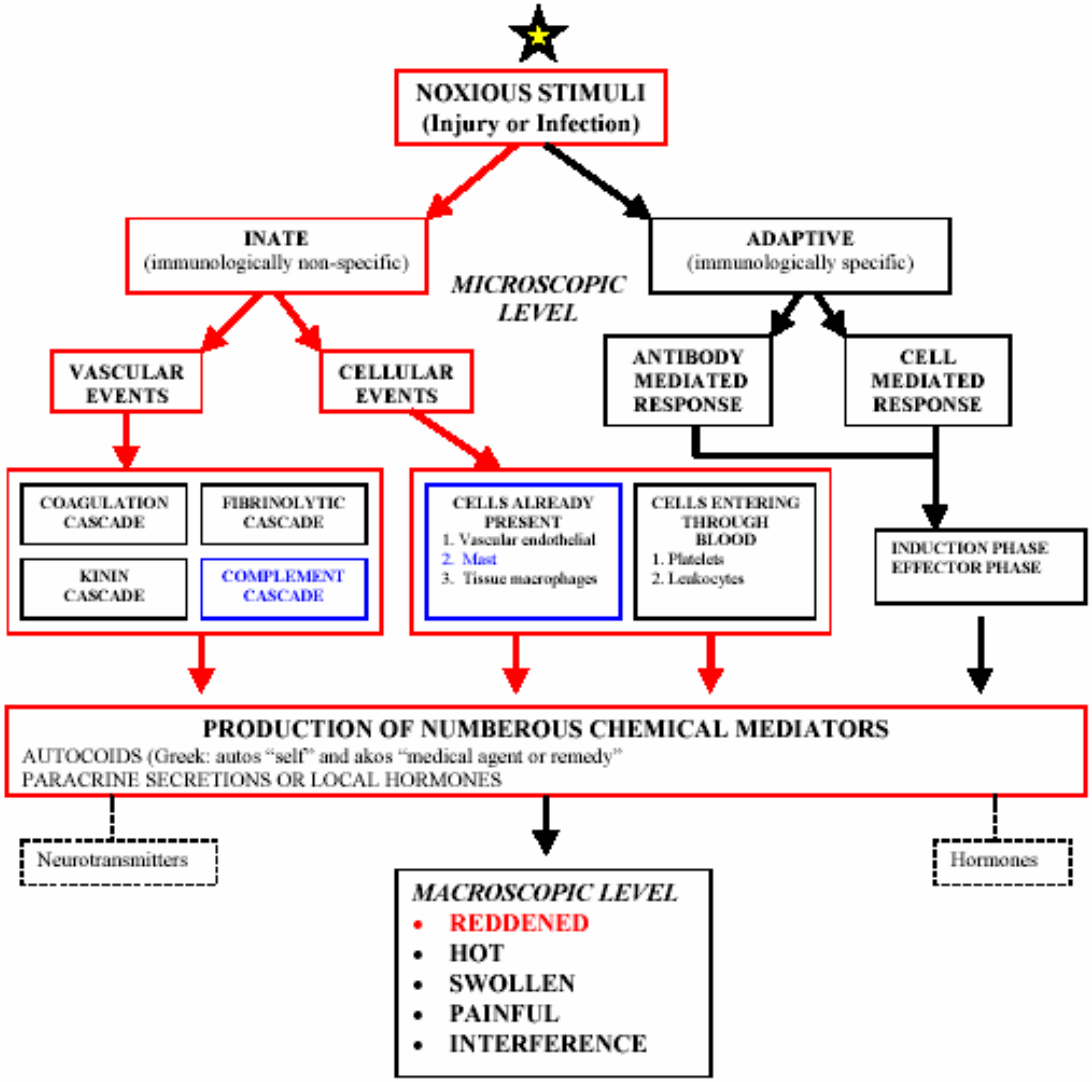
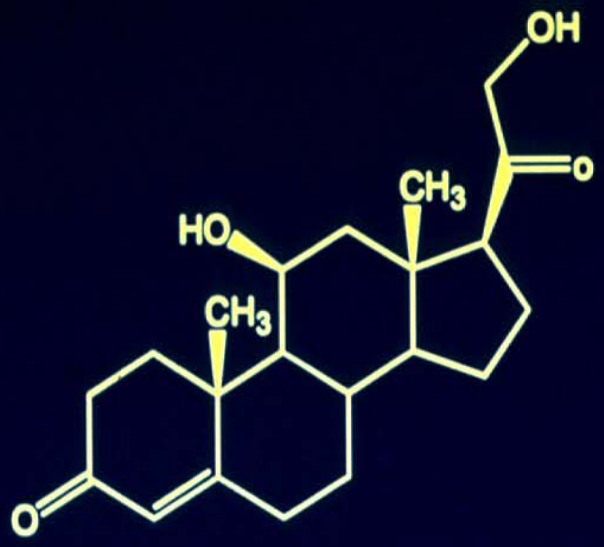
From Aspirin to Coxibs and Beyond

Inflammation is the response of the body to invasion by a pathogen (infection) or injury, directed at destroying the infectious agents and repairing the damaged areas.



STEROIDAL AND NON-STEROIDAL ANTIINFLAMATORY DRUGS (NSAIDS)



Synthesis of cortisone, Sarett et al., J. Biol. Chem.,162, 601 (1946)

- Introduction of cortisone® was followed by many other improved steroidal antiinflamatory drugs but side effects were many including effects on salt and calcium balance
- Aspirin, the butazones and related compounds came to be known as non-steroidal antiinflamatory drugs (or NSAIDS)

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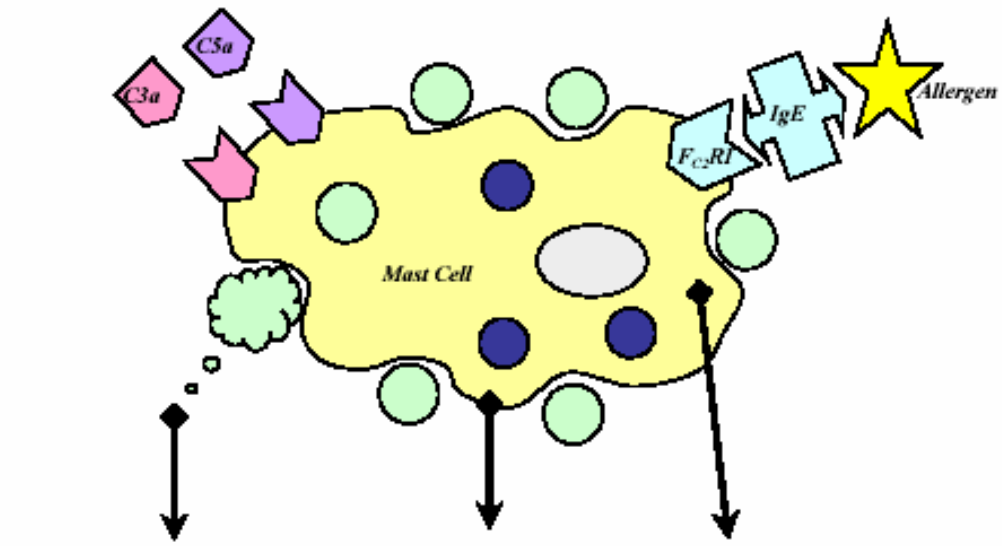
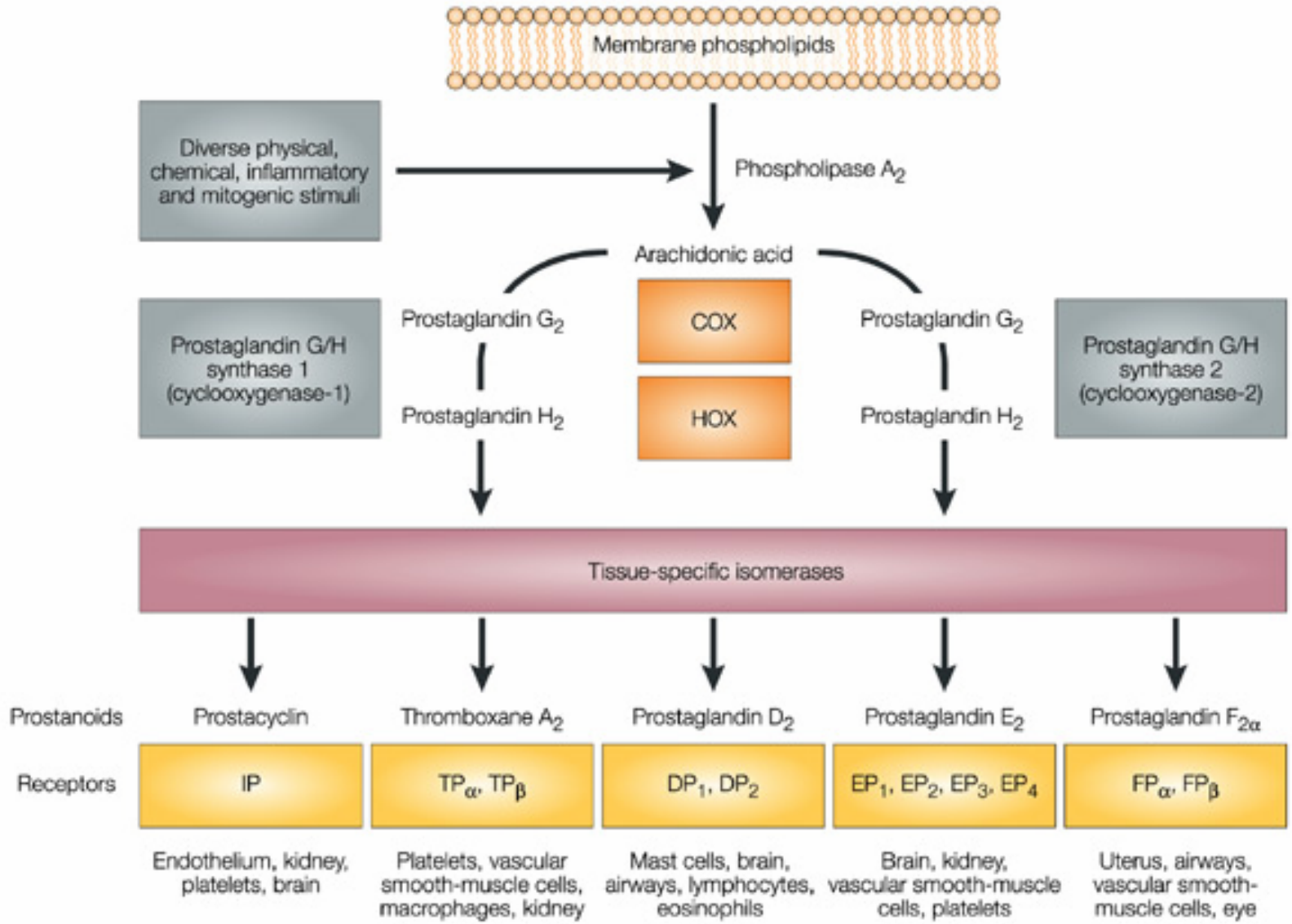
Inflammatory Mediators Released by Mast Cell

