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Facile synthesis and characterization of some new 5-arylidene-thiazolidine-2,4-diones and their antimicrobial evaluation

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A series of novel 5-arylidene-thiazolidine-2,4-diones derivatives have been synthesized by the Knoevenagel condensation of aromatic aldehydes and N-substituted thiazolidinedione-2,4-diones. Use of $KAl(SO_4)_2 \cdot 12(H_2O)$ *i.e.* Alum for the reaction makes this synthesis facile because of several advantageous factors *viz.* non-toxicity, efficient catalytic ability and cheap cost of alum. Substitution of arylidene moiety at the position 5 on the thiazolidine-2,4-diones nucleus has occured using this facile approach. Synthesized derivatives have been characterized using various analytical tools and antimicrobial evaluation thereof has been performed against Gram-positive, Gram negative bacteria and a fungal strain. All synthesized compounds show moderate to very good activity against the microorganisms that they have been tested against.

Keywords: Alum, antimicrobial evaluation, 5-arylidene-thiazolidine-2,4-diones, Knovenagel condensation

Thiazolidine is privileged class of compounds having many biological activities. Thiazolidine-2,4-dione derivatives found as basic pharmacophore for variety of biological activities such as antibacterial and antifungal[1,2], anticancer, analgesic, aldose reductase inhibitors[3], antiinflammatory, antituberculosis, antitumor[4], cardiotonic[5] and antidiabetic activity[6]. Now a days the group of heterocyclic compounds such as thiazolidine-2,4-dione, 2-imino-4-thiazolidinone, 4-thioxo-thiazolidine-2,4-dione and 2-thioxo-1,3-thiazolidine- 4-one (rhodanine) have been found as an interesting candidate to work on because of their pharmacological properties[7-12].

In the recent years, bacterial infections have increased considerably. In human civilization, bacteria are the main source of most deadly diseases and widespread epidemics. Some of the bacterial diseases such as tuberculosis, typhus, plague, diphtheria, typhoid fever, cholera, dysentery, and pneumonia have taken a high toll on humanity[13]. Improper and repeated use of certain antibiotics is the main cause of bacterial resistance. Bacteria become resistant to chemotherapeutic agents by destruction or inactivation of the drug, prevention of the penetration of the target site within the microbe and alteration of drug target sites[14, 15]. Furthermore, antimicrobial therapy for infection control has been focused due to increasing bacterial resistance to antibiotics[16]. Antibiotics are formed synthetically by bacteria and fungi which suppressing the growth of microorganisms[17]. At present, new antibiotics are to be discovered which are active against multidrug-resistant.

Several literature reports are there on thiazolidine conjugates where in they showed number of biological activities on getting various substituents on different positions of thiazolidine ring[18]. Medicinal chemistry is an important field of research for the discovery of new active molecules using heterocyclic moieties that increases biological activity[19]. Introducing arylidene groups at the position 5 of the thiazolidine ring by using alum has also been reported where in alum was used as catalyst and was found as an effective promoter for the synthesis of 5- arylidine-2,4- thiazolidinediones[20]. With this in view strategy has been made to synthesize some novel compounds based on heterocyclic moiety *i.e.* thiazolidine and evaluation thereof for antimicrobial activity against different Gram positive bacteria *e.g. Staphylococcus Aureus* and *Streptococcus Pyogenes* bacteria, Gram negative bacteria *e.g. Escherichia Coli* and *Pseudomonas Aeruginosa*. Further the synthesized conjugates were also studied for their anti fungal activity against *Candida albicans*. To the best of our knowledge this is the first time that modification of the thiazolidine is carried out using various

substituents and derivatives were studied for antimicrobial evaluation.

Results and Discussion

Physicochemical properties

Study indicates the use of solvents is huge in Knoevenagel condensation, but herein present study the conditions of Knovenagel condensation have been customized. The proposed derivatives were synthesized by condensation of aromatic aldehydes and N-substituted thiazolidinedione-2,4-diones using alum yielding 5-arylidene-thiazolidine-2,4-dione derivatives. The reactions were carried out in water using 10 mol% alum as a catalyst, use of an alum is to avoid some hazardous bases and catalyst *e.g.* piperdine, pyridine and some solvents *i.e.* DMF, DMSO and toluene. Several literatures reported the use of alum in organic synthesis since it is non toxic, cost-effective, eco-friendly and easy to handle as a catalyst (Scheme I). The study was carried out using different catalytic amount of alum as shown in Table I. There was no change in time but % yield decreased with 15 mol% alum. On decreasing the amount of alum (5 mol%), the reaction time increases and the %yield decreases. The final products given in Table II with their physical and chemical characteristics which highlights that the compound having hydroxyl groups, nitro, methoxy and halogen groups, go through smooth reactions.

