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Synthesis and biological evaluation of *N*-difluoromethyl-1,2-dihydropyrid-2-one acetic acid regioisomers: Dual Inhibitors of cyclooxygenases and 5-lipoxygenase

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ABSTRACT

A new group of acetic acid (**7a**–**c**, R^1 = H), and propionic acid (**7d**–**f**, R^1 = Me), regioisomers wherein a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety is attached via its C-3, C-4, and C-5 position was synthesized. This group of compounds exhibited a more potent inhibition, and hence selectivity, for the cyclooxygenase-2 (COX-2) relative to the COX-1 isozyme. Attachment of the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system to an acetic acid, or propionic acid, moiety confers potent 5-LOX inhibitory activity, that is, absent in traditional arylacetic acid NSAIDs. 2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**) exhibited the best combination of dual COX-2 and 5-LOX inhibitory activities. Molecular modeling (docking) studies showed that the highly electronegative CHF_2 substituent present in **7c**, that showed a modest selectivity for the COX-2 isozyme, is oriented within the secondary pocket (Val523) present in COX-2 similar to the sulfonamide (SO_2NH_2) COX-2 pharmacophore present in celecoxib, and that the *N*-difluoromethyl-1,2-dihydropyrid-2-one pharmacophore is oriented close to the region containing the LOX enzyme catalytic iron (His361, His366, and His545). Accordingly, the *N*-difluoromethyl-1,2-dihyrdopyrid-2-one moiety possesses properties suitable for the design of dual COX-2/5-LOX inhibitory drugs.

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The eicosanoid family of inflammatory mediators arise from the biotransformation of arachidonic acid (AA) via the cyclooxygenase (COX) and lipoxygenase (LOX) pathways.[1] In this regard, the prostanoids (prostaglandins, prostacyclin, and thromboxanes) and leukotrienes (LTs) are produced via their respective COX and LOX pathways. Traditional non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effect by inhibition of the *constitutive* COX-1 and *inducible* COX-2 isozymes.[2] Adverse side effects, in particular gastrointestinal (GI) irritation, ulcerogenicity and renal toxicity attributed to inhibition of the cytoprotective COX-1 isozyme, are frequent deterrents to the chronic use of NSAIDs.[3] Alternatively, LTs produced via the 5-LOX enzyme catalyzed pathway are known to play a role in the pathogenesis of inflammatory and allergic disorders.[1] The related isozyme 15-LOX is linked to cardiovascular complications since it is known to participate in oxidative modification of low-density lipoproteins (LDL) leading to the development of atherosclerosis.[4] In addition, the 5-LOX pathway is up-regulated during COX blockade that may be due to a shift in the metabolism of AA to the uninhibited LOX pathway. Accordingly, increased levels of LTs may induce undesirable adverse effects such as bronchial constriction. Therefore, dual inhibitors of COXs and LOXs represent an attractive safer clinical alternative to COX inhibitors in view of their potentially greater anti-inflammatory efficacy due to a synergistic block of both the COX and LOX metabolic pathways in the AA cascade.[5]

In an earlier study, we reported that replacement of the tolyl ring in celecoxib (**1**) by a *N*-difluoromethyl-1,2-dihydropyrid-2-one 5-LOX pharmacophore[6] furnished a novel class of dual COX/5-LOX inhibitors (**2**) which exhibited effective AI activity.[7] It was subsequently discovered that phenylacetic acid regioisomers (**3**) possessing a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety attached to its C-2, C-3, or C-4 position also exhibited dual COX/5-LOX inhibitory activities (see structures in Fig. 1).[8] It was anticipated that replacement of the aryl ring in a classical arylacetic acid NSAID template by a *N*-difluoromethyl-1,2-dihydropyrid-2-one 5-LOX pharmacophore would similarly provide dual 5-LOX/COX inhibitors. Accordingly, we now describe the synthesis of a novel class of acetic acid regioisomers having a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety (**7a**–**f**), their in vitro evaluation as

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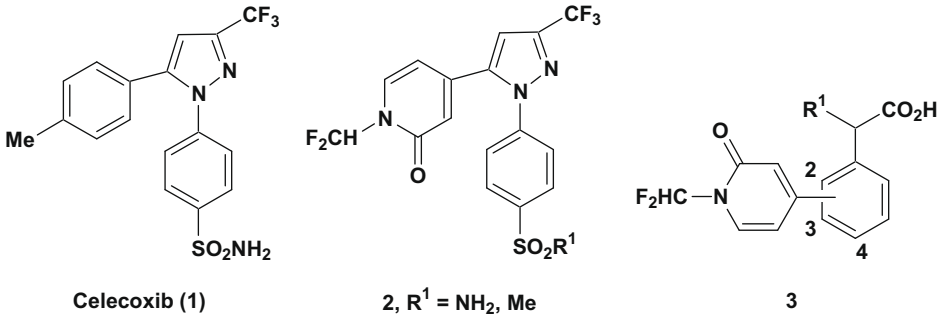
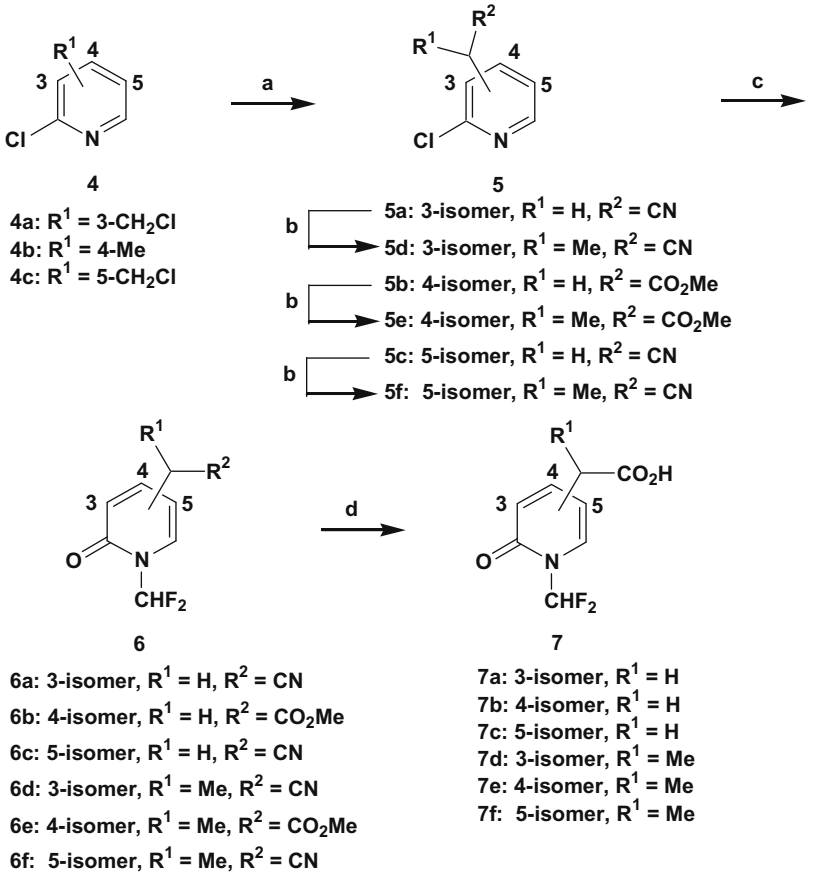