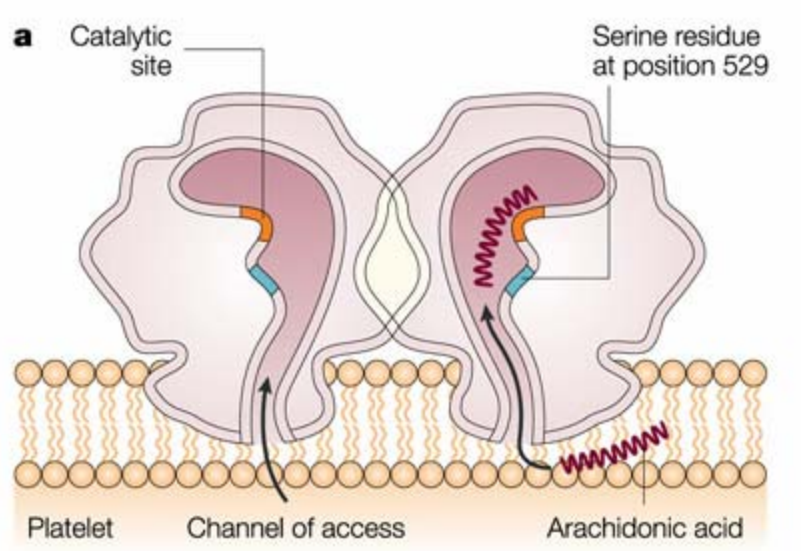


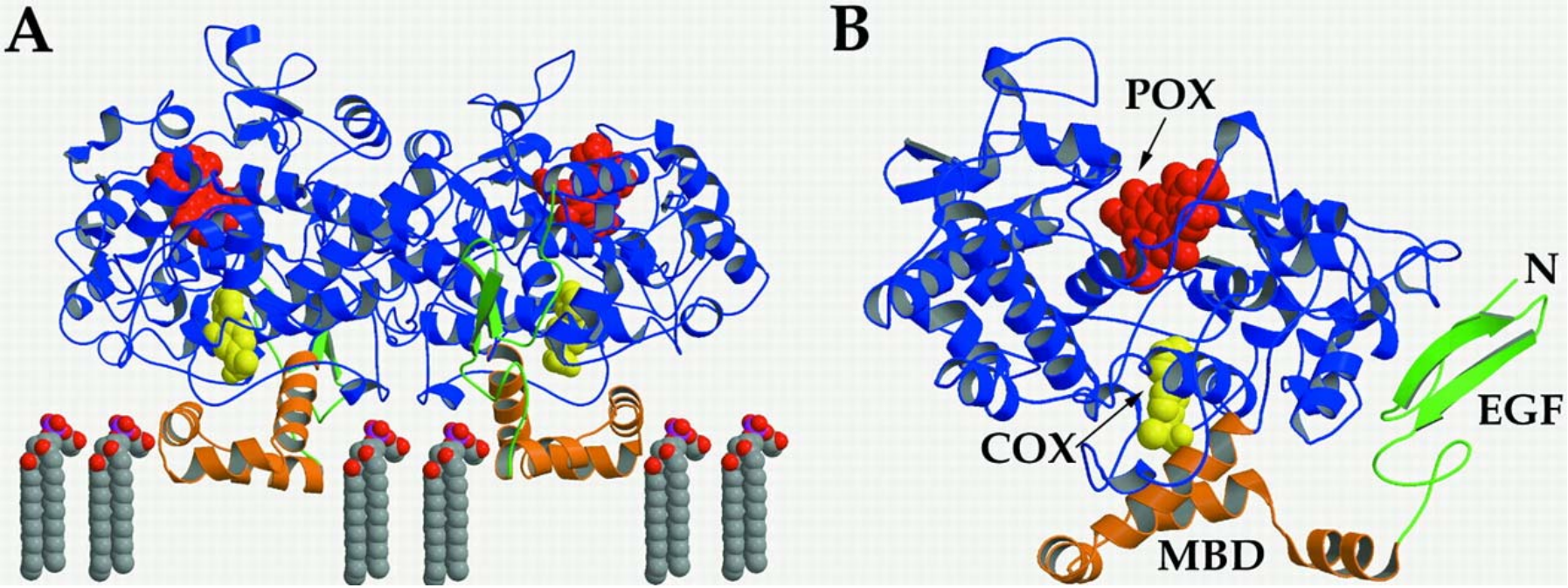
GRANULE CONTENT RELEASE	MEMBRANE-DERIVED LIPID MEDIATORS	CYTOKINE PRODUCTION
(Immediate) release of preformed mediators	*Lipid metabolism (over minutes)*	*New mRNA and protein synthesis (over hours)*

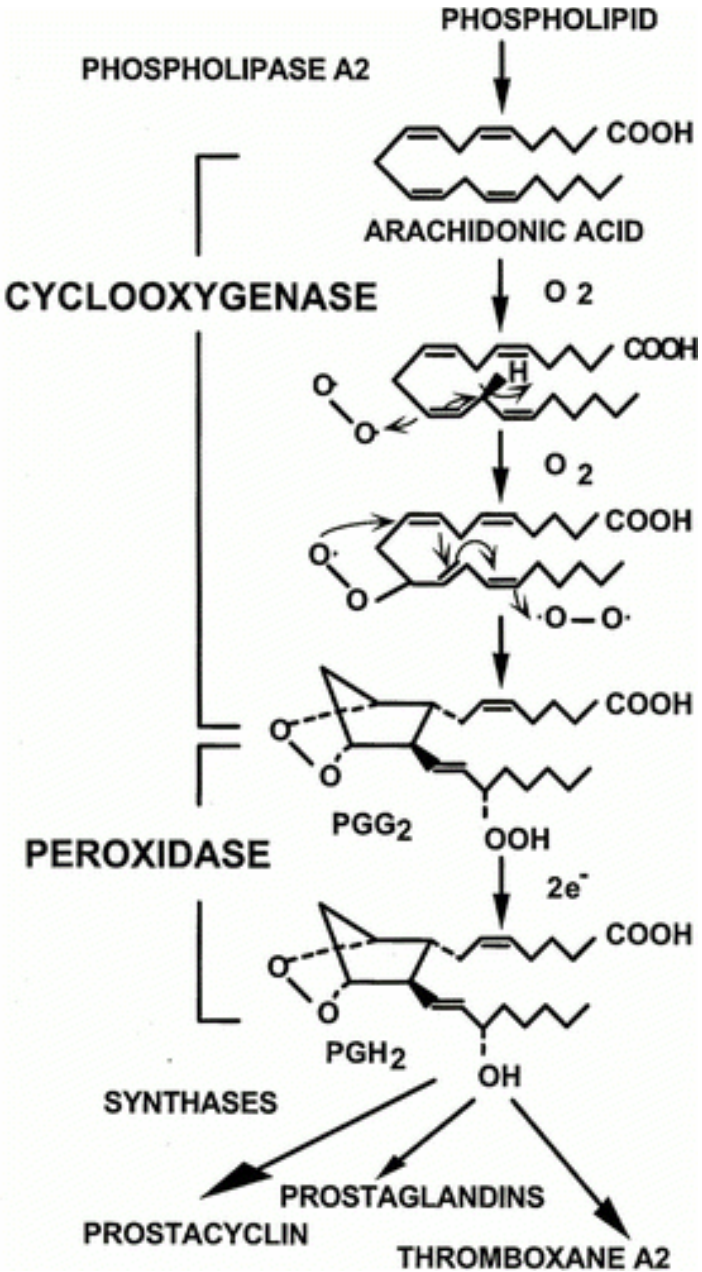
• Histamine
• Proteases
• heparin
• Tumor Necrosis Factor (TNF)
	• Prostaglandins
	• Leukotrienes
	• Platelet activation factor (PAF)
		• Interleukins
		  • IL-1 IL-3
		  • IL-4 IL-5
		  • IL-6 IL-8
		• TNF

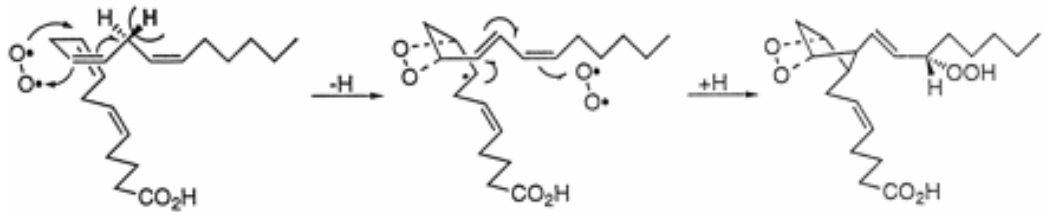
The Mast Cells are uniquely placed at the sites of possible entry into the tissues of pathogen or noxious agent: near skin surfaces, close to the mucous membranes lining body cavities and around blood vessels. They are capable of secreting or generating an array of mediators which have the ability to modify vascular and cellular reactions as well as to affect some of the plasma factors.

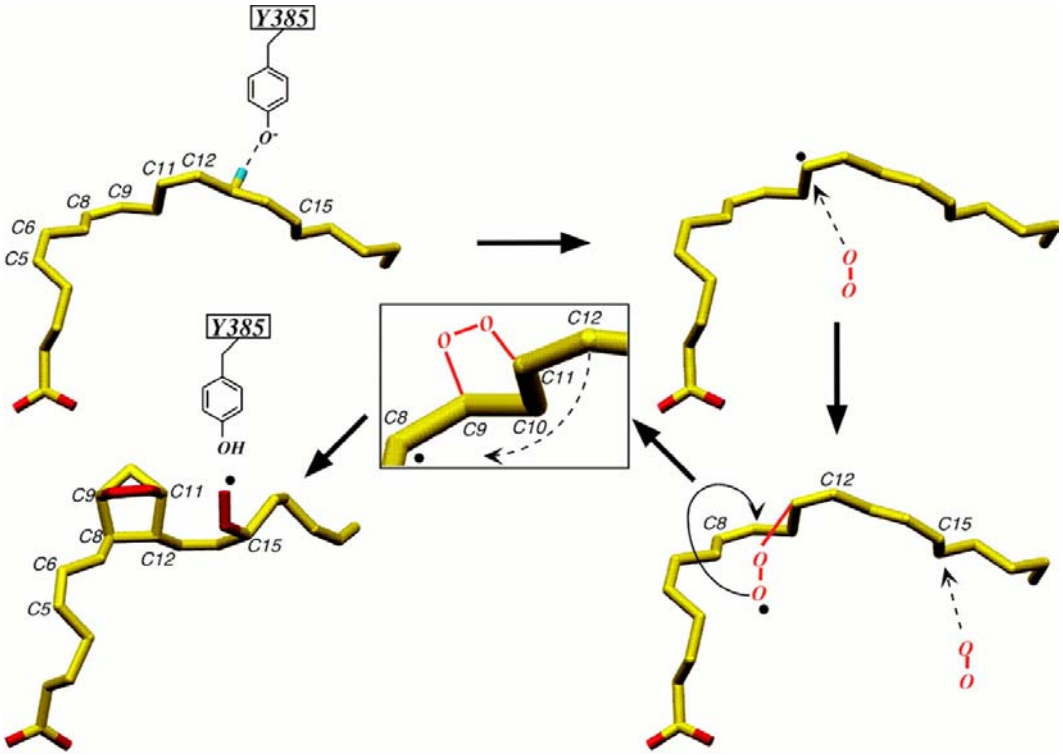

Membrane phospholipids
Diverse physical, chemical, inflammatory and mitogenic stimuli
Phospholipase A2
Arachidonic acid
Prostaglandin G/H synthase 1 (cyclooxygenase-1)
Prostaglandin G2
Prostaglandin H2
COX
HOX
Prostaglandin G2
Prostaglandin H2
Prostaglandin G/H synthase 2 (cyclooxygenase-2)
Tissue-specific isomerases
Prostanoids
Prostacyclin
Thromboxane A2
Prostaglandin D2
Prostaglandin E2
Prostaglandin F2α
Receptors
IP
TPα, TPβ
DP1, DP2
EP1, EP2, EP3, EP4
FPα, FPβ
Endothelium, kidney, platelets, brain
Platelets, vascular smooth-muscle cells, macrophages, kidney
Mast cells, brain, airways, lymphocytes, eosinophils
Brain, kidney, vascular smooth-muscle cells, platelets
Uterus, airways, vascular smooth-muscle cells, eye

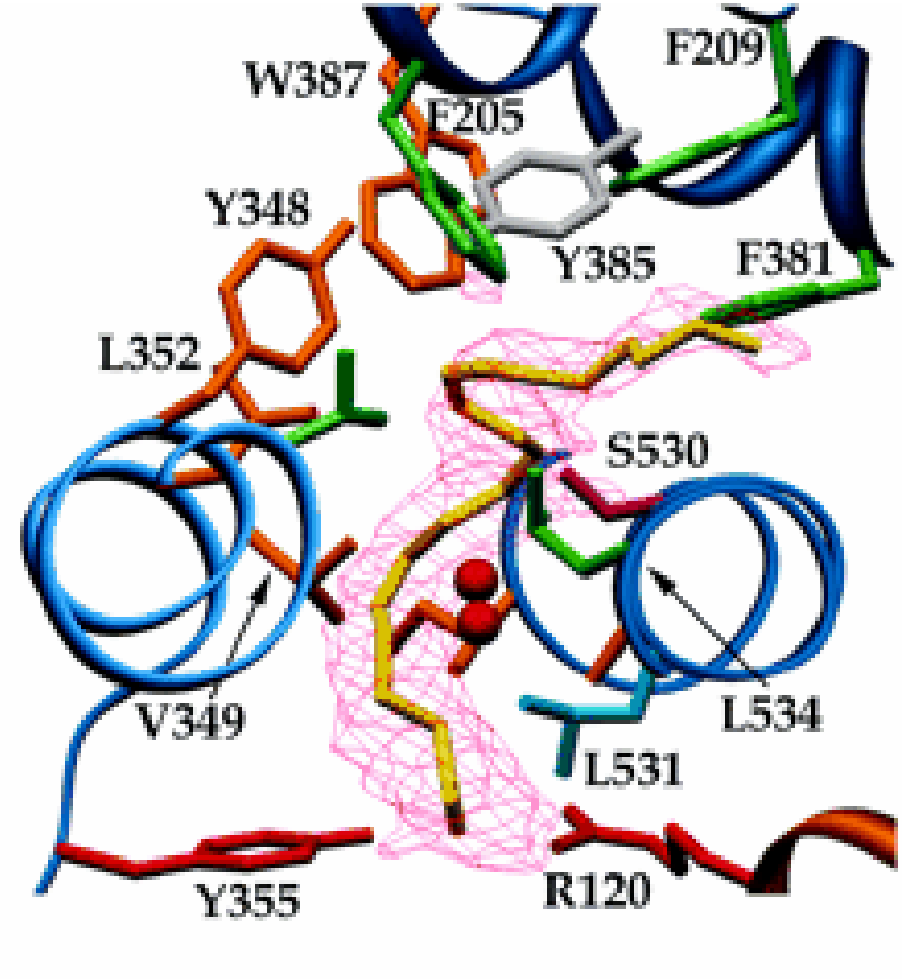

a
Catalytic site
Serine residue at position 529
Platelet
Channel of access
Arachidonic acid

