Synthesis of New 1-(2-, 3-, Or 4-Methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene Regioisomers: A Search for Novel Cyclooxygenase and Lipoxygenase Inhibitors

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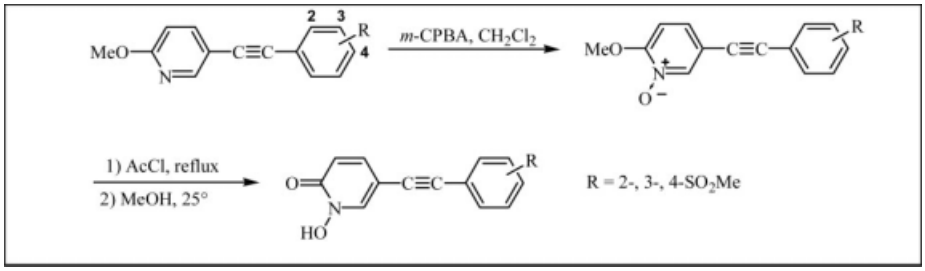

A group of acetylene regioisomers were designed such that a cyclooxygenase-2 (COX-2) SO_2Me pharmacophore was located at the ortho-, meta-, or para-position of the acetylene C-1 phenyl ring, and an iron-chelating 5-lipoxygenase (5-LOX) *N*-hydroxypyridin-2(1*H*)-one moiety was attached *via* its C-5 position to the C-2 position on an acetylene template (scaffold). These target linear acetylene regioisomers were synthesized *via* a palladium-catalyzed Sonogashira cross-coupling reaction. Structure-activity data acquired using *in vitro* cell-based inhibition assays indicated that this novel class of 1-(2-, 3-, or 4-methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers did not inhibit the COX-2 or (5-LOX) enzymes, and that they are devoid of *in vivo* anti-inflammatory activities.

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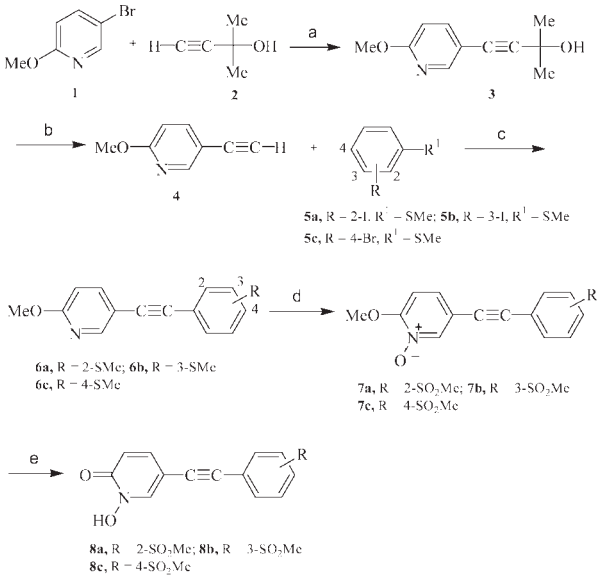
INTRODUCTION

Dual inhibitors of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) represent an attractive safer alternative to selective COX-2 inhibitors. This view is based on a potentially greater anti-inflammatory efficacy because of their ability to synergistically block both metabolic pathways of the arachidonic acid (AA) cascade [1]. A group of 1-(2-, 3-, and 4-methanesulfonylphenyl)-2-(2-, 3-, and 4-pyridyl)acetylene regioisomers, which are effective COX-1/COX-2 inhibitors that exhibit *in vivo* anti-inflammatory activities, were recently reported [2]. The most successful effort to develop 5-LOX inhibitors has been in the area of hydroxamic acids and related *N*-hydroxyureas that likely chelate iron present in the 5-LOX enzyme [3]. The recently described 1-(methanesulfonlylphenyl)-2-(pyridyl)acetylene regioisomers possess a suitable scaffold (template) to design novel acyclic dual inhibitors of the COX and LOX enzymes [2]. It was anticipated that replacement of the pyridyl ring in these parent acetylenes by a *N*-hydroxypyridin-2(1*H*)one moiety, which has the potential to chelate iron, may provide a hitherto unknown class of dual COX/5-LOX inhibitory anti-inflammatory agents. Accordingly, we now describe the synthesis of a novel group of 1-(2-, 3-, or 4-methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers (**8a–c**), to determine their potential utility as inhibitors of the COX-2 and 5-LOX enzymes.

