

Design and Synthesis of Some 5-Substituted-2-(4-(azido or methylsulfonyl)phenyl)-1H-indole Derivatives as Selective Cyclooxygenase (COX-2) Inhibitors

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Abstract

A group of 5-substituted-2-(4-azido or (methylsulfonyl)phenyl)-1H-indoles were designed and synthesized as selective cyclooxygenase (COX-2) inhibitors. In vitro COX-1 and COX-2 isozyme inhibition studies were carried out to investigate the effect of different substituents (H, F, Cl, Me, OMe) at C-5 position and different pharmacophore groups (azido or methylsulfonyl) at *para* position of phenyl ring at C-2 position of the 1H-indole ring on COX-2 selectivity and potency. The structure-activity relationship study of these compounds indicated that the introduction of a methoxy substituent at C-5 position and 4-(methylsulfonyl) phenyl group at C-2 position of the 1H-indole ring (compound **4e**) had the best COX-2 selectivity (S.I = 291.2). A molecular modeling study where **4e** was docked in the binding site of COX-2 showed that the methylsulfonyl group at *para* position of phenyl ring is oriented in the vicinity of the COX-2 secondary pocket.

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Keywords

2-Phenyl-1*H*-indoles • COX-2 inhibitors • SAR

Introduction

Cyclooxygenase (COX) also known as prostaglandin synthase (PGH) is a potent mediator of inflammation. Nonsteroidal anti-inflammatory drugs (NSAIDs) bind to cyclooxygenase thereby inhibiting the production of prostaglandins. However, inhibition of COXs may lead to undesirable side effects. Nowadays, it is well established that there are at least two COX isozymes, COX-1 and COX-2 [1]. The constitutive COX-1 isozyme is produced in a variety of tissues and appears to be important to the maintenance of physiological functions such as gastric protection and vascular homeostasis [2]. Alternatively, the COX-2 isozyme is induced by mitogenic and proinflammatory stimuli linking its involvement to inflammatory processes [3]. Thus, selective inhibition of COX-2 over COX-1 is useful for the treatment of inflammation and inflammation-associated disorders with reduced gastrointestinal toxicities when compared with NSAIDs. In addition to the role of COX-2 in rheumatoid arthritis and osteoarthritis, it is also implicated in colon cancer and angiogenesis [4, 5]. Recent studies have shown that the progression of Alzheimer's disease is reduced among some users of NSAIDs. Chronic treatment with selective COX-2 inhibitors may therefore slow the progress of Alzheimer's disease without causing gastrointestinal damage [6]. Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as selective COX-2 inhibitors. All these molecules possess 1,2 diaryl substitution on a central heterocyclic or carbocyclic ring system (see structures **A–F** in Chart 1) [7–13]. Recently, we reported several investigations describing the design, synthesis and COX inhibitory activities of a novel class of compounds possessing an acyclic 1,3-diarylprop-2-en-1-one structural template [14, 15]. For example, the acyclic (*E*) 1,3-diphenylprop-2-en-1-ones possessing a 4-methylsulfonyl or 4-azido COX-2

pharmacophore group at the C-1 phenyl ring (see structure **F**) exhibited highly selective COX-2 inhibition.

