Bioorganic & Medicinal Chemistry Letters 19 (2009) 3271–3274

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The influence of double bond geometry in the inhibition of cyclooxygenases by sulindac derivatives

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ARTICLE INFO

Article history:
Received 5 March 2009
Revised 15 April 2009
Accepted 20 April 2009
Available online 23 April 2009

Keywords:
COX inhibition
Photoisomerization
NSAIDs
2′-*des*-Methyl sulindac sulfide

ABSTRACT

Sulindac sulfide is a benzylidene–indene that is a potent, time-dependent inhibitor of cyclooxygenases-1 and -2. Removal of the 2′-methyl group from the indene ring dramatically reduces time-dependent inhibition of both enzymes but also changes the geometry of the benzylidene double bond from *Z* to *E*. Herein, we explore the importance of double bond geometry on cyclooxygenase inhibition. The *Z*-isomer of 2′-*des*-methyl sulindac sulfide was synthesized by reduction of a bromoindene precursor or by photoisomerization of the *E*-isomer. The *Z*-isomer inhibited both cyclooxygenases, but with diminished potency compared to sulindac sulfide. Thus, although the 2′-methyl group is a major determinant of time-dependent cyclooxygenase inhibition, the geometry of the benzylidene double bond plays a role as well.

Sulindac sulfide and indomethacin are non-steroidal anti-inflammatory drugs (NSAIDs) that exert anti-inflammatory, analgesic, and anti-pyretic activities by inhibition of cyclooxygenase (COX) enzymes[1,2] (Fig. 1). Both compounds are slow, tight-binding inhibitors of COX-1 and COX-2 and the kinetics of inhibition are consistent with a rapid reversible interaction with the enzymes followed by a slow transition to a much more tightly bound complex[3,4] (Eq. 1). We have shown that an important determinant of the tight binding of sulindac sulfide and indomethacin is insertion of the 2′-methyl group of the indene or indole rings into a hydrophobic depression in the side of the COX active site.[5,6] Removal of the methyl group nearly completely eliminates the time-dependent inhibition of COX enzymes and transforms these compounds into weaker competitive inhibitors.[5,6] In the case of sulindac sulfide, removal of the 2′-methyl group also changes the geometry of the benzylidene double bond, which is introduced in the last step of synthesis, from *Z* to *E*.[6]

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{-}I \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E^*{-}I, \quad K_1 = k_{-1}/k_1 \tag{1}$$

Equation 1. Multi-step rate equation in which an initial rapid, reversible step is followed by a slow reversible step for COX.

Models of sulindac sulfide–COX-2 interactions based on the crystal structure of an indomethacin–COX-2 complex[7] suggest this geometric isomerism may also contribute significantly to the reduction of inhibition. To determine the importance of the geometry of the benzylidene double bond in COX inhibition, we have synthesized the *Z*-isomer of 2′-*des*-methyl sulindac sulfide and compared the kinetics of its inhibition of COX-1 and COX-2 with those of (*E*)-2′-*des*-methyl sulindac sulfide.

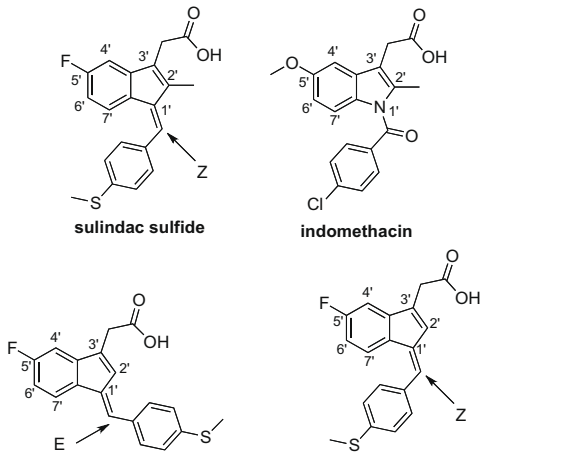

Figure 1. The structures of sulindac sulfide and indomethacin along with the two isomers of 2′-*des*-methyl sulindac sulfide.

