Arch. Pharm. Res. (2013) 36:832–839
DOI 10.1007/s12272-013-0100-z

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Synthesis of novel derivatives of murrayafoline A and their inhibitory effect on LPS-stimulated production of pro-inflammatory cytokines in bone marrow-derived dendritic cells

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Received: 22 October 2012 / Accepted: 13 March 2013 / Published online: 6 April 2013

Abstract A series of *N*-substituted-1,2,3-triazole murrayafoline A derivatives were successfully synthesized using click azide–alkyne Huisgen cycloaddition reaction between 1-methoxy-3-methyl-9-(3-azido)-propyl-9*H*-carbazole and substituted alkynes. Their chemical structures were confirmed by ^{1}H, ^{13}C NMR and HR-ESI–MS spectral data. In addition, the interested effects on LPS-stimulated production of pro-inflammatory cytokines in bone marrow-derived dendritic cells of synthetic murrayafoline A derivatives were also investigated. Our results indicated that murrayafoline A derivatives containing 1,2,3-triazole nucleus potentially possessed anti-inflammatory action through inhibiting production of IL-6, IL-12 p40 and TNF-α.

Keywords Murrayafoline A · 1,2,3-triazole · TNF-α · IL-6 · IL-12 p40

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Introduction

The first isolation of naturally carbazole alkaloid murrayanine was reported in 1964 from the leaves of curry-leaf tree (*Murraya koenigii*) (Burman et al. 1964). Since then a large number of carbazole alkaloids have been isolated from higher plants, fungi, microorganisms and recently from other natural sources, for example, the blue–green algae (Knoelker and Reddy 2002). This class of structure possesses a variety of biological activities including anti-microbial, anti-fungal, anti-tumor, anti-inflammatory, anti-platelet aggregation, anti-oxidative and so on. (Ito et al. 2000; Itoigawa et al. 2000). The synthesis of carbazole derivatives has been a strong interest of many research groups because of their potentially useful biological activities. The anti-inflammatory activity of carbazole derivatives has attracted widespread attention. Among these derivatives (Fig. 1), 1-ethyl-8-*n*-propyl-1,2,3,4-tetrahydrocarbazole-1-acetic acid (**1**) was discovered as a novel anti-inflammatory agent. 6-Chloro-1,2,3,4-tetrahydrocarbazole-2-carboxylic acid (**2**) was clinically active in the treatment of acute gout. Carprofen (**3**), a cyclooxygenase inhibitor, was approved for use in animals as an anti-inflammatory drug with weak ulcerogenic activity. Murrayafoline A (**4**), isolated from the root of several species of the genus *Murraya* (Itoigawa et al. 2000), *Glycosmis* (Cuong et al. 2008) and *Clausena* (Rutaceae) (Cui et al. 2002), exhibits strong fungicidal activity against *Cladosporium cucumerinum* and growth inhibitory activity on human fibrosarcoma HT-1080 cells, cell cycle M-phase inhibitory, and apoptosis inducing activities on mouse tsFT210 cells (Cui et al. 2002). Murrayafoline A was found to attenuate the Wnt/β-catenin pathway by promoting the degradation of intracellular β-catenin proteins (Choi et al. 2010). However, there are few of researches on the synthesis and inflammatory activity of murrayafoline A derivatives

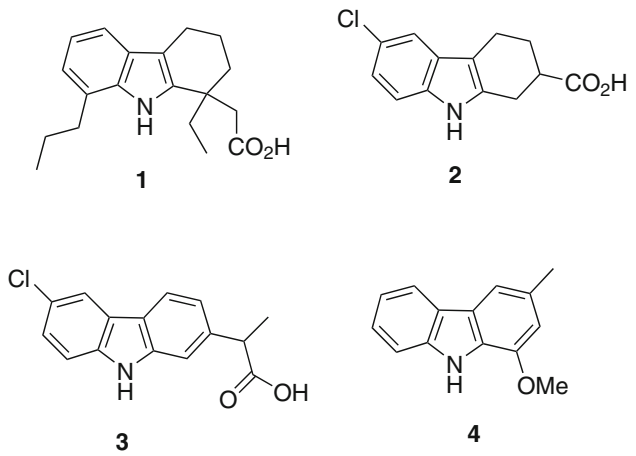


Fig. 1 Several carbazole derivatives possessing highly biological activities

(Cuong et al. 2008). Otherwise, a wide variety of pharmacological properties have been shown to be associated with 1,2,3- and 1,2,4-triazole nucleus (Choi et al. 2010) which including antibacterial (Prakash et al. 2004; Sztanke et al. 2006), antifungal (Lebouvier et al. 2007; Liu et al. 2008), antioxidant (Kamotra et al. 2007), anticancer (Sztanke et al. 2008), anticonvulsant (Almasirad et al. 2004), analgesic (Gilani et al. 2008), antiviral (De Clercq 2002; Dong et al. 2000), and anti-inflammatory (Buckler et al. 1978; Shafi et al. 2012) activities. Recently, the synthesis, anti-bacterial and antifungal activities of imidazole and 1,2,4-triazole-based *N*-substituted carbazole derivatives have been reported (Zhang et al. 2010). However, their anti-inflammatory effects of them have not been extensively studied. Therefore, we describe herein the synthesis and anti-inflammatory activity of *N*-substituted-1,2,3-triazole murrayafoline A derivatives (**8a-h**) which have not been reported in the literature.

Materials and methods

General experimental and procedures

Melting points were recorded on Buchi B-540 melting point appraratus. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker AV 500 using TMS as an internal standard at 500 and 125 MHz respectively. NMR spectra were recorded in $CDCl_3$ or CD_3OD at room temperature. *J* values refer to coupling constants, and signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin.), multiplet (m), or combination thereof. The HR-ESI–MS were obtained from an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. TLC was performed on silica gel plates 60 F254 (0.25 mm, Merck) and column chromatography using silica gel (230–400 mesh, Merck).

