RESEARCH PAPER

Synthesis and In Vitro Evaluation of Anti-Diabetic Potential of Novel Some Novel Thiazole Derivatives

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ABSTRACT

Background

Diabetes Mellitus is a chronic metabolic disorder characterized typically with increased blood glucose levels and which has seen to be causing several complications like neuropathy, retinopathy, nephropathy etc and although medications and insulin are available for treatment and controlling blood glucose levels this line of treatment is cumbersome and expensive. This study was designed to synthesize and evaluate newer Thiazole derivatives.

Materials and Methods

Ten Thiazole derivatives were synthesized and subjected to in vitro Baker's Yeast Cell method and α-Amylase enzyme evaluation, amongst these THI-6 and THI-9 have shown promising activity against both these methods hence these two compounds were subjected to study against α-glucosidase enzyme. The synthesized compounds were also subjected to TLC and spectral analysis.

Results

The results of the pharmacological evaluation of the synthesized Thiazole derivatives show THI-6 and THI-9 compounds have maximum glucose utilization capacity and these compounds also have shown good percentage inhibition capacity against α-Amylase enzyme, however when these compounds were subjected to study against α-glucosidase enzyme the results of percentage inhibition were not very promising.

Conclusion

Thus we may conclude that among the ten synthesized derivatives of Thiazole THI6 and THI9 have shown more promising activity in Yeast Cell method and α-Amylase method indicating promising anti diabetic potential through these mechanisms but they have not shown positive and promising activity on α-Glucosidase enzyme. Further subjecting these three compounds to in vivo studies using different animal models could help us in validating the mechanism of action and lead us to a newer series of anti-diabetic agents.

Key Words: diabetes, thiazoles, in vitro, α-amylase, α-glucosidase.

How to cite this article: Patil S, Kognole PM, Pattanshetti L, Ankalikar AA, Ronad PM, Swami B. Synthesis and In Vitro Evaluation of Anti-Diabetic Potential of Novel Some Novel Thiazole Derivatives. Int J Drug Deliv Technol. 2026;16(23s): 880-889. DOI: 10.25258/ijddt.16.23s.94

Source of support: Nil.

Conflict of interest: None

Introduction:

A chronic metabolic disorder typically characterized with persistent elevated levels of glucose in the blood either due to insufficient insulin secretion or due to failure of the body to utilize the available insulin for maintaining blood glucose levels or both combined is known to us as Diabetes Mellitus. Most of the times a genetically predisposed disorder, an unhealthy lifestyle and environmental factors also play a vital role in contributing towards the development of insulin resistance and eventually Diabetes mellitus. Development of insulin resistance could be attributed to low levels of C-peptide in blood, elevated levels of pro inflammatory cytokines and excessive hepatic production of glucose and increased fat breakdown. Amongst the two types of diabetes that exist the adult onset diabetes which is also known as age onset diabetes is the most common accounting to 90% of the cases

