Bioorganic & Medicinal Chemistry Letters 20 (2010) 5776–5780

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and biological evaluation of indomethacin analogs possessing a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system: A search for novel cyclooxygenase and lipoxygenase inhibitors

Morshed A. Chowdhury, Zhangjian Huang, Khaled R. A. Abdellatif, Ying Dong, Gang Yu, Carlos A. Velázquez, Edward E. Knaus *

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alta, Canada T6G 2N8

ARTICLE INFO

Article history:
Received 16 July 2010
Revised 29 July 2010
Accepted 30 July 2010
Available online 3 August 2010

Keywords:
Indomethacin analogs
N-Difluoromethyl-1,2-dihydropyrid-2-one
5-Lipoxygenase pharmacophore
Cyclooxygenase and 5-lipoxygenase inhibition
Anti-inflammatory activity

ABSTRACT

A novel class of indomethacin analogs were synthesized wherein a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety (5-LOX pharmacophore) was attached at its C-4 or C-5 position via either a C=O (**14a**–**b**) or CH_2 (**19a**–**b**) linker to the indole N^1-position. In this regard, replacement of the 4-chlorobenzoyl group present in indomethacin by *N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)carbonyl and *N*-difluoromethyl-1,2-dihydropyrid-2-one-4-yl(or 5-yl)methylene moieties furnished compounds showing no inhibitory activities against the COX-2/5-LOX enzymes (except for the weak but selective COX-2 inhibitor **19a**, COX-2 IC_{50} = 31 μM), and moderate in vivo anti-inflammatory activities (except for the methylene compound **19a** that was inactive). These structure–activity data indicate replacement of the 4-chlorobenzoyl group present in indomethacin by a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system connected by a C=O or CH_2 linker is not a suitable approach for the design of dual COX-2/5-LOX inhibitory analogs of indomethacin.

Arachidonic acid (AA), following its release from membrane-bound phospholipids, undergoes biotransformation via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways. Proinflammatory prostaglandins (PGs) produced via the COX pathway, and leukotrienes (LTs) produced via the LOX pathway, are implicated in physiological processes such as inflammation, fever, arthritis and bronchospasm.[1,2] PGs that cause contraindicated inflammation, fever, and pain are formed via the inducible COX-2 isozyme whereas, PGs that regulate beneficial gastrointestinal cytoprotection and renal effects are produced via the constitutive COX-1 isozyme.[1–3] Alternatively, 5-LOX is associated with the production of LTs that cause inflammatory, bronchoconstrictor, hypersensitivity, anaphylactic and asthmatic actions. On the other hand, 15-LOX is implicated in atherosclerosis because it catalyzes the oxidation of lipoproteins (LDL, HDL) to atherogenic forms.[4,5]

Dual inhibitors of the LOX/COX enzymatic pathways[6] constitute a rational approach for the design of more effective anti-inflammatory agents with a superior safety profile relative to ulcerogenic nonsteroidal anti-inflammatory drugs (NSAIDs), and selective COX-2 inhibitors that increase the incidence of adverse cardiovascular thrombotic effects.[7,8] This view is based on a potentially greater anti-inflammatory (AI) efficacy because of their ability to synergistically block both metabolic pathways of the arachidonic acid (AA) cascade.[1,9] It has been pointed out that inhibition of only one of the COX/LOX pathways could shift the metabolism of AA towards the other pathway, thereby inducing potential side effects.[10] One of the more successful strategies to develop 5-LOX inhibitors utilized hydroxamic acids and related *N*-hydroxyureas that act by chelation of iron present in the 5-LOX enzyme.[11] Two representative examples of iron chelating 5-LOX inhibitors include zileuton (**1**)[12] and tepoxalin (**2**)[11] (see structures in Fig. 1). A potent hybrid COX-2/5-LOX inhibitor (**3**) in which the C-3 trifluoromethyl substituent present in the COX-2 inhibitor celecoxib (**4**)[13] was replaced by a non-redox competitive 4-(3-fluoro-5-oxymethyl)phenyl-4-methoxytetrahydropyran 5-LOX pharmacophore was reported by Henichart and co-workers[2] In recent studies, we described novel classes of dual COX/5-LOX inhibitors that include celecoxib analogs (**5**)[14] and 1,2-diarylacetylene regioisomers (**6**).[15] These compounds **5** and **6**, that possess a *N*-difluoromethyl-1,2-dihydropyrid-2-one 5-LOX pharmacophore, exhibited effective AI activity. It was subsequently discovered that salicylic acid derivatives **7** possessing a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety also exhibited dual COX-2/5-LOX inhibitory activities.[16] Accordingly, it was anticipated that replacement of the 4-chlorobenzoyl moiety in indomethacin (**8**) by *N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)carbonyl, or *N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)ylmethylene, regioisomeric moieties may provide a hitherto unknown class of dual COX-2/5-LOX inhibitory AI agents. As part of this ongoing research program, we now describe the synthesis of

* Corresponding author. Tel.: +1 780 492 5993; fax: +1 780 492 1217.
E-mail address: eknaus@pharmacy.ualberta.ca (E.E. Knaus).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.132

