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EVALUATE THE *IN VITRO* ANTI-INFLAMMATORY ACTIVITY OF SYNTHETIC 1, 3 THIAZINE DERIVATIVES

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ABSTRACT

The large number of biologically active moleules that contain heterocyclic rings have play important roles in the drug discovery process and exhibit various biological activities. A series of novel compounds of 1,3- thiazine derivatives namely , CMT1 CMT2, CCT were synthesized and characterized by ^{1}HNMR and ^{13}C NMR. Thiazines are heterocyclic compounds having four carbon atoms, one nitrogen, one sulphur atom at various positions in the six member ring and exist as 1,2; 1,3 and 1,4 isomers [1-3]. However their derivatives having N-C-S linkage have been used in the fields of medicinal and pharmaceutical chemistry and reported to exhibit a variety of biological activities. Anti-inflammatory activity of CMT1, CMT2 and CCT were dose dependent manner. The increases in the concentrations are directly proportional to the activity. Anti-inflammatory activity was nearest to the standard diclofenac sodium were observed.

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1. INTRODUCTION

A number of synthetic products are used in the allopathic medical systems in many countries. Allopathic medicine for treatment of various diseases is getting more popular. Making synthetic compounds provide immediate relief of symptoms comparable to that obtained from ayurdedic medicines. The majority of clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutic for treatment of various inflammatory diseases. Though these drugs have potent activity and therefore, agents of synthetic origin with very little side effects are required as substitute of chemical therapeutics. The present investigations describe about the synthesis, characterization and antioxidant activity of novel 1,3-thiazinederivatives. These compounds are synthesized by performing reaction between thiourea and various chalcones derived by claisen-schimidt reaction between various schiff bases of p-NH_2-acetophenone and p-Cl-benzaldehyde.

MATERIALS AND METHODS:

In order to evaluate the *in vitro* anti-inflammatory activity of CMT1, CMT2 and CCT, the following aspects were analyzed.

- To synthesize the 1, 3-thiazine derivatives.
- To investigate anti-inflammatory activity by Inhibition of albumin denaturation method
- To investigate anti-inflammatory activity by Inhibition of Bovine serum albumin denaturation

General: 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 CDCl3 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.

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

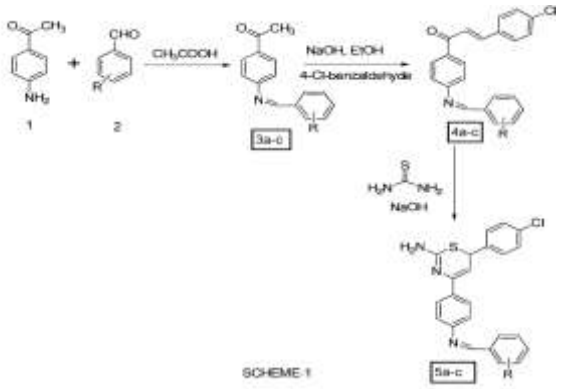
A mixture of 4-NH_2-acetophenone (0.01mole) and substituted benzaldehyde (0.01mole) were taken in pestle and morter with catalytic amount of acetic acid. The mixture was grinded continuously for 10-15 min at room temperature. The progress of the reaction was monitored by using TLC-technique. After completion 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. 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 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.

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.



SCHEME 1

IN-VITRO ANTI-INFLAMMATORY ACTIVITY

Inhibition of albumin denaturation:

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*. (2012)

Reagent

1. Egg albumin
2. Phosphate Buffer (pH 6.4)
3. Diclofenac sodium as standard

Procedure

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of CMT1, CMT2 and CCT (100, 200, 300, 400 and 500 µg/ mL respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in a incubator for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100-500µg/ ml) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 x (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Inhibition of Bovine serum albumin denaturation:

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra *et al*. (2012)

Reagent

1. Bovine albumin
2. Phosphate Buffer (pH 6.4)
3. Diclofenac sodium as standard

Procedure

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of CMT1, CMT2 and CCT (100, 200, 300, 400 and 500 µg/ ml respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in a incubator for 15 min and then heated at 70

°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100-500µg/ ml) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 x (Vt / Vc - 1)

Where, Vt = absorbance of test sample, Vc = absorbance of control.

RESULTS

The schiff bases 3a-c were synthesized by using condensation reaction between p-NH2-acetophenone and substituted benzaldehyde in the presence of catalytic amount of acetic acid at room temperature by grinding technique. The schiff base obtained from the above step was allowed to react with p-Cl-benzaldehyde in ethanol with aqueous NaOH produced chalcones 4a-c. The target product 1, 3-thiazine derivatives 5a-c were obtained by the cyclyzation reaction between chalcones and thiourea with catalytic amount of NaOH in ethanol medium under reflux condition for 3-4 hours (Scheme-1).

