Bioorganic & Medicinal Chemistry Letters 18 (2008) 4195–4198

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological evaluation of 1-(benzenesulfonamido)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers: A novel class of 5-lipoxygenase inhibitors

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ARTICLE INFO

Article history:
Received 4 April 2008
Revised 16 May 2008
Accepted 16 May 2008
Available online 22 May 2008

Keywords:
Linear acetylenes
N-hydroxypyridin-2(1*H*)-ones
Lipoxygenase inhibition
Anti-inflammatory activity

ABSTRACT

A hitherto unknown class of linear acetylene regioisomers were designed such that a SO_2NH_2 group was located at the *ortho*-, *meta*-, or *para*-position of the acetylene C-1 phenyl ring, and a *N*-hydroxypyridin-2(1*H*)-one moiety was attached via its C-5 position to the C-2 position on an acetylene template (scaffold). All three regioisomers inhibited 5-lipoxygenase (5-LOX), where the relative potency order was 2-SO_2NH_2 (IC_{50} = 10 μM) >3-SO_2NH_2 (IC_{50} = 15 μM) >4-SO_2NH_2 (IC_{50} = 68 μM) relative to the reference drug nordihydroguaiaretic acid (NDGA; IC_{50} = 35 μM). The 2-SO_2NH_2 regioisomer (ED_{50} = 86.0 mg/kg po) exhibited excellent oral anti-inflammatory (AI) activity that was more potent than aspirin (ED_{50} = 128.9 mg/kg) and marginally less potent than ibuprofen (ED_{50} = 67.4 mg/kg). The *N*-hydroxypyridin-2(1*H*)one moiety provides a novel pharmacophore for the design of cyclic hydroxamic mimetics capable of chelating 5-LOX iron for exploitation in the design of 5-LOX inhibitory AI drugs.

The most successful effort to develop 5-lipoxygenase (5-LOX) inhibitors encompasses hydroxamic acids and related *N*-hydroxyureas that likely chelate iron present in the 5-LOX enzyme.[1] Some representative examples of iron-chelating 5-LOX inhibitors (**1**,[2] **2**,[1] **3**[3]) are shown in Figure 1. In a recent study, we described a group of 1-aryl-2-(pyridyl)acetylene regioisomers (**4**) that constitute a suitable scaffold (template) to design novel acyclic inhibitors of the 5-LOX isozyme.[4] It was anticipated that replacement of the pyridyl ring in the acetylenes **4** by a *N*-hydroxypyridin-2(1*H*)one moiety, that has the potential to chelate iron, may provide a hitherto unknown class of 5-LOX inhibitory anti-inflammatory agents. Accordingly, we now describe the synthesis of a novel exploratory group of 1-(2-, 3-, and 4-benzenesulfonamido)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers (**12a**–**c**), their in vitro evaluation as 5-LOX inhibitors, and in vivo assessment as anti-inflammatory (AI) agents.

The 1-(benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylenes (**10a–c**) were prepared using two consecutive palladium-catalyzed Sonogashira cross-coupling reactions. The subsequent transformation of **10a–c** to the target 1-(2-, 3, and 4-benzenesulfonamido)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers (**12a–c**) was carried out using the synthetic methodologies shown in Scheme 1. A modified procedure[5] was used to synthesize 5-ethynyl-2-methoxypyridine (**8**). Thus, Sonogashira coupling of 5-bromo-2-methoxypyridine (**5**) with 2-methylbut-3-yn-2-ol (**6**) in the presence of Et_3N, CuI, and $PdCl_2(PPh_3)_2$ catalyst afforded 4-(2-methoxypyridin-5-yl)-2-methyl-but-3-yn-2-ol (**7**) in 75% yield. Subsequent removal of the isopropanol moiety using sodium hydride furnished 5-ethynyl-2-methoxypyridine (**8**) in 82% yield. A second Sonogashira cross-coupling reaction of **8** with the bromobenzenesulfonamide (**9a–c**) regioisomers was carried out under an argon atmosphere in Et_3N-THF (1:1, v/v) using $PdCl_2(PPh_3)_2$/CuI as catalyst to furnish the respective 1-(benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylenes **10a–c** in 26–72%

