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Optimization of 5-hydroxytryptamines as dual function inhibitors targeting phospholipase A_2 and leukotriene A_4 hydrolase

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ABSTRACT

Dual function inhibitors targeting phospholipase A_2 (PLA_2) and leukotriene A_4 hydrolase (LTA_4H) may balance the arachidonic acid (AA) metabolic network and be used as new anti-inflammatory drugs. In previous study, we discovered multi-target drugs towards the AA metabolic network, among which a dual-target inhibitor (**JMC08-4**) for human nonpancreatic secretory phospholipase A_2 (hnps-PLA_2) and human leukotriene A_4 hydrolase (LTA_4H-h) was found. Based on the structure of compound **JMC08-4**, new dual-target inhibitors were designed assisted by molecular docking. In this report, a series of 5-hydroxytryptamine compounds were synthesized; and most of these title compounds showed more potent inhibitory activity than compound **JMC08-4** in the *in vitro* bioassay against these two enzymes. The best one inhibited hnps-PLA_2 and LTA_4H-h with IC_{50} values of 9.2 ± 0.5 μM and 2.4 ± 1.4 μM, respectively.

1. Introduction

Phospholipase A_2 (PLA_2) and leukotriene A_4 hydrolase (LTA_4H) enzymes are important anti-inflammatory targets. PLA_2 hydrolyzes the acylester bond at the sn-2 position of phosphoglycerides and liberates free fatty acids (predominantly arachidonic acid) and lysophospholipids. Over expression of PLA_2 plays an important role in the inflammatory process [1,2]. The proinflammatory substance leukotriene B4 (LTB_4) derived from arachidonic acid (AA) via 5-lipoxygenase (5-LOX) pathway. LTA_4H catalyzes the rate-limiting step in the biosynthesis of LTB_4, and therefore is a potent inducer of inflammation [3]. PLA_2, LTA_4H, and other key inflammation-related proteins in the AA metabolic network, such as cyclo-oxygenase 2 (COX-2) and 5-LOX, have been studied as targets for anti-inflammatory drugs. Many inhibitors of these targets have been used in treating acute and chronic inflammation. However, inhibiting only one target of the metabolic pathways can possibly shift the metabolism of AA towards other pathways, which may cause side effects. For example, selective COX-2 inhibitors were reported to have cardiovascular side effects in clinical trials [4]. The reason is that the inhibitors increased the production of leukotrienes (LTs) on one hand and decreased the generation of prostaglandins (PGs) on the other hand. Multi-target drug discovery has become an increasingly perspective to overcome disadvantages of single-target based drugs [5,6]. In anti-inflammatory drug research, some multi-target inhibitors have been reported, including combinations of COX/5-LOX [7–10], 5-LOX/thromboxane A_2 synthase (TxA_2S) [11], thromboxane A_2 receptor (TxA_2R)/TxA_2S [12], 5-LOX/microsomal prostaglandin E synthase (mPGEs) [13], and COX/LTA_4H [14].

Although multi-target drugs have advantages, there have been no interchangeable strategies to design this new kind of drugs and the discovery of it was often serendipitous [15]. In a previous study, we developed a computational algorithm for finding multiple target optimal intervention (MTOI) solutions in a disease network. We applied MTOI to the AA network and a number of optimal combinations of target intervention (MTOI solutions) were found, such as: COX-2/LTA_4H, COX-2/5-LOX, PGES/LTA_4H, PLA_2/LTA_4H, PLA_2/COX-2/LTA_4H, and PLA_2/5-LOX/COX-2, which are both effective in controlling inflammation mediators and safe with minimal side effects [16]. We also developed a strategy that combined receptor-based common pharmacophores with molecular docking, which was successfully applied to screen for dual-target inhibitors against hnps-PLA_2 and LTA_4H-h [17]. Compound 1,2-amino-3-[5-(benzyloxy)-1H-indol-3-yl] propanoic acid (**JMC08-4**) (Fig. 1), whose binding conformations in proteins accommodate the common pharmacophores, is used as lead compound against the two enzymes. We explored structural optimizations of the **JMC08-**

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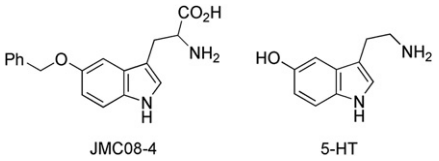


Fig. 1. Structure of **JMC08-4** and 5-HT.

4, in relation to the binding pocket requirements of both enzymes, 5-hydroxytryptamine (serotonin, 5-HT, Fig. 1) analogs were then selected for studying.

The 5-HT analogs are less difficulty in synthesis and possess potential druggability. The compound 5-HT is a neurotransmitter that modulates neural activity and a wide range of neuropsychological processes [18]. Some of 5-HT analogs have been reported as agonists or antagonists of serotonin receptors. Peroutka et al. reported that 5-benzyloxytryptamine (**4a** in this article) is a known ligand of 5-hydroxytryptamine receptors [19], and Buzzi et al. found it can mediate blockage of neurogenic plasma extravasation in rat duramater [20]. We hope to develop 5-HT analogs as new anti-inflammatory drugs. A series of 5-HT analogs have been designed and tested for their inhibition against LTA_4H-h and hnps-PLA_2.

2. Results and discussion

2.1. Design of 5-HT analogs

Compound **JMC08-4** was docked into the substrate binding site of LTA_4H-h and hnps-PLA_2 using Autodock 4.0 (Fig. 2). In Fig. 2A, we found that there was hydrophobic space in the L-shaped pocket of LTA_4H-h when compound **JMC08-4** bound there. It suggested that increasing the length of compound **JMC08-4's** hydrophobic chain could enhance the interaction between this compound and LTA_4H-h. Fig. 2B is the binding pocket of hnps-PLA_2. We can see that using a larger aromatic group to replace the 5-benzyloxy group of compound **JMC08-4** (e.g. compound in Fig. 3B), the binding of the ligand to hnps-PLA_2 was almost unchanged.

Molecular docking results of compound **JMC08-4** gave clues on the further optimization of LTA_4H/PLA_2 dual function inhibitors. Using compound **JMC08-4** backbone, a series of 5-HT analogs were designed as LTA_4H/PLA_2 inhibitors. First, 5-hydroyl group of 5-HT was combined with different aromatic groups to enhance the hydrophobicity. One of them is **4f**, which was docked into both enzymes as shown in Fig. 3. The larger aromatic group of **4f** interacted with the residues Leu369, Leu367 and Pro382 of the pocket, the interaction increased hydrophobicity as we prediction. To further enhance the activity of these compounds, the 3-ethylamine

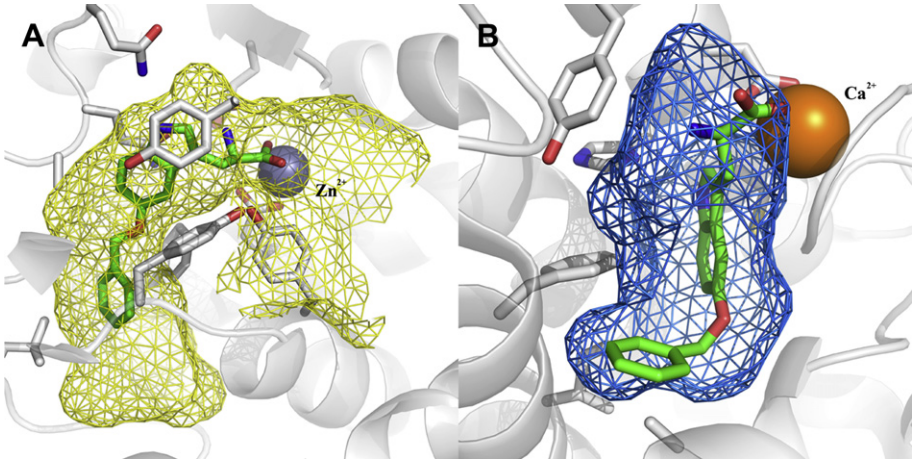


Fig. 2. The binding models of compound **JMC08-4**. A, compound **JMC08-4** with LTA_4H; B, compound **JMC08-4** with $sPLA_2$.

