

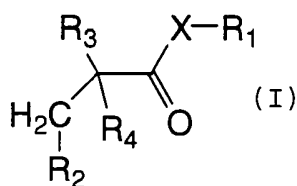
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(54) Title: SUBSTANCES AND PHARMACEUTICAL COMPOSITIONS FOR THE INHIBITION OF GLYOXALASES AND THEIR USE AGAINST PROTOZOA AND HELMINTHES



(57) Abstract: The present invention pertains to substances of the formula (I) wherein X is O or S; and R1 is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxy-carbonylalkyl, aryl or a sugar residue; and R2 is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxy-carbonylalkyl or aryl residue; and R3 and R4 together are =O, or R3 is OH and R4 is H; or R3 is H and R4 is OH. The invention relates to compounds of the

general formula (I) for the inhibition of glyoxalase I and/or II, pharmaceutical compositions comprising one or more compounds according to formula (I), the use of one or more compounds according to formula (I) for the preparation of a medicament, and methods of treatment comprising the administration of one or more compounds according to formula (I). In particular, the present invention relates to the effects of a substance of formula (I) against parasites selected from protozoa and helminthes. The compound of formula (I), pharmaceutical composition, medicament or method of treatment related to said compound of the invention are for the treatment of diseases associated with increased glycolytic metabolism, comprising diseases associated with one or more of: increased formation of methylglyoxal, increased activity of glyoxalase I and/or II activity, and enhanced cell growth/proliferation. In one embodiment, the disease is a parasitic disease, wherein the parasite is selected from protozoa and helminthes.

Substances and pharmaceutical compositions for the inhibition of glyoxalases and their use against protozoa and helminthes

FIELD OF THE INVENTION

5 The invention relates to compounds of the general formula (I) as an anti-parasitic agent, wherein said parasite is selected from protozoa and helminthes. In one embodiment, the compounds of the general formula (I) are for the inhibition of glyoxalase I and/or II. The invention also relates to,
10 pharmaceutical compositions comprising one or more compounds according to formula (I), the use of one or more compounds according to formula (I) for the preparation of a medicament, and methods of treatment comprising the administration of one or more compounds according to formula (I).

15 The compound, pharmaceutical composition, medicament or method of treatment of the invention are for the treatment of diseases associated with increased glycolytic metabolism, comprising diseases associated with one or more of: increased
20 formation of oxoaldehydes such as methylglyoxal, increased activity of glyoxalase I and/or II activity and enhanced cell growth/proliferation. In one embodiment, the disease is a parasitic disease, wherein said parasite is selected from protozoa and helminthes.

25 BACKGROUND OF THE INVENTION

Parasites are organisms that live off another, typically higher metazoa. There are protozoal parasites, as well as higher organisms, like helminthes, which live as parasites.

30 Many parasites are characterized by several different developmental stages, which may require a change of host. The parasites that are pathogenic for higher animals, including man, oftentimes have a blood-borne or tissue borne stage in
35 said animal, and may have a different developmental stage in other animals, such as blood sucking insects, etc. For example, Plasmodium falciparum, which causes malaria, has a

complex life cycle encompassing human hosts as well as blood sucking flies. Similarly, many parasitic helminthes change hosts during their life cycle.

5 There are many devastating diseases of animals, including man, which are caused by protozoal or metazoal parasites. Such diseases include many tropical diseases, such as malaria, leishmaniasis, bilharziosis, toxoplasmosis, sleeping sickness, Chagas disease, and others. Protozoa such as
10 trypanosomes and Leishmania belong to a family of ubiquitous parasites of insects, plants, fish, amphibians, reptiles, birds, and mammals.

Trypanosomes are extracellular protozoan parasites that are
15 transmitted by the bite of the blood-sucking tsetse fly. In sub-Saharan Africa, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* cause the East and West/Central forms of human sleeping sickness, which are responsible for up to 500,000 deaths/year and *Trypanosoma cruzi* is the
20 causative agent for Chagas disease in South America. *Leishmania major* is responsible for a variety of leishmaniasis.

In human sleeping sickness, there are two clinical
25 manifestations, namely the early hemolympathic stage of parasite proliferation in the blood and the lymphatic system, and as a result of brain-blood-barrier penetration, the late meningoencephalitic stage, when the CNS is involved.

30 All these parasitic protozoa have a unique thiol metabolism. Instead of the ubiquitous glutathione, the main non-protein thiol is trypanothione [bis(glutathionyl)spermidine] which is kept reduced by the flavoenzyme trypanothione reductase (Krauth-Siegel et al., 2003, Fairlamb, 2003). Consequently,
35 one method of treatment is aimed on inhibition of the trypanothione reductase by clomipramine (Rivarola et al. 2005)

Other therapeutic approaches for treatment of trypanosomiasis involve pentamidine and suramin which are used prior to CNS involvement however their mechanism of action is unknown (Gao et al., 1998). In former time, the highly toxic and inadequate drugs based on arsenic or antimony such as melarsoprol have been used to combat trypanosoma infection (Keiser et al., 2000).

A promising drug eflornithine (alpha-difluoromethylornithine) is used effectively against late stage disease caused by *Trypanosoma brucei gambiense* but is ineffective against *Trypanosoma brucei rhodesiense* (Burchmore et al., 2002; Chappuis et al., 2005). The mechanism of action is the irreversible inhibition of ornithine decarboxylase involved in polyamine biosynthesis which has been also applied as anti-cancer drug (Huang et al., 2005). Another drug is Nifurtimox which seems to be helpful against melarsoprol-refractory late stage disease (Olliaro et al., 2002).

A further large class of important pathogenic parasites are helminthes, such as *Schistosoma*, which comprise worms infecting the gastrointestinal tract, as well as such that leave the gut and settle in various organs. In particular the latter are difficult to treat and cause severe pathologies.

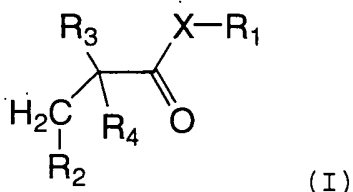
To date, there are no adequate therapies available for most parasites. Lack of efficacy, high toxicity and quickly spreading resistances inflict severe limitations on the clinical efficacy of controlling parasitic diseases. Thus, millions of people are in need for better therapies against parasites.

SUMMARY OF THE INVENTION

The problem underlying the invention thus resides in providing new substances, compositions, and medicaments for use as anti-parasitic agents and methods of treatment using

the same, in particular for parasites selected from protozoa and helminthes.

Accordingly, the present invention provides compounds of the
5 general formula (I)



wherein X is O or S; and

- 10 R1 is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and
R2 is H or a branched or non-branched alkyl, cycloalkyl,
15 branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and
R3 and R4 together are =O,
or R3 is OH and R4 is H; or R3 is H and R4 is OH

20

for use as an anti-parasitic agent, in particular for the treatment of parasitic diseases, wherein the parasite is selected from protozoa or helminthes, with the proviso that when the disease is cerebral malaria,
25 R3 or R4 are OH;
and when the parasite is a parasite of fish, X is O, and R3 and R4 together are =O, R1 is not methyl, ethyl, propyl or butyl.

- 30 The anti-parasitic effects encompass cytotoxic and cytostatic action. Specific examples of parasites against which the substances of the invention can be used comprise one or more of the genus Flagellats (Trichomonads, Trypanosoma spp., Leishmania spp, Gardia lamblia); Rhizopods (Entamoeba spp.),

Sporozoa (*Isospora belli*, *Sarcocystis bovi-hominis*,
Toxoplasma, in particular *Toxoplasma gondii*, *Plasmodium* spp.,
Cryptosporidium spp., *Giardia* spp.), Ciliates, *Pneumocystis*
carinii, *Microspora*, *Ichthyophthirius multifiliis* and
5 helminthes comprising one or more selected from Trematodea
(trematodes, flukes, *Schistosoma*, *Bilharziella*, etc),
Cestoidea (tapeworms, *Taenia*, *Echinococcus*, *Hymenolepis*),
Nematodes (roundworms, *Strongyloides*, *Ancylostoma*, *Necator*,
Trichostrongylus, *Angiostrongylus*, *Ascaris*, *Toxocara* spp),
10 cestodes, and trichinella.

One embodiment relates to substances according to formula
(I), wherein R1 comprises 1 to 8 carbon atoms and R2 is H or
comprises 1 to 8 carbon atoms, or wherein R1 comprises 1 to 4
15 carbon atoms and R2 is H or comprises 1 to 2 carbon atoms.

In a further embodiment, the substance according to formula
(I) is a substance wherein R1 and/or R2 is methyl, ethyl,
propyl, isopropyl, butyl, or isobutyl. Particular embodiments
20 according to the invention comprise one or more selected from
methyl pyruvate, ethyl pyruvate, isopropyl pyruvate, butyl
pyruvate, isobutyl pyruvate, ethyl 2-oxobutyrate, or the
said pyruvate compound according to formula (I) wherein X =
S, as well as compounds exemplified in table 1.

25 The invention also relates to substances according to formula
(I), wherein in said substance R3 or R4 is OH and it is
selected from the group comprising the D-, L- enantiomer, and
the racemic mixture thereof (equimolar as well as non-
30 equimolar), as exemplified in table 2.

The invention further relates to pharmaceutical compositions
comprising one or more substances according to formula (I),
the use of said substances for the manufacture of a
35 medicament, and methods of treatment comprising the
administration of said substances. The treatment with and/or
administration of substances of the invention to an animal in

need thereof, including humans, with a therapeutically effective amount of said substance is also encompassed by the present invention.

5 The invention further relates to the use of said substance, pharmaceutical composition, medicament or method of treatment in the treatment of a disease associated with an increased glycolytic metabolism. Said disease can be further associated with one or more selected from: increased production of oxo-
10 aldehydes such as methylglyoxal, increased activity of glyoxalase I and/or II, and increased cell proliferation and growth. In one embodiment, the disease is a parasitic infection, which is caused by a parasite selected from protozoa and helminthes as detailed above.

15 In one embodiment, the pharmaceutical composition or medicament comprises one or more additional pharmaceutically active ingredients. Such ingredients can be selected from anti parasitic agents, in particular one or more selected
20 from abendazol, antimonials, clomipramine, eflornithine, fluconazol, ivermectin, mebendazol, melarsoprol, metronidazole, nifurtimox, nitrazoxanide, paromomycin, pentamidine, praziquantel, suramin, thrimethoprim or tinidazole.

25 The pharmaceutical composition or medicament can further comprise one or more auxiliary substances, including, but not limited to, fillers, flavouring agents and stabilizers. The pharmaceutical composition or medicament of the invention can
30 be prepared in the form of galenic formulations commonly known in the art, including sustained release or controlled release galenic formulation.

The pharmaceutical composition or medicament of the invention
35 is for topic or systemic administration, more particularly, for oral, intravenous, subcutaneous, intramuscular, intradermal, intraperitoneal, rectal, intranasal, epidural,

percutaneous, transdermal, or pulmonary administration, or for administration as an aerosol, via mini-pumps, as mouth lavage, cream, ointment, spray, gel, plaster, and/or via microbubbles. The pharmaceutical composition or medicament
5 can also be in the form of a food supplement and/or beverage supplement.

The pharmaceutical composition or medicament is for the treatment and/or prophylaxis of parasitic infections in
10 animals, including invertebrates, non-mammalian vertebrates, mammals and humans. The parasitic infection can be resistant to treatment with known anti-parasitic agents, such as abendazol, antimonials, clomipramine, eflornithine, fluconazol, ivermectin, mebendazol, melarsoprol,
15 metronidazole, nifurtimox, nitrazoxanide, paromomycin, pentamidine, praziquantel, suramin, thrimethoprim or tinidazole.

In one embodiment, the infection is in an immunosuppressed
20 animal, including man, wherein said immunosuppression is associated with hereditary or acquired immune-defects, comprising acquired immune defect associated with HIV, organ transplantation, chemotherapy or exposure to radiation.

25 In one embodiment, the animal, including man, is concomitantly suffering from a fungal or bacterial infection caused by one or more selected from Acrobacter, Actinobacillus, Actinomyces, Bacteroides, Brucella, Clamydia, Clostridium, Campylobacter, Escherichia, Enterobacter,
30 Enterococcus, Eubacterium, Fusobacterium, Helicobacter, Hemophilus, Legionella, Listeria, Mycobacteria, Mycoplasma, Neissaria, Pasteurella, Peptostreptococcus, Pneumococcus, Pneumocystis, Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella, Spirochetes, Staphylococcus,
35 Streptococcus, Treponema, Vibrio, Yersinia, Escherichia coli or Pneumocystis carinii. Some of these infectious diseases may be an opportunistic infection, and/or may be

characterized by antibiotic resistance. In a further embodiment, the animal has a reduced blood glucose level.

According to the invention, the animal can be concomitantly suffering from cancer, and can be going to receive, is currently receiving, or has received conventional cancer therapy, comprising one or more of chemotherapy, surgery, radiotherapy or brachytherapy.

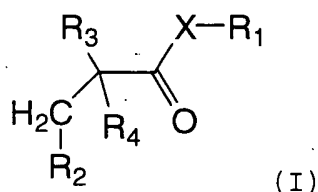
It is to be understood that all embodiments described in the context of substances, pharmaceutical compositions or medicaments of the invention equally apply to methods of treatment, and vice versa. Thus, the mentioning of a particular embodiment in the context of one or more of a substance, pharmaceutical composition, medicament or method of treatment describes this embodiment for all these kinds of subject matter.

DETAILED DESCRIPTION OF THE INVENTION

A. Substances of the invention

In the context of this application, the terms "substances" or "compounds" are used interchangeably.

The present invention relates to compounds of the general formula (I),



wherein X is O or S,
wherein R₁ is a branched or non-branched alkyl, branched or non-branched alkenyl, branched or non-branched alkynyl, alkoxyalkyl, or alkoxycarbonylalkyl, each preferably with a

chain length of C1 to C10, more preferably C1 to C8, more preferably C1 to C4, in particular C1, C2, C3 or C4; or a cycloalkyl, cycloalkenyl, cycloalkinyl, aryl or a sugar residue, each preferably with a chain length of C3 to C10, more preferably C3 to C8, more preferably C3, C4, C5 or C6; and

R2 is H or a branched or non-branched alkyl, branched or non-branched alkenyl, branched or non-branched alkynyl, alkoxyalkyl, or alkoxycarbonylalkyl, each preferably with a chain length of C1 to C10, more preferably C1 to C8, more preferably C1 to C4, in particular C1, C2, C3 or C4; or a cycloalkyl, cycloalkenyl, cycloalkinyl or aryl residue, each preferably with a chain length of C3 to C10, more preferably C3 to C8, more preferably C3, C4, C5 or C6; and

R3 and R4 together are =O, or R3 is OH and R4 is H; or R3 is H and R4 is OH.

In one embodiment the sugar in position R1 is substituted or non-substituted sugar.

In one embodiment R1 comprises 1 to 4 carbon atoms and R2 is H or comprises 1 or 2 carbon atoms. In a further embodiment of the substance according to formula (I), R1 and/or R2 is methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

In one embodiment R2 is H, and R1 is methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

Specific examples of compounds of the invention are listed in the following table 1, however, it is to be understood that this is not a limiting list. The skilled person can readily devise a large variety of additional compounds according to formula (I):

Table 1: specific examples of compounds according to formula (I)

R1	R2	R3 and R4	X	Name (I)
Alkyl				
Methyl	H	=O	=O	Methyl pyruvate
Ethyl	H	=O	=O	Ethyl pyruvate
Ethyl	H	=O	=S	S-Ethyl 2-oxopropanethionate
Propyl	H	=O	=S	S-Propyl 2-oxopropanethionate
Butyl	H	=O	=S	S-Butyl 2-oxopropanethionate
Propyl	H	=O	=O	Propyl pyruvate
Butyl	H	=O	=O	Butyl pyruvate
Pentyl	H	=O	=O	Pentyl pyruvate
Hexyl	H	=O	=O	Hexyl pyruvate
Heptyl	H	=O	=O	Heptyl pyruvate
Octyl	H	=O	=O	Octyl pyruvate
Branched Alkyl				
Isopropyl	H	=O	=O	Isopropyl pyruvate
Isobutyl	H	=O	=O	Isobutyl pyruvate
Isopentyl	H	=O	=O	Isopentyl pyruvate
Isohexyl	H	=O	=O	Isohexyl pyruvate
Isoheptyl	H	=O	=O	Isoheptyl pyruvate
Isooctyl	H	=O	=O	Isooctyl pyruvate
Cycloalkyl				
Cyclohexyl	H	=O	=O	Cyclohexyl pyruvate
Cyclohexyl methyl	H	=O	=O	Cyclohexylmethyl pyruvate
Cyclopentyl	H	=O	=O	Cyclopentyl pyruvate
Cyclopentyl methyl	H	=O	=O	Cyclopentylmethyl pyruvate
Alkenyl residues				
Vinyl	H	=O	=O	Ethenyl pyruvate

