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-(oxoindolinyl)-2-thioxothiazolidinone derivatives were synthesized. The C2-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 (oxonidolyl)thiazolidine derivatives (3a, 6f, 8b, 10 and 12) showed significant COX-2 inhibitory activity exhibiting IC50values 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.

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 enzyme2- 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 function5. 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 Celecoxib6, Rofecoxib7and Valdecoxib8have been marketed as a new generation of NSAIDs. However, Rofecoxib was banned in 2004 because of cardiac toxicity9.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 heterocycles10,11 (II-IV, Fig. 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-112.A number of indole incorporating compounds have also been revealed as potent and selective COX-2 inhibitors13,14. Furthermore, several derivatives of thiazole10,15 and thiazolidinedione16,17 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 (V and VI, Fig. 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 derivatives18-22, 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 (60 F254), visualizing with ultraviolet light. Column chromatography was performed on silica gel (230–400 mesh) using distilled petroleum ether, ethyl acetate, dichloromethane, chloroform, and methanol. Infra red 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 (d) are relative to tetramethylsilane (TMS, d = 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 C H N S 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,b were prepared following a previously reported method23.

Synthesis of 5-(5-methyl-2-oxoindolin-3-ylidene)-3-Substituted-2-thioxothia- zolidin-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 (20 ml) 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. > 300oC (reported m.p. >295oC)

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 oC. IR (KBr, cm- 1): νmax 3208 (NH), 1711, 1680 (2 C=O), 1281 (C=S); 1H-NMR (400 MHz, DMSO - d6): δ ppm: 2.32 (s, 3H, CH3), 7.69-6.91 (m, 8H, Ar-H), 11.24 (s, 1H, NH exchangeable). GC-MS: m/z [M]-1 352.03 (14.5%),189 (100%), 146 (1.5%), 133

(10.8%), 105 (4.6%), 89(0.8%); Anal. Calcd. for C18H12N2O2S2 (352.43): C, 61.35; H,

3.43; N, 7.95; found: C, 61.66; H, 3.32; N, 7.80.

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 > 300oC. IR (KBr, ν

/cm-1): νmax 3175(NH), 1697, 1657 (2C=O), 1248 (C=S).1HNMR (400 MHz, DMSO- d6); δ ppm:2.30 (s, 3H, CH3), 8.451-7.478 (m, 7H, Ar-H), 11.16 (s, 1H, NH).GC-MS:

m/z [M]+ 386 (14.4 %), 189 (100%),146 (4%), 134 (31.6%), 105 (6.3%); 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.

5-(5-Methyl-2-oxoindolin-3-yl)-3-substitutedphenyl-2-thioxothiazolidin-4-one (4a-c).

General procedure: A mixture of the thioxothiazolidine-4-one derivatives 3a-c (0.001 mole) and 0.5 g of zinc powder and glacial acetic acid (15 ml), the mixture was wormed 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.

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

White crystals, recrystallized from ethanol; yield 60%, m.p. 260-262oC. IR (KBr, /cm- 1): νmax 3422, 3181 (NH), 1694, 1621 (2 C=O), 1250 (C=S); 1H-NMR (400 MHz, DMSO - d6): δ ppm 2.30 (s, 3H, CH3), 4.16, 5.33 (d, d, 2H, J1 = 16.6, J2 = 6, CH-CH), 7.20-6.71 (m, 3H, Ar-H), 10.49,10.81 (2s, 2H, 2 NH); Anal. Calcd. for C12H10N2O2S2 (278.34) C, 51.78; H, 3.62; N, 10.06; found: C, 51.42; H, 3.36; N, 9.78.

