European Patent 13,376 discloses N-(4-trifluoromethyl)-5-methylisoxazole-4-carboxanilide (compound 1) as being anti-inflammatory. Processes for the preparation of this compound are also described therein.

It is additionally known that the compound 1 and its metabolite N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonamide (compound 2) have immunomodulating properties, so that they are suitable as pharmaceuticals against chronic graft-versus-host diseases and against autoimmune disorders, in particular systemic lupus erythematosus (EP-A-217,206).

US Patent 4,061,767 describes the use of 2-hydroxyethylidenecyanoacetanilide derivatives for the preparation of pharmaceuticals having anti-inflammatory and analgesic action.

In the publication Agents and Actions (32, 1991, pages 10 to 21) it is described that the occurrence of the acute rejection reaction is slowed down by compound 1 and 2. In the publication by Küchle et al. (Transplantation Proceedings, 23, 1991, pages 1083 to 1086) the action of compound 1 on rejection reactions which occur more than 8 days after transplantation is described.

In the USA, 15,000 organ transplantations were performed in 1990. The majority of the transplantations relate to the kidney, but hearts, skin, lungs, liver and pancreas are also increasingly transplanted. In a large number of the patients, there is a rejection reaction of the body to the transplanted organ, which is derived from another person. A differentiation is made here between three forms of rejection reaction:
hyperacute, acute and chronic rejection.

Hyperacute rejection is essentially caused by circulating antibodies in the blood, which are directed against the tissue of the transplanted organ (transplant), and in a

very short time - often in minutes - lead to necroses in the transplant.

In acute rejection of the transplant, the rejection reaction is delayed. In addition, there is the chronic form of the course of the disease. The transplants survive the first year of transplantation, but can be rejected in the course of the next few years. It is additionally known that the transplant-host relationship is not restricted to rejection by the host organism alone; in certain cases an immune reaction originating from the transplant and directed against the host tissue can occur (EP-A-217,206). A differentiation is therefore made between a rejection reaction between transplant and host and between host and transplant.

It is additionally known that there are also rejection reactions if organs of various animal species are transplanted, for example from the mouse to the rat (Roitt et al., Immunology, Gower Medical Publishing Ltd., 1985).

To date, no pharmaceuticals are known which offer effective protection against the hyperacute rejection reaction. In the clinic, until now donors and recipients of the organ have been tested for incompatibility. In 20 to 40% of the patients it turns out to be the case that they cannot receive any donor organ. The acute rejection reaction can be treated, but the medicaments show nephrotoxic side effects during treatment. To date, there are also no medicaments known which can treat the cause of the chronic rejection reaction.

The essential pathogenic factor for tissue death in the transplant is regarded to be allophilic and xenophilic antibodies (Auchincloss H., Transplantation 46, 1, 1988). These antibodies are essentially responsible for rejection in organ transplants within an animal species (allo) or between two different species (xeno).

Surprisingly, compound 1 and its metabolite, the above-mentioned compound 2, show a potent inhibition of the formation of allophilic or xenophilic antibodies. There is thus the possibility of effectively treating the hyperacute, acute and chronic rejection reaction of the recipient to the transplanted organ.

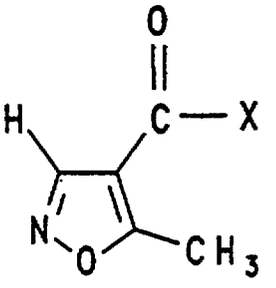
The invention therefore relates to the use of N-(4-trifluoromethylphenyl)-5-methylisoxazole-4-carboxanilide and N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide and/or their physiologically tolerable salts for the preparation of pharmaceuticals for the treatment of hyperacute rejection reactions of the organ recipient to the transplanted organ.

The invention furthermore relates to the use of the compound of the formula 1 and/or 2, where compound 2 is present as such or in the form of a physiologically tolerable salt, for the preparation of pharmaceuticals for the treatment of rejection reactions of the organ recipient to the transplanted organ, the organ being tranferred from one species of the organism to another species.

Suitable physiologically tolerable salts of compound 2 are, for example, alkali metal, alkaline earth metal or ammonium salts, including those of physiologically tolerable organic ammonium bases. The term organ is understood as meaning all organs in mammals, in particular the human, for example kidney, heart, skin, liver, pancreas, muscle, bone, intestine or stomach, but also blood or hair. Rejection reaction means all defence mechanisms of the recipient organism which in the end lead to cell or tissue death of the transplanted organ or affect the viability of the transplanted organ.

