Synthesis and Biological Evaluation of Biarylamide Derivatives as Inhibitors of Phagocytosis in Macrophages

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Macrophages are a kind of immune cells that play important roles in the innate immune system. Their phagocytic ability contributes not only to the removal of pathogens and dead cells, but also to tissue remodeling for tissue homeostasis.[1] Phagocytosis is a highly complicated process that involves a number of biomolecules including cell surface receptors and cytoskeletal proteins are involved.[2] Great efforts have been made to discover novel factors involved in phagocytosis to elucidate its molecular mechanism.[3] Immune thrombocytopenia (ITP) is an autoimmune disorder, characterized by an increased tendency to bleed and is caused by the low number of platelet. Patients with ITP produce antibodies against their own platelets thereby destroying most of them.[4] It has been suggested that platelet destruction mainly results from phagocytosis, and that inhibition of phagocytosis may prevent the loss of platelets.[5] Now therefore, it is suggested that small molecule inhibitors for phagocytosis could be developed as first-in-class drugs to combat the increased platelet destruction that occurs in ITP.[6]

With regard to discover small molecules that regulate phagocytosis, we have recently reported biarylamide compounds, including MPS03, inhibit macrophage phagocytosis of zymosan (Figure 1).[7,8] In addition, we demonstrated that the biarylamides reduce the expression level of Rac1, Rac2, and Cdc42, which are the key regulators of phagocytosis.[7] Furthermore, biarylamides suppressed the production of pro-inflammatory cytokines by downregulation of dectin-1 in macrophages.[8]

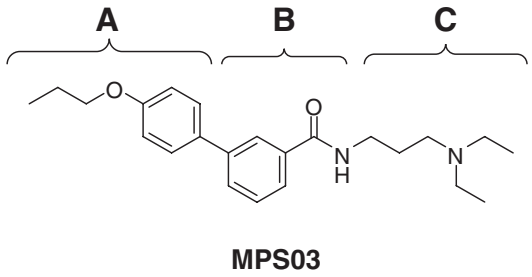
We attempted to find additional potent small molecules against phagocytosis to further explore structure–activity relationship (SAR) of the various biaryl amide compounds. A brief SAR for region C of MPS03 has been previously reported.[7] In this study, we describe inhibitory effect of small molecules, derived from MPS03, on phagocytosis, with a focus on modification within region A. The synthetic procedures for MPS03 derivatives are outlined in Schemes 1. and 2. Various alkyl groups were introduced to region A by *O*-alkylation of bromophenol. The alkoxy bromobenzene **1** was converted to nitrile **2** through Suzuki coupling reaction. Hydrolysis of the nitrile group with KOH gave acid **3**. Conversion of acid **3** to acid chloride and subsequent amide formation with diethylaminopropylamine provided the desired biarylamides **4**. Dibutoxy derivative **8** was synthesized according to the same procedure as that used to obtain compounds **4a–e**.

Flow cytometry-based phagocytosis assay was used to evaluate the effects of all synthesized MPS03 derivatives in RAW264.7 cells, a mouse microphage cell line, as our previously reported method.[7,8] All derivatives had increased activity compared to the parent compound MPS03 and showed no detectable cytotoxicity at 20 μM (Figure 2). Pentyl derivative **4a** exhibited most potent activity among synthesized compounds. Cyclohexylmethyl compound **4c** was equipotent to compound **4a**. However, hexyl derivative **4d** and heptyl compound **4e** showed less activity than pentyl compound **4a**, indicating that inhibitory activity for phagocytosis decrease as the chain length of region A increases. We have previously reported that butyl derivative was slightly less potent compared to 5 μM of cytochalasin D[8] and methyl derivative almost no inhibitory activity.[7] Therefore, the optimal alkyl group of region A deemed most effective is the pentyl group. Dibutoxy derivative **8** showed activity similar to 5 μM of cytochalasin D. Additional introduction of alkoxy group at *meta*-position did not have any significant effect on activity. Although further optimization, including modification for region B, remains to be carried out, the finding of this study provide useful information to establish SAR of this scaffold as a basis to develop more potent compounds. We believe that this series of compounds deserve further development as inhibitors of phagocytosis.

