

Figure 51 Changes of biochemical parameters in blood of mice treated by Fullereneol particles. a) Blood samples were collected from BALB/c mice treated with Fullereneol particles or water at 24 h intravenously. Plasma was recovered by centrifuging blood at 1750 g for 15 min. Biochemical parameters were analyzed using FUJI DRI-CHEM 7000. Data are expressed as the mean ± S.E. (n = 6; **P < 0.01, *P < 0.05 versus value for control (water) group by Tukey). b) Caco-2 cells were treated with 0.2 mM H₂O₂ or Fullereneol particles. DNA strand breaks were detected by alkaline comet assay according to the Comet Assay Kit.

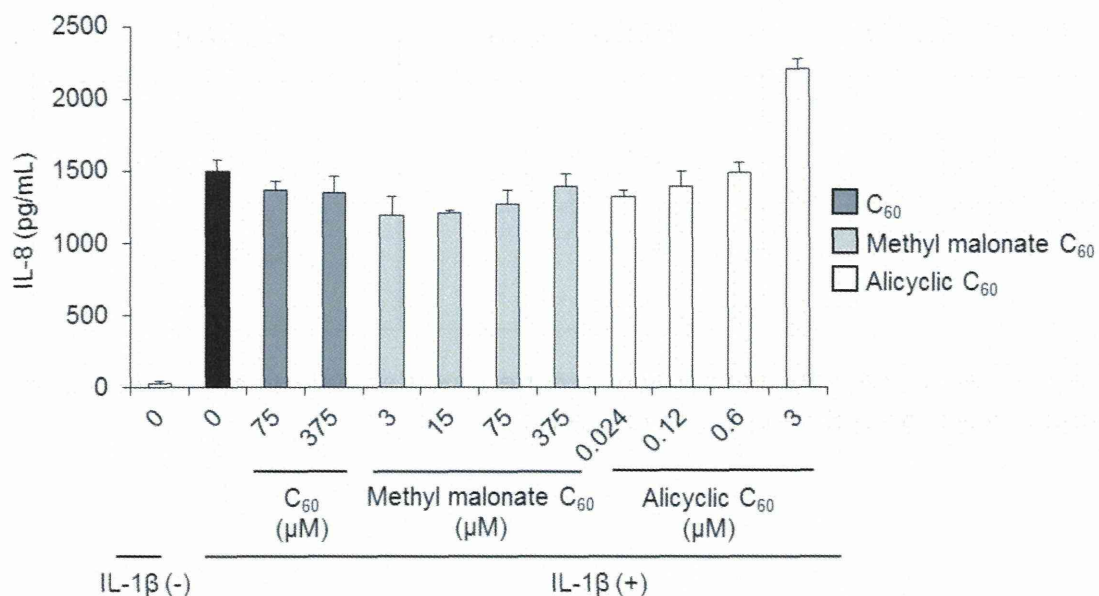
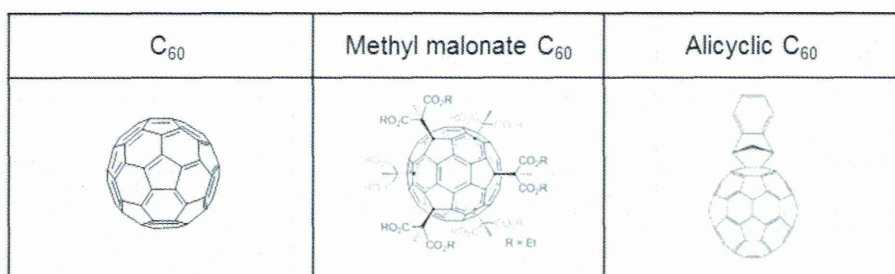


Fig 52 Inhibitory effects of fullerene C_{60} derivatives on IL-1 β -induced IL-8 secretion in the Caco-2 cells. Caco-2 cells were treated with fullerene C_{60} or fullerene C_{60} derivatives for 30 min, and then, stimulated with IL-1 β (125 ng/ml) for 24 h. Secreted IL-8 protein level in the culture supernatant was measured by IL-8 ELISA kit. Data are expressed as the mean \pm S.D. (n = 4)

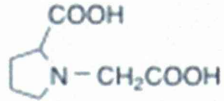
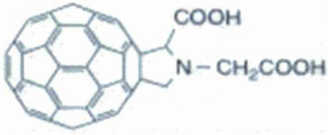
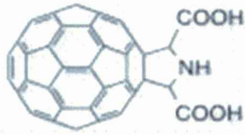
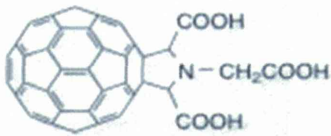
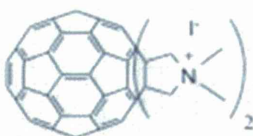

Proline (1)'	Proline C ₆₀ (1)	
		
Proline C ₆₀ (3)	Proline C ₆₀ (4)	C ₆₀ (OH) ₃₆
		

图 53 The chemical structure formula of proline-modified fullerene C₆₀ derivatives, functional group of proline C₆₀(1) and C₆₀(OH)₃₆.

	Particle size In solution (nm)	Zeta potential (mV)
Proline C₆₀ (1)	114 (608)	-26.5
Proline C₆₀ (2)	152 (629)	-29.2
Proline C₆₀ (3)	32.6	-17.1
Proline C₆₀ (4)	52.7	+40.0
C₆₀(OH)₃₆	2.1	-28.9
C₆₀	338	-14.0

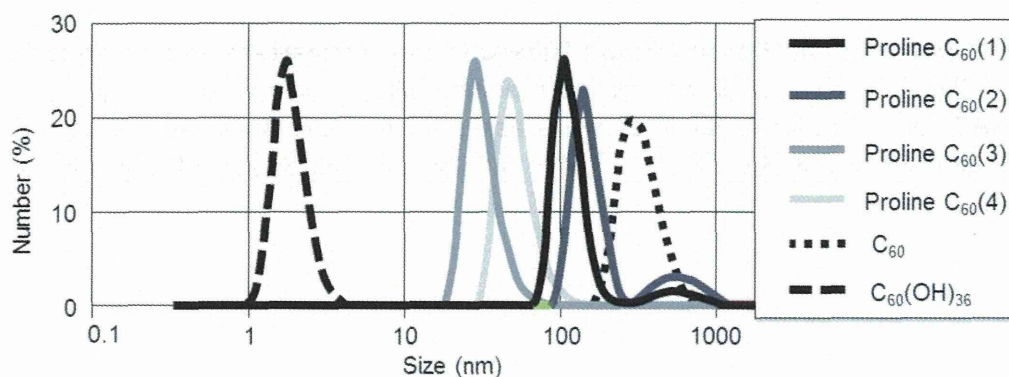


Fig 54 Particle size of proline-modified fullerene C₆₀ derivatives. Proline-modified fullerene C₆₀ derivatives used in this study were suspended in DMSO and then suspended in distilled water and their sizes (proline C₆₀ (1), proline C₆₀ (2), proline C₆₀ (3), proline C₆₀ (4), C₆₀ or C₆₀(OH)₃₆) were measured by dynamic light scattering (DSL).

