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Structure-based parallel medicinal chemistry approach to improve metabolic stability of benzopyran COX-2 inhibitors

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ABSTRACT

Combination of the structure-based design and solid-phase parallel synthesis provided an integrated approach to rapidly develop the structure–activity relationship of benzopyran COX-2 inhibitors. Binding free energies predicted by free energy perturbation theory yielded good agreement with experimental results. New potent and selective lead compounds with improved metabolic properties were identified.

Produced as one of the major metabolites from the arachidonic acid metabolism pathway, prostaglandin E_2 (PGE_2) is a key mediator of the pain and edema associated with inflammation. The production of PGE_2 is regulated by cyclooxygenase (COX) which catalyzes the oxygenation of arachidonic acid to prostaglandin G_2 (PGG_2) and further reduces it to prostaglandin H_2 (PGH_2) in a peroxidation reaction. PGH_2 is then converted by various downstream enzymes into important inflammatory and cardiovascular modulators such as PGE_2, PGD_2, PGI_2, and thromboxane (TXA_2).[1–3]

Cyclooxygenase exists in mammalian cells in two isoforms, COX-1 and COX-2. Although both isoforms share about 60% amino acid sequence, a high degree of structural topology, and an identical catalytic mechanism, they have very different biological implications.[3,4] COX-1 is constitutively expressed in healthy cells responsible for regulating thrombogenesis and protecting gastrointestinal tract, while COX-2 is induced during inflammation process in response to different types of cytokines or toxins in many cell types.[5–7] Traditional nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacine, ibuprofen, and aspirin have provided positive treatment for pain and edema associated with arthritis and inflammation. However, due to their non-selective inhibition of both COX-1 and COX-2 that depletes tissues of prostaglandins and thromboxanes,[6,8,9] long term use of NSAIDs often causes gastrointestinal ulcers and potentially life-threatening bleeds.[9–11]

It was substantiated by specific COX-2 inhibitors, including **Celecoxib**, Rofecoxib and Valdecoxib, that selective inhibition of COX-2 was sufficient for efficacy while avoiding the gastrointestinal side effects of NSAIDs.[12–14] Although the gastrointestinal benefits of Coxibs were shadowed by observation of increased incidences of adverse cardiovascular effects,[15] the results of large scale of clinical trial indicated that cardiovascular risk not only happened to COX-2 selective inhibitors but also occurred with traditional NSAIDs.[16] It was also observed that there was marked variability in how each individual response to COX-selective inhibitor or traditional NSAIDs, depending on their unique genetic make-up.[17] These findings implicate the need to discover novel COX-2 inhibitors which could provide better treatment options for each individual patient.

As part of our continued effort to discover novel anti-inflammatory agents to treat arthritis, pain, and other inflammatory conditions, a novel COX-2 inhibitor lead was identified from in-house high throughput screening.[18] In comparison to the preceding COX-2 inhibitors, the new lead **SD-8381** as shown in Figure 1 has unique physicochemical and pharmacological properties. Despite its low molecular weight (313 Da) compared to the Coxibs it showed decent activity for COX-2 (IC_{50} = 0.3 μM) and selectivity against COX-1 (IC_{50} = 32 μM). Furthermore, it had very high solubility (>30 μM), good in vivo efficacy (ED_{50} = 0.034 mg/kg in

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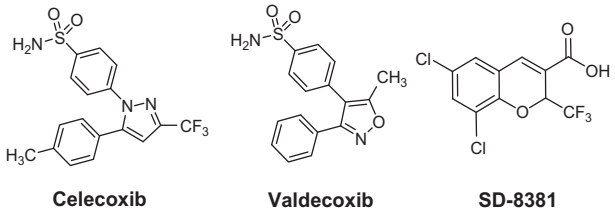


Figure 1. Some COX-2 inhibitors discovered from Pfizer laboratories.

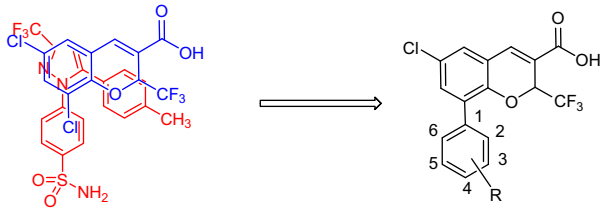


Figure 3. Overlay of **Celecoxib** (red) and benzopyran lead SD-8381 (blue) led to the design of new benzopyran analogues.

adjuvant arthritis model) and it does not employ the sulfonamide group that could be potentially associated with the allergic reaction for certain patient populations. However, advancement of this compound was significant hindered by its extremely high metabolic stability ($t_{1/2}$ = 360 h in human). Therefore our goal was to overcome the metabolic deficiency of **SD-8381** and discover new lead compounds that could demonstrate improved metabolic properties. This Letter disclosed our efforts on the utilization of parallel medicinal chemistry under the guidance of structure-based molecular modeling design to rapidly develop the structure–activity relationship and identify novel benzopyran-based COX-2 inhibitors with improved metabolic stability and pharmacokinetic properties.

