Synthesis of phenylacetic acid regioisomers possessing an N-substituted 1,2-dihydropyrid-2-one pharmacophore — Evaluation as inhibitors of cyclooxygenases and 5-lipoxygenase

Gang Yu, Morshed A. Chowdhury, Zhangjian Huang, Khaled R. A. Abdellatif, and Edward E. Knaus

Abstract: The Suzuki–Miyaura cross-coupling reaction provides a useful method for the synthesis of methyl 2-(2-chloropyridyl or 2-methoxypyridyl)phenylacetates. The 2-chloropyridyl or 2-methoxypyridyl ring system can be readily elaborated to N-substituted (OH, Me, CHF_2, H) 1,2-dihydropyrid-2-one ring systems that are useful synthons for use in bioorganic and medicinal chemistry applications.

Key words: Suzuki–Miyaura cross-coupling reaction, 1,2-dihydropyrid-2-ones, cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) inhibition.

Résumé : La réaction de couplage croisé de Suzuki–Miyaura est une méthode utile pour la synthèse d'esters 2-(2-chloropyridyl)- ou de 2-(méthoxypyridyl)phénylacétates. Ce système cyclique, portant des groupes 2-chloropyridyle ou 2-méthoxypyridyle, peut facilement être transformé en systèmes cycliques à base de 1,2-dihydropyrid-2-one N-substituée (OH, Me, CHF_2, H), des synthons utiles dans des applications de chimie bioorganique ou médicinale.

Mots-clés : réaction de couplage croisé de Suzuki–Miyaura, 1,2-dihydropyrid-2-ones, inhibition de la cyclooxygénase (COX) et de la 5-lipoxygénase (5-LOX).

[Traduit par la Rédaction]

Introduction

Biotransformation of arachidonic acid gives rise to prostaglandin and leukotriene inflammatory mediators that are produced via their respective cyclooxygenase (COX) and lipoxygenase (LOX) pathways.[1] Dual inhibition of the LOX and COX enzymatic pathways[2] provides a rational approach for the design of superior anti-inflammatory agents with a better safety profile relative to ulcerogenic nonsteroidal anti-inflammatory drugs (NSAIDs) or selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects.[3,4] The most successful effort to develop 5-LOX inhibitors was focused on *N*-hydroxyureas such as zileuton (**1**, see structure in Fig. 1), which is believed to chelate iron present in the 5-LOX enzyme.[5,6] In earlier studies, we showed that linear acetylenes possessing cyclic hydroxamic acid, *N*-hydroxy-1,2-dihydropyrid-2-one (**2**),[7] or *N*-difluoromethyl-1,2-dihydropyrid-2-one (**3**,[8] **4**[9]) 5-LOX pharmacophores exhibited dual COX and 5-LOX inhibitory activities. In a continuation of our ongoing program to design novel anti-inflammatory agents and acquire additional structure–activity relationship data, we now describe the synthesis of a group of arylacetic acids possessing an N-substituted (OH, Me, CHF_2, H) 1,2-dihydropyrid-2-one moiety, and their evaluation as inhibitors of the COX-1, COX-2, and 5-LOX enzymes.

Results and discussion

Reaction of methyl 2-iodophenylacetate (**5**)[9] with a 4- or 5-pyridineboronic acid (**6a**–**6c**) using a Suzuki–Miyaura[10,11] cross-coupling reaction in the presence of the tetrakis(triphenylphosphine)palladium (0) catalyst and 2 mol/L aqueous sodium carbonate furnished the respective diaryl products **7a**–**7c** in good yields (58%–77%) (Scheme 1). Oxidation of **7a** and **7c** using *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane afforded the target methyl 2-(1-oxido-2-chloropyridin-4-yl)phenylacetate (**8a**, 42%) or methyl 2-(1-oxido-2-methoxypyridin-5-yl)phenylacetate (**8c**, 59%). Hydrolysis of the methyl ester **8a** using 2 N sodium hydroxide at reflux for 24 h afforded the target phenylacetic acid product **9a** in 41% yield. Reaction of the *N*-oxide **8c** with acetyl chloride at reflux, and then methanolysis, afforded the *N*-hydroxy-1,2-dihydropyrid-2-one **9c** in 96% yield.[12] Subsequent depro-

