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Synthesis and Biological Evaluation of *N*-Aryl-5-aryloxazol-2-amine Derivatives as 5-Lipoxygenase InhibitorsJee Hee Suh,^a Eul Kgun Yum,^b and Young Sik Cho^{*c}

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We describe the synthesis and biological evaluation of *N*-aryl-5-aryloxazol-2-amine derivatives that are able to inhibit 5-lipoxygenase (5-LOX), a key enzyme of leukotriene synthesis, for the treatment of inflammation-related diseases including asthma and rheumatoid arthritis. A novel structural moiety containing oxazole was initially identified from a chemical library using an *in vitro* enzymatic and cell-based assay, and its synthesized oxazole derivatives were further examined to develop a structure–activity relationship (SAR). SAR analysis demonstrated that a hydroxyl or amino group at the *p*-position on *N*-phenyl was essential for the 5-LOX-inhibitory activities of the derivatives, and that other halogen and methyl group-substituted derivatives affected the potency, positively or negatively. As a result, derivatives selected through first-round screening were further optimized using a cell-based assay and an *in vivo* assay to develop a potent, selective 5-LOX inhibitor. A final hit exhibited an improved efficacy in arachidonic acid-induced ear edema when applied topically but not orally. Moreover, it showed the additional advantage of sustainable antiinflammatory activity over a reference compound, zileuton. Taken together, chemical entities bearing an oxazole scaffold could be promising as therapeutic drugs for the treatment of chronic inflammatory skin disorders.

Key words 5-lipoxygenase; antiinflammatory activity; oxazol-2-amine; leukotriene

In response to external stresses, several enzymes are involved in the production of inflammatory chemical mediators from arachidonic acid (AA) that is released from the cytoplasmic membrane.¹⁾ Specifically, prostaglandins (PGs) and leukotrienes (LTs) are, respectively, formed from an AA substrate, catalyzed by two individual enzymes, cyclooxygenase (COX) and lipoxygenase (LOX), at the bifurcation of the metabolic pathway.^{2,3)} Initial approaches targeting COX-2 selectively in an effort to develop anti-inflammatory drugs proved to be inappropriate due to their adverse cardiac effects.⁴⁾ Alternative ways to control inflammatory mediators have been pursued for the treatment of inflammation-related diseases. The 5-lipoxygenase (5-LOX) is a key enzyme in the first step of leukotriene (LT) synthesis, and its dysregulation causes various inflammatory diseases, such as atopy and asthma.^{5,6)} Among the LTs, leukotriene B₄ (LTB₄) is a key chemoattractant for neutrophils recruitment, a hallmark of acute inflammation⁷⁾ and the others, termed collectively the “cysteinyl LTs,” act as the slowly reacting substances of anaphylaxis.⁸⁾ Thus, 5-LOX inhibitors that are able to block LTs production could be effective therapeutic agents to treat inflammatory diseases. Presently, only zileuton has been approved by the Food and Drug Administration (FDA) as a direct 5-LOX inhibitor.⁹⁾ However, there are many anti-inflammatory drugs based on the abrogation of the ligand–receptor interaction or indirect interference in the process of 5-LOX activation.^{10–12)}

Recently, there has been special interest on thiazole, imidazole, and oxazole moieties due to their roles as pharmacophores in various biologically active natural products or potential medicinal agents. It has been already reported that oxazole derivatives have anti-inflammatory activity, and leading eventually to the synthesis of the novel bicyclic system oxazole

[3,2-*a*]-s-triazine-5,7-dione, which shows anti-inflammatory activity in a carrageenan-induced edema test.¹³⁾ Additionally, 4,5-diaryloxazole has been designed as a novel anti-inflammatory drug, based on the docking method of interaction between oxaprozin and cyclooxygenase-2.¹⁴⁾ In addition to the anti-inflammatory activity of oxazoles, their derivatives have been documented to have a wide range of biological activities, such as analgesic, antibacterial, antiproliferative, and hypoglycemic activity.^{15–18)}

In an effort to develop small molecules against 5-LOX, chemicals bearing oxazole scaffolds as a bioisostere of thiazole were explored from chemical libraries deposited at Korea Research Institute of Chemical Technology (KRICT, Daejeon, South Korea) using an enzymatic screening assay as described previously.^{19,20)} As a result, we identified *N*-aryl-5-aryloxazol-2-amine derivatives that could inhibit 5-LOX catalytic activity potently. Accordingly, a series of *N*-aryl-5-aryloxazol-2-amine derivatives was synthesized, and tested in an *in vitro* enzymatic assay to determine the structure–activity relationship (SAR) of those novel 5-LOX inhibitors. Some compounds with potent inhibitory activity were finally confirmed in an ear-edema mouse model after topical or oral administration, and they were assessed for sustained efficacy after topical application.

