Design, synthesis and anti-inflammatory activity of novel 5-(Indol-3-yl)-thiazolidinone derivatives as COX-2 inhibitors.

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Abstract

New N-substituted 5-(oxindolyl)-2-thiooxothiazolidinone derivatives were synthesized. The C⁵-substituted thiazolidinone derivatives with piperidinyl and morpholinyl moieties in addition to the tetracyclic [(oxindolo) pyrazino] thiazolidine, the chloro- and amino-derivatives of the (indolyl) thiazolidinone ring system were also prepared. The COX-2 inhibition activity of the synthesized compounds was investigated by studying their ability to inhibit the conversion of arachidonic acid to prostaglandin H₂ (PGH₂). Five of the tested candidates, substituted (oxindolyl) thiazolidine derivatives (3a, 6f, 8b, 10 and 12) showed significant COX-2 inhibitory activity exhibiting IC₅₀ values better than or close to the reference celecoxib. The anti-inflammatory activity was studied revealing that a number of compounds have shown good activities and compound 10 produced no significant mucosal injury. Molecular docking study was implemented to interpret the variable inhibitory activity of the newly synthesized compounds against COX enzyme. The results suggested that some of these derivatives could be active COX inhibitors possessing a high preference for COX-2.

Keywords: Docking, 4-Thiazolidinones, COX-2 inhibitors, Catalyst, Indole-2,4-dione.

Accepted April 29, 2020

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are considered the most excessively particular drugs for inflammation treatment including pain releasing, anti-pyretic and rheumatoid arthritis. They inhibit synthesis of prostaglandin by blocking the cyclooxygenation of arachidonic acid (AA) to prostaglandin G₂ (PGG₂) [1]. This inhibition process is catalyzed by means of the enzyme cyclooxygenase (COX) of which (COX-1) and (COX-2) are two similar but diverse isoforms of the enzyme [2-4]. COX-2 is prompted upon inflammatory motivators and is responsible for advancement of inflammation process, whereas COX-1 is a constitutively expressed isoform and is responsible for the servicing of physiological homestasis, such as gastrointestinal integrity and renal function [5]. Thus inhibition of COX-2 over COX-1 enzymes selectively will be beneficial for the treatment of inflammation and related turmoil with diminished gastrointestinal toxicities when compared with the conventional NSAIDs. Current research has focused on the development of more secure NSAIDs-selective COX-2 inhibitors. Recently, several selective COX-2 inhibitors such as Celecoxib, Rofecoxiband Valdecoxib have been marketed as a new generation of NSAIDs [6-8]. However, Rofecoxib was banned in 2004 because of cardiac toxicity [9]. One of the important templates widely used in drug design is indole ring system which constitutes the classical nonselective NSAID indomethacin I. Several strategies have been studied on the amendment of indomethacin which included replacing the acid radical and/or the 4-chlorobenzoyl group by more bulky groups and heterocycles[10,11] (II-IV, Figure 1). The previous strategies planned for production of lead compounds able to fit favorable into COX-2 active site, but less in COX-1, considering the supposition that COX-2 enzyme might have a wider active site than COX-1 [12]. A number of indole incorporating compounds have also been revealed as potent and selective COX-2 inhibitors [13,14]. Furthermore, several derivatives of thiazole [10,15] and thiazolinedione [16,17]. Derivatives have been known with their established anti-inflammatory activity and COX-2 inhibition revealing that the activity of these compounds was proved to be attributed to these moieties (Vand VI, Figure 1). Motivated by the aforementioned findings and aiming to design new selective COX-2 inhibitors and continuing the previous work for discovering of new effective indole and thiazole derivatives [18-22]. We report the synthesis of a series of hybrid compounds having two active pharmacophores namely indole and rhodanine studying their activity as anti-inflammatory agents and COX-2 inhibitors.

Experimental

Chemistry

General methods: All the solvents used were commercially purchased and distilled before use. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60F254), visualizing with ultraviolet light. Column chromatography was performed on silica gel (230-400 mesh) using distilled petroleum ether, ethyl acetate, dichloromethane, chloroform, and methanol. Infrared spectra (KBr) were recorded on FT-IR 5300 spectrophotometer and Perkin Elmer spectrum RXI FT-IR system (ν, cm⁻¹). ¹H NMR spectra were recorded on Varian Gemini spectrophotometer (400 MHz) in DMSO-d₆ or CDCl₃ as solvent. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ=0.00) as internal standard and expressed in ppm. Coupling constants (J) are given in hertz. Melting points were determined by using melting point apparatus and are uncorrected. MS spectra were obtained on a
GC-Ms-QP 1000 EX mass spectrometer at 70 eV. Microanalyses were performed using a CHNS analyzer. Elemental data are within ± 0.4% of the theoretical values. All yields reported are unoptimized. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Aldrich. Compounds 5a, 5b were prepared following a previously reported method [23].

**Synthesis of 5-(5-methyl-2-oxoindolin-3-ylidene)-3-substituted-2-thioxothiazolidin-4-one (3a-c)**

**General procedure:** A mixture of 5-methylindol-2.3-dione (1) (1.61 g, 0.01 mole), 3-substituted 4-thiozolidinone (2a-c) (0.01 mol) and fused sodium acetate (2.46 g, 0.02 mol) in glacial acetic acid (20ml) was refluxed for 2 hrs. The reaction mixture was cooled and poured onto 150 mL ice-cold water, the red precipitated solid that formed was filtered off, washed with water and recrystallized from the proper solvent to give (3a-c). Compound was obtained as grey powder; m.p>300°C (reported m.p>295°C) [24].

