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Novel 1,3-Thiazine derivatives bearing schiff base moiety as potential antidiabetic and antiproliferative agents.

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Abstract

Heterocyclic chemistry comprises at least half of all organic chemistry research worldwide. The large number of biologically active moleules that contain heterocyclic rings have play an important roles in the drug discovery process and exhibit various biological activities.Conventional antidiabetic(insulin, thiazolidinediones , sulfonylureas, metformin etc,) and anticancer drugsare available in the market but these sufferfrom one or the other side effects. Hence, it becomes essential to search for novel antidiabetic and anticancer agents in order to develop new improved antidiabetic and anticancer drugs having lesser side effects as compared to the other antidiabetic drugs.A series of novel compounds of 1,3- thiazine derivatives namely , CMT1 CMT2, CCT were synthesized and characterized by ^{1}H and ^{13}C NMR. These compounds were screened for their invitroantidiabetic and antiproliferative activities. The obtained data indicated that the tested compounds exhibited both antidiabetic and antiproliferative activities particularly at higher concentrations. Thepercentage of inhibitions were found to be in the order CCT >CMT2 >CMT1 for antidiabetie activity and CCT>CMT1> CMT2 for antiproliferative activity.

Key words: *1, 3-thiazine, Antidiabetic, Antiproliferative agents*

1. Introduction

Medicinal or pharmaceutical chemistry is a discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Thiazines are heterocyclic compounds having four carbon atoms, one nitrogen, one sulphur atom at variouspositions in the six member ring and exist as 1,2; 1,3 and 1,4 isomers [1-3]. However their derivatives having N-C-Slinkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety ofbiological activities.

2 .Materials and Methods

Chemicals were procured from E. Merck (India), S. D. Fine Chemicals (India) and reagent/solvents were used without distillation procedure. Melting points were taken in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Perkin-Elmer 157 infrared spectrometer (ν in cm–1) and NMR spectra were recorded on a Bruker spectrometer DPX-300MHz (Bruker, Germany) by using $CDCl_3$ as solvent with TMS as an internal standard. All the spectral data are consistent with the assigned structures of the desired product and the progress of the reactions was monitored on silica gel G plates using iodine vapour as visualizing agent.

2.1. General procedure for preparation of schiff bases (3a-c):

A mixture of p-NH_2-acetophenone (0.01mole) and substituted benzaldehyde (0.01mole) were taken in pestle and morter with catalytic amount of acetic acid. The mixture was grinded continuously for 10-15 min at room temperature. The progress of the reaction was monitored by using TLC-technique. After completion of the reaction indicated by TLC, the mixture was poured in crushed ice and acidified with dilute NH_4OH if needed. The solid separated was filtered and recrystalyzed from ethanol.

2.2. General procedure for preparation of chalcones (4a-c):

A mixture of schiff base (0.01mole) and p-chlorobenzaldehyde (0.01mole) were stirred in ethanol for 2-3 hour with aqueous NaOH in distilled

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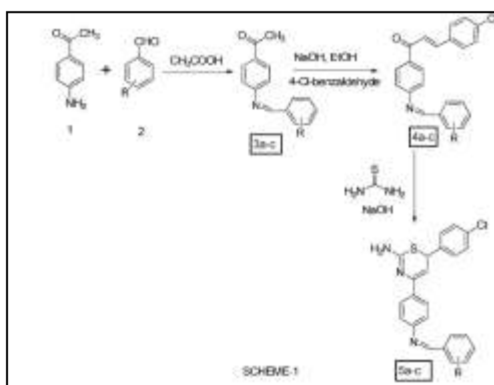
ethanol (20 mL). The progress of the reaction was monitored by using TLC-technique. After completion of the reaction, the mixture was poured in ice cold water, solid formed was filtered off, dried and recrystalyzed from ethanol.

2.3. General procedure for preparation of 1, 3-thiazine derivatives (5a-c):

The mixture of chalcone (0.01mole) and thiourea (0.01mole) was refluxed in ethanol with catalytic amount of NaOH by using round bottom flask. The reaction was monitored by TLC and after completion of reaction, the content were cooled to room temperature and poured into beaker containing crushed ice, the solid obtained was filtered, washed with water and finally recrystalyzed from ethanol.