Figure 1. Some representative examples of the selective COX-2 inhibitor celecoxib (**1**), and dual COX/5-LOX inhibitors having a *N*-difluoromethyl-1,2-dihydropyrid-2-one pharmacophore (**2**–**3**).

COX-1/COX-2, 5-LOX inhibitors, and some molecular modeling studies.

A group of acetic acid (**7a**–**c**, R^1 = H), and propionic acid (**7d**–**f**, R^1 = Me), regioisomers in which a *N*-difluoromethyl-1,2-dihydropyrid-2-one moiety was attached via its C-3, C-4, and C-5 position was synthesized as illustrated in Scheme 1. Reaction of the chloromethylpyridines (**4a**, **4c**) with NaCN furnished the respective cyanomethylpyridines (**5a**, 85%; **5c**, 70%).[9,10] 2-Chloro-4-methylpyridine (**4b**) was elaborated to methyl 2-chloroisonicotinate (**5b**, 53% yield) upon reaction with lithium diisopropylamide (LDA) and methyl chloroformate.[11] The subsequent methylation of **5a**–**c** using iodomethane in the presence of LDA at low temperature under argon afforded the respective products (**5d**–**f**) in 75–83% yields.[12,13] Reaction of the 2-chloropyridyl compounds **5a**–**f** in acetonitrile with 2,2-difluoro-2-(fluorosulfonyl)acetic acid ($FSO_2CF_2CO_2H$)[7,14] in the presence of $NaHCO_3$ under an argon atmosphere at reflux temperature afforded the respective *N*-difluoromethyl-1,2-dihydropyrid-2-one product (**6a**–**f**) in 39–65% yields. Acid hydrolysis of the cyano substituent present in the acetonitrile compounds (**6a**, **6c**–**d**, **6f**) using 30% HCl in 1,4-dioxane at 80 °C afforded the respective carboxylic acid target products **7a**, **7c**–**d**, or **7f** in 57–79% yield. Alkaline hydrolysis of the methyl ester moiety present in compounds **6b** and **6e** using aqueous 2 N NaOH in MeOH at reflux furnished the respective target acetic acid (**7b**, 75%), and propionic acid (**7e**, 74%), product.[15]

Traditional arylacetic acid NSAIDs share a number of similar structural features that include a carboxyl group separated by one-carbon atom from a flat aromatic nucleus, and one or more



Scheme 1. Reagents and conditions: (a) NaCN, $EtOH/H_2O$, reflux, 12 h for **5a**, **5c**; LDA, $ClCO_2Me$, THF, −78 °C, 1 h; then 0 °C, 30 min for **5b**; (b) LDA, MeI, THF, −78 °C, 30 min; then 0 °C, 30 min; (c) $FSO_2CF_2CO_2H$, $NaHCO_3$, MeCN, argon atmosphere, reflux, 6 h; (d) 30% HCl, 1,4-dioxane, reflux, 8 h for **7a**, **7c**–**d**, **7f**; aqueous 2 N NaOH, MeOH, reflux, 3 h for **7b**, **7e**.

large lipophilic groups attached to the aromatic nucleus, that is, two, three, or four carbon atoms removed from the point of attachment of the acetic acid side chain.[16] Similarly, the lipophilic CHF_2 substituent present in the 1-difluoromethyl-2-oxo-1,2-dihydropyridyl derivatives of acetic acid (**7a**–**c**) and propionic acid (**7d**–**f**) is separated from the point of attachment of the acetic acid side chain by either 2 or 3 carbon atoms and 1 N atom that are embedded in the nearly planar 2-oxo-1,2-dihydropyridyl ring. In this regard, the rational for the design of compounds (**7a**–**f**) was based on the belief that replacement of the aromatic ring with an attached lipophilic substituent in an arylacetic acid NSAID by a lipophilic *N*-difluoromethyl-2-oxo-1,2-dihydropyridyl moiety may furnish a new class of dual COX-2/5-LOX inhibitors. This expectation is based on a drug design concept that the $CONCHF_2$ fragment of the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring present in **7a**–**f** can be viewed as a cyclic hydroxamic acid mimetic. If this concept is true, there are two plausible mechanisms by which *N*-difluoromethyl-1,2-dihydropyrid-2-ones **7a**–**f** could inhibit the 5-LOX enzyme. For example, **7a**–**f**, like acyclic hydroxamic acids, may chelate iron present in 5-LOX to induce inhibitory activity. There is a substantial localized negative potential around the two fluorine atoms of a CHF_2 group.[17] However, aliphatic fluorine atoms, in spite of this high electron-density, seldom act as a hydrogen-bond acceptor, that is, most likely due to their high electronegativity and low polarizability.[18,19] Therefore, it is also plausible that the CHF_2 group could interact with a positively charged region on the enzyme that may provide enhanced affinity and competitive reversible inhibition of the COX and/or 5-LOX enzymes.[20]

