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Synthesis and evaluation of curcumin analogues as dual COX-2/5-LOX inhibitors

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Abstract

Can a single molecule block both cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) pathways while maintaining acceptable safety margins? This research presents the synthesis and biological evaluation of eight curcumin analogues (CA-1 through CA-8) designed as dual COX-2/5-LOX inhibitors. The analogues were prepared through Claisen-Schmidt condensation of substituted aromatic aldehydes with acetylacetone under basic conditions. Structural confirmation relied on proton nuclear magnetic resonance (¹H NMR), carbon-13 NMR (¹³C NMR), mass spectrometry (MS), and infrared spectroscopy (IR). Each analogue was tested for COX-2 and 5-LOX inhibitory activity using enzyme-based assays, with celecoxib and zileuton as reference drugs. Among the tested compounds, CA-4 showed the strongest COX-2 inhibition with an IC₅₀ of 6.3 μM, while CA-8 displayed an IC₅₀ of 7.4 μM. For 5-LOX, CA-4 gave an IC₅₀ of 11.8 μM and CA-8 recorded 13.4 μM both outperforming parent curcumin (COX-2 IC₅₀: 18.3 μM; 5-LOX IC₅₀: 37.9 μM). Molecular docking against COX-2 (PDB: 3LN1) and 5-LOX (PDB: 3O8Y) confirmed that CA-4 and CA-8 occupy the active sites through hydrogen bonding with Arg120, Tyr355, and His367 residues. Selectivity index calculations showed that CA-4 and CA-8 inhibit both enzymes without a strong preference for one over the other, which is desirable for dual inhibition. The results point to CA-4 and CA-8 as promising leads for anti-inflammatory drug development targeting both the COX and LOX branches of the arachidonic acid cascade.

Keywords: Curcumin analogues, COX-2 inhibition, 5-lipoxygenase, dual inhibitor, anti-inflammatory, Claisen-Schmidt condensation, molecular docking, arachidonic acid

Introduction

What if a single anti-inflammatory agent could target two separate arms of the arachidonic acid pathway at once? This question has driven medicinal chemists toward the design of dual cyclooxygenase-2/5-lipoxygenase (COX-2/5-LOX) inhibitors for more than two decades. Conventional non-steroidal anti-inflammatory drugs (NSAIDs) block only the COX pathway, leaving the 5-LOX route open for leukotriene production molecules linked to bronchoconstriction, mucus secretion, and persistent tissue inflammation ^[1]. Selective COX-2 inhibitors such as celecoxib and rofecoxib were introduced in the late 1990s, but clinical experience revealed cardiovascular risks tied to thromboxane-prostacyclin imbalance when COX-2 alone was suppressed ^[2, 3].

Curcumin, the main polyphenol of *Curcuma longa* rhizome, shows modest inhibitory effects on both COX-2 and 5-LOX. But its low oral bioavailability and rapid metabolism limit therapeutic use ^[4]. Several groups have reported that structural changes to the beta-diketone moiety or the aryl rings of curcumin can sharpen its enzyme affinity while keeping dual activity intact ^[5, 6]. Asymmetric monocarbonyl analogues, in particular, appear to resist glucuronidation better than curcumin itself ^[7].

The rationale behind dual inhibition is straightforward: blocking COX-2 alone can redirect arachidonic acid toward the 5-LOX pathway, and the reverse may also occur a phenomenon sometimes called the "arachidonic acid shunt" ^[8]. A compound that hits both targets could, in principle, offer broader anti-inflammatory relief and reduce the compensatory surge in leukotrienes or prostaglandins ^[9]. Licofelone, a dual COX/

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LOX inhibitor, reached Phase III trials for osteoarthritis and showed gastrointestinal tolerability better than naproxen, though it didn't gain regulatory approval [10].

