Published in final edited form as:

Bioorg Med Chem Lett. 2011 October 15; 21(20): 6071–6073. doi:10.1016/j.bmcl.2011.08.050.

Efficient synthesis of apricoxib, CS-706, a selective cyclooxygenase-2 inhibitor, and evaluation of inhibition of prostaglandin E2 production in inflammatory breast cancer cells

Pijus K. Mandal, Eric M. Freiter, Allison L. Bagsby, Fredika M. Robertson, and John S. McMurray

^aDepartment of Experimental Therapeutics, M.D. Anderson Cancer Center, 1862 East Road, Houston, Texas-77054-3005, U.S.A

Abstract

An efficient synthesis of apricoxib (CS-706), a selective cyclooxygenase inhibitor, was developed using copper catalysed homoallylic ketone formation from methyl 4-ethoxybenzoate followed by ozonolysis to an aldehyde, and condensation with sulphanilamide. This method provided multi-gram access of aprocoxib in good yield. Apricoxib exhibited potency equal to celecoxib at inhibition of prostaglandin E₂ synthesis in two inflammatory breast cancer cell lines.

Keywords

Apricoxib; CS-706; Cox-2 inhibitor; Cyclooxygenase 2; Homoallylic ketone

Cyclooxygenase (Cox) enzymes, also known as prostaglandin H synthases (PGHS), catalyze the rate limiting step in the formation of inflammatory prostaglandins, most notably the inflammatory mediator, prostaglandin E₂ (PGE₂).^{1, 2} Cox-1 is constitutively expressed and PGE₂ derived from this isoform is associated with survival of specific populations of epithelial cells such as crypt stem cells in the gastrointestinal tract.³ Cox-2 is transcribed from an inducible immediate early gene primarily responsible for the production of PGE₂. Since this isoform was first cloned and sequenced in 1992,⁴ numerous studies have documented the association between elevated gene expression and proliferation, invasion, angiogenesis and metastasis in human tumors from different organ sites, thus validating Cox-2 as a useful therapeutic target.^{5, 6} In invasive breast cancer, Cox-2 mRNA and protein are elevated regardless of hormone receptor or Her-2 neu status.^{7, 8, 9}

Selective Cox-2 inhibitors, such as celecoxib (Celebrex®, Pfizer, Inc), in addition to treating pain and inflammation, showed great promise as inhibitors of tumor growth and angiogenesis.^{10, 11} Unfortunately, due to the observation of unacceptably high rate of cardiovascular side effects,^{12, 13} the Cox-2 selective inhibitor rofecoxib (Vioxx®, Merck, Inc) was removed from the market on September 30, 2004, with a black box warning issued for Celebrex®. Consequently, efforts to develop next generation Cox2 inhibitors with

© 2011 Elsevier Ltd. All rights reserved.

Supplementary Material

¹H, ¹³C, and COSY NMR spectra of compounds **1** and **8**.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

different toxicity profiles that can be used clinically as anti-tumor, anti-angiogenesis and chemopreventive agents^{10, 11} continue.

Apricoxib, (CS-706, **1**) 2-(4-ethoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-pyrrole, a small-molecule, orally active, selective COX-2 inhibitor was discovered by investigators at Daiichi Sankyo in 1996.^{14, 15} Clinical studies demonstrated potent analgesic activity^{16, 17} and preclinical studies demonstrated good pharmacokinetics, pharmacodynamics and gastrointestinal tolerability.¹⁸ As an anticancer agent, preclinical studies demonstrated efficacy in biliary tract cancer models¹⁹ and colorectal carcinoma,²⁰ and Recamp et al.²¹ recently reported a Phase I trial of **1** in combination with erlotinib in lung cancer.