Isolation of murrayafoline A

Dried powdered roots (3 kg) of *Glycosmis stenocarpa* were extracted with MeOH, filtered and evaporated *in vacuo*. The suspension of the methanol residue in MeOH/H_2O (1:1) was successively fractioned with *n*-hexane, chloroform, and butanol to give hexane (80 g), chloroform (50 g), and butanol fractions. The hexane fraction (80 g) was chromatographed on silica gel (350 g, Merck silica 80–120 mesh) using a gradient of hexane and EtOAc as eluent to give 12 fractions. Fraction 4 was further rechromatographed on flash silica gel (hexane/EtOAc 10/1 as eluent) to yield murrayafoline A (9 g, 3 %).

Synthesis of 1-methoxy-3-methyl-9-(3-bromo)-propyl-9*H*-carbazole (**5**) and 1-methoxy-3-methyl-9-(propen-2-yl)-9*H*-carbazole (**6**)

A solution of murrayafoline A (2 g, 9.5 mmol) in anhydrous THF (50 mL), NaH (0.3 g, 11.9 mmol), and 1,3-dibromopropane (6 mL, 28.5 mmol) were added into a 100 mL round bottom flask under inert gas. Reaction mixture was stirred at room temperature for about 72 h under inert gas until the starting carbazole had been completely consumed (by TLC monitoring). THF was removed *in vacuo* and then the mixture was cooled. Water (50 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 40 mL). All the combined organic phases were washed by water (30 mL), dried over Na_2SO_4 and then evaporated under reduced pressure. The residue was separated with a silica gel column chromatography (*n*-hexane/ethyl acetate, 6/1) to give **5** (2.6 g, 83 %) and **6** (0.26 g, 11 %) as white solids. **5**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.99 (d, $J = 7.5$ Hz, 1H), 7.48 (s, 1H), 7.43 (m, 2H), 7.18 (dd, $J = 7.5$, 1.5 Hz), 6.73 (s, 1H), 4.69 (t, $J = 6.5$ Hz, 2H), 3.96 (s, 3H), 3.37 (t, $J = 6.5$ Hz, 2H), 2.50 (s, 3H), 2.39 (quin., $J = 6.5$ Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.4, 140.9, 129.2, 127.7, 125.6, 125.0, 122.9, 120.3, 118.8, 112.7, 108.8, 108.7, 55.5, 43.6, 33.9, 30.9, 21.6. **6**: ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (d, $J = 7.5$ Hz, 1H), 7.49 (s, 1H), 7.40 (d, $J = 7.5$ Hz, 1H), 7.33 (d, $J = 7.5$ Hz, 1H), 7.18 (d, $J = 7.5$ Hz, 1H), 6.74 (s, 1H), 6.01 (m, 1H), 5.21 (d, $J = 5.0$ Hz, 2H), 5.06 (dd, $J = 10.0$, 1.5 Hz, 1H), 4.93 (dd, $J = 17.0$, 1.5 Hz, 1H), 3.95 (s, 3H), 2.51 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.6, 140.9, 134.4, 129.1, 128.0, 125.4, 124.9, 123.1, 120.2, 118.7, 115.6, 112.7, 109.2, 109.1, 55.8, 47.5, 21.7.

Synthesis of 1-methoxy-3-methyl-9-(3-azido)-propyl-9*H*-carbazole (**7**)

A solution of **5** (2.5 g, 7.5 mmol) in acetonitrile (80 mL), sodium azide (0.75 g, 11.25 mmol) was added into a

250 mL round bottom flask. The mixture was heated under reflux conditions for 72 h until the starting material (**5**) had been completely consumed (by TLC monitoring). The mixture was cooled and ethyl acetate (50 mL) was added. The obtained mixture was washed with Na_2CO_3 saturated solution (3 × 50 mL), brine (40 mL) and water (50 mL). The combined organic layers were then dried with Na_2SO_4 and evaporated under reduced pressure. The residue was purified on a silica gel column chromatography (*n*-hexane/ethyl acetate, 5:1) to give **7** (2.11 g, 96 %) as white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.42 (dd, J = 8.0, 7.5 Hz, 1H), 7.37 (d, J = 8 Hz, 1H), 7.18 (dd, J = 8.0, 7.5 Hz, 1H), 6.73 (s, 1H), 4.64 (t, J = 6.5 Hz, 2H), 3.97 (s, 3H), 3.25 (t, J = 6.5 Hz, 2H), 2.51 (s, 3H), 2.10 (quin., J = 6.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.4, 140.9, 129.2, 127.7, 125.6, 124.9, 122.9, 120.3, 118.8, 112.7, 108.7, 108.6, 55.5, 48.9, 42.3, 29.9, 21.6.

Protection of propargyl amine

Propargyl amine (0.4 g, 7.26 mmol) and $(Boc)_2O$ (1.58 g, 7.26 mmol) were dissolved in THF (8 mL). Distilled water (8 mL) were added and the mixture was stirred at r.t. in 48 h. Ethyl acetate (3 × 10 mL) was added and the organic layer was separated. The combined organic layers were washed with 5 % $NaHCO_3$ solution (10 mL), water (2 × 10 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The residue was purified with a column of silica gel (*n*-hexane/EtOAc, 95:5) to give a white solid (94 %).

Protection of propargyl alcohol

A solution of propargyl alcohol (0.9 mmol) in DMF (0.5 mL), *t*-butyldiphenylsilyl chloride (0.3 mL) and imidazole (74 mg) were added into a 10 mL round bottom flask. The mixture was stirred overnight at room temperature and water (0.5 mL) was added. After extraction with diethyl ether (4 × 1 mL), the combined organic extracts were dried (Na_2SO_4), the solvent was removed under reduced pressure and the residue was purified by column chromatography (*n*-hexane/ethyl acetate; 95:5) to give a colorless oil (96 %).

