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Syntheses and characterization of nimesulide derivatives for dual enzyme inhibitors of both cyclooxygenase-1/2 and 5-lipoxygenase

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ABSTRACT

Cyclooxygenase-1/2 (COX-1/2) and 5-lipoxygenase (5-LOX) are enzymes in two different pathways in the inflammatory process. In the present study, a variety of new nimesulide derivatives were synthesized through incorporation of a 5-LOX pharmacophore into nimesulide followed with some structural modifications, which were then characterized for dual enzyme inhibitors for these two types of enzymes. Their structure–activity relationships (SARs) were studied, and compound **20f** was found to be an excellent dual enzyme inhibitor. Its binding conformation and interaction mode were studied with molecular docking experiments. Compound **20f** could become a lead compound for further development for potential anti-inflammatory drugs.

1. Introduction

Cyclooxygenases (COXs) or prostaglandin endoperoxide synthases (PGHSs) are key enzymes in the first step for the biosynthesis of the prostaglandins (PGs) from the substrate arachidonic acid (AA) during the inflammatory process.[1,2] Two isoforms of COX enzymes, COX-1 and COX-2[3] are known to catalyze the rate-limiting step of prostaglandins synthesis, which are the targets of nonsteroidal anti-inflammatory drugs (NSAIDs).[4–6] However, NSAIDs have been reported to be associated with gastrointestinal adverse effects, and many users of traditional NSAIDs have been suffering from gastrointestinal ulcers and bleeding.[7–9] The COX-2 hypothesis has suggested that selective COX-2 inhibitors share the anti-inflammatory properties of traditional NSAIDs without the gastrointestinal toxicity,[10] which has been validated by the successful human clinical trials of celecoxib (**1**) and other selective COX-2 inhibitors.[11] However, selective COX-2 inhibitors have also been failed to fully satisfy the criteria of new safer anti-inflammatory agents for the increased risk of cardiovascular complications,[12–14] for example, rofecoxib (**2**) has been withdrawn from the global market. Meanwhile, the post-marketing surveillances have revealed that nimesulide (**3**) may cause more severe adverse effect of hepatic damage.[15,16] Therefore, recent studies are mainly focusing on strategies towards reducing the adverse effects of anti-inflammatory drugs and searching for new safer drugs.

In addition to the COX pathway, AA is also metabolized via the lipoxygenase (LOX) pathway. Lipoxygenases (LOXs) belong to a class of non-heme iron-containing enzymes, which catalyze the hydroperoxidation reaction of fatty acids to peroxides.[17] 5-LOX is associated with the production of leukotrienes (LTs) from AA, which causes inflammatory, bronchoconstrictor, hypersensitivity, anaphylactic, and asthmatic actions.[18,19] Thus, 5-LOX inhibitors have also been used as potential therapeutic agents for the treatment of inflammatory and allergic diseases.[20] Furthermore, among three types of known 5-LOX inhibitors (redox, iron chelators, and non redox), non-redox compounds such as licofelone (**4**) and ZD-2138 (**5**), which are the active-site directed inhibitors, have shown fewer side effects than redox and iron chelating agents.[21]

It has been believed that the design and discovery of a dual inhibitor for both COX and LOX enzymatic pathways should provide a new rational approach for effective anti-inflammatory agents with better safety profiles than NSAIDs and selective COX-2 inhibitors.[22] The inhibition of only one of the enzymatic pathways can possibly shift the metabolism of AA toward the other pathway, which causes side effects. Therefore, searching for a balance in the inhibition of two enzymatic pathways is essential in the development of superior and safer anti-inflammatory agents.[23] One of the successful strategies to develop COX/5-LOX inhibitors is to modify NSAIDs and selective COX-2 inhibitors by introducing a 5-LOX pharmacophore, and several successful examples are shown in Figure 1. A potent hybrid COX/5-LOX inhibitor (**6**) has been reported, in which the C-3 trifluoromethyl substituent present in the COX-2 inhibitor celecoxib (**1**) has been replaced by a non-redox competitive 5-LOX pharmacophore, 4-(3-fluoro-

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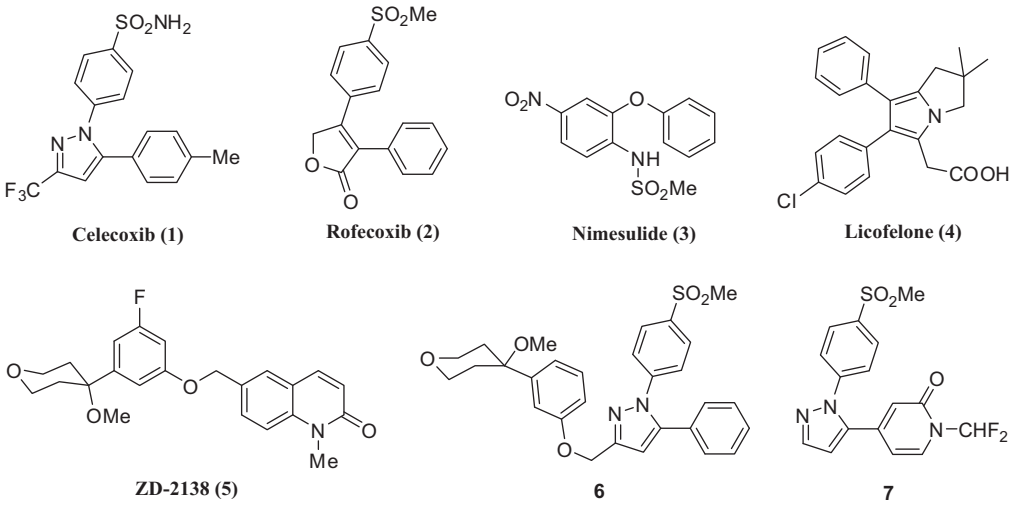


Figure 1. Chemical structures of the selective COX-2 inhibitors celecoxib (**1**), rofecoxib (**2**), nimesulide (**3**), non-redox 5-LOX inhibitors licofelone (**4**) and ZD-2138 (**5**), and representative COX/LOX inhibitors (**6** and **7**).

5-oxymethyl)phenyl-4-methoxytetrahydropyran.[24,25] Another type of novel dual COX/5-LOX inhibitor celecoxib analogues (**7**)[26] that possess *N*-difluoromethyl-1,2-dihydropyrid-2-one 5-lipoxygenase pharmacophore, has also shown acceptable in vitro COX-2 inhibitory potency and selectivity, and potent inhibition of the enzyme 5-LOX. These dual COX/5-LOX inhibitors have key pharmacophoric elements in their structures for optimal interactions with binding sites of both enzyme systems, and have shown certain antitumor activity. Accordingly, we anticipated that introduction of a 5-LOX pharmacophore to the selective COX-2 inhibitor nimesulide (**3**) may provide a type of effective dual COX/5-LOX inhibitory anti-inflammatory agents. In this paper, we report our syntheses of nimesulide derivatives with incorporation of a 5-LOX pharmacophore, 4-methoxytetrahydropyran, and their characterization through dual enzyme inhibition and molecular docking experiments.

2. Results and discussion

2.1. Syntheses of nimesulide derivatives

A new type of COX/5-LOX dual enzyme inhibitors was synthesized through incorporation of a 5-LOX pharmacophore to a selective COX-2 inhibitor, nimesulide. According to the structural requirement for selective COX-2 inhibition by nimesulide reported previously, the nitro group and methane sulfonamide group are crucial to its selectivity and inhibition activity.[27] Therefore, modifications of these two groups may be helpful to make a balance between COX and 5-LOX inhibition. On the other hand, our binding models showed that the replacement of the nitro group with other functional groups at different positions might give good dual COX/5-LOX inhibitors. As shown in Figure 2, nimesulide derivative was systematically modified in both position A and position B, which include the replacement of a nitro group in position A with various other substitutive groups, and the change of the linkage between 5-LOX pharmacophore and nimesulide analogue in position B.

The preparation of synthetic intermediate compounds **11** and **12** was shown in Scheme 1. The Grignard reagent was prepared from 1-bromo-3-methylbenzene, which was then reacted with tetrahydro-4*H*-pyran-4-one to give a tertiary alcohol compound **9**. Its methylation followed with bromination afforded intermediate compound **11**. Finally, another intermediate compound **12** with a phenylmethanesulfonyl chloride functional group was prepared following a general method reported previously.[28]

The synthetic pathway for compounds **16a–f** and **17a–e** is shown in Scheme 2. Various substituted *o*-aminophenols were reacted with phthalic acid anhydride to afford compounds **14a–f**, which could protect their amino group from reacting with benzyl bromide in the next step. After the reactions of compounds **14a–f** with benzyl bromide and subsequent deprotection reactions with hydrazine, compounds **16a–f** were obtained in good yield. Then, the reactions of compounds **16a–f** with methanesulfonyl chloride gave products **17a–e** as methane sulfonamides.