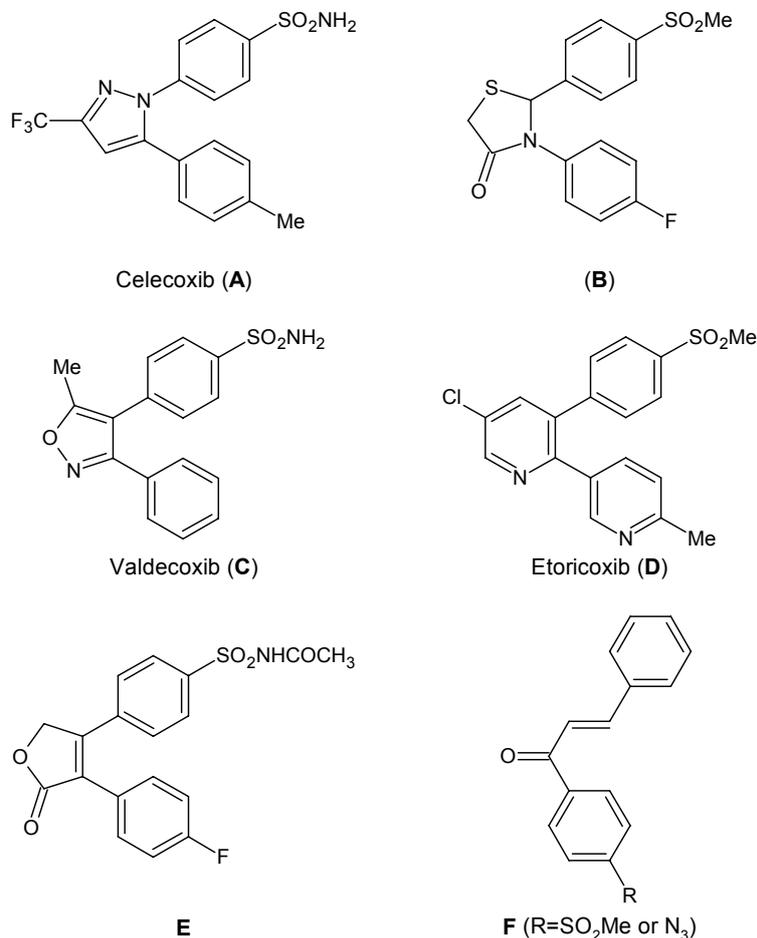


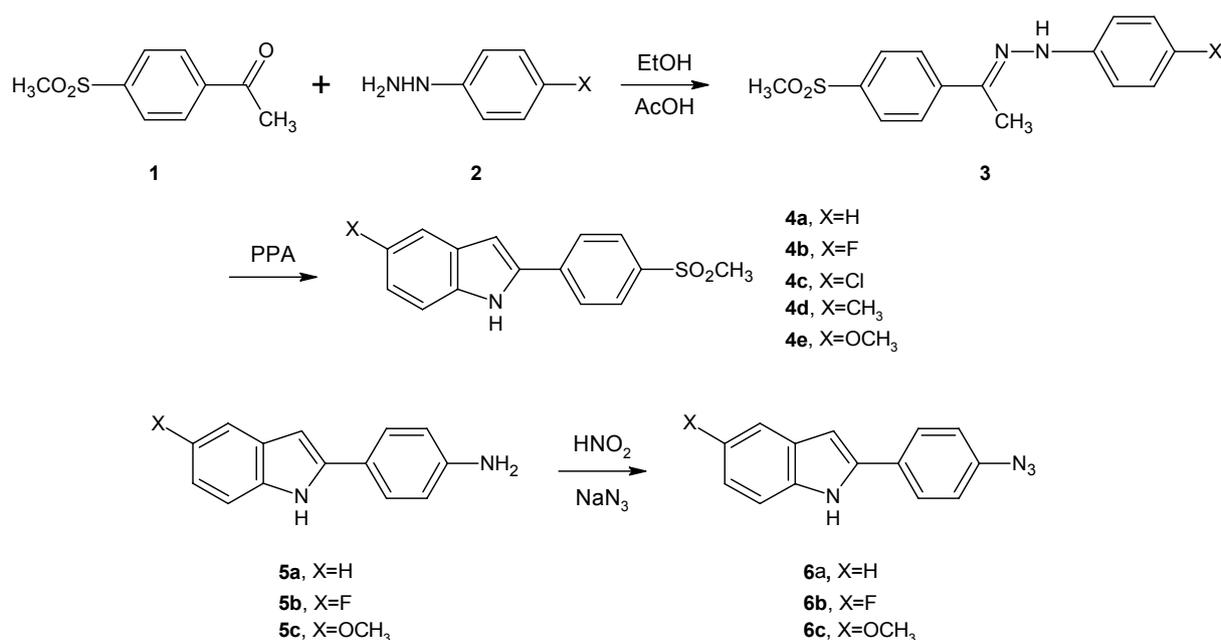
Chart 1. Representative examples of selective COX-2 inhibitors

As part of our ongoing program to design new types of selective COX-2 inhibitors, we now report the synthesis, some structure-activity relationships, and a molecular modeling study for a group of 2-phenyl-1*H*-indoles possessing a COX-2 azido or SO₂Me pharmacophore at the *para*-position of phenyl ring in conjunction with an 1*H*-indole ring having different substituents at C-5 position.

