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Brief Article

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Modified Acidic Nonsteroidal Anti-Inflammatory Drugs as Dual Inhibitors of mPGES-1 and 5-LOX

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Supporting Information

ABSTRACT: mPGES-1 is a promising target for development of new anti-inflammatory drugs. We aimed to create mPGES-1 inhibitors by modifying the structure of NSAIDs by replacing the carboxylic acid functionality by sulfonamide moieties. Compounds were also tested for 5-LOX inhibition. The most potent mPGES-1 inhibitor was lonazolac derivative **22** (IC_{50} = 0.16 μM), while the best 5-LOX inhibition was attained by indomethacin derivative **17** (IC_{50} = 0.9 μM). Inhibition of COX-1 activity was completely removed.

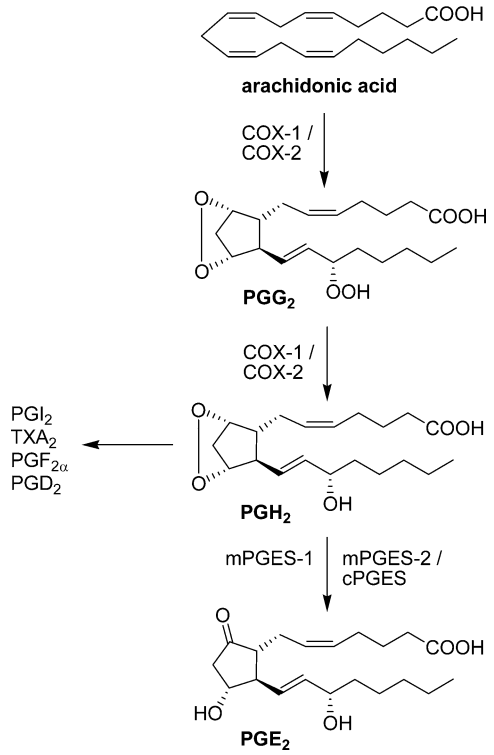
INTRODUCTION

Prostaglandin E_2 (PGE_2) is a prostanoid with diverse biological activities and plays major roles in inflammation and various stages of tumorigenesis.[1−3] Inhibition of the biosynthesis of PGE_2 is therefore a promising approach for anti-inflammatory therapy that circumvents adverse effects due to long-term use of common nonsteroidal anti-inflammatory drugs (NSAIDs), which still represent the medication of choice. The adverse effects of NSAIDs include gastrointestinal toxicity and increased risk of cardiovascular events.[4] The biosynthetic pathway of PGE_2 consists of three steps[5] (Scheme 1). First, arachidonic acid (AA) is released by the action of phospholipase A_2 (PLA_2), followed by the conversion of AA to PGH_2 by the action of cyclooxygenase (COX) 1 or COX-2. Finally, PGH_2 is converted to PGE_2 by the action of PGE_2 synthase (PGES) enzymes. There are three identified types of PGES, (i) microsomal prostaglandin E synthase 1 (mPGES-1), (ii) mPGES-2, and (iii) cPGES.[5] Inducible enzyme mPGES-1 is associated with inflammatory processes, stroke, and bone disorders.[6] Several types of human cancer have been correlated with elevated levels of mPGES-1 in the respective tissues.[1−3] This observation together with the increased levels of protumorigenic PGE_2 highlights the relevance of mPGES-1 in tumorigenesis. Currently, the development of selective inhibitors of mPGES-1 is an active area of investigation.

In this study, our aim was to synthesize derivatives of different acid-modified NSAIDs as new leads for inhibitors of mPGES-1 and 5-LOX that at the same time show reduced activity on COX. In previous work we showed that modification of the acidic carboxylic group of licofelone (a mPGES-1, COX, and LOX inhibitor) to sulfonamides impaired the COX activity while simultaneously increasing the potency against mPGES-1 and 5-LOX.[7−10]

Here, we report the use of this approach in general on NSAIDs. Among various sulfonamide derivatives of ibuprofen, ketoprofen, naproxen, indomethacin, and lonazolac, the derivatives of lonazolac and indomethacin displayed promising results as dual inhibitors of mPGES-1/5-LOX. On the basis of this, we further optimized the series of indomethacin and lonazolac derivatives. As a result, we improved the inhibitors'