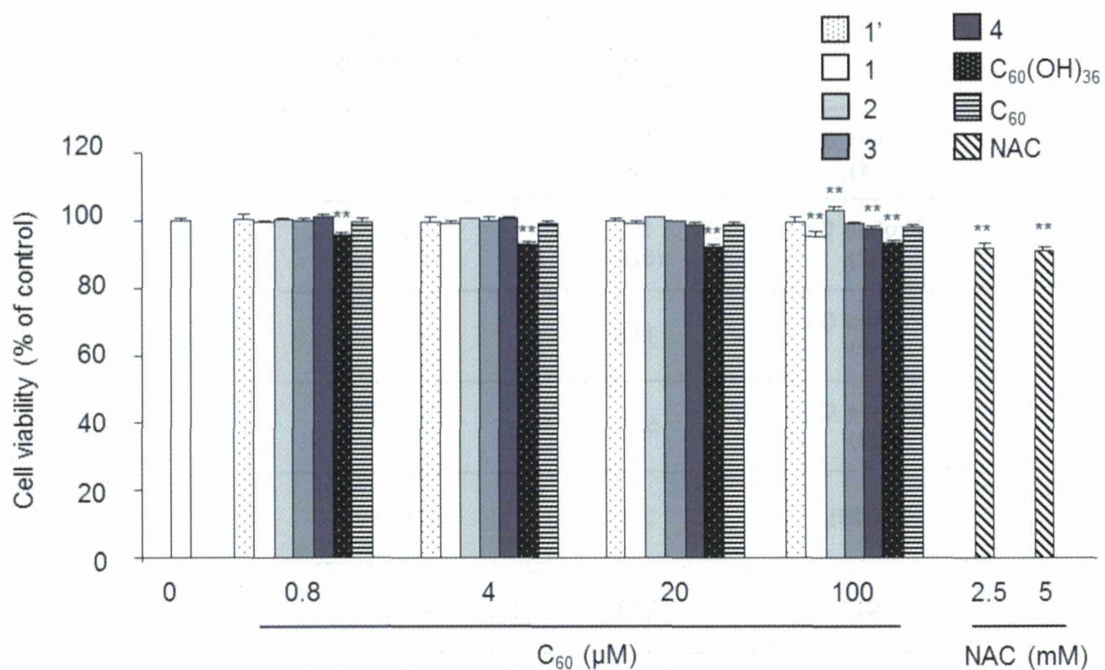


Fig 55 Cytotoxicity of proline-modified fullerene C₆₀ derivatives in the Caco-2 cells. Caco-2 cells were treated with proline-modified fullerene derivatives, C₆₀(OH)₃₆, C₆₀ and NAC for 24 h. Cell viabilities were assessed by LDH Assay kit. Data are expressed as the mean ± S.D. (n = 4; **P < 0.01 versus value for control group by Bonferroni)

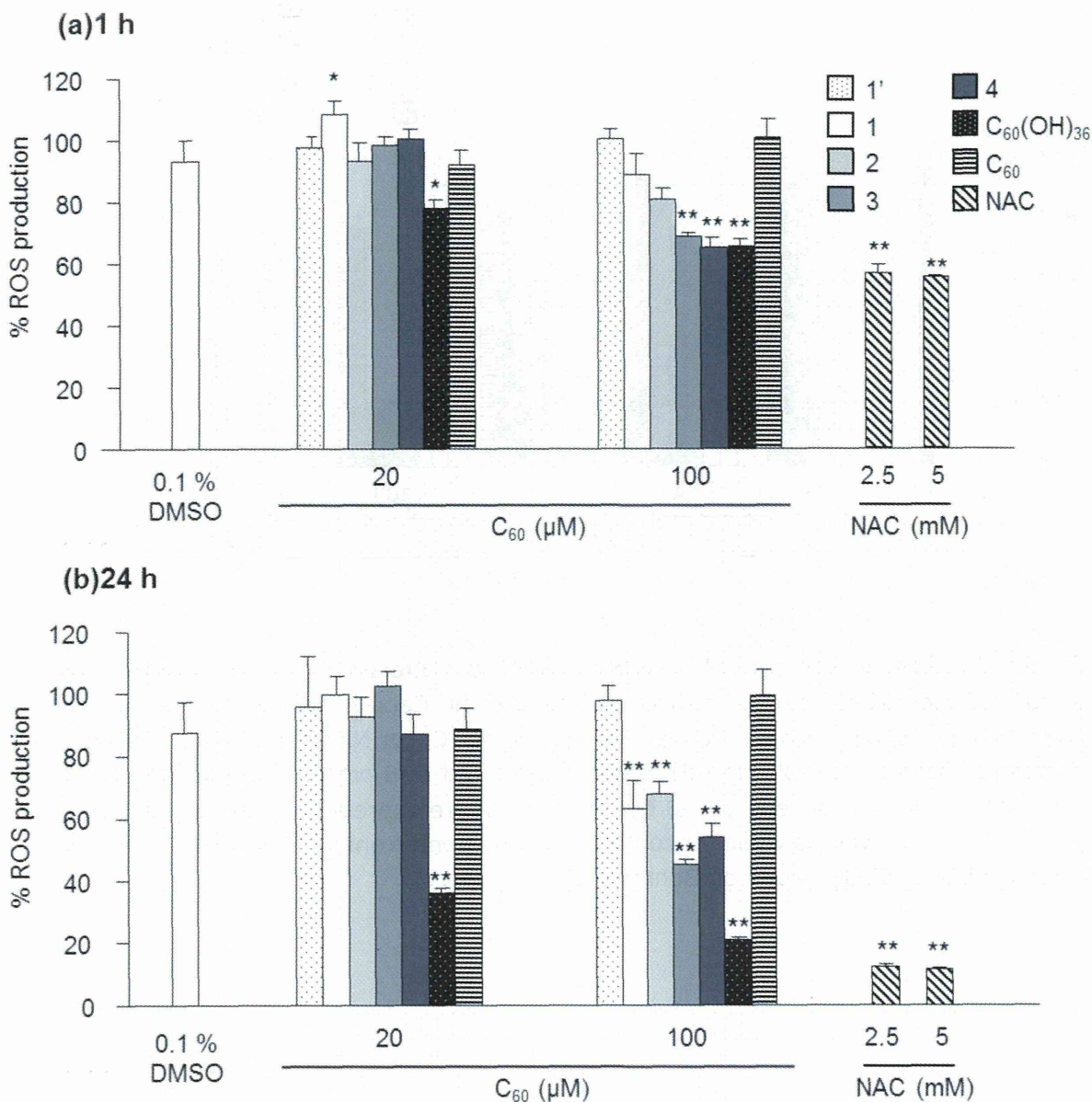


Figure 56 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on intracellular ROS production in the Caco-2 cells. Intracellular ROS production was measured as DCF-fluorescence intensity. Caco-2 cells were treated with 20 μM DCFH-DA for 20 min then treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 1 h (a) or 24 h (b). Data are expressed as the mean ± S.D. (n = 4; **P < 0.01, *P < 0.05 versus value for 0.1 % DMSO group by Bonferroni)

