Selective Cyclooxygenase Inhibitors: Novel 1,2-Diarylcyclopentenes Are Potent and Orally Active COX-2 Inhibitors

David B. Reitz,*,† James J. Li,† Monica B. Norton,†
Emily J. Reinhard,† Joe T. Collins,†
Gary D. Anderson,‡ Susan A. Gregory,‡
Carol M. Koboldt,‡ William E. Perkins,‡
Karen Seibert,‡ and Peter C. Isakson‡

Searle Research & Development, c/o Monsanto Company, 700 Chesterfield Parkway North, Chesterfield, Missouri 63198

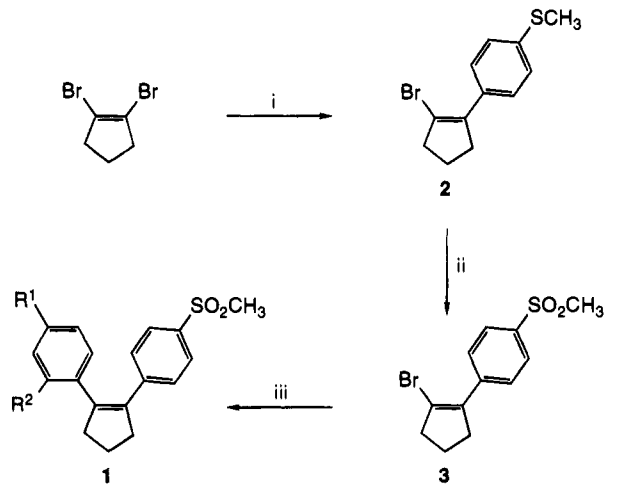
Received August 5, 1994

Prostaglandins (PGs) play a major role in the inflammation process, and the inhibition of PG production has been a common target of antiinflammatory drug discovery.[1,2] Nonsteroidal antiinflammatory drugs (NSAIDs) that are active in reducing the pain and swelling associated with inflammation also affect other prostaglandin-regulated processes not associated with inflammation. Thus, ingestion of high doses of most common NSAIDs can produce side effects, including life-threatening ulcers, that may limit their therapeutic potential.[3] An alternative to NSAIDs is the use of corticosteroids, which have even more severe side effects, especially when long-term therapy is involved.[4]

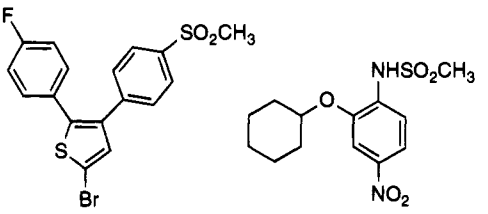
NSAIDs have been found to prevent the production of prostaglandins by inhibiting conversion of arachidonic acid to PGs by the constitutive cyclooxygenase enzyme (COX-1).[1,2] More recently, a previously unknown enzyme in the human arachidonic acid/prostaglandin pathway was discovered[5-7] and designated "cyclooxygenase II (COX-2)" or "prostaglandin G/H synthase II". Cytokines and endotoxins have been reported[8] to induce COX-2 expression, and such induction is inhibited by glucocorticoids. The discovery of an inducible enzyme (COX-2) associated with inflammation provides a novel target for therapeutic intervention with the potential for more effective reduction of inflammation with fewer side effects.

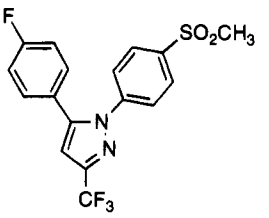
Due to the novelty of this approach, the literature contains very few documented examples of selective cyclooxygenase inhibitors. Gans et al.[9] have reported that the thiophene DuP 697 (ED_{50} = 0.18 mpk) shows antiinflammatory activity in the rat adjuvant-induced arthritis model without the concomitant formation of gastric lesions at 400 mpk, which is the pharmacological profile expected of a selective COX-2 inhibitor. Similarly, Futaki et al.[10] have reported that the methanesulfonamide NS-398 (ED_{30} = 4.7 mpk) also shows antiinflammatory activity in the rat adjuvant-induced arthritis model without the concomitant formation of gastric lesions at 1000 mpk. Moreover, several groups have now reported that NS-398 is a selective inhibitor of COX-2.[11,12] Recently, Isakson et al.[13] reported that the pyrazole SC-58125 (COX-1 IC_{50} > 100 μM, COX-2 IC_{50} = 0.09 μM) is a selective inhibitor of the inducible form of human recombinant cyclooxygenase and is orally active (ED_{50} = 0.4 mpk) in rat adjuvant-induced arthritis.

