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Synthesis and Anti-platelet Activity of Obovatol Derivatives

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Obovatol derivatives were synthesized and evaluated for anti-platelet activity. Three derivatives (**1, 2, 4i**) displayed equipotent activity to obovatol in arachidonic acid-induced platelet aggregation. An initial SAR study revealed that the introduction of alkoxy group in B ring could enhance inhibitory activity.

Key words: Obovatol, Anti-platelet activity, Derivatives, Small molecules

INTRODUCTION

Platelet aggregation is a physiological mechanism that seals off the damaged blood vessel wall to prevent blood loss. Various endogenous factors such as collagen, thrombin, ADP, serotonin, vasopressin, and ephineprine induce platelet activation followed by shape change and aggregation. In addition, arachidonic acid is converted to TXA_2 and 12-HETE via the COX pathway, which plays a major role in aggregation (Cowan, 1981; Eynard et al., 1986; Pollock, 1986). Under pathological conditions, however, platelets also play a key role in the pathogenesis of coronary artery disease and arthrosclerosis. Platelet hyperaggregation has been observed in pathological conditions such as diabetes mellitus, hypercholesterolaemia, and hypertension, all of which cause significantly coronary artery disease (Willoughby et al., 2002; Michelson, 2010). Development of platelet activity modulators has been intensively investigated, as platelet aggregation inhibition is therapeutically beneficial (Jackson and Schoenwaelder, 2003). Among natural resources of our interest, obovatol has been reported to show inhibitory effects on either collagen- or arachidonic acid-induced platelet aggregation (Pyo et al., 2002; Jin et al., 2008), as well as other biological effects, such as antiproliferative activity for vascular smooth muscle cells and cancer cells (Kwak et al., 2009; Lee et al., 2009; Yu et al., 2009; Lim et al., 2010). Several of the obovatol derivatives we synthesized were evaluated for effect on platelet aggregation activity. Herein, we describe anti-platelet activity and structure-activity relationship of obovatol derivatives.

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MATERIALS AND METHODS

Saturated obovatol (1)

To a solution of obovatol (20 mg, 0.070 mmol) in ethanol was added Pd/C (10 mg). The reaction flask was purged with hydrogen. The reaction mixture was stirred for 1 day at room temperature. The reaction mixture was filtered through celite. The filtrate was concentrated *in vacuo* and purified by flash column chromatography (EtOAc-Hexanes = 1 : 4) to afford saturated compound **1** (19 mg, 95%) as an oil. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.14 (d, 2H, J = 8.4 Hz), 6.93 (d, 2H, J = 8.4 Hz), 6.56 (s, 1H), 6.28 (s, 1H), 2.57 (t, 2H, J = 7.6 Hz), 2.41 (t, 2H, J = 7.6 Hz), 1.70 - 1.42 (m, 4H), 0.95 (t, 3H, J = 7.3 Hz), 0.88 (t, 3H, J = 7.3 Hz).

Diacetylated obovatol (2)

To a solution of obovatol (30 mg, 0.106 mmol) in

methylene chloride, was added 4-dimethylaminopyridine (3.0 mg, 0.021 mmol), triethylamine (53 mg, 0.53 mmol), acetic anhydride (16 mg, 0.159 mmol). The reaction mixture was stirred for 20 min at room temperature. The reaction mixture was neutralized by sodium bicarbonate, extracted with EtOAc (3×). The extract was concentrated *in vacuo*. Purification by flash column chromatography (EtOAc-Hexanes = 1 : 5) gave acetylated obovatol **2** (32 mg, 82%). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.14 (d, 2H, *J* = 8.4 Hz), 6.95 (d, 2H, *J* = 8.4 Hz), 6.76 (s, 1H), 6.65 (s, 1H), 6.03 - 5.82 (m, 2H), 5.06 (d, 4H, *J* = 15.2 Hz), 3.38 (d, 2H, *J* = 6.6 Hz), 3.29 (d, 2H, *J* = 6.6 Hz), 2.29 (s, 3H), 2.26 (s, 3H).

Methyl 3-hydroxy-4,5-dimethoxybenzoate (3)

To a solution of methyl gallate **7** (2.00 g, 10.86 mmol) in DMF anhydrous was added potassium carbonate (1.20 g, 8.69 mmol) under nitrogen atmosphere. The mixture was stirred for 30 min at room temperature. To the mixture, was added methyl iodide (3.39 g, 23.89 mmol) drop by drop. The reaction mixture was stirred for 2 h at room temperature and then extracted with ether. The extract was concentrated *in vacuo*. The crude product was purified by flash column chromatography (EtOAc-Hexanes = 1 : 3) to afford methyl ether **3** (1.84 g, 80%) as a white solid. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.28 (s, 1H), 7.18 (s, 1H), 6.02 (s, 1H), 3.94 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H).