Experimental

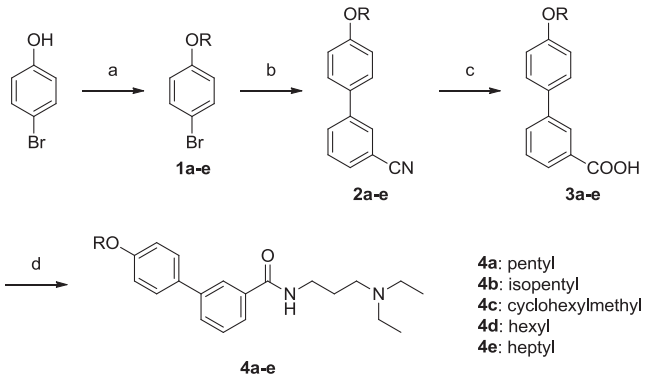
General Procedure for Synthesis of 4a–e and 8

Synthesis of 1a–e and 5. A mixture of bromophenol (1.0 equiv.), alkylbromide (2.0 equiv.), and K_2CO_3 (excess) in DMF was stirred for 1–3 h at 90 °C. The reaction mixture was diluted with acetone, filtered, and the filtrate was concentrated in vacuo. The crude mixture was purified by flash column chromatography on silica gel to afford title compounds **1a–e** and **5**.



MPS03

Figure 1. Structure of MPS03.



Scheme 1. Reagents and conditions: (a) alkyl bromide, K_2CO_3, DMF, 90 °C, 1–3 h, **1a** (78%), **1b** (83%), **1c** (69%), **1d** (79%), **1e** (93%); (b) 3-cyanophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, H_2O, THF, reflux, 12–24 h, **2a** (43%), **2b** (35%), **2c** (43%), **2d** (47%), **2e** (20%); (c) KOH, H_2O, EtOH, microwave 150 °C, 20–60 min, **3a** (75%), **3b** (54%), **3c** (50%), **3d** (87%), **3e** (87%); (d) (i) oxalyl chloride, DMF, CH_2Cl_2, 1–2 h; (ii) *N*,*N*-diethyl-1,3-diaminopropane, Et_3N, THF, 12 h, **4a** (93%), **4b** (99%), **4c** (64%), **4d** (92%), **4e** (15%) for two steps.

Synthesis of 2a–e and 6. A mixture of 3-cyanophenyl boronic acid (1.2–1.5 equiv.), appropriate aryl bromide (1.0 equiv.), and 2 N-K_2CO_3 (2.0–5.0 equiv.) in THF was degassed for 5 min. To the mixture, $Pd(PPh_3)_4$ (0.02 equiv) was added and the mixture was refluxed for 12–24 h. The reaction mixture was diluted with EtOAc, washed by water and brine, dried over Na_2SO_4 anhydrous, and filtered. The filtrate was concentrated and purified by flash column chromatography on silica gel to afford title compounds **2a–e** and **6**.

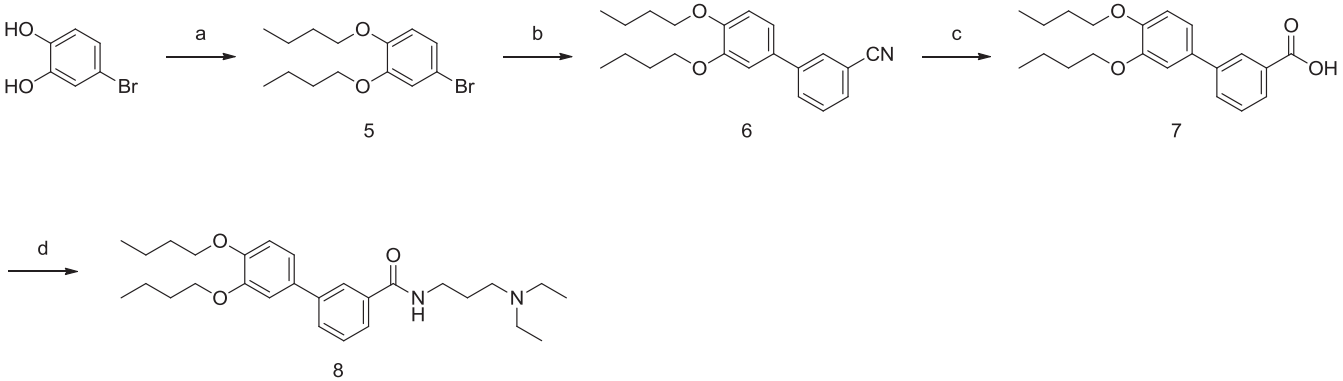
Synthesis of 3a–e and 7. Biphenyl-3-carbonitrile compound was dissolved in EtOH, and mixed with KOH in water. The mixture was stirred for 20–60 min at 150 °C under microwave irradiation. The reaction mixture was concentrated to remove EtOH, acidified by HCl, and extracted by DCM. Combined organic layer was washed by brine, dried over Na_2SO_4 anhydrous, filtered, and concentrated in vacuo to afford biphenyl-3-carboxylic acid **3a–e** and **7**. The crude product was used next reaction without further purification.