Scheme 1[a]



[a] Reagents: (i) Pd^0, 4-$CH_3SC_6H_4B(OH)_2$, Na_2CO_3, $PhCH_3$, Δ; (ii) Oxone, THF, H_2O; (iii) Pd^0, 2-R^2-4-$R^1C_6H_3B(OH)_2$, Na_2CO_3, $PhCH_3$, Δ.





Our interest in this area has been to develop novel selective inhibitors of cyclooxygenase that have improved therapeutic properties relative to currently used NSAIDs. Toward that goal, we have investigated a series of 1,2-diaryl-substituted cyclopentenes and now report our preliminary results.

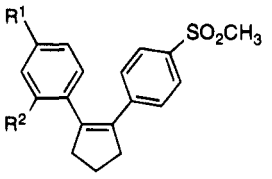
The spatial disposition of the 1,2-diaryl rings of DuP 697 and SC-58125, relative to the carbon–carbon double bond of their respective heterocyclic rings, was thought to play an important role in cyclooxygenase inhibition. It was believed that the function of the heterocyclic ring was to provide the necessary double bond geometry and that the heterocycle itself was not essential for good activity. To test this hypothesis, carbocycles (cyclopentenes) were investigated as heterocyclic surrogates; this substitution in both DuP 697 and SC-58125 would produce the same 1,2-diarylcyclopentene analog, i.e., 1-[2-(4-fluorophenyl)cyclopenten-1-yl]-4-(methylsulfonyl)benzene (**1a**, Table 1).

The synthesis of **1a** (SC-57666) from commercially available 1,2-dibromocyclopentene in three relatively simple steps is outlined in Scheme 1. Suzuki coupling[14]

† Department of Medicinal Chemistry.
‡ Department of Inflammatory Diseases Research.

0022-2623/94/1837-3878$04.50/0

Table 1. Cyclopentene Cyclooxygenase Inhibitors



compd[a]	R1	R2	IC50 (μM) COX-1[b]	IC50 (μM) COX-2[b]	n[c]	selectivity
1a	F	H	>100	0.026	11	>3800
1b	OCH_3	H	9.92	0.005	3	1980
1c	Cl	H	>100	0.003	4	>33,000
1d	CH_3	H	>100	0.003	2	>33,000
1e	H	H	>100	2.25	2	>44
1f	CF_3	H	>100	0.865	2	>120
1g	Cl	Cl	>100	0.053	2	>1900
1h	CN	H	>100	77.9	3	>1.3
1i	CH_2OH	H	>100	3.20	1	>31
1j	CH_2OCH_3	H	>100	6.60	2	>15
1k	SCH_3	H	>100	0.221	4	>450
1l	F	CH_3	>100	0.075	3	>1300
NS-398			>10	0.01	3	>1000
indomethacin			0.2	1.2	10	0.167

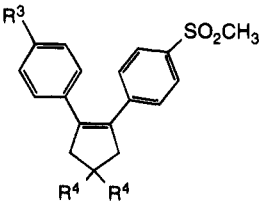
[a] See ref 20. [b] See ref 21. [c] Number of assays conducted.

of [4-(methylthio)phenyl]boronic acid (prepared from 4-bromothioanisole, *n*-butyllithium, and trimethyl borate) with an excess of commercially available 1,2-dibromocyclopentene in the presence of tetrakis(triphenylphosphine)palladium(0) provided a mixture of monocoupled and dicoupled material that could be separated by silica gel chromatography to give **2** in 45% yield. Selective oxidation of the sulfide **2** to the corresponding sulfone **3** in the presence of the cyclopentene double bond was conveniently accomplished with potassium peroxymonosulfate (Oxone) in 90–95% yield. A second Suzuki coupling with commercially available (4-fluorophenyl)boronic acid gave **1a** in 89% yield.