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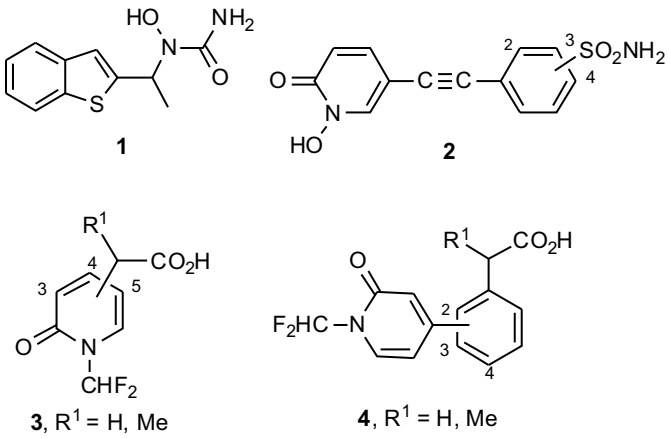
Fig. 1. Structures of the iron-chelating 5-LOX inhibitor zileuton (**1**) and dual inhibitors of the COX and LOX enzymes that possess an *N*-hydroxy-1,2-dihydropyrid-2-one (**2**) or *N*-difluoromethyl-1,2-dihydropyrid-2-one (**3**, **4**) 5-LOX pharmacophore.

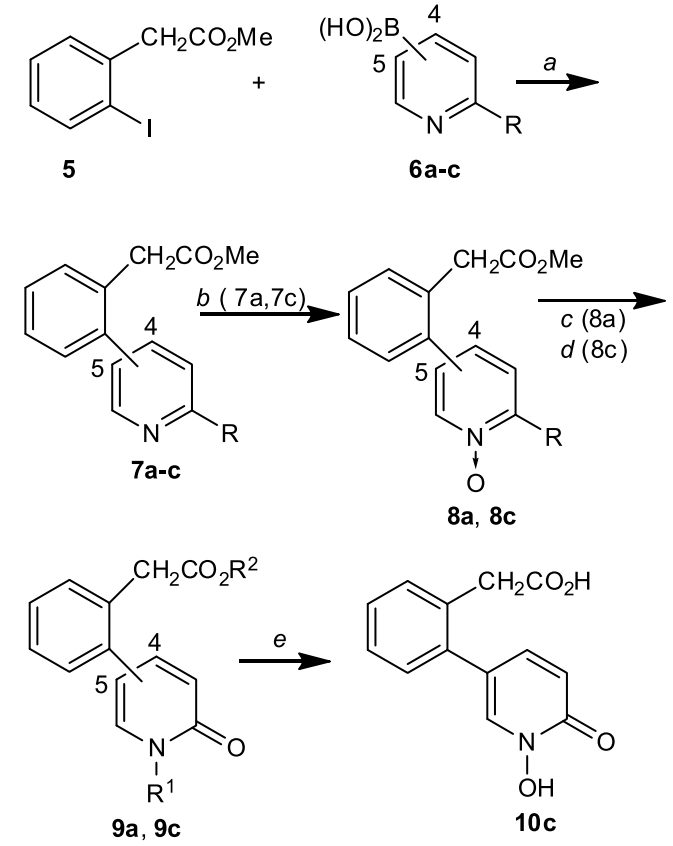


tection of the methyl ester group present in **9c** using lithium hydroxide in MeOH and tetrahydrofuran (THF) at reflux temperature afforded the target phenylacetic acid product **10c** in 74% yield.

The 2-chloropyridine compounds **7a** and **7b** were converted to their respective *N*-methylpyridinium methylsulfate salts (**11a** and **11b**) upon reaction with dimethyl sulfate at reflux in acetonitrile (see Scheme 2).[13] Subsequent treatment of the salts **11a** and **11b** with 2 N aqueous lithium hydroxide at reflux proceeded smoothly, resulting in simultaneous hydrolysis of the methyl ester and conversion of the *N*-methyl-2-chloropyridinium ring to an *N*-methyl-1,2-dihydropyrid-2-one ring, to furnish the corresponding target products **12a** and **12b** in 70%–89% yield.[14] Alternatively, reaction of the 2-chloropyridyl compound **7b** with $FSO_2CF_2CO_2H$[15] in the presence of $NaHCO_3$ afforded the *N*-difluoromethyl-1,2-dihydropyrid-2-one product **13** in 53% yield. Selective hydrolysis of the methyl ester group in **13** using 2 N LiOH in a MeOH–THF–H_2O (1:1:1, *v*/*v*/*v*) solvent system afforded the phenylacetic acid product **14**.[14] In contrast, hydrolysis of **13** under more basic reaction conditions using aqueous 2 N NaOH and a longer reaction time of 5 h resulted in concomitant cleavage of the methyl ester substituent and removal of the *N*-difluoromethyl substituent to afford 2-(2-oxo-1,2-dihydropyridin-5-yl)phenylacetic acid (**15**) in 65% yield.

