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2,3-Diarylbenzopyran Derivatives as a Novel Class of Selective Cyclooxygenase-2 Inhibitors

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Abstract—A new series of cyclooxygenase-2(COX-2) inhibitors with naturally occurring flavone as the main skeleton has been synthesized and their biological activities were evaluated for cyclooxygenase inhibitory activity. Rational structural modifications were applied to potent COX-2 inhibitors to obtain the desired pharmacokinetic profiles for improved oral anti-inflammatory activity.

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used over a century for the treatment of inflammation, pain and inflammation-associated disorders. Their anti-inflammatory activity results from inhibition of cyclooxygenases (COXs), which convert arachidonic acid into prostaglandins. However, inhibition of COXs may lead to undesirable side effects such as life-threatening gastrointestinal perforation, ulcer and bleeding (PUB) as well as renal toxicity.[1]

Nowadays, it is well established that there are at least two isoforms of cyclooxygenases, COX-1 and COX-2.[2] COX-1 is constitutively expressed in a variety of tissues including the gastrointestinal tract, the kidneys, and the platelets, and is known to be responsible for bodily homeostasis. On the other hand, COX-2 is induced upon inflammatory stimuli and responsible for aggravation of inflammation. Thus, selective inhibition of COX-2 should be useful for treatment of inflammation and inflammation-associated disorders without disrupting the bodily homeostasis maintained by COX-1. Indeed, selective COX-2 inhibitors including celecoxib and rofecoxib manifested the therapeutic efficacy comparable to that of traditional NSAIDs but with improved gastrointestinal safety.[3]

COX-2 inhibitors could be regarded to have evolved from aspirin over a 100 years. The main theme in this century-long evolution process has been in the improvement of safety for chronic use. Even though COX-2 inhibitors have shown an improved gastrointestinal safety profile, they are not a perfect choice for long term use for arthritis. Recently the cardiotoxicity issue echoed around COX-2 inhibitors. Even now there are enough reasons for discovery of new classes of COX-2 inhibitors to meet the unmet safety issues. Herein we report the synthesis and the biological activity of 2,3-diarylbenzopyran derivatives as a novel class of selective COX-2 inhibitors and also we

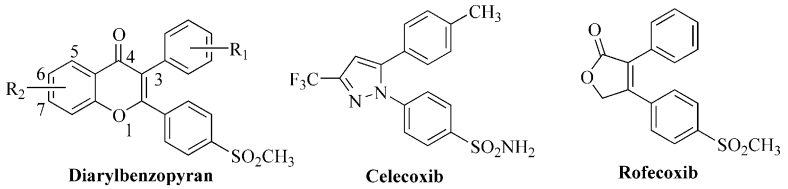

Figure 1.

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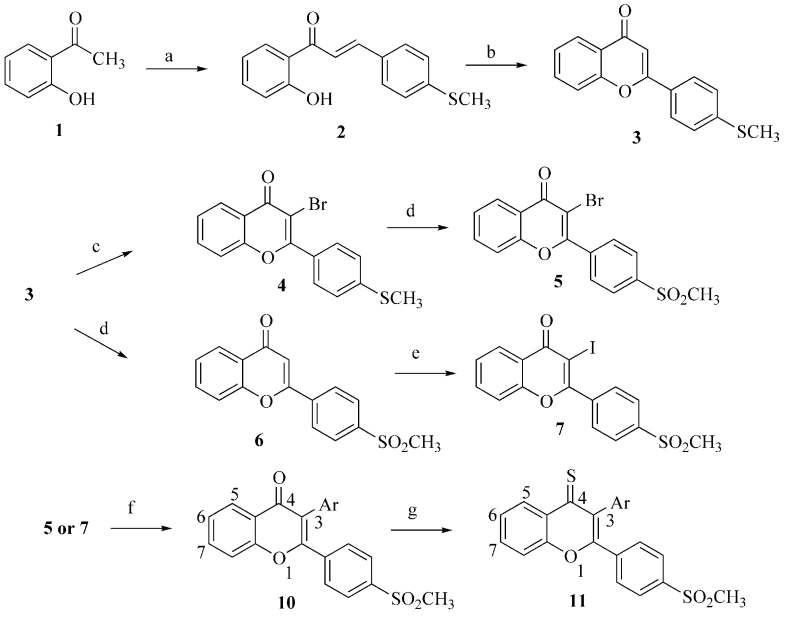
wish to discuss a rationale for the modification of benzopyran structure together with an issue for translation of in vitro activity into in vivo effect (Fig. 1).