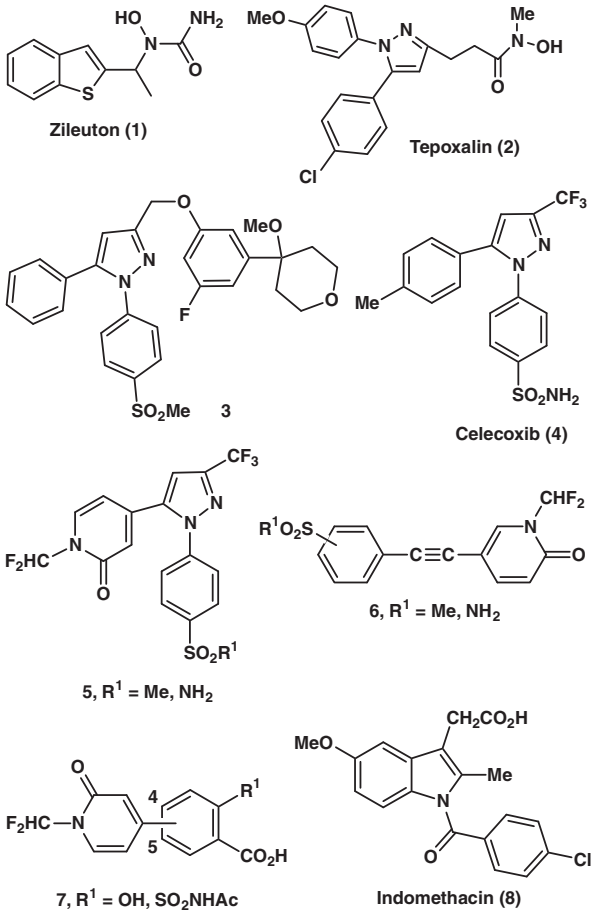
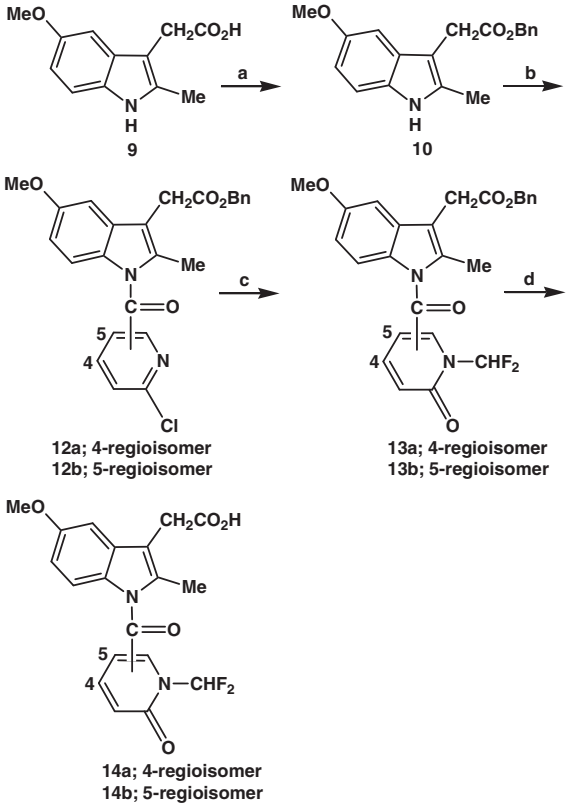


Figure 1. Chemical structures of some representative iron-chelating 5-LOX inhibitors (**1**–**2**), a COX-2/5-LOX inhibitor (**3**), a selective COX-2 inhibitor (**4**), dual COX/5-LOX inhibitors (**5**–**7**) and the non-selective COX-1/COX-2 inhibitor (**8**).



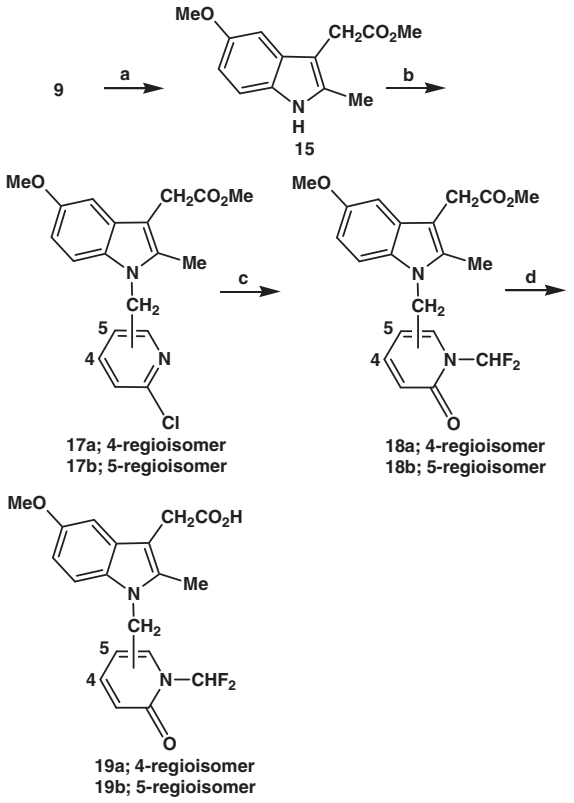
Scheme 1. Reagents and conditions: (a) benzyl chloroformate, DMAP, Et_3N, CH_2Cl_2, 0 °C, 0.5 h; (b) 2-chloropyridine-4-carbonyl chloride (**11a**) or 2-chloropyridine-5-carbonyl chloride (**11b**), DMAP, Et_3N, CH_2Cl_2, 25 °C, 60 h; (c) FSO_2CF_2COOH, $NaHCO_3$, MeCN, reflux, 40 h; (d) 10% Pd/C, EtOAc, MeOH, H_2 (45 psi), 25 °C, 24 h.

a novel class of indomethacin analogs wherein a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety (5-LOX pharmacophore) was attached at its C-4 or C-5 position via either a C=O (**14a**–**b**) or CH_2 (**19a**–**b**) linker to the indole N^1-position, their in vitro evaluation as COX-1, COX-2, and 5-LOX inhibitors, and in vivo assessment as AI agents.

