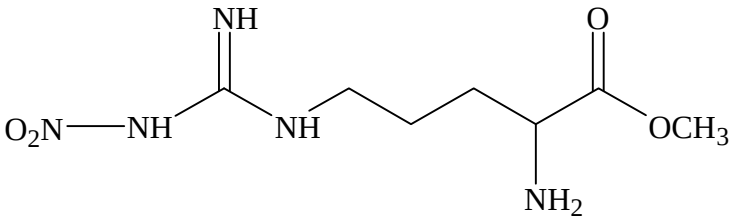
肆、NO (nitric oxide)**及** TNF-α**實驗**

將前述合成出及經結構判定正確之化合物 21-33、41-48、50-53、61-68、70-73、81-93、101-109、111-114、116-118 及 120-123 提供 NO **及** TNF-α**實驗，測試之方法分別採用化合物對於** LPS **刺激細胞培養液中亞硝酸鹽蓄積作用抑制試驗** (Cell line: RAW 264.7 cells)**、化合物對於** LPS+IFN-γ **刺激細胞培養液中亞硝酸鹽蓄積作用抑制試驗** (Cell line: N9 cells)**、化合物對於** LPS **刺激細胞培養液中** TNF-α**形成作用抑制試驗** (Cell line: RAW 264.7 cells)**及化合物對於** LPS+IFN-γ **刺激細胞培養液中** TNF-α**形成作用抑制試驗** (Cell line: N9 cells)**，依其抑制百分率來判定其活性強度，篩選結果分別如：**Table 31 **至** Table 36、Table 37 **至** Table 42、Table 43 **至** Table 48 **及** Table 49 **至** Table 54 **所示。**

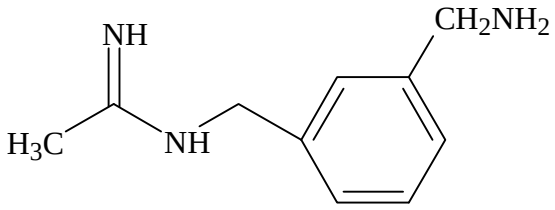
LPS[36]**之全名為** lipopolysaccharide **是屬於一種細菌性內毒素** (bacterial endotoxin)**類。**LPS **其藥理作用是促使** nitric oxide (NO)**及** tumor necrosis factor-alpha (TNF-α)**的生成，而且，**NO **及** TNF-α**兩者皆會引起發炎反應，故** LPS **可作為化合物測定抗發炎的藥理活性試驗時之誘導劑。另外，**IFN-γ[91]**是屬於一種細胞素** (cytokine)**，也會引起發炎反應，故** IFN-γ**亦可作為化合物測定抗發炎的藥理活性試驗時之誘導劑。**

選用 *N* **ω** -nitro-L-arginine methyl ester[92] (L-NAME) **或** *N*-(3-amino- methyl)benzylacetamidine[93] (1400W)**當作** positive control **的原因是** L-NAME **和** 1400W **皆屬於** nitric oxide synthetase (NOS)**的抑制劑，它們可以減少** nitric oxide (NO)**的生成。**

基於上述原因，因此在實驗上選用 L-NAME **或** 1400W **當作** positive control**。然而，由於最初使用** L-NAME **的濃度單位為** mM**，對於實驗結果而言，其濃度明顯過高，因此後來改用** 1400W **的濃度單位為**μM **當作** positive control**。**

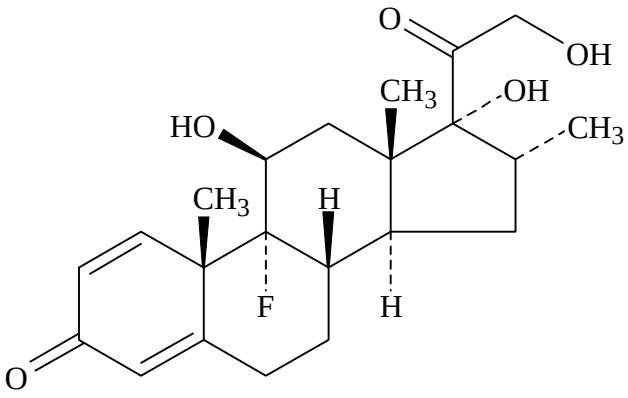


L-NAME



1400W

選用 dexamethasone[(94)] **當作** positive control **的原因是** dexamethasone **可以阻斷** LPS **誘導** TNF-**α的生成，使得** TNF-**α的含量減少，因此在實驗上選用** dexamethasone **當作** positive control。



dexamethasone

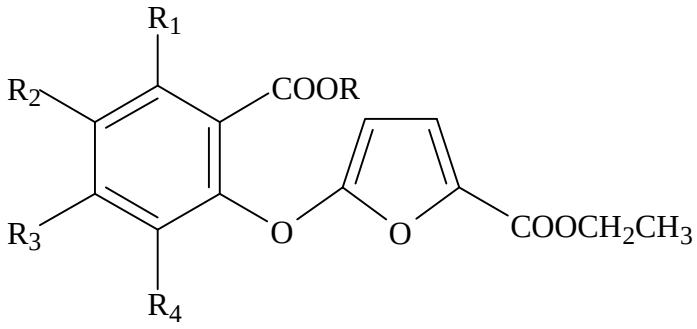
由測試的結果發現：

(一)對於LPS**刺激細胞培養液中亞硝酸鹽蓄積作用抑制試驗** (Cell line: RAW 264.7 cells)

從化合物25-33、45-48、50-53、65-68、70-73、81-93、101-109、111-114、116-118及120-123對以LPS**刺激細胞培養液中亞硝酸鹽蓄積作用之體外試驗中，由**nitrite accumulation**的抑制百分率** (**見**Table 31 **至**Table 36)**看來，在濃度**30 μM**時，化合物25、81、82、85-89、101-105、113及114分別呈現弱的抑制活性** (**具有約**20-48%**的抑制百分率**)**，但是發現化合物83、84及90-93呈現明顯的抑制活性，其抑制**nitrite accumulation**的**IC_{50}**值分別為**30.4±1.1 μM**、**30.2±1.4 μM**、**23.0±1.3 μM**、**15.1±0.2 μM**、**18.7±0.5 μM**及**22.3±0.9 μM**。其他化合物則無明顯的抑制活性。**

綜合上述，發現5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (**81-93**)**類衍生物的活性較明顯。在**5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (**81-93**)**類衍生物中將甲基、氯原子、溴原子或碘原子導入苯環時，具有較高的活性，相較之下，若將甲氧基導入苯環，則其活性降低。**

Table 31. The inhibitory effect of ethyl 5-(2'-alkoxycarbonyl substituted phenoxy)-furan-2-carboxylates on accumulation of nitrite in medium



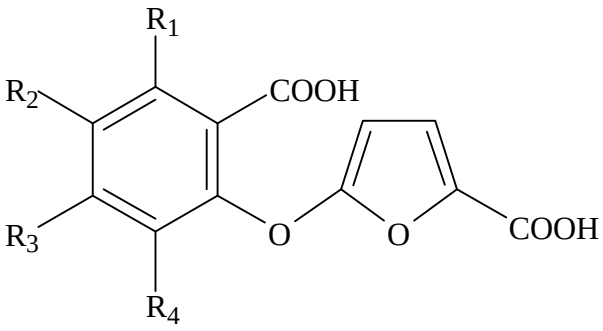
25: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
26: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
27: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
28: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
29: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3
30: R=CH_3, R_1=R_2=R_4=H, R_3=Cl
31: R=CH_3, R_1=R_3=R_4=H, R_2=Cl
32: R=CH_3, R_1=R_3=R_4=H, R_2=Br
33: R=CH_3, R_1=R_3=R_4=H, R_2=I

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	37.6±0.5	--
25	(10)	21.5±0.9**	42.3±2.5
	(30)	29.2±2.9**	22.2±7.7
L-NAME	(0.1 mM)	27.6±1.4**	26.4±3.7
	(0.3 mM)	17.1±3.0**	54.6±8.0
	(1 mM)	8.2±1.2**	78.1±3.2
	IC_{50}	0.42±0.07 mM	
	Control	55.7±0.2	--
26	(10)	54.2±0.6	2.5±1.2
	(30)	54.2±1.1	2.7±2.0
27	(10)	54.3±0.8	2.4±1.4
	(30)	52.2±1.0	6.2±1.8
28	(10)	54.8±0.8	1.5±1.4
	(30)	55.7±1.1	-0.1±2.0
L-NAME	(0.3 mM)	35.4±0.3**	36.4±0.5
	(1 mM)	23.4±0.3**	58.0±0.6
	(3 mM)	10.3±0.3**	81.5±0.7
	IC_{50}	0.91±0.03 mM	
	Control	40.5±0.4	--
29	(10)	38.5±0.4	5.1±1.1
	(30)	34.6±0.2**	14.5±0.5
1400W	(0.1 μM)	31.6±0.2**	21.8±0.7
	(1 μM)	21.9±0.3**	46.0±0.7

	(10 μM)	9.6±0.1**		76.2±0.2
IC_{50}			1.2±0.03 μM	
	Control	55.7±0.2		--
30	(10)	57.0±0.4		-2.4±0.8
	(30)	60.8±2.1		-9.2±3.8
31	(10)	55.0±0.4		1.1±0.8
	(30)	56.8±0.6		-2.0±1.1
32	(10)	54.5±0.3		2.1±0.6
	(30)	57.1±2.9		-2.5±5.2
L-NAME	(0.3 mM)	35.4±0.3**		36.4±0.5
	(1 mM)	23.4±0.3**		58.0±0.6
	(3 mM)	10.3±0.3**		81.5±0.7
	IC_{50}		0.91±0.03 mM	
	Control	49.2±0.5		--
33	(10)	45.1±0.5		8.5±1.1
	(30)	43.7±0.6		11.3±1.4
L-NAME	(0.1 mM)	38.1±0.1**		21.2±0.2
	(0.3 mM)	29.5±0.2**		39.1±0.6
	(1 mM)	19.7±0.2**		59.3±0.5
	IC_{50}		0.71±0.001 mM	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N*ω-nitro-L-arginine methyl ester) or 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean±S.E., N=3-4. *: P<0.05, **: P<0.01.

