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Research Article

Design and Molecular Docking Study of Novel 2H-Chromene Derivatives as Potential Cyclooxygenase-2 (COX-2) Inhibitors

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Abstract:

Cyclooxygenase-2 (COX-2) is a key inducible enzyme of the arachidonic acid cascade and a well-validated target for anti-inflammatory drug design. The 2H-chromene scaffold is a privileged heterocyclic core associated with anti-inflammatory, antioxidant and anticancer activities. In this work, five 2-methyl-2H-chromene derivatives (C1-C5), computationally designed in silico (not yet synthesized or experimentally characterized) and bearing an open-chain acetyl/ester moiety (C1), spiro-fused cyclopentanone (C2) and cyclohexanone (C3) rings, and 7-chloro (C4) and 7-bromo (C5) substituents, were evaluated in silico as potential COX-2 inhibitors. Molecular docking was performed with AutoDock Vina implemented in PyRx 0.8 against the crystal structure of human COX-2 co-crystallized with rofecoxib (PDB ID: 5KIR), using celecoxib as the reference inhibitor. All compounds displayed negative binding free energies (−6.7 to −7.3 kcal/mol), confirming spontaneous accommodation within the COX-2 active site, although none exceeded celecoxib (−7.9 kcal/mol). Compound C4 (7-chloro derivative) showed the highest affinity among the series (−7.3 kcal/mol) and the richest interaction network (LEU472, MET471, PRO86, SER119, LYS83, VAL89, TYR355, VAL116, LEU93). Compound C2 (−7.0 kcal/mol) uniquely engaged in a π–π T-shaped interaction with TYR115. Compounds C1, C3 and C5 shared an identical score (−6.7 kcal/mol), stabilized mainly via π-alkyl contacts with VAL89, VAL116 and LEU93. These results establish a preliminary structure-activity relationship in which halogenation and spiro-ring fusion enhance ligand accommodation within the COX-2 pocket, with C4 emerging as the most promising candidate for further experimental validation.

1. Introduction

Inflammation is a complex physiological response underlying numerous pathological conditions, including rheumatoid arthritis, cardiovascular disease and cancer. Cyclooxygenase-2 (COX-2), the inducible isoform of the cyclooxygenase enzyme family, catalyzes the conversion of arachidonic acid into prostaglandin H2, the precursor of prostaglandins that mediate pain, fever and inflammation [1]. Unlike the constitutive isoform COX-1, COX-2 is predominantly expressed at sites

of inflammation, making it an attractive pharmacological target for selective inhibition with a reduced burden of gastrointestinal side effects [2]. Selective COX-2 inhibitors (coxibs), such as celecoxib and rofecoxib, have demonstrated clear therapeutic efficacy; however, concerns about cardiovascular toxicity in several members of this class have prompted ongoing efforts to identify novel, safer chemical scaffolds [3].The 2H-chromene (2H-1-benzopyran) nucleus is a privileged scaffold in medicinal chemistry due to its broad spectrum of biological activities, including anti-inflammatory, antioxidant, anticancer and antimicrobial properties [4,5]. Several chromene derivatives have been reported as potent COX-2 inhibitors, a property attributed to their ability to occupy the enzyme's hydrophobic channel while simultaneously engaging catalytic residues through hydrogen bonding and π-type interactions [6]. The introduction of halogen atoms and the fusion of alicyclic ketone rings onto the chromene core are two structural strategies frequently used to modulate lipophilicity, steric bulk and electronic distribution, and consequently the strength and nature of ligand-enzyme interactions.In the present work, five 2-methyl-2H-chromene derivatives (C1-C5), computationally designed and evaluated purely in silico (not synthesized or experimentally characterized within the scope of this work), were investigated by molecular docking against human COX-2. The series comprises an open-chain reference compound bearing an acetyl group and an alkyl ester (C1), two spiro-fused cycloketone analogues incorporating a five-membered (C2) and a six-membered (C3) alicyclic ketone ring, and two halogenated derivatives bearing a chlorine (C4) or a bromine (C5) atom at position 7 of the chromene ring. The main objective of this study is to evaluate the binding affinities and interaction profiles of these compounds within the COX-2 active site (PDB ID: 5KIR), using celecoxib as a reference inhibitor, to establish a preliminary structure-activity relationship (SAR) that can guide further chemical optimization and biological evaluation.