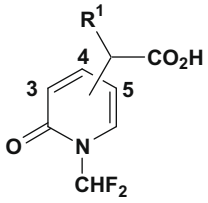
In vitro COX-1 and COX-2 enzyme inhibition studies (see data in Table 1) showed that the *N*-difluoromethyl-1,2-dihydropyrid-2-one regioisomers **7b**–**f** exhibited a more potent inhibition, and hence selectivity, for the COX-2 isozyme (COX-1 IC_{50} = inactive to 16.5 μM range; COX-2 IC_{50} = 1.3–12.9 μM range). In comparison, the acetic acid regioisomer **7a** was a nearly equiactive inhibitor of COX-1 and COX-2. The point of attachment of the acetic acid (**7a**–**c**, R^1 = H) substituent to the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring system was a greater determinant of COX-2 inhibitory activity relative to that observed for the propionic acids (**7d**–**f**, R^1 = Me). All compounds **7a**–**f** were less potent inhibitors of COX-1 and COX-2 than the reference drug ibuprofen. The most potent COX-2 inhibitor **7c** (IC_{50} = 1.3 μM) was marginally less potent than ibuprofen (IC_{50} = 1.1 μM).

In vitro 5-LOX inhibition studies showed that attachment of the *N*-difluoromethyl-1,2-dhydropyrid-2-one ring system to an acetic acid moiety confers 5-LOX inhibitory activity, that is, absent in traditional arylacetic acid NSAIDs. The group of compounds **7a**–**f** exhibited more potent 5-LOX inhibitory activity (IC_{50} = 0.25–3.7 μM range) than the reference drug caffeic acid (IC_{50} = 4.0 μM). The point of attachment of the acetic acid (R^1 = H) substituent was not a determinant of activity for the nearly equipotent 3-, 4-, and 5-regioisomers **7a**–**c** (IC_{50} = 0.44–0.77 μM range). In contrast, the point of attachment of the propionic acid (R^1 = Me) substituent modulated 5-LOX potency where the 4-regioisomer **7e** (IC_{50} = 0.25 μM) was greater than 10-fold more potent than the 3- and 5-regioisomers **7d** and **7f** showing IC_{50} values of 3.7 and 3.0 μM, respectively. The R^1 substituent was a determinant of 5-LOX inhibitory potency for the C-3 and C-5 regioisomers (**7a** and **7c**, R^1 = H > **7d** and **7f**, R^1 = Me), but not the C-4 regioisomers (**7b**, R^1 = H approximately equipotent to **7e**, R^1 = Me).

Some physicochemical comparisons indicate that the acetic acids **7a**–**c** (R^1 = H) are less lipophilic (calculated log *P* = 0.08–0.21 range) and smaller in size (calculated volume of 154 $Å^3$) than the corresponding propionic acids **7d**–**f** (R^1 = Me; calculated log *P* = 0.65–0.82 range; calculated volume of 171 $Å^3$; see data in Table 1).

The binding interactions of an acetic acid derivative possessing a novel *N*-difluoromethyl-1,2-dihydropyrid-2-one pharmacophore was investigated by enzyme–ligand molecular modeling (docking) studies. The binding interaction of 2-(1-difluoromethyl-2-oxo-1,2-

Table 1
In vitro COX-1, COX-2, and 5-LOX enzyme inhibition data for 1-difluoromethyl-2-oxo-1,2-dihydropyridyl derivatives of acetic acid (**7a**–**c**) and propionic acid (**7d**–**f**)



7: a,d = 3-isomer; b,e = 4-isomer; c,f = 5-isomer

Compound	R^1	COX-1 IC_{50}[a] (μM)	COX-2 IC_{50}[a] (μM)	5-LOX IC_{50}[b] (μM)	Log *P*[c]	Volume[d] ($Å^3$)
7a	H	6.0	8.0	0.77	0.21	154.2
7b	H	Inactive	12.9	0.44	0.08	154.9
7c	H	7.9	1.3	0.46	0.08	154.7
7d	Me	16.5	5.9	3.7	0.82	171.0
7e	Me	10.7	5.5	0.25	0.65	170.9
7f	Me	9.8	6.4	3.0	0.65	171.0
Ibuprofen		2.9	1.1[e]	—	3.68	211.8
Caffeic acid[f]		—	—	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] The log *P* value was calculated using the ChemDraw Ultra program, Version 6.0, CambridgeSoft company.

[d] The volume of the molecule, after minimization using the MM3 force field, was calculated using the Alchemy 2000 program, Tripos Inc.

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

[f] Caffeic acid: 3,4-dihydroxycinnamic acid.