2.4 *In vitro* antidiabetic activity

In vitro α-amylase inhibition study

In vitro α-amylase inhibition assay was carried out by the method of Apostolidis (Apostolidis et al, 2007; Kwon YI et al, 2007).

Various concentrations of the CMT1, CMT2 and CCT were prepared i.e; 10 µg/ml, 20 µg/ml, 30 µg/ml, 40µg/ml & 50 µg/ml using phosphate buffer (pH 6.9). 500 µl of CMT ,CMT2 and CCTto separate test tube and 500 µl of 20 % phosphate buffer pH 6.9, containing α-amylase at a concentration of 0.5 mg/ml were incubated at 25°C for 10 min. After preincubation, 500µl of 0.5% starch solution in 20 % phosphate buffer, pH 6.9, was added. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1000µl of 96 % 3, 5-dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and cooled to room temp. Absorbance (A) was measured at 540 nm. A carbose was used as positive control and the inhibitory activity of α-amylase and percent of inhibition was calculated as follows:

% of inhibition = (Control O.D. – Test O.D.) / Control O.D.) × 100

Control incubations represent 100% enzyme activity and were conducted in a similar way by replacing sample. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. Separate incubation carried out for reaction t = 0 was performed by adding samples to DNS solution immediately after addition of the enzyme. Each experiment was done in triplicate. IC_{50} value was calculated by using regression analysis.

2.5 *In vitro* antiproliferative activity

Evaluation of the antiproliferative activity of CMT1, CMT2 and CCTwere done by yeast *Saccharomyces cerevisiae*model according to Shwetha *et al.* (Shweta S, Khadabadi S, Ganesh TG et al 2012.)

Yeast inoculums preparation

The yeast was inoculated with sterilized PDB (Potatoes Dextrose Broth) and incubated at 37° C for 24 h and it was referred as seed broth.

Determination of cell viability

The cell viability assay was performed with 2.5 ml of PDB and 0.5 ml of yeast inoculums in five separate test tubes. In the first test tube distilled water, in second test tube quercetin (Sigma Aldrich) as standard (1mg/ml), in third, fourth and fifth test tubes CMT1, CMT2 and CCT (10mg/ml respectively) were added. All tubes were incubated at 37° C for 24h. In the above cell suspension, few drops of 0.1% methylene blue dye was added in all tubes and they were observed under low power microscope. The number of viable cells, those that do not stain and look transparent with oval shape while dead cells get stained and appeared blue in color, were counted in 16 chambers of hemocytometer and the average number of cells was calculated. Each experiment was done in triplicate. The percentage of cell viability was calculated using the formula (Subhadradevi V, Jagannath P et al. 2011).

% of Cytotoxicitycalculated by

$$\frac{\text{No.of dead cells}}{\text{No. of viable cell + No. of dead cells}} \times 100$$

3. Results and discussion

The schiff bases **3a-c**were synthesized by using condensation reaction between p-NH_2-acetophenone andsubstituted benzaldehyde in the presence of catalytic amount of acetic acid at room temperature by grindingtechnique. The schiff base obtained from the above step was allowed to react

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with p-Cl-benzaldehyde in ethanolwith aqueous NaOH produced chalcones**4a-c**. The target product 1, 3-thiazine derivatives **5a-c**were obtained by thecyclyzation reaction between chalcones and thiourea with catalytic amount of NaOH in ethanol medium underreflux condition for 3-4 hours **(Scheme-1).**All the compounds were characterized by using UV-Vis, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Table 1: Physical data of synthesized compound 5a-c

Code	R	Yield	M.Pt	R_f Value
5a	p-cl	85	160-162	0.65
5b	p-Ome	85	132-134	0.75
5c	p-Me	90	173-175	o.75

3.1 Antidiabetic activity of CMT1, CMT2 and CCT

α-Amylase inhibitory activity

The *in vitro*antidiabetic activity of the CMT1, CMT2 and CCTwere investigated through α-Amylase inhibitory activity. The inhibitory activities of CMT1, CMT2 and CCT reported in Table 1. CMT1, CMT2 and CCT were comparable with standard antidiabetic drug viz. Acarbose. All the compounds showed inhibitory effect on α-amylase with varying degrees of inhibition. The maximum inhibition was seen with the standard drug acarbose. Among the various doses (10 µg/ml, 20 µg/ml, 30 µg/ml, 40µg/ml & 50 µg/ml) of CMT1, CMT2 and CCT, the highest dose (500 µg/ml) of all CMT1, CMT2 and CCT has greatest inhibition activity.

