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FULL PAPER

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Design, synthesis, and biological evaluation of novel 1,2-diaryl-4-substituted-benzylidene-5(4*H*)-imidazolone derivatives as cytotoxic agents and COX-2/LOX inhibitors

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Abstract

A new series of 1,2-diaryl-4-substituted-benzylidene-5(4*H*)-imidazolone derivatives **4a–l** was synthesized. Their structures were confirmed by different spectroscopic techniques (IR, ^{1}H NMR, DEPT-Q NMR, and mass spectroscopy) and elemental analyses. Their cytotoxic activities *in vitro* were evaluated against breast, ovarian, and liver cancer cell lines and also normal human skin fibroblasts. Cyclooxygenase (COX)-1, COX-2 and lipoxygenase (LOX) inhibitory activities were measured. The synthesized compounds showed selectivity toward COX-2 rather than COX-1, and the IC_{50} values (0.25–1.7 μM) were lower than that of indomethacin (IC_{50} = 9.47 μM) and somewhat higher than that of celecoxib (IC_{50} = 0.071 μM). The selectivity index for COX-2 of the oxazole derivative **4e** (SI = 3.67) was nearly equal to that of celecoxib (SI = 3.66). For the LOX inhibitory activity, the new compounds showed IC_{50} values of 0.02–74.03 μM, while the IC_{50} of the reference zileuton was 0.83 μM. The most active compound **4c** (4-chlorobenzoxazole derivative) was found to have dual COX-2/LOX activity. All the synthesized compounds were docked inside the active site of the COX-2 and LOX enzymes. They linked to COX-2 through the N atom of the azole scaffold, while C═O of the oxazolone moiety was responsible for the binding to amino acids inside the LOX active site.

KEYWORDS
anti-inflammatory, cytotoxicity, diaryl imidazolone derivatives, molecular docking study

1 | INTRODUCTION

Developing new anti-inflammatory drugs is still a challenge. This can be attributed to a variety of reasons related to undesirable side effects of anti-inflammatory agents such as ulcerogenic, hepatic, and renal toxicity as well as increasing cardiovascular disorders. Besides searching for new drugs with high safety profiles to relieve inflammation.[1,2]

The most common way for treatment of inflammatory symptoms (pain, redness, heat, and swelling) is non-steroidal anti-inflammatory drugs (NSAIDs).[3] These act by inhibiting the biotransformation of arachidonic acid (AA) – a membrane bound phospholipid – to prostaglandins (PGs), prostacyclin (PGI2), and thromboxane A2 (TXA2) via cyclooxygenase (COX) enzymes (COX-1, -2, -3).[4–6] Either non-selective NSAIDs (such as aspirin, phenazone, and indomethacin) or selective COX-2 inhibitors like celecoxib, rofecoxib, and valdecoxib (Figure 1) produce unwanted side effects by decreasing the cytoprotective action of constitutive COX-1 isoform or by increasing cardiovascular disorders related to inducible COX-2 isoform.[7]

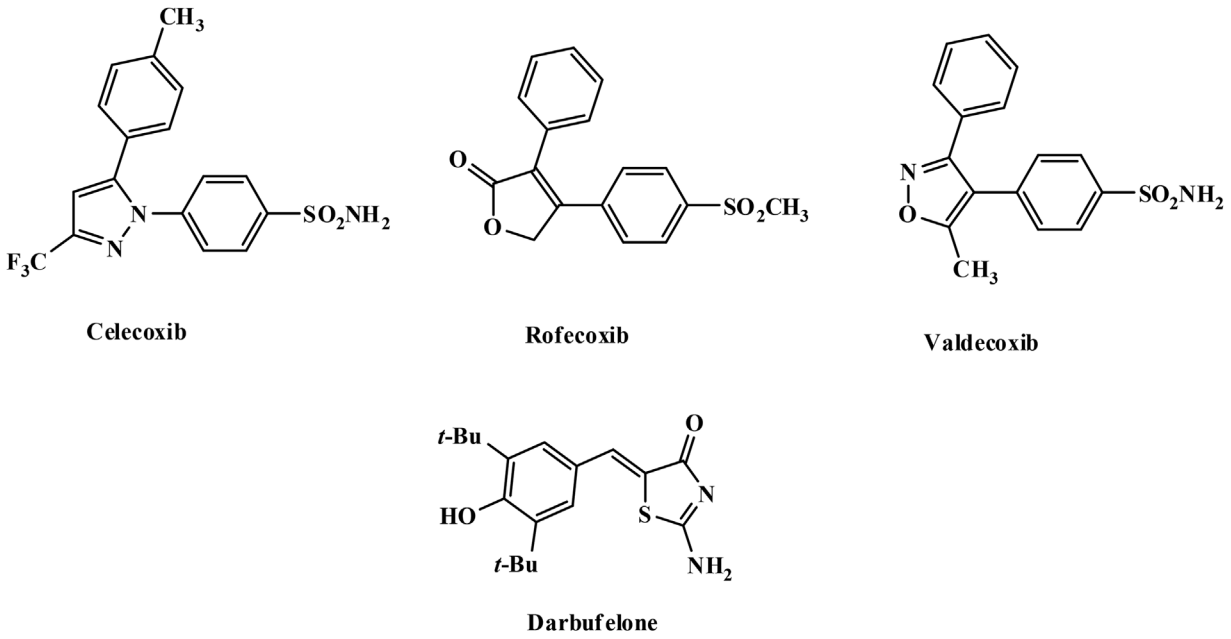


FIGURE 1 Chemical structures of some known selective COX-2 inhibitors and a dual COX-2/LOX inhibitor

Another metabolic pathway of AA to leukotrienes (LTs) is associated to lipoxygenase (LOX) enzymes (5-, 8-, 12-, 15-LOX). 5-LOX is accompanied by inflammation, bronchoconstriction, hypersensitivity, anaphylaxis, and asthma. While 15-LOX is involved in atherosclerosis.[8,9] Monotherapy of a drug with dual inhibitory activity for COX/LOX enzymatic pathways is highly desirable for developing more effective anti-inflammatory agents with low side effects.

By searching in literature, it was observed that compounds with dual COX/5-LOX inhibitory activity are found to have the vinyl bridge between the phenyl and the heterocyclic five membered ring as in drabufelone.[4]

Derivatives of imidazole as example for five membered heterocyclic ring were reported to have multiple therapeutic and pharmacological actions such as antimicrobial,[10] anticonvulsant,[11] anticancer,[12] anti-inflammatory,[13] and analgesic activities.[14] Many drugs in the market have imidazole nucleus in their structures like metronidazole as antifungal, flumazenil as benzodiazepine antagonist, dacarbazine as anticancer, cimetidine as antihistaminics and ketoconazole and flutrimazole were found to have anti-inflammatory activity in addition to their antifungal activity.[15,16]

Flumizole, (2-(2,4-difluorophenyl)-4,5-bis(4-methoxyphenyl)imidazole), one of the early known NSAI agents, was reported to have similar anti-inflammatory, analgesic, and anti-pyretic activities as indomethacine but with reduced side effects. This compound was in clinic but its pharmacokinetics was very complex in humans.[1,17] Another diarylimidazole derivative is triflamizole which is more potent than indomethacin by eight times in the rat adjuvant-induced arthritis assay. Clinically, this compound showed dose-limiting incidence of GI ulceration probably due to its non-selective inhibition of COX enzymes.[18]

Additionally, azole derivatives (benzoxazole and benzothiazole), heterocyclic compounds, are widely distributed in nature and essential to life. The therapeutic importance of azole derivatives has been reported.[19–22]

2-(2-Arylphenyl)benzoxazole derivatives **I** and **II** were evaluated as selective inhibitors of COX-2 and have better anti-inflammatory activity when compared to diclofenac and celecoxib.[23]

Additionally, benzothiazole derivative with 6-sulfonamide moiety **III** was screened for its anti-inflammatory activity by carrageenan-induced paw edema method in rats and showed considerable anti-inflammatory activity.[24]