Table 32. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)furan-2-carboxylic acids on accumulation of nitrite in medium



45: R_2=R_3=R_4=H, R_1=CH_3 **50**: R_1=R_2=R_4=H, R_3=Cl

46: R_1=R_2=R_3=H, R_4=OCH_3 **51**: R_1=R_3=R_4=H, R_2=Cl

47: R_1=R_2=R_4=H, R_3=OCH_3 **52**: R_1=R_3=R_4=H, R_2=Br

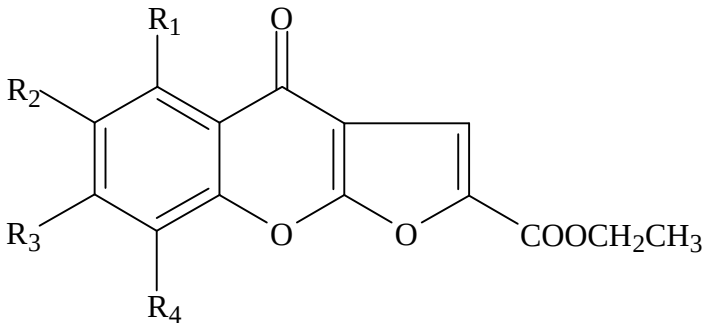
48: R_1=R_3=R_4=H, R_2=OCH_3 **53**: R_1=R_3=R_4=H, R_2=I

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	37.6±0.5	--
45	(10)	35.5±4.0	5.5±10.9
	(30)	34.2±1.3	8.9±3.5
L-NAME	(0.1 mM)	27.6±1.4**	26.4±3.7
	(0.3 mM)	17.1±3.0**	54.6±8.0
	(1 mM)	8.2±1.2**	78.1±3.2
	IC_{50}	0.42±0.07 mM	
	Control	55.7±0.2	--
46	(10)	57.1±0.4	-2.6±0.7
	(30)	57.4±0.4	-3.1±0.7
47	(10)	53.1±0.4	4.5±0.8
	(30)	53.5±0.5	3.9±0.9
48	(10)	54.0±0.5	3.0±0.9
	(30)	54.4±0.4	2.6±0.8
50	(10)	52.9±0.6	5.0±1.2
	(30)	54.4±0.8	2.2±1.4
51	(10)	58.2±0.6	-4.6±1.1
	(30)	58.3±0.7	-4.7±1.4
52	(10)	54.9±0.3	1.3±0.6
	(30)	55.4±0.2	0.5±0.4
L-NAME	(0.3 mM)	35.4±0.3**	36.4±0.5
	(1 mM)	23.4±0.3**	58.0±0.6
	(3 mM)	10.3±0.3**	81.5±0.7
	IC_{50}	0.91±0.03 mM	

	Control	49.2±0.5		--
53	(10)	52.4±0.3		-6.5±0.7
	(30)	49.3±0.7		-0.1±1.4
L-NAME	(0.1 mM)	38.1±0.1**		21.2±0.2
	(0.3 mM)	29.5±0.2**		39.1±0.6
	(1 mM)	19.7±0.2**		59.3±0.5
	IC_{50}		0.71±0.001 mM	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N*ω-nitro-L-arginine methyl ester) is a positive control. Values are presented as mean±S.E., N=3-4. *: P<0.05, **: P<0.01.

Table 33. The inhibitory effect of substituted furo[2,3-*b*]chromone-2-carboxylic acid ethyl esters on accumulation of nitrite in medium



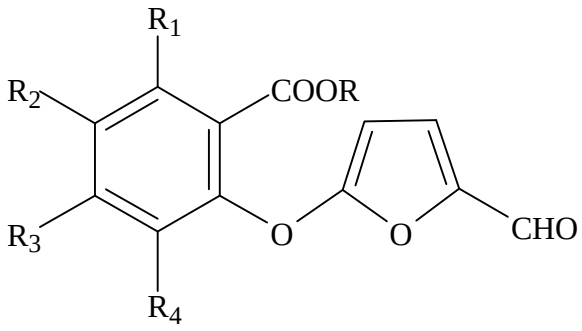
65: R_2=R_3=R_4=H, R_1=CH_3 **70**: R_1=R_2=R_4=H, R_3=Cl
66: R_1=R_2=R_3=H, R_4=OCH_3 **71**: R_1=R_3=R_4=H, R_2=Cl
67: R_1=R_2=R_4=H, R_3=OCH_3 **72**: R_1=R_3=R_4=H, R_2=Br
68: R_1=R_3=R_4=H, R_2=OCH_3 **73**: R_1=R_3=R_4=H, R_2=I

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	34.1±1.4	--
65	(10)	39.4±1.7*	-15.1±5.0
	(30)	37.9±1.1	-10.9±3.3
66	(10)	42.0±2.1**	-23.1±6.2
	(30)	38.5±1.3	-12.7±3.9
67	(10)	40.1±1.3*	-17.5±4.0
	(30)	35.2±1.3	-3.0±4.0
68	(10)	37.8±1.1	-10.7±3.4
	(30)	36.7±1.6	-7.5±4.7
L-NAME	(0.1 mM)	30.9±2.0	9.5±5.8
	(0.3 mM)	20.5±1.6**	39.9±4.7
	(1 mM)	9.4±1.0**	72.5±3.0
	IC_{50}	0.59±0.02 mM	
	Control	60.6±0.7	--
70	(10)	62.5±0.2	-3.1±0.4
	(30)	59.3±0.4	2.1±0.7
71	(10)	60.8±0.2	-0.4±0.3
	(30)	56.5±0.9	6.7±1.5
72	(10)	54.3±0.9*	10.2±1.6
	(30)	52.3±0.5**	13.6±0.9
73	(10)	57.2±0.5	5.5±0.8
	(30)	49.5±1.0**	18.3±1.7
1400W	(0.1 μM)	60.6±0.2	-0.1±0.5
	(1 μM)	40.2±0.4**	33.8±0.9

	(10 μM) 19.7±0.1**	67.3±0.1
IC_{50}		6.1±0.06 μM

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N*ω-nitro-L-arginine methyl ester) or 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 34. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals on accumulation of nitrite in medium



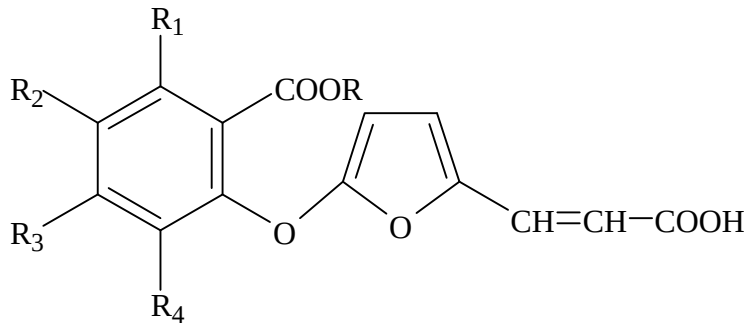
81: R=CH_3, R_1=R_2=R_3=R_4=H
82: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
83: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
84: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
85: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
86: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
87: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
88: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
89: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3
90: R=CH_3, R_1=R_2=R_4=H, R_3=Cl
91: R=CH_3, R_1=R_3=R_4=H, R_2=Cl
92: R=CH_3, R_1=R_3=R_4=H, R_2=Br
93: R=CH_3, R_1=R_3=R_4=H, R_2=I

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	53.7±0.3	--
81	(10)	51.3±0.8	4.8±1.5
	(30)	39.6±0.6**	26.5±1.0
82	(10)	48.4±0.8*	10.2±1.9
	(30)	37.4±1.1**	30.5±2.1
83	(3)	53.8±0.6	0.2±1.5
	(10)	44.3±0.3**	17.8±0.9
	(30)	26.1±0.5**	51.5±1.3
	IC_{50}	30.4±1.1	
84	(3)	54.1±1.5	-0.4±3.0
	(10)	45.2±0.4**	16.1±0.9
	(30)	26.0±0.9**	51.7±1.6
	IC_{50}	30.2±1.4	
85	(10)	42.0±1.5*	22.1±3.2
	(30)	34.1±0.3**	36.6±0.6
1400W	(1)	34.9±0.8**	35.1±1.9
	(3)	27.2±0.2**	49.6±0.6
	(10)	17.2±0.3**	68.0±0.8

	IC_{50}		2.8±0.1	
	Control	40.5±0.4		--
86	(10)	34.4±0.2**		15.0±0.7
	(30)	25.6±0.2**		36.8±0.6
87	(10)	32.4±1.1**		20.1±2.9
	(30)	21.2±0.1**		47.6±0.3
88	(10)	33.1±0.4**		18.2±1.0
	(30)	22.3±0.2**		45.0±0.5
89	(10)	35.0±0.4*		13.6±1.0
	(30)	28.4±0.5**		29.7±1.4
90	(3)	37.5±0.6		7.6±1.7
	(10)	29.8±0.4**		26.4±1.1
	(30)	17.1±0.5**		57.8±1.4
	IC_{50}		23.0±1.3	
91	(3)	34.3±0.7**		15.5±1.7
	(10)	27.0±0.6**		33.4±1.6
	(30)	12.8±0.4**		68.3±1.0
	IC_{50}		15.1±0.2	
92	(3)	35.7±0.6*		11.9±1.5
	(10)	31.0±0.5**		23.4±1.3
	(30)	14.5±0.4**		64.1±1.2
	IC_{50}		18.7±0.5	
93	(3)	37.0±0.3		8.7±0.8
	(10)	31.2±0.4**		23.1±1.1
	(30)	16.5±0.5**		59.2±1.2
	IC_{50}		22.3±0.9	
1400W	(0.1)	31.6±0.2**		21.8±0.7
	(1)	21.9±0.3**		46.0±0.7
	(10)	9.6±0.1**		76.2±0.2
	IC_{50}		1.2±0.03	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean± S.E., N=3. *: P<0.05, **: P<0.01.