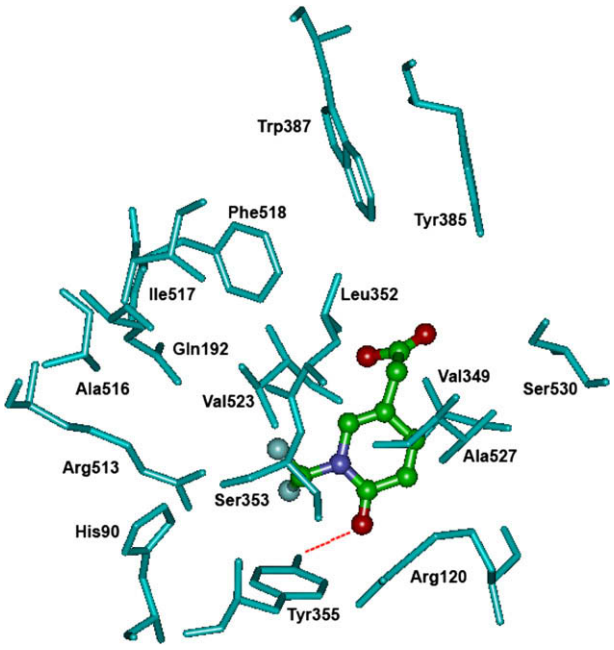


Figure 2. Docking 2-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**) (ball-and-stick) in the active site of mammalian COX-2 ($E_{intermolecular}$ = −23.08 kcal/mol). Red dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity.

dihydropyridin-5-yl)acetic acid (**7c**, COX-2 IC_{50} = 1.30 μM) within the COX-2 binding site is illustrated in Figure 2. The enzyme–ligand complex observed shows that the 1,2-dihydropyrid-2-one ring is oriented such that the $CONCHF_2$ fragment is positioned in a region close to both the entrance of the COX-2 active site (Arg120 and Tyr355) and the COX-2 secondary pocket (His90, Arg513, and Val523). The C=*O* oxygen atom ($CONCHF_2$), that undergoes a favorable hydrogen bonding interaction with the O*H* of Tyr355 (distance = 1.87 Å), is located about 2.57 Å from Arg120. The highly electronegative CHF_2 substituent is oriented within the COX-2 secondary pocket (Val523) in a polar region comprised of His90 and Arg513. The CHF_2 fluorine atoms are located about 4.54 and 3.82 Å from the N*H* of His90 and the NH_2 ofArg513, respectively. The C-5 acetic acid (CH_2COOH) side chain is oriented in a region consisting of Val349, Leu352, Tyr385, Ala527, and Ser530 (distance <5 Å). The observation that the C=*O* (CH_2COOH) is oriented closer to the apex of the COX-2 active site (Tyr385 and Ser530) where it is positioned about 4.26 and 4.54 Å from the O*Hs* of Ser530 and Tyr385, respectively, is consistent with previously reported crystal structure data for the NSAID diclofenac [ortho-(2,6-dichloroanilino)phenylacetic acid] bound to the COX-2 enzyme.[21]

The closely related mammalian lipoxygenase enzymes (5-, 12-, and 15-LOX) share 35–80% sequence identity. X-ray data for mammalian 15-LOX, but not 5-LOX, has been reported.[22] Accordingly, 15-LOX X-ray data was used as a model to investigate the binding interactions of 2-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**, 5-LOX IC_{50} = 0.46 μM) within a LOX binding site (Fig. 3). This docking study indicated that the LOX *N*-difluoromethyl-1,2-dihydropyrid-2-one pharmacophore is oriented close to the region containing the enzyme catalytic iron (His361, His366, and His545). The $CONCHF_2$ fragment is located in the vicinity of a polar region where the distance between the fluorine atoms (CHF_2) and the N*Hs* of His361 and His366 are about 4.41 and 4.23 Å, respectively. In addition, the C=*O* (dihydropyrid-2-one) is located about 3.94 Å from the N*H* of His361. The distance between the CHF_2 and the NH_2 (guanidine) group of Arg403, that is, closer to the mouth of the 15-LOX binding site is about 10.48 Å. The 1,2-dihydropyrid-2-one ring is surrounded by His361, His362, Leu408, Phe415, and Leu597 (distance <5 Å). The C-5 acetic acid (CH_2COOH) side chain is oriented toward the hydrophobic base region of the 15-LOX active site in the vicinity of Leu408, Ile414, Phe415, Met419, Ile593, and Leu597 (distance <5 Å). The COOH substituent is oriented in a polar region comprised of Glu357 and Gln548 (distance <5 Å). The C=*O* (CH_2COOH) forms a favorable hydrogen bonding interaction with the NH_2 of Gln548 (distance = 1.92 Å). This docking study supports our hypothesis that the $CONCHF_2$ fragment present in the *N*-difluoromethyl-1,2-dihydropyrid-2-one ring can be viewed as a cyclic hydroxamic acid mimetic.

In conclusion, a new class of C-3, C-4, and C-5 regioisomeric 1-difluoromethyl-2-oxo-1,2-dihydropyridyl derivatives of acetic acid (**7a**–**c**) and propionic acid (**7d**–**f**) was synthesized[23] for evaluation as dual COX-1/COX-2[24] and 5-LOX[25] enzyme inhibitors. Biological data acquired indicated that (1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**) exhibits the best combination of in vitro COX-2 and 5-LOX inhibitory potency. Molecular modeling (docking) studies[26] showed that the highly electronegative CHF_2 substituent present in **7c** is oriented within the secondary pocket (Val523) present in COX-2 similar to the sulfonamide (SO_2NH_2) COX-2 pharmacophore present in celecoxib (**1**) to confer a modest selectivity for the COX-2 isozyme, and that the *N*-difluoromethyl-1,2-dihydropyrid-2-one pharmacophore is oriented close to the region containing the enzyme catalytic iron (His361, His366, and His545). Accordingly, the *N*-difluoromethyl-1,2-dihyrdopyrid-2-one moiety possesses properties suitable for the design of dual COX-2/5-LOX inhibitory AI drugs.

