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Design, synthesis, and biological evaluation of 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) inhibitors

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Abstract—A series of 20 novel 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines were designed, synthesized, and screened in vitro for anti-inflammatory activity. These compounds were designed for evaluation as dual inhibitors of cyclooxygenases (COX-1 and COX-2) and lipoxygenases (LOX-5, LOX-12, and LOX-15) that are responsible for inflammation and pain. All pyrazoline molecules prepared are optically active and compounds that are more potent in COX-2 inhibitory activity (**5a** and **5f**) were resolved by chiral column and each enantiomer was tested for cyclooxygenase inhibitory activity. Molecular modeling and comparison of molecular models of **5a** enantiomers with that of celecoxib model shows that **5a** (enantiomer-1) and **5a** (enantiomer-2) have more hydrogen bonding interactions in the catalytic domain of COX-2 enzyme than celecoxib. Compounds **5a**, **5e**, and **5f** showed moderate to good LOX-5 and LOX-15 inhibitory activity and this is comparable to that of celecoxib and more potent than rofecoxib.

1. Introduction

Cyclooxygenases (COXs) are key enzymes in the synthesis of prostaglandin H_2 which is a precursor for the biosynthesis of prostaglandins, thromboxanes, and prostacyclins.[1] COX enzymes exist in two isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2).[2] The COX-1 enzyme is constitutively expressed and is critical for protection of gastric mucosa, platelet aggregation, and renal blood flow whereas the COX-2 enzyme is inducible and expressed during inflammation, pain and oncogenesis.[3] Since COX-2 is involved in inflammation and pain, molecules that inhibit it's enzymatic activity would be of therapeutic value. Many non-steroidal anti-inflammatory drugs (NSAIDs) were found to interact with these enzymes and inhibit their enzymatic activity.[4] These molecules include aspirin and indomethacin which are non-selective and inhibit both COX-1 and COX-2. Aspirin inhibits COX-1 more strongly than COX-2[4] and inhibition of COX-1 by aspirin reduces the production of PGE_2 and PGI_2 which has an adverse ulcerogenic effect.[5]

Recently several new inhibitors were developed[6–8] which selectively inhibit the COX-2 enzyme without interfering with COX-1 enzymatic activity. These molecules include celecoxib,[9] rofecoxib,[10] and valdecoxib,[11] all of which inhibit COX-2 without the side effects observed with traditional NSAIDs. These selective inhibitors take advantage of the larger enzymatic pocket in the COX-2 active site where valine at amino acid position 523 has a smaller side chain that accommodates the sulfur containing side chains of the inhibitors, whereas the isoleucine residue at position 523 in COX-1 has a larger side chain that prevents docking of the inhibitor at the active site.[12] This preferential binding of selective inhibitors to the COX-2 enzyme over COX-1 enzyme prevents the side effects as seen in non-selective inhibitors.[13]

Aspirin is a unique NSAID that interacts with both cyclooxygenases but inhibits COX-1 10- to 100-fold

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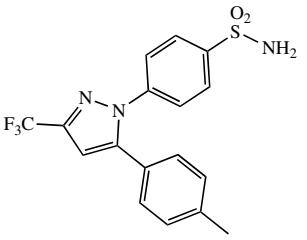
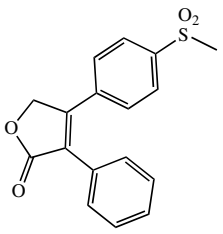
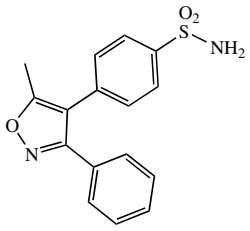


celecoxib



rofecoxib



valdecoxib

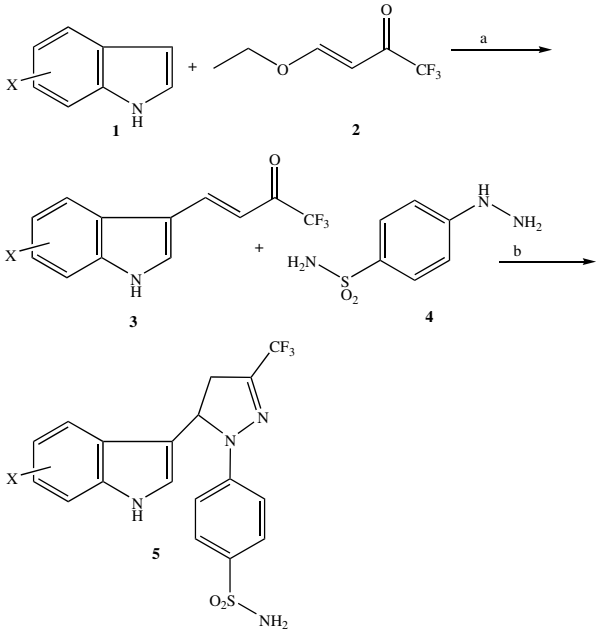
more effectively than the COX-2 enzyme.[14] Aspirin's inhibitory activity is due to its ability to acetylate serine residues positioned at 530 and 516 in COX-1 and COX-2, respectively.[15,16] Acetylation of these residues prevents the positioning of arachidonic acid to its binding site, thereby blocking the substrate to the active site of oxygenation. Because of its dual inhibitory activity, some of aspirin's therapeutic advantages can be attributed to its ability to reduce inflammation by acetylating COX-2 and prevent platelet aggregation and antithrombosis by acetylating COX-1.[17] Recent studies[18] on selective COX-2 inhibitors revealed that patients with heart disease are more prone to myocardial infarction and this may be due to the TxA_2/PGI_2 imbalance created by selective COX-2 inhibitors.[19] Recently, Kalgutkar et al.[20,21] have synthesized novel aspirin like analogs with variations at acyl group, alkyl group, aryl substitution pattern, and heteroatom substitution and discovered a lead molecule that selectively acetylated, and irreversibly inactivated, COX-2.

Lipoxygenases (LOXs) belong to a class of non-heme iron-containing enzymes which catalyze the hydroperoxidation reaction of fatty acids to peroxides. Lipoxygenases exist in three isoforms, lipoxygenase-5, lipoxygenase-12, and lipoxygenase-15. Of the three isoforms, LOX-5 and LOX-15 have been implicated in several undesirable physiological effects. LOX-5 has been shown to be involved in the production of leukotrienes which are known to contribute to the progression of osteoarthritis, asthma, and inflammation.[22,23] Lipoxygenase-15 has been implicated in the oxidation of low-density lipoprotein (LDL), which ultimately causes atherosclerosis.[24] Licofelone (ML-3000), which is a dual COX/LOX-5 inhibitor, is a potent anti-inflammatory agent without gastrointestinal side effects and demonstrating platelet inhibitory and anti-thrombotic effect.[25] It clearly shows that a dual inhibitor of the COX/LOX enzymatic pathways offers a better alternate approach in designing a new drug with excellent safety profile and least side effects.