Results and Discussion

1. Chemistry

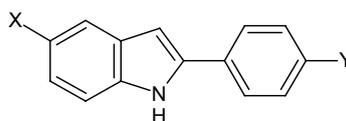
The target 2-(4-(methylsulfonyl or azido)phenyl)-1*H*-indole derivatives were synthesized using a Fischer-indole synthesis as shown in Scheme 1. Accordingly, warming an ethanolic mixture of 4-methylsulfonylacetophenone (**1**) [16] and an appropriate phenylhydrazine (**2**) on a water bath in presence of few amounts of acetic acid gave 4-methylsulfonylacetophenone phenylhydrazone (**3**) (50–89%). Cyclization of **3** by polyphosphoric acid at 120 °C afforded the 2-(4-(methylsulfonyl)phenyl)-1*H*-indoles (**4**, 60–92%) [17, 18]. Similarly, 2-(4-aminophenyl)-1*H*-indole derivatives **5** (40–62%) were synthesized and the amino group was converted to azido group by diazotation with sodium nitrite and hydrochloric acid and then treatment with sodium azide to obtain 2-(4-(azido)phenyl)-1*H*-indoles **6** (55–68%) [15]. The purity of all products was determined by thin layer chromatography using several solvent systems of different polarity. All compounds were pure and stable. The compounds were characterized by ¹H nuclear magnetic resonance, infrared, mass spectrometry and CHN analysis.



Sch. 1.

2. Enzyme Inhibitory Activity

The ability of the 2-phenyl-1*H*-indoles **4a–e** and **6a–c** to inhibit the COX-1 and COX-2 isozymes was determined using chemiluminescent enzyme assays as previously described [13] (see enzyme inhibition data in Table 1.). In vitro COX-1/COX-2 inhibition studies showed that compounds containing methylsulfonyl pharmacophore group **4a–e** were selective inhibitors of the COX-2 isozyme with IC_{50} values in the highly potent 0.08 to 0.26 μ M range, and COX-2 selectivity indexes (S.I.) in the 30.5 to 291.2 range. The structure-activity relationship study of these compounds indicated that the order of COX-2 selectivity was OMe>F >Me,Cl >H. These results showed that the nature of substituent at C-5 position of the 1*H*-indole ring has an important role on selectivity and potency. Replacing the methylsulfonyl group with an azido pharmacophore group in these compounds (**6a–6c**) decreased either selectivity or potency. According to these results, 5-methoxy-2-(4-(methylsulfonyl) phenyl)-1*H*-indole **4e** was the most potent ($IC_{50} = 0.08 \mu$ M), and selective (S.I. >291) COX-2 inhibitor among the synthesized compounds. These data suggest that the compound **4e** should inhibit the synthesis of inflammatory prostaglandins via the cyclooxygenase pathway at sites of inflammation and be devoid of ulcerogenicity due to the low COX-1 inhibitory activity.

Tab. 1. In vitro COX-1 and COX-2 Enzyme Inhibition Data

Compound	X	Y	IC ₅₀ (μM) ^a		COX-2 S.I. ^b
			COX-1	COX-2	
4a	H	SO ₂ CH ₃	7.9	0.26	30.5
4b	F	SO ₂ CH ₃	12.5	0.10	125
4c	Cl	SO ₂ CH ₃	20.1	0.22	91.3
4d	Me	SO ₂ CH ₃	13.5	0.14	96.4
4e	OMe	SO ₂ CH ₃	23.3	0.08	291.2
6a	H	N ₃	10.8	4.25	2.54
6b	F	N ₃	10.5	1.85	5.68
6c	OMe	N ₃	15.5	1.62	9.57
Celecoxib			24.3	0.06	405

^a Values are mean values of two determinations acquired using an ovine COX-1/COX-2 assay kit, where the deviation from the mean is < 10% of the mean value.
^b *In vitro* COX-2 selectivity index (COX-1 IC₅₀/ COX-2 IC₅₀).