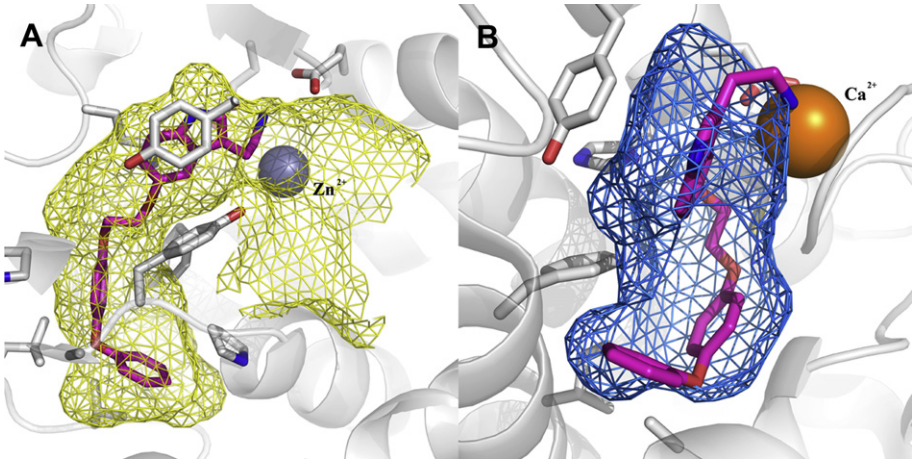


Fig. 3. The binding models of a molecule with a longer hydrophobic group (**4f**) (predicted by molecular docking). A, compound **4f** with LTA_4H; B, compound **4f** with $sPLA_2$.

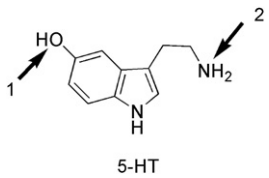


Fig. 4. Design of new inhibitors. 1, modification with large hydrophobic groups; 2, modification with functional groups that coordinate with metal atoms.

group of 5-subtituied-tryptamine was combined with either functional groups possessing negative charge or atoms coordinating with metal atoms (e.g. carboxyl).

For designed potential 5-HT analogs, the two major ways of optimization are: 1) modification with large hydrophobic groups; 2) addition of functional groups that coordinate with metal atoms (Fig. 4).

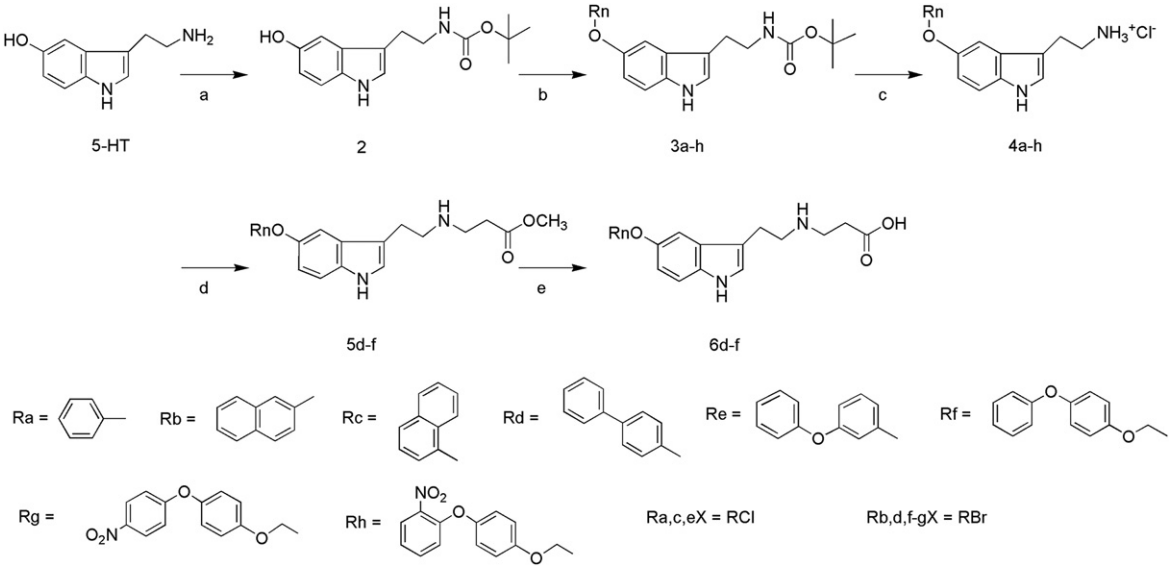
2.2. Synthesis of 5-HT analogs

The fourteen 5-HT analogs (**4a**–**h**, **5d**–**f**, and **6d**–**f**) were prepared by multi-step organic synthesis (Scheme 1). Primary amino group of 5-HT was first protected by BOC [21]. Then, in the presence of Cs_2CO_3, the intermediate N-BOC-5-HT (**2**) and different electrophiles R_nX (X is a halogen) reacted in DMF to get intermediates **3a**–**h**. The title compounds **4a**–**h** were obtained by removing the BOC anhydride in the ethyl acetate solution of hydrochloride. These monohydrochloride compounds were converted to the title compounds **5d**–**f** through nucleophilic conjugate addition by treating with methacrylate in methanol. Another kind of title compounds **6d**–**f** were obtained from **5d**–**f** by hydrolysis in an acetone solution of NaOH.

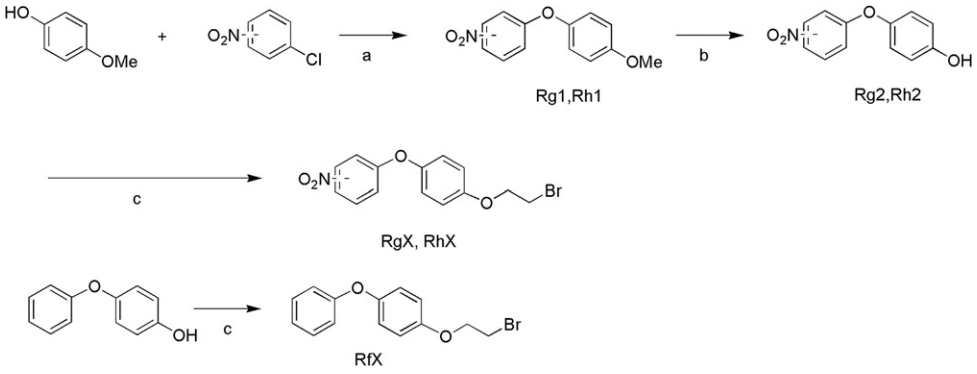
In the synthesis of compounds **3a**–**h**, some electrophilic reagents (**Rf-hX**) were not commercially available. Scheme 2 shows the preparations of these complicated electrophiles. In the presence of KOH, 4-methoxyphenol and chloronitrobenzene was melt together and condensed to get compounds $\mathbf{R_{g1}}$ and $\mathbf{R_{h1}}$. Stirred in dichloromethane solution of BBr_3, compounds $\mathbf{R_{g1}}$ and $\mathbf{R_{h1}}$were converted to $\mathbf{R_{g2}}$ and $\mathbf{R_{h2}}$. When $\mathbf{R_{g2}}$, $\mathbf{R_{h2}}$ and phenoxyphenol were further refluxed in 1,2-dibromoethane of KOH, corresponding intermediates **RfX**, and **Rg-hX** were got.

