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Design, Synthesis And *In-Vitro* Anti-inflammatory, Antimicrobial Activities of Some Novel 2, 3-Disubstituted -1,3-Thiazolidin-4-One Derivatives Containing Thiazole Moiety

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Abstract

In Present work, the Reduction of ethyl 2-(4-phenyl substituted)-4-methylthiazole-5-carboxylate**(1)** by Lithium Aluminium hydride in diethyl ether Solvent yield (2-(4-phenyl substituted)-4-methylthiazol-5-yl) methanol **(2)** followed by Oxidation using IBX in the DMSO solvent, to yield 2-(4-phenyl substituted)-4-methylthiazole-5-carbaldehyde**(3)** which on further reaction with2-(4-phenyl substituted)-4-methylthiazole-5-carbohydrazide**(4)** in the presence of conc. sulphuric acid in ethanol solvent to yield N-((2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-4-methyl-2-(phenyl substituted)thiazole-5-carbohydrazide**(5)** which further cyclisation with mercaptoacetic acid in presence of zinc chloride catalyst in DMF Solvent afforded series of thiazolidin-4-one derivatives namely N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methyl thiazole-5-carboxamide**(6a-h).** The structure of all the synthesized compounds was characterized by FT-IR, ^{1}H NMR, DIP MS data. Furthermore, compounds **(6a-h)** were screened for their antibacterial activity against gram negative (*E. coli* and *P. aeruginosa*) and gram positive (*S. aureus* and *B. subtilis*) bacteria, antifungal activity against pathogenic fungal strains and anti-inflammatory activities. Some of the compounds exhibited promising antibacterial, antifungal and anti-inflammatory activities.

Key words: thiazole, hydrazide, acyl-hydrazone, 4-thiazolidinone, antiinflammatory (*in-vitro*), antibacterial, antifungal activity.

Introduction

There are numerous biologically active molecules which contain various heteroatoms such as nitrogen, sulphur and oxygen, always drawn the attention of chemist over the years mainly because of their biological importance. Similarly 1,3-thiazolidin-4-ones are heterocyclic nucleus that have an atom of

sulfur and nitrogen at position 1 and 3, respectively and a carbonyl group at position 4 have been subjected to extensive study in the recent years. The 4-thiazolidinone scaffold is very versatile and has featured in a number of clinically used drugs.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of pain, inflammation, fever and a number of arthritic diseases such as rheumatoid arthritis and osteoarthritis.[1,2] However, their remedial use are often limited by common side effects, such as gastrointestinal (GI) hemorrhage, perforation and ulceration[3,4]. The incident of clinically significant GI side manifestation due to NSAIDs is high (30%) and causes some patients to cede NSAID therapy.[5] Hence in spite of abundance of NSAIDs in the market, an ideal agent is still a dream and the search continues to develop new drugs that have potent anti-inflammatory activity with minimum side effects. All such problems raise the extremity and make the noteworthy interest of medicinal apothecary in the discovery and evolution of new lead structures.

Thiazoles are an important set of heterocyclic compounds, found in many potent biologically active molecules such as Nizatidine, Sulfatiazole, Ritonavir, Meloxicam, Bleomycine, Fentiazac, and Tiazofurin.[6] Thiazole scaffold is an essential pharmacophore and its shackle with other rings could furnish novel biologically active compounds. Thiazole derivatives confine compounds exhibit a broad stroll of biological properties, such as anti-inflammatory[7], antimicrobial[8], antitumor[9], anticonvulsant[10], cardiotonic[11], anti-biofilm[12], analgesic[13] and anticancer[14].

Similarly, there has been a considerable interest in the chemistry of thiazolidin-4-one ring system, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities.[15] Thiazolidinone ring also occurs in nature; thus actithiazic acid isolated from *Streptomyces* strains exhibits highly specific *in vitro* activity against *Mycobacterium tuberculosis*.[16] Thiazolidinone derivatives are also known to exhibit diverse bioactivities such as anti-convulsant[17], antidiarrheal[18], anti-platelet activating factor[19], antihistaminic[20], anti-diabetic[21], cyclooxy genase (COX) inhibitory[22], Ca^{2+}-channel blocker[23], platelet activating factor (PAF) antagonist[24], cardioprotective[25], anti-ischemic[26], anti-cancer[27], tumor necrosis factor-α antagonist[28] and nematicidal activities[29]. The synthesis of heterocycles containing multistructure in a molecule has received much attention in recent years[30]. However, literature survey revealed that linked heterocycles containing thiazole and thiazolidinone have seldom been reported.