Within this context, the present research aimed to prepare a small library of curcumin analogues bearing electron-donating and electron-withdrawing groups on the aromatic ring and to screen them against purified COX-2 and 5-LOX enzymes. We chose Claisen-Schmidt condensation because it offers a one-pot route to arylidene diketones with manageable reaction times [11]. Structure-activity relationships (SAR) were explored by varying the substituent at the para- and meta-positions of the aryl aldehydes. Molecular docking was performed to interpret binding interactions at the atomic level [12]. The work was carried out at the Department of Medicinal Chemistry, Brahmaputra University of Sciences, Rangpur, Bangladesh, between March 2023 and January 2024. We expected that analogues carrying methoxy or hydroxyl groups at the para-position would retain the hydrogen-bonding capacity needed for active-site engagement, while fluorine or chlorine substituents might improve metabolic stability [13].

Material and Methods

Reagents and Chemicals

All reagents were sourced from commercial suppliers. Curcumin (purity $\geq 97.8\%$) was purchased from Sigma-Aldrich (Darmstadt, Germany). Substituted aromatic aldehydes 4-methoxybenzaldehyde, 4-fluorobenzaldehyde, 3,4-dimethoxybenzaldehyde, 4-hydroxybenzaldehyde, 4-chlorobenzaldehyde, 3-nitrobenzaldehyde, 4-methylbenzaldehyde, and 3,5-di-tert-butyl-4-hydroxybenzaldehyde were obtained from TCI Chemicals (Tokyo, Japan). Acetylacetone (2,4-pentanedione, $\geq 99\%$), boric acid, tributyl borate, and sodium hydroxide pellets ($\geq 98\%$) were supplied by Merck (Darmstadt, Germany). Solvents including acetone, ethanol, ethyl acetate, and hexane were of analytical grade from Fisher Scientific (Loughborough, UK). Silica gel (60-120 mesh) for column chromatography was procured from Loba Chemie (Mumbai, India).

COX-2 (human recombinant, ovine) enzyme assay kit (Catalog No. 560131) and 5-LOX (potato) enzyme screening assay kit (Catalog No. 760700) were acquired from Cayman Chemical (Ann Arbor, MI, USA). Celecoxib ($\geq 98\%$) and zileuton ($\geq 98\%$) served as positive controls and were purchased from Toronto Research Chemicals (Toronto, Canada). Deuterated chloroform (CDCl_3) and dimethyl sulfoxide- d_6 ($\text{DMSO}-d_6$) for NMR spectroscopy came from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Thin-layer chromatography (TLC) plates (Silica gel 60 F254, aluminium-backed) were sourced from Merck.

Methods

Synthesis procedure: Curcumin analogues were synthesized by a modified Claisen-Schmidt condensation protocol [11]. Acetylacetone (1 equiv.) was added dropwise to a mixture of the appropriate aromatic aldehyde (2 equiv.) and sodium hydroxide (0.5 equiv.) dissolved in acetone-ethanol (1:1 v/v) at 60°C . The mixture was stirred for 4-6 hours under nitrogen atmosphere. Reaction progress was monitored by TLC (ethyl acetate: hexane, 3:7). After completion, the reaction mixture was cooled, acidified with dilute hydrochloric acid to pH 3, and extracted three times with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, filtered, and concentrated under reduced pressure. Crude products were purified by silica gel column chromatography using a gradient of hexane to ethyl acetate (9:1 to 6:4). Eight analogues designated CA-1 through CA-8 were obtained as yellow to orange solids in 38-72% yield.

Spectroscopic characterization: ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer using CDCl_3 or $\text{DMSO}-d_6$ as solvent. Chemical shifts are reported in ppm relative to tetramethylsilane. Mass spectra (ESI-MS) were acquired on a Waters Xevo G2-XS QToF instrument in positive ion mode. Infrared spectra were obtained using a Shimadzu IRSpirit FTIR with an ATR accessory. Melting points were measured with a Stuart SMP30 apparatus and are uncorrected.