Table 35. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)-2-furanacrylic acids on accumulation of nitrite in medium



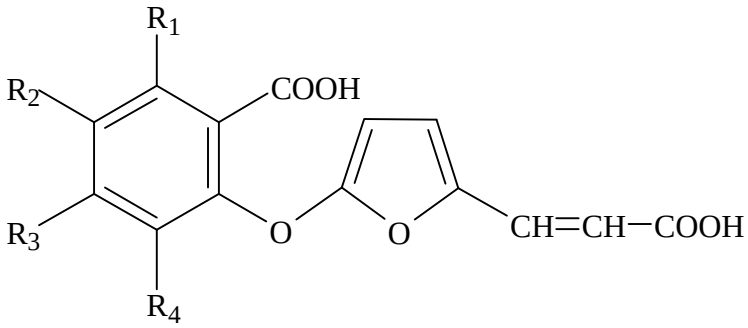
101: R=CH_3, R_1=R_2=R_3=R_4=H
102: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
103: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
104: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
105: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
106: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
107: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
108: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
109: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	53.7±0.3	--
101	(10)	43.2±0.6*	19.9±1.0
	(30)	40.1±1.4**	25.6±2.4
102	(10)	43.4±0.5**	19.5±1.3
	(30)	43.1±0.7**	20.1±1.6
103	(10)	42.6±1.3**	21.0±2.8
	(30)	35.4±0.9**	34.3±2.1
104	(10)	41.8±1.4**	22.5±2.9
	(30)	43.1±0.7**	20.0±1.7
105	(10)	42.7±0.8*	20.8±1.2
	(30)	39.8±1.4**	26.3±2.3
1400W	(1)	34.9±0.8**	35.1±1.9
	(3)	27.2±0.2**	49.6±0.6
	(10)	17.2±0.3**	68.0±0.8
	IC_{50}	2.8±0.1	
	Control	40.5±0.4	--
106	(10)	39.0±1.2	3.6±3.0
	(30)	38.5±0.1	4.9±0.1
107	(10)	37.1±1.0	8.5±2.4
	(30)	34.4±0.9**	15.2±2.2

108	(10)	38.3±0.2	5.5±0.6
	(30)	35.6±0.5*	12.3±1.3
109	(10)	37.4±0.3	7.6±0.8
	(30)	36.4±0.5	10.3±1.3
1400W	(0.1)	31.6±0.2**	21.8±0.7
	(1)	21.9±0.3**	46.0±0.7
	(10)	9.6±0.1**	76.2±0.2
	IC_{50}	1.2±0.03	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean± S.E., N=3. *: P<0.05, **: P<0.01.

Table 36. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)-2-furanacrylic acids on accumulation of nitrite in medium



111: $R_1=R_2=R_3=R_4=H$
112: $R_1=R_2=R_3=H$, $R_4=CH_3$
113: $R_1=R_2=R_4=H$, $R_3=CH_3$
114: $R_1=R_3=R_4=H$, $R_2=CH_3$
116: $R_1=R_2=R_3=H$, $R_4=OCH_3$
117: $R_1=R_2=R_4=H$, $R_3=OCH_3$
118: $R_1=R_3=R_4=H$, $R_2=OCH_3$
120: $R_1=R_2=R_4=H$, $R_3=Cl$
121: $R_1=R_3=R_4=H$, $R_2=Cl$
122: $R_1=R_3=R_4=H$, $R_2=Br$
123: $R_1=R_3=R_4=H$, $R_2=I$

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	53.7±0.3	--
111	(10)	55.1±1.1	-2.1±2.4
	(30)	50.4±1.2	6.6±1.8
112	(10)	41.7±0.7**	22.7±1.0
	(30)	43.8±1.6**	18.7±2.6
113	(10)	39.7±1.2**	26.3±1.8
	(30)	42.2±0.8**	21.7±1.1
114	(10)	41.4±0.3**	23.2±0.8
	(30)	42.7±1.6	20.7±3.0
1400W	(1)	34.9±0.8**	35.1±1.9
	(3)	27.2±0.2**	49.6±0.6
	(10)	17.2±0.3**	68.0±0.8
	IC_{50}	2.8±0.1	
	Control	40.5±0.4	--
116	(10)	38.2±0.2	5.7±0.7
	(30)	35.4±0.3*	12.7±0.9
117	(10)	36.8±0.4	9.2±1.1
	(30)	35.8±0.6*	11.6±1.4
118	(10)	38.7±0.3	4.4±0.8

	(30)	36.2±0.4	10.7±1.0
120	(10)	39.6±1.0	2.4±1.4
	(30)	37.0±0.3	8.6±0.9
121	(10)	39.8±0.2	1.9±0.6
	(30)	37.0±0.3	8.8±0.7
122	(10)	38.5±0.1	5.0±0.4
	(30)	38.2±2.1	5.8±5.2
123	(10)	34.9±1.2*	14.0±3.0
	(30)	37.3±0.6	8.0±1.7
1400W	(0.1)	31.6±0.2**	21.8±0.7
	(1)	21.9±0.3**	46.0±0.7
	(10)	9.6±0.1**	76.2±0.2
	IC_{50}	1.2±0.03	

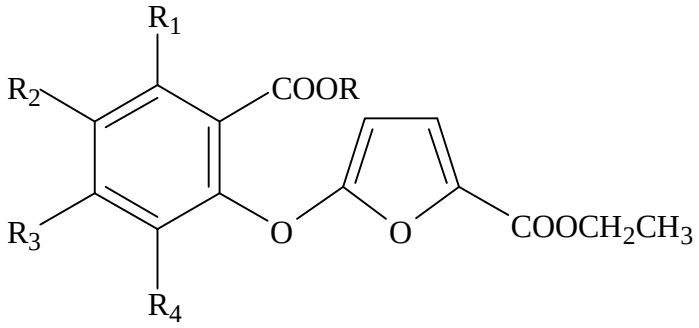
Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean± S.E., N=3. *: P<0.05, **: P<0.01.

(二)對於LPS+IFN-**γ刺激細胞培養液中亞硝酸鹽蓄積作用抑制試驗**
(Cell line: N9 cells)

從化合物25-33、45-48、50-53、65-68、70-73、81-93、101-109、111-114、116-118及120-123對以LPS+IFN-**γ刺激細胞培養液中亞硝酸鹽蓄積作用之體外試驗中，由**nitrite accumulation**的抑制百分率**(見Table 37**至**Table 42)**看來，在濃度**30 μM**時，化合物26、30、66、82、86、103及105分別呈現弱的抑制活性**(具有約21-45%**的抑制百分率)，但是發現化合物27、28、32、83、84、87、88、90及91呈現明顯的抑制活性，其抑制**nitrite accumulation**的**IC_{50}**值分別為**28.1±0.5 μM、18.8±1.2 μM、20.0±0.7 μM、21.6±0.8 μM、21.9±0.2 μM、17.1±0.3 μM、17.5±0.3 μM、15.1±0.5 μM及**10.8±0.2 μM。**其他化合物則無明顯的抑制活性。**

綜合上述，發現ethyl 5-(2'-alkoxycarbonyl substituted phenoxy)-furan-2-carboxylates (**21-33**) **類及** 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (**81-93**)**類衍生物的活性較明顯。在**ethyl 5-(2'-alkoxy- carbonyl substituted phenoxy)furan-2-carboxylates (**21-33**) **類衍生物中將甲氧基或溴原子導入苯環時，具有較高的活性，相較之下，若將氯原子導入苯環，則其活性降低，此外，若將碘原子導入苯環，則其活性降得更低。另外，在**5-(2'-alkoxycarbonyl substituted phenoxy)-furfurals (**81-93**)**類衍生物中將甲基、甲氧基或氯原子導入苯環時，具有較高的活性，但是若將溴原子或碘原子導入苯環，則在濃度**30 μM**時，呈現細胞毒性。**

Table 37. The inhibitory effect of ethyl 5-(2'-alkoxycarbonyl substituted phenoxy)-furan-2-carboxylates on accumulation of nitrite in medium



25: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3 **30**: R=CH_3, R_1=R_2=R_4=H, R_3=Cl

26: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3 **31**: R=CH_3, R_1=R_3=R_4=H, R_2=Cl

27: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3 **32**: R=CH_3, R_1=R_3=R_4=H, R_2=Br

28: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3 **33**: R=CH_3, R_1=R_3=R_4=H, R_2=I

29: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

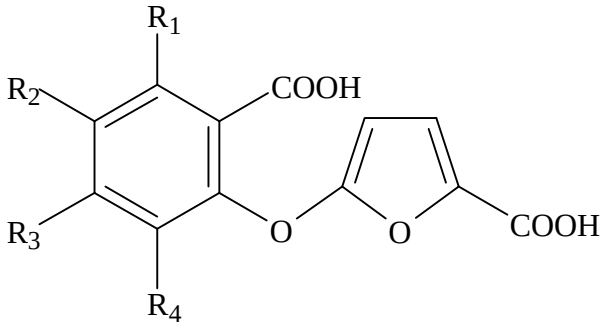
		Nitrite accumulation		
Compound	conc.			
	(μM)	μM		(%inh)
	Control	52.0±0.7		--
25	(3)	52.8±0.5		-1.5±1.0
	(10)	48.6±0.2		6.6±0.4
L-NAME	(0.1 mM)	44.8±1.6*		23.4±5.8
	(0.3 mM)	32.3±0.8**		44.4±3.2
	(1 mM)	21.1±0.5**		64.8±2.0
	IC_{50}		0.59±0.002 mM	
	Control	23.7±0.2		--
26	(10)	19.3±0.2**		18.8±1.3
	(30)	13.2±2.2**		44.7±8.9
27	(3)	22.2±0.8		6.6±4.1
	(10)	19.8±0.5**		16.3±2.7
	(30)	11.1±0.2**		53.4±0.7
	IC_{50}		28.1±0.5	
28	(3)	21.9±1.5		7.9±6.6
	(10)	16.6±0.6**		30.3±2.2
	(30)	6.1±0.8**		74.5±3.0
	IC_{50}		18.8±1.2	
L-NAME	(0.1 mM)	12.6±0.9**		46.8±4.4
	(0.3 mM)	7.5±0.5**		68.6±2.1
	(1 mM)	2.0±0.5**		91.4±2.1
	IC_{50}		0.3±0.01 mM	
	Control	37.6±0.4		--