3. Docking study

The orientation of the highly potent and selective COX-2 inhibitor, 5-methoxy-2-(4-(methylsulfonyl)phenyl)-1*H*-indole **4e** in the COX-2 active site was examined by a docking experiment (Fig. 1) [18]. This molecular modeling shows that it binds in the primary binding site such that the C-2 *para*-SO₂Me substituent inserts into the secondary pocket present in COX-2. One of the O-atoms of *p*-SO₂Me forms a hydrogen binding interaction with hydroxyl group (OH) of Tyr³⁵⁵ (distance = 4.8 Å). The NH of the central 1*H*-indole ring forms a hydrogen bond (distance = 3.4 Å) with the C=O group of Val³⁴⁹. In addition, the oxygen atom of methoxy substituent at C-5 position of the 1*H*-indole ring can form hydrogen binding interaction with hydroxyl group (OH) of Tyr³⁴⁸ (distance < 6 Å) which may explain the high potency of compound **4e** compared with other derivatives. These observations together with

experimental results provide a good explanation for the potent and selective inhibitory activity of **4e**.

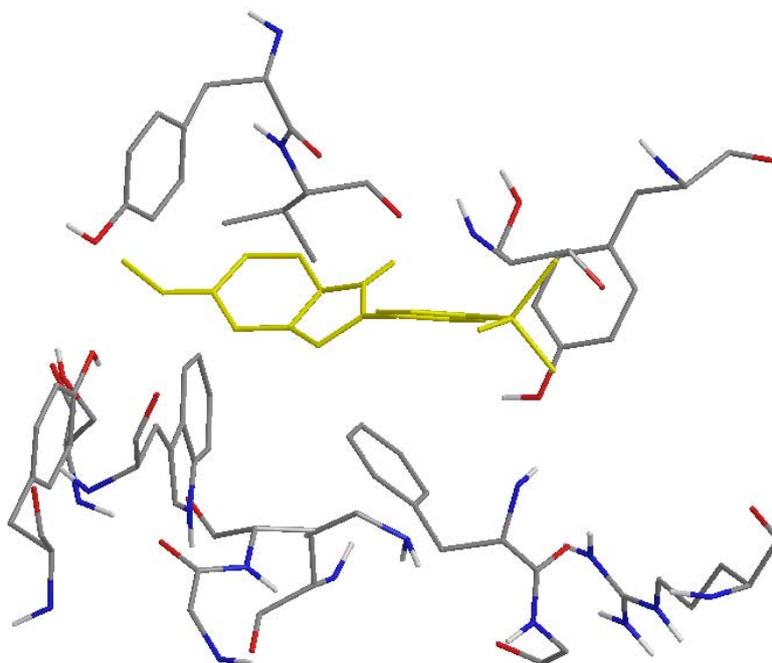


Fig. 1. Compound **4e** 5-methoxy-2-(4-(methylsulfonyl)phenyl)-1*H*-indole docked in the active site of murine COX-2 isozyme.

Experimental

1. Chemistry

Melting points (mp) were determined using a Thomas Hoover capillary apparatus (Philadelphia, USA). Infrared spectra were acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Bruker Biosciences, USA) was used to acquire ^1H NMR spectra; chloroform- D , $\text{DMSO-}\text{D}_6$ and methanol- D_4 were used as solvents. Mass spectra were acquired with a Finnigan TSQ-70 mass spectrometer. Electron-impact ionization was performed at an ionizing energy of 70 eV; the source temperature was 250 °C. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C,H,N) were

within $\pm 0.4\%$ of the calculated amounts. All chemicals and reagents were obtained from Aldrich (USA) or Merck (Germany) and were used without further purification.

