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Discovery of Dual Target Inhibitors against Cyclooxygenases and Leukotriene A_4 Hydrolyase

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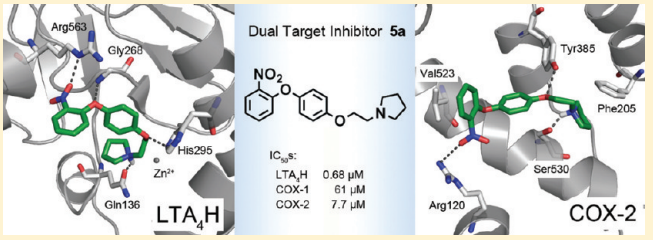
Supporting Information

ABSTRACT: Dual target inhibitors against COX-2 and LTA_4H were designed by adding functional groups from a marketed COX-2 inhibitor, Nimesulide, to an existing LTA_4H inhibitor 1-(2-(4-phenoxyphenoxy) ethyl) pyrrolidine. A series of phenoxyphenyl pyrrolidine compounds were synthesized and tested for their inhibition activities using enzyme assays and human whole blood assay. Introduction of small electron withdrawing groups like NO_2 and CF_3 in the ortho-position of the terminal phenyl ring was found to change the original single target LTA_4H inhibitor to dual target LTA_4H and COX-2 inhibitors. Compound **5a** and **5m** showed dual LTA_4H and COX-2 inhibition activities in the enzyme assays and the HWB assay with IC_{50} values in the micromolar to submicromolar range. As their activities in HWB assay were comparable to the two starting single target inhibitors, the two compounds are promising for further studies. The strategy used in the current study may be generally applicable to other dual target drug designs.



INTRODUCTION

Nonsteriodal anti-inflammatory drugs (NSAIDs) have been used for many years in treating acute and chronic inflammation. It is believed that the main mechanism of action of NSAIDs is blocking the formation of prostaglandins by inhibiting the cyclooxygenase (COX) enzymes. As traditional NSAIDs cause side effects such as gastrointestinal toxicity and mild bleeding by inhibition of COX-1,[1] selective inhibition of COX-2 was found to be a safer way to gastrointestinal toxicity and mild bleeding.[2] A large number of selective COX-2 inhibitors have been developed and approved for clinical use, such as Nimesulide (Figure 1),[3] 5-(4-fluorophenyl)-1-[4-(methylsulfonyl)phenyl]-3-(trifluoromethyl)-1*H*-pyrazole (SC-58125),[4] and Rofecoxib (Figure 1),[5] which represent significant advances in treating inflammatory diseases. However, selective COX-2 inhibitors have been subsequently reported to have cardiovascular side effects in clinical trials.[6] Rofecoxib, Merck's selective COX-2 inhibitor, was voluntarily withdrawn from the market in 2004,[7] and other marketed COX-2 inhibitors, like Celecoxib (Figure 1),[8] were reappraised.[9] The reasons for the toxicity of these drugs have not been fully revealed. One hypothesis suggests that these inhibitors induce an imbalance between prostacyclin (PGI_2), an antiaggregant and vasodilator mainly synthesized via the action of COX-2, and thromboxane A_2 (TXA_2), a molecule with strong pro-aggregant and vasoconstrictor activity mainly produced through the COX-1 pathway.[10]

The activity of drug molecules in the disease network can be complex, and problems occur when a single-target-based molecule is involved in multiple targets. The failure of unselective and selective COX inhibitors provides an example of the limitations in using single-target-based drugs in treating a complex disease. One possibility to overcome the limitations of single-target-based drugs is to design and/or find multitarget drugs. Complex diseases like cancer, AIDs, and atherosclerosis can benefit from complex treatment that modulates multiple targets.[11] In order to understand the possible side effects of NSAIDs, we have built mathematical models for the inflammation-related arachidonic acid metabolic network in human polymorphous neutrophil,[12]

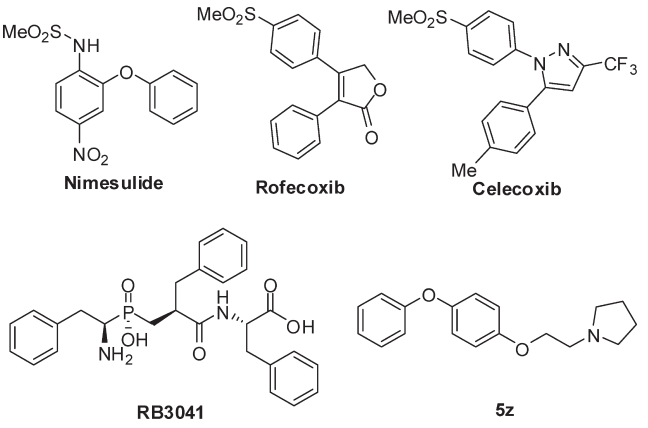


Figure 1. Chemical structures of COX-2 inhibitors Nimesulide, Rofecoxib, Celecoxib, and LTA_4H inhibitor RB3041, 1-(2-(4-phenoxyphenoxy)ethyl)pyrrolidine (**5z**).

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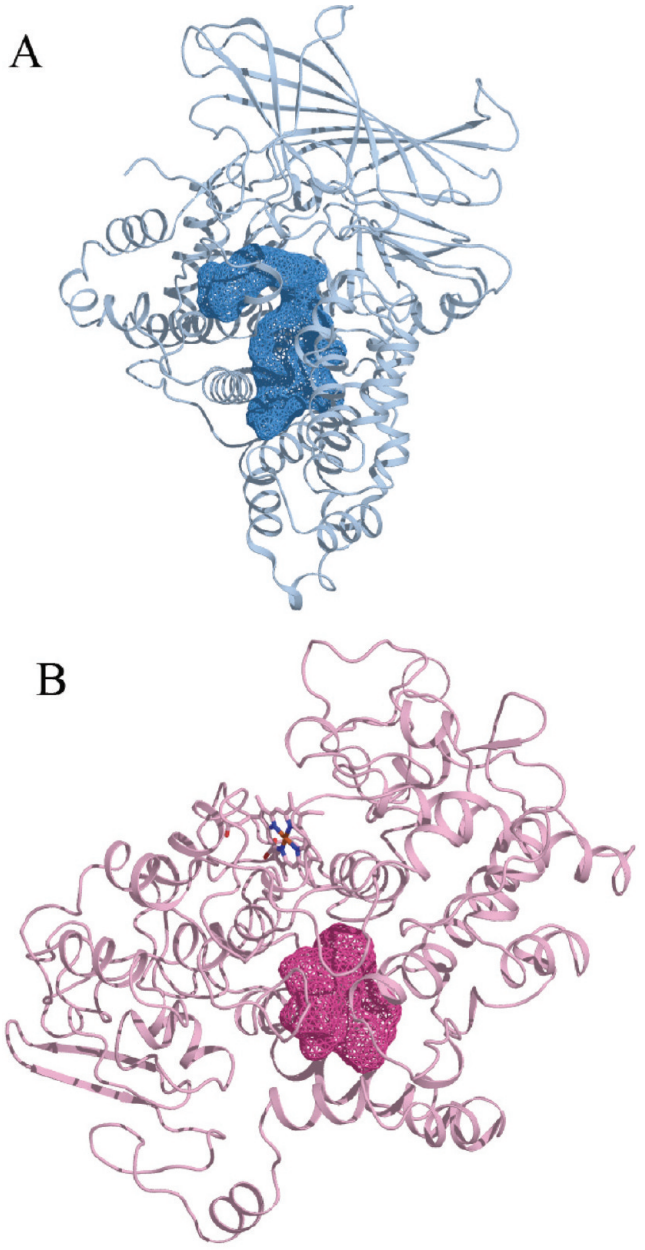


Figure 2. Substrate binding pockets of LTA_4H (A) and COX-2 (B).

and in a mixture of three cell types simulating a stretch of human blood vessel.[13] The two major inflammatory mediators, prostaglandins and leukotrienes, are produced through the COX pathway and the 5-lipoxygenase (5-LOX) pathway, respectively. Our simulation verified that there are five key enzymes for inhibitor design to reduce the production of inflammatory mediators, which include COX (COX-2/COX-1), 5-LOX, prostaglandin E synthase (PGES), LTA_4H, and phospholipase A_2 (PLA_2). We also searched for multiple target optimum intervention solutions that can simultaneously reduce the production of both inflammatory mediators and keep a good balance of PGI_2 and TXA_2 (minimizing possible side effects). All of the solutions include at least inhibition of two targets at the same time, for example, the inhibition of COX-2/COX-1 and 5-LOX, the inhibition of COX-2/COX-1 and LTA_4H, and the inhibition of PLA_2, LTA_4H, and COX-2/COX-1.

