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Short communication

Inhibitory effects of L-arginine derivatives on endothelium-dependent vasorelaxing response to acetylcholine of the rat aorta

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Abstract

N^{ω}-nitro-L-arginine alkyl esters (**A-1–A-B**) and L-arginine alkyl esters (**E-1–E-B**) were synthesized, and the vasorelaxing effects of acetylcholine were studied in the absence or presence of these compounds in rat aortic rings with intact endothelium that was precontracted with phenylephrine. These compounds revealed that the nitro group is an essential inhibiting group of these inhibitors, and that hydrophobic functional groups can fine-tune the binding effects. Among them, **A-3** is the most potent inhibitor.

Keywords: Nitric oxide synthase; N^{ω}-nitro-L-arginine alkyl esters

1. Introduction

Nitric oxide (NO) with odd numbers of valence electrons is a reactive, gaseous molecule that has various roles in many physiological processes [1]. NO at high concentration plays a role as a defensive cytotoxin against tumor cells and pathogens, and at low concentration as a signal in many diverse physiological processes, such as blood flow regulation, neurotransmission, learning, and memory [3,7–10]. The biochemical production of NO results from the oxidation of L-arginine by nitric oxide synthases (NOSs) to form N^{G}-hydroxy-L-arginine (NHA) at the heme active site, then NHA is further oxidized at the heme site to NO and L-citrulline [2,3]. The three mammalian NOS isoforms are neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS) have a similar modular architecture [4,5]. All three isoforms share a sequence identity of up to 50% [6], including three-component construction [3,5,7–11]: (i) an NH_2-terminal catalytic oxygenase domain that binds heme, tetrahydrobiopterin (H_4B) and L-arginine; (ii) a COOH-terminal reductase domain that binds flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and reduced nicotine adenine dinucleotide phosphate (NADPH), and (iii) an intervening calmodulin-binding region that regulates electronic communication between NOS_{ox} and NOS_{red} [5,12].

The heme environment of NOS [10] exhibited an arc of conserved hydrophobic residue curves near the heme coordination site. Although the immediate distal heme pocket in NOS is largely hydrophobic, devoid of hydrogen-donating groups, polar residues at the pocket edge may affect L-arginine or H_4B-binding and dimerization. The closest hydrophilic residue to the heme iron, glutamate, forms a hydrogen bond with the second imidazole and is necessary for L-arginine-binding, localizing the substrate-binding site above heme pyrrole rings A and B.

The crystal structure of the eNOS heme domain complexed with NO reveals close hydrogen bonding interactions between NO and the terminal guanido nitrogen of the substrate, L-arginine [6]. The structure of the NO complex can mimic the oxy complex because the extremely short lifetime of NOS–O_2 adduct precludes it from solving the oxy complex structure [13]. Dioxygen is expected to bind in a similar mode, which facilitates proton abstraction from L-arginine to dioxygen, a step required for cleaving O–O bonds [6].

The NO released by the vascular endothelium sustains the vasodilator tone and suppresses the functioning of platelets, the adhesion of leukocytes and the proliferation of smooth muscle cells [14–17]. The hypertension, hyperchloesterolemia and atherosclerosis share an endothelial dysfunction that involves the abnormal synthesis of NO [18–20]. The N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced blockade of NO synthesis provides a model of specific vascular

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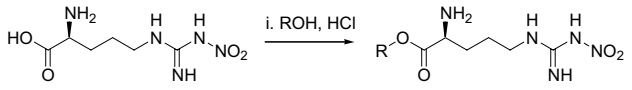
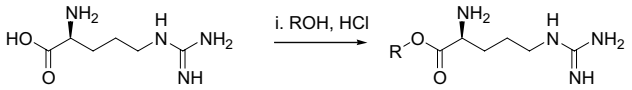
dysfunction responsible for systemic arterial hypertension and structural alterations of the heart and the arterial walls [21–23]. This complex model of hypertension would also be suitable for testing the vascular protective effect of drugs in improving the function of the endothelial vasodilator [24]. Based on these premises, to yield further insight into the endothelial dysfunction caused by the inhibition of NOS, the effects of N^{ω}-nitro-L-arginine alkyl esters (**A-1–A-B**) and L-arginine alkyl esters (**E-1–E-B**) on the relaxant activity of acetylcholine is examined. Acetylcholine is an endothelial NO-mediated vasodilator in rat thoracic aorta. The effects of N^{ω}-nitro-L-arginine (L-NNA; an NOS inhibitor) and aminoguanidine (AG; a selective iNOS inhibitor) on acetylcholine-evoked vasorelaxation in phenylephrine-precontracted endothelium-intact aortic rings were used as positive and negative controls, respectively. The procedures for measuring tension have been described in detail elsewhere [26].



A-1: R = CH_3, (99%)
A-2: R = CH_2CH_3, (99%)
A-3: R = $(CH_2)_2CH_3$, (99%)
A-4: R = $(CH_2)_3CH_3$, (99%)
A-5: R = $(CH_2)_4CH_3$, (94%)
A-6: R = $(CH_2)_5CH_3$, (99%)
A-7: R = $(CH_2)_6CH_3$, (90%)
A-8: R = $(CH_2)_7CH_3$, (80%)
A-9: R = $(CH_2)_8CH_3$, (67%)
A-10: R = $(CH_2)_9CH_3$, (35%)
A-B: R = $CH_2C_6H_5$, (94%)

Fig. 1. Synthesis of N^{ω}-nitro-L-arginine alkyl ester and L-arginine alkyl ester. (i) Alkanol, HCl(g). R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}, $C_{10}H_{21}$, $C_6H_5CH_2$, **A-1–A-B**.



