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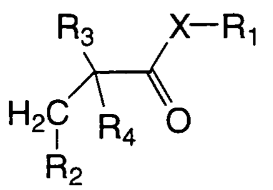
(54) **Title:** SUBSTANCES AND PHARMACEUTICAL COMPOSITIONS FOR THE INHIBITION OF GLYOXALASES AND THEIR USE AS ANTI-FUNGAL AGENTS



(I)

(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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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. 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 anti-fungal effects of a substance of formula (I). 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 fungal infection.

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Substances and pharmaceutical compositions for the inhibition of glyoxalases and their use as anti-fungal agents

FIELD OF THE INVENTION

The invention relates to compounds of the general formula (I) as an anti-fungal agent. 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 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).

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 formation of oxo-aldehydes such as methylglyoxal, increased activity of glyoxalase I and/or II activity and enhanced cell growth/proliferation. In one embodiment, the disease is a fungal infection.

BACKGROUND OF THE INVENTION

A multitude of fungi which do not cause any problems live on and partially in the body of all human beings and animals. About 100 of the more than 100.000 species of fungi described so far are known to be capable of causing disease in human beings and animals. Moreover, many fungi are known to infect and damage plants.

Fungal infections, in particular when they remain superficial and locally circumscribed, such as e.g. athletes foot, can be relatively benign. Nevertheless, such infections can cause discomfort and require treatment.

Systemic fungal infections are life threatening diseases that are difficult to treat with available medication.

Many fungal infections (mycosis) are the result of an opportunistic infection on the basis of a weakened immune defence. The weakening of the immune defence is often the result of chemotherapy, an organ transplantation accompanied by immune suppression or an HIV infection. In such situations, fungi populating the mucosa (mucosal fungi) can enter the blood or vessels and thus can become highly dangerous for the patient (systemic mycosis). The most common fungi that are pathogenic to human beings are species of Candida and Aspergillus.

In Germany alone, about 40.000 people are affected by an invasive Candida infection each year. Amongst hospital infections this yeast is in the meanwhile ranked fourth amongst the most dangerous pathogens and necessitates high costs for therapy. Antimycotic therapy also has a high significance for the reduction of candidiasis and aspergillosis in case of transplantations (Higashiyama and Kohno, 2004). Fungal infections represent the main cause of death of recipients of bone marrow transplantation. About half of all recipients of transplants fall ill with a systemic fungal infection and about one third dies due to a non controllable infection (Epstein et al., 2003).

Candida belongs to yeasts. The transmittal of yeasts is possible via contaminated food but also by direct contact with other human beings and animals, particularly pets. Besides the affection of the mucosa in mouth, esophagus or vagina, a Candida infection can also result in a spread into liver, kidney, spleen and other organs. Candida albicans is the most commonly found species, but lately Candida glabrata and Candida krusei are found more often in isolates, often exhibiting resistance against common antifungal agents.

Apart from infections by Candida, Aspergillus spp. (moulds), in particular Aspergillus fumigatus, can cause the life threatening disease invasive aspergillosis. The fungus grows from the lung, the location of the primary infestation following the inhalation of fungal spores, into the tissue and can than affect all other organs of the body. Only modest possibilities are available for therapy of an invasive infection by A. fumigatus. Consequently, the disease leads to the death of the patient in more than 70 percent of the cases. But even if the infection spreads only on the surface of the body, the fungal infections are not only unpleasant but also dangerous in such location. Fungi take in glucose from the blood with their radices and segregate partially toxic metabolites, so called mycotoxins, damaging the liver. Thus, fungal infections have to be treated.

Fungal infections also represent a big problem in animals (e.g. farm animals, birds, fish, small animals and zoo animals as well as invertebrates like bees) where they can cause diseases that have to be treated.

Fungi, like their hosts, are eukaryotic organisms and thus, they are considerably more difficult to combat without compromising the human or animal organism as compared to bacteria. The treatment of Candida infections is particularly difficult as this microorganism is particularly flexible and permanently changes its appearance. In doing so it changes from a small spheroidal cell form, the yeast type, to a long multi cellular filaments, the hypha form. In particular this hypha form is particularly dangerous as it is able to penetrate tissue.

The treatment of fungal diseases often takes a long time. The duration of treatment depends on the type of fungus causing the infection. Therefore, therapy is accompanied by the identification of the fungus and the description of its characteristics, in particular its resistance against antifungal agents, in laboratory experiments.

Antifungal agents (antimycotica) can in principle have two different effects. They can lead to inhibition of fungal proliferation and thus act fungistatically. But they can also have fungicidal action and thus, exert a killing effect on the fungi. Most of the known antifungal agents interfere with the synthesis of the substance ergosterol, which is an important component of the fungal cell membrane. Ergosterol synthesis takes place in several steps, wherein different antifungal agents can interfere with different stages of synthesis. The interference with the ergosterol synthesis can lead to inhibition of fungal growth and can even lead to killing of the fungi.

Azole derivatives inhibit the biosynthesis of ergosterol, an important component of the fungal cell membrane, by interfering with the cytochrome P450 system. The effect of most azole derivatives is fungistatic, some also have fungicidal properties. Azole-derivatives are mainly used locally but are also suitable for systemic treatment. In this context one must pay attention that the active ingredients must not reach the bone tissue or the central nervous system.

Locally applied active ingredients are e.g. clotrimazole, bifonazole and econazole. All three substances are effective against yeasts, moulds and dermatophytes.

The systemic intake is oral or in form of injections or infusions. Indications are for example systemic mycosis, onychomycosis and severe mucocutanic Candida mycosis, in particular in case of AIDS and immune defects. For this purpose medicaments like fluconazole, ketoconazole and others are available. Amongst experts fluconazole these days represents the agent of first choice when Candida is detected in blood culture even if the species is not identified yet and the test for resistances is still missing.

Inhibitors of squalene epoxidase also interfere with ergosterol synthesis. Their effect is fungistatic. In case of dermatophytes, these substances are also fungicidal. Terbinafine and naftifine belong to these medicaments.

Medicaments belonging to the group of morpholine derivatives (amorolfine) are preferably used for the treatment of dermatophyte diseases.

The polyene derivatives form complexes (bonding) with sterols of the fungal membranes and thus disturb membrane functions. In case of the systemic administration of polyene derivatives it is problematic that the fungal sterols are very similar to sterols of the human cell membrane. Therefore, only such polyene derivatives can be used for a systemic treatment that have only a low binding capacity to the human scaffolds, like cholesterol, to avoid damaging these substances. The most widely used agents are amphotericin B and nystatin.

Amphotericin B is a broadband antimycotic agent whose effect and function is amply described in patent US 2,908,611. However, its use is limited by its high kidney, liver and myelotoxicity. Because of its poor solubility it can be applied in form of colloids or liposome formulations (US 4,663,167; EP 0 421 733).

The prophylactic and therapeutic treatment of Candida infections by administration of probiotic agents is also known. Such probiotics contain lactate producing bacteria, which bacteria, or their metabolites, respectively, are thought to be responsible for the curative effect (WO99/17788; Perdigon et al., 1990). In particular, an immune stimulatory effect is presumed, which is desirable for defence reactions against fungal diseases (De Simone et al., 1993). Such an antimycotic therapy is indicated after administration of antibiotics against bacterial infections, as Candida pathogens damage the mucosa and suppress the immune defence.

Further, so called non-classical antimycotics have different mechanisms of action. US 6,414,035 describes the use of polyols like mannitol, sorbitol, or xylitol for the treatment and prophylaxis of fungal diseases of the mucosa.

WO97/07802 describes the use of an inhibitor of chitin synthesis (nikkomycin Z) against Candida pathogens.

In a further patent the use of complexing substances is recommended to combat fungi (GB 2033220). Complexing substances deprived fungi of zinc ions, required for their metabolism.

New approaches of antimycotic therapy are based on activating the body's own defence. By activation of human dendritic cells by fungal proteins or nucleic acids a cellular immune response is meant to be caused. The method has the disadvantage of being species specific (Bozza et al., 2004) and not having a broad antimycotic reaction.

A general problem in using antimycotics is the development of resistances (Sanglard and Odds, 2002). Resistances are closely related to the number of AIDS cases, bone marrow transplantations and chemotherapies (Randhawa, 2000). Candida glabrata and C. krusei are often resistant against fluconazole (Marr, 2004). Occurrence of resistance is attributed to increased expression of ABC-transporters transporting the medicament fluconanzole back out of the fungal cell (Bennett et al., 2004).

Different tests are available for the determination of fungal resistance. Amongst others, the standardized disc test according to Kirby-Bauer (Qin et al., 2004) or the determination of the minimal inhibition concentration (MIC) by VITEK 2 (bioMerieux, Marcy l'Etoile, France) are used for this purpose. The disc test according to Kirby-Bauer is based on the impregnation of filter discs and measuring of the corona of inhibition after applying the filters to inoculated agar plates. For the MIC test dilution series of the test substance are added to the nutrition medium containing defined concentrations of the fungus to be tested. The lowest inhibitor concentration which inhibits growth of the microorganism is determined. The tests should be in accordance with the NCCLS-guidelines (NCCLS, 1997).

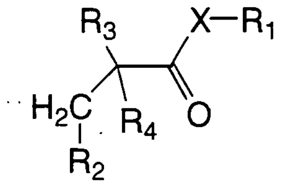
Some antimycotics show severe side effects, in particular in the context of long term or high dose intake (Schwarze et al., 1998). In particular the administration of amphotericin B can be accompanied by angioedema, allergies, shaking chill, want of appetite, nausea, vomiting, partially severe anaemia, kidney and liver dysfunction, anaphylactic shock, loss of hearing or tinnitus. The application of antimycotics together with cisplatin, pentamidine, aminoglycosides can cause severe kidney damage.

New options for therapy are therefore more than desirable, in particular as amongst pathogenic fungi antibiotic resistance is increasing. For example, amongst the Candida species an increasing number of C. glabrata and C. krusei strains is resistant primarily to fluconazole (13th European Congress of Clinical Microbiology and Infectious Diseases, Glasgow, June 2003).

SUMMARY OF THE INVENTION

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

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



(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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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 as an anti-fungal agent, in particular for the treatment of fungal infections
with the proviso that when X=O, R2 is H and R3 or R4 is OH, R1 is not C1-C12 alkyl.

The anti-fungal effects encompass fungicidal and fungistatic action.

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 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 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.

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

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-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 fungal infection.

In one embodiment, the pharmaceutical composition or medicament comprises one or more additional pharmaceutically active ingredients. Such ingredients can be selected from antifungal agents, such as one or more selected from fluconazole, ketoconazole, clotrimazole, bifonazole, econazole, itraconazole, terbinafine, naftifine, amorolfine, amphotericin B, nystatin, and nikkomycin, miconazolemiconazole, oxiconazole, sulconazole, flucytosine , griseofulvin, and biocidal peptides including defensines. 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 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 is for topic or systemic administration, more particularly, for oral, intravenous, subcutaneous, intramuscular, intradermal, intraauricular, intraperitonal, rectal, intranasal, epidural, percutanous, transdermal, or pulmonary administration, or for administration as an aerosol, via mini-pumps, as mouth lavage, oil, cream, ointment, spray, gel, plaster, and/or via microbubbles. The pharmaceutical composition or medicament 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 fungal infections in animals, including invertebrates, non-mammalian vertebrates, mammalians and humans. The fungal infection can be resistant to antibiotic treatment, such as fluconazole, and may be an opportunistic infection. The fungal infection may be caused by one or more selected from the list comprising Candida spp., Aspergillus spp., Cryptococcus spp., Pneumocystis spp., Zygomyces spp., Dermatophytes, Blastomyces spp., Histoplasma spp., Coccidoides spp., Sporothrix spp., Microsporidia spp, Malassezia spp and Basidiomycetes. Some of these infectious diseases may be an opportunistic infection, and/or may be characterized by antibiotic resistance.