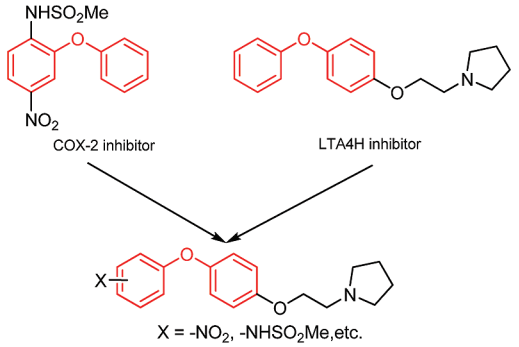


Figure 3. Design strategy for COX-2/LTA_4H dual inhibitors.

Among the multitarget control solutions, inhibitors that can act on both COX-2 and 5-LOX have been explored for some time and were shown to possess a wide range of anti-inflammatory activity.[14] Recent research has focused on the development of COX-2/5-LOX dual inhibitors with a superior safety profile. One of the most successful strategies is to design a hybrid molecule, a COX-2 inhibitor analogue possessing a 5-LOX pharmacophore, or a compound containing a COX-2 fragment and a 5-LOX fragment, such as some celecoxib analogues[15] and Tepoxalin.[16]

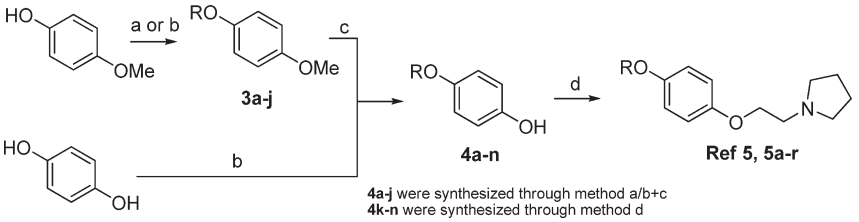
In the current study, we tried to design inhibitors that can inhibit both COX-2/COX-1 and LTA_4H at the same time. As COX-1 and COX-2 have quite similar structures, most COX inhibitors act on both of them. From our previous simulations and literature, around 10× selectivity of COX-2 to COX-1 is good for maintaining the balance between PGI_2 and TXA_2.[13]

To our knowledge, no COX-2/LTA_4H dual target inhibitors have been reported. A possible difficulty in designing dual target inhibitors for these enzymes is the large difference between their substrate binding pockets. A cavity search showed that the LTA_4H enzyme contains a long narrow substrate binding channel (Figure 2, A), so most inhibitors have a long chain without ramification; while the pocket of COX-2 enzyme is much wider (Figure 2, B), and typical COX-2 inhibitors are in a V-shape. However, some compounds with a branched structure can also bind to LTA_4H at the entrance of the substrate binding pocket, such as RB3041 complexed to LTA_4H in the crystal structure (PDB code 2R59, Figure 1).[17] This implies that it is possible to design compounds that bind to both COX-2 and LTA_4H.

Multitarget inhibitor design has attracted much attention recently.[18−21] Both target structure-based and ligand-based design strategies have been tested. For target structure-based design, sequential docking and multiple cross docking are the straightforward strategies, which are all computationally demanding. We have developed a docking combined with a common pharmacophore matching strategy for multitarget inhibitor design and successfully used it to discover dual functional inhibitors for $sPLA_2$ and LTA_4H.[22] Compared to target structure-based discovery, ligand-based multitarget inhibitor design learns from the chemical structures of known inhibitors and tries to combine them.[23,24] The chances of deriving compounds that are active for one of the targets are high, while it is difficult to retain all the desirable activities at the same time.

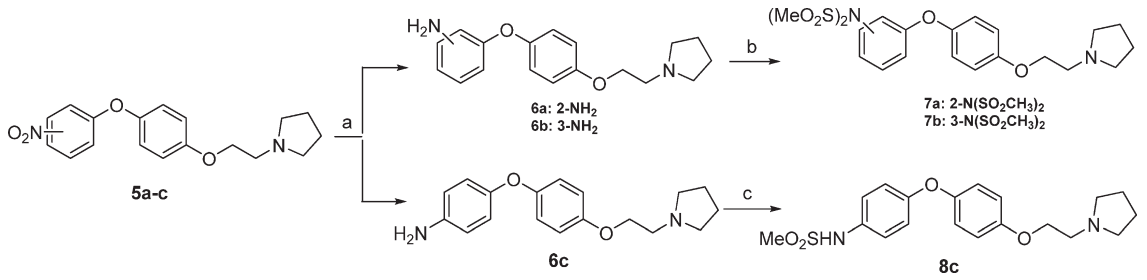
Our rational design of COX-2/LTA_4H dual inhibitors was mainly ligand-based, while molecular docking of the designed compounds to both enzymes was also performed to compare their interaction affinities. We started from the fusion

Scheme 1[a]



[a] Reagents: (a) RX, KOH, 170 °C; (b) RX, K_2CO_3 or KOH, TDA, CuI or CuBr, Anisole or NMP, N_2, 100−170 °C; (c) BBr_3, CH_2Cl_2, 0 °C ∼rt,H_2O; (d) 1-(2-chloroethyl)pyrrolidine, KOH, DMF, 100 °C.

Scheme 2[a]



[a] Reagents: (a) 85% NH_2NH_2, IPA, Pd/C, reflux; (b) $MeSO_2Cl$, TEA, CH_2Cl_2, rt; (c) $MeSO_2Cl$, H_2O, rt.

of 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine (Figure 1, **5z**), a LTA_4H inhibitor reported by Pennings,[25] and Nimesulide (Figure 1),[3] a marketed NSAID targeting COX-2. Though these two compounds are different in shape, they share the same scaffold phenoxyphenyl. We used the slim-shaped LTA_4H inhibitor as the parent compound and added functional groups in Nimesulide to it (Figure 3). Other substituted groups in *o*-, *m*-, and *p*-position of the first phenyl were also considered.

A series of 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine derivatives were synthesized, and their inhibition activities to purified enzymes and in human whole blood assay were tested. Molecular docking of these compounds to COX-2, COX-1, and LTA_4H was done to analyze their structure and activity relationship.

RESULTS

Chemistry. The 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine derivatives were synthesized by the routes shown in Schemes 1′−3.

Substituted 1-phenoxyethylpyrrolidine compounds (**5a**−**r**) were prepared according to Scheme 1 starting from commercially available aryl halides or substituted phenoxyphenol. 1-(4-Methoxyphenoxy)-2-nitrobenzene (**3a**) and 1-(4-methoxyphenoxy)-4-nitrobenzene (**3c**) were obtained through direct heating to couple the chloronitrobenzene with 4-methoxyphenol, and other compounds need to be coupled through Goldberg reaction using CuBr/TDA. Demethylation of **3** yielded a series of substituted phenoxyphenol key intermediates, including 4-(2-nitrophenoxy) phenol (**4a**) and similar compounds (**4b**−**j**) by boron tribromide, and 4-(pyrazin-2-yloxy) phenol (**4k**) and similar compounds **4l**−**n** by Goldberg reaction through monocoupling commercial aryl halide with hydroquinone. Then, these substituted phenoxyphenols were alkylated to produce **5a**−**n**. **5z** and other **5** compounds were prepared directly from corresponding **4** compounds in one step.

Nitro groups in **5a**−**c** could be further reduced to amine groups to form (4-(2-(pyrrolidin-1-yl) ethoxy)phenoxy) aniline (**6a**−**c**). *N*-(Methylsulfonyl)-*N*-(2-(4-(2-(pyrrolidin-1-yl) ethoxy)-phenoxy)phenyl) methanesulfonamide (**7a**) and *N*-(methylsulfonyl)-*N*-(3-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)phenyl) methanesulfonamide (**7b**) were obtained by dimethylsulfonylation of **6a** or **6b**, and *N*-(4-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)-phenyl) methanesulfonamide (**8c**) was provided by monomethylsulfonylation of **6c** (Scheme 2).

