International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 8, Issue 12, 2016, pp.-807-812.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

SYNTHESIS, CHARACTERIZATION AND SCREENING OF THIAZOLIDINE-2,4-DIONE DERIVATIVES AS ANTIMICROBIAL AGENTS

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Received: September 27, 2016; Revised: October 28, 2016; Accepted: October 29, 2016; Published: November 28, 2016

Abstract-Thiazolidine-2,4-dione derivatives (4a-4e) have been synthesized and characterized by physico-chemical, elemental (C,H,N,S), FT-IR, mass and ^{1}HNMR spectral analysis. In this context, the aim was to synthesized thiazolidine-2,4-dione derivatives and were screened for antimicrobial activity. Antimicrobial activity was screened using agar well diffusion assay method for determination of the diameter of zone of inhibition in millimeter (mm) and solid dilution method for determination of minimum inhibitory concentration (MIC) in µg/ml against Gram-positive(*Staphylococcus aureus* MTCC 1430, *Bacillus subtilis* MTCC 0441), Gram- negative(*Escherichia coli* MTCC 1573, *Pseudomonas aeruginosa* MTCC 2453) and fungal strain (*Aspergillus tubingensis* MTCC 2546). The antimicrobial activity of compound 4c 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(4-fluorophenyl)acetamide was found to be most potent against all the tested strains of microorganisms with the zone of inhibition 17.1-19.5 mm at 200µg/ml and minimum inhibitory concentration value 0.6-0.8 µg/ml.

Keywords- Antimicrobial agents, Thiazolidinedione, Molecular sieves and minimum inhibitory concentration

Citation: Shukla Karuna S., et al., (2016) Synthesis, Characterization and Screening of Thiazolidine-2,4-Dione Derivatives as Antimicrobial Agents. Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 8, Issue 12, pp.-807-812.

Academic Editor / Reviewer: Akash Ved

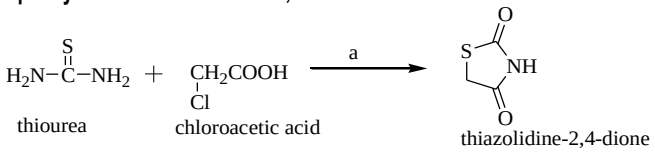
Introduction

Antibiotic compounds have become need to current health care system and complementing the immune system against different microbial strains. As conventional antibiotics are often used to cure microbial infections, some microorganisms have developed resistant to these antibiotics. Due to the appearance of antibiotic-resistant strains, the continuous development of efficient antibiotic agents is more crucial than ever [1-3]. The medical researcher faces a lot of problem against diseases caused by the pathogen bacteria, fungi and needs effective treatment and development for novel antimicrobial agents. Thiazolidinedione which are five membered ring containing three carbon atoms, one nitrogen and one sulfur atom are of more interest in different fields of medicinal chemistry [4].Thiazolidinedione is derivative of thiazolidine with two carbonyl groups at the second and fourth positions. Thiazolidine-2, 4-dione and their derivatives have been reported to have therapeutic potential including antidiabetic [5-6], anticancerous [7-8], anti-inflammatory [9-10], antioxidant [11], hypolipidemic[12], aldose reductase inhibitor[13-14], antiobesity [15], antimicrobial [16] etc. Different possibilities of heterocyclic modifications with a wide range of biological properties are the most important for design of this class of compounds [17-18]. Having such diverse range of pharmacological activities, thiazolidine-2,4-dione derivatives attracted medicinal chemists and consequently a number of strategies have been originated to synthesize them. In this context a series of thiazolidine-2, 4-dione derivatives were designed to synthesize and evaluated as antimicrobial agents **(4a-4e)**.

