Dual Inhibitors of COX-2 and Soluble Epoxide Hydrolase (sEH)

Vijaykumar D. Nimbarte*, Murtuza Hadianawala and Krishnam Raju Atcha

Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER) Hyderabad, Balanagar, Hyderabad-500037, India

Abstract: A series of novel Dual Inhibitors of COX-2 and sEH containing an *N*-urea substituted pyrazole as a lead for sEH inhibition have been synthesized and screened as novel analogues to reduce blood pressure elevation by acting as sEH inhibitors. The synthesized compounds having varying degrees of selectivity towards the sEH enzymes. Particularly compounds **6g** and **6i** emerged as the most potent sEH inhibitor displaying IC_{50} values of 0.114 ± 0.014 µM and 0.120 ± 0.03 µM for *in-vitro* sEH inhibition and the selectivity for COX-2 enzyme pave the way for discovery of novel Dual Inhibitors.

Keywords: AUDA, (1, 5)-Diarylpyrazole derivatives, Hypertension, *N*-Acyl, sEH inhibition, Urea, etc.

INTRODUCTION

The major target for the various conditions like Cardiovascular, Inflammatory and asthmatic diseases is arachidonic acid cascade (AA). For example the conversion of AA to prostaglandins (PGs) which is important in regulation of inflammation and pain can be targeted by means of Nonsteroidal anti-inflammatory drugs (NSAIDs) and selective Cycloxygenase (COX-2). In addition the conversion of AA to leukotrienes (Lts) which is playing important role in allergic reactions can be inhibited by means of Lipoxygenase (LOX) inhibitors in particular 5-LOX inhibitors.

The dual inhibition approach of both COX & LOX enzymes proven to be advantageous in various cardiovascular complications & cancer therapy. Already reported dual inhibitors acts by inhibiting both Cycloxygenase (either COX-2 or both COX-1 & COX-2) and 5-LOX are used as potential agents for the treatment of arthritis. Licofelone (ML-3000) is an example of the above mentioned dual COX-2 and 5-LOX inhibitory approach (Figure **1**). These approaches of dual inhibition already have shown importance in curing Inflammation [1], pain [2] and cancer [3].

In addition to the above mentioned COX and LOX pathways. There is an important major metabolic pathway in the AA cascade which involves cytochrome –P450 metabolism. This leads 20-Hydroxyeicatrienoic acid (20-HETE) and epoxyeicosatrienoic acid formation [4]. The conversion of these epoxyeicosatrienoic acid (EEts) to corresponding diols is facilitated by an enzyme Soluble epoxy Hydrolase (sEH). EEts exhibits vasodilatory [5], cardioprotective [6], antihypertensive and antiinflammatory properties, while DHETS have key roles in reduced activity in most ways.

Biosynthesis of prostaglandin from AA mediated an enzyme cyclooxygenase which are the major target of NSAIDS. However, this drug class (NSAIDS) use was limited because of the NSAIDS induced gastrointestinal toxicity. To overcome this side effect primarily cause by COX-1 inhibition, selective COXIBs (COX-2 inhibitors) such as celecoxib and rofecoxib were designed [7]. This selectivity of COXIBs enhances the GI tolerance & retains beneficial antiinflammatory and antihyperalgesic properties. In spite of this selectivity of COXIBs at higher concentration, COX-2 inhibitor retains some gastro intestinal toxicity. In addition to this at higher concentration COX-2 inhibitors will loses selectivity and inhibits COX-1 enzyme which cause undesirable sideeffects. Moreover the shift in plasma thromboxanes/prostacyclin ratio [7] and increase in eicasonide 20-HETE, leads to thrombic events and hypertension due to COX-2 selective inhibitors. It was already reported in the literature that the low doses of NSAIDS and sEH inhibitors produces synergistic effects in terms of anti inflammation and antihyperalgesic outcomes [8]. This synergistic approach helps in improving pain and inflammation. In addition it decreases the cardiotoxicity caused by COX-2 inhibitors.

The sEH playing key role in the metabolism of arachidonic acid, 4 linoleic acid [8] and other lipid epoxides, which are important endogenous chemical mediators. Epoxides arachidonic acid (epoxyeicosatrienoic acids or EETs) are known effectors of blood pressure [9] and modulators vascular permeability [10]. Hydrolysis of the epoxides by sEH diminishes this activity [11]. It is already reported in that treatment with selective sEH inhibitors significantly

*Address correspondence to this author at the Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research (NIPER) Hyderabad, Balanagar, Hyderabad-500037, India;
Tel: +91-9652118722; E-mail: vnimbarte@gmail.com