Based on the wide spectrum of biological profile of thiazole and thiazolidin-4-one and their increasing importance in pharmaceutical, and biological field, and in continuation of our ongoing research on biologically active heterocycles[31,32], it was thought of interest to accommodate thiazole and thiazolidin-4-one moieties in a single molecular frame work to synthesize some new heterocyclic compounds with potential biological activity. The present investigation deals with the synthesis of some new N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide(6a-h) in good yields, from N-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-2-(4-phenyl substituted)-4-methylthiazole-5-carbohydrazide **(5a-h)**. The antibacterial and antifungal and activities and *in-Vitro* anti-Inflammatory activity of the compounds **(6a-h)** have also been evaluated. The structure of newly synthesized compounds was confirmed by IR, ^{1}HNMR, DIP Mass spectrometry and Elemental analysis data.

Materials and Methods

Melting points were determined in open capillarie and are uncorrected. FT-IR spectra were recorded using Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on Brucker Advance II 400 spectrometer in $CDCl_3$ solvent by using TMS as internal standard. Chemical shift values are reported in ppm units, relative to TMS as internal standard. Mass spectra were recorded on DIP Mass spectrometer. Thin layer chromatography was performed with E. Merk precoated TLC plates, silica gel 60 F_{254} with thickness of 0.25 mm and spots were visualized by irradiation with ultraviolet light (254 nm)

or by exposing to I_2.

Synthesis of (2-(4-phenyl substituted)-4-methylthiazol-5-yl)methanol ***(2) :***

To a cold solution of lithium aluminium hydride (0.04 mol) in dry diethyl ether (50 mL), ethyl 2-(4-phenyl substituted)-4-methylthiazole-5-carboxylate (0.02 mol) in diethyl ether (50 mL) was added dropwise over a period of 30 minutes and the reaction mixture was further stirred for 1 hour at 0 °C. After completion of the reaction (TLC), the reaction mixture was quenched by saturated solution of sodium sulphate. The reaction mixture was filtered and the aqueous layer was extracted with diethyl ether (2 x 50 mL), the combined organic layer was washed with water, brine and dried over sodium sulphate.(2-(4-phenyl substituted)-4-methylthiazol-5-yl)methanol(2) was obtained by removing the solvent by distillation.

(4-Methyl-2-phenylthiazol-5-yl)methanol ***(2a):***

Yield 84%. m.p: 93-96 ^oC. ^{1}HNMR($CDCl_3$, 400MHz): δ 7.50-7.35 (m, 5H, Ar-H), 4.80 (s, 2H, -CH_2OH), 2.46(s, 3H, thiazolic-CH_3). IR(KBr pellets Cm^{-1}) : 3297(-OH), 3130(C-H, Aromatic), 3003-2932(-CH_3), 1496(C=C str. in Ar), 1092(C-O). Mass(DIP MS): m/z- 205. Elemental analysis Calcd for ($C_{11}H_{11}NOS$): C, 64.30; H, 5.40; N, 6.80; found:C, 64.30; H, 5.38; N; 6.80 %.

(2-(4-chlorophenyl)-4-methylthiazol-5-yl)methanol ***(2b):***

Yield 87%. m.p: 134-138 ^oC. ^{1}HNMR($CDCl_3$, 400MHz): δ7.80(dd,2H,Ar-H),7.35(dd,2H,Ar H),4.82(s,2H, -CH_2OH),2.46(s,3H,thiazolic-CH_3). IR(KBr pellets Cm^{-1}): 3270(-OH), 3140 (Ar-C-H), 3000-2940 (-CH_3),1494 (C=C str.in Ar),1090 (C-O). Mass (DIP MS): m/z-239. Elemental analysis Calcd. For ($C_{11}H_{10}ClNOS$): C, 55.11; H, 4.20; N, 5.84; found: C, 55.04; H, 4.10; N, 5.80%.

Synthesis of 2-(4-phenyl substituted)-4-methylthiazole-5-carbaldehyde ***(3) :***

To a solution of (2-(4-phenyl substituted)-4-methylthiazol-5-yl)methanol(2) (0.02 mol) in DMSO (50 mL), IBX (0.022 mol) was added and the reaction mixture was stirred at room temperature for 3hrs.Then,water(30 mL) was added, reaction mixture was filtered. The filtrate was extracted with diethyl ether (3x50 mL). The organic layer was washed with water, brine and dried over sodium sulphate.The solvent was distilled to afford pure aldehyde.

4-methyl-2-phenylthiazole-5-carbaldehyde ***(3a):***

Yield 93%. m.p: 110-114 ^oC. ^{1}HNMR ($CDCl_3$, 400MHz): δ7.99-7.50(m, 5H, Ar-H), 2.80(s, 3H, thiazolic-CH_3), 10.04(s, 1H, -CHO). IR(KBr pellets Cm^{-1}): 3160(Ar-C-H,), 2942(-CH_3), 2732(-CH in –CHO), 1690(CO in –CHO), 1605(C=C str.in Ar). Mass (DIP MS): m/z-203. Elemental analysis Calcd for ($C_{11}H_9NOS$): C, 65; H, 4.46, N, 6.89; found: C, 64.90; H, 4.40; N, 6.90%.

