Anti-inflammatory, anti-bacterial activity and structure-activity relationships of substitutions on 4-thiazolidinone derivatives - Part-1

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Abstract: Environmentally benign and economically feasible procedures have been adopted for the synthesis of novel biologically potential 4-thiazolidinone derivatives. Purpose built microwave oven and ionic liquids (PTCs) showed wrack improvements in yield, time and cost. The yield of 1st series (01-08) obtained in the ranged from 82.4-94.2% and for 2nd series (09-16) obtained 80.6-92.8%. The compounds (01-16) were applied for anti-inflammatory activity at concentrations of 0.5 and 01 mg/kg in carrageenan induced acute and formalin induced chronic inflammatory procedures in mice and better results were obtained at 0.5 mg/kg dose. Some of the compounds 03, 04, 07, 12, 13 showed remarkable anti-inflammatory activity in both procedures as compared to the standard reference drug 2-(2,6-dichloranilino) phenyl acetic acid (diclofenac). Particularly compound 12 and 13 may be used as a non-steroidal anti-inflammatory drug (NSAID) to reduce inflammation. The compounds (01-16) were screened for their antimicrobial activity (*in-vivo*) and found that the compounds 12, 13 and 14 exhibited comparable or higher antibacterial activity then ciprofloxacin (standard) against *E. coli, S. enteritidis*, *P. aeruginosa, S. aureus* and *B. subtilis.* The compounds of series-2 showed significant activity as compared with ciprofloxacin. These compounds could be lead to the selection and use as efficient antimicrobial agents, especially for the treatment of multi-drug resistant infections.

Keywords: Carrageenan, diclofenac, phase transfer catalyst, 4-thiazolidinone, anti-inflammatory and formalin

INTRODUCTION

Thiazolidinone is an important precursor known to be associated with several biological applications (Allen, 2004 and Barreca, 2001). These observations served as an impetus for the extension of investigation in the field of synthesis of 4-thiazolidione derivatives in the hope of discovering compounds with good pharmacological properties using cost effective and environmentally benign synthetic procedure.

Synthesis

Several different synthetic approaches to synthesize these heterocyclic compounds are being used such as condensation of aromatic aldehyde with pipradinium benzoate refluxing in toluene, the reaction of ammonia or primary amines with carbon disulfide, condensation of aromatic aldehydes, sodium acetate in refluxing glacial acetic acid, by heating the reactants dissolved in toluene at 110°C for 3 days. Unfortunately these developments were made with the presence of varieties of solvents, involving long reaction time, using large quantities of organic solvents but generally give unsatisfactory yields. It is need of the hour to carry out reaction in non toxic solvent or solvent free conditions using cost effective methods. To serve this purpose microwave reaction and environment friendly catalyst (PTCs) has been applied for the formation of different products under simple operational conditions.

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Anti-inflammatory activity

For anti-inflammatory activity; carrageenan induces for acute and formalin induces for chronic inflammation and these are applied to paw edema. A long term administration of anti-inflammatory drugs is necessary for chronic diseases such as osteoarthritis and rheumatoid arthritis. The most widely used non-steroidal anti-inflammatory drugs (NSAID) or steroids suffer from several side effects. Hence, the search for effective anti-inflammatory agents that could be safely used on a long-term basis is a priority (Chen, 2001). Jose, 2002 investigated anti-inflammatory activity of two different extracts of *Uncaria tomentosa* (Rubiaceae) by using carrageenan-induced paw edema model in mice. He used high performance liquid chromatography (HPLC) with a Phenomenex Luna column C18, (particle size 5 µm, length 250 mm and ID 4.6 mm). He used two extracts that were diluted in 1 ml of distilled water in the following concentrations; 500, 200, 100, and 50 mg/kg of the specimen's body weight. Soniamol, 2009 studied anti-oxidative and anti-inflammatory activities of *Ganoderma lucidum* (Aphyllophoromycetideae) from tropical South India.

Anti-bacterial activity

For antibacterial; five member heterocyclic compounds and their derivatives have proven to be attractive compounds due to their outstanding biological activities and have undergone rapid development as antibacterial, anticonvulsant, antiviral, antidiabetic, anti-helminthic,

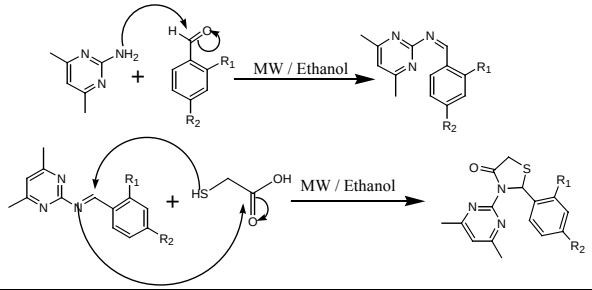
analgesic, anti-inflammatory, diuretic herbicidal and bactericidal agents (Naeem, 2008). Bacteria are resistant over existing antibiotics and there is always need to develop new antibiotics. For this reason, a variety of 4-thiazolidinone based compounds and its intermediates have been prepared and tested for antibacterial activities. Alloum reported the successful synthesis of 5-arylalkylidene rhodanines on solid inorganic support under microwave irradiation (Alloum, 1989). Naeem, 2008 and 2009 synthesized two series of thiazolidinone based derivatives in solvent free conditions and reported the promising results of antibacterial activities.

In this study, the synthesis of thiazolidinone derivatives has been carried out with different aromatic aldehyde, in the presence of ionic liquids (Phase Transfer Catalysts) under microwave radiation for the particular temperature (100-110 ºC), power (200-300 watt), rpm (200) and time (8-16 min). FTIR, ^{1}HNMR, ^{13}CNMR are used for qualitative and final confirmation is made by the elemental analyzer and mass spectrometer. The synthesized compounds have been used for biological studies (anti-bacterial and anti-inflammatory).