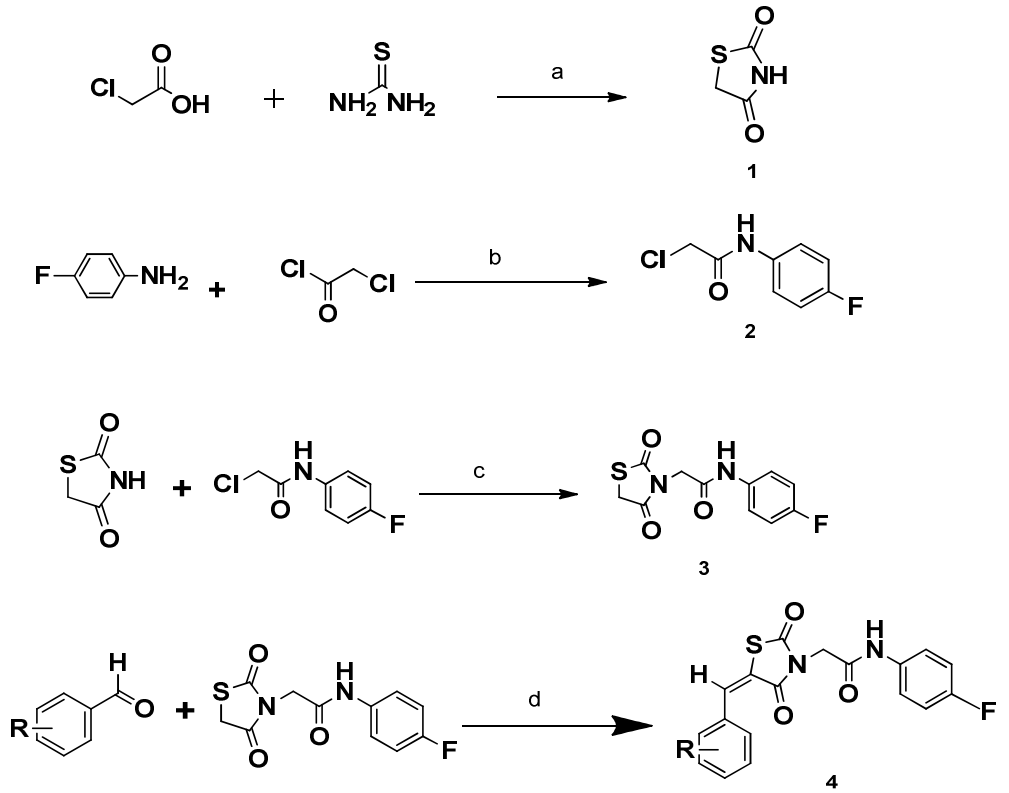
The prograss of reaction was monitored by thin layer chromatography (TLC) which is step wise given in the general synthesis procedure. All the melting points were taken after the recryslization of compound and are given in Table II.

Antimicrobial evaluation

The synthesized compounds were evaluated for their antimicrobial activity against Gram positive and Gram negative bacteria. The results of antibacterial and antifungal activity of 5- arylidene-thiazolidine-2,4-dione derivatives are reported in Table III, in

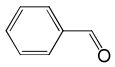
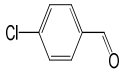
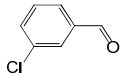
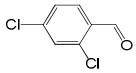
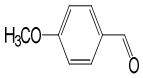
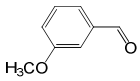
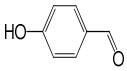
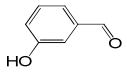
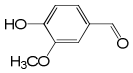
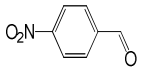
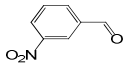
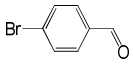
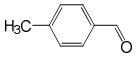
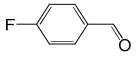
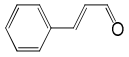
Table I — Reaction condition for the synthesized compound with catalyst under different mol%

Entry	Catalyst (mol%)	Solvent	Time (min)	Yield (%)
1	Alum (5)	H_2O	90	78
2	Alum (10)	H_2O	60	90
3	Alum (15)	H_2O	60	84



