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Short communication

Synthesis of novel cinnamanilides as potential immunosuppressive agents

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ABSTRACT

A series of new cinnamanilides (**6**–**40**) were synthesized and their immunosuppressive activity and cytotoxicity were evaluated. Most of the cinnamanilides showed good immunosuppressive activity. Among the synthesized compounds, (*Z*)-N-(4-bromophenyl)-2-methoxy-3-(4-methoxyphenyl)acrylamide (**37**) and (*Z*)-2-methoxy-3-(4-methoxyphenyl)-N-p-tolylacrylamide (**38**) exhibited potent immunosuppressive activity (IC_{50} = 1.77 ± 0.33 and 0.94 ± 0.13 μM) without significant cytotoxicity.

1. Introduction

Immunosuppressive drugs are widely used for the treatment of some autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, psoriasis and glomerulonephritis, and in organ transplantations [1,2]. T-lymphocytes play an integral role in transplant rejection and autoimmune diseases. Cyclosporine A (CsA), [3] tacrolimus, [4] and sirolimus (rapamycin) [4,5] are clinically important therapeutic immunosuppressants, which exert their immunosuppressive effects by inhibiting the proliferation of T-lymphocytes [6,7]. However, despite their undeniable clinical advantages, the immunosuppressive drugs in current clinical use such as glycocorticoids [8], CsA, tacrolimus, and sirolimus, etc., had rather serious side effects including renal toxicity, liver toxicity, infection, malignancy, cosmetic consequences, and other unwanted effects [9–16]. Thus there is a pressing need for novel potential immunosuppressive agents with high efficacy and low toxicity. A program to produce synthetic compounds is therefore ongoing in our laboratory to identify new immunosuppressants [17].

Cinanserin (2′-(3-dimethylaminopropylthio)cinnamanilide) has been shown to be a potent antagonist of serotonin [18], that also exhibits analgesic activity [19] and possess immunosuppressive activity [20]. Some other compounds containing the cinnamide template also showed potent immunosuppressive activity [21,22].

In this study, 35 novel cinnamanilides were synthesized and screened for their immunosuppressive activity. Most of the synthetic compounds exhibited potent immunosuppressive activity.

2. Results and discussion

2.1. Chemistry

The route that enabled the synthesis of the 2-methoxy cinnamanilides is outlined in Scheme 1. The synthesis of these cinnamanilides started from a series of commercially available *p*-substituted benzaldehydes **1a**–**1e**. Condensation of **1a**–**1e** with N-acetylglycine in refluxing acetic anhydride and sodium acetate for 1 h gave the oxazole intermediates **2a**–**2e** in 72–86% yield. Hydrolysis of these oxazole intermediates **2a**–**2e** with 3 M HCl under reflux for 3 h provided the pyruvic acid derivatives **3a**–**3e** in 62–76% yield. Methylation of **3a**–**3e** with dimethyl sulfate in 10% NaOH at room temperature for 4 h and followed by acidification with 3 M HCl for 0.5 h furnished methylated products **4a**–**4e** in 80–86% yield. Condensation of **4a**–**4e** with a series of *p*-substituted anilines **5f**–**5l** with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) in refluxing CH_2Cl_2 for 6–8 h gave the 2-methoxy cinnamanilides **6**–**40** in 68–84% yield. The structures and chemical features of these newly synthesized cinnamanilides were summarized in Table 1.

Cinnamanilides **10**, **14** and **32** were successfully crystallized and their structures were determined by single-crystal X-ray diffraction analysis. The molecular structures of cinnamanilides **10**, **14** and **32**

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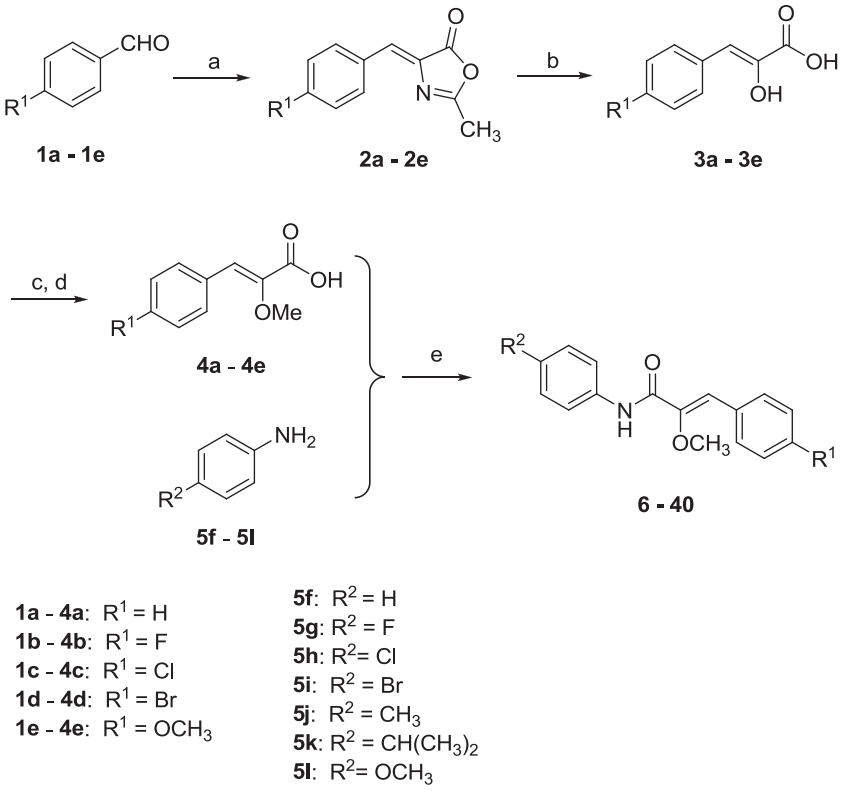


Scheme 1. Synthesis of 2-methoxy cinnamanilides: (a) N-acetylglycine, Ac_2O, CH_3COONa, reflux, 1 h, 72–86% yield; (b) 3 M HCl, reflux, 3 h, 62–76% yield; (c) 10% NaOH, $(CH_3)_2SO_4$, rt, 4 h; (d) 3M HCl, rt, 0.5 h, 80–86%; (e) EDC, CH_2Cl_2, reflux, 6–8 h, 68–84% yield.

are shown in Fig. 1a, b and c, respectively. The collection data details are condensed in Table 2, and the selected geometrical parameters are given in Table 3. Hydrogen bonds for cinnamanilides **10**, **14** and **32** are listed in Table 4. Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre Nos. 698515 for **10**, 698516 for **14** and 698517 for **32**. Copies of this information can be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

2.2. Biological activity

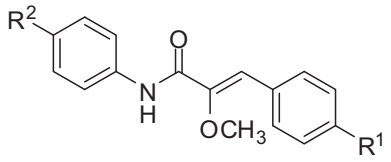
The spleen cells from 6 to 8 week-old BALB/c mice were co-stimulated by CD3/CD28 at the presence of 5 μg/mL of different newly synthesized compounds for 48 h. The cell survival rate was taken by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide) assay (Fig. 2). Some of the synthesized cinnamanilides (compounds **6**–**8**, **10**, **11**, **15**, **18**, **19**, **21**, **23**, **24**, **31** and **36**–**38**) showed good immunosuppressive activity with the inhibition rate over 50% at 5 μg/mL. The inhibition rate of the other cinnamanilides (**9**, **12**–**14**, **16**, **17**, **20**, **22**, **25**-**30**, **32**-**35**, **39** and **40**) was below 50% at 5 μg/mL.

The IC_{50} values of compounds **6**–**8**, **10**, **11**, **15**, **18**, **19**, **21**, **23**, **24**, **31** and **36**–**38** were tested on CD3/CD28 co-stimulated mouse spleen cells in the presence of different concentrations of compound. The results are listed in Table 5. Among these compounds, **37** and **38** exhibited the most potent immunosuppressive activity with IC_{50}'s 1.77 ± 0.33 and 0.94 ± 0.13 μM, respectively. Compounds **7**, **11** and **18** also showed potent immunosuppressive activity with the IC_{50}'s ranging from 3.46 ± 0.68 to 4.94 ± 0.66 μM. The other compounds showed moderate immunosuppressive activity with the IC_{50}'s ranging from 9.48 ± 1.02 to 17.00 ± 1.99 μM.

