Bioorganic & Medicinal Chemistry Letters 19 (2009) 459–461

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of dithiolethiones as novel cyclooxygenase inhibitors

Shannon D. Zanatta [a,b], David T. Manallack [c], Bevyn Jarrott [a,d], Spencer J. Williams [b,*]

[a] Howard Florey Institute, Parkville, Vic. 3010, Australia
[b] School of Chemistry and Bio21 Molecular Science and Biotechnology Institute, University of Melbourne, 30 Flemington Rd, Parkville, Vic. 3010, Australia
[c] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Pde Parkville, Vic. 3052, Australia
[d] Department of Pharmacology, University of Melbourne, Parkville, Vic. 3010, Australia

ARTICLE INFO

Article history:
Received 16 September 2008
Revised 12 November 2008
Accepted 13 November 2008
Available online 18 November 2008

Keywords:
Cyclooxygenase
Dithiolethiones
Inhibitor
Modelling

ABSTRACT

3*H*-1,2-Dithiole-3-thiones substituted with a 3,5-di-*tert*-butyl-4-hydroxyphenyl (DTBHP) or a 3,5-di-*tert*-butyl-4-methoxyphenyl group at the C5 position were prepared and their ability to inhibit the cyclooxygenase isoenzymes, COX-1 and COX-2 was evaluated. Both compounds were potent inhibitors of COX-2 (relative to rofecoxib), and while the phenol was a weak inhibitor of COX-1, the methyl ether gave no measurable inhibition. Docking studies of the two compounds into the COX-1 and -2 active sites showed that the methyl ether could only fit in the COX-2 active site whereas the phenol could be docked into both COX-1 and -2. This study reports a new mode for inhibitor binding to COX-1 and -2 and a novel structural scaffold for the development of COX-2 selective inhibitors.

Inflammation is a major cause of pain and contributes to tissue injury and dysfunction, in particular for arthritis and inflammatory bowel syndrome.[1] Inflammation has also been implicated in the pathogenesis of neurodegenerative conditions such as Alzheimer's and Parkinson's diseases.[2] Non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin and diclofenac) are some of the most commonly used and prescribed drugs. Most NSAIDs prevent the synthesis of prostaglandins through inhibiting the constitutive enzyme cyclooxygenase-1 (COX-1) as well as cyclooxygenase-2 (COX-2), an inducible enzyme that is abundant in activated macrophages and other cells at sites of inflammation. Associated with their clinical use are side effects that include gastrointestinal ulceration, bleeding and impaired renal function. Non-selective inhibition of COX enzymes has been highlighted as a potential cause of these adverse side effects, as COX-1 is responsible for the synthesis of endogenous prostaglandins that are believed to be important for maintenance of gastric mucosa.[3] A series of COX-2 selective inhibitors, the coxibs (eg celecoxib and rofecoxib) were developed and introduced over the last 10 years. Coxibs have a reduced incidence of gastrointestinal ulceration and bleeding; however, there have been recent concerns over cardiovascular adverse effects of rofecoxib.[4] These concerns have resulted in all but one coxib, celecoxib, being voluntarily withdrawn from the market. Some studies have suggested that rofecoxib's adverse cardiac events may not be a class effect but rather an intrinsic chemical property related to its metabolism.[5] For this reason novel scaffolds with COX-2 selective inhibitory activity need to be found and evaluated for their anti-inflammatory effects.

It has been demonstrated that the 3,5-di-*tert*-butyl-4-hydroxyphenyl containing drug **1** is a potent anti-inflammatory, which acts by dual inhibition of COX-2 and lipooxygenase-5 (Fig. 1).[6] As well, conjugates of non-selective NSAIDs with hydrogen sulfide releasing dithiolethiones such as the diclofenac ester **2**, displayed enhanced anti-inflammatory activities and reduced gastrointestinal toxicity.[7] It was suggested that release of hydrogen sulfide from the dithiolethione fragment might interfere with early stages of inflammation. Hydrogen sulfide has been identified as an important signaling molecule in the stomach and nervous system and acts to suppress adherence of leukocytes to the vascular

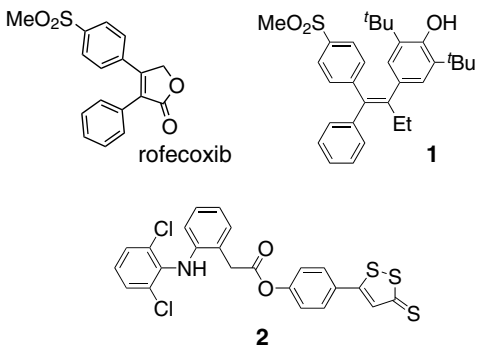

Figure 1. Assorted non-steroidal anti-inflammatory drugs.