The *N*-hydroxy-1,2-dihydropyrid-2-one cyclic hydroxamic acid mimetics **9a** and **10c** were designed with the expectation[7] that they would act as iron chelators to inhibit the 5-LOX enzyme. Although the C-5 regioisomer **10c** did exhibit weak COX-2 inhibition (IC_{50} = 10.1 μmol/L), **9a** and **10c** were both inactive as inhibitors of the 5-LOX enzyme (see data in Table 1). Similar enzyme inhibition studies showed that the C-4 (**12a**) and C-5 (**12b**) *N*-methyl-1,2-dihydropyrid-2-one regioisomers were inactive as inhibitors of the 5-LOX and COX-2 enzymes (IC_{50} > 100 μmol/L), but the C-4 regioisomer **12a** exhibited weak inhibition (IC_{50} = 52.4 μmol/L) of the COX-1 enzyme. In spite of its weak in vitro COX-1 inhibitory activity, **12a** showed good in vivo anti-inflammatory activity in a rat model, where a 45% reduction in inflammation was observed for a 100 mg/kg oral dose relative to the reference drug ibuprofen, which furnished a 50% reduction in inflammation for a 67 mg/kg oral dose. The structurally related *N*-difluoromethyl-1,2-dihydropyrid-2-one **14** and 1,2-dihydropyrid-2-one **15** compounds, which failed to inhibit the COX-1 and 5-LOX enzymes (IC_{50} > 100 μmol/L), showed weak inhibition of the COX-2 enzyme relative to the reference drugs aspirin and ibuprofen. In view of their weak COX-2 inhibitory potency and failure to inhibit 5-LOX, the anti-inflammatory activities for **14** and **15** were not determined. These enzyme inhibition structure–activity data show that none of the compounds investigated in this study exhibited the desired dual inhibition of the COX-2 and 5-LOX enzymes.

Scheme 1. Synthesis of *N*-hydroxy-1,2-dihydropyrid-2-one derivatives of phenylacetic acid. Reagents and conditions: (*a*) $Pd(PPh_3)_4$, 2 mol/L Na_2CO_3 (**6a** and **6b**, reflux in THF for 16 h; **6c**, reflux in 1,4-dioxane for 4 h); (*b*) *m*-CPBA, CH_2Cl_2, 25 °C, 48 h; (*c*) 2 N NaOH, reflux, 24 h (**8a**); (*d*) (*i*) AcCl, reflux, 1 h, (*ii*) MeOH, 25 °C, 16 h (**8c**); (*e*) 2 N LiOH, MeOH–THF–H_2O (1:1:1, *v*/*v*/*v*), reflux, 3 h.



Conclusion

The Suzuki–Miyaura cross-coupling reaction of methyl 2-iodophenylacetate with a 2-chloro- or 2-methoxypyridine-

Scheme 2. Synthesis of N-substituted (Me, CHF_2, H) 1,2-dihydropyrid-2-one derivatives of phenylacetic acid. Reagents and conditions: (*a*) Me_2SO_4, MeCN, reflux, 6 h; (*b*) 2 N LiOH, MeOH–THF–H_2O (1:1:1, *v/v/v*), reflux, 2 h; (*c*) $FSO_2CF_2CO_2H$, MeCN, $NaHCO_3$, reflux under argon, 12 h; (*d*) 2 N NaOH, reflux, 5 h.