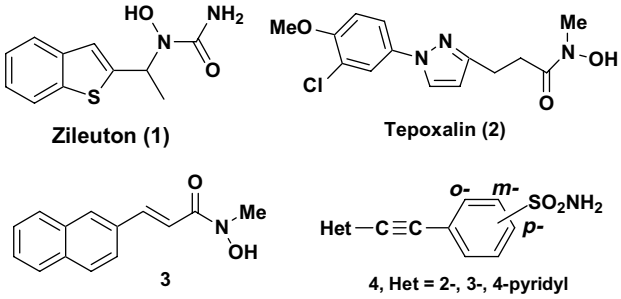

Figure 1. Some representative iron-chelating 5-LOX inhibitors (**1–3**).

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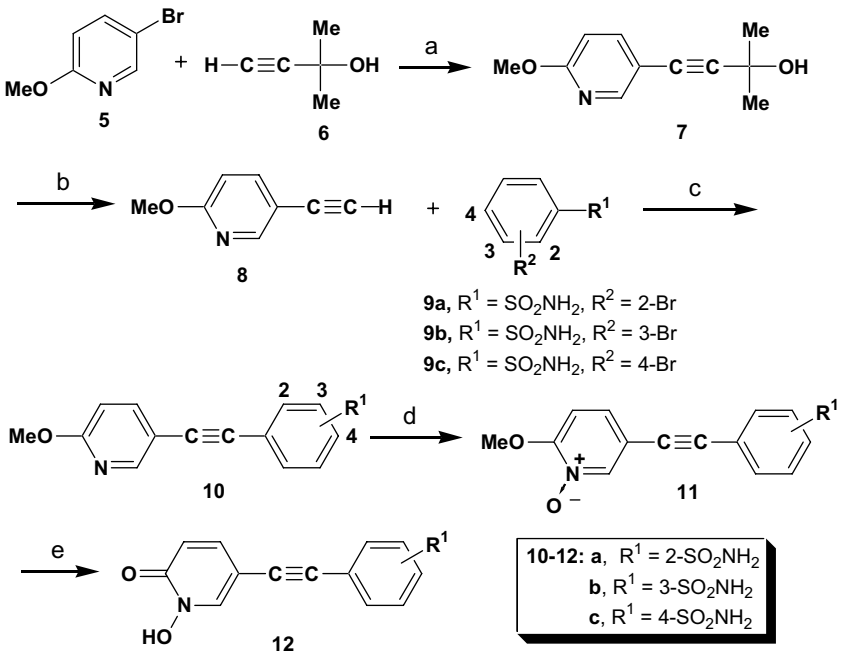
doi:10.1016/j.bmcl.2008.05.071



Scheme 1. Reagents and conditions: (a) Et_3N, $Pd(PPh_3)_2Cl_2$, CuI, 70–75 °C, 3 h; (b) benzene, NaH, 105–110 °C, 1 h; (c) Et_3N/THF, $Pd(PPh_3)_2Cl_2$, CuI, 90 °C, 5 h; (d) *m*-chloroperoxybenzoic acid, CH_2Cl_2, 25 °C, overnight; (e) i—acetyl chloride, reflux, 1 h, ii—MeOH, 25 °C, overnight.

yield. Oxidation of **10a–c** with *meta*-chloroperbenzoic acid in dichloromethane[6] afforded the *N*-oxides **11a–c** in 22–42% yield. Finally, reaction of **11a–c** with acetyl chloride at reflux, and then methanolysis in place of hydrolysis,[6] furnished the target *N*-hydroxypyridin-2(1*H*)-ones (**12a–c**) in 33–94% yield.

