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Synthesis and biological evaluation of α,β-unsaturated lactones as potent immunosuppressive agents

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ABSTRACT

Compounds having α,β-unsaturated lactones display a variety of biological activities. Many research groups have tested both natural and unnatural α,β-unsaturated lactones for as-yet undiscovered biological properties. We synthesized α,β-unsaturated lactones with various substituents at the δ-position and studied their immunosuppressive effects, that is, the inhibition of Interleukin-2 (IL-2) production. Among the compounds synthesized, the benzofuran-substituted α,β-unsaturated lactone **4h** showed the best inhibitory activity toward IL-2 production in Jurkat e6-1 T lymphocytes (IC_{50} = 66.9 nM) without cytotoxicity at 10 μM. The results indicated that **4h** may be useful as a potent immunosuppressive agent, as well as in IL-2-related studies.

α,β-Unsaturated lactone moieties display a variety of biological activities, including in the context of HIV[1], as protein phosphatase inhibitors[2], as antioxidants[3], as antiprotozoals[4], as antibacterials[5], and as anticancer agents.[6] Goniothalamin[7,8], Kazusamycin A[9], fostriecin[10], and kavalactone[3] are naturally occurring α,β-unsaturated lactones that show antibacterial, antifungal, anticancer, and protective activity against oxidative stress-induced neuronal cell death. Some previously described α,β-unsaturated lactones have displayed anti-inflammatory and immunosuppressive effects in immune-related cells.[11–13] Because the pharmacological effects of α,β-unsaturated lactones have not been fully established, many research groups have explored these properties. Herein, we investigate the relationship between α,β-unsaturated lactones and immunosuppressive effect by measuring activity toward inhibition of IL-2 production.[14–16]

IL-2 is produced by activated T cells during an immune response. IL-2/IL-2 receptor interactions then stimulate the growth, differentiation, and survival of T cells by inducting of the expression of specific genes. Thus, IL-2 is a cytokine targeted as a means by which the T lymphocytes-mediated immune response may be modulated, including the auto-reactive T cells.

We began the synthesis of α,β-unsaturated lactones by treating allyl magnesium bromide or allyl zinc bromide with various aldehydes **1** to obtain an allyl alcohol **2**. The R groups included naphthalene, quinoline, benzene, substituted benzene (methyl and methoxy groups at the *ortho*-, *meta*-, and *para*-positions), benzofuran, benzothiophene, furan, thiophene, benzyloxy, styryl, diphenyl, and adamantine (Table 1). Acrylic acid under basic conditions introduced an acrylate group to **2** to yield **3**. A second-generation Grubb's catalyst completed the formation of the α,β-unsaturated lactone **4** via a ring-closing metathesis[17] (Scheme 1).[18]

We measured the production of IL-2 by enzyme-linked immunosorbent assay (ELISA) to examine the immunosuppressive activity of the α,β-unsaturated lactones synthesized. Jurkat T lymphocytes (1×10^6 cells/mL) were seeded into 24-well plates. Cells in each well were treated with 10 μM or 1 μM final concentration of test compounds, and 5 nM ionomycin and 500 nM Phorbal 12-myristate 13-acetate (PMA) were added to stimulate the cells. Cells were incubated for 13 h at 37 °C. The cells were centrifuged at 4800 rpm for 2 min, and supernatants from each well were collected. IL-2 was measured by ELISA using an anti-human IL-2 antibody as the capture antibody and a biotinylated anti-human IL-2 antibody for detection (BD, NJ, USA).

An MTT assay was performed to measure the cytotoxicity of the compounds (**4a**–**4x**) in Jurkat e6-1 cells. These results were used to select potent immunosuppressive and non-cytotoxic compounds. The Jurkat cells were seeded into 96-well plates at a density of 1×10^5 cells/mL/well. Cells were incubated with or without 10 μM of each compound for 3 days at 37 °C. After 3 days, 10 μL of an MTT solution were added to each well, and the absorbance at 450 nm was read. The optical density was directly correlated with cell concentration.