MATERIALS AND METHODS

Microwave procedure-I: Multi-component reaction in ethanol (01-08)

4,6-dimethylpyrimidin-2-amine (0.739 g, 6 mmoles) was treated with 2,4-dimethylbenzaldehyde (0.805 g, 6 mmoles) in ethanol (10.0 mL) and irradiated under microwave radiation at 105^{o}C for 10 minutes to get the Schiff base (*N*-[(*Z*)-(2,4-dimethylphenyl)methylidene]-4,6-dimethylpyrimidin-2-amine) as an intermediate. Schiff base was reacted with sulfanyl acetic acid (0.552 g, 6 mmoles) and again irradiated for 8 min. The crude product was cooled to room temperature, washed with ethanol, filtered under suction and dried. The final product (**01**) was purified with preparative TLC (ethyl acetate: n-hexane, 6:4, v/v) followed by recrystallization in ethanol: hexane (50:50, v/v) to obtain the compound 2-(2,4-dimethylphenyl)-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one (**01**) (yield, 94.2%).

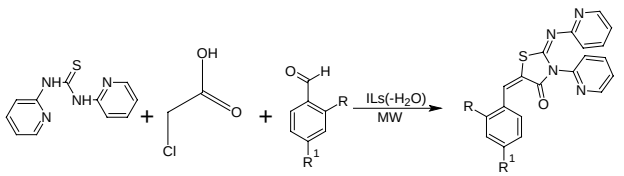


Compounds	R	R^1	Compounds	R	R^1
01	$-CH_3$	$-CH_3$	05	-H	-F
02	-OH	$-CH_3$	06	-H	$-NO_2$
03	-OH	-OH	07	-F	-F
04	-Cl	-Cl	08	-H	$-N(CH_3)_2$

Scheme-1

Microwave procedure-II: Multi-Component Reaction using Ionic Liquids (PEG, TBAB and TEBAC) (09-16)

Equimolar quantities of compounds 1,3-dipyridin-2-yl-thiourea (0.920 g, 4 mmoles), chloroacetic acid (0.378 g, 4 mmoles), benzaldehyde (0.424 g, 4 mmoles) and Ionic liquids in water (15 mL) were mixed and irradiated under microwave oven at 106^{o}C for 12 min. The irradiated product was cooled to room temperature. The crude mixture was subjected to solvent extraction using dichloromethane. The product was dried under suction followed by recrystallization with ethanol: water (80:20, v/v) to obtain 5-benzylidene-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one (**09**) (yield, 80.6%).



Compounds	R	R^1	Compounds	R	R^1
09	-H	-H	13	-H	$-NO_2$
10	-OH	$-OCH_3$	14	-F	-F
11	-H	$-N(CH_3)_2$	15	-H	$-C_2H_5$
12	-Cl	-Cl	16	-H	$-O(CH_3)$

Scheme-II

Method for anti-inflammatory activity

Anti-inflammatory activities of acute and chronic were evaluated by carrageenan induced for acute and formalin induced for chronic inflammatory in mouse paw edema. The dosage was administered orally. The mice of 40-60 days of age, weighing between 30-38 g were used.

Carrageenan-induced paw edema

Male Swiss albino mice were divided into nine groups of three animals in each group. In all groups the inflammation was induced by single sub-plantar injection of 20 µl of freshly prepared 1% carrageenan suspension in normal saline Ajith and Janardhanan, 2001. Group 1 treated with carrageenan alone served as control. Other groups received synthesized compounds at concentrations of 0.5 and 01 mg/kg body wt. orally 1 h before the carrageenan injection.

The compounds were pre-solubilized in 0.2% dimethyl sulfoxide (DMSO) and a fine suspension was prepared in phosphate buffered saline. One group was administered with reference drug (2,6-dichloranilino) phenyl acetic acid (0.5 mg/kg body weight) also orally 1 h before carrageenan injection. The paw thickness of animals in all groups was measured using digital vernier calipers before one, 3 and 5 h after carrageenan injection.

Formalin – induced paw edema

Experimental procedure was the same as described above except that single dose of 0.02 µl of formalin (2%) was

used to induce inflammation (Ajith and Janardhanan, 2001). The compounds were administered once daily for 6 consecutive days (Nitha 2007).

In the above two models, the degree of edema formation was determined as increase in paw thickness. In the case of acute anti-inflammatory activity, paw thickness was measured once daily for 6 days. The increase in paw thickness and percent inhibition were calculated as follows:

Increase in paw thickness in control/treatment $P_c/P_T=P_t-P_i$

$$\text{Percent inhibition} = \frac{(P_c - P_T)}{P_c} \times 100$$

Where Pt is paw thickness at time t, P_i is initial paw thickness, Pc is the increase in paw thickness of the control group and P_T is the increase in paw thickness of the treatment groups Ohkawa, 1979.

Animal Experiments

All animal experiments were carried out according to the guidelines of the Committee for the Purpose of Control of Experiments on Animals (Reg. No. 149/1999/CPCSEA) and approval of the Institutional Animal Ethics Committee was obtained.