29	(10)	34.4±0.5		8.3±1.5
	(30)	33.5±0.3		10.9±1.0
1400W	(0.1 μM)	32.0±0.6**		15.1±1.8
	(1 μM)	25.6±0.2**		32.3±0.2
	(10 μM)	13.7±0.1**		63.8±0.3
	IC_{50}		3.3±0.1 μM	
	Control	23.7±0.2		--
30	(10)	22.8±0.1		3.9±0.9
	(30)	15.6±1.2**		34.3±5.1
31	(10)	22.6±0.9		4.7±4.8
	(30)	19.3±2.3**		18.4±10.4
32	(3)	22.9±0.2		3.5±1.5
	(10)	20.2±0.1*		14.9±1.2
	(30)	4.5±0.6**		80.8±3.0
	IC_{50}		20.0±0.7	
L-NAME	(0.1 mM)	12.6±0.9**		46.8±4.4
	(0.3 mM)	7.5±0.5**		68.6±2.1
	(1 mM)	2.0±0.5**		91.4±2.1
	IC_{50}		0.3±0.01 mM	
	Control	51.5±1.6		--
33	(10)	51.1±0.6		1.0±1.2
	(30)	45.9±0.8*		11.1±1.6
L-NAME	(0.1 mM)	44.8±1.3**		12.9±2.5
	(0.3 mM)	32.8±0.3**		36.2±0.6
	(1 mM)	25.4±0.4**		50.6±0.9
	IC_{50}		0.84±0.001 mM	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N* ω -nitro-L-arginine methyl ester) or 1400W (*N*-(3-aminomethyl)benzyl-acetamidine) is a positive control. Values are presented as mean±S.E., N=3-4. *: P<0.05, **: P<0.01.

ps：**凡最高濃度小於** 30 μM **者，皆為於高濃度時有細胞毒性。**

Table 38. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)furan-2-carboxylic acids on accumulation of nitrite in medium



45: $R_2=R_3=R_4=H$, $R_1=CH_3$ **50**: $R_1=R_2=R_4=H$, $R_3=Cl$

46: $R_1=R_2=R_3=H$, $R_4=OCH_3$ **51**: $R_1=R_3=R_4=H$, $R_2=Cl$

47: $R_1=R_2=R_4=H$, $R_3=OCH_3$ **52**: $R_1=R_3=R_4=H$, $R_2=Br$

48: $R_1=R_3=R_4=H$, $R_2=OCH_3$ **53**: $R_1=R_3=R_4=H$, $R_2=I$

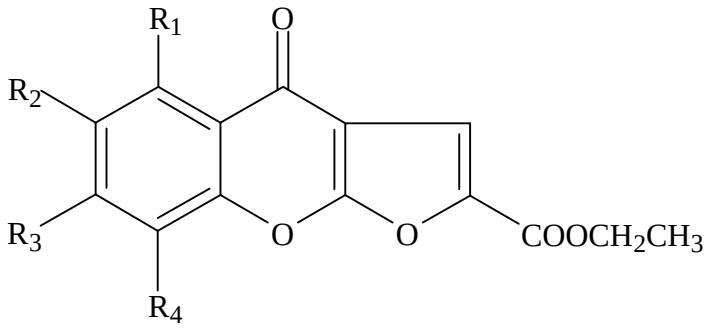
Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	52.0±0.7	--
45	(3)	55.6±0.8	-7.0±1.7
	(10)	54.0±1.1	-3.8±2.1
L-NAME	(0.1 mM)	44.8±1.6*	23.4±5.8
	(0.3 mM)	32.3±0.8**	44.4±3.2
	(1 mM)	21.1±0.5**	64.8±2.0
	IC_{50}	0.59±0.002 mM	
	Control	24.3±0.5	--
46	(10)	24.3±0.7	0.04±2.0
	(30)	23.4±0.5	3.5±4.3
47	(10)	23.7±0.8	2.7±1.0
	(30)	21.9±0.6	9.6±4.1
48	(10)	23.9±1.6	1.9±4.2
	(30)	22.8±0.2	6.2±1.7
50	(10)	24.7±1.4	-1.4±4.5
	(30)	21.4±0.6	11.8±4.0
51	(10)	22.3±1.0	8.1±5.3
	(30)	24.5±0.1	-0.7±2.0
52	(10)	24.1±0.7	1.0±1.7
	(30)	24.3±0.7	0.01±2.7
L-NAME	(0.1 mM)	12.6±0.9**	46.8±4.4
	(0.3 mM)	7.5±0.5**	68.6±2.1
	(1 mM)	2.0±0.5**	91.4±2.1

	IC_{50}	0.3±0.01 mM	
	Control	51.5±1.6	--
53	(10)	48.0±0.7	7.1±1.4
	(30)	49.1±0.6	5.0±1.3
L-NAME	(0.1 mM)	44.8±1.3**	12.9±2.5
	(0.3 mM)	32.8±0.3**	36.2±0.6
	(1 mM)	25.4±0.4**	50.6±0.9
	IC_{50}	0.84±0.001 mM	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N*ω-nitro-L-arginine methyl ester) is a positive control. Values are presented as mean±S.E., N=3-4. *: P<0.05, **: P<0.01.

ps：**凡最高濃度小於** 30 μM **者，皆為於高濃度時有細胞毒性。**

Table 39. The inhibitory effect of substituted furo[2,3-*b*]chromone-2-carboxylic acid ethyl esters on accumulation of nitrite in medium



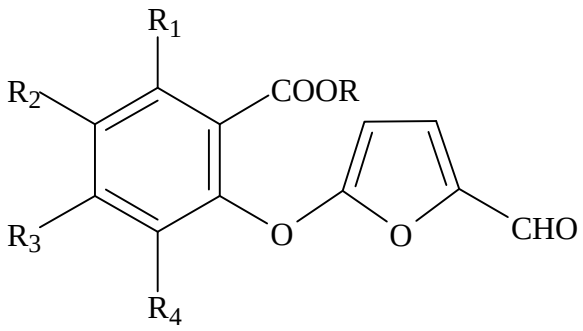
65: R_2=R_3=R_4=H, R_1=CH_3 **70**: R_1=R_2=R_4=H, R_3=Cl
66: R_1=R_2=R_3=H, R_4=OCH_3 **71**: R_1=R_3=R_4=H, R_2=Cl
67: R_1=R_2=R_4=H, R_3=OCH_3 **72**: R_1=R_3=R_4=H, R_2=Br
68: R_1=R_3=R_4=H, R_2=OCH_3 **73**: R_1=R_3=R_4=H, R_2=I

		Nitrite accumulation	
Compound	conc.		
	(μM)	μM	(%inh)
	Control	34.2±0.7	--
65	(10)	30.4±1.6**	11.0±4.8
	(30)	27.3±0.1**	19.9±0.4
66	(10)	29.6±1.8**	13.5±5.5
	(30)	26.9±4.3**	21.1±12.6
67	(10)	33.2±0.4	2.5±1.3
	(30)	31.4±0.5*	8.1±1.5
68	(10)	30.5±0.6**	10.7±2.0
	(30)	29.2±0.9**	14.4±2.7
L-NAME	(0.1 mM)	32.2±1.3	5.6±3.9
	(0.3 mM)	20.7±0.3**	39.4±1.0
	(1 mM)	12.2±0.3**	64.3±1.0
	IC_{50}	0.67±0.003 mM	
	Control	48.6±0.7	--
70	(10)	47.2±0.4	2.9±0.9
	(30)	46.2±0.5	5.0±1.2
71	(10)	45.7±1.1	6.0±2.3
	(30)	44.4±0.8*	8.6±1.6
72	(10)	45.3±0.5	6.7±1.1
	(30)	44.5±0.4	8.5±0.8
73	(10)	45.5±0.3	6.4±0.7
	(30)	41.1±0.3**	15.4±0.6
1400W	(0.1 μM)	46.5±1.0	4.1±2.8

(1 μM)	34.3±0.3**	29.1±0.9
(10 μM)	16.8±0.1**	65.3±0.4
IC_{50}	6.6±0.1 μM	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. L-NAME (*N* ω -nitro-L-arginine methyl ester) or 1400W (*N*-(3-aminomethyl)benzyl-acetamidine) is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 40. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals on accumulation of nitrite in medium



81: R=CH_3, R_1=R_2=R_3=R_4=H
82: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
83: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
84: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
85: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
86: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
87: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
88: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
89: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3
90: R=CH_3, R_1=R_2=R_4=H, R_3=Cl
91: R=CH_3, R_1=R_3=R_4=H, R_2=Cl
92: R=CH_3, R_1=R_3=R_4=H, R_2=Br
93: R=CH_3, R_1=R_3=R_4=H, R_2=I

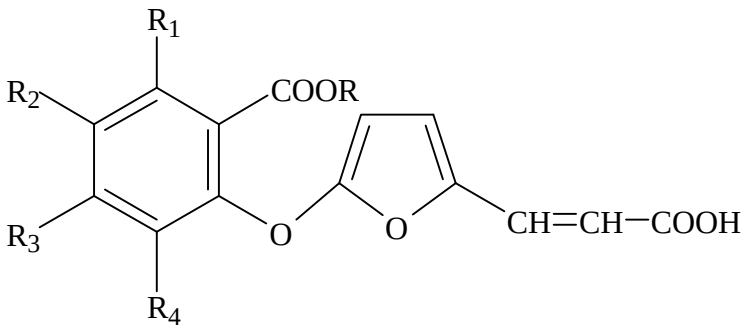
Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	38.5±0.3	--
81	(10)	39.2±0.4	-1.8±1.2
	(30)	31.9±0.6**	17.0±1.7
82	(10)	33.3±1.4**	13.3±3.6
	(30)	27.9±0.3**	27.5±0.9
83	(3)	39.0±0.4	-1.4±1.2
	(10)	28.9±0.5**	24.8±1.5
	(30)	15.1±0.3**	60.7±0.8
	IC_{50}	21.6±0.8	
84	(3)	38.9±0.4	-1.1±1.2
	(10)	27.5±0.1**	28.3±0.2
	(30)	15.8±0.1**	58.8±0.2
	IC_{50}	21.9±0.2	
85	(10)	38.2±0.5	0.6±1.4
	(30)	35.3±0.3	8.1±0.9
1400W	(0.1)	34.9±0.4*	9.4±1.1

