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Synthesis and PGE_2 production inhibition of 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives

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ABSTRACT

3,4-Diphenyl-substituted 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives were synthesized and evaluated for the inhibitory activities on LPS-induced PGE_2 production in RAW 264.7 macrophage cells. Both 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione rings as main scaffolds were easily obtained using one of three synthetic methods. Among the compounds investigated, 1*H*-3-(4-sulfamoylphenyl)-4-phenyl-pyrrole-2,5-dione (**6l**) showed a strong inhibitory activity (IC_{50} = 0.61 μM) of PGE_2 production.

New generations of anti-inflammatory drugs have been developed to enhance the anti-inflammatory and analgesic activities of classic nonsteroidal anti-inflammatory drugs (NSAIDs), and to reduce the adverse effects of these agents. Selective COX-2 inhibitors are viewed enthusiastically because they match traditional NSAIDs in terms of efficacy, but circumvent constitutively active COX-1 and are comparatively free of stomach-associated complications. Diarylheterocycles, and other central ring pharmacophore templates, have been extensively studied as selective COX-2 inhibitors.[1] All these tricyclic molecules have 1,2-diaryl substitutions on their central hetero- or carbocyclic ring systems. The recent withdrawal of the selective COX-2 inhibitors rofecoxib and valdecoxib because of their adverse cardiovascular side effects demonstrates the need to identify new scaffolds with COX-2 inhibitory activity, but without the side effects of known agents.[2] PGE_2 has long been considered the principal prostaglandin of acute inflammation and of chronic diseases such as rheumatoid arthritis[3] and inflammatory bowel disease.[4] Macrophages play particularly important roles in inflammation because they produce many pro-inflammatory molecules such as PGE_2. Therefore, the pharmacological interference of PGE_2 production has been postulated as a means of alleviating a number of disease states mediated by excessive and/or protracted macrophage activation. As an attempt to discover novel compound with potent anti-inflammatory activity, therefore, we have synthesized 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives and evaluated their inhibitory activities against LPS-induced PGE_2 production in RAW 264.7 macrophages.

The synthetic procedures and reaction conditions for 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives are shown in Schemes 1 and 2. As a general synthetic procedure, the condensation reaction of benzoylformic acid (**3**) with phenylacetic acid (**4**) under acetic anhydride reflux condition provided 1*H*-furan-2,5-dione (**5**).[5] Meanwhile, some commercially unavailable benzoylformic acids (**3**) were prepared by Friedel–Crafts acylation and subsequent NaOH hydrolysis.[6,7] 1*H*-Furan-2,5-dione (**5**) was easily converted into 1*H*-pyrrole-2,5-dione (**6**) on treatment with hexamethyldisilazane (HMDS) in MeOH/DMF solution.[8] For the structure–activity relationship (SAR) study, deprotection of compounds **6** containing methoxy group with BBr_3 gave 1*H*-pyrrole-2,5-diones (**6f**–**6h** and **6m**) containing free hydroxy groups[9] and also the oxidation of 1*H*-furan-2,5-dione (**5**) containing thiomethoxy group with *m*-CPBA (1 or 2 equiv) gave 1*H*-furan-2,5-dione (**5e**, **5g**, **5h**, **5j**, and **5k**) containing methanesulfinyl or methanesulfonyl groups as shown in Scheme 1. As another synthetic procedure for 1*H*-pyrrole-2,5-diones (**6**), phenylacetic acid (**4**) was converted into phenylacetamide (**7**), which was condensed with ethyl 4-methanesulfanylbenzoylformate (**2**) and NaH to