General procedure for preparation of (E)-1-(4-substituted-phenyl)-2-(1-(4-(methylsulfonyl)phenyl)ethylidene)hydrazines (3)

An ethanolic mixture of 4-methylsulfonylacetophenone **1** (4 mmol) and an appropriate phenylhydrazine **2** (4 mmol) was heated on a water bath in presence of few amounts of acetic acid for 1 hour to give 4-methylsulfonyl acetophenone phenylhydrazone **3**. The obtained product was separated, dried and recrystallized from ethanol (Yields: 50–89%).

(1E)-1-{1-[4-(Methylsulfonyl)phenyl]ethylidene}-2-phenylhydrazine (3a)

Yield: 89%; mp: 175 °C; IR (KBr): ν (cm⁻¹) 3310 (NH), 1300, 1150 (SO₂), Mass, m/z (%): 288.3 (M⁺, 70), 273.1 (20), 208.1 (20), 196.1 (40), 117.1 (50), 92.1(100), 77.1 (50).

(2E)-1-(4-Fluorophenyl)-2-{1-[4-(methylsulfonyl)phenyl]ethylidene}hydrazine (3b)

Yield: 68%; mp: 179 °C; IR (KBr): ν (cm⁻¹) 3300 (NH), 1300, 1160 (SO₂), Mass, m/z (%): 306.2 (M⁺, 30), 264.5 (20), 227.0 (20), 149.1 (30), 109.1 (100), 83.1 (90).

(2E)-1-(4-chlorophenyl)-2-{1-[4-(methylsulfonyl)phenyl]ethylidene}hydrazine (3c)

Yield: 70%; mp: 194–195 °C; IR (KBr): ν (cm⁻¹) 3280 (NH), 1310, 1150 (SO₂), Mass, m/z (%): 322.2 (M⁺, 10), 279.6 (20), 213.1 (20), 156.1 (30), 138.1 (100), 111.1 (50), 77.1 (50).

(2E)-1-(4-methylphenyl)-2-{1-[4-(methylsulfonyl)phenyl]ethylidene}hydrazine (3d)

Yield: 62%; mp: 138–139 °C; IR (KBr): ν (cm⁻¹) 3290 (NH), 1300, 1150 (SO₂), Mass, m/z (%): 302.2 (M⁺, 10), 273.2 (30), 222.1 (20), 156.1 (40), 119.2 (100), 91.2

(60), 77.1 (30).

(2E)-1-(4-methoxyphenyl)-2-{1-[4-(methylsulfonyl)phenyl]ethylidene}hydrazine (3e)

Yield: 50%; mp: 142–143 °C; IR (KBr): ν (cm⁻¹) 3300 (NH), 1300, 1150 (SO₂), Mass, m/z (%): 318.3 (M⁺, 10), 303.3 (20), 239.1 (30), 165.6 (30), 122.2 (80), 92.1 (50), 79.1 (100).

General procedure for preparation of 5-substituted-2-(4-(methyl sulfonyl)-phenyl)-1H-indoles (4)

4 mmol of **3** was added to polyphosphoric acid (5g) and the mixture was heated at 120 °C for 30 min. After this time, the mixture was cooled and poured in 100 ml cold water. The solid product was filtered and boiled with 100 ml of ethanol. The hot mixture is treated with a little decolourising carbon and filtered through a pre-heated Buchner funnel; wash the residue with 40 ml of hot ethanol. Cool the combined filtrates to room temperature, filter off the 2-phenylindole and wash it with 20 ml of cold alcohol. The obtained product was dried and recrystallized from ethanol (Yields: 60–92%).

