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SYNTHESIS AND EVALUATION OF NOVEL THIAZOLIDINEDIONES FOR ANTI INFLAMMATORY ACTIVITY

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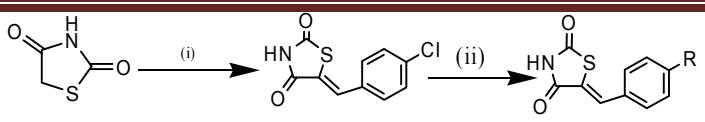
ABSTRACT
The aim of our study was to review on synthesis and various pharmacological activities associated with thaizolidinediones. Thiazolidinediones (TZDs) or glitazones forms a significant class of drugs which exhibit an array of biological activities ranging from anti diabetic, anti-inflammatory, antibacterial, antifungal, antiviral and anticancer. 1, 3-Thiazolidine-2, 4-dione contains basic skeleton of thiazole or thiazolidine. Presence of one carbonyl group in thiazole at 4th position makes it thiazolidine-4-one which is known for various activities, such as anti-inflammatory and presence of another carbonyl group at 2nd position makes it thiazolidine-2, 4-dione which is basically known for its antidiabetic activity. Various studies report the effects of TZDs on the metabolism of lipids, on cell differentiation and on some cardiovascular risk factors. Success of thiazolidinediones (TZDs), the ligand of peroxisome proliferator-activated receptor γ (PPARγ) has generated a lot of interest for new drug discovery. In the present study we were reporting a systematic synthesis of thiazolidinediones analogues. The basic ring of thiazolidinedione was prepared by 1, 3 dipolar cycloaddition reaction using thiourea and monochloroaceticacid. Knoevenagel condensation with substituted aromatic aldehydes was carried out to yield various substituted benzylidenethiazolidine-2, 4-dione. These were subjected to further alkylation and/or substitution reaction to yield our targets compound as described in scheme **1.** The synthesized molecules were screened for bovine serum denaturation (in vitro). Characterization was done by using various physicochemical and spectral techniques. All molecules were weakly active in bovine serum denaturation assay.
Keywords: Anti inflammatory**,** Protein denaturation, Thiazolidinediones, PPARγ

INTRODUCTION

Inflammation is a basic way in which the body reacts to infection, irritation or other injury, the key feature being redness, warmth, swelling and pain. In the early 1990s, it was discovered that Cyclooxygenase is the key enzyme for biosynthesis of prostaglandins, which catalyses the conversion of arachidonic acid to prostaglandins and thromboxanes. Cyclooxygenase enzymes exists as 2 isoforms i.e. COX-1 and COX-2. COX-1 produced in many tissues such as the kidney and the GIT, while COX-2 is inducible and is expressed during inflammation at a site of injury[1-3]. Heterocycles are important components of biomolecules such as proteins, DNA, RNA and vitamins and also found in cell lining. Among the heterocyclic compounds, five membered heterocyclic moieties fused with aromatic ring systems containing various heteroatoms such as N, S, and O, exhibited wide spectrum of pharmacological activities. Many thiazolidinedione and their derivatives serve as basic pharmacophore for various biological profiles i.e. antidiabetic, anticancer[4], antimalarial[5] and anti-inflammatory[6] and so on. These observations promoted us to synthesise a new series of thiazolidinedione with higher biological activity. We report here in the synthesis and evaluation of anti-inflammatory activity of some novel structure hybrids. The structures of the various synthesized compounds were assigned on the basis of elemental analysis, IR and ^{1}H NMR spectral data. These compounds were also screened for their bovine serum denaturation (*in vitro*).

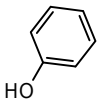
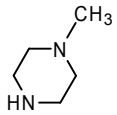
MATERIALS AND METHODS

The chemicals used in the present project work were purchased from Rankem, Merck and Spectrochem. The melting point of the synthesised compound was determined by open capillary with Thiel's melting point tube (capillary tube method) or Thermonik melting point apparatus and was uncorrected. TLC plates were prepared by using Merck Silica Gel 60 GF 254. Visualization was done in UV light chamber at 254 nm, iodine chamber and visualizing agent (Ninhydrin, 2, 4 DNP reagents). The IR spectra of the synthesized compounds were recorded on a Fourier Transform Infra Red spectrometer (model Shimadzu 8400 S) in the range of 400-4000 cm^{-1} as KBr pellets. (^{1}H NMR) data of the compound was carried out in Bruker 200 spectrospin NMR at Astra Zeneca Pharma India Limited, Bangalore and Bruker 400 spectrospin NMR at Indian Institute of Science, Bangalore. The solvent used for NMR was $CDCl_3$.



(I) **(II)** **(IIIa-e)**

Reactants

R= H	**III a**	
	**III b**	Phenol
	**III c**	Morpholine
	**III d**	4-methylpiperazine
	**III e**	Aniline

Scheme I

Scheme 1. Reagents and conditions: (i) 4-chlorobenzaldehyde, benzoicacid , piperidine, toluene, stirred at 80 ˚C, 16 - 20 h. (ii) N-methyl Pyrrolidone, CuI, Anhydrous K_2CO_3, stirred, reflux.