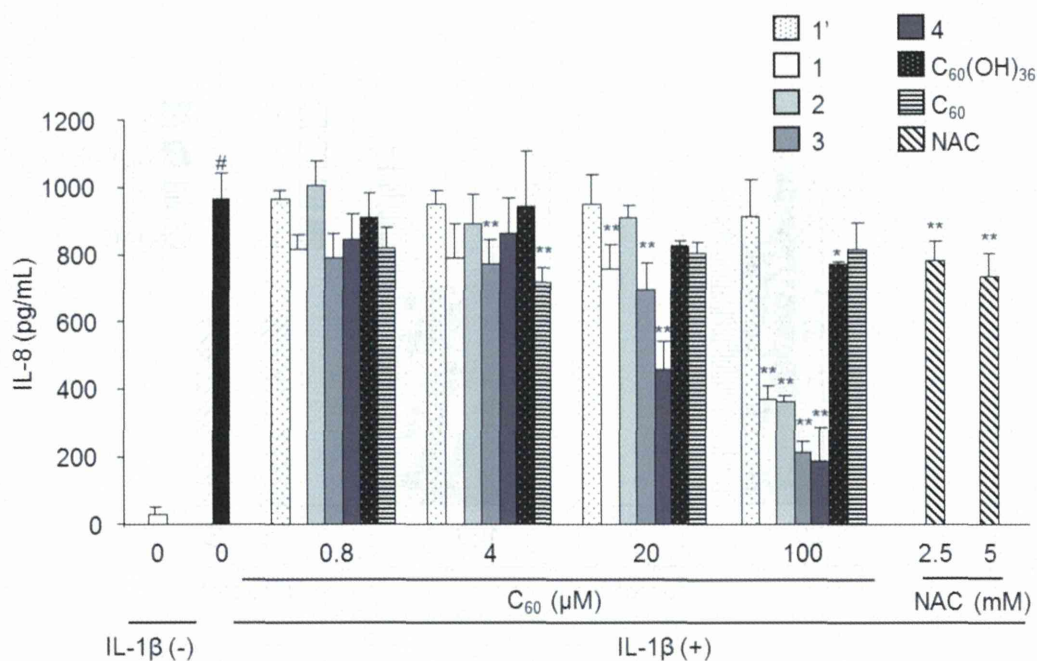


图 57 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on IL-1β-induced IL-8 secretion in the Caco-2 cells. Caco-2 cells were treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with IL-1β (125 ng/ml) for 24 h. Secreted IL-8 protein level in the culture supernatant was measured by IL-8 ELISA kit. Data are expressed as the mean ± S.D. (n = 4; #P < 0.01 versus value for control group by Bonferroni ; **P < 0.01, *P < 0.05 versus value for IL-1β group by Bonferroni)

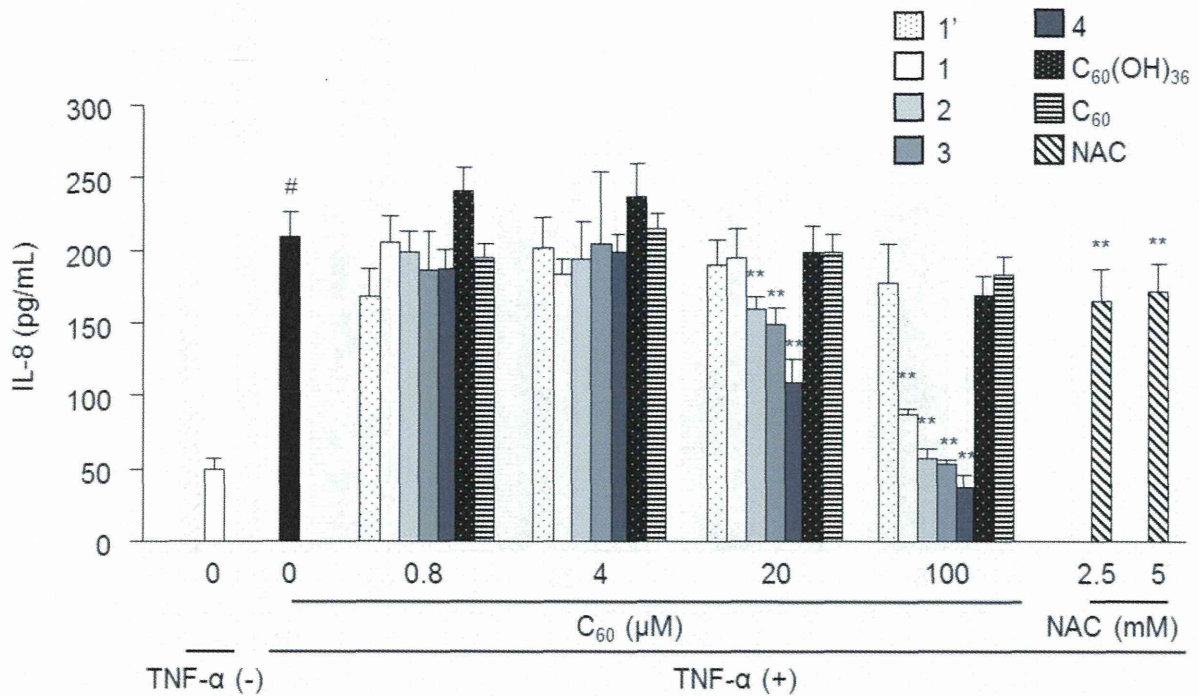


图 58 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on TNF- α -induced IL-8 secretion in the Caco-2 cells. Caco-2 cells were treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with TNF- α (10 ng/ml) for 24 h. Secreted IL-8 protein level in the culture supernatant was measured by IL-8 ELISA kit. Data are expressed as the mean \pm S.D. (n = 4; #P < 0.01 versus value for control group by Bonferroni ; **P < 0.01, *P < 0.05 versus value for TNF- α group by Bonferroni)

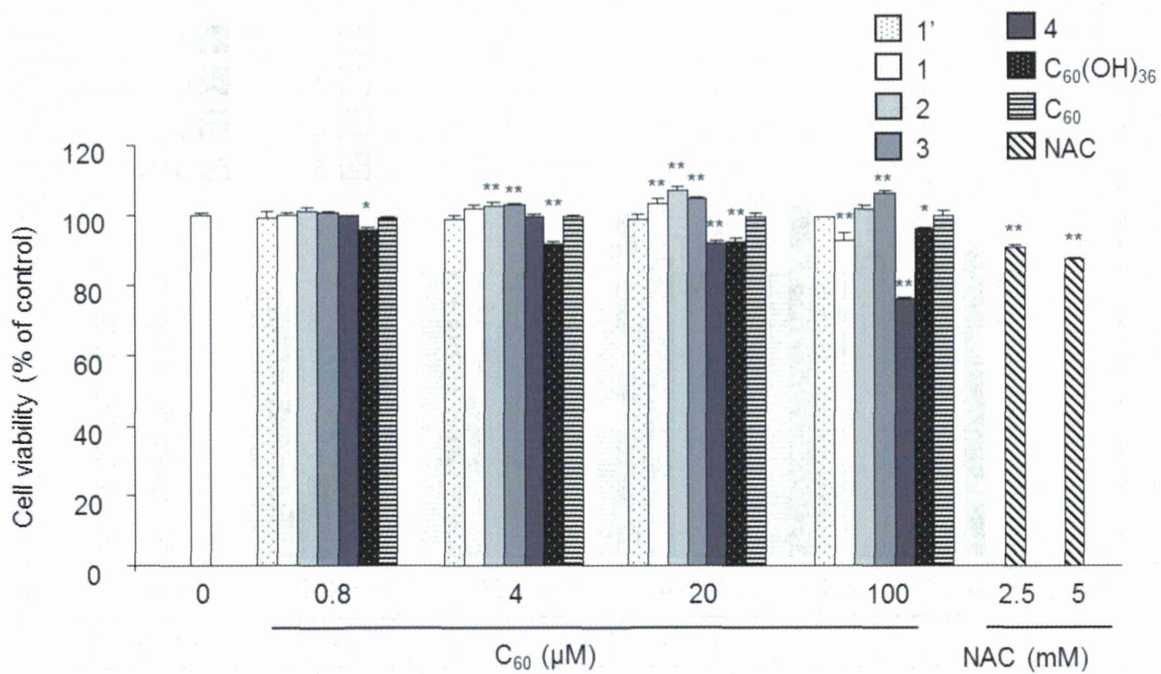


Fig 59 Cytotoxicity of proline-modified fullerene C₆₀ derivatives in the RAW264.7 cells. RAW264.7 cells were treated with proline-modified fullerene derivatives, C₆₀(OH)₃₆, C₆₀ and NAC for 24 h. Cell viabilities were assessed by LDH Assay kit. Data are expressed as the mean ± S.D. (n = 4; **P < 0.01 versus value for control group by Bonferroni)