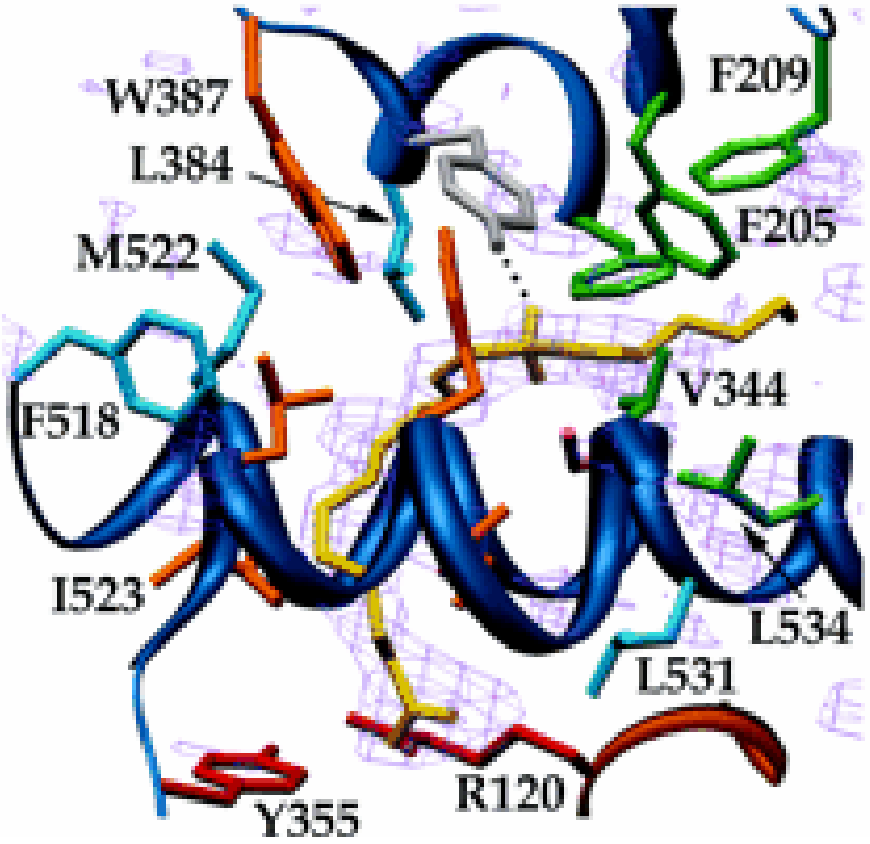

A
B
POX
N
EGF
COX
MBD


PHOSPHOLIPID
PHOSPHOLIPASE A2
COOH
ARACHIDONIC ACID
CYCLOOXYGENASE
O 2
COOH
O 2
COOH
COOH
PGG2
OOH
PEROXIDASE
2e-
COOH
PGH2
OH
SYNTHASES
PROSTAGLANDINS
PROSTACYCLIN
THROMBOXANE A2

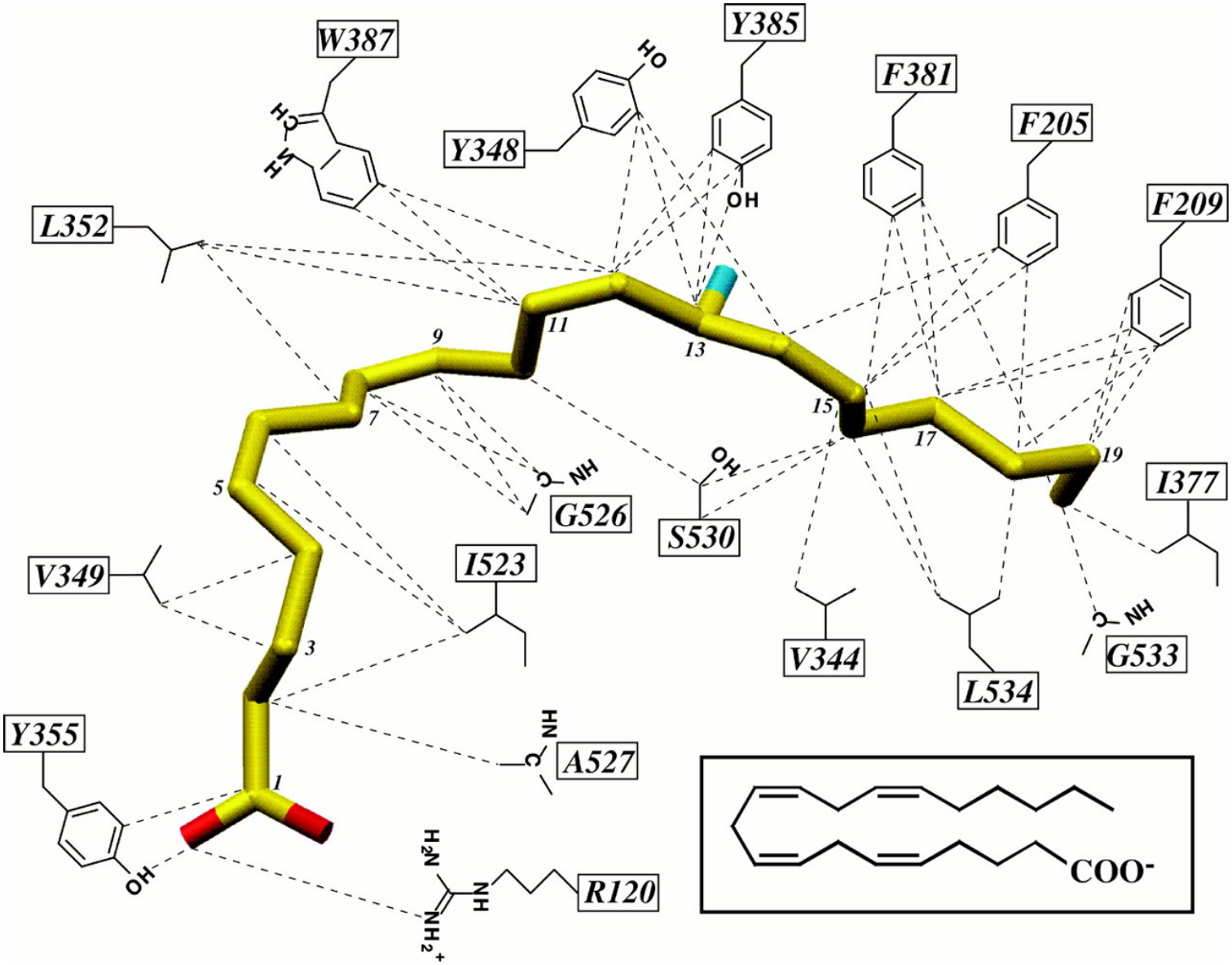

-H
+H
CO2H
CO2H
CO2H
OOH


Y385
Y385
OH
C5
C6
C8
C9
C10
C11
C12
C15





M. G. Malkowski,[1] S. L. Ginell,[2] W. L. Smith,[1] R. M. Garavito[1]*


W387
Y385
Y348
F381
F205
F209
L352
I377
G526
S530
V349
I523
V344
L534
G533
Y355
A527
R120
COO-
1
3
5
7
9
11
13
15
17
19

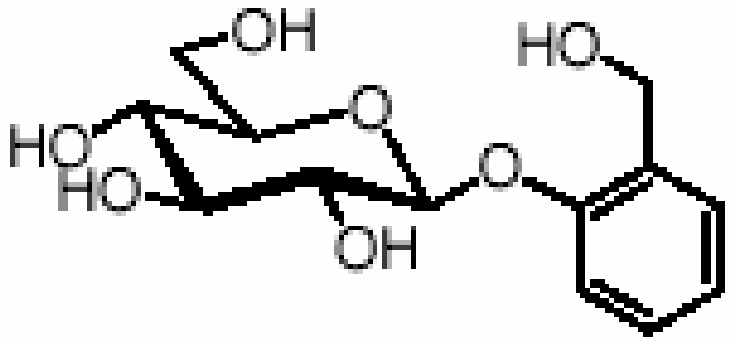
Chemical Classification of Analgesic, Antipyretic, and Nonsteroidal Anti-inflammatory Drugs.

- ***Salicylic acid derivatives*** (e.g. Aspirin, sodium salicylate, choline magnesium trislicylate, salsalate, diflunisal, salicylsalicylic acid, sulfasalazine, olsalazine).
- ***Para-aminophenol derivatives*** (e.g. Acetaminophen)
- ***Indole and indende acetic acids*** (e.g. Indomethacin, sulindac, etodolac).
- ***Heteraryl acetic acids*** (e.g. Tolmetin, diclofenac, ketorolac).
- ***Arylpropionic acids*** (e.g. Ibuprofen, naproxen, flubiprofen, ketoprofen, fenoprofen, oxaprozin).
- ***Anthranilic acids*** (fenamates) (e.g. Mefenamic acid, meclofenamic acid).
- ***Enolic acids*** (e.g. Oxicams (piroxican, tenoxicam), pyrazolidinediones (phenylbutazone, oxyphenthratrazone)).
- ***Alkanones*** (e.g. Nabumetone).

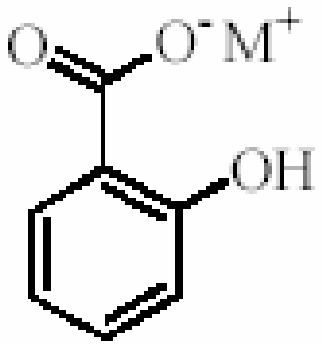
Development of NSAIDs

- 1500 B.C. : Dried Myrtle leaves for rheumatic pain of the womb - Egypt
- 500 B.C. : Hippocrates used willow bark to relieve pain of childbirth and fever
- 1763 : 1st "clinical trial" reported on the use of willow bark in fever*
- 1859 : 1st synthesis of salicylic acid
- 1899 : Discovery of acetylsalicylic acid (aspirin)
- 1971 : Elucidation of the mechanism of action of aspirin, inhibition of PGs production

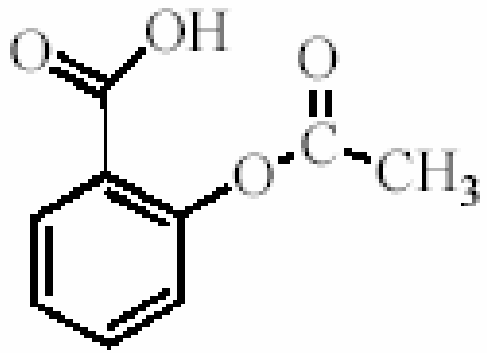
* Edward Stone, Philosophical Transactions of the Royal Society 1763;53:95-200



Salicin



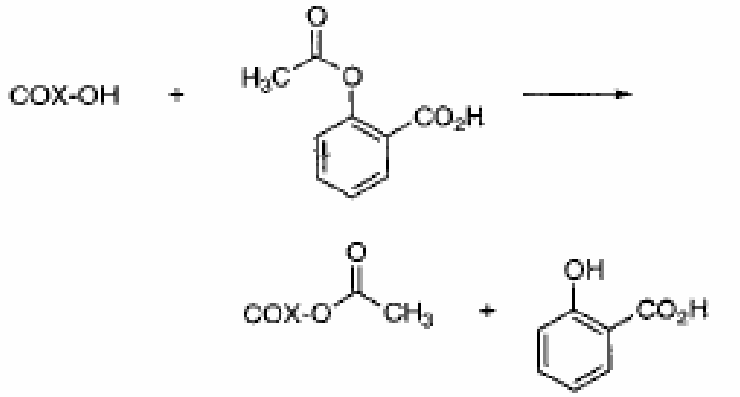
Salicylate



Acetylsalicylate

The Aspirin and Heme-binding Sites of Ovine and Murine Prostaglandin Endoperoxide Synthases*