	(1)	27.6±0.4**		28.1±1.0
	(10)	14.1±0.4**		63.4±1.0
	IC_{50}		3.9±0.1	
Control		37.6±0.4		--
86	(10)	35.3±0.5		6.4±0.8
	(30)	24.5±0.4**		35.0±0.9
87	(3)	37.8±0.6		-0.03±1.6
	(10)	31.0±0.1**		17.8±0.9
	(30)	10.3±0.2**		72.8±0.7
	IC_{50}		17.1±0.3	
88	(3)	37.9±0.3		-0.2±1.1
	(10)	32.2±0.2*		14.8±0.5
	(30)	10.4±0.5**		72.4±1.3
	IC_{50}		17.5±0.3	
89	(10)	36.1±0.1		3.7±1.9
	(30)	33.2±1.4*		11.6±1.9
90	(3)	34.5±0.5		8.5±1.3
	(10)	29.6±0.5**		21.6±1.0
	(30)	9.2±0.5**		75.4±1.5
	IC_{50}		15.1±0.5	
91	(3)	35.8±0.8		5.2±1.6
	(10)	28.4±0.6**		24.8±1.3
	(30)	0.05±0.3**		99.8±0.9
	IC_{50}		10.8±0.2	
92	(3)	37.3±0.2		1.3±0.9
	(10)	27.3±0.7**		27.9±1.6
	(30)		Cytotoxic	
93	(3)	36.1±0.5		4.4±0.8
	(10)	24.7±0.9**		34.8±2.5
	(30)		Cytotoxic	
1400W	(0.1)	32.0±0.6**		15.1±1.8
	(1)	25.6±0.2**		32.3±0.2
	(10)	13.7±0.1**		63.8±0.3
	IC_{50}		3.3±0.1	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are

presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 41. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)-2-furanacrylic acids on accumulation of nitrite in medium



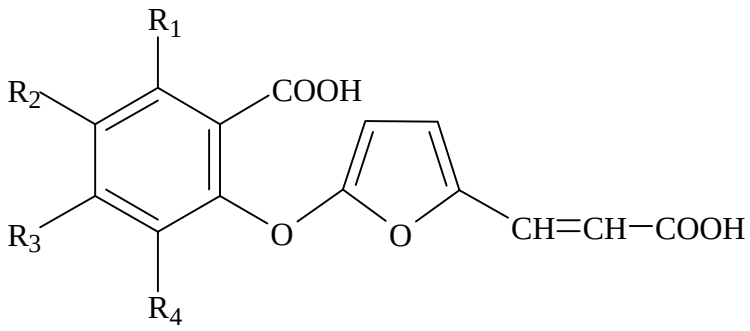
101: R=CH_3, R_1=R_2=R_3=R_4=H
102: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
103: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
104: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
105: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
106: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
107: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
108: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
109: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	38.5±0.3	--
101	(10)	38.2±0.2	0.6±0.5
	(30)	36.2±0.1	6.0±0.4
102	(10)	35.9±0.6	6.6±1.6
	(30)	35.1±1.2	9.0±3.1
103	(10)	30.8±0.9**	19.9±2.4
	(30)	27.0±0.5**	29.8±1.3
104	(10)	39.9±1.7	-3.5±4.5
	(30)	34.5±1.7*	10.1±4.4
105	(10)	32.1±0.9**	16.6±2.4
	(30)	29.6±0.5**	23.1±1.5
1400W	(0.1)	34.9±0.4*	9.4±1.1
	(1)	27.6±0.4**	28.1±1.0
	(10)	14.1±0.4**	63.4±1.0
	IC_{50}	3.9±0.1	
	Control	37.6±0.4	--
106	(10)	41.4±2.3	-10.1±3.5
	(30)	37.3±2.6	1.0±4.6

107	(10)	35.0±0.9	6.7±0.2
	(30)	34.9±2.3	7.2±4.1
108	(10)	36.1±1.1	3.9±3.0
	(30)	35.4±0.7	5.8±1.8
109	(10)	34.1±0.2	9.3±0.6
	(30)	34.6±0.2	7.9±0.7
1400W	(0.1)	32.0±0.6**	15.1±1.8
	(1)	25.6±0.2**	32.3±0.2
	(10)	13.7±0.1**	63.8±0.3
	IC_{50}	3.3±0.1	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 42. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)-2-furanacrylic acids on accumulation of nitrite in medium



111: $R_1=R_2=R_3=R_4=H$
112: $R_1=R_2=R_3=H$, $R_4=CH_3$
113: $R_1=R_2=R_4=H$, $R_3=CH_3$
114: $R_1=R_3=R_4=H$, $R_2=CH_3$
116: $R_1=R_2=R_3=H$, $R_4=OCH_3$
117: $R_1=R_2=R_4=H$, $R_3=OCH_3$
118: $R_1=R_3=R_4=H$, $R_2=OCH_3$
120: $R_1=R_2=R_4=H$, $R_3=Cl$
121: $R_1=R_3=R_4=H$, $R_2=Cl$
122: $R_1=R_3=R_4=H$, $R_2=Br$
123: $R_1=R_3=R_4=H$, $R_2=I$

Compound	conc. (μM)	Nitrite accumulation μM	(%inh)
	Control	38.5±0.3	--
111	(10)	42.7±0.4	-11.1±1.1
	(30)	40.0±0.2	-4.1±0.7
112	(10)	40.9±0.2	-6.2±0.7
	(30)	37.5±0.4	2.4±1.1
113	(10)	39.1±0.2	-1.7±0.7
	(30)	34.5±0.8	10.1±2.3
114	(10)	39.5±0.2	-2.6±0.5
	(30)	35.9±0.6	6.7±1.7
1400W	(0.1)	34.9±0.4*	9.4±1.1
	(1)	27.6±0.4**	28.1±1.0
	(10)	14.1±0.4**	63.4±1.0
	IC_{50}	3.9±0.1	
	Control	37.6±0.4	--
116	(10)	33.5±0.2	10.7±0.6
	(30)	34.5±0.2	8.2±0.7
117	(10)	34.3±0.6	8.8±1.7

	(30)	35.6±0.1	5.3±0.3
118	(10)	35.9±0.7	4.5±2.0
	(30)	34.3±0.8	8.8±2.2
120	(10)	36.1±0.3	3.8±0.8
	(30)	35.0±0.6	6.8±1.6
121	(10)	35.4±0.4	5.6±1.2
	(30)	34.1±0.1	9.3±0.3
122	(10)	34.1±0.7	8.9±2.0
	(30)	33.6±0.5	10.5±1.5
123	(10)	38.9±1.4	-3.5±3.9
	(30)	34.5±1.1	2.9±3.0
1400W	(0.1)	32.0±0.6**	15.1±1.8
	(1)	25.6±0.2**	32.3±0.2
	(10)	13.7±0.1**	63.8±0.3
	IC_{50}	3.3±0.1	

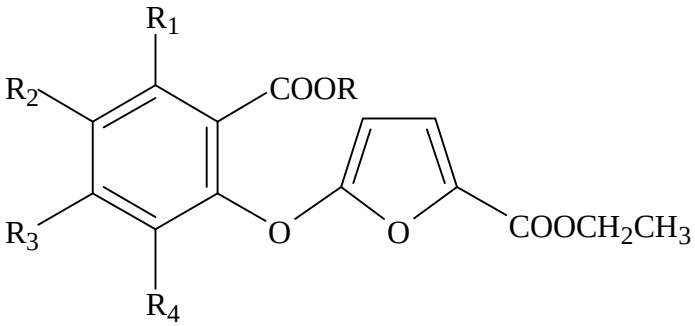
Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. The production of NO was determined in cell medium by measuring the content of nitrite based on the Griess reaction. 1400W (*N*-(3-aminomethyl)benzylacetamidine) is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

(三)對於LPS刺激細胞培養液中TNF-α形成作用抑制試驗 (Cell line: RAW 264.7 cells)

從化合物25-33、45-48、50-53、65-68、70-73、81-93、101-109、111-114、116-118及120-123對以LPS刺激細胞培養液中TNF-α形成作用之體外試驗中，由TNF-α formation的抑制百分率 (見Table 43至Table 48)看來，在濃度30 μM時，化合物30-33、46、51、66、68、82、84、86、88、89、101-104、112及120分別呈現弱的抑制活性 (具有約30-49%的抑制百分率)，但是發現化合物81、83、87及90-93呈現明顯的抑制活性，其抑制TNF-α formation的IC_{50}值分別為10.3±0.9 μM、17.3±2.4 μM、18.4±4.1 μM、14.4±0.5 μM、17.9±3.1 μM、10.4±0.6 μM及21.8±9.1 μM。其他化合物則無明顯的抑制活性。

綜合上述，發現5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (81-93)類衍生物的活性較明顯。在5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (81-93)類衍生物中溴原子導入苯環時，具有較高的活性，相較之下，若將甲基或氯原子導入苯環，則其活性降低，此外，將甲氧基或碘原子導入苯環，則其活性降得更低。

Table 43. The inhibitory effect of ethyl 5-(2'-alkoxycarbonyl substituted phenoxy)-furan-2-carboxylates on TNF-α formation in medium



25: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3 **30**: R=CH_3, R_1=R_2=R_4=H, R_3=Cl

26: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3 **31**: R=CH_3, R_1=R_3=R_4=H, R_2=Cl

27: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3 **32**: R=CH_3, R_1=R_3=R_4=H, R_2=Br

28: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3 **33**: R=CH_3, R_1=R_3=R_4=H, R_2=I

29: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	(μM)	% inhibition
25	(10)	28.2±3.9
Dexamethasone	(0.01)	22.0±4.2*
	(1)	41.0±4.7**
	(10)	49.9±1.7**
26	(30)	16.9
27	(30)	6.2
28	(30)	12.8
Dexamethasone	(1)	41.1±4.75
	(10)	49.9±1.71
29	(30)	-2.5±6.9
Dexamethasone	(0.01)	22.4±4.8
	(0.1)	42.5±2.0**
	(1)	55.4±9.5**
	IC_{50}	0.42±0.12
30	(30)	41.0
31	(30)	38.5
32	(30)	42.9
Dexamethasone	(1)	41.1±4.75
	(10)	49.9±1.71
33	(30)	30.6±2.8**
Dexamethasone	(0.1)	48.1±8.2**
	(1)	61.7±8.1**
	(10)	64.9±10.5**

	IC_{50}	1.25±0.83

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Control value: 73.2±10.5 ng/ml TNF-α (compound: **25**)

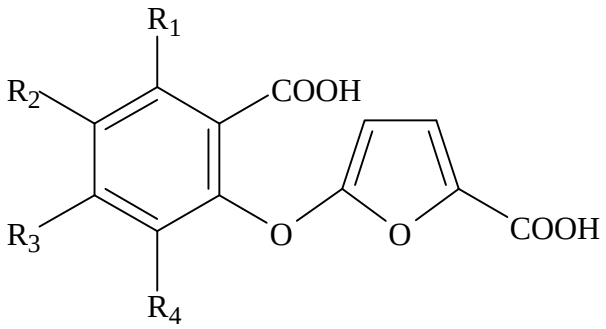
Control value: 60.7 ng/ml TNF-α (compound: **26, 27, 28, 30, 31, 32**)

