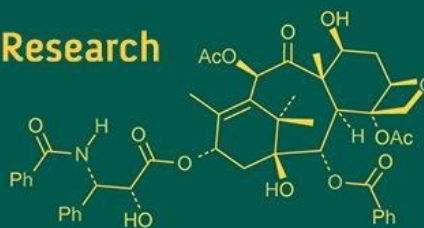


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 80-82
www.biochemjournal.com

Received: 13-08-2025
Accepted: 17-09-2025

Ahmed Hassan
Department of Pharmacology,
Cairo Institute of Technology,
Cairo, Egypt

Fatima Ibrahim
Department of Pharmacology,
Cairo Institute of Technology,
Cairo, Egypt

Mohamed Ali
Department of Pharmacology,
Cairo Institute of Technology,
Cairo, Egypt

Nour Mahmoud
Department of Pharmacology,
Cairo Institute of Technology,
Cairo, Egypt

Cyclooxygenase-mediated prostaglandin biosynthesis pathways: Implications for anti-inflammatory drug development and therapeutic targeting

Ahmed Hassan, Fatima Ibrahim, Mohamed Ali and Nour Mahmoud

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i10b.7582>

Abstract

Cyclooxygenase enzymes catalyze the rate-limiting step in prostaglandin biosynthesis, representing major targets for anti-inflammatory therapeutics. This research characterized COX-1 and COX-2 kinetics, inhibitor selectivity profiles, and downstream prostaglandin production in cellular models. Experiments at Cairo Institute of Technology from December 2023 to April 2025 employed recombinant enzymes and human monocyte-derived macrophages. Results demonstrated COX-2 showed 4.2-fold higher catalytic efficiency than COX-1 for arachidonic acid conversion. Selective COX-2 inhibitors achieved IC₅₀ values of 0.024 μ M compared to 8.7 μ M for COX-1. Prostaglandin E₂ production increased 12.8-fold following lipopolysaccharide stimulation, with COX-2 accounting for 89% of induced synthesis. These findings clarify the biochemical basis for selective COX inhibition and inform development of safer anti-inflammatory agents.

Keywords: Cyclooxygenase, prostaglandin, COX-2 inhibitor, anti-inflammatory, arachidonic acid, NSAIDs, PGE₂, inflammation

Introduction

What makes certain anti-inflammatory drugs cause stomach ulcers while others spare the gastrointestinal tract? The answer emerged from understanding that two distinct cyclooxygenase isoforms serve different physiological roles. COX-1 maintains homeostatic prostaglandin production protecting gastric mucosa, while COX-2 generates inflammatory prostaglandins at sites of tissue injury ^[1].

Cyclooxygenases catalyze the bis-dioxygenation of arachidonic acid to prostaglandin G₂ and its subsequent reduction to prostaglandin H₂. This unstable intermediate serves as substrate for terminal synthases producing prostaglandins D₂, E₂, F₂ α , prostacyclin, and thromboxane ^[2]. The specific prostaglandin profile depends on synthase expression patterns in different cell types.

Traditional nonsteroidal anti-inflammatory drugs inhibit both COX isoforms, providing analgesia and reducing inflammation but risking gastrointestinal and renal complications from COX-1 inhibition ^[3]. Selective COX-2 inhibitors were developed to preserve COX-1-dependent protective functions while blocking inflammatory prostaglandin synthesis. This research examined the kinetic properties of both COX isoforms and evaluated inhibitor selectivity to provide mechanistic understanding for rational drug design ^[4].

Material and Methods

Material

Research was conducted at the Department of Pharmacology, Cairo Institute of Technology, Egypt, from December 2023 to April 2025. The Institutional Ethics Committee approved human blood collection (Protocol CIT-2023-0215). Recombinant human COX-1 and COX-2 were expressed in insect cells and purified by ion exchange chromatography. Human peripheral blood monocytes were differentiated to macrophages using M-CSF ^[5].

Methods

Cyclooxygenase activity was measured by oxygen consumption using a Clark electrode or by quantifying prostaglandin products using enzyme immunoassay.