Results and Discussion

Chemistry *N*-Aryl-5-aryloxazol-2-amine derivatives were prepared by cyclization of isothiocyanate (**1**) with α -azidoacetophenones (**2**) in the presence of triphenyl phosphine in refluxing dioxane, as shown in Chart 1. The compound **3–24** were synthesized with substituent variations at the phenyl groups.

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(a) Triphenyl phosphine, dioxane, reflux, 40–82%.

Chart 1

Table 1. SAR Data on *N*-Aryl-5-aryloxazol-2-amine Derivatives for 5-LOX Inhibitors

Compounds	R ₁	R ₂	X	<i>In vitro</i> assay ^{a)}	
				% inhibition at 1 μM	IC ₅₀ ^{b)} (nM)
Zileuton				78	180
3	4-OH	H	H	88	92
4	4-OH	4-Cl	H	76	120
5	4-OH	3,4-(Cl) ₂	H	91	140
6	4-OH	4-OCH ₃	H	89	84
7	4-OH	4-F	H	97	330
8	4-OH	2,4-(F) ₂	H	97	172
9	4-OH	4-F	3,5-(CH ₃) ₂	82	120
10	4-OH	4-F	3,5-(Cl) ₂	82	65
11	4-NH ₂	H	H	85	154
12	4-NH ₂	4-Cl	H	91	105
13	4-NH ₂	3,4-(Cl) ₂	H	79	220
14	4-NH ₂	4-F	H	94	95
15	4-NH ₂	2,4-(F) ₂	H	100	110
16	4-NH ₂	4-F	3,5-(Cl) ₂	50	790
17	3-NH ₂	4-Cl	H	58	690
18	4-NHAc	4-Cl	H	47	1250
19	4-NHAc	4-F	H	49	907
20	4-OAc ₃	4-F	H	84	180
21	4-OAc	2,4-(F) ₂	H	89	190
22	4-OAc	3,4-(Cl) ₂	H	51	560
23	3-CO ₂ H	3-NO ₂	H	2	ND
24	3-CO ₂ H	3-NH ₂	H	4	ND ^{c)}

^{a)} 5-LOX enzymatic activities were examined in the reaction mixtures including chemical derivatives at 1 μM, and compared with the vehicle control. ^{b)} IC₅₀ values were calculated by fitting the dose–response curves for % inhibition using the GraFit software. ^{c)} ND, not determined.

Biological Activity The synthesized *N*-aryl-5-aryloxazol-2-amine derivatives were preliminarily tested with an *in vitro* enzymatic assay. The SAR results of the derivatives are summarized in Table 1. Our previous SAR studies on *N*-aryl-4-aryl-1,3-thiazol-2-amine derivatives showed that either –OH or –NH₂ group at the 4-position of *N*-phenyl group was essential for inhibitory activity against 5-LOX.²¹⁾ In that article, we first presented 4-aryl-*N*-(4-hydroxyphenyl)thiazol-2-amine derivatives as a specific inhibitor of 5-LOX, subsequently leading to the considerable suppression of inflammatory responses when given topically, but not orally. Likewise, a series of *N*-(4-hydroxyphenyl)oxazol-2-amine derivatives bearing 5-phenyl substituents showed good inhibitory effects against 5-LOX (compounds **3–8**), indicating that electronic effects of 5-phenyl substituents did not significantly affect inhibitory potencies of oxazole scaffolds.