N-Methyl-*N*-(2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)phenyl) methanesulfonamide (**13a**) was achieved starting from **3a** in five steps (Scheme 3): **3a** returned its 2-nitro group to 2-amine group to form 2-(4-methoxyphenoxy) aniline (**9a**), then methylsulfonylation of 2-amine group **9a** produced *N*-(2-(4-methoxyphenoxy)phenyl) methanesulfonamide (**10a**), methylation of 2-methanesulfonamide group **10a** produced *N*-(2-(4-methoxyphenoxy)phenyl)-*N*-methylmethanesulfonamide (**11a**), demethylation of **11a** produced *N*-(2-(4-hydroxyphenoxy)-phenyl)-*N*-methylmethanesulfonamide (**12a**), and alkylation of **12a** reached **13a**.

***In Vitro* Enzyme Inhibition Study.** The 25 synthesized target compounds were tested for their inhibition activity against purified COX-1, COX-2, and LTA_4H (shown in Table 1).

The LTA_4H hydrolase activity was measured using an ELISA assay to quantify the amount of LTB_4 generated. The known LTA_4H inhibitor, 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine (**5z**) was used as a positive control.

COX-2 is one of the two distinct isoforms of COX, which can convert arachidonic acid to a hydroperoxy endoperoxide, PGG_2, and subsequently reduce it to corresponding alcohol, PGH_2. The enzyme activity of the purified COX-2 was measured by a chromogenic assay based on monitoring the absorption of oxidized *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2 at 610 nm. A known COX-2 inhibitor, Nimesulide, was used as a positive control. Suitable

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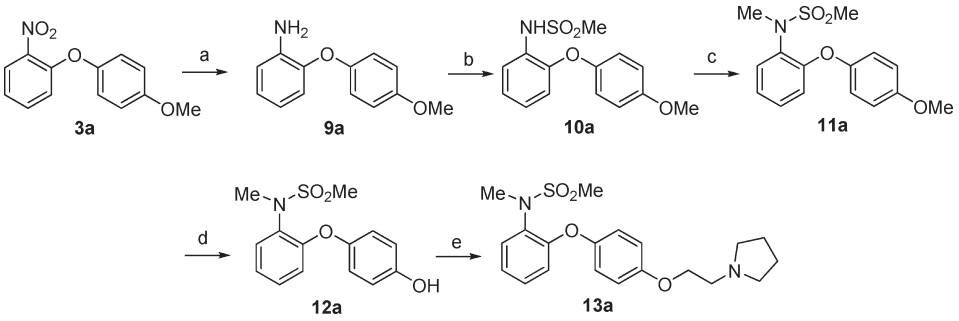
Scheme 3[a]



[a] Reagents: (a) Sn, HCl, EtOH, reflux; (b) $MeSO_2Cl$, Pyridine, 90 °C; (c) $(MeO)_2SO_2$, K_2CO_3, acetone, reflux; (d) BBr_3, CH_2Cl_2, 0 °C; (e) 1-(2-chloroethyl)pyrrolidine, KOH, K_2CO_3, DMF, 100 °C.

absorption could be observed by adding surfactant Genapol X-100.[26] The IC_{50} value of Nimesulide derived under this condition was 23 nM, which was close to the reported value of 30 nM.[27]

Human Whole Blood Assay Study. We tested the inhibition activity of the title compounds using human whole blood (HWB) assay (shown in Table 1).

In order to monitor the production of both PGE_2 and LTB_4 at the same time, we developed an assay that can simultaneously stimulate both the COX and 5-LOX pathways.

E. coli lipopolysaccharide (LPS) was used to induce the COX-2/PGES pathway in human whole blood, and the production of PGE_2 was monitored, while the 5-LOX/LTA_4H pathway was stimulated using calcium ionophore A23187 and the production of LTB_4 was measured. Heparinized HWB was first treated with LPS for 23.5 h, then ionophore A23187 was added to the sample for 0.5 h stimulation. As a control, we also tested whether LTB_4 can be produced when stimulating with LPS and whether PGE_2 can be produced with A23187, which turned out to be negligible compared to their respective main products.

DISCUSSION

***In Vitro* Enzyme Inhibition Study.** For the first step, we tested whether the title compounds could retain the LTA_4H inhibition activity. Inhibition testing against LTA_4H hydrolase activity indicated that most compounds maintained the LTA_4H inhibition activity. IC_{50} values of 14 compounds were less than 1 μM and that of 3 compounds were in the nanomolar range. This encouraged us to do further study. From Table 1, we can see that the three most potent inhibitors were 4-halogenphenoxy-substituted compounds (**5p**, **5q**, and **5r**), with IC_{50} values between 0.04 and 0.11 μM. In two series of compounds **5a**−**c** (nitro-substituted) and **6a**−**c** (amino-substituted), which only differed in the position of substituted group in the terminal aryl ring; inhibition activities of *p*-substituted compounds (**5c** and **6c**) were stronger than the others; while for the trifluoromethyl-substituted series **5m**−**o**, *o*-substituted compound **5m** had the strongest inhibition effect. When the aryl group was pyridine (**5e** and **5f**), the compounds also had strong inhibition activity. However, compounds with large substituted groups in the terminal aryl ring had weak or no inhibition activity, except the *p*-methanesulfonamide substituted compound **8c** with moderate inhibition activity.

We then tested the COX-2 and COX-1 inhibition activity of the title compounds (Table 1). Seven compounds, with a NO_2 (**5a**), CF_3 (**5m**), NMe (SO_2Me) as the *o*-substituent, and NO_2 (**5c**), NH_2 (**6c**) as the *p*-substituent, pyridin-2-yl (**5e**), or pyrazin-2-yl (**5k**) in the first phenyl ring, exhibited moderate COX-2 inhibitory activities. Compound **5a** having an *o*-NO_2 showed selective COX-2 inhibitory activity (COX-2, IC_{50} = 7.7 μM; COX-1, IC_{50} = 61.0 μM). In contrast, compound **6c** having *p*-NH_2 tends to be a selective COX-1 inhibitor (COX-2, IC_{50} = 36.3 μM; COX-1, IC_{50} = 1.9 μM).

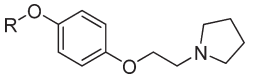
As the purpose of the current study was to design multitarget inhibitors of COX-1, COX-2, and LTA_4H, we compared the inhibition activities of the title compounds against COX-1, COX-2, and LTA_4H. Three compounds (**5a**, **5c**, and **6c**) showed significant inhibition activity toward the three enzymes. In particular, **5a** inhibits LTA_4H with an IC_{50} of 0.7 μM and inhibits COX-1 and COX-2 with IC_{50} of 61 μM and 7.7 μM, respectively.

We estimated possible side effects of **5a** using the mathematical model for the arachidonic acid metabolic network we previously developed for the human blood vessel.[13] Compound **5a** was introduced into the network as inhibitor for LTA_4H, COX-1, and COX-2 with its IC_{50} values measured in the enzyme assays. Concentration of **5a** used could reduce the PGE_2 and LTB_4 formation to less than 10% compared to no inhibition. The ratio of PGI_2/TXA_2 was used as an index of side effects, which was calculated to be 0.7820 here, quite close to the normal value of 0.6817. Thus, **5a** has good target selectivity and might maintain the balance between PGI_2 and TXA_2 *in vivo*.

Human Whole Blood Assay Study. From the HWB assay, compound **5a** showed moderate inhibition to both PGE_2 and LTB_4 with IC_{50} values of 8.4 μM and 6.9 μM, respectively, indicating that the introduction of *o*-NO_2 can successfully make the compound inhibit the formation of PGE_2, though its inhibition activity of LTB_4 production was also slightly reduced. The introduction of an *o*-CF_3 (**5m**) led to a notable PGE_2 formation inhibition, while keeping the inhibition activity to the biosynthesis of LTB_4. In fact, IC_{50} values of **5m**, 5.0 μM for PGE_2 and 0.73 μM for LTB_4 formation, were comparable to those of the two reference molecules. Though the introduction of a *p*-Br moiety (**5r**) also led to a notable PGE_2 formation inhibition, its functions might be more complex, as we did not detect any notable COX-1 and COX-2 inhibition in our enzyme assay; it is possible that inhibition of upstream enzymes like PLA_2, or downstream enzyme PGES, may be involved.

