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Synthesis and antimicrobial activity of 5-arylidene-3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-ones

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ABSTRACT

The condensation of 3-chloro-4-fluoroaniline **(1)** with chloroacetyl chloride in refluxing benzene in the presence of anhydrous K_2CO_3 gives 2-chloro-N-(3-chloro-4-fluorophenyl)acetamide **(2)**. Compound **(2)** on treatment with KSCN in refluxing acetone yield 3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one **(3)**. Compound **(3)** on condensation with various aromatic aldehydes affords a series of 5-arylidene-3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one **(4a-i)**. The structures of all the synthesized compounds were confirmed by spectral data and have been screened for antibacterial and antifungal activity.

KEYWORDS

Thiozolidin-4-one;
3-chloro-4-fluoroaniline;
Antimicrobial activity.

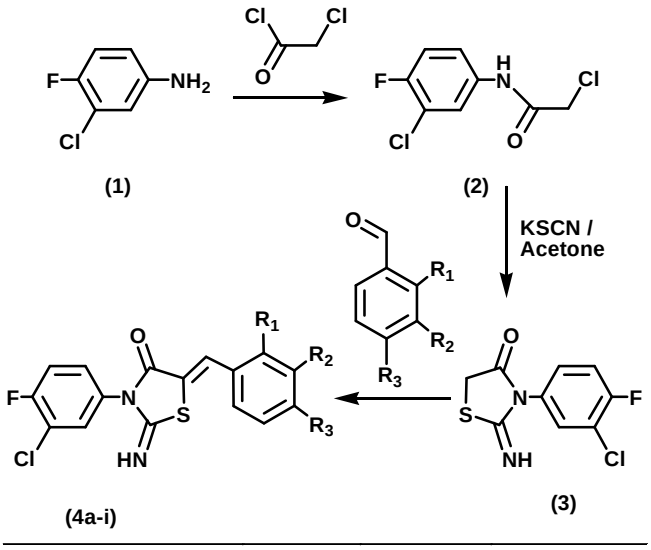
INTRODUCTION

Heterocycles bearing nitrogen, sulphur and thiazole moieties constitute the core structure of a number of biologically interesting compounds. The chemistry of thiazolidin-4-one ring system is of considerable interest as it is a basic structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities[1]. Thiazolidin-4-one derivatives are known to exhibit diverse bioactivities such as antidiarrheal[2], anticonvulsant[3], antimicrobial[4], antidiabetic[5], antihistaminic[6], anticancer[7], antiHIV[8], Ca^{2+} channel blocker[9], PAF antagonist[10], cardioprotective[11], antiischemic[12], cycloxygenase inhibitory[13], anti-platelet activating factor[14], non-peptide thrombin receptor antagonist[15] and tumor necrosis factor-α antagonist activities[16]. Also 2-imino-thiazolidin-4-ones have been found to have antifungal activity[17-19]. Moreover, literature survey reveals that substituted benzothiazole possess antimicrobial and various other pharmacological activities like diuretic[20], anticancer[21], antiulcer[22] and antihistamine[23]. Hence it is thought of interest to accommodate thiazolidin-4-one and 3-chloro-4-fluoroaniline moieties in single molecular framework and screen them for their antimicrobial activity.

RESULTS AND DISCUSSION

3-Chloro-4-fluoroaniline **(1)** on condensation with chloroacetyl chloride in the presence of anhydrous K_2CO_3 as base and benzene as solvent gives 2-chloro-N-(3-chloro-4-fluorophenyl)acetamide **(2)**. The IR spectrum of compound **(2)** exhibited a peak at 3220 cm^{-1} due to NH stretching, 1712 cm^{-1} due to CONH and 1200 cm^{-1} due to C-F groups. ^1H NMR spectrum of compound **(2)** exhibited a peak at δ 3.98 due to two CH_2 protons, a broad peak at δ 10.21 due to NH proton (D_2O exchangeable) and a multiplet in between 7.12