Scheme 1. Biosynthetic Pathway of PGE_2



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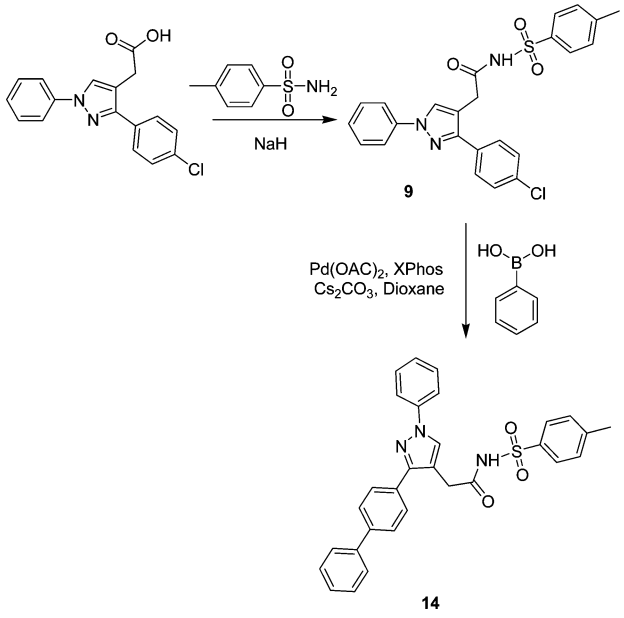
potency against mPGES-1 and retained 5-LOX inhibitory activity while at the same time diminishing COX inhibitory efficiency.

CHEMISTRY

Modification of acidic NSAIDs was carried out as shown in Schemes 2−4. Compounds **1**−**13** were synthesized by coupling

Scheme 2. Synthesis of 1−14, Exemplified by 9 and 14



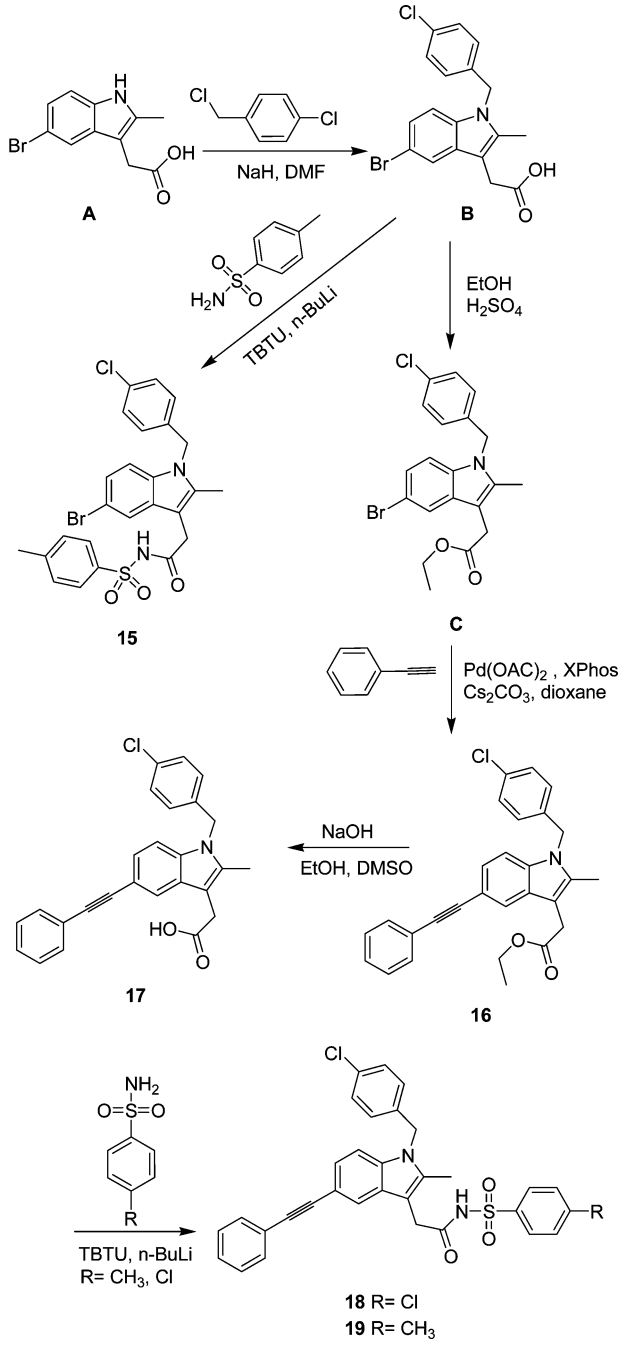
of the respective NSAIDs with different sulfonamides under basic conditions (NaH, THF) after activation with CDI[8] (see Supporting Information for all synthetic details). Lonazolac derivative (**9**) was further modified by Suzuki coupling with benzeneboronic acid[11] to yield **14** (Scheme 2). The syntheses of **15**−**19** were accomplished in five or six steps as displayed in Scheme 3. Intermediate **B** was synthesized by N-substitution of indole derivative **A** (a Fischer synthesis product of 4-bromophenylhydrazine and levulinic acid) with 4-chlorobenzyl chloride.[12] Coupling of **B** to *p*-toluenesulfonamide gave **15**. Compound **16** was prepared from ethyl ester **C** in a Sonogashira coupling reaction with phenyl acetylene. Subsequent hydrolysis of **16** led to **17**. Sulfonamidation of **17** gave **18** and **19**.

