# Biological Evaluation of 2-Aryl-2-fluoropropionic Acids as Possible Platforms for New Medicinal Agents

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**We investigated the cyclooxygenase (COX) inhibitory and anticancer activities of 2-aryl-2-fluoropropionic acids 1a—e. These fluorinated compounds showed lower inhibitory activity toward COX-1 than the corresponding non-fluorinated compounds 2a—e with retained inhibitory activity against COX-2 resulting in modification of the balance of COX-1/COX-2 inhibitions, and they showed little anticancer activity. Interesting differences of the activities between (*S*)- and (*R*)-enantiomers were observed in some cases.**

**Key words** 2-aryl-2-fluoropropionic acid; non-steroidal anti-inflammatory drug; epimerization; chiral fluorinated structure

We have been studying the design, synthesis, and biological evaluation of chiral fluorine-containing organic compounds as effective analogues of pharmaceutically important molecules. Introduction of fluorine into a prototype molecule results in minimal steric alterations, which can facilitate interactions of fluorinated biomolecules or medicinals with enzyme active sites, receptor recognition sites, transport mechanisms, and other biological systems.[1)] On the other hand, the presence of fluorine can alter the biological consequences of these interactions, often in a useful way. As a part of our research, we recently reported the synthesis of optically active 2-aryl-2-fluoropropionic acids **1**, which possess a unique structure with the fluorine atom located at the stereogenic center (Fig. 1).[2)]

The non-steroidal anti-inflammatory drugs (NSAIDs) inhibit COX-1 and COX-2 activities. An increasing number of epidemiological, clinical, and laboratory studies have suggested that NSAIDs, including the series of 2-arylpropionic acids **2** (Fig. 2), may also be able to inhibit the initiation and proliferation of some tumors.[3—5)] For the following reasons we thought that the fluorinated analogues of **2** could be possible platforms for multi-dimensional, clinically effective therapeutic agents. First, there have been reported a few potent leads having a chiral fluorine-containing structure, such as fluorothalidomide, fluorodonepezil, *etc.*[6—9)] These compounds were found to be pharmacologically more effective than the fluorine-free parent compounds. This suggests that the screening of biological activities of chiral fluorine-containing acids **1** could guide the development of new kinds of medicinal agents. Secondly, there have been literature reports describing an *in vivo* inversion of less active (*R*)-enantiomer into more active (*S*)-enantiomer in some 2-arylpropionic acids **2** (Fig. 2).[10—13)] Such *in vivo* epimerization of chiral medicinal agents is quite rare although there are several examples of *in vivo* racemization. It is apparent that non-epimerizable 2-aryl-2-fluoropropionic acids **1** could serve as useful tools to clarify the roles of stereochemical lability in living organisms.

To examine the pharmacological potential of chiral 2-aryl-2-fluoropropionic acids **1** as pharmacological leads, we have evaluated them with respect to both COX inhibitory activities and antiproliferative activities of cancer cell lines. In this paper, we present the results of this biological evaluation and discuss the results in terms of the potential for further drug development.

## Results and Discussion

We first investigated the inhibitory activity of the racemic and optically active fluorinated acids **1a**—**e** toward COX-1 and COX-2. The corresponding non-fluorinated racemates **2a**—**e**, indomethacin, and NS-398 were also investigated for reference. Their inhibitory activities are shown in Table 1.

The inhibitory activities of (±)-**1c** and (±)-**1e** toward COX-1 were much lower than those of the corresponding acids (±)-**2c** and (±)-**2e**, while the activity of (±)-**1c** toward COX-2 was almost the same as that of (±)-**2c**. Although the COX-2 inhibitory activity of (±)-**1e** was lower than that of

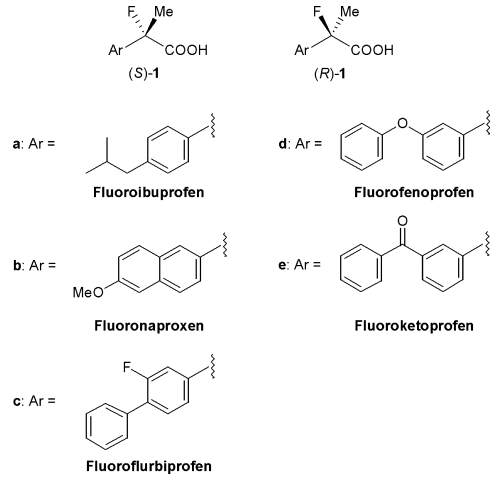


Fig. 1

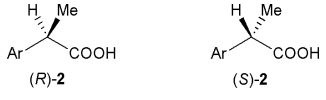


Fig. 2

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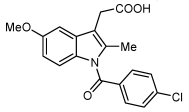
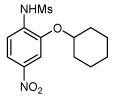
Table 1

