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RESEARCH ARTICLE

Microwave Assisted Rapid and Efficient Synthesis and Screening of Halogenated Anilin-yl-Substituted Thiazolidin-4-One Derivatives for Antimicrobial Activities.

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ABSTRACT:

3-chloro-4-fluoro phenyl hydrazine was synthesized from 3-chloro 4-fluoro aniline in the presence of hydrazine hydrate. Compound upon treatment with substituted aromatic aldehydes yielded some Schiff's bases(2,2'-disubstituted-1(3-chloro-4-fluoro)phenyl hydrazones)which upon treatment with mercapto acetic acid afforded some disubstituted thiazolidin-4-ones. These thiazolidin-4-ones were treated with some substituted aromatic aldehydes to arrive at 5-arylidene derivatives of substituted thiazolidin-4-ones. The compounds were structurally confirmed by IR, ^{1}H NMR and Mass spectral data. The synthesized compounds were screened for their antibacterial, antifungal and antitubercular activities.

KEYWORDS: Microwave, Schiff's bases, Thiazolidin-4-ones, Phenyl hydrazones, arylidenes.

INTRODUCTION:

There has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core 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 anticonvulsant[2], antimicrobial[3], antifungal[4], anti-inflammatory[5] and anticancer[6] activities. Based upon these facts we embarked upon the synthesis of some halogenated thiazolidin-4-ones.

MATERIALS AND METHOD:

Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was assessed by TLC using chloroform along with methanol, ethyl acetate (9:1) and benzene (8:2) as solvent system and iodine vapours as visualizing agent. FT-IR spectra were recorded on a JascoV410 FT-IR spectrometer by KBr pellet method. ^{1}H NMR were recorded on a Bruker Ultraspec 500 MHz/ AMX 400 MHz spectrometer using DMSO and $CDCl_3$ as solvents and TMS as internal standard. Mass spectra was recorded on a Shimadzu LC-MS 2010.

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a) 3-chloro-4-fluoro phenyl hydrazine (2)
Conventional method:

Concentrated HCl was added dropwise with stirring to hydrazine hydrate which was previously maintained at a temp. of 5-10^{0}C followed by ethylene glycol(25 ml). To the above reaction mixture 3-chloro-4-fluoro aniline(0.01 mol) was added in small portions. The mixture was then refluxed for 2 hrs and then poured into crushed ice. The separated solid was then filtered and recrystallized from ethanol-water mixture.

Microwave method:

Concentrated HCl was added dropwise with stirring to hydrazine hydrate which was previously maintained at a temp. of 5-10^{0}C followed by ethylene glycol(22 ml). To the above reaction mixture 3-chloro-4-fluoro aniline (0.01 mol) was added in small portions. The mixture was charged under microwave for 6 minutes and allowed to cool, on separation of a solid it was recrystallized from ethanol-water mixture.

b) 2,2'-disubstituted-1-(3-chloro-4-fluoro)phenyl hydrazones[Schiff's base] (3a-f).
Conventional method:

To a solution of substituted aldehydes (0.01 mol) in methanol/ethanol (15 ml) ,compound 2(0.01 mol) was added along with few drops of acetic acid and the mixture

was refluxed for 3h. The reaction mixture was cooled, poured into ice-cold water and the separated solid was filtered, dried and recrystallized from ethanol.

Microwave method:
To a solution of substituted aldehydes (0.01 mol) in methanol/ethanol(15 ml) ,0.01 mol of the amine and a few drops of acetic acid were added and the mixture was charged under microwave for 3-4 minutes. The reaction mixture was then cooled to separate out the solid, the solid separated was filtered, dried and recrystallized from ethanol.

1-(3-chloro-4-fluorophenyl)-2-(4-chlorobenzylidene) hydrazine (**3**b)- IR(KBr, cm^{-1}): 3310 (NH); 3028.6 (Ar, C-H); 1705.7 (C=N); 1259.7 (halogens, C-F). ^1H NMR($CDCl_3$,TMS): 4 (s, 1H, NH); 7.1-8.03 (m, 8H, Ar-H); 8.6 (s, 1H, CH).