RESULTS AND DISCUSSION

1-(Methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylenes (**6a–c**) were prepared in 28–72% yield using two consecutive palladium-catalyzed Sonogashira cross-coupling reactions [2,4–7]. The subsequent transformation of **6a–c** to the target 1-(2-, 3-, or 4-methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene regioisomers (**8a–c**) was carried out using the synthetic methodologies shown in Scheme 1. A modified procedure [8] was used to synthesize 5-ethynyl-2-methoxypyridine (**4**). Thus, Sonogashira coupling of 5-bromo-2-methoxypyridine (**1**) with 2-methylbut-3-yn-2-ol (**2**) in the presence of Et_3N, cuprous iodide (CuI), and dichloro-bis(triphenylphosphine)palladium(0) ($[PdCl_2(PPh_3)_2]$) catalyst afforded 4-(2-methoxypyridin-5-yl)-2-methyl-but-3-yn-2-ol (**3**) in 75% yield. Subsequent removal of the isopropanol moiety using sodium hydride furnished 5-ethynyl-2-methoxypyridine (**4**) in 82% yield.

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, $Pd(PPh_3)_2Cl_2$, 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.



A second Sonogashira cross-coupling reaction of **4** with the halothioanisole (**5a–c**) regioisomers was carried out under an argon atmosphere in Et_3N using [$PdCl_2(PPh_3)_2$]/CuI as catalyst to furnish the respective 1-(methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylenes **6a–c** in 28–72% yield. Oxidation of **6a–c** with *meta*-chloroperbenzoic acid in dichloromethane [9] afforded the 1-(methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylenes (**7a–c**) in 44–51% yield. Finally, reaction of the *N*-oxides **7a–c** with acetyl chloride at reflux, and then methanolysis in place of hydrolysis [9] furnished the target *N*-hydroxypyridin-2(1*H*)-ones (**8a–c**) in 69–91% yield.

Replacement of the carboxyl (CO_2H) in traditional arylacetic acid nonsteroidal anti-inflammatory drugs (NSAIDs) by a hydroxamic acid (CONHOH) moiety provided potent orally active 5-LOX inhibitory agents [10]. NSAIDs having a CONHOH or CON(Me)OH (pKa 9–11 range) in place of the CO_2H in traditional NSAIDs (pKa generally in the 4–5 range) are much less acidic, which decreases ulcerogenicity [11].

The rational for the design of the acyclic acetylenes **8a-c** was based on the expectations that (i) the phenyl ring bearing the SO_2Me pharmacophore will confer COX-2 inhibitory activity, and (ii) 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 **8a–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 carboxylic acids, are expected to be metabolically stable with improved oral efficacy.

In vitro cell-based inhibition assays were carried out to determine the biological effect of compounds **8a–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) and prostaglandin E_2 (PGE_2) secreted into the culture medium of human brain cancer cells. The assay to determine the ability of **8a–c** to inhibit *in vitro* cell-based 5-LOX activity showed that all three regioisomers failed to inhibit the 5-LOX enzyme ($IC_{50} > 50$ μM) relative to the reference drug nordihydroguaiaretic acid (NDGA, $IC_{50} = 35$ μM). Compounds **8a–c** were similarly inactive ($IC_{50} > 100$ μM) inhibitors of the COX-2 isozyme relative to the reference drug celecoxib ($IC_{50} = 2.5$ μM) in a cell-based assay.