The preparations of compounds **19a–d** are shown in Scheme 3. The reactions of pyrocatechols with benzyl bromide gave compounds **19a** and **19d**. The following chlorination and bromination of 2-(benzyloxy) phenol (**19a**) gave compounds **19b** and **19c**, respectively.

The synthetic scheme for the preparation of final products **20a–f, 21a–e**, **22a–e**, and **23a–d** with different linkages are shown

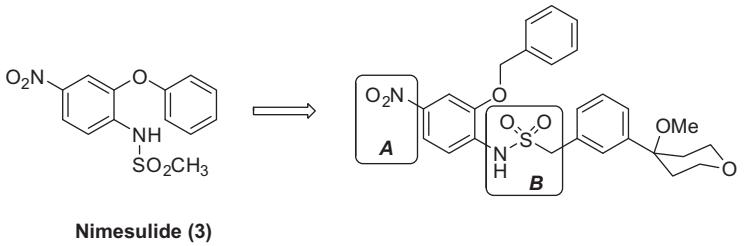
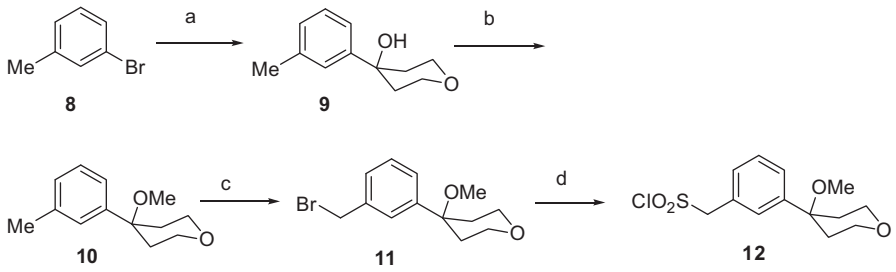
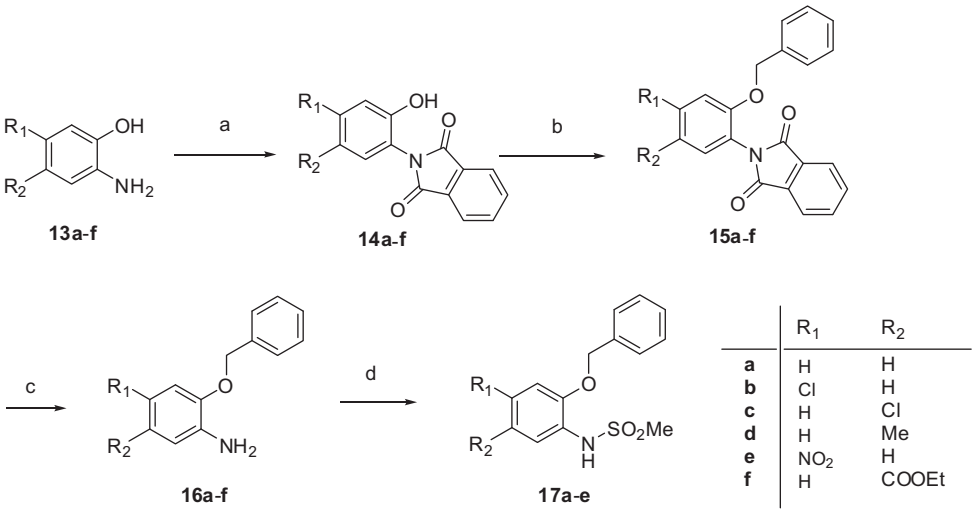


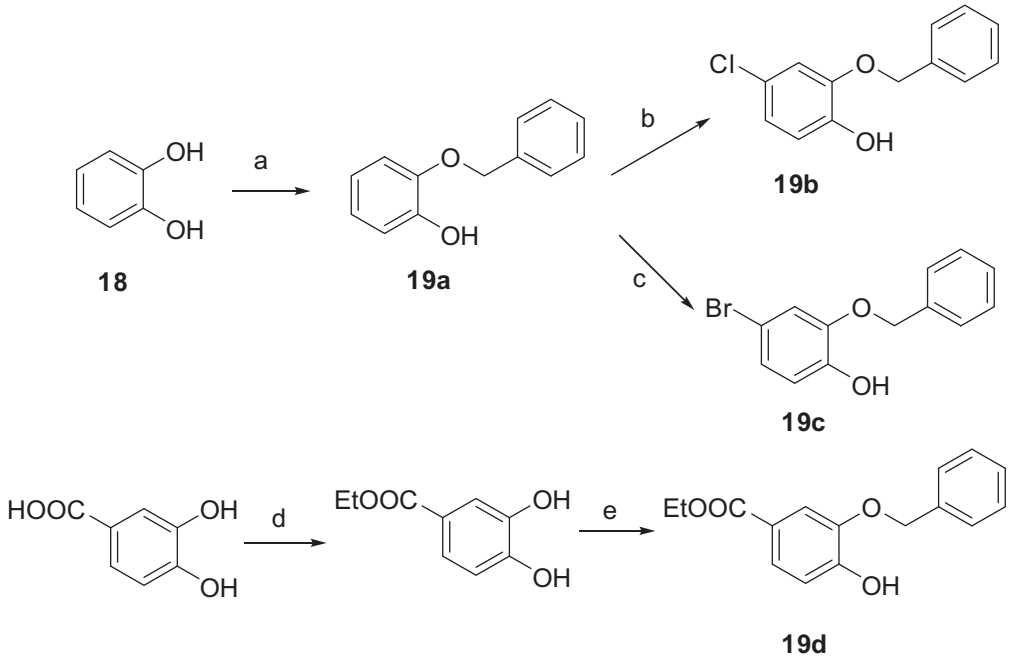
Figure 2. Design of dual COX/5-LOX enzyme inhibitors through incorporation of a 5-LOX pharmacophore to nimesulide followed with some structural modifications in position A and B.



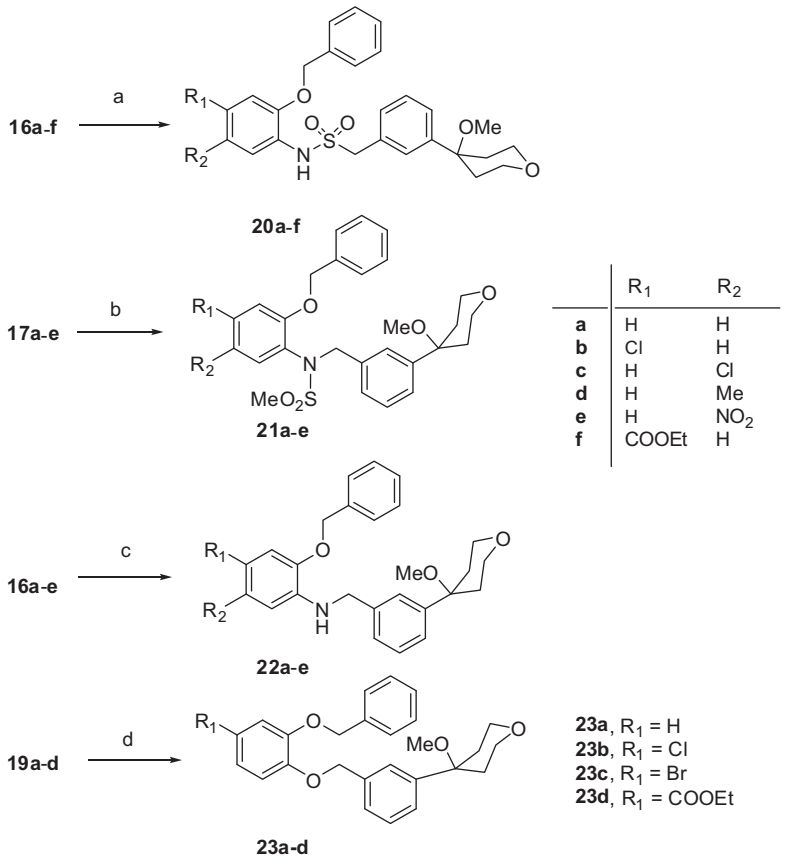
Scheme 1. Organic syntheses of compounds **11** and **12**. Reagents and conditions: (a) (i) Mg, THF, 65 °C; (ii) tetrahydro-4*H*-pyran-4-one, THF, reflux, 2 h yield 71%; (b) NaH, CH_3I, THF, 50 °C, 2 h, yield 89%; (c) NBS, CCl_4, reflux, 2 h, yield 85%; (d) (i) Na_2SO_3, H_2O, C_2H_5OH, reflux, overnight; (ii) $SOCl_2$, DMF, CH_2Cl_2, rt, 3 h yield 69%.