Table 2: Effect of CMT1, CMT2 and CCT on α Amylase activity

Concentrations (µg/ml)	CMT1	CMT2	CCT	Std Acarbose
10	23.14± 0.09	17.37±0. 046	12.92± 0.06	22.45 ± 1.57
20	30.43± 0.11	27.60±0. 10	21.11± 0.06	39.61 ± 2.74
30	30.43± 0.10	38.30±0. 06	35.10± 0.03	65.74 ±4.67
40	35.17± 0.22	61.04±0. 15	53.83± 0.03	78.31 ± 5.48
50	39.10± 0.10	76.48±1. 45	93.35± 0.03	92.84 ± 6.49
IC_{50}	35.12	33.86	37.94	27.09

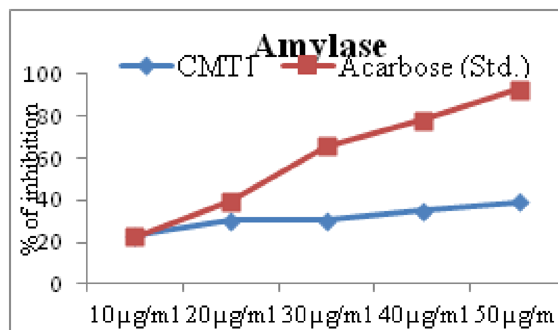

Fig 1:Effect of CMT1on inhibition of α Amylase activity

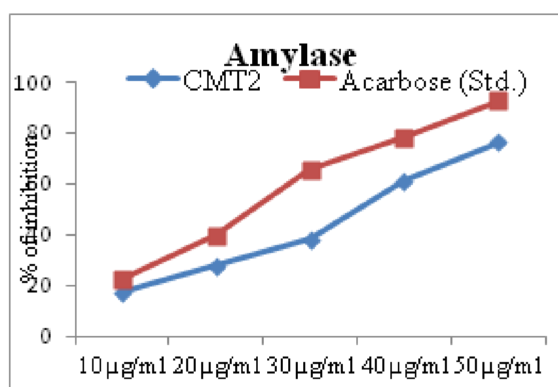

Fig 2: Effect of CMT2 on inhibition of Amylase activity

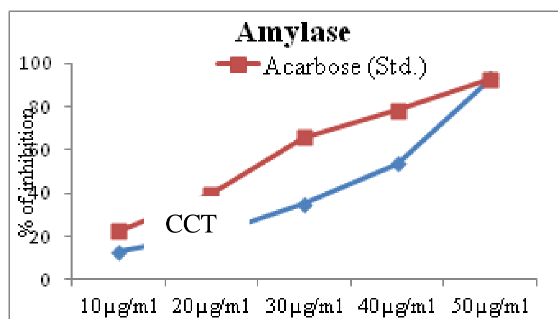

Fig 3: Effect of CCT on inhibition of α Amylase activity

α-amylase is one of the main enzymes in human body that is responsible for the breakdown of starch to more simple sugars.

α-Amylases hydrolyze complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by α -glycosidase to monosaccharide which are absorbed through the small intestines into the hepatic portal vein and increase postprandial glucose levels (Ranilla et al., 2010, El-Kaissi et al., 2010.) Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have

beneficial effects on insulin resistance and glycemic index control in people with diabetes (Barret and Udaniet al 2011 ,Tundis et al., Tundis R, Loizzo MR, Menichini F, **2010**,Nair et al., Tundis R, Loizzo MR, Menichini F. , **2010**.)

. In our investigation we found that CMT1, CMT2 and CCT moderately inhibited α-amylase. The inhibition was in the following order: CMT2< CMT1< CCT.