Our goal is to evaluate the clinical potential of apricoxib in inflammatory breast cancer models, which requires multi-gram quantities. Daiichi Sankyo reported the synthesis of **1** in a patent¹⁴ and appears to have provided the compound for the clinical and pre-clinical studies mentioned above. The original synthetic route is outlined in Scheme 1. Though the initial two steps were accomplished with decent yields, the final step of pyrrolidine formation followed by dehydration and dehydrocyanation produced only 3% of **1** as a brown powder. The yield in the last step of the synthesis of the 2-(4-methoxyphenyl) analog, 2-(4-methoxyphenyl)-4-methyl-1-(4-sulfamoylphenyl)-pyrrole, was 6%,¹⁴ indicating that this synthesis route is problematic.

The route used to prepare the 4-ethyl analog, 2-(4-ethoxyphenyl)-4-ethyl-1-(4-sulfamoylphenyl)-pyrrole, in the same patent employed the well known Paal-Knorr condensation of the intermediate γ -ketoaldehyde **7** and sulfanilamide (Scheme 2).¹⁴ The process involved conventional enamine alkylation with the appropriate phenacyl bromide in an inert solvent followed by acid hydrolysis leading to the desired dicarbonyl compound. In our hands, coupling of 4-ethoxyphenacyl bromide (**5**)²² and 1-(*N,N*-diisopropylamino)-1-propene (**6**) in solvents such as toluene, CH₂Cl₂, dry THF at 0°C, room temperature, or reflux produced at best 18% yield of **7**. Purification of **6** through distillation was fruitless so crude enamine was used, which likely contributed to the low yields. The corresponding 1-piperidino-1-propene also did not give high yields of **7**. In a later patent researchers at Daiichi Sankyo used γ -ketoaldehyde protected as a ketal²³ and condensed this with sulfanilamide under acid conditions.²² Note that **5** and **6** also had to be synthesized which add further steps to the preparation of **1**.

We envisioned that **7** could be prepared by ozonolysis of homoallylic ketone (**8**) (Route B). A recent development in the synthesis of homoallylic ketones by Dorr *et al.* via copper-catalyzed cascade addition of alkenylmagnesium bromide to an ester²⁴ was examined. Treatment of commercially available methyl 4-ethoxybenzoate with 1-propenylmagnesium bromide (4.0 equiv) in presence of CuCN (0.6 equiv) resulted in 95% yield of desired ketone **8** after silica gel chromatography, along with a minor amount of unreacted ester (Scheme 3).²⁵

The product was a mixture of *cis/trans R/S* stereoisomers, as detected in the ¹H NMR spectrum, and was used directly in the next step without separation. Ozone was bubbled through a solution of **8** in MeOH/CH₂Cl₂ at -78°C, until all starting materials were consumed. The ozonide was then reduced to aldehyde **7** by treatment with Me₂S overnight. Removal of volatiles and subsequent addition and evaporation of toluene gave the crude 1,4-dicarbonyl compound **7** which was sufficiently pure for the following condensation step. The ¹H NMR signal at 9.78 ppm of the crude product confirmed the formation of the aldehyde. No attempt was made to characterize the enantiomeric ratio of **7** since the dehydration/aromatization reaction of the next step removes the chirality of the product. Treatment of **7** with sulfanilamide in 40% acetic acid-acetonitrile at 70°C for three hours

resulted in a brown product. Purification by silica gel flash chromatography yielded 71% of pure **1** as a white solid.²⁶

Our lots of apricoxib were assayed for the ability to inhibit production of PGE₂ in two inflammatory breast cancer lines, SUM149 and SUM190.²⁷ As shown in Table 1, after stimulation with arachidonic acid, SUM149 cells produced 3 pg/mL/1000 cells of PGE₂. Apricoxib at 100 nM resulted in 70% inhibition (1 pg/mL/1000 cells). Concentrations of 1 and 10 μ M resulted in 91% and 97% inhibition, respectively. Although SUM190 cells produced double the amount of PGE₂, the degree of inhibition with apricoxib was the same. The well known Cox-2 inhibitor, celecoxib was equally potent as **1**.