Allyl	H	=0	=0	Propenyl pyruvate
Butenyl	H	=0	=0	Butenyl pyruvate
Pentenyl	H	=0	=0	Pentenyl pyruvate
Hexenyl	H	=0	=0	Hexenyl pyruvate
Heptenyl	H	=0	=0	Heptenyl pyruvate
Octenyl	H	=0	=0	Octenyl pyruvate
Branched Alkenyl				
Isopropenyl	H	=0	=0	Isopropenyl pyruvate
Isobutenyl	H	=0	=0	Isobutenyl pyruvate
Isopentenyl	H	=0	=0	Isopentenyl pyruvate
Isohexenyl	H	=0	=0	Isohexenyl pyruvate
Isoheptenyl	H	=0	=0	Isoheptenyl pyruvate
Isooctenyl	H	=0	=0	Isooctenyl pyruvate
Cycloalkenyl				
Cyclohexenyl	H	=0	=0	Cyclohexenyl pyruvate
Cyclohexenyl methyl	H	=0	=0	Cyclohexenylmethyl pyruvate
Cyclopentenyl	H	=0	=0	Cyclopentenyl pyruvate
Cyclopentenyl methyl	H	=0	=0	Cyclopentenylmethyl pyruvate
Alkynyl				
Ethinyl	H	=0	=0	Ethinyl pyruvate
Propinyl	H	=0	=0	Propinyl pyruvate
Butinyl	H	=0	=0	Butinyl pyruvate
Pentinyl	H	=0	=0	Pentinyl pyruvate
Hexinyl	H	=0	=0	Hexinyl pyruvate
Heptinyl	H	=0	=0	Heptinyl pyruvate
Octinyl	H	=0	=0	Octinyl pyruvate
Branched Alkynyl				
Isopentinyl	H	=0	=0	Isopentinyl pyruvate
Isohexinyl	H	=0	=0	Isohexinyl pyruvate
Isoheptinyl	H	=0	=0	Isoheptinyl pyruvate
Isooctinyl	H	=0	=0	Isooctinyl pyruvate

Cycloalkinyl				
Cyclooctinyl	H	=0	=0	Cyclooctinyl pyruvate
Alkoxyalkyl				
Methoxymethyl	H	=0	=0	Methoxymethyl pyruvate
Ethoxymethyl	H	=0	=0	Ethoxymethyl pyruvate
Methoxyethyl	H	=0	=0	Methoxyethyl pyruvate
Alkoxy carbonylalkyl				
Methoxycarbonylmethyl	H	=0	=0	Methoxycarbonylmethyl pyruvate
Ethoxycarbonylmethyl	H	=0	=0	Ethoxycarbonylmethyl pyruvate
Aryl				
Phenyl	H	=0	=0	Phenyl pyruvate
Naphthyl	H	=0	=0	Naphthyl pyruvate
Sugar	H			
Glucosyl	H	=0	=0	Gucosyl pyruvate
Galactosyl	H	=0	=0	Galactosyl pyruvate
Mannosyl	H	=0	=0	Mannosyl pyruvate
Alkyl	Alkyl			
Methyl	Methyl	=0	=0	Methyl 2-oxobutanoate
Ethyl	Methyl	=0	=0	Ethyl 2-oxobutanoate
Ethyl	Ethyl	=0	=S	S-Ethyl 2-oxopentaneethionate
Propyl	Methyl	=0	=0	Propyl 2-oxobutanoate
Butyl	Methyl	=0	=0	Butyl 2-oxobutanoate
Methyl	Ethyl	=0	=0	Methyl 2-oxopentanoate
Ethyl	Ethyl	=0	=0	Ethyl 2-oxopentanoate
Propyl	Ethyl	=0	=0	Propyl 2-oxopentanoate
Butyl	Ethyl	=0	=0	Butyl 2-oxopentanoate
Methyl	Propyl	=0	=0	Methyl 2-oxohexanoate
Ethyl	Propyl	=0	=0	Ethyl 2-oxohexanoate
Propyl	Propyl	=0	=0	Propyl 2-oxohexanoate
Butyl	Propyl	=0	=0	Butyl 2-oxohexanoate
Methyl	Butyl	=0	=0	Methyl 2-oxoheptanoate

Ethyl	Butyl	=0	=0	Ethyl 2-oxoheptanoate
Propyl	Butyl	=0	=0	Propyl 2-oxoheptanoate
Butyl	Butyl	=0	=0	Butyl 2-oxoheptanoate
Branched Alkyl	Alkyl			
Isobutyl	Methyl	=0	=0	Isobutyl 2-oxobutanoate
Isobutyl	Ethyl	=0	=0	Isobutyl 2-oxopentanoate
Isobutyl	Propyl	=0	=0	Isobutyl 2-oxohexanoate
Isobutyl	Butyl	=0	=0	Isobutyl 2-oxoheptanoate
Cyclo Alkyl	Alkyl			
Cyclohexyl	Ethyl	=0	=0	Cyclohexyl 2-oxopentanoate
Cyclohexyl methyl	Ethyl	=0	=0	Cyclohexylmethyl 2-oxopentanoate
Sugar	Alkyl			
Glucosyl	Methyl	=0	=0	Glucosyl 2-oxobutanoate
Glucosyl	Ethyl	=0	=0	Glucosyl 2-oxopentanoate
Alkyl	Alkenyl			
Propyl	Butenyl	=0	=0	Propyl 2-oxoheptenoate
Cycloalkyl	Alkenyl			
Cyclohexyl	Butenyl	=0	=0	Cyclohexyl 2-oxoheptenoate
Alkyl	Alkynyl			
Propyl	Butinyl	=0	=0	Propyl 2-oxoheptinoate
Butyl	Butinyl	=0	=0	Butyl 2-oxoheptinoate
Cycloalkyl	Alkynyl			
Cyclohexyl	Butinyl	=0	=0	Cyclohexyl 2-oxoheptinoate
Alkoxyalkyl	Alkyl			
Methoxymethyl	Ethyl	=0	=0	Methoxymethyl 2-

1				oxopentanoate
Ethoxymethyl	Ethyl	=0	=0	Ethoxymethyl 2-oxopentanoate
Methoxyethyl	Ethyl	=0	=0	Methoxyethyl 2-oxopentanoate
Alkoxy carbon ylalkyl	Alkyl			
Methoxycarbonylmethyl	Ethyl	=0	=0	Methoxycarbonylmethyl 2-oxopentanoate
Ethoxycarbonylmethyl	Ethyl	=0	=0	Ethoxycarbonylmethyl 2-oxopentanoate
Alkyl	Alkoxy carbon ylalkyl			
Ethyl	Methoxycarbonylmethyl	=0	=0	Ethyl-4-methoxycarbonyl-2-oxobutanoate
Ethyl	Ethoxycarbonylmethyl	=0	=0	Ethyl-4-ethoxycarbonyl-2-oxobutanoate
Alkyl	Alkoxyalkyl			
Ethyl	Methoxymethyl	=0	=0	Ethyl 4-methoxy-2-oxobutanoate
Ethyl	Ethoxymethyl	=0	=0	Ethyl 4-ethoxy-2-oxobutanoate
Ethyl	Methoxyethyl	=0	=0	Ethyl 5-methoxy-2-oxopentanoate

Particular examples of substances according to formula (I) comprise methyl pyruvate, ethyl pyruvate, propyl pyruvate, butyl pyruvate, pentyl pyruvate, hexyl pyruvate, octyl pyruvate, isobutyl pyruvate, isopentyl pyruvate, isohexyl pyruvate, isoheptyl pyruvate, isooctyl pyruvate, cyclopentyl pyruvate, cyclopentylmethyl pyruvate, cyclohexyl pyruvate, cyclohexylmethyl pyruvate, butenyl pyruvate, hexenyl pyruvate, isobutenyl pyruvate, isohexenyl pyruvate, butinyl pyruvate, hexinyl pyruvate, methoxymethyl pyruvate,

ethoxymethyl pyruvate, ethoxycarbonylmethyl pyruvate, methyl-2-oxobutanoate, ethyl 2-oxobutanoate, butyl-2-oxobutanoate, methyl-2-oxopentanoate, ethyl-2-oxoheptanoate, butyl-2-oxopentanoate, methyl-2-oxohexanoate, ethyl-2-oxohexanoate, butyl-2-oxohexanoate, methyl-2-oxoheptanoate, ethyl-2-oxoheptanoate, butyl-2-oxoheptanoate, isobutyl-2-oxobutanoate, isobutyl-2-oxohexanoate, cyclohexyl-2-oxopentanoate, cyclohexylmethyl-2-oxopentanoate, propyl-2-oxoheptenoate, cyclohexyl-2-oxoheptenoate, butyl-2-oxoheptenoate, methoxymethyl-2-oxopentanoate, ethoxycarbonylmethyl-2-oxopentanoate, ethyl-4-methoxycarbonyl-2-oxobutanoate, ethyl-4-methoxy-2-oxobutanoate, , or the said compounds wherein X = S, and/or the said compound wherein R3 or R4 is OH.

Preferred examples of substances of the invention comprise methyl pyruvate, ethyl pyruvate, propyl pyruvate, butyl pyruvate, pentyl pyruvate, hexyl pyruvate, isopropyl pyruvate, isobutyl pyruvate, isopentyl pyruvate, isohexyl pyruvate, methyl-2-oxobutanoate, methyl-2-oxopentanoate, ethyl-2-oxobutanoate, butyl-2-oxobutanoate, ethyl-2-oxopentanoate, cyclohexylmethyl pyruvate, or the said compounds wherein X = S, and/or the said compound wherein R3 or R4 is OH.

More preferred compounds of the invention comprise methyl pyruvate, ethyl pyruvate, propyl pyruvate, butyl pyruvate, isobutyl pyruvate, ethyl-2-oxobutanoate, ethyl-2-oxopentanoate, cyclohexylmethyl pyruvate, or the said compounds wherein X = S, and/or the said compound wherein R3 or R4 is OH.

Particularly preferred compounds are methyl pyruvate, ethyl pyruvate, butyl pyruvate, isobutyl pyruvate and ethyl-2-oxobutyrate or the said compounds wherein X = S, in particular S-ethyl pyruvate, and/or the said compounds wherein R3 or R4 together are -OH.

a) Substances according to formula (I) inhibit glyoxalases

Surprisingly it was found that the substances according to formula (I) inhibit glyoxalase I and/or II.

5

Parasites such as parasitic protozoa and helminthes generate energy by the degradation of different food stuffs, and store it as chemical energy in energy rich compounds, particularly in the form of ATP. These energy rich compounds are subject to extensive turnover interconnected with anabolic and catabolic processes, by being used, for example, in the synthesis of proteins, nucleic acids, sugars, lipids etc., the transport of substances against concentration gradients and regulatory activities, and are formed anew in certain metabolic pathways. A plurality of compounds can serve as energy providing substances, the most important being sugars and fatty acids. After metabolising the different monosaccharides and their di-, oligo- and polymers extra- or intracellularly into corresponding derivatives, sugar degradation takes place in glycolysis. Glycolysis allows anaerobic as well as, in combination with oxidative phosphorylation, aerobic energy generation.

10

15

20

Glycolysis, however, is always accompanied by the formation of oxoaldehydes, in particular of methylglyoxal. These compounds are highly toxic as they easily form adducts with cellular proteins and nucleic acids and lead to their inactivation. Therefore, all cells using glycolysis employ detoxification systems, in most cases consisting of the enzymes glyoxalase I and II.

25

30

Both glyoxalases I and II are responsible for the degradation of the side product of glycolysis, methylglyoxal. Methylglyoxal is cytotoxic (e.g. by the formation of adducts with cellular proteins and nucleic acids). Inhibition of the degradation of methylglyoxal leads to inhibition of cell proliferation and cell death by different mechanisms.

35

Thus, in one embodiment the substances according to formula (I) are for the inhibition of glyoxalase I and / or II, advantageously I and II. The inhibition of multiple enzymes
5 drastically reduces the probability of developing resistance within the therapeutic period.

The compounds of the invention inhibit glyoxalase I and / or II, advantageously I and II. The inhibition of multiple
10 enzymes drastically reduces the probability of developing resistance within the therapeutic period.

Surprisingly it was found that compounds of the present invention like e.g. ethyl pyruvate are capable of inhibiting
15 glyoxalase I as well as glyoxalase II. Inhibition of glyoxalases by compounds of the present invention inhibits the cellular detoxification of methylglyoxal and via various mechanisms leads to the inhibition of cell proliferation and to cell death.

20 Advantageously, compounds of the invention inhibit such cells showing a clearly increased rate of glycolysis whereas the metabolism of cells with a normal rate of glycolysis is not or only slightly affected.

25 Glyoxalase I (GLOI, alternatively abbreviated as Gly I,) is also known as (R)-S-lactoylglytythione methyl-glyoxal-lyase EC4.4.1.5), glyoxalase II (GLOII, alternatively abbreviated as Gly II) is also known as S-2-hydroxy-acylglutathione
30 hydrolase (EC 3.1.2.6).

Glyoxalases are phylogenetically highly conserved at the amino acid and genetic level. As used herein, the term "glyoxalase" refers to the mammalian enzymes glyoxalase I
35 and/or II, as well as to the respective glyoxalases of non-mammalian eukaryotic and prokaryotic organisms, such as

glyoxalase I and II of bacteria, fungi like yeast or other microorganisms.

Thus, the term "inhibiting glyoxalase I and/or II"

5 encompasses the inhibition of the mammalian as well as the respective non-mammalian enzymes.

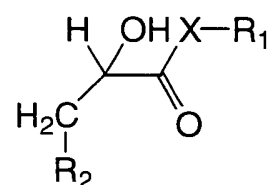
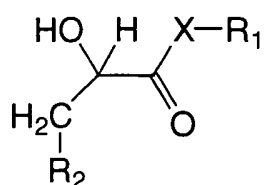
According to the invention, the substances according to formula (I) are for direct inhibition of glyoxalase I and/or
10 II when R3 and R4 together are =O.

In contrast, when R3 or R4 are -OH, said substances according to formula (I) do not directly inhibit glyoxalase I and/or II. Rather, said substances, also called "prodrugs", are
15 transformed, i.e. oxidized, to a substance wherein R3 and R4 together are =O.

Said transformation/oxidation can be effected ex vivo, e.g. by means of a chemical oxidant, such as potassium
20 permanganate. Other suitable oxidants are for example hydrogen peroxide, iodine, iodide benzoic acid and others.

Alternatively, said transformation takes place in the organism, or on the skin or mucosa of the mammal upon
25 administration of said compound. Such transformation is effected e.g. via dehydrogenases, in particular via lactate dehydrogenase (Lluís and Bozal 1976).

Compounds of formula (I), wherein R3 or R4 is -OH, and which
30 undergo transformation such that R3 and R4 together are =O are for example compounds of the general formula (II) and/or (III),



(II)

(III)

wherein X, R1 and R2 are defined as in formula (I), above.

5 Specific compounds of the general formula (II) and/or (III) are for example methyl lactate, propyl lactate, butyl lactate, ethyl lactate, and ethyl-2-hydroxybutanoate, which are transformed into, e.g. butyl pyruvate, ethyl pyruvate, and ethyl-2-oxobutanoate, respectively,

10

When in a substance according to formula (I) R3 or R4 is OH, the invention encompasses the D-, L- enantiomer and the racemic mixture thereof. In the context of this invention, equimolar as well as non-equimolar mixtures of corresponding
15 enantiomers are to be considered as racemic mixtures.

In other words, in case the compounds of the invention are compounds with one or more chiral centres, for example ethyl lactate or butyl lactate, the corresponding D- and L- isomers
20 can be used as well as racemic mixtures, for example ethyl D-lactate (DEL), ethyl L-lactate (LEL) or racemic mixtures of DEL and LEL, and butyl D-lactate (DBL), butyl L-lactate (LBL) or racemic mixtures of DBL and LBL, respectively.