As can been seen from the immunosuppressive activity of all the synthesized compounds (Fig. 2 and Table 5), compounds **13**–**33** with halogenated R^1 substituent generally showed weak to moderate activity except compound **18** (R^1 = F, R^2 = isopropyl), which exhibited potent immunosuppressive activity with $IC_{50} = 4.66 \pm 0.70$ μM. This result indicated that electron withdrawing R^1 substituents such as halogen are not benefit for the activity. Compounds **34**–**40** with the same electron donating R^1 substituent (R^1 = methoxyl) showed distinct activity due to their different R^2 substituents. Among them, compounds **38** with electron donating R^2 substituent (R^2 = methyl) exhibited the most potent immunosuppressive activity with $IC_{50} = 0.94 \pm 0.13$ μM, which suggested that electron donating R^2 substituents are favorable for the activity. However, Compounds **39** and **40** with electron donating R^2 substituent (isopropyl or methoxyl) showed weak activity, which suggested that the activity may also be relevant to the size of molecule. Compounds **35**–**37** with different halogenated R^2 substituent showed distinct activities in the following order: Br (**37**) > Cl (**36**) > F (**35**). Compounds **6**–**12** with the same R^1 substituent (-H) also showed distinct activity due to their different R^2 substituents in the following order: isopropyl (**11**) > F (**7**) > methyl (**10**) and Cl (**8**) > H (**6**) > Br (**9**) > methoxyl (**12**).

In conclusion, the immunosuppressive activity of cinnamanilides with R^1 substituents of methoxy group or hydrogen were more potent than those with a R^1 substituent of a halogen. The data indicated that an electronic-donating R^1 substitute was favorable for the immunosuppressive activity generally. The electronic-donating R^2 substitute (methyl group and isopropyl) was also conducive to the activity generally. The activity may also be

Table 1
Physical properties of 2-methoxy cinnamanilides.



Compound	R^1	R^2	Formula	mp., °C	Yield, %
6	H	H	$C_{16}H_{15}NO_2$	108–110	80
7	H	F	$C_{16}H_{14}FNO_2$	142–143	68
8	H	Cl	$C_{16}H_{14}ClNO_2$	118–120	82
9	H	Br	$C_{16}H_{14}BrNO_2$	139–141	75
10	H	CH_3	$C_{17}H_{17}NO_2$	111–112	78
11	H	$CH(CH_3)_2$	$C_{19}H_{21}NO_2$	145–147	83
12	H	OCH_3	$C_{17}H_{17}NO_3$	135–137	76
13	F	H	$C_{16}H_{14}FNO_2$	132–133	76
14	F	F	$C_{16}H_{13}F_2NO_2$	149–150	82
15	F	Cl	$C_{16}H_{13}ClFNO_2$	153–154	80
16	F	Br	$C_{16}H_{13}BrFNO_2$	162–163	84
17	F	CH_3	$C_{17}H_{16}FNO_2$	139–140	75
18	F	$CH(CH_3)_2$	$C_{19}H_{20}FNO_2$	183–185	80
19	F	OCH_3	$C_{17}H_{16}FNO_3$	141–142	77
20	Cl	H	$C_{16}H_{14}ClNO_2$	95–97	79
21	Cl	F	$C_{16}H_{13}ClFNO_2$	145–146	80
22	Cl	Cl	$C_{16}H_{13}Cl_2NO_2$	172–173	83
23	Cl	Br	$C_{16}H_{13}BrClNO_2$	177–179	75
24	Cl	CH_3	$C_{17}H_{16}ClNO_2$	145–146	72
25	Cl	$CH(CH_3)_2$	$C_{19}H_{20}ClNO_2$	173–175	76
26	Cl	OCH_3	$C_{17}H_{16}ClNO_3$	178–180	81
27	Br	H	$C_{16}H_{14}BrNO_2$	139–141	77
28	Br	F	$C_{16}H_{13}BrFNO_2$	158–159	75
29	Br	Cl	$C_{16}H_{13}BrClNO_2$	172–173	78
30	Br	Br	$C_{16}H_{13}Br_2NO_2$	175–177	80
31	Br	CH_3	$C_{17}H_{16}BrNO_2$	157–158	69
32	Br	$CH(CH_3)_2$	$C_{19}H_{20}BrNO_2$	165–167	82
33	Br	OCH_3	$C_{17}H_{16}BrNO_3$	180–182	72
34	OCH_3	H	$C_{17}H_{17}NO_3$	88–90	73
35	OCH_3	F	$C_{17}H_{16}FNO_3$	151–153	82
36	OCH_3	Cl	$C_{17}H_{16}ClNO_3$	113–115	78
37	OCH_3	Br	$C_{17}H_{16}BrNO_3$	118–120	80
38	OCH_3	CH_3	$C_{18}H_{19}NO_3$	114–116	75
39	OCH_3	$CH(CH_3)_2$	$C_{20}H_{23}NO_3$	130–132	82
40	OCH_3	OCH_3	$C_{18}H_{19}NO_4$	162–164	69

relevant to the size of molecule. The bigger molecule is not benefit for the activity.

In order to examine the cytotoxicity of the synthesized cinnamanilides, we treated activated or un-activated mouse spleen cells with different concentrations of the potent immunosuppressive compounds **11**, **18**, **37** and **38** for 48 h. The results are shown in Fig. 3. These compounds did not affect the survival rate of un-activated cells significantly, which suggested that they should not be toxic for normal tissues, and the inhibitory effect on activated mouse spleen cells by these compounds should be specific.

3. Conclusion

A series of new cinnamanilides were synthesized and their immunosuppressive activity and cytotoxicity were evaluated. Compounds **37** and **38** exhibited potent immunosuppressive activity with IC_{50}'s 1.77 ± 0.33 and 0.94 ± 0.13 μM, respectively. In general, electronic-donating substituents at R^1 and R^2 were favorable for the immunosuppressive activity of the synthesized cinnamanilides. The synthesized cinnamanilides with smaller molecular size showed more potent activity. Cytotoxicity assaying of the compounds with potent immunosuppressive activity indicated that these compounds did not exhibit significant cytotoxicity to un-activated mouse spleen cells.

4. Experimental section

4.1. Chemistry

All chemicals (reagent grade) used were purchased from Sigma Aldrich (U.S.A) and Sinopharm Chemical Reagent Co., Ltd (China). TLC was run on the silica gel coated aluminum sheets (silica gel 60 GF_{254}, E. Merk, Germany) and visualized in UV light (254 nm). Melting points (uncorrected) were determined on an XT4 MP apparatus (Taike Corp., Beijing, China). ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer, and ^{1}H NMR spectra were recorded on a Bruker DPX-300, AV-300 or AV-500 spectrometer at 25 °C with TMS and solvent signals allotted as internal standards. Chemical shifts were reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument.

4.2. Crystal structure determination

Crystal structure determination of compounds **10**, **14** and **32** were carried out on a Nonius CAD4 diffractometer equipped with graphite-monochromated MoKα (λ = 0.71073 Å) radiation. The structure was solved by direct methods and refined on F^2 by full-matrix least-squares methods using SHELX-97 [23]. All the non-hydrogen atoms were refined anisotropically. All the hydrogen atoms were placed in calculated positions and were assigned fixed isotropic thermal parameters at 1.2 times the equivalent isotropic U of the atoms to which they are attached and allowed to ride on their respective parent atoms. The contributions of these hydrogen atoms were included in the structure-factors calculations.

4.3. General procedure for the preparation of the oxazole derivatives **2a**–**2e**

A mixture of *p*-substituted benzaldehydes **1a**–**1e** (30 mmol, 1 eq), *N*-acetylglycine (4.56 g, 39 mmol, 1.3 eq) and sodium acetate (3.20 g, 39 mmol, 1.3 eq) in acetic anhydride (15 mL) was stirred at reflux for 1 h. The reaction was quenched with ice water and vigorously stirred for 1 h in an ice bath to allow precipitation. Filtration afforded **2a**–**2e**.

4.3.1. (Z)-4-benzylidene-2-methyloxazol-5(4H)-one (**2a**)

Yellow powder, yield: 72%. ^{1}H NMR (300 MHz, d_6-DMSO): 2.49 (s, 3H, CH_3); 7.22 (s, 1H, CH); 7.48–7.52 (m, 3H, ArH); 8.16–8.19 (m, 2H, ArH). MS (ESI^+) *m/z* 188 $(M + H)^+$. Anal. Calcd for $C_{11}H_9NO_2$: C, 70.58; H, 4.85; N, 7.48; Found: C, 70.75; H, 4.90; N, 7.46.