A group of compounds where the 4-chlorobenzoyl group present in indomethacin was replaced by a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system attached via a carbonyl, or methylene, linking group was synthesized using the reaction sequence illustrated in Schemes 1 and 2, respectively. The carboxylic group of 5-methoxy-2-methyl-3-indoleacetic acid (**9**) was protected using a procedure analogous to a literature method[17] that furnished the benzyl ester (**10**)[18] in 97% yield (Scheme 1). Reaction of **10** with 2-chloropyridine-4-carbonyl chloride (**11a**), or its 5-regioisomer **11b**, in the presence of 4-dimethylaminopyridine (DMAP) in triethylamine and dichloromethane[19] afforded the respective coupled product **12a** or **12b** in 95–97% yield. Subsequent transformation of the 2-chloropyridine ring in compounds **12a** and **12b** upon treatment with 2,2-difluoro-2-(fluorosulfonyl)acetic acid (FSO_2CF_2COOH)[20] afforded the respective *N*-difluoromethyl-1,2-dihydropyrid-2-one regioisomer **13a** or **13b** in 31–32% yields. Deprotection of the CO_2Bn group in **13a** and **13b** with H_2 (45 psi) in the presence of 10% Pd on carbon in methanol–ethyl acetate[19] afforded the target [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(and 5-)carbonyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid regioisomers (**14a** and **14b**) in 46–75% yields.

For the synthesis of the target compounds **19a**–**b** having a methylene linker, the carboxyl group in **9** was protected by reaction with acetyl chloride and methanol[21] that afforded the methyl ester (**15**)[22] in 95% yield (Scheme 2). N^1-alkylation of **15** with a 2-chloro-4-(or 5-)chloromethylpyridine **16a** or **16b** in THF using potassium *t*-butoxide as base[19] furnished the respective coupled product **17a** or **17b** in 34–49% yields as illustrated in Scheme 2. Subsequent transformation of the 2-chloropyridine ring in **17a** or **17b** to a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring present in **18a** or **18b** was carried out in 34–53% yield using the same procedure described previously for the preparation of **13a**–**b**. Deprotection of the CO_2Me group in **18a** and **18b** with aqueous 2 N lithium hydroxide in methanol-tetrahydrofuran[23] furnished the target [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)ylmethyl-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid regioisomers (**19a** and **19b**) in 59–85% yields.

The rational for the design of the [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)carbonyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acids (**14a**–**b**) and [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4-(or 5-)ylmethylene-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acids (**19a**–**b**) was based on the expectation that replacement of the 4-chlorobenzoyl moiety in indomethacin by a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system in conjunction with a carbonyl or methylene linker may provide a hitherto unknown class of compounds with dual COX-2/5-LOX inhibitory activities. The $CONCHF_2$ fragment of the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring present in **14a**–**b** and **19a**–**b** can be viewed as a cyclic hydroxamic acid mimetic. These *N*-difluoromethyl-1,2-dihydropyrid-2-ones **14a**–**b** and **19a**–**b** could inhibit the 5-LOX enzyme by two possible mechanisms. In this regard **14a**–**b** and **19a**–**b**, like acyclic hydroxamic acids, may act as effective iron chelators to exhibit 5-LOX inhibitory activity. It has been reported that there is a substantial build-up of negative potential



Scheme 2. Reagents and conditions: (a) acetyl chloride, MeOH, 0 °C and then 60 °C, 16 h; (b) 2-chloro-4-chloromethylpyridine (**16a**) or 2-chloro-5-chloromethylpyridine (**16b**), *t*-BuOK, THF, reflux under argon, 60 h; (c) FSO_2CF_2COOH, $NaHCO_3$, MeCN, reflux, 40 h; (d) LiOH, THF–MeOH–H_2O, reflux, 3 h.

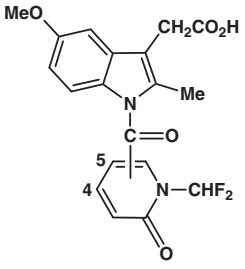
around the two fluorine atoms of a CHF_2 group.[24] Despite this high electron-density, an aliphatic fluorine seldom acts as a hydrogen-bond acceptor, presumably due to its high electronegativity and low polarizability.[25,26] Therefore, it is also plausible that the CHF_2 group may interact with a positively charged region on the enzyme that may contribute to enhanced affinity and competitive reversible inhibition of the COX and/or 5-LOX enzymes.[27] 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 increased 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 4-chlorobenzoyl moiety present in indomethacin **8** resulting in retention of COX-2 inhibitory activity.

In vitro COX-1 and COX-2 enzyme inhibition studies (see data in Table 1) showed that three indomethacin analogs (**14a**–**b**, **19b**) did not inhibit either the COX-1 or COX-2 isozyme at a 100 μM test compound concentration. Although the C-4 regioisomer **19a** did not inhibit COX-1 (IC_{50} >100 μM), it is a weak inhibitor of the COX-2 isozyme (COX-2 IC_{50} = 31 μM).

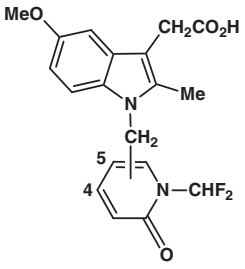
In vitro 5-LOX inhibition studies showed that all four indomethacin analogs (**14a**–**b**, **19a**–**b**) failed to inhibit the 5-LOX enzyme (IC_{50} >100 μM) relative to the reference drug nordihydroguaiaretic acid (NDGA) (IC_{50} = 13 μM).

The AI activities exhibited by the indomethacin analogs **14a**–**b** and **19a**–**b** were determined using a carrageenan-induced rat foot paw edema model at a 100 mg/kg oral dose. In this assay, compounds **14a**–**b**, **19b** showed moderate AI activity (16.0–25.5% inhibition). In contrast, compound **19a** showed no AI activity. The

Table 1
In vitro COX-1, COX-2, 5-LOX enzyme inhibition, and in vivo anti-inflammatory activity, data for indomethacin analogs containing a *N*-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-carbonyl moiety (**14a**–**b**) or *N*-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-ylmethyl moiety (**19a**–**b**)



14a; 4-regioisomer
14b; 5-regioisomer



19a; 4-regioisomer
19b; 5-regioisomer

Compound	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	5-LOX IC_{50} (μM)[b]	AI activity % Inhibition
14a	>100	>100	>100	25.5 ± 7[c]
14b	>100	>100	>100	18.9 ± 6.4[c]
19a	>100	31	>100	Inactive[c]
19b	>100	>100	>100	16.0 ± 0.5[c]
Indomethacin	0.1	5.7[d]	—	50[e]
NDGA[f]	—	—	13	—

[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 immunoassay kit (catalog no. 560131, Cayman Chemicals Inc., Ann Arbor, MI), 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), 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 % inhibition of inflammation at 3 h following a 100 mg/kg oral dose of the test compound.