25 Specific examples of compounds of the invention are listed in the following table 2, however, it is to be understood that this is not a limiting list. The skilled person can readily devise a large variety of additional compounds according to formula (II/III). The following table is understood to
30 encompass the D- or L- Isomers or racemic mixtures of the listed substances. In other words, substances according to either formula II or formula III are specifically disclosed:

Table 2: specific compounds according to formula II / III:

35

R1	R2	R3/R4	X	Name
Alkyl				

Methyl	H	H / OH	=O	Methyl lactate
Ethyl	H	H / OH	=O	Ethyl lactate
Propyl	H	H / OH	=O	Propyl lactate
Butyl	H	H / OH	=O	Butyl lactate
Pentyl	H	H / OH	=O	Pentyl lactate
Hexyl	H	H / OH	=O	Hexyl lactate
Heptyl	H	H / OH	=O	Heptyl lactate
Octyl	H	H / OH	=O	Octyl lactate
Ethyl	H	H / OH	=S	S-Ethyl 2-hydroxypropanethionate
Propyl	H	H / OH	=S	S-propyl 2-hydroxypropanethionate
Butyl	H	H / OH	=S	S-Butyl 2-hydroxypropanethionate
Branched Alkyl				
Isopropyl	H	H / OH	=O	Isopropyl lactate
Isobutyl	H	H / OH	=O	Isobutyl lactate
Isopentyl	H	H / OH	=O	Isopentyl lactate
Isohexyl	H	H / OH	=O	Isohexyl lactate
Isoheptyl	H	H / OH	=O	Isoheptyl lactate
Isooctyl	H	H / OH	=O	Isooctyl lactate
Cycloalkyl				
Cyclohexyl	H	H / OH	=O	Cyclohexyl lactate
Cyclohexylmethyl	H	H / OH	=O	Cyclohexylmethyl lactate
Cyclopentyl	H	H / OH	=O	Cyclopentyl lactate
Cyclopentyl methyl	H	H / OH	=O	Cyclopentylmethyl lactate
Cyclopropyl	H	H / OH	=O	Cyclopropyl lactate
Cyclopropyl methyl	H	H / OH	=O	Cyclopropylmethyl lactate
Alkenyl				
Vinyl	H	H / OH	=O	Vinyl lactate
Allyl	H	H / OH	=O	Propenyl lactate
Butenyl	H	H / OH	=O	Butenyl lactate
Pentenyl	H	H / OH	=O	Pentenyl lactate

Hexenyl	H	H / OH	=0	Hexenyl lactate
Heptenyl	H	H / OH	=0	Heptenyl lactate
Octenyl	H	H / OH	=0	Octenyl lactate
Branched Alkenyl				
Isopropenyl	H	H / OH	=0	Isopropenyl lactate
Isobutenyl	H	H / OH	=0	Isobutenyl lactate
Isopentenyl	H	H / OH	=0	Isopentenyl lactate
Isohexenyl	H	H / OH	=0	Isohexenyl lactate
Isoheptenyl	H	H / OH	=0	Isoheptenyl lactate
Isooctenyl	H	H / OH	=0	Isooctenyl lactate
Cycloalkenyl				
Cyclohexenyl	H	H / OH	=0	Cyclohexenyl lactate
Cyclohexenylmeth yl	H	H / OH	=0	Cyclohexenylmethyl lactate
Cyclopentenyl	H	H / OH	=0	Cyclopentenyl lactate
Cyclopentenylmet hyl	H	H / OH	=0	Cyclopentenylmethyl lactate
Alkinyl				
Ethinyl	H	H / OH	=0	Ethinyl lactate
Propinyl	H	H / OH	=0	Propinyl lactate
Butinyl	H	H / OH	=0	Butinyl lactate
Pentinyl	H	H / OH	=0	Pentinyl lactate
Hexinyl	H	H / OH	=0	Hexinyl lactate
Heptinyl	H	H / OH	=0	Heptinyl lactate
Octinyl	H	H / OH	=0	Octinyl lactate
Branched Alkinyl				
Isopentinyl	H	H / OH	=0	Isopentinyl lactate
Isohexinyl	H	H / OH	=0	Isohexinyl lactate
Isoheptinyl	H	H / OH	=0	Isoheptinyl lactate
Isooctinyl	H	H / OH	=0	Isooctinyl lactate
Cycloalkinyl				
Cyclooctinyl	H	H / OH	=0	Cyclooctinyl lactate
Alkoxyalkyl				
Methoxymethyl	H	H / OH	=0	Methoxymethyl lactate
Ethoxymethyl	H	H / OH	=0	Ethoxymethyl lactate
Methoxyethyl	H	H / OH	=0	Methoxyethyl lactate

Alkoxycarbonylalkyl				
Methoxycarbonylmethyl	H	H / OH	=O	Methoxycarbonylmethyl lactate
Ethoxycarbonylmethyl	H	H / OH	=O	Ethoxycarbonylmethyl lactate
Aryl				
Phenyl	H	H / OH	=O	Phenyl lactate
Naphthyl	H	H / OH	=O	Naphthyl lactate
Sugar				
Glucosyl	H	H / OH	=O	Glucosyl lactate
Galactosyl	H	H / OH	=O	Galactosyl lactate
Mannosyl	H	H / OH	=O	Mannosyl lactate
Alkyl	Alkyl			
Methyl	Methyl	H / OH	=O	Methyl 2-hydroxybutanoate
Ethyl	Methyl	H / OH	=O	Ethyl 2-hydroxybutanoate
Propyl	Methyl	H / OH	=O	Propyl 2-hydroxybutanoate
Butyl	Methyl	H / OH	=O	Butyl 2-hydroxybutanoate
Methyl	Ethyl	H / OH	=O	Methyl 2-hydroxypentanoate
Ethyl	Ethyl	H / OH	=O	Ethyl 2-hydroxypentanoate
Ethyl	Ethyl	H / OH	=S	S-Ethyl 2-hydroxypentanethionate
Propyl	Ethyl	H / OH	=O	Propyl 2-hydroxypentanoate
Butyl	Ethyl	H / OH	=O	Butyl 2-hydroxypentanoate
Methyl	Propyl	H / OH	=O	Methyl 2-hydroxyhexanoate
Ethyl	Propyl	H / OH	=O	Ethyl 2-hydroxyhexanoate

Propyl	Propyl	H / OH	=0	Propyl 2-hydroxyhexanoate
Butyl	Propyl	H / OH	=0	Butyl 2-hydroxyhexanoate
Methyl	Butyl	H / OH	=0	Methyl 2-hydroxyheptanoate
Ethyl	Butyl	H / OH	=0	Ethyl 2-hydroxyheptanoate
Propyl	Butyl	H / OH	=0	Propyl 2-hydroxyheptanoate
Butyl	Butyl	H / OH	=0	Butyl 2-hydroxyheptanoate
Branched Alkyl				
Isobutyl	Methyl	H / OH	=0	Isobutyl 2-hydroxybutanoate
Isobutyl	Ethyl	H / OH	=0	Isobutyl 2-hydroxypentanoate
Isobutyl	Propyl	H / OH	=0	Isobutyl 2-hydroxyhexanoate
Isobutyl	Butyl	H / OH	=0	Isobutyl 2-hydroxyheptanoate
Cycloalkyl				
Cyclohexyl	Ethyl	H / OH	=0	Cyclohexyl 2-hydroxypentanoate
Cyclohexylmethyl	Ethyl	H / OH	=0	Cyclohexylmethyl 2-hydroxypentanoate
Sugar	Alkyl			
Glucosyl	Methyl	H / OH	=0	Glucosyl 2-hydroxybutanoate
Glucosyl	Ethyl	H / OH	=0	Glucosyl 2-hydroxypentanoate
Alkyl	Alkenyl			
Propyl	Butenyl	H / OH	=0	Propyl 2-hydroxyheptenoate
Cycloalkyl	Alkenyl			
Cyclohexyl	Butenyl	H / OH	=0	Cyclohexyl 2-

				hydroxaheptenoate
Alkyl	Alkynyl			
Propyl	Butynyl	H / OH	=0	Propyl 2-hydroxyheptinoate
Butyl	Butynyl	H / OH	=0	Butyl 2-hydroxyheptinoate
Cycloalkyl	Alkynyl			
Cyclohexyl	Butynyl	H / OH	=0	Cyclohexyl 2-hydroxyheptinoate
Alkoxyalkyl	Alkyl			
Methoxymethyl	Ethyl	H / OH	=0	Methoxymethyl 2-hydroxypentanoate
Ethoxymethyl	Ethyl	H / OH	=0	Ethoxymethyl 2-hydroxypentanoate
Methoxyethyl	Ethyl	H / OH	=0	Methoxyethyl 2-hydroxypentanoate
Alkoxycarbonylalkyl	Alkyl			
Methoxycarbonylmethyl	Ethyl	H / OH	=0	Methoxycarbonylmethyl 2-hydroxypentanoate
Ethoxycarbonylmethyl	Ethyl	H / OH	=0	Ethoxycarbonylmethyl 2-hydroxypentanoate
Alkyl	Alkoxy carbonyl alkyl			
Ethyl	Methoxy carbonyl methyl	H / OH	=0	Ethyl-4-methoxycarbonyl-2-hydroxybutanoate
Ethyl	Ethoxy carbonyl methyl	H / OH	=0	Ethyl-4-ethoxycarbonyl-2-hydroxybutanoate
Alkyl	Alkoxy alkyl			
Ethyl	Methoxy methyl	H / OH	=0	Ethyl 4-methoxy-2-hydroxybutanoate
Ethyl	Ethoxy	H / OH	=0	Ethyl 4-ethoxy-2-

	methyl			hydroxybutanoate
Ethyl	Methoxy ethyl	H / OH	=O	Ethyl 5-methoxy 2- hydroxypentanoate

The D- or L- enantiomers or the racemic mixtures thereof of the following substances are further particular examples of substances of the invention: methyl lactate, ethyl lactate, propyl lactate, butyl lactate, pentyl lactate, hexyl lactate, 5 octyl lactate, isobutyl lactate, isopentyl lactate, isohexyl lactate, isoheptyl lactate, isooctyl lactate, cyclopentyl lactate, cyclopentylmethyl lactate, cyclohexyl lactate, cyclohexylmethyl lactate, butenyl lactate, hexenyl lactate, 10 isobutenyl lactate, isohexenyl lactate, butinyl lactate, hexinyl lactate, methoxymethyl lactate, ethoxymethyl lactate, ethoxycarbonylmethyl lactate, methyl-2-hydroxybutanoate, ethyl 2-hydroxybutanoate, butyl-2-hydroxybutanoate, methyl-2-hydroxypentanoate, ethyl-2-hydroxyheptanoate, butyl-2- 15 hydroxypentanoate, methyl-2-hydroxyhexanoate, ethyl-2-hydroxyhexanoate, butyl-2-hydroxyhexanoate, methyl-2-hydroxyheptanoate, ethyl-2-hydroxyheptanoate, butyl-2-hydroxyheptanoate, isobutyl-2-hydroxybutanoate, isobutyl-2-hydroxyhexanoate, cyclohexyl-2-hydroxypentanoate, 20 cyclohexylmethyl-2-hydroxypentanoate, propyl-2-hydroxyheptenoate, cyclohexyl-2-hydroxyheptenoate, butyl-2-hydroxyheptinoate, methoxymethyl-2-hydroxypantanoate, ethoxycarbonylmethyl-2-hydroxypentanoate, ethyl-4-methoxycarbonyl-2-hydroxybutanoate, ethyl-4-methoxy-2- 25 hydroxybutanoate,
or the said compounds, wherein X=S.

If ethyl lactate is used, ethyl L-lactate (LEL) as well as ethyl D-lactate (DEL) are effective. The effect of esters of 30 D-lactate is surprising as D-lactate is considered to be non-metabolizable in metazoan cells (Murray et al., 1993) and the same had to also be presumed for esters of D-lactate. Hence it could not have been expected that those compounds could be

applied at all according to the invention and that they would exhibit such good effectiveness.

The inventors' own measurements confirm the interconversion
5 of ethyl lactate and ethyl pyruvate by NAD-dependent lactate
dehydrogenases. Butyl lactate can, to a lesser degree than
ethyl lactate, also be transformed by NAD-dependent lactate
dehydrogenases. When butyl lactate is used according to the
invention only cells with a particularly high activity of
10 lactate dehydrogenase will reach therapeutically effective
concentrations of butyl pyruvate.

Thus, compounds of the general formula (II) and (III) act as
prodrugs, as exemplified in Examples 2 and 3.

15

Lactate and alkyl lactate, respectively, are transported over
the cell membrane by a lactate shuttle (monocarboxylate
transporters (MCT's)) (Garcia et al., 1994; von Grumbckow et
al., 1999) in combination with a proton transporter. For the
20 transport into mitochondria mitochondrial MCTs are available.
Addition of lactate and its alkyl esters, respectively, to
blood leads to slight alkalization due to the proton-
connected lactate transporters whereas the application of
pyruvate and its alkyl esters, respectively, leads to an
25 acidosis of blood, caused by enzymatic ester cleavage.
Lactate and alkyl lactate are transported stereo selectively
and better through the membrane as compared to pyruvate and
alkyl pyruvate (Roth and Brooks, 1990). Alkyl pyruvates
administered to blood have to be transformed into alkyl
30 lactates before they can enter cells.

Moreover, the rate of hydrolysis of compounds according to
formula (I) wherein R3 or R4 is -OH is lower as compared to
compounds wherein R3 and R4 together are =O, leading to an
35 improved in vivo stability.

Therefore, it is advantageous to use compounds wherein R3 or R4 is -OH, and in particular, therapeutically active, physiologically compatible alkyl lactates.

5 In the context of this application, the compounds according to general formula (I), including direct glyoxalase inhibitors and their prodrugs, and the specific examples of compounds, including the compounds according to formula (II) and (III), are also summarily referred to as "compounds of
10 the invention".

A particular advantage of the substances of the invention resides in the fact that toxicity of said substances and their metabolites is only very low (Clary et al., 1998).
15 After saponification by esterases they are metabolized to equally non- or only slightly toxic alcohols and to carboxylic acids which are also produced in normal cell metabolism (e.g. pyruvate and lactate). For example, the concentration of lactate in human blood is 2-20 mM. Lactate
20 is contained in many foods, is generated in metabolism and can be metabolised.

This also explains the low or even absent ecotoxicity of these compounds (Bowmer et al., 1998) in tests with
25 *Selenastrum capricornutum*, *Daphnia magna*, *Pimephales promelas* and *Brachydanio rerio*. These compounds are also devoid of mutagenic potential in normal cells as demonstrated in an established test system (Andersen and Jensen, 1984).

30 b) The substances of formula (I) differ from known inhibitors of glyoxalases

The inhibition of glyoxalases by compounds of the present invention has so far been unknown.

35 On the basis of the substrate of glyoxalase I, the hemithioacetal of methylglyoxal and glutathione, peptidic glyoxalase inhibitors are widely described in the literature

(Creighton et al, 2003; Hamilton & Creighton, 1992; Hamilton and Batist, 2004; Johansson et al., 2000; Kalsi et al., 2000; Kamiya et al, 2005; Ranganathan et al, 1995; Sharkey et al., 2000; Thornalley, 1993; Thornalley et al, 1996; Thornalley, 5 1996; Vince and Daluge, 1970, Fairlamb, 2003; D'Silva and Daunes, 2000).

US 4,898,870 describes pyrroloquinoline quinone compounds in the context of inhibition of glyoxalase I. WO 99/035128 also 10 describes compounds for inhibition of glyoxalase I. WO 04/101506 describes a further class of non-peptidic inhibitors of glyoxalase I, as does Douglas et al (1985).

However, the glyoxalase inhibitors known so far exhibit a 15 relatively high or very high toxicity and are metabolized to compounds which in turn have manifold pharmacological effects, some of which lead to severe side effects.

Furthermore, the glyoxalase inhibitors known so far only 20 inhibit either glyoxalase I or glyoxalase II, respectively. However, when inhibitors are directed to a single protein target only, resistance can develop very quickly, as for example mutations appear in the relevant protein, which make the inhibitor ineffective.

25

Therefore, the glyoxalase inhibitors of the present invention are advantageous over known inhibitors.

c) The known effects of substances according to formula (I)
30 do not encompass glyoxalase inhibition

From the known effect of methyl pyruvate its influence on glyoxalases was not predictable. For years methyl pyruvate has been intensely investigated as an insulinotropic compound (Düfer et al., 2002; Valverde et al., 2001; Lember et al., 35 2001). This effect is mediated by influencing potassium channels and mitochondrial effects. Inhibitory effects on LDH have also been proposed (Lluís and Bozal 1976).

Furthermore, it has been described that the administration of ethyl pyruvate can improve inflammatory states, reperfusion injury and ischemia (WO 03/088955; WO 02/074301; WO 01/024793, WO 05/044299). In patent US2004/110833 ethyl pyruvate is used to influence cytokine mediated diseases. This is attributable to abolishing the effect of NF- κ B (Han et al., 2005; Yang et al., 2004; Fink et al., 2004; Miyaji et al., 2003; Ulloa et al., 2002). However, opposite observations also exist in this respect (Mulier et al., 2005).

However, by no means these mechanisms indicate an inhibition of glyoxalases. Moreover, they can not be used to explain an inhibition of cell growth, because according to the findings of the present invention the growth of yeast cells is also inhibited by ethyl pyruvate, which cells neither have NF- κ B nor cytokines nor other inflammatory mediators.

Additionally, it could be shown that protein adducts of methylglyoxal, the concentration of which is increased after inhibition of glyoxalases, even increase the release of TNF- α and the activation of NF- κ B (Fan et al., 2003). In particular, this mechanism can not be used to explain the inhibitory effect of ethyl pyruvate on proliferation as the effect of ethyl pyruvate on cytokines is also detectable when cells are not proliferating.

The inhibitory effect of ethyl pyruvate on proliferation mediated via the inhibition of glyoxalases is the more surprising as ethyl pyruvate, due to its known effect as "scavenger" of reactive oxygen radicals should rather have a growth enhancing effect (Varma et al., 1998). As a matter of fact, this has been described for normal human T-lymphocytes (Dong et al., 2005). In this report it has furthermore been described that the formation of the cytokine interleukin-2 was enhanced in these cells.

B. Pharmaceutical composition/manufacture of a
medicament/methods of treatment

The present invention relates to the medical use of compounds
5 of the invention, their use for the preparation of
medicaments, pharmaceutical compositions comprising said
compounds and methods of treatment comprising administering
said compounds or compositions.

10 In the following, particular embodiments will be described in
the context of pharmaceutical compositions. However, it is to
be understood that these embodiments also apply to the
medical use of compounds of the invention, the manufacture of
a medicament, and a method of treatment. In other words, any
15 disclosure of an embodiment in the context of a
pharmaceutical composition is not to be understood as being
limited thereto, but also relates to the manufacture of a
medicament or a method of treatment. Thus, the terms "medical
use of a compound of the invention", "pharmaceutical
20 composition", "use for the manufacture of a medicament" and
"method of treatment" in the context of this application are
interchangeable. This applies to the entirety of the present
application.

25 The basic embodiment of the invention is a pharmaceutical
composition comprising at least one substance of the
invention.

In one embodiment, the pharmaceutical composition of the
30 invention comprises the substance according to the invention
as the sole active ingredient. Thus, in one embodiment the
combination of the substance of the present invention with a
further active ingredient is excluded. This does not exclude
the presence of more than one substance of the present
35 invention. This does also not exclude the presence of non-
pharmaceutically active additives, i.e. substances which

contribute to preparing a galenic formulation, such as fillers, flavouring agents, stabilizers, etc.

In one embodiment the pharmaceutical composition can comprise
5 a combination of one or more compounds of the general formula (I) wherein R3 and R4 together are =O, e.g. ethyl pyruvate, and one or more compounds wherein R3 or R4 is -OH like compounds of the general formula (II) and (III), e.g. ethyl lactate, (ethyl D- and/or L-lactate).

10 The pharmaceutical composition of the invention can further comprise one or more additional pharmaceutically active ingredients. In the context of combinations with further active ingredients the low toxicity of the compounds of the
15 present invention as well as their metabolites is of particular advantage.

As further pharmaceutical compounds, preferably chemotherapeutics, immunosuppressive agents, common agents
20 against worms and fungi, antibiotics, substances favoring cell differentiation like transcription- and growth factors, inhibitors of glycolysis or substrates for glycolysis are used.

