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Sulfonamide derivatives of styrylheterocycles as a potent inhibitor of COX-2-mediated prostaglandin E_2 production

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ABSTRACT

The overproduction of prostaglandin E_2 (PGE_2) plays an important role in a variety of pathophysiological processes including inflammation and carcinogenesis. Therefore, the modulation of PGE_2 production is a promising target in the design of chemotherapeutic agents. In the present study, the inhibitory effects of a series of styrylheterocycles having either a *p*-SO_2NH_2 or *p*-SO_2Me group on the production of cyclooxygenase-2-mediated PGE_2 were evaluated in lipopolysaccharide-stimulated RAW264.7 murine macrophages. Among the series of styrylheterocycle derivatives, (*E*)-4-(2-(thiophen-3-yl)vinyl)benzenesulfonamide exhibited a potent inhibitory activity, with an IC_{50} value of 0.013 μM. The inhibitory activity against the overproduction of PGE_2 by the active compound was found to be due in part to the suppression of COX-2 mRNA expression.

Accumulating evidence suggests that the overproduction of prostaglandins (PGs) influences a variety of pathophysiological processes, including inflammation, Alzheimer's disease, hypertension, heart failure, and carcinogenesis.[1–5] Consequently, inhibition of PG synthesis has been investigated as a means of developing new therapeutic agents for the treatment of numerous diseases mediated by PGs.[6–8]

The rate limiting step in PG biosynthesis is the conversion of arachidonic acid to PGH_2, which is the precursor of primary PGs.[1,9,10] This necessary step is catalyzed by the enzyme cyclooxygenase (COX). Two isoforms of the COX enzyme, which are encoded by two different genes, have been identified. COX-1 is constitutively expressed in virtually all tissues, whereas COX-2 is expressed only in response to growth factors, tumor promoters, or various physiological and inflammatory stimuli.[11,12] Because COX-2-mediated metabolites, including PGE_2, are involved in the induction of inflammation, pain, and carcinogenesis,[1–3] COX-2 inhibitors have received much attention as effective anti-inflammatory or cancer chemopreventive agents. In addition, selective COX-2 inhibitors are postulated to be beneficial in the prophylactic treatment of neurodegenerative disorders.[13]

Several classes of COX-2 inhibitors have been reported.[14] Of these, vicinal diaryl heterocycles that contain a *p*-SO_2NH_2 or *p*-SO_2Me substituent on one of the phenyl rings represent a major class of selective COX-2 inhibitors. Extensive structure–activity studies indicate that the SO_2NH_2 or SO_2Me substituents interact with the COX-2 side pocket through tight-binding kinetics, providing COX-2 selectivity and potency.[15] Typical examples of this class of the selective COX-2 inhibitors are Coxibs such as celecoxib and rofecoxib. However, the recent market withdrawal of rofecoxib due to its increase risk of cardiovascular side effects with its long-term use in clinic[16] clearly depicts the need to explore new class of compounds having reduced cardiovascular adverse effects.[17,18] Several natural compounds have been shown to inhibit COX without causing cardiac toxicities, probably by acting on other pathways that compensate for the cardiac toxicity of pure COX inhibition.[18] The dual inhibition of COX and LOX enzymes was suggested as potential therapeutic approaches for anti-inflammatory agents with reduced GI and cardiovascular side effects.[18]

Several compounds with acylic central systems have been suggested as potential COX-2 modulators.[19] It has also been shown that stilbenoids exhibit COX inhibitory activity. Synthetic *cis*-stilbene derivatives that possess a *p*-SO_2NH_2 or *p*-SO_2Me pharmacophore exhibit COX-2 inhibitory activities with appreciable potency.[20] A number of naturally occurring and synthetic *trans*-stilbenoids were also revealed as inhibitors of COX, although this compound class was somewhat less active than the representative COX inhibitors.[21]

Recently, we reported on the preparation of a series of *trans*-stilbenoids, including styrylheterocycles, and evaluated their inhibitory effects on the production of COX-2 mediated PGE_2.[22]

