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SUBSTITUTED HETEROCYCLIC ANALOGS AS SELECTIVE COX-2 INHIBITORS IN THE FLOSULIDE CLASS

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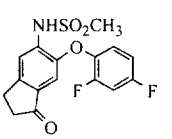
Abstract: Substituted heterocyclic analogs in the Flosulide class were investigated as potential selective cyclooxygenase-2 inhibitors. 6-(4-Ethyl-2-thiazolylthio)-5-methanesulfonamido-3*H*-isobenzofuran-1-one 14 was found to be the optimal compound in the series with superior in vitro and in vivo activities.

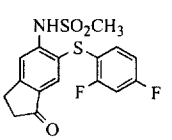
Introduction

The cyclooxygenase enzyme exists as two isoforms,[1] a constitutive form, COX-1 and an inducible form, COX-2. The COX-1 isoform is believed to be important in the maintenance of normal physiological functions, such as gastric cytoprotection and the COX-2 isoform can be induced by a wide variety of inflammatory mediators, and appears to play a major role in the production of prostaglandins associated with inflammation responses. It is likely that a selective COX-2 inhibitor will have useful antiinflammatory therapeutic effects without the ulcerogenic side effects associated with currently available nonsteroidal antiinflammatory drugs, all of which inhibit both COX-1 and COX-2.[2]

The aryl sulfonamide flosulide (CGP 28238) **1** is a potent and selective COX-2 inhibitor.[3] We have reported that the thioether analog of flosulide, **L-745,337**, is a potent and more selective COX-2 inhibitor, with superior pharmacokinetic and in vivo profiles.[4] In these earlier studies, the 2-pyridyl and 2-thiazolyl analogs were found to be slightly less potent in vitro than **L-745,337**.[4] In this communication, we report a more detailed SAR study undertaken for these two classes of heterocycles in the indanone and also in a related benzofuranone series and show that appropriate substitutions can improve both in vitro and in vivo potency.



Flusolide



L-745,337

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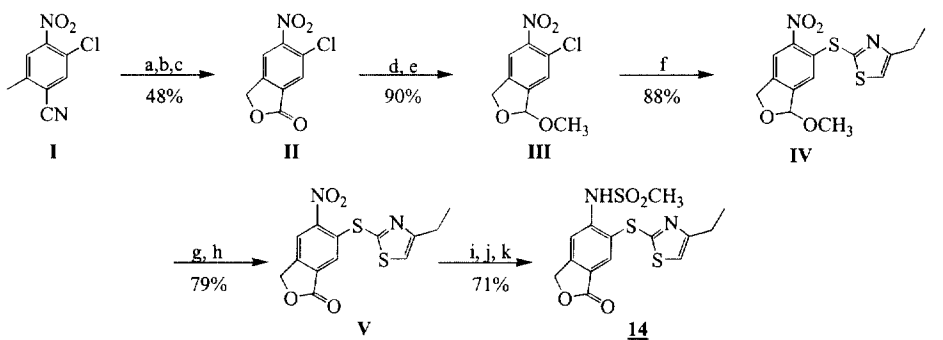
Chemistry

The indanone derivatives were prepared according to our reported route,[4] while the benzofuranone analogs were prepared as illustrated for the ethyl substituted thiazole **14** (Scheme 1). The known nitrotoluene **I**[5] was brominated and then selectively displaced by the acetate. After hydrolysis, the resulting alcohol was cyclized in situ to give the lactone **II**. It was necessary that the lactone be protected as an acetal such as **III** to prevent nucleoplilic displacement of the nitro group in the subsequent coupling reaction. The acetal **III** was then used as a common intermediate for the synthesis of other analogs. Displacement of the chlorine atom of compound **III** with the potassuim salt of the 2-mercapto thiazole proceeded in high yield to give the compound **IV**. Conventional deprotection and lactol oxidation provided intermediate **V**. The nitro group was then transformed to the sulfonamide **14** by standard reduction of the nitro with iron, bis-mesylation followed by selective hydrolysis.