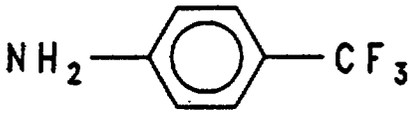
The compounds 1 and 2 can be prepared by the following process:

A compound of the formula I



(I)

in which X represents a halogen atom, preferably chlorine or bromine, is reacted with the amine of the formula II



(II)

to give the compound 1, and this can then be reacted in the presence of a basic agent to give the compound 2.

The abovementioned reactions are carried out under standard conditions in a known manner (EP-B-13,376, US 4,061,767).

The starting substances for the reactions are known or can be easily prepared by methods known from the literature.

The invention also relates to pharmaceuticals which contain an effective amount of compound 1 or compound 2 and/or physiologically tolerable salts of compound 2, in addition to pharmaceutically suitable and physiologically tolerable excipients, diluents and/or other active substances and auxiliaries.

The invention also relates to a process for the preparation of a pharmaceutical for the treatment of rejection reactions of the organ recipient against the transplanted organ, which comprises bringing compound 1 or 2 and/or a

physiologically tolerable salt of compound 2 into a suitable administration form using a pharmaceutically suitable and physiologically acceptable excipient and, if appropriate, other suitable active substances, additives or auxiliaries.

The pharmaceutical according to the invention can be administered orally, topically, rectally, intravenously or alternatively parenterally. Administration is carried out before, during and after organ transplantation in the recipient and/or donor.

Suitable solid or liquid pharmaceutical administration forms are, for example, granules, powders, coated tablets, tablets, (micro)capsules, suppositories, syrups, juices, suspensions, emulsions, drops or injectable solutions and preparations having a protracted release of active substance, in whose preparation customary auxiliaries, such as excipients, disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are used. Commonly used auxiliaries which may be mentioned are, for example, magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, cellulose and its derivatives, animal and vegetable oils, polyethylene glycols and solvents, such as, for example, sterile water and mono- or polyhydric alcohols, for example glycerol.

Preferably, the pharmaceutical preparations are prepared and administered in dosage units, each unit containing as the active constituent a certain dose of compound 1 or 2 and/or physiologically tolerable salts of compound 2. In the case of solid dosage units, such as tablets, capsules or suppositories, this dose can be up to about 300 mg, but preferably 10 to 200 mg.

For the treatment of a patient (70 kg) to whom an organ has been transplanted, in the early phases after

transplantation an intravenous infusion treatment of at most 1200 mg per day and in the later rehabilitation phases an oral administration of 3 times 300 mg per day of compound 1 or 2 and/or of the corresponding salts of compound 2 are indicated.

Under certain circumstances, however, higher or lower doses may also be appropriate. The administration of the dose can be carried out both by single administration in the form of an individual dosage unit or else several smaller dosage units and by multiple administration of subdivided doses at specific intervals.

Finally, compound 1 or 2 and/or its corresponding salts can also be combined during the preparation of the abovementioned pharmaceutical administration forms together with other suitable active substances, for example antiuricopathics, thrombocyte aggregation inhibitors, analgesics and steroidal or non-steroidal anti-inflammatories.

Example 1

Pharmacological tests and results

2 to 3 month-old rats (LEW) are intraperitoneally (i.p.) sensitized with 2×10^7 human peripheral blood lymphocytes. The i.p. administration of compound 2 starts 4 days before sensitization and ends 10 days after sensitization of the rats. Serum samples are taken from the tail vein and stored at -80°C; the function of complement is avoided by heat deactivation.

The naturally occurring xenophilic antibodies (NXA) and the xenophilic antibodies induced by sensitization (SXA) are titrated and live human peripheral blood lymphocytes are added (45 min. at 20°C). After intensive washing, FITC-labeled goat-anti-rat IgG or IgM antibodies are added and the binding to sXA or nXA is quantitatively

determined by flow cytometry (FACScan, Becton Dickinson).

A) Non-sensitized rats

Male LEW rats contain in their serum NXA which bind to vital human peripheral blood lymphocytes, to be precise usually with an average IgM titer of 1:4 and an average IgG titer which is less than 1:1.

The rats (n=8) which are treated every day with 10 mg/kg of compound 2, show a 30% reduction in the IgG titer and a 50% reduction in the IgM titer on the 11th day.

B) Sensitized rats

In sensitized rats there are greatly increased amounts of SXA. The IgG titer is 1:1024 to 1:16384 and the IgM titer 1:4 to 1:256. The IgG titer remains stable for over 50 days, while the IgM titer slowly decreases after the 10th day.

The sensitized rats (n=5) are treated with 3 or 10 mg/kg of compound 2. The following SXA titers result on the 11th day:

Compound (mg/kg)	IgG	IgM
0	1:2048	1:90
3	1:25	1:16
10	1:1	1:1.5

The action of compound 2 on the formation of allophilic antibodies

Male Brown-Norway rats having a weight of 200 to 250 g are used as heart donors. The hearts are transplanted into male Lewis rats of the same weight. All rats are

supplied with a standard diet and water and kept in an environment with 12 hours of light and darkness.