In summary, we present a highly efficient synthesis of the promising COX-2 inhibitor, apricoxib, in only three steps from commercially available starting materials. The key is the improved synthesis of the γ -ketoaldehyde intermediate, **7**. Multigram quantities have been prepared for use in preclinical studies. Our lots of apricoxib potently inhibit COX-2 activity in inflammatory breast cancer cells. Details of further biological evaluation will be published under separate cover.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the National Cancer Institute for support (PKM, JSM) CA096652, the Cancer Center Support Grant CA016672 for support of both our NMR facility and the Translational Chemistry Core facility which provided HRMS, the American Airlines-Komen For the Cure Foundation Promise Grant KGO81287 (FMR, EMF, and ALB) and The State of Texas Fund for Rare and Aggressive Breast Tumors (FMR, EMF, and ALB).

References and notes

1. Rouzer CA, Marnett LJ. *J Lipid Res.* 2009; 50(Suppl):S29. [PubMed: 18952571]
2. Hla T, Bishop-Bailey D, Liu CH, Schaeffers HJ, Trifan OC. *Int J Biochem Cell Biol.* 1999; 31:551. [PubMed: 10399316]
3. Cohn SMZ, Schloemann S, Tessner T, Seibert K, Stenson WF. *J Clin Invest.* 1997; 99:1367. [PubMed: 9077547]
4. Hla T, Neilson K. *Proc Natl Acad Sci USA.* 1992; 89:7384. [PubMed: 1380156]
5. Wang D, Dubois RN. *Semin Oncol.* 2004; 31(1 Suppl 3):64. [PubMed: 15052544]
6. Menter DG, Schilsky RL, DuBois RN. *Clin Cancer Res.* 2010; 16:1384. [PubMed: 20179228]
7. Parrett M, Harris R, Joarder F, Ross M, Clausen K, Robertson F. *Int J Oncol.* 1997; 10:503. [PubMed: 21533404]
8. Hwang D, Scollard D, Byrne J, Levine E. *J Natl Cancer Inst.* 1998; 90:455. [PubMed: 9521170]
9. Soslow RA, Dannenberg AJ, Rush D, Woerner BM, Khan KN, Masferrer J, Koki AT. *Cancer.* 2000; 89:2637. [PubMed: 11135226]
10. Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA, Flickinger AG, Moore RJ, Seibert K. *Cancer Res.* 2000; 60:1306. [PubMed: 10728691]
11. Abou-Issa HM, Alshafie GA, Seibert K, Koki AT, Masferrer JL, Harris RE. *Anticancer Res.* 2001; 21:3425. [PubMed: 11848504]
12. Psaty BM, Furberg CD. *N Engl J Med.* 2005; 352:1133. [PubMed: 15713946]
13. Graham DJ, Campen D, Hui R, Spence M, Cheetham C, Levy G, Shoor S, Ray WA. *Lancet.* 2005; 365:475. [PubMed: 15705456]
14. Kimura, T.; Noguchi, Y.; Nakao, A.; Suzuki, K.; Ushiyama, S.; Kawara, A.; Miyamoto, M. *EP* 799823. 1997. p. A1
15. Rao PP, Grover RK. *IDrugs.* 2009; 12:711. [PubMed: 19844858]