and 8.11 for aromatic proton. The structure of **(2)** was evident by the appearance of molecular ion peak at m/z 222 in its mass spectrum. The compound **(2)** on reaction with KSCN in refluxing acetone yield 3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one **(3)**. The IR spectrum of compound **(3)** exhibited a peak at 2980 cm^{-1} due to NH stretching, 1732 cm^{-1} due to C=O and 1520 cm^{-1} due to C=NH groups. The ^1H NMR spectrum of same compound exhibited a peak at δ 3.60 due to two CH_2 protons, a broad peak at δ 4.70 due to C=NH proton (D_2O exchangeable) and a multiplet in between 7.12 and 8.10 for aromatic proton. The structure of **(3)** was evident by the appearance of molecular ion peak at m/z 244.17 in its mass spectrum. Condensation of compound **(3)** with various aromatic aldehydes afforded a series of 5-arylidene-3(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one **(4a-i)**. The IR spectrum of compound **(4a)** which exhibited peaks at 2985, 1729 and 1521 cm^{-1} corresponds to N-H, C=O and C=NH stretching absorption frequencies respectively. The ^1H NMR spectrum of compound **(4a)** which exhibited a multiplet in the region 7.12 and 8.12 δ ppm due to nine aromatic protons and a singlet at 4.63 δ ppm corresponds to one protons of C=NH groups. As an additional proof the mass spectrum of compound **(4a)** which exhibited a molecular ion peak at m/z 332.05 was indicated for its formation (Scheme 1).



Compound	R_1	R_2	R_3
4a	-H	-H	-H
4b	-H	-H	$-OCH_3$
4c	-OH	-H	-H
4d	-H	-H	-OH
4e	-Cl	-H	-H
4f	-H	-H	-Cl
4g	-H	-Br	-H
4h	-H	$-NO_2$	-H
4i	-H	-H	$-NO_2$

Scheme 1

Antibacterial activity

A Cup plate method using Hi-Media agar medium was employed to study the antibacterial activity of the synthesized compounds against two Gram-positive bacteria, *Staphylococcus aureus*-ATCC 25923, and *Bacillus subtilis*-ATCC 6633 and Gram-negative bacteria, *Pseudomonas aeruginosa*-ATCC 10145, and *Escherichia coli*-ATCC 35218. Preparation of nutrient broth, subculture, base layer medium, agar medium and peptone water is done as per the standard procedure[24]. Each test compound (50 mg) was dissolved in dimethyl formamide (50 mL, 1000 µg/mL) to obtain a sample solution. Sample volume for all the compounds was fixed at 0.1 mL. The cups were made by scooping out agar medium with a sterilized cork borer in a petri dish, which was previously inoculated with the microorganisms. The solution of each test compound (0.1 mL) was added to the cups and petri dishes and were subsequently incubated at 37°C for 48 h. Ampicillin and Streptomycin were used as reference drugs and dimethyl formamide as a control. The zone of inhibition produced by each compound was measured in mm. As shown in the

TABLE 1 : Antibacterial activity of the tested compounds

Compound	Zone of inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
3	13	12	16	16
4a	16	15	11	16
4b	15	15	18	17
4c	12	15	11	11
4d	17	11	12	17
4e	13	15	14	13
4f	11	13	11	11
4g	16	13	15	14
4h	13	16	11	16
4i	10	15	16	11
DMF (control)	00	00	00	00
Ampicillin	22	25	22	19
Streptomycin	23	21	20	24

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TABLE 1. The tested compound showed moderate antibacterial activity then the standard drugs against all microorganisms.

Antifungal activity

The antifungal activity of the synthesized compounds were tested against four different fungi, *i.e. Candida albicans*, *Crysosporium pannical* and *Aspergillus niger* by the filter paper disc technique[25]. The concentration of test compounds were 1000 µg/mL. After 48 h treatment, the zone of inhibition produced by each compound was measured in mm. Griseofulvin was used as the standard antifungal agent and dimethyl formamide as a control. The results of antifungal activity are depicted in TABLE 2. Tested compounds showed slight to moderate antifungal activity.

TABLE 2 : Antifungal activity of the tested compounds

Compound	Zone of inhibition (mm)		
	C. albicans	*C. pannical*	*A.niger*
3	15	16	15
4a	14	14	12
4b	13	13	11
4c	11	09	17
4d	12	10	15
4e	14	09	16
4f	12	16	14
4g	15	12	12
4h	16	17	10
4i	19	15	14
DMF (Control)	00	00	00
Griseofulvin	23	24	22

EXPERIMENTAL

All chemicals were analytical grade, purchased from commercial suppliers and used as received without further purification. Melting points were determined in open capillary and were uncorrected. FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instrument Technologies, Inc. WI, USA). Infrared spectra were recorded between 400 cm^{-1} to 4,000 cm^{-1} in transmittance mode. ^{1}H-NMR and ^{13}C-NMR were obtained in DMSO-d_6 at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei (Varian Company, USA). All chemical shifts were reported in parts per million (ppm) using residual proton or carbon signal in deuterated solvents as internal references. Mass spectra were obtained using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF) by using dithranol as a matrix. Elemental analysis (C, H, N and S) was performed on Perkin Elmer 240 analyzer. The purity of the compound was checked by TLC on silica gel and further purification was performed through column chromatography (silica gel, 60–120 mesh).

