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Research Article

SYNTHESIS OF 5-ARYLIDINE-2-(3, 4, 5-TRIMETHOXYPHENYL)-3-(4-PHENYLTHIAZOL-2-YL) THIAZOLIDIN-4-ONE DERIVATIVES AS A NOVEL CLASS OF ANTIMICROBIAL AGENTS

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ABSTRACT

The present work describes the synthesis and *in vitro* antimicrobial evaluation of 5-arylidine derivatives of 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**). The reaction of 2-amino-4-phenylthiazole, 3,4,5 trimethoxybenzaldehyde and mercaptoacetic acid in presence of DCC yielded 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**) and further 5-arylidne derivatives (**5a-5k**) were synthesized by the subsequent reaction of **4** with different aryl aldehydes. All the synthesized compounds were characterized by standard spectroscopic techniques, evaluated their antibacterial and antifungal activity by agar well diffusion method. The compounds showed some interesting antibacterial activity. The substitution of 5-arylidne groups on new thiazolidinone (**4)** have resulted enhanced antibacterial activity. The compounds showed moderate antifungal activity in a scattered manner.

Keywords: 2-Amino-4-arylthiazole, Antimicrobial activity, 5-arylidine, 4-Thiazolidinone and 3,4,5 trimehoxybenzaldehyde.

INTRODUCTION

4-Thiazolidinone, a mimic of bioactive β-lactam with additional sulphur atom is most privileged scaffolds among vast array of thiazolidine heterocycles. It has been widely recognised as wonder class in the field of medicinal chemistry due its ability to accommodate a wide variety of bioactive motifs in its unique structural framework[1]. It was explored that the substitution of different bioactive entities at each position (1, 2, 3 and 5) of 4-thiazolidinone motif imparts preferential specificities in their biological responses[2]. In turn, 5-benzylidene derivatives of 4-thiazolidinone are of great interest for the medicinal chemists because of their potential biological activities *viz* antiviral[3], antimicrobial[4], cardiac[5] and anti-inflammatory activities[6]. This variety in the biological responses and their diverse reactions has attracted the attention of many researchers to explore this skeleton for multiple potential biological activities.

In search of bioactive entities for the new series of 4-thiazilidinone, we found that thiazole is a versatile motif comprising biocidal unit (S-C=N) which is easily metabolised inside the body and is already been a parent structure in many synthetic drugs that have been used for the treatment of infective diseases[7]. Especially, 2-amino-4-arylthiazole has significant place in research areas in synthetic as well as in pharmaceutical chemistry because of its potent and significant pharmacological activities[8]. On the other hand, the synthesis of 3,4,5 trimethoxybenzene derivatives become increasingly important in organic synthesis with respect to their widespread potential application such as antibacterial activity [9], antitumor activity[10], antiviral activity[11], antinucleoplastic[12], antipsychotic activity [14], and antagonistic activity[15]. It is likely that the number of drugs containing the trimethoxybenzene group will continue to increase and would facilitate broaden clinical applications.

Henceforth, considering the huge biological importance of 2-amino-4-arylthiazoles and 3, 4, 5-trimethoxybenzene ring system, we have decided to synthesize a new series of 4-thiazolidnone comprising 2-amino-4-arylthiazoles and 3, 4, 5 -trimethoxybenzene ring system in a single structural framework of 4-thiazolidinone and to study effect of different 5-benzylidine substitution on their antibacterial and antifungal activity.

MATERIALS AND METHODS

All the starting materials and reagents were obtained from Aldrich (USA), Spectrochem Pvt. Ltd (India) and Rankem Pvt. Ltd. (India) and were used without further purification. The course of reaction and purity were ascertained by performing TLC. Melting points were determined in open capillaries and are uncorrected. IR spectra were recorded in JASCO FT-IR 4100 spectrophotometer with KBr and only significant absorption levels (reciprocal centimeter) are listed. 1H-NMR spectra were recorded at 300 MHz Bruker FT-NMR Spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as internal standard.