**5-(5-Methyl-2-oxoindolin-3-ylidene)-3-phenyl-2-thioxothiazolidin-4-one (3b)**

Pink crystals, recrystallized from acetic acid; yield 60%, m.p.>300 C. IR (KBr, cm⁻¹): νmax 3208 (NH), 1694, 1621 (2C=O), 1250 (C=S); ¹H-NMR (400 MHz, DMSO-d₆); δppm: 2.32 (s, 3H, CH₃), 7.69-6.91 (m, 8H, Ar-H), 10.49, 10.81 (2s, 2NH); Anal.Calcd. for C₁₈H₁₁ClN₂O₂S₂ (386.87) C, 55.88; H, 2.87; N, 7.24; found: C, 55.59; H, 3.02; N, 6.98.

**3-(4-Chlorophenyl)-5-(5-methyl-2-oxoindolin-3-ylidene)-2-thioxothiazolidin-4-one (3c)**

Dark pink powder, recrystallized from ethanol, yield 75%, m.p.>30 C. IR (KBr, cm⁻¹): νmax 3175 (NH), 1697, 1657 (2C=O), 1248 (C=S); ¹H-NMR (400 MHz, DMSO-d₆); δppm: 2.30 (s, 3H, CH₃), 8.45, 17.478 (m, 7H, Ar-H), 11.16 (s, 1H, NH). GCMS: m/z[M]+ 386 (14.4%), 189 (100%), 146 (4%), 134 (31.6%), 105 (6.3%); Anal.Calcd. for C₁₈H₁₁CN₂O₂S₂ (386.87) C, 55.88; H, 2.87; N, 7.24; found: C, 55.59; H, 3.02; N, 6.98.

**5-(5-Methyl-2-oxoindolin-3-yl)-3-substituted phenyl-2-thioxothiazolidin-4-one (4a-4c)**

**General procedure:** A mixture of the thioxothiazolidine-4-one derivatives 3a-3c (0.001 mole) and 0.5 g of zinc powder and glacial acetic acid (15 ml), the mixture was warmed on steam bath till the reaction was completed (the red color of the solution is completely discharged). The mixture was cooled and poured onto 150 ml ice water; the white precipitate was collected and crystallized from proper solvent.

**Figure 1. Examples of indole and thiazole derivatives as COX-2 inhibitors.**
5-(5-Methyl-2-oxoindolin-3-yl)-3-phenyl-2-thioxothiazolidin-4-one (4b)

White crystals, recrystallized from ethanol, yield 65%, m.p. 240-242 C; IR (KBr,v/cm⁻¹): ν max 3210 (NH), 1760, 1700 (C=O), 1320 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.30 (s, 3H, CH₃), 3.58-4.53 (d, d, 2H, J=16.6, 6.0, CH-CH), 7.67-6.88 (m, 8H, Ar-H), 10.84 (s, 1H, NH exchangeable); Anal. Calc. for C₁₄H₁₇NO₂S (354.45) C, 61.00; H, 3.98; N, 7.90; found: C, 60.71; H, 3.82; N, 7.72.

5-(5-Methyl-2-oxoindolin-3-yl)-3-(4-chlorophenyl)-2-thioxothiazolidin-4-one (4c)

White powder, recrystallized from ethanol, yield 70%, m.p. 224-226 C; IR (KBr, v/cm⁻¹): ν max 3311 (NH), 1757, 1700 (C=O), 1319 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.30 (s, 3H, CH₃), 5.59-4.53 (d, d, 2H, J=16.6, 6.0, CH-CH), 7.67-6.88 (m, 7H, Ar-H), 10.67 (d, 1H, N exchangeable); Anal. Calc. for C₁₉H₁₇NO₂S (388.89) C, 55.59; H, 3.37; N, 7.20; found: C, 55.35; H, 3.24; N, 6.84.

5-Methyl-1-(piperyrid-1-ylmethyl)-indoline-2,3-dione (5c)

To a solution of 5-methylindol-2,3-dione (1) (1.61 g, 0.01 mol) dissolved in DMSO (25 ml) was added formaldehyde (40%, 1.5 ml) and piperazine (0.86 g, 0.01 mol), and the mixture was stirred for 3-4 hrs, at room temperature. The resulting solid was collected by filtration and recrystallized from suitable solvent. The physical properties of compounds 5a and 5b were in accordance with those reported earlier. Red powder; recrystallized from ethanol; yield 75%, m.p. 155-157 C; IR (KBr/cm²): ν max 3218 (NH), 3073 (C-H aromatic), 2925 (C-H aliphatic), 1754, 1733 (C=O); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.30 (s, 3H, CH₃), 3.75-3.27 (m, 8H, piperazine-CH₂), 3.96 (s, 2H, CH₂), 6.91-7.69 (m, 8H, Ar-H); Anal. Calc. for C₂₃H₂₀N₂O₃S (373.49) C, 61.42; H, 4.28; N, 8.39.

Synthesis of Mannich bases; 5-(5-methyl-2-oxo-1-(2-amino-1-phenylindolin-3-ylidene)-3-substituted-2-thioxothiazolidin-4-one (6a-6g)

General procedure: A solution of 5-(5-methyl-2-oxoindolin-3-ylidene)-3-substituted 2-thioxothiazolidin-4-one derivatives 3a-3c (0.01 mol), formaldehyde (40%, 1.5 ml) and appropriate amine (0.01 mole) in DMSO (25 ml) was stirred for 5-6 h, at room temperature. The resulting solid was collected by filtration and recrystallized from suitable solvent.