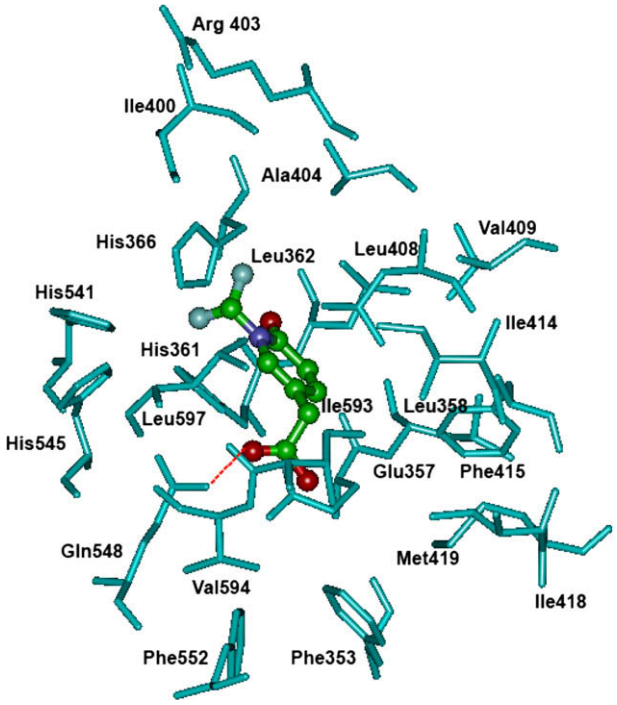


Figure 3. Docking 2-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**) (ball-and-stick) in the active site of mammalian 15-LOX ($E_{intermolecular}$ = −33.52 kcal/mol). Red dotted line represents hydrogen bonding. Hydrogen atoms are not shown for clarity.

Acknowledgments

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23. *Experimental procedures and spectral data for compounds* **5**, **7–9**. *General:* Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on NaCl plates using a Nicolet 550 Series II Magna FT-IR spectrometer. ^{1}H NMR spectra were measured on a Bruker AM-300 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hz. Spin multiples are given as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Microanalyses were performed for C, H, N (MicroAnalytical Service Laboratory, Department of Chemistry, University of Alberta) and were within ±0.4% of theoretical values. Silica gel column chromatography was performed using Merck Silica Gel 60 ASTM (70–230 mesh). 2-Chloro-3-chloromethylpyridine (**4a**) was prepared starting from commercially available 2-chloronicotinic acid.[27] All other reagents, purchased from the Aldrich Chemical Company (Milwaukee, WI), were used without further purification.

2-Chloro-3-cyanomethylpyridine (**5a**): Sodium cyanide (1.3 g, 26.5 mmol) was added to a solution of **4a** (4.0 g, 24.7 mmol) in EtOH (45 mL) and water (5 mL) at 25 °C with stirring. The reaction was allowed to proceed at reflux for 12 h, the reaction mixture was cooled to 25 °C, and the solvent was removed in vacuo. The residue was partitioned between EtOAc and water, the EtOAc fraction was washed with water and then brine, the EtOAc fraction was dried ($MgSO_4$), filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography (hexane/EtOAc) to afford **5a** (3.2 g, 85%) as a pale brown solid, mp 84–85 °C (lit.[9b] mp 85–86 °C); ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 2H, C*H*$_2$CN), 7.34 (dd, *J* = 8.5, 4.9 Hz, 1H, H-5), 7.90 (dd, *J* = 8.5, 1.8 Hz, 1H, H-4), 8.41 (dd, *J* = 4.9, 1.8 Hz, H-6).

2-Chloro-4-methoxycarbonylmethylpyridine (**5b**): *n*-Butyllithium (17.6 mL of 2.5 M in hexane, 44.0 mmol) was added to a solution of diisopropylamine (6.22 mL, 44.0 mmol) in dry THF (50 mL) at −78 °C under an atmosphere of argon, and the mixture was stirred for 30 min. A solution of 2-chloro-4-methylpyridine **(4b)** (5.11 g, 40.0 mmol) in dry THF (20 mL) was added drop wise during 20 min, and the mixture was stirred for 1 h at −78 °C. A solution of methyl chloroformate (3.69 mL, 48.0 mmol) in dry THF (10 mL) was added drop wise during 20 min, and the reaction was allowed to proceed with stirring for 1 h at −78 °C and then at 0 °C for 30 min. The mixture was quenched with aqueous saturated $NaHCO_3$ solution, the organic fraction was extracted with ether (3 × 80 mL), the combined organic extracts were washed with brine, and the organic fraction was dried ($MgSO_4$). Removal of the solvent in vacuo gave a residue that was purified by silica gel column chromatography using hexane–ethyl acetate (4:1, v/v) as eluent to afford **5b** (3.92 g, 53%) as a pale yellow oil, IR (film): 2948, 1733, 1593, 1227 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 2H, C*H*$_2$), 3.67 (s, 3H, O*Me*), 7.17 (dd, *J* = 4.9, 1.8 Hz, 1H, H-5), 7.29 (d, *J* = 1.8 Hz, 1H, H-3), 8.35 (d, *J* = 4.9 Hz, H-6).

2-Chloro-5-cyanomethylpyridine (**5c**): The title compound was prepared by reaction of **4c** (2.0 g, 12.35 mmol) with NaCN (0.67 g, 13.59 mmol) according to a reported procedure[12] as colorless plates (1.31 g, 70%), mp 52–54 °C (lit.[9b] mp 51–52 °C); ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 2H, C*H*$_2$CN), 7.40 (d, *J* = 8.5 Hz, 1H, H-3), 7.70 (dd, *J* = 8.5, 2.4 Hz, 1H, H-4), 8.41 (d, *J* = 2.4 Hz, H-6).

*General procedure for the synthesis of the propionitriles (***5d**, **5f***) and methyl propanoate (***5e***):* *n*-Butyllithium (4.40 mL of 2.5 M in hexane, 11.0 mmol) was added to a solution of diisopropylamine (1.55 mL, 11.0 mmol) in dry THF (20 mL) at −78 °C under an atmosphere of argon, and the mixture was stirred for 30 min. A solution of either **5a**, **5b**, or **5c** (10.0 mmol) in dry THF (10 mL) was added drop wise during 20 min, and the solution was stirred at −78° for 30 min prior to warming to 0 °C. Methyl iodide (0.75 mL, 12.0 mmol) was added and the reaction was allowed to proceed with stirring for 30 min. The reaction mixture was washed with aqueous saturated NH_4Cl (**15** mL) and the THF layer was extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with brine and dried over $MgSO_4$. Removal of the solvent in vacuo afforded a residue that was purified by silica gel column chromatography using hexane–ethyl acetate (4:1, v/v) as eluent to furnish the respective product **5d**, **5e**, or **5f**. The physical and spectral data for **5d–f** are listed below.