General procedure for the preparation of (4a-4i)

To a mixture of **3** (1.0 eq), arylboronic acid (1.2 eq), copper acetate (0.1 eq) and triethyl amine (5.0 eq) in methylene chloride was added activated 4 molecular sieve. The reaction flask was sealed with septa stuck by a 18 gauge needle. The reaction mixture was stirred for 2.5 h at room temperature, and then filtered to remove molecular sieve and copper salt. The filtrate was concentrated *in vacuo* and purified by flash column chromatography (EtOAc-Hexanes = 1 : 3 → 1 : 5) to afford biaryl ether.

Methyl 3-(4-cyanophenoxy)-4,5-dimethoxybenzoate (4a)

Yield 60%; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.60 (d, 2H, *J* = 8.8 Hz), 7.52 (s, 1H), 741 (s, 1H), 6.97 (d, 2H, *J* = 8.8 Hz), 3.96 (s, 3H), 3.90 (s, 3H), 3.83 (s, 3H).

Methyl 3,4-dimethoxy-5-(4-vinylphenoxy)benzoate (4f)

Yield 89%; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.47 (s, 1H), 7.40 (d, 2H, *J* = 8.7 Hz), 7.25 (s, 1H), 6.88 (d, 2H, *J* = 8.7 Hz), 6.74-6.64 (m, 2H), 5.68 (d, 1H, *J* = 17.7 Hz), 5.16 (d, 1H, *J* = 10.8 Hz), 3.92 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H).

Methyl 3-(4-ethoxyphenoxy)-4,5-dimethoxybenzoate (4h)

Yield 71%; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.37 (s, 1H), 7.20 (s, 1H), 6.94 (d, 2H, *J* = 9.0 Hz), 6.85 (d, 2H, *J* = 9.0 Hz), 4.01 (q, 2H, *J* = 7.0 Hz), 3.94 (s, 6H), 3.84 (s, 3H), 1.41 (t, 3H, *J* = 7.0 Hz).

Methyl 3,4-dimethoxy-5-(4-propoxyphenoxy)benzoate (4i)

Yield 80%; IR (KBr) 1722, 1211, 1095 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.37 (s, 1H), 7.20 (s, 1H), 6.94 (d, 2H, *J* = 9.0 Hz), 6.85 (d, 2H, *J* = 9.0 Hz), 4.01 (q, 2H, *J* = 7.0 Hz), 3.94 (s, 6H), 3.84 (s, 3H), 1.41 (t, 3H, *J* = 7.0 Hz).

Spectral data of **4b-4e, 4g** and **5a-5h** were reported in our previous report (Lee et al., 2007).

3,4-Dimethox-5-(4-propoxyphenoxy)benzoic acid (6)

The compound **1** (0.110 mmol) was dissolved in KOH (*aq*.) (1 N, 2 mL)/ethanol (2 mL). The reaction mixture was refluxed for 2 h. After cooling to room temperature, the mixture was acidified with 1 N HCl, extracted with Ethyl Acetate. The extract was dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The resulting acid was used in next reaction without further purification. Yield 99%; ^{1}H-NMR (Acetone-d_6, 300 MHz): δ 7.43 (d, 1H, *J* = 1.8 Hz), 7.18 (d, 1H, *J* = 1.8 Hz), 6.95 (d, 4H, *J* = 3.6 Hz), 3.84-3.95 (m, 8H), 1.78 (s, 2H, *J* = 7.2 Hz), 1.02 (t, 3H, *J* = 7.2 Hz).

General procedure for the preparation of compounds (7a-c)

To a solution of **2** (0.040 mmol) in dry methylene chloride (3 mL) was added oxalyl chloride (0.400 mmol) and DMF (catalytic amount). The reaction mixture was stirred for 1 h at room temperature. After completion, the mixture was evaporated completely and then dissolved in dry methylene chloride (1 mL). To the solution, alcohol (0.040 mmol) was added and stirred for 30 min at room temperature. The reaction mixture was extracted with Ethyl Acetate, washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated. The residue was purified by flash column chromatography to afford ester **7**.