The 2,3-diarylbenzopyran derivatives were synthesized via the route outlined in Scheme 1. First, condensation of 2-hydroxyacetophenone (**1**) and 4-(methylthio)-benzaldehyde under basic condition gave chalcone **2**, which was followed by oxidative cyclization to afford the desired flavone **3**.[4] Flavone **3** was subjected to bromination by *N*-bromosuccinimide (NBS) to yield bromide **4**, which was then oxidized to methylsulfone compound **5** by reaction with OXONE®. Alternatively, compound **3** was subjected to the OXONE® reaction and then reaction with bis(trifluoroacetoxyiodo)benzene (BTI) to give iodide compound **7**. Haloflavone **5** or **7** was then coupled with an aromatic boronic acid by Suzuki coupling[5] to yield 2,3-diarylbenzopyran derivatives **10** with a pharmacophore for selective COX-2 inhibition. A detailed procedure for synthesis of 2-{4-(methylsulfonyl)phenyl}-3-(3-pyridinyl)-(*4H*)-1-benzopyran-4-one (**10p**) is presented in ref 6 in order to illustrate the generality of Scheme 1. The benzothiopyran derivatives **11** were prepared via the reaction of diarylbenzopyran **10** with Lawesson's reagent in toluene at reflux.

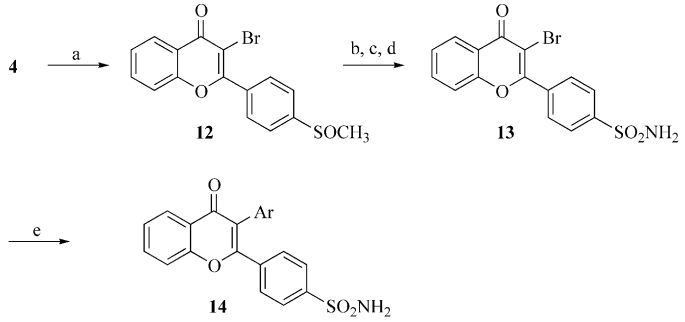
Another key functional moiety for selective COX-2 inhibition, aminosulfonyl group, was introduced as illustrated in Scheme 2.

Compound **4** was converted to sulfoxide **12** using OXONE® or MCPBA, which was then subjected to in a sequential manner (1) a Pummerer-type reaction,[7] (2) treatment with chlorine in AcOH, and (3) reaction with NH_4OH to yield sulfonamide **13**. Then the Suzuki coupling of sulfonamide **13** with boronic acid **8** or boronate **9** afforded the desired sulfonamide **14**.

The benzopyran analogues synthesized in this study were tested for their ability to inhibit COX-2 and COX-1 by using freshly harvested mouse peritoneal macrophages as described in the literature.[8] The in vitro inhibitory



Scheme 1. Reagents and conditions: (a) 4-(methylthio)benzaldehyde, KOH, EtOH–H_2O; (b) iodine/DMSO, reflux; (c) NBS,$CHCl_3$; (d) oxone; (e) BTI (=$PhI(OCOCF_3)_2$), I_2; (f) $ArB(OH)_2$(**8**) or $ArB^-(OCH_3)_3Li^+$(**9**), $Pd(PPh_3)_4$, 2 M Na_2CO_3, EtOH–H_2O–toluene; (g) Lawesson's reagent, toluene. Ar = substituted or unsubstituted aryl, heteroaryl.



Scheme 2. Reagents and conditions: (a) oxone or MCPBA; (b) TFAA, reflux; (c) Cl_2, AcOH; (d) NH_4OH; (e) arylboronic acid, $Pd(PPh_3)_4$, 2 M–Na_2CO_3, EtOH–H_2O–toluene. Ar = substituted or unsubstituted aryl, heteroaryl.