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doi:10.1016/j.bmcl.2009.04.078

6-Fluoro-1-indanone was treated with zinc and ethyl bromoacetate in a Reformatsky reaction to give the desired alcohol product.[6] The alcohol was then dehydrated under acidic conditions[6] and the indene was treated with bromine to introduce a bromide in the 2′-position to serve as a temporary steric unit. Under basic conditions, the ethyl ester was cleaved to the acid along with the condensation of the bromoindene with *p*-methylthiobenzaldehyde to give a single product. In the final step, debromination was accomplished using tributyltin hydride. The final product resulted in an 80:20 mixture of *E* and *Z* isomers (Fig. 2).

The *E*- and *Z*-isomers of 2′-*des*-methyl sulindac sulfide were separated by reverse-phase HPLC using a Phenyl–Hexyl column (Phenomenex, Inc. 25 × 4.6 cm) with gradient elution (Fig. 3). The mobile phase consisted of water with 0.5% acetic acid (*v*/*v*) (A) and 1:1 acetonitrile/methanol with 0.5% acetic acid (B). Isomer separation was also achieved on an analytical C8 column, but separation on the Phenyl–Hexyl column was more robust. The NMR spectra of the two compounds was helpful in their structural assignment. The triplet–doublet at 7.04 ppm on the aromatic ring of the *E*-isomer shifted upfield to 6.89 ppm in the *Z*-isomer whereas the indene proton at 7.11 ppm of the *E*-isomer shifted upfield to 6.66 ppm and the external double bond proton shifted from 7.57 ppm to 7.31 ppm (Fig. 4). The two isomers have slightly different UV properties (Fig. 3, inset). The *E*-isomer has a λ_{max} of 370 nm whereas the *Z*-isomer has a λ_{max} at a lower wavelength (350 nm). During HPLC purification of both isomers, it was observed that if the *Z*-isomer was exposed to natural light it photoisomerized to the *E*-isomer (Fig. 4). Upon further study, an equilibrium was found to exist between the two isomers if exposed to natural light for 10 h (approximately a 70:30 ratio of *E*/*Z*). If kept in the dark, each isomer remained stable.

We attempted to photoisomerize the *E*-isomer of 2′-*des*-methyl sulindac sulfide to the *Z*-isomer at 370 nm for 24 h using a high-pressure mercury lamp (1000 W, Hanovia 528B-1) and a Spectral Energy GM 252 high intensity grating monochromator. The percentage conversion was only 1–2%, which compared poorly to the conversion under natural light (10–15%) and indicated that photoisomerization has a low quantum efficiency at 370 nm (Fig. 4). We also tried to photoisomerize the sulfoxide and sulfone derivatives of 2′-*des*-methyl sulindac sulfide[8] and sulindac sulfide itself under natural light and ultraviolet conditions but no interconversion was observed.

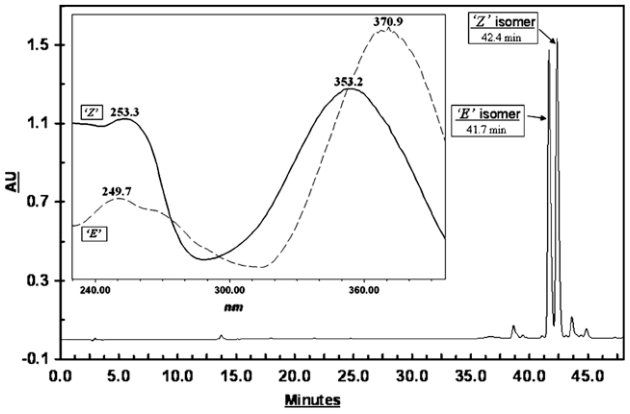

Figure 3. HPLC separation of the *E*- and *Z*-isomers of 2′-*des*-methyl sulindac sulfide.