The introduction of dimethyl or dichloro group into *N*-(4-

hydroxyphenyl)-5-(4-fluorophenyl)oxazol-2-amine derivatives increased inhibitory activity significantly when compared with corresponding unsubstituted derivatives (compounds **7**, **9**, **10**). Second, the inhibitory potencies of derivatives bearing 4-amino-*N*-phenyl moiety (compounds **11–15**) were further examined for varying R₂ substituents. In fact, the inhibitory activities of the series of 5-phenyl substituents were not significantly altered. However, the replacement of 4-aminophenyl with 3-aminophenyl negatively affected its inherent inhibitory activity, leading to a 6-fold increase in IC₅₀ compared with 4-aminophenyl derivatives (compound **17**). Additionally, the placement of NHAc at position R₁ decreased severely the ability to inhibit 5-LOX (compounds **18**, **19**). However, a 4-OAc group attached to position R₁ did not affect the inhibitory activities so much (compounds **20–22**), although compound **22** bearing 3,4-dichloro at R₂ position exhibited a higher IC₅₀ value than **20** and **21**. The 4-OAc substituents at R₁ position

were still potent, unlike the counterpart of thiazole scaffold as described above. Moreover, placing a 3-CO₂H group at the R₁ position abrogated completely the 5-LOX inhibitory potency (compounds **23**, **24**). Accordingly, substituent variations of R₁ bonded to *N*-phenyl were important for conferring 5-LOX inhibitory activity on the skeleton, while various substituents at R₂ or X may affect the inhibitory activity.

Some derivatives drawn from the first-round *in vitro* screening were further examined to see whether such compounds could prevent cells from generating leukotriene B₄ (LTB₄) after stimulation of rat basophilic leukemia-1 (RBL-1) cells with A23187, a calcium ionophore (Table 2). The cell-based assays were carried out according to methods reported previously.²²⁾

Cell-based assay showed that some selected derivatives (compounds **7**, **9–11**) were comparable or superior to zileuton by criteria of IC₅₀s, demonstrating that modifications of the R₂ side group suppressed effectively the production of LTB₄ in A23187-stimulated cells. In contrast with *in vitro* enzymatic results, the cell-based assay showed that *N*-(4-hydroxyphenyl)oxazol-2-amine derivative was less potent than *N*-(4-aminophenyl)oxazol-2-amine derivatives counterpart with respect to interfering with LTB₄ production (compounds **3**, **11**; data not shown). Intriguingly, cellular efficacies of chemical derivatives were examined with substituent groups varied at R₂, reasoning that compounds' cellular uptake and intracellular metabolism might be of primary concern in keeping the *in vitro* potency in a cellular format. In effect, addition of a methyl group to the aminophenyl or hydroxyphenyl moiety enhanced the inhibitory activity of the parent compound whereas substitution with a halogen group less-

ened anti-inflammatory potency, compared with the methyl group-bearing derivatives (compounds **9**, **10**), indicating that the introduction of the halogen group may interfere with cell membrane permeability or may facilitate cellular metabolism of those compounds although its underlying mode of action remains to be determined.

Subsequently, *in vivo* efficacy studies demonstrated that some compounds under investigation showed suppressive effects on AA-induced edema when administered topically, but not orally. To induce inflammatory responses, AA (2 mg in 20 μ L acetone) was painted to the inner surface of the right ear of a BALB/c mouse. The administration scheme was performed in the same way as described before.²³⁾ As an example, oral administration of compound **9** to mice exerted lower anti-inflammatory activity than that of zileuton (Table 3), suggesting that derivatives bearing oxazole as a pharmacophore moiety have poor bioavailability as those bearing thiazole in rats.²³⁾ Despite potent *in vitro* inhibitory profiles of selected compounds that were comparable to zileuton, their relatively low *in vivo* efficacies with oral administration may be explained partly by their poor bioavailability. In contrast, topical application of these same derivatives in mice improved the inflammatory conditions considerably, such as ear swelling and myeloperoxidase (MPO) activity following AA painting onto the ear (Table 3). In particular, compound **9** had more potent anti-inflammatory activity than any other compounds when given topically. Similarly, as shown previously in *N*-aryl-4-aryl-1,3-thiazol-2-amine derivatives, compound **9** showed a more sustainable effect against AA-induced inflammation than zileuton. In fact, the anti-inflammatory activity of compound **9** was still substantial (approximately 50% inhibition) at 2 d after topical treatment, whereas the effects of zileuton were significantly reduced even at 1 d of treatment (Fig. 1). It is suggested that compound **9** keeps staying longer at the painting spot or was metabolically stable, compared with zileuton.