Table 1. In vitro COX-2 and COX-1 enzyme inhibitory activities of benzopyran derivatives **10**, **11** and **14**

Compd	Ar	COX-2 (IC_{50}, μg/mL)[a]	COX-1 (% inhibition at 10 μg/mL)[a]
10a	Phenyl	0.35	<5
10b	4-Fluorophenyl	0.08	<5
10c	2-Fluorophenyl	0.03	<5
10d	3-Fluorophenyl	0.21	<5
10e	4-Chorophenyl	0.14	<5
10f	3-Chlorophenyl	0.15	<5
10g	2,4-Dichlorophenyl	0.16	<5
10h	3,4-Dichlorophenyl	0.12	<5
10i	3,5-Dichlorophenyl	7%[b]	<5
10j	4-Methoxyphenyl	0.21	<5
10k	2-Methylphenyl	0.37	<5
10l	1-Naphthyl	1.62	<5
10m	2-Benzo[*b*]thiophenyl	0.14	<5
11a	Phenyl	0.04	<5
11b	4-Fluorophenyl	0.03	<5
11c	2-Fluorophenyl	0.08	<5
11h	3,4-Dichlorophenyl	0.24	<5
11j	4-Methoxyphenyl	0.06	<5
14a	Phenyl	0.04	61
14b	4-Fluorophenyl	0.05	67
14c	2-Fluorophenyl	0.07	60
14j	4-Methoxyphenyl	0.16	84
14n	4-Methylthiophenyl	0.12	90
14o	3,4-Methylenedioxyphenyl	0.39	75
	Celecoxib	0.01	76

[a]Values are means of at least two measurements.
[b]% Inhibition at 3 μg/mL.

activities against COX-2 and COX-1 are summarized in the Table 1.

Methylsulfone compounds **10** and thiobenzopyran compounds **11** showed moderate to good COX-2 inhibitory activities. No meaningful COX-1 inhibition was observed for these COX-2 inhibitors at 10 μg/mL. Introduction of a halogen atom on the 3-aryl ring often resulted in improved COX-2 potency, however, a big steric bulk on the 3-aryl ring appeared to be inappropriate for COX-2 activity as in 3,5-dichlorophenyl derivative **10i** and 1-naphthyl derivative **10l**. **10c** showed COX-2 inhibitory potency comparable to that of celecoxib. Sulfonamide derivatives **14** showed some improvement in COX-2 inhibitory potency from the corresponding methylsulfone derivatives **10**, however, the improvement was not significant. Instead sulfonamide derivatives **14** showed a decreased COX-2 selectivity

Table 2. In vitro COX-2 and COX-1 enzyme inhibitory activities of benzopyran derivatives with *N*-heterocyclic substituents

Compd	COX-2 (IC_{50}, μg/mL)[a]	COX-1[a,b]
10p	0.5	13
10q	24%[b]	<5
10r	0.94	18
10s	44%[b]	17
10t	13%[b]	<5
15	0.5	12

[a]Values are means of at least two measurements.
[b]% Inhibition at 10 μg/mL.

over COX-1, replicating the observations in other classes of COX-2 inhibitors with pharmacophore of 1,2-diaryl alkene type.[9]

When the benzopyran derivatives were evaluated against carrageenan-induced rat paw edema model, there were no meaningful anti-inflammatory effects observed. Pharmacokinetic evaluation for some of the benzopyran derivatives suggested that their oral bioavailability is very poor. The benzopyran COX-2 inhibitors in Table 1 have three aromatic groups and tend to be highly lipophilic. If oral anti-inflammatory activity is to be pursued for the benzopyran derivatives, there should be some structural variation to overcome the issue of poor bioavailability.

A nitrogen-containing aromatic group such as pyridine would be a reasonable choice for reducing overt lipophilicity of an aromatic group. The substitution of the phenyl moiety with a pyridinyl group on the 3-position of benzopyran tended to show reduction of COX-2 inhibitory potency when compared to **10a** (see Tables 1 and 2). However, their solubility increased to the level of 100–200 μg/mL in 1% aqueous DMSO solution to meet a premise for pharmacokinetic purpose. Among them, the 3-pyridinyl compounds **10p**, **10r**, and **15** showed in vitro COX-2 inhibitory activity similar to that of the corresponding phenyl-containing parent COX-2 inhibitor **10a** (Fig. 2).