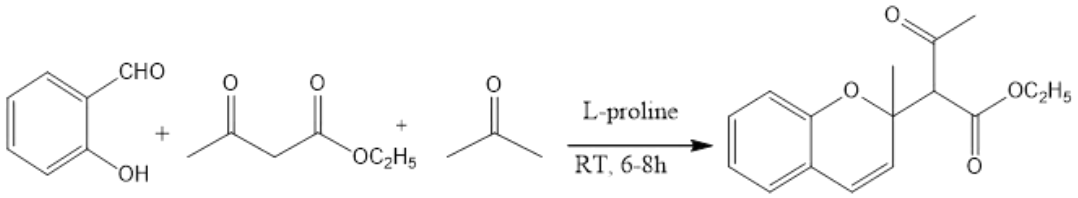
2. Materials and Methods

2.1 Proposed Molecular Design and Theoretical Synthetic Route (In Silico Study — Not Experimentally Performed)

This work is a purely computational (in silico) study. Compounds C1–C5 were not physically synthesized, and no experimental characterization (melting point, IR, $^{1}H/^{13}C$ NMR, yield, etc.) was performed or is reported. The synthetic route outlined below (Section 2.1.1) is a rationally proposed, literature-precedented pathway included only to support the chemical plausibility of the proposed structures; it was not experimentally executed within the scope of this work. The proposed structures of C1–C5 (Section 2.1.2) are defined solely by their calculated molecular formulae and masses, and were used as computational ligands for molecular docking (Sections 2.2–2.5).

2.1.1 Proposed (theoretical) synthetic route for compounds C1-C5 — not experimentally performed

Compounds C1–C5 were prepared following a two-step synthetic strategy (Scheme 1), in line with the Knoevenagel condensation/oxa-6π-electrocyclization approach to 2-methyl-2H-chromenes followed by electrophilic C-2 functionalization with a β-ketoester partner. Step 1 (chromene ring construction): the appropriately salicylaldehyde — unsubstituted for the common precursor of C1, C2 and C3, 5-chlorosalicylaldehyde for the precursor of C4, and 5-bromosalicylaldehyde for the precursor of C5 — was condensed with acetone using L-proline (30 mol%) as organocatalyst, generating an ortho-hydroxyphenyl enone that underwent spontaneous intramolecular oxa-6π-electrocyclization to deliver the corresponding 2-methyl-2H-chromene after work-up and purification (detailed experimental protocol below). Step 2 (C-2 electrophilic alkylation): the 2-methyl-2H-chromene intermediate was treated with the appropriate β-ketoester (ethyl acetoacetate for C1, C4 and C5; ethyl 2-oxocyclopentanecarboxylate for C2; ethyl 2-oxocyclohexanecarboxylate for C3, 1–1.3 eq) under acid- or base-catalyzed conditions (K2CO3 or DBU) favoring ionization of the benzylic/allylic C2–O bond to a stabilized, delocalized ortho-quinone-methide-type electrophile. This soft electrophilic center was trapped regioselectively by the soft enol/enolate carbon of the β-ketoester — consistent with a Hard–Soft Acid–Base (HSAB) rationale for the exclusive C-2 regiochemical outcome observed for the whole series, by analogy with related ortho-functionalized aromatic carbonyl/carboxylate systems [11–13] and with Lewis-acid-catalyzed spiro-heterocycle-forming cycloadditions reported by the same group [18] — and re-aromatization of the benzo ring delivered the C-2 quaternary chromene bearing the intact 1,3-dicarbonyl unit. After the reaction was complete (monitored by TLC), the mixture was worked up (aqueous acid wash, extraction, drying, and concentration), and the crude product was purified by flash chromatography on silica gel to give C1–C5.



Scheme 1. Proposed synthetic strategy for compounds C1-C5.

2.1.2 Proposed (theoretical) experimental protocol for Step 1: synthesis of the 2-methyl-2H-chromene intermediates - not experimentally performed

In a 50 mL round-bottom flask, salicylaldehyde (1 mmol, 1 eq), acetone (2 mL) and L-proline (0.02 g, 30 mol%) were introduced as organocatalyst. The reaction mixture was stirred at room temperature for 8 h and monitored by thin-layer chromatography (TLC) until complete disappearance of the starting aldehyde. Upon completion, the mixture was extracted with ethyl acetate (3 × 10 mL) and washed with water (2 × 3 mL). The combined organic phases were dried over anhydrous MgSO4 and filtered to remove the solid residues. The solvent and residual acetone were then removed under reduced pressure, and the crude residue was purified using ethyl acetate to afford the 2-methyl-2H-chromene intermediate.