[d] Data acquired using ovine COX-2 (catalog no. 56101, Cayman Chemicals Inc.).

[e] Inhibitory activity in a carrageenan-induced rat paw edema assay. The results are expressed as the % inhibition of inflammation at 3 h following a 4.2 mg/kg oral dose of the reference drug indomethacin.

[f] NDGA, nordihydroguaiaretic acid.

observation that compounds **14a**–**b** and **19b** did not inhibit either the COX-1 or COX-2 isozyme suggests that their weak AI effect may occur by a non-COX mechanism to reduce inflammation.

In conclusion, a hitherto unknown class of [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4 or 5-carbonyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acids (**14a**–**b**) and [1-(*N*-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-ylmethyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acids (**19a**–**b**), was synthesized[28] for evaluation as dual 5-LOX[29] and COX-1/COX-2[30] isozyme inhibitors of inflammation.[31] The structure–activity data acquired indicate that coupling of a *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system via a carbonyl (C=O), or methylene (CH_2), linker to the N^1-position of 5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acids is not a suitable strategy for the design of indomethacin analogs as AI agents that act by inhibition of the 5-LOX and/or COX-2 enzymes. The observation that **14a**–**b** and **19a**–**b** are not effective inhibitors of the COX and LOX enzymes indicates how structural changes can unexpectedly abolish activity since previous studies showed that celecoxib (**5**), linear acetylene (**6**) and salicylic acid (**7**) analogs having a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety exhibited effective in vitro dual COX/LOX inhibitory, and in vivo anti-inflammatory, activities.

Acknowledgment

We are grateful to the Canadian Institutes of Health Research (Grant No. MOP-14712) for financial support of this research.