Compound **1a** was found to be a very potent COX-2 inhibitor (IC_{50} = 0.026 μM),[15] essentially devoid of COX-1 activity (IC_{50} > 100 μM). On the basis of these results, a structure–activity relationship (SAR) study was conducted by varying only the substituents on the 4-fluorophenyl ring of **1a** to determine if COX-2 potency and/or selectivity could be increased. The results of this study are summarized in Table 1 for analogs **1a–1l** along with reference compounds NS-398 and indomethacin.

Substitution at the 4-position of the 4-fluorophenyl ring of **1a** had little effect on the inhibition of the constitutive COX-1 enzyme. With the exception of **1b** (R^1 = OCH_3, COX-1 IC_{50} = 9.92 μM), all 1,2-diarylcyclopentene analogs in Table 1 are essentially inactive on COX-1 (COX-1 IC_{50} > 100 μM). On the other hand, substituents did have a dramatic effect on the inhibition of the inducible COX-2 enzyme, and thus on selectivity. Replacing fluorine with chlorine or a methyl group gave **1c** (COX-2 IC_{50} = 0.003 μM) or **1d** (COX-2 IC_{50} = 0.003 μM), respectively, which were almost an order of magnitude more potent than **1a**. Moreover, both analogs had impressive enzyme selectivity ratios of >33,000. Some type of substituent at the 4-position of the 4-fluorophenyl ring of **1a** appears to be necessary for good COX-2 inhibition, since removal of the substituent altogether gave the phenyl analog **1e** (COX-2 IC_{50} = 2.25 μM), which was almost 2 orders of magnitude less potent than **1a**.

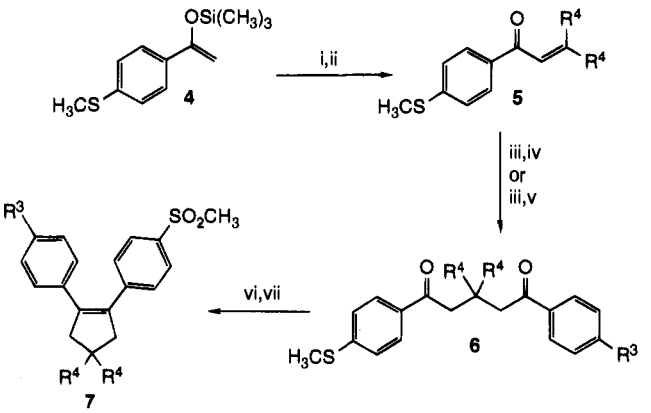
Table 2. 4,4-Disubstituted Cyclopentene Cyclooxygenase Inhibitors



compd[a]	R3	R4	IC50 (μM) COX-1[b]	IC50 (μM) COX-2[b]	n[c]	selectivity
1	F	H	>100	0.026	11	>3800
7a	F	CH_3	18.3	0.015	3	1200
7b	Cl	CH_3	1.6	0.007	3	230
7c	F	CF_3	>100	0.067	3	>1500
7d	F	C_2H_5	>100	65	2	>1.5
7e	F	CH_2F	58	0.051	1	1100

[a] See ref 20. [b] See ref 21. [c] Number of assays conducted.

Scheme 2[a]