| Compound | $IC_{50}$, $\mu$M (% inhibition at 100 $\mu$M) | | | | COX-1/-2[a] ratio |
|---|---|---|---|---|---|
| | COX-1 | | COX-2 | | |
| (±)-**1a** | >100 | (19) | >100 | (20) | |
| (*S*)-**1a** | >100 | (14) | >100 | (10) | |
| (*R*)-**1a** | >100 | (26) | >100 | (25) | |
| (±)-**2a** | >100 | (48) | >100 | (25) | |
| (±)-**1b** | 37.2 | (55) | >100 | (48) | <0.37 |
| (*S*)-**1b** | >100 | (46) | >100 | (38) | |
| (*R*)-**1b** | 13.1 | (70) | 63.5 | (52) | 0.21 |
| (±)-**2b** | 2.70 | (75) | >100 | (45) | <0.027 |
| (*S*)-**2b** | 0.828 | (76) | 69.9 | (51) | 0.012 |
| (*R*)-**2b** | 8.50 | (79) | 28.8 | (60) | 0.30 |
| (±)-**1c** | 0.142 | (94) | 0.347 | (80) | 0.41 |
| (*S*)-**1c** | 1.10 | (79) | 1.18 | (84) | 0.93 |
| (*R*)-**1c** | 0.0698 | (97) | 0.297 | (>99) | 0.24 |
| (±)-**2c** | 0.00893 | (98) | 0.509 | (50) | 0.018 |
| (±)-**1d** | >100 | (21) | >100 | (16) | |
| (*S*)-**1d** | >100 | (7) | >100 | (17) | |
| (*R*)-**1d** | >100 | (25) | >100 | (23) | |
| (±)-**2d** | 27.0 | (65) | >100 | (36) | <0.27 |
| (±)-**1e** | 2.09[b] | (63) | 10.3 | (81) | 0.20 |
| (*S*)-**1e** | 6.31 | (75) | 23.6 | (82) | 0.27 |
| (*R*)-**1e** | 1.45 | (83) | 6.73 | (91) | 0.22 |
| (±)-**2e** | 0.0437 | (99) | 1.66 | (60) | 0.026 |
| Indomethacin[e] | 0.0138 | (85[c]) | 1.03 | (92[d]) | 0.013 |
| NS-398[e] | >100 | (−2) | 1.33 | (80) | >75 |

*a*) The values were calculated from each $IC_{50}$ value. *b*) The value was not significant. *c*) The value was obtained in concentration of 0.1 $\mu$M. *d*) The value was obtained in concentration of 10 $\mu$M. *e*)



indomethacin

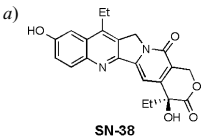


NS-398

Table 2

| Compound | $IC_{50}$, $\mu$g/ml (% inhibition at 100 $\mu$g/ml) | | | |
|---|---|---|---|---|
| | A549 | | HT-29 | |
| (*S*)-**1a** | >100 | (11) | >100 | (4) |
| (*R*)-**1a** | >100 | (15) | >100 | (1) |
| (±)-**2a** | >100 | (18) | >100 | (6) |
| (*S*)-**1b** | >100 | (2) | >100 | (−2) |
| (*R*)-**1b** | >100 | (5) | >100 | (−1) |
| (±)-**2b** | >100 | (2) | >100 | (13) |
| (*S*)-**1c** | >100 | (11) | >100 | (2) |
| (*R*)-**1c** | >100 | (10) | >100 | (4) |
| (±)-**2c** | >100 | (20) | >100 | (11) |
| (*S*)-**1d** | >100 | (19) | >100 | (9) |
| (*R*)-**1d** | >100 | (11) | >100 | (6) |
| (±)-**2d** | >100 | (10) | >100 | (11) |
| (*S*)-**1e** | >100 | (26) | >100 | (13) |
| (*R*)-**1e** | >100 | (4) | >100 | (5) |
| (±)-**2e** | >100 | (21) | >100 | (17) |
| SN-38[a] | 0.0580 | | 0.304 | |
| CDDP[a] | 4.75 | | 10.0 | |

*a*)



