



Synthesis and Antimicrobial Evaluation of N-Substituted-5-Benzylidene-2,4-Thiazolidinedione Derivatives

Neeru Malik*, D.N. Prasad

Shivalik College of Pharmacy, Nangal, Ropar, Punjab, India

Abstract

A series of N-substituted-2,4-thiazolidinedione derivatives (TZDs) were prepared via N-alkylation of 2,4-TZD at position 3 using substituted benzyl halides. Synthesized N-substituted-2,4-TZD was then substituted at position 5 with substituted aromatic aldehyde according to Knoevenagel condensation method. Structures of the compounds were elucidated using various spectral techniques viz. IR, ¹HNMR. The synthesized compounds were evaluated for their antimicrobial activity.

Keyword: Antimicrobial activity; Knoevenagel condensation; Thiazolidinedione.

Received: February 2, 2012; *Accepted:* May 19, 2012.

1. Introduction

The presence of thiazolidine ring in penicillin and related derivatives was the first recognition of its occurrence in the nature. Thiazolidine derivatives reported to show variety of biological activities such as antibacterial, antifungal [1-3], anti-inflammatory, antituberculostatic, antitumor, anticonvulsant [6], cardiogenic [5], besides showing promising antidiabetic [4] activity. The medication class of thiazolidinediones (TZDs; also called as “glitazones”) was introduced in late 1990’s as an adjunct therapy for type II diabetes mellitus and related diseases. Ciglitazone, which became a prototype of this class was synthesized in 1982, but was withdrawn because of low potency and appearance of cataracts in animals receiving long term exposure. Troglitazone

in January 1997 was approved as a glucose lowering therapy for patients in US with type II diabetes, but the drug was withdrawn due to hepatotoxicity in 2000 [7-8]. The valuable properties of “glitazones” initiated us to synthesize N-substituted-5-benzylidene-2,4-TZD derivative. The synthesized 2,4-thiazolidinedione ring was substituted at position 3 with substituted benzyl halides via N-alkylation method using NaOH in ethanolic solution. The synthesized N-substituted-2,4-TZD product was then substituted at position 5; i.e. at the nucleophilic methylene carbon with substituted aromatic aldehydes by Knoevenagel condensation using piperidine as catalyst. The progression of the reaction was monitored with the help of TLC. The structure elucidation of the compound was done by IR and ¹HNMR spectroscopy. The synthesized compound was then evaluated for the antimicrobial activity.

*Corresponding Author:

Tel: (+91)9465168557, E.Mail: malik.neeru@ymail.com

2. Materials and methods

2.1. Chemicals and instruments

All of the chemicals used were procured from CDH, Acros and Himedia. Melting point (m.p) was recorded on Veego melting point apparatus and is uncorrected. Infra red (IR) spectra were taken using FTIR thermo Scientific; NICOLET Is10, KBR disk spectrophotometer. Only principle peaks of interest are reported and expressed in cm^{-1} . The ^1H NMR spectra were recorded on sophisticated multinuclear FT-NMR spectrometer model Avance-II (Bruker) 400 NMR Spectrometer, using dimethylsulfoxide- d_6 and CdCl_2 as solvent. Chemical shifts are expressed as δ value (ppm), downfield from tetramethylsilane used as internal standard. While citing ^1H NMR data, the following abbreviation have been used. s (singlet), d (doublet), t(triplet), q (quartet), m (multiplet). All reaction were monitored by thin layer chromatography (TLC) using silica gel G (Rankem) and activated at 110°C for 30 min. The plates were developed by exposing to iodine vapours. All reagent and solvents were purified and dried by standard techniques.

2.2. General synthesis procedure

2.2.1. Synthesis of 2, 4 thiazolidinedione

In a 250 ml three-necked flask, a solution containing 56.4 g (0.6 mol) of chloroacetic acid in 60 ml of water and 45.6 g (0.6 moles) of thiourea was dissolved in 60 ml of water (Figure 1). The mixture was stirred for 15 min till occurrence of white precipitates. To the contents of the flask was now added slowly 60 ml concentrated hydrochloric acid from dropping funnel to dissolve the

Table 1. Illustrative substituted aromatic aldehydes.