Replacement of the carboxyl (CO_2H) in traditional non-steroidal anti-inflammatory drugs (NSAIDs) by a hydroxamic acid (CONHOH) moiety provided potent orally active 5-LOX inhibitory agents.[3] NSAIDs having a CONHOH or CON(Me)OH (pK_a 9–11 range) in place of the CO_2H in traditional NSAIDs (pK_a generally in the 4–5 range) are much less acidic which decreases ulcerogenicity.[7]

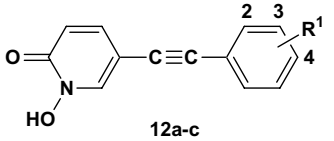
The rational for the design of the acyclic acetylenes **12a–c** is based on the expectations that the *N*-hydroxypyridin-2(1*H*)one moiety will confer 5-LOX inhibitory activity. The CONOH part of the *N*-hydroxypyrid-2(1*H*)-one ring present in **12a–c** can be viewed as a cyclic hydroxamic acid mimetic. These *N*-hydroxypyridin-2(1*H*)-ones, like acyclic hydroxamic acids, are expected to serve as effective iron chelators to exhibit 5-LOX inhibitory activity. However, these cyclic *N*-hydroxypyridin-2(1*H*)-ones, unlike acyclic hydroxamic acids which undergo facile biotransformation to the acids, are expected to be metabolically stable with improved oral efficacy. Compounds that possess a *N*-hydroxypyridin-2(1*H*)-one moiety, in view of their low acidity, are expected to be non-ulcerogenic.

An in vitro cell-based inhibition assay was used to determine the biological effect of compounds **12a–c** on eicosanoid synthesis/release by measuring the amounts of cysteinyl leukotrienes (collectively referred to as a group of 5-LOX derived metabolites LTC_4, LTD_4, and LTE_4) secreted into the culture medium of human brain cancer cells. In this 5-LOX inhibition assay, all three SO_2NH_2 regioisomers **12a–c** inhibited 5-LOX, where the relative potency order was 2-SO_2NH_2 (**12a**, IC_{50} = 10 μM) >3-SO_2NH_2 (**12b**, IC_{50} = 15 μM) >4-SO_2NH_2 (**12c**, IC_{50} = 68 μM) relative to the reference drug NDGA (IC_{50} = 35 μM).

The oral AI activities (ED_{50} values) exhibited by the cyclic *N*-hydroxypyridin-2(1*H*)-one regioisomers **12a–c** were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). The structure-activity data acquired showed that the active 2-SO_2NH_2 regioisomer **12a** (ED_{50} = 86.0 mg/kg po), unlike the inactive 3-SO_2NH_2 **12b** and 4-SO_2NH_2 **12c** regioisomers, exhibited an AI activity that was more potent than the aspirin (ED_{50} = 128.9 mg/kg po) and marginally less potent than ibuprofen (ED_{50} = 67.4 mg/kg po). Both aspirin and ibuprofen are over-the-counter (OTC) non-prescription drugs used extensively to treat minor pain and inflammation.

In conclusion, a previously unknown class of 1-(2-, 3-, and 4-benzenesulfonamido)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers[8] were designed as 5-LOX inhibitors of inflammation. The structure-activity data acquired indicate that (i) a linear acetylene spacer between *vicinal* 2-benzenesulfonamide and

Table 1
In vitro cell-based 5-LOX inhibition data and anti-inflammatory (AI) activities, for 1-(benzenesulfonamido)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylenes (**12a–c**)



12a-c

Compound	R^1	5-LOX inhibitory activity: IC_{50} (μM)[a]	AI activity:[b] ED_{50} (mg/kg)
12a	2-SO_2NH_2	10	86.0
12b	3-SO_2NH_2	15	Inactive
12c	4-SO_2NH_2	68	Inactive
Aspirin	—	—	128.9
Ibuprofen	—	—	67.4
NDGA[c]	—	35	—

[a] Values are the mean of two determinations and the deviation from the mean is <10% of the mean value.

[b] Anti-inflammatory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the ED_{50} value (mg/kg) at 3 h after oral (po) administration of the test compound.

[c] NDGA, nordihydroguaiaretic acid was used as the Ref. 5-LOX inhibitory drug.