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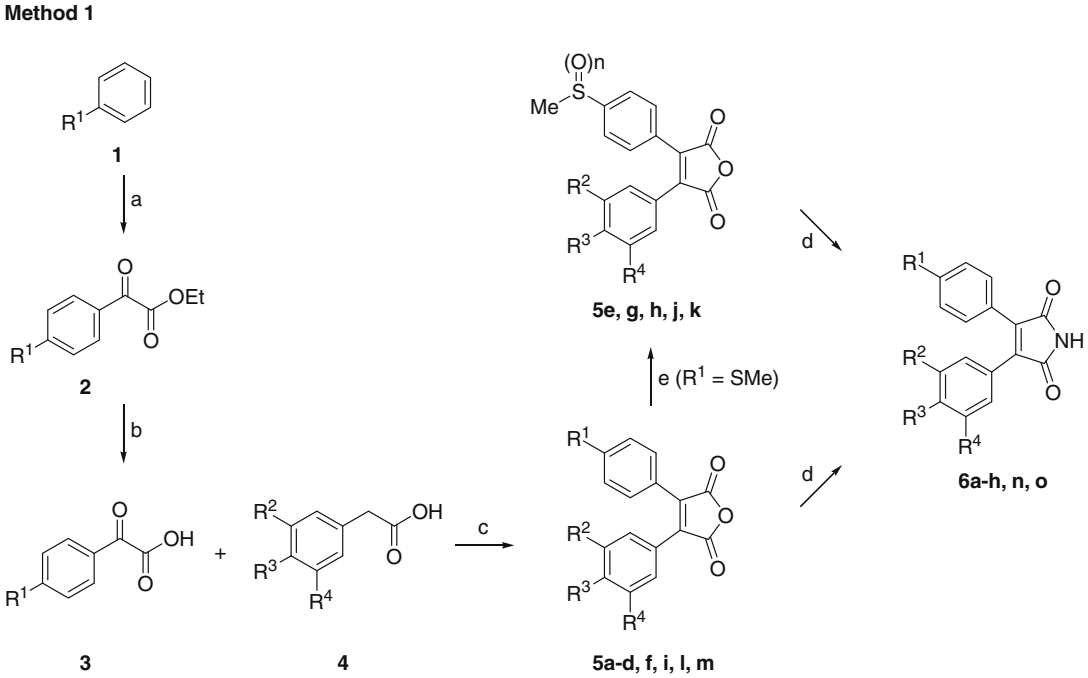
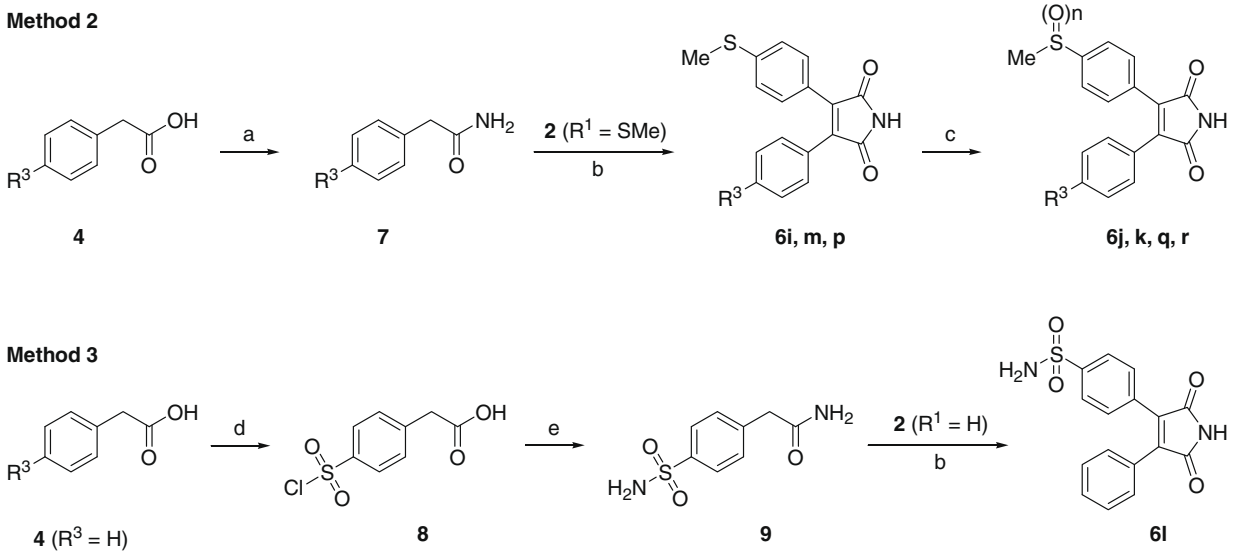


Scheme 1. Reagents and conditions: (a) $AlCl_3$, $ClCOCO_2Et$, DCM, 0 °C; (b) 2 N NaOH, H_2O, rt; (c) Ac_2O, reflux; (d) (i) HMDS, MeOH, DMF, rt; (ii) BBr_3, DCM, −78 °C to rt; (e) *m*-CPBA (1 or 2 equiv), DCM, −20 °C.



Scheme 2. Reagents and conditions: (a) (i) $SOCl_2$, DMF, rt to reflux; (ii) NH_4OH, rt; (b) NaH, THF, 0 °C; (c) *m*-CPBA, DCM, −20 °C; (d) $ClSO_3H$, 0 °C to rt; (e) (i) $SOCl_2$, DMF, rt; (ii) NH_3 (*g*), CH_3CN, rt.