E-ISSN: 2308-8044/13

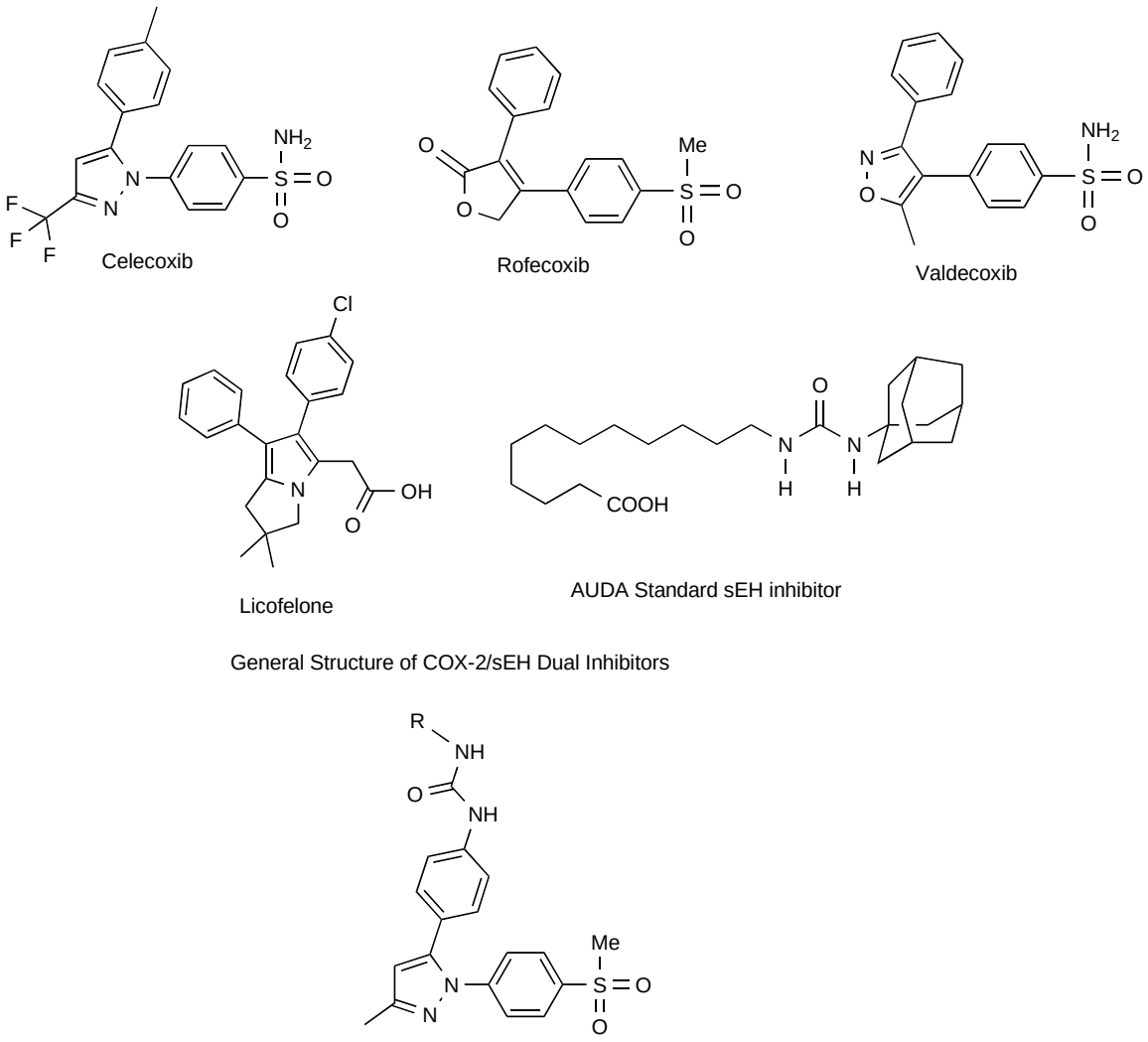


Figure 1: Reference COX-2 and sEH Inhibitor structures.

reduces the blood pressure of spontaneous hypertensive rats (SHRs) or angiotensin II induced hypertension in rats [12, 13] further supporting the role of sEH in regulation of blood pressure. The EETs have also demonstrated anti-inflammatory properties in endothelial cells [14, 15]. In contrast, diols derived from epoxylinoleate (leukotoxin) perturb membrane permeability and calcium homeostasis [16], which is responsible for results in inflammation that is modulated by nitric oxide synthase and endothelin-1 [17]. Micro molar concentrations of leukotoxin reported in association with inflammation and hypoxia [18] depress mitochondrial respiration *in vitro* [19] and cause mammalian cardiopulmonary toxicity *in vivo* [20]. Leukotoxin toxicity presents symptoms suggestive of multiple organ failure and acute respiratory distress syndrome (ARDS) [21]. In cellular and organismal models, leukotoxin-mediated toxicity is mediated through epoxide hydrolysis, suggesting sEH have a role in the regulation of inflammation.

The therapeutic targeting of the P450 branch of the AA cascade remains to be thoroughly explored and even less so using dual inhibitors. To date, there is only one current example of a dual inhibitor related to sEH in the literature, a sEH/ 11ß-HSD1 dual inhibitor designed by GlaxoSmithKline. Herein, we report COX-2/sEH dual inhibitors as a new class of DMLs active in the AA cascade.

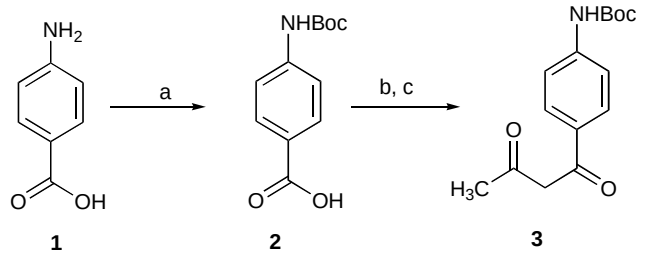
Biological screening of all analogues were carried out by using *In vitro* Cayman's fluorescence-based sEH inhibitor screening assay kit containing mammalian recombinant sEH enzyme and compounds which showed greater than 80% inhibition were further screened at 3 different concentration and IC_{50} values were determined for them [22]. The obtained results were showing that the compounds are having potential sEH inhibition. Selectivity of these compounds towards COX-2 over COX-1 enzyme inhibition activity has been

evaluated by using *In vitro* Cayman's fluorescence-based COX inhibitor screening assay kit [23].

RESULTS AND DISCUSSION

Chemistry

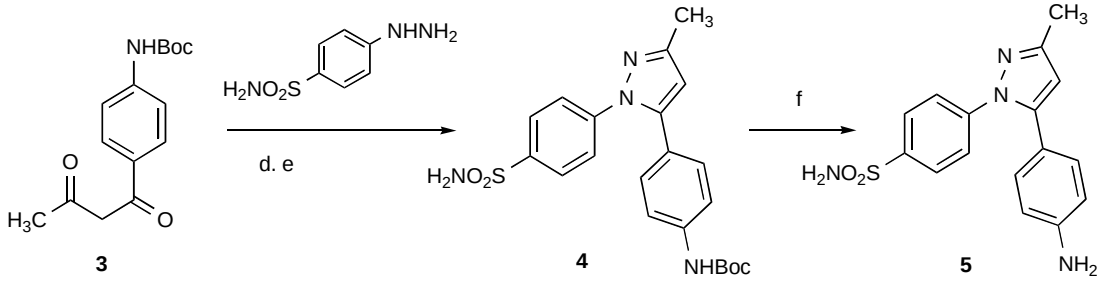
The novel amide derivatives of substituted pyrazoles were synthesized starting from 4-aminobenzoic acid **1**. The NH group of the 4-aminobenzoic acid **1** was protected [24] using $(Boc)_2O$ and NaOH as base to give Boc protected acid **2** in 97% yield. The acid **2** was coupled with 1,1'-carbonyldi-1*H*-imidazole (CDI) in DMF solvent to give imidazole coupled intermediate which was in-situ further treated with acetone under Claisen reaction conditions [25] using NaH as base and DMF as solvent at room temperature to afford the requisite intermediate 1,3 diketone **3** in 70% yield (Scheme **1**). Condensation of the diketone **3** with methylsulfonyl phenyl hydrazine/sulphonamide phenyl hydrazine in the presence of ethanol gave compounds **4** in 85% yields. The compounds **4** on Boc deprotection [26] using HCl gave required pyrazole intermediates **5** in 85% yield (Scheme **2**). The pyrazole intermediates **5** was treated with various isocyanate in the presence of triethylamine as base and solvent THF afforded corresponding novel urea derivatives of substituted pyrazoles (**6a-l)** 85 – 90% yields (Scheme **3**).