Click reaction using for synthesis of 1,2,3-triazole derivatives of murrayafoline A (**8a**, **8b**, **8d**, **8f**-**h**)

A solution of azide **7** (1 eq, 0.1 g), alkyne (1.2 eq) in *t*-BuOH (2 mL), solution of sodium ascorbate (200 mol%) in water (1 mL) and $CuSO_4{\cdot}5H_2O$ (20 mol%) in water (1 mL) were added into a 10 mL round bottom flask under inert gas. The mixture was stirred overnight at room temperature. After the reaction came to the end (by TLC monitoring), solution of NH_4OH (5 mL) was added and the mixture was extracted with ethyl acetate (2 × 4 mL). The combined organic layers were washed by water (5 mL), dried with Na_2SO_4, and evaporated under reduced pressure. The residue was purified with a silica gel column chromatography (*n*-hexane/ethyl acetate) to give **8a**, **8b**, **8d**, **8f**-**h** (86–94 %).

*1-(3-(1-Methoxy-3-methyl-9H-carbazol-9-yl)propyl)-1H-1,2,3-triazole-4-carboxylic acid (**8a**)*

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (d, J = 7.5 Hz, 1H), 7.70 (s, 1H), 7.49 (s, 1H), 7.41 (m, 2H), 7.25 (d, J = 7.5 Hz, 1H), 7.19 (d, J = 7.0 Hz, 1H), 6.74 (s, 1H) 4.69 (t, J = 7.0 Hz, 2H), 4.37 (t, J = 7.0 Hz, 2H), 3.91 (s, 3H), 2.51 (overlapped, 5H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.3, 140.6, 129.5, 127.6, 125.7, 125.0, 123.5, 123.1, 120.4, 119.0, 112.8, 108.8, 108.6, 55.5, 47.9, 42.3, 30.9, 21.7.

*t-Butyl 1-(3-(1-methoxy-3-methyl-9H-carbazol-9-yl)propyl)-1H-1,2,3-triazol-4-ylcarbamate (**8b**)*

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (d, J = 7.5 Hz, 1H), 7.49 (s, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.32 (br, 1H), 7.26 (d, J = 7.5 Hz, 1H), 7.19 (t, J = 7.5 Hz, 1H), 6.74 (s, 1H), 4.69 (t, J = 7 Hz, 2H), 4.35 (d, J = 6 Hz, 2H), 4.30 (t, J = 7 Hz, 2H), 3.93 (s, 3H), 2.51 (s, 3H), 2.49 (quin., J = 7.0 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.3, 145.3, 140.6, 129.5, 127.6, 125.67, 125.0, 123.0, 121.8, 120.4, 119.0, 112.8, 108.8, 108.6, 55.5, 48.1, 42.3, 36.1, 30.8, 28.4, 21.6. HR-ESI–MS: 484.2143 $[M+Cl]^-$ (calcd. 484.2115 for $C_{25}H_{31}N_5O_3Cl$).

*1-(3-(1-Methoxy-3-methyl-9H-carbazol-9-yl)propyl)-1H-1,2,3-triazol-4-amine (**8c**)*

Solution of **8b** (150 mg, 0.34 mmol) in CH_2Cl_2 (1 mL) and TFA (1 mL) was added into a 10 mL round bottom flask. The mixture was stirred in 2 h at room temperature and evaporated under reduced pressure. EtOAc (3 mL) were added to dissolve the residue and the solution obtained was washed with 0.3 N NaOH solution (2 mL), brine (2 mL) and water (3 mL). The organic layer was dried with Na_2SO_4 and evaporated of EtOAc. The residue was purified with a column of silica gel (*n*-hexane : EtOAc, 4:1) to give **8c** as colorless oil (75 %). ^{1}H NMR (500 MHz, CD_3OD) δ_H 8.00 (d, J = 8.0 Hz, 1H), 7.76 (s, 1H), 7.48 (s, 1H), 7.40 (m, 2H), 7.16 (m, 1H), 6.83 (s, 1H), 4.72 (t, J = 7.0 Hz, 2H), 4.44 (t, J = 7.0 Hz, 2H), 3.98 (s, 2H), 3.95 (s, 3H), 2.50 (s, 3H), 2.46 (quin., J = 7.0 Hz, 2H). ^{13}C NMR (125 MHz, CD_3OD) δ_C 147.8, 142.1, 130.7, 128.9,

126.6, 126.2, 124.3, 124.0, 121.1, 119.9, 113.5, 109.9, 109.8, 56.1, 43.4, 36.8, 31.9, 21.7.

*9-(4((tert-Butyldiphenylsilyloxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)-1-methoxy-3-methyl-9H-carbazole (**8d**)*

8d was prepared by click reaction and directly used to the deprotection without any purification.

*(1-(3-(1-Methoxy-3-methyl-9H-carbazol-9-yl)propyl)-1H-1,2,3-triazol-4-yl)methanol (**8e**)*

The solutions of silylated alcohol **8d** (0.4 mmol) in THF (4 mL), and TBAF 1 M in THF (800 μL) were added into a 10 mL round bottom flask. The reaction mixture was stirred at room temperature for 30 min then saturated NH_4Cl and ethyl acetate were added. The organic layer was separated, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography with dichloromethane/methanol to give **8e** as colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00 (d, $J = 7.5$ Hz, 1H), 7.47 (s, 1H), 7.39 (t, $J = 7.5$ Hz, 1H), 7.31 (s, 1H), 7.23 (d, $J = 7.5$ Hz, 1H), 7.17 (t, $J = 7.5$ Hz, 1H), 6.72 (s, 1H), 4.73 (s, 2H), 4.65 (t, $J = 7.0$ Hz, 2H), 4.28 (t, $J = 7.0$ Hz, 2H), 3.90 (s, 3H), 2.53 (br, 1H), 2.50 (s, 3H), 2.46 (quin., $J = 7.0$ Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 147.6, 146.2, 140.6, 129.5, 127.6, 125.6, 125.0, 123.0, 121.6, 120.4, 119.0, 112.7, 108.9, 108.5, 56.4, 55.5, 48.0, 42.2, 30.7, 21.6; HR-ESI–MS: 385.1071 $[M+Cl]^-$ (calcd. 385.1431 for $C_{20}H_{22}N_4O_2Cl$).