Compound	R'	R
1a	H	H
1b	Cl	H
1c	p-OH	H
1d	$\text{N}(\text{CH}_3)_2$	H
1e	o-OH	H
1f	4-OH, 3-OCH ₃	H

precipitates, after which the reaction mixture was stirred and refluxed for 10-12 h at $100-110^\circ\text{C}$. On cooling the contents of the flask were solidified to a mass of clusters of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was re-crystallized from ethanol [11]. Yield: 85%. m.p: $123-125^\circ\text{C}$. TLC: Chloroform: Methanol (4.5:0.5), Rf: 0.6.

2.2.2. Synthesis of N-substituted 2, 4- thiazolidinedione

In a 250 ml RBF, NaOH (0.8 g, 0.02 moles) was dissolved in 20 ml of ethanol. To this solution, 2,4-thiazolidinedione (0.02 mol) and benzyl chloride (0.02 mol) was added. The resulting reaction mixture was then refluxed for 20 h (Figure 2). Precipitation of salt was observed during the reaction. At the end of the reflux period, ethanol was distilled out and the residue was extracted from ether and water. The ethereal layer was removed, dried over sodium sulfate sulphate and concentrated. The product was dried under reduced pressure and was re-crystallized from ethanol [11]. Yield: 70%, m.p.: $60-62^\circ\text{C}$. TLC: Benzene: Ethyl acetate (4:1), Rf: 0.43.

2.2.3. Synthesis of N-substituted-5-benzylidene-2, 4 thiazolidinedione derivatives

In a 250 ml 3-necked round bottom flask

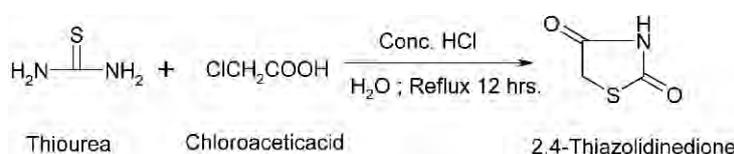


Figure 1. Method of synthesis of 2,4-thiazolidinedione.

Table 2. Characterization data of the synthesized compounds.

Compound	Yield %	Melting point (°C)	R _f Value
1a	70	198-200	0.67
1b	63	200-202	0.6
1c	76	212-215	0.5
1d	80	250-253	0.7
1e	69	185-186	0.45
1f	78	190-194	0.52

provided with a Dean-Stark apparatus, aromatic aldehyde (Table 1) (0.188 mol) and N-substituted-benzyl-2,4-thiazolidinedione (0.188 mol) were together suspended in dry toluene (Figure 3). To this catalytic amount of piperidine (1 ml) was added. The mixture was refluxed with stirring. After complete removal of water and when the temperature crossed 110 °C the reaction mixture was stirred for further 1 h. On cooling, the product precipitated out of toluene. The compound was filtered and washed with cold, dry toluene and dry ethanol and then re-crystallized from dry ethanol [12-14].

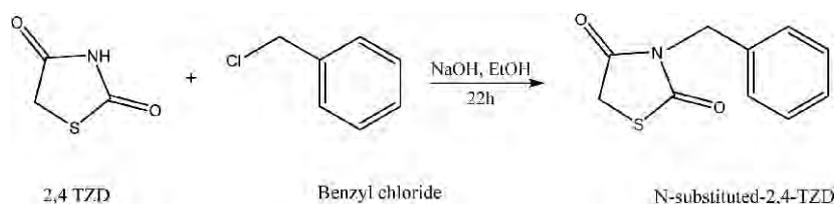
2.3. Spectral data

A series of N-substituted-5-benzylidene-2,4-thiazolidinedione derivatives (1a-1f) were synthesized and the structures were established by means of IR and ¹H-NMR. The yields, melting point of synthesized compounds are illustrated (Table 2).