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

White crystals, recrystallized from ethanol, yield 65 %, m.p. 240 – 242oC; IR (KBr, ν

/cm-1): νmax 3210 (NH), 1760, 1700 (2 C=O), 1320 (C=S);1H-NMR (400 MHz, DMSO-d6): δ ppm 2.30 (s, 3H, CH3), 5.58, 4.53 (d, d, 2H, J1 = 16.6, J2 =6, CH-

CH),7.67-6.88 (m, 8H, Ar-H), 10.84 (s, 1H, NH exchangeable); Anal. Calcd. for C18H14N2O2S2 (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 – 226oC; IR (KBr, ν

/cm-1): νmax 3311 (NH), 1757, 1700 (2 C=O), 1319 (C=S); 1H-NMR (400 MHz, DMSO - d6); δ ppm 2.30 (s, 3H, CH3),5.59, 4.53 (d, d, 2H, J1 = 16.6, J2 =6, CH-

CH),7.72-6.81 (m, 7H, Ar-H), 10.67 (s, 1H, NH exchangeable); Anal. Calcd. for C18H13ClN2O2S2 (388.89) C, 55.59; H, 3.37; N, 7.20; found: C, 55.35; H, 3.24; N,

6.84.

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

To a solution of 5-methylindol-2.3-dione (1) (1.61g, 0.01mol) 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 5bwere in accordance with those reported earlier. Red powder; recrystallized from ethanol; yield 75%, m.p. 155-157oC; IR (KBr/cm-1): νmax 3218 (NH), 3073 (C-H aromatic), 2925 (C-H aliphatic), 1754, 1733 (2C=O); 1H-NMR (400MHz, DMSO-d6); δppm: 2.30 (s, 3H, CH3),3.75-3.27 (m, 8H, piperazine-CH2),

3.96 (s, 2H, CH2), 6.81-8.83 (s, d, d, 3H, Ar-CH),11.03 (s,

1H, NH exchangeable);

GC-MS: m/z [M]+2 261 (73.95 %), 228 (20.31 %), 218 (100 %), 144 (33.84 %), Anal.

calcd. for C14H17N3O2 (259.30) C, 64.85; H, 6.61; N, 16.20; found: C, 65.02; H, 6.82;

N, 15.86.

Synthesis of Mannich bases;5-(5-methyl-2-oxo-1-(2o amine-1-ylmethyl)indolin-3- ylidene)-3-substituted-2-thioxothiazolidin-4-one (6a-g)

General procedure: A solution of 5-(5-metheyl-2-oxoindolin-3-ylidine 3-substituted

/or 2-thioxothiazolidine-4-one derivatives3a-c (0.01mol), 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-thioxothiazoli- din-4-one (6a).

Yellowish Brown powder, recrystallized from toluene, yield 60%, m.p. 150-152oC. IR (KBr, ν /cm-1): νmax 3116 (NH),3013, 3066 (C-H aromatic), 2944, 2854(C-H aliphatic),1701, 1685 (2C=O), 1263 (C=S); 1H-NMR (400 MHz, DMSO-d6): δ 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) : δ ppm 21.9 (CH3), 24.2, 29.7, 56.0 (piperidinyl-C), 74.1 (CH2), 114.1-153.6 (Ar-C and C=C), 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 C18H19N3O2S2 (373.49) C, 57.89; H, 5.13; 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. >300oC. IR (KBr, ν /cm-1): νmax 3007(C-H aromatic),2934 (C-H aliphatic), 1686, 1658 (2C=O), 1302 (C=S); 1H-NMR (400 MHz, DMSO - d6): δ 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); Anal. Calcd. for C24H23N3O2S2 (449.59) C, 64.12; H, 5.16; N, 9.35; found: C, 63.96; H, 4.89; N, 9.71;

S, 13.80.

5–(5-Methyl-2-oxo-1-(piperidin-1-ylmethyl) indolin-3-yl)-4-chlorophenyl) -2- thioxothiazolidin-4-one (6c).

Brown powder, recrystallized from xylene, yield 55%, m.p. 132-134oC. IR (KBr, ν

/cm-1): νmax 3061 (C-H aromatic), 2920, 2856 (C-H aliphatic), 1729, 1658 (2C=O), 1224 (C=S),1H-NMR (400MHz, DMSO-d6); δppm2.30 (s, 1H, CH3), 3.44-3.35 (m,

10H, piperidine-CH2),3.90 (s, 2H, CH2),9.29-7.31 (m, 7H, Ar-CH);GC-MS: m/z

[M]+2486(6.55%);[M]+484 (2.59 %); 3 8 5 (8.75%), 243 (8.7%), 146 (1.84%), 118

(8.49%). Anal. Calcd. for C24H22ClN3O2S2, (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).

