

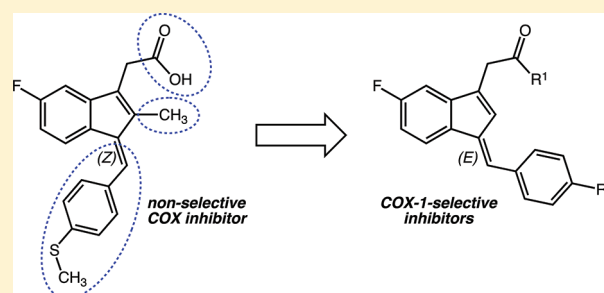
Cyclooxygenase-1-Selective Inhibitors Based on the (*E*)-2'-Des-methyl-sulindac Sulfide Scaffold

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Supporting Information

ABSTRACT: Prostaglandins (PGs) are powerful lipid mediators in many physiological and pathophysiological responses. They are produced by oxidation of arachidonic acid (AA) by cyclooxygenases (COX-1 and COX-2) followed by metabolism of endoperoxide intermediates by terminal PG synthases. PG biosynthesis is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). Specific inhibition of COX-2 has been extensively investigated, but relatively few COX-1-selective inhibitors have been described. Recent reports of a possible contribution of COX-1 in analgesia, neuroinflammation, or carcinogenesis suggest that COX-1 is a potential therapeutic target. We designed, synthesized, and evaluated a series of (*E*)-2'-des-methyl-sulindac sulfide (*E*-DMSS) analogues for inhibition of COX-1. Several potent and selective inhibitors were discovered, and the most promising compounds were active against COX-1 in intact ovarian carcinoma cells (OVCAR-3). The compounds inhibited tumor cell proliferation but only at concentrations >100-fold higher than the concentrations that inhibit COX-1 activity. *E*-DMSS analogues may be useful probes of COX-1 biology in vivo and promising leads for COX-1-targeted therapeutic agents.



INTRODUCTION

Cyclooxygenases (COX-1 and COX-2) catalyze the oxygenation of arachidonic acid (AA) to form prostaglandins and thromboxane, which mediate a range of physiological and pathophysiological responses.¹ The discovery in 1971 that nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit prostaglandin synthesis in guinea pig lung and human platelets established COX-1 as a molecular target for this ancient class of drugs.^{2–4} The subsequent discovery in 1991 of COX-2, which is induced by cytokines during inflammation, suggested that this form of the enzyme represents the molecular target for the anti-inflammatory effects of NSAIDs.^{5–8} The differential expression of COX-1 and COX-2, and in particular the finding that COX-1 is the principal form expressed in the gastrointestinal tract, led to the search for COX-2-selective inhibitors as potential anti-inflammatory agents that might lack the gastrointestinal side effects of traditional NSAIDs.^{9–11} A number of highly selective inhibitors were introduced to the market including celecoxib (6), rofecoxib (7), valdecoxib, etoricoxib, and lumiracoxib (Figure 1).^{12–17} These compounds exhibited reduced gastrointestinal side effects in human testing but did not completely eliminate this liability.^{18,19} In addition, long-term placebo-controlled studies revealed cardiovascular side effects that led to the withdrawal of rofecoxib and valdecoxib from the market in the United States and Europe.^{20–23}

The “COX-2” hypothesis posited that COX-1 inhibition is responsible for the gastrointestinal toxicity of NSAIDs. However, animal studies suggest that the total extent of COX inhibition is more important than the inhibition of the individual enzymes and that highly selective COX-1 inhibitors do not exhibit gastrointestinal toxicity analogous to highly selective COX-2 inhibitors.^{24,25} COX-1-dependent prostaglandin synthesis has been implicated in many pathophysiological processes including atherosclerosis, endothelial dysfunction, neuroinflammation, preterm labor, pain, and cancer.^{26,27} The role of COX-1 in neurodegenerative diseases is of particular interest. Neurodegenerative disorders that exhibit a strong inflammatory component produce prostaglandins (PGs) in neurons and glial cells.²⁸ The extent of neurodegeneration is reduced in COX-1 knockout mice or by administration of COX-1-selective inhibitors.^{29–31} Very recent results indicate that COX-1 is a major source of pro-inflammatory PGs in the brains of both LPS-treated mice and mice treated with the Parkinsonism-inducing compound, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.³² Therefore, COX-1-selective inhibitors represent a potentially useful class of drugs that has not been extensively investigated.

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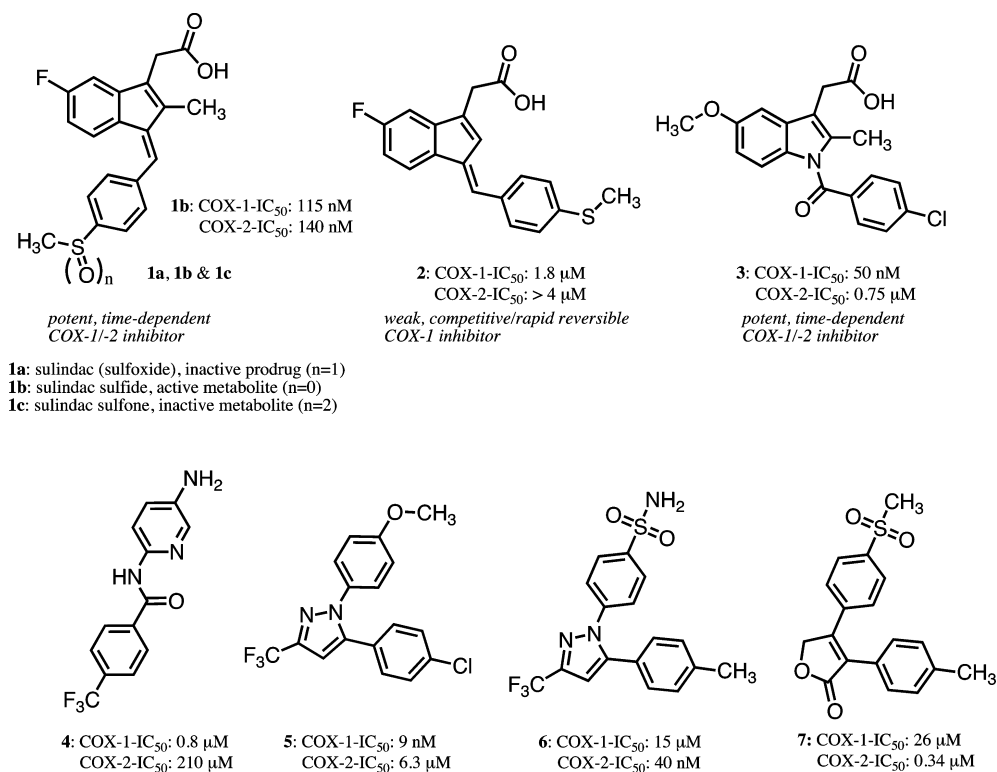


Figure 1. Upper row: the structures of sulindac (sulfoxide, **1a**), its *in vivo* metabolites (**1b** and **1c**), and 2'-*des*-methyl sulindac sulfide (**2**) as well as indomethacin (**3**, nonselective COX-1/-2 inhibitor). Lower row: *N*-(5-amino-2-pyridinyl)-4-(trifluoromethyl) benzamide (TFAP, **4**), SC-560 (**5**) (selective COX-1 inhibitors), celecoxib (**6**), and rofecoxib (**7**) (selective COX-2 inhibitors).