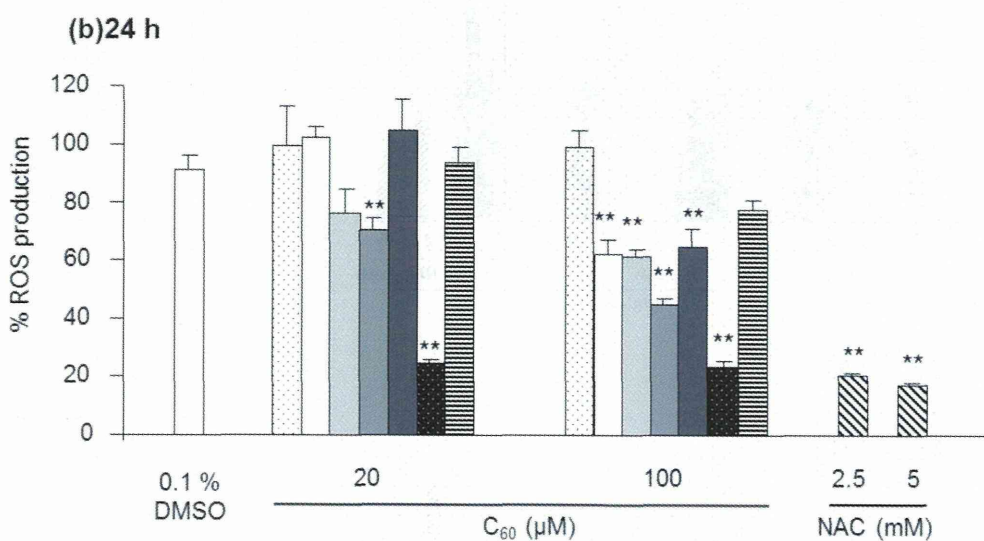
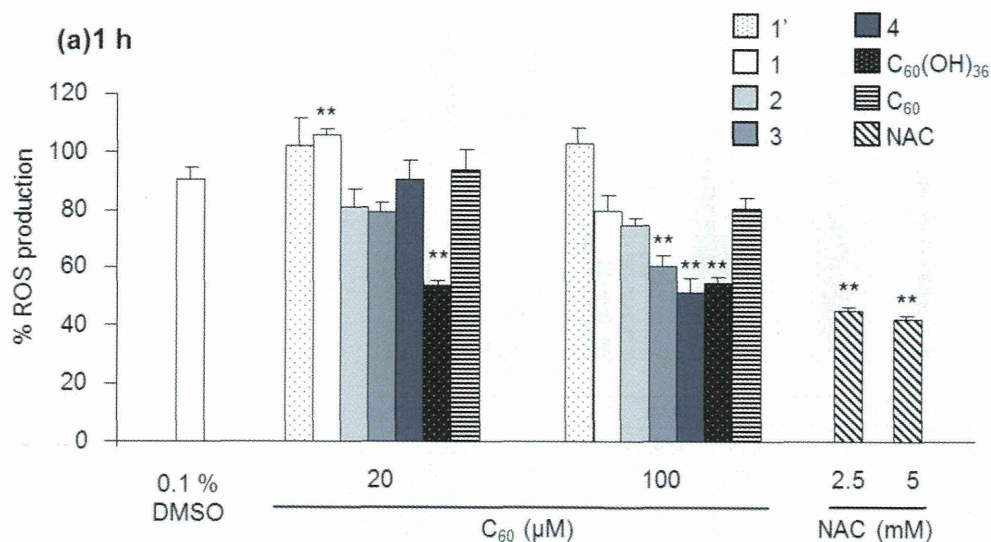


Fig. 60 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on intracellular ROS production in the RAW264.7 cells. Intracellular ROS production was measured as DCF-fluorescence intensity. RAW264.7 cells were treated with 20 μM of DCFH-DA for 20 min then treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 1 or 24 h. Data are expressed as the mean ± S.D. (n = 4; **P < 0.01, *P < 0.05 versus value for 0.1 % DMSO group by Bonferroni)

