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Atropisomerism Observed in Indometacin Derivatives

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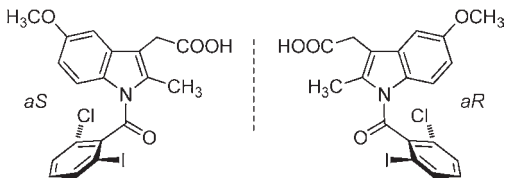
ABSTRACT



To elucidate the active conformation of indometacin that differentiates between cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), the stereochemistry around the *N*-benzoylated indole moiety of indometacin was studied. Resolution of stable atropisomers as representative conformations was found to be possible by restricting rotation about the N−C7′ and/or C7′−C1′ bond. Only the a*R*-isomer showed specific inhibition of COX-1, and COX-2 was not inhibited by either atropisomer.

Indometacin, a "classical" nonsteroidal antiinflammatory drug (NSAID), acts at the cyclooxygenase (COX) active site and inhibits two isoforms of COX, i.e., COX-1 and COX-2, with little specificity, leading to serious side effects.[1−7] Therefore COX-1/2-selective indometacin analogues have been extensively explored in attempts to develop a new generation of NSAIDs.[8−11]

In the design of new COX-1/2-selective indometacin analogues, the conformation of the *N*-benzoyl moiety has been considered. The benzoyl (phenyl) group can adopt either a *cis* or *trans* conformation with respect to the indole (Figure 1a). In 1996, Loll et al. proposed that indometacin interacts with COX-1 in two possible binding modes corresponding to the *cis* or *trans* form.[12] Independently, the group of Miyashiro and Penning reported that indometacin adopts a *cis* conformation in its complex with COX-2.[13] Since then, the *cis*/*trans* conformations of

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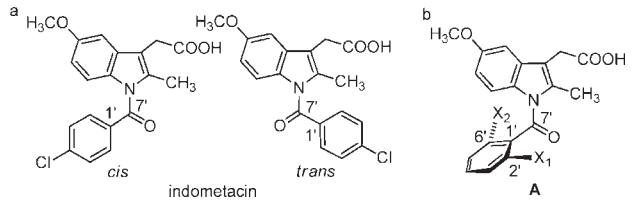
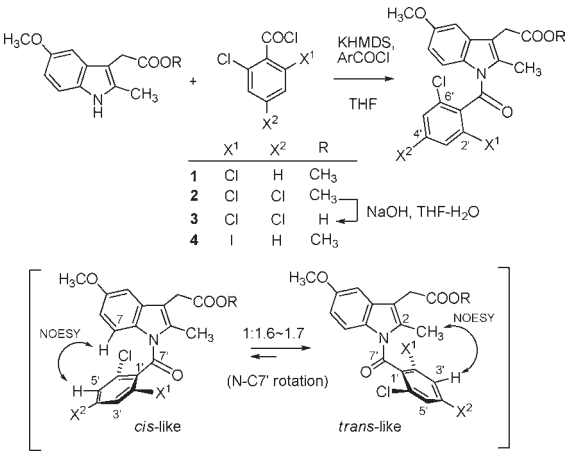


Figure 1. (a) Conformations traditionally used for indometacin. (b) 2′,6′-Disubstituted indometacin derivative **A**.

indometacin have been regarded as a key feature for improved drug design.[14,15] However, "*cis*/*trans*" terminology is associated with a planar structure in which the indole and benzoyl (phenyl) groups are coplanar. Such vague terminology could prove misleading in the present case. X-ray crystallographic analysis has shown that indometacin, even in a crystal state, adopts several different conformations, in which the indole and benzoyl (phenyl) groups are twisted relative to each other.[16,17]