Bright Brown powder, recrystallized from toluene, yield 50%, m.p. >300oC. IR (KBr, ν /cm-1): νmax 3003 (C-H aromatic),2957, 2837 (C-H aliphatic), 1694, 1657 (2C=O), 1244 (C=S);1H-NMR (400MHz, DMSO-d6); δ ppm 2.30 (s, 1H, CH3),3.55-3.30 (m,

8H, morpholine-CH2),4.54 (s, 2H, CH2), 9.29-7.31 (m, 8H, Ar-H); Anal. calcd. for

C23H21N3O3S2 (451.56): C, 61.18; H, 4.69; N, 9.31; found: C, 61.01; H, 4.37; N, 8.98.

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 75%, m.p. >300oC; IR (KBr, ν /cm-1): νmax 3062, 3035 (C-H aromatic), 2928, 2854 (C-H aliphatic), 1732, 1686 (2C=O), 1225 (C=S); 1H-NMR (400MHz, DMSO-d6); δppm2.30 (s, 3H, CH3), 3.55-

3.30 (m, 8H, morpholine-CH2), 4.54 (s, 2H, CH2),7.60-6.84 (m, 7H, Ar-H); Anal. Calcd. for C23H20ClN3O3S2 (486.01) C, 56.84; H, 4.15; N, 8.65.found: C, 56.70; H,

4.02; N, 8.32.

5-(5-Methyl-2-oxo-1-(piperazin-1-ylmethyl) indolin-3-ylidene)-2-thioxothia- zolidin-4-one (6f).

Bright pink powder, recrystallized from toluene, yield 70%, m.p >300oC. IR (KBr, ν

/cm-1): νmax 3264, 3193 (NH), 3090 (C-H aromatic),2938, 2854 (C-H aliphatic) 1725, 1666 (2C=O), 1313 (C=S); 1H-NMR (400MHz, DMSO-d6); δppm 2.30 (s, 3H, CH3),

3.75-3.27 (d, 8H, piperazine-CH2), 3.96 (s, 2H, CH2), 6.8-8.83(s, d, d, 3H, Ar- H),11.96, 11.03 (2s, 2H, 2 NH exchangeable); GC-MS: m/z [M]+ 374 (1.53%), 145

(100%), 133(10.91%), Anal. Calcd. for C17H18N4O2S2 (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-(piperazin-1-ylmethyl)indolin-3-ylidene)-3-(4-chlorophenyl- 2-thioxothiazolidin-4-one (6g).

Bright Brown powder, recrystallized from toluene, yield 65%, m.p. >300oC. IR (KBr, ν /cm-1): νmax 3173 (NH), 2917, 2852(C-H aliphatic), 2085 (C-H aromatic), 1697, 1656 (2C=O),

1247 (C=S),1H-NMR (400MHz, DMSO-d6); δppm 2.30 (s, 3H, CH3),

3.32 (m, 8H, piperazine-CH2),3.36 (s, 2H, CH2), 7.59-7.00 (m, 7H, Ar-H), 11.44 (s,

1H, NH exchangeable); Anal. calcd. for C23H21ClN4O2S2 (485.02) C, 56.96; H, 4.36; N, 11.55; found: C, 56.74; H, 4.18; N, 11.30.