2.3. Evaluation of inhibitory activities

All the title compounds were subjected to *in vitro* inhibitory activities against LTA_4H-h and hnps-PLA_2 using the following

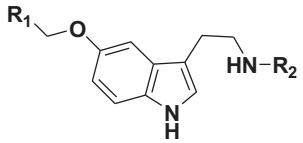


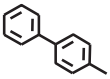
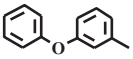
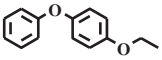
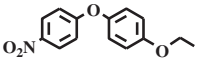
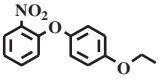
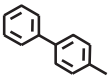
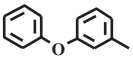
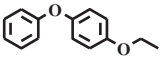
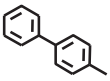
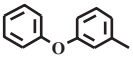
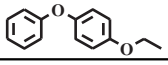
Scheme 1. Synthesis of compounds **4a**–**h**, **5d**–**f**, and **6d**–**f**. (a), BOC anhydride, $NaHCO_3$, NaCl, DCM; (b), R_nX, Cs_2CO_3, DMF; (c), HCl, EtOAc, rt; (d), methyl acrylate, Na_2CO_3, MeOH; (e), NaOH, acetone, water.



Scheme 2. Preparation of complicated electrophiles **Rf-hX**. (a), KOH; (b), BBr_3, CH_2Cl_2; (c), 1,2-dibromoethane, KOH.

Table 1
Inhibitory activity of compounds **4a**–**h**, **5d**–**f**, and **6d**–**f**.



Compound	R1	R2	LTA_4H IC_{50} (μM)		PLA_2 IC_{50} (μM)
			Aminopeptidase	Epoxide hydrolase	
Bestatin	–	–		4.2 ± 0.5	>100
NI101	–	–	>100	>100	0.88 ± 0.15
JMC08-4	–	–	17.3 ± 0.2	231 ± 4	42.1 ± 1.9
4a		H	13 ± 1	>100	52 ± 5
4b		H	8.7 ± 0.8	>100	25 ± 5
4c		H	30 ± 5	>100	27 ± 9
4d		H	2.1 ± 0.2	18 ± 4	4.4 ± 0.9
4e		H	8.9 ± 0.5	>100	15.3 ± 0.4
4f		H	1.3 ± 0.2	15 ± 3	11.5 ± 0.9
4g		H	41.8 ± 0.9	>100	5.3 ± 0.6
4h		H	6.8 ± 0.6	>100	8.6 ± 0.9
5d		COOMe	5.8 ± 0.5	7.6 ± 0.5	38 ± 5
5e		COOMe	9.27 ± 0.09	15.8 ± 5.3	25 ± 6
5f		COOMe	0.67 ± 0.03	2.4 ± 1.4	9.2 ± 0.5
6d		COOH	7.08 ± 0.01	3.8 ± 0.7	53 ± 12
6e		COOH	9.9 ± 0.7	>100	58 ± 9
6f		COOH	5.2 ± 0.1	>100	71 ± 12

methods. The inhibitory activity results are summarized in Table 1. Limited by solubility, only compounds with 50% inhibition below 100 μM were tested for IC_{50} values.

2.3.1. Against LTA_4H

LTA_4H is a ubiquitously distributed 69 kDa zinc-containing cytosolic enzyme. It possesses both aminopeptidase activity and epoxide hydrolase activity [22], and the substrate binding pockets of these two activities are largely overlap. The activity assay of LTA_4H aminopeptidase is more sensitive to inhibitor binding and less expensive. When the weak inhibitors showed no reliable response in the epoxide hydrolase activity assay, the activity data of LTA_4H aminopeptidase could be used to evaluate inhibitors' binding affinities. Using previously reported methods [23,24], we measured LTA_4H inhibitory activities of these compounds against both aminopeptidase and epoxide hydrolase, with bestatin as the positive control.

2.3.2. Against PLA_2

Testing inhibitory activity of the compounds on hnps-PLA_2 was based on a continuous fluorescence assay and carried out in a 96-well plate, using 1-anilinonaphthalene-8-sulfonate (ANS) as an interfacial probe [25]. 2-(1-Benzyl-5-methoxy-2-methyl-1H-indol-3-yl) acetamide (**NI101**), one reported hnps-PLA_2 inhibitor [26], was used as the positive control.

2.4. Structure activity relationships

As shown in Table 1, most compounds displayed more potent inhibitory activity against LTA_4H-h and hnps-PLA_2, than compound **JMC08-4** did.

In the series of secondary amine compounds (compounds **4a**–**f**), compounds **4d** and **4f** show more potent inhibitory activity than the other compounds against LTA_4H-h and hnps-PLA_2, with IC_{50} values in the magnitude of 10 μM. These results suggested that compounds with longer hydrophobic groups would show higher activities than those with smaller ones, which were consistent with the molecular docking results. However, compounds **4g** and **4h** with nitro-substituted aromatic groups did not show good inhibition against LTA_4H because these groups were not fit well into the L-shaped narrow pocket.

Compounds **5d**–**f** showed stronger LTA_4H inhibitory activities than compounds **4a**–**h** due to their functional groups coordinating with metal atoms. The interaction between the zinc ion and oxygen atoms of the methyl propionate group may cause the enhancement of their binding affinities. In contrast, compounds **6d**–**f**, with the propionic acid group at position 2 showed little improvement in inhibitory activity against LTA_4H. Propionic acid group possessed both negative charge and atoms that could coordinate with metal atoms and was supposed to improve the binding affinity of compounds **6d**–**f**. It may have been caused by the intramolecular hydrogen bonds between the carboxyl group and the amino group.

Except for compound **5f**, all compounds with a substitution at position 2 had decreased inhibitory activity against PLA_2 about one order of magnitude. It is probably caused by unwanted collisions appeared between the inhibitors and some residues of PLA_2.

Though multi-target inhibitors generally do not have high affinities, they have potential beneficial effects, such as avoiding drug resistance and toxicity, and stabilizing "sick" cells [27]. For example, noncompetitive antagonists of N-methyl-D-aspartate receptors only inhibit multiple targets weakly, but exhibit advantages of reducing side effects [28–31]. We would therefore expect that these multi-target inhibitors may be more effective to control inflammation than single-target inhibitors. Of course, these compounds need to be further studied to characterize their overall effects at system level.-

3. Conclusion

In this report, we aimed to identify potent dual function inhibitors by improving the binding affinities of a known moderate inhibitor of PLA_2 and LTA_4H, compound **JMC08-4**. Following docking result and structural information of this compound, two major optimizations were made: 1) hydrophobic group at position 5 of indole backbone was enlarged to increase the hydrophobic interaction; and 2) functional group that could coordinate with metal atoms was added at proper site. These optimizations turned out to be effective. Fourteen 5-HT analogs were successfully designed and synthesized. Most of these compounds showed more potent inhibitory activity against LTA_4H-h and hnps-PLA_2 than compound **JMC08-4**. The best potential compound is **5f**, which inhibited LTA_4H-h with an IC_{50} value of 2.4 ± 1.4 μM, increasing nearly three orders of magnitude than compound **JMC08-4**. It also improved inhibitory activity against hnps-PLA_2, the IC_{50} value is reach to 9.2 ± 0.5 μM.