The anti-inflammatory activities exhibited by the regioisomers **8a–c** were determined using a carrageenan-induced rat foot paw edema model at a 50 mg/kg oral dose. In this assay, compounds **8a–c** were all inactive anti-inflammatory (AI) agents compared with the reference drug celecoxib (79.9 ± 1.9% inhibition at 50 mg/kg po; $ID_{50} = 10.8$ mg/kg po).

The biological data acquired in this study indicate that the 1-(methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene structure is not a suitable template for the design of anti-inflammatory agents that act by inhibition of the 5-LOX and/or COX-2 enzymes.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Unless otherwise noted, infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FTIR spectrometer. ^1H NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$ or $CDCl_3$ + DMSO-d_6 with TMS as the internal standard. Microanalyses were performed for C, H, N (Micro-Analytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ±0.4% of theoretical values. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70-230 mesh). 2-Iodothioanisole (**5a**) [12] and 3-iodothioanisole (**5b**) [13] were synthesized in 91% and 76% yields, respectively, starting from 2-(methylthio)aniline and 3-(methylthio)aniline using the procedure of Ullmann [14]. All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

4-(2-Methoxypyridin-5-yl)-2-methylbut-3-yn-2-ol (3). $PdCl_2(PPh_3)_2$ (63 mg, 0.09 mmoles) and CuI (19 mg, 0.10 mmoles) were added to a stirred solution of 5-bromo-2-methoxypyridine

(**1**) (2.75 mL, 21.39 mmoles) and 2-methylbut-3-yn-2-ol (**2**) (2.20 mL, 22.61 mmoles) 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 **3** 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 (4). Sodium hydride (26 mg, 1.08 mmoles) was added to a solution of 4-(2-methoxypyridin-5-yl)-2-methylbut-3-yn-2-ol (**3**) (1.52 g, 7.96 mmoles) 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 **4** 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-(methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylenes (6a–c). CuI (46 mg, 0.24 mmoles) was added with stirring to a solution containing $PdCl_2(PPh_3)_2$ (85 mg, 0.12 mmoles), 5-ethynyl-2-methoxypyridine (**4**) (6 mmoles), and a halothioanisole **5a**, **5b**, or **5c** (4 mmoles), in 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-EtOAc (10:1, v/v) as eluent to furnish the respective product **6a–c**. Some physical and spectroscopic data for **6a–c** are listed below.

1-(2-Methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylene (6a). The product was obtained as a pale yellow oil using the Sonogashira coupling reaction of **4** with 2-iodothioanisole (**5a**) in 72% yield; IR (film): 2213 (C≡C), 1615, 1580, 1488 (Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.52 (s, 3H, S*Me*), 4.00 (s, 3H, O*Me*), 6.77 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.12 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H, phenyl H-5), 7.19 (dd, J = 7.6, 1.2 Hz, 1H, phenyl H-3), 7.31 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H, phenyl H-4), 7.48 (d, J = 7.6, 1.2 Hz, 1H, phenyl H-6), 7.77 (dd, J = 8.5, 2.1 Hz, 1H, pyridyl H-4), 8.41 (d, J = 2.1 Hz, 1H, pyridyl H-6).

1-(3-Methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylene (6b). The product was obtained as a pale yellow oil using the Sonogashira coupling reaction of **4** with 3-iodothioanisole (**5b**) in 44% yield; IR (film): 2229 (C≡C), 1588, 1560, 1495 (Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, S*Me*), 3.99 (s, 3H, O*Me*), 6.77 (d, J = 8.9 Hz, 1H, pyridyl H-3), 7.20–7.31 (m, 3H, phenyl H-4, H-5, H-6), 7.39 (s, 1H, phenyl H-2), 7.72 (dd, J = 8.9, 2.1 Hz, 1H, pyridyl H-4), 8.36 (d, J = 2.1 Hz, 1H, pyridyl H-6).