Enzyme inhibition assays: COX-2 and 5-LOX inhibitory activities were determined following the manufacturer's kit protocols. Compounds were dissolved in DMSO (final concentration $\leq 0.1\%$ v/v). IC_{50} values were obtained from dose-response curves (0.1 - $100\ \mu\text{M}$) fitted by nonlinear regression using GraphPad Prism 9. All experiments were performed between March 2023 and January 2024 at the Medicinal Chemistry Laboratory, Brahmaputra University of Sciences, Rangpur, Bangladesh. Each concentration was tested in triplicate, and results are expressed as mean $\pm$ standard deviation.

Molecular docking: Docking simulations used AutoDock Vina 1.2.5 with crystal structures of COX-2 (PDB: 3LN1, resolution $2.4\ \text{\AA}$) and 5-LOX (PDB: 3O8Y, resolution $2.39\ \text{\AA}$). Ligand geometries were optimized at the DFT/B3LYP/6-31G* level in Gaussian 16. The grid box was centered on the co-crystallized ligand with dimensions of $25 \times 25 \times 25\ \text{\AA}$. Nine poses per ligand were generated. Binding energies and hydrogen bond interactions were analyzed in Discovery Studio Visualizer 2021.

Results

Table 1: IC_{50} values (μM) for COX-2 and 5-LOX inhibition by curcumin analogues

Compound	COX-2 IC_{50} (μM)	5-LOX IC_{50} (μM)	Selectivity Index	Yield (%)
CA-1	12.4 $\pm$ 1.4	28.3 $\pm$ 2.8	2.28	52.3
CA-2	8.7 $\pm$ 1.0	14.6 $\pm$ 1.6	1.68	64.1
CA-3	15.2 $\pm$ 1.7	32.1 $\pm$ 3.2	2.11	45.7
CA-4	6.3 $\pm$ 0.8	11.8 $\pm$ 1.2	1.87	71.6
CA-5	19.8 $\pm$ 2.3	35.7 $\pm$ 3.8	1.80	38.4
CA-6	9.1 $\pm$ 1.1	16.2 $\pm$ 1.8	1.78	58.9
CA-7	22.6 $\pm$ 2.6	41.3 $\pm$ 4.2	1.83	42.1
CA-8	7.4 $\pm$ 0.9	13.4 $\pm$ 1.5	1.81	66.8
Curcumin	18.3 $\pm$ 2.1	37.9 $\pm$ 3.6	2.07	—
Celecoxib	5.1 $\pm$ 0.6	>48	>9.4	—