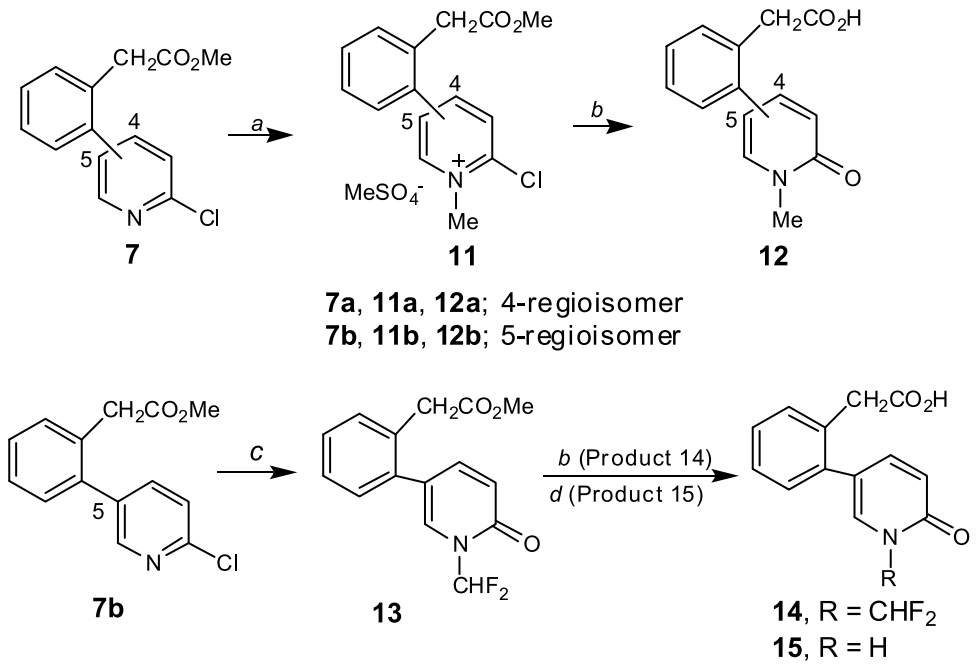


Table 1. In vitro COX-1, COX-2, and 5-LOX enzyme inhibition data for N-substituted (OH, Me, CHF_2, H) 1,2-dihydropyrid-2-one derivatives of phenylacetic acid.

Entry	Compound	COX-1 IC_{50} (μmol/L)[a]	COX-2 IC_{50} (μmol/L)[a]	5-LOX IC_{50} (μmol/L)[b]
1	**9a**	—	—	>100
2	**10c**	>100	10.1	>100
3	**12a**	52.4	>100	>100
4	**12b**	>100	>100	>100
5	**14**	>100	187	>100
6	**15**	>100	18.2	>100
7	Aspirin	0.2	2.4[c]	—
8	Ibuprofen	2.9	1.1[c]	—
9	Zileuton	—	—	1.1

[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}, μmol/L) is the mean of two determinations acquired using an enzyme immunoassay kit (catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, Michigan), 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}, μmol/L) is the mean of two determinations acquired using a LOX assay kit (catalog No. 760700, Cayman Chemicals Inc.), and the deviation from the mean is <10% of the mean value.

[c]Data taken from a previous report (ref. 16). COX-2 inhibition was determined using ovine COX-2.

boronic acid provides a useful method for the synthesis of methyl 2-(pyridinyl)phenylacetates. The 2-chloropyridyl and 2-methoxypyridyl ring systems present in this group of compounds can be readily elaborated to *N*-hydroxy-1,2-dihydropyrid-2-one (a cyclic hydroxamic acid), *N*-methyl-1,2-dihydropyrid-2-one, and 1,2-dihydropyrid-2-one ring systems that may serve as useful synthons in bioorganic and medicinal chemistry applications.

Experimental

Instrumentation, analysis, and starting material

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. IR spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FT–IR spectrometer. ^1H NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard. Compounds **7**–**8**, **9c**, **10c**, **11**, and **13** showed a single spot on Macherey–Nagel Polygram Sil G/UV254 silica gel plates (0.2 mm) using a low, medium, or highly polar solvent system; no residue remained after combustion, indicative of high purity. Microanalyses for all other compounds were performed for C, H, and 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). All reagents were purchased from the Sigma-Aldrich Chemical Company (Milwaukee, Wisconsin), with the exception of 2-chloropyridine-4-boronic acid, purchased from Combi-Blocks Inc., and were used without further purification.

General Suzuki–Miyaura cross-coupling synthesis of 7a–7c

Methyl 2-iodophenylacetate (**5**, 1.20 g, 4.35 mmol) and a pyridineboronic acid (**6a**–**6c**, 4.35 mmol) were dissolved in a polar solvent (THF, 50 mL for **6a** and **6b** or 1,4-dioxane, 50 mL for **6c**). Aqueous 2 mol/L Na_2CO_3 (6.5 mL, 13.05 mmol) and then $Pd(PPh_3)_4$ (115 mg, 0.10 mmol) were added. The reaction was allowed to proceed at reflux under argon (16 h for **6a** and **6b** and 4 h for **6c**) and then cooled to 25 °C; water (50 mL) was added, and the mixture was extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water (2 × 30 mL), the organic

fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The residue obtained was purified by silica gel column chromatography using hexanes–EtOAc (3:1, *v*/*v*) as eluent to afford the respective products **7a**–**7c**. Some spectroscopic data for **7a**–**7c** are listed below.