Scheme 2. Synthetic pathway for the preparations of compounds **16a–f** and **17a–e**. Reagents and conditions: (a) phthalic acid anhydride, HOAc, reflux, 3 h, yield 91–98%; (b) benzyl bromide, K_2CO_3, acetone, reflux, 3 h, yield 79–89%; (c) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 2 h, yield 57–86%; (d) $MeSO_2Cl$, pyridine, CH_2Cl_2, rt, 1 h, yield 46–88%.



Scheme 3. Synthetic pathways for the preparation of compounds **19a–d**. Reagents and conditions: (a) benzyl bromide, K_2CO_3, acetone, reflux, 3 h, yield 90%; (b) SO_2Cl_2, toluene, rt, 1 h, yield 76%; (c) Br_2, HOAc, 0 °C, 1 h, yield 55%; (d) H_2SO_4, EtOH, 50 °C, 3 h, yield 98%; (e) benzyl bromide, K_2CO_3, acetone, reflux, 3 h, yield 89%.



Scheme 4. Syntheses of compounds **20a–f**, **21a–e**, **22a–e**, and **23a–d**. Reagents and conditions: (a) compound **12**, pyridine, CH_2Cl_2, rt, overnight, yield 71–75%; (b) compound **11**, K_2CO_3, MeCN, reflux, 3 h, yield 43–66%; (c) compound **11**, K_2CO_3, acetone, reflux, 3 h, yield 33–77%; (d) compound **11**, K_2CO_3, acetone, reflux, 3 h, yield 43–88%.

in Scheme 4. Compounds **20a–f** were obtained through the reactions of compounds **16a–f** with compound **12**, while compounds **21a–e** were synthesized through the reactions of compounds **17a–e** with compound **11**. The reactions of compounds **16a–e** with compound **11** gave products **22a–e**, while the reactions of compounds **19a–d** with compound **11** afforded compounds **23a–d**.

2.2. Enzyme inhibition studies of nimesulide derivatives

Compounds **20a–f**, **21a–e**, **22a–e**, and **23a–d** were characterized for their COX-1/2 and 5-LOX inhibitory activity using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI, USA) and a LOX assay kit (Catalog No. 760700, Cayman Chemicals Inc., Ann Arbor, MI, USA), and the results are shown in Table 1. The IC_{50} values were measured, which indicated the required concentration of tested compound for 50% inhibition of the enzymatic activity. The average of two experimental data was used, and the deviation from the mean was about 10%.

In general, sulfonamide group was found to be essential for their COX inhibition activity. The substitution of methane sulfonamide with other linkages caused almost complete loss of their COX inhibition activity, except the compounds with polar substitution in position A, which showed weak COX inhibition activity. Compounds **21a–e** modified in position B lost their COX inhibition activity, which was consistent with previous reports that the ionization process of N–H in position B is important for their COX-2 inhibitory activity.[29,30] Compounds **23a–d** with an ether linkage also had much weak inhibition for COX-1 and COX-2. Polar substitutions in position A gave good inhibition activity for compounds **20e** and **20f**, while other substitutions generally caused certain loss of their COX inhibition activity. The primary structure–activity relationship study suggested that the combination of a sulfonamide linkage and a polar substitutive group in position A gave compounds **20e** and **20f** with strong inhibition activity for COX. However, the selectivity of compound **20f** between COX-1 and COX-2 was slightly lower than that of nimesulide, and most of above compounds did not show significant selectivity between COX-1 and COX-2.

For 5-LOX inhibition activity, the linkage of 5-LOX pharmacophore was found to be less important, and most above compounds showed certain inhibition activity towards 5-LOX, except the compounds with secondary amine linkage (**22a**, **22c**, **22d** and **22e**). However, the compounds with sulfonamide and ether linkage had better 5-LOX inhibition activity. Interestingly, like the COX inhibition, the combination of a sulfonamide or an ether linkage with a polar substitutive group in position A showed good 5-LOX inhibition. Compounds **23d** and **20f** were found to be the best 5-LOX inhibitors, which indicated that a proper polar group such as ester group in the position A could effectively improve 5-LOX inhibition activity.

Compound **20a–f** with a sulfonamide linkage showed dual inhibition activity against COX and 5-LOX, and the inhibition activity varied among different substituted groups in position A. It was obviously that polar group like nitro (**20e**) and benzoate (**20f**) were better than other groups (methyl or halogen) in both COX and 5-LOX inhibition activity. Meanwhile, benzoate substituted in position A led to certain selectivity between COX-1 and COX-2 inhibition activity, though the selective index was still lower than nimesulide. It suggested that further modifications in position A may result in a good selectivity between COX-1 and COX-2 inhibition activity.

Table 1
Inhibition study results for compounds **20a–f**, **21a–e**, **22a–e**, and **23a–d**

Compound	R_1	R_2	IC_{50} (μM)			Selectivity COX-1/COX-2
			COX-1[a]	COX-2[a]	5-LOX[b]	
20a	H	H	14.8	2.3	3.4	6.4
20b	Cl	H	24.4	3.7	2.4	6.6
20c	H	Cl	15.6	2.9	3.3	5.4
20d	H	Me	16.5	4.9	1.9	3.4
20e	NO_2	H	7.9	1.5	1.1	5.3
20f	H	COOEt	11.6	0.97	0.87	12
21a	H	H	>100	>100	7.5	–
21b	H	Cl	>100	>100	6.9	–
21c	Cl	H	>100	>100	8.5	–
21d	H	Me	>100	>100	4.6	–
21e	NO_2	H	13.5	>100	3.2	–
22a	H	H	>100	>100	>10	–
22b	Cl	H	34.6	>100	7.8	–
22c	H	Cl	>100	>100	>10	–
22d	H	Me	>100	>100	>10	–
22e	NO_2	H	88.8	>100	>10	–
23a	H		>100	>100	3.6	–
23b	Cl		>100	>100	1.6	–
23c	Br		>100	>100	1.9	–
23d	COOEt		44.2	38.8	0.98	1.1
Celecoxib			8.1	0.05	>10	160
Nimesulide			6.2	0.4	>10	15
Caffeic acid			–	–	4.5	–

[a] The concentration of in vitro tested compound required to produce 50% inhibition of ovine COX-1 or COX-2. The result (IC_{50}, μM) is the mean of two determinations using an ovine COX-1/COX-2 assay kit (Catalog No. 560101, Cayman Chemicals Inc., Ann Arbor, MI), and the deviation from the mean is 10% of the mean value.

[b] The concentration of in vitro tested compound required to produce 50% inhibition of potato 5-LOX (Cayman Chemicals Inc. Catalog No. 60401). The result (IC_{50}, μM) is the mean of two determinations using a LOX assay kit (Catalog No. 760700, Cayman Chemicals Inc., Ann Arbor, MI), and the deviation from the mean is 10% of the mean value.

Among these synthetic compounds, compound **20f** had the best overall inhibition activity and selectivity. The modifications of position A and B for nimesulide derivatives successfully made some dual COX/5-LOX inhibitors. Based on the result of our structure–activity relationship study, the sulfonamide part should be kept due to its key contribution to COX inhibition, and further modifications at position A with addition of more polar groups might give better inhibitors.