In one embodiment, the infection is in an immunosuppressed animal, 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.

In one embodiment, the animal is concomitantly suffering from a bacterial or protozoal infection or worms, such as Trypanosoma, Leishmania, Plasmodium, Toxoplasma, helmithes, Acrobacter, Actinobacillus, Actinomyces, Bacteroides, Brucella, Clamydia, Clostridium, Campylobacter, Escherichia, Enterobacter, Enterococcus, Eubacterium, Fusobacterium, Helicobacter, Hemophilus, Legionella, Listeria, Mycobacteria, Mycoplasma, Neissaria, Pasteurella, Peptostreptococcus, Pneumococcus, Pneumocystis, Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella, Spirochetes, Staphylococcus, 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.

The substances of the invention can also be used for anti-fungal applications related to plants, including the treatment of fungal infections in plants. Moreover, the invention relates to contacting substrates comprising glass, metal, plastic or wood with the substances of the invention as anti-fungal agents, or incorporating the substances into compositions, wherein they act as anti-fungal agents.

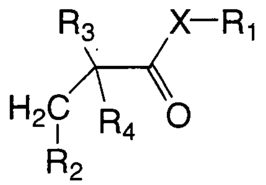
It is to be understood that all embodiments described in the context of substances, pharmaceutical compositions or medicaments of the invention equally apply to methods of treatment, and vice versa. Thus, the mentioning of a particular embodiment in the context of one or more of a substance, pharmaceutical 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),



(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 alkinyl, 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 alkinyl, 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, R1 is not C1-C12 alkyl when X=O, R2 is H and R3 or R4 is OH.

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

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 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 pyrvate
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 pyrvate
Cyclopentyl methyl	H	=O	=O	Cyclopentylmethyl pyruvate
Alkenyl residues				
Vinyl	H	=O	=O	Ethenyl pyruvate
Allyl	H	=O	=O	Propenyl pyruvate
Butenyl	H	=O	=O	Butenyl pyruvate
Pentenyl	H	=O	=O	Pentenyl pyruvate
Hexenyl	H	=O	=O	Hexenyl pyruvate
Heptenyl	H	=O	=O	Heptenyl pyruvate

Octenyl	H	=O	=O	Octenyl pyruvate
Branched Alkenyl				
Isopropenyl	H	=O	=O	Isopropenyl pyruvate
Isobutenyl	H	=O	=O	Isobutenyl pyruvate
Isopentenyl	H	=O	=O	Isopentenyl pyruvate
Isohexenyl	H	=O	=O	Isohexenyl pyruvate
Isoheptenyl	H	=O	=O	Isoheptenyl pyruvate
IsooctenyL	H	=O	=O	Isooctenyl pyruvate
Cycloalkenyl				
Cyclohexenyl	H	=O	=O	Cyclohexenyl pyruvate
Cyclohexenyl methyl	H	=O	=O	Cyclohexenylmethyl pyruvate
Cyclo pentenyl	H	=O	=O	Cyclopentenyl pyruvate
Cyclopenteny lmethyl	H	=O	=O	Cyclopentenylmethyl pyruvate
Alkinyl				
Ethinyl	H	=O	=O	Ethinyl pyruvate
Propinyl	H	=O	=O	Propinyl pyruvate
Butinyl	H	=O	=O	Butinyl pyruvate
Pentinyl	H	=O	=O	Pentinyl pyruvate
Hexinyl	H	=O	=O	Hexinyl pyruvate
Heptinyl	H	=O	=O	Heptinyl pyruvate
Octinyl	H	=O	=O	Octinyl pyruvate
Branched Alkinyl				
Isopentinyl	H	=O	=O	Isopentinyl pyruvate
Isohexinyl	H	=O	=O	Isohexinyl pyruvate
Isoheptinyl	H	=O	=O	Isoheptinyl pyruvate
Isooctinyl	H	=O	=O	Isooctinyl pyruvate
Cycloalkinyl				
Cyclooctinyl	H	=O	=O	Cyclooctinyl pyruvate
Alkoxyalkyl				
Methoxymethy l	H	=O	=O	Methoxymethyl pyruvate

Ethoxymethyl	H	=O	=O	Ethoxymethyl pyruvate
Methoxyethyl	H	=O	=O	Methoxyethyl pyruvate
Alkoxycarbonylalkyl				
Methoxycarbonylmethyl	H	=O	=O	Methoxycarbonylmethyl pyruvate
Ethoxycarbonylmethyl	H	=O	=O	Ethoxycarbonylmethyl pyruvate
Aryl				
Phenyl	H	=O	=O	Phenyl pyruvate
Naphthyl	H	=O	=O	Naphthyl pyruvate
Sugar	H			
Glucosyl	H	=O	=O	Gucosyl pyruvate
Galactosyl	H	=O	=O	Galactosyl pyruvate
Mannosyl	H	=O	=O	Mannosyl pyruvate
Alkyl	**Alkyl**			
Methyl	Methyl	=O	=O	Methyl 2-oxobutanoat
Ethyl	Methyl	=O	=O	Ethyl 2-oxobutanoat
Ethyl	Ethyl	=O	=S	S-Ethyl 2-oxopentanethionate
Propyl	Methyl	=O	=O	Propyl 2-oxobutanoat
Butyl	Methyl	=O	=O	Butyl 2-oxobutanoat
Methyl	Ethyl	=O	=O	Methyl 2-oxopentanoate
Ethyl	Ethyl	=O	=O	Ethyl 2-oxopentanoate
Propyl	Ethyl	=O	=O	Propyl 2-oxopentanoate
Butyl	Ethyl	=O	=O	Butyl 2-oxopentanoate
Methyl	Propyl	=O	=O	Methyl 2-oxohexanoate
Ethyl	Propyl	=O	=O	Ethyl 2-oxohexanoate
Propyl	Propyl	=O	=O	Propyl 2-oxohexanoate
Butyl	Propyl	=O	=O	Butyl 2-oxohexanoate
Methyl	Butyl	=O	=O	Methyl 2-oxoheptanoate
Ethyl	Butyl	=O	=O	Ethyl 2-oxoheptanoate
Propyl	Butyl	=O	=O	Propyl 2-oxoheptanoate
Butyl	Butyl	=O	=O	Butyl 2-oxoheptanoate
Branched Alkyl	**Alkyl**			

Isobutyl	Methyl	=O	=O	Isobutyl 2-oxobutanoate
Isobutyl	Ethyl	=O	=O	Isobutyl 2-oxopentanoate
Isobutyl	Propyl	=O	=O	Isobutyl 2-oxohexanoate
Isobutyl	Butyl	=O	=O	Isobutyl 2-oxoheptanoate
Cyclo Alkyl	**Alkyl**			
Cyclohexyl	Ethyl	=O	=O	Cyclohexyl 2-oxopentanoate
Cyclohexyl methyl	Ethyl	=O	=O	Cyclohexylmethyl 2-oxopentanoate
Sugar	**Alkyl**			
Glucosyl	Methyl	=O	=O	Glucosyl 2-oxobutanoate
Glucosyl	Ethyl	=O	=O	Glucosyl 2-oxopentanoate
Alkyl	**Alkenyl**			
Propyl	Butenyl	=O	=O	Propyl 2-oxoheptenoate
Cycloalkyl	**Alkenyl**			
Cyclohexyl	Butenyl	=O	=O	Cyclohexyl 2-oxoheptenoate
Alkyl	**Alkinyl**			
Propyl	Butinyl	=O	=O	Propyl 2-oxoheptinoate
Butyl	Butinyl	=O	=O	Butyl 2-oxoheptinoate
Cycloalkyl	**Alkinyl**			
Cyclohexyl	Butinyl	=O	=O	Cyclohexyl 2-oxoheptinoate
Alkoxyalkyl	**Alkyl**			
Methoxymethyl	Ethyl	=O	=O	Methoxymethyl 2-oxopentanoate
Ethoxymethyl	Ethyl	=O	=O	Ethoxymethyl 2-oxopentanoate
Methoxyethyl	Ethyl	=O	=O	Methoxyethyl 2-oxopentanoate

Alkoxycarbonylalkyl	**Alkyl**			
Methoxycarbonylmethyl	Ethyl	=O	=O	Methoxycarbonylmethyl 2-oxopentanoate
Ethoxycarbonylmethyl	Ethyl	=O	=O	Ethoxycarbonylmethyl 2-oxopentanoate
Alkyl	**Alkoxycarbonylalkyl**			
Ethyl	Methoxycarbonylmethyl	=O	=O	Ethyl-4-methoxycarbonyl-2-oxobutanoate
Ethyl	Ethoxycarbonylmethyl	=O	=O	Ethyl-4-ethoxycarbonyl-2-oxobutanoate
Alkyl	**Alkoxyalkyl**			
Ethyl	Methoxy methyl	=O	=O	Ethyl 4-methoxy-2-oxobutanoate
Ethyl	Ethoxymethyl	=O	=O	Ethyl 4-ethoxy-2-oxobutanoate
Ethyl	Methoxyethyl	=O	=O	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 pyrvate, 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-oxo-butanoate, 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-oxo-heptanoate,

isobutyl-2-oxobutanoate, isobutyl-2-oxohexanoate, cyclohexyl-2-oxopentanoate, cyclohexylmethyl-2-oxopentanoate, propyl-2-oxoheptenoate, cyclohexyl-2-oxoheptenoate, butyl-2-oxoheptinoate, methoxymethyl-2-oxopantanoate, 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-oxo-butanoate, 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-oxopantanoate, 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-oxo-butyrate
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.

Fungal cells 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.

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.

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.

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 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 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 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.

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.

Glyoxalase I (GLO1, alternatively abbreviated as Gly I,) is also known as (R)-S-lactoylglytythione methyl-glyoxal-lyase EC4.4.1.5), glyoxalase II (GLO2, alternatively abbreviated as Gly II) is also known as S-2-hydroxy-acylglutathione 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 and/or II, as well as to the respective glyoxalases of non-mammalian eukaryotic and prokaryotic organisms, such as glyoxalase I and II of fungi like yeast or other microorganisms.

Thus, the term "inhibiting glyoxalase I and/or II" encompasses the inhibition of the mammalian as well as the respective non-mammalian enzymes.

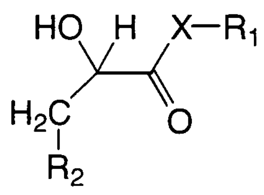
According to the invention, the substances according to formula (I) are for direct inhibition of glyoxalase I and/or II when R3 and R4 together are =O.