References and notes

1. Charlier, C.; Michaux, C. *Eur. J. Med. Chem.* **2003**, *38*, 645.
2. Barbey, S.; Goossens, L.; Taverne, T.; Cornet, J.; Choesmel, V.; Rouaud, C.; Gimeno, G.; Yannic-Arnoult, S.; Michaux, C.; Charlier, C.; Houssin, R.; Henichart, J.-P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 779.
3. Dannhardt, G.; Laufer, S. *Curr. Med. Chem.* **2000**, *7*, 1101.
4. Vila, L. *Med. Res. Rev.* **2004**, *24*, 399.
5. Zhao, L.; Funk, C. D. *Trends Cardiovasc. Med.* **2004**, *14*, 191.
6. Praveen Rao, P. N.; Chen, Q.-H.; Knaus, E. E. *J. Med. Chem.* **2006**, *49*, 1668; and references cited therein
7. Scheen, A. J. *Rev. Med. Liege* **2004**, *59*, 565.
8. Dogné, J.-M.; Supuran, C. T.; Pratico, D. *J. Med. Chem.* **2005**, *48*, 2251.
9. Fiorucci, S.; Meli, R.; Bucci, M.; Cirino, G. *Biochem. Pharmacol.* **2001**, *62*, 1433.
10. Asako, H.; Kubes, P.; Wallace, J.; Gaginella, T.; Wolf, R. E.; Granger, N. *Am. J. Physiol. Gastrointest. Liver Physiol.* **1992**, *262*, 903.
11. Muri, E. M. F.; Nieto, M. J.; Sindelar, R. D.; Williamson, J. S. *Curr. Med. Chem.* **2002**, *9*, 1631.
12. Carter, G. W.; Young, P. R.; Albert, D. H.; Bouska, J.; Dyer, R.; Bell, R. L.; Summers, J. B.; Brooks, D. W. *J. Pharmacol. Exp. Ther.* **1991**, *256*, 929.
13. Penning, T. D.; Talley, J. J.; Bertenshaw, S. R.; Carter, J. S.; Collins, P. W.; Docter, S.; Graneto, M. J.; Lee, L. F.; Malecha, J. W.; Miyashiro, J. M.; Rogers, R. S.; Rogier, D. J.; Yu, S. S.; Anderson, G. D.; Burton, E. G.; Cogburn, J. N.; Gregory, S. A.; Koboldt, C. M.; Perkins, W. E.; Seibert, K.; Veenhuizen, A. W.; Zhang, Y. Y.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1347.
14. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Das, D.; Suresh, M. R.; Knaus, E. E. *J. Med. Chem.* **2009**, *52*, 1525.
15. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Rahman, M.; Das, D.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 584.
16. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Das, D.; Yu, G.; Velázquez, C. A.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6855.
17. Kim, S.; Lee, J. I.; Kim, Y. C. *J. Org. Chem.* **1985**, *50*, 560.
18. Kasaya, Y.; Hoshi, K.; Terada, Y.; Nishida, A.; Shuto, S.; Arisawa, M. *Eur. J. Org. Chem.* **2009**, 4606.
19. Wey, S.-J.; Augustyniak, M. E.; Cochran, E. D.; Ellis, J. L.; Fang, X.; Garvey, D. S.; Janero, D. R.; Letts, L. G.; Martino, A. M.; Melim, T. L.; Murty, M. G.; Richardson, S. K.; Schroeder, J. D.; Selig, W. M.; Trocha, A. M.; Wexler, R. S.; Young, D. V.; Zemtseva, I. S.; Zifcak, B. M. *J. Med. Chem.* **2007**, *50*, 6367.
20. Ando, M.; Wada, T.; Sato, N. *Org. Lett.* **2006**, *8*, 3805.
21. Gou, X.; Lo, H. Y.; Man, C. C.; Takahashi, H. PCT WO 2009023655, 2009; *Chem. Abstr.* **2009**, *150*, 237629.
22. Kalgutkar, A. S.; Crews, B. C.; Saleh, S.; Prudhomme, D.; Marnett, L. J. *Bioorg. Med. Chem.* **2005**, *13*, 6810.
23. Adamski-Werner, S. L.; Palaninathan, S. K.; Sacchettini, J. C.; Kelly, J. W. *J. Med. Chem.* **2004**, *47*, 355.
24. 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.
25. Dunitz, J. D.; Taylor, R. *Chem. A Eur. J.* **1997**, *3*, 89.
26. Howard, J. A. K.; Hoy, V. J.; O'Hagan, D.; Smith, G. T. *Tetrahedron* **1996**, *52*, 12613.
27. Chavatte, P.; Yous, S.; Marot, C.; Baurin, N.; Lesieur, D. *J. Med. Chem.* **2001**, *44*, 3223.
28. Experimental procedures and spectral data for compounds **10**, **12**–**14**, **15**, **17**–**19**. *General*. 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 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AM-300 spectrometer. Microanalyses (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) were performed for C, H and N and were within ±0.4% of theoretical values for all elements listed. Compounds **10**, **12a**–**b**, **13a**–**b**, **15**, **17a**–**b** and **18a**–**b** showed a single spot on Macherey-Nagel Polygram Sil G/UV254 silica gel plates (0.2 mm) using a low, medium, and highly polar solvent system, and no residue remained after combustion, indicating a purity of >95%. Silica gel column chromatography was performed using Merck silica gel 60 ASTM (70–230 mesh). 2-Chloro-4-chloromethylpyridine (**16a**) was synthesized in 43% overall yield by an analogous reduction of 2-chloropyridine-4-carbonyl chloride (**11a**) with $NaBH_4$ in water[32] followed by the reaction with $SOCl_2$ in toluene.[33] All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification. The in vivo anti-inflammatory assay was carried out using protocols approved by the Health Sciences Animal Welfare Committee at the University of Alberta.
(5-Methoxy-2-methyl-1H-indol-3-yl)acetic acid benzyl ester (**10**):[18] Benzyl chloroformate (0.65 mL, 4.6 mmol) was added to a solution of 5-methoxy-2-methylindole-3-acetic acid (**9**, 1.0 g, 4.6 mmol) and triethylamine (0.7 mL, 5.0 mmol) in dichloromethane (15 mL) at 0 °C and the mixture was stirred for 5 min. DMAP (55 mg, 0.45 mmol) was added at 0 °C and the reaction was allowed to proceed with stirring for 0.5 h. The reaction mixture was diluted with dichloromethane (30 mL), washed successively with saturated $NaHCO_3$ (30 mL), 0.1 N HCl (15 mL) and brine (30 mL), and the organic fraction was dried ($MgSO_4$). Filtration and then removal of the solvent from the organic fraction in vacuo afforded the benzyl ester **10** as a yellow oil in 97% yield; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.71 (s, 2H, CH_2CO_2Bn), 3.78 (s, 3H, OCH_3), 5.12 (s, 2H, CH_2Ph), 6.77 (dd, J = 2.4, 8.5 Hz, 1H, H-6), 6.98 (d, J = 2.4 Hz, 1H, H-4), 7.15 (d, J = 8.5 Hz, 1H, H-7), 7.27–7.40 (m, 5H, C_6H_5), 7.80 (br s, 1H, N*H*, exchangeable with D_2O).
General procedure for the synthesis of [1-(2-chloropyridine-4- or 5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl esters (**12a**–**b**): A solution of the benzyl ester **10** (0.5 g, 1.62 mmol), 2-chloropyridine-4-(or 5-)carbonyl chloride **11a** or **11b** (0.34 g, 1.94 mmol), DMAP (104 mg, 0.85 mmol), and triethylamine (1.20 mL, 8.61 mmol) in dichloromethane (10 mL) was stirred at 25 °C for 60 h. Removal of solvents from the reaction mixture in vacuo gave crude product that was purified by silica gel column chromatography using hexanes–ethyl acetate (1:1, v/v) as eluent to furnish the respective title compound **12a** or **12b**. Some physical and spectroscopic data for **12a**–**b** are listed below.
