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SYNTHESIS OF SOME NEW THIADIAZOLYL-THIAZOLIDIN-4-ONE DERIVATIVES AND BIOLOGICAL EVALUATION FOR *IN-VITRO* ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT
4- Thiazolidinones are important members of heterocyclic compounds It contains sulfur and nitrogen along with carbonyl functional group arranged in a saturated heterocyclic ring system which incorporates many biological activities such as anti-inflammatory, antioxidant, antitubercular, antibacterial activities. It has been considered as a magic moiety by many researchers because of its important contribution in the field of drug discovery and its property of possessing several various types of biological activities including antitumor activity. It has been a nucleus of interest because of its usefulness as intermediate for the synthesis of many new heterocyclic compounds. In the present study, an attempt has been made to synthesize 2,4-substituted-1,3-thiazolidin-4-one derivatives by cyclisation of schiff base derivatives. The newly synthesized thiazolidinone derivatives have been characterized by UV, IR, ^{1}HNMR, ^{13}CNMR, Mass spectra and elemental analysis and evaluated for their *in-vitro* anti-inflammatory and antioxidant activities. It was found that the derivatives containing methyl substitution has exhibited good anti-inflammatory and antioxidant activity at low concentrations as compared to the standard drugs.

KEYWORDS: Thiadiazole, 1,3-Thiazolidin-4-one, Antioxidant, anti-inflammatory.

INTRODUCTION
Inflammation is associated with pain and fever, commonly prescribed drug for the treatment of acute and chronic inflammation includes non steroidal anti-inflammatory drugs (NSAIDs). However, there long term use is associated with significant side effects like gastro-intestinal irritations, hypertension, ulcers and nephrotoxicity. Therefore there exists a need for discovery of new and safer anti-inflammatory agents.[1,2,3,4]

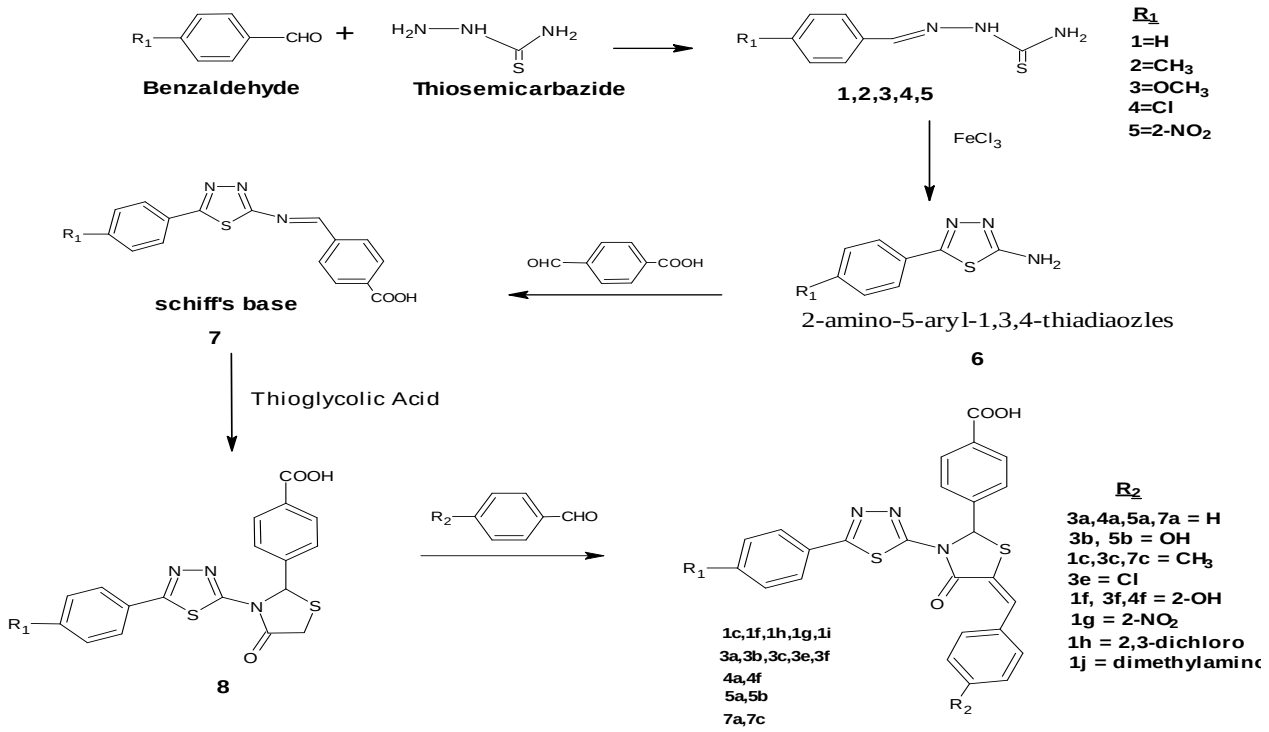
Antioxidants are agents inhibit the oxidation of other molecules. Oxidation of the biomolecules may lead to generation of Reactive oxygen species, which causes oxidative damage to cells leading to age related degenerative diseases and many other diseases including cancer[5,6]. Antioxidant drugs acts by terminating these chain reactions and hence protect the cellular damage. Many antioxidant agents are already there in pharmaceutical practice. However, there is a need to develop new and alternative antioxidant agents because of the side effects caused by existing regimen.[7,8]