Synthesis:

General procedure for the synthesis of 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (4):

In a 100 mL round bottom flask, 2-amino- 4- phenyl-thiazole (4 g, 0.027 mol) and 3,4,5-trimethoxybenzaldehyde (10.70 g, 0.0546 mol) were stirred in THF under ice-bath for 5 min, followed by addition of mercaptoacetic acid (7.54 mL, 0.082mol). After 5 min, DCC (6.8 g, 0.033 mol) was added to the reaction mixture at 0°C and the reaction mixture stirred for additional 1-3 hours at room temperature. Formed DCU was removed by filtration, filtrate was concentrated to dryness under reduced pressure and the residue was taken up with ethyl acetate. The organic layer was washed with 5 % aq. citric acid, water, 5 % aq. sodium hydrogen carbonate and then with brine. The organic layer was dried over sodium sulphate and the solvent removed under vacuum to give the crude product, which was purified by recrystallization from 2:1 petroleum ether-diethyl ether.

General procedure for the preparation of 5-arylidine-2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (5a-5k):

A solution of 4-thiazolidinone (1g, 0.0027mol) and aryl aldehyde (0.0054 mol)) in glacial acetic acid (25 mL) was refluxed for about 4-8 hrs, in the presence of sodium acetate (0.57 g, 0.0070mol), cooled, poured into ice cold water to give crude product which was recrystalised using benzene/ethyl acetate.

Antimicrobial assay

Antibacterial assay

All the synthesized compounds *viz* 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**) and their respective 5-arylidine derivatives (**5a-5k)** were evaluated for their *in vitro* antibacterial activity against gram +ve and gram -ve bacterial strains *viz, Escherichia coli, Staphylococcus aereus, Klebeliessa pneumoniae,* and *Pseudomonas areginosa* by using the agar well diffusion method[16]. The bacterial strains were maintained on LB agar medium at 28 °C. The bacteria were grown in LB broth, centrifuged at 10,000 rpm for 5 minutes; a pellet was dissolved in double distilled water and used to inoculate the plates. The autoclaved molten media (20

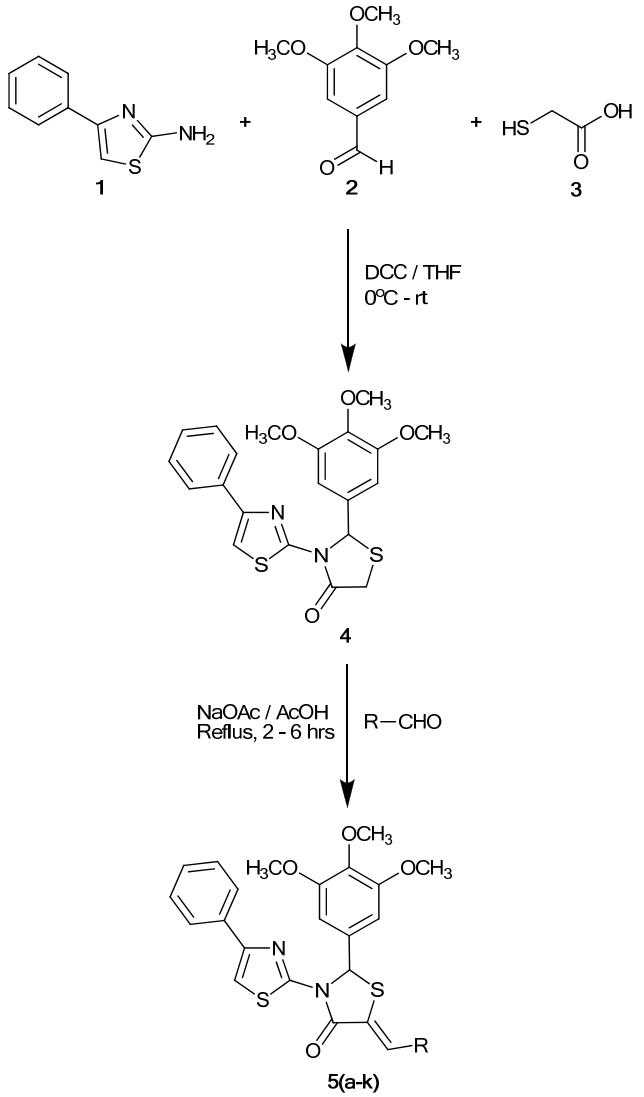
mL) was poured in each 90 mm sterilized petriplate and allowed to solidify. A circular well of diameter 6 mm was made exactly at the center of the plates by using cork borer and each well was filled with 0.1 mL of the test solution (10mg/mL). Streptomycin and DMSO were used as positive control and negative control respectively. All the compounds were tested in triplicate and inhibition zones were measured in mm after 24 hrs of incubation.