2.4. Antibacterial activity

The antibacterial activity of the compounds was studied systematically against strains of bacteria *Bacillus subtilis* and *Escherichia coli* using cup plate method. The zone of inhibition in (mm) was measured and recorded accordingly. A standard drug, ciprofloxacin

100 µg/ml in sterile water was subjected for the same. The compound solutions were made in 100 µg/ml in DMSO. The test organism was subcultured using agar media. The tube containing sterilized medium were inoculated with bacterial strain. After incubation at 34-37 °C for 48 h, it was stored in refrigerator. The stock culture was maintained. Bacterial inoculum was prepared by transferring a loop full of stock culture to the nutrient broth in conical flask. The flask was incubated at 34-37 °C for 24 h before experimentation. The sub-sterilized agar medium was sterilized by autoclaving at 121 °C (15 lb/sq inch) petridish, tubes and flasks, plug with cotton wool were sterilized in hot air oven at 160 °C for an h. Into each sterilized petridish, about 30 ml of molten nutrient media inoculated with the respective strain of bacteria (6 ml inoculated to 30 ml of nutrient media) was transferred aseptically. The plates were left at room temperature to solidify. One hundred µl of each of the dilution of compounds were transferred to the wells. One of the well was diluted with standard in each palate. The plates were kept undisturbed for at least 2 h at room temperature to allow diffusion of solution properly in nutrient media. After incubation of the plate at 34-37 °C for 48 h, the diameter of the zone of inhibition

**Figure 2.** Method of synthesis of N-benzyl 2,4-thiazolidinedione.

surrounding each of the well was measured. All the experiment was carried out in triplicates and values were averaged and display in the Table 3. A set of negative and positive controls of growth were also kept for incubation along with test plates. In the plate for negative growth the highest volume of DMSO and sterile distilled water were used with no culture being seeded, while in the plate representing maximum growth (positive control), the highest volume of DMSO and sterile distilled water were used along with the organism [9].

3. Result and discussion

A series of N-substituted-5-benzylidene-2,4-thiazolidinedione derivatives were synthesized using different substituted benzyl halides and aromatic aldehydes (Figures 1-3). Synthesized compounds were characterized by chromatographic methods, IR spectroscopy and ¹HNMR spectra's. The chemical and physical characteristics of the compounds are shown in Tables 1 and 2. The IR and NMAR characteristic of the compounds were as follows:

IR spectra of 5-benzylidene 3-benzyl-2,4-thiazolidinedione (1a) shows the following peaks: 2955 cm⁻¹ (aliphatic -CH stretching), 3036 cm⁻¹ (aromatic -CH stretching), 1732 cm⁻¹ (aliphatic C=O), 566 cm⁻¹ (C-S stretching), 1139 cm⁻¹ (C-N stretching).

¹HNMR, signal appeared at 4.8 ppm (2H, s, CH₂ of benzyl), 7.8 ppm (1H, s, CH of benzylidene), 7.2-7.5 ppm (10H, m, aromatic protons).

IR spectra of 5-(4-chlorobenzylidene)-3-benzyl-2,4-thiazolidinedione (1b) shows THE

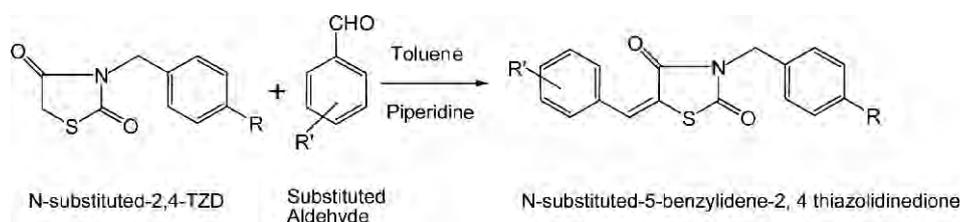


Figure 3. Method of synthesis of N-substituted-5-benzylidene-2,4-thiazolidinedione derivatives.

Table 3. *In vitro* antibacterial activity of synthesized compound against *Bacillus subtilis* and *Escherichia coli*.

