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Synthesis of 1-(methanesulfonyl- and aminosulfonylphenyl)acetylenes that possess a 2-(*N*-difluoromethyl-1,2-dihydropyridin-2-one) pharmacophore: Evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity

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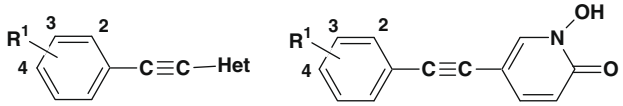
ABSTRACT

A hitherto unknown class of linear acetylene regioisomers were designed such that a SO_2Me or SO_2NH_2 group was located at the *ortho-*, *meta-* or *para*-position of the acetylene C-1 phenyl ring, and a *N*-difluoromethyl-1,2-dihydropyridin-2-one moiety was attached via its C-5 position to the C-2 position on an acetylene template (scaffold). All three SO_2Me regioisomers, and the 4-SO_2NH_2 analog, were potent inhibitors of 5-lipoxygenase (5-LOX IC_{50} = 3.2–3.5 μM range) relative to the reference drug caffeic acid (IC_{50} = 4.0 μM). The SO_2Me regioisomers exhibited weak cyclooxygenease-1 (COX-1) and -2 (COX-2) inhibitory activity with a modest COX-2 selectivity index. The most potent 3-SO_2Me, 4-SO_2Me and 4-SO_2NH_2 compounds, with respective ED_{50} values of 66.1, 68.5 and 86.5 mg/kg po, exhibited comparable oral anti-inflammatory (AI) activity to that of the reference drug ibuprofen (ED_{50} = 67.4 mg/kg po). The *N*-difluoromethyl-1,2-dihydropyridin-2-one moiety provides a novel pharmacophore for the design of cyclic hydroxamic mimetics capable of inhibiting 5-LOX for exploitation in the development of 5-LOX inhibitory AI drugs.

A group of 1-(aryl)-2-(pyridyl)acetylene regioisomers (**1**) were recently reported that are inhibitors of the cyclooxygenase-1 (COX-1) and/or cyclooxygenase-2 (COX-2) isozymes (see structure in Fig. 1).[1] Subsequent replacement of the pyridyl ring in the acetylenes **1** by a *N*-hydroxy-1,2-dihydropyridin-2-one cyclic hydroxamic acid moiety, that has the potential to chelate iron, provided a novel class of 5-lipoxygenase (5-LOX) inhibitors (**2**).[2] It was anticipated that elaboration of the pyridyl ring present in the 1-(aryl)-2-(pyridyl)acetylene regioisomers (**1**) to a *N*-difluoromethyl-1,2-dihydropyridin-2-one moiety would provide a potential 5-LOX pharmacophore. This concept is based on the premise that the *N*-difluoromethyl-1,2-dihydropyridin-2-one group may bind to, or chelate iron present in the 5-LOX enzyme. Hybrid compounds of this type also containing a suitably positioned methanesulfonyl ($MeSO_2$) or sulfonamide (H_2NSO_2) COX-2 pharmacophore constitute a potential class of dual COX/5-LOX inhibitors. Accordingly, we now describe the synthesis of a novel group of linear acetylenes (**9a–d**) that possess COX-2 and 5-LOX pharmacophores, their in vitro evaluation as 5-LOX, COX-1/COX-2 inhibitors, and in vivo assessment as anti-inflammatory agents.

The target 1-(2-, 3- and 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]-acetylenes (**9a–d**) were prepared using the reaction sequence illustrated in Scheme 1. 2-Iodothioanisole (**3a**)[3] and 3-iodothioanisole (**3b**)[4] were synthesized in 91% and 76% yield starting from the respective 2-(methylthio)aniline and 3-(methylthio)aniline using the procedure of Ullmann.[5] The Sonogashira cross-coupling reaction of 2-amino-5-ethynylpyridine (**4**)[6] with a halothioanisole (**3a–c**), or 4-bromobenzenesulfonamide (**3d**),[7] in the presence of cuprous iodide (CuI), dichlorobis(triphenylphosphine)palladium(II)



1, Het = 2-, 3-, 4-pyridyl
R^1 = 2-, 3-, 4-SO_2NH_2
R^1 = 2-, 3-, 4-SO_2Me

2, R^1 = 2-, 3-, 4-SO_2NH_2

Figure 1. Linear acetylenes that exhibit COX-1 and COX-2 (**1**), and 5-LOX (**2**), inhibitory activities.