5-(5-Methyl-2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene)-2-thioxothiazolidin-4-one (6a)

Yellowish Brown powder, recrystallized from toluene, yield 60%, m.p. 150-152 C; IR (KBr, v/cm⁻¹): ν max 3116 (NH), 3013, 3066 (C-H aromatic), 2944, 2854 (C=H aliphatic), 1701, 1685 (C=O), 1263 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.32 (s, 3H, CH₃), 3.35-3.44 (m, 10H, piperidine-CH₂), 3.90 (s, 2H, CH₂), 6.85-7.22, 8.60 (s, d, d, 3H, Ar-CH), 13.98 (s, 1H, NH exchangeable); 13C NMR (DMSO-d6); 6 ppm 21.9 (CH₂), 24.2, 29.7, 56.0 (piperidinyl-CH), 74.1 (CH), 114.1-153.6 (Ar-CH and C=CH²), 172.0, 172.1 (2C=O), 187.1 (C=S); GC-MS: m/z [M] 373 (0.06%), 145 (100%), 134 (13.30%); Anal. Calcd. for C₁₈H₁₉N₂O₂S (373.49) C, 57.61; H, 5.11; N, 11.25; found: C, 57.61; H, 5.11; N, 11.02.

5-(5-Methyl-2-oxo-1-(piperidin-1-ylmethyl)-indolin-3-ylidene)-3-phenyl-2-thioxothiazolidin-4-one (6b)

Brownish red powder, recrystallized from toluene, yield 60%, m.p. 300 C; IR (KBr, v/cm⁻¹): ν max 3061 (C-H aromatic), 2920, 2856 (C-H aliphatic), 1729, 1658 (2C=O), 1224 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.32 (s, 3H, CH₃), 3.44-3.35 (m, 10H, piperidine-CH₂), 3.90 (s, 2H, CH₂), 6.91-7.69 (m, 8H, Ar-CH); GC-MS: m/z [M] 486 (65%), [M]+ 484 (2.59%), 385 (8.75%), 243 (8.47%), 146 (1.84%), 118 (8.49%); Anal. Calcd. for C₂₃H₂₀N₂O₂S (484.03) C, 59.56; H, 4.58; N, 8.68; found: C, 59.54; H, 4.28; N, 8.39.

5-(5-Methyl-2-oxo-1-(morpholin-1-ylmethyl)indolin-3-ylidene)-3-phenyl-2-thioxothiazolidin-4-one (6d)

Brown powder, recrystallized from xylene, yield 55%, m.p. 132-134 C; IR (KBr, v/cm⁻¹): ν max 3061 (C-H aromatic), 2920, 2856 (C-H aliphatic), 1729, 1658 (2C=O), 1224 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.30 (s, 1H, CH₃), 3.55-3.30 (m, 8H, morpholine-CH₂), 4.54 (s, 2H, CH₂), 9.29-7.31 (m, 7H, Ar-CH); GC-MS: m/z[M] 486 (65%), [M]+ 484 (2.59%), 385 (8.75%), 243 (8.47%), 146 (1.84%), 118 (8.49%); Anal. Calcd. for C₂₃H₂₀N₂O₂S (484.03) C, 59.56; H, 4.58; N, 8.68; found: C, 59.54; H, 4.28; N, 8.39.

5-(5-Methyl-2-oxo-1-(morpholin-1-ylmethyl)indolin-3-ylidene)-3-(4 chlorophenyl)-2-thioxothiazolidin-4-one (6e)

Bright Brown powder, recrystallized from toluene, yield 50%, m.p. 300 C; IR (KBr, v/cm⁻¹): ν max 3062, 3035 (C-H aromatic), 2928, 2854 (C-H aliphatic), 1732, 1686 (2C=O), 1225 (C=S); H-NMR (400 MHz, DMSO-d6): 6 ppm 2.30 (s, 1H, CH₃), 3.55-3.30 (m, 8H, morpholine-CH₂), 4.54 (s, 2H, CH₂), 7.60-6.84 (m, 7H, Ar-H); GC-MS: m/z[M] 373 (1.53%), 145 (100%), 133 (10.91%); Anal. Calcd. for C₁₈H₁₉N₂O₂S (374.48) C, 54.53; H, 4.85; N, 14.96; found: C, 54.61; H, 4.71; N, 14.90.
5-(5-Methyl-2-oxo-1-(piperazine-1-ylmethyl)indolin-3-ylidene)-3-(4-chlorophenyl-2-thioxothiazolidin-4-one (6g)

Bright Brown powder, recrystallized from toluene, yield 65%, m.p.>300°C. IR (KBr, cm⁻¹): νס 3173 (NH), 2917, 2852 (C-H aliphatic), 2085 (C-H aromatic), 1697, 1656 (2C=O), 1247 (C=S), 1141 (NH), 1340 (C-O), 1247 (C=S), 8.61; found: C, 56.77; H, 4.42; N, 8.94.

1702, 1687 (2C=O); GC-MS: m/z[M⁺] 331 (82.02%), 189 (11.24%), 155 (11.24%), 146 (65.17%), 102 (0.79%); Anal. Calcd. for C₂₃H₁₇N₁₀O₂S (392.33) C, 58.35; H, 4.59; N, 12.76; found: C, 58.08; H, 4.32; N, 12.86.

5-(5-Methyl-2-oxoindolin-3-yl)-2-morphinothiazol-4(5H)-one (9)

Zinc powder (0.5 gm) was added to a solution of compound 8b (0.01 mole) in glacial acetic acid (20 ml) and the mixture was heated on steam bath for 6 hrs. till the reaction completed (the color of the solution is completely disappear). The reaction mixture was cooled and poured onto ice cold water (150 ml); then the precipitated solid was collected by filtration and recrystallized from methanol. White crystals, recrystallized from toluene, yield 55%, m.p.260-262°C. IR (KBr, cm⁻¹): νς 3194 (NH), 1703, 1682 (2C=O), 1557 (C=N); 1H-NMR (400 MHz, DMSO-d₆): δppm 2.30 (s, 3H, CH₃), 3.99 (m, 8H, morpholine-CH₂), 4.30 (m, 10H, piperidin-CH), 7.72-6.74 (m, 7H, Ar-H); 13C NMR (DMSO-d₆): δppm 21.4 (CH₃), 24.2, 31.7, 45.5 (piperidinyl-C), 116.1-161.6 (Ar-C and C=C), 172.1, 172.2 (2C=O); GC-MS: m/z[M⁺] 326 (13.4%), 224 (11.7%), 189 (100%), 154 (16%), 134 (21.1%), Anal. Calcd. for C₁₆H₁₇N₂O₂S (329.30) C, 58.70; H, 5.23; N, 21.39; found: C, 58.83; H, 4.99; N, 21.33.

