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In vitro antiproliferative activity against human colon cancer cell lines of representative 4-thiazolidinones. Part I☆

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Abstract—The characterization of two cyclooxygenase isoforms (COX), the rate-limiting enzyme for the synthesis of prostaglandins (PGs) from arachidonic acid, has allowed the development of COX-2 selective inhibitors as non-steroidal anti-inflammatory drugs (NSAIDs) with significant gastric tolerability. However, PGs are also important in cancer pathogenesis. Thus, there is an increasing interest in studying COX-2 inhibitors as potential drugs aimed at the prevention and treatment of cancer, especially colorectal cancer. The purpose of this study was to determine the inhibitory effects of some representative 4-thiazolidinones, already widely investigated as potential NSAIDs, on the growth of five human colon carcinoma cell lines with a different COX-2 expression, and to correlate them with COX-2 inhibitory properties. Our results preliminarily revealed that 2-phenylimino derivative **3** and 2,4-thiazolidindione **4** were the most active compounds. In particular, **3** mainly inhibited the HT29 cell line characterized by a high COX-2 expression, whereas **4** showed antiproliferative properties on all tested cell lines, suggesting molecular targets other than COX-2 inhibition.

Cyclooxygenase (COX) is a well-known enzyme that catalyzes the conversion of arachidonic acid to prostaglandins (PGs) in the cells. Two different isoforms have been discovered, COX-1 and COX-2, that catalyze the same chemical transformation but have a different genetic expression.[1] While constitutive COX-1 is present in most mammalian tissues and mediates the synthesis of PGs required for many physiological functions, such as maintenance of gastric and renal functions, vascular homeostasis,[2–4] COX-2 is mainly induced in response to many pro-inflammatory stimuli, cytochines, growth factors and mitogens.[5,6]

In addition, COX-2 is overexpressed in several human tumors, including colorectal, gastric, prostatic and bladder carcinomas. Besides, COX-2 upregulation in transformed cells and in tumors was reported to be associated with increased production of PGs, which prove to be important in cancer pathogenesis since they affect mitogenesis, cellular adhesion, immune surveillance and apoptosis.[7,8]

In recent years, epidemiological reports have indicated that the prolonged administration of non-steroidal anti-inflammatory drugs (NSAIDs) to people with familial adenomatous polyposis (FAP) results in a lower incidence of colorectal adenomas.[9] The mechanism of such an antiproliferative effect of NSAIDs remains to be clarified, but most hypotheses have been focused on their ability to reduce PG levels through the inhibition of COX-2.[10]

The expanding body of evidence as to the relationship between COX-2 upregulation and the onset of human colorectal carcinoma has encouraged us to investigate the in vitro antiproliferative activity of some representative 4-thiazolidinones (**1–5**) (belonging to some classes that we have already studied as potential NSAIDs) on cellular lines of human colorectal carcinoma (Fig. 1).[11–13]

In particular, we are now investigating (2*R*,2′*S*) 3,3′-(1,2-ethanediyl)bis[2-(4-methoxyphenyl)-4-thiazolidinone] (**1**) that displayed interesting anti-inflammatory