Antifungal activity

In vitro antifungal assays of all the synthesized compounds *viz* 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**) and their respective 5-arylidine derivatives (**5a-5k)** were performed against fungal strains *Aspergillus niger* and *Aspergillus flavus* using agar well diffusion method[17]. The fungal cultures were raised by growing on potato dextrose agar media at pH 7.4 for six days at 25 °C. The spores were harvested in sterilized normal saline (0.9% NaCl in distilled water) and its concentration was adjusted to 1 x 10^6 / mL with a Haemometer. The autoclaved molten media (20 mL) was poured in each 90 mm sterilized petriplate and allowed to solidify. To study the growth response of fungi species, 0.4 mL of the synthesized compound solution (5mg/mL) was poured into each plate and spread over the agar media. 10 µL spore suspension was poured in to small depression made at the center of the plate and kept for 6 days at 25 °C. After six days of incubation, the fungal growth were measured and compared with the control. The control plates contained only DMSO for which fungal growth is taken as 100% (without inhibition). The fungal activity of all the synthesized compounds was assessed by comparing the zone of fungal growth in treated plates with that of control plates in mm.

RESULTS AND DISCUSSION

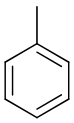
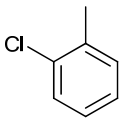
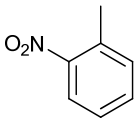
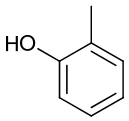
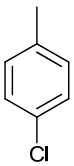
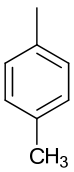
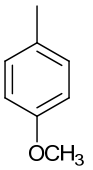
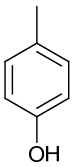
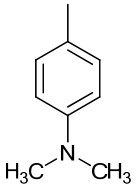
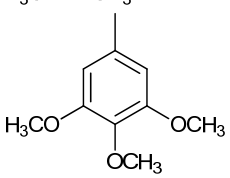
The synthesis of the new compounds was carried out as outlined in **Scheme 1**. The starting compounds 2-amino-4-phenylthiazole (**1**) was prepared according to the literature method by refluxing acetophenone, thiourea and iodine. Then, 2-amino-4-phenylthiazole (**1**) was reacted with 3,4,5 trimethoxybezaldehyde (**2**) and mercaptoacetic acid (**3**) in presence of DCC to afford 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**). Further, reaction of **4** with various aryl aldehydes in presence of glacial acetic acid and sodium acetate under reflux condition gave corresponding 5-arylidine derivatives (**5a-k**). The structures of all the synthesised compounds were confirmed by the m.p., IR, ^{1}H-NMR and data are presented in **Table 1**.

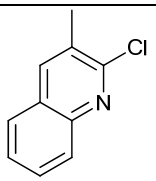


Scheme 1: Synthesis of 5-arylidine-2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-ones (5a-5k)

Where R = various aryl moieties

Table 1: Physical and analytical data of 2- (3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-ones (4) and their 5-arylidine derivatives (5a-5k)