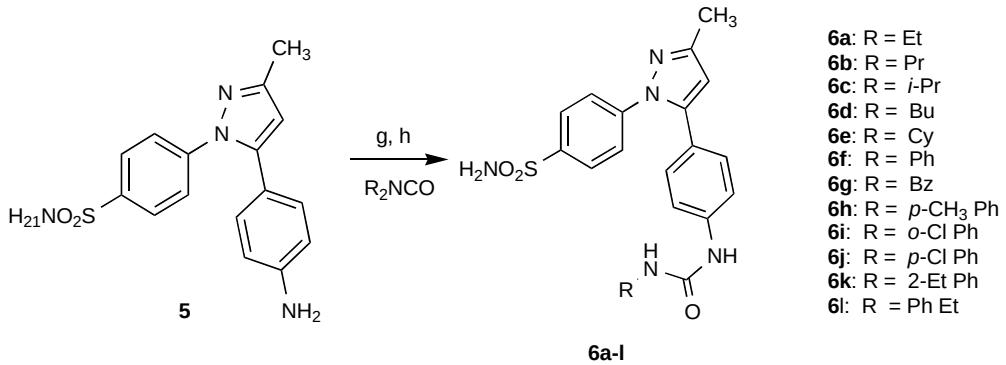
Scheme 1: Reagents and conditions**:** Synthesis of amide derivatives of substituted pyrazoles. (a) NaOH, $(Boc)_2O$, THF, 0 ^oC to rt, 24 h, 97%; (b) CDI, DMF, 0 ^oC, 1 h; (c) i. NaH (60% oil dispersion), DMF, 0 ^oC, ii. Acetone, ^oC to rt, 24 h, 70%.

sEH Inhibitory Assay

The ability of the synthesized compounds to inhibit sEH enzyme was evaluated by *In-vitro* biological evaluation of all compounds for sEH activity is planned to be done using Soluble Epoxide Hydrolase Inhibitor Screening Assay kit (10011671). The assay utilizes (3-phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphtalen-2-yl)-methyl ester (PHOME) as substrate. When the epoxide moiety of PHOME is hydrolysed by epoxide hydrolase, an intramolecular cyclization occurs which results in the release of cyanohydrins under basic conditions. The cyanohydrins quickly decompose into cyanide ion and highly fluorescent 6-methoxy-2-methoxy-2-naphthaldehyde which can be analyzed



Scheme 2: Reagents and conditions**:** (d) EtOH, rt, 20 h, 83%; (e) HCl, EtOH, rt, 4 h.



Scheme 3: Reagents and conditions**:** (g) THF (h) Et_3N, 0^oC to rt, 3 h (85-90%).

Table 1:

Compound Code.	R	IC_{50} Value (μM)[c*] sEH	IC_{50} Value((μM)[a]		COX-2 SI [b]
			COX-1	COX-2	
6a	Ethyl	-	3900	180	21.66
6b	Propyl	-	8600	49	175.51
6c	Iso-Propyl	0.250±1.32	450	2.0	225.00
6d	Butyl	9.082±0.84	3800	176	21.59
6e	Cyclohexane	0.121 ± 0.03	8200	43	190.69
6f	Phenyl	12.699±2.11	460	1.9	242.10
6g	Benzyl	0.122±0.014	-	-	-
6h	p-CH3 Ph	16.607±0.68	-	-	-
6i	p-Cl Ph	0.144 ± 0.014	-	-	-
6j	o-Cl Ph	3.471±0.52	-	-	-
6k	2-Et Ph	0.220±0.03	-	-	-
Celecoxib	-	-	9.4	0.03	313.33
AUDA	-	0.0065±0.002	-	-	-

[a]Values are means of two determinations. *In vitro* COX-2 selectivity index (IC_{50} COX-1/IC_{50}COX-2).
[b]*In vitro* COX-2 selectivity index (IC_{50} COX-1/IC_{50} COX-2).
[c]Values are the means (SD of three independent experiments with sEH Fluorescent Inhibitor Screening Assay Kit (catalogue no. 10011671, Cayman Chemicals Inc., Ann Arbor, MI). [a]Determined *via* a kinetic fluorescent assay, results are means (SD of three separate experiments. Percent inhibition at 100 μM concentration. [b]Data points are triplicate average. We observed coefficient variation between 5 and 10%.
*Enzymes (5μl human sEH) were incubated with (2μl) inhibitors for 15 min in Assay buffer at 25 °C before substrate introduction ([S]) 5μl). Results are means (SD of three separate experiments).

using an excitation wavelength of 330 nm and an emission wavelength of 465nm [24].

The preliminary *in vitro* findings of sEH enzyme inhibition studies showed that, all the compounds **(6a-l)** series were shown 54-100% inhibition of the sEH enzyme at 100 μM concentration & compounds **6g** and **6i** emerged as the most potent sEH inhibitors, displaying 0.114 ± 0.014μM and 0.120 ± 0.03μM for *in-vitro* sEH inhibition respectively, which is comparable to that of standard AUDA 0.0065±0.002 (Table **1**). These results clearly indicate that all the compounds are showing potent sEH inhibition. This activity may be due to the additional interactions of urea linked functionalities.

COX-2 Inhibitory Assay

Novel urea derivatives of substituted pyrazoles (**6a-l)** were screened by *in vitro* cayman's fluorescence-based COX assay [28] to determine the different steric and electronic effects upon COX-1 and COX-2 inhibitory potency and selectivity. Compounds (**6a–e)** possess a aromatic nucleus containing a central heterocyclic ring system with two vicinal aryl substituent's as typically found in numerous selective and potent COX-2 inhibitors. One of the aryl rings bears a methylsulfonyl (SO_2Me) group, which shown COX-2 selectivity and potency. Selective COX-2 inhibitor Celecoxib was used as reference in the COX assay. The determined enzyme inhibitory data, the respective COX-2 selectivity index (COX-2 SI), (Table **1**).

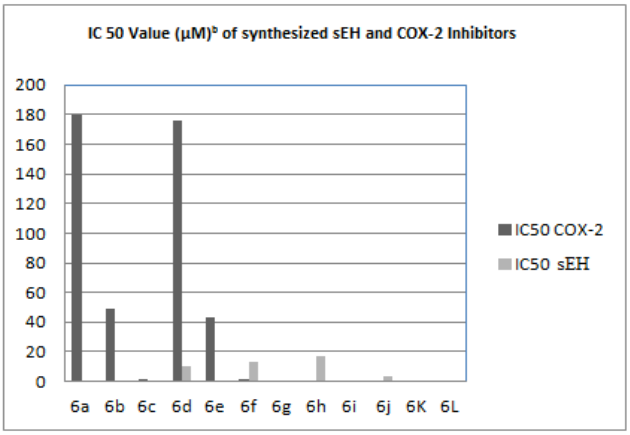


Figure 2: Represents IC_{50} Values of synthesized compound.