E-1: R = CH_3, (71%)
E-2: R = CH_2CH_3, (72%)
E-3: R = $(CH_2)_2CH_3$, (72%)
E-4: R = $(CH_2)_3CH_3$, (68%)
E-5: R = $(CH_2)_4CH_3$, (71%)
E-6: R = $(CH_2)_5CH_3$, (68%)
E-7: R = $(CH_2)_6CH_3$, (37%)
E-8: R = $(CH_2)_7CH_3$, (35%)
E-9: R = $(CH_2)_8CH_3$, (37%)
E-10: R = $(CH_2)_9CH_3$, (38%)
E-B: R = $CH_2C_6H_5$, (58%)

Fig. 2. Synthesis of L-arginine alkyl ester. (i) Alkanol, HCl(g). R = CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, C_7H_{15}, C_8H_{17}, C_9H_{19}, $C_{10}H_{21}$, $C_6H_5CH_2$, **E-1–E-B**.

2. Chemistry

The hydrophobic residue curves around the heme coordination site [11]. N^{ω}-nitro-L-arginine alkyl esters (**A-1–A-B**) and 11 L-arginine alkyl esters (**E-1–E-B**) were synthesized as inhibitors [25] (Fig. 1 and Fig. 2) to optimize the van der Waals' interaction between inhibitor and NOS.

3. Results and discussion

In this vasorelaxing bioassay, each test compound given individually did not alter the baseline tension of the aortic rings (Tables 1 and 2). Figs. 3 and 4 show the vasorelaxing effects of acetylcholine (10 nM–10 μM) in the absence or presence of **A-1–A-B** in aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM). When the concentration of acetylcholine reached to 1.0 μM a marked relaxation occurred (96.09% ± 1.05%). The maximal vasorelaxation of the negative control AG was 98.08% ± 1.92%. In the presence of **A-1–A-B** (100 μM) for 20 min, the vasorelaxation-induced by acetylcholine was markedly inhibited, whereas the positive control L-NNA (100 μM) produced a significant inhibition of 89.98% ± 6.57% at acetylcholine (10 μM). Among these test compounds, the largest

Table 1
Vasorelaxing effects of acetylcholine in the presence of **A-1–A-10** and **A-B** in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM). The concentrations of acetylcholine are 0.01, 0.05, 0.10, 0.50, 1.0, 5.0 and 10 μM. (or 0.01–10 μM)

Vasorelaxing effects of acetylcholine in the presence of **A-1–A-10** and **A-B**							
Compound	Acetylcholine concentration (μM)						
	0.01	0.05	0.10	0.50	1.00	5.00	10.00
Control	23.08 ± 2.00	56.07 ± 0.57	79.56 ± 2.12	94.16 ± 0.89	96.09 ± 1.05	96.00 ± 1.33	97.46 ± 0.81
AG	9.58 ± 0.98	51.40 ± 6.49	77.78 ± 9.72	92.65 ± 3.83	98.08 ± 1.92	98.08 ± 1.92	98.08 ± 1.92
L-NNA	0.99 ± 2.06	–1.69 ± 2.35	–2.11 ± 2.86	1.03 ± 6.43	3.82 ± 6.50	8.35 ± 6.38	10.02 ± 6.57
A-1	–1.39 ± 1.13	–2.92 ± 2.21	–1.84 ± 4.13	–0.94 ± 4.23	1.91 ± 6.05	1.86 ± 7.01	8.57 ± 11.44
A-2	–4.76 ± 2.56	–7.24 ± 2.4	–5.93 ± 2.89	–5.26 ± 5.12	–2.85 ± 6.35	–0.67 ± 8.82	2.31 ± 9.22
A-3	–3.90 ± 1.64	–5.51 ± 1.47	–5.99 ± 1.84	–5.42 ± 2.62	–6.08 ± 2.01	–4.55 ± 2.22	0.10 ± 2.91
A-4	–2.04 ± 1.07	–2.96 ± 1.02	–1.42 ± 3.10	–0.06 ± 4.61	3.07 ± 7.83	6.31 ± 10.59	10.98 ± 11.94
A-5	–1.70 ± 1.76	–1.07 ± 2.13	–5.16 ± 3.99	–4.92 ± 2.04	–4.59 ± 3.95	–2.68 ± 3.79	1.38 ± 4.66
A-6	–0.95 ± 1.38	0.45 ± 3.02	–0.07 ± 2.25	0.91 ± 3.56	0.14 ± 3.27	3.80 ± 4.47	7.13 ± 5.23
A-7	–2.35 ± 0.56	–3.40 ± 1.57	–3.38 ± 2.20	–4.04 ± 3.14	–1.77 ± 3.92	0.39 ± 4.94	5.64 ± 5.41
A-8	–2.54 ± 1.11	–4.12 ± 2.06	–5.11 ± 3.64	–3.64 ± 5.16	–1.97 ± 6.10	–0.38 ± 7.00	6.81 ± 7.05
A-9	–1.28 ± 1.03	–1.56 ± 3.04	–3.34 ± 6.91	–1.72 ± 8.63	2.35 ± 10.28	3.91 ± 10.84	11.25 ± 9.09
A-10	–0.53 ± 3.94	0.59 ± 6.17	1.54 ± 7.79	5.49 ± 9.41	12.53 ± 11.36	18.23 ± 11.45	29.76 ± 10.24
A-B	–0.57 ± 0.67	–3.29 ± 2.47	–2.60 ± 3.84	–0.24 ± 5.36	2.59 ± 7.09	5.57 ± 8.22	12.34 ± 7.07