As with the poor bioavailability of 4-[4-(4-fluoro-phenyl)-thiazol-2-ylamino]-2,6-dimethyl-phenol (KR-33749) (2% bioavailability in rat), small molecules with aromatic amino and hydroxyl groups are subject to extensive metabolism in the liver.^{23–25)} For example, one of the non-specific LOX inhibitors, nordihydroguaiaretic acid (NDGA), has been reported to display only topical activity in reducing both AA-induced edema and infiltration of inflammatory cells into inflamed areas.²⁶⁾ Thus, anti-inflammatory compounds designed for potent topical application can be further tested in the inflammation-related skin lesions, such as atopic dermatitis and other chronic inflammatory diseases.

Table 2. Inhibitory Activities of Potent Derivatives Selected through the First-Round Screening against LTB₄ Production in A23187-Stimulated RBL Cells

Compounds	% inhibition at 1 μ M ^{a)}	IC ₅₀ (nM)
Zileuton	77	430
3	31	ND
7	90	279
9	97	104
10	70	352
11	85	384

a) Cell-based assay to determine LTB₄ amounts released in RBL-1 cells stimulated with A23187. Cells were preloaded with selected compounds prior to A23187 stimulation to compare their inhibitory potencies. LTB₄ levels, indicative of 5-LOX activity, were measured quantitatively according to the protocol provided by the manufacturer.

Table 3. Comparative *in Vivo* Efficacy of Selected Compounds against AA-Induced Edema *via p.o.* or Topical Route

Compounds	Administration routes ^{a)}			
	Oral administration (% inhibition)		Topical administration (% inhibition)	
	Ear thickness	Myeloperoxidase	Ear thickness	Myeloperoxidase
Zileuton	75 $\pm$ 12	82 $\pm$ 20	43 $\pm$ 7	63 $\pm$ 8
3	18 $\pm$ 6	12 $\pm$ 10	39 $\pm$ 6	58 $\pm$ 13
7	30 $\pm$ 10	20 $\pm$ 5	49 $\pm$ 7	66 $\pm$ 12
9	44 $\pm$ 16	34 $\pm$ 8	58 $\pm$ 10	80 $\pm$ 10

a) For *p.o.* administration, compounds and zileuton (50 mpk) were given to the mice 30 min before AA painting (2 mg in 20 μ L acetone) on the right ear. Also, test compounds were applied topically at a dose of 500 μ g. At 1 h after AA application, the ear thickness of the inflamed right ear was measured, and then the inflamed ears were taken for MPO activity measurement. Efficacy of the drug was evaluated by relative suppression of ear thickness and MPO activity in inflamed ears of treated group vs. vehicle group.

Fig. 1. The Sustainable Anti-inflammatory Potency of Compound 9 in an AA-Induced Ear Edema Model

To determine the sustainable action of compound 9, test compounds (500 μ g) were applied and for 48, 24, or 0.5 h. Following the indicated incubation times, ears were stimulated with AA and then inflammatory indicators, such as ear thickness and MPO activity, were measured 1 h later. Duration of test and reference compound was monitored by % anti-inflammatory activity with pretreatment times.

Conclusion

We identified an oxazole-bearing skeleton with anti-inflammatory activity targeting 5-LOX. A few compounds (compounds 3, 7, 9) were more effective against AA-induced ear edema than zileuton when applied topically, but not orally. Judged from *in vivo* topical efficacy data of compound 9 with its sustainable activity, it may be applicable to a wide range of skin models associated with 5-LOX in the future. To define more comprehensively molecular mechanism of a compound 9, a controlled pilot study for clarifying redox or non-redox inhibitory types will be carried out and its selectivity for 5-LOX over other LOX isozymes will be further examined.

Experimental

General Information All the commercially available chemicals were obtained from Aldrich, Sigma, Fluka, and Junsei Chemical Company and used generally without further purification. Solvents including ethyl acetate and *n*-hexane for column chromatography were of technical grade and distilled before use.

The ^1H -NMR spectra (300 MHz ^1H) were recorded on Bruker Fourier Transform AG-300 and Varian Fourier Transform-300 instrument in chloroform- d_3 (CDCl_3) or dimethyl sulfoxide- d_6 ($\text{DMSO}-d_6$). Chemical shifts were reported in δ units (ppm), using tetramethylsilane (TMS) as internal standard. The following abbreviations were used to describe peak patterns when appropriate: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constant (J) was reported in Hertz unit (Hz). Mass spectra were recorded on a Varian 1200L GC/MS using electron impact (EI) method.

