Bioorganic & Medicinal Chemistry Letters 20 (2010) 7155–7158

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

The novel benzopyran class of selective cyclooxygenase-2 inhibitors-part I: The first clinical candidate

Jane L. Wang *, Jeffery Carter, James R. Kiefer, Ravi G. Kurumbail, Jennifer L. Pawlitz, David Brown, Susan J. Hartmann, Matthew J. Graneto, Karen Seibert, John J. Talley

Pfizer Global Research and Development, 700 Chesterfield PKWY West, Chesterfield, MO 63017, United States

ARTICLE INFO

Article history:
Received 14 January 2010
Revised 12 July 2010
Accepted 14 July 2010
Available online 23 July 2010

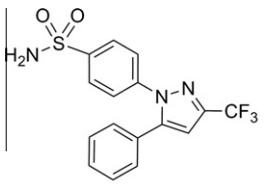
Keywords:
Cyclooxygenase
COX-2 inhibitor
Clinical candidate
Benzopyran
Half-life
Plasma protein bound
X-ray crystal structure

ABSTRACT

In this manuscript, we report the discovery of the substituted 2-trifluoromethyl-2*H*-benzopyran-3-carboxylic acids as a novel series of potent and selective cyclooxygenase-2 (COX-2) inhibitors. **5c**-(*S*) (SD-8381) was advanced into clinical studies due to its superior in vivo potency. The high plasma protein binding (>99% bound) of **5c**-(*S*) has resulted in a surprisingly long human half life $t_{1/2}$ = 360 h.

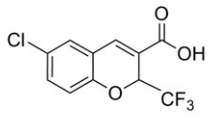
Non-steroidal anti-inflammatory agents (NSAIDs) are the most widely used drugs world wide for the treatment of pain and edema associated with arthritis and inflammation. However, one-third of patients suffer severe side effects from treatment, including ulcers and potentially life-threatening bleeds.[1] Alternative therapies for the treatment of arthritis include use of anti-inflammatory steroids that, although efficacious, cause other serious side effects, including muscle wasting, osteoporosis, and immune system suppression.[2] In 1970 Vane[3] and colleagues identified cyclooxygenase, the key enzyme responsible for the first two steps in the conversion of arachidonic acid to prostaglandins. The group also discovered that aspirin (acetyl salicylic acid) irreversibly acetylates the side chain hydroxyl of Ser530 of cyclooxygenase (now termed COX-1) and devised improved enzyme assays for evaluation of new compounds. A second COX isoform (COX-2), expressed in response to inflammatory stimuli, was subsequently discovered.[4] Unlike COX-2, the COX-1 isoform is constitutively produced in tissues such as the gastrointestinal (GI) tract and blood. The COX-1 isoform in these tissues provides the basal level of prostaglandins and thromboxanes required for GI tissue homeostasis and platelet clotting ability, respectively.[5] Although traditional NSAIDs are efficacious in treating the pain and edema associated with inflammation, their GI side effect profile derives from their non-selective inhibition of both COX-1 and COX-2. Selective inhibition of COX-2 is sufficient for efficacy while avoiding the gastrointestinal side effects of non-selective COX inhibitors. Approved uses for the specific COX-2 inhibitor celecoxib (Fig. 1) now extends beyond treatment of pain and inflammation and includes cancer chemoprevention in the treatment of familial adenomatous polyposis, a precancerous condition that previously was treatable only by colonectomy.[6]

During our ongoing chemistry program aimed at identifying potent and selective COX-2 inhibitors, we discovered a compound that was efficacious in in vivo animal models[7,8] of inflammation containing the core of benzopyran **1** (Fig. 1). Due to its potency



celecoxib

[a]AirPouch	94 % inh.
[b]Hyperalgesia ED_{50}	35 mg/kg
[c]Adj. Arth. ED_{50}	0.3 mg/kg



1

AirPouch	98 % inh.
Hyperalgesia ED_{50}	6 mg/kg
Adj. Arth. ED_{50}	0.4 mg/kg

Figure 1. Celecoxib and lead COX-2 inhibitor. (a) See Ref. 8a–c sec. 2.9; (b) see Ref. 8a–c sec. 2.10; (c) see Ref. 8a–c sec. 2.11.