2-(2-Chloropyridin-3-yl)propionitrile (**5d**): Product **5d** was obtained as a pale yellow oil (1.24 g, 75%); IR (film): 2986, 2242 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.67 (d, *J* = 6.7 Hz, 3H, CH*Me*), 4.32 (q, *J* = 6.7 Hz, 1H, C*H*Me), 7.36 (dd, *J* = 7.9, 4.9 Hz, 1H, H-5), 7.95 (dd, *J* = 7.9, 1.8 Hz, 1H, H-4), 8.41 (dd, *J* = 4.9, 1.8 Hz, H-6). ^{13}C NMR ($CDCl_3$) δ 18.3, 54.2, 122.3, 123.9, 147.7, 151.9, 153.4, 160.3 Hz.

Methyl 2-(2-chloropyridin-4-yl)propanoate (**5e**): Product **5e** was obtained as a pale yellow oil (1.65 g, 83%); IR (film): 2950, 1735, 1592, 1246 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (d, *J* = 6.7 Hz, 3H, CH*Me*), 3.68 (q, *J* = 6.7 Hz, 1H, C*H*Me), 3.69 (s, 3H, O*Me*), 7.16 (dd, *J* = 4.9, 1.2 Hz, 1H, H-5), 7.27 (d, *J* = 1.2 Hz, 1H, I H-3), 8.33 (d, *J* = 4.9 Hz, H-6); ^{13}C NMR ($CDCl_3$) δ 17.8, 44.6, 52.5, 121.6, 123.4, 149.8, 151.9, 152.4, 173.0 Hz.

2-(2-Chloropyridin-5-yl)propionitrile (**5f**): Product **5f** was obtained as a pale yellow solid (1.27 g, 77%); mp 69–70 °C (lit[12] mp 67–69 °C); ^{1}H NMR ($CDCl_3$) δ 1.68 (d, *J* = 7.3 Hz, 3H, CH*Me*), 3.96 (q, *J* = 7.3 Hz, 1H, C*H*Me), 7.40 (d, *J* = 8.5 Hz, 1H, H-3), 7.95 (dd, *J* = 8.5, 2.4 Hz, 1H, H-4), 8.41 (d, *J* = 2.4 Hz, H-6).

General procedure for synthesis of the 1-difluoromethyl-2-oxo-1,2-dihydropyridine analogs (**6a–f**): 2,2-Difluoro-2-(fluorosulfonyl)acetic acid (FSO_2CF_2COOH, 1.85 mL, 18.0 mmol), and then $NaHCO_3$ (0.56 g, 6.6 mmol), was added to a solution of each 2-chloropyridine compound **5a–f** (6.0 mmol) in dry acetonitrile (30 mL) with stirring. The reaction was allowed to proceed at reflux under an argon atmosphere for 6 h. After cooling to 25 °C, the solvent was removed in vacuo, a saturated solution of aqueous $NaHCO_3$ (25 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with 10 N HCl (2 × 15 mL) to remove any starting material **5** that was still present. The CH_2Cl_2 extract was washed with water (50 mL) and then brine (25 mL), the organic fraction was dried ($MgSO_4$), and the solvent was removed in vacuo to afford the respective title product **6**. Some physical and spectral data for **6a–f** are listed below.

(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-3-yl)acetonitrile (**6a**): Product **6a** was obtained as a pale yellow solid (0.47 g, 43%); mp 76–78 °C; IR (film): 2924, 2252, 1672 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 2H, C*H*$_2$), 6.40 (dd, *J* = 6.7, 6.7 Hz, 1H, H-5), 7.51 (dd, *J* = 6.7, 1.8 Hz, 1H, H-4), 7.65 (dd, *J* = 6.7, 1.8 Hz, 1H, H-6), 7.70 (t, *J* = 59.8 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 19.1, 106.5 (t, *J* = 234 Hz), 106.6, 116.4, 123.6, 129.1, 138.6, 159.7.

Methyl (1-difluoromethyl-2-oxo-1,2-dihydropyridin-4-yl)acetate (**6b**): Product **6b** was obtained as a yellow solid (0.69 g, 53%); mp 74–76 °C; IR (film): 2959, 1733, 1683 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 2H, C*H*$_2$), 3.74 (s, 3H, O*Me*), 6.28 (dd, *J* = 7.9, 1.8 Hz, 1H, H-5), 6.45 (d, *J* = 1.8 Hz, 1H, H-3), 7.49 (d, *J* = 7.9 Hz, 1H, H-6), 7.66 (t, *J* = 59.8 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 40.6, 56.8, 107.3 (t, *J* = 234 Hz), 108.8, 121.2, 129.3, 148.3, 161.3, 169.4.

