Bioorganic & Medicinal Chemistry Letters 19 (2009) 4509–4514

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification and optimisation of a novel series of pyrimidine based cyclooxygenase-2 (COX-2) inhibitors. Utilisation of a biotransformation approach

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ARTICLE INFO

Article history:
Received 28 November 2008
Revised 2 February 2009
Accepted 2 February 2009
Available online 27 February 2009

Keywords:
Cyclooxygenase
COX-2
Pyrimidine
Biotransformation

ABSTRACT

Many years of work have been invested in the identification of potent and selective COX-2 inhibitors for the treatment of chronic inflammatory pain. One issue faced by workers is the balance between the lipophilicity required for potent enzyme inhibition and the physical properties necessary for drug absorption and distribution in vivo. Frequently approaches to reduce lipophilicity through introduction of polar functionality is hampered by highly challenging chemistry to prepare key molecules. We have complemented traditional synthetic chemistry with a biotransformations approach which efficiently provided access to an array of key target molecules.

At the outset of our work the majority of selective COX-2 inhibitors reported were 1,2 diaryl heterocycles, for example, DUP6971 (**1**)[1] and SC58152 (**2**)[2] (Fig. 1). The core heterocycle was most commonly a five-membered ring with only a few examples of six-membered cores being reported. We were interested in investigating a series of pyrimidines **6**. Pyrimidine being the core of choice as the two nitrogen atoms help to control the lipophilicity of the final molecule.

Compounds were prepared by the general route shown in Scheme 1. Perkin condensation of an appropriately substituted phenyl acetic acid with a benzaldehyde gave the intermediate acid **3**. The acids were efficiently converted to the corresponding ketones **4** by a Curtius reaction, and concomitant chlorination and Vilsmeir formylation gave the versatile chloroaldehyde intermediates **5** which were condensed with amidines to give the final compounds **6**.

The first two compounds prepared were the two isomeric benzyl derivatives (**6a** and **6b**, R = benzyl). Encouragingly both isomers had measurable activity at COX-2, but did not inhibit COX-1.

Encouraged by this result we decided to investigate further structure activity relationships (SAR) around this novel template, initially focusing on modification of, or replacement of the benzyl group R. The SAR observed were similar in each isomeric series and in this paper we will focus on the series represented by **6a**. Data from an initial set of compounds is shown in Table 1. All of these molecules were prepared by the general route depicted in Scheme 1.

Substitution of the benzyl unit with hydrophobic groups was generally well tolerated as illustrated by compounds **6b** and **6c**. Heterocyclic replacements were accepted, the optimal activity residing in the less polar rings (cf. compounds **6h** and **6i**). Removal of the benzyl group and replacement with hydrogen or smaller alkyl entities resulted in a loss of activity (compounds **6d–g**), as did increasing the length of the linker (compound **6j**). Encouragingly saturated carbocyclic rings resulted in compounds of increased potency (compound **6k**).

In addition to SAR around the benzyl group we also examined the effect of replacing the linking methylene group with a heteroatom. The molecules were prepared by a modification of the route shown in Scheme 1 (Scheme 2).

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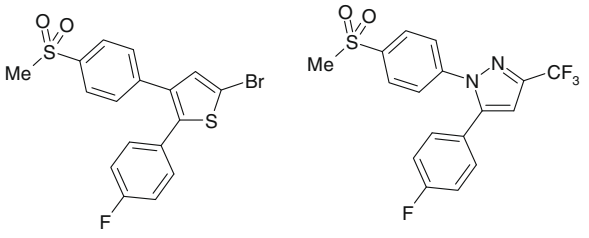
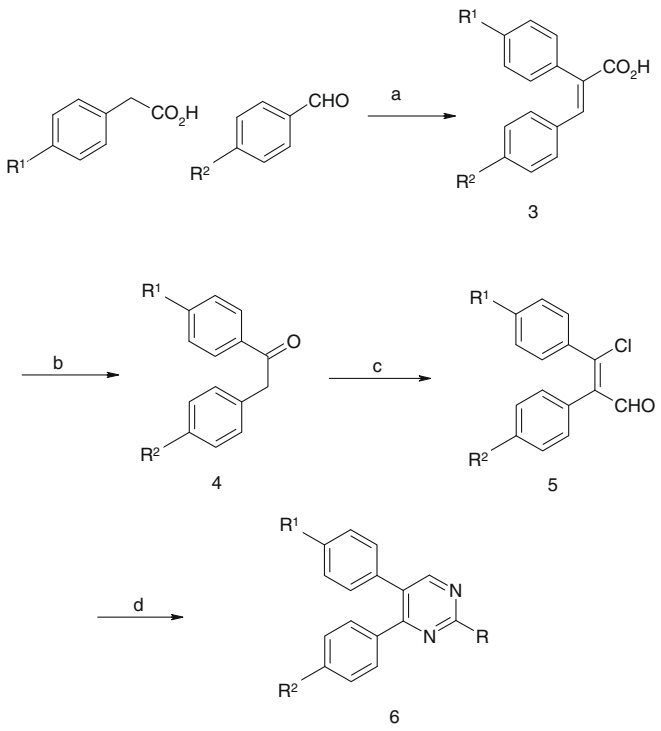
doi:10.1016/j.bmcl.2009.02.089