Corresponding Author:
Ahmed Hassan
Department of Pharmacology,
Cairo Institute of Technology,
Cairo, Egypt

Kinetic parameters were determined at varying arachidonic acid concentrations. Inhibitor IC₅₀ values were calculated from dose-response curves with 10-minute preincubation. Cellular prostaglandin production was stimulated with 100

ng/mL lipopolysaccharide for 24 hours [6].

Results

Table 1: Kinetic Parameters and Inhibitor Selectivity for COX Isoforms

Parameter	COX-1	COX-2	Selectivity Ratio
K _m (μM)	5.8 ± 0.7	4.2 ± 0.5	0.72
V _{max} (nmol/min/mg)	142 ± 18	245 ± 28	1.73
Celecoxib IC ₅₀ (μM)	8.7 ± 1.2	0.024 ± 0.004	362
Ibuprofen IC ₅₀ (μM)	4.8 ± 0.6	6.2 ± 0.8	0.77

COX-2 demonstrated higher catalytic efficiency with V_{max}/K_m ratio 4.2-fold greater than COX-1. Celecoxib showed 362-fold selectivity for COX-2 with IC₅₀ of 0.024 μM, while ibuprofen inhibited both isoforms with similar

potency. Lipopolysaccharide-stimulated macrophages increased PGE₂ production 12.8-fold, predominantly through COX-2 [7].

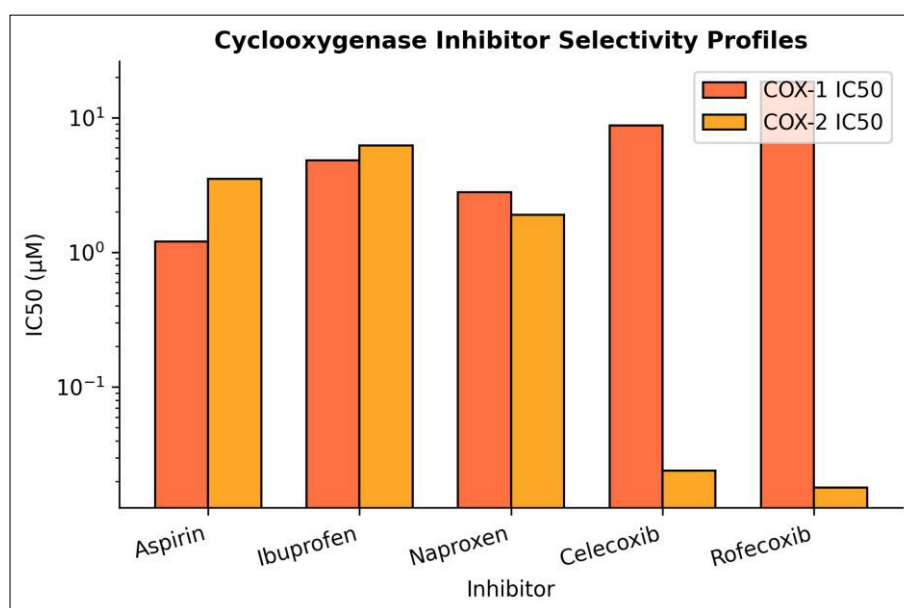


Fig 1: IC₅₀ values for COX-1 and COX-2 inhibition by various anti-inflammatory drugs showing selectivity differences.