(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetonitrile (**6c**): Product **6c** was obtained as a pale yellow solid (0.66 g, 60%); mp 86–87 °C; IR (film): 2971, 2245, 1699 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.54 (s, 2H, C*H*$_2$), 6.63 (d, *J* = 9.8 Hz, 1H, H-3), 7.34 (dd, *J* = 9.8, 2.4 Hz, 1H, H-4), 7.47 (d, *J* = 2.4 Hz, 1H, H-6), 7.67 (t, *J* = 59.8 Hz, 1H, C*H*F$_2$); ^{1}C NMR ($CDCl_3$) δ 20.3, 107.3 (t, *J* = 253 Hz), 109.7, 116.1, 122.8, 128.0, 140.8, 159.9.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-3-yl)propionitrile (**6d**): Product **6d** was obtained as a yellow oil (0.46 g, 39%); IR (film): 2999, 2245, 1671 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (d, *J* = 7.3 Hz, 3H, CH*Me*), 4.15 (q, *J* = 7.3 Hz, 1H, C*H*Me), 6.34 (t, *J* = 7.3 Hz, 1H, H-5), 7.49 (dd, *J* = 7.3, 1.8 Hz, 1H, H-4), 7.65 (dd, *J* = 7.3, 1.8 Hz, 1H, H-6), 7.70 (t, *J* = 60.4 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 18.4, 26.3, 106.7, 107.5 (t, *J* = 253 Hz), 120.4, 129.2, 129.8, 137.7, 159.3.

Methyl 2-(1-difluoromethyl-2-oxo-1,2-dihydropyridin-4-yl)propanoate (**6e**): Product **6e** was obtained as a pale yellow oil (0.73 g, 53%); IR (film): 2950, 1734, 1683 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.45 (d, *J* = 6.7 Hz, 3H, CH*Me*), 3.55 (q, *J* = 6.7 Hz, 1H, C*H*Me), 3.71 (s, 3H, O*Me*), 6.27 (dd, *J* = 7.3, 1.8 Hz, 1H, H-5), 6.44 (d, *J* = 1.8 Hz, 1H, H-3), 7.40 (d, *J* = 7.3 Hz, 1H, H-6), 7.65 (t, *J* = 60.4 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 16.8, 45.0, 52.5, 107.2, 107.3 (t, *J* = 252 Hz), 119.3, 129.1, 154.3, 160.9, 172.5.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)propionitrile (**6f**): Product **6f** was obtained as a yellow gum (0.77 g, 65%); IR (film): 2992, 2241, 1693 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.62 (d, *J* = 7.3 Hz 3H, CH*Me*), 3.72 (q, *J* = 7.3 Hz 1H, C*H*Me), 6.63 (d, *J* = 9.7 Hz, 1H, H-3), 7.38 (dd, *J* = 9.7, 2.4 Hz, 1H, H-4), 7.45 (d, *J* = 2.4 Hz, 1H, H-6), 7.66 (t, *J* = 59.8 Hz, 1H, C*H*F$_2$); ^{13}C NMR ($CDCl_3$) δ 19.8, 27.9, 107.3 (t, *J* = 253 Hz), 116.3, 119.6, 123.0, 126.9, 139.7, 160.0.

General procedure for the synthesis of 1-difluoromethyl-2-oxo-1,2-dihydropyridyl derivatives of acetic acid (**7a**, **7c**) *and propionic acid* (**7d**, **7f**): Hydrochloric acid (15 mL of 10 N) was added to a solution of **6a**, **6c**, **6d**, or **6f** (3.0 mmol) in 1,4-dioxane (10 mL). The mixture was heated at 80 °C for 8 h, cooled to 25 °C, and the solvent was removed in vacuo. Cold water (30 mL) was added to the residue, the mixture was adjusted to pH 5 using 5% HCl, and this mixture was extracted with EtOAc (3 × 30 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 residue was purified by silica gel column chromatography (hexanes–EtOAc = 2:1, v/v) to afford the respective product **7a**, **7c**, **7d**, or **7f**. Physical and spectral data are listed below.

General procedure for synthesis of 1-difluoromethyl-2-oxo-1,2-dihydropyridyl derivatives of the acetic acid (**7b**) *and propionic acid* (**7e**): A solution of aqueous NaOH (10 mL of 2 N) was added to a solution of either **6b** or **6e** (3.0 mmol) in MeOH (10 mL), and the mixture was stirred at gentle reflux for 3 h prior to cooling to 25 °C. After nearly complete removal of the MeOH in vacuo, water (30 mL) was added, the mixture was adjusted to pH 3 using aqueous 5% HCl solution, and the mixture was extracted with EtOAc (3 × 30 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 residue was purified by silica gel column chromatography (hexanes–EtOAc, 2:1, v/v) to furnish the respective product **7b** or **7e**. Physical and spectral data for **7b** and **7e** are listed below.

(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-3-yl)acetic acid (**7a**): Product **7a** was obtained as a pale brown solid (0.48 g, 79%), mp 98–100 °C; IR (film): 3100 (br), 2919, 1734, 1667 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 2H, C*H*$_2$), 6.39 (dd, *J* = 6.7, 6.7 Hz, 1H, H-5), 7.45 (dd, *J* = 6.7, 1.2 Hz, 1H, H-4), 7.58 (dd, *J* = 6.7, 1.2 Hz, 1H, H-6), 7.73 (t, *J* = 60.4 Hz, 1H, C*H*F$_2$), 9.37 (br s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 36.5, 107.4, 107.5 (t, *J* = 253 Hz), 126.7, 128.7 (t, *J* = 3 Hz), 140.1, 161.5, 174.1. Anal. Calcd for $C_8H_7F_2NO_3$: C, 47.30; H, 3.47; N, 6.89. Found: C, 47.70; H, 3.71; N, 6.87.

(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-4-yl)acetic acid (**7b**): Product **7b** was obtained as a yellow solid (0.45 g, 75%); mp 112–114 °C; IR (film): 3100 (br), 2926, 1723, 1677 cm^{-1}; ^{1}H NMR ($CDCl_3$ + DMSO) δ 3.41 (s, 2H, C*H*$_2$), 6.28 (dd, *J* = 7.3, 1.2 Hz, 1H, H-5), 6.42 (d, *J* = 1.2 Hz, 1H, H-3), 7.36 (d, *J* = 7.3 Hz, 1H, H-6), 7.66 (t, *J* = 59.8 Hz, 1H, C*H*F$_2$), 8.62 (br s, 1H, COO*H*); ^{1}C NMR ($CDCl_3$) δ 40.6, 107.3 (t, *J* = 234 Hz), 108.8, 121.2, 129.3, 148.3, 161.3, 169.4. Anal. Calcd for $C_8H_7F_2NO_3$: C, 47.30; H, 3.47; N, 6.89. Found: C, 47.59; H, 3.20; N, 6.67.