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 transformed, i.e. oxidized, to a substance wherein R3 and R4 together are =O.

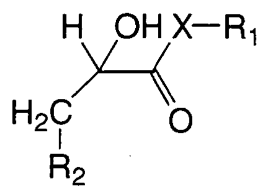
Said transformation/oxidization can be effected ex vivo, e.g. by means of a chemical oxidant, such as potassium permanganate. Other suitable oxidants are for example hydrogen peroxide, iodine, iodide benzoic acid and others.

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

Compounds of formula (I), wherein R3 or R4 is -OH, and which 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.

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,

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 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 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.

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 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:

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	=O	Hexenyl lactate
Heptenyl	H	H / OH	=O	Heptenyl lactate
Octenyl	H	H / OH	=O	Octenyl lactate
Branched Alkenyl				
Isopropenyl	H	H / OH	=O	Isopropenyl lactate
Isobutenyl	H	H / OH	=O	Isobutenyl lactate
Isopentenyl	H	H / OH	=O	Isopentenyl lactate

Isohexenyl	H	H / OH	=O	Isohexenyl lactate
Isoheptenyl	H	H / OH	=O	Isoheptenyl lactate
IsooctenyL	H	H / OH	=O	Isooctenyl lactate
Cycloalkenyl				
Cyclohexenyl	H	H / OH	=O	Cyclohexenyl lactate
Cyclohexenylmethyl	H	H / OH	=O	Cyclohexenylmethyl lactate
Cyclopentenyl	H	H / OH	=O	Cyclopentenyl lactate
Cyclopentenylmethyl	H	H / OH	=O	Cyclopentenylmethyl lactate
Alkinyl				
Ethinyl	H	H / OH	=O	Ethinyl lactate
Propinyl	H	H / OH	=O	Propinyl lactate
Butinyl	H	H / OH	=O	Butinyl lactate
Pentinyl	H	H / OH	=O	Pentinyl lactate
Hexinyl	H	H / OH	=O	Hexinyl lactate
Heptinyl	H	H / OH	=O	Heptinyl lactate
Octinyl	H	H / OH	=O	Octinyl lactate
Branched Alkinyl				
Isopentinyl	H	H / OH	=O	Isopentinyl lactate
Isohexinyl	H	H / OH	=O	Isohexinyl lactate
Isoheptinyl	H	H / OH	=O	Isoheptinyl lactate
Isooctinyl	H	H / OH	=O	Isooctinyl lactate
Cycloalkinyl				
Cyclooctinyl	H	H / OH	=O	Cyclooctinyl lactate
Alkoxyalkyl				
Methoxymethyl	H	H / OH	=O	Methoxymethyl lactate
Ethoxymethyl	H	H / OH	=O	Ethoxymethyl lactate
Methoxyethyl	H	H / OH	=O	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	Gucosyl 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-hydrxypentanethionate
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	=O	Propyl 2-hydroxyhexanoate
Butyl	Propyl	H / OH	=O	Butyl 2-hydroxyhexanoate
Methyl	Butyl	H / OH	=O	Methyl 2-hydroxyheptanoate
Ethyl	Butyl	H / OH	=O	Ethyl 2-

				hydroxyheptanoate
Propyl	Butyl	H / OH	=O	Propyl 2-hydroxyheptanoate
Butyl	Butyl	H / OH	=O	Butyl 2-hydroxyheptanoate
Branched Alkyl				
Isobutyl	Methyl	H / OH	=O	Isobutyl 2-hydroxybutanoate
Isobutyl	Ethyl	H / OH	=O	Isobutyl 2-hydroxypentanoate
Isobutyl	Propyl	H / OH	=O	Isobutyl 2-hydroxyhexanoate
Isobutyl	Butyl	H / OH	=O	Isobutyl 2-hydroxyheptanoate
Cycloalkyl				
Cyclohexyl	Ethyl	H / OH	=O	Cyclohexyl 2-hydroxypentanoate
Cyclohexylmethyl	Ethyl	H / OH	=O	Cyclohexylmethyl 2-hydroxypentanoate
Sugar	**Alkyl**			
Glucosyl	Methyl	H / OH	=O	Glucosyl 2-hydroxybutanoate
Glucosyl	Ethyl	H / OH	=O	Glucosyl 2-hydroxypentanoate
Alkyl	**Alkenyl**			
Propyl	Butenyl	H / OH	=O	Propyl 2-hydroxyheptenoate
Cycloalkyl	**Alkenyl**			
Cyclohexyl	Butenyl	H / OH	=O	Cyclohexyl 2-hydroxaheptenoate
Alkyl	**Alkinyl**			
Propyl	Butinyl	H / OH	=O	Propyl 2-hydroxyheptinoate
Butyl	Butinyl	H / OH	=O	Butyl 2-hydroxyheptinoate
Cycloalkyl	**Alkinyl**			

Cyclohexyl	Butinyl	H / OH	=O	Cyclohexyl 2-hydroxyheptinoate
Alkoxyalkyl	**Alkyl**			
Methoxymethyl	Ethyl	H / OH	=O	Methoxymethyl 2-hydroxypentanoate
Ethoxymethyl	Ethyl	H / OH	=O	Ethoxymethyl 2-hydroxypentanoate
Methoxyethyl	Ethyl	H / OH	=O	Methoxyethyl 2-hydroxypentanoate
Alkoxycarbonylalkyl	**Alkyl**			
Methoxycarbonylmethyl	Ethyl	H / OH	=O	Methoxycarbonylmethyl 2-hydroxypentanoate
Ethoxycarbonylmethyl	Ethyl	H / OH	=O	Ethoxycarbonylmethyl 2-hydroxypentanoate
Alkyl	**Alkoxycarbonylalkyl**			
Ethyl	Methoxycarbonylmethyl	H / OH	=O	Ethyl-4-methoxycarbonyl-2-hydroxybutanoate
Ethyl	Ethoxycarbonylmethyl	H / OH	=O	Ethyl-4-ethoxycarbonyl-2-hydroxybutanoate
Alkyl	**Alkoxyalkyl**			
Ethyl	Methoxymethyl	H / OH	=O	Ethyl 4-methoxy-2-hydroxybutanoate
Ethyl	Ethoxymethyl	H / OH	=O	Ethyl 4-ethoxy-2-hydroxybutanoate
Ethyl	Methoxyethyl	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, octyl lactate, isobutyl lactate, isopentyl lactate, isohexyl lactate, isoheptyl lactate, isooctyl lactate, cyclopentyl lactate, cyclopentylmethyl lactate, cyclohexyl lactate, cyclohexylmethyl lactate, butenyl lactate, hexenyl lactate, 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-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, 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-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 D-lactate is surprising as D-lactate is considered to be non-metabolizable in mammalian 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 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 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.

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 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 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 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 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.

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 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). 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 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 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).

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.

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, 1996; Vince and Daluge, 1970).

US 4,898,870 describes pyrroloquinoline quinone compounds in the context of inhibition of glyoxalase I. WO 99/035128 also 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 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 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.

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

<u>c) The known effects of substances according to formula (I) do not encompass glyoxalase inhibition</u>

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; Lembert et al., 2001). This effect is mediated by influencing potassium channels and mitochondrial effects. Inhibitory effects on LDH have also been proposed (Lluis and Bozal, 1976).

Furthermore, it has been described that the administration of ethyl pyruvate can improve inflammatory states, reperfusion injury, acute renal failure and ischemia (WO 03/088955; WO 02/074301; WO 01/024793, WO 05/044299, WO02/081020, US2003/232884). In patent US2004/110833 ethyl pyruvate is used to influence cytokine mediated diseases. This is attributable to abolishing the effect of NF-kß (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-kß 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-a and the activation of NF-kß (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.

<u>B. Pharmaceutical composition/manufacture of a medicament/methods of treatment</u>

The present invention relates to the medical use of compounds 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.

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 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 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.

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 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 invention. This does also not exclude the presence of non-pharmaceutiaclly 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 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).

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 present invention as well as their metabolites is of particular advantage.

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

For example, a combination of a compound of the present invention, such as ethyl pyruvate with common antifungal agents, such as one or more selected from fluconazole, ketoconazole, clotrimazole, bifonazole, econazole, itraconazole, terbinafine, naftifine, amorolfine, amphotericin B, nystatin, and nikkomycin, miconazolemiconazole, oxiconazole, sulconazole, flucytosine , griseofulvin, and biocidal peptides including defensines is preferably used.

A preferred combination consists of compounds of the present invention and an inhibitor of glycolysis wherein the 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.

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 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 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.

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 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 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 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 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 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, 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 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 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.

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 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.

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 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, 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 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 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 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.

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, intraauricular, subcutaneous, intramuscular, intradermal, intraperitonal, rectal, intranasal, epidural, percutanous, or transdermal administration, or administration as an aerosol, via mini-pumps, as mouth lavage, gel, cream, oil, ointment, 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 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.

C. Medical indications

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

The invention encompasses the administration or use in invertebrates, 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, 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 glyocolytic metabolism. Such diseases may further be associated with one or more selected form increased formation of oxoaldehydes, in particular of methylglyoxal, increased activity of glyoxalase I and/or II and increased cellular growth and/or proliferation. Preferably, the diseases associated with increased glycolytic metabolism are associated with enhanced methylglyoxal formation. Specific examples of such diseases include fungal infections, including the various specific examples of fungal infections discussed below.

According to the invention the cells proliferation of which is inhibited are mainly infectious organisms, in particular, fungi. Such fungi cause infectious diseases, such as various fungal diseases.

a) Fungal infections

It was surprisingly found that the substances of the invention can be used for the treatment and/or prophylaxis of fungal infections, and more specifically, inhibition and/or killing of fungi.

The term "treatment and/or prophylaxis of fungal infections" encompasses fungicidal and fungistatic effects.

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

US 5,580,902 discloses certain substances of the present invention as auxiliary agents in pharmaceutical compositions, in particular as enhancers for a multitude of active ingredients. This document does not disclose a fungicidal effect of substances of the invention. WO 02/102366 describes certain pyruvate esters for the treatment of fish-parasites, such as plathelmintes.

US 2005/0020678 discloses substances, which are not encompassed by the substances of the present invention, as fungicides. It provides no teaching that could be generalized to other substances, however.

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

According to EP 0 717 984 and JP 8 208 422 proliferation of normal human cells, for example keratinocytes, is even 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 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 3,920,835, US 4,246,261 and US 7,33,815.

Moreover, ethyl lactate can be added to shampoos used in the treatment of canine superficial bacterial pyoderma (de Jaham, 2003). 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 found, connected to the effect of pyruvate formed by hydrolysis of ethyl pyruvate. Moreover, methyl pyruvate has been suggested to treat fish parasites (WO 02/102366).

CN 1175632 describes ethyl lactate as an auxiliary substance in the manufacture of Spirulina wine, but does not disclose ethyl lactate as an active ingredient. WO 03/088955 and WO 02/074301 deal with reperfusion injury 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.

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

Living fungal 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.

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.

Thus, most parasites, such as pathogenic fungi, are specialized in the utilization of glucose in blood, body cavities and on the skin, and show a high rate of glycolysis. 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).

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).

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

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

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 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.