worldwide. The disease is seen to cause both macro as well as micro vascular complications like nephropathy, neuropathy, retinopathy, cardiovascular as well as cognitive complications. It is also steadily increasing morbidity and mortality. Posing a major health challenge in developed as well as developing countries the treatment for the disease is available in form of oral medications, like Biguanides ex: Metformin, sulphonylureas which help in enhancing insulin secretions, thiazolidinediones which have the capacity to exogenously provide insulin, GLP-1 receptor agonists Meglitinides are some of the oral anti-diabetic drugs available for treating type-II Diabetes. For treating Type-I diabetes where insulin is completely absent insulin therapy in the form of injectable is available. Advancements' in biotechnology have helped us establish and clone the human insulin gene and manufacture the same on large scale for the purpose of treatment. This insulin is available in many forms of preparations depending upon the duration of action namely: Rapid acting, Short acting, Intermediate acting and Long acting. Available as insulin pens or insulin pumps these preparations of insulin are injected subcutaneously to mimic the action of normal insulin of being slow steady and long lasting in action. Sometimes aggravated or long standing type-II diabetes also requires to be supplemented with injectable of insulin along with oral hypoglycaemic agents to manage the blood glucose levels. A combination of one or more of these options is put into practice for controlling and treating the disease. Along with this lifestyle modification like diet control, exercise, physical activity, weight management are implemented to control the elevated blood glucose levels[1-6]. Since Diabetes Mellitus is a chronic disorder which requires prolonged and lifelong treatment with either insulin injections, anti-diabetic agents and/ or both, it is cumbersome and often inconvenient to consume medicines for a prolonged period. Many of the anti-diabetic class of drugs which belong to varied pharmacological classes are unable to prevent long-term complications and have limited durability of efficacy. Use of insulin injections increase risk of lipohypertrophy at injection site, fear of injections leads to poor patience adherence and injection regimens are complex. Drugs belonging to class of Sulphonylureas and Meglitinides which work by stimulating insulin secretions are known to cause weight gain, secondary drug failure and cardiovascular risks. Biguanides like Metformin lead to Vitamin B12 deficiency, anaemia and neuropathy. The thiazolidinedione class of anti-diabetics' increase bone fractures risk, edema, fluid retention and hepatoxicity. Alpha-glucosidase inhibitors lead to gastrointestinal side effects while Dipeptidyl peptidase-4 (DPP-4) inhibitors which enhance Incretin levels exhibit pancreatitis, severe joint pain, and potential immune modulation. These limitations highlight the urgent need for discovering and developing newer class of anti-diabetic class of drugs which minimize risks, provide durable glucose level control, give renal and more efficacious, safe and with disease modifying potential[7-13].

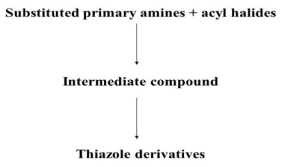
Materials and Methods:

Chemicals: Ethanol, Chloroform, Hexane, Ethyl acetate, DMSO, Chloroacetylchloride, Triethylamine, Thiourea, Aniline Derivatives (LOBA Chem, HYMA Chem & Spectrochem) D-Glucose, NaOH, Sodium potassium tartrate, Sodium dihydrogen phosphate, α-amylase enzyme solution, starch, phosphate buffer 6.9, Acarbose, 3,5-dinitrosalicylic acid (DNSA), Baker's yeast & p-nitrophenyl-α-D-glucopyranosid

Instrument: UV-VIS spectrophotometer (Shimadzu UV-1800), IR (Shimadzu), UV chamber, Magnetic Stirrer, Chiller, Fuming Chamber, TLC plates (Sigma), Centrifuge, Hot air oven, Incubator, Hot plate, Stuart SMP10 Melting & Weighing balance

Scheme for Synthesis of title compounds:

The below mentioned scheme was implemented for synthesizing the Thiazole derivatives.



Using this scheme the Ten novel title compounds were synthesized (THI-1 to THI-10). The reaction progress was monitored using TLC at specific intervals, melting points of the compounds were determined using Stuart SMP10 Melting apparatus and IR spectra were recorded using QATR-S IRSpirit (Shimadzu).

***in-vitro* Antidiabetic Activity assay:**

Baker's Yeast method[14-15]:

The yeast was centrifuged in distilled water to get clear supernatant liquid further a 10%v/v suspension was prepared in distilled water. Various concentrations of the synthesized thiazole derivatives (THI-1 to THI-10) as

well as std drug (Acarbose) were prepared viz 100,200,300,400,500 µg/mL and to each of these 1mL. of Glucose solution was added followed by incubation for 10min at 37ºC. The reaction was triggered by adding 100µL of yeast suspension vortexed and then further incubated for 60min at 37ºC. On completing 60min the test tubes were centrifuged, supernatant liquid separated and glucose was estimated in the same by measuring absorbance at 540nm by taking readings in triplicate.

The percentage uptake by yeast cells was calculated by the below mentioned formula:

Activity% = [(Abs control-Abs sample)/ Abs control] x 100

where:

Abs control is the absorbance of the control

Abs sample is the absorbance of the test samples

The IC $_{50}$ values were also calculated for all the test samples and were compared with std. Acarbose as positive control.