4.3.2. (Z)-4-(4-fluorobenzylidene)-2-methyloxazol-5(4H)-one (**2b**)

Yellow powder, yield: 85%. ^{1}H NMR (300 MHz, d_6-DMSO): 2.39 (s, 3H, CH_3); 7.25 (s, 1H, CH); 7.35 (t, J = 8.8 Hz, 2H, ArH); 8.23–8.28 (m, 2H, ArH). MS (ESI^+) *m/z* 206 $(M + H)^+$. Anal. Calcd for $C_{11}H_8FNO_2$: C, 64.39; H, 3.93; N, 6.83; Found: C, 64.26; H, 3.96; N, 6.86.

4.3.3. (Z)-4-(4-chlorobenzylidene)-2-methyloxazol-5(4H)-one (**2c**)

Yellow powder, yield: 83%. ^{1}H NMR (300 MHz, d_6-DMSO): 2.40 (s, 3H, CH_3); 7.24 (s, 1H, CH); 7.58 (d, J = 8.6 Hz, 2H, ArH); 8.20 (d, J = 8.8 Hz, 2H, ArH). MS (ESI^+) *m/z* 222 $(M + H)^+$. Anal. Calcd for $C_{11}H_8ClNO_2$: C, 59.61; H, 3.64; N, 6.32; Found: C, 59.78; H, 3.62; N, 6.35.

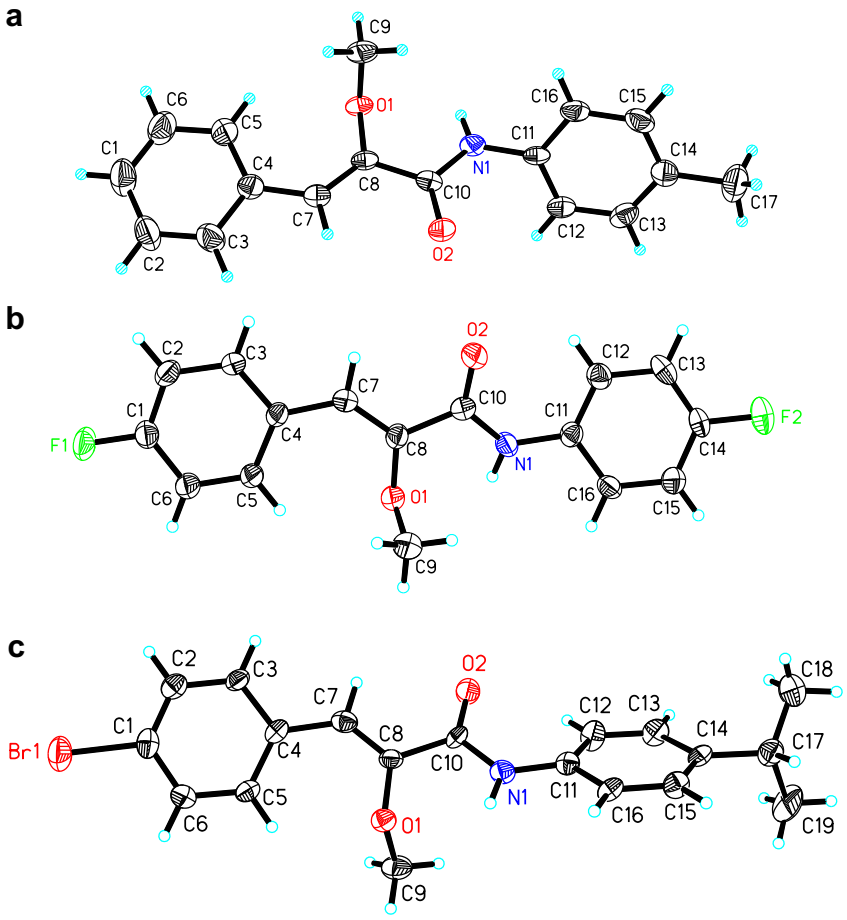


Fig. 1. Molecular structures of compounds a) **10**, b) **14** and c) **32**. Displacement ellipsoids are drawn at the 30% probability level.

Table 2
Crystallographical and experimental data for cinnamanilides **10**, **14** and **32**.

Compound	**10**	**14**	**32**
Formula	$C_{17}H_{17}NO_2$	$C_{16}H_{13}F_2NO_2$	$C_{19}H_{20}BrNO_2$
Mr	267.32	289.27	374.27
Crystal size [mm^3]	0.30 × 0.10 × 0.10	0.30 × 0.20 × 0.10	0.40 × 0.15 × 0.15
Crystal system	Monoclinic	Monoclinic	Triclinic
Space group	$P2_1/c$	$P2_1/c$	$P-1$
a [Å]	6.2470(12)	11.328(2)	5.5840(11)
B [Å]	31.964(6)	5.4930(11)	9.3800(19)
C [Å]	8.935(3)	22.269(5)	17.889(4)
α [°]	90	90	103.23(3)
β [°]	126.24(2)	98.63(3)	95.01(3)
γ [°]	90	90	99.62(3)
V [Å^3]	1439.0(6)	1370.0(5)	891.6(3)
Z	4	4	2
Dc [g cm^{-3}]	1.234	1.402	1.394
μ [mm^{-1}]	0.081	0.111	2.314
F(000)	568	600	384
Max/min trans.	0.9920/0.9762	0.9890/0.9675	0.7228/0.4579
θ range [°]	1.27/25.00	1.82/25.24	1.18/25.17
Index ranges (*h*, *k*, *l*)	−7/0,−38/0, −8/10	−13/13,0/6,0/26	0/6, −11/11, −21/21
Reflections collected/unique	2745/2590	2658/2473	3567/3205
Data/restraints/ parameters	2509/0/181	2473/0/190	3205/0/208
R_{int}	0.0523	0.0356	0.0335
Goodness-of-fit on F^2	1.029	1.172	1.112
R_1, ωR_2 [$I > 2\sigma(I)$][a]	0.0861/0.1946	0.0742/0.1920	0.1065/0.2588
R_1, ωR_2[a]	0.1896/0.2604	0.1283/0.2796	0.1645/0.3186
Largest diff. peak and hole (eÅ^{-3})	0.231/-0.277	0.316/-0.308	1.496/-1.607

[a] $R_1 = \Sigma||Fo|-|Fc||/\Sigma|Fo|$, $wR_2 = [\Sigma w(Fo^2-Fc^2)^2/\Sigma w(Fo^2)^2]^{1/2}$.

*4.3.4. (Z)-4-(4-bromobenzylidene)-2-methyloxazol-5(4H)-one (**2d**)*

Yellow powder, yield: 86%. ^{1}H NMR (300 MHz, d_6-DMSO): 2.40 (s, 3H, CH_3); 7.24 (s, 1H, CH); 7.58 (d, J = 8.6 Hz, 2H, ArH); 8.20 (d, J = 8.8 Hz, 2H, ArH). MS (ESI$^+$) *m/z* 266 (M + H)$^+$. Anal. Calcd for $C_{11}H_8BrNO_2$: C, 49.65; H, 3.03; N, 5.26; Found: C, 49.72; H, 3.02; N, 5.23.

*4.3.5. (Z)-4-(4-methoxybenzylidene)-2-methyloxazol-5(4H)-one (**2e**)*

Yellow powder, yield: 75%. ^{1}H NMR (300 MHz, d_6-DMSO): 2.37 (s, 3H, CH_3); 3.83 (s, 3H, OCH_3); 7.08 (d, J = 8.6 Hz, 2H, ArH); 7.19 (s, 1H, CH); 8.18 (d, J = 8.8 Hz, 2H, ArH). MS (ESI$^+$) *m/z* 218 (M + H)$^+$. Anal. Calcd for $C_{12}H_{11}NO_3$: C, 66.35; H, 5.10; N, 6.45; Found: C, 66.63; H, 5.07; N, 6.49.

*4.4. General procedure for the preparation of the pyruvic acid derivatives **3a**–**3e***

A suspension of oxazole derivatives **2a**–**2e** (20 mmol) in aq. HCl (3 M, 20 mL) was stirred at reflux for 3 h. The mixture was cooled to room temperature to allow crystallization. Filtration afforded the title compound **3a**–**3e**.