Methyl 2-(2-chloropyridin-4-yl)phenylacetate (7a)

Yield, 74%; yellow oil. IR (film): 2920, 1736, 1536 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.72 (s, 2H, C*H*$_2$), 3.73 (s, 3H, O*Me*), 7.39–7.55 (overlapping multiplets, 6H total, phenyl H-3, H-4, H-5, H-6, pyridyl H-3, H-5), 8.44 (d, J = 5.5 Hz, 1H, pyridyl H-6). ^{13}C NMR ($CDCl_3$) δ: 41.0, 52.2, 120.5, 122.1, 125.8, 128.0, 129.5, 130.6, 135.1, 137.2, 150.0, 151.2, 152.2, 171.5.

Methyl 2-(2-chloropyridin-5-yl)phenylacetate (7b)

Yield, 58%; pale yellow oil. IR (film): 2918, 1734, 1590 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.56 (s, 2H, C*H*$_2$), 3.64 (s, 3H, O*Me*), 7.24 (d, J = 6.1 Hz, 1H, phenyl H-6), 7.36–7.41 (overlapping multiplets, 3H, phenyl H-3, H-4, H-5), 7.37 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.68 (dd, J = 8.5, 2.4 Hz, 1H, pyridyl H-4), 8.37 (d, J = 2.4 Hz, 1H, pyridyl H-6). ^{13}C NMR ($CDCl_3$) δ: 38.6, 52.1, 123.7, 127.6, 128.7, 130.3, 130.7, 132.1, 135.6, 137.3, 139.5, 149.7, 150.5, 171.7.

Methyl 2-(2-methoxypyridin-5-yl)phenylacetate (7c)

Yield, 77%; pale yellow syrup. IR (film): 2919, 1736, 1086 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.60 (s, 2H, C*H*$_2$), 3.65 (s, 3H, O*Me*), 3.99 (s, 3H, pyridyl-O*Me*), 6.80 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.23–7.39 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.57 (dd, J = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.12 (d, J = 1.8 Hz, 1H, pyridyl H-6). ^{13}C NMR ($CDCl_3$) δ: 38.7, 52.0, 53.5, 110.2, 127.2, 127.4, 127.9, 129.7, 130.4, 132.3, 138.7, 139.6, 146.6, 163.3, 172.0.

General procedure for the synthesis of methyl 2-(1-oxido-2-chloropyridin-4-yl)phenylacetate (8a) and methyl 2-(1-oxido-2-methoxypyridin-5-yl)phenylacetate (8c)

m-Chloroperoxybenzoic acid (77% maximum purity) (12 mmol) was added to a stirred solution of a pyridine (**7a** or **7c**, 3.0 mmol) in CH_2Cl_2 (30 mL), and the reaction was allowed to proceed with stirring at 25 °C for 48 h. The solvent was washed with a saturated solution of aqueous $NaHCO_3$ (2 × 20 mL), then washed with water (40 mL) and brine, and the organic fraction was dried ($MgSO_4$). Removal of the solvent from the organic fraction in vacuo gave a residue that was purified by silica gel column chromatography using methanol–EtOAc (10:1, *v*/*v*) as eluent to afford the respective product **8a** or **8c**. Some physical and spectroscopic data for **8a** and **8c** are listed below.

Methyl 2-(1-oxido-2-chloropyridin-4-yl)phenylacetate (8a)

Yield, 42%; pale yellow oil. IR (film): 3060, 3030, 2955, 1741, 1461, 1267 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.59 (s, 2H, C*H*$_2$), 3.69 (s, 3H, O*Me*), 7.26 (dd, J = 6.7, 2.4 Hz, 1H, pyridyl H-5), 7.27–7.55 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.55 (d, J = 2.4 Hz, 1H, pyridyl H-3), 8.40 (d, J = 6.7 Hz, 1H, pyridyl H-6). ^{13}C NMR ($CDCl_3$) δ: 38.5, 52.3, 124.7, 127.6, 127.9, 129.3, 129.5, 131.1, 131.5, 137.1, 139.5, 139.9, 141.6, 171.5.