16. Moberly JB, Xu J, Desjardins PJ, Daniels SE, Bandy DP, Lawson JE, Link AJ, Truitt KE. Clin Ther. 2007; 29:399–412. [PubMed: 17577461]
17. Rohatagi S, Kastrissios H, Sasahara K, Truitt K, Moberly JB, Wada R, Salazar DE. Br J Clin Pharmacol. 2008; 66:60. [PubMed: 18522627]
18. Ushiyama S, Yamada T, Murakami Y, Kumakura S, Inoue S, Suzuki K, Nakao A, Kawara A, Kimura T. Eur J Pharmacol. 2008; 578:76. [PubMed: 17920584]
19. Kiguchi K, Ruffino L, Kawamoto T, Franco E, Kurakata S, Fujiwara K, Hanai M, Rumi M, DiGiovanni J. Mol Cancer Ther. 2007; 6:1709. [PubMed: 17575102]
20. Senzaki M, Ishida S, Yada A, Hanai M, Fujiwara K, Inoue S, Kimura T, Kurakata S. Int J Cancer. 2008; 122:1384. [PubMed: 18027868]
21. Reckamp K, Gitlitz B, Chen LC, Patel R, Milne G, Syto M, Jezior D, Zaknoen S. Cancer. 2011; 117:809. [PubMed: 20922800]
22. Kojima, S.; Ooyama, J. WO. 2008020617A. 2008.
23. Okazaki, R.; Kojima, S. US. 20050148780. 2005.
24. Dorr AA, Lubell WD. Can J Chem. 2007; 85:1006.
25. **Synthesis of 1-(4-ethoxy-phenyl)-3-methyl-hex-4-en-1-one (8):** To a stirred suspension of CuCN (1.8 g, 20.0 mmol) in 50 mL of dry THF at -78°C under argon, a solution of 1-propenylmagnesium bromide (133.2 mmol, 265 mL of 0.5 M solution in THF) was added dropwise. The slurry was stirred for an additional 30 min and then a solution of methyl 4-ethoxybenzoate (6.0 g, 33.3 mmol) in 60 mL of dry THF was added slowly. The stirred reaction mixture was allowed to warm to room temperature overnight. The reaction was quenched with ice cold saturated aqueous NaH_2PO_4 (100mL) and the mixture was extracted with ether (4×100 mL). The combined ether extracts were washed with brine (2×100 mL), dried (MgSO_4), filtered, and evaporated to dryness. The crude homoallylic ketone was purified by silica gel flash chromatography using a gradient of ethyl acetate in hexane as the eluent to give **8** (7.4 g, 95%) as a colorless oil. ^1H NMR (CDCl_3 , 300.0 MHz) δ 1.04–1.07 (m, 3H), 1.44 (t, $J = 6.9$ Hz, 3H), 1.6–1.64 (m, 3H), 2.8–2.96 (m, 2.5H), 3.2 (m, 0.5H), 4.1 (q, $J = 6.9$ Hz, 2H), 5.25 (m, 0.5 H), 5.34–5.46 (m, 1.5H), 6.92 (d, $J = 9.0$ Hz, 2H), 7.92 (d, $J = 9.0$ Hz, 2H). ^{13}C NMR (CDCl_3 , 75.0 MHz) δ 12.9, 14.6, 17.9, 20.4, 21.0, 28.4, 33.0, 45.4, 45.5, 63.7, 114.1, 123.1, 123.4, 130.2, 130.3, 135.5, 136.0, 141.9, 162.7, 198.1. M+H Calcd: 233.1542; Found, 233.2482.
26. **Synthesis of Apricoxib (1):** Homoallylic ketone (**8**) (5.0 g, 21.53 mmol) in 180 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1:5) was treated with ozone bubbles at -78°C until a blue coloration persisted. The solution was purged with argon, 8.0 mL of dimethylsulphide (21.5 mmol) was added, and the reaction mixture then warmed slowly to rt overnight. The solvent was evaporated under vacuum to give **7** which was then diluted with 100 mL of 40 % acetic acid in acetonitrile, (v/v) and sulphanilamide (4.0 g, 23.2 mmol) was added. The mixture was refluxed until complete consumption of 1,4-dicarbonyl compound was detected by TLC (ca 3 h). After cooling to room temperature, the product was concentrated under vacuum and diluted with 250 mL of ethyl acetate. The organic layer then washed with saturated Na_2CO_3 solution (3×50 mL) followed by brine (1×50 mL), dried (MgSO_4), and evaporated to dryness. The crude brown material was purified by silica gel flash chromatography using a gradient of EtOAc in hexane to give apricoxib as white solid (5.5 g, 15.43 mmol, 71%). m.p. 161–163 $^{\circ}\text{C}$ (lit. 135–139 $^{\circ}\text{C}^{14}$). ^1H NMR (CDCl_3 , 300.0 MHz) δ 1.32 (t, $J = 6.9$ Hz, 3H), 2.1 (s, 3H), 3.92 (q, $J = 6.9$ Hz, 2H), 4.95 (s, 2H), 6.14 (m, 1H), 6.63 (m, 1H), 6.69 (d, $J = 6.6$ Hz, 2H), 6.94 (d, $J = 6.6$ Hz, 2H), 7.13 (d, $J = 6.6$ Hz, 2H), 7.74 (d, $J = 6.6$ Hz, 2H). ^{13}C NMR (CDCl_3 , 75.0 MHz) δ 11.7, 14.8, 63.4, 82.4, 113.2, 114.4, 121.0, 121.1, 124.9, 125.2, 127.4, 129.7, 133.6, 138.7, 144.2, 158.0 M+H Calcd: 357.1273; Found, 357.1252.
27. **PGE₂ production in inflammatory breast cancer cells.** SUM149 or SUM190 cells were cultured in F12 media (Invitrogen) supplemented with 10% fetal bovine serum, 5 $\mu\text{g/mL}$ insulin (Sigma-Aldrich, St Louis, MO), 1 $\mu\text{g/mL}$ hydrocortisone (Sigma-Aldrich), and antibiotic/antimycotic (Invitrogen/Life Technologies, Carlsbad, CA). Cells were seeded in triplicate in six-well tissue culture plates in a humidified cell culture incubator at 37°C with 5% CO_2 . When cells reached 80% confluence, culture media was removed by aspiration, cells were washed once with sterile PBS and fresh medium containing the indicated COX-2 inhibitor, either Apricoxib, or Celecoxib at concentrations of 0.1, 1, and 10 μM , or an equivalent amount of DMSO used as the vehicle control was added. Cells were pre-incubated for 2 hours in the presence of either the COX-2