Scheme 1

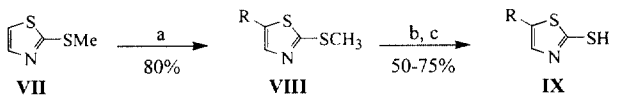


I II III IV V 14

(a) NBS PhCl, reflux; (b) NaOAc; (c) NaOH, then HCl; (d) DIBAH; (e) $HC(OCH_3)_3$, PPTS; (f) 4-ethyl-2-mercaptothiazole, KOH / DMF, 100°C; (g) HCl; (h) PDC; (i) Fe, NH_4Cl, EtOH:H_2O, reflux; (j) MsCl, Et_3N; (k) NaOH.

The required 4-substituted thiazoles were prepared according to a procedure described by Buchmann.[6] For the 5-alkyl substituted thiazole, a metalation procedure inspired by the work of Dondoni was used to introduce the substituent.[7] Using this approach (Scheme 2), the 2-thiomethylthiazole **VII** was deprotonated regioselectively at the 5-position, by simple addition of n-butyllithium at -60 °C followed by the addition of the corresponding electrophile. Yields were high with both primary and secondary alkyl halides as well as with simple aldehydes. Oxidation of the thioether **VIII** gave the sulfoxide intermediate which underwent Pummerer rearrangement with trifluoroacetic anhydride. The Pummerrer intermediate was then hydrolysed with a mixture of methanol and triethylamine to provide the 5-substituted-2-mercaptothiazole intermediate such as **IX.**[8]

Scheme 2



VII VIII IX

(a) nBuLi / THF, -60°C then R-X; (b) MMPP; (c) TFAA, Δ, then $(CH_3CH_2)_3N$: MeOH.

In the pyridine series, the mercapto pyridines analogs were synthesized from the nucleophilic displacements of 2-halopyridine with ethanethiol. The thioether intermediate was then converted to the corresponding thiol as described for the thiazole **VIII**, in Scheme 2.

Results and Discussion

Compounds were tested for the inhibition of purified recombinant human COX-2 and COX-1 activities.[9] Potent and selective compounds from these primary screens were then tested in a hCOX-2 transfected CHO (Chinese hamster ovary) cell assay.[10] Inhibition of COX-1 activity was also determined using a highly sensitive U937 microsomal assay at low arachidonic acid concentration.[11] All data represent the average of at least two determinations. Promising compounds were studied further in vivo using a rat paw edema assay[9] and a rat pyresis assay.[9]

Two series of 2-mercapto heterocycles, the pyridine and the thiazole, were studied in an attempt to improve the lead compound **L-745,337**. For the pyridine analogs, only the indanone series was studied. Halogen substitution at the 3/5 positions on the pyridine ring led to improved in vitro COX-2 inhibitor potency compared to the unsubstituted analog **3** or the methyl analog **4** (Table 1).

Table 1

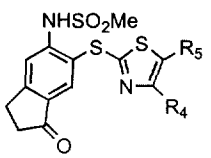
No.	R	Purified enzyme[9] COX-2 IC_{50} (μM)	Purified enzyme[9] COX-1 IC_{50} (μM)	CHO cells[10] COX-2 IC_{50} (μM)	U937 micr.[11] COX-1 IC_{50} (μM)
L-745,337		0.14	>100	0.029	1.6
3	H	0.81	>100	0.50	>30
4	5-CH_3	1.0	>100	0.64	>100
5	3-Cl	0.52	>100	0.22	6.1
6	**3,5-Cl_2**	**0.36**	**>100**	**0.12**	**4.9**

One of the most potent analog in this series, the 3,5-dichloropyridyl **6**, was tested in the rat paw edema assay. The ED_{30} in this assay was 0.7 mg/kg, but unfortunately this study also showed that inhibition of the edema

reached a plateau of approximately 50% inhibition between 1 and 3 mg/kg. The same trend of moderate in vivo efficacy was observed with other pyridine analogs and the series was not further investigated.