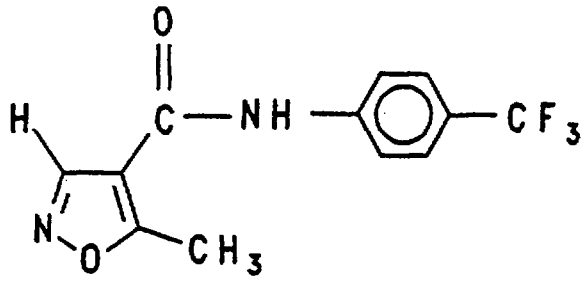
On the day of heart transplantation and then at intervals of 4 days, 1 ml of blood in each case is taken from the tail vein of the recipient rats (Lewis rats). The titer of allophilic antibodies in the blood of the recipient rats is determined by incubating different dilutions of the recipient blood with spleen cells of the donor rats (Brown-Norway rats) and rabbit complement. The cytotoxicity is determined by the Trypan Blue exclusion method. The pt value for the antibodies is determined using the standard t-test and compared with the untreated control animals.

Treatment with compound 2 begins on the 4th day after transplantation. 10 mg/kg of compound 2 in each case are administered i.p. once per day. The number of animals which are simultaneously treated is 5 (n = 5). Treatment ends on the 17th day. The following table shows the mean of the cytotoxicity values (%) during the treatment.

Serum dilutions	Days after transplantation						p value on the 17th day
	0	4	7	11	14	17	
1:5	3	63	36	19	22	11	0.001
1:25	3	58	31	16	11	6	0.001
1:125	4	34	20	9	6	5	0.050

Example 2

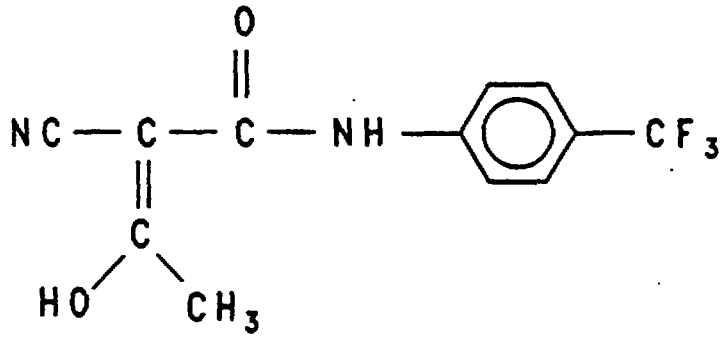
Preparation of N-(4-trifluoromethyl)-5-methylisoxazole-4-carboxanilide
(Compound 1)



A solution of 0.05 mol of 5-methylisoxazole-4-carbonyl chloride (7.3 g) in 20 ml of acetonitrile are added dropwise at room temperature to a solution of 0.1 mol of 4-trifluoromethylaniline (16.1 g) in 150 ml of acetonitrile. After stirring for 20 minutes, the precipitated 4-trifluoromethylaniline hydrochloride is filtered off with suction and washed twice with 20 ml of acetonitrile each time, and the combined filtrates are concentrated under reduced pressure. 12.8 g of white, crystalline N-(4-trifluoromethyl)-5-methylisoxazole-4-carboxanilide (compound 1) are thus obtained.

Example 3

Preparation of N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide (compound 2)



0.1 mol of N-(4-trifluoromethyl)-5-methylisoxazole-4-carboxanilide is dissolved in 100 ml of methanol and

treated at +10°C with a solution of 0.11 mol (4.4 g) of sodium hydroxide solution in 100 ml of water. The mixture is stirred for 30 minutes and, after diluting with water, is acidified with concentrated hydrochloric acid. The precipitated crop of crystals is filtered off with suction, washed with water and dried in air.
The yield is 24.4 g of N-(4-trifluoromethylphenyl)-2-cyano-3-hydroxycrotonamide (compound 2).
Melting point from methanol 205 to 206°C.