* Corresponding author. Tel.: +61 3 8344 2422; fax: +61 3 9347 8124.
E-mail address: sjwill@unimelb.edu.au (S.J. Williams).

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doi:10.1016/j.bmcl.2008.11.045

endothelium and their subsequent migration into underlying tissue.[7] Accordingly, these two studies led us to ask whether a hybrid 3*H*-1,2-dithiole-3-thione bearing a 3,5-di-*tert*-butylhydroxyphenyl (DTBHP) group at the C5 position might act as novel COX inhibitors.

We targeted the synthesis of **3**, which bears a dithiolethione group directly joined to the 4-position of the di-*tert*-butylphenol, and its methyl ether **4**. Compounds **3** and **4** were prepared from 2,6-di-*tert*-butylphenol as shown in Scheme 1. 2,6-Di-*tert*-butylphenol was converted to the acetophenone **5** by treatment with acetic acid and trifluoroacetic anhydride.[8] Towards **3**, the phenol of **5** was protected by a MOM (methoxymethyl) group by treatment with MOMCl, prepared in situ from dimethoxymethane, acetyl chloride and $ZnCl_2$ according to Berliner,[9] affording the acetal **6**. Methoxycarbonylation[10] of **6** using dimethyl carbonate in the presence of NaH afforded the β-ketoester **7**. Treatment of **7** with phosphorus pentasulfide (P_4S_{10}) and elemental sulfur in the presence of hexamethyldisiloxane according to Curphey[11] smoothly afforded the dithiolethione **3**[12] with concomitant removal of the MOM group. Towards **4**, the phenol was converted to the methyl ether **8** by treatment with methyl iodide and potassium carbonate. Methoxycarbonylation of **8** as above afforded the β-ketoester **9**. Treatment of **9** with P_4S_{10} and elemental sulfur in the presence of hexamethyldisiloxane afforded the dithiolethione **4**.[13]

The potencies of **3** and **4** as inhibitors of COX-1 and COX-2 enzymes were determined using microsomal COX-1 isolated from human platelets and recombinant human COX-2 expressed in Sf21 insect cells (Table 1).[14] These data reveal that both compounds are potent inhibitors of COX-2 (relative to rofecoxib), and exhibit good selectivity relative to COX-1. Most interestingly, modification of **3** with a methyl group gives **4**, which no longer inhibits COX-1 activity up to a concentration of 30 μM.

To understand the binding of compounds **3** and **4** to COX-1 and COX-2 we performed an initial docking study using Glide[15,16] within the Maestro package.[17]

This preliminary experiment revealed that compounds **3** and **4** could only bind into the COX-2 structure. However, if the van der Waals (VDW) radii were reduced to below 0.8 Å during the docking run then it was possible to fit each compound into COX-1, highlighting the smaller size of this binding site.

In order to more fully investigate the docking of **3** and **4** we allowed protein flexibility within the docking protocol. Table 2 shows the results of the induced fit docking experiment giving the best energy pose for each case. Each docking experiment provided multiple ligand poses; however, these were essentially identical to one another. The docking studies demonstrate that compound **4** is unable to fit into the COX-1 binding cavity unless the VDW constraints are relaxed.

Table 1
COX-1 and COX-2 inhibition of **3**, **4** rofecoxib and indomethacin

Compound	IC_{50} (nM)	
	COX-1	COX-2
3	3640	7.2
4	>30,000	47.2
Rofecoxib	–	74.5
Indomethacin	28.2	–

Table 2
Glide scores for binding of **3** and **4** to COX-1 and COX-2

Compound	GlideScore	
	1ht5[a] (COX-1)	4cox[a] (COX-2)
3	−14.50	−14.48
4	Unable to dock	−14.08

[a] Protein data bank ID.

Broadly speaking, the inability of compound **4** to dock into COX-1 without relaxation of VDW constraints, and the ability of **3** to dock without such constraint relaxation, are in agreement with the enzyme inhibition study.