Table 2
Vasorelaxing effects of acetylcholine in the presence of **E-1–E-10** and **E-B** in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM). The concentrations of acetylcholine are 0.01, 0.05, 0.10, 0.50, 1.0, 5.0 and 10 μM. (or 0.01–10 μM)

Vasorelaxing effects of acetylcholine in the presence of **E-1–E-10** and **E-B**							
Compound	Acetylcholine concentration (μM)						
	0.01	0.05	0.10	0.50	1.00	5.00	10.00
Control	23.08 ± 2.00	56.07 ± 2.57	79.56 ± 2.12	94.16 ± 0.89	96.09 ± 1.05	96.00 ± 1.33	97.46 ± 0.81
AG	9.58 ± 0.98	51.40 ± 6.49	77.78 ± 9.72	92.65 ± 3.83	98.08 ± 1.92	98.08 ± 1.92	98.08 ± 1.92
L-NNA	0.99 ± 2.06	–1.69 ± 2.35	–2.11 ± 2.86	1.03 ± 6.43	3.82 ± 6.50	8.35 ± 6.38	10.02 ± 6.57
E-1	19.09 ± 3.18	61.29 ± 7.05	80.34 ± 6.50	93.65 ± 3.42	96.24 ± 2.19	97.65 ± 1.62	95.72 ± 2.57
E-2	29.95 ± 7.71	53.61 ± 12.71	74.39 ± 13.08	87.25 ± 7.16	92.07 ± 3.47	92.65 ± 4.03	96.00 ± 3.22
E-3	17.61 ± 5.19	46.40 ± 7.13	68.84 ± 4.94	88.95 ± 3.88	90.32 ± 4.84	94.74 ± 0.71	96.13 ± 1.97
E-4	37.64 ± 12.57	67.27 ± 7.40	81.97 ± 6.81	89.40 ± 3.55	97.41 ± 1.63	97.09 ± 2.35	97.02 ± 2.98
E-5	13.74 ± 6.60	49.08 ± 3.71	68.05 ± 3.03	76.72 ± 6.96	85.37 ± 3.71	86.65 ± 5.96	86.81 ± 4.59
E-6	9.58 ± 4.77	33.08 ± 9.52	52.03 ± 9.00	80.20 ± 4.40	93.98 ± 3.30	95.97 ± 2.06	97.18 ± 1.75
E-7	22.15 ± 6.40	55.22 ± 6.12	79.01 ± 3.05	90.86 ± 3.39	97.30 ± 1.56	97.25 ± 2.25	96.94 ± 3.07
E-8	10.97 ± 4.97	34.31 ± 9.64	52.86 ± 11.45	71.48 ± 8.78	82.56 ± 8.61	79.57 ± 8.58	83.69 ± 9.56
E-9	11.12 ± 2.94	27.60 ± 5.79	40.39 ± 8.42	68.98 ± 7.14	74.86 ± 5.08	78.60 ± 6.38	83.17 ± 5.98
E-10	8.90 ± 4.32	50.93 ± 8.49	66.19 ± 8.46	79.29 ± 9.28	78.87 ± 10.34	80.43 ± 16.20	80.11 ± 16.28
E-B	15.50 ± 4.19	41.71 ± 11.02	72.40 ± 10.39	91.67 ± 3.28	94.46 ± 2.62	95.66 ± 1.69	95.85 ± 2.53

inhibition evoked by **A-3** was observed with a maximal relaxation of 0.10% ± 2.91%. In the presence of **A-10**, the inhibition of acetylcholine-induced relaxation exhibited a minimum value of 70.24% ± 10.24%, perhaps due to this compound's cytotoxicity (data not shown). Compounds **A-2, A-3, A-5**, and **A-7** in the inhibition of acetylcholine-induced relaxation exhibited better than that of **A-1** (L-NAME) as shown in Figs. 3 and 4. These results have never been reported. The alkyl group of N^{ω}-nitro-L-arginine monoalkyl esters with odd number of methylene group have stronger in the inhibition of acetylcholine-induced relaxation than those of with even number of methylene group. This may be demonstrated by the more symmetry of alkyl group with even number of methylene group. The size of alkyl group with three, five and seven methylene group exhibited better in the inhibition of acetylcholine-induced relaxation. The inhibition of vasorelaxation ranged from 2.98% to 19.89% caused by **E-1–E-B**, as shown in Figs. 5 and 6. Compounds **A** and **E** differed, only difference by a nitro group, which may replace the position of NO or O_2 in the heme domain of eNOS in the presence of L-arginine. When compound **A** was used as a NOS inhibitor, it bonded with the NOS's heme

