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Oroxylin A analogs exhibited strong inhibitory activities against *i*NOS-mediated nitric oxide (NO) production

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ABSTRACT

A number of oroxylin A analogs were prepared and evaluated for their inhibitory activities against *i*NOS-mediated nitric oxide (NO) production from LPS-stimulated BV2 cells. The analogs were synthesized from purchased 2′-hydroxy-4,5,6-trimethoxyacetophenone and aldehydes in 3 steps. Among the tested compounds, several analogs (**3b**, **3c**, **3d**, **3f**) exhibited strong inhibitory activities. Especially, the analog with 4-nitrophenyl group (**3b**) showed stronger inhibitory activity (IC_{50} = 4.73 μM) than that of wogonin (IC_{50} = 7.80 μM).

Oroxylin A (5,7-dihydroxy-6-methoxyflavone) is a plant originated flavone from *Scutellaria baicalensis*, which has been traditionally used as medicinal herb to improve memory and cognition. Several major components of this herb were isolated and identified as flavones such as wogonin, baicalin, baicalein, oroxylin A and so forth.[1,2] Behavior studies have demonstrated that water extract of *S. baicalensis* exhibited potent anxiolysis in mice without sedative and myorelaxant effects.[3] Flavones from *S. baicalensis* may possess diverse biological activities such as anxiolysis, anticonvulsion, muscle-relaxation and sedative effects due to their binding affinity with benzodiazepine or GABA receptors.[4] Recently, oroxylin A was reported to improve attention-deficit hyperactivity disorder (ADHD) related behaviors in spontaneously hypertensive rats.[5]

Recently wogonin (5,7-dihydroxy-8-methoxyflavone), one of major constituents of *S. baicalensis*, was reported to possess strong anti-inflammatory activity.[6] Wogonin and oroxylin A have similar chemical structures except the position of methoxy group in their structures as shown in Figure 1. The structural difference may result in different biological activities of wogonin and oroxylin A.

Our previous results showed that 6,8-disubstituted chrysin analogs (5,7-dihydroxy-6,8-disubstituted flavones) retained strong inhibitory activities against COX-2 catalyzed PGE_2 and *i*NOS-mediated NO production.[7]

These results showed that di-substitutions at C6 and C8 of chrysin were tolerable for anti-inflammatory activity. Deletion of the methoxy group at C8 of wogonin produced chrysin, which exhibited significantly reduced anti-inflammatory activity.[8,9] These results imply that the C8 methoxy group of wogonin plays very important roles for strong anti-inflammatory activity. Oroxylin A also possesses a methoxy group at C6 of its structure. Since the C6 methoxy group of oroxylin A was also expected to contribute for strong anti-inflammatory activity, a number of oroxylin A analogs were prepared and evaluated their inhibitory activities against *i*NOS-mediated NO production, a major chemical mediator for inflammation.

Oroxylin A and its analogs were synthesized from commercial starting materials in 3 steps. The reaction of 2′-hydroxy-4′,5′,6′-trimethoxyacetophenone, purchased aldehydes and KOH in methanol yielded the corresponding chalcones in 65%–96% yields. Reaction of chalcones (**1a**∼**1i**) in iodine/DMSO condition afforded flavones (**2a**∼**2i**) in 81%–98% yields. Demethylation of flavones (**2a**∼**2i**) in 48% HBr/AcOH produced oroxylin A (**3a**) and its analogs (**3b**∼**3i**) in 36%–97% yields (Scheme 1).[7,10]

Inhibition of *i*NOS mediated NO production from LPS-stimulated BV2 cells by oroxylin A analogs was determined according to the published procedure.[6] RAW 264.7 cells obtained from American Type Culture Collection were cultured in DMEM supplemented with 10% FBS and 1% CO_2 at 37 °C and activated with LPS. Briefly, cells were plated in 96-well plates (2×10^5 cells/well). Oroxylin A analog and LPS (200 ng/mL) were added and incubated for 24 h. Cell viability was assessed with MTT assay based on the experimental procedures described previously. All experiments were carried out at least twice and they gave similar results. The inhibitory activities of analogs on *i*NOS mediated NO production from LPS-stimulated BV-2 cells were estimated and the results are shown in Table 1.