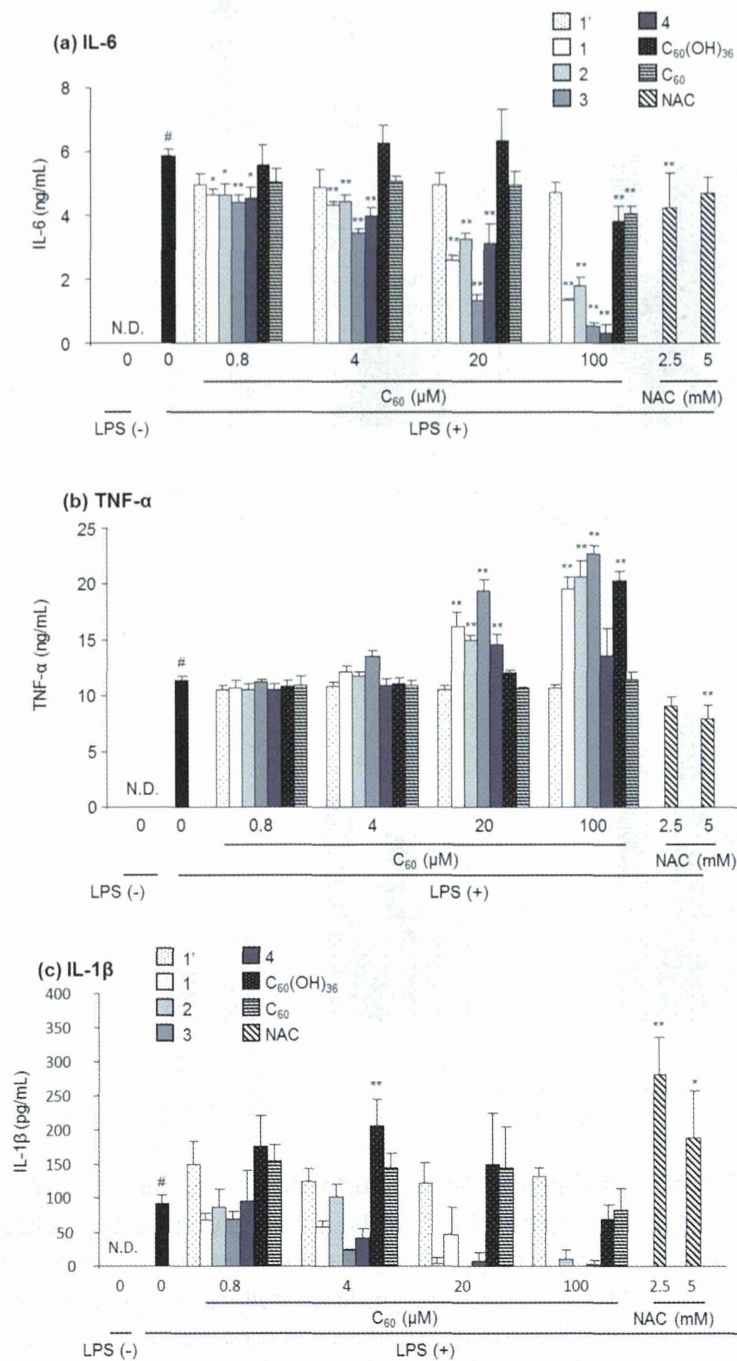


图 61 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on LPS-induced IL-6, TNF-α, or IL-1β secretion in the RAW264.7 cells. RAW264.7 cells were treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with LPS (1 μg/ml) for 24 h. Secreted IL-6 (a), TNF-α (b), IL-1β (c) protein level in the culture supernatant was measured by IL-6, TNF-α, IL-1β, IFN-β or IP-10 ELISA kit. Data are expressed as the mean ± S.D. (n = 4; #P < 0.05 versus value for control group by Bonferroni; **P < 0.01, *P < 0.05 versus value for LPS group by Bonferroni)

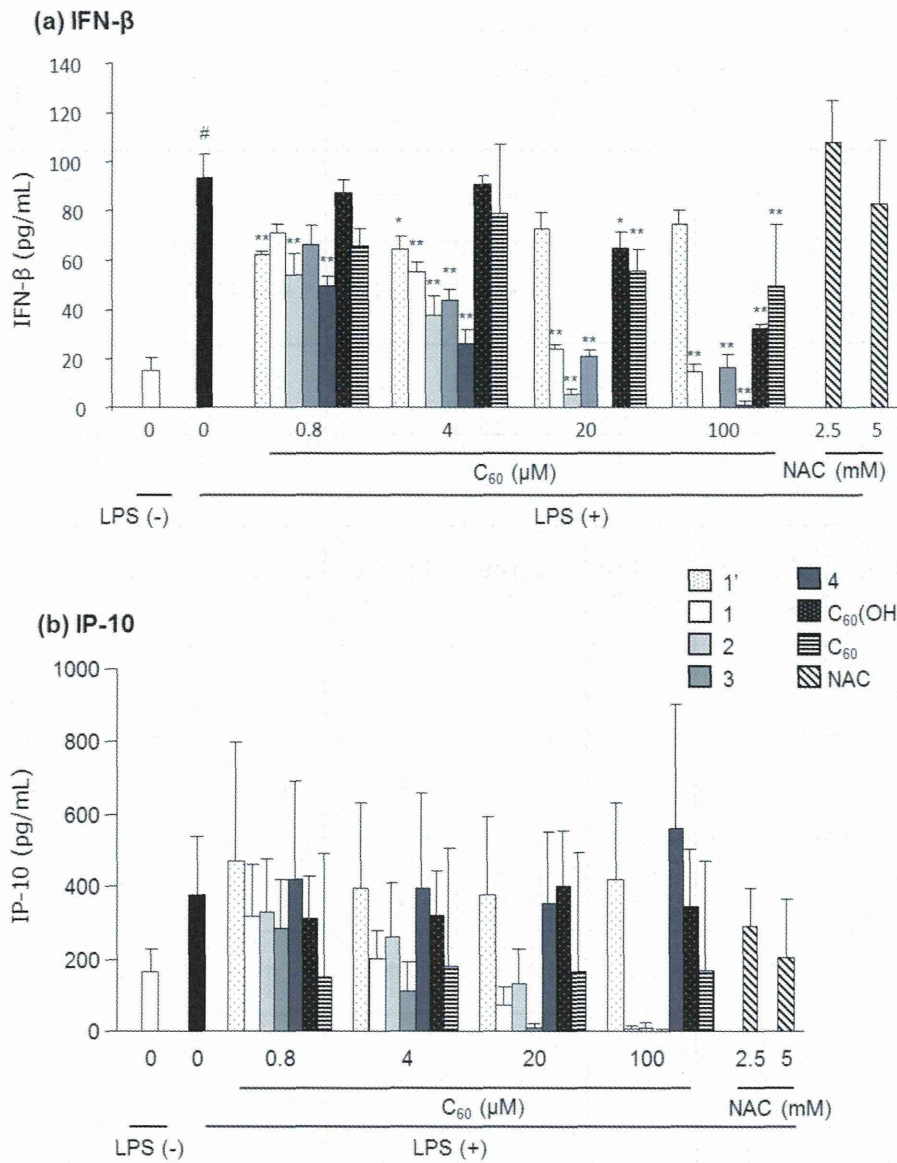


图 62 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on LPS-induced IFN-β or IP-10 secretion in the RAW264.7 cells. RAW264.7 cells were treated with proline-modified fullerene C₆₀ derivatives, C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with LPS (1 μg/ml) for 24 h. Secreted IFN-β (a) or IP-10 (b) protein level in the culture supernatant was measured by IL-6, TNF-α, IL-1β, IFN-β or IP-10 ELISA kit. Data are expressed as the mean ± S.D. (n = 4; #P < 0.05 versus value for control group by Bonferroni ; **P < 0.01, *P < 0.05 versus value for LPS group by Bonferroni)

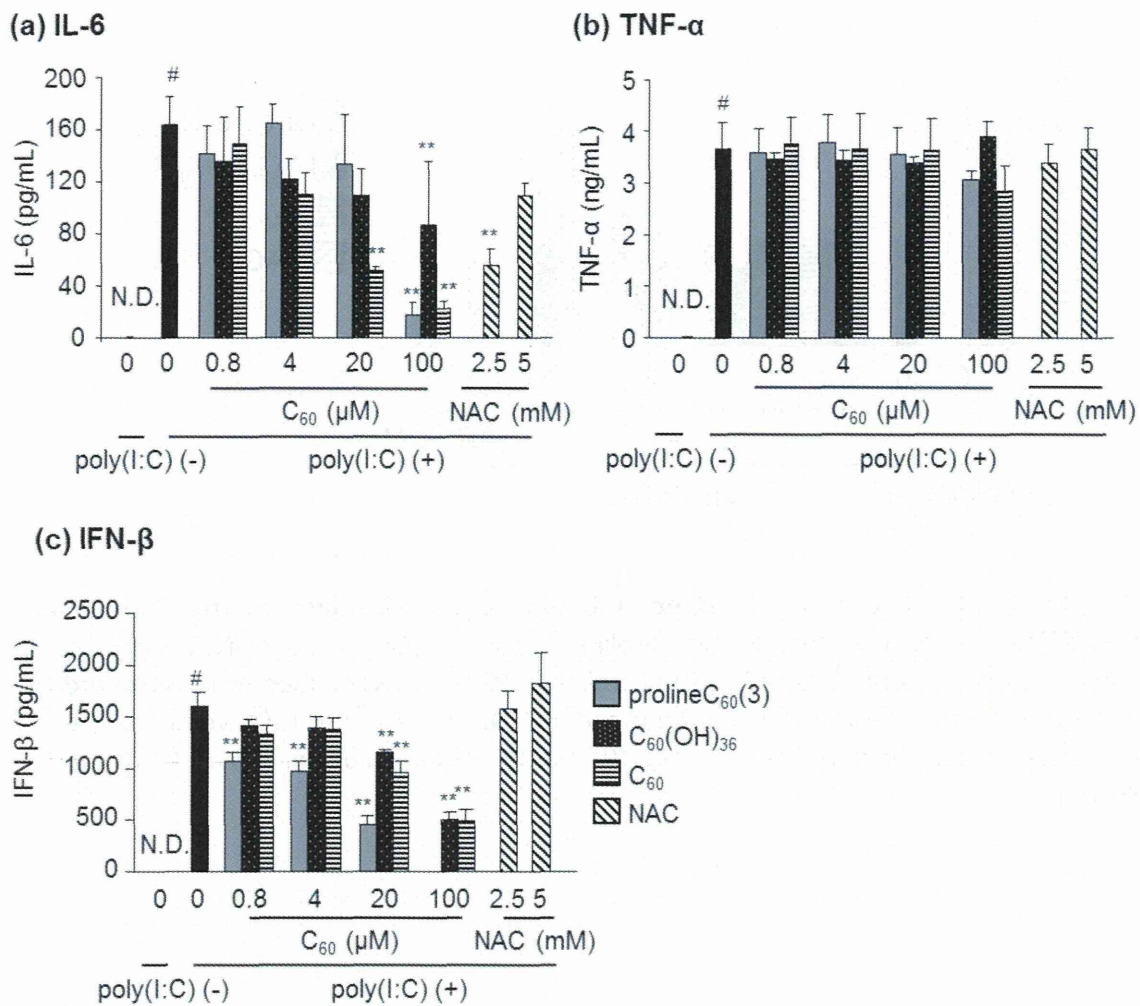
Caco-2 cells.