Butyl 3,4-dimethoxy-5-(4-propoxyphenoxy)benzoate (7a)

Yield 90%; ^{1}H-NMR (Acetone-d_6, 300 MHz): δ 7.41 (d, 1H, *J* = 1.8 Hz), 7.17 (d, 1H, *J* = 1.8 Hz), 6.95 (s, 4H),

4.25 (t, 2H, J = 6.6 Hz), 3.94 (t, 6H, J = 6.6 Hz), 3.83 (s, 3H), 1.66-1.79 (m, 4H), 1.42 (s, 2H, J = 7.8 Hz), 1.02 (t, 3H, J = 7.2 Hz), 0.93 (t, 3H, J = 7.5 Hz).

Allyl 3,4-dimethoxy-5-(4-propoxyphenoxy)benzoate (7b)

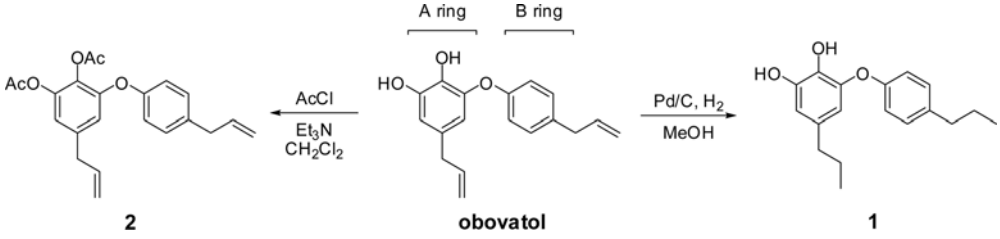
Yield 55%; ^{1}H-NMR (Acetone-d_6, 300 MHz): δ 7.39 (d, 1H, J = 1.8 Hz), 7.25 (d, 1H, J = 1.8 Hz), 6.92 (d, 2H, J = 6.3 Hz), 6.87 (d, 2H, J = 6.3 Hz), 5.97 (m, 1H), 5.34 (d, 1H, J = 17.1 Hz), 5.25 (d, 1H, J = 10.5 Hz), 4.76 (d, 2H, J = 5.7 Hz), 3.88-3.94 (m, 8H), 1.80 (s, 2H, J = 13.8 Hz), 1.04 (t, 3H, J = 7.2 Hz).

Benzyl 3,4-dimethoxy-5-(4-propoxyphenoxy)benzoate (7c)

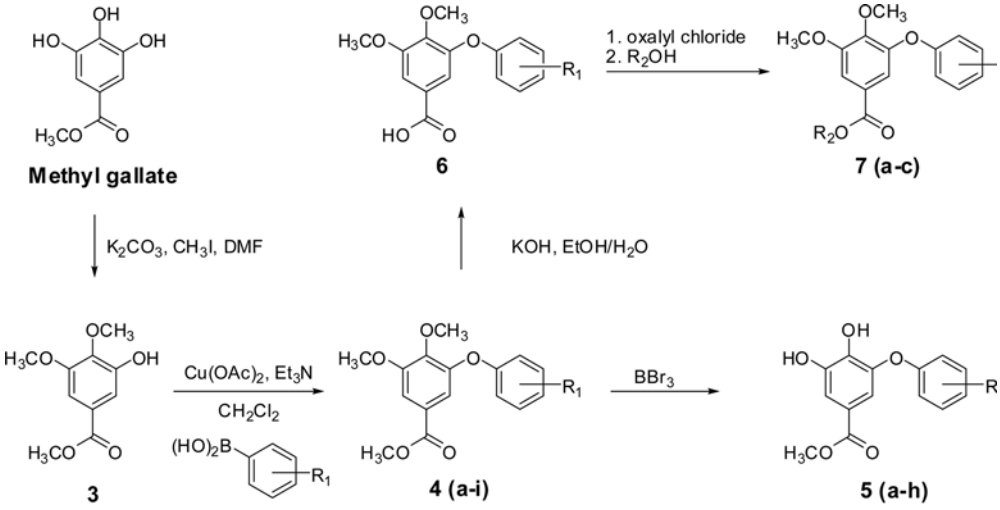
Yield 50%; ^{1}H-NMR (Acetone-d_6, 300 MHz): δ 7.31-7.40 (m, 5H), 7.26-7.27 (m, 2H), 6.92 (d, 2H, J = 6.6 Hz), 6.85 (d, 2H, J = 6.6 Hz), 5.30 (s, 2H), 3.84-3.94 (m, 8H), 1.80 (s, 2H, J = 14.4 Hz), 1.04 (t, 3H, J = 7.5 Hz).