Similarly, in solution the 1-benzoyl-2-methylindole moiety of indometacin is not expected to adopt a completely planar conformation because of the large steric hindrance between the phenyl and indolyl groups. The *N*-benzoyl moiety has two sp^2-sp^2 axes along the N−C7′ and C7′−C1′ bonds that can provide numerous conformations of indometacin in solution. To elucidate the active conformation of indometacin, the resolution of stable atropisomers as a representative conformation by restricting the rotation of the N−C7′ and/or C7′−C1′ bond could provide a new means to study the molecular origins of COX-1/2 selectivity. At present, atropisomerism is most common in biphenyl or biaryl C−C bond stereochemistry, although atropisomers arising from C−N bonds have recently attracted much attention.[18−25] However, to the best of our knowledge, reports on the chirality of *N*-benzoylindoles, with chirality arising from the two sp^2-sp^2 axes of the aromatic N−CO−Ar bonds, have not yet been published. Here we show presence of atropisomers based on the C7′−C1′ axis in the *N*-benzoyl moiety of 2′,6′-disubstituted indometacin derivatives (**A**, Figure 1b), which possess high stereochemical stability and are recognized by COX-1. This novel chirality is produced by fixing the C7′−C1′ axis alone, and hence the other N−C7′ axis independently rotates to form an equilibrium between *cis*- and *trans*-like conformations in each enantiomer.

Scheme 1. Synthesis of C-2′/C-6′ Disubstituted and C-2′/C-4′/C-6′ Trisubstituted Indometacin Derivatives (**1**−**4**), Which Exist As Equilibrium Mixtures of *cis*-Like and *trans*-Like Conformers



Through the examination of various indometacin derivatives, we found that the C-2′ and C-6′ disubstituted indometacin derivatives (**1**−**4**, Scheme 1) exist in *cis*−*trans* equilibrium: the ^{1}H NMR spectrum of **1** in $CDCl_3$ was observed as two sets of resonances in a 1:1.7 ratio (see Supporting Information). In order to assign the *cis*/*trans* conformations, the NOESY method was used for the trisubstituted (at C-2′, C-4′, and C-6′) benzoyl derivative **2**. In the NOESY specrum of the minor form, H7 showed a correlation peak to H3′/5′, which confirmed the position of the benzoyl (phenyl) ring to be *cis*. On the contrary, for the major isomer of **2**, 2-CH_3 showed a correlation peak to H3′/5′, which confirmed the position of the benzoyl ring to be *trans* (see Supporting Information). We therefore reasoned that compound **1** exists as a mixture of *cis*-like and *trans*-like[26] forms (*cis*/*trans* = 1:1.7) in solution. Compound **1** was observed as an inseparable single peak on nonchiral HPLC at room temperature, indicating that conformers are at equilibrium as the result of rotation around the N−C7′ axis.[27] For **1**−**4**, either or both conformations (*cis*-like and *trans*-like) could be adopted when it binds to an enzyme. However, the ratio of *cis*-like and *trans*-like (1:1.6−1.7) may be taken to indicate a tendency to adopt the *trans*-like conformer.

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(26) These "*trans*-like/*cis*-like" terms have the implication that the carbonyl group takes a type of perpendicular position with respect to the plane of the phenyl group of the benzoyl moiety.
(27) In order to examine the equilibrium between the *cis*-/*trans*-conformers, VT-NMR has been measured (see: Supporting Information).

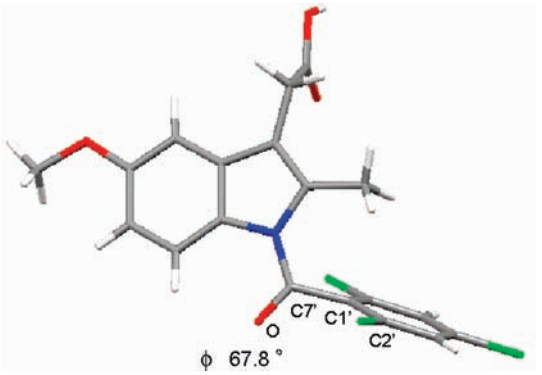


Figure 2. Structure of **3** determined using X-ray crystallographic analysis.

