Biological Evaluation and Molecular Docking Study of 3-(4-Sulfamoylphenyl)-4-phenyl-1*H*-pyrrole-2,5-dione as COX-2 Inhibitor

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Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids, including prostaglandins, prostacyclin and thromboxane. Three COX isoenzymes are known: COX-1, COX-2, and COX-3 which is a splice variant of COX-1.[1] COX-1 is considered a constitutive enzyme, being found in most mammalian cells. On the other hand, COX-2, undetectable in normal tissues, and induced during inflammation, hypoxia and Wnt-signalling, is present in many cancers.[2] Classical non-steroidal *anti*-inflammatory drugs (NSAIDs) such as aspirin block both COX-1 and COX-2. When the COX-1 is inhibited, inflammation is reduced, but the protection of the lining of the stomach is also lost. This can cause stomach upset as well as ulceration and bleeding from the stomach and even the intestines.[3] Therefore, selective COX-2 inhibitors such as celecoxib and rofecoxib had been developed and prescribed.[4] As COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibition together with the decreased risk of peptic ulceration.[5] However, coxib drugs such as rofecoxib (Vioxx®) and valdecoxib (Bextra®) were withdrawn from the market in 2004 and 2005, respectively, because they excessively increased the risk of heart attacks and strokes with long term use.[6] Celecoxib (Celebrex®) is the only COX-2 inhibitor available in the United States. On the other hand, some studies have suggested that rofecoxib's adverse cardiac events may not be a class effect but rather an intrinsic chemical property related to its metabolism.[7] For this reason, novel scaffolds with selective COX-2 inhibitory activity needs to be found and evaluated for their *anti*-inflammatory effects. Recently, we reported that 1*H*-pyrrole-2,5-dione derivative **2l** from our compound library showed an inhibitory activity, with IC$_{50}$ value of 0.61 μM against LPS-induced PGE$_2$ production in RAW 264.7 macrophages (Fig. 1).[8] In this study, compound **2l** was further biologically evaluated in the hope that this may be further explored as selective COX-2 inhibitor and non-adverse *anti*-inflammatory lead compound.

The synthetic procedures and reaction conditions for 3-phenyl-4-(4-sulfonamido)-1*H*-pyrrole-2,5-dione **2l** are shown in Scheme 1: 4-Chlorosulfonylphenylacetic acid (**4**) was prepared from the reaction of phenylacetic acid (**3**) with ClSO$_3$H, and then treated with SOCl$_2$ and subsequent NH$_3$ gas in CH$_3$CN to provide 4-chlorosulfonylphenylacetamide (**5**), which was condensed with ethyl benzoylformate (**2**) using NaH condition to yield 3-(4-sulfamoylphenyl)-4-phenyl-1*H*-pyrrole-2,5-dione (**2l**).[8]

Compound **2l** was further evaluated for an inhibitory activity against both peptidoglycan- and poly(I:C)-induced PGE$_2$ productions in RAW 264.7 macrophages together with NS-398 as positive control as described previously.[9] This compound generally showed the inhibitory trend similar to those of LPS-related assay on both dose- and time-dependent responses, respectively. It exhibited particularly equal or superior activity to ibuprofen and meloxicam at high concentrations (> 5 μM) against three TLR ligand-induced assay systems (Supplementary materials: Fig. 1). The IC$_{50}$ values of ibuprofen and meloxicam were determined to be 0.86 and 0.09 μM, respectively, compared to 0.61 μM of **2l** under our assay system (Table 1). The study of the ability of compound **2l** to inhibit ovine COX-1 and human recombinant COX-2 activity were carried out using an enzyme immunoassay (EIA) kit (kit catalog number 560101, Cayman Chemical, Ann Arbor, MI, USA).[10] The efficacy of compound **2l** was determined as the concentration causing 50% enzyme
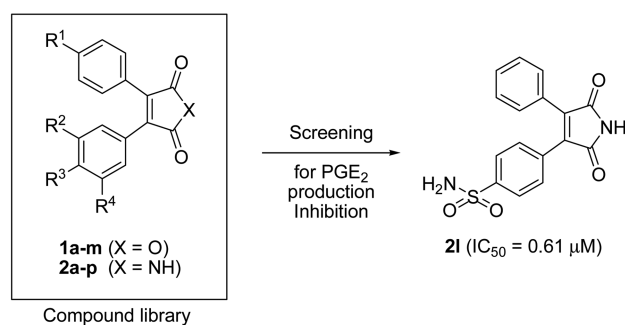
Figure 1. Selection of **2l** from 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione library.