*4.4.1. (Z)-2-hydroxy-3-phenylacrylic acid (**3a**)*

Pale yellow crystal, yield: 68%. ^{1}H NMR (300 MHz, d_6-DMSO): 6.39 (s, 1H, CH); 7.23–7.37 (m, 3H, ArH); 7.75 (d, J = 7.2 Hz, 2H, ArH); 9.24 (s, 1H, OH); 13.21 (s, 1H, COOH). MS (ESI$^+$) *m/z* 165 (M + H)$^+$. Anal. Calcd for $C_9H_8O_3$: C, 65.85; H, 4.91; Found: C, 66.01; H, 4.94.

Table 3
Selected bond lengths/Å and angles/° for cinnamanilides **10**, **14** and **32**.

Compound	**10**	**14**	**32**
Bond lengths/Å			
C(4)–C(7)	1.461(7)	1.445(6)	1.462(10)
C(7)–C(8)	1.319(7)	1.327(6)	1.316(11)
C(8)–O(1)	1.375(5)	1.390(5)	1.387(8)
C(8)–C(10)	1.483(7)	1.503(6)	1.501(10)
C(9)–O(1)	1.431(6)	1.425(7)	1.425(10)
C(10)–O(2)	1.233(5)	1.218(5)	1.236(9)
C(10)–N(1)	1.354(6)	1.351(6)	1.364(9)
C(11)–N(1)	1.418(6)	1.404(5)	1.394(9)
C(14)–C(17)	1.513(8)	–	1.519(11)
C(1)–F(1)	–	1.333(5)	–
C(14)-F(2)	–	1.350(5)	–
Br(1)–C(1)	–	–	1.899(8)
C(17)–C(18)	–	–	1.479(15)
C(17)–C(19)	–	–	1.512(14)
Bond angles/°			
C(3)–C(4)–C(7)	117.6(5)	118.7(4)	118.8(7)
C(5)–C(4)–C(7)	124.9(5)	124.8(4)	124.1(6)
C(8)-C(7)-C(4)	131.1(5)	132.2(4)	131.4(7)
C(7)–C(8)–O(1)	123.7(5)	122.3(4)	123.9(7)
C(7)–C(8)–C(10)	120.3(4)	121.1(4)	119.9(6)
O(1)–C(8)–C(10)	116.0(4)	116.3(4)	116.2(6)
O(2)–C(10)–N(1)	122.1(5)	124.4(4)	123.4(7)
O(2)–C(10)–C(8)	122.3(4)	121.5(4)	121.8(6)
N(1)–C(10)–C(8)	115.6(4)	114.1(4)	114.8(6)
C(12)–C(11)–N(1)	121.2(5)	123.7(4)	123.6(7)
C(16)–C(11)–N(1)	119.2(5)	117.4(4)	118.0(7)
C(15)–C(14)–C(17)	121.7(7)	–	121.3(8)
C(13)–C(14)–C(17)	120.6(7)	–	122.5(8)
C(10)–N(1)–C(11)	123.8(4)	128.1(4)	126.6(6)
C(8)–O(1)–C(9)	114.8(4)	113.0(4)	115.7(6)
F(1)–C(1)–C(2)	–	118.7(4)	–
F(1)–C(1)–C(6)	–	119.3(5)	–
F(2)–C(14)–C(15)	–	119.6(5)	–
F(2)–C(14)–C(13)	–	118.3(4)	–
C(2)–C(1)–Br(1)	–	–	120.5(7)
C(6)–C(1)–Br(1)	–	–	117.9(6)
C(18)–C(17)–C(19)	–	–	113.6(11)
C(18)–C(17)–C(14)	–	–	114.1(8)
C(19)–C(17)–C(14)	–	–	110.4(8)

*4.4.2. (Z)-3-(4-fluorophenyl)-2-hydroxyacrylic acid (**3b**)*

Pale yellow crystal, yield: 72%. ^{1}H NMR (300 MHz, d_6-DMSO): 6.41 (s, 1H, CH); 7.17 (t, J = 8.9 Hz, 2H, ArH); 7.78–7.83 (m, 2H, ArH); 9.27 (s, 1H, OH). MS (ESI$^+$) m/z 183 (M + H)$^+$. Anal. Calcd for $C_9H_7FO_3$: C, 59.35; H, 3.87; Found: C, 59.46; H, 3.85.

*4.4.3. (Z)-3-(4-chlorophenyl)-2-hydroxyacrylic acid (**3c**)*

White powder, yield: 70%. ^{1}H NMR (300 MHz, d_6-DMSO): 6.40 (s, 1H, CH); 7.40 (d, J = 8.6 Hz, 2H, ArH); 7.78 (d, J = 8.6 Hz, 2H, ArH); 9.48 (s, 1H, OH); 13.30 (s, 1H, COOH). MS (ESI$^+$) m/z 199 (M + H)$^+$. Anal. Calcd for $C_9H_7ClO_3$: C, 54.43; H, 3.55; Found: C, 54.48; H, 3.52.

*4.4.4. (Z)-3-(4-bromophenyl)-2-hydroxyacrylic acid (**3d**)*

White powder, yield: 76%. ^{1}H NMR (300 MHz, d_6-DMSO): 6.37 (s, 1H, CH); 7.53 (d, J = 8.6 Hz, 2H, ArH); 7.70 (d, J = 8.6 Hz, 2H, ArH); 9.45 (s, 1H, OH); 13.33 (s, 1H, COOH). MS (ESI$^+$) m/z 243 (M + H)$^+$. Anal. Calcd for $C_9H_7BrO_3$: C, 44.47; H, 2.90; Found: C, 44.69; H, 2.88.

Table 4
Hydrogen bonds for cinnamanilides **10**, **14** and **32** [Å and °].

Compound	D-H…A	D-H	H…A	D…A	D-H…A
10[a]	N(1)–H(1A)…O(2)#1	0.86	2.17	2.944(5)	149.6
14[b]	N(1)–H(1A)…O(1)	0.86	2.25	2.682(5)	111.4
	N(1)–H(1A)…O(2)#1	0.86	2.50	3.292(5)	152.9
32[c]	N(1)–H(1A)…O(2)#1	0.86	2.57	3.356(8)	152.7

[a] Symmetry transformations used to generate equivalent atoms: #1 $x-1$, $-y+1/2$,$z-1/2$.
[b] Symmetry transformations used to generate equivalent atoms: #1 x,$y+1$,z.
[c] Symmetry transformations used to generate equivalent atoms: #1 $x-1$,y,z.

*4.4.5. (Z)-2-hydroxy-3-(4-methoxyphenyl)acrylic acid (**3e**)*

White powder, yield: 62%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.76 (s, 3H, OCH_3); 6.37 (s, 1H, CH); 6.92 (d, J = 8.7 Hz, 2H, ArH); 7.71 (d, J = 8.7 Hz, 2H, ArH); 8.96 (s, 1H, OH); 13.02 (s, 1H, COOH). MS (ESI$^+$) m/z 195 (M + H)$^+$. Anal. Calcd for $C_{10}H_{10}O_4$: C, 61.85; H, 5.19; Found: C, 62.04; H, 5.23.

*4.5. General procedure for the preparation of methylation products **4a**–**4e***

Dimethyl sulfate (5 mL) was added dropwise into a suspension of pyruvic acid derivatives **3a**–**3e** (10 mmol) in 10% NaOH (15 mL) in ice-water bath and then the mixture was stirred at room temperature for 4 h. After that, 3 M HCl (15 mL) was added and stirred for another 0.5 h to precipitate. Filtration afforded the title compound **4a**–**4e**.

*4.5.1. (Z)-2-methoxy-3-phenylacrylic acid (**4a**)*

Pale yellow powder, yield: 80%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.74 (s, 3H, OCH_3); 6.93 (s, 1H, CH); 7.34–7.45 (m, 3H, ArH); 7.78 (d, J = 7.1 Hz, 2H, ArH); 13.00 (s, 1H, COOH). MS (ESI$^+$) m/z 179 (M + H)$^+$. Anal. Calcd for $C_{10}H_{10}O_3$: C, 67.14; H, 5.66; Found: C, 67.01; H, 5.69.