After confirming that the N−C7′ axis of *N*-benzoylated indole derivatives allows rotation in solution, the next step was to examine the C7′−C1′ axis. The structural analysis was supported by single-crystal X-ray analysis of **3**, in which the dihedral angle (ϕ) C2′−C1′−C7′−O was shown to be 67.8°, confirming that the benzene ring is essentially orthogonal to the indole ring (Figure 2). This geometry convinced us that the atropisomeric property arises from the C7′−C1′ axis in C-2′ and C-6′ disubstituted indole derivatives. As expected, compound **4**, in which Cl and I are present at C-2′ and C-6′, respectively, gave important insight into this. On the basis of the ^{1}H NMR spectrum, it is clear that **4** also exists as an equilibrium mixture of *cis*-like and *trans*-like conformation (*cis*-like:*trans*-like = 1:1.6) in $CDCl_3$. It was also observed as a single peak on nonchiral HPLC at room temperature. In short, rotation about the N−C7′ axis of **4** is similar to that of **1**. To our surprise, however, **4** could be resolved by chiral HPLC (CHIRALPAK IB) into enantiomers at room temperature, indicating that it exists as a racemate of the atropisomers. It is clear that the steric hindrance provided by the C-2′ and C-6′ substituents forms a higher rotational barrier for the C7′−C1′ axis than for the N−C7′ axis. It should be noted that each *cis*-like and *trans*-like conformer of **4** exists as the racemate of a*R* and a*S* isomers, as shown in Figure 3.

We managed to obtain each enantiomer of **4** using preparative chiral HPLC at about 96% ee, with high stereochemical stability at room temperature (see Supporting Information). It was confirmed that each enantiomer exists as an equilibrium mixture of *cis*-like and *trans*-like conformers. The isolated atropisomers have opposite $[\alpha]_D$ values: **4A** (with shorter retention time in HPLC using CHIRALPAK IB) as 96% ee showed $[\alpha]^{24}_D$ −25.7 (*c* 0.1, $CHCl_3$) and **4B** (with longer retention time in HPLC using CHIRALPAK IB) as 96% ee showed $[\alpha]^{23}_D$ +23.1 (*c* 0.15, $CHCl_3$), confirming that they are enantiomers. The isolation of each enantiomer with high stereochemical stability provides evidence that rotation about the C7′−C1′ axis can be fully restricted by substituents at C-2′ and C-6′. It is worth noting that the N−C7′ axis can allow rotation even though the neighboring C7′−C1′ axis is blocked to

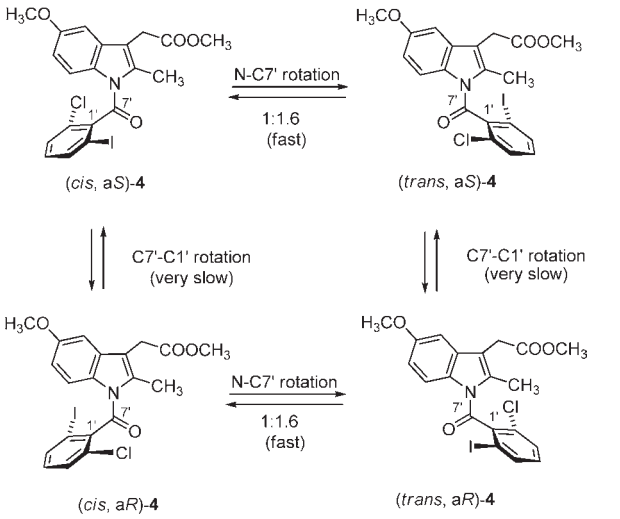
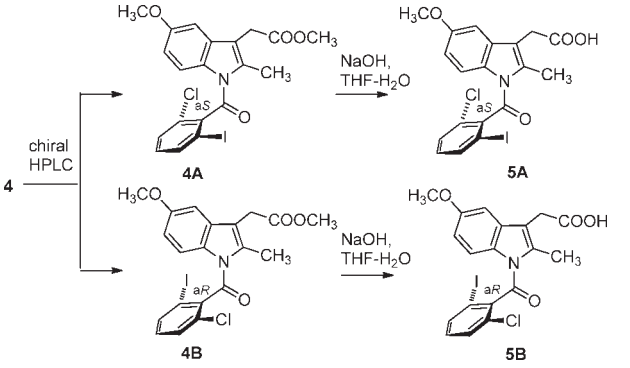


Figure 3. Conformers including atropisomers of the indometacin analogue **4**.

rotation. Judging from the above, these two axes behave independently for **4** in solution. To the best of our knowledge, this type of atropisomer has not been previously reported. The separated enantiomers of **4** (**4A** and **4B**) were hydrolyzed to afford the corresponding carboxylic acid derivatives **5** (**5A**, **5B**) (Scheme 2). We fortunately succeeded in obtaining the enantiomer **5A** as a single crystal.