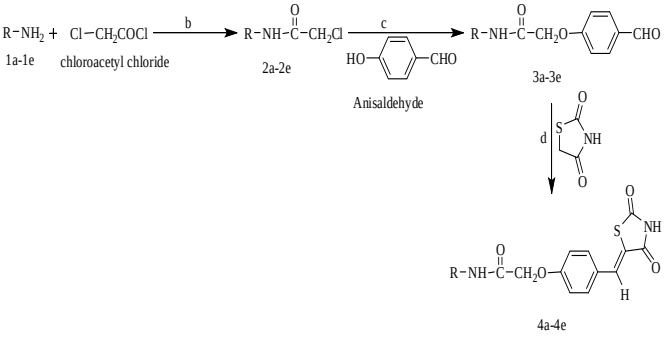
Materials and Methods

Thiazolidine-2, 4-dione derivatives **(4a-4e)** were synthesized according to the synthetic pathways described in Scheme A.

Step-1 Synthesis of thiazolidine-2, 4- dione



Step-2 Synthesis of thiazolidine-2,4-dione derivatives (4a-4e)



Scheme A. Synthetic scheme for the synthesis of thiazolidine-2,4-dione derivatives (**4a-4e**). Reagents and conditions: **a.** Conc. HCl, reflux (10-11h), **b.** triethylamine, $CHCl_3$, stirr (26-28h), $NaHCO_3$, Na_2SO_4, **c.** K_2CO_3, dimethyl formamide, stirr at room temperature, **d.** toluene, piperidine, acetic acid, 4-6 dried molecular sieves, reflux (10-14h).

The final synthesized compounds were substituted by different groups/atoms (R) as shown in [Table-1].

Table-1 *Substituted groups/atoms (R) for synthesized compounds* ***(4a-4e)***

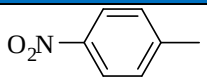
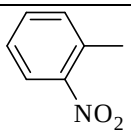
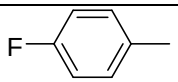
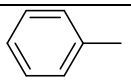
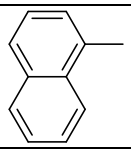
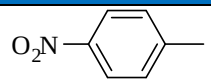
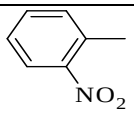
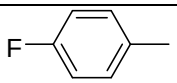
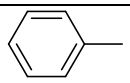
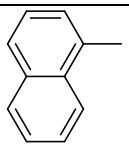
Compound	R
4a	
4b	
4c	
4d	
4e	

Table-2 *Physico-chemical and elemental data of synthesized compounds (****4a-4e****)*

Compound	R	Molecular formula	M.P. (ºC)	Yield (%)	Log P	Elemental analysis
4a		$C_{18}H_{13}N_3O_6S$	190-192	65.14	2.25	C, 54.03 H, 3.14 N, 10.31 O, 24.34 S, 8.20
4b		$C_{18}H_{13}N_3O_6S$	189-191	56.13	1.75	C, 54.02 H, 3.14 N, 10.43 O, 24.31 S, 8.20
4c		$C_{18}H_{13}FN_2O_4S$	204-206	59.19	2.44	C, 58.16 H, 3.32 F, 5.14 N, 7.41 O, 17.39 S, 8.51
4d		$C_{18}H_{14}N_2O_4S$	208-210	64.23	2.44	C, 61.11 H, 3.88 N, 7.81 O, 18.21 S, 9.15
4e		$C_{22}H_{16}N_2O_4S$	198-200	58.32	2.71	C, 65.12 H, 3.86 N, 6.81 O, 15.91 S, 7.85

Method for synthesis of thiazolidine-2,4-dione

Equimolar amount of chloroacetic acid (0.6M) and thiourea (0.6M) were dissolved each in 60 ml of water separately and mixed slowly at 0-5ºC and stirred for 20 minute to form a white precipitate of 2-imino-thiazolidine-4-one. Conc. Hydrochloric acid (60 ml) was added and refluxed for about 10-11hrs. Reaction was monitored through thin layer chromatography (chloroform: methanol) and reaction was allowed to cool to form white solid crystals then washed with water and dried.