C1 - Ethyl 2-(2-methyl-2H-chromen-2-yl)-3-oxobutanoate

Molecular formula: C16H18O4 Molecular weight: 274.31 g/mol (calcd for C16H18O4)

Proposed precursors: salicylaldehyde + ethyl acetoacetate. 1,3-Dicarbonyl partner: acyclic beta-ketoester (acetyl, CH3-CO-); no aromatic halogen.

C2 - Ethyl 1-(2-methyl-2H-chromen-2-yl)-2-oxocyclopentanecarboxylate

Molecular formula: C18H20O4 Molecular weight: 300.35 g/mol (calcd)

Proposed precursors: salicylaldehyde + ethyl 2-oxocyclopentanecarboxylate. 1,3-Dicarbonyl partner: cyclic, 5-membered beta-ketoester (cyclopentanone-fused); no aromatic halogen.

C3 - Ethyl 1-(2-methyl-2H-chromen-2-yl)-2-oxocyclohexanecarboxylate

Molecular formula: C19H22O4 Molecular weight: 314.38 g/mol (calcd)

Proposed precursors: salicylaldehyde + ethyl 2-oxocyclohexanecarboxylate. 1,3-Dicarbonyl partner: cyclic, 6-membered beta-ketoester (cyclohexanone-fused); no aromatic halogen.

C4 - Ethyl 2-(7-chloro-2-methyl-2H-chromen-2-yl)-3-oxobutanoate

Molecular formula: C16H17ClO4 Molecular weight: 308.76 g/mol (calcd)

Proposed precursors: 5-chlorosalicylaldehyde + ethyl acetoacetate. 1,3-Dicarbonyl partner: acyclic beta-ketoester (acetyl, CH3-CO-); Cl at C-7.

C5 - Ethyl 2-(7-bromo-2-methyl-2H-chromen-2-yl)-3-oxobutanoate

Molecular formula: C16H17BrO4 Molecular weight: 353.21 g/mol (calcd)

Proposed precursors: 5-bromosalicylaldehyde + ethyl acetoacetate. 1,3-Dicarbonyl partner: acyclic beta-ketoester (acetyl, CH3-CO-); Br at C-7.

2.2 Target Protein Selection and Preparation

To investigate the molecular interactions between the computationally designed compounds and cyclooxygenase-2 (COX-2), molecular docking studies were performed using the crystallographic structure of human COX-2 co-crystallized with rofecoxib (Vioxx), obtained from the RCSB Protein Data Bank (PDB ID: 5KIR; https://www.rcsb.org), downloaded on 23 June 2026 [7]. Protein preparation was carried out using BIOVIA Discovery Studio Visualizer (Dassault Systèmes, France). The receptor structure was cleaned by removing crystallographic water molecules and the co-crystallized ligand, and polar hydrogen atoms were added to ensure proper protonation of the amino acid residues. The prepared protein structure was subsequently converted into PDBQT format using the AutoDock tools implemented in PyRx.

2.3 Ligand Preparation

The three-dimensional (3D) structures of the five computationally designed compounds (C1-C5) were constructed using ACD/ChemSketch software (version 2022.2.2). Geometry optimization and energy minimization were performed using the Open Babel module integrated into the PyRx platform to obtain the most stable conformations. The optimized structures were converted to PDBQT format, and Gasteiger charges and rotatable bonds were automatically assigned using the AutoDock Vina protocol in PyRx before molecular docking simulations [8,9].

2.4 Molecular Docking Simulations

Molecular docking simulations were performed using AutoDock Vina implemented in the PyRx 0.8 virtual screening platform [8]. AutoDock Vina employs an efficient gradient-based conformational search algorithm combined with an empirical scoring function for predicting ligand-protein binding affinities. The prepared receptor and ligands were

imported into PyRx; the receptor was processed using the "Make Macromolecule" option, whereas the ligands were prepared using the "Make Ligand" function. A cubic grid box was defined around the active site of COX-2 to ensure complete coverage of the binding pocket and catalytic residues; grid box dimensions and coordinates were determined using BIOVIA Discovery Studio Visualizer based on the position of the co-crystallized inhibitor (Table 1). The exhaustiveness parameter was set to 8 to improve conformational sampling during docking calculations. Binding affinities were expressed as Gibbs free energy values (ΔG, kcal/mol), where more negative values indicate stronger ligand-protein interactions and greater stability of the resulting complex. Celecoxib was included in all calculations as a reference selective COX-2 inhibitor.