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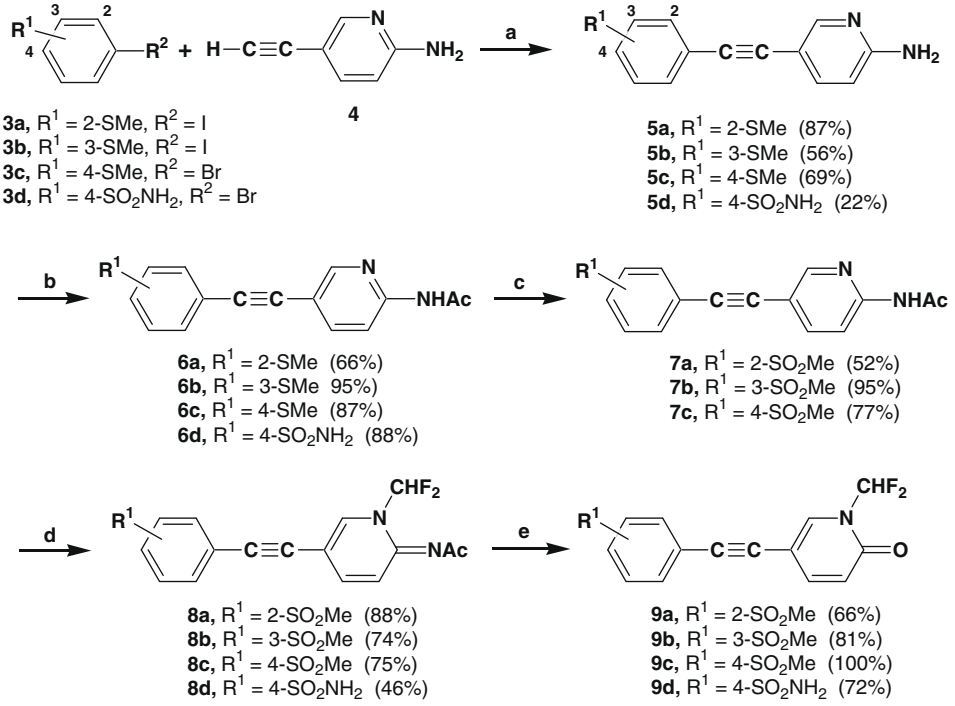


Scheme 1. Reagents and conditions: (a) Et_3N–THF, PPh_3, $Pd(PPh_3)_2Cl_2$, CuI, 75–80 °C, overnight; (b) Ac_2O, 80 °C, 4 h; (c) Oxone®, MeOH–THF–H_2O, 25 °C, 2 h, (**6a–c**); (d) $ClCF_2COONa$, MeCN, reflux, 18 h (**6d**, **7a–c**); (e) 1% $KHSO_4$, MeCN, reflux, 2 h.

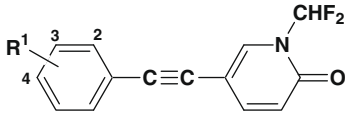
($[Pd(PPh_3)_2Cl_2]$) catalyst and triphenylphosphine (PPh_3) in Et_3N–THF under an argon atmosphere[6] afforded the 1-(2-, 3- or 4-methylthiophenyl and 4-aminosulfonylphenyl)-2-(2-aminopyrid-5-yl)acetylenes (**5a–d**) in 22–87% yield. Acetylation of the amino group in **5a–d** using acetic anhydride[8] at 80 °C afforded the 1-(2-, 3- or 4-methylthiophenyl and 4-aminosulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylenes (**6a–d**) in 66–95% yields. Oxidation of the thiomethyl substituent in **6a–c** to a methylsulfonyl group using an aqueous solution of Oxone® (potassium peroxymonosulfate)[1] in MeOH–THF afforded the 1-(2-, 3- or 4-methanesulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene regioisomers (**7a–c**) in 52–95% yields. The difluoromethylation of **6d** and **7a–c** with 1.2 equiv of sodium chlorodifluoroacetate[9] ($ClCF_2COONa$) proceeded smoothly in refluxing acetonitrile to furnish the expected 1-(2-, 3- or 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyridin-2-acetylimino]-acetylenes (**8a–d**) in 46–88% yields. Subsequent hydrolysis of **8a–d** with 1% aq potassium hydrogen sulfate[9] afforded the target 1-(2-, 3- and 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]acetylenes (**9a–d**) in 66–100% isolated yield.

The rational for the design of the 1-(2-, 3- and 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]acetylenes (**9a–d**) was based on the expectation that replacement of a pyridyl ring in the 1-(aryl)-2-(pyridyl)acetylene regioisomers (**1**), or replacement of the *N*-hydroxypyridin-2(1*H*)one moiety present in the 1-(benzenesulfonamido)-2-[5-(*N*-hydroxypyrid-2(1*H*)-one)]acetylene regioisomers (**2**), would furnish a novel class of compounds with dual 5-LOX/COX-2 inhibitory activities. The $CONCHF_2$ part of the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring present in **9a–d** can be viewed as a cyclic hydroxamic acid mimetic. These *N*-difluoromethyl-1,2-dihydropyrid-2-ones, like acyclic hydroxamic acids, are expected to serve as effective iron chelators to exhibit 5-LOX inhibitory activity. It has been reported that there is a substantial build-up of negative potential around the two fluorine atoms of a CHF_2 group.[10] Despite this high electron-density, an aliphatic fluorine seldom acts as a hydrogen-bond acceptor, presumably due to its high electronegativity and low polarizability.[11,12] Instead, it is plausible that the CHF_2 group may interact with a positively charged region on the enzyme that may contribute to enhanced affinity and competitive reversible inhibition of the COX and/or 5-LOX enzymes.[13] In addition, these cyclic *N*-difluoromethyl-1,2-dihydropyrid-2-ones, unlike acyclic hydroxamic acids which undergo facile biotransformation to the acids, are expected to have a greater metabolic stability with improved oral efficacy. Although there is some distortion from planarity at the N^1-nitrogen atom of the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system, the relatively flat diene portion of this quasi-planar ring system has the potential to serve as a suitable replacement for the pyridyl group in the 1-(aryl)-2-(pyridyl)acetylene regioisomers (**1**) resulting in retention of selective COX-2 inhibitory activity.