The COX-1 inhibitors that have been described in the literature fall into two main structural classes.²⁶ One class comprises diarylheterocycles discovered during the COX-2 inhibitor programs that led to the development of **6** and **7** (Figure 1). Noteworthy among this class of inhibitors is SC-560 (**5**, Figure 1), which exhibits COX-1 selectivity greater than 700-fold and which has been extensively used as an *in vitro* and *in vivo* probe, for example, for investigating inhibition of PGE₂ synthesis in rat primary spinal cord neurons or in (mouse) models of human ovarian cancer (OVCAR-3).^{33–36} The other class of COX-1 inhibitors comprises substituted benzamides related to *N*-(5-amino-2-pyridinyl)-4-(trifluoromethyl)-benzamide (TFAP, **4**; Figure 1).^{25,37} This compound exhibits high COX-1 selectivity but has not been used extensively as a probe. TFAP and its analogues have been suggested as candidates for analgesics that do not cause stomach erosions or ulcers.²⁵ Other compounds related to resveratrol, a noncompetitive COX inhibitor with anti-inflammatory, cardiovascular protective, and cancer chemopreventive properties, and curcumin, the active component of turmeric that possesses anti-inflammatory and anticancer activity, are reported to exhibit modest COX-1 selectivity. A recent study of 92 stilbene analogues, whose biological activities were assessed with various chemoprevention targets, revealed a handful of nitro- or amino-substituted resveratrol derivatives that inhibit COX-1 with potency roughly comparable to the parent compound but without affecting COX-2.³⁹ Finally, valeryl salicylate, a derivative of aspirin, selectively inhibits COX-1 by covalent modification of an active site serine residue.^{26,38} Biological effects of valeryl salicylate have been, for example, studied in models of acute inflammation in mice.³⁹

Sulindac (compound **1a**, Figure 1) is an NSAID that exhibits cancer chemopreventive activity in animal models, induces polyp regression in familial polyposis patients, and inhibits polyp recurrence in patients when combined with α -difluoromethylornithine.^{40–46} Sulindac is a pro-drug that is reduced to the active sulfide metabolite by colonic microflora.⁴⁷ Sulindac sulfide (compound **1b**, Figure 1) is a slow, tight-binding inhibitor of both COX-1 and COX-2. We recently reported that the 2'-*des*-methyl derivative of **1b** (compound **2**, Figure 1) does not inhibit COX-2 but selectively inhibits COX-1 (Table 1 and Figure S5 in the Supporting Information).⁴⁸ The absence of the 2'-methyl group during indene synthesis quantitatively directs the geometry of the introduced benzylidene double bond to the (*E*) rather than (*Z*), which is formed when the 2'-methyl group is present;⁴⁹ compound **2** is a rapid, reversible inhibitor of COX-1. The novelty of this scaffold prompted us to investigate its potential for the construction of more potent and selective COX-1 inhibitors.

In the present manuscript, we describe the synthesis of a series of compounds designed to probe the importance of the various functionalities in **2** with regard to selective inhibition of COX-1. We find that modification of the benzylidene group leads to improved inhibitors, but alterations in the 5'-fluoro or carboxyl group are less well tolerated. The most active compounds are potent inhibitors of COX-1 in cultured human ovarian cells but are weak inhibitors of the growth of the cells *in vitro*.

RESULTS

The indene template is a widely used pharmacophore that has found many applications in medicinal chemistry, for example, in NSAIDs.^{50–53} Recent reports have indicated that the

Table 1. COX-1 and COX-2 Inhibition by Arylmethylidene Variants of *E*-DMSS*

Compd #	R	oCOX-1	mCOX-2	Compd #	R	oCOX-1	mCOX-2
		%-inhibition @ 4 μ M ^a	%-inhibition @ 4 μ M ^a			%-inhibition @ 4 μ M ^a	%-inhibition @ 4 μ M ^a
<i>benzylidene derivatives</i>				<i>benzylidene derivatives</i>			
2		65 ± 9 (4)	10 ± 6 (4)	11m		68 ± 2	20 ± 3
11a		27 ± 8	8.5 ± 2	11n		43 ± 4 (4)	0 ± 2 (4)
11b		n.d.	n.d.	<i>naphthylmethylidene- and biphenylmethylidene derivatives</i>			
11c		77 ± 2	35 ± 2	12a		75 ± 1	34 ± 5
11d		51 ± 1	15 ± 8	12b		58 ± 6 (4)	16 ± 3 (4)
11e		34 ± 3	16 ± 0	12c		31 ± 7	29 ± 15
11f		74 ± 2	34 ± 1	12d		53 ± 4	7 ± 4
11g		52 ± 2 (4)	13 ± 5 (4)	12e		61 ± 5	22 ± 9
11h		81 ± 9 (4)	60 ± 3 (4)	12f		51 ± 9 (4)	0 ± 1 (4)
11i		73 ± 1 (4)	34 ± 5 (4)	12g		14	42
11j		62 ± 9	17 ± 3	13a		75 ± 8 (8)	1 ± 7 (6)
11k		62 ± 10	22 ± 9	13b		68 ± 5 (4)	18 ± 7 (4)
11l		17 ± 5	5 ± 5				

*Compounds were screened at 4 μ M. A concentration of 5 μ M of AA-substrate was employed; n.d., not determined. ^aMean \pm SEM of two (*n*) experiments.

benzylidene-indene acetic acid derivative **1b** can be structurally re-engineered to improve isoform-specific COX binding inter alia.^{43,54,55} In the present study, compound **2**, the *E*-2'-des-

methyl-sulindac sulfide [(*E*)-DMSS] analogue of **1b**, served as the lead scaffold for construction of potential COX-1-selective inhibitors. Modifications were made at the carboxyl, benzyli-

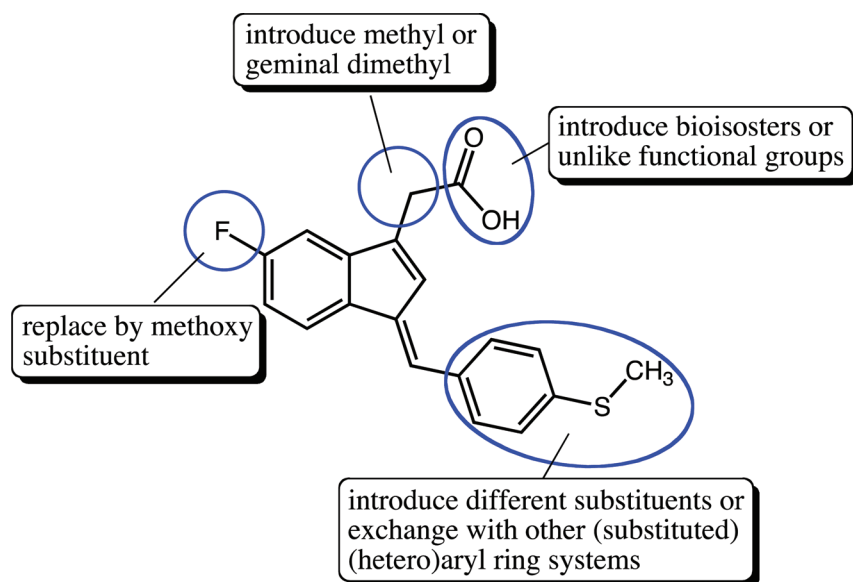
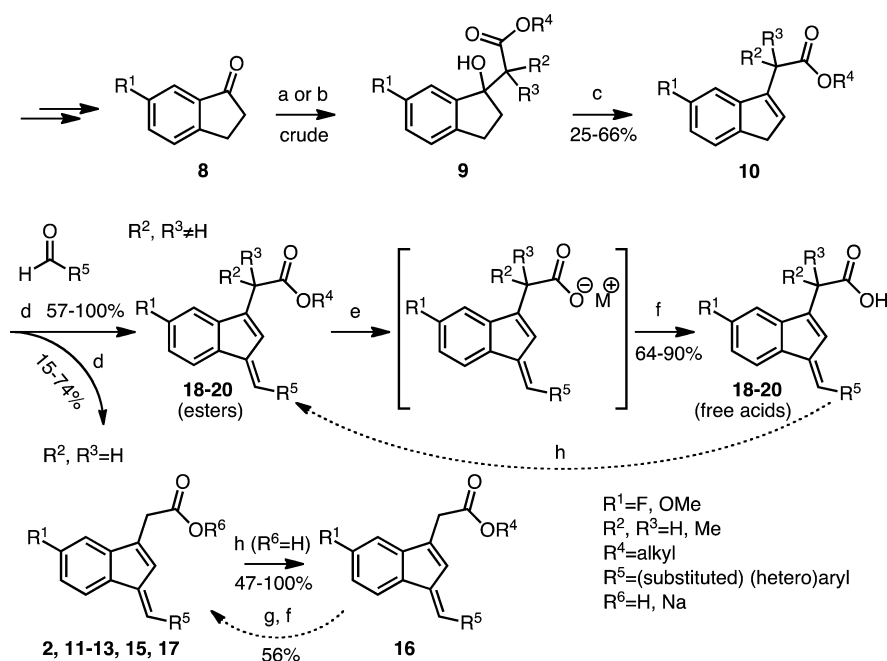


Figure 2. Intended chemical modifications on 2 and 13.