All reactions were monitored using thin layer chromatography (TLC) on silica gel glass plates (E. Merck, TLC silica gel 60F $_{254}$). Visualization on TLC was achieved by UV light (254 nm), typical TLC indicating solution (*p*-anisaldehyde), iodine and polymolybdic acid indicator. Flash column chromatography was carried out using Merck 230-400 mesh ASTM.

Synthetic Procedure for the Preparation of *N*-Aryl-5-aryloxazol-2-amine Derivatives

(a) Preparation of 2-Azido-1-phenylethan-1-one

Small amount of 2-bromoacetophenone (1.00 g, 5.02 mmol) was first dissolved in 15 mL of acetone, and 0.39 g (6.03 mmol) of sodium azide was added, and then kept at room temperature for 2 h. After the reaction was completed, the reaction mixture was diluted with 20 mL of ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and finally concentrated under a reduced pressure. The residues were purified using silica gel column chromatography (hexane:ethyl acetate=10:1) to obtain the 2-azido-1-phenylethan-1-one (0.66 g, 82%). ^1H -NMR (300 MHz, CDCl_3) δ : 4.57 (s, 2H), 7.50 (dd, J =7.5 Hz, 7.4 Hz, 2H), 7.63 (dd, J =7.4 Hz, 7.4 Hz, 1H), 7.91 (d, J =7.5 Hz, 2H).

(b) Synthesis of 4-((5-Phenyloxazol-2-yl)amino)phenol (3)

The resulting compound 2-azido-1-phenylethan-1-one (0.31 g, 1.91 mmol) was dissolved in 10 mL of 1,4-dioxane. The triphenyl phosphine (0.55 g, 2.09 mmol) and 4-hydroxy phenyl isothiocyanate (0.26 g, 1.74 mmol) were added at room temperature, and the mixture was refluxed at 80°C for 1 h. After the reaction was completed, the reaction mixture was diluted with 10 mL of ethyl acetate, washed with water, dried over anhydrous magnesium sulfate, and concentrated under a reduced pressure. The residues were purified using silica gel column chromatography (hexane:ethyl acetate=2:1) to obtain the final product 3 (0.25 g, 56%). ^1H -NMR (300 MHz, $\text{DMSO}-d_6$) δ : 6.71 (d, J =9.0 Hz, 2H), 7.25 (dd, J =7.5 Hz, 7.5 Hz, 1H), 7.40 (m, 5H), 7.55 (d, J =7.5 Hz, 2H), 9.03 (s, 1H), 9.86 (s, 1H); MS (EI) m/z 252 [M] $^+$.

The following oxazole-bearing derivatives were prepared according to the general procedures described above by employing the appropriate 2-azido-1-phenylethan-1-one and phenyl isothiocyanate.

4-((5-(4-Chlorophenyl)oxazol-2-yl)amino)phenol (4)

^1H -NMR (300 MHz, $\text{DMSO}-d_6$) δ : 6.64 (d, J =8.9 Hz, 2H), 7.32 (d, J =8.9 Hz, 2H), 7.38 (s, 1H), 7.39 (d, J =8.6 Hz, 2H), 7.48 (d, J =8.6 Hz, 2H), 8.98 (s, 1H), 9.90 (s, 1H); MS (EI) m/z 287 [M] $^+$.

4-((5-(3,4-Dichlorophenyl)oxazol-2-yl)amino)phenol (5)

^1H -NMR (300 MHz, $\text{DMSO}-d_6$) δ : 6.73 (d, J =8.7 Hz, 2H), 7.40 (d, J =8.7 Hz, 2H), 7.50 (d, J =8.4 Hz, 1H), 7.52 (s, 1H), 7.67

(d, $J=8.4$ Hz, 1H), 7.78 (s, 1H), 9.11 (s, 1H), 10.04 (s, 1H); MS (EI) m/z 321 [M]⁺.