5-(5-methyl-1-(morpholinomethyl)-2-oxoindolin-3-yl)-3-(4-chlorophenyl-2- thioxothiazoldi-4-one (7)

a) A solution of the thiazolidinyl indole 4c (0.01mol), formaldehyde (40%, 1.5 ml) and morpholine (0.01 mole) in ethanol was stirred for 5-6 h. at room temperature. The solvent was reduced under vacuum and the resulting solid was collected by filtration and recrystallized from ethanol to give compound 7.

b) A mixture of the N-substituted thiazolidinylindole 6e (0.01 mole) and 0.5g zinc metal (powder) in acetic acid glacial (25 ml) was refluxed for 60 min. The reaction mixture was then poured into ice cold water and the formed precipitate was collected by filtration and recrystallized from ethyl acetate to give compound 7. Bright brown powder, yield 75%, m.p. 165-167oC; IR (KBr, cm-1): νmax 2916, 2849(C-H aliphatic),1739, 1699 (2 C=O), 1243(C=S); 1H NMR (400 MHz, DMSO-d6): δppm 2.30 (s, 3H, CH3), 3.50 (m, 8H, morpholine-CH2),4.42, 5.19 (d, d, 2H, J1 = 16.6, J2

=6.0, CH-CH), 7.72-6.74 (m, 7H, Ar-H); Anal. Calcd. For C23H22ClN3O3S2 (488.02) C, 56.61; H, 4.54; N, 8.61; found: C, 56.77; H, 4.42; N, 8.94.

5-(5-Methyl-2-oxoindolin-3-yl)-2-2o amine-1-yl) thiazol-4(5H)-one (8a,b).

General procedure: A mixture of 5-(5-methyl-2-oxoindolin-3-yl)-2-thioxothiazolidin- 4-one (3a) and piperidine or morpholine (0.01 mole) in ethanol (25mL)was heated under reflux for 6 h. and the precipitated solid was filtered off and recrystallized from the suitable solvent to give compounds 8a and 8b, respectively.

5-(5-Methyl-2-oxoindolin-3-yl)-2-piperidin-1-yl)thiazol-4(5H)-one (8a)

Red crystals, recrystallized from ethanol, yield 70%, m.p. 300oC. IR (KBr, cm-1): νmax 3197 (NH), 1703, 1687 (2C=O); 1H-NMR (400MHz, DMSO-d6); δppm2.31 (s, 3H, CH3), 3.99 (m, 10H, piperidine-CH2), 6.81-9.05 (s, d, d, 3H, Ar-H),11.25 (s, 1H, NH exchangeable); 13C NMR (DMSO-d6): δ ppm 22.4 (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]-1 326 (13.4%), 224 (1.7%), 189 (100%), 154 (16%),134 (21.1%), Anal. calcd. for

C17H17N3O2S (327.40) C, 62.37; H, 5.23; N, 12.83; found C, 62.09; H, 4.98; N, 12.98.

5-(5-Methyl-2-oxoindolin-3-ylidene)-2-morpholinothiazol-4(5H)-one (8b)

able); Anal. calc Pink crystals, recrystallized from ethanol, yield 75%, m.p. >300oC. IR (KBr, cm-1): νmax 3120 (NH), 2972(CH aliphatic), 1702, 1687 (2C=O);1H-NMR (400 MHz,

DMSO-d6): δppm 2.30 (s, 3H, CH3), 3.99-3.71 (m, 8H, morpholine),6.94, 7.04, 9.04

(d, d. s, 3H, Ar-H), 11.19 (s, 1H, NH exchangeable); GC-MS: m/z [M]+2

331(82.02%), 189 (11.24%),155(11.24%), 146 (65.17%),102 (0.79%); Anal. Calcd.

For C16H15N3O3S (329.37) 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-morpholinothiazol-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 h. 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 -262oC.IR (KBr, cm-1) : νmax 3194 (NH), 1703, 1688(2 C=O), 1557 (C=N); 1H-NMR (400MHz, DMSO-d6); δppm 2.30 (s, 3H, CH3),

4.30 (m, 8H, morpholin-CH2),4.80-5.08 (d, d, 2H, J1 = 16.6, J2 =6, CH-CH), 6.94,

7.41, 9.04 (d, d, s, 3H, Ar-H), 10.76(s, 1H, NH exchangeable); GC-MS: m/z [M]+ 331 (82.02%), 92 (100%), 146 (65.17%), 178 (61.80%); Anal. Calcd. For C16H17N3O3S

(331.39) C, 57.99; H, 5.17; N, 12.68; found: C, 57.71; H, 5.03; N, 12.54.