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 inactivation.

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

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 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).

Cells which cover their energy consumption exclusively by glycolysis in the presence of glucose are for example yeast cells. Similarly, pathogenic fungi, which can be treated according to the invention comprise, but are not limited to, Candida spp., Aspergillus spp., Cryptococcus spp., Pneumocystis spp., Zygomyces spp., Dermatophytes, Blastomyces spp., Histoplasma spp., Coccidoides spp., Sporothrix spp., Microsporidia spp., Malassezia spp. and Basidiomycetes, which also cover their energy consumption primarily via glycolysis when glucose is available in their human or animal hosts.

These organisms switch off other energy producing processes by glucose and use glycolysis (catabolite repression) or grow under hypoxic conditions.

As pathogenic fungi 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 fungal infections.

The term "fungal infection" encompasses superficial colonisation by fungi, e.g. of the skin or mucosa as well as systemic infections. Also encompassed are infections of the gastrointestinal tract. Compounds of the invention are used for the treatment of mucosal (topic) and/or systemic diseases. The mucosal diseases can be caused by oral or vaginal infections. The oral or vaginal infections are for example the consequence of AIDS, chemotherapy or an immune suppressive therapy or immune suppressive conditions.

The term "treatment" encompasses subjects suffering from any of the various disease stages, such as acute or chronic 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-fungal agents) is also part of the present invention.

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 disease has not fully evolved or has not been diagnosed.

The term "treating fungal infections" in the stricter sense relates to the treatment of clinically manifest disease. It is meant to encompass both cytotoxic and cytostatic effects. Thus, "inhibition of fungal cells" encompasses the inhibition of cell proliferation (fungistatic action) as well as the killing of the cells (fungicidal action). The killing of cells by necrosis or apoptosis is encompassed by the invention. The terms "proliferation" and "growth" are used interchangeably.

In one embodiment of the invention, the fungal infections are caused by a strain that is resistant to conventional antifungal agents, for example, fluconazole, ketoconazole, clotrimazole, bifonazole, econazole, itraconazole, terbinafine, naftifine, amorolfine, amphotericin B, nystatin, and nikkomycin, miconazolemiconazole, oxiconazole, sulconazole, flucytosine , griseofulvin, and biocidal peptides including defensins, in particular fluconazole. Thus, the invention in one embodiment relates to the treatment of fluconazole resistant Candida infections. Importantly, the compounds of the present invention also affect fungi that are resistant to conventional anti-fungal therapy, such as fluconazole resistant Candida, because they act via a different mechanism (Bennett et al, 2004).

b) Treatment of infections in immunosuppressed subjects

Fungal infections represent a significant problem in patients in an immunosuppressed state, and are amongst the leading cause of death, e.g. in transplant patients.

Thus, in one embodiment of the invention, the infection is in an immunosuppressed animal, 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.

Infections on an immunsuppressed 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.

c) Treatment of infections in cancer patients

Tumor patients are often in addition suffering from infectious diseases, due to a weakened immune defence, which results in a high sensitivity to infections.

Oftentimes cancer patients suffer from infectious disease caused by an ubiquitous, typically non-pathogenic organisms because of this weakened immune defence, also known as opportunistic infections.

It is therefore part of the invention to treat fungal infections 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 fungal infections, comprising opportunistic infections, in such patients.

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 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 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 fungal 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.

<u>d) Treatment of concomitant infectious disease</u>

Fungal infections may be accompanied by other infections, such as bacterial infections or protozoal 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, 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.

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, Iwami et al, 1995). Cells which cover their energy consumption solely by glycolysis when glucose is available are for example yeast cells, but also cells of multicellular organisms like Helminthes (e.g. Schistosoma). 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 by compounds of the present invention.

Specific examples of infectious organisms which can be treated according to the invention comprise Trypanosoma, Leishmania, Plasmodium, Toxoplasma, helmithes, Acrobacter, Actinobacillus, Actinomyces, Bacteroides, Brucella, Clamydia, Clostridium, Campylobacter, Escherichia, Enterobacter, Enterococcus, Eubacterium, Fusobacterium, Helicobacter, Hemophilus, Legionella, Listeria, Mycobacteria, Mycoplasma, Neissaria, Pasteurella, Peptostreptococcus, Pneumococcus, Pneumocystis, Porphyromonas, Prevotella, Pseudomonas, Salmonella, Shigella, Spirochetes, Staphylococcus, Streptococcus, Treponema, Vibrio, Yersinia, Escherichia coli or Pneumocystis carinii.

Importantly, the compounds of the present invention can also inhibit bacteria that are resistant against antibiotics, such as methicillin-resistant staphylococcus aureus (MRSA), which poses severe problems in the clinical setting (Cunha, 2005).

Prokaryotes including aerobic, anaerobic and facultative anaerobic bacteria, like fungi, perform glycolysis. Mechanisms exist in most of the bacteria even preferring the metabolism of the glucose (catabolite repression). Particularly in anaerobic organisms glycolysis is the most important way for generating energy.

Similarly, pathogenic protozoa, such as the bloodstream forms of trypanosoma, leishmania, plasmodium or toxoplasma, but also bilharzia, depend exclusively on glucose as energy source and undergo glycolysis.

In one embodiment of the invention, therefore, the animal, including man, is concomitantly suffering from a bacterial or protozoal infection as discussed above, including 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 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 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.

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 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) Anti-fungal applications

a) Plants

Fungal infections represent a major health and economic problem in plants.

The substances of the invention can also be used for anti fungal applications related to plants, including the treatment of fungal infections in plants. The substances can be used in an agricultural setting, or for plants kept indoors, both including culturing plants in liquid nutrient solutions. In the agricultural context the low toxicity of the substances of the present invention is a particular advantage. Thus, extended periods of rest to allow the active agent to decay are not necessary.

Moreover, the substances of the invention can be used to combat or prevent fungal growth when harvesting, storing, or transporting plants, or parts of plants, including fruits, and cereals like corn or maize, rice, potatoes, tomatoes or hops. Of particular advantage is the low toxicity of the substances of the invention. It can therefore be envisaged to contact plants or parts thereof, including fruit, which are intended for human or animal consumption, with the substance of the invention.

They can also be used in biotechnological plant culturing techniques.

b) Anti-fungal applications

Moreover, the invention relates to contacting substrates comprising metals, plastics, glass, concrete, limestone, soil particles, medical material and living substrates such as teeth with the substances of the invention as anti-fungal agents, or incorporating the substances into compositions, wherein they act as anti-fungal agents (i.e. fungistatic and/or fungicidal agents).

Thus, the compounds of the invention can serve as anti-fungal agents in all settings where antiseptic treatment is desired, and moreover serve as inhibitors of fungal contamination of compositions, such as compositions containing organic substances. These include, but are not limited to, drinkable compositions, food compositions, non-food compositions, such as decorative paints, wallpaper paste; coating agents comprising paint; glues; and cleaning agents comprising household and industrial cleaning agents etc. In one embodiment the substances of the invention can be incorporated into conventional cleaning agents, comprising e.g. tensids. Such cleaning agents can be for domestic or industrial use, in particular for use in a clinical setting.

c) Biofilms

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.

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

Fungi, like bacteria 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.

Sessile fungal cells within the biofilm are resistant to a range of antifungal agents currently in clinical use, such as triazoles and polyenes. 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).

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, 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 MS. Appl Environ Microbiol. 2000).

Biofilms containing microbes can be dangerous in space stations. In long-term missions fungi formation in closed systems can affect the astronauts health and water-thin 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 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).

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 bacteria due to low pH-value (3.8-4.7). Most important beer-spoiling organisms belong to the genera Lactobacillus, Pediococus, Pectinatus, and to yeast representing Saccharomyces or Dekkera. Beer-spoiling bacteria are facultative or obligate anaerobes and are acidophilic or at least acidotolerent.

Biofilms have been found in brewery pasteurizers and conveyor systems. Bacteria associated with biofilms with conveyor tracks and bottles and can warmers belong to Pseudomonas, Enterobacter, Bacillus, and to yeast representing Saccharomyces, Candida, Rhodotorula and others (Storgards E, et al, J. Am. Soc. Brew. Chem. 2006).

Consequently, the present invention encompasses the combating of biofilms in all of the above settings, comprising 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, 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 fungi attached to a substrate by means of an extracellular matrix produced by said bacteria, and comprising said bacteria. 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 are 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 fungi, but also other organisms, such as e.g. bacteria and protozoa. Biofilms are typically colonized by other organisms in addition to fungi. 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 cathers, 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 others).

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.

Particular applications of the substances of the invention to combat fungi, 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.

d) 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 fungi. As a matter of fact, many fungal diseases are mainly transmitted by contaminated drinking water.

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 fungi from the drinking water.

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

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).

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

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).

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.

Fig. 8: Inhibition of growth of yeast cells by ethyl pyruvate (EP).

Fig. 9: Effect of ethyl pyruvate (EP) on growth of different microbial species.

EXPERIMENTS

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 e = 2,86 mM -1 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.

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 (e = -3,1 mM -1 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 Saccharomyces cerevisiae HD 65-5a

A colony of strain HD 65-5a (Saccharomyces cerevisiae) was inoculated in 5 ml YPD-medium [(2% glucose (Fluka), 1 % yeast extract (BD, Sparks), 2% peptone (BD, Sparks)] in a rotating

20 ml glass tube and was incubated overnight at 30°C. At an O.D. of 12,9 (580 nm) a starting density of O.D. 0,19 was adjusted by dilution with a culture medium.

The culture was aliquoted to 10 ml (in 20 ml glass tubes), and the respective test substances were added. The aliquots were incubated at 30°C under continuous rotation of the tubes (200 U/min). At the indicated times, samples were removed and optical density was determined at a wavelength of 600 nm.

<u>e) Protocol for experiments using LNCaP cells (androgen dependent prostate carcinoma cells; DSMZ No ACC 256)</u>
An identical number of LNCaP cells (androgen dependent prostate carcinoma cells; DSMZ No ACC 256) were routinely cultured in 75 cm^2 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 humidified atmosphere (relative humidity >95%) of 5% CO_2 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 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 of the plate by trypsin/EDTA (Gibco; No. 25300-054) and were pelleted. After resuspension and homogenization the cells were counted using a hemocytometer.

<u>B. Data</u>

Example 1

Inhibition of the enzymatic activity of glyoxalase I of yeast 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) to the measuring reagent.

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

Example 2

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 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 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 IC_{50}s, while the alkyl 2-hydroxy-derivatives revealed no 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

Alkyl 2-Oxo-		IC_{50} GLOI*

Derivatives		
Ethyl 2-oxobutyrate	CH3-CH2-O-CO-CO-CH2-CH3	1,8 mM
Butyl pyruvate	CH3-CH2-CH2-CH2-O-CO-CO-CH3	7,5 mM
Ethyl pyruvate	CH3-CH2-O-CO-CO-CH3	10 mM
Methyl pyruvate	CH3-O-CO-CO-CH3	15 mM
Diethyl oxalate	CH3-CH2-O-CO-CO-OCH2-CH3	>50mM
Alkyl-2-Hydroxy Derivatives		
(-)-Butyl L-Lactate	CH3-CH2-CH2-CH2-O-CO-CHOH-CH3	no inhibition
(-)-Ethyl L-Lactate	CH3-CH2-O-CO-CHOH-CH3	no inhibition
(+)-Ethyl D-Lactate	CH3-CH2-O-CO-CHOH-CH3	no inhibition
Methyl L-Lactate	CH3-O-CO-CHOH-CH3	no inhibition
(-)-Isopropyl L-Lactate	CH3-CH(CH3)-O-CO-CHOH-CH3	no inhibition
(+)-Isobutyl L-Lactate	CH3-CH(CH3)-CH2-O-CO-CHOH-CH3	no inhibition

*GLO I = glyoxalase I

<u>Example 3</u>

Effect of prodrugs

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.