*9-(3-(4-(3,5-Difluorophenyl)-1H-1,2,3-triazol-1-yl)propyl)-1-methoxy-3-methyl-9H-carbazole (**8f**)*

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00 (d, $J = 7.5$ Hz, 1H), 7.47 (s, 1H), 7.42 (s, 1H), 7.40 (t, $J = 7.5$ Hz, 1H), 7.27 (m, 3H), 7.19 (t, $J = 7.5$ Hz, 1H), 6.76 (m, 1H), 6.72 (s, 1H), 4.73 (t, $J = 7.0$ Hz, 2H), 4.33 (t, $J = 7.0$ Hz, 2H), 3.89 (s, 3H), 2.56 (quin., $J = 7.0$ Hz, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 164.4 (d, $J_{C-F} = 12.6$ Hz), 162.4 (d, $J_{C-F} = 13.1$ Hz), 146.3, 140.6, 129.6, 127.6, 125.7, 125.0, 123.1, 120.5, 119.1, 112.8, 108.9, 108.6, 108.5, 108.4, 108.3, 103.5, 103.3, 103.1, 55.5, 48.2, 42.2, 30.6, 21.6. MS: 432.1762. HR-ESI–MS: 467.1472 $[M+Cl]^-$ (calcd. 467.1450 for $C_{25}H_{22}F_2N_4OCl$).

*1-Methoxy-3-methyl-9-(3-(4-(3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-1-yl)propyl)-9H-carbazole (**8g**)*

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.00 (d, $J = 8.0$ Hz, 1H), 7.98 (s, 1H), 7.95 (d, $J = 7.5$ Hz, 1H), 7.57 (d, $J = 7.5$ Hz, 1H), 7.50 (m, 2H), 7.46 (s, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 7.28 (s, 1H), 7.25 (d, $J = 8.0$ Hz, 1H), 7.19 (t, $J = 8.0$ Hz, 1H), 6.72 (s, 1H), 4.73 (t, $J = 7.0$ Hz, 2H), 4.34 (t, $J = 7.0$ Hz, 2H), 3.89 (s, 3H), 2.56 (quin., $J = 7.0$ Hz, 2H), 2.49 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 146.3, 140.6, 131.5, 131.4, 131.2, 129.6, 129.3, 128.7, 127.6, 125.7, 125.1, 125.0, 124.7, 124.6, 123.1, 123.0, 122.4, 122.4, 120.5, 119.1, 112.8, 108.9, 108.6, 55.5, 48.2, 42.2, 30.7, 21.6. HR-ESI–MS: 499.1535 $[M+Cl]^-$ (calcd. 499.1512 for $C_{26}H_{23}F_3N_4OCl$).

*9-(3-(4-((4-(2,5-Difluorobenzyl)piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)propyl)-1-methoxy-3-methyl-9H-carbazole (**8h**)*

^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.01 (d, $J = 7.5$ Hz, 1H), 7.49 (s, 1H), 7.40 (t, $J = 7.5$ Hz, 1H), 7.33 (s, 1H), 7.25 (d, $J = 7.5$ Hz, 1H), 7.19 (t, $J = 7.5$ Hz, 1H), 7.09 (m, 1H), 6.95 (ddd, $J = 9.0, 9.0, 4.5$ Hz, 1H), 6.89 (m, 1H), 6.74 (s, 1H), 4.70 (t, $J = 7.0$ Hz, 2H), 4.32 (t, $J = 7.0$ Hz, 2H), 3.94 (s, 3H), 3.67 (s, 2H), 3.54 (s, 2H), 2.49 (s, 3H), 2.48 (m, overlapped, 10H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 158.5 (d, $J_{C-F} = 12.6$ Hz), 156.5 (d, $J_{C-F} = 13.0$ Hz), 146.3, 140.6, 132.6, 129.5, 127.7, 126.8, 125.7, 125.0, 123.1, 122.7, 120.4, 119.0, 117.5, 116.3, 115.0, 112.8, 108.9, 108.6, 55.6, 54.8, 53.2, 52.8, 52.8, 52.8, 52.8, 52.6, 48.0, 42.3, 30.9, 21.6.

Synthesis of 3-(1-methoxy-3-methyl-9*H*-carbazol-9-yl)propane-1,2-diol (**9**)

A solution of alkene **6** (45 mg, 0.18 mmol) in a THF/H_2O mixture (10/1, 1 mL), NMP (63 mg, 3 eq) and 4 % aqueous solution of OsO_4 (23 μL, 0.36 mmol) were added into a 10 mL round bottom flask. The mixture was stirred for 2 h at 30 °C. 20 % solution of $NaHSO_3$ was added and the solution was extracted with EtOAc, and washed with brine. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified with a silica gel column chromatography (CH_2Cl_2/MeOH, 9:1) to give **9** as white solid (36 mg, 70 %). ^{1}H NMR (500 MHz, CD_3OD) δ_H 7.98 (d, $J = 8.0$ Hz, 1H), 7.54 (d, $J = 8.0$ Hz, 1H), 7.48 (s, 1H), 7.40 (dd, $J = 7.0$, 1.0 Hz, 1H), 7.15 (dd, $J = 7.0$, 0.5 Hz), 6.84 (s, 1H), 4.72 (dd, $J = 6.0$, 14.5 Hz, 1H), 4.53 (dd, $J = 7.0$, 14.5 Hz, 1H), 4.15 (m, 1H), 4.00 (s, 3H), 3.54 (m, 2H), 2.50 (s, 3H). ^{13}C NMR (125 MHz, CD_3OD) δ_C 147.9, 142.9, 130.4, 126.4, 126.3, 124.1, 120.8, 119.7, 113.5, 110.6, 109.9, 73.7, 65.4, 56.1, 49.3, 21.7. HR-ESI–MS 320.0710 $[M+Cl]^-$ (calcd. $C_{17}H_{19}NO_3Cl$ for 320.1053).