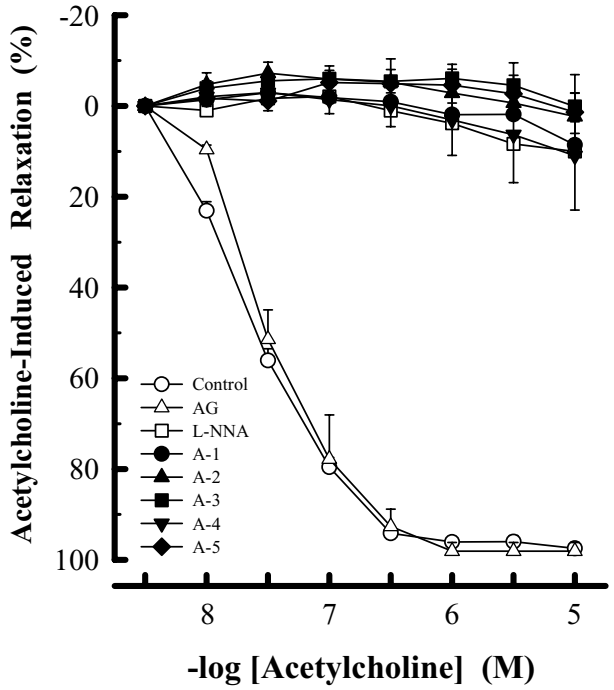


Fig. 3. Vasorelaxing effects of acetylcholine (10 nM–10 μM) in the absence or presence of **A-1–A-5** (100 μM) for 20 min in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM) (n = 5–6).

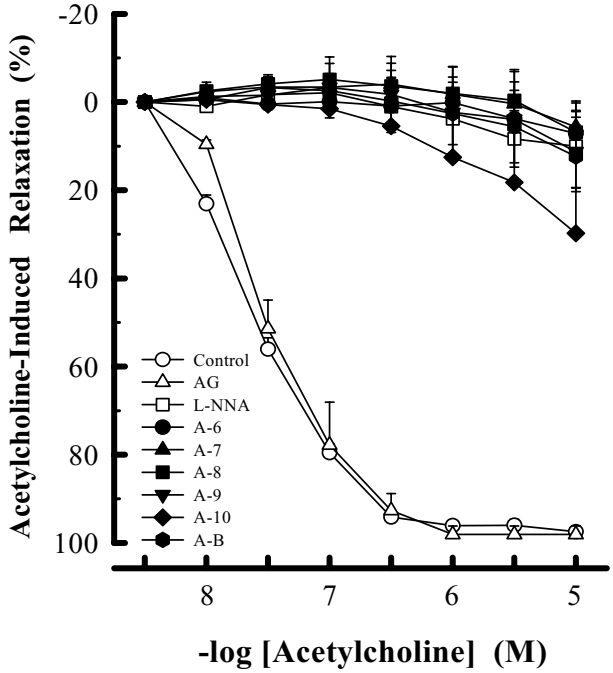


Fig. 4. Vasorelaxing effects of acetylcholine (10 nM–10 μM) in the absence or presence of **A-6–A-B** (100 μM) for 20 min in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM) (n = 4–6).

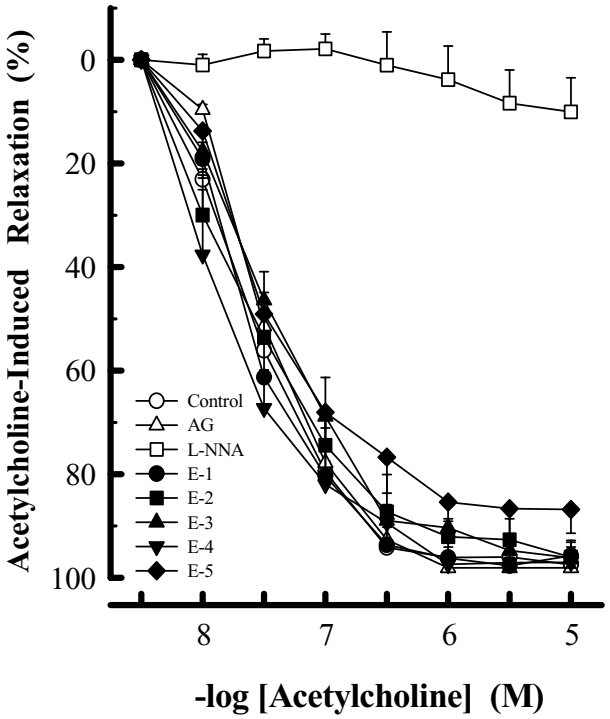


Fig. 5. Vasorelaxing effects of acetylcholine (10 nM–10 μM) in the absence or presence of **E-1–E-5** (100 μM) for 20 min in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM) (n = 4–5).

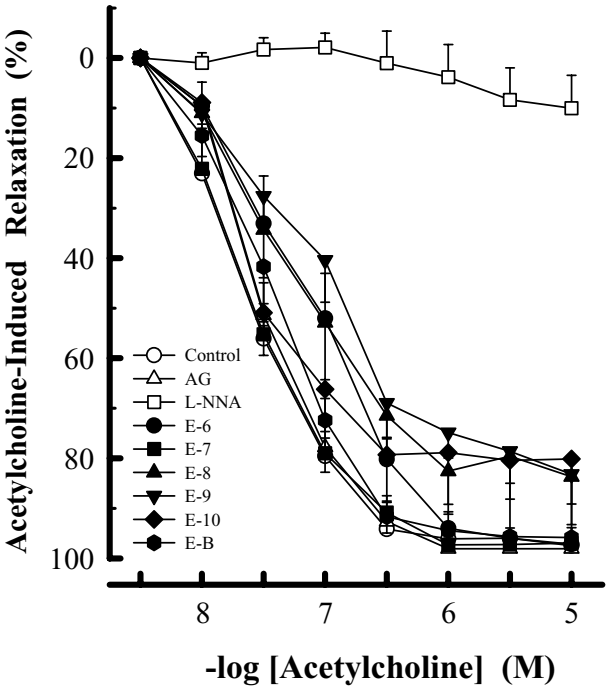


Fig. 6. Vasorelaxing effects of acetylcholine (10 nM–10 μM) in the absence or presence of **E-6–E-B** (100 μM) for 20 min in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM) (n = 4–5).

domain more strongly than did L-arginine and the binding site of O_2 was replaced by the NO_2 group. Therefore, vasorelaxation is inhibited, while N^{ω}-nitro-L-arginine alkyl esters may have inhibited the production of NO (Fig. 7).

