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A NEW SERIES OF SELECTIVE COX-2 INHIBITORS: 5,6-DIARYLTHIAZOLO[3,2-b][1,2,4]TRIAZOLES

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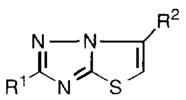
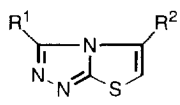
Abstract: A series of 5,6-diarylthiazolo[3,2-b][1,2,4]triazoles was prepared for evaluation of potency and selectivity against human COX-1 and COX-2 enzymes. This lead to the discovery of L-768,277, a potent and selective COX-2 inhibitor that also demonstrated good in vivo activity.

In the previous paper,[1] we reported the discovery of a series of diarylimidazolothiazole cyclooxygenase-2 (COX-2) inhibitors, as represented by L-766,112 (**1**). This compound was shown to be a potent, selective inhibitor in both in vitro and in vivo assays. Effort was also directed towards the synthesis of other fused 5,5 - heterocyclic templates that could give rise to selective COX-2 inhibitors having a better metabolic profile than L-766,112. In a recent report by Mazzone et al.[2] thiazolo[3,2-*b*][1,2,4]triazoles **A** and the isomeric thiazolo[2,3-*c*][1,2,4]triazoles **B** were shown to possess moderate antiinflammatory, analgesic, and antipyretic activitiy. However, no examples of 5,6-diaryl sustituted analogues were presented, particularly those containing a methyl sulfone moiety that is known to confer enhanced COX-2 activity and selectivity.[3] Based on recent knowledge of COX-1 and COX-2 inhibitors, we felt that appropriately substituted diaryl analogues would give enhanced activity against the enzyme if binding occurs in a similar manner to the known tricyclic compounds.[3-5]



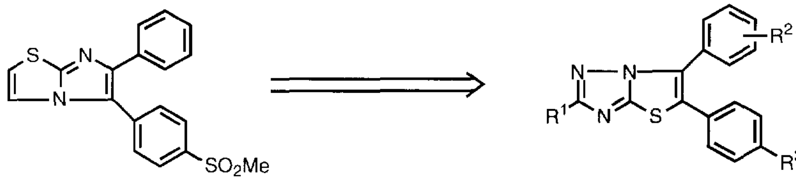
eg. R^1 = 3,4-(O-CH_2-O)-C_6H_3
R^2 = 4-Br-Ph



A **B**

The current paper describes our results with the diarylthiazolo [3,2-*b*][1,2,4]triazole system **2** (Figure 1), as well as two examples of the [2,3-c][1,2,4] system. Various analogues with different substitution patterns were prepared in order to study the structure activity relationship (SAR) of these series.

Figure 1

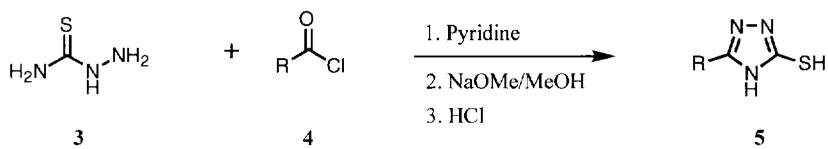


1 L-766,112 **2**

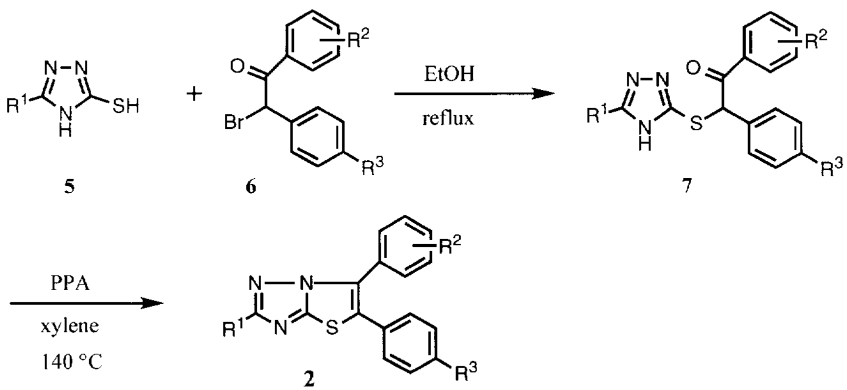
The 2-mercaptotriazoles used in the present work were either commercially available (R = H, CF_3) or were prepared as shown in Scheme 1.[6] Thiosemicarbazide **3** was treated with the appropriate acid chloride **4** in pyridine. The intermediate was isolated, and cyclization was effected with sodium methoxide/methanol. Treatment with HCl then liberated the desired mercaptotriazole in modest yield.

The thiazolo[3,2-*b*][1,2,4]triazole ring system was constructed as shown in Scheme 2. Compound **5** was condensed with diaryl bromoketone **6**[5,7] by refluxing overnight in EtOH. Removal of the solvent gave **7** which was cyclized by stirring overnight in a two-phase mixture of polyphosphoric acid (PPA) and xylene at 140 °C. After cooling to room temperature, the PPA was neutralized by careful addition of saturated $NaHCO_3$ solution.