25 For example, a combination of a compound of the present invention, such as ethyl pyruvate with common anti-parasitic agents, such as abendazol, antimonials, clomipramine, eflornithine, fluconazol, ivermectin, mebendazol, melarsoprol, metronidazole, nifurtimox, nitrazoxanide,
30 paromomycin, pentamidine, praziquantel, suramin, thrimethoprim or tinidazole is preferably used.

A preferred combination consists of compounds of the present invention and an inhibitor of glycolysis wherein the
35 inhibitor of glycolysis interferes with glycolysis downstream of the triosephosphate isomerase reaction. The rationale of such a combination is to increase the concentration of

triosephosphates from which methylglyoxal evolves parametabolically or paracatalytically, and thus, to improve the efficacy of therapy.

5 Particularly preferred is the combination of compounds of the present invention, in particular ethyl pyruvate or the corresponding thioester, and oxamate, an inhibitor of lactate dehydrogenase. Also particularly preferred is the combination of compounds of the present invention and an inhibitor of the
10 glycerol aldehyde phosphate dehydrogenase, such as iodide acetate, and/or the lactate dehydrogenase inhibitor oxamate.

Furthermore, chemotherapeutics in the context of a standard chemotherapy which generally exist for example for carcinomas
15 and sarcomas can be used. Some representative examples of standard chemotherapeutic agents are cyclophosphamide and doxorubicin for the treatment of breast cancer and leukemia, taxol for the treatment of ovary cancer, and 5-fluorouracil or cisplatin for sarcoma.

20 In addition to the compounds according to the invention further compounds may be preferably applied which stimulate the metabolism of infectious organisms, such as bacteria, fungi or protozoa, like substrates of glycolysis, in
25 particular glucose, or for example 2,4-dinitrophenol acting as uncoupler of the respiratory-chain. In this manner advantageously the concentration of methylglyoxal is increased further and the efficacy of the compounds of the invention is increased further, resulting in an enhanced
30 efficacy of the pharmaceutical composition.

A further aspect of the invention is the use of compounds of the present invention in combination with known or novel genetic methods like siRNA and antisense nucleotides for the
35 targeted inhibition of enzymes or proteins to increase the sensitivity of tumors (Nesterova and Cho-Chung, 2004).

The pharmaceutical composition or medicament can further comprise one or more auxiliary substances useful for the galenic formulations of drugs, including, but not limited to, fillers, flavouring agents, stabilizers and agents that
5 prevent microbial growth in the pharmaceutical composition.

The pharmaceutical composition can be in any suitable galenic formulation, depending on the kind of disease to be treated and the chosen route of administration. The skilled person
10 can readily select and prepare a suitable preparation on the basis of common general knowledge. Pharmaceutical compositions of the invention can be prepared according to known methods e.g. by mixing one or more effective substances with one or more carriers, and forming of e.g. tablets,
15 capsules, or solutions. Where appropriate, solutions can be e.g. encapsulated in liposomes, microcapsules or other forms of containment.

Examples of suitable formulations comprise aqueous solutions
20 which can optionally be buffered, water in oil emulsions, oil in water emulsions, creams, ointments and formulations comprising any of the foregoing.

The invention encompasses a pharmaceutical composition
25 prepared in the form of a sustained release or controlled release galenic formulation. Such formulations allow the targeted release in e.g. a certain location, such as a certain part of the gut, or a certain tissue or organ, and/or allow the sustained release over a defined period of time.

30

A pharmaceutical preparation can also be prepared by mixing the ester components of the compounds of the invention under conditions at which compounds of the general formula (I) are formed. The pharmaceutical preparation can also be prepared
35 by assembling ester components of the compounds of the invention such that in the organism, for example in the acidic environment of the stomach, the compounds of the

general formula (I), (II) or (III) are formed. Ester components are for example an alkanol like for example ethanol and an organic acid like for example lactic acid.

5 The pharmaceutical composition of the invention comprises at least one compound of the invention in a therapeutically effective amount. The skilled person can readily determine the therapeutically effective amount in standard in vitro or in vivo experiments. For example, the effective amount can be
10 estimated on the basis of an extrapolation from in vitro data, such as enzyme inhibition or cellular assays.

For example a dosage can be formulated in animal models which corresponds to the IC50 in cell culture experiments. Hence,
15 according to commonly known methods, the optimal dosage for the vertebrate to be treated, such as humans, can be deduced from animal experiments. The amount of the agent to be administered naturally depends on the person to be treated, his body weight, his genetic and physical constitution, the
20 disease state, the route of administration, the galenic formulation and other parameters.

Furthermore, dosage and the interval of administration can be guided by the individual plasma concentrations of the agent
25 that guarantee a therapeutic effect.

Useful effective concentrations, i.e. concentrations to be achieved at the level of cellular exposure, range from at least 0.05 mM, preferably from 0.05 mM to 50 mM, more
30 preferably 1 mM to 40 mM, more preferably 1 mM to 20 mM, most preferably 1 mM, 2.5 mM, 5 mM, or 7.5 mM in systemic application. In topic applications higher concentrations may be useful. Preferred are 0.2 to 200 mM, more preferred are 0.2 to 50 mM and 50 to 200 mM.

35

In other words, the concentrations above refer to desired blood and/or tissue concentrations, or local concentrations.

Thus, the invention relates to pharmaceutical preparations suitable to achieve such concentrations upon administration.

To achieve a therapeutic effect the pharmaceutical composition of the present invention is generally applied for several days or weeks as repeated bolus doses (e.g. injections) or continuous administration (e.g. infusion), or any time period required to achieve a therapeutic effect, at the respective therapeutically effective dosage.

The pharmaceutical composition of the present invention can be administered topically or systemically. In both topical and systemic administration a local administration to a selected site can be performed. Because of their nature, esters are of limited stability, necessitating the use of higher and/or repeated doses for systemic application. This can be circumvented by local application.

Pharmaceutical compositions comprising the compounds of the invention can be administered according to generally known methods - including but not limited to oral, intravenous, intraarterial, subcutaneous, intramuscular, intradermal, intraperitoneal, intraauricular, rectal, intranasal, epidural, percutaneous, or transdermal administration, or administration as an aerosol, via mini-pumps, as mouth lavage, gel, oil, ointment, cream, spray, plaster, via microbubbles and/or pulmonary application (e.g. by inhalation).

Administration is for example systemic, e.g. by single or repeated oral or parenteral application, or via methods wherein the medicament is administered systemically in an inert vehicle and is only released at the desired location by respective manipulation. An example thereof is, amongst others, so called microbubbles as described in Bekeredjian et al. (2005) and Bekeredjian et al. (2003).

The pharmaceutical composition can also be a food supplement or beverage supplement. In the context of the present invention, "food supplement" or "beverage supplement" means a pharmaceutical composition that is administered together with the standard daily diet, or a special medical diet. It also means "health food", i.e. food of a particular composition that is consumed by subjects without medical supervision to achieve a prophylactic or therapeutic effect.

10 C. Medical indications

The substance, pharmaceutical composition, medicament or method of treatment of the present invention is for the following medical indications.

15 The invention encompasses the administration or use in non-mammalian vertebrates, mammals and humans in need thereof. In one embodiment the non-mammalian vertebrates are not fish. In the context of this application, the term "animal" is meant to encompass non-vertebrate animals, vertebrate animals,
20 comprising non-mammalian vertebrates and mammals, which mammals comprise man. Thus, the term animal encompasses humans.

In particular, the pharmaceutical composition is for an animal, including man, suffering from a disease associated with increased glycolytic metabolism. Such diseases may further be associated with one or more selected form increased formation of methylglyoxal, increased activity of glyoxalase I and/or II and increased cellular growth and/or proliferation. Preferably, the diseases associated with
30 increased glycolytic metabolism are associated with enhanced methylglyoxal formation. Specific examples of such diseases include parasitic diseases, wherein the parasite is selected from protozoa and helminthes. Particular examples of
35 protozoal parasites include Trypanosoma (like e.g. Trypanosoma cruzi), Leishmania, Plasmodium (like the malaria pathogen Plasmodium falciparum), Flagellates (Trichomonads,

Trypanosoma spp., Leishmania spp, Gardia lamblia); Rhizopods (Entamoeba spp.), Sporozoa (Isospora belli, Sarcocystis bovi-hominis, Toxoplasma, in particular Toxoplasma gondii, Plasmodium spp., Cryptosporidium spp., Giardia spp.),
5 Ciliates, Pneumocystis carinii, Microspora, Ichthyophthirius multifiliis.

Specific examples of helminthes include Trematodea (trematodes, flukes, Schistosoma, Bilharziella, etc),
10 Cestoidea (tapeworms, Taenia, Echinococcus, Hymenolepis), Nematodes (roundworms, Strongyloides, Ancylostoma, Necator, Trichostrongylus, Angiostrongylus, Ascaris, Toxocara spp), cestodes, and trichinella.

15 In one embodiment, the parasite is Trypanosoma. Substances of the invention encompassing EP, LEL and BL are very effective in the inhibition of growth and proliferation of Trypanosoma, and in particular Trypanosoma brucei, as demonstrated in the experimental section.

20 As used herein the term "parasite" encompasses the above mentioned examples of parasites.

According to the invention the cells proliferation of which
25 is inhibited are mainly parasitic organisms, in particular, parasites selected from protozoa and helminthes. Such parasites cause parasitic diseases, such as various parasitic diseases.

30 a) Parasitic diseases

It was surprisingly found that the substances of the invention can be used for the treatment and/ or prophylaxis of parasitic diseases, wherein the parasite is selected from protozoa and helminthes, comprising the inhibition and/or
35 killing of the parasites.

In one embodiment, R3 or R4 are OH when the disease is cerebral malaria.

5 In one embodiment R1 is not methyl, ethyl, propyl or butyl, when the treated subject is a fish, X is O, and R3 and R4 together are =O.

In one embodiment, R3 or R4 are OH when the disease is pancreatitis.

10

In the context of this invention the term "parasite" relates to an organism which spends at least part of its life-cycle within a higher organism, e.g. within the blood, body cells or tissues of the host organism. Host organism relates to a multicellular organism, such as a non-vertebrate animal, a vertebrate, including mammals, including humans.

15

It is a characteristic feature of many parasites to be highly specialized with respect to their host organism. Such specialisation can be e.g. to mammals in general, or can be as specific as certain body areas on one particular species. Because the host organism provides a typically highly stable environment and nutrient supply, the respective protozoal and metazoal parasites can be specialists concerning the metabolic pathways for energy generation.

20

25

The term "treatment and/or prophylaxis of parasitic diseases" encompasses cytotoxic and cytostatic effects on the parasite in at least one of its different developmental stages, e.g. its blood borne or tissue borne stage, in particular where these stages are metabolising glucose.

30

The effects on glyoxalases, and the associated anti-parasitic effects (i.e. cytostatic and/or cytotoxic effects) of substances of the present invention have previously been unknown.

35

It has previously been suggested, that glyoxalase I inhibitors may be useful for the treatment of malaria (Thornalley et al, 1994, Thornalley, 1996). However, these documents contain no teaching or suggestion regarding
5. compounds of the present invention.

WO 02/102366 discloses the use of certain pyruvate esters, which are not part of the claimed invention, for the treatment of fish parasites. However, this document provides
10 no teaching which can be generalized to other substances, or other organisms (hosts or parasites) apart from the specific examples mentioned.

WO 03/088955, WO 02/074301, US 2003/0232884 and
15 US2004/0110833 relate to the treatment of inflammatory, cytokine mediated conditions. There is no teaching or disclosure to treat parasites per se.

US 5,580,902 discloses certain substances of the present
20 invention as auxiliary agents in pharmaceutical compositions, in particular as enhancers for a multitude of active ingredients. This document does not, however, disclose any anti-parasitic effects of substances of the invention per se.

25 de Jaham (2003) discloses that ethyl lactate can be added to shampoos used in the treatment of canine superficial bacterial pyoderma. Prottey et al (1984), mentions ethyl lactate in the context of the treatment of acne. A number of documents mentions substances of the invention in the context
30 of cytokine mediated diseases, including sepsis or septic shock (e.g. Fink et al, 2004, Miyaji et al, 2003, Ulloa et al, 2002, Han et al, 2005).

Therefore, these documents discuss an effect of substances of
35 the invention on cytokine mediated pathologies. They do not, however, disclose or suggest any effects on parasites per se.

US 2005/0020678 discloses substances of the invention as fungicides. It provides no teaching that could be generalized to other substances or applications, however.

5 Other known medical applications of substances of the invention equally provide no link to the treatment of parasitic infections.

WO05/004857 relates to the treatment of pancreatitis, but
10 does not disclose any direct anti-parasitic activity of substances of the invention.

According to EP 0 717 984 and JP 8 208 422 proliferation of normal human cells, for example keratinocytes, is even
15 stimulated by compounds of the present invention, which effect is used to improve the appearance of the skin. Similarly, in several patents (US 5,580,902; US 4,234,599; US 4,105,783) compounds of the present invention have been described as agents to improve skin consistency and smoothen
20 wrinkles. Effects on keratosis, and several diseases characterized by defective keratinisation (dandruff, acne, palmar and plantar hyperkeratosis, dry skin, Darier's disease, lichen simplex chronicus, psoriasis, eczema, pruritus, warts and herpes) are described in US 3,879,537, US
25 3,920,835, US 4,246,261 and US 7,33,815.

Ethyl pyruvate has been used to improve cataracts (Devamanoharan et al., 1999). In this connection a lowering of dulcitol and glycated proteins by ethyl pyruvate has been
30 found, connected to the effect of pyruvate formed by hydrolysis of ethyl pyruvate.

CN 1175632 describes ethyl lactate as an auxiliary substance in the manufacture of Spirulina wine, but does not disclose
35 ethyl lactate as an active ingredient. WO 03/088955, WO 02/081020, US 2003/0232884 and WO 02/074301 deal with clinical situations such as reperfusion injury, kidney

failure, ischemia and inflammatory disorders. Marx et al (1988) suggests the inhibition of cancer cells by lactate. Stanko et al (1994), discusses a role of pyruvate in the treatment of cancer.

5

Though not intending to be bound by theory, it is suggested that the anti parasitic activity of the compounds of the invention is due to their inhibition of glyoxalase I and/or II.

10

Parasitic cells generally require significant amounts of energy for cell division and general metabolism, provided in the form of ATP. Glycolysis allows anaerobic as well as, in combination with oxidative phosphorylation, aerobic energy generation.

15

Animals and humans regulate glucose concentration in their organs and body fluids in a narrow concentration range. The glucose concentration for example in human blood is constant at about 5 mM. Animals show a similar glucose homoeostasis.

20

Thus, most parasites are specialized in the utilization of glucose in blood, body cavities and on the skin, and show a high rate of glycolysis. As a matter of fact, the bloodstream forms of parasites depend exclusively on glucose as energy source.

25

Degradation of glucose by glycolysis and the formation of oxoaldehydes are ubiquitous metabolic pathways, which are phylogenetically highly conserved (Heymans and Singh, 2003; Clugston et al., 1997).

30

Thus, glycolysis per se has been discussed as a possible therapeutic target (Brady and Cameron, 2004; Kavanagh et al., 2004; Lakhdar-Ghazal et al., 2002, Iwami et al., 1995).

35

However targeting glycolysis for therapeutic purposes has so far remained elusive, as glycolysis is a central metabolic pathway of parasites as well their hosts.

5 US 2004/0167079 describes for example a method for treatment of cancer by use of 2-deoxyglucose (2-DG), an inhibitor of glycolysis.

10 US 2003/0181393 discloses the glycolysis inhibitors 2-deoxyglucose, oxamat and iodide-acetate. Iodide acetate inhibits glycerolaldehyde-3-phosphate dehydrogenase and oxamate inhibits lactate dehydrogenase.

The main shortcoming of inhibiting glycolysis is that
15 glycolysis is used for energy generation in almost all cells, such that healthy cells are also affected by inhibition of glycolysis. In particular the influence on the brain is dramatic as the brain is an obligatory consumer of glucose and thus is highly dependent on glycolysis.

20 However, glycolysis is always accompanied by the formation of glyoxal compounds, in particular methylglyoxal. These compounds are highly toxic as they easily form adducts with cellular proteins and nucleic acids and lead to their
25 inactivation.

Therefore, all glycolyzing cells use detoxification systems which are mostly consisting of the enzymes glyoxalase I and II.

30 An alternative approach to therapeutic intervention aiming at influencing modified and increased glycolysis is therefore no longer related to the inhibition of glycolysis, but to the inhibition of glyoxalases, which has extensively been
35 discussed in the context of cancer (Thornalley et al., 1994; Vander Jagt et al., 1990; Pemberton and Barrett, 1989; Creighton et al., 2003; Hamilton and Batist, 2004).

As pointed out above, parasites perform glycolysis, which may be their main metabolic pathway for energy generation.

- 5 As pathogenic parasites utilize glycolysis, as well as detoxifying glyoxalases, it is suggested by this invention that the inhibition of glycoxalases can serve as a "universal" therapy for a plurality of parasitic diseases.
- 10 Thus, the present invention relates to the treatment and/or prevention of parasitic diseases.

The term "parasitic disease" encompasses systemic colonisation by parasites, including infections of blood,
15 tissues and organs. Also encompassed are infections of the gastrointestinal tract.

The term "treatment" encompasses subjects suffering from any of the various disease stages, such as acute or chronic
20 infection, and encompasses after-treatment as well as prophylaxis. "After-treatment" means a treatment following conventional therapy. Treatment concomitantly with conventional therapy (e.g. known anti-parasitic agents) is also part of the present invention.