Methodology for antibacterial screening

Mueller-Hinton agar (MHA) medium (United State Pharmacopia, 2008) was prepared by dissolving agar in 250 ml distilled water with slow heating and stirring to dissolve the medium completely. It was sterilized in autoclave at 15 PSI pressure and 121°C temperature for 15 min. The sterilized medium was immediately poured into petri dishes to form a uniform layer (2 mm to 5 mm thick). The petri dishes were stored in incubator so that no appreciable growth of the microorganisms was observed before the dishes were used British Pharmacopeia, 2003. Solutions of synthesized compounds (50 mg/ml) and reference substances were prepared that were presumed to be of equal concentration. The solutions of 10 µl of synthesized compound and reference standard were applied to the surface of the medium (6 mm in diameter) in triplicate, in cavities prepared in the agar. Negative controls were prepared by using N,N-dimethylformamide (DMF) which was employed to dissolve the test compounds. The inoculated plates were incubated at 37°C for 24 to 48 h (United State Pharmacopia, 2008). Antibacterial activities of synthesized compounds were calculated quantitatively (bioassay) by measuring the zone of inhibition (mm) against test organisms of synthesized compounds and compared with the zone of inhibition of reference standards (measured the diameters with a precision of at 0.1 mm). All bacterial strains were cultured on their respective medium for further bacterial propagation (Cruickshank, 1979).

RESULTS

2-(2,4-dimethylphenyl)-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one

Yield: 94.2%; mp: 218-220°C; IR (υ cm^{-1}): 3420, 2968, 1715, 1614; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 6.84-6.74 (m, 4H, aromatic), 5.92 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.38, 3.28 (s, 2H, $C_{5\text{-thiazolidinone}}$), 2.36 (s, 12H, $4CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 166.7($C{=}O_{\text{thiazolidinone}}$), 165.6, 159.9, 138.0, 136.0, 129.8, 128.7, 126.1, 116.1, 51.5($C_{2\text{-thiazolidinone}}$), 36.3, 21.2, 14.3, 36.3($C_{5\text{-thiazolidinone}}$); MS *m/z:* 313.12 ($M^{+1}$, 100%), 207, 107, 101; Anal.:$C_{16}H_{15}NOS$: (313.42): Calculated: C, 65.15; H, 6.11; N, 13.41; O, 5.10; S, 10.23; found: C, 65.13; H, 6.10; N, 13.38; O, 5.08; S, 10.20.

3-(4,6-dimethylpyrimidin-2-yl)-2-(2-hydroxy-4-methylphenyl)-thiazolidin-4-one

Yield: 82.4%;mp: 218-220°C; IR (υ cm^{-1}): 3400, 3200, 2968, 1720, 1664; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 7.01 (s, 1H), 6.80 (s, 1H), 6.60-6.40 (d, 2H, *J=6.8Hz*), 5.96 (s, 1H, $C_{2\text{-thiazolidinone}}$), 5.0 (s, 1H, OH), 3.33 (d, 2H, $C_{5\text{-thiazolidinone}}$), 2.36 (s, 9H, $3CH_3$);^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 166.6 ($C{=}O_{\text{thiazolidinone}}$), 165.6, 159.9, 157.6, 137.5, 130.2, 122.5, 121.7, 116.3, 116.1, 47.8 ($C_{2\text{-thiazolidinone}}$), 36.3 ($C_{5\text{-thiazolidinone}}$), 21.2; MS *m/z:* 315.1(M^{+1}, 100%),209, 107, 102; Anal.: $C_{16}H_{15}NOS$: (315.39): Calculated: C, 60.93; H, 5.43; N, 13.32; O, 10.15; S, 10.17; found: C, 60.90; H, 5.42; N, 13.29; O, 10.13; S, 10.15.

2-(2, 4-dihydroxyphenyl)-3-(4, 6-dimethylpyrimidin-2-yl)-thiazolidin-4-one

Yield: 92.4%; mp: 224-226°C; IR (υ cm^{-1}): 3346, 3180, 2968, 1718, 1624; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 6.96 (s, 1H), 6.72 (s, 1H), 6.17-6.08 (d, 2H, *J=6.7Hz*), 5.95 (s, 1H, $C_{2\text{-thiazolidinone}}$), 5.0 (s, 1H, OH), 3.34 (d, 2H, $C_{5\text{-thiazolidinone}}$), 2.35 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 166.7 ($C{=}O_{\text{thiazolidinone}}$), 165.6, 159.9, 159.1, 157.1, 131.7, 118.1, 116.1, 108.2, 102.8, 47.8 ($C_{2\text{-thiazolidinone}}$), 36.3 ($C_{5\text{-thiazolidinone}}$), 21.2; MS *m/z:* 317.08 (M^{+1}, 100%), 211, 109, 107, 101 Anal.: $C_{16}H_{15}NOS$: (317.36): Calculated: C, 56.77; H, 4.76; N, 13.24; O, 14.11; S, 10.12; found: C, 56.79; H, 4.72; N, 13.22; O, 14.11; S, 10.12

2-(2,4-dichlorophenyl)-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one

Yield: 88.4%; mp: 224-226°C, IR (υ cm^{-1}):3410, 3170, 2970, 1724, 1619; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 7.20-7.16 (d, 2H, *J=7.2Hz*), 6.98 (s, 1H), 6.84 (s, 1H), 5.94 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.34 (d, 2H, $C_{5\text{-thiazolidinone}}$), 2.37 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 166.7 ($C{=}O_{\text{thiazolidinone}}$), 165.6, 159.9, 136.8, 135.6, 133.6, 131.7, 129.2, 126.9, 116.1, 48.9($C_{2\text{-thiazolidinone}}$), 36.3 ($C_{5\text{-thiazolidinone}}$), 21.2 ($CH_3$); MS *m/z:* 353.02 ($M^{+1}$, 100%), 247, 145, 107; Anal.: $C_{16}H_{15}NOS$: (354.25): Calculated:

C, 50.86; H, 3.70; N, 11.86; O, 4.52; S, 9.05; found: C, 50.88; H, 3.66; N, 11.88; O, 4.50; S, 9.06