This human whole blood assay can provide inhibition activity data under more realistic conditions. However, the inhibition activities to COX-1 cannot be assayed at the same time, since the measurement

Table 1. COX-1, COX-2, and LTA_4H Inhibition Activities of the Title Compounds



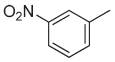
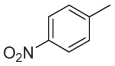
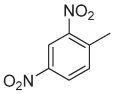
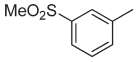
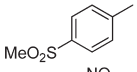
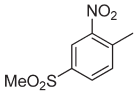
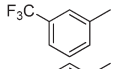
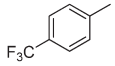
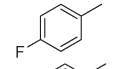
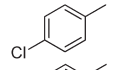
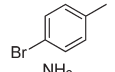
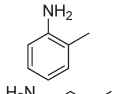
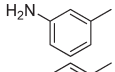
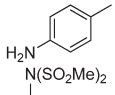
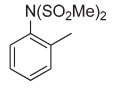
compd	R	IC_{50} (μM)				
		COX-1	COX-2	LTA_4H	PGE_2 (HWB)	LTB_4 (HWB)
	Nimesulide [a]	32.2±1.2	0.023±0.02	> 100	8.7±2.0	> 100
5z [a]		> 100	> 100	0.03±0.01	> 100	0.78±0.14
5a		61.0±0.4	7.7±0.3	0.68±0.27	8.4±0.7	6.9±0.1
5b		> 100	> 100	0.47±0.32	13±1	1.4±0.5
5c		20.4±0.1	35.3±0.2	0.37±0.23	> 100	0.69±0.12
5d		> 100	> 100	> 100	> 100	> 100
5e		> 100	34.4±0.4	0.77±0.14	> 100	0.18±0.04
5f		> 100	> 100	0.58±0.43	> 100	0.67±0.22
5g		> 100	> 100	> 100	> 100	> 100
5h		> 100	> 100	> 100	> 100	> 100
5i		> 100	> 100	> 100	> 100	> 100
5j		> 100	> 100	> 100	> 100	> 100
5k		> 100	61.6±0.3	4.3±0.2	> 100	> 100
5l		> 100	> 100	2.5±0.2	> 100	34±1
5m		> 100	41.4±0.1	0.22±0.03	5.0±0.8	0.73±0.03
5n		> 100	> 100	0.54±0.15	> 100	> 100
5o		> 100	> 100	0.39±0.04	> 100	2.0±0.1
5p		> 100	> 100	0.08±0.02	> 100	0.99±0.15
5q		> 100	> 100	0.04±0.02	22±4	0.73±0.08
5r		> 100	> 100	0.11±0.04	5.1±1.0	0.29±0.01
6a		> 100	> 100	0.63±0.24	> 100	0.11±0.03
6b		> 100	> 100	0.69±0.16	> 100	0.97±0.23
6c		1.9±0.1	36.3±1.1	0.32±0.16	> 100	0.029±0.009
7a		> 100	> 100	> 100	> 100	> 100

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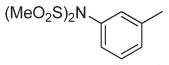
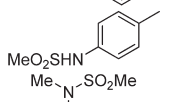
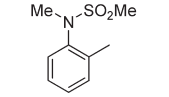
Table 1. Continued

7b		> 100	> 100	> 100	> 100	> 100
8c		> 100	> 100	3.4±0.5	> 100	2.1±0.3
13a		> 100	92±1	> 100	> 100	> 100

[a] The structures of these known inhibitors as positive control are provided in Figure 1.

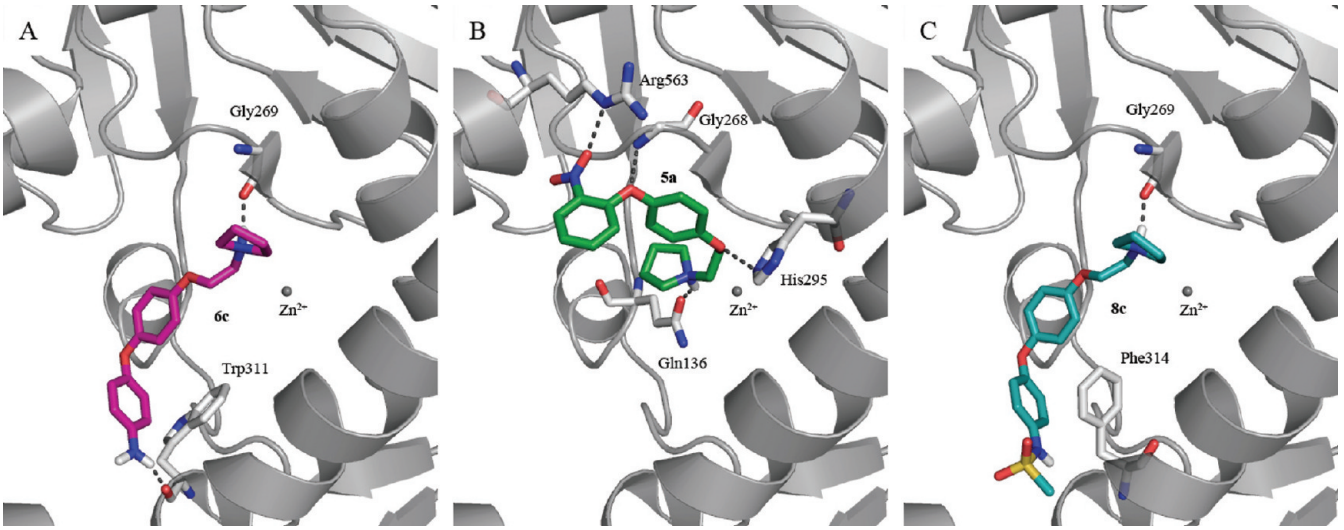


Figure 4. Inhibitors in the LTA_4H substrate binding pocket (predicted by molecular docking). A. **6c** (magenta). B. **5a** (green). C. **8c** (cyan).

of COX-1 in HWB is typically based on TXB_2 production through the COX-1/TXAS pathway, either in spontaneously clotting, unheparinized blood[28] or in only A23187 stimulated, heparinized blood; however, the addition of LPS could also induce the production of TXB_2 through the COX-2/TXAS pathway.[29]

Combining the enzyme assay and HWB assay data, two compounds, **5a** and **5m**, were found to meet the requirements for dual COX-2 and LTA_4H inhibition and a good selectivity for COX-2 over COX-1.

Molecular Docking. Molecular docking was performed to study the structure−function relationship of the title compounds toward the three enzymes.

For LTA_4H, docking results showed that position and size of substituted groups played a key role in the binding conformation of the compounds. There are four cases: (1) the parent compound **5z**, compounds without branches (**5e**, **5f**, **5k**), and *p*-substituted compounds (**5c**, **5o**, **5p**, **5q**, **5r**, **6c**) tended to bind LTA_4H in the channel, forming a hydrogen bond between the pyrrolidine nitrogen atom and Gly269 main-chain oxygen atom (Figure 4A). Compounds may also interact with the residues at the end of the channel, such as hydrogen bond between **6c** amino group and Trp311 main-chain oxygen atom. This binding mode of Figure 4A and C is a typical conformation in LTA_4H and can be found in many crystal structures including the first reported structure (PDB code 1HS6).[30] (2) *o*- or *m*-Substituted compounds (**5a**, **5b**, **5l**, **5m**, **5n**, **6a**, **6b**) can stay at the entrance part of the pocket in a reversed orientation with three hydrogen bonds (nitrogen atom to Gln136, the oxygen atom connected to alkyl group to His295, and the oxygen atom connected to bibenzyl to Gly268 main-chain nitrogen atom (Figure 4B). Some compounds may form extra hydrogen bonds to the enzyme using their substituted groups. For example, NO_2 of **5a** forms a hydrogen bond to Arg563. This binding mode corresponded with other peoples' docking results[31] and can be seen in crystal structures (PDB code 2R59 and 3B7R).[17] (3) Compounds possessing both *o*- and *m*-substituted groups on the terminal aryl ring (**5d**, **5j**) cannot enter the long and narrow substrate channel of LTA_4H, so they did not show inhibition activities to LTA_4H. (4) When the compounds have large substituted group such as methylsulfonyl or aminomethanesulfonyl (**5g**, **5h**, **5i**, **7a**, **7b**, **8c**, **13a**), inhibition activities to LTA_4H of the compounds were lower except **8c** gave an IC_{50} value 3.4 μM. Molecular docking result showed that **5g**, **5h**, **5i**, **7a**, **7b**, and **13a** would collide with the pocket, and only compound **8c** entered into the narrow cleft of the LTA_4H pocket and stayed in the same conformation as **6c** (Figure 4C). The large methylsulfonyl group of **8c** was a little deeper than the position of Phe314. In comparison, the methylsulfonyl group of compound **5i** was directly connected to the phenyl ring and would collide with the side chain of Phe314. Compounds in case (1) and (2) all maintained the inhibition activity of the parent compound **5z**, while those in cases (3) and (4) lost inhibition activity.