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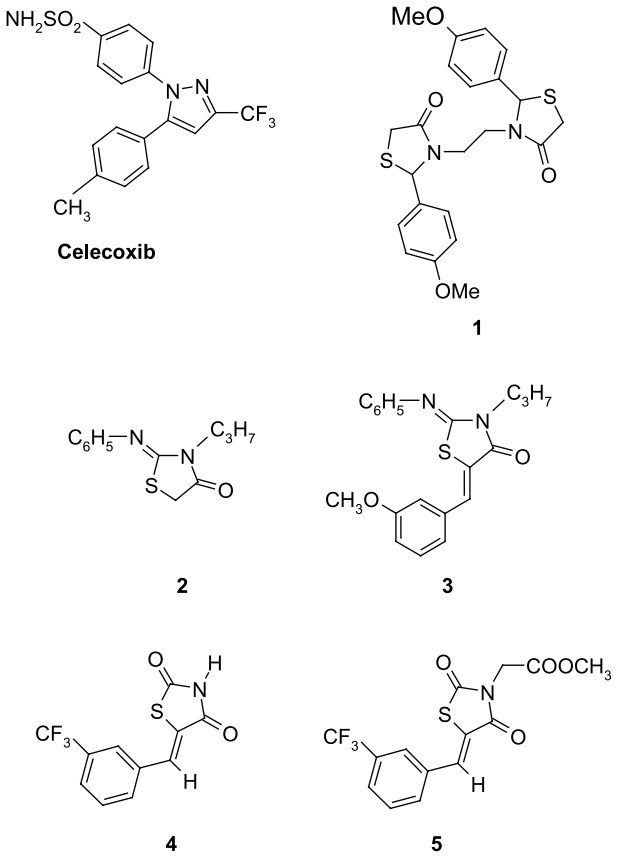

Figure 1.

properties with greater gastrointestinal safety than conventional NSAIDs.[14] It was found to preferentially inhibit inducible COX-2 with a IC_{50} COX-1/IC_{50} COX-2 ratio of about 50 (Table 1). Molecular modeling study allowed such an enzymatic inhibition to be conceivably rationalized.[13]

Based on an increasing knowledge about the structure of a COX-2 active site through co-crystallized complexes with known inhibitors,[15] we have thus designed and similarly explored some classes of simplified congeners of **1** to which compounds **2–5**, chosen as representative in the present paper, belong. In particular, 2-phenylimino-5-(3-methoxyphenylmethylidene)-3-propyl-4-thiazolidindione (**3**), which has a coxib-like structure (Fig. 1), showed significant in vivo anti-inflammatory activity along with COX-2 selective inhibition (Table 1).[16] Its parent compound **2** was inserted for comparison.

Table 1. In vitro assays for inhibition of COX-1 and COX-2 activity

Compound	COX-1	COX-2	COX-1/COX-2
Nimesulide[a]	17.4	0.60	29
1[a]	53.7	1.12	47.9
2[b]	8	0	—
3[b]	0	24	—
4[b]	0	0	—
5	n.d.	n.d.	—
Celecoxib[b]	62	93	—

Standard errors <10%.
n.d., not determined.
[a] Data are expressed as IC_{50} (μM); assay performed according to Ref. 19.
[b] Data are expressed as percentage of COX-1 and COX-2 inhibition at 10 μM; assay performed according to Ref. 18.

On the other hand, 5-(3-trifluoromethylbenzylidene)-2,4-thiazolidinedione (**4**) and [5-(3-trifluoromethylbenzylidene)-2,4-dioxothiazolidin-3-yl]acetic acid methyl ester (**5**),[17] although never assayed as anti-inflammatory agents, have been included in this study to acquire some suggestions about the possible relationships between structural modifications of the 4-thiazolidinone skeleton and antiproliferative activity against colorectal tumours.

COX-1 and COX-2 inhibition assays were carried out following reported procedures;[18,19] the results are given in Table 1.

The antiproliferative in vitro screening was performed on five cell lines of human colon cancers, such as DLD-1,[20] HCT-116,[21] HT29,[22] HCT-8[23] and H630,[24] obtained from the American Type Culture Collection (Manassas, VA); among them, HT29 cell line expresses high COX-2 levels[25] while, as far as we know, HCT-116 and DLD-1 do not express this isoform.[26,27]

The compounds were diluted in DMSO and tested at concentrations ranging from 0.001 to 100 μM. Growth inhibition was evaluated after continuous exposures of 72, 96 and 168 h, and also after exposures of 6, 24 and 72 h, followed by a 72 h, period in drug-free medium.[28]

The treatment of the five cell lines with all compounds did not cause a decrease in cell number with 6 h exposure (data not shown). Instead, the results obtained with the most prolonged exposure time (i.e., 168 h) were affected by the high cytotoxicity induced by DMSO (up to 80%) and thus not reported.

