ACS Medicinal Chemistry Letters

Letter

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Design of Fluorine-Containing 3,4-Diarylfuran-2(5*H*)-ones as Selective COX-1 Inhibitors

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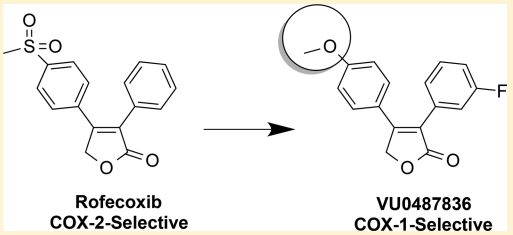
Supporting Information

ABSTRACT: We report the design and synthesis of fluorine-containing cyclooxygenase-1 (COX-1)-selective inhibitors to serve as prototypes for the development of a COX-1-targeted imaging agent. Deletion of the SO_2CH_3 group of rofecoxib switches the compound from a COX-2- to a COX-1-selective inhibitor, providing a 3,4-diarylfuran-2(5*H*)-one scaffold for structure–activity relationship studies of COX-1 inhibition. A wide range of fluorine-containing 3,4-diarylfuran-2(5*H*)-ones were designed, synthesized, and tested for their ability to selectively inhibit COX-1 in purified protein and human cancer cell assays. Compounds containing a fluoro-substituent on the C-3 phenyl ring and a methoxy-substituent on the C-4 phenyl ring of the 3,4-diarylfuran-2(5*H*)-one scaffold were the best COX-1-selective agents of those evaluated, exhibiting IC_{50}s in the submicromolar range. These compounds provide the foundation for development of an agent to facilitate radiologic imaging of ovarian cancer expressing elevated levels of COX-1.



KEYWORDS: *Cyclooxygenase-1 (COX-1), rofecoxib, furanone, structure–activity relationship, imaging*

The cyclooxygenase enzymes (COX-1 and COX-2), which catalyze the first two steps in the biosynthesis of prostaglandins from arachidonic acid, are the primary targets of the nonsteroidal anti-inflammatory drugs, such as indomethacin, ibuprofen, and naproxen. The inducible isoform, COX-2, is strongly expressed in response to inflammatory and mitogenic stimuli, leading to the widely accepted belief that this enzyme plays an important role in inflammation and carcinogenesis.[1] However, growing evidence suggests that the constitutively expressed COX-1 also contributes to some disease processes, including neuroinflammation, thrombosis, and some cancers.[2−6] Of the cancers reported to overexpress COX-1, the strongest case has been made for epithelial ovarian cancer. Indeed, recent evidence suggests that COX-1 contributes to the pathophysiology of ovarian cancer and that COX-1 inhibition may have both preventive and therapeutic benefits in this disease.[7−11]

We have shown that COX-2-selective inhibitors bearing fluorescent, ^{18}F, or ^{123}I tags can be used in conjunction with optical, positron emission tomography (PET), or single-photon emission computerized tomography imaging modalities, respectively, to visualize COX-2 expressed in tumors and inflammatory sites in vivo.[12−16] These findings led us to hypothesize that COX-1 could serve as an imaging target to detect ovarian cancer, a disease for which better diagnostic modalities are sorely needed. To that end, selective uptake of an [^{18}F]-labeled analogue of the COX-1-selective inhibitor P6 (3-(5-chlorofuran-2-yl)-5-(fluoromethyl)-4-phenylisoxazole) by COX-1-expressing ovarian carcinoma xenografts was recently reported.[17] These studies provided proof-of-concept for COX-1 targeting in ovarian cancer; however, it has been difficult to achieve adequate potency, selectivity, and pharmacokinetic properties for in vivo imaging using the P6 scaffold.[18] To date, only a very few COX-1-selective inhibitors have been reported. Although a few have been built on benzamide or sulindac sulfide scaffolds,[19−21] most have employed a pyrazole-, thiazole-, triazole-, or isoxazole-containing diaryl heterocycle scaffold similar to that of the COX-2-selective inhibitors, celecoxib, rofecoxib, and valdecoxib (Figure 1).[22−28] Here, we report that the 3,4-diphenylfuran-2(5*H*)-one obtained from desulfurization of rofecoxib exhibits a weak COX-1-selective inhibitory activity. Furthermore, we describe the structure–activity relationships obtained from the modification of that