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 150ml ice cold water. The precipitated solid was filtered off, dried and recrystallized from ethanol to give compound 10. Orange powder, recrystallized from methanol, yield 60%, m.p264-265oC; IR (KBr, cm-1): νmax 3229 (NH), 2921 (C-H aliphatic), 1617 (C=N); 1H-NMR (300 MHz, DMSO-d6); δppm 2.30 (s, 3H, CH3),3.47-3.2 (m, 8H,morpholine-CH2),4.99-5.11 (d, d, 2H, J1 =

16.6, J2 =6, CH-CH), 8.42,7.30, 6.83 (d, d, s, 3H, Ar-H), 10.7 (s, 1H, NH); 13C NMR

(DMSO-d6): δ ppm 21.4 (CH3), 24.2 (CH), 39.3 (CH), 49.4,67.1 (morpholine-C),

116.4-143.5 (Ar-C), 161.7, 163.7, 167.2 (3C=N);GC-MS: m/z [M]+ 327 (24.3%) , 189

(100%) , 134 (21.1%) , 110 (10.9%). Anal. Calcd. For C16H17N5OS (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-(piperazin-1-ylmethyl)indolin-3-yl)-2-morpholinothiazol- 4(5H)-one (11)

A mixture of compound (9) (0.01mole) and (0.01 mole) of 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

ethanol, yield 71%, m.p. 233-235oC; IR (KBr, cm-1): νmax 2939 (CH2), 1690 (C=O);1H-NMR (400 MHz, DMSO-d6); δppm 2.30 (s, 3H, CH3), 3.36-2.08 (m, 16H,

piperdine and piprazin-H), 4.50-5.18 (d, d, 1H, 1H, J1 = 16.6, J2 =6, CH-CH),8.76– 7.02 (m, 3H, Ar-H), 9.12 (s, 1H, NH exchangeable); GC-MS: m/z [M]-1 428 (6.8%);

386 (29.9%), 189 (100%), 134 (29.1%), 75 (17.9%); Anal. Calcd. For C21H27N5O3S

(429.54) C, 58.72; H, 6.34; N, 16.30; found: C, 58.52; H, 6.15; N, 15.98.

3-(3-(4-Chlorophenyl)-2,4-dithioxothiazolidin-5-ylidene)-5-methylindolin-2-one (12)

A solution of the thioxothiazolidin-4-one derivative 4c (0.01mole) and tetraphosphorus decasulfide (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. 187-188oC; IR (KBr, cm-1): νmax 3106 (NH), 3062, 3003 (=C-H), 1692 (C=O), 1279, 1250 (C=S); 1H-NMR (400 MHz, DMSO-d6); δ ppm; 2.30 (s, 3H, CH3),4.48-4.39 (d, d, 1H, 1H, J1 = 16.6, J2 =6.0 Hz, CH-CH),7.92-

7.37 (m, 7H, Ar-CH), 10.25 (s, 1H, NH exchangeable); GC-MS: m/z [M]+2 406 (2.802 %), [M]+ 404 (4.102 %), 205 (32.98%), 169 (100%), 127 (24.62%), 76 (17.89%). Anal. calcd. for C18H13ClN2OS3 (404.95) C, 53.39; H, 3.24; N, 6.92; found C, 53.20; H, 3.42; N, 6.69.

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), was covered by POCl3 (0.01mole) then refluxed on a water bath for 6 hrs. The mixture was cooled to room temperature and poured onto 150 ml of ice cold water. The precipitated solid substance was collected by filtration then recrystallized from ethanol to give compound 13. Black crystals, yield 60%, m.p. 206-207oC, IR (KBr, cm-1): νmax2919(C-H aliphatic),1709(C=O), 1249(C=S); 1H-NMR (400 MHz, DMSO-d6); δ ppm: 2.30 (s, 3H, CH3), 3.96-4.15 (d, d, 1H, 1H, J1 = 16.6, J2 =6, CH-CH), 8.84 -7.40

(m, 7H, Ar-H); GC-MS: m/z [M]+2 408 (2.14%), [M]+ 406 (3.15%), 205

(11.44%),169 (100%), 153 (29.9%), 134 (9.7%); Anal. Calcd. for C18H12Cl2N2OS2.