Control value: 92.5±8.5 ng/ml TNF-α (compound: **29**)

Control value: 182.5±14.2 ng/ml TNF-α (compound: **33**)

ps：**凡最高濃度小於** 30 μM **者，皆為於高濃度時有細胞毒性。**

Table 44. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)furan-2-carboxylic acids on TNF-α formation in medium



45: R_2=R_3=R_4=H, R_1=CH_3 **50**: R_1=R_2=R_4=H, R_3=Cl

46: R_1=R_2=R_3=H, R_4=OCH_3 **51**: R_1=R_3=R_4=H, R_2=Cl

47: R_1=R_2=R_4=H, R_3=OCH_3 **52**: R_1=R_3=R_4=H, R_2=Br

48: R_1=R_3=R_4=H, R_2=OCH_3 **53**: R_1=R_3=R_4=H, R_2=I

Compound	(μM)	% inhibition
45	(10)	33.2±11.2
Dexamethasone	(0.01)	22.0±4.2*
	(1)	41.0±4.7**
	(10)	49.9±1.7**
46	(30)	49.1
47	(30)	59.5
48	(30)	52.6
50	(30)	55.3
51	(30)	40.5
52	(30)	52.1
Dexamethasone	(1)	41.1±4.75
	(10)	49.9±1.71
53	(30)	-5.4±2.0
Dexamethasone	(0.1)	48.1±8.2**
	(1)	61.7±8.1**
	(10)	64.9±10.5**
	IC_{50}	1.25±0.83

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

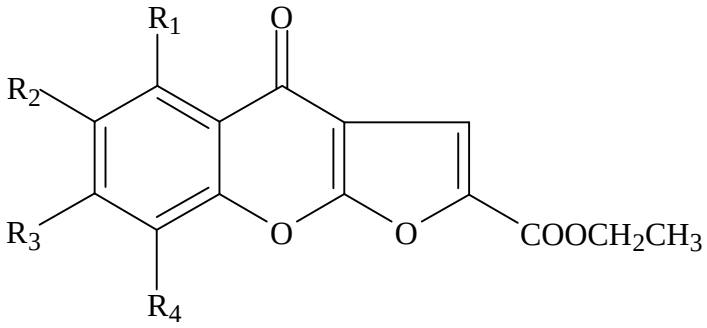
Control value: 73.2±10.5 ng/ml TNF-α (compound: **45**)

Control value: 60.7 ng/ml TNF-α (compound: **46, 47, 48, 50, 51, 52**)

Control value: 182.5±14.2 ng/ml TNF-α (compound: **53**)

ps：**凡最高濃度小於** 30 **μ**M **者，皆為於高濃度時有細胞毒性。**

Table 45. The inhibitory effect of substituted furo[2,3-*b*]chromone-2-carboxylic acid ethyl esters on TNF-α formation in medium



65: R_2=R_3=R_4=H, R_1=CH_3 **70**: R_1=R_2=R_4=H, R_3=Cl
66: R_1=R_2=R_3=H, R_4=OCH_3 **71**: R_1=R_3=R_4=H, R_2=Cl
67: R_1=R_2=R_4=H, R_3=OCH_3 **72**: R_1=R_3=R_4=H, R_2=Br
68: R_1=R_3=R_4=H, R_2=OCH_3 **73**: R_1=R_3=R_4=H, R_2=I

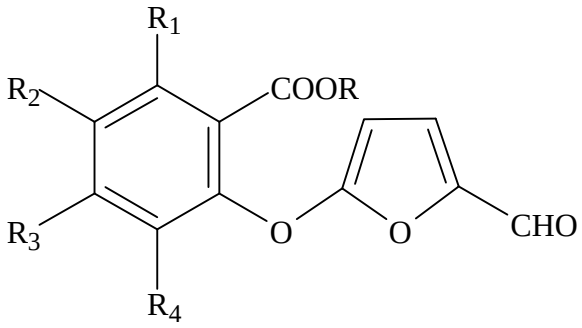
Compound	(μM)	% inhibition
65	(30)	15.6±15.7
66	(30)	43.9±4.6**
67	(30)	25.1±11.4*
68	(30)	30.4±11.5**
Dexamethasone	(0.01)	22.0±4.2*
	(1)	41.0±4.7**
	(10)	49.9±1.7**
70	(30)	23.4±10.8
71	(30)	21.7±7.1
72	(30)	9.4±1.0
73	(30)	8.5±3.8
Dexamethasone	(0.01)	22.0±4.2*
	(1)	41.0±4.7**
	(10)	49.9±1.7**

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Control value: 102.5±10.4 ng/ml TNF-α (compound: **65, 66, 67, 68**)

Control value: 58.4±1.0 ng/ml TNF-α (compound: **70, 71, 72, 73**)

Table 46. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals on TNF-α formation in medium



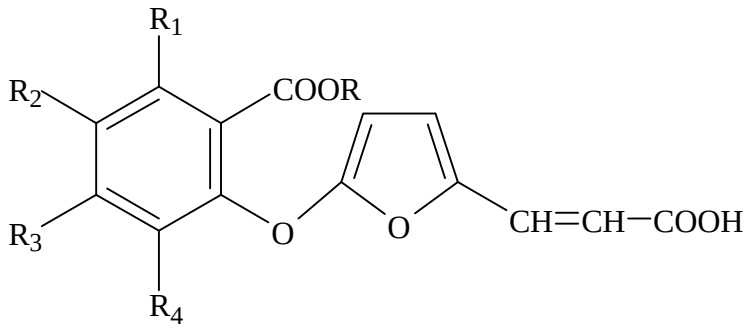
81: R=CH_3, R_1=R_2=R_3=R_4=H
82: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
83: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
84: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
85: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
86: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
87: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
88: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
89: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3
90: R=CH_3, R_1=R_2=R_4=H, R_3=Cl
91: R=CH_3, R_1=R_3=R_4=H, R_2=Cl
92: R=CH_3, R_1=R_3=R_4=H, R_2=Br
93: R=CH_3, R_1=R_3=R_4=H, R_2=I

Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	55.8±1.1	--
81	(3)	35.8±0.3**	35.1±3.5
	(10)	27.6±1.3**	50.2±3.0
	(30)	21.0±0.1**	61.8±1.7
	IC_{50}	10.3±0.9	
82	(30)	29.2±0.3**	47.3±1.0
83	(3)	62.7±2.9	-13.5±0.6
	(10)	42.5±2.3**	22.6±4.7
	(30)	23.7±1.1**	57.5±1.5
	IC_{50}	17.3±2.4	
84	(10)	54.0±2.4	2.2±2.2
	(30)	30.6±0.7**	44.5±1.2
85	(30)	39.0±0.8**	29.6±2.7
Dexamethasone	(0.01)	43.0±3.9**	22.4±4.8
	(0.1)	31.0±2.9**	42.5±2.0
	(1)	24.9±6.0**	55.4±9.5

	IC_{50}		0.42±0.12	
	Control	92.5±8.5		--
86	(30)	51.4±6.0**		44.4±0.5
87	(3)	71.4±4.9		21.7±7.8
	(10)	57.1±2.2**		37.8±4.2
	(30)	37.8±0.8**		58.7±3.0
	IC_{50}		18.4±4.1	
88	(30)	46.5±4.3**		49.1±5.2
89	(30)	49.0±7.9**		45.8±10.6
90	(3)	73.1±14.6		21.4±4.6
	(10)	60.1±13.9*		35.6±5.0
	(30)	29.4±3.9**		67.6±4.4
	IC_{50}		14.4±0.5	
91	(3)	94.9±1.7		-3.3±6.1
	(10)	60.1±9.0*		33.8±9.6
	(30)	31.9±1.2**		65.1±2.5
	IC_{50}		17.9±3.1	
92	(3)	66.5±7.5		28.3±2.7
	(10)	56.3±8.6**		39.9±3.8
	(30)	21.1±1.6**		76.5±4.1
	IC_{50}		10.4±0.6	
93	(3)	72.4±4.6		20.7±7.9
	(10)	63.2±0.3		30.5±6.6
	(30)	34.8±4.0**		61.3±7.6
	IC_{50}		21.8±9.1	
Dexamethasone	(0.01)	71.4±2.1		22.4±4.8
	(0.1)	51.2±6.0**		42.5±2.0
	(1)	41.1±2.9**		55.4±9.5
	IC_{50}		0.42±0.12	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 47. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)-2-furanacrylic acids on TNF-α formation in medium



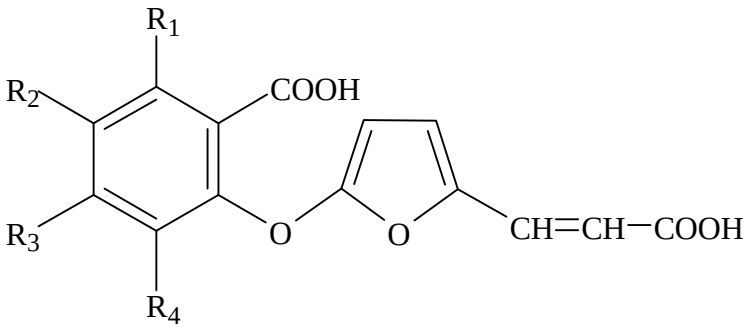
101: R=CH_3, R_1=R_2=R_3=R_4=H
102: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
103: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
104: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
105: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
106: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
107: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
108: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
109: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	55.8±1.1	--
101	(30)	33.9±1.3**	38.9±2.5
102	(30)	36.3±0.7**	34.7±1.7
103	(30)	37.3±1.0**	32.4±5.1
104	(30)	30.7±1.4**	44.6±3.5
105	(30)	43.7±0.8**	20.6±6.7
Dexamethasone	(0.01)	43.0±3.9**	22.4±4.8
	(0.1)	31.0±2.9**	42.5±2.0
	(1)	24.9±6.0**	55.4±9.5
	IC_{50}	0.42±0.12	
	Control	92.5±8.5	--
106	(30)	66.6±3.2	26.1±10.3
107	(30)	85.1±4.9	7.4±3.3
108	(30)	84.3±5.5	7.7±7.9
109	(30)	87.1±1.4	4.3±8.3
Dexamethasone	(0.01)	71.4±2.1	22.4±4.8
	(0.1)	51.2±6.0**	42.5±2.0