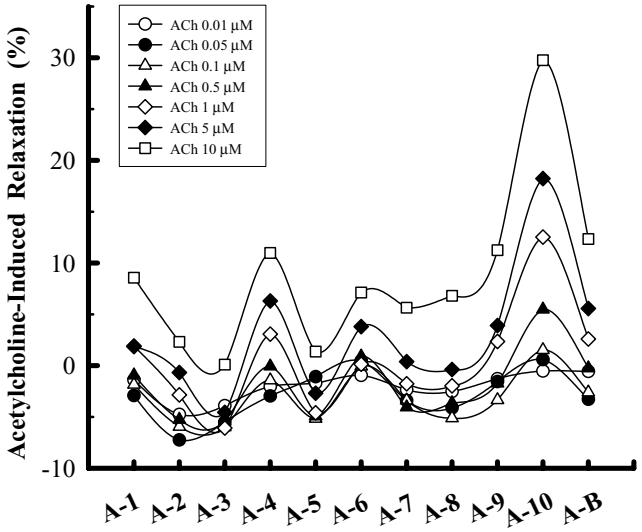


Fig. 7. Vasorelaxing effects of acetylcholine (10 nM–10 μM) in the presence of **A-1–A-10** and **A–B** in rat aortic rings with intact endothelium-precontracted with phenylephrine (0.3 μM). The concentrations of acetylcholine are 0.5, 1.0, 5.0, 10, and 50 μM.

4. Conclusions

In summary, N^{ω}-nitro-L-arginine alkyl esters (**A-1–A-B**) and L-arginine alkyl esters (**E-1–E-B**) were synthesized. These compounds demonstrated that the nitro group is an essential inhibiting group in these inhibitors, and that hydrophobic functional groups can fine-tune binding effects. Of these, **A-3** is the most potent inhibitor. The functions of **A-3** in other biological systems are currently under investigation.

5. Experimental protocols

5.1. Chemistry

All ^{1}H-NMR spectra were obtained on a Varian Gemini-200, VXR-200s spectrometer. The chemical shift values are reported in ppm (d) and coupling constants (J) in Hertz (Hz). Fast atom bombardment mass spectra (FABMS) and electron ionization (EI) were obtained on a JEOL JMS-DX303HF or PerSeptive Voyager Elite spectrometer. Atmospheric pressure chemical ionization (APCI) was determined on a Hitachi M1200H spectrometer. Matrix assisted laser desorption ionization-time of flight high-resolution mass spectra (MALDI-TOF HRMS) were obtained on a PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin–Elmer FTIR 1760X or JASCO FTIR-430 spectrometer.

5.1.1. Starting materials

L-Arginine is commercially available.

5.1.2. Preparation of compound *A*

Nitro-L-arginine (1.0 g) was suspended in alkanol (15 ml) and hydrogen chloride was passed through the solution for

10 min. The mixture was heated up to 100–110 °C (oil bath) or refluxed for 15 min, and the solvent was removed in vacuo. The residue was redissolved in methanol (15 ml) and the procedure was repeated twice. The solvent was removed in vacuo, the resulting foam was redissolved in methanol (4 ml), and ether was added to the solution until it become slightly turbid. The mixture was kept at room temperature for 4–5 h and was then placed in a refrigerator to complete the crystallization. After the solvent was removed produced **A**·HCl.

5.1.2.1. N$^{\omega}$*-nitro-L-arginine methyl ester (**A-1**).* **A-1**·HCl: Yield 99%. $[\alpha]_D^{20}$ +10.6° (c 0.36, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 1.64 (b, 2H), 1.91 (m, 2H), 3.23 (t, 2H, J = 6.7 Hz), 3.74 (s, 3H), 4.10 (t, 1H, J = 6.4 Hz). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.0, 23.4, 40.2, 52.4, 53.9, 158.8, 170.3. MS (FAB) *m/e* 234 (M^+ + H). HRMS (FAB) calc. for $C_7H_{16}N_5O_4$ (M^+ + H) 234.1202, found 234.1207.

5.1.2.2. N$^{\omega}$*-nitro-L-arginine ethyl ester (**A-2**).* **A-2**·HCl: Yield 99%. $[\alpha]_D^{21}$ +8.9° (c 0.62, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 1.89 (t, 3H, J = 7.1 Hz), 1.64 (b, 2H), 1.91 (m, 2H), 3.24 (t, 2H, J = 6.3 Hz), 4.02 (t, 1H, J = 7.1 Hz), 4.08 (q, 2H, J = 6.1 Hz). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.1, 23.1, 26.9, 40.2, 52.4, 63.5, 158.8, 169.8. MS (FAB) *m/e* 248 (M^+ + H). HRMS (FAB) calc. for $C_8H_{18}N_5O_4$ (M^+ + H) 248.1539, found 248.1351.