Cell cultures and measurement of cytokine production

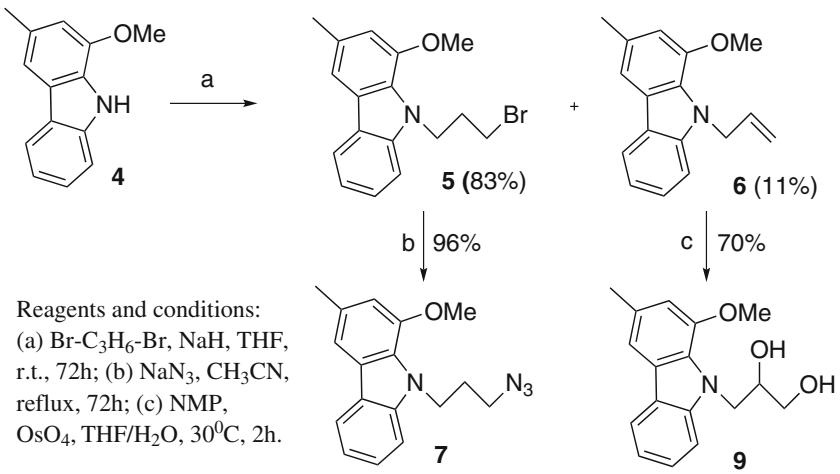
Bone marrow-derived dendritic cells (BMDCs) were grown from wild-type C57BL/6 mice (Taconic Farm, NY,

USA). Briefly, bone marrow from tibia and femur was obtained by flushing with DMEM, and bone marrow cells were cultured in RPMI 1640 medium containing 10 % heat-inactivated fetal bovine serum (FBS) (Gibco, NY, USA), 50 μM of 2-ME, and 2 mM of glutamine supplemented with 3 % J558L hybridoma cell culture supernatant containing granulocyte–macrophage colony-stimulating factor (GM-CSF). The culture medium containing GM-CSF was replaced every other day. At day 6 of culture, nonadherent cells and loosely adherent DC aggregates were harvested, washed, and resuspended in RPMI 1640 supplemented with 5 % FBS. DCs were incubated in 48-well plates at a density of 2×10^5 cells/mL, and then treated with the isolated compounds at the concentration of 0–50 μM for 1 h before stimulation with 10 ng/mL of LPS from *Salmonella minnesota* (Alexis, NY, USA). Supernatants were harvested 16 h after stimulation. Concentrations of murine IL-12 p40, IL-6, and TNF-a in the culture supernatants were determined by ELISA (BD PharMingen, CA, USA) according to the manufacture's instructions. All experiments were performed at least three times. The data were presented as mean ± standard deviation (SD) of three independent experiments in triplicate.

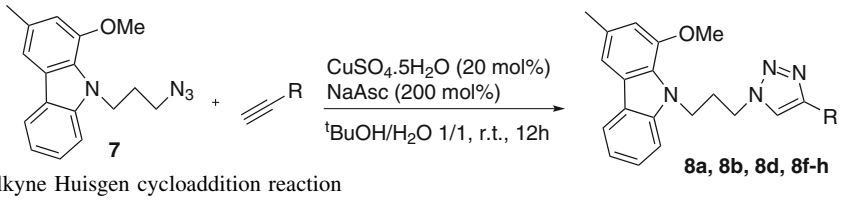
Results and discussion

The key step to introduce the 1,2,3-triazolyl groups into the murrayafoline A molecule was Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction (click reaction) (Rostovtsev et al. 2002) between azide derivatives and terminal alkynes. The 1,2,3-triazole-based murrayafoline A derivatives were synthesized as in the synthetic route showed in Scheme 1. The starting material, murrayafoline A, was isolated and purified from the roots of *Glycosmis stenocarpa* (see "Experimental" section). Murrayafoline A bromide **5** was prepared according to the method described by Zhang (Zhang et al. 2010). Beside the main bromide compound **5** (83 %), the side-product **6** (11 %) was also obtained due to the elimination reaction in small proportion. Dihydroxylation (Marsac et al. 2005) of *N*-allyl-murrayafoline A (**6**) by OsO_4/NMP in the mixture of THF/H_2O gave the dihydroxyl compound **9** with isolated yield of 70 %. The azide **7** was obtained with good yield (96 %) by treatment of **5** with NaN_3 in acetonitrile under reflux conditions (Scheme 1). The cycloaddition reactions between azide **7** and various terminal alkynes (Scheme 2) under standard conditions (cat. $CuSO_4 \cdot 5H_2O$, sodium ascorbate, mixture *t*-BuOH/H_2O: 1/1, inert gas) proceeded in good yield (86–94 %) to afford the 1,2,3-triazole derivatives of murrayafoline A (**8a**, **8b**, **8d**, **8f-h**) (Table 1). In case of propargyl amine (in **8c**) and propargyl alcohol (in **8e**), protections of amino and alcohol group were required before this cycloaddition reaction. Protecting groups (Boc in **8b** and TBDPS in **8d**) were removed by using TFA and TBAF respectively after click reaction. The structures of these new compounds were confirmed by HR-MS, ^1H and ^{13}C NMR spectra (See "Experimental" section). To evaluate these synthesized compounds for anti-inflammatory activity, all of products, intermediate compounds, and initial material were tested for their effects in the inflammatory response on BMDCs. Particularly, each compound was examined for their effects to the interleukin-6 (IL-6), IL-12 p40, and tumor necrosis factor-α (TNF-α) production in BMDCs since they were well known as one of the important chemical mediators of inflammation (Kim et al. 2010). Briefly, TNF-α, a cytokine involved in systemic inflammation, plays a central role in the inflammatory response and implicates in the pathogenesis of both acute and chronic inflammatory diseases (Beutler and Cerami 1986). IL-6 has both pro- and anti-inflammatory effects. It was a key player in chronic inflammation, and IL-6 production levels are important factors in inflammatory diseases in humans (Cem 2006). IL-12, a pro-inflammatory cytokine, stimulates the production of interferon-gamma (IFN-γ), TNF-α. It is rapidly produced by