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.

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.

<u>Example 4</u>

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

<u>Synthesis of 2-Oxopropanethioic acid S-ethyl ester</u>

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 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 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 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).

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.

<u>Determination of glyoxalase I activity</u>

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.

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

Example 5

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

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) (triangle), BP (prepared according to the instructions of patent JP 11080089; 98 %) (circle) and BL (Fluka, 69819; lot. 443090/1 21503090) (squares) to the measuring reagent (Fig. 3).

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 influence the reaction of glyoxalase when it is not transformed into BP.

Example 6

Influence of ethyl pyruvate (EP) on the enzymatic activity of 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 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 with 0.1 M MES buffer, pH 6,5 to an O.D. of 4 and were then disrupted in a glass mill (Schwock 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, 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 (ε =13.6 mM^{-1} cm^{-1}). The activity of the glyoxalase II (IU) correlates with the amount of enzyme hydrolysing 1µmol of S-D-lactoyl-glutathion/min.

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.

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.

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.

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).

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 measuring reagent.

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

Example 9

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

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 (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 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), 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 µl 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.

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.

Example 10

Inhibition of growth of yeast cells by ethyl pyruvate (EP).

A colony of Saccharomyces strain HD65-5a was cultured as describe above.

Increasing concentrations of ethyl pyruvate were added and the culture continued as described above. After defined intervals 1 ml samples were removed and the O.D. was measured at 600 nm.

The experiment (Fig. 8) shows that EP inhibits the growth of yeast cells in a concentration dependent manner.

Example 11

Inhibition of the growth of yeast cells (Saccharomyces cerevisiae) by ethyl pyruvate (EP), butyl pyruvate (BP), ethyl L-lactate (LEL) and oxidized ethyl L-lactate (LEL).

A colony of Saccharomyces strain HD65-5a was cultured as describe above. Increasing concentrations of EP, BP and LEL (without pre-treatment) were added. LEL, which had been oxidized with potassium permanganate (KMnO4), was added to another aliquot. Samples of 1 ml were taken after 8 hours and were measured at 600 nm.

The experiment (Table 5) shows that EP and BP inhibit the growth of yeast cells in a concentration dependent manner. LEL did not influence growth, whereas in contrast, oxidized LEL affects proliferation.

Table 5:

	Concentration of the inhibitor				
Inhibitor	without	1 mM	10 mM	20 mM	50 mM
	Number of cells in [%] of the total cell number in the sample without inhibitor				
Ethyl pyruvate (EP)	100	94	23	11	9.4
Butyl pyruvate (BP)	100	96	88	73	14
Ethyl L-lactate (LEL) + $KMnO_4$	100	101	94	75	62
Ethyl L-lactate (LEL)	100	102	98	91	95

Example 12

Inhibition of a fluconazole resistant strain of Candida albicans by ethyl pyruvate (EP), butyl pyruvate (BP), and ethyl 2-oxobutyrate (EOB).

A colony of the fluconazole resistant Candida albicans strain A435 5 was cultured in the same medium and under the same conditions as used for Saccharomyces HD65-5a.

At an O.D. of 14.4 (600 nm) a starting density of O.D. 0.20 was set by dilution with culture medium. The preparation was aliquoted to 10 ml (in 20 ml glass tubes), increasing concentrations of EP, BP and EOB were added and were incubated at 30°C with continuous rotation of the tubes (200 U/min).

Samples of 1 ml were taken after 5 hours and the O.D. at 600 nm was determined as a standard for the cell number. The resistance against fluconazole was determined according to the guidelines of NCCLS (1997). Resistance is defined as growth at a dosage of ≥ 64 µg/ml of fluconazole.

The experiment (Table 6) shows that EP, BP and EOB inhibit the growth of fluconazole resistant Candida albicans cells.

Table 6

	Concentration of the inhibitor				
Inhibitor	without	1 mM	10 mM	25 mM	50 mM
	Number of cells in [%] of the total cell number in the sample without inhibitor				
Ethyl 2-oxobutyrate (EOB)	100	81	5,7	1,4	1,7
Ethyl pyruvate (EP)	100	94	1.8	0.9	0.9
Butyl pyruvate (BP)	100	100	42	15	7

<u>Example 13</u>

Inhibition of the growth of a fluconazole sensitive strain of Candida albicans by ethyl pyruvate (EP) and butyl pyruvate (BP).

A colony of the fluconazole sensitive Candida albicans strain R/HO13 was cultured as described above.

After aliquoting, increasing concentrations of EP and BP were added and were incubated at 30°C under continuous rotation of the tubes (200 U/min). Samples of 1 ml were taken after 9 hours and the O.D. was measured at 600 nm. The strain was sensitive against fluconazole at a dosage of ≤ 0.13 µg/ml.

The experiment (Table 7) shows that EP and BP inhibit the growth of fluconazole sensitive Candida albicans cells.

Table 7

	Concentration of the inhibitor				
Inhibitor	without	1 mM	10 mM	25 mM	50 mM
	Number of cells in [%] of the total cell number in the sample without inhibitor				
Ethyl pyruvate (EP)	100	89	3	1	1
Butyl pyruvate (BP)	100	100	55	15	7

<u>Example 14</u>

Effect of ethyl pyruvate (EP) on growth of different microbial species

The agar dilution method was used for antimicrobial susceptibility test. A series of plates (petri dishes; Greiner, CatNo. 933161) were prepared with 20 ml agar medium (Iso-Sensitest-Agar Ca.No. CM474, OXOID, Germany) to which various concentrations of EP (12.5mM to 40 mM) were added. Control agar plates were prepared without EP. The plates are then inoculated with a suitable standardized suspension of the test microbial species (10^4 cfu) using an automated spotter (spots 1-21). The agar plates were incubated under aerobic conditions at 37°C for 20 hours. At the respective positions of the spots growth (if permissive under the selected conditions) will result in the formation of cell discs. The evaluation of the test results is performed by inspecting the sizes of these discs.

The following microbials were spotted to the agar dilution: (1) Candida albicans ATCC 90028 (2) Candida albicans (Patient isolate), (3) Candida krusei, ATCC 6258, (4) Candida krusei (Patient isolate), (5) Candida tropicalis (Patient isolate), (6) Candida glabrata (patient isolate), (7) Candida parapsilosis, ATCC 22019, (8-21) prokaryotic controls.

The experiment (Fig. 9) shows that EP effectively inhibits the growth and proliferation of different fungi (1-7).

<u>Example 15</u>
Mouth lavage in a patient suffering from candiasis

A male patient at the age of 54 with a diagnosis of diabetes mellitus (presenting with a fasting blood sugar level of 6.88 mM) complained about dryness in the mouth, chapped lips and white blotchy alterations of the oral mucosa. An oral smear test showed an infection with Candida albicans. Determination of the germ number in the lavage of the oral cavity (10 ml physiological sodium chloride solution, 30 seconds) showed a value of > 100 CFU/ml of lavage.

The patient treated his mouth 3 times daily with a lavage of 10 ml ethyl pyruvate solution (75 mM ethyl pyruvate, 1 % glucose in physiological sodium chloride solution) over 4 days. A further determination of the number of germs after a lavage with 10 ml sodium chloride solution showed a reduction of germ number to < 5%.

The results show that the application of ethyl pyruvate is helpful for oral candidiasis.

<u>Example 16:</u>

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:

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

Example 17:

Pharmaceutical composition for bolus injection

A solution for bolus injection can be prepared according to Example 16, wherein the concentration of the substance of the invention is adapted accordingly.

Example 18:

Cream

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

aqueous phase:	butyleneglycol	4%
	substance of the invention	25%
	water	to 100%
lipid phase:	steareth-2	3%

steareth-21	2%
glycol-15-stearylether	9%
cetearylalcohol	2,5%

therafter addition of:

phenoxyethanol, methylparaben, ethylparaben, propylparaben,	
butylparaben	0,5%
butylenglycol	0,5%
tocopherole	0,2%

Example 19:

Ointment

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

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

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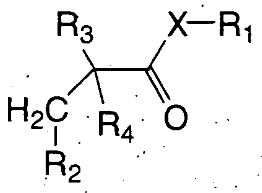
List of abbreviations

2-DG	2-deoxyglucose
AIDS	Aquired immunodeficiency syndrom
ATP	Adenosine triphosphate
ATCC	American Type Culture Collection
BBB	Blood Brain Barrier
BL	butyl lactate
BP	butyl pyruvate
CFU	Colony forming unit
CNS	Central nervous system
CSF	colony stimulation factor
DBL	butyl D-lactate
DEL	Ethyl D-lactate
DMEM	Dulbecco's modified Eagle Medium
DMSO	dimethyl sulfoxide
DSMZ	German Collection of Microorganisms and Cell Cultures GmbH (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH)
DTNB	5,5`-dithiobis(2-nitro benzoic acid)
E.C.	Enzyme Commission
EDTA	ethylene diamine tetraacetate
EOB	ethyl -2-oxo-butyrate
EP	ethyl pyruvate
FGF	fibroblast growth factor
FCS	fetal calf serum
GLO I	Glyoxalase 1
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IC-50	Inhibitor concentration to reach 50% of the maximal effect
IU	international unit
LBL	Butyl L-lactate
LEL	Ethyl L-lactate
MES	4-morpholine ethanesulfonic acid
MCT	monocarboxylate transporter
MIC	Minimal inhibition concentration
MRSA	Methicillin-resistant Staphylococcus Aureus
MSSA	Methicillin-sensitive Staphylococcus Aureus

NFkB	nuclear factor kappa B
INF	interferon
O.D.	optical density
PBS	phosphate buffered saline
RPMI	Rosswell Park Memorial Institute
SF	serum free
YPD	yeast peptone dextrose medium

Claims

1. Substance according to the general formula (I)



(I)

for use as an anti-fungal agent; 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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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 X is O, R2 is H and R3 or R4 is OH, R1 is not C1-C12 alkyl.

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-oxo-butyrate, 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. The substance according to any one of claims 1 to 6, having fungicidal effects.

8. The substance according to any one of claims 1 to 6, having fungistatic effects

9. The substance according to any one of claims 1 to 8 for the treatment of fungal infections.

10. The substance according to any one of claims 1 to 9 for contacting substrates as an anti-fungal agent.

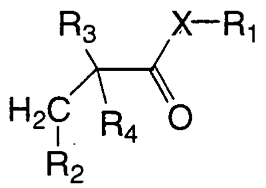
11. The substance according to claim 10, wherein said substrate is one or more selected from the group comprising metal, glass, plastic, wood, medical devices, medical material, concrete, limestone, plants or parts of plants including fruit and cereals.