	Proline (1)	Proline C ₆₀ (1)	Proline C ₆₀ (2)	Proline C ₆₀ (3)	Proline C ₆₀ (4)	C ₆₀ (OH) ₃₆	C ₆₀	NAC
Cytotoxicity	—	—	—	—	—	—	—	—
Antioxidant effect	—	+	+	++	++	+++	—	+++
IL-8	→	↓↓	↓↓	↓↓↓	↓↓↓	↓	→	↓

RAW cells.

	Proline (1)	Proline C ₆₀ (1)	Proline C ₆₀ (2)	Proline C ₆₀ (3)	Proline C ₆₀ (4)	C ₆₀ (OH) ₃₆	C ₆₀	NAC
Cytotoxicity	—	—	—	—	+	—	—	—
Antioxidant effect	→	+	++	++	++	+++	+	+++
IL-6	→	↓↓	↓↓	↓↓↓	↓↓	↓	↓	→
TNF-α	→	↑↑	↑↑	↑↑	↑	↑↑	→	→
IL-1β	→	↓	↓	↓	↓	→	→	↑
IFN-β	→	↓↓	↓↓	↓↓	↓↓	↓	↓	→
IP-10	→	↓	↓	↓	→	→	→	→

☒ 63 The cytotoxicity, antioxidant effect and anti-inflammatory effect of proline-modified fullerene C₆₀ derivatives in the Caco-2 cells and RAW264.7 cells.



▣ 64 Inhibitory effects or cytotoxicity of proline-modified fullerene C₆₀ derivatives on poly(I:C)-induced IL-6, TNF-α or IFN-β secretion in the RAW264.7 cells. RAW264.7 cells were treated with proline C₆₀(3), C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with poly(I:C) (20 μg/ml) for 24 h. Secreted IL-6 (a), TNF-α (b) or IFN-β (c) protein level in the culture supernatant was measured by IL-6, TNF-α or IFN-β or ELISA kit. Data are expressed as the mean ± S.D. (n = 4; [#]P < 0.01 versus value for control group by Bonferroni; ^{**}P < 0.01, ^{*}P < 0.05 versus value for poly(I:C) group by Bonferroni)

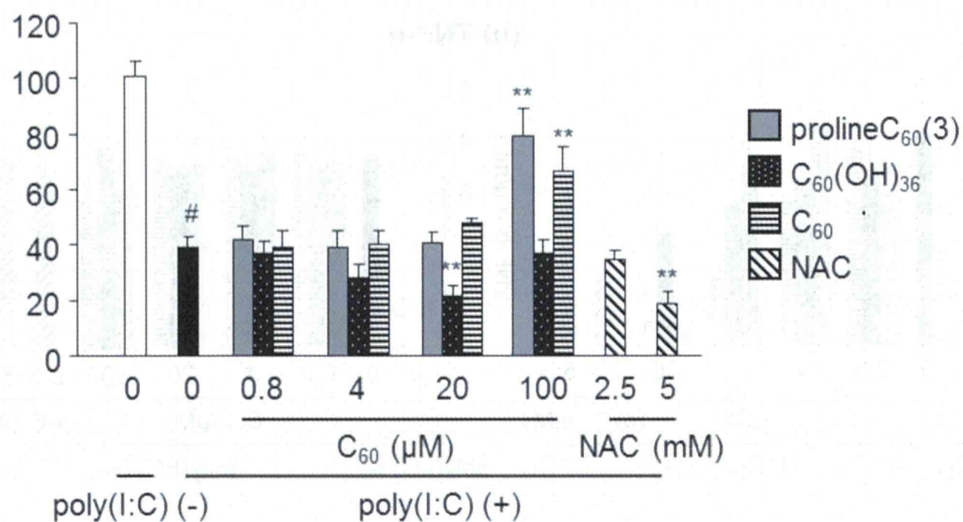
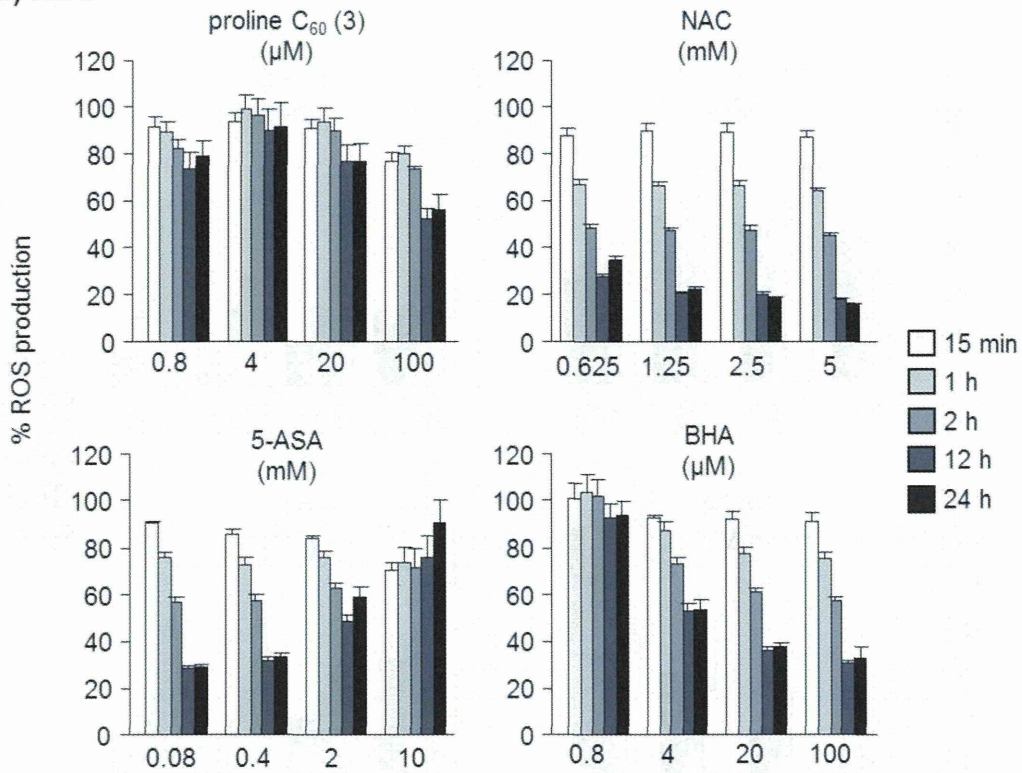


