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The novel benzopyran class of selective cyclooxygenase-2 inhibitors. Part III: The three microdose candidates

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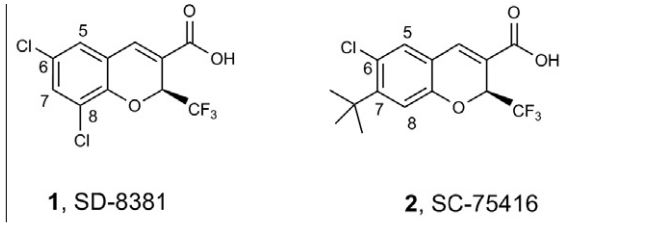
ABSTRACT

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. We provide the structure–activity relationships, optimization of design, testing criteria, and human half-life data. The challenge of a surprisingly long half-life ($t_{1/2}$ = 360 h) of the first clinical candidate **1** and human $t_{1/2}$ had been difficult to predict based on allometric scaling for this class of highly ppb compounds. We used a microdose strategy which led to the discovery of clinical agents **18c**-(*S*), **29b**-(*S*), and **34b**-(*S*) with human half-life of 57, 13, and 11 h.

In Part I of this Letter we described 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. We discussed the discovery of the first clinical candidate **1** (SD-8381)[1] that had excellent potency and efficacy as an analgesic and anti-inflammatory agent.[1] In Part II of this Letter, we described the incorporation of metabolically labile moieties to the first clinical candidate **1** to provide a path to shorten its half-life, which led to discovery of second clinical candidate **2** (SC-75416) (Fig. 1). Compound **2** (SC-75416) also had excellent potency and efficacy as an analgesic and anti-inflammatory agent. It displayed a much shorter human half-life ($t_{1/2}$ = 34 h) compared to compound **1** ($t_{1/2}$ = 360 h), a half-life was appropriate for once-a-day dosing.

During our ongoing chemistry program, our goal was to identify potent and selective COX-2 inhibitors as backup candidates of compound **2** (SC-75416) with good PK and metabolism. In both Parts I and II of these Letters, we reported the results of exploration of the 5-position and the 7-position of the benzopyran while keeping the 6-choro substituent constant. We also described the exploration of the 8-position of the benzopyran that extended into and made contacts within the side-pocket binding region based on celecoxib and compound **1** (SD-8381)'s X-ray structures. These early modifications of substituents about the pyran nucleus showed that the 2-trifluoromethyl, 3-carboxy, and 4-H substitutes were key elements of the pharmacophore, and these compounds



$t_{1/2}$ rat = 10.2 h, human = 360 h	$t_{1/2}$ rat = 4 h, human = 34 h
hCOX-1[a] IC_{50} = 0.69 uM	hCOX-1[a] IC_{50} = 1.02 uM
hCOX-2[a] IC_{50} = 0.01 uM	hCOX-2[a] IC_{50} = 0.06 uM
Air pouch[b] ED_{50} = 0.54 mg/kg	Air pouch[b] ED_{50} = 0.43 mg/kg
Edema[c] ED_{50} = 7.1 mg/kg	Edema[c] ED_{50} = 2.7 mg/kg
Hyperalgesia[d] ED_{50} = 2.6 mg/kg	Hyperalgesia[d] ED_{50} = 4 mg/kg
Adj. Arth.[e] ED_{50} = 0.03 mg/kg	Adj. Arth.[e] ED_{50} = 0.08 mg/kg

Figure 1. **1** (SD-8381), a lead COX-2 inhibitor (see Part I of this Letter) and **2** (SC-75416), a lead COX-2 inhibitor (see Part II of this Letter). (a) See Ref. 2c sec. 2.4 and Ref. 3. (b) See Ref. 2c sec. 2.9. (c and d) See Ref. 2a–c sec. 2.10. (e) See Ref. 2a–c sec. 2.11.