*4.5.2. (Z)-3-(4-fluorophenyl)-2-methoxyacrylic acid (**4b**)*

Pale yellow powder, yield: 83%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.73 (s, 3H, OCH_3); 6.93 (s, 1H, CH); 7.24 (t, J = 9.0 Hz, 2H, ArH); 7.81–7.86 (m, 2H, ArH); 13.09 (s, 1H, COOH). MS (ESI$^+$) m/z 197 (M + H)$^+$. Anal. Calcd for $C_{10}H_9FO_3$: C, 61.22; H, 4.62; Found: C, 61.45; H, 4.65.

*4.5.3. (Z)-3-(4-chlorophenyl)-2-methoxyacrylic acid (**4c**)*

Pale yellow powder, yield: 86%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.73 (s, 3H, OCH_3); 6.89 (s, 1H, CH); 7.47 (d, J = 8.6 Hz, 2H, ArH); 7.78 (d, J = 8.6 Hz, 2H, ArH); 13.07 (s, 1H, COOH). MS (ESI$^+$) m/z 213 (M + H)$^+$. Anal. Calcd for $C_{10}H_9ClO_3$: C, 56.49; H, 4.27; Found: C, 56.38; H, 4.25.

*4.5.4. (Z)-3-(4-bromophenyl)-2-methoxyacrylic acid (**4d**)*

Pale yellow powder, yield: 85%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.73 (s, 3H, OCH_3); 6.87 (s, 1H, CH); 7.59 (d, J = 8.6 Hz, 2H, ArH); 7.71 (d, J = 8.6 Hz, 2H, ArH); 13.09 (s, 1H, COOH). MS (ESI$^+$) m/z 257(M + H)$^+$. Anal. Calcd for $C_{10}H_9BrO_3$: C, 46.72; H, 3.53; Found: C, 46.75; H, 3.52.

*4.5.5. (Z)-2-methoxy-3-(4-methoxyphenyl)acrylic acid (**4e**)*

Pale yellow powder, yield: 80%. ^{1}H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 6.87 (s, 1H, CH); 6.95 (d, J = 8.4 Hz, 2H, ArH); 7.71 (d, J = 8.4 Hz, 2H, ArH); 13.05 (s, 1H, COOH). MS (ESI$^+$) m/z 209(M + H)$^+$. Anal. Calcd for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Found: C, 63.72; H, 5.78.

*4.6. General procedure for the preparation of 2-methoxy cinnamanilides **6**–**40***

A solution of **4a**–**4e** (2 mmol) and EDC.HCl (3 mmol) in CH_2Cl_2 (10 mL) was added phenylamine **5f**–**5l** (2 mmol), respectively. Stirred at refluxing for 6–8 h and evaporated of the solvent, the residue was purified by column chromatography on silica gel, eluting with petroleum ether/EtOAc (3/1) to furnish title compound **6**–**40**.

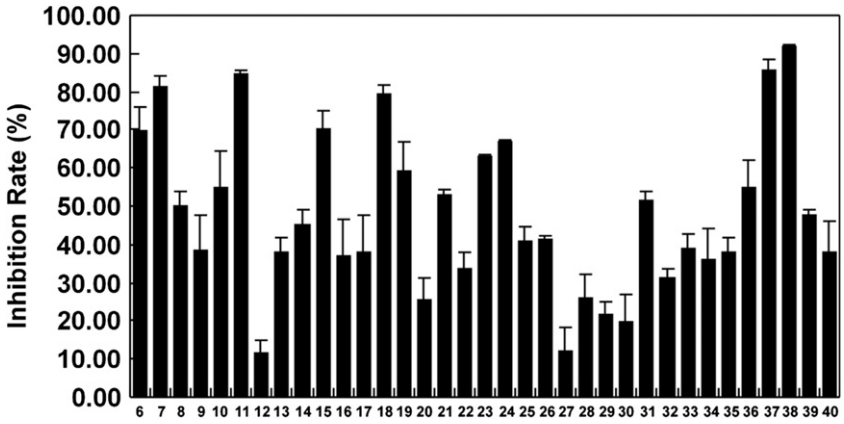


Fig. 2. The spleen cells from 6 to 8 week-old BALB/c mice were co-stimulated by CD3/CD28 at the present of 5 μg/ml of different compounds for 48 h. The cell survival rate was taken by MTT assay.

*4.6.1. (Z)-2-methoxy-N,3-diphenylacrylamide (**6**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.80 (s, 1H, CH); 7.08–7.12 (m, 1H, ArH); 7.32–7.45 (m, 5H, ArH); 7.74–7.79 (m, 4H, ArH); 10.11 (s, 1H, NH). MS (ESI^+) *m/z* 254 $(M + H)^+$. Anal. Calcd for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53; Found: C, 75.78; H, 5.91; N, 5.61.

*4.6.2. (Z)-N-(4-fluorophenyl)-2-methoxy-3-phenylacrylamide (**7**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.70 (s, 3H, OCH_3); 6.81 (s, 1H, CH); 7.16–7.22 (m, 2H, ArH); 7.32–7.36 (m, 1H, ArH); 7.40–7.45 (m, 2H, ArH); 7.74–7.82 (m, 4H, ArH); 10.19 (s, 1H, NH). MS (ESI^+) *m/z* 272 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}FNO_2$: C, 70.84; H, 5.20; N, 5.16; Found: C, 71.13; H, 5.25; N, 5.14.

*4.6.3. (Z)-N-(4-chlorophenyl)-2-methoxy-3-phenylacrylamide (**8**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.70 (s, 3H, OCH_3); 6.82 (s, 1H, CH); 7.34–7.43 (m, 5H, ArH); 7.74–7.84 (m, 4H, ArH); 10.26 (s, 1H, NH). MS (ESI^+) *m/z* 288 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}ClNO_2$: C, 66.79; H, 4.90; N, 4.87; Found: C, 66.93; H, 4.95; N, 4.94.

*4.6.4. (Z)-N-(4-bromophenyl)-2-methoxy-3-phenylacrylamide (**9**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.70 (s, 3H, OCH_3); 6.82 (s, 1H, CH); 7.34–7.45 (m, 3H, ArH); 7.52–7.55 (m, 2H, ArH); 7.74–7.79 (m, 4H, ArH); 10.25 (s, 1H, NH). MS (ESI^+) *m/z* 332 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}BrNO_2$: C, 57.85; H, 4.25; N, 4.22; Found: C, 57.81; H, 4.27; N, 4.25.

Table 5
In vitro inhibitory effects of the synthetic cinnamanilides on mouse spleen cells co-stimulated by CD3/CD28.

Compound	R^1	R^2	IC_{50} [μM]
6	H	H	15.22 ± 1.38
7	H	F	4.94 ± 0.66
8	H	Cl	17.00 ± 1.99
10	H	CH_3	16.59 ± 1.72
11	H	$CH(CH_3)_2$	3.46 ± 0.68
15	F	Cl	9.48 ± 1.02
18	F	$CH(CH_3)_2$	4.66 ± 0.70
19	F	OCH_3	13.46 ± 1.20
21	Cl	F	14.00 ± 1.80
23	Cl	Br	11.01 ± 0.74
24	Cl	CH_3	12.72 ± 1.20
31	Br	CH_3	12.87 ± 1.74
36	OCH_3	Cl	14.73 ± 1.39
37	OCH_3	Br	1.77 ± 0.33
38	OCH_3	CH_3	0.94 ± 0.13

*4.6.5. (Z)-2-methoxy-3-phenyl-N-p-tolylacrylamide (**10**)*

Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 2.28 (s, 3H, CH_3); 3.70 (s, 3H, OCH_3); 6.78 (s, 1H, CH); 7.13 (d, J = 7.4 Hz, 2H, ArH); 7.31–7.36 (m, 1H, ArH); 7.40–7.45 (m, 2H, ArH); 7.65 (d, J = 8.4 Hz, 2H, ArH); 7.74 (d, J = 7.3 Hz, 2H, ArH); 10.03(s, 1H, NH). MS (ESI^+) *m/z* 268 $(M + H)^+$. Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24; Found: C, 76.51; H, 6.47; N, 5.18.