4-(9-Methyl-10b, 10c-dihydro-6H-thiazolo[5',4':5,6]pyridazino[3,4-b]indol-2-yl)morpholine (10)

To a solution of compound 9 (0.01 mole) and hydrazine hydrate (0.01 mole) in ethanol (25 ml) was added few drops of piperidine and the reaction mixture was refluxed for 2 hrs, then poured onto 150 ml ice cold water. The precipitated solid was filtered, dried and recrystallized from ethanol to give compound 10. Orange powder, recrystallized from methanol, yield 60%, m.p.264-265°C; IR (KBr, cm⁻¹): νς 3229 (NH), 2912 (C-H aliphatic), 1617 (C=O); 1H-NMR (300 MHz, DMSO-d₆): δppm 3.00 (s, 3H, CH₃), 4.30-2.00 (m, 16H, piperidine and morpholine exchangeable); 13C NMR (DMSO-d₆): δppm 21.4 (CH₃), 24.2, 31.7, 45.5 (piperidinyl-C), 116.1-161.6 (Ar-C and C=C), 172.1, 172.2 (2C=O); GC-MS: m/z[M⁺] 327 (24.3%), 189 (100%), 134 (21.1%), 110 (10.9%); Anal. Calcd. for C₁₆H₁₇N₂O₂S (327.41) C, 58.70; H, 5.23; N, 21.39; found: C, 58.83; H, 4.99; N, 21.33.

5-(5-Methyl-2-oxo-1-(piperazine-1-ylmethyl)indolin-3-yl)-2-morphinothiazol-4(5H)-one (11)

A mixture of compound 9 (0.01 mole) and morpholine with formaldehyde (40%, 1.5 ml) in 30 ml ethanol, the mixture was stirred for 2-3 hrs., at room temperature. The resulting solid was collected by filtration and recrystallized from suitable solvent to give 11. Yellowish brown powder, recrystallized from methanol, yield 55%, m.p.260-262°C; IR (KBr, cm⁻¹): νς 3229 (NH), 2912 (C-H aliphatic), 1617 (C=O); 1H-NMR (300 MHz, DMSO-d₆): δppm 3.00 (s, 3H, CH₃), 4.30-2.00 (m, 16H, piperidine and morpholine exchangeable); 13C NMR (DMSO-d₆): δppm 21.4 (CH₃), 24.2, 31.7, 45.5 (piperidinyl-C), 116.1-161.6 (Ar-C and C=C), 172.1, 172.2 (2C=O); GC-MS: m/z[M⁺] 327 (24.3%), 189 (100%), 134 (21.1%), 110 (10.9%); Anal. Calcd. for C₁₆H₁₇N₂O₂S (327.41) C, 58.70; H, 5.23; N, 21.39; found: C, 58.83; H, 4.99; N, 21.33.
A solution of the thioxothiazolidin-4-one derivative 4c (0.01 mole) and tetraphosphorusdecaulfide (0.01 mole) in toluene (30 ml) was heated under reflux for 4 hrs., then poured onto ice cold water. The precipitate solid was then collected by filtration, dried and recrystallized from ethanol to give compound 12. Yellowish brown powder, yield 70%, m.p. 187-188°C. 1H-NMR (400 MHz, DMSO-d6); δppm: 2.30 (s, 3H, CH3), 4.48-4.39 (d,d,1H,1H, J1 = 16.6 Hz, 6.0 Hz, CH-CH), 7.92-7.37 (m, 7H, Ar-CH), 10.25 (s, 1H, NH exchangeable); GC-MS: m/z[M]+ 406 (2.802%), [M] 404 (4.102%), 205 (32.98%), 169 (100%), 127 (24.62%), 76 (17.89%).

5-(2-Chloro-5-methyl-3H-indol-3-yl)-3-(4-chlorophenyl)-2-thioxothiazolidin-4-one (13)

The thioxothiazolidin-4-one derivatives 4c (0.01 mol, 3.52 gm), covered by POCl3 (0.01 mole) then refluxed on a water bath for 6 hrs. The mixture was cooled to room temperature and poured onto 150 ml of icc cold water. The precipitated solid substance was collected by filtration then recrystallized from ethanol to give compound 13. Black crystals, yield 60%, m.p. 206-207°C; IR (KBr, cm-1): νmax 3106 (NH), 3062, 3033 (C=H), 1692 (C=O), 1279, 1250 (C=S); 1H-NMR (400 MHz, DMSO-d6); δppm: 2.30 (s, 3H, CH3), 4.48-4.39 (d,d,1H,1H, J1 = 16.6 Hz, 6.0 Hz, CH-CH), 7.92-7.37 (m, 7H, Ar-CH), 10.25 (s, 1H, NH exchangeable); GC-MS: m/z[M]+ 406 (2.802%), [M] 404 (4.102%), 205 (32.98%), 169 (100%), 127 (24.62%), 76 (17.89%).

