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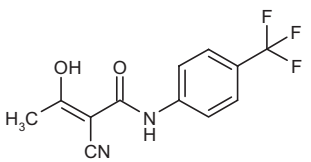
Teriflunomide

Prop INN

Agent for Multiple Sclerosis
Dihydroorotate Dehydrogenase Inhibitor

A-771726
HMR-1726
RS-61980
SU-0020

(*Z*)-2-Cyano-3-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-2-butenamide



$C_{12}H_9F_3N_2O_2$
Mol wt: 270.2073

CAS: 108605-62-5

CAS: 282716-73-8 (as monosodium salt)

EN: 178777

Abstract

Teriflunomide, a dihydroorotate dehydrogenase (DHODH) inhibitor, is the active metabolite of leflunomide a synthetic, low-molecular-weight drug currently used in the treatment of rheumatoid arthritis. The mechanisms by which teriflunomide exerts its antiinflammatory, antiproliferative and immunosuppressive effects are not yet completely understood, although inhibition of pyrimidine biosynthesis (via suppression of DHODH) and interference with tyrosine kinase activity both appear to be involved. Based on its efficacy shown in animal models of experimental allergic encephalomyelitis, teriflunomide was tested in a phase II study in patients with multiple sclerosis with relapses. Recruitment is ongoing for a phase III study to determine the efficacy of teriflunomide in reducing the frequency of relapses and accumulation of disability in multiple sclerosis patients.

Synthesis

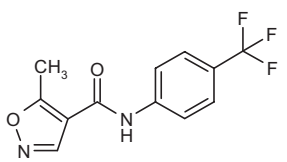
Teriflunomide can be prepared by several ways:

1) Reaction of 5-methylisoxazole-4-carboxylic acid (I) with refluxing $SOCl_2$ gives the corresponding acid chloride (II), which is coupled with 4-(trifluoromethyl)aniline (III) in acetonitrile to produce anilide (IV). Finally, the isoxazole ring in (IV) is opened by means of NaOH in refluxing MeOH/H_2O (1). Scheme 1.

2) Reaction of ethyl 2-cyanoacetate (V) with 4-(trifluoromethyl)aniline (III) by heating at 180 °C affords the corresponding cyanoacetamide (VI) (2), which can also be obtained by condensation of 2-cyanoacetic acid (VII) with aniline (III) by means of DIC (3). Finally, cyanoacetamide (VI) is then condensed with acetyl chloride (VIII) by means of NaH in THF (2-4), or alternatively condensed with acetic anhydride (IX) by means of NaOH in diethoxymethane in the presence of dimethylditetradecylammonium bromide (4). Scheme 2.

Introduction

Leflunomide [I] is a synthetic, low-molecular-weight drug of the isoxazole class. Due to its potent antiinflammatory and immunosuppressive activity, leflunomide is currently used in the treatment of rheumatoid arthritis,



[I]