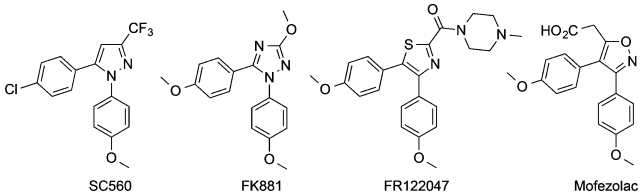


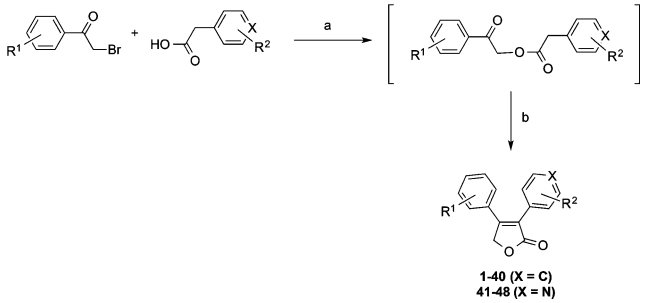
Figure 1. Nitrogen-containing diaryl heterocyclic class of COX-1-selective inhibitors.

Received: August 25, 2014
Accepted: October 12, 2014
Published: October 12, 2014

scaffold to obtain potent and selective fluorine-containing COX-1 inhibitors suitable for use as a prototype for the development of a PET imaging agent.

The key determinant of the COX-2-selectivity of the diaryl heterocycle-based COX-2 inhibitors is the presence of a sulfonamide or a methylsulfone on one of the aromatic rings. This sulfur-containing functional group inserts into a side-pocket in the cyclooxygenase active site that is only accessible in COX-2. Interestingly, the COX-1-selective inhibitor SC-560 was derived from celecoxib via replacement of the sulfonamide group with a methoxy group.[29] Similarly, deletion of the sulfonylmethyl group of rofecoxib affords 3,4-diphenylfuran-2(5*H*)-one (**1**), which exhibits a weak COX-1 inhibitory activity, suggesting that it could serve as a scaffold for the discovery of novel selective COX-1 inhibitors. We employed an efficient one-pot parallel synthetic method for the synthesis of fluorinated 3,4-diarylfuran-2(5*H*)-one derivatives involving condensation of a group of substituted-phenacyl bromides with substituted-phenylacetic acids followed by intramolecular cyclization of the acetate intermediate using 1,8-diazabicyclo[5.4.0]undec-7-ene (Scheme 1).[30] The IC_{50} values

Scheme 1. One-Pot Synthesis of Fluorine-Containing 3,4-Diarylfuran-2(5*H*)-one 1−40 or 3-Pyridyl-4-arylfuran-2(5*H*)-one derivatives 41−48[a]



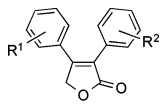
[a]Reagents and conditions: (a) acetonitrile, triethylamine, room temperature, 20 min; (b) 1,8-diazabicyclo[5.4.0]undec-7-ene, room temperature, 20 min.

for inhibition of purified murine COX-2 or ovine COX-1 by test compounds were determined by a thin layer chromatography (TLC)-based assay that measures the conversion of [1-^{14}C]-arachidonic acid to radiolabeled prostaglandins.[13]

The first series of compounds that were synthesized by this approach possessed halogen substituents at the 2-, 3-, or 4-positions of the C-4 phenyl ring of 3,4-diphenyl-2(5*H*)-furanone. Compounds possessing a fluoro substituent at these positions (compounds **2**−**4**) exhibited no COX inhibitory activity. Attachment of methyl, hydroxymethyl, methoxy, dimethylamino, bromo, or chloro substituents to the C-3 phenyl ring of these fluorinated derivatives similarly produced inactive compounds (compounds **5**−**16**, Table 1). Thus, we concluded that compounds bearing a fluoro-substituent on the C-4 phenyl ring of 3,4-diphenyl-2(5*H*)-furanone are inactive as COX inhibitors.

The second series of compounds possessed halogen-containing substituents at the 2-, 3-, or 4-positions of the C-3 phenyl ring and a range of substituents in the para-position of the C-4 phenyl ring of the scaffold (Table 2). Of these, the most potent selective COX-1 inhibitors possessed a 4-methoxy group in the C-4 phenyl ring. Compounds containing this substituent along with a 3-fluoro (**27**), 4-fluoro (**28**), 4-iodo

Table 1. In Vitro Biochemical Properties of 3-Aryl-4-(2-, 3-, or 4-fluorophenyl)-furan-2(5*H*)-one Derivatives



1-16

no.	R^1	R^2	oCOX-1 IC_{50} (μM)[a]	mCOX-2 IC_{50} (μM)[a]
1	H	H	5.90	>25
2	2-F	H	>25	>25
3	3-F	H	>25	>25
4	4-F	H	>25	>25
5	2-F	4-CH_3	>25	>25
6	3-F	4-CH_3	>25	>25
7	4-F	4-CH_3	>25	>25
8	2-F	4-CH_2OH	>25	>25
9	4-F	4-CH_2OH	>25	>25
10	4-F	4-OH	>25	>25
11	2-F	4-$N(CH_3)_2$	>25	>25
12	4-F	4-$N(CH_3)_2$	>25	>25
13	2-F	4-Br	>25	>25
14	4-F	4-Br	>25	>25
15	2-F	4-Cl	>25	>25
16	4-F	4-Cl	>25	>25

