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Synthesis, structure determination, and biological evaluation of phenylsulfonyl hydrazide derivatives as potential anti-inflammatory agents

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ABSTRACT

In our previous research, a novel series of phenylsulfonyl hydrazide derivatives were found to reduce LPS-induced PGE_2 levels in RAW 264.7 macrophage cells via an inhibition of mPGES-1 enzyme. Recently, it was found that a regioisomeric mixture of phenylsulfonyl hydrazide was formed depending on the reaction conditions, which favor either of two regioisomers. One regioisomer corresponds to a kinetic product (**7a**–**7c**) and the other regioisomer corresponds to a thermodynamic product (**8a**–**8c**). Among them, the structure of kinetic product **7b** was confirmed by measuring single X-ray crystallography. In vitro PGE_2 assay studies showed that the kinetic product (**7a** and **7b**; IC_{50} = 0.69 and 0.55 μM against PGE_2) is generally more potent than the thermodynamic product (**8a** and **8b**; IC_{50} = >10 and 0.79 μM against PGE_2). A molecular docking study also exhibited that the kinetic product (**7a**) has a higher MolDock Score (−147.4) than that of **8a** (−142.4), which is consistent with the PGE_2 assay results. A new potent phenylsulfonyl hydrazide (**7d**; IC_{50} = 0.06 μM against PGE_2) without affecting COX-1 and COX-2 enzyme activities was identified based on these overall results.

PGE_2 (prostaglandin E_2) has long been considered the principal prostaglandin of acute inflammation and of chronic diseases such as rheumatoid arthritis[1] and inflammatory bowel disease.[2] As PGE_2 is relatively over-produced by COX-2 (cyclooxygenase-2) induced by pro-inflammatory stimuli,[3] selective COX-2 inhibitors (coxibs) such as celecoxib (Celebrex®) and rofecoxib (Vioxx®) reduce PGE_2 levels relative to other prostaglandins.[4] However, these coxibs prevent the production of all prostaglandins [in particular, prostacyclin (PGI_2)] downstream of PGH_2 as well as PGE_2, which results in adverse cardiovascular effects of rofecoxib.[5–7] Meanwhile, microsomal prostaglandin E_2 synthase (mPGES)-1 enzyme catalyzes the terminal step in the biosynthesis of COX-2-derived PGE_2 from PGH_2.[8] Thus, the inhibition of mPGES-1 has been expected to retain the same anti-inflammatory effect as coxibs without the side effects of classical non-steroidal anti-inflammatory drugs (NSAIDs) and coxibs.[9] Therefore, small molecule inhibitors of mPGES-1 have been developed for anti-inflammatory therapy with reduced side effects.[10–22] Among them, only a few of compounds such as PF-4693627 and LY3023703 have been advanced to clinical trials until now.[23,24] Our group also reported that a novel series of phenylsulfonyl hydrazide derivatives were found to suppress LPS-induced PGE_2 production in RAW 264.7 macrophage cells via the inhibition of mPGES-1 enzyme.[25]

In the course of synthesis of new phenylsulfonyl hydrazide derivatives for structure–activity relationship (SAR) study, we had found that two regioisomers possessing phenoxycarbonyl group attached to its N-1 or N-2 position were formed depending on the reaction conditions, which favor either of two regioisomers as shown in Figure 1. Interestingly, their biological evaluation showed that one regioisomer was systematically more potent than the other regioisomer. Therefore, synthetic conditions had to be adapted and optimized to yield the desired regioisomer. Thus, this study aims to characterize and evaluate each regioisomer, and compare experimental results to theoretical results by using docking studies for the optimization of reaction condition and the focused SAR study.

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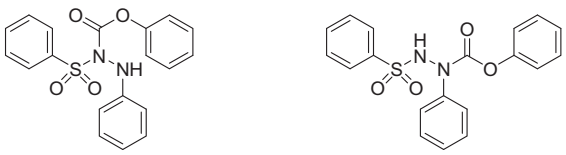


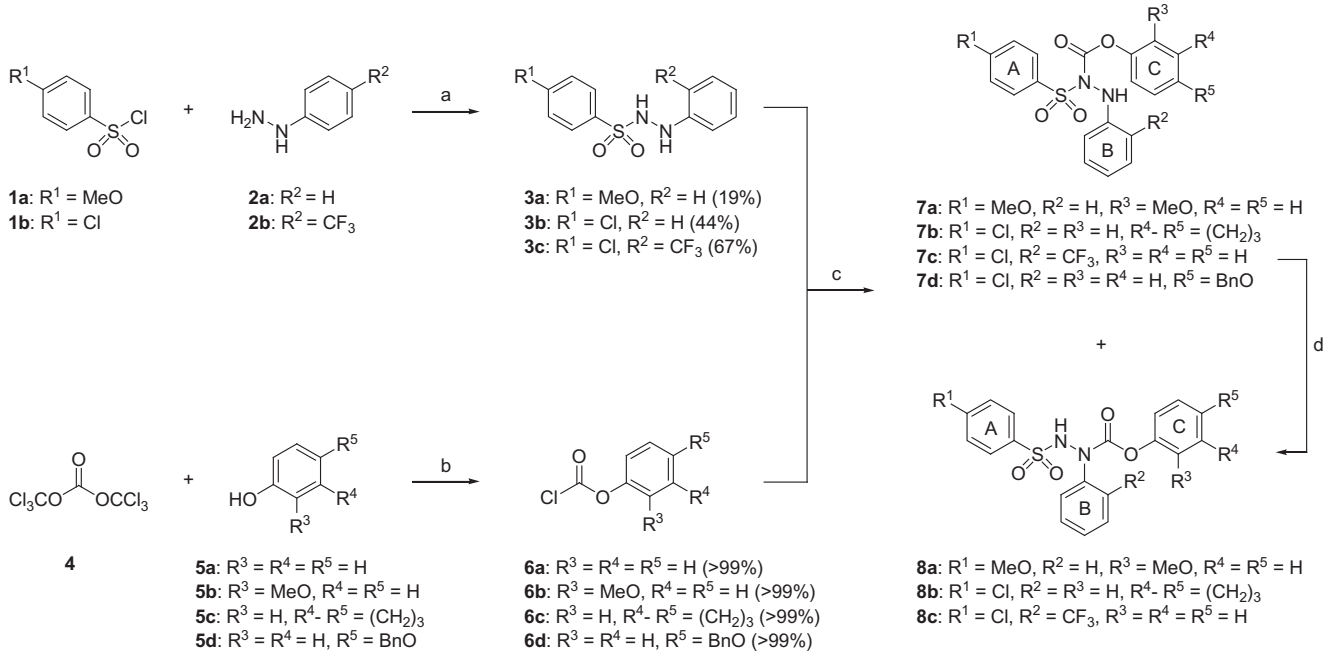
Figure 1. Two regioisomers of phenylsulfonyl hydrazide derivative in this work.