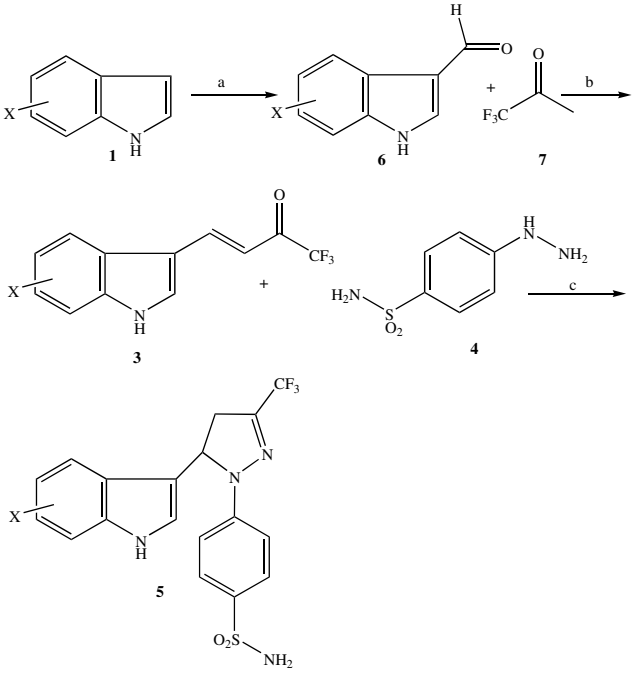
In this paper, we describe the synthesis and biological evaluation of a novel class of 5-(3-indolyl)-1-(4-sulfamylphenyl)-3-trifluoromethyl pyrazolines as dual inhibitors of COXs and LOXs.

2. Chemistry

The synthesis of 5-(3-indolyl)-1-(4-sulfamylphenyl)-3-trifluoromethyl pyrazolines is outlined in Schemes 1 and 2.



Scheme 1. Reagents and conditions: (a) $ZnCl_2/CH_2Cl_2$, 3 h; (b) ethanol, reflux 8 h.



Scheme 2. Reagents and conditions: (a) $POCl_3$, DMF; (b) piperidine-AcOH, THF, 3 h; (c) ethanol, reflux 8 h.

The starting material for the synthesis of substituted pyrazolenes **5** in Scheme 1 is (*E*)-1,1,1-trifluoro-4-(1*H*-indol-3-yl)-but-3-en-2-one **3**. Treatment of substituted indoles **1** with 4-ethoxy-1,1,1-trifluoro-3-buten-2-one **2** in the presence of zinc chloride in dichloromethane produced **3** in quantitative yield. The NMR spectra of **3** revealed that the vinylic fragments are in *trans* configuration. Condensation of **3** with 4-sulfamylphenylhydrazine hydrochloride **4** in ethanol gave 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines **5** in quantitative yields.

Because trifluoroacetylvinylation of methoxy, amino, carboxy, and nitro substituted indoles was unsuccessful with **2**, alternate method was developed to synthesize 1,1,1-trifluoro-4-(3-substituted indolyl)-3-buten-2-ones. In this method, amino, nitro, carboxy, and methoxy substituted indole-3-carboxaldehydes **6** were treated with 1,1,1-trifluoroacetone **7** in tetrahydrofuran in presence of catalytic amounts of piperidine and acetic acid to give trans 1,1,1-trifluoro-4-(3-substituted indolyl)-3-buten-2-ones **3**. Condensation addition of 4-sulfamylphenylhydrazine hydrochloride to **3** produced 1-(4-sulfamylphenyl)-3-trifluoromethyl-5-indolyl pyrazolines **5** (Scheme 2).

Because all these molecules (**5**) have a chiral center at 5th position, they exist as optical isomers. Two of the most active molecules were resolved by preparative HPLC passing through a chiral column. The optical rotation of the separated enantiomers was determined on a polarimeter (Table 3). Usually the hydrogen on the chiral carbon atom has interaction with the hydrogens adjacent to the chiral center. If the carbon adjacent to the chiral center has one hydrogen, then the resulting two enantiomers will have these two hydrogen atoms in *cis* or *trans* position and can easily be distinguished by NMR spectral analysis. But in this case, the carbon atom next to the chiral center has two hydrogens and would not be distinguished as *cis* and *trans* forms and could not be distinguished by ^{1}H NMR spectral data (Table 4).

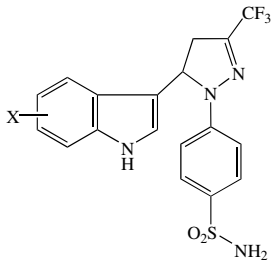
3. Results and discussion

We have synthesized a series of 5-(3-indolyl)-1-(4-sulfamylphenyl)-3-trifluoromethyl pyrazolines (**5**) and evaluated their ability to inhibit COX-1, COX-2, LOX-5, LOX-12, and LOX-15 enzymes in vitro using recombinant enzymes. IC_{50} values for inhibition of ovine COX-1 and COX-2 enzymes by these compounds were determined by an Enzyme Immuno Assay (Table 1).

Inhibition of human LOX-5 from human PBML cells, LOX-12 from human platelets, and LOX-15 from rabbit reticulocytes were determined by EIA and spectrophotometric quantitation (Table 2).

A comparison of the SAR data for the 5-(3-indolyl)-1-(4-sulfamylphenyl)-3-trifluoromethyl pyrazolines (**5a–5s**) against COX-1 and COX-2 enzyme inhibition showed that the presence of a polar group such as nitro, carboxy, and cyano moiety on the indole ring totally inactivates the molecule. When the nitro group at 7th position in **5r** is replaced by an amino group (**5s**) or by a chlorine atom (**5f**) the molecules show significant improvement in COX-2 inhibition. Similarly when 5-nitro group in the indole moiety of **5p** is converted to 5-amino (**5i**), the molecule became highly potent and showed excellent inhibition of COX-2 enzyme with out affecting the activity of COX-1. Comparison of **5b** and **5c** showed that smaller halogen atom at 5th position on the indole ring enhances COX-2 enzyme inhibition activity, whereas the presence of a larger bromine atom at 6th position of the ring improves the inhibitory activ-

Table 1. In vitro COX-1 and COX-2 enzyme inhibition assay data for the pyrazolines **5**



Compound	X	IC_{50}[a] (μ)		Selectivity index[b]
		COX-1	COX-2	
5a	H	>100	1.4	>72
5b	5-F	>100	13.5	>8
5c	5-Cl	>100	38.7	>3
5d	6-F	>100	>100	0
5e	6-Cl	>100	>100	0
5f	7-Cl	>100	3.9	>26
5g	5-CN	>100	>100	0
5h	6-Br	>100	4.4	>23
5i	5-NH_2	>100	0.85	>118
5j	4-NH_2	>100	>100	0
5k	6-CN	>100	>100	0
5l	6-NO_2	>100	>100	0
5m	2-CH_3, 6-Cl	>100	6	>17
5n	4-OCH_3	>100	8.7	12
5o	6-NH_2	>100	7.5	14
5p	5-NO_2	>100	57.2	2
5q	5-COOH	>100	>100	0
5r	7-NO_2	>100	>100	0
5s	7-NH_2	>100	2.4	>42
8	H	>100	>100	0
Celecoxib		>100	1.71	>60