The synthesis of **21**−**26** was accomplished in 9 or 10 steps as outlined in Scheme 4. Substituted hydrazone **D** (from phenylhydrazine and 4-bromoacetophenone) was treated with $POCl_3$ and DMF to give the respective formylated pyrazole **E**.[13] Reduction of **E**,[14] followed by chlorination, cyanide substitution,[15] hydrolysis,[15] and esterification yielded the pyrazoloacetic acid ester derivatives **J**. Sonogashira reaction in the presence of $Pd(OAC)_2$, XPhos, Cs_2CO_3, and dry dioxane led to ester **K**, and by hydrolysis, **21**−**23** were obtained. Compounds **24**−**26** were produced by sulfonamidation in the presence of TBTU and *n*-butyllithium/*n*-hexane.

BIOLOGICAL TESTING

All synthesized compounds were first screened in a cell-free mPGES-1 assay at 10 μM (see Table 2). In this assay, direct inhibition of mPGES-1 was determined in microsomes from

Scheme 3. Synthesis of 15−19



human A549 cells by assessment of the enzymatic generation of PGE_2 from 20 μM PGH_2 as substrate.[16]

The well-recognized mPGES-1 inhibitor MK-886 was used as a reference inhibitor, and results are expressed as percentage of remaining PGE_2 formation measured in the presence of the vehicle control. Inhibition of 5-LOX by the test compounds (10 μM, each) was determined in a cell-based assay using human neutrophils that were stimulated with A23187 plus 20 μM exogenous AA as substrate; BWA4C was used as a reference inhibitor.[16] Inhibition of COX activity was assessed in a cell-free assay using isolated ovine COX-1 and 5 μM AA as substrate.[17]

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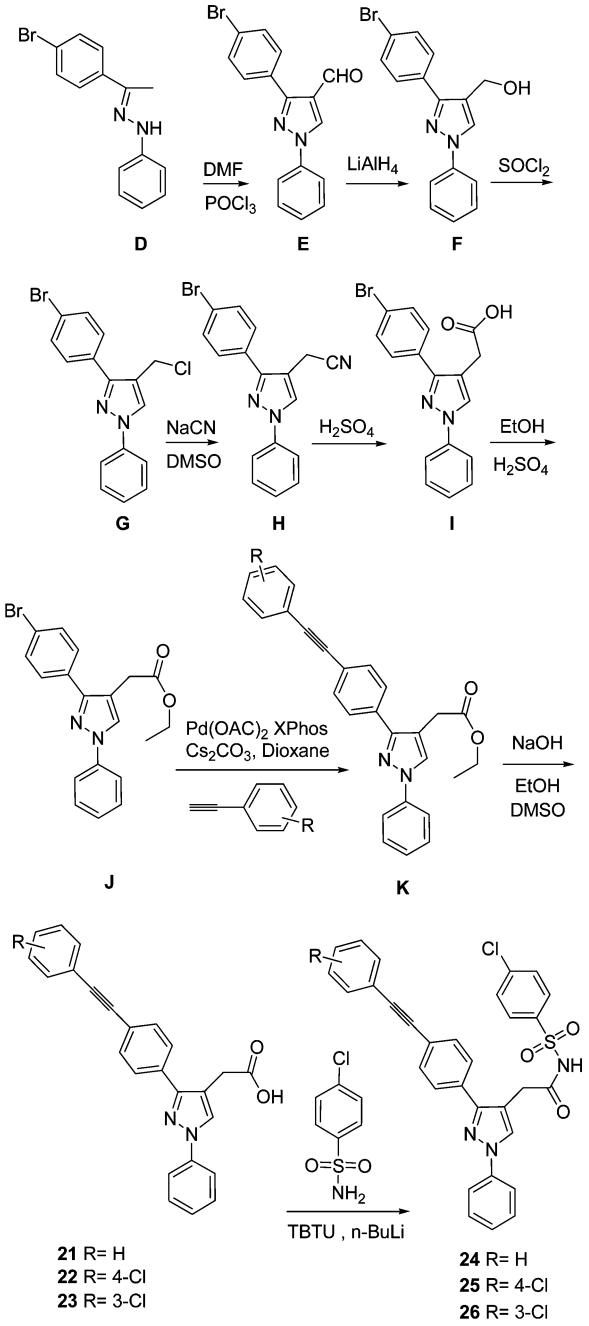
Scheme 4. Synthesis of 21−26