2-(4-chlorophenyl)-4-methylthiazole-5-carbaldehyde ***(3b) :***

Yield 90%. m.p: 140-144 ^oC. ^{1}H NMR($CDCl_3$, 400MHz): δ7.90 (dd, 2H, Ar-H), 7.34 (dd, 2H,Ar-H), 2.78 (s, 3H, thiazolic-CH_3), 10.08 (s, 1H, -CHO). IR (KBr pellets Cm^{-1}): 2932 (Ar-C-H), 2920 (CH_3), 2730 (-CH in –CHO), 1695 (CO in –CHO), 1608 (C=C str.in Ar), 840 (C-Cl). Mass (DIP MS): m/z-237. Elemental analysis Calcd for ($C_{11}H_8ClNOS$): C, 55.58; H, 3.39, N, 5.89; found: C, 55.50; H, 3.40; N, 5.80 %.

Synthesis of N-((2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-4-methyl-2 (phenylsubstituted)thiazole-5-carbohydrazide ***(5a-h):***

To a solution of 2-(4-phenyl substituted)-4-methylthiazole-5-carbaldehyde**(3)** (0.01mol) in absolute ethanol (30 mL), (0.01mol) of 2-(4-phenyl substituted)-4-methylthiazole-5-carbohydrazide**(4)** and two-three drops of concentrated sulphuric acid were added. The mixture was heated for 5hrs. The residue was filtered and recrystallized from ethanol in order to give compounds **(5a-h)**.

2-(4-fluorophenyl)-4-methyl-N-((4-methyl-2-phenylthiazol-5-yl)methylene)thiazole-5-carbohydrazide ***(5a) :***

Yield 88 %. m.p: 180-184 ^oC. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.55 7.40 (m, 5H, Ar-H), 7.48 (dd, 2H, Ar-

H),7.10 (dd, 2H, Ar-H), 2.58 (s, 3H, thiazolic CH_3), 2.65 (s, 3H, thiazolic-CH_3), 8.30 (s, 1H, -CH=N), 11.40 (s, 1H, -NH-). IR (KBr pellets Cm^{-1}): 3185 (-NH), 3040 (Ar-C-H), 2980 (CH_3), 1661(-CONH), 1602 (-CH=N), 1590 (C=C str.in Ar), 1240 (-CF). Mass (DIP MS): m/z-436. Elemental analysis Calcd for ($C_{22}H_{17}FN_4OS_2$): C, 60.53; H, 3.93, N; 12.83; found: C, 60.40; H, 3.90; N, 12.78 %.

(4-methyl-N-((4-methyl-2-phenylthiazol-5-yl)methylene)-2-(4-nitrophenyl)thiazole-5-carbohydrazide ***(5b) :***

Yield 72%. m.p: 175-178 °C. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.40-7.50 (m, 5H, Ar-H), 8.30 (dd, 2H, Ar-H), 7.80 (dd, 2H, Ar-H), 2.80 (s, 3H, thiazolic-CH_3), 2.78 (s, 3H, thiazolic-CH_3), 8.30 (s, 1H, -CH=N), 12.10 (s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3150 (-NH), 2970 (Ar-C-H), 2932 (CH_3), 1698 (-CONH), 1513 (-CH=N), 1588 (C=C str. in Ar), 1580, 1369 (-NO_2). Mass (DIP MS): m/z- 463. Elemental analysis Calcd for ($C_{22}H_{17}N_5O_3S_2$): C, 57.00; H, 3.70, N; 15.11; found: C, 56.80; H, 3.60; N, 15.08 %

2-(4-methoxyphenyl)-4-methyl-N-((4-methyl-2-phenylthiazol-5-yl)methylene)thiazole-5-carbohydrazide ***(5c) :***

Yield 84 %. m.p: 199-204°C. ^{1}H NMR($CDCl_3$, 400MHz): δ7.42-7.30 (dd, 2H, Ar-H), 7.40-6.83(dd, 2H, Ar-H), 2.75(s, 3H, thiazolic-CH_3), 2.64 (s, 3H, thiazolic-CH_3), 8.40 (s, 1H, CH=N), 11.00 (s, 1H, -NH), 3.74 (s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3150 (-NH), 3020 (Ar-C-H), 2970 (CH_3), 1692(-CONH), 1540(CH=N), 1567(C=C, str.in Ar), 1180 (OCH_3), 860(C-Cl). Mass (DIP MS): m/z- 448. Elemental analysis Calcd for ($C_{23}H_{20}N_4O_2S_2$): C, 61.59; H, 4.49, N; 12.49; found: C, 61.50; H, 4.40; N, 12.40%

2-(4-chlorophenyl)-4-methyl-N-((4-methyl-2-phenylthiazol-5-yl)methylene)thiazole-5-carbohydrazide ***(5d) :***

