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Synthesis and biological evaluation of 2-aroylbenzofurans, rugchalcones A, B and their derivatives as potent *anti*-inflammatory agents

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ABSTRACT

An efficient synthesis of 2-aroylbenzofurans, rugchalcones A, B and their derivatives was accomplished in excellent yields by the Rap–Stoermer reaction between substituted salicylaldehydes and phenacyl bromides. Later their *anti*-inflammatory effects were evaluated in lipopolysaccharide (LPS)-induced RAW-264.7 macrophages. The compounds were exhibited exceptional potency against inflammatory mediated NO production with no cytotoxicity at 10 μM concentration and IC_{50} values are found in the range from 0.75 to 13.27 μM. Among the 2-aroylbenzofurans prepared in this study, compounds **4** (99.6%; IC_{50} = 0.57), rugchalcone B (**2**) (99.3%; IC_{50} = 4.13), **7** (96.8%; IC_{50} = 1.90) and **8** (74.3%; IC_{50} = 0.99) were showed the maximum inhibitory activity. This study suggests that compounds **2**, **4**, **7** and **8** which are having 4-hydroxyphenyl group and/or hydroxy (–OH) group at 5- and/or 6-position of benzofuran motif could be considered as a promising scaffolds for the further development of iNOS inhibitors for potential *anti*-inflammatory applications.

In multi-cellular organisms, inflammation is a cardinal host defense response to tissue damage, injury, infectious agents or autoimmune responses and is an integral part of the immune response.[1] Symptoms of inflammation include swelling, redness of the area, pain, and sometimes loss of function.[2] Based on time and pathological features, it can be either acute or chronic. Inflammation is present in several disorders and diseases like atherosclerosis, diabetes and cancer. Increased blood supply, enhanced vascular permeability and migration of immune cells occur at damaged sites. In this process, activated inflammatory cells (neutrophils, eosinophils, mononuclear phagocytes and macrophages) secrete increased amounts of nitric oxide (NO), prostaglandins (PGs) and cytokines, such as interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF). Among these, two of the most prominent are PGs and NO. PGs are produced by cyclooxygenase (COX, which mainly having two forms COX-1 and COX-2) by arachidonic acid pathway. NO is a small, lipophilic, diffusible and transient free-radical species generated from L-arginine by three types of nitric oxide synthase (NOS) enzymes. It acts as a double-edged sword. Physiologically vital amount of NO produced by the endothelial (eNOS) and neuronal (nNOS) enzymes which is crucial for signaling, including vasodilatation, thermoregulation, and neuromodulation. High levels of NO is produced 'on-demand' by the inducible (iNOS) enzyme, to help kill tumors, viruses and bacteria. Both underproduction and overproduction of NO have been linked to various human pathologies. Insufficient NO production from eNOS and nNOS can lead to hypertension, atherosclerosis, and cardiovascular disease, whereas excess NO production by iNOS can cause inflammation, inflammatory bowel disease (IBD), rheumatoid arthritis, asthma, diabetes, stroke, cancer and neurodegenerative disorders.[3] Therefore, control of the excess NO production by inhibition of iNOS may exert *anti*-inflammatory effects.

Traditional non-steroidal *anti*-inflammatory drugs (tNSAIDs) and aspirin usage is general practice in the therapeutic approach to alleviate the symptoms associated with both acute and chronic inflammatory diseases. Their activity is most likely mediated through their ability to inhibit COX enzymes. However, their long-term oral administration is restricted because of the high incidence of side effects, particularly those relating to the gastrointestinal (GI) tract, renal and cardiovascular systems due to the inhibition of the housekeeping enzyme COX-1 along with COX-2.[4] Later, selective COX-2 inhibitors (COXIBs) were introduced to reduce the risks. While these COXIBs did reduce the risk of GI injury, like the tNSAIDs, they are also appeared to increase the risk of cardiovascular events, such as heart attack and stroke. Hence,

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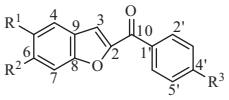
there remains an appeal to search for more effective *anti*-inflammatory drugs with minimal side-effects.