* Corresponding author. Tel.: +1 636 527 5037; fax: +1 636 247 2086.
E-mail address: jl47wang@yahoo.com (J.L. Wang).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.053

as an analgesic and anti-inflammatory agent, oral bioavailability, and unique physicochemical properties relative to the diaryl heterocyclic series of COX-2 inhibitors (e.g., celecoxib), we proceeded with optimization of this template.

The initial medicinal chemistry efforts around the lead molecule **1** demonstrated that replacing the carboxylic acid or trifluoromethyl moieties with other functional groups lead to reduced COX potency. Exchanging oxygen with thiol diminished COX selectivity while exchanging with nitrogen reduced enzyme potency. Finally, saturation of the pyran double bond generated one pair of isomers with slightly decreased potency, but this increased chemistry complexity. These early modifications of the pyran nucleus showed the 2-trifluoromethyl, 3-carboxy, and 4-H substitutes were key elements of the pharmacophore. These elements were readily incorporated by efficient chemistry methodology[9a] allowing us to optimize the substitution at the 5, 6, 7, and 8 positions (benzenoid nucleus) for potency and pharmacokinetic parameters. The key SAR was summarized in the Figure 2.

First, we explored the importance of the substituted positions on the benzopyran ring via preparation of compounds **5a**–**h**. As shown in Scheme 1, substituted salicylaldehydes **2a**–**h**, synthesized via known procedures,[9] were the key intermediates that were reacted with commercially available ethyl trifluorocrotonate **3** to produce the substituted benzopyran nucleus (**4a**–**h**) in an efficient, one-step procedure via either heated in K_2CO_3 and DMF or in Et_3N and DMSO. The key pyran ring formation step is formally a Michael addition of the phenol followed by an aldol condensation and elimination. The trifluoromethyl group activates the crotonate, thus greatly enhancing the efficiency of this regio-specific condensation. The resulting benzopyran-3-carboxylic acid esters **4a**–**h** were hydrolyzed with sodium hydroxide yielding the free acids **5a**–**h**.

The SAR of analogs **5a**–**h** (Table 1) revealed that the substitution at the 6 position was the key to retain hCOX-2 potency. Compounds **5d**, **5e**, and **5f** lacked the 6-substitution and showed dramatic loss of hCOX-2 activity. Analogs with 6-chloro **5a**, **5b**, and **5c** displayed excellent potency and selectivity for hCOX-2. Both of the tri chloro analogs **5g** and **5h** with 6-Cl substituent also displayed excellent hCOX-2 inhibition and selectivity.

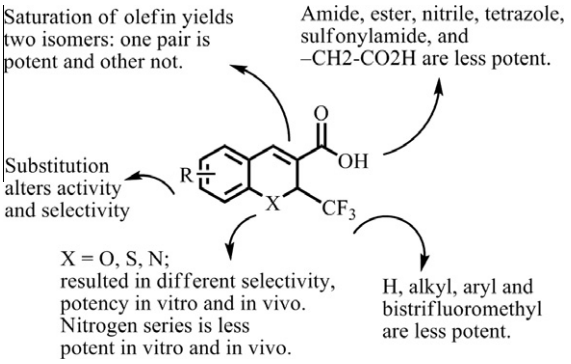
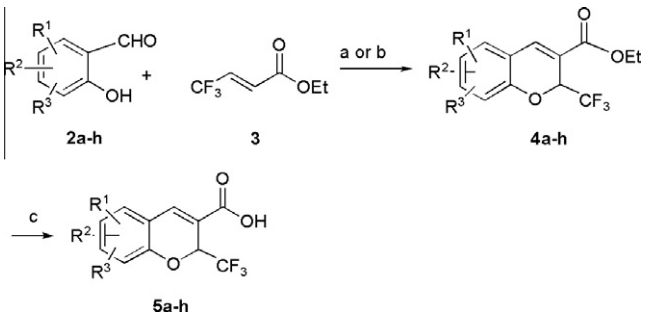
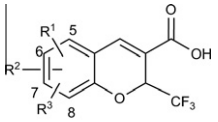


Figure 2. Chromene template: the initial SAR study results.



Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 80–100 °C; (b) Et_3N, DMSO; (c) NaOH, THF, CH_3OH, H_2O.

Table 1
Exploration the substituted pattern of benzopyran



1,5a-h

Compds	R group's position	Mod human IC_{50}[a] (μM)		hCOX-1/hCOX-2
		COX-1	COX-2	
1	6-Cl	29.2	0.32	90
5a	5,6-Di-Cl	1.51	0.003	4747
5b	6,7-Di-Cl	94.5	0.062	1522
5c	6,8-Di-Cl	0.74	0.018	41.8
5d	5,7-Di-Cl	145	12	12
5e	5,8-Di-Cl	11	0.1	110
5f	7,8-Di-Cl	300	300	1
5g	5,6,7-Tri-Cl	0.88	0.006	136
5h	6,7,8-Tri-Cl	2.7	0.043	63

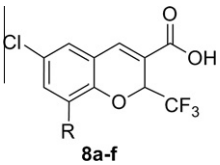
IC_{50} curves were generated with each test concentration run in duplicate, each curve was done $n \geqslant 2$. The high concentration was 500 μM.
[a] See note 10.

In order to examine the specific binding interaction imparted by the chirality of the trifluoromethyl moiety at the 2-position of the benzopyran, compound **5c** was resolved.[9] Separation of the enantiomers was accomplished via classical diastereomeric salt formation (selective crystallization using chiral amines, such as α-methylbenzylamine) or by chiral chromatography.[9]

The *S*-stereoisomer **5c**-(*S*) (SD-8381) (hCOX-2 IC_{50} = 0.0098 μM, hCOX-1 IC_{50} = 0.69 μM) was the most potent and selective inhibitor of COX-2 in vitro; whereas, its *R*-stereoisomer **5c**-(*R*) was far less potent in the enzyme assay (hCOX-2 IC_{50} = 333 μM, hCOX-1 IC_{50} = 50.3 μM). These data show that the binding interaction between the inhibitor and the enzyme is very specific, recognizing the chirality at the C-2 center. The potency of the two enantiomers in the COX-2 enzyme assay correlated well with the in vivo biomarker (PGE_2) levels (prophylactic air pouch) and in vivo efficacy (pain and arthritis models) as shown in Table 2.

We determined the crystal structure of celecoxib bound to the COX-2 active site at 2.4 Å resolution, and similar to the previously

Table 2
SAR of 6-Cl-8 substituted benzopyran



8a-f

Compds	R	Mod human IC_{50}[a] (μM)		*c* log *P*
		COX-1	COX-2	
8a	$-HCCH_2$	0.14	0.018	4.59
8b	–CCH	<0.35	0.043	4.14
8c	–Ph	3.11	0.11	5.76
8d	–CCPh	33	0.14	6.51
8e	$-PhSO_2NH_2$	1.74	0.76	3.96
8f	$-CCPhSO_2NH_2$	0.41	18.6	4.67

IC_{50} curves were generated with each test concentration run in duplicate, each curve was done n ⩾ 2. The high concentration was 500 μM.
[a] See notes 10.

