

## Synthesis and immunomodulation of human lymphocyte proliferation and cytokine (interferon- $\gamma$ ) production of four novel malonitrilamides

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### Abstract

Leflunomide is an immunomodulator drug with applications in the management of arthritis rheumatoid. In this study, four novel analogs (1a-d) of A771226, the active metabolite of leflunomide were synthesized and examined in vitro for their immunomodulation activity by examining human lymphocyte proliferation and determination of the cytokine interferon- $\gamma$  concentrations in human lymphocyte cell culture. For this purpose,  $2 \times 10^5$  human lymphocyte cells were incubated at 37 °C in 5% CO<sub>2</sub> with phytohemagglutinin and one of the analogs (concentrations 1-100  $\mu$ M), negative controls or cyclosporine (10  $\mu$ M). Effects of the compounds on lymphocyte proliferation and interferon- $\gamma$  production were determined by MTT assay and enzyme-linked immunosorbent assay, respectively. Our results showed that all four compounds dose-dependently suppressed lymphocyte proliferation. Moreover, these compounds at some concentrations reduced interferon- $\gamma$  production which is an indicator of the immune response. Generally, the most potent analog was 1b with an amide linkage (X=NH) and the weakest analog was 1a with an ester linkage (X=O). Compound 1a has little similarity with the leflunomide active metabolite which has an amide linkage. In this study, four novel compounds were synthesized that showed considerable immunosuppressive effects that deserve further investigations. © 2009 John Wiley & Sons A/S.

### Reaxys Database Information

### Author keywords

Immunomodulation; Interferon- $\gamma$ ; Leflunomide; Malonitrilamides; MTT assay; Synthesis

### Indexed Keywords

**EMTREE drug terms:** 2,2-dimethyl-2-thiazolyl)-2-diphenyltetrazolium bromide; amide; carbon dioxide; cyclosporin; cytokine; drug metabolite; ester; gamma interferon; immunosuppressive agent; leflunomide; malonitrilamide derivative; n-(2'-cyanobiphenyl-5-yl)-2-cyano-3-hydroxycrotonate; n-(2'-cyanobiphenyl-5-yl)-2-cyano-3-hydroxycrotonate; n-(biphenyl-5-yl)-2-cyano-3-hydroxycrotonate; o-(biphenyl-5-yl)-2-cyano-3-hydroxycrotonate; phytohemagglutinin; teriflunomide; undassified drug

**EMTREE medical terms:** article; concentration response; controlled study; cytokine production; drug structure; drug synthesis; enzyme linked immunosorbent assay; human; human cell; immune response; immunomodulation; in vitro study; lymphocyte; lymphocyte culture; lymphocyte proliferation; priority journal

**MeSH:** Amides; Cell Proliferation; Humans; Immunosuppressive Agents; Interferon-gamma; Isoxazoles; Lymphocyte Activation; Lymphocytes; Nitriles

Medline is the source for the MeSH terms of this document.

**Chemicals and CAS Registry Numbers:** 2,2-dimethyl-2-thiazolyl)-2-diphenyltetrazolium bromide, 298-93-1; amide, 13700-21-1; carbon dioxide, 124-38-9, 080611-67-4; cyclosporin, 79217-60-0; gamma interferon, 82110-62-6; leflunomide, 70706-12-6; phytohemagglutinin, 9008-97-3; teriflunomide, 108700-62-0, 282716-73-8; Amides;