4-((5-(4-Methoxyphenyl)oxazol-2-yl)amino)phenol (6) ¹H-NMR (300 MHz, DMSO- d_6) δ : 3.78 (s, 3H), 6.70 (d, $J=8.8$ Hz, 2H), 6.98 (d, $J=8.8$ Hz, 2H), 7.22 (s, 1H), 7.39 (d, $J=8.7$ Hz, 1H), 7.47 (d, $J=8.7$ Hz, 1H), 9.01 (s, 1H), 9.83 (s, 1H); MS (EI) m/z 282 [M]⁺.

4-((5-(4-Fluorophenyl)oxazol-2-yl)amino)phenol (7) ¹H-NMR (300 MHz, DMSO- d_6) δ : 6.72 (d, $J=8.9$ Hz, 2H), 7.05–7.42 (m, 5H), 7.56–7.61 (m, 2H), 9.05 (s, 1H), 9.91 (s, 1H); MS (EI) m/z 270 [M]⁺.

4-((5-(2,4-Difluorophenyl)oxazol-2-yl)amino)phenol (8) ¹H-NMR (300 MHz, DMSO- d_6) δ : 6.73 (d, $J=9.0$ Hz, 2H), 7.22–7.24 (m, 2H), 7.37–7.43 (m, 3H), 7.60 (m, 1H), 9.08 (s, 1H), 10.03 (s, 1H); MS (EI) m/z 288 [M]⁺.

4-((5-(4-Fluorophenyl)oxazol-2-yl)amino)-3,5-dimethylphenol (9) ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.21 (s, 6H), 7.28 (m, 2H), 7.44 (s, 1H), 7.58–7.65 (m, 4H), 9.68 (s, 1H), 10.41 (s, 1H); MS (EI) m/z 298 [M]⁺.

4-((5-(4-Fluorophenyl)oxazol-2-yl)amino)-3,5-dichlorophenol (10) ¹H-NMR (300 MHz, DMSO- d_6) δ : 7.28 (m, 2H), 7.44 (s, 1H), 7.58–7.65 (m, 4H), 9.68 (s, 1H), 10.41 (s, 1H); MS (EI) m/z 339 [M]⁺.

***N*¹-(5-Phenylloxazol-2-yl)benzene-1,4-diamine (11)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.75 (br, 2H), 6.54 (d, $J=8.7$ Hz, 2H), 7.21–7.29 (m, 3H), 7.36–7.43 (m, 3H), 7.53 (d, $J=8.0$ Hz, 2H), 9.72 (s, 1H); MS (EI) m/z 251 [M]⁺.

***N*¹-(5-(4-Chlorophenyl)oxazol-2-yl)benzene-1,4-diamine (12)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.76 (br, 2H), 6.52 (d, $J=8.6$ Hz, 2H), 7.24 (d, $J=8.6$ Hz, 2H), 7.41 (s, 1H), 7.47 (d, $J=9.3$ Hz, 2H), 7.52 (d, $J=9.3$ Hz, 2H), 9.76 (s, 1H); MS (EI) m/z 285 [M]⁺.

***N*¹-(5-(3,4-Dichlorophenyl)oxazol-2-yl)benzene-1,4-diamine (13)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.80 (br, 2H), 6.54 (d, $J=8.6$ Hz, 2H), 7.25 (d, $J=8.6$ Hz, 1H), 7.43 (d, $J=8.4$ Hz, 1H), 7.56 (s, 1H), 7.66 (d, $J=8.4$ Hz, 1H), 7.77 (s, 1H), 9.83 (s, 1H); MS (EI) m/z 320 [M]⁺.

***N*¹-(5-(4-Fluorophenyl)oxazol-2-yl)benzene-1,4-diamine (14)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.76 (br, 2H), 6.54 (d, $J=8.6$ Hz, 2H), 7.23–7.32 (m, 4H), 7.34 (s, 1H), 7.55–7.59 (m, 2H), 9.71 (s, 1H); MS (EI) m/z 269 [M]⁺.

***N*¹-(5-(2,4-Difluorophenyl)oxazol-2-yl)benzene-1,4-diamine (15)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.78 (br, 2H), 6.55 (d, $J=8.6$ Hz, 2H), 7.19–7.29 (m, 4H), 7.37 (m, 1H), 7.58 (m, 1H), 9.83 (s, 1H); MS (EI) m/z 287 [M]⁺.