From all the above findings, we focused in the present work on designing and synthesizing new 1,2-diaryl-4-substituted-benzylidene-5(4*H*)-imidazolone derivatives **4a–l** by merging benzoxazole/benzothiazole scaffold with imidazolone nucleus (Figure 2). The new candidates were related to selective COX-2 inhibitors by maintaining vicinal diarylheterocycles of coxibs, and as 5-LOX inhibitors through presence of vinyl bridge between phenyl and the heterocyclic five membered ring. All the synthesized compounds were *in vitro* evaluated as COX-1/COX-2 inhibitors. *In vitro* cytotoxic activity was also measured. Molecular docking study was used to explore the mechanism of action of the new compounds.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthetic pathway for the target imidazolones **4a–l** is presented in Scheme 1. Substituted hippuric acids (*N*-acyl glycines) **1a**,**b** were prepared from aromatic acyl halides (benzoyl chloride and 4-methyl benzoyl chloride) and glycine by Schotten–Baumann benzoylation reaction.[25] Coupling hippuric acid derivatives **1a**,**b** with different aromatic aldehydes, namely, benzaldehyde, 4-methoxybenzaldehyde,

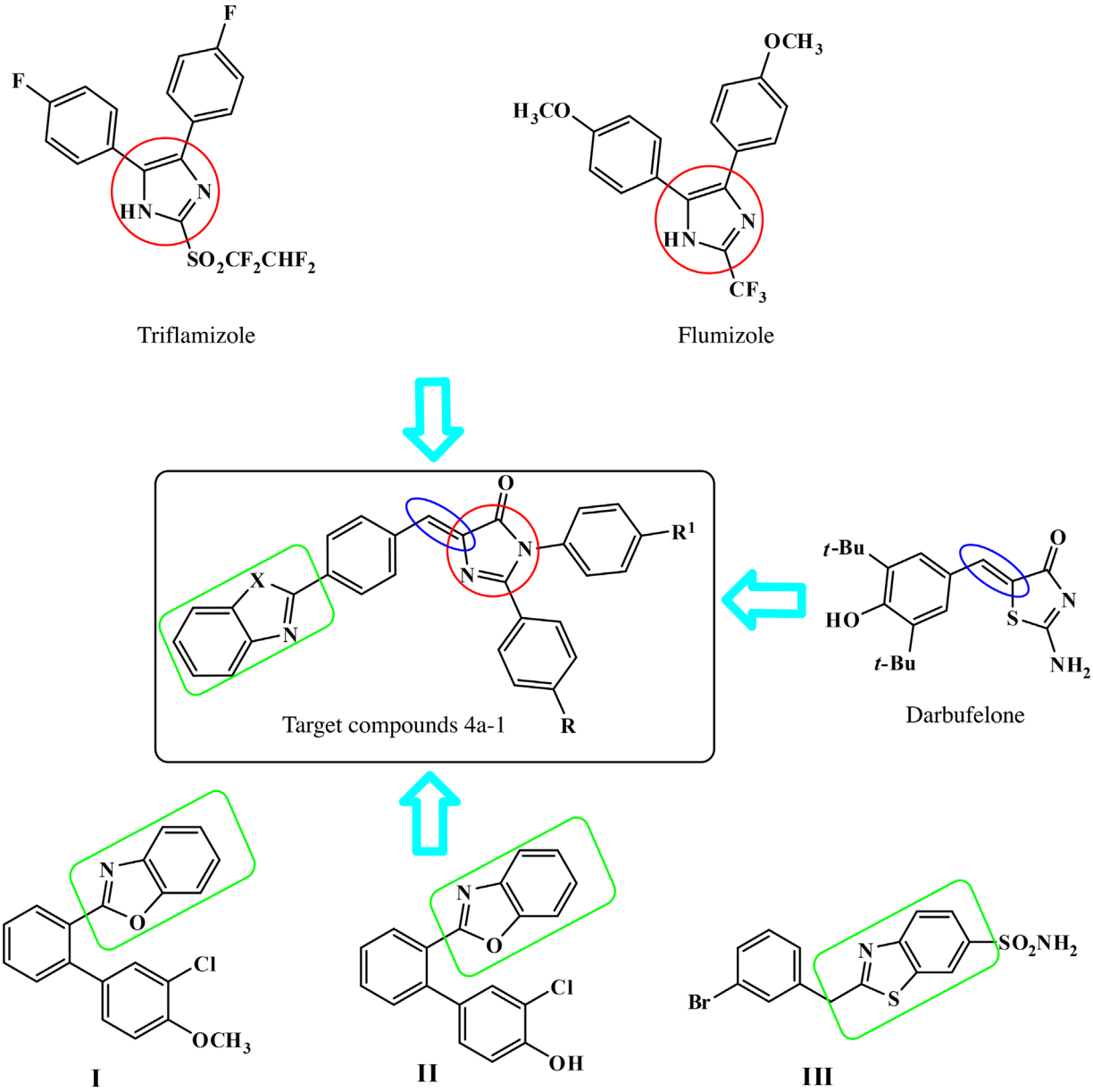


FIGURE 2 Design for the new synthesized compounds **4a–l** on the bases of previously known anti-inflammatory agents

and 4-chlorobenzaldehyde, using sodium acetate as a base and a small amount of acetic anhydride (to increase the yield of *Z*-isomer) occurred by Erlenmeyer–Plochl azalactone synthesis for compounds **2a–f**.[25]

(4-Aminophenyl-2-yl)benzoxazole and benzothiazole derivatives **3a**,**b** were synthesized from 4-aminobenzoic acid and 2-aminophenol (for benzoxazole derivative **3a**) or 2-aminothiophenol (for benzothiazole derivative **3b**) using polyphosphoric acid (PPA).[26]

Refluxing **2a–f** with **3a**,**b** in glacial acetic acid and anhydrous sodium acetate for 10–12 h afforded the target imidazolones **4a–l** in high yields, 64–87%. TLC was run throughout the reaction to check the completion and purity of the reaction.

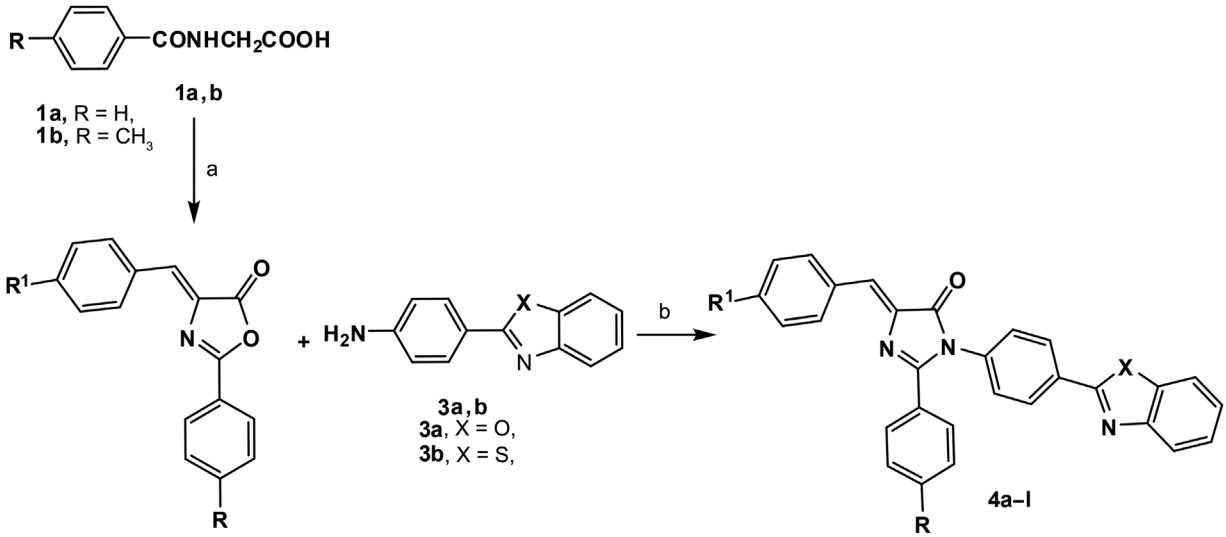
Physical and spectroscopic data for the target compounds **4a–l** are listed in Section 4.