Compound	Inhibition zone (mm)	
	<i>B. subtilis</i>	<i>E. coli</i>
1a	11	7
1b	7	-
1c	13	11
1d	-	-
1e	8	6
1f	16	13
Ciprofloxacin	25	25

following peaks: 2924 cm⁻¹ (aliphatic -CH stretching), 3034 cm⁻¹ (aromatic -CH stretching), 1731 cm⁻¹ (aliphatic C=O), 693 cm⁻¹ (C-S stretching), 1141 cm⁻¹ (C-N stretching), 736 cm⁻¹ (C-Cl stretching).

¹HNMR, signal appeared at 4.8 ppm (2H, s, CH₂ of benzyl), 7.8 ppm (1H, s, CH of benzylidene), 7.2-7.5 ppm (9H, m, aromatic protons).

IR spectra of 5-(4-hydroxybenzylidene)-3-benzyl-2,4-thiazolidinedione (1c) shows the following peaks: 2925 cm⁻¹ (aliphatic -CH stretching), 3065 cm⁻¹ (aromatic -CH stretching), 3346 cm⁻¹ (-OH stretching), 1723 cm⁻¹ (aliphatic C=O), 533 cm⁻¹ (C-S stretching), 1174 cm⁻¹ (C-N stretching).

¹HNMR, signal appeared at 4.8 ppm (2H, s, CH₂ of benzyl), 8.2 ppm (1H, s, CH of benzylidene), 7.7 ppm (-OH of aldehyde), 6.8-7.3 ppm (9H, m, aromatic protons).

IR spectra of 5-(4-N,Ndimethylbenzylidene)-3-benzyl-2,4-thiazolidinedione (1d) shows the following peaks: 2947 cm⁻¹ (aliphatic -CH stretching), 1723 cm⁻¹ (aliphatic C=O), 547 cm⁻¹ (C-S stretching), 1197 cm⁻¹ (C-N stretching).

¹HNMR, signal appeared at 4.8 ppm (2H,

s, CH₂ of benzyl), 7.8 ppm (1H, s, CH of benzylidene), 3 ppm (6H, s, -(CH₃)₂ of aldehyde), 6.7-7.4 ppm (9H, m, aromatic protons).

IR spectra of 5-(2-hydroxybenzylidene)-3-benzyl-2,4-thiazolidinedione (1e) shows the following peaks: 2923 cm⁻¹ (aliphatic -CH stretching), 3025 cm⁻¹ (aromatic -CH stretching), 3267 cm⁻¹ (-OH stretching), 1723 cm⁻¹ (aliphatic C=O), 581 cm⁻¹ (C-S stretching), 1139 cm⁻¹ (C-N stretching).

¹HNMR, signal appeared at 4.8 ppm (2H, s, CH₂ of benzyl), 8.2 ppm (1H, s, CH of benzylidene), 7.7 ppm (-OH of aldehyde), 6.8-7.3 ppm (9H, m, aromatic protons).

IR spectra of 5-(4-hydroxy-3-methoxybenzylidene)-3-benzyl-2,4-thiazolidinedione (1f) shows the following peaks: 2949 cm⁻¹ (aliphatic -CH stretching), 3112 cm⁻¹ (aromatic -CH stretching), 3437 cm⁻¹ (-OH stretching), 1726 cm⁻¹ (aliphatic C=O), 568 cm⁻¹ (C-S stretching), 1197 cm⁻¹ (C-N stretching), 1140 cm⁻¹ (C-O-C stretching).

¹HNMR, signal appeared at 4.8 ppm (2H, s, CH₂ of benzyl), 3.8 ppm (3H, s, -OHC₃ of aldehyde), 7.8 ppm (1H, s, CH of benzylidene), 6.7 ppm (-OH of aldehyde), 6.8-7.4 ppm (9H, m, aromatic protons).