Scheme 1. General Synthetic Route to Multisubstituted (*E*)-2'-Des-methyl Sulindac Sulfide Analogues Including Procedures for Ester Cleavage and (Re)Esterification Reactions^a



^aReagents and conditions: (a) For compound 2: ethyl bromoacetate, activated zinc dust, benzene/ether, reflux, 16 h. (b) 2 M LDA, THF, (substituted) ethyl alkanolate, -75°C , 2 h. (c) TsOH \cdot 2H₂O, CaCl₂ \cdot 2H₂O, toluene, reflux, overnight. (d) 1 N NaOH, MeOH, 65–70 $^{\circ}\text{C}$. (e) Potassium trimethylsiloate (90% material), THF, microwave radiation, 90–120 $^{\circ}\text{C}$, 15 min. (f) Water, 2–4 M HCl. (g) 1 M LiOH, THF, RT, 5–6 h. (h) Alkyl alcohol, H₂SO₄, reflux, overnight.

dene, and 5'-position of the indene ring (Figure 2). The synthetic scheme followed the general approaches previously described for assembly of sulindac analogues with some modifications (Scheme 1).^{53,56} In contrast to the standard procedures, which employ "activated" zinc and expensive α -haloalkanoyl esters, we reacted the indanone precursors, 8, with inexpensive ethyl alkanolates at low temperatures using lithium diisopropylamide in tetrahydrofuran.⁵⁷ This method generated the intermediate alcohols, 9, after an acidic quench in a much shorter time (2 h). The quantities of side products detected by

HPLC were less than with the lengthy Reformatsky reaction (overnight with Zn⁰ catalyst).⁵⁸ The applied alkylation reaction tolerated different substituents at the core aromatic annulus (e.g., 6'-F, 6'-OCH₃) and allowed the introduction of α -unsubstituted (9a,b), monomethylated (9c), or geminal-dimethylated (9d) carboxymethyl moieties at position 1 of the indanone ring. Compounds 9a, 9c, and 9d hold a 6'-F substituent at the 1-hydroxy-2,3-dihydro-1H-inden-1-yl partial structure, whereas 9b has a methoxy group at its 6'-position. HPLC analysis of 9a, 9b, and 9d, which contain a single

asymmetric carbon, afforded a single peak, whereas compound **9c**, which has two chiral centers, afforded two substantial peaks due to the presence of diastereomers. Attempts to prepare a cyclopropyl analogue using ethyl cyclopropanecarboxylate as an alkylation reagent failed due to the instability of the intermediate cycloalkyl carbanion.^{59,60}

Dehydration of **9a** and **9b** produced a mixture of *exo*- and *endo*-olefins for both **10a** and **10b** (Table S1 in the Supporting Information).^{50,57} Steric hindrance from the exocyclic alkyl chain of the racemic mixture **10c** favored conversion to the *endo*-olefin as indicated by HPLC analysis and by comparison to the UV spectrum of **10d**, which cannot exist in the *exo*-form (Table S1 in the Supporting Information). Unseparated isomeric mixtures of ethyl esters, **10a–c**, were employed in the subsequent condensation step. Knoevenagel (aldol) condensation between **10a–d** and the appropriate aldehydes proceeded under basic conditions to afford a single *endo*-olefin in all cases. Considerable amounts of byproduct, most likely from the Cannizzaro reaction, could be detected by HPLC, MS, and NMR. Together with the recognized light instability of the *E*-DMSS analogues (UV-induced isomerization about the exocyclic double bond from *E* to *Z*), this complicated the purification process and decreased the isolatable yields for some compounds.^{48,50,61} The basic conditions of the condensation led to ester hydrolysis for compounds derived from **10a** and **10b**. In addition, the lipophilic nature of the final *E*-DMSS analogues occasionally allowed their quantitative isolation as pure sodium salts directly from the aqueous-alkaline mother liquor (Table S2 in the Supporting Information). The acids could be esterified with alcohols under acidic conditions and hydrolyzed with LiOH.⁵⁵ Hydrolysis was not observed for compounds derived from **10c** and **10d**. The ester products either crystallized from the reaction mixture or were extracted and purified by chromatography. The stereochemistry of the exocyclic double bond of all of the *E*-DMSS analogues was *E* as anticipated by the absence of a methyl group at the 2'-position of the indenyl ring (compare ¹H NMR and NOESY spectrum of **20b** in Figures S2 and S3 in the Supporting Information).⁴⁸ Detailed experimental conditions and spectral properties of all intermediate and final compounds are provided in the Supporting Information.

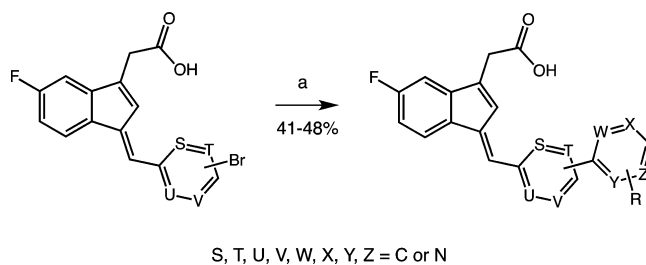
Individual compounds were preincubated with purified ovine COX-1 or murine COX-2 for 17 min followed by addition of [1-¹⁴C]-AA (Figure S4 in the Supporting Information: experimental timeline). After 3 min, the reaction was quenched, and radioactive products were extracted and quantified as described in Supporting Information. For initial screening, a single concentration of 4 μM inhibitor was employed, and the concentration of [1-¹⁴C]-AA was 5 μM. The 4 μM inhibitor concentration was chosen based on the previously determined IC₅₀ of 1.8 μM for compound **2**. The AA concentration of 5 μM represents the K_m for COX-1 and COX-2, which enables rapid, reversible, and slow, tight-binding inhibitors to be detected. Compound **2** was used as a reference for all new *E*-DMSS analogues.

Table 1 lists the percent inhibition of COX for the initial series of arylmethylidene analogues of **2**.⁵¹ We determined the effects of different steric and electronic properties upon COX-1 and COX-2 inactivation. Oxidation of the sulfide (**11a** and **11b**) reduces activity as previously reported for sulindac sulfide analogues. Substitution with trifluoromethylsulfide (**11c**) maintains some COX-1 selectivity, while oxidation of the sulfur again reduces activity (**11d** and **11e**). A range of *para*

substituents exhibited modest COX-1 selectivity (**11f–m**). Substitution of carboxyl at the *para* position reduced the potency against COX-1 but more substantially reduced inhibition of COX-2. Conversion of the phenyl ring to a naphthyl or aza-naphthyl ring maintained COX-1 selectivity and in some cases increased it (**12d** and **12f**). The most potent and selective COX-1 inhibitor in this series was the biphenyl analogue **13a** (COX-1 IC₅₀, 570 nM; COX-2 IC₅₀ > 4 μM). Such hydrophobic biphenyl systems are a common structure template seen in other small molecule inhibitors of the AA pathway (e.g., flurbiprofen [NSAID] or MK-866 analogues [microsomal prostaglandin E₂ synthase-1 (mPGES-1) inhibitors]).^{60,62} Concentration dependences were determined for the most potent compounds, which led to the determination of IC₅₀ values for a subset of the inhibitors (Table 1 and Figure S5 and Table S4 in the Supporting Information).

To build on the discovery of **13a**, a series of substituted biphenyls were synthesized by either Knoevenagel condensation or Suzuki–Miyaura coupling of brominated benzylidene precursors with (hetero)aryl boronic acids (e.g., **13f** and **13k**; Scheme 2 and Figure S1 in the Supporting Information).⁶³

Scheme 2. Synthesis of New “Biphenyl Derivatives” of 2'-Des-methyl Sulindac Sulfide Using Suzuki–Miyaura Coupling Reactions^a

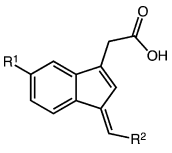


^aReagents and conditions: Pd(PPh₃)₄, K⁺-carbonate, DME/H₂O, 90 °C, 14 h. Suzuki–Miyaura couplings were performed on submillimolar scale in a sealed glass tube under argon.⁶³

Evaluation of this series (Table 2) indicated that multiple substitutions were tolerated, although none dramatically increased either the potency or the selectivity of COX-1 inhibition over compound **13a**. Interestingly, introduction of a 2-aza substituent into a *meta*-substituted biphenyl scaffold reversed the selectivity of inhibition, yielding compounds with modest COX-2 selectivity (**17a–e**; compare Figure S6 in the Supporting Information).