[a] IC_{50} are means of two determinations.
[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 2. In vitro LOX-5, LOX-12, and LOX-15 enzyme inhibition assay data for **5**

Compound	% Inhibition (10 μM)		
	LOX-5	LOX-12	LOX-15
5a	41	5	31
5e	51	−2	36
5f	94	3	36
Celecoxib	89	7	22
Rofecoxib	11	−2	6

ity (**5d**, **5e**, and **5h**) of the compound. Also it is interesting to compare **5i**, **5j**, **5o**, and **5s,** which reveals that the amino group at any position on the ring except at 4th (**5j**) renders the molecules quite active.

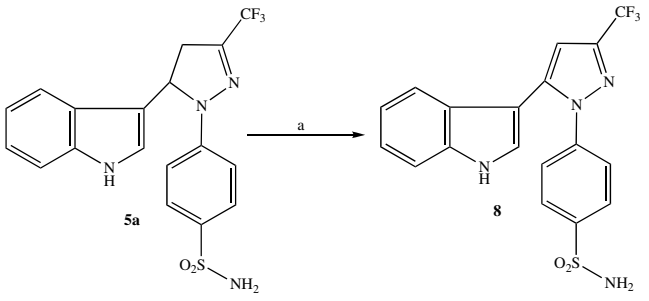
In this series of compounds, the pyrazoline ring has a chiral center on the 5th carbon atom and exists as optical isomers. In order to evaluate the activity of the individual enantiomers, we have taken the racemic mixtures of **5a** and **5f** and subjected them to chiral separation on a high throughput HPLC system using a Chiralpak AD 250X4.6 mm column. Both enantiomers were isolated and their optical rotations $[\alpha]_D$ were calculated. The separated optically pure isomers (**5a**–**A** and **5f**–**A** levorotatory-isomers, **5a**–**B** and **5f**–**B** dextrorotatory-isomers) were then analyzed for COX-1 and COX-2 enzyme inhibitory activity. In both cases, the *l*-isomers exhibited an increased potency of several folds than their corresponding racemic mixtures (Table 3). These data clearly show that a pure single enantiomer may have a greater binding affinity toward the catalytic domain of the enzyme than the racemic mixture.

To understand the role of chiral center and its significance in contribution of COX-2 inhibitory activity in these pyrazolines, **5a** is aromatized to pyrazole (**8**) (Scheme 3) which led to the loss of chiral center. Analysis of the compound **8** in COX-1 and COX-2 in an in vitro enzyme inhibition assay showed that aromatization of pyrazoline **5a** to pyrazole **8** inactivated the molecule thus preventing COX-2 inhibition (Table 1).

In vitro enzymatic assays using LOX-5, LOX-12, and LOX-15 studies (Table 2) with **5a**, **5e**, and **5f** showed that these molecules have a moderate to good activity toward LOX-5 and LOX-15 and very low activity toward LOX-12. The LOX enzyme inhibition of these compounds is comparable to celecoxib and better than rofecoxib (Table 2).

Table 3. Optical activity and enzyme inhibition of **5a–A**, **5a–B**, **5f–A**, and **5f–B**

Compound	$[\alpha]_D$ (degrees)	IC_{50} (μM)		Selectivity index
		COX-1	COX-2	
5a–A	−94.690	>100	0.85	>119
5a–B	+91.640	>100	12.5	>8
5f–A	−70.769	>100	0.29	>345
5f–B	−70.816	>100	84	>1



Scheme 3. Reagents and conditions: (a) 5% bromine-water, rt, 8 h.

A molecular modeling study was performed where the most selective COX-2 inhibitor **5a** was docked in the binding site of the COX-2 isoenzyme (Fig. 1). All molecular modeling was performed on a Silicon Graphics Personal IRIS 4D/25 workstation. X-ray crystal structures for COX-1 and COX-2 described by Loll and coworkers[26] and Kurumbail and coworkers,[27], respectively, were obtained from the Protein Data Bank. All calculations were performed using the DREIDING II all atom force field and biograf® software (BIOSYM/Molecular Simulations, San Diego, CA). Modified crystal structures for COX-1 and COX-2 were obtained by the addition of all heterogeneous hydrogen atoms in optimized positions according to the biograf® software protocols.

Models of celecoxib and **5a** and its enantiomers (Fig. 1) were constructed using the organic builder contained within the main biograf® program and were individually docked into the active site of cyclooxygenase-2 by overlaying the structure of each analog with the structure of indomethacin derived from the original crystal structure. Modeling calculations were performed only allowing for the movement of each analog within the active site, while the atomic coordinates of COX-2 were held constant. The calculations involved energy minimization to convergence, limited molecular dynamics calculations (5 ps), followed again by energy minimization to convergence. By overlaying the crystal structure of COX-1 with COX-2, the models of the analog:cyclooxygenase complexes could be evaluated for binding efficiency to COX-2 by evaluating hydrogen bonding interactions and for a lack of binding efficiency to COX-1 as a result of the steric hindrance in COX-1 due to the presence of the longer side chain of Ile 523 compared to Val 523 in COX-2. All inhibitors examined were sterically selective for COX-2 over COX-1. As an example, the presence of Arg 513 and Val 523 in COX-2 do not inhibit the binding of analog **5a** (Fig. 1A), while the presence of His 513 and the longer side chain of Ile 523 in COX-1 resulted in increased van der Waals interactions accounting for the steric inhibition of the binding of **5a** to COX-1 (Fig. 1B).

The protein main chain for COX-2 (Fig. 1A) and COX-1 (Fig. 1B) is shown in light blue. Space filling models of **5a** and residues involved in isotype selectivity are shown in red and blue, respectively.

Examination of the molecular models for each analog docked into COX-2 established a rubric for assessing the binding efficiency of COX-2 specific analogs based on the number and type of the possible hydrogen bonding interactions suggested by the models (Table 5). The data shown in Table 5 were constructed by analyzing active site snapshots of molecular models of analog:COX-2 complexes, similar to those shown in Figure 2. Note the absence of hydrogen bonding interactions to His 90 and Q192 when celecoxib was docked with COX-2 (Fig. 2A) compared to the complex containing **5a** (enantiomer1) docked with COX-2 (Fig. 2B).