12. The substance according to any one of claims 1 to 11, for use in a composition.

13. The substance according to claim 12, wherein said composition further comprises one or more surface active agents.

14. The substance according to claim 12 or 13, wherein said composition is selected from the group comprising wallpaper paste; coating agents comprising paint; glues; and cleaning agents comprising household and industrial cleaning agents.

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



(I)

for the treatment and/or prophylaxis of a fungal infection in an animal; 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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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 X=O, R2 is H and R3 or R4 is OH, R1 is not C1-C12 alkyl.

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

17. The pharmaceutical composition according to claim 15, wherein R1 comprises 1 to 4 carbon atoms and R2 is H or comprises 1 to 4 carbon atoms.

18. The pharmaceutical composition according to any one of claims 15 to 17, wherein R1, R2, or R1 and R2 are methyl, ethyl, propyl, isopropyl, butyl, or isobutyl.

19. The pharmaceutical composition according to any one of claims 15 to 18, 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.

20. The pharmaceutical composition according to any one of claims 15 to 18, 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.

21. The pharmaceutical composition according to any one of claims 15 to 20, wherein said substance according to formula (I) is present in a therapeutically effective concentration.

22. The pharmaceutical composition according to any one of claims 15 to 21, which comprises one or more additional pharmaceutically active ingredients.

23. The pharmaceutical composition according to claim 22, wherein said additional pharmaceutically active ingredient is selected from antifungal agents.

24. The pharmaceutical composition according to claim 23, wherein said antifungal agent is one or more selected from fluconazole, ketoconazole, clotrimazole, bifonazole, econazole, itraconazole, terbinafine, naftifine, amorolfine, amphotericin B, nystatin, and nikkomycin, miconazolemiconazole, oxiconazole, sulconazole, flucytosine , griseofulvin, and biocidal peptides including defensines.

25. The pharmaceutical composition according to any one of claims 15 to 24, which further comprises one or more auxiliary substances, comprising fillers, flavouring agents and stabilizers.

26. The pharmaceutical composition according to any one of claims 15 to 25, wherein said composition is in the form of a sustained release or controlled release galenic formulation.

27. The pharmaceutical composition according to any one of claims 15 to 26, which is for topic or systemic administration.

28. The pharmaceutical composition according to any one of claims 15 to 27, which is for one or more selected from the group comprising oral, intravenous, subcutaneous, intramuscular, intradermal, intraperitonal, intraauricular, rectal, intranasal, epidural, percutanous, transdermal, pulmonary administration, or administration as an aerosol, via mini-pumps, as mouth lavage, as an oil, cream, ointment, spray, gel, plaster, and via microbubbles.

29. The pharmaceutical composition according to any one of claims 15 to 28, which is for use as a food supplement and/or beverage supplement.

30. The pharmaceutical composition according to any one of claims 15 to 29, wherein said animal is selected from the

group comprising invertebrates, non-mammalian vertebrates, and mammals including humans.

31. The pharmaceutical composition according to any one of claims 15 to 30, which is for the treatment, prophylaxis, or treatment and prophylaxis of fungal infections resistant to antibiotic treatment.

32. The pharmaceutical composition according to claim 31, wherein said resistance is to one or more selected from the group comprising fluconazole, ketoconazole, clotrimazole, bifonazole, econazole, itraconazole, terbinafine, naftifine, amorolfine, amphotericin B, nystatin, and nikkomycin, miconazolemiconazole, oxiconazole, sulconazole, flucytosine , griseofulvin, and biocidal peptides including defensines.

33. The pharmaceutical composition according to any one of claims 15 to 32, which is for the treatment of infections of one or more of the genus Candida., Aspergillus, Cryptococcus, Pneumocystis, Zygomyces, Dermatophytes, Blastomyces, Histoplasma, Coccidoides, Sporothrix, Microsporidia, Malassezia and Basidiomycetes.

34. The pharmaceutical composition according to any one of claims 15 to 33, wherein said fungal infection is an opportunistic infection.

35. The pharmaceutical composition according to any one of claims 15 to 34, which is for use in an immunosuppressed animal including man.

36. The pharmaceutical composition according to claim 35, wherein said immunosuppression is associated with one or more selected from hereditary and acquired immune-defects.

37. The pharmaceutical composition according to claim 36, wherein said acquired immune defect is associated with one or

more selected from the group comprising HIV, organ transplantation, chemotherapy and exposure to radiation.

38. The pharmaceutical composition according to any one of claims 15 to 37, which is for use in an animal, including man, concomitantly suffering from one or more selected from bacterial and protozoal infection.

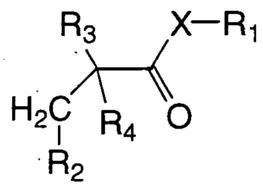
39. The pharmaceutical composition according to any one of claims 15 to 38, which is for use in an animal, including man, having a reduced blood glucose level.

40. The pharmaceutical composition according to any one of claims 15 to 39, which is for use in an animal, including man, concomitantly suffering from cancer.

41. The pharmaceutical composition according to any one of claims 15 to 40, wherein said animal, including man, is going to receive, is currently receiving, or has received conventional cancer therapy.

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

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

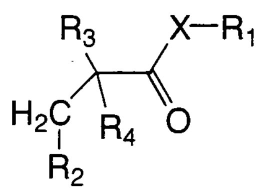


(I)

for the preparation of a medicament for the treatment, prophylaxis, or treatment and prophylaxis of a fungal infection; 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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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 X=O, R2 is H and R3 or R4 is OH, R1 is not C1-C12 alkyl.

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



(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 alkinyl, cycloalkinyl, 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 alkinyl, cycloalkinyl, 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.

to a mammal including humans in need thereof, wherein said mammal is suffering from a fungal infection,
with the proviso that when X=O, R2 is H and R3 or R4 is OH, R1 is not C1-C12 alkyl.

Fig. 1

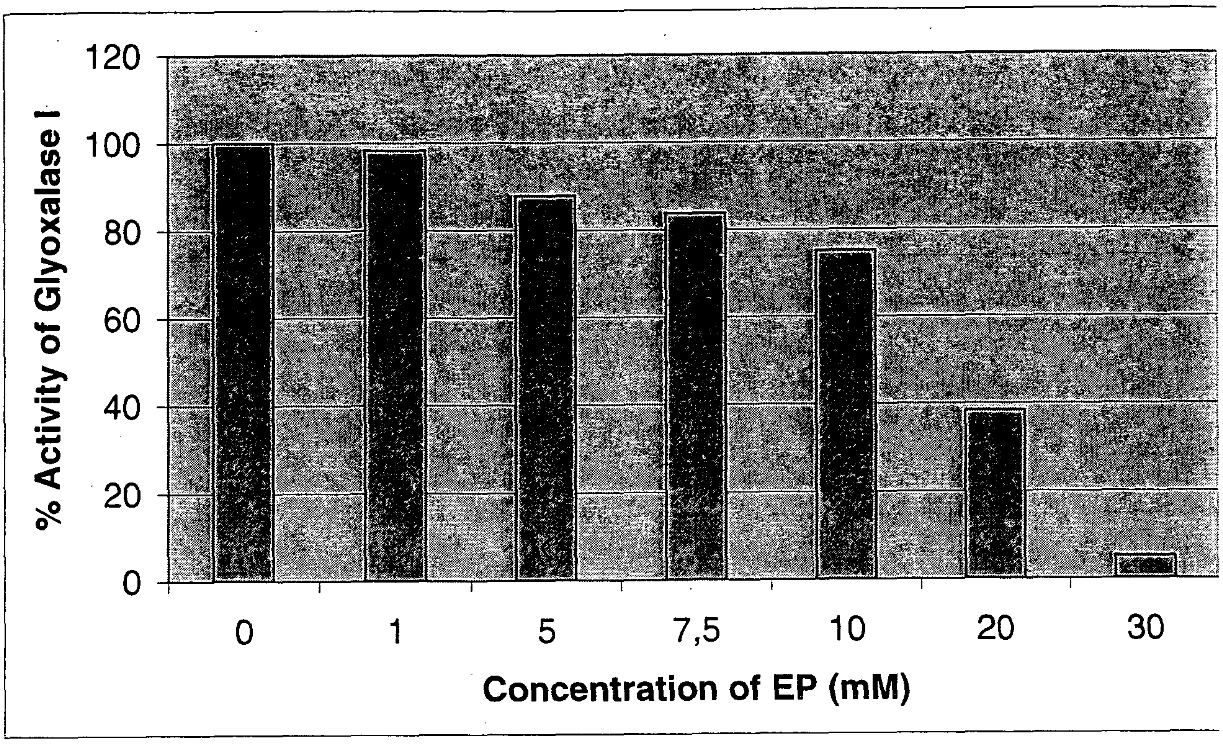

120
100
80
60
40
20
0
% Activity of Glyoxalase I
0
1
5
7,5
10
20
30
Concentration of EP (mM)

Fig. 2

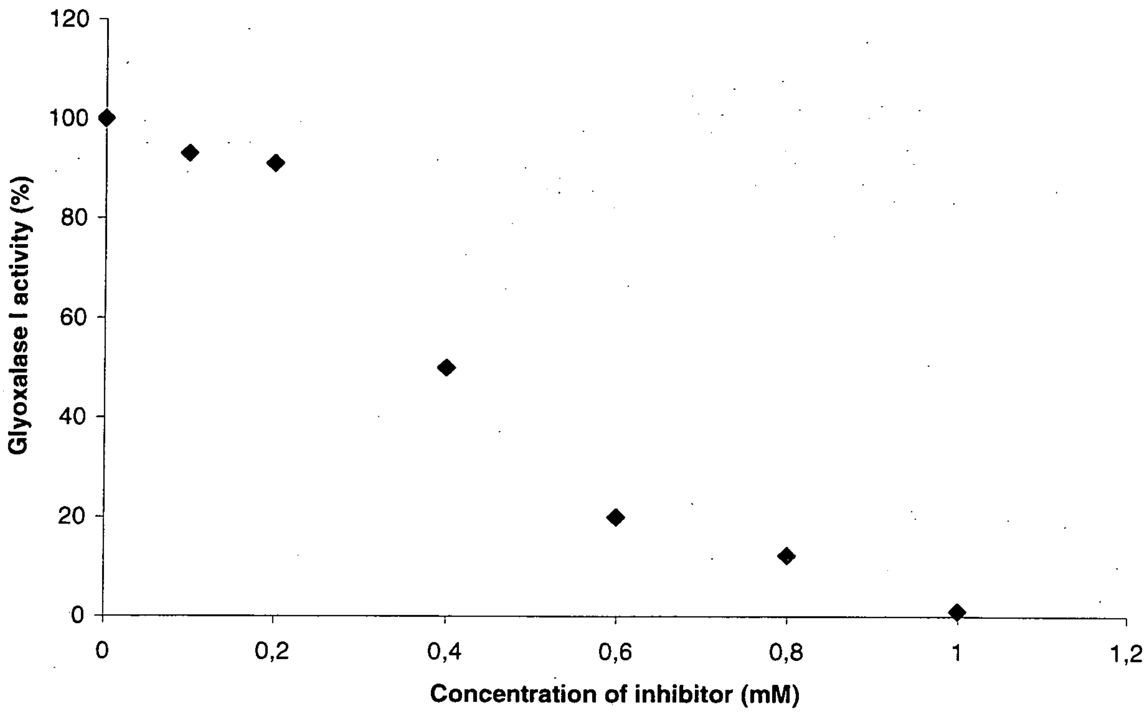

120
100
80
60
40
20
0
Glyoxalase I activity (%)
0
0,2
0,4
0,6
0,8
1
1,2
Concentration of inhibitor (mM)