4. Experimental

4.1. Chemistry

The reagents and solvents were commercially available and purified according to conventional methods. All reactions were monitored by thin layer chromatography; using silica gel 60 F-254 aluminum sheets and UV light (254 and 366 nm) for detection. All title compounds gave satisfactory ^1H NMR, ^{13}C NMR, elemental analyses, and mass spectrometry analyses. The ^1H NMR and ^{13}C NMR spectra were measured on a Bruker-400 M spectrometer using TMS as internal standard. Elemental analyses were performed on an ElementarVario EL instrument. Mass spectra were recorded on a VG-ZAB-HS spectrometer. Title compounds **4a**–**f** possessed a purity of >99.5% as verified by elemental analyses after comparison with calculated values.

4.1.1. General procedure for the synthesis of N-(butyloxycarbonyl) serotonin (*2*)

Serotonin hydrochloride (1.07 g, 5 mmol) was suspended in 10 mL of chloroform. Sodium bicarbonate (0.42 g, 10 mmol) in 7 mL of water, 1 g of sodium chloride, and di-tert-butyloxycarbonyl anhydride (1.1 g, 5 mmol) in 2.5 mL of chloroform were added. The mixture was refluxed for 3 h. The reaction mass was filtered and aqueous phase was washed with chloroform. The combined organic phases were washed with water and brine and dried over magnesium sulfate. The solvent was removed under reduced pressure to produce a solid product. Yield: 90%. ^1H NMR ($CDCl_3$): δ 7.97 [s (br), 1H], 7.26 (d, 1H), 7.01 (d + s, 2H), 6.80 (dd, 1H), 4.66 (s (br), 1H), 3.42 (t + m, 2H), 2.87 (t, 2H), 1.44 (s, 9H).

4.1.2. General procedure for the preparation of 1-(4-methoxyphenoxy)-2-nitrobenzene (*R_{h1}*)

A mixture of 4-methoxyphenol (2.0 g, 16 mmol) and anhydrous potassium hydroxide (1.0 g, 18 mmol) was heated at 150 °C and stirred. After the mixture melted (about 10 min), 1-chloro-2-nitrobenzene (2.0 g, 13 mmol) was added and the mixture was stirred at 170 °C for 2 h, while being monitored by TLC. When the reaction was complete, the reaction mass 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 to afford yellow solid 2.8 g (yield 90%). ^1H NMR (400 MHz, DMSO-d6): δ 7.93 (1H, d), 7.44 (1H, m), 7.26–6.90 (6H, m), 3.82 (3H, s).

R_{g1} was synthesized by a similar procedure, which was monitored by TLC.

4.1.3. General procedure for the preparation of 4-(2-nitrophenoxy) phenol (*R_{h2}*)

R_{h1} (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 approximately 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. The residue was separated by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 1:2) as a yellow solid which weighed 0.87 g (yield 92%). ^1H NMR (400 MHz, DMSO-d6): δ 9.50 (1H, s), 8.00 (1H, d), 7.60 (1H, m), 7.24 (1H, m), 6.97–6.80 (5H, m).

R_{g2} was synthesized by a similar procedure, which was monitored by TLC.

4.1.4. General procedure for the preparation of 1-(4-(2-bromoethoxy) phenoxy) benzene (*R_fX*)

The compound 4-phenoxyphenol (250 mg, 1.34 mmol) and anhydrous potassium hydroxide (150 mg, 2.66 mmol) were refluxed in 1,2-dibromoethane (2 mL) for 8 h. The mixture was washed with water and brine, and then dried over magnesium sulfate. After being concentrated at reduced pressure, the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from hexane to EtOAc/hexane = 3:7) as a colorless

liquid and weighed 313 mg (yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (2H, t + s), 7.06 (1H, t), 6.98 (6H, m), 4.28 (2H, t), 3.65 (2H, t).

R_gX and **R_hX** were synthesized by a similar procedure, which was monitored by TLC.

*4.1.5. General procedure for the preparation of tert-butyl 2-(5-(2-(4-phenoxyphenoxy) ethoxy)-1H-indol-3-yl) ethylcarbamate (**3f**)*

A mixture of **2** (126 mg, 0.457 mmol) and **R_fX** (147 mg, 0.502 mmol) was stirred in anhydrous DMF (4 mL) in the presence of Cs_2CO_3 (140 mg, 0.430 mmol) at 90 °C for 8 h. The mixture was poured into ethyl acetate (20 mL) and washed with water and brine, then dried over magnesium sulfate. After being concentrated at reduced pressure, the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from EtOAc/hexane = 1:9 to EtOAc/hexane = 2:3) as a colorless oil which weighed 120 mg (yield 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (s (br), 1H), 7.26 (m, 2H), 7.10 (s, 1H), 7.04 (m, 2H), 6.97 (m, 7H), 4.61 (s (br), 1H), 4.35 (dd, 4H), 3.45 (s (br), 2H), 2.91 (t, 2H), 1.43 (s, 9H).

The compounds **3a**, **3c**, and **3e**–**h** were synthesized by a similar procedure, which was monitored by TLC.

*4.1.6. General procedure for the preparation of tert-butyl 2-(5-(naphthalen-2-ylmethoxy)-1H-indol-3-yl) ethylcarbamate (**3b**)*

A mixture of compound **2** (292 mg, 1.06 mmol) and 2-(bromomethyl) naphthalene (141 mg, 1.00 mmol) was stirred in anhydrous DMF (4 mL) in the presence of Cs_2CO_3 (420 mg, 1.5 mmol) at room temperature for 8 h. The mixture was poured into ethyl acetate (20 mL) and washed with water and brine, then dried over magnesium sulfate. After being concentrated at reduced pressure, the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from EtOAc/hexane = 1:9 to EtOAc/hexane = 2:3) as a colorless oil which weighed 290 mg (yield 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11 (s (br), 1H), 7.92 (s, 1H), 7.84 (m, 3H), 7.58 (d, 1H), 7.47 (m, 2H), 7.22 (d, 1H), 7.18 (s, 1H), 6.97 (m, 2H), 5.25 (s, 2H), 4.65 (s (br), 1H), 3.43 (d, 2H), 2.89 (t, 2H), 1.44 (s, 9H).

The compound **3d** was synthesized by a similar procedure, which was monitored by TLC.

*4.1.7. General procedure for the preparation of 2-(5-(benzyloxy)-1H-indol-3-yl) ethanaminium chloride (**4a**)*

The compound **3a** (183 mg, 0.5 mmol) was dissolved in anhydrous ethyl acetate saturated with hydrogen chloride (6 mL). A white precipitate appeared while the solution was stirred and bubbled with dry HCl gas for 45 min. The solid was filtered with suction and washed with ethyl acetate, and the crude product was recrystallized by water as a white powder which weighed 166 mg (yield 90%).

The compounds **4b**–**h** were synthesized by a similar procedure.