RESULTS AND DISCUSSION

Replacement of the carboxylic acid moiety of ibuprofen, ketoprofen, or naproxen by the sulfonamide moieties did not lead to marked inhibition of mPGES-1 or of 5-LOX (see Table 2). However, these substitutions resulted in a complete loss of COX-1 inhibition activity (assay described in ref 17; no inhibition at 10 μM, details not shown.)

Modification of indomethacin and lonazolac yielded two *p*-tolylsulfonamides (**7**, **9**) that at 10 μM showed significant (>50%) inhibition of mPGES-1 and 5-LOX. The IC_{50} values for **7** were 2.9 μM (5-LOX) and 6.4 μM (mPGES-1), and for **9**, they were 2.5 μM (5-LOX) and 3.4 μM (mPGES-1). Remarkably, these simple sulfonamide derivatives of indomethacin and lonazolac were about equipotent to MK-886, an efficient inhibitor of human mPGES-1 (IC_{50} = 2.3 μM). Methanesulfonamide analogues **6** and **10** showed less potency, suggesting that a rather lipophilic and bulky moiety is required at this position. Thus, substitution of the carboxylic group by sulfonamide was effective but depended more on the structure of the substituent; **1**− **6**, **8**, **10** were not as efficient as indomethacin derivative **7** and lonazolac derivative **9**. Compounds **15**−**26**) were initially screened in the cell-free mPGES-1 assay at 10 μM; all of them were active except **16** and **20**. In addition, we analyzed **7**, **19**, **22**, **25** for inhibition of COX activity; all of the compounds displayed $IC_{50} \gg 10$ μM indicating loss of COX inhibition (see Table 1).

Table 1. Effects of Test Compounds on COX-1 Activity in a Cell-Free Assay Using Isolated Ovine COX-1 and 5 μM AA as Substrate

compd[a]	COX-1[b]
7	89.4 ± 6.1
14	99.1 ± 5.7
19	83,1 ± 2.8
22	77.8 ± 0.74
25	70.1 ± 5.2
indomethacin	23.3 ± 1.8

[a]For all structures, refer to Table 2. [b]Inhibition of COX-1 is expressed as % activity 12-HHT formation compared to vehicle control value. Compounds were screened at a fixed concentration of 10 μM. Data are expressed as the mean ± SE, n = 3.

Compounds **12** and **13** showed that removing the methyl group from toluenesulfonamide led to loss in inhibitory activity but replacing the methyl group with chlorine resulted in even greater inhibition of mPGES-1. Introducing a biphenyl moiety to **9** was inspired by previous work reported by Wang et al.[18] Compound **14** displayed better binding efficacy as a dual inhibitor, with IC_{50} of 1.7 μM for mPGES-1 and of 3.5 μM for 5-LOX. Compound **9** was modified at two different positions to yield **21**−**26**. Introducing the phenylacetylene moiety led to significantly improved mPGES-1 enzyme inhibition (**21**, IC_{50} = 0.4 μM). Further substitution of the para and meta positions of the phenylacetylene moiety by chlorine led to **22** and **23**, with IC_{50} as low as 0.16 μM for **22** and 0.18 μM for **23**. Obviously, *p*-chloro substitution is superior to *m*-chloro or unsubstituted phenyl. Substitution of the carboxylic acid of **21**−**23** by 4-chlorobenzenesulfonamide led to **24**−**26**. The improved IC_{50} of **24** indicated tolerance of the sulfonamide moiety at this position. Analogues **25** and **26**, however, showed loss of activity.