(407.33) C, 53.08; H, 2.97; N, 6.88; found: C, 52.90; H, 2.92; N, 6.80.

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

A solution of the thioxothiazolidin-4-onederivative 13 and p-hydroxyl aniline (0.01 mole) in ethanol (25 mL) was heated under reflux for 3 hrs.; then allowed to cool at room temperature. The formed precipitate was filtered off, dried and recrystallized from ethanol to give 14. Bright brown crystals, yield 50%, m.p. 250-251oC; IR (KBr, cm-1): νmax 3430 (OH),3151 (NH),3024 (C-H Aromatic), 2915 (C-H aliphatic),1703 (C=O), 1295 (C=S); 1H-NMR (400 MHz, DMSO-d6); δ ppm 2.30 (s, 3H, CH3),4.5-

5.18 (d, d, 1H, 1H, J1 = 16.6, J2 =6, CH-CH),7.92-7.37 (m, 11H, Ar-H),9.50 (bs, 1H,

OH),10.25 (s, 1H, NH exchangeable); MS: m/z [M]+ 481 (17.5%), [M]+ 479 (52.5%),

189 (39.8%), 161 (41.03%), 144 (51.4%), 134 (2.17%), 109 (100%). Anal. calcd. For

C24H18ClN3O2S2 (480.0) C, 60.06; H, 3.78; N, 8.75; found: C, 59.80; H, 3.92; N, 9.06.

Docking studies

Docking studies were performed using Software version. 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 package. The invalid or missing residues were added and the structures were aligned using the protein structure alignment module. Hydrogen atoms were added and the structure was minimized to relax the backbone and to remove the clashes. The protein was inspected visually for accuracy in the X2 dihedral angle of Asn and His residues and the X3 angle of Gln, and rotated by 180o when needed to maximize hydrogen bonding. The proper His tautomer was also manually selected to maximize hydrogen bonding. The proposed compounds were optimized by semiempirical method (AM1) using Chem3D to eliminate bond length and bond angle biases and saved to be used for docking and binding energy calculations, which were carried out by Schrodinger Suite 2009.We delineate our approach for the design of specific COX2 inhibitors. It starts with the description of generating a proposed library of indole 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 in Maestro.

Biological assays

In vitro biochemical assays, COX-inhibition-EIA assay

The ability of the test compounds to inhibit ovine COX-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 10μM in a 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 were 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 PGs 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 PGs in the wells since the concentration of PG tracer is held constant while the concentration of PGs varies. This antibody-PG complex binds to a mouse anti-rabbit monoclonal antibody that had been previously attached to the well. The plate was washed to remove any unbound reagents and then Ellman's reagent, which contains the substrate to acetylcholine esterase, was added to the well. The product of this enzymatic reaction produces a distinct yellow color that absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of the PG tracer bound to the well which is inversely proportional to the amount of free PGs present in the well during the incubation; or Absorbance α [Bound PG Tracer] α 1/PGs. Percent inhibition was calculated by the comparison of the compounds treated to control

incubation. The concentration of the test compounds causing 50% inhibitions (IC50,

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 et al.25 Male albino rats (120–140 g) were fasted for 16 h before the experiment. The animals were kept in the groups (control, treated, standard) under constant temperature (25oC) and 12-hours light/dark cycle. 30 min later, 0.2mL 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 plethysmometersocrel and then measured again 3 h 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:

$$\% \text{ oedema inhibition} = \frac{Vc - Vt}{Vc} \times 100$$

Where Vc is the volume of the leg injected with carrageenan and Vt is the volume of the leg injected with the tested compounds.