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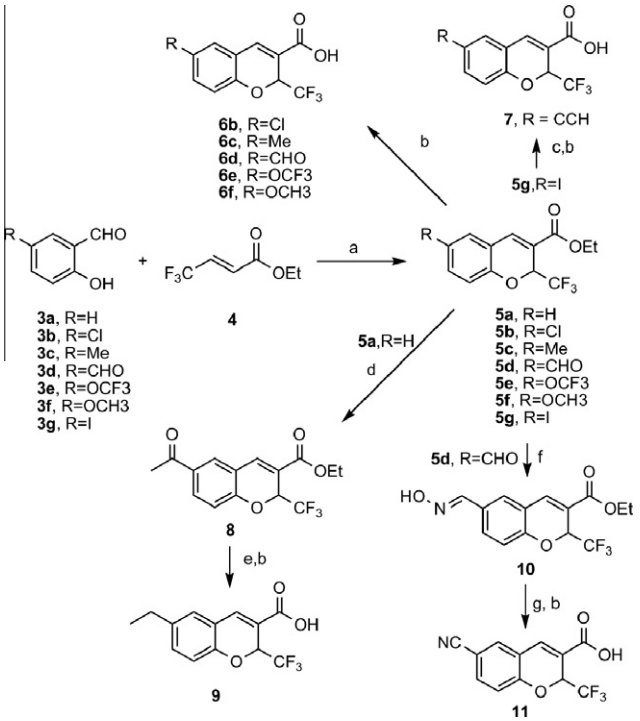
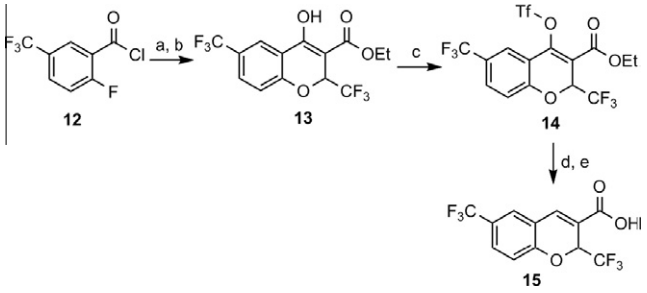
exhibited very considerable in vivo potency. In addition, we demonstrated that incorporation of metabolically labile moieties in this series provided a method for reducing the half-life, a strategy we continued to apply to achieve our goal in this work.

In Part I of this Letter, we described that the substituent at the 6-position was very important for COX potency both in vitro and in vivo (air pouch model), so we maintained the 6-chloro as a cornerstone in our initial SAR. We designed several compounds containing alkyl, alkoxy, acetylene, and cyano, moieties to explore the role of these substituents in COX-2 blockade. As described in Scheme 1, salicylaldehydes **3a–g** were reacted with commercially available ethyl trifluorocrotonate **4** to produce the substituted benzopyran ester (**5a–g**) in an efficient, one-step procedure[4] in K_2CO_3 and DMF. After hydrolysis of **5b–f**, the desired acids **6b–f** were obtained. 6-Iodo **5g** was coupled with TMS acetylene using tetrakis(triphenylphospine)Pd(0) and CuI, followed by hydrolysis to provide 6-ethynyl **7**. Friedel–Crafts reaction of **5a** using $AlCl_3$ and acetyl chloride formed 6-acetyl **8**, which was reduced by triethylsilane followed by hydrolysis to provide 8-ethyl **9**. The 6-formyl **5d** was reacted with hydroxylamine to form 5-aldoxime **10**, which was dehydrated by treatment with trifluoroacetic anhydride to give 6-cyano **11**. In Scheme 2, commercially available benzoyl chloride **12** was reacted with 4-trifluoro-3-oxobutanoate and sodium hydride to form the 4-oxo benzopyran, which was reduced by sodium borohydride to provide the 4-hydroxy chromene ester **13**. Reaction of **13** with trifluoromenthanesulfonic anhydride produced the triflate **14**. Subsequent treatment of **14** with tributyltin hydride, followed by ester hydrolysis, provided the acid **15**.