General Procedure for synthesis of compounds (2a-2e)

An equimolar solution of substituted amines (1a-1e,0.2M) and triethyl amine (0.2M) in dry chloroform (50 ml) was stirred at 0-5ºC. Chloroacetyl chloride (0.21M) was added dropwise for a period of about 40-50 minutes and later stirred for 26-28 hrs. The reaction was monitored through TLC. After completion of the reaction, the product obtained was washed with sodium carbonate later with water and then dried over anhydrous Na_2SO_4 and was recrystallized by chloroform.

General Procedure for synthesis of compounds (3a-3e)

Substituted aldehyde (p-hydroxy benzaldehyde, 1.0 mol) along with 1.5 mol of potassium carbonate were taken in dimethyl formamide and added to this solution of chloroacylated product (1.5 mol 2a-2e) in dimethyl formamide. The stirring was continued at room temperature till the completion of reaction and monitored by thin layer chromatography. Water was added to precipitate product.

General Procedure for synthesis of compounds (4a-4e)

To a suspension of thiazolidine-2,4-dione and 3-(4-formyl phenoxy)-N-phenylpropanamide (0.01 M 3a-3e) in dry toluene, anisaldehyde (0.01M), catalytic amounts of piperidine (0.0005M), acetic acid (0.0005M) and 4-6 dried molecular sieves were added. The reaction mixture was stirred for about 10 minute and refluxed at 110^{0}C with occasional stirring for 10-14 hr. The reaction mixture was monitored through thin layer chromatography. The reaction mixture was allowed to cool, precipitate was obtained which was filtered and recrystallized with aqueous ethanol [19-20].

***In vitro* screening of antimicrobial activity**

The antimicrobial activity of the synthesized compounds (**4a-4e**) was determined by two methods viz. agar well diffusion method and solid dilution method.The standard strains were attained from Institute of Microbial Technology, Chandigarh, India. Culture media used for the activation of microorganisms were according to Microbial type culture collection protocol. The synthesized compounds were screened for antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus* MTCC 1430, *Bacillus subtilis* MTCC 0441), Gram-negative bacteria (*Escherichia coli* MTCC 1573, *Pseudomonas aeruginosa* MTCC 2453) and fungal strain (*Aspergillus tubingensis* MTCC 2546).

Agar well diffusion method

Agar well diffusion method depends upon diffusion of the antibiotic from a cavity through a solidified agar layer in a petridish to an extent such that growth of added microorganisms was prevented entirely in a zone around the cavity containing a solution of the antibacterial agents. 2-10% of the nutrient broath suspension of the micro-organisms were added to sterile molten nutrient agar which has been cooled to 45^{0}C, mixed well and poured into sterile petridish. The agar was allowed to solidify and five wellswas made by sterile cork borer. Test samples of concentration 50,100 and 200 µg/ml by dissolving in dimethyl sulphoxide, standard drug and control were poured into the corresponding well by micropipettes. Inoculated plates in triplicate and petridish were left at room temperature. To allow the diffusion of the samples petridish were incubated at corresponding temperature of each organism for 24 hrs. The diameter of the zones of inhibition was measured to the nearest millimeter.

Solid dilution method

This method was used for determining minimum inhibitory concentration value of synthesized compounds. The minimum inhibitory concentration was defined as the lowest concentration of compound required for a complete inhibition of the microbial growth after incubation time. In this method the dilutions of the samples under test were made in agar instead of broath. The agar containing the samples under test was subsequently poured onto a petriplate. Nutrient agar was melted, the solution under test and the mixture poured into a sterile petriplate and allowed to set in the form of a wedge. After this second amount of agar was then pour onto the wedge and allowed to set with the petriplate on the bench. To allow diffusion of the drugthe plates were incubated overnight. The streaking of microorganism was in a direction running from the highest concentration to the lowest concentration.

The result of this method was calculated by measuring the length of growth of micro-organism and the total length of the agar surface streaked; then if total length of possible growth was x cm and total length of actual growth was y cm, the minimum inhibitory concentration of compounds was determined by using the formula:

$$\frac{c \times y}{x} \text{ mg ml}^{-1}$$

where c is the final concentration, in µg or mg ml^{-1}of the drug in the total volume of the medium [21-23].