α-amylase Enzyme estimation[16-18]**:**

A 1 µ/ml of α-amylase enzyme solution and 1% w/v of starch solution were prepared separately in 20 mM phosphate buffer (pH 6.9). Using the same buffer, a series of solutions of different concentrations ranging from 100,200,300,400,500 µg/mL of standard drug (Acarbose) and synthesized thiazoles (THI-1 to THI-10) were prepared. 100 µL of each of these concentration of std as well as test solutions were separately mixed with 200 µL of enzyme solution and kept for 10 min for incubation at 30°C.

Further, 100 µL of starch solution of 1% w/v was added to each of the above solutions and the mixture was further kept for incubation for 10min at 30°C to initiate the reaction. The enzyme was allowed to react with the samples at 37°C for 30 min and then this reaction was stopped by using 200 µL 3,5-dinitrosalicylic acid (DNSA) which was added in each of the sample mixtures which were further heated in a boiling water bath for 5min. These mixtures were then cooled and diluted with 5mL of water. The absorbance of these mixtures was then measured using a UV-VIS spectrophotometer (Shimadzu UV-1800) at 540 nm. The readings were then taken in triplicate and IC_{50} values were calculated using the below formula:

The percentage inhibition of α-amylase was calculated by using the equation below:

Percentage Inhibition = $\frac{\text{Absorbance Control} - \text{Absorbance Test}}{\text{Absorbance Control}} \times 100$

The IC_{50} values were also calculated for all the test samples and were compared with std. Acarbose as positive control.

α-Glucosidase Enzyme estimation[19-23]**:**

A 50 µL of solution of α-Glucosidase was prepared using 1U/mL Yeast (SRL, Bangalore, India) in 50 mM of phosphate buffer at pH 6.9 which was then added to pre-treated samples of different concentrations for 10 min at 37°C. Further to this buffer 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside was added and conducive environment for the enzyme reaction to occur was made available at 37°C for 30 min. After this stipulated time the reaction was ended by adding a solution of 1M Na2CO3 followed by recording of absorbance at 405 nm.

The percentage of inhibition was calculated by the formula:

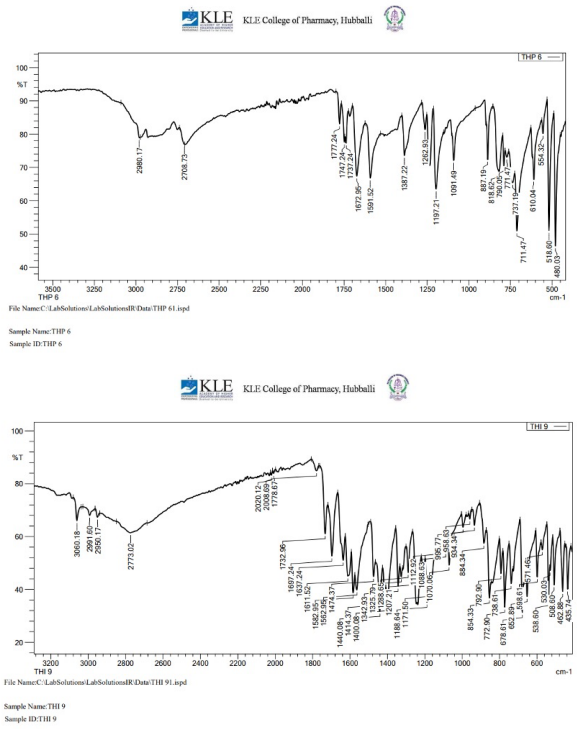
$\frac{\text{OD of Blank - OD of Test}}{\text{OD of Blank}} \times 100$

The results were expressed as IC 50 values and were compared with std. Acarbose as positive control.