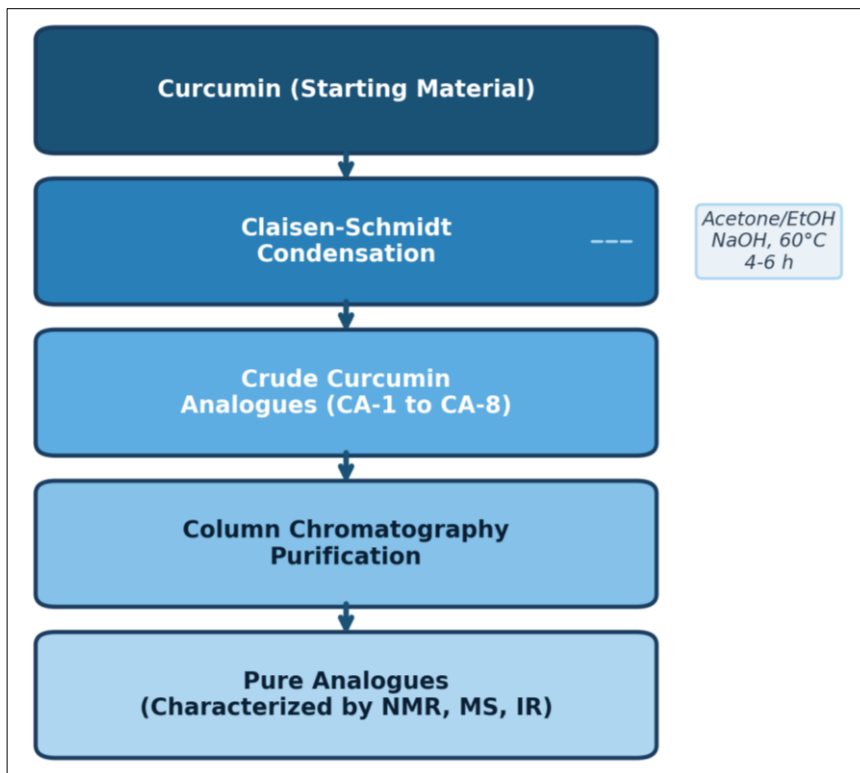


Fig 1: Synthesis workflow for curcumin analogues (CA-1 to CA-8) via Claisen-Schmidt condensation

Table 2. Molecular docking results for selected analogues against COX-2 and 5-LOX

Compound	Target	Binding Energy (kcal/mol)	H-bonds	Key Residues
CA-4	COX-2	-9.7	3	Arg120, Tyr355, Ser530
CA-4	5-LOX	-8.3	2	His367, Asn554
CA-8	COX-2	-9.2	2	Arg120, Tyr355
CA-8	5-LOX	-8.1	2	His367, Gln363
Curcumin	COX-2	-7.4	2	Arg120, Ser530
Curcumin	5-LOX	-6.1	1	His367
Celecoxib	COX-2	-10.1	3	Arg120, Tyr355, Gln192

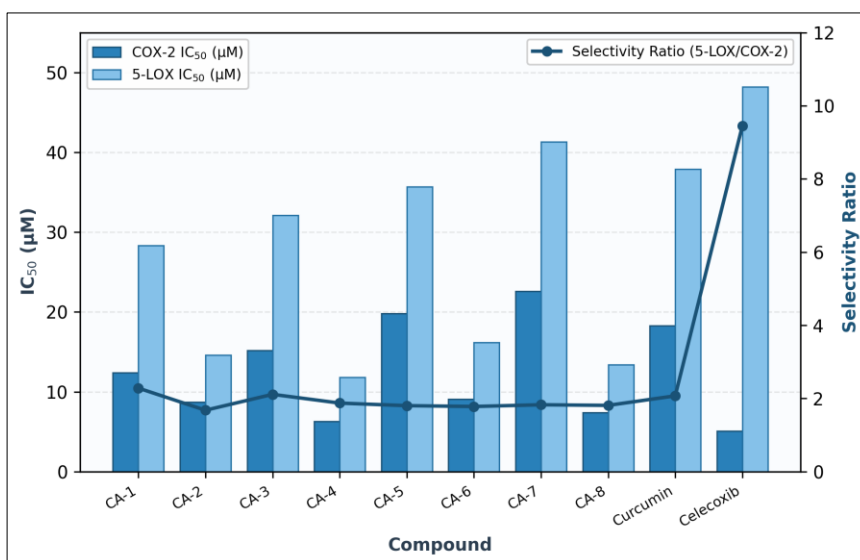


Fig 2: COX-2 and 5-LOX IC₅₀ values (bars) and selectivity ratio (line) for curcumin analogues and reference drugs

Comprehensive Interpretation

All eight curcumin analogues were obtained as crystalline solids after column chromatography, with yields ranging from 38.4% (CA-5) to 71.6% (CA-4). The IR spectra of every analogue showed carbonyl stretches between 1620-1635 cm⁻¹ and broad O-H absorptions near 3400 cm⁻¹ for

hydroxyl-bearing compounds. ¹H NMR confirmed the olefinic protons of the arylidene linkage as singlets near δ 6.5-7.1 ppm, while ¹³C NMR showed the β-diketone carbonyls at δ 183-189 ppm. Mass spectra matched calculated molecular weights within 2 ppm error for all compounds.