[1-(2-Chloropyridine-4-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl ester (**12a**): Product **12a** was obtained as a yellow oil in 95% yield; IR (film) 1735 (ester), 1685 (amide) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.71 (s, 2H, CH_2CO_2Bn), 3.77 (s, 3H, OCH_3), 5.15 (s, 2H, CH_2Ph), 6.72 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 6.93 (d, J = 2.4 Hz, 1H, indole H-4), 7.01 (d, J = 9.1 Hz, 1H, indole H-7), 7.27–7.39 (m, 5H, C_6H_5), 7.43 (dd, J = 1.2, 4.9 Hz, 1H, pyridyl H-5), 7.59 (d, J = 1.2 Hz, 1H, pyridyl H-3), 8.58 (d, J = 4.9 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 13.9, 30.4, 55.6, 66.9, 101.7, 112.3, 114.1, 115.2, 121.2, 123.5, 128.2, 128.4, 128.6, 130.2, 131.0, 135.3, 135.6, 146.0, 150.6, 152.6, 156.6, 166.0, 170.3.
[1-(2-Chloropyridine-5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl ester (**12b**): Product **12b** was obtained as a yellow oil in 97% yield; IR (film) 1735 (ester), 1683 (amide) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H, CH_3), 3.72 (s, 2H, CH_2CO_2Bn), 3.77 (s, 3H, OCH_3), 5.15 (s, 2H, CH_2Ph), 6.71 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 6.93 (d, J = 2.4 Hz, 1H, indole H-4), 6.94 (d, J = 9.1 Hz, 1H, indole H-7), 7.27–7.39 (m, 5H, C_6H_5), 7.47 (d, J = 8.5 Hz, 1H, pyridyl H-3), 7.96 (dd, J = 2.4, 8.5 Hz, pyridyl H-4), 8.70 ((d, J = 2.4 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 13.7, 30.4, 55.6, 66.9, 101.6, 112.1, 113.4, 114.9, 124.4, 128.2, 128.4, 128.5, 130.3, 130.5, 130.8, 135.5, 135.6, 139.5, 150.7, 155.3, 156.4, 166.0, 170.4.
General procedure for the synthesis of [1-(N-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl esters (**13a**–**b**): $FSO_2CF_2CO_2H$ (0.72 g, 4.02 mmol), and then $NaHCO_3$ (113 mg, 1.34 mmol), was added to a solution of a 2-chloropyridine **12a** or **12b** (0.60 g, 1.34 mmol) in MeCN (10 mL). This mixture was then heated at reflux under argon for 40 h, cooled to 25 °C, a saturated solution of aqueous $NaHCO_3$ (10 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic extracts were successively washed with water (25 mL) and brine, and the organic fraction was dried ($MgSO_4$). Filtration and then removal of the solvent from the organic fraction in vacuo afforded the impure product which was purified by silica gel chromatography using hexanes–ethyl acetate (2:1, v/v) as eluent to furnish the title compound **13a** or **13b**. Some physical and spectroscopic data for **13a**–**b** are listed below.
[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-4-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl ester (**13a**): Product **13a** was obtained as a yellow oil in 32% yield; IR (film) 1741 (ester), 1689 (amide) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 3H, CH_3), 3.69 (s, 2H, CH_2CO_2Bn), 3.78 (s, 3H, OCH_3), 5.14 (s, 2H, CH_2Ph), 6.46 (dd, J = 1.8, 7.3 Hz, 1H, pyridone H-5), 6.78 (d, J = 1.8 Hz, 1H, pyridone H-3), 6.79 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 6.93 (d, J = 2.4 Hz, 1H, indole H-4), 7.27–7.39 (m, 5H, C_6H_5), 7.38 (d, J = 9.1 Hz, 1H, indole H-7), 7.59 (d, J = 7.3 Hz, 1H, pyridone H-6), 7.70 (t, J = 60 Hz, 1H, CHF_2); ^{13}C NMR ($CDCl_3$) δ 13.9, 30.3, 55.6, 66.9, 101.8, 105.6, 112.4, 114.3, 115.4, 122.1, 128.2, 128.3, 128.5, 130.0, 130.8, 131.0, 134.9, 135.6, 147.9, 156.7, 160.3, 165.4, 170.2.
[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid benzyl ester (**13b**): Product **13b** was obtained as a yellow oil in 31% yield; IR (film) 1736 (ester), 1697 (amide) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 3H, CH_3), 3.67 (s, 2H, CH_2CO_2Bn), 3.72 (s, 3H, OCH_3), 5.10 (s, 2H, CH_2Ph), 6.54 (d, J = 9.7 Hz, 1H, pyridone H-3), 6.71 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 6.91 (d, J = 2.4 Hz, 1H, indole H-4), 7.04 (d, J = 9.1 Hz, 1H, indole H-7), 7.22–7.35 (m, 5H, C_6H_5), 7.60 (dd, J = 2.4, 9.7 Hz, 1H, pyridone H-4), 7.64 (t, J = 60 Hz, 1H, CHF_2), 8.06 (d, J = 2.4 Hz, 1H, pyridone H-6); ^{13}C NMR ($CDCl_3$) δ 13.0, 30.4, 55.6, 66.9, 101.6, 112.2, 112.9, 114.2, 115.8, 121.2, 128.1, 128.3, 128.5, 130.2, 130.7, 135.6, 135.7, 136.2, 140.4, 156.2, 160.1, 164.4, 170.5.
General procedure for the synthesis of [1-(N-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acids (**14a**–**b**): Palladium-on-charcoal (25 mg of 10% w/w) was added to a solution of a benzyl ester **13a** or **13b** (0.27 g, 0.56 mmol) in EtOAc (22 mL) and MeOH (4 mL). The resulting suspension was flushed with argon followed by three consecutive flushes with H_2 gas to remove any air or argon from the hydrogenation flask. The pressure in the hydrogenation flask was maintained at 45 psi with H_2 gas using a Parr apparatus. After shaking for 24 h at 25 °C, the H_2 gas was released from the hydrogenation flask, and the reaction mixture was filtered through a Celite pad to remove any Pd/C catalyst. The filtrate was concentrated in vacuo to afford the crude product which was purified by silica gel column chromatography using hexanes–ethyl acetate (1:3, v/v in 1% acetic acid) as eluent to furnish the title compound **14a** or **14b**. Some physical and spectroscopic data for **14a**–**b** are listed below.
[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-4-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid (**14a**): Product **14a** was obtained as a yellow solid in 75% yield; mp 177–179 °C; IR (film) 1728 (ester), 1653 (amide) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.29 (s, 3H, CH_3), 3.67 (s, 2H, CH_2CO_2H), 3.78 (s, 3H, OCH_3), 6.60 (dd, J = 1.8, 7.3 Hz, 1H, pyridone H-5), 6.79 (d, J = 1.8 Hz, 1H, pyridone H-3), 6.83 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 7.06 (d, J = 2.4 Hz, 1H, indole H-4), 7.51 (d, J = 9.1 Hz, 1H, indole H-7), 7.89 (t, J = 60 Hz, 1H, CHF_2), 8.01 (d, J = 7.3 Hz,