Results:

Synthesized thiazole derivatives (TH1-TH12) were preliminarily identified by TLC, and Rf values were calculated which was followed by determination of melting points and the IR spectra of the compounds. The IUPAC names, melting points and Rf values are represented (**Table No:01)**

IR spectra of only **TWO** compounds which demonstrated promising *in vitro* anti-diabetic activity namely: N4-(2-chlorophenyl)thiazole-2,4-diamine **(THI-6)**, N4-(3-bromophenyl)thiazole-2,4-diamine **(THI-9)** is shown in **Fig No: 1 & 2.**



Product Code	IUPAC Name of Compound	Melting Point	Rf Value
THI-1	N4-(4-chlorophenyl)thiazole-2,4-diamine	210-215° C	0.4
THI-2	N4-(4-bromophenyl)thiazole-2,4-diamine	208-210° C	0.54
THI-3	N4-(2,5-dichlorophenyl)thiazole-2,4-diamine	205-210° C	0.6
THI-4	N4-(2-chloro-4-nitrophenyl)thiazole-2,4-diamine	201-207° C	0.69
THI-5	N4-(3-chlorophenyl)thiazole-2,4-diamine	215-220° C	0.64
THI-6	N4-(2-chlorophenyl)thiazole-2,4-diamine	210-214° C	0.67
THI-7	N4-(2,5-dinitrophenyl)thiazole-2,4-diamine	208-213° C	0.68
THI-8	N4-(4-chloro-2-nitrophenyl)thiazole-2,4-diamine	205-212° C	0.69
THI-9	N4-(3-bromophenyl)thiazole-2,4-diamine	210-215° C	0.65
THI-10	N4-(2,6-dimethylphenyl)thiazole-2,4-diamine	210-215° C	0.69

Table No:01: The IUPAC names, melting points and Rf values of Ten Title Compounds

Spectral Analysis of compounds by IR Spectra

Fig 01, and 02 show the IR spectra of those three Thiazole derivatives which have shown promising *in vitro* anti-diabetic activity. The IR spectrum of compound **THI-6 (Fig No. 01)** shows an Aromatic stretch of C-H at 2980 cm^{-1}, NH_2 stretching at 2808 cm^{-1}, C=N stretching at 1679 cm^{-1} and an aromatic C=C stretching at 1597 cm^{-1}. For compound **THI-9 (Fig No. 02**) it shows NH stretching at 3060 cm^{-1}, NH_2 stretching at 2991 & 2950 cm^{-1} and C–N C–S and C–Br stretching at 1474–1400 cm^{-1}, 678–652 cm^{-1}, 508–480 cm^{-1} respectively.

Fig 01: IR spectrum of N4-(2-chlorophenyl)thiazole-2,4-diamine

Fig 02: IR spectrum of N4-(3-bromophenyl)thiazole-2,4-diamine

Evaluation of inhibitory activity against Yeast:

Table No:02 & Fig No 03 represents the percentage inhibition capacity of the synthesized Thiazole derivatives of glucose uptake in yeast cells. Both show the with increase in concentration the percentage inhibition of glucose uptake in the yeast cells is increasing. Amongst the ten derivatives of Thiazole which were synthesized THI-6 & THI-9 have shown promising and exponential increase in the percentage inhibition capacity of glucose uptake in the yeast cells. While THI-6 showed 5.12% inhibition at 100µL, it has shown 53.84% inhibition at 500 µL. Similarly, THI-9 has shown 18.75% inhibition at 100µL and at 500 µL it has shown inhibition of 81.25%. Amongst all the ten synthesized Thiazole derivatives this is the maximum percentage inhibition activity depicted. The standard drug Acarbose on the other has shown 9.6% inhibition at 100 µL and 45.1% inhibition at 500 µL. Both THI-6 and THI-9 have shown better percentage inhibitory activity as compared to the std drug. The IC50 values for the std drug as well as the synthesized Thiazole derivatives was also calculated and it was seen that the std. drug Acarbose has IC50 value of 489.96 while the two derivatives which have shown promising and exponential increase in percentage inhibition i.e. THI-6 and THI-9 have IC 50 values of 361.74 & 258.33 respectively thus implying that at lower concentration also the THI-9 derivative will show higher glucose inhbibitory potency compared to THI-6, other Thiazole derivatives and the std. drug as well.

