European Journal of Medicinal Chemistry 47 (2012) 585–593

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis of novel cinnamanilides as potential immunosuppressive agents

Lei Shi [a,*], Lu Wang [b], Zhi Wang [a], Hai-Liang Zhu [b,**], Qiao Song [a]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, People's Republic of China
[b] State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, People's Republic of China

ARTICLE INFO

Article history:
Received 29 July 2011
Received in revised form
12 October 2011
Accepted 12 October 2011
Available online 20 October 2011

Keywords:
Cinnamanilides
Synthesis
Immunosuppressive activity

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

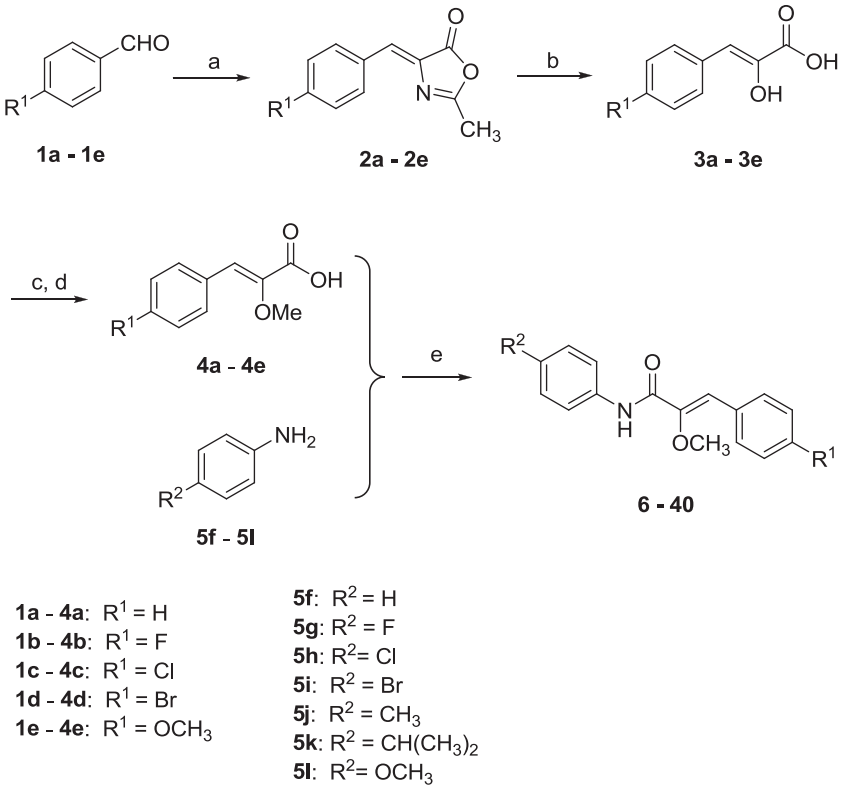
2.1. Chemistry

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**

* Corresponding author. Tel./fax: +86 25 83 27 1445.
** Corresponding author. Tel.: +86 25 8359 2572; fax: +86 25 8359 2672.
E-mail addresses: shilei0828@gmail.com (L. Shi), zhuhl@nju.edu.cn (H.-L. Zhu).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.10.027



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