Neutralization of the carboxylic acid by conversion to an ester or amide in the benzylidene series reduced potency but maintained selectivity for COX-1, except for the biphenyl methylidene analogue **16e**, which revealed potent anti-COX-1 activity but also increased COX-2 inhibition (Table 3). No significant differences were observed on changing the ester from methyl to isopropyl (**16a–c**).⁶⁴

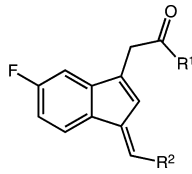
Substitution α - to the carboxyl was explored by synthesis of a series of esters and acids (Table 4). The general route outlined in Scheme 1 was employed using ethyl esters of the indene acetic acids, **10c** and **10d**, as building blocks. It was not possible to make a complete series of monomethyl-substituted analogues because of low yields and the generation of multiple side products (Scheme S1 in the Supporting Information). The only monomethyl analogue prepared was the ethyl ester, **18a** (Table 4), which demonstrated reduced COX-1 inhibitory

Table 2. COX-1 and COX-2 Inhibition by Substituted Biphenylmethylidene Derivatives of *E*-DMSS*


Compd #	R ¹	R ²	oCOX-1 %-inhibition @ 4 μM ^a	mCOX-2 %-inhibition @ 4 μM ^a
13c	F		70 ± 1	26 ± 9
13d	F		44 ± 2	34 ± 0
13e	F		33 ± 1	16 ± 1
13f	F		31 ± 3	15 (1)
13g	F		55 ± 8	5 ± 2
13h	F		35 ± 0	8 (1)
13i	F		45 ± 4	25 ± 2
13j	F		19 ± 6	19 ± 2
13k	F		27 ± 2	10 ± 6
17a	F		17 ± 8 (4)	81 ± 6 (4)
17b	F		35 ± 3	56 ± 7
17c	F		59 ± 11	43 ± 14
17d	F		13 ± 6 (5)	54 ± 13 (3)
17e	F		15 (1)	28 (1)

Table 2. continued

* Compounds were screened at 4 μM. A concentration of 5 μM of AA-substrate was employed. ^aMean ± SEM of two (*n*) experiments.

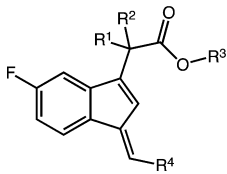
Table 3. COX-1 and COX-2 Inhibition by Ester and Amide Derivatives of *E*-DMSS*


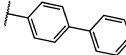
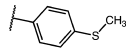
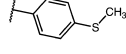
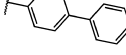
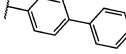
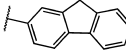
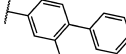
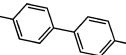
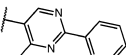
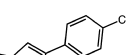
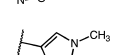
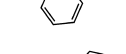
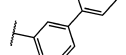
Compd #	R ¹	R ²	oCOX-1 %-inhibition @ 4 μM ^a	mCOX-2 %-inhibition @ 4 μM ^a
14	NH ₂		21 ± 1	5 ± 5
16a	Me		39 ± 2	9 ± 0
16b	Et		20 ± 7	0 ± 0
16c	<i>i</i> -Pr		27 ± 0	0 ± 11
16d	Et		39 ± 8	0 ± 3
16e	Et		64 ± 5	28 ± 7

* Compounds were screened at 4 μM. A concentration of 5 μM of AA-substrate was employed. ^aMean ± SEM of two (*n*) experiments.

activity (30% inhibition at 4 μM) as compared to the ethyl ester of the unsubstituted biphenyl methylidene compound, **16e** (COX-1 IC₅₀, 1.1 μM) (Table 3 and Figure 7).⁶⁵ Dimethyl analogues were prepared in high yield with minimal side products. The steric hindrance introduced by α,α-disubstitutions dramatically reduced the ease of hydrolysis to the acids. Thus, a nonaqueous hydrolytic strategy was employed in which THF solutions of individual esters were heated in a microwave with potassium trimethylsilanoate (Scheme 1).⁶⁶ This afforded nearly quantitative conversion of the esters to the acids. The two initially prepared acids, **19a** and **19c**, were less active than the unsubstituted free acids, **2** and **13a** (Table 1), so no further acids were synthesized. The ethyl esters of the α,α-disubstituted compounds, **19b** and **19d–20b** (Table 4) retained COX-1 selectivity but exhibited reduced potency. The most selective inhibitor was the oxazole, **19i**.

A series of substituted alkyl- and arylsulfonimides as carboxylic acid isosteres were synthesized as depicted in Scheme 3.^{60,67,68} Bioisosteric replacements represent a common approach for the rational modification of lead compounds into safer and more clinically effective agents with similar biological properties to the parent compound. Moderate COX-1 selectivity was observed for compounds **21a**, **21b**, and **21d–n** (Table 5), but the trifluoromethylsulfonimide, **21c**, with biphenylmethylidene substituent, was the most potent and selective compared of all of the analogues

Table 4. COX-1 and COX-2 Inhibition by α -Substituted *E*-DMSS Analogues*


Compd #	R ¹	R ²	R ³	R ⁴	α COX-1 %-inhibition @ 4 μ M ^a	mCOX-2 %-inhibition @ 4 μ M ^a
alpha-monomethylated acetic acid ester						
18a	Me(H)	H(Me)	Et		30 \pm 4	0 \pm 0
alpha-dimethylated acetic acids and esters						
19a	Me	Me	H		17 \pm 2 (4)	16 \pm 7
19b	Me	Me	Et		43 \pm 3	0 \pm 1
19c	Me	Me	H		58 \pm 6 (3)	8.5 \pm 4 (4)
19d	Me	Me	Et		26 \pm 3	2 \pm 5
19e	Me	Me	Et		34 (1)	18 \pm 1
19f	Me	Me	Et		40 \pm 2	15 \pm 6
19g	Me	Me	Et		10 \pm 2 (4)	0 \pm 3
19h	Me	Me	Et		25 \pm 5 (4)	9 \pm 10
19i	Me	Me	Et		37 \pm 2	0 \pm 1
19j	Me	Me	Et		47 \pm 1	22 \pm 7
20a	Me	Me	Et		24 \pm 2	4 \pm 6
20b	Me	Me	Et		24 \pm 10	21 \pm 0

*Compounds were screened at 4 μ M. A concentration of 5 μ M of AA-substrate was employed. ^aMean \pm SEM of two (*n*) experiments.

synthesized in the present study (COX-1 IC₅₀, 470 nM; COX-2 IC₅₀, 14 μ M; compare Figure S6 in the Supporting Information). This meant that it exhibited a more than 4-fold improvement in activity against COX-1 as compared with our early lead and reference compound **2**. Analogue **21c** was approximately 30-fold more selective for COX-1 over COX-2.

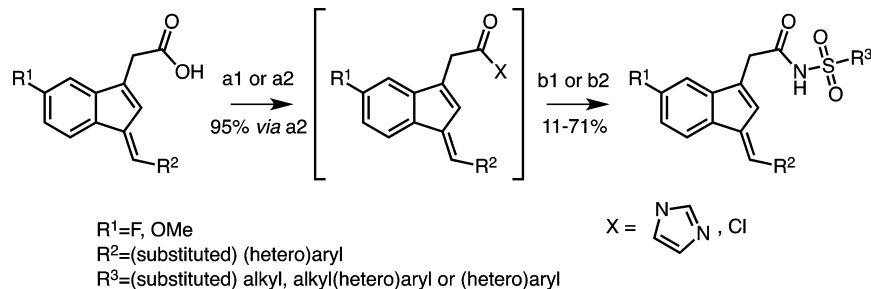
Substitution of methoxy for fluoro at the indene 5'-position in analogues **15a** and **15b**, respectively (like in compound **3**), led to a substantial loss of inhibitory potency for both the phenylmethylsulfide and the biphenyl derivatives (Figure 3 and Table S3 in the Supporting Information).⁶⁹ The differential was most dramatic for the phenylmethylsulfide derivative, **15a**, which was a poor COX-1 inhibitor. The 5'-methoxy derivative of the biphenyl, **15b**, exhibited modest COX-1 inhibitory activity, although the presence of a plateau at higher inhibitor concentrations suggests that inhibition is readily reversible (also see Figures S7 and S8 in the Supporting Information).⁴⁹

The ability of the various *des*-methylsulindac sulfide analogues to inhibit COX-1 activity in intact cells was evaluated using the human ovarian cancer cell line, NIH-OVCAR-3.^{35,36} This cell line expresses high levels of COX-1 but no detectable COX-2 as demonstrated by the Western blot in Figure 4a.³⁶ Individual compounds were incubated with the cells for 30 min followed by the addition of [1-¹⁴C]-AA and another 30 min incubation. Compound **2**, **13a**, and **21c** inhibited COX-1-dependent [1-¹⁴C]-AA oxidation with potencies comparable to that of the known COX-1 inhibitor, SC-560 (compound **5**).³³ Minimal inhibition of COX-1 in OVCAR-3 cells was detected with celecoxib (compound **6**) (Figure 4a). When parallel experiments were conducted in the COX-2 expressing human head and neck cancer cell line, 1483, no inhibition of [1-¹⁴C]-AA oxygenation was observed by **2** and **13a**, whereas **6** potently inhibited oxygenation (IC₅₀ = 54 nM) (Figure 4b).