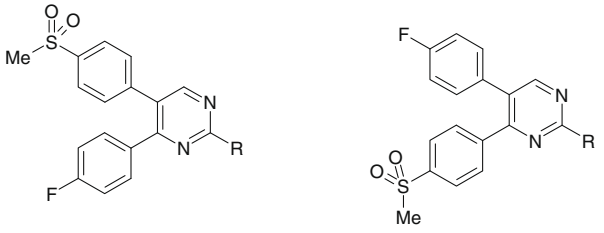


(1) (2)

Figure 1. Examples of early selective COX2 inhibitors.



Scheme 1. Reagents: (a) Acetic anhydride, sodium acetate, reflux; (b) (i) $SOCl_2$; (ii) NaN_3, toluene; (iii) Δ; (iv) HCl, H_2O; (c) $POCl_3$, DMF; (d) amidine, MeCN.



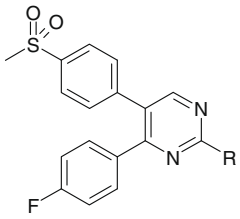
6a
COX-2 IC_{50} = 104nM
COX-1 IC_{50} = >34,000nM

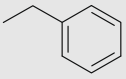
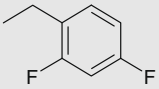
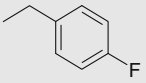
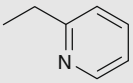
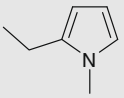
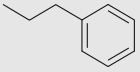
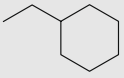
6b
COX-2 IC_{50} = 266nM
COX-1 IC_{50} = >100,000nM

The chloroaldehyde **5** was condensed with *S*-methylisothiourea to give the 2-methylthiopyrimidine **7**, this was oxidised to the corresponding sulfone **8**, which when treated with a variety of nucleophiles and was readily displaced to give the final compounds **9** activities are shown in Table 2.

Replacing the methylene with a nitrogen linker was generally well tolerated (**6l** and **6o**), however oxygen and sulfur linkers resulted in a loss of activity (**6m** and **6n**).

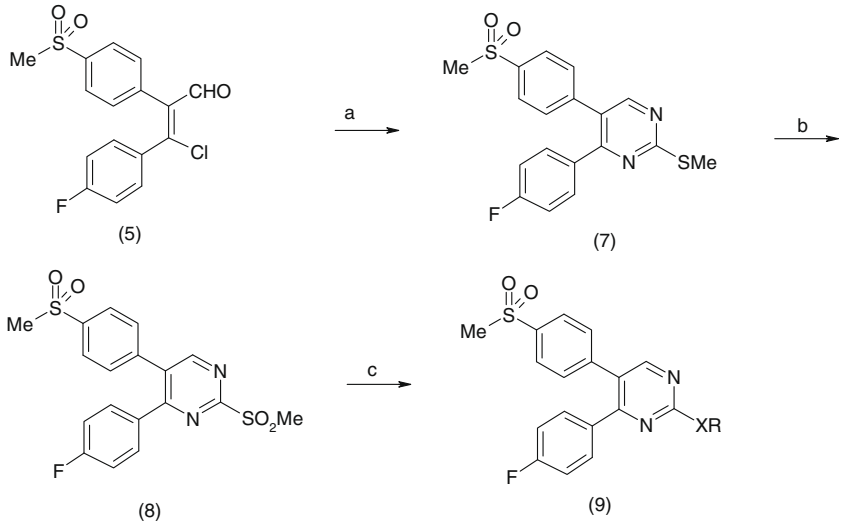
Table 1
COX-2 inhibition and selectivity data for initial set of compounds