Washed rabbit platelet preparation and aggregation assay

Rabbits were fixed in a cage. Blood was drawn from the ear artery of conscious rabbits and collected directly in the vacutainer tubes containing anticoagulant solution (comprised of 0.8% citric acid, 2.2% trisodium citrate, and 2% dextrose (w/v)). PRP was prepared by centrifugation at 230 g for 10 min at room temperature. Platelets were sedimented by centrifugation of PRP at 2100 g for 10 min and then washed twice with HEPES buffer (137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 5.6 mM glucose, 0.35% bovine serum albumin, and 3.8 mM HEPES, pH 6.5) containing 0.4 mM EGTA. After centrifugation, the pellets were re-suspended in HEPES buffer (pH 7.4). The platelet concentration was counted using a Coulter Counter (Coulter Electronics) and adjusted to 4×10^8 platelets per milliliter. Platelet aggregation was mea sured as previously described (Jin et al., 2008). Briefly, washed platelet suspensions were incubated at 37°C in the aggregometer with stirring at 1000 rpm. After incubation with various concentration of obovatol (50 μM and 10 μM), DMSO as a control, for 5 min, platelet aggregation was induced by the addition of collagen (10 μg/mL), arachidonic acid (100 μM), respectively. The resulting aggregation measured as the change in light transmission was recorded for 10 min.



Scheme 1. Synthesis of acetylated and saturated obovatol derivatives



Scheme 2. Synthesis of obovatol derivatives

RESULTS AND DISCUSSION

Synthesis of obovatol derivatives

The obovatol derivatives were prepared as shown in Scheme 1. Most compounds were obtained according to the known procedure we previously reported (Lee et al., 2007; Kwak et al., 2009). Copper catalyzed etherification was used as the key step to prepare diaryl ethers. Selective dimethylation of methyl gallate and subsequent etherification catalyzed by $Cu(OAc)_2$ provided the diversed diaryl ethers. Demethylation of aryl methyl ethers by BBr_3 gave catechol derivatives. Synthesis of ester derivatives was started with hydrolysis of **4**. The resulting carboxylic acid **6** was transformed to other esters **7** via acid chloride intermediate (Scheme 2).

Anti-platelet activities

All obovatol derivatives prepared in this study were evaluated for their anti-platelet aggregation activity. Collagen and arachidonic acid were used as platelet aggregation inducers. Anti-platelet activity of the obovatol derivatives at 50 μM and 10 μM is summarized in Table I. Obovatol inhibited collagen- and