Preparation of 2-chloro-*N*-(3-chloro-4-fluorophenyl)acetamide (2)

A solution of 3-chloro-4-fluoroaniline **(1)** (2 mmol) in dry benzene (30 ml) was cooled to 0-5°C. Chloroacetyl chloride (2 mmol) dissolved in dry benzene (10 ml) was slowly added to the solution with vigorous stirring. When the addition was complete, the reaction mixture was refluxed for 3 hr. Benzene was removed *in vacuo*. The residue was washed with 5% $NaHCO_3$ and subsequently with water. The crude product was dried and recrystallised from methanol to give colourless solid **(2)**.

Yield 90%; mp 154-156ºC; IR *v* (cm^{-1}): 3220 (-NH stretching of secondary amine), 1712 (CONH str.), 1200 (C-F str.); ^{1}H-NMR δ (ppm): 10.21 (1H, s, NH, D_2O exchangeable), 7.12-8.11 (3H, m, ArH), 3.98 (2H, s, CH_2); MS, m/z: 222.07(M^+). Analysis. Calcd. For $C_8H_6Cl_2FNO$: C, 43.27%; H, 2.72%; N, 6.31%; found: C, 43.28%; H, 2.68%; N, 5.28%.

Preparation of 3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one (3)

A mixture of 2-chloro-*N*-(3-chloro-4-fluorophenyl)acetamide **(2)** (3 mmol) and KSCN (6 mmol) and dry acetone (100 ml) was refluxed for 9 hr. Excess of acetone was removed *in vacuo* and the residue was stirred with water (50 ml). The solid product was filtered, washed with water and dried. The thiazolidinone **(3)** was obtained by recrystallization from methanol to give colourless solid.

Yield 60%; mp 164-166ºC; IR *v* (cm^{-1}): 2980 (N-H stretching of secondary amine), 1732 (C=O str.), 1520 (C=NH str.); ^{1}H-NMR δ (ppm): 7.12-8.10 (3H, m, ArH), 4.70 (1H, s, C=NH, D_2O exchangeable), 3.60 (2H, s, CH_2); MS, m/z: 244.17 (M^+). Analysis. Calcd. For $C_9H_6ClFN_2OS$: C, 44.18%; H, 2.47%; N, 11.45%; S, 13.11%; found: C, 44.17%; H, 2.48%; N, 11.42%; S, 13.10%.

General procedure for the synthesis of 5-arylidene-3-(3-chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one (4a-i)

3-(3-Chloro-4-fluorophenyl)-2-imino-1,3-thiazolidin-4-one **(3)** (1 mmol) and aromatic aldehydes (2 mmol) are added to a solution of anhydrous sodium acetate (2 mmol) in glacial acetic acid (30 ml). The mixture is refluxed for 5 hr at 110^0C and cooled to room temperature. The solid product is filtered from the mixture, washed with water, dried and recrystallised from 1,4-dioxane.

(4a): Yield 65%; mp 199-202ºC; IR ν (cm^{-1}): 2985 (N-H stretching of secondary amine), 1729 (C=O str.), 1521 (C=NH str); ^{1}H-NMR δ (ppm): 7.12-8.12 (9H, m, ArH and CH-Ar), 4.63 (1H, s, C=NH D_2O exchangeable); MS, m/z: 332.05 (M^+). Analysis. Calcd. For $C_{16}H_{10}ClFN_2OS$: C, 57.75%; H, 3.03%; N, 8.42%; S, 9.64%; found: C, 57.70%; H, 3.00%; N, 8.39%; S, 9.59%.

(4b): Yield 75%; mp 229-231ºC; IR ν (cm^{-1}): 2967 (N-H stretching of secondary amine), 1720 (C=O str.), 1561 (C=NH str.); ^{1}H-NMR δ (ppm): 10.90 (1H, s, CH-Ar), 7.50-8.30 (7H, m, ArH), 4.70 (1H, s, C=NH, D_2O exchangeable), 3.71 (3H, s, Ar-OCH_3); MS, m/z: 362.70 (M^+). Analysis. Calcd. For $C_{17}H_{11}ClFN_2O_2S$: C, 56.28%; H, 3.33%; N, 7.72%; S, 8.84%; found: C, 56.22%; H, 3.39%; N, 7.70%; S, 8.80%.