N-hydroxypyridin-2(1*H*)-one rings constitutes a potential template for the design of new acyclic 5-LOX inhibitors, (ii) the presence and position of a SO_2NH_2 substituent on the phenyl ring is a determinant of 5-LOX inhibitory activity[9] where the relative potency profile is 2- >3- and 4-SO_2NH_2, (iii) the *N*-hydroxypyrid-2(1*H*)-one moiety provides a new 5-LOX pharmacophore for the design of cyclic hydroxamic mimetics and (iv) 1-(2-benzenesulfonamidol)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene (**12a**) that exhibits excellent oral anti-inflammatory activity,[10] relative to the reference drugs aspirin and ibuprofen, is a useful lead-compound to further develop the 5-LOX drug design strategy described herein.

Acknowledgment

We are grateful to the Canadian Institutes of Health Research (CIHR) (MOP-14712) for financial support of this research.

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8. Experimental and spectral data for compounds **7–12**. *4-(2-Methoxypyridin-5-yl)-2-methylbut-3-yn-2-ol* (**7**). $PdCl_2(PPh_3)_2$ (63 mg, 0.09 mmol) and CuI (19 mg, 0.10 mmol) were added to a stirred solution of **5** (2.75 mL, 21.39 mmol) and **6** (2.20 mL, 22.61 mmol) in Et_3N (40 mL) under an argon atmosphere at 25 °C, and the reaction was allowed to proceed at 70–75 °C for 3 h. The reaction mixture was allowed to cool to 25 °C, filtered, and excess Et_3N was removed from the filtrate in vacuo. The dark brown residue obtained was purified by silica gel column chromatography using hexane-EtOAc (3:1, v/v) as eluent to afford **7** in 75% yield; yellowish oil; IR (film): 3368 (OH), 2235 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.62 (s, 6H, C*Me*$_2$), 2.44 (br s, 1H, O*H*), 3.94 (s, 3H, O*Me*), 6.69 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.59 (dd, *J* = 8.5, 2.1 Hz, 1H, pyridyl H-4), 8.27 (d, *J* = 2.1 Hz, 1H, pyridyl H-6).
5-Ethynyl-2-methoxypyridine (**8**). Sodium hydride (26 mg, 1.08 mmol) was added to a solution of **7** (1.52 g, 7.96 mmol) in benzene (7 mL), and the reaction mixture was heated at 105–110 °C for 1 h. Removal of the solvent in vacuo gave a dark brown oil which was purified by silica gel column chromatography using hexane-EtOAc (3:1, v/v) as eluent to afford **8** in 82% yield; brown oil; IR (film): 2230 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.12 (s, 1H, C≡C*H*), 3.96 (s, 3H, O*Me*), 6.72 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.66 (dd, *J* = 8.5, 2.1 Hz, 1H, pyridyl H-4), 8.27 (d, *J* = 2.1 Hz, 1H, pyridyl H-6).
General procedure for the synthesis of 1-(benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylenes (**10a–c**). CuI (46 mg, 0.24 mmol) was added with stirring to a solution containing $PdCl_2(PPh_3)_2$ (85 mg, 0.12 mmol), **8** (6 mmol), and a bromobenzenesulfonamide **9a**, **9b**, or **9c** (4 mmol) in dry THF (10 mL) and Et_3N (10 mL) under an argon atmosphere. The reaction mixture was heated at 90 °C for 5 h, cooled to 25 °C, and filtered to remove the inorganic salts. The solvent from the filtrate was removed in vacuo, and the residue obtained was purified by silica gel column chromatography using hexane-acetone (1:1, v/v) as eluent to furnish the respective products **10a–c**.