*4.1.7.1. 2-(5-(Benzyloxy)-1H-indol-3-yl) ethanaminium chloride (**4a**).* White solid, yield 90%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.87 (s, 1H), 8.16 (s, 3H), 7.49 (d, 2H), 7.29 (m, 6H), 6.82 (dd, 1H), 5.11 (s, 2H), 3.01 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.08 (CH_2), 69.83 (O–CH_2), 101.74, 109.27, 111.81, 112.14, 124.04, 127.15 (C_{indole}), 127.60 (C_{ph}), 127.69, 128.31 (2C, C_{ph}), 131.57 (C_{indole}), 152.08 (O–C_{indole}); ESI-HRMS *m*/*z* 267.14865. Anal. Calcd for $C_{17}H_{19}ClN_2O$: C, 67.43; H, 6.32; N, 9.25. Found: C, 67.09; H, 6.27; N, 9.21.

*4.1.7.2. 2-(5-(Naphthalen-2-ylmethoxy)-1H-indol-3-yl) ethanaminium chloride (**4b**).* White solid, yield 67%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.88 (s, 1H), 8.15 (s, 3H), 8.04 (s, 1H), 7.93 (m, 3H), 7.63 (dd, 1H), 7.52 (m, 2H), 7.29 (dd, 2H), 7.21 (d, 1H), 6.87 (dd, 1H), 5.29 (s, 2H), 3.03 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.09 (CH_2), 70.00 (O–CH_2), 101.95, 109.28, 111.84, 112.15, 124.09 (C_{indole}), 125.85, 125.98, 126.16, 126.23 (C_N), 127.18 (C_{indole}), 127.56, 127.75, 127.90 (C_N), 131.63 (C_{indole}), 132.46, 132.80, 135.38 (C_N), 152.09 (O–C_{indole}); ESI-HRMS *m*/*z* 317.16435. Anal. Calcd for $C_{21}H_{21}ClN_2O$: C, 71.48; H, 6.00; N, 7.94. Found: C, 71.01; H, 6.00; N, 7.83.

*4.1.7.3. 2-(5-(Naphthalen-1-ylmethoxy)-1H-indol-3-yl) ethanaminium chloride (**4c**).* White solid, yield 89%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.85 (s, 1H), 8.15 (d, 1), 7.92–8.02 (m, 6H), 7.72 (d, 1H), 7.55 (m, 3H), 7.36 (s, 1H), 7.28 (d, 1H), 7.21 (d, 1H), 6.86 (m, 1H), 5.29 (s, 2H), 3.01 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.12 (CH_2), 69.50 (O–CH_2), 101.84, 109.29, 111.85, 112.17, 124.09 (C_{indole}), 125.36, 125.88, 126.17, 126.59 (C_N), 127.21 (C_{indole}), 127.56, 127.75, 127.90, 128.40, 131.23 (C_N), 131.65 (C_{indole}), 133.20 (C_N), 152.08 (O–C_{indole}); ESI-HRMS *m*/*z* 317.16454. Anal. Calcd for $C_{21}H_{21}ClN_2O$: C, 71.48; H, 6.00; N, 7.94. Found: C, 71.00; H, 6.02; N, 7.75.

*4.1.7.4. 2-(5-(4-Phenylbenzyloxy)-1H-indol-3-yl) ethanaminium chloride (**4d**).* White solid, yield 91%, ^{1}H NMR (400 MHz, DMSO-d6). δ 10.88 (s, 1H), 8.16 (s (br), 3H), 7.68 (dd, 4H), 7.59 (d, 2H), 7.47 (t, 2H), 7.37 (t, 1H), 7.28 (d, 1H), 7.21 (d, 2H), 6.84 (dd, 1H), 5.17 (s, 2H), 2.98 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.11 (CH_2), 69.45 (O–CH_2), 101.76, 109.29, 111.81, 112.16, 124.06 (C_{indole}), 126.63 (4C, C_{ph}), 127.17 (C_{indole}), 127.56 (C_{ph}), 128.31, 128.91 (2C, C_{ph}), 131.59 (C_{indole}), 136.97, 139.47, 139.85(C_{ph}), 152.05 (O–C_{indole}); ESI-HRMS *m*/*z* 343.18014. Anal. Calcd for $C_{23}H_{23}ClN_2O$: C, 72.91; H, 6.12; N, 7.39. Found: C, 72.89; H, 6.10; N, 7.27.

*4.1.7.5. 2-(5-(3-Phenoxybenzyloxy)-1H-indol-3-yl) ethanaminium chloride (**4e**).* White solid, yield 88%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.87 (s, 1H), 8.11 (s (br), 3H), 7. 43 (m, 3H), 7.18 (m, 6H), 6.98 (dd, 3H), 6.80 (d, 1H), 5.11 (s, 2H), 2.96 (dd, 4H); ^{13}C NMR (DMSO-d6): δ 23.07 (CH_2), 69.42 (O–CH_2), 101.95, 109.26, 111.77, 112.12 (C_{indole}), 117.40, 117.61 (C_{ph}), 118.69 (2C, C_{ph}), 122.47, 123.51 (C_{ph}), 124.10, 127.17 (C_{indole}), 129.99 (C_{ph}), 130.04 (2C, C_{ph}), 131.66 (C_{indole}), 140.10 (C_{ph}), 151.92 (O–C_{indole}), 156.47, 156.73(O–C_{ph}); ESI-HRMS *m*/*z* 359.17468. Anal. Calcd for $C_{23}H_{23}ClN_2O_2$: C, 69.95; H, 5.87; N, 7.09. Found: C, 69.62; H, 5.86; N, 7.09.

*4.1.7.6. 2-(5-(2-(4-Phenoxyphenoxy) ethoxy)-1H-indol-3-yl) ethanaminium chloride (**4f**).* White solid, yield 90%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.88 (s, 1H), 8.14 (s, 3H), 7.32 (m, 3H), 7.32 (m, 3H), 7.20 (dd, 2H), 7.04 (m, 5H), 6.93 (d, 2H), 6.79 (dd, 1H), 4.00 (s, 4H), 3.03 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.07 (CH_2), 66.96, 79.00 (O–CH_2), 101.37, 109.32, 111.65, 112.20 (C_{indole}), 115.78, 117.29, 120.69 (2C, C_{ph}), 122.59 (C_{ph}), 124.09, 127.19 (C_{indole}), 129.85 (2C, C_{ph}), 131.60 (C_{indole}), 149.53 (C_{ph}), 152.05 (O–C_{indole}), 154.77, 157.94(O–C_{ph}); ESI-HRMS *m*/*z* 389.18504. Anal. Calcd for $C_{24}H_{25}ClN_2O_3$: C, 67.84; H, 5.93; N, 6.59. Found: C, 67.48; H, 5.94; N, 6.53.

*4.1.7.7. 2-(5-(2-(4-(4-Nitrophenoxy) phenoxy) ethoxy)-1H-indol-3-yl) ethanaminium chloride (**4g**).* Pale yellow solid, yield 63%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.88 (s, 1H), 8.26 (d, 2H), 8.21 (s, 3H), 7.28 (d, 1H), 7.13 (m, 8H), 6.80 (d, 1H), 4.36 (s, 4H), 3.03 (m, 4H); ^{13}C NMR (DMSO-d6): δ 23.07 (CH_2), 66.85, 79.06 (O–CH_2), 101.38, 109.32, 111.64, 112.20 (C_{indole}), 116.11, 116.58, 121.97 (2C, C_{ph}), 124.09 (C_{indole}), 126.12 (2C, C_{ph}), 127.19, 131.61 (C_{indole}), 141.42, 147.42 (C_{ph}), 152.03 (O–C_{indole}), 154.97, 163.72(O–C_{ph}); ESI-HRMS *m*/*z* 434.17094.