In vitro COX-1 and COX-2 enzyme inhibition studies showed that the *N*-difluoromethyl-1,2-dihydropyrid-2-ones (**9a–d**) were much weaker inhibitors (COX-1 IC_{50} = 5.2 to >100 μM range; COX-2 IC_{50} = 3.7–9.4 μM range) than the selective COX-2 inhibitor celecoxib (COX-1 IC_{50} = 7.7 μM; COX-2 IC_{50} = 0.12 μM) (see data in Table 1). The $MeSO_2$ regioisomers **9a–c** are more potent inhibitors of COX-2 than COX-1 thereby showing some COX-2 selectivity. The 4-SO_2Me (**9c**) and 4-SO_2NH_2 (**9d**) compounds exhibited relatively similar COX-1/COX-2 inhibitory activity. In contrast, all *N*-difluoromethyl-1,2-dihydropyrid-2-ones (**9a–d**) were more potent in vitro inhibitors of the potato 5-LOX enzyme (IC_{50} = 3.2–3.5 μM range) than the reference drug caffeic acid (IC_{50} = 4.0 μM). This small difference in 5-LOX inhibitory potency for compounds **9a–d** suggests that the *N*-difluoromethyl-1,2-dihydropyrid-2-one, rather than the position (*ortho*, *meta* or *para*) of the methanesulfonyl or aminosulfonyl COX-2 pharmacophore, is the major determinant of 5-LOX inhibitory activity.

The oral AI activities (ED_{50} values) exhibited by the *N*-difluoromethyl-1,2-dihydropyrid-2-ones (**9a–d**) were determined using a carrageenan-induced rat foot paw edema model (see data in Table 1). The AI structure–activity data acquired showed that the 3-SO_2Me (**9b**, ED_{50} = 66.1 mg/kg po), 4-SO_2Me (**9c**, ED_{50} = 68.5 mg/

Table 1
In vitro COX-1, COX-2, 5-LOX enzyme inhibition, and in vivo anti-inflammatory activity, data for the 1-(2-, 3- and 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]acetylenes (**9a–d**)



Compound	R^1	COX-1 IC_{50}[a] (μM)	COX-2 IC_{50}[a] (μM)	5-LOX IC_{50}[b] (μM)	AI activity[c] ED_{50} (mg/kg)
9a	2-SO_2Me	>100	9.4	3.4	>100
9b	3-SO_2Me	32.4	3.7	3.2	66.1
9c	4-SO_2Me	14.1	5.1	3.3	68.5
9d	4-SO_2NH_2	5.2	6.0	3.5	86.5
Celecoxib		7.7	0.12	—	10.8
Ibuprofen		2.9	1.1[d]	—	67.4
Aspirin		0.35	2.4[d]	—	128.9
Caffeic acid		—	—	4.0	—

[a] The in vitro test compound concentration required to produce 50% inhibition of ovine COX-1 or human recombinant COX-2. The result (IC_{50}, μM) is the mean of two determinations acquired using the enzyme immuno assay kit (Catalog No. 560131, Cayman Chemicals, Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

[b] The in vitro test compound concentration required to produce 50% inhibition of potato 5-LOX (Cayman Chemicals, Inc., Catalog No. 60401). The result (IC_{50}, μM) is the mean of two determinations acquired using a LOX assay kit (Catalog No. 760700, Cayman Chemicals, Inc., Ann Arbor, MI, USA) and the deviation from the mean is <10% of the mean value.

[c] Inhibitory 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 administration of the test compound.

[d] Data acquired using ovine COX-2 (Catalog No. 560101, Cayman Chemicals, Inc.).

kg po) and 4-SO_2NH_2 (**9d**, ED_{50} = 86.5 mg/kg po), compounds exhibited AI activities that were more similar to that of the reference drug ibuprofen (ED_{50} = 67.4 mg/kg po) than the selective COX-2 inhibitor celecoxib (ED_{50} = 10.8 mg/kg po) or the non-selective COX inhibitor aspirin (ED_{50} = 128.9 mg/kg po). The in vitro and in vivo structure–activity data acquired suggest that the *N*-difluoromethyl-1,2-dihydropyrid-2-ones (**9b–d**), that are weak inhibitors of the COX-1 and COX-2 isozymes, exhibit their AI effect to a significant degree by preventing the biosynthesis of proinflammatory leukotrienes (LTs) produced via the LOX pathway.

The *N*-hydroxypyrid-2(1*H*)-one regioisomers (**2**, R^1 = 2-, 3- and 4-SO_2NH_2) reported previously exhibited excellent 5-LOX inhibitory activities (IC_{50} = 10–68 μM range) relative to the reference drug nordihydroguaiaretic acid (NDGA, IC_{50} = 35 μM) determined using a cell-based enzyme immuno assay. In spite of this high in vitro 5-LOX inhibitory activity, only the 2-SO_2NH_2 regioisomer (**2**) exhibited in vivo AI activity (ED_{50} = 86 mg/kg po).[2] In comparison, the *N*-difluoromethyl-1,2-dihydropyrid-2-ones **9a–d**, as indicated above, were potent in vitro inhibitors of the isolated potato 5-LOX enzyme (IC_{50} = 3.2–3.5 μM range) similar to the reference drug caffeic acid (IC_{50} = 4.0 μM). In contrast, all of the *N*-difluoromethyl-1,2-dihydropyrid-2-ones **9**, with the exception of the 2-SO_2Me regioisomer **9a**, exhibited good AI activity. These latter differences in AI structure–activity relationships suggests the *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety may have more favorable bioavailability, biodistribution and/or metabolic properties making it more suitable than the *N*-hydroxypyrid-2(1*H*)-one moiety as a 5-LOX pharmacophore.