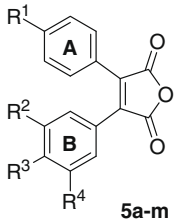
afford 1*H*-pyrrole-2,5-diones (**6i**, **6m**, and **6n**) as shown in method 2 of Scheme 2. In the case of 1*H*-pyrrole-2,5-dione containing 4-sulfamoylphenyl group (**6l**), 4-chlorosulfonylphenylacetic acid (**8**) was prepared from the reaction of phenylacetic acid (**4**) with $ClSO_3H$, and then treated with $SOCl_2$ and subsequent NH_3 gas in CH_3CN to provide 4-sulfamoylphenylacetamide (**9**), which was condensed with ethyl benzoylformate (**2**) and NaH to yield 1*H*-3-(4-sulfamoylphenyl)-4-phenyl-pyrrole-2,5-dione (**6l**) as shown in method 3 of Scheme 2. The synthesized compounds were purified by flash column chromatography and analyzed for the structures based on ^1H NMR and ^{13}C NMR spectra.

Initially, we examined the cytotoxicity of synthetic compounds in RAW 264.7 cells in the presence of LPS using MTT assays: RAW 264.7 cells were plated at a density of 10^5 cells/well in 96-well plates. To determine the appropriate concentration not toxic to cells, cytotoxicity studies were performed 24 h after treating cells with various concentrations of synthetic compounds. Viabilities were determined using colorimetric MTT assays as described previously.[10] These compounds did not affect the viabilities of RAW 264.7 cells at concentrations up to 100 μM in the presence of LPS over 24 h (Table 1), indicating that their suppressive effects on PGE_2 production could not be attributable to non-specific cytotoxicity. The inhibitory activity of COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells was performed according to the published procedure:[11] The RAW 264.7 macrophage cell line was obtained from the Korea Cell Line Bank (Seoul, Korea). Cells

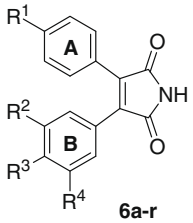
were grown at 37 °C in DMEM supplemented with 10% FBS, penicillin (100 units/ml), and streptomycin sulfate (100 μg/ml) in a humidified 5% CO_2 atmosphere. Cells were incubated with the tested samples at increasing concentrations or positive control chemical (NS398) and then stimulated with LPS 1 μg/ml for the indicated time. PGE_2 concentration in the medium was quantified using EIA kits (R&D Systems, Minneapolis, MN). In addition, the inhibitory activity against COX-2 was also measured for compounds showing less than IC_{50} of PGE_2 production = 10 μM and then Dup-697 was used as a positive control COX-2 inhibitor.[12] All experiments were carried out at least three times and the data of bioassays were summarized in Table 1.

As shown in Table1, both 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives showed the broad inhibitory spectrum against PGE_2 production. With respect to a series of 1*H*-furan-2,5-dione derivatives (**5a**–**5m**), we could not find a clear structure–activity relationship (SAR) based on the substituents of phenyl rings. However, 1*H*-furan-2,5-dione derivatives with sulfide moiety in ring A (**5d**, **5f**, and **5i**) were generally more active than those with sulfoxide or sulfone moiety (**5e**, **5g**, and **5j**) except for **5h** and **5k**. Among this series, 1*H*-furan-2,5-dione with 4-methanesulfanyl group (**5d**) showed good inhibitory activity (IC_{50} = 3.58 μM). With respect to a series of 1*H*-pyrrole-2,5-dione derivatives (**6a**–**6r**), we could find a general structure–activity relationship (SAR) when compared to 1*H*-furan-2,5-dione series (**5**). In the case of compounds without sulfur-substituent (**6a**–**6h**) in ring A, compounds having appropriate hydrophobic or hydrophilic moiety (**6d** and **6g**) revealed considerable PGE_2 inhibitory activities. For compounds with sulfur-substituent (**6i**–**6k** and **6m**–**6r**) in ring A, compounds with sulfoxide moiety (**6j**, **6n**, and **6q**) showed reduced bioactivity compared to those with sulfide or sulfone moiety (**6i**, **6k**, **6m**, **6p**, and **6r**) except for **6o**. The introduction of hydroxyl group at 4-position in ring B led to the increased inhibitory activity (**6i** vs **6m**) but the introduction of the fluoride atom at 4-position in ring B showed the decreased bioactivity (**6i**–**6k** vs **6p**–**6r**) regardless of the oxidation state of sulfur atom. Among all compounds