Methyl 2-(1-oxido-2-methoxypyridin-5-yl)phenylacetate (8c)

Yield, 59%; pale yellow oil. IR (film): 2961, 1740, 1438, 1257 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.58 (s, 2H, C*H*$_2$), 3.67 (s, 3H, O*Me*), 4.14 (s, 3H, pyridyl-O*Me*), 6.96 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.23–7.39 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.32 (dd, J = 8.5, 1.8 Hz, 1H, pyridyl H-4), 8.29 (d, J = 1.8 Hz, 1H, pyridyl H-6). ^{13}C NMR ($CDCl_3$) δ: 38.5, 52.2, 57.4, 107.3, 127.7, 128.8, 129.0, 130.1, 130.8, 132.1, 136.0, 139.9, 157.8, 171.6.

2-(1-Hydroxy-2-oxo-1,2-dihydropridin-4-yl)phenylacetic acid (9a)

A mixture of methyl 2-(1-oxido-2-chloropyridin-4-yl)phenylacetate (**8a**, 0.75 mmol) and 2 N NaOH (3.0 mL, 6.0 mmol) was refluxed for 24 h. The reaction mixture was cooled to 25 °C and washed with CH_2Cl_2 (30 mL). The water phase was then acidified to pH 2 by addition of 3 N hydrochloric acid prior to extraction with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water and then brine, the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash silica gel column chromatography using EtOAc–MeOH (9:1, *v*/*v*) as eluent to furnish **9a** in a 41% yield as an off-white solid, mp >335 °C. IR (film): 3084, 3018, 2922, 1722, 1651, 1575, 1229 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.56 (s, 2H, C*H*$_2$), 6.63 (dd, J = 6.7, 1.8 Hz, pyridone H-5), 6.85 (dd, J = 6.7, 1.8 Hz, 1H, pyridone H-3), 7.18–7.34 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 8.02 (d, J = 6.7 Hz, 1H, pyridone H-6), 8.65 (br s, 2H, NO*H* and COO*H*). Anal. Calcd for $C_{13}H_{11}NO_4 \cdot 1/8H_2O$: C, 63.03; H, 4.55; N, 5.65. Found: C, 63.21; H, 4.64; N, 5.62.

Methyl 2-(1-hydroxy-2-oxo-1,2-dihydropridin-5-yl) phenylacetate (9c)

Acetyl chloride (5 mL) was added to methyl 2-(1-oxido-2-methoxypyridin-5-yl)phenylacetate (**8c,** 82 mg, 0.30 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 for 16 h. Methanol was removed in vacuo to afford **9c**; yield, 96%; pale yellow oil. IR (film): 3026, 2961, 2921, 1746, 1656, 1582, 1168 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.61 (s, 2H, C*H*$_2$), 3.68 (s, 3H, O*Me*), 6.87 (d, J = 9.1 Hz, 1H, pyridyl H-3), 7.23–7.37 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.49 (dd, J = 9.1, 2.4 Hz, 1H, pyridyl H-4), 7.90 (d, J = 2.4 Hz, 1H, pyridyl H-6), 8.67 (br s, 1H, *N*-O*H*). ^{13}C NMR ($CDCl_3$) δ: 38.7, 52.2, 116.8, 119.8, 127.7, 128.7, 130.4, 130.6, 130.7, 132.4, 136.3, 139.5, 156.7, 171.9.

2-(1-Hydroxy-2-oxo-1,2-dihydropridin-5-yl)phenylacetic acid (10c)

A mixture of **9c** (0.75 mmol) and 2 N LiOH (3.0 mL, 6.0 mmol) in THF (3.0 mL) and MeOH (3.0 mL) was stirred at reflux temperature for 3 h. The reaction mixture was cooled to 25 °C and washed with CH_2Cl_2 (30 mL). The water phase was acidified to pH 2 by addition of 3 N hydrochloric acid prior to extraction with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water and then brine, the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified

by flash silica gel column chromatography using EtOAc–MeOH (9:1, *v*/*v*) as eluent to yield **10c**; yield, 74%; pale yellow solid, mp 147–149 °C. IR (film): 3081, 3041, 2924, 2859, 1703, 1663, 1562 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.70 (s, 2H, CH_2), 6.57 (d, J = 6.7 Hz, pyridone H-3), 7.24–7.39 (overlapping multiplets, 5H total, phenyl H-3, H-4, H-5, H-6 and pyridone H-4), 7.88 (d, J = 2.4 Hz, 1H, pyridone H-6), 12.22 (br s, 2H, NO*H* and COO*H*). Anal. Calcd for $C_{13}H_{11}NO_4 \cdot 1/10H_2O$: C, 63.21; H, 4.57; N, 5.67. Found: C, 63.25; H, 4.55; N, 5.61.