Entry	R	Mol. formula Mol. weight	m. p. (°C)	Yield (%)	IR cm^{-1}	^{1}H-NMR ($CDCl_3$) δ ppm
4	---	$C_{21}H_{20}N_2O_4S_2$ 428.09	146-149	92	1717, 1510, 1460, 1045	7.30-7.82 (m, 6H, Ar-H), 7.03 (s, 2H, Ar-H), 6.43 (1H, s, CH-thiazolidinone ring), 3.73 (6H, s, *m*-OCH_3), 3.60 (3H, s, *p*-OCH_3), 4.01-4.06 (d, $1H_a$, CH_2), 4.44 -4.50 (d, $1H_b$, CH_2).
5a		$C_{28}H_{24}O_4N_2S_2$ 514.45	181-184	68	1720, 1579, 1511, 1462, 1044	7.21-7.85 (m, 11H, Ar-H), 7.72 (s, 1H, CH-arylidine), 7.03 (s, 2H, Ar-H), 6.05 (1H, s, CH-thiazolidinone ring), 3.80 (6H, s, *m*-OCH_3), 3.74 (3H, s, *p*-OCH_3).
5b		$C_{28}H_{23}O_4N_2S_2Cl$ 548.90	185-187	67	1721, 1580, 1512, 1463, 1047, 816	8.03 (s, 1H, CH-arylidine), 7.21-7.85 (m, 10H, Ar-H), 7.03 (s, 2H, Ar-H), 6.10 (1H, s, CH-thiazolidinone ring), 3.84 (6H, s, *m*-OCH_3), 3.77 (3H, s, *p*-OCH_3).
5c		$C_{28}H_{24}O_6N_3S_2$ 559.45	185-188	53	3400, 1722, 1582, 1511, 1462	8.32 (s, 1H, CH-arylidine), 7.35-8.21 (m, 10H, Ar-H), 7.03 (s, 2H, Ar-H), 6.15 (1H, s, CH-thiazolidinone ring), 3.85 (6H, s, *m*-OCH_3), 3.76 (3H, s, *p*-OCH_3).
5d		$C_{28}H_{24}O_5N_2S_2$ 530.44	195-198	60	3416, 1722, 1570, 1512, 1461,	11.83 (s, 1H, -OH), 7.93 (s, 1H, CH-arylidine), 6.85-7.73 (m, 10H, Ar-H), 7.03 (s, 2H, Ar-H), 6.09 (1H, s, CH-thiazolidinone ring), 3.84 (6H, s, *m*-OCH_3), 3.76 (3H, s, *p*-OCH_3).
5e		$C_{28}H_{23}O_4N_2S_2Cl$ 548.90	180-183	75	1720, 1572, 1510, 1461, 1044, 815	7.21-7.85 (m, 10H, Ar-H), 7.72 (s, 1H, CH-arylidine), 7.03 (s, 2H, Ar-H), 6.06 (1H, s, CH-thiazolidinone ring), 3.82(6H, s, *m*-OCH_3), 3.74 (3H, s, *p*-OCH_3).
5f		$C_{29}H_{26}O_4N_2S_2$ 530.30	169-172	72	1720, 1571, 1511, 1464, 1045	7.21-7.85 (m, 10H, Ar-H), 7.72 (s, 1H, CH-arylidine), 7.03 (s, 2H, Ar-H), 6.02 (1H, s, CH-thiazolidinone ring), 3.79 (6H, s, *m*-OCH_3), 3.73 (3H, s, *p*-OCH_3), 2.34 (t, 3H, *p*-CH_3)
5g		$C_{29}H_{26}O_5N_2S_2$ 544.53	161-164	80	1719, 1573, 1513, 1462, 1047, 1025	7.02-7.85 (m, 10H, Ar-H), 7.72 (s, 1H, CH-arylidine), 7.03 (s, 2H, Ar-H), 6.00 (1H, s, CH-thiazolidinone ring), 3.86 (3H, s, *p*-OCH3), 3.78 (6H, s, *m*-OCH_3), 3.72 (3H, s, *p*-OCH_3).
5h		$C_{28}H_{24}O_5N_2S_2$ 530.44	181-185	69	3418, 1721, 1570, 1510, 1460	9.43 (s, 1H, -OH), 7.21-7.65 (m, 11H, Ar-H), 7.12 (2H, s, benzylidine), 6.62 (s, 2H, Ar-H), 6.05 (1H, s, CH-thiazolidinone ring), 3.82 (6H, s, *m*-OCH_3), 3.75 (3H, s, *p*-OCH_3).
5i		$C_{30}H_{28}O_4N_3S_2Cl$ 591.90	180-183	52	1723, 1571, 1513, 1464, 1042	6.85-7.73 (m, 10H, Ar-H), 7.12 (2H, s, benzylidine), 7.03 (s, 2H, Ar-H), 6.05 (1H, s, CH-thiazolidinone ring), 3.80 (6H, s, *m*-OCH_3), 3.74 (3H, s, *p*-OCH_3), 3.02 (s, 6H, $(CH_3)_2N$)
5j		$C_{31}H_{30}O_7N_2S_2$ 604.48	183-186	78	1724, 1569, 1512, 1461, 1041, 1028	7.02-7.85 (m, 10H, Ar-H), 7.72 (s, 1H, CH-arylidine), 7.07 (s, 2H, Ar-H), 6.79 (s, 2H, Ar-H), 6.00 (1H, s, CH-thiazolidinone ring, 3.86-3.79 (18H, m, - $(OCH_3)_6$)

5k		$C_{31}H_{24}N_3O_4S_2Cl$ 601.09	172-176	73	1726, 1573, 1512, 1463, 1046	8.62 (s, 1H, CH-arylidine), 7.35-8.04 (m, 10H, Ar-H), 7.03 (s, 2H, Ar-H), 6.05 (1H, s, CH-thiazolidinone ring), 3.81 (6H, s, *m*-OCH_3), 3.75 (3H, s, *p*-OCH_3).