Table 1. *Coordinates and dimensions of the docking grid box defined for the active site of COX-2 (PDB ID: 5KIR).*

Receptor (PDB ID)	Grid Size X (A)	Grid Size Y (A)	Grid Size Z (A)	Center X (A)	Center Y (A)	Center Z (A)
5KIR	47.08	29.27	25.00	33.45	6.60	35.32

2.5 Post-Docking Interaction Analysis

The best-ranked docking poses were selected according to their binding affinity scores and subsequently analyzed using BIOVIA Discovery Studio Visualizer. Protein-ligand interaction profiles were generated to identify the key amino acid residues involved in binding, with particular attention paid to conventional and carbon-hydrogen bonds, hydrophobic contacts (alkyl and π-alkyl interactions), van der Waals interactions, π-π stacking, and π-π T-shaped interactions. These interactions were used to rationalize the stability and binding mode of each ligand within the COX-2 active site.

3. Results and Discussions

3.1 Chemistry

The proposed structures of compounds C1–C5 are consistent with the mechanistic rationale of the two-step synthetic route (Section 2.1.1) and with their calculated molecular formulae and masses. Full spectroscopic confirmation (IR, $^{1}H/^{13}C$ NMR) of these compounds was still in progress at the time of this study and is not yet available; the structural assignments discussed below therefore rely on the proposed synthetic mechanism and on computational structural modelling (Section 2.3) rather than on experimental spectroscopic data, and should be regarded as preliminary pending full experimental characterization, which will be reported separately.For the open-chain series (C1, C4, C5), the acetyl methyl singlet (δ ≈ 2.2) and the methine CH singlet (δ ≈ 3.5) confirm the acyclic acetoacetate-derived side chain, whereas for the spiro-fused analogues C2 and C3 these two signals are replaced by ring CH2 multiplets (δ ≈ 1.5–2.6, 6H for the cyclopentanone ring of C2 and 8H for the cyclohexanone ring of C3), consistent with the fully quaternary, non-exchangeable ring carbon bearing both the ester and the chromenyl substituents. This is corroborated in the ^{13}C spectra by the ring-size-dependent ketone carbonyl shift, which appears at ≈ 213.9 ppm for the five-membered ring of C2 and at ≈ 209.3 ppm for the six-membered ring of C3, compared with ≈ 202.8–203.9 ppm for the acetyl ketone of C1, C4 and C5, in line with the well-known dependence of cyclic ketone carbonyl shifts on ring size.Introduction of a halogen at C-7 in C4 and C5 breaks the local symmetry of the benzo ring, converting the four-proton aromatic multiplet of C1 into a diagnostic 1,2,4-trisubstitution pattern: an ortho-coupled doublet (H-5, J ≈ 8.5 Hz), a doublet of doublets (H-6, ortho + meta coupling) and a meta-coupled doublet for the proton peri to the halogen (H-8, J ≈ 2.2 Hz). Consistent with the anisotropic/heavy-atom effect, H-8 appears further downfield for the bromo derivative C5 (δ 7.24) than for the chloro analogue C4 (δ 7.12). At the same time, the corresponding ^{13}C spectra show the expected ipso C–Cl (≈ 126.3 ppm, C4) and C–Br (≈ 113.8 ppm, C5) resonances. Taken together, the IR and NMR data are fully consistent with the proposed structures of C1–C5 and support the C-2 regiochemical outcome expected from the electrophilic alkylation of the chromene intermediate by the corresponding β-ketoester (Section 2.1.1).

3.2 Molecular Structures of the Designed Compounds

The chemical structures of the five computationally designed compounds (C1-C5) are presented in Figure 1. Structural analysis revealed that all compounds possess an aromatic 2-methyl-2H-chromene framework substituted with oxygenated functional groups (ketone and ester). At the same time, C4 and C5 additionally contain a halogen atom (chlorine and bromine, respectively) at position 7 of the aromatic ring. Such structural features are known to modulate molecular lipophilicity, electronic distribution, and steric bulk, which may ultimately influence binding affinity for the COX-2 catalytic pocket. The structural diversity introduced through the different substituents — an open acetyl/ester

chain (C1), spiro-fused cyclopentanone (C2) and cyclohexanone (C3) rings, and halogen substitution (C4, C5) — provides a suitable basis for evaluating the structure-activity relationship (SAR) of this series.