(*E*)- and (Z)-2′-*des*-methyl sulindac sulfide were evaluated as inhibitors of murine COX-2 or ovine COX-1 in assays performed with a saturating concentration of arachidonic acid substrate (50 μM). The presence of saturating substrate concentrations precludes competitive inhibition and biases the assay to detect time-dependent inhibitors. The data summarized in Figure 5 confirms our previous report that (*E*)-2′-*des*-methyl sulindac sulfide exhibits no time-dependent inhibition of COX-1 or COX-2.[5,6] Very weak COX-1 and COX-2 inhibition was detected with (*Z*)-2′-*des*-methyl sulindac sulfide, but the maximal extent of inhibition was only 20%. The inability to achieve complete inhibition suggests that the binding of the (*Z*)-isomer is readily reversible and consistent

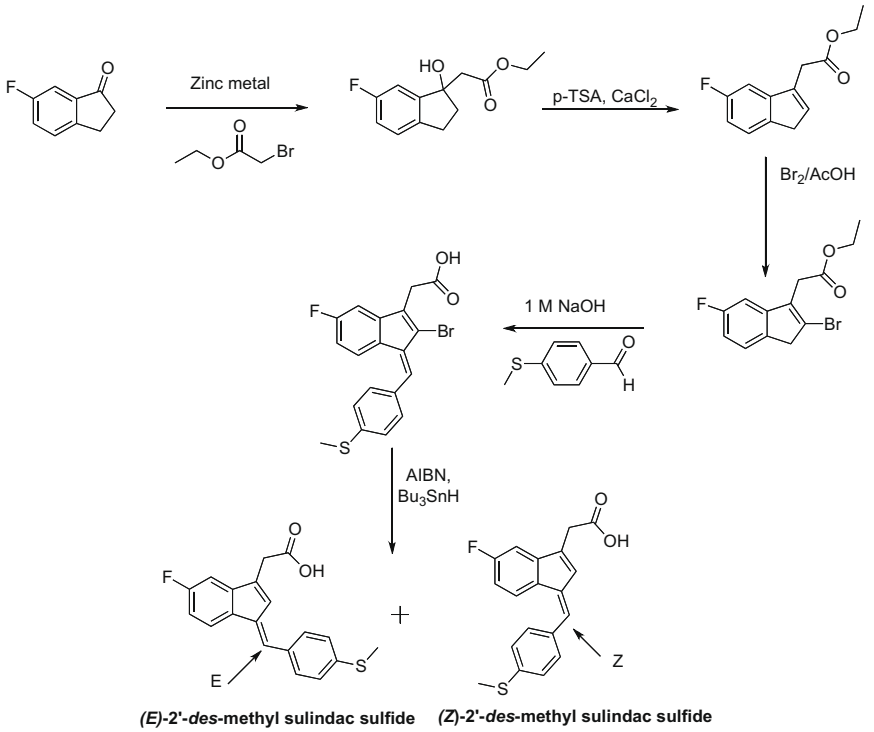

Figure 2. Synthesis of (*Z*)- and (*E*)-2′-*des*-methyl sulindac sulfide.

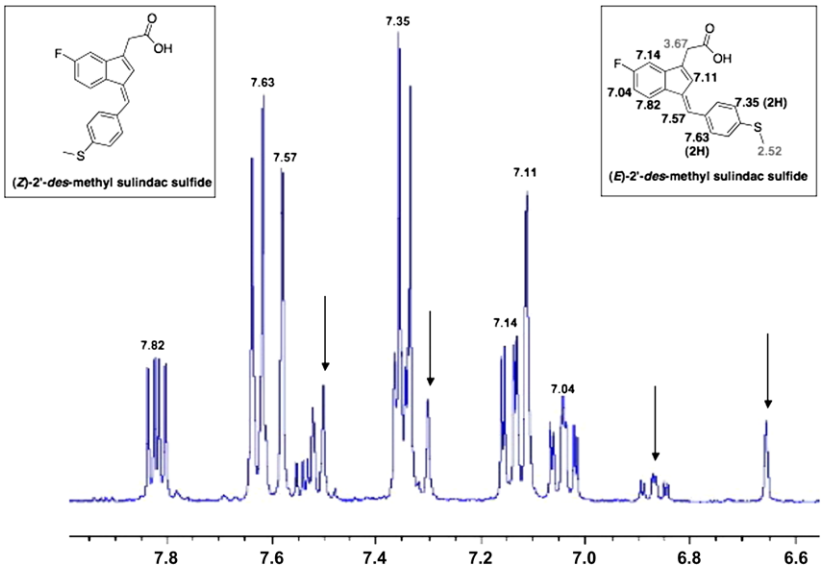

Figure 4. Photoisomerization of (*E*)-2′-*des*-methyl sulindac sulfide to (*Z*)-2′-*des*-methyl sulindac sulfide under natural light for 10 h. The arrows indicate the appearance of new peaks for (*Z*)-2′-*des*-methyl sulindac sulfide.