Results and Discussion:

Chemistry

5-Methylisatin 1was reacted with 3-substituted or unsubstituted2-thioxo-4- thiazolidinone2a-cin the presence of anhydrous sodium acetate and glacial acetic acid and gave the corresponding 5-(5-methyl-2-oxoindolin-3-ylidene)-3-substituted or unsubstituted 2-thioxothiazolidin-4-one derivatives 3a-c.The spectral analyses of the afforded indolyl-thiazolidinoneshowed 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 1744cm-1 of isatin and appearance of another amidic carbonyl of 4-thiazolidine. Their 1H 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 unsubstituted -2- thioxothiazolidin-4-one 3a-c to form 5-(5-

methyl-2-oxoindolin-3-yl)-3-substituted or/ unsubstituted -2- thioxothiazolidin-4-one 4a-c was performed using Zn/AcOH. The breakdown of the extended conjugation in 4a-c between the indole and the 4-thiazolidinone rings by reducing the exocyclic double bond, is evidenced by the fading in the color of the solution of the sample, the bathochromic shift of stretching vibration of the two amidic C=O in the IR spectra, the presence of two doublets of vicinal coupling (CH-CH) of the newly formed ethylenic protons in the 1H NMR. The Mannich

bases 5a-c were prepared by reaction of 5-methylisatine 1 with formaldehyde and secondary amine under the Mannich conditions, which in turn condensed with 2-thioxo-4-thiazolidinone or its substituted analogs to produce 6a-g. The intermediates 5a,b are known and were prepared according to previously reported method26. The IR spectrum of 5c showed high value of stretching vibration of amidic C=O group due to methylation of NH group23. The 1H NMR spectrum showed the singlet peak of the methylinic protons and the assigned signals of the aliphatic protons. The indolyl-thiazolidinone compounds 3a-cwere also converted to the Mannich bases 6a-g via reaction with formaldehyde and secondary amine under the Mannich reaction conditions. The IR spectra of the latter products showed a high frequency of the ν C=O of the lactam bond due to methylation27.The 1HNMRspectra showed in addition to the aromatic protons, multiplet signals of the secondary amine moiety at δ 3.27–3.75ppmas well as a singlet at 3.96 ppm of the methylene protons (CH2). The EI-MS spectra showed that the molecular ion peak and the fragmentation pattern agreed with the suggested structure (cf. Scheme 1 & Experimental part). These mentioned findings proved evidence for confirmation of the proposed structures 6a-g. (cf. Scheme 1 & 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).

Scheme 1

irmation (Scheme 1). Reaction of the thiazolyl-oxoindoline derivative 3a with piperidine or morpholine produced the piperidinyl- or morpholinyl-thiazoline derivatives 8a,b via elimination of H2S molecule. Compound 8b was converted to its reduced form 9 for which the IR

spectrum showed a band for NH at 3194 cm-1 correlated to NH of isatin and absorption bands related to the two C=O at 1703 and 1688 cm-1. Its 1H 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 hydrazine hydrate on steam bath gave compound 10. The IR Spectra showed complete disappearances of any absorption correlate to the carbonyl group and its 1H NMR agreed with the assigned structure. Furthermore, reaction of 9 with either 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 1H NMR showed signals for all protons in the assigned structure (Scheme 2 & Experimental part)

Scheme 2

Reaction of the 2-thioxothiazolidin-4-one 4c with tetraphosphoursdecasulfide in dry toluene produced 4-(chlorophenyl)-2,4-dithioxothiazolidin derivative 12. In addition, compound 4c was allowed to react with phosphorous oxychloride to give the (indol-3- yl)-3(4-chlorophenyl)-2-thioxothiazolidine derivative13, 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 amidic carbonyl of indole moiety and displaced by a band of C=N at 1624 cm-1 and the MS spectrum showed the molecular ion peak of the structure. IR spectrum of 14 shows only one amidic C=O at ν 1703 cm-1 and the MS showed the exact molecular ion peak agreeing with the proposed structure. The presence of only one carbonyl group in the IR spectrum of 12 means that the amidic C=O (NH-CO), did not react with P4S10 under applied condition and the MS spectrum showed the exact molecular ion peak (Scheme 3).