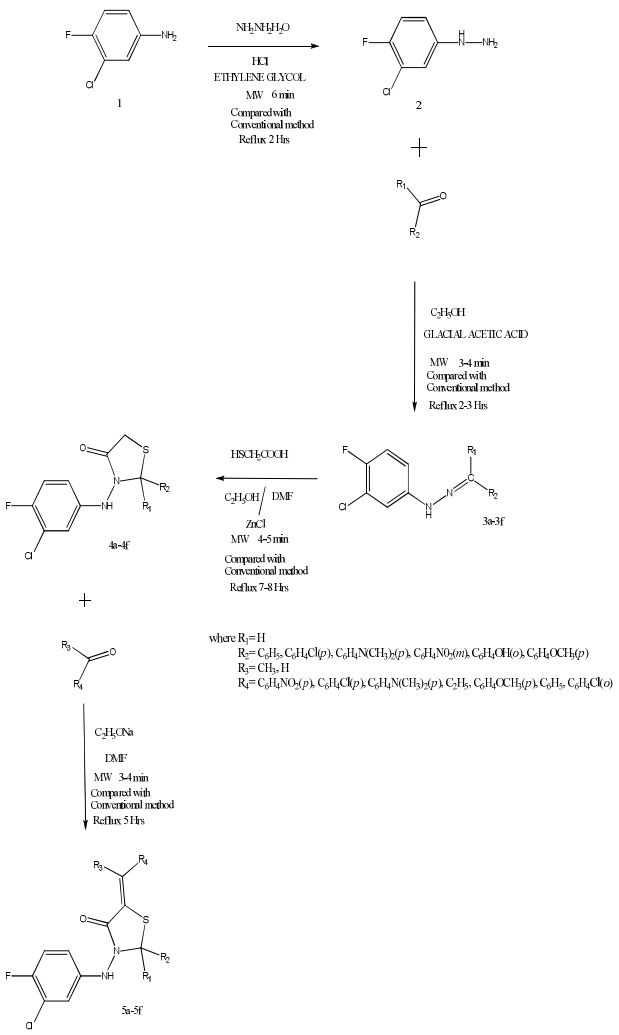
c) 2,2'-disubstituted thiazolidin-4-ones(4a-f)
Conventional method:
A mixture of the schiff's base and mercapto acetic acid(0.01 mol) with a pinch of anhyd. $ZnCl_2$ was refluxed with DMF(10-15 ml) for 7-8 hrs. The mixture was then cooled and poured into crushed ice, the separated solid was then washed with water and recrystallized with a suitable solvent.

Microwave method:
A mixture of the schiff's base and mercapto acetic acid(0.01 mol) with a pinch of anhyd. $ZnCl_2$ was taken in DMF(12 ml) and the mixture was charged under microwave for 5-6 minutes. The mixture was then cooled, and poured into crushed ice if the solid has not separated and washed with water and recrystallized with a suitable solvent.

3-(3-chloro-4-fluorophenylamino)-2-(4-cholorophenyl)thiazolidin-4-one(**4**b)- IR (KBr, cm^{-1}): 3310.2 (NH); 3028.6 (Ar-CH); 1594.8 (C=O); 1351.8 (C-N); 1137.8 (Halogens, C-F). ^1H NMR ($CDCl_3$, TMS): 3.75,3.85 (s, 2H, CH_2); 4 (s, 1H, NH); 5.92 (s, 1H, Methine CH); 6.62-7.70 (m, 7H, Ar-H).

d) 5-arylidene derivatives of thiazolidin-4-ones(5a-f)
Conventional method:
Equimolar amount of thiazolidin-4-ones(0.01 mol) and substituted aldehydes(0.01 mol) in DMF(15-20 ml) was taken in a reaction vessel along with sodium ethoxide(small amount) and the mixture was refluxed for 5-6 hrs. The mixture was then cooled and the solid that precipitates out was recrystallized from ethanol.

Microwave method:
Equimolar amount of thiazolidin-4-ones(0.01 mol) and substituted aldehydes(0.01 mol) in DMF was taken in a reaction vessel along with sodium ethoxide(small amount) and the mixture was charged under microwave for about 2-3 minutes. The mixture was cooled and the solid that precipitates out is recrystallized from ethanol.



Scheme : 5-arylidene derivatives of thiazolidin-4-ones(5a-f)

RESULT AND DISCUSSION:

3-Chloro-4-Fluoro Phenyl hydrazine **2** was prepared by 3-Chloro-4-Fluoro aniline with hydrazine hydrate which was previously maintained at a temp of $5\text{-}10^0$c, in a domestic microwave at 350 watts for 6 mn. The yield was good in comparison with conventional method and time required was comparatively very less. The solid precipitate obtained was purified and recrystalized. Melting points for all the compounds were taken (Table 1). The formation of **2** was confirmed by IR spectrum which has exhibited characteristic N-H stretch at 3300 cm^{-1} (Table-2) , proton NMR (Table-3) and Mass spectra shows the presence of M+1 ion (Table-4).

Antibacterial activity was carried out by cup plate method using sterile nutrient agar medium against *Escherichia coli* and *Staphylococcus aureus*. Ciprofloxacin was used as reference standard. The results revealed that compounds 5b(1), 5d, 5e, 5f showed good antibacterial activity at 75 µg/ml against *E. coli* while compound 5e has shown moderate activity at 75 µg/ml against *S. aureus* (Table 5).