CONCLUSION

Structural modification of NSAIDs, like replacement of the carboxylic acid functionality by substituted sulfonamide moieties, is a useful approach to create dual mPGES-1/5-LOX inhibitors that also show reduced COX inhibition compared to the parent compounds. The most potent mPGES-1 inhibitor of this series was lonazolac derivative **22** (IC_{50} = 0.16 μM), while indomethacin derivative **17** was most efficient with respect to 5-LOX inhibition (IC_{50} = 0.9 μM).

EXPERIMENTAL SECTION

NMR data (^{1}H and ^{13}C) were recorded on a Bruker Avance 200. Melting points were determined on a Büchi B-545 apparatus. IR spectra were recorded on a Perkin-Elmer Spectrum One spectrometer. Purity of compounds was determined by HPLC on a HP 1090 series II

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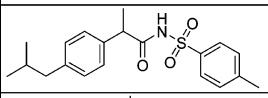
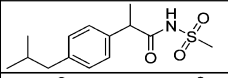
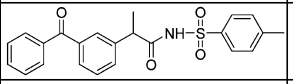
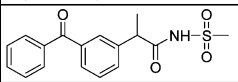
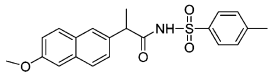
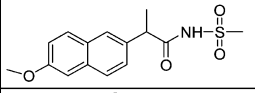
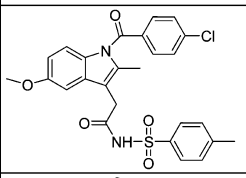
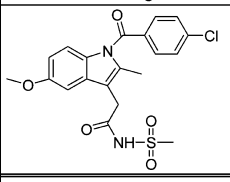
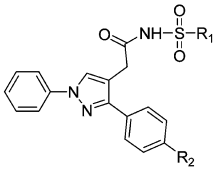
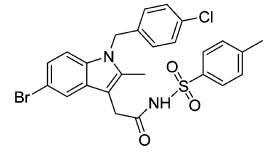
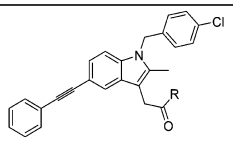
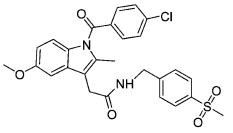
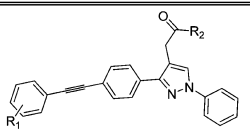
Table 2. Effects of Test Compounds on mPGES-1 Activity in Microsomes of A549 Cells and 5-LOX Activity in Human Neutrophils[e]

#	Compound / Structure	mPGES-1[a]	5-LOX[b]
1		78.6 ± 6.4	67.7 ± 8.9
2		67.9 ± 15.8	91.2 ± 4.3
3		76.3 ± 10	78.9 ± 12.7
4		78.6 ± 2.1	90.7 ± 10.6
5		72.8 ± 6.9	n. i.[c]
6		92.5 ± 4.8	n. i.
7		(*) 6.4 ± 1.4	(*) 2.9 ± 0.8
8		73.6 ± 14.5	64.9 ± 10.3
			
9	R_1 = p-tolyl R_2 = Cl	(*) 3.4 ± 0.8	(*) 2.5 ± 0.3
10	R_1 = CH_3 R_2 = Cl	98.0 ±26.9	72.1 ± 13.9
12	R_1 = phenyl R_2 = Cl	(*) 5.9 ± 0.1	(*) 6.8 ± 2.3
13	R_1= 4-Cl-phenyl R_2 = Cl	(*) 2.3 ± 0.2	(*) 2.9 ± 1.1
14	R_1 = p-tolyl R_2 = phenyl	(*) 1.7 ± 0.2	(*) 3.5 ± 1.5
15		(*) 1.75 ± 0.6	(*) 1.7 ± 0.7

#	Compound / Structure	mPGES-1[a]	5-LOX[b]
			
16	R = OEt	86.1 ± 3.09	n. d.[d]
17	R = OH	(*) 2.5 ± 0.6	(*) 0.9 ± 0.4
18	R= 4-Cl-Ph-SO_2NH-	(*) 1.8 ± 0.5	(*) 2.0 ± 0.7
19	R= 4-CH_3-Ph-SO_2NH-	(*) 2.8 ± 0.6	(*) 1.5 ± 0.6
20		68.7 ± 3.5	n. d.
			