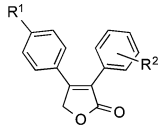
[a]IC_{50} values were determined by incubating several concentrations of inhibitors or DMSO vehicle with purified murine COX-2 (63 nM) or ovine COX-1 (22.5 nM) for 20 min, followed by treatment with [1-^{14}C]-arachidonic acid (50 μM) at 37 °C for 30 s. Assays were run in duplicate.

(**30**), or 3-chloro-2-fluoro (**32**) group in the C-3 phenyl ring all exhibited submicromolar IC_{50}s against COX-1, while residual activity of COX-2 in the presence of 25 μM of the compounds was higher than 50% (IC_{50} > 25 μM). A *p*-bromo-substituted compound (**29**) was also a potent COX-1 inhibitor, but demonstrated some activity against COX-2, while 3- and 4-trifluoromethyl-substituted compounds (**39** and **40**) exhibited weak COX-1-selective activity, and unsubstituted (**25**), 2-fluoro-substituted (**26**), and 4-fluorophenoxy-substituted (**31**) compounds were inactive. Of four compounds bearing no substituent on the C-4 phenyl ring (**17**−**20**), only one, with a 4-fluoro substituent in the C-3 phenyl ring, demonstrated weak COX-1 inhibitory activity. Two out of five compounds (**21**−**24**) bearing a 4-methyl group in the C-4 ring exhibited selective COX-1 inhibitory activity with IC_{50}s in the low micromolar range. These compounds contained 2-fluoro (**21**) and 4-fluoro (**23**) substituents in the C-3 phenyl ring. Compounds bearing a 3-fluoro substituent in the C-3 phenyl ring with 4-cyano (**35**), 4-ethyl (**37**), and 4-hydroxyl (**38**) groups in the C-4 phenyl ring were selective COX-1 inhibitors with a range of IC_{50}s from 0.4 to 10 μM. A single compound bearing a 4-fluoro group in the C-4 phenyl ring and a 4-thiomethyl group in the C-3 phenyl ring (**36**) was inactive.

The third series of compounds possessed a substituted phenyl ring at the C-4 position and a substituted-4-pyridyl ring at the C-3 position on the furanone core. Compounds **41** through **48** were synthesized from the reaction of methyl-, methoxy-, chloro-, or cyano-substituted phenacyl bromides and 2-chloro- or 2-fluoro-4-pyridylacetic acids, followed by a cyclization reaction. These pyridyl analogues showed COX-1-selective inhibition with very low levels of potency (Scheme 1 and Table 3).

dx.doi.org/10.1021/ml500344j | *ACS Med. Chem. Lett.* 2014, 5, 1254−1258

Table 2. In Vitro Biochemical Properties of 3-(2-, 3-, or 4-Fluorophenyl)-4-arylfuran-2(5*H*)-one Derivatives

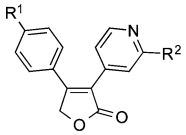


17-40

no.	R^1	R^2	oCOX-1 IC_{50} (μM)[a]	mCOX-2 IC_{50} (μM)[a]
17	H	2-F	>25	>25
18	H	3-F	>25	>25
19	H	4-F	6	>25
20	H	4-OPhF	>25	>25
21	CH_3	2-F	1.00	>25
22	CH_3	3-F	>25	>25
23	CH_3	4-F	0.95	>25
24	CH_3	4-OPhF	>25	>25
25	OCH_3	H	>25	>25
26	OCH_3	2-F	>25	>25
27	OCH_3	3-F	0.36	>25
28	OCH_3	4-F	0.48	>25
29	OCH_3	4-Br	0.12	0.45
30	OCH_3	4-I	0.09	>25
31	OCH_3	4-OPhF	>25	>25
32	OCH_3	2-F, 3-Cl	0.30	>25
33	CF_3	3-F	>25	>25
34	OCF_3	3-F	>25	>25
35	CN	3-F	0.47	>25
36	SCH_3	4-F	>25	>25
37	CH_2CH_3	3-F	9.75	>25
38	OH	3-F	1.75	>25
39	OCH_3	3-CF_3	8.80	>25
40	OCH_3	4-CF_3	1.00	>25
R	SO_2CH_3	H	>25	0.06

[a]IC_{50} values were determined by incubating several concentrations of inhibitors or DMSO vehicle with purified murine COX-2 (63 nM) or ovine COX-1 (22.5 nM) for 20 min followed by treatment with [1-^{14}C]-AA (50 μM) at 37 °C for 30 s. Assays were run in duplicate. Compound **R** is rofecoxib.