1H, pyridone H-6), 12.42 (s, 1H, CO_2H, exchangeable with D_2O). Anal. Calcd for $C_{19}H_{16}F_2N_2O_5$: C, 58.46; H, 4.13; N, 7.18. Found: C, 58.26; H, 4.20; N, 7.02.

[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-5-carbonyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid (**14b**): Product **14b** was obtained as a yellow solid in 46% yield; mp 163–165 °C; IR (film) 1725 (ester), 1676 (amide) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.33 (s, 3H, CH_3), 3.66 (s, 2H, CH_2CO_2H), 3.76 (s, 3H, OCH_3), 6.64 (d, J = 9.7 Hz, 1H, pyridone H-3), 6.75 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 7.04 (d, J = 2.4 Hz, 1H, indole H-4), 7.23 (d, J = 9.1 Hz, 1H, indole H-7), 7.70 (dd, J = 2.4, 9.7 Hz, 1H, pyridyl H-4), 7.86 (t, J = 60 Hz, 1H, CHF_2), 8.19 (d, J = 2.4 Hz, 1H, pyridone H-6), 12.37 (s, 1H, CO_2H, exchangeable with D_2O); Anal. Calcd for $C_{19}H_{16}F_2N_2O_5$: C, 58.46; H, 4.13; N, 7.18. Found: C, 58.29; H, 4.23; N, 7.23.

(5-Methoxy-2-methyl-1H-indol-3-yl)acetic acid methyl ester (**15**):[22] Acetyl chloride (0.23 mL, 3.12 mmol) was added to a solution of the acetic acid **9** (0.5 g, 2.28 mmol) in methanol (5 mL) at 0 °C, and the reaction was allowed to proceed with stirring at 60 °C for 16 h. The mixture was then concentrated in vacuo to dryness to which a solution of saturated aqueous $NaHCO_3$ (20 mL) was added. The solution was extracted with EtOAc (3 × 20 mL), the combined EtOAc extracts were successively washed with water and brine, and the organic fraction was dried ($MgSO_4$). Filtration and then removal of the solvent in vacuo from the organic fraction afforded the methyl ester **15** as a pale yellow oil in 95% yield; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 3.67 (s, 2H, $CH_2CO_2CH_3$), 3.68 (s, 3H, CO_2CH_3) 3.86 (s, 3H, OCH_3), 6.78 (dd, J = 2.4, 8.5 Hz, 1H, H-6), 6.99 (d, J = 2.4 Hz, 1H, H-4), 7.16 (d, J = 8.5 Hz, 1H, H-7), 7.77 (br s, 1H, N*H*, exchangeable with D_2O).

General procedure for the synthesis of [1-(2-chloropyridin-4- or 5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl esters (**17a–b**): A solution of *t*-BuOK (254 mg, 2.15 mmol) in dry THF (3 mL) was added slowly via a syringe to a stirred solution of 5-methoxy-2-methyl-1*H*-indol-3-yl)acetic acid methyl ester (**15**, 0.5 g, 2.15 mmol) and either 2-chloro-4-chloromethylpyridine (**16a**) or 2-chloro-5-chloromethylpyridine (**16b**) (0.35 g, 2.15 mmol) in dry THF (30 mL) at 25 °C under argon. The reaction mixture was heated at reflux under argon atmosphere for 60 h. The reaction mixture was cooled to 25 °C, poured onto ice water, acidified with 5% aqueous HCl, and extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed successively with water and brine, and the organic fraction was dried ($MgSO_4$). Filtration and removal of the solvent from the organic fraction in vacuo afforded the impure product which was purified by silica gel flash column chromatography using hexanes–ethyl acetate (2:1, v/v) as eluent to furnish the respective title compound **17a** or **17b**. Some physical and spectroscopic data for **17a–b** are listed below.

[1-(2-Chloropyridin-4-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl ester (**17a**): Product **17a** was obtained as a brown oil in 34% yield; IR (film) 1734 (ester) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H, CH_3), 3.69 (s, 3H, CO_2CH_3) 3.73 (s, 2H, $CH_2CO_2CH_3$), 3.87 (s, 3H, OCH_3), 5.24 (s, 2H, NCH_2), 6.75 (dd, J = 1.3, 5.5 Hz, 1H, pyridyl H-5), 6.80 (dd, J = 2.4, 8.5 Hz, 1H, indole H-6), 6.92 (d, J = 1.3 Hz, 1H, pyridyl H-3), 6.99 (d, J = 8.5 Hz, 1H, indole H-7), 7.06 (d, J = 2.4 Hz, 1H, indole H-4), 8.27 (d, J = 5.5 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 10.4, 30.5, 45.4, 52.0, 55.9, 100.8, 105.2, 109.3, 111.3, 119.7, 121.4, 128.4, 131.2, 134.3, 150.1, 150.5, 152.2, 154.6, 172.2.

[1-(2-Chloropyridin-5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl ester (**17b**): Product **17b** was obtained as a brown oil in 49% yield; IR (film) 1735 (ester) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 3.68 (s, 3H, CO_2CH_3) 3.71 (s, 2H, $CH_2CO_2CH_3$), 3.87 (s, 3H, OCH_3), 5.27 (s, 2H, NCH_2), 6.80 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 7.01–7.11 (m, 3H, indole H-4, H-7, pyridyl H-4), 7.19 (d, J = 7.9 Hz, 1H, pyridyl H-3), 8.20 (d, J = 1.8 Hz, 1H, pyridyl H-6); ^{13}C NMR ($CDCl_3$) δ 10.4, 30.5, 43.8, 51.9, 55.9, 100.8, 105.1, 109.4, 111.3, 124.5, 128.4, 131.2, 132.4, 134.3, 136.6, 147.6, 150.6, 154.5, 172.2.

General procedure for the synthesis of [1-(N-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl esters (**18a–b**): Compounds **18a** and **18b** were synthesized using the same procedure used for the synthesis of **13a–b**. The product was purified by silica gel flash chromatography using hexanes–ethyl acetate (1:2, v/v) as eluent to furnish the respective title compound **18a** or **18b**. Some physical and spectroscopic data for **18a–b** are listed below.