1-(4-Methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylene (6c). The product was obtained as a pale yellow solid using the Sonogashira coupling reaction of **4** with 4-bromothioanisole (**5c**) in 28% yield; mp 85–87°C; IR (film): 2230 (C≡C), 1602, 1560, 1495 (Ar) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, S*Me*), 4.00 (s, 3H, O*Me*), 6.77 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.21 (d, J = 8.5 Hz, 2H, phenyl H-3, H-5), 7.43 (d, J = 8.5 Hz, 2H, phenyl H-2, H-6), 7.72 (dd, J = 8.5, 2.1 Hz, 1H, pyridyl H-4), 8.36 (d, J = 2.1 Hz, 1H, pyridyl H-6).

General procedure for the synthesis of 1-(methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylenes (7a-c). *m*-Chloroperoxybenzoic acid (77% max.) (12 mmoles) was added to a stirred solution of a 1-(methylthiophenyl)-2-(2-methoxypyrid-5-yl)acetylene (**6a**, **6b**, or **6c**, 2 mmoles) in dry CH_2Cl_2 (25 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 product **7a–c**. Some physical and spectroscopic data for **7a–c** are listed below.

1-(2-Methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (7a). Yield, 48%; pale yellow solid; mp 140–142°C; IR (film): 2220 (C≡C), 1669, 1602, 1521 (Ar), 1313, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.25 (s, 3H, SO_2*Me*), 4.16 (s, 3H, O*Me*), 6.98 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.52–7.70 (m, 3H, pyridyl H-4, phenyl H-4, H-5), 7.74 (dd, J = 7.6, 1.5 Hz, 1H, phenyl H-6), 8.15 (dd, J = 7.6, 1.5 Hz, 1H, phenyl H-3), 8.54 (d, J = 1.8 Hz, 1H, pyridyl H-6).

1-(3-Methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (7b). Yield, 51%; pale yellow solid; mp 172–174°C; IR (film): 2227 (C≡C), 1609, 1528 (Ar), 1307, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.09 (s, 3H, SO_2*Me*), 4.14 (s, 3H, O*Me*), 6.93 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.47 (dd, J = 8.5, 1.8 Hz, 1H, pyridyl H-4), 7.61 (dd, J = 7.6, 7.6 Hz, 1H, phenyl H-5), 7.77 (ddd, J = 7.6, 1.5, 1.2 Hz, 1H, phenyl H-6), 7.95 (ddd, J = 7.6, 1.5, 1.2 Hz, 1H, phenyl H-4), 8.10 (dd, J = 1.5, 1.2 Hz, 1H, phenyl H-2), 8.45 (d, J = 1.8 Hz, 1H, pyridyl H-6).

1-(4-Methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (7c). Yield, 44%; white solid; mp 175–177°C; IR (film): 2227 (C≡C), 1602, 1522 (Ar), 1300, 1146 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2*Me*), 4.14 (s, 3H, O*Me*), 6.93 (d, J = 8.9 Hz, 1H, pyridyl H-3), 7.48 (d, J = 8.9, 1.8 Hz, 1H, pyridyl H-4), 7.71 (d, J = 8.5 Hz, 2H phenyl H-2, H-6), 7.95 (d, J = 8.5 Hz, 2H, phenyl H-3, H-5), 8.46 (d, J = 1.8 Hz, 1H, pyridyl H-6).

General procedure for the synthesis of 1-(methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylenes (**8a-c**). Acetyl chloride (6 mL) was added to a 1-(methanesulfonylphenyl)-2-(1-oxido-2-methoxypyrid-5-yl)acetylene (7a, 7b or 7c, 2 mmoles) and the reaction was allowed to proceed at reflux for 1 hour. 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 product (**8a-c**). The spectral and microanalytical data for compounds **8a-c** are listed below.