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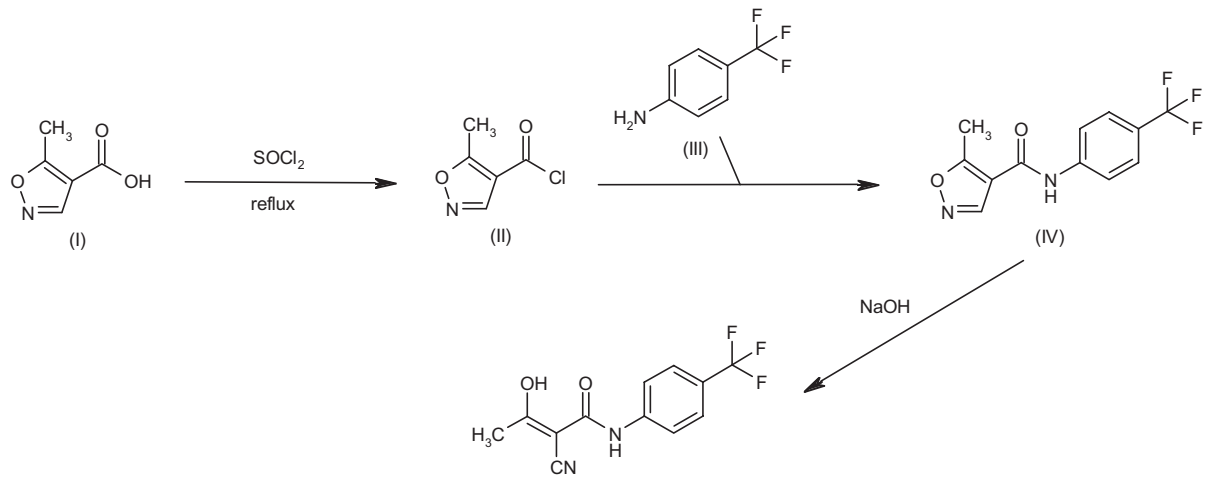
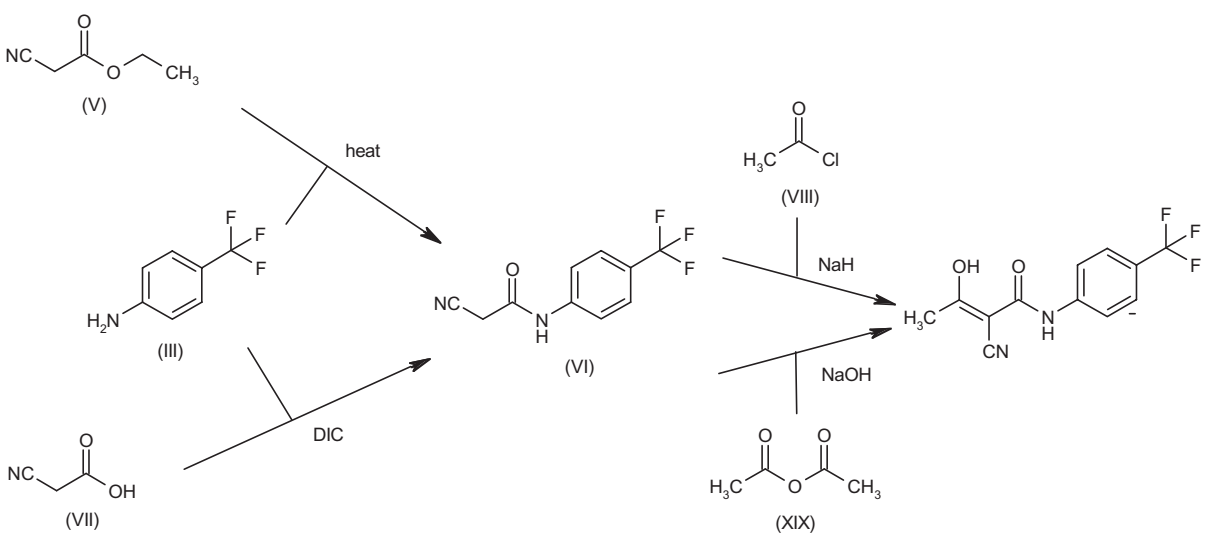
Scheme 1: Synthesis of Teriflunomide



Scheme 2: Synthesis of Teriflunomide



and is also undergoing testing for use in the treatment of inflammatory bowel disease and chronic allograft rejection. Once administered, leflunomide is quickly converted to its active metabolite, teriflunomide (HMR-1726; also referred to as RS-61980, A-771726, SU-0020).

The mechanisms by which teriflunomide exerts its antiinflammatory, antiproliferative and immunosuppressive effects are not yet completely understood, although inhibition of pyrimidine biosynthesis (via suppression of dihydroorotate dehydrogenase [DHODH]) and interference with tyrosine kinase activity both appear to be involved.