Examining binding modes of **Celecoxib** and **SD-8381** in the crystal structures of their respective complexes with COX-2 revealed that both inhibitors bound to the main pocket of COX-2 enzyme. Based on X-ray crystallography the Cl atom at the 8-position of **SD-8381** pointed to the same side pocket of COX-2 that the arylsulfonamide moiety of **Celecoxib** occupied.[19,20] As illustrated in Figure 2 molecular modeling suggested that the side pocket could accommodate much bigger and more diverse set of groups than Cl. A phenyl group was introduced as spacer to form a new template, 8-phenyl benzopyran core. The para or ortho positions of 8-phenyl group of benzopyran thus provided suitable places to further modify the pharmacological and physicochemical properties of **SD-8381** as shown in Figure 3. Considering the steep structure–activity-relationship of benzopyran analogues observed in our preliminary research, a library of compounds with a variety of physicochemical properties was designed and synthesized to explore the binding space occupied by sulfonamide group of **Celecoxib** in COX-2 enzyme.

The analog synthesis of **SD-8381** was carried out on solid phase in a parallel approach to accelerate the program progression. As illustrated in Figure 4, the starting material **1** was reacted with ethyl trifluoro-methylcrotonate in the presence of potassium carbonate under nitrogen at 70 °C. The resulting intermediate **2** was produced in 90% yield. After hydrolysis of intermediate **2** with lithium hydroxide in the mixture of THF and water, intermediate **3** was obtained in 80% yield. The intermediate **3** was then loaded on bromo Wang resin (4-(bromomethyl)phenoxymethyl-polystyrene) in the presence of cesium carbonate at 60 °C; DMA was found to be a better solvent than DMF for the loading. Subsequently the bromo group on benzopyran template was converted into various aromatic substituents via Suzuki coupling chemistry catalyzed by tetrakistriphenylphosphinopalladium (0) with a diverse set of aromatic boronic acids.[21] The product **6** was cleaved from resin with 95% trifluoroacetic acid in DCM using triisopropylsilane as scavenger. The final product was purified on reverse-phase LC before submitted for biological evaluation.

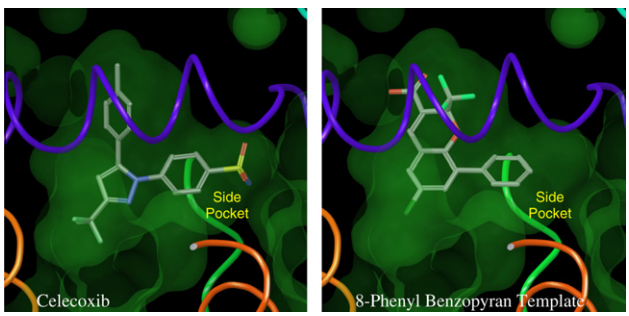


Figure 2. Comparison of binding modes of **Celecoxib** (PDB code 3LN1) and the 8-phenylbenzopyran template modeled in COX-2.

The in vitro COX enzymatic inhibition and metabolic stability data were listed in Table 1. In agreement with modeling prediction, the hybrid analogue **6a** with sulfonamide group at the para position had reasonable binding affinity and selectivity against COX-2, albeit it had weaker binding affinity and less selectivity than **Celecoxib**. However, replacing the sulfonamide with methyl sulfone (SO_2Me) resulted in 30-fold loss of the binding potency against COX-2 and produced a weak COX-1 selective inhibitor **6b**, while the SAR studies on **Celecoxib** had shown that a SO_2Me or SO_2NH_2 substituent at the para position of the phenyl ring on the pyrazole template often provided optimum COX-2 selectivity and inhibitory potency.[14] Substitution of the para position with polar and hydrogen bond-donating groups such as NH_2, methyl hydroxyl, OH, and carboxylic acid all generated analogues (**6c**–**6g**) with poor binding affinity and selectivity against COX-2; apparently, this position did not prefer polar group for desirable binding and selectivity for COX-2.

Para substitution of the phenyl ring with lipophilic group often improved potency and selectivity. Small substituent like H, Me, and Cl except F could generate reasonable COX-2 binding affinity as shown by analogues **6h, 6j,** and **6k**. Para Et and SEt substitution significantly improved the selectivity up to over 200-folds for COX-2 and maintained similar binding affinity as shown in analogue **6l** and **6n.** Comparing to **6n**, larger substituent such as 4-$CH_2CH(CH_3)_2$ in **6o**, reduced binding affinity by more than 50-folds. Such bulky groups started to exceed the spatial limit of the side pocket thus exhibited poor activity. On the other hand, di-substitution on the phenyl ring with small lipophilic groups produced analogues with decent binding affinity to COX-2. Compounds **6p** and **6q** demonstrated better binding potency and selectivity than lead compound **SD-8381** against COX-2.