All the synthesized compounds *viz* 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-one (**4**) and their respective 5-arylidine derivatives (**5a-k**) were evaluated for their *in vitro* antibacterial activity and antifungal activity. The results are presented in **table 2**. The synthesized series of 5-arylidine-2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-ones (**5a-k**) have showed some interesting antibacterial activity against bacterial strains tested. The starting compound **4** which comprises bioactive 2-amino-4-phenyl thiazole and 3, 4, 5-trimethoxybenzene moieties together in a single 4-thiazilidinone structural framework has shown moderate antibacterial activity against all the bacterial strains tested. However, subsequent introduction of different arylidine group at position 5 (**5a-5j**) has resulted in enhanced activity of 4-thiazilidinone. Among the 5-benzylidine derivatives, the benzyl group having substitution at *para* position (**5e-5j**) has shown to be more active than the *ortho* substituted derivatives. Even so, some of the *ortho* substituted derivatives (**5b-5d**) have showed highest activity than *para* substituted benzylidene derivatives against certain bacterial strains *e.g.,* **5d** against *Staphylococcus aereus* and *Klebsiella pneumoniae,* **5c** against *Pseudomonas areginosa*.

In vitro antifungal activity was performed against *Aspergillus niger* and *Aspergillus flavus*. The starting compound *ie* 2-(3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-ones (**4**) has shown some good fungicidal activity. However, in contrary to the antibacterial activity, 5-benzylidiene derivatives showed scattered fungicidal activity and have very minor as well as dual effect (both negative and positive) on antifungal activity of **4** against *aspergillus flavus* and *aspergillus niger*. Further, *ortho* substituted benzylidene derivatives (**5b-5d**) comparatively more active against *aspergillus flavus* compare to *aspergillus niger* where as conversely, *para* substituted benzylidene derivatives (**5e-5j**) were found to be more active against *aspergillus niger* compare to *aspergillus flavus.*

Table 2: Antibacterial and antifungal activity of 2- (3, 4, 5-trimethoxyphenyl)-3-(4-phenylthiazol-2-yl) thiazolidin-4-ones (4) and their 5-arylidine derivatives (5a-5k)

Entry	Zone of Inhibition (mm)					
	Antibacterial Activity				Antifungal Activity	
	S. aureus	*K. pneumoniae*	*P. aeruginosa*	*E. coli*	*A. flavus*	*A. niger*
4	09	09	10	06	12	15
5a	09	11	13	10	10	12
5b	13	11	13	10	13	11
5c	10	10	18	08	15	12
5d	18	13	13	16	14	15
5e	11	12	12	12	12	12
5f	10	16	17	14	12	16
5g	19	12	16	06	10	17
5h	13	11	19	14	14	16
5i	12	10	18	14	09	17
5j	15	10	16	06	09	16
5k	23	11	18	16	13	18
Gentamycin	21	12	19	20	---	---
Fluconazole	---	---	---	---	19	20

[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

Lastly, while identifying the most active compound from the set of compounds tested, compound **5k**, bearing 2-chloroquinine substitution at 5-aryldine position has emerged as a most active molecule. The compound **5k** has exhibited almost equipotent antibacterial and fungal activity with that of standard antibiotics**.** Indeed, **5k** is found be more active than antibiotic gentamycine against gram-positive bacteria *staphylococcus aureus.*

CONCLUSION

On whole, a new series of 4-thiazolidnone comprising 2-amino-4-arylthiazoles and 3, 4, 5 -trimethoxybenzene ring system in a single structural framework has showed good antibacterial activity and antifungal activity. The substitution of arylidine at position 5 has the potential to impart better activity. Further, any systematic modification on this structural unit might lead to the highly potent antimicrobials.

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