In our enzyme inhibitory assay, Celecoxib showed high COX-2 inhibitory potency and selectivity with IC_{50} values of 0.03 μM for COX-2 and 9.4 μM for COX-1, which is in the same range as previously reported in the literature [29, 30]. All prepared substituted pyrazoles **6a–e** are selective COX-2 inhibitors as reflected by the COX-2, SI values ranging from 1.9 (**6c**) to 180 (**6a**).

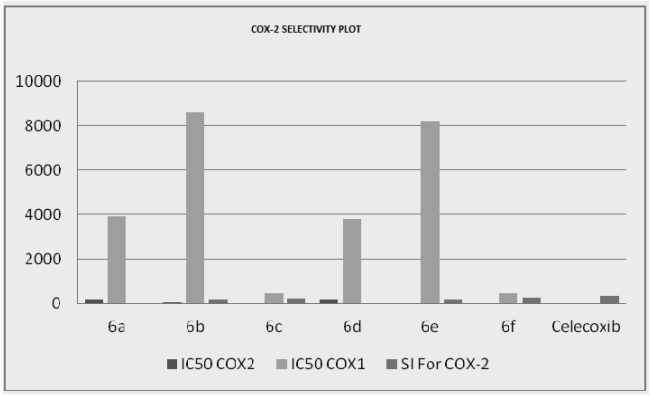


Figure 3: COX-2 Inhibition selectivity Plot.

Structure Activity Relationship

Comparison of Dual COX-2/sEH Inhibitors having Variable-Length Spacer and Effect of ortho and para Substitution on the Aryl Side Chain

These results showing that 1; 5-diarylpyrazole group is essential for COX-2 inhibition activity, which also has been proven to decrease sEH inhibition. In an attempt to improve sEH inhibition activity were initiated by increasing the length of the linker between the 1,5-diarylpyrazole linked urea group to improve hydrogen bonding. A compound with urea linked alkyl side chain of 6c-k series were reported to show improved COX-2 and sEH inhibition, and with para substituted compounds with Methyl, Methoxy and Chloro group on the urea linked aryl side chain shows potentially improved activity as compared to ortho substituted urea pharmacophore linked compounds (6i) (Figure **3**). The above observation on the effect of urea pharmacophore and para substituted side chain on the urea linked pharmacophore on inhibition of COX-2 and sEH.

CONCLUSION

A series of novel dual COX-2 and sEH inhibitors were synthesized. The compounds synthesized to demonstrate significant inhibition sEH. This study has demonstrated that a grouping of amide substituted pyrazole (sEH pharmacophore) side chain can improve the inhibitory activity of the resulting molecule on sEH *in vitro*. This study has demonstrated that the sEH inhibitor **6g** & **6i** displays improved *in vitro* efficacy in fluorescence based sEH assay and all prepared substituted pyrazoles **6a–e** are selective COX-2 inhibitors as reflected by the COX-2, SI values ranging from 1.9 (**6c**) to 180 (**6a**).

This novel dual inhibition approach will leads to new design of analogues which will pave way for novel selective COX-2 Inhibitors with reduced cardiac cytotoxicity.

ACKNOWLEDGEMENTS

Author thank National Institute of Pharmaceutical Education and Research (NIPER)-Hyderabad; and Indian Institute of Chemical Technology (IICT)-Hyderabad for providing facilities and Department of Pharmaceuticals (DoP); Government of India (GoI) for financial support.

EXPERIMENTAL

General Materials and Methods

Reagents and solvents were purchased from commercial suppliers (Across, Sigma–Aldrich, Avra) and used as provided, unless indicated otherwise. All the solvents used for reactions were analytical grade and used as provided. Reactions were carried out in oven-dried glassware under nitrogen atmosphere and stirred at room temperature, unless indicated otherwise. AUDA was purchased from Prolab.

^{1}H NMR was recorded on either a Varian Unity 500 or Bruker Avance 300 MHz. The samples were made in

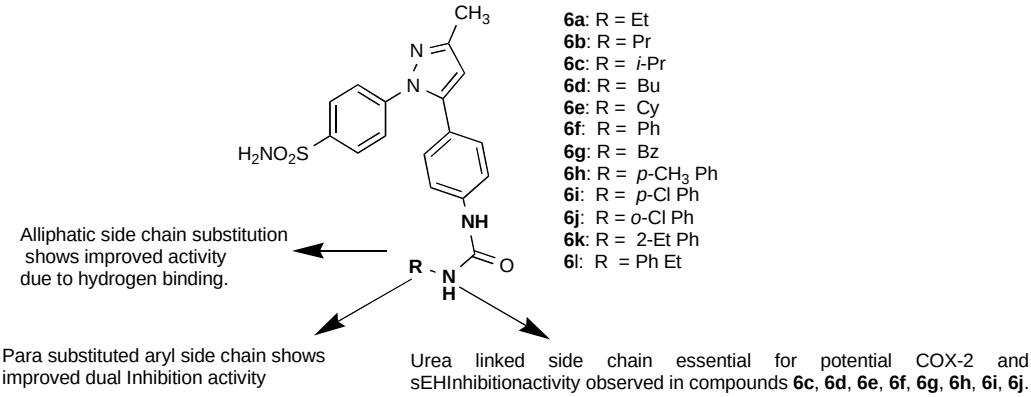


Figure 4: Structure activity relationship representation of synthesised COX-2-sEH Inhibitors analogues.

$CDCl_3$ and/or DMSO-d_6 using TMS (tetramethylsilane) as the internal standard. The chemical shifts are expressed as d values in parts per million (δ), using the residual solvent peaks (chloroform: ^{1}H, 7.26 δ; DMSO: ^{1}H, 2.50 δ) as a reference. Coupling constants are given in Hertz (Hz). The peak patterns are indicated by the following abbreviations: brs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet and m = multiple.

High resolution mass spectra were recorded on Micro Mass VG-7070H Mass spectrometer for ESI. Infrared spectra were recorded on Perkin-Elmer Infrared spectrophotometer with NaCl optics. Spectra were calibrated against the polystyrene absorption at 1610 cm^{-1}. Samples were scanned in neat KBr pellets. Melting points were determined on an SMP3 Stuart melting point apparatus and are uncorrected.

For TLC, precoated aluminium sheets were used (Merck, Silica Gel 60 F_{254}). The spots were visualized by UV light. Column chromatography was performed using silica gel (60-120, 100-200 mesh) and the column was usually eluted with EtOAc/Hexanes. All evaporation of solvents was carried out under reduced pressure on Heidolph Laborota-4000 rotary evaporator below 45 ^{o}C. All compounds were characterized by NMR and MS.