Compound	R	COX-1[b] IC_{50} (nM)	COX-2[a] IC_{50} (nM)	Selectivity[c]
6a		>34,000	104	>326
6b		>52,124	114	>457
6c		>100,000	277	>361
6d	H	NSE	NSE	–
6e	Me	NSE	NSE	–
6f	*i*Pr	10,000	1135	8.8
6g	CH_2CONH_2	NSE	NSE	–
6h		>100,000	6323	>16
6i		6541	23	284
6j		29,371	5263	5.6
6k		2969	12	247
1	–	870	2	435
2	–	32,000	66	485

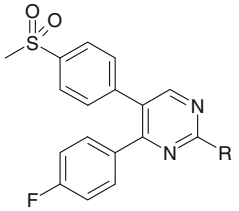
[a] IC_{50} values for inhibition of PGE2 produced by arachidonic acid stimulated COS cells stably expressing human COX-2 as described in Ref. 7.
[b] IC_{50} values for inhibition of PGE2 produced by arachidonic acid stimulated COS cells stably expressing human COX-1 as described in Ref. 7.
[c] IC_{50} value for COX-1 divided by IC_{50} value against COX-2.

We were encouraged by the activity seen amongst key members of this novel series of inhibitors, however when the more potent examples were studied in vivo poor efficacy was observed when compared with standard molecules. We had previously observed in other series of COX-2 inhibitors that high lipophilicity often impaired in vivo efficacy[3], and thus focussed the chemistry on synthesising analogues with reduced lipophilicity. Introducing polar groups around the template proved chemically challenging. We were particularly interested in hydroxylating the methylene linker of the original benzyl compound **6a**, to give compound **10**, as observations in related series suggested that polar groups were generally poorly tolerated, but we hypothesized that the potential for internal hydrogen bonding may 'mask' the polarity of the hydroxyl group in **10** thus retaining potency. We were initially unable to prepare this compound by conventional synthetic methodology.



Scheme 2. Reagents and conditions: (a) *S*-Methylisothiourea, K_2CO_3, MeCN, reflux; (b) Oxone®, MeOH, H_2O, rt; (c) RX, MeCN, rt.

Table 2
COX2 inhibition and selectivity data for compounds with a modified linker



Compound	R	COX-1[b] IC_{50} (nM)	COX-2[a] IC_{50} (nM)	Selectivity[c]
6l	NHPh	>100,000	56	>1785
6m	OPh	>100,000	>4640	—
6n	SPh	>100,000	>7200	—
6o	N(Me)Ph	4340	85	51
6p	NHBenzyl	>100,000	686	>145

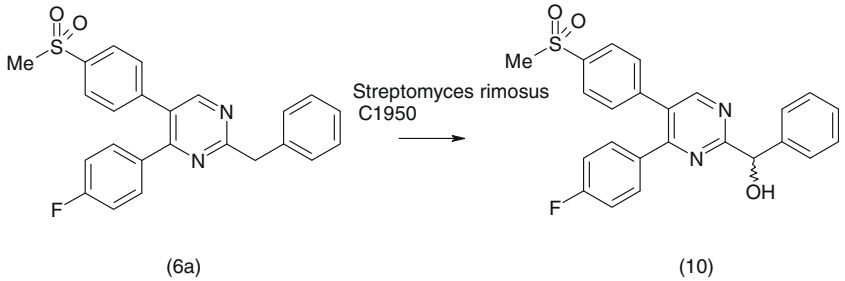
[a] IC_{50} values for inhibition of PGE2 produced by arachidonic acid stimulated COS cells stably expressing human COX-2 as described in Ref. 7.
[b] IC_{50} values for inhibition of PGE2 produced by arachidonic acid stimulated COS cells stably expressing human COX-1 as described in Ref. 7.
[c] IC_{50} value for COX-1 divided by IC_{50} value against COX-2.

Direct oxidation using a variety of reagents failed to give significant oxidation. We prepared the compound utilising a biotransformation approach from the parent **6a** (Scheme 3). This is described in the following section.