Assessment of the anti-inflammatory activity of **10p** by carrageenan-induced rat paw edema[10] in Sprague–Dawley (SD) rats exhibited a modest oral activity (18% inhibition at 3 mg/kg, 25% inhibition at 10 mg/kg, 41% inhibition at 30 mg/kg body weight) compared to cel-

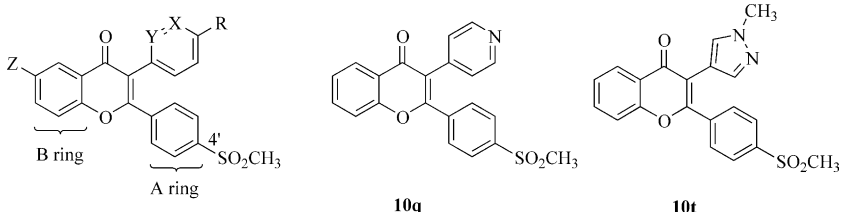


Figure 2.

ecoxib (38% inhibition at 3 mg/kg). However, **10p** failed to show any appreciable oral anti-inflammatory activity at 3 mg/kg/day, QD (daily once), when evaluated by a subchronic inflammatory animal model of adjuvant-induced arthritis.[11] It was perceived that such a poor anti-inflammatory effect by **10p** would be from its rapid degradation in the body after oral intake. Indeed **10p** was metabolized very fast in the SD rat with a plasma clearance half-life of 0.5 h. It has been well-documented that the 4′-position of flavonoid A ring is highly susceptible to hydroxylation by CYP 450 isozymes.[12] However, owing to the presence of 4′- methylsulfonyl group, the A ring metabolism of compound **10p** must be structurally hindered. Furthermore, reduced electron density in the pyridine ring makes the pyridine moiety of **10p** resistant to metabolic degradation by CYP 450 isozymes. Therefore, it is likely that the metabolic event should occur in the B ring of flavonoid in this specific case. In order to extend the plasma clearance half-life, was designed and prepared compound **15**, in which the 6-position of the flavone B ring is substituted with fluorine atom. Indeed, the effect of the substitution at the 6-position by fluorine atom showed up as a significant increase in plasma clearance half-life: $T_{1/2} = 7.4$ h; $T_{max} = 5.0$ h; and $C_{max} = 6.9$ μg/mL at 10 mg/kg, po Compound **15** showed a modest anti-inflammatory effect by carrageenan-induced rat paw edema, comparable to that of **10p** (20% inhibition at 3 mg/kg po, 26% inhibition at 10 mg/kg po, 41% inhibition at 30 mg/kg po). As noted previously compound **10p** failed to show any notable anti-inflammatory effect at 3 mg/kg/day, QD (daily once) by adjuvant-induced arthritis, possibly due to its very short plasma clearance half-life. However, the extended plasma clearance half-life of compound **15** allowed to show a modest oral activity against adjuvant-induced arthritis by the therapeutic model: 63% inhibition at 3 mg/kg/day, QD ($n = 7$ per group) for **15**; 51% inhibition at 0.3 mg/kg/day, QD for positive comparator celecoxib.

In summary, we prepared a class of selective COX-2 inhibitors having the central scaffold with naturally occurring flavonoids. In order to improve the inappropriate pharmacokinetic properties of the benzopyran COX-2 inhibitors, rational considerations were applied for the modification of the benzopyran scaffold as well as the pendant 3-position aromatic group. Bioavailability was improved by modification of the 3-position aromatic pendant. In the meantime the extension of plasma clearance half-life became possible by modulating the metabolically susceptible flavone ring. Compound **15** is an orally potent COX-2 inhibitor obtained through such rational structural modifications.