Scheme 1 Synthesis of derivatives **5–9**

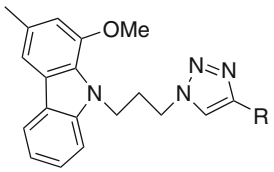


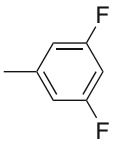
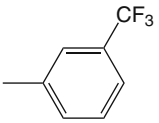
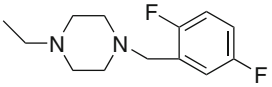
Reagents and conditions: (a) Br-C_3H_6-Br, NaH, THF, r.t., 72h; (b) NaN_3, CH_3CN, reflux, 72h; (c) NMP, OsO_4, THF/H_2O, 30^0C, 2h.



Scheme 2 Click azide–alkyne Huisgen cycloaddition reaction

Table 1 Structure of 1,2,3 triazole *N*-substituted murrayafoline A derivatives and their reaction yields



No.	R	Product	Yield (%)
1	–COOH	**8a**	86
2	–CH_2–NHBoc	**8b**	93
3	–CH_2–NH_2	**8c**	75
4	–CH_2–OTBDPS	**8d**	94
5	–CH_2–OH	**8e**	61
6		**8f**	94
7		**8g**	88
8		**8h**	91

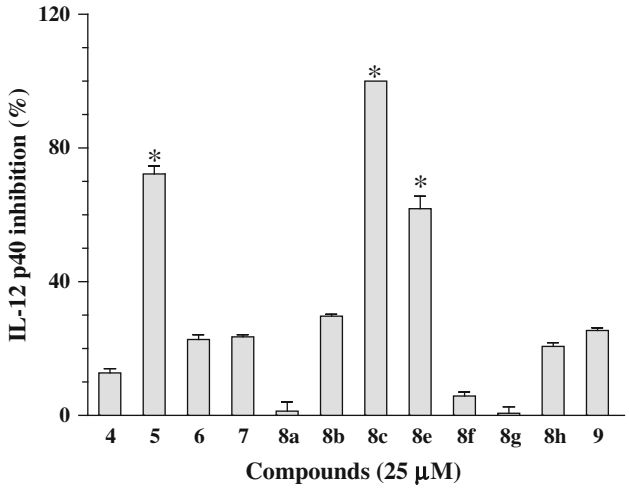


Fig. 2 Initial single point concentration screening and selection for candidate compounds in synthetic compounds series. BMDCs were pre-treated with indicated compounds for 1 h, and then stimulated with LPS (10 ng/ml) for 16 h. The cytokine IL-12 p40 was measured by ELISA. The compounds were diluted in DMSO. The candidate compounds **5**, **8c**, and **8e** are indicated with *asterisk* (*)

activated inflammatory cell and plays an important role in the activities of cellular immunity. It also involved in the differentiation of naive T cell into type-1 helper T cell and hence leads to generation of inflammatory disease (Trinchieri et al. 2003). As our study, we first used a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, MO, USA) to confirm that these compounds have no or little effect on the cell viability (data not shown). Next, the synthetic compounds were tested their effects on the production TNF-α, IL-6, and IL-12 p40 in BMDCs induced by LPS stimulator. In brief, BMDCs were incubated in 48-well plates at a density of 2×10^5 cells/mL. After that, they were treated for 1 h with the compounds at the various concentrations, and then stimulated with LPS (10 ng/mL). Supernatants were harvested 16 h after stimulation. The IL-12 p40, IL-6 and TNF-α production were determined by ELISA (BD PharMigen, CA, USA) according to the manufacture's instructions. SB203580, an inhibitor of cytokine suppressive binding protein/p38 kinase, was used as a positive control. The preliminary results on the inhibitory effects on cytokine IL-12 p40 production showed that at a concentration of 25 μM, compounds **5**, **8c**, and **8e** highly inhibited the production of cytokine IL-12 p40 in DCs induced by LPS stimulator with the inhibitory values 72.3, 100 and 61.8 %, respectively. Other compounds weakly demonstrated inhibitory effects (inhibitory values lower than 30 %) (Fig. 2). Therefore, compounds **5**, **8c**, **8e** were then subjected for further studies. As showed in Fig. 3, compounds **8c**, **8e** exhibited strong inhibitory effects in all IL-12 p40, IL-6 and TNF-α production meanwhile compound **5** did not significantly inhibited the production of TNF-α. All of them significantly inhibited higher than positive control with the exception of TNF-α inhibitory activity of **5**. Especially, at concentration of 12.5 μM, compound **8c** potentially inhibited the production of IL-12 p40, IL-6 and TNF-α with inhibitory values of 100, 88.7 and 79.3 %, respectively. It also should be noted that, with the exception of compounds **8a**, **8f**, and **8g**, all of synthetic compounds showed higher inhibitory activity in compared with initial material (**4**)