Fig. 3

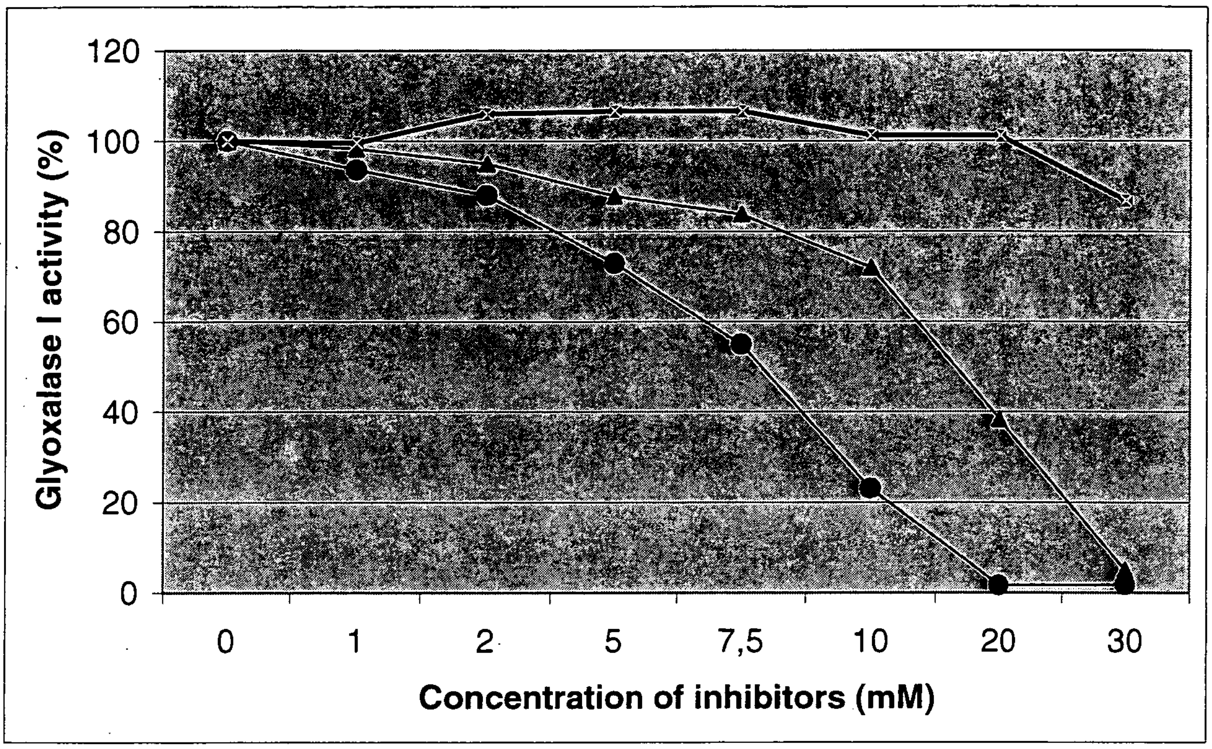

Glyoxalase I activity (%)
120
100
80
60
40
20
0
0
1
2
5
7,5
10
20
30
Concentration of inhibitors (mM)

Fig. 4

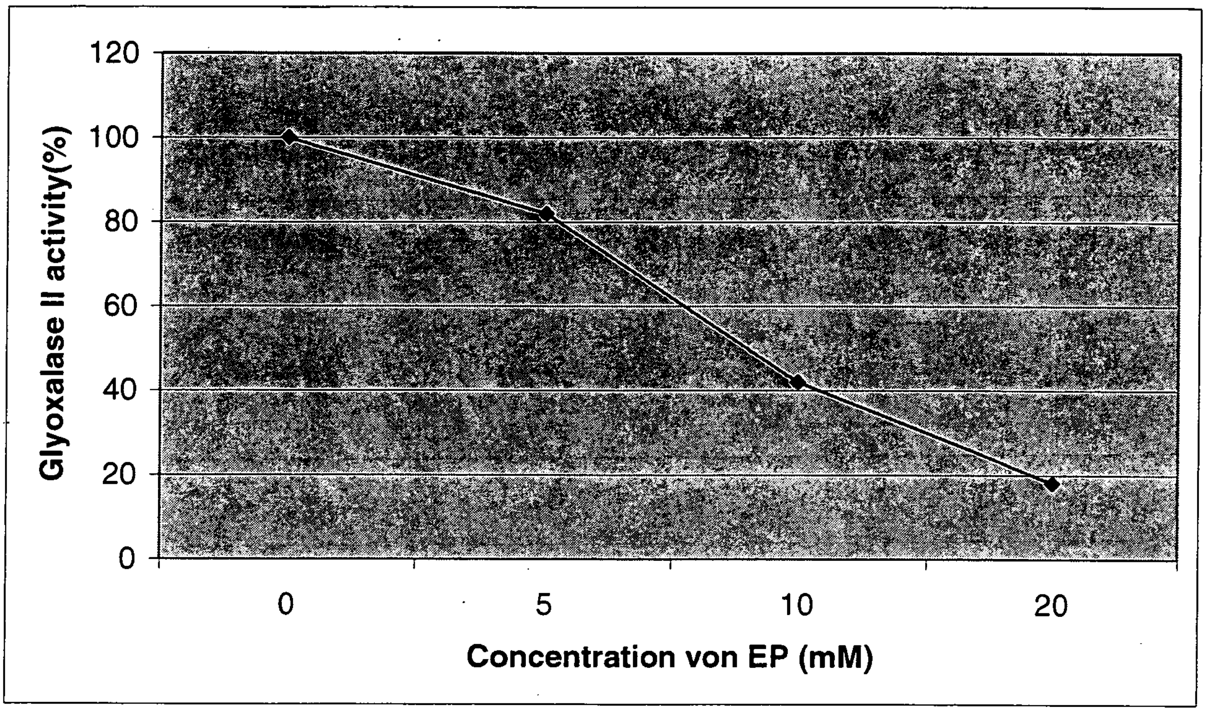

120
100
80
60
40
20
0
Glyoxalase II activity(%)
0
5
10
20
Concentration von EP (mM)

Fig. 5

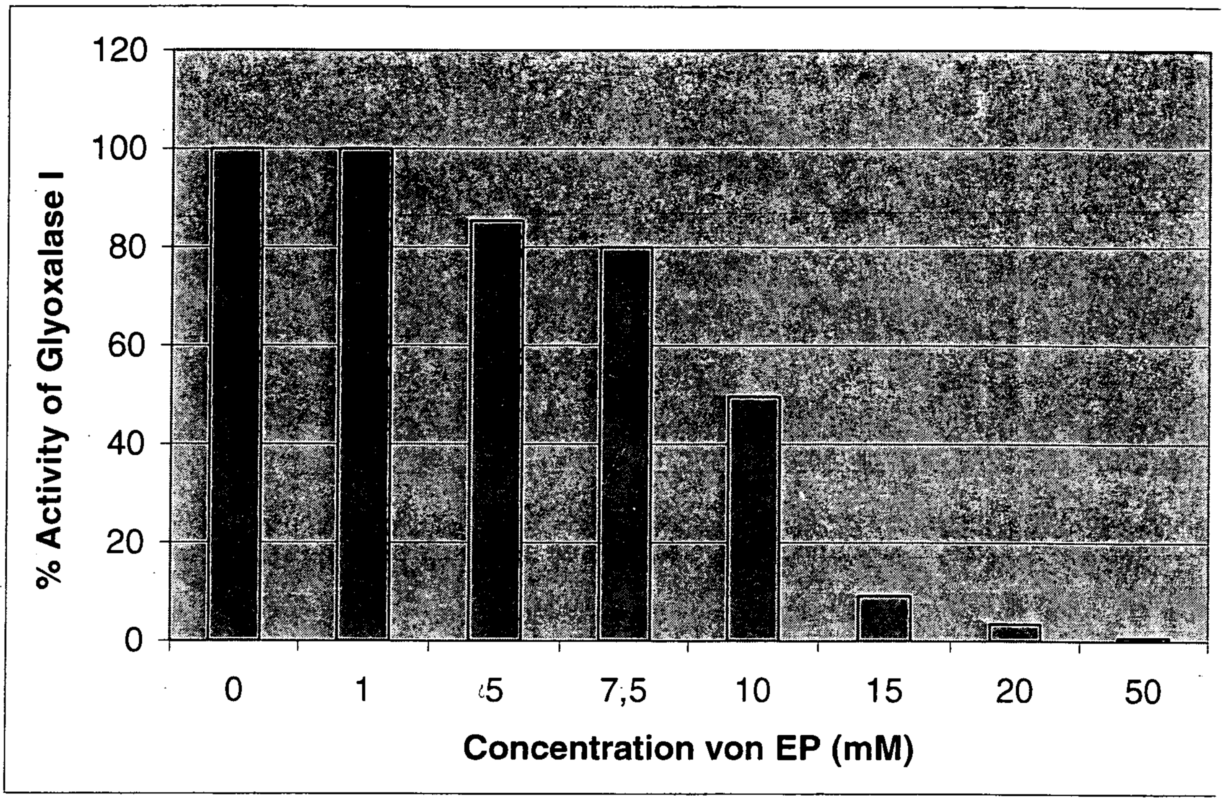

120
100
80
60
40
20
0
% Activity of Glyoxalase I
0
1
5
7,5
10
15
20
50
Concentration von EP (mM)