Biotransformation approaches have been used to good effect in the pharmaceutical industry over many years. Enzyme-catalysed reactions and whole-cell-mediated transformations can often provide mild and high yielding alternatives to chemical syntheses, functional group modifications and chiral synthesis.[4] Micro-organisms can also be employed to mimic mammalian metabolism (so-called 'microbial models of mammalian metabolism[5]) generating valuable metabolites to aid drug metabolism studies. In this study, we employed a whole-cell biotransformation approach looking to identify micro-organisms capable of hydroxylating the methylene linker of a key benzyl compound **6a**. Conventional synthetic chemistry had failed in this regard.

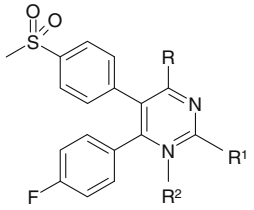
A broad range of bacteria and fungi (>220 taxonomically diverse organisms selected from the GSK culture collection) were initially screened for their ability to biotransform compound **6a**. Cultures were grown at 2 ml scale in 24-well plates (7 ml volumes) employing conditions previously reported.[6] Cultures were held on porus beads (Microbank™, Prolab Inc., Ontario, Canada) and a single bead was used to inoculate each 2 ml aliquot of culture medium. Cultures were incubated on a rotary shaker (250 rpm; 50 mm throw) at 25 °C (fungi) or 28 °C (bacteria) for three days prior to addition of compound **6a (0.125 mg/ml** final concn in 200 μl 50% methanol). Following three days additional incubation, 1 ml samples were removed and added to 1 ml 0.6% TFA in MeOH, stood for 1 h, centrifuged and supernatant was subsequently evaluated by LC–MS. Comparison of these data with those obtained for unfed controls allowed an assessment of the extent and nature of the metabolism of **6a** by each organism.

Based on this initial analysis, nine micro-organisms (seven bacteria and two fungi) were selected for scale up because they showed significant substrate conversion to products of potential interest. Scale-up was successfully carried out at the 2 × 50 ml scale under conditions that mimicked those shown above. The biotransformation products were isolated in mg amounts by

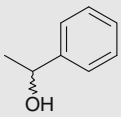
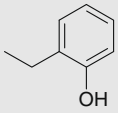
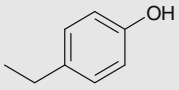
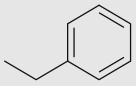
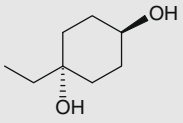
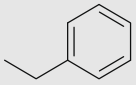
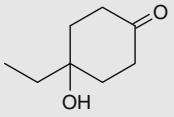
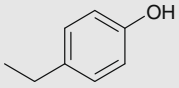
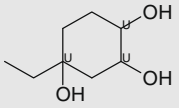
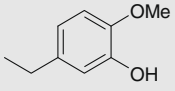
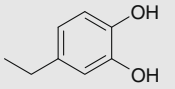


Scheme 3.

Table 3
Structures and COX-2 inhibition data for phase 1 modifications of compound **6a**

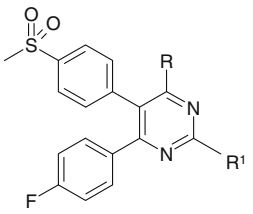


(11)

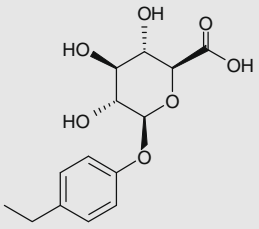
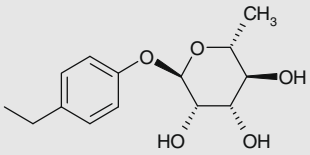
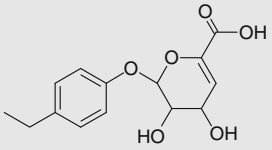
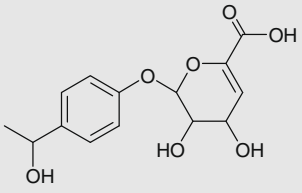
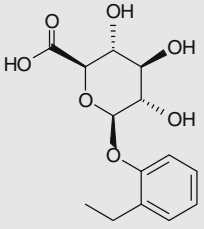
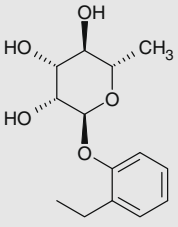
No.	R	R^1	R^{2b}	COX-2 IC_{50} (nM) [a]	Organism
10[c]	H		—	57	*Streptomyces rimosus* C1950
11a	H		—	43	*Mortierella isabellina* 4278E
11b	H		—	NSEb	*Beauvaria bassiana* C2592
11c	OH		—	NSE	*Streptomyces* sp. 4789E
11d[c]	H		—	NSE	*Beauvaria bassiana* C2592
11e	H		O	717	*Streptomyces* sp. 4789E
11f[c]	H		—	NSE	*Streptomyces mashuensis* 5221I
11g	OH		—	NSE	*Streptomyces mashuensis* 5221I
11h[c]	H		—	NSE	*Streptomyces armentosus* C2162
11i	H		—	NSE	*Streptomyces griseus* 4321E
11j	H		—	NSE	*Streptomyces armentosus* C2162