Results and Discussion

The structure of the synthesized thiazolidine-2, 4-dione derivatives (**4a-4e**) were determined by physico-chemical and elemental analysis was shown in [Table-2] and FTIR, ^{1}HNMR and mass spectral analysis. IR spectrum of all the final thiazolidinedione derivatives showed characteristic peaks for -N-H stretching in the range of 3402-3415cm^{-1} and -C=O stretching in the range of 1705-1720 cm^{-1},-C=O stretching for cyclic amide in the range of 1660-1675 cm^{-1}and –C=C- in the range of 1560-1575cm^{-1} was shown in [Table-3]. ^{1}H NMR showed characteristic peak in the range of 10.91-10.95 δ ppm to confirm the presence of -NH proton of thiazolidinedione derivatives. This large deshielding effect on -NH proton was attributed to the presence of electron withdrawing carbonyl groups. Thiazolidine-2,4-dione derivatives (**4a-4e**) showed a characteristic peak of benzylidene proton (=CH) between 7.15-8.15 δ ppm. The molecular weights of synthesized compounds were confirmed by mass spectral analysis. The observed molecular weights of particular compounds were showing (M+1) molecular ion peak which was summarized in [Table-4] [24-25].

Table-3 IR spectral analysis of synthesized compounds

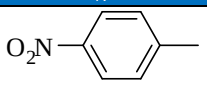
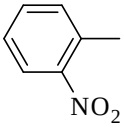
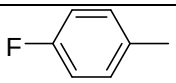
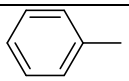
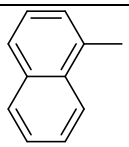
Compound	R	IR peaks(in cm^{-1})
4a		3412 (-NH), 1716 (-C=O), 1680 (amide –C=O), 1540 (-C=C)
4b		3410 (-NH), 1710 (-C=O), 1670 (amide –C=O), 1530 (-C=C)
4c		3405 (-NH), 1726 (-C=O), 1670 (amide –C=O), 1550 (-C=C)
4d		3402 (-NH), 1715 (-C=O), 1660 (amide –C=O), 1560 (-C=C)
4e		3408 (-NH), 1710 (-C=O), 1670 (amide –C=O), 1555 (-C=C)

Table-4 *Mass and ^{1}H NMR Spectral analysis of synthesized compounds*

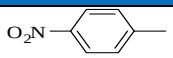
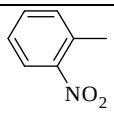
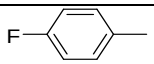
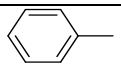
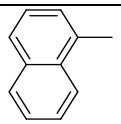
Compound	R	Mass spectra value (ESI)	NMR value (δ ppm)
4a		400.15 (M + 1)$^+$	10.94 (s, 1H, -CO-NH-CO-), 10.14 (s, 1H,-NH), 4.41 (s, 2H, -CH_2), 7.25 (s, 1H, benzylidene), 8.41 (d, 2H, Ar), 8.11 (d, 2H, Ar),7.71 (d, 2H, Ar);
4b		400.10 (M + 1)$^+$	10.95 (s, 1H, -CO-NH-CO-), 10.17 (s, 1H,-NH), 4.44 (s, 2H, -CH_2), 8.15 (s, 1H, benzylidene), 8.41 (d, 2H, Ar), 7.75 (t, 2H, Ar),7.45 (d, 2H, Ar), 6.99 (d, 2H, Ar);
4c		373.06 (M + 1)$^+$	10.94 (s, 1H, -CO-NH-CO-), 10.14 (s, 1H,-NH), 4.45 (s, 2H, -CH_2), 7.25 (s, 1H, benzylidene), 8.25 (d, 2H, Ar), 7.77 (d, 2H, Ar), 6.79 (d, 2H, Ar);
4d		355.08 (M + 1)$^+$	10.95 (s, 1H, -CO-NH-CO-), 10.26 (s, 1H,-NH), 4.43 (s, 2H, -CH_2), 7.15 (s, 1H, benzylidene), 8.25 (d, 2H, Ar), 7.75 (d, 2H, Ar), 7.25 (d, 2H, Ar);
4e		405.15 (M + 1)$^+$	10.91 (s, 1H, -CO-NH-CO-), 10.21 (s, 1H,-NH), 4.42 (s, 2H, -CH_2), 7.15 (s, 1H, benzylidene), 7.75 (d, 2H, Ar), 8.15 (d, 2H, Ar), 7.25 (d, 2H, Ar), 7.74 (d, 2H, Ar);