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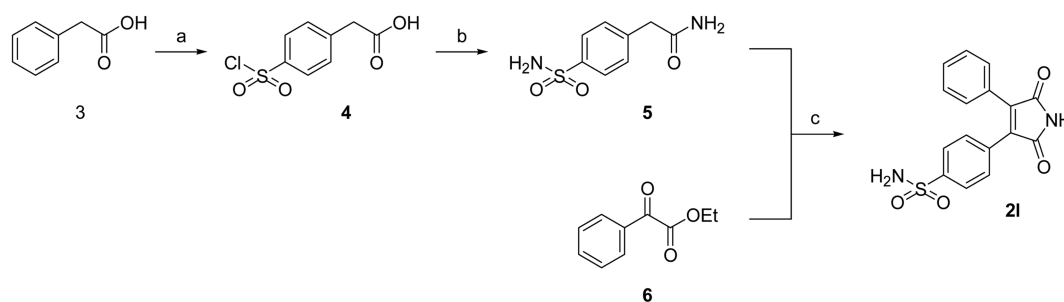
Scheme 1. Reagents and conditions for compound **2l**: (a) ClSO$_3$H, 0 °C to rt, 12 h, 91%; (b) i. SOCl$_2$, DMF, rt, 16 h; ii. NH$_3$ (*g*), CH$_3$CN, 0 °C to rt, 3 h, 33%; (c) NaH, THF, 0 °C, 24 h, 68%.

Table 1. PGE$_2$ production, COX-1 and COX-2 inhibitory activities of compound **2l**

| Compound | IC$_{50}$^{a} | | | Selectivity index (SI) [COX-1: IC$_{50}$/COX-2: IC$_{50}$] |
	PGE$_2$^{b} (μM)	COX-1 (μM)	COX-2 (μM)	
2l	0.61	126.78	9.10	13.93
Ibuprofen	0.86	26.21	30.22	1.15
Meloxicam^c	0.09	73.18	0.31	236.06 (14.00)^d

^{a}IC$_{50}$ value is the compound concentration required to produce 50% inhibition of PGE$_2$ production, COX-1 and COX-2 enzymatic activity, respectively. ^{b}LPS-induced PGE$_2$ production. ^{c}Positive control for COX-2 used. ^{d}Selectivity index of meloxicam in whole-cell assay and in the microsomal assay, meloxicam showed 74.69- fold selectivy.[1]

Table 2. Docking results of compound **2l**, ibuprofen, meloxicam and celecoxib into the active sites of COX-1 and COX-2

| Compounds | Against COX-1 (PDB: 1EQH) | | Against COX-2 (PDB: 3LN1) | |
	No. H-bond	Rerank Score^a	No. H-bond	Rerank Score^a
2l	2	-67.93	5	-133.03
Ibuprofen	3	-85.27	1	-85.89
Meloxicam	4	-69.02	4	-79.26
Celecoxib^b	3	-22.72	6	-135.08

^{a}Rerank Score in MVD2010.4.2. ^{b}Celecoxib was docked for comparison.

inhibition (IC$_{50}$) (Table 1). The selectivity index (SI values) was calculated as IC$_{50}$ (COX-1)/IC$_{50}$ (COX-2), and compared with those of ibuprofen and meloxicam. As a result, compound **2l** exhibited time-dependent inhibition at 1 μM concentration but time-independent inhibition of human recombinant COX-2 at higher concentrations (10 and 50 μM) (Supplementary materials: Fig. 2). In our assay system, the IC$_{50}$ values of **2l** on COX-1 and COX-2 were determined to be 126.78 and 9.10 μM, respectively. Thus, the selectivity index (SI) for **2l** was found to be 13.93, indicating that **2l** is slightly potent and selective COX-2 inhibitor compared to 1.15 of ibuprofen, 236.06 of meloxicam, respectively (Table 1).

The COX-1 and COX-2 inhibitory activity of **2l** prompted us to perform molecular docking studies to understand the ligand-protein interactions, and COX-1/COX-2 selectivity in detail.[12-15] All the calculations were performed using Molegro Virtual Docker (MVD) 2010.4.2 for Windows.[16] The docking studies were carried out using the crystal structures of COX-1 (1EQH)[17] and COX-2 (3LN1)[18] complexed with flurbiprofen and celecoxib, respectively. The active site of the enzyme was defined to include residues within a 10.0 Å radius to each inhibitor atoms. The docking wizard of MVD2010.4.2 was used to dock all compounds in Table 1 on the active sites of COX-1 and COX-2 enzymes. The most stable docking model was selected according to the best Rerank score conformation predicted by the MVD scoring function (Table 2) for each crystal structure. The compound **2l** was found to dock into the active site of COX-2 with higher Rerank score of −133.03 compared to −67.93