Benzofurans and their derivatives in particular are important scaffolds for drug development.[5] Several natural and non-natural 2-substituted benzofurans have been noted for their antioxidant,[6] antifungal,[7] antimicrobial,[8] *anti*-inflammatory,[9] PPAR-δ agonists,[10] antitubercular,[11] anti-HIV, anti-tumor and anti-platelet activity.[12] Radiolabeled benzofuran derivatives were used as molecular imaging probes for β-amyloid plaques in Alzheimer's Disease (AD).[13] Besides this, few derivatives were find application as fluorescent sensors and organic semiconductors.[14] Their wide range of pharmacological and physical properties created special interest to researchers towards the design and synthesis of these important heterocyclic compounds.



R^1 = OH, R^2, R^3 = OMe; **Rugchalcone A (1)**

R^1, R^3 = OH, R^2 = OMe; **Rugchalcone B (2)**

R^1, R^3 = OMe, R^2 = OH; (**3**)

R^1, = OMe, R^2, R^3 = OH; (**4**)

R^1, R^2, R^3 = OMe; (**5**)

R^1, R^2 = OMe, R^3 =OH; (**6**)

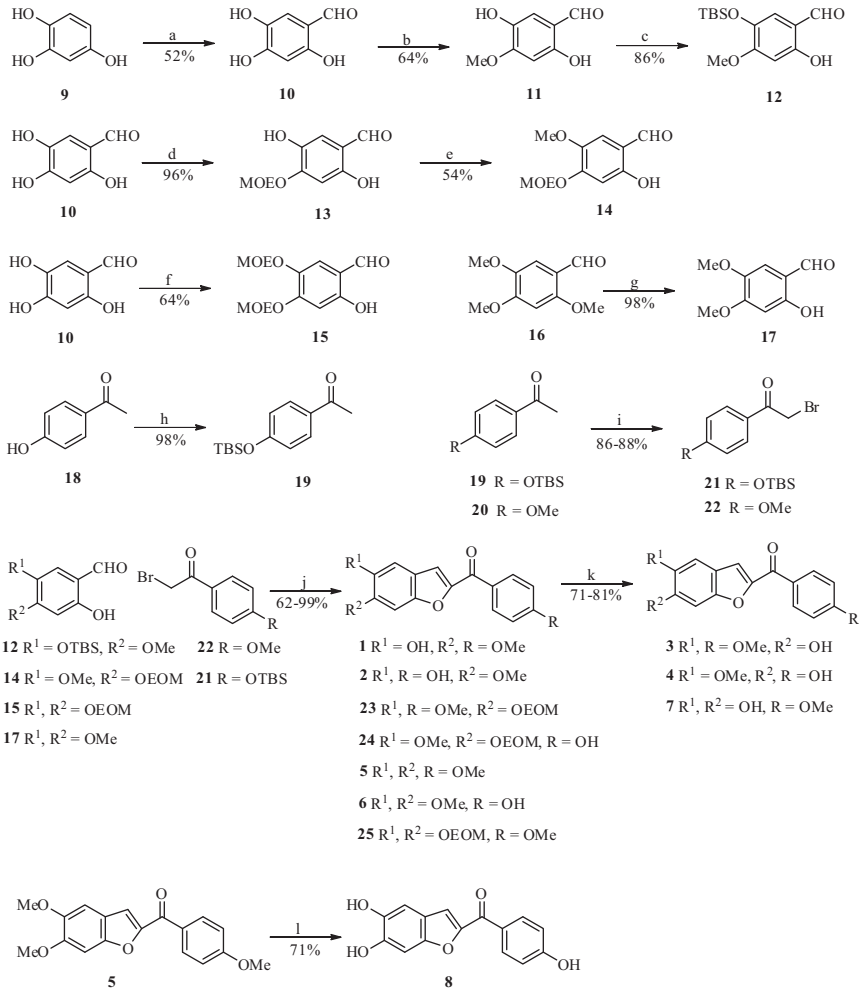
R^1, R^2 = OH, R^3 =OMe; (**7**)

R^1, R^2, R^3 = OH; (**8**)

Figure 1. Structures of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**).