For COX enzymes, 7 compounds (**5a**, **5c**, **5e**, **5k**, **5m**, **6c**, **13a**) showed inhibition activity. **5a**, **5c**, and **6c** inhibit both COX-1 and COX-2, but with different selectivity. **5a** was 8 times more selective toward COX-2, with IC_{50} of 7.7 ± 0.3 μM and

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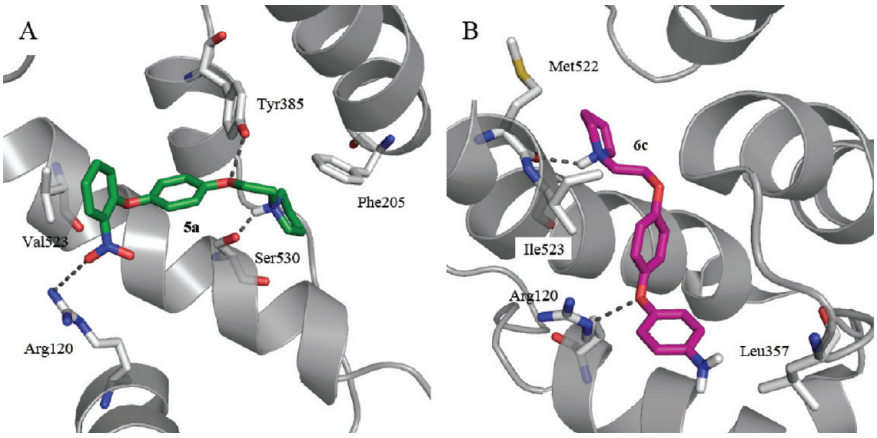

Figure 5. Inhibitors in the substrate binding pocket of COX enzymes (predicted by molecular docking). A. **5a** (green) in COX-2. B. **6c** (magenta) in COX-1.

61.0 ± 0.4 for COX-2 and COX-1, respectively. **6c** displayed more inhibition activity toward COX-1, with IC_{50} of 1.9 ± 0.1 and 36.3 ± 1.1 μM for COX-1 and COX-2, respectively. Molecular docking can well explain the activity difference due to different interactions of **5a** and **6c** to COX-1 and COX-2. The pyrrolidine group of **5a** extended to a hydrophobic pocket around Phe205 in COX-2 (Figure 5A) in a similar binding mode with other reported COX inhibitors.[32] Three hydrogen bonds were formed between the inhibitor and the enzyme. The terminal phenyl ring of **5a** was close to Val523 and might collide with Ile523 in COX-1, explaining COX-2 selectivity of **5a**. Val523 also prevents the binding of *p*-substituted compounds COX-2 in this conformation. Compound **6c** bound to COX-1 in a different conformation with two hydrogen bonds (Figure 5B). The amino group of **6c** was close to Leu357, while in COX-2, the larger side chain of Phe357 might collide with **6c** and reduce its binding affinity. There was no space for *o*- or *m*-substituted group on the terminal ring so these compounds cannot bind to COX-1 in this conformation. Thus, compounds with an electron-drawing substituted group in the *p*-position have the potential for COX-2 selectivity, while *p*-substituted compounds may bind to COX enzyme in a different conformation that fit the pocket of COX-2 better and have COX-1 selectivity. In either situation, the substituted group cannot be too large, for no compounds with substituted groups larger than methylsulfonyl had high inhibition activity to COX enzymes.

CONCLUSIONS

We have successfully designed and synthesized a series of COX-2/LTA_4H dual inhibitors by adding groups from known COX-2 inhibitors to an existing LTA_4H inhibitor 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine backbone. The inhibition activities of the compounds indicate that introducing small electron-withdrawing groups like NO_2 and CF_3 in the ortho-position of the phenyl ring can make the original LTA_4H inhibitor turn into a dual functional COX-2/LTA_4H inhibitor. Compounds **5a** and **5m** showed dual COX-2/LTA_4H inhibition activities in the enzyme assay, as well as in the HWB assay with good COX-2/COX-1 selectivity, which are promising for further studies. The strategy used in the current study for designing dual functional inhibitors may be generally applicable in dual functional drug design against other targets.

EXPERIMENTAL SECTION

Chemistry. The reagents and solvents were commercially available and purified according to conventional methods. All reactions were monitored by thin layer chromatography, carried out on silica gel 60 F-254 aluminum sheets using UV light (254 and 366 nm). All new compounds gave satisfactory ^{1}H NMR, elemental analyses, and mass spectrometry analyses. ^{1}H NMR spectra and ^{13}C NMR were measured on a Bruker-400 M spectrometer using TMS as internal standard. Elemental analyses were preformed on an Elementar Vario EL instrument. Mass spectra were recorded on a VG-ZAB-HS spectrometer. All target compounds possessed a purity of ≥95% as verified by elemental analyses by comparison with the theoretical values.

*General Procedure for the Preparation of 1-(4-Methoxyphenoxy)-2-nitrobenzene (**3a**).* A mixture of 4-methoxyphenol (2.0 g, 16 mmol) and anhydrous potassium hydroxide (1.0 g, 18 mmol) were heated at 150 °C and stirred for 10 min. After the mixture was melted, 1-chloro-2-nitrobenzene (2.0 g, 13 mmol) was added and the mixture was stirred at 170 °C for 2 h (TLC monitor). The reaction mixture was poured into 50 mL of 3% potassium hydroxide aqueous solution and stirred at room temperature for about 2 h and then cooled to 4 °C. The solid was filtered with suction and washed with water. The crude product was recrystallized by ethanol as a yellow solid and weighed 2.8 g (yield 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (1H, d), 7.44 (1H, m), 7.26−6.90 (6H, m), 3.82 (3H, s).

3c was synthesized by a similar procedure.

*1-(4-Methoxyphenoxy)-3-nitrobenzene (**3b**).* A solution of 1-bromo-2-nitrobenzene (2.0 g, 9.9 mmol), 4-methoxyphenol (3.7 g, 30 mmol), anhydrous potassium carbonate (7.4 g, 54 mmol), cuprous bromide (0.15 g, 1 mmol), and TDA (0.34 g, 1 mmol) in 15 mL of anisole was stirred at 170 °C under a nitrogen atmosphere for 48 h. The solution was cooled and diluted with ethyl ether, washed with 1 M KOH twice and water. The organic layer was evaporated on a rotary evaporator and then vacuum distilled. The residue was purified by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane is 1:15) as a pale-yellow solid and weighed 1.3 g (yield 54%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.94 (1H, d), 7.64 (1H, t), 7.59 (1H, t), 7.42 (1H, d), 7.14 (2H, m), 7.04 (2H, m), 3.79 (3H, s).

*General Procedure for the Preparation of 1-(4-Methoxyphenoxy)-2,4-dinitrobenzene (**3d**).* Under the protection of nitrogen, a solution of 1-chloro-2,4-dinitrobenzene (1 g, 4.9 mmol), 4-methoxyphenol (0.75 g, 6 mmol), anhydrous potassium carbonate (3.4 g, 24 mmol), cuprous bromide (0.15 g, 1 mmol), and TDA (0.34 g, 1 mmol) in 10 mL NMP was heated to 110 °C and stirred for 1 h. The reaction mixture was cooled and diluted with ethyl ether, washed with 1 M KOH twice and

water, dried over Na_2SO_4, concentrated at reduced pressure, and the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:5) as a yellow solid and weighed 1.0 g (yield 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (1H, d), 8.43 (1H, d), 7.24 (2H, m), 7.08 (3H, m), 3.77 (3H, s).

3e−**j** were synthesized using a similar procedure.

*General Procedure for the Preparation of 4-(2-Nitrophenoxy)phenol (**4a**).* **3a** (1.0 g, 4 mmol) was stirred in CH_2Cl_2 (20 mL) at 0 °C, and 0.57 mL (6 mmol) BBr_3 in 20 mL CH_2Cl_2 was slowly dropped into the solution above. After being stirred at room temperature for about 3 h, the mixture was poured into H_2O. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated at reduced pressure and the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:2) as a yellow solid and weighed 0.87 g (yield 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.50 (1H, s), 8.00 (1H, d), 7.60 (1H, m), 7.24 (1H, m), 6.97−6.80 (5H, m).