Scheme I — Synthesis of derivatives 4. Reagents and conditions: (a) H_2O, 1 h stirring, 4 h reflux; (b) K_2CO_3, DCM, addition at 0 ° and stirring, 6 h RT stirring; (c) K_2CO_3, Acetone, reflux, DMF, $NaHCO_3$, RT, 6-7 h; (d) Alum (10 mol%), H_2O

Table II — Characterization data of the synthesized compounds

Compd	Aldehyde	Time (min)	Yield (%)	m.p. (°C)
4a		60	90	210
4b		70	87	225
4c		70	85	212
4d		60	88	220
4e		80	85	258
4f		70	86	240
4g		80	83	200
4h		90	80	215
4i		80	83	265
4j		60	89	230
4k		70	87	240
4l		90	82	250
4m		80	85	262
4n		60	89	276
4o		90	80	235

comparison with standard drugs, ciprofloxacin and ketoconazole. It has been demonstrated that the placing of arylidine moieties at position 5 of the thiazolidine ring enhanced the antimicrobial activity. Inclusion of electron-withdrawing group such as halogen and nitro on phenyl ring increase the activity. Compounds **4j, 4k, 4l, 4n** which have halogens and nitro groups showed moderate activity towards *S. Aureus* and *S. Pyogenes* whereas good activity against *E. Coli* and *P. Aeruginosa.* Compounds **4e, 4f, 4g, 4h, 4i** having methoxy and hydroxyl group exhibited moderate activity against *E.Coli* and *P.Aeruginosa.* Compounds **4a, 4m, 4o** showed poor activity against all organisms. The compounds **4b, 4c, 4d** have chlorine atoms which improving the lipophilicity of the compound showed excellent activities against all tested strains. Most of the compounds exhibited moderate antifungal activity.

Experimental Section

Materials and methods

All the chemicals (chloro acetic acid, thiourea, 4- fluroaniline, chloroacetyl chloride, potassium carbonate, DCM, DMF, Sodium bicarbonate, acetone, methanol, ethyl acetate) used were supplied by SD fine and were used directly without further purification. Thin layer chromatography (TLC) was performed to monitor the reaction progress by using silica gel 60F254 plates. Melting points of the synthesizes compound were measured in an open capillary in a paraffin bath.

Instrumental analysis

IR spectra of the synthesized derivatives were recorded on perkin-Elmer FT Spectrophotometer. ^{1}H NMR spectra of the compounds were recorded on FT-NMR spectrometer model Bruker Avance-II 400 NMR Spectrometer, using dimethylsulfoxide-d_6 as solvent. The chemical shifts were reported in δ units (ppm). The following abbreviation have been used s (singlet), d (doublet), t (triplet), m (multiplet) for indicate the peak multiplicity in ^{1}H NMR data.

General synthesis procedure

Synthesis of 2,4-thiazolidinedione, 1

To a 250 mL three neck flask outfitted with a thermometer, a stirrer and a condenser were added 2-chloroacetic acid (37.8 g, 0.4 mol), thiourea (30.4 g, 0.4 mol) and water (100 mL). This mixture was stirred at RT for at least 1 h, and then was heated to reflux. The progress of the reaction was monitored by TLC (elution: chloroform-methanol: 20:1) until the reaction was complete (3 h). Then the solution was allowed to cool down at RT. The large amount of pale yellow crystalline solid was filtered and washed with water. It was recrystalized from water (decolorized with active carbon) to give pure white crystals. Yield: 79%, mp. 124 –125˚C.

Synthesis of 2-chloro-N-(4-fluorophenyl)a cetamide, 2

To a well stirred solution of *p*-fluoro aniline (1.13 mL, 0.0106 mol) and K_2CO_3 (5.24 g, 0.038 mol) in dry CH_2Cl_2 (40 mL), chloroacetyl chloride (1 mL, 0.0126 mol) was added drop wise at 0˚C. The mixture was stirred at RT for 6-7 h. After completion of the reaction, excess DCM was evaporated in rotary

Table III — Antimicrobial activity of the synthesized compound **4a-o** as Minimum inhibitory concentration (MIC) in µg/mL