3-(4,6-dimethylpyrimidin-2-yl)-2-(4-fluorophenyl)-thiazolidin-4-one

Yield: 90.3%; mp: 202-204°C, IR (υ cm^{-1}): 3424, 3234, 2982, 1720, 1664, 1130; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 7.04-6.85 (m, 4H), 6.86 (s, 1H), 5.92 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.38 (d, H, $C_{5\text{-a thiazolidinone}}$), 3.28 (d, H, $C_{5\text{-b thiazolidinone}}$), 2.35 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{\text{thiazolidinone}}$), 164.8, 161.3, 159.9, 134.8, 130.4, 115.4, 109.6, 65.6 ($C_{2\text{-thiazolidinone}}$), 33.6 ($C_{5\text{-thiazolidinone}}$), 25.1 ($CH_3$); MS *m/z:* 303.08 ($M^{+1}$, 100%), 197, 107, 95; Anal.: $C_{15}H_{14}FN_3OS$: (303.08): Calculated: C, 59.39; H, 4.65; N, 13.85; O, 5.27; S, 10.57; found: C, 59.33; H, 4.63; N, 13.81; O, 5.22; S, 10.59

3-(4, 6-dimethylpyrimidin-2-yl)-2-(4-nitrophenyl)-thiazolidin-4-one

Yield: 82.9%; mp: 202-204°C; IR (υ cm^{-1}): 3336, 3190, 2974, 1722, 1644; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.07-7.32 (m, 4H), 6.86 (s, 1H), 5.96 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.42 (d, H, $C_{5\text{-a thiazolidinone}}$), 3.30 (d, H, $C_{5\text{-b thiazolidinone}}$), 2.35 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{\text{thiazolidinone}}$), 164.8, 159.9, 146.8, 145.3, 129.7, 121.0, 109.6, 65.8 ($C_{2\text{-thiazolidinone}}$), 33.7 ($C_{5\text{-thiazolidinone}}$), 25.3 ($CH_3$); MS *m/z:* 330.08 ($M^{+1}$, 100%), 122, 107, 102; Anal.: $C_{15}H_{14}N_4O_3S$: (333.36): Calculated: C, 54.53; H, 4.27; N, 16.96; O, 14.53; S, 9.71; found: C, 54.51; H, 4.25; N, 16.92; O, 14.49; S, 9.67

2-(2,4-difluorophenyl)-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one

Yield: 86.6%; mp: 224-226°C; IR (υ cm^{-1}): 3402, 3175, 2956, 1726, 1668, 1145; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 7.02-6.90 (m, 3H aromatic), 6.86 (1s, 1H), 5.94 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.38 (s, H, $C_{5\text{-a thiazolidinone}}$), 3.28(s, H, $C_{5\text{-b thiazolidinone}}$), 2.35 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{\text{thiazolidinone}}$), 164.8, 162.9, 160.6, 159.9, 132.0, 111.0, 109.6, 104.6, 97.5, 54.8 ($C_{2\text{-thiazolidinone}}$), 33.6 ($C_{5\text{-thiazolidinone}}$), 25.1 ($CH_3$); MS *m/z:* 321.07 ($M^{+1}$, 100%), 225, 113, 107, 101; Anal.: $C_{16}H_{17}N_3O_2S$: (321.35): Calculated: C, 56.06; H, 4.08; N, 13.08; O, 4.98; S, 9.98; found: C, 56.08; H, 4.06; N, 13.06; O, 4.97; S, 9.96

2-(4-(dimethylamino)phenyl)-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one

Yield: 88.8%; mp: 218-200°C; IR (υ cm^{-1}): 3360, 3160, 2928, 1724, 1652; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 6.96-6.40 (m, 4H), 6.88 (s, 1H), 5.90 (s, 1H, $C_{2\text{-thiazolidinone}}$), 3.40 (d, H, $C_{5\text{-a thiazolidinone}}$), 3.31(d, H, $C_{5\text{-b thiazolidinone}}$), 2.85 (s, 6H, $N(CH_3)_2$), 2.38 (s, 6H, $2CH_3$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 171.2 ($C{=}O_{\text{thiazolidinone}}$), 164.8, 159.9, 149.5, 140.1, 129.6, 118.3, 113.7, 112.7, 109.6, 65.9 ($C_{2\text{-thiazolidinone}}$), 40.3, 33.6 ($C_{5\text{-thiazolidinone}}$), 25.2 ($CH_3$); MS *m/z:* 328.43 ($M^{+1}$, 100%) , 222, 120, 107, 101; Anal.: $C_{17}H_{20}N_4OS$: (328.43): Calculated: C, 62.17; H, 6.14; N, 17.06; O, 4.87; S, 9.76; found: C, 62.15; H, 6.16; N, 17.02; O, 4.85; S, 9.72

5-benzylidene-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 80.6%; mp: 238-240°C; IR (υ cm^{-1}): 3140, 2868, 1712, 1662; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.55 (d, 2H, *J*=6.7*Hz*), 8.11 (d, 2H, *J*=4.3*Hz*), 7.74 (d, 2H, J=5.6*Hz*), 7.30 (m, 5H), 6.80 (s, 1H, $CH{=}C5_{\text{rhod}}$), 6.60 (d, 2H, *J* = 4.3 *Hz*); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164, 163, 161.1, 151.1, 148.9, 142, 138.0, 137.0, 134.9, 128.4, 127.7, 126.2, 122.1, 120, 117.1, 113.0, 108.9; MS *m/z:* 358 (M+1, 100%), 280, 203, 90, 78; Anal.: $C_{20}H_{14}N_4OS$: (358.42): Calculated: C, 67.02; H, 3.94; N, 15.63; O, 4.46; S, 8.95; found: C, 67.06; H, 3.96; N, 15.61; O, 4.44; S, 8.97