1-(2-Benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylene (**10a**). The product was obtained as a pale yellow oil using the Sonogashira coupling reaction of **8** with **9a** in 26% yield; IR (film): 3380, 3250 (NH_2), 2160 (C≡C), 1295, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.97 (s, 3H, O*Me*), 5.28 (br s,2H, SO_2NH_2), 6.75 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.45 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H, phenyl H-5), 7.55 (ddd, *J* = 7.6, 7.6, 1.2 Hz, 1H, phenyl H-4), 7.72 (dd, *J* = 7.6, 1.2 Hz, 1H, phenyl H-6), 7.77 (dd, *J* = 8.5, 2.1 Hz, 1H, pyridyl H-4), 8.05 (dd, *J* = 7.6, 1.2 Hz, 1H, phenyl H-3), 8.41 (d, *J* = 2.1 Hz, 1H, pyridyl H-6).
1-(3-Benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylene (**10b**). The product was obtained as a dark oil using the Sonogashira coupling reaction of **8** with **9b** in 72% yield; IR (film): 3360, 3270 (NH_2), 2200 (C≡C), 1315, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.98 (s, 3H, O*Me*), 5.08 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 6.76 (d, *J* = 8.6 Hz, 1H, pyridyl H-3), 7.51 (dd, *J* = 7.9, 7.6 Hz, 1H, phenyl H-5), 7.65-7.72 (m, 2H, pyridyl H-4, phenyl H-6), 7.87 (ddd, *J* = 7.9, 1.5, 1.5 Hz, 1H, phenyl H-4), 8.08 (dd, *J* = 1.5, 1.5 Hz, 1H, phenyl H-2), 8.36 (d, *J* = 2.1 Hz, 1H, pyridyl H-6).
1-(4-Benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylene (**10c**). The product was obtained as a white solid using the Sonogashira coupling reaction of **8** with **9c** in 70% yield; mp 185–187 °C; IR (film): 3340, 3250 (NH_2), 2190 (C≡C), 1290, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 3.87 (s, 3H, O*Me*), 6.74 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.20 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.56 (d, *J* = 8.5 Hz, 2H, phenyl H-2, H-6), 7.69 (dd, *J* = 8.5, 2.4 Hz, 1H, pyridyl H-4), 7.82 (d, *J* = 8.5 Hz, 2H, phenyl H-3, H-5), 8.27 (d, *J* = 2.4 Hz, 1H, pyridyl H-6).
General procedure for the synthesis of 1-(benzenesulfonamido)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (**11a–c**). *m*-Chloroperoxybenzoic acid (77% max.) (12 mmol) was added to a stirred solution of a 1-(benzenesulfonamido)-2-(2-methoxypyrid-5-yl)acetylene (**10**) (2 mmol) in dry CH_2Cl_2 (50 mL), and the reaction was allowed to proceed with stirring at 25 °C overnight. The solvent CH_2Cl_2 was removed in vacuo to give a crude product which was purified by silica gel column chromatography using methanol-EtOAc (2:3, v/v) as eluent to afford the respective products **11a–c**.
1-(2-Benzenesulfonamido)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (**11a**). Yield, 22%; pale yellow solid; mp 190–192 °C, IR (film): 3150–2800 (broad) (NH_2), 2150 (C≡C), 1325, 1165 (SO_2) cm^{-1}; ^1H NMR($CDCl_3$+DMSO-d_6) δ 4.08 (s, 3H, O*Me*), 7.26 (d, *J* = 8.9 Hz, 1H, pyridyl H-3), 7.29 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.45–7.58 (m, 2H, pyridyl H-4, phenyl H-5), 7.60–7.69 (m, phenyl H-4, H-6), 7.95 (dd, *J* = 7.6, 1.2 Hz, 1H, phenyl H-3), 8.62 (d, *J* = 1.8 Hz, 1H, pyridyl H-6).