(4c): Yield 65%; mp 203-205ºC; IR ν (cm^{-1}): 3580 (Ar-OH), 2975 (N-H stretching of secondary amine), 1711 (C=O str.), 1562 (C=NH str.); ^{1}H-NMR δ (ppm): 7.00-8.50 (8H, m, ArH and CH-Ar), 5.06 (1H, s, Ar-OH), 4.69 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 377 (M^+). Analysis. Calcd. For $C_{16}H_{10}ClFN_2O_2S$: C, 55.10%; H, 2.89%; N, 8.03%; S, 9.19%; found: C, 55.11%; H, 2.88%; N, 8.04%; S, 9.17%.

(4d): Yield 70%; mp 210-212ºC; IR ν (cm^{-1}): 3572 (Ar-OH), 2990 (N-H stretching of secondary amine), 1721 (C=O str.), 1552 (C=NH str.); ^{1}H-NMR δ (ppm): 7.40-8.40 (8H, m, ArH and CH-Ar), 5.00 (1H, s, Ar-OH), 4.64 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 377.10 (M^+). Analysis. Calcd. For $C_{16}H_{10}ClFN_2O_2S$: C, 55.10%; H, 2.89%; N, 8.03%; S, 9.19%; found: C, 55.08%; H, 2.90%; N, 8.01%; S, 9.20%.

(4e): Yield 77%; mp 178-180ºC; IR ν (cm^{-1}): 2985 (N-H str.), 1702 (C=O str.), 1531 (C=NH stretching of secondary amine); ^{1}H-NMR δ (ppm): 7.00-8.50 (8H, m, ArH and CH-Ar), 4.72 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 367.06 (M^+). Analysis. Calcd. For $C_{16}H_9Cl_2FN_2OS$: C, 52.33%; H, 2.47%; N, 7.63%; S, 8.73%; found: C, 52.29%; H, 2.23%; N, 7.60%; S, 8.70%.

(4f): Yield 75%; mp 197-199ºC; IR ν (cm^{-1}): 2960 (N-H stretching of secondary amine), 1712 (C=O str.), 1537 (C=NH str.); ^{1}H-NMR δ (ppm): 7.00-8.50 (8H, m, ArH and CH-Ar), 4.77 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 367.16 (M^+). Analysis. Calcd. For $C_{16}H_9Cl_2FN_2OS$: C, 52.33%; H, 2.47%; N, 7.63%; S, 8.73%; found: C, 52.30%; H, 2.33%; N, 7.62%; S, 8.69%.

(4g): Yield 62%; mp 208-210ºC; IR ν (cm^{-1}): 2965 (N-H stretching of secondary amine), 1702 (C=O str.), 1542 (C=NH str.); ^{1}H-NMR δ (ppm): 7.00-8.60 (8H, m, ArH and CH-Ar), 4.65 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 411.60 (M^+). Analysis. Calcd. For $C_{16}H_9BrClFN_2OS$: C, 46.68%; H, 2.20%; N, 6.80%; S, 7.79%; found: C, 46.65%; H, 2.15%; N, 6.76%; S, 7.75%.

(4h): Yield 70%; mp 209-212ºC; IR ν (cm^{-1}): 2979 (N-H stretching of secondary amine), 1712 (C=O str.), 1532 (C=NH str.), 1341-1491 (C-NO_2); ^{1}H-NMR δ (ppm): 7.00-7.41 (8H, s, Ar-H and CH-Ar), 4.75 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 377.17 (M^+). Analysis. Calcd. For $C_{16}H_9ClFN_3O_3S$: C, 50.87%; H, 2.40%; N, 11.12%; S, 8.49%; found: C, 50.84%; H, 2.35%; N, 11.09%; S, 8.51%.

(4i): Yield 73%; mp 219-221ºC; IR ν (cm^{-1}): 2976 (N-H stretching of secondary amine), 1700 (C=O str.), 1542 (C=NH str.), 1355-1498 (C-NO_2); ^{1}H-NMR δ (ppm): 7.40-8.50 (8H, m, ArH and CH-Ar), 4.70 (1H, s, C=NH, D_2O exchangeable); MS, m/z: 377.10 (M^+). Analysis. Calcd. For $C_{16}H_9ClFN_3O_3S$: C, 50.87%; H, 2.40%; N, 11.12%; S, 8.49%; found: C, 50.89%; H, 2.31%; N, 11.10%; S, 8.47%.

CONCLUSION

In conclusion, a new class of thiazolidin-4-one derivatives were synthesized and evaluated as antibacterial agents. The newly synthesized heterocycles exhibited mordarate antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* and significant antifungal activity against *C. albicans*, *C. pannical* and

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A. niger. It can be concluded that these classes of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

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