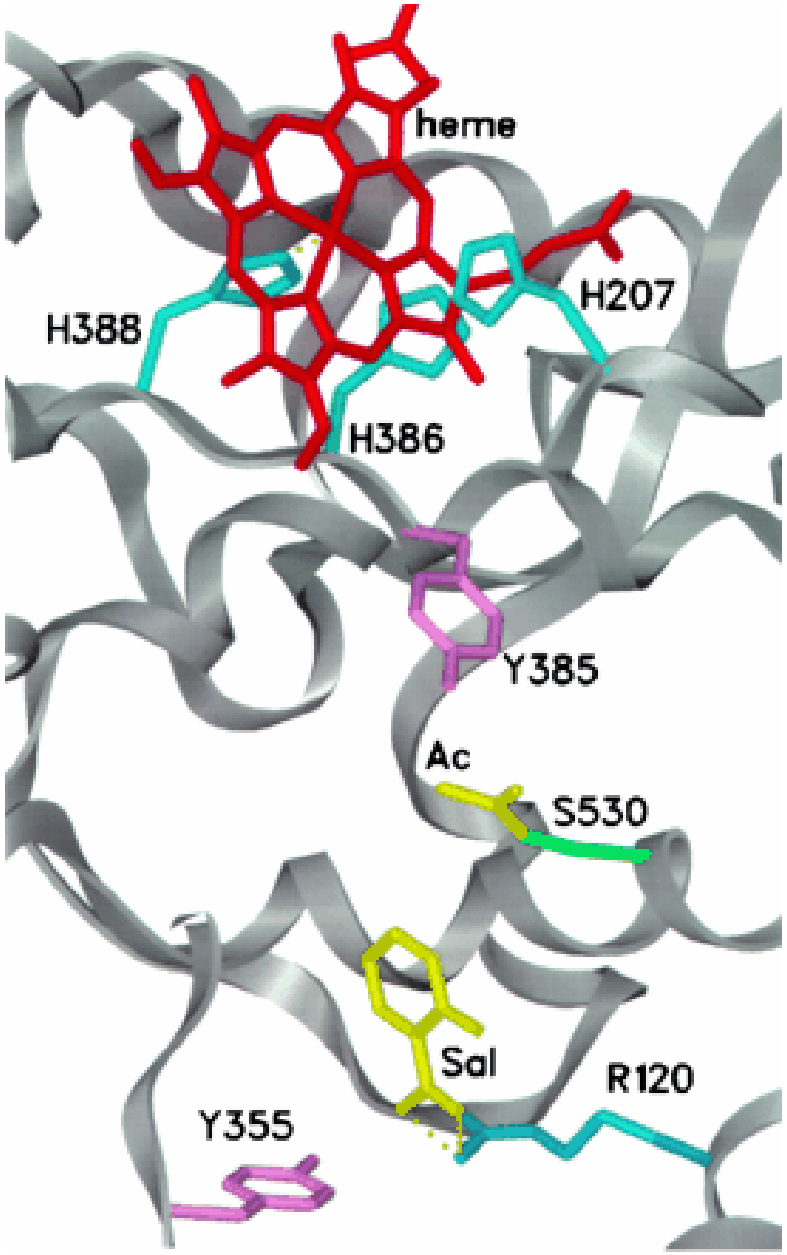
David L. DeWitt, E. A. El-Harith, Stacey A. Kraemer, Martha J. Andrews, Eveline F. Yao, Robert L. Armstrong, and William L. Smith

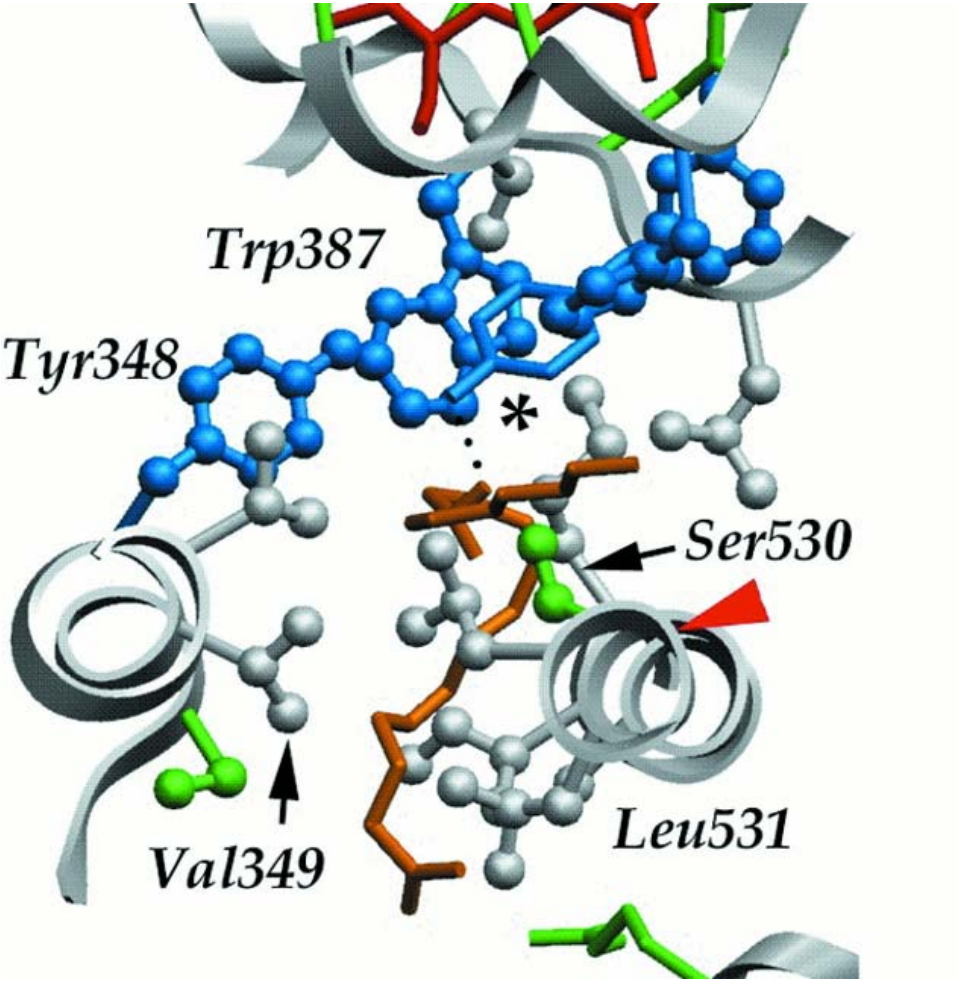


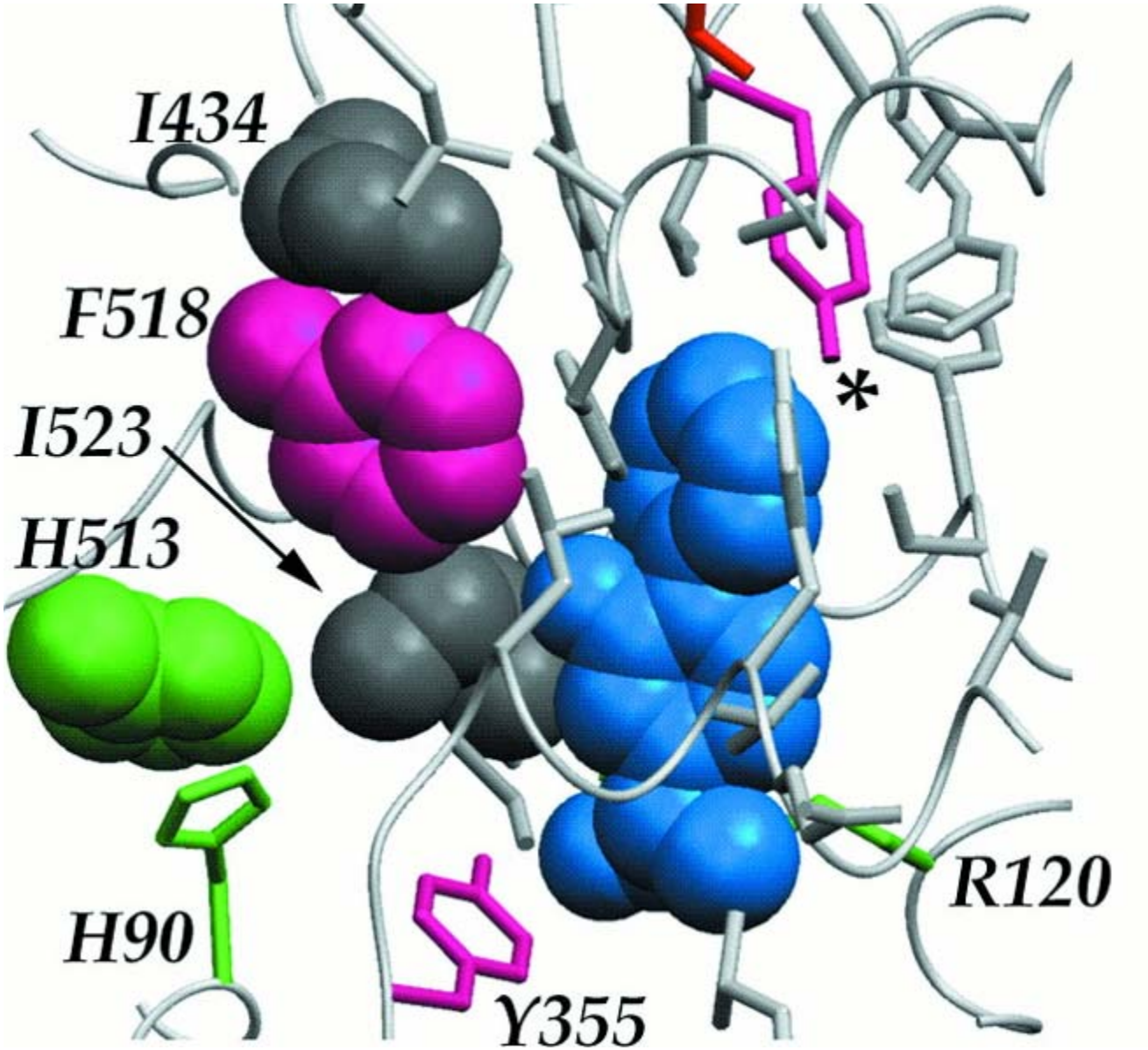
Acetylation of Ser-530 of sheep prostaglandin endoperoxide (PGG/H) synthase by aspirin causes irreversible inactivation of the cyclooxygenase activity of the enzyme. To determine the catalytic function of the hydroxyl group of Ser-530, we used site-directed mutagenesis to replace Ser-530 with an alanine. *Cos*-1 cells transfected with expression vectors containing the native (Ser-530) or mutant (Ala-530) cDNAs for sheep PGG/H synthase expressed comparable cyclooxygenase and hydroperoxidase activities. K_m values for arachidonate (8 μM) and ID_{50} values for reversible inhibition by the cyclooxygenase inhibitors, flurbiprofen (5 μM), flufenamate (20 μM), and aspirin (20 mM), were also the same for both native and mutant PGG/H synthases; however, only the native enzyme was irreversibly inactivated by aspirin. Thus, the "active site" Ser-530 of PGG/H synthase is not essential for catalysis or substrate binding. Apparently, acetylation of native PGG/H synthase by aspirin introduces a bulky sidechain at position 530 which interferes with arachidonate binding. In related studies, a cDNA for mouse