2.3. Docking studies of nimesulide derivatives

High-resolution structure of COX–inhibitor complex has been solved, which provides detailed information about the COX active site.[31] The COX channel narrows at the interface between the membrane binding domain and the catalytic domain, to form a constriction composed of three residues (Arg-120, Tyr-355, and Glu-524) that separate the 'lobby' from the active site. The solvent accessible surface in the COX-2 active site is larger than that of COX-1, because of the Val-523 to Ile substitution in the active site and several key substitutions in the secondary shell (Arg-513 to His and Val-434 to Ile). This difference in the side pocket is very essential for the selective COX-2 inhibition.[31]

One main binding mode was considered for the inhibitors **20a–f**, which was very similar to that of nimesulide, as shown in Figure 3.[27] The benzyloxy group was inserted into the selective side pocket of COX-2, and the aryl ring substituent was close to Ser530 that was the key amino acid for the interaction between NSAIDs and COXs.[31] The additional 5-LOX pharmacophoric group filled a hydrophobic cavity at the entrance of the active site. This was the most favorable binding mode given by AUTODOCK VINA[32] for six good COX inhibitors (**20a–f**). Two hydrogen bonds were crucial for selective COX-2 inhibition, including one hydrogen bond formed between methanesulfonyl and Arg120, and another one between ethyl benzoate and Ser530. The interaction between the benzyloxy group and the selective COX-2 pocket formed by Arg513, His90, and Val523, was also important. Therefore, it was reasonable that compounds **20e** and **20f** with polar substituent group on their aryl ring were the best COX-2 inhibitors. Further modifications on the benzyloxy group, which interacted with the selective COX-2 pocket, might improve the selectivity of these compounds.

Meanwhile, another possible binding mode was also provided by AUTODOCK VINA. The additional 5-LOX pharmacophoric group got into the upper part of the channel, and the substituted aryl ring was close to Ser530. The amide linkage of the compounds **20e** and **20f** formed hydrogen bonds with residues Arg120 and Tyr355. These key interactions might provide an explanation why introduction of any other linkage in the position B caused almost complete loss of their COX inhibition activity. The difference between these two binding modes was the location of the benzyloxy group and 5-LOX pharmacophoric group. The two key interactions were similar, including the interaction of methanesulfonyl with Arg120 and the interaction of ethyl benzoate with Ser530. There was no key interaction observed in the COX-2 pocket for selective binding in both binding modes, which may explain why these compounds had relatively low selectivity between COX-1 and COX-2.

The three dimensional structure of the enzyme 5-LOX has been well studied. The 5-LOX active site is consisted of a deep bent-shaped cleft containing the non-heme iron cofactor. Two hydrogen bond acceptors exist in the entrance and exit of cleft. It's interesting that a small side pocket also exists, which could accommodate lipophilic groups.[34] In our study, two binding modes for the excellent 5-LOX inhibitors (**20f** and **23d**) were provided by AUTODOCK, which were slightly different according to the site of substitutions in aryl ring (Fig. 4), while their general binding modes were very similar. The 5-LOX pharmacophore of the inhibitor filled the entrance of the active site, while the polar substituent formed hydrogen bonds with hydrogen bond acceptors. The difference between compounds **20f** and **23d** was the orientation of their benzoxy group, which was deepen into the non-heme iron cofactor site for compound **20f** and a small side pocket for compound **23d,** respectively. They both had the hydrogen bonds formed between ethyl benzoate and hydrogen bond acceptors at the bottom of the active site, leading to their stronger 5-LOX inhibition activity than other compounds. It should be noted that an ester substitutive group in position A might form hydrogen bond with Ser530 in the COX active site, which could also form hydrogen bonds with the hydrogen bond acceptors at the bottom of the 5-LOX active site in our docking studies. This could be a key substitutive group for further modifications for dual enzyme inhibitors. On the other hand, the benzoxy group, which got deep into the COX-2 active site in our docking studies, might be further modified to improve the selectivity of the inhibitors.

3. Conclusions

In conclusion, a novel class of dual COX/5-LOX inhibitors derived from selective COX-2 inhibitor nimesulide was synthesized, and their structure–activity relationship was studied. Compounds **20e** and **20f** were found to be overall the best dual enzyme inhibitors. Further computational docking studies provided rational explanation for their binding affinity with above two enzymes. These studies shed light on their further modifications for better dual COX/5-LOX inhibitors as anti-inflammatory drugs.

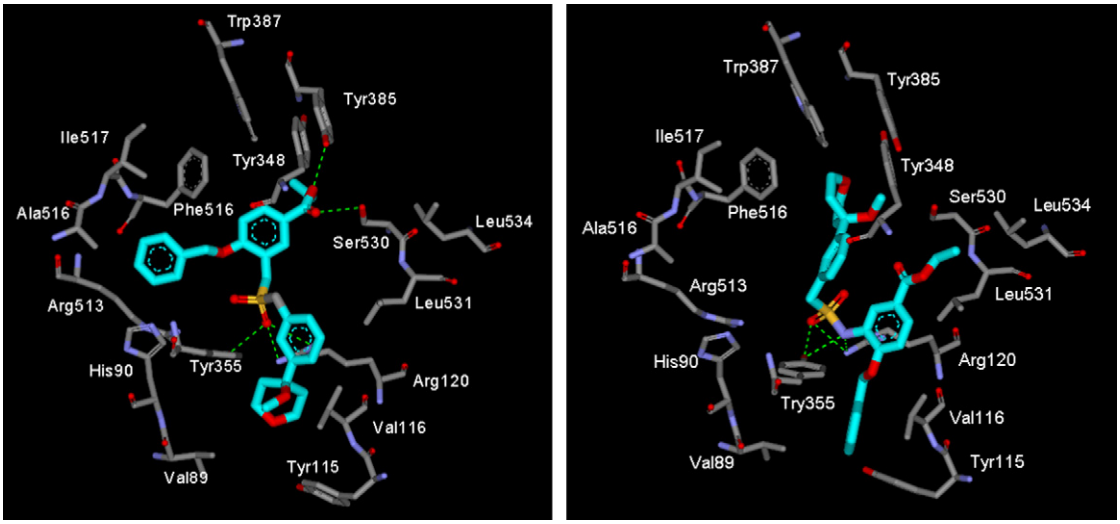


Figure 3. Two different favorable docking modes of compound **20f** in the COX-2 active site were provided by AUTODOCK VINA. Hydrogen bonds were marked with dashed green lines. Pictures were generated with PyMOL.[33]

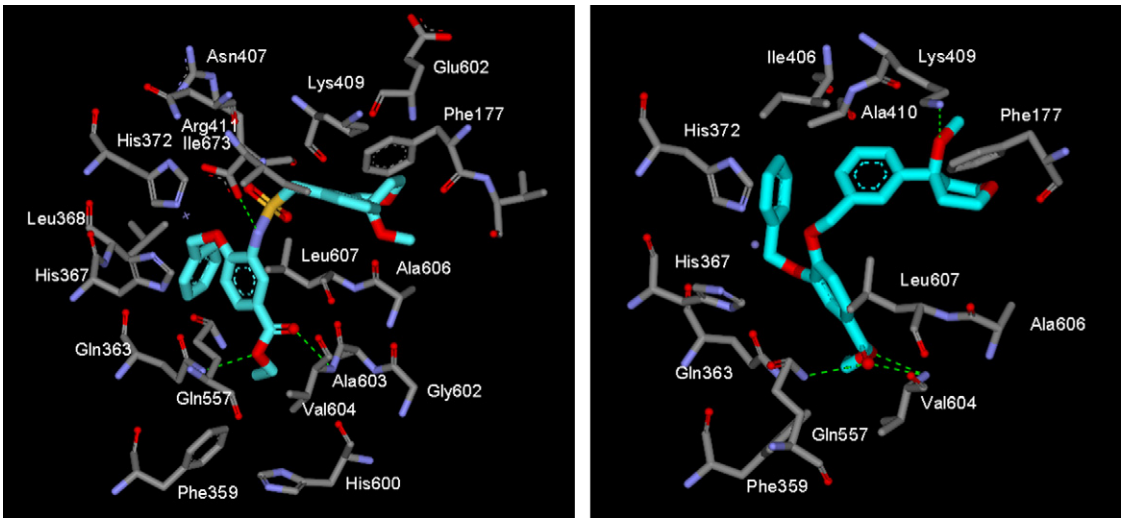


Figure 4. Docking of compounds **20f** (left) and **23d** (right) inside the 5-LOX active site, given by AUTODOCK VINA. Hydrogen bonds were marked with dashed green lines. Pictures were generated with PyMOL.[33]

4. Experimental

4.1. Chemistry

Commercially available chemicals and solvents were used without further purification, unless otherwise stated. NMR was recorded on a Bruker AvanceII 400 spectrometer, and TMS was used as an internal standard for recorded spectra. HRMS was recorded on an Agilent Iron-TOF-LC/MS spectrometer. Melting points (mp) were determined using a SRS-OptiMelt automated melting point instrument without correction. ESI-MS spectra were obtained using a Thermo LCQ DECA XP LC–MS spectrometry. HPLC analysis was performed on a HP1100 system (Hewlett–Packard, Palo Alto, CA), and the purity of all target compounds used in the biophysical and biological studies was more than 95%. All reactions were routinely monitored using TLC with Merck Silica Gel 60F-254 glass plates.