*4.6.6. (Z)-N-(4-isopropylphenyl)-2-methoxy-3-phenylacrylamide (**11**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.16 (d, J = 6.9 Hz, 6H, CH_3); 2.81–2.88 (m, 1H, CH); 3.70 (s, 3H, OCH_3); 6.78 (s, 1H, CH); 7.21 (d, J = 8.6 Hz, 2H, ArH); 7.31–7.36 (m, 1H, ArH); 7.40–7.45 (m, 2H, ArH); 7.67 (d, J = 8.6 Hz, 2H, ArH); 7.73–7.77 (m, 2H, ArH); 10.04 (s, 1H, NH). MS (ESI^+) *m/z* 296 $(M + H)^+$. Anal. Calcd for $C_{19}H_{21}NO_2$: C, 77.26; H, 7.17; N, 4.74; Found: C, 77.21; H, 7.23; N, 4.72.

*4.6.7. (Z)-2-methoxy-N-(4-methoxyphenyl)-3-phenylacrylamide (**12**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.69 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 6.78 (s, 1H, CH); 6.92 (d, J = 8.7 Hz, 2H, ArH); 7.33–7.45 (m, 3H, ArH); 7.66–7.75 (m, 4H, ArH); 10.00 (s, 1H, NH). MS (ESI^+) *m/z* 284 $(M + H)^+$. Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; Found: C, 71.83; H, 6.08; N, 5.01.

*4.6.8. (Z)-3-(4-fluorophenyl)-2-methoxy-N-phenylacrylamide (**13**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.70 (s, 3H, OCH_3); 6.79 (s, 1H, CH); 7.11 (d, J = 7.3 Hz, 1H, ArH); 7.23–7.37 (m, 4H, ArH); 7.76–7.84 (m, 4H, ArH); 10.11 (s, 1H, NH). MS (ESI^+) *m/z* 272 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}FNO_2$: C, 70.84; H, 5.20; N, 5.16; Found: C, 70.92; H, 5.24; N, 5.13.

*4.6.9. (Z)-N,3-bis(4-fluorophenyl)-2-methoxyacrylamide (**14**)*

Colorless crystal, 1H NMR (300 MHz, d6-DMSO): 3.70 (s, 3H, OCH_3); 6.80 (s, 1H, CH); 7.15–7.28 (m, 4H, ArH); 7.77–7.83 (m, 4H, ArH); 10.16 (s, 1H, NH). MS (ESI+) *m/z* 290 (M + H)+. Anal. Calcd for $C_{16}H_{13}F_2NO_2$: C, 66.43; H, 4.53; N, 4.84; Found: C, 66.58; H, 4.54; N, 4.78.

*4.6.10. (Z)-N-(4-chlorophenyl)-3-(4-fluorophenyl)-2-methoxyacrylamide (**15**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.70 (s, 3H, OCH_3); 6.82 (s, 1H, CH); 7.26 (t, J = 8.9 Hz, 2H, ArH); 7.39–7.42 (m, 2H, ArH); 7.79–7.84 (m, 4H, ArH); 10.26 (s, 1H, NH). MS (ESI^+) *m/z* 306 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}ClFNO_2$: C, 62.86; H, 4.29; N, 4.58; Found: C, 62.90; H, 4.32; N, 4.55.

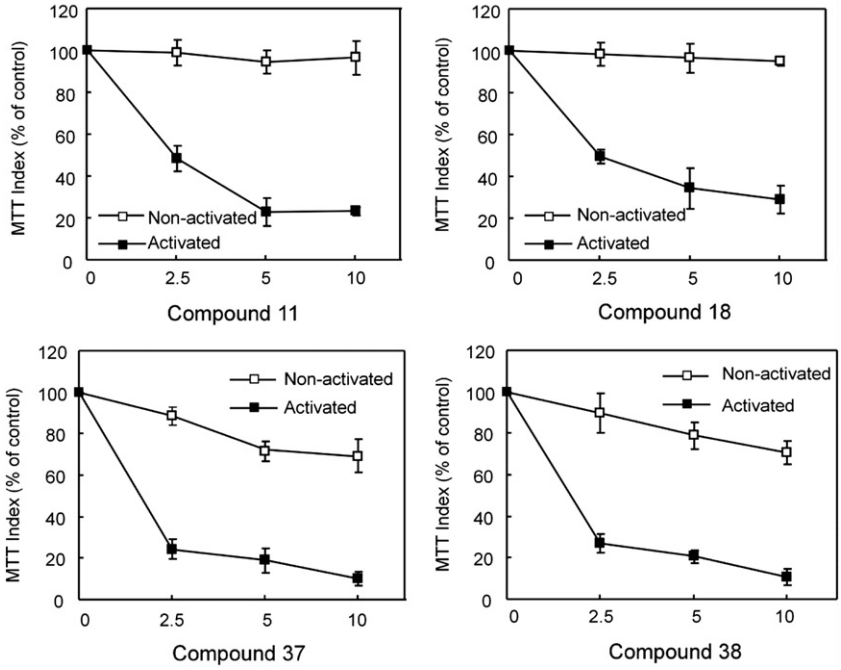


Fig. 3. Stimulated or non-stimulated spleen cells from 6 to 8 week-old BALB/c mice were incubated with different amount of compound **11**, **18**, **37** and **38** for 48 h, the cell toxicity of these compounds were measured by MTT assay.

*4.6.11. (Z)-N-(4-bromophenyl)-3-(4-fluorophenyl)-2-methoxyacrylamide (**16**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 6.79 (s, 1H, ArH); 7.24 (t, J = 8.9 Hz, 2H, ArH); 7.50 (d, J = 8.8 Hz, 2H, ArH); 7.72–7.81 (m, 4H, ArH); 10.23 (s, 1H, NH). MS (ESI$^+$) m/z 350 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}BrFNO_2$: C, 54.88; H, 3.74; N, 4.00; Found: C, 55.02; H, 3.72; N, 4.04.

*4.6.12. (Z)-3-(4-fluorophenyl)-2-methoxy-N-p-tolylacrylamide (**17**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 2.27 (s, 3H, CH_3); 3.69 (s, 3H, OCH_3); 6.77 (s, 1H, CH); 7.14 (d, J = 8.2Hz, 2H, ArH); 7.25 (t, J = 9.0 Hz, 2H, ArH); 7.64 (d, J = 8.4 Hz, 2H, ArH); 7.77–7.82 (m, 2H, ArH); 10.01(s, 1H, NH). MS (ESI$^+$) m/z 286 $(M + H)^+$. Anal. Calcd for $C_{17}H_{16}FNO_2$: C, 71.56; H, 5.65; N, 4.91; Found: C, 71.83; H, 5.61; N, 5.02.

*4.6.13. (Z)-3-(4-fluorophenyl)-N-(4-isopropylphenyl)-2-methoxyacrylamide (**18**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.17 (d, J = 7.0 Hz, 6H, CH_3); 2.79–2.88 (m, 1H, CH); 3.67 (s, 3H, OCH_3); 6.75 (s, 1H, CH); 7.17–7.26 (m, 4H, ArH); 7.64 (d, J = 8.4 Hz, 2H, ArH); 7.75–7.80 (m, 2H, ArH); 9.99 (s, 1H, NH). MS (ESI$^+$) m/z 314 $(M + H)^+$. Anal. Calcd for $C_{19}H_{20}FNO_2$: C, 72.82; H, 6.43; N, 4.47; Found: C, 72.95; H, 6.45; N, 4.43.

*4.6.14. (Z)-3-(4-fluorophenyl)-2-methoxy-N-(4-methoxyphenyl) acrylamide (**19**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.69 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 6.78 (s, 1H, CH); 6.92 (d, J = 9.1 Hz, 2H, ArH); 7.25 (t, J = 9.0 Hz, 2H, ArH); 7.67 (d, J = 9.1 Hz, 2H, ArH); 7.77–7.82 (m, 2H, ArH); 9.99(s, 1H, NH). MS (ESI$^+$) m/z 302 $(M + H)^+$. Anal. Calcd for $C_{17}H_{16}FNO_3$: C, 67.76; H, 5.35; N, 4.65; Found: C, 67.63; H, 5.41; N, 4.63.

*4.6.15. (Z)-3-(4-chlorophenyl)-2-methoxy-N-phenylacrylamide (**20**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.72 (s, 3H, OCH_3); 6.75 (s, 1H, CH); 7.11 (t, J = 7.3 Hz, 1H, ArH); 7.32–7.37 (m, 2H, ArH); 7.48 (d, J = 8.4 Hz, 2H, ArH); 7.77 (d, J = 8.6 Hz, 4H, ArH); 10.16 (s, 1H, NH). MS (ESI$^+$) m/z 288 $(M + H)^+$. Anal. Calcd for $C_{16}H_{14}ClNO_2$: C, 66.79; H, 4.90; N, 4.87; Found: C, 68.07; H, 4.84; N, 4.91.