Yield 86%. m.p: 98-102 °C. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.40-7.30 (m, 5H, Ar-H), 7.45(dd, 2H, Ar-H), 7.35(dd, 2H, Ar-H), 2.70(s, 3H, thiazolic-CH_3), 2.50(s, 3H, thiazolic-CH_3), 8.10(s, 1H, -CH=N), 11.80(s, 1H, -NH). IR(KBr pellets Cm^{-1}): 3180 (-NH), 3000 (Ar-C-H), 2930 (CH_3), 1670 (-CONH), 1640(-CH=N), 1560(C=C, str.in Ar), 840 (C-Cl). Mass(DIP MS): m/z-452. Elemental analysis Calcd for ($C_{22}H_{17}ClN_4OS_2$): C, 58.33; H, 3.78, N; 12.37; found: C, 58.30; H, 3.70; N, 12.30%.

N-((2-(4-chlorophenyl)-4-methylthiazol-5-yl) methylene)-2-(4-fluorophenyl)-4-methyl thiazole-5-carbohydrazide ***(5e) :***

Yield 86%. m.p: 208-210 °C . ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.46(dd, 2H, Ar-H), 7.30(dd, 2H, Ar-H), 2.70(s, 3H, thiazolic-CH_3), 2.62(s, 3H, thiazolic-CH_3), 8.50 (s, 1H,-CH=N), 11.84 (s, 1H, -NH-). IR (KBr pellets Cm^{-1}): 3195 (-NH), 3050 (Ar-C-H), 2970 (CH_3), 1670 (-CONH), 1610 (-CH=N), 1596 (C=C str.in Ar), 1245(C-F). Mass (DIP MS): m/z- 470. Elemental analysis Calcd for ($C_{22}H_{16}ClFN_4OS_2$): C, 56.10; H, 3.42, N; 11.90; found: C, 56; H, 3.36; N, 11.85 %.

N-((2-(4-chlorophenyl)-4-methylthiazol-5-yl)methylene)-4-methyl-2-(4-nitrophenyl) thiazole-5-carbohydrazide ***(5f) :***

Yield 80%. m.p:140-150 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 8.40-7.80 (dd, 4H, Ar-H), 7.50-7.40 (dd, 4H, Ar-H), 2.84 (s, 3H, thiazolic-CH_3), 2.80(s, 3H, thiazolic-CH_3), 8.90 (s, 1H, -CH=N), 12.08 (s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3180 (-NH), 2990 (Ar-C-H), 2950 (CH_3), 1700 (-CONH), 1520 (-CH=N), 1568 (C=C str.in Ar), 1584, 1370 (-NO_2). Mass (DIP MS): m/z- 497. Elemental analysis Calcd for ($C_{22}H_{16}ClN_5O_3S_2$): C, 53.06; H, 3.24, N; 14.06; found: C, 53; H, 3.20; N, 13.90 %.

N-((2-(4-chlorophenyl)-4-methylthiazol-5-yl) methylene)-2-(4-methoxyphenyl)-4-methylthiazole-5-carbohydrazide ***(5g) :***

Yield 78%. m.p:110-114 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.48-7.22 (m, 5H, Ar-H), 7.40 (dd, 2H, Ar-H), 6.83 (dd, 2H, Ar-H), 2.70(s, 3H, thiazolic-CH_3), 2.65(s, 3H, thiazolic-CH_3), 8.30(s, 1H, -CH=N), 10.20(s, 1H, -NH), 3.70(s, 3H, -OCH_3). IR (KBr pellets Cm^{-1}): 3130 (-NH), 2950 (Ar-C-H), 2920 (CH_3), 1690 (-CONH), 1530 (-CH=N), 1490 (C=C, str.in Ar), 1170 (OCH_3). Mass (DIP MS): m/z- 482. Elemental analysis Calcd for ($C_{23}H_{19}ClN_4O_2S_2$): C, 57.19; H, 3.96, N; 11.60; found: C, 57.10;

H, 3.90; N, 11.50 %

2-(4-chlorophenyl)-N'-((2-(4-chlorophenyl)-4-methylthiazol-5-yl)methylene)-4-methyl thiazole-5-carbohydrazide ***(5h) :***

Yield 90 %. m.p:164-168 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.42-6.90 (dd, 2H, Ar-H), 7.40-7.30 (dd, 2H, Ar-H), 2.70(s, 3H, thiazolic-CH_3), 2.68(s, 3H, thiazolic-CH_3), 8.20(s, 1H, -CH=N), 11.78(s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3170 (-NH), 3010 (Ar-C-H), 2940 (CH_3), 1660(-CONH), 1635(-CH=N), 1530(C=C str.in Ar), 830(C-Cl). Mass(DIP MS): m/z- 486. Elemental analysis Calcd for ($C_{22}H_{16}Cl_2N_4OS_2$): C, 54.21; H, 3.31, N; 11.49; found: C, 54.15; H, 3.28; N, 11.40 %.