Table 1
Inhibition of PGE_2 production from LPS-induced RAW 264.7 cells by synthetic 1*H*-furan-2,5-dione (**5a**–**5m**) and 1*H*-pyrrole-2,5-dione (**6a**–**6r**) derivatives



5a-m



6a-r

Compound	R^1	R^2	R^3	R^4	Cell viability IC_{50} (μM)	Inhibition of PGE_2 production IC_{50} (μM)	Inhibition of COX-2 activity IC_{50} (μM)
5a	H	H	OCH_2O		328.68	7.13	18.52
5b	H	H	OMe	OMe	257.40	71.29	
5c	H	OMe	OMe	OMe	312.56	37.10	
5d	SMe	H	H	H	194.77	3.58	18.45
5e	$S(O)_2Me$	H	H	H	265.89	15.42	
5f	SMe	H	OAc	H	333.94	30.46	
5g	S(O)Me	H	OAc	H	261.90	68.75	
5h	$S(O)_2Me$	H	OAc	H	261.82	4.41	16.63
5i	SMe	H	OMe	H	266.08	12.60	
5j	S(O)Me	H	OMe	H	283.66	23.75	
5k	$S(O)_2Me$	H	OMe	H	271.24	Inactive	
5l	SMe	H	NHAc	H	249.75	5.71	20.11
5m	SMe	H	NAc_2	H	276.61	78.48	
6a	H	H	H	H	269.90	27.94	
6b	H	H	H	OMe	259.63	9.95	
6c	H	H	OMe	OMe	305.41	131.53	
6d	H	H	OCH_2O		195.36	7.96	
6e	H	OMe	OMe	OMe	150.55	25.08	
6f	H	H	H	OH	158.01	13.83	
6g	H	H	OH	OH	206.59	2.69	16.04
6h	H	OH	OH	OH	261.45	8.19	17.51
6i	SMe	H	H	H	151.88	4.73	16.34
6j	S(O)Me	H	H	H	303.22	21.52	
6k	$S(O)_2Me$	H	H	H	268.74	2.71	17.50
6l	$S(O)_2NH_2$	H	H	H	158.18	0.61	15.62
6m	SMe	H	OH	H	124.74	0.84	21.01
6n	S(O)Me	H	OH	H	212.89	98.12	
6o	$S(O)_2Me$	H	OH	H	171.20	Inactive	
6p	SMe	H	F	H	121.22	25.50	
6q	S(O)Me	H	F	H	242.04	45.20	
6r	$S(O)_2Me$	H	F	H	260.76	10.76	
NS398[a]						4.80	
Dup-697[b]							0.17

[a] Positive control compound for assay of PGE_2 production inhibition.
[b] Positive control compound for assay of COX-2 inhibition.

tested, 1*H*-pyrrole-2,5-dione (**6l**) containing 4-sulfamoyl group at 4-position in ring A showed the strongest inhibitory activity (IC_{50} = 0.61 μM) of PGE_2 production. This result indicated that the sulfamoyl group at 4-position in ring A plays very an important role on the inhibition of PGE_2 production. In addition, compound **6l** showed the highest therapeutic index (TI = cytotoxicity/PGE_2 = 259.3) although the TI data were not inserted in Table 1.

Therefore, this preliminary result indicated that the inhibition of PGE_2 production by 1*H*-pyrrole-2,5-dione (**6l**) was not associated with its cytotoxicity. With respect to activity against COX-2 enzyme, however, all compounds having less than IC_{50} = 10 μM showed the similar inhibitory activity range (IC_{50} = 16.62–21.01 μM) against COX-2 enzyme regardless of IC_{50} values of PGE_2 production. Thus, this result means that further experiments should be performed to evaluate the effect of 1*H*-pyrrole-2,5-dione compound on PGE_2 production and to investigate the related mechanism involved in RAW 264.7 cells, and thus the mechanism study is currently under the progress.

In summary, we prepared 1*H*-furan-2,5-dione and 1*H*-pyrrole-2,5-dione derivatives, and evaluated their inhibitory activities on PGE_2 production from LPS-induced RAW264.7 cells. We found that 1*H*-3-(4-sulfamoylphenyl)-4-phenyl-pyrrole-2,5-dione (**6l**) possessed strong inhibitory activity compared to that of NS398, a positive control. Further SARs study on the A ring and B ring is currently under progress as well as a mechanism study.

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