Sulindac (compound **1a**) and sulindac sulfide (compound **1b**) inhibit the growth of tumor cells in culture, and there has been some controversy about the role of COX inhibition (especially COX-2 inhibition) in this antiproliferative activity.^{43,70–75} Therefore, we evaluated the effect of **2**, **13a**, **1b**, and **1a** on the viability of OVCAR-3 cells (Figure 5 and Supporting Information).⁷⁶ Following a 2 day treatment, comparable EC₅₀ values for inhibition of cell growth were observed with **2** (223 μ M), **13a** (132 μ M), and **1a** (210 μ M), but no inhibition was observed with **1b** up to 250 μ M. Comparison of Figures 4a and 5 reveals that inhibition of cell growth requires concentrations of **2** and **13a** that are more than 100-fold higher than the concentrations required for inhibition of COX-1 activity in the OVCAR-3 cells. Therefore, it appears that the antiproliferative effects of this series of compounds on OVCAR-3 cells grown in culture are independent of their ability to inhibit COX-1 activity. Evaluation of sulindac sulfide and compounds **13a** and **21b** in the breast cancer cell line, MDA-MB231, yielded EC₅₀ values of 209, 141, and 215 μ M (Figure S11 in the Supporting Information).

DISCUSSION

Figure 6 summarizes the structure–activity relationships (SARs) for the series of compounds evaluated in the present study. Increasing the size and hydrophobicity of the aryl group from methylsulfanylbenzylidene to biphenylmethylidene significantly improved the potency and selectivity of COX-1 inhibition, so a number of analogues were made (Figure 7). Substitutions on the biphenyl ring (e.g., fluoro, trifluoromethyl) did not increase potency. Introduction of nitrogen into either biphenyl ring to increase hydrophilicity decreased potency, and the presence of multiple nitrogens in one ring reduced it further. Fusing the biphenyl (fluorene) retained potency against COX-1 but introduced a higher activity against COX-2. Alkyl substitution α - to the carboxyl group reduced activity in all cases. Conversion of the carboxylic acid to an ester or amide

Scheme 3. Preparation of Sulfonimide Derivatives of 2'-Des-methyl Sulindac Sulfide^a

^aSyntheses were performed on a parallel synthesis apparatus using a straightforward one-pot, two-step CDI carbodiimide coupling reaction (route A).⁶⁷ Alternatively, the two-step sulfonamide couplings could be accomplished with oxalyl chloride as the carboxylic acid activator, according to a disclosed procedure, as established for compound **21c** (route B).^{65,86,87} Reagents and conditions: (Route A): (a1) CDI/DCM, 0–5 °C, 2 h; (b1) alkyl-/arylsulfonamide, DBU, RT, overnight. (Route B): (a2) oxalyl chloride/DCM, RT, overnight; (b2) alkyl-/arylsulfonamide, 1,2-DCE, pyridine, RT, overnight.

derivative significantly reduced potency, but introduction of the sulfonimide isostere in place of the carboxylate generated the most potent and selective COX-1 inhibitor in the series. Substitution on the sulfanyl sulfur was tolerated from methyl to substituted aryl. Perhaps the most surprising result was the finding of significantly reduced activity on substitution of a 5'-methoxy for the 5'-fluoro.

The *in vitro* screen utilized ovine COX-1 and mouse COX-2. Ovine COX-1 is the closest in sequence to human COX-1 among several mammalian species and is commonly used as a surrogate for human COX-1.^{77–79} The human enzyme has proven difficult to routinely express and purify with high activity. To verify the *in vitro* results, we tested the most active compounds against human COX-1 in cultured human ovarian cancer cells, OVCAR-3, and against human COX-2 in cultured head and neck cancer cells, 1483. All of the compounds inhibited COX-1 in the OVCAR cells to an extent that was comparable to that of the established COX-1 inhibitor, **5**. None of the compounds inhibited COX-2 in the 1483 cells. In contrast, **6** potently inhibited COX-2 in the 1483 cells but had no effect on COX-1 in the OVCAR-3 cells. Thus, the lead compounds identified in the *in vitro* screen were effective inhibitors of human COX-1 in intact cells.

There was a dramatic differential in the ability of the lead compounds to inhibit PG synthesis by COX-1 in the OVCAR-3 cells and their ability to reduce proliferation in the same cell type. Whereas complete COX-1 inhibition was observed below 1 μM , none of the *E*-DMSS analogues reduced proliferation below 50 μM , and the EC₅₀ values were on the order of 130–220 μM (Figures S9a,b and S10 in the Supporting Information). Thus, it appears that the ability of compound **1b** and the analogues described herein to inhibit proliferation is independent of their ability to inhibit COX-1. Because these experiments were performed in cell culture, one cannot conclude that COX-1 inhibition would not inhibit tumor growth *in vivo*.^{74,80} Tumor growth and metastasis are complex processes involving multiple steps, such as angiogenesis, motility, invasion etc., which are not components of growth *in vitro*.^{36,81} Indeed, literature compound **5** inhibits tumor growth in different mouse models of ovarian carcinoma.^{35,82} Thus, *E*-DMSS analogues may have antitumor activity as well.⁷⁶

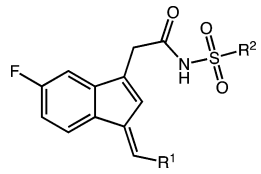
Compound **3** (Figure 1) is a structurally related COX inhibitor to **1b** (and **2**) and was used for modeling of COX-1 inhibitor interactions.²⁵ In **3**, the *p*-chlorobenzoyl group at the indole nitrogen can rotate around the connecting σ -bond. This

allows the molecule to take up an *S-trans*-conformation, besides its preferred and lower energy *S-cis*-conformation, to interact with COX. Whereas the *cis* conformer of **3** had been found to bind to both COX forms, the *trans* conformer was only present in cocrystal complexes with COX-1.²⁵ This *trans*-conformation of **3** emulates the molecule geometry of the rigid *E*-2'-DMSS derivatives described in the present study (see Figure 1).

The nearly identical 3D structures of the two COX isoforms constitute a substantial obstacle for the design of selective COX-1 inhibitors.^{78,83} This is particularly true because in COX-1, the cavity of the selectivity pocket is less accessible due to the presence of Ile523. The same cavity is more spacious in COX-2, having a Val at the same amino acid position.¹ It is assumed that compound **2** and its analogues described herein exert an atypical binding mode with COX, as the orientation of the *p*-methylsulfanylphenylidene group is predicted to cause steric clashes when the compounds are modeled into a similar configuration as **1b** or **3** in the active site.⁸⁴ Unfortunately, the attempt to cocrystallize one of the more potent *E*-DMSS analogues (**2** or **13a**) together with oCOX-1 has not succeeded thus far.⁸³

There has been a report proposing an optional binding mode of COX-1-selective inhibitors derived from **3**.⁸⁴ Moreover, a recent docking study on the COX binding of 2-methyl-3-indolylacetic acid derivatives that preferentially inactivate COX-1 suggested that the binding specificity of the reported compounds resulted from individual plasticities of the 3D protein structure close to the binding site (“selective induced fit”).⁸⁵ These data further point out the formation of a new lipophilic interaction site by COX-1 structural rearrangements triggered by the ligand that favors a boost in inhibitory activity for accurately fitting compounds. The less flexible COX-2 protein does not allow such conformational changes to take place and thus confines binding affinity.⁸⁵ This hypothesis could be taken into consideration to rationalize the benefit of the rather bulky and lipophilic biphenyl substituent as part of our optimized COX-1 selective *E*-DMSS analogues (e.g., **13a** or **21c**).