General method of synthesis of thiazolidine-2, 4-dione (I): By 1, 3 dipolar cyclo addition as reported procedure.[7]

Preparation of 5-(4-chlorobenzylidene) thiazolidine-2,4-dione (IIa-b):

Knoevenagel condensation with p-chloro benzaldehyde using weakly basic amine as a catalyst (piperidine) and toluene as a solvent. [7]

General procedure for the preparation of compounds (IIIa-e) by replacement of chlorine

In a 100 ml of round bottom flask was placed 5-(4-chlorobenzylidene) thiazolidine-2, 4-dione (0.250 g, 0.000975 mol, and 1 eq.), anhydrous potassium carbonate (0.271 g, 0.00195 mol, and 3 eq.) and N-Methyl pyrrolidone in (10 ml) was placed and stirred at room temperature for 30 min. To this were added phenol or amine (1.5 eq.) and a small pinch of cuprous iodide and heated to 160 ˚C for 16 - 24 hrs with stirring. The reaction mixture cooled to room temperature and poured into 100 ml of water and filtered. The filtrate was washed with ammonium chloride and brine followed by extraction with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and solvent removed under vacuum to get the desired product. The completion of the reaction was checked by TLC using used (4:1 and 2:1) ethylacetate: hexane as mobile phase.

***IN VITRO* SCREENING FOR PROTEIN DENATURATION**

Denaturation of protein assay by bovine serum albumin

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutically important in the treatment of rheumatoid arthritis and in various types of inflammatory conditions. But they are found to be of limited use in treatment of patients with chronic inflammation because of their frequently observed gastrointestinal side effects.[8]

Many in vitro assays, each based on a specific biochemical or cellular mechanism have been developed for the initial screening of the anti-inflammatory compounds. Denaturation of proteins is one of the causes for inflammation which is well documented. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins. In vitro test which causes denaturation of proteins may be used as a preliminary screening for anti-inflammatory activity.

B) Preparation of Phosphate Buffer (pH 7.4)

Place 50 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask. To this add 39.1 ml of 0.2 M sodium hydroxide solution. Make up the volume using distilled water.

Materials: Bovine serum albumin (Loba Chem)

Drug: Ibuprofen

Procedure

1. The compound was dissolved in minimum amount of Dimethylformamide (DMF) & diluted with phosphate buffer (0.2 M, pH 7.4).
2. Final concentration of DMF in all solution was 2%, test solution (1 ml) containing different concentration of drug was mixed with 1 ml of 1%mM bovine albumin serum in phosphate buffer & incubated at 27±1°C for 15 minutes.
3. Keeping the reaction mixture at 60 ± 1 °C in water bath for 10 minutes induce denaturation.
4. After cooling the turbidity was measured at 660 nm.
5. The % of inhibition of denaturation was calculated from control, where no drug was added.
6. Each experiment was done in triplicate & average is taken.
7. Percentage inhibition of denaturation was calculated from the formula :

$$\text{Inhibition (\%)} = \frac{\text{Abs. of control- Abs. of sample}}{\text{Abs. of control}}$$

8. All compound tested at 0.2 mm concentration.[9]

RESULTS AND DISCUSSION

The synthesis of parent ring was done by 1, 3 cycloaddition of thiourea and monochloroaceticacid using H_2O and Conc. HCl as a solvent. It was proved by comparing observed m.p with literature m.p. ^{1}H NMR spectra which clearly show two singlets at 4.31 and 11.98 indicating the presence of $–CH_2$ and –NH. Further Knoevenagel condensation reaction with p-chloro benzaldehyde leads to structure **II** . The formation of compounds were confirmed by visualizing agents (2, 4 DNP, Phosphomolybdicacid) and IR. The chlorine was replaced by various phenols and amides using anhydrous potassium carbonate, N-Methyl pyrrolidone and catalytic amount of cuprous iodide to obtain compounds **IIIa-e**. Physical and spectroscopical data described in Table **2-3** and Figure **1-2.**

CONCLUSION

The purpose of the present work was to synthesize, characterize and evaluate the biological activity of substituted thiazolidinedione derivatives. The compounds were studied for antiinflammatory activity. The activity was not favourable when compared to standard.

ACKNOWLEDGEMENT

I take this opportunity with much pleasure to thank the AstraZeneca Pharma India Ltd., Bangalore and Indian Institute of Sciences, Bangalore for providing me the ^{1}H-NMR spectra of the synthesized compounds.