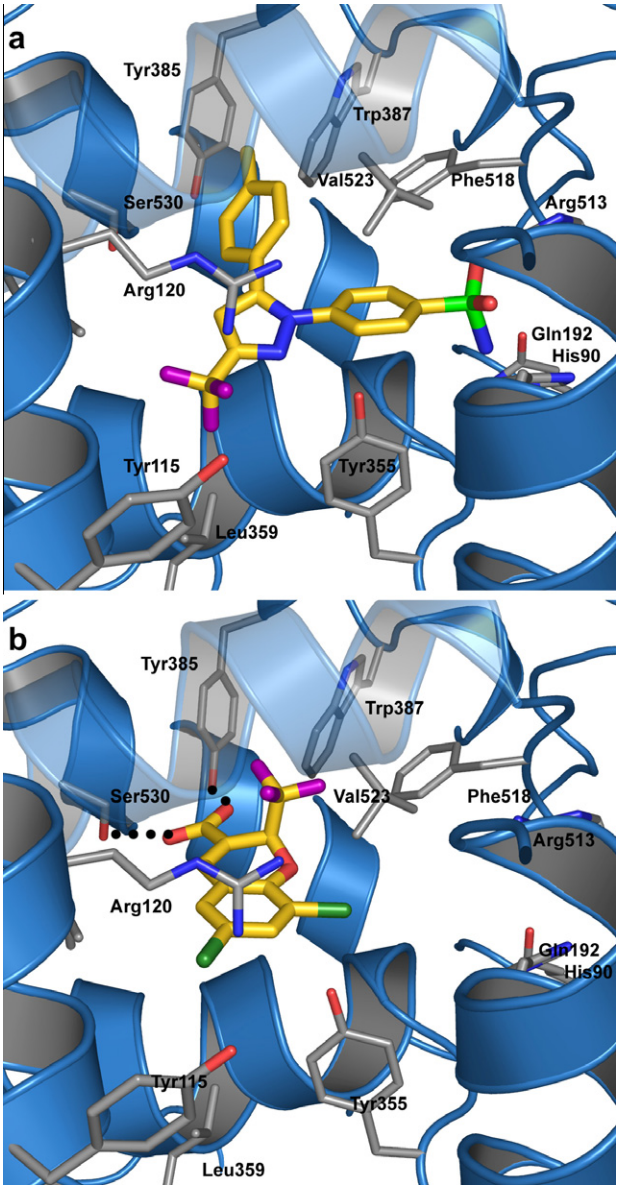


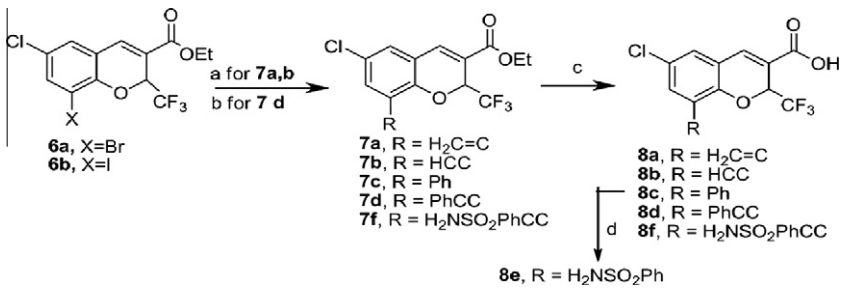
Figure 3. Crystal structures of inhibitors (a) celecoxib; (b) **5c**-(*S*) bound at the COX-2 active site [composed with PyMol]. Key crystal structures of this chemical series were determined at 2.8 Å or better resolution and were compared to the structure of celecoxib bound to COX-2.[11]

reported structure of SC-558,[11] celecoxib binds to COX-2 and inserts its phenyl sulfonamide moiety into the so-called 'side pocket' (Fig. 3a and Supplementary data), adjacent to residue Val523 (ovine COX-1 numbering convention). Mutation of V523 to the equivalent COX-1 residue (Ile)-singly or in combination with other neighboring amino acids alters the kinetic profile of the inhibition and reduces inhibitor affinity,[12] paralleling the potency of the compound versus COX-1 itself. Side pocket interaction is necessary but not sufficient for selectivity in this chemical series. While the binding site of COX enzymes is predominantly hydrophobic, clusters of hydrophilic amino acids are found in the side pocket. The constriction (Arg120 and Tyr355) leading to the membrane binding helices, and the apex of the binding site (Tyr385 and Ser530). Celecoxib forms three hydrogen bonds with the hydrophilic side chains (His90 and Gln192) in the side pocket and the main chain carbonyl at residue 338, but most typical NSAIDs form an ion pair between their carboxylic acid and Arg120 at the constriction.[11,13] Van der Waals interactions dominate the remainder of the celecoxib interactions with the protein.