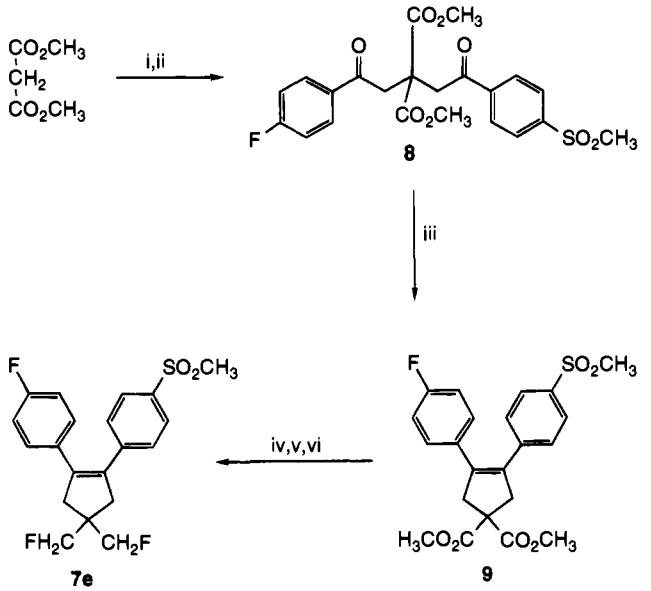


[a] Reagents: (i) $TiCl_4$, $(R^4)_2CO$, CH_2Cl_2; (ii) $(CF_3CO)_2O$, $N(C_2H_5)_3$, CH_2Cl_2, 0 °C; (iii) −78 °C, 4-$R^3C_6H_4C{=}CH_2OSi(CH_3)_3$; (iv) $TiCl_4$, CH_2Cl_2; (v) $[(CH_3)_2N]_3S[(CH_3)_3SiF_2]$, THF; (vi) $TiCl_4$, Zn^0, THF; (vii) Oxone, THF, H_2O.

Substituents at the 4-position of cyclopentene ring of COX-2 inhibitors are oriented above and below the plane of the double bond and therefore offer a unique opportunity to probe the spatial requirements of the enzyme in these areas. By comparision, substituents at the 5-position of the thiophene DuP 697 and/or the 3-position of the pyrazole SC-58125 lie in the plane of the heterocycle. A second SAR study was conducted to ascertain the effects of geminal substitution at the 4-position of the cyclopentene ring of **1a**. The results of this study are summarized in Table 2.

The 4,4-disubstituted cyclopentene analogs shown in Table 2 were synthesized by the procedures outlined in Schemes 2 and 3. Scheme 2 was used to prepare analogs **7a–7d**. The silyl enol ether **4** (prepared from 4-(methylthio)acetophenone, chlorotrimethylsilane, sodium iodide, and triethylamine in acetonitrile) was reacted with the appropriate ketone and titanium(IV) chloride to give the corresponding β-hydroxy ketone intermediate which was dehydrated with trifluoroacetic anhydride and pyridine to give the α,β-unsaturated ketone **5**. Subsequent reaction with 4-$R^3C_6H_4C{=}CH_2$-$OSi(CH_3)_3$ (prepared as above for **4**) and titanium(IV) chloride (for analogs **7a–7c**) or tris(dimethylamino)-(trimethylsilyl)sulfur difluoride (TAS-F) (for analog **7d**) provided the 1,5-diketones **6**. McMurry coupling[16] of **6** with titanium(IV) chloride and metallic zinc in THF

Scheme 3[a]



[a] Reagents: (i) NaH, DMF, 4-$FC_6H_4COCH_2Br$, −20 °C; (ii) NaH, DMF, 4-$(CH_3SO_2)C_6H_4COCH_2Br$, −20 °C; (iii) $TiCl_4$, Zn^0, THF; (iv) DIBAL-H, THF; (v) C_6H_5N, 0 °C, 4-$CH_3C_6H_4SO_2Cl$; (vi) $(C_4H_9)_4NF$, THF, Δ.

gave the methyl sulfide cyclopentene analogs which were subsequently oxidized with Oxone to the corresponding methyl sulfone analogs shown in Table 2. Scheme 3 was used to prepare analog **7e**. Dimethyl malonate was successively dialkylated with 2-bromo-4-fluoroacetophenone and 2-bromo-4-(methylsulfonyl)-acetophenone to give the 1,5-diketone **8**, which was cyclized under McMurry conditions to give the cyclopentene analog **9**. Reduction with diisobutylalumunim hydride (DIBAL-H) provided the bis(hydroxymethyl) analog. Treatment with *p*-toluenesulfonyl chloride in pyridine gave the corresponding ditosylate, which was subsequently converted to the bis(fluoromethyl) analog **7e** by reaction with tetrabutylammonium fluoride in THF at reflux.