4b−**j** were synthesized by a similar procedure.

*General Procedure for the Preparation of 4-(pyrazin-2-yloxy)phenol (**4k**).* Under the protection of nitrogen gas, a solution of 2-chloropyrazine (0.4 g, 3.5 mmol), hydroquinone (1.92 g, 17.5 mmol), anhydrous potassium carbonate (3.0 g, 23.6 mmol), cuprous bromide (0.25 g, 1.8 mmol), and TDA (0.6 g, 1.8 mmol) in 25 mL of NMP was stirred at 120 °C for about 1 h (TLC monitor). The solution was diluted with ethyl ether, washed with saturated $KHCO_3$ aqueous solution and water, dried over Na_2SO_4, and concentrated at reduced pressure, and the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:1) as a pale-yellow solid and weighed 0.59 g (yield 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34 (1H, s), 8.11 (1H, d), 7.79 (1H, t), 7.01 (2H, t), 6.91 (2H, m), 6.78 (2H, m).

4l−**n** were synthesized by a similar procedure.

*General Procedure for the Preparation of 1-(2-(4-(2-Nitrophenoxy)-phenoxy)ethyl) pyrrolidine (**5a**).* A solution of **4a** (0.2 g, 0.87 mmol) and anhydrous potassium hydroxide (0.32 g, 5.7 mmol) in DMF was stirred at 80−90 °C for 10 min. Then, 1-(2-chloroethyl) pyrrolidine·HCl (0.36 g, 2.1 mmol) was added and the solution was stirred at 100 °C for about 0.5 h (TLC monitor). The solution was cooled, poured into water, and extracted with EtOAc twice. The combined organic extracts were washed with 1 M KOH and water, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, the eluting solvent ranged from CH_2Cl_2 to CH_2Cl_2/MeOH = 9:1) as a yellow oil and weighed 0.19 g (yield 68%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.02 (1H, d), 7.64 (1H, t), 7.29 (1H, t), 7.07−6.98 (5H, m), 4.06 (2H, t), 2.80 (2H, t), 2.53 (4H, m), 1.70 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ 154.60, 150.31, 149.07, 140.61, 134.85, 125.50, 123.36, 120.64, 119.42, 116.25, 63.74, 53.52, 52.49, 22.62. ESI-HRMS m/z 329.1492 [M + H]. Anal. ($C_{18}H_{20}N_2O_4\cdot 0.3H_2O$).

5z and **5b**−**r** were synthesized by a similar procedure.

*1-(2-(4-Phenoxyphenoxy)ethyl) pyrrolidine (**5z**).* The title compound was prepared from 4-phenoxyphenol as a light yellow oil (yield 82%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.26 (2H, d), 7.08 (2H, d), 7.17−6.95 (5H, m), 4.06 (2H, t), 2.82 (2H, t), 2.57 (4H, m), 1.70 (4H, m). Anal. ($C_{18}H_{21}NO_2$).

*1-(2-(4-(3-Nitrophenoxy)phenoxy)ethyl) pyrrolidine (**5b**).* The title compound was prepared from **4b** as a yellow solid (yield 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.93 (1H, m), 7.64 (1H, m), 7.60 (1H, m), 7.43 (1H, m), 7.11 (2H, m), 7.05 (2H, m), 4.09 (2H, t), 2.81 (2H, t), 2.54 (4H, m), 1.69 (4H, m). ESI-HRMS m/z 329.1491 [M + H]. Anal. ($C_{18}H_{20}N_2O_4\cdot 0.4H_2O$).

*1-(2-(4-(4-Nitrophenoxy)phenoxy)ethyl) pyrrolidine (**5c**).* The title compound was prepared from **4c** as a yellow solid (yield 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.20 (2H, m), 7.00 (4H, m), 6.82 (2H, m), 4.08 (2H, t), 2.74 (2H, t), 2.66 (4H, m), 1.74 (4H, s). ^{13}C NMR (400 MHz, DMSO-d_6) δ 163.75, 156.04, 147.25, 141.84, 126.11, 121.88, 116.55, 115.96, 67.12, 54.28, 53.98, 23.14. Anal. ($C_{18}H_{20}N_2O_4$).

*1-(2-(4-(2,4-Dinitrophenoxy)phenoxy)ethyl) pyrrolidine (**5d**).* The title compound was prepared from **4d** as a yellow oil (yield 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.88 (1H, d), 8.45 (1H, m), 7.24 (2H, m), 7.11 (3H, m), 4.22 (2H, t), 3.24 (2H, t), 2.99 (4H, m), 1.84 (4H, m). Anal. ($C_{18}H_{19}N_3O_6\cdot 0.4H_2O$).

*2-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy)pyridine (**5e**).* The title compound was prepared from **4e** as a yellow oil (yield 48%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.12 (1H, d), 7.82 (1H, t), 7.07 (3H, m), 6.97 (3H, m), 4.08 (2H, t), 2.85 (2H, t), 2.59 (4H, s), 1.71 (4H, s). Anal. ($C_{17}H_{20}N_2O_2\cdot 0.5H_2O$).

*4-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy)pyridine (**5f**).* The title compound was prepared from **4f** as a yellow oil (yield 22%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42 (2H, d), 7.02 (4H, m), 6.82 (2H, d), 4.08 (2H, t), 2.76 (2H, t), 2.65 (4H, m), 1.73 (4H, s). Anal. ($C_{17}H_{20}N_2O_2\cdot 0.3H_2O$).

*1-(2-(4-(2-(Methylsulfonyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5g**).* The title compound was prepared from **4g** as a white solid (yield 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.90 (1H, d), 7.64 (1H, t), 7.29 (1H, t), 7.11 (2H, d), 7.11 (2H, d), 7.03 (2H, d), 6.88 (1H, d), 4.07 (2H, t), 3.36 (3H, s), 2.79 (2H, t), 2.51 (4H, m), 1.69 (4H, m). Anal. ($C_{19}H_{23}NO_4S$).

*1-(2-(4-(3-(Methylsulfonyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5h**).* The title compound was prepared from **4h** as a white solid (yield 74%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (1H, s), 7.63 (2H, d), 7.34 (1H, m), 7.11−7.02 (4H, m), 4.08 (2H, t), 3.22 (3H, s), 2.81 (2H, t), 2.54 (4H, m), 1.69 (4H, m). ESI-HRMS m/z 362.1415 [M + H]. Anal. ($C_{19}H_{23}NO_4S$).

*1-(2-(4-(4-(Methylsulfonyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5i**).* The title compound was prepared from **4i** as a white solid (yield 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.88 (2H, d), 7.11−7.02 (6H, m), 4.08 (2H, t), 3.36 (3H, s), 2.80 (2H, t), 1.69 (4H, s). ESI-HRMS m/z 362.1418 [M + H]. Anal. ($C_{19}H_{23}NO_4S$).

*1-(2-(4-(4-(Methylsulfonyl)-2-nitrophenoxy)phenoxy)ethyl) pyrrolidine (**5j**).* The title compound was prepared from **4j** as a yellow solid (yield 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (1H, d), 8.11 (1H, d), 7.20 (2H, m), 7.12−7.06 (3H, m), 4.10 (2H, t), 3.30 (3H, s), 2.85 (2H, t), 2.58 (4H, m), 1.71 (4H, m). Anal. ($C_{19}H_{22}N_2O_6S$).

*2-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy) pyrazine (**5k**).* The title compound was prepared from **4k** as a light red solid (yield 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.56 (1H, d), 8.40 (1H, d), 7.35 (1H, m), 7.13 (2H, d), 7.00 (2H, d), 4.08 (2H, t), 2.82 (2H, t), 2.53 (4H, m), 1.70 (4H, s). Anal. ($C_{16}H_{19}N_3O_2\cdot 0.2H_2O$).

*3-Nitro-2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)pyridine (**5l**).* The title compound was prepared from **4l** as a yellow solid (yield 73%).^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55 (1H, d), 8.39 (1H, t), 7.35 (1H, m), 7.13 (2H, d), 6.99 (2H, d), 4.08 (2H, t), 2.82 (2H, t), 1.70 (4H, s). Anal. ($C_{17}H_{19}N_3O_4$).