The structures and biological activities of the synthesized α,β-unsaturated lactones are listed in Table 1. All derivatives contained aromatic or heteroaromatic moieties except for the adamantane-substituted **4x**. Among the compounds containing naphthalene (**4a**–**4e**), compounds **4a**, **4d**, and **4e** showed IL-2 inhibitory activity

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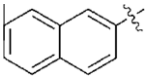
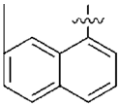
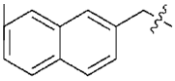
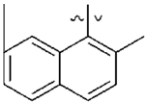
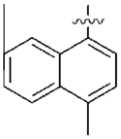
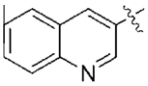
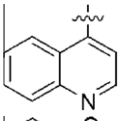
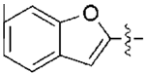
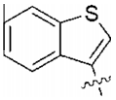
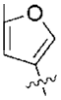
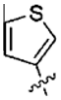
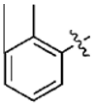
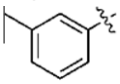
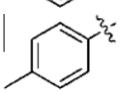
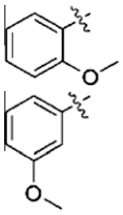
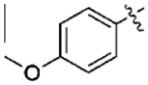
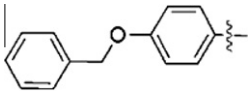
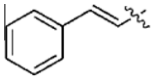
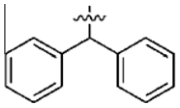
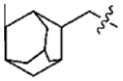
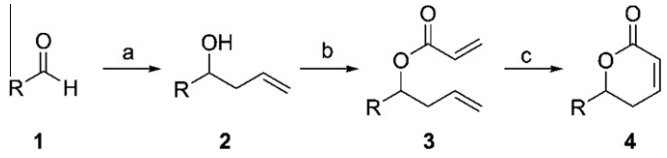
Table 1
Percent inhibition of IL-2 production and cell viability in the presence of α,β-unsaturated lactones

Compound number	R	10 μM[a]	1 μM[a]	Cell viability[b]
DMSO		0.00	0.00	100.00
Cyclosporin A		100.00	100.00	81.06
4a		97.20	37.53	123.45
4b		100.00	72.55	59.20
4c		100.00	67.72	77.73
4d		75.51	6.40	123.22
4e		100.00	57.35	87.52
4f		98.69	41.46	78.53
4g		100.00	27.75	140.49
4h		100.00	100.00	118.73
4i		100.00	77.08	41.66
4j		21.66	N/I	114.13
4k		N/I	N/I	103.97
4l		59.61	N/I	113.68
4m		46.16	N/I	116.69
4n		82.62	89.70	127.86
4o		86.11	15.19	62.29
4p		64.52	47.93	79.73
4q		80.61	89.03	103.27
4r		89.18	10.96	132.19

(*continued on next page*)

Table 1 (*continued*)

Compound number	R	10 μM[a]	1 μM[a]	Cell viability[b]
4s		90.36	N/I [c]	122.39
4t		87.22	39.84	144.69
4u		98.90	27.99	118.95
4v		90.13	12.72	145.15
4w		89.18	18.74	91.79
4x		100.00	57.39	111.91



Scheme 1. Synthetic method of α,β-unsaturated lactones. Reagents and conditions: (a) Allyl MgBr or allyl ZnBr, tetrahydrofuran (THF), 0 °C, 1 h then rt 2–12 h; (b) triethylamine (TEA), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), acrylic acid, dichloromethane (DCM), rt 12 h; (c) Grubb's catalyst 2nd generation, DCM, rt 8 h.