Figure 65 Cytotoxicity of proline-modified fullerene C₆₀ derivatives in the RAW264.7 cells. RAW264.7 cells were treated with proline C₆₀(3), C₆₀(OH)₃₆, C₆₀ or NAC for 30 min, and then, stimulated with poly(I:C) (20 μg/ml) for 24 h. Cell viabilities were assessed by LDH Assay kit. Data are expressed as the mean ± S.D. (n = 4; #*P* < 0.01 versus value for control group by Bonferroni; ***P* < 0.01, **P* < 0.05 versus value for poly(I:C) group by Bonferroni)

(a) ROS



(b) IL-8

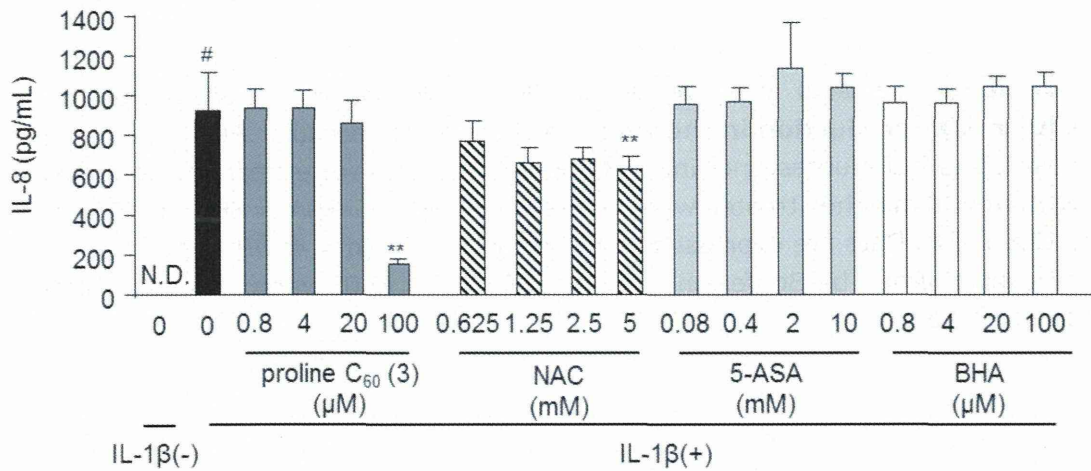


图 66 Inhibitory effects of proline-modified fullerene C₆₀ derivatives and antioxidants on intracellular ROS production and IL-1β-induced IL-8 secretion in the Caco-2 cells. Intracellular ROS production was measured as DCF-fluorescence intensity. Caco-2 cells were treated with 20 μM of DCFH-DA for 20 min then treated with proline C₆₀(3), NAC, 5-ASA or BHA for 15 min, 1h, 2h, 12h or 24h(a). Caco-2 cells were treated with proline C₆₀(3), NAC, 5-ASA or BHA for 30 min, and then, stimulated with IL-1β(125 ng/ml) for 24 h. Secreted IL-8 (b) protein level in the culture supernatant was measured by IL-8 ELISA kit. Data are expressed as the mean ± S.D. (n = 4; [#]P < 0.05 versus value for control group by Bonferroni; ^{**}P < 0.01, ^{*}P < 0.05 versus value for IL-1β group by Bonferroni)

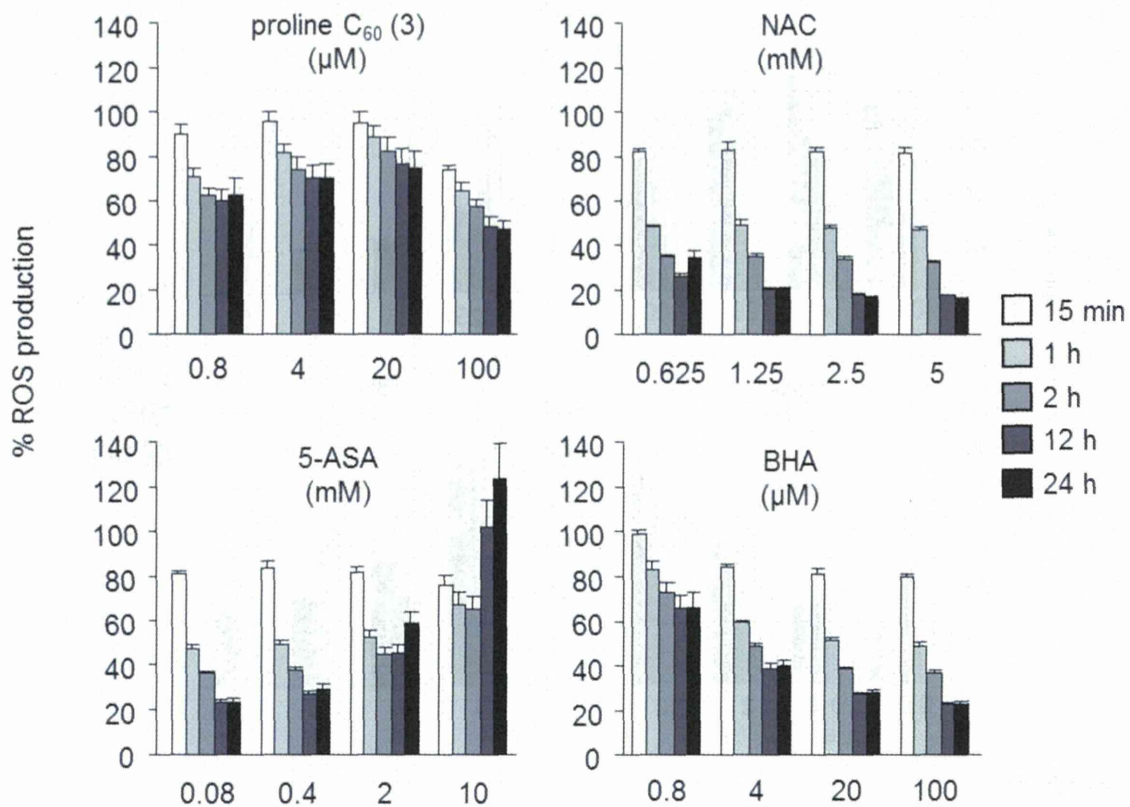


Figure 67 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on intracellular ROS production in the RAW264.7 cells. Intracellular ROS production was measured as DCF-fluorescence intensity. RAW264.7 cells were treated with 20 μM of DCFH-DA for 20 min then treated with proline C₆₀(3), NAC, 5-ASA or BHA for 15 min, 1h, 2h, 12h or 24h. Data are expressed as the mean ± S.D. (n = 4; #P < 0.01 versus value for control group by Bonferroni ; **P < 0.01, *P < 0.05 versus value for LPS group by Bonferroni)