3,5-Dichloro-*N*¹-(5-(4-fluorophenyl)oxazol-2-yl)benzene-1,4-diamine (16) ¹H-NMR (300 MHz, DMSO- d_6) δ : 5.13 (br, 2H), 7.27 (m, 2H), 7.41 (s, 1H), 7.56–7.61 (m, 4H), 10.21 (s, 1H); MS (EI) m/z 337 [M]⁺.

***N*¹-(5-(4-Chlorophenyl)oxazol-2-yl)benzene-1,3-diamine (17)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 5.07 (br, 2H), 6.18 (d, $J=7.8$ Hz, 1H), 6.70 (d, $J=7.8$ Hz, 1H), 6.89–6.94 (m, 2H), 7.47–7.60 (m, 5H), 10.00 (s, 1H); MS (EI) m/z 286 [M]⁺.

***N*-(4-((5-(4-Chlorophenyl)oxazol-2-yl)amino)phenyl)acetamide (18)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.00 (s, 3H), 7.48–7.61 (m, 9H), 9.82 (s, 1H), 10.24 (s, 1H); MS (EI) m/z 327 [M]⁺.

***N*-(4-((5-(4-Fluorophenyl)oxazol-2-yl)amino)phenyl)acetamide (19)** ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.02 (s, 3H), 7.29 (dd, $J=8.9$ Hz, 8.7 Hz, 2H), 7.42 (s, 1H), 7.49–7.64 (m, 6H), 9.82 (s, 1H), 10.20 (s, 1H); MS (EI) m/z 311 [M]⁺.

4-((5-(4-Fluorophenyl)oxazol-2-yl)amino)phenyl Acetate (20) ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.09 (s, 3H), 7.07 (d, $J=8.7$ Hz, 2H), 7.29 (m, 2H), 7.43 (s, 1H), 7.60–7.70 (m, 4H), 10.38 (s, 1H); MS (EI) m/z 312 [M]⁺.

4-((5-(2,4-Difluorophenyl)oxazol-2-yl)amino)phenyl Acetate (21) ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.26 (s, 3H), 7.09 (s, 2H), 7.21–7.31 (m, 2H), 7.44 (m, 1H), 7.61–7.67 (m, 3H), 10.49 (s, 1H); MS (EI) m/z 330 [M]⁺.

4-((5-(3,4-Dichlorophenyl)oxazol-2-yl)amino)phenyl Acetate (22) ¹H-NMR (300 MHz, DMSO- d_6) δ : 2.25 (s, 3H), 7.09 (d, $J=8.7$ Hz, 2H), 7.53 (d, $J=8.4$ Hz, 1H), 7.63–7.71 (m, 4H), 7.83 (s, 1H), 10.49 (s, 1H); MS (EI) m/z 363 [M]⁺.

3-((5-(3-Nitrophenyl)oxazol-2-yl)amino)benzoic Acid (23) ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.35 (br, 2H), 7.25 (m, 1H), 7.36 (d, $J=7.5$ Hz, 1H), 7.55 (m, 1H), 7.62 (s, 1H), 7.64 (m, 1H), 7.84 (d, $J=7.8$ Hz, 1H), 7.91 (d, $J=9.6$ Hz, 1H), 8.13 (s, 1H), 8.18 (s, 1H), 10.50 (s, 1H); MS (EI) m/z 325 [M]⁺.

3-((5-(3-Aminophenyl)oxazol-2-yl)amino)benzoic Acid (24) ¹H-NMR (300 MHz, DMSO- d_6) δ : 4.35 (br, 2H), 7.25 (m, 1H), 7.36 (d, $J=7.8$ Hz, 1H), 7.55 (m, 1H), 7.62 (s, 1H), 7.63 (m, 1H), 7.84 (d, $J=7.8$ Hz, 1H), 7.91 (d, $J=9.6$ Hz, 1H), 8.13 (s, 1H), 8.18 (s, 1H), 10.50 (s, 1H); MS (EI) m/z 295 [M]⁺.