3-(4-Chlorophenyl)-5-(4-(4-hydroxyphenyl)amino)-5-methyl-3H-indol-3-yl)-2-thioxothiazolidin-4-one (14)


Docking Studies

Docking studies were performed using the Schrödinger Suite software. The coordinate for the protein structure was obtained from the RCSB Protein Data Bank (PDB ID: 3kk6 and 1CX2). Protein Structure was prepared using Schrodinger Suite 2009 software. The thioxothiazolidin-4-one derivatives, followed by the approaches used to optimize the chemotype requirements for the COX2 conformations. Finally, a section on the in silico validation based on docking has been given. Grids for molecular docking with Glide were calculated with no constraints and the newly proposed compounds were docked using Glide in extra-precision mode, with up to ten poses saved per molecule. The docked poses were then minimized using the local optimization feature in Prime, and the energies were calculated using the OPLS-AA force field and the GBSA continuum model.

Biological Assays

In vitro biochemical assays, COX-inhibition-EIA assay

The ability of the test compounds to inhibit tov ineCOX-1 and COX-2 (IC50 values, µM) was determined using an enzyme immunoassay (EIA) kit (catalog number 560131, Cayman Chemical, USA). Cyclo-oxygenase catalyses the first step in the biosynthesis of arachidonic acid (AA) to PGH2. Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (950 µL; 0.1 M Tris-HCl, pH 8.0 containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 µL) enzyme in the presence of heme (10 µL) were added 10 µL of two concentrations of test drug solutions (1 and 10 µM in final volume of 1 mL). These solutions were incubated for a period of 10 minutes at 37°C after which 10 µL of AA (100 µL) solution was added and the solutions further incubated for another 2 minutes then the COX reaction was stopped by the addition of 50 µL of 1 M HCl. Saturated stannous chloride solution (100 µL) was added to each test tube then the tubes were incubated for 5 minutes at room temperature. PGF2α, produced from PGH2 by reduction with stannous chloride, is measured by enzyme immunoassay. This assay is based on the competition between PGSs and a PG-acetylcholinesterase conjugate (PG tracer) for a limited amount of PG antiserum. The amount of PG tracer that is able to bind to the PG antiserum is inversely proportional to the concentration of PGSs in the wells since the concentration of PG in the well during the incubation; or Absorbance α [Bound PG-Tracer] / [PG-Tracer]. Percent inhibition was calculated by comparing the compounds treated to control (incubation). The concentration of the test compounds causing 50% inhibition...

(1C<sub>0</sub>,μM) was calculated from the concentration inhibition response curve (duplicate determinations).

**In vivo screening methods (carrageenan-induced rat paw edema)**

Paw oedema inhibition test was performed on albino rats by adopting the method of Winter [25]. Male albino rats (120-140 g) were fasted for 16 hrs before the experiment. The animals were kept in the groups (control, treated, standard) under constant temperature (25°C) and 12 hours light/dark cycle. 30 min later, 0.2 mL of 1% carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the plantar aponeurosis of the hind paw, and the paw volume was measured by a water plethysmometersorel and then measured again 3 hrs later. The mean increase of paw volume at each time interval was compared with that of control group at the same time intervals and percent inhibition values were calculated by the formula given below:

\[
\% \text{ oedema inhibition} = \frac{V_c - V_t}{V_c} \times 100
\]

Where V<sub>c</sub> is the volume of the leg injected with carrageenan and V<sub>t</sub> is the volume of the leg injected with the tested compounds.

**Results and Discussion**

**Chemistry**

5-Methylisatin 1 was reacted with 3-substituted or unsubstituted2-thioxo-4-thiazolidinone2a-c in the presence of anhydrous sodium acetate and glacial acetic acid and gave the corresponding 5-(5-methyl-2-oxoindolin-3-ylidene)-3-substituted or unsubstituted2-thioxothiazolidin-4-one derivatives 3a-c. The spectral analyses of the afforded indolyl-thiazolidinone showed the incorporation of one molecule of isatin and one molecule of 4-thiazolidinone. The IR spectra, showed disappearance of the high frequency of ketonic carbonyl group at ν 1744 cm<sup>-1</sup> of isatin and appearance of another amidic carbonyl of 4-thiazolidine. Their <sup>1</sup>H NMR spectra showed the acidic protons of NH at δ 11.24 and 11.16 ppm for 3b and 3c, respectively in addition to the signals attributed to the methyl and aromatic protons (cf. Scheme 1 and the experimental section). Reduction of the exocyclic double bond of 5-(5-methyl-2-oxoindolin-3-ylidene)-3-substituted or unsubstituted2-thioxothiazolidin-4-one derivatives 3a-c with zinc in acetic acid led to the formation of the thiazolyl-indole derivative 7 for which the spectral data confirmed the assigned structure. Compound 7 was prepared by another pathway via the reaction of 4c with formaldehyde and morpholine providing additional evidence for structure confirmation (Scheme 1).

Reaction of the thiazolyl-o xoindoline derivative 3a with piperidine or morpholine produced the piperidinyl- or morpholinyl-thiazolidinone derivatives 8a, b via elimination of H<sub>2</sub>S molecule. Compound 8b was converted to its reduced form 9 for which the IR spectrum showed a band for NH at 3194 cm<sup>-1</sup> correlated to NH of isatin and absorption bands related to the two C=O at 1703 and 1688 cm<sup>-1</sup>. Its <sup>1</sup>H NMR spectrum showed the ethylenic protons of vicinal coupling (CH-CH) and revealed the presence of the morpholine part signals in addition to methyl, NH and aryl signals. Reaction of compound 9 with hydration hydrate on steam bath gave compound 10. The IR Spectra showed complete disappearances of any absorption correlate to the carbonyl group and its <sup>1</sup>H NMR agreed with the assigned structure. Furthermore, reaction of 9 with morpholine in the presence of formaldehyde furnished the corresponding Mannich base 11 for which the IR spectrum showed bands for the C=O and NH functions and its <sup>1</sup>H NMR showed signals for all protons in the assigned structure (Scheme 2 and Experimental part).