Synthesis of N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide ***(6a-h).***

A mixture of N-((2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-4-methyl-2-(phenyl substituted)thiazole-5-carbohydrazide(5a-h) (0.01 mol) in DMF(30 ml) and mercaptoacetic acid (0.02 mole) with a pinch of anhydrous Zinc chloride was added reflux the mixture for 8-10hrs. The reaction mixture was cooled, and poured it in to an beaker containing 50ml crushed ice, the obtained precipitate was filtered, dried and re-crystallized by absolute alcohol and obtained compounds**(6a-h)**.

2-(4-fluorophenyl)-4-methyl-N-(2-(4-methyl-2-phenylthiazol-5-yl)-4-oxothiazolidin-3-yl) thiazole-5-carboxamide ***(6a) :***

Yield 78 %. m.p:186-190 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.50-7.22 (m, 5H, Ar-H), 7.45 (dd, 2H, Ar-H), 7.05 (dd, 2H, Ar-H), 2.28 (s, 3H, thiazolic-CH_3), 2.10(s, 3H, thiazolic-CH_3), 3.62 (AB, quartet, 2H, thiazolidinone-CH_2), 6.23 (s, 1H, -SCH), 11.50 (s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3232 (-NH), 3030 (Ar-C-H), 2940 (CH_3), 1730 (CO, cyclic), 1665 (-CONH), 1580 (C=C str.in Ar), 1160 (C-F). Mass (DIP MS): m/z- 510. Elemental analysis Calcd for ($C_{24}H_{19}FN_4O_2S_3$): C, 56.45; H, 3.75, N; 10.97; found: C, 56.40; H, 3.74; N, 11.01 %.

4-methyl-N-(2-(4-methyl-2-phenylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-nitrophenyl) thiazole-5-carboxamide ***(6b) :***

Yield 87%. m.p: 210-214 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 8.0-7.40 (m, 5H, Ar-H), 8.30 (dd, 2H, Ar-H), 7.80 (dd, 2H, Ar-H), 2.38(s, 3H, thiazolic-CH_3), 2.30 (s, 3H, thiazolic-CH_3), 4.10 (AB, quartet, 2H, thiazolidinone-CH_2), 6.40 (s, 1H, -SCH), 10.90 (s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3240 (-NH), 3030 (Ar-C-H), 2910 (CH_3), 1725(CO, cyclic), 1663(-CONH), 1570(C=C str.in Ar), 1540, 1345(NO_2). Mass (DIP MS): m/z- 537. Elemental analysis Calcd for ($C_{24}H_{19}N_5O_4S_3$): C, 53.62; H, 3.56, N; 13.03; found: C, 53.58; H, 3.48; N, 12.90 %.

2-(4-methoxyphenyl)-4-methyl-N-(2-(4-methyl-2-phenylthiazol-5-yl)-4-oxothiazolidin-3-yl)thiazole-5-carboxamide **(6c) :**

Yield 74%. m.p: 167-170 °C. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.42-7.23 (m, 5H, Ar-H), 7.38 (dd, 2H, Ar-H), 6.84 (dd, 2H, Ar-H), 2.20(s, 3H, thiazolic-CH_3), 2.10(s, 3H, thiazolic-CH_3), 3.62(AB, quartet, 2H, thiazolidinone-CH_2), 6.10(s, 1H, -SCH), 9.90 (s, 1H, NH), 3.70(s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3260 (-NH), 3017 (Ar-C-H), 2930 (CH_3), 1718 (CO, cyclic), 1668 (-CONH), 1582(C=C, str.in Ar), 1160(OCH_3). Mass(DIP MS): m/z- 522. Elemental analysis Calcd for ($C_{25}H_{22}N_4O_3S_3$): C, 57.45; H, 4.24, N; 10.72; found: C, 57.40; H, 4.18; N, 10.60 %.

2-(4-chlorophenyl)-4-methyl-N-(2-(4-methyl-2-phenylthiazol-5-yl)-4-oxothiazolidin-3-yl) thiazole-5-carboxamide ***(6d) :***

Yield 80%. m.p: 188-192 °C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.45-7.39 (m, 5H, Ar-H), 7.12 (dd, 2H, Ar-H), 6.98 (dd, 2H, Ar-H), 2.28(s, 3H, thiazolic-CH_3), 2.20(s, 3H, thiazolic-CH_3), 3.87 (AB, quartet, 2H, thiazolidinone-CH_2), 6.20 (s, 1H, -SCH), 10.65(s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3216 (-NH), 3029 (Ar-C-H), 2930 (CH_3), 1721(CO, cyclic), 1660(-CONH), 1550 (C=C str.in Ar), 845 (C-Cl). Mass (DIP MS): m/z- 526. Elemental analysis Calcd for ($C_{24}H_{19}ClN_4O_2S_3$): C, 54.69; H, 3.63, N; 10.63; found: C, 54.60; H, 3.58; N, 10.58%.