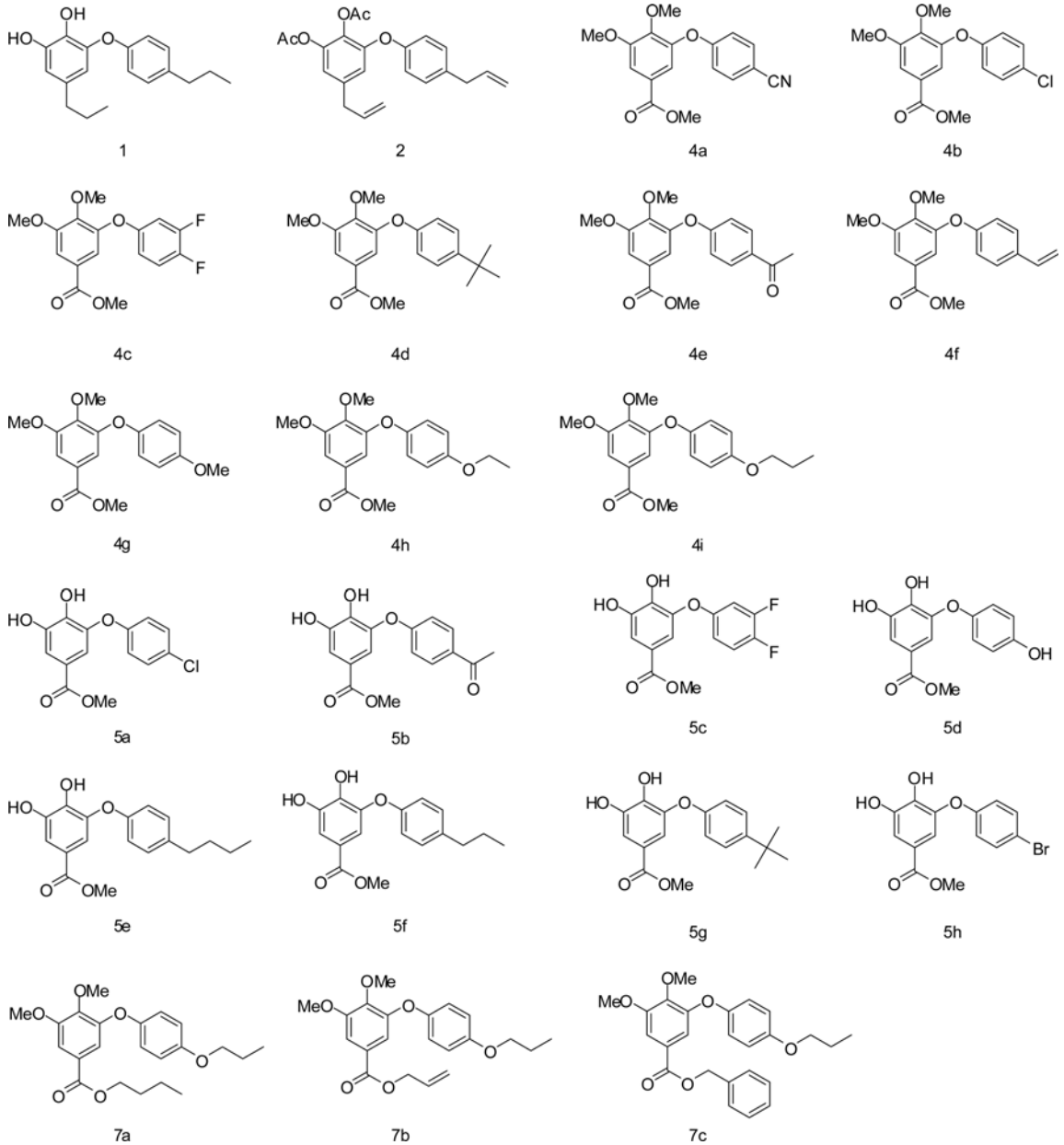


Fig. 1. Structures of synthesized compounds

Table I. Anti-platelet aggregation activity of obovatol derivatives

obovatol derivatives	Aggregation rate (% of control)			
	Collagen (10 μg/mL)		Arachidonic acid (100 μM)	
	at 50 μM	at 10 μM	at 50 μM	at 10 μM
obovatol	5.8	9.9	2.5	1.2
1	1.2	38.8	2.5	1.0
2	9.2	24.4	2.5	2.4
4a	91.2	ND*	89.3	ND
4b	1.1	83.1	0.0	81.9
4c	2.3	97.6	49.1	ND
4d	57.7	ND	69.2	ND
4e	0.0	79.5	0.0	94.0
4f	86.6	ND	79.2	ND
4g	0.0	100.0	0.0	91.6
4h	0.0	98.5	1.3	100.0
4i	0.0	22.0	0.0	1.0
5a	47.3	ND	0.0	88.2
5b	100.0	ND	83.0	ND
5c	100.0	ND	74.2	ND
5d	80.8	ND	1.3	91.8
5e	91.2	ND	2.5	83.5
5f	71.6	ND	2.5	89.4
5g	100.0	ND	81.8	ND
5h	2.3	84.9	6.3	88.2
7a	ND	88.9	ND	ND
7b	ND	95.7	ND	ND
7c	ND	90.4	ND	ND

*ND, not determined

arachidonic acid-induced rabbit platelet aggregation in a dose-dependent manner, with IC_{50} values of 2.4 ± 0.8 and 4.8 ± 0.9 μM, respectively. Saturated obovatol **1** and acetylated compound **2** exerted equipotent effects to obovatol in arachidonic acid-induced platelet aggregation, although they showed a somewhat decreased inhibitory effect compared to obovatol in treatment with collagen (Table I). Unfortunately, most catechol analogues and dimethoxyphenyl analogues displayed loss of anti-aggregation activity at 10 μM, even though a few compounds showed strong activities at 50 μM. Only one derivative, **4i**, displayed excellent activity. However, ester analogues of compound **4i** exhibited loss of activity. This SAR study revealed that anti-platelet aggregation activity of obovatol is highly sensitive to minor structural changes, indicating obovatol may have a limitation as a lead compound. Interestingly, derivatives possessing alkoxy group in the B ring provided useful information. Effect of chain length of alkoxy group was observed; the longer alkoxy group in the B ring resulted in better activity. The longest propyl derivatives showed the best inhibitory activities in this study. Therefore, further structural modifications might lead to the discovery of more potent anti-platelet aggregation agents.

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