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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis and biological evaluation of new tricyclic spiroisoxazoline derivatives as selective COX-2 inhibitors and study of their COX-2 binding modes via docking studies

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Abstract A new series of 3′-(4-substitutedphenyl)-4′-(4-(methylsulfonyl)phenyl) spiroisoxazoline derivatives containing naphthalenone and chromanonespiro-bridge were synthesized for evaluation as selective cyclooxygenase-2 (COX-2) inhibitors. A synthetic reaction based on the 1,3-dipolar cycloaddition mechanism was used for the regiospecific formation of various spiroisoxazolines. One of the analogs, i.e., compound **7h**, as the representative of the series was recrystallized and characterized structurally by single-crystal X-ray diffraction method. Moreover, the 3D structures of the synthesized compounds were docked into the COX-2 binding site to determine their most probable binding modes once the drug-receptor complexes are formed.

Keywords Spiroisoxazoline · 1,3-Dipolar cycloaddition · Molecular modeling · Docking

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Introduction

Cyclooxygenase (COX) (prostaglandin synthase), the key enzyme of inflammatory process, exists at least in two isoforms (COX-1 and COX-2). COX-2 is induced in response to proinflammatory conditions, while COX-1 is constitutive and responsible for the maintenance of physiological homeostasis. This finding led to the theory that inhibition of COX-1 causes the side effects of non-selective COX inhibitors such as NSAIDs like gastric ulceration, bleeding, and renal dysfunction, whereas inhibition of COX-2 is responsible for their therapeutic effects (McAdam *et al.,* 1999; Vane *et al.,* 1998; WANG *et al.,* 2005). In normal condition, prostacyclin and thromboxane balance each other's opposing effects. Accordingly, introducing the selective COX-2 inhibitor may alter the balance dangerously toward thromboxane, raising blood pressure, cardiotoxicity and certainly promoting heart attack in some cases (Hinz *et al.,* 2006). This could describe the cardiovascular side effects caused by rofecoxib (Prasit *et al.,* 1999) and subsequently valdecoxib (Talley *et al.,* 2000) which were withdrawn from the market (Dogne' *et al.,* 2005). Thus, there is a need to explore and evaluate alternative selective COX-2 inhibitors with a mild therapeutic effect on COX-1, which could theoretically reduce cardiovascular side effects due to their antiplatelet and antithrombotic activities, and raise the safety profiles (Black 2004). Additionally, COX-2 inhibitors have emerged in recent years as candidates for drugs due to their anti-cancer activity (Koki and Masferrer 2002; Rizzo 2011; Ye *et al.,* 2004). Afterward COX-2 appears to be expressed at high levels in many different types of cancer cells, but not in bordering normal tissue, it appears plausible that the model developed for colon cancer can be expanded to other organs (Marnett and DuBois 2002). COX-2 is an enzyme

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that is upregulated in colorectal cancer adenomas and carcinoma (Eberhart *et al.,* 1994). It has been shown that PGE2 concentration is increased in colon cancer where COX-2 overexpression (Chell *et al.,* 2006; Yang *et al.,* 1998) leads to increased angiogenic and metastatic potential of tumor cells (Li *et al.,* 2002). These findings are supported by anticancer effect of selective COX-2 inhibitor, celecoxib (Koki and Masferrer 2002; Ye *et al.,* 2004). Another well-documented cancer therapeutic target is spiroisoxazoline compounds (**1**, **2** and **3**, Fig. 1) that are earning a lot of importance because of their extraordinary biological properties such as anticancer activities (Al Houari *et al.,* 2008; Kang *et al.,* 2000; Najim *et al.,* 2010) (CSID:13611429). Furthermore, Bennani and co-workers have recently investigated the anti-breast cancer activity of some spiroisoxazoline derivatives (Bennani *et al.,* 2004; Howe and Shelton 1990; Smietana *et al.,* 1999). On the other hand, the majority of selective COX-2 inhibitors belong to diarylheterocycles that contain vicinal diaryl substitution attached to a central ring system mainly mono or bicyclic ring (van Ryn *et al.,* 2000; Talley *et al.,* 2000; Zarghi *et al.,* 2007a, b; Zarghi *et al.,* 2009; Zebardast *et al.,* 2009) (Fig. 1). Extensive structure–activity relationship (SAR) studies for this class of COX-2 inhibitors have shown that a SO_2NH_2, or a SO_2Me substituent at the *para* position of one of the phenyl rings often provides optimum selective COX-2 inhibitory potency (Palomer *et al.,* 2002; Talley *et al.,* 2000; Zarghi *et al.,* 2007a, b). In the current work, we report the design and synthesis of a new series of 3′-(4-substituted phenyl)-4′-(4-(methylsulfonyl)phenyl)

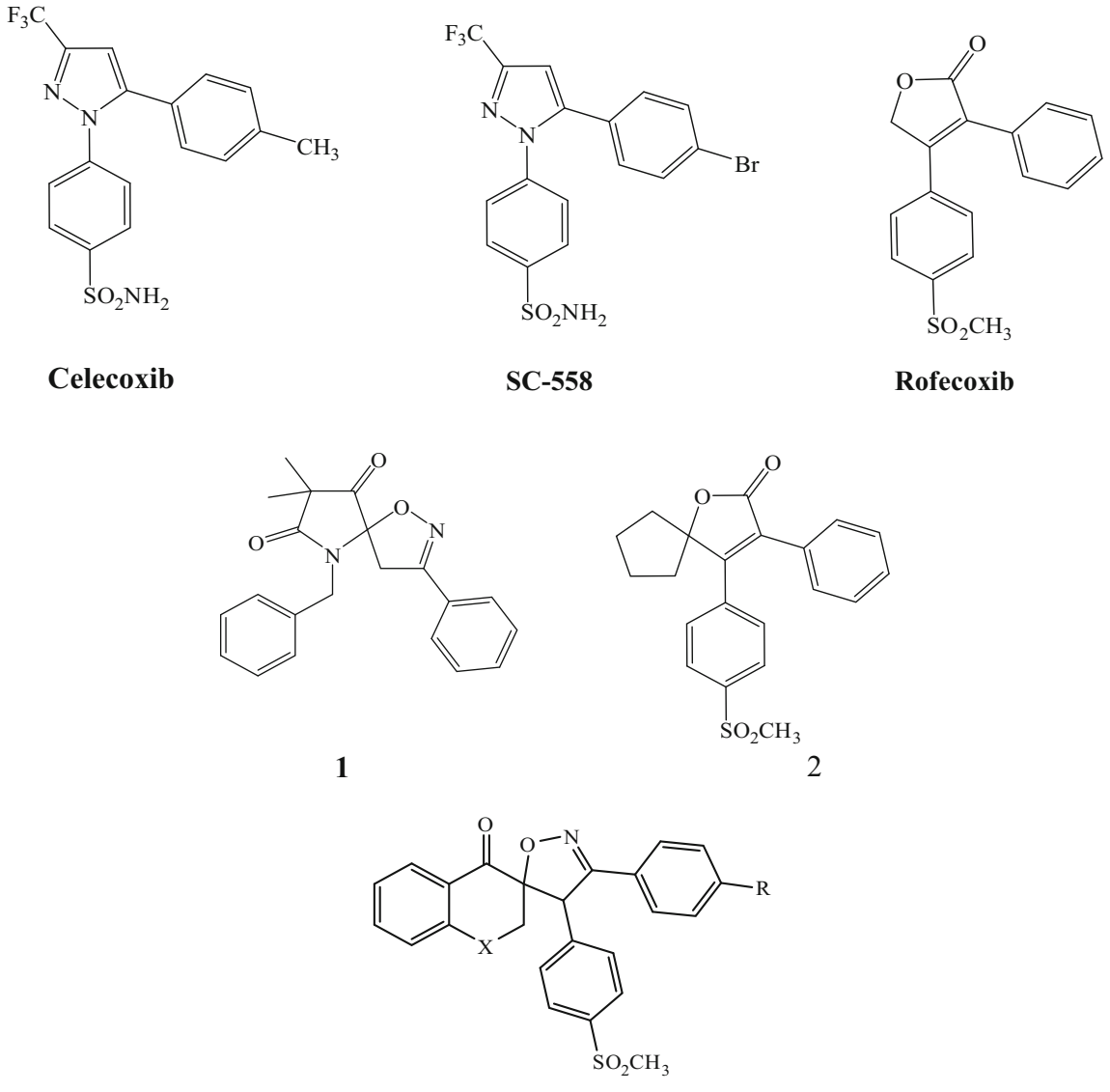


Fig. 1 Chemical structures of some known selective COX-2 inhibitors, spiro lead compounds (**1**, **2**) and our designed scaffold

spiroisoxazoline derivatives containing naphthalenone and chromanonespiro-bridge were synthesized for evaluation as selective COX-2 inhibitors. The design strategy was to combine pharmacophoric elements from COX-2 inhibitors with added extra anticancer and anti-inflammatory properties as the result of bioisosteric replacement of the central tricyclic bridge with spiroisoxazoline motif. Correspondingly, it is desirable to develop safe, potent, profit selective COX-2 inhibitors that show not only anti-inflammatory but also anticancer effects.

Result and discussion

Crystal structure of compound

Among these compounds (**7a**–**h**), the crystal structure of compound (**7h**) was determined by single-crystal X-ray diffraction method. The crystal data of compound (**7h**) are presented in Table 1, and the corresponding perspective view is shown in Fig. 2.

Molecular modeling

Docking simulation was performed to predict interaction of compounds (**7a–h**) with COX-2 binding site (PDB code: 1CX2). All docking calculations were performed with the GOLD program. Docking of compound **7b** into the COX-2 binding site (Fig. 3) shows four hydrogen bonds His90 (angle O–H–N = 173.98°, distance = 2.10 Å) and Arg513 (angle O–H–N = 157.79°, distance = 2.02 Å), Arg120 (angle O–H–N = 120.18°, distance = 2.20 Å) and Tyr355 (angle O–H–O = 143.29°, distance = 2.44 Å). As shown in Fig. 3, two extra hydrogen bounds in compound **7b** by interaction of oxygen atom of chromanone with Arg120 and Tyr355 are generated. Furthermore the *para*-substituted phenyl ring of docked compound was embedded in a hydrophobic pocket created by amino acids Tyr385, Trp387 and Phe518. In addition, docking shows the other hydrophobic pocket surrounding tricyclic spiroisoxazoline by the side chains of residues Leu531, Leu359 and Val349. The mentioned residues were also found to be involved in the binding of SC-558 with COX-2 binding site. Figure 4 shows different three-dimensional superimposition of the SC-558 and synthesized compounds while docked into the COX-2 binding site and reveals that all compounds were well incorporated in the selective COX-2 active pocket. The docking scores of synthesized compounds and SC-558 are mentioned in Table 2.

Biological activity

The result of COX-1/COX-2 inhibition is summarized in Table 3. SAR data (IC_{50} values) obtained by the determination of the in vitro ability (Zarghi *et al*., 2007a, b) of the title compounds to inhibit the COX-1 and COX-2 isozymes showed that all compounds (**7a**–**h**) were selective inhibitors of the COX-2 isozyme with IC_{50} values in the 0.09–0.20 μM range, and COX-2 selectivity indexes in the 63.2–116.2 range. Our results demonstrated that the COX inhibition was affected by the type of substituent at the *para* position of C-3′ phenyl ring. Accordingly, compounds having smaller groups (**7a**, **7b** and **7g**) were more potent and selective COX-2 inhibitors compared with other analogs which had larger ones. Based upon that, compound **7h** having chlorine group showed the lowest potency and selectivity this may be explained by steric parameter for interaction with COX-2 active binding site. These results also indicated that the spiroisoxazoline derivatives containing chromanone showed better both selectivity and potency for COX-2 inhibitory activity compared with naphthalenone analogs. This may be explained by the ability of hydrogen binding ability of oxygen atom in chromanones for better interaction with the COX-2 active site. Therefore, the introduction of suitable substituents at the *para* position of C-3′ phenyl ring combined with chromanone moiety improved the selectivity and potency for COX-2 inhibitory activity. These data showed that the size of substituent attached to *para* position of C-3′ phenyl ring and type of atom at position 1 of bicyclic ring can influence both selectivity and potency for COX-2 inhibitory activity. Our results indicated that 4′-(4-(methylsulfonyl)phenyl)3′-phenyl-4′H-spiro [chroman-3,5′-isoxazol]-4-one **7b** showed the highest COX-2 selectivity (SI = 116.2) among the synthesized compounds which may be due to better interaction with the COX-2 active site.

Experimental

Materials

All reagents purchased from the Aldrich (USA) or Merck (Germany) Chemical Company and were used without further purifications.

General

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 ^{1}HNMR spectra, chloroform-D and DMSO-d_6 used as solvents. Coupling constant (*J*) values are estimated in hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The

Table 1 Crystal data and structure refinement for compound **7h**

Compound	**7h**
Empirical formula	$C_{25}H_{20}ClNO_4S$
Formula weight	465.95
Temperature (K)	120(2)
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P-1$
a (Å)	8.1577(16)
b (Å)	10.200(2)
c (Å)	14.947(3)
α (°)	97.83(3)
β (°)	90.88(3)
γ (°)	106.41(3)
Volume (Å^3)	1180.0(5)
Z	2
D_{calcd}/(Mg/m^{-3})	1.401
Absorption coefficient (mm^{-1})	0.289
Crystal size (mm)	0.35 × 0.30 × 0.25
θ range for data collection (°)	2.34–29.13
F(0 0 0)	520
Limiting indices	$11 \le h \le 8$, $-13 \le k \le 13$, $-20 \le l \le 20$
Reflections collected/unique	13224/6315 [R(int) = 0.0853]
Completeness to θ = 29.13°	99.5 %
Absorption correction	Numerical
Max. and min. transmission	0.9312 and 0.9056
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	6315/1/313
Final R indices $[I > 2\sigma(I)]$	$R^1 = 0.0620$, $wR^2 = 0.1554$
R indices (all data)	$R^1 = 0.0859$, $wR^2 = 0.1690$
Goodness-of-fit on F^2	1.008
Largest diff. peak and hole (e Å^{-3})	0.545 and −0.594

mass spectral measurements were performed on a 6410 Agilent LCMS triple quadruple mass spectrometer (LCMS) with an electrospray ionization (ESI) interface.

Chemistry

In the present work, the synthesis of 3′-(4-substituted phenyl)-4′-(4-(methylsulfonyl)phenyl) spiroisoxazoline derivatives containing naphthalenone and chromanone (**7a–h**) from (E)-2-(4-(methylsulfonyl)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (**4a**) or (E)-3-(4-(methyl sulfonyl)benzylidene)chroman-4-one (**4b**) and (E)-4-substituted-benzaldehyde oxime (oximes) (**6a–e**) employing 1,3-dipolar cycloaddition reaction was carried out. Reaction of aldehydes (**5a–e**) with hydroxylamine hydrochloride in absolute ethanol and NaOH 10 % solution produced the corresponding oximes (**6a–e**). Methylsulfonylbenzylidene chalcones (**4a–b**) were obtained by aldol condensation reaction, where 4-(methylthio)benzaldehyde (**2**) were reacted with 3,4-dihydronaphthalen-1(2H)-one(**1a**) or chroman-4-one (**1b**) in the presence of sodium hydroxide and methanol to obtain methylthiobenzylidene and chalcones (**3a–b**) following by oxidation by Oxon®. Subsequently the methylsulfonylbenzylidene chalcones (**4a–b**) were reacted with oximes (**6a–e**) in biphasic medium of aqueous sodium hypochlorite 5 % and chloroform at cold temperature (−10 to 0 °C) to produce the corresponding spiroisoxazolines (**7a–h**) using 1,3-dipolar cycloaddition reaction. The procedures and the conditions for the synthesis of 3′-(4-substituted phenyl)-4′-(4-(methyl sulfonyl)phenyl)spiroisoxazoline derivatives containing naphthalenone and chromanone (**7a–h**) and their structures are shown in Scheme 1. Some of the basic physicochemical properties as well as the production yields of the synthesized compounds (**7a–h**) are shown in Table 4. Figure 2 shows the crystal structure determined by single-crystal X-ray diffraction method for one of compounds as the representative of the series (i.e., **7h**). Also the structures of the synthesized compounds have been verified using IR, MS and NMR (^{1}H and ^{13}C) spectroscopic methods.

General Procedure for Preparation of Chalcones (3a, 3b)

One of the (**1a**) 3, 4 dihydronaphthalen-1(2H)-one (0.146 g, 1 mmol) or (**1b**) chroman-4-one (0.148 g, 1 mmol) and 4-methylthiobenzaldehyde (**2**) (0.152 g, 1 mmol) were dissolved in the methanol (10 mL), a single NaOH pellet (100 mg, 2.5 mmol) was then added to this solution, and the reaction mixture was stirred at room temperature. In most cases, a cream to dark yellow solid was formed within a few minutes, but the reaction was allowed to proceed for 3 h. The solid product **3a** or **3b** was collected on a filter, washed with cold methanol, and the product was recrystallized from methanol. (Yields: 65–72 %). The physical and spectral data for **3a** and **3b** are listed below.

*(E)-2-(4-(Methylthio)benzylidene)-3,4-dihydronaphthalen-1(2H)-one (**3a**)* Yield: 72 %; yellow crystalline powder; mp: 191 °C; IR (KBr): ν (cm^{-1}) 1590 (C=O); LC–MS (ESI) m/z: 281.1 (M + 1)), 303.1 (M + 23); ^{1}HNMR ($CDCl_3$): δ ppm 2.94 (s, 3H, SMe), 2.99 (t, 2H, CH_2), 3.17 (t, 2H, CH_2), 7.29–7.30 (d, 1H, phenyl H_5, $J = 7.7$ Hz), 7.32 (d, 2H, 4-methylthiophenyl H_3 & H_5, $J = 8.3$ Hz), 7.39–7.41 (t, 1H, phenyl H_7), 7.42–7.44 (d, 2H, 4-methylthiophenyl H_2 & H_6, $J = 8.3$ Hz), 7.53 (t, 1H, phenyl H_6), 7.87 (s, 1H, CH), 8.16 (d, 1H, phenyl H_8, $J = 7.0$ Hz).

*(E)-3-(4-(Methylthio)benzylidene)chroman-4-one (**3b**)* Yield: 65 %; yellow crystalline powder; mp: 137–138 °C;

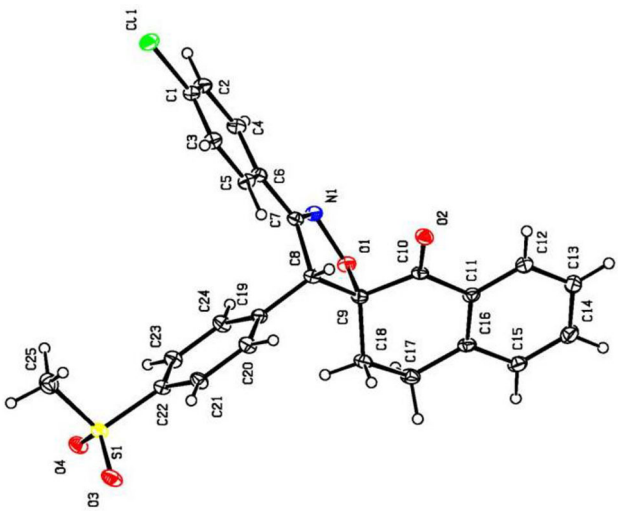


Fig. 2 Crystal structure diagrams of compound **7h**

IR (KBr): ν (cm^{-1}) 1600 (C=O); LC–MS (ESI) m/z: 283.1 (M + 1); ^{1}HNMR ($CDCl_3$): δ ppm 2.57 (s, 3H, SO_2Me), 5.40 (s, 2H, CH_2), 7.01 (d, 1H, phenyl H_8, J = 8.2 Hz), 7.11 (t, 1H, phenyl H_6), 7.29 (d, 4-methyl thiophenyl H_3 & H_5, J = 8.4 Hz), 7.33 (d, 2H, 4-methylthiophenyl H_2 & H_6, J = 8.4 Hz), 7.52 (t, 1H, phenyl H_7), 7.65 (s, 1H, CH), 8.06 (d, 1H, phenyl H_5, J = 7.1 Hz).

General Procedure for Preparation of Methylsulfonyl chalcones (4a, 4b)

One gram of **3a** or **3b** was dissolved in 20 ml of THF and 5 g Oxone in THF/water (30 ml) was added. The mixture was stirred at room temperature for 2 h. After evaporation of THF, the residue was extracted with chloroform, washed with 10 % aqueous sodium bicarbonate and dried with anhydrous sodium sulfate and then the solvent evaporated. In most cases, off-white to pale yellow solid was formed. Yield: (70–94 %). The physical and spectral data for **4a** and **4b** are listed below.

*(E)-2-(4-(methylsulfonyl) benzylidene)-3, 4-dihydronaphthalen-1(2H)-one (**4a**))* Yield: 70 %; yellow crystalline powder; mp: 200–202 °C; IR (KBr): ν (cm^{-1}) 1592 (C=O); 1301, 1157 (SO_2); LC–MS (ESI) m/z: 313.1 (M + 1); 335.1 (M + 23). ^{1}HNMR ($CDCl_3$): δ ppm 3.01–3.04 (t, 2H, CH_2), 3.13 (s, 1H, SO_2Me), 3.13–3.15 (m, 2H, CH_2), 7.31 (d, J = 7.7 Hz, 1H, phenyl H_5), 7.45 (t, 1H, phenyl H_7), 7.58 (t, 1H, phenyl H_6), 7.64 (d, J = 8.2 Hz, 2H, 4-methyl sulfonylphenyl H_2 & H_6), 7.88 (s, 1H, CH), 8.03 (d, J = 8.2 Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5), 8.18 (d, J = 6.7 Hz, 1H, phenyl H_8).

*(E)-3-(4-(methylsulfonyl)benzylidene) chroman-4-one (**4b**)* Yield: 94 %; white crystalline powder; mp: 180–182 °C; IR (KBr): ν (cm^{-1}) 1594 (C=O), 1306, 1141 (SO_2); LC–MS (ESI) m/z: 315.1 (M + 1); 337.1 (M + 23); ^{1}HNMR ($CDCl_3$): δ ppm 3.14 (s, 3H, SO_2Me), 5.33 (s, 2H, CH_2), 7.03 (d, J = 8.3 Hz, 1H, phenyl H_8), 7.13 (t, 1H, phenyl H_6), 7.53 (d, J = 7.5 Hz, 2H, 4-methylsulfonylphenyl H_2 & H_6), 7.57–7.58 (t, 1H, phenyl H_7), 7.91 (s, 1H, CH), 8.06–8.08 (m, 3H, 4-methylsulfonylphenyl H_3 & H_5, phenyl H_5).

General Procedure for Preparation of p-substituted-benzaldoximes (6a–e)

One gram of compounds **5a–e** was dissolved in 20 ml of 95 % ethanol. After the mixture was heated to 60 °C,

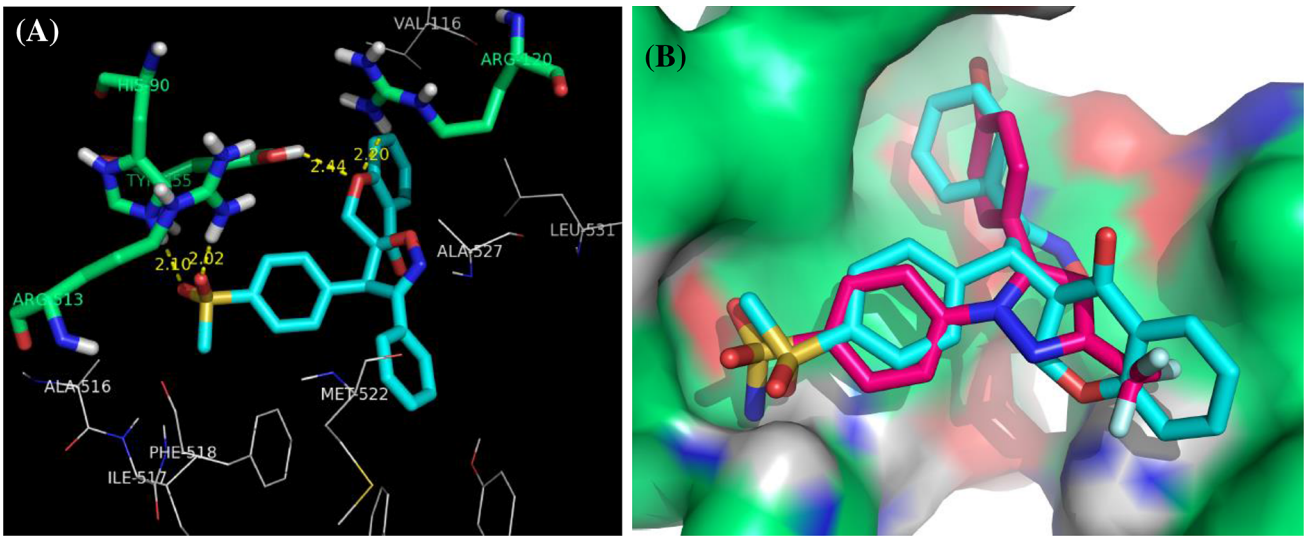
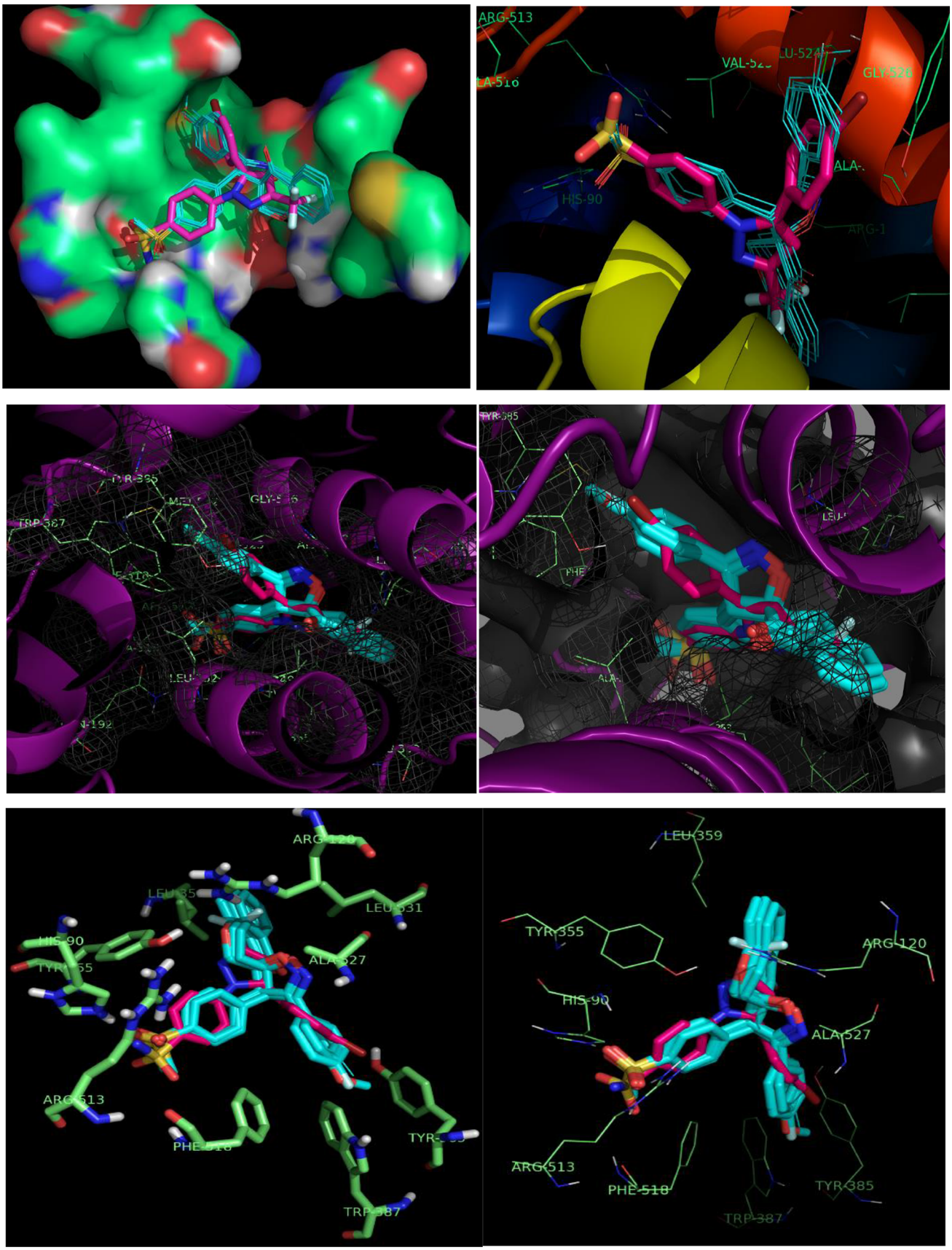


Fig. 3 3D representations of the binding of compound **7b** (colored according to the atom type) in the active site of murine COX-2 (COX-2 structure with PDB code 1CX2 was used for docking). **a** The *yellow dotted lines* show the hydrogen bonds in protein–ligand complex. **b** The protein is represented by colored surface and shows superimposition of SC-558 (hot pink and stick) and **7b** (*cyan* and stick) with COX-2 by the essential amino acid residues at the active site. All pictures were prepared with PyMOL (Color figure online)


ARG-513
HIS-90
ARG-120
LEU-359
TYR-355
ALA-527
PHE-518
TRP-387
TYR-385

◀**Fig. 4** Different three-dimensional superimpose representations of the SC-558(*hot pink* and *stick*) and synthesized compounds (**7a**, **7b**, **7c**, **7d**, **7f**, **7g** and **7h**) (*cyan* and *stick*) with COX-2 using PyMOL program with the essential amino acid residues at the active site (Color figure online)

NaOH aq 10 % (10 ml) and $NH_2OH{\cdot}HCl$ (4 g) were added. The mixture was refluxed for 2 h at 70 °C. Then the reaction was terminated, and the majority of solvent was evaporated under reduced pressure. Cool distilled water and 2 ml HCl 6 M was added into the reaction mixture. The solid product **6b–e** was collected on a filter, washed with cold water, and the product was recrystallized from ethanol. But compound **6a** was extracted with diethyl ether. The organic layer dried over Na_2SO_4 and evaporated to obtain the oily product.

Yield: (60–92 %). The physical and spectral data for **6a–e** are listed below.

(E)-benzaldehydeoxime(Benzaldoxime) ***(6a)*** Yield: 62 %; yellow oily liquid; IR (neat): ν (cm^{-1}) 1720 (C=N), 3406 (O–H), 955 (N–O); LC–MS (ESI) m/z: 122.1 (M + 1).

Table 2 Docking scores for compounds **7a–7h**

Compound	R	X	Docking score
7a	H	CH2	31.84
7b	H	O	33.71
7c	CH3	CH2	32.82
7d	CH3	O	32.80
7f	OCH3	O	33.94
7g	F	CH2	30.60
7h	Cl	CH2	32.78
SC-558	–	–	15.09

Table 3 In vitro COX-1 and COX-2 enzyme inhibition assay data for compounds **7a–7h**

Compound	COX-1 IC^a_{50} ± SD (μM)	COX-2 IC^a_{50} ± SD (μM)	Selectivity index[b] (SI)
7a	11.65	0.12	97.1
7b	10.46	0.09	116.2
7c	10.90	0.16	68.4
7d	10.11	0.12	84.2
7e	13.65	0.18	75.8
7f	9.11	0.11	82.8
7g	10.23	0.10	102.3
7h	12.65	0.20	63.2
Celecoxib	24.30	0.06	405

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit and the deviation from the mean is <10 % of the mean value

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50})

(E)-4-fluorobenzaldehyde oxime) 4-fluorobenzaldoxime ***(6b)*** Yield: 69 %; white crystalline powder; mp: 70–72 °C; IR (KBr): ν (cm^{-1}) 1681 (C=N), 3084 (O–H), 859 (N–O); LC–MS (ESI) m/z: 140 (M + 1).

(E)-4-chlorobenzaldehyde oxime) 4-chlorobenzaldoxime ***(6c)*** Yield: 82 %; white crystalline powder; mp: 100–113 °C; IR (KBr): ν (cm^{-1}) 1588 (C=N), 3308 (O–H), 972 (N–O); LC–MS (ESI) m/z: 156 (M + 1).

(E)-4-methylbenzaldehyde oxime(4-methyl benzaldoxime) ***(6d)*** Yield: 62 %; cream crystalline powder; mp: 70–74 °C;IR (KBr): ν (cm^{-1}) 1598 (C=N), 3273 (O–H), 954 (N–O); LC–MS (ESI) m/z: 136.1 (M + 1).

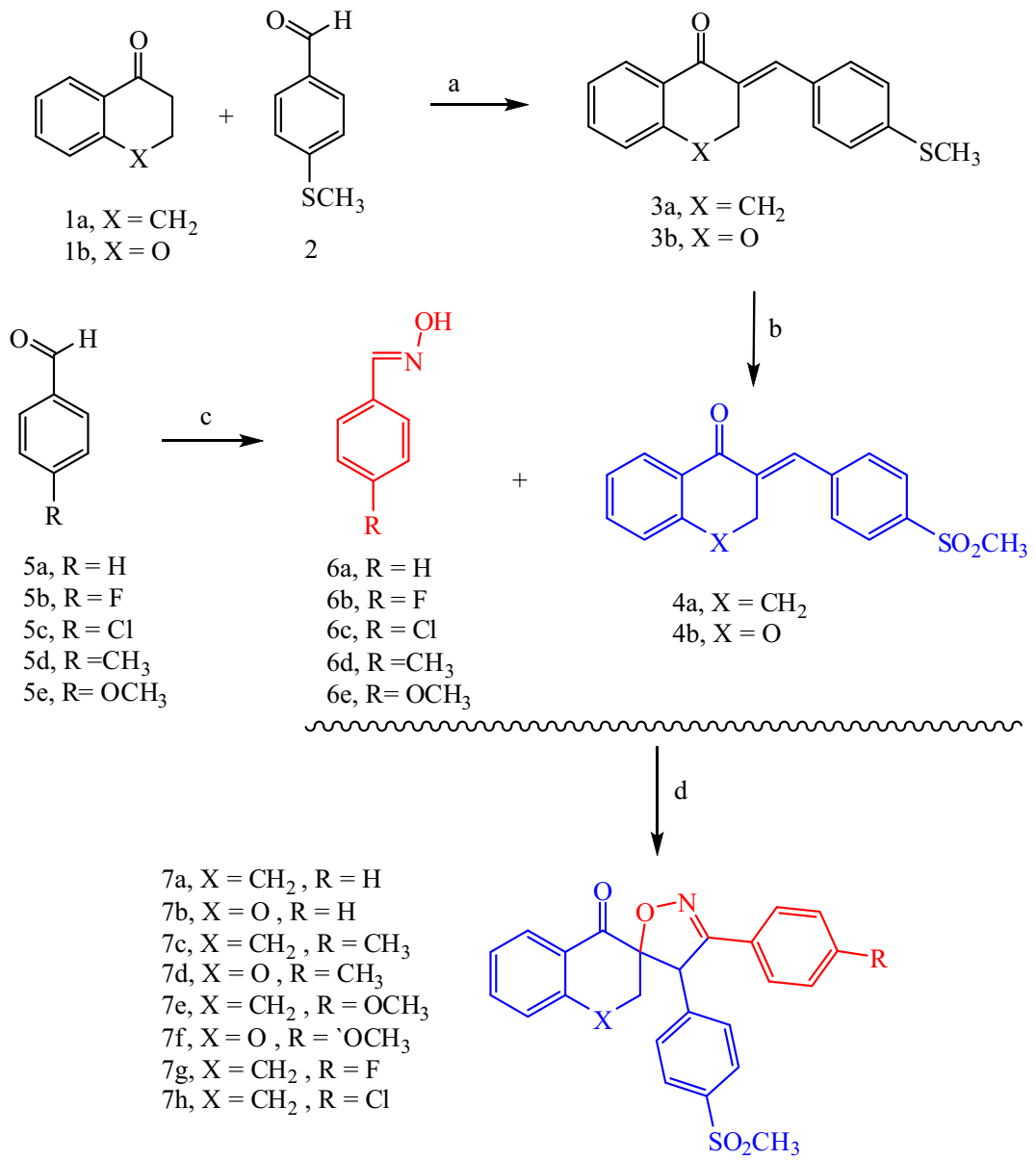
(E)-4-methoxybenzaldehyde oxime) 4-methoxy benzaldoxime) ***(6e)*** Yield: 65 %; cream crystalline powder; mp: 55–57 °C; IR (KBr): ν (cm^{-1}) 1682 (C=N), 3228 (O–H), 1170 (C–O), 950 (N–O); LC–MS (ESI) m/z: 152.3 (M + 1).

General Procedure for Preparation of Spiroisoxazoline Compounds (7a-h)

A mixture of a dipolarophile (**4a** or **4b**; 1 mmol) and *p*-substituted-benzaldoxime (**6a–e**; 2.5 mmol) was dissolved in chloroform (15 ml) at cold temperature (−10 °C) that placed in an ice-salt bath. To this mixture was added 2 ml of sodium hypochlorite (5 %) that diluted in 5 ml water, drop ways over 20 min. The temperature was controlled and has not to excess (−5 °C) along addition of sodium hypochlorite. After complete addition of NaOCl, the mixture was stirred 1–2 h at room temperature. Compounds were extracted by chloroform, washed several times with water, and dried on anhydrous sodium sulfate. White solid obtained after evaporation of the solvent and crystallization in methanol.

Yield: (32–56 %). The physical and spectral data for **7a–h** are listed below.

4-(4-(Methylsulfonyl)phenyl)-3-phenyl-3′,4′-dihydro-1′H,4H-spiro[isoxazole-5,2′-naphthalen]-1′-one ***(7a)*** Yield: 37 %; white crystalline powder; mp: 130–132 °C; IR (KBr): ν (cm^{-1}) 1686 (C=O), 1310, 1153 (SO_2); LC–MS (ESI) m/z: 432.2 (M + 1), 454.1 (M + 23); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 1.79–1.93 (m, 2H, CH_2, naphtalenon $H_{4'}$), 2.82–2.87 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.36–3.41 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.09 (s, 3H, SO_2CH_3), 5.67 (s, 1H, isoxazolin H_4), 7.27–7.44 (m, 7H, phenyl H_3 & H_4 & H_5, 4-methylsulfonylphenyl H_2 & H_6, naphtalenon $H_{5'}$ & $H_{7'}$), 7.54–7.57 (t, 1H, naphtalenon $H_{6'}$), 7.62–7.64 (d, J = 8.5 Hz, 2H, phenyl H_2 & H_6), 7.95–7.97 (d, J = 8.1 Hz, 2H, 4-methylsulfonylphenyl H_3& H_5), 8.11–8.13 (d, J = 7.7 Hz, 1H, naphtalenon $H_{8'}$); ^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 24.40, 28.91 (CH_2-CH_2), 43.13 (SO_2CH_3), 54.36 (CH), 88.01 (Spiro $C^{2',5}$), 123.96, 126.11,

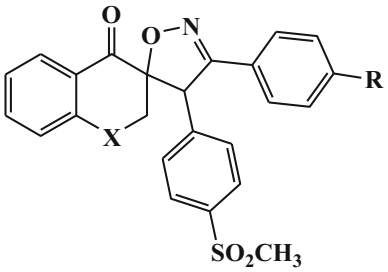


Scheme 1 Synthesis of spiroheterocycles **7a**–**h**. Reagents: **a** NaOH, CH_3OH, stir, r.t; **b** Oxone, THF,H_2O, stir, r.t.; **c** NH_2OHCl, NaOH aq 10 %, Ethanol, Reflux; **d** NaOCl 5 %, H_2O, $CHCl_3$, stir, −10 to 0 °C

126.13, 126.45, 127.10, 127.74, 127.88, 128.24, 128.54,, 129.22, 133.21, 139.29, 139.81, 140.19 (Aromatic C), 158.67 (C=N), 190.05 (C=O). Anal. Calcd for $C_{25}H_{21}NO_4S$: C, 69.59; H, 4.91; N, 3.25. Found: C, 69.71; H, 5.02; N, 3.01.

*4′-(4-(Methylsulfonyl)phenyl)3′-phenyl-4′H-spiro[chroman-3,5′-isoxazol]-4-one (**7b**)* Yield: 35 %;white crystalline powder; mp: 168–170 °C; IR (KBr): ν (cm^{-1}) 1709 (C=O), 1331, 1171 (SO_2); LC–MS (ESI) m/z: 433.8 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 3.12 (s, 3H, SO_2CH_3), 4.01–4.03 (d, J = 12.7 Hz, 1H, CH_2, chromanon H_2), 4.23–4.26 (d, J = 12.7 Hz, 1H, CH_2, chromanon H_2), 5.53 (s, 1H, isoxazolin $H_{4'}$), 7.02–7.03 (d, 1H, chromanon H_8), 7.14–7.17 (t, 1H, chromanon H_6), 7.33–7.47 (m, 5H, phenyl H_3 & H_4 & H_5 & 4-methylsulfonylphenyl H_2& H_6), 7.58–7.62 (m, 3H, phenyl H_2 & H_6 &chromanon H_7), 7.99–8.00 (d, J = 8 Hz, 2H, 4-methylsulfonylphenyl H $_3$& H_5), 8.01–8.03 (d, J = 7.5 Hz, 1H, chromanon H_5);^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 43.55 (SO_2CH_3), 54.66 (CH), 60.9 (CH_2), 89.88 (Spiro $C^{2',5}$), 115.56, 126.31, 126.53, 126.88, 127.60, 127.84, 128.08, 128.34, 128.66, 130.12, 135.44, 142.11, 143.21, 146.24 (Aromatic C), 160.17 (C=N), 191.10 (C=O). Anal. Calcd for $C_{24}H_{19}NO_5S$: C, 66.50; H, 4.42; N, 3.23. Found: C, 66.71; H, 4.63; N, 3.39.

Table 4 Structure and characteristics of the synthesized compounds **7a–7h**



Compound	X	*R*	Empirical formula	MW	mp (°C)	Yield (%)	Color
7a	CH_2	H	$C_{25}H_{21}NO_4S$	431	130–132	37	White
7b	O	H	$C_{24}H_{19}NO_5S$	433	168–170	35	White
7c	CH_2	CH_3	$C_{26}H_{23}NO_4S$	445	140–142	41	White
7d	O	CH_3	$C_{25}H_{21}NO_5S$	447	138–140	38	White
7e	CH_2	OCH_3	$C_{26}H_{23}NO_5S$	461	150–152	50	White
7f	O	OCH_3	$C_{25}H_{21}NO_6S$	463	140–141	48	White
7g	CH_2	F	$C_{25}H_{20}FNO_4S$	449	122–123	32	White
7h	CH_2	Cl	$C_{25}H_{20}ClNO_4S$	465	171–173	54	White

*4-(4-(Methylsulfonyl)phenyl)-3-p-tolyl-3′,4′-dihydro-1′H,4H-spiro[isoxazole-5,2′-naphthalen]-1′-one (**7c**)* Yield: 41 %; white crystalline powder; mp: 140–142 °C; IR (KBr): ν (cm^{-1}) 1697 (C=O), 1320, 1162 (SO_2); LC–MS (ESI) m/z: 445.8 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 1.78–1.92 (m, 2H, CH_2, naphtalenon $H_{4'}$), 2.35 (s, 3H, CH_3, 4-methylphenyl), 2.81–2.86 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.35–3.42 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.09 (s, 3H, SO_2CH_3), 5.65 (s, 1H, isoxazolin H_4), 7.13–7.15 (d, J = 8.1 Hz, 2H, 4-methylphenyl H_3 & H_5), 7.26–7.43 (m, 4H, 4-methylsulfonylphenyl H_2& H_6, naphtalenon $H_{5'}$ & $H_{7'}$), 7.51–7.53 (d, J = 8.2 Hz, 2H, 4-methylphenyl H_2 & H_6), 7.54–7.57 (t, 1H, naphtalenon $H_{6'}$), 7.94–7.96 (d, J = 8.2 Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5), 8.11–8.13 (d, J = 8 Hz, 1H, naphtalenon $H_{8'}$); ^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 20.51 (CH_3), 24.38, 28.85 (CH_2-CH_2), 43.33 (SO_2CH_3), 54.16 (CH), 87.81 (Spiro $C^{2',5}$), 124.06, 126.13, 126.47, 127.17, 127.55, 127.85, 128.32, 128.47, 128.66, 129.19, 133.16, 139.78, 140.22, 142.33 (Aromatic C), 158.49 (C=N), 189.97 (C=O). Anal. Calcd for $C_{26}H_{23}NO_4S$: C, 70.09; H, 5.20; N, 3.14. Found: C, 70.21; H, 5.43; N, 2.99.

*4′-(4-(Methylsulfonyl)phenyl)3′-p-methylphenyl-4′H-spiro[chroman-3,5′-isoxazol]-4-one (**7d**)* Yield: 38 %;white crystalline powder; mp: 138–140 °C; IR (KBr): ν (cm^{-1}) 1677 (C=O), 1300, 1142 (SO_2); LC–MS (ESI) m/z: 448.1 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 2.36 (s, 3H, CH_3, 4-methylphenyl), 3.11 (s, 3H, SO_2CH_3), 4.00–4.02 (d, J = 12.7 Hz, 1H, CH_2, chromanon H_2), 4.22–4.25 (d, J = 12.7 Hz, 1H, CH_2, chromanon H_2), 5.51 (s, 1H, isoxazolin $H_{4'}$), 7.01–7.02 (d, 1H, chromanon H_8), 7.13–7.16 (m, 3H, chromanon H_6& 4-methylphenyl H_3 & H_5), 7.42–7.49 (m, 4H, 4-methylphenyl H_2 & H_6 & 4-methylsulfonylphenyl H_2& H_6), 7.57–7.61 (t, 1H, chromanon H_7), 7.97–7.99 (d, J = 8.2 Hz, 2H, 4-methylsulfonylphenyl H_3& H_5), 8.01–8.03 (d, J = 7.9 Hz, 1H, chromanon H_5); ^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 21.11 (CH_3), 44.05 (SO_2CH_3), 54.46 (CH), 60.2 (CH_2), 88.24 (Spiro $C^{2',5}$), 115.26, 126.41, 126.33, 126.77, 127.55, 127.91, 128.33, 128.48, 129.44, 129.92, 135.14, 141.33, 142.31, 145.51 (Aromatic C), 159.10 (C=N), 190.25 (C=O). Anal. Calcd. for $C_{25}H_{21}NO_5S$: C, 67.10; H, 4.73; N, 3.13. Found: C, 67.26; H, 4.51; N, 3.15.

*3-(4-Methoxyphenyl)-4-(4-(methylsulfonyl)phenyl)-3′,4′-dihydro-1′H,4H-spiro[isoxazole-5,2′-naphthalen]-1′-one (**7e**)* Yield: 50 %; white crystalline powder; mp: 150–152 °C; IR (KBr): ν (cm^{-1}) 1688 (C=O), 1304, 1148 (SO_2); LC–MS (ESI) m/z: 462.1 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 1.79–1.92 (m, 2H, CH_2, naphtalenon $H_{4'}$), 2.82–2.87 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.36–3.42 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.1 (s, 3H, SO_2CH_3), 3.82 (s, 3H, OCH_3, 4-methoxyphenyl), 5.63 (s, 1H, isoxazolin H_4), 6.85–6.88 (d, J = 8.8 Hz, 2H, 4-methoxylphenyl H_3 & H_5), 7.27–7.44 (m, 4H, 4-methylsulfonylphenyl H_2& H_6, naphtalenon $H_{5'}$ & $H_{7'}$), 7.57–7.58 (d, J = 8.8 Hz, 2H, 4-methoxylphenyl H_2 & H_6), 7.54–7.57 (t, 1H, naphtalenon

$H_{6'}$), 7.96–7.97 (d, J = 8.0 Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5), 8.12–8.13 (d, J = 7.8 Hz, 1H, naphtalenon $H_{8'}$);^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 24.39, 28.85 (CH_2-CH_2), 43.32 (SO_2CH_3), 54.20 (CH), 54.29 (OCH_3), 87.67 (Spiro $C^{2',5}$), 113.11, 119.37, 126.02, 126.96, 127.15, 127.63, 127.83, 129.20, 129.42, 133.12, 139.33, 140.29, 142.34, 158.15 (Aromatic C), 160.25 (C=N), 190.04 (C=O). Anal. Calcd. for $C_{26}H_{23}NO_5S$: C, 67.66; H, 5.02; N, 3.03. Found: C, 67.90; H, 5.23; N, 3.21.

*4′-(4-(Methylsulfonyl)phenyl)3′-p-methoxyphenyl-4′H-spiro [chroman-3,5′-isoxazol]-4-one (**7f**)* Yield: 48 %; white crystalline powder; mp: 140–142 °C; IR (KBr): ν (cm^{-1}) 1693 (C=O), 1308, 1150 (SO_2); LC–MS (ESI) m/z: 463.7 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δ ppm 3.11 (s, 3H, SO_2CH_3), 3.82 (s, 3H, OCH_3, 4-methoxyphenyl), 4.00–4.02 (d, J = 12.7 Hz, 1H, chromanon H_2), 4.22–4.24 (d, J = 12.7 Hz, 1H, chromanon H_2), 5.48 (s, 1H, isoxazolin $H_{4'}$), 6.84–6.86 (d, 2H, 4-methoxyphenyl H_3 & H_5), 7.01–7.02 (d, J = 8 Hz, 1H, chromanon H_8), 7.13–7.16 (t, 1H, chromanon H_6), 7.47–7.52 (d, 2H, J = 8 Hz, 4-methylsulfonylphenyl H_2 & H_6), 7.52–7.54 (d, J = 8.9 Hz, 2H, 4-methoxyphenyl H_2 & H_6), 7.57–7.61 (t, 1H, chromanon H_7), 7.98–8.00 (d, J = 8 Hz, 2H, 4-methylsulfonylphenyl H_3 & H_5), 8.01–8.03 (d, J = 7.9 Hz, 1H, chromanon H_5); ^{13}CNMR ($CDCl_3$, 125 MHz): δ ppm 44.05 (SO_2CH_3), 54.39 (CH), 54.61 (CH_3), 59.86.2 (CH_2), 88.55 (Spiro $C^{2',5}$), 114.87, 126.31, 126.55, 126.95, 127.75, 128.12, 128.43, 128.85, 129.33, 129.88, 135.44, 141.56, 142.51, 144.88 (Aromatic C), 158.92 (C=N), 191.05 (C=O). Anal. Calcd. for $C_{25}H_{21}NO_6S$: C, 64.78; H, 4.57; N, 3.02. Found: C, 64.99; H, 4.65; N, 3.22.

*3-(4-Fluorophenyl)-4-(4-(methylsulfonyl)phenyl)-3′,4′-dihydro-1′H,4H-spiro[isoxazole-5,2′-naphthalen]-1′-one (**7g**)* Yield: 32 %; white crystalline powder; mp: 122–124 °C; IR (KBr): ν (cm^{-1}) 1685 (C=O), 1317, 1152 (SO_2); LC–MS (ESI) m/z: 449.8 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δppm 1.80–1.93 (m, 2H, CH_2, naphtalenon $H_{4'}$), 2.83–2.88 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.37–3.43 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.11 (s, 3H, SO_2CH_3), 5.64 (s, 1H, isoxazolin H_4), 7.03–7.09 (t, 2H, 4-fluorophenyl H_3 &H_5), 7.28–7.51 (m, 4H, 4-methylsulfonylphenyl H_2& H_6, naphtalenon $H_{5'}$ & $H_{7'}$), 7.56–7.59 (t, 1H, naphtalenon $H_{6'}$), 7.62–7.65 (m, 2H, 4-fluorophenyl H_2 & H_6), 7.98–7.99 (d, J = 8.1 Hz, 2H, 4-methylsulfonylphenyl H_3& H_5), 8.12-8.14 (d, J = 6.9 Hz, 1H, naphtalenon $H_{8'}$); ^{13}CNMR ($CDCl_3$, 100 MHz): δ ppm 24.33, 28.79 (CH_2–CH_2), 43.29 (SO_2CH_3), 53.92 (CH), 88.34 (Spiro $C^{2,5'}$), 125.46, 126.05, 127.21, 127.62, 127.74, 127.84, 128.12, 129.21, 129.28, 133.22, 135.40, 139.47, 139.71, 142.36 (Aromatic C), 157.71 (C=N), 189.97 (C=O). Anal. Calcd. for $C_{25}H_{20}FNO_4S$: C, 66.80; H, 4.48; N, 3.12. Found: C, 66.61; H, 4.27; N, 3.25.

*4-(4-(Methylsulfonyl) phenyl)-3-p-chlorophenyl-3′, 4′-dihydro-1′H, 4H-spiro [isoxazole-5, 2′-naphthalen]-1′-one (**7h**)* Yield: 54 %; white crystalline powder; mp: 171–173 °C; IR (KBr): ν (cm^{-1}) 1690 (C=O), 1315, 1160 (SO_2); LC–MS (ESI) m/z: 465.8 (M + 1); ^{1}HNMR ($CDCl_3$, 500 MHz): δppm 1.80–1.93 (m, 2H, CH_2, naphtalenon $H_{4'}$), 2.83–2.88 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.36–3.43 (m, 1H, CH_2, naphtalenon $H_{3'}$), 3.11 (s, 3H, SO_2CH_3), 5.64 (s, 1H, isoxazolin H_4), 7.28–7.29 (d, J = 8.6 Hz, 2H, 4-chlorophenyl H_3 &H_5), 7.28–7.49 (m, 4H, 4-methylsulfonylphenyl H_2& H_6, naphtalenon $H_{5'}$ & $H_{7'}$), 7.56–7.59 (t, 1H, naphtalenon $H_{6'}$), 7.56–7.58 (d, J = 8.6 Hz, 2H, 4-chlorophenyl H_2 & H_6), 7.97–7.99 (d, J = 8.1 Hz, 2H, 4-methylsulfonylphenyl H_3& H_5), 8.12–8.13 (d, J = 8 Hz, 1H, naphtalenon $H_{8'}$); $_{13}$CNMR ($CDCl_3$, 100 MHz): δ ppm 24.31, 28.78 (CH_2–CH_2), 43.32 (SO_2CH_3), 53.89 (CH), 88.27 (Spiro $C^{2,5'}$), 125.40, 126.11, 127.31, 127.70, 127.71, 127.86, 128.09, 129.13, 129.19, 133.32, 135.46, 139.50, 139.66, 142.30 (Aromatic C), 157.63 (C=N), 189.75 (C=O). Anal. Calcd. for $C_{25}H_{20}ClNO_4S$: C, 64.44; H, 4.33; N, 3.01. Found: C, 64.59; H, 4.45; N, 3.20.

Crystal structure determination

Crystal structure determination of compound **7h** was carried out by single-crystal X-ray diffraction method. The crystal data, data collection, and refinement parameter for the compound **7h** is listed in Table 1.

Molecular modeling (docking) studies

3D structures of the synthesized compounds were generated using the Hyper Chem (version 7.0). The initial structures were first minimized using molecular mechanics MM + force field (Allinger1977). Then, those structures were fully optimized based on the semi-empirical quantum mechanics AM1 method, available in HyperChem (Dewar and Thiel 1977). The output structures were converted to SYBYL Cartesian coordinate files (mol2 file format) using Open Babel program (version 2.3.2) in order to be used as an acceptable format in GOLD program (version 5.0) with the aim of docking (Jones *et al.,* 1995, Morris *et al.,* 1998). Flexible docking of all synthesized compounds was carried out using GOLD program running under Linux OS. The crystal structure of SC-558 (PDB code: 1CX2) was obtained from Protein Data Bank at RCSB (http://www.rcsb.org/pdb/home/home.do). The protein structure was

prepared for docking using GOLD program. Docking was performed by applying a point assigned based on the important residues involved in the binding. All atoms within a 10Å radius were selected, and flexible docking was carried out. Chem PLP method of GOLD suite was selected as the scoring function. The binding cavity was determined based on the binding location of SC-558 co-crystallized with cyclooxygenase-2, and then SC-558 molecule was removed and the synthesized compounds were docked. The interactions between ligands and COX-2 have been visualized via the 3D representation using PyMOL (v0.99) programs. The results from docking study using GOLD program indicates specific interactions. Superimposition of the Sc-558 and the synthesized compounds once bound into the COX-2 binding site has been illustrated in Fig. 4 in surface and cartoon representations.

In vitro cyclooxygenase (COX) inhibition assays

The ability of the test compounds listed in Table 3 to inhibit ovine COX-1 and COX-2 (IC50 value, lM) was determined using chemiluminescent enzyme assays kit (Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method (Zarghi *et al.,* 2007a, b).

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