Thiazolidinone is an important member of heterocyclic compounds. It is a saturated form of thiazole having a carbonyl group at fourth carbon atom. It has been considered as a magic moiety by many researchers because of its important contribution in the field of drug discovery and its property of possessing several various types of biological activities including antitumor activity. It has been a nucleus of interest because of its usefulness as intermediate for the synthesis of many new heterocyclic compounds. Thiazolidinone have been reported to exhibit good anti-inflammatory and antioxidant activities by the researchers.[9-13]

MATERIALS AND METHODS
All the melting points were determined in a Thermonik melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds was recorded on a Fourier Transform IR spectrometer (model Shimadzu 8700) in the range of 400 -4000 using KBr pellets and the value of λmax were reported in cm-1. 1HNMR spectra was recorded on Amx - 400 MHz NMR spectrometer using CDCl3 and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as internal reference. 13CNMR spectra was recorded on Amx - 400 MHz NMR spectrometer using CDCl3 and the chemical shifts (δ) reported are in parts per million downfield using tetramethylsilane (TMS) as an internal reference. A mass spectrum was recorded on Mass spectrophotometer

(model Shimadzu) by LC-MS 2010A. The purity of the compounds was checked by thin-layer chromatography on silica gel-G plates of 0.5mm thickness as stationary phase and combination of n-hexane: ethyl acetate in different ratios as mobile phase. The UV spectra of the synthesized compounds were recorded on UV–Visible spectrophotometer (model Shimadzu 1601) using methanol and the values of wave length (λ max) were reported in nm. Elemental analysis was done by Thermo Finnigan Flash EA 1112 Series.

SCHEME



General procedure for synthesis of thiosemicarbazone (1,2,3,4,5): 0.2M substituted aromatic benzaldehyde was dissolved 300 ml of warm ethanol. 0.2M of thiosemicarbazide was dissolved in 300 ml of hot water. Both the solutions were mixed slowly with continuous stirring. After cooling, the shiny white needle shaped crystals of product were filtered, washed and dried.

Synthesis of 2-amino-5-substituted-phenyl-1,3,4-thiadiaozles (6): 0.1M of thiosemicarbazone was suspended in 500 ml distilled wateter in a one-litre beaker. Ferric chloride (0.3M), dissolved in 500 ml of distilled water was added to it. The solution was heated to 80-90˚C and maintained for 45 minutes and filtered. A mixture of citric acid (0.22M) and sodium citrate (0.1M) was added to the solution and stirred. After cooling, the solution was neutralized with aqueous ammonia solution (10%). The precipitate then obtained was filtered and washed with distilled water and dried. It was recrystallized using ethanol.

Synthesis of 4-{3-[5-(substituted-phenyl)-[1,3,4]thiadiazol-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid(Schiff base derivatives) (7): 0.02M of the amino-thiadiazole was dissolved in 10 ml of absolute ethanol by heating and stirring. A solution of 0.02M p-carxoylbenzaldehyde in 10ml ethanol was added to it, stirred and whole solution was refluxed for 3-4 hours (77 %) M.P. 212˚C. The crystals obtained on cooling, were filtered and dried.