1-(3-Benzenesulfonamido)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (**11b**). Yield, 41%; yellowish solid; mp 150–152 °C; IR (film): 3200–2700 (broad) (NH_2), 2150 (C≡C), 1325, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 4.05 (s, 3H, O*Me*), 7.18 (d, *J* = 8.9 Hz, 1H, pyridyl H-3), 7.34 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.40 (dd, *J* = 7.9, 7.6 Hz, 1H, phenyl H-5), 7.45–7.55 (m, 2H, pyridyl H-4, phenyl H-6), 7.66 (ddd, *J* = 7.9, 1.5, 1.5 Hz, 1H, phenyl H-4), 7.97 (dd, *J* = 1.5, 1.5 Hz, 1H, phenyl H-2), 8.40 (d, *J* = 1.8 Hz, 1H, pyridyl H-6).
1-(4-Benzenesulfonamido)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (**11c**). Yield, 42%; white solid; mp 205–207 °C; IR (film): 3250–2700 (broad) (NH_2), 2225 (C≡C), 1365, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 4.05 (s, 3H, O*Me*), 7.23 (d, *J* = 8.9 Hz, 1H, pyridyl H-3), 7.37 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.52 (dd, *J* = 8.9, 1.8 Hz, 1H, pyridyl H-4), 7.65 (d, *J* = 8.5 Hz, 2H, phenyl H-2, H-6), 7.85 (d, *J* = 8.5 Hz, 2H, phenyl H-3, H-5), 8.42 (d, *J* = 1.8 Hz, 1H, pyridyl H-6).
General procedure for the synthesis of 1-(benzenesulfonamido)-2-[5-(N-hydroxypyridin-2(1H)-one)]acetylenes (**12a–c**). Acetyl chloride (6 mL) was added to **11a**, **11b**, or **11c** (2 mmol) and the reaction was allowed to proceed at reflux for 1 h. The reaction mixture was cooled to 25 °C, and excess acetyl chloride was removed in vacuo. The residue was dissolved in methanol prior to stirring at 25 °C overnight. Methanol was removed in vacuo to give a solid product which was then mixed with Et_2O (10 mL) to form a slurry. Finally, the product was filtered out and dried under vacuum to give the respective products (**12a–c**).
1-(2-Benzenesulfonamido)-2-[5-(N-hydroxypyridin-2(1H)-one)]acetylene (**12a**). Yield, 94%; pale yellow solid; mp 221–223 °C (decomp.); IR (film): 3350–2900 (broad) (NH_2), 2200 (C≡C), 1662 (CO), 1350, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 6.14 (d, *J* = 9.8 Hz, 1H, pyridone H-3), 6.45 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 6.97 (d, *J* = 6.7, 6.7 Hz, 1H, phenyl H-5), 7.02–7.13 (m, 2H, pyridone H-4, phenyl H-4), 7.18 (d, *J* = 6.7 Hz, 1H, phenyl H-6), 7.56 (d, *J* = 6.7 Hz, 1H, phenyl H-3), 7.74 (br s, 1H, pyridone H-6); ^{13}C NMR (DMSO-d_6) δ 86.3 (acetylene C-2), 92.5 (acetylene C-1), 99.2 (pyridone C-5), 119.1 (pyridone C-3), 119.7 (phenyl C-1), 127.2 (pyridone C-6), 128.3 (phenyl C-3), 131.7 (phenyl C-4), 133.6 (phenyl C-5), 139.9 (pyridone C-4), 140.0 (phenyl C-6), 144.0 (phenyl C-2), 157.0 (C=O); MS (ES^+) *m/z*: Calcd for $C_{13}H_{10}N_2O_4S$ (MH^+): 291.04. Found: 290.84.
1-(3-Benzenesulfonamido)-2-[5-(N-hydroxypyridin-2(1H)-one)]acetylene (**12b**). Yield, 33%; yellowish solid; mp 180-182 °C (decomp.); IR (film): 3300–2850 (broad) (NH_2), 2200 (C≡C), 1660 (CO), 1350, 1160 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 6.51 (d, *J* = 8.9 Hz, 1H, pyridone H-3), 7.28 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.40 (dd, *J* = 8.9 Hz, 1H, pyridone H-4), 7.48 (dd, *J* = 7.9, 7.6 Hz, 1H, phenyl H-5), 7.59 (d, *J* = 7.6 Hz, 1H, phenyl H-6), 7.79 (d, *J* = 7.9 Hz, 1H, phenyl H-4), 7.90 (s, 1H, phenyl H-2), 8.09 (s, 1H, pyridone H-6), 12.05 (br s, 1H, N–O*H*); ^{13}C NMR (DMSO-d_6) δ 86.7 (acetylene C-2), 87.6 (acetylene C-1), 98.7 (pyridone C-5), 119.2 (pyridone C-3), 122.9 (phenyl C-1), 125.4 (phenyl C-4), 127.9 (pyridone C-6), 129.5 (phenyl C-5), 133.9 (phenyl C-2), 139.7 (phenyl C-6), 139.9 (pyridone C-4), 144.5 (phenyl C-3), 156.9 (C=O); MS (ES^+) *m/z*: Calcd for $C_{13}H_{10}N_2O_4S$ (MH^+): 291.04. Found: 290.84.