In conclusion, a hitherto unknown class of 1-(2-, 3- and 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]acetylenes (**9a–d**)[14] was designed for evaluation as dual 5-LOX[15] and COX-1/COX-2 isozyme[16] inhibitors of inflammation. The structure–activity data acquired indicate that (i) the relative AI potency order with respect to the aryl substituent is 3-SO_2Me (**9b**) ≈ 4-SO_2Me (**9c**) > 4-SO_2NH_2 ≫ 2-SO_2Me (**9a**), (ii) the *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety provides a novel 5-LOX pharmacophore for the design of cyclic hydroxamic mimetics and (iii) the title compounds **9b–d**, that are very weak inhibitors of the COX-1/COX-2 isozymes, exhibit anti-inflammatory activity[17] that is dependent upon inhibition of proinflammatory leukotriene biosynthesis in the lipoxygenase pathway.

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References and notes

1. Chowdhury, M. A.; Dong, Y.; Chen, Q.-H.; Abdellatif, K. R. A.; Knaus, E. E. *Bioorg. Med. Chem.* **2008**, *16*, 1948.
2. Chowdhury, M. A.; Chen, H.; Abdellatif, K. R. A.; Dong, Y.; Petruk, K. C.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4195.
3. Larock, R. C.; Harrison, W. *J. Am. Chem. Soc* **1984**, *106*, 4218.
4. Mongin, O.; Papamicaël, C.; Hoyler, N.; Gossauer, A. *J. Org. Chem.* **1998**, *63*, 5568.
5. Ullmann, F. *Justus Liebigs Ann. Chem.* **1904**, *332*, 69.
6. Aakeröy, C. B.; Schultheiss, N.; Desper, J. *Dalton Trans.* **2006**, 1627.
7. Anana, R.; Rao, P. N. P.; Chen, Q.-H.; Knaus, E. E. *Bioorg. Med. Chem.* **2006**, *14*, 5259.
8. Sollogoub, M.; Fox, K. R.; Powers, V. E. C.; Brown, T. *Tetrahedron Lett.* **2002**, *43*, 3121.
9. Ando, M.; Wada, T.; Sato, N. *Org. Lett.* **2006**, *8*, 3805.
10. Narjes, F.; Koehler, K. F.; Koch, U.; Gerlach, B.; Colarusso, S.; Steinkühler, C.; Brunetti, M.; Altamura, S.; De Francesco, R.; Matassa, V. G. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 701. and references cited therein.
11. Dunitz, J. D.; Taylor, R. *Chem. Eur. J.* **1997**, *3*, 89.
12. Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613.
13. Chavatte, P.; Yous, S.; Marot, C.; Baurin, N.; Lesieur, D. *J. Med. Chem.* **2001**, *44*, 3223.
14. Experimental procedures and spectral data for compounds **5a–d**, **6a–d**, **7a–c**, **8a–d** and **9a–d**. *General procedure for the synthesis of 1-(2-, 3- or 4-methylthiophenyl and 4-aminosulfonylphenyl)-2-(2-aminopyrid-5-yl)acetylenes* (**5a–d**). Bis(triphenylphosphine)palladium(II) dichloride (0.22 g, 0.32 mmol), copper(I) iodide (0.05 g, 0.26 mmol) and triphenylphosphine (0.22 g, 0.84 mmol) were added to a stirred solution of 2-amino-5-ethynylpyridine (**4**, 1.03 g, 8.70 mmol) and a bromo- or iodothioanisole (**3a**, **3b** or **3c**), or 4-bromobenzenesulfonamide (**3d**), (10.12 mmol) in Et_3N–THF (1:1 v/v, 60 mL) at 25 °C. Dry argon was bubbled through the resultant mixture for 10 min. The reaction was allowed to proceed at 80 °C overnight under an argon atmosphere, the mixture was 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 hexanes/EtOAc (1:3, v/v) as eluent to furnish the respective product **5a–d**. Some physical and spectroscopic data for **5a–d** are listed below.
1-(2-Methylthiophenyl)-2-(2-aminopyrid-5-yl)acetylene (**5a**). The product was obtained as a pale yellow solid using the Sonogashira cross-coupling reaction of 2-iodothioanisole (**3a**) with **4** in 87% yield; mp 103–105 °C; IR (film): 3370, 3300 (NH_2), 2200 (C≡C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, S*Me*), 4.62 (br s, 2H, NH_2 that exchanges with D_2O), 6.48 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.11 (ddd, *J* = 7.3,

7.3, 1.2 Hz, 1H, phenyl H-5), 7.18 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-3), 7.30 (ddd, *J* = 7.3, 7.3, 1.2 Hz, 1H, phenyl H-4), 7.46 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-6), 7.61 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.32 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.1, 87.3, 93.5, 107.9, 109.8, 121.4, 124.0, 124.2, 128.5, 131.9, 140.3, 141.2, 151.4, 157.5.

1-(3-Methylthiophenyl)-2-(2-aminopyrid-5-yl)acetylene (**5b**). The product was obtained as a pale yellow solid using the Sonogashira cross-coupling reaction of 3-iodothioanisole (**3b**) with **4** in 56% yield; mp 115–117 °C; IR (film): 3300, 3250 (NH_2), 2200 (C≡C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, S*Me*), 4.68 (br s, 2H, N*H*$_2$ that exchanges with D_2O), 6.48 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.15–7.30 (m, 3H, phenyl H-4, H-5, H-6), 7.37 (s, 1H, phenyl H-2), 7.57 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.28 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.7, 87.4, 89.4, 107.9, 109.6, 124.0, 126.2, 127.9, 128.6, 128.7, 138.8, 140.3, 151.4, 157.5.