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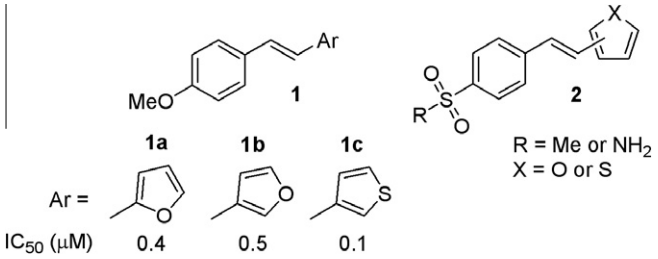


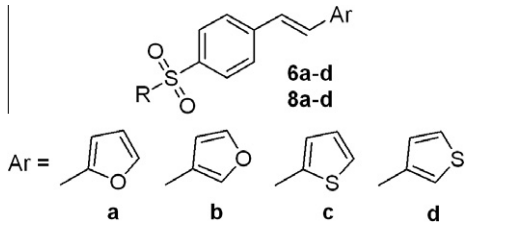
Figure 1. Chemical structures of compounds **1** and **2**.

We found that styrylheterocycles **1** (Fig. 1) containing a *para*-methoxy group on the phenyl ring showed submicromolar inhibitory activity against the overproduction of the inflammatory mediator PGE_2 in LPS-stimulated RAW264.7 cells. This result led us to consider styrylheterocycles **1** as a new lead compound for further chemical optimization. As part of our ongoing program to develop inhibitors of PG production, we now describe the synthesis and biological evaluation of styrylheterocycles of general structure **2** (Fig. 1) that possess a *p*-SO_2NH_2 or *p*-SO_2Me COX-2 pharmacophore on the phenyl ring.

The designed compounds were synthesized from the commercially available 4-methylthiobenzaldehyde (**3**), as shown in Scheme 1. A Wittig reaction was performed with phosphonium bromides **4a**–**d** and aldehyde **3** in the presence of *n*-BuLi to yield a mixture of olefins **5a**–**d** with an *E*/*Z* ratio of ca. 2:1. The *E*/*Z* mixture **5** was converted to the *E*-isomer by heating with catalytic amounts of iodine in refluxing heptane. Oxidation of the SMe substituent of *E*-isomer **5** using an aqueous Oxone solution[23] afforded the methylsulfone products **6a**–**d**. Conversion of the methylsulfone substituent to the corresponding sulfonamide group was accomplished according to a previously reported two-step process.[24] Treatment of **6a**–**d** with LDA and (iodomethyl)trimethylsilane led to the formation of the trimethylsilylethyl sulfone intermediate **7**. Desilylation with TBAF and subsequent treatment of the resulting sulfinic acid salts with hydroxylamine-*O*-sulfonic acid provided the desired sulfonamides (**8a**–**d**) in moderate yields.

The prepared compounds, **6a**–**d** and **8a**–**d**, were evaluated for their inhibitory activities on the PGE_2 production in LPS-stimulated RAW264.7 cells.[25] As shown in Table 1, the replacement of the *p*-OMe substituent with a *p*-SO_2Me group resulted in a loss in inhibitory activity. For example, aryl sulfones **6a** and **6d** were found to be almost three or five times less potent than their corresponding parent compounds **1a** and **1c**, respectively (Fig. 1). The

Table 1
Inhibitory effects of styrylheterocycles on the PGE_2 production in LPS-stimulated RAW264.7 cells[a]



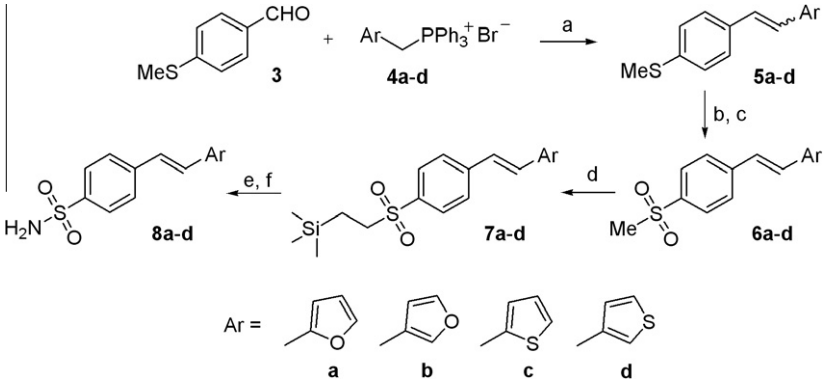
Compd	R	IC_{50}[b] (μM)
6a	Me	1.18
6b	Me	>50
6c	Me	0.38
6d	Me	0.52
8a	NH_2	18.27
8b	NH_2	>50
8c	NH_2	0.013
8d	NH_2	0.44