Table 2 shows that geminal substitution at the 4-position of 1,2-diarylcyclopentene cyclooxygenase inhibitors produced analogs which were generally less selective due to increased COX-1 activity. Furthermore, the decrease in COX-2 activity of the series **7a** (methyl) > **7e** (fluoromethyl) > **7c** (trifluoromethyl) > **7d** (ethyl) also suggests that the enzyme domain binding this region is highly sensitive to inhibitor steric bulk. In fact, over 3 orders of magnitude of COX-2 activity is lost by replacing methyl with ethyl (**7a** vs **7d**).

Figure 1 shows the dose−response curves for **1a** and **1c** in the rat adjuvant-induced arthritis model. While **1c** (COX-2 IC_{50} = 0.003 μM) is almost 1 order of magnitude more potent than **1a** (COX-2 IC_{50} = 0.026 μM) in enzyme inhibition, **1a** (ED_{50} = 1.7 mpk) is almost twice as active as **1c** (ED_{50} = 3.2 mpk) in the arthritis model. The superior *in vivo* performace of **1a** relative to **1c** is likely due to a combination of better absorption, longer half-life, lower first-pass clearance, and/or differential distribution;[17] however, at this time the exact cause is unknown. Additional *in vivo* testing was conducted in both mice and rats to address the central issue of GI toxicity. No gastric lesions were observed in mice after 5 h when **1a** was administered intragastrically at 600 mpk. Similarly, no intestinal damage

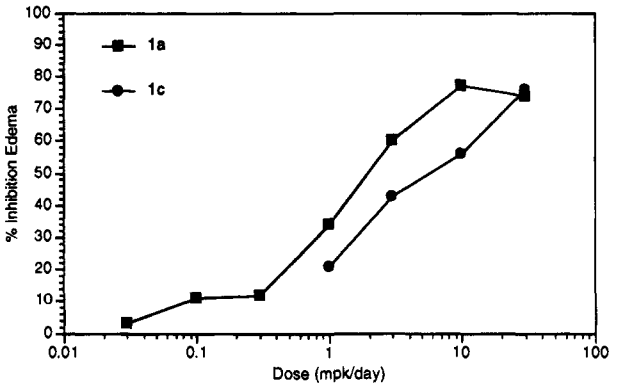


Figure 1. Rat adjuvant-induced arthritis dose−response curves for **1a** and **1c**. Each point represents an average of eight animals; see ref 9 for assay procedure.

was observed in rats after 72 h when **1a** was administered intragastrically at 200 mpk.[18]

In summary, novel 1,2-diarylcyclopentenes have been shown to be very potent COX-2 inhibitors with inhibition (IC_{50}) in the low nanomolar range and enzyme selectivity ratios as high as 4 orders of magnitude. *In vivo* testing in the rat adjuvant-induced arthritis model has established that 1,2-diarylcyclopentenes are orally active with edema inhibition (ED_{50}) in the low mpk range. Compound **1a** (COX-1 IC_{50} > 100 μM, COX-2 IC_{50} = 0.026 μM) has been shown to be orally active (ED_{50} = 1.7 mpk) in the adjuvant-induced arthritis model and therefore is a selective 1,2-diarylcyclopentene cyclooxygenase inhibitor of particular interest.[19] Moreover, no gastric or intestinal lesions were observed in mice at 600 mpk or rats at 200 mpk, respectively. Studies with selective 1,2-diarylcyclopentene cyclooxygenase inhibitors are continuing, and a more detailed report will appear subsequently.

Supplementary Material Available: Biological procedures for the *in vitro* human recombinant COX-1 and COX-2 assays and GI toxicity studies conducted in both mice and rats are available, detailed procedural examples for the synthesis of **1a** and **7e**, as well as the physical properties, spectral data, and elemental analyses for all analogs synthesized (10 pages). Ordering information is given on any current masthead page.