THE JOURNAL OF BIOLOGICAL CHEMISTRY

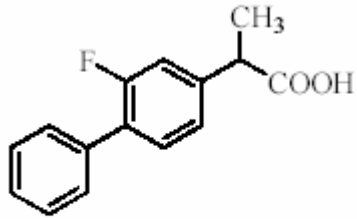
Vol. 265, No. 9, Issue of March 25, pp. 5192–5198, 1990


heme
H388
H207
H386
Y385
Ac
S530
Sal
R120
Y355


Trp387
Tyr348
*
Ser530
Val349
Leu531

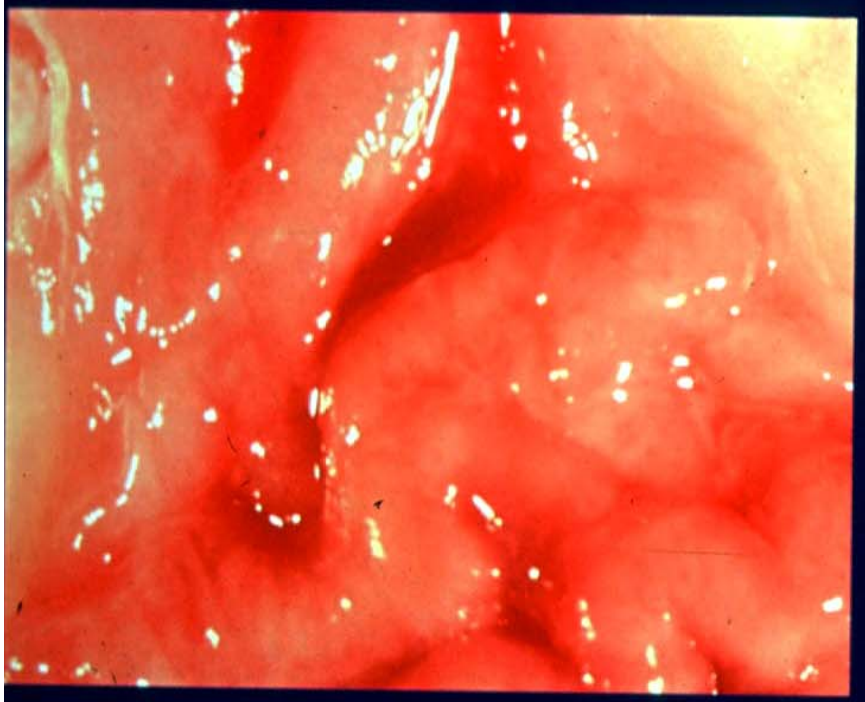


Flurbiprofen

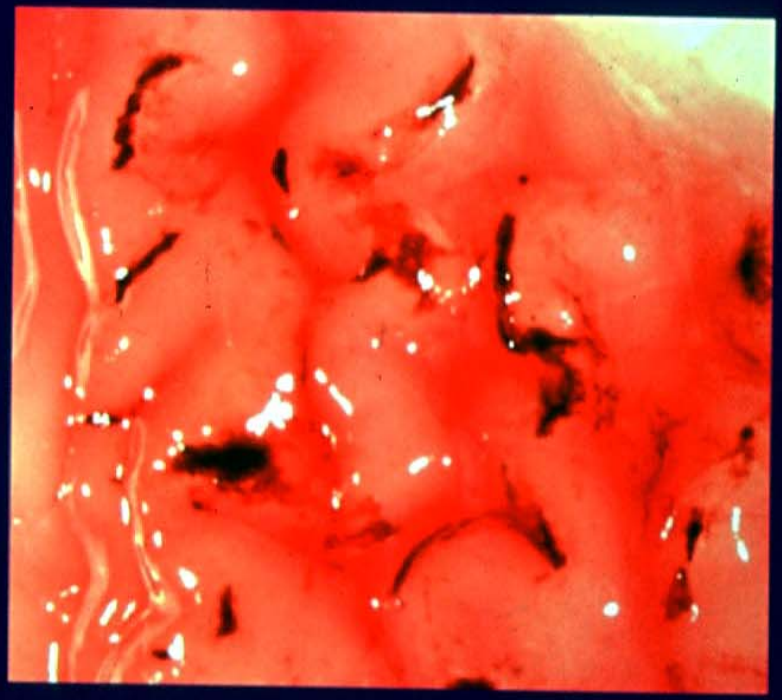


Flurbiprofen

NSAID in Rat Stomach



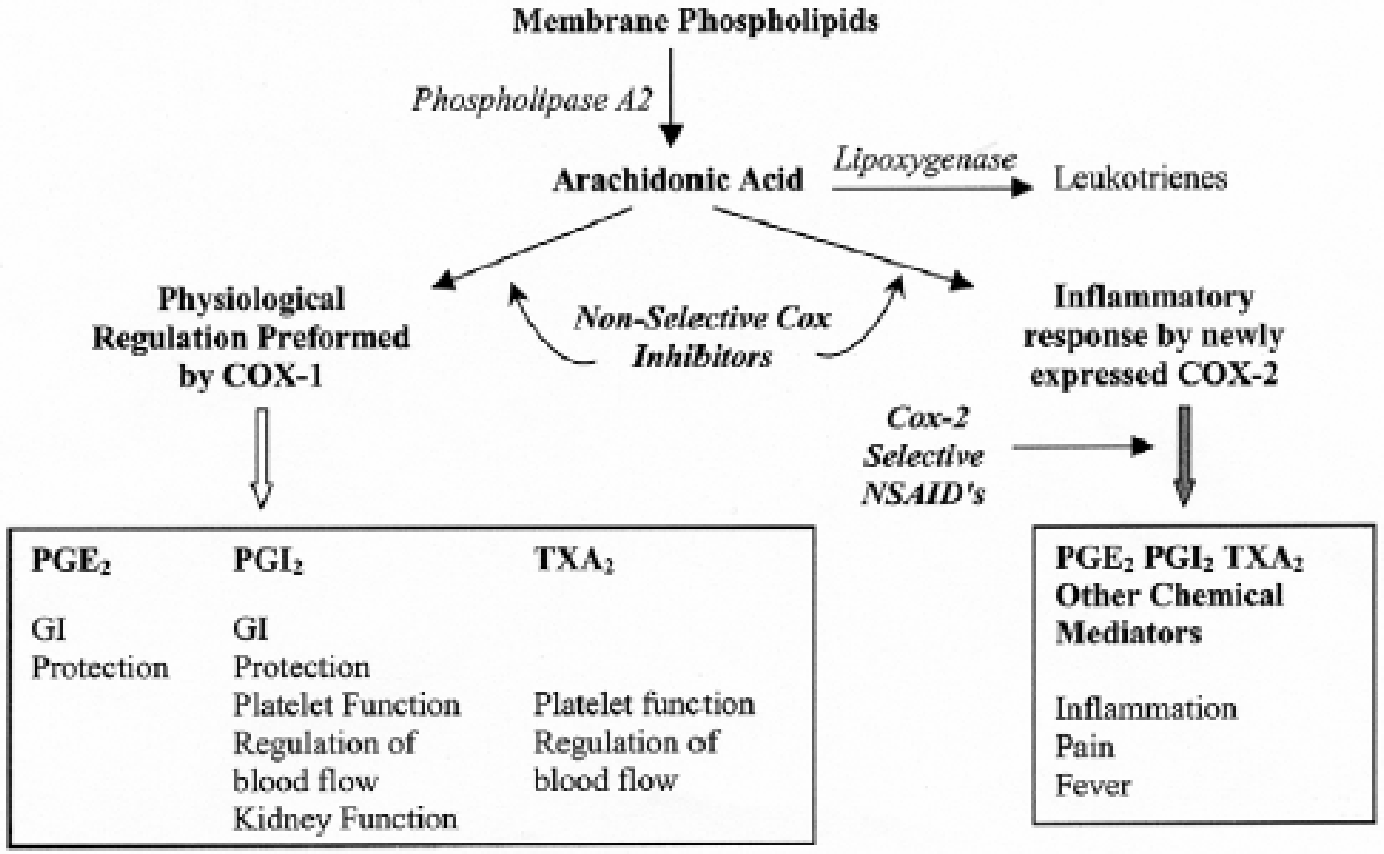
Control



Indomethacin

(3 mg/kg, 5 hr postdose)

Cox-1 Good / Cox-2 Bad Theory



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Rationale for Development of COX-2 Specific Inhibitor

- NSAIDs are believed to inhibit both the COX-1 and the COX-2 enzymes
- Toxicity of NSAIDs due to inhibition of constitutive enzyme (COX-1)
- Therapeutic utilities of NSAIDs due to inhibition of inducible enzyme (COX-2)
- A COX-2 specific inhibitor will effectively reduce inflammation, pain and fever with reduced risk of GI toxicity

Varne et al. Inflam Res. 1995;44:1-10
Donnelly et al. Ailment Pharmacol Ther.11, 227-236.
Klippel, Primer on Rheum Diseases 1997; 422-426.

Bakhle et al. Med Inflamm. 1996;5:305-323
Simon. J Clin Rheumatol 1996;2(3):135-140.
Lane. J Rheumatol 1997;24 (suppl 49):20-24.

KEY ISSUES

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- Will selective Cox-2 inhibitors have the same anti-inflammatory, analgesic and anti-pyretic activities as standard NSAIDs ?

  Is there a component of the inflammation / pain / fever response mediated by Cox-1 derived products?

- Will selective Cox-2 inhibitors be G.I. sparing compared to standard NSAIDs ?

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Potential Indications for Highly Selective Cox-2 Inhibitors

- Osteoarthritis
- Analgesia
- Rheumatoid Arthritis and Other Inflammatory Conditions
- Colon Cancer
- Alzheimer's Disease

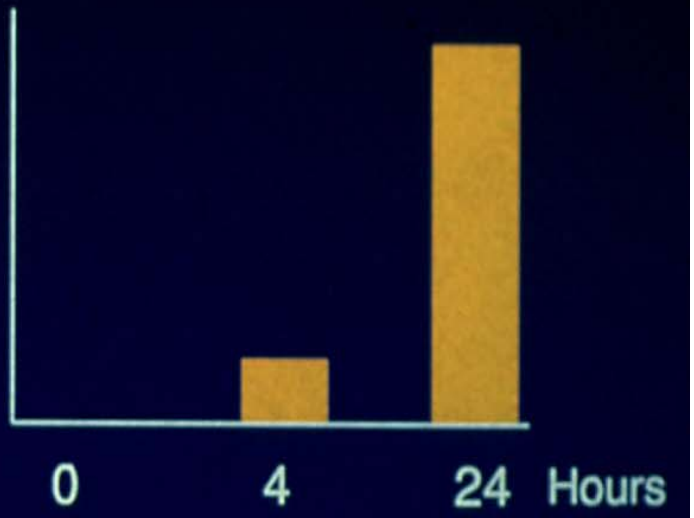
Whole-Blood Assay for COX-2 Activity

24 hour
37°C

Monocyte COX-2

Heparinized Whole Blood + Lipopolysaccharide (LPS)

Plasma PGE_2



LPS → PGE_2

Monocyte COX-2

Patrignani et al. *J Pharmacol Exp Ther.* 1994;271:1705-1712.

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Whole-Blood Assay for COX-1 Activity

Whole Blood

Allowed to Clot for 1 Hour

37°C

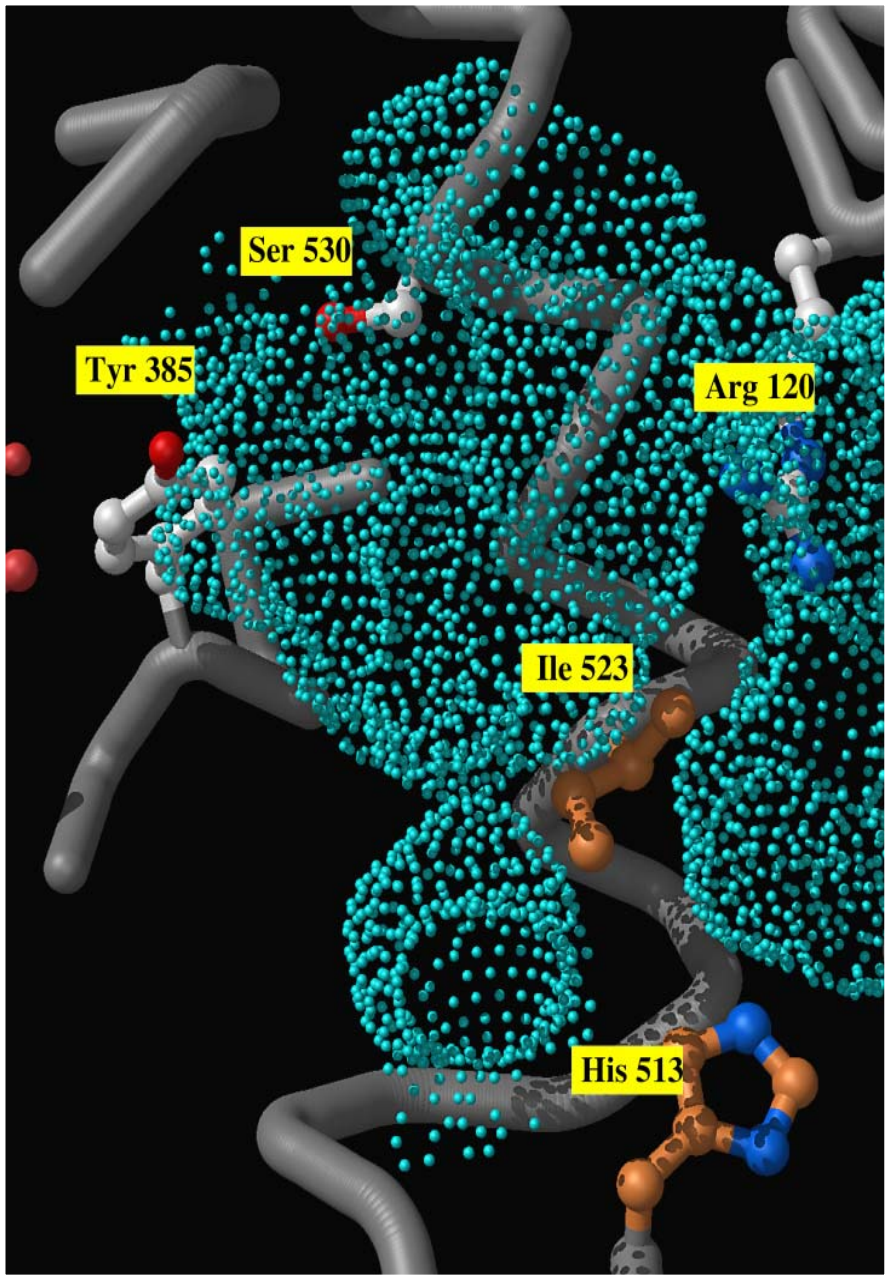
Thromboxane B_2 (TxB_2) Release Into Serum