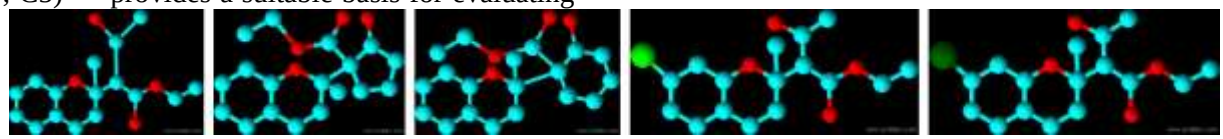


Figure 1. *Molecular structures of the five computationally designed compounds: (A) C1; (B) C2; (C) C3; (D) C4 and (E) C5, generated using ACD/ChemSketch software.*

3.3 Molecular Docking Results and Binding Affinities

The molecular docking scores of celecoxib and the five designed compounds toward COX-2 (PDB ID: 5KIR) are summarized in Table 2. Celecoxib, used as the reference selective COX-2 inhibitor, exhibited the strongest binding affinity with a docking score of −7.9 kcal/mol, confirming the reliability of the docking protocol. Among the synthesized compounds, C4 displayed the highest affinity (−7.3 kcal/mol), followed by C2 (−7.0 kcal/mol), whereas C1, C3 and C5 all produced an identical docking score of −6.7 kcal/mol. The superior performance of C4 may be attributed to its chlorinated aromatic scaffold, which increases hydrophobicity and facilitates favorable van der Waals interactions within the predominantly hydrophobic COX-2 active pocket. Conversely, the slightly weaker affinities of C1, C3 and C5 suggest that although these compounds fit within the binding site, they establish fewer stabilizing interactions than both celecoxib and C4. Overall, all synthesized molecules exhibited negative binding energies, indicating spontaneous binding toward COX-2 and supporting their potential anti-inflammatory activity.

Table 2. *Molecular formulae, molecular weights and docking scores of celecoxib and compounds C1-C5 toward COX-2 (PDB ID: 5KIR).*

No	Name	Mol. Formula	MW (g/mol)	Binding Affinity, PyRx (ΔG, kcal/mol)
1	Celecoxib	C17H14F3N3O2S	381.37	−7.9
2	C4	C16H17ClO4	308.76	−7.3
3	C2	C18H20O4	300.35	−7.0
4	C1	C16H18O4	274.31	−6.7
5	C3	C19H22O4	314.38	−6.7
6	C5	C16H17BrO4	353.21	−6.7

3.4 Analysis of Protein-Ligand Interactions

Detailed interaction profiles are presented in Table 3 and Figures 2-7. Celecoxib formed the most complex interaction network within the COX-2 active site (Figure 2). In addition to multiple hydrophobic contacts, it formed conventional hydrogen bonds, carbon-hydrogen bonds, a halogen interaction involving a fluorine atom, a π-sulfur interaction, and a π-anion contact with residues ILE274, GLU290, LYS211, ASN222, VAL291, and HIS214. The coexistence of several complementary interaction types explains its superior docking score and high binding stability.Among the designed compounds, C4 produced the richest interaction profile (Figure 3). It established π-alkyl interactions, along with alkyl and carbon-hydrogen bonds, involving LEU472, MET471, PRO86, SER119, LYS83, VAL89, TYR355, VAL116, and LEU93. The large number of hydrophobic contacts likely accounts for its relatively strong binding affinity and suggests a stable accommodation within the enzyme pocket.Compound C2 exhibited the second-best docking performance (Figure 4). Unlike C4, it formed carbon-hydrogen bonds and a π-π T-shaped interaction with ARG120, SER119, TYR115 and VAL89. Aromatic π-π interactions are known to enhance ligand stabilization and may explain the improved affinity of C2 relative to C1, C3 and C5.Compound C1 interacted predominantly through π-alkyl contacts involving VAL116, LEU93 and VAL89 (Figure 5). The absence of hydrogen bonds or aromatic stacking interactions likely limited its overall binding strength.Compound C3 showed an additional conventional hydrogen bond with LYS83, together with carbon-hydrogen and π-alkyl interactions involving VAL116 and VAL89 (Figure 6). Although hydrogen bonding generally enhances complex stability, the limited number of interacting residues may explain why its docking score remained comparable to C1's.Similarly, C5 mainly relied on hydrophobic π-alkyl interactions with VAL116, LEU93 and VAL89 (Figure 7), producing the same docking score as C1. Replacing chlorine with

bromine did not significantly improve binding, suggesting that the larger bromine atom may introduce steric constraints within the active pocket. Collectively, these findings indicate that the docking affinity is governed not only by the number of interactions but also by their nature. Ligands combining hydrophobic contacts with hydrogen bonding and aromatic interactions generally exhibit enhanced binding stability.