score for COX-1(Supplementary data: Fig. 3). It formed five hydrogen bonds with Ser516 (3.13 Å, C=O), Ser339 (3.13 Å, -NH$_2$), Gln178 (3.10 Å, -NH$_2$), Arg499 (3.29 Å, SO$_2$) and Phe504 (3.57 Å, SO$_2$) into the active site of COX-2 (Fig. 2(a); Hydrogen bonds are shown in blue). On the other hand, docked-celecoxib showed the Rerank score of −22.72 and −135.08 into the active site of COX-1 and COX-2, respectively and it formed six hydrogen bonds with His75 (3.48 Å, -NH$_2$) Ser339 (3.15 Å, -NH$_2$), Gln178 (3.13 Å, -NH$_2$), Arg499 (3.50 Å, SO$_2$), Ile503(3.55 Å, SO$_2$) and Phe504 (3.19 Å, SO$_2$) into the active site of COX-2 [Fig. 2(b)]. In addition, its docked-conformation was nearly overlapped with that of celecoxib (red color) in COX-2 complex in PDB 3LN1. These Rerank score differences between COX-1 and COX-2 structures appropriately support the experimental selectivity indexes in Table 1 with exceptions of meloxicam. On the other hand, the comparison of docking conformation of **2l** with celecoxib, co-crystallized with COX-2, illustrated in Figure 2(b), shows that compound **2l** can bind into the active site of COX-2 enzyme in almost the same fashion as celecoxib with exceptions of two van der Waals interactions (blue circles) of celecoxib, which can provide rationale for higher potency and selectivity of celecoxib against COX-2.

Finally, compound **2l** was investigated for its potential effect on both LPS-induced nitric oxide (NO) production and iNOS expression in RAW 264.7 cells. In addition, effects of compound **2l** on LPS-induced production of TNF-α and mRNA expression of TNF-α, IL-1β, and IL-6 in RAW 264.7 cells was also investigated. Compound **2l** showed little effect on these biological events (Supplementary materials: Fig. 4 and 5). These overall results mean that

(a)   (b)
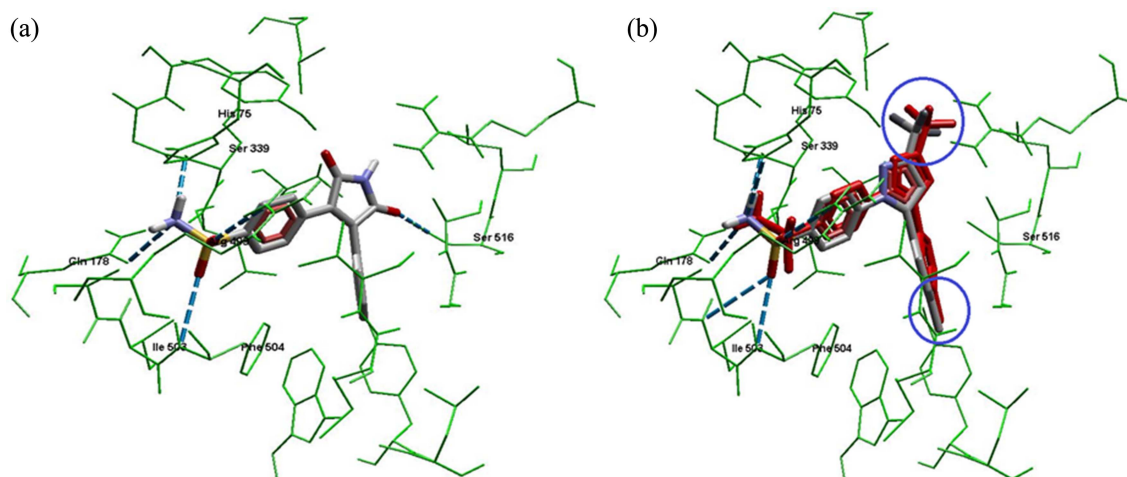
Figure 2. (a) Docking of compound **2l** into the active site of COX-2. Hydrogen bonds are shown in blue (b) Docking of celecoxib into the active site of COX-2 compared with real celecoxib (red color) of 3LN1 (PDB Code). Two blue circles show more van der Waals interactions of celecoxib compared to **2l**. The only polar hydrogens are shown for the simplicity.

compound **2l** prohibited the PGE_2 production in RAW 264.7 cells by inhibiting only the COX-2 enzyme.

In conclusion, 1*H*-pyrrole-2,5-dione **2l** were synthesized and screened for COX-1/COX-2 inhibition. The biological results showed that **2l** showed moderate inhibitory and selective profiles against COX-2, which is consistent with the molecular docking result of **2l** inside the COX-2 active site. Thus, the molecular docking results suggest us that 1*H*-pyrrole-2,5-dione derivatives with appropriate substitutions which can fill the adjunct pocket and interact with the other residues may be useful to propose new molecules with enhanced selectivity towards COX-2. The overall findings of this study inferred that moderate inhibition of compound **2l** rendered it as a hit molecule for further development of more potent and selective COX-2 inhibitor. We are in progress of synthesizing a number of new derivatives based on this molecular docking result.