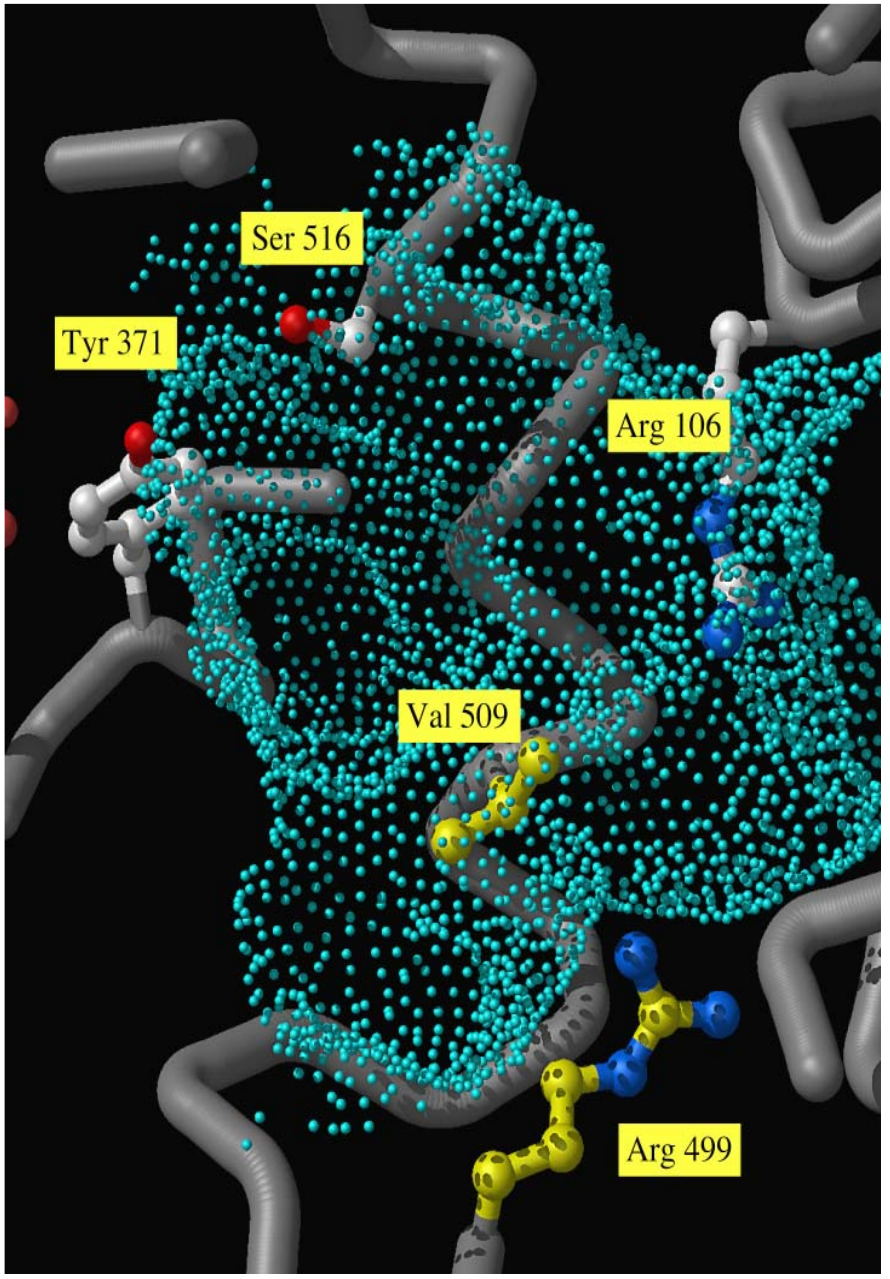
Endogenously Generated Thrombin →

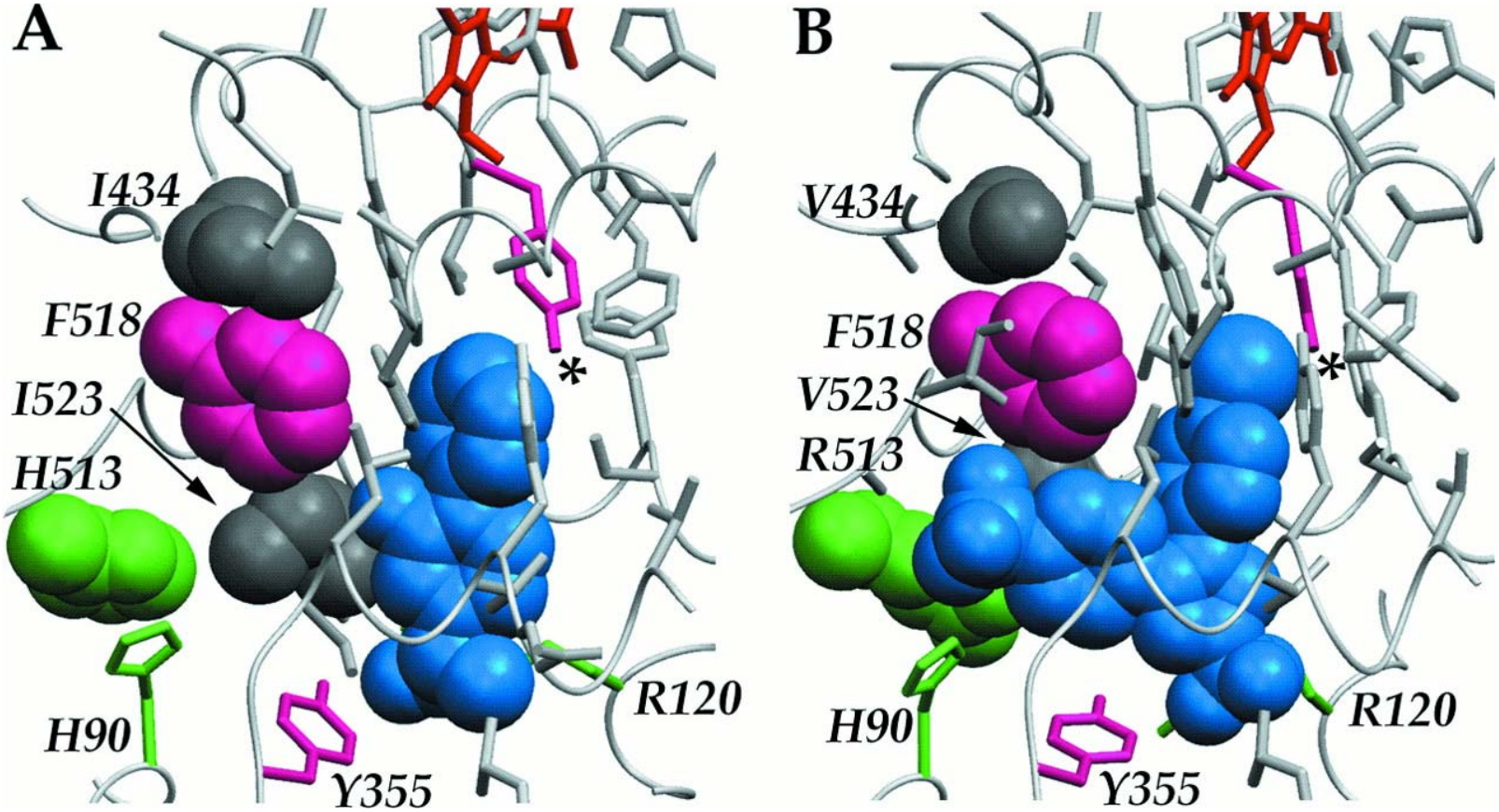
Platelet Arachidonate → (COX-1) Thromboxane A_2 (TxA_2) → TxB_2

atrignani et al. *J Pharmacol Exp Ther.* 1994;271:1705-1712.

Drug or Compound	IC50 Cox-1 (μM)	IC50 Cox-2 (μM)	Cox-2 / Cox1
Piroxicam	0.0005	0.3	600
			10
	0.00527	0.175	33
Aspirin	1.67	278	166
			25-60
Indomethacin	0.028	1.68	60
			22-60
			30
Sulindac Sulfide			31
Ibuprofen			0.7-50
Tenoxicam	0.0201	0.322	16
Meclofenamic			7
Flurbiprofen			6
Naproxen			1
Diclofenac	1.57	1.1	0.70
			0.70
6-MNA <= Nabumetone	278	187	0.67
Etodolac	34	3.4	0.100
Meloxicam	4.8	0.43	0.090
Nimesulide	9.2	0.52	0.057
DuP-697			0.017
SC 58125	38.7	0.27	0.007
NS 398	16.8	0.1	0.006
L-745,337	369	1.5	0.004
DFU	>50	0.04	<0.001
Celecoxib, SC 58635	15	0.04	0.003
	6.3	1	0.159
Refecoxib, MK 966	19	0.5	0.026


Ser 530
Tyr 385
Arg 120
Ile 523
His 513


Ser 516
Tyr 371
Arg 106
Val 509
Arg 499



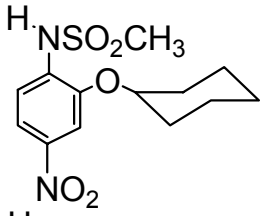
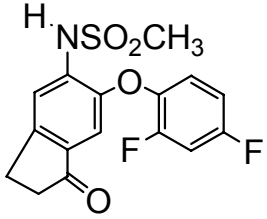
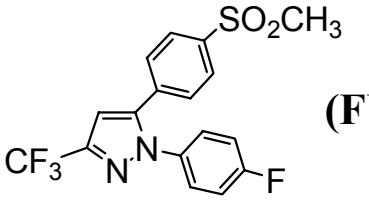
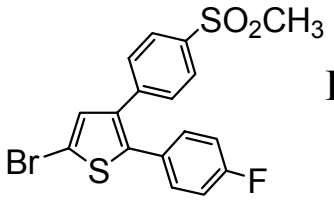
Flurbiprofen