*4.6.16. (Z)-3-(4-chlorophenyl)-N-(4-fluorophenyl)-2-methoxyacrylamide (**21**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.77 (s, 1H, CH); 7.19 (t, J = 9.0 Hz, 2H, ArH); 7.48 (d, J = 8.6 Hz, 2H, ArH); 7.76–7.81 (m, 4H, ArH); 10.24 (s, 1H, NH). MS (ESI$^+$) m/z 306 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}ClFNO_2$: C, 62.85; H, 4.29; N, 4.58; Found: C, 62.80; H, 4.31; N, 4.62.

*4.6.17. (Z)-N,3-bis(4-chlorophenyl)-2-methoxyacrylamide (**22**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.78 (s, 1H, CH); 7.41 (d, J = 8.8 Hz, 2H, ArH), 7.48 (d, J = 8.4 Hz, 2H, ArH); 7.76–7.83 (m, 4H, ArH); 10.30 (s, 1H, NH). MS (ESI$^+$) m/z 322 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}Cl_2NO_2$: C, 59.65; H, 4.07; N, 4.35; Found: C, 59.87; H, 4.18; N, 4.32.

*4.6.18. (Z)-N-(4-bromophenyl)-3-(4-chlorophenyl)-2-methoxyacrylamide (**23**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.77 (s, 1H, CH); 7.47–7.55 (m, 4H, ArH), 7.74–7.78 (m, 4H, ArH); 10.29 (s, 1H, NH). MS (ESI$^+$) m/z 366 $(M + H)^+$. Anal. Calcd for $C_{16}H_{13}BrClNO_2$: C, 52.41; H, 3.57; N, 3.82; Found: C, 52.48; H, 3.54; N, 3.86.

*4.6.19. (Z)-3-(4-chlorophenyl)-2-methoxy-N-p-tolylacrylamide (**24**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 2.27 (s, 3H, CH_3); 3.70 (s, 3H, OCH_3); 6.73 (s, 1H, CH); 7.15 (d, J = 8.4 Hz, 2H, ArH); 7.47(d, J = 8.4 Hz, 2H, ArH); 7. 64 (d, J = 8.4 Hz, 2H, ArH); 7.76 (d, J = 8.6 Hz, 2H, ArH); 10.07 (s, 1H, NH). MS (ESI$^+$) m/z 302 $(M + H)^+$. Anal. Calcd for $C_{17}H_{16}ClNO_2$: C, 67.66; H, 5.34; N, 4.64; Found: C, 57.59; H, 5.39; N, 4.65.

*4.6.20. (Z)-3-(4-chlorophenyl)-N-(4-isopropylphenyl)-2-methoxyacrylamide (**25**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.20 (d, J = 7.0 Hz, 6H, CH_3); 2.82–2.90 (m, 1H, CH); 3.71 (s, 3H, OCH_3); 6.74 (s, 1H, CH); 7.21 (d, J = 8.4 Hz, 2H, ArH); 7.48 (d, J = 8.4 Hz, 2H, ArH); 7.67 (d, J = 8.4 Hz, 2H, ArH); 7.76 (d, J = 8.6 Hz, 2H, ArH); 10.08 (s, 1H, NH). MS (ESI$^+$) m/z 330 (M + H)$^+$. Anal. Calcd for $C_{19}H_{20}ClNO_2$: C, 69.19; H, 6.11; N, 4.25; Found: C, 69.35; H, 6.06; N, 4.29.

*4.6.21. (Z)-3-(4-chlorophenyl)-2-methoxy-N-(4-methoxyphenyl) acrylamide (**26**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 3.75 (s, 3H, OCH_3); 6.73 (s, 1H, CH); 6.92 (d, J = 8.8 Hz, 2H, ArH); 7.47 (d, J = 8.4 Hz, 2H, ArH); 7.67 (d, J = 8.8 Hz, 2H, ArH); 7.75 (d, J = 8.4 Hz, 2H, ArH); 10.01(s, 1H, NH). MS (ESI$^+$) m/z 318 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}ClNO_3$: C, 64.24; H, 5.08; N, 4.41; Found: C, 64.56; H, 5.03; N, 4.35.

*4.6.22. (Z)-3-(4-bromophenyl)-2-methoxy-N-phenylacrylamide (**27**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.72 (s, 1H, CH); 7.08–7.13 (m, 1H, ArH); 7.32–7.37 (m, 2H, ArH); 7.61 (d, J = 8.6 Hz, 2H, ArH); 7.70 (d, J = 8.6 Hz, 2H, ArH); 7.76 (d, J = 7.9Hz, 2H, ArH); 10.16 (s, 1H, NH). MS (ESI$^+$) m/z 332 (M + H)$^+$. Anal. Calcd for $C_{16}H_{14}BrNO_2$: C, 57.85; H, 4.25; N, 4.22; Found: C, 57.92; H, 4.24; N, 4.28.

*4.6.23. (Z)-3-(4-bromophenyl)-N-(4-fluorophenyl)-2-methoxyacrylamide (**28**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.74 (s, 1H, CH); 7.16–7.22 (m, 2H, ArH); 7.61 (d, J = 9.0 Hz, 2H, ArH); 7.69 (d, J = 9.0 Hz, 2H, ArH); 7.76–7.81 (m, 2H, ArH); 10.24 (s, 1H, NH). MS (ESI$^+$) m/z 350 (M + H)$^+$. Anal. Calcd for $C_{16}H_{13}BrFNO_2$: C, 54.88; H, 3.74; N, 4.00; Found: C, 54.75; H, 3.79; N, 4.06.

*4.6.24. (Z)-3-(4-bromophenyl)-N-(4-chlorophenyl)-2-methoxyacrylamide (**29**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.74 (s, 1H, CH); 7.40 (d, J = 8.8 Hz, 2H, ArH); 7.61 (d, J = 8.4 Hz, 2H, ArH); 7.69 (d, J = 8.4 Hz, 2H, ArH); 7.80 (d, J = 8.9 Hz, 2H, ArH); 10.27 (s, 1H, NH). MS (ESI$^+$) m/z 366 (M + H)$^+$. Anal. Calcd for $C_{16}H_{13}BrClNO_2$: C, 52.41; H, 3.57; N, 3.82; Found: C, 52.53; H, 3.62; N, 3.79.

*4.6.25. (Z)-N,3-bis(4-bromophenyl)-2-methoxyacrylamide (**30**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.71 (s, 3H, OCH_3); 6.75 (s, 1H, CH); 7.53 (d, J = 8.8 Hz, 2H, ArH); 7.61 (d, J = 8.6 Hz, 2H, ArH); 7.69 (d, J = 8.6 Hz, 2H, ArH); 7.75 (d, J = 8.8 Hz, 2H, ArH); 10.27 (s, 1H, NH). MS (ESI$^+$) m/z 410 (M + H)$^+$. Anal. Calcd for $C_{16}H_{13}Br_2NO_2$: C, 46.75; H, 3.19; N, 3.41; Found: C, 46.94; H, 3.25; N, 3.33.

*4.6.26. (Z)-3-(4-bromophenyl)-2-methoxy-N-p-tolylacrylamide (**31**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 2.28 (s, 3H, CH_3); 3.70 (s, 3H, OCH_3); 6.71 (s, 1H, CH); 7.15 (d, J = 8.4 Hz, 2H, ArH); 7.59–7.71 (m, 6H, ArH); 10.09 (s, 1H, NH). MS (ESI$^+$) m/z 346 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}BrNO_2$: C, 58.97; H, 4.66; N, 4.05; Found: C, 59.18; H, 4.60; N, 4.03.

*4.6.27. (Z)-3-(4-bromophenyl)-N-(4-isopropylphenyl)-2-methoxyacrylamide (**32**)*

Colorless crystal, ^{1}H NMR (300 MHz, d_6-DMSO): 1.19 (d, J = 6.9 Hz, 6H, CH_3); 2.81–2.88 (m, 1H, CH); 3.70 (s, 3H, OCH_3); 6.71 (s, 1H, CH); 7.21 (d, J = 8.6 Hz, 2H, ArH); 7.59–7.71 (m, 6H, ArH); 10.09 (s, 1H, NH). MS (ESI$^+$) m/z 374 (M + H)$^+$. Anal. Calcd for $C_{19}H_{20}BrNO_2$: C, 60.97; H, 5.39; N, 3.74; Found: C, 60.90; H, 5.43; N, 3.79.