5.1.2.3. N$^{\omega}$*-nitro-L-arginine propyl ester (**A-3**).* **A-3**·HCl: Yield 99%. $[\alpha]_D^{21}$ +8.4° (c 0.58, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.80 (t, 3H, J = 7.2 Hz), 1.56–1.89 (b, 6H), 3.21 (b, 2H), 4.09 (b, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 9.5, 21.2, 23.2, 26.9, 40.1, 52.5, 68.5, 158.9, 170.0. MS (FAB) *m/e* 262 (M^+ + H). HRMS (FAB) calc. for $C_9H_{20}N_5O_4$ (M^+ + H) 262.1515, found 262.1517.

5.1.2.4. N$^{\omega}$*-nitro-L-arginine butyl ester (**A-4**).* **A-4**·HCl: Yield 99%. $[\alpha]_D^{22}$ +8.6° (c 0.93, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.79 (t, 3H, J = 4.2 Hz), 1.22–1.89 (b, 8H), 3.22 (b, 2H), 4.14 (b, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 12.8, 18.4, 23.8, 27.0, 29.4, 40.2, 52.5, 67.0, 158.9, 170.0. MS (FAB) *m/e* 276 (M^+ + H). HRMS (FAB) calc. for $C_{10}H_{22}N_5O_4$ (M^+ + H) 276.1672, found 276.1677.

5.1.2.5. N$^{\omega}$*-nitro-L-arginine pentyl ester (**A-5**).* **A-5**·HCl: Yield 94%. $[\alpha]_D^{22}$ +6.8° (c 0.45, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.75 (t, 3H, J = 3.0 Hz), 1.19–1.89 (b, 10H), 3.22 (b, 2H), 4.06–4.21 (b, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.2, 21.6, 23.4, 27.0, 27.1, 27.2, 40.2, 52.5, 67.5, 158.9, 170.1. MS (FAB) *m/e* 290 (M^+ + H). HRMS (FAB) calc. for $C_{11}H_{24}N_5O_4$ (M^+ + H) 290.1828, found 290.1820.

5.1.2.6. N$^{\omega}$*-nitro-L-arginine hexyl ester (**A-6**).* **A-6**·HCl: Yield 99%. $[\alpha]_D^{23}$ +7.6° (c 0.72, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.71 (b, 3H), 1.13–1.87 (b, 12H), 3.20 (b, 2H), 4.04–4.19 (b, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.3, 21.9, 23.4, 24.8, 27.0, 27.5, 30.7, 40.2, 52.5, 67.4, 158.9, 170.1. MS (FAB) *m/e* 304 (M^+ + H). HRMS (FAB) calc. for $C_{12}H_{26}N_5O_4$ (M^+ + H) 304.1895, found 304.1985.

5.1.2.7. N$^{\omega}$*-nitro-L-arginine heptyl ester (**A-7**).* **A-7**·HCl: Yield 90%. $[\alpha]_D^{23}$ +9.5° (c 1.11, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.82 (b, 3H), 1.28 (b, 10H), 1.63 (b, 2H), 2.02 (b, 2H), 3.33 (b, 2H), 4.22 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.0, 22.7, 25.8, 27.5, 28.3, 28.9, 31.8, 40.8, 52.9, 67.5, 159.4, 170.4. MS (FAB) *m/e* (M^+ + H) 318.1. HRMS (FAB) calc. for $C_{13}H_{28}N_5O_4$ (M^+ + H) 318.2141, found 318.2145.

5.1.2.8. N$^{\omega}$*-nitro-L-arginine octyl ester (**A-8**).* **A-8**·HCl: Yield 80%. $[\alpha]_D^{23}$ +10.2° (c 1.26, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.82 (b, 3H), 1.23 (b, 12H), 1.60 (b, 2H), 2.02 (b, 2H), 3.33 (b, 2H), 4.16 (b, 1H), 4.23 (b, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.1, 22.8, 26.0, 27.4, 27.6, 28.5, 29.4, 32.1, 41.0, 52.9, 67.3, 159.4, 170.4. MS (FAB) *m/e* (M^+ + H) 332.2. HRMS (FAB) calc. for $C_{14}H_{30}N_5O_4$ (M^+ + H) 332.2298, found 332.2306.

5.1.2.9. N$^{\omega}$*-nitro-L-arginine nonyl ester (**A-9**).* **A-9**·HCl: Yield 67%. $[\alpha]_D^{24}$ +14.2° (c 0.71, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.83 (b, 3H), 1.27 (b, 14H), 1.63 (b, 2H), 2.00 (b, 2H), 3.33 (b, 2H), 4.15 (b, 1H), 4.26 (b, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.3, 23.0, 26.2, 27.5, 27.7, 28.6, 29.8, 30.0, 32.3, 40.8, 53.0, 67.3, 159.5, 170.4. MS (FAB) *m/e* (M^+ + H) 346.2. HRMS (FAB) calc. for $C_{15}H_{32}N_5O_4$ (M^+ + H) 346.2454, found 346.2465.