2.2 | Biological evaluation

2.2.1 | Anti-inflammatory activity

Inhibition of COX-1, COX-2, and 5-LOX – biological assays

The COX activity assay kit directly measures PGF_{2a} by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoid product is quantified via enzyme immune assay (ELISA) using a broadly specific antiserum that binds to all the major PG compounds. The target of the *in vitro* biological activity assay was to explore the ability of tested compounds to inhibit both human recombinant COX-1 and COX-2 using Cayman Human (EIA) kit by Robomik P2000 ELISA reader at 450 nm. The kit includes isozyme-specific inhibitors for distinguishing COX-2 activity from COX-1 activity. The COX assay is an excellent tool that can be



2a, R = H, R^1 = H;
2b, R = H, R^1 = OCH_3;
2c, R = H, R^1 = Cl;
2d, R = CH_3, R^1 = H;
2e, R = CH_3, R^1 = OCH_3;
2f, R = CH_3, R^1 = Cl

4a, X = O, R = H, R^1 = H;
4b, X = O, R = H, R^1 = OCH_3;
4c, X = O, R = H, R^1 = Cl;
4d, X = O , R = CH_3, R^1 = H;
4e, X = O, R = CH_3, R^1 = OCH_3;
4f, X = O, R = CH_3, R^1 = Cl
4g, X = S, R = H, R^1 = H;
4h, X = S, R = H, R^1 = OCH_3;
4i, X = S, R = H, R^1 = Cl;
4j, X = S , R = CH_3, R^1 = H;
4k, X = S, R = CH_3, R^1 = OCH_3;
4l, X = S, R = CH_3, R^1 = Cl

SCHEME 1 Synthetic procedure for compounds **4a–l**. Reagents and conditions: (a) aromatic aldehydes, Ac_2O, NaAc, 3–5 h.; (b) gl. acetic acid, NaAc, reflux 10–12 h

used for general inhibitor screening. The potency of tested compounds was determined as the concentration causing 50% enzyme inhibition (IC_{50}). Also, the COX-2 selectivity index (SI values) which is defined as IC_{50} (COX-1)/IC_{50} (COX-2) was calculated and compared with that of the standard drug celecoxib.

Caymen's lipoxygenase inhibitor screening assay kit detects and measures hydroxyperoxides produced in lipoxygenation reactions using a purified lipoxygenase. Compounds **4a–l** were tested and the data are listed in Table 1.

The synthesized compounds **4a–l** showed low selectivity to COX-1 (IC_{50}: 0.49–4.6 μM) when compared to indomethacin (IC_{50} = 1.42 μM) and celecoxib (IC_{50} = 0.26 μM).

Regarding COX-2, the most active compound was *p*-chlorophenyl derivative of benzoxazole **4c** (IC_{50} = 0.25 μM). Most of new compounds had certain selectivity toward COX-2 (IC_{50}: 0.38–1.7 μM), much better than indomethacin (IC_{50} = 9.47 μM) but lower than celoecoxib (IC_{50} = 0.071 μM). Benzoxazole derivative **4e** bearing electron donating groups (CH_3 and OCH_3) had SI = 3.67, which was nearly equal to that of celecoxib (SI = 3.66). Compounds **4b**, **4c**, **4f**, **4h**, **4k**, and **4l** exhibited good selectivity indices ranging from 2.16 to 3.06. It was observed that SI increased for benzoxazole or benzothiazole derivatives bearing *p*-methoxy aldehydic group (**4h** and **4b**).

By inspecting data obtained from LOX, it was found that benzoxazole derivative **4c**, with *p*-chlorophenyl part, showed excellent selectivity to LOX enzyme (IC_{50} = 0.02 μM), while that of zileuton, the reference drug, was 0.83 μM. Compounds **4b**, **4d**, **4f**, **4i**, and **4k** exhibited certain selectivity with IC_{50} ranging from 0.12 to 0.51 μM. While unsubstituted benzoxazole/or benzothiazole derivatives **4a** and **4g** showed lower selectivity (IC_{50} = 1.05 and 2.35, respectively). Four compounds **4e** (IC_{50} = 74.03 μM), **4h** (IC_{50} = 21.15 μM), **4j** (IC_{50} = 25.26 μM), and **4l** (IC_{50} = 16.82 μM) were not selective toward LOX. From these results, we can say that by increasing the electron donating groups, decreasing LOX activity of the compounds will be produced.

The results obtained from *in vitro* data were in consistence with molecular modeling study.

2.2.2 | *In vitro* cytotoxic activity

Twelve tested imidazolone derivatives were evaluated for their cytotoxicity in four cancer cell lines (MCF7, Hep3B, PLC/PRF5, A2780) and normal human fibroblasts (BJ). Five substances (**4d**, **4e**, **4g**, **4j**, and **4k**) showed moderate cytotoxicity in hepatocellular carcinoma cells (Hep3B) and also in normal human fibroblasts. The most interesting compound **4f** was strongly cytotoxic (IC_{50} 12.8 ± 3.0 μM) only in hepatocellular carcinoma cells, but not in normal human cells (BJ) (Table 2).

TABLE 1 *In vitro* COX-1, COX-2, and LOX inhibition, and selectivity index (SI) data for **4a–l** and the reference drugs indomethacin, celecoxib, and zileuton

	IC_{50} (μM)[a]			IC_{50} (μM)[a]
Compound no.	COX-1	COX-2	COX-2 SI[b]	5-LOX
Indomethacin	1.42	9.47	0.14	n.d.
Celebrex	0.26	0.071	3.66	n.d.
Zileuton	n.d.	n.d.	n.d.	0.83
4a	0.73	0.4	1.82	1.05
4b	1.82	0.66	2.75	0.12
4c	0.54	0.25	2.16	0.02
4d	0.49	0.6	0.81	0.29
4e	3.09	0.84	3.67	74.03
4f	1.02	0.46	2.21	0.51
4g	0.96	0.49	1.95	2.35
4h	4.6	1.5	3.06	21.15
4i	1.47	1.7	0.86	0.48
4j	0.73	0.39	1.87	25.26
4k	0.84	0.38	2.21	0.42
4l	1.72	0.7	2.45	16.82

[a]The *in vitro* test compound concentration required to produce 50% inhibition of COX-1 or COX-2. The result (IC_{50}, μM) is the mean of two determinations acquired using an ovine COX-1/COX-2 assay Kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.
[b]The *in vitro* COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

2.3 | Docking study

In this study, all new designed compounds were docked using MOE into COX-2 and 5-LOX receptors. It was noticed that: (i) docking inside COX-2 active site occurred mainly between N benzoxazole/benzothiazole and His90 amino acid, (ii) while in the case of docking the target compounds inside 5-LOX active site, most of the compounds showed a hydrogen bond between C═O imidazolone and Asn180 amino acid. Thus, 4-chloro derivative of benzoxazole **4c**, the most active compound was docked inside both COX-2 and 5-LOX active sites, it was found to have one hydrogen bond between His90 amino acid and N atom of benzoxazole nucleus when docked into COX-2 active site. However, it made a different hydrogen bond between Asn180 amino acid and C═O of imidazolone moiety when docked inside active site of 5-LOX enzyme. Docking of compound **4c** inside the active site of COX-2 and 5-LOX is illustrated in Figures 3–6. Results of binding free energies for best poses are tabulated in Table 3.

3 | CONCLUSION

In conclusion, a new series of vicinal diarylimidazolone derivatives **4a–l** was synthesized. They were tested for both cytotoxic and *in vitro* anti-inflammatory activities. Five substances (**4d**, **4e**, **4g**, **4j**, and **4k**) showed moderate cytotoxicity in hepatocellular carcinoma cells (Hep3B) and also in normal human fibroblasts. The most interesting compound **4f** was strongly cytotoxic only in hepatocellular carcinoma cells, but not in normal human cells (BJ). The target compounds **4a–l** were evaluated against COX-1, COX-2, and LOX enzymes using indomethacin, celecoxib, and zileuton as references, respectively. The synthesized compounds showed selectivity toward COX-2 rather than COX-1, IC_{50}s (0.25–1.7 μM) were less than indomethacin (IC_{50} = 9.47 μM) and somewhat more than celecoxib (IC_{50} = 0.071 μM). SI for COX-2 was nearly equal to celecoxib (SI = 3.66) in oxazole derivative **4e** (SI = 3.67). In thiazole derivative **4h** SI was equal to 3.06. The rest of compounds were found to have SIs in the range of 0.81–2.75. For LOX inhibitory activity the new compounds showed IC_{50}s 0.02–74.03 μM, while IC_{50} of the reference zileuton was 0.83 μM. The most active compound **4c** (4-chlorobenzoxazole derivative) was found to have dual COX-2/LOX activity. From molecular docking study, it was observed that compounds linked to COX-2 through N atom of azole scaffold, while

TABLE 2 Cytotoxicity *in vitro* in cancer cell lines and normal human fibroblasts (BJ) after 72 h