The phenylsulfonyl hydrazide **7** could be obtained according to our previously reported procedure as illustrated in Scheme 1.[25] *N′*-Mono-substituted phenylsulfonyl hydrazide **3** was prepared from the reaction of phenylsulfonyl chloride **1** with phenylhydrazine **2** in presence of TEA. The reaction of phenol **5** with 0.6 equiv of triphosgene **4** in the presence of *N*,*N*-diisopropylethylamine (Hünig's base) gave phenyl chloroformate **6**. At the final synthetic step for *N*,*N′*-di-substituted compound, two regioisomers **7a**–**b** and **8a**–**b** were formed as major and minor products (less than 5%), respectively, as shown in Scheme 1. Synthesized compounds were characterized by different spectroscopic techniques ^{1}H NMR, ^{13}C NMR and HRMS (the Supplementary data). However, compound **8a**–**b** was unexpectedly obtained as a major product when reacted under THF reflux condition or longer reaction time (>24 h). In addition, pure separated compound **7a**–**b** was completely converted into pure compound **8a**–**b** when treated with TEA under THF reflux condition. This overall result implies that compound **7a**–**b** and **8a**–**b** could be regarded as a kinetic product and a thermodynamic product, respectively. In order to confirm this hypothesis, a regioisomeric mixture of **7c** and **8c** (95:5) was dissolved in DMSO and left on workbench for 3 days at room temperature to result in a mixture with 70:30 ratio based on the integral of each NH peak in ^{1}H NMR (the data is not shown here). Therefore, we decided to separate two pure regioisomers, elucidate their exact structures, and compare their biological activities for our future work.

Two regioisomers of phenylsulfonyl hydrazide could be separated due to their small difference in polarity and characterized based on ^{1}H NMR and X-ray analysis. Compound **7a** was polar than compound **8a** based on normal TLC and the characteristic difference in chemical shifts of their NH groups was clearly observed in ^{1}H NMR (in particular DMSO solution). The NH of compound **7a** was appeared in upfield (δ 9.44) compared to that (δ 10.82) of compound **8a** (Table 1), which is assumed to be the combined influence of SO_2 and NCO_2 groups of compound **8a**. Each NH group of other regioisomers **7b** and **8b** was also appeared at δ 9.23 and 10.91, respectively. Each NH group of regioisomers **7c** and **8c** containing 2-CF_3-phenyl ring at *N′*-position was similarly appeared at δ 9.06 and 11.02, respectively.

In order to elucidate the exact structure of one regioisomer, pure compound **7b** was prepared by carefully controlling the reaction condition (i.e., one equiv. TEA and 2 h reaction time). The NH group of compound **7b** was appeared in δ 9.23, which approximately corresponds to the chemical shifts of other kinetic products (**7a** and **7c**). More importantly, the single yellowish needle crystal of **7b** was obtained and the corresponding structure was identified by X-ray crystallographic analysis. Crystal parameters belonging to $C_{22}H_{19}ClN_2O_4S$ molecule (**7b**), details of data collection and the refinement processes are given in Table 2 and Supplementary data. ORTEP diagram of the molecule drawn with 40% probability ellipsoids is given in Figure 2, which strongly confirms the structure of a kinetic product (**7b**).

In order to check that the suppressive effects of regioisomers on PGE_2 level could be attributable to non-specific cytotoxicity, initially, we examined the cytotoxicity of all synthetic compounds in RAW 264.7 cells in the presence of LPS using MTT assays.[26] None of the compounds affected the viabilities of RAW 264.7 cells at 10 μM concentration over 24 h (Table 1). Therefore, all compounds were screened for their ability to suppress PGE_2 level in LPS-induced RAW 264.7 cells at a concentration of 1 or 10 μM concentration over 24 h using NS398 (@ 3 μM) as a positive control. Then, active compounds exhibiting >50% suppression of PGE_2 level at 1 μM concentration were pushed forward for IC_{50} determinations.[27] All experiments were carried out at least twice and they gave similar results. The biological activities of all compounds are summarized as cell viability, %inhibition, and IC_{50} value of PGE_2 production in Table 1 with NS398 utilized as a positive control in PGE_2 assays. All kinetic products (**7a**–**b**) generally exhibited good reduction of



Scheme 1. Reagents and conditions: (a) TEA, THF, 0 °C to rt, 3–6 h; (b) DIPEA, THF, 0 °C, 2 h; (c) TEA, THF, rt, 2–4 h; (d) TEA, THF, reflux, 4 h for **7a-c.**