1-(2-Methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene (8a). Yield, 71%; brown solid; mp 198–200°C; IR (film): 2210 (C≡C), 1650 (CO), 1300, 1140 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.18 (s, 3H, SO_2*Me*), 6.54 (d, J = 9.2 Hz, 1H, pyridone H-3), 7.39 (dd, J = 9.2, 1.2 Hz, 1H, pyridone H-4), 7.46 (dd, J = 7.6, 7.6 Hz, 1H, phenyl H-

5), 7.55 (dd, J = 7.6, 7.6 Hz, 1H, phenyl H-4), 7.60 (d, J = 7.6 Hz, 1H, phenyl H-6), 7.93 (d, J = 1.2 Hz, 1H, pyridone H-6), 7.99 (d, J = 7.6 Hz, 1H, phenyl H-3). Anal. Calcd for $C_{14}H_{11}NO_4S \cdot 1/2H_2O$: C, 56.32; H, 4.02; N, 4.69. Found: C, 56.30; H, 4.10; N, 4.83.

1-(3-Methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene (8b). Yield, 69%; brown solid; mp 205–207°C; IR (KBr): 2210 (C≡C), 1650 (CO), 1300, 1145 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 3.01 (s, 3H, SO_2Me), 6.54 (d, J = 9.4 Hz, 1H, pyridone H-3), 7.35 (dd, J = 9.4, 2.4 Hz, 1H, pyridone H-4), 7.50 (dd, J = 7.6, 7.6 Hz, 1H, phenyl H-5), 7.65 (ddd, J = 7.6, 2.7, 1.5 Hz, 1H, phenyl H-6), 7.80 (ddd, J = 7.6, 2.7, 1.5 Hz, 1H, phenyl H-4), 7.89 (d, J = 2.4 Hz, 1H, pyridone H-6), 7.93 (dd, J = 1.5, 1.5 Hz, 1H, phenyl H-2). Anal. Calcd for $C_{14}H_{11}NO_4S \cdot 1/2H_2O$: C, 56.32; H, 4.02; N, 4.69. Found: C, 56.71; H, 3.80; N, 4.83.

1-(4-Methanesulfonylphenyl)-2-[5-(*N*-hydroxypyridin-2(1*H*)-one)]acetylene (8c). Yield, 91%; pale yellow solid; mp 234–236°C; IR (film): 2211 (C≡C), 1651 (CO), 1300, 1144 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.20 (s, 3H, SO_2Me), 6.55 (d, J = 9.5 Hz, 1H, pyridone H-3), 7.50 (dd, J = 9.5, 2.4 Hz, 1H, pyridone H-4), 7.70 (d, J = 8.5 Hz, 2H phenyl H-2, H-6), 7.93 (d, J = 8.5 Hz, 2H, phenyl H-3, H-5), 8.33 (d, J = 2.4 Hz, 1H, pyridone H-6), 12.1 (br s, 1H, N-O*H*). Anal. Calcd for $C_{14}H_{11}NO_4S$: C, 58.12; H, 3.83; N, 4.84. Found: C, 57.80; H, 3.87; N, 4.95.

***In vitro* cell-based enzyme immunoassays for determination of prostaglandin E_2 (COX-2) and cysteinyl leukotrienes (5-LOX).** The biological effects of the test compounds **8a–c** on eicosanoid synthesis/release were determined by measuring the amounts of prostaglandin E_2 and 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 [15]. 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) supplemented with 10% heat-inactivated fetal calf serum, 100 units/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 controls celecoxib (LKT Laboratories, St. Paul, MN) for COX-2 activity and NDGA (Cayman Chemical, Ann Arbor, MI) 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 prostaglandin E_2 (catalog number 514010) and cysteinyl leukotriene (catalog number 520501) enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) according to a previously reported method [16].

***In vivo* anti-inflammatory assay.** The test compounds **8a–c** and the reference drug celecoxib were evaluated using the in vivo carrageenan-induced foot paw edema model reported previously [17].

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