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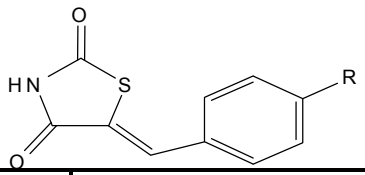
Table 1: List of synthesized compounds



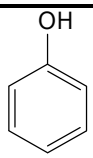
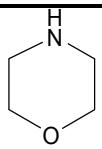
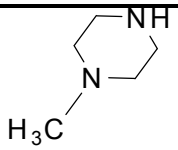
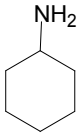
Comp. Code	R	Molecular Formula	Chemical Name
III b		$C_{16}H_{11}NO_3S$	5-(4-Phenoxy-benzylidene)-thiazolidine-2,4-dione
IIIc		$C_{14}H_{14}N_2O_3S$	5-(4-Morpholin-4-yl-benzylidene)-thiazolidine-2,4-dione
IIId		$C_{15}H_{17}N_3O_2S$	5-[4-(4-Methyl-piperazin-1-yl)-benzylidene]-thiazolidine-2,4-dione
IIIe		$C_{16}H_{12}N_2O_2S$	5-(4-Phenylamino-benzylidene)-thiazolidine-2,4-dione

Table 2: Physical properties of synthesized compounds

1.	**IIIb**	$C_{16}H_{11}NO_3S$	297.05	46.7 %	liquid	0.57	n-Hex : EA 4 : 1
2.	**IIIc**	$C_{15}H_{17}N_3O_2S$	290.34	53.2%	semisolid	0.69	n-Hex : EA 4 : 1
3.	**IIId**	$C_{15}H_{17}N_3O_2S$	303.38	55.7 %	112	0.81	n-Hex : EA 2 : 1
4.	**IIIe**	$C_{16}H_{12}N_2O_2S$	296.06	61 %	136	0.72	n-Hex : EA 2 : 1

Table 3: Elemental analysis and spectral data of synthesized compounds

III b	C= 64.63, H= 3.73, N= 4.71, O= 16.14, S= 10.78	3147.93 (N-H str.), 3051.49 (aromatic C-H str.), 1767.21, 1722.49 (C=O str.), 1219.05, (C-O str.).
IIIc	C= 57.92, H= 4.86, N= 9.65, O= 16.53, S= 11.04	3375.54 (N-H str.), 2956.97 (aliphatic C-H str.), 1732.13, 1664.62 (C=O str.), 1224.34 (C-O str.).
IIId	C= 59.38, H= 5.65, N= 13.85, O= 10.55, S= 10.5	3171.05 (N-H str.), 2942.56 (aliphatic C-H str.), 1770.72, 1703.20 (C=O str.).

Sl.no	**Comp.code**	**NMR In (DMSO-d6) (δ) values in ppm from TMS**
Fig 2	**I**	4.130 (s, 2H,-S-CH_2-C=O) 11.98(1s, 1H, -NH-).

Table 4: Bovine Serum Albumin Denaturation Assay

Sl.No	**Compound Code**	**IC $_{50}$ value µM**
1	24 a	>500 µM
2	24 b	>500 µM
3	24 c	>500 µM
4	24 d	>500 µM
5	STD	300 µM

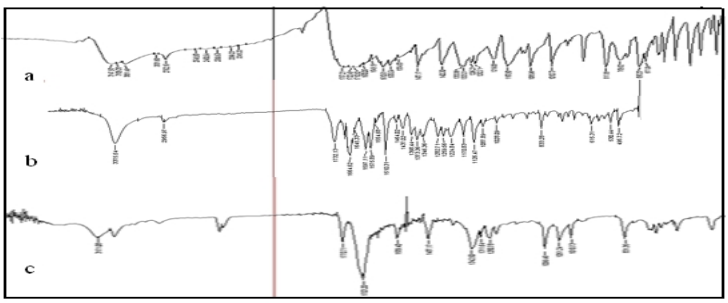


Figure no 1

a. FTIR Spectra of 5-(4-phenoxybenzylidene) thiazolidine-2, 4-dione.
b. FTIR Spectra of 5-(4-morpholinobenzylidene) thiazolidine-2, 4-dione.
c. FTIR Spectra of 5-(4-(4-methylpiperazin-1-yl) benzylidene) thiazolidine-2, 4-dione.

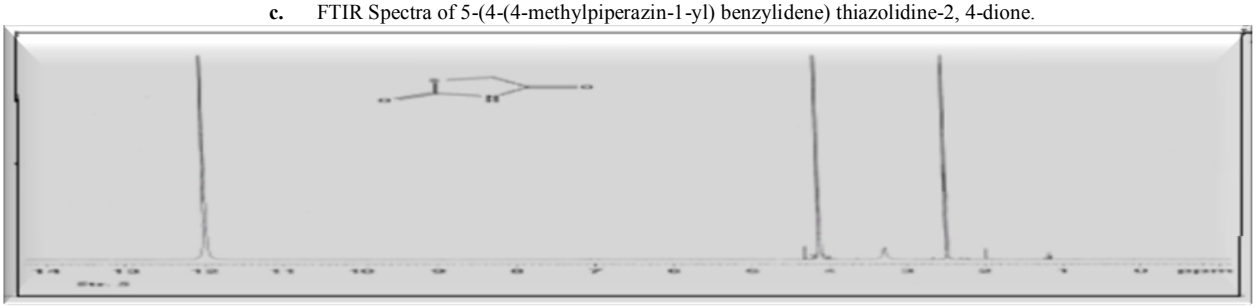


Figure no 2 ^{1}H NMR Spectra of Thiazolidine-2, 4-dione **(I).**

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