4.1.1. 4-*m*-Tolyltetrahydro-2*H*-pyran-4-ol (9)

1-Bromo-3-methylbenzene (1.7 g, 10 mmol) was added dropwise to Mg (0.36 g, 15 mmol) in dry THF under N_2 at such a rate that the solution was kept at gentle reflux. After the addition was completed, the mixture was heated under reflux for an additional 1–3 h. Then a solution of tetrahydro-4*H*-pyran-4-one (1.0 g, 10 mmol) in THF was added slowly to keep the reaction solution at a gentle reflux. The mixture was heated under reflux for 1 h after the addition was finished. The reaction was then stopped with the addition of satd NH_4Cl followed with extraction using EtOAc (3×50 mL). The organic layers were combined, washed with brine (3×50 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product as oil. The product was purified using flash column chromatography (EtOAc/hexane, 10:90) to give a white solid (1.4 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28–7.16 (m, 3H), 7.11 (m, 1H), 3.94–3.78 (m, 4H), 2.96 (s, 3H), 2.08–1.94 (m, 4H), 1.60 (s, 1H).

4.1.2. 4-Methoxy-4-*m*-tolyltetrahydro-2*H*-pyran (10)

A solution of alcohol **9** (1.9 g, 10 mmol) in THF (100 mL) were added sodium hydride (60% dispersion in mineral oil, 0.44 g, 11 mmol) and methyl iodide (1.0 mL, 11 mmol). The mixture was stirred for 15 h at room temperature before water (100 mL) was added. The aqueous phase was extracted with EtOAc (3 × 50 mL), and the combined organic phases were washed with brine, dried with $MgSO_4$, and evaporated. The crude product was purified using flash chromatography with hexane–EtOAc (80:20) elution to give a colorless liquid (1.9 g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.23 (m, 1H), 7.22–7.16 (m, 2H), 7.10 (d, J = 7.4 Hz, 1H), 3.92–3.77 (m, 4H), 2.97 (s, 3H), 2.37 (s, 3H), 2.05–1.92 (m, 4H).

4.1.3. 4-(3-(Bromomethyl)phenyl)-4-methoxytetrahydro-2*H*-pyran (11)

A mixture of compound **10** (2.1 g, 10 mmol), *N*-bromosuccinimide (2.0 g, 11 mmol), and azoisobutyronitrile (100 mg, 0.6 mmol) in carbon tetrachloride (80 mL) was heated under reflux for 1.5 h. Then, the reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to give a crude product, which was purified using flash chromatography with hexane–EtOAc (80:20) elution to give a colorless liquid (2.4 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29–7.22 (m, 3H), 7.10 (m, 1H), 4.51 (s, 2H), 3.94–3.77 (m, 4H), 2.98 (s, 3H), 2.36 (s, 3H), 2.06–1.94 (m, 4H).

4.1.4. (3-(4-Methoxytetrahydro-2*H*-pyran-4-yl)phenyl)methanesulfonyl chloride (12)

A mixture of compound **11** (1.0 equiv) and sodium sulfite (1.2 equiv) in H_2O (0.1 M) and EtOH (0.2 M) was heated under reflux overnight. The mixture was cooled to room temperature, and concentrated until a precipitate began to form. The product was collected through filtration to give a white solid. To a suspension of this white solid (1.0 equiv) in CH_2Cl_2 (0.1 M) were added DMF (0.1 equiv) and $SOCl_2$ (5 equiv). After 1.5 h, the white suspension was concentrated. The sulfonyl chloride compound **12** thus formed was used for the next step without further purification (yield 69%).

4.1.5. General procedure for the preparation of anilines 16a–f

4.1.5.1. 2-(2-Hydroxyphenyl) isoindoline-1,3-dione (14a). A mixture of 2-aminophenol **13a** (2.2 g, 20 mmol) and phthalic anhydride (3.0 g, 20 mmol) in acetic acid (100 mL) was heated under reflux for two hours, and put in cooled water. The suspension was filtered, and the aqueous filtrate was extracted with chloroform. The organic phase was dried with $MgSO_4$, and the solvent was removed. The residue and the solid, obtained through filtration, were used in the next step without further purification (yield 93%).

4.1.5.2. 2-(2-(Benzyloxy)phenyl) isoindoline-1,3-dione (15a). K_2CO_3 (1.4 g, 10 mmol) and benzyl bromide (2.1 g, 12 mmol) were sequentially added to a solution of compound **14a** (2.4 g, 10 mmol) in acetone, and the mixture was stirred under reflux for 3 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to give the desired product, which was used for the next step without further purification (yield 86%).

4.1.5.3. 2-(Benzyloxy) aniline (16a). A solution of dioxolan **15a** (1.7 g, 5 mmol) and $NH_2NH_2{\cdot}H_2O$ (2 mL) in ethanol (30 mL) was heated under reflux for 2 h. The reaction mixture was cooled to room temperature, and the mixture was filtered through Celite. The solvent was removed, and the crude product was purified using column chromatography with hexane–EtOAc (90:10) elution to give a red oil (0.83 g, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.27 (m, 5H), 6.91–6.77 (m, 2H), 6.76–6.64 (m, 2H), 5.08 (s, 2H), 3.70 (s, 2H).

4.1.5.4. 2-(Benzyloxy)-4-chloroaniline (16b). Following the above procedure, the desired product was obtained as a red oil with a yield of 79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.30 (m, 5H), 6.74 (s, 1H), 6.57 (d, J = 1.6 Hz, 1H), 6.50 (d, J = 1.6 Hz, 1H), 5.05 (s, 2H), 3.60 (s, 2H).

4.1.5.5. 2-(Benzyloxy)-5-chloroaniline (16c). Following the above procedure, the desired product was obtained as a red oil with a yield of 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.30 (m, 5H), 6.76 (s, 1H), 6.57 (d, J = 1.8 Hz, 1H), 6.51 (d, J = 1.8 Hz, 1H), 5.07 (s, 2H), 3.65 (s, 2H).

4.1.5.6. 2-(Benzyloxy)-5-methylaniline (16d). Following the above procedure, the desired product was obtained as a brown oil with a yield of 73%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.25 (m, 5H), 6.68 (s, 1H), 6.54 (d, J = 2.0 Hz, 1H), 6.46 (d, J = 2.0 Hz, 1H), 5.08 (s, 2H), 3.65 (s, 2H), 2.17 (s, 3H).

4.1.5.7. Ethyl 3-amino-4-(benzyloxy) benzoate (16e). Following the above procedure, the desired product was obtained as a red oil with a yield of 57%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48–7.30 (m, 5H), 6.70 (d, J = 8.7 Hz, 1H), 6.66 (d, J = 2.5 Hz, 1H), 6.63 (dd, J = 8.7, 2.5 Hz, 1H), 5.06 (s, 2H), 4.39–4.27 (q, J = 2.3 Hz, 2H), 3.72 (s, 2H) ,1.42 (t, J = 2.3 Hz, 3H).

4.1.5.8. 2-(Benzyloxy)-5-nitroaniline (16f). Following the above procedure, the desired product was obtained as a yellow solid with a yield of 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.30 (m, 5H), 6.74 (d, J = 8.5 Hz, 1H), 6.70 (d, J = 2.4 Hz, 1H), 6.63 (dd, J = 8.5, 2.5 Hz, 1H), 5.05 (s, 2H), 3.84 (s, 2H).

4.1.6. General procedure for the preparation of methane sulfonamides 17a–e

4.1.6.1. N-(2-(Benzyloxy)phenyl)methanesulfonamide (17a). A solution of pyridine (1 mL) and compound **16a** (1.0 g, 5 mmol) was stirred at room temperature. The solution of $MeSO_2Cl$ (1.6 mL, 20 mmol) in CH_2Cl_2 was added slowly to keep the reaction solution at a gentle reflux. After the addition was finished, the reaction mixture was stirred at room temperature for 1 h. Then the mixture was washed with saturated $NaHCO_3$, 1 M HCl, and brine, which was then dried with $MgSO_4$ and evaporated to give a crude product. This product was purified through recrystallization in ethanol to give a white crystal with a yield of 88%. Mp: 78–82 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.31 (m, 7H), 6.95–6.85 (m, 2H), 6.70 (s, 1H), 5.08 (s, 2H), 2.89 (s, 3H).