SC-588
Precursor of Celecoxib

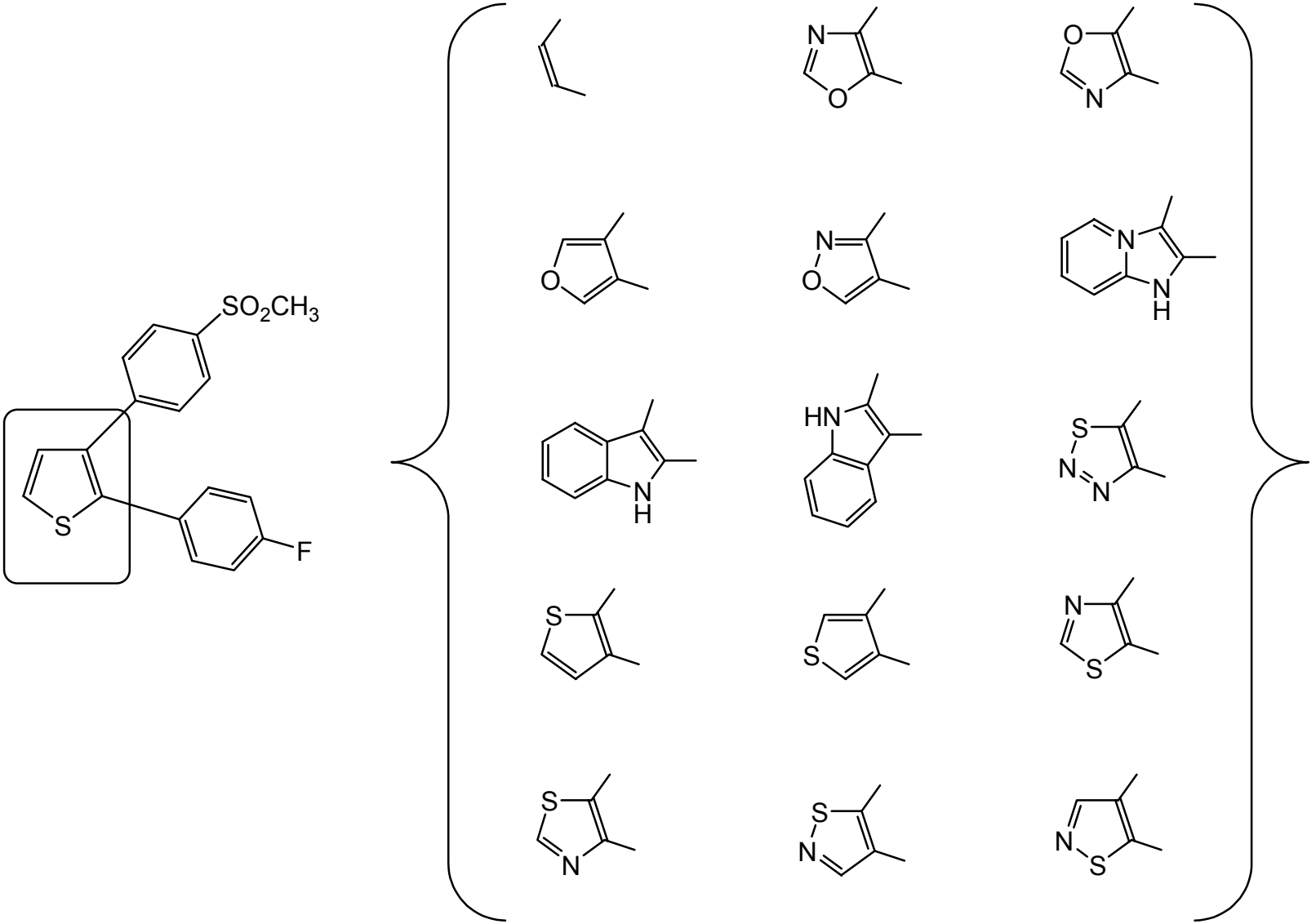
Story of Rofecoxib

Merck-Frosst Centre for Therapeutic Research

SELECTIVE LEADS

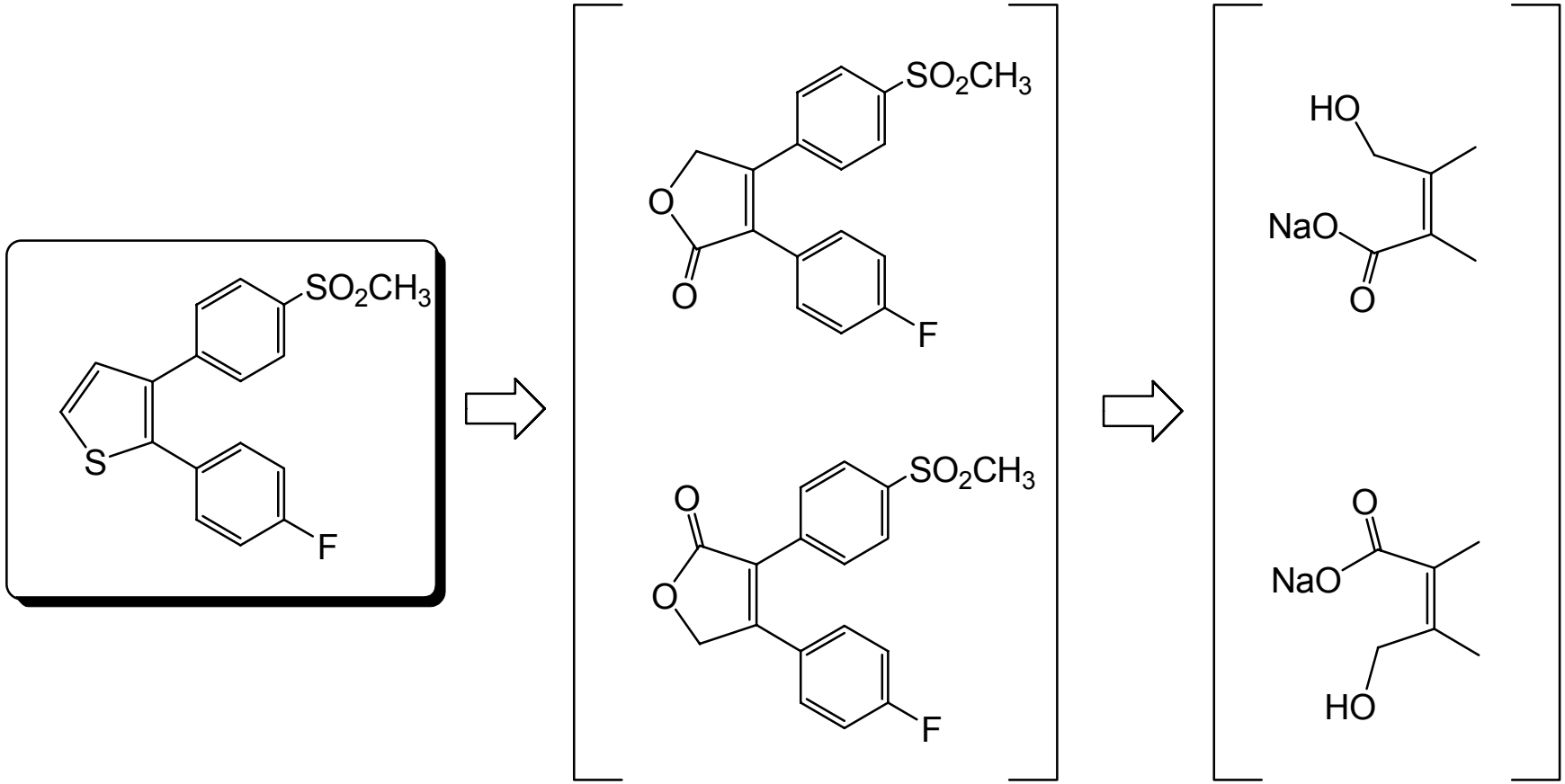
	HUMAN WHOLE BLOOD IC_{50} VALUES (μM)		RATIO
	COX-1	COX-2	
 **NS 398**	5.0	0.05	10
 **FLOSULIDE**	31	1.0	31
 **(FUJISAWA)**	n.d.	n.d.	n.d.
 **DuP 697**	0.8	0.06	13

THIOPHENE REPLACEMENTS



Most are inactive or not bioavailable

SOLVING THE BIOAVAILABILITY ISSUE: PRODRUG APPROACH



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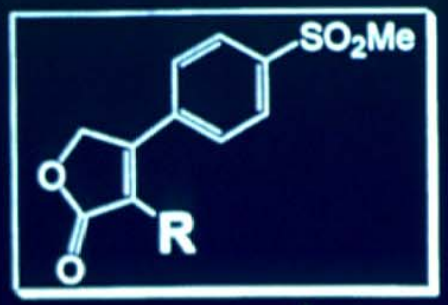
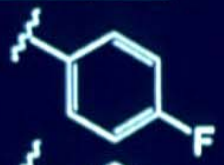
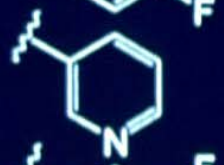
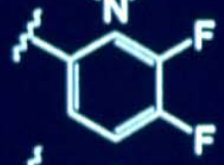
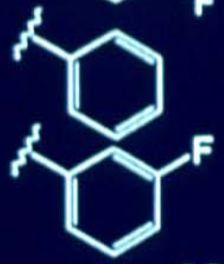
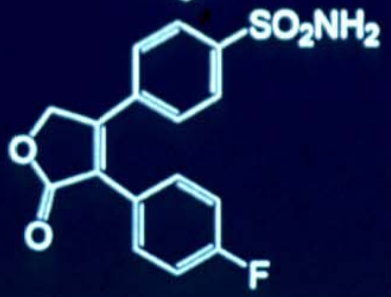
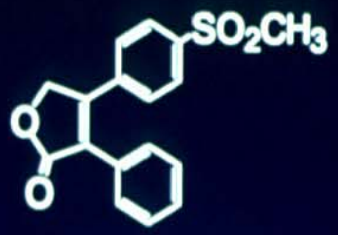
REPRESENTATIVE LACTONE COMPOUNDS



	COX-2 whole blood $IC_{50}(\mu M)$	COX-2 whole blood	RATIO
	0.6	10	17
	33	ND	ND
	0.9	13	14
 (Rofecoxib)	0.5	19	38
	1.8	86	48
	0.8	5.8	7
INDOMETHACIN	0.4	0.2	0.5

Merck-Frosst

Comparison of in vivo activity of Rofecoxib and Indomethacin

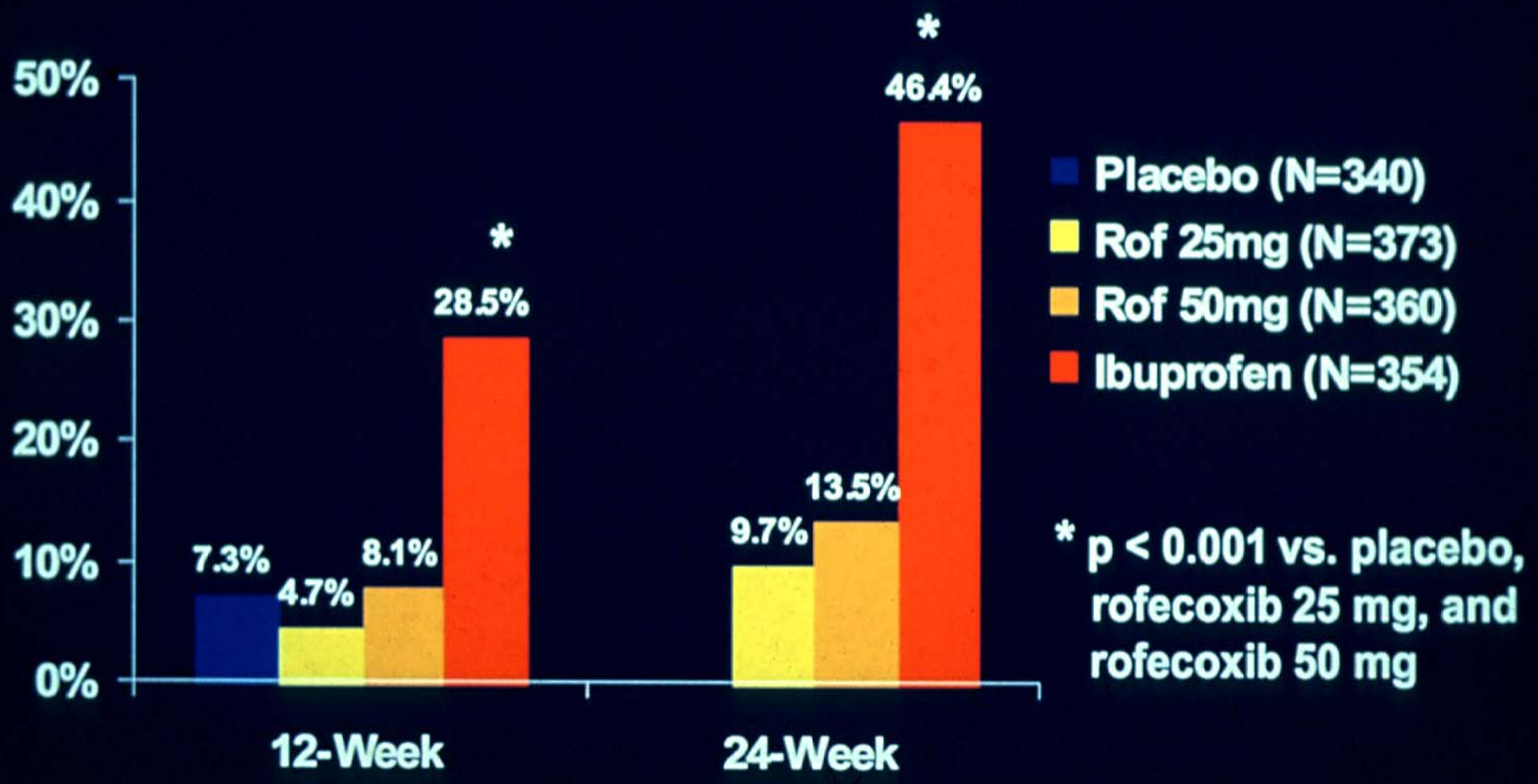


Rofecoxib

INDOMETHACIN

	ED_{50}, mg/kg	
Rat Paw Edema	1.5	2.0
Rat Pyresis	0.2	1.0
Rat Paw Hyperalgesia	1.0	1.5
Adjuvant Arthritis	0.7 (b.i.d.)	0.2