Synthesis of 4a–e and 8. To a solution of appropriate biphenyl-3-carboxylic acid in DCM, was added oxalyl chloride (1.0–2.0 equiv.) and DMF (0.02 equiv.) in DCM successively. The mixture was stirred for 1–2 h at room temperature. From the reaction mixture, volatiles were evaporated to dryness under reduced pressure. The crude product was used next reaction without further purification.

To a solution of appropriate biphenyl-3-carbonyl chloride (1.0 equiv.) n DCM, *N*,*N*-diethyl-1,3-diaminopropane (1.5 equiv.), and trimethylamine (3.0 equiv.) was added. The mixture was stirred for 12 h at room temperature. the reaction mixture was diluted by DCM, washed by water and brine, dried over Na_2SO_4 anhydrous, filtered, and concentrated under reduced pressure. The crude mixture was purified by flash column chromatography on silica gel to afford title compounds **4a–e** and **8**.

Compound **4a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.68 (br, 1H), 8.05 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.44 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 3.99 (t, J = 6.6 Hz, 2H), 3.57–3.63 (m, 2H) 2.67–2.77 (m, 6H), 1.76–1.90 (m, 4H), 1.33–1.49 (m, 4H), 1.1 (t, J = 7.2 Hz, 6H), 0.94 (t, J = 6.9 Hz, 3H).

Compound **4b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (br, 1H), 8.05 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.44 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 4.02



Scheme 2. Reagents and conditions: (a) 1-bromobutane, K_2CO_3, DMF, 90 °C, 2 h, 83 %; (b) 3-cyanophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, H_2O, THF, reflux, 12 h, 45 %; (c) KOH, H_2O, EtOH, microwave 150 °C, 20 min, 96 %; (d) (i) oxalyl chloride, DMF, CH_2Cl_2, 1–2 h; (ii) *N*,*N*-diethyl-1,3-diaminopropane, Et_3N, THF, 12 h, 32 % for two steps.