Title Compound/ % of glucose uptake capacity by Yeast method	100µL	200 µL	300 µL	400 µL	500 µL	IC50 values (µM)
THI-1	22.9	31.4	37.1	40	51.4	504.45
THI-2	21.21	24.24	30.30	36.36	48.48	567.9
THI-3	17.14	22.85	28.57	40.00	51.42	510.08

THI-4	31.81	36.36	45.45	50.00	59.09	379.89
THI-5	28.57	39.28	50.00	60.71	71.42	300.12
THI-6	**5.12**	**17.94**	**30.76**	**41.02**	**53.84**	**361.74**
THI-7	10.52	13.15	21.05	28.94	42.10	627.04
THI-8	21.95	29.26	39.02	51.21	58.53	405.22
THI-9	**18.75**	**46.87**	**62.5**	**71.87**	**81.25**	**258.33**
THI-10	8.82	47.05	50.00	55.88	64.70	338.99
Acarbose	9.6	12.9	25.8	38.7	45.1	489.86

Table No 02: % glucose uptake capacity by Yeast method

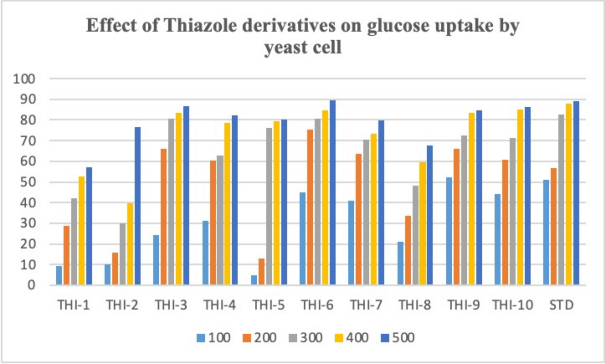


Fig No 03: Graph depicting Effect of Thiazole derivatives on glucose uptake by yeast cell

Table No:03 & Fig No 04 represent the percentage inhibition capacity of the synthesized Thiazole derivatives on the α-Amylase enzyme. The results show that the thaizole derivatives have inhibition capacity over the activity of α-Amylase enzyme and that with increasing concentration the inhibitory activity is also increasing exponentially. Amongst the ten synthesized derivatives of Thiazole the compounds THI-6 and THI-9 have shown promising exponential inhibitory activity and when compared to the std drug Acarbose these two compounds have shown promising inhibitory activity. Amongst these two compounds THI-6 & THI-9, THI-9 shows better percentage inhibition at lowest as well as highest concentration. At 100µL concentration THI-6 has shown 5.12% inhibition while THI-9 has shown 18.75% inhibition. While at 500 µL concentration as well the percentage inhibition of THI-9 is better namely 81.25% while that of THI-6 is 53.84%. On calculating and comparing the IC_{50} values of all the Thiazole derivatives and the std drug Acarbose it is seen that amongst the Thiazole derivatives THI-6 and THI-9 have IC_{50} values of 361.74 and 258.33 respectively which are comparatively better than std drug Acarbose which has IC_{50} value of 489.86. On comparing amongst the Thiazole derivatives as well, THI-9 has better and lower IC_{50} value than as compared to THI-6.