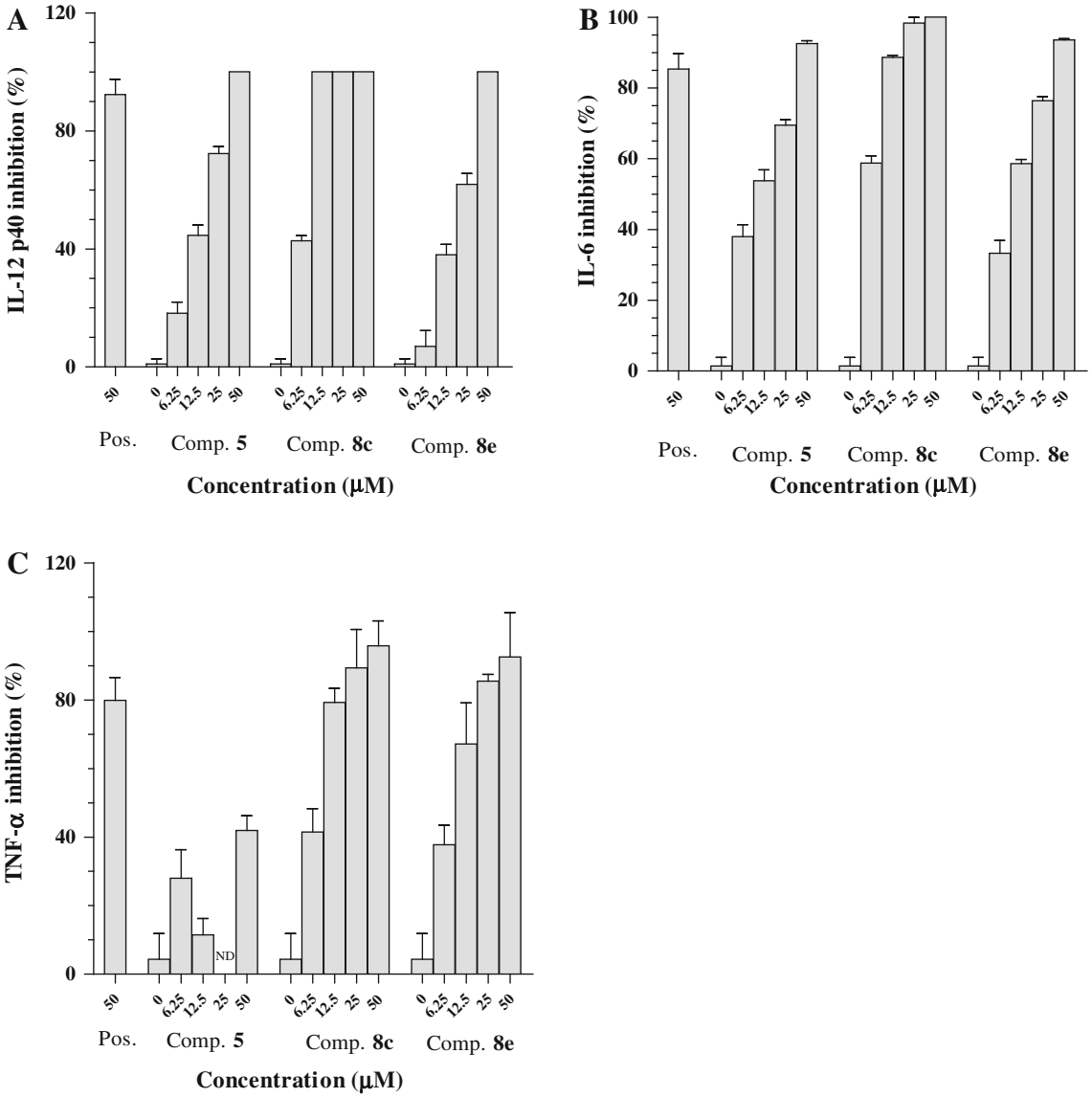


Fig. 3 Effect of compounds **5**, **8c**, and **8e** on IL-12 p40 (**a**), IL-6 (**b**), and TNF-α (**c**) production by LPS-stimulated BMDCs. DCs were treated with the compounds (0, 6.25, 12.5, 25, and 50 μM) for 1 h before stimulation with LPS (10 ng/mL). Supernatants were harvested 16 h after stimulation. Concentrations of murine IL-12 p40, IL-6, and TNF-α in the culture supernatants were determined by ELISA. The data were presented as inhibition rate (%) compared to the value of vehicle-treated DCs. SB203580 was used as positive control (pos.), *ND* not detected

(Fig. 2). This results warranted that carbazole alkaloids, particularly murrayafoline A derivatives, containing 1,2,3-triazole nucleus may be potent anti-inflammatory action and further studies should be needed to elucidate the mechanism of anti-inflammatory action of those compounds.

Acknowledgments This study was supported by National Foundation for Science & Technology Development (NAFOSTED of Vietnam, project 104.01.91.09), Vietnam Ministry of Science and Technology under International Project (52/2011/HD-NDT) and by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0093815), Republic of Korea.

References

Almasirad, A., S.A. Tabatabai, M. Faizi, A. Kebriaeezadeh, N. Mehrabi, A. Dalvandi, and A. Shafiee. 2004. Synthesis and anticonvulsant activity of new 2-substituted-5-[2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. *Bioorganic & Medicinal Chemistry Letters* 14: 6057–6059.

Beutler, B., and A. Cerami. 1986. Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature* 320: 584–588.

Buckler, R.T., H.E. Hartzler, E. Kurchacova, G. Nichols, and B.M. Phillips. 1978. Synthesis and antiinflammatory activity of some 1,2,3- and 1,2,4-triazolepropionic acids. *Journal of Medicinal Chemistry* 21: 1254–1260.

Burman, S.R., N. Sen, and J.J. Ghosh. 1964. Hepatic monoamine oxidase activity in rats fed a high level of tyrosine under toxic conditions. *Science and Culture* 30: 445–446.

Cem, G. 2006. Interleukin-6 and chronic inflammation. *Arthritis Research & Therapy* 8: S3.

Choi, H., J. Gwak, M. Cho, M.J. Ryu, J.H. Lee, Y.H. Kim, G.W. Lee, M.Y. Yun, N.M. Cuong, J.G. Shin, G.Y. Song, and S. Oh. 2010. Murrayafoline A attenuates the Wnt/β-catenin pathway by promoting the degradation of intracellular β-catenin proteins. *Biochemical and Biophysical Research Communications* 391: 915–920.