*4.6.28. (Z)-3-(4-bromophenyl)-2-methoxy-N-(4-methoxyphenyl) acrylamide (**33**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.68 (s, 3H, OCH_3); 3.71 (s, 3H, OCH_3); 6.68 (s, 1H, CH); 6.89 (d, J = 9.0 Hz, 2H, ArH); 7.58 (d, J = 8.6 Hz, 2H, ArH); 7.62–7.67 (m, 4H, ArH); 10.01 (s, 1H, NH). MS (ESI$^+$) m/z 362 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}BrNO_3$: C, 56.37; H, 4.45; N, 3.87; Found: C, 56.45; H, 4.44; N, 3.83.

*4.6.29. (Z)-2-methoxy-3-(4-methoxyphenyl)-N-phenylacrylamide (**34**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.68 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 6.82 (s, 1H, CH); 7.0 (d, J = 8.9 Hz, 2H, ArH); 7.07–7.12 (m, 1H, ArH); 7.30–7.36 (m, 2H, ArH); 7.70–7.79 (m, 4H, ArH); 9.99 (s, 1H, NH). MS (ESI$^+$) m/z 284 (M + H)$^+$. Anal. Calcd for $C_{17}H_{17}NO_3$: C, 72.07; H, 6.05; N, 4.94; Found: C, 72.29; H, 6.14; N, 4.92.

*4.6.30. (Z)-N-(4-fluorophenyl)-2-methoxy-3-(4-methoxyphenyl) acrylamide (**35**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 6.83 (s, 1H, CH); 7.00 (d, J = 8.8 Hz, 2H, ArH); 7.18 (t, J = 9.0 Hz, 2H, ArH); 7.72 (d, J = 8.8 Hz, 2H, ArH); 7.78–7.82 (m, 2H, ArH); 10.07 (s, 1H, NH). MS (ESI$^+$) m/z 302 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}FNO_3$: C, 67.76; H, 5.35; N, 4.65; Found: C, 67.65; H, 5.31; N, 4.62.

*4.6.31. (Z)-N-(4-chlorophenyl)-2-methoxy-3-(4-methoxyphenyl) acrylamide (**36**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 6.84 (s, 1H, CH); 7.00 (d, J = 8.8 Hz, 2H, ArH); 7.38–7.41 (m, 2H, ArH); 7.72 (d, J = 9.0 Hz, 2H, ArH); 7.80–7.84 (m, 2H, ArH); 10.15 (s, 1H, NH). MS (ESI$^+$) m/z 318 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}ClNO_3$: C, 64.26; H, 5.08; N, 4.41; Found: C, 64.40; H, 5.09; N, 4.35.

*4.6.32. (Z)-N-(4-bromophenyl)-2-methoxy-3-(4-methoxyphenyl) acrylamide (**37**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 3.80 (s, 3H, OCH_3); 6.83 (s, 1H, CH); 7.00 (d, J = 8.7 Hz, 2H, ArH); 7.52 (d, J = 8.7 Hz, 2H, ArH); 7.70–7.79 (m, 4H, ArH); 10.13 (s, 1H, NH). MS (ESI$^+$) m/z 362 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}BrNO_3$: C, 56.37; H, 4.45; N, 3.87; Found: C, 56.43; H, 4.47; N, 3.85.

*4.6.33. (Z)-2-methoxy-3-(4-methoxyphenyl)-N-p-tolylacrylamide (**38**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 2.27 (s, 3H, CH_3); 3.70 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 6.80 (s, 1H, CH); 6.99 (d, J = 8.8 Hz, 2H, ArH); 7.13 (d, J = 8.4 Hz, 2H, ArH); 7.65 (d, J = 8.4 Hz, 2H, ArH); 7.70 (d, J = 8.8 Hz, 2H, ArH); 9.88 (s, 1H, NH). ^{13}C NMR (d_6-DMSO): 198.6, 159.9, 158.8, 135.5, 133.9, 131.1, 129.6, 127.4, 120.5, 114.7, 114.3, 56.0, 44.3, 20.9. MS (ESI$^+$) m/z 298 (M + H)$^+$. Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.71; H, 6.44; N, 4.71; Found: C, 72.46; H, 6.48; N, 4.80.

*4.6.34. (Z)-N-(4-isopropylphenyl)-2-methoxy-3-(4-methoxyphenyl)acrylamide (**39**)*

White powder, ^{1}H NMR (300 MHz, d_6-DMSO): 1.19 (d, J = 6.9 Hz, 6H, CH_3); 2.80–2.89 (m, 1H, CH); 3.67 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 6.80 (s, 1H, CH); 6.99 (d, J = 8.8 Hz, 2H, ArH); 7.20 (d, J = 8.6 Hz, 2H, ArH); 7.67 (d, J = 8.6 Hz, 2H, ArH); 7.71 (d, J = 8.8 Hz,

2H, ArH); 9.91 (s, 1H, NH). MS (ESI^+) *m/z* 326 $(M + H)^+$. Anal. Calcd for $C_{20}H_{23}NO_3$: C, 73.82; H, 7.12; N, 4.30; Found: C, 73.98; H, 7.07; N, 4.32.

*4.6.35. (Z)-2-methoxy-N,3-bis(4-methoxyphenyl)acrylamide (**40**)*

White powder, ^1H NMR (300 MHz, d_6-DMSO): 3.67 (s, 3H, OCH_3); 3.74 (s, 3H, OCH_3); 3.79 (s, 3H, OCH_3); 6.80 (s, 1H, CH); 6.90 (d, $J = 9.0$ Hz, 2H, ArH); 6.99 (d, $J = 8.8$ Hz, 2H, ArH); 7.66–7.72 (m, 4H, ArH); 9.88 (s, 1H, NH). MS (ESI^+) *m/z* 326 $(M + H)^+$. Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47; Found: C, 68.85; H, 6.08; N, 4.51.

4.7. Biological assay

4.7.1. Materials

Stock solutions of compounds were prepared with dimethylsulfoxide (DMSO, Sigma) and diluted with RPMI-1640 medium containing 10% fetal bovine serum (FBS). MTT was purchased from Sigma (St Louis, Mo). CD28 and CD3 were purchased from BD Pharmingen (San Diego, CA).

4.7.2. Animals

Female BALB/c mice, used at 6–8 weeks of age, were purchased from Shanghai Experimental Animal Center (Shanghai, China) They were maintained with free access to pellet food and water in plastic cages at 21 ± 2 °C and kept on a 12 h light–dark cycle. Animal welfare and experimental procedures were carried out strictly in accordance with the 'Guide for the Care and Use of Laboratory Animals' (National Research Council, 1996) and the related ethical regulations of our university. All efforts were made to minimize the animals' suffering and to reduce the number of animals used.

4.7.3. Preparation of spleen cell from mice

BALB/c mice were sacrificed and the spleens were removed aseptically. A single-cell suspension was prepared after cell debris and clumps were removed. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed three times with PBS (phosphate buffer solution) containing 2% FBS and were resuspended in RPMI 1640 medium at the indicated concentration.

4.7.4. Immunosuppressive activity and cytotoxicity

Spleen cells were cultured in 96-well flat-bottomed microplates (Falcon) at a density of 5×10^5 cells/well in RPMI 1640 medium (0.2 mL) containing 10% FBS, 100 U mL^{-1} penicillin, and 100 U mL^{-1} streptomycin. They were co-stimulated or non-stimulated by CD3/CD28 for 48 h at 37 °C in 5% CO_2-air in the presence or absence (control group) of various concentrations of compounds (0, 1, 2, 5 and 10 μg/mL). 20 μL MTT (5 mg/mL) reagent was added 4 h before the end of culture. Then 90 μL of lysis buffer (10% SDS, 50% DMF, pH 7.2) was added to each well for 6 h and the absorbance value at 570 nm was collected by microplate reader. The percentage of cell growth inhibition was determined using the following formula:

$$\begin{aligned}\text{Inhibitory rate (\%)} = \{1 - [&\text{Compounds (OD570)} \\ &- \text{Background (OD570)}]/ \\ &\times [\text{Control (OD570)} \\ &- \text{Background (OD570)}]\} \times 100\end{aligned}$$

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