The 6-methyl **6c** and 6-ethyl **9** demonstrated similar COX-2 inhibitory potency to 6-chloro **6b**. The more polar 6-cyano analog **11** showed a twofold increase in potency and a 10-fold increase in selectivity, but 6-formyl **6d** exhibited a decreased potency. As shown in Table 1, COX-2 inhibition by the electron-donating

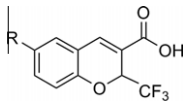


Scheme 1. Reagents and conditions: (a) K_2CO_3, DMF, 80–100 °C; (b) NaOH, THF, CH_3OH, H_2O; (c) acetylene, tetrakis(PPh_3)Pd(0), Cu(I)I, TEA, toluene; (d) $AlCl_3$, acetyl chloride, CH_2Cl_2, 0 °C to reflux, 6 days, 68% yield; (e) Et_3Si, CH_2Cl_2, 89% yield; (f) hydroxylamine HCl, CH_3CO_2Na, EtOH, H_2O, 18 h, 55% yield; (g) $(CF_3CO)_2O$, dioxane, 25–85 °C, 16 h, 40% yield.



Scheme 2. Reagents and conditions: (a) 4-trifluoro-3-oxobutanoate, NaH (60% oil disp.), toluene, 105 °C, 24 h, 39% yield; (b) $NaBH_4$, EtOH, THF, 0 °C, 1 h, 87% yield; (c) 2,6-di-*tert*-butylpyridine, $(CF_3SO_2)_2O$, CH_2Cl_2, 48 h, 66% yield; (d) LiCl, tetrakis(PPh_3)Pd(0), $[CH_3(CH_2)_3]_3SnH$, 50–65 °C, 3 h, 19% yield; (e) NaOH, THF, CH_3OH, H_2O.

Table 1
In vitro activity of 6-substituted benzopyrans



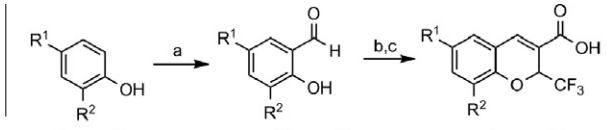
Compd	R	Mod human IC_{50}[a] (μM)		hCOX-1/hCOX-2
		COX-1	COX-2	
6b	Cl	29.2	0.32	91
6c	Me	224	0.25	896
6d	CHO	500	0.73	685
6e	OCF_3	17	0.029	773
6f	OCH_3	100	100	1
7	CCH	94.2	0.073	1290
9	Et	0.12	0.33	0.36
11	CN	115	0.11	1045
15	CF_3	4.39	0.041	107

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.

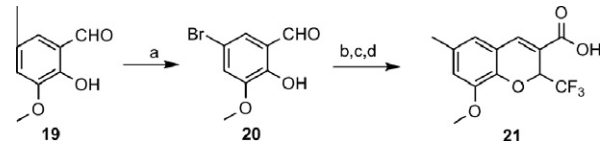
[a] see Ref. 2c sec. 2.4 and note 3.

6-methoxy substituted analog **6f** was significantly diminished. 6-OCF_3 **6e**, 6-ethynyl **7**, and 6-CF_3 **15** analogs demonstrated improved COX-2 inhibition. In the prophylactic rat air pouch assay,[2c] the 6-ethynyl **7** provided 60% inhibition, whereas the 6-OCF_3 **6e** and 6-CF_3 **15** provided complete inhibition, a difference postulated to be due to different metabolic fates of these analogs. The analog **6c** suggests a similar metabolic trend, with the methyl in the 6-position performing poorly (13%) in prophylactic rat air pouch assay. However in the therapeutic rat air pouch assay,[5] which was developed by modification of the prophylactic rat air pouch assay to identify compounds that were metabolically labile, the 6-ethyl analog **9** exhibited 90% inhibition of PGE_2 production.