4.1.6.2. N-(2-(Benzyloxy)-4-chlorophenyl)methanesulfonamide (17b). Following the above procedure, the desired product was obtained as a white solid with a yield of 86%. Mp: 81–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.39 (m, 6H), 6.95 (s, 1H), 6.92 (d, J = 2.4 Hz, 1H), 6.75 (d, J = 2.4 Hz, 1H), 5.15 (s, 2H), 2.83 (s, 3H).

4.1.6.3. N-(2-(Benzyloxy)-5-chlorophenyl)methanesulfonamide (17c). Following the above procedure, the desired product was obtained as a white solid with a yield of 76%. Mp: 80–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.29 (m, 6H), 6.97 (s, 1H), 6.96 (d, J = 2.4 Hz, 1H), 6.70 (d, J = 2.4 Hz, 1H), 5.17 (s, 2H), 2.89 (s, 3H).

4.1.6.4. N-(2-(Benzyloxy)-5-methylphenyl)methanesulfonamide (17d). Following the above procedure, the desired product was obtained as a white solid with a yield of 77%. Mp: 78–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43–7.33 (m, 6H), 6.93–6.87 (m, 2H), 6.77 (s, 1H), 5.10 (s, 2H), 2.88 (s, 3H), 2.29 (s, 3H).

4.1.6.5. N-(2-(Benzyloxy)-5-nitrophenyl)methanesulfonamide (17e). Following the above procedure, the desired product was

obtained as a pale yellow solid with a yield of 46%. Mp 89–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, J = 2.5 Hz, 1H), 7.55–7.28 (m, 5H), 7.06 (dd, J = 8.8, 2.5 Hz, 1H), 6.91 (d, J = 8.8 Hz, 1H), 6.86 (s, 1H), 5.09 (s, 2H), 2.95 (s, 3H).

4.1.7. General procedure for the preparation of sulfonamides 20a–f

To a solution of the amine (1.0 mmol) in CH_2Cl_2 (10 mL) were added sulfonyl chloride (1.2 mmol) and saturated $NaHCO_3$ (10 mL). The resulting suspension was stirred until the amine was consumed through TLC analysis. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (20 mL) and brine, dried, and concentrated. Purification of the crude product using flash chromatography (EtOAc–hexanes) afforded the sulfonamide.

4.1.7.1. N-(2-(Benzyloxy)phenyl)-1-(3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methanesulfonamide (20a). Following the above procedure, the desired product was obtained as a white solid with a yield of 73%; mp: 130–133 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.26 (m, 5H), 7.25–7.11 (m, 5H), 7.05–6.86 (m, 3H), 5.11 (s, 2H), 4.92 (s, 1H), 4.57 (s, 2H), 3.85–3.74 (m, 4H), 2.81 (s, 3H), 1.94–1.83 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.71, 140.77, 137.19, 136.10, 134.69, 131.62, 129.36, 128.32, 128.23, 128.17, 128.16, 125.72, 125.32, 123.02, 122.45, 114.21, 75.14, 71.13, 67.62, 61.10, 50.19, 34.95; ESI-MS: m/z 480 $[M-H]^-$.

4.1.7.2. N-(2-(Benzyloxy)-4-chlorophenyl)-1-(3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methane-sulfonamide (20b). Following the above procedure, the desired product was obtained as a white solid with a yield of 71%; mp: 132–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.12 (m, 9H), 6.78–6.53 (m, 3H), 5.12 (s, 2H), 4.94 (s, 1H), 4.59 (s, 2H), 3.89–3.78 (m, 4H), 2.92 (s, 3H), 2.03–1.94 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.18, 144.57, 136.19, 135.49, 134.08, 129.58, 128.95, 128.90, 128.87, 128.08, 127.98, 127.78, 126.67, 125.45, 125.78, 113.55, 74.70, 70.91, 63.56, 55.44, 49.83, 35.24; ESI-MS: m/z 514 $[M-H]^-$.

4.1.7.3. N-(2-(Benzyloxy)-5-chlorophenyl)-1-(3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methane-sulfonamide (20c). Following the above procedure, the desired product was obtained as a white solid with a yield of 75%; mp: 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54–7.15 (m, 9H), 6.88–6.55 (m, 3H), 5.11 (s, 2H), 4.98 (s, 1H), 4.63 (s, 2H), 3.82–3.75 (m, 4H), 2.94 (s, 3H), 1.99–1.90 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.10, 140.54, 136.09, 135.10, 134.48, 130.62, 129.56, 128.72, 128.67, 128.16, 127.90, 127.66, 126.72, 125.75, 125.45, 113.40, 74.54, 70.43, 63.32, 55.00, 50.19, 34.98; ESI-MS: m/z 514 $[M-H]^-$.

4.1.7.4. N-(2-(Benzyloxy)-5-methylphenyl)-1-(3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methane-sulfonamide (20d). Following the above procedure, the desired product was obtained as a white solid with a yield of 74%; mp 123–125 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.27 (m, 8H), 6.85–6.77 (m, 2H), 6.70–6.63 (m, 2H), 5.11 (s, 2H), 4.84 (s, 1H), 4.51 (s, 2H), 3.84–3.76 (m, 4H), 2.95 (s, 3H), 2.21 (s, 3H), 2.06–1.95 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.91, 140.74, 136.89, 135.10, 134.62, 129.76, 129.36, 128.32, 128.17, 128.16, 128.15, 127.72, 126.63, 125.45, 125.14, 112.61, 75.54, 71.43, 63.42, 55.19, 52.19, 34.88, 21.21; ESI-MS: m/z 518 $[M+Na]^+$.

4.1.7.5. N-(2-(Benzyloxy)-5-nitrophenyl)-1-(3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methanesulfonamide (20e). Following the above procedure, the desired product was obtained as a white solid with a yield of 72%; mp 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64–7.43 (m, 9H), 7.08–6.83 (m, 3H), 5.12 (s, 2H), 4.99 (s, 1H), 4.64 (s, 2H), 3.90–3.88 (m, 4H), 2.98 (s, 3H), 2.01–1.95 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.81, 141.37, 140.74, 137.09, 136.10, 130.07, 129.62, 129.36, 128.12, 128.19, 128.17, 125.82, 124.47, 122.45, 119.82, 109.27, 75.64, 71.33, 67.32, 61.00, 52.22, 34.78; ESI-MS: m/z 527 $[M+H]^+$.

4.1.7.6. Ethyl 4-(benzyloxy)-3-((3-(4-methoxytetrahydro-2H-pyran-4-yl)phenyl)methylsulfonamido) benzoate (20f). Following the above procedure, the desired product was obtained as a white solid with a yield of 73%; mp: 120–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52–7.35 (m, 5H), 7.28–7.20 (m, 3H), 7.19 (s, 1H), 7.11 (d, J = 4.8 Hz, 1H), 6.98 (d, J = 4.8 Hz, 2H), 5.10 (s, 2H), 4.97 (s, 1H), 4.51 (s, 2H), 4.37–4.21 (q, J = 2.7 Hz, 2H), 3.90–3.69 (m, 4H), 2.87 (s, 3H), 1.96–1.84 (m, 4H) ,1.35 (t, J = 2.7 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.21, 150.43, 148.25, 143.73, 136.56, 134.54, 128.46, 127.97, 127.30, 125.88, 124.66, 124.12, 123.45, 122.59, 114.77, 112.79, 73.91, 70.38, 69.89, 62.70, 59.77, 48.80, 34.27, 13.33; HRMS (ESI): $[M+Na]^+$ ($C_{29}H_{33}NNaO_7S$) calcd 562.1875, found 562.1861.

4.1.8. General procedure for the preparation of compounds 21a–e, 22a–e, and 23a–d

K_2CO_3 (1 equiv) and substituted benzyl bromine (1.1 equiv) were sequentially added to a solution of phenols **19a–d**, anilines **16a–e,** or methanesulfonamides **17a–e** (1.0 equiv) in acetone/acetonitrile, and the mixture was stirred under reflux from 3 h to overnight. Then, the reaction mixture was cooled to room temperature and filtered. The filtrate was evaporated to give a crude product, which was purified using flash chromatography with hexane–EtOAc to give the desired final product.