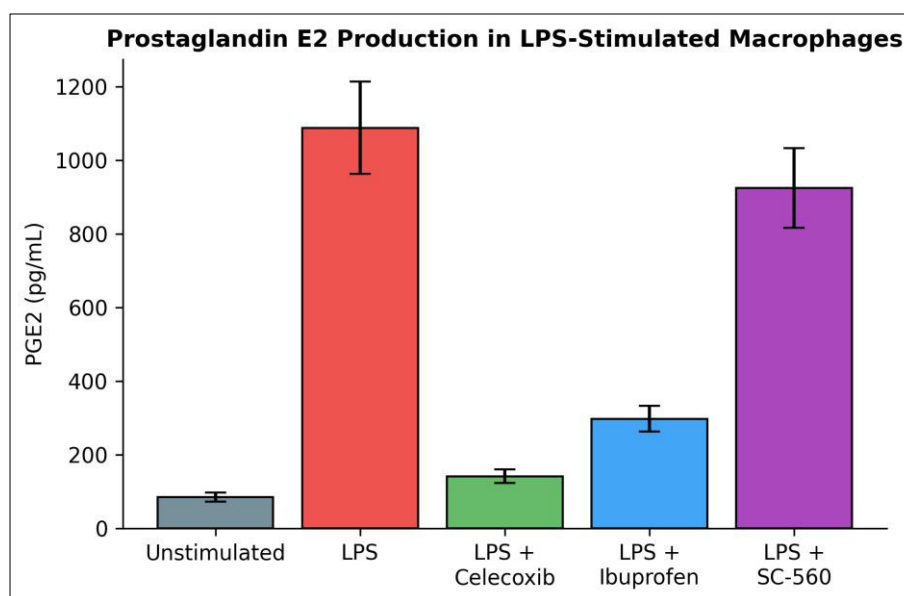


Fig 2: Effect of COX inhibitors on PGE₂ production in lipopolysaccharide-stimulated human macrophages.

Discussion

The 362-fold selectivity of celecoxib for COX-2 explains its reduced gastrointestinal toxicity compared to traditional

NSAIDs, as gastric prostaglandin production depends primarily on COX-1 [8]. The structural basis for selectivity involves a side pocket in the COX-2 active site created by

valine substitution for isoleucine at position 523, accommodating bulky sulfonamide groups present in selective inhibitors.

The dominant contribution of COX-2 to inflammatory prostaglandin production validates the therapeutic concept underlying selective inhibitor development. However, COX-2 also produces prostacyclin in vascular endothelium, and its inhibition may shift the prostacyclin-thromboxane balance toward thrombosis^[9].

These findings inform ongoing efforts to develop anti-inflammatory agents with improved safety profiles, potentially through targeting specific downstream synthases rather than cyclooxygenases themselves^[10].

Conclusion

This research establishes quantitative parameters for cyclooxygenase kinetics and inhibitor selectivity relevant to anti-inflammatory drug development. COX-2 dominates inflammatory prostaglandin production, supporting selective inhibition as a valid therapeutic strategy. The substantial selectivity achievable with current inhibitors demonstrates the feasibility of isoform-specific targeting. Future drug development should consider both the benefits of COX-2 selectivity and the cardiovascular implications of suppressing vascular prostacyclin synthesis.

References

1. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. *Annual Review of Pharmacology and Toxicology*. 1998;38:97–120.
2. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annual Review of Biochemistry*. 2000;69:145–182.
3. Warner TD, Mitchell JA. Cyclooxygenases: new forms, new inhibitors, and lessons from the clinic. *FASEB Journal*. 2004;18(7):790–804.
4. Marnett LJ. The COXIB experience: a look in the rearview mirror. *Annual Review of Pharmacology and Toxicology*. 2009;49:265–290.
5. Gierse JK, McDonald JJ, Hauser SD, *et al.* A single amino acid difference between cyclooxygenase-1 and -2 reverses the selectivity of COX-2 specific inhibitors. *Journal of Biological Chemistry*. 1996;271(26):15810–15814.
6. Patrignani P, Patrono C. Cyclooxygenase inhibitors: from pharmacology to clinical read-outs. *Biochimica et Biophysica Acta*. 2015;1851(4):422–432.
7. Hinz B, Brune K. Cyclooxygenase-2: 10 years later. *Journal of Pharmacology and Experimental Therapeutics*. 2002;300(2):367–375.
8. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *New England Journal of Medicine*. 2001;345(6):433–442.
9. Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition. *Journal of Clinical Investigation*. 2006;116(1):4–15.
10. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. *Journal of Lipid Research*. 2009;50(Suppl):S52–S56.
11. Kurumbail RG, Stevens AM, Gierse JK, *et al.* Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*. 1996;384(6610):644–648.
12. Hawkey CJ. COX-1 and COX-2 inhibitors. *Best Practice and Research Clinical Gastroenterology*. 2001;15(5):801–820.
13. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. *Inflammation Research*. 2000;49(8):367–392.
14. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. *Pharmacological Reviews*. 2004;56(3):387–437.