Purity of the compounds were detected by HPLC system [Waters e2695 (alliance)] consisting of low pressure gradient pump plus auto sampler and Photo Diode Array (PDA) detector. The column used was GRACE - C_{18} (250 mm x 4.6 mm x 5 mm). The mobile phase was 1% TFA (pH 3.25) and ACN (gradient elution) with a flow rate of 1 mL/min.

Starting Material

The 4-aminobenzoic acid **1** was commercially obtained from Avra lab and used as provided.

4-((tert-butoxycarbonyl)amino)benzoic Acid (2)

This compound was prepared by addition of drop wise 1N NaOH (20 mL) to a solution of acid **1** (1 g, 4.21 mmol) in THF (5 mL) at 0 ºC. To this was added di-*tert*-butyl dicarbonate (4 mL, 16.7 mmol) drop-wise over a period of 5 min with vigorous stirring. After 30 min, the mixture was brought to room temperature and allowed to stir overnight. The resulting mixture was concentrated to half of the original volume and then neutralized with dilute HCl (pH 5-6). The precipitated compound was filtered, washed with water and air dried to give the titled compound **2** (1.73 g, 97%) and it was obtained as a White color solid; mp 142-145 ^{o}C; IR (KBr): v 3214, 1734, 1662 cm^{-1}. TLC: Methanol, R_f ~ 0.7; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.3 (Br, s), 9.0 (Br, s), 8.06 (d, 2H, J 9.1 Hz), 7.63 (d, 2H, J 9.1 Hz), .71-1.56 (m, 2H), 1.45 (s, 9H); MS (ESI): m/z 238 $[M+H]^+$.

tert-butyl (4-(3-oxobutanoyl)phenyl)carbamate (3)

This compound was prepared by addition of 1,1'-Carbonyldiimidazole (1.7 g, 10.5 mmol) to a solution of 4-((tert-butoxycarbonyl)amino)benzoic acid (2) (2 g, 8.4 mmol) in Dimethyl formamide (5 mL) was added at 0 ^{o}C and the mixture was stirred vigorously for 1 h. Further, to a solution of NaH (60% oil dispersion) (0.5 g, 13 mmol) in Dimethylformamide (10 mL) was added acetone (1 mL, 13 mmol) at 0 ^{o}C and stirred for about 1 h. Subsequently, 1,1'-Carbonyldiimidazole coupled boc-protected isonipecotic acid was added to the mixture and stirred for 30 min. The reaction mixture was allowed to warm to room temperature and stirred for overnight. The resulting mixture was quenched with cool water and acidified to pH 5-6 with 5M citric acid. It was extracted with EtoAc/hexanes (1:1) (2 x 100 mL, then 2 x 50 mL) and the combined organic extracts were washed with brine and dried over anhydrous $NaSO_4$. The solvent was evaporated under reduced pressure which was then purified by silica gel column chromatography using EtoAc/hexanes (1:9) as an eluent to get compound the diketone **3** (1.04 g, 70%) and it was obtained as a white solid; mp 52-53 ^{o}C; IR (KBr): v 3358, 1686, 1605 cm^{-1}; TLC: EtoAc/ Hexanes (3:7), R_f ~ 0.5; ^{1}H NMR ($CDCl_3$, 300 MHz):): δ 9.3 (Br, 1H), 9.0 (Br, 1H), 8.06 (d, 2H, J 9.1 Hz), 7.63 (d, 2H, J 9.1 Hz), 71-1.56 (m, 2H), 1.45 (s, 9H); 2.07 (s, 3H), 1.58 – 1.52 (m, 2H), 1.45 (s, 9H), MS (ESI): m/z 278 $[M+1]^+$.

General Procedure for the Synthesis of Boc Protected Sulphanilamide Phenyl Pyrazoles (4)

This compounds were prepared by addition 4-hydrazinylbenzenesulfonamide (1.0 g, 5.34 mmol) a solution of tert-butyl 4-(3-oxobutanoyl)piperidine-1-carboxylate **3** (2.0 g, 7.4 mmol) in ethanol (75 mL) was and refluxed for about 20 h. The resulting mixture was allowed to cool to room temperature and solvent was evaporated under reduced pressure. The residue was extracted with ethyl acetate (2 x 50 ml) followed by brine wash (2 x 30 ml). The combined organic layer was then dried over anhydrous $NaSO_4$ and the solvent was evaporated under reduced pressure which was then purified by silica gel column chromatography using EtoAc/ Hexanes (3:7) as an eluent to give compounds **4**.

tert-butyl (4-(3-methyl-1-(4-sulfamoylphenyl)-1H-pyrazol-5-yl)phenyl)carbamate (4)

This compound was prepared from compound **3** (2.0 g, 4.68 mmol) by the general procedure detailed above and it was obtained as a yellow solid (2.7 g, 83% yield); mp 160 - 163 ^{o}C; TLC: EtoAc/ Hexanes (6:4), R_f ~ 0.5. IR (KBr): v 3150,1703, 1415, 1315, 1151 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.3(Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 1H), 4.22 – 4.14 (m, 2H), 2.07 (s, 3H), 1.47 (s, 9H); MS (ESI): m/z 429 $[M+1]^+$.

General Procedure for the Synthesis of 4-(5-(4-aminophenyl)-3-methyl-1H-pyrazol-1-yl)benzene-sulfonamide (5)

This compound was prepared by the addition of HCl (0.5 ml) to a solution of tert-butyl 4-(3-methyl-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-5-yl)piperidine-1-carboxylate (**4)** in ethanol (10 ml) was and stirred vigorously for about 4 h at room temperature. The solvent was evaporated under reduced pressure and the obtained residue was washed with Methanol: EtoAc (1:9) (2 x 30 ml) to give compounds **5a**.

4-(5-(4-aminophenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (5)

This compound was prepared from compound **4** (2.0 g, 4.28 mmol) by the general procedure detailed above and it was obtained as a white solid (2.0 g, 97% yield); mp 183-187 ^{o}C. TLC: Methanol, R_f ~ 0.2; IR (KBr): v 3401, 1433, 1289, 1150 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs, 2H), 2.07 (s, 3H); MS (ESI): m/z 329 $[M+1]^+$.

General Procedure for Synthesis of the Piperidine Urea Disubstituted Pyrazoles (6a-6l)

To a solution of Compound **4** (0.4 g, 0.936 mmol) in THF (15 mL) was added Et_3N (2 mL) and stirred for about 20 min. To the resulting mixture, the corresponding isocyanate was added at 0 ^{o}C and allowed to come to room temperature and stirred for 3 h. The solvent was evaporated under vacuum to give respective urea derivatives which was further purified by column chromatography using EtoAc/hexanes as an eluent.