inhibitor or DMSO at 37°C. Following the 2 hour pre-incubation time period, PGE₂ production was stimulated by the addition of sodium arachidonate (Cayman Chemical, Ann Arbor, MI), added directly to growth medium, with gentle agitation, to a final concentration of 10 µM, and incubated for an additional 2 hours. Conditioned supernatant was then isolated from cells and stored at -80°C until further analysis. Following removal of supernatants, cells were immediately trypsinized and cell number was assessed. PGE₂ was assayed from conditioned supernatant using a competitive enzyme immunoassay specific to PGE₂ (Cayman Chemical), as recommended by the manufacturer. The concentration of PGE₂ was normalized to total cell number per well, expressed as pg PGE₂/mL conditioned supernatant/1000 cells. Assays were run three times and are expressed as mean ± standard deviation.

Scheme 1.
Original synthetic route to Apricoxib (**1**)¹⁴

Scheme 2.
Retro synthetic route to **1**

Scheme 3.
Efficient synthesis of apricoxib (**1**):

Table 1

Apricoxib inhibits PGE₂ in inflammatory breast cancer cells.

SUM-149	PGE ₂ ^a (pg/mL/1000 cells)	SUM-190	PGE ₂ ^a (pg/mL/1000 cells)
DMSO	3.15 ± 0.19	DMSO	6.43 ± 1.87
0.1 μM Apricoxib	0.97 ± 0.24	0.1 μM Apricoxib	2.10 ± 0.81
1 μM Apricoxib	0.28 ± 0.07	1 μM Apricoxib	0.50 ± 0.15
10 μM Apricoxib	0.11 ± 0.01	10 μM Apricoxib	0.22 ± 0.05
0.1 μM Celecoxib	1.19 ± 0.13	0.1 μM Celecoxib	2.87 ± 0.74
1 μM Celecoxib	0.43 ± 0.08	1 μM Celecoxib	0.81 ± 0.13
10 μM Celecoxib	0.13 ± 0.04	10 μM Celecoxib	0.18 ± 0.01

^aPGE₂—average PGE₂ production of triplicate samples ± standard deviation.