In contrast to the most common NSAID binding mode,[13] the 2.2 Å resolution crystal structure of compound **5c**-(*S*) shows that it binds in an inverted mode, with its carboxylate group forming hydrogen bonds to the side chains of Tyr355 and Ser530 at the top of the active site (Fig. 3b).[14] This orientation resembles the binding mode observed for diclofenac and the inverted arachidonic acid binding mode[15] and positions the 8-Cl pointed towards the side pocket. Unlike the diaryl heterocycle series, mutation of the side pocket residues to their COX-1 counterpart has no effect on selectivity in this series (data not shown), presumably because these analogs did not sufficiently enter the side-pocket. In the benzopyran complex, the trifluoromethyl group packs between Tyr385, Trp387, and Phe518. Retention of COX-2 selectivity in the benzopyran series without significant entry into the side pocket underscores the fact that features within the main cavity of the binding site also impact selectivity.

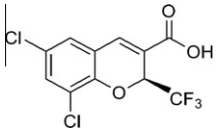
Based upon the crystal structures of **5c**-(*S*), we explored the 8-position extension to make contacts within the side-pocket binding region. Utilizing Pd-mediated coupling chemistry and 6-chloro-8-bromo **6a** or 6-chloro-8-iodo **6b** as intermediates shown in Scheme 2, we were able to rapidly explore this region of the enzyme. Analogs having small, lipophilic 8-substituents such as vinyl **8a** and acetylene **8b** were potent, but exhibited diminished selectivity. Larger 8-substituents such as 8-phenyl **8c** showed moderate selectivity; whereas, 8-phenylacetylene **8d** was among the most potent and selective compounds discovered, but performed poorly in vivo in the air pouch assay. Similar chemistry provided sulfonamide analogs **8e** and **8f**, which exhibited greatly decreased COX inhibition. Thus, the 8-phenylacetylene analog **8d** demonstrated that 8-substituted analogs could enhance potency and selectivity as predicted by the crystal structure. The fact that sulfonamide containing analogs were less potent underscores difference in binding mode of the diaryl heterocyclic series and the benzopyrans, where a phenylsulfonamide substitution at the 8-position can not achieve similar registration within the side pocket.

Due to the excellent potency and efficacy of **5c**-(*S*) as an analgesic (Table 3) and anti-inflammatory agent, it was advanced into a



Scheme 2. Reagents: (a) $(Ph_3P)_4PPd[0]$, Bu_3SnR, toluene, reflux, yield 16–65%; (b) $(Ph_3P)_4PPd[0]$, CuI, Et_3N,TMSCCR, toluene, rt, yield 47–76%; (c) NaOH, THF, CH_3OH, H_2O; (d) $ClSO_3H$, −20 °C, NH_4OH, 1 h, yield 85–95%.

Table 3
Pharmacology of **5c**-(*S*) isomer



5c-S
SD-8381

Air pouch ED_{50}[a]	Edema ED_{50}[b]	Hyperalgesia ED_{50}[c]	Adj. Arthritis ED_{50}[d]	hCOX-2 IC_{50}[e]
0.54	7.1	2.6	0.03	0.0098

[a] See Ref. 8 sec. 2.9.
[b&c] See Ref. 8a–c sec. 2.10.
[d] See Ref. 8a–c sec. 2.11.
[e] See notes 10.