*1-(2-(4-(2-(Trifluoromethyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5m**).* The title compound was prepared from **4m** as a colorless oil (yield 75%).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (1H, t), 7.42 (1H, d), 7.22−7.18 (2H, m), 7.08−7.00 (4H, m), 4.07 (2H, t), 2.79 (2H, t), 2.53 (4H, m), 1.69 (4H, m). Anal. ($C_{19}H_{20}F_3NO_2$).

*1-(2-(4-(3-(Trifluoromethyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5n**).* The title compound was prepared from **4n** as a colorless oil (yield 73%).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.58 (1H, t), 7.42 (1H, d), 7.22−7.18 (2H, m), 7.08−7.00 (4H, m), 4.07 (2H, t), 2.80 (2H, t), 2.53 (4H, m), 1.69 (4H, m). Anal. ($C_{19}H_{20}F_3NO_2$).

*1-(2-(4-(4-(Trifluoromethyl)phenoxy)phenoxy)ethyl) pyrrolidine (**5o**).* The title compound was prepared from 4-(4-(trifluoromethyl) phenoxy) phenol as a white solid (yield 76%).^{1}H NMR (400 MHz, DMSO-d_6) δ 7.69 (2H, d), 7.11−7.00 (6H, m), 4.08 (2H, t), 2.85 (2H, t), 2.58 (4H, m), 1.71 (4H, m). Anal. ($C_{19}H_{20}F_3NO_2$).

*1-(2-(4-(4-Fluorophenoxy)phenoxy)ethyl) pyrrolidine (**5p**).* The title compound was prepared from 4-(4-fluorophenoxy) phenol as a white solid (yield 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (2H, t), 6.96 (6H, m), 4.04 (2H, t), 2.77 (2H, t), 2.51 (4H, m), 1.68 (4H, m). Anal. ($C_{18}H_{20}FNO_2 \cdot 0.1H_2O$).

*1-(2-(4-(4-Chlorophenoxy)phenoxy)ethyl) pyrrolidine (**5q**).* The title compound was prepared from 4-(4-chlorophenoxy) phenol as a white solid (yield 60%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.28 (2H, t), 7.06 (6H, m), 4.03 (2H, t), 2.78 (2H, t), 2.56 (4H, m), 1.68 (4H, m). Anal. ($C_{18}H_{20}ClNO_2$).

*1-(2-(4-(4-Bromophenoxy)phenoxy)ethyl) pyrrolidine (**5r**).* The title compound was prepared from 4-(4-bromophenoxy) phenol as a light brown solid (yield 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.36 (2H, d), 7.26 (2H, d), 7.12 (2H, d), 7.04 (2H, d), 4.03 (2H, t), 2.78 (2H, t), 2.56 (4H, m), 1.71 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ 157.49, 155.17, 148.80, 132.54, 120.89, 119.22, 115.72, 114.00, 67.03, 54.27, 53.96, 23.12. ESI-HRMS m/z 362.0746 [M + H]. Anal. ($C_{18}H_{20}BrNO_2$).

*General Procedure for the Preparation of 2-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy) aniline (**6a**).* A solution of **5a** (0.17 g, 0.52 mol) and 10% Pd/C (0.008 g) in propan-2-ol was heated to 70 °C. 85% Hydrazine hydrate solution (0.1 mol, 1 mol) was added and the solution was further stirred for 0.5−1 h (TLC monitor). The solution was cooled, filtered by 0.2 μm filter, and evaporated in vacuo. The product was a white solid weighed 0.15 g (yield 97%) and was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92−6.84 (5H, m), 6.79 (1H, d), 6.67 (1H, d), 6.50 (1H, t), 4.90 (2H, s), 4.00 (2H, t), 2.75 (2H, t), 2.49 (4H, m), 1.66 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ 153.98, 150.78, 143.00, 140.04, 124.22, 118.95, 118.44, 116.24, 115.56, 115.36, 67.12, 54.39, 54.01, 23.14. Anal. ($C_{18}H_{22}N_2O_2$).

6b−c were synthesized by a similar procedure.

*3-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy) aniline (**6b**).* The title compound was prepared from **5b** as a colorless oil (yield 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.94 (5H, m), 6.24 (1H, d), 6.09−6.03 (2H, m), 5.16 (2H, s), 4.04 (3H, t), 2.80 (2H, t), 2.54 (4H, m), 1.69 (4H, m). Anal. ($C_{18}H_{22}N_2O_2$).

*4-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy) aniline (**6c**).* The title compound was prepared from **5c** as a colorless oil (yield 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.85 (2H, m), 6.34 (4H, d), 6.09 (2H, m), 5.26 (2H, s), 4.07 (3H, t), 2.85 (2H, t), 2.58 (4H, m), 1.70 (4H, m). ^{13}C NMR (400 MHz, DMSO-d_6) δ 157.23, 154.20, 149.69, 144.89, 126.46, 124.80, 120.85, 116.17, 63.76, 53.57, 52.57, 22.60. ESI-HRMS m/z 299.1747 [M + H]. Anal. ($C_{18}H_{22}N_2O_2$).

*General Procedure for the Preparation of N-(Methylsulfonyl)-N-(2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)phenyl) methanesulfonamide (**7a**).* A solution of **6a** (0.15 g, 0.50 mol) and triethylamine (1.5 mL) in 2.5 mL CH_2Cl_2 was cooled to 0 °C. Methanesulfonyl chloride (0.14 g, 1.2 mmol) diluted with 1.5 mL CH_2Cl_2 in 0 °C was slowly dropped into the solution above. After being stirred at 0 °C for 10 min and room temperature for 0.5 h, the mixture was poured into H_2O, and extracted with EtOAc twice. The combined organic extracts were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, the eluting solvent ranged from CH_2Cl_2 to CH_2Cl_2/MeOH = 9:1) as a yellow solid and weighed 0.12 g (yield 53%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.59 (1H, d), 7.41 (1H, t), 7.13 (1H, t), 7.10−7.01 (4H, m), 6.76 (1H, d), 4.10 (2H, t), 3.54 (6H, s), 2.93 (2H, t), 2.64 (4H, m), 1.73 (4H, m). ESI-HRMS m/z 455.1304 [M + H]. Anal. ($C_{20}H_{26}N_2O_6S_2 \cdot 0.1H_2O$).

7b was synthesized by a similar procedure.

*N-(Methylsulfonyl)-N-(3-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)-phenyl) methanesulfonamide (**7b**).* The title compound was prepared from **6b** according to the procedure for **7a** as a yellow solid (yield 36%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (1H, m), 7.23 (1H, m), 7.11 (1H, m), 7.03 (1H, m), 6.97 (2H, m), 6.85 (2H, m), 4.11 (2H, t), 3.52 (6H, s), 2.86 (2H, t), 2.59 (4H, s), 1.72 (4H, m). Anal. ($C_{20}H_{26}N_2O_6S_2 \cdot 0.3H_2O$).

*General Procedure for the Preparation of N-(4-(4-(2-(Pyrrolidin-1-yl)ethoxy)phenoxy)phenyl) methanesulfonamide (**8c**).* A solution of **6c** (0.23 g, 0.78 mmol) in 4 mL H_2O was slowly added in methanesulfonyl chloride (0.12 g, 1.0 mmol) and stirred at room temperature for 2 h. The mixture was extracted with EtOAc twice. The combined organic extracts were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, the eluting solvent ranged from CH_2Cl_2 to CH_2Cl_2/MeOH = 9:1) as a yellow solid and weighed 0.20 g (yield 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (2H, d), 6.96−6.91 (6H, m), 4.04 (3H, t), 2.93 (3H, s), 2.79 (2H, t), 2.53 (4H, m), 1.69 (4H, m). ESI-HRMS m/z 377.1528 [M + H]. Anal. ($C_{19}H_{24}N_2O_4S \cdot 0.1H_2O$).

*General Procedure for the Preparation of N-Methyl-N-(2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy)phenyl) methanesulfonamide (**13a**).*

*Step 1: Preparation of 2-(4-methoxyphenoxy) aniline (**9a**).* Tin granular (11 g) was added in the solution of **3a** (1 g, 4 mmol) in ethanol (18 mL). A mixture of concentrated HCl (9 mL) and ethanol (9 mL) was then added into the solution above and refluxed for 16 h. The solution was evaporated on a rotary evaporator to remove the ethanol, alkalized with NaOH aqueous solution, and extracted with EtOAc. The combined organic extracts were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo to provide 0.74 g (86%) of a yellow oil, which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.94−6.88 (5H, m), 6.72 (1H, d), 6.57 (1H, d), 6.52 (1H, t), 4.95 (2H, s).