2-[4-(Methylsulfonyl)phenyl]-1H-indole (4a)

Yield: 92%; mp: 219–220 °C; IR (KBr): ν (cm⁻¹) 3290 (NH), 1300, 1150 (SO₂); ¹H NMR (chloroform-D): δ (ppm) 3.01 (s, 3H, SO₂CH₃), 6.89 (s, 1H, indole H-3), 7.01 (m, 1H, indole H-5), 7.13 (m, 1H, indole H-6), 7.37 (d, 1H, Indole H-4, J= 8.1 Hz), 7.55 (d, 1H, indole H-7, J= 8.1 Hz), 7.88 (d, 2H, 4-methylsulfonyl phenyl H-2 & H-6, J = 8.5 Hz), 7.93 (d, 2H, 4-methylsulfonyl phenyl H-3 & H-5, J = 8.5 Hz), 10.76 (s, 1H, NH); Ms: m/z (%): 271.2 (M⁺, 100), 208.1 (35), 192.2 (95), 165.1 (40), 139.1 (20), 95.7 (30), 89.1 (40). Anal. Calcd. for C₁₅H₁₃NO₂S: C, 66.40; H, 4.83; N, 5.16. Found: C, 66.65; H, 4.62; N, 5.19.

5-Fluoro-2-[4-(methylsulfonyl)phenyl]-1H-indole (4b)

Yield: 85%; mp: 243 °C; IR (KBr): ν (cm⁻¹) 3260 (NH), 1300, 1160 (SO₂); ¹H

NMR (chloroform-D): δ (ppm) 3.02 (s, 3H, SO₂CH₃), 6.84 (s, 1H, indole H-3), 7.09–7.17 (m, 3H, indole H-4, H-6 & H-7), 7.89 (d, 2H, 4-methylsulfonyl phenyl H-2 & H-6, J = 8.4 Hz), 7.90 (d, 2H, 4-methylsulfonyl phenyl H-3 & H-5, J = 8.4 Hz), 11.06 (s, 1H, NH); Ms: *m/z* (%): 289.2 (M⁺, 70), 226.2 (50), 210.2 (100), 198.2 (40), 183.2 (70), 158.2 (30), 107.1 (50), 91.2 (40). Anal. Calcd. for C₁₅H₁₂NO₂FS: C, 62.27; H, 4.18; N, 4.44. Found: C, 62.55; H, 4.32; N, 4.35.

5-Chloro-2-[4-(methylsulfonyl)phenyl]-1H-indole (4c)

Yield: 88%; mp: 254 °C; IR (KBr): ν (cm⁻¹) 3230 (NH), 1300, 1150 (SO₂); ¹H NMR (chloroform-D): δ (ppm) 3.01 (s, 3H, SO₂CH₃), 6.76 (s, 1H, indole H-3), 6.99 (d, 1H, indole H-7, J = 8.4 Hz), 7.25 (d, 1H, indole H-6, J = 8.4 Hz), 7.44 (s, 1H, indole H-4), 7.83 (d, 2H, 4-methylsulfonyl phenyl H-2 & H-6, J = 8.5 Hz), 7.87 (d, 2H, 4-methylsulfonyl phenyl H-3 & H-5, J = 8.5 Hz), 11.09 (s, 1H, NH); Ms: *m/z* (%): 305.1 (M⁺, 40), 242.2 (40), 226.2 (50), 191.2 (100), 163.2 (60), 149.2 (30), 158.2 (30), 95.6.1 (60), 76.1 (50). Anal. Calcd. for C₁₅H₁₂NO₂ClS: C, 58.92; H, 3.96; N, 4.58. Found: C, 59.15; H, 4.12; N, 4.35.

5-Methyl-2-[4-(methylsulfonyl)phenyl]-1H-indole (4d)