One major goal of our investigation was to reduce the metabolic stability of **SD-8381**. The clearance of the benzopyran analogues due to phase I metabolism was evaluated in human and rat microsomal stability assays.[22] **SD-8381** was highly stable and was 100% remained after 30 min incubation in both human and rat microsomal assays. The analogues substituted with polar functions of hydrogen bonding capacity, for example, **6c**–**6g**, were also very stable against phase I metabolism, displaying more than 90% remaining in human microsomal assay. However, analogues with lipophilic alkyl substituent significantly increased the phase I metabolism, in keeping with the general trend of metabolic observation.

Analogues **6k**, **6l,** and **6p** had very low stability with around 30% or less remaining in human microsomal assay. Both **6l** and **6P**

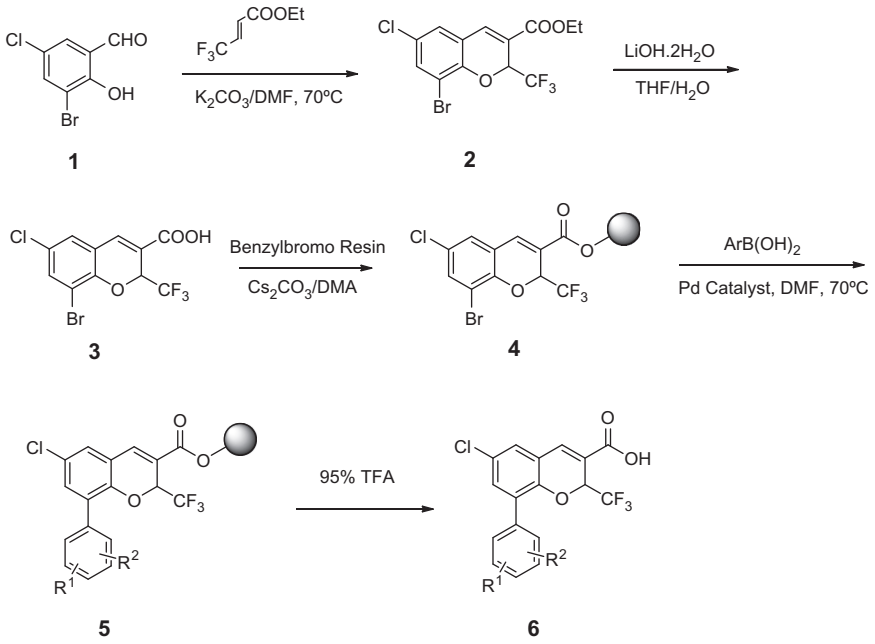
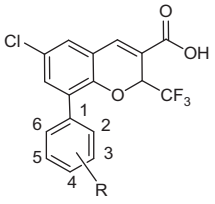


Figure 4. The solid phase synthetic scheme.

Table 1
COX enzyme binding affinities and metabolic stability having diverse substitution on aromatic ring at position 8



Compds	R	COX inhibition IC_{50} (μM)		Selectivity ratio	Microsomal metabolic stability, % remaining	
		hCox-1	hCox-2		Human	Rat
Celecoxib		15	0.04	375		
SD-8381	Cl	32	0.3	107	100	100
6a	4-SO_2NH_2	91.4	0.758	120		
6b	4-SO_2CH_3	3.87	21.3	0.182		
6c	4-NH_2	1.39	1.82	0.764	93	53
6d	4-$NHSO_2$–CH_3	>100	40.1	>2.50	100	53
6e	4-CH_2OH	2.44	6.38	0.38	93	75
6f	4-OH	2.91	3.48	0.84	91	94
6g	4-COOH	>100	>100	1.00	90	91
6h	H	1.54	0.188	8.20		
6i	4-F	0.740	>100	<0.007		
6j	4-Cl	0.242	0.0211	11.4	53	57
6k	4-Me	4.34	0.103	42.1	18	60
6l	4-Et	17.7	0.0784	226	18	60
6m	4-OEt	32.0	0.355	90.1	91	82
6n	4-SEt	75.9	0.242	314	75	47
6o	4-CH_2CH–$(CH_3)_2$	26.5	13.4	2.00	88	96
6p	3-F, 4-Me	24.7	0.066	375	31	55
6q	3-Me, 4-OMe	>100	0.339	>295	84	61

retained high COX-2 binding affinity and over 200-folds COX-2 selectivity. Surprisingly, analogs **6m**, **6o**, and **6q** were highly stable in microsomal assay (80% remaining) since OEt, 4-$CH_2CH(CH_3)_2$, and OMe moieties were generally considered to be metabolically labile in phase I metabolism. It was possible that the unique molecular conformation of benzopyran template combined with the aryl ring bridge either hindered molecular binding to the active site of cytochrome P450, or prevented the presentation of the metabolically labile groups to the catalytic machinery of cytochrome P450.