(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)acetic acid (**7c**): Product **7c** was obtained as an off-white solid (0.44 g, 73%); mp 128–130 °C; IR (film): 3069 (br), 2903, 1734, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.46 (s, 2H, CH_2), 6.60 (d, J = 9.2 Hz, 1H, H-3), 7.38 (dd, J = 9.2, 2.4 Hz, 1H, H-4), 7.40 (d, J = 2.4 Hz, 1H, H-6), 7.82 (t, J = 60.4 Hz, 1H, CHF_2), 9.91 (br s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 36.4, 107.0 (t, J = 253 Hz), 113.7, 120.8, 127.5, 143.3, 160.0, 171.8. Anal. Calcd for $C_8H_7F_2NO_3$: C, 47.30; H, 3.47; N, 6.89. Found: C, 47.53; H, 3.59; N, 6.64.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-3-yl)propanoic acid (**7d**): Product **7d** was obtained as a pale yellow solid (0.39 g, 64%); mp 102–104 °C; IR (film): 3098 (br), 2940, 1718, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.51 (d, J = 7.3 Hz, 3H, CH*Me*), 3.94 (q, J = 7.3 Hz, 1H, C*H*Me), 6.43 (dd, J = 6.7, 6.7 Hz, 1H, H-5), 7.38 (dd, J = 6.7, 1.2 Hz, 1H, H-4), 7.40 (dd, J = 6.7, 1.2 Hz, 1H, H-6), 7.74 (t, J = 60.4 Hz, 1H, CHF_2), 9.73 (br s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 15.5, 39.8, 107.6 (t, J = 253 Hz), 107.8, 128.1, 132.3, 137.9, 161.2, 177.5. Anal. Calcd for $C_9H_9F_2NO_3$: C, 49.78; H, 4.18; N, 6.45. Found: C, 49.53; H, 4.14; N, 6.35.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-4-yl)propanoic acid (**7e**): Product **7e** was obtained as an off-white solid (0.48 g, 74%); mp 64–66 °C; IR (film): 3100 (br), 2986, 1682, 1667 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (d, J = 7.3 Hz, 3H, CH*Me*), 3.60 (q, J = 7.3 Hz, 1H, C*H*Me), 6.34 (dd, J = 7.3, 1.8 Hz, 1H, H-5), 6.54 (d, J = 1.8 Hz, 1H, H-3), 7.44 (d, J = 7.3 Hz, 1H, H-6), 7.67 (t, J = 60.4 Hz, 1H, CHF_2), 8.65 (br s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 16.7, 44.5, 107.3 (t, J = 253 Hz), 107.8, 119.3, 129.2, 154.3, 161.3, 176.1. Anal. Calcd for $C_9H_9F_2NO_3$: C, 49.78; H, 4.18; N, 6.45. Found: C, 49.55; H, 4.20; N, 6.64.

2-(1-Difluoromethyl-2-oxo-1,2-dihydropyridin-5-yl)propanoic acid (**7f**): Product **7f** was obtained as a white solid (0.37 g, 57%); mp 90–92 °C; IR (film): 3074 (br), 2986, 1734, 1688 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.49 (d, J = 7.3 Hz, 3H, CH*Me*), 3.54 (q, J = 7.3 Hz, 1H, C*H*Me), 6.62 (d, J = 9.7 Hz, 1H, H-3), 7.37 (d, J = 2.4 Hz, 1H, H-6), 7.49 (dd, J = 9.7, 2.4 Hz, 1H, H-4), 7.68 (t, J = 60.4 Hz, 1H, CHF_2), 9.95 (br s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$) δ 17.2, 41.6, 107.4 (t, J = 253 Hz), 119.4, 121.7, 127.1, 141.8, 160.9, 178.0. Anal. Calcd for $C_9H_9F_2NO_3$: C, 49.78; H, 4.18; N, 6.45. Found: C, 49.46; H, 4.22; N, 6.55.

24. *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.[28]
25. *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.[29]
26. *Molecular modeling (docking) studies*: Docking experiments were performed using Discovery Studio Client v2.5.0.9164 (2005-09), Accelrys Software Inc. running on a HP xw4600 workstation (Processor x86 family 6 model 23 stepping 10 GenuineIntel 2999 ~ Mhz). The coordinates for the X-ray crystal structure of the enzyme COX-2 and 15-LOX were obtained from the RCSB Protein Data Bank and hydrogens were added. The ligand molecules were constructed using the Build Fragment tool and energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The docking experiment on COX-2 was carried out by superimposing the energy minimized ligand on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The coordinates for 15-LOX was obtained from PDB file 1lox and the energy minimized ligand was superimposed on the inhibitor RS75091 after which RS75091 was deleted. In all these experiments the resulting ligand–enzyme complex was subjected to docking using the Libdock command in the receptor–ligand interactions protocol of Discovery Studio after defining subsets of the enzyme within 10 Å sphere radius of the ligand. The force field, Chemistry at HARvard Macromolecular Mechanics (CHARMM) was employed for all docking purposes. The ligand–enzyme assembly was then subjected to a molecular dynamics (MD) simulation using Simulation protocol at a constant temperature of 300 K with a 100 step equilibration for over 1000 iterations and a time step of 1 fs using a distance dependent dielectric constant 4*r*. The optimal binding orientation of the ligand–enzyme assembly obtained after docking was further minimized for 1000 iterations using the conjugate gradient method until a convergence of 0.001 kcal/mol Å was reached after which $E_{\text{intermolecular}}$ (kcal/mol) of the ligand–enzyme assembly was evaluated.
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