*Step 2: Preparation of N-(2-(4-Methoxyphenoxy)phenyl) methanesulfonamide (**10a**).* A solution of **9a** (1 g, 4.7 mmol) in 20 mL pyridine was heated to 70 °C. Methanesulfonyl chloride (0.69 g, 6.0 mmol) was slowly dropped into the solution above. After being stirred at 90 °C for 1 h, the mixture was poured into18% HCl aqueous solution, filtered, and extracted with EtOAc twice. The combined organic extracts were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:5) as a white solid and weighed 1.08 g (yield 73%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (1H, s), 7.37 (1H, d), 7.12 (1H, t), 7.02 (1H, t), 6.90 (2H, d), 6.78 (2H, d), 6.70 (1H, d), 3.76 (3H, s), 3.03 (3H, s).

*Step 3: Preparation of N-(2-(4-Methoxyphenoxy)phenyl)-N-methylmethanesulfonamide (**11a**).* A solution of **10a** (1 g, 3.4 mmol), dimethyl sulfate (0.86 g, 6.8 mmol) and anhydrous potassium carbonate (2.6 g, 20 mmol) in 20 mL acetone was reflux for 3 h. 1 M KOH aqueous solution was added and stirred at 60 °C for 1 h. The mixture was cooled to room temperature and extracted with EtOAc twice. The combined organic extracts were washed with brine and water, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:3) as a white solid and weighed 1.08 g (yield 72%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.42 (1H, d), 7.28 (1H, t), 7.12−6.98 (5H, m), 6.74 (1H, s), 3.76 (3H, s), 3.22 (3H, s), 3.05 (3H, s).

*Step 4: Preparation of N-(2-(4-Hydroxyphenoxy)phenyl)-N-methylmethanesulfonamide (**12a**).* The title compound was prepared from **11a**, using the general procedure for the preparation of **4a**, to give **12a** as a white solid (yield 81%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (1H, s), 7.32 (1H, d), 7.18 (1H, t), 7.11−6.94 (5H, m), 6.72 (1H, s), 3.22 (3H, s), 3.05 (3H, s).

*Step 5: Preparation of N-Methyl-N-(2-(4-(2-(pyrrolidin-1-yl)ethoxy)phenoxy) phenyl) methanesulfonamide (**13a**).* The title compound was prepared from **12a**, using the general procedure for the preparation of **5a**, to give **13a** as a light yellow solid (yield 86%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.43 (1H, d), 7.27 (1H, m), 7.11−6.98

(5H, m), 6.74 (1H, d), 4.05 (2H, t), 3.20 (3H, s), 3.05 (3H, s), 2.79 (2H, t), 2.52 (4H, m), 1.69 (4H, m). ESI-HRMS m/z 391.1685 [M + H]. Anal. ($C_{20}H_{26}N_2O_4S$).

BIOLOGICAL METHODS

Inhibition Assay of Human LTA_4H Hydrolase. The LTA_4H hydrolase activity was measured using an ELISA assay to quantify the amount of LTB_4 produced. LTA_4 substrate was prepared from hydrolyzation of LTA_4 methyl ester (Cayman Chemical) in cold acetone with 50 mM NaOH (20%, v/v) under an inert atmosphere of nitrogen at room temperature for 40 min, and the substrate solution was stored at −80 °C until used. 300 ng of enzyme was diluted into 180 μL reaction buffer (10 mM sodium phosphate, pH 7.4, 4 mg/mL BSA) and incubated with test compounds (5% v/v DMSO, final) for 15 min at 37 °C. 20 μL LTA_4 substrate (150 nM final concentrations) was added to initiate the reaction and it was incubated for 10 min at 37 °C. A 25 μL sample was diluted 20−100 times in EIA buffer to stop the reaction. The concentration of LTB_4 was quantified in the diluted sample by a commercially available LTB_4 ELISA kit (Cayman Chemical).

Inhibition Assay of COX-2 and COX-1. The enzyme activity of the purified COX was measured using a chromogenic assay based on the oxidation of TMPD during the reduction of PGG_2 to PGH_2 in 96-well plates (Costar, Corning Incorporated). The assay mixture (180 μL) contained 100 mM Tris, pH 8, 2 mM genapol X-100,[26] 1 μM hematin, and approx 20 units purified COX-1 (Cayman Chemical, Cat. No. 60100) or COX-2 (Cayman Chemical, Cat. No. 60120). One unit of enzyme activity is defined as the amount of enzyme required to cause a change in TMPD absorbance at 610 nm of 0.001 OD per min. The TMPD solution was prepared in 0.1 M HCl with the concentration of 1 mM and the arachidonic acid was prepared in ethanol with the concentration of 1.1 mM. Inhibitors were prepared in DMSO and added for the final DMSO concentration was always 5% of the final sample volume. The assay mixture was preincubated at 25 °C with inhibitors for 15 min and 20 μL of the TMPD solution was added before initiation of the enzymatic reaction by the addition of 20 μL of a solution of arachidonic acid in assay buffer. The enzyme activity was measured by estimation of the initial velocity of TMPD oxidation over the first 36 s of the reaction as followed by the increase in absorbency at 610 nm (V_{max}; Molecular Devices). A low rate of nonenzymatic oxidation in the absence of enzyme was subtracted before the calculation of the percentage of inhibition. IC_{50} values were obtained from fitting the data to a four-parameter logistical model of the graph of log dose against percentage inhibition. All reported IC_{50} values are the results of averages of independent experiments conducted at least twice.

Human Whole Blood Assay. Fresh blood was collected into tubes containing heparin (10 IU/mL) and used within 1 h after collection. 0.5 mL of blood were aliquoted into PP tubes preloaded with either 2 μL of vehicle (DMSO) or 2 μL of test compounds DMSO solutions at final concentrations ranging from 0.01 μM to 100 μM. The tubes were vortexed gently and incubated for 15 min. 10 μL LPS (PBS solution as the final concentration of 100 μg/mL) was added to the blood and incubated for 23.5 h at 37 °C to induce COX-2 expression. Calcium ionophore A23187 (final concentration of 20 μg/mL) was added and the incubation was continued under the same conditions for an additional 30 min. The incubation was terminated by centrifugation (3200 g 15 min, 4 °C) to obtain plasma. Plasma was kept at −80 °C until it was assayed for PGE_2 and LTB_4 production using an ELISA kit (Cayman Chemical) according to the manufacturer's instructions.

Molecular Docking. All the synthesized 1-(2-(4-phenoxyphenoxy)ethyl) pyrrolidine derivatives were docked into the cyclooxygenase sites of COX-1 and COX-2, and substrate binding site of LTA_4H. The crystal structures of the three enzymes were retrieved from Protein Data Bank (RCSB PDB, http://www.rcsb.org/pdb/, entry codes: COX-1, 1Q4G;[33] COX-2, 1PXX;[34] LTA_4H, 3CHR[35]). The structures of ovine COX-1 and murine COX-2 were used, which have conserved substrate binding sites compared to those in human enzymes. 3D models of the small molecules were built in Schrödinger software using the LigPrep command. Molecular docking with flexible ligands and rigid receptors was performed using software *AutoDock 4.00*. Conformational searching used the Lamarckian genetic algorithm (LGA). Number of GA runs was set to 256, population size to 300, maximum number of energy evaluations to 25 000 000, and maximum number of generations to 27 000. Docked states of each small molecule were clustered with rms of 2.0 Å. The lowest energy conformation in the largest cluster was taken as the binding state.

ASSOCIATED CONTENT

Supporting Information. Elemental analyses data of all the compounds; scanned spectrum of the ^1H NMR and ^{13}C NMR data, and high-resolution mass spectra of representative compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS

NSAIDs, nonsteriodal anti-inflammatory drugs; COX, cyclooxygenase; 5-LOX, 5-lipoxygenase; LTA_4H, leukotriene A_4 hydrolase; LTB_4, leukotriene B_4; PGES, prostaglandin E synthase; PGE_2, prostaglandin E_2; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; TXB_2,Thromboxane B_2TXAS, Thromboxane synthetase; PLA_2, phospholipase A_2

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