4-(5-(4-(3-ethylureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6a)

This compound was prepared by treating compound **4** with ethyl isocyanate using the procedure detailed above and it was obtained as a white solid in 93% as yield; mp 245-246 ^{o}C; IR (KBr): v 3414, 3189, 1627, 1333, 1162 cm^{-1}; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.2; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs, 2H), 2.23 (s, 3H), 1.62 – 1.48 (m, 2H), 1.11 (t, 3H, *J* 7.2 Hz); MS (ESI): m/z 400 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{19}H_{21}N_5O_3S$: 400.17480 and found: 400.17509; Purity: 98.83% (R_t = 8.56 min).

4-(3-methyl-5-(4-(3-propylureido)phenyl)-1H-pyrazol-1-yl)benzenesulfonamide (6b)

This compound was prepared by treating compound **4** with propyl isocyanate using the procedure detailed above and it was obtained as a white solid in 95% as yield; mp 251-253 ^{o}C; IR (KBr): v 3435, 3187, 1623, 1331, 1164 cm^{-1}; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.2; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs, 2H), 2.23 (s, 3H), 1.62 – 1.48 (m, 2H), 1.11 (t, 3H, *J* 7.2 Hz), 0.9 (t, 3H, *J* 7.3 Hz); MS (ESI): m/z 414 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{20}H_{23}N_5O_3S$: 414.18994 and found: 414.18994; Purity: 97.61% (R_t = 9.52 min).

4-(5-(4-(3-isopropylureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6c)

This compound was prepared by treating compound **4** with isopropyl isocyanate using the procedure detailed above and it was obtained as a white solid in 96% as yield; mp 257-259 ^{o}C; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.2. IR (KBr): v 3421, 3188, 1627, 1330, 1164 cm^{-1}. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs, 2H), 2.23 (s, 3H), 1.62 – 1.48 (m, 2H), 1.11 (t, 6H, *J* 7.2 Hz); MS (ESI): m/z 414 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{20}H_{23}N_5O_3S$: 414.18994 and found: 414.18990; Purity: 99.01% (R_t = 9.49 min).

4-(5-(4-(3-butylureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6d)

This compound was prepared by treating compound **4** with butyl isocyanate using the procedure detailed above and it was obtained as a white solid in 95% as yield; mp 180-182 ^{o}C; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.3. IR (KBr): v 3430, 3413, 1615, 1322, 1161 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs,

2H), 2.23 (s, 3H), 1.62 – 1.48 (m, 2H), 0.90 (t, 3H, *J* 7.1 Hz); MS (ESI): m/z 428 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{21}H_{25}N_5O_3S$: 428.20517 and found: 428.20302; Purity: 90.62% (R_t = 10.57 min).

4-(5-(4-(3-cyclohexylureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6e)

This compound was prepared by treating compound **4** with cyclohexyl isocyanate using the procedure detailed above and it was obtained as a white solid in 94% as yield; mp 259-260 ^oC; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.2; IR (KBr): v 3407, 3187, 1620, 1327, 1161 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 7.63 (d, 2H, *J* 9.1 Hz), 6.07 (s, 2H), 4.22 – 4.14 (Brs, 2H), 2.23 (s, 3H), 1.83 - 1.67 (m, 4H), 1.61 - 1.43 (m, 4H), 1.29 - 1.12 (m, 3H); MS (ESI): m/z 454 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{23}H_{27}N_5O_3S$: 454.22055 and found: 454.22204; Purity: 93.75% (R_t = 11.59 min).

4-(5-(4-(3-benzylureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6f)

This compound was prepared by treating compound **4** with phenyl isocyanate using the procedure detailed above and it was obtained as a white solid in 95 % as yield; mp 231-233 ^oC; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.3. IR (KBr): v 3294, 1633, 1340, 1165 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.02 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 8.5 Hz), 7.88 – 7.80 (m, 2H), 7.55 – 7.51 (m, 2H), 7.29 – 7.26 (m, 2H), .1 (s, 1H), 4.36 (d, 2H, *J* 12.86 Hz), 4.18 – 4.13 (Brs, 2H), 2.89 – 2.83 (m, 2H), 2.78 – 2.69 (m, 1H), 2.26 (s, 3H), 1.59 – 1.51 (m, 2H); MS (ESI): m/z 462 $[M+1]^+$. HRMS (ESI *m/z*) Calcd for $C_{24}H_{23}N_5O_3S$ 462.17911 and found: 462.17984; Purity: 96.24% (R_t = 11.03 min).

4-(3-methyl-5-(4-(3-(p-tolyl)ureido)phenyl)-1H-pyrazol-1-yl)benzenesulfonamide (6g)

This compound was prepared by treating compound **4** with *p*-tolyl isocyanate using the procedure detailed above and it was obtained as a white solid in 95% as yield; mp 213 – 215 ^oC. TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.3; IR (KBr): v 3302, 1633, 1594, 1513, 1247, 1151 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.02 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 8.5 Hz), 7.88 – 7.80 (m, 2H), 7.55 – 7.51 (m, 2H), 7.29 – 7.26 (m, 2H), 6.1 (s, 1H), 4.27 (d, 2H, *J* 13.6 Hz), 2.27 (s, 3H), 1.72 - 1.55 (m, 4H); MS (ESI): m/z 462 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{24}H_{23}N_5O_3S$: 462.191510 and found: 462.191510; Purity: 97.7 % (R_t = 9.7 min).

4-(5-(4-(3-(2-chlorophenyl)ureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6h)

This compound was prepared by treating compound **4** with *p*-chlorophenyl isocyanate using the procedure detailed in above and it was obtained as a white solid in 96% as yield; mp 179 - 181 ^oC; TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.3, IR (KBr): v 3319, 1630, 1593, 1530, 1299, 1149 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.02 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 8.5 Hz), 7.88 – 7.80 (m, 2H), 7.55 – 7.51 (m, 2H), 7.29 – 7.26 (m, 2H), 6.1 (s, 1H), 4.27 (d, 3H, *J* 13.6 Hz), 2.27 (s, 3H); MS (ESI): m/z 482 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{23}H_{20}ClN_5O_3S$: 482.14130 and found: 482.14087; Purity: 98.5 % (R_t = 10.0 min).

4-(5-(4-(3-(4-chlorophenyl)ureido)phenyl)-3-methyl-1H-pyrazol-1-yl)benzenesulfonamide (6i)

This compound was prepared by treating compound **4** with 4-chloro isocyanate using the procedure detailed above and it was obtained as a white solid in 93% as yield; mp 245-246 ^oC. IR (KBr): v 3414, 3189, 1627, 1333, 1162 cm^{-1}. TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.3; ^1H NMR (DMSO-d_6, 500 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.02 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 8.5 Hz), 7.88 – 7.80 (m, 2H), 7.55 – 7.51 (m, 2H), 7.29 – 7.26 (m, 2H), 6.1 (s, 1H), 4.27 (d, 3H, *J* 13.6 Hz), 2.27 (s, 3H); MS (ESI): m/z 481 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{23}H_{20}ClN_5O_3S$: 482.14130 and found: 482.14087; Purity: 98.1 % (R_t = 9.5 min).