Pharmacological Actions

Given the results from cellular assays demonstrating that the antiproliferative effects of teriflunomide can be antagonized by uridine, researchers postulated that the compound might inhibit one of the enzymes in the *de novo* pyrimidine biosynthetic cascade. *In vitro* studies designed to explore this possibility found that teriflunomide is a potent inhibitor of DHODH, which catalyzes the fourth step in *de novo* pyrimidine biosynthesis (5-7). The K_i values reported were 2.7 ± 0.7 μM for inhibition of DHODH in human cells (5) and 179 ± 19 nM for inhibition

of purified recombinant human enzyme (6, 7). The observation that exogenous uridine counteracted the teriflunomide-induced inhibition of graft-*versus*-host reaction in B6C3F1 hybrid mice confirmed this mechanism of action (8). Leflunomide, the parent compound, did not inhibit DHODH at concentrations up to 1 μM. Further assessment showed that teriflunomide binds competitively to the ubiquinone cofactor binding site of DHODH. Teriflunomide's potency as a DHODH inhibitor is 100-1,000-fold greater than that reported for inhibition of protein tyrosine kinases (6, 7).

In other experiments, researchers examined the relative influence of teriflunomide's inhibition of pyrimidine nucleotide synthesis and protein tyrosine phosphorylation on the drug's capacity to inhibit proliferation of the murine leukemia cell line LSTRA. Results showed that teriflunomide inhibited LSTRA cell growth and proliferation with an IC_{50} of about 10-30 μM. Similar to previous findings, exogenous uridine reversed this inhibitory activity. Teriflunomide directly inhibited DHODH activity, with an IC_{50} of about 220 nM, and it reduced the intracellular levels of tyrosine phosphorylated proteins in LSTRA cells, with IC_{50} values of 50-100 μM. Teriflunomide also inhibited $p56^{lck}$ activity in LSTRA membrane preparations and immunoprecipitates, with an IC_{50} value of 80 μM for inhibition of immunoprecipitated $p56^{lck}$ autophosphorylation and an IC_{50} value of 40 μM for inhibition of exogenous substrate histone 2B. Uridine did not interfere with the drug's inhibition of tyrosine phosphorylation (9).

A similar study in murine cytotoxic T-cell lines (CTLLs) demonstrated that the two biochemical mechanisms of action reported for teriflunomide (*i.e.*, inhibition of pyrimidine biosynthesis and tyrosine phosphorylation) both play a role in the drug's immunosuppressive activity in these cell lines (10). The compound's inhibitory effects on DHODH, together with the resultant antiproliferative activity, were also demonstrated *in vitro* in U-937 cells and human spleen membrane preparations (11).

Teriflunomide also concentration-dependently inhibited the proliferation of human Jurkat leukemia cells, with IC_{50} values of 20-25 μM. This inhibition could be reversed by the addition of exogenous uridine. Additionally, incubation with teriflunomide caused potent inhibition of DHODH, with a consequent concentration- and time-dependent intracellular accumulation of dihydroorotate in these cells. Cell cycle analysis revealed that teriflunomide caused cell cycle arrest at the G0/G1/S phase boundary in Jurkat cells *in vitro*. Incubation of these cells with the *de novo* pyrimidine biosynthesis inhibitor brequinar produced similar results (12).

In another study, teriflunomide was shown to concentration-dependently inhibit the proliferation of platelet-derived growth factor (PDGF)-stimulated rat smooth muscle cells, with an IC_{50} of about 3 μM. Uridine (1 mM) reversed this antiproliferative effect. Similar results were found for the two teriflunomide derivatives HMR-1279 and HMR-1715, which belong to a pharmacological class known as the malononitrilamides (MNAs) (13).

An additional study using teriflunomide, HMR-1279 and HRM-1715 also found that all three MNAs inhibited the generation of oxygen radicals in mouse macrophages and in human and rat peripheral blood monocytes. This inhibition was demonstrated to be concentration-dependent, and the compounds were not cytotoxic at the concentrations used. *In vivo* administration to mice and rats resulted in strong, long-lasting inhibition of mononuclear phagocytes. In nonobese diabetic (NOD) mice, these compounds caused a delay in the development of insulin-dependent diabetes mellitus and suppressed macrophage-mediated oxygen radical generation. Based on these results, the researchers suggested that the inhibition of macrophage functions may also play a role in the activities of teriflunomide and its malononitrilamide derivatives (14).