	IC_{50} (μM)				
Compound	MCF7	Hep3B	PLC/PRF5	A2780	BJ
4a	>50	>50	>50	>50	>50
4b	>50	>50	>50	>50	>50
4c	>50	>50	>50	>50	>50
4d	>50	29.3 ± 1.0	>50	>50	40.9 ± 8.2
4e	>50	25.1 ± 0.2	>50	>50	28.5 ± 0.9
4f	>50	12.8 ± 3.0	>50	>50	>50
4g	>50	15.2 ± 1.7	>50	>50	7.8 ± 0.8
4h	>50	>50	>50	>50	>50
4i	>50	>50	>50	>50	>50
4j	>50	27.4 ± 4.7	>50	>50	11.2 ± 0.8
4k	>50	26.7 ± 0.4	>50	>50	9.0 ± 0.7
4l	>50	>50	>50	>50	>50

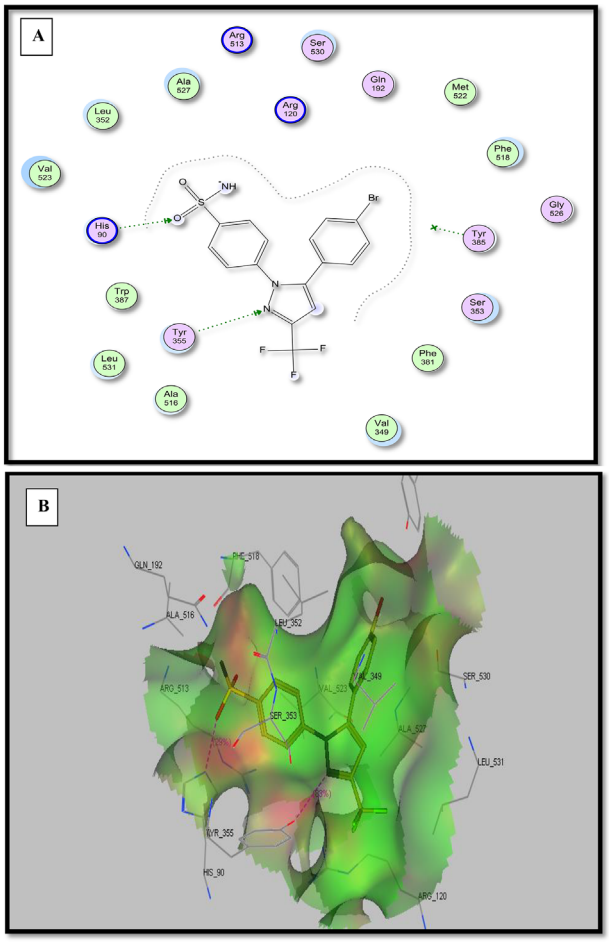


FIGURE 3 Binding of celecoxib inside COX-2 active site, the most important amino acids are shown together with their respective numbers. (A) 2D interaction; (B) 3D interaction

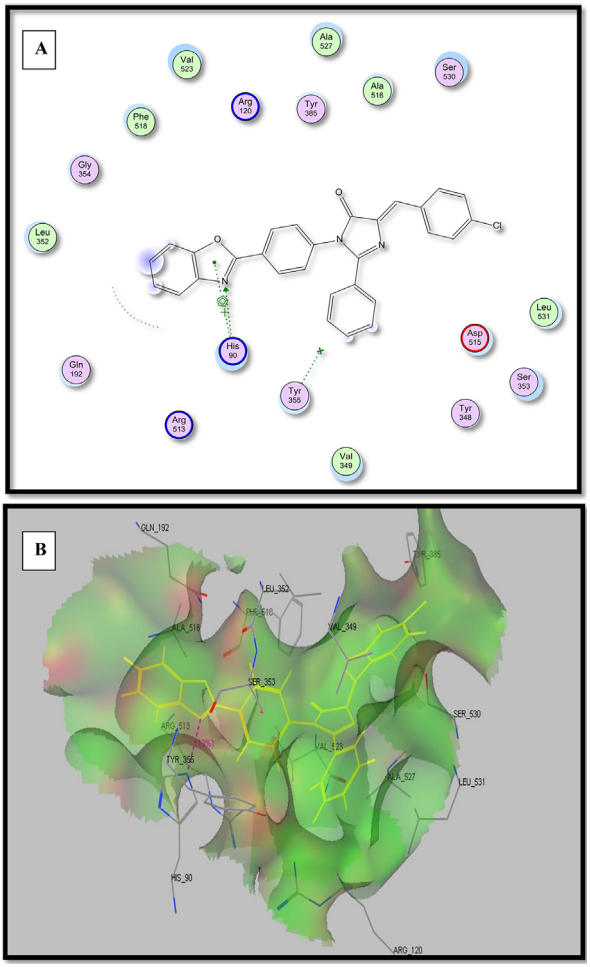


FIGURE 4 Binding of **4c** inside COX-2 active site, the most important amino acids are shown together with their respective numbers. (A) 2D interaction; (B) 3D interaction

C═O of oxazolone moiety was the one responsible for the binding to amino acids inside 5-LOX active site.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Melting points were determined using a Griffin apparatus and were uncorrected. IR spectra were recorded on a Shimadzu IR-435 spectrophotometer using KBr discs and values were represented in cm^{-1}. ^{1}H NMR and ^{13}C NMR (DEPT-Q) were carried out using the Bruker instrument at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR spectrophotometer (Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt), in DMSO-d_6, D_2O using TMS as an internal standard and chemical shifts were recorded in ppm on the δ scale using DMSO-d_6 (2.5) as a solvent. Coupling constant (J) values were estimated in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet, t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. The electron impact (EI) mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Palo Alto, CA). Microanalysis was performed for C, H, N on Perkin-Elmer 2400 at the Microanalytical Center, Cairo University, Egypt and was within ±0.4% of theoretical values. Analytical thin layer chromatography (TLC) with pre-coated plastic sheets, 0.2 mm silica gel with UV indicator (Macherey-Nagel) was employed routinely to follow the course of reactions and to check the purity of products. All other reagents and solvents were purchased from the Aldrich Chemical Company (Milwaukee, WI) and were used without further purification.

Original spectra of the investigated compounds are provided as Supporting Information, as are the InChI codes of the compounds together with some biological activity data.

4.1.2 | General procedure for the synthesis of compounds 4a–l

A mixture of the appropriate azalactone **2a–f** (10 mmol), the appropriate azole derivative **3a**,**b** (12 mmol) and anhydrous sodium acetate (0.3 g) in glacial acetic acid (10 mL) was heated under reflux

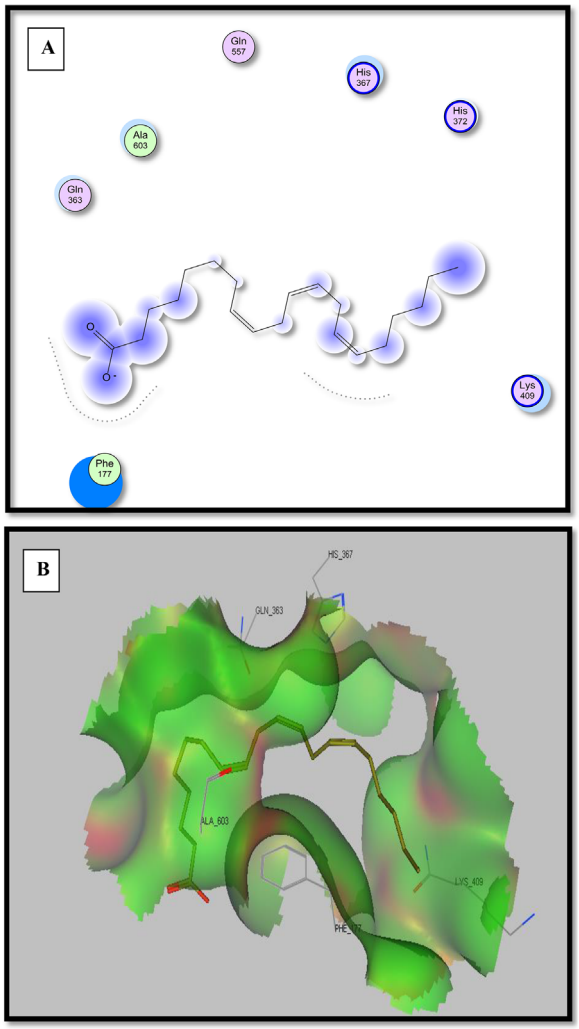


FIGURE 5 Binding of ACD inside 5-LOX active site. (A) 2D interaction; (B) 3D interaction