The main chain atoms of the COX-2 protein are shown in blue. Residues previously demonstrated to be in-

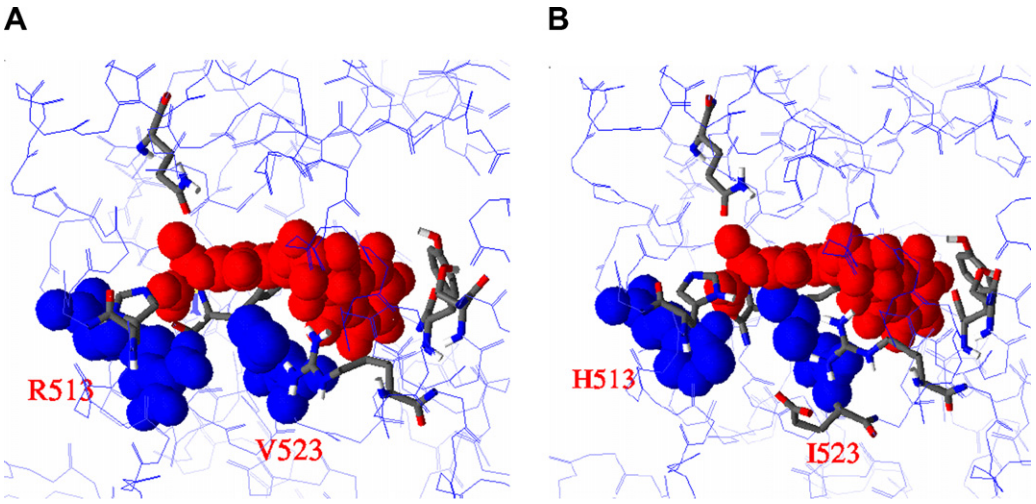


Figure 1. Steric selectivity of COX-2 analogs.

Table 4. ^{1}H NMR data of **5a–A**, **5a–B**, **5f–A**, and **5f–B**

Compound	NMR data ($CDCl_3$)
5a–A	δ 3.21(dd, J = 18.0, 6.8 Hz, 1H), 3.73 (dd, J = 18.0, 12.8 Hz, 1H), 4.64 (bs, 2H), 5.78 (dd, J = 12.8, 6.0 Hz, 1H), 7.07–7.67 (m, 9H), 8.20 (bs, 1H)
5a–B	δ 3.21 (dd, J = 18.0, 6.8 Hz, 1H), 3.73 (dd, J = 18.0, 12.8 Hz, 1H), 4.64 (bs, 2H), 5.79 (dd, J = 12.8, 6.8 Hz, 1H), 7.07–7.67 (m, 9H), 8.21 (bs, 1H)
5f–A	δ 3.02 (dd, J = 18.0, 6.8 Hz, 1H), 3.71 (dd, J = 18.0, 12.8 Hz, 1H), 4.63 (bs, 2H), 5.91 (dd, J = 12.8, 6.8 Hz, 1H), 6.85–7.60 (m, 8H), 8.01(bs, 1H)
5f–B	δ 3.02 (dd, J = 18.0, 6.8 Hz, 1H), 3.71 (dd, J = 18.0, 12.8 Hz, 1H), 4.62 (bs, 2H), 5.91 (dd, J = 12.8, 6.8 Hz, 1H), 6.86–7.61 (m, 8H), 8.00 (bs, 1H)

Table 5. Hydrogen bonding interactions for COX-2 analogs

Analog	Q192	H90	R120	F518(αNH_2)
5a (ent1)	2.83A($\mathbf{SO_2}NH_2$)[a]	2.13A($SO_2\mathbf{NH}_2$)	2.56A($\mathbf{CF_3}$)	2.15A($\mathbf{SO_2}NH_2$)
5a (ent2)	1.91A($SO_2\mathbf{NH}_2$)	***[b]	2.29A($\mathbf{CF_3}$)	2.58A($SO_2\mathbf{NH}_2$)
Celecoxib	***	***	2.55A($\mathbf{CF_3}$)	1.94A($\mathbf{SO_2}NH_2$)

[a] Bold letters indicate the sulfamide group to which the hydrogen bond is formed.
[b] ***, no hydrogen bond observed within 4 A of the designated residue.

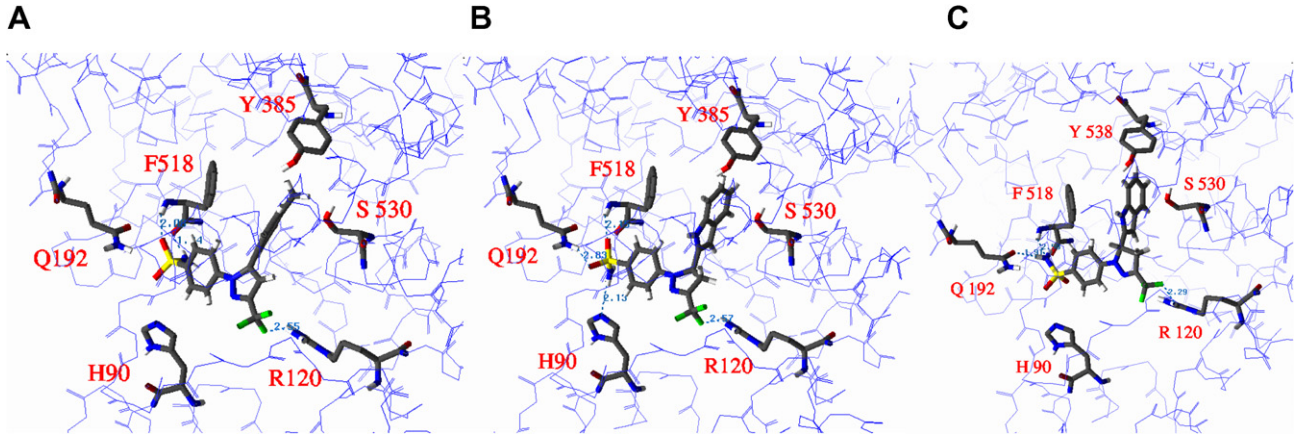


Figure 2. Active site snapshots of molecular models of analog:COX-2 complexes.

volved in ligand binding are identified. 2A: celecoxib, 2B: **5a** (enantiomer-1), 2C: **5a** (enantiomer-2). Hydrogen bonds are represented as dotted lines.