Table 3. Biochemical Properties of 3-(2-Chloro or 2-Fluoropyridin-4-yl)-4-arylfuran-2(5*H*)-one Derivatives



41-48

no.	R^1	R^2	oCOX-1 IC_{50} (μM)[a]	mCOX-2 IC_{50} (μM)[a]
41	OCH_3	F	4.60	>25
42	OCH_3	Cl	4.40	>25
43	Cl	Cl	>25	>25
44	Cl	F	15.70	>25
45	CH_3	Cl	5.00	>25
46	CH_3	F	>25	>25
47	CN	F	4.40	>25
48	CN	Cl	3.00	>25

[a]IC_{50} values were determined by incubating several concentrations of inhibitors or DMSO vehicle with purified murine COX-2 (63 nM) or ovine COX-1 (22.5 nM) for 20 min followed by treatment with [1-^{14}C]-AA (50 μM) at 37 °C for 30 s. Assays were run in duplicate.

The ability of the promising fluorine-containing furanone derivatives to inhibit COX-1 and COX-2 in intact cells was evaluated using COX-1-expressing human ovarian cancer cells (OVCAR3) and COX-2-expressing human head and neck squamous cell carcinoma cells (1483 HNSCC).[13,17] Selected compounds were incubated with these cells in the presence of [1-^{14}C]-arachidonic acid, and COX-mediated formation of prostaglandin products was monitored by a TLC assay.[13,17] Compounds **19**, **23**, **27**, and **28** inhibited COX-1 in OVCAR3 cells but not COX-2 in 1483 HNSCC cells (Table 4). Although

Table 4. In Vitro Inhibition of COX-1 in OVCAR3 and COX-2 in 1483 HNSCC Cell Line Assay Data of Promising Compounds

no.	OVCAR3 COX-1 IC_{50} (μM)[a]	1483 HNSCC COX-2 IC_{50} (μM)[a]
19	2.80	>5
23	0.78	>5
27	0.18	>5
28	0.36	>5
30	>4	>5

[a]IC_{50} values were determined as described previously[13,17] for OVCAR3 or 1483 HNSCC cells.

compound **30** inhibited COX-1 in the purified protein assay, it did not inhibit COX-1 in OVCAR3 cells. The remaining fluoro-compounds in Table 2 that exhibit low to moderate COX-1 inhibitory potency and selectivity in the purified COX enzyme assay were not evaluated in the OVCAR3 or 1483 HNSCC cell line assays.

Compound **27** was the most potent of those tested against COX-1 in OVCAR3 cells. We further characterized this compound to determine whether its inhibitory potency is time-dependent. In the standard TLC assay, which includes a 20 min preincubation, **27** exhibited an IC_{50} of 0.36 μM. Elimination of the preincubation resulted in only a small change in potency (IC_{50} of 1.25 μM). Thus, **27** may be an example of a rapid reversible inhibitor of COX-1. We also evaluated the effect of plasma proteins on inhibitor potency in the OVCAR3 cell assay, demonstrating a mild loss of potency when cells were treated with **27** in the presence of 10% FBS (IC_{50} of 0.87 μM) as compared to its potency in the absence of serum (IC_{50} of 0.18 μM).

In conclusion, we describe the SAR of a series of COX-1-selective small molecules, which indicates that the regiochemical disposition of alkyl, thioalkyl, alkoxy, phenoxy, trifluoromethyl, halo, or other substituents on the 3,4-diphenylfuran-2(5*H*)-one core controls COX inhibitory activity, selectivity, and potency. In general, 4-methoxy substitution on the C-4 phenyl ring combined with 3- and 4-substitution with fluorine-containing substituents in the C-3 phenyl ring was the most productive approach to potent and selective COX-1 inhibitors that may serve as prototypes for PET imaging agents. Further work will be required to develop the radiochemistry to incorporate an [^{18}F] label and evaluate the compounds as in vivo imaging agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full synthetic procedures and analytical and spectral characterization data of the synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

dx.doi.org/10.1021/ml500344j | ACS Med. Chem. Lett. 2014, 5, 1254−1258

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This study is supported by research grants from the National Institutes of Health (CA89450, CA136465, and S10 RR019022).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

Spectroscopic analysis of all new molecules was conducted in the Small Molecule NMR Facility and Mass Spectrometry Research Center at the Vanderbilt Institute of Chemical Biology.

ABBREVIATIONS

COX, cyclooxygenase; PET, positron emission tomography; TLC, thin layer chromatography

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