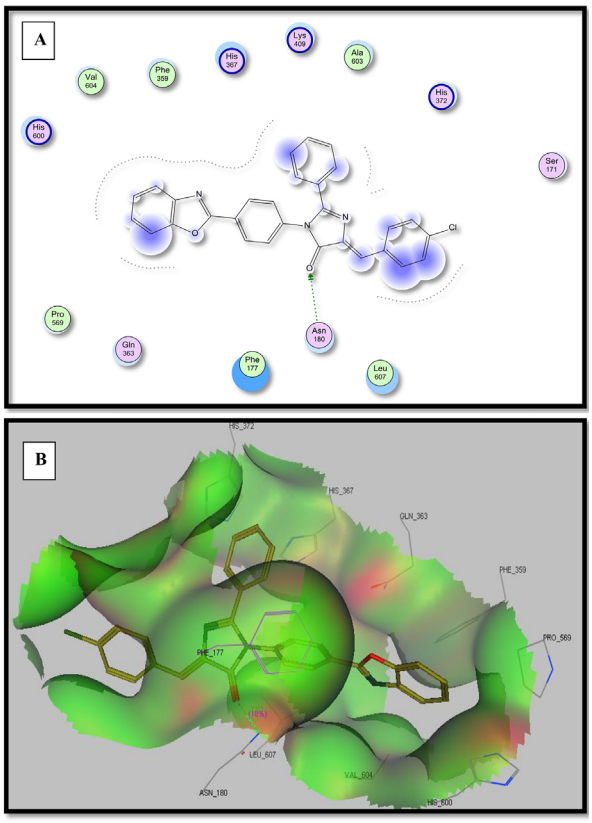


FIGURE 6 Binding of **4c** inside 5-LOX active site, the most important amino acids are shown together with their respective numbers. (A) 2D interaction; (B) 3D interaction

temperature for 10–12 h. The solid separated on cooling was filtered, dried, and crystallized from DMF/ethanol (1:2) to give **4a–l**.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-benzylidene-2-phenyl-1*H*-imidazol-5(4*H*)-one (4a)

Brown solid; yield 77%; m.p. 245–247°C; IR (KBr, cm^{-1}) 3063 (aromatic CH), 2947 (aliphatic CH), 1721 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 7.40 (s, 1H, ═CH), 7.41–7.53 (m, 11H, benzoxazole H-4, H-7, phenyl H-2, H-3, H-5, H-6 and benzylidene H-2, H-3, H-4, H-5, H-6), 7.61–7.64 (m, 4H, benzoxazole H-5, imidazole-1-phenyl H-2, H-6, phenyl H-4), 7.80–7.82 (m, 1H, benzoxazole H-6), 8.34 (d, J = 8.8 Hz, 2H, imidazole-1-phenyl H-3, H-5); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 111.51 (benzoxazole C-7), 120.46 (═CH), 125.52 (benzoxazole C-4), 126.29 (benzoxazole C-6), 126.60 (imidazole-1-phenyl C-4), 128.53 (benzoxazole C-5), 128.64 (imidazole-1-phenyl C-2, C-6), 128.93 (imidazole-1-phenyl C-3, C-5), 129.00 (imidazole C-4), 129.03 (benzylidene C-4), 129.38 (phenyl C-2, C-6), 129.51 (benzylidene C-2, C-6), 131.15 (benzylidene C-3, C-5), 132.11 (phenyl C-3, C-5), 132.88 (phenyl C-4), 134.52 (phenyl C-1), 137.8 (benzylidene C-1), 138.80 (imidazole-1-phenyl C-1), 141.93 (benzoxazole C-3a), 150.81 (benzoxazole C-7a), 160.90 (imidazole C-2), 161.99 (benzoxazole C-2), 169.84 (C═O). Anal. calcd. for $C_{29}H_{19}N_3O_2$ (441.48): C, 78.90; H, 4.34; N, 9.52. Found: C, 79.07; H, 4.38; N, 9.56.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-(4-methoxybenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (4b)

Yellow solid; yield 70%; m.p. 264–266°C; IR (KBr, cm^{-1}) 3063 (aromatic CH), 2924 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 3.86 (s, 3H, OCH_3), 7.11 (d, J = 8.4 Hz, 2H, p-methoxybenzylidene H-3, H-5), 7.23 (s, 1H, ═CH), 7.35–7.66 (m, 9H, phenyl H-2, H-3, H-4, H-5, H-6, imidazole-1-phenyl H-2, H-6 and benzoxazole H-4, H-7), 7.81–7.86 (m, 2H, benzoxazole H-5, H-6), 8.31 (d, J = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.38 (d, J = 8.4 Hz, 2H, p-methoxybenzylidene H-2, H-6); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 55.92 (OCH_3), 111.52 (benzoxazole C-7), 115.09 (p-methoxyphenyl C-3, C-5), 120.43 (═CH), 124.81 (benzoxazole C-4), 125.48 (benzoxazole C-6), 126.32 (phenyl C-4), 127.00 (p-methoxybenzylidene C-1), 127.51 (imidazole-1-phenyl C-4), 128.01 (imidazole-1-phenyl C-3, C-5), 128.63 (phenyl C-2, C-6), 129.00 (phenyl C-3, C-5), 129.86 (p-methoxybenzylidene C-2, C-6), 130.01 (imidazole C-4), 131.88 (benzoxazole C-5), 135.02 (phenyl

TABLE 3 Results of binding free energies for best poses

Compound no.	COX2 ΔG_{bind} best (kcal/mol)	5-LOX ΔG_{bind} best (kcal/mol)
4a	−12.3213	−16.7653
4b	−10.7318	−19.5523
4c	−10.4570	−25.9524
4d	−10.9451	−20.6572
4e	−10.3987	−11.3131
4f	−10.6680	−18.8222
4g	−10.4543	−17.6090
4h	−9.7986	−13.6775
4i	−9.8134	−21.2467
4j	−12.4140	−12.0961
4k	−11.2761	−19.5916
4l	−10.5202	−13.6775

C-1), 138.02 (imidazole-1-phenyl C-2, C-6), 138.60 (imidazole-1-phenyl C-1), 142.53 (benzoxazole C-3a), 151.01 (benzoxazole C-7a), 159.28 (imidazole C-2), 162.11 (benzoxazole C-2), 169.77 (*p*-methoxybenzylidene C-4), 172.60 (C═O); EIMS (*m/z*) 471 ($M^{+\cdot}$, 100%). Anal. calcd. for $C_{30}H_{21}N_3O_3$ (471.51): C, 76.42; H, 4.49; N, 8.91. Found: C, 76.37; H, 4.51; N, 8.89.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-(4-chlorobenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (4c)

Brown solid; yield 64%; m.p. 265–267°C; IR (KBr, cm^{-1}) 3232 (aromatic CH), 2924 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 7.32 (s, 1H, ═CH), 7.35–7.56 (m, 9H, phenyl H-2, H-3, H-4, H-5, H-6, imidazole-1-phenyl H-2, H-6 and *p*-chlorobenzylidene H-3, H-5), 7.61–7.63 (m, 3H, benzoxazole H-4, H-5, H-7), 7.79, 7.81 (dd, *J* = 3.2 Hz, 7.2 Hz, 1H, benzoxazole H-6), 8.27 (d, *J* = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.33 (d, *J* = 8.4 Hz, 2H, *p*-chlorobenzylidene H-2, H-6); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 110.70 (benzoxazole C-7), 120.21 (═CH), 124.82 (benzoxazole C-4), 125.50 (benzoxazole C-6), 126.96 (phenyl C-4), 127.54 (imidazole-1-phenyl C-3, C-5), 128.23 (benzoxazole C-5), 128.47 (imidazole-1-phenyl C-4), 128.59 (phenyl C-2, C-6), 128.60 (phenyl C-3, C-5), 129.15 (*p*-chlorobenzylidene C-3, C-5), 129.26 (imidazole-1-phenyl C-2, C-6), 131.77 (imidazole C-4), 132.72 (*p*-chlorobenzylidene C-1), 133.81 (*p*-chlorobenzylidene C-2, C-6), 136.74 (phenyl C-1), 137.17 (*p*-chlorobenzylidene C-4), 138.60 (imidazole-1-phenyl C-1), 142.05 (benzoxazole C-3a), 150.86 (benzoxazole C-7a), 160.40 (imidazole C-2), 161.98 (benzoxazole C-2), 169.91 (CO); EIMS (*m/z*) 477 ($M+2^{+\cdot}$, 25.30), 475 ($M^{+\cdot}$, 66.26%), 105 (100%). Anal. calcd. for $C_{29}H_{18}ClN_3O_2$ (475.93): C, 73.19; H, 3.81; N, 8.83. Found: C, 72.97; H, 3.88; N, 8.85.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-benzylidene-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4d)