		Gram positive bacteria		Gram negative bacteria		Fungi
Compd	Ar	S.Aureus	S.Pyogenes	E.Coli	P.Aeruginosa	C.albicans
4a	H	64	32	64	64	16
4b	4-Cl	4	4	8	8	16
4c	3-Cl	8	8	8	8	16
4d	2,4-Cl	4	4	4	8	8
4e	4-OCH_3	32	64	16	32	32
4f	3-OCH_3	32	64	16	32	16
4g	4-OH	64	32	32	16	32
4h	3-OH	64	32	32	16	16
4i	4-OH, 3-OCH_3	32	64	16	16	16
4j	4-NO_2	16	16	8	8	16
4k	3-NO_2	16	16	8	8	16
4l	4-Br	16	16	8	8	32
4m	4-CH_3	32	32	64	32	16
4n	4-F	8	8	16	16	16
4o	$CH=CH_2$	32	64	32	64	16
Ciprofloxacin		4	4	4	4	-
Ketoconazole		-	-	-	-	4

S.A. : S.Aureus, S.P. : S.P.yogenes, E.C. : E.Coli, P.A. : P.Aeruginosa, C.a. : C.albicans - Not tested

evaporator. The solid formed was collected and washed with excess of water and crystallized by using methanol/acetone. Yield: 80%, mp. 134 - 136˚C.

Synthesis of 2- (2,4-dioxothiazolidin-3-yl) -N-(4-fluorophenyl) acetamide, 3

A mixture of thiazolidine-2,4-dione (0.88 g, 0.0075 mol), 2-chloro-N-(4-fluorophenyl)acetamide (0.93 g, 0.005mol) and sodium bicarbonate (0.84 g, 0.01mol) were dissolved in DMF (15 mL) and stirred at RT for overnight. After completion of the reaction as confirmation by TLC (eluent 7:3/hexane–ethyl acetate) the reaction mixture was poured into water (125 mL), extracted with ethyl acetate and the solvent distilled under vacuum to obtain solid and crystallized by using methanol/acetone. Yield: 80%, mp. 189 - 190˚C.

Synthesis of 5-benzylidene-2,4-thiazolidinedione derivatives, 4

A mixture of aromatic aldehyde (0.001 mol), 2-(2,4-dioxothiazolidin-3-yl)-N-(4-fluorophenyl) acetamide (0.001 mol), and water (10 mL) were taken in flask and to this alum (10 mol%) was added. The reaction mixture was heated to reflux for the appropriate time given in Table II. The progress of reaction was monitored by TLC using ethyl acetate: n-hexane (1:9) as a solvent system. After the completion of the reaction, the mixture was cooled to RT and poured into crushed ice, stirred, and the solid product obtained, was filtered, and recrystalized from ethanol to give 5-benzylidene-2,4-thiazolidenedione **4a-o** in good to excellent yield.

Spectral analysis

FT-IR and ^{1}H NMR spectral analysis

Spectroscopic analysis were carried out for the optimized product and spectra for the synthesized compounds were compared to that of reference. Detailed discussion on the spectral analysis of some selected compounds given below.

2-(5-Benzylidene-2,4-dioxothiazolidin-3-yl)-N-(4-fluorophenyl)acetamide, 4a: IR (KBr): 2957 (CH, aliphatic), 3038 (CH, aromatic), 1735 (C=O), 1580 (C=C), 690 (CS), 1175 (CN), 3250 cm^{-1} (NH); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.52 (s, 2H, CH_2), 7.98 (s, 1H, -CH=), 10.34 (s, 1H, NH), 7.33-7.60 (m, 5H, Ar-H), 7.60 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H).

2-(5-(4-Chlorobenzylidene)-2,4-dioxothiazolidin-3-yl)-N-(4-fluorophenyl)acetamide, 4b: IR (KBr): 2945 (CH, aliphatic), 3035 (CH, aromatic), 1733 (C=O), 1590 (C=C), 695 (CS), 1160 (CN), 3220 (NH), 738 cm^{-1} (CCl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.64 (s, 2H, CH_2), 8.32 (s, 1H, -CH=), 10.45 (s, 1H, NH), 7.69 (d, 2H, Ar-H), 7.34 (d, 2H, Ar-H), 7.68 (d, 2H, Ar-H), 7.44 (d, 2H, Ar-H).

2-(5-(2,4-Dichlorobenzylidene)-2,4-dioxothiazolidin-3-yl)-N-(4-fluorophenyl)acetamide, 4d: IR (KBr): 2955 (CH, aliphatic), 3064 (CH, aromatic), 1745 (C=O), 1590 (C=C), 685 (CS), 1170 (CN), 3235 (NH), 748 cm^{-1} (CCl); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.75 (s, 2H, CH_2), 8.36 (s, 1H, -CH=), 10.34 (s, 1H, NH), 7.48 (s, 1H, Ar-H), 7.32 (d, 1H, Ar-H), 7.61 (d, 1H, Ar-H), 7.60 (d, 2H, Ar-H), 7.22 (d, 2H, Ar-H).