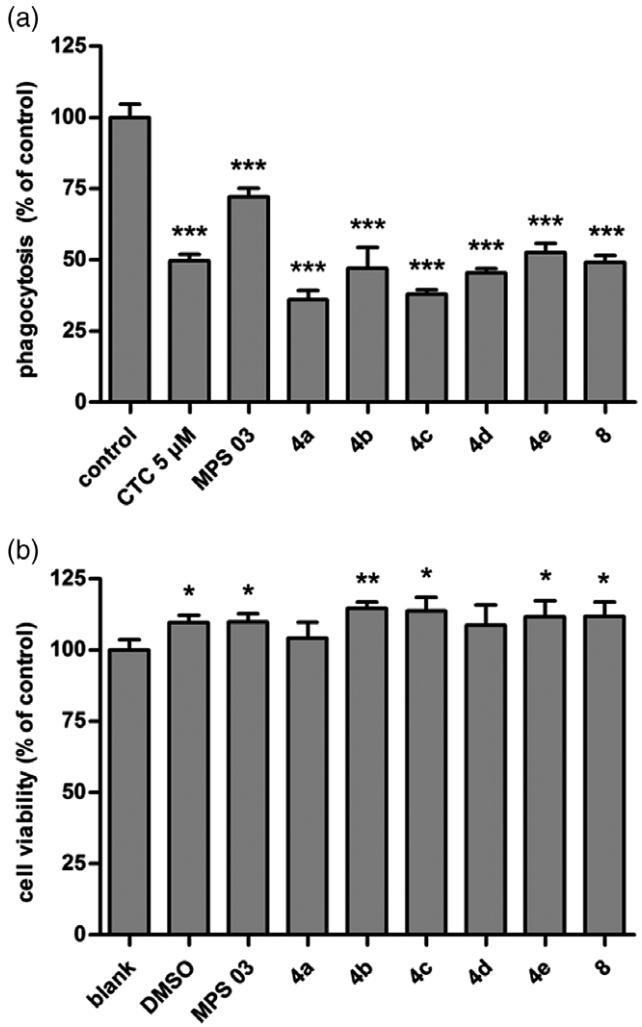


Figure 2. Inhibitory effect of derivatives on phagocytosis of zymosan by RAW264.7 cells. (a) Phagocytosis of zymosan was analyzed using flow cytometry. All compounds were evaluated at 20 μM except cytochalasin D (CTC). (b) Cytotoxicity of all test compounds was evaluated at 20 μM. Error bars represent ±SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$; Student's *t*-test).

(t, J = 6.6 Hz, 2H), 3.59 (q, J = 5.4 Hz, 2H), 2.64–2.74 (m, 6H), 1.81–1.87 (m, 3H), 1.70 (q, J = 6.6 Hz, 2H), 1.08 (t, J = 7.2 Hz, 6H), 0.98 (d, J = 6.6 Hz, 6H).

Compound **4c**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.70 (br, 1H), 8.05 (s, 1H), 7.75 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 9 Hz, 2H), 7.44 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 9 Hz, 2H), 3.79 (d, J = 6 Hz, 2H), 3.60 (q, J = 5.7 Hz, 2H), 2.65–2.75 (m, 6H), 1.69–1.91 (m, 8H), 1.19–1.33 (m, 5H), 1.09 (t, J = 7.2 Hz, 6H).

Compound **4d**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.69 (br, 1H), 8.06 (s, 1H), 7.76 (d, J = 7.8 Hz, 1H), 7.66 (d, J = 7.8 Hz, 1H), 7.56(d, J = 8.7 Hz, 2H), 7.45 (t, J = 7.8 Hz, 1H), 6.96 (d, J = 8.7 Hz, 2H), 3.99 (t, J = 6.6 Hz, 2H), 3.58–3.64 (m, 2H), 2.67–2.77 (m, 6H), 1.76–1.92 (m, 4H), 1.30–1.50 (m, 6H), 1.11 (t, J = 7.2 Hz, 6H), 0.91 (t, J = 7.2 Hz, 3H).

Compound **4e**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (s, 1H), 8.09 (s, 1H), 7.80 (d, J = 7.5 Hz, 1H), 7.67 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.46 (t, J = 7.5 Hz, 1H), 6.97 (d, J = 8.7 Hz, 2H), 3.99 (t, J = 6.3 Hz, 2H), 3.60–3.65 (m, 2H), 2.78–2.85 (m, 4H), 1.94–2.00 (m, 2H), 1.76–1.85 (m, 2H), 1.25–1.47 (m, 8H), 1.17 (t, J = 7.2 Hz, 6H), 0.90 (t, J = 6.3 Hz, 3H).

Compound **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (s, 1H), 8.13 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.45 (t, J = 8.1 Hz, 1H), 7.16–7.19 (m, 2H), 6.95 (d, J = 8.7 Hz, 1H), 4.02–4.11 (m, 4H), 3.60–3.66 (m, 2H), 2.82–2.89 (m, 6H), 2.00 (quint, J = 6.0 Hz, 1H), 1.78–1.87 (m, 4H), 1.49–1.59 (m, 4H), 1.20 (t, J = 7.2 Hz, 6H), 0.96–1.01 (m, 6H).

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