Yellow solid; yield 75%; m.p. 261–263°C; IR (KBr, cm^{-1}) 3063 (aromatic CH), 2920 (aliphatic CH), 1721 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.33 (s, 3H, CH_3), 7.25 (d, *J* = 8 Hz, 2H, *p*-tolyl H-3, H-5), 7.30 (s, 1H, ═CH), 7.41–7.54 (m, 8H, benzylidene H-2, H-3, H-4, H-5, H-6, imidazole-1-phenyl H-2, H-6 and benzoxazole H-4), 7.81–7.86 (m, 3H, benzoxazole H-5, H-6, H-7), 8.29 (d, *J* = 8 Hz, 2H, *p*-tolyl H-2, H-6), 8.38 (d, *J* = 7.6 Hz, 2H, imidazole-1-phenyl H-3, H-5); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 21.49 (CH_3), 111.53 (benzoxazole C-7), 119.67 (benzoxazole C-4), 120.38 (═CH), 125.80 (benzoxazole C-6), 126.20 (imidazole-1-phenyl C-4), 126.42 (benzoxazole C-5), 128.70 (imidazole-1-phenyl C-3, C-5), 129.06 (benzylidene C-4), 129.42 (benzylidene C-2, C-6), 129.45 (benzylidene C-3, C-5), 129.64 (*p*-tolyl C-3, C-5), 131.20 (*p*-tolyl C-2, C-6), 132.79 (imidazole-1-phenyl C-2, C-6), 130.02 (imidazole C-4), 132.01 (*p*-tolyl C-1), 133.20 (benzylidene C-1), 135.70 (imidazole-1-phenyl C-1), 138.50 (*p*-tolyl C-4), 141.50 (benzoxazole C-3a), 150.01 (benzoxazole C-7a), 155.82 (imidazole C-2), 162.71 (benzoxazole C-2), 169.87 (C═O). Anal. calcd. for $C_{30}H_{21}N_3O_2$ (455.16): C, 79.10; H, 4.65; N, 9.22. Found: C, 78.95; H, 4.54; N, 9.17.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-(4-methoxybenzylidene)-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4e)

Yellow solid; yield 78%; m.p. 261–263°C; IR (KBr, cm^{-1}) 3063 (aromatic CH), 2951 (aliphatic CH), 1709 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 2.35 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.02 (d, *J* = 8 Hz, 2H, *p*-methoxybenzylidene H-3, H-5), 7.17 (d, *J* = 8 Hz, 2H, *p*-tolyl H-3, H-5), 7.35–7.45 (m, 5H, ═CH, imidazole-1-phenyl H-2, H-6 and benzoxazole H-4, H-7), 7.50 (d, *J* = 8 Hz, 2H, *p*-tolyl H-2, H-6), 7.62 (t, *J* = 8.8, 1H, benzoxazole H-5), 7.81 (t, *J* = 8.8 Hz, 1H, benzoxazole H-6), 8.30–8.34 (m, 4H, imidazole-1-phenyl H-3, H-5 and *p*-methoxybenzylidene H-2, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, δ = ppm) δ = 21.58 (CH_3), 55.44 (OCH_3), 110.68 (benzoxazole C-7), 114.44 (*p*-methoxybenzylidene C-3, C-5), 120.18 (═CH), 124.78 (benzoxazole C-4), 125.43 (benzoxazole C-6), 125.92 (*p*-methoxybenzylidene C-1), 126.67 (imidazole-1-phenyl C-4), 127.33 (benzoxazole C-5), 127.63 (*p*-tolyl C-2, C-6), 128.53 (*p*-tolyl C-3, C-5), 129.12 (*p*-methoxybenzylidene C-2, C-6), 129.23 (imidazole-1-phenyl C-3, C-5), 133.80 (imidazole C-4), 134.71 (imidazole-1-phenyl C-2, C-6), 136.52 (*p*-tolyl C-1), 137.23 (*p*-tolyl C-4), 137.65 (imidazole-1-phenyl C-1), 142.07 (benzoxazole C-3a), 150.86 (benzoxazole C-7a), 158.83 (imidazole C-2), 161.75 (benzoxazole C-2), 162.14 (*p*-methoxybenzylidene C-4), 170.12 (C═O); Anal. calcd. for $C_{31}H_{23}N_3O_3$ (485.53): C, 76.69; H, 4.77; N, 8.65. Found: C, 76.73; H, 4.71; N, 8.63.

(*ZE*)-1-[4-(Benzo[d]oxazol-2-yl)phenyl]-4-(4-chlorobenzylidene)-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4f)

Yellow solid; yield 72%; m.p. 277–279°C; IR (KBr, cm^{-1}) 3051 (aromatic CH), 2924 (aliphatic CH), 1721 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 2.39 (s, 3H, CH_3), 7.18 (d, *J* = 8 Hz, 2H, *p*-tolyl H-3, H-5), 7.29 (s, 1H, ═CH), 7.36 (d, *J* = 8.4 Hz, 2H, imidazole-1-phenyl H-2, H-6), 7.39–7.41 (m, 2H, benzoxazole H-4, H-7), 7.46 (d, *J* = 8.4 Hz, 2H, *p*-chlorobenzylidene H-3, H-5), 7.50 (d, *J* = 8 Hz, 2H, *p*-tolyl H-2, H-6), 7.61, 7.63 (dd, *J* = 3.6 Hz, 7.2 Hz, 1H,

benzoxazole H-5), 7.80, 7.81 (dd, *J* = 3.6 Hz, 6.8 Hz, 1H, benzoxazole H-6), 8.26 (d, *J* = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.33 (d, *J* = 8.4 Hz, 2H, *p*-chlorobenzylidene H-2, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, *δ* = ppm) *δ* = 21.62 (CH_3), 110.70 (benzoxazole C-7), 120.20 (═CH), 124.42 (benzoxazole C-4), 125.49 (benzoxazole C-6), 125.56 (benzoxazole C-5), 126.89 (imidazole-1-phenyl C-4), 127.61 (imidazole C-4), 127.64 (imidazole-1-phenyl C-3, C-5), 128.59 (*p*-chlorobenzylidene C-3, C-5), 129.11 (*p*-chlorobenzylidene C-2, C-6), 129.24 (*p*-tolyl C-3, C-5), 129.31 (*p*-tolyl C-2, C-6), 132.83 (*p*-chlorobenzylidene C-1), 133.73 (imidazole-1-phenyl C-2, C-6), 136.57 (*p*-tolyl C-1), 137.34 (*p*-chlorobenzylidene C-4), 138.69 (imidazole-1-phenyl C-1), 142.04 (*p*-tolyl C-4), 142.53 (benzoxazole C-3a), 150.86 (benzoxazole C-7a), 160.48 (imidazole C-2), 162.02 (benzoxazole C-2), 170.00 (C═O); EIMS (*m/z*) 491 ($M+2^{+\cdot}$, 29.78), 489 ($M^{+\cdot}$, 77.86%), 119 (100%). Anal. calcd. for $C_{30}H_{20}ClN_3O_2$ (489.12): C, 73.54; H, 4.11; N, 8.58. Found: C, 73.38; H, 4.09; N, 8.45.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-benzylidene-2-phenyl-1*H*-imidazol-5(4*H*)-one (4g)

Yellow solid; yield 86%; m.p. 282–284°C; IR (KBr, cm^{-1}) 3063 (aromatic CH), 2924 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, *δ* = ppm) *δ* = 7.34 (s, 1H, ═CH), 7.43–7.61 (m, 12 H, phenyl H-2, H-3, H-4, H-5, H-6, benzylidene H-2, H-3, H-4, H-5, H-6 and imidazole-1-phenyl H-2, H-6), 8.10 (d, *J* = 8 Hz, 1H, benzothiazole H-6), 8.19–8.21 (m, 3H, benzothiazole H-4, H-5, H-7), 8.39 (d, 2H, imidazole-1-phenyl H-3, H-5); ^{13}C NMR (100 MHz, DMSO-d_6, *δ* = ppm) *δ* = 115.05 (═CH), 124.50 (benzothiazole C-4), 125.30 (benzothiazole C-7), 127.90 (benzothizole C-6), 128.25 (benzothiazole C-5), 128.36 (benzylidene C-4), 128.52 (benzylidene C-2, C-6), 128.80 (benzylidene C-3, C-5), 129.10 (phenyl C-2, C-6), 129.40 (phenyl C-3, C-5), 129.50 (imidazole-1-phenyl C-4), 130.43 (imidazole-1-phenyl C-3, C-5), 132.89 (imidazole C-4), 133.01 (imidazole-1-phenyl C-2, C-6), 133.23 (benzothiazole C-7a), 135.14 (phenyl C-1), 135.20 (imidazole-1-phenyl C-1), 137.69 (benzylidene C-1), 151.90 (benzothiazole C-3a), 159.59 (imidazole C-2), 167.04 (benzothiazole C-2), 171.62 (C═O). Anal. calcd. for $C_{29}H_{19}N_3OS$ (457.55): C, 76.13; H, 4.19; N, 9.18. Found: C, 75.99; H, 4.17; N, 9.20.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-(4-methoxybenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (4h)