25 The term "prophylaxis" or "chemo-preventivum" relates to administration of a pharmaceutical composition of the invention when a subject is at risk to develop a disease, or a disease is suspected or is present subclinically, but said
30 disease has not fully evolved or has not been diagnosed.

The term "treating parasitic disease" in the stricter sense relates to the treatment of clinically manifest disease. It is meant to encompass both cytotoxic and cytostatic effects
35 on parasites. Thus, "inhibition of parasites" encompasses the inhibition of cell proliferation (cytostatic action) as well as the killing of the cells (cytotoxic action). The killing

of parasites by necrosis or apoptosis is encompassed by the invention. The terms "proliferation" and "growth" are used interchangeably in the context of this application.

5 In one embodiment of the invention, the parasitic infections are caused by an organism that is resistant to conventional anti parasitic agents, for example, abendazol, antimonials, clomipramine, eflornithine, fluconazol, ivermectin, mebendazol, melarsoprol, metronidazole, nifurtimox,
10 nitrazoxanide, paromomycin, pentamidine, praziquantel, suramin, thrimethoprim or tinidazole.

b) Treatment of infections in immunosuppressed subjects

Parasitic infections represent a significant problem in
15 patients in an immunosuppressed state. Furthermore, parasites achieve long-term infection via suppression or modulation of the host immune system (Toman et al., 1998)

Thus, in one embodiment of the invention, the treatment is in
20 an immunosuppressed animal, wherein said immunosuppression is associated with hereditary or acquired immune-defects, comprising acquired immune defect associated with HIV, organ transplantation, chemotherapy, exposure to radiation or is associated with parasite-induced immunosuppression.

25 Infections on an immunosuppressed background are oftentimes opportunistic infections by organisms that are non-pathogenic in the normal individual. Treatment of opportunistic infections is encompassed by the present invention.

30

c) Treatment of parasites in cancer patients

Tumor patients are often suffering from additional diseases, such as parasites, due to a weakened immune defence.

35 It is therefore part of the invention to treat parasitic diseases in cancer patients, and to apply the substances, pharmaceutical composition, medicament or method of treatment

of the invention for the treatment and/or prophylaxis against parasitic diseases.

As it is known that most tumors show a high rate of glycolysis (Gatenby RA and Gillies RJ, 2004), the growth of tumor cells and infectious agents, such as bacteria, protozoa or fungi can be simultaneously inhibited, which represents an increase in efficacy for the treatment of such patients. Thus, it is a particular advantage of the present invention that cancer cells, like fungal, protozoal and bacterial cells, exhibit an increased glycolysis, accompanied by high activity of glyoxalases. Hence, cancer cells can be targeted by the substances of the invention, just like protozoal, fungal and bacterial cells. In other words, the substances of the invention at the same time inhibit both kinds of cells.

Glyoxalase I is up-regulated in many tumors. Generally, it is presumed that increasing concentrations of glyoxalase I correlate with the malignant phenotype of tumors. The increased concentration of glyoxalase I in tumor tissue in comparison to normal tissue is said to increase the resistance of tumors to chemotherapeutics like mitomycin C and other anti-cancer agents (Ranganathan et al., 1995; Ayoub et al., 1993). Inhibition of the glyoxalase I reaction by compounds of the present invention, such as ethyl pyruvate, alone or in combination with conventional cancer therapy, such as radiation or chemotherapy is therefore advantageous for the treatment of cancer.

According to the invention, the animal can be concomitantly suffering from cancer, and can be going to receive, is currently receiving, or has received conventional cancer therapy, comprising one or more of chemotherapy, surgery, radiotherapy or brachytherapy. It is meant to encompass treatment following a completed conventional therapy (e.g. a full regimen of chemotherapy comprising several individual treatment periods, or following surgery), and treatment that

is intermittent with the conventional therapy, e.g. taking place in the intervals between individual courses of chemotherapy. It is also meant to relate to a therapy that is started after the conventional therapy (e.g. after the first course of chemotherapy) and then continues concomitantly with the first therapy (e.g. throughout the further courses of chemotherapy).

Advantageously, the substances of the invention also are effective in cancer cells that are resistant against conventional therapy, such as chemotherapy and/or radiation therapy.

Moreover, the known effect of substances of the invention, such as ethyl pyruvate as scavenger of reactive oxygen radicals represents a desired side effect for cells which do not have a high rate of glycolysis (non-cancer-cells) as such cells are additionally protected. In a combination therapy with a chemotherapeutic agent this is an additional advantage as also normal cells are stressed, which is reduced by compounds of the present invention.

In one embodiment the treatment of actinic keratoses with methyl- or ethylpyruvate is excluded.

The simultaneous inhibition of glyoxalases by compounds of the present invention such as ethyl or butyl pyruvate, ethyl or butyl lactate etc. in cancer cells as well as infectious organisms (in particular bacteria, fungi and protozoa) is particularly advantageous, as cancer cells and parasites are killed simultaneously.

d) Treatment of concomitant infectious disease

Parasitic infections may be accompanied by other infections, such as fungal infections or bacterial infections. Such multiple infections are of particular significance in immune-compromised individuals, and pose a significant clinical

problem in transplant patients, cancer patients or HIV patients.

For example, the weakening of the patient by cancer per se,
5 as well as by cancer therapy, such as chemotherapy, which weakens the immune system, favors fungal as well as bacterial infections. For such patients, even ubiquitous bacteria that are non-pathogenic for healthy human beings can be dangerous (opportunistic infections), the more so pathogenic bacteria.

10

Degradation of glucose by glycolysis and the formation of glyoxal compounds are ubiquitous metabolic pathways, which are phylogenetically highly conserved (Heymans and Singh, 2003; Clugston et al., 1997). Cells which cover their energy
15 consumption solely by glycolysis when glucose is available are for example fungal cells (e.g. *Candida* spp., *Aspergillus* spp., *Cryptococcus* spp., *Zygomycetes* spp., *Dermatophytes*, *Blastomyces* spp., *Histoplasma* spp., *Coccidioides* spp., *Sporothrix* spp.), bacteria such as *Actinobacillus*,
20 *Actinomyces*, *Bacteroides*, *Clostridium*, *Campylobacter*, *Enterobacter*, *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Helicobacter*, *Peptostreptococcus*, *Pneumococcus*, *Porphyromonas*, *Prevotella*, *Pseudomonas*, *Spirochetes*, *Staphylococcus*, *Streptococcus*, *Treponema*, *Porphyromonas*
25 *gingivalis*, *Prevotella intermedia*, *Actinomyces*, *Eubacterium nodatum*, *Peptostreptococcus micros*, *Peptostreptococcus anaerobius* and *Fusobacterium nucleatum*, and hence can be treated according to the present invention.

30

Specific examples of infectious organisms which can be treated according to the invention comprise *Trypanosoma*, *Leishmania*, *Plasmodium*, *Toxoplasma*, helminthes, *Acrobacter*, *Actinobacillus*, *Actinomyces*, *Bacteroides*, *Brucella*, *Chlamydia*, *Clostridium*, *Campylobacter*, *Escherichia*, *Enterobacter*,
35 *Enterococcus*, *Eubacterium*, *Fusobacterium*, *Helicobacter*, *Hemophilus*, *Legionella*, *Listeria*, *Mycobacteria*, *Mycoplasma*, *Neisseria*, *Pasteurella*, *Peptostreptococcus*, *Pneumococcus*,

Pneumocystis, Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella, Spirochetes, Staphylococcus, Streptococcus, Treponema, Vibrio, Yersinia, Escherichia coli or Pneumocystis carinii.

5

They turn off other energy producing processes by glucose and use glycolysis (catabolite repression). It is therefore not surprising that many infectious organisms, such as fungi, bacteria and protozoa can also be inhibited in their growth
10 by compounds of the present invention.

In one embodiment of the invention, therefore, the animal, including man, is concomitantly suffering from a fungal or bacterial infection as discussed above, including
15 opportunistic infections and infections by organisms showing antibiotic resistance.

Therefore, it is a particular advantage of the present invention that fungal infections as well as bacteria and
20 protozoa can be effectively treated with the compounds of the present invention. Where in conventional therapies multiple active ingredients are required, the present invention provides substances that are effective against all these pathogens simultaneously. Consequently, the invention
25 provides for a reduction in side effects, because only a single active ingredient is necessary, where the state of the art requires several different ingredients. In this context, the low toxicity of the compounds of the invention is a further particular advantage.

30

e. "Postprandial state"

In one embodiment the pharmaceutical composition or medicament is for use in a vertebrate having a reduced blood glucose level. The use of the compounds of the present
35 invention for the treatment in such a postprandial state is also part of the invention. In postprandial states the utilization of glucose is reduced in normal cells. These

states can be reached for example by long-term fasting and can be accelerated by administration of hormones or can be forced by administration of hormones. Characteristic for such states is a low blood level of glucose and a high blood level of ketone bodies. Ketone bodies can be used by the brain to generate energy such that metabolic states of the patient can be generated under control of a medical practitioner prior to therapy wherein infectious organisms and/or tumors represent the primary consumers of glucose, under conditions of reduced blood glucose levels (Sugden and Holness, 2002).

Thus, in a postprandial state the selectivity of the therapy is enhanced, because of the reduced glucose metabolism in normal cells.

D) Further applications

a) Methods of treating drinking water

The present invention encompasses methods for treating water, in particular drinking water, with substances of the invention.

Drinking water is oftentimes contaminated by parasitic organisms. As a matter of fact, many parasites are mainly transmitted by contaminated drinking water.

For example, the microorganisms Giardia and Cryptosporidium have become the two of the largest concerns for the water treatment industry, due to their resistance to chlorine disinfection. Recent widespread outbreaks of disease caused by these two microorganisms have infected thousands of individuals, causing severe gastrointestinal disorders. The most severe epidemic case occurred in Wisconsin in 1993 when 400,000 people were infected by Cryptosporidium, causing more than 60 deaths.

Giardia lamblia is a tiny protozoan that makes its way into surface water sources primarily through the contamination of

water via animal feces. Cryptosporidium is a protozoan, similar in size and effect to Giardia, which also enters drinking water sources through animal fecal matter. Protozoa are uniquely designed to survive in water. They are larger in size than either bacteria or viruses. Some species can secrete a protective covering and enter a resting stage called a "cyst". These cysts are then protected from disinfection, and they continue to be powerful disease-causing pathogens.

Scientists presume that Giardia and Cryptosporidium have always maintained a presence in drinking water sources. Due to recent improvements in sanitation, most people now have a low immunity to the protozoa. This low immunity makes them all the more vulnerable to the effects of the microorganisms when sanitation breaks down.

Exposure to Giardia and Cryptosporidium can cause symptoms similar to the flu. Severe diarrhea, vomiting, abdominal pain, and/or fever often accompany the diseases. Though the majority of people recover from the disease within a week, individuals with low or developing immune systems (AIDS patients, children, and the elderly) can die from the effects of exposure to these pathogenic protozoa. There is currently no disinfection technique that can eliminate the presence of these two microorganisms in drinking water (Savioli et al., 2006).

Thus, the present invention encompasses a method for treating drinking water, wherein water is contacted with substances of the invention. Such method comprises e.g. dosing of drinking water with such substances, or their use in filters and other devices or procedures for water purification. In a particular embodiment, the treatment is for the elimination of protozoa from the drinking water, comprising elimination of Giardia and/or Cryptosporidium.

b) Aquaculture

A further application of the compounds of the invention is for the treatment of water for aquaculture, and/or the treatment of aquatic organisms kept in aquaculture, in particular fish. Such organisms are oftentimes affected by parasitic diseases, in particular diseases caused by protozoa, such as Ichthyophthirius.

Ichthyophthirius multifiliis is one of the most important, and probably the most harmful parasite, which infects freshwater fish, and is a cosmopolitan organism of worldwide importance. Ichthyophthirius multifiliis can cause extensive lesions due to intensive infection. In cultures of Ictalurus punctatus (catfish) in the USA and carps in Israel the parasite infection can cause annual loss of millions of dollars.

Thus, in a particular application of the compounds of the invention fish, or water for keeping fish is treated with the compounds of the invention.

c) Industrial biofouling / biofilms

Yet another application of the compounds of the invention is the prevention and/or combat of protozoal biofouling.

A number of organisms including bacteria, protozoa, amoebae, fungi, barnacles and other organisms have been shown to form biofilms that create problems in man-made environments. Biofilms occur and are a problem in the water service utilities, many industrial processes including the food, beverages, pharmaceutical, paint, oil processing and manufacturing, and engineering industries. In addition they may create havoc in medicine and dentistry. Industrial biofouling covers biofouling/biofilms in domestic, industrial and health related fields in which they are a persistent problem.

Thus, the present invention provides methods to prevent and/or combat industrial biofouling comprising protozoa.

5 The present invention relates to the prevention of the formation of biofilms, as well as combating biofilms by use of substances according to the invention.

10 More specifically, the invention relates to methods for preventing and/or combating protozoa comprised in biofilms, comprising contacting a substrate comprising glass, metal, plastics, wood, medical material, medical devices, soil particles, concrete, limestone, with a compound of the invention.

15 Protozoa can produce biofilms after attachment to a surface, which is a mixture of cells that coexist as an organized community. Biofilms represent a protective environment and thus biofilm formation carries important consequences, both in industrial and clinical settings.

20 Sessile protozoa within the biofilm are resistant to a range of antiprotozoal agents currently in clinical or industrial use. Biofilms can stick to plastic, are able to coat medical implants causing serious complications in patients with hip and valve replacements, shunt tubing weavers and contact lens weavers, vascular bypass crafts and urinary catheter (Reynolds TB, and Fink GR. Science 2001).

30 Microbes, such as bacteria, fungi, protozoa, or nematodes are found in dental unit waterlines. The presence of various microorganisms is a potential source of microbial contamination of dental aerosols, and thus a potential threat to the health of patients and dental staff. In particular under conditions of decreased immunity of an organism, 35 opportunistic infections constitute a health risk (Szymanska J. Ann Agric Environ Med. 2005)

The presence of microbes in drinking water and within biofilms of water distribution systems are associated with taste and odor problems, contamination in food and beverage preparation, and a variety of health-related effects (Doggett
5 MS. Appl Environ Microbiol. 2000).

Biofilms containing microbes can be dangerous in space stations. In long-term missions bacteria formation in closed systems can affect the astronauts health and water-thin
10 biofilms can attack inaccessible cable harnesses causing electric breakdown.

Microorganisms have been implicated in the attack of both natural limestone materials and concrete. For example, the
15 fungus *Fusarium* plays an important role in concrete deterioration. Treatment of microbial growth as constituents of biofilms is therefore promising for the protection of historical buildings and gravestones. (Gu, et al, International Biodeterioration & Biodegradation. 1998).

20 Microbial spoilage is a constant concern in the food processing industry. Even breweries have a risk of contamination of their products, even though there is a predisposition of beer per se to be contaminated with
25 bacteria due to low pH-value (3.8-4.7). Biofilms have been found in brewery pasteurizers and conveyor systems.

Consequently, the present invention encompasses the combating of biofilms in all of the above settings, comprising
30 contacting surfaces with substances of the invention to avoid biofilm formation and/or combat existing biofilms.

In one embodiment, the substance of the invention can be encompassed in a composition the substrate is covered with,
35 e.g. a paint or coating layer. Such paint or coating layer may optionally result in the retarded liberation of the substances of the invention.

In the context of the present invention the term "biofilm" relates to microorganisms, including protozoa, attached to a substrate by means of an extracellular matrix produced by said microorganisms, and comprising said microorganisms. Biofilms can anchor microorganisms to substrates formed by all kinds of materials e.g. metals, plastics, glass, concrete, limestone, soil particles, medical material and living substrates such as teeth. Thus, the invention relates to the prevention and or combating biofilms on such kinds of substrates.

In one embodiment, the substrate is teeth, and the biofilm may optionally be associated with the formation of dental plaque.

It is of particular advantage in this connection that substances of the invention not only affect protozoa, but also other organisms, such as e.g. fungi and bacteria. Biofilms are typically colonized by other organisms in addition to protozoa. Thus, the substances of the present invention can influence (i.e. inhibit their growth, and/or exert cytostatic and/or cytotoxic effects) many organisms typically found in biofilms (Armitage, 2004; Leclerc, 2005; Coetser and Cloete, 2005; Chandra et al., 2005).

Thus, the invention relates to, but is not limited to, the prevention and combating of biofilm-formation in tube systems like pumps and filter systems (biofouling) for water distribution, conveyor tracks, bottles, can warmers in breweries, water tanks, clinical equipment like catheters, wound dressing material, colonisation of heat exchangers, paper machines, ship hulls, cooling water systems, oil recovery systems and corrosion of pipes (biocorrosion).

Moreover, the present invention relates to the treatment of infections associated with biofilms, such as infections

caused by the inhalation of fragments of biofilms (e.g. inhaled biofilm fragments derived from contaminated inhalation devices, such as in dental unit waterlines or other setc).

5

It is a further particular advantage of the substances of the present invention that they can attack biofilms, which are difficult to combat with conventional antibiotics, such as colonisation of a cow's udder with biofilms.

10

Particular applications of the substances of the invention to combat protozoa, in particular in the context of biofilms, comprise the addition of said substances to fluid for storing and/or treating contact lenses, as an additive to or cleaning solution for dental unit waterlines, for use in the prevention of electric short circuits in electronic industry, for the cleaning of air ventilation systems by volatile substances of the invention, and/or their use as sprays/as a fog, their use in the protection of historical buildings and gravestones and monuments, as well as the use of the compounds of the invention to stabilize beer by preventing colonization with beer-spoiling organisms (e.g. by use of ethyl pyruvate or ethyl lactate, which degrades into ethanol and pyruvate/lactate) or to clean conveyor systems and bottles in breweries.