Synthesis of 2-substitued-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one derivatives (8): A mixture of Schiff base derivative 5-phenyl-n-phenylmethylidene-1,3,4-thiadiazol-2-amine (0.01 mol) and thioglycolic acid (0.01 mol) was refluxed in DMF for 8- 10 hours in presence of anhydrous $ZnCl_2$. The precipitate was filtered, washed, dried and recrystallized using DMF: water as solvent system. It was purified by column chromatography using ethyl acetate: hexane as mobile phase (yield: 80%) M.P.198˚C.

Synthesis of 4-{5-(substituted-benzylidene)-3-[(substituted phenyl)-[1,3,4]thiadiazol-2-yl]-4-oxo-thiazolidin-2-yl)-benzoic acid derivatives (final compounds): 1 mmol of the ***1,3-thiazolidin-4-one derivatives (8)*** was refluxed with 1.5 mmol of substitued aromatic aldehyde in 10 ml of acetic acid with 2 mmol of sodium acetate for 5-7 hours. The mixture was poured on crushed ice. Precipitate was filtered, washed with water, dried and recrystallized from methanol: dioxane (4:1).

4-[5-(4-mehtylbenzylidene)-4-oxo-3-(5-phenyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoic acid ***(1c):*** brown solid; m.p. 166-168ºC; Yield: 53.58 %%; λmax= 213.40; IR (KBr) cm-1: 3480 cm-1 (broad trough of carboxyl group), 2922 cm-1 (Ar C-H str.), 1608cm-1 (

C=O str), 1604.81 cm-1 (Ar C=C), 1457 (C-N str.) and 803 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [13H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 162 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 146 (C, benzoic acid),140-125 Ar-C), 118, 115(C, thiadiazole), 63 (C_2, Thiazolidinone), 20 (C, methyl); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=64.15%, H=4.10% and N=10.02%. Calculated C=64.31%, H=3.94 and N= 8.65%.

*4-[5-(2-Hydroxybenzylidene)-4-oxo-3-(5-phenyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-2-yl]-benzoic acid **(1f):*** brown solid; m.p. 212-214ºC; Yield: 48%; λmax= 213.40; IR (KBr) cm-1: 3416 (-OH str.), 2920 (-CH= str.), 1691 (C=O str.), 1317 (C-N str.)761 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COOH] 8.2 [1H (s,OH)], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 488 (Molecular ion), CHNS: Found C=62.15%, H=3.89% and N=8.11%. Calculated C=61.59%, H=3.31 and N= 8.62%.

[5-(2-Nitro-benzylidene)-4-oxo-3-(5-phenyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-2-yl]-benzoic acid **(1g):** yellowish-brown solid; m.p. 234-236ºC; Yield: 56%; λmax= 213.40; IR (KBr) cm-1: 3328 (broad trough of carboxyl group), 2945(-CH= str.), 1608 (C=O str.), 1532 (-NO_2 str.), 1263 (C-N str.), 798 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.6 [1H (s, COO**H**] 8.1-7.22[14H, (m, Ar H)], 5.80 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 170 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 147 (C-NO_2) 142-120 (13 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 514 (Molecular ion); CHNS: Found C=57.92%, H=3.86% and N=11.12%. Calculated C=58.13%, H=3.12 and N= 10.85%.

[5-(2,3-dichloro-benzylidene)-4-oxo-3-(5-phenyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-2-yl]-benzoic acid **(1h):** brown solid; m.p. 260-262ºC; Yield: 46%; λmax= 213.40; IR (KBr) cm-1: 3448 (broad trough of carboxyl group), 2924(-CH= str.), 1593 (C=O str.),1456 (-NO_2 str.), 1319 (C-N str.), 761 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.5 [1H (s, COO**H**] 8.1-7.22 [12H, (m, Ar H)], 7.65 [1H, s, =CH-], 5.92 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 142-120 15C, Ar-C), 132-130(2C-Cl), 118-115 2(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 540(Molecular ion), 541, 542; CHNS: Found C=56.05%, H=3.16% and N=7.10%. Calculated C=55.56%, H=2.80 and N= 7.78%.