Rugchalcones A and B (Fig. 1) are 2-aroylbenzofuran derivatives isolated from the flowers of *Rosa rugosa* and displayed *anti*-tobacco mosaic virus (*anti*-TMV) activities.[15] In continuation of our work[16] on the synthesis of bioactive natural products and their analogs, herein we wish to describe an efficient synthesis and *anti*-inflammatory activity evaluation of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**).

Our approach for the synthesis of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**) is outlined in Scheme 1. The synthesis commenced with the preparation of 2,4,5-trihydroxybezaldehyde (**10**) from 1,2,4-benzenetriol (**9**). Regioselective methylation of phenolic 4-OH group of **10** was carried out using equimolar amounts of methyl iodide (MeI) and KOH in *N*,*N*-dimethylformamide (DMF) to afford compound **11** in 64% yield which was subsequently protected using *tert*-butyldimethylsilyl chloride (TBDMSCl) to furnish substituted salicylaldehyde, **12** in 86% yield. While, phenolic 4-OH group of **10** regioselectively protected with ethoxymethyl (EOM) group using chloromethyl ethyl ether (EOM-Cl), triethylamine (Et_3N) and catalytic tetrabutylammonium iodide (TBAI) in acetone and we were pleased to isolate compound **13** in 96% yield which



Scheme 1. Reagents and conditions: (a) triethyl orthoformate, $AlCl_3$, benzene, rt, 1 h; (b) MeI, KOH, anhyd DMF, 0–40 °C, 8 h; (c) TBDMSCl, imidazole, DMF, 40 °C, 2.5 h; (d) chloromethyl ethyl ether, Et_3N, TBAI, acetone, 0 °C–rt, 4 h; (e) dimethyl sulfate, K_2CO_3, acetone, 0 °C–rt, 12 h; (f) chloromethyl ethyl ether, K_2CO_3, TBAI, acetone, 0 °C–rt, 2.5 h; (g) BCl_3, CH_2Cl_2, −78 °C to rt, 12 h; (h) TBDMSCl, imidazole, DMF, 40 °C, 12 h; (i) $CuBr_2$, EtOAc, reflux, 2 h; (j) K_2CO_3, acetonitrile, reflux, 1–2 h; (k) Dowex® resin, MeOH, THF, rt, 2–4 days; (l) BBr_3, CH_2Cl_2, 0 °C–rt, 28 h.

upon methylation of 5-OH group gave compound **14**. Substituted salicylaldehyde **15** was also obtained from compound **10** by the two phenolic–OH groups protection using EOM-Cl, catalytic TBAI and K_2CO_3 as base in acetone. Next, 4,5-dimethoxysalicylaldehyde (**17**) was obtained in excellent yield from 2,4,5-trimethoxybenzaldehyde by demethylation using BCl_3.

With the substituted salicylaldehydes **12**, **14**, **15** and **17** in hand, our attention shifted to preparation of required phenacyl bromides. Protection of 4-hydroxyacetophenone (**18**) with *tert*-butyldimethylsilyl (TBS) group gave compound **19** in 98% yield. Next, α-bromination of compound **19** and 4-methoxyacetophenone (**20**) was carried out using copper(II) bromide ($CuBr_2$) in ethyl acetate and the corresponding phenacyl bromides **21** and **22** were obtained in good yields.