*4.1.7.8. 2-(5-(2-(4-(2-Nitrophenoxy) phenoxy) ethoxy)-1H-indol-3-yl) ethanaminium chloride (**4h**).* Pale yellow solid, yield 74%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.87 (s, 1H), 8.11 (m, 3H), 7.40 (q, 3H), 7.18 (m, 6H), 6.98 (dd, 3H), 6.81 (d, 1H), 5.11 (s, 4H), 3.00 (dd, 4H); ^{13}C NMR (DMSO-d6): δ 23.06 (CH_2), 66.87, 79.06 (O–CH_2), 101.38, 109.32, 111.64, 112.20 (C_{indole}), 115.98 (2C, C_{ph}), 119.20 (C_{ph}), 120.71 (2C, C_{ph}), 123.17 (C_{ph}), 124.07 (C_{indole}), 125.45 (C_{ph}),

127.19, 131.61 (C_{indole}), 134.78, 140.50, 148.49, 150.48 (C_{ph}), 152.03 (O—C_{indole}), 155.48 (O—C_{ph}); ESI-HRMS *m*/*z* 434.17016.

*4.1.8. General procedure for the preparation of methyl 3-(2-(5-(4-phenylbenzyloxy)-1H-indol-3-yl) ethylamino) propanoate (**5d**)*

Compound **4d** (378 mg, 0.679 mmol), Cs_2CO_3 (111 mg, 0.575 mmol), and methyl acrylate (68 μL, 0.68 mmol) were stirred in methanol (2 mL) at room temperature for 12 h. The mixture was poured into ethyl acetate and washed with water and brine, then dried over magnesium sulfate. After being concentrated at reduced pressure, the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from CH_2Cl_2 to MeOH/CH_2Cl_2 = 1:5) as a colorless oil which weighed 226 mg (yield 78%). The compounds **5e** and **5f** were synthesized by a similar procedure.

*4.1.8.1. Methyl 3-(2-(5-(4-phenylbenzyloxy)-1H-indol-3-yl) ethylamino) propanoate (**5d**).* Yield 78%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (s, 1H), 7.59 (m, 6H), 7.44 (t, 2H), 7.34 (t, 1H), 7.25 (m, 1H), 7.17 (d, 1H), 6.97 (m, 2), 5.15 (s, 2H), 3.62 (s, 3H), 2.93 (m, 6H), 2.50 (t, 2H); ^{13}C NMR ($CDCl_3$): δ 25.79, 34.63, 44.98, 49.76 (CH_2), 51.55, 70.77 (O—C), 102.46, 111.86, 112.92, 113.73, 122.82 (C_{indole}), 127.13 (4C, C_{ph}), 127.29 (C_{indole}), 127.87 (C_{ph}), 128.10, 128.78 (2C, C_{ph}), 131.78 (C_{indole}), 136.75, 140.75, 140.93(C_{ph}), 153.11 (O—C_{indole}), 173.17 (C═O); ESI-HRMS *m*/*z* 429.21640 [M + H].

*4.1.8.2. Methyl 3-(2-(5-(3-phenoxybenzyloxy)-1H-indol-3-yl) ethylamino) propanoate (**5e**).* Yield 72%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.28 (m, 5H), 7.11 (dd, 3H), 6.96 (t, 5H), 5.08 (s, 2H), 3.62 (s, 3H), 2.91 (m, 6H), 2.50 (t, 2H); ^{13}C NMR ($CDCl_3$): δ 25.73, 34.59, 44.96, 49.71 (CH_2), 51.56, 70.61 (O—C), 102.53, 111.84, 112.91, 113.67 (C_{indole}), 117.94, 118.09 (C_{ph}), 118.94 (2C, C_{ph}), 122.28, 122.83 (C_{ph}), 123.28, 127.83 (C_{indole}), 129.75 (2C, C_{ph}), 129.83 (2C, C_{ph}), 131.79 (C_{indole}), 139.79 (C_{ph}), 152.94 (O—C_{indole}), 156.13, 156.45 (O—C_{ph}), 173.17 (C═O); ESI-HRMS *m*/*z* 445.21206 [M + H].

*4.1.8.3. Methyl 3-(2-(5-(4-phenylbenzyloxy)-1H-indol-3-yl) ethylamino) propanoate (**5f**).* Yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H), 7.27 (m, 3H), 7.12 (d, 1H), 6.97 (m, 9H), 4.35 (dq, 4H), 3.62 (s, 3H), 2.94 (m, 6H), 2.51 (t, 2H); ^{13}C NMR ($CDCl_3$): δ 25.74, 34.60, 44.97, 49.77 (CH_2), 51.57, 67.36, 67.64 (O—C), 102.42, 111.88, 112.91, 113.70 (C_{indole}), 115.88, 117.68, 120.78 (2C, C_{ph}), 122.48 (C_{ph}), 122.86, 127.86 (C_{indole}), 129.62 (2C, C_{ph}), 131.86 (C_{indole}), 150.46 (C_{ph}), 152.89 (O—C_{indole}), 155.08, 158.44 (O—C_{ph}), 173.17 (C═O); ESI-HRMS *m*/*z* 475.22250 [M + H].

*4.1.9. General procedure for the preparation of 3-(2-(5-(4-phenylbenzyloxy)-1H-indol-3-yl) ethylamino) propanoic acid (**6d**)*

The compound **5d** (226 mg, 0.527 mmol) and sodium hydroxide (80 mg, 2 mmol) were dissolved in a mixture solution (acetone and water, *v*:*v* = 3:1) and stirred at room temperature for 12 h. The solution was acidified to pH = 1with 1 M hydrochloric acid and extracted with ethyl acetate three times. The organic layer was washed with brine, then dried over Na_2SO_4, concentrated at reduced pressure, and the residue was separated by flash chromatography (silica gel, the eluting solvent ranged from CH_2Cl_2/MeOH = 5:1 to MeOH) as white solid which weighed 107 mg (yield 47%). ^{1}H NMR (400 MHz, DMSO-d6): δ 10.84 (s, 1H), 7.68 (t, 4H), 7.58 (d, 2H), 7.47 (t, 2H), 7.37 (t, 1H), 7.28 (d, 2H), 7.20 (s, 1H), 6.85 (d, 1H), 5.17 (s, 2H), 3.15 (d, 4H), 3.04 (m, 2H), 2.68 (t, 2H); ^{13}C NMR (DMSO-d6): δ 21.89, 30.61, 42.66, 47.11 (CH_2), 69.50 (O—C), 101.90, 109.14, 111.82, 112.15, 124.03 (C_{indole}), 126.62 (4C, C_{ph}), 127.12 (C_{indole}), 127.42 (C_{ph}), 128.30, 128.91 (2C, C_{ph}), 131.60 (C_{indole}), 136.97, 139.48, 139.84 (C_{ph}), 152.06 (O—C_{indole}), 172.08 (C═O); ESI-HRMS *m*/*z* 415.20104 [M + H].

The compounds **6e** and **6f** were synthesized by a similar procedure.