Based upon these data and our confidence in the therapeutic air pouch model, we were gratified to identify efficacious compounds possessing sites for metabolism such as 6-methyl (**6c**) and 6-ethyl (**9**). Both **6c** and **9** had previously failed to meet our potency criterion, (100% inhibition at 2 mpk), for advancement as judged by their performance in the prophylactic air pouch assay. Modification of the testing scheme allowed a broader range of analogs to be considered for advanced in vivo assessment. Phenols **16a–d** were converted to salicylaldehydes **17a–d**, and further elaborated to provide **18a–d** as is shown in Scheme 3. In Scheme 4, salicylaldehyde **19** was treated with bromine to form bromo salicylaldehyde **20**, which was converted to the chromene by our standard procedure. Suzuki coupling with trimethylboroxine and hydrolysis provided 6-Me-7-MeO analog **21**. Stille coupling of **22** with tributyl(ethynyl)stannane, followed by hydrolysis gave acid **23**, see



Scheme 3. Reagents and conditions: (a) CH_3CH_2MgBr, toluene, HMPA, *p*-formaldehyde, 90 °C, 3 h, ~40% yield; (b) **3**, K_2CO_3, DMF, 80–100 °C; (c) NaOH, THF, CH_3OH, H_2O.

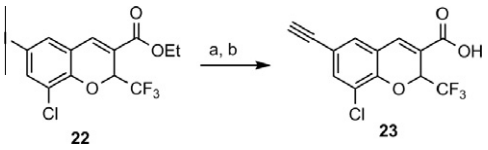


Scheme 4. Reagents and conditions: (a) bromine, HOAc, 0 °C, 2 h, 80% yield; (b) **3**, K_2CO_3, DMF, 85 °C,18 h, 63% yield; (c) trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, DMF, 90 °C, 18 h, 79% yield; (d) NaOH, THF, CH_3OH, H_2O.

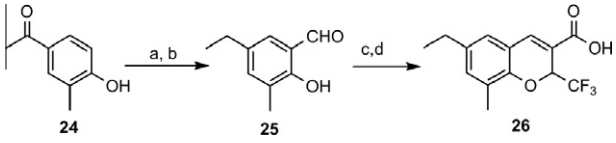
Scheme 5. 4-Acetyl phenol **24** was hydrogenated and treated with $MgCl_2$ and *p*-formaldehyde to form salicylaldehyde **25** which upon further elaboration provided **26**, see Scheme 6.

The favorable in vitro and in vivo activity of 6-chloro (**6b**), 6-trifluoromethyl (**6e**), and 6-trifluoromethoxy (**15**) substituted analogs suggested retaining these preferred 6-substituents along with a metabolically labile group. Salicylaldehyde **27** was iodinated with NIS, followed by formation of the chromene to provide **28**, which were converted to analogs **29a–d**, see Scheme 7. Analog **29a** was obtained by coupling **28** with trimethylboroxine followed by hydrolysis. The 8-ethyl **29b** was prepared in four steps; by coupling with TMS acetylene in the presence of CuI and Pd(0), followed by removal the TMS group, hydrogenation, and saponification. The 8-propyl **29c** was prepared by coupling of **28** with propyne using CuI and $PdCl_2(dppf)_2CH_2Cl_2$ as catalyst, followed by hydrolysis. 8-Iodo **28** was converted to 8-boron ester by treating with pinicol-diborane and $PdCl_2(dppf)_2CH_2Cl_2$, then converted to the 8-OH ester, which was then alkylated and hydrolyzed to provide **29d**.

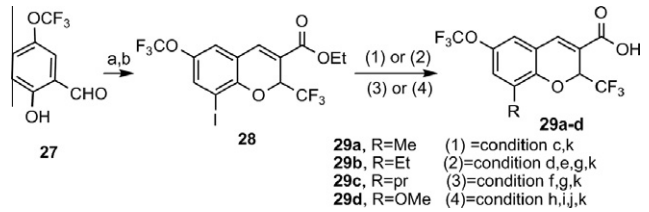
The SAR indicated that the 6-Cl-8-Me **18a** was a very potent but not a highly selective COX-2 inhibitor. The analogs 6-Me-8-Cl **18b**, 6-Me-8-Me **18c**, and 6-OMe-8-Cl **18d** displayed good potency in



Scheme 5. Reagents and conditions: (a) $(CH_3CH_2)_3SnCCH$, tetrakis(PPh_3)Pd(0), toluene, reflux, 3 h, 76% yield; (b) NaOH, THF, CH_3OH, H_2O.