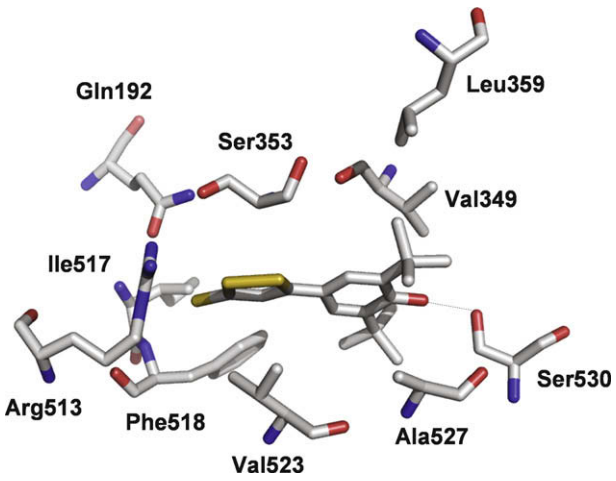
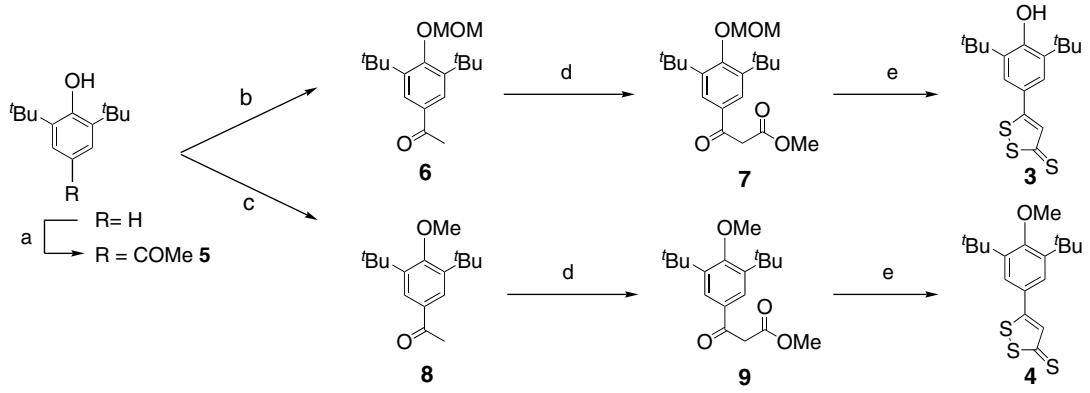


Figure 2. Diagram showing selected residues of COX-2 adjacent to compound **3**.



Scheme 1. Reagents and conditions: (a) TFAA, HOAc, 85%; (b) (i) AcCl, $(MeO)_2CH_2$, $ZnCl_2$, toluene (ii) **5**, $(iPr)_2EtN$, 71%; (c) MeI, K_2CO_3, acetone, 83%; (d) Me_2CO_3, NaH, THF, 97%; (e) P_4S_{10}, S, $(Me_3Si)_2O$, 43%.

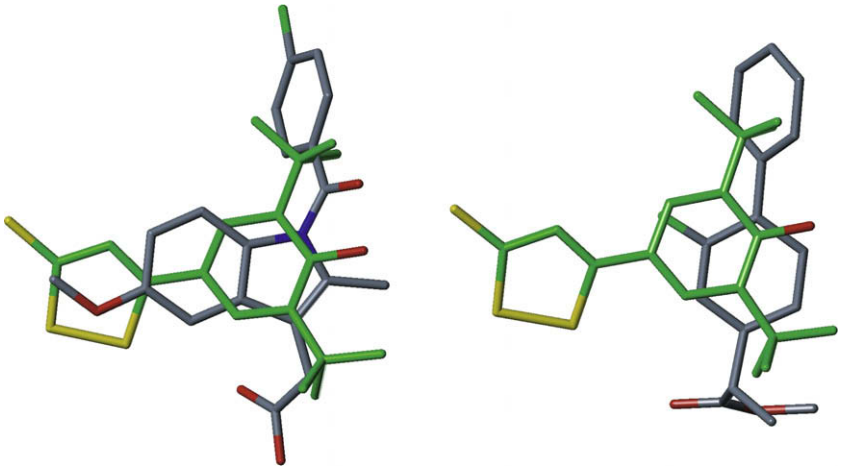


Figure 3. Left: Overlay of indomethacin (carbon atoms colored grey) and **3** (carbon atoms colored green) in the binding site of COX-2; Right: Overlay of flurbiprofen methyl ester (carbon atoms colored grey) and **3** (carbon atoms colored green) in the binding site of COX-1.