We were now ready to subject the Rap–Stoermer reaction[17] between substituted salicylaldehydes (**12**, **14**, **15** and **17**) and phenacyl bromides (**21** and **22**), a prominent synthetic protocol employed for the synthesis of 2-aroylbenzofurans. Treatment of 1.0 equiv of phenacyl bromide, 1.1 equiv of substituted salicylaldehyde and 1.3 equiv of K_2CO_3 in acetonitrile under reflux condition for 1–2 h led to the isolation of 2-aroylbenzofurans **1**, **2**, **5**, **6** and **23**–**25** in moderate to excellent yields. Compounds **23**–**25** were further subjected to EOM-group deprotection with Dowex® resin and we were delighted to obtain the desired rugchalcone derivatives **3**, **4** and **7** in 71–81% yields. Demethylation of **5** using excess BBr_3 in CH_2Cl_2 afforded the product **8** in good yield. The structures of all the 2-aroylbenzofurans were settled from their spectral (^1H and ^{13}C NMR and MS) data (see the Supplementary data).

In order to evaluate the *anti*-inflammatory effects of the prepared rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**), we measured the amount of nitric oxide (NO) which is one of the essential mediators of inflammation, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages.[18]

anti-Inflammatory activity: Effect of compounds **1**–**8** on NO generation by induced macrophages was monitored (Table 1). Lipopolysaccharide (LPS) treated RAW 264.7 has been used to stimulate the production of NO through the activation of iNOS and N^G-monomethyl-L-arginine acetate (L-NMMA)[19] was employed as positive control. Of the 2-aroylbenzofurans prepared in the present study, four compounds, that is, rugchalcone B, compounds **4**, **7** and **8** showed significant activities at 10 μM (Fig. 2). Among the 8 compounds, the maximum inhibitory activity was observed with compound **4** (99.6%) followed by rugchalcone B (99.3%), compounds **7** (96.8%) and **8** (74.3%). The cell viability assay at 10 μM concentration was not affected by any compound indicating no cytotoxicity as shown in Table 2. Next, we investigated NO inhibition by these compounds further to determine whether it was caused by a reduced expression of iNOS. As shown in Figure 3, levels of iNOS cells treated with compound **4** was dramatically decreased which is consistent with the findings shown in NO production (Fig. 2 and Table 1). This indicates that the reduced expression of iNOS due to these compounds exposure was responsible for the inhibition of NO production.

IC_{50} values of compounds **1**–**8** were evaluated by using GraphPad Prism 4.0 software and showed 10.79, 4.13, 13.27, 0.57, 11.84, 10.42, 1.90 and 0.99 μM, respectively (Table 2). From the aforementioned pharmacological results, we can conclude that 2-aroylbenzofurans having 4-hydroxyphenyl moiety and/or hydroxy (–OH) group at 5- and/or 6-position of benzofuran scaffold are fruitful to show potent *anti*-inflammatory activity by effective inhibition of iNOS with no cytotoxicity.

In summary, we have described an efficient synthesis of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**) using substituted salicylaldehydes and phenacyl bromides as building blocks and Rap–Stoermer reaction as a key step. Later, their *anti*-inflammatory effects were evaluated in lipopolysaccharide (LPS)

Table 1
anti-Inflammatory activities of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**)

Compound	NO production (% inhibition)	
	1 μM	10 μM
Medium (MED)	1.0 ± 0.0 (99.0)***	1.0 ± 0.0 (99.0)***
1	83.8 ± 6.0 (16.2)	57.0 ± 6.5 (43.0)***
2	100.0 ± 2.7 (0.0)	0.7 ± 0.7 (99.3)***
3	92.2 ± 0.5 (7.8)	70.3 ± 3.4 (29.7)**
4	45.8 ± 1.8 (54.2)***	0.4 ± 0.4 (99.6)***
5	90.5 ± 6.5 (9.5)	70.2 ± 4.7 (29.8)**
6	91.4 ± 8.4 (8.6)	64.2 ± 3.5 (25.8)***
7	80.3 ± 1.7 (19.7)	3.2 ± 1.9 (96.8)***
8	57.1 ± 1.1 (42.9)***	25.7 ± 0.2 (74.3)***
L-NMMA	79.1 ± 4.1 (20.9)	7.6 ± 4.0 (92.4)***

The results are reported as mean value ± SEM for $n = 3$. Statistical significance is based on the difference when compared with LPS-treated groups (**P <0.01, ***P <0.001).
% inhibition is based on LPS as shown in parenthesis.