N-(2-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-fluorophenyl) -4-methylthiazole-5-carboxamide ***(6e) :***

Yield 88%. m.p: 220-224 ^{0}C. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.40-7.10(dd, 2H, Ar-H), 7.50-7.0 (dd, 2H, Ar-H), 2.30(s, 3H, thiazolic-CH_3), 2.18(s, 3H, thiazolic-CH_3), 3.64 (AB, quartet, 2H, thiazolidinone-CH_2), 6.30 (s, 1H, -SCH), 12.00(s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3240 (-NH), 3020 (Ar-C-H), 2930(CH_3), 1728(CO, cyclic), 1670(-CONH), 1562(C=C str.in Ar), 847(C-Cl), 1165(C-F). Mass (DIP MS): m/z- 544. Elemental analysis Calcd for ($C_{24}H_{18}ClFN_4O_2S_3$): C, 52.88; H, 3.33, N; 10.28; found: C, 52.80; H, 3.30; N, 10.30 %.

N-(2-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-4-methyl-2-(4-nitrophenyl) thiazole-5-carboxamide ***(6f) :***

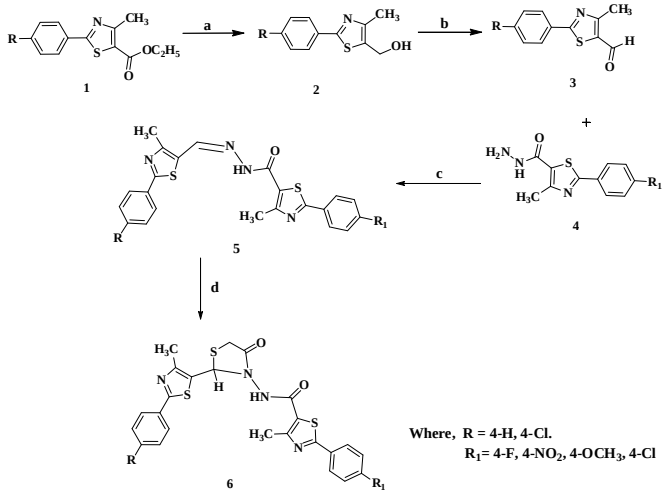
Yield 82 %. m.p: 260-265 ^{o}C. ^{1}H NMR($CDCl_3$, 400MHz): δ 8.45-7.70(dd, 2H, Ar-H), 7.40-7.30 (dd, 2H, Ar-H), 2.84(s, 3H, thiazolic-CH_3), 2.80(s, 3H, thiazolic-CH_3), 4.25 (AB, quartet, 2H, thiazolidinone-CH_2), 6.50(s, 1H, -SCH),11.20(s, 1H, -NH). IR(KBr pellets Cm^{-1}) : 3245 (-NH), 3040 (Ar-C-H), 2920 (CH_3), 1728(CO, cyclic), 1680 (-CONH), 1594 (C=C, str.in Ar), 851(C-Cl), 1550, 1350 (NO_2). Mass (DIP MS): m/z-571. Elemental analysis Calcd for ($C_{24}H_{18}ClN_5O_4S_3$): C, 50.39; H, 3.17, N; 12.24; found: C, 50.25; H, 3.10; N, 12.20 %.

N-(2-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-methoxy phenyl)-4-methylthiazole-5-carboxamide ***(6g) :***

Yield 90%. m.p:222-226 ^{o}C. ^{1}H NMR($CDCl_3$, 400MHz): δ 7.40-7.35(dd, 2H, Ar-H), 7.30-6.80 (dd, 2H,Ar-H), 2.25(s, 3H, thiazolic-CH_3), 2.18(s, 3H, thiazolic-CH_3), 3.80 (AB, quartet, 2H, thiazolidinone-CH_2), 5.12(s, 1H, -SCH), 10.0(s, 1H, -NH), 3.73(s, 3H, OCH_3). IR (KBr pellets Cm^{-1}): 3268 (-NH), 3010 (Ar-C-H), 2940 (CH_3), 1720(CO, cyclic), 1672 (-CONH), 1495(C=C str.in Ar), 1170(OCH_3), 840 (C-Cl). Mass (DIP MS): m/z- 557. Elemental analysis Calcd for ($C_{25}H_{21}ClN_4O_3S_3$): C, 53.90; H, 3.80, N; 10.06; found: C, 53.85; H, 3.84; N, 9.90 %.

2-(4-chlorophenyl)-N-(2-(2-(4-chlorophenyl)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl) -4-methylthiazole-5-carboxamide ***(6h) :***

Yield 75 %. m.p: 136-140 ^{o}C. ^{1}H NMR ($CDCl_3$, 400MHz): δ 7.48-7.30(dd, 4H, Ar-H), 2.25(s, 3H, thiazolic-CH_3), 2.15(s, 3H, thiazolic-CH_3), 4.00(AB, quartet, 2H, thiazolidinone-CH_2), 6.10(s, 1H, -SCH), 9.98 (s, 1H, -NH). IR (KBr pellets Cm^{-1}): 3220(-NH), 3030(Ar-C-H), 2940 (CH_3), 1723(CO, cyclic), 1622 (-CONH), 1545(C=C str.in Ar), 880 (C-Cl). Mass (DIP MS): m/z-559. Elemental analysis Calcd for ($C_{24}H_{18}Cl_2N_4O_2S_3$): C, 51.33; H, 3.23, N; 9.98; found: C, 51.30; H, 3.18; N, 9.90 %.