5.1.2.10. N$^{\omega}$*-nitro-L-arginine decyl ester (**A-10**).* **A-10**·HCl: Yield 35%. $[\alpha]_D^{21}$ +7.9° (c 0.70, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.84 (b, 3H), 1.24 (b, 16H), 1.65 (b, 2H), 2.04 (b, 2H), 3.26 (b, 2H), 4.06 (b, 1H), 4.23 (b, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.2, 23.0, 26.2, 27.4, 27.6, 28.6, 29.7, 29.8, 30.1, 32.4, 40.7, 53.1, 67.2, 159.4, 170.3. MS (FAB) *m/e* (M^+ + H) 360.2. HRMS (FAB) calc. for $C_{16}H_{34}N_5O_4$ (M^+ + H) 360.2611, found 360.2604.

5.1.2.11. N$^{\omega}$*-nitro-L-arginine benzyl ester (**A-B**).* **A-B**·HCl: Yield 94%. $[\alpha]_D^{21}$ +1.7° (c 0.36, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 1.62–2.04 (b, 4H), 3.21 (b, 2H), 4.10 (t, 1H, J = 6.3 Hz), 5.21–5.37 (dd, 2H, J = 82.5, 11.9 Hz), 7.42 (m, 5H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 23.2, 26.9, 40.1, 52.4, 68.6, 128.6, 128.8, 128.9, 129.1, 129.5, 134.5, 158.8, 171.9. MS (FAB) *m/e* 310 (M^+ + H). HRMS (FAB) calc. for $C_{13}H_{20}N_5O_4$ (M^+ + H) 310.1515, found 310.1511.

*5.1.3. Preparation of compound **E***

L-Arginine (1.0 g) was suspended in alkanol (15 ml) and hydrogen chloride was passed through the solution for 10 min. The mixture was heated up to 100–110 °C (oil bath) or refluxed for 15 min, and the solvent was removed in vacuo. The residue was redissolved in methanol (15 ml) and the procedure was repeated twice. The solvent was removed in vacuo, the resulting foam was redissolved in methanol (4 ml), and ether was added to the solution until it become slightly turbid. The mixture was kept at room temperature for 4–5 h and was then placed in a refrigerator to complete the crystallization. After the solvent was removed produced **E**·HCl.

*5.1.3.1. L-Arginine methyl ester (**E-1**).* **E-1**·HCl: Yield 71%. $[\alpha]_D^{21}$ +16.9° (c 1.01, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 1.68–1.79 (m, 2H), 1.97–2.05 (m, 2H), 3.26 (t, J = 6.8 Hz, 2H), 3.86 (s, 3H), 4.21 (t, J = 6.5 Hz, 1H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 24.2, 27.3, 40.7, 52.9, 54.1, 157.2, 170.8. MS (FAB) *m/e* (M^+ + H) 189.1. HRMS (FAB) calc. for $C_7H_{17}N_4O_2$ (M^+ + H) 189.1352, found 189.1362.

*5.1.3.2. L-Arginine ethyl ester (**E-2**).* **E-2**·HCl: Yield 72%. $[\alpha]_D^{21}$ +10.3° (c 0.58, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 1.30 (t, J = 7.2 Hz, 3H), 1.68–1.76 (m, 2H), 1.98–2.03 (m, 2H), 3.25 (t, J = 6.9 Hz, 2H), 4.17 (t, J = 6.4 Hz, 1H), 4.31 (m, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.1, 23.7, 26.8, 40.2, 52.4, 63.6, 156.7, 169.8. MS (FAB) *m/e* (M^+ + H) 203.3. HRMS (FAB) calc. for $C_8H_{19}N_4O_2$ (M^+ + H) 203.1508, found 203.1514.

*5.1.3.3. L-Arginine propyl ester (**E-3**).* **E-3**·HCl: Yield 72%. $[\alpha]_D^{21}$ +9.9° (c 0.76, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.94 (t, J = 7.4 Hz, 3H), 1.68–1.80 (m, 4H), 2.03 (m, 2H), 3.27 (m, 2H), 4.19–4.26 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 10.1, 21.7, 24.3, 27.4, 40.7, 53.0, 69.5, 157.2, 170.5. MS (FAB) *m/e* (M^+ + H) 217.1. HRMS (FAB) calc. for $C_9H_{21}N_4O_2$ (M^+ + H) 217.1665, found 217.1658.

*5.1.3.4. L-Arginine butyl ester (**E-4**).* **E-4**·HCl: Yield 68%. $[\alpha]_D^{21}$ +6.6° (c 0.90, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.89 (t, J = 7.4 Hz, 3H), 1.37 (m, 2H), 1.67 (m, 2H), 1.77 (m, 2H), 3.01 (m, 2H), 3.26 (m, 2H), 4.19 (t, J = 6.3 Hz, 1H), 4.26–4.31 (m, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.3, 18.9, 24.3, 27.4, 30.1, 40.7, 53.0, 67.7, 157.1, 170.4. MS (FAB) *m/e* (M^+ + H) 231.1. HRMS (FAB) calc. for $C_{10}H_{23}N_4O_2$ (M^+ + H) 231.1821, found 231.1829.

*5.1.3.5. L-Arginine pentyl ester (**E-5**).* **E-5**·HCl: Yield 71%. $[\alpha]_D^{21}$ +7.3° (c 0.60, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.89 (t, J = 7.1 Hz, 3H), 1.35 (m, 4H), 1.69–1.80 (m, 4H), 2.03 (m, 2H), 3.28 (t, J = 6.8 Hz, 2H), 4.20 (t, J = 6.3 Hz, 1H), 4.26–4.33 (m, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.7, 22.0, 24.3, 27.4, 27.5, 27.8, 40.7, 53.0, 68.0, 157.2, 170.5. MS (FAB) *m/e* (M^+ + H) 245.1. HRMS (FAB) calc. for $C_{11}H_{25}N_4O_2$ (M^+ + H) 245.1978, found 245.1968.