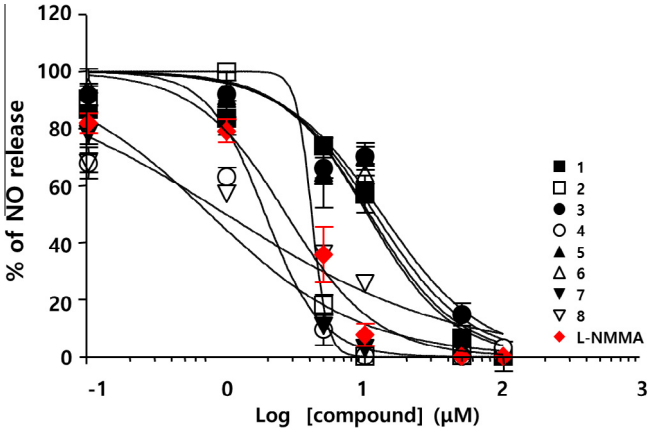


Figure 2. Inhibition of iNOS mediated NO production by compounds **1**–**8**.

Table 2
Proliferation effect of rugchalcones A (**1**), B (**2**) and their derivatives (**3**–**8**)

Compound	Proliferation[a]		IC_{50} (μM)
	1 μM	10 μM	
Medium (MED)	100.1 ± 6.7	100.1 ± 6.7	
1	98.1 ± 5.2	100.1 ± 3.9	10.79
2	97.9 ± 4.6	93.1 ± 1.7	4.13
3	93.2 ± 3.3	91.8 ± 3.6	13.27
4	98.1 ± 2.1	95.9 ± 2.1	0.57
5	105.4 ± 5.7	98.6 ± 2.6	11.84
6	105.7 ± 2.0	101.7 ± 1.8	10.42
7	95.1 ± 3.9	101.2 ± 1.5	1.90
8	106.3 ± 3.1	100.3 ± 3.3	0.99
L-NMMA	98.6 ± 2.9	97.6 ± 5.6	2.69

[a] The results are reported as mean value ± SEM for $n = 3$.

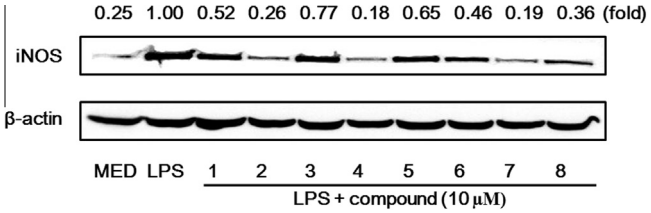


Figure 3. Effects of compounds **1**–**8** on iNOS expression (Western blot).

stimulated RAW-264.7 macrophages. Of the 2-aroylbenzofurans prepared in this study, four compounds, that is, compound **4** (99.6%; IC_{50} = 0.57 μM), rugchalcone B (**2**) (99.3%; IC_{50} = 4.13 μM), **7** (96.8%; IC_{50} = 1.90 μM) and **8** (74.3%; IC_{50} = 0.99 μM) showed exceptional inhibitory activity at 10 μM with no cytotoxicity. This study revealed that compounds **2**, **4**, **7** and **8** which are having 4-hydroxyphenyl group and/or hydroxy (–OH) group at 5- and/or 6-position of benzofuran motif could be considered as a promising scaffolds for the further development of NO inhibitors for potential *anti*-inflammatory applications.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.02.023.

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