21	R_1 = H R_2 = OH	(*) 0.4 ± 0.07	(*) 5.0 ± 2.78
22	R_1 = 4-Cl R_2 = OH	(*) 0.16 ± 0.03	(*) 3.7 ± 1.2
23	R_1 = 3-Cl R_2 = OH	(*) 0.18 ± 0.025	(*) 3.9 ± 1.5
24	R_1 = H R_2 = 4-Cl-Ph-SO_2NH	(*) 0.2 ± 0.05	(*) 1.6 ± 0.5
25	R_1 = 4-Cl R_2 = 4-Cl-Ph-SO_2NH	(*) 0.35 ± 0.07	(*) 4.7 ± 1.3
26	R_1 = 3-Cl R_2 = 4-Cl-Ph-SO_2NH	(*) 0.8 ± 0.15	(*) 2.3 ± 0.4
--	ibuprofen	81.2 ± 2.7	n. i.
--	ketoprofen	n. i.	n. i.
--	naproxen	n. i.	n. i.
--	indomethacin	(*) 40.6 ± 6.9	n. i.
--	lonazolac-Ca	(*) 45.0 ± 3.2	59.7 ± 5.7
--	MK-886	(*) 2.3 ±0.8	n. d.
--	BWA4C	n. d.	(*) 0.16 ±0.09

[a]Inhibition of mPGES-1 is expressed as % activity remaining compared to vehicle control value. Compounds were screened at a fixed concentration of 10 μM for promising compounds. (*) IC_{50} [μM]: calculated for promising compounds. mPGES-1 activity was determined in microsomes prepared from human A549 cells. [b]Inhibition of 5-LOX is expressed as % activity remaining compared to vehicle control value. Compounds were screened at a fixed concentration of 10 μM for promising compounds. (*) IC_{50} [μM]: calculated for promising compounds. 5-LOX activity was assayed in whole cell preparations from human neutrophils. [c]n.i.: no inhibitory activity observed. [d]n.d.: not determined. [e]Data are expressed as the mean ± SE, n = 3−5.

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C_8-RP stationary phase (150 mm × 4.6 mm, d_p = 5 μm) with UV-DAD, based on the area under the curve as calculated by Agilent ChemStation revision A.09.03 for individual measurement at 230 and 254 nm and by recording HR-MS data on a Bruker Apex II FT-ICR-MS or a Finnigan MAT95 (EI) instrument. All compounds have a purity of >95%.

General Procedure for Activation of Carboxylic Acids and Coupling with Sulfonamides. Carboxylic acid (1 mmol) and CDI (1.5 mmol) in dry THF (15 mL) were stirred for 3 h. To sulfonamide (2.5 mmol) in dry THF was added NaH (mineral oil suspension, 1.2 mmol). The mixture was stirred for 3 h and was slowly added to the activated carboxylic acid. After being stirred overnight, the mixture was poured into cold water, adjusted to pH 1, and extracted with EtOAc. Purification was by recrystallization or flash chromatography.

2-(3-(4-Chlorophenyl)-1-phenyl-1*H*-pyrazol-4-yl)-*N*-tosylacetamide (9) was obtained from lonazolac (1.59 mmol, 0.5 g). The crude product was purified using methanol to yield 0.45 g (60%) of white solid product. ^{1}H NMR (200 MHz, DMSO-d_6): δ [ppm] = 2.3 (s, 3H), 3.69 (s, 2H), 7.35 (m, 5H), 7.49 (4H), 7.7 (4H), 8.3 (s, 1H), 12.2 (s, 1H, NH). IR (ATR) 3253, 2924, 2160, 1707, 1598, 1504, 1437, 1177, 1121, 1086, 1011, 860, 834, 817, 751, 686, 673 cm^{-1}. Purity (HPLC) 98%. Mp 186.6 °C. HR-MS (FT-ICR-MS) for $C_{24}H_{20}ClN_3O_3S$ $[M + H]^+$: 466.098.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis details and analytical data (NMR, IR, HPLC, MS) of all compounds; biological assay details. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

AA, arachidonic acid; CDI, 1,1-carbonyldiimidazole; 12-HHT, 12(*S*)-hydroxyheptadeca-5*Z*,8*E*,10*E*-trienoic acid; TBTU, 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate; XPhos, 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl

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