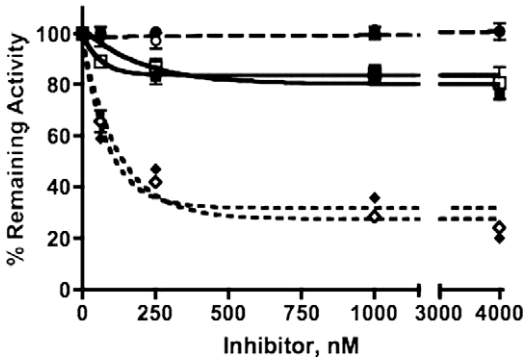

Figure 5. Time-dependent inhibition of murine COX-2 and ovine COX-1 under saturating substrate conditions by sulindac sulfide and its 2′-*des*-methyl sulindac sulfide *E*- and *Z*-isomers. (○) COX-2 and (*E*)-2′-*des*-methyl sulindac sulfide, (●) COX-1 and (*E*)-2′-*des*-methyl sulindac sulfide, (□) COX-2 and (*Z*)-2′-*des*-methyl sulindac sulfide, (■), COX-1 and (*Z*)-2′-*des*-methyl sulindac sulfide, (◇) COX-2 and sulindac sulfide, (◆) COX-1 and sulindac sulfide.

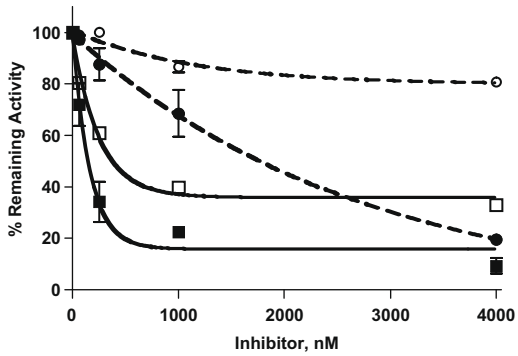

Figure 6. Competitive inhibition of murine COX-2 and ovine COX-1 by the *E*- and *Z*-isomers of 2′-*des*-methyl sulindac sulfide under low arachidonate conditions. (○) COX-2 and (*E*)-2′-*des*-methyl sulindac sulfide, (●) COX-1 and (*E*)-2′-*des*-methyl sulindac sulfide, (□) COX-2 and (*Z*)-2′-*des*-methyl sulindac sulfide, (■), COX-1 and (*Z*)-2′-*des*-methyl sulindac sulfide.

with competitive inhibition. The parent molecule, sulindac sulfide, inhibits both COX enzymes potently (115 nM and 140 nM, for COX-1 and COX-2, respectively), although it reaches a plateau around 75% inhibition. These results indicate that the 2′-methyl group on the indene nucleus is a major determinant of time-dependent inhibition of both COX-1 and COX-2. The effect of removing the 2′-methyl group is greater for sulindac sulfide than for indomethacin.[5] Indomethacin potently inhibits wild-type COXs with IC_{50} values of 40 nM and 250 nM against COX-1 and COX-2, respectively.[5] However, the 2′-*des*-methyl indomethacin has no activity against COX-1, but is still able to inhibit COX-2 with an IC_{50} of 4 μM.[5]

To evaluate the ability of (*E*)- or (Z)-2′-*des*-methyl sulindac sulfide to act as competitive inhibitors, each isomer was tested in an assay with reduced substrate concentrations (5 μM) near the K_m of COX-1 and COX-2 for arachidonate. Interestingly, (*E*)-2′-*des*-methyl sulindac sulfide showed weak inhibition of COX-1 (IC_{50} of 1.8 μM), but no inhibition of COX-2 at the concentration ranges tested (Fig. 6). In contrast, (*Z*)-2′-*des*-methyl sulindac sulfide strongly inhibited both COX-1 and COX-2 under these conditions (IC_{50}s of 147 nM and 375 nM, respectively) (Fig. 6). The ability of sulindac sulfide to inhibit COX-1 and COX-2 was not significantly affected by the reduced substrate concentrations (data not shown). Thus, whereas the 2′-methyl group is a critical determinant of time-dependent inhibition of COX enzymes by both (*E*)- and (*Z*)-isomers, it is less important for competitive inhibition. The geometry of the