Fig. 6

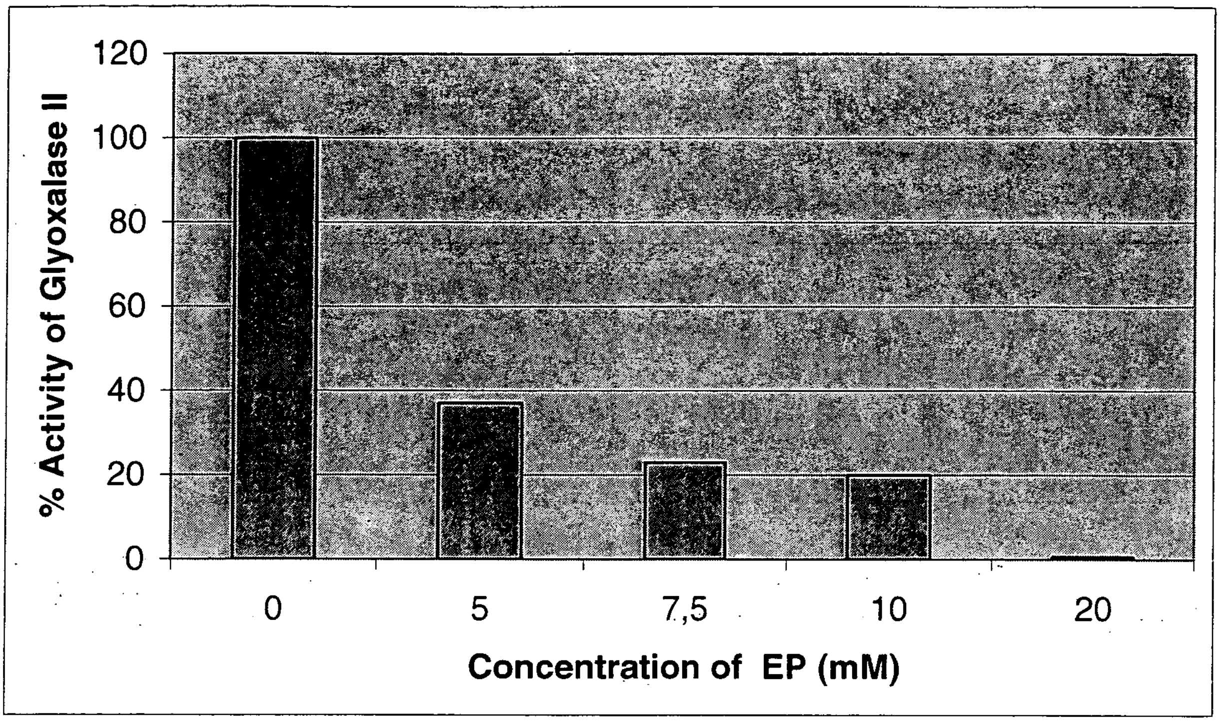

120
100
80
60
40
20
0
% Activity of Glyoxalase II
0
5
7,5
10
20
Concentration of EP (mM)

Fig. 7

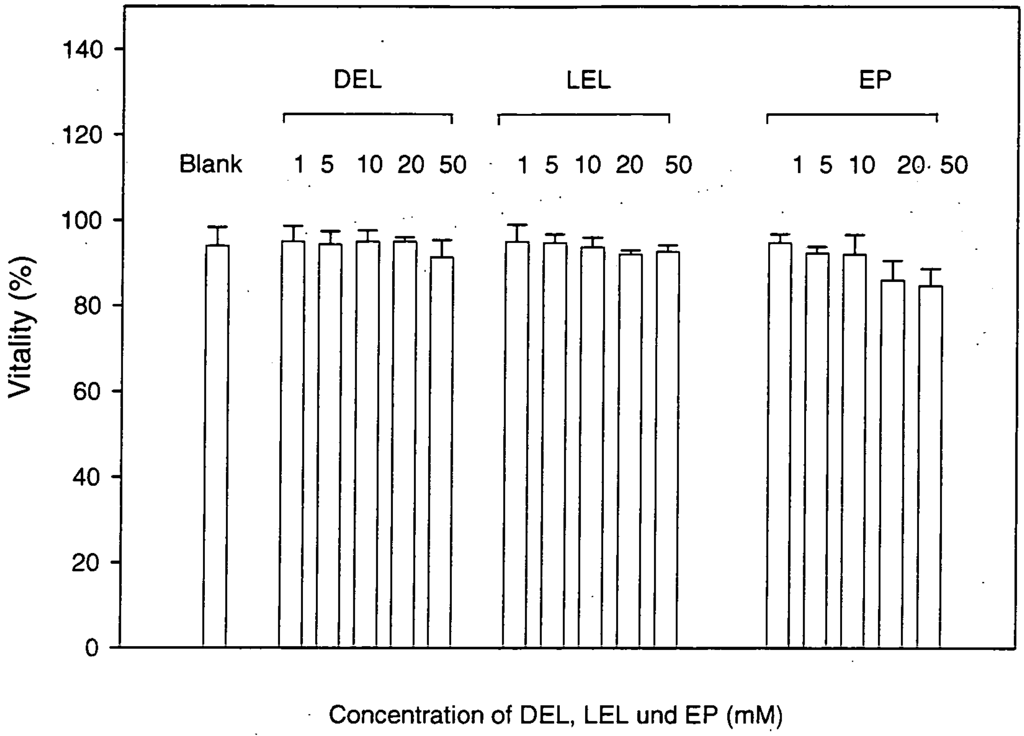

Vitality (%)
140
120
100
80
60
40
20
0
DEL
LEL
EP
Blank
1 5 10 20 50
1 5 10 20 50
1 5 10 20 50
Concentration of DEL, LEL und EP (mM)

Fig. 8

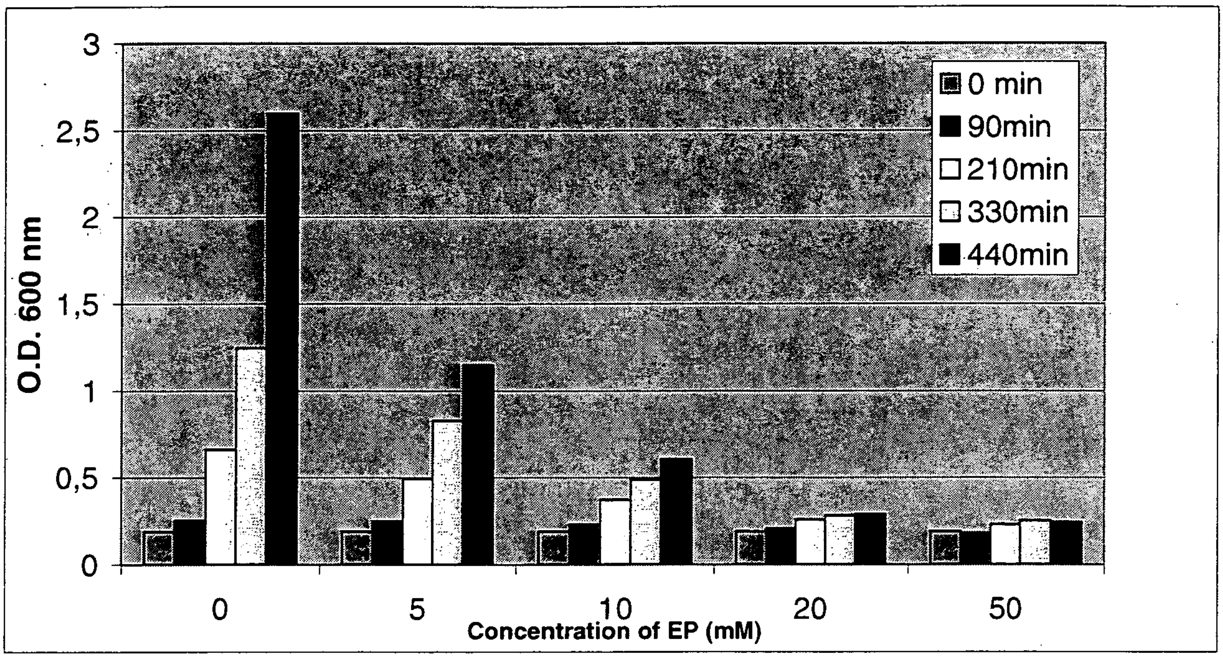
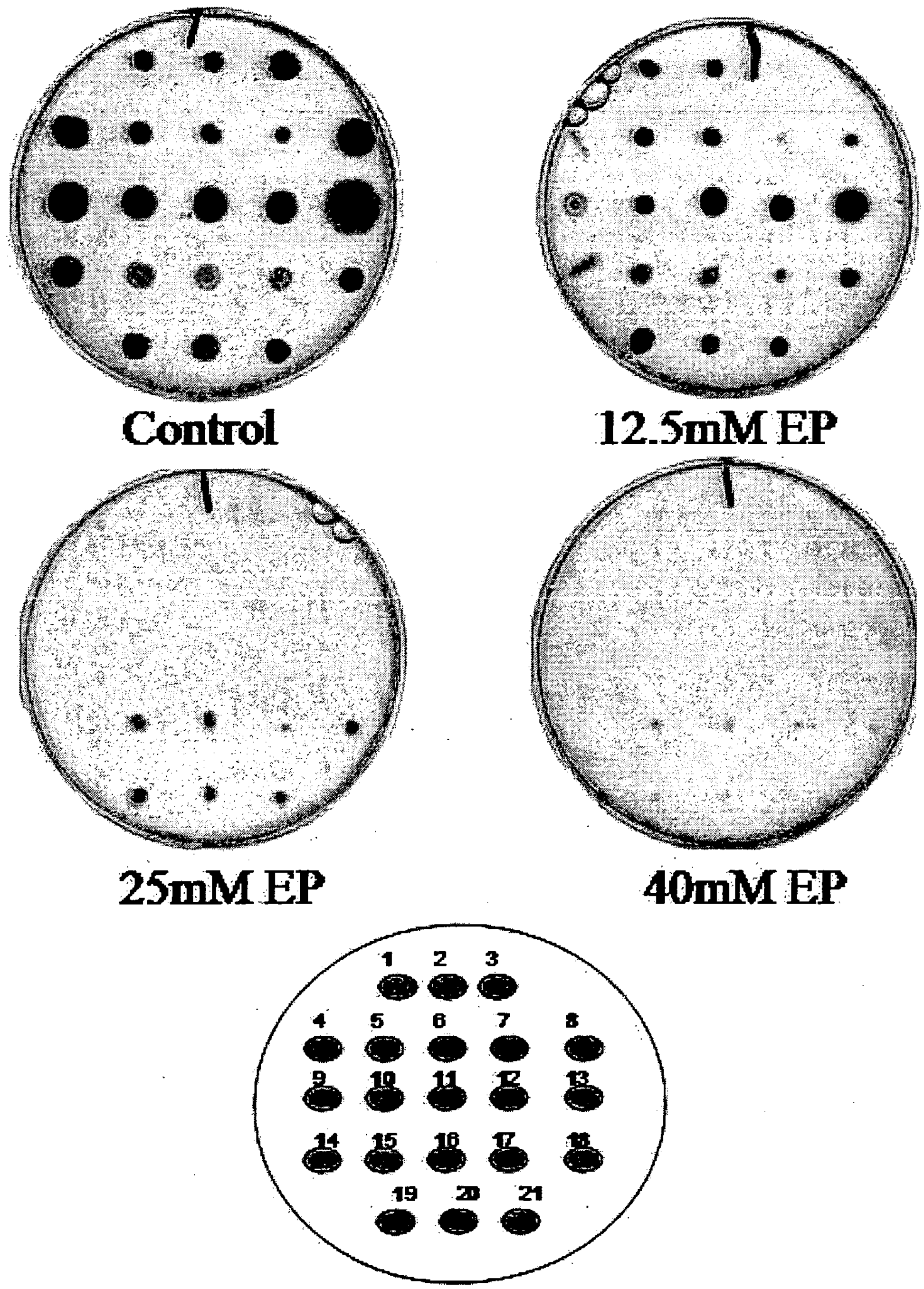

3
2,5
2
1,5
1
0,5
0
O.D. 600 nm
0 min
90min
210min
330min
440min
0
5
10
20
50
Concentration of EP (mM)

Fig. 9


Control
12.5mM EP
25mM EP
40mM EP
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21