Scheme 6. Reagents and conditions: (a) 20% $Pd(OH)_2$/C, HOAc, H_2, 60 psi, 16 h, 93% yield; (b) $MgCl_2$, TEA, *p*-formaldehyde, ACN, reflux, 3 h, 53% yield; (c) **3**, K_2CO_3, DMF, 80–100 °C, 78% yield; (d) NaOH, THF, CH_3OH, H_2O.



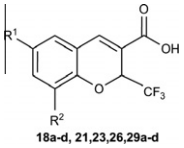
Scheme 7. Reagents and conditions: (a) NIS, DMF, 2 days, 97% yield; (b) **3**, TEA, DMF, 85 °C, 66 h, 90% yield; (c) trimethylboroxine, $PdCl_2(dppf)_2$ –CH_2Cl_2, Cs_2CO_3, dioxane, 110 °C, 6 h, 83% yield; (d) TMS-CCH, CuI, $Pd(PPh_3)_4$, TEA, 4 days, 100% yield; (e) TBAF, THF, 10 min, 73% yield; (f) CuI, $PdCl_2(dppf)_2CH_2Cl_2$, TEA, toluene, propyne, −78 °C to 25 °C, 24 h, 89% yield; (g) 10%Pd/C, EtOH, 30 psi, 3 h, 100% yield; (h) pinicolborane, $PdCl_2(dppf)_2CH_2Cl_2$, TEA, dioxane, 80 °C, 2 days, 59% yield; (i) 30% H_2O_2, THF aq NaOH, 0 °C to 25 °C, 3.5 h, 91% yield; (j) CH_3I, KI, K_2CO_3, DMF, 17 h, 100% yield; (k) NaOH, THF, CH_3OH, H_2O.

the COX-2 assay, but only the 6-Me-8-Me **18c** exhibited very high selectivity for COX-2 (Table 2). The 6-Me-8-OMe **21** exhibited weak COX-2 inhibition, while the 6-ethyynl-8-Cl **23** and 6-Et-8-Me **26** demonstrated good potency but poor selectivity for COX-2. Analogs of 6-OCF_3 **29a–d** demonstrated high COX-2 potency and intermediate selectivity, except **29d**, which displayed low selectivity. Analogs **18c** and **29a–c** displayed good potency, selectivity, and evidence of metabolism (data not shown) were subjected to chiral resolution. The data (Table 3) indicated that the *S*-isomers were more potent COX-2 inhibitors and possessed in vivo efficacy in the therapeutic air pouch model.

As mentioned previously, compounds **30** (in Part I **5g**) and **31** (in Part I **5h**) (Fig. 2) were very potent and selective COX-2 inhibitors. To explore the utility of these tri-substituted COX-2 inhibitors with reduced half-lives, we replaced two chloro groups with methyl groups. Di-methyl substituted phenols **32a–c** were converted to salicylaldehydes **33a–c**, followed by condensation with ethyl trifluorocrotonate, chlorination, and ester hydrolysis to provide acids **34a–c**, see Scheme 8.