*4.1.9.1. 3-(2-(5-(3-Phenoxybenzyloxy)-1H-indol-3-yl) ethylamino) propanoic acid (**6e**).* White solid, yield 37%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.77 (s, 1H), 7.39 (dd, 3H), 7.24 (m, 2H), 7.13 (dd, 4H), 7.01 (d, 2H), 6.94 (d, 1H), 6.78 (dd, 1H), 5.10 (s, 2H), 2.98 (t, 4H), 2.89 (m, 2H), 2.20 (t, 2H); ^{13}C NMR (DMSO-d6): δ 23.37, 44.32, 47.18 (CH_2), 69.36 (O—C), 101.93, 110.35, 111.70, 112.03 (C_{indole}), 117.40, 117.60 (C_{ph}), 118.67 (2C, C_{ph}), 122.44, 123.49 (C_{ph}), 123.82, 127.24 (C_{indole}), 129.97 (C, C_{ph}), 130.03 (3C, C_{ph}), 131.62 (C_{indole}), 140.16 (C_{ph}), 151.80 (O—C_{indole}), 156.48, 156.71(O—C_{ph}), 173.66 (C═O); ESI-HRMS *m*/*z* 431.19692 [M + H].

*4.1.9.2. 3-(2-(5-(2-(4-Phenoxyphenoxy) ethoxy)-1H-indol-3-yl) ethylamino) propanoic acid (**6f**).* White solid, yield 40%, ^{1}H NMR (400 MHz, DMSO-d6): δ 10.75 (s, 1H), 7.35 (t, 2H), 7.25 (d, 1H), 7.17 (s, 1H), 7.05 (d, 6H), 6.93 (d, 2H), 6.77 (d, 1H), 4.32 (s, 4H), 2.99 (t, 2H), 2.89 (t, 4H), 2.19 (t, 2H); ^{13}C NMR (DMSO-d6): δ 23.44, 30.64, 43.58, 44.32, 47.23 (CH_2), 66.85, 66.98 (O—C), 110.43, 111.56, 112.09, 114.53 (C_{indole}), 115.78, 117.29, 120.69 (2C, C_{ph}), 122.56 (C_{ph}), 123.82, 127.29 (C_{indole}), 129.85 (2C, C_{ph}), 132.60 (C_{indole}), 149.51 (C_{ph}), 151.94 (O—C_{indole}), 154.45, 157.95(O—C_{ph}), 173.03 (C═O); ESI-HRMS *m*/*z* 461.20681 [M + H].

4.2. Biochemical methods

4.2.1. Inhibition assay of aminopeptidase activity of LTA_4H-h

The aminopeptidase activity was determined as described previously [17]. The enzyme was incubated at 37 °C in 96-well microtiter plates in 50 mM Tris—HCl, pH 8.0, containing 100 manacles. The reactions were initiated by the addition of Ala-p-nitroanilides as substrate. The formation of p-NA was then monitored at 37 °C for 15 min at 405 nm using a multi well ultraviolet spectrometer (Spectra Max 190, Molecular Devices). For IC_{50} determinations, 0.125 mM substrate was used. All reactions containing inhibitors were performed after incubating inhibitors with enzymes for 2 min. The final concentration of DMSO was 5% (*v*/*v*). The initial reaction rates at different inhibitor concentrations were used for calculating the IC_{50} values.

4.2.2. Inhibition assay of hydrolase activity of LTA_4H-h

The epoxide hydrolase activity was determined using an ELISA assay to quantify the amount of LTB_4 [17]. LTA_4 methyl ester (Cayman Chemical) was hydrolyzed in cold acetone with 50 mM NaOH (20%, *v*/*v*) under an inert atmosphere of nitrogen. To determine the effect of small molecules on the epoxide hydrolase activity of LTA_4H-h, 300 ng of enzyme was incubated with compounds in reaction buffer (10 mM sodium phosphate, pH 7.4, 4 mg/mL BSA, 5% *v*/*v* DMSO) for 15 min at 37 °C The reaction was initiated by the addition of LTA_4 (150 nM final assay concentration) and incubated for another 10 min at 37 °C. 25 μL sample was added to 500 μL assay buffer without DMSO to stop the reaction and then further diluted fivefold. LTB_4 was quantified in the diluted sample by a commercially available LTB_4 ELISA kit (Cayman Chemical).

4.2.3. Inhibitory activity assay of hnps-PLA_2

Reaction buffer (150 μL in 50 mM Tris—HCl, pH 8.0, 100 mM NaCl, 5 μg/mL bovine serum albumin (BSA), and 10 mM ANS), 20 μL of substrate stock solution [2 mM 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC)] and 2 mM of sodium deoxycholate in water, sonicated for 2 min, 10 μL of $CaCl_2$ stock solution (100 mM), and 10 μL of inhibitor stock solution (dissolved in DMSO) were incubated at 25 °C for 10 min. Reactions were started by adding 10 μL of hnps-PLA_2 stock solution (3.2 μg/mL) and monitored by

excitation at 377 nm and emission at 470 nm using a multiwall fluorometer (SYNERGY 4, BioTek). The initial reaction rates at different inhibitor concentrations were used for calculating the IC_{50} value.

4.3. Molecular docking

All new compounds were docked into the substrate binding site of LTA_4H and PLA_2. The crystal structures of the two enzymes were retrieved from the Protein Data Bank (LTA_4H, PDB ID: 3CHR [32]; PLA_2, PDB ID: 1DB4 [33]). Molecular docking with flexible ligands and rigid receptors was performed using AutoDock 4.00 software. Conformational searching used the Lamarckian genetic algorithm (LGA). Number of GA runs was set to 100, population size to 300, maximum number of energy evaluations to 12,500,000, 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.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.10.057. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