3.2 Antiproliferative activity of CMT1, CMT2 and CCT

Table 3: % Cell viability of CMT1, CMT2 and CCT using the *Saccharomycescerevisiae* yeast model assay

Concentrations (µg/ml)	CMT1	CMT2	CCT	Std. ml)
10	79.13	87.37	74.54	95.32
20	65.57	74.57	63.93	82.14
30	51.16	60.07	45.74	67.87
40	35.00	34.65	34.34	41.35
50	20.58	20.85	17.89	22.65

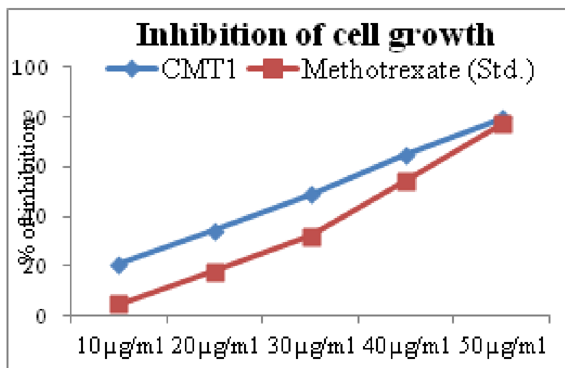

Fig 4 . Anti proliferative activity of CMT1

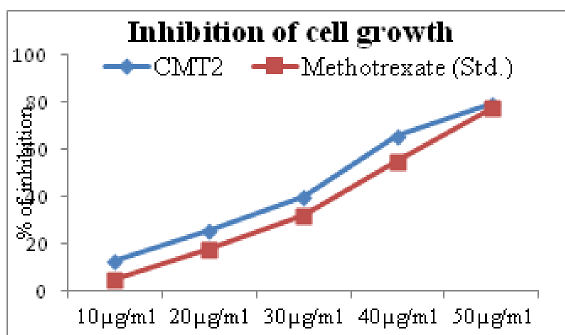

Fig 5. Anti proliferative activity of CMT2

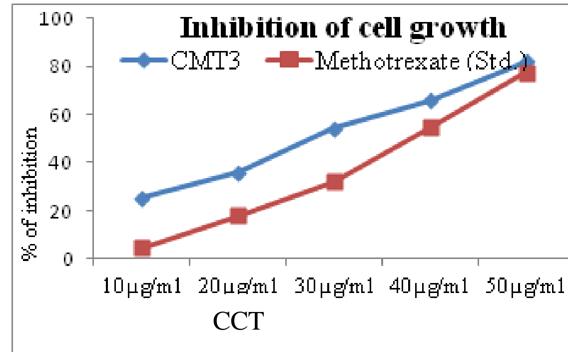

Fig 6. Anti proliferative activity of CMT2

Saccharomycescerevisiae is one of species of yeast in the Saccharomycetaceae family .This yeast possibly is the best intensively studied eukaryote organism that is referred to as an ideal model organism since its gens are significantly conserved during the evolution. *Saccharomycescerevisiae* yeast is a nonpathogenic organism that is used as a model for pathogenic yeasts. This organism is used to identify some compounds with antiproliferative characteristic and also to study the performance mechanism of these compounds.

4. Conclusion

Synthesized a series of new 1, 3,-thiazine derivatives obtained with good yield. All the compounds were characterized by using UV-Vis, IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

Among the three compounds, CMT1has grater activity than CMT2 and CCT. The inhibition was in the following order: CMT2> CMT1> CCT. Thus the result obtained suggests CMT1, CMT2 and CCTare bioactive compounds which inhibit the activity of α-amylase enzyme therapy can be used as an oral hypoglycemic agent to control diabetic. This study justifies the hypoglycemic activity of CMT1, CMT2 and CCT can be used in the management of diabetes.

In the case of invitroantiproliferative study, CMT2 has grater activity than CMT1 and CCT. The cytotoxicity was in the following order: CMT2> CMT1> CCT. Regarding the results of this study it can be demonstrated that CMT1, CMT2 and CMT3 has the antiproliferative effect on the *saccharomycescerevisiae*. In order to validate the antiproliferative property of CMT1, CMT2 and CMT3, it is recommended to study the effectiveness of the substance and possible molecular mechanism of this compound performance for functional usages in the treatment of cancer.

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