The SAR indicated (Table 4) that tri-substituted chromene analogs **34a–c** were very potent and very selective COX-2 inhibitors. Analogs **34b** and **34c** displayed evidence of metabolism (data not shown) and were advanced to chiral resolution. The data (Table 5)

Table 2
In vitro activity of selected 6,8-disubstituted benzopyrans



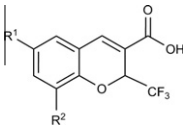
Compd	R^1, R^2	Mod human IC50[a] (μM)		hCOX-1/hCOX-2
		COX-1	COX-2	
18a	R^1 = Cl, R^2 = Me	0.47	0.084	5.6
18b	R^1 = Me, R^2 = Cl	1.08	0.19	5.7
18c	R^1 = Me, R^2 = Me	169[b]	<0.24[b]	704
18d	R^1 = OMe, R^2 = Cl	25.6	0.24	107
21	R^1 = Me, R^2 = OMe	9.62	3.33	2.9
23	R^1 = CCH, R^2 = Cl	0.14	0.026	5.4
26	R^1 = Et, R^2 = Me	0.14	0.27	0.5
29a	R^1 = OCF_3, R^2 = Me	0.14	0.016	9
29b	R^1 = OCF_3, R^2 = Et	0.50	0.074	7
29c	R^1 = OCF_3, R^2 = Pr	7	0.043	163
29d	R^1 = OCF_3, R^2 = *i*Pr	0.32	0.68	0.5

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 Ref. 2c sec. 2.4 and Ref. 3.

[b] The traditional COX enzyme assay was run with longer reaction time compared with the modified assay see Ref. 2c, sec. 2.4.

Table 3
SAR of *S* and *R* isomers



Compd	R^1, R^2	*S* isomer			*R* isomer		
		Mod hIC_{50}[a] (μM)		Air pouch	Mod hIC_{50}[a] (μM)		Air pouch
		COX-1	COX-2	Inhibition	COX-1	COX-2	Inhibition
18c	R^1 = Me, R^2 = Me	100	0.29	80%	n.d.	n.d.	n.d.
29a	R^1 = OCF_3, R^2 = Me	2.69	0.014	>68%	62.7	100	n.d.
29b	R^1 = OCF_3, R^2 = Et	19.9	0.06	>72%	100	0.86	n.d.
29c	R^1 = OCF_3, R^2 = Pr	71.7	0.042	>68%	100	1.7	n.d

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 Ref. 2c sec. 2.4 and notes 3.

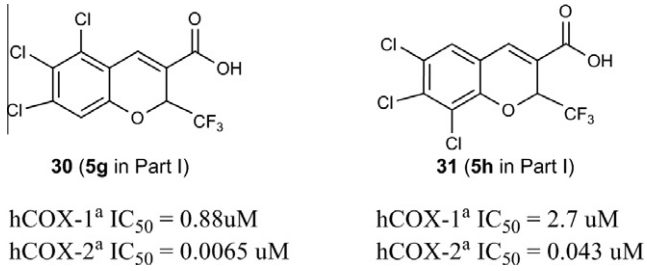
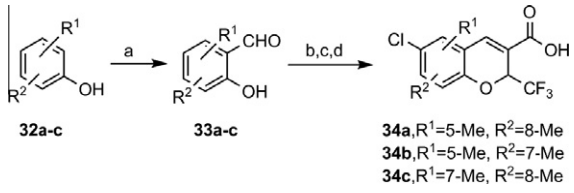
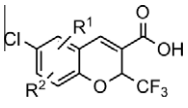


Figure 2. Examples of COX-2 inhibitors **30** and **31** (see Part 1 of this Letter). (a) See Ref. 2c sec. 2.4 and Ref. 3.



Scheme 8. Reagents and conditions: (a) $MgCl_2$, ACN, TEA, *p*-formaldehyde, 90 °C, 6 h, ~40% yield; (b) **3**, K_2CO_3, DMF, 80–100 °C; (c) HOAc, Cl_2(g), Zn dust; (d) NaOH, THF, CH_3OH, H_2O.

Table 4
Exploration of 6-chloro di-substituted patterns of benzopyrans



Compd	R^1, R^2	Human IC_{50}[a] (μM)		hCOX-1/hCOX-2
		COX-1	COX-2	
34a	5-Me, 8-Me	2.58	0.21	12
34b	5-Me, 7-Me	29.8	0.13	229
34c	7-Me, 8-Me	36.3	0.12	302

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] The traditional COX enzyme assay was run with a longer reaction time compared with the modified assay see Ref. 2c, sec. 2.4 and note 3.