Table 3. *Molecular docking scores and non-covalent interaction profiles of celecoxib and compounds C1-C5 toward COX-2.*

No	Binding Affinity, PyRx (ΔG, kcal/mol)	Interaction Types Observed	Key Interacting Residues
1	−7.9	π-Alkyl, Conventional H-bond, Alkyl, Carbon H-bond, Halogen (F), π-Sulfur, π-Anion	ILE274, GLU290, LYS211, ASN222, VAL291, HIS214
2	−7.3	π-Alkyl, Alkyl, Carbon H-bond	LEU472, PRO86, MET471, SER119, LYS83, VAL89, TYR355, VAL116, LEU93
3	−7.0	Carbon H-bond, π-Alkyl, π-π T-shaped	ARG120, SER119, TYR115, VAL89
4	−6.7	π-Alkyl	VAL116, LEU93, VAL89
5	−6.7	π-Alkyl, Conventional H-bond, Carbon H-bond	LYS83, VAL116, VAL89
6	−6.7	π-Alkyl	VAL116, LEU93, VAL89

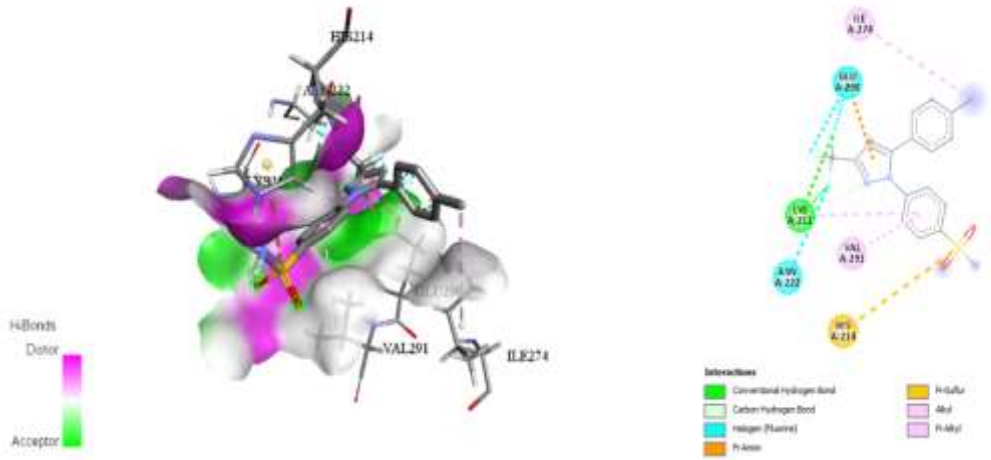


Figure 2. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of celecoxib within the COX-2 active site (PDB ID: 5KIR).*

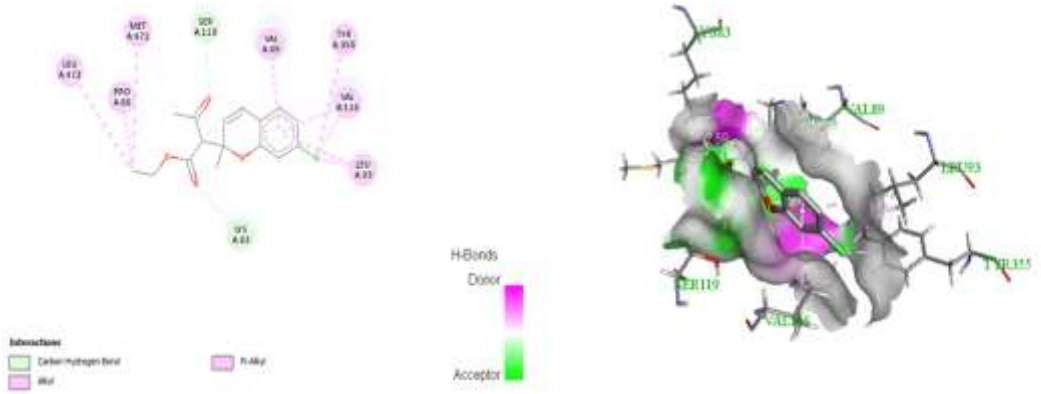


Figure 3. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of compound C4 within the COX-2 active site.*

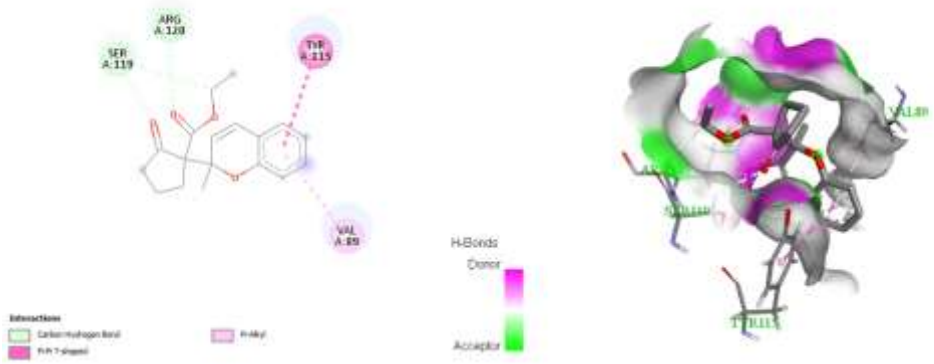


Figure 4. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of compound C2 within the COX-2 active site.*