5-(2-hydroxy-4-methoxybenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 86.2%; mp: 224-226°C; IR (υ cm^{-1}):3142, 2874, 1716, 1667; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.42-7.68 (m, 8 H of two pyridine molecule), 7.07 (s, 1H, $CH{=}C_{5\text{-rhod}}$), 7.02 (s, 1H aromatic, *J*=6.28*Hz*), 6.68 (d, 2H aromatic, *J*=5.2*Hz*), 5.02(s, 1H , OH), 3.72 (s, 3H, CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164, 163, 162.6, 161.1, 156.0, 151.1, 148.9, 142, 138.0, 137.0, 128.6, 122.1, 120, 117.1, 114.4, 113.0, 108.9, 106.6, 101.2, 56.0 MS *m/z:* 404 (M^{+1}, 100%) 327, 281, 136, 92; Anal.: $C_{21}H_{16}N_4O_3S$: (404.44): Calculated: C, 62.36; H, 3.99; N, 13.85; O, 11.87; S, 7.93; found: C, 62.38; H, 4.02; N, 13.84; O, 11.86; S, 7.94

5-[4-(dimethylamino)benzylidene]-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 89.7%; mp: 234-236°C; IR (υ cm^{-1}): 3144, 2869, 1722, 1669; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.40-7.72 (m, 8 H of two pyridine molecule), 7.22 (d, 2H, *J*=6.2*Hz*), 6.98 (d, 2H, *J*=5.7*Hz*), 6.78 (s, 1H, $CH{=}C_{5\text{-rhod}}$), 2.85 (s, 6H, $(CH_3)_2$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164, 163,161.1, 155.9, 151.1, 148.9, 145.1, 142, 138.0, 137.0, 128.5, 122.1, 120, 117.1, 113.0, 111.6, 108.9, 105.6, 101.2, 100.2, 43.6, 43.6; MS *m/z:* 334 (M^{+1}, 100%) 309, 281,133, 92; Anal.: $C_{22}H_{19}N_5OS$: (401.48): Calculated: C, 65.81; H, 4.77; N, 17.44; O, 3.99; S, 7.99; found: C, 65.82; H, 4.78; N, 17.46; O, 3.96; S, 7.98

5-(2,4-dichlorobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)- thiazolidin-4-one

Yield: 92.8%; mp: 228-230°C; IR (υ cm^{-1}):3146, 2868, 1718, 1667; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.36-7.68 (m, 8 H of two pyridine molecule), 7.23 (d, 2H, *J*=6.6*Hz*), 7.18 (s, 1H), 6.90 (s, 1H, $CH{=}C_{5\text{-rhod}}$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164.0, 163.0, 161.1, 151.1, 148.9, 142.0, 138.0, 137.0, 134.4, 133.4, 132.9, 129.2, 129.0, 126.9, 122.1, 120.0, 117.1, 113.0, 108.9; MS *m/z:*427 (M^{+1}, 100%) 347, 281, 157, 92; Anal.: $C_{20}H_{12}Cl_2N_4OS$:

(427.30): Calculated: C, 56.22; H, 2.83; N, 13.11; O, 3.74; S, 7.50; found: C, 56.24; H, 2.84; N, 13.12; O, 3.72; S, 7.48.

5-(4-nitrobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4- one

Yield: 86.2%; mp: 228-230^{o}C; IR (υ cm^{-1}): 3144, 2865, 1716, 1663; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.32-7.85 (m, 8 H of two pyridine molecule), 7.65 (d, 2H, *J*=5.5*Hz*), 7.56 (d, 2H, *J*=5.5*Hz*), 6.87 (s, 1H, C<u>H</u>=$C_{5\text{-rhod}}$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164.0, 163.0, 161.1, 151.1, 148.9, 147.6, 142.0, 141.0, 138.0, 137.0, 127.1, 123.5, 122.1, 120.0, 117.1, 113.0, 108.9; MS *m/z:* 403 (M^{+1}, 100%) 325, 281, 135, 92; Anal.: $C_{20}H_{13}N_5O_3S$: (403.41): Calculated: C, 59.55; H, 3.25; N, 17.36; O, 11.90; S, 7.95; found: C, 59.54; H, 3.26; N, 17.34; O, 11.92; S, 7.96

5-(2,4-difluorobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 88.1%; mp: 232-234^{o}C; IR (υ cm^{-1}): 3148, 2866, 1724, 1662; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.42-7.68 (m, 8 H of two pyridine molecule), 7.36 (d, 2H, *J*=5.3*Hz*), 7.14 (s, 1H, *J*=6.9*Hz*), 6.87 (s, 1H, CH=$C_{5\text{-rhod}}$); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164.0, 163.0, 161.1, 151.1, 148.9, 147.6, 142.0, 141.0, 138.0, 137.0, 127.1, 123.5, 122.1, 120.0, 117.1, 113.0, 108.9; MS *m/z:* 394 (M^{+1}, 100%) 281, 126, 92; Anal.: $C_{20}H_{12}F_2N_4OS$: 394.0; Calculated; C, 60.91; H, 3.07; N, 14.21; O, 4.06; S, 8.13; Found: C, 60.90; H, 3.05; N, 14.19; O, 4.04; S, 8.11

5-(4-ethylbenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 84.2%; mp: 212-214^{o}C; IR (υ cm^{-1}): 3152, 2868, 1726, 1660; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.36-7.28 (m, 8 H of two pyridine molecule), 7.21 (d, 2H, *J*=6.5*Hz*), 7.01 (s, 1H), 6.79 (s, 1H, CH=$C_{5\text{-rhod}}$), 2.59 (d, 2H, CH_2CH_3), 1.28 (t, 2H, CH_2CH_3); ^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 166.9, 163.0, 156.5, 150.4, 148.2, 147.7, 142.0, 138.3, 138.7, 137.3, 132.4, 127.7, 126.3, 122.4, 116.2, 115.9, 113.3, 109.9, 32.4, 14.6; MS *m/z:* 386.12(M^{+1}, 100%) 281, 203,118, 92; Anal.: $C_{22}H_{18}N_4O_4S$: 386.47; Calculated: C, 68.37; H, 4.69; N, 14.50; O, 7.4.14, S, 8.30; Found: C, 68.37; H, 4.69; N, 14.50; O, 7.4.14, S, 8.28.