Scheme: 1. Synthesis of N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide(6a-h)**.** Reagents and condition: (a) $LiAlH_4$, Diethyl ether, 1.30Hrs, 0°C; (b) IBX,DMSO,3Hrs, RT; (c) Ethanol, Conc. H_2SO_4, reflux, 5Hrs; (d) 2-mercaptoacetic acid, anhy. $ZnCl_2$, DMF, 8-10 Hrs

Biological activity

Antibacterial and Antifungal studies :

The synthesized of N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)- 2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide**(6a-h**).were screened for the antibacterial activity against two Gram-positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus* two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* by using the disc diffusion method.[33] Ciprofloxacin was used as reference standard for comparing the results and DMSO as a control Solvent. Newly synthesized compounds were screened for their antifungal activity against *Aspergillus niger, Aspergillus flavus, penicillium chrysogenum, Fusarium moneliforme,* by standard agar disc diffusion method.[34] using Griseofulvin as reference standard and DMSO as control solvent. The antibacterial and antifungal activity of the N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)- 2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide**(6a-h)**. **Table-1 and Table-2** respectively. Investigation of the structure activity relationship study revealed that compound with **6a, 6d, 6e, 6h** electron withdrawing (fluoro,chloro) group on phenyl rings showed significant activity against both Gram-negative and Gram-positive bacteria. Compound **6b, 6c, 6f, 6g** showed moderate activity against both Gram-negative and Gram-positive bacteria. The compounds with electron donating (methoxy) group on phenyl ring showed less activity as compared standard by zone of inhibition data. The investigation of antifungal activity data revealed that compounds **6a,6e,** show inhibitory effect against foure fungal steins and compounds **6b,6d,6e,6h** show inhibitory effect against *Aspergillusniger.* Compounds **6c, 6e, 6f, and 6h** show inhibitory effect against *Aspergillus flavus*. Similarly most of the compounds are active against Fusarium *moneliforme*.

Table-1. In-Vitro Antibacterial activity for compounds 6(a-h)

Sr. No	Compounds	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
1	6a	23	20	19	24
2	6b	15	16	14	17
3	6c	16	14	11	15
4	6d	20	18	17	20
5	6e	21	19	18	22
6	6f	17	16	15	18
7	6g	14	15	12	16
8	6h	21	18	16	20
9	Ciprofloxacin	27	24	22	30
10	DMSO	-ve	-ve	-ve	-ve

-ve no antibacterial activity

In-vitro anti-inflammatory activity :

The synthesized compounds are screened for anti-inflammatory activity by using inhibition of albumin denaturation technique, which was studied according to Muzushima and Kabayashi with slight modification. [35-36]

The standard drug and synthesized compounds **(6a-h)** were dissolving in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solutions was less than 2.0%. Test solution (1 ml) containing different concentration of drugs was mixed with 1 ml of 1% mM albumin solution in phosphate buffer and incubated at 27±1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60±1°C in water bath for 10 min. After cooling, the turbidity was measured at 660nm (UV-Visible Shimadzu Spectrophotometer). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. The Ibuprofen was used as standard drugs. Results are tabulated in **table 3.**

Table-2. Antifungal screening results of the compounds (6a-h).

Sr.No	Compounds	*Aspergillus niger*	*Aspergillus flavus*	*Penicillum chrysogenum*	*Fusarium moneliforme*
1	6a	-ve	-ve	-ve	-ve
2	6b	-ve	RG	+ve	-ve
3	6c	RG	-ve	RG	+ve
4	6d	-ve	RG	-ve	-ve
5	6e	-ve	-ve	-ve	-ve
6	6f	+ve	-ve	RG	-ve
7	6g	RG	+ve	-ve	RG
8	6h	-ve	-ve	RG	-ve
9	Griseofulvin	-ve	-ve	-ve	-ve
10	DMSO	+ve	+ve	+ve	+ve

-ve : No growth Antifungal activity present
+ve : Growth Antifungal activity absent RG : Reduced growth

Table-3- Anti-inflammatory activity of synthesized compounds (6a-h)

Sr. No	Compounds	Mean absorbance value ± SEM	Inhibition of denaturation (in %)
1	Control	0.195±0.04	-
2	Ibuprofen	0.372±0.02	90.76
3	6a	0.359±0.06	84.10
4	6b	0.302±0.08	54.87
5	6c	0.290±0.01	48.71
6	6d	0.332±0.05	70.26
7	6e	0.340±0.03	74.36
8	6f	0.294±0.01	50.76
9	6g	0.280±0.04	43.58
10	6h	0.326±0.07	67.18

Anti-inflammatory activity data **table 3** of compound **(6a-h)** revealed that compound 6a with electron withdrawing fluoro substituent exhibited excellent anti-inflammatory activity. Whereas, compounds **6d**, **6e, and 6h** showed very good anti-inflammatory activity compared to that of standard drug Ibuprofen. Compounds **6b, 6c, 6f, and 6g** showed moderate activity.