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6. Selected compounds were prepared as follows.
3-Bromo-2-(4-(methylthio)phenyl)-4*H*-1-benzopyran-4-one(4). A solution of (4-(methylthio)phenyl)-4*H*-1-benzopyran-4-one (0.50 g,1.86 mmol) and NBS (0.36 g, 2.05 mmol) in $CHCl_3$ (30 mL) was heated to reflux for 5 h. The resulting mixture was washed with saturated $NaHCO_3$, brine and dried over anhydrous $MgSO_4$, and filtered, and concentrated under reduced pressure. The residue was subjected to flash chromatography (SiO_2, hexane/ethyl acetate, 4:1) to yield the title compound as a pale yellow solid (0.6 g, 93%). Mp; 162–163 °C (CH_2Cl_2/petroleum ether): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.30–8.27 (1H, m), 7.84–7.80 (2H, m), 7.74–7.69 (1H, m), 7.51–7.43(2H, m), 7.38–7.34 (2H, m), 2.55 (3H, s): IR (KBr); 1658, 1611, 1463, 1331, 1065, 753 cm^{-1}: MS(EI); 346 (M^+), 348 ($M^+ + 2$): HRMS(EI); calcd for $C_{16}H_{11}O_2SBr$ 345.9663, found 345.9669.
3-Bromo-2-(4-(methylsulfonyl)phenyl)-4*H*-1-benzopyran-4-one(5). To a solution of 3-bromo-2-(4-(methylthio)phenyl)-4*H*-1-benzopyran-4-one (0.6 g, 1.73 mmol) in MeOH (10 mL) and THF (10 mL) was added a solution of OXONE® (1.59 g, 2.59 mmol) in H_2O (10 mL) dropwise at 0 °C. The resulting mixture was stirred for 3 h. And the solution was extracted two times with CH_2Cl_2 (20 mL per each), and the organic layer was washed with brine and dried over anhydrous $MgSO_4$. The resulting solution was filtered and concentrated under reduced pressure. Recrystallization (CH_2Cl_2/petroleum ether) of the resulting residue yielded the title compound as a solid (0.59 g, 90%). Mp 211–213 °C (CH_2Cl_2/petroleum ether): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.34–8.30 (1H, m), 8.15–8.05 (4H, m), 7.80–7.74 (1H, m), 7.54–7.49 (2H, m), 3.15 (3H, s): IR (KBr); 1646, 1310, 1146, 1075 cm^{-1}: MS(EI); 378 (M^+), 380($M^+ + 2$): HRMS(EI); calcd for $C_{16}H_{11}O_4SBr$ 377.9561, found 377.9561.
2-(4-(Methylsulfonyl)-phenyl)-3-(3-pyridinyl)-4*H*-1-benzopyran-4-one(10p). To a solution of 3-bromo-2-(4-(methylsulfonyl)phenyl)-4*H*-1-benzopyran-4-one (2.5 g, 6.6 mmol), lithium trimethoxy-3-pyridinylboronate (1.62 g, 8.5 mmol) in toluene (100 mL) and ethanol (100 mL) was added 2 M aqueous sodium carbonate (6 mL) and then tetrakis (triphenylphosphine) palladium (0.38 g, 0.33 mmol) and was stirred at 100 °C for 27 h. After being concentrated under reduced pressure, it was dissolved in CH_2Cl_2 (50 mL) and washed with water, brine. The organic layer was dried over anhydrous $MgSO_4$. And the resulting solution was filtered and concentrated under reduced pressure. The residue was subjected to flash chromatography (SiO_2, CH_2Cl_2/ethyl acetate, 1:4) to yield the title compound as a pale yellow solid (1.04 g, 42%). Mp 220–221 °C (CH_2Cl_2/petroleum ether): ^{1}H NMR ($CDCl_3$, 300MHz) δ 8.57–8.54 (1H, m), 8.33–8.29 (2H, m), 7.92–7.88 (2H, m), 7.82–7.70 (2H, m), 7.62–7.48 (4H, m), 7.37–7.34 (1H, m), 3.07 (3H ,s): IR (KBr); 3052, 2923, 1639, 1446, 1381, 1301, 1156, 787 cm^{-1}: Anal. calcd for $C_{22}H_{17}FO_4S$: C, 66.83; H, 4.01; N, 3.71; S, 8.50. Found: C, 66.67; H, 4.02; N, 3.65; S, 8.47.
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