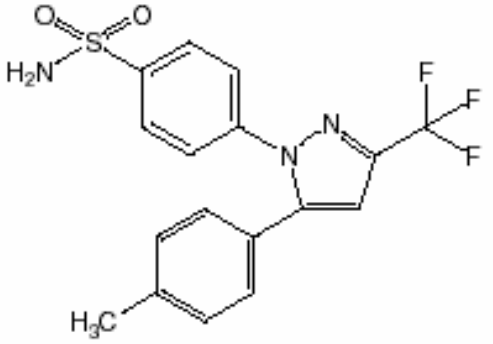
-month Endoscopy Studies: Cumulative Incidence of Gastric/Duodenal Ulcer ≥ 3 MM



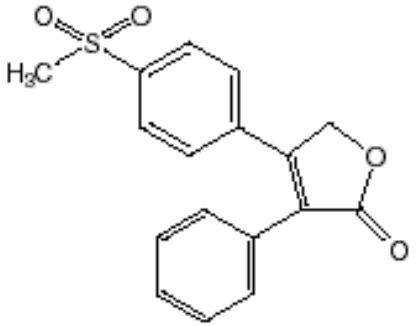
Merck-Frosst

Laine et al. Gastro 1999

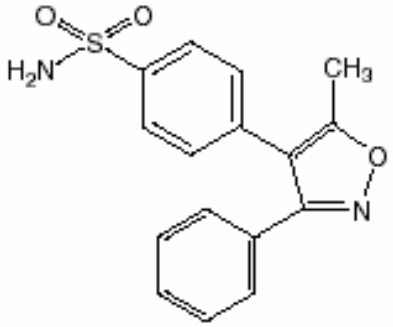
Second Generation of Cox-2 Inhibitors



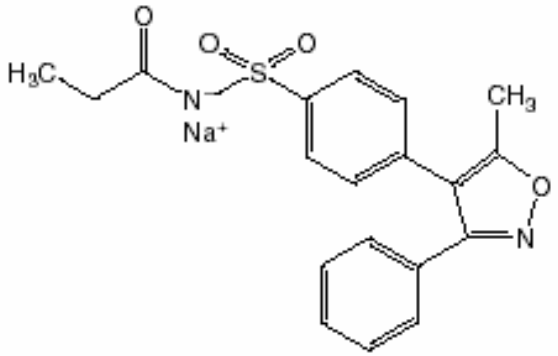
Celecoxib



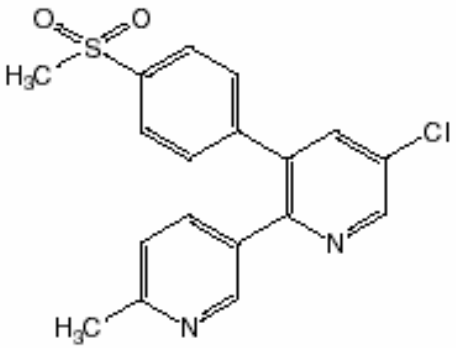
Rofecoxib



Valdecoxib



Parecoxib



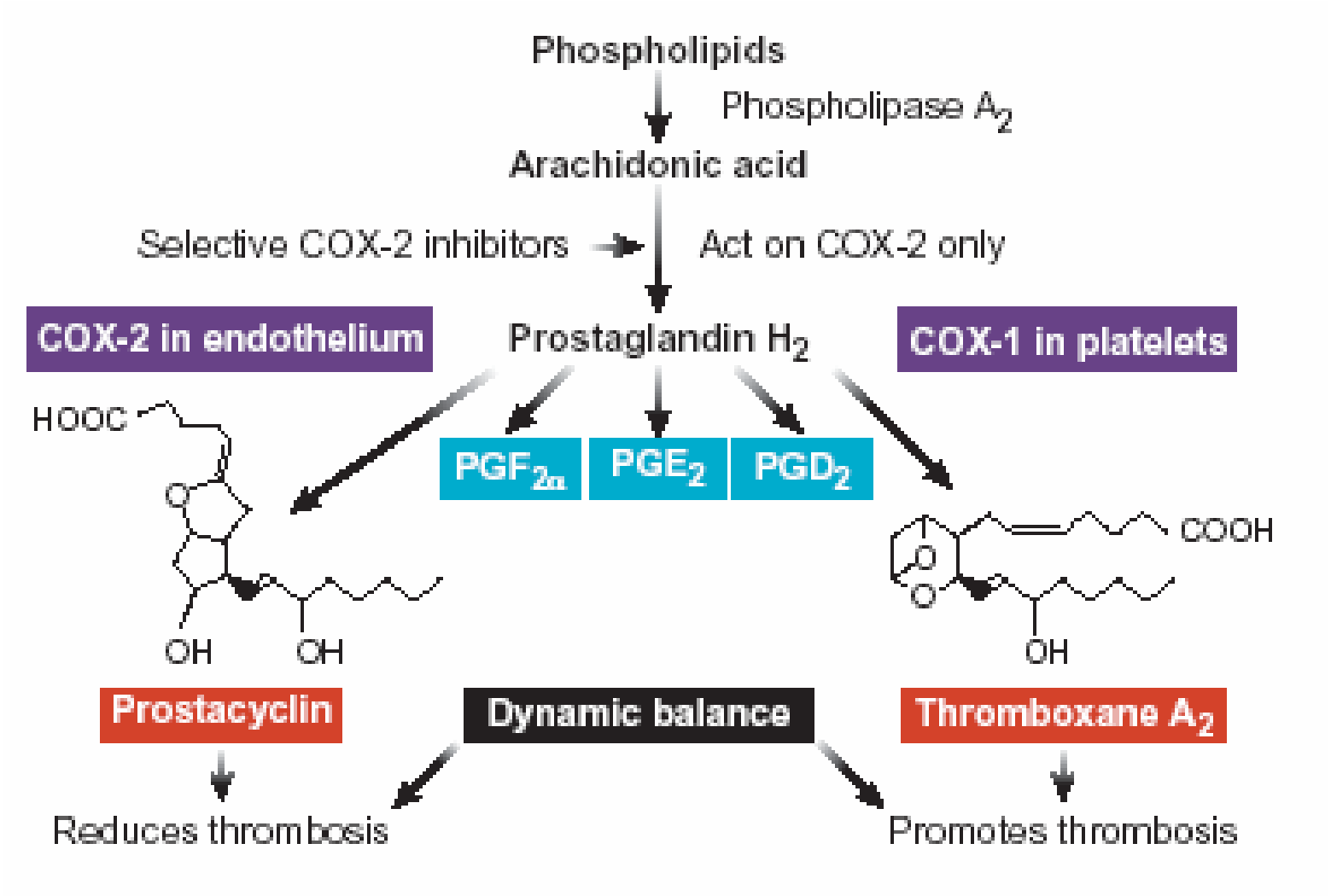
Etoricoxib

End of Medicinal Chemistry?

However…

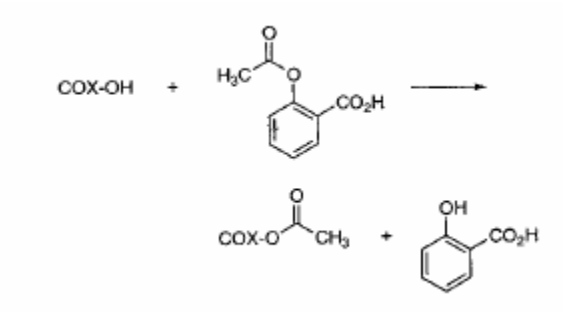
Problems with Bad/Good Approach

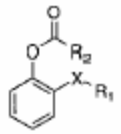
- Small effect in one study (2% vs. 4%)
- Very narrow choice of patients
- In a study vs. Naproxen, cardiovascular side effects significantly increased
- Renal side effects
- Cox-3 and beyond
- Upregulation of isozymes
- Unexplained effects of some NSAIDS


Phospholipids
Phospholipase A_2
Arachidonic acid
Selective COX-2 inhibitors
Act on COX-2 only
COX-2 in endothelium
Prostaglandin H_2
COX-1 in platelets
$PGF_{2\alpha}$
PGE_2
PGD_2
HOOC
OH
OH
COOH
OH
Prostacyclin
Dynamic balance
Thromboxane A_2
Reduces thrombosis
Promotes thrombosis

Aspirin-like Molecules that Covalently Inactivate Cyclooxygenase-2

Amit S. Kalgutkar, Brenda C. Crews, Scott W. Rowlinson, Carlos Garner,* Karen Seibert, Lawrence J. Marnett†





R_1	R_2	X	IC_{50} (μM) COX-2	IC_{50} (μM) COX-1	IC_{50} (COX-1)/ IC_{50} (COX-2)
CH_3	CH_3	S	250	>5000	>20
$(CH_2)_6CH_3$	CH_3	S	2.0	6.0	3.0
$(CH_2)_6CH_3$	CH_3	$N(CH_3)$	>40	>40	–
$(CH_2)_6CH_3$	CH_3	CH_2	>40	>40	–
$(CH_2)_6CH_3$	CH_3	Se	12	12	1.0
$(CH_2)_6CH_3$	CH_3	SO	>40	>40	–
$(CH_2)_6CH_3$	CH_3	SO_2	>40	>40	–
$CH_2C{\equiv}C(CH_2)_3CH_3$ (APHS)	CH_3	S	0.8	17	21.0
$CH_2C{\equiv}C(CH_2)_3CH_3$	CH_3	O	>40	>40	–
$CH_2C{\equiv}C(CH_2)_3CH_3$	CH_2CH_3	S	>40	>40	–

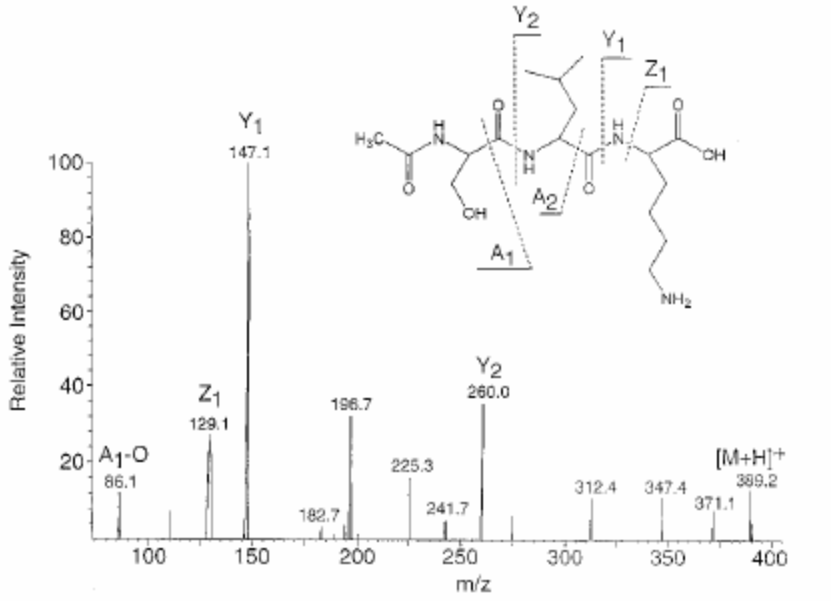


Table 2. Effect of APHS and indomethacin on PGE_2 and TxB_2 synthesis in rat air pouch and blood platelets. Protocols are described in (*29*). Each group contained six animals.

Treatment	Dose (mg/kg)	Pouch PGE_2 (ng/ml)	Platelet TxB_2 (ng/ml)
Vehicle	–	82 ± 6	108 ± 12
APHS	5	4 ± 1	118 ± 19
APHS	50	0	96 ± 14
Indomethacin	2	0	11.3 ± 0.6