Result and Discussion

Literature survey reveals that synthesis of N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)- 2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide(6a-h) derivatives was not reported. Hence it was though worthwhile to synthesized these compounds. Synthesis of (2-(4-phenyl substituted)-4-methylthiazol-5-yl)methanol**(2)** by reduction of ethyl 2-(4-phenyl substituted)-4-methylthiazole-5-carboxylate**(1)** using Lithium Aluminium hydride[37] in diethyl ether solvent at 0^0C.synthesis of 2-(4-phenyl substituted)-4-methylthiazole-5-carbaldehyde(**3)** by oxidation of (2-(4-phenyl substituted)-4-methylthiazol-5-yl)methanol**(2)** using IBX[38] in DMSO solvent at RT. synthesis of N-((2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-4-methyl-2-(phenyl substituted)thiazole-5-carbohydrazide**(5a-h)** from

reaction between 2-(4-phenyl substituted)-4-methylthiazole-5-carbaldehyde(3) and 2-(4-phenyl substituted)-4-methylthiazole-5-carbohydrazide**(4)** in presence of sulphuric acid in ethanol solvent under reflux conditions. synthesis of thiazolidin-4-one derivatives namely N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide**(6a-h)** by reaction of N-((2-(4-phenyl substituted)-4-methylthiazol-5-yl)methylene)-4-methyl-2-(phenyl substituted)thiazole-5-carbohydrazide(5a-h) cyclisation with mercaptoacetic acid in presence of zinc chloride catalyst in DMF solvent under reflux conditions.

In the present study a series of compounds **(6a-h)** were synthesized and evaluated for antibacterial, antifungal and anti-inflammatory activity. The compounds **(6a-h)** were synthesized in good yield and their structures were characterized by spectral data.

The IR spectrum of compound (**3a)** showed a characteristic absorption bands appeared at 1690 cm^{-1}, C=O functional group for aldehyde. ^{1}H-NMR spectrum of compound **(3a)** exhibit singlet at δ 10.04 for –CHO group. IR spectrum of compound **(5a)** absorption band appeared at 1661 cm^{-1}, for amide (-CONH) and 1602 cm^{-1} for imine (-CH=N) functional group. ^{1}H NMR spectrum of compound (5a) exhibit singlet at δ 8.30 for imine (-CH=N) and for singlet at δ 11.40 for (-NH). The mass spectrum showed molecular ion peak at *m/z* - 436(M^+). The IR spectrum of compound (**6a)** showed a characteristic absorption bands appeared at 3232 cm^{-1} for (-NH),1730 cm^{-1} for cyclic CO functional group for thiazolidinone ring and 1665 cm^{-1} for amide (-CONH). ^{1}H-NMR spectrum of compound(6a) exhibit quartet at δ 3.62 for thiazolidinone-CH_2 functional group and singlet at δ 6.23 for SCH functional group of thiazolidinone ring. The mass spectrum showed molecular ion peak at *m/z* - 510(M^+). It is a confirmatory for the synthesis of thiazolidinone derivatives.

Conclusion

In this study we have reported an effective and convenient synthesis of a new series of N-(2-(2-(4-phenyl substituted)-4-methylthiazol-5-yl)-4-oxothiazolidin-3-yl)-2-(4-phenyl substituted)-4-methylthiazole-5-carboxamide **(6a-h).** The structures of these new heterocyclic compounds bearing both thiazole and thiazolidinone ring arrangements were supported by spectral (IR, ^{1}H and Mass) analysis and were evaluated for their antibacterial, antifungal and anti-inflammatory activities. The results proved that many of the synthesized derivatives exhibited significant antibacterial, antifungal and anti-inflammatory activities. The compounds with the electron withdrawing (fluoro) group on 4-position of the phenyl ring supported the antibacterial, antifungal and anti-inflammatory activities. While the electron donating (methoxy) group showed moderate activity. Compounds with electron withdrawing substituents (fluoro and chloro) on phenyl ring encouraged the antifungal activity. The results of anti-inflammatory studies revealed that substitutions with the electron withdrawing (fluoro) group on the phenyl ring exhibited potential activity. Thus, the significant antibacterial, antifungal and anti-inflammatory profiles of some derivatives offer them as promising lead molecules for further optimization using molecular modelling techniques.

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