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Structure–activity relationship of celecoxib and rofecoxib for the membrane permeabilizing activity

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) achieve their anti-inflammatory effect by inhibiting cyclooxygenase activity. We previously suggested that in addition to cyclooxygenase-inhibition at the gastric mucosa, NSAID-induced gastric mucosal cell death is required for the formation of NSAID-induced gastric lesions in vivo. We showed that celecoxib exhibited the most potent membrane permeabilizing activity among the NSAIDs tested. In contrast, we have found that the NSAID rofecoxib has very weak membrane permeabilizing activity. To understand the membrane permeabilizing activity of coxibs in terms of their structure–activity relationship, we separated the structures of celecoxib and rofecoxib into three parts, synthesized hybrid compounds by substitution of each of the parts, and examined the membrane permeabilizing activities of these hybrids. The results suggest that the sulfonamidophenyl subgroup of celecoxib or the methanesulfonylphenyl subgroup of rofecoxib is important for their potent or weak membrane permeabilizing activity, respectively. These findings provide important information for design and synthesis of new coxibs with lower membrane permeabilizing activity.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently used classes of medicines.[1] NSAIDs are inhibitors of cyclooxygenase (COX), a protein essential for the synthesis of prostaglandins (PGs), which have a strong ability to induce inflammation. However, NSAID use is associated with gastrointestinal complications, such as gastric ulcers and bleeding. In the United States, about 16,500 people per year die as a result of NSAID-associated gastrointestinal complications.[2] Thus, understanding the mechanism of NSAID-induced gastric lesions and its application to design and synthesis of new NSAIDs with reduced adverse effects on the gastric mucosa is important.

The inhibition of COX by NSAIDs was initially thought to be responsible for the adverse gastric side effects manifested by such treatment, because PGs have a strong protective effect on the gastric mucosa. Thus, after the identification of two subtypes of COX (COX-1 and COX-2), which are responsible for the majority of COX activity at the gastric mucosa and in inflammatory tissues, respectively,[3,4] selective COX-2 inhibitors (most of which are coxibs, such as celecoxib and rofecoxib) were developed as NSAIDs with reduced adverse gastric side effects.[5–7] However, due to the observation that rofecoxib was associated with an increased potential risk of cardiovascular thrombotic events,[8,9] this NSAID was withdrawn from the market. At first, this increased risk was believed to be due to the class effect of selective COX-2 inhibitors, because prostacyclin, a potent anti-aggregator of platelets and a vasodilator, is mainly produced by COX-2.[10–12] However, some clinical studies showed that the potential risk of cardiovascular thrombotic events was indistinguishable between celecoxib users and classic NSAID users.[13,14] Thus, it is possible that the increased potential risk of cardiovascular thrombotic events is not due to the class effect of selective COX-2 inhibitors, but rather is a specific characteristic of rofecoxib. While mechanisms to explain this rofecoxib-specific increase in the potential risk of cardiovascular thrombotic events have been proposed,[15–17] a definitive explanation for this increase has not yet been forthcoming.

It is now believed that the inhibition of COX by NSAIDs is not the sole explanation for the adverse gastric side effects of NSAIDs, given that the increased incidence of gastric lesions and the

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decrease in PG levels induced by NSAIDs do not always occur in parallel.[18–20] We proposed that, in addition to COX-inhibition at the gastric mucosa, NSAID-induced gastric mucosal cell death is required for the formation of NSAID-induced gastric lesions in vivo.[21,22] Furthermore, we reproduced NSAID-induce cell death in cultured gastric mucosal cells in vitro[22–26] and showed that the primary target of NSAIDs for the induction of cell death is the cytoplasmic membrane. Moreover, a close relationship between membrane permeabilizing activity and cell death-inducing activity among various NSAIDs was shown.[23,25] Thus, decreasing the membrane permeabilizing activity of NSAIDs may be another strategy to synthesize safer NSAIDs for the gastric mucosa. In fact, we recently reported that screening for NSAIDs with lower membrane permeabilizing activity resulted in the identification of an interesting new NSAID, fluoro-loxoprofen, which has much lower membrane permeabilizing and gastric ulcerogenic activities compared with clinically used NSAIDs.[27–31] These results suggest that NSAIDs with lower membrane permeabilizing activity could be therapeutically beneficial. Thus, it is important to understand how the membrane permeabilizing properties of NSAIDs are affected by their structure–activity relationship.

We previously reported that celecoxib showed the most potent membrane permeabilizing and cytotoxic activities among the NSAIDs we tested.[23,25] We also reported that the cytotoxic activity of rofecoxib is much lower than that of celecoxib.[21] As these results suggested that the membrane permeabilizing activity of rofecoxib is lower than that of celecoxib, our objective here was to confirm this hypothesis.

Furthermore, to identify how the structure–activity relationship of coxibs affects their membrane permeabilizing activity, we synthesized hybrid compounds from celecoxib and rofecoxib and examined their membrane permeabilizing activities. The results suggest that the sulfonamidophenyl subgroup of celecoxib and the methanesulfonylphenyl subgroup of rofecoxib are important for determining the membrane permeabilizing activities of these NSAIDs.