Yellow solid; yield 84%; m.p. 304–306°C; IR (KBr, cm^{-1}) 3059 (aromatic CH), 2859 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, *δ* = ppm) *δ* = 3.91 (s, 3H, OCH_3), 7.02 (d, *J* = 8.8 Hz, *p*-methoxybenzylidene H-3, H-5), 7.33–7.39 (m, 5H, ═CH, imidazole-1-phenyl H-2, H-6 and phenyl H-3, H-5), 7.42–7.49 (m, 2H, benzothiazole H-5, H-6), 7.54 (t, *J* = 8 Hz, 1H, phenyl H-4), 7.63 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 7.95 (d, *J* = 8.4 Hz, 1H, benzothiazole H-7), 8.10 (d, *J* = 8 Hz, 1H, benzothiazole H-4), 8.17 (d, *J* = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.32 (d, *J* = 8.8 Hz, *p*-methoxybenzylidene H-2, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, *δ* = ppm) *δ* = 55.44 (OCH_3), 114.47 (*p*-methoxybenzylidene C-3, C-5), 121.71 (═CH), 123.37 (benzothiazole C-4), 125.51 (benzothiazole C-7), 126.55 (benzothizole C-6), 127.27 (*p*-methoxybenzylidene C-1), 127.64 (phenyl C-2, C-6), 128.46 (phenyl C-3, C-5), 128.51 (*p*-methoxybenzylidene C-2, C-6), 128.86 (imidazole-1-phenyl C-4), 129.16 (imidazole-1-phenyl C-3, C-5), 130.07 (benzothiazole C-5), 131.30 (phenyl C-4), 133.14 (imidazole C-4), 134.78 (imidazole-1-phenyl C-2, C-6), 135.60 (benzothiazole C-7a), 136.50 (phenyl C-1), 137.03 (imidazole-1-phenyl C-1), 154.12 (benzothiazole C-3a), 158.78 (imidazole C-2), 161.84 (*p*-methoxybenzylidene C-4), 166.77 (benzothiazole C-2), 170.13 (C═O); EIMS (*m/z*) 487 ($M^{+\cdot}$, 31.53%), 105 (100%). Anal. calcd. for $C_{30}H_{21}N_3O_2S$ (487.57): C, 73.90; H, 4.34; N, 8.62. Found: C, 73.87; H, 4.28; N, 8.66.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-(4-chlorobenzylidene)-2-phenyl-1*H*-imidazol-5(4*H*)-one (4i)

Green solid; yield 87%; m.p. 299–301°C; IR (KBr, cm^{-1}) 3055 (aromatic CH), 2924 (aliphatic CH), 1716 (CO); ^{1}H NMR (400 MHz, DMSO-d_6, *δ* = ppm) *δ* = 7.32–7.54 (m, 10H, ═CH, phenyl H-2, H-3, H-4, H-5, H-6, imidazole-1-phenyl H-2, H-6 and benzothiazole H-5, H-6), 7.64 (d, *J* = 8 Hz, 2H, *p*-chlorobenzylidene H-3, H-5), 7.95 (d, *J* = 8 Hz, 1H, benzothiazole H-7), 8.04 (d, *J* = 8 Hz, 1H, benzothiazole H-4), 8.18 (d, *J* = 8 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.27 (d, *J* = 8 Hz, 2H, *p*-chlorobenzylidene H-2, H-6); ^{13}C NMR (100 MHz, DMSO-d_6, *δ* = ppm) *δ* = 122.97 (═CH), 123.50 (benzothiazole C-4), 126.27 (benzothiazole C-7), 127.01 (imidazole-1-phenyl C-4), 127.30 (benzothizole C-6), 128.53 (phenyl C-2, C-6), 128.87 (phenyl C-3, C-5), 129.01 (imidazole C-4), 129.05 (*p*-chlorobenzylidene C-3, C-5), 129.11 (imidazole-1-phenyl C-2, C-6), 129.54 (*p*-chlorobenzylidene C-2, C-6), 130.07 (benzothiazole C-5), 131.30 (*p*-chlorobenzylidene C-1), 132.23 (imidazole-1-phenyl C-3, C-5), 133.07 (benzothiazole C-7a), 134.39 (phenyl C-1), 135.00 (*p*-chlorobenzylidene C-4), 135.03 (phenyl C-4), 137.31 (imidazole-1-phenyl C-1), 152.03 (benzothiazole C-3a), 159.02 (imidazole C-2), 166.17 (benzothiazole C-2), 169.13 (C═O). Anal. calcd. for $C_{29}H_{18}ClN_3OS$ (491.99): C, 70.80; H, 3.69; N, 8.54. Found: C, 70.75; H, 3.63; N, 8.47.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-benzylidene-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4j)

Yellow solid; yield 85%; m.p. 288–290°C; IR (KBr, cm^{-1}) 3059 (aromatic CH), 2920 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, *δ* = ppm) *δ* = 2.33 (s, 3H, CH_3), 7.26 (d, *J* = 8 Hz, 2H, *p*-tolyl H-3, H-5), 7.30 (s, 1H, ═CH), 7.48–7.60 (m, 9H, benzylidene H-2, H-3, H-4, H-5, H-6, *p*-tolyl H-2, H-6 and imidazole-1-phenyl H-2, H-6), 8.10 (d, *J* = 8 Hz, 1H, benzothiazole H-6), 8.18–8.21 (m, 3H, benzothiazole H-4, H-5, H-7), 8.38 (d, *J* = 7.6 Hz, 2H, imidazole-1-phenyl H-3, H-5); ^{13}C NMR (100 MHz, DMSO-d_6, *δ* = ppm) *δ* = 21.55 (CH_3), 114.60 (═CH), 122.97 (benzothiazole C-4), 123.50 (benzothiazole C-7), 125.30 (benzothizole C-6), 127.90 (benzthiazole C-5), 128.01 (imidazole-1-phenyl C-1), 128.54 (benzylidene C-4), 128.80 (imidazole C-4), 129.12 (benzylidene C-2, C-6), 129.38 (benzylidene C-3, C-5), 129.49 (*p*-tolyl C-3, C-5), 129.64 (imidazole-1-phenyl C-3, C-5), 131.05 (imidazole-1-phenyl C-2, C-6), 132.00 (*p*-tolyl C-1), 132.81 (*p*-tolyl C-2, C-6), 133.05 (benzothiazole C-7a), 135.03 (phenyl C-1), 137.07 (imidazole-1-phenyl C-4), 139.31 (*p*-tolyl C-4), 154.02 (benzothiazole C-3a), 157.09 (imidazole C-2), 168.02 (benzothiazole C-2), 169.18 (C═O). EIMS (*m/z*) 471 ($M^{+\cdot}$, 87.55%), 119 (100%). Anal.

calcd. for $C_{30}H_{21}N_3OS$ (471.14): C, 76.41; H, 4.49; N, 8.91. Found: C, 76.38; H, 4.50; N, 8.88.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-(4-methoxybenzylidene)-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4k)