Table 5
SAR of *S* and *R* isomers of tri-substituted benzopyrans **33b** and **33c**

Compd	*S* isomer			*R* isomer		
	Mod hIC_{50}[a] (μM)		Air pouch	Mod hIC_{50}[a] (μM)		Air pouch
	COX-1	COX-2	Inhibition	COX-1	COX-2	Inhibition
34b	2.28	0.046	92%	30.8	1.28	n.d.
34c	4.99	0.03	60%	100	100	n.d.

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 Ref. 2c sec. 2.4 and Ref. 3.

Table 6
Pharmacology of selected *S* isomers

Compd	Air pouch ED_{50}[a] (mg/kg)	Edema ED_{50}[b] (mg/kg)	Hyperalgesia ED_{50}[c] (mg/kg)	Arthritis ED_{50}[d] (mg/kg)	Hmicr[e]%
18c	2	8.6	20.7	10	41
29b	1.87	3.8	5.6	0.8	99
34b	0.41	8	7.6	3.2	15

[a] See Ref. 2c sec. 2.9.
[b] See Ref. 2c sec. 2.10
[c] See Ref. 2c sec. 2.10
[d] See Ref. 2c sec. 2.11.
[e] Human microsomal assay, percent metabolized.

Table 7
PK of selected *S* isomers

Priority	Compd	Rat $t_{1/2}$ (h)	Dog $t_{1/2}$ (h)	Cyno $t_{1/2}$ (h)	Projected[a] human $t_{1/2}$ (h)
Ref. Part I	SD-8381	10	20	17	115
Ref. Part II	SC-75416	16	23	24	13
1	**29b**	2	7.7	12	5
2	**18c**	8.7	19	28	67
3	**34b**	4.7	23	5.4	169

[a] Projected human $t_{1/2}$ was generated by allometric scaling.

indicated that the *S*-isomers were more potent COX-2 inhibitors and displayed in vivo efficacy in the therapeutic air pouch model. These data (Tables 3 and 5) demonstrated that analogs with small groups attached to the 6,8-positions of benzopyran, containing the *S*-isomers were more potent COX-2 inhibitors. This result was consistent with our previous findings (see Parts I and II of this Letter).

Selected compounds from Tables 3 and 5 were advanced for additional in vivo studies. All analogs in Table 6 demonstrated evidence of in vitro metabolism and good rodent in vivo efficacy. In vivo PK studies were conducted in multiple species with the objective of predicting human half-life by allometric scaling, see Table 7. Although empirical, the approach has been widely used to extrap-

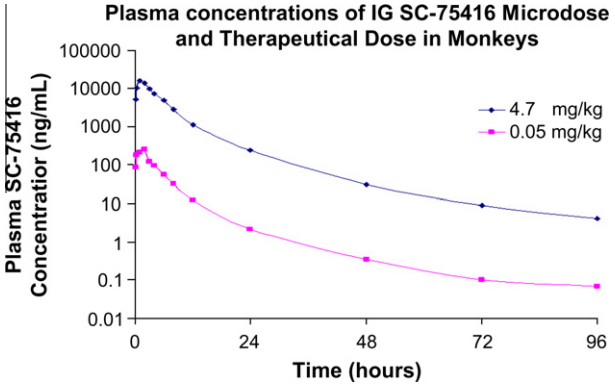


Figure 3. Plasma concentration of compound **2** (SC-75416) in microdose and therapeutic dose in monkeys.