[1] R.H. Schaloske, E.A. Dennis, The phospholipase A2 superfamily and its group numbering system, Biochim. Biophys. Acta. 1761 (2006) 1246–1259.
[2] E.A. Dennis, D.A. Six, The expanding superfamily of phospholipase A(2) enzymes: classification and characterization, Biochim. Biophys. Acta. 1488 (2000) 1–19.
[3] J.Z. Haeggstrom, Structure, function, and regulation of leukotriene A4 hydrolase, Am. J. Respir. Crit. Care Med. 161 (2000) S25–S31.
[4] K.D. Rainsford, Leukotrienes in the pathogenesis of NSAID-induced gastric and intestinal mucosal damage, Agents Actions 39 (1993) C24–C26. Spec No.
[5] M.C. Fishman, J.A. Porter, Pharmaceuticals – a new grammar for drug discovery, Nature 437 (2005) 491–493.
[6] E. Werner, Systems biology: the new darling of drug discovery? Drug Discov. Today 7 (2002) 947–949.
[7] G.G. Moore, K.F. Swingle, 2,6-Di-tert-butyl-4-(2′-thenoyl)phenol(R-830): a novel nonsteroidal anti-inflammatory agent with antioxidant properties, Agents Actions 12 (1982) 674–683.
[8] P.C. Unangst, D.T. Connor, W.A. Cetenko, R.J. Sorenson, C.R. Kostlan, J.C. Sircar, C.D. Wright, D.J. Schrier, R.D. Dyer, Synthesis and biological evaluation of 5-[[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]methylene]oxazoles, -thiazoles, and -imidazoles – novel dual 5-lipoxygenase and cyclooxygenase inhibitors with antiinflammatory activity, J. Med. Chem. 37 (1994) 322–328.
[9] S. Barbey, L. Goossens, T. Taverne, J. Cornet, V. Choesmel, C. Rouaud, G. Gimeno, S. Yannic-Arnoult, C. Michaux, C. Charlier, R. Houssin, J.P. Henichart, Synthesis and activity of a new methoxytetrahydropyran derivative as dual cyclooxygenase-2/5-lipoxygenase inhibitor, Bioorg. Med. Chem. Lett. 12 (2002) 779–782.
[10] S.A. Laufer, J. Augustin, G. Dannhardt, W. Kiefer, (6,7-Diaryldihydropyrrolizin-5-Yl)acetic acids, a novel class of potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase, J. Med. Chem. 37 (1994) 1894–1897.
[11] S. Hibi, Y. Okamoto, K. Tagami, H. Numata, N. Kobayashi, M. Shinoda, T. Kawahara, M. Murakami, K. Oketani, T. Inoue, et al., Novel dual inhibitors of 5-lipoxygenase and thromboxane A2 synthetase: synthesis and structure-activity relationships of 3-pyridylmethyl-substituted 2-amino-6-hydroxybenzothiazole derivatives, J. Med. Chem. 37 (1994) 3062–3070.
[12] R.P. Dickinson, K.N. Dack, J. Steele, Thromboxane modulating agents. 1. Design of 1-[(arylsulfonyl)amino] alkylindole derivatives as dual thromboxane synthase inhibitor thromboxane receptor antagonists, Bioorg. Med. Chem. Lett. 5 (1995) 3017–3022.
[13] O. Werz, A. Koeberle, H. Zettl, C. Greiner, M. Wurglics, M. Schubert-Zsilavecz, Pirinixic acid derivatives as novel dual inhibitors of microsomal prostaglandin E(2) synthase-1 and 5-lipoxygenase, J. Med. Chem. 51 (2008) 8068–8076.
[14] Z. Chen, Y.R. Wu, Y. Liu, S.J. Yang, Y.J. Chen, L.H. Lai, Discovery of dual target inhibitors against cyclooxygenases and leukotriene A(4) hydrolyase, J. Med. Chem. 54 (2011) 3650–3660.
[15] R. Morphy, C. Kay, Z. Rankovic, From magic bullets to designed multiple ligands, Drug Discov. Today 9 (2004) 641–651.
[16] K. Yang, H.J. Bai, Q. Ouyang, L.H. Lai, C. Tang, Finding multiple target optimal intervention in disease-related molecular network, Mol. Syst. Biol. 4 (2008) 228.
[17] D.G. Wei, X.L. Jiang, L. Zhou, J. Chen, Z. Chen, C. He, K. Yang, Y. Liu, J.F. Pei, L.H. Lai, Discovery of multitarget inhibitors by combining molecular docking with common pharmacophore matching, J. Med. Chem. 51 (2008) 7882–7888.
[18] M. Berger, J.A. Gray, B.L. Roth, The expanded biology of serotonin, Annu. Rev. Med. 60 (2009) 355–366.
[19] S.J. Peroutka, B.G. McCarthy, X.M. Guan, 5-benzyloxytryptamine: a relatively selective 5-hydroxytryptamine 1D/1B agent, Life Sci. 49 (1991) 409–418.
[20] M.G. Buzzi, M.A. Moskowitz, S.J. Peroutka, B. Byun, Further characterization of the putative 5-HT receptor which mediates blockade of neurogenic plasma extravasation in rat dura mater, Br. J. Pharmacol. 103 (1991) 1421–1428.
[21] H.G. Breitinger, R. Wieboldt, D. Ramesh, B.K. Carpenter, G.P. Hess, Synthesis and characterization of photolabile derivatives of serotonin for chemical kinetic investigations of the serotonin 5-HT(3) receptor, Biochemistry 39 (2000) 5500–5508.
[22] J.Z. Haeggstrom, Leukotriene A4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B4 biosynthesis, J. Biol. Chem. 279 (2004) 50639–50642.
[23] I.R. Ollmann, J.H. Hogg, B. Munoz, J.Z. Haeggstrom, B. Samuelsson, C.H. Wong, Investigation of the inhibition of leukotriene A4 hydrolase, Bioorg. Med. Chem. 3 (1995) 969–995.
[24] T.D. Penning, N.S. Chandrakumar, B.B. Chen, H.Y. Chen, B.N. Desai, S.W. Djuric, S.H. Docter, A.F. Gasiecki, R.A. Haack, J.M. Miyashiro, M.A. Russell, S.S. Yu, D.G. Corley, R.C. Durley, B.F. Kilpatrick, B.L. Parnas, L.J. Askonas, J.K. Gierse, E.I. Harding, M.K. Highkin, J.F. Kachur, S.H. Kim, G.G. Krivi, D. Villani-Price, E.Y. Pyla, W.G. Smith, Structure-activity relationship studies on 1-[2-(4-phenylphenoxy)ethyl]pyrrolidine (SC-22716), a potent inhibitor of leukotriene A(4) (LTA(4)) hydrolase, J. Med. Chem. 43 (2000) 721–735.
[25] C.K. Huang, L. Zhou, Y. Liu, L.H. Lai, A continuous fluorescence assay for phospholipase A2 with nontagged lipid, Anal. Biochem. 351 (2006) 11–17.
[26] R.D. Dillard, N.J. Bach, S.E. Draheim, D.R. Berry, D.G. Carlson, N.Y. Chirgadze, D.K. Clawson, L.W. Hartley, L.M. Johnson, N.D. Jones, E.R. McKinney, E.D. Mihelich, J.L. Olkowski, R.W. Schevitz, A.C. Smith, D.W. Snyder, C.D. Sommers, J.P. Wery, Indole inhibitors of human nonpancreatic secretory phospholipase A2. 1. Indole-3-acetamides, J. Med. Chem. 39 (1996) 5119–5136.
[27] P. Csermely, T. Korcsmaros, M.S. Szalay, B. Csaba, I.A. Kovacs, How to design multi-target drugs: target search options in cellular networks, Expert Opin. Drug Dis. 2 (2007) 799–808.
[28] M.A. Rogawski, Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents – toward an understanding of their favorable tolerability, Amino Acids 19 (2000) 133–149.
[29] G.C. Palmer, E.F. Cregan, P. Bialobok, S.G. Sydserff, T.J. Hudzik, D.J. McCarthy, The low-affinity, use-dependent NMDA receptor antagonist AR-R 15896AR – an update of progress in stroke, Ann. N.Y. Acad. Sci. 890 (1999) 406–420.
[30] A.L. Mueller, L.D. Artman, M.F. Balandrin, E. Brady, Y. Chien, E.G. Delmar, K. George, A. Kierstead, T.B. Marriott, S.T. Moe, M.K. Newman, J.L. Raszkiewicz, E.L. Sanguinetti, B.C. van Wagenen, D. Wells, NPS 1506, a novel NMDA receptor antagonist and neuroprotectant. Review of preclinical and clinical studies, Ann. N.Y. Acad. Sci. 890 (1999) 450–457.
[31] S.A. Lipton, Turning down, but not off, Nature 428 (2004) 473.
[32] T.A. Kirkland, M. Adler, J.G. Bauman, M. Chen, J.Z. Haeggstrom, B. King, M.J. Kochanny, A.M. Liang, L. Mendoza, G.B. Phillips, M. Thunnissen, L. Trinh, M. Whitlow, B. Ye, H. Ye, J. Parkinson, W.J. Guilford, Synthesis of glutamic acid analogs as potent inhibitors of leukotriene A4 hydrolase, Bioorg. Med. Chem. 16 (2008) 4963–4983.
[33] R.W. Schevitz, N.J. Bach, D.G. Carlson, N.Y. Chirgadze, D.K. Clawson, R.D. Dillard, S.E. Draheim, L.W. Hartley, N.D. Jones, E.D. Mihelich, et al., Structure-based design of the first potent and selective inhibitor of human non-pancreatic secretory phospholipase A2, Nat. Struct. Biol. 2 (1995) 458–465.