Reaction of the 2-thioxothiazolidin-4-one with tetraphosphorus decasulfide in dry toluene produced 4-(chlorophenyl)-2,4-dithioxothiazolidine derivative 12. In addition, compound 4c was allowed to react with phosphorous oxychloride to give the (indol-3-yl)-3-(4-chlorophenyl)-2-thioxothiazolidine derivative 13, which in turn was reacted with p-hydroxyaniline to form the (hydroxyphenyl)amino derivative 14. The IR spectrum of compound 13 showed the disappearance of amide carbonyl of indole moiety and displaced by a band of C=O at 1624 cm<sup>-1</sup> and the MS spectrum showed the molecular ion peak of the structure. IR spectrum of 14 shows only one amidic C=O group due to methylation of NH group [23]. The <sup>1</sup>H NMR spectrum showed the singlet peak of the methyllic protons and the assigned signals of the aliphatic protons. The indolyl-thiazolidinone compounds 3a-c were also converted to the Mannich bases 6a-g and 6v-gvi and the MS showed the exact molecular ion peak and the fragmentation pattern agreed with the suggested structure (cf. Scheme 1 and Experimental part). These mentioned findings proved evidence for confirmation of the proposed structures 6a-g. (cf. Scheme 1 and Experimental part). Reduction of compound 6e using zinc in acetic acid led to the formation of the substituted thiazolyl-indole derivative 7 for which the spectral data confirmed the assigned structure. Compound 7 was prepared by another pathway via the reaction of 4c with formaldehyde and morpholine providing additional evidence for structure confirmation (Scheme 1).

\[10\] under applied condition and the MS spectrum showed the exact molecular ion peak (Scheme 3). The carbonyl group in the IR spectrum of 12 means that the amidic C=O (NH-CO), did not react with P<sub>S<sub>4</sub>-<sub>10</sub></sub> under applied condition.

**Scheme 1:** Synthesis of N-Substituted thiazolyloxoindole derivatives.

**Scheme 2.** Synthesis of disubstituted thiazolyl-oxoindole and thiazolopyrazinoindole derivatives.

condition and the MS spectrum showed the exact molecular ion peak (Scheme 3).

**Biological Evaluation**

The biological assays for the newly synthesized compounds were carried out to evaluate the inhibitory activity against COX-2 and the anti-inflammatory effect on carrageenan-induced edema.

**COX-2 Enzyme inhibition**

The efficiency of the novel synthesized (oxindolyl) thiadiazolidine compounds to inhibit the transformation of arachidonic acid to prostaglandin H$_2$ (PGH$_2$) was investigated using a colorimetric Cox (ovine) inhibitor screening assay kit. The inhibitory effects of the tested compounds are expressed as IC$_{50}$ (µM) (concentrations that produce reduction of 50%
of the enzymatic activity of COX control isoform) adapting reported method usingCelecoxib as a reference compound (Table 1) [27-30]. Fourteen test candidates (3a, 4a-c, 6b, 6c, 6e, 6f, 7, 8b, 9, 10, 12 and 14) were screened for their COX-2 inhibitory activity. From the observed results (Table 1), it has been concluded that most of the screened compounds had good inhibitory activity against COX-2. Moreover, five of the tested candidates revealed potent and promising activity. These are thiadiazolidine derivatives 3a, 6f, 8b, 10, and 12 (IC50=5.91, 5.85, 5.40, 5.63 and 5.87 µM, respectively) comparable to that of reference celecoxib (IC50=5.94 µM). Other derivatives as 7, 4a and 6b were possessed moderate inhibitory action compared to the reference IC50 values are 6.77, 7.09 and 7.32 µM, respectively.

**Scheme 3. Synthesis of thiazolyl-chloro-and aminoindole derivatives.**

**Structure activity relationships (SAR)**

Based on the observed COX-2 inhibitory activity of the synthesized compounds it was concluded that, replacement of 2-thione in compound 3a (IC50=5.91 µM) with morpholine as in compound 8b (IC50=5.40 µM) had no significant effect on the inhibitory action. However, reduction of these compounds to the 5-(indol-3-yl)-2-thioxothiazolidinone as exhibited in pairs 9 and 4c (lower inhibitory effect). Introduction for additional cycle as tetracyclic derivative 10 possessed a high inhibitory action (IC50=5.63 µM). Additionally, the bioisosteric replacement of the oxygen atom in 4c by sulfur introduce the bioisostericdithioxothiazolidine derivative 12 with approximately one and a half more potent than 4c (IC50 values 5.87 and 7.67 µM, respectively). The N-substituted indole derivatives 6b-f exhibited varying degrees of COX inhibition with 6e showing low potency (IC50=15.40 µM), while compound 6f which lacked the aryl substitution on N-thiazole was as potent as the celecoxib (IC50=5.85 µM) (Table 1).

**Anti-inflammatory activity**

The anti-inflammatory activity of the synthesized derivatives and indomethacin on carrageen an induced oedema assay at 1, 2, 3 and 4 h, is depicted in Table 2. Percent edema inhibition (Table 2) was calculated in regard to control group and the potency (%) was calculated respect to the indomethacin response.