(1)	41.1±2.9**	55.4±9.5
IC_{50}		0.42±0.12

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 48. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)-2-furanacrylic acids on TNF-α formation in medium



111: $R_1=R_2=R_3=R_4=H$
112: $R_1=R_2=R_3=H$, $R_4=CH_3$
113: $R_1=R_2=R_4=H$, $R_3=CH_3$
114: $R_1=R_3=R_4=H$, $R_2=CH_3$
116: $R_1=R_2=R_3=H$, $R_4=OCH_3$
117: $R_1=R_2=R_4=H$, $R_3=OCH_3$
118: $R_1=R_3=R_4=H$, $R_2=OCH_3$
120: $R_1=R_2=R_4=H$, $R_3=Cl$
121: $R_1=R_3=R_4=H$, $R_2=Cl$
122: $R_1=R_3=R_4=H$, $R_2=Br$
123: $R_1=R_3=R_4=H$, $R_2=I$

Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	55.8±1.1	--
111	(30)	41.0±1.9**	26.0±3.2
112	(30)	35.4±1.7**	36.2±5.6
113	(30)	39.6±0.4**	28.5±3.4
114	(30)	42.9±0.3**	22.6±3.9
Dexamethasone	(0.01)	43.0±3.9**	22.4±4.8
	(0.1)	31.0±2.9**	42.5±2.0
	(1)	24.9±6.0**	55.4±9.5
	IC_{50}	0.42±0.12	
	Control	92.5±8.5	--
116	(30)	83.6±2.3	8.4±6.9
117	(30)	86.7±3.3	4.7±9.2
118	(30)	66.1±1.1	27.0±8.1
120	(30)	60.4±2.2*	33.0±9.0
121	(30)	70.5±0.8	22.4±7.8

122	(30)	83.1±7.2	10.1±0.5
123	(30)	70.1±4.3	29.7±8.2
Dexamethasone	(0.01)	71.4±2.1	22.4±4.8
	(0.1)	51.2±6.0**	42.5±2.0
	(1)	41.1±2.9**	55.4±9.5
	IC_{50}	0.42±0.12	

Pretreatment of RAW 264.7 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (1 μg/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

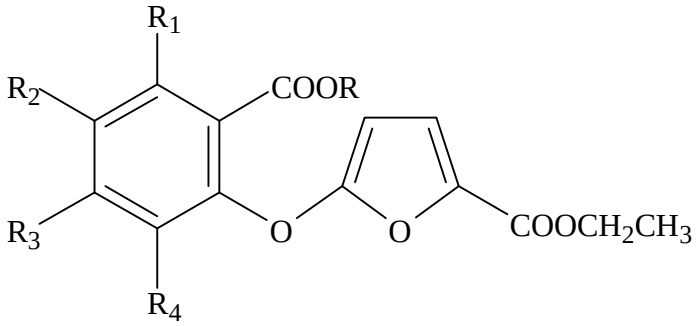
(四)對於LPS+IFN-**γ刺激細胞培養液中**TNF-**α形成作用抑制試驗**

(Cell line: N9 cells)

從化合物 25-33、45-48、50-53、65-68、70-73、81-93、101-109、111-114、116-118 及 120-123 對以 LPS+IFN-**γ刺激細胞培養液中** TNF-**α形成作用之體外試驗中，由** TNF-**α** formation **的抑制百分率** (**見** Table 49 **至** Table 54)**看來，在濃度** 30 μM **時，化合物 26-30、32、33、72、73、81、82、85、86 及 103 分別呈現弱的抑制活性** (**具有約** 30-49% **的抑制百分率**)**，但是發現化合物 83、84、87、88 及 90-93 呈現明顯的抑制活性，其抑制** TNF-**α** formation **的** IC_{50} **值分別為** 17.7±1.2 μM、20.7±2.3 μM、14.7±1.6 μM、22.1±3.0 μM、12.1±0.3 μM、14.5±1.6 μM、13.0±5.9 μM **及** 10.6±1.7 μM**。其他化合物則無明顯的抑制活性。**

綜合上述，發現 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (**81-93**)**類衍生物的活性較明顯。在** 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals (**81-93**)**類衍生物中將氯原子、溴原子或碘原子導入苯環時，具有較高的活性，相較之下，若將甲基或甲氧基導入苯環，則其活性降低，但是若將溴原子或碘原子導入苯環，則在濃度** 30 μM **時，呈現細胞毒性。**

Table 49. The inhibitory effect of ethyl 5-(2'-alkoxycarbonyl substituted phenoxy)-furan-2-carboxylates on TNF-α formation in medium



25: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3 **30**: R=CH_3, R_1=R_2=R_4=H, R_3=Cl

26: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3 **31**: R=CH_3, R_1=R_3=R_4=H, R_2=Cl

27: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3 **32**: R=CH_3, R_1=R_3=R_4=H, R_2=Br

28: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3 **33**: R=CH_3, R_1=R_3=R_4=H, R_2=I

29: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	(μM)	% inhibition
25	(10)	-9.4±11.6
Dexamethasone	(0.01)	20.1±6.1*
	(0.1)	74.3±3.1**
	(10)	82.0±3.8**
	IC_{50}	0.25±0.14
26	(30)	31.3
27	(30)	49.0
28	(30)	48.4
Dexamethasone	(0.01)	15.2±3.2
	(0.1)	51.7±4.6
	(1)	59.0±3.2
	IC_{50}	0.50±0.03
29	(30)	37.9±5.0*
Dexamethasone	(0.01)	31.1±0.9*
	(0.1)	55.6±2.4**
	(1)	72.9±0.9**
	IC_{50}	0.074±0.01
30	(30)	37.4
31	(30)	29.8
32	(30)	46.3
Dexamethasone	(0.01)	15.2±3.2
	(0.1)	51.7±4.6

	(1)	59.0±3.2
	IC_{50}	0.50±0.03
33	(30)	45.0±3.9**
Dexamethasone	(0.1)	32.0±1.6**
	(1)	35.9±0.6**
	(10)	48.6±3.8**

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Control value: 1.4±0.2 ng/ml TNF-α (compound: **25**)

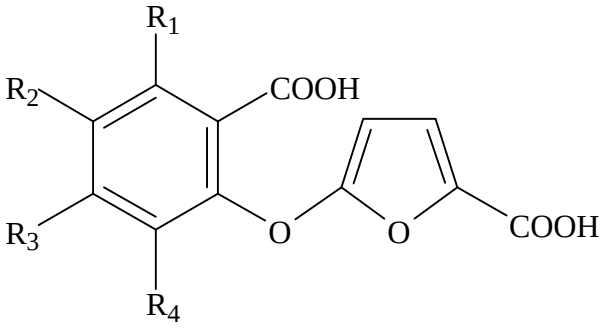
Control value: 4.2 ng/ml TNF-α (compound: **26, 27, 28, 30, 31, 32**)

Control value: 1.93±0.08 ng/ml TNF-α (compound: **29**)

Control value: 13.8±1.35 ng/ml TNF-α (compound: **33**)

ps：**凡最高濃度小於** 30 μM **者，皆為於高濃度時有細胞毒性。**

Table 50. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)furan-2-carboxylic acids on TNF-α formation in medium



45: R_2=R_3=R_4=H, R_1=CH_3 **50**: R_1=R_2=R_4=H, R_3=Cl

46: R_1=R_2=R_3=H, R_4=OCH_3 **51**: R_1=R_3=R_4=H, R_2=Cl

47: R_1=R_2=R_4=H, R_3=OCH_3 **52**: R_1=R_3=R_4=H, R_2=Br

48: R_1=R_3=R_4=H, R_2=OCH_3 **53**: R_1=R_3=R_4=H, R_2=I

Compound	(μM)	% inhibition
45	(10)	-23.3±1.6
Dexamethasone	(0.01)	20.1±6.1*
	(0.1)	74.3±3.1**
	(10)	82.0±3.8**
	IC_{50}	0.25±0.14
46	(30)	6.9
47	(30)	10.3
48	(30)	-17.2
50	(30)	20.7
51	(30)	12.1
52	(30)	15.0
Dexamethasone	(0.01)	15.2±3.2
	(0.1)	51.7±4.6
	(1)	59.0±3.2
	IC_{50}	0.50±0.03
53	(30)	19.9±1.4
Dexamethasone	(0.1)	32.0±1.6**
	(1)	35.9±0.6**
	(10)	48.6±3.8**

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the

manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

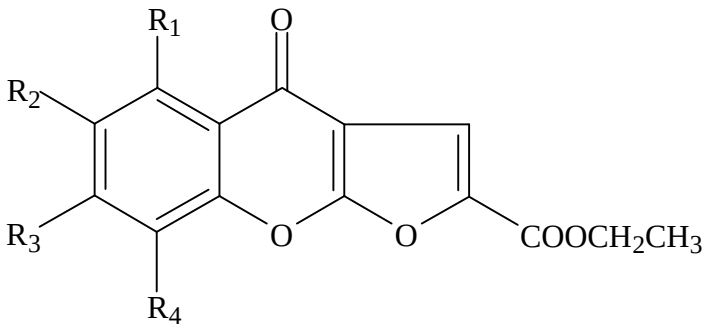
Control value: 1.4±0.2 ng/ml TNF-α (compound: **45**)

Control value: 4.2 ng/ml TNF-α (compound: **46, 47, 48, 50, 51, 52**)

Control value: 13.8±1.35 ng/ml TNF-α (compound: **53**)

ps：**凡最高濃度小於** 30 **μ**M **者，皆為於高濃度時有細胞毒性。**

Table 51. The inhibitory effect of substituted furo[2,3-*b*]chromone-2-carboxylic acid ethyl esters on TNF-α formation in medium



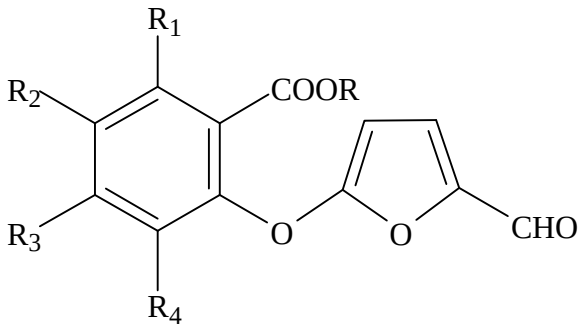
65: $R_2=R_3=R_4=H$, $R_1=CH_3$ **70**: $R_1=R_2=R_4=H$, $R_3=Cl$
66: $R_1=R_2=R_3=H$, $R_4=OCH_3$ **71**: $R_1=R_3=R_4=H$, $R_2=Cl$
67: $R_1=R_2=R_4=H$, $R_3=OCH_3$ **72**: $R_1=R_3=R_4=H$, $R_2=Br$
68: $R_1=R_3=R_4=H$, $R_2=OCH_3$ **73**: $R_1=R_3=R_4=H$, $R_2=I$

Compound	(μM)	% inhibition
65	(30)	23.2±4.2*
66	(30)	4.8±14.3
67	(30)	18.7±8.0
68	(30)	21.7±1.6*
Dexamethasone	(0.01)	20.1±6.1*
	(0.1)	74.3±3.1**
	(10)	82.0±3.8**
	IC_{50}	0.25±0.14
70	(30)	24.2±2.8**
71	(30)	27.7±3.4**
72	(30)	30.2±2.1**
73	(30)	33.9±3.3**
Dexamethasone	(0.01)	20.1±6.1*
	(0.1)	74.3±3.1**
	(10)	82.0±3.8**
	IC_{50}	0.25±0.14

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: $P<0.05$, **: $P<0.01$.