TABLE NO-1: PHYSICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

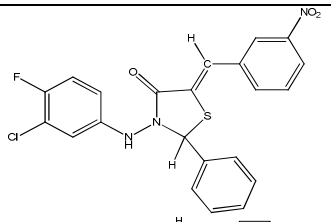
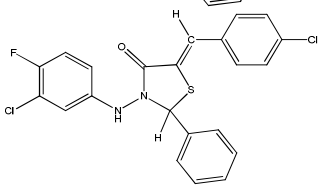
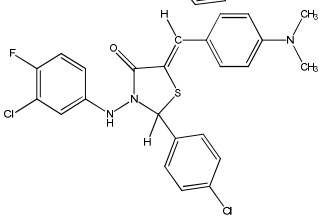
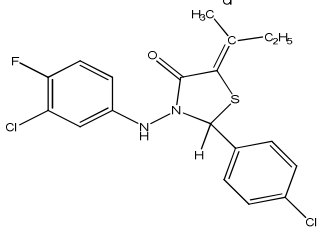
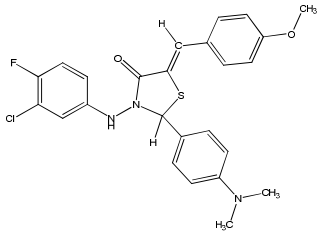
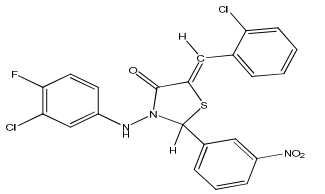
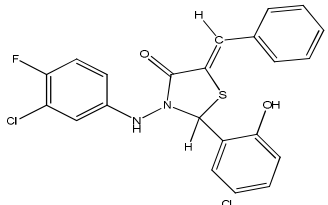
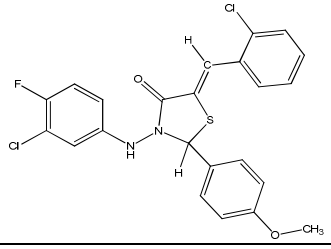
Code	Structural Formula	Physical State	% Yield	M.P ^{0}C	M.F	Mol.Wt.
5a		Yellow Powder	58	183-186	$C_{22}H_{15}Cl_2FN_3O_3S$	455.89
5b		White crystals	59	182-184	$C_{22}H_{15}Cl_2FN_2OS$	445.34
5c		Light Brown Powder	60	184-186	$C_{24}H_{20}Cl_2FN_3OS$	488.40
5d		Brown Transparent crystals	64	185-188	$C_{19}H_{17}Cl_2FN_2OS$	411.32
5e		Brownish White Powder	59	180-183	C25H23Cl FN3O2S	483.99
5f		Yellowish White crystals	60	181-185	C22H14Cl2 FN3O3S	490.33
5g		Brown Powder	63	182-185	C22H16Cl FN2O2S	426.89
5h		Yellow Powder	64	183-187	$C_{23}H_{17}Cl_2FN_2O_2S$	475.36

Table No-2: Spectral Data(I.R)

Code	I.R	Functional Group
5a	3060.48	N-H [Amines(1^0 and 2^0)]
	3002.62	-C-H(Aromatic)
	1764.55	-C=O
	1586.16	R-NO_2(Nitro)
	1494.56	-C=C
	1186.97	-F(Halogens- C-X)
	750.174	-Cl(Halogens- C-X)
5b	3092.30	N-H [Amines(1^0 and 2^0)]
	2917.77	-C-H(Aromatic)
	1699.94	-C=O
	1532.17 and 1347.03	-R-NO_2(Nitro)
	1190.83	-F(Halogens- C-X)
	731.853	-Cl(Halogens- C-X)
5c	3405.67	N-H [Amines(1^0 and 2^0)]
	3050.83	-C-H(Aromatic)
	1695.15	-C=O
	1466.60	-C=C
	1174.44	-F(Halogens- C-X)
	726.066	-Cl(Halogens- C-X)
5d	3588.88	-N-H [Amines(1^0 and 2^0)]
	3091.33	-C-H(Aromatic)
	2984.30	-C-H(Aliphatic)
	1731.76	-C=O
	1493.60	-C=C
5e	3408.57	N-H [Amines(1^0 and 2^0)]
	3147.26	-C-H(Aromatic)
	2886.91	-C-H(Aliphatic)
	1824.33	-C=O
	1492.63	-C=C
	1309.43	-C-N
	1182.15	-F(Halogens- C-X)
	695.212	-Cl(Halogens- C-X
5f	3444.24	N-H [Amines(1^0 and 2^0)]
	3040.23	-C-H(Aromatic)
	1658.48	-C=O
	1604.48	-C=C
	1165.76	-F(Halogens- C-X)
	743.42	-Cl(Halogens- C-X)