*4-[5-(4-Dimethylamino-benzylidene)-4-oxo-3-(5-phenyl-[1,3,4]thiadiazol-2-yl)-thiazolidin-2-yl]-benzoic acid **(1j):*** brown solid; m.p. 190-192ºC; Yield: 60%; λmax= 213.40; IR (KBr) cm-1: 3491 (broad trough of carboxyl group), 2992(-CH= str.), 1608(C=O str.), 1320 (C-N str.), 1289 (C-N of amino group), 808 (cyclic C-S of thiazolidinone), 1HNMR (400 MHz, DMSO D6): δ= 10.6 [1H (s, COO**H**] 7.1-7.22 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 4.2[2H, (s, NH_2]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 145(C, C-NH_2), 142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 540 (Molecular ion), 541, 542; CHNS: Found C=62.17%, H=3.18% and N=11.25%. Calculated C=61.71%, H=3.73 and N= 11.51%.

4-5[-benzylidene-4-oxo-3-(5-p-tolyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoic acid **(3a):** off-white solid; m.p. 210-212ºC; Yield: 47%, λmax= 213.40; IR (KBr) cm-1: 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [13H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 162 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 146 (C, benzoic acid),140-125 Ar-C), 118, 115(C, thiadiazole), 63 (C_2, Thiazolidinone), 20 (C, methyl); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=62.31%, H=3.94% and N=9.12%. Calculated C=62.89%, H=4.00 and N= 9.12%.

4-5[4-hydroxy-benzylidene-4-oxo-3-(5-p-tolyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoicacid **(3b):** Off-white solid; m.p. 140-142 ºC; Yield: 53.58 %%; λmax= 213.40; IR (KBr) cm-1: 3481(COOH), 3161 (-OH str.), 2916 (C=O of carboxyl group), 2783 (C-H str. of CH_3), 1693 (C=O str.), 1313 (C-N), 823 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.8[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone), 20 (C, methyl); m/e 501 (Molecular ion); CHNS: Found C=2.81%, H=43.42% and N=8.38%. Calculated C=642.26%, H=3.82 and N= 8.38%.

4-5[4-methyl-benzylidene-4-oxo-3-(5-p-tolyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoic acid **(3c):** Off-white solid; m.p. 152-154ºC; Yield: 58%; λmax= 213.40; IR (KBr) cm-1: 3481(COOH), 3153 (-OH str.), 2916 (C=O of carboxyl group), 2783 (C-H str. of CH_3), 1693 (C=O str.), 1313 (C-N), 823 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[6H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 118,

115(C, thiadiazole), 63(C_2, Thiazolidinone), 20 (2C, methyl); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=64.28%, H=4.63% and N=8.35%. Calculated C=64.63%, H=4.24 and N= 8.41%.

4-5[4-chloro-benzylidene-4-oxo-3-(5-p-tolyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoic acid **(3e):** Off-white solid; m.p. 172-174ºC; Yield 60%; λmax= 213.40; IR (KBr) cm-1: 3480 cm-1 (broad trough of carboxyl group), 2922 cm-1 (Ar C-H str.), 1608cm-1 (C=O str), 1604.81 cm-1 (Ar C=C), 1457 (C-N str.) and 803 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**], 7.94-6.87 [13H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 156 (C, C-CH_3), 151 (C, benzoic acid),142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone), 24 (C, methyl); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=60.79%, H=3.85% and N=7.58%. Calculated C=60.05%, H=3.49 and N= 8.08%.

4-5[2-hydroxy-benzylidene-4-oxo-3-(5-p-tolyl-[1,3,4]thiadiazole-2-yl)-thiazolidin-2-yl]-benzoic acid **(3f):** Off-white solid; m.p. 187-188ºC; Yield: 64%; λmax= 213.40; IR (KBr) cm-13481(COOH), 3161 (-OH str.), 2916 (C=O of carboxyl group), 2783 (C-H str. of CH_3), 1693 (C=O str.), 1313 (C-N), 823 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone), 25 (C, methyl); m/e 501(Molecular ion); CHNS: Found C=62.18%, H=3.82% and N=8.38%. Calculated C=62.26%, H=3.64 and N= 8.42%.