1-(4-Methylthiophenyl)-2-(2-aminopyrid-5-yl)acetylene (**5c**). The product was obtained as a pale yellow solid using the Sonogashira cross-coupling reaction of 4-bromothioanisole (**3c**) with **4** in 69% yield; mp 155–157 °C; IR (film): 3435, 3305 (NH_2), 2200 (C≡C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, S*Me*), 4.59 (br s, 2H, N*H*$_2$ that exchanges with D_2O), 6.48 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 7.20 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-3, H-5), 7.41 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-2, H-6), 7.55 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.27 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.4, 87.0, 89.6, 107.9, 110.0, 119.6, 125.9, 131.6, 139.0, 140.3, 151.3, 157.3.

1-(4-Aminosulfonylphenyl)-2-(2-aminopyrid-5-yl)acetylene (**5d**). The product was obtained as a pale yellow solid using the Sonogashira cross-coupling reaction of **3d** with **4** in 22% yield; mp 248–250 °C; IR (film): 3480, 3367, 3323 (NH_2), 2217 (C≡C), 1311, 1167 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.46 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 6.51 (br s, 2H, N*H*$_2$ that exchanges with D_2O), 7.43 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.53 (dd, *J* = 8.5, 2.4 Hz, 1H, pyridyl H-4), 7.65 (d, *J* = 8.5 Hz, 2H, phenyl H-2, H-6), 7.81 (d, *J* = 8.5 Hz, 2H, phenyl H-3, H-5), 8.15 (d, *J* = 2.4 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 88.3, 89.7, 106.9, 108.6, 125.8, 126.6, 130.9, 140.2, 142.6, 149.4, 157.9.

General procedure for the synthesis of 1-(2-, 3- or 4-methylthiophenyl and 4-aminosulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylenes (**6a–d**). A solution of a 1-(2-, 3- or 4-methylthiophenyl or 4-aminosulfonylphenyl)-2-(2-aminopyrid-5-yl)acetylene (**5a–d**) (5 mmol) in acetic anhydride (50 mL) was heated at 80 °C for 2 h. The reaction was cooled to 25 °C, the solvent was removed in vacuo, and the crude product was purified by silica gel column chromatography using hexanes/EtOAc (2:1, v/v) as eluent to furnish the respective product **6a–d**. Some physical and spectroscopic data for **6a–d** are listed below.

1-(2-Methylthiophenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6a**). The product was obtained as a white solid in 66% yield; mp 138–140 °C; IR (film): 3250 (NH), 2200 (C≡C), 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CO*Me*), 2.53 (s, 3H, S*Me*), 7.14 (ddd, *J* = 7.3, 7.3, 1.2 Hz, 1H, phenyl H-5), 7.20 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-3), 7.34 (ddd, *J* = 7.3, 7.3, 1.2 Hz, 1H, phenyl H-4), 7.49 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-6), 7.88 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.15 (br s, 1H, N*H* that exchanges with D_2O), 8.23 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.48 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.0, 24.8, 89.2, 92.2, 113.1, 116.0, 120.7, 124.0, 124.3, 129.1, 132.2, 140.9, 141.7, 150.4, 168.5.

1-(3-Methylthiophenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6b**). The product was obtained as a yellow solid in 95% yield; mp 125–127 °C; IR (film): 3250 (NH), 2200 (C≡C), 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CO*Me*), 2.51 (s, 3H, S*Me*), 7.20–7.35 (m, 3H, phenyl H-4, H-5, H-6), 7.40 (s, 1H, phenyl H-2), 7.83 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.23 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.26 (br s, 1H, N*H* that exchanges with D_2O), 8.42 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.6, 24.7, 85.9, 91.4, 113.6, 115.9, 123.2, 126.7, 128.0, 128.6, 128.8, 139.0, 141.3, 149.7, 150.5, 168.8.

1-(4-Methylthiophenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6c**). The product was obtained as a pale yellow solid in 87% yield; mp 200–202 °C; IR (film): 3250 (NH), 2200 (C≡C), 1700 (CO) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CO*Me*), 2.51 (s, 3H, S*Me*), 7.22 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-3, H-5), 7.44 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-2, H-6), 7.82 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.05 (br s, 1H, N*H* that exchanges with D_2O), 8.21 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.42 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 15.3, 24.8, 85.9, 91.6, 113.1, 116.2, 118.9, 125.8, 131.8, 139.8, 140.8, 150.1, 150.3, 168.5.

1-(4-Aminosulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6d**). The product was obtained as a pale yellow solid in 88% yield; mp 225–227 °C; IR (film): 3321 (NH), 2217 (C≡C), 1700 (CO), 1334, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 2.10 (s, 3H, CO*Me*), 7.21 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.56 (d, *J* = 8.5 Hz, 2H, phenyl H-2, H-6), 7.75 (dd, *J* = 8.5, 2.4 Hz, 1H, pyridyl H-4), 7.82 (d, *J* = 8.5 Hz, 2H, phenyl H-3, H-5), 8.13 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.39 (d, *J* = 2.4 Hz, 1H, pyridyl H-6), 10.48 (br s, 1H, N*H* that exchanges with D_2O); ^{13}C NMR ($CDCl_3$) δ 22.9, 88.5, 89.3, 125.5, 125.6, 127.5, 131.1, 138.4, 140.1, 143.2, 151.4, 169.1.