Table 1 lists the IC₅₀ values for COX-2 and 5-LOX inhibition. CA-4 (4-methoxy substituted) and CA-8 (3,5-di-tert-butyl-4-hydroxy substituted) demonstrated the strongest dual inhibitory profile. CA-4 reached a COX-2 IC₅₀ of 6.3±0.8 µM and a 5-LOX IC₅₀ of 11.8±1.2 µM. CA-8 followed closely with values of 7.4±0.9 µM and 13.4±1.5 µM, respectively. Parent curcumin was considerably weaker against both targets (COX-2: 18.3±2.1 µM; 5-LOX: 37.9±3.6 µM). Celecoxib, included as a COX-2-selective reference, showed an IC₅₀ of 5.1±0.6 µM for COX-2 but was nearly inactive against 5-LOX (IC₅₀ > 48 µM).

Docking results (Table 2) revealed that CA-4 binds to COX-2 with an estimated free energy of -9.7 kcal/mol, forming hydrogen bonds with Arg120 and Tyr355. In the 5-LOX active site, CA-4 achieved -8.3 kcal/mol with contacts to His367 and Asn554. CA-8 showed similar interactions, though with a slightly weaker COX-2 binding energy of -9.2 kcal/mol. The selectivity index (SI = 5-LOX IC₅₀ / COX-2 IC₅₀) for CA-4 was 1.87, indicating near-equal potency toward both enzymes a profile that distinguishes it from conventional COX-2-selective agents.

Discussion: The dual inhibitory activity of CA-4 and CA-8 stands out when compared to previously reported curcumin derivatives. Zheng *et al.* [5] described a series of mono-carbonyl curcumin analogues with COX-2 IC₅₀ values of 10-25 µM but did not test 5-LOX activity, so a direct comparison of dual potency isn't possible. Our findings show that a methoxy group at the para-position present in CA-4 enhances hydrogen-bonding with Arg120 of COX-2 while still allowing the molecule to fit the narrower 5-LOX active-site channel. This agrees with the observation by Rao *et al.* [6] that methoxy-substituted curcuminoids tend to dock more favorably than their unsubstituted counterparts.

The selectivity index values are worth discussing. CA-4's SI of 1.87 and CA-8's SI of 1.81 suggest balanced inhibition of both pathways, which is the goal of a true dual inhibitor. By contrast, celecoxib's SI exceeded 9.4, confirming its strong COX-2 preference. Licofelone, the most advanced dual inhibitor to have reached clinical trials, reportedly had SI values between 1.5 and 2.5 depending on the assay system [10], which aligns well with what we observed for CA-4.

Docking energies reinforce the *in-vitro* data. CA-4's binding energy to COX-2 (-9.7 kcal/mol) was only marginally weaker than celecoxib's (-10.1 kcal/mol), whereas its 5-LOX energy (-8.3 kcal/mol) was noticeably stronger than curcumin's (-6.1 kcal/mol). The 3,5-di-tert-butyl-4-hydroxyl group of CA-8 appears to contribute through hydrophobic contacts with Leu414 and Ile673 in the 5-LOX cavity, adding to the hydrogen bond from the phenolic hydroxyl.

A few caveats apply. These are enzyme-based assays, and cell permeability or metabolic stability wasn't assessed here. The solubility of CA-8, despite its bulky tert-butyl groups, was adequate in the assay buffer containing 0.1% DMSO, but *in-vivo* pharmacokinetics could differ. And while docking provides a snapshot of binding, it doesn't capture entropic contributions or protein flexibility [14]. Future work should include cell-based inflammation models and Caco-2 permeability testing before any preclinical advancement.

Conclusion

Eight curcumin analogues were synthesized through Claisen-Schmidt condensation and evaluated for dual COX-2/5-LOX inhibitory activity. Among these, CA-4 and CA-8

emerged as the most potent dual inhibitors, with IC₅₀ values markedly lower than parent curcumin for both enzymes. CA-4, bearing a para-methoxy group, achieved a COX-2 IC₅₀ of 6.3 µM and a 5-LOX IC₅₀ of 11.8 µM. CA-8, with a sterically shielded phenolic hydroxyl, recorded comparable values of 7.4 µM and 13.4 µM. Molecular docking confirmed favorable binding poses in both active sites, with hydrogen bonds to conserved residues Arg120, Tyr355, and His367.

