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Research Article

IN-VITRO ANTIMICROBIAL SCREENING OF 2-(4-((2, 4-DIOXOTHIAZOLIDIN-5-YLIDENE) METHYL) PHENOXY)-N-PHENYLACETAMIDE DERIVATIVES

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Abstract-A series of thiazolidine-2,4-dione derivatives (**4a-4f**) have been designed and synthesized by appropriate route and further characterized by physico-chemical, elemental (nitrogen and sulphur) and FT-IR spectral analysis. The purpose of this study was to screened these title compounds for antimicrobial activity against some selected microbial strains, 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) using agar well diffusion assay method for screening zone of inhibition and solid dilution method for minimum inhibitory concentration. The antimicrobial activity expressed as the diameter of zone of inhibition in millimeter and minimum inhibitory concentration in µg/ml. Amongst the series of synthesized compound screened, the compound (**4e)** 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-(4-bromophenyl) acetamide was found to be most active with the zone of inhibition 16.6-18.5 mm and MIC value 0.8-0.9 µg/ml against all the tested strains of microorganisms.

Keywords- Thiazolidine-2,4-dione derivatives, antibacterial activity, antifungal activity, piperidine and Knoevenagel condensation.

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Introduction

The crisis due to the development of multi drug resistance in contemporary antimicrobial agents is the major challenges for contemporary researchers worldwide. To address the problem of multi drug resistance, the researchers are making their continuous efforts to develop novel agents, which can overcome problem of multi drug resistance with significant activity. The need to design new compounds to overcome this multi drug resistance has become one of the most important areas of research today. Heterocyclic which are five membered ring containing three carbon atoms, one nitrogen and one sulfur atom known as thiazoles are of more interest in different fields of medicinal chemistry [1]. Thiazolidine-2,4-dione is derivative of thiazolidine with two carbonyl groups at the 2nd and 4th positions. Various substitution may be take place at position 3rd& 5th [2]. Thiazolidine-2, 4-dione is a five membered heterocyclic ring system with multiple applications. Thiazolidine-2, 4-dione and their derivatives have been reported to have wide therapeutic potential including anti-inflammatory [3], anti-diabetic [4-5], anticancer [6], neuroprotective [7], antioxidant [8], hypolipidemic [9], aldose reductase inhibitor [10], antimalarials [11], anti-obesity [12], wound healing activity [13], antimicrobial [14], anti-plasmodial [15], PTP1B inhibitor [16] *etc*. Thiazolidine-2,4-dione derivatives are well known chemical class of compounds with antimicrobial, anti-tuberculosis, and anti-HIV activities etc. Having such wide range of biological activities, these compounds attracted medicinal chemists and consequently a number of strategies have been design to synthesize them. The introduction of antibiotics in the 1940's was thought to have eliminated all infectious diseases. However, due to the widespread use and misuse of antibiotics, the resistance develop by bacterial strains to antibiotics, has become a serious public health problem. With the increase in resistance of bacterial strains to antibiotic treatment, main focused on developing novel approaches to antimicrobial therapy [17]. The study was aimed to design and develop some novel compounds derived from thiazolidine-2,4-dione moiety and to screen them against a wide range of microbes.

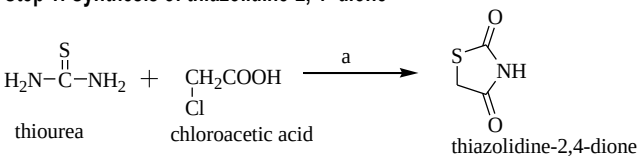
Herein we have been designed and synthesized a series of total 6 compounds as 2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-phenylacetamide derivatives **(4a-4f)** by appropriate route and further characterized by physico-chemical, elemental (carbon, hydrogen, nitrogen and sulphur) and FT-IR spectral analysis and evaluated for their antibacterial and antifungal activities.

Materials and Methods

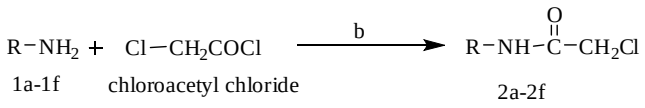
2-(4-((2,4-dioxothiazolidin-5-ylidene)methyl)phenoxy)-N-phenylacetamide derivatives **(4a-4f)** were synthesized according to the synthetic pathways described in Scheme A. Firstly synthesis of thiazolidine-2,4-dione was done by reaction of thiourea and chloroacetic acid. Chloroacetylated amines **(2a-2f)** were prepared by chloroacetylation of substituted amines **(1a-1f)** with chloroacetyl chloride in presence of base triethyl amine.