Example 4

Acute toxicity after intraperitoneal administration

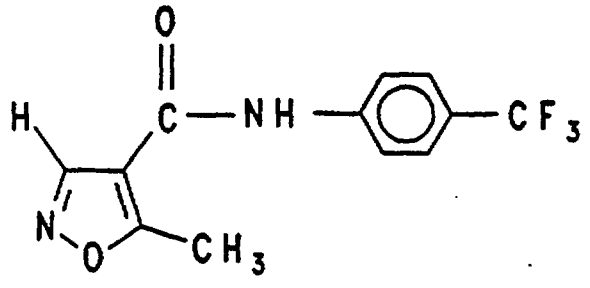
The acute toxicity after intraperitoneal administration of the test substances was determined with NMRI mice (20 to 25 g) and SD rats (120 to 195 g). The test substance was suspended in a 1% strength sodium carboxymethylcellulose solution. The different dosages of the test substance were administered to the mice in a volume of 10 ml/kg of body weight and to the rats in a volume of 5 ml/kg of body weight. Per dosage, 10 animals were used. After 3 weeks, the acute toxicity was determined by the method of Litchfield and Wilcoxon. The results are summarized in the table.

Table

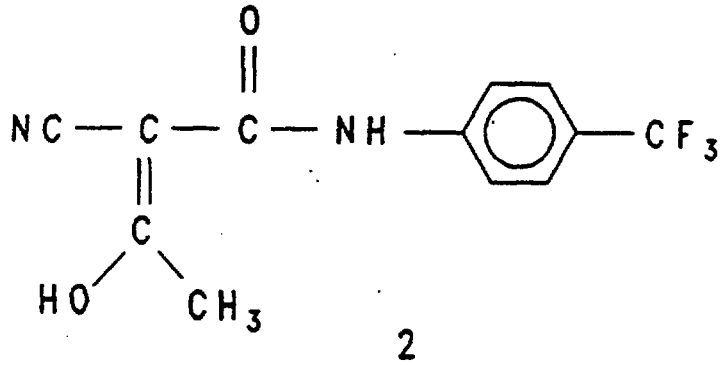
	Compound 1 acute toxicity intraperitoneal LD_{50} (mg/kg)	Compound 2 acute toxicity intraperitoneal LD_{50} (mg/kg)
NMRI mouse SD rat	185 (163 - 210) 170 (153 - 189)	150 (100 - 200)

Patent Claims for the Contracting States
AT,BE,CH,DE,DK,ES,FR,GB,GR,IE,IT,LI,LU,MC,NL,PT,SE

1. The use of the compound of the formulae 1 and/or 2



1



2

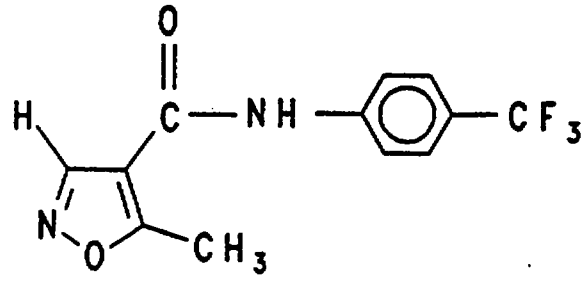
where compound 2 is present as such or in the form of a physiologically tolerable salt, for the preparation of pharmaceuticals for the treatment of hyperacute rejection reactions of the organ recipient to the transplanted organ.

2. The use as claimed in claim 1, wherein the pharmaceutical is administered to the recipient or donor of the organ before, during and/or after transplantation.

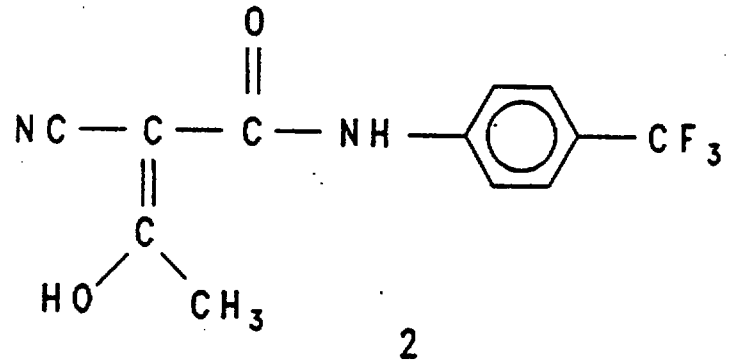
3. The use as claimed in claim 1 or 2, wherein the amount of allophilic or xenophilic antibodies is decreased.

4. The use as claimed in one or more of claims 1 to 3, wherein the pharmaceutical is employed in mammals, in particular the human.

5. The use of the compound of the formulae 1 and/or 2



1



2

where compound 2 is present as such or in the form of a physiologically tolerable salt, for the preparation of pharmaceuticals for the treatment of rejection reactions of the organ recipient to the transplanted organ, the organ being transferred from one species of the organism to another species.

6. The use as claimed in claim 5, wherein the pharmaceutical is administered to the recipient or donor of the organ before, during and/or after transplantation.

7. The use as claimed in claim 5 or 6, wherein the amount of allophilic or xenophilic antibodies is decreased.

8. The use as claimed in one or more of claims 5 to 7, wherein the pharmaceutical is employed in mammals, in particular the human.