[a] The activities were measured as described previously.[22,25]
[b] The IC_{50} values were determined from triplicate tests.

sulfone analogue **6b**, which contains a 3-furyl group, did not show any inhibitory activities up to 50 M, while its parent styrylheterocycle (**1b**) had an IC_{50} of 0.5 μM. Among these sulfone analogues, compound **6c**, which contains a 2-thienyl group, exhibited the most appreciable inhibitory activity (IC_{50} = 0.38 μM).

The effects of styrylheterocycles with a *p*-SO_2NH_2 group were revealed to also be highly dependent on the structure of the heterocyclic ring. The sulfonamide analogue possessing a 3-thienyl group **8d** was approximately equipotent to its corresponding sulfone analogue **6d** in this assay. The sulfonamide analogue possessing a 3-furyl group **8b** was not effective. The inhibitory activity of the 2-furyl containing compound **8a** was significantly decreased compared to the 2-furyl sulfone analogue **6a**. On the other hand, the 2-thienyl sulfonamide analogue **8c** exhibited the most potent inhibitory activity, with an IC_{50} value of 0.013 μM. This potency was 30-fold greater than that of the corresponding sulfone analogue **6c** and approximately 8- to 40-fold greater than that of the *p*-OMe substituted styrylheterocycles **1**.

We performed molecular modeling studies on the interaction of COX-2 with the most promising analogue **8c**.[25] A docking study with compound **8c** and COX-2 (Protein Data Bank code 1CX2) showed that the spatial location of the sulfonamide groups on **8c**



Scheme 1. A solution phase synthetic approach was employed for the preparation of the *trans*-stilbenoids as described in Table 1. Reagents and conditions: (a) *n*-BuLi, THF, rt; aqueous workup (**5a**, *E*/*Z* = 2:1, 68%; **5b**, *E*/*Z* = 2.1:1, 65%; **5c**, *E*/*Z* = 2.2:1, 75%; **5d**, *E*/*Z* = 2.3:1, 70%); (b) I_2, heptane, reflux; (c) Oxone, THF, rt; isolation (**6a**, 75%; **6b**, 70%; **6c**, 100%; **6d**, 80%); (d) LDA, (iodomethyl)trimethylsilane, THF, −78 °C to rt; aqueous workup (**7a**, 42%; **7b**, 42%; **7c**, 50%; **7d**, 51%); (e) TBAF, THF, reflux; (f) H_2NOSO_3H, rt (**8a**, 52%; **8b**, 48%; **8c**, 60%; **8d**, 48%).

and SC-558 (a celecoxib analogue) are partially overlapped, and their benzenesulfonamide groups are nearly superimposed (Fig. 2). The Ar-SO_2NH_2 group of **8c** interacts with the amino acid residues lining the COX-2 binding site (Gly519, Phe518, Ala516, Gln192, Arg513, and His90). The two oxygen atoms of the SO_2NH_2 group form two hydrogen bonds with the backbone NHs of His90 and Arg513 (distance = 2.87 Å). In addition, other possible hydrogen bonds are observed between the nitrogen of the SO_2NH_2 moiety and the backbone carbonyl in Gln192 (distance = 3.72 Å)/Phe518 (distance = 3.84 Å)/Leu352 (distance = 4.12 Å). These observations suggest that styrylheterocyclic sulfonamides interact favorably within the COX-2 binding site.