only at 10 μM, and **4b** displayed activity both at 10 μM and 1 μM. However, **4b**, **4c**, and **4e** caused cell death due to cytotoxicity. When we compared the methyl-substituted naphthalene with the simple naphthalene, the unsubstituted naphthalene derivatives (**4a**, **4b**) were more potent than the methyl-substituted naphthalene derivatives (**4d**, **4e**). Compound **4c**, which includes a methylene group between the α,β-unsaturated lactones and the naphthalene, showed around 70% inhibitory effects over the range 1–10 μM. This result may be derived from the cytotoxicity of **4c**. The compounds containing quinoline (**4f**, **4g**) yielded almost the same inhibitory activity toward IL-2 production, but the position of the substituents resulted in large differences in cytotoxicity. Benzofuran **4h** and benzophiophene **4i** were very potent and showed IC_{50}s of 66.9 nM and 907.8 nM for IL-2 production, respectively, as shown in Table 2. Compound **4h** showed 100% inhibition at both concentrations, and the cell viability was better than that observed in the presence of cyclosporine A under the given conditions. On the other hand, compound **4i** showed a high cytotoxicity with less than 50% cell viability. Furan (**4j**, **4k**) and thiophene (**4l**, **4m**) derivatives displayed neither inhibitory activity nor cytotoxicity. It should be noted that **4m** and **4i** yielded very different inhibitory activities and cytotoxicities because they include a thiophene moiety at the same position. Among the methyl- and methoxy-substituted benzyl derivatives (**4n**–**4t**), the unsubstituted benzyl and 4-methyl benzyl-substituted compounds (**4n**, **4q**) were active, non-cytotoxic, and showed good IC_{50}s (165.9 nM and 141.5 nM).

Table 2
IC_{50} of IL-2 production inhibition

Compound number	10 μM[a]	1 μM[a]	Cell viability[b]	IC_{50} (nM)
4c	100.00	67.72	77.73	395.9
4h	100.00	100.00	118.73	66.9
4i	100.00	77.08	41.66	907.8
4n	82.62	89.70	127.86	165.9
4q	80.61	89.03	103.27	141.5
4x	100.00	57.39	111.91	1574

[a] Percent inhibition of IL-2 production at each concentration comparing with DMSO, negative control.
[b] Cell viability was measured by an MTT assay, higher value means less cytotoxicity. All biological evaluation were performed at least three times.

The position of the substituent appeared to minimally affect the activity in this class of compounds. Although the methoxy benzyl derivatives (**4r**–**4t**) displayed weak activity at 1 μM, low cytotoxicity and good activity were observed at 10 μM. Compounds **4u** (benzyloxy moiety), **4v** (styryl moiety), **4w** (diphenyl moiety), and **4x** (adamantane moiety) were potent at 10 μM but inactive at 1 μM without cytotoxicity.

The IC_{50}s of selected compounds (**4c**, **4h**, **4i**, **4n**, **4q**, and **4x**) were measured, as shown in Table 2. The simple aromatic compounds **4c**, **4n**, and **4q** showed moderate or good IC_{50}s. The IC_{50} of benzothiophene **4i** was not good, even with 100% inhibition at 10 μM and more than 70% inhibition at 1 μM. We expected compound **4x** to provide good inhibitory effects because its structure was relatively wide. Introduction of other nonaromatic substituents may be productive, but is outside the scope of this study.

As mentioned above, benzofuran-substituted α,β-unsaturated lactones **4h** displayed the best inhibitory activity toward IL-2 production (IC_{50} = 66.9 nM, Fig. 1) without cytotoxicity at 10 μM. The results indicated that **4h** may be a potent immunosuppressive agent, and it could be used in other IL-2 related studies. Although the other compounds showed less potency toward inhibiting IL-2 production or less cell viability than that observed in the presence of **4h**, some compounds may potentially be developed as potent immunosuppressive agents.

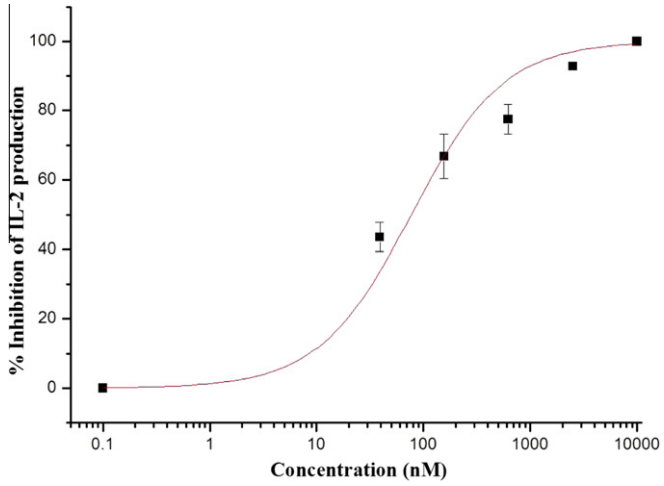


Figure 1. IC_{50} plot for compound **4h**.