IN VITRO ANTI-INFLAMMATORY ACTIVITY

The organic compounds obtained by chemical synthesis as model compounds have useful anti-inflammatory and antimicrobial activities. There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationable for their use when other suitable methods are available or could be investigated. Hence, in the present study the protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory property of CMT1, CMT2 and CCT. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain inflammatory diseases may be due to *in vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al*., 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein (Egg & Bovine albumin) denaturation and reference diclofenac sodium (Jagtap *et al.*, 2011). The anti-inflammatory activity of CMT1, CMT2 and CCT represented in table 1and 2. Anti-inflammatory activity of CMT1, CMT2 and CCT were dose dependent manner. The increase in the concentrations are directly proportional to the activity. Anti-inflammatory activity was nearest to the standard diclofenac sodium were observed.

CONCLUSION

In the present study, the anti- inflammatory activity of CMT1, CMT2, CCT. Were investigated. The following conclusion obtained from the study Anti-inflammatory activity of CMT1, CMT2 and CCT were dose dependent manner. The increase the concentrations are directly proportional to the activity. Highest concentrations of CMT1, CMT2 and CCT activities were nearest to the standard as Diclofenac sodium. The results of this study, it clearly indicates that 1, 3-thiazine derivatives had powerful *in vitro* anti-inflammatory activity.

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

Table 2: *In vitro* anti-inflammatory activity of CMT1, CMT2 CCT and standard (Egg albumin)

Doses (µg/ml)	CMT1	CMT2	CCT	Standard (Diclofenac sodium)
100	18.52±1.29	16.95±1.18	20.15±1.41	21.37±1.98
200	27.76±1.94	23.12±1.61	27.61±1.93	36.45±2.37
300	51.65±3.61	50.78±3.55	52.48±3.67	55.94±3.47
400	59.9±4.19	75.89±5.31	77.36±5.41	79.45±4.65
500	62.49±4.37	86.12±6.02	91.24±6.38	93.45±6.84

Values are expressed as Mean ± SD for triplicates

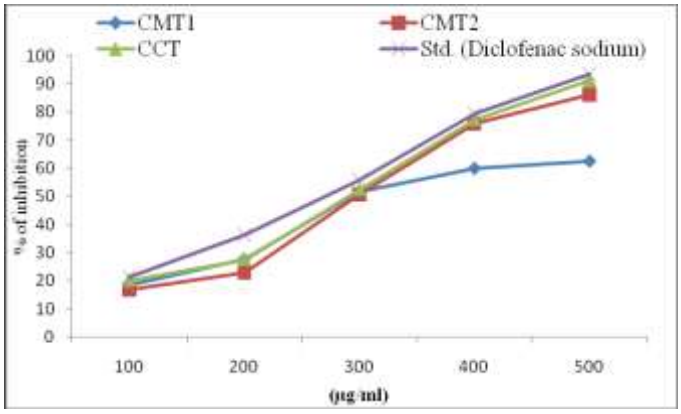


Fig 1 *In vitro* anti-inflammatory activity of CMT1, CMT2, CCT and standard

Table 3 *In vitro* anti-inflammatory activity of CMT1, CMT2, CCT and standard (Bovine serum albumin)

Doses (µg/ml)	CMT1	CMT2	CCT	Standard (Diclofenac sodium)
100	15.70±1.09	13.23±0.92	17.52±1.22	19.20±1.56
200	28.75±2.01	26.16±1.83	30.08±2.10	32.75±2.14
300	46.56±3.25	42.02±2.94	46.39±3.24	51.25±2.95
400	64.23±4.49	60.11±4.20	68.54±4.79	75.42±4.44
500	83.77±5.86	79.06±5.53	87.16±6.10	90.68±6.11

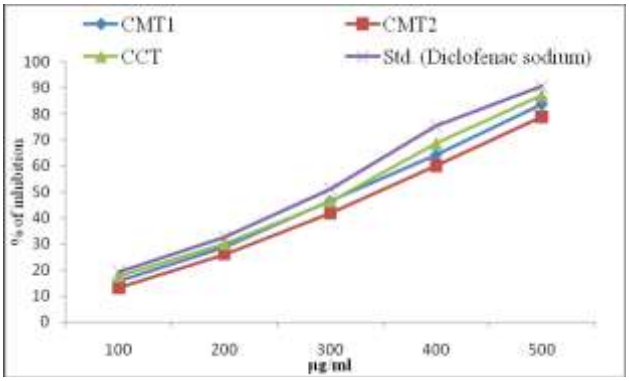


Fig 2 *In vitro* anti-inflammatory activity of CMT1, CMT2, CCT and standard

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