Substituted aldehyde **(3a-3f)** were synthesized by condensation of **(2a-2f)** with anisaldehyde in presence of K_2CO_3. Knoevenagel condensation between substituted aldehyde **(3a-3f)** and thiazolidine-2, 4-dione using piperidine as a base, dried molecular sieves in refluxing toluene provide corresponding 2-(4-((2, 4-dioxothiazolidin-5-ylidene) methyl) phenoxy)-N-phenylacetamide derivatives **(4a-4f)** in good yields [18-19].

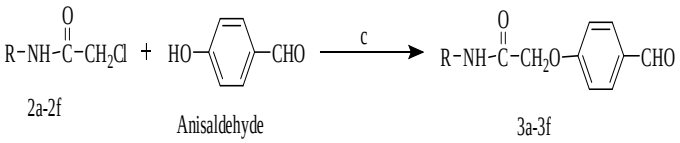
Step 1: Synthesis of thiazolidine-2, 4- dione



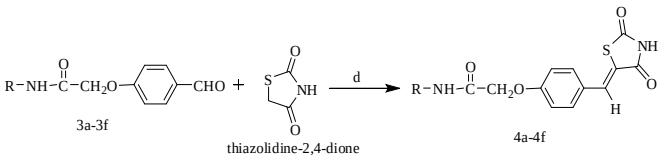
Step 2: Synthesis of compounds 2a-2f (chloroacetylation)



Step 3: Synthesis of compounds 3a-3f



Step 4: Synthesis of compounds 4a-4f (Knoevenagel condensation)



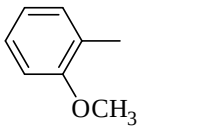
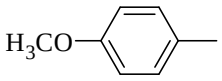
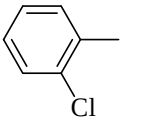
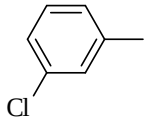
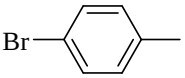
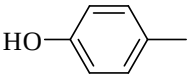
Scheme A. Synthetic scheme for the synthesis of compounds **4a-4f**. Reagents and conditions: **a.** Conc. HCl, reflux (11-12h), **b.** triethylamine, $CHCl_3$, stir (27-30h), $NaHCO_3$, Na_2SO_4, **c.** K_2CO_3, DMF, stirr at room temperature, **d.** toluene, piperidine, acetic acid, 4-5 dried molecular sieves, reflux (10-15h).

The final synthesized compounds **(4a-4f)** R groups were substituted which were shown in [Table-1].

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

With respect to the antimicrobial activity, the standard strains were procured from Institute of Microbial Technology, Chandigarh, India. The antimicrobial activity of the thiazolidinedione derivatives (**4a-4f**) was determined by agar well diffusion method. The compounds were screened for antibacterial and antifungal 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 depends upon diffusion of the antibiotic from a cavity by 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. Culture media used for the activation of microorganisms were according to MTCC protocol. 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 it was punched five wells by sterile cork borer. Test samples of 50,100 and 200 µg/ml by dissolving in DMSO, control and standard drug were poured into the corresponding well by micropipettes. Inoculated plates in triplicate and petridish were left at room temperature.

Table-1 *R groups for compounds(**4a-4f**).*

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

To allow the diffusion of the sample, all the 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 [20-23]. Solid dilution method was used for determining minimum inhibitory concentration (MIC) value. The minimum inhibitory concentration of compound was defined as the lowest concentration of compound required for a complete inhibition of the bacterial and fungal growth after incubation time. In this method, the dilutions of the substances under test were made in agar instead of broath. The agar containing the substances under test was subsequently poured onto a petridish. To perform the test, nutrient agar was melted, the solution under test and the mixture poured into a sterile petridish and allowed to set in the form of a wedge. A second amount of agar was then pour onto the wedge and allowed to set with the petridish on the bench. The plates were incubated overnight to allow

diffusion of the drug and to dry the surface. The microorganisms were streaked in a direction running from the highest to the lowest concentration. To calculate the result, the length of growth and the total length of the agar surface streaked was measured, then if total length of possible growth was x cm and total length of actual growth was y cm, the inhibitory concentration as determined by this method was:

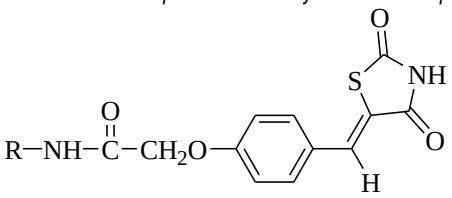
$$\frac{c \times y}{x} \text{ mg ml}^{-1}$$

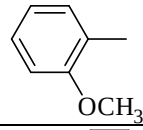
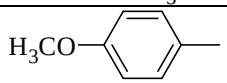
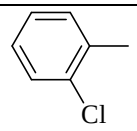
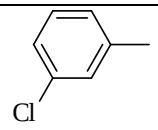
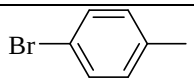
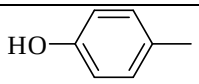
Where c was the final concentration in µg or mg ml^{-1} of the drug in the total volume of the medium [24].

Results and Discussion

The structure of the synthesized compounds were determined by physico-chemical, elemental analysis (C,H,N, analysis) and FT-IR spectral analysis was shown in [Table-2]. IR spectrum of all the final compounds, showed characteristic peaks for N-H stretching in the range of 3402-3412 cm^{-1}, C=O stretching in the range of 1710-1726 cm^{-1}, C=O stretching of cyclic amide in the range of 1665-1680 cm^{-1} and benzylidene -C=C in the range of 1530-1560 cm^{-1} [25-26].

Table-2 *Physico-chemical and spectral data of synthesized compounds (**4a-4f**)*



Compound	R	Molecular formula	M.P. (°C)	Yield (%)	Log P	IR(in cm^{-1})	Elemental Analysis
4a		$C_{19}H_{16}N_2O_5S$	190-192	65.14	2.25	3412(-NH), 1716(-C=O), 1680(amide –C=O), 1540(C=C)	C, 59.27; H,4.30; N,7.31 O, 20.70; S, 8.33.
4b		$C_{19}H_{16}N_2O_5S$	189-191	56.13	1.75	3410(-NH), 1710(-C=O), 1670(amide –C=O), 1530(C=C)	C,59.27; H,4.30; N,7.31 O, 20.70; S, 8.33.
4c		$C_{18}H_{13}ClN_2O_4S$	204-206	59.19	2.44	3405(-NH), 1726(-C=O), 1670(amide –C=O), 1550(C=C)	C,54.60; H,4.30; Cl,9.11 N,7.21 O, 16.44; S, 8.23.
4d		$C_{18}H_{13}ClN_2O_4S$	208-210	64.23	2.44	3402(-NH), 1715(-C=O), 1660(amide –C=O), 1560(C=C)	C,54.60; H,4.30; Cl,9.11 N,7.21 O, 16.44; S, 8.23.
4e		$C_{18}H_{13}BrN_2O_4S$	198-200	58.32	2.71	3408(-NH), 1710(-C=O), 1670(amide –C=O), 1555(C=C)	C,49.80; H,3.05; Br,18.33 N,6.56 O, 14.70; S, 7.47.
4f		$C_{18}H_{14}N_2O_5S$	220-222	60.36	1.41	3405(-NH), 1715(-C=O), 1665(amide –C=O), 1560(C=C)	C,58.27; H,3.70; N,7.58 O, 21.70; S, 8.53.

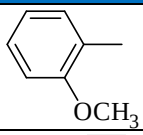
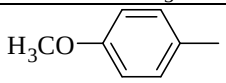
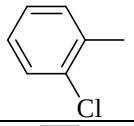
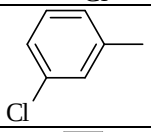
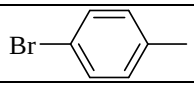
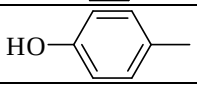
Antimicrobial activity

The micro-organisms used in the present study were 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) were procured from Institute of Microbial Technology, Chandigarh, India. Culture media used for the activation of microorganisms were according to MTCC protocol.