The effects of teriflunomide on T-cell integrin activation, immunological synapse formation and the antigen-specific formation of stable T-cell/antigen-presenting cell (APC) conjugates were also assessed in a recent study. Results showed that teriflunomide did not alter most critical T-cell signaling events, including mitogen-activated protein kinase (MAPK) and NF-κB activation. However, the compound did inhibit T-cell receptor (TCR)/CD3-mediated calcium mobilization, which led to impaired $\beta_{1,2}$ integrin avidity and integrin-mediated signaling. This impaired integrin activation occurred independently of altered pyrimidine synthesis. Furthermore, teriflunomide profoundly disrupted the formation of mature immunological synapses, which led to an abrogation of antigen-specific conjugate formation between APCs and T-cells. These results suggest a novel mechanism of action that may contribute to teriflunomide's activity in disorders involving activated T-cells (15).

Another study examined the activity of teriflunomide on the proliferation of spleen colony-forming units (CFU) in mice on day 12 during the recovery of hemopoiesis after nonlethal damage by irradiation. Teriflunomide at doses of 25-250 μmol/ml dose-dependently inhibited CFU cycling (16).

The effects of teriflunomide on the behavioral consequences of experimental allergic encephalomyelitis (EAE) were assessed in female Lewis rats to determine the drug's potential utility in multiple sclerosis. Oral treatment with teriflunomide at 3 and 10 mg/kg or with dexamethasone at 1 mg/kg began 10 days after inoculation with guinea pig spinal cord and Freund's adjuvant, when clonal expansion of inflammatory and immune cells had already occurred. Both compounds caused a significant delay in the onset of disease and in symptom severity (17).

Clinical Studies

Based on its efficacy in EAE, teriflunomide was tested in a phase II study in 179 patients with multiple sclerosis with relapses. Once-daily oral treatment with teriflunomide (7 and 14 mg) for 36 weeks led to a reduced number of unique active lesions as compared to placebo. The

adjusted mean number of unique active lesions per scan was 2.69 in the placebo group, 1.06 ($p < 0.024$) in the 7-mg dose group and 0.98 ($p < 0.006$) in the 14-mg dose group. The median values for the three groups were 0.6, 0.17 and 0.33 active lesions per scan, respectively. In addition, teriflunomide at either dose was associated with a reduction in new T1 lesions ($p < 0.05$) and new T2 lesions ($p < 0.01$), as well as fewer subjects with new T2 lesions ($p < 0.05$), compared to placebo. The higher dose was also associated with reduced disease burden ($p < 0.03$). A trend towards fewer patients with relapses was seen for teriflunomide compared to placebo. Additionally, fewer patients receiving the higher dose of teriflunomide exhibited an increase in Expanded Disability Status Scale (EDSS) compared to those receiving placebo ($p < 0.04$). Adverse events were similar among groups, with headache, nasopharyngitis and upper respiratory tract infection being the most common (18).

A pilot study investigated the *in vitro* functioning of peripheral blood mononuclear cells from the 9 multiple sclerosis patients in the Vancouver cohort of a double-blind, placebo-controlled phase II trial of teriflunomide (7 or 14 mg). A variable number of tests were performed in a varying number of patients due to organizational constraints. Results from this study showed a 30-40% reduction in immobilized CD3-stimulated DNA and RNA synthesis in the patients treated with teriflunomide. Pokeweed-stimulated IgG secretion was higher in 7 multiple sclerosis patients than in 12 controls, with values of 1527 ± 375 ng/10^6 cells/7 days and 1147 ± 140 ng/10^6 cells/7 days, respectively. Teriflunomide reduced this increase to 469 ± 97 ng/10^6 cells/7 days in the patients receiving 7 mg/day and to 641 ± 148 ng/10^6 cells/7 days in those receiving 14 mg/day. Lymphocyte adhesion was higher in untreated multiple sclerosis patients than in same-day healthy controls, with an observed dose-dependent reduction of 26 ± 2.6% with the lower dose and of 39 ± 3% with the higher dose of teriflunomide (19).

Recruitment is ongoing for a phase III study to determine the efficacy of teriflunomide in reducing the frequency of relapses and accumulation of disability in multiple sclerosis patients (20).

Source

Sanofi-Aventis (FR).

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