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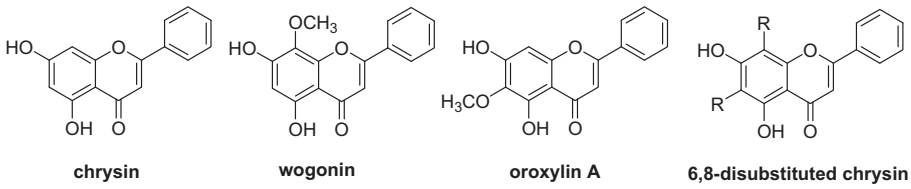
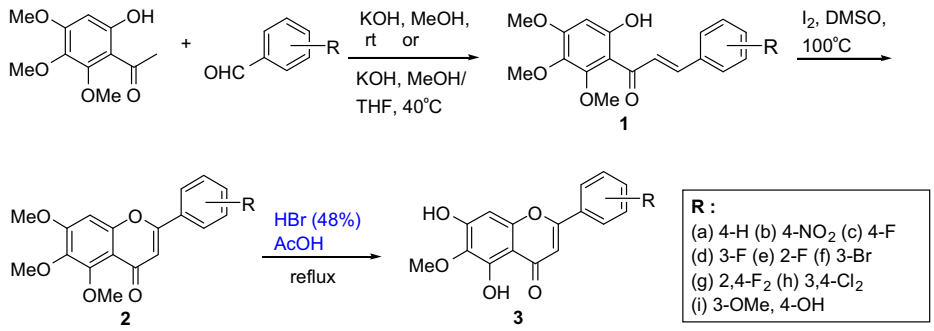


Figure 1. Structures of chrysin, oroxylin A, wogonin and 6,8-disubstituted chrysin analogs.



Scheme 1. Synthesis of oroxylin A and its analogs.

Table 1
Inhibition of NO production from LPS-stimulated BV2 cells by oroxylin A analogs at 10 μM[a,b,c]

No	% of Inhibition (IC_{50}, μM)	No	% of Inhibition (IC_{50}, μM)
3a	26.0	**3f**	45.2 (10.84)
3b	45.7 (4.73)	**3g**	10.6
3c	40.8 (8.58)	**3h**	13.1
3d	39.9 (11.42)	**3i**	8.2
3e	14.2	wgn	40.0 (7.80)

[a] All compounds were treated at 10 μM. Treatment of LPS to BV2 cells increased NO production from the basal level.
[b] % inhibition = 100 × [1 − (NO of LPS with the analogs treated group − NO of the basal)/(NO of LPS stimulated group − NO of the basal)].
[c] Wogonin (wgn) was used as the reference compound.

As demonstrated in Table 1, synthetic oroxylin A (**3a**) exhibited less potent inhibitory activity against *i*NOS-mediated NO production from LPS-stimulated BV2 cells compared to that of wogonin. However, several analogs **(3b**, **3c**, **3d**, **3f)** showed equivalent to more potent inhibitory activities than that of wogonin. Mono-substitutions at 4′ (**3b**, **3c**), or 3′ (**3d**, **3f**) position increased the bioactivities, while mono-substitution at 2′ position exhibited significantly reduced bioactivity (**3e**) compared to those of analogs at 3′ (**3d**) and 4′ (**3c**) positions. Moreover, di-substitutions at 2′/4′ (**3g**) and 3′/4′ (**3h**, **3i**) positions resulted in significantly decreased bioactivities regardless of atoms or functional group. Our present results imply that anti-inflammatory activity of oroxylin A is largely dependent on structural modification of B ring. Also the methoxyl group of oroxylin A is essential for strong inhibitory activity since deletion of the methoxyl group resulted in significantly reduced bioactivity as shown in the previous results.[8,9]

In conclusion, structural modification of oroxylin A provided synthetic oroxylin A analogs possessing strong inhibitory activities against *i*NOS-mediated NO production from LPS-stimulated BV2 cells. Strong inhibitory activity was observed from the analogs with mono-substitution at 3′ or 4′ position of oroxylin A regardless of atoms or functional groups. Further SAR study with a large number of analogs possessing diverse functional groups with different electronic and physical parameters is under progress.

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