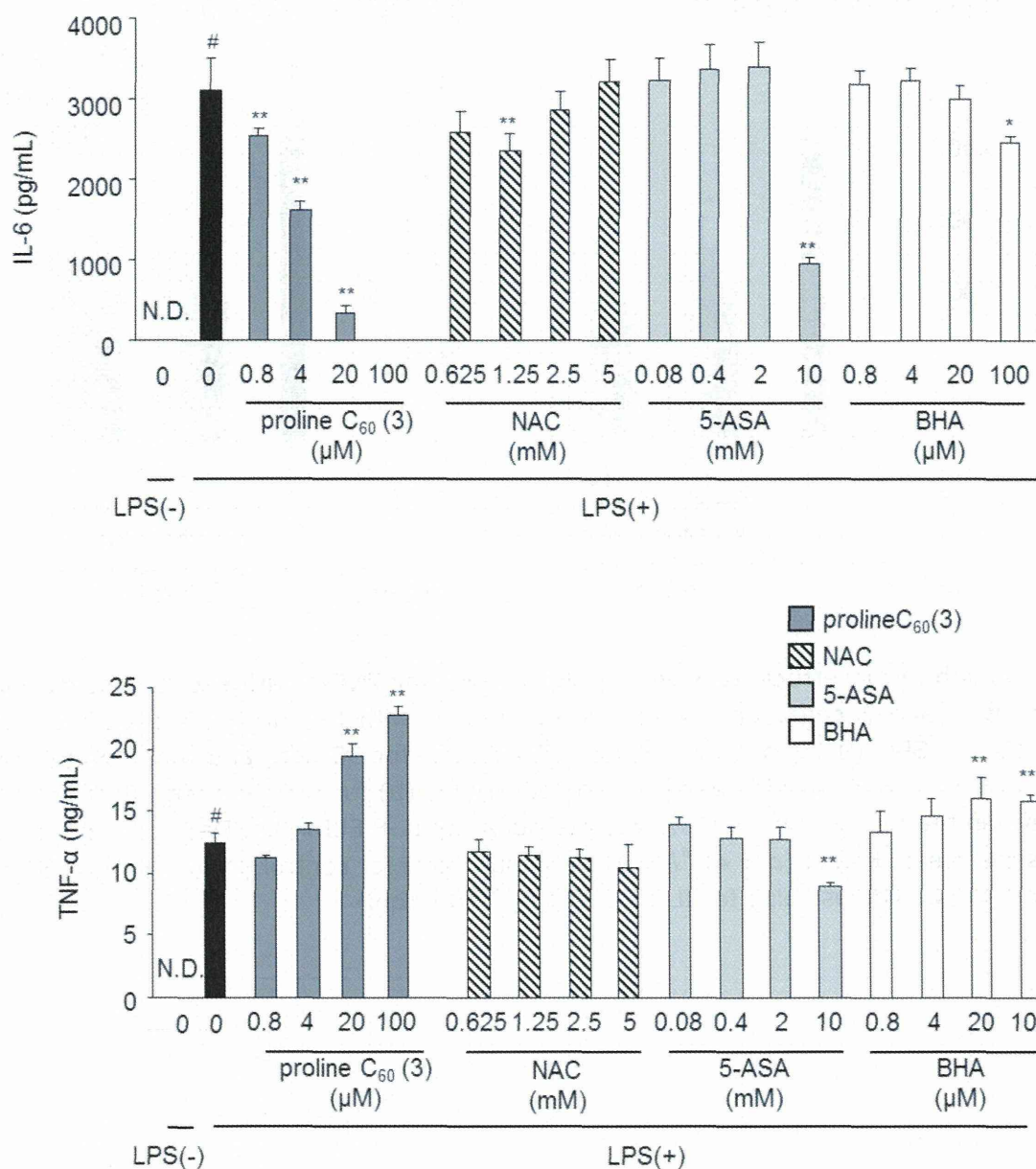


图 68 Inhibitory effects of proline-modified fullerene C₆₀ derivatives on LPS-induced IL-6 or TNF-α secretion in the RAW264.7 cells. RAW264.7 cells were treated with proline C₆₀(3), NAC, 5-ASA or BHA for 30 min, and then, stimulated with LPS (1 μg/ml) for 24 h. Secreted IL-6 or TNF-α protein level in the culture supernatant was measured by IL-6 or TNF-α ELISA kit. Data are expressed as the mean ± S.D. (n = 4; #P < 0.01 versus value for control group by Bonferroni; **P < 0.01, *P < 0.05 versus value for LPS group by Bonferroni)

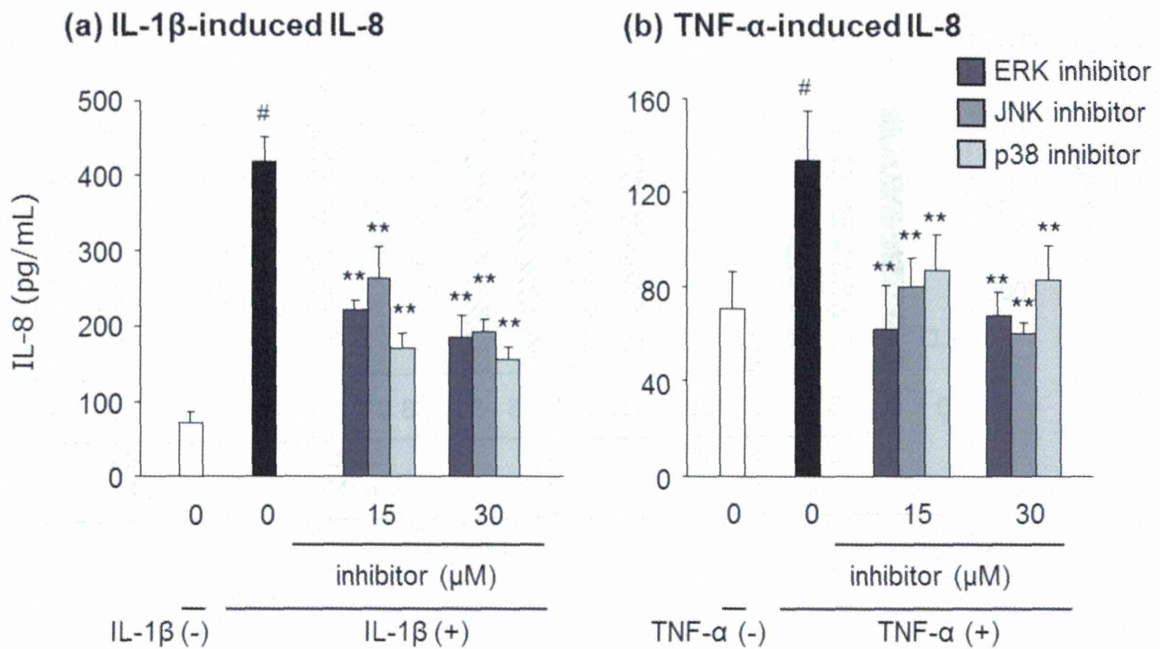


Figure 69 Inhibitory effects of MAPK inhibitor IL-1 β or TNF- α -induced IL-8 secretion in the Caco-2 cells. Caco-2 cells were treated with ERK inhibitor (u0126), JNK inhibitor (SP600125) or p38 inhibitor (SB203580) for 30 min, and then, stimulated with IL-1 β (125 ng/mL) (a) or TNF- α (10 ng/ml) (b) for 6 h. Secreted IL-8 protein level in the culture supernatant was measured by IL-8 ELISA kit. Data are expressed as the mean \pm S.D. (n = 4; [#]*P* < 0.01 versus value for control group by Bonferroni; ^{**}*P* < 0.01 versus value for IL-1 β group by Bonferroni)

