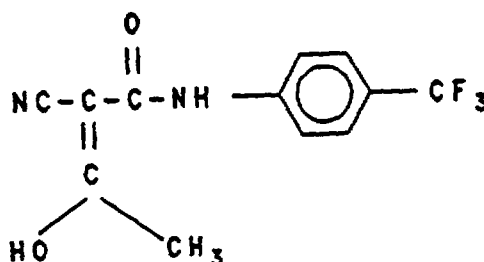


Description

Medicaments to combat chronic graft-versus-host diseases and to combat autoimmune diseases, in particular systemic lupus erythematosus

- 5 The (4-trifluoromethyl)anilide of 5-methylisoxazole-4-carboxylic acid is known to be antiinflammatory from European Patent 13,376. This patent likewise describes processes for the preparation of this compound.

10 It has now been found that N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide



- 15 [Stecher and Carlson, Ann. Report Med. Chem. 18, 171-179 (1983)] has immunomodulating properties such that it is suitable as a medicament to combat chronic graft-versus-host diseases (cGvH) and to combat autoimmune diseases, in particular systemic lupus erythematosus (SLE).

20 Thus the invention relates to the use of N-(4-trifluoromethylphenyl)-2-cyanno-3-hydroxycrotonamide, it being possible to use it as such or in the form of a physiologically tolerated salt, for the preparation of medicaments to combat chronic graft-versus-host diseases and to combat autoimmune diseases, in particular systemic lupus erythematosus. Examples of suitable salts are

alkali metal, alkaline earth metal and ammonium salts, including those of physiologically tolerated organic ammonium bases.

A) Chronic graft-vs.-host (cGvH) diseases

5 With transplantations there is, relatively frequently, rejection of the transplant. The transplant-host relation is, however, not confined merely to the rejection by the host organism; in certain cases there may be an immune reaction originating from the transplant and directed
10 against the host tissue. A distinction is made between an acute and a chronic reaction. The features of the acute graft-vs.-host reaction are spleen enlargement, liver swelling, lymph node hypertrophy, hemolytic anemia, low levels of immunoglobulins and complement, and diminished
15 immunological reactivity. The reaction which has an acute course almost always has a fatal outcome.

There is also the chronic form of the disease process. It results in lymphadenopathy, immune complex glomerulonephritis and in the formation of many antibodies. This
20 form of the disease is milder than the acute form and does not always result in death within a short time. Symptoms produced by cGvH reaction very closely resemble those of systemic lupus erythematosus.

B) Systemic lupus erythematosus (SLE)

25 Systemic lupus erythematosus is an autoimmune disease which is not specific to any organ. This disease affects a large number of organs and has a chronic course with acute episodes. The external manifestations of SLE are lesions on the facial skin. In most cases, other areas of
30 skin and the mucosa are affected. Also observed are nephritis, endocarditis, hemolytic anemia, leukopenia and involvement of the central nervous system.

Many immunological phenomena have been observed with SLE.

There is formation of antibodies against certainly endogenous antigens. These antibodies which can be detected in SLE patients are directed against, for example, the basement membrane of the skin, and against lymphocytes, erythrocytes and nuclear antigens. In the first place, the antibodies which are directed against double-stranded DNA (ds-DNA) form with the latter complexes which are deposited together with complement on small blood vessels and frequently result in vasculitis. These deposits are especially dangerous when they occur in the renal glomeruli because they result in glomerulonephritis and kidney failure. The incidence of clinically detectable involvement of the kidneys is reported in the literature to be 50 to 80 %.

Glucocorticoids and other immunosuppressive medicaments, for example cyclophosphamide (CPA), are of crucial importance for the survival of patients with systemic lupus erythematosus. There is as yet no specific curative agent. To date, therapy has been aimed at preventing or overcoming acute exacerbation and averting recurrences. For this purpose, the patients have been treated with glucocorticoids and other immunosuppressants, but these themselves have hazardous side effects.

There is a variety of animal models for research into SLE. A few strains of mice spontaneously develop SLE, such as New Zealand mice or MRL/l mice, which are animals which originated from the Jackson Laboratories, Maine, USA, and which have been reared further in our own animal rooms under specific pathogen-free (SPF) conditions. However, it is also possible to induce a disease resembling SLE by experimental operation on non-autoimmune mice.

In the text which follows the quantities stated in mg/kg relate to kg of body weight; CMC denotes the sodium salt of carboxymethylcellulose, and N.S. denotes "no test substance". In Figure 3 N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide is designated anilide 2. The

active substances were administered orally in a mixture with CMC. "N.C." denotes negative control.

C) Pharmacological tests and results

1. Chronic graft-vs.-host disease

5 This model has been described in various publications by
GLEICHMANN et al. SLE is induced by initiating a GvH
reaction by an abnormal T/B-cell cooperation [GLEICHMANN
et al. Euro. J. Immunol. 12; 152-159 (1982)]. The cGvH
disease was initiated by two injections of spleen and
10 thymus cells. The mice, F1 generation in accordance with
GLEICHMANN et al. (DBA/2 x C57B1/6), each received
70 x 10⁶ DBA/2 cells, injected intravenously in 0.2 ml of
culture medium, on day 1 and day 8. The animals were
treated for the first time on the 17th day after the
15 first injection of the donor cells. Experiment 1.1 was
carried out, use being made in this experiment of only
female animals as donors and recipients. Each animal
received oral administration of 1 ml which in each case
contained the amount of active compound indicated in
20 experiment 1.1, plus CMC in a concentration of 100 mg/l.