Therefore, Table 2 displays the results, expressed as IC_{50} values, relative to the other experimental times after having subtracted the cytotoxicity of DMSO at the same concentrations.

A clear correlation between concentration and cytotoxic effect was experimentally obtained in the 10–100 μM range. On the contrary, no correlation appeared between inhibition levels and exposure times in a 24–96 h range.

Compounds **1**, **2** and **5**, which did not display any activity, have not been included in Table 2. It is to be noted that bisthiazolidinone **1** had proved to be a good COX-2 inhibitory compound;[13] thus, we expected it to inhibit at least the COX-2 expressive HT29 cell line.

Only compounds **3** and **4** were active on a HT29 line (Table 2). In particular, compound **3**, a selective COX-2 inhibitor (Table 1), displayed inhibitory effect at 72 (IC_{50} = 64.1 μM) and 96 h (IC_{50} = 23.4 μM); interestingly with 72 + 72 h its efficacious dose proved to be halved with respect to that shown after 72 h. It also showed activity at 96 h exposure against DLD-1 and HCT-116, cell lines that do not express COX-2,[20,21] and

Table 2. Inhibitory effects, expressed as IC_{50} (μM), on the growth of human colon carcinoma cell lines at different exposure times

Cell line	Compound **3**				Compound **4**			
Time (h):	72	96	24 + 72	72 + 72	72	96	24 + 72	72 + 72
HCT-8	—	—	n.d.	n.d.	100	82.2	n.d.	n.d.
H630	—	—	n.d.	n.d.	97.6	83.2	n.d.	n.d.
DLD-1	—	13.1	—	—	37.4	17.8	40.1	31.4
HCT-116	—	34.2	—	—	48.8	45.5	91.7	36.8
HT29	64.1	23.4	92.8	36.2	38.8	59.7	71.7	31.4

—, >100; n.d., not determined.

reached good levels of activity, as indicated by its IC_{50} values (13.1 and 34.2 μM, respectively).

On the other hand, analogue **4**, which does not interact with COX enzymes, inhibited the growth of HT29 cells, reaching activity levels with IC_{50} ranging between 38.8 and 59.7 μM (exposure times 72 and 96 h), while regrowth (72 + 72 h) improved the inhibitory effect further. Compound **4**, differently from **3**, displayed activity on all cell lines, mainly on the DLD-1 (IC_{50} = 17.8 μM at 96 h).

On the contrary, as mentioned above, compound **2**, a weak COX-1 inhibitor, compound **1**, a preferential COX-2 inhibitor, and compound **5** proved to be totally inactive.

In conclusion, from a preliminary screening these results demonstrated that 5-(3-trifluoromethylbenzylidene)-2,4-thiazolidindione (**4**) exerted inhibitory effects on the growth of HT29 cell line at all exposure times, reaching the best level at 72 + 72 h regrowth. 2-Phenylimino-5-(3-methoxyphenylmethylidene)-3-propyl-4-thiazolidindione (**3**) on the same COX-expressing cell line proved to induce antiproliferative effects at all exposure times mainly after 96 h. They were also active on DLD-1 and HCT-116 cell lines but only after prolonged exposure without regrowth.

The observed antiproliferative profile of **3** might or might not be dependent on the expression status of COX-2 enzyme, in agreement with previous observations of some authors for other COX-2 inhibitors.[25–27,29] The mechanism of action of COX non-interfering compound **4**, which displayed the best antiproliferative profile on all cell lines, could be due to interaction with other unidentified cellular targets. Recently, AA demonstrated that some 2,4-thiazolidinediones, structurally similar to **4** and **5**, possess in vitro antiproliferative activity by acting as inhibitors of translation initiation process.[30]

Further studies are needed to clarify the mechanism of action of the active 4-thiazolidinones **3** and **4**, while other analogues will be similarly investigated and the SAR picture outlined. These forthcoming studies will be published in a future note.

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