Because a chiral center is present in **5a** at the point of attachment of the pyrazoline group, this analog exists in two different enantiomeric forms with the pyrazoline

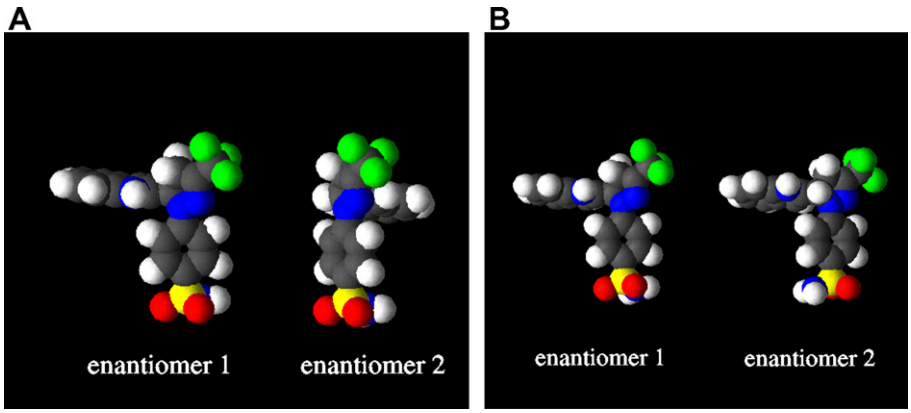


Figure 3. Molecular models of the enantiomeric forms of analog **5a**.

ring pointing out (enantiomer-1) or in (enantiomer-2) as shown in Figure 3. Initial docking of **5a** (enantiomer-2) with the COX-2 protein resulted in major Van der Waals contacts between the ligand and residues Glu 524, Arg 120, and Val 523 due to the opposite orientation of the pyrazoline group on **5a** (enantiomer-1). In order to successfully dock this analog into the active site of COX-2, the intact enantiomer-2 was rotated to more closely align the pyrazoline ring of this enantiomer with that of enantiomer-1 as shown in Figure 3B. The molecular model of the resulting enantiomer-2:COX-2 complex was subjected to energy calculations and its active site snapshot is shown in Figure 2C. Comparison of Figure 2B with Figure 2C suggests that the binding efficiency of the **5a** enantiomer-2 should be less than that of enantiomer-1 for two reasons. First, a hydrogen bond to His 90 is not observed when enantiomer-2 was docked into the COX-2 active site. In addition, in the presence of COX-2, the hydrogen bonds demonstrated to be present between enantiomer-2 and Gln 192 or Phe 518 involve the less electronegative sulfonamide NH_2 group as compared to the more electronegative sulfonamide SO_2 group in the presence of enantiomer-1.

Molecular models are shown for the enantiomers of **5a** before (Fig. 3A) and after (Fig. 3B) docking into the COX-2 protein.

4. Conclusions

In this report we have synthesized a series of novel 5-(3-indolyl)-1-(4-sulfamylphenyl)-3-trifluoromethyl pyrazolines and examined their activity against cyclooxygenases and lipoxygenases. Our results show that some of these compounds show moderate to good activity in COX-2, LOX-5, and LOX-15 inhibition assays. Single enantiomers resolved from the racemate mixture by a chiral column have exhibited much higher inhibitory activity than the parent compound. Aromatization of the pyrazoline ring in **5a** to pyrazole **8** resulted in total loss of activity indicating the importance of non-planar ring structure. Finally, the molecular modeling suggests that active enantiomer establishes more hydrogen bonding interactions with the catalytic domain of COX-2 enzyme than the inactive or less active isomer.

5. Experimental

5.1. COX-inhibition-EIA assay

Cyclooxygenase activity of ovine COX-1 and COX-2 was assayed using COX inhibitory screening assay kit (Cayman Chemicals, MI). This assay directly measures $PGF_{2\alpha}$ that was produced by stannous chloride reduction of COX derived PGH2 by enzyme immunoassay (EIA). This assay is more accurate and reliable than the peroxide inhibition assay as shown by Gierse et al.[28] All assays were conducted in duplicate and IC_{50} values are the average of duplicate determinations for each compound. In brief, for the inhibition assay, hematin reconstituted purified COX-1 and COX-2 enzymes (six units) in a reaction buffer containing Tris–HCl (0.1 M, pH 8.0), 5 mM EDTA, and 2 mM phenol were pre-incubated at room temperature for 1 h with inhibitor concentrations ranging from 0.001 to 100 mM in DMSO followed by the addition of arachidonic acid (100 μM) for 2 min at 37 °C. Reactions were terminated by adding 50 μL of 1 M HCl followed by the addition of 100 μL of stannous chloride. The final product $PGF_{2\alpha}$ formed was measured by EIA and IC_{50} values were determined following the instructions given in the kit manual.

5.2. LOX-inhibition assay

Lipoxygenase 5[29] (LOX-5), Lipoxygenase 12[30] (LOX-12), and Lipoxygenase 15[31] (LOX-15) activities were performed by MDS Pharma Services, 158 Li-Teh Road, Peitou, Taipei, Taiwan, following the procedures as described. In these assays, the enzyme sources for LOX-5, LOX-12, and LOX-15 are human PBML cells, human platelets and rabbit reticulocytes, respectively. The substrates for LOX-5, LOX-12, and LOX-15 used in the assays are endogenous arachidonic acid, 30 mM arachidonic acid and 256 mM linoleic acid, respectively. The enzyme inhibition was quantitated by measuring LTB4, 12-HETE, and 15-HETE by EIA and spectrophotometrically.

5.3. Chemistry

5.3.1. General information. Melting points were determined in an open capillary tube using a Mel-Temp electro thermal melting point apparatus and are uncorrected. Proton NMR (^{1}H NMR) spectra were determined in

$CDCl_3$, or DMSO-d_6 solution on a Bruker Avance 400 spectrometer. Proton chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard. Spin multiplicities are given as s (singlet), d (doublet), bs (broad singlet), m (multiplet), and q (quartet). Coupling constants (J) are given in hertz (Hz). Liquid chromatography was performed with a forced flow (flash chromatography) with Merck Grade Silica gel (70–230 mesh). The solvents used for elution varied depending on the compound and included either one or a combination of the following: petroleum ether, ethyl acetate, chloroform, and methanol. Analytical thin-layer chromatography (TLC) was performed with Sigma–Aldrich 0.25 mm silica gel plates (60 Å), visualized under 254 nm ultraviolet light or iodine spray. Elemental analyses were obtained by Quantitative Technologies Inc. (White House, NJ) and the results were mentioned individually. All Yields were of purified product and were not optimized. Celecoxib was prepared according to the literature procedure.[6] Reagents and solvents were purchased from common suppliers and were used without further purification. 5-Nitroindole carboxaldehyde, 5-carboxyindole carboxaldehyde, 7-nitroindole carboxaldehyde, 4-methoxyindole carboxaldehyde, 6-methoxyindole carboxaldehyde, and 7-methoxyindole carboxaldehyde were synthesized according to the literature methods.[32] For molecular modeling, all calculations were performed using the DREIDING II all atom force field and biograf® software (BIOSYM/Molecular Simulations, San Diego, CA). Modified crystal structures for COX-1 and COX-2 were obtained by the addition of all heterogeneous hydrogen atoms in optimized positions according to the biograf® software protocols.