Free energy perturbation (FEP) methods had demonstrated promise in predicting relative binding affinities with accuracies that were usually better than docking and MM-GBSA calculations.[23–26] The sources of COX2 binding affinities, for example, contributions from the desolvation energy and the binding enthalpy

Table 2
Comparison of experimental and calculated relative binding free energies (kcal/mol)

A→B	Calculated		$\Delta\Delta G_{binding}$	
	ΔG_{aq}	ΔG_{COX2}	Calcd	Exptl[a]
6i→6h	1.5454	0.4513	−1.0941	<−3.718
6j→6h	1.1312	1.1578	0.0266	1.096
6j→6i	−0.8280	−0.1158	0.7122	>5.014
6k→6h	1.7415	4.3390	2.5975	0.356
6k→6i	0.3697	−0.8550	−1.2247	>4.075
6k→6j	0.9269	−0.4958	−1.4227	−0.9393
6k→6f	−10.5302	−11.3169	−0.7867	2.085
6k→6c	−10.7945	−9.9471	0.8474	1.701
6l→6k	−0.3905	−0.4782	−0.0877	0.1617
6l→6e	−6.4125	−5.9378	0.4747	2.606
6m→6l	−0.076	−6.6389	−5.9313	−0.895
6n→6m	2.5271	5.9868	3.4597	0.2270

ΔG_{aq} is the entropy change in the aqueous phase upon desolvation, and ΔG_{COX2} is the enthalpy change upon binding to COX2 protein.

[a] Relative free energies are derived from the IC_{50} values given in Table 1 using the approximation $\Delta\Delta G = \Delta G_2 - \Delta G_1 \approx RT \ln(IC_{50,2}/IC_{50,1})$.

separately to the total binding free energy, could provide insights into understanding the complex event of ligand–protein binding. We studied this set of benzopyran analogs by employing an approach that combined docking and Monte Carlo (MC) simulations. Using the docking-predicted starting conformations, relative changes in binding free energies were computed by the MC FEP simulations.[24] Results of the predictions along with experimental data were shown in Table 2.

It was noted that the FEP calculations produced trends in activity changes that was in keeping with the experimental values for most of the pairs mutating from compound A to compound B. The absolute values of binding affinities still awaited improvement in accuracy. In particular, the calculation for the 4-fluoro-phenylchromene (**6i**) and its 4-methyl analog (**6k**) failed to predict the abolishment of its COX-2 activity, which was a very contradictive SAR data point. It had been reported that the affinity of certain COX-2 inhibitors were largely driven by slow kinetics rather than thermodynamic equilibrium, thus relative binding affinity prediction could be more challenging for COX-2 enzyme than the other biological targets.[27] Approximations were made to derive binding affinities from IC_{50} values. Error in theoretical prediction could also be introduced by limitations in FEP calculations, for example, chopping of the protein–inhibitor complex to gain calculation speed, deficiency in force field parameters, insufficient sampling, etc.

It was revealed by the calculation though, that difference in solvation might play an important role between relatively similar compounds. Mutations from methyl to amino (**6k→6c**) and hydroxyl (**6k→6f**) groups both resulted in large free energy changes in the free ligand states. The trend was carried to a less extent by the ethyl to hydroxyl methyl mutation (**6l→6e**). Two molecules might have similar interactions with a protein, but different solvation properties in water, leading to solvation-driven differences in binding free energies which might not be always intuitive.

In summary, application of parallel medicinal chemistry and structure-based design yielded libraries of compounds that addressed diverse spaces of physicochemical properties. In the current study we demonstrated that the combination of computer modeling design and parallel medicinal chemistry could rapidly explore the interesting binding pocket space and develop structural–activity and structure–property relationships. In particular, the substitution on phenyl group at position 8 of benzopyran COX-2 lead **SD-8381** preferred hydrophobic groups, but disfavored hydrogen bond bearing polar functions, to retain high binding affinity and selectivity against COX-2. Reduction of metabolic stability in phase I metabolism was achieved by installing lipophilic moieties on the 8-phenyl off benzopyran. A few analogs such as **6l** and **6p** were carried on for further investigation in vivo. New lead compounds had emerged from our efforts to advance the COX2 drug discovery program.

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