The synthesized compounds were then evaluated for their antimicrobial activity against the strains *Bacillus subtilis* and *E. coli*. using cup plate method. The results obtained showed that out of 6 compounds, (1a, 1b, 1c, 1d, 1e, 1f) 1f, 1c and 1d exhibit significant activity (Table 3), while the rest of the compounds showed moderate activity. Further modifications on benzylidene ring may produce effective compounds. Attachment of more lipophilic agents may increase the bioavailability and efficacy of the drug.

Acknowledgment

The authors thank all the faculty of the

college for their valuable support and encouragement. Our sincere thanks to Dr. Avatar Singh, Sophisticated Instrument Analytical Facility, Punjab University, Chandigarh for his help in spectral studies.

Reference

- [1] Labouta IM, Salma HM, Ehsba O, Kader E, El-Churbini. Potential anti-microbial: synthesis and in vitro anti-microbial evaluation of some 5-arylazo-thiazolidones and related compounds. *Eur J Med Chem* 1987; 22: 485-89.
- [2] Goes AJS, DeLima MCA, Galdino SL, Pitta IR, Luu-Ducc C. Synthese et activite antifongique des chlorobenzyl benzylidene thiazolidinediones et imidazolinediones substituees. *Ann Pharm Francaises* 1991; 49: 92-8.
- [3] DeLima MCA, Costa DLB, Goes AJS, Galdino SL, Pitta IR, Luu-Ducc C. Synthese et activite antimicrobienne dederives chlorobenzyl benzylidene imidazolinediones et thiazolidinediones substituees. *Pharmazie* 1992; 47: 182-4.
- [4] Cantello BCC, Cawthorne MA, Cottam GP, Duff PT, Haigh D, Hindley RM Lister CA, Smith SA, Thurlby PL. [[ω-(Heterocycllamino)alkoxy]benzyl]-2,4-thiazolidinediones as potent antihyperglycemic agents. *J Med Chem* 1994; 37: 3977-85.
- [5] Andreani A, Rambaldi M, Locatelli A, Leoni A, Bossa R, Chiericozzi M, Galatulas I, Salvatore G. Synthesis of lactams with potential cardiotoxic activity. *Eur J Med Chem* 1993; 28: 825-9.
- [6] Eel-Feky SAH. Synthesis and anticonvulsant properties of some novel quinazolinone thiazolidine and 4-thiazolidine derivatives. *Pharmazie* 1993; 48: 28-34.
- [7] Timothy M, Peter J, Brown, Daniel DS, Brad RH. The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000; 43: 527-50.
- [8] Shiva PS, Surinder SP. Chemistry and biological activity of thiazolidinediones. *Chem Rev* 1981; ISSUE NUMBER: 175-203.
- [9] Kucukguzel GS, Oruc E, Sevim R, Sahin F, Ozbek A. Synthesis, characterization and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. *Eur J Med Chem* 2002; 37:197-206.
- [10] Frances CB. 4-thiazolidinones. *Chem Rev* 1961; ISSUE NUMBER: 464-521.
- [11] Pattan SR, Kerkare P, Nikalje A, Hole MB. Synthesis and evaluation of some new thiazolidinedione derivatives for their antidiabetic activity. *Asian J Res Chem* 2009; 2: 123-6.

- [12] Pattan SR, Khade AB, Pawar PD, Tarnalli AD, Kittur BS, Borkar SD. Synthesis of 2-amino [5'-(4-sulphonyl benzylidene)-2,4-thiazolidinedione]-6-fluoro benzothiazoles as anti-inflammatory agents. *Indian J Heterocyclic Chem* 2007; 16: 299-300.
- [13] Pattan SR, Suresh Ch, Pujar VD, Reddy VVK, Rasal VP, Koti BC. Synthesis and antidiabetic activity of 2-amino[5(4-sulphonylbenzylidene)-2,4-thiazolidinedione]-7-chloro-6-fluorobenzothiazole. *Indian J Chem* 2005; 44B: 2404-8.
- [14] Shelke KF, Sapkal SB, Shingare MS. Ionic liquid promoted efficient synthesis of 5-arylidene-2, 4-thiazolidinedione. *Bulletin Catalysis Society India* 2009; 8: 30-4.