Yield: 68%; mp: 110–111 °C; IR (KBr): ν (cm⁻¹) 3270 (NH), 1310, 1150 (SO₂); ¹H NMR (chloroform-D): δ (ppm) 2.28 (s, 3H, CH₃), 3.03 (s, 3H, SO₂CH₃), 6.64 (s, 1H, indole H-3), 6.79 (s, 1H, indole H-4), 6.86 (d, 1H, Indole H-6, J= 8.3 Hz), 6.99 (d, 1H, indole H-7, J= 8.3 Hz), 7.81 (d, 2H, 4-methylsulfonyl phenyl H-2 & H-6, J = 8.5 Hz), 7.84 (d, 2H, 4-methylsulfonyl phenyl H-3 & H-5, J = 8.5 Hz), 10.72 (s, 1H, NH); Ms: *m/z* (%): 285.2 (M⁺, 30), 238.2 (20), 209.1 (20), 183.0 (30), 134.1 (100), 107.1 (40), 92.1 (60), 77.1 (50). Anal. Calcd. for C₁₆H₁₅NO₂S: C, 67.34; H, 5.30; N, 4.91. Found: C, 67.65; H, 5.12; N, 4.85.

5-Methoxy-2-[4-(methylsulfonyl)phenyl]-1H-indole (4e)

Yield: 60%; mp: 118–120 °C; IR (KBr): ν (cm⁻¹) 3250 (NH), 1300, 1150 (SO₂); ¹H NMR (chloroform-D): δ (ppm) 3.03 (s, 3H, SO₂CH₃), 3.65 (s, 3H, OCH₃), 6.61 (s, 1H, indole H-3), 6.74 (s, 1H, indole H-4), 6.81 (d, 1H, Indole H-6, J= 8.3 Hz), 6.97

(d, 1H, indole H-7, $J = 8.3$ Hz), 7.81 (d, 2H, 4-methylsulfonyl phenyl H-2 & H-6, $J = 8.5$ Hz), 7.84 (d, 2H, 4-methylsulfonyl phenyl H-3 & H-5, $J = 8.5$ Hz), 10.72 (s, 1H, NH); Ms: m/z (%): 301.5 (M^+ , 30), 273.7 (20), 219.5 (30), 183.0 (40), 152.1 (50), 104.0 (100), 89.1 (50). Anal. Calcd. for $C_{16}H_{15}NO_3S$: C, 63.77; H, 5.02; N, 4.65. Found: C, 63.95; H, 4.82; N, 4.37.

General procedure for preparation of 5-substituted-2-(4-aminophenyl)-1H-indoles (5)

The compounds **5a–5c** were prepared according to the procedure as described for **4**. The obtained products were crystallized from ethanol (Yields: 40–62%).

4-(1H-Indol-2-yl)aniline (5a)

Yield: 60%; mp: 201–202 °C; IR (KBr): ν (cm^{-1}) 3300, 3250 (NH_2); 1H NMR (chloroform- D): δ (ppm) 6.65 (d, 2H, 4-aminophenyl H-3 & H-5, $J = 8.3$ Hz), 6.81 (s, 1H, indole H-3), 7.01 (m, 1H, indole H-5), 7.12 (m, 1H, indole H-6), 7.30–7.48 (m, 2H, indole H-4 & H-7), 7.24 (d, 2H, 4-amino phenyl H-2 & H-6, $J = 8.3$ Hz), 10.61 (s, 1H, NH).

4-(5-Fluoro-1H-indol-2-yl)aniline (5b)

Yield: 43%; mp: 215–216 °C; IR (KBr): ν (cm^{-1}) 3310, 3260 (NH_2); 1H NMR (chloroform- D): δ (ppm) 6.68 (d, 2H, 4-aminophenyl H-3 & H-5, $J = 8.2$ Hz), 6.76 (s, 1H, indole H₃), 7.02–7.21 (m, 3H, indole H-4, H-6 & H-7), 7.25 (d, 2H, 4-aminophenyl H-2 & H-6, $J = 8.2$ Hz), 10.73 (s, 1H, NH).

4-(5-Methoxy-1H-indol-2-yl)aniline (5c)

Yield: 40%; mp: 171–172 °C; IR (KBr): ν (cm^{-1}) 3340, 3250 (NH_2); 1H NMR (chloroform- D): δ (ppm) 3.53 (s, 3H, OCH_3), 6.55 (d, 2H, 4-aminophenyl H-3 & H-5, $J = 8.2$ Hz), 6.66 (s, 1H, indole H-3), 6.70 (s, 1H, indole H-4), 6.79 (d, 1H, indole H-6, $J = 8.3$ Hz), 6.92 (d, 1H, indole H-7, $J = 8.3$ Hz), 7.23 (d, 2H, 4-aminophenyl H-2 & H-6, $J = 8.2$ Hz), 10.53 (s, 1H, NH).