5.3.2. General procedure for the synthesis of substituted (*E*)1,1,1-trifluoro-4-(1*H*-indol-3-yl)-but-3-en-2-one (3).[33]

Method A: To a solution of indole (5 mmol) and 4-ethoxy-1,1,1-trifluoro-3-buten-2-one (5 mmol) in anhydrous dichloromethane (10 mL) was added $ZnCl_2$ (0.5 mmol). The reaction mixture was stirred at rt for 3 h. Reaction progress was monitored with TLC and, after completion, the contents were cooled to 10 °C and the precipitated product was filtered, washed with CH_2Cl_2, and dried. The crude products were recrystallized from hexane/ethanol to obtain pure **3**.

5.3.2.1. 1,1,1-Trifluoro-4-(5-fluoro-1*H*-indol-3-yl)-but-3-en-2-one (3a). Yield 50%; mp 193–196 °C; ^{1}H NMR (DMSO-d_6) δ 7.44 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.66 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{12}H_7F_4NO$: C, 56.04; H, 2.74; N, 5.45. Found: C, 55.88; H, 2.61; N, 5.23.

5.3.2.2. 1,1,1-Trifluoro-4-(5-chloro-1*H*-indol-3-yl)-but-3-en-2-one (3b). Yield 62%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ 7.44 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.65 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{12}H_7ClF_3NO$: C, 52.67; H, 2.58; N, 5.12. Found: C, 53.00; H, 2.67; N, 5.29.

5.3.2.3. 1,1,1-Trifluoro-4-(6-fluoro-1*H*-indol-3-yl)-but-3-en-2-one (3c). Yield 48%; mp 191–194 °C; ^{1}H NMR (DMSO-d_6) δ 7.43 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.66 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{12}H_7F_4NO$: C, 56.04; H, 2.74; N, 5.45. Found: C, 56.09; H, 2.69; N, 5.34.

5.3.2.4. 1,1,1-Trifluoro-4-(6-chloro-1*H*-indol-3-yl)-but-3-en-2-one (3d). Yield 77%; mp 137–139 °C; ^{1}H NMR (DMSO-d_6) δ 7.44(d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.66 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{12}H_7ClF_3NO$: C, 52.67; H, 2.58; N, 5.12. Found: C, 52.44; H, 2.29; N, 5.01.

5.3.2.5. 1,1,1-Trifluoro-4-(7-chloro-1*H*-indol-3-yl)-but-3-en-2-one (3e). Yield 55%; mp 166–168 °C; ^{1}H NMR ($CDCl_3$) δ 6.78 (d, J = 15.7, 1H), 6.88–7.67 (m, 4H), 8.04 (d, J = 15.5, 1H), 10.90 (bs, 1H). Anal. calcd for $C_{12}H_7ClF_3NO$: C, 52.67; H, 2.58; N, 5.12. Found: C, 52.31; H, 2.27; N, 5.07.

5.3.2.6. 1,1,1-Trifluoro-4-(5-cyano-1*H*-indol-3-yl)-but-3-en-2-one (3f). Yield 62%; mp 95–97 °C; ^{1}H NMR (DMSO-d_6) δ 7.43 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.66 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{13}H_7F_3N_2O$: C, 59.10; H, 2.67; N, 10.60. Found: C, 58.77; H, 2.30; N, 10.44.

5.3.2.7. 1,1,1-Trifluoro-4-(5-bromo-1*H*-indol-3-yl)-but-3-en-2-one (3g). Yield 50%; mp 170–172 °C; ^{1}H NMR (DMSO-d_6) δ 7.44 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.65 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd for $C_{12}H_7BrF_3NO$: C, 45.31; H, 2.22; N, 4.40. Found: C, 44.99; H, 1.99; N, 4.18.

5.3.2.8. 1,1,1-Trifluoro-4-(5-amino-1*H*-indol-3-yl)-but-3-en-2-one (3h). Yield 58%; mp 161–165 °C; ^{1}H NMR ($CDCl_3$) δ 5.62–7.42 (m, 4H), 7.69 (d, J = 13.5, 1H), 7.71 (d, J = 13.5, 1H), 8.3 (bs, 1H). Anal. calcd for $C_{12}H_9F_3N_2O$: C, 56.70; H, 3.57; N, 11.02. Found: C, 56.26; H, 3.12; N, 10.77.

5.3.2.9. 1,1,1-Trifluoro-4-(4-amino-1*H*-indol-3-yl)-but-3-en-2-one (3i). Yield 70%; mp 102–104 °C; ^{1}H NMR ($CDCl_3$) δ 5.72–7.95 (m, 4H), 7.88 (d, J = 13.6, 1H), 7.90 (d, J = 13.7, 1H), 8.40 (bs, 1H). Anal. calcd for $C_{12}H_9F_3N_2O$: C, 56.70; H, 3.57; N, 11.02. Found: C, 57.13; H, 3.83; N, 11.13.

Method B.[34] To a solution of Indole carboxaldehyde (7.5 mmol) in dry THF (15 mL) was added glacial acetic acid (0.5 mL) followed by piperidine (0.5 mL) under Argon atmosphere. Trifluoroacetone (30 mmol) was later added to this reaction mixture over a period of 2–5 min and stirred at room temperature for 2–3 h. The progress of the reaction was monitored by TLC. After completion, the reaction was quenched with saturated NH_4Cl solution and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated. Purification by column chromatography eluting with 3:1 hexane/ethyl acetate afforded pure **3**.

5.3.2.10. 1,1,1-Trifluoro-4-(5-nitro-1*H*-indol-3-yl)-but-3-en-2-one (3j). Yield 50%; mp 246–249 °C; ^{1}H NMR (DMSO-d_6) δ 7.43 (d, J = 15.7, 1H), 7.95–9.16 (m, 4H), 8.66 (d, J = 15.7, 1H), 13.09 (bs, 1H). Anal. calcd

for $C_{12}H_7F_3N_2O_3$: C, 50.71; H, 2.48; N, 9.86. Found: C, 51.09; H, 2.70; N, 10.11.

5.3.2.11. 1,1,1-Trifluoro-4-(5-carboxy-1*H*-indol-3-yl)-but-3-en-2-one (3k). Yield 40%; mp 264–266 °C; ^{1}H NMR ($CDCl_3$) δ 6.79 (d, J = 16.0, 1H), 7.25–8.40 (m, 4H), 8.01 (d, J = 15.5, 1H), 8.40 (bs, 1H, OH). Anal. calcd for $C_{13}H_8F_3NO_3$: C, 55.13; H, 2.85; N, 4.95. Found: C, 54.89; H, 2.33; N, 4.49.

5.3.2.12. 1,1,1-Trifluoro-4-(7-nitro-1*H*-indol-3-yl)-but-3-en-2-one (3l). Yield 55%; mp 228–230 °C; ^{1}H NMR ($CDCl_3$) δ 6.98 (d, J = 16.0, 1H), 7.36–8.22 (m, 4H), 8.18 (d, J = 16.5, 1H). Anal. calcd for $C_{12}H_7F_3N_2O_3$: C, 50.71; H, 2.48; N, 9.86. Found: C, 50.19; H, 2.21; N, 9.11.