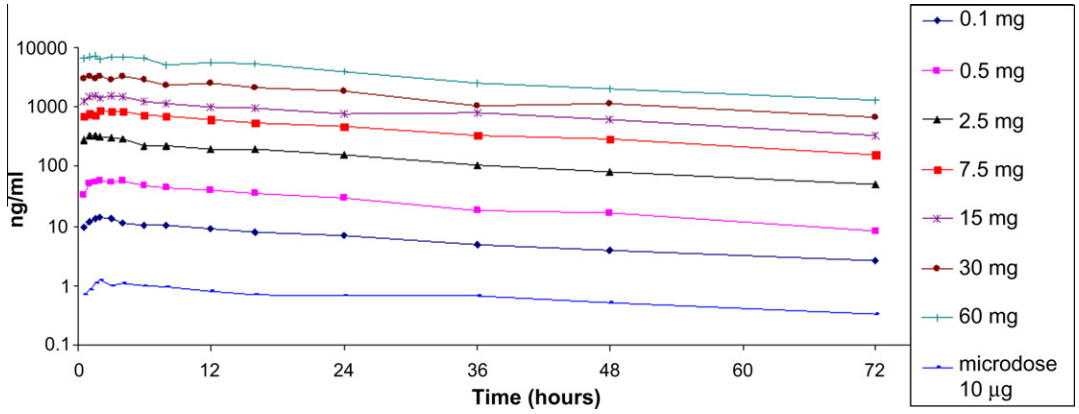


Figure 4. Utility of the microdose: plasma levels in man after oral doses of compound **2** (SC-75416).

Table 8
Human half-life of microdose candidates

Study	Compd	Human hepta. CL int	Human $t_{1/2}$ (h)	Projected[a] human $t_{1/2}$ (h)
Microdose	**29b**-(*S*)	67	13	5
Microdose	**18c**-(*S*)	23	57	67
Microdose	**33b**-(*S*)	10	11	169

[a] Projected human $t_{1/2}$ was generated by allometric scaling.

olate from animal to human.[6] Historically human $t_{1/2}$ had been difficult to predict based on allometric scaling for this class of highly ppb compounds, since neither the microsomal data nor the hepatocyte data simply predicted clearance. The data of **1** (SD-8381) (pred. human $t_{1/2}$ = 115 h, actual human $t_{1/2}$ = 340 h) and **2** (SC-75416) (pred. human $t_{1/2}$ = 13 h, actual human $t_{1/2}$ = 34 h) indicated that allometric scaling in this series was largely ineffective.

We further sought to decreased risk in our development strategy by developing and employing a screening microdose clinical study.[7] This method was thought to permit us to efficiently obtain human pharmacokinetic data (sub-therapeutic dose) prior to selection of an agent for full clinical development. In the microdose clinical trial, compounds were dosed at 1/100th the preclinical efficacious dose, which streamlined safety profiling required (low dose) and the amount of compound required. To determine if the PK profile for this class was similar across species at both micro and therapeutic dose levels, compound **2** (SC-75416) was dosed at both micro and therapeutic dose levels in monkey and man. The data demonstrated a similar PK profile in monkey and man (Figs. 3 and 4). The PK profile was unchanged, suggesting that **2** had the same clearance mechanism at both micro and therapeutic doses and in both monkey and man. Three backup candidates predicted to have an acceptable human $t_{1/2}$ (Table 8) were advanced for a clinical microdose study. Analogs **18c**-(*S*), **29b**-(*S*), and **34b**-(*S*) displayed human half-life of 57, 13, and 11 h.

In summary, we successfully applied a filter requiring Phase I metabolism as a cornerstone in our compound design and advancement criteria. We validated compounds in lower species (cyno) the relative microdose to therapeutic dose by showing the same PK profiles (data not shown). We applied a microdose strategy to rapidly obtain human PK parameters. We successfully discovered **18c**-(*S*), **29b**-(*S*), and **34b**-(*S*) as back up candidates for compound **2** (SC-75416). **18c**-(*S*), **29b**-(*S*), and **34b**-(*S*) showed human half-lives of 57, 13, and 11 h and possess superior efficacy in in vivo animal models. The microdose strategy permitted us to identify **29b**-(*S*) and **34b**-(*S*) as candidates for advancement.

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References and notes

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