*5.1.3.6. L-Arginine hexyl ester (**E-6**).* **E-6**·HCl: Yield 68%. $[\alpha]_D^{21}$ +7.9° (c 0.75, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.87 (t, J = 4.9 Hz, 3H), 1.30–1.38 (m, 6H), 1.69–1.85 (m, 4H), 2.03 (m, 2H), 3.27 (t, J = 6.9 Hz, 2H), 4.20 (t, J = 6.4 Hz, 1H), 4.26–4.33 (m, 2H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.8, 22.3, 24.3, 25.2, 27.5, 28.0, 31.0, 40.8, 53.0, 68.0, 157.2, 170.5. MS (FAB) *m/e* (M^+ + H) 259.2. HRMS (FAB) calc. for $C_{12}H_{27}N_4O_2$ (M^+ + H) 259.2134, found 259.2136.

*5.1.3.7. L-Arginine heptyl ester (**E-7**).* **E-7**·HCl: Yield 37%. $[\alpha]_D^{21}$ +6.6° (c 0.66, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.69 (t, J = 6.2 Hz, 3H), 1.11–1.20 (m, 8H), 1.52–1.61 (m, 4H), 1.84–1.87 (m, 2H), 3.10 (t, J = 6.8 Hz, 2H), 4.07–4.15 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.9, 22.4, 24.3, 25.5, 27.4, 28.1, 28.4, 31.5, 40.7, 52.9, 67.9, 157.1, 170.5. MS (FAB) *m/e* (M^+ + H) 273.2. HRMS (FAB) calc. for $C_{13}H_{29}N_4O_2$ (M^+ + H) 273.2291, found 273.2296.

*5.1.3.8. L-Arginine octyl ester (**E-8**).* **E-8**·HCl: Yield 35%. $[\alpha]_D^{21}$ +6.0° (c 0.72, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.83 (t, J = 7.0 Hz, 3H), 1.16–1.37 (m, 10H), 1.62–1.81 (m, 4H), 1.92–2.51 (m, 2H), 3.23 (t, J = 6.9 Hz, 2H), 4.16–4.29 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 13.9, 14.6, 22.5, 24.3, 25.5, 27.5, 28.1, 28.7, 31.5, 40.8, 53.0, 68.0, 157.2, 170.5. MS (FAB) *m/e* (M^+ + H) 287.2. HRMS (FAB) calc. for $C_{14}H_{31}N_4O_2$ (M^+ + H) 287.2447, found 287.2441.

*5.1.3.9. L-Arginine nonyl ester (**E-9**).* **E-9**·HCl: Yield 37%. $[\alpha]_D^{21}$ +6.3° (c 0.42, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.83 (t, J = 7.0 Hz, 3H), 1.21–1.37 (m, 12H), 1.69–1.77 (m, 4H), 1.96–2.01 (m, 2H), 3.24 (t, J = 6.8 Hz, 2H), 4.17–4.31 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.0, 14.6, 22.6, 24.2, 25.5, 27.6, 28.0, 28.7, 28.8, 31.5, 40.8, 53.0, 68.0, 157.2, 170.5. MS (FAB) *m/e* (M^+ + H) 301.1. HRMS (FAB) calc. for $C_{15}H_{33}N_4O_2$ (M^+ + H) 301.2604, found 301.2607.

*5.1.3.10. L-Arginine decyl ester (**E-10**).* **E-10**·HCl: Yield 38%. $[\alpha]_D^{21}$ –2.7° (c 0.28, H_2O). ^{1}H-NMR (600 MHz, D_2O) δ (ppm) 0.80 (t, J = 6.9 Hz, 3H), 1.40–2.02 (m, 20H), 3.20 (t, J = 6.8 Hz, 2H), 4.15-4.30 (m, 3H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 14.0, 14.4, 22.6, 24.2, 25.5, 27.6, 28.1, 28.7, 28.8, 31.5, 40.8, 53.2, 68.1, 157.0, 170.5. MS (FAB) *m/e* (M^+ + H) 315.2. HRMS (FAB) calc. for $C_{16}H_{35}N_4O_2$ (M^+ + H) 315.2760, found 315.2762.

*5.1.3.11. L-Arginine benzyl ester (**E-B**).* **E-B**·HCl: Yield 58%. $[\alpha]_D^{21}$ –0.40° (c 0.52, H_2O). ^{1}H-NMR ^{13}C-NMR (600 MHz, D_2O) δ (ppm) 1.95 (m, 4H), 3.20 (t, J = 6.9 Hz, 2H), 4.17 (t, J = 6.3 Hz, 1H), 5.20–5.34 (dd, J = 71.9, 11.9 Hz, 2H), 7.41 (m, 5H). ^{13}C-NMR (151 MHz, D_2O) δ (ppm) 24.1, 27.3, 40.5, 52.8, 70.0, 129.1, 129.2, 129.4, 135.0, 157.0, 170.0. MS (FAB) *m/e* (M^+ + H) 265.1. HRMS (FAB) calc. for $C_{13}H_{21}N_4O_2$ (M^+ + H) 265.1665, found 265.1666.

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