Materials Xylenol, iron sulfate, ATP, hexadecyltrimethyl ammonium bromide (HTAB), α -phosphatidyl choline (PC), and zileuton were obtained from Sigma (St. Louis, MO, U.S.A.). Arachidonic acid (AA) and A23187 were obtained from Calbiochem (La Jolla, CA, U.S.A.). The leukotriene B₄ (LTB₄) enzyme immunoassay (EIA) kit was obtained from Amersham (NJ, U.S.A.). A series of chemical derivatives was synthesized as described below. All other reagents were of analytical grade and from Sigma.

Cell Culture and Animal Care RBL-1 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.) and maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum, glutamine, penicillin, and streptomycin. For *in vivo* efficacy, BALB/c mice (males, 8 weeks old) were bred, and cared for in a barrier system in a temperature- and humidity-controlled room with a 12/12-h light/dark cycle, and acclimated for 1 week before experiments. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Chemical Technology (Daejeon, South Korea).

***In Vitro* 5-LOX Assay** A 5-LOX enzyme assay was carried out with some modifications of the ferric oxidation of xylenol orange (FOX) assay, which is based on the complex formation of Fe³⁺/xylenol orange with absorption at visible wavelengths 19, 20). The enzyme sources were prepared from insect cell (Sf21) lysates highly expressing rat 5-LOX as described previously. Cell lysates (15 μ g protein) were preincubated with various concentrations of either some selected chemicals or zileuton (in DMSO) in 50 μ L of 50 mM Tris-HCl buffer (pH 7.4) containing 0.4 mM CaCl₂, 24 μ g/mL PC and 20 μ M ATP at room temperature for 4 min. The reactions were started by the addition of 40 μ M AA, kept for another 4 min, and terminated by the addition of 100 μ L FOX reagent: sulfuric acid (25 mM), xylenol orange (100 μ M), iron(II) sulfate (100 μ M), methanol–water (9:1, v/v). A blank assay was done with no substrate in the incubation.

LTB4 Secretion in RBL-1 Cells Activated by A23187

A cell-based assay was carried out as reported previously.²²⁾ Briefly, RBL-1 cells were plated at a density of 4×10^4 cells in 96-well plates. Increasing doses of compounds or zileuton dissolved in DMSO (final 0.5%) were added to plates and incubation for 10 min at 37°C prior to the stimulation with the Ca^{2+} ionophore A23187 (final $10 \mu\text{M}$ in DMSO). After further incubation for 15 min, culture media were collected and LTB4 content in the supernatant was measured using an EIA kit (Amersham, NJ, U.S.A.).

Arachidonic Acid-Induced Ear Inflammation in Mice

To induce an inflammatory response, 2 mg of AA in $20 \mu\text{L}$ of acetone was painted to the inner surface of the right ear of a BALB/c mouse (8 weeks of age, male, $n=5-6$ per group). Some potent derivative (**3**, **7**, **9**) and zileuton were painted directly on the ears ($500 \mu\text{g}/\text{ear}$) or given orally to mice (50 mpk) 1 h before AA treatment (2 mg in $20 \mu\text{L}$ acetone). For vehicle control for *per os* (*p.o.*) administration, an equal volume of 0.5% carboxymethyl cellulose (CMC) was given. At 1 h after AA stimulation, ear thickness of the treated- and untreated-ears was measured with a dial thickness gauge (Mitutoyo, Tokyo, Japan). To further examine the long-lasting effect of a potent compound against an ear-edema animal model, mouse ears were preloaded topically with either zileuton or compound **9** over the indicated times and then the skin was inflamed with AA treatment. The effects of chemical derivatives were calculated as the percent difference in ear thickness from the mean values of the contralateral untreated ear. Myeloperoxidase (MPO) activity in the inflamed ear tissue was determined according to the method described before.²⁷⁾ In brief, punched ears (2 mm in diameter) were chopped, and homogenized in $300 \mu\text{L}$ of 50 mM potassium phosphate buffer, pH 6.0, containing 0.5% HTAB. The homogenate was then centrifuged and the resulting supernatant ($100 \mu\text{L}$) was allowed to react with 0.167 mg/mL *o*-dianisidine and 0.0005% H_2O_2 in order to determine the absorbance at 450 nm.

Statistical Analysis Results were expressed as means $\pm$ standard error of the mean (S.E.M.) of two experiments, each carried out in triplicate. Statistical significance was evaluated using Student's *t*-test, and $p < 0.05$ was considered to indicate statistical significance.

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Conflict of Interest The authors declare no conflict of interest.

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