N-(4-Fluorophenyl)-2-(5-(3-methoxy–benzylidene)-2,4-dioxothiazolidin-3-yl)acetamide, 4f: IR (KBr): 2935 (CH, aliphatic), 3055 (CH, aromatic), 1735 (C=O), 1595 (C=C), 690 (CS), 1175 (CN), 3240 (NH), 1270 cm^{-1} (COC); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.86 (s, 2H, CH_2), 8.36 (s, 1H, -CH=), 10.55 (s, 1H, NH), 3.87 (s, 3H, OCH_3), 7.20 (s,1H, Ar-H), 7.13 (d,1H, Ar-H), 7.44 (t, 1H, Ar-H), 7.19 (d, 1H, Ar-H), 7.65 (d, 2H, Ar-H), 7.26 (d, 2H, Ar-H).

N-(4-Fluorophenyl)-2-(5-(4-nitrobenzylidene)-2,4-dioxothiazolidin-3-yl)acetamide, 4j: IR (KBr): 2945 (CH, aliphatic), 3044 (CH, aromatic), 1733 (C=O), 1590 (C=C), 695 (CS), 1170 (CN), 3250 (NH), 1530 cm^{-1} (NO); ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.77 (s, 2H, CH_2), 8.22 (s, 1H, -CH=), 10.82 (s, 1H, NH), 8.15 (d, 2H, Ar-H), 8.34 (d, 2H, Ar-H), 7.58 (d, 2H, Ar-H), 7.34 (d, 2H, Ar-H).

Antimicrobial activity

The newly synthesized compounds were evaluated for their antibacterial activity by Broth Dilution Method. It is one of the non automated *in vitro* bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in tubes. *Staphylococcus Aureus* (MTCC 96) and *Streptococcus Pyogenes* (MTCC 96) from Gram positive group of bacteria and *Escherichia Coli* (MTCC 443) and *Pseudomonas Aeruginosa* (MTCC 1688) from Gram negative group of bacteria for antibacterial activity and yest *Candida albicans* (MTCC 227) used for evaluation of antifungal activity. All MTCC Cultures were tested against synthesized and reference standard drugs. Mueller Hinton Broth was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum size for test strain was adjusted to 108 Cfu [Colony Forming Unit] per milliliter by comparing the turbidity. Ciprofloxacin was used as the reference standard for antibacterial activity and Ketoconazole was used as the reference standard for antifungal activity. DMSO was used as diluents to get desired concentration of drugs to test upon standard bacterial strains.

Serial dilutions were prepared in primary and secondary screening. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37ºC overnight. The tubes are then incubated overnight. The MIC of the control organism was read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. The amount of growth from the control tube before incubation [which represents the original inoculum] was compared.

Each synthesized drug was diluted obtaining 2000 microgram /mL concentration, as a stock solution. In primary screening 1000 micro/mL, 500 micro/mL, and 250 micro/mL concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further diluted to obtain 200 micro/mL 100 micro/mL, 50 micro/mL, 25 micro/mL, 12.5 micro/mL, 6.250 micro/mL, and concentrations for secondary screening. The highest dilution showing at least 99 % inhibition zone was taken as MIC.

Conclusions

A series of innovative 5-arylidene-thiazolidine-2,4-diones were synthesized by the knoevenagel condensation using alum as a catalyst. All compounds were tested against Gram-positive, Gram-negative bacteria and *C. albicans*. Synthesized compounds were characterized in terms of physicochemical characterization and also by using some different analytical techniques *i.e.* IR and ^{1}H NMR spectroscopy. Present study showed a sort of structural function relationship amongst the synthesized derivatives. It was noted that the compounds containing chlorine in the molecule showed the excellent antibacterial activity that indicates the potential of chlorine towards the bacteria. Furthermore, it is to be noted that attaching an electron-withdrawing functional group *e.g.* halogen or nitro on phenyl ring played a crucial role to enhance the activity. In conclusion, the present study revealed a facile method to synthesize a proposed 5-arylidene-thiazolidine-2,4-diones conjugates using

alum as a catalyst. In addition the proposed method is having many advantages *e.g*. easy work up procedure, better yields, reduced reaction time. Furthermore, the catalyst used is easily available, cost-effective, non-toxic and eco-friendly.

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