Table 3: Spectral Data (NMR)

Code	Chemical Shift Value(δ) in ppm	Proton Nature and No. of protons
5a	8.60	1 H, C-H
	8.03-7.10	8 H, Ar-H
	4.00	1 H, N-H
5b	7.70-6.62	7 H, Ar-H
	5.92	1 H, Methine
	4.0	1 H, N-H
	3.75-3.85	2 H, CH_2
5c	8.70-6.65	11 H, Ar-H
	7.60	1H, CH
	5.92	1 H, Methine
	3.06	4 H, CH_3(Methyl)
5d	7.50-6.88	11 H, Ar-H
	5.92	1 H, Methine
	5.40	1 H, O-H
	4.0	1 H, N-H

Table no-4: MASS SPECTRA

Code	m/z value
5c	489(M+1)

Table no- 5 ANTIBACTERIAL ACTIVITY

Code	Concentration (µg/ml)	Zone of Inhibition (mm)	
		E.Coli	**S.Aureus**
NSS-1(a)		R	R
NSS-2(b)		R	R
NSS-3	50	R	R
NSS-4		6	R
NSS-5		8	R
NSS-6		6	R
STD (Ciprofloxacin)	10	28	
NSS-1(a)		R	R
NSS-2(b)		10	R
NSS-3		R	R
NSS-4	75	12	R
NSS-5		10	7
NSS-6		10	R
STD (Ciprofloxacin)	10	28	

Antifungal activity was carried out by cup plate method (diffusion method) using sabouand dextrose broth medium against *Aspergillus fumigatus* and *Candida albicans*. Fluconazole was used as a reference standard. The results revealed that compounds 5b(1) and 5e showed good antifungal activity at 75 and 50 µg/ml against *A. fumigates* and *C. albicans,* while compounds 5a(1), 5c, 5d, 5e and 5f have shown moderate antifungal activity at all concentrations (Table 6).

Table No-6: ANTIFUNGAL ACTIVITY

CODE	CONCENTRATION (IN µg/ml)	ZONE OF INHIBITION (IN mm)	
		A.Fumigatus	C.Albicans
NSS-1(a)		R	R
NSS-2(b)		9	R
NSS-3	5	R	R
NSS-4		R	R
NSS-5		R	R
NSS-6		R	R
STD-Fluconazole	30	24	
NSS-1(a)		R	R
NSS-2(b)	10	11	13
NSS-3		R	R
NSS-4	10	R	R
NSS-5		10	7
NSS-6		R	R
STD Fluconazole	30	24	

Table No-7: ANTITUBERCULAR ACTIVITY

CODE	CONCENTRATION IN µg/ml		
	25	10	5
NSS-1(a)	--	--	++
NSS-2(a)	--	--	++
NSS-3	--	--	--
NSS-4	--	--	--
NSS-5	--	--	--
NSS-6	--	--	--
CONTROL	Concentration 7.5 µg/ml		
Ciprofloxacin	---		
Pyrazinamide	---		

--- = High sensitivity -- = Sensitive ++= Resistant

Antitubercular activity of the synthesized compounds was carried out on Lowenstein-Jensen egg medium(LJ medium) against *Mycobacterium tuberculosis* -H_{37} RV strain. Ciprofloxacin and Pyrazinamide was used as reference standard. The results revealed that the organism *M. tuberculosis* was sensitive towards the compounds 5c, 5d, 5e and 5f at various concentrations (i.e., 5, 10, 25 µg/ml) and towards compounds 5a(1) and 5b(1) only at concentrations of 10 and 25 µg/ml (Table 7).

CONCLUSION:

A simple, efficient and environmental friendly method for the synthesis of substituted Thiazolidin-4-one derivatives is developed under microwave irradiation technique. This technique is comparatively better than the conventional method in terms of time required and yield obtained.

Screening results have revealed that many of our compounds have shown promising antimicriobial activities. Among all the compounds 5b(1), 5d, 5e, 5f showed good antibacterial activity at 75 µg/ml against *E. coli* while compound 5e has shown moderate activity at 75 µg/ml against *S. aureus.* The results also revealed that compounds 5b(1) and 5e shown good antifungal activity at 75 and 50 µg/ml against *A. fumigatus* and C. *C. albicans,* while compounds 5a(1), 5c, 5d, 5e and 5f have shown moderate antifungal activity at all concentrations.

The compounds 5c, 5d, 5e and 5f at various concentrations (i.e., 5, 10, 25 µg/ml) and compounds 5a(1) and 5b(1) only at the concentrations of 10 and 25 µg/ml have shown good antitubercular activity.

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