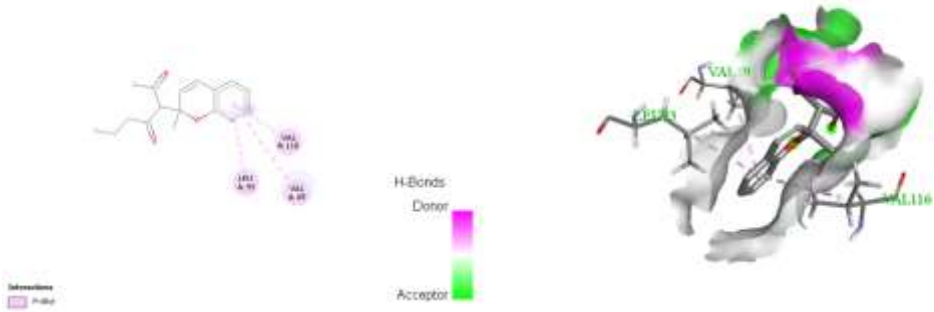


Figure 5. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of compound C1 within the COX-2 active site.*

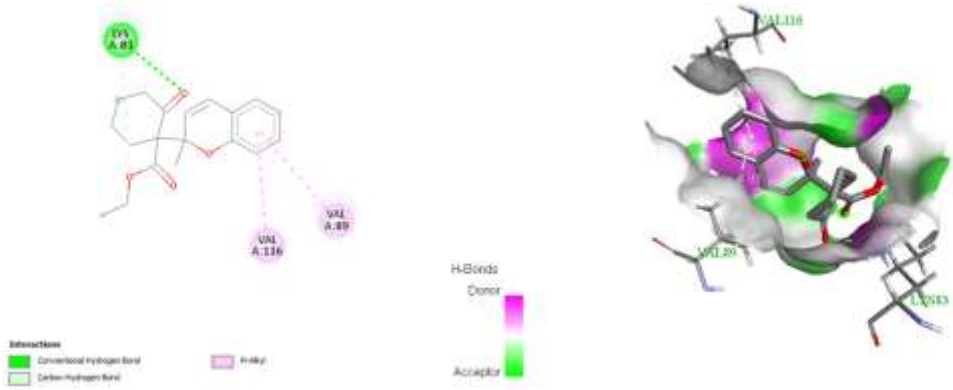


Figure 6. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of compound C3 within the COX-2 active site.*

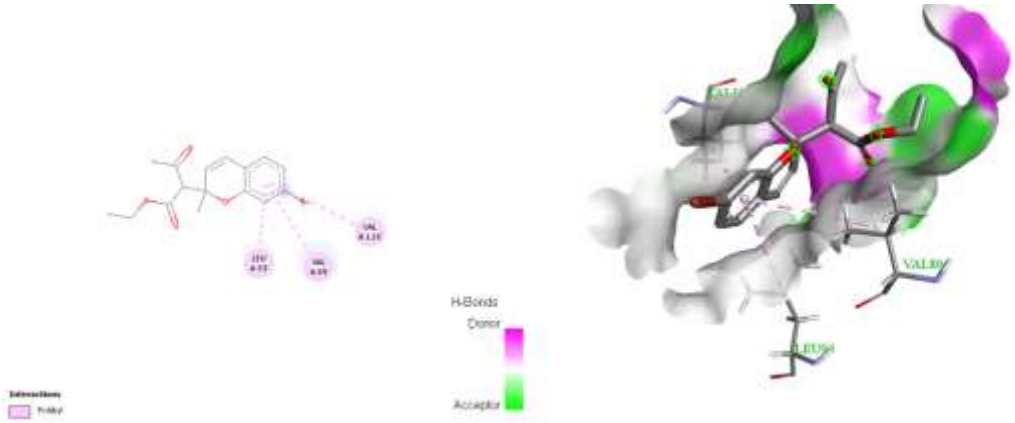


Figure 7. *Two-dimensional (2D) and three-dimensional (3D) binding conformations of compound C5 within the COX-2 active site.*