COX-1 has emerged as a potential pharmacological target for the treatment of several clinical conditions, and preclinical and clinical data suggest that selective COX-1 inhibitors may not exhibit significant gastrointestinal and cardiovascular side effects.^{24,25,56} Thus, the *E*-DMSS analogues described in the present report may be useful leads for novel COX-1 inhibitors or useful probes for the involvement of COX-1 in physiological

Table 5. COX-1 and COX-2 Inhibition by Sulfonimide Derivatives of *E*-DMSS*


Compd #	R ¹	R ²	oCOX-1 %-inhibition @ 4 μM ^a	mCOX-2 %-inhibition @ 4 μM ^a
21a		Me	21 ± 8 (4)	7 ± 10 (4)
21b		CF ₃	65 ± 8 (6)	15 ± 5 (4)
21c		CF ₃	99 ± 6	15 ± 6
21d		2-tolyl	60 ± 8 (4)	36 ± 5
21e		4-tolyl	55 ± 8 (4)	20 ± 5 (4)
21f		2-naphthyl	74 ± 5 (4)	26 ± 4 (3)
21g			44 ± 3	20 ± 2
21h			48 ± 2	19 ± 4
21i			(47) ± 1	32 ± 11
21j			59 ± 6 (4)	34 ± 10 (4)
21k			40 ± 4 (3)	(30) (1)
21l			3.5 ± 8 (3)	26 ± 3
21m			45 ± 4	2 ± 4
21n			58 ± 1	8 ± 1

*Differently substituted alkyl- and arylsulfonimides (21a–n) were prepared as bisoesters of 2 and 13a, respectively, to alter acidity or modify lipophilicity without making significant changes to the core structure or affecting pK_a (Scheme 3; see also the Experimental Section). Compounds were screened at 4 μM. A concentration of 5 μM of AA-substrate was employed. ^aMean ± SEM of two (n) experiments.

or pathophysiological processes. However, some of the inhibitors may have off-target effects that would limit their utility in vivo. For example, compound 13a is a potent activator of peroxisome proliferator-activated receptor (PPAR) γ in cell culture.⁵¹ Interestingly, the SAR for activation of PPAR γ indicates that a free carboxyl group is absolutely required for activity. Thus, analogues such as the trifluoromethylsulfonimide, 21c, may be more useful as probes for COX-1 inhibition in vivo.

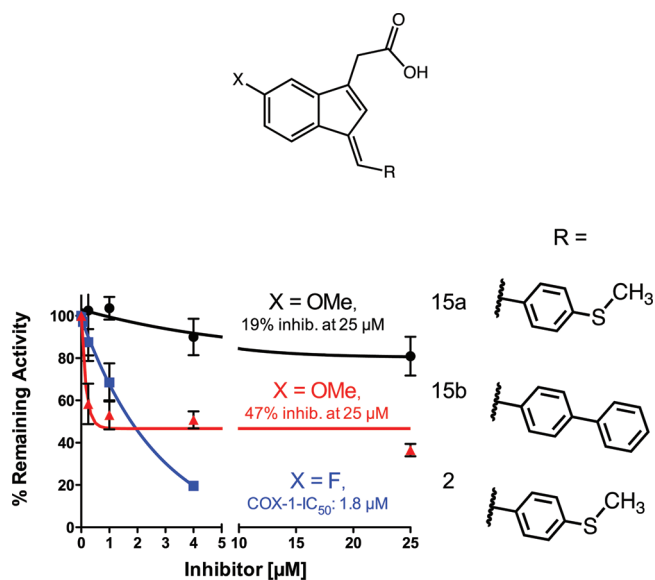


Figure 3. Comparison of the inhibition of ovine COX-1 by 15a (black circle), 15b (red triangle), and 2 (blue square).

EXPERIMENTAL SECTION

General. Reagents and solvents were of commercial quality and were used without further purification. All synthesized intermediates and final compounds were structurally characterized using ¹H NMR (optionally ¹⁹F NMR, ¹³C NMR) and electrospray ionization mass spectrometry. The purity of the test compounds was determined using HPLC [UV detection at 220 and 254 nm (optionally 288 and 347 nm) along with ELSD detection] and was generally ≥95%, if not denoted otherwise. The general synthetic procedures A–J are specified in the Supporting Information.

Preparation and Analytical Characterization of Exemplified Test Compounds. (*E*)-2-(5-Fluoro-1-((2-fluorobiphenyl-4-yl)methylene)-1H-inden-3-yl)acetic Acid (13d). According to general procedure C, the title compound was obtained from the isomer mixture 10a (0.05 g, 0.23 mmol) and 2-fluorobiphenyl-4-carbaldehyde (0.05 g, 0.25 mmol) after heating to 65 °C for 3 h and subsequent stirring at ambient temperature overnight. After acidification of the reaction mixture and repeated extraction with dichloromethane, the organic layers were combined and concentrated in vacuo. The remaining yellow solid material revealed good analytical quality and required no further purification. Yield, 63 mg (74%): C₂₄H₁₆F₂O₂, M_r = 374.39. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.70 (s, 2H), 7.08 (dtd, J = 1.2/2.4/8.4 Hz, 1H), 7.16–7.19 (m, 2H), 7.41–7.45 (m, 1H), 7.49–7.53 (m, 2H), 7.58–7.67 (m, 6H), 7.84 (dd, J = 5.2/8.0 Hz, 1H). HPLC (method 1) t_R: 11.30 min (>99%, UV347). ESI/MS: calcd, 373; found, 373.13 ([M – 1]⁺, 22%), 329.27 ([M – 1]⁺ – CO₂, 100%).

(*E*)-2-(5-Fluoro-1-((4-methyl-2-phenylpyrimidin-5-yl)methylene)-1H-inden-3-yl)acetic Acid (13j). According to general procedure C, the title compound was obtained from the isomer mixture 10a (0.05 g, 0.23 mmol) and 4-methyl-2-phenylpyrimidine-5-carbaldehyde (0.050 g, 0.25 mmol) after heating to 70 °C for 3 h and subsequent stirring at ambient temperature overnight. The crude product was purified by preparative thin-layer chromatography (SiO₂, EtOAc/hexane, 0.5% AcOH) to afford 43 mg (51%) of 13j: C₂₃H₁₇FN₂O₂, M_r = 372.40. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 2.69 (s, 3H), 3.67 (s, 2H), 6.87 (s, 1H), 7.11 (td, J = 2.4/8.4 Hz, 1H), 7.18 (dd, J = 2.4/9.2 Hz, 1H), 7.54–7.56 (m, 3H), 7.75 (s, 1H), 7.98 (dd, J = 5.2/8.4 Hz, 1H), 8.44–8.47 (m, 2H), 8.82 (s, 1H). HPLC (method 1) t_R: 10.16 min (97%, UV347). LCMS (ESI) (method 2) t_R: 2.95 min (97%, ELSD); m/z: 373.2 [M + 1]⁺.

(*E*)-2-(5-Fluoro-1-(4-(6-fluoropyridin-3-yl)benzylidene)-1H-inden-3-yl)acetic Acid (13k). According to general procedure E, the title