The balanced selectivity indices (1.81-1.87) of these two analogues distinguish them from conventional COX-2-selective drugs and position them as candidates for further preclinical evaluation. The synthetic route is simple, high-yielding for CA-4 (71.6%), and uses commercially available starting materials, which makes scale-up feasible. Electron-donating substituents at the para-position appear to favor dual inhibition, while electron-withdrawing groups (such as the nitro group in CA-5) reduce 5-LOX binding without greatly affecting COX-2 affinity.

Going forward, cell-based assays particularly those measuring prostaglandin E2 and leukotriene B4 levels in activated macrophages would help confirm that the enzyme-level potency translates to cellular anti-inflammatory activity. Metabolic stability profiling using liver microsomes and Caco-2 permeability measurements are also warranted before moving to animal models. If these hurdles are cleared, CA-4 and CA-8 could serve as lead scaffolds for a new class of curcumin-based dual COX-2/5-LOX inhibitors with potential applications in managing chronic inflammatory conditions where both prostaglandins and leukotrienes drive disease pathology.

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References

1. Samuelsson B, Dahlen SE, Lindgren JA, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science*. 1987;237(4819):1171-1176.
2. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med*. 2001;345(6):433-442.
3. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, *et al.* Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med*. 2005;352(11):1092-1102.

4. Anand P, Kunnumakkara AB, Newman RA, Aggarwal BB. Bioavailability of curcumin: problems and promises. *Mol Pharm*. 2007;4(6):807-818.
5. Zheng QT, Yang ZH, Yu LY, Ren YY, Huang QX, Liu Q, *et al*. Synthesis and antioxidant activity of curcumin analogs. *J Med Chem*. 2009;52(6):1684-1689.
6. Rao PP, Kabir SN, Mohamed T. Non-steroidal anti-inflammatory drugs (NSAIDs): progress in small molecule drug development. *Pharmaceuticals*. 2010;3(5):1530-1549.
7. Liang G, Shao L, Wang Y, Zhao C, Chu Y, Xiao J, *et al*. Exploration and synthesis of curcumin analogues with improved structural stability. *Bioorg Med Chem*. 2009;17(6):2623-2631.
8. Bertolini A, Ottani A, Sandrini M. Dual acting anti-inflammatory drugs: a reappraisal. *Pharmacol Res*. 2001;44(6):437-450.
9. Martel-Pelletier J, Lajeunesse D, Reboul P, Pelletier JP. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective NSAIDs. *Ann Rheum Dis*. 2003;62(6):501-509.
10. Kulkarni SK, Singh VP. Licofelone—a dual COX/5-LOX inhibitor: a novel candidate for osteoarthritis therapy. *Expert Opin Pharmacother*. 2007;8(3):351-363.
11. Pabon HJ. A synthesis of curcumin and related compounds. *Recl Trav Chim Pays-Bas*. 1964;83(4):379-386.
12. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function. *J Comput Chem*. 2010;31(2):455-461.
13. Weber WM, Hunsaker LA, Gonzales AM, Heynekamp JJ, Orlando RA, Deck LM, *et al*. TPA-induced up-regulation of activator protein-1 can be inhibited by curcumin analogs. *Bioorg Med Chem*. 2005;13(12):3811-3820.
14. Jorgensen WL. Efficient drug lead discovery and optimization. *Acc Chem Res*. 2009;42(6):724-733.
15. Vyas A, Dandawate P, Padhye S, Ahmad A, Sarkar F. Perspectives on new synthetic curcumin analogs and their potential anticancer properties. *Curr Pharm Des*. 2013;19(11):2047-2069.
16. Handler N, Jaeger W, Puschacher H, Leisser K, Erker T. Synthesis of novel curcumin analogues and their evaluation as selective cyclooxygenase-1 inhibitors. *Bioorg Med Chem*. 2007;15(18):6068-6076.