General procedure for the synthesis of 2-(1-methyl-2-oxo-1,2-dihydropyridyl)phenylacetic acids (12a–12b)

Dimethyl sulfate (420 mg, 3.33 mmol) in MeCN (10 mL) was added to a solution of **7a** or **7b** (290 mg, 1.11 mmol) in MeCN (10 mL). The mixture was stirred at reflux for 6 h. Removal of excess dimethyl sulfate and MeCN in vacuo afforded the respective 1-methylpyridinium methylsulfate salt **11a** or **11b**. Compound **11a**, ^1H NMR (DMSO-d_6) δ: 3.62 (s, 3H, *Me*SO_4^-), 3.67 (s, 2H, CH_2), 3.68 (s, 3H, O*Me*), 4.53 (s, 3H, N*Me*), 7.41–7.47 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.98 (dd, J = 6.7, 1.8 Hz, 1H, pyridyl H-5), 8.09 (d, J = 1.8 Hz, 1H, pyridyl H-3), 9.19 (d, J = 6.7 Hz, 1H, pyridyl H-6). Compound **11b**, ^1H NMR (DMSO-d_6) δ: 3.65 (s, 3H, O*Me*), 3.67 (s, 3H, *Me*SO_4^-), 3.68 (s, 2H, CH_2), 4.46 (s, 3H, N*Me*), 7.34–7.50 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 8.07 (d, J = 8.5, Hz, 1H, pyridyl H-3), 8.48 (dd, J = 8.5, 1.8 Hz, 1H, pyridyl H-4), 9.12 (d, J = 1.8 Hz, 1H, pyridyl H-6). A solution of the 1-methylpyridinium methylsulfate **11a** or **11b** in THF (3.0 mL) and MeOH (3.0 mL) was added to 2 N LiOH (3.0 mL, 6.0 mmol), and the mixture was stirred at reflux temperature for 2 h. The reaction mixture was cooled to 25 °C and washed with CH_2Cl_2 (30 mL). The water phase was then acidified to pH 4 by addition of 3 N hydrochloric acid prior to extraction with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water and then brine, the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash silica gel column chromatography using EtOAc–MeOH (9:1, *v*/*v*) to yield the respective product **12a** or **12b**. Some physical and spectral data of **12a** and **12b** are listed below.

2-(1-Methyl-2-oxo-1,2-dihydropridin-4-yl)phenylacetic acid (12a)

Yield, 89%; off-white solid, mp 182–184 °C. IR (film): 3068, 2943, 1723, 1658, 1558 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 3.52 (s, 3H, N*Me*), 3.53 (s, 2H, CH_2), 6.22 (dd, J = 6.7, 1.8 Hz, 1H, pyridone H-5), 6.52 (d, J = 1.8 Hz, 1H, pyridone H-3), 7.15–7.33 (overlapping multiplets, 5H total, phenyl H-3, H-4, H-5, H-6, pyridone H-6), 9.98 (br s, 1H, COO*H*). Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C, 68.64; H, 5.42; N, 5.66.

2-(1-Methyl-2-oxo-1,2-dihydropridin-5-yl)phenylacetic acid (12b)

Yield, 70%; pale yellow solid, mp 171–173 °C. IR (film): 3075, 2941, 1719, 1659, 1564 cm^{-1}. ^1H NMR ($CDCl_3$ + DMSO-d_6) δ: 3.47 (s, 3H, N*Me*), 3.48 (s, 2H, CH_2), 6.46 (d, J = 9.2 Hz, 1H, pyridone H-3), 7.12–7.28 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.34 (dd, J = 9.2, 2.4 Hz, 1H, pyridone H-4), 7.46 (d, J = 1.8 Hz, 1H, pyridone H-6), 10.1 (br s, 1H, COO*H*). Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.12; H, 5.39; N, 5.76. Found: C, 68.78; H, 5.51; N, 5.55.