General procedure for the synthesis of 1-(2-, 3- or 4-methylsulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylenes (**7a–c**). An aqueous solution of Oxone® (6.14 g, 10 mmol, 18 mL) was added to a stirred solution of a 1-(2-, 3- or 4-methylthiophenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6a–c**) (2 mmol) in methanol (30 mL) and THF (30 mL), and the reaction was allowed to proceed with stirring at 25 °C for 3 h. The reaction mixture was diluted with water (200 mL), extracted with EtOAc (3 × 75 mL), the organic phase was washed successively with water and brine, and dried ($MgSO_4$). After filtration, the solvent from the organic fraction was removed in vacuo to give a crude product which was purified by silica gel column chromatography using hexanes/EtOAc (1:3, v/v) as eluent to furnish the respective title compound **7a–c**. Some physical and spectroscopic data for **7a–c** are listed below.

1-(2-Methanesulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**7a**). The product was obtained as a white solid in 52% yield; mp 180–182 °C; IR (film): 3330 (NH), 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 3H, CO*Me*), 3.31 (s, 3H, SO_2*Me*), 7.55 (ddd, *J* = 7.3, 7.3, 1.2 Hz, 1H, phenyl H-5), 7.65 (ddd, *J* = 7.3, 7.3, 1.2 Hz, 1H, phenyl H-4), 7.74 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-6), 7.90 (dd, *J* = 9.2, 1.8 Hz, 1H, pyridyl H-4), 8.15 (dd, *J* = 7.3, 1.2 Hz, 1H, phenyl H-3), 8.27 (d, *J* = 9.2 Hz, 1H, pyridyl H-3), 8.31 (br s, 1H, N*H* that exchanges with D_2O), 8.50 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 24.8, 42.5, 87.7, 94.8, 113.3, 114.9, 121.6, 128.8, 128.9, 133.2, 134.2, 141.0, 141.1, 150.6, 151.1, 168.7.

1-(3-Methanesulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**7b**). The product was obtained as a yellow solid in 95% yield; mp 170–172 °C; IR (film): 3325 (NH), 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CO*Me*), 3.09 (s, 3H, SO_2*Me*), 7.59 (dd, *J* = 7.9, 7.9 Hz, 1H, phenyl H-5), 7.79 (ddd, *J* = 7.9, 1.2, 1.2 Hz, 1H, phenyl H-6), 7.85 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 7.92 (dd, *J* = 7.9, 1.2 Hz, 1H, phenyl H-4), 8.12 (dd, *J* = 1.2, 1.2 Hz, 1H, phenyl H-2), 8.15 (br s, 1H, N*H* that exchanges with D_2O), 8.26 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.45 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 24.8, 44.4, 88.3, 89.4, 113.3, 115.1, 124.5, 126.9, 129.5, 130.4, 136.2, 141.0, 141.1, 150.5, 150.8, 168.7.

1-(4-Methanesulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**7c**). The product was obtained as a pale yellow solid in 77% yield; mp 205–207 °C; IR (film): 3360 (NH), 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CO*Me*), 3.09 (s, 3H, SO_2*Me*), 7.71 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-2, H-6), 7.85 (dd, *J* = 8.5, 1.8 Hz, 1H, pyridyl H-4), 7.95 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-3, H-5), 8.10 (br s, 1H, N*H* that exchanges with D_2O), 8.25 (d, *J* = 8.5 Hz, 1H, pyridyl H-3), 8.46 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 24.8, 44.5, 89.9, 113.2, 115.0, 127.5, 128.6, 132.2, 139.8, 141.1, 150.7, 150.9, 168.6.

General procedure for the synthesis of 1-(2-, 3- or 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-acetylimino)] acetylenes (**8a–d**). Sodium chlorodifluoroacetate (0.68 g, 4.46 mmol) was added to a stirred solution of a 1-(2-, 3- or 4-methanesulfonylphenyl or aminosulfonylphenyl)-2-(2-acetamidopyrid-5-yl)acetylene (**6d**, **7a–c**, 3.66 mmol) in dry acetonitrile (75 mL), and the mixture was heated at reflux for 18 h under an argon atmosphere. At this time, the mixture was concentrated and saturated aqueous $NaHCO_3$ (25 mL) was added and this mixture was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with brine, and dried ($MgSO_4$). After filtration, the solvent from the organic fraction was removed in vacuo to give a crude product which was purified by silica gel column chromatography using hexanes/EtOAc (1:3, v/v) as eluent to afford the respective product **8a–d**. The spectral data for compounds **8a–d** are listed below.

1-(2-Methanesulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-acetylimino)]acetylene (**8a**). The product was obtained as a brown solid in 88% yield; mp 95–97 °C; IR (film): 2200 (C≡C), 1670 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CO*Me*), 3.24 (s, 3H, SO_2*Me*), 7.45 (dd, *J* = 9.8, 1.8 Hz, 1H, pyridyl H-4), 7.56 (ddd, *J* = 7.9, 7.9, 1.2 Hz, 1H, phenyl H-5), 7.64 (ddd, *J* = 7.9, 7.9, 1.2 Hz, 1H, phenyl H-4), 7.70 (dd, *J* = 7.9, 1.2 Hz, 1H, phenyl H-6), 7.75 (d, *J* = 9.8 Hz, 1H, pyridyl H-3), 7.87 (d, *J* = 1.8 Hz, 1H, pyridyl H-6), 8.01 (t, *J* = 60.5 Hz, 1H, C*H*F$_2$), 8.14 (dd, *J* = 7.9, 1.2 Hz, 1H, phenyl H-3); ^{13}C NMR ($CDCl_3$) δ 28.0, 42.6, 87.4, 92.2, 105.0, 119.8, 121.0, 129.1, 129.1, 133.1, 133.3, 134.1, 140.7, 141.0, 153.7, 182.3.