4.1.8.1. 2-(Benzyloxy)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl) benzyl) aniline (22a). Following the above procedure, the desired product was obtained as a brown oil with a yield of 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.26 (m, 9H), 6.88–6.81 (m, 2H), 6.70–6.56 (m, 2H), 5.10 (s, 2H), 4.73 (s, 1H), 4.38 (s, 2H), 3.89–3.76 (m, 4H), 2.92 (s, 3H), 2.05–1.91 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.83, 144.56, 139.52, 136.72, 128.81, 128.62, 128.16, 127.59, 127.54, 126.85, 126.31, 124.91, 124.88, 115.72, 111.92, 110.36, 74.95, 70.87, 63.65, 49.77, 47.86, 35.31; ESI-MS: m/z 402 $[M-H]^-$.

4.1.8.2. 2-(Benzyloxy)-4-chloro-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl) benzyl) aniline (22b). Following the above procedure, the desired product was obtained as red oil with a yield of 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.24 (m, 9H), 6.78 (s, 1H), 6.68 (d, J = 4.9 Hz, 1H), 6.63 (d, J = 4.9 Hz, 1H), 5.08 (s, 2H), 4.68 (s, 1H), 4.43 (s, 2H), 3.85–3.76 (m, 4H), 2.93 (s, 3H), 2.05–1.95 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 154.21, 144.78, 144.23, 136.16, 135.57, 129.16, 129.34, 128.32, 127.97, 127.47, 127.61, 126.56, 125.87, 119.09, 117.23, 116.67, 74.35, 73.79, 70.56, 63.04, 48.97, 34.63; HRMS (ESI): $[M+Na]^+$ ($C_{26}H_{28}ClNNaO_3$) calcd 460.1655, found 460.1641.

4.1.8.3. 2-(Benzyloxy)-5-chloro-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)aniline (22c). Following the above procedure, the desired product was obtained as red oil with a yield of 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.22 (m, 9H), 6.71 (s, 1H), 6.58–6.53 (m, 2H), 5.10 (s, 2H), 4.74 (s, 1H), 4.39 (s, 2H), 3.88–3.78 (m, 4H), 2.91 (s, 3H), 2.07–1.92 (m, 4H); ^{13}C NMR (101 MHz, DMSO) δ 154.22, 144.71, 144.25, 136.12, 135.47, 129.15, 129.34, 128.31, 127.95, 127.44, 127.60, 126.57, 125.88, 119.11, 117.22, 116.61, 74.38, 73.78, 70.55, 63.04, 48.98, 34.62; ESI-MS: m/z 460 $[M+Na]^+$.

4.1.8.4. 2-(Benzyloxy)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)-5-methylaniline (22d). Following the above proce-

dure, the desired product was obtained as brown oil with a yield of 55%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46–7.26 (m, 8H), 6.84–6.80 (m, 2H), 6.75–6.66 (m, 2H), 5.10 (s, 2H), 4.75 (s, 1H), 4.41 (s, 2H), 3.87–3.75 (m, 4H), 2.92 (s, 3H), 2.17 (s, 3H), 2.06–1.91 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.84, 144.55, 139.42, 136.72, 128.81, 128.62, 128.16, 127.59, 127.54, 126.85, 126.31, 124.91, 124.88, 115.72, 111.92, 110.36, 74.95, 70.87, 63.65, 49.77, 47.86, 35.31, 20.15; ESI-MS: *m/z* 418 $[M+H]^+$.

4.1.8.5. 2-(Benzyloxy)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)-5-nitroaniline (22e). Following the above procedure, the desired product was obtained as a yellow solid with a yield of 56%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44–7.22 (m, 9H), 6.71 (s, 1H), 6.58 (d, J = 5.2 Hz, 1H), 6.53 (d, J = 5.2 Hz, 1H), 5.08 (s, 2H), 4.78 (s, 1H), 4.40 (s, 2H), 3.89–3.78 (m, 4H), 2.91 (s, 3H), 2.06–1.92 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.11, 141.77, 140.71, 137.19, 136.11, 130.11, 129.67, 129.99, 128.12, 128.19, 128.24, 125.67, 124.57, 122.46, 119.88, 109.29, 75.64, 71.33, 67.52, 61.09, 52.48, 34.77; ESI-MS: *m/z* 461 $[M+Na]^+$.

4.1.8.6. N-(2-(Benzyloxy)phenyl)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)methanesulfonamide (21a). Following the above procedure, the desired product was obtained as a white solid with a yield of 77%. Mp: 98–103 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.36 (m, 5H), 7.29–7.21 (m, 5H), 7.05–6.86 (m, 3H), 5.10 (s, 2H), 4.77 (s, 2H), 3.88–3.74 (m, 4H), 2.87 (s, 3H), 2.82 (s, 3H), 1.96–1.84 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.16, 144.56, 136.24, 135.52, 134.05, 129.57, 128.94, 128.74, 128.70, 127.96, 127.77, 127.63, 126.61, 125.64, 125.52, 113.54, 74.73, 70.98, 63.59, 53.57, 49.69, 39.96, 35.20; ESI-MS: *m/z* 482 $[M+H]^+$.

4.1.8.7. N-(2-(Benzyloxy)-4-chlorophenyl)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)methanesulfonamide (21b). Following the above procedure, the desired product was obtained as a white solid with a yield of 64%. Mp 90–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50–7.36 (m, 5H), 7.36–7.09 (m, 5H), 6.98 (d, J = 5.8 Hz, 1H), 6.92 (d, J = 5.8 Hz, 1H), 5.10 (s, 2H), 4.75 (s, 2H), 3.99–3.71 (m, 4H), 2.88 (s, 3H), 2.83 (s, 3H), 1.96–1.85 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.13, 144.14, 136.88, 136.22, 134.73, 130.55, 130.12, 128.83, 128.48, 128.46, 127.98, 127.69, 126.61, 126.19, 125.23, 112.46, 74.75, 70.53, 63.61, 53.63, 49.61, 39.78, 35.44; HRMS (ESI): $[M+H]^+$ ($C_{27}H_{31}ClNO_5S$) calcd 516.1533, found 516.1511.

4.1.8.8. N-(2-(Benzyloxy)-5-chlorophenyl)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)methanesulfonamide (21c). Following the above procedure, the desired product was obtained as a white solid with a yield of 62%. Mp: 94–97 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.34 (m, 5H), 7.35–7.09 (m, 5H), 6.88 (d, J = 5.6 Hz, 1H), 6.72 (d, J = 5.6 Hz, 1H), 5.10 (s, 2H), 4.75 (s, 2H), 3.96–3.70 (m, 4H), 2.88 (s, 3H), 2.85 (s, 3H), 1.98–1.86 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.11, 144.15, 136.89, 136.22, 134.73, 130.54, 130.12, 128.81, 128.49, 128.46, 127.96, 127.69, 126.61, 126.19, 125.20, 112.45, 74.77, 70.53, 63.61, 53.63, 49.61, 39.77, 35.45; ESI-MS: *m/z* 516 $[M+H]^+$.

4.1.8.9. N-(2-(Benzyloxy)-5-methylphenyl)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)methanesulfonamide (21d). Following the above procedure, the desired product was obtained as a white solid with a yield of 58%. Mp: 88–91 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.49–7.35 (m, 5H), 7.29–7.20 (m, 3H), 7.15 (s, 1H), 7.01 (d, J = 4.8 Hz, 1H), 6.86 (d, J = 4.8 Hz, 2H), 5.10 (s, 2H), 4.78 (s, 2H), 3.90–3.69 (m, 4H), 2.87 (s, 3H), 2.82 (s, 3H), 2.12 (s, 3H), 1.96–1.84 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.08, 144.19,136.90, 136.22, 134.70, 130.54, 130.13, 128.81, 128.48, 128.46, 127.95, 127.68, 126.61, 126.18, 125.20, 112.40, 74.73, 70.52, 63.60, 53.62, 49.59, 39.69, 35.20, 20.11; ESI-MS: *m/z* 496 $[M+H]^+$.

4.1.8.10. N-(2-(Benzyloxy)-5-nitrophenyl)-N-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyl)methanesulfonamide (21e). Following the above procedure, the desired product was obtained as a pale yellow solid with a yield of 33%. Mp: 102–105 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45–7.36 (m, 5H), 7.29–7.21 (m, 5H), 7.15 (s, 1H), 7.02 (d, J = 5.1 Hz, 1H), 6.86 (d, J = 5.1 Hz, 1H), 5.08 (s, 2H), 4.98 (s,2H), 3.98–3.72 (m, 4H), 2.86 (s, 3H), 2.81 (s, 3H), 1.98–1.85 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.13, 144.54, 136.23, 135.50, 134.08, 129.61, 128.97, 128.77, 128.73, 127.98, 127.80, 127.53, 126.61, 125.62, 125.53, 113.52, 74.73, 70.96, 63.59, 53.54, 49.73, 39.95, 35.18; ESI-MS: *m/z* 527 $[M+H]^+$.