Evaluation of inhibitory activity against α-Amylase:

Title Compound/ % inhibition by the compounds for *in vitro* activity	100 µL	200 µL	300 µL	400 µL	500 µL	IC50 values (µM)
THI-1	9.30	28.59	42.30	52.79	57.19	399
THI-2	10.03	15.83	30.11	39.76	76.44	238
THI-3	24.21	66.06	80.80	83.52	86.73	171
THI-4	31.28	60.26	62.57	78.69	82.34	191
THI-5	4.88	12.88	76.0	79.5	80.4	163.23
THI-6	**44.8**	**75.18**	**80.55**	**84.81**	**89.44**	**47.56**
THI-7	41.05	63.60	70.29	73.40	79.62	120
THI-8	20.89	33.56	48.12	59.50	67.60	333
THI-9	**52.36**	**65.83**	**72.63**	**83.61**	**84.58**	**34.8**
THI-10	44.32	60.74	71.44	84.89	86.21	119.147
Std. Drug Acarbose	50.86	56.65	82.62	87.79	89.20	82.74

Table No 03: % Inhibition of Thiazole derivatives against α-Amylase

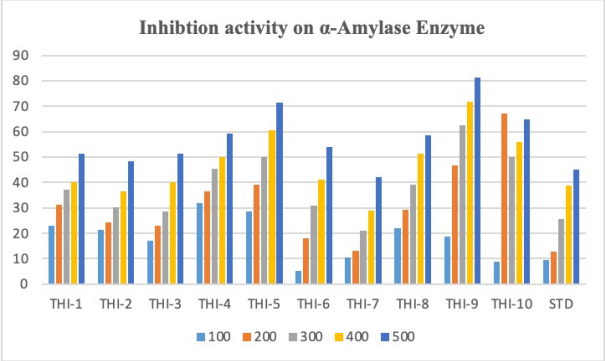


Fig No 04: Graph depicting % Inhibition of Thiazole derivatives against α-Amylase

Amongst the ten derivatives synthesized of Thiazole those compounds which have higher percentage inhibition in yeast cells and against **α-Amylase** were subjected to percentage inhibition studies against **α-Glucosidase enzyme. Table No. 04 & Fig No 5** shows the percentage inhibitory activity of the synthesized Thiazole derivatives against **α-Glucosidase** enzyme. It is seen that the percentage inhibitory activity of the std. drug as well as the three derivatives of Thiazole is unpredictable and hence inconclusive. Although THI-6 has shown good inhibition of activity of the **α-Glucosidase enzyme** the inhibitory activity is not seen to be dose dependent i.e. it does not increase or decrease with increase in concentration of the drug. For compound THI-9 the results seem to be inconclusive and are not dose dependent. We could interpret from this the synthesized Thiazole derivatives have little or no effect on the functioning of the **α-Glucosidase**
enzyme and that their probable mechanism of action is through enhancing effective glucose utilization and by inhibiting the activity of **α-Amylase** enzyme.

Title Compound/ % inhibition by the compounds for *in vitro* activity	10µL	20 µL	30 µL	40 µL	50 µL
THI-6	71.32	66.94	64.55	71.55	78.91
THI-9	62.74	69.5	42.98	47.66	36.95
Std. Drug Acarbose	66.81	66.67	56.02	75.62	75.11

Table No 04: % Inhibition of Thiazole derivatives against α-Glucosidase

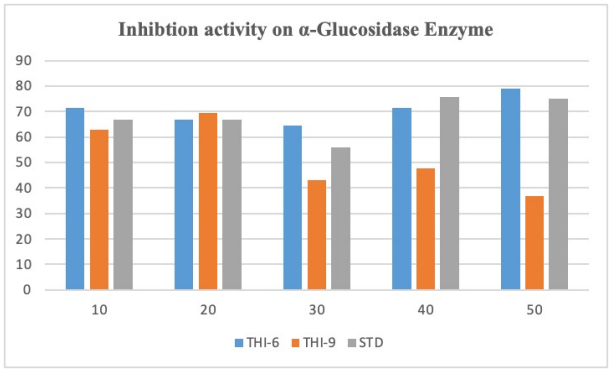


Fig No 04: Graph depicting % Inhibition of Thiazole derivatives against α-Glucosidase

Discussion:

The negative impact Diabetes Mellitus has on human life and its quality, the potential of the disease to debilitate the patient financially and the prevalence of this endocrine disorder globally provoked the research for newer and better anti-diabetic drugs with lesser adverse effects. Heterocyclic compounds fused heterocyclics, heterocyclics with various linkages have always been associated with several pharmacological activities. The titled thiazole derivatives which have a unique heterocyclic ring containing nitrogen and sulphur display varied pharmacological efficiency namely anti-convulsant, antibiotic, anti-inflammatory etc. and since they play a pivotal part of those pipeline drugs which are in process of exploration this present study of synthesizing novel Thiazole derivatives was initialized. The Thiazole scaffolds is an active biological moiety and structural modifications at position C-2, C-4 and C-5 has seen to improve enzyme inhibition activity and receptor binding. On correlating this to anti-diabetic activity the thiazole moiety have demonstrated DPP-4 inhibitory activity, improving insulin sensitivity via PPAR-γ activation or mere inhibition of activity of enzymes like α – Amylase or α-Glucosidase.

There are several mechanisms proposed through which anti-diabetic drugs can exert their pharmacological action. Similarly, there are several in vitro assays available for evaluating the ability of the newly synthesized Thiazole compounds as anti-diabetic agents. Namely Glucose Uptake Assay, α – Amylase, α-

Glucosidase, Aldolase reductase inhibitory assay, DPP-4 Inhibition Assay etc. Both the enzymes α-Amylase and α-Glucosidase play an imperative role in handling carbohydrate metabolism and further in regulating blood glucose levels. While α-Amylase is an enzyme which interacts at a very initial stage in carbohydrate metabolism as it is present in the saliva and is involved in breaking down complex carbohydrates like starch to simpler disaccharides like dextrin and maltose, α-Glucosidase which is present in the small intestine converts oligosaccharides and disaccharides to glucose thus it is involved in the final metabolism step of carbohydrates[24-30].

To obtain the title compounds substituted anilines were treated with halide chlorides to obtain a halide acetamide intermediate which then in presence of thiourea was converted to Thiazole derivatives. Ten such Thiaozle derivatives (THI-1 to THI-10) were synthesized and subjected to *in vitro* anti-diabetic studies.

The ten derivatives of Thiazoles were evaluated for their *in vitro* antidiabetic activity using three different models namely: Yeast Cell Method, the α-Amylase method and the α-Glucosidase method since these are simple, inexpensive, yet sensitive and physiologically relevant.

Yeast Cell Method has gained wide popularity as a method to screen the anti-diabetic potential of synthetic and natural compounds mainly for its simplicity and accuracy in predicting and helping in concluding on the efficacy of the drug/s as anti-diabetic agents. The anti-diabetic potential is evaluated depending on the ability of the derivatives to uptake glucose in the yeast cells. After a specific interval the amount of glucose remaining in the medium serves as an indicator about the capacity of the synthetic derivatives to facilitate diffusion of glucose across the yeast cell membrane and this effective facilitated transport of glucose is correlated to glucose removal from the blood plasma which in turn serves as a parameter for effective glucose utilization thereby controlling blood glucose levels. Amongst the ten synthesized derivatives of Thiazoles compounds THI-6 and THI-9 have shown exponential and steady increase in glucose utilization capacity. For the derivative THI-6 the glucose utilization capacity at 100 µL was found to be 5.12% and it is seen that with increase in concentration to 500 µL the utilization capacity increased to 53.84%. Similarly, for the derivative THI-9 the glucose utilization capacity at 100 µL was 18.75% which showed an exponential growth to 81.25% at 500 µL. Thus amongst the derivatives, the derivative THI-9 has shown maximum glucose utilization capacity even better than the std. drug Acarbose which showed 45% glucose uptake capacity at 500 µL. At the same time the IC_{50} values of these two compounds and the std drug was evaluated and among the two Thiazole derivatives THI9 has shown lower and better IC_{50} value of 258.8 than as compared to THI6 as well as std. drug Acarbose which was found to be 361.7 and 489.8 respectively[31-34].