2. Chemistry

The synthetic route for target compounds **3**–**5** is outlined in Scheme 1. Pyrazole compounds **3**–**5** were synthesized by the condensation of appropriate 1,3-diketones and hydrazine. The reaction of 4,4,4-trifluoro-1-*p*-tolylbutane-1,3-dione **9** with 4-methylphenylhydrazine hydrochloride **11**, 4,4,4-trifluoro-1-phenylbutane-1,3-dione **10** with **11**, or **10** with 4-sulfamoylphenylhydrazine hydrochloride **12** afforded target compounds **3**, **4** or **5**, respectively.

The synthetic route for target compounds **6**–**8** is outlined in Scheme 2. Furanone compounds **6**–**8** were synthesized by the condensation of a phenylacetic acid analog and phenacyl bromide. The reaction of **13** with **15**, **14** with **17** or **14** with **16** in the presence of triethylamine afforded the phenacyl phenylacetate products **18**, **19** or **20**, respectively. Treatment of intermediates **18**–**20** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) provided 3,4-diphenyl-2(5*H*)furanone **21** or target compounds **7** or **8**. chlorosulfonylation of **21** by the reaction with chlorosulfonic acid followed by sulfonamidation using ammonium hydroxide gave target compound **6**.

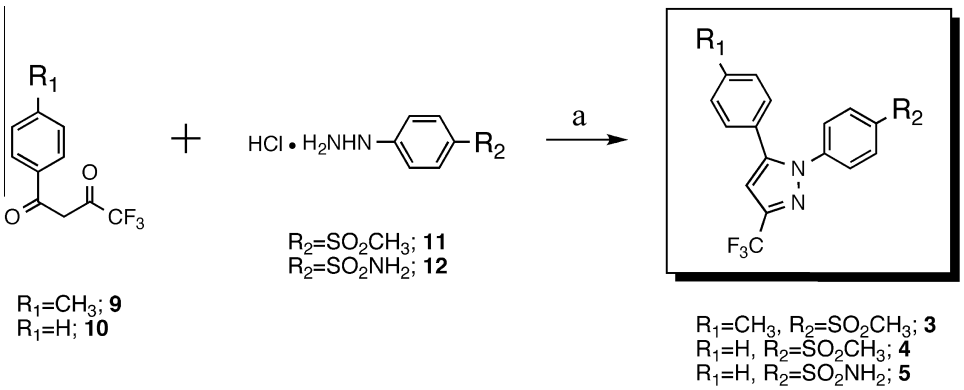
The final compounds were characterized by nuclear magnetic resonance (NMR), infrared spectroscopy (IR), high resolution mass spectra (HR-MS) and elemental analysis.

3. Results and discussion

The chemical structures of celecoxib and rofecoxib exhibit some similarities (Fig. 1) and can be divided into three parts (A–C in Table 1); part A, methylphenyl for celecoxib, phenyl for rofecoxib; part B, trifluoromethylpyrazole for celecoxib, furanone for rofecoxib; part C, sulfonamidophenyl for celecoxib, methanesulfonylphenyl for rofecoxib. Thus, in addition to celecoxib and rofecoxib, there are six possible combinations of these three parts that could be used to obtain hybrid compounds of celecoxib and rofecoxib (compounds **3**–**8** in Table 1). We synthesized these six compounds and tested their membrane permeabilizing and COX-inhibitory activities.

To begin with, we used calcein-loaded liposomes to compare the membrane permeabilizing activities of celecoxib and rofecoxib. As calcein fluorescence is very weak at high concentrations due to self-quenching, the addition of membrane-permeabilizing drugs to a medium containing calcein-loaded liposomes causes an increase in fluorescence by diluting the calcein.[25] As shown in Figure 2, celecoxib and rofecoxib increased the calcein fluorescence in a dose-dependent manner. Compared with celecoxib, however, a rofecoxib concentration about 100 times higher was required to increase the fluorescence by the same amount. Figure 2 shows that rofecoxib has a much lower membrane permeabilizing activity than celecoxib.

We next examined the membrane permeabilizing activities of the six hybrid compounds in a similar manner. As shown in Figure 3, all of the hybrid compounds increased the calcein fluorescence in a dose-dependent manner. To compare the membrane permeabilizing activity of these compounds, we used the EC_{50} (half-maximal effective concentration) index, which is defined as the concentration of each compound required for 50% of the calcein in loaded liposomes to be released (Table 2). Comparison of the EC_{50} index of **3**, **5** and **8** (compounds with one part substitution from celecoxib) showed that the membrane permeabilizing activity of **3** was much lower than that of **5** or **8**, suggesting that part



R_1=CH_3; **9**
R_1=H; **10**

R_2=SO_2CH_3; **11**
R_2=SO_2NH_2; **12**

R_1=CH_3, R_2=SO_2CH_3; **3**
R_1=H, R_2=SO_2CH_3; **4**
R_1=H, R_2=SO_2NH_2; **5**

Scheme 1. Synthesis of pyrazole compounds **3**–**5**.