5-(4-methoxybenzylidene)-3-(pyridin-2-yl)-2-(pyridin-2-ylimino)-thiazolidin-4-one

Yield: 92.3%; mp: 238-240^{o}C; IR (υ cm^{-1}):3138, 2870, 1710, 1665; ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ: 8.22-7.54 (m, 8 H of two pyridine molecule), 7.19 (d, 2H, J=5.7*Hz*), 7.02 (d, 2H, J=6.3*Hz*), 6.80 (s, 1H, CH=$C_{5\text{-rhod}}$), 6.72 (d, 2H, J=4.8*Hz*), 6.62 (d, 2H , *J* = 4.5*Hz*), 3.73 (s, 3H, CH_3);^{13}CNMR (75.5 MHz, $CDCl_3$) δ: 170.3, 164, 163, 161.1, 151.1, 148.9, 142, 138.0, 137.0, 127.2, 122.1, 120, 117.1, 114.0, 113.0, 108.9, 56.0; MS *m/z:* 388 (M^{+1}, 100%), 281, 203, 120, 92; Anal.: $C_{21}H_{16}N_4O_2S$: (388.44): Calculated: C, 64.93; H, 4.15; N, 14.42; O, 8.24; S, 8.25; found: C, 64.95; H, 4.12; N, 14.44; O, 8.26; S, 8.22

DISCUSSION

A facile, cost effective and efficient procedure was adopted for the synthesis of potentially biologically active 4-thiazolidinone derivatives. In this procedure ionic liquids were used as phase transfer catalysts with cavitations energy (microwave irradiation) and offer convenient isolation of the product. Environment friendly solvent water and ethanol were used due have high dielectric constant. These prepared compounds were applied for anti-inflammatory and antibacterial activities. Bacterial strains are resistant over existing antibiotic and represent the emerging health problem that has moved the efforts to develop new antibacterial agents. Many compounds in this study showed significant inhibition against different bacterial strains and viable alternate to the existing antibiotics. This study was also performed to develop a compound which has a potent anti-inflammatory activity by oral administration. An optimum concentration of synthesis compounds was determined comparing the anti-inflammatory effect among the different reference drugs. Some of the compounds were no significant difference in the anti-inflammatory activities even at high concentration. Some of the compounds showed valuable anti-inflammatory activity as compared to the standard reference drug 2-(2,6-dichloranilino) phenyl acetic acid.

Chemistry

First phase of reaction; lone pair of nitrogen atom attack on the carbon center of substituted aromatic aldehyde in the presence of ethanol and irradiated under microwave irradiation to get the Schiff base as an intermediate product. In second phase the intermediates were sequentially treated with thioglycolic acid under microwave irradiation (106^{o}C). Ethanol was used as reaction medium for the compounds (**01-08**) and recrystallized from ethanol: hexane (50:50, v/v) to obtain the yields ranged from 82.4-94.2%. Ionic liquids (TBAB, PEG and TEBAC) were used as phase transfer catalyst in water for the compounds (**09-16**) in Scheme-II. The yield of compounds (**09-16**) ranged from 80.6-92.8% was obtained with shorter time and environmentally benign procedure. TLC was used to monitor the reaction and final product was recrystallized from ethanol. In such case tetra-alkylammonium cations are preferred in heterogeneous two-phase system. The $(C_2H_5)_4N^+Br^-$ serves both as a phase-transfer catalyst as will as base. The main advantage of a PTC is its environmentally benign nature. Three types of PTC were used, which gave varying yields of the products. It is worth mentioning that reaction in TBAB afforded better yields, as compared with respective yields obtained by using PEG & TEBAC. Increase in the quantity of TBAB had no effect on yield and time.

Conventional techniques require longer reaction time, toxic solvents like pyridine, dimethyl formamide, DMSO, etc., and use considerable amount of the catalysts. However, in the present investigation microwave radiation and very small amount of catalysts were used which considerably enhanced reaction rate, provided cleaner products and simplified the whole process under non polluting condition. It is pertinent to mention that the present reaction under microwave radiation dramatically reduced the reaction time from 12-24 h to only few min.

The compounds were characterized by using FTIR, ^{1}HNMR and ^{13}C NMR for qualitative determination and final confirmation was made by mass spectrometer and elemental analyzer. In FTIR spectra, peaks which appeared at 1715 cm^{-1} and 1614 cm^{-1} due to C=O (4-thiazolidinone) and C=N (pyrimidine). In compounds 02, 03 and 10 Ar-OH bands displayed at 3180-3200 cm^{-1} and bands of Ar-F were displayed in the range 1130-1145 cm^{-1} for the compounds 05, 07 and 14. In ^{1}H-NMR (300M*Hz*, $CDCl_3$) δ (01) signals at 6.84-6.74 ppm multiplet for four aromatic, a singlet at 5.92 ppm for one proton at $C_{2\text{-thiazolidinone}}$, two peaks appear at 3.38, 3.28 of two proton on $C_{5\text{-thiazolidinone}}$, a high intensity singlet appear at 2.36 for twelve protons of $4CH_3$ (six protons of $(CH_3)_2$ on phenyl and six proton on 4,6 dimethylpyrimidine). On the other hand ^{13}CNMR signal were recorded at 166.7 (C=O thiazolidinone), 51.5 ($C_{2\text{-thiazolidinoe}}$) and 36.3 ($C_{5\text{-thiazolidinoe}}$). Final confirmation was made by Mass spectrometer; molecular ion peak appear at 313.42 m/z (M^{+1}, 100%) and other fragmentations were at 209, 108, 106 and 103. Elemental analysis showed the results very close to the thoratical value.