4-{5-benzylidene-3-[5-(4-mehtoxy-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(4a):** yellowish solid; m.p. 196-198ºC; Yield: 58 %; λmax= 213.40; IR (KBr) cm-1: 3431(COOH), 3161 (C=O of carboxyl group), 2985 (OH str.), 2873 (C-H str. of CH_3), 1701 (C=O str.), 1313 (C-N), 1255 (C-O-C str.), 823 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 3.8 [3H, d, OCH_3]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 142-120 (Ar-C), 118-115(C, thiadiazole), 63(C_2, Thiazolidinone), 58 (C, methoxy); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=62.12%, H=3.95% and N=8.43%. Calculated C=62.26%, H=3.82 and N= 8.38%.

4-{5-hydroxy-benzylidene-3-[5-(4-mehtoxy-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(4f):** yellowish-white solid; m.p. 220-222ºC; Yield: 78%; λmax= 213.40; IR (KBr) cm-1: 3431(COOH), 3163 (C=O of carboxyl group), 2985 (OH str.of COOH), 2873 (C-H str. of CH_3), 2783(OH str.), 1701 (C=O str.), 1313 (C-N), 1178 (C-O-C str.), 823 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 3.8 [3H, d, OCH_3] ; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid), 142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone), 58 (C, methoxy); m/e 547(Molecular ion); CHNS: Found C=61.28%, H=3.70% and N=8.12%. Calculated C=61.28%, H=3.24 and N= 8.88%.

4-{5-chloro-benzylidene-3-[5-(4-mehtoxy-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(5a):** white solid; m.p. 260-262ºC; Yield: 46 %%; λmax= 213.40; IR (KBr) cm-1: 3431(COOH), 2922 (OH str. of COOH),1685 (C=O str. of COOH), 1317 (C-N), 823 (cyclic C-S of thiazolidinone), 688(Cl str.); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 142-120 (Ar-C), 133(C, C-Cl), 118-115(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=58.36%, H=2.78% and N=8.65%. Calculated C=59.34%, H=3.19 and N= 8.30%.

4-{5-hydroxy-benzylidene-3-[5-(4-mehtoxy-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(5b):** White solid; m.p. 190-192ºC; Yield: 60 %%; λmax= 213.40; IR (KBr) cm-1: 3431(broad trough of COOH), 3285 (OH str.), 2787 (OH str. of COOH), 1685 (C=O str. of COOH), 1317 (C-N), 823 (cyclic C-S of thiazolidinone), 688(Cl str.); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**] 8.2 [1H (s,O**H**)], 7.94-6.87 [13H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 133(C, C-Cl), 118-115(C, thiadiazole), 63(C_2, Thiazolidinone); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=57.46%, H=3.15% and N=8.10%. Calculated C=57.52%, H=3.09 and N= 8.05%.

4-{5-benzylidene-3-[5-(4-nitro-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(7a):** yellow solid; m.p. 188-190ºC; Yield: 60%; λmax= 213.40; IR (KBr) cm-1: 3431 (broad trough of COOH), 2918 (OH str. of COOH), 1701 (C=O str. of COOH), 1533 (-NO_2), 1379 (C-N), 773 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**], 7.94-6.87 [14H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 151 (C, benzoic acid), 142-120 (Ar-C), 118, 115(C, thiadiazole), 63 (C_2, Thiazolidinone); m/e 517(Molecular ion); CHNS: Found C=58.02%, H=3.26%

and N=10.66%. Calculated C=58.13%, H=3.12 and N= 10.85%.

4-{5-methyl-benzylidene-3-[5-(4-nitro-phenyl)-1,3,4]thiadiazole-2-yl]-4-oxo-thiazolidin-2-yl}-benzoic acid **(7c):** Off-white solid; m.p. 196-198ºC; Yield: 67 %%; λmax= 213.40; IR (KBr) cm-1: 3380 (broad trough of COOH), 3012 (C-H str. of CH_3), 2777 (OH str. of COOH), 1701 (C=O str. of COOH), 1533 (-NO_2), 1377 (C-N), 773 (cyclic C-S of thiazolidinone); 1HNMR (400 MHz, DMSO D6): δ= 10.3 [1H (s, COO**H**], 7.94-6.87 [13H, (m, Ar H)], 5.92 [1H, (s, Thiazolidinone,CH], 2.4[3H (C**H**$_3$)]; 13CNMR(400 MHz CDCl3, ppm): δ= 169 (C, Carboxyl), 161 (C, carbonyl of thiazolidinone), 158 (C, C-OH), 151 (C, benzoic acid),142-120 Ar-C), 118, 115(C, thiadiazole), 63(C_2, Thiazolidinone), 21 (C, methyl); m/e 485(Molecular ion), 478, 460, 301(100); CHNS: Found C=58.72%, H=3.54% and N=10.62%. Calculated C=58.86%, H=3.42 and N= 10.56%.