[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-4-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl ester (**18a**): Product **18a** was obtained as a brown oil in 34% yield; IR (film) 1734 (ester), 1684 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.32 (s, 3H, CH_3), 3.69 (s, 3H, CO_2CH_3) 3.71 (s, 2H, $CH_2CO_2CH_3$), 3.87 (s, 3H, OCH_3), 5.03 (s, 2H, NCH_2), 5.96 (d, J = 7.9 Hz, 1H, pyridone H-5), 5.97 (s, 1H, pyridone H-3), 6.80 (dd, J = 2.4, 8.5 Hz, 1H, indole H-6), 7.00 (d, J = 8.5 Hz, 1H, indole H-7), 7.04 (d, J = 2.4 Hz, 1H, indole H-4), 7.36 (d, J = 7.9 Hz, 1H, pyridone H-6), 7.61 (t, J = 60 Hz, 1H, CHF_2); ^{13}C NMR ($CDCl_3$) δ 10.3, 30.5, 45.7, 51.9, 55.9, 100.9, 105.2, 105.4, 109.3, 111.4, 117.3, 128.4, 129.8, 131.2, 134.2, 152.6, 154.6, 160.7, 172.2.

[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid methyl ester (**18b**): Product **18b** was obtained as a greenish oil in 53% yield; IR (film) 1734 (ester), 1690 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 3.67 (s, 3H, CO_2CH_3) 3.70 (s, 2H, $CH_2CO_2CH_3$), 3.87 (s, 3H, OCH_3), 5.01 (s, 2H, NCH_2), 6.49 (d, J = 9.7 Hz, 1H, pyridone H-4), 6.83 (dd, J = 2.4, 8.5 Hz, 1H, indole H-6), 7.01–7.11 (m, 4H, indole H-4, H-7, pyridone H-3, H-6), 7.62 (t, J = 60 Hz, 1H, CHF_2); ^{13}C NMR ($CDCl_3$) δ 10.5, 30.5, 43.5, 51.9, 55.9, 100.8, 105.3, 109.3, 111.4, 117.2, 122.5, 125.9, 128.4, 131.2, 134.2, 140.0, 154.5, 160.4, 172.2.

General procedure for the synthesis of [1-(N-difluoromethyl-1,2-dihydropyrid-2-one-4- or 5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acids (**19a–b**): Aqueous LiOH (2 N, 5 mL) was added to a solution of a methyl ester **18a** or **18b** (0.18 g, 0.46 mmol) in MeOH (5 mL) and THF (5 mL), and the reaction was allowed to proceed at reflux for 3 h. The mixture was cooled to 25 °C, and acidified to pH 3 using 5% HCl prior to extraction with EtOAc (3 × 25 mL). The combined organic extracts were successively washed with water (50 mL) and brine, and the organic fraction was dried ($MgSO_4$). Filtration and then removal of the solvent from the organic fraction in vacuo afforded the crude product which was purified by silica gel column chromatography using hexanes–ethyl acetate (1:3, v/v in 1% acetic acid) as eluent to furnish the title compound **19a** or **19b**. Some physical and spectroscopic data for **19a–b** are listed below.

[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-4-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid (**19a**): Product **19a** was obtained as a brown solid in 59% yield; mp 165–167 °C; IR (film) 1716 (ester), 1682 (amide) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.25 (s, 3H, CH_3), 3.62 (s, 2H, CH_2CO_2H), 3.87 (s, 3H, OCH_3), 5.28 (s, 2H, NCH_2), 5.61 (d, J = 1.8 Hz, 1H, pyridone H-3), 6.09 (dd, J = 1.8, 7.9 Hz, 1H, pyridone H-5), 6.70 (dd, J = 2.4, 9.1 Hz, 1H, indole H-6), 6.98 (d, J = 2.4 Hz, 1H, indole H-4), 7.24 (d, J = 9.1 Hz, 1H, indole H-7), 7.75 (d, J = 7.9 Hz, 1H, pyridone H-6), 7.76 (t, J = 60 Hz, 1H, CHF_2), 12.10 (br s, 1H, CO_2H, exchangeable with D_2O). Anal. Calcd for $C_{19}H_{18}F_2N_2O_4$: C, 60.64; H, 4.82; N, 7.44. Found: C, 60.28; H, 4.89; N, 7.38.

[1-(N-Difluoromethyl-1,2-dihydropyrid-2-one-5-ylmethyl)-5-methoxy-2-methyl-1H-indol-3-yl]acetic acid (**19b**): Product **19b** was obtained as a yellow solid in 85% yield; mp 145–147 °C; IR (film) 1714 (ester), 1688 (amide) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.34 (s, 3H, CH_3), 3.71 (s, 2H, CH_2CO_2H), 3.87 (s, 3H, OCH_3), 5.00 (s, 2H, NCH_2), 6.48 (d, J = 9.1 Hz, 1H, pyridone H-4), 6.82 (dd, J = 2.4, 8.5 Hz, 1H, indole H-6), 7.01–7.15 (m, 4H, indole H-4, H-7, pyridone H-3, H-6), 7.61 (t, J = 60 Hz, 1H, CHF_2), 11.01 (br s, 1H, CO_2H, exchangeable with D_2O). Anal. Calcd for $C_{19}H_{18}F_2N_2O_4$: C, 60.64; H, 4.82; N, 7.44. Found: C, 60.32; H, 4.84; N, 7.31.

29. *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.[34]
30. *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.[35]
31. *Anti-inflammatory assay*: The test compounds **14a–b**, **19a–b**, and the reference drug indomethacin were evaluated using the in vivo carrageenan-induced rat foot paw edema model reported previously.[36]

32. Read, M. W.; Ray, P. S. *J. Heterocycl. Chem.* **1995**, *32*, 1595.
33. Zhuang, L.; Wai, J. S.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P., Jr.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J., Jr.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453.
34. Chowdhury, M. A.; Abdellatif, K. R. A.; Dong, Y.; Das, D.; Suresh, M. R.; Knaus, E. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6138.
35. Rao, P. N. P.; Amini, M.; Li, H.; Habeeb, A.; Knaus, E. E. *J. Med. Chem.* **2003**, *46*, 4872.
36. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.