Anti-bacterial evaluations

In this study sixteen novel compounds of 4-thiazolidinone derivatives were tested for their anti-inflammatory and antibacterial activity. For anti-inflammatory studies two procedures were adopted; carrageenan for acute inflammation and formalin for chronic inflammation. The results of anti-inflammatory study were compared with reference standard drug 2-(2,6-dichloranilino) phenyl acetic acid (diclofenac). For Anti-bacterial activity, nine cultures namely *S. aureus, B. subtilis, B. pumilus, L. monocytogenes, E. coli, S. typhi, P. aeruginosa, P. vulgaris, P. shigella* were applied on synthesized novel compounds containing 4-thiazolidinone. Well dip method was used and results compared with reference standards drugs ciprofloxacin and sulfamethoxazole.

Anti-inflammatory activity

The in-vivo carrageenan and formalin induced paw edema assay to evaluate their anti-inflammatory activity and to investigate their structure activity relationships. Some of the synthesized compounds showed significant inhibitory effect against induced inflammation in both carrageenan and formalin procedures. The carrageenan induced acute and formalin induced chronic inflammation were significantly inhibited by compounds. The results from the inhibition of the paw edema were presented graphically in figs. 1 and 2. The effect of some compounds was significantly high at concentration 0.5 mg/kg body weight and compared with standard reference drug diclofenac. The compounds of 2nd series reduced carrageenan induced paw edema by 74% to 84% at concentrations of 0.5 mg/kg body weight respectively compared to that of the control group. Where as the inhibitory effect of same compounds was 48% and 63% for formalin induced paw edema at concentration of 0.5 mg/kg body weight respectively. Reference standard drug diclofenac showed an inhibition of 58% and 42% of carrageenan and formalin induced inflammation respectively.

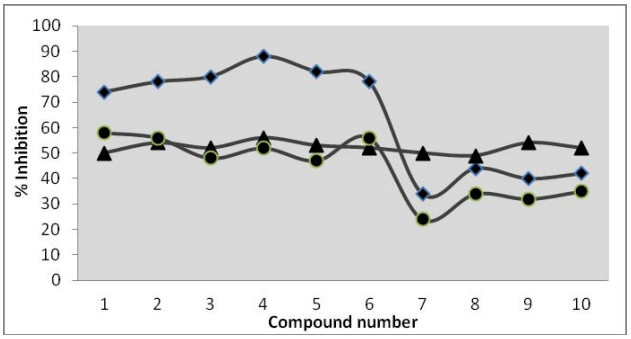


Fig. 1: Inhibition activity of acute inflammation by synthesized compounds of 0.5 and1.0 mg/kg administered

- ■ Inhibition activity at 1.0 mg/kg concentration of synthesized compounds (1-10)
- ● Inhibition activity at 0.5 mg/kg concentration of synthesized compounds (1-10)
- ▲ Inhibition activity of standard drug diclofenac at 0.5 mg/kg concentration

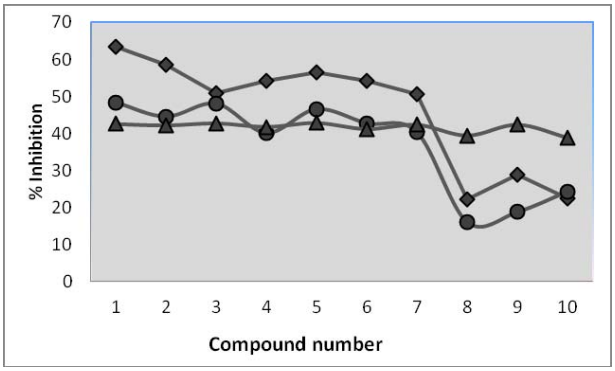


Fig. 2: Inhibition activity of chronic inflammation by synthesized compounds of 0.5 and 1.0 mg/kg administered

- ■ Inhibition activity at 1.0 mg/kg concentration of synthesized compounds (1-10)
- ● Inhibition activity at 0.5 mg/kg concentration of synthesized compounds (1-10)
- ▲ Inhibition activity of standard drug diclofenac at 0.5 mg/kg concentration

Table 1: Anti-inflammatory effects of 4-thiazolidinone derivatives 01-16 and reference standards to reduce inflammation

Compound ID	Substitution		Decrease inflammation with respect to time (mm)		
	R	R^1	after 1 h	after 3 h	after 5 h
Diclofenac			2.30	1.88	0.28
01	$-CH_3$	$-CH_3$	1.04	0.90	0.10
02	-OH	$-CH_3$	1.50	1.13	0.14
03	-OH	-OH	1.32	1.34	0.18
04	-Cl	-Cl	1.52	1.42	0.20
05	-H	-F	1.32	1.33	0.22
06	-H	$-NO_2$	1.62	1.40	0.18
07	-F	-F	2.38	1.98	0.34
08	-H	$-N(CH_3)_2$	1.64	1.53	0.12
09	-H	-H	1.02	0.93	0.04
10	-OH	$-OCH_3$	1.22	1.08	0.08
11	-H	$-N(CH_3)_2$	1.34	1.35	0.14
12	-Cl	-Cl	2.18	1.78	0.22
13	-H	$- NO_2$	2.12	1.48	0.16
14	-F	-F	2.78	2.02	0.38
15	-H	$-C_2H_5$	1.30	0.98	0.05
16	-H	$-O(CH_3)$	1.34	1.04	0.08

Each value represents the mean value of 3 animals (mm)

The anti-inflammatory activity of synthesized compounds was evaluated by adopting one of the most suitable test procedures "carrageenan induced acute inflammation in animals" to screen anti-inflammatory agents. The carrageenan induced edema is mediated by activation of platelet activating factor (PAF), prostaglandins and other inflammatory mediators (Hwang, 1986). The first phase is attributed to the release of histamine 5-HT and kinins. The second phase is related to the release of prostaglandins Vane and Booting (1987).

