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

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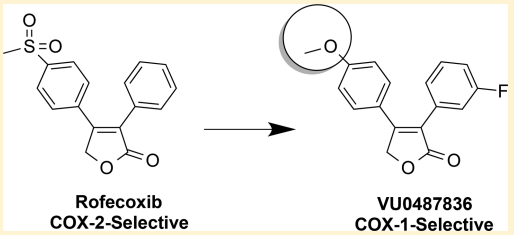
Supporting Information

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

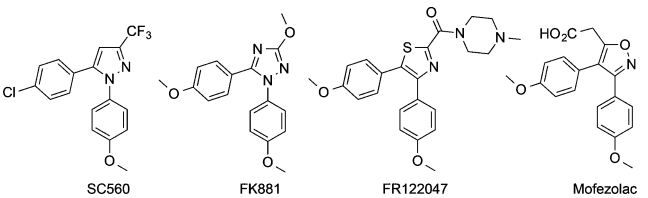


Figure 1. Nitrogen-containing diaryl heterocyclic class of COX-1-selective inhibitors.

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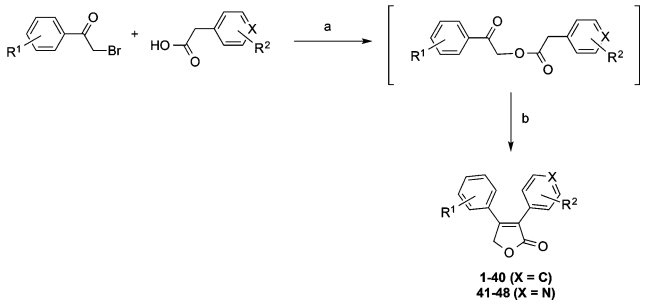
scaffold to obtain potent and selective fluorine-containing COX-1 inhibitors suitable for use as a prototype for the development of a PET imaging agent.

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]



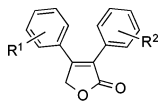
[a]Reagents and conditions: (a) acetonitrile, triethylamine, room temperature, 20 min; (b) 1,8-diazabicyclo[5.4.0]undec-7-ene, room temperature, 20 min.

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).

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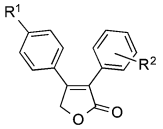
Table 2. In Vitro Biochemical Properties of 3-(2-, 3-, or 4-Fluorophenyl)-4-arylfuran-2(5*H*)-one Derivatives

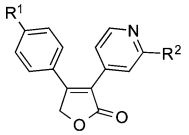


17-40

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 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.

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 **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.

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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.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

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

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