Cui, C.B., S.Y. Yan, B. Cai, and X.S. Yao. 2002. Carbazole alkaloids as new cell cycle inhibitor and apoptosis inducers from *Clausena dunniana* Levl. *Journal of Asian Natural Products Research* 4: 233–241.

Cuong, N.M., H. Wilhelm, A. Porzel, N. Arnold, and L. Wessjohann. 2008. 1-O-substituted derivatives of murrayafoline A and their antifungal properties. *Natural Product Research* 22: 1428–1432.

De Clercq, E. 2002. New anti-HIV agents and targets. *Medicinal Research Reviews* 22: 531–565.

Dong, H.S., K. Wei, Q.L. Wang, B. Quan, and Z.Y. Zhang. 2000. Synthesis of 3-[5-methyl-1-(4-methylphenyl)-1,2,3-triazol-4-yl]-6-substituted-*S*-triazolo[3,4-b]-1,3,4-thiadiazoles. *Journal of the Chinese Chemical Society* 47: 343–346.

Gilani, S.J., S.A. Khan, O. Alam, and H. Kumar. 2008. Synthesis and biological evaluation of some substituted-1,2,3-triazole derivatives. *Indian Journal of Heterocyclic Chemistry* 17: 245–248.

Ito, C., S. Katsuno, M. Itoigawa, N. Ruangrungsi, T. Mukainaka, M. Okuda, Y. Kitagawa, H. Tokuda, H. Nishino, and H. Furukawa. 2000. New carbazole alkaloids from *Clausena anisata* with antitumor promoting activity. *Journal of Natural Products* 63: 125–128.

Itoigawa, M., Y. Kashiwada, C. Ito, H. Furukawa, Y. Tachibana, K.F. Bastow, and K.H. Lee. 2000. Antitumor agents. 203. Carbazole alkaloid murrayaquinone A and related synthetic carbazolequinones as cytotoxic agents. *Journal of Natural Products* 63: 893–897.

Kamotra, P., A.K. Gupta, R. Gupta, P. Somal, and S. Singh. 2007. Microwave-assisted synthesis and biological activity of 3-alkyl/aryl-6-(1-chloro-3,4-dihydronaphth-2-yl)-5,6-dihydro-*S*-triazolo[3,4-b][1,3,4]thiadiazoles. *Indian Journal of Chemistry B Organic and Medicinal Chemistry* 46B: 980–984.

Kim, J.A., S.Y. Yang, J.E. Koo, Y.S. Koh, and Y.H. Kim. 2010. Lupane-type triterpenoids from the steamed leaves of *Acanthopanax koreanum* and their inhibitory effects on the LPS-stimulated pro-inflammatory cytokine production in bone marrow-derived dendritic cells. *Bioorganic & Medicinal Chemistry Letters* 20: 6703–6707.

Knoelker, H.J., and K.R. Reddy. 2002. Isolation and synthesis of biologically active carbazole alkaloids. *Chemical Reviews* 102: 4303–4427.

Lebouvier, N., F. Pagniez, M. Duflos, P. Le Pape, Y.M. Na, G. Le Baut, and M. Le Borgne. 2007. Synthesis and antifungal activities of new fluconazole analogues with azaheterocycle moiety. *Bioorganic & Medicinal Chemistry Letters* 17: 3686–3689.

Liu, P., S. Zhu, P. Li, W. Xie, Y. Jin, Q. Sun, Q. Wu, P. Sun, Y. Zhang, X. Yang, and D. Zhang. 2008. Synthesis and SAR studies of biaryloxy-substituted triazoles as antifungal agents. *Bioorganic & Medicinal Chemistry Letters* 18: 3261–3265.

Marsac, Y., A. Nourry, S. Legoupy, M. Pipelier, D. Dubreuil, A.M. Aubertin, N. Bourgougnon, R. Benhida, and F. Huet. 2005. Synthesis of mono- and polyhydroxylated cyclobutane nucleoside analogs. *Tetrahedron* 61: 7607–7612.

Prakash, O., V. Bhardwaj, R. Kumar, P. Tyagi, and K.R. Aneja. 2004. Organoiodine(III)-mediated synthesis of 3-aryl/heteroaryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines as antibacterial agents. *European Journal of Medicinal Chemistry* 39: 1073–1077.

Rostovtsev, V.V., L.G. Green, V.V. Fokin, and K.B. Sharpless. 2002. A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. *Angewandte Chemie International Edition* 41: 2596–2599.

Shafi, S., M.M. Alam, N. Mulakayala, C. Mulakayala, G. Vanaja, A.M. Kalle, R. Pallu, and M.S. Alam. 2012. Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: their anti-inflammatory and anti-nociceptive activities. *European Journal of Medicinal Chemistry* 49: 324–333.

Sztanke, K., K. Pasternak, A. Sidor-Wojtowicz, J. Truchlinska, and K. Jozwiak. 2006. Synthesis of imidazoline and imidazo[2,1-*c*][1,2,4]triazole aryl derivatives containing the methylthio group as possible antibacterial agents. *Bioorganic & Medicinal Chemistry* 14: 3635–3642.

Sztanke, K., T. Tuzimski, J. Rzymowska, K. Pasternak, and M. Kandefer-Szerszen. 2008. Synthesis, determination of the lipophilicity, anticancer and antimicrobial properties of some fused 1,2,4-triazole derivatives. *European Journal of Medicinal Chemistry* 43: 404–419.

Trinchieri, G., S. Pflanz, and R.A. Kastelein. 2003. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. *Immunity* 19: 641–644.

Zhang, F.F., L.L. Gan, and C.H. Zhou. 2010. Synthesis, antibacterial and antifungal activities of some carbazole derivatives. *Bioorganic & Medicinal Chemistry Letters* 20: 1881–1884.