25

On the basis of the following figures and examples the present invention is illustrated further.

30 BRIEF DESCRIPTION OF THE FIGURES

Fig. 1: Inhibition of the enzymatic activity of glyoxalase I of yeast by ethyl pyruvate (EP).

Fig. 2: Inhibition of the enzymatic activity of yeast glyoxalase I by 2-Oxopropanethioic acid S-ethyl ester (SE).

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Fig. 3: Influence of ethyl pyruvate (EP), butyl pyruvate (BP) and butyl L-lactate (BL) on the enzymatic activity of yeast glyoxalase I.

5 Fig. 4: Influence of ethyl pyruvate (EP) on the enzymatic activity of yeast glyoxalase II.

Fig. 5: Inhibition of the enzymatic activity of glyoxalase I of human erythrocytes by ethyl pyruvate (EP).

10

Fig. 6: Inhibition of the enzymatic activity of glyoxalase II of human erythrocytes by ethyl pyruvate (EP).

Fig. 7: Effect of ethyl D-lactate (DEL), ethyl L-lactate (LEL) and ethyl pyruvate (EP) on the vitality of primary human fibroblasts.

15

EXPERIMENTS

20 A. General experimental procedures

a) Determining enzymatic activity of yeast glyoxalase I

Measuring glyoxalase I (E.C.4.4.1.5, lactoyl-glutathione lyase Sigma, G-4252) was performed according to the instructions of McLellan and Thornalley (1989). The principle is based on measuring the initial rate of formation of S-D-lactoyl-glutathione from methylglyoxal (Sigma, MO252, 40%) and reduced L-glutathione (Aldrich, G-4251, >99%). The formation of the product is observed using the extinction coefficient $\epsilon = 2,86 \text{ mM}^{-1} \text{ cm}^{-1}$ at 240 nm. The measurement was performed in 50 mM sodium phosphate buffer, pH 7,0. For that purpose 2 mM methylglyoxal and 2 mM reduced glutathione were incubated for 2 minutes at 30°C for the formation of the hemithioacetal. Thereafter 20 μl of a 1 to 1000 dilution of the glyoxalase I (Sigma, G-4252) was added to 1 ml of the measuring reagent to start the reaction.

30

35

Glyoxalase activity (IU) corresponds to the amount of enzyme forming 1 μmol of S-D-lactoyl-glutathione/min.

b) Determining enzymatic activity of human erythrocyte glyoxalase I

The determination of glyoxalase I was performed as described above for the yeast enzyme. After the formation of the hemithioacetal, suitable amounts (5 to 100 μl) of an erythrocyte lysate were added to 1 ml of the measuring reagent to start the reaction. The erythrocyte lysate was prepared according to the instructions of Mannervik et al. (1982).

Glyoxalase activity (IU) corresponds to the amount of enzyme forming 1 μmol of S-D-lactoyl-glutathione/min.

c) Determining enzymatic activity of human erythrocyte glyoxalase II

The determination of erythrocyte glyoxalase II (E.C.3.1.2.6., hydroxyacyl glutathione hydrolase) was performed according to the instructions of McLellan and Thornalley (1989). The principle is based on determining the initial hydrolysis of S-D-lactoyl-glutathione (L 7140, Sigma - Aldrich Chemie GmbH) in the presence of low amounts of a cell extract. Hydrolysis is observed on the basis of the reduction of the extinction at 240 nm ($\epsilon = -3,1 \text{ mM}^{-1} \text{ cm}^{-1}$). Glyoxalase II activity (IU) correlates to the amount of enzyme hydrolysing 1 μmol of S-D-lactoyl-glutathione/min. The measurement was performed in 100 mM Tris-HCl buffer, pH 7,4. For this purpose 0.4 mM S-D-lactoyl-glutathione was added to 1 ml of the measuring reagent and the reaction was started by addition of suitable amounts (5-100 μl) of an erythrocyte hemolysate. The erythrocyte lysate was prepared according to the instructions of Mannervik et al. (1982).

d) Protocol for experiments using LNCaP cells (androgen dependent prostate carcinoma cells; DSMZ No ACC 256)

An identical number of LNCaP cells (androgen dependent prostate carcinoma cells; DSMZ No ACC 256) were routinely
5 cultured in 75 cm² culture flasks in RPMI-1640 medium (Gibco; Nr. 21875-034), penicillin/streptomycin (100 units penicillin/ml; 100 µg streptomycin/ml; Gibco; Nr. 15140/122) in the presence of 10% fetal calf serum (Biochrom; Nr. S0113/5; RPMI-FKS). The flasks were incubated at 37°C in a
10 humidified atmosphere (relative humidity >95%) of 5% CO₂ in air. After reaching 50% confluency the medium was removed and the adherent cells were washed twice with PBS (phosphate buffered sodium chloride; 50 mM sodium phosphate, 150 mM NaCl, pH 7,4). Thereafter, the cells were incubated with
15 serum free RPMI-medium (RPMI-SF) comprising the experimental supplements in five flasks each (i.e. five replicates each). The culture was continued at 37°C, 5% CO₂ and 95% humidity for 24 hours. Thereafter the respective supernatants were removed and the adherent cells were detached from the bottom
20 of the plate by trypsin/EDTA (Gibco; No. 25300-054) and were pelleted. After resuspension and homogenization the cells were counted using a hemocytometer.

B. Data

25

Example 1

Inhibition of the enzymatic activity of glyoxalase I of yeast by ethyl pyruvate (EP).

30 Determination of glyoxalase I activity was performed according to the general protocol as described above. The influence of ethyl pyruvate on enzyme activity was investigated by addition of increasing concentrations of EP (Sigma; no. E4,780-8; lot. S18972-513) to the measuring
35 reagent.

The experiments (Fig. 1) show that EP inhibits the reaction of yeast glyoxalase I in a concentration dependent manner.

Example 2

- 5 Inhibition of yeast glyoxalase I by compounds of the general formula (I), (II), and (III), respectively

Determination of glyoxalase I activity was performed according to the general protocol as described above. The
 10 influence of compounds of the general formula (I), (II), and (III) on the activity of the enzyme was investigated by addition of increasing concentrations of these compounds to the preparation. The IC₅₀-values were calculated from
 15 inhibition curves of each compound.

The data (Table 3) show that alkyl 2-oxo-derivatives inhibit the enzymatic activity of yeast glyoxalase I with different IC₅₀s, while the alkyl 2-hydroxy-derivatives revealed no
 20 inhibitory effect at all. Thus, the experiments demonstrate that alkyl 2-hydroxy-derivatives are prodrugs which must be activated to the respective 2-oxo-derivatives in living cells or organisms.

Table 3

25

Alkyl 2-Oxo-Derivatives		IC ₅₀ GLOI*
Ethyl 2-oxobutyrate	CH ₃ -CH ₂ -O-CO-CO-CH ₂ -CH ₃	1,8 mM
Butyl pyruvate	CH ₃ -CH ₂ -CH ₂ -CH ₂ -O-CO-CO-CH ₃	7,5 mM
Ethyl pyruvate	CH ₃ -CH ₂ -O-CO-CO-CH ₃	10 mM
Methyl pyruvate	CH ₃ -O-CO-CO-CH ₃	15 mM
Diethyl oxalate	CH ₃ -CH ₂ -O-CO-CO-OCH ₂ -CH ₃	>50mM
Alkyl-2-Hydroxy Derivatives		
(-)-Butyl L-Lactate	CH ₃ -CH ₂ -CH ₂ -CH ₂ -O-CO-CHOH-	no

	CH ₃	inhibition
(-)-Ethyl L-Lactate	CH ₃ -CH ₂ -O-CO-CHOH-CH ₃	no inhibition
(+)-Ethyl D-Lactate	CH ₃ -CH ₂ -O-CO-CHOH-CH ₃	no inhibition
Methyl L-Lactate	CH ₃ -O-CO-CHOH-CH ₃	no inhibition
(-)-Isopropyl L-Lactate	CH ₃ -CH(CH ₃)-O-CO-CHOH-CH ₃	no inhibition
(+)-Isobutyl L-Lactate	CH ₃ -CH(CH ₃)-CH ₂ -O-CO-CHOH-CH ₃	no inhibition

*GLO I = glyoxalase I

Example 3

Effect of prodrugs

5

The compounds of the general formula (II) and/or (III) can act as prodrug in the sense that the compounds are activated by enzymes within cells or in the organism, or are oxidized in vitro by addition of a suitable oxidant.

10

In cells or organisms lacking such activation systems the compounds of the general formula (II) and/or (III) remain inefficient. This is demonstrated by the effect of EP and LEL on the activity of glyoxalase I and the proliferation of tumor cells as well as yeast cells.

15

Table 4

	Inhibition of enzyme	Inhibition of cell proliferation	
	yeast GLO I	LNCaP	Yeast cells
Ethyl pyruvate	+	+	+
Ethyl L-lactate	-	+	-

The experiment (Table 4) shows that alkyl 2-hydroxy derivatives have to be activated into alkyl 2-oxo-derivatives by endogenous activation systems to inhibit cell proliferation via inhibition of GLO1. In contrast to human tumor cells (LNCaP), yeast cells lack enzyme systems for transformation of alkyl 2-hydroxy derivatives into alkyl 2-oxo-derivatives and therefore proliferation can not be inhibited by alkyl 2-hydroxy derivatives directly.

10 Example 4

Inhibition of the enzymatic activity of yeast glyoxalase I by 2-Oxopropanethioic acid S-ethyl ester (SE).

Synthesis of 2-Oxopropanethioic acid S-ethyl ester

15 In a 500 ml three necked flask equipped with a reflux condenser and dropping funnel N,N'-dicyclohexylcarbodiimide (20.6 g, 0.1 mol; Cat.No. 36650, Lot. RA 13160, Fluka, Germany) was dissolved in dry tetrahydrofuran (200 mL; Cat.No. AE 07.1, Lot. 2121/5CR, Roth, Germany). Then, a
20 solution of ethanethiol (6.2 g, 0.1 mol; Cat.No. EC 200-837-3, Lot. AO200018001, Acros Organics) in tetrahydrofuran (25 mL) was added. Under stirring a solution of 2-oxopropanoic acid (8.9 g, 0.1 mol) in tetrahydrofuran (25 mL) was added dropwise within 10 min without external heating. The solution
25 warmed up to 45°C, turned yellow and a colourless precipitate of N, N'-dicyclohexylurea appeared. After complete addition the suspension was heated to reflux for 3 min and then cooled to 0° C. The urea was filtered off and the solvent removed from the filtrate by distillation. Without other
30 manipulations the remaining orange oily residue was rapidly distilled in the air stream of a heat gun at normal pressure yielding a yellow fraction between boiling point (bp) 145 and 165 °C at normal pressure (amount of crude product 4.0 g, n_D 1.4802 (21 °C)) together with a considerable amount of brown

resin in the distillation flask. The yellow crude product was redistilled in the same manner to yield 2.3 g of 2-oxopropanethioic acid S-ethyl ester as yellow oil, bp 153-157 °C, nD 1.4750 (21 °C), purity > 95 % (NMR).

5

¹H-NMR (300.06 MHz, CDCl₃): δ 1.29 (-CH₂-CH₃), 2.41 (H₃C-CO-), 2.92 (-CH₂-CH₃); ¹³C-NMR (75.45 MHz, CDCl₃): δ 14.2 (-CH₂-CH₃), 23.2 (-CH₂-CH₃), 23.9 (H₃C-CO-), 191.4 (-CO-), 193.4 (-CO-). NMR spectra were measured with a Varian Mercury-300BB spectrometer. Chemical shifts are reported at the δ scale in ppm.

10

Determination of glyoxalase I activity

Determination of glyoxalase I activity was performed according to the general protocol as described above. The influence of 2-oxopropanethioic acid S-ethyl ester on the activity of the enzyme was investigated by addition of increasing concentrations of SE to the preparation for the measurement.

20

The experiment (Fig. 2) shows that SE inhibits the reaction of the glyoxalase I of yeast in a concentration dependent manner.

25 Example 5

Influence of ethyl pyruvate (EP), butyl pyruvate (BP) and butyl L-lactate (BL) on the enzymatic activity of yeast glyoxalase I.

30 Determination of glyoxalase I activity was performed according to the general protocol as described above. The influence of effectors on the enzymatic activity was investigated by addition of increasing concentrations (0 - 30

mM) of EP (Sigma; no. E4,780-8; lot. S18972-513) (triangles), BP (prepared according to the instructions of patent JP 11080089; 98 %) (circles) and BL (Fluka, 69819; lot. 443090/1 21503090) (squares) to the measuring reagent (Fig. 3).

5

The experiments show that EP (triangles) as well as BP (circles) inhibit the activity of glyoxalase I in a concentration dependent manner wherein the effect of BP is stronger than the effect of EP. BL (squares) does not
10 influence the reaction of glyoxalase when it is not transformed into BP.

Example 6

Influence of ethyl pyruvate (EP) on the enzymatic activity of
15 yeast glyoxalase II.

A colony of strain HD65-5a (*Saccharomyces cerevisiae*) was incubated in 5 ml YPD-medium [(2% glucose (Fluka), 1% yeast extract (BD, Sparks), 2% peptone (BD, Sparks)] over night at
20 30°C under rotation. A 10 ml aliquot was added to 200 ml culture medium in a 500 ml glass flask and was incubated at 30°C on a shaker (250 U/min). The yeast cells were harvested in the stationary growth face (O.D. 1cm/600nm = 2-4) by centrifugation (15 min, 3000 x g). The cells were diluted
25 with 0.1 M MES buffer, pH 6,5 to an O.D. of 4 and were then disrupted in a glass mill (Schwack et al., 2004). Subsequently the disrupted cells were centrifuged at 23000 x g, 4°, 30 min. Protein concentration of the cell free extract was determined according to the Bradford method (Bradford,
30 1976).

The activity of the glyoxalase II (Hydroxyacyl glutathione hydrolase, E.C. 3.1.2.6.) was determined according to the

instructions of Martins et al. (1999) in 0.1 M MES buffer, pH 6.5, 1.5 mM S-D-lactoyl-glutathione (L7140, Sigma-Aldrich Chemistry GmbH) and 0.75 mM DTNB (Sigma, D8130). After addition of suitable amounts of cell extract and an incubation period of 15 min at 25°C the formation of glutathione was measured at 412 nm ($\epsilon = 13.6 \text{ mM}^{-1} \text{ cm}^{-1}$). The activity of the glyoxalase II (IU) correlates with the amount of enzyme hydrolysing 1 μmol of S-D-lactoyl-glutathion/min.

10 The influence of ethyl pyruvate on the activity of the enzyme was investigated by adding increasing concentrations of EP (Sigma, No. E4,780-8; Lot. S18972-513) (0-20mM) to the measurement reagent.

15 The relative activities of glyoxylase II in presence or absence of EP are illustrated in Fig. 4. The experiment shows that EP inhibits the reaction of yeast glyoxalase II in a concentration dependent manner.

20 Example 7

Inhibition of the enzymatic activity of glyoxalase I of human erythrocytes by ethyl pyruvate (EP).

Determination of glyoxalase I activity was performed according to the general protocol as described above. The influence of ethyl pyruvate on enzyme activity was investigated by addition of increasing concentrations of EP (Sigma; no. E4,780-8; lot. S18972-513) (0-50mM) to the measuring reagent.

30

The experiment (Fig. 5) shows that EP inhibits the reaction of glyoxalase I of human erythrocytes in a concentration dependent manner.

Example 8

Inhibition of the enzymatic activity of glyoxalase II of human erythrocytes by ethyl pyruvate (EP).

5 Determination of glyoxalase II activity was performed according to the general protocol as described above. The influence of ethyl pyruvate on the activity of the enzyme was investigated by addition of increasing concentrations of EP (Sigma, no. E 4,780-8; lot. S18972-513) (0-20 mM) to the
10 measuring reagent.

The experiment (Fig. 6) shows that EP inhibits the reaction of glyoxalase II in a concentration dependent manner.

15 Example 9

Effect of ethyl D-lactate (DEL), ethyl L-lactate (LEL) and ethyl pyruvate (EP) on the vitality of primary human fibroblasts.

20 An identical number (104 cells per well) of primary human skin fibroblasts were inoculated in 24-well plates (Greiner, no. 662160) and were cultured in DMEM (Gibco; no. 41966-092) in the presence of 10 % calf serum (Biochrom, S0113/5), 2 mM L-glutamine (Gibco; no. 25030-024), penicillin/streptomycin
25 (100 units penicillin/ml; 100 µg streptomycin/ml, Gibco; no. 15140/122), 5 mg% ascorbic acid (Serva, no. 14030.02) at 37°C, 5%CO₂ and 95% humidity. The primary human fibroblasts were prepared according to the instructions of Birkenmeier et al. (1998). After reaching 50 % confluency the medium was by
30 fresh serum free medium. Thereafter the following supplements were added to the cells: preparation 1 (equivalent volume of serum free (SF) medium, blank); preparation 2 (1 mM DEL or 1 mM LEL or 1 mM EP); preparation 3 (5 mM DEL or 5 mM LEL or 5 mM EP), preparation 4 (10 mM DEL or 10 mM LEL or 10 mM EP),
35 preparation 5 (20 mM DEL or 20 mM LEL or 20 mM EP), preparation 6 (50 mM DEL or 50 mM LEL or 50 mM EP). The culture was continued at 37°C, 5% CO₂ and 95% humidity for 24

hours. Thereafter the supernatants were removed and 100 μ l of a 50 % thymol blue solution was added to the wells. After washing the cells with medium the unstained and stained cells were counted under the light optical microscope comprising a coordinate plane. Cells stained blue were assessed as avital, unstained cells as vital. The percentage of unstained cells of the total number of cells corresponds to the vitality of the cells.