[a] IC_{50} values for inhibition of PGE2 produced by arachidonic acid stimulated COS cells stably expressing human COX-2 as described in Ref. 7.
[b] No effect observed up to a concentration of 100 μM.
[c] Relative or undefined stereochemistry.

Table 4
Structures of phase 2 modifications

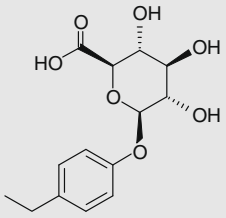
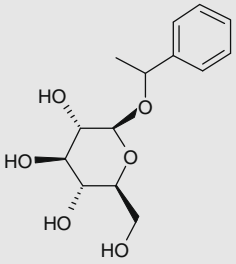


(12)

No.	R	R^1	Organism
12a	H		*Streptomyces* sp. 4789E
12b[a]	H		*Actinomyces* sp. 3986E
12c[a]	H		*Streptomyces rimosus* C1950
12d[a]	H		*Streptomyces rimosus* C1950
12e[a]	H		*Streptomyces eurythermus* 5014I
12f[a]	H		*Streptomyces* sp. 4789E

(*continued on next page*)

Table 4 (*continued*)

No.	R	R^1	Organism
12g[a]	OH		*Streptomyces griseus* 4321E
12h[a]	H		*Streptomyces mashuensis* 5221I

[a] Relative or undefined stereochemistry.

preparative HPLC and characterised by a combination of electrospray MS, ^{1}H NMR spectroscopy and, when necessary, 2D NMR data.

In addition to the target compound **10,** an array of additional analogues was identified (represented by general structures: **11** and **12**. Many of these compounds would have been extremely challenging to prepare and included hydroxylation of the benzyl group (**11a**, **11b**, **11i** and **11j**)) and both C and N oxidation of the core pyrimidine ring (**11c**, **11e** and **11g**) together with some chemically very interesting transformations of the benzyl group (**11d**, **11f** and **11h**). Modified compounds can be divided into so-called phase 1 (Table 3) and phase 2 (Table 4) products. It was often the case that multiple micro-organisms produced the same product however only representative species are shown.

Encouragingly the target compound **10** was a highly potent and selective COX-2 inhibitor (no activity at COX-1 up to 100 μM) and was progressed into a model inflammatory pain (Freund's Complete Adjuvant (FCA)—induced arthritis model).[7] When administered orally the compound showed similar activity to standard compounds in the same model (ED_{50} = 1.6 mg/kg).

Whilst the majority of the phase 1 products showed little or no activity in the COX-2 enzyme inhibition assay we believe the is due the increased polarity this observation is consistent with previously observed SAR amongst COX-2 inhibitors which demonstrates that the enzyme is generally not tolerant of polar ligands.

Data for the phase 2 products is shown in Table 4. None of the compounds had any significant activity in the COX-2 inhibition assay, again this is consistent with their physico-chemical properties, however such species have potential utility in the identification of in vivo metabolites arising from future pharmacodynamic studies with lead molecules from this series.

In summary we have prepared a novel series of COX2 inhibitors, early examples of this series were highly potent and selective but devoid of in vivo efficacy. We hypothesised that the high lipophilicity of these molecules was contributing to an unfavourable pharmacokinetic profile and focussed on preparing more hydrophilic compounds, as apart of this exercise we employed a biotransformations approach which identified a highly potent and selective inhibitor with an excellent in vivo profile.

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