Table-5 *Antimicrobial activities of thiazolidinedione derivatives (**4a-4e**)*

Zone of inhibition by compounds at various concentrations against different strains (mm)

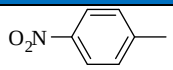
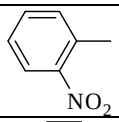
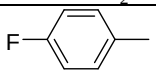
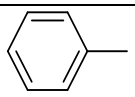
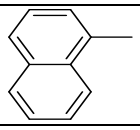
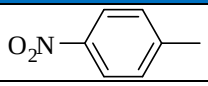
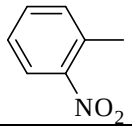
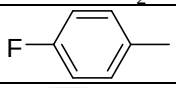
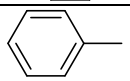
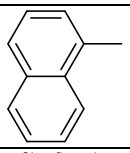
Compound	R	Conc. µg/ml	Gram positive Bacteria		Gram negative Bacteria		Fungus
			Bs(Mean±SEM)	Sa(Mean±SEM)	Ec(Mean±SEM)	Pa(Mean±SEM)	At(Mean±SEM)
4a		50 100 200	5.5±0.5 9.6±0.5 12.1±1.0	5.3±0.2 9.3±0.2 11.8±0.5	5.1±0.2 9.0±0.3 11.5±0.6	5.0±0.0 8.8±0.2 11.1±0.4	4.1±0.2 8.5±0.2 10.6±0.4
4b		50 100 200	4.6±0.4 7.5±0.5 10.5±0.5	4.6±0.2 7.3±0.2 10.3±0.2	4.6±0.2 7.0±0.0 9.8±0.5	4.3±0.2 6.8±0.2 10.0±0.6	4.0±0.0 6.1±0.2 8.6±0.4
4c		50 100 200	7.1±1.0 13.6±0.5 19.5±0.5	7.0±0.2 13.3±0.2 19.1±0.2	6.8±0.5 12.6±0.4 18.1±0.2	6.6±0.5 12.3±0.2 17.6±0.2	6.1±0.2 10.6±0.4 17.1±0.2
4d		50 100 200	4.6±0.4 7.5±0.5 10.5±0.5	4.6±0.2 7.3±0.2 10.3±0.2	4.6±0.2 7.0±0.0 9.8±0.5	4.3±0.2 6.8±0.2 10.0±0.6	4.0±0.0 6.1±0.2 8.6±0.4
4e		50 100 200	4.6±0.4 7.5±0.5 10.5±0.5	4.6±0.2 7.3±0.2 10.3±0.2	4.6±0.2 7.0±0.0 9.8±0.5	4.3±0.2 6.8±0.2 10.0±0.6	4.0±0.0 6.1±0.2 8.6±0.4
Std.	Ciprofloxacin	50 100 200	7.6±0.2 14.1±0.2 21.6±0.5	7.5±0.2 14.0±0.2 21.4±0.5	7.6±0.2 14.1±0.2 21.5±0.5	7.0±0.2 14.1±0.2 21.3±0.5	–
Std.	Norfloxacin	50 100 200	7.5±0.2 14.0±0.2 21.4±0.5	7.5±0.2 14.0±0.2 21.4±0.5	7.5±0.2 14.0±0.2 21.4±0.5	7.5±0.2 14.0±0.2 21.4±0.5	–
Std.	Fluconazole	50 100 200	–	–	–	–	7.8±0.2 12±1.0 18.5±1.0
Con.	DMSO (1%)		–	–	–	–	–