Scheme 1



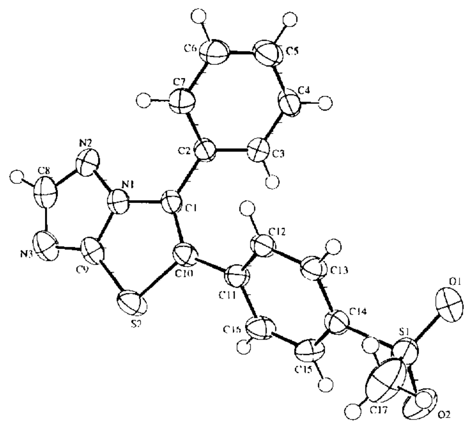
Scheme 2



The product was extracted with CH_2Cl_2 and the crude material was purified either by flash chromatography ($MeOH$:CH_2Cl_2) or by vigourous stirring in a mixture of CH_2Cl_2:ethyl acetate:hexane (2:1:2). The desired diaryl heterocycle was generally obtained in 50 to 80% yield. In order to prove definitively that the PPA cyclization had proceeded as described, crystals of compound **10** were submitted for X-ray crystallographic analysis. As can be seen in the ORTEP projection[8] (Figure 2), the regiochemistry of the cyclization was indeed consistent with that reported in the literature.[2]

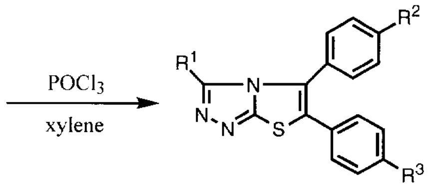
For comparison purposes, the isomeric thiazolo[2,3-*c*][1,2,4]triazoles **8** and **9** were prepared as described by Mazzone et al.[2] (Scheme 3). Intermediate **7** was treated with $POCl_3$/xylene under reflux to give the final product in moderate yield. In this case, the regiochemistry of the cyclization was determined by NOE experiments.

Figure 2. Perspective view of 10 generated by ORTEP-II using 50% probability elipsoids for the nonhydrogen atoms. The crystallographic labelling scheme is shown.



Scheme 3

7



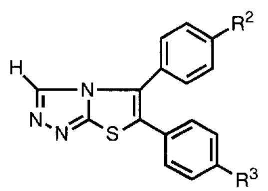
8 $R^1 = R^2 =$ H, $R^3 = SO_2Me$

9 $R^1 = R^3 =$ H, $R^2 = SO_2Me$

Discussion

Table 1 shows the IC_{50}s of the isomeric thiazolo[2,3-*c*][1,2,4]triazoles **8** and **9** in inhibiting COX-1 and COX-2 in chinese hamster ovary (CHO) cells,[9] as well as the ratio of COX-1/COX-2 to indicate selectivity. The two compounds were moderately potent against COX-2, with compound **8** showing good selectivity.

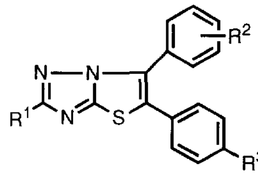
Table 1

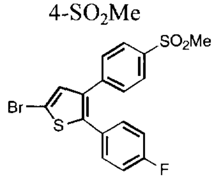
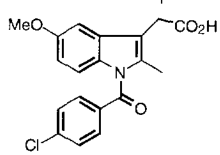


Compound	R^2	R^3	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX1/COX2
8	H	SO_2Me	>50	0.45	>108
9	SO_2Me	H	2.1	0.23	9

Table 2 shows the IC_{50}s of the thiazolo[3,2-*b*][1,2,4]triazole compounds. From these data it is clear that most of the analogues in this series are very potent against the COX-2 enzyme, with IC_{50}s varying from 0.003 to 0.08 μM. Exceptions are entries **20** (IC_{50} = 0.26 μM) and **25** (IC_{50} > 50 μM). Furthermore, good selectivity was observed in this series, especially in those analogues where R^1 was H, Me, or vinyl. A notable loss of selectivity was seen when R^1 was CF_3, Et, or isopropyl. When R^2 was 3,5-difluoro or 3-methoxy, reduced selectivity was also noted. As illustrated by compound **24**, the reverse methyl sulfone isomer was also very selective.