SN-38

CDDP

(±)-**2e**, the difference of the activity between (±)-**1e** and (±)-**2e** was much smaller than that of the COX-1 inhibitory activity between these two acids. These results strongly indicate that introduction of fluorine into the 2-position of 2-arylpropionic acid structure affects the balance of COX-1 and COX-2 inhibition by lowering selectively the activity toward COX-1 (see the column of COX-1/-2 ratio in Table 1). Similar results were also observed for the other fluorinated acids (±)-**1a**, (±)-**1b**, and (±)-**1d** although their COX inhibitory activities were relatively small. It should be noted that (*R*)-**1b**, **c**, **e** strongly inhibited COX compared to (*S*)-**1b**, **c**, **e**. The relationship between the activities and the stereochemistry of **1a**—**e** also seemed to be the same as that reported for **2a**—**e**.[10—13] However, the effect of absolute stereochemistry on the inhibitory activities of the enantiomer of **1b** is greater than that of the corresponding acid **2b** (Table 1). Thus the introduction of fluorine into the 2-position of 2-arylpropionic acid is considered to contribute to the modification of balance of COX-1/COX-2 inhibitions, and the 2-aryl-2-fluoropropionic acid structural unit may be leads for effective profens with sufficient anti-inflammatory activity and without severe side effects such as gastrointestinal injury and increased risk of cardiovascular events caused by COX-1 and COX-2 inhibition, respectively. However, the observed potencies for COX-2 inhibition of series of **1a**—**e** are much lower than that of NS-398, one of the representative COX-2 selective NSAIDs, and retaining and/or increasing activity against COX-2 inhibition remain to be furthermore investigated.

We next investigated antiproliferative activities of (*R*)- and (*S*)-**1a**—**e** against A549 and HT-29 cancer cell lines from human lung and colon, respectively (Table 2). The non-fluorinated racemates (±)-**2a**—**e**, SN-38,[14] an active component of CPT-11, and cisplatin (CDDP) were also examined for reference. Since NSAIDs, including profens **2**, have been reported to have lower antiproliferative activities than the existing anticancer agents, quite high concentrations of NSAIDs are required to produce the sufficient activity to inhibit the proliferation of cancer cells.[5] Then we expected that introduction of fluorine into **2** would lead to increase of the antiproliferative activity and the activity of **2** would be superior or equal to that of the existing anticancer agents. Unfortunately, similar to the behavior of (±)-**2a**—**e**, (*R*)- and (*S*)-**1a**—**e** showed smaller antiproliferative activities than did SN-38 and CDDP. However, in the case of **1e**, considerable difference in the activities was observed between the enantiomers.

All of these data were obtained from preliminary experiments. It will be necessary to carry out *in vivo* examination to gain further information on the effectiveness of the chiral fluorinated structures as leads for further development.

## Experimental

2-Aryl-2-fluoropropionic acids **1** were prepared by our published procedure.[2] 2-Arylpropionic acids **2**, indomethacin, and NS-398 were commercially available. SN-38 and CDDP were prepared by Yakult Honsha (Tokyo, Japan).

**General Procedure for Measurement of COX Inhibitory Activities** COX-1 and COX-2 activities were measured with a chemiluminescent COX inhibitor screening assay system (Cayman chemical, Ann Arbor, MI, U.S.A.) according to the manufacturer's instructions. In brief, COX-1 (12.5 units/well) or COX-2 (9.5 units/well) was added to 0.1 mol/l Tris–HCl buffer (pH 8.0) containing 0.2 mmol/l KOH and 1 $\mu$M hematin. The mixture was pre-incubated with drugs at 37 °C for 15 min, and then a chemiluminescent reagent was added to the mixture. Reactions of COX-1 and COX-2 were ini-

tiated by adding arachidonic acid (20 $\mu$M). Chemiluminescence was measured for 20 s with a microplate luminometer (TR717, Applied Biosystems, Foster city, CA, U.S.A.). Percent inhibition of a test compound at each concentration was calculated on the basis of its area under the curve of chemiluminescence as compared with that of vehicle control.

**General Procedure for Measurement of Antiproliferative Activities against A549 and HT-29 Cancer Cell Lines** A549 and HT-29 cancer cell lines (obtained from American Type Culture Collection, Manassas, VA, U.S.A.) were maintained in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C under 5% $CO_2$ atmosphere. The cells were harvested by a solution of 0.25% trypsin and 1 mM EDTA, and resuspended in fresh media at a density of 40000 cells/ml. The resuspended cells were then seeded in Falcon® 96-well culture plates (2000 cells/well). After overnight incubation, fresh media with test compounds were added to the wells. Cell viability was determined after 48 h by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS) method. Absorbance was measured at 490 nm using a SPECTRA Max 250 mini plate reader (Molecular Devices, Sunnyvale, CA, U.S.A.). Cell growth at each drug concentration was expressed as percentage of untreated controls.

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