General procedure for preparation of 5-substituted-2-(4-(azido) phenyl)-1H-indoles (6)

6 mmol of **5** was added to 10 ml of cold water and 5 ml of hydrochloric acid (12N) and the mixture was stirred at r.t. for 1 hr. After this time, the mixture was cooled (0 °C) and a solution of 6 mmol sodium nitrite in 5 ml water was added slowly to the mixture. Then 6 mmol sodium azide in 5 ml water was added to reaction medium. The solid product was filtered, washed with water and crystallized from ethanol (Yields: 55-68%).

2-(4-Azidophenyl)-1H-indole (6a)

Yield: 68%; mp: 120–121 °C; IR (KBr): ν (cm⁻¹) 3270 (NH), 2120 (N₃); ¹H NMR (chloroform-D): δ (ppm) 6.85 (s, 1H, indole H-3), 7.01 (m, 1H, indole H-5), 7.13 (m, 1H, indole H-6), 7.32-7.50 (m, 2H, indole H-4 & H-7), 7.55 (d, 2H, 4-azidophenyl H-2 & H-6, J = 8.3 Hz), 7.85 (d, 2H, 4-azidophenyl H-3 & H-5, J = 8.5 Hz), 10.56 (s, 1H, NH) ; Anal. Calcd. for C₁₄H₁₀N₄: C, 71.78; H, 4.30; N, 23.92. Found: C, 71.65; H, 4.02; N, 23.79.

2-(4-Azidophenyl)-5-fluoro-1H-indole (6b)

Yield: 60%; mp: 135–136 °C; IR (KBr): ν (cm⁻¹) 3260 (NH), 2140 (N₃); ¹H NMR (chloroform-D): δ (ppm) 6.80 (s, 1H, indole H₃), 7.05-7.25 (m, 3H, indole H-4, H-6 & H-7), 7.56 (d, 2H, 4-azidophenyl H-2 & H-6, J = 8.4 Hz), 7.88 (d, 2H, 4-azidophenyl H-3 & H-5, J = 8.4 Hz), 10.95 (s, 1H, NH); Anal. Calcd. for C₁₄H₉N₄F: C, 66.66; H, 3.60; N, 22.21. Found: C, 66.85; H, 3.82; N, 22.35.

2-(4-Azidophenyl)-5-methoxy-1H-indole (6c)

Yield: 55%; mp: 70–71 °C; IR (KBr): ν (cm⁻¹) 3250 (NH), 2110 (N₃); ¹H NMR (chloroform-D): δ (ppm) 3.55 (s, 3H, OCH₃), 6.61 (s, 1H, indole H-3), 6.71 (s, 1H, indole H-4), 6.80 (d, 1H, indole H-6, J= 8.2 Hz), 6.95 (d, 1H, indole H-7, J= 8.2 Hz), 7.60 (d, 2H, 4-azidophenyl H-2 & H-6, J = 8.4 Hz), 7.80 (d, 2H, 4-azidophenyl H-3 & H-5, J = 8.4 Hz), 10.72 (s, 1H, NH); Anal. Calcd. for C₁₅H₁₂N₄O: C, 68.17; H, 4.58; N, 21.20. Found: C, 68.45; H, 4.72; N, 21.36.

2. *In vitro* cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and COX-2 (IC₅₀ value, μM) was determined using chemiluminescent enzyme assays kit (catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method [13].

3. *Molecular modeling (docking) studies*

Docking studies were performed using Autodock software Version 3.0. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. Searching is conducted within a specified 3D docking box using annealing based on the Monte Carlo method and MMFF94 molecular mechanics force field for 8000 iterations. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures was evaluated by measuring the intermolecular energy of the ligand-enzyme assembly [19].

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