References

(1) Vane, J. R. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-Like Drugs. *Nature [New Biol.]* **1971**, *231*, 232−235.
(2) Smith, J. B.; Willis, A. L. Aspirin Selectively Inhibits Prostaglandin Production in Human Platelets. *Nature [New Biol.]* **1971**, *231*, 235−237.
(3) Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russell, R. I. Gastrointestinal Damage Associated with the Use of Nonsteroidal Antiinflammatory Drugs. *N. Engl. J. Med.* **1992**, *327*, 749−754.
(4) Haynes, R. C., Jr. Adrenocorticotropic Hormones; Adrenocortical Steroids and Their Synthetic Analogs; Inhibition of the Synthesis and Actions of Adrenocortical Hormones. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; McGraw-Hill: New York, 1993; pp 1442−1452.
(5) Hla, T.; Neilson K. Human Cyclooxygenase-2 cDNA. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7384−7388.
(6) Xie, W.; Chipman, J. G.; Robertson, D. L.; Erikson, R. L.; Simmons, D. L. Expression of a Mitogen-response Gene Encoding Prostaglandin Synthase is Regulated by mRNA Splicing. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2692−2696.
(7) Kujubu, D. A.; Fletcher, B. S.; Varnum, B. C.; Lim, R. W.; Herschman, H. R. TIS10, a Phorbol Ester Tumor Promoter-inducible mRNA from Swiss 3T3 Cells, Encodes a Novel Prostaglandin Synthase/Cyclooxygenase Homologue. *J. Biol. Chem.* **1991**, *266*, 12866−12872.

(8) Masferrer, J. L.; Seibert, K.; Zweifel, B.; Needleman, P. Endogenous Glucocorticoids Regulate an Inducible Cyclooxygenase Enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 3917–3921.
(9) Gans, K. R.; Galbraith, W.; Roman, R. J.; Haber, S. B.; Kerr, J. S.; Schmidt, W. K.; Smith, C.; Hewes, W. E.; Ackerman, N. R. Antiinflammatory and Safety Profile of DuP 697, a Novel Orally Effective Prostaglandin Synthesis Inhibitor. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 180–187.
(10) Futaki, N.; Yoshikawa, K.; Hamasaka, Y.; Arai, I.; Higuchi, S.; Iizuka, H.; Otomo, S. NS-398, A Novel Non-steroidal Antiinflammatory Drug with Potent Analgesic and Antipyretic Effects, which Causes Minimal Stomach Lesions. *Gen. Pharmacol.* **1993**, *24*, 105–110.
(11) Futaki, N.; Takahashi, S.; Yokoyama, M.; Arai, I.; Higuchi, S.; Otomo, S. NS-398, A New Antiinflammatory Agent, Selectively Inhibits Prostaglandin G/H Synthase/Cyclooxygenase (COX-2) Activity *in vitro*. *Prostaglandins* **1994**, *47*, 55–59.
(12) Masferrer, J. L.; Zweifel, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P. C.; Seibert, K. Selective Inhibition of Inducible Cyclooxygenase 2 *in vivo* is Antiinflammatory and Nonulcerogenic. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3228–3232.
(13) Isakson, P.; Seibert, K.; Masferrer, J.; Salvemini, D., Lee, L.; Needleman, P. Discovery of a Better Asprin. Presented at the Ninth International Conference on Prostaglandins and Related Compounds, Florence, Italy, June 1994.
(14) Miyaura, N.; Yanagi, T.; Suzuki, A. The Palladium-catalyzed Cross-coupling Reaction of Phenylboronic Acid with Haloarenes in the Presence of Base. *Synth. Commun.* **1981**, 513–515.
(15) Human recombinant enzymes were used in both COX-1 and COX-2 assays to determine inhibition (IC_{50}), see supplementary material for details.
(16) McMurry, J. E. Titanium-induced Dicarbonyl-coupling Reactions. *Acc. Chem. Res.* **1983**, *16*, 405–411.
(17) log *P* values for **1a** and **1c** were determined to be 3.89 and 4.73, respectively.
(18) See supplementary materials for experimental details.
(19) Reitz, D. B.; Li, J. U.S. Pat. 5 344 991, 1994.
(20) All new compounds were fully characterized spectrally; purity was established by a combination of analytical HPLC and HRMS and/or combustion analysis; see supplementary material for data.
(21) Seibert, K.; Zhang, Y.; Leahy, K. M.; Hauser, S. D.; Masferrer, J. L.; Isakson, P. Procedings of the Third International Conference on Eicosanoids and Other Bioactive Lipids. In Cancer, Inflammation and Radiation Injury; Kluwar Academic Publishers, in press. The *in vitro* human COX-1 and human COX-2 Assays used are also detailed in the supplementary material.