3.5 Binding Mode Analysis and Structure-Activity Relationship

The predicted binding conformations, illustrated in Figures 2-7, corroborate the docking scores discussed above. Celecoxib occupied the catalytic pocket in a highly favorable orientation, allowing simultaneous formation of hydrogen bonds, hydrophobic contacts, and aromatic interactions, which together account for its excellent docking score. Compound C4 adopted a binding pose that engaged an extended hydrophobic sub-pocket while maintaining an extensive network of contacts with surrounding residues, a feature that supports its ranking as the most promising synthesized compound. Compound C2 displayed a distinct orientation but remained deeply inserted into the catalytic cavity, allowing aromatic stacking with TYR115, which contributed to its relatively high affinity. In contrast, C1, C3, and C5 adopted less-optimized conformations characterized by fewer stabilizing contacts and a reduced interaction density, which explains their comparatively lower binding affinities.Taken together, these results allow the outline of a preliminary structure-activity relationship for this chromene series: (i) fusion of an alicyclic ketone ring onto the open-chain scaffold of C1 (giving C2 and C3) introduces additional van der Waals contact surface and, in the case of the cyclopentanone-fused analogue C2, enables an aromatic π-π T-shaped interaction with TYR115 that is absent in C1 and C3; (ii) halogenation at position 7 of the chromene ring markedly influences binding, with the chloro-substituted C4 recruiting a substantially larger number of hydrophobic residues (LEU472, MET471, PRO86, TYR355, in addition to the common VAL89/VAL116/LEU93 triad) than the parent compound C1, whereas the bromo-substituted analogue C5 reproduces only the interaction pattern of C1, suggesting that the bulkier bromine atom does not confer the same favorable pocket complementarity as chlorine in this particular scaffold. None of the synthesized derivatives surpassed the reference drug celecoxib, whose superior affinity is associated with a qualitatively richer interaction network combining conventional hydrogen bonds, a halogen bond and π-sulfur/π-anion contacts — interaction types not observed for any of the chromene derivatives investigated here. This observation indicates that, while the chromene scaffold is well accommodated within the COX-2 pocket, further structural optimization — for example through the introduction of polar hydrogen-bond-donor/acceptor groups capable of engaging Arg120, Tyr355 or Ser530 more directly — will likely be required to approach or exceed the affinity of clinically used coxibs. This conclusion is in line with recent reports on chromone- and chromene-related scaffolds, where the introduction of additional hydrogen-bond-donor/acceptor or electron-withdrawing groups was similarly found to improve COX-2 binding and selectivity relative to simple halogenated or alkyl-substituted analogues [14–16], in line with the broader value of combined in silico pharmacokinetic/toxicity and molecular docking approaches for prioritizing candidate structures before experimental validation [17].It is important to acknowledge the inherent limitations of molecular docking as a static, single-point method. The docking scores represent approximate binding free energies and do not account for entropic contributions, receptor flexibility or solvation effects. Future work should therefore include molecular dynamics simulations to assess the stability of these complexes over time, followed by MM-PBSA or MM-GBSA binding free energy calculations for more rigorous affinity estimates, together with in vitro COX-2 inhibition assays to experimentally validate the present computational predictions.

4. Conclusions

This study presents a purely computational (in silico) design and molecular docking evaluation of five newly proposed 2-methyl-2H-chromene derivatives (C1-C5) as potential COX-2 inhibitors, using AutoDock Vina/PyRx against the crystal structure of human COX-2 (PDB ID: 5KIR), with celecoxib as reference. Compounds C1–C5 were not physically synthesized or experimentally characterized; they exist only as computationally modelled ligands. All compounds displayed negative, thermodynamically favorable binding energies (−6.7 to −7.3 kcal/mol), although none exceeded the reference drug (−7.9 kcal/mol). Compound C4, bearing a chlorine atom at position 7, emerged as the most promising candidate of the series, combining the highest binding affinity (−7.3 kcal/mol) with the richest hydrophobic interaction network within the active site. Spiro-fusion of a cyclopentanone ring (C2) additionally enabled a favorable π-π T-shaped interaction with TYR115, improving its affinity relative to the open-chain and cyclohexanone-fused analogues. These findings provide a preliminary, computationally-derived structure-activity basis for this chromene series. As this is an in silico screening study only, the actual synthesis of C1–C5, their full spectroscopic and physical characterization (IR, $^{1}H/^{13}C$ NMR, melting point, yield), and subsequent in vitro COX-2 inhibition assays and molecular dynamics/MM-GBSA studies constitute necessary future work to confirm and refine the present computational predictions.

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- **Ethical approval:** The research conducted does not involve the use of human or animal subjects.

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