The DMSO (1%) alone was used as a control. Ciprofloxacin and Norfloxacin for bacterial micro-organism and fluconazole for fungal micro-organism were used as standard drug for comparison. The results of antimicrobial activity of the synthesized compounds (**4a-4f**) against selected Gram-positive, Gram-negative and fungal strains were illustrated in [Table-3 & 4]. Some of the compounds showed moderate to excellent *in vitro* activity against different Gram-positive, Gram-negative and fungal strain with MIC values between 0.6 and 1.7 µg/mL and zone of inhibition

values between 10.6 to 18.5 mm. Compounds **4e**, **4b** and **4d** exhibited better activity (MIC = 0.8-1.1 µg/mL) and zone of inhibition values between 16.5 to 18.5 mm against different strains. Compound **4e** showed broad spectrum activity against four bacterial strains (MIC = 0.8-0.9 µg/mL, zone of inhibition values 17.1-18.5 mm) and one fungal strains (MIC = 0.9 µg/mL, zone of inhibition value 16.6 mm) and was proved to be the most active compound of the series. Agar plates for diameter of zone of inhibition and MIC were shown in [Fig-1] & [Fig-2] for most active compound **4e** and showed that compound **4e** has almost similar activity as compared with standard drug. The preliminary structure activity relationship was discussed. The compounds substituted with halogens on the phenyl ring at *para* & *meta* positions enhanced the antibacterial activity (Br>Cl) as seen in the case of compounds **4e, 4c & 4d**.

Table-3 *Zone of inhibition (mm) of microbial strains by different synthesized compounds (**4a-4f**)*

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	4.8±0.2 9.8±0.2 13.6±0.2	4.8±0.2 9.5±0.2 13.5±0.2	4.6±0.2 9.3±0.2 13.3±0.2	4.5±0.2 9.1±0.2 13.1±0.2	4.1±0.2 8.5±0.3 12.0±0.0
4b		50 100 200	7.8±0.2 11.3±1.1 17.5±0.5	7.8±0.2 11.3±1.1 17.5±0.5	7.5±0.0 10.5±0.6 17.1±0.2	7.5±0.0 10.5±0.6 17.1±0.2	7.0±0.0 10.0±0.6 16.5±0.3
4c		50 100 200	5.6±0.5 9.5±0.5 14.0±1.0	5.5±0.2 9.3±0.2 13.6±0.2	5.5±0.2 9.1±0.2 13.0±0.3	5.1±0.2 9.0±0.0 12.8±0.2	4.5±0.0 8.1±0.2 12.1±0.2
4d		50 100 200	7.8±0.2 11.3±1.1 17.5±0.5	7.1±0.2 10.6±0.2 17.3±0.2	7.5±0.2 10.5±0.6 17.1±0.2	7.3±0.2 10.3±0.5 16.8±0.2	7.0±0.0 10.0±0.6 16.5±0.3
4e		50 100 200	7.8±0.2 12±1.0 18.5±1.0	7.1±0.2 11.8±0.2 18.1±0.2	7.1±0.2 11.5±0.3 17.5±0.3	7.0±0.0 11.1±0.2 17.1±0.2	6.3±0.2 10.5±0.3 16.6±0.3
4f		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
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%)		_	_	_	_	_

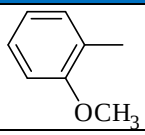
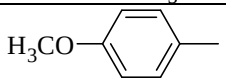
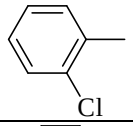
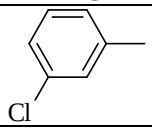
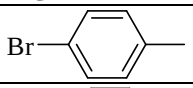
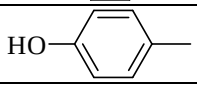
Compound 4e *B. subtilis*

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

Compound 4e *B. subtilis*

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

Table 4 *Minimum inhibitory activity (MIC, µg/ml) of synthesized compounds (**4a-4f**)*

Compound	R	Gram positive Bacteria		Gram negative Bacteria		Fungus
		Bs	Sa	Ec	Pa	At
4a		1.3	1.3	1.4	1.4	1.5
4b		0.9	0.9	1.0	1.0	1.1
4c		1.1	1.1	1.2	1.2	1.3
4d		0.9	0.9	1.0	1.0	1.1
4e		0.8	0.8	0.8	0.8	0.9
4f		1.5	1.5	1.6	1.6	1.7
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%)	_	_	_	_	_

It is noteworthy that compound **4b** (p-OCH_3) having electron donating substituents on the phenyl ring at *para* positions was found to enhance the antibacterial activity. A decrease in the antimicrobial activity was due to presence of –OH group on the phenyl ring at *para* position as seen in the case of compound **4f**. An increase in the antifungal activity was also observed for the compounds having halogen substitution but the level of activity in many cases was found to be less than that of the antibacterial activity.

Compound **4e** was found to have the most potent inhibitory capacity as it has *para* bromo substitution at phenyl ring. These results indicate that further optimization of thiazolidine-2,4-dione derivatives may provide a new class of broad spectrum antimicrobial agents.

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, DMSO- dimethyl sulphoxide.

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

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