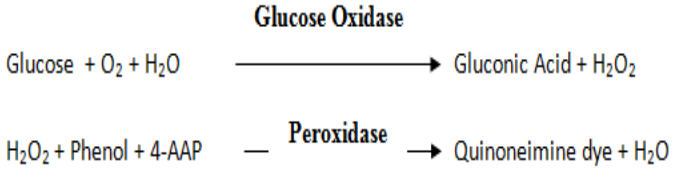


Table 2: Procedure for estimation of Blood Glucose

Pipette into tubes marked	Blank	Standard	Test
Serum/Plasma	-	-	10µL
Reagent 2	-	10µL	-
Reagent 1	1000µL	1000µL	1000µL
Mix well. Incubate at 37°C for 10 minutes.			
Distilled Water	2000µL	2000 µL	2000 µL

The absorbance of standard followed by the Test was measured at 505 nm. The Fasting Blood Sugar was then calculated using following formula:

$$\text{Serum/PlasmaGlucoseconcentration(mg/dL)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 100$$

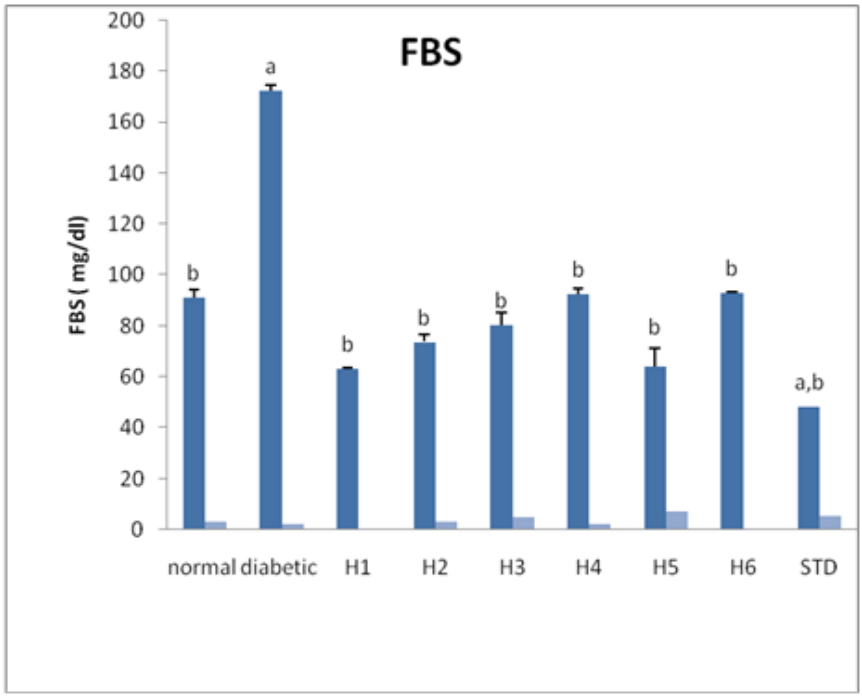
Drugs and Chemicals: Alloxan used to induce the diabetes was procured form Sigma aldrich, USA. Metformin was used as the standard drug was a gift sample from Jackson Pharmaceautical Pvt Ltd , Majitha Road,Amritsar(Punjab).

Statistical analysis: One specific group of rats was assigned to one specific drug treatment condition and each group comprised of five rats (n=5). The data was analyzed using tukey test . In all the tests the criterion for the statistical significance was $p<0.05$.Data are expressed as mean ± SEM and analyzed for significance by ANOVA(comparing all Vs control) using Sigmastat Software.P value < 0.05 was considered significant.

TABLE 3: FASTING BLOOD GLUCOSE AFTER 14th DAY

Group	Normal	Diabetic	H1	H2	H3	H4	H5	H6	Metformin
Mean	91.26	2.98322	63.2	73.8	80.2	92.4	92.8	92.8	48.2
SEM	2.98322	2.135416	0.583095	3.006659	4.933559	2.293469	0.374166	0.374166	5.351635

Fig 16 : Effects of test compounds H1-H5 on serum glucose in alloxan induced diabetes in rats (n=5)



a, $p<0.5$ as compare to normal

b, $p<0.05$ as compare to diabetic

CONCLUSION.

In our present study, we have synthesized and evaluated azadiene as antidiabetic agent .The substituent of the phenyl ring are altered randomly to find a lead compound for further studies. The diabetic control group developed significant($p<0.05$) hyperglycemia as compared to normal control group.After the treatment with test compounds all the compounds were found to decrease the serum glucose levels significantly($p<0.05$)when compared with normal control group.However, these compounds showed a lesser activity than metformin used as reference standard in the study.The order of anti-hyperglycemic activity for test compounds was found to be H1>H5>H2>H3>H4>H6(Fig16) The effects of all the Six test compounds are found to be significant and comparable with the standard group. H1 is found to give results more closer to the standard(Metformin) than any other compound. From the results, H1 appears to be good hypoglycaemic agent.The compound having chlorine group on benzene ring was found to be more active than other derivative.From all the compound, S-ethyl derivative have been found to be more active as compared to S-methyl derivative.But further work on the structure–activity relationship is needed to to find out more active compounds..

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