To understand the underlying molecular mechanisms that lead to the inhibition of PGE_2 production, the suppressive effect of **8c** on the COX-2 mRNA expression was investigated using reverse transcription-polymerase chain reaction (RT-PCR) analysis.[25] Treatment with LPS for 5 h dramatically increased the expression of the COX-2 mRNA levels, and the induction was suppressed by the treatment of **8c** in a concentration-dependent manner as depicted in Figure 3. Based on our observations, we hypothesize that the inhibitory effects of styrylheterocycles **8c** against LPS-stimulated PGE_2 production may in part be correlated with the suppression of COX-2 mRNA expression. However, because the suppressive effect of **8c** on the COX-2 mRNA expression was not reached completely at the IC_{50} value of PGE_2 production, other mechanisms such as direct COX-2 inhibitory activity could not be excluded. Therefore, the inhibition of PGE_2 production in LPS-activated RAW264.7 cells by styrylheterocyles might be due to either suppression of COX-2 expression or direct inhibition of COX-2 enzyme activity.

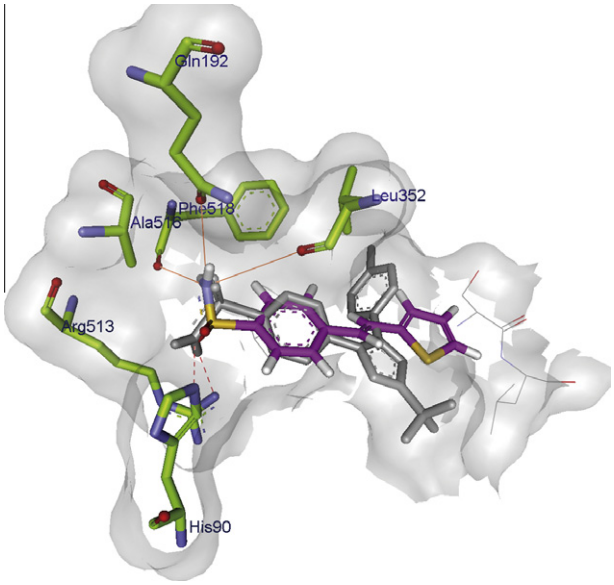


Figure 2. Docking model of compound **8c** within the active site of COX-2 (Protein Data Bank code 1CX2). Compound **8c** (shown in magenta) and SC-558 (shown in gray) are overlaid and hydrogen bonds and possible hydrogen bonds are indicated by red dashed and orange solid lines, respectively. The key amino acid residues are shown in green. For clarity, only active site residues are designated.

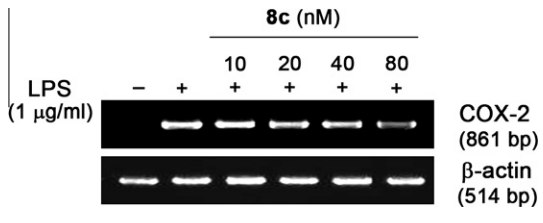


Figure 3. Effects of **8c** on the expression of COX-2 mRNA in LPS-stimulated RAW264.7 cells using RT-PCR analysis. Modulation of COX-2 mRNA expression by **8c** in LPS-stimulated macrophage cells. RAW264.7 cells (5×10^5 cells/ml) were incubated for 24 h and then treated with 1 μg/ml LPS with or without various concentrations of **8c** for an additional 5 h. Total RNA was isolated and 1 μg of RNA was used for reverse transcription. The cDNA obtained was used in the polymerase chain reaction using Taq DNA polymerase and specific primers. PCR products were separated by 2% agarose gel electrophoresis, stained with SYBR Gold, and visualized under a UV transilluminator.

In summary, although the caution with the cardiovascular side effect by vicinal diaryl heterocycle class compounds targeting COX-2 activity was recently raised, studies are still required to explore new templates having reduced cardiovascular adverse effects. In this study, a series of styrylheterocycle derivatives have been prepared and evaluated as to their effects on COX-2-mediated PGE_2 production, with the goal of identifying a potent modulator. Our previous lead compound **1** was optimized by the incorporation of a *p*-SO_2NH_2 COX-2 pharmacophore. We found that (*E*)-4-(2-(thiophen-3-yl)vinyl)benzenesulfonamide (**8c**) exhibited a potent inhibitory activity against the overproduction of the inflammatory mediator PGE_2 and could serve as a new therapeutic agent for the treatment of diseases mediated by PGs. We have also gained insight into the structure–activity relationship of styrylheterocycles that is valuable in the design and development of new class of inhibitors of PG synthesis. Further studies are currently in progress, including a more detailed biological evaluation and chemical optimizations.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.136.

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25. See Supplementary data for detailed experimental methods.