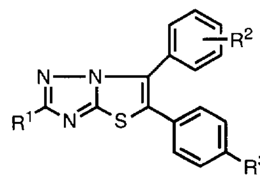
Table 2



Compound	R^1	R^2	R^3	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1/COX-2
10	H	H	SO_2Me	43	0.010	4300
11	Me	H	SO_2Me	>50	0.020	>2500
12	Et	H	SO_2Me	2.4	0.015	160
13	CF_3	H	SO_2Me	1.1	0.003	370
14	vinyl	H	SO_2Me	9.6	0.006	1600
15	i-Pr	H	SO_2Me	0.60	0.011	55
16	H	3-F	SO_2Me	>50	0.022	>2300
17	H	4-F	SO_2Me	15	0.036	420
18	H	3,4-di F	SO_2Me	30	0.048	630
19	H	3,5-di F	SO_2Me	>50	0.080	>630
20	H	3-OMe	SO_2Me	>50	0.26	>190
21	Me	3-F	SO_2Me	>50	0.15	>330
22	CF_3	3-F	SO_2Me	8.50	0.013	650
23	CF_3	3-Me	SO_2Me	0.61	0.003	200
24	H	4-SO_2Me	H	>50	0.032	>1600
25	CF_3	4-SO_2Me	H	9	>50	<0.18
DuP 697				0.059	0.002	28
Indomethacin				0.018	0.026	0.7

All of the compounds showing good selectivity were then tested in the COX-2 human whole blood assay,[10] which showed compound **10** (L-768,277) to be the most potent, with an IC_{50} of 2.3 μM (see Table 3). The low activity of compounds **18** and **24** in this assay may be due to protein binding in the plasma.

Table 3



Compound	R^1	R^2	R^3	COX-2 IC_{50} (μM)	COX-2 human whole blood IC_{50} (μM)
10	H	H	SO_2Me	0.010	2.3
11	Me	H	SO_2Me	0.020	10
14	vinyl	H	SO_2Me	0.006	4.1
16	H	3-F	SO_2Me	0.022	4.2
18	H	3,4-di F	SO_2Me	0.048	>30
21	Me	3-F	SO_2Me	0.15	14
24	H	4-SO_2Me	H	0.032	>30
DuP 697					0.06
Indomethacin					0.46

L-768,277 has good pharmacokinetics in the rat with a c_{max} of 7.2 μM at 4 h after a single dose of 5 mg/kg PO. The oral bioavailability is ~100% with a clearance of 13 mL/min/kg. The good intrinsic activity of L-768,277 combined with its bioavailability made this compound a good candidate for further in vivo studies. The compound is potent in the rat paw edema model[11] (ED_{50} = 1.7 mg/kg), the rat pyresis assay[11] (ED_{50} = 1.0 mg/kg), and the rat hyperalgesia assay[11] (ID_{50} = 1.0 mg/kg). The low COX-1 activity of L-768,277 was also reflected in the rat ^{51}Cr assay,[11] where no chromium leakage was observed after 5 days of treatment at 100 mg/kg bid.

As well, in vitro studies with rhesus monkey liver microsomes have shown that L-768,277 is much less prone to metabolism, giving a 95% recovery of the parent compound after a typical 1 h incubation.[12] A study carried out with freshly prepared rat hepatocytes showed a >95% recovery of L-768,277 after a 3 h incubation, as compared to <10% recovery for L-766,112 under the same conditions.

In conclusion, the SAR obtained in the thiazolotriazole series has lead to the discovery of L-768,277, a potent and selective oral COX-2 inhibitor. The in vivo efficacy observed with L-768,277 is similar to that of indomethacin,[10] but unlike indomethacin it appears to be free of gastrointestinal side effects. The compound also demonstrates excellent pharmacokinetic and metabolic properties.

References and Notes

1. See preceding two papers.

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8. Crystal structure details: $C_{17}H_{13}N_3O_2S_2$, M_r = 355.440, monoclinic space group $P2_1/n$, a = 9.8526(7), b = 11.534(1), c = 15.0139(9) Å, β = 109.0(3)°, V = 1613(3) Å^3, Z = 4, D_x = 1.463 g cm^{-3}, monochromatized radiation λ(Cu K_α) = 1.541838 Å, μ =3.07 mm^{-1}, F(000) = 736, T = 294 K. Data were collected on a Rigaku AFC5 diffactometer to a θ limit of 71°. There are 3196 unique reflections out of 3380 measured with 1656 observed at the $I \geq 3\sigma(I)$ level. The structure was solved by direct methods (SHELXS*) and refined using full-matrix least-squares (SDP**) on F using 218 parameters and the observed data. All nonhydrogen atoms were refined with anisotropic thermal displacements and the H atoms were included at their calculated positions. Final agreement statistics are: R = 0.045, wR = 0.045, S = 1.77, $(\Delta/\sigma)_{max}$ = 0.02. Weighting scheme is $1/\sigma^2(F)$. The maximum peak height in final difference Fourier map is 0.24(6) eÅ^{-3} and it has no chemical significance. The authors have deposited the atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK. *Sheldrick, G.M., *Acta Crystallogr.* **1990**, *A46*, 467-473. **Structure Determination Package Ver. 3, Enraf-Nonius, Delft, 1985.

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12. See preceding paper.

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