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 H2 (PGH2) was investigated using a colorimetric Cox (ovine) inhibitor screening assay kit. The inhibitory effects of the tested compounds are expressed asIC50 (μM) (concentrations that produce reduction of 50% of the enzymatic activity of COX control isoform) adapting reported method using Celecoxib as a reference compound (Table 1)28-30. 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

Table 1:-*In vitro* enzyme inhibition,docking scores and binding energy data of the new synthesized compounds.

Compd. No.	IC50 (μM)	-C-DOCKER Interaction energy (kcal/mol)	-C-DOCKER Interaction energy (kcal/mol)	RMSD on COX-2(A°)
	Cox-2a	COX-1	COX-2	
3a	5.91	32.4	50.4	0.69
4a	7.09	36.5	46.2	0.89
4b	10.02	39.9	39.6	1.02
4c	7.67	34.5	47.2	1.1
6b	7.32	36.1	48.3	0.75
6c	9.31	37.5	41.2	1.2
6e	15.40	35.1	32.7	1.35
6f	5.85	33.4	51.7	0.77
7	6.77	32.2	48.1	0.84
8b	5.40	27.2	53.2	0.66

9	9.49	35.2	41.2	1.12
10	5.63	28.9	51.8	0.74
12	5.87	31.2	51.0	0.65
14	10.63	-32.5	38.9	0.77
Celecoxib	5.94	37.1	55.1	0.45
Indomethacine	-	59.1	49.5	0.64

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

= 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.

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=5.91μM) with morpholine as in compound 8b (IC50=5.40μM) 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 bioisostere dithioxothiazolidine derivative 12 with approximately one and a half more potent than 4c (IC50 values 5.87 and 7.67μM), 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.

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

Compd.	% Inhibition at 30 mg/kg (rat paw edema) a 1 hr	% Inhibition at 30 mg/kg (rat paw edema) a 2 hr	% Inhibition at 30 mg/kg (rat paw edema)a 3 hr	% Inhibition at 30 mg/kg (rat paw edema) a 4 hr
3a	17.2	32.5	48.84	62.63
4a	20.23	35.81	46.92	51.57
4c	-10.69	6.83	18.26	28.77
6b	3.02	5.59	3.26	4.73
6c	-11.62	8.90	20	0.7
6e	17.2	43.06	55.19	61.4
6f	10.93	19.04	23.07	45.08
7	3.25	23.39	35.96	41.57
8b	12.55	28.98	45.57	45.61
9	6.27	10.35	10.38	9.47
10	6.27	7.66	9.03	10
12	0.93	0.62	13.46	24.03
Indomethacin	20.93	42.85	52.3	73.68

aThe 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.

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 hour treatment. For the inhibition of compound 6e, interestingly, after two and 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 hours treatment.

Table 2

Fig. 2-11

Molecular docking study

Molecular docking studies represent a useful approach in understanding the diverse interactions between the ligands 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 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 added33.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-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 domain34. In the upper region of the channel both isozymes possess a tyrosine (Tyr 385) 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 enzyme35. This opens an additional 2o- polar side pocket which is a prerequisite for COX-2 drug selectivity; access of ligands to the 2o- pocket is controlled by histidine (His 90), glutamine (Gln

192) 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 2o-pocket and compound 9 also formed a hydrogen bond with Ser 530 in the active site of COX-2 (Fig. 3). Moreover, the SAR studies results as well as the IC50 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 (Fig. 5). However, compound 4c (Fig. 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, (Fig. 6). While its unreduced rigid analogue 3a (IC50 = 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 (Fig. 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 (Fig. 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 (Fig. 10). Compound 10 interacts with the enzyme active sites via formation of a hydrogen bond with Arg 120 with a high binding energy (Fig. 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 (Fig 12 and13).

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 (IC50 = 5.40μM) comparable with celecoxib, so it appears promising in addition to 3a, 10 and 12. The structure–activity relationships (SAR) acquired showed that appropriately (morpholinyl-oxinidolyl)thiazolidinestructure has the necessary geometry to provide potent and selective inhibition of the COX-2 isozyme. Furthermore analysis of the obtained results for newly prepared compounds opens the possibility for further optimization of studied compounds.

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