Carrageenan also induces a protein rich exudate containing large number of neutrophils (Greenwald, 1991). For the screening of chronic anti-inflammatory agent "formalin induced paw edema" is also one of the most suitable test procedures because it closely resembles human arthritis. The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by tissue mediated response (Soniamol, 2009). The compounds 03, 04, 07, 12, 13 significantly showed significant anti-inflammatory activity and after pharmacological screenings, compounds bearing electron-withdrawing groups such as 2/4-Cl, 2/4-F, and 4-NO_2 are responsible to control the inflammatory activities.

Antibacterial activity in vitro of compounds 01-16

The effects of newly synthesized 4-thiazolidinone analogues were studied against nine bacterial strains and data is presented in table 2. The activities against gram negative bacterial strains of 4-thiazolidinone derivatives (09-16) showed better results due to presence of pyrimidine molecule but the compounds (01-08) did not show better result. From the data obtained (table 2), the compounds 12, 13 and 14 exhibited high activity against negative bacterial strains as compared to the other compounds in this study that might be due to presence of electron withdrawing group with pyrimidine molecule. The substituted dimethyl amino group was found to increase the zone of inhibition against gram positive bacterial strains *S. aureus* and *B. subtilis* (08 and 11) particularly at forth position because it is very stable position. The substituted methoxy, ethoxy and hydroxyl groups participated a little in the zone of inhibition against the gram positive bacterial growth as shown in Table 2 for the compounds 02, 03, 10 and 16. Other substituents *i.e.* methanol, ethanol and benzene did not affect the zone of inhibition against the most of bacterial strains under study. In general, 4-thiazolidinone derivatives showed different activities due to the presence of different groups. The compounds 07 and 14 showed excellent results due to the presence of Fluoro group; the compounds 04 and 12 showed significant results against *S. aureus a*nd *B. subtilis* due to the presence of chloro group and the compounds 06 and 13 produced good results against *P. vulgaris* and *P. shigella* due to the presence of nitro group. The compounds 09-16 showed significant results as compared with standard drug ciprofloxacin. The groups 4-methoxy, methyl and ethoxy did not significantly effect on the inhibition of bacterial growth. ciprofloxacin and sulphamethoxazole were used as reference standard for comparison with synthesized compounds. The results of two series are presented in table 2.

Table 2: Antibacterial response of compounds 01-16

Compound No.	Antibacterial activity of compounds 01-16								
	Gram positive				Gram negative				
	S. aureus	*B. subtilis*	*B. pumilus*	*L. monocytogenes*	*E. coli*	*S. typhi*	*P. aeruginosa*	*P. vulgaris*	*P. Shigella*
01	-	-	-	-	+	+	+	+	-
02	++	++	-	-	+	+	+	+	-
03	++	++	+	-	+	+	+	+	-
04	+	+	-	-	+	+	+	+	-
05	++	++	+	+	++	++	+	+	-
06	++	++	++	-	++	++	+	++	++
07	++	++	+	-	++	-	++	+	-
08	++	++	++	+	++	++	++	+	++
09	+	+	+	-	-	-	-	-	-
10	++	++	+	-	-	+	-	-	-
11	++	++	++	+	+	+	-	-	-
12	+++	+++	+	+	+++	++	+++	-	-
13	+++	+++	+	+	+++	++	+++	++	++
14	+++	+++	+	+	+++	++	+++	-	-
15	+	+	+	+	-	-	-	-	-
16	+++	+++	++	+	++	++	+	-	-
CIP.	+++	+++	++	-	++	+++	+++	+	+
SMZ	++++	+++	+++	-	++	+++	++	+	+

Key to symbols:

Highly active	=	++++	(inhibition zone > 20 mm)
Highly active	=	+++	(inhibition zone 15-20 mm)
Active	=	++	(inhibition zone 10-15 mm)
Slightly active	=	+	(inhibition zone 5-10 mm)
Inactive	=	-	(inhibition zone < 5mm)

Reference Standards

- CIP = Ciprofloxacin
- SMZ = Sulphamethoxazole

CONCLUSION

Novel derivatives of 4-thiazolidinone were synthesized under microwave irradiation and phase transfer catalysts (TBAB, PEG and TEBAC). A significant yield (80.6-92.8%) was obtained with TBAB as compared to others. The compounds (01-16) were applied for anti-inflammatory activity at concentrations of 0.5 and 1.0 mg/kg in carrageenan induced acute and formalin induced chronic inflammatory procedures in mice and better results were obtained at 0.5 mg/kg dose. Some of the compounds 03, 04, 07, 12, 13 showed remarkable anti-inflammatory activity in both procedures as compared to the standard reference drug 2-(2,6-dichloranilino) phenyl acetic acid (diclofenac). Particularly compound 12 and 13 may be used as a NSAID to reduce inflammation. The compounds (01-16) were also checked for their antibacterial activity (*in-vivo*) and found that the compounds 12, 13 and 14 exhibited comparable or higher antibacterial activity then ciprofloxacin (standard) against *E. coli, S. typhi*, *P. aeruginosa, S. aureus* and *B. subtilis.* Most of the compounds of series-2 showed significant activity as compared with ciprofloxacin. These compounds could lead to the selection and may be used as efficient antimicrobial agents, especially for the treatment of multi-drug resistant infections.

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