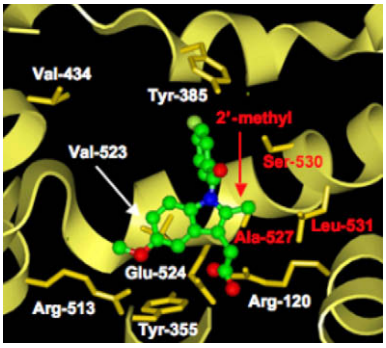

Figure 7. Indomethacin bound in the active site of murine COX-2 (model based on Kurumbail et al.).[7] The figure illustrates where the 2′-methyl of indomethacin inserts into a small, hydrophobic depression in the COX active site comprised of Ala527, Ser530, Leu531, and Val349. (PDB ID: 4COX).

benzylidene double bond seems important for competitive inhibition as isomerization from (*Z*) to (*E*) significantly reduces the potency against COX-1 and nearly abolishes inhibition of COX-2.

The 2′-methyl of the indole ring of indomethacin inserts into a hydrophobic pocket of COX-2 comprised of residues Val349, Ala527, Ser530, and Leu531[5] (Fig. 7). These residues are responsible for the tight-binding and time-dependence of COX inhibition by indomethacin.[5] Whereas indomethacin is released only very slowly once it is tightly-bound to COX-2, 2′-*des*-methyl indomethacin is readily competed off the enzyme ($k_{-2} = 0.006\ s^{-1}$).[5] The binding of indomethacin results in the 4-chlorobenzoyl chloride being out of the plane compared to the indole ring where the carbonyl forms a hydrogen bond with Ser530.[5] The carboxylate of indomethacin interacts with Arg120 while the *o*-methoxy group protrudes into a large cavity surrounded by residues Ser353, Tyr355, and Val523.[5] It seems possible that (*Z*)-2′-*des*-methyl sulindac sulfide is oriented similarly to the parent compound, sulindac sulfide, and to 2′-*des*-methyl indomethacin in the COX active site. The carboxylate group and fluorine are likely oriented similarly to that of indomethacin. The key differences between the binding of the sulindac sulfide derivatives is the absence of a hydrogen bond between the substrate and Ser530 as well as the planarity of the indene ring and the benzylidene double bond. The absence of the 2′-methyl group makes the *des*-methyl derivatives very poor time-dependent COX inhibitors. The *E*-isomer of 2′-*des*-methyl sulindac sulfide must bind in a completely different orientation in the COX active site because the orientation of the thiomethoxyphenyl group should introduce numerous steric clashes as it attempts to adopt a configuration similar to that of sulindac sulfide. There has been a recent report suggesting a novel binding mode of indomethacin derived COX-1 selective inhibitors.[9]

In conclusion, we have found that the *E*- and *Z*-isomers of 2′-*des*-methyl sulindac have unique inhibitory profiles when tested against COX-1 and COX-2. Importantly, we have shown that the 2′-methyl group of sulindac sulfide is a major determinant of tight-binding as it is for indomethacin.[5] In addition, we have established the importance of the geometry of the benzylidene double bond in competitive inhibition of COX-1 and COX-2. Our discovery that (*E*)-2′-*des*-methyl sulindac sulfide is a selective COX-1 inhibitor represents the first report of selective COX-1 inhibition by a member of the arylacetic acid class of inhibitors. This may not only be useful for evaluating the importance of COX-1 inhibition in the pharmacological action of sulindac sulfide, but it may represent an opportunity to define a new binding mode for an arylacetic acid to a COX enzyme.

Acknowledgements

This work was supported by the National Institutes of Health (CA89450).

Supplementary data

Procedures for the synthesis of (*Z*)-2′-*des*-methyl sulindac sulfide under non-photoisomerization and photoisomerization conditions. Enzymatic assay conditions for both time-dependent and competitive inhibition of COX are available. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.078.

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