The observed data revealed that, the activity of the tested compounds varied from moderate to significant inhibition of developing paw edema induced by carrageenan after one, two, three and four hours of treatment. Compounds 3a, 4a and 6e exhibited maximum inhibition with 62.63%, 51.57% and 61.4%, respectively after 4 hours at the end of the experiment whereas Indomethacin showed reduction in oedema volume by 73.68%. Compound 6f showed increased moderate activity with 45.03% inhibition which was weekly active after 1 hr treatment. For the inhibition of compound 6e, interestingly, after two to three hour's treatment, its results revealed inhibition activity of 43.06 and 55.19% which is higher than that of the standard Indomethacin at the same interval times. Compound 7 showed little effect on the volume of paw thickness after one hour, and then its inhibition gradually increased to achieve moderate activity after four hour treatment (Figures 2-11).

**Molecular docking study**

Molecular docking studies represent a useful approach in understanding the diverse interactions between the ligands
Table 1. In vitro enzyme inhibition, docking scores and binding energy data of the new synthesized compounds.

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>IC50 (μm)</th>
<th>-C-DOCKER Interaction energy (kcal/mol)</th>
<th>-C-DOCKER Interaction energy (kcal/mol)</th>
<th>RMSD on COX-2(A°)</th>
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<tbody>
<tr>
<td></td>
<td>Cox-2a</td>
<td>COX-1</td>
<td>COX-2</td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>5.91</td>
<td>32.4</td>
<td>50.4</td>
<td>0.69</td>
</tr>
<tr>
<td>4a</td>
<td>7.09</td>
<td>36.5</td>
<td>46.2</td>
<td>0.89</td>
</tr>
<tr>
<td>4b</td>
<td>10.02</td>
<td>39.9</td>
<td>39.6</td>
<td>1.02</td>
</tr>
<tr>
<td>4c</td>
<td>7.67</td>
<td>34.5</td>
<td>47.2</td>
<td>1.1</td>
</tr>
<tr>
<td>6b</td>
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<td>36.1</td>
<td>48.3</td>
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<tr>
<td>6c</td>
<td>9.31</td>
<td>37.5</td>
<td>41.2</td>
<td>1.2</td>
</tr>
<tr>
<td>6e</td>
<td>15.4</td>
<td>35.1</td>
<td>32.7</td>
<td>1.35</td>
</tr>
<tr>
<td>6f</td>
<td>5.85</td>
<td>33.4</td>
<td>51.7</td>
<td>0.77</td>
</tr>
<tr>
<td>7</td>
<td>6.77</td>
<td>32.2</td>
<td>48.1</td>
<td>0.84</td>
</tr>
<tr>
<td>8b</td>
<td>5.4</td>
<td>27.2</td>
<td>53.2</td>
<td>0.66</td>
</tr>
<tr>
<td>9</td>
<td>9.49</td>
<td>35.2</td>
<td>41.2</td>
<td>1.12</td>
</tr>
<tr>
<td>10</td>
<td>5.63</td>
<td>28.9</td>
<td>51.8</td>
<td>0.74</td>
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<tr>
<td>12</td>
<td>5.87</td>
<td>31.2</td>
<td>51</td>
<td>0.65</td>
</tr>
<tr>
<td>14</td>
<td>10.03</td>
<td>-32.5</td>
<td>38.9</td>
<td>0.77</td>
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<tr>
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<td>37.1</td>
<td>55.1</td>
<td>0.45</td>
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<tr>
<td>Indomethacin</td>
<td>-</td>
<td>59.1</td>
<td>49.5</td>
<td>0.64</td>
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</table>

NOTE: *Value are means of two determinations using an ovine COX-2 assay kit and deviation from the mean is <10% of the mean value.

Table 2. In vivo anti-inflammatory results of the newly synthesized compounds and indomethacin on carrageenan-induced edema of the hind paw in rats.

<table>
<thead>
<tr>
<th>Compd.</th>
<th>%Inhibition at 30 mg/kg (rat Paw edema)a</th>
<th>%Inhibition at 30 mg/kg (rat Paw edema)b</th>
<th>%Inhibition at 30 mg/kg (rat Paw Edema) c</th>
<th>%Inhibition at 30 mg/kg (rat Paw edema) d</th>
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<tbody>
<tr>
<td>3a</td>
<td>17.2</td>
<td>32.5</td>
<td>48.84</td>
<td>62.63</td>
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<tr>
<td>4a</td>
<td>20.23</td>
<td>35.81</td>
<td>46.92</td>
<td>51.57</td>
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<tr>
<td>4c</td>
<td>-10.69</td>
<td>6.83</td>
<td>18.26</td>
<td>28.77</td>
</tr>
<tr>
<td>6b</td>
<td>3.02</td>
<td>5.59</td>
<td>3.26</td>
<td>4.73</td>
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<tr>
<td>6c</td>
<td>-11.62</td>
<td>8.9</td>
<td>20</td>
<td>0.7</td>
</tr>
<tr>
<td>6e</td>
<td>17.2</td>
<td>43.06</td>
<td>55.19</td>
<td>61.4</td>
</tr>
<tr>
<td>6f</td>
<td>10.93</td>
<td>19.04</td>
<td>23.07</td>
<td>45.08</td>
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<tr>
<td>7</td>
<td>3.25</td>
<td>23.39</td>
<td>35.96</td>
<td>41.57</td>
</tr>
<tr>
<td>8b</td>
<td>12.55</td>
<td>28.98</td>
<td>45.57</td>
<td>45.61</td>
</tr>
<tr>
<td>9</td>
<td>6.27</td>
<td>10.35</td>
<td>10.38</td>
<td>9.47</td>
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<td>10</td>
<td>6.27</td>
<td>7.66</td>
<td>9.03</td>
<td>10</td>
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<tr>
<td>12</td>
<td>0.93</td>
<td>0.62</td>
<td>13.46</td>
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<tr>
<td>Indomethacin</td>
<td>-</td>
<td>42.85</td>
<td>52.3</td>
<td>73.68</td>
</tr>
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</table>

NOTE: *The carrageenan-induced rat paw edema assay was carried out using six animals (male rats)/group following IP of the test compound. The results are expressed as means ± SEM (n=4-6) following a 30 mg/kg IP of the tested compounds.