Table-6 *Minimum inhibitory activity (MIC, µg/ml) of thiazolidinedione derivatives (**4a-4e**) against different strains*

Compound	R	Gram positive Bacteria		Gram negative Bacteria		Fungus
		Bs	Sa	Ec	Pa	At
4a		1.5	1.5	1.6	1.6	1.7
4b		1.6	1.6	1.7	1.7	1.8
4c		0.6	0.6	0.7	0.7	0.8
4d		1.8	1.8	1.9	1.9	1.9
4e		1.8	1.8	1.9	1.9	1.9
Std.	Ciprofloxacin	0.3	0.3	0.3	0.3	_
Std.	Norfloxacin	0.3	0.3	0.3	0.3	_
Std.	Fluconazole	_	_	_	_	0.4
Con.	DMSO (1%)	_	_	_	_	_

Antimicrobial activity

The micro-organisms used for screening antimicrobial activity in the present study were *S. aureus* MTCC 1430, *B. subtilis* MTCC 0441 (Gram-positive bacteria), *E. coli* MTCC 1573, *P. aeruginosa* MTCC 2453 (Gram negative bacteria)and *A. tubingensis* MTCC 2546 (fungal strain) were procured from Institute of Microbial Technology, Chandigarh, India. Culture media used for the activation of micro-organisms were according to microbial type culture collection (MTCC) protocol. Norfloxacinand ciprofloxacin for bacterial strains and fluconazole for fungal strain were used as standard drug.

The dimethyl sulphoxide (1%) was used as a control. The results of antimicrobial activity of the synthesized compounds (**4a-4e)** against Gram-positive, Gram-negative and fungal strains were illustrated in [Table-5 & 6]. Compound **4c** showed maximum activity against four bacterial strains (MIC = 0.6-0.7 µg/mL, zone of inhibition values 17.6-19.5 mm) and one fungal strains (MIC = 0.8 µg/mL, zone of inhibition value 17.1mm) and was proved to be the most active compound of the series.

Agar plate for zone of inhibition and MIC values were shown in [Fig-1] & [Fig-2] for most active compound **4c** and showed that compound **4c** has almost similar activity as compared with standard drug. The compounds substituted with halogen on the phenyl ring at *para*positions enhanced the antibacterial activity as seen in the case of compound **4c** (*para fluro*).The compounds substituted with electron withdrawing groups have not showing promising activity as seen in the case of compounds **4a** (*para*-NO_2) and **4b** (*Ortho*-NO_2).

A decrease in the antimicrobial activity was attributed when phenyl ring was unsubstituted as seen in the case of compound **4d**. An increase in the antifungal activity was also observed for the thiazolidinedione derivatives which have halogen substitution but the potency of activity in many cases was found to be less than that of the antibacterial activity. Compound **4c** was found to have the most potent inhibitory capacity as it has *para* fluoro substitution at phenyl ring. These results indicate that further optimization of thiazolidinedione derivatives may provide a new class of broad spectrum antimicrobial agents.

Compound 4c *B. subtilis*

Fig-1 Compound 4c showing antibacterial activities (diameter of zone of inhibition) against *B. subtilis*.

Compound 4c *B. subtilis*

Fig-2 Compound 4c showing agar plate for calculation of MIC against *B. subtilis*

Bs: *Bacillus subtilis* (MTCC 0441), Sa: *Staphylococcus aureus* (MTCC 1430), Ec: *Escherichia coli* (MTCC 1573), Pa: *Pseudomonas aeruginosa* (MTCC 2453), At: *Aspergillus tubingensis* (MTCC 2546), Std.- Standard, Con.- Control

Conflicts of Interest The author(s) confirm that this article content has no conflict of interest.

Acknowledgement: One of the authors is greatly acknowledge Institute of Microbial Technology, Chandigarh, India for providing microbial strains. Authors would like to acknowledge Dean School of Pharmacy Babu Banarasi Das University, Lucknow for providing necessary facilities during research.

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