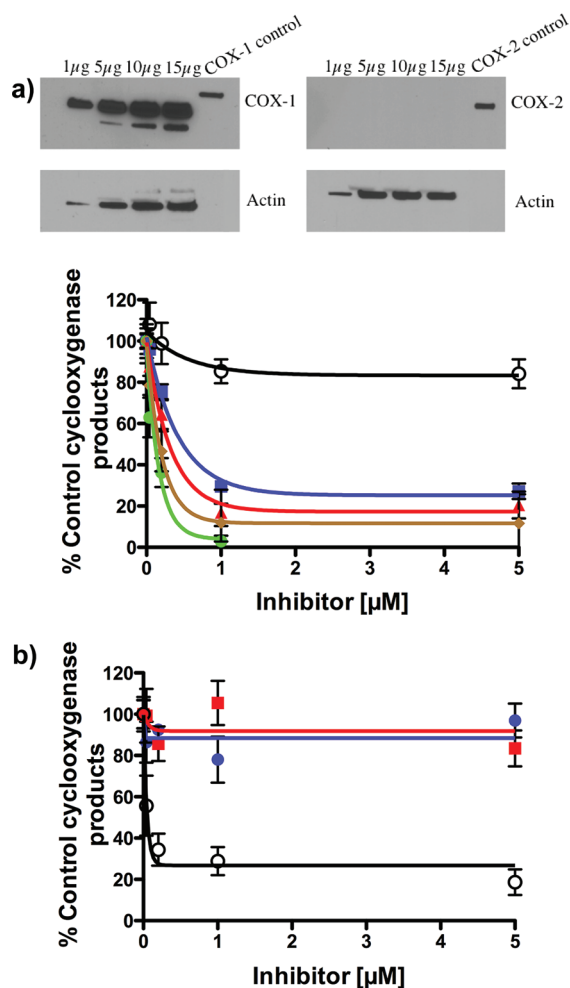


Figure 4. (a) Inhibition of COX-1 in ovarian cancer cells. The OVCAR-3 human ovarian epithelial cancer cell line expresses high levels of catalytically active COX-1 but no COX-2 (see Western blot above).³⁶ Whole-cell lysates from the OVCAR-3 human ovarian epithelial cancer cell lines were fractionated on a 10% PAGE and probed with polyclonal antibodies specific for COX-1 and COX-2. The control lane for the COX-1 blot is a standard solution of 0.3 μg/μL purified oCOX-1 in M-PER and Laemmli sample buffer, whereas the control lane for the COX-2 blot is a standard solution of 0.3 μg/μL purified mCOX-2 in M-PER and Laemmli sample buffer. Lower graph: Inhibition of hCOX-1 in OVCAR-3 cells (8 μM ¹⁴C-AA, 30 min at 37 °C) by *E*-DMSS analogues **2** (green circle; IC₅₀, 116 nM), **13a** (red triangle; IC₅₀, 300 nM), and **21c** (blue square; IC₅₀, 495 nM) in comparison with literature reference compounds **5** (brown diamond; positive control; IC₅₀, 160 nM) and **6** (○; negative control 16% inhibition at 5 μM). (b) Inhibition of hCOX-2 in HNSCC 1483 cells by *E*-DMSS analogues **2** (blue circle, n.i. at 5 μM) and **13a** (red square, n.i. at 5 μM) in comparison with literature reference compound **6** (○; positive control; IC₅₀, 54 nM).

compound was obtained from (*E*)-2-(1-(4-bromobenzylidene)-5-fluoro-1*H*-inden-3-yl)acetic acid **11i** (0.50 g, 0.14 mmol) and 2-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (0.037 g, 0.17 mmol) after 14 h at 90 °C. The crude product was purified by recrystallization from MeOH/hexanes. Yield, 25 mg (48%), bright yellow solid: C₂₃H₁₅F₂NO₂, M_r = 375.37. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.69 (s, 2H), 7.07 (td, *J* = (1.2)2.4/8.8 Hz, 1H), 7.15–7.18 (m, 2H), 7.31 (dd, *J* = 2.8/8.8 Hz, 1H), 7.69 (s, 1H), 7.79–7.88 (m, 5H), 8.36 (td, *J* = 2.8/8.2 Hz, 1H), 8.63 (d, *J* = 2.8 Hz, 1H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ: –112.96 (d, 1F, C5'-F), –68.78 (d,

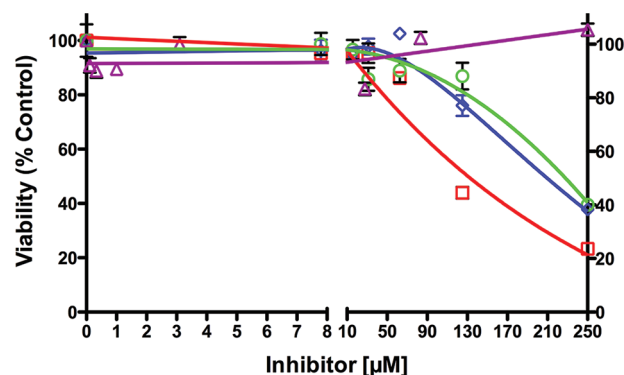


Figure 5. OVCAR-3 cell growth inhibition by **1a** (purple triangle, n.i. at 250 μM), **1b** (blue diamond; EC₅₀, 210 μM), **2** (green circle; EC₅₀, 223 μM), and **13a** (red square; EC₅₀, 132 μM).

1F, Pyr-F). HPLC (method 1) *t*R: 9.75 min. LCMS (ESI) (method 2) *t*R: 2.70 min (≥95%, ELSD); *m/z*: 376.0 [M + 1]⁺.

*Sodium (E)-2-(1-([1,1'-Biphenyl]-4-ylmethylene)-5-methoxy-1*H*-inden-3-yl)acetate [15b(Na)]*. The title compound was prepared by the aldol condensation reaction specified in general procedure D (step 1) from the isomer mixture **10b** (0.050 g, 0.22 mmol) and commercially available [1,1'-biphenyl]-4-carbaldehyde (0.043 g, 0.24 mmol). After stirring for 72 h at room temperature, the accumulated product precipitate was filtered and washed first with a little ice-cold methanol and then with diethylether. Compound **15b** was obtained as a yellow solid (Na-salt) in 37% yield (31.5 mg): C₂₅H₁₉NaO₃, M_r = 390.41. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 3.23 (s, 2H), 3.79 (s, 3H), 6.74 (dd, *J* = 2.22/8.22 Hz, 1H), 6.89 (s, 1H), 6.99 (d, *J* = 2.22 Hz, 1H), 7.34–7.41 (m, 2H), 7.47–7.52 (m, 2H), 7.65 (d, *J* = 8.22 Hz, 1H), 7.73–7.79 (m, 6H).

*(E)-2-(1-([1,1'-Biphenyl]-4-ylmethylene)-5-methoxy-1*H*-inden-3-yl)acetic Acid (15b)*. The title compound was prepared according to general procedure D (step 2) using 200 μL of 1.5 N HCl, starting from **15b(Na)** (0.016 g, 0.041 mmol) in 1 mL of H₂O. Yield, 13 mg (86%): C₂₅H₂₀O₃, M_r = 368.42. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.64 (s, 2H), 3.80 (s, 3H), 6.81 (dd, *J* = 2.4/8.2 Hz, 1H), 6.92 (d, *J* = 2.0 Hz, 1H), 7.05 (s, 1H), 7.39 (tt, *J* = 0.8/2.0/7.2 Hz, 1H), 7.47–7.51 (m, 3H), 7.72–7.79 (m, 7H). LCMS (ESI) (method 2) *t*R: 3.14 min (≥95%, ELSD); *m/z*: 369.2 [M + 1]⁺.

*(E)-Isopropyl 2-(5-fluoro-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetate (16c)*. According to general procedure F, (*E*)-2-(5-fluoro-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)acetic acid (**2** (100 mg, 0.31 mmol) was subjected to reaction with 2-propanol (5 mL) to afford **16c** as a fluffy yellow solid. Yield, 118 mg (100%). C₂₂H₂₁FO₂S, M_r = 368.46. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 4.00 MHz: 1.19 (d, *J* = 6.4 Hz, 6H), 2.52 (s, 3H), 3.72 (s, 2H), 4.94 (sept, *J* = 6.4 Hz, 1H), 7.05 (td, *J* = 8.9/2.4 (1.2) Hz, 1H), 7.13–7.15 (m, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.59–7.64 (m, 3H), 7.83 (dd, *J* = 8.4/5.2 Hz, 1H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ: –113.39 (1F, C5'-F). LCMS (ESI) 3.60 min (>99%, ELSD); *m/z*: 369.2 [M + 1]⁺.

*(E)-2-(5-Fluoro-1-(4-(methylthio)benzylidene)-1*H*-inden-3-yl)-2-methylpropanoic Acid (19a)*. Ester cleavage as described in general procedure G_{variant2} was performed on **19b** (15 mg, 0.039 mmol). The crude product was purified by flash chromatography (ethyl acetate/hexane 1:1, 0.5% HOAc, gradient) to produce **19a**. Yield, 12.5 mg (90%). C₂₁H₁₉FO₂S, M_r = 354.44. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 1.53 (s, 6H), 2.53 (s, 3H), 6.98 (dd, *J* = 2.4/10.0 Hz, 1H), 7.02–7.07 (m, 2H), 7.36 (d, *J* = 8.8 Hz, 2H), 7.60 (s, 1H), 7.66 (d, *J* = 8.4 Hz, 2H), 7.86 (dd, *J* = 5.4/8.2 Hz, 1H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ: –113.31 (q, 1F, C5'-F). LCMS (ESI) (method 2) *t*R: 3.12 min (98%, ELSD); *m/z*: 355.2 [M + 1]⁺.