Phase-I clinical trial. Despite its favorable half life in rats ($t_{1/2}$ = 10.1 h), dogs ($t_{1/2}$ = 20.4 h), and cyno ($t_{1/2}$ = 14.2 h), its half-life in humans was strikingly longer ($t_{1/2}$ = 360 h). Clearance was not consistent across species. The primary clearance pathway in rat and dog was as parent (feces); whereas, in the cyno primary clearance was as the acyl-glucuronide phase-2 metabolite (urine).

In summary, we have discovered a new series of potent and selective COX-2 inhibitors structurally distinct from the diaryl heterocycle classes. In vivo it is among the most potent and efficacious anti-inflammatory and analgesic COX-2 inhibitors yet described. Additionally, compounds from this series possess high water solubility as their corresponding carboxylate salts providing the possibility of alternative formulation and dosing strategies. In parts II and III of this series, we described our strategy and results in discovering clinical candidates with reduced human half-lives relative to **5c**-(*S*).

Acknowledgments

We would like to thank C.M. Kobldt, Y. Zhang, B.S. Zweifel for providing in vitro and in vivo data., J. Collins, P. Kleine, A. Libby, K. Palmquist for scale up and chiral purification, M. Baratta, R. Ridgewell, A. Breau for in vitro metabolism and PK data, J.L. Pierce for protein expression, J.K. Gierse for protein purification, and W.C. Stallings for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.053.