1-(2-ethylphenyl)-3-(4-(3-methyl-1-(4-(methylsulfonyl)phenyl)-1H-pyrazol-5-yl)phenyl)urea (6j)

This compound was prepared by treating compound **4** with 2-ethylphenyl isocyanate using the procedure detailed above and it was obtained as a white solid in 94% as yield; mp 222-224 ^oC. IR (KBr): v 3343, 1620, 1595, 1532, 1296, 1150 cm^{-1}. TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.4. ^1H NMR (DMSO-d_6, 500 MHz): δ 9.3 (Br, 1H), 8.06 (d, 2H, *J* 9.1 Hz), 8.02 (d, 2H, *J* 9.1 Hz), 8.0 (d, 2H, *J* 8.5 Hz), 7.88 – 7.80 (m, 2H), 7.55 – 7.51 (m, 2H), 7.29 – 7.26 (m, 2H), 6.1 (s, 1H), 4.27 (d, 2H, *J* 13.6 Hz), 2.27 (s, 3H); 1.63 - 1.48 (m, 3H), 1.25 (t, 3H, *J* 7.2 Hz); MS (ESI): m/z 476 $[M+1]^+$. HRMS (ESI *m/z*) Calcd for $C_{25}H_{25}N_5O_3S$: 476.21005 and found: 476.21114; Purity: 99.3 % (R_t = 9.2 min).

4-(3-methyl-5-(4-(3-phenethylureido)phenyl)-1H-pyrazol-1-yl)benzenesulfonamide (6k)

This compound was prepared by treating compound **4** with *N*-phenyl ethyl isocyanate using the procedure detailed above and it was obtained as a white solid in 93% as yield; mp 72 - 74 ^oC. IR (KBr): v 3349, 1629,

1594, 1534, 1298, 1150 cm^{-1}. TLC: EtoAc/ Hexanes (9:1), R_f ~ 0.5. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 9.3 (Br, 1H), 8.07 (d, 2H, *J* 8.5 Hz), 7.67 (d, 2H, *J* 8.5 Hz), 7.31 - 7.17 (m, 4H), 6.08 (s, 1H), 5.98 (brs, 1H), 4.05 (d, 2H, *J* 13.2 Hz), 2.95 - 2.86 (m, 1H), 2.81 (t, 2H, *J* 7.2 Hz), 2.69 (t, 2H, *J* 12.1 Hz), 2.28 (s, 3H), 1.80 (d, 2H, *J* 12.3 Hz), 1.61 - 1.47 (m, 2H); MS (ESI): m/z 476 $[M+1]^+$; HRMS (ESI *m/z*) Calcd for $C_{25}H_{25}N_5O_3S$: 476.21005 and found: 476.21005; Purity: 94.3 % (R_t = 9.2 min).

sEH IC_{50} Assay Procedure

4-(3-methyl-5-(4-(3-phenethylureido)phenyl)-1H-pyrazol-1-yl)benzenesulfonamide Inhibition at 100μM concentration) was determined using Soluble Epoxide Hydrolase Inhibitor Screening Assay kit (Catalog. no.10011671), Cayman Chemical, Ann Arbor, MI, USA). Enzymes (5μl human sEH) were incubated with 2μl inhibitors for 15 min in 25 mM Bis-Tris/HCl buffer (188μl; pH 7.0) at 25 ^oC before 5μl of substrate (cyano(2-methoxynaphthalen-6-yl)methyl trans-(3-phenyl-oxyran-2-yl)methyl carbonate (CMNPC) was added. Activity was assessed by measuring the appearance of the fluorescent 6-methoxynaphth-aldehyde product (λ_{em} = 330 nm, λ ex = 465 nm) at 25 ^oC during a 15 min incubation (Spectramax M2; Molecular Device, Inc., Sunnyvale, CA). The IC_{50}s were calculated from at least three separate runs, each in triplicate, to obtain the standard deviation given in the Results section. The IC_{50} was determined from at least four points in the linear region of the inhibition curve with at least one point above and one below the IC_{50}.

In Vitro Cyclooxygenase (COX) Inhibition Assay

The ability of the test compounds 6a-k to inhibit ovine COX-1 and human recombinant COX-2 (% inhibition at 100 μM and IC_{50} values (μM), respectively) was determined using an COX Fluorescent Inhibitor Screening Assay Kit (catalogue no. 700100, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Stock solutions of test compounds were dissolved in a minimum volume of DMSO. Briefly, to a series of supplied reaction buffer solutions (150 μL, 100 mM Tris-HCl, pH 8.0) with either COX-1 or COX-2 (10 μL) enzyme in the presence of Heme (10 μL) and fluorometric substrate (10 μL) were added 10 μL of various concentrations of the test compound solutions ([I] between 0.01 and 100 μM). The reactions were initiated by quickly adding 10 μL of arachidonic acid solution and then incubated for 2 min at room temperature. Fluorescence of resorufin that is produced by the reaction between PGG final and the fluorometric substrate, 10-acetyl-3,7-dihydroxyphen-oxazine (ADHP), were analyzed with an excitation wavelength of 535 nm and an emission wavelength of 590 nm. The intensity of this fluorescence is proportional to the amount of resorufin, which is proportional to the amount of PGG2 present in the well during the incubation. Percent inhibition was calculated by comparison from the 100% initial activity sample value (no inhibitor). The concentration of the test compound causing 50% inhibition of COX-2 (IC50, μM) was calculated from the concentration inhibition response curve (triplicate determinations).

The ability of compounds 6a–e and Celecoxib to inhibit ovine COX-1 and recombinant human COX-2 was determined using a COX fluorescence inhibitor assay (catalog number 700100), Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's assay protocol. Compounds 6a–e was assayed in concentrations ranging from 10 M to10^{-3} M. PRISM5 software was used for the calculation of IC_{50} values.