10 The experiment (Fig. 7) shows that DEL, LEL and EP are not toxic over the concentration range investigated and that they do not significantly influence vitality of primary human fibroblasts.

15 Example 10

Inhibition of the proliferation of *Trypanosoma brucei* by ethyl pyruvate (EP), ethyl L-lactate (LEL) and butyl L-lactate (BL).

20 The effect of drugs on growth and proliferation of *Trypanosoma brucei* TC 221 was determined by a cytotoxicity and proliferation assay measuring the reduction of the tetrazolium dye AlamarBlue™ (Sigma Resazurin Salt R-7017) (O'Brien et al., 2000). The medium used throughout the test was Baltz-Medium (Baltz et al., 1985) without phenol red supplemented with FCS (20%), penicillin (100 μ g/ml) and streptomycin (100 U/ml). A volume of 160 μ l medium was placed into the cavities of 96-well plates (Nunc) and 20 μ l trypanosomes stock containing 10^5 parasites/ml were added.

30 Increasing concentrations of EP, LEL or BL in a volume of 20 μ l were added to the parasites to have final concentrations between 0.156 mM to 20 mM, respectively. Samples were then incubated in a humidified atmosphere at 37°C, 5% CO₂ for 24h. Control samples contained no test substances, blanks

35 contained no cells. After that 20 μ l of AlamarBlue® reagent was added and the incubation was continued for 24h and 48h. Finally, absorbance at 550/630 nm was determined and

corrected for the blank value. For the compounds tested, GI₅₀-values (the concentration of a compound at which only 50% of living cells are found compared to the control) were calculated from cell growth curves. Data are given in Table 5.

As positive control pentamidine (Sanofi-Aventis) was used. All experiments were done in triplicates.

The data show that EP, LEL and BL inhibit the growth of parasites with GI₅₀-values in the range between 3.6 and 10.3 mM.

Table 5:

Test No.	GI ₅₀ - values (mM)	Mean GI ₅₀ value (mM)
EP-1	3,86	3,66
EP-2	3,71	
EP-3	3,4	
LEL-1	14,1	14,52
LEL-2	14,4	
LEL-3	15,06	
BL-1	10,16	10,29
BL-2	10,2	
BL-3	10,5	

Example 11:

Pharmaceutical composition for infusion

A solution for infusion comprising the substances of the invention is prepared as follows:

The compound of the invention, e.g. sterile ethyl pyruvate and/or ethyl lactate, is mixed with sterile 250 ml Lactated Ringers Balanced Salt Solution, pH 7.5, to achieve a final concentration of 0.05% to 10% per volume, e.g. 0.05%, 0.5%, 1%, 5%, or 10%, per volume. The pH of the solution is

adjusted to 7.5 with NaOH, if necessary. After sterilization, the solution is packed in plastic containers and stored at 4°C. The composition of lactated Ringers Balanced Salt Solution is as follows:

5		
	Sodium	130 mM,
	Calcium	3,7 mM,
	Potassium	5,4 mM,
	Chloride	111,7 mM
10	Lactate	27,2 mM.

Example 12:

Pharmaceutical composition for bolus injection

- 15 A solution for bolus injection can be prepared according to Example 11, wherein the concentration of the substance of the invention is adapted accordingly.

Example 13:

20 Cream

A cream comprising a substance of the invention is prepared from the following ingredients:

	aqueous phase:	butyleneglycol	4%
25		substance of the invention	25%
		water	to 100%
	lipid phase:	steareth-2	3%
		steareth-21	2%
		glycol-15-stearylether	9%
30		cetearylalcohol	2,5%
	thereafter addition of:		
		phenoxyethanol, methylparaben,	
		ethylparaben, propylparaben,	
		butylparaben	0,5%
35		butylenglycol	0,5%
		tocopherole	0,2%

Example 14:

Ointment

An ointment of the oil-in-water-emulsion type, comprising a compound of the invention is prepared from the following ingredients.

10	A	product of the invention	10-20%
		butyleneglycol	5%
		glycerol	4%
		sodium dihydroxy cetylphosphate,	
		isopropyl hydroxy cety ether	2%
		water	to 100%
15	B	glycolstearate SE	15%
		octylcocoate	11%
	C	butyleneglycol, metylparabene	
		ethylparabene, propylparabene,	
		pH: adjusted to 5,5	2%

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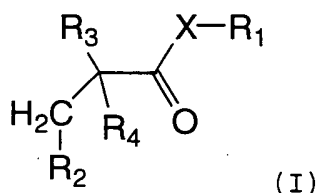
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List of abbreviations

	AIDS	Acquired immunodeficiency syndrom
	ATP	Adenosine triphosphate
	ATCC	American Type Culture Collection
5	BBB	Blood Brain Barrier
	BL	butyl lactate
	BP	butyl pyruvate
	CNS	Central nervous system
	DBL	butyl D-lactate
10	DEL	Ethyl D-lactate
	DMEM	Dulbecco's modified Eagle Medium
	DMSO	dimethyl sulfoxide
	DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
15	DTNB	5,5'-dithiobis(2-nitro benzoic acid)
	ECACC	European Collection of Cell Cultures
	E.C.	Enzyme Commission
	EDTA	ethylene diamine tetraacetate
	EOB	ethyl -2-oxo-butyrat
20	EP	ethyl pyruvate
	FCS	fetal calf serum
	GLO I	Glyoxalase I
	HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
	HIV	human immunodeficiency virus
25	IC-50	Inhibitor concentration to reach 50% of the maximal effect
	IU	international unit
	LBL	Butyl L-lactate
	LEL	Ethyl L-lactate
30	MES	4-morpholine ethanesulfonic acid
	MCT	monocarboxylate transporter
	MIC	Minimal inhibition concentration
	O.D.	optical density
	PBS	phosphate buffered saline
35	RPMI	Roswell Park Memorial Institute
	SF	serum free
	YPD	yeast peptone dextrose medium

Claims

1. Substance according to the general formula (I)



for use against parasitic diseases, wherein the parasite is selected from protozoa or helminthes; wherein

X is O or S; and

R₁ is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and

R₂ is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and

R₃ and R₄ together are =O,

or R₃ is OH and R₄ is H; or R₃ is H and R₄ is OH,

with the proviso that when the disease is cerebral malaria, R₃ or R₄ is OH;

and when the parasite is a parasite of fish, X is O, and R₃ and R₄ together are =O, R₁ is not methyl, ethyl, propyl or butyl.

2. The substance according to claim 1, wherein R₁ comprises 1 to 8 carbon atoms and R₂ is H or comprises 1 to 8 carbon atoms.

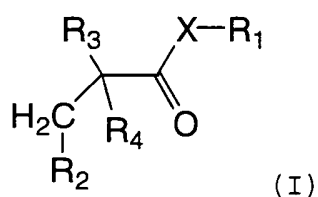
3. The substance according to claim 1 or 2, wherein R1 comprises 1 to 4 carbon atoms and R2 is H or comprises 1 to 4 carbon atoms.

4. The substance according to any one of claims 1 to 3, wherein R1, R2, or R1 and R2 are selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

5. The substance according to any one of claims 1 to 4, wherein said substance is one or more selected from methyl pyruvate, ethyl pyruvate, isopropyl pyruvate, butyl pyruvate, isobutyl pyruvate, ethyl 2-oxobutyrate, butyl-2-oxobutyrate, cyclohexylmethyl pyruvate or the said compound wherein X = S.

6. The substance according to any one of claims 1 to 4, wherein R3 or R4 is OH and it is selected from the group comprising the D- enantiomer, L- enantiomer, and the racemic mixture thereof.

7. Substance according to the general formula (I)



wherein X is O or S; and

R1 is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and

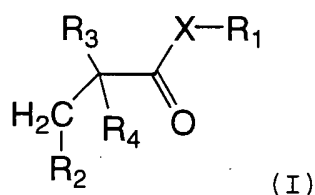
R2 is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and

R3 and R4 together are =O,

or R₃ is OH and R₄ is H; or R₃ is H and R₄ is OH

for use against protozoa comprised in biofilms.

- 5 8. Method for treating water, comprising contacting water with a substance according to the general formula (I)



wherein

- 10 X is O or S; and

R₁ is a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and

- 15 R₂ is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and

R₃ and R₄ together are =O,

- 20 or R₃ is OH and R₄ is H; or R₃ is H and R₄ is OH, with the proviso that when X is O, and R₃ and R₄ together are =O, and R₁ is selected from methyl, ethyl, propyl or butyl, said treatment is not for the treatment of parasites of fish.

- 25 9. The method according to claim 8 wherein said water is selected from drinking water or water for industrial use.

10. The method according to claims 8 or 9, wherein said treatment is for preventing colonisation by protozoa, comprising one or more selected from Giardia and
30 Cryptosporidium.

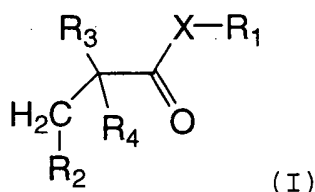
11. The method according to any one of claims 8 to 10, wherein said method is for combating protozoa, comprising one or more selected from Giardia and Cryptosporidium.

5 12. The method according to any one of claims 8 to 11 which is to prevent or combat biofouling comprising protozoa.

13. The method according to any one of claims 8 to 12, wherein when X is O, and R3 and R4 together are =O, R1 is not
10 methyl, ethyl, propyl or butyl.

14. Pharmaceutical composition comprising at least one substance according to the general formula (I)

15



for the treatment and/or prophylaxis of parasitic diseases,
20 wherein the parasite is selected from protozoa or helminthes in an animal; wherein

X is O or S; and

R1 is a branched or non-branched alkyl, cycloalkyl, branched
25 or non-branched alkenyl, cycloalkenyl, branched or non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl, aryl or a sugar residue; and

R2 is H or a branched or non-branched alkyl, cycloalkyl, branched or non-branched alkenyl, cycloalkenyl, branched or
30 non-branched alkynyl, cycloalkynyl, alkoxyalkyl, alkoxycarbonylalkyl or aryl residue; and

R3 and R4 together are =O; or R3 is OH and R4 is H; or R3 is H and R4 is OH

with the proviso, that when the disease is cerebral malaria, R3 or R4 is OH;

and when the parasite is a parasite of fish, X is O, and R3 and R4 together are =O, R1 is not methyl, ethyl, propyl or butyl.

15. The pharmaceutical composition according to claim 14, wherein R1 comprises 1 to 8 carbon atoms and R2 is H or comprises 1 to 8 carbon atoms.

16. The pharmaceutical composition according to claim 14, wherein R1 comprises 1 to 4 carbon atoms and R2 is H or comprises 1 to 4 carbon atoms.

17. The pharmaceutical composition according to any one of claims 14 to 16, wherein R1, R2, or R1 and R2 are selected from the group comprising methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

18. The pharmaceutical composition according to any one of claims 14 to 17, wherein said substance is one or more selected from methyl pyruvate, ethyl pyruvate, propyl pyruvate, isopropyl pyruvate, butyl pyruvate, isobutyl pyruvate, ethyl 2- oxobutyrate, butyl-2-oxo-butyrate, cyclohexylmethyl pyruvate or the said compounds wherein X = S.

19. The pharmaceutical composition according to any one of claims 14 to 17, wherein in said substance R3 or R4 is OH, and it is selected from the group comprising the D-enantiomer, L- enantiomer and the racemic mixture thereof.

20. The pharmaceutical composition according to any one of claims 14 to 19, wherein said substance according to formula (I) is present in a therapeutically effective concentration.

21. The pharmaceutical composition according to any one of claims 14 to 20, which comprises one or more additional pharmaceutically active ingredients.

5 22. The pharmaceutical composition according to claim 21, wherein said additional pharmaceutically active ingredient is selected from anti parasitic agents.

23. The pharmaceutical composition according to claim 22,
10 wherein said anti parasitic agent is one or more selected from to clomipramine, suramin, melarsoprol, eflornithine, or nifurtimox, benzimidazole, ivermectin, abendazole, nitroimidazole, metronidazole, pyrimethamine, chloroquine, amodiaquine, miltefosine, paromomycin, primaquine and
15 tafenoquine.

24. The pharmaceutical composition according to any one of claims 14 to 23, which further comprises one or more auxiliary substances, comprising fillers, flavouring agents
20 and stabilizers.

25. The pharmaceutical composition according to any one of claims 14 to 24, wherein said composition is in the form of a sustained release or controlled release galenic formulation.

26. The pharmaceutical composition according to any one of claims 14 to 25, which is for topic or systemic administration.

30 27. The pharmaceutical composition according to any one of claims 14 to 26, which is for one or more selected from the group comprising oral, intravenous, subcutaneous, intramuscular, intradermal, intraperitoneal, rectal, intranasal, intraauricular, epidural, percutaneous,
35 transdermal, pulmonary administration, or administration as an aerosol, via mini-pumps, as mouth lavage, cream, ointment, spray, gel, plaster, and via microbubbles.

28. The pharmaceutical composition according to any one of claims 14 to 27, which is for use as a food supplement and/or beverage supplement.

5

29. The pharmaceutical composition according to any one of claims 14 to 28, wherein said animal is selected from non-mammalian vertebrates, and mammals including humans.

10 30. The pharmaceutical composition according to any one of claims 14 to 29, wherein said parasites are resistant to conventional treatment.

31. The pharmaceutical composition according to claim 30,
15 wherein said resistance is to clomipramine, suramin, melarsoprol, eflornithine, or nifurtimox. chloroquine, amodiaquine, miltefosine, paromomycin, primaquine and tafenoquine, benzimidazole, macrocyclic lactones, levamisole, mebendazole, thiabendazole, diethylcarbazine.

20

32. The pharmaceutical composition according to any one of claims 14 to 31, which is against one or more parasites belonging to the genus Trypanosoma, Leishmania, Plasmodium, Toxoplasma, or is against helminthes comprising one or more
25 selected from echinococcus, tremadodes, zestodes, trichinella, ascaris, and schistosoma.

33. The pharmaceutical composition according to any one of claims 14 to 32, wherein said infection is an opportunistic
30 infection.

34. The pharmaceutical composition according to any one of claims 14 to 33, which is for use in an immunosuppressed animal.

35

35. The pharmaceutical composition according to claim 34, wherein said immunosuppression is associated with hereditary or acquired immune-defects.

5 36. The pharmaceutical composition according to claim 35, wherein said acquired immune defect is associated with HIV, organ transplantation, chemotherapy or exposure to radiation.

10 37. The pharmaceutical composition according to any one of claims 14 to 36, which is for use in an animal, including man, concomitantly suffering from a bacterial or fungal infection.

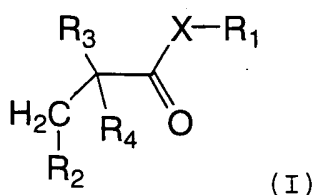
15 38. The pharmaceutical composition according to any one of claims 14 to 37, which is for use in an animal, including man, having a reduced blood glucose level.

20 39. The pharmaceutical composition according to any one of claims 14 to 38, which is for use in an animal, including man, concomitantly suffering from cancer.

25 40. The pharmaceutical composition according to any one of claims 14 to 39, wherein said animal, including man, is going to receive, is currently receiving, or has received conventional cancer therapy.

30 41. The pharmaceutical composition according to claim 40, wherein said conventional cancer therapy is one or more of chemotherapy, surgery, radiotherapy or brachytherapy.

42. Use of a substance according to the general formula (I)



for the preparation of a medicament for the treatment of
 parasitic diseases, wherein the parasite is selected from
 5 protozoa or helminthes; wherein

X is O or S; and

R1 is a branched or non-branched alkyl, cycloalkyl, branched
 or non-branched alkenyl, cycloalkenyl, branched or non-
 10 branched alkynyl, cycloalkynyl, alkoxyalkyl,
 alkoxycarbonylalkyl, aryl or a sugar residue; and

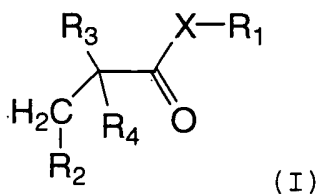
R2 is H or a branched or non-branched alkyl, cycloalkyl,
 branched or non-branched alkenyl, cycloalkenyl, branched or
 non-branched alkynyl, cycloalkynyl, alkoxyalkyl,
 15 alkoxycarbonylalkyl or aryl residue; and

R3 and R4 together are =O; or R3 is OH and R4 is H; or R3 is
 H and R4 is OH

with the proviso, that when the disease is cerebral malaria,
 R3 or R4 are OH;

20 and when the parasite is a parasite of fish, X is O, and R3
 and R4 together are =O, R1 is not methyl, ethyl, propyl or
 butyl.