Methyl 4-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl) phenylacetate (13)

To a solution of **7b** (522 mg, 2.0 mmol) in acetonitrile (15 mL) was added $FSO_2CF_2CO_2H$ (1.12 g, 6.0 mmol) followed by $NaHCO_3$ (186 mg, 2.2 mmol). This mixture was heated at reflux under argon for 12 h and cooled to 25 °C; a saturated solution of aqueous $NaHCO_3$ (40 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 40 mL). The organic fraction was washed with water (50 mL) and then brine, and the organic fraction was dried ($MgSO_4$). Removal of the solvent from the organic fraction in vacuo afforded **13** in 53% yield; pale yellow oil. IR (film): 3010, 2966, 1744, 1694, 1619, 1153 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.60 (s, 2H, CH_2), 3.67 (s, 3H, O*Me*), 6.61 (d, J = 9.1 Hz, 1H, pyridone H-3), 7.23–7.39 (overlapping multiplets, 4H, phenyl H-3, H-4, H-5, H-6), 7.43 (dd, J = 9.1, 2.4 Hz, 1H, pyridone H-4), 7.48 (d, J = 2.4 Hz, 1H, pyridone H-6), 7.74 (t, J = 60.4 Hz, 1H, C*H*F_2). ^{13}C NMR ($CDCl_3$) δ: 38.7, 52.2, 107.5 (t, J = 250 Hz), 120.4, 121.1, 127.8, 128.5, 128.8, 130.0, 130.8, 132.4, 136.3, 143.3, 160.2, 171.8.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl) phenylacetic acid (14)

A mixture of methyl 2-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)phenylacetate (**13**, 0.75 mmol) and 2 N LiOH (3.0 mL, 6.0 mmol) in THF (3.0 mL) and MeOH (3.0 mL) was stirred at reflux temperature for 2 h. The reaction mixture was cooled to 25 °C and washed with CH_2Cl_2 (30 mL). The water phase was then acidified to pH 2 by addition of 3 N hydrochloric acid prior to extraction with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water and then brine, the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash silica gel column chromatography using EtOAc–MeOH (9:1, *v*/*v*) as eluent to furnish **14** in 90% yield; pale yellow gum. IR (film): 3063, 2948, 1737, 1692, 1592, 1152 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.64 (s, 2H, CH_2), 6.68 (dd, J = 7.3, 1.8 Hz, 1H, pyridone H-3), 7.25–7.42 (overlapping multiplets, 4H total, phenyl H-3, H-4, H-5, H-6), 7.47 (dd, J = 7.3, 2.4 Hz, 1H, pyridone H-4), 7.56 (d, J = 2.4 Hz, 1H, pyridone H-6), 7.75 (t, J = 60.4 Hz, 1H, C*H*F_2), 10.2 (br s, 1H, COO*H*). Anal. Calcd for $C_{14}H_{11}F_2NO_3$: C, 60.22; H, 3.97; N, 5.02. Found: C, 59.94; H, 4.30; N, 4.79.

2-(2-Oxo-1,2-dihydropyridin-5-yl)phenylacetic acid (15)

A mixture of **13** (0.75 mmol) and 2 N NaOH (3.0 mL, 6.0 mmol) was heated at reflux for 5 h. The reaction mixture was cooled to 25 °C and washed with CH_2Cl_2 (30 mL). The water phase was then acidified to pH 2 by addition of 3 N hydrochloric acid prior to extraction with EtOAc (3 × 50 mL). The combined EtOAc extracts were washed with water and then brine, the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash silica gel column chromatography using EtOAc–MeOH (9:1, *v*/*v*) as eluent to afford **15**;

yield, 56%; pale yellow solid, mp 242–244 °C. IR (film): 3060, 2981, 1663, 1614 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 3.46 (s, 2H, CH_2), 6.43 (d, J = 9.1 Hz, 1H, pyridone H-3), 7.18–7.33 (overlapping multiplets, 5H total, phenyl H-3, H-4, H-5, H-6 and pyridone H-6), 7.38 (dd, J = 9.1, 2.4 Hz, 1H, pyridone H-4), 7.60 (s, 1H, N*H*), 10.1 (br s, 1H, COO*H*). Anal. Calcd for $C_{13}H_{11}NO_3$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.23; H, 4.86; N, 5.98.

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, μmol/L) were determined using an enzyme immunoassay kit (catalog No. 560131, Cayman Chemical, Ann Arbor, Michigan, USA) according to our previously reported method.[17]

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, Michigan, USA) (IC_{50} values, μmol/L) were determined using an enzyme immunoassay kit (catalog No. 760700, Cayman Chemical) according to our previously reported method.[18]

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