Our attention was then focused on the thiazole analogs. The SAR was extensively studied in the indanone and the benzofuranone series and was found to be very similar for both series. For the indanone analogs, the result of in vitro activity suggest that small alkyl substituents at the 4-position on the 2-mercapto thiazole ring improved COX-2 potency and substitutions at the 5-position decreased it (Table 2).

Table 2

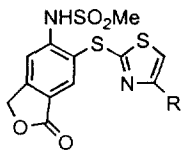


No.	R_4	R_5	Purified enzyme[9] COX-2 IC_{50} (μM)	Purified enzyme[9] COX-1 IC_{50} (μM)	CHO cells[10] COX-2 IC_{50} (μM)	U937micr.[11] COX-1 IC_{50} (μM)
7	H	H	0.48	>100	0.32	13
8	CH_3	H	0.38	>100	0.03	0.62
9	CH_3	CH_3	1.7	>100	>1.8	>100
10	H	CH_3	1.5	>100	0.87	>100
11	**CH_2CH_3**	**H**	**0.28**	**~100**	**0.049**	**14**

In this indanone series, the 4-ethylthiazolyl, **11**, had the best in vitro overall profile. The in vivo efficacy study showed an ED_{30} of 0.3 mg/kg in rat paw edema assay. Unfortunately, as in the pyridine series, a maximum of 43 % inhibition was observed at 3 mg/kg. In addition, compound **11** was less potent in the rat pyresis model, with an ED_{50} of 8.1 mg/kg (5 h post dosing). For these reasons, this compound was not studied further.

Our interest was subsequently shifted to a related benzofuranone series. In this case, the SAR was focused toward the size and the orientation of the 4-substituents on thiazole ring. Many larger 4-alkyl subtituents like propyl, isopropyl, *tert*-butyl, or phenyl were inactive against COX-2. Superior inhibitor activity and selectivity was observed with substituents having comparable size to the ethyl substituent **14**, as shown in Table 3.

Table 3



No.	R	Purified enzyme[9] COX-2 IC_{50} (μM)	Purified enzyme[9] COX-1 IC_{50} (μM)	CHO cells[10] COX-2 IC_{50} (μM)	U937 micr.[11] COX-1 IC_{50} (μM)
12	H	0.61	>100	0.027	1.3
13	CH_3	6.9	>100	0.043	0.35
14	**CH_2CH_3**	**1.0**	**>100**	**0.013**	**6.3**
15	CH_2CH_2	0.31	>100	0.14	12
16	SCH_3	0.29	>100	11.0	9.3

The 4-ethylthiazole **14** was the best analog prepared in the benzofuranone series. Compound **14** showed superior in vitro potency and selectivity than **L-745,337**. In the rat paw edema assay, an ED_{30} of 0.16 mg/kg was observed and this time, the ED_{50} was 0.93 mg/kg. At 3 mg/kg, 65% inhibition was obtained. Compound **14** was also active in rat pyresis with an ED_{50} of 2.7 mg/kg (5 h post dosing). Compound **14** was further studied in the rat ^{51}Cr gastrointestinal permeability assay,[9] to verify the hypothesis that a COX-2 selective compound would be GI sparing. Rats were dosed with compound **14** for 5 days at 100 mg/kg/day BID with no evidence of increased ^{51}Cr excretion in the feces, indicating a high level of GI tolerance.

Conclusion

We have shown that appropriately substituted 2-mercaptopyridine and 2-mercaptothiazole were suitable replacements of the thiophenol moiety of **L-745,337** in the flosulide class of COX-2 inhibitors. Although many of these compounds were equipotent to **L-745,337** in vitro, the potency didn't always translate well *in vivo*. However, from the numerous compounds prepared in this SAR study, we have identified compound **14** as a very potent antiinflammatory agent with a comparable in vitro and in vivo profile to **L-745,337**. The 6-(4-Ethyl-2-thiazolylthio)-5-methanesulfonamido-3*H*-isobenzofuran-1-one **14**, exhibited a high in vivo efficacy in rat models of pain and fever and no significant GI toxicity in rats.

Acknowledgments

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