43. Method of treatment comprising administering a
 25 therapeutically effective amount of at least one substance
 according to the general formula (I)



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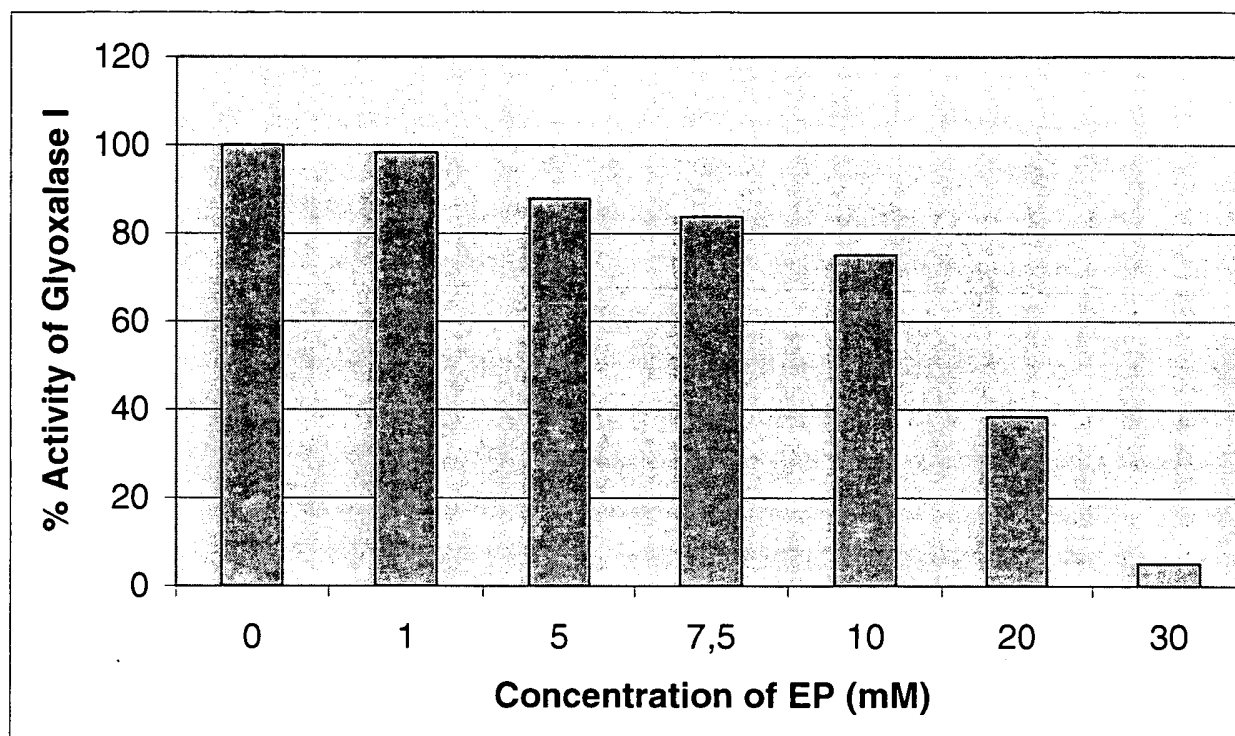
wherein X is O or S; and

R1 is a branched or non-branched alkyl, cycloalkyl, branched
 or non-branched alkenyl, cycloalkenyl, branched or non-

- branched alkynyl, cycloalkynyl, alkoxyalkyl,
alkoxycarbonylalkyl, aryl or a sugar residue; and
R2 is H or a branched or non-branched alkyl, cycloalkyl,
branched or non-branched alkenyl, cycloalkenyl, branched or
5 non-branched alkynyl, cycloalkynyl, alkoxyalkyl,
alkoxycarbonylalkyl or aryl residue; and
R3 and R4 together are =O; or R3 is OH and R4 is H; or R3 is
H and R4 is OH.
- 10 to a mammal including humans in need thereof, wherein said
mammal is suffering from a parasitic disease, wherein the
parasite is selected from protozoa or helminthes,
with the proviso, that when the disease is cerebral malaria,
R3 or R4 are OH;
- 15 and when the parasite is a parasite of fish, X is O, and R3
and R4 together are =O, R1 is not methyl, ethyl, propyl or
butyl.

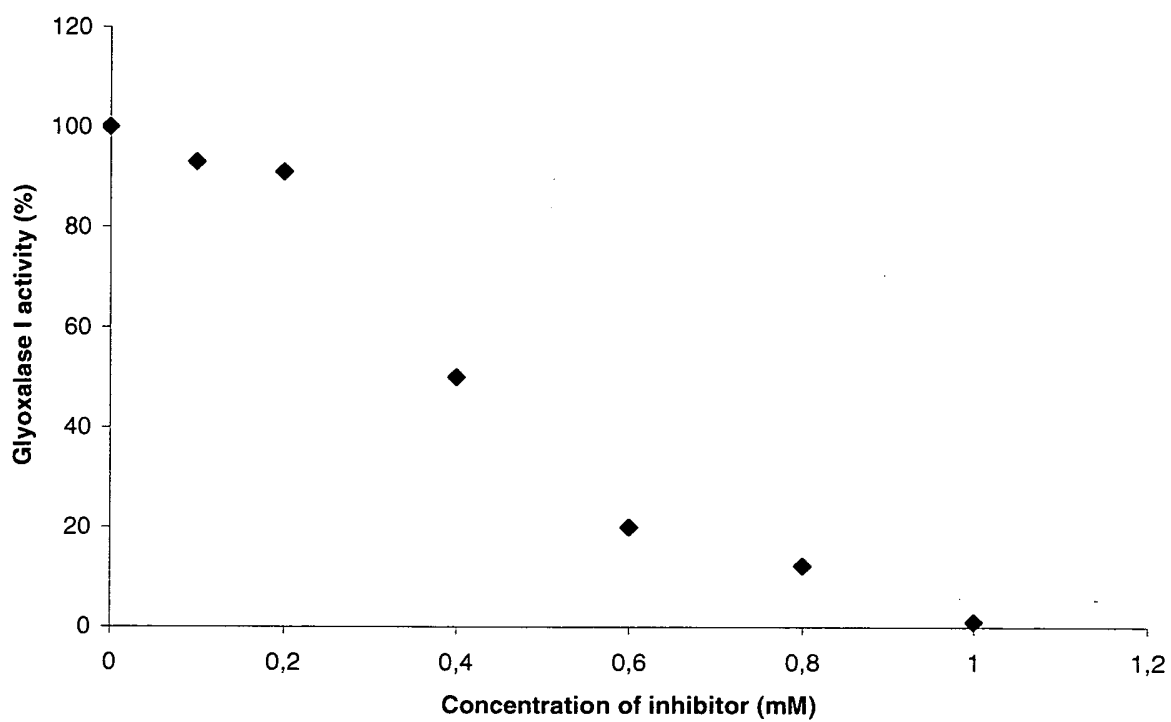
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Fig. 1



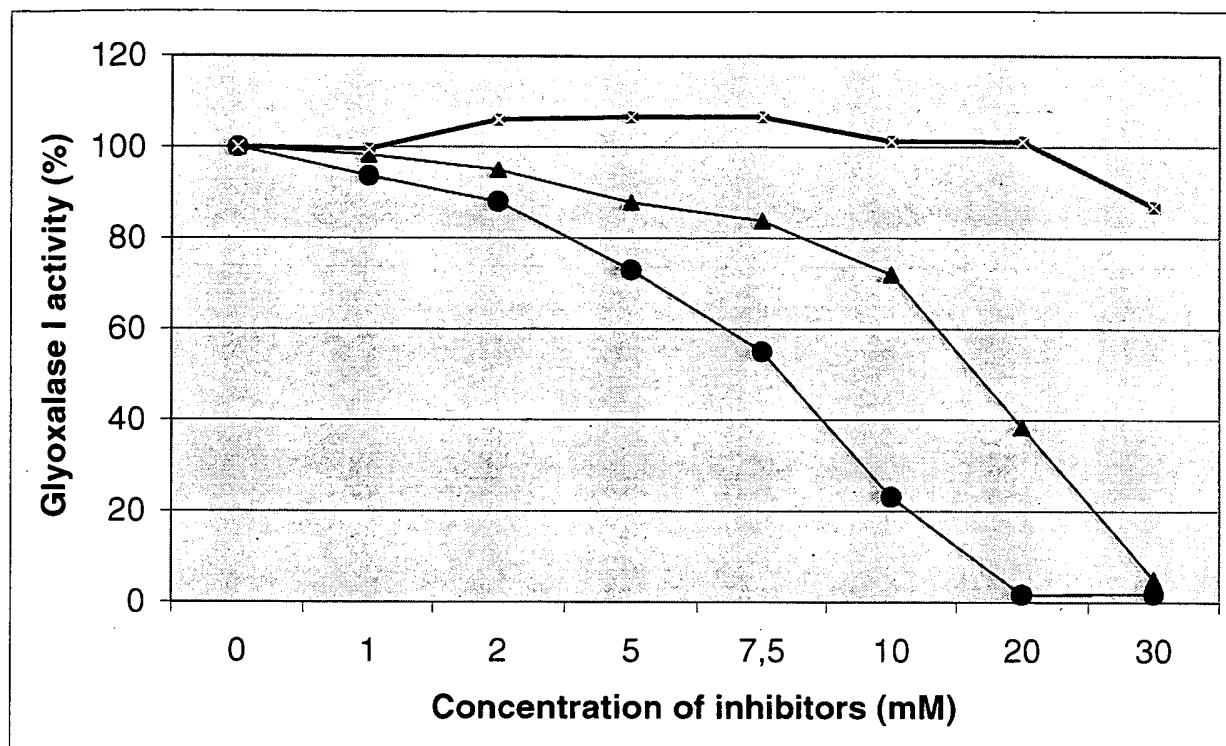
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Fig. 2



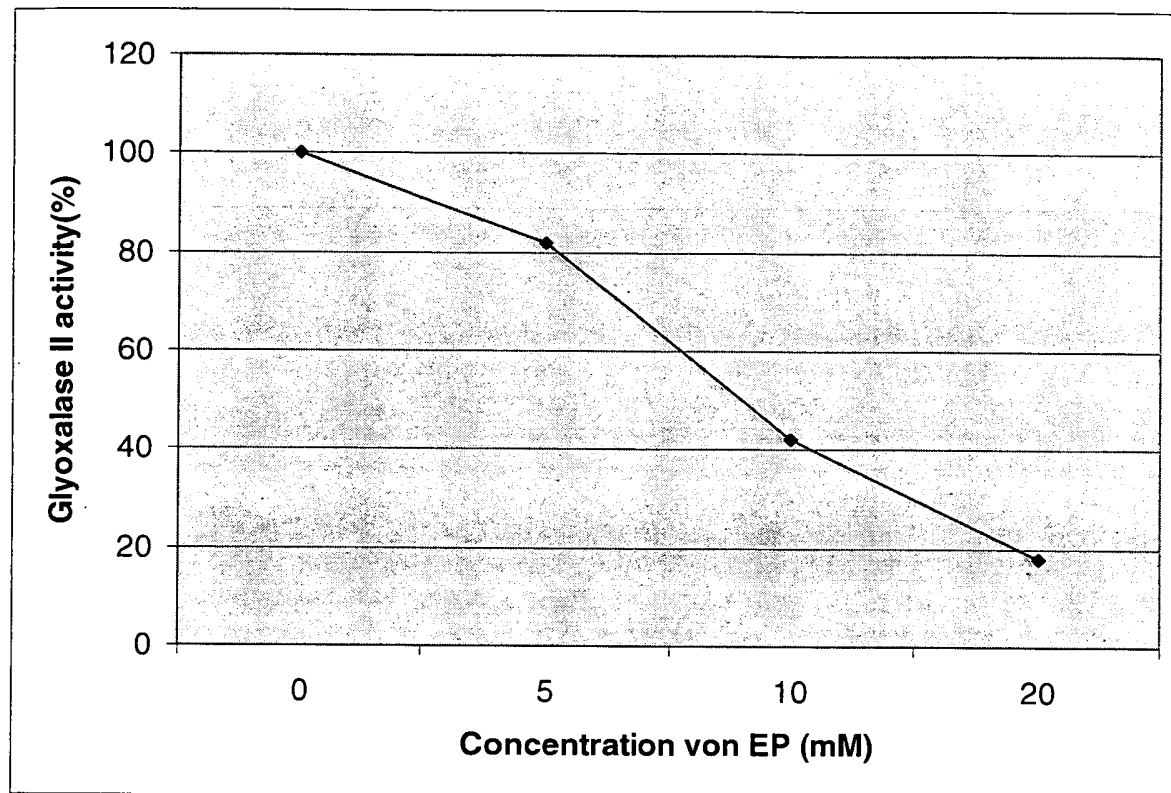
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Fig. 3



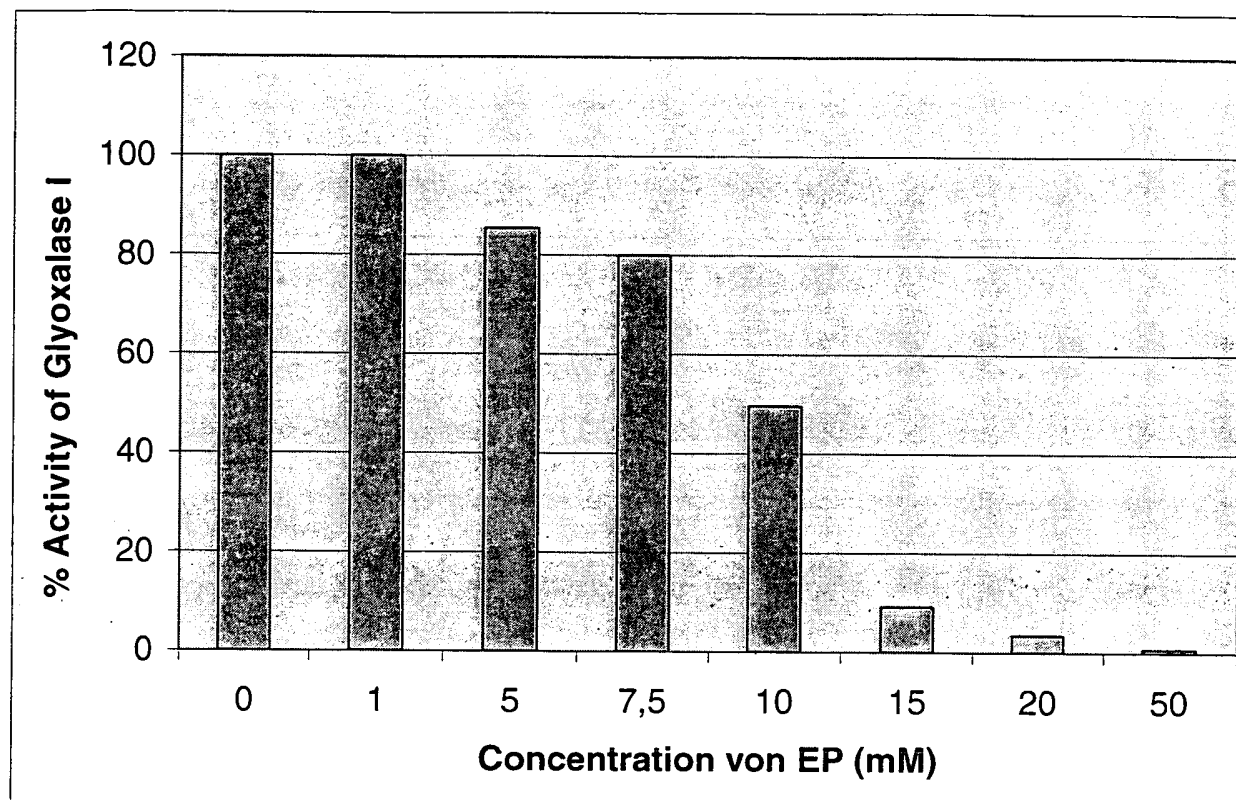
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Fig. 4



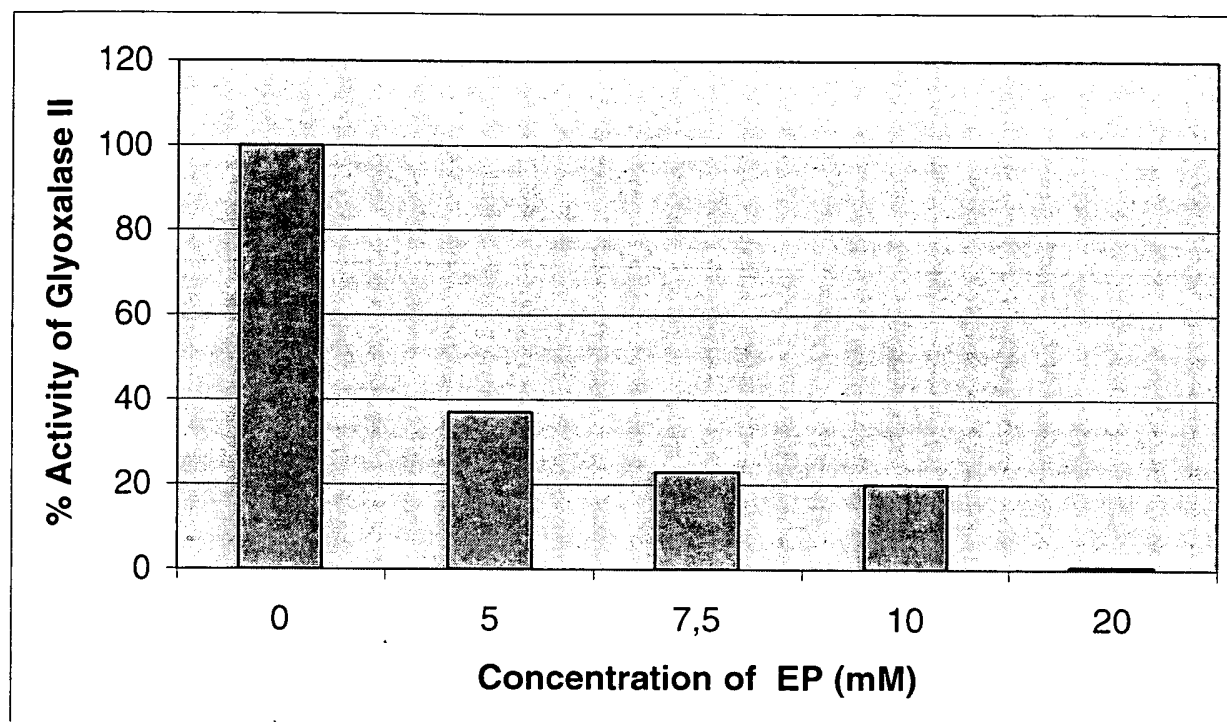
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Fig. 5



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Fig. 6



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Fig. 7