1-(3-Methanesulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-acetylimino)]acetylene (**8b**). The product was obtained as a yellow solid in 74% yield; mp 132–134 °C; IR (film): 2200 (C≡C), 1670 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H, CO*Me*), 3.09 (s, 3H, SO_2*Me*), 7.40 (dd, *J* = 9.8, 2.5 Hz, 1H, pyridyl H-4), 7.59 (dd, *J* = 7.9, 7.9 Hz, 1H, phenyl H-5), 7.75 (ddd, *J* = 7.9, 1.8, 1.8 Hz, 1H, phenyl H-6), 7.78 (d, *J* = 9.8 Hz, 1H, pyridyl H-3), 7.83 (d, *J* = 2.5 Hz, 1H, pyridyl H-6), 7.93 (ddd, *J* = 7.9, 1.8, 1.8 Hz, 1H, phenyl H-4), 8.02 (t, *J* = 60.5 Hz, 1H, C*H*F$_2$), 8.08 (dd, *J* = 1.8, 1.8 Hz, 1H, phenyl H-2); ^{13}C NMR ($CDCl_3$) δ 27.9, 44.3, 85.7, 89.2, 105.4, 119.8, 123.8, 127.2, 129.6, 130.3, 133.0, 136.1, 141.0, 141.1, 153.5, 181.9.

1-(4-Methanesulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-acetylimino)]acetylene (**8c**). The product was obtained as a pale yellow solid in 75% yield; mp 160–162 °C; IR (film): 2200 (C≡C), 1670 (CO), 1300, 1150 (SO_2) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 3H, CO*Me*), 3.09 (s, 3H, SO_2*Me*), 7.41 (dd, *J* = 9.8, 1.8 Hz, 1H, pyridyl H-4), 7.68 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-2, H-6), 7.78 (d, *J* = 9.8 Hz, 1H, pyridyl H-3), 7.85 (d, *J* = 1.8 Hz, 1H, pyridyl H-6), 7.95 (dd, *J* = 8.5, 1.8 Hz, 2H, phenyl H-3, H-5), 8.03 (t, *J* = 60.5 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 28.0, 44.4, 87.4, 89.5, 105.0, 119.8, 127.5, 127.9, 132.2, 133.1, 140.2, 140.9, 153.7, 182.3.

1-(4-Aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-acetylimino)] acetylene (**8d**). The product was obtained as a pale yellow solid in 46% yield; mp 208–210 °C; IR (film): 3300 (broad NH_2), 2219 (C≡C), 1700 (CO), 1294, 1163 (SO_2) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.11 (s, 3H, CO*Me*), 7.47 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.59 (d, *J* = 9.8 Hz, 1H, pyridyl H-3), 7.67 (dd, *J* = 9.8, 1.8 Hz, 1H, pyridyl H-4), 7.73 (d, *J* = 8.5 Hz, 2H, phenyl H-2, H-6), 7.86 (d, *J* = 8.5 Hz, 2H, phenyl H-3, H-5), 8.10 (t, *J* = 60.5 Hz, 1H, C*H*F$_2$), 8.42 (d, *J* = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 27.3, 85.8, 89.6, 102.5, 119.1, 124.9, 125.7, 131.1, 133.3, 141.3, 143.7, 152.5, 180.1.

General procedure for the synthesis of 1-(2-, 3- or 4-methanesulfonylphenyl and 4-aminosulfonylphenyl)-2-[5-(1-difluoromethyl-1,2-dihydropyrid-2-one)]acetylenes (**9a–d**). A solution of 1% aq $KHSO_4$ (45 mL) was added to a stirred solution of a 1-difluoromethyl-1,2-dihydropyrid-2-acetylimino compound (**8a–d**, 3.10 mmol) in acetonitrile (45 mL), and the mixture was heated at reflux for 3 h. At this time, the mixture was concentrated in vacuo, 0.5 N HCl (25 mL) was

added, and the mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed successively with saturated aqueous $NaHCO_3$ and brine, and dried ($MgSO_4$). After filtration, the solvent from the organic fraction was removed in vacuo to give a crude product which was purified by silica gel column chromatography using hexanes/EtOAc (1:2, v/v) as eluent to afford the respective product **9a–d**. The spectral and microanalytical data for compounds **9a–d** are listed below.

1-(2-Methylsulfonylphenyl)-2-[5-(1-difluoromethylpyrid-2-one)]acetylene (**9a**). The product was obtained as a yellow solid in 66% yield; mp 155–157 °C; IR (film): 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.25 (s, 3H, SO_2Me), 6.61 (d, J = 9.8 Hz, 1H, pyridone H-3), 7.55 (dd, J = 9.8, 1.8 Hz, 1H, pyridone H-4), 7.59 (ddd, J = 7.9, 7.9, 1.2 Hz, 1H, phenyl H-5), 7.65–7.75 (m, 2H, phenyl H-4, H-6), 7.68 (t, J = 60.5 Hz, 1H, CHF_2), 7.80 (d, J = 1.8 Hz, 1H, pyridone H-6), 8.15 (dd, J = 7.9, 1.2 Hz, 1H, phenyl H-3); ^{13}C NMR ($CDCl_3$) δ 42.6, 86.9, 92.4, 103.2, 107.1, 121.1, 121.9, 129.0, 133.2, 133.3, 134.0, 140.9, 142.6, 159.4. Anal. Calcd for $C_{15}H_{11}F_2NO_3S$: C, 55.72; H, 3.43; N, 4.33. Found: C, 55.78; H, 3.66; N, 4.32.