Figure 2. Anti-inflammatory activity of synthesized compounds using rat paw edema method.
Figure 3. The crystal structure conformation of celecoxib (green C-atoms, 1CX2) is superimposed for referee (as a reference).

Figure 4. Docking of compound 9 into the active site of COX-2 (red for hydrogen bond acceptor, blue for hydrogen bond donor, grey for mild polar or hydrophob and yellow dotted lines for hydrogen bonding).

Figure 5. Docking of compound 4b into the active site of COX-2.

Figure 6. Docking of compound 4a into the active site of COX-2.
and enzyme active sites in detail and thereby help in designing novel potent inhibitors. The important COX-2 inhibition results and anti-inflammatory activities of the prepared substituted (oxoindolyl) thiazolidine derivatives prompted us to perform
Figure 9. Docking of compound 8 into the active site of COX-2.

Figure 10. Docking of compound 12 into the active site of COX-2.
molecular docking studies to understand the ligand–protein interactions. Docking study was carried out for the target compounds into COX-1 and COX-2 using Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, USA). In the present study, celecoxib that was co-crystallized with the 3D-structure of COX-1 and COX-2 obtained from the protein data bank (Code: 3KK6) [31] and (Code: 1CX2) [32], respectively was used a reference compound to evaluate the molecular modeling docking study results. Interactive docking using was carried out for all the conformers of each compound of the tested set (3a, 4a-c, 6b, 6c, 6e, 6f, 7, 8b, 9, 10, 12 and 14) to the selected active site of COX-1 and COX-2, after energy minimization using prepared ligand protocol. Protein Structure was prepared and the invalid or missing residues were added [33]. In order to validate the docking algorithm on the target enzyme, the RMSD value was calculated for each compound. Each docked compound was assigned a score according to its binding mode onto the binding site and listed in Table 2.

The reported molecular modeling studies based on x-ray crystallography of the 3D structures of COX-1 and COX-2 indicated that COX binding site can be considered as a hydrophobic channel expanding from the membrane binding domain [34]. In the upper region of the channel both isozymes possess a tyrosine (Tyr385) and a serine (Ser 530), the amino acid acetylated by aspirin. The main variation between the two COX active sites is the replacement of the relatively bulky isoleucine (Ile) residue in COX-1 by Valine (Val) at position 523 of the active site of the enzyme [35]. This opens an additional 2°-polar side pocket which is a prerequisite for COX-2 drug selectivity; access of ligands to the 2°-pocket is controlled by histidine (His 90), glutamine (Gln192) and tyrosine (Tyr 355) [36].
The results obtained from the study showed that compounds 9 and 4b possessed high binding energy equal to -100.76 Kcal/mol and -75.5 Kcal/mol respectively; both compounds did not have substituents on the N-indole. The compounds formed a hydrogen bond interaction with Tyr 355 in the hydrophilic 2°-pocket and compound 9 also formed a hydrogen bond with Ser 530 in the active site of COX-2 (Figure 3). Moreover, the SAR studies results as well as the IC$_{50}$ value (10 µM) of compound 4b could be attributed to the phenyl ring superimposed on the p-triflourophenyl of the co-crystallized inhibitor celecoxib, occupying the “hydrophobic pocket” along with its fitting in the lateral pocket (Figure 5). However, compound 4c (Figure 8), the chloro counterpart of compound 4b formed a hydrogen bond with Ser 530 and exhibited only one third of its activity. Compound 4a formed a hydrogen bond interaction between the 2-indole carbonyl and Tyr 355, (Figure 6). While its unreduced rigid analogue 3a (IC$_{50}=5.91$ µM) did not form such hydrogen bond interactions but was embedded in the hydrophobic region, allowing the possibility of lipophilic contacts with the side chains of both Leu352 and Val523 (Figure 7). The structural features of compound 8b obviously contributed to its docking result. The double bond linking thiazolidinone and indole moieties restricts the rotation of the molecules in space, in spite of its interaction by means of hydrogen bonds with the backbone NH group of Phe518 as well as hydrogen bond interaction with Tyr 355 (Figure 9). The results obtained for compounds 4c and 12 showed that they have the same binding mode with binding energies -47.2 and -51.0 Kcal/mol, respectively (Figure 10). Compound 10 interacts with the enzyme active sites via formation of a hydrogen bond with Arg 120 with a high binding energy (Figure 11).

These observations are consistent with the inability of derivatives 7, 6e, 6c, 6b and 14; these structures bear a large moiety on the N-indole so they were sterically hindered, from entering into the enzyme active site. In addition, most of docked compound revealed low docking score on COX-1 enzyme indomethacin which support the selectivity of these compounds to COX-2 (Figures 12 and 13).

**Conclusion**

Thioxothiazolidin-4-one derivatives of the oxindoline ring system as well as their N-substituted analogs were synthesized and screened for COX-2 inhibition and anti-inflammatory activity in addition to related docking studies. Compounds which showed significant COX-2 inhibition were subjected to anti-inflammatory studies and docking studies. Compound 8b was found to exhibit optimal COX-2 inhibitory potency (IC$_{50}=5.40$ µM) comparable with celecoxib, so it appears promising in addition to 3a, 10 and 12. The structure-activity relationships (SAR) acquired showed that appropriately (morpholinyl-oxindolyl) thiazolidine structure has the necessary geometry to provide potent and selective inhibition of the COX-2 isozyme. Further, more analysis of the obtained results for newly prepared compounds opens the possibility for further optimization of studied compounds.

**References**

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