Examination of the binding modes of compounds **3** and **4** into COX-1 and COX-2 reveals them to be almost identical. The only significant difference was seen for compound **4** where R513 was moved allowing binding to occur. In all cases the oxygen atom made a hydrogen bond with S530, the length of which varied from 2.82 to 3.39 Å. Figure 2 shows a diagram of the residues in close proximity to compound **3** highlighting the hydrogen bond with S530

An overlay of compound **3** with the bound structure of indomethacin reveals that the dithiolethione ring coincides with the location of the methoxy group of indomethacin while the *tert*-butyl groups are placed in a similar location to the ethanoic acid and chlorophenyl side chains of indomethacin (Fig. 3). This figure also shows the overlay of compound **3** with flurbiprofen methyl ester (FME). In this case the dithiolethione ring does not overlap with the other structure while the *tert*-butyl groups are oriented towards the binding locations of the propanoate and 4-phenyl side chains of FME.

In conclusion we have reported the design and discovery of two novel dithiolethiones that display potent anti-COX-2 activity. Molecular modelling of these compounds in the active sites of COX-1 and COX-2 provide a good explanation for their selectivity. These compounds are interesting lead structures for the development of hydrogen sulfide releasing anti-inflammatory drugs.

Acknowledgments

We are grateful to Kamani R. Subasinghe for early synthetic studies towards **3** and **4**. We acknowledge the support of the National Health and Medical Research Council of Australia and the Australian Research Council.

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12. *Compound **3**:* The crude reaction mixture was applied directly to silica gel and purified by flash chromatography (5% EtOAc/petrol). The residue was recrystallised from EtOAc/petrol to afford 5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione **3** as a yellow-brown solid (540 mg, 43%); mp 180–183 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.47 (s, 18H, *t*-Bu ×2), 5.72 (s, 1H, OH), 7.41 (s, 1H, H4), 7.48 (s, 2H, Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.3 (**C**$(CH_3)_3$), 34.8 (CH_3), 123.5 (C4), 124.5 (C5), 134.7, 137.5, 158.1, 175.1 (Ar), 215.2 (CS); IR ν 3429, 2960, 1593, 1514, 1419, 889, 715 cm^{-1}; HRMS ESI$^+$ $[M+H]^+$ = 339.0908, requires 339.0911 for $C_{17}H_{22}OS_3$; Microanalysis: Found C, 60.37; H, 6.65. $C_{17}H_{22}OS_3$, requires C, 60.31; H, 6.55%.
13. *Compound **4**:* The crude reaction mixture was applied directly to silica gel and purified by flash chromatography (5% EtOAc/petrol). The residue was recrystallised from EtOAc/petrol to afford 5-(3,5-di-*tert*-butyl-4-methoxyphenyl)-3*H*-1,2-dithiole-3-thione **4** as an orange solid, (117 mg, 51%); mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 18H, *t*-Bu ×2), 3.74 (s, 3H, OCH_3), 7.41 (s, 1H, H4), 7.53 (s, 2H, Ar); ^{13}C NMR (100 MHz, $CDCl_3$) δ 31.8 (**C**$(CH_3)_3$), 36.2 (CH_3), 64.6 (OCH_3), 125.5 (C4), 126.2 (C5), 135.2, 145.6, 163.5, 174.2 (Ar), 215.2 (CS); IR ν 2948, 2865, 1744, 1587, 1498, 1304, 1110, 782 cm^{-1}; HRMS ESI$^+$ $[M+H]^+$ = 353.1063, requires 353.1062 for $C_{18}H_{24}OS_3$; Microanalysis: Found C, 61.33; H, 6.85; S, 27.34. $C_{18}H_{23}OS_3$, requires C, 61.32; H, 6.86; S, 27.28%.
14. *COX-1 and COX-2 assays:* Aliquots either of a microsomal preparation of human platelets or insect Sf21 cells were preincubated with dilutions of the compounds in 1% DMSO for 15 min at 37 °C. The enzyme reaction was started by the addition of arachidonic acid (100 μM for COX-1 or 0.3 μM for COX-2) and incubated for 15 min at 37 °C. The reaction was then stopped and the amount of prostaglandin E_2 formed was quantitated using an ELISA assay.
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