Experiments

1.1) From day 17 onwards, the following were administered
once a day:
CMC, N.S.,
25 1 mg/kg indomethacin or
2 mg/kg prednisolone or
20 mg/kg N-(4-trifluoromethylphenyl)-2-cyano-3-
hydroxycrotonamide
30 mg/kg N-(4-trifluoromethylphenyl)-2-cyano-3-
30 hydroxycrotonamide.
There were 10-11 animals in each group.

a) Proteinuria

For the duration of the experiment (9-10 weeks) the amount of protein in the urine of the animals was established each week (Albu-sticks reagent sticks, Ames Division, Miles Laboratories, Elkhart). The results of these tests are shown in Figure 3.

b) Inhibitions of the graft-vs.-host index

During the course of cGvH disease there is a considerable enlargement of the spleen as a consequence of the immunological defensive activity. If the weight of the spleen is related to the body weight of the diseased animal, and this ratio is compared with the corresponding ratio for the healthy animal, the result is the graft-vs.-host index:

$$\text{GvH index} = \frac{\text{spleen weight X} / \text{body weight X}}{\text{spleen weight h} / \text{body weight h}}$$

where X = (diseased) animal investigated, and h = healthy animal.

It is possible with the GvH index to establish the severity of the disease: the larger the index the greater the severity of the disease. The results are shown in Table 1.

Table 1

Experiment	Substance	GvH index	% change
1.1	CMC, N.S.	2.99	0
1.1	1 mg/kg indomethacin per day	3.44	115
1.1	2 mg/kg prednisolone per day	1.27	-58
30 1.1	20 mg/kg N-(4-trifluoromethylphenyl-2-cyano-3-hydroxycrotonamide	1.99	-33
1.1	30 mg/kg N-(4-trifluoromethylphenyl-2-cyano-3-hydroxycrotonamide	1.27	-56

The results of the abovementioned tests show that N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide inhibit the development of cGvH diseases and SLE in mice.

This substance results in

- 5 1) prevention of the development of glomerulonephritis in both diseases; this has been shown by the diminution in the protein excreted in the urine and by histological tests on the kidneys;
- 2) a reduction in the anti-ds-DNA antibody titer;
- 10 3) a decrease in the GvH index;
- 4) a dose-dependent improvement in the diminished proliferation of T-lymphocytes.

15 N-(4-Trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide has advantages compared with immunosuppressants such as cyclophosphamide or glucocorticoids, since it does not cause general suppression of the immune system and, in fact, allows regeneration of the diminished T-cell function. It is all the more surprising that it very effectively combats chronic GvH diseases and SLE.

20 N-(4-Trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide can be administered either alone, where appropriate in the form of microcapsules, or mixed with customary physiologically tolerated excipients, diluents and/or ingredients. The agents can be administered orally, 25 rectally, intravenously or parenterally, oral or rectal administration being preferred. Examples of suitable solid or liquid pharmaceutical presentations are granules, powders, tablets, coated tablets, capsules, suppositories, syrups, emulsions, suspensions, aerosols, 30 drops or injectable solutions presented in ampules, this also including the dry ampule as a special presentation, as well as products with protracted release of active compound, in whose preparation use is customarily made of auxiliaries such as excipients, disintegrants, binders, 35 coating agents, swelling agents, lubricants, flavorings, sweeteners, buffers, antioxidants and/or solubilizers.

Examples of auxiliaries which are frequently used are magnesium or calcium carbonate, calcium phosphates, titanium dioxide, mannitol, lactose and other sugars, talc, lactalbumin, gelatin, starch, vitamins, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols, and physiologically acceptable solvents such as sterile water, alcohols, glycerol and other polyhydric alcohols.

The pharmaceutical products are preferably prepared and administered in dosage units, each unit containing as active ingredient a defined dose of N-(4-trifluoromethylphenyl)2-cyano-3-hydroxycrotonamide. This dose can be from 10 to 200 mg, but preferably 50 to 100 mg, for solid dosage units, such as tablets, capsules and suppositories, 1 to 30 mg, preferably 5 to 10 mg, for injection solutions presented in ampules (intravenous), especially those based on N-(4-trifluoromethylphenyl)2-cyano-3-hydroxycrotonamide or a salt thereof, and 50 to 300 mg, preferably 100 to 200 mg, for rectal administration.

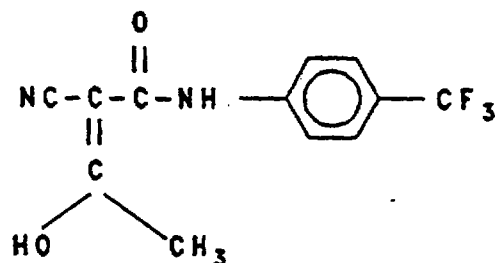
In humans, daily doses of 50 to 200 mg of active compound on oral administration, of 10 to 30 mg on intravenous administration and of 100 to 300 mg on rectal administration are indicated for the treatment of an adult patient. However, in certain circumstances higher or lower daily doses may be advisable. The daily dose may be administered either as one dose in the form of a single dosage unit or as several smaller dosage units, as well as by administration of several divided doses at defined intervals.

Another use of the compounds comprises combination with other suitable active compounds, for example antiuricopathics, platelet aggregation inhibitors, analgesics and steroidal or non-steroidal antiinflammatory agents.



Patent claims:

1. A compound of the formula



the compound being present as such or in the form of a physiologically tolerated salt.

- 5 2. A medicament containing an effective amount of the compound as claimed in claim 1, the compound being present as such or in the form of a salt.