REFERENCES

[1] Vane JR. The mode of action of aspirin and similar compounds. J Allergy Clin Immunol 1976; 58: 691-12. http://dx.doi.org/10.1016/0091-6749(76)90181-0

[2] Rao BN, Synthesis of 1,5-Diarylpyrazoles as Potential COX-2 Inhibitors with Nitric Oxide Releasing Ability. Lett Drug Design Discov 2013; 10: 594-603. http://dx.doi.org/10.2174/1570180811310070006

[3] Moncada S, Ferreira SH, Vane JR, Prostaglandins, aspirin-like drugs and the oedema of inflammation. Nature 1973; 246: 217-19. http://dx.doi.org/10.1038/246217a0

[4] Tamblyn R, Berkson L, Dauphinee WD, Gayton D, Grad RA, *et al*. Unnecessary prescribing of NSAIDs and the management of NSAID related gastropathy in medical practice. Ann Intern Med 1997; 127: 429-38. http://dx.doi.org/10.7326/0003-4819-127-6-199709150-00003

[5] Masferrer JL, Zweifel BS, Seibert K, Needleman P. Selective regulation of cellular cyclooxygenase by dexamethasone and endotoxin in mice. J Clin Invest 1990; 86: 1375-79. http://dx.doi.org/10.1172/JCI114850

[6] Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of amitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 1991; 88: 2692-96. http://dx.doi.org/10.1073/pnas.88.7.2692

[7] Kujubu DA, Herschman HR. Dexamethasone inhibits mitogen induction of the TIS10 prostaglandin synthase/cyclooxygenase gene. J Biol Chem 1992; 267: 7991-94.

[8] Meade EA, Smith WL, DeWitt D L. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1993; 268: 6610-14.

[9] Vane JR, Bakhle YS, Botting RM. Cyclooxygenase 1 and 2. Ann Rev Pharmacol Toxicol 1998; 38: 97-120. http://dx.doi.org/10.1146/annurev.pharmtox.38.1.97

[10] Newman JW, Morisseau C, Hammock BD. Epoxide hydrolases: Their roles and interactions with lipid metabolism. Prog Lipid Res 2005; 44: 1-51. http://dx.doi.org/10.1016/j.plipres.2004.10.001

[11] Morisseau C, Hammock BD. Epoxide hydrolases: Mechanisms, inhibitor designs, and biological roles. Annu Rev Pharmacol Toxicol 2005; 45: 311-33. http://dx.doi.org/10.1146/annurev.pharmtox.45.120403.095920

[12] Fretland AJ, Omiecinski CJ. Epoxide hydrolases: biochemistry and molecular biology. Chem Biol Interact 2000; 129: 41-59. http://dx.doi.org/10.1016/S0009-2797(00)00197-6

[13] Xiang F. Recent Patents on Cardiovascular Drug Discovery. 2006; 1: 1-7.

[14] Falck JR, Reddy LM, Reddy YK. 11, 12-epoxyeicosatrienoic acid (11, 12-EET): structural determinants for inhibition of TNF-alpha induced VCAM-1 expression. Bioorg Med Chem Lett 2003; 13: 4011-14. http://dx.doi.org/10.1016/j.bmcl.2003.08.060

[15] Node K, Ruan XL, Dai J. Activation of G as mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoicacids. J Biol Chem 2001; 276: 15983-89. http://dx.doi.org/10.1074/jbc.M100439200

[16] Node K, Huo Y, Ruan X. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science 1999; 285: 1276-79. http://dx.doi.org/10.1126/science.285.5431.1276

[17] Fitzpatrick FA, Ennis MD, Baze ME. Inhibition of cyclooxygenase activity and platelet aggregation by epoxyeicosatrienoic acids. Influence of stereochemistry. J Biol Chem 1986; 261: 15334-38.

[18] Iber H, Chen Q, Cheng PY. Suppression of CYP2C11 gene transcription by interleukin-1 mediated by NF-kappaB binding at the transcription start site. Arch Biochem Biophys 2000; 377: 187-94. http://dx.doi.org/10.1006/abbi.2000.1772

[19] Kessler P, Popp R, Busse R. Proinflammatory mediators chronically downregulate the formation of the endothelium-derived hyperpolarizing factor in arteries *via* a nitric oxide/cyclic GMP-dependent mechanism. Circulation 1999; 99: 1878-84. http://dx.doi.org/10.1161/01.CIR.99.14.1878

[20] Wallace JL, Ignarro LJ, Fiorucci S. Potential cardioprotective actions of NO-releasing aspirin. Nat Rev Drug Discov 2002; 1: 375-82. http://dx.doi.org/10.1038/nrd794

[21] Dailey LA, Imming P. 12-Lipoxygenase: Classification, possible therapeutic benefits from inhibition and inhibitors. Curr Med Chem 1999; 6: 389-98.

[22] Zalewski A, Nelson JJ, Heg JJ, Macphee C. Lp-PLA22: A new kid on the block. Clin Chem 2006; 52: 1645-49. http://dx.doi.org/10.1373/clinchem.2006.070672

[23] Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: A novel therapeutic target. Pharmacol Rev 2007; 59: 207-24. http://dx.doi.org/10.1124/pr.59.3.1

[24] Friesen RW, Mancini JA. Microsomal prostaglandin E synthase-1 (mPGES-1): A novel antiinflammatory therapeutic target. J Med Chem 2008; 51: 4059-67. http://dx.doi.org/10.1021/jm800197b

[25] Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein protein interfaces. Nature 2007; 450: 1001-1009. http://dx.doi.org/10.1038/nature06526

[26] Taylor PC. The future of TNF-α antagonism. Future Rheumatol 2007; 2: 233-36. http://dx.doi.org/10.2217/17460816.2.3.233

[27] Chan DS, Lee HM, Yang F, Che CM, Wong CCL, Abagyan R, *et al*. Structure-based discovery of natural-product-like TNF-*a* inhibitors. Angew Chem Int Ed 2010; 49: 2860-64. http://dx.doi.org/10.1002/anie.200907360

[28] Smith AS, Oslob JD, Flanagan WM, Braisted AC, Whitty A, Cancilla MT, *et al*. Small-molecule inhibition of TNF-alpha. Science 2005; 310: 1022-25. http://dx.doi.org/10.1126/science.1116304

[29] Magda AA, El Sayed, Naglaa I, Abdel Aziz, Alaa AM, Abdel Aziz, *et al*. Design, synthesis, and biological evaluation of substituted hydrazone and pyrazole derivatives as selective COX-2 inhibitors: Molecular docking study. Bioorg Med Chem 2011; 19: 3416-24. http://dx.doi.org/10.1016/j.bmc.2011.04.027

[30] Hwang S H, Wagner K M, Morisseau C, Liu J Y, Dong H, Aaron T, Wecksler A T, Hammock B D. Synthesis and Structure Activity Relationship Studies of Urea-Containing Pyrazoles as Dual Inhibitors of Cyclooxygenase-2 and Soluble Epoxide Hydrolase. J Med Chem 2011; 54: 3037–3050. http://dx.doi.org/10.1021/jm2001376

Received on 08-08-2013 Accepted on 11-09-2013 Published on 30-12-2013

DOI: http://dx.doi.org/10.12970/2308-8044.2013.01.02.5