Control value: 1.8±0.01 ng/ml TNF-α (compound: **65, 66, 67, 68**)
Control value: 3.9±0.27 ng/ml TNF-α (compound: **70, 71, 72, 73**)

Table 52. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)furfurals on TNF-α formation in medium



81: R=CH_3, R_1=R_2=R_3=R_4=H
82: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
83: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
84: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
85: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
86: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
87: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
88: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
89: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3
90: R=CH_3, R_1=R_2=R_4=H, R_3=Cl
91: R=CH_3, R_1=R_3=R_4=H, R_2=Cl
92: R=CH_3, R_1=R_3=R_4=H, R_2=Br
93: R=CH_3, R_1=R_3=R_4=H, R_2=I

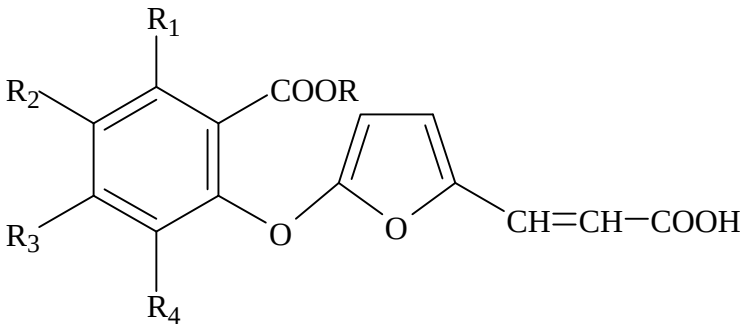
Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	2.14±0.06	--
81	(30)	1.14±0.02**	46.7±2.3
82	(30)	1.43±0.02**	33.0±3.6
83	(3)	1.85±0.07	13.3±4.5
	(10)	1.38±0.01**	35.0±0.7
	(30)	0.79±0.03**	62.5±2.1
	IC_{50}	17.7±1.2	
84	(3)	1.92±0.05	10.2±3.4
	(10)	1.42±0.06**	33.1±3.9
	(30)	0.88±0.03**	58.6±2.0
	IC_{50}	20.7±2.3	
85	(30)	1.21±0.07**	43.5±4.6
Dexamethasone	(0.01)	1.50±0.01**	31.1±0.9
	(0.1)	0.97±0.04**	55.6±2.4

	(1)	0.59±0.02**		72.9±0.9
	IC_{50}		0.074±0.009	
Control		1.93±0.08		--
86	(30)	1.11±0.11**		42.6±4.0
87	(3)	1.56±0.19		18.5±12.2
	(10)	1.17±0.06**		39.2±0.6
	(30)	0.66±0.10**		65.9±3.6
	IC_{50}		14.7±1.6	
88	(3)	1.76±0.07		8.9±3.7
	(10)	1.37±0.12		29.2±3.6
	(30)	0.80±0.08**		58.6±2.7
	IC_{50}		22.1±3.0	
89	(30)	1.34±0.15		29.7±10.9
90	(3)	1.50±0.12		22.2±3.2
	(10)	1.06±0.08**		45.0±1.8
	(30)	0.60±0.01**		68.4±1.6
	IC_{50}		12.1±0.3	
91	(3)	2.21±0.18		-14.1±8.0
	(10)	1.31±0.08*		32.4±3.1
	(30)	0.36±0.08**		81.1±4.5
	IC_{50}		14.5±1.6	
92	(1)	1.78±0.01		7.4±3.6
	(3)	1.54±0.01		19.9±3.8
	(10)	0.91±0.11**		52.1±7.5
	(30)		Cytotoxic	
	IC_{50}		13.0±5.9	
93	(1)	2.03±0.14		-5.1±7.8
	(3)	1.81±0.11		6.0±7.3
	(10)	0.95±0.07**		50.6±4.3
	(30)		Cytotoxic	
	IC_{50}		10.6±1.7	
Dexamethasone	(0.01)	1.33±0.01*		31.1±0.9
	(0.1)	0.82±0.04**		55.6±2.4
	(1)	0.51±0.02**		72.9±0.9
	IC_{50}		0.074±0.01	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the

manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 53. The inhibitory effect of 5-(2'-alkoxycarbonyl substituted phenoxy)-2-furanacrylic acids on TNF-α formation in medium



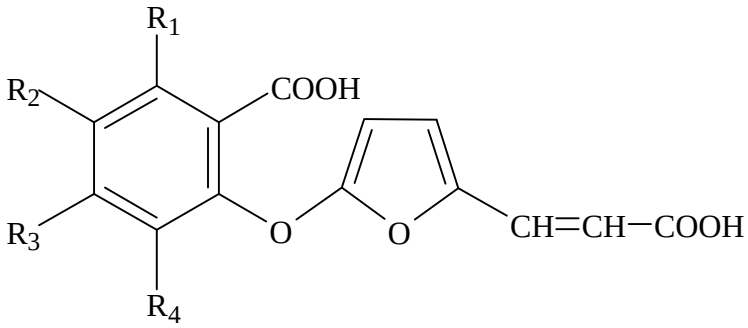
101: R=CH_3, R_1=R_2=R_3=R_4=H
102: R=CH_3, R_1=R_2=R_3=H, R_4=CH_3
103: R=CH_3, R_1=R_2=R_4=H, R_3=CH_3
104: R=CH_3, R_1=R_3=R_4=H, R_2=CH_3
105: R=C_2H_5, R_2=R_3=R_4=H, R_1=CH_3
106: R=CH_3, R_1=R_2=R_3=H, R_4=OCH_3
107: R=CH_3, R_1=R_2=R_4=H, R_3=OCH_3
108: R=CH_3, R_1=R_3=R_4=H, R_2=OCH_3
109: R=CH_3, R_2=R_3=R_4=H, R_1=OCH_3

Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	2.14±0.06	--
101	(30)	1.56±0.06**	27.1±4.1
102	(30)	1.89±0.04	11.5±4.4
103	(30)	1.22±0.07**	42.9±2.3
104	(30)	1.50±0.06**	29.9±4.3
105	(30)	1.95±0.03	8.8±0.9
Dexamethasone	(0.01)	1.50±0.01**	31.1±0.9
	(0.1)	0.97±0.04**	55.6±2.4
	(1)	0.59±0.02**	72.9±0.9
	IC_{50}	0.074±0.009	
	Control	1.93±0.08	--
106	(30)	1.93±0.13	-0.1±5.3
107	(30)	1.51±0.23	22.3±9.9
108	(30)	1.56±0.22	17.5±12.1
109	(30)	1.92±0.17	0.6±8.6
Dexamethasone	(0.01)	1.33±0.01*	31.1±0.9
	(0.1)	0.82±0.04**	55.6±2.4

(1)	0.51±0.02**	72.9±0.9
IC_{50}	0.074±0.01	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.

Table 54. The inhibitory effect of 5-(2'-carboxyl substituted phenoxy)-2-furanacrylic acids on TNF-α formation in medium



111: $R_1=R_2=R_3=R_4=H$
112: $R_1=R_2=R_3=H$, $R_4=CH_3$
113: $R_1=R_2=R_4=H$, $R_3=CH_3$
114: $R_1=R_3=R_4=H$, $R_2=CH_3$
116: $R_1=R_2=R_3=H$, $R_4=OCH_3$
117: $R_1=R_2=R_4=H$, $R_3=OCH_3$
118: $R_1=R_3=R_4=H$, $R_2=OCH_3$
120: $R_1=R_2=R_4=H$, $R_3=Cl$
121: $R_1=R_3=R_4=H$, $R_2=Cl$
122: $R_1=R_3=R_4=H$, $R_2=Br$
123: $R_1=R_3=R_4=H$, $R_2=I$

Compound	conc. (μM)	TNF-α formation ng/ml	(%inh)
	Control	2.14±0.06	--
111	(30)	2.80±0.04	-30.7±5.4
112	(30)	2.36±0.05	-10.4±4.9
113	(30)	1.85±0.06	13.4±5.0
114	(30)	2.18±0.06	-1.7±5.0
Dexamethasone	(0.01)	1.50±0.01**	31.1±0.9
	(0.1)	0.97±0.04**	55.6±2.4
	(1)	0.59±0.02**	72.9±0.9
	IC_{50}	0.074±0.009	
	Control	1.93±0.08	--
116	(30)	1.85±0.16	4.2±9.4
117	(30)	1.72±0.20	10.8±10.1
118	(30)	1.74±0.10	9.3±7.1
120	(30)	1.65±0.05	14.1±5.9
121	(30)	1.59±0.10	17.7±3.3
122	(30)	1.66±0.03	13.7±2.0

123	(30)	1.72±0.06	10.1±7.2
Dexamethasone	(0.01)	1.33±0.01*	31.1±0.9
	(0.1)	0.82±0.04**	55.6±2.4
	(1)	0.51±0.02**	72.9±0.9
	IC_{50}	0.074±0.01	

Pretreatment of N9 cells with test drugs at 37 ℃ for 1h before stimulation with LPS (10 ng/ ml) plus IFN-γ (10 U/ml) for 24h, and then the medium was collected and stored at –70 ℃ until used. TNF-α in medium was measured by use of a EIA kit according to the procedure described by the manufacturers. Dexamethasone is a positive control. Values are presented as mean±S.E., N=3. *: P<0.05, **: P<0.01.