References and notes

1. (a) Rainsford, K. D.; D, K.. In *Side-effects of Anti-inflammatory Drugs*; Rainsford, V., Ed.; MTP Press: Norwell, Massachusetts, 1985; Vol. 2, pp 343–362; (b) Allison, M. C.; Howatson, A. G.; Torrance, C. J.; Lee, F. D.; Russel, R. I. G. *N. Eng. J. Med.* **1992**, *327*, 749.
2. *The Pharmacological Basis of Therapeutics*; Haynes, R. C., Jr., Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds., 8th ed.; McGraw-Hill: New York, 1993; pp 1442–1452.
3. Vane, J. R. *Nat. New Biol.* **1971**, *231*, 232.
4. (a) Talley, J. J. *Prog. Med. Chem.* **1999**, *36*, 201; (b) Masferrer, J. L.; Zweifel, B. S.; Manning, P. T.; Hauser, S. D.; Leahy, K. M.; Smith, W. G.; Isakson, P. C.; Seibert, K. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 3228; (c) Seibert, K.; Zhang, Y.; Leahy, K.; Hauser, S.; Masferrer, J. L.; Perkins, W.; Lee, L.; Isakson, P. C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12013; (d) Peptiboon, P.; Riendea, D. *Annu. Rep. Med. Chem.* **1997**, *32*, 211; (e) Vane, J. R.; Bakhle, Y. S.; Botting, R. M. *Annu. Rev. Pharmacol. Toxicol.* **1998**, *38*, 97; (f) Simon, L. S.; Lanza, F. L.; Lipsky, P. E.; Hubbard, R. C.; Talwalker, S.; Schwartz, B. D.; Isakson, P. C.; Geis, G. S. *Arthritis Rheum.* **1998**, *41*, 1591; (g) Crofford, L. J.; Oates, J. C.; McCune, W. J.; Gupta, S.; Kaplan, M. J.; Catella-Lawson, F.; Morrow, J. D.; McDonagh, K. T.; Schmaier, A. H. *Arthritis Rheum.* **2000**, *43*, 1891.
5. Radi, Z. A.; Khan, N. K. *Exp. Toxicol. Pathol.* **2006**, *58*, 163.
6. (a) Jacoby, R. F.; Seibert, K.; Cole, C. E.; Kelloff, G.; Lubet, R. A. *Cancer Res.* **2000**, *60*, 5040; (b) Baek, S. J.; Eling, T. E. *Prog. Lipid Res.* **2006**, *45*, 1.
7. The Pfizer Institutional Animal Care and Use Committee reviewed and approved the animal use in these studies. The animal care and use program is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.
8. (a) Zhang, Y.; Shaffer, A.; Portanova, J.; Seibert, K.; Isakson, P. C. *J. Pharmacol. Exp. Ther.* **1997**, *283*, 1069; (b) Portanova, J. P.; Zhang, Y.; Anderson, G. D.; Hauser, S. D.; Masferrer, J. L.; Seibert, K.; Gregory, S. A.; Isakson, P. C. *J. Exp. Med.* **1996**, *184*, 883; (c) Gierse, J.; Nickols, M.; Leahy, K.; Warner, J.; Zhang, Y.; Cortes-Burgos, L.; Carter, J.; Seibert, K.; Masferrer, J. *Eur. J. Pharmacol.* **2008**, *588*, 93.
9. (a) Aston, K. W.; Brown, D. L.; Carter, J. S.; Deprow, A. M.; Fletcher, T. R.; Hallinan, E. A.; Hamper, B. C.; Huff, R. M.; Kiefer, J. R., Jr.; Koszyk, F.; Kramer, S. W.; Liao, S.; Limburg, D.; Springer, J. R.; Tsymbalov, S.; Wang, L. J.; Xing, L.; Yu, Y. WO 04087687 A1, 2004.; (b) Carter, J.; Obukowicz, M.; Talley, J.; Brown, D.; Graneto, M.; Bertenshaw, S.; Rogier, D.; Nagarajan, S.; Hanau, C.; Hartmann, S.; Ludwig, C.; Metz, S. US006034256A, 2000.; (c) Carter, J.; Obukowicz, M.; Devadas, B.; Talley, J.; Brown, D.; Graneto, M.; Bertenshaw, S.; Rogier, D.; Nagarajan, S.; Hanau, C.; Hartmann, S.; Ludwig, C.; Metz, S.; Korte, D. US006077850A, 2000.
10. The modified enzyme assays (Ref. 9a) was created to identify inhibitors that exhibit slow 'off-rate' kinetics due to tight binding to their target enzyme, the traditional enzyme assay (Ref. 8c) may not be optimal for characterization of compounds from alternative classes that exhibit different kinetics for inhibition. Further, such assays may provide substantially different IC_{50} values with subtle changes in assay protocol.
11. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature* **1996**, *384*, 644.
12. Gierse, J. K.; McDonald, J. J.; Hauser, S. D.; Rangwala, S. H.; Koboldt, C. M.; Seibert, K. *J. Biol. Chem.* **1996**, *271*, 15810.
13. (a) Picot, D.; Loll, P. J.; Garavito, R. M. *Nature* **1994**, *367*, 243; (b) Mancini, J. A.; Riendeau, D.; Falgueyret, J. P.; Vickers, P. J.; O'Neill, G. P. *J. Biol. Chem.* **1995**, *270*, 29372.
14. X-ray structure coordinates for all complexes have been deposited into the Protein Data Bank with accession codes 3LN1 (celecoxib) and 3LN0 (**5c**-(*S*)). Data for the crystal structures described herein were collected at IMCA-CAT (17-ID) beamline at the Advanced Photon Source and was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with the Center for Advanced Radiation Sources at the University of Chicago.
15. (a) Rowlinson, S. W.; Kiefer, J. R.; Prusakiewicz, J. J.; Pawlitz, J. L.; Kozak, K. R.; Kalgutkar, A. S.; Stallings, W. C.; Kurumbail, R. G.; Marnett, L. J. *J. Biol. Chem.* **2003**, *278*, 45763; (b) Kiefer, J. R.; Pawlitz, J. L.; Moreland, K. T.; Stegeman, R. A.; Hood, W. F.; Gierse, J. K.; Stevens, A. M.; Goodwin, D. C.; Rowlinson, S. W.; Marnett, L. J.; Stallings, W. C.; Kurumbail, R. G. *Nature* **2000**, *405*, 97.