The ten Thiazole derivatives were then also subjected to study their inhibitory activity against α-Amylase enzyme which is responsible for breaking the glycosidic bonds in starch and glycogen to convert them into glucose and maltose. Amongst the ten Thiazole derivatives synthesized, derivatives THI6 and THI-9 have shown promising inhibitory action against α-Amylase enzyme. By this we can interpret that these three compounds namely: THI6 and THI-9 slow down the process of carbohydrate digestion, helps in controlling sudden spike in post prandial glucose level and improves glycaemic control. Among these two Thiazole derivatives THI-6 has shown 44.8% inhibitory activity at 100 µL while at the same concentration THI-9 has shown 52.36% inhibitory activity. With increase in concentration both the compounds have shown exponential increase in α-Amylase enzyme inhibitory activity. THI-6 at 500 µL concentration has shown 52.36% inhibition while THI-9 has shown 84.58% inhibition. Their IC_{50} values were also calculated and it was found that IC_{50} value of THI-6 is more promising than IC_{50} value of THI-9. On comparing the inhibitory activity and IC_{50} values of these compounds to that of std drug Acarbose it was seen that both the drugs are at par with the std drug in terms of percentage inhibition as well as IC_{50} values. Thus showing potentially strong anti-diabetic activity similar to std. drugs like Acarbose and Voglibose[35-37].

Amongst the ten Thiazole derivatives synthesized since three compounds namely: THI-6 and THI9 showed promising activity in Yeast Cell method as well as the α-Amylase enzyme inhibitory method only these three compounds were studied for their potential to inhibit the α-Glucosidase enzyme activity. The α-Glucosidase enzyme is present at the brush border of the small intestine and is responsible for the final step of carbohydrate digestion. If a synthetic compound decreases or inhibits the activity of the enzyme it is delaying glucose formation, absorption and controlling a spike in post prandial glucose levels. On studying the

compounds THI-6 and THI9 for their inhibitory activity against α-Glucosidase enzyme it was observed that compound THI-6 has shown 71.32% inhibition at 10µL and on increasing the concentration of the drug to 50 µL the inhibitory effect was constant and stable at 78.91%. However, the compound THI-9 has not displayed a constant or exponential rise in the inhibitory activity on increasing the dose. Thus demonstrating that these three compounds although have an inhibitory action against α-Amylase enzyme activity they are not effective against inhibiting the activity of α-Glucosidase enzyme[38-40].

Conclusion:

In conclusion we may say that on determining the melting points, Rf value and IR of the ten synthesized derivatives of Thiazole the reactions were completed and the desired products were formed. Further on subjecting these ten derivatives for their *in vitro* anti diabetic activity by Yeast Cell method and α-Amylase method amongst the ten derivatives of Thiazole synthesized THI6 and THI9 have shown promising activity depicting that the probable mechanism through which the compounds are demonstrating *in vitro* anti-diabetic activity is by preventing the uptake of glucose as seen in the Yeast Cell method and simultaneously they have also shown inhibitory activity against the α-Amylase enzyme which is consistent and exponential with increase in concentration of the drug. However, these compounds when subjected to analysing their *in vitro* inhibitory activity against α-Glucosidase enzyme the results are not promising and consistent both at lower as well as higher concentrations. Thus we may conclude that the ten synthesized derivatives of Thiazole have shown anti-diabetic activity, among them THI6 and THI9 have shown more promising activity in Yeast Cell method and α-Amylase method indicating promising anti diabetic potential through these mechanisms but they have not shown positive and promising activity on α-Glucosidase enzyme thus demonstrating limited inhibitory action against α-Glucosidase. Further subjecting these three compounds to *in vivo* studies using different animal models pertaining to anti-diabetic activity could lead us to a newer series of anti-diabetic agents which are more potent, less cumbersome, economical and affordable than the existing anti-diabetic agents.

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ACKNOWLEDGEMENT: The authors are thankful to The Principal KLE College of Pharmacy for providing research facility for the mentioned research work.

CONFLICT-OF-INTEREST:
The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS:
All authors have accepted responsibility for the manuscript's content and consented to its submission. They have thoroughly reviewed all results and unanimously approved the final version of the manuscript.

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