Yellow solid; yield 86%; m.p. 282–284°C; IR (KBr, cm^{-1}) 3059 (aromatic CH), 2924 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, $CDCl_3$, δ = ppm) δ = 2.39 (s, 3H, CH_3), 3.91 (s, 3H, OCH_3), 7.02 (d, J = 8.8 Hz, *p*-methoxybenzylidene H-3, H-5), 7.17 (d, J = 8 Hz, 2H, *p*-tolyl H-3, H-5), 7.33–7.35 (m, 3H, ═CH and imidazole-1-phenyl H-2, H-6), 7.42–7.44 (t, J = 8 Hz, 1H, benzothiazole H-5), 7.50–7.54 (m, 3H, *p*-tolyl H-2, H-6 and benzothiazole H-6), 7.95 (d, J = 8 Hz, 1H, benzothiazole H-7), 8.10 (d, J = 8 Hz, 1H, benzothiazole H-4), 8.16 (d, J = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.31 (d, J = 8.8 Hz, 2H, *p*-methoxybenzylidene H-2, H-6); ^{13}C NMR (100 MHz, $CDCl_3$, δ = ppm) δ = 21.58 (CH_3), 55.44 (OCH_3), 114.44 (*p*-methoxybenzylidene C-3, C-5), 121.72 (═CH), 123.32 (benzothiazole C-4), 125.56 (benzothiazole C-7), 125.90 (imidazole-1-phenyl C-4), 126.60 (*p*-methoxybenzylidene C-1), 127.30 (benzothizole C-6), 127.32 (imidazole C-4), 127.72 (*p*-methoxybenzylidene C-2, C-6), 128.48 (*p*-tolyl C-3, C-5), 129.15 (*p*-tolyl C-2, C-6), 129.25 (benzothiazole C-5), 129.55 (imidazole-1-phenyl C-3, C-5), 132.94 (*p*-tolyl C-1), 134.72 (imidazole-1-phenyl C-2, C-6), 136.47 (benzothiazole C-7a), 137.23 (*p*-tolyl C-4), 141.98 (imidazole-1-phenyl C-1), 153.82 (benzothiazole C-3a), 158.88 (imidazole C-2), 161.75 (*p*-methoxybenzylidene C-4), 167.01 (benzothiazole C-2), 170.16 (C═O). Anal. calcd. for $C_{31}H_{23}N_3O_2S$ (501.15): C, 74.23; H, 4.62; N, 8.38. Found: C, 74.35; H, 4.57; N, 8.31.

(*ZE*)-1-[4-(Benzo[d]thiazol-2-yl)phenyl]-4-(4-chlorobenzylidene)-2-(*p*-tolyl)-1*H*-imidazol-5(4*H*)-one (4l)

Yellow solid; yield 81%; m.p. 281–283°C; IR (KBr, cm^{-1}) 3048 (aromatic CH), 2924 (aliphatic CH), 1717 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ = ppm) δ = 2.40 (s, 3H, CH_3), 7.13 (s, 1H, ═CH), 7.25 (d, J = 8 Hz, *p*-tolyl H-3, H-5), 7.43–7.59 (m, 4H, *p*-chlorobenzylidene H-3, H-5 and imidazole-1-phenyl H-2, H-6), 7.66 (d, J = 8.4 Hz, 2H, *p*-chlorobenzylidene H-2, H-6), 7.93–7.97 (m, 4 H, benzothiazole H-5, H-6 and *p*-tolyl H-2, H-6), 8.04 (d, J = 8 Hz, 1H, benzothiazole H-7), 8.09 (d, J = 8.4 Hz, 2H, imidazole-1-phenyl H-3, H-5), 8.14 (d, J = 7.6 Hz, 1H, benzothiazole H-4); ^{13}C NMR (100 MHz, DMSO-d_6, δ = ppm) δ = 21.52 (CH_3), 120.59 (═CH), 122.75 (benzothiazole C-4), 123.06 (benzothiazole C-7), 125.74 (benzothizole C-6), 127.07 (benzothiazole C-5), 128.18 (imidazole-1-phenyl C-4), 128.31 (*p*-chlorobenzylidene C-3, C-5), 128.47 (*p*-chlorobenzylidene C-2, C-6), 128.85 (imidazole-1-phenyl C-3, C-5), 129.07 (*p*-tolyl C-3, C-5), 129.43 (imidazole-1-phenyl C-2, C-6), 129.55 (imidazole C-4), 130.98 (*p*-tolyl C-2, C-6), 131.61 (*p*-tolyl C-1), 132.17 (*p*-chlorobenzylidene C-1), 133.50 (*p*-chlorobenzylidene C-4), 134.77 (benzothiazole C-7a), 142.46 (imidazole-1-phenyl C-1), 142.73 (*p*-tolyl C-4), 154.13 (benzothiazole C-3a), 165.16 (imidazole C-2), 166.36 (benzothiazole C-2), 167.50 (C═O); EIMS (*m/z*) 507 ($M+2^{+\cdot}$, 26.06), 505 ($M^{+\cdot}$, 60.64%), 119 (100%). Anal. calcd. for $C_{30}H_{20}ClN_3OS$ (505.10): C, 71.21; H, 3.98; N, 8.30. Found: C, 71.16; H, 4.03; N, 8.36.

4.2 | Biological activity

4.2.1 | Anti-inflammatory activity

In vitro cyclooxygenase (COX) inhibition assay

The ability of the test compounds listed in Table 1 to inhibit human recombinant COX-1 and COX-2 (IC_{50} value, μM) was tested using an enzyme immune assay (EIA) kit (Cayman Chemical, Ann Arbor, MI) according to a reported method.[27,28]

In vitro lipoxygenase (LOX) inhibition assay

The ability of the test compounds listed in Table 1 to inhibit LOX enzyme (IC_{50} value, μM) was determined using the Cayman Human Lipoxygenase Inhibitor Screening Assay (EIA) kit (catalog no 760700, Cayman Chemical). Before use, stock solutions were freshly prepared. Buffer solution used was (0.1 M Tris-HCl, pH 7.4). A total of 10 μL of different compounds were dissolved in at least the amount of DMSO and diluted with the stock solution concentrations of 0.001, 01, 1, 5, 10 μM in a final volume of 210 μL. The IC_{50} of test compounds were determined according to the manufacturer's instructions and according to reported method.[29]

4.2.2 | *In vitro* cytotoxicity

Cell culture

Stock solutions (10 mmol/L) of the tested compounds were prepared by dissolving an appropriate quantity of each substance in dimethylsulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin were purchased from Sigma (MO, USA). Calcein AM was obtained from Molecular Probes (Life Technologies, CA, USA). The screening cell lines (breast carcinoma cell line MCF7, hepatocellular carcinoma cell line Hep3B, liver hepatoma cell line PLC/PRF5, ovarian carcinoma cell line A2780, and human fibroblasts BJ) were obtained from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in DMEM medium (Sigma, MO, USA), supplemented with 10% fetal bovine serum, L-glutamine (2 mmol/L), penicillin (100 U), and streptomycin (100 μg/mL). The cell lines were maintained under standard cell culture conditions at 37 °C and 5% CO_2 in a humid environment. Cells were subcultured twice or three times a week using the standard trypsinization procedure.

Calcein AM assay

Suspensions of tested cell lines (ca. 1.0×10^5 cells/mL) were placed in 96-well microtiter plates and after 24 h of stabilization (time zero), the tested compounds were added (in three 20 μL aliquots) in serially diluted concentrations in dimethylsulfoxide (DMSO). Control cultures were treated with DMSO alone, and the final concentration of DMSO in the incubation mixtures never exceeded 0.6%. The test compounds were typically evaluated at six threefold dilutions and the highest final concentration was generally 50 μM. After 72 h incubation, Calcein AM solution (100 μL, Molecular Probes, Life Technologies, CA, USA) was added, and incubation was continued for another hour. The fluorescence of viable cells was then quantified using a Fluoroskan Ascent instrument (Labsystems, Finland). The percentage of surviving cells in each well was

calculated by dividing the intensity of the fluorescence signals from the exposed wells by the intensity of signals from control wells and multiplying by 100. These ratios were then used to construct dose–response curves from which IC_{50} values, the concentrations of the respective compounds that were lethal to 50% of the tumor cells, were calculated.

4.3 | Docking study

Docking was performed to obtain the binding free energies for best poses between COX2 receptor (PDB ID: 1CX2)[30] and 5-LOX receptor (PDB ID: 3V99)[31] and the designed compounds **4a–l**. The docking studies were carried out using Molecular Operating Environment (MOE, Version 2005.06, Chemical Computing Group Inc., Montreal, Quebec, Canada).[32]

The co-crystallized ligands, celecoxib (SC-558) in COX-2 receptor and arachidonic acid (ACD), the natural ligand of 5-LOX receptor, were docked first. The best crystal-like poses of each ligand were analyzed. Docking was performed using London dG force and force field energy was used to refine the results.

The test compounds were prepared for docking using 3D structure built by MOE and by selecting the least energetic conformer. The same docking protocol used with ligand was also applying for the designed compounds. The amino acid His90 was located in COX-2 active site and made a hydrogen bond with ligand celecoxib and most of the test compounds. The 5-LOX active site contains the amino acid Asn180 which was important for formation of hydrogen bonds with the target compounds (Table 3 and Figures 3–6).

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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SUPPORTING INFORMATION

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