1-(3-Methylsulfonylphenyl)-2-[5-(1-difluoromethylpyrid-2-one)]acetylene (**9b**). The product was obtained as a yellow solid in 81% yield; mp 158–160 °C; IR (film): 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2Me), 6.59 (d, J = 9.8 Hz, 1H, pyridone H-3), 7.46 (dd, J = 9.8, 2.5 Hz, 1H, pyridone H-4), 7.58 (dd, J = 7.9, 7.9 Hz, 1H, phenyl H-5), 7.67 (t, J = 60.5 Hz, 1H, CHF_2), 7.74 (ddd, J = 7.9, 1.8, 1.8 Hz, 1H, phenyl H-6), 7.75 (d, J = 2.5 Hz, 1H, pyridone H-6), 7.92 (ddd, J = 7.9, 1.8, 1.8 Hz, 1H, phenyl H-4), 8.07 (dd, J = 1.8, 1.8 Hz, 1H, phenyl H-2); ^{13}C NMR ($CDCl_3$) δ 44.4, 86.1, 88.6, 103.3, 107.2, 121.9, 124.1, 127.1, 129.6, 130.3, 133.3, 136.1, 141.1, 142.8, 159.5. Anal. Calcd for $C_{15}H_{11}F_2NO_3S$: C, 55.72; H, 3.43; N, 4.33. Found: C, 55.85; H, 3.58; N, 4.26.

1-(4-Methylsulfonylphenyl)-2-[5-(1-difluoromethylpyrid-2-one)]acetylene (**9c**). The product was obtained as a white solid in 100% yield; mp 159–161 °C; IR (film): 2200 (C≡C), 1700 (CO), 1300, 1150 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.08 (s, 3H, SO_2Me), 6.60 (d, J = 9.8 Hz, 1H, pyridone H-3), 7.47 (dd, J = 9.8, 1.8 Hz, 1H, pyridone H-4), 7.67 (dd, J = 8.5, 1.8 Hz, 2H, phenyl H-2, H-6), 7.68 (t, J = 60.5 Hz, 1H, CHF_2), 7.77 (d, J = 1.8 Hz, 1H, pyridone H-6), 7.94 (dd, J = 8.5, 1.8 Hz, 2H, phenyl H-3, H-5); ^{13}C NMR ($CDCl_3$) δ 44.4, 87.5, 88.9, 103.2, 107.1, 121.8, 127.5, 128.0, 132.1, 133.4, 140.0, 142.7, 159.4. Anal. Calcd for $C_{15}H_{11}F_2NO_3S$: C, 55.72; H, 3.43; N, 4.33. Found: C, 55.67; H, 3.59; N, 4.27.

1-(4-Aminosulfonylphenyl)-2-[5-(1-difluoromethylpyrid-2-one)]acetylene (**9d**). The product was obtained as a white solid in 72% yield; mp 190–192 °C; IR (film): 3315 (broad NH_2), 2225 (C≡C), 1675 (CO), 1352, 1165 (SO_2) cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.54 (d, J = 9.8 Hz, 1H, pyridone H-3), 7.33 (br s, 2H, SO_2NH_2 that exchanges with D_2O), 7.56 (dd, J = 9.8, 1.8 Hz, 1H, pyridone H-4), 7.60 (d, J = 8.5 Hz, 2H, phenyl H-2, H-6), 7.76 (t, J = 60.5 Hz, 1H, CHF_2), 7.83 (d, J = 8.5 Hz, 2H, phenyl H-3, H-5), 8.00 (d, J = 1.8 Hz, 1H, pyridone H-6); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 85.9, 88.8, 102.8, 106.9, 121.1, 125.2, 125.7, 131.1, 132.8, 142.5, 143.3, 158.7. Anal. Calcd for $C_{14}H_{10}F_2N_2O_3S \bullet 1/4H_2O$: C, 51.14; H, 3.22; N, 8.52. Found: C, 51.05; H, 3.46; N, 8.32.

15. *5-Lipoxygenase inhibition assay:* The ability of the test compounds listed in Table 1 to inhibit potato 5-LOX (Catalog No. 60401, Cayman Chemical, Ann Arbor, MI, USA) (IC_{50} values, μM) were determined using an enzyme immuno assay (EIA) kit (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Das, D.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6138.
16. *Cyclooxygenase inhibition assays.* The ability of the test compounds listed in Table 1 to inhibit ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) were determined using an enzyme immuno assay (EIA) kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to our previously reported method. Rao, P. N. P.; Amini, M.; Li, H.; Habeeb, A.; Knaus, E. E. *J. Med. Chem.* **2003**, *46*, 4872.
17. *In vivo anti-inflammatory assay.* The test compounds **9a–d** and the reference drugs celecoxib, ibuprofen and aspirin were evaluated using the in vivo carrageenan-induced rat foot paw edema model reported previously. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.