Experimental Section

***In vitro* Cyclooxygenase (COX) Inhibition Assay.** The ovine COX-1 and human recombinant COX-2 activity directly measures $PGF_{2\alpha}$ produced by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoid product is quantified *via* enzyme immunoassay (EIA) using a broadly specific antibody that binds to all the major prostaglandin compounds using COX Inhibitor Screening Assay (Cayman Chemical, Ann Arbor, MI, USA). Briefly, control value was obtained in the absence of compound. COX enzyme was mixed with different concentration of each tested compound and heme, and incubated for 10 min at 37 °C. The reaction was initiated by adding arachidonic acid and all tubes were incubated for another 2 min at 37 °C. NS-398 (100 nM) were used as a positive control COX-2 inhibitor. The efficacy of compound was determined as the concentration causing 50% enzyme inhibition (IC_{50}). The selectivity index (SI values) was calculated as IC_{50} (COX-1)/IC_{50} (COX-2).

Docking Methodology. Docking studies have been performed using MVD 2010.4.2. With this purpose, crystal structures of COX-1/flurbiprofen and COX-2/celexocib complex (PDB codes: 1EQH and 3LN1) were obtained from the Protein Data Bank in order to prepare the protein for docking studies. Docking procedure was followed using the standard protocol implemented in MVD 2010.4.2 and the geometry of resulting complexes was studied using the MVD's Pose Viewer utility.

References and Notes

1. Chandrasekharan, N. V.; Dai, H.; Roos, K. L.; Evanson, N. K.; Tomsik, J.; Elton, T. S.; Simmons, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 13926.
2. Lee, S. H.; Kim, M. H.; Han, H. J. *Am. J. Physiol. Cell Physiol.* **2009**, *297*, C207.
3. Watson, D. J.; Harper, S. E.; Zhao, P.-L.; Quan, H.; Bolognese, J. A.; Simon, T. J. *Arch. Intern. Med.* **2000**, *160*, 2998.
4. Hawkey, C. J. *Lancet* **1999**, *353*, 307.
5. Chan, F. K.; Hung, L. C.; Suen, B. Y.; Wu, J. C.; Lee, K. C.; Leung, V. K.; Hui, A. J.; To, K. F.; Leung, W. K.; Wong, V. W.; Chung, S. C.; Sung, J. J. *N. Engl. J. Med.* **2002**, *347*, 2104.
6. McGettigan, P.; Henry, D. *JAMA* **2006**, *296*, 1633.
7. Mason, R. P.; Walter, M. F.; McNulty, H. P.; Lockwood, S. F.; Byun, J.; Day, C. A.; Jacob, R. F. *J. Cardiovasc. Pharmacol.* **2006**, *47*, S7.
8. Moon, J. T.; Jeon, J. Y.; Park, H. A.; Noh, Y. S.; Lee, K. T.; Kim, J.; Choo, D. J.; Lee, J. Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 734.
9. Yun, K. J.; Shin, J. S.; Choi, J. H.; Back, N. I.; Chung, H. G.; Lee, K. T. *Int. Immunopharmacol.* **2009**, *9*, 1323.
10. Uddin, M. J.; Praveen Rao, P. N.; Knaus, E. E. *Bioorg. Med. Chem.* **2004**, *12*, 5929.
11. Churchill, L.; Graham, A. G.; Shih, C.-K.; Pauletti, D.; Farina, P. R.; Grob, P. M. *Inflammopharmacology* **1996**, *4*, 125.
12. Manivannan, E.; Chaturvedi, S. C. *Bioorg. Med. Chem.* **2011**, *19*,

4520.

13. Abdel-Aziz, A. A.; ElTahir, K. E.; Asiri, Y. A. *Eur. J. Med. Chem.* **2011**, *46*, 1648.

14. Gautam, R.; Jachak, S. M.; Kumar, V.; Mohan, C. G. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1612.

15. Singh, P.; Bhardwaj, A.; Kaur, S.; Kumar, S. *Eur. J. Med. Chem.* **2009**, *44*, 1278.

16. MVD 2010.4.2 for Windows in Molegro ApS.

17. Selinsky, B. S.; Gupta, K.; Sharkey, C. T.; Loll, P. J. *Biochemistry* **2001**, *40*, 5172.

18. Wang, J. L; Limburg, D.; Graneto, M. J.; Springer, J.; Hamper, J. R.; Liao, S.; Pawlitz, J. L.; Kurumbail, R. G.; Maziasz. T.; Talley, J. J.; Kiefer, J. R.; Carter, J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7159.