The mechanism by which **4h** functions as an IL-2 inhibitor in this study was not elucidated. However, we demonstrated that our compound potently inhibited IL-2 production in stimulated T cells without cytotoxicity. Thus, new α,β-unsaturated lactones may potentially be useful in the discovery of immunosuppressants for the treatment of T cell-mediated inflammatory disorders.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.020.

References and notes

1. Hagen, S. E.; Vara Prasad, J. V. N.; Boyer, F. E.; Domagala, J. M.; Ellsworth, E. L.; Gajda, C.; Hamilton, H. W.; Markoski, L. J.; Steinbaugh, B. A.; Tait, B. D.; Lunney, E. A.; Tummino, P. J.; Ferguson, D.; Hupe, D.; Nouhan, C.; Gracheck, S. J.; Saunders, J. M.; Vanderroest, S. *J. Med. Chem.* **1997**, *40*, 3707.
2. Buck, S. B.; Hardouin, C.; Ichikawa, S.; Soenen, D. R.; Gauss, C.-M.; Hwang, I.; Swingle, M. R.; Bonness, K. M.; Honkanen, R. E.; Boger, D. L. *J. Am. Chem. Soc.* **2003**, *125*, 15694.
3. Tanaka, A.; Hamada, N.; Fujita, Y.; Itoh, T.; Nozawa, Y.; Iinuma, M.; Ito, M. *Bioorg. Med. Chem.* **2010**, *18*, 3133.
4. Carmona, D.; Saez, J.; Granados, H.; Perez, E.; Blair, S.; Angulo, A.; Figadere, B. *Nat. Prod. Res.* **2003**, *17*, 275.
5. Hester, Jr. et al. U.S. Patent 5 708 169, 1998.
6. Zhou, F. S.; Tang, W. D.; Mu, Q.; Yang, G. X.; Wang, Y.; Liang, G. L.; Lou, L. G. *Chem. Pharm. Bull.* **2005**, *53*, 1387.
7. Shi, B.; Greaney, M. F. *Chem. Commun.* **2005**, *41*, 886.
8. Fatima, A.; Kohn, L. K.; Carvalho, J. E.; Pilli, R. A. *Bioorg. Med. Chem.* **2006**, *14*, 622.
9. Fujimoto, H.; Sumino, M.; Nagano, J.; Natori, H.; Okuyama, E.; Yamazaki, M. *Chem. Pharm. Bull.* **1999**, *47*, 71.
10. Takeuchi, T.; Takahashi, N.; Ishi, K.; Kusayanagi, T.; Kuramochi, K.; Sugawara, F. *Bioorg. Med. Chem.* **2009**, *17*, 8113.
11. Penissi, A. B.; Vera, M. E.; Mariani, M. L.; Rudolph, M. I.; Ceñal, J. P.; de Rosas, J. C.; Fogal, T. H.; Tonn, C. E.; Favier, L. S.; Giordano, O. S.; Piezzi, R. S. *Eur. J. Pharmacol.* **2009**, *612*, 122.
12. Lyb, G.; Knorre, A.; Schmidt, T. J.; Pahl, H. L.; Merfort, I. *J. Biol. Chem.* **1998**, *273*, 33508.
13. Ramachandran, P. V.; Yip-Schneider, M.; Schmidt, C. M. *Future Med. Chem.* **2009**, *1*, 179.
14. Yun, C.-H.; Son, C. G.; Jung, U.; Han, S. H. *Toxicology* **2006**, *217*, 31.
15. Dejica, D. *Roum. Arch. Microbiol. Immunol.* **2001**, *60*, 183.
16. Mortellaro, A.; Songia, S.; Gnocchi, P.; Ferrari, M.; Fornasiero, C.; D'Alessio, R.; Isetta, A.; Colotta, F.; Golay, J. *J. Immunol.* **1999**, *162*, 7102.
17. Kasaplar, P.; Yılmazer, O.; Cağır, A. *Bioorg. Med. Chem.* **2009**, *17*, 311.
18. Full experimental procedures and spectral data (^{1}H NMR and Mass) of the final compounds (**4a**–**4x**) are described in the Supplementary data.