4.1.8.11. 4-(3-((2-(Benzyloxy)phenoxy)methyl)phenyl)-4-methoxytetrahydro-2H-pyran (23a). Following the above procedure, the desired product was obtained as a white oil with a yield of 78%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53–7.22 (m, 10H), 7.05–6.79 (m, 3H), 5.16 (s, 2H), 5.11 (s, 2H), 3.94–3.75 (m, 4H), 2.97 (s, 3H), 2.04–1.92 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 148.23, 147.98, 143.64, 136.59, 127.58, 127.44, 126.75, 126.28, 125.48, 124.41, 124.25, 124.01, 120.82, 120.61, 120.03, 114.54, 73.91, 70.49, 70.25, 62.64, 48.75, 34.28; ESI-MS: *m/z* 427 $[M+Na]^+$.

4.1.8.12. 4-(3-((2-(Benzyloxy)-4-chlorophenoxy)methyl)phenyl)-4-methoxytetrahydro-2H-pyran (23b). Following the above procedure, the desired product was obtained as a colorless solid with a yield of 81%. Mp: 55–57 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55–7.33 (m, 12H), 5.22 (s, 2H), 4.95 (s, 2H), 3.70–3.66 (m, 4H), 3.36 (s, 3H), 1.94–1.86 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.23, 144.39, 144.10, 136.08, 135.46, 128.46, 128.44, 128.14, 127.87, 127.55, 127.51, 126.29, 125.74, 118.08, 116.99, 116.49, 74.35, 73.83, 70.69, 62.79, 49.17, 34.73; HRMS (ESI): $[M+Na]^+$ ($C_{26}H_{27}ClNaO_4$) calcd 461.1496, found 461.1481.

4.1.8.13. 4-(3-((2-(Benzyloxy)-4-bromophenoxy)methyl)phenyl)-4- methoxytetrahydro-2H-pyran (23c). Following the above procedure, the desired product was obtained as a white solid with a yield of 88%. Mp: 58–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58–7.30 (m, 12H), 5.22 (s, 2H), 4.94 (s, 2H), 3.72–3.68 (m, 4H), 3.33 (s, 3H), 1.97–1.88 (m, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.35, 144.29, 144.14, 136.09, 135.56, 128.56, 128.47, 128.11, 127.81, 127.56, 127.53, 126.49, 125.78, 118.11, 117.08, 116.47, 74.37, 73.79, 70.69, 62.78, 49.20, 34.75; ESI-MS: *m/z* 505 $[M+Na]^+$.

4.1.8.14. Ethyl 3-(benzyloxy)-4-(3-(4-methoxytetrahydro-2H-pyran-4-yl)benzyloxy)benzoate (23d). Following the above procedure, the desired product was obtained as a colorless oil with a yield of 43%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69–7.62 (m, 2H), 7.56–7.30 (m, 9H), 6.95 (d, J = 4.9 Hz,1H), 5.2 (s, 4H), 4.39–4.28 (q, J = 2.8 Hz ,2H), 3.94–3.73 (m, 4H), 2.92 (s, 3H), 2.11–1.83 (m, 4H), 1.37 (t, J = 2.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.20, 151.93, 147.23, 143.72, 136.10, 135.54, 127.64, 127.57, 127.00, 125.48, 124.56, 124.05, 123.04, 122.50, 114.72, 112.12, 73.90, 70.34, 69.84, 62.64, 59.75, 48.76, 34.28, 13.36; HRMS (ESI): $[M+Na]^+$ ($C_{29}H_{32}NaO_6$) calcd 499.2097, found 499.2071.

4.2. Cyclooxygenase-1/2 inhibition assay

All compounds were tested for their activity to inhibit COX-1 and COX-2 using a COX (ovine) inhibitor screening kit (Catalog No. 560101, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. The COX (ovine) Inhibitor Screening Assay directly measures $PGF_{2\alpha}$ through $SnCl_2$ reduction

of COX-derived PGH_2 produced in COX reaction. The prostanoid product was measured with enzyme immunoassay (EIA) using a broadly specific antiserum that binds to all major PG compounds. COX reagent preparation was carefully performed according to instructions before COX reaction. Reaction buffer solution (960 μL, 0.1 M Tris–HCl, pH 8.0, containing 5 mM EDTA and 2 mM phenol) with either COX-1 or COX-2 (10 μL) enzyme in the presence of heme (10 μL) was added 10 μL of various concentrations of testing drug solutions (0.1, 1, 10, 50 μM) in a final volume of 1 mL. This solution was incubated for a period of 2 min at 37 °C, and then 10 μL of AA (100 μM) was added. The COX reaction was stopped through the addition of 50 μL of 1 M HCl after 2 min. $PGF_{2\alpha}$, which was produced from PGH_2 through reduction with stannous chloride, was measured with enzyme immunoassay. After the addition of EIA reagent, the plate was covered with plastic film and incubated for 18 h at room temperature on an orbital shaker. The plate was then washed to remove any unbound reagents, and Ellman's Reagent was added to the well. The product of this enzymatic reaction had a yellow color. The COX activity was determined by measuring the absorbance at a wavelength of 410 nm. The percentage inhibition was calculated through comparison of enzymatic activity with and without inhibitor. The IC_{50} value was determined as the concentration of the testing compound causing 50% inhibition of enzymatic activity, and the average of two measured data was reported.

4.3. 5-Lipoxygenase inhibition assay

The IC_{50} values of all compounds was determined using potato 5-LOX (Catalog No. 60401, Cayman Chemical, Ann Arbor, MI, USA) and an enzyme immuno assay (EIA) kit (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) according to manufacturer's instructions. The lipoxygenase inhibitor screening assay detects and measures the hydroperoxides produced in the lipoxygenation reaction. Pre-assay preparation was carefully performed according to the instructions. To a 90 μL solution of 5-LOX enzyme in 0.1 M Tris–HCl buffer, pH 7.4, 10 μL of various concentrations of testing drug solutions (0.1, 1, 10 and 50 μM in a final volume of 210 μL) were added, and the lipoxygenase reaction was initiated by the addition of 10 μL (100 μM) of linoleic acid (LA). After putting the 96-well plate on a shaker for 5 min, 100 μL of chromogen was added, and the plate was on the shaker for another 5 min. The lipoxygenase activity was determined by measuring absorbance at a wavelength of 490 nm. The percentage inhibition was calculated through the comparison of enzymatic activity with and without inhibitor. The IC_{50} value was the concentration of the testing compound causing 50% inhibition of enzymatic activity, and the average of two measured data was reported.

4.4. Molecular modeling

Since the 3D structures of human COX-2 and 5-LOX were not available in the Protein Data Bank, the model structure of human 5-LOX was obtained through homology modeling using rabbit 15-LOX (PDB entry LOX1)[35] and the docking study of COX-2 used murine COX-2 (PDB entry 6COX)[36] instead. We repeated the homology modeling with Discovery Studio (DS 2.1 Accelrys, San Diego, CA)[37] to get a human 5-LOX model. With the highest sequence similarity, rabbit 15-LOX was used as template for the homology model of human 5-LOX. Five models were done with slightly difference and model with the best verify score was chosen as the candidate. Then the candidate model was energy-minimized using the CVFF force field to make the final model.

Docking studies were performed using the program AUTODOCK VINA 1.0.3. The crystallographic structures of murine COX-2 (PDB entry 6COX) and rabbit 15-LOX (PDB entry LOX1) complexed with their ligands were used to check the AUTODOCK program for the binding modes of compounds in the binding sites of the enzymes.[38] For this purpose, the complexes were manipulated to extract the inhibitors, which were in turn computationally re-docked by using AUTODOCK within the binding sites. The grid was narrowed to an area slightly larger than the enzyme binding sites ($15 \times 18 \times 18$ Å) and was centered on the mass center of the crystallographic inhibitors for both COX-2 and 15-LOX. Docking was carried out with a maximum output of 25 structures. By using these settings, the program successfully reproduced the X-ray coordinates of the inhibitor's binding conformations. The same set of parameters was also used to perform docking simulations of all the remaining inhibitors. The most favorable conformations of inhibitors were chosen based on the combination of affinity energy and structures given by the program. The candidate models were energy-minimized with Discovery Studio to get the final models.

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