Scheme 2. Separation of **4** into Atropisomers **4A** and **4B** and Hydrolysis to the Corresponding Carboxylic Acids **5A** and **5B**



On the basis of its X-ray analysis, **5A** was assigned to be (a*S*), and hence, **5B** to be (a*R*) (see Supporting Information). We examined the stereochemical stability of the enantiomers (**5A** and **5B**) and found that it was estimated to be high: racemization occurred after storage for 48 h at 37 °C in EtOH. Thus, the activation free-energy barrier to rotation ($\Delta G^{\ddagger}$) was calculated to be 106 kJ/mol[28] (see Supporting Information).

We next examined the activities of the racemate **5** and each pure enantiomer (**5A** and **5B**) against COX-1/2

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Table 1. In Vitro Cyclooxygenase Assay of **5** (Racemate and Atropisomers)

		IC_{50} (μM)	
	$[\alpha]^{22}_D$ ($CHCl_3$)	COX-1	COX-2
5		13.4 ± 3.4	>500
5A (a*S*)	-21.2 (*c* 0.13, as 96% ee)	>200	>500
5B (a*R*)	$+20.4$ (*c* 0.10, as 94% ee)	15.6 ± 1.8	>500
indometacin		0.106 ± 0.042	3.50 ± 041

(Table 1). It was found that the racemate **5** or enantiomers **5A** and **5B** did not show COX-2-inhibitory activity at a concentration of 500 μM. On the other hand, the racemate **5** exhibited potent COX-1-selective inhibitory activity with an IC_{50} value of 13.4 ± 3.4 μM. The result is attractive because COX-1 inhibitors have been revaluated recently.[14] It appears reasonable to assume that the COX-1 versus COX-2 selectivity of **5** is closely linked with its conformation. As pointed out above, there is a difference in the conformation of indometacin relevant to the interactions with COX-1 and COX-2. Although the interaction of indometacin with COX-2 has been determined to be only the *cis* conformation,[13] indometacin interacts with COX-1 in the *cis* or *trans* conformation.[12] On the basis of ^{1}H NMR spectroscopy, compound **5** exists as an equilibrium mixture of *cis*-like and *trans*-like conformation (*cis*-like:*trans*-like = 1:1.6) in $CDCl_3$. Such an equilibrium biased toward the *trans*-like conformer should be advantageous to COX-1. In addition, it was revealed that the enantiomer **5B** (a*R*) shows potent activity against COX-1 with an IC_{50} value of 15.6 ± 1.8 μM. The other enantiomer **5A** (a*S*) showed no inhibitory activity at a concentration of 200 μM. The results clearly indicate that COX-1 recognizes the stereochemistry of the axial chirality arising from the C7′−C1′ axis. We assumed that C-2′ and C-6′ disubstituted indole derivative **5** provided an atropisomeric property that should be a clue for the design of COX-1/2-selective indometacin derivatives.

In conclusion, one of the active conformations of indometacin for COX-1 among the numerous possible conformations has been revealed by the introduction of different substituents at the 2′- and 6′-positions of indometacin (twisted conformation). This is the first example of introducing atropisomerism to *N*-benzoylated indole derivatives. We hope that our newly discovered atropo-selective bioactivity triggers the development of pure atropisomers as new drug candidates in medicinal chemistry.

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Supporting Information Available. NMR data of **1**−**5**, NOESY data of **2**, analytical study of enantiomers of **4** (**4A** and **4B**) and **5** (**5A** and **5B**), stereochemical stability of the enantiomers of **5** (**5A** and **5B**), crystal data (CIF file) for **3** and **5A**, in vitro cyclooxygenase assay. This material is available free of charge via the Internet at http://pubs.acs.org.