1-(4-Benzenesulfonamido)-2-[5-(N-hydroxypyridin-2(1H)-one)]acetylene (**12c**). Yield, 48%; dark brown solid; mp 125–127 °C; IR (film): 3150–2900 (board) (NH_2), 2180 (C≡C), 1650 (C=O), 1340, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6) δ 6.23 (d, *J* = 9.2 Hz, 1H, pyridone H-3), 6.65 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.06 (d, *J* = 9.2 Hz, 1H, pyridone H-4), 7.18 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 7.51 (d, *J* = 7.6 Hz, 2H, phenyl H-3, H-5), 7.60 (s, 1H, pyridone H-6), 11.55 (br s, 1H, N–O*H*); ^{13}C NMR (DMSO-d_6) δ 87.8 (acetylene C-2), 88.0 (acetylene C-1), 98.7 (pyridone C-5), 119.2 (pyridone C-3), 125.6(phenyl C-1), 126.0 (phenyl C-2, C-6), 127.8 (pyridone C-6), 131.4 (phenyl C-3, C-5), 140.0 (pyridone C-4), 143.5 (phenyl C-4), 156.9 (C=O); MS (ES^+) *m/z*: Calcd for $C_{13}H_{10}N_2O_4S$ (MH^+): 291.04. Found: 290.84; Anal. Calcd for $C_{13}H_{10}N_2O_4S \cdot H_2O$: C, 50.65; H, 3.92. Found: C, 50.79; H, 3.96.
9. *In vitro cell-based enzyme immunoassay for determination of cysteinyl leukotrienes (5-LOX).* The biological effects of the test compounds **12a–c** on eicosanoid synthesis/release were determined by measuring the amounts of cysteinyl leukotrienes (collectively referred to as a group of 5-LOX derived metabolites LTC_4, LTD_4, and LTE_4) secreted into the culture medium of human brain cancer cells. Primary culture of ED 273b-BT human glioblastoma cells derived from patient was established and characterized in our laboratory as previously described (Farr-Jones, M. A.; Parney, I. F.;

Petruk, K. C. *J. Neurooncology* **1999**, *43*, 399). Cells were seeded in 12-well plates (2×10^5cells/well) and cultured in Dulbecco's modified Eagle's medium and F-12 nutrition mixture (Invitrogen, Grand Islands, NY, USA) supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 units/mL streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. The cells were stimulated with the appropriate concentrations of the test compounds and the positive control NDGA (Cayman Chemical, Ann Arbor, MI, USA) for 5-LOX activity. After a 24 h incubation, supernatants were harvested, centrifuged for 10 min at 2000 rpm, and stored at −80 °C until assayed. The concentrations of eicosanoids were determined using a cysteinyl leukotriene (catalog number 520501) enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA) according to a previously reported method (Uddin, M. J.; Rao, P. N. P.; Knaus, E. E. *Bioorg. Med. Chem.* **2004**, *12*, 5929).

10. *In vivo anti-inflammatory assay*. The test compounds **12a–c** and the reference drugs aspirin and ibuprofen were evaluated using the in vivo carrageenan-induced foot paw edema model reported previously (Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544).