*(E)-Ethyl 2-(1-([1,1'-biphenyl]-4-ylmethylene)-5-fluoro-1*H*-inden-3-yl)-2-methylpropanoate (19d)*. The title compound was prepared by the aldol condensation reaction specified in general procedure D (step 1) from **10d** (0.10 g, 0.40 mmol) and commercially available [1,1'-biphenyl]-4-carbaldehyde (0.081 g, 0.44 mmol). After stirring for

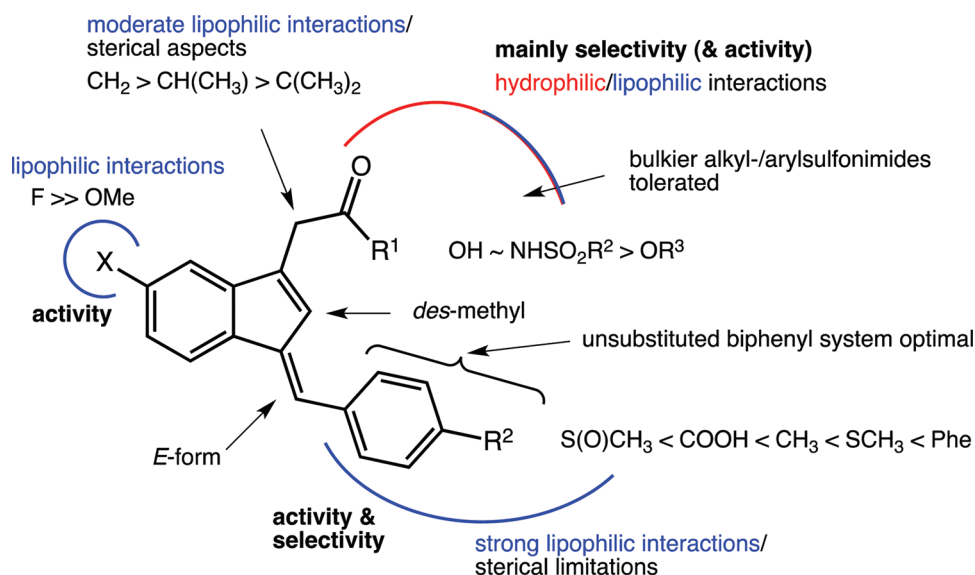


Figure 6. SAR for selective COX-1 inhibition.

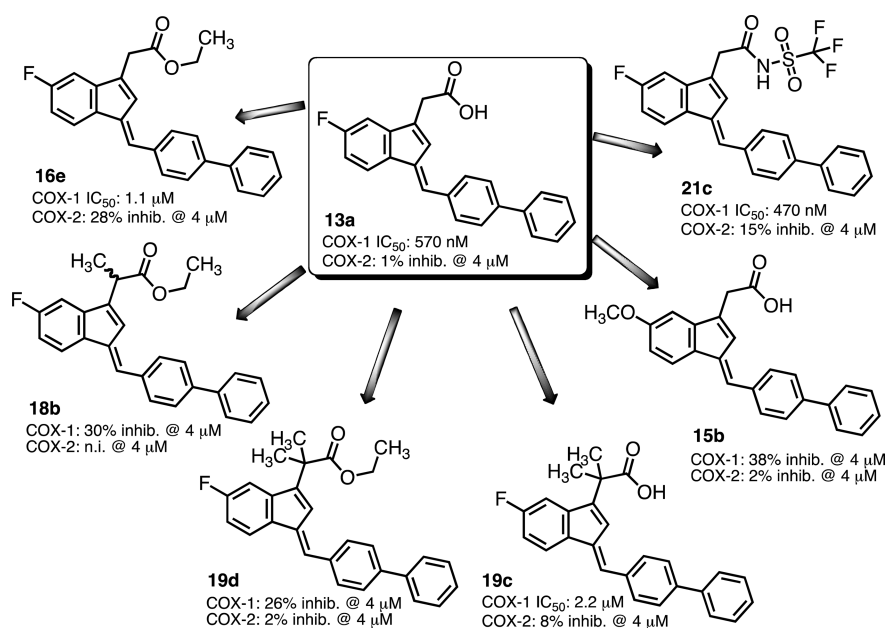


Figure 7. Derivatization and COX activity data of lead compound 13a.

72 h at room temperature, the accumulated product precipitate was filtered and carefully washed with water. Compound **19d** was obtained as a yellow solid (ethyl ester) in 98% yield (163 mg): C₂₈H₂₅FO₂, M_r = 412.50. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 1.07 (t, J = 7.2 Hz, 3H), 1.57 (s, 6H), 4.09 (q, J = 7.2 Hz, 2H), 6.90 (dd, J = 2.4/9.6 Hz, 1H), 7.05–7.10 (m, 2H), 7.40 (tt, J = 7.2 Hz, 1H), 7.50 (t, J = 7.2 Hz, 1H), 7.72–7.76 (m, 3H), 7.81 (s, 4H), 7.91 (dd, J = 5.2/8.4 Hz, 1H). LCMS (ESI) (method 2) t_R: 3.89 min (>99%, ELSD); m/z: 413.2 [M + 1]⁺.

(*E*)-Ethyl 2-(5-fluoro-1-((6-(4-fluorophenyl)pyridin-2-yl)methylene)-1H-inden-3-yl)-2-methyl-propanoate (**20b**). The title compound was prepared by the aldol condensation reaction specified in general procedure D (step 1) from **10d** (0.050 g, 0.20 mmol) and commercially available 6-(4-fluorophenyl)picolin-aldehyde (0.045 g, 0.22 mmol). After stirring for 72 h at room temperature, the accumulated product precipitate was filtered and carefully washed with water. The title compound was obtained as a yellow solid (Na salt) in 83% yield (72.5 mg): C₂₇H₂₃F₂NO₂, M_r = 431.47. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 1.06 (t, J = 7.2 Hz, 3H), 1.59 (s, 6H), 4.08 (q, J =

7.2 Hz, 2H), 6.91 (dd, J = 2.0/9.6 Hz, 1H), 7.07 (td, J = 2.4/8.8 Hz, 1H), 7.38 (pseudo t, J = 8.8 Hz, 2H), 7.61–7.63 (m, 2H), 7.87–8.00 (m, 3H), 8.07 (s, 1H), 8.20–8.24 (dd, J = 5.6/8.8 Hz, 2H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ: -111.98 (s, 1F), -110.74 (s, 1F). LCMS (ESI) (method 2) t_R: 3.79 min (>99%, ELSD); m/z: 432.2 [M + 1]⁺.

(*E*)-2-(1-([1,1'-Biphenyl]-4-ylmethylene)-5-fluoro-1H-inden-3-yl)-N-((trifluoromethyl)sulfonyl)-acetamide (**21c**). According to general procedure J, (*E*)-2-(1-([1,1'-biphenyl]-4-ylmethylene)-5-fluoro-1H-inden-3-yl)acetyl chloride (20 mg, 0.053 mmol) was subjected to reaction with trifluoromethanesulfonamide (11.9 mg, 0.080 mmol). The crude product was purified by flash chromatography (SiO₂, EtOAc + hexane: 1 + 1 (0.5% AcOH, gradient) to afford 13.5 mg (52%) of **21c** as bright yellow solid. C₂₅H₁₇F₄NO₃S, M_r = 487.47. ¹H NMR (400 MHz, DMSO-*d*₆) δ: 3.47 (s, 2H), 7.02 (td, J = 2.4/8.8 Hz, 1H), 7.09 (s, 1H), 7.15 (dd, J = 2.4/9.4 Hz, 1H), 7.39 (tt, J = 1.6/7.2 Hz, 1H), 7.49 (t, J = 7.2 Hz, 2H), 7.59 (s, 1H), 7.74 (dd, J = 1.6/7.2 Hz, 2H), 7.78 (s, 4H), 7.82 (dd, J = 5.2/8.4 Hz, 1H). ¹⁹F NMR (282 MHz, DMSO-*d*₆) δ: -115.25 (s, 1F, C⁵-F), -77.31 (s, 3F, -CF₃).

LCMS (ESI) (method 2) *t*R: 3.62 min (>99%, UV254, ELSD); *m/z*: 488.0 [M + 1]⁺.

■ ASSOCIATED CONTENT

■ Supporting Information

Synthetic procedures, routine spectroscopic and spectrometric data, HPLC data, exemplified NMR spectra, and biochemical and biological testing methods. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

NSAID, nonsteroidal anti-inflammatory drug; PPAR, peroxisome proliferator-activated receptor; COX, cyclooxygenase; SAR, structure–activity relationship; PG, prostaglandin; AA, arachidonic acid; RPMI, Roswell Park Memorial Institute medium; HBSS, Hank's balanced salt solution; FBS, fetal bovine serum; mPGES-1, microsomal prostaglandin E₂ synthase-1; PBS, phosphate-buffered saline; M-PER, mammalian protein extraction reagent

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