Anti-Inflammatory Activity

a) Protein denaturation assay: A solution of 0.2% w/v of BSA was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Stock solutions of 1000µg/ml of all test samples were prepared by using methanol as a solvent. From the stock solutions two different concentrations of 100µg/ml and 200µg/ml were prepared by using methanol as a solvent. 100µg/ml (0.1ml) of each test sample was transferred to volumetric flask (10ml) using 1ml micropipette. 5ml of 0.2% BSA was added to all of the above flasks. The control consists of 5ml 0.2% w/v BSA solution with 0.1ml methanol. The 0.1ml standard consisted 100µg/ml of indomethacin in methanol with 5ml 0.2% w/v BSA solution. The volumetric flasks were heated at 72°C for five minutes and then cooled for 10 min. the absorbance of these solutions was determined by using spectrophotometer at a wavelength of 660 nm. The % denaturation of the protein (% inhibition) was determined.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

b) Trypsin (Proteinase) inhibitory assay

The reaction mixtures (2.0 ml) contained 0.06 mg trypsin, 1.0 ml. 25 mM tris-HCl buffer (pH 7.4) and 1.0 ml of different concentrations of compounds (10, 25 and 50 µg/ml). The mixtures were incubated at 37°C for 5 minutes then 1.0 ml of 0.8% (w/v) casein was added. The mixtures were incubated for an additional 20 minutes. Then 2.0 ml of 70% (v/v) perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and absorbance of the supernatant was read at 280 nm against buffer as blank[16-18].

The percentage of inhibition was calculated as follows.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Antioxidant Activity

a) 2, 2-diphenyl-1-picryl hydrazine (DPPH method)

10 mg of standard ascorbic acid was dissolved in methanol. From this stock solution dilutions were made to obtain concentrations of 10 to 40 µg/ ml. 1 ml from each of these solutions was taken in different volumetric flasks to which 1 ml of DPPH solution was added and volume was made up to 10 ml. The test solution were prepared in similar manner as that of standard ascorbic acid and the absorbance were recorded at 516 nm after duration of 30 min.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

b) Nitric oxide scavenging assay

Sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations (5 - 200µg/ml) of test compounds and incubated at 300 C for 2 hours. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3P0_4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance of the resulted chromophore was recorded at 550nm. Inhibition of nitrite formation by the compounds and the standard antioxidant ascorbic acid were calculated relative to the control and the percentage inhibition was calculated.

$$\% \text{ inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISSCUSSION

Anti-inflammatory activity: Compounds exhibited good *in vitro* antiinflammatory activity as compared to standard indomethacin. Two different mechanism of antiinflammtory activity were studied and compounds were found to exhibit good activity via both the mechanisms.

Protein denaturation assay: Compounds 1f, 3a, 4c and 5a were found to be most active while others exhibited moderate activity.

S. N.	Compound code	% Inhibition	
		100µg/ml	200µg/ml
1.	1c	68.69	84.82
2.	1f	76.24	88.19
3.	1g	69.12	79.09
4.	1h	64.53	77.28
5.	1j	77.24	80.90
6.	3a	87.51	90.54
7.	3b	76.36	88.20
8.	3c	67.37	74.28
9.	3e	77.28	82.54
10.	3f	78.84	85.62
11.	4c	80.88	89.63
12.	4f	78.84	86.72
13.	5a	80.19	90.14
14.	5b	54.58	70.11
15.	7a	68.81	74.48
16.	7c	63.58	74.48
	Indomethacin	**90%**	**91.81%**

Trypsin (Proteinase) inhibitory assay: Compounds 1h, 3c, 4c, 5a were found to be most active while others exhibited moderate activity.

S. N.	Compound code	% Inhibition		
		10µg/ml	25µg/ml	50µg/ml
1.	1c	75.5	77.3	84.0
2.	1f	36.3	48.19	56.2
3.	1g	59.1	62.3	64.4
4.	1h	78.8	82.2	85.8
5.	1j	77.2	80.9	82.6
6.	3a	57.6	69.9	80.3
7.	3b	76.3	82.2	84.6
8.	3c	47.3	82.3	88.6
9.	3e	45.6	52.6	66.8
10.	3f	48.8	60.6	77.8
11.	4c	77.7	80.5	88.4
12.	4f	55.8	62.7	70.4
13.	5a	74.2	80.4	87.2
14.	5b	54.58	60.11	66.8
15.	7a	52.3	54.48	58.6
16.	7c	68.6	74.48	80.3
	Indomethacin	88.10	89.15	90.18

Antioxidant Activity

Antioxidant assay was performed using two different methods to study the mechanism of antiinflammtory action of the compounds. The compounds exhibited better results in DPPH method as compared to nitric oxide scavenging method.

2, 2-diphenyl-1-picryl hydrazine (DPPH method)

All the compounds exhibited high antiinflammatory potential while 1c, 1h, 3a, 3c, 3f, 4c, 4f and 5a exhibited highest percentage inhibition.

S. N.	Compound code	% Inhibition			
		10µg/ml	25µg/ml	50µg/ml	100µg/ml
1.	1c	92.2	92.7	92.8	94.3
2.	1f	84.8	87.4	90.4	90.8
3.	1g	81.4	84.3	87.2	90.7
4.	1h	88.2	89.7	90.8	94.2
5.	1j	82.8	85.4	86.1	86.7
6.	3a	92.8	93.1	94.2	94.6
7.	3b	80.1	84.7	85.8	90.7
8.	3c	87.5	90.5	92.3	93.6
9.	3e	85.6	85.7	86.3	86.7
10.	3f	85.1	88.0	90.9	93.6
11.	4c	89.8	90.5	92.1	93.4
12.	4f	88.8	90.5	93.2	93.5
13.	5a	79.4	80.5	83.3	97.4
14.	5b	76.9	77.6	83.5	84.8
15.	7a	83.0	85.6	87.4	87.9
16.	7c	80.9	83.5	85.6	89.9
	Indomethacin	92.3	96.5	97.1	99.9

Nitric oxide scavenging assay: The compounds were found to be moderately active via nitric oxide scavenging method. 4c and 4f exhibited highest percentage inhibition.

S. N.	Compound code	% Inhibition			
		10µg/ml	25µg/ml	50µg/ml	100µg/ml
1.	1c	62.8	66.7	68.5	71.3
2.	1f	54.6	57.4	58.0	60.8
3.	1g	39.4	44.3	47.2	52.7
4.	1h	48.6	49.9	52.8	54.2
5.	1j	68.7	71.7	74.6	76.7
6.	3a	46.8	53.0	57.2	59.3
7.	3b	50.0	54.8	65.8	70.7
8.	3c	37.6	39.5	43.3	48.6
9.	3e	65.5	54.6	56.8	58.2
10.	3f	55.8	58.7	63.3	67.0
11.	4c	78.5	80.1	82.1	83.4
12.	4f	74.8	76.8	78.9	80.9
13.	5a	39.5	43.6	45.7	47.4
14.	5b	26.5	37.6	43.5	54.8
15.	7a	53.6	55.9	57.4	57.9
16.	7c	70.9	73.2	75.6	79.5
	Indomethacin	89.7	90.3	92.2	97.4

CONCLUSION

Thiazolidinone derivatives were synthesized and the structure was verified with spectral characterization. The final derivatives were evaluated for their antiinflammatory activity. The complunds were found to exhibit good antiinflammatory and antioxidant activity. The compounds were found to exhibit better antioxidant activity via DPPH method as compared to NO scavanging method. It can be said that the thiazolidinone would react better with oxygen free radical than the nitrogen free radicals. Thiazolidinones may be a potential drug moiety for designing of new anti-inflammatory and antioxidant agents.

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