5.3.2.13. 1,1,1-Trifluoro-4-(4-methoxy-1*H*-indol-3-yl)-but-3-en-2-one (3m). Yield 60%; mp 188–192 °C; ^{1}H NMR ($CDCl_3$) δ 3.93 (s, 3H), 7.16 (d, J = 16.0, 1H), 6.61–7.64 (m, 4H), 8.50 (d, J = 15.7, 1H). Anal. calcd for $C_{13}H_{10}F_3NO_2$: C, 58.00; H, 3.74; N, 5.20. Found: C, 57.59; H, 3.41; N, 5.01.

5.3.3. General procedure for the synthesis of substituted 4-[5-(1*H*-Indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5). To a stirred solution of substituted (*E*)1,1,1-trifluoro-4-(1H-indol-3-yl)-but-3-en-2-one (**3**) (2.5 mmol) in ethanol (10 mL) was added (4-sulfamylphenyl)hydrazine hydrochloride (**4**) (3 mmol) in small portions and the reaction mixture was refluxed for 8–10 h. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was cooled to 25 °C and the solid separated out in most cases was filtered, washed with ethanol, and dried. In some instances where solid did not separate, the reaction mixture was poured into cold water, extracted with ethyl acetate (2 × 100 mL), washed with brine, dried over anhydrous Na_2SO_4, and evaporated the solvent under reduced pressure. The oily product obtained was triturated with 2-propanol and the solid separated was filtered and dried. The crude products were recrystallized from ethanol to give pure 5.

5.3.3.1. 4-[5-(1*H*-Indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5a). Yield 53%; mp 160–162 °C; ^{1}H NMR ($CDCl_3$) δ 3.22 (dd, J = 8.5 Hz, 1H), 3.74 (dd, J = 8.5 Hz, 1H), 4.60 (bs, 2H), 5.80 (dd, J = 6.5 Hz, 1H), 7.08–7.69 (m, 9H), 8.19 (bs, 1H). Anal. Calcd for $C_{18}H_{15}F_3N_4O_2S$: C, 52.94; H, 3.70; N, 13.72. Found: C, 53.09; H, 3.81; N, 13.90.

5.3.3.2. 4-[5-(5-Fluoro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5b). Yield 60%; mp 193–196 °C; ^{1}H NMR (DMSO-d_6) δ 2.58 (dd, J = 8.5 Hz, 1H), 3.41 (dd, J = 8.5 Hz, 1H), 5.95 (dd, J = 6.5 Hz, 1H), 6.40–7.20 (m, 8H), 10.78 (bs, 1H). Anal. Calcd for $C_{18}H_{14}F_4N_4O_2S$: C, 50.70; H, 3.30; N, 13.14. Found: C, 50.26; H, 3.09; N, 12.70.

5.3.3.3. 4-[5-(5-Chloro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5c). Yield 62%; mp 163–165 °C; ^{1}H NMR (DMSO-d_6) δ 2.44 (dd, J = 8.5 Hz, 1H), 3.23 (dd, J = 8.5 Hz, 1H), 5.46 (dd, J = 6.5 Hz, 1H), 6.32–7.18 (m, 8H), 10.70 (bs, 1H). Anal. calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.49; H, 3.09; N, 12.45.

5.3.3.4. 4-[5-(6-Fluoro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5d). Yield 60%; mp 194–196 °C; ^{1}H NMR (DMSO-d_6) δ 2.59 (dd, J = 8.5 Hz, 1H), 3.38 (dd, J = 8.5 Hz, 1H), 5.59 (dd, J = 8.0 Hz,, 1H), 6.35–7.20 (m, 8H), 10.75 (bs, 1H). Anal. calcd for $C_{18}H_{14}F_4N_4O_2S$: C, 50.70; H, 3.30; N, 13.14. Found: C, 50.22; H, 2.88; N, 12.54.

5.3.3.5. 4-[5-(6-Chloro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5e). Yield 62%; mp 96–98 °C; ^{1}H NMR (DMSO-d_6) δ 3.09 (dd, J = 8.5 Hz, 1H), 3.90 (dd, J = 8.5 Hz, 1H), 6.11 (dd, J = 8.0 Hz, 1H), 7.03–8.12 (m, 8H), 11.29 (bs, 1H). Anal. Calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.44; H, 2.86; N, 12.24.

5.3.3.6. 4-[5-(7-Chloro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5f). Yield 55%; mp 166–168 °C; ^{1}H NMR (DMSO-d_6) δ 3.03 (dd, J = 8.5 Hz, 1H), 3.71 (dd, J = 8.5 Hz, 1H), 5.91 (dd, J = 8.0 Hz, 1H), 6.86–7.60 (m, 8H), 8.0 (bs, 1H). Anal. Calcd for $C_{18}H_{14}ClF_3N_4O_2S$: C, 48.82; H, 3.19; N, 12.65. Found: C, 48.33; H, 2.80; N, 12.31.

5.3.3.7. 4-[5-(5-Cyano-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5g). Yield 60%; mp 241–244 °C; ^{1}H NMR (DMSO-d_6) δ 3.15 (dd, J = 8.5 Hz, 1H), 3.93 (dd, J = 8.5 Hz, 1H), 6.18 (dd, J = 8.0 Hz, 1H), 7.09–8.11 (m, 8H), 11.73 (bs, 1H). Anal. calcd for $C_{19}H_{14}F_3N_5O_2S$: C, 52.65; H, 3.25; N, 16.15. Found: C, 52.29; H, 3.39; N, 15.99.

5.3.3.8. 4-[5-(6-Bromo-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5h). Yield 68%; mp 112–114 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (dd, J = 8.5 Hz, 1H), 3.90 (dd, J = 8.5 Hz, 1H), 6.12 (dd, J = 8.0 Hz, 1H), 7.06–7.62 (m, 8H), 11.37 (bs, 1H). Anal. calcd for $C_{18}H_{14}BrF_3N_4O_2S$: C, 44.36; H, 2.89; N, 11.49. Found: C, 43.34; H, 2.22; N, 10.73.

5.3.3.9. 4-[5-(5-Amino-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5i). Yield 50%; mp 152–156 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 (bs, 2H), 2.94 (dd, J = 8.5 Hz, 1H), 3.70 (dd, J = 8.5 Hz, 1H), 6.15 (dd, J = 8.0 Hz, 1H), 6.81–8.17 (m, 8H), 11.66 (bs, 1H). Anal. calcd for $C_{18}H_{16}F_3N_5O_2S$: C, 51.05; H, 3.80; N, 16.53. Found: C, 50.99; H, 3.69; N, 16.33.

5.3.3.10. 4-[5-(4-Amino-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5j). Yield 40%; mp 140–144 °C; ^{1}H NMR (DMSO-d_6) δ 2.50 (bs, 2H), 3.24 (dd, J = 8.5 Hz, 1H), 3.54 (dd, J = 8.5 Hz, 1H), 6.58 (dd, J = 8.0 Hz, 1H), 6.90–7.70 (m, 8H), 8.30 (bs, 1H). Anal. calcd for $C_{18}H_{16}F_3N_5O_2S$: C, 51.05; H, 3.80; N, 16.53. Found: C, 51.22; H, 3.69; N, 16.49.

5.3.3.11. 4-[5-(6-Cyano-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5k). Yield 55%; mp 222–224 °C; ^{1}H NMR (DMSO-d_6) δ 3.10 (dd, J = 8.5 Hz, 1H), 3.74 (dd, J = 8.5 Hz, 1H), 6.40 (dd,

J = 8.0 Hz, 1H), 6.99–8.15 (m, 8H), 11.65 (bs, 1H). Anal. Calcd for $C_{19}H_{14}F_3N_5O_2S$: C, 52.65; H, 3.25; N, 16.15. Found: C, 53.01; H, 3.36; N, 16.05.

5.3.3.12. 4-[5-(6-Nitro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5l). Yield 65%; mp 189–193 °C; ^{1}H NMR (DMSO-d_6) δ 3.20 (dd, J = 8.5 Hz, 1H), 3.94 (dd, J = 8.5 Hz, 1H), 6.23 (dd, J = 8.0 Hz, 1H), 7.00–8.03 (m, 8H), 11.55 (bs, 1H). Anal. Calcd for $C_{18}H_{14}F_3N_5O_4S$: C, 47.68; H, 3.11; N, 15.45. Found: C, 47.88; H, 3.01; N, 15.62.

5.3.3.13. 4-[5-(6-Chloro-2-methyl-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydro-pyrazol-1-yl]-benzenesulfonamide (5m). Yield 60%; mp 120–124 °C; ^{1}H NMR (DMSO-d_6) δ 2.5 (s, 3H), 3.05 (dd, J = 8.5 Hz, 1H), 3.84 (dd, J = 8.5 Hz, 1H), 6.06 (dd, J = 8.0 Hz, 1H), 6.97–7.60 (m, 8H), 11.15 (bs, 1H). Anal. calcd for $C_{19}H_{16}ClF_3N_4O_2S$: C, 49.95; H, 3.53; N, 12.26. Found: C, 49.45; H, 3.23; N, 12.06.

5.3.3.14. 4-[5-(4-Methoxy-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5n). Yield 68%; mp 156–158 °C; ^{1}H NMR (DMSO-d_6) δ 3.39 (dd, J = 8.5 Hz, 1H), 3.79 (dd, J = 8.5 Hz, 1H), 3.98 (s, 3H), 6.15 (dd, J = 8.0 Hz, 1H), 6.66–7.80 (m, 8H), 10.37 (bs, 1H). Anal. calcd for $C_{19}H_{17}F_3N_4O_3S$: C, 52.05; H, 3.90; N, 12.77. Found: C, 52.44; H, 3.76; N, 11.57.

5.3.3.15. 4-[5-(6-Amino-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5o). Yield 50%; mp 208–210 °C; ^{1}H NMR (DMSO-d_6) δ 2.54 (bs, 2H), 2.94 (dd, J = 8.5 Hz, 1H), 3.95 (dd, J = 8.5 Hz, 1H), 6.19 (dd, J = 8.0 Hz, 1H), 6.55–8.40 (m, 8H), 11.05 (bs, 1H). Anal. calcd for $C_{18}H_{16}F_3N_5O_2S$: C, 51.05; H, 3.80; N, 16.53. Found: C, 50.04; H, 4.07; N, 13.69.

5.3.3.16. 4-[5-(5-Nitro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5p). Yield 55%; mp 230–233 °C; ^{1}H NMR (DMSO-d_6) δ 3.02 (dd, J = 8.5 Hz, 1H), 3.85 (dd, J = 8.5 Hz, 1H), 6.11 (dd, J = 8.0 Hz, 1H), 6.91–8.23 (m, 8H), 11.72 (bs, 1H). Anal. Calcd for $C_{18}H_{14}F_3N_5O_4S$: C, 47.68; H, 3.11; N, 15.45. Found: C, 47.71; H, 3.16; N, 14.75.

5.3.3.17. 4-[5-(5-Carboxy-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5q). Yield 65%; mp 157–160 °C; ^{1}H NMR (CD_3CN) δ 3.03 (dd, J = 8.5 Hz, 1H), 3.65 (dd, J = 8.5 Hz, 1H), 5.83 (dd, J = 8.0 Hz, 1H), 7.03–7.98 (m, 8H), 9.45 (bs, 1H). Anal. calcd for $C_{19}H_{15}F_3N_4O_4S$: C, 50.44; H, 3.34; N, 12.38. Found: C, 49.48; H, 4.02; N, 12.12.

5.3.3.18. 4-[5-(7-Nitro-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5r). Yield 50%; mp 115–119 °C; ^{1}H NMR (DMSO-d_6) δ 3.14 (dd, J = 8.5 Hz, 1H), 3.90 (dd, J = 8.5 Hz, 1H), 6.20 (dd, J = 8.0 Hz, 1H), 7.11–8.25 (m, 8H), 11.70 (bs, 1H). Anal. calcd for $C_{18}H_{14}F_3N_5O_4S$: C, 47.68; H, 3.11; N, 15.45. Found: C, 47.98; H, 3.31; N, 15.46.

5.3.3.19. 4-[5-(7-Amino-1*H*-indol-3-yl)-3-trifluoromethyl-4,5-dihydropyrazol-1-yl]-benzenesulfonamide (5s). Yield 50%; mp 220–224 °C; ^{1}H NMR (DMSO-d_6) δ 2.64 (bs, 2H), 3.12 (dd, J = 8.5 Hz, 1H), 3.92 (dd, J = 8.5 Hz, 1H), 6.13 (dd, J = 8.0 Hz, 1H), 6.45–7.80 (m, 8H), 10.32 (bs, 1H). Anal. calcd for $C_{18}H_{16}F_3N_5O_2S$: C, 51.05; H, 3.80; N, 16.53. Found: C, 50.99; H, 3.61; N, 16.66.

5.4. Computational details

For molecular modeling, all calculations were performed using the DREIDING II all atom force field and biograf® software (BIOSYM/Molecular Simulations, San Diego, CA). Modified crystal structures for COX-1 and COX-2 were obtained by the addition of all heterogeneous hydrogen atoms in optimized positions according to the biograf® software protocols.

Models of celecoxib and **5a** and its enantiomers (Fig. 1) were constructed using the organic builder contained within the main biograf® program and were individually docked into the active site of cyclooxygenase-2 by overlaying the structure of each analog with the structure of indomethacin, derived from the original crystal structure. Modeling calculations were performed only allowing for the movement of each analog within the active site, while